LabAutomation2011 Table of Contents

Plenary Speaker Series

Monday, January 31, 9 am; Opening Keynote Address

Location: Primrose Ballroom B, Palm Springs Convention Center

Chad A. Mirkin, Ph.D.

George B. Rathmann Professor of Chemistry and Director of International Institute for Nanotechnology at Northwestern University; Member of the President's Council of Advisors on Science and Technology

The Polyvalent Oligonucleotide Nanoparticle Conjugate: A New Frontier in In Vitro Diagnostics and Intracellular Gene Regulation

Over the past decade, we have developed methods for modifying nanoparticles with oligonucleotides and explored how they can be used as designer constructs for preparing highly ordered, highly functional materials. Over the course of these studies, we have discovered many unusual fundamental properties that make these materials particularly useful in biodiagnostics and intracellular gene regulation. This session focuses on the rules that govern the use of these conjugates and sequence specific crystallization, high selectivity and sensitivity nucleic acid and protein detection, and "antisense" therapy. Specifically, the concept of the "antisense particle," as well as similarly functionalized siRNA particles, which exhibit a range of unique properties that make them very well-suited for gene regulation are introduced. In particular, the particles are highly resistant to nuclease digestion, have high and tailorable binding constants for target mRNA, and exhibit high entry efficiency into multiple cell types. Further, we can tailor the chemistry on the nanoparticle surface, and thus control the particles' binding strength to complementary target sequences, ultimately demonstrating that changing the binding strength or surface chemistries offers a means to control the degree of protein expression.

Tuesday, February 1, 8:30 am; Featured Plenary Speaker

Location: Primrose Ballroom B, Palm Springs Convention Center

Daryl Lund, Ph.D.

Editor-in-Chief, Journal of Food Science, Institute of Food Technologists; Emeritus Professor at the University of Wisconsin, Madison

Laboratory Sensitivity and Automation: Essential for Utilizing the Full Value of Food

From the beginning of commerce, those who could measure quantity were a part of the business team. That we have come a long way is a gross understatement. The food industry in particular required from the earliest days instrumentation that could be used to measure content. From my own university (UW-Madison) came the Babcock Test, used universally to determine the butterfat content of milk, a basis upon which farmers were paid. Our regulatory agencies have come to depend on the ability to measure ingredients and contaminants to assure consumers of value and safety and eliminate fraud. As knowledge has expanded to the point of marketing food because of its functional properties of promoting good health, it has become even more crucial to have laboratory analytical techniques that accurately measure chemical constituents. Now, as our ability to measure constituents becomes more and more sensitive, it calls into question, "What is a safe level?" These issues and the relationship between food and drugs are examined in this presentation. Progress in developing analytical laboratory equipment is essential if we are to realize the full potential of food for health.

Wednesday, February 2, 12:45 pm; Awards Luncheon & Closing Ceremony

Location: Primrose Ballroom B, Palm Springs Convention Center

John M. Butler, Ph.D.

NIST Fellow & Group Leader; Applied Genetics Group; Biochemical Science Division; Chemical Science & Technology Laboratory; National Institute of Standards and Technology

Lab Automation: A Necessary Part of the Future of Forensic DNA Testing

While TV shows like CSI: Crime Scene Investigation and NCIS make crime solving appear trivial and show scientists making conclusions at high speeds with full resolution, real forensic DNA laboratories today are overwhelmed with evidence needing to be analyzed. Budgets are shrinking while case backlogs are growing. Quality must not suffer in spite of demands for higher quantities of sample processing. Improved automation of laboratory processes is an obvious solution particularly in the area of data interpretation. This presentation reviews the current state-of-the-science and show where automation improvements have been made and can still be made to handle a growing number of DNA samples that need to be processed. Applications of DNA testing beyond forensic analysis are shown.

MP03

Christopher Baker, Florida State University, cbaker@chem.fsu.edu

Co-Author: Michael G. Roper

Automated Microfluidic Approaches to Fraction Collection for HPLC and Electrophoresis

Low cost fabrication methods, automated operation and a high degree of integration make microfluidic devices a convenient and economical alternative to commercially-available fraction collection systems. Two novel microfluidic fraction collection devices will be discussed which achieve automated fractionation of separated samples in zone electrophoresis and high performance liquid chromatography (HPLC). In the first device, effluent from a 3.5 cm electrophoretic separation channel was focused via two sheath flow channels into one of seven collection channels. By holding the collection channels at ground potential and varying the voltage ratio at the two sheath flow channels, the separation effluent was directed to either specific collection channels, or could be swept past all channels in a defined time period. As the sum of the voltages applied to the two sheath flow channels was constant, the electric field remained at 275 V/cm during the separation regardless of the collection channel used. The constant potential in the separation channel allowed uninterrupted separation for late-migrating peaks while early-migrating peaks were being collected. To minimize the potential for carryover between fractions, the device geometry was optimized using a three-level factorial model. The optimum conditions were a 22.5° angle between the sheath flow channels and the separation channel, and a 350 µm length of channel between the separation outlet and the fraction channels. Using these optimized dimensions, the device performance was evaluated by separation and fraction collection of a fluorescently-labeled amino acid mixture. In the second fraction collector, the outlet of a conventional HPLC column was connected to a nine-channel microfluidic device. Eight of the nine microfabricated channels were connected to 3 mL collection reservoirs in a poly(methyl methacrylate) manifold, while the last channel was connected to a 50 mL waste reservoir. These reservoirs were sealed and incorporated pressure outlets connected to computer-actuated solenoid valves. To direct the eluate from the HPLC column to a specific reservoir, all solenoid valves, except one, were closed, restricting the flow to the collection reservoir with the open solenoid valve. Control software, developed in-house, detected peaks in real time and automatically performed fraction collection based on signal threshold and timing parameters controlled by the user. The use of computer simulations in the development of microfluidic device geometries, as well as the technical challenges involved in scaling these devices from the flow rates typical of microchip electrophoresis (nL/min) to those typical of HPLC (mL/min) will also be discussed. The devices described present economical alternatives to conventional fraction collection technology while also offering the potential for integration into more complex automated analysis systems.

MP04

Krzysztof Churski, Institute of Physical Chemistry of the Polish Academy of Sciences, churski@ichf.edu.pl

Co-Authors: Tomasz Kaminski, Slawomir Jakiela, Piotr Garstecki

A Droplet Microfluidic Device for High-Throughput Screening of Reaction Conditions

Current standard in high throughput screening is set by the microtitier technology that provides a minimum reaction volume of ~ 2 µL and a minimum filling time of few seconds per well. From the very advent of droplet microfluidics the vision has been put forward for the droplets to serve as reaction beakers. This technology offers several attractive features: i) lack of dispersion of time of residence, ii) fast mixing, iii) control over kinetics of reactions, iv) improved statistics through repeated experiments, and v) minute consumption of reagents. These characteristics (fast mixing, temporal resolution and ability to integrate detection in-situ) have the potential of forming a basis for technology competitive with the microtiter platform. To date, the true potential of droplet microfluidics is largely unexplored, as it requires flexibility to perform arbitrary protocols of compositions of the reaction mixtures. For example, such integrated systems must allow for samples of reagents to be drawn from multiple sources, mixed, reacted and analyzed in a high-throughput combinatorial manner. We demonstrate a new droplet on demand (DOD) technique and an integrated system for scanning of arbitrary combinations of N solutions in ~ 1 µL droplets at 3 Hz. The system generates synchronized packets of droplets and merges them into reaction mixtures. The DOD system that we developed i) uses standard electromagnetic valves that are external to the microfluidic chip, ii) is compatible with virtually any microfluidic chip, iii) can generate arbitrarily large range of volumes of droplets, iv) has a maximum operational frequency of ~ 30 Hz, and v) has an on-chip footprint of less than 1 mm2. The integrated system that we demonstrate can be used to scan up to 10 000 conditions of chemical and biochemical reactions per hour using ~ 10 mL of solutions in total. The system that we developed can be particularly useful in microbiology in screening of epistatic interactions among genes and biologically active compounds. We have applied this technology to screening interactions between antibiotics acting on bacteria E. coli and demonstrated a scan revealing an antagonistic interaction between chloramphenicol and tetracycline within three hours, with the preparation time of the sequence of incubation mixtures spanning only 40 seconds. The system offers slightly higher rates and slightly smaller volumes of reaction mixtures than those offered by the microtitier technology. Development of a similar technology using piezoelectric valves provides a tenfold increase in the rate and a greater than tenfold decrease in the volumes of the samples extending the capabilities of automated droplet microfluidic chips well above the standard set by the well plate technology.

MP05

Caroline A. Davis, San Diego State University, carolinedavis44@yahoo.com

Co-Author: Christopher R. Harrison, San Diego State University

Paper Based Chromatographic Devices

The recent development of paper based diagnostic systems has inspired further investigations into the utility of paper based structures for analytical work. Here we present our development of a low-cost alternative to traditional liquid chromatography systems. Although liquid chromatography is a commonly used separation technique, its applicability can be limited due to the expense and large in size of the instruments; in particular, this makes offsite analysis very difficult. For our device the chromatographic support is cellulose based paper; this allows us the freedom to derivatize the support with the desired stationary phase through the use of various borate compounds. To achieve pressurized, directional flow through the paper chromatographic support the paper is embedded in polydimethylsiloxane (PDMS). This affords us a number of advantages, particularly with respect to the detection systems. As PDMS is transparent and non-conductive we can incorporate spectrophotometric and electrical detection systems directly into the structure of the device. Furthermore, proper design of the device, including T injection designs used in traditional lab-on-a-chip devices frees us from the need for an expensive, switching valve injection system. Finally, the low back pressure that results from the monolithic nature of the paper chromatographic support means that the entire system can be operated with inexpensive syringe or peristaltic pumps. We will present our advances on these designs and the results of our trials with developing ion exchange stationary phases.

MP06

Gerard Dumancas, Oklahoma State University, gerard.dumancas@okstate.edu

Co-Authors: Neil Purdie, Oklahoma State University; Lisa Reilly, Bethany College

Partial Least Squares (PLS1) Algorithm in Obtaining Meaningful Concentrations of Cholesterol and Polyunsaturated Fatty Acids in Human Serum

In the modern era, biomedical research plays a very critical role in human health. Within the biomedical research, scientists are searching for new biomarkers that would serve to identify the causes of obesity, coronary heart disease, diabetes, hypercholesterolemia, and cancer among others. Cholesterol and polyunsaturated fatty acids (PUFAs) are among the biomarkers associated with the previously mentioned diseases. Currently, several methods exist for the determination of cholesterol and PUFAs levels in human serum. Gas chromatography (GC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC) are the methods commonly used for PUFAs level determination in human serum. However, these methods are complicated, quite laborious, and suffer from the difficulty of obtaining meaningful concentrations. We have previously exploited various chemometric algorithms for the direct determination of cholesterol and PUFAs molar concentrations in synthetic mixtures and human serum. The simple colorimetric assay used is rapid, rugged, inexpensive, and specific to the -CH=CH-CH2- group that accomplishes, in a single assay the simultaneous quantitation of cholesterol, omega-3 (methyl esters of linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids), and omega-6 (methyl esters of linoleic, conjugated linoleic (CLA), and arachidonic fatty acids). Previously, ridge regression (RR), P-matrix regression (PM), principal component regression (PCR), and partial least squares (PLS2) successfully out-performed the K-matrix regression (KM) approach when applied to the study of prepared mixtures (synthetic sera) in chloroform solutions. In this paper, partial least squares in the form of PLS1 is investigated and applied to obtain meaningful concentrations in actual human serum samples. Results show that PLS1 yielded lesser root mean square error of prediction in the calibration model and more meaningful concentrations than PLS2 in the actual human serum samples. This technology offers the advantages of being time and cost saving as compared to other instrumental methods and would be most appropriate in its application in the actual clinical diagnostic settings.

MP07

Victoria Echeverria, BellBrook Labs, victoria.echeverria@bellbrooklabs.com

Co-Authors: Ivar Meyvantsson, Allyson Skoien, Casey Lamers, Daniella Echeverria, Tracy Worzella, Steven Hayes

An Automated High-Content Assay for Tumor Cell Migration Through 3-Dimensional Matrices

High-content tumor cell migration assays in 3-dimensional extracellular matrix are a powerful tool for modeling and understanding the biology of this critical step in the process of metastasis. Currently available methods are not amenable to increased throughput required by studies of comparative pharmacology or small scale screening. We present here an automated and miniaturized approach to high-content tumor cell migration assays. A standard screening-sized plate with an array of embedded microchannels was designed and constructed from common thermoplastics. After filling the channels with 3D matrix, cells were placed at one end of the channel and migration into the channel was monitored via an imaging system. All liquid handling steps were performed by standard liquid handling robotics. Tumor cell migration in the channel was truly 3-dimensional. The information-rich data from these assays was used to rank the potency of migration inhibitors through 3D collagen, as well as gain additional insights into the compounds' activities related to cell proliferation and health. Based on these insights, we have identified 3 classes of inhibitors that range from cytotoxic to purely anti-invasive. This approach is compatible with a variety of multiparametric, morphological and/or kinetic readouts, and the reduced cell number requirement is compatible with primary cell studies. Preliminary work with the laminin-rich matrix, Matrigel, exhibits a contrasting phenotype consistent with collective migration, as compared to collagen where the cells move individually.

MP08

Mikael Evander, Stanford University, evander@stanford.edu

Co-Authors: Burak Dura, Antonio J. Ricco, Gregory T. A. Kovacs, Laurent Giovangrandi, Stanford University

Dielectric Sheath Flows in Microfluidic Impedance Cytometers

Impedance cytometers are being increasingly used within the biomedical community for counting and measuring the sizes and/or dielectric properties of particles and cells. One of the benefits of miniaturized impedance cytometers is the possibility to use microfluidics to achieve dielectric sheathing of the sample in a simple fashion. We show here that dielectric sheathing can be used to improve the signal amplitude and signal-to-noise ratio (SNR) for impedance detection of single particles and cells. A microfluidic impedance cytometer with sensing electrodes, dielectrophoretic focusing electrodes and fluid inlets to allow for dielectric focusing was used to perform a characterization on how low-conductivity aqueous and two-phase dielectric sheathing affect the detection sensitivity and SNR. De-ionized water or oil was used as dielectric sheath together with a conductive core of phosphate buffered saline. The relative signal (deltal/l) from single 10 µm particles was measured and analyzed for core sizes ranging from 30 to 150 µm. A fivefold increase in the relative signal can be seen over the aqueous core widths. Utilizing a two-phase system, another fourfold increase is achieved. The SNR shows no significant change for the different aqueous cores. However, an improvement above 20 dB can be achieved by using the two-phase system. Dielectric sheathing enables the use of wider channels that are less prone to clogging and easier to manufacture. The precision in cell and particle size measurements can be increased and the limit for the smallest detectable object size lowered. The technique can be taken one step further by the using two-phase dielectric sheathing. As the two-phase flow is not limited by diffusion, it is possible to achieve even higher signal amplitudes and SNR. This will be especially useful when analyzing small particles and cells that normally are difficult to detect.

MP09

Paige Feikert, Wichita State University, pnfeikert@wichita.edu

Co-Authors: Krishna Vattipall, Shalini Prasad, Wichita State University

A Detailed Study of the Nano-Porous Membranes With Applications in the Enhanced Detection of Cardiovascular Biomarker Proteins

The goal of this work is to understand the role of nano confinement in designing biosensors. We have been investigating silicon based micro devices incorporated with nanoporous membranes in designing sensors. We have observed that nanoporous membranes enable nanoscale size based confinement of biomolecules such as proteins onto micro platforms. This in turn enhances the concentration of the biomolecules which in turn enhances the sensitivity in detecting biomolecules. It is critical that ultralow detection of biomolecules be achieved as they have significant impact in designing diagnostics platforms for early disease diagnosis. Commercially available nano-porous membranes made out of anodized alumina and polycarbonate are evaluated for their role in nano-confinement and enhancing sensitivity of detection. In this biosensor configuration sandwich assay, an electrical double layer is formed between a test protein (C-reactive protein) and the gold surface underneath the porous membrane. Using electrical impedance spectroscopy, the capacitance changes in the electrical double layer, translating to the sensitivity and the linear dose response over a large dynamic range will be analyzed for each of the physical characteristic of the porous membrane – pore densities, height of the pore and diameter of pore.

MP10

Laura A. Filla, Saint Louis University, fillal@slu.edu

Co-Authors: Laura C. Mecker, R. Scott Martin, Saint Louis University

Signal Enhancement and Improved Temporal Resolution for Microchip Electrophoresis With Electrochemical Detection

Microdialysis sampling is an in vivo sampling technique that has been used to continuously monitor neurotransmitter release in the brain. Our group has described a valving-based microchip that integrates microdialysis sampling with microchip electrophoresis and electrochemical detection to separate and detect any sampled neurotransmitters. While this approach offers fast analysis times, detection limits and temporal resolution must be further improved to study biological systems. Initially, the use of an electrode array was investigated to improve the limits of detection; it was found that two optimally-spaced palladium electrodes offer a 2.7X improvement in signal over a single electrode of the same total area. A new method to fabricate an eight electrode carbon-ink microarray was developed with the spacing between the electrodes being such that fresh analyte can diffuse across the surface of each of the eight electrodes. Although the use of more electrodes increases the length of the detection zone and thus decreases resolution, the array has been optimized so that the best compromise between signal and resolution is achieved, with the limit of detection for norepinephrine improving from 2.8 μM to 170 nM. This poster will also address the issue of minimizing Taylor dispersion and thus conserving temporal resolution in the microchip by segmenting the aqueous microdialysate with oil droplets, which confines the sample and prevents analyte diffusion within the flowstream. A corona treatment system is used to generate a hydrophobic/hydrophilic interface downstream, which desegments the droplets into a continuous aqueous flowstream prior to analysis. This approach enables the coupling of segmented flow with microdialysis sampling, microchip electrophoresis, and electrochemical detection to provide optimal temporal resolution throughout the device. The implications of segmented flow include the ability to monitor rapid concentration changes of neurotransmitters in near real-time, which is crucial in monitoring concentration dynamics for in vivo studies.

MP11

Santaneel Ghosh, Southeast Missouri State University, sghosh@semo.edu

Co-Author: Tong Cai, University of North Texas

Controlled Bending of Biocompatible, Multifunctional Magnetic Hydrogel Nanocomposites for Lab-on-a-Chip Applications

Externally tunable magnetic hydrogel nanocomposites hold great therapeutic potential for magnetic resonance imaging, organelle specific binding, intracellular spatial control, sequential release of drug molecules and flow path regulation in micro-fluidic devices. Smart polymers, conventionally poly(N-isopropylacrylamide) (PNIPAM) or polyethylene glycol (PEG) analogue-based systems are attractive for device fabrication because of their perceived intelligence to external stimuli, i.e., possession of lower critical solution transition (LCST) behavior at ~30-40C, close to normal physiological temperature. Alternating magnetic field can be applied to change the phase of the nano-magnet doped polymeric structures as the magnetic nanodots act as nano sources of heat when exposed to the oscillating magnetic field. Efficiency of the induced heat generation inside the colloidal medium can be controlled intrinsically by changing the size, concentration and composition of the nano-magnets or extrinsically by tuning the frequency and intensity of the applied magnetic fields. Recent studies have reported various magnetic hydrogel nano-composites and temperature regulation induced by remotely applied ac magnetic excitations. DC magnetic field induced bending has also been investigated. However, although, dc field initiates bending, it is not possible to change the phase of the polymer monolith, i.e., bending and shrinkage are not simultaneous. Remote controlled bending actuation is one of the key requirements for practical use of these systems in lab-on-a-chip applications or as soft actuators in switching devices. As ac magnetic field induced controlled modulation of the polymer networks can directly transform the absorbed energy into bending and shrinkage simultaneously, this novel approach may lead to a new category of magnetically responsive polymeric structures for potential applications in the field of smart gel based devices, such as sensors, artificial muscles, drug delivery systems, and film separation devices. In this work, a performance enhanced, alternating magnetic field responsive hydrogel nanocomposite system has been designed. The system is capable of producing fast mechanical response in an alternating magnetic field (bending from sides) over a wide range of field and frequency domains. The equilibrium bending angle of the polymer monolith was influenced by several factors: mechanical strength of the monolith, ac field induced temperature regulation and the surface evaporation. In addition, we found that both de-swelling and LCST were not affected due to the nanoparticle incorporation. Combined bending and shrinkage response, especially at the micro-scale indicates its great potential for application in micro-sensors and actuators, particularly in lab-on-a-chip applications. To our knowledge, this is the first time that quantitative oscillating field modulated bending actuation has been investigated for a tunable nano-composite system.

MP12

Siegfried F. Graf, CSEM SA, sgf@csem.ch

Co-Authors: Helmut F. Knapp; Ruoya Lee, Biopredic International, France; Thierry Madigou, Centre de Ressources Biologiques Xénope, Université de Rennes; Sebastian Hötzel, Institute for Toxicology and Genetics, Karlsruhe Institute of Technology

Image-Based Fluidic Sorting System for Large Biological Entities Demonstrated for Zebrafish Larvae Sorting Into 96-Well Plates and Automated Microinjection of Xenopus Oocytes

In drug discovery, ADMET or toxicity testing often high numbers of large single cells or small single organisms, such as Xenopus oocytes or Zebrafish embryos and larvae, are used. Usually, these biological entities have to be singled out from suspension, then subjected to some action, and finally analyzed. Currently, the main bottleneck is the cost-efficient removal of single unstained biological entities from a suspension, which precedes all other steps. Either the conventional manual removal significantly slows down the whole process or current automated removal methods make it very expensive. The low-cost cell sorter module presented here uses CMOS-cameras and self-learning, generic vision algorithms for real-time analysis of individual, transparent or opaque, and also fluorescently labeled biological entities with a size range of 200 to 2000 microns. These entities are circulated and stored in a semi-closed fluidic channel which allows continuous feeding with additional entities or exchange of fluid. The result of the vision algorithm triggers the actions of the compact fluidic system, containing miniature pumps and valves, in order to remove selected biological entities from the suspension. Criteria for the vision algorithm can easily be set by the user and can consist of size, shape, color, contrast but also more complex spatial combinations. Consequently, single biological entities can be delivered-on-demand to a subsequent system, which makes the cost-efficient cell sorter module ideally suitable for integration into an automated process. In a first example, the cell sorter is combined with a customized well plate feeder to demonstrate sorting of wild type and transgenic Zebrafish embryos, with and without chorion, into 96 multi-well plates. This feat is accomplished in only 9 minutes (less than 6 seconds per embryo), with a survival rate comparable to the control (6.6% control, 7.6% cell sorter).—(see video at http://www.youtube.com/watch?v=1ZtMQ-dI52E)—In another example, the same cell sorter is combined with an automated microinjection carousel. This combination can be used to automatically sort and remove individual Xenopus oocytes, based on their quality, and dose the viable ones into a dedicated microinjection carousel, where the oocytes are immobilized, microinjected, and finally collected in containers or, again, multi-well plates. The cycle time for one oocyte is about 10 seconds compared to the manual 2 minutes (average for quality control, sorting, injecting and collecting).—(see video at http://www.youtube.com/watch?v=eaURVbc13Y8)—These examples demonstrate two possible uses of the cell sorter in lab automation. The savings in time and money enables researchers to concentrate on the characterization phase of their experiments and to increase the statistical value of their results.

MP13

Björn Hammarström, Lund University, b.hammarstrom@gmail.com

Co-Authors: Johan Nilsson, Thomas Laurell, Simon Ekström

An Acoustic Trapping System for Monitoring Kinases in Small Cell Populations

A novel method for acoustic non-contact trapping of small cell populations (500 000 individuals) utilizing disposable glass capillaries is presented here as a viable option for automated handling and analysis of small cell/bead populations. By exiting the cross-sectional resonance of a large aspect ratio rectangular capillary (200x2000 µm2) with a small external ultrasonic transducer cells are trapped inside the capillary. Here, the cells quickly form a 3D cluster that is levitated in the vicinity of the transducer. The resonating capillary causes a strong acoustic force buildup making it possible to retain a fixed number of cells against high flow rates in the capillary.The acoustic trapping capillaries are operated in aspirate/dispense mode and automated using XYZ-robotics to address a 96-well-plate where automated fluid control allows samples to be deposited for analysis or aspirated to the trapping site for cell treatment. Interfacing the capillary with a standard plate format streamlines subsequent interrogation of analyte from the trapped population using standard equipment such as plate-readers or mass spectrometers. The system design enables a future high-throughput set-up using parallel trapping capillaries with 9 mm pitch (corresponding to the 96-well-plate format). The result is a highly versatile system closely resembling a pipetting robot and with the integrated feature of non-contact cell trapping. Successful application of the system for accessing drug uptake in red blood cells and platelets as well as monitoring kinase phosphorylation and inhibition has been shown by using miniaturized solid-phase extraction with MALDI-MS read-out. In the recent years many improved assays for screening kinase inhibition has emerged. Most of these assays are based on scintillation or fluorescence (e.g. ELISA, TRF and FRET) using phosphoantibodies or fluorescent substrates. While many of these techniques have high sensitivity and throughput addressing the complex cellular environment and the multidirectional pathways involved in kinase phosphorylation is a challenge. Here, mass-spectrometric monitoring of small cell populations has a clear niche as it provides label free molecular information, complementary to many standard assays. Our measurement system based on acoustic trapping easily facilitates label free monitoring of kinase-substrate/inhibition kinetics by use of isotope dilution as well as easy re-use of rare/expensive kinases located in the acoustic trap for e.g. IC50 determination for inhibitors. The systems greatest potential is cell-based analysis, the topic for our continued work. Here, discovery of kinase phosphorylation in small cell populations using isotope labeled ATP (O418–ATP) to track down phosphorylation substrates is a primary target. The small cell population should also allow for analysis of pathway activation and functional effects. Furthermore, simultaneous assessment of drug penetration and toxicity is possible on the small cell population.

MP14

Amy Hsiao, University of Michigan, ahsiao@umich.edu

Co-Authors: Yi-Chung Tung, Steven G. Allen, Yu-suke Torisawa, University of Michigan; Mitchell Ho, National Cancer Institute, National Institutes of Health; Shuichi Takayama, University of Michigan

High-Throughput 3D Spheroid Culture and Drug Testing Using a 384 Hanging Drop Array

Three-dimensional (3D) culture of cells is motivated by the need to work with accurate in vitro models that closely mimic physiological tissues. Culture of cells as 3D aggregates is well-known to provide better accuracy in in vitro tests for basic biological research as well as for therapeutics development. Such 3D culture models, however, are often more complicated, cumbersome, and expensive than typical two-dimensional (2D) cultures. We have developed a versatile 384-well format 3D cell culture plate that makes spheroid formation, culture, and subsequent drug testing on the obtained 3D cellular constructs as straightforward to perform and adapt to existing high-throughput screening (HTS) instruments as conventional 2D cultures. The technology is based on the scientifically proven but traditionally tedious hanging drop method to form 3D spheroids from multiple cell types. The developed hanging drop array platform allows for efficient formation of uniformly-sized spheroids, their long-term culture, and drug testing using liquid handling robots and plate readers. Utilizing this platform, we show that drugs with different modes of action produce distinct responses in the physiological 3D cell spheroids compared to conventional 2D cell monolayers. Specifically, the anti-cancer drug 5-fluorouracil (5-FU) has higher anti-proliferative effects on 2D cultures whereas the hypoxia activated drug commonly referred to as tirapazamine (TPZ) are more effective against 3D cultures. The multiplexed 3D hanging drop culture and testing plate provides an efficient way to obtain biological insights that are often lost in 2D platforms.

MP15

Songyu Hu, City University of Hong Kong, songyuhu2@student.cityu.edu.hk

Co-Authors: Dong Sun,Gang Feng, City University of Hong Kong

Dynamics Analysis and Closed-Loop Control of Biological Cells in Transportation Using Robotic Manipulation System With Optical Tweezers

Increasing demand for both accuracy and productivity in cell manipulation highlights the need for automation process that integrates robotics and micro/nano manipulation technologies. Optical tweezers, which use low power laser beams to trap and manipulate particles at micro/nano scale, have provided a revolutionary solution to manipulate biological objects in a noninvasive way. In this paper, we propose to use a robot-tweezer manipulation system for automatic transportation of biological cells. Dynamics equation of the trapped cell during the movement is analyzed. Closed-loop controllers are designed for transporting single cell as well as multiple cells. Experiments are performed on transporting live cells to verify the effectiveness of the proposed approach.

MP16

Elzbieta Jedrych, Warsaw University of Technology, Department of Microbioanalytics, ejedrych@ch.pw.edu.pl

Co-Authors: Michal Chudy, Artur Dybko, Zbigniew Brzozka, Warsaw University of Technology

Evaluation of Cytostatic Effect on the Normal and Cancer Cells in the Microfluidic System

Nowadays, a lot of people suffer from a cancer. Only in 2008, 12.7 million people worldwide have detected tumors, of which approximately 60% of patients die every year. Available methods, which are used for anticancer therapy, causes appearance side effects to patients. Usually, anticancer drugs are toxic for both normal and carcinoma cells. Therefore, the scientists are still searching new methods for cancer treatment, which will be less invasive for normal cells. The investigation of chemotherapeutic agents and new compounds are performed using 96-well plates, but those methods are not satisfactory now. In our research we examined a new tool, which enables fast, easy investigation of the tested drugs influence on the normal and carcinoma cells. The aim of the studies was evaluation of the usefulness of microsystems (fabricated in our laboratory) for fast assessment of cytotoxicity performed on the normal and carcinoma cells. The hybrid microdevice was fabricated using glass, PDMS and consisted of microchannels network for medium dosing and the microchambers for cells' seeding and culture. The cell culture microchambers were integrated with concentration gradient generator (CGG), which created five different substance concentrations achievement in a single step. Toxic effect of cytostatic drug (5-fluorouracil: 5-FU) was examined after 24 and 48 hours of incubation with cells cultured in microsystem. The normal cells BALB/3T3 (mouse embryo cells) were cultured and tested using fabricated microsystem. Obtained results were compared with tests performed in the same conditions, using carcinoma cells (A549 human lung carcinoma cells). Viability tests performed after incubation with 5-FU show, that 5-FU has a toxic effect on both cell lines. However, observed toxic effect of 5-FU was higher for carcinoma (A549) than for BALB/3T3 (normal) cells for all tested conditions. Normal cells are more immunity to toxic effect of chemotherapeutic drug. Obtained results confirmed that the microsystem can be used for fast and easy tests of new drugs. The microsystem provides conditions similar to in vivo and it decreases costs and waste amount, which is important for the natural environment protection.

MP17

Kayla Kaiser, University of California, Riverside, khame001@ucr.edu
Co-Authors: Christian Paetz, Cynthia K. Larive, Bernd Schneider

Natural Product Isolation for Chemical Ecology: Rapid Screening, Analysis, and Characterization

Bottlenecks in the field of chemical ecology arise in isolation and structure elucidation of molecules responsible for biological interactions. The utilization of on-line high-pressure liquid chromatography, solid-phase extraction, and nuclear magnetic resonance with a cryoprobe (HPLC-SPE-NMR) can overcome challenges of sample complexity and sensitivity while seeking new chemical and biological knowledge. In this research, culture medium of Xiphidium caeruleum root was found to contain a set of phenylpropanoid glycosides, of interest because this class of compounds possesses an extensive bioactivity profile. Liquid growth medium was first treated by solid-phase extraction (SPE) to remove hydrophilic constituents of the sample matrix, reducing sample complexity. Rapid screening for molecular identity was carried out using high-pressure liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometry (ESI-MS). This powerful combination of high-resolution separation and information-rich detection allowed us to focus our further studies only on molecules containing functional groups of interest. Automated peak-trapping of novel phenylpropanoid glycosides on SPE cartridges following multiple injections of growth media accumulated a sufficient quantity of material of reproducible chemical content for generating structural hypotheses by heteronuclear two-dimensional nuclear magnetic resonance (NMR). Automated sample handling from SPE cartridges to a cryogenically-cooled flow probe reduced volume of deuterated solvent necessary and hence cost of analysis, in addition to providing structural information from a single root culture flask (80 mL). Without having to expose the compounds to light, oxygen or acidic conditions, we could be more confident that the structure we observed reflected the bioactive molecules excreted by the plant. By fractionating the molecules contained in the medium into single components, the problem of dynamic range can be addressed. Dereplication of identified compounds was assisted by in-house (ACD/Labs) and publicly available (NMRShiftDB) databases as well as comparison with literature. For complete structure elucidation, precision isolation of milligram quantities of pure material was achieved by preparative HPLC and computerized fraction collection. Large scale SPE (10g sorbent) was applied to post-column effluent to prevent sample degradation prior to concentration. Finally, isolates were partitioned and a portion (5%) was reconstituted in ultrapure protonated solvent for high-resolution mass measurement using double sector double focusing MS while the remainder (95%) was reconstituted in deuterated solvent for full NMR characterization. This approach has utilized efficient handling of limited quantities of bioactive molecules excreted by roots of Xiphidium caeruleum, ostensibly for the purposes of chemical communication, perhaps to manipulate partners, competitors, and ecosystems. Automation has contributed at various points throughout this discovery approach to speed identification of chemical substances encoding biological information, ultimately allowing further studies of the developmental, behavioral, and ecological consequences of these previously unexplored chemical signals.

MP18

Kelly Karns, University of California, Berkeley, kelly.karns@berkeley.edu
Co-Author: Amy E. Herr, University of California, Berkeley

Human Tear Fluid-Based Point-of-Care Diagnostics Enabled by Integrated Microfluidic Systems

Human tear fluid holds enormous promise as a non-invasive diagnostic fluid for ocular and systemic diseases. Here, for the first time, we introduce technologies critically needed for non-invasive tear-based diagnostics which could impact medical care in both developed and developing nations. Important to realizing near-patient diagnostics, we report on the first microfluidic homogeneous electrophoretic immunoassay for endogenous lactoferrin quantification in human tears with highly efficient protein extraction from limited-volume tear fluid. Our electrophoretic immunoassays complete in tens of seconds with dynamic detection ranges commensurate with anticipated clinical expression levels. To our knowledge, no similar efforts exist. Owing to the unrivaled performance of homogeneous electrophoretic immunoassays in diagnostic medicine, we introduce a microfluidic immunoassay for lactoferrin, a tear-specific biomarker down-regulated in several ocular and systemic diseases (e.g. Sjögren's syndrome). Our microfluidic approach is uniquely suited to rapidly quantify endogenous proteins in volume-limited tear samples. By combining standardized sample preparation and microfluidic immunoassays for endogenous tear protein quantification which are highly specific, multiplexed, automated, sensitive (nM), rapid (sec), and consume low sample volumes (<5μL), we aim to expand the use of tear fluid in diagnostic medicine which is currently limited to rudimentary volumetric and surface chemistry measurements. Our assay utilizes on-chip native PAGE separations of unbound antibody and antibody-antigen complexes to specifically quantify clinically relevant concentrations of endogenous lactoferrin (nanomolar range) in <5uL of human tears. We also employ a newly introduced discontinuous polyacrylamide gel pore-size to enable ultra-short separation distances (350μm), making the assay amenable to low power, point-of-care use. Critical for point-of-care diagnostics, our lactoferrin immunoassay completes in <15s and is quantitative. Gold-standard ELISA validation on clinical samples supports our on-chip assay measurements. Further and importantly, we have developed a highly efficient protein extraction protocol using Schirmer strips, the clinical de facto standard for tear collection and storage in large patient registries (e.g. UCSF). The utility of any assay output requires standardized upstream sample preparation; yet no standard methodology for tear sample collection and preparation currently exists. We have developed off-chip diffusion-based protein extraction that enables 70-100% efficiencies; on par with the most efficient reported extraction protocols. Our approach is compatible with on-chip integration, which is currently underway, as is important for fully integrated clinical devices and handling of the vanishingly small fluid volumes. Taken together, this work represents major advances toward first-in-kind tear-based point-of-care diagnostic tests relevant to ocular and systemic diseases and opens the possibility of a new, non-invasive sample modality for point-of-care diagnosis. Ongoing work focuses on extending multiplexing capabilities to a panel of biomarkers and quantifying protein levels in a cohort of Sjogren's syndrome patients, with the aim of developing the first point-of-care biochemical test for this serious autoimmune disease.

MP19

Joong Kim, Korea Research Institute of Bioscience and Biotechnology, jhkimweb@gmail.com

Co-Author: Bong Chung, Korea Research Institute of Bioscience and Biotechnology

Instrument-Free and High-Throughput Screening of Mutagenic/Carcinogenic DNA Sensitizing Drugs Using Gold Nanoparticles and Functional DNAs

Phototoxic responses after administration of photosensitive pharmaceutics have been recognized as undesirable side effects, and predicting potential hazardous side effects is gaining importance as new drugs are introduced to the market. One of the most harmful influences of the photosensitive drugs is pyrimidine dimer sensitizing effect, because the frequency of the formation is highest among other DNA photolesions and they are the main cause of skin cancer. However, conventional methods for the dimer analysis, such as immunological assay, gel electrophoresis and chromatic assays are not feasible for a high throughput screening of the dimer sensitizing drugs because they are time consuming and require additional manipulations such as purification, radio-labeling, enzymatic digestion, or chemical modification of DNA. In this presentation, we report a colorimetric method to detect DNA photodimer and screening of the dimer photosensitizing drugs by the naked eye using gold nanoparticles and functional DNAs. The stability of gold nanoparticles (AuNPs) in a high ionic strength solution is maintained by straight ssDNA adsorbed physically on the AuNPs. However, we found that UV-irradiated DNA was less adsorptive onto gold nanoparticles because of a conformational change of UV-irradiated DNA and thus triggered aggregation of the gold nanoparticles resulting in red to purple color changes of the mixture and allowing colorimetric detection of the DNA photodimers by the naked eye. We successfully applied the colorimetric dimer detecting method to visually qualify the photosensitizing effect of Non-steroidal anti-inflammatory drugs in parallel within only ten minutes. Since our method does not require any chemical or biochemical treatments or special instruments for purifying and qualifying the DNA photolesions, it should provide a feasible tool to accelerate screening of a large number of drug candidates.

MP20

Douglas Kirkpatrick, Saint Louis University, dkirkpa3@slu.edu

Co-Author: R. Scott Martin, Saint Louis University

Improving the Versatility of Microchip-Based Electrochemical Detection: Use of Compact Discs for Electrode Fabrication and Integration of Segmented Flow

This poster will present two different strategies to increase the versatility of electrochemical detection in microchip-based systems. First, the integration of electrodes fabricated from gold-layered compact discs (CDs) for amperometric detection in microchip-based systems will be presented. This approach greatly reduces the device cost when compared to commonly used gold-sputtered glass plates. A unique fabrication procedure was developed that allows for electrodes as small as 10 ?m to be patterned. These electrodes show wide versatility in electrochemical applications. Unmodified single and dual electrodes used with microchip-based flow analysis were able to detect the oxidation and reduction of catechol. The ability to form a mercury amalgam is also demonstrated, which selectively detects thiols at low potentials. Lastly, palladium was electrodeposited on the surface of an electrode, which allowed for the integration of a decoupler for microchip capillary electrophoresis. This poster will also describe how an increase in temporal resolution was obtained using droplets of aqueous analyte suspended in an immiscible oil phase. In microdialysis, temporal resolution is lost by dispersion of analyte throughout the capillary while in transit to the detector. The utilization of an additional phase maintains a concentration gradient for long periods by preventing dispersion outside of the aqueous phase. Droplets were created off-chip in a simple micro-tee, connected by perfluoroalkoxy (PFA) tubing to a microchip using a unique transfer method. Once on chip, droplets were desegmented via a hydrophilic/hydrophobic interface created prior to chip operation by a one second corona discharge. Hydrophilic droplets travel down a special tapered channel completely isolated from any oil, allowing analysis with electrochemical detection.

MP21

Radoslaw Kwapiszewski, Warsaw University of Technology, r.kwapiszewski@gmail.com

Co-Authors: Karina Ziólkowska, Michal Chudy, Artur Dybko, Zbigniew Brzózka, Warsaw University of Technology

A Microfluidic System for Studying Cellular Markers of Metabolic Diseases

Development of novel, effective diagnostic strategies and procedures based on intracellular components analysis is strongly related with applying micro total analysis systems (μTAS). Apart from low reagents consumption, short reaction time, integration, automation, versatility, possibility of diagnosis monitoring and stimulation of each step of the procedure, the use of miniaturized microdevices in progressive clinical medicine affects the patients' comfort. However, there are still a few key challenges in this field to be solved before the chips can be accepted for real applications. Currently more than 3000 congenital metabolic diseases are known (e.g. mitochondrial, peroxisomal, lysosomal). Most of them are caused by the abnormal value of enzyme activity. The problem is that there is no biochemical/enzymatic method that reliably identifies all carriers of many of these disorders. New methods and tools for medical diagnostics are still explored and in request. The goal of this work was to design and develop a first microdevice for clinical diagnostics of lysosomal storage diseases. The main advantage of microchip presented is possibility for intracellular components analysis directly after the cell lysis process. This approach reduces the effect of proteases released in a lysis process on determined enzymes. We are carrying out researches into integration of such modules as: a microflow cytometer, a module for a chemical cell lysis process, a passive micromixer, an optical detection zone, and a temperature control system on one chip. There is still no fully integrated, easy to fabricate and relatively inexpensive microdevices adapted to cell-based assays. The microsystem presented was fabricated in PDMS with photolithography and replica molding techniques. The usability of the microsystem was verified by performing assays of ß-glucocerebrosidase activity (a marker of Gaucher's disease) and a-galactosidase activity (a marker of Fabry's disease) using L929 fibroblasts as a source of enzymes. The curve of the enzymatic reaction progression was prepared for three reaction times obtained from application of different flow rates of solutions introduced to the microsystem. Afterwards, enzyme activity determined was recalculated with regard to 105 fibroblasts present in samples (mouse fibroblasts) used for the tests (developed cell counting system lets for detection over 88% cells introduced to the microsystem). The results obtained were compared with a cuvette-based measurements. The calculated ß-glucocerebrosidase activity, 95.0 ± 15.0 μU/105 cells, and a-galactosidase activity, 34.0 ± 6.0 μU/105 cells, determined in the microsystem were in good correlation with the values determined during macro-scale measurements: 110.2 ± 19.6 μU/105 cells and 40.5 ± 8.5 μU/105 cells, respectively. After the validation of the analytical procedure using fibroblasts from patients suffering from Gaucher's and Fabry's diseases and control group (in cooperation with the Institute of Psychiatry and Neurology in Poland) microsystem presented will be ready for preliminary tests in clinical laboratories.

MP22

Cari Launiere, University of Illinois at Chicago, clauni2@uic.edu

Co-Authors: Gregory Czaplewski; Ja Hye Myung, University of Illinois at Chicago;
Seungpyo Hong, University of Illinois at Chicago; David T. Eddington

Biomimetic System for Circulating Tumor Cell (CTC) Isolation

Microfluidic CTC isolation devices (MCIDs) are used to remove cancer cells from patient blood samples for the purposes of cell enumeration or molecular characterization. The information collected can be used for the monitoring of disease progression or for basic research. While some devices are already being used in a clinical setting, there is still much room for improvement in terms of cost, efficiency and ease of use. We have developed a system that combines biomimetic protein combinations, hydrophoretic cell focusing, and microscope interface features within one inexpensive and easily fabricated device. Improvements to the validation procedure, such as the use of biomimetic rheological test solutions and automation of the enumeration procedure, allow for rapid benchmarking of new device designs.

MP23

Thomas Linz, University of Kansas, twixx@ku.edu

Co-Authors: Christa Snyder, Susan Lunte

Development of a Microfluidic-Based Screening Device for the Detection of Methylarginines in Plasma

The methylated arginines (MAs) monomethylarginine (MMA), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) have been shown to be independent predictors of cardiovascular disease. Because these biomarkers could potentially track disease onset and progression, an analytical method capable of quantifying MAs in human plasma has been developed utilizing capillary electrophoresis (CE) with laser induced fluorescence (LIF) detection. The effects of borate concentration, sulfobutylether-cyclodextrin concentration, and voltage were evaluated to determine the optimal CE separation conditions. Baseline resolution was achieved between naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized arginine, ADMA, MMA and SDMA in less than nine minutes with the optimized method. The limits of detection for these species were determined to be 5 nM, 20 nM, 40 nM and 20 nM, respectively, which are well below the expected plasma concentrations. MAs from plasma samples were selectively preconcentrated using cation exchange solid phase extraction (SPE) pipette tips and quantified with CE-LIF. The CE separation method was then transferred to a microchip electrophoresis (MCE) device where MAs were baseline resolved in four minutes on a 14 cm glass microchip. To our knowledge, this is the first report of the separation of MAs by MCE.

MP24

Ying Liu, University of California Riverside, yliu021@student.ucr.edu

Co-Authors: Quan Cheng, University of California; Hui Li, Danke Xu, Nanjing University

Conjugated Nanoparticles Based Enhancement for High-Throughput Protein Detection via Surface Plasmon Resonance Imaging

Sensitive monitoring of multiple biological interactions in a multiplexed array format has numerous advantages. Surface plasmon resonance imaging (SPRi), an optical technique allowing label-free detection of thin organic films, has emerged as an excellent tool to provide real-time analysis for array-based methodologies. However, current microarray based SPRi methods have limited detection sensitivity. We report here an effective SPRi signal amplification method using conjugated nanoparticles. Goat anti-human immunoglobulin E (IgE) is immobilized on SPRi gold film by nanoliter deposition to form an antibody array where the spare area is blocked by bovine serum albumin (BSA). IgE is captured to the anti-IgE surface through incubation in a humidity chamber. SPRi signal amplification is achieved via consecutive binding of two different kinds of nanoparticles: silver nanoparticle and magnetic iron oxide nanopaticle. The former is functionalized with an IgE-binding aptamer and oligonucleotide A12 (Ag -Apt-A12), while the latter with oligonucleotide T12. Fe3O4-T12 sufficiently filled the gap area between the building blocks Ag-Apt-A12, significantly enhance SPR signal due to its high refractive index and stacking density. Control experiments are conducted in the absence of IgE and from direct binding of aptamer functionalized Fe3O4 only. The high specificity of protein-aptamer interaction eliminates cross reaction and allows adaptation of this strategy for simultaneously in situ multiplexed detection, opening new avenues for sensitive high-throughput detection of DNA, RNA, and peptides.

MP25

Joe Fu-Jiou Lo, University of Illinois at Chicago, joef.lo@gmail.com

Co-Authors: Yong Wang, David T. Eddington, University of Illinois at Chicago

High-Throughput Hypoxic Modulation of Islet Calcium Response and Preconditioning

Type I diabetes can be treated with the transplant of islets of Langerhans in the hepatic portal vein. However, this environment represents a decrease in available oxygen (hypoxia), and the islets must adapt and later recruit microvasculature post-transplantation. Moreover, the insulin response, correlated with intracellular calcium concentration, is unknown for islets under hypoxia (~8% volumetric O2 for hepatic portal). While there are large number of studies on the success and the complications of islet transplants, there is no demonstration of how islets can operate or even recover under hypoxic transplant conditions. Quantifying islet response to oxygen levels can elucidate the role respiration has on glucose metabolism, and may provide critical understanding of diabetes beyond transplant research. Islet trapping and perfusion were achieved using high-throughput microfluidic structures previously published by our group. Furthermore, novel diffusion-based oxygen delivery was integrated into the microfluidic device. Highly controlled oxygen concentrations of 0%, 5%, 10%, 21% and 100% volumetric concentrations (balance air) were delivered to islets across a 100 μm gas-permeable polydimethylsiloxane, PDMS membrane. Islets were monitored for their intracellular calcium fluxes via ratiometric FURA-2 AM intensities on an Olympus IX71 inverted microscope. Using this system, oxygen can be delivered and modulated in timescale of several minutes. Islet exposure to hypoxia predictably showed impairment in calcium response to 14mM glucose, and thus insulin secretion. Furthermore, oxygen concentration exhibits a dose response with 10% oxygen exposure leading to a 50% depressed calcium response but retaining the phasic overshoot and oscillation seen in normal glucose response. 5% oxygen was taken as the “hypoxic standard” and islet response under this hypoxia showed no phasic response under 7, 14, and 25mM glucose exposure, showing hypoxic response is neither improved nor worsened by short-term elevated glucose exposure. Furthermore, preconditioning of islets was tested to recover islet response under 5% hypoxia. Preconditioning of islets to hypoxia and 100% hyperoxia for 20 minutes does not improve calcium response. Also hyperoxic exposure might be related to poor antioxidant capacity of islet cells. However, cycling of 5% and 21% oxygen with 5 minute intervals for 4 cycles, totally 20 minutes of hypoxia precondition, recovers both maginitude (~70%) and phasic response including oscillations related to membrane calcium ion channels. The response of islet glucose stimulation in low oxygen shows that hypoxic impairment critically affects islet performance. Preconditioning using constant hypoxia or hyperoxia cannot prepare islets for hypoxic operations. Intermittent hypoxia at 5 minute intervals showed promise to recover calcium responses for islet operation under 5% hypoxia. These results show both insights to islet oxygen/glucose metabolism, as well as provide a possible enhancement to islet transplant therapy using high-throughput microfluidic devices.

MP26

Zhenda Lu, University of California, Riverside, zlu002@ucr.edu

Co-Author: Yadong Yin, University of California, Riverside

Nanocrystal Clusters for Highly Efficient Bioseparation

Nanometer-sized particles have been extensively used for the analysis of biomolecules, such as biolabelling, drug delivery and hyperthermia treatment. Another application area of importance is the bioseparation. Due to their small size and high surface area, nanoparticles have many superior characteristics for bioseparation compared to those of the conventional micrometer-sized resins or beads, such as good dispersability, the fast and effective binding of biomolecules. However, there are several intrinsic difficulties of the use of nanoparticles in separation. First, they cannot be conveniently separated from the mixture by centrifugation because of their samll sizes. Second, these monodisperse nanoparticles are normally dispersed in nonpolar solvents, which is a great limitation for the bioapplications. Third, the surfaces of these nanoparticles are always coated with a layer of protecting ligands, which prevent nanoparticles from trapping the biomolecules. In this work, we propose a general strategy for the fabrication of novel porous nanostructured materials for efficient separation of biomolecules such as proteins and peptides. Briefly, nanoparticles of various oxide materials with uniform sizes and shapes are synthesized, and then self-assembled into three-dimensional submicrometer clusters containing well-defined mesoscale pores. As the first example of this general strategy, we demonstrate the self-assembly of superparamagnetic gamma-Fe2O3 nanocrystals into densely packed clusters in the form of submicrometer spheres. Based on the hydrophobic-hydrophobic interactions between the protection ligands of nanocrystals and the analytes, the clusters can be directly used as a powerful absorbent for enrichment of peptides and proteins in solution and easily separated from solution by an external magnetic field. The nanocrystal clusters containing proteins/peptides can be directly used for MS measurements without the need of additional elusion steps. The cluster structures can possess clean internal surface to selectively capture the biomolecules after calcinations, and the great separation properties such as high surface area, good stability, controllable size and fast mass transfer can be well maintained. We report here the fabrication of mesoporous TiO2 nanocrystal clusters and demonstrate their use for selective enrichment of phosphorylated proteins from complex biosamples by taking advantage of both specific affinity offered by the metal oxide and the size exclusion mechanism enabled by the mesoporous structures. The high specificity and capacity of these mesoporous TiO2 clusters have been also demonstrated by effectively enriching phosphopeptides from digests of phosphoprotein (alpha- or beta-casein), protein mixtures of ?-casein and bovine serum albumin, milk and human serum samples. We also demonstrate that the self-assembly process brings the flexibility of incorporation of multiple components, such as superparamagnetic nanocrystals to further facilitate the peptide separation. This self-assembly approach opens the door to a new class of mesoporous materials that may have wide applications in isolating biomolecules by selecting the nanocrystal materials with appropriate composition, size and shape.

MP27

Debkishore Mitra, University of California, Berkeley, debkishore.mitra@gmail.com

Co-Authors: Hinesh Patel, Luke P. Lee, University of California, Berkeley

Integrated Molecular Diagnostic Systems (iMDs): A Breast Cancer Theranostic Platform

Breast cancer comprises 10.4% of all cancer incidences among women worldwide, making it the most common type of 'non-skin' cancer in women and the fifth most common cause for cancer-related death. Early diagnosis is important so that early treatment and care can be initiated. At present, both histological and imaging-based methods are used for diagnosis. Fine needle aspiration (FNA) is a diagnostic technique which can be used to probe and sample suspected malignant tumors, cysts or lesions (identified by imaging techniques). It uses a fine-bore needle (22-27 gauges) to collect cellular samples from these areas, which are later analyzed for cancer biomarkers like the Her-2/neu receptor. However the present methods to do the biomarker analysis (ELISA, cytometry, immunostaining) are limited by the small volumes available, absence of multiple biomarker analysis, cost and portability. We are designing and developing a portable, robust, microfluidic-based integrated molecular diagnostic system (iMDs) for breast cancer theranostics. Such a system would require small sample volumes and would be able to perform multiplexed analysis of a panel of biomarkers , for improved specificity, as well as integrated drug screening. This device would detect the presence of breast cancer biomarkers in breast aspirates by employing a microfluidic sandwich immunoassay with a fluorescent readout. The device would include an upstream sample preparation module that will pre-concentrate the biomarker for improved sensitivity. The sample preparation module will have two components; for cell trapping and cell lysis. Cell trapping is done to pre-concentrate the cellular biomarkers of interest and would be achieved using the novel 'Hydraulic Jump' trapping modality. Using this method, >90% of all cells in the aspirate can be trapped and extraneous proteins in the aspirate removed. Once the cells are trapped, they would be lysed so that both intracellular and surface proteins can be probed downstream. Cell lysis would be achieved using on-chip electrochemical lysis, which would remove the need for any external reagents for lysis. This device, which can detect cancer biomarkers in microliters of breast aspirate, will be important for the detection, prevention, and treatment of breast cancer as well as other non-skin cancers. The described iMDs has potential beyond cancer in the theranostics and prognostics of other diseases as well.

MP28

Ahsan Munir, Worcester Polytechnic Institute, ahsan.munir@wpi.edu

Co-Authors: Jianlong Wang, Zanzan Zhu, Susan Zhou, Worcester Polytechnic Institute

A Time-Dependent Magnetic Nanoparticle Enhanced Actuation Strategy for Mixing and Tagging Biomolecules in a Point-of-Care Microfluidic Lab-on-a-Chip System

Magnetic nanoparticle (MNP) tagged with biomolecules in a microfluidic system can be efficiently used in various applications that involve separation and detection. These systems can be realized for DNA and protein analysis, bio-defense, drug delivery, and pharmaceutical development. However, for microfluidic devices to be used for point-of-care analysis, it is often necessary to tag biomolecule of interest with MNPs in situ. Tagging various fluid contents continuously in the micro-scale is often difficult due to slow diffusion process where length scale of mixing is large. Therefore, extended time is required for the biomolecules to be thoroughly mixed and combined with MNPs. In this work, we report a CFD model based on finite element technique to demonstrate a novel method of tagging biomolecules with MNPs on-chip especially for point-of-care devices. The process require time-dependent magnetic field which is produced due to the electrodes embedded in the device substrate beneath the microchannel. A time-dependent magnetic body forces produces oscillation in MNPs causing agitation in the surrounding fluid that otherwise follow laminar profile and overall speeds up the reaction kinetics of the tagging process. This strategy is easy to implement and can be integrated on a lab-on-a-chip device especially designed for point-of-care testing. The model was used to quantify the effect of convection, diffusion, reaction and magnetic field on the mixing as well as tagging performance. Species Concentration Distribution (SCD) and Residence Time Distribution (RTD) are used to analyze the performance of this novel scheme both quantitatively and qualitatively. Overall, the developed CFD model demonstrates that time-dependent magnetic actuation is an efficient tool to mix or tag MNPs with biomolecules in situ for the development of efficient point-of-care microfluidic testing devices.

MP29

Frank B. Myers, University of California, Berkeley, fbm@berkeley.edu

Co-Authors: Oscar J. Abilez, Chris K. Zarins, Stanford University; Luke P. Lee, University of California, Berkeley

Electrophysiological Cell Sorting: Label-free Purification of Differentiated Stem Cells via Functional Response to Stimulus in a Microfluidic Platform

We describe a new technology for non-genetic, label-free cell purification which classifies cells based on their electrophysiological response to stimulus. As many of the cell types relevant for regenerative medicine are electrically-excitable (e.g. cardiomyocytes, neurons, smooth muscle), this technology is well-suited for generating highly-pure populations of desired cell phenotypes from heterogeneous stem cell progeny. It is a particularly promising technology for purifying cardiomyocytes, which do not have reliable surface markers suitable for fluorescent labeling. Furthermore, labeling molecules and genetic selection methods pose toxicity and tumorigenic risks in a clinical application. We have developed a prototype cell sorter and have demonstrated its capacity to distinguish undifferentiated induced pluripotent stem cell (iPSC) embryoid bodies from iPSC-derived cardiomyocyte clusters. The system utilizes a microfluidic device with integrated electrodes for electrical stimulation and recording of extracellular field potential signals from suspended cells in constant or intermittent flow. Based on automated analysis of these signals, the system directs cells into one of several outlet reservoirs. This modular microfluidic device can be parallelized to achieve throughputs relevant for clinical applications. This concept represents an entirely different approach to cell sorting, in which a cell's functionality is assessed rather than its physical characteristics or expression profile. Besides its clinical applications, electrophysiological sorting of stem cell-derived cardiomyocytes would be useful in the development of in vitro drug screening assays by enabling the rapid production of highly pure cardiomyocyte populations from different pathological genotypes.

MP30

Vinay J. Nagaraj, The Biodesign Institute at Arizona State University, vinay.nagaraj@asu.edu

Co-Authors: Manish Bothara, Portland State University; Seron Eaton, Peter Wiktor, Center for Bioelectronics and Biosensors, The Biodesign Institute at Arizona State University; Shalini Prasad, Wichita State University

Miniature Electronic Biosensor for the Detection of Glycan Biomarkers

Glycans (oligosaccharide chains attached to proteins) hold great promise as a new class of biomarkers for the early diagnosis of cancer and other diseases. To realize the potential of glycans as biomarkers and to overcome the inherent limitations of current laboratory analytical techniques, we are developing a novel ultrasensitive diagnostic platform called 'NanoMonitor' to enable rapid label-free glycosylation analysis from human samples in a clinical setting. The operation of the NanoMonitor is based on the principles of electrochemical impedance spectroscopy. The device consists of a silicon chip with an array of gold electrodes forming multiple sensor sites. Each sensor site is overlaid with a nanoporous alumina membrane that forms a high density of nanowells. Lectins, proteins that bind to and recognize specific glycan structures, are conjugated to the surface of the electrode. When specific glycans from a test sample bind to lectins at the base of each nanowell, a perturbation to the electrical double-layer occurs, which results in a change in the impedance. In order to test the ability of NanoMonitor to identify defined glycan structures, as well as distinguish between closely related ones, we analyzed glycoform variants of the serum protein fetuin for binding to a panel of lectins on the NanoMonitor. The highly specific binding pattern of lectins to fetuin glycoforms on the NanoMonitor correlated very well with results from a conventional laboratory technique: lectin-based enzyme linked immunosorbent assay (ELISA). However, compared to ELISA, the analysis of protein glycosylation was quick (about 10 min), completely label free, required just 10 µl of sample, and was several orders of magnitude more sensitive. Analysis of protein extracts from cultured human pancreatic cells on the NanoMonitor indicated the presence of significantly higher amount of alpha-(2,3) linked rather than alpha-(2,6) linked sialic acids. Our results indicate that the NanoMonitor is capable of rapid label-free protein glycosylation analysis with high sensitivity and selectivity over a broad dynamic range of glycoprotein concentrations. This system can be easily integrated into a handheld electronic instrument for use as a point-of-care diagnostic device for routine detection of glycan biomarkers from clinical samples.

MP31

Johnbull Sonny Ogboi, Ahmadu Bello University, ogboijb@yahoo.com

Co-Authors: Kemgni Raoul Da'si, John Idoko, Ahmadu Bello University Teaching Hospital, Zaria

A Faster and Safer Staining Technique for Acid Fast Bacilliin Resource-Poor Setting

The traditional Ziehl-Neelsen (ZN) method used for the staining of acid fast bacilli contains phenol and because of the hazardous nature and inherent danger of phenol, this study was carried out to develop a faster and safer method in our environment that will exclude toxic phenol from the staining solution. "Morning fresh", a commercial liquid dish washing solution with super grease cutting power and citrus extract distributed by PZ Industry PLC, Nigeria was substituted for phenol in preparing carbol fuchsin. This study was carried out between September, 2006 – May, 2007 at the Public Health laboratory, Dept of Community Medicine, Ahmadu Bello University and Histopathology Department, Ahmadu Bello University Teaching Hospital, Zaria Kaduna, Nigeria. Departmental slides known to contain acid fast bacilli were stained in duplicates using both the traditional carbol fuchsin and the staining solution containing "morning fresh" following the standard procedures. The modified and the traditional ZN stained red for acid fast bacilli, nuclei, cytoplasm and cytoplasmic element stained blue on a clear background. The results were compared with departmental control slides for acid fast bacilli stained by traditional ZN method. The liquid dish washing solution "morning fresh" with super grease cutting power and citrus extract was found to be very efficient because of its affinity for lipids, its ease to handle and safety when compared to phenol. This study method stains acid fast bacilli more efficiently than the traditional carbol fuchsin method using phenol and it is also cheaper and readily available for use in our environment, and in the light of this finding, this may be a more suitable alternative in the detection of acid fast bacilli especially in resource poor and constrained countries where Mycobacterial infections are a major public health problem.

MP32

Tim Ruckh, Colorado State University, ruck0036@lamar.colostate.edu

Co-Authors: Derek Carroll, Ketul Popat, Colorado State University

Soluble and Insoluble Cues Incorporated Into Polymer Nanofiber Scaffolds for Tissue Engineering and Regenerative Medicine in Orthopaedic Tissue

Biodegradable bone tissue scaffolds have the potential to impact patients with numerous ailments. Starting with fabrication techniques that produce nano-scale features, the ability to manipulate architecture, alter surface chemistry, and deliver biological molecules allows for the design of elegant and highly effective bone scaffolds. This work aimed to develop a porous, nanofiber scaffold with osteogenic design features the capability to deliver an antibiotic molecule from within the nanofibers. Two osteogenic design factors with unique mechanisms of action were selected; hydroxyapatite nanoparticles and oleic acid. Hydroxyapatite (HAp) is the primary inorganic phase of natural bone tissue and has been used to more closely mimic the extracellular environment of synthetic bone tissue scaffolds. Oleic acid (OLA) is an w-9 fatty acid with suspected osteogenic effects due to activation of peroxisome proliferator-activator receptors (PPARs). In separate in vitro evaluations, OLA significantly increased osteoblast phenotypic behaviors and led to differential expression of the three PPAR isoforms, suggesting that the OLA is activating its anticipated receptor. HAp produced mixed results by inducing a small increase in alkaline phosphatase activity, but decreasing expression levels of bone matrix proteins. An in vivo evaluation of biocompatibility revealed that neither design factor increased the inflammatory response over control nanofiber scaffolds in paravertebral muscle pouches. However, both factors separately increased new osteoid production. Scaffolds with both HAp and OLA elicited the greatest osteogenic response in vivo, suggesting positive synergy between the two design factors. Finally, rifampicin (RIF), an antibiotic molecule was loaded into the nanofibers, and its release into static bacterial culture was effective in inhibiting bacterial population growth for both a Gram-positive and Gram-negative bacterial strain, separately. Overall, these nanofiber scaffolds were demonstrated to be effective carriers of soluble (OLA, RIF) and insoluble signals (HAp) which can modulate cell behaviors.

MP33

Thiago Segato, University of São Paulo, segato22@yahoo.com.br

Co-Authors: Thiago P. Segato, Renato S. Lima, Emanuel Carrilho, University of São Paulo; Nathan V. de Castro, José A. F. da Silva, University of Campinas

Liquid Electrodes for Contactless Conductivity Detection in Microchip

The aim of this research was to integrate the Ga-In eutectic (EGaIn, > 99.99%) as electrodes for contactless conductivity detection (C4D) in microchip, obtaining an innovative platform to replace the use of irreproducible tapes, or expensive vapor-deposited films as electrodes for analysis in miniaturized systems with C4D. Conversely, the proposed platform provides inherent advantages of the two methods mentioned, namely: (i) ease of microfabrication (present in cases that use tapes as electrodes), and (ii) satisfactory reproducibility (obtained when vapor deposited films are used as electrodes). Microdevices used in this study consisted of two layers of PDMS with channels fabricated by soft lithography. The channels for the electrodes were filled with EGaIn using a simple syringe, resulting in a continuous filling of channels by the liquid metal. Analytical signals associated to a KCl solution (deionized water was used as blank) were monitored under a flow analysis procedure. Analysis were conducted by simply switching between two syringe-pumps containing KCl solution and deionized water, both operating at a flow rate of 100 µL min-1. The first study was performed to select the optimal excitation signal parameters (frequency and peak-to-peak amplitude) for detection in two different designs (aligned and non-aligned electrodes). The best results were obtained when high amplitude potential (10 V) and low frequencies (50 and 40 kHz, for the aligned and non-aligned designs, respectively) were chosen. These parameters were used to perform the sensitivity studies. Measurements performed under flow analysis using different concentration of KCl solutions provided calibration curves and the limits of detection (LOD) for both electrode configurations. The following experimental conditions were adopted: 100 µL min-1 flow rates with 15 s sampling time. The values of LOD for the aligned and non-aligned designs, calculated using parameters of standard curves, were 3230.0 and 20.7 µmol L-1. The non-aligned electrodes showed significantly lower LODs (about three orders of magnitude) compared to those obtained with the aligned electrodes, which can be attributed to the lower stray capacitance between the detection electrodes. Additional experiments are being carried out to predict the influent of the position of one electrode relative to the other, and the distance between the solution in the analytical channel and the electrode. The new fabrication procedure proposed here provides a simple alternative to integrate the detection electrodes to microchip-C4D in a reproducible and inexpensive fashion.

MP34

Asmira Selimovic, Saint Louis University, asmiras@slu.edu

Co-Authors: Alicia Johnson, Istvan Z. Kiss, R. Scott Martin, Saint Louis University

Use of Epoxy-Embedded, High-Aspect Ratio Pillar Electrodes for Microchip-Based Systems

This presentation will describe a new approach for microelectrode fabrication of planar and pillar electrodes that can be integrated into microfluidic channels. The electrodes sizes can be easily varied from 1mm to 25 microns in diameter? and the resulting embedded electrodes can be reversibly sealed against a PDMS-based fluidic network for introduction of pressure-based flow. Gold planar electrodes have been used to detect catechol samples for characterization and the selective detection of glutathione was achieved via a mercury amalgamation. While the planar electrodes are easy to fabricate and enable the use of different electrode materials, electro-deposition of gold or gold-silver mixtures onto the epoxy embedded electrodes allows for creation of high aspect pillar electrodes. This is a first time a group has developed an easy, inexpensive, fast, and reproducible way of making 3D electrodes for in-channel detection. Gold depositions for creation of pillar electrodes were optimized to achieve tenfold higher surface area and signal than planar electrodes of the same diameter. These pillars can be made in a 4-electrode array, resulting in a quadrupling of analyte signal compared to use of a signal pillar. Electrical deposition of gold-silver mixture give rise to branched pillars, allowing a tenfold increase in surface area over a gold pillar of the same height. Characterization of electrode depositions, flow performance, life time stability, reproducibility, and electrode surface area calculations will be presented in this poster.

MP35

Barbara Smith, Colorado State University, barb.smith@colostate.edu

Co-Authors: Sorachon Yoriya, Pennsylvania State University; Laura Grissom; Craig Grimes, Pennsylvania State University; Ketul Popat

Hemocompatibility of Titania Nanotube Arrays

Hemocompatibility and inflammation remain a serious concern for the long-term success of blood-contacting biomaterials, eliciting a need for an improved understanding of the mechanisms behind blood/nano-biomaterial interactions. Thus, in this study, we have examined one of the earliest stages in the physiological immune response by considering the in vitro adsorption of proteins, adhesion and activation of platelets, and clotting kinetics of whole blood. Titanium and titanium-based alloys are the most widely used implantable biomaterials due to their mechanical strength, biocompatibility, non-toxicity, corrosion resistance, and ease of process-ability. These materials have been used extensively in orthopaedic and dental implants. Recent studies have reported on the hemocompatibility of biomaterials, however, little is known about the hemocompatibility of nano-biomaterials. Previous studies have shown that material surfaces which mimic the natural physiological hierarchy of in vivo tissue may provide one possible solution for enhancing biomaterial integration. Thus, the hemocompatibility of titania nanotube arrays has been investigated to identify its potential role in implantable biomedical devices. These titania nanotube arrays can be fabricated using a simple anodization technique, and provide a favorable template for increased cellular functionality and localized drug delivery at a hierarchy similar to that of natural tissue. In addition, titania nanotube arrays have elicited minimal levels of monocyte activation and cytokine secretion, thus exhibiting a very low degree of immunogenicity. In this study, we have investigated the in vitro adsorption of key blood serum proteins, adhesion and activation of platelets, and clotting kinetics of whole blood on titania nanotube arrays (diameter: 70-90 nm, length 1 μm). Driven by a need for reduced material rejection, and thus obtaining an enhanced biocompatibility between the implant/body interactions, a study focusing on an improved understanding of the physiological response to nanomaterials, specifically hemocompatibility, is considered here.

MP36

Nickolaj F. Starodub, National University of Life and Environmental Sciences of Ukraine, nikstarodub@yahoo.com

Co-Authors: Oxana M. Brezvin, State Sci.-Res., Control Inst. Vet. Preparat. and Feed Add

Immune Biosensor Based on the Oxide Cerium IsFETs for the Determination of Mycotoxin Level in Environmental Objects

IsFET's with the oxide cerium (CeO2) dielectric layers intended for the creation of very sensitive, stable and reliable immune biosensors were studied. Input and output characteristics of such IsFET's shown the increasing of the pH-sensitivity in comparison with the Si3N4 layer. It is due to high density of the surface sensitive centers, large level of the permittivity (e = 26) and the band-gap energy (3.6 eV) of cerium. It leads to decrease current losses through the dielectric. The CeO2 IsFETs have the increased pH-sensitivity (about 58 mV/pH) that is near to the maximal possible index (59 mV/pH). We tested the efficiency of this structure at the control of T-2 mycotoxin. It was shown that the maximal sensor output (about 100 mV) correspond to 0.1 μg ml-1 of mycotoxin labeled by horse radish peroxidase (HRP) when binding sites of the specific Ab were saturated. It was used a special approach when the active oxygen (formed during the enzymatic reaction) oxidizes ascorbic acid and causes a local basic pH shift. The linearity of signal changes at the competitive mycotoxin analysis is in the range of concentrations from 5 to 190 ng ml-1. In this range the potential of the IsFET gate varies from 95 to 5 mV. The standard deviation was on average about 5%. In case of the analysis by "to saturated way" (when at first the immobilized Ab interacted with the mycotoxin in the analyzed sample and then with the solution of this analyte but labeled by HRP) the sensitivity is 0.5 ng ml-1 and linearity in the range 1.0-200 ng ml-1. The overall time including preparation of sample (from corn) and competition between the labeled and native mycotoxin for binding with Ab (~10 min) was ~30 min. The sensitivity of the developed biosensor based on the CeO2 IsFETs is much more high (up to 2 orders) than that which is based on the SPR and is close to that which is inherent for biosensor based on the total internal reflection ellipsometry. Both parameters (the sensitivity and stability) are much better for biosensor based on the CeO2 than Si3N4 IsFETs. Taking into account the high chemical stability of the CeO2 and the simplicity of obtaining of this dielectric thin layer such structure may be recommended for the creation of the multi-biosensors for the practical application in medical diagnostics and environmental monitoring.

MP37

See on-site Conference Addendum for poster title and author updates.

MP38

Krishna Vattipalli, Wichita State University, v_mohank@yahoo.com

Co-Authors: Tom Barrett, Oregon Health and Sciences University; John Carruthers, Portland State University; Shalini Prasad, Wichita State University

Nanoporous Alumina Membranes Based Microdevices for Ultrasensitive Protein Detection

Robust diagnosis of a disease can be accomplished by reliable detection of multiple protein biomarkers. Traditional assay methods for protein detection such as enzyme-linked immunosorbent assay (ELISA) have several limitations – need for use of labels, time of detection is several hours, large volume of reagents, multiple proteins cannot be detected simultaneously and they are expensive. This poster discusses the development of nanomonitors, which are electrical immunoassays for label-free, sensitive, fast, reliable and cost effective detection of multiple protein biomarkers. Thus, novel nanotechnology has been successfully employed to obtain significant enhancements over ELISA. The biosensors designed by our lab use the electrical and chemical properties of nanoporous alumina membranes to improve the sensitivity and performance of Si-based microdevices for protein sensing. The integration of nanoporous membranes with the microdevice results in nanoscale well-like structures, also known as nanowells which exhibit size matching with respect to proteins. The trapping of proteins within the nanowells is an experimental demonstration of “molecular crowding” phenomenon whereby the functionality of the proteins is retained due to confinement in small spaces i.e. nanowells. These alumina nanopores are electrically insulating and isolated resulting in grouping of nanowells for capacitance based detection of proteins. Conjugation of proteins into the nanowells results in a charge perturbation in the electrical double layer at the interface between the biomolecule and the gold electrode, thus causing a measurable change in the capacitance. The device performance has been demonstrated for simultaneous detection of two inflammatory markers - C-reactive protein (CRP) and Myeloperoxidase (MPO) from complex fluids samples i.e. patient human serum. These two proteins are biomarkers of vulnerable coronary vascular plaque rupture and detection of these proteins enables the pre-operative identification of the disease. The performance parameters of the nanomonitors are compared with the traditional assay methods. Apart from being a label-free technique, it was also concluded that the nanomonitors can provide several improvements such as highly increased speed of detection on the order of minutes as compared to several hours for ELISA, significant reduction in volume of reagents to a few µl, large reduction in cost per assay and the reduction in the size of assay thus making it a candidate for a clinical diagnostic “lab-on-a-chip” device.

MP39

Ming Zhong, University of Illinois at Urbana-Champaign, mzhong3@illinois.edu

Co-Author: Jonathan V. Sweedler

Quantitation and Spatial Control of Peptide Release From Neuronal Cells Within Microfluidic Devices

Within the brain, communication between neuronal cells relies on a variety of signaling molecules, one important category of which is neuropeptides. Different regions of an individual neuron experience distinct chemical environments and the overall cellular response depends on these chemical microenvironments. Measuring these peptides of low abundance and providing chemical and spatial control of these small-volume regions around cultured neurons remain challenges. Microfluidics is a suitable solution because of the minimal sample consumption and the ability to manipulate microenvironments. First, we demonstrate a microfluidic device capable of quantifying peptide release by measuring the length of channel covered by each peptide. In this device, neurons are placed in small chambers, and the cellular releasates are collected as they flow along a serpentine channel that contains a surface derivatized with octadecyltrichlorosilane designed to collect the peptides released from chemically-stimulated neurons. In order to minimize peptide loss due to nonspecific adsorption, the sample chamber surface is patterned with oligo(ethylene glycol). Next the derivatized surfaces are characterized by matrix-assisted laser desorption/ionization (MALDI)-based mass spectrometry (MS) imaging to determine the part of channel covered by a peptide. By carefully controlling the flow rate, experiments with known amounts of acidic peptide (AP) show a linear correlation between the length of channel occupied by AP and its amount. We then use our approach with neurons from Aplysia californica, a model organism for neurochemistry research. For one example using a small number of Aplysia bag cell neurons stimulated by elevated potassium, the expected signaling peptides, including AP and alpha-bag cell peptide (BCP) are detected. The amounts of released peptides determined by the channel coverage length match other measures of peptide release amounts. Therefore, this microfluidic device offers a unique label-free solution to quantifying mass-limited samples. In order to selectively manipulate the environments surrounding sub-regions of a single neuron, we are adapting a new microfluidic platform, which has the cells loaded and cultured inside a main channel and contains two other parallel channels in which the liquid flows can be separately manipulated. Those secondary channels are interconnected to the main channel using a large number of small-diameter interconnections that have controlled surface chemistry and can selectively guide the axons and dendrites into these secondary channels. Fluidic isolation of a channel is achieved by creating a minute hydrostatic pressure difference from the adjacent channels. This type of microfluidic device allows controlled delivery of reagents to isolated subcellular regions and collection of peptide releasate locally.

MP40

Karina Ziolkowska, Warsaw University of Technology, kziolkowska@ch.pw.edu.pl

Co-Authors: Radoslaw Kwapiszewski, Michal Chudy, Artur Dybko, Zbigniew Brzozka, Warsaw University of Technology

A Microfluidic Chip for Multicellular Tumor Spheroid Formation and Analysis: a Prospective System for Anticancer Drug Screening

Nowadays drug development and pre-clinical tests are performed using animals and simple in vitro cellular models. Animal participation is questionable both for ethical reasons and differences between species occurrence. On the other hand, available in vitro models, based on monolayer cell culture, do not give answers for essential aspects concerning organism's response to drugs. These limitations cause the long evaluation process of new drugs, which is expensive and may cost life of many patients suffering from cancer. Thus, there is a need for new, fast and reliable alternative drug testing methods. The best cellular model for anticancer therapy testing developed so far is Multicellular Tumor Spheroid (MCTS). The MCTS presents morphology and physiology similar to tumor in vivo with the network of cell-cell interactions, three dimensional structure, ECM presence and nutrients, metabolites and oxygen gradients – factors inaccessible for monolayer cell culture. Number of methods of MCTS formation were described in literature and several found their final applications. However, most of them causes variation in size or is labor and energy consuming, which prevents automation and standardization of methods. First attempts of spheroid formation using microfluidic devices have been reported, but the solutions proposed are not fully suitable for MCTS cultivation and testing, e.g. rapid spheroid formation methods do not mimic original tumor genesis and microchambers of diameters of 70-200μm are too tight for MCTS, which diameter often exceeds 200μm. In this work a 3D microfluidic system of microwells for MCTS cultivation is presented. The poly(dimethylsiloxane) was chosen for the fabrication, due to its hydrophobicity preventing cell adhesion. Separate layers were fabricated using low-cost soft lithography and replica molding method. An array of microwells (volumes of 0.2μL) was connected with the network of microchannels for medium supply. Alignment and plasma bonding of the three layers resulted in the 3D structure which enabled cultivation of MCTS of diameters up to 300μm with the medium perfusion around. HT-29 cells' suspension was introduced into the microsystem and after 16 hours of incubation cell aggregation was observed. Unaggregated cells were washed out from microchannels with the fresh medium flow. Within the next 24 hours loose cell aggregates remained in the microwells formed compact spheroids. In each experiment, 72 hour cultivation gave obtainment of uniform spheroids of required properties (diameter over 150μm, necrotic core presence). The growth rate of MCTS observed in the microsystem was lower than in the nonadhesive plates, which most likely corresponds with the tumor growth in vivo. The presented MCTS microsystem can be an inexpensive and easy to handle alternative for current spheroids' cultivation methods. The microfluidic array can be easily coupled with the concentration gradient generator forming an integrated system for anticancer drug screening.

MP41

Carl Hull, UNIConnect, carl.hull@uniconnect.com

How to Use a Generic Platform to Track and Control Specific Lab Processes

Commercial laboratory software systems are designed to track and control specific processes. This creates two fundamental problems:

1. Keeping up with change—Detailed process specifications change over time: Process details are where a laboratory differentiates itself from competitors, and must be accommodated in tracking and control software. Yet software providers need to treat customers the same to leverage their programming investment and minimize costs. Thus labs often end up with software that doesn't fit.
2. Supporting a patchwork—the many types of a lab's processes requires many specific programs from different providers: This is unaffordable because of license fees and integration issues. It's frustrating to have workers login and enter the same information into multiple programs. What laboratories really need is a universal, comprehensive, process tracking and control software – a system that documents completed units of work, records chain of custody and enforces process quality controls.

Solution:

1. Change the way we think about the problem: Stop looking for specific software for specific types of processes. Instead, think of the problem generically and solve it with a general purpose tracking and control system. When laboratory processes are viewed through a universal model, one worker's output becomes the next worker's input. Products can include a calibrated machine, qualified reagent, customer request, training document, project, job candidate, proposal, order, etc. An intermediate product becomes part of another product, resulting in genealogical relationships which can be traced from beginning to end, including the who, what, when, and where of each action. Generic software that tracks 'products' and 'units of work', makes it simple to define workflows. This shift in perspective is called an abstraction. From this elevated perspective labs see that all of the specific product solutions are doing the same things; only the labels and the combinations of the parts are different.
2. Use a power tool to deal with the programming. Use a domain-specific language to define the necessary details needed for product tracking and control. Put this language in the hands of the laboratory. The high-level language radically changes the cost of programming and the skills required by eliminating the need to communicate the detailed requirements to the software provider. The process expert interacts with the tool directly, receiving instant feedback. It puts the lab in control of its destiny and lowers the risk and cost of depending on the software provider for change.
3. Use a single comprehensive program where possible, and create bridges to existing programs where appropriate. Replacing existing programs that are doing a good job is not necessary. Use a tool that provides a variety of effective high-level ways to integrate with these programs and multiply their value to the company.

MP42

Li Liu, Beckman Coulter, li.liu@beckman.com

Co-Authors: Michael Kowalski, Laura Pajak, David Schumm, Renuka de Silva, Barry A. Schoenfelner, Beckman Coulter, Inc.

cAMP Assay Using Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL*) Technology

G protein-coupled receptors (GPCRs) remain the dominant target class of modern drugs and drug discovery programs due to their vital role in both physiological processes and pathological conditions. We used SPARCL (Spatial Proximity Analyte Reagent Capture Luminescence) technology from Beckman Coulter to measure intracellular cAMP levels as an indicator of the functional activity of a specific GPCR, the ß2-adrenergic receptor, in response to stimuli in the human epithelial carcinoma cell line, A431. SPARCL is a luminescent homogeneous assay technology which is amenable to automation. The cAMP assay is based on the competition between endogenous cAMP and exogenous HRP-labeled cAMP for binding to an acridan-labeled anti-cAMP antibody. The SPARCL cAMP assay** provides high sensitivity to intracellular cAMP and detects levels as low as 10 pM, enabling miniaturization by requiring fewer cells for reliable detection. In this report, SPARCL cAMP assay data were compared with the data from Perkin Elmer's AlphaScreen* cAMP assay and Cisbio Bioassays' HTRF* cAMP assay. Cells, compounds, and reagents for the assays were dispensed in a 384-well microplate format using the BioRAPTR* FRD workstation. The SPARCL cAMP assay was more sensitive than the competitor assays with comparable precision and accuracy. Therefore, the homogeneous SPARCL cAMP assay kit by Beckman Coulter enabled high-throughput, miniaturized screens in 384-well plates without compromising data integrity as indicated by robust Z' factors. Note: BioRAPTR FRD For research use only; not for use in diagnostic procedures.

*All trademarks are property of their respective owners.

** Internal feasibility assay, not for commercial distribution or diagnostic use.

MP43

Karen Poulter, Life Technologies, karen.poulter@lifetech.com

Co-Authors: John Bishop, Adam Harris, Clarence Lee, Rhonda Meredith, Kamini Varma

Walk-Away Automation of the SOLiD™ Low Input Fragment Library Preparation Protocol Utilizing the Tecan® Freedom EVO®75

A great deal of research is limited by the quantity of DNA samples, especially, medical and clinical research; this is particularly true for library construction intended for next generation sequencing (NGS) technology. SOLiD™ Low Input Fragment Library Preparation Protocol that has been successfully used manually to prepare SOLiD fragment libraries with pre-fragmented DNA samples from sub-nanogram up to 10 ng of DNA from clinical sources. To help with through-put, multiplexing adaptors have been used so that libraries can be pooled together, allowing for a single round of emulsion PCR and then simultaneous sequencing of multiple samples with a single SOLID run. This protocol, utilizing Agencourt® AMPure® XP beads, has been successfully automated on the inexpensive Tecan® Freedom EVO®75. The protocol has been split up into three scripts: 1) End-repair and clean-up; 2) Ligation, clean-up and reaction set-up; and 3) Reaction clean-up. All three scripts can handle a maximum of 24 samples. It takes one person less than 1.5 days of processing to go from 24 low input fragmented or sheared DNA samples to produce high quality, high coverage sequencing libraries.

MP44

Zorayr Khalapyan, Global Bio Lab, zkhalapyan@gbl.ucla.edu

Co-Authors: Alexander Roth, Lee Borenstein, Global Bio Lab

Automated Data Import and Validation Framework for a High-Throughput Laboratory

The consequences from emerging infectious diseases as well as the proliferation of established diseases demand a new task force ready for early identification, rapid response, and widespread surveillance. The current obstacles that impede an establishment of such an entity include lack of systems that allow information sharing across a network of local, national, and international institutions amongst other difficulties. To address this issue, a fully automated data import and validation system has been constructed that allows preregistered users to upload and validate data via secure, centralized, service based, online data interchange framework. The tool enables scientific groups and organizations to upload their survey, collection, or study data virtually from anywhere and anytime and receive instant validation and confirmation. The constructed software provides temporary data repository where uploaded data undergoes validation, quality control, modification if necessary, and final review by project managers. A part of the validation procedure includes tests for certain value ranges, permissible values, and existence of certain keys. All validation is done against a pre-specified and project-specific data format descriptions managed and assigned by administrators. Upon completion of QC and validation process, the data may be released to the final data repository. For assuring a high level of security, an access control system will allow only preregistered users to login with a username and a password. The authentication and authorization is based on user roles and permissions enabling users to have rights for only certain operations such as importing, validating, or managing projects. Additionally, each registered user in the system is bound by a profile which includes personal and organization information. To further achieve data integrity and traceability a complex trace system has been implemented to log an audit trail that identifies who did what, when, and why. Allowing automated validation upon data import and an immediate validation feedback, the framework will increase efficiency and manageability of projects with high data volume and allow better information sharing amongst institutions and organizations. Future developments may include integration with various surveillance and surveying devices.

MP45

Daniel Gossett, University of California, Los Angeles, daniel.gossett@gmail.com

Co-Authors: Henry Tse, Serena Lee, Amander Clark, Dino Di Carlo, University of California, Los Angeles

A Powerful New High-Throughput Tool for Measuring the Deformability of Single Cells

There is growing evidence that cell deformability (i.e. the ability to change shape under load) is a useful indicator of cytoskeletal variations and may provide a label-free biomarker for determining cell states or properties such as metastatic potential, cell cycle stage, degree of differentiation, and leukocyte activation. Clinically, a measure of metastatic potential could guide treatment decisions, or a measure of degree of differentiation could prevent transplantation of undifferentiated and tumorigenic stem cells in regenerative therapies. However, in order for deformability measurements to be clinically valuable, given the heterogeneity within a population of single cells, there exists a need for high-throughput automated assays of these mechanical properties. Here, we present a novel microfluidic device for the continuous deformation of cells in flow (~ 2,000 cells/sec, throughputs comparable to traditional flow cytometry and greater than 3 orders of magnitude over the current state-of-the-art methods). We have devised and fabricated a microfluidic system which uses previously reported inertial focusing techniques to precisely position cells in flow where they can be hydrodynamically stretched in an extensional flow. We have applied our system to the problem of screening cancer cells for their metastatic potential, measuring the deformability of normal (MCF10A), benign (MCF7), and malignant (MCF7 with enhanced motility) cell lines. Indeed, measureable differences in whole cell deformability exist in this model. We also explored a potential application in regenerative medicine and stem cell technologies as a tool for rapidly screening differentiation methodologies and identifying residual undifferentiated subpopulations in various embryonic stem cell differentiation protocols. Again, differences exist and preliminary results suggest that whole cell deformability may be an earlier biomarker for differentiation than currently used biochemical markers in some cell lines. Lastly, we have evaluated deformability of cells treated with cytoskeleton-acting drugs. Detectable differences from untreated, control cells suggest a potential application in rapid screening of cancer drug candidates or combinations for personalized medicine.

MP46

Albert J. Mach, University of California, Los Angeles, ajmach@ucla.edu

Co-Authors: Armin Arshi, Jaehyun Kim, Dino Di Carlo, University of California, Los Angeles

Centrifuge-on-a-Chip: Rapid and Automated Sample Preparation for Cell Suspensions

Labeling and washing target cells are common protocols for many cell biology assays. Typically, this time-consuming process requires multiple centrifugation steps for sample preparation. Additionally, the standard bench top centrifuge is a bulky instrument that limits many diagnostics assays performed in remote settings. Here, we have developed a miniaturized system that replicates the function of a centrifuge without moving parts. The system can concentrate and resuspend cells to low liquid volumes from dilute and concentrated samples at rates of milliliters per minute. Operating using purely fluid dynamic phenomenon, the centrifuge-on-a-chip consists of a microchip with straight channels followed by expansion-contraction arrays to create microscale vortices with recirculating fluid that can be used to trap and exchange fluid around cells. We investigate this novel phenomenon of cell trapping by identifying the hydrodynamic forces responsible for cell trapping, determining the critical particle size required for trapping in specific geometries, and presenting potential biological applications. Using polydisperse polymer beads, we demonstrate that particles larger than the critical particle size migrate laterally through fluid streamlines and orbit within the vortex while smaller particles follow fluid streamlines and travel out of the device. As a proof of concept, we injected blood spiked with MCF-7 breast cancer cells and demonstrated selective trapping of MCF-7 cells (~20 microns) spiked within blood (cell sizes 2-15 microns). Once the blood sample was completely loaded, we flushed out blood cells via rapid solution exchange and collected the trapped cancer cells for analysis. In another application, we demonstrate on-chip cell fluorescent labeling techniques using rapid solution exchange, including cell fixation, permeabilization and labeling of intracellular antigens. We performed all operations of cell labeling within the microchip without the need for manual input and washing steps. Since the cell traps can be deactivated by lowering the flow rate, we retrieved these cells for downstream assays. We envision this system to be used in-line with downstream cell-based assays performed by flow cytometry, and may critically address the need for low cost centrifugation steps for point-of-care or resource poor settings.

MP47

Kevin P. Armendariz, University of Kansas, kparmenda@ku.edu

Co-Authors: Heath Huckabay, Robert C. Dunn, The University of Kansas

Multiplexed Biosensing Using Fiber Optic Imaging of Microsphere Whispering Gallery Mode Resonators

Localized, multiplexed, label-free detection of biomolecules with low detection limits continues to be challenge for many biological sensors currently available. Microsphere whispering gallery mode (WGM) resonators provide an opportunity to create sensing elements for a novel biological sensor. Originally used to describe acoustic phenomenon in domed structures, WGMs are now used to describe the resonant confinement of light within circular dielectric structures. The wavelength of light resonantly stored in a microsphere is highly dependent on the radius of the sphere and the effective refractive index experienced by the sphere. By placing a recognition element on the sphere, changes in effective refractive index experienced upon analyte binding can be quantified through shifts in the wavelength of light resonantly stored in the sphere. Extensive progress with WGM sensors has been made over the past decade, with successes in attaining detection limits down to the single molecule level. A variety of optical resonator designs including glass microspheres, microfabricated waveguides, and liquid core optical ring resonators have been implemented for biosensing applications in the past. While microsphere resonators have been shown to offer superior optical qualities, their implementation in biosensing devices is typically hindered by difficulties in multiplexing and integrating photonic and fluidic designs. In this work, we have developed a prototype sensor based WGM resonance changes in microspheres on the end of a fiber optic bundle. Each fiber of the fiber bundle is used to transfer the fluorescence from an individual microsphere to a CCD camera. Spatial orientation of individual microspheres is maintained throughout the fiber bundle and used to provide a spatial address on the CCD for each microsphere. Sequential loading and addressing of spheres with unique recognition elements allows for localized, multiplexed, label-free detection of biomolecules. Progress in this new approach combining fluorescence detection with spatially addressed readout through a fiber bundle will be discussed.

MP48

Moran Bercovici, Stanford University, moranb@stanford.edu

Co-Authors: Moran Bercovici, Govind V. Kaigala, Kathy E. Mach, Joesph C. Liao, Juan G. Santiago, Stanford University

Novel Assay and System for Rapid Diagnostics of Urinary Tract Infections Using on-Chip Isotachophoresis and Molecular Beacons

Moran Bercovici is a PhD Candidate in his final year in the Microfluidics Laboratory at Stanford University. He received his M.Sc. (2006) and B.Sc.(2001) in Engineering from Technion, Israel Institute of Technology. Between ‘01-‘06 he served as a Research Engineer at RAFAEL, Advanced Defense Systems. He was awarded the Office of Technology Licensing Stanford Graduate Fellowship (‘06-‘10), and a Fulbright Fellowship from the US Department of State (’06-’08). His research focuses on developing novel diagnostic tool for medical applications, and he has published numerous journal and conference papers on development of on-chip detection techniques, sample concentration methods, and numerical techniques in microfluidics.

MP49

Alicia Johnson, Saint Louis University, ajohn140@slu.edu

Co-Authors: A. Selimovic, R. Scott Martin, Saint Louis University

Integration of Microchip Electrophoresis With Electrochemical Detection Using an Epoxy-Based Molding Method to Embed Multiple Electrode Materials

The fabrication of multiple electrode materials onto planar substrates for integration with microfluidic channels is a challenging and expensive process. Multiple electrodes of different compositions have been used to integrate microchip electrophoresis with a palladium decoupler and a downstream electrode for detection. This poster describes a novel approach for multiple electrode material fabrication into an epoxy base that is simple, inexpensive, fast, and reproducible. This new approach has been used to integrate gold, glassy carbon, palladium, and platinum electrodes. The epoxy surface can be polished and used for separation and in-channel detection of electroactive species. The embedded electrodes, ranging from 25 microns to 1 millimeter in diameter, can be modified to improve selectivity or for signal enhancement to decrease limits of detection. This poster will also describe how these epoxy embedded electrodes can be used with injection valves to make discreet injections into a high voltage electrophoresis channel. Peristaltic pumps can be utilized to monitor and detect cellular release and to allow the cells to be located directly on-chip away from the high voltage electrophoresis channel.

MP50

Javier Lopez Prieto, University of California, Irvine, lopezprj@uci.edu

Co-Authors: Jami Nourse, Jente Lu, Lisa Flanagan, Department of Pathology and Laboratory Medicine, University of California, Irvine and Abraham P. Lee, University of California, Irvine

Automated Dielectrophoretic Separation of Heterogeneous Cell Populations

Dielectrophoresis (DEP) has shown promising results as a tool for the separation and trapping of cells. These results are specially relevant in some applications such as the isolation of stem cells from their differentiated progeny where the lack of unique surface markers make traditional methods challenging. As an alternative DEP is a non-invasive method that avoids the use of surface markers. Varying electric fields at different frequencies interact differently with cells with different electric phenotypes allowing for their separation and selective trapping based on the cell polarizability. Here we present an automated DEP cell trapping and cell separation device for the characterization and enrichment of heterogeneous cell populations. The device consists of an inlet, where an initial mixture of cells is loaded, connected to a main channel with three multiplexed DEP trapping regions. Each region is intersected by a dedicated perpendicular collection channel leading to three separated wells. Loading and unloading of cells are easily done by conventional methods such as pipetting. The fabrication of the device is done using soft lithography and bonding together two layers of poly(dimethyl) siloxane (PDMS) to a glass slide with patterned Ti-Au electrodes. Flow direction is controlled by the use of pneumatic valves that occlude the collection channels during cell trapping and separate the different regions during cell recovery. Trapping cycles consisting of a trapping step, a washing step and a collection step is automated and controlled through a LabView interface. The user can setup the different parameters such as number of trapping cycles, duration of each step, frequencies of each trapping zone and flow rates. Operation of the entire platform is therefore simplified and reduced to loading and unloading of the cells and parameter setting through a graphical interface. In order to prove the ability of the system to enrich different cell populations, two different mixed populations were used, the first consisting of mouse NSPCs and neurons while the second contained mouse NSPCs and astrocytes. A low frequency of 100 kHz and a higher frequency of 1 Mhz and an amplitude of 8 Vpp were used for trapping in the different regions. By staining the different populations we could determine after separation an enrichment of cells of up to 10-20% depending on frequency and cell type. This platform has the potential to automate the separation and enrichment of different cells types where differences in electrical phenotypes are apparent.

MP51

Molly Allen, CellASIC Corporation, mollyallen@cellasic.com

Co-Authors: Terry Gaige, Paul Hung, Philip J. Lee

A Mircrofluidic Liver Array for Long Term Culture of Fresh and Cryopreserved Primary Hepatocytes

Primary human hepatocyte cultures serve as important pre-clinical in vitro liver models and are used extensively for the screening of drugs and other xenobiotics. However, the current 2D or overlay culture methods are labor-intensive, costly, and fail to sustain key phenotypic gene expression and liver-like cell behavior such as P450 enzymatic activity, metabolism, and albumin production. Taking into consideration both the highly perfusive nature of the liver and its complex sinusoidal architecture, we have developed a single-use microfluidic plate consisting of 32 independent cell culture units, all within the confines of a standard 96-well-plate footprint. Approximately 50,000 hepatocytes are loaded into each microenvironment of a given plate using a novel flow control system called the PEARL. It creates pressure differentials between the inlets and outlets of the microfluidic chambers to drive fluid flow. Once loaded, multiple plates of primary human hepatocytes can be cultured and maintained in parallel without the PEARL, using standard laboratory equipment. Each unit only requires the daily addition of 125 ul medium and aspiration of waste to maintain adequate nutrient perfusion. Thus, our plates offer a high-throughput, cost-effective, and easily automatable platform to work with. Both fresh and cryopreserved primary hepatocytes in our plate consistently retain their liver-like functions in the long-term, which is especially important when examining the effects of slow-acting compound. We have compared 3 cryopreserved human hepatocyte lots, one rat cryopreserved lot, and freshly isolated rat hepatocytes. Depending on the lot, cells recovered viability and CYP3A4 activity between days 3-6 in microfluidic culture, and by day 14 retained 50-300% of initial activity. We have demonstrated stable and robust CYP3A4 enzyme activity and albumin secretion, healthy hepatic morphology, and low lactose dehydrogenase levels over 4 weeks of culture, whereas present methods limit this window from a few hours in suspension to a few weeks in overlay culture. Additionally, CYP3A4 enzyme activity of hepatocytes in our plates was successfully doubled by induction with rifampicin and halved by inhibition with ketoconazole, as is commonly demonstrated. Moreover, this induction and inhibition was carried out on primary human hepatocyte cultures that were over 30 days old, demonstrating that hepatocytes remain liver-like in our microfluidic plate even after a month of culture. The PEARL flow control system can also be used at higher pressures to quickly expose hepatocytes to specific solutions, as is necessary in fixing cells with paraformaldehyde or lysing cells for mRNA and protein analysis. The plate's thin glass bottom allows for excellent cell imaging of both live and fixed/stained hepatocytes. In conclusion, our microfluidic plates enable long-term, multiplexed, automatable primary human hepatocyte culture, which will help streamline a variety of applications including drug metabolite analysis and mechanistic toxicity screening.

MP52

Joseph Barco, Labcyte, jbarco@labcyte.com

Co-Authors: Joseph Barco, Maria Sonntag, Royal Huang, Steve Hinkson, Luke Ghislain, Sammy Datwani, Labcyte Inc.

Rapid Liquid Transfer From a 1536-Well Microplate Using Acoustic Droplet Ejection

Assay miniaturization is often limited by the ability of multichannel liquid handlers to accurately address higher density microplates. It is also limited by the quality of the 1536-well plate itself: increased well density often causes microplates to be less rigid, which in turn makes automation solutions challenging. Labcyte has developed and released a new 1536-well, low dead volume microplate composed of cyclic olefin copolymer. The low profile microplate design reduces the dead volume to 1 µL, and acoustic transfer with the microplate is up to 30% faster than previous 1536-well microplates. The microplate is manufactured to stringent rigidity and flatness specifications, ensuring reliable performance with high-throughput automation. Microplate performance is demonstrated in conjunction with acoustic liquid transfer on the Echo 500 series liquid handlers, with accuracy and precision better than 95 percent.

MP53

Paul Held, BioTek Instruments, heldp@biotek.com

Co-Authors: Jason Greene, Peter Banks, BioTek Instruments

Automated Washing of Multiplex Bead Based Assays for the MagPix® Reader System

Next generation xMAP technology based on the use of magnetic MagPlex microspheres has been available for now for a number of years. The use of MagPlex® eases sample processing through the use of magnets to immobilize the microspheres during wash processes. Recently, a new paradigm of xMAP Reader has been provided by Luminex which does not utilize flow cytometry principles for detection. The MagPix® Reader System has been developed by Luminex exclusively for MagPlex® microspheres. The washing of MagPlex® beads used with the MagPix® reader technology has been accomplished using a manual hand-held magnet or vacuum apparatus. The wash steps can be automated using an appropriately configured microplate washer equipped for biomagnetic separation. The spatial relationship between the localization of the magnetic beads by the magnet and the aspiration tubes is paramount for bead recovery. Here we describe the washer parameters associated with low residual volumes, good bead recovery and optimal assay performance. Human Multiplex assay kits from Millipore were was used to optimize the ELx50, ELx405 and ELx406 washers. Washer settings and resultant assay data will be provided.

MP54

Brian Kozlowski, Merck-WP-PSCS-Analytics Sciences, brian_kozlowski@merck.com

Co-Author: Jessica Kogan, Merck- WP-PSCS-Analytical Sciences

Full Automation of the Composite Assay and Content Uniformity Test by Sotax Tablet Processing Workstation II – HPLC Interface (TPW-HPLC)

Objective: Utilize a union between the Sotax TPWII Tablet Processing Station (TPW) and an HPLC for the direct injection of samples following extraction.

Experimental Procedure: The Sotax Tablet Processing Workstation (TPWII) is a powerful robotic platform which allows automated sample preparation for composite assay or content uniformity testing of solid dosage forms. The instrument has the ability to dispense solvents, perform extraction of active components, impurities, and degradates from solid pharmaceutical products via a high powered homogenizer, perform filtration of solutions, and dilute samples to their desired concentrations. Following dilution of sample solutions to method concentration, the TPWII can dispense a portion of the sample to a HPLC vial for off-line analysis by conventional stand-alone HPLC. Alternately, the TPWII is equipped with a Rheodyne injection valve which can be coupled directly to a dedicated HPLC instrument for direct injection. Utilized in conjunction with the sample preparation functionalities of the TPWII, the HPLC interface allows front-to-back automation of composite assay or content uniformity sample preparation and assay.

Results: In order to directly challenge the TPWII-HPLC interface for content uniformity analysis, five samples were prepared manually and were then placed on the TPWII-HPLC platform with system suitability injections for comparison to assay by stand-alone HPLC. Results generated by the TPWII-HPLC interface were equivalent to stand-alone HPLC (1% claim difference or less in injections on TPW-HPLC as compared to conventional HPLC for active component). A similar equivalency for composite assay testing was performed using an analytical method which utilizes gradient chromatography that is selective for both active ingredient and degradate products. Results for TPWII-HPLC were equivalent to stand-alone HPLC in this instance as well (less than 1% claim difference in TPWII-HPLC injections for active component and less than 0.05% claim for degradates/impurities as compared to conventional HPLC in all sample preparations). For both content uniformity and composite assay tests, system suitability criteria were well within limits established by SOPs.

Conclusion: The Sotax TPWII is a powerful automated platform that is useful in the full automation of the content uniformity and/or composite assay test. A time-saving component of the platform is evident as sample injection occurs immediately following extraction, and additional sample preparation can be performed while injection is underway. Additionally, the platform may be employed in situations involving unstable compounds which need HPLC analysis directly following extraction to avoid sample degradation.

MP55

Christa Snyder, s11.csnyder@wittenberg.edu

Co-Author: Thomas Linz, Susan M. Lunte, Ralph N. Adams, University of Kansas

Analyzing Methylated Arginnes Using Capillary Electrohoresis and Laser Induced Fluorescence Detection

The methylated arginines monomethylarginine (MMA), asymmetrical dimethylarginine (ADMA) and symmetrical dimethylarginine (SDMA) have been shown to be independent predictors of cardiovascular disease (CVD). As a result, an analytical method was developed to determine the concentrations of MAs in human plasma in order to track the onset and progression of CVD. Capillary electrophoresis (CE) with laser induced fluorescence detection (LIF) was used for the analysis of naphthalene-2,3-dicarboxaldehyde (NDA)-derivatized MAs. The optimization of run buffer composition, focusing on sodium borate and sulfylbutylether ?-cyclodextrin (SBE??-CD) concentrations, was crucial in obtaining adequate resolution between the analytes of interest. Under optimal conditions, SDMA, ADMA, MMA and arginine were baseline resolved and the limits of detection for those species were 41 amol, 30 amol, 41 amol and 10 amol, respectively. Plasma analysis was performed using strong cation exchange solid phase extraction (SPE) tips to preconcentrate the analytes of interest prior to derivatization. Recovery of MAs from the SPE tips was determined to be 43 percent.

MP56

Pavithra Wijethunga, The University of Texas at Arlington, pavithra.wijethunga@mavs.uta.edu

Co-Authors: Yasith Nanayakkara, Chemistry and Biochemistry, The University of Texas at Arlington;
Praveen Kunchala, Mechanical Engineering, The University of Texas at Arlington; Daniel Armstrong, Chemistry and Biochemistry, The University of Texas at Arlington; Hyejin Moon, Mechanical Engineering, The University of Texas at Arlington

High-Throughput Drop-to-Drop Liquid-Liquid Microextraction Method Coupled With Real Time Concentration Monitoring

Liquid-Liquid Extraction (LLE) is among the most important pretreatment techniques in basic sciences. Although, various miniaturized LLE methods were emerged to overcome drawbacks in macro scale LLE,, multiplexing LLE processes has never been considered. Therefore, we introduced a new drop-to-drop liquid microextraction (DTD-LLME) method that is capable of achieving high throughput multiplexed LLE. The DTD-LLME device was built as an electrowetting on a dielectric (EWOD) based digital microfluidic (DMF) device, which can easily be automated. Due to its multiplexing capability, this DTD-LLME technique is an attractive solution for the need for high throughput micro total analysis (μTAS) applications, for instance preparing and screening samples from complicated biological fluids. In this paper, we completely demonstrate the proposed DTD-LLME technique experimentally, along with two further studies related to the device: (i) developing an image based real time concentration measurement technique to study extraction kinetics in the device and (ii) Investigating the potential use of AC frequency to enhance the extraction kinetics of the proposed DTD-LLME technique. For the demonstration of DTD-LLME, Acid Green 25, a dye soluble in both DI water and a chosen ionic liquid (IL), [bmim][PF6], was selected as a solute. DI water and [bmim][PF6] were served as solvent and extractant, respectively. Four major steps in DTD-LLME (i.e., dispensing droplets, moving and merging, mixing to effect extraction, and separating extractant phase from donor phase) are demonstrated experimentally. Specially, a successful phase separation of two immiscible droplets is presented for the first time on a digital microfluidic device. To study the extraction performance, we developed an image based technique to read real time donor concentration while the extraction is in progress. Images of the droplets captured from the digital microscope system (HIROX KH 1300) were processed and color information was extracted (using MATLAB R2009a). The relationship between the color parameter and solute concentration of droplet was used as a calibration plot. An evaluation of the use of color parameters from three color models (RGB, HSV and CIELab) was carried out. Further, the technique was compared with a typical UV absorption based concentration measurement technique. Finally, we investigated the effect of AC frequency on the extraction kinetics. The DTD-LLME technique was successfully implemented. The image based technique showed to be a precise method for real time concentration measurement. Results on the study of AC frequency effect showed that the application of high frequency (> 150 kHz) is an effective factor to accelerate the extraction. Overall, the DTD-LLME technique can be extended toward high throughput μTAS for various processes that involve liquid-liquid extraction, such as screening for drug development and bio marker preparation for point of care devices.

MP57

Carl Hilliker, Precision System Science, Carl.Hilliker@pssbio.com

Co-Authors: Tetsuya Ueda, Osamu Segawa, Hidegi Tajima, Kimimichi Obata, Precision System Science Co., Ltd.

Gene-Lead and Gene-Typist: Two New Fully Automated Sample to Answer Systems

Nucleic acid amplification tests (NATs) are powerful tools for the molecular diagnostic laboratory, such as virus detection, mRNA profiling, SNPs (single nucleotide polymorphism) typing and detection of somatic cell mutation. However, operations of NATs are time consuming and require technical experience, since NATs utilize several processes (extraction of nucleic acids, amplification, detection and analysis of data). Moreover, NATs should be processed by highly trained laboratory technicians in special laboratory environments for assuring the quality of NATs results. These factors seem to be limiting factors for further expansion of NATs in molecular diagnostic testing, especially for point-of-care (POC) and on-site test. To eliminate these difficulties, easy to use, fully-automated and -integrated instruments are necessary. We have developed two platforms which are fully-automated and –integrated instruments for NATs. They are the 'Gene-LEAD' and 'Gene-TYPIST'. These instruments can process all steps for NATs, including extraction of DNA/RNA from various samples, reaction set up, amplification of target gene and detection/analysis of the products. These instruments use Magtration technology for nucleic acid extraction and incorporate different methods for analysis of the amplified products. Gene-LEAD can measure fluorescence signals from amplified products and can be used for endpoint or real time monitoring of amplified products. Gene-TYPIST uses a capillary bead array 'BIST' technology for multiplex SNPs typing (up to 9 genes). We will present data obtained from these instruments for detection of mRNA in simple and multiplex SNPs typing. With the Ease-of-Use and robust platform design, these fully-automated and integrated systems show great promise for highly accurate NATs in the molecular diagnostic testing fields.

MP58

Marie-Elena Brett, University of Illinois at Chicago, mebrett@uic.edu

Co-Authors: Shuping Zhao, Jonathan L. Stoia, David T. Eddington

Pin Valve for Microfluidics

There is a need for a simple method to control fluid flow within microfluidic channels. To meet this need, a simple push pin with a polydimethylsiloxane (PDMS) tip has been integrated into microfluidic networks to obstruct flow. This new valve design can attain on/off control of fluid flow without an external power source using readily-available, low-cost materials. The valve consists of a 14 gauge (1.6mm) one inch piece of metal tubing filled with PDMS and a PDMS pad at the tip to achieve a fluidic seal. The metal tubing or pin is then manually actuated to achieve valving. The valve was validated using a pressure transducer and fluorescent dye to determine the breakthrough pressure and simultaneously monitor leakages. Each valve was tested over multiple cycles. In the first cycle, the median value for pressure withstood by the vale was 8.8 psi with a maximum value of 17.5 psi and minimum value of 2.7 psi. The pressure the valves were able to withstand during successive trials was lower. These valves can achieve flow regulation within microfluidic devices, have a small dead volume, and are simple to fabricate and utilize making this technique is widely suitable for a range of applications.

MP59

See on-site Conference Addendum for poster title and author updates.

MP60

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MP61

Frederic Bottausci, CEA, frederic.bottausci@cea.fr

Co-Authors: Arnaud Rival, Yann Bettassa, Yves Fouillet, Dorothée Jary, Manuelle Quinaud, Claude Chabrol, Gaël Castellan, Manuel Alessio, Xavier Gidrol, Patrice Caillat, and Cyril Delattre

Fully Integrated EWOD Based Bio-Analysis Device

The goal of this study is to elaborate a reliable fully integrated Lab On Chip device for bio-analysis applications. Various activated micro electro mechanism systems (MEMS) have been used in the past to control, move and mix microliters volumes. Compared to complex systems involving moving parts like valves and pumps (activated for instance by magnetic, electrical or electrothermal systems involving complex and time consuming fabrication process) Electrowetting on Dielectric (EWOD) enables the basic operations without moving parts making it suitable for applications like in the area of optical lens, microfluidics and bio-technologie. EWOD allows digital fluidics and then offers the basics set of functions that are moving, mixing, merging, and separation of droplets on a single platform. The EWOD actuation of a droplet is controlled by an applied electrical potential between insulated electrodes, which induces an electric field and modifies the wetting behaviour of the droplet in contact with the electrodes. In the past decade lot of attention have been made on electrodes design and the influence of the hydrophobic-insulating layers on EWOD. In part of this study we go further and focus on solutions to make the EWOD device compatible with highly parallelized assembling keeping it reliable for bio-analysis. In LETI laboratories, we developed a fully integrated and automated EWOD Lab On Chip for biology and healthcare. The microfluidics device is a silicon chip coated with a SiOC hydrophobic layer. A pattern of SiOC coated metallic electrodes is designed to precisely form and control the droplets. The whole device is made in classic cleanrooms environment on 200mm wafer scale to parallelize the production. In order to facilitate the fluid motion and to eliminate any evaporation, the device is filled with silicon oil and closed. The assembly of the device is a crucial step in the process because it must integrate the fluidic and mechanical constraints and be compatible with very sensible bio-analysis. After packaging, the chip is fully sealed and ready to be used. A novel experimental setup enables direct and reliable fluidic and electrical connections. The set-up includes a thermal cycling element for Polymer Chain Reaction (PCR) analysis coupled with an optical detection system. In this study we present a reliable digital microfluidics chip that offers very well reduced, controlled, parallelized and reproducible droplets for quantitative bio-analysis. The volume of the reaction chambers ranges from 1µL down to a few tens of nanoliters. The chip is designed to perform PCR from solutions and qRT-PCR (quantitative Reverse Transcription – Polymerase Chain Reaction) from cells loading. Results on PCR or qRT-PCR presented have been validated and compared to conventional genomic tools.

MP62

Peter J. Brescia Jr., BioTek Instruments, Inc., bresciap@biotek.com

Co-Authors: Gary Barush, Peter Brescia, Brad Larson, Peter Banks, BioTek Instruments; Peter F. Stecha, Brock Binkowski, Neal Cosby, Promega Corporation

Automation of a Bioluminescent Live-Cell cAMP Assay for the Pharmacological Evaluation of GPCRs

GPCR responses to extracellular signaling events remain a major focus of both academic research and drug discovery efforts as pharmacological targets. Hit to lead applications typically require the pharmacological evaluation of hits from screening campaigns where their dose-response is quantified. This secondary screening usually incorporates a functional assay where the GPCR is expressed in a cell line of relevance. Here we demonstrate the automation of the workflow for the assessment of agonist and antagonist activity for the ß2-adrenergic receptor endogenously expressed in HEK293 cells using a stably transfected bioluminescent protein that binds cAMP. Data obtained using automated methods were consistent with data generated when using manual methods including data quality and EC50/IC50 precision.

MP63

Eric Bro, Mayo Clinic, bro.eric@mayo.edu

Co-Author: Brian C. Netzel, Mayo Clinic

Utilizing Robotic Sample Preparation and Result Reporting for Automation of the 25-Hydroxyvitamin D2 and D3 Assay

Background: Testing volumes continue to increase at a rate that is rapidly pushing potential instrument and staffing capacity. The ability to manage such active growth while maintaining the efficiency of the assay becomes progressively more difficult. The clinical surge in 25-hydroxyvitamin D2 and D3 by LC-MS/MS testing has pushed the throughput limitations of many clinical laboratories. To overcome these boundaries, we automated a derivatization-based sample-multiplex LC-MS/MS assay for detection of 25-hydroxyvitamin D2 and D3, which eliminates potential errors in the preparative and reporting phases.

Methods: The 25-hyrdoxyvitamin D extraction procedure utilizes separate differential mass tagging derivatization steps, followed by combination of calibrators, controls, or patient specimens and injecting them simultaneously into a LC-MS/MS based platform. The sample preparation for the 25-hydroxyvitamin D2 and D3 relies on customized robotic programming for LIM system communication via worklist building and result reporting, creating sample templates for LC-MS/MS operation, labware location confirmation based on a systematic barcode database, and automation of sample transfer, extraction, derivatization, reconstitution, and combination of specimens.

Results: Automation of the 25-hydroxyvitamin D assay allows for increased accuracy and precision across multiple software and instrument platforms while improving assay efficiency. Implementation of a specimen-to-plate tracking system eliminates the possibility of specimen mix-up and transfer errors that may otherwise occur in a more manual-based process. The laboratory section dedicated to the assay and the staffing required to perform test specific responsibilities has been significantly downsized. The conversion to a tailored automated-based system permits for increased potential in instrument capacity and sample volume growth while maintaining the reliability of the assay.

Conclusion: The automated protocol eliminates manual interaction and increases accuracy and reproducibility of the 25-hydroxyvitamin D2 and D3 assay. In combination with the derivatization-based method, the automation of sample preparation and reporting has increased throughput over standard methods and allowed for accommodation of potential sample volume growth.

MP64

Andrew Brooks, Rutgers University, brooks@eohsi.rutgers.edu

Co-Authors: Theresa Giesler, Robert Gaglione, Rutgers University Cell and DNA Repository (RUCDR), Rutgers University; David D'Ambrosia, Bionomics Research and Technology Center (BRTC), Rutgers University; Jennifer Qin, David Kobi, Qiagen Inc.

DNA Extraction From Frozen Serum Samples: An Automated Approach for Genetic Analysis Utilizing QIASymphony and Repli-g Whole Genome Amplification

Tremendous resources are used for the collection of clinical samples in research trials worldwide. Occasionally the analytical criteria for some studies are not well defined based on fiscal and technological uncertainty at the time of project initiation. To this end there are large numbers of samples stored from various clinical protocols which are currently not considered for mainstream analyses. One example of this is serum and plasma samples which are typically stored for specific analyte interrogation where no other molecular components were collected for additional analyses. This presentation describes the use of serum (and plasma) derived from whole blood for in DNA based genetic and genomic analyses. Given the quantity and quality of DNA in these, mostly cell free, preparations the extraction approach and validation of nucleic acid following processing is of paramount importance. We will describe a fully automated, high throughput, extraction and amplification workflow for these precious clinical isolates. The protocol details and QIASymphony implementation will be discussed specifically in the context of a biorepository setting. Additionally, every nucleic acid sample is subjected to Repli-g amplification in an automated process to maximize the utility of these biological resources for future analyses. The technical issues addressed in these protein rich, cell free fractions include and are not limited to: shearing and nicking of gDNA, clotting of samples during the extraction process, low molecular weight DNA retention and variable amounts of gDNA yields per sample. All of these issues are addressed in the automated protocol to be presented. Additionally, a fully automated solution for whole genome amplification has been engineered to complete an integrated workflow for analysis of genomic DNA from serum resulting in a nucleic acid resource for large scale genetic analysis. Both analytical and functional analysis of DNA derived from these processes will be presented.

MP65

Richard Kim, TTP Labtech

Co-Authors: Joby Jenkins, Ben Schenker, Rob Lewis, Chloe Carter

Flexible Nanoliter Liquid Handling for Reliable Assay Miniaturisation

Serial dilutions, plate replications and reformatting into higher density plates are important and often routine processes in drug discovery but, when assay volumes are miniaturised to nanolitre levels, there are some significant challenges to be met in the process of automating them. Miniaturised assays have important cost benefits in the reduction of precious compounds and reagents required, but it is paramount to maintain speed, accuracy and precision at these volumes or the efficiencies achieved become outweighed by the disadvantages. Miniaturising assays also allows users to draw on a wider range of high density plates for further efficiencies, but these often present challenges for liquid handling systems. mosquito® HTS (TTP LabTech) is a nanolitre liquid handler flexible enough to automate the miniaturisation of all standard HTS assays without needing special labware or system set-up changes. It offers extremely fast and accurate plate replication and serial dilutions. It is also capable of reformatting between different plate types in the same protocol – even from standard 96-well formats direct to high density 1536-well plates.

MP66

Larry Chin, RTS Life Science, larry.chin@rts-group.com

Co-Author: Martin Schwalm, RTS Life Science

Implementing Digital Video Recording to Increase Laboratory Efficiency

Every laboratory is under pressure to increase efficiency and take on a greater load of experimentation and testing. To do this scientists have turned to automated processing systems to grind out the mundane and often time consuming tasks, while they concentrate on the design and interpretation of the studies being performed. This all sounds fine, except that real world automation systems and robotics need a level of care and supervision to keep running well, and many experiments still need to be qualitatively viewed and analyzed during processing. To enable labs to increase their productivity, while keeping headcount the same or lower, there needs to be an enabling technology to allow the scientists to multitask their time amongst several critical daily duties. This technology is video monitoring and recording. Advances in CCD cameras and processing technologies have made the implementation of video recording affordable to most operations. The RTS Life Science LabEye 210 system incorporates up to 8 digital cameras with a digital video recording system that is PC based. The LabEye software has been written to facilitate the type of qualitative analysis tasks that are prevalent in all labs. This poster will examine as a case study the use of digital video recording with solid dose tablet dissolution. This case will look at the application and benefits achieved by using six camera system to monitor and record an extended release dosage during typically USP testing. The paper will also outline the broader use of this technology in auditing and debugging automation systems used in testing laboratories.

MP67

Douglass Fahnoe, Pfizer, douglass.fahnoe@pfizer.com

Co-Authors: Michael Barron, Craig Kent, Eric Benvenuti, Ann Janssen

Continuous Improvement Through the Development of Equipment Scheduling and Issue Reporting Tools

When managing an in vitro pharmacology Open Access Screening Lab (OASL), there are many challenges presented regarding instrumentation for use in plate-based screening. Historically, equipment failures and status were communicated by word of mouth, e-mail, notes on instrument, or not at all. Instrument failures and downtime were captured using multiple tools and organizational groups which were not seamlessly integrated. Inefficiencies in the system made it a challenge to provide reliable and timely service to users. Additionally, users had been using a global, conference room scheduling system to block time on equipment. It was independent and inaccessible to other software tools, making it very difficult to query for meaningful metrics necessary to facilitate capacity planning. We saw an opportunity to create a more efficient process through the implementation of a strategical, well-designed, fit-for-purpose, tool. In August 2010, the Pfizer Groton Primary Pharmacology Group (PPG) rolled out the PPG Equipment Dashboard. This Microsoft™ Access 2007-based software allows OASL users to visually see the status and inventory of the OASL equipment in real time. In the Dashboard, OASL support staff can include comments so users know if there are minor issues of which they should be aware. In addition, users can report issues from their desktop or directly from the instrument on which they are working. Upon reporting an issue, an e-mail is sent to the OASL support staff for quick and timely resolution. Finally, all instruments listed on the Dashboard have a link to a custom scheduling interface so that the equipment can be easily reserved. One month later, Groton PPG implemented the BookIt component of the PPG Equipment Dashboard. This tool allows users to reserve equipment directly from the Equipment Dashboard. We have integrated several OASL-required features into BookIt which were lacking in the legacy scheduling system. First, users have the ability to pool equipment, allowing them to easily see which instruments may be available. Reservations can be added to the users' Outlook calendar and marked as free or busy. If an instrument issue is reported, an e-mail is sent to anyone that has a reservation on that piece within the next 72 hours so that they may modify plans. Additionally, we now have the ability to gather metrics for usage and downtime which allows us to optimize equipment usage, anticipate user needs, determine equipment lifecycle, and drive process improvement. The development of the Equipment Dashboard and BookIt scheduling tool allows Groton PPG to be more responsive to OASL user needs while improving the issue reporting and scheduling processes. Furthermore, continued usage will allow us to collect and mine meaningful data on the instruments which can be applied to the development of an automation strategy designed to support our Research Portfolio.

MP68

Z. Hugh Fan, University of Florida, hfan@ufl.edu

Co-Authors: Ruba Khnouf, Qian Mei, Shouguang Jin

Microfluidic Array for High-Throughput Protein Expression

Protein expression is often required for studying genes' functions since the corresponding proteins are needed to characterize their structures and biological properties. As more and more new genes are being identified, there is a considerable need to have high-throughput methods for the production of a large number of proteins in parallel, matching the throughput and scale of gene discovery. Protein expression is primarily implemented using over-expression in E. coli cells, but cell-based methods are difficult and cost-prohibitive to implement in a high-throughput format. An alternative approach is cell-free protein synthesis (CFPS), which has been developed to address the limitations encountered in E. coli-based protein production, including cytotoxicity and susceptibility to proteolysis. We have developed a microfluidic array device for high-throughput CFPS. The device consists of an array of 96 units and each unit contains a reaction chamber and a feeding chamber. Protein expression (gene transcription and protein translation) took place in the reaction chamber while the feeding chamber functioned as the nutrient reservoir. A microfluidic channel connecting them provided a means to supply nutrients continuously, ensure proper mixing, and remove the reaction byproducts. The function of the array device was demonstrated by expression of a variety of proteins, with more than 2 orders of magnitude reduction in reagent consumption compared to a commercially available CFPS instrument. The protein expression yield in the device was up to 87 times higher than that in a conventional microplate. The feasibility of using the device for drug screening was demonstrated by measuring the inhibitory effects of compounds on synthesized ß-lactamase without the need for harvesting proteins, which enabled us to reduce the analysis time from days to hours.

MP69

Celeste Glazer, Labcyte, celeste.glazer@labcyte.com

Co-Authors: Maria Sonntag, Howard Lee, Carl Jarman, Siobhan Pickett, Sammy Datwani, Labcyte, Inc.

Miniaturized Quantitative PCR in 1536-Well Plate Format Using the Echo Liquid Handler

Miniaturizing quantitative PCR (qPCR) reactions into 1536-well plates holds great promise for increased throughput and reagent savings. However, delivering reagents into such high-density plates can be challenging for conventional tip-based liquid handling systems, leading to inaccurate assay volumes and contamination errors across wells. This study utilized the Labcyte Echo® 555 liquid handler to prepare miniaturized qPCR reactions in total reaction volumes ranging from 0.25 to 1.0 µL. The resulting amplification curves yielded excellent crossing point precision and accuracy, with CV's ranging from 0.95 to 1.25%. Tests with positive and negative controls dispensed into alternating wells revealed zero cross-contamination. These results demonstrate the Echo liquid handler as an ideal platform for preparing miniaturized qPCR reactions in 1536-well plate format.

MP70

Mark Herrmann, ARUP Laboratories, 18233@aruplab.com

Co-Authors: Jacob Durtschi, Tanya Sandrock, Elizabeth Pattison, ARUP Institute for Clinical and Experimental Pathology; Carl Wittwer, ARUP Laboratories, University of Utah School of Medicine

Automated Solid Phase Extraction for a High Through-Put Vitamin D 1, 25-Dihydroxy Assay

The Vitamin D 1, 25-Dihydroxy assay has a testing through-put in excess of 20,000 samples a month. The assay is performed in two parts, the first comprising of protein precipitation of serum or plasma followed by solid phase extraction (SPE) and purification of the Vitamin D 1, 25-Dihydroxy on C18OH columns. The second half is a competitive RIA procedure, using an I125 labeled Vitamin D 1, 25-Dihydroxy analog. None of the SPE extractors on the market met all of our laboratories requirements of small foot print, organic solvent compatibility, multiple solvent capability, 1 mL column compatibility, 96-sample through-put, SBS format compatibility, positive pressure displacement, and dry down. An automated SPE extractor was developed in house. The extractor was developed around KD Scientific's UltraSpense 2000 reagent dual plate dispenser. The UltraSpense has a small footprint and can pipette organic solvents with high accuracy into SBS formatted consumables. Multiple reagents were accessed with the addition of a 6 channel selector valve. The dispenser has positions for two plates, one in front of the other. Above each position a 96-port manifold was placed for positive displacement of the reagents through the columns. The back position was designed to handle vacuum removal of the wash waste. The front position was designed for sample elution and drying, with a heated base and retractable heated needle dryer. Software programming for walk away extraction was done in LabView. Total time for the processing of 96 samples for column activation, 3 washes, elution and dry down was approximately 40 minutes. The automated extractor was evaluated against the manual method for validation. Studies for precision, accuracy, linearity, sensitivity and carry-over were performed and results reported. Five sample pools were created for precision determination, spanning the range of 17 to 90 pg/mL. Within run CV's varied from 8.2% at 17 pg/mL to 10.5% at 90 pg/mL. Higher CV's were observed at higher concentrations due to the inverse relationship between counts and concentration in the RIA procedure. Correlation between values obtained through the manual and automated were good ($y = 0.928x + 1.876$, $r=0.893$). Better correlation was seen with samples prepared for LC-MS/MS ($y = 1.19x + 0.841$, $r = 0.95$). The assay was found to be linear over the standard range of 6 to 274 pg/mL with the lowest level of detection at 6 pg/mL. No evidence of cross contamination was observed when samples were extracted in a "checker board" layout with samples in excess of 250 pg/mL. Based on these results, the extractor has been placed into the production lab for daily use. We conclude that this design for automated solid phase extraction may be of greater benefit for a clinical laboratory then those currently available.

MP71

Shahriar Hessami, Hessami Plant Tissue Culture Laboratory(HPTCL), sh.hessami@hptcl.com

Co-Author: Alireza Babaei, Tarbiat Modares University

Development of an Efficient and Exclusive Temporary Immersion Bioreactor for Plant Production in Commercial Scale

Application of usual methods in plant micropropagation in comparison with new techniques is expensive and time consuming. Therefore development of new methods for production of less expensive in vitro plants has steadily increased in recent years. In this study development of an efficient and exclusive Temporary Immersion Bioreactor (TIB) has been carried out. In order to decrease the primary investment, application of disposable polyethylene terephthalate (PET)-based tank instead of glass based tank considered. As this kind of container is not autoclavable, a special chemical sterilization has been applied. It does not require much time for preparing medium and transplanting of multiplied plantlets under aseptic condition. Therefore it does not need any special equipment such as air flow cabinet and autoclave. In addition the mentioned TIB system is light and easy transferable. Using this bioreactor is a great step forward in lab automation and increase lab efficiency. Already a pilot scale of this TIB system has been setup for the commercial production of Banana in multiplication and rooting phases in HPTC laboratory.

MP72

Matthew Hymes, Pall Life Sciences, matthew_hymes@pall.com

Co-Authors: Tarbiat Modares University; Lisa Bradbury, Director R&D, Pall Corporation;
Alana Lerch, Todd Allen, Astoria-Pacific International

Diagnostic Determination of Biotinidase Activity Using AcroPrep™ Advance Filter Plates

Newborn screening for genetic disorders that can affect the physical and mental development of a newborn baby is preformed for approximately 97% of more than four million babies born in the US. Biotinidase Deficiency (BIOT) is an autosomal recessive disorder caused by the lack of an enzyme called biotinidase. This deficiency leads to the inability to metabolize biotin, a water-soluble B-complex vitamin. BIOT is easily treated with vitamin supplementation to alleviate or even stop symptoms. Without treatment, this disorder can lead to seizures, developmental delay, eczema, and hearing loss. AcroPrep™ Advance filter plates have been optimized for use in neonatal screening assays and play an integral role in the sample preparation procedure for the diagnostic determination of BIOT. AcroPrep Advance plates can be used in conjunction with the two 510(k) approved kits for the semi-quantitative determination of biotinidase activity in dried whole blood spots available from Astoria-Pacific International. Both of Astoria-Pacific's SPOTCHECK Biotinidase Reagent Kits are intended for in vitro screening of decreased biotinidase activity, primarily for the diagnosis and treatment of BIOT in newborns. One reagent kit is used solely on the SPOTCHECK Continuous Flow Analyzer while the other kit utilizes only standard microplates. The results can be collected with a colorimetric plate reader. The benefits of the AcroPrep Advance filter plate with the Astoria-Pacific 510(k) approved kits include rapid and consistent filtration times and excellent assay performance in part due to good fiber retention, no assay interference from extractables, and very low probability of cross contamination.

MP73

Chatura Jayakody, University of North Carolina at Chapel Hill, jayakody@unc.edu

Co-Authors: Samantha G. Pattenden, William P. Janzen, Center for Integrative Chemical Biology & Drug Discovery, University of North Carolina at Chapel Hill

Automated F.A.I.R.E. for Chromatin-Based Cancer Diagnosis and Drug Screening

Chromatin consists of DNA, histones and other associated proteins that make up chromosomes. Some of the functional attributes associated with chromatin include transcription of RNA and miRNA, DNA repair, recombination, cell cycle regulation, embryonic development and the role played by aberrant states of chromatin in diseases such as cancer. Thus, techniques that promote a better understanding of chromatin are an essential tool for science. Formaldehyde-Assisted Isolation of Regulation Elements (FAIRE) is a technique used to isolate regions of "open" chromatin, which are associated with gene regulatory activity. Access to these regions of "open" chromatin makes it possible to gain an understanding of DNA-dependent processes such as transcription, recombination, and DNA repair. Despite its robustness, the FAIRE technique is currently low-throughput and labor intensive. Our group is interested in the development of small molecule inhibitors to various chromatin readers and writers. Specifically, we focus on inhibitors of methyltransferases and methyl binding proteins, disruption of which is associated with large-scale changes in chromatin architecture. We have adapted the FAIRE technique to high-throughput automation. Automation of this technique allows us to reduce sample size and screen for changes in chromatin profiles in multiple cell lines treated with various small molecule inhibitors. Some of our future work will focus on miniaturizing sample sizes to the point where individual cells could be analyzed, thereby making FAIRE the first diagnostic tool capable of interrogating "single cell chromatin states".

MP74

Junhoe Cha, Institute of Bioengineering and Nanotechnology, jcha@ibn.a-star.edu.sg

Co-Authors: Jaehong Lim, Yi Li Ang, Jessica Oon, Mei Wei Ang, Su Seong Lee, Institute of Bioengineering and Nanotechnology

High-Throughput Screening and Validation of High Affinity of Protein Capture Agents

Combinatorial one-bead-one-compound (OBOC) peptide libraries are widely used for affinity screening, and the sequencing of peptides from hit beads is a key step in the process. For rapid sequencing, CNBr cleavage of the peptides from the beads, followed by de novo sequencing by MALDI-TOF/TOF is explored. We been successively demonstrated a semi-automatic sequencing algorithm resulting in sequencing success rate to nearly 100%. We tested the algorithm by using MALDI-TOF/TOF to identify a peptide biligand affinity agent against the protein bovine carbonic anhydrase II (bCAII), starting from comprehensive one-bead-one-compound peptide libraries comprised of nonnatural and artificial amino acid components and using the strategy of in situ click/OBOC library screening. The sequencing results displayed strong homology to those by Edman degradation. Several positive peptides had the same motif sequence and characterized those affinities by surface plasmon resonance (SPR) and dot blot.

MP75

Michael Kowalski, Beckman Coulter, mkowalski@beckman.com

Co-Authors: Amy Yoder, Li Liu, Laura Pajak

Automated Optimization of Murine Embryonic Stem Cell Differentiation Into Cardiomyocytes

Pure populations of cardiomyocytes derived from embryonic stem cells offer great promise as potential cell replacement therapies, as well as for use in pharmaceutical studies. The differentiation process, however, is frequently inefficient and nonspecific. We sought to improve upon the current processes by coupling automation and design of experiment (DOE). DOE-generated combinations of pro-cardiomyocyte compounds and media components were converted to dispensing volumes using Automated Assay Optimization (AAO) for BioRAPTR* software, and reagents and cells were dispensed into 384-well plates using the BioRAPTR FRD* microfluidic workstation. After four or five days of culture, the resulting embryoid bodies (EBs) were transferred to 96-well gelatin-coated plates using the Biomek FXP* liquid handler. After an additional three to seven days, the wells were observed for beating colonies and the differentiated cells were further analyzed using flow cytometry. This work illustrates the potential of a system that can both automate and optimize the differentiation of murine embryonic stem cells.

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MP76

Arja Lamberg, Thermo Fisher Scientific, arja.lamberg@thermofisher.com

Co-Authors: Marika Suomalainen, Noora Lindholm, Sini Suomalainen, Virpi Puro

Optimizing the Automated High-Throughput Purification of Nucleic Acids With Thermo Scientific KingFisher Flex

The pure and intact DNA or RNA is an important starting point for various experiments. Thermo Scientific KingFisher technology offers an efficient and rapid automated system for isolation of small particles, such as nucleic acids, proteins and cells. Magnetic particle kits enable purification of nucleic acids from various starting materials, for example from blood and tissue. The chemistry of the reagents generate the optimal environment where nucleic acids bind to the magnetic particles in the beginning of the purification protocol and are released to the elution buffer after sequential wash steps. In the nucleic acid purification sample preparation is an important step. Efficient lysing of the sample is essential and ensures gaining of the maximum yield of the purified DNA. However, if saving time is necessary it may be possible to shorten the processing time and find a balance between time-saving and gaining adequate yield of DNA for downstream analyses. We compared the lysing time and the yield of the purified DNA in order to speed up the purification process using Thermo Scientific KingFisher Flex. The results indicate that sufficient yield of DNA can be obtained with surprisingly short lysis step, offering an adequate material for PCR, and enabling to obtain for example genotyping results during the same day. On the other hand, longer lysing time ensured larger yield of DNA, thus providing material for downstream applications where the quantity of DNA is important. The DNA purification was also quickened and modified more automatic by using Thermo Scientific Versette and Multidrop Combi for dispensing reagents on the Microtiter deep well 96 plates. Occasionally the best possible quality and quantity of purified DNA are not the prime goals of the purification, but instead more hands-free time is required. Shortening the purification protocol and gaining more hands-free time when using KingFisher Flex can be achieved by removing the dispense step and instead adding all the required reagents on the plates in the beginning of the purification protocol. We compared these two protocols, conventional and walkaway protocols, for DNA purification from blood. The results show that although DNA isolated with the conventional protocol had better quality and quantity than DNA isolated with the walkaway protocol, the latter proved to have sufficient quality for several down stream applications, such as PCR. The KingFisher Flex together with the easily modified Thermo Scientific BindIt Software 3.1 provides large variety of options to create different purification protocols. The sample preparation as well as the purification protocols can and should be modified according to the sample material and the requirements of the user to offer suitable sample material for the downstream applications as conveniently as possible.

MP77

Hunter Rogers, Auburn University, hbr0002@tigermail.auburn.edu

Co-Authors: Juncheng Liu, Steven R. Saunders, Christopher B. Roberts, Auburn University

The Effects of Morphology on the Catalytic Activities of Platinum Nanoparticles Synthesized Using Green Chemistry Methods

Within the past few years, much interest has been taken in the research of nanoparticles due to their potential use as catalysts for the production of alternative fuel and energy sources. Catalysis has long relied on noble metal nanocrystals for a wide variety of organic and inorganic reactions. Noble metal nanocrystals are very effective as catalysts due to their high surface-to-volume ratios and high surface energies which cause their surface atoms to be highly active. Catalysis requires the use of noble metal nanocrystals in an optimal state, both in terms of size and shape, to maximize their catalytic activity. Integration of green chemistry principles into the overall process of the nanoparticles production is another formidable challenge. This requires not only the generation of favorable functional nanocrystals, but also the elimination of the chemical hazards to human health and the environment during the creation and use of these materials. Given these situations, the objective of this work is to investigate the effect of morphology on the catalytic activity and efficiency of platinum nanocatalysts synthesized using "green" chemistry methods. In synthesizing the platinum nanoparticles, we have employed the seed-mediated growth method, using only water as the solvent and carboxymethyl cellulose (CMC) as an ecologically benign capping agent. We conducted the synthesis of the nanoparticles under varying temperatures (25°C, 100°C) as well as under varying CMC concentrations (0.01wt%, 0.05wt%, 0.15wt%, 0.30wt%). Upon the completion of the synthesis of the nanoparticles, we determined that the Pt nanoparticles synthesized at 25°C were "nearly spherical" in shape while the Pt nanoparticles synthesized at 100°C had an even ratio of cubic and "nearly spherical" structures. In order to investigate their catalytic capabilities, the nanoparticles were used as catalysts in the reduction of 4-nitrophenol. By observing the rate in which the 4-nitrophenol was reduced, we were able to compare the catalytic efficiency of the synthesized Pt nanoparticles. We observed that the cubic structures synthesized at 100°C had higher catalytic rates per surface area than did the "nearly spherical" structures of similar size synthesized at 25°C. We also observed that the nanoparticles synthesized with a CMC concentration of 0.30wt% at 100°C had the highest initial catalytic rate of the concentrations tested , but the Pt nanoparticles synthesized with a concentration of 0.15wt% had the highest rate over the entire course of the experimental reaction. From this work we were able to illustrate the morphological effects of nanoparticles on their catalytic activity using green chemistry methods, which will allow for further work to be done in the optimization of nanocatalysts' morphology.

MP78

Brad Larson, BioTek Instruments, Inc., larsonb@biotek.com

Co-Authors: Peter Banks, Gary Barush, Wendy Goodrich, BioTek Instruments, Inc.; Timothy A. Moeller, Celsis In Vitro Technologies; Mary Sobol, Tracy Worzella, Dongping Ma, James J. Cali, Promega Corporation

Automated 384-Well Cell-Based Cytochrome P450 Inhibition Assays Using Cryopreserved Human Hepatocytes in Suspension

Drug-drug interactions (DDI) are of serious concern to the pharmaceutical industry and associated regulatory agencies. Cytochrome P450 (CYP) enzymes are key players in the metabolism of drugs within the body, and modulations in their activity have been implicated in many known DDI. Therefore, it is essential to understand how these enzymes can be affected by xenobiotics with regards to inhibition to avoid potential drug-drug interactions. Numerous formats currently exist to monitor the potential effects that a lead compound may have on a CYP. These include the use of recombinant CYP isoforms, microsomes, and immortalized cell lines with hepatocyte-like function. While each of these formats has its advantages, there is an increasing realization that data generated using these methods do not provide a complete picture of the effects a compound would have in an in vivo setting. Therefore, assays using primary hepatocytes are becoming increasingly important to accurately determine potential DDI. This is due to the fact that hepatocytes, the primary cell of the liver, possess the full complement of enzymes, nuclear factors and co-factors at physiological levels. Because of the increased desire to perform hepatocyte-based assays, coupled with the need to perform ADME-Tox assays earlier in the drug discovery process, it is essential to be able to perform these assays in a higher-throughput setting, using appropriate laboratory automation. Here we present an application that demonstrates the ability to monitor CYP inhibition using cryopreserved human hepatocytes in an automated format. Using this setup, the inhibitory effects of lead compounds on multiple CYP isoforms can be tested in a single 384-well plate, in a profiling format. Multiple luminescent assays, each using a luminogenic substrate specific for the isoform being analyzed, were employed for this application. The assay procedure was automated using a microplate dispenser as well as an 8-channel liquid handler. Four known inhibitors were tested with each of the three isoforms included in the profile, CYP1A2, -2C9, and -3A4. IC50 values were derived from a similar 11-point titration curve. Inhibition data was compared to that generated using human liver microsomes in a similar assay format, as well as to IC50 values for select inhibitors listed in the current FDA Draft Guidance.

MP79

Brad Larson, BioTek Instruments, Inc., larsonb@biotek.com

Co-Authors: Peter Banks, BioTek Instruments, Inc.; Stephanie Nichles, George Klarmann, Lonza, Inc.; Sylvie Crawford, Cisbio US; Mark Rothenberg, Corning Life Sciences

A Homogeneous Assay to Quantify Endogenous AKT Phosphorylation in Human Umbilical Endothelial Cells

AKT is a serine/threonine protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. AKT is involved in cellular survival pathways, by inhibiting apoptotic processes. AKT is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, AKT has been implicated as a major factor in many types of cancer. AKT is a key downstream intracellular point of convergence for a number of cellular signaling pathways. These diverse signaling pathways are activated by a variety of growth factors (including vascular endothelial growth factors (VEGFs), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin-like growth factor 1 (IGF-1)). One or more of these signaling pathways may be abnormally activated in patients with many different types of cancer, resulting in deregulated cell proliferation, tumor angiogenesis, and abnormal cell metabolism. Here we provide results for an automated HTRF® assay for measuring AKT phosphorylation at its serine(473) residue, using primary human umbilical vein epithelial (HUVEC) cells. Due to the increased need to generate the most biologically relevant data during drug discovery, primary cells are gaining in popularity for use in target based cellular assays. HUVEC cells are a robust primary endothelial cell type which is widely used for in vitro studies, including angiogenesis. Cells were plated on tissue culture treated polystyrene plates. 96-well plates were collagen coated to promote cell adhesion during media exchanges. The entire assay procedure was automated including cell manipulations, compound titration and transfer, and reagent dispensing. Optimization and validation experiments demonstrate how the combination of assay, cells, and instrumentation can provide an easy to use method to examine the function of this important signal transduction pathway.

MP80

Mark Rothenberg, Corning Incorporated Life Sciences, mark_rothenberg@yahoo.com

Co-Author: Michael W. Briggs, Corning Incorporated Life Sciences

Microplates and Automation—An Interesting Relationship

The past thirty years has seen an astonishing rate of growth of automation to aid in research and drug development. From low throughput instruments such as microplate readers to highly advanced instrumentation such as High Content Imaging, and the automation involved in running the assays, microplates have always played a central role. The relationship between microplates and the automated equipment is fundamentally critical to any High-Throughput Screen (HTS). By not appreciating this dynamic the incidence of screen failures becomes all too relevant. This presentation will provide a basis for understanding this relationship as well as guidance on choosing the correct microplate for your high-throughput screen.

MP81

Chris Walsh, RTS Life Science, chris.walsh@rts-group.com

Co-Authors: Julio Maher, Mel Whiteside, RTS Life Science

High-Speed Identification of Problem Samples In Your Compound Collection

New samples are regularly submitted to compound collections, either as a result of internal synthesis or external acquisition programs. Many samples may come from remote sites or a variety of suppliers, not all of which will adhere to the same quality standards as a central facility. Allowing these samples to be loaded into an automated store, without undergoing some form of quality check can ultimately lead to problems further along the drug discovery/development process. Data gathered by RTS suggests that in some organisations as many as 5% of plate wells may be erroneously empty, and 3-5% of wells may have samples at the wrong concentration; this is generally ascribed to poor quality sample input. Checking there is sufficient quantity of sample, and whether it is correctly solubilised, is thus commonly undertaken, and although these checks can be performed manually, they are somewhat subjective, as well as being onerous if many samples are being checked. The RTS Tube Auditor™ enables high-speed non-contact volume measurement and precipitate detection in SBS format microtubes to be undertaken rapidly and accurately, offering significant benefits over manual and other semi-automated solutions. This poster presents the latest data showing how vision technology used within the Tube Auditor™ is helping customers rapidly identify problem samples, and thus improve the quality of their compound collection, at a variety of stages within the compound management lifecycle.

MP82

Ken Ward, ken.ward@hp.com

Co-Authors: Michael Day, Christie Dudenhoefer, Jeff Nielsen, Heather Paris, Kevin F. Peters, Debora Thomas, Hewlett-Packard Company

Picoliter Dispense Performance for Dose-Response Assays by Direct Digital Titration

Picoliter droplet dispensing is used to perform direct titration for dose-response studies using Hewlett-Packard's digital dispenser. By placing the proper number of ultrasmall droplets into wells, the intended dose is directly generated without resorting to serial or intermediate dilution. The numerous pitfalls of serial dilution are avoided using this noncontact method. In addition, time-consuming serial dilution workflows are greatly simplified in order to reduce time-to-results which is critical for lead optimization. The performance of this technology will be demonstrated both using bioassay results from biopharma collaborations and using HP quality assurance methodology for liquid handling. For QA, it will be shown how fluorescence methods have been optimized for picoliter to nanoliter dispense volumes. Absorption spectroscopy is utilized for volumes greater than five nanoliters. Preliminary results demonstrate CV's less than 10% for single 15pL drops, and much less for larger volumes. Additionally, results demonstrate accuracies better than +/-15% across the dispense range. Other advantages of the technology will be shown as well, including reduced dead volume and reduced compound usage for dose-response titrations. Results from beta program experiments in collaboration with pharmaceutical researchers will be used to demonstrate improvements in dose-response assays. One of the dramatic improvements enabled with digital dispense is a reduction in the error of EC50 value determinations. This enhanced ability to compare candidate efficacies for SAR (structure-activity relationship) studies is a major advantage of this technology. Another improvement enabled by digital dispense is the ability to put any dose into any well, which significantly reduces edge effects that are common in traditional serial dilutions. Overall, the performance and benefits of HP picoliter dispense for direct digital titrations will be clearly demonstrated.

MP83

Ruth Zhang, Beckman Coulter, Inc., rzhang@beckman.com

Co-Authors: Kelly Marshall, Beckman Coulter, Inc.; Zach Smith, The Center for Genomics and Bioinformatics, Indiana University; Keithanne Mockaitis, John Colbourne, The Center for Genomics and Bioinformatics, Indiana University; Alisa Jackson, Beckman Coulter, Inc.

Complete Solution for Next-Generation Sequencing Sample Preparations of Roche GS FLX Titanium* Series Kits Using Biomek® FXP and Biomek NXP Laboratory Automation Workstation With REM e Integration

Next-generation sequencing (NGS) revolutionized the field of genomic sequencing since 2005 due to its massive-parallel nature. However, many research labs, including most genome centers, still manually prepare NGS library samples using laborious and expensive processes. Our complete NGS sample preparation solution increases sample throughput, consistency and accuracy; and enables genomics research to address population-level questions and large-scale screening for DNA polymorphisms in the fields of medicine, evolutionary biology, environmental toxicology, and others. This poster presents a suite of high-throughput NGS sample preparation methods that automated the Roche 454* GS FLX Titanium* Series Kits in a 96well plate format with samples starting from random sheared gDNA to the enriched emPCR+ beads that are ready-to-load onto Roche FLX Genome Sequencer. This complete solution consists of a pre-PCR system using Biomek FXP dual hybrid with Trobot and a post-PCR system using Biomek NXP Span-8 with integrated REM e. The Pre-PCR system has three major applications: (1) Preparation of up to 96 libraries using Roche GS FLX Titanium* Rapid Library Preparation Kit (< 3 hours). (2) On deck NGS Library Quantitation and Normalization using Rapid Library (RL) Standard and PicoGreen Assay Kit (Invitrogen) (< 1 hour). (3) emPCR Reaction Setup using Roche GS FLX Titanium* LV and SV emPCR Kits (1-1.5 hours). The Post-PCR system is designed for LV or SV emPCR enrichment and sequencing primer annealing processes (<4 hours). Random sheared DNA (500 ng) samples from clonal isolates of Daphnia pulex provided by Indiana University were used to generate libraries for sequencing on the Roche/454* Life Science* platform. The DNA sequencing data generated from this model species for testing environmental water was obtained from both manual and automated library preparation processes. The data shows that comparing to libraries prepared manually, libraries prepared using automation was proportionally equal across libraries but the yield was slightly better, and the size distribution of DNA fragments fitted within acceptable boundaries yet with a shortened average length of the reads (361 bp automate vs. 391 bp manual). Refinements were made to maximize sequence with automation sample preparations and additional data will be presented.

Note: The Biomek FXP and NXP are for Laboratory Use only; not for use in diagnostic procedures.

MP84

Justin Murray, Merck & Co. Inc., justin_murray@merck.com

Co-Authors: Kurtis Berry, Jeff Doto, Xinnian Li, Oleg Kornienko

Automated Dispense Quality Control and the Active Monitoring of Liquid Handlers

With today's compound libraries growing to such large collection sizes, High Throughput Screening an entire compound collection could involve multiple weeks of plate processing. Screening over multiple days or weeks introduces day to day variations in the assay data statistics. Sometimes data is comparatively unacceptable resulting in the need to reprocess plates, which can be costly and time consuming. These fluctuations in statistics can arise from slight variations in the daily reagent preparation to minor variations in the volumes dispensed during the liquid handling steps. In an effort to obtain consistent assay data statistics, to obtain optimal assay quality, to minimize unnecessary reagent use, and to minimize the number of reprocessed plates, we have developed automated processes that actively monitor, regulate, and calibrate the liquid handlers before and routinely during a screen.

MP85

Tim Schroeder, Atoll GmbH, t.schroeder@atoll-bio.com

Co-Authors: Jürgen Friedle

Innovative Automated Small Scale Parallelized Biochromatography for High-Throughput Method Development in Downstream Processing

A new platform technology has been developed which enables 96 array format column chromatography. The design allows the user to select any chromatographic material which is packed with due consideration to individual material compression requirements. Bed containment between two filter frits ensures high efficiency and peak symmetry similar to that of preparative and process separation columns, and distinguishes the system from the current filter based systems for simple on/off sample equilibration operation. Liquid flow in the columns was driven by positive pressure liquid displacement, like in columns individually connected to a one channel stand-alone chromatography system. 8 columns are operated in parallel and pre-programmed buffer preparation resulting in fast completion of 12 column rows. Fractions from step elution were collected into standard microplates, utilizing an automated microplate transport system and subsequently submitted to a next separation step for a further chromatographic dimension or analysis like UV, ELISA, MS, HPLC or SDS-PAGE. This resulted in fully automated, walk-away procedures with a drastic reduction in processing time (up to 70%) and significantly increased process security. Applications shown were successfully implemented for parameter elucidation and optimization in process development of therapeutic protein production, in-process monitoring of fermentation broth for mAb-production, protein drug discovery and depletion of abundant components in screening experiments.

MP86

Sara Saedinia, University of California, Irvine, ssaedini@uci.edu

Co-Authors: Liang(Lily) Wu, Kent Nastiuk, John Krolewski, Mark Bachman, G.P. Li

Microscale immunoblotting for small sample protein analysis

Western blot, electrophoretic separation followed by immunoblotting, is a well understood and popular technique for analyzing and quantifying protein composition in a complex mixture. This technique can potentially be useful for identifying the protein expression of cancer cells in resected tumor samples, thus providing important information for applications in personalized medicine for cancer treatment. However, conventional embodiments of this technique typically require large quantities of proteins, making it unsuitable for anayzing small samples of tissue containing tumor cells of interest, which often results in proteins yields measured in nanograms. We are developing a micro-western blot system that can work on a very small quantity of protein in solution, making it suitable for use with small samples such as cancer cells separated from biopsy tissue samples (such as by laser capture microdissection). The device employs microfluidic gels and electrophoretic transport to pre-concentrate the proteins in a stacking region, then performs protein separation in a long microgel. The small gel dimensions ensure that proteins remain in high concentration during separation, even though the total quantity of the proteins is small. After separation, microfluidic side channels pull the proteins into hydrophobic capture regions (micro-blotting), where immunostaining is subsequently performed. This device is thus capable of running a small volume sample through a complete western blot analysis on an integrated chip. The small integrated device may be driven by external electronics, and so may be completely automated, making it suitable for use by non-specialists, for example, in the field or at the point of care.

TP01

Vibha Chawla, Patni Life Sciences, vibha.chawla@patni.com

Co-Authors: John Petrakis, Donald Hart, Smita Nayak

Stability Management: Journey From Requirements to LIMS Prototype

Stability plays an important role in drug development process and helps establish recommended storage conditions, re-test period, product shelf-life and expiration dating. Managing all the stability information on paper is risky since it involves a lot of information compilation and review through email exchanges and personal communications. Searching for a piece of data can take hours of looking through piles of paper reports. Reporting usually involves transcribing key information into new document. As a result, information retrieval and reporting can be extremely complex and time consuming for stability teams, and hence, the need for a Laboratory Information Management System (LIMS). This presentation will summarize key findings from recent LIMS prototyping projects for Stability processes in mid to top tier consumer, biotechnology and pharmaceutical companies. Some projects involved consolidating laboratories with a variety legacy LIMS and paper system. Development of a properly designed corporate-wide stability system required a lot of design decisions that ultimately resulted in simplified and more efficient processes.

TP02

Ming-Jhy Hseu, BiOptic, Inc., varoujamirkhanian@yahoo.com

Co-Authors: Shou-Kuan Tsai, Varoujan D. Amirkhanian, BiOptic, Inc.

Compact and inexpensive Capillary Gel-Electrophoresis Fluorescence System for Antibody-Antigen Interactions

We introduce the world's first smallest and very inexpensive high performance capillary gel-electrophoresis fluorescence immunoassay analyzer. The new bench-type instrument provides 96-well sample capacity that uses pen-size disposable micro-fluidic gel-cartridges, providing high-speed electrophoretic separations of bio-molecules (Protein & Carbohydrate) at less than $0.1 USD per sample run. Using commercially available bio-markers as indicators we have demonstrated that the novel CE system provides high fluorescence detection sensitivity with fast separations. The rugged CE system can be applied potentially for routine clinical chemistry applications as well as for research purposes as a highly sensitive and low-cost type bio-analyzer for rapid screening of antibody – antigen interactions.

TP03

Emir Aznakayev, National Aviation University, aznakayev1@hotmail.com
Co-Authors: Vladimir Shutko, Valeriy Barzinski

Spectral Correction Complex SCC-Bars

The Spectral Correction Complex (SCC) device is designed for medical purposes. It allows obtaining the objective readings of information received from an object, evaluation of the object's state, the affected spectral detection, correction of the object's state, and bringing the human organism into the normal state. The SCC-BARS device influences on the biologically active points (BAP) and changes their electric potentials. This new method provides the advanced evaluation of homeostasis. For diagnosing a disease, it is important to know about existing correlation between organism dysfunction and individual cells pathology, since any disease changes the metabolic processes in cells, which in its turn, changes the electromagnetic fields (EMF) spectra of the cells. The suggested method can be used for both direct and indirect detection of functional and organic changes as well as their causes of pre-pathologic and pathologic processes. It can localize these processes at the pre-clinical and clinical stages. It can be used for dynamic monitoring and treatment of the disease. Testing and correction can be carried out at medical centers and laboratories. SCC-BARS device allows solve of some health problems at less costs and less time. Theory of Method. According to theoretical consideration of the lower resonance-wave and electromagnetic field oscillations, it has been found that their relationship and interaction occurs in the form of the resonance-wave processes of certain frequency and wavelengths of cells. Interference of these processes creates the new characteristic of cells' frequencies. Any pathogenic process occurring in organism and its treatment can be corrected by controlling its characteristic frequencies. Diagnostic-therapeutic equipment receives information about processes in organism through computer information processing of signals from BAPs. Therefore, for the analysis of the E?F therapeutic effects, we should study spectrums of radiation from cells interacted with external (therapeutic) E?Fs. Any pathological process has its own individual spectrum. The computer memory contains information about pathologic events which take into account age, sex and other variations. After computer reads the organ's frequency parameters, the diagnostic device compares their spectral similarity with standard processes (healthy tissues, tissues with pathologic changes, infection agents) stored in computer and defines the closest pathologic process or tendency in organism. In case of multiple processes, the spectral diagnostic device allows provides the differential diagnostics of each process. Results and conclusions. The SCC-BARS device allows quick reading and analysis of information received from an object, detection of affected mono-spectra and their correction thus bringing an organism into compliance. Detection and correction of organism may take place in clinics and outside. SCC-BARS device has small cost and short time for human health diagnostics.

TP04

Robyn Barnes, Invetech, rmb@invetech.com.au
Co-Author: Rob Danby, Invetech

Novel Plate Preparation Technology Enables High-Throughput Application

Microbiology is one of the last areas of clinical pathology to widely embrace automated processing systems. One key process in microbiology; agar plate inoculation, is a repetitive, manually intensive process that has not changed significantly since the 1880s, when Robert Koch first introduced gelatine as a solid culture medium and Richard Petri developed the Petri dish. Manual streaking of agar plates has high inter-operator variability, and is therefore not suited to standardization or quantitation, rendering the scoring of test results a complex task requiring interpretation from skilled microbiologists. The invention of the MicroStreak® applicator embodied a novel concept for streaking biological samples onto agar plates. The applicator was modified to enable robotic handling without changing its essential streaking properties, and its commercialization was achieved by housing this process within a custom-designed instrument, the PREVI™ Isola. The applicator consists of a flexible tined comb that streaks a line of sample around an agar plate. Different levels of sample dilution are achieved by controlling the material properties, geometry, and the applicator's placement on the agar surface along with the speed of plate rotation. This provides operator independent control over colony formation. The streaking pattern is highly uniform and reproducible, and leads to improved colony production and isolation for sample types such as: urine, swabs, feces and enrichment broths. The goal of achieving these results and proving an automated protocol that reliably replicates the work of skilled lab technicians, drove some interesting choices in the original system definition and posed a series of comparison exercises, ranging from the hourly plate throughput rate, to the capacity to deal with variability in Petri dishes and agar mediums. Petri Dishes, consumables created for hand manipulation in the 19th century, were now to be adopted unchanged for 21st century automation. The choice to retain the industry "standard" Petri Dish as the consumable, rather than design a more automation compatible vessel, was one of the key system definition decisions underlying the Previ Isola's acceptance in the microbiology lab. Other decisions influencing the instrument's design improved the chain of custody in the laboratory. The PREVI automatically reads sample barcodes, and labels the output agar plates, and records protocol detail, providing a level of quality control not previously available. Despite being a revolutionary step away from the traditional manual process, the automated process is instantly recognizable and familiar, enabling microbiologists to continue to use their accumulated expertise to determine patient results. It provides laboratories new flexibility for routine processing, allowing resources and expertise to be concentrated where they are needed most.

TP05

Jean Shieh, Microsonic Systems, jean.shieh@microsonics.com

Co-Authors: Lauren Frick, BIOCIUS; Jim O'Keefe, Microsonic Systems; Jennifer Rossi, BIOCIUS

Ultrasonic Fluid Processing Improves Triolein Measurement Accuracy in Mass Spectrometry Assays

Development of therapeutics for obesity is the focus of many pharmaceutical research and development programs, particularly the identification of potential inhibitors of the triacylglycerol (TAG) synthesis pathway. The reaction catalyzed by the enzyme diaceylglycerol aceyl transferase (DGAT) in the TAG pathway produces triolein, and because triolein remains in the lipid bilayer of the cell membrane rather than in the assay buffer making it difficult to accurately measure the compound activity and to reliably detect triolein in the mass spectrometer. In the protocol described in this poster, we used the Microsonic Systems HENDRIX SM100 ultrasonic fluid processor to release triolein from the membrane bilayer, and then used the BIOCIUS RapidFire® Mass Spectrometry (RF-MS) platform to accurately detect and quantify native compounds. We compared the results of various HENDRIX SM100 settings to determine the optimal operating conditions and achieved a 50% improvement in the triolein signal-to-noise ratio in the RF-MS assay.

TP06

Justin Murray, Merck & Co. Inc., justin_murray@merck.com

Co-Authors: Jack Dawson, High Res Biosolutions; Oleg Kornienko

The Single 9-Sided Pod—Ultra High-Throughput Multi-Function Robotic Screening Platform

Working with High Res Biosolutions (HRB) we have built an Ultra High-Throughput Multi-Function Robotic screening platform that can process any plate density (384, 1536, and 3456) in a compact foot print. This screening platform consists of a single robotic arm and the instrumentation necessary to perform many assay technologies. This single 9-sided pod system has a modular design and the throughput capabilities that rival multi-pod systems. We recently upgraded this system to include the addition of three new HRB AmbiStores. These new carousels provide robust high speed random access plate storage that have out performed the two Cytomat random access carousels we previously had integrated. We now have our full compound collection stored online. The plate storage capacity for both assay and compound plates are now increased. And plate throughput has increased by eliminating the bottleneck caused from loading and unloading plates.

TP07

Amy Yoder, Beckman Coulter, amy.yoder@beckman.com

Co-Authors: Michael Kowalski, Li Liu, Laura Pajak, Beckman Coulter Inc.

Analysis of Differentiation of Embryonic Stem Cells by Automated Flow Cytometry Sample Preparation on the Biomek® NXP

The well-documented ability of stem cells to differentiate into various cell lineages generates tremendous potential for cell-based treatments. For example, differentiated cardiomyocytes from embryonic stem cells can be used in drug discovery processes and therapeutic cardiac treatments. Experimentation to optimize differentiation to enhance the yield of cardiomyocytes is enabled by efficient analysis of differentiation. Downstream detection technologies for such experiments vary in time, complexity, and the ability to quantitatively determine the efficiency of differentiation. Flow cytometry is a common method for detection of various cell types, but requires automated sample preparation for use in higher throughput analyses of differentiation. We have employed the Biomek NXP Span-8 Automation Workstation for high throughput flow cytometry sample preparation to determine the efficiency of cardiomyocyte differentiation from murine embryonic stem cells. The workflow includes fixation, permeabilization, blocking, and antibody staining in a 96-well plate format. The Agilent Microplate Centrifuge has been integrated to the system to reduce user interaction for executing the multiple washes required. The automated workflow and results from the analysis are described.

TP08

Jens Beator, Invitek GmbH, jbeator@invitek.de

Co-Authors: Ralf Griebel, STRATEC Biomedical Systems AG; Curtis Kautzer, Navigenics; Hans Joos, Invitek GmbH

Novel Concept for Walk-Away Automated Sample Preparation for Direct-to-Consumer Genetics and Pharmacogenomic Studies

The analysis of genetic information continues to gain importance in all aspects of life ranging from individual ancestry research and personal genetic analysis to pharmacogenomic studies in drug development. Here we introduce a novel method of sample collection specifically designed for high DNA yields from saliva samples. The novel saliva stabilization buffer supersedes cooling of samples and stabilizes genomic DNA for months at room temperature. The non-invasive saliva collection method reduces puncturing-associated infection risks from blood collection, offers improved handling and consistency capabilities compared to swab collection, and is designed to integrate with automated nucleic acid extraction procedures. In this study the saliva samples are purified using a new magnetic particle processor for variable sample volumes from 50 µl to 5000µl. Utilizing a unique magnetic separation tool combined with reliable pipetting technology as well as a newly developed integrated incubator the instrument automates diagnostic extraction protocols for nucleic acids with full in-process control. Sensitivity of extraction and purification is shown by the detection of as little as 100 copies of viral nucleic acids in 200 µl of serum/plasma. Furthermore, reproducibility of high yields is achieved in extraction protocols of DNA from blood and saliva. As much as 90 % of contained nucleic acids are recovered in the automated process. The yield and purity of the extracted DNA is compliant e.g. with single nucleotide polymorphism (SNP) based methods such as the clinically guided genetic analysis offered by Navigenics.

TP09

Daniel Bezdek, Life Technologies, Dan.bezdek@lifetech.com

BenchPro 2100 Automated Maxiprep Purification System

Traditional plasmid purification technologies have changed little since the advent of column purification. While improvements have been made in the chemistry as to which the DNA is captured and purified, the most common method relies upon purification on an individual column or a 96 well plate. The hands on time for column based purifications can be significant and although automated solutions do exist they have been historically been limited to small “mini” scale purifications. Customers requiring larger “Maxi” scale plasmid purification are relegated to performing column purifications requiring up to 4 hours of hands on time. The BenchPro 2100 is the first of its kind fully automated plasmid purification system for Maxi scale plasmid purification available on the market. The system includes a small affordably priced benchtop instrument and two consumables consisting of a disposable card and reagent tray. Powered by pneumatically driven pumps and valves each purification card performs the complete plasmid purification from capturing cells to final elution in a little less than 90 minutes. The result is a concentrated sample of high quality transfection grade plasmid DNA suitable for most downstream applications with less than 5 minutes of setup time. From 125 mL of E. coli culture containing a high copy plasmid each card can consistently purify a minimum of 500 ug of plasmid DNA with an endotoxin level below 10 EU/ug. The simplicity of the pneumatic technology used in the BenchPro 2100 opens the door to other inexpensive and reliable automated instruments. Couple with that the robustness of encapsulating membranes in a disposable card and you have a very flexible platform for many other purification applications.

TP10

Simon Tullett, TTP LabTech

Co-Authors: Brian Everatt, Novartis; C.Chem., FRSC; Tim Bedford, Grant Gardner, TTPLabTech

Enabling Remote Open Access for Biochemical Analysis of the Future

Currently submission of chemical and biological samples to analytical equipment such as HPLC and GC/MS requires substantial manual investment. Typically samples are formulated and collated into racks before manually entering these into the analytical instrumentation. This can be a time consuming, error prone and an inefficient process, time that scientists can better spend in the laboratory. Lab2Lab from TTP LabTech is a novel approach to submitting and transporting samples for analysis across an entire site. Sample tubes are registered and methods selected, an ELN reference is assigned and the sample tube is placed into the “Sender”. The system then transports the samples using low pressure compressed air and directs them to the most appropriate analytical instrumentation available. The analytical results are then automatically returned to the originators ELN. The system is unique in that it allows samples to be sent from anywhere within building, from disparate laboratories to collated or disparate analytical instrumentation. This poster will demonstrate how it is possible to rationalise expensive equipment, reducing the cost of maintenance and support whilst increasing availability. In addition, the system has the capability to buffer submitted samples whilst the analytical equipment is busy or otherwise unavailable, meaning analyses can take place overnight. This poster will describe the system recently installed at Novartis, Horsham (UK) as part of their “Open Access” initiative for the “Lab of the Future” and demonstrate the typical throughput capability and time savings this introduces to the users. It will show how the system can cope with failure of the analytical instrumentation by being able to redirect samples to working equipment. In addition, this poster will illustrate how the analytical system can be expanded to incorporate NMR and comPOUND libraries. This allows full follow up analysis of biochemical samples or 100% QC of samples pre and post HTS.

TP11

Jas Sanghera, TTP LabTech

Co-Authors: Richard Kim, Ben Schenker, Simon Tullett, TTP LabTech

The Future of Compound Management

"Current compound management practices have evolved to support both primary and secondary screening projects from a centralized repository storing a combination of plates and tubes. Storage of both tube and plate formats is an inefficient design and adds to the complexity of these repositories, making them expensive to implement and more importantly difficult to expand or relocate. Also, new sample submission becomes a protracted process, which can lead to delays of several months until the samples are available for HTS.

Plates are considered the only way to support the throughput requirements of primary screening, but they also compromise sample integrity by introducing uncontrolled exposure to the environment and multiple freeze/thaw cycles. In addition, quality control of plate based material is almost impossible causing false positives and negatives, which are not picked up until the secondary screening stage. Plates also limit the flexibility of a library, requiring the entire library to be screened in each HTS campaign. A more efficient approach to primary screening would be to only screen against library subsets that are chemically relevant to the target, but this would require ultra high throughput cherry-picking of tubes. In this poster we will describe an innovative large-scale tube-based compound management approach to maximize lab space; improve compound stability; enable rapid generation of custom screening sets; while providing background QC and real time library integration of new chemical entities."

TP12

Joby Jenkins, TTP LabTech

Co-Authors: Rob Lewis, Stephen Starkie, Aidan Hird, TTP LabTech

An Integrated Solution for Automated Nanoliter Hit-Picking

A prerequisite for efficient primary screening is rapid, automated selection of "hits" for confirmation and secondary profiling. The mosquito® X1 offers precision sampling of any individual well in 48-, 96-, 384-or 1536-well plates. This enables researchers to quickly select small volumes of hits from a variety of primary screening plates and transfer them directly to the next screening stage without further dilution. Mosquito X1's disposable pipette tips guarantee zero cross-contamination, and their unique positive displacement pipetting technology ensures accurate and reproducible pipetting throughout the 25 nL -1.2 µL range. This poster describes a mosquito X1 integration with the Thermo RapidStak plate stacker using TTP LabTech's specially designed CherryPicker software, which allows the instrument to work unattended for extended periods. The CherryPicker software drives the system automatically by converting pick lists provided by LIMS systems or Library databases into mosquito protocols, and feeding appropriate plates via the RapidStak. The software optimises each protocol for efficient pipette use, and can also track the volumes of the source plates for subsequent reporting back to the LIMS system.

TP13

Lee Borenstein, UCLA School of Public Health Global Bio Laboratory, lborenstein@gbl.ucla.edu

Co-Authors: Alex Roth, Rahul Oliver, Savanna L. Carson, UCLA School of Public Health; Tracy H. Erkkila, Craig Blackheart, Jennifer Foster-Harris, Helen Cui, Chris Detter, Los Alamos National Laboratory; Jeffery Miller, Hilary Godwin, UCLA School of Public Health

Global Bio Lab: Enabling Technologies for High-Throughput Screening of Infectious Disease Samples

The Global Bio Lab at UCLA is a new high speed, high volume (high-throughput) laboratory for analyzing infectious disease samples. This facility was designed to meet a national need to be able to process, characterize, and analyze hundreds to thousands of infectious disease samples per day. As of Fall 2010, the lab will have online Automated Systems that will allow conventional samples to be reformatted and aliquoted into racks of compact tubes for storage ("Automated Accessioning System"), an Automated Biobanking System to allow for archiving of infectious disease samples at -80°C, and an Automated genotyping System to allow for full genome sequences to be determined. Two additional systems – an Automated Screening System and an Automated Culturing System – have been designed and will be procured and delivered in the upcoming year. The concept for the high throughput screening facility and the automated systems were developed as part of an ongoing partnership with Los Alamos National Laboratory (LANL). Also in partnership with LANL, we are developing a highly integrated information technology system that will allow the Global Bio Lab to be linked seamlessly into a network of collaborating laboratories and organizations (the "High-Throughput Laboratory Network", or HTLN). The automated, networked capability will provide an unprecedented capacity for processing and analyzing infectious disease samples enabling cutting edge research that will support enhanced biosurveillance and will make us stronger against natural diseases and bioterrorist attacks. We are actively seeking new partners and collaborators who are interested in taking advantage of this important new capability and in working with us to drive forward the frontiers of science and technology in this arena.

TP14

See on-site Conference Addendum for poster title and author updates.

TP15

Tobias Brode, Fraunhofer Institute Manufacturing Engineering and Automation, brode@ipa.fhg.de

Co-Authors: Andreas Traube, Jan Stallkamp, Christopher Laske

Analysis of Volume Precision and Accuracy of a New Device and Method for Nanoliter Liquid Handling in Micro Well Plates

Trust in nanoliter dispensing technologies has been on the decrease in the recent years. A new technology has to prove its functionality and robustness to get any acceptance on the market. The i-dot (immediate drop on demand technology) is now at that point. In this XXXXX the principle and idea of this dispensing technology will be presented followed by the results of experiments on volume precision and accuracy for single wells, 96 and 384 well plates. The principle of the new method consists of equipping the well grounds of a corrugated plate with very thin holes in a way that the capillary-pressure is greater than the pressure in the drilling hole induced by the liquid-level in the well. Thus, a leakage is being prevented. By a pneumatic pressure pulse a sample of ~5 nl may be taken, whereby the total dispensed volume is controlled by the number of drops and the pulse duration. With this technology it is possible to transfer probes between several formats of MWPs without any pipetteting steps. With molded micro well plates it is possible to achieve a cheap disposable with good nozzle parameters. These could be used for high-throughput applications like plate reformation or cherry-picking. Even 1536 or higher formats can be filled with this technology. The presentation will focus on the results of reformatting tests between several plate formats for high throughput applications via precision heat maps produced with the accredited Artel MVS. Later on, some special applications e.g., cell dispensing, cell sorting and microarray applications will be discussed using the example of ongoing projects at Fraunhofer.

TP16

Annette Brodte, Genedata, Annette.Brodte@genedata.com

Co-Authors: Stephan Heyse, Oliver Leven

A Platform for the Analysis of Time-Resolved Data From FLIPR, Label-free, and Ion-flux Technologies, in High-Throughput Style: Automated Analysis, Systematic Quality Control and Multiplexed Hit List Generation

Time-dependent responses are measured in many assay technologies, including Calcium flux assays, label-free binding / receptor activation assays, and ion channel assays. These experiments produce rich information for distinguishing multiple modes of action, for separating biologically relevant effects from artifacts, and optimizing quantification of responses on the level of complete screens. However, time-resolved experiments on a larger scale typically are narrowed down to one or two output variables already at the instrument, to facilitate analysis and fit with standard HTS workflows and infrastructure. While conceptually simple, this procedure tends to eliminate precious biological information measured in such time-resolved screens. In our case study, we demonstrate a framework which overcomes these limitations. Combining integration and business logic specific to different time-resolved screens with HTS-style quality assurance, automation and standardization on a single platform, we show how full-deck time-resolved screens are analyzed and results are generated without loss of information or fidelity.

TP17

Andrew Brooks, brooks@eohsi.rutgers.edu

Co-Authors: Robert Gaglione, Rutgers University Cell and DNA Repository (RUCDR); Michael DiCola, Bionomics Research and Technology Center (BRTC), Rutgers University; Alex Lopez; Chris Piccirillo, Hamilton Robotics

High-Throughput Extraction of Compromised Whole Blood Samples for Genetic Analysis: Implementation of the Chemagic STAR in a Genetics Repository

Genetic analysis continues to evolve with the utilization of novel technological approaches that require specific sources of genomic DNA. DNA from whole blood is becoming more important in the biomedical research environment with the introduction of epigenetic analyses. To this end, all whole blood sources of DNA, including compromised samples and archived resources, are critical for analysis. In addition, existing large collections (based on retrospective sampling) require a robust high-throughput approach to maximize the yield of DNA from each sample while retaining the highest quality nucleic acid. The unique challenges of extracting DNA from various volumes of compromised and frozen whole blood samples require extraction chemistries and automated solutions that ensure a high level of success with these precious samples. This presentation describes the protocol development and implementation of the chemagic STAR for frozen whole blood samples. The integration of chemagen's bead-based chemistry (with modifications for sample source and volume) with the Hamilton STAR creates a robust workflow that maximizes the yield and quality of compromised blood samples, provides a fully integrated "walk away" workflow that catalogues all DNA stocks, and prepares samples for analytical and functional quality control. This platform is tunable from 1ml to 10ml in single extractions providing the flexibility needed for large biorepository projects where samples are processed across many different collections. Both analytical and functional analyses are performed on all DNA samples and are described in this presentation to document the quality and downstream utility of this extraction approach.

TP18

John Warzeka, Pfizer, Inc., john.warzeka@pfizer.com

Co-Authors: Alex Opio, Beverly Nickerson, Gang Xue, Ken Norris, Pfizer Inc.

Expanding the Application of a Tablet Processing Workstation to Support the Sample Preparation of Liquid Formulations

In the pharmaceutical industry, sample preparation is often a very time consuming and tedious process. There are several commercially available systems which can automate the sample preparation steps. These systems have historically been used for the extraction of drug from solid oral dosage forms, however, they typically contain all of the necessary components to perform the sample preparation for liquid formulations. The system chosen for this research utilized a very robust filter station, a vortexer for mixing, and the ability to perform serial dilutions with gravimetric delivery confirmation. It also contained a fixed loop injector valve to provide online HPLC capability. Methods were developed for the potency, purity, and preservative assay for a powder for oral suspension (POS), as well as a potency assay for an oral solution. The results obtained compared favorably to the manual methods and demonstrate that the automated technique is suitable for this application. With the efficiencies gained it is possible to analyze a large number of samples with minimal human intervention.

TP19

Walter Cedeno, Johnson & Johnson Pharma R&D, wcedeno@its.jnj.com

Co-Authors: Edward Jaeger, Johnson & Johnson Pharma R&D; Dimitris Agrafiotis, Johnson & Johnson Pharma R&D

An Efficient System for End to End Automation of Discovery Processes

The need for systems that can provide seamless environment for the flow of information from the internal and external laboratories to decision makers has been an ongoing challenge as new tools and technologies evolve. We have developed a laboratory informatics platform based on a service-oriented architecture to efficiently and transparently integrate processes and information in the laboratories across multiple sites worldwide with our Advanced Biological and Chemical Discovery Informatics platform. The integration allows the flow of information about compounds, biological assay requests, biological assay results, and other data that aid in the day-to-day tasks scientists perform and provide timely information to decision makers. The platform consists of well-defined services and applications for reagent management, compound registration, compound management, assay request & fulfillment, data reduction and upload, data mining, data analysis and reporting.
Key to the success of the platform was the ability to define standard interfaces, standard terminology and separate key services for people management, security, and instrument automation into different frameworks that can be reused, scaled up, or replaced as needed. The laboratory informatics system represents a departure from a centralized approach normally used in LIMS systems to successfully accommodate the ever changing needs of scientists and accommodate new technologies and systems as they evolve. The laboratory informatics platform allows for easier integration of disparate applications and systems, automation of tasks done manually in the past, and use of detailed process information to make informed decisions about current processes and future direction.

TP20

Emory Chan, Lawrence Berkeley National Laboratory, emory.commerce@gmail.com

Combinatorial Discovery and Characterization of Upconverting Nanocrystal Probes for Biological Imaging

Lanthanide-doped nanocrystals can exhibit visible upconversion luminescence when excited with near-infrared (NIR) laser excitation, enabling their use as non-toxic, non-photobleaching probes in cells and tissue. Existing upconverting materials, however, have narrow excitation ranges and exhibit multiple emission peaks that are not optimal for biological imaging. Tuning the multi-photon excitation of upconverting materials is hindered by the insensitivity of lanthanide f-orbitals, and emission wavelengths are dependent on the energy transfer between multiple dopant ions as well as on the crystal structure of the surrounding nanocrystal matrix. Optimizing upconverting probes over this expansive parameter space presents sizeable synthetic and analytical challenges. We describe the high-throughput discovery and optimization of upconverting nanoparticle (UCNP) probes with tunable excitation and emission spectra. To perform combinatorial screening of these materials, we used an automated platform capable of synthesizing a library of high quality upconverting nanocrystals (e.g. NaYF4) with various inorganic matrices and lanthanide dopant combinations and concentrations. To characterize the optical properties of these materials, we constructed a high-throughput spectroscopic apparatus that can acquire upconversion spectra of samples in a 96-well microplate using excitation from eight NIR laser diodes. We demonstrate that by tuning the matrix material, we can alter the excitation spectra of UCNP probes to avoid absorption by water and to overlap with common laser lines. We demonstrate that using non-standard pairs of lanthanide dopants, we can enhance the spectral selectivity of upconverting probes by selectively quenching undesired transitions via energy transfer. Finally, we will discuss the underlying mechanisms behind the tunability and selectivity of these materials and will illustrate the advantages of these properties in robust biological probes.

TP21

Daniel Chen, University of California, Irvine, dchen2@uci.edu

Co-Authors: Mike Xie, UCLA; Yahya Elshimali, Charles Drew University

Application of Microarrayer for Yeast Cell Array in Drug Screening

Build-your-own microarrayer has been a tool for genomics. Here, we are describing our process of converting an xyz microarrayer for drug screening using systemic biology principle. Specifically, yeast deletion set is arrayed onto a growth agar plate containing small molecules investigated. Each yeast deletion strain has one known gene deleted. Together the whole yeast deletion set contains all 6,400 yeast deleted gene strain. By analyzing the growth patterns of all 6,400 yeast strains, one can infer the potential gene target of the unknown small molecules in questions. Since 80% of human genes has analogous in yeast genome, this strategy is a fast and cheap way to analyze small molecules in the context of complex biochemical pathways.

TP22

Gabriela Chirica, Sandia National Laboratory, gschiri@sandia.gov

Co-Authors: Geun-Cheol Gil, Mrowka Stanley, Steve Branda

Comparison of Workflows for Mouse Serum Peptidome Analysis in an Automated Mesofluidic Platform

The search for biomarkers in biological fluids requires extensive fractionation to increase the signal of biologically informative proteins from the noise of higher abundance proteins. The particular workflow used in each study is limited by the nature and volume of the sample, its chemical compatibility with the fractionation method, as well as duration, laboriousness and cost considerations. We developed a modular automated processing system (MAPS), a mesofluidic platform which enables simultaneous analysis in sample customized workflows. We will present a comparison of the mouse serum peptidome and proteome analysis achieved using restricted access media with hydrophobic, and cation exchange functionalities. A third processing workflow including treatment with proteinase K to reduce the amount of non-specifically retained higher molecular weight proteins is also reported. Our results indicate that each method reveals essentially distinct sets of proteins and combining these workflows is significantly improving proteome coverage. Automated multistep processing is therefore essential for a more complete proteome description at a practical cost. Equally important for biomarker applications, is the standardized processing of large numbers of samples enabled by automation.

TP23

Kjersten Cook, Douglas Scientific, kjersten.cook@douglasscientific.com

Array Tape™ Platform Provides HTP Screening with Reduced Plastic, Reagent and Energy Consumption

The Array Tape Platform is an automated, high-throughput (HTP) technology based on a continuous strip of a light and flexible polymer that is serially embossed with reaction wells in customized volumes and formats. Automation is achieved by indexing holes running along each edge of the Array Tape, which guides reaction wells through liquid handling (Nexar) and detection (Araya) instruments. Array Tape has many potential applications, but the Platform has proven to increase throughput, flexibility and cost savings in HTP SNP genotyping laboratories that previously used robotically driven, microplate-based technology. We will demonstrate how the properties of Array Tape and customized Platform also provide environmental benefits based on a comparison of plastic consumption, reaction volumes and energy efficiency. Both Array Tape and microplates are single use consumables in most HTP laboratories; therefore, plastic consumption is based on the amount of material required to manufacture an SBS array of reactions wells in Array Tape vs. microplates. For comparison, we have chosen a commonly used 384-well PCR microplate (Axygen Biosciences, PCR-384-RGD-C), which weighs 21.3 g. This is nearly 7 times more plastic than the 3.1 g required to manufacture a 384-well equivalent in Array Tape. The Array Tape Platform is optimized for small volume reactions, usually < 800 nL. This fluid volume is 80 to 90 percent less than 384-well plates, which have a minimum practical reagent volume of approximately 5 µL per well. In scalable assays, these smaller reaction volumes allow the Array Tape Platform to reduce the amount of reagents consumed, minimizing the need to store, handle and dispose of potentially hazardous chemicals. Laboratories utilizing the Array Tape Platform are more energy efficient because they require a smaller laboratory footprint and less instrumentation to complete the same processes. The inline modular Array Tape Platform reduces the laboratory footprint by eliminating the need for robotic handlers to move microplates from station to station. In addition, Array Tape is thin and flexible, which allows 200 microplate equivalents (76,800 reaction wells) to be spooled onto a single, compact reel (90 mm wide by 560 mm diameter). Beyond the obvious storage benefits before and after processing, the spooled Array Tape may be thermocycled in a single, commercially available water bath. Processing a similar number of microplates in the same amount of time would require 6 water baths (32 microplate capacity) and an estimated 3.5 times more power (26.4 kWh Array Tape, 92.4 kWh microplate). Thermocycling in standard block heaters (4 microplate capacity) would require 50 instruments and 12 times more power (313.6 kWh). The Array Tape Platform transforms the landscape of microplate-based HTP automation and allows space-limited laboratories to achieve HTP goals with environmental benefits including reduced plastic, reagent and energy consumption.

TP24

Evan Cromwell, Molecular Devices, evan.cromwell@moldev.com

Co-Authors: Oksana Sirenko, Pierre Turpin, Jayne Hesley, Molecular Devices; Juan L. Almara, Daniel Zimmerman, David Novo, De Novo Software; H Roger Tang, Molecular Devices

Homogeneous Multiplexed Assay for Hematopoietic Stem Cell Toxicity

Many potential pharmaceuticals fail in clinical trials due to unacceptable toxicity, thus early discovery and elimination of toxic compounds is important to the drug discovery process. For example, in chemotherapy toxicity to hematopoietic stem cells (HSC) or bone marrow often limits dosing and duration and can cause anemia, neutropenia, or other side effects. One way to improve information on toxicity is to introduce more biologically relevant assays early in development. Hematopoietic progenitors are extremely sensitive to the toxic side effects of many compounds and can provide valuable information about both general and lineage-specific toxicity making them useful for screening of therapeutics for cancers and other diseases. However, primary cells, especially non-adherent hematopoietic progenitors, are more challenging to use in automated high-throughput assays compared to adherent cell lines. Current HSC assays using flow cytometry are labor intensive and less amenable to high-throughput. Imaging based assays can require multiple wash and centrifugation steps making them less automation friendly. Here we report on the use of IsoCyte™ cytometer (Molecular Devices) and FCS Express software (De Novo Software) to automate the study of cell differentiation, cytotoxicity, and carcinogenesis in 96 or 384 multi-well plate formats at throughputs of 3-5 minutes per plate. The IsoCyte™ cytometer is used to quantify expression of cell surface markers in a homogeneous multiplexed assay by using appropriate fluorophore-conjugated antibodies. The unique optics of the IsoCyte™ cytometer allows it to measure lineage and abundance of HSCs in a "mix-and-read" format. The FCS Express software provides tools for visualization and reporting of multicolor data analysis. Protocols will be presented for cell culture, drug treatment, and lineage-specific marker analysis that contain minimum steps and are suitable for a high-throughput assay. We have tested the effect of different combinations of hematopoietic growth factors (SCF, IL-3, GM-CSF, EPO) and the impact of cytotoxic agents on expansion and differentiation of human bone marrow CD34+ cells. We have shown that SCF Flk3 and TPO combination resulted in greater expansion of CD34+ cells, while GM-CSF and EPO promoted increase of myeloid or erythroid progenitors respectively. Cytotoxic effects of several anti-cancer drugs (DNA-intercalating agents or kinase inhibitors) Imatinib, Fluoroadenin, Cyclophosphamide, and PD98058 were measured. A number of different markers were studied as indicators of myeloid (CD13, CD15, CD45) or erythroid (GlycophorinA) development and cytotoxicity. The number of marker-positive, and total viable cells were measured as a function of compound dose and IC50 values were obtained. The ability to monitor expansion, differentiation and toxicity of hematopoietic progenitors in a high-throughput, homogeneous multi-well plate based format provides new tools to researchers in this area.

TP25

Cheryl Dlhos, Amgen Inc., chedlhos@amgen.com

Co-Authors: Peter Grandsard, Bill Gigante, Craig Schulz, Amgen Inc; Walt Caldwell, Ballista Inc.

Volume Detection Through Pressure Measurement

Given the complex operations in the Compound Management (CM) group at Amgen, it is critical to have quality checkpoints inserted into processes to identify and eliminate process failures. A critical step in many of the CM processes is compound transfer done by various automated liquid handlers to and from multiple labware types. CM would like to add a checkpoint into the compound transfer process to determine if the volume expected in the transfer was completed successfully. A liquid detection device would ensure that accurate volumes of compound are transferred to the operational inventory stored in single use REMP storage tube racks (STbRs). Volume accuracy of compound in STbRs is critical for use in making downstream plates for research experiments. After meeting Ballista Inc. at ALA in 2009, the Research and Automation Technology group at Amgen began working with them on possible solutions for development of a liquid detection device for plates and tubes. With Ballista's core competency being in optics, the use of vision to detect liquid in wells was explored first. The use of optical feedback between an empty and non-empty well was explored. This approach performed well under ideal circumstances; however, it became clear that due to varying labware density (96 and 384-well) and shape of well bottoms, obtaining exact volume would not be possible. Having ruled out using vision technology as an option, Ballista presented us with a proposal to use a pressure measurement to obtain an accurate well volume. A proof of concept experiment was set up to obtain data regarding accuracy, repeatability, and resolution of the system. The experiment showed promising results, so a single channel prototype was developed. The prototype was optimized and a three axis robot was added to allow for automated data collection. The data results showed the single channel prototype to be a promising solution to liquid detection in plates and tubes, yielding %CV of less than 2% for each volume tested with the ability to detect down to 2µL in a well. In order to meet throughput requirements, development of a multi-channel device is in progress. The completed development and implementation of this device into CM processes will improve the quality of compound plates requested for research.

TP26

John Eschelbach, Amgen Inc., jeschelb@amgen.com

Co-Authors: David Wernick, University of California, Los Angeles; Elizabeth Doherty, James Petersen, Amgen Inc.

Characterization of the H-Cube Flow Chemistry Reactor for Discovery Scale Hydrogenation

The HCube was recently introduced as a commercially available reactor for performing hydrogenation chemistry via a flow paradigm. Several advantages of this type of chemistry by flow have been noted, including the ability to operate at pressures up to 100bar, increased safety, and enhanced reaction rates. While there have been a variety of reports in the literature demonstrating the applicability of the HCube to various chemotypes, very little has been reported to characterize the fundamentals of the reactor and how various flow-chemistry parameters can affect the overall reaction yield, rate, and purity. Additionally, most reports are not focused on discovery-scale reactions, where starting material can be significantly limited. In this study, we aimed to characterize the ThalesNano HCube in a Medicinal Chemistry workflow and understand several of these flow chemistry parameters. The importance of online analytical techniques for both characterization of the reactor and reaction optimization has also been recognized. The reduction of styrene to ethylebenzne was selected as a benchmark reaction and used to evaluate the performance of the HCube. We have evaluated the impact that reactor dispersion has on the overall reaction, potential options for reaction optimization using minimal material, and the ability to scale-up an optimized reaction using a flow chemistry model. Simple online analytical techniques such as UV and HPLC have been employed throughput the study, and the potential impact on the overall flow-chemistry workflow will also be presented.

TP27

Scott Fall, Genefluidics, sfall@genefluidics.com

Co-Authors: May Chiu, Matthew Davis, Quyen Ho, Genaro Sepulveda, Steven Snyder, Matthew Wu, Vincent Gau, Genefluidics

A Fully Automated Robotic System for 96 Multiplexed Electrochemical Based Assays in Less Than 70 Minutes

The increased activity in areas such as assay optimization and high throughput screening has fueled a need for detection methods that can satisfy high throughput demands. The absence of sample purification and very limited sample preparation requirements of electrochemical detection opens up new possibilities in the area of high throughput molecular analysis. When sample preparation is taken into account, an ELISA assay can take over 2 hours. Using electrochemical detection technology and a small precision X Y Z robot to run multiplexed assays, 96 different assay conditions can be verified in less than 70 minutes to obtain optimal assay conditions. This robot utilizes 6 arrays of 16 electrochemical biosensors to accomplish fast multiplexed assays. The multiplexing ability comes from the versatile surface functionalizations of the sensor. Each sensor can be treated with a different capture probe or antibody for a specific target. Alternately a single sensor can be treated with a mixture of capture probes / antibodies to detect multiple targets. Sensor treatment for capture takes around 30 minutes prior to testing. The sample can contain multiple target molecules for detection and a cocktail of detector probes / antibodies can be added to the sample while the sensor is being pre-coated with the capture molecules. The sample is then placed on the sensor allowing the target molecules to be captured onto it. This step typically takes 15 minutes. The sensor is then treated with a detection enzyme for 15 minutes and the results from each array of sensors are available within 1 minute by means of an integrated electrochemical array reader. All reagent incubation times are dependent on passive diffusion and can be greatly reduced with active diffusion. The elimination of sample purification and low sample preparation requirements allows the automated system to surpass much more complicated or labor intensive optical detection based systems.

TP28

Olaf Galuba, Novartis Pharma AG, olaf.galuba@novartis.com

Co-Authors: Stephane Laurent, Solange Vidal

High-Throughput Technologies Evaluation & Comparison of Kinetic Detectors

Readers capable of monitoring rapid biological events, such as ion fluxes, e.g. calcium, have become essential drug discovery tools. Such readers are capable of monitoring the biological response of cells following receptor stimulation, capturing a time course of activity. The exact profile of activity will then determine the dynamic range and reproducibility of the reader for a particular assay. Such a complex data response makes the comparision of different machines more difficult than just comparing readers that generate a single assay readout. In order to compare these two readers a number of different assay and conditions were used. For example “wash” and”no wash” conditions were compared, as well a assays using mouse and human cell lines. Reader performance was compared using a simple correlation as well as the Bland-Altman plot. Using these data analysis methods it was possible to compare the activity results obtained with two commonly available kinetic assay plate readers, using a test set of compounds. This has allowed the sensitivity and reproducibility of the two readers to be compared. In addition the time and effort required to set up, run and analyze the results obtained from these two readers was compared. While both readers were capable of monitoring the activity of different compounds in this assay they showed differences in their ease of use and the time required for assay analysis and data reporting.

TP29

Cory Gerdts, Emerald BioSystems, cgerdts@embios.com

Co-Authors: Jeff Christiansen, Shelley Dietrich, Emerald BioStructures

The MPCS Plug Maker Enables Screening to Obtain Diffraction-Ready Crystals of Infectious Disease Targets

The MPCS Plug Maker is a microcapillary-based protein crystallization system for generating diffraction-ready crystals from nanovolumes of protein. Comparitive crystallization screening is reported between sitting drop vapor diffusion using traditional crystallization plates and nanovolume microbatch using the Plug Maker and its associated CrystalCards. The Plug Maker demonstrates its ability to enable crystallization screening and crystal production with nanoliter volumes of protein that lead to diffraction-ready crystals of infectious diseases protein targets from the Seattle Structural Genomics Center of Infectious Disease.

TP30

Cory Gerdts, Emerald BioSystems, cgerdts@embios.com

Co-Authors: Mark Mixon, Emerald BioSystems; Lance Stewart, Emerald BioStructures

Web-Based E-Wizard Screen Builder and Desktop Instrument Facilitate Automated Chemical Screen Design

Designing crystallization screens is a task all crystallographers must perform, but generating the conditions and pipetting is labor-intensive and time consuming. The scientists at Emerald BioSystems have created a software module that does the work of designing optimization screens for any commercial index screen. Located at www.emeraldbiosystems.com/ewizard, the E-Wizard screen builder allows users to generate a 96-well optimization screen for protein crystallization with just a few mouse clicks. The user can the download the condition information for free in spreadsheet format, order the screen with fast delivery from Emerald, or transfer the generated screen design to the new Opti-Matrix Maker screen preparation desktop instrument for simple preparation of screens containing up to 12 stock solutions. These innovations help researchers save the time it takes to design and create optimization screens so they can focus on science, not pipetting.

TP31

Klaus Rehfeldt, STRATEC Biomedical Systems AG, k.rehfeldt@stratec-biomedical.de

Co-Authors: Dirk Herrmann, Ralf Griebel, STRATEC Biomedical Systems AG

Volume Verification in Steel Probe for Enhanced Process Control

In automated analytical systems a high number of pipetting steps are executed to dispense various reagents. As the test results are sensitive to the amount of the dispensed reagents, the liquid volumes have to be verified by sensors in the dispensing line. For disposable tips, pressure sensor systems are commonly used, but they are limited in volume resolution. In general, bubbles or foam in the fluidic path may affect the dispensed volumes as well as the accuracy and precision of the aspirating and dispensing pump, the elasticity of the tubing, disrupted liquid plugs, clogged probes or tubes, any leakage in the fluidic system (loss of liquid or bubble intrusion), invalid liquid level detection (LLD) and empty sample or reagent reservoirs. An ideal volume verification system should change the conventional pipetting pattern as little as possible. Any extended airgaps or other main changes to already optimised sequences should be avoided. Now, for advanced process control, an optical sensor system has been completely integrated into a steel probe. All electronic and active optical elements are situated on one single printed circuit board (PCB) in the housing of the probe. The measuring point itself is situated inside the probe close to its tip. Optical fibres routed inside the steel probe connect the measuring point to the PCB. All signals are processed and analysed on the integrated PCB. The only connections from the probe to the automated analytical system are a ribbon cable to hand over the measurement results, and a flexible tube to build a seamless connection in the fluidic system from the pump down to the probe tip. With each pipetting step, the system will recognise the liquid-gas sequence, ready to compare it to the expected volumes. The sensor layout ensures even cloudy or highly absorptive liquids (e.g. blood) or suspensions to be clearly distinguished from gas. The measuring point situated close to the tip of the probe minimises the dead volume and the airgap volumes, thus reducing needed sample or reagent amount, pipetting and wash cycle time and the risk of disturbed phase boundaries. In addition to the new optical liquid verification system, the steel probe is equipped with a conventional capacitive LLD system. The width of the probe housing allows to use several probes side by side in a 96 well MTP grid. The probe itself fits into a 384 well MTP grid. Volumetric error recognition has been tested successfully by offering underdosed liquid amounts for aspiration. The resolution of the integrated optical liquid verification system has been tested to be at least 1 µl for liquid-gas-liquid as well as for gas-liquid-gas phase boundaries. Failures that may be generated by pump defects, clogging, empty sample or reagent reservoirs or tubing leakage can be detected immediately to avoid false results and to save time, reagent cost and irretrievable samples.

TP32

Man Bock Gu, Korea University, mbgu@korea.ac.kr

Co-Authors: Joo-Myung Ahn, Ji Hoon Kim; Joong Hyun Kim, BioNano Research Center, Korea Research Institute of Bioscience and Biotechnology

Smart Fabrication of the Cell Array Chips Using Optically Coded Functional Microbeads

We have successfully developed optically coded functional microbeads by co-encapsulating both bioluminescent reporter bacterial cells and fluorescent microspheres within a common alginate microbead. This smart functional microbead has a specific stress-specific bacterial strain and, as an its identification optical code, one of five optical codes generated from fluorescence microspheres such as yellow, green, red, yellow + green, or no fluoresce. These smart microbeads could be randomly scattered on any multi-well chip plate with the result that, since the cell types are identified on the basis of fluorescent color, the microbead arrays were fabricated without pre-designation of an individual well. As an example of this method, five different stress specific bioluminescent bacterial strains, each with a different optical code, were successfully implemented to make five different types of optically coded functional microbeads, with a speed of about 30 microbeads/ min. This final randomly scattered functional microbeads array biochip, with a fast fabrication of each chip at every 2 min, successfully demonstrated its ability in toxicity screening and monitoring for samples with a few examples for five different stress chemicals. This simple and fast, but not tedious and complicated procedure should be widely and practically used in making the cell array chips for the monitoring of environmental toxicity, new-borne chemicals, pharmaceutical drugs and the cosmic rays in space station or spaceships in future.

TP33

Reija-Riitta Harinen, Thermo Fisher Scientific, reija-riitta.harinen@thermofisher.com

Co-Authors: Marika Raitio, Hanna Grano-Fabritius, Jorma Lampinen, Thermo Fisher Scientific

High-Throughput Determination of Enzyme Kinetic Parameters With High Speed Microplate Photometry

Determination of enzyme kinetic parameters Km and Vmax as well as inhibition constant Ki are very common assays in modern drug discovery. These assays require by their nature a measurement of a concentration series of either the enzyme substrate or the possible inhibitor, or both. Therefore, these assays are ideal to be performed in high density microplate format, where high number of samples can be easily measured. Anyhow, when performing kinetic measurements with high number of samples one easily has to sacrifice the quality of the resulting kinetic curves due to low sampling frequency of each sample. If the time difference between two consecutive kinetic readings of the same sample becomes too long, the reaction rate calculations easily loose their accuracy. These kinds of enzyme kinetic assays should therefore be performed with an instrument that can read the plate very fast. The obvious solutions are CCD based imaging devices that can read all the wells of the plate simultaneously, but these devices are quite expensive, therefore not ideal choices for those who need this kind of capacity only every now and then. This paper shows how enzyme kinetic assays can be performed with high throughput using 384-well microplates and affordable microplate photometers with a sampling frequency sufficient for excellent result quality. The instruments used were Thermo Scientific Multiskan GO and Multiskan FC that can read the whole 384-well plate in ten seconds. The performance was tested with two common enzyme reactions. The first assay was the reaction between beta-galactosidase enzyme and ONPG substrate, and was inhibited with PETG (2-phenylethyl ß-D-thiogalactoside) or lactose. The second assay was the enzyme reaction of Cytochrome C reductase that was inhibited by KCN. Both assays were performed in standard 384-well plates and in special low volume 384-well plates. 96-well plates were also used for comparison. All results were compared to the results obtained with very high precision and accuracy multitechnology microplate reader, Thermo Scientific Varioskan Flash. The results show that these microplate readers offer high enough sampling frequency for accurate results. Calculated kinetic parameters correlate well with the literature references and using much more expensive readers does not improve the result quality. It is also possible to reach high throughput with these low cost photometers. For example, when performing inhibition efficiency measurements it is possible to have 16 inhibitors per 384-well plate (6 inhibitor concentrations + blank + zero inhibition control, all in triplicate). As the assays can be performed in low assay volume with low volume 384-well plates, it is also possible to keep the reagent consumption and costs reasonable.

TP34

Christopher Harrison, San Diego State University, harrison@sciences.sdsu.edu

Co-Authors: Sarah Dobson, San Diego State University; Devin Wakefield, Cornell University

Simplified Nanodroplet Formation and Injection for Capillary Electrophoretic Separations

The utility of segmented droplet streams for time resolved sampling and analysis is clear. The ability to contain, manipulate and process samples without contamination or diffusion problems is of great benefit to biological analyses, where samples, such as neurotransmitters can be in very small volumes. The challenge however remains in how best to process these droplets, without the ability to combine the droplet sampling with a separation technique only a small amount of information can be obtained from the samples. Other groups have had some success with the injection and separation of nanoliter sized droplets on custom microfluidic devices. We are approaching the process of both forming and sampling nanoliter sized droplets through the use of conventional chromatographic supplies (e.g. mixing tees, teflon tubing,...) to produce low cost, but highly reproducible droplets. Our process for the formation and injection of nanodroplets centers around coaxial fluid flows. We have devised a simple device to generate nanoliter sized droplets based on the coaxial mixing of a perfluorocarbon and aqueous stream. We are also developing a method for the coaxial injection of the generated droplets for capillary electrophoretic separation. The heart of the coaxial injection system lies in the use a dialysis membrane through which the injections will take place. This work will present the results of our droplet generation system as well as our advances in the development of our coaxial injection system.

TP35

Randy Hice, STARLIMS, randy.hice@starlims.com

A Call for Unification in Laboratory Informatics

Economic pressures affect us all in ways we are only beginning to fathom. The world of laboratory informatics is not immune to the economic downturn in the world, and companies are demanding that mission critical informatics software solutions take on more and more of the burden of the modern scientific enterprise. What were once simple Laboratory Information Management Systems (LIMS) have now expanded into domains not formally associated with them. Once a tool to track samples and facilitate result entry, modern LIMS now wear many hats. Clinical Laboratory Information Systems (LIS), Scientific Document Management Systems (SDMS) Electronic Laboratory Notebooks (ELN), as well as reagent and media preparation, employee training, instrument calibration, and a host of related informatics functions now reside in the once-humble domain of LIMS. Indeed, LIMS have eclipsed the outdated perception that they are “quality systems”, and now are uniting the R&D and manufacturing arms of major companies. This presentation will trace the remarkable evolution of LIMS from the early 1980’s to the present day where these systems now reside as centrally-served, web browser-accessed applications that have transformed multi-site/multi-national companies into massive virtual laboratory environments where management and users can treat satellite laboratories sitting thousands of miles away as though they are a few feet away. We will also examine how the modern scientific enterprise has dictated the evolutionary path of these applications, and what is coming in the next few years. The coming decade promises a dizzying array of functionality and an unprecedented access to information that we have never seen before.

TP36

SoonGweon Hong, University of California, Berkeley, gweon1@berkeley.edu

Co-Authors: Qiong Pan, Luke P. Lee, University of California, Berkeley

Quantitative Studies of Long-Term Culture and Development of Embryoid Bodies Regulated by Reactive Oxygen Species (ROS)

Embroid body (EB) from embryonic stem cell has been highlighted as an in-vitro model of early development of embryogenesis. Due to its strong pluripotency derived from its origin, inner cell mass of blastocytes, natural culture conditions can result in various differentiations including all of germ layers (endoderm, mesoderm and ectoderm) and further detail developments happening in animal bodies. Even with the promising aspects, the complexity, variation and inconsistency during EBs' growth hinder its usages as models for biological answering or regenerative medicine. EB development is influenced by simultaneous multiple factors, intracellular interactions and the number of consisting cells. In order to obtain statistical and quantitative data collection, a large amount of uniform EB formation is a main prerequisite. Previous uniform EB formation chips were only able to sustain limited EB viability or short time culture on chip, which is probably due to mechanical stress on EB formation or growth. In other cases, the chips are used only as a template for EB formation and replacing is followed for a long-time culture. To overcome these limitations, here we demonstrate a biochip for long-time EB array culture enabling tracking of each individual EB and providing multiple conditions during culture. As one of the most important biological questions, reactive oxygen species (ROS) were examined for ROS-modulatable EB development. Comparatively long period of culturing (up to 2 weeks) was executed under various extracellular oxidative factors and metabolic factors. Uniform sizes (200 to 250 μm diameters) of EBs' array showed different but repeating responses to different chemicals and concentrations. Due to transparent platform of our chip, microscope imaging (bright field and fluorescence) was taken. The uniformity of EBs even made possible for time-response invasive methods (immunostaining and PCR). In brief, the percentage of differentiated third germ layer is much higher under lower oxidative stress, while yolk sac formation with distinguishable percentage was observed under certain culture conditions. Also, haematopoietic cell differentiation is preferable under low oxygen supplies. In summary, we demonstrate a promising platform to study EB development, enabling a long-term culture with various concentrations of various chemicals, individual EB tracking, microscope imaging and successive invasive characterizations. An experiment on this chip showed that ROS is an important factor in early development of EBs. Likewise, this platform can be believed to approach to quantitative large-amount EB studies for a variety of biological questions.

TP37

Olaf Hoyer, Q.Instruments - QUANTIFOIL Instruments GmbH, Olaf.Hoyer@QInstruments.com

Optimization of Mixing Processes in Microplates—A Methodology and Study of Microplate Mixing Techniques Including BioShake 3000

The significance of microplate mixing has largely been ignored as a serious problem, although potentially it could undermine the generation of meaningful data. We analyzed orbital microplate mixing technologies to gain a better understanding of the current requirements for mixing technologies that can be applied to compounds or bioassays in microplates. This poster reviews some results of studies with BioShake 3000, such as where the greatest need for improved mixing exists. It also discusses the microplate formats and applications where there is greatest concern about achieving improved mixing.

TP38

Heath Huckabay, The University of Kansas, huckabay@ku.edu

Co-Authors: Kevin Armendariz, Robert Dunn (Corresponding Author)

Multiplexed Biosensing Using Whispering Gallery Mode Imaging

Historically used to explain the travel of sound in domed structures, the term whispering gallery modes (WGMs) now commonly describes the resonant confinement of light in circular dielectrics. The wavelength confined in dielectrics is dependant primarily on both the radius of the structure and the effective refractive index it experiences. If a recognition element is bound to such a structure, changes in refractive index may be exploited for biosensing, as analyte capture results in a shift of the resonant condition. Since light coupled into these structures circumnavigates the resonator many times, the light-sample interaction is greatly increased, improving signal considerably. WGM sensors have developed tremendously over the past decade, with detection limits reaching single molecule levels. These resonators include a variety of designs, such as glass microspheres, microfabricated waveguides, and liquid core optical ring resonators. While these methods all show promise in biosensor capabilities, microsphere-based WGM sensors offer advantages due to their superior quality (Q)-factor and ease of fabrication. Disadvantages with microsphere sensors include their difficulty to multiplex and integrate into photonics and fluidics designs. In this work, we introduce the development of a WGM-based sensing platform which incorporates microsphere resonators with an evanescent excitation source for multiplexed biomolecule detection. Although the resonant wavelength in these sensors is determined by a wavelength dependent increase in fluorescence, analyte specificity is encoded in microsphere size. Using traditional microscopy techniques, these sphere sizes are easily distinguishable, lending to highly scalable multiplexed biomolecule detection. Progress in this new approach combining fluorescence detection with size encoded specificity will be discussed.

TP39

Carl Hull, UNIConnect, carl.hull@uniconnect.com

New Software Technology That Enables Scientists to Design and Build Their Own Assays

Scientists know how to design assays. They also know how to optimize and improve assays after they start processing samples. However, the process of getting the LIMS to catch up and stay current with the science is painful. New technology has been created which puts the power to design and configure new assays and change existing assays, into the hands of the experts – the scientists. This session will detail an HTS implementation of this technology. The traditional method of creating LIMS software to track and control the steps of an assay has involved multiple people. The scientist designs the assay. The process expert creates the specification for the LIMS and passes it to a programmer who either modifies existing software or creates custom software to meet the specification. There is insufficient bandwidth between the parties for successful communication which results in multiple iterations and numerous hours spent trying to get it right. Contention often arises between the process expert and the IT team trying to support them. Often, the process expert makes changes to the specification during the development process which exacerbates the communication issues and further frustrates the IT team. A new software platform has been created to eliminate this problem. This technology enables non-programmers to create their own assay tracking systems. It is comparable in difficulty to designing formulas in a spreadsheet. Non-programmers routinely use spreadsheets to create formulas that formerly were only able to be created by programmers. In a similar fashion, non-programmers now use new technology to create their own process tracking and control systems. When the person who understands the requirements also has the tool to create the system, the bandwidth issue goes away and assay development time is reduced by 90%. This session will provide case studies of an HTS lab and a diagnostics lab that are now successfully designing and building their own assays in their LIMS. Objectives: 1.Show scientists how to turn their LIMS into an asset rather than a liability. 2. Show screen shots of assays designed and created by scientists 3. Present ideas on how and when to implement process improvements. Teaching Methods: Presentation will consist of a brief technology overview followed by 2 case studies. The case studies will lay out the problems faced by the labs and show in detail how they used technology to solve them.

TP40

Jim Schools, Zinsser Analytic, jimschools@zinsserna.com

Co-Authors: Clifford Olson, Zinsser NA; Werner Zinsser, Zinsser Analytic

Automated Protein Crystallization of Membrane Proteins With ProCrys Meso

Dealing with membrane proteins in lipidic mesophases is not an easy task. Until now the sample preparation has been a very tedious and time-consuming task. Zinsser Analytic has developed ProCrys Meso, the new liquid handling platform for the preparation of 96 in-meso (or cubic phase) proteins. It offers accurate and precise dispensing of the protein and the screening solution and also can be used to prepare your own screening solutions on the workbench instead of using the more expensive commercial kits. An integrated module ensures the ideal humidity conditions for the process. ProCrys Meso is based on a 1 meter liquid handling platform with 2 pipetting arms. The first pipetting arm is equipped with pipetting probes for the preparation of the screening solutions and its distribution to the crystallization plate. The second arm is used for the transfer of the nanoliter volumes of the protein solution. The protein and the screening solution, as well as the crystallization plate are placed on the workbench. Clever tools have been incorporated to set up a run more easily, including precise and reproducible dispensing of the mesophase. The positioning of the protein is controlled by a high precision laser. The shape and travel length of the needle of the protein dispenser is sensor controlled. The software automatically adjusts the coordinates of dispense height and position in the well to the reading of the laser and needle sensors. The WinProCrys software allows maximum flexibility of method development. Work lists can be imported from Excel® or the customer's LIMS system. ProCrys Meso not only increases the throughput and the reproducibility of the experiments, it also reduces costs.

TP41

Sun-Young Jung, Chungju National University, jung.sunyoung7@gmail.com

Co-Author: Jeong-Won Kang, Chungju National University

Study on Tunable Resonator Using a Cantilevered Carbon Nanotube Encapsulating a Copper Nanocluster

We investigated an ultrahigh frequency carbon nanotube resonator encapsulating a nanocluster, as another tunable resonator, via classical molecular dynamics simulations and continuum models. The fundamental frequency of cantilevered carbon nanotube resonator encapsulating a copper nanocluster could be adjusted by controlling the position of the encapsulated copper nanocluster. Data obtained from the molecular dynamics simulations were analyzed with continuum theory, and we found that, in the statistically, the change of the effective mass factor was greatly correlated with the position change of the encapsulated nanocluster.

TP42

Kevin Khovananth, Artel Inc., kkhovananth@artel-usa.com

Co-Authors: Jim Neilsen, Keith J. Albert

Measuring Residual Volumes Remaining in a Microplate After Sample Aspiration

The purpose of this application note is to demonstrate an alternative use for the Artel MVS® Multichannel Verification System. As opposed to measuring the accuracy and precision for a volume transfer of sample into a microtiter plate, the MVS is instead employed to measure the volume of sample left behind, i.e., residual volume of sample, after attempting to aspirate off the entire sample from each well of a microplate. Measuring the residual volume after sample aspiration is a direct way for assessing the performance of the sample removal step. Using one or more aspiration steps to remove reagents and contaminants in wells of a microplate can be an important step in some assays, such as with the removal of ethanol during some DNA purification procedures. In this case, incomplete removal of ethanol likely leads to insufficient elution of the DNA decreasing its overall yield and purity. The experiments discussed herein show how residual volumes can be measured for volumes as low as 100 nL in a 96-well plate or as low as 30 nL in a 384-well plate.

TP43

Tanya Knaide, Artel, tknaide@artel-usa.com

Co-Authors: Tanya R. Knaide, John Thomas Bradshaw, Richard H. Curtis, Geary Ritter, Rachel Parshley, Artel

Do Plate Readers Agree? Understanding Performance Differences Between Different Plate Reader Makes/Models

Microplate readers are used for numerous laboratory applications to measure results of chemical assays in microplates. Readers may be used to collect one or several types of measurements including absorbance, fluorescence, luminescence, or a combination thereof. However, like any type of laboratory equipment, not all plate readers are created equal, nor do all readers of the same type always report the same answer. Stated uncertainties for a given model of reader are meant to cover the native variation of all readers of that model. But, how does this uncertainty relate to a completely different reader type? Some reader types are superior in performance than others and each lends different amounts of uncertainty to measurements collected, and therefore the overall results of the assays conducted. Characterization of plate reader performance enables users to harmonize groups of instruments, making results agree regardless of the plate reader employed, as well as to understand how the uncertainty of the plate reader measurements translates to their assay results. In this poster we will discuss methods for characterizing differences in optical performance of one reader type versus another, as well as between various readers of a given type. Uncovering differences between absorbance measurements made from liquids in a microplate versus differences measured for solid artifacts can aide in aligning the performance of multiple reader types.

TP44

Ja Choon Koo, Sungkyunkwan University, jckoo@me.skku.ac.kr

Co-Authors: Seung Hoon Shin, Hyouk Ryeol Choi, Hyung Pil Moon, Sungkyunkwan University;
Sung Moo Ryew, KNR System; Suk Joong Kim, ED Corporation; Jinhyun Kim, Seoul National University of Technology;
Wankyun Chung, Pohang University of Science and Technology

A Job Scheduling Algorithm Development for a Small Size Clinical Test Platform Using a Virtual Prototype Machine

As there are many different clinical laboratory test equipments are being developed, it is also required to develop the proper job schedulers for each test platform. Noting difficulties in the job scheduling development without a particular physical machine, the present work addresses a process for job scheduling development for a small size lab test machine using a virtual test machine. With this capability, an optimized job scheduler can be provided while the actual machine is in build. The machine includes every testing modules such as the cuvettes, washing module, robotic sample dispensing and reagent dispensing module, spectro-photometer, and incubator tray so that a full scale job scheduling should be followed by an optimization of process and module arrangement. The final machine configuration is to be compared to the other many possible candidates in terms of throughput of the test. Meanwhile an attempt is made for yielding an efficient job scheduling for the machine configuration.

TP45

Michael Kowalski, Beckman Coulter, mkowalski@beckman.com

Co-Authors: John Snider, Li Liu, Laura Pajak

Utilizing Automated Assay Optimization for BioRAPTR to Maximize Antibody Production by a Hybridoma Cell Line

Cell culture consists of numerous processes that can benefit from optimization. One example is optimizing media conditions to maximize the production of a protein of interest. We used design of experiment (DOE) to generate an array of media component combinations to determine which conditions could produce the most IgG molecules from a hybridoma culture. The Beckman Coulter Automated Assay Optimization (AAO) software transformed the output from the statistical design software into dispensing volumes for use with the BioRAPTR FRD* microfluidic workstation. With a single experimental design, we increased the IgG titer by 45% over standard growth conditions and established serum-free cultures that produced >90% of the titer in standard culture conditions. These results illustrate the rapid and powerful returns that can be achieved through process optimization using AAO for BioRAPTR. For Laboratory Use Only; not for use in diagnostic procedures. BioRAPTR and FRD are registered trademarks of Beckman Coulter.

TP46

Thomas Kowski, Amgen, kowskit@amgen.com

Automation Experiences From A Centralized Bio-Analytical Testing Group

At Amgen, for greater work efficiency and speed, a core support group for evaluating samples was established in Process & Product Development. This core analytical group supports the analysis of in-process samples for the cell culture and purification work streams of protein therapeutics. We have automated methods for an ELISA to quantitate Host Cell Protein, sample and reagent preparation for Amino Acid Analysis and Reduced Capillary Electrophoresis on a common automation platform. We have found that the automation of these methods has improved assay precision, reduced repetitive motion tasks and allowed the staff to focus on additional tasks. Some of the challenges were deciding if automation was worth the investment and modifying the methods to accommodate a 96-well plate format. The decision processes and data will be presented demonstrating the advantages and disadvantages of automating an analytical sample testing laboratory.

TP47

Brad Larson, BioTek Instruments, Inc., larsonb@biotek.com

Co-Authors: Peter Banks, Gary Barush, BioTek Instruments, Inc.; Hilary Sherman, Mark Rothenberg, Corning Life Sciences

Utility of Automated Drug Transport Assays in 96-Well Format, Using Permeable Support Systems

Drug transport assays play an important part in determining how a compound is absorbed into the body. Therefore, the performance of these assays is essential to help determine the ADME/Tox profile of a new drug entity (NDE). Typically, these assays have been carried out in a manual format, using colorectal carcinoma (Caco-2) cells, or Madin-Darby Canine Kidney (MDCK) cells in 24-well plates. However, due to the fact that ADME/Tox testing is moving further upstream in the drug discovery process, a greater number of lead compounds are now being tested in an effort to fail NDEs with negative profiles earlier and in a more cost effective manor. To meet the demands for higher throughput and reduced processing time we present an automated drug transport assay using either Caco-2 or MDCK cells in 96-well Permeable Supports. The entire assay process was automated using a combination microplate washer/dispenser and an 8-channel liquid handler. This included cell dispensing, media exchanges, compound addition and compound transfer to the detection microplates. A two-part permeable support system, incorporating an insert plate, and receiver plate, was used in order for manipulations to be performed without the need to separate the parts of the system. The metrics used to validate the automated process were Transepithelial Electrical Resistance (TEER), and Lucifer Yellow and Rhodamine 123 permeability. All automated methods were done in parallel to manual for comparison. Results show that the automated assay is able to deliver results that are more consistent than manual processing, while also reducing the overall experimental time. Thus, by automating the drug transport assay, one increases efficiency, and also attains the high quality data needed from today's ADME/Tox laboratory.

TP48

Brad Larson, BioTek Instruments, Inc., larsonb@biotek.com

Co-Authors: Peter Banks, BioTek Instruments, Inc.; Timothy A Moeller, Celsis In Vitro Technologies; James J. Cali, Promega Corporation

Automated Triplexed Hepatocyte-Based Viability and CYP1A and CYP3A Induction Assays in 96 and 384-well Microplates

Drug-drug interactions (DDI) are of serious concern to the pharmaceutical industry and associated regulatory agencies. One parameter of DDI is induction, the upregulation of enzyme levels that increase metabolism, reducing a drug's therapeutic efficacy. Aryl hydrocarbon receptor (AHR), constitutively active receptor (CAR) and pregnane X receptor (PXR) are nuclear receptors responsible for transcription of the majority of enzymes and transporters associated with drug metabolism and excretion. However, all the enzymes and transporters do not need to be measured. To quantify this risk, the FDA guidance states that in vitro CYP1A and CYP3A activities may be used to monitor induction potential of all drug metabolizing CYPs. Though there are cell lines and reporter gene assays that can be used, the gold standard is the use of hepatocytes to assess the induction potential of a drug by measuring the enzymatic activity of CYP1A and CYP3A with specific substrates and comparing the activity to the basal rate of the enzyme. Typically this information has been gathered from separate wells of cultured hepatocytes and by combining the data from multiple experiments. This process is time-consuming and labor intensive. And it may lead to misinterpretation when combining the data due to the variability within each assay. An automated multiplexed format, combining complimentary methods to obtain multiple readouts from a single microplate well, with liquid handling instrumentation which can create a robust, and repeatable assay process, may attenuate these concerns. Here we demonstrate the ability to monitor CYP1A and CYP3A induction, combined with cytotoxicity measurements, from a single well using cryoplateable hepatocytes. The assay procedure was automated in 96- and 384-well formats using a combination microplate washer/dispenser and an 8-channel liquid handler. This included cell manipulations, compound titration and transfer, and reagent dispensing. Four known inducers of CYP1A and four known inducers of CYP3A were used as control compounds to validate the triplex assay. Results show how the ability to measure three critical responses from a single sample streamlines work flow, derives maximum value from costly hepatocytes, and provides a means for reaching more biologically relevant conclusions.

TP49

Walson Lawi, Genefluidics, Inc., wlawi@genefluidics.com

Co-Authors: Steven Snyder, Pavan Narsai, Casey Chiou, Scott Fall, May Chiu, Vincent Gau

Multiplexed Point-of-Care Diagnostics With Fluid Delivery Control and Electrochemical Detection

A microfluidic system has the potential to expand the scope of point-of-care (POC) testing to encompass more clinically significant applications. However, the development of microfluidic systems faces many engineering difficulties, including unwanted air pockets in fluidic channels, improper sealing, and limited production capability. In addition, current gold-standard assays require separate equipment for sample preparation and additional analyte-specific detection systems for different analytes. We have demonstrated a POC system consisting of a disposable microfluidic cartridge and a portable sensing and control instrument with embedded software. The microfluidic cartridge is comprised of an electrochemical sensor array as well as reagent storage and fluid-handling components. The portable instrument controls the cartridge through pneumatic and hydraulic manifolds. Integrated data acquisition and potentiostat circuits within the instrument enable quantitative measurement from the electrochemical sensor array. Each disposable cartridge consists of only four parts, simplifying the assembly process and reducing manufacturing costs. A low-cost injection-moldable elastomer gasket layer is used to construct internal channels and normally-closed membrane valves for fluid delivery control. The fluid delivery system utilizes positive displacement hydraulics, allowing precise control of delivery at microliter resolution. The cartridge utilizes electrochemical detection technology capable of detecting femtomolar genetic targets and pg/mL protein targets in raw samples without the need for purification or target amplification steps. With this technology, typical assay time from raw sample to results is less than an hour. The electrochemical sensor layer is designed as a modular component of the cartridge such that the end user has the flexibility to functionalize the sensor surface if necessary. The ability to perform simultaneously multiplexed immunoassays and genetic assays in a disposable cartridge was demonstrated by the quantitative measurement of cytokines spiked into a buffer solution. Six electrochemical sensors were functionalized with duplicates for interleukin-8 (IL-8) protein, IL-8 RNA and negative control. A sample consisting of buffer spiked with both IL-8 protein and IL-8 RNA was loaded into the cartridge. The measured current outputs had means of 392 nA for the IL-8 RNA, 105 nA for the IL-8 protein, and 35 nA for the negative control sample. The % CV for IL-8 RNA and IL-8 protein were 1.3% and 13.5%, respectively. A preliminary study of the microfluidic system shows a 350 pg/mL LOD for IL-8 protein using spiked buffer. The viability of the low-cost microfluidic system has been demonstrated by the multiplexed immunoassays and genetic assays of IL-8 protein and IL-8 RNA. Further studies will be performed to validate this cartridge system for other clinically significant applications such as urinary tract infection and bacteremia.

TP50

Howard Lee, Labcyte, hlee@labcyte.com

Co-Authors: Celeste Glazer, David Harris, Richard Stearns, Royal Huang, Siobhan Pickett, Sammy Datwani, Labcyte Inc.

Accurate and Precise Low Volume Transfer of Antibody and Enzyme Solutions Stored in Glycerol Using Acoustic Droplet Ejection

The lifetime and stability of antibody stock solutions, antibody conjugate solutions and enzymes are increased by the addition of cryoprotectants such as glycerol, sorbitol or ethylene glycol. Aqueous solutions containing glycerol have historically been problematic to dispense in tip-based liquid handlers. Glycerol increases both the solution viscosity and the tendency for sample to adhere to the inside and outside of the tip. Increased solution viscosity and sample sticking reduces pipetting accuracy and precision, which can be especially significant in nanoliter volume transfers. Acoustic droplet ejection (ADE) is a completely touchless transfer technology that can precisely and accurately transfer viscous materials. The Echo® 555 liquid handler (Labcyte Inc., Sunnyvale, CA) was used to transfer glycerol stock solutions in the viscosity range from 1 – 10.8 cP (corresponding to 0 – 60% weight of glycerol). Nanoliter volume transfers are confirmed with a fluorescent marker dye and a series of standard curves which were created on a Synergy H4 microplate reader (BioTek Inc., Winooski, VT). The Echo liquid handler was then used to dispense highly viscous aqueous solutions (stored in glycerol), as well as a variety of ELISA, kinase, cyclic AMP and cytokine assay kit components, with excellent accuracy and precision. The results for these applications illustrate the new capabilities of the Echo liquid handler that enable assay setup with flexible plate layout and volume reduction for reagent and consumables savings and higher throughput.

TP51

Li Liu, Beckman Coulter, li.liu@beckman.com

Co-Authors: Michael Kowalsk, Laura Pajak, Beckman Coulter, Inc.

mES Cell-Derived Cardiomyocytes Characterization Using the xCELLigence RTCA System

Cardiac toxicity, drug-induced adverse effects, accounts for most drug recalls and delays in gaining regulatory approvals, and is critical to uncover in the early stage of the drug discovery process. Primary cultures of cardiomyocytes are tedious to establish for screening purposes, and embryonic stem (ES) cell-derived cardiomyocytes provide a novel approach as an in vitro model for studying drug-induced cardiac toxicity.

In this study, we characterize cardiomyocytes derived from mES cells, and examine the effects of various drugs or compounds on ES cell-derived cardiomyocytes by monitoring the cellular events in a real time manner with the xCELLigence* RTCA system from Roche Applied Science. Cells and compounds were dispensed into the sensor-coated 96-well E-Plates using Biomek® FXP workstation.

Note: Biomek® FXP workstation is For Laboratory Use Only; not for use in diagnostic procedures.

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TP52

Leela maheshwari, ACS, nleela.maheshwari1@gmail.com

Cytogenetical Studies of Oil Crops

Staining techniques, microscopic observation, resolution.

TP53

Brian Majors, Biogen Idec, brian.majors@biogenidec.com

Automation of Cell Line Development Workflow and Data Analysis for Increased Efficiency

Cell line development is a crucial first step in the production process for biological therapeutics. In an effort to find the rare high expressing clones expressing protein with the desired product quality characteristics, thousands of cell lines are typically screened. Additionally, the history of each of these cell lines must be documented, since they may eventually be used in a commercial process. The combination of the hands on laboratory manipulations to create and isolate a commercial cell line coupled with the vast amount of cell line tracking data makes the process time consuming and complex. In an effort to improve the efficiency of the cell line development process, Biogen Idec has implemented a number of automation steps in the process, both from the laboratory and information management sides. Automation helps decrease errors, improves consistency, and saves time for scientists in the lab and at the desk. This presentation will discuss the Biogen Idec cell line development workflow and how we have been able to integrate automation, including liquid handling robotics, instruments with plate stacking abilities, and automated data processing. This establishment of this technology in the process comes poised at a time when electronic documentation and notebooks are making headway into biologics laboratories.

TP54

Frank Masur, University of California, Los Angeles School of Public Health, fmasur@gbl.ucla.edu

Co-Authors: Alexander Roth; Lee Borenstein, Global Bio Lab, University of California, Los Angeles; Craig Blackhart, Los Alamos National Laboratory

Integration of Heterogeneous Automated Systems in a High-Throughput Laboratory Using Web Services by the Example of a LIMS and an Accessioning System

Integration of a multitude of scientific devices into a laboratory's data processing infrastructure remains a challenging task. As there is no single widely used standard for data interchange, all vendors implement their own approaches, which almost exclusively leads to incompatibility with other devices, often even within devices of the same manufacturer. Manual data transfer and manipulation is not feasible, due to the fact that manpower is expensive and processing speed is vital, especially in a high throughput environment. In addition to that, reliability and robustness are also important factors that need to be addressed appropriately. With the purpose of providing a solution for these issues, we developed an expandable integration infrastructure for our laboratory, which consists of various devices such as an accessioning system and a genotyping system as well as a Laboratory Information Management System as the central information and control hub. Our general approach was to utilize well-established standards such as XML, SOAP and HTTPS in combination with leveraging integration features of the devices themselves, such as application programming interfaces (APIs) and data exchange functionality in order to minimize both development efforts and manipulation of the device's software. Key components are two classical, XML-based web services, offering standardized methods that reflect general requirements for both data exchange and instrument control. Furthermore, using XML-Schema, the web services offer a set of expressive data types that enhance data integrity, because they can be easily validated. By implementing these web services virtually any device can be attached to the network. Essentially, the creation of information providers and consumers (web service servers and clients) can be done quite rapidly, since modern technologies such as Windows Communication Foundation offer a broad set of ready-to-use scaffolds to work with the current standards. Moreover, due to strict decoupling, the web service part of the integration interface can be reused for every system, while the device part requires only minor adaptations, for instance to incorporate a device specific data access API. Thanks to the use of XML and open standards, the actual implementation remains transparent to the data exchange, thus allowing the utilization of not only .NET but various programming languages and system platforms. All in all this results in an infrastructure providing high flexibility and rapid integration paired with minimal dependencies among the software components and maximal reliability both in terms of data security and integrity.

TP55

Randy Dyer, Labcyte Inc., rdyer@labcyte.com

Co-Authors: Brent Browning; Harry Vlahos, Labcyte Inc.

The Labcyte Access™ Laboratory Workstation: A Novel System That Quickly and Easily Integrates the Echo Liquid Handler® Into Any Laboratory Workflow

As researchers begin to utilize the Echo liquid handler® in areas new to the use of automation, a need for a small scale automation platform that is easy to use and implement is critical. The Access™ laboratory workstation is a novel solution to quickly transform protocols created with the Echo software applications into fully optimized automation routines. With a compact footprint, the Access system can be configured to integrate the Echo liquid handler with plate handling devices and accessories directly on the bench top. This poster explores the implementation of the Access system into areas new to automation and examines the key attributes allowing researchers to quickly gain comfort with the system and achieve a greater utilization of the Echo liquid handler.

TP56

Vaughn Miller, BIOCIUS Life Sciences, vmiller@biocius.com

Co-Authors: Michelle V. Romm; Nikunj Parikh; William A. LaMarr; Can C. Özbal

Ultrafast SPE Integrated With TOF-MS Streamlines Workflow and Increases the Throughput of ADME Assay Analysis

Many analytical labs are challenged with the goal to more quickly and efficiently deliver assay results without compromising data integrity. The throughput of ADME assay analysis has gained an almost equal footing with data quality as a determinant of laboratory workflow. Improvements to conventional LC-MS/MS, the most common ADME assay analysis technology, have significantly reduced sample cycle times (~1 min/sample). However, it is clear that innovative new technologies are necessary to produce a several fold further reduction in run times. The major workflow bottleneck for tandem MS analysis is its requisite MRM method development that requires several minutes per sample of processing time. The use of accurate mass offered by high resolution (time of flight: TOF) mass spectrometers, which eliminates the need for MRM optimization, in conjunction with a high throughput solid phase extraction (SPE) system, which eliminates the need for liquid chromatography (LC), was investigated to assess its ability to enable a faster and more efficient assay analysis workflow. We compared the assay results for a panel of in vitro ADME assays (CYP inhibition, metabolic stability, PAMPA and plasma protein binding) using ultrafast SPE-MS/MS and SPE-TOF systems for analysis. A chemically diverse set of 50 compounds was used for each assay and all analyses utilized the same generic SPE conditions. All SPE-TOF analyses were performed using the same generic MS conditions with a RapidFire RF360 interfaced to an Agilent 6530 Q-TOF run in ESI-TOF mode. The experimental results obtained for all four of the ADME assays by each analysis system were comparable (R2 >0.9). Using a generic SPE condition, the ultrafast SPE-MS systems consistently produced sample analysis cycle times of 7 seconds/sample with no changes to standard laboratory workflow and no significant sample carryover. Using a generic MS method, the TOF analysis gave comparable results to conventional triple quadruple analysis. Assay results with the SPE-TOF system were comparable to the SPE-MS/MS system for a variety of ADME assays indicating that MRM method development could be eliminated for these assays. The SPE-TOF system enabled a 10x increase in workflow efficiencies in sample analysis across a panel of ADME assays without compromising the quality of results. This SPE-TOF system may also have utility for the analysis of other assays, for example whole protein modifications or the simultaneous analysis of multiple analytes.

TP57

Keith Miller, Pfizer, keith.miller@pfizer.com

Co-Author: Chi-Kin Chan

The Impact of Low-Volume Liquid-Handling Technologies on Sample Management and Screening Workflows

The introduction of low-volume liquid transfer instrumentation such as the TTP LabTech's Mosquito, Agilent Bravo, and Labcyte Echo to the Compound Management group at Pfizer has greatly extended the range of plate deliverables and revolutionized assay workflows. Historically it had been difficult to reliably dispense volumes below 500nL, which often required an additional dilution by biologists in order to achieve desired final assay conditions. This poster will demonstrate how these three liquid-handlers have broken through these barriers and the resultant impacts they have generated for both Compound Management and downstream screening groups. We will highlight unique features & functionality for each instrument, and discuss how Pfizer has incorporated these instruments into a production environment to optimize identified comparative advantages. The scope of production covered will include spotting of single concentration assay ready plates as well as creation of serialized plates for dose-response determinations. Implications to screening data integrity, production throughput, research cost, and compound conservation will be considered.

TP58

Chris Morrow, Genentech, chrism@gene.com

High-Throughput Sample Dilution in a GLP Environment – a Case Study

What if it was not sufficient to get your complex laboratory robot working correctly? What if you also had to prove beyond a shadow of a doubt, at a moment's notice, that every single function of your robot is doing exactly what it is supposed to do? This is the challenge of implementing lab automation in a regulated environment. These regulatory requirements necessitate a very specific approach to lab automation that is comprehensive and data-driven. This presentation describes the experience of implementing a Hamilton STAR sample dilutor in a high-throughput GLP environment at Genentech, Inc, as well as some of the key challenges that had to be overcome. These challenges include: How do you establish the accuracy of the system? How do you detect, recover from, and report errors during the run? How do you ensure a complex dilution method is free from all errors? This presentation will also cover lessons learned and suggestions for future projects.

TP59

Tal Murthy, Thermo Fisher Scientific, tal.murthy@thermofisher.com

Liquid Handling Options for Low Volume Scientific Applications

Laboratory experimentation involves numerous types of applications including different volumes, reagents, devices and labware. It is well known that automating repetitive and multi sample processes at both small and large scales will help save time, alleviate manual errors, offer consistency and can also miniaturize experimental volumes. We envisage that the result of any automated experiment is dependent on two factors, one is being able to achieve precise liquid handling and the second is addressing the inherent variables in the experimental assay. The former factors can be optimized significantly using the controls of the device and the latter factors are usually addressed during the pilot validation of the assay. From the device standpoint, there are several liquid handling instruments with different types of engineering mechanisms that are capable of dispensing a wide range of volumes from nano liter to milli liter levels in different configurations. Depending on the need, researchers choose the appropriate device and associated labware for their experimentation. In this presentation, I will discuss a variety of options with different capabilities, for use in scientific applications. Although it is not practically possible to address the need of every specific application, this presentation will discuss different types of liquid handling solutions, help researchers understand some of the issues, and address them appropriately. I will also discuss our experience with device optimization and our attempts to develop versatile platforms.

TP60

Kelechi Eluwa, Beckman Coulter, k.eluwa@beckman.com

Co-Author: Valentin Quesada, Beckman Coulter, Inc.

Cell Signaling Assay—Hardware integration on the Beckman Coulter Biomek

Cell signaling is a complex mechanism that directs the basic cellular processes and directs corresponding actions. The ability of the cell to respond correctly to its microenvironment is the basic element of healthy cell growth, tissue repair, effective immune responses as well as maintaining the proper tissue environmental operating point. Errors in cell signaling responses will cause the tissue's environmental operating point to change. This change in operating point is responsible for multiple disease states. By understanding this cellular signaling mechanism, such diseases can be treated effectively. A great benefit to patients is potentially available from drugs that treat the aberrant signal paths while preserving the healthy cellular responses. The flow of information within a cellular organism (system) is a complex process. A complex assay is required to study the complex signaling process. Traditional approaches to the study of cell signaling pathways involve laborious and rigorous sample preparations. The complex nature of these assays tends to limit the throughput of the studies, and is responsible for results that may depend on the technique and the manual skills of the investigator. Automation of assay preparation is the intended resolution for the complex preparation burden. We have automated the process of assay preparation for cell signaling. The automation solution uses a Beckman Coulter Biomek NXp laboratory workstation, fitted with standard automated labware positioners (ALPS) and custom adapters. The resulting automated assay is a method implemented in the Biomek programming environment to distribute the reagents, aliquot the sample, incubate the reaction plate, wash the resulting preparation, and re-suspend the cell pellet. Because the incubation temperature affects the reaction rates (kinetics) for the various signaling epitopes, it is a principle component of the reaction variation. Automation focused on the successful hardware integration for two key areas: reagent volume and reaction temperature. The reaction temperature was successfully modeled and optimized with an onboard Peltier module to mimic the manual temperature profile, while the low-high volume reagent requirements of the assay was successfully achieved using an intermediate volume syringe system. Results of the optimized automated method were comparable to the manual method, and generated reproducible results with less than 10% coefficient of variance. The resulting automated method is faster, produces repeatable results and relieves the burdens on the researcher for the intensely manual labor of assay preparation. Further, automation adds the ability to prepare more complex assays and assay more samples per day.

TP61

John Bradshaw, Artel, jbradshaw@artel-usa.com

Co-Author: Keith J. Albert, Artel

Do You Really Know What Your Robot is Doing? —The Importance of Paying Attention to Liquid Handling Details

The introduction of automation into biology and chemistry labs has arguably lead to significant advances in testing capabilities over the past 20+ years. Automation has certainly led to increased numbers of experiments, as compared to manual testing, particularly for pipetting operations. Because of this advantage, liquid handling robots have become commonplace even in small laboratories. However, in spite of all the advantages that something like a liquid handling robot brings to a laboratory, it also brings a different set of commonly overlooked challenges. It may be argued that the largest challenge presented by using a liquid handling robot is the potentially incorrect assumption that the robot is doing what it is "supposed" to be doing. The robot may in fact be doing exactly what the user told it to do, but is that really what the user wanted? One may say that the real question is, do you really know how your robot is behaving, and particularly, do you really know how your robot is performing your assays? This presentation is a follow-up to a poster we presented last year that discussed real case studies of how liquid handlers were performing, or rather miss-performing, certain commonly employed test procedures. Herein we will present even more examples of the importance of monitoring various commonly employed tasks, which are likely considered mundane and often assumed to have little bearing on overall robot performance. Specific examples that will be presented include; 1) pre-wetting tips, 2) using a blowout volume after pre-wet, 3) protocol transfer from one robot to a sibling, 4) protocol differences between high volume and low volume dispenses, 5) etc. The examples presented herein will help users to think more about the specific tasks they are asking their robots to perform, and hopefully uncover certain steps that, if observed and controlled, will result in better performance.

TP62

Rudy Potenzone, SciencePoint Solutions, rudy@sciencepoint.net

SharePoint as a Lab Infirmatics Platform

SharePoint has become a widely used platform for document sharing and corporate web sites across the PHARMA and Chemical Industry. With the addition of scientific components and related workflow it is possible to create exciting new capabilities within a SharePoint environment. These can provide the basis of scientific collaboration portals and even cloud hosted workspaces. These give the opportunity to operate virtual project teams that span geographies, organizations and academic partnerships.

TP63

Karen Poulter, Life Technologies, karen.poulter@lifetech.com

Co-Authors: John Bishop, John Adam Harris, John Bodeau, Rhonda Meredith, Kamini Varma

Automation of SOLID® Standard Fragment Library Preparation With Size Selection by Utilizing Agencourt® AMPure® XP Beads for Cleanup and a Tecan® Freedom EVO® 75 for the Mechanization

There will always be a need to have genomic DNA library samples processed at as high a quality as possible; especially when you have a next generation sequencer (NGS) that runs for several days at a time. It's also nice to have samples ready to go onto the instrument when the NGS is finished running. To help with through-put and cost, multiplexing adaptors have been used so that libraries can be pooled together, allowing for a single round of emulsion PCR and then simultaneous sequencing of multiple samples with a single SOLiD run. Applied Biosystems SOLiD™ Express Fragment Library Preparation Using the Tecan® Freedom EVO® 75 was modified to do size selection with Agencourt® AMPure® XP beads during the End-repair purification step of the first script. The robot went from a maximum of 24 to 12 samples with the first script while keeping everything automated. The second script can still process up to 24 samples and the third script 12 samples. The protocol has been validated to work with 0.5ug to 4ug of sheared genomic DNA to produce high quality, high coverage sequencing libraries from multiple samples in a pool.

TP64

Victor Rosso, Bristol-Myers Squibb, victor.rosso@bms.com

Co-Authors: Jose Tabora, James Bergum, Erik Rubin, Robert Waltermire, Bristol-Myers Squibb Company

Productivity Enhancement and Acceleration of Chemical Process Development Through Utilization of Laboratory Automation

Automated workflows such as crystallization and solubility are enhancing the productivity of our Process Research and Development organization. Experience with these established workflows helped enable the introduction of a new workflow, Highly Automated Design of Experiments (HAD). The HAD provides a powerful approach for rapid detailed optimization of chemical processes especially when combined with data exploration analysis and statistical modeling. This workflow provides a glimpse at the future state of laboratory automation at BMS which requires information management across a global organization. The concept of using fungible resources across workflows and a centralized data repository has eased the introduction of new automated technology and increased the impact these tools have on the Process Research and Development organization at Bristol-Myers Squibb.

TP65

Drew Sauter, NanoLiter LLC, adsauterjr@gmail.com

Induction Based Fluidics

At last years meeting along with the US Army, JEOL and Douglas we introduced induction based fluidic (IBF) technology that was for the first time applied to pipette/MS for the analysis of small molecules and 384 channel, parallel, non-touch nL,uL dispensing. In the past few months Jarecki, Vestling, et al of the University of Wisconsin published single cell MALDI of a neuron using IBF nanoLiter dispensing. In the same month NIST also published that by using IBF to dispense nL quantities of glycerol, they could increase the sensitivity of SIMS for the analysis of RDX and cocaine by factors of 100 to 1000,literally. Also, at the annual ASMS meeting JEOL demonstrated using IBF and a DART TOF/MS that small molecule drugs could be detected with 10 to 100 x increase in sensitivity using IBF nanoliter devices as compared to older sample introduction technology. These applications followed peer review publications in 2008 and 2009 by M.L. Gross and J. Harmon showing increases in MALDI TOF MS for the analysis of proteins, peptides and synthetic polymers where in case at NIH an increase in MALDI sensitivity of 100x was estimated by for PTM's of tublins. In this presentation, we review the fundamentals of IBF. We discuss how via the proposed Beer's Law of mass spectrometry that we reiterated last year, why parallel nanoliter IBF depositions from dispensers, SPE devices, LC columns yield enhanced sensitivity and reproducibility for DART, SIMS and MALDI MS and how IBF could potentially replace ESI for MS sample introduction.

TP66

Tim Schroeder, Atoll GmbH, t.schroeder@atoll-bio.com

Co-Author: Jürgen Friedle

Automated Parallel Chromatographic Separations in Process Development

Optimization of chromatographic separation conditions is normally a time consuming task in the large scale production of proteins such as monoclonal antibodies. In this study a 96 well formatted MediaScout® RoboColumn® array was adapted for automated operation in a modified commercial Tecan Freedom Evo liquid handling workstation and used for the fast testing of different chromatographic parameters of a three-step mAb purification process. This outline is focussed on the intermediate purification step by AIEX chromatography. Therefore the Protein A capture eluate was applied on 8 different AIEX media under non binding conditions (negative chromatography). The most suited candidate out of this experiment was optimized regarding protein binding by varying salt concentration and pH. All experiments were first performed manually with PipetColumns (MediaScout® StarterKit), then transferred to automated RoboColumns and finally scaled up to 10 ml MediaScout® MiniChrom columns for proof of concept. The combined approach of Atoll's MediaScout® chromatography tools and Tecan's liquid handling workstation Freedom EVO enables to speed up the development of a purification process by nearly one order of magnitude.

TP67

Cristopher Cowan, Promega Corporation, ccowan@promega.com

Co-Authors: Steve Krueger, Promega Corporation; Joe Kennedy, Susan Lee, Hamilton Robotics; Christine Helt, Promega Corporation; Aaron Bonk, Hamilton Robotics, Eric Vincent, Promega Corporation

Automated Isolation of Genomic DNA From Large Volumes of Whole Blood

A common laboratory sample for genomics research is blood drawn into a standard 10ml Vacutainer® tube. The Promega ReliaPrep™ Large Volume HT gDNA Isolation System integrated on the Hamilton MICROLAB® STARplus liquid handling workstation provides a unique and dependable system for isolating genomic DNA from large volumes of blood. The novel chemistry and instrumentation resolve many challenges encountered when processing large-volume samples in a high-throughput format such as: loss of yield from frozen or mistreated samples, loss of DNA pellets during decanting of waste fluids, transport of full 50 mL tubes to various locations on a liquid handling robot, and manual re-suspension of final DNA pellets. The ReliaPrep™ Large Volume HT gDNA Isolation System lyses the entire sample and binds genomic DNA overcoming limitations of processing frozen or mishandled blood samples. Liquid handler resource constraints have been removed by the design of a unique new instrument accessory, the ReliaPrep HSM 32 LV instrument, which provides heating, shaking and magnetization of samples at one deck position. The combination of this new device, the MICROLAB STARplus workstation and the ReliaPrep Large Volume HT gDNA Isolation System allows fully automated recovery of gDNA from up to 96 ten milliliter blood samples within 8 hours. We present verification studies demonstrating automated system performance. 3 to 10 ml blood samples were processed on the automated system, which detects input blood volumes and scales the chemistry for each sample. Comparisons between the ReliaPrep™ Large Volume HT gDNA Isolation System and a precipitation-based method were made for duplicate blood samples from multiple donors. Yield, purity, and integrity of the extracted gDNA were assessed using UV absorbance spectroscopy and gel electrophoresis. Genomic DNA yields from normal 10 mL whole blood samples were 200 to 400 µg (depending on white blood cell count) in an eluted volume of 1mL. Recovered DNA exhibited good purity with A260/A280 ratios greater than 1.7 and A260/A230 ratios between 1.8 and 2.2. Isolated DNA was suitable for storage and many typical downstream analysis applications including restriction digestion, PCR amplification, and real-time PCR amplification. Linearity of genomic DNA recovery from the same sample at multiple volumes was confirmed. Data is presented to show the reproducibility of the purification from multiple aliquots of the same sample and intra-sample CV's were determined to be =10%. Results of genomic DNA purification from frozen (hemolysed) blood samples as well as blood collected using common anticoagulants (EDTA, heparin, citrate) are also compared to demonstrate the efficacy of the new system.

TP68

Hsiung Suz Kai, Fooyin University and Hospital, henrisky@gmail.com

Co-Authors: Hui Jen Chang, Fooyin University Hospital; Ming Je Yang, Kaohsiung Medical Universit; Ming Sung Chang, Fooyin University Hospital, Pingtung; Der An Tsao, Fooyin University Hospital; Hua Hsien Chiu, Fooyin University, Koahsiung; Yi Fang Chen, Gene Target Technology Co.Ltd; Tian Lu Cheng, Kaohsiung Medical University; Shiu Ru Lin, Fooyin University Hospital

A Novel Technique for Detecting the Therapeutic Target, Kras Mutant, From Peripheral Blood Using the Automatic Genechip Analyzer Device With Weighted Enzymatic Chip Array

RT-PCR and real-time PCR are widely used for the detection of gene overexpression. However, various disadvantages and limitations arise when the detection of multiple genetic targets is required. In previous studies, our laboratory successfully established a membrane array operation platform with a diagnostic biochip for the screening of gene overexpression by circulating tumor cells in cancer patients. In order to effectively shorten the reaction time, we have improved the conventional RNA extraction method. The concept of weightedness was included in the reading procedure of the chip array and a weighted enzymatic chip array (WEnCA) platform was established. This study uses fluidic engineering to develop a fully automatic genechip analyzer, which runs automatically on the WEnCA platform. The combination of the two systems is called the WEnCA-automatic system. Furthermore, in order to understand the actual differences between the operations of WEnCA-automatic and WEnCA-manual, we used the WEnCA-manual to analyze KRAS associated gene overexpression in 200 samples from cancer patients to establish a cut-off-value for Activating KRAS Detection Chip. Specifically, the activated KRAS expression in blood samples of 209 lung cancer patients were analyzed by both WEnCA-manual and WEnCA-automatic and compared. The clinical applicability of WEnCA-automatic was defined, including the sensitivity, specificity and the accuracy. The results showed that, among 209 samples, 71 of the patients were positive for activated KRAS expression by WEnCA-automatic with a sensitivity of 89%, specificity of 94%, and accuracy of 92%. In addition, the average total score of WEnCA-automatic was 4.7 lower than that of the WEnCA-manual. The WEnCA-automatic required an operation time of only 7.5hr, which was approximately 1/9 of the WEnCA-manual operation time and was 1/5 of the cost of WEnCA-manual. No significant difference was found between the detection limitations of the two systems. Hence, great strides have been made in this development with the potential of the WEnCA-automatic operation system being used for clinical applications.

TP69

Markus Tarin, MoviMED, m.tarin@movimed.com

Using Graphical Programming Tools With Fexible Design Hardware to Speed Time to Market

In this session, Markus Tarin, President & CEO of MoviMED (Irvine, CA, (www.movimed.com) demonstrates how today's system design tools allow for rapid product development cycles in a highly dynamic product development environment. The medical and pharmaceutical markets demand new development approaches, high throughput and greater flexibility. These demands are often not met, due to the inherent complexity of laboratory automation processes as well as point of care diagnostic equipment. System design engineering usually encompasses a multi-faceted engineering approach. Inherent to that process are vendors that offer specialized development tools. These tools usually are good for their specific target application, but often do not play nice with other development tools, hence requiring an army of specialized engineers. The end result is often a complex system that has to work seamlessly, constructed by engineers and tool chains that lack the interface to make this task easy and intuitive. To address these design challenges and to ultimately shorten the time to market and therefore reduce the overall development cost, Mr. Tarin will introduce the concept of a so-called _Starter Kit_. This starter kit is a bundle of useful hardware, graphical design software, firmware and intellectual property (IP) that allows rapid prototyping and a segway into production with one and the same tool chain.

TP70

Max Tella, Pfizer Inc., max.tella@pfizer.com

Co-Author: Justin Walton, Pfizer Inc.

Leveraging SharePoint and InfoPath Technologies to Perform Harmonized Study Tracking Across a Complex Global Organization

Background: In order to perform accurate forecasting of resources, the Business Resources Management group (BRM) requires accurate and timely data about project work carried out at each R&D site and at each out-sourcing partner (CRO). Bio-Analytical (BA) analysis, whether performed on site or by CROs, funnels through the majority of both in vivo and in vitro studies thus becoming a natural data collection point. Consumers of these data are also BA discipline leaders and C.I. experts which use it for their own forecasting and work-load planning needs.

Project Description: Modern day business trends emphasize collaborative tools as means for improving workflows and communications. The larger the company, such as with organizations that are distributed across many sites around the world, the larger the benefits of the using these tools. In recent times, local groups at Pfizer have put increasingly more emphasis on controlling processes and implementing metrics based system. As a product of this new paradigm, group leaders have been required to produce reports and often relied on the tools they were more familiar with. With each group taking on a different path it is easy to reach circumstances where common goals, as simple as producing a quarterly report, are achieved with diverse solutions. This led to non standardized deliverables and ultimately the realization of the problem at hand. A harmonization of systems and processes was the only way to address the need of consistent metrics and accurate reports.SharePoint technologies had in the mean time emerged as the best candidate for a common data repository so the choice of this tool was natural. This poster describes the realization of a project aimed at harmonizing data collection systems and nomenclature across global sites using SharePoint as a repository. The project also covers the design of a business layer and an interface layer. Data aggregation and filtering is performed via the use of a Pipeline Pilot protocol. The script connects to the databases located across the world; it reorganizes the data and provides a dash-boarding solution for visualization. The protocol also creates ad hoc spreadsheet based reports that are used for further dynamic analysis via desktop based applications such as Spotfire, which is the most common tool used by line managers.

The novelty of the project consists on having obtained for the first time in our department a means for collecting, storing and visualizing process data coming from diverse global sites, thereby producing consistent and accurate reports enabling the business units and line managers to obtain meaningful forecasting and workload analysis reports.

TP71

Marc Valer, Agilent Technologies Inc., marc_valer@agilent.com

Co-Authors: Manuel Gomez, Nick Howells, David Bracey, Cyrille Habis, Kimberly Subrahmanyan, Agilent Technologies Inc.

A New Automation Platform Ideally Suited for Small to Mid Size Automated Workstations in the Life Science Laboratory

Laboratory automation platforms have traditionally been divided between standalone device automation (i.e. a stacker mounted on a reader or a small, simple robot feeding it from multiple stackers), large liquid handling platforms that can access one or two additional off deck devices and finally largely integrated systems, with central or rail mounted robotic arms, often derived from other industrial disciplines. In recent years, laboratories handling cells, proteins and genetic samples have been looking at mid size solutions that can easily be reconfigured as demands for automation in the lab shift over time. Such solution typically requires more than the 2-3 devices that most small automation solution permit today and less than ten devices that could arbitrarily be defined as a highly integrated system. One would expect this versatile automation powerhouse to integrate a liquid handler, plate washers /dispensers or common microplate handling devices (like a sealer, labeler, centrifuge or vacuum station), in addition to a variety of plate storage/incubation options that can be either placed on a standard bench, a laminar flow cabinet or a small integration table. In addition adding a reader to the system would permit moving from a sample prep station to a completely assay or screening platform. The needs for such a platform would point to automation solutions that are not readily available today, requiring the performance of industrial robotic platforms adapted to the safety and ease of use required in laboratory environments as well as the constricted real state for today's laboratories. Additionally user's would want to have intuitive and complete control on the setup, transparent robot teaching, and an automation protocol writing that resembles the manual protocols. We have developed a new robotic platform to address these needs. A SCARA (Selective Compliant Articulated Robot Arm) robot, designed from scratch for the life science laboratory is at the core of a new automation platform that uses novel methods of device attachment, position teaching, and utilization of the robot workspace to ensure a flexible, safer and intuitive use of the robot while retaining performance figures similar to those of larger more complex, industrial platforms.

TP72

Jolanda Van Putten, Lab Services BV, j.vanputten@lab-services.nl

Co-Author: Ferry de Vugt, Lab Services BV

Lab Services PlateButler 3.0 Robotic Control Software

Investigating the customer's wishes and fitted to the demands of a modern laboratory Lab Services developed the PlateButler Software.

In today's market Software needs to be flexible, adaptable and based on actual needs. Lab Services is now presenting and releasing version 3.0 of its robotic control software which has al key benefits and is fitted to work in a Windows 7 environment: PlateButler 3.0. State of the art .NET compatible development tools such as Embarcadero® RAD Studio 2010 are used to create the software. As a result of this the Layout is sophisticated and easy to understand.

Key benefits:

- Dashboard view to see the system's status at a quick glance
- Modular architecture
- Multiple robot systems control on just one PC
- Access level security
- Color themed client status sidebar
- Easy programming through plate path
- Integrated simulation module to check the assay
- Throughput calculation from sequence programming
- Automated Runtime calculation with self learning timings
- Time limiting system component analyser.
- Integrated, free definable checklist wizard when starting assay
- High versatility; No limitations in brands of system components
- SiLA compatible
- Extreme reliability

A large versatility is reached because PlateButler can easily be connected to any device or software. By using the client-server principle a multi instrument system can be setup in a distributed environment. Also there are no restrictions on brands or makes of these instruments. The main application manages the server. The instruments are controlled by it's clients. The user can control all devices from the main application. However the clients can also be accessed separately, if desired. This creates maximum flexibility and full control of your system. The system is also able to work in local environments.

TP73

Alona Vizel, Amgen Inc., avizel@amgen.com

Co-Authors: Kate Hutterer, Amgen Inc.; Eleanor Le, Amgen Inc.

Automated rCE-SDS Fc-Fusion Sample Preparation Using PhyNexus Robotic Platform

Reduced capillary electrophoresis sodium dodecyl sulfate (rCE-SDS) sample analysis is used with an increased frequency to characterize protein samples in the biopharmaceutical industry. Analysis of in-process protein samples is often time consuming due to the need for manual clean up steps necessary to make samples suitable for rCE-SDS injection. Desalting, protein concentration normalization, and pH adjustment performed manually can often lead inconsistent electropherograms and variable peak signals. In the current work we automate the Fc-fusion sample preparation with the use of PheNexus system. Optimization of buffer compositions and liquid handling parameters allowed us to successfully normalize protein concentrations ranging from 0.5 mg/ml to 70 mg/ml as well as consistently buffer exchange samples which ranged widely in pH and salt compositions. Sample recoveries of 95% have been regularly achieved using the automation platform. The automated protocol resulted in improved assay capacity and superior, more consistent sample signals.

TP74

Sandy Weinberg, Clayton State University, sweinberg@clayton.edu

A Comparison of International Laboratory Regulatory Requirements

This presentation compares the laboratory regulatory requirements as established int he United States by USFDA with the standards estalbished by equivalent agencies in Australia (TGA); Brazil (Anvisa); China (SFDA); the European Union (EMEA); France (AFSSAPS); Germany (BfArM); India (CDSCO); Israel (IMOH); Japan (PMDA); South Korea (KFDA); Switzerland (Swissmed); and the United Kingdom (MHRA). The comparison considers five (5) categories of regulations: electronics/automation; quality assurance and control; role of risk assessment and alanysis; audits, visits, inspections and enforcment; and acceptance of a Quality by Design (QbD) approach. Trends, commoanlities, and areas of harmonization are explored.

TP75

Jan Prochnow, Mettler Toledo

Co-Authors: Fraser McLeod, Dionex Corporation, Bruno Nufer, Michalis Meyer, Quantos Perfect Dosing, Mettler Toledo

An Ultrafast Workflow for ICH Linearity Studies Using Automated Standard Preparation and UHPLC

Introduction: According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), it is necessary to test analytical methods for their linearity as part of a method validation study. The typical process for this is to prepare standards for 5 different calibration levels, and to make triplicate injections of each of these standards. The concentration range used is typically 70-130% of the nominal concentration. Statistically it is recommended that each concentration level is prepared individually. This has the effect of randomizing potential sources of error. However, this is typically not done as it is a time consuming process. Instead, many laboratories prepare a stock solution, and then dilute this down to the 5 different concentration levels. This has the benefit of being the fastest way of preparing standards, but has a major disadvantage: any error in the stock solution will be carried through to the diluted standards.

Methods: Some chromatography methods have more than 1 analyte, and each one must be tested for linearity. This substantially increases the time (and chance of error) for manual preparation and also increases the chance of error for the stock standard/dilution approach. This means that all approaches have drawbacks. A better way is to automate the preparation of the standards. This can be achieved using the new Quantos QB1-L system from Mettler-Toledo. This can automatically dispense analytes into HPLC vials or volumetric flasks and also weigh in the appropriate amount of diluents. In this experiment we tested the linearity of 5 analytes in a soft drinks analysis. The analytes studied were Acesulfame K, Saccharin, Caffeine, Vanillin, and Benzoate. The Quantos system automatically weighed the correct amount of each analyte, and then the correct amount of diluent (90:10 Water:Methanol) to provide the concentrations. This analysis was performed on the Dionex UltiMate® 3000 Basic Automated System – an entry level system that is fully UHPLC compatible. It supports pressures up to 620 bar and flow rates as high as 10 mL/min, and is ideal for running fast, routine analyses. ICH requires that the following values are reported; correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares. Laboratories also need to check that the correlation coefficient is within the limits expected of the method (typically = 0.999). The Chromeleon® Chromatography Data System from Dionex can fully automate this particular analysis, and immediately generate all results for all analytes.

Results: The combination of automated powder dispensing systems, UHPLC systems produced outstanding results for the linearity experiment. It shows the correlation coefficient for all analytes – each of which as an R2 value greater than 0.999 and allows laboratories to realize significant productivity benefits."

TP76

Mark Bayliss, Virscidian Inc., mbayliss@virscidian.com

Co-Authors: Mark Bayliss, Joseph Simpkins, Virscidian Inc.; Martin Fuhr, Utz-Peter Jagusch, Josephine Archinal, Grünenthal GmbH

Experiences and Details of High-Throughput, Multi-User, Multiple Instrument Hardware Vendor Screening Solution for Library QC and Target Purification

Screening high volumes of analytical results for quality and consistency of results when it comes to library compound management QC, small to medium automated synthesis support and purification of targets is tedious and costly in terms of required experienced manpower. Our laboratories for analyzing incoming samples are comprised of a heterogeneous array of instrument types and instrument vendors. Our goals at the start of the project were multi-fold. Improve quality of results, reduce the number of false positive results, reduce the number of samples requiring manual reprocessing, and decrease the throughput time from initial QC, purification and post purification QC. Automate the processing of raw data and to create a single consistent output of results that are integrated with our existing inter/intra departmental workflows and corporate infra-structure. In this presentation we would like to share our practical experiences in achieving our primary goals, some of the challenges that we faced prior to implementing the automated approach and how the new workflow has impacted the departmental workflow in a positive way. Already we have seen the cycle time from initial QC of samples to final QC of purified fractions reduced from between 3 and 4 days to around 24 hours.

TP77

Jorma Lampinen, Thermo Fisher Scientific, jorma.lampinen@thermofisher.com

Co-Authors: Marika Raitio, Hanna Grano-Fabritius, Reija-Riitta Harinen

Comparison of Monochromator and Filter Based Fluorometers in the Measurement of Narrow Stoke's Shift Labels; Fluorescent Proteins as Practical Examples

Fluorescent proteins are such a group of fluorometric labels that their Stoke's shifts are normally quite small. Good examples of the labels with very narrow differences in excitation and emission peaks are Renilla reniformis GFP and EYFP. Renilla GFP has excitation and emission maxima at 498 nm and 507 nm and YFP has 514 and 527 nm. These about 10-15 nanometer Stoke's shifts are the smallest one can normally find but there are several other labels with 15-20 nm Stoke's shifts. This paper describes the general instrumentational difficulties that are present when these small Stoke's shift labels are measured. Excitation and emission wavelengths can not be selected using label's maximum efficiencies because placing excitation and emission so close to each others would cause excitation light leakage through the reader's optical system directly to the detector, resulting in very high background. When the wavelength used are moved outside from the peak wavelengths both excitation and emission efficiencies might decrease drastically, resulting in low signal to background values. This paper shows the general principles how excitation and emission wavelengths and filters should be optimally selected. The effect of this optimization with narrow Stoke's shift labels is shown on the assay performance parameters (detection limit, dynamic range, Z-prime values etc.). Optimization procedure is described for the most common fluorescent proteins including Renilla GFP, eGFP, YFP. CFP and mCherry. During the optimization calibration series of the pure labels were measured with different excitation emission wavelength pairs using Thermo Scientific Varioskan Flash microplate spectrofluorometer. Wavelength combinations were selected based on shape of the fluorometric spectra of each label so that as high excitation and emission efficiency as possible was used without any straylight leakage through the optics. Then the detection limits of the labels were determined according to IUPAC principle and other central assay parameters were analyzed. As a comparison, all labels were also measured with Thermo Scientific Fluoroskan Ascent microplate fluorometer using optimal filter sets for each label. The results will show that the monochromator based spectrofluorometer is clearly more powerful in measuring these small Stoke's shift labels than traditional filter fluorometer, even with the optimal filters. Monochromator technology makes it possible to use excitation and emission wavelengths closer to each others and therefore both higher excitation and emission efficiency can be used in the assay.

TP78

Paul Held, BioTek Instruments, heldp@biotek.com

Co-Authors: Dee Shen, Enzo Life Sciences; Peter Banks, BioTek Instruments; Wayne Patton, Enzo Life Sciences

A Fluorescence Microplate-Based Assay Workflow for High-Throughput Screening of Compounds Modulating Autophagy in Living Cells

Autophagy is a normal degradative pathway that involves the sequestration of entire organelles, protein complexes, and misfolded proteins in a membrane vacuole called the autophagosome. The autophagosomal vacuole is subsequently delivered to the lysosome where it is degraded into its essential constituents and recycled back to the cytoplasm. Autophagy plays important roles in diverse biological events, including responses to nutrient limitation, tumor suppression, the immune response, and aspects of neurodegeneration. However, experimental methods to monitor this process in mammalian cells are limited and often highly qualitative. For example, LC3, a mammalian homologue of the ubiquitin-like (UBL) protein Atg8, has been used as a specific marker of autophagosomes in mammals. However, current methods to quantify autophagic activity using LC3 are time-consuming and labor-intensive, involving the physical counting of fluorescent punctate signal forming within the cells. We have developed and validated a novel cell-based autophagy assay using a 488 nm-excitable green-emitting fluorescent probe to highlight the various vacuolar components of the autophagy pathway. We demonstrated that the accumulation of autophagy probe was specifically induced by amino acid deprivation and was inhibited by 3-methlyadenine, a classical inhibitor of the autophagic pathway. Furthermore, a population of this dye-labeled vesicle co-localizes with LC3. We have validated this fluorescent probe under a wide range of conditions known to modulate autophagy pathways. We have optimized the use of the EL406™ Combination Washer Dispenser to automatically aspirate media, wash cells and dispense reagents for the assay, allowing, for the first time, easy quantitation of autophagy using a convenient fluorescence microplate-based HTS format that generates Z' factor values greater than 0.5. This assay enables kinetic analysis of the autophagy pathway and is able to distinguish between increases in autophagic flux vs. autophagic vacuole accumulation.

TP79

Matthew Hymes, Pall Life Sciences, matthew_hymes@pall.com

Co-Authors: Victor Chen, John Frenna, Pall Corporation, Port Washington, New York;
Lisa Bradbury, Hongshan Li, Laura Pueschel, Pall Corporation, Woburn, Massachusetts

Streamlined Purification of Plasmid DNA From Prokaryotic Cultures

Plasmid DNA has become an essential element of molecular biology and protein based research. This invaluable tool is required for expression of recombinant proteins in a variety of host organisms. Advances in cloning techniques have greatly increased the number of samples requiring small-scale plasmid preparation, or mini preps. The use of 96-well culture plates for the growth of E Coli expressing plasmids of interest benefits from clarification and purification tools also in a 96-well format. The challenge however, is to have protocols that adapt well to robotic systems and plates that show very good well-to-well and plate-to-plate consistency to avoid unexpected complications or variability in the results, during a largely hands free operation. The new Pall AcroPrep Advance lysate clarification and DNA purification plates were tested using an epMotion automated pipetting system. Single pools of crude lysate and clarified lysate were used to demonstrate high reproducibility of these plates and protocols. The clarification protocol was simple and resulted in particulate free lysate samples having very low OD320 values (<0.03). The purified plasmid DNA quantity (average of ~5µg/well) and quality (OD260/OD280 average 1.9) was highly reproducible. Contributors to strong performance include consistently low hold up volume, absence of cross-talk, and high recovery of eluate fraction due to combined effects of plate design and high performance DNA binding membrane.

TP80

Jason Luo, University of California, Irvine, jtluo@uci.edu

Co-Authors: Edward Nelson, Michael Lilly, G.-P. Li, Mark Bachman

A Microfluidic Device for the Capture, Enumeration, and Isolation of Circulating Tumor Cells

Circulating tumor cells (CTCs) represent an opportunity for noninvasively obtaining tumor tissue and quantifying disease progression, and are thus potentially useful to scientists and healthcare professionals. However, their isolation and enumeration has proved challenging due to their rarity in human blood, even in patients at advanced stages of metastasis. Microfluidics presents an attractive solution to this obstacle by allowing researchers and technicians to search through blood at the microliter level. We propose a microfluidic device for processing milliliter volumes of whole blood and isolating CTCs using both techniques from immunology and microfabrication. Briefly, a blood sample from a patient is pumped into the device and flowed into a preliminary microchannel. A non-uniform AC electric field is generated across the sidewalls of the channel, resulting in a lateral field gradient through the blood, a conductive media. For select frequencies, different cell types in the sample will experience different lateral dielectrophoretic forces due to the the field gradient, and are sorted as a function of their position along the channel width. Laminar flow maintains the sorted pools and prevents any mixing, and healthy blood cells, confounding leukocytes in particular, are eliminated. To further enhance separation between cell types and increase sorting efficiency, the blood sample is stained with polystyrene beads selective for EpCAM, a cell surface marker upregulated in tumor cells. Because the dielectric properties of polystyrene beads differ sharply from those of both tumor cells and healthy blood cells, these bead-tumor cell conjugates display unusual dielectrophoretic properties in the device and can be sorted at electric frequencies other than those used for either bare tumor cells or blood cells.This isolated population of CTCs has applications in personalized drug regimen design, whereby a clinician can determine specific mutations in the captured CTC and treat the consequences of those mutations, and in immunotherapy, using the cells as non-self antigens in an animal host, isolating the resultant antibodies produced by the animal, and using them in the patient to defeat the tumor cells.

TP81

James Brady, MaxCyte, Inc., jamesb@maxcyte.com

Advancing Drug Discovery with the MaxCyte® STX™ Scalable Transient Transfection System: Expression of Intracellular, Membrane-Bound and Secreted Proteins in Physiologically Relevant Cell Lines, Primary Cells and Stem Cells

The MaxCyte® STX™ Scalable Transfection System is a bench-top instrument that can transfect small scale (5x10^5 cells) to HTS scale (1x10^10 cells) using its proprietary flow electroporation technology with DNA, mRNA, siRNA or protein in less than thirty minutes. The cell type specific electroporation protocols allow users to consistently transfect cells on large and small scale with high viability, transfection efficiency, and cell membrane integrity for high quality results in downstream HTS/HCS functional assays for ion channels, GPCRs, and other targets of interest. The technology works very well with commonly used cell lines, such as HEK 293 and CHO, but one of its primary advantages is that it allows efficient transfection of challenging cell lines and primary cells that are not normally amenable to transient transfection. We will present data on how to use the MaxCyte STX system to transfect various cell types at small and large scale for rapid development of cell based assays. And, we will illustrate the capabilities of the MaxCyte STX system for protein production at both small and large scale.

Podium Abstracts

Detection and Separation—Track 1

10:30 am Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Susan Olesik, Ohio State University, olesik@chemistry.ohio-state.edu

Co-Authors: Joseph Zewe, Toni Newsome, Tian Lu, Ohio State University

Self-Assembled Nanomaterials for Separation Science

Separation Science significantly impacts fields as diverse as disease detection, drug discovery, warfare agent detection, environmental pollutant detection and remediation. Marked improvements in separation efficiency and selectivity would benefit all of these areas of science significantly because many of the current challenges in these fields are the separation of highly complex mixtures. Our research group recently develop a new separation device capable of provide as many as 50,000 plates /cm of separation media using nanoscale ordering. More recently a method to molecular imprint these highly efficient surface was develop. High selectivity for model compounds have been documented to date. This presentation will describe the further development of this new separation technology along with specific examples of challenging separations that can only be addressed with this new nanomaterials based technology.

11:00 am Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Michael Natan, Cabot Security Materials Inc., michael.natan@cabotcorp.com

Encapsulated SERS-Active Au Nanotags: New Applications for Old Nanomaterials

We have developed a series of encapsulated SERS-active labels (SERS nanotags) and several different assay formats that are designed to be used with small or handheld Raman readers for chemical and biochemical sensing applications. We will describe the basic nanomaterials-driven compositions, highlighting the challenges and successes in synthesis, characterization, and scale-up. We will then highlight current applications, ranging from in vivo imaging to ultrasensitive detection of proteins and DNA to detection of low molecular weight species such as aflatoxin and even TNT.

Detection and Separation—Track 1

11:30 am Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Linda Sargent, CDC/National Institute for Occupational Safety and Health, lqs1@cdc.gov

Co-Authors: WV, Ann F. Hubbs, Stanley A. Benkovic, Anna A. Shvedov, Michael .L. Kashon, David T. Lowry, National Institute for Occupational Safety and Health; J.L. Salisbury, Mayo Clinic; Ashley Murray, Elena Kisin, Loir Battelli National Institute for Occupational Safety and Health; John Mastovich, PA Jacqueline Sturgeon, Kristin Bunker, RJLGroup

Induction of Centrosome Fragmentation, Mitotic Spindle Aberrations and Aneuploidy by Occupationally Relevant Doses of Single Walled Carbon Nanotubes and Multi-Walled Carbon Nanotubes, Implications for Monitoring of Acutely Exposed Workers

Engineered carbon nanotubes are newly emerging manufactured particles with potential applications in electronics, computers, aerospace and medicine. The low density and small size of these biologically persistent particles makes respiratory exposures to workers likely during the production or use of commercial products. We have previously shown mitotic spindle aberrations in cultured primary and immortalized human airway epithelial cells exposed to 96, 48 and 24 ?g/cm2 single-walled carbon nanotubes (SWCNT). In order to investigate whether the mitotic spindle damage was unique to SWCNT, we examined mitotic spindle aberrations following dosing of cells to multi-walled carbon nanotubes (MWCNT) and SWCNT at concentrations anticipated in workers exposed at the current permissible exposure limit. Immortalized and primary respiratory epithelial cells were exposed to 24, 2.4, .24 and .024??g/cm2 nanotubes, doses equivalent to permissible worker exposures. SWCNT induced fragmented centrosomes, multiple mitotic spindle poles and aneuploid chromosome number at these doses. By contrast, similar doses of MWCNT induced predominately monopolar mitotic spindles. The SWCNT bundles and MWCNT are similar to the size of microtubules that form the mitotic spindle and may be incorporated into the mitotic spindle apparatus. Our results show significant disruption of the mitotic spindle by SWCNT and MWCNT at occupationally relevant doses. The integrity of the centrosome number and morphology are tightly regulated in normal cell populations. Disruption of the centrosome is common in many solid tumors including lung cancer. The resulting aneuploidy is a key event in the progression of cancer and is correlated with tumor stage. The acute disruption of the centrosome that was observed in this study resulted in errors in chromosome number. Future studies are in progress to determine if the chromosome number and the integrity of the centrosome are disrupted in vivo and if chronic exposure results in cancer.

12:00 pm Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Cerasela Zoica Dinu, West Virginia University, czdinu01@gmail.com

Co-Authors: A. Campbell, T. Sobray, C. Xiang, West Virginia University: J. S. Dordick, Rensselaer Polytechnic Institute; N. Wu, West Virginia University

Enzyme-Nanomaterial Conjugates for Decontamination of Biological Warfare Agents

Biological warfare agents can be engineered for ease in production and mass dissemination as well as for increased virulence. Thus, there is a critical need to develop and deploy safe and effective means for recognition and decontamination of such agents when used against military, civilian, or other targets. We developed “smart” coatings capable of detecting and eliminating biological warfare agent simulants, such as bacteria, microbes and spores. Our strategy is cost-effective and environmentally friendly and is based on immobilization of biocatalysts onto nanosupports of carbon or titanium and further entrapment of such enzyme-based conjugates into universally applicable coatings. By taking advantage of the unique properties of the nanoscale support we ensure high enzyme loading in the coatings and thus increased efficiency against the biological warfare target. Decontamination capabilities of the enzyme-based coatings are reported relative to standard decontamination units achieved when chemical agents are used. The advantages of the coatings containing “green” namely enzyme-based technology are that they can eliminate the risks associated with other decontamination methods that use corrosive chemicals and are less efficient while generating a substantial amount of residual waste.

Detection and Separation—Track 1

3:00 pm Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Linda McGowan, Rensselaer Polytechnic Institute, mcgowl@rpi.edu

Co-Authors: Tian Zhang, Huiping Zhang

A Bio-Inspired Pathway to Aptamer Discovery

Antibodies have long been unrivaled as affinity reagents for proteins due to their strong and selective binding; however, drawbacks associated with their production, stability and manipulation have prompted researchers to seek alternatives. Foremost among alternatives are aptamers, which offer affinity on par with that of antibodies, but with important advantages: first, once an aptamer to a target protein has been identified, it can be synthesized, chemically modified and manipulated with ease; second, aptamers are chemically stable and can be reversibly folded and unfolded for capture and release of the target protein, allowing indefinite reuse of aptamer-modified surfaces. A limitation of aptamers is the dependence on combinatorial routes to their discovery that are often tedious, lengthy, laborious and not always successful. We recently introduced a different approach to aptamer discovery based on genomic DNA sequences from gene promoter regions that form well-defined secondary structures. We hypothesize that these ligands will exhibit high affinity towards proteins that bind to them in vivo and may play a role in regulation or misregulation of the corresponding gene. Since this path to aptamer discovery is based on biologically significant protein-DNA interactions, it has potential to uncover mechanisms of diseases related to gene regulation while at the same time yielding an aptamer to the protein(s) involved in the regulatory mechanism. This talk will describe the investigation of G-quadruplex-forming DNA sequences from promoter regions of human genes for discovery of target proteins that bind to these structures in vitro and possible in vivo as potential aptamers for the target proteins.

3:30 pm Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Claudimir do Lago, Universidade de Sao Paulo, claudemi@iq.usp.br

Co-Author: Kelliton José Mendonça Francisco, Universidade de Sao Paulo

Capacitively Coupled Contactless Conductivity Detection: An Open Project

Since the introduction of the axial form of a capacitively coupled contactless conductivity detector (C4D) in 1998, different implementations were suggested. Although the conceptual design has been kept almost the same, a lot of studies about shape and size of the electrodes as well as amplitude and frequency of the oscillator has been carried out. Because of this continuous improvement, the detector evolved to commercial versions, which spread the use of C4D. However, the number of options to the users is still modest when compared to optical detectors. Thus, we decided to create a new version of a C4D that intend to be compact, simple, and robust enough to be made by the researchers and, at the same time, could inspire new commercial versions with the same features. The volume of the complete circuit is only 6.5 cm3 including oscillator, transimpedance amplifier, rectifier, filter and analog-to-digital converter (ADC). Another important feature lies on this converter: a 21-bit effective ADC dismisses a baseline compensation step, because the compensation may be obtained by software. This approach did not only diminish the number of moving parts, but rendered a complete new feature. The baseline shift, when the capillary is alternatively filled with air and water, may be used to determine the inner diameter of this capillary. The limits of detection under realistic conditions of electrophoretic separation were bellow 1O-6 mol/L for alkaline and earth alkaline metals, which indicates that this compact version is comparable to other high-quality C4Ds. The entire project is available as open project, which means that one can not only construct this C4D, but also contribute to its improvement based on his/her own experience. Similarly to what is occurring to other areas, the fact that it is an open project does not preclude that an industrial implementation based on it is marketed.

Detection and Separation—Track 1

4:00 pm Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Yolanda Fintschenko, Labsmith, dr_yf@yahoo.com

Co-Authors: Eric Cummings, Kirsten Pace, LabSmith, Inc.; Holger Becker, Claudia Gaertner, microfluidic-ChipShop

Automated Modular Interface for Microfluidic Separations and Fluorescent Detection

Microfluidics can be an attractive tool to automating separations for many applications in in vitro and in vivo analyses. Connecting and controlling fluid and electric fields to microfluidic chips in the development stage of separation-based microfluidic devices can be a challenge. In addition, it is often desired to view the separation chips on a microscope both for real-time visualization of the effect of the design on the experimental results. The challenge of making simple, reliable, and reusable connections is often overwhelming to users new to microfluidics. Discussed here is a design that couples the convenience of off-the-shelf, relatively inexpensive polymer chips with an interface that connects to leak-free, zero-dead-volume CapTite connectors, pumps, electrodes, and heaters for full control of the world-to-chip interface. This manifold mounts on a unique inverted fluorescence microscope, the SVM340, used to image the chip in this interface. Direct connections from the instrumentation controlling the experiment to the chip can be made with this breadboardable system. Sample preparation fluid circuits using valves, crosses, tees, and other sample preparation and handling components also connect directly to the chip itself. Results demonstrate real time visualization of on-chip phenomena in this automated, breadboardable system.

4:30 pm Monday, January 31

Location: Madera, Renaissance Palm Springs Hotel

Jean-Marc Busnel, Beckman Coulter Inc., jmbusnel@beckman.com

Significant Advances in Peptide/Protein Analysis by Mass Spectrometry

In this presentation we will discuss our most recent work on the advancements of a novel sheathless interface that combines the resolving power and low flow characteristics of capillary electrophoresis (CE) with electrospray ionization mass spectrometry (ESI-MS). Considering the miniaturized format of CE and the intrinsic properties of the ESI process, the sheathless design can be regarded as the most natural way to perform the coupling. While CE separations are usually performed at very low flow rates the ESI process is known to provide enhanced performance with increased sensitivity and decreased ion suppression at these low flow rates. We have have found that the interface is capable of generating stable spray with flow rates ranging from below 10 nL/min to >340 nL/min, enabling its use in either the mass or concentration-sensitive regions of the ESI process. We further assessed the potential of this platform for the analysis of peptide samples of increasing complexity. Particular attention has been dedicated to parameters such as sensitivity and peak capacity. To increase the mass loading abilities of the platform, various online preconcentration methodologies have been integrated, providing concentration sensitivity down to the low picomolar level while achieving peak capacities above 300. We also explore the power of the mass-sensitive region of the ESI process to improve the analysis of phosphorylated and/or heavily sialylated or glycosylated peptides. Examples of using this approach for intact proteins as well as protein complex analysis will also be discussed. As compared to conventional approaches, this sheathless CE-MS platform represents a real breakthrough in terms of bioanalytical performance.

Detection and Separation—Track 1

10:30 am Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Frank Gomez, California State University, Los Angeles, fgomez2@calstatela.edu

Co-Authors: Mark Goldberg, Roger Lo, Maria Ortega, Amy Wat

Development of Microfluidic Chips for Heterogeneous Receptor-Ligand Interaction Studies

The understanding of interactions between receptors and ligands in biological systems holds important information on the initiation, progression, and harmful effects of human diseases, such as AIDS/HIV, Alzheimer's, Parkinson's and cancer. To develop new drugs and screen potential candidates, there is a great demand for analytical technologies that can rapidly and reliably investigate biological interactions by measuring affinity parameters. A simple microfluidic-based technique to quantitate the binding affinity between the glycopeptide antibiotics teicoplanin from Actinoplanes teicomyceticus and vancomycin from Streptomyces orientalis to 5-carboxyfluorescein-D-Ala-D-Ala-D-Ala (FAM-(D-Ala)3 is described. In this work, 3-aminopropyltriethoxysilane (APTES) is used to modify the surfaces of a series of microchannels and each channel is subsequently exposed to a solution of antibiotic for a few minutes. The antibiotic is retained after washing through electrostatic interactions and the series of channels are subsequently exposed to an increasing concentration of FAM-(D-Ala)3 followed by washing to exclude any non-specific binding. Extent of fluorescence is quantified using a microscope fitted with a CCD camera. The binding constants for the interaction of teicoplanin and vancomycin with the fluorescent peptide were in good agreement with previous data. The ease of quantifying extent of interaction in this microchip technique may prove powerful for exploration of a myriad of receptor-ligand pairs. A competitive assay and further work utilizing this concept are also detailed.

11:00 am Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Norberto Guzman, Princeton Biochemicals, Inc., norbertog67@gmail.com

A Modular, Multi-Task Immunoaffinity Device Connected to Capillary Electrophoresis for the Enrichment, Separation and Identification of Protein Biomarkers

In recent years, increased emphasis has been placed on predictive biomarkers to forecast the origins or future course of toxic events or diseases caused by inflammation. Many organ-specific diseases are preceded by a long preclinical phase that is left undetected by the lack of specific and sensitive assays capable of assessing the early and advanced molecular changes that may cause dysfunction of one or more organs. Several observational studies have shown that patients may carry unique biomarkers for many years before they manifest clinical symptoms. Detecting a panel of "inflammatory" biomarkers in biological fluids, tissues and cells can have important predictive and confirmatory value depending on the disease and test method under consideration. When respect to pharmaceutical safety and efficacy, the incorporation of biomarkers into the drug development process will improve understanding of how new therapies work and allow for more accurate identification of patients who will benefit most from innovative treatments. A number of current technologies and methodologies are available for the characterization and quantification of protein/peptide biomarkers (e.g., ELISA, immunoblotting, HPLC, MS, etc.). Unfortunately, in most cases these protocols rely on separate, large-scale instruments based on completely different principles. They also employ high-cost, time-consuming methods that may generate false positive or false negative data. This presentation will describe an effective approach to enrich, separate, quantify and identify proteins of low- and medium-abundance in biological fluids, tissues and cells. It will also feature the description of a modular device that can be attached to commercial capillary electrophoresis instruments. The device is specifically based on the technology of immunoaffinity capillary electrophoresis (IACE). IACE is a unique two-dimensional platform that is emerging as the most promising method for biomarker research because it can enrich, quantify and identify proteins and peptides found at a wide range of concentrations in biological fluids, cells and tissues. Unlike nucleic acids, which use polymerase chain reaction, there is no enzymatic technique for the amplification of low abundance proteins. Equivalent amplification can be provided by a proprietary analyte concentrator device containing selective ligands immobilized to a solid support. These affinity ligands will capture and purify one or more biomarkers of interest. This unique two-dimensional, multi-task concept will provide a rapid, convenient, cost-effective, and accurate (free of alse positive and false negative results) routine assay for diagnosing physiological and pathophysiological conditions. At the technical level, we anticipate that the IACE platform will accommodate comprehensive, high-speed, high-throughput, cost effective analysis on a proteome scale. This comprehensive protocol will generate a new level of understanding for many diseases. A description of a modular, interchangeable unit containing an analyte concentrator device adjacent to miniaturized valves and inlet/outlet passages connected to a commercial separation instrument (e.g., CE and LC) will be presented. Applications will be discussed.

Detection and Separation—Track 1

11:30 am Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Z. Hugh Fan, University of Florida, hfan@ufl.edu

Co-Authors: Champak Das, Ke Liu, Pan Gu

Two-Dimensional Protein Separation in a Device With Microvalve Arrays

Among the approaches being developed for proteomics, two-dimensional (2D) gel electrophoresis is an essential tool. It consists of isoelectric focusing (IEF) as the first dimension and polyacrylamide gel electrophoresis (PAGE) as the second dimension. One major advantage of the conventional 2D gel electrophoresis is its enormous separation resolution, whereas its key limitations include poor reproducibility and time-consuming procedure. To address the limitations, we have developed a microfluidics-enabled 2D separation device. The device was made in cyclic olefin copolymers, consisting of one IEF channel and 29 parallel PAGE channels that were orthogonal to the IEF channel. An array of microfluidic valves was created for introducing different separation media, without cross-contamination, in both dimensions; it also allowed transfer of proteins from the first to the second dimension. Fabrication of microvalves was achieved by either photo-initiated, in situ gel polymerization or chemically-assisted multilayer bonding. In the presence of microvalves, IEF separation of proteins was first implemented in the device. The IEF analysis time was in minutes, much shorter than hours typically needed in the traditional slab gel format. Further, we demonstrated 2D separation by separating fluorescently-labeled proteins with either similar pI values or close molecular weight in the device. Detection in the device was carried out using a laser-induced fluorescence, multiple-channel imaging system. The total separation time was less than 10 minutes for IEF and PAGE, an improvement of 2 orders of magnitude over the conventional 2D slab gel electrophoresis

12:00 pm Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Stephanie Archer-Hartmann, West Virginia University, stephanie.archer@gmail.com

Co-Author: Lisa Holland, West Virginia University

On-Line Biomolecule Characterization Using Phospholipid-Based "Nanodisk" Additives in Capillary Electrophoresis

The ability to capture, manipulate, and assay biomolecules on a micro scale is of great interest in fields such as drug discovery, pharmaceutical research, and proteomic studies. A number of groups utilize self-assembled phospholipid "nanodisk" bicelles for capillary electrophoresis due to their inherent biocompatability and ease of use as coatings and thermally responsive additives. We have recently demonstrated the use of phospholipid additives for the the improved separations of glycans cleaved from glycoprotein sources, as well as for the incorporation of lectins and enzymes. The addition of a protein of interest to the phospholipid allows for quick and simple immobilization within capillary without the need for covalent modification providing a method for online analysis and interaction assays. This presentation describes the use of lectins and enzymes to probe complex solutions of glycans to quickly determine structural features and affinity for biomolecules of interest.

Detection and Separation—Track 1

3:00 pm Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Peter Willis, Caltech/Jet Propulsion Laboratory, willis@jpl.nasa.gov

Co-Authors: Maria F. Mora, Anita M. Fisher, H. Frank Greer, Jet Propulsion Laboratory; Thomas Chiesl, University of California, Berkeley; Hong Jiao, Los Gatos Research

Development of Automated Micro-Total-Analysis Systems for Planetary Exploration

Ongoing efforts in the design, fabrication, and implementation of lab-on-a-chip systems intended for the payloads of future robotic explorers to destinations such as Mars or Titan are summarizes. The purpose of these chemical analyses is to determine the extent of past or present life on these worlds, or the extent of prebiotic chemical evolution that may have taken place. Samples are to be provided by the robotic platform (i.e. rover or boat) carrying the instrument, either via drilling into regolith and treatment with liquid water (Mars and Titan) or possibly direct sipping from hydrocarbon lakes (Titan). Organic species are mixed with stored buffer solutions, and then separated from one another on-chip electrophoretically (via capillary electrophoresis or capillary electrochromatography) and detected via laser-induced fluorescence, or alternatively via nanoelectrospray ionization and mass spectrometric analysis. Automated capillary electrophoresis analysis of this type for Mars exploration was brought to an intermediate level of automation by Skelley using fluorescamine dye for labeling of amino acids. Current work focuses on the use of Pacific Blue dye, but now in an automated fashion, in which the derivitization process is performed on chip using reagents stored in dedicated reaction reservoirs. Capillary electrochromatography (CEC) analysis is also performed for the determination of polycyclic aromatic composition, utilizing porous polymer monoliths as the separation medium. We demonstrate all required liquid sampling handling operations (mixing, derivitization, dilution, etc.) required for these automated analyses using circuits comprised of monolithic diaphragm valves.

3:30 pm Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Carlos Garcia, University of Texas at San Antonio, carlos.garcia@utsa.edu

Co-Authors: Claudimir L. do Lago, Carlos A. Neves, Eric Tavares da Costa, Guilherme Minoru Otta, Marcelo Fagundes Barros, Thiago Garcia, University of Sao Paulo; Arturo A. Ayon, The University of Texas, San Antonio

Remote Chemical Analysis of Volatile Compounds Using Microchip —Capillary Electrophoresis and Electrochemical Detection

Due to increasing demands at the industrial and military levels, there has been a recent trend towards the development of miniaturized or portable sensors. Among others, sensors based on optical responses, surface plasmon resonance, metal oxide semiconductors, fluorescence, mass spectrometry, or surface acoustic waves, have been recently reported. Although most of these sensors are able to detect minute amounts of analytes, one common shortcoming is their versatility. In this regard, separation-based sensors have the potential to quantify a larger number of important analytes in the same sample without modifications of the hardware. Among those, capillary electrophoresis – based sensors have caught much attention recently. Capillary electrophoresis (CE) is very attractive for miniaturized devices because it offers high separation efficiency, low cost, fast analysis, and minimal waste generation. An additional advantage of CE is the simplicity of the required instrumentation, which requires no moving parts and therefore can be easily miniaturized. Consequently, CE and microchip-CE (?chip-CE) have the potential to combine most of the advantages of CE –sample handling capabilities, custom design, and portability– and also offer similar analytical performance as standard bench-top instrumentation. Additional advantages of CE-based sensors include the possibility of using a wide variety of well-established separation techniques, performing the analysis with low-consumption of power, and controlling the entire system electronically. These advances are particularly relevant in the analysis of environmental, forensic, and military samples that have to be physically acquired, transported, and then processed in the laboratory. Exposure of personnel to untested environments, sample degradation, contamination, and labor-intensive analytical protocols obviate the necessity for testing systems capable to perform on-site analysis and transmit the results autonomously. These problems, associated with current systems, suggest that the next evolutionary stage of instrumentation for microchip-capillary electrophoresis (?chip-CE) should compete with commercial systems in sensitivity, versatility, and user interface, while merging low cost, low-power consumption, small size, and wireless data acquisition. Aiming to address the aforementioned goals, this presentation will address the most recent results related to the development of an integrated device to perform remotely operated chemical analysis performed by ?chip-CE-ECD. Since these devices have the possibility of performing an entire analysis (sample acquisition, preconcentration, injection, separation of the components, detection, data processing, and data transmission) are referred to as lab-on-a-robot (LOAR). The presentation will discuss the performance of the first prototype (LOAR1), which was conceived as a proof-of-concept and demonstrated the possibility of performing an entire chemical analysis from a distance of up to 1 mile. Then, the advantages of the second prototype (LOAR2), which was built featuring a tougher design, autonomy, and integration with cartography will be presented. Finally, the progress achieved in the design of LOAR3 will be briefly discussed. References: - Electrophoresis 31 (2010), 2469–2486. - Electrophoresis 29 (2008) 4914 – 4921.

Detection and Separation—Track 1

4:00 pm Tuesday, February 1

Location: Madera, Renaissance Palm Springs Hotel

Suzanne DeMarco, Pfizer Inc., suzanne.c.demarco@pfizer.com

Co-Authors: Corey Johnston, Allison Kowalski, Abigail Lee, Claudia Posadas, Tammy Thurman, Xing Wang, Pfizer Inc.

Thinking Out of the Box: Automated Magnetic Particle Purification Method for Purifying Picogram Quantities of DNA From Biologics

The analysis and clearance of residual host cell DNA is one major focus of therapeutic biologics production. Host cell DNA is typically very large, coiled, with complex random structures, but may also exist as smaller segments post-extraction when exposed to shearing and DNAse enzymes in the environment (500 base pairs to >1x106 base pairs). The presence of residual host cell DNA could pose potential unwanted side effects and may indicate a lack of process control. Therefore, it is imperative to have a sensitive and robust method to monitor the removal of residual host cell DNA and to demonstrate log DNA clearance throughout the process. The current method for residual DNA analysis includes an extremely labor intensive NaI/Isopropanol extraction method followed by detection using QPCR. The purpose of this research was to develop a higher throughput alternative for quantitatively purifying pg quantities of DNA with acceptable precision. A magnetic particle DNA purification method that combines the MagMax™-96 Viral RNA Isolation Kit with Promega MagneSil® PMPs was developed for the evaluation of residual DNA from in-process development samples. Magnetic particle DNA purification in a 96-well format can be performed manually or robotically using the KingFisher Flex magnetic particle processor in under 40 minutes. The in-process method was subsequently optimized and validated as a release assay for drug substance. Magnetic particle DNA purification provides comparable sensitivity, precision, working range, repeat rates and reduced reagent price when compared to Wako extraction.

4:30 pm Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Madera, Renaissance Palm Springs Hotel

Scott Fulton, BioSystem Development, LLC, scott.fulton@biosystemdevelopment.com

A New High-Throughput Micro-Chromatography Platform for Quantitative Analytical Protein Sample Prep

Biopharmaceutical development has created an increasing demand for high precision, high sensitivity and high throughput protein analysis for process development, process control and analysis of clinical trial and biomarker trial samples. Several techniques are widely used for sensitive and precise specific protein quantitation, including affinity HPLC and immunoassays, but these methods suffer from low throughput and complexity of automation, respectively. A wide range of other analytical instrumentation (such as LC-MS and NMR) is used for structural analyses of proteins to examine characteristics such as post-translational modification, but the protein of interest must first be highly purified intact from complex sample matrices (such as culture supernatant or plasma) and often requires other complex preparation methods (such as enzymatic treatment or labeling). Overall workflows can involve highly complex combinations of purification and other sample processing methods. Although throughput requirements can range into hundreds or even thousands of samples at a time, many assays are only run occasionally, and the traditional high throughput screening paradigm has proven to be of limited utility. Liquid chromatography is a very well-developed general technique which can address most of the sample preparation challenges in protein analysis. Even enzymatic digestion can be carried out in a column format very efficiently. However, it has been a major challenge to develop a microliter-scale, multiplexed chromatography system which is flexible, quantitative and high throughput. This talk will describe a new high throughput micro-chromatography platform based on disposable cartridge devices with 5 µL packed bed contained between two insert-molded bed support filters. The packed bed can contain any media in the 15 – 100 µm particle size range. The cartridges are operated on a modified 96-channel, microplate-compatible liquid handler which incorporates ultra-low dead volume syringes that connect to the cartridges through a special probe. The liquid handler provides highly precise positive-displacement flow control through the cartridges in either direction to well under 1 µL/min, and can dispense liquids through the cartridges at 20 bar pressure. Applications to be discussed include the use of affinity chromatography media to quantitatively capture and purify target proteins (such as MAbs) for either direct quantitative analysis or further structural analysis by other methods. UV absorbance can be used for quantitation of 10 – 100 µL samples from mg/mL down to the low µg/mL range. Through the addition of fluorescently-labeled reagents, the same system can quantitate 10 µL samples to the low ng/mL range. Other chromatographic methods demonstrated with the system include ion exchange, reversed-phase, hydrophobic and hydrophilic interaction. Enzymatic digestion methods have also been developed on the system. Using large diameter, non-porous beads, microplate-ELISA reagents can be used to perform 30 minute immunoassays. Examples including host cell protein ELISAs will be shown.

Detection and Separation—Track 1

9:00 am Wednesday, February 2

Location: Madera, Renaissance Palm Springs Hotel

James P. Landers, University of Virginia, landers@virginia.edu

Exploiting the Microfluidic "Pinwheel Effect" for Label-Free DNA Quantitation, Cell Counting and Bacterial Detection

Many applications involving genetic analysis require quantitation of DNA mass, particularly highly multiplexed PCR amplifications that have template mass requirements that are narrow. We describe the development of a 'pinwheel assay as a means of quantitating the mass of DNA in raw samples. The effect is rooted in the DNA-mediated aggregation of magnetic-cored silica particles exposed to a rotating magnetic field (RMF). With a magnetic field of the appropriate strength, 3-D configuration, and rotational speed, the pinwheels formed define the presence of DNA in the presence of other cellular components (incl. protein) that exceed the mass of DNA by orders of magnitude. In addition, the mass of DNA present in the sample correlates with the extent of pinwheel formation, which can be determined with simple still capture images of the pinwheel in concert with the appropriate image processing software. DNA detection sensitivity down to a mass of tens of picograms is shown to bw possible, with the bound polynucleic acids that cause the effect representing DNA purified from the sample. This represents the first DNA quantitation technique that does not require that the DNA be purified prior to quantitation, with samples as complex as whole blood amenable to direct analysis. In addition, we show evidence for the pinwheel effect as a 'cell counter' and an ultrasensitive bacterial detection system.

9:30 am Wednesday, February 2

Location: Madera, Renaissance Palm Springs Hotel

Jeff Chapman, Beckman Coulter Inc., jdchapman@beckman.com

Co-Authors: Yong Wu, Handy Yowanto, Beckman Coulter Inc.

Multiplexing Respiratory Virus Surveillance and Screening

As viruses co-circulate they have the potential to reassort, creating a need for highly specific, yet adaptable methods that can screen for novel influenza viruses against other known respiratory viruses. Although RT-PCR has been a valuable tool for the identification of viral pathogens, the complexity in optimizing PCR conditions for a multiplex of primers across several targets has made it difficult add new viral reassortants to existing viral screens. eXpress Profiling (XP) PCR allows for the simultaneous and uniform amplification of multiple genetic targets from a single set of universal primers, providing a format where we can rapidly add new targets to a given panel. In this paper we present our research in pairing XP PCR with capillary gel electrophoresis (CGE) to develop a multiplexed method for respiratory virus screening and surveillance. Specifically we have designed a 24-target multiplex panel to subtype the Influenza A virus for the identification of the novel 2009 H1N1, including mutations for therapeutic resistance, with other respiratory viruses of interest to public health agencies. By utilizing markers for a conserved region of the H1 gene, as well as targeting the seasonal and 2009 H1N1 sequences, we propose a strategy for potentially flagging new H1N1 viral mutations. We will present an update on our results from a number of blind clinical trials, and assess the concordance of this method with RT-PCR based upon the CDC Protocols. For Investigational Use Only.

Detection and Separation—Track 1

10:00 am Wednesday, February 2

Location: Madera, Renaissance Palm Springs Hotel

Vincent Gau, Genefluidics, vgau@genefluidics.com

Co-Authors: Quyen Ho Pavan, Narsai Casey, Chiou May Chiu

Rapid Detection Method Utilizing Raw Specimen Without Sample Purification or Target Amplification

The standard optical detection methods used to measure analyte concentration are currently subject to stringent sample purity requirements in order to yield accurate results. These sample purification requirements add steps prior to the detection process. In most cases considerable time is taken to complete these steps, in addition, investment in costly equipment and their operators are necessary as well. In the case of detecting genetic material, an amplification step such as Polymerase Chain Reaction (PCR) is needed to amplify the target molecule prior to detection. This too adds time and infrastructure to the detection process. We have demonstrated that electrochemical detection can have the specificity and reproducibility necessary to detect a target quantitatively at clinically significant concentrations in a biological liquid matrix. This eliminates the need for purification of raw specimens, as they can be used directly on an electrochemical sensor. In addition, the detection method is sensitive enough to eliminate the need to perform PCR amplification in most genetic assays. Our multiplexed genetic assays and immunoassays utilize a standard sandwich assay binding mechanism to detect the target molecule. Using this simple assay, the entire process from raw sample to result can be completed in less than 60 minutes. The electrochemical detection method and the electrochemical sensor have been tested using various clinical specimens and have shown the ability to handle some of the most difficult sample media without the need for purification and target amplification. The electrochemical sensor has been used to detect Troponin I spiked in both plasma and whole blood. Troponin spiked plasma yielded a limit of detection of 18.9 pg/mL with N=96 for each concentration (of 4 concentrations tested). Troponin spiked whole blood yielded a limit of detection of 23.8 pg/mL with N = 24 for each concentration (of 4 concentrations tested). Each study was conducted over 3 days by 3 operators. Clinically significant concentrations of Troponin I are >250 pg/mL. For most genetic assays, the need for sample purification is only half the procedure. A target molecule also needs to be amplified many times before standard detection methods can be applied. Our electrochemical sensor has shown a limit of detection for E. coli directly from Mueller Hinton culture media of 1x10^5 CFU/mL without extracting or amplifying any genetic material from the sample. This is a clinically significant detection limit for E. coli concentration for infections such as urinary tract infection. Due to the simplicity of the sample preparation requirements of the electrochemical assays, it is a perfect candidate for adapting to an automated process. With fewer steps than assays that use optical detection, electrochemical assays can be automated from start to finish.

10:30 am Wednesday, February 2

Location: Madera, Renaissance Palm Springs Hotel

Rosalee Rasmussen Hellberg, Oregon State University

Co-Authors: Michael T. Morrissey, Oregon State University; Robert H. Hanner, University of Guelph

Development of a Multiplex PCR Assay for the Identification of Commercial Salmon and Trout Species (Oncorhynchus and Salmo) in North America

Although commercial salmon and trout species are similar in appearance, they command markedly different prices, making them susceptible to market substitution. The purpose of this study was to develop a species-specific multiplex polymerase chain reaction (PCR) assay for the detection of salmon and trout species substitution on the commercial market. Species-specific primers and TaqMan minor grove binder (MGB) probes were developed using a comprehensive collection of cytochrome c oxidase subunit I (COI) DNA barcode sequences. Primers and probes were optimized in multiplex assays and tested for specificity against 94-112 reference samples representing 19-25 species. Sensitivity and linearity tests were carried out for single-species mixtures using 10-fold dilutions of the target DNA and for DNA admixtures containing the target species at levels of 0.1, 1.0, and 10% mixed with DNA from a secondary species. Strong signals were detected for the target DNA in specificity tests with both real-time and conventional PCR systems. Nonspecific amplification in both systems was minimal; however, false positives were detected at low levels (1.2-8.3%) in conventional PCR. Sensitivity testing showed similar detection levels for the target species in admixtures compared to single-species mixtures. Conventional PCR detection limits were determined to be 0.25 to 2.5 ng DNA (1.0 to 10%) and limits for real-time PCR detection were 0.05 to 5.0 ng DNA (0.1 to 10%). A small-scale test with food samples showed promising results, with species identification possible in a variety of products, including heavily processed food items. Overall, this study presents a rapid, specific and sensitive method for salmon species identification that can be applied to mixed-species and heavily processed samples in either a conventional or real-time format. Furthermore, this method could readily be adapted for high-throughput operations through the use of 'ready-to-use' 96-well reaction plates.

Micro- and Nanotechnologies—Track 2

10:30 am Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

Samuel A. Wickline, Washington University, wicklines@aol.com

Co-Authors: Hua Pan, Paul H. Schlesinger, Washington University

Lipid Membrane Editing With Peptide Cargo Linkers In Cells and Synthetic Nanostructures: New Approaches to Cancer Therapy

The optimal design of nanotechnologies for diagnosis and treatment of complex diseases will require the incorporation of multiplexed targeting ligands and drug combinations to improve cell specific delivery and therapeutic efficacy. To date, typical cargos such as targeting ligands, imaging agents, or drugs are incorporated into the nanocarriers during the formulation process, which requires a dedicated particle design for each individual application. However, the potential of targeted nanocarriers to achieve improved selectivity and combinatorial therapy against complex diseases may not be fully exploited if flexible multiply targeted and/or drug loadings are not developed and deployed. A strategy designed to accommodate swapping and/or combining multiple cargos in generic base nanocarriers would enable customization of the nanocarriers for application to specific pathologies and disease stages. To fully exploit existing lipidic nanoparticle carriers, we now report the design, characterization, and evaluation of a peptide linker that can quickly load cargoes into lipidic nanoparticles after they are formulated and stored. The source for this linker derives from the bee venom peptide component, melittin, which inserts into lipid membranes and causes membrane disruption. We have demonstrated that this modified melittin-derived linker can insert into both perfluorocarbon (PFC) nanoparticles and liposomes without affecting the integrity of these nanocarriers. By using this linker strategy, VCAM-1-targeted PFC nanoparticles were generated and their specific molecular targeting was quantified by magnetic resonance spectroscopy; and the VCAM-1-targeted Doxil™ presents enhanced cellular drug delivery. Moreover, by loading cargo into avb3-integrin targeted PFC nanoparticles, we achieved in vivo cargo delivery, using this linker approach. This approach preserves the integrity of both the manufacturing process and the product for the base nanocarrier, while enabling the addition of myriad cargos after formulation. Furthermore, we showed that this strategy could also load cargos onto living cells for in vivo tracking or cell-based Trojan horse therapeutic applications. Next we sought to employ this strategy to elicit metered control the NF-kB signaling axis in tumors with the use of an inhibitory peptide/linker fusion construct that could be delivered into cells. The inhibitor peptide candidate for NF-kB inhibition is the Nemo Binding Domain (NBD) inhibitory peptide that has been shown by others to regulate the signaling events that release NF-kB from the cytoplasmic compartment to translocate to the nucleus and stimulate inflammatory responses. We generated NBD peptide-Linker fusions (NBD-Linker) and incorporated them into PFC nanoparticles. As an example of bioactivity of the NBD nanoparticles against HTLV-Tax leukemia/lymphoma cancer cells that exhibit constitutively activated NF-kB signaling, we demonstrated that NBD nanoparticles down-regulated NF-kB nuclear translocation, and inhibited expression of the NF-kB dependent gene, Inter-Cellular Adhesion Molecule 1(ICAM-1). This post-formulation cargo loading strategy enables the use of a generic synthetic or biologic lipidic nanostructure for drug conjugation that permits flexible specification of types and doses of peptides and/or other materials as diagnostic or therapeutic agents for metered incorporation and cellular delivery.

11:00 am Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

David Giljohann, AuraSense LLC, dgiljohann@aurasense.com

Nano-Flares for Detecting and Quantifying mRNA and Small Molecules in Living Cells by Fluorescence

Detecting a small number of diseased cells in a large population of healthy cells is one of the main challenges in research of cancer and other diseases. Moreover, assessment of the amount of genetic material or proteins is often impossible inside living cells. AuraSense has developed a new technology for intracellular assays termed “Nano-Flares.” These non-toxic nanoparticle-based gene probes are designed to enter living cells and are equipped with fluorescent “flares” to provide a robust and discernable signal when they encounter specific molecular targets (e.g., mRNA or small molecules). Nano-Flares provide exceptional benefits and opportunities for studying and detecting disease in living systems.

Micro- and Nanotechnologies—Track 2

11:30 am Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

SoonGweon Hong, University of California, Berkeley, gweon1@berkeley.edu

Co-Authors: Mi Yeon Lee, Andrew O. Jackson, Luke P. Lee, University of California at Berkeley

Plasmonic Eu-Virus for Targeting, Delivery, and Molecular Imaging

Multifunctional nanoprobes have been highlighted due to their potential in revolutionizing understanding and treatment of diseases. For nanoprobes to be truly effective, they must possess three functionalities: targeting, delivery and sensing. While the targeting functionality allows nanoprobes to reach specific cells or even subcellular regions like nucleus, the delivery function adding to nanoprobe allow on-demand drug or gene releasing in a required cellular region. A further localization inside cells can be accomplished in the case of the sensing functionality of nanoprobe. When nanoprobes are combined with selective optical antenna, it can provide enormous potential for molecular level imaging in living cells through electron absorption and vibration spectroscopic imaging. However, beside a difficulty of combining the other two functions, nanoscale fabrication, single-molecule sensitivity, and practical applications need to be resolved to realize optimal formation of optical antenna on nanoprobe. When investigating nature's architectures, we can find a best answer toward these technical limitations. Highly organized viral structures are the one of this nature's present. Even the simplest viruses have evolved the ability to enter cells, either directly or via various vectors, and to co-opt the host cellular processes needed to replicate their genomes and assemble viable progeny virions. During the last century, these processes have been intensively studied to understand virus structure and replication and to control viral diseases of importance to human health and agriculture. More recently nano/biotechnology approaches have attempted to engineer viruses for approaching diagnostic and therapeutic applications. These new engineering approaches are designed to eliminate the viral disease-causing activities, while maintaining the inherent structure and cellular targeting capabilities for applications design. Here, for the multifunctional nanoprobe we demonstrate another promising paradigm of virus engineering by adding nanospectroscopic antenna on the highly ordered viral capsids. Used representative plant viruses (BdMV, BMV, CPMV, TBSV) are a simple but perfectly regular icosahedral shape consisting of a few repeating proteins, while their detail three dimensional structures increase plasmonic phenomena through thin metal layer imprinted on capsids (so it is called plasmonic virus). An electromagnetic simulation study suggests a plasmonic virus more enhance localized and focused optical field near the particle than similar-sized smooth spheres, guaranteeing localized optical field based sensor applications. Through two experiments for surface-enhanced Raman spectroscopy (SERS) and plasmon resonance energy transfer (PRET), the viral particles were shown to increase the sensitivity by a factor of 101 to ~106, compared to the smooth spheres. Therefore, we believe this study increases the potential for engineering viruses as resources for powerful research and medical applications involving optical antenna molecular spectroscopy.

12:00 pm Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

Dean Ho, Northwestern University, d-ho@northwestern.edu

Nanodiamond-Based Therapeutic Delivery Platforms for Cancer Treatment

Nanodiamond (ND) surface properties mediate clinically-relevant improvements to drug delivery which can be realized through enhanced cancer treatment efficiency. Additional characteristics that enable their application as versatile drug delivery vehicles include their functionalization with a broad array of therapeutics which includes small molecules, proteins, antibodies, and RNA/DNA for applications in cancer treatment, cardiovascular medicine, wound healing, and beyond. In addition, NDs possess uniform dimensions (~4nm in diameter per particle) and material stability that are coupled with observed biocompatibility in vitro and in vivo. Furthermore, NDs can be batch purified and functionalized for scalable and high yield processing. Among other functional groups, NDs also possess an abundance of surface-bound carboxyl groups which are conducive towards facile, application-dependent molecular linking/conjugation onto the diamond surface. Furthermore, NDs can be functionalized with additional chemical species to enable direct drug conjugation. Our previous studies have confirmed robust drug binding to NDs through transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR) coupled with in vitro tracking of cellular internalization and quantitative demonstration of bio-amenable cell response through quantitative real time polymerase chain reaction (RT-PCR) assays of inflammatory and apoptosis-regulating gene expression programs. Furthermore ND-mediated drug release against several cell lines has also been observed. Towards the broadening of ND applicability in clinically-significant treatment scenarios, recent work pertaining to simultaneous high-efficacy/high biocompatibility gene delivery, ND-based microfilm device formation for localized chemotherapy, pH-dependent therapeutic protein release, and pre-clinical studies will be discussed.

Micro- and Nanotechnologies—Track 2

3:00 pm Monday, January 31

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Thomas Laurell, Lund Institute of Technology, Lund University, thomas.laurell@elmat.lth.se

Acoustophoretic Cell Handling in Microfluidic Systems—Towards Clinical Applications

Acoustophoresis and controlled acoustic standing wave forces in microfludic systems have in recent years become a viable strategy for advanced cell manipulation, including cell separation, buffer switching, valving, affinity bead extraction, cell interaction studies etc. Applications in the bioanalytical field and in clinical diagnostics are being explored at an increasing rate. Early work on clinical applications in our group demonstrated lipid microemboli depletion from recovered blood during surgery prior to autologous retransfusion. In the past years we have reported a range of microfluidic unit operations based on acoustophoresis. Our recent developments will be overviewed, where e.g. continuous flow buffer exchange of a cell/particle population has emerged to be a simple but powerful modality and has direct implications in clinical applications, elimination time-consuming manual centrifugation steps. Cells undergoing acoustophoresis experience a low mechanical stress, which opens the route to a wide range of clinical applications. Recent work describes whole blood plasmapheresis based on acoustophoresis, which subsequently was coupled to protein micro arrays for PSA-diagnostics. Acoustophoresis has also been developed to purify/extract components from complex matrices using microbeads with an affinity to a targeted species, i.e. Affinity Acoustophoresis. Affinity bead extraction of specific bacteriophages from a phage library will be presented, demonstrating an optional and improved purification modality as compared to magnetic bead based phage display selection. We have also developed Free Flow Acoustophoresis, FFA, utilizing the fact that cells display individual migration velocities in the acoustic field. This allows for cell specific differentiation and examples of separations of erythrocytes, leukocytes and thrombocytes will be given. Preprocessing of cell samples in clinical diagnostics and therapeutics is a key target area of our group. The simplicity of implementing acoustophoresis, and its ability to be tethered to downstream analytics has recently been addressed by our team. We have for the first time reported the linkage of acoustophoretic buffer switching of cells as a modality for down stream sample processing in FACS analysis of labelled cells, still in their labelling buffer. The buffers switching eliminates the centrifugation step prior to FACS analysis. Acoustophoresis is an evident development route for a set of modalities in preprocessing cell samples prior to FACS analysis and hence eliminating the influence of the human factor in the centrifugation step, and to speed up the process. Recent developments of acoustophoresis in clinical applications in our group also covers purification of circulating tumor cells from blood and removal of trombocytes from aperesis products and production of WBC-free trombocyte fractions. Acoustic standing wave resonators can also be designed in microfluidic systems to retain/trap and enrich cellular, bacterial or other bioparticle species prior to down stream diagnostic readout. This has been demonstrated in the enrichment and purification of sperm cells in forensic samples.

3:30 pm Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

Joan Bienvenue, Lockheed Martin, joan.bienvenue@lmco.com

Co-Authors: Jessica V. Norris, Brian E. Root, Orion N. Scott, Michael Egan, MicroLab Diagnostics; David Saul, ZyGEM; Peter Trost, Doug South, Lockheed Martin; Paul Kinnon, ZyGEM; Abby Mackness, Lockheed Martin; James P. Landers, ZyGEM-MicroLab

Defining Integrated and Portable Microfluidic Systems for Automated STR Analysis Applicable to Forensic DNA Analysis: the RapID™ System

Integrated systems for STR typing have become the accepted gold standard for human identification over the past two decades, and is now successfully employed in forensic, civil, and military laboratories. Although highly successful and reliable, current methodologies require 8-10 hours to complete under routine conditions, use large sample volumes, costly reagents, and are labor-intensive. A transition of these sample processing and analytical methods to the microscale format will permit automation, miniaturization, and integration that will provide the end user with a system that provides expedited, cost-effective analysis in a closed system that reduces sample handling and possible contamination. A system capable of fully-automated processing and analysis of STR loci directly from buccal swab samples (RapID™ system) will be presented The system utilizes a single, integrated, and disposable microfluidic chip, and encompasses liquid DNA extraction, PCR amplification, and electrophoretic separation of STR loci. Expedited liquid extraction of DNA from crude samples is performed in less than ten minutes, and guided into a microfluidic PCR chamber. The PCR process can be completed in less than forty minutes, with efficient amplification of 16-18 loci in sub-microliter volumes. Separation is then carried out using electrophoresis in a short channel, using an optimized polymer, with baseline resolution and with 5-color detection. With the RapID™ System, the multistep process that consumes 8-10 hours for conventional forensic STR analysis is carried out in as little as 69 minutes. The design and function of the integrated instrument capable of accepting the microfluidic device will be detailed, with data supporting the capability of the microfluidic system for rapid, automated, end-to-end genetic analysis for human identification.

Micro- and Nanotechnologies—Track 2

4:00 pm Monday, January 31

Location: Pasadena, Renaissance Palm Springs Hotel

Silja Senkbeil, Technical University of Denmark, silja.senkbeil@nanotech.dtu.dk

Co-Authors: Jørn Smedsgaard, University of Denmark; Albert Romano-Rodríguez IN_UB Universitat de Barcelona; Jörg P. Kutter, Technical University of Denmark

Tackling Challenges in Food Safety With Lab-on-Chip Technologies

We are developing a prototype lab-on-chip system to separate and simultaneously analyze different dithiocarbamate (DTC) pesticides. DTCs are a group of organo sulfur compounds, which are widely used in agriculture and some of the most frequently detected pesticides in the European Union. Generally, dithiocarbamates are determined by acidic hydrolysis which involves the generation of gaseous carbon disulfide. The carbon disulfide, which can be detected by spectrophotometric or chromatographic methods, can arise from different dithiocarbamates, however. This means that at this time there are no specific analytical methods that can differentiate one dithiocarbamate from another. This paper describes the design and performance of a polymeric microfluidic system for the analysis of DTCs using cyclic olefin polymer (COP) as the substrate material. COPs are a group of thermoplastic polymers which, apart from good optical properties and low water absorption, are highly resistant to chemicals including polar organic solvents. We take advantage of the chemical stability and optical transparency of COP for performing electrochemical and UV absorbance detection of DTCs in organic media. The microfluidic channel system with an integrated pillar array for chromatographic separation is realized by hot embossing on a 188µm thick COP sheet, serving as the top part of the chip. Unlike other systems, the detection unit with an integrated gold microband electrode array for various electrochemical detection approaches is directly deposited on the COP bottom sheet of the chip. The final closed channel configuration is achieved by thermally bonding the two parts together. Additionally, optical fibers for simultaneous UV absorbance detection are embedded into the chip as well. DTC containing samples dissolved in organic media and diluted with buffer solutions are injected into the channel system and can be separated by either pressure driven reverse phase HPLC or electrochromatography. The analytes are detected by passing a detection cell where UV light (_=254nm) from a mercury lamp is guided into the cell by an optical fiber. The transmitted light is collected by another fiber on the opposite side of the cell, connected to a photomultiplier tube. At the same time, different electrochemical measurements can be performed, e.g., by using the on chip gold microband electrodes as a three electrode detection cell. Differential pulse anodic stripping voltammetry and pulsed amperometric detection are potential complementary detection methods, taking advantage of the adsorptive behavior of sulphur compounds on noble metals. Preliminary tests indicate possible detection pathways for the dithiocarbamates Ziram and Ferbam. In this work, a polymer-based lab on chip device with a great potential to separate and identify samples of DTC pesticides is developed. By combining chromatographic separations with a subsequent optical and/or electrochemical detection on a single chip we provide a platform suitable for many different monitoring tasks in food control.

4:30 pm Monday, January 31

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Elliot Hui, University of California, Irvine, eehui@uci.edu

Self-Contained Microfluidic Systems Enabled by On-Chip Pneumatic Control Circuits

Although microfluidics promises the low-cost automation of complex multiplexed chemical reactions, this goal has continued to remain elusive. One significant limitation is the extensive control hardware that is required to operate a microfluidic chip that is otherwise small and affordable. For point-of-care diagnostics, it would be advantageous to integrate a self-contained system onto a single chip. One elegant approach is to attempt to build essentially a microfluidic computer. The idea is to use microfluidic components to build digital logic circuits capable of executing control programs. This is attractive from a manufacturing standpoint because control and chemistry modules could then manufactured together in a single process. In addition, a single-chip system would be much easier for the user to set up and operate. A number of digital components have been reported in the literature, supporting the feasibility of this approach. Still, a significant hurdle has been the lack of on-chip timing references. Complex microfluidic handling often requires rapid and precisely timed valve switching. For example, a peristaltic pump requires the high-frequency sequential actuation of three in-series valves. Up until this point, off-chip computers have been required to generate the timing signals for such precisely controlled operations. In this work, we report self-oscillating microfluidic circuits suitable for timing and control of fluid handling and chemistry. The frequency can be tuned from less than 1 Hz up to 100 Hz via circuit design. The precision is excellent, with a variation of less than 0.3 percent. Long-term drift is in the range of a few percent per hour, meaning that a 1-hour chemical reaction can be timed to an accuracy of a couple minutes. Power is supplied by a simple vacuum source, which we have demonstrated can even be hand-powered. To demonstrate system integration, the oscillators were used to create a control circuit for driving a peristaltic pump. On-chip pumping at 1 uL per minute was thus accomplished without off-chip control. In summary, our timing references provide a critical building block towards the goal of fully self-contained lab-on-a-chip systems.

Micro- and Nanotechnologies—Track 2

10:30 am Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Guillermo Garcia-Cardena, Harvard Medical School, ggarcia-cardena@rics.bwh.harvard.edu

A Novel High-Throughput Hemodynamic System for Biological and Drug Discovery

Recent advances in cell culture modalities and high-throughput screening technology have vastly expanded our potential for biological discovery, and have triggered new efforts towards the identification of new pharmacological spaces and the implementation of cell-based therapies for treatment of a wide range of diseases. Nevertheless, current systems do not have the capability to probe cellular function in the context of complex hemodynamic environments that dictate the fate and phenotype of cells comprising diverse tissue milieus in vivo. Here we report the development and implementation of a dynamic high-throughput flow system (HTFS) that delivers programmable, time-varying hemodynamic waveforms to cultured cells grown in individual wells of a commercially available 96-well plate, and demonstrate its utility in interrogating biologically diverse tissues. To this end, we have begun mapping the signalling pathways activated in endothelial cells by a shear stress waveform derived from atherosclerosis resistant regions of the human vasculature. These efforts have led to the identification of novel signaling pathways critical for the expression of the transcription factor KLF2, and ultimately to the establishment and maintenance of endothelial atheroprotection. In addition, using the HTFS, we have optimized the microenvironmental conditions required to promote the biomechanical induction of hematopoiesis from murine embryonic stem cells, and have documented that certain ECM components play an important role in this process. Collectively, our data indicates that this novel high-throughput platform should enable a better understanding of human pathophysiology, and its use could lead to the discovery of new therapeutic targets and drugs for clinical interventions in a broad range of diseases.

11:00 am Tuesday, February 1

Location: Pasadena, Renaissance Palm Springs Hotel

Philip Lee, CellASIC Corporation, pjlee@cellasic.com

Co-Authors: Molly Allen, Terry Gaige, Paul Hung

An Automation Compatible Microfluidic Liver Array for Metabolite Screening

We have developed a microfluidic perfusion array plate for bio-mimetic culture of primary hepatocytes for use in drug screening. Primary hepatocytes possess many liver-specific properties that are critical for drug safety and efficacy evaluation. However, current in vitro methods are unable to maintain these biological functions adequately, and there is no commercially acceptable long term hepatocyte culture system. In most cases, hepatocytes de-differentiate after isolation, and no longer serve as useful physiologic models for drug screening. As a consequence, many clinical failures due to hepatotoxicty, drug-drug interactions, and unforeseen metabolites are not screened out prior to clinical trials. The microfluidic liver array (MLA) is designed using an industry standard 96 well plate, with every 3 wells making up a single flow unit (32 units/plate). The plates are fabricated via integration of a microfluidics layer under an open-bottom 96-well plate. The single flow unit consists of an inlet well, a microfluidic culture region, and an outlet well. Medium flows from the inlet well to the outlet well (past the cells) via gravity at a rate of 100 ul/day. The outlet is collected and additional medium added daily. The microfluidic hepatocyte culture region was designed to mimic the human liver microstructure. Hepatocytes are cultured in parallel 3D cords roughly 60 um in diameter with sinusoid channels between each cord to mimic blood flow. An artificial endothelial-like barrier (constructed via microfabrication) localizes hepatocytes to the cords while enabling diffusion of soluble factors through 4 um pores. About 20-30,000 hepatocytes from suspension are loaded into each flow unit using a pressure driven cell loading apparatus, creating the 3D configuration in roughly 1 minute. The cells are fed by continuous nutrient perfusion, resulting in sustained CYP450 metabolic activities, albumin production, and cell viability for many weeks. Once the cells are loaded, no external equipment is needed to operate the plate, allowing the system to be run using existing 96 well automation tools. We have demonstrated assays in this format including: toxicity, metabolite identification, mRNA quantification, and cell staining. The culture format has been validated in human and rat hepatocytes, freshly isolated as well as cryopreserved. In summary, we present a microfluidic technology for primary hepatocyte culture combining improved biological functionality, reduced cell consumption, and automation compatibility.

Micro- and Nanotechnologies—Track 2

11:30 am Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Paul van Midwoud, University of Groningen, P.M.van.Midwoud@RuG.nl

Co-Authors: Marjolijn T. Merema, Geny M.M. Groothuis, Elisabeth Verpoorte

Microfluidics Enables Small-Scale Tissue-Based Metabolism Studies With Scarce Human Tissue

Early information on the metabolism and toxicity properties of new drug candidates is crucial for selecting the right candidates for further development. Preclinical trials rely on cell-based in vitro tests and animal studies to characterize the in vivo behavior of drug candidates, though neither are ideal predictors of drug behavior in humans. While several animal species share some similarity with humans with respect to drug metabolism, species differences remain substantial enough that animal-derived data are still often difficult to extrapolate back to humans. In vitro tests with cells of human origin, on the other hand, suffer from a different drawback, namely that cell monocultures lack the tissue structure and, importantly, endogenous enzymes and other compounds which dictate metabolism in vivo. Improving in vitro systems for preclinical studies thus remains a major challenge, motivated both by a desire to reduce the use of animals for practical and ethical reasons and improve the quality of data obtained with human cells. This contribution describes how microfluidics can be exploited to come closer to this goal, in combination with precision-cut liver slices (PCLS) as an improved organomimetic system. One of the advantages of PCLS over hepatocytes or cell lines for in vitro studies on liver function is that all different cell types are present in their natural tissue-matrix configuration. Clearly, however, human tissue is not readily available, and it is worthwhile to consider how to perform a maximum number of informative experiments with small amounts of material. We developed a novel microfluidic-based (biochip) system to perform metabolism and toxicity studies with mammalian PCLS under continuous flow. A poly(dimethylsiloxane) (PDMS) biochip was developed which contains a 25-µL microchamber to incorporate 4-mm-diameter, 100-µm-thick PCLS. The chamber is continuously perfused with fresh medium to keep the environment stable during incubations. The viability of human PCLS was assessed by morphological evaluation and measurement of the leakage of liver-specific enzymes. All experiments were verified with well plates, an excellent benchmark for these experiments. The metabolism of four different substrates, lidocaine, 7-hydroxycoumarin, 7-ethoxycoumarin and testosterone, were tested in both systems. The ability to form phase I and II metabolites after 24 hour incubation was studied by measuring the metabolism of 7-ethoxycoumarin and 7-hydroxycoumarin after 24 h of pre-incubation. We have shown that the use of microfluidics makes more sophisticated experimentation possible in this system when compared to conventional well-plate formats. For example this system enables the in vitro measurement of interorgan effects, by connecting the outlet of one chamber to the inlet of another chamber containing a slice from a different organ. Direct coupling of the biochip to an HPLC system allows on-line monitoring and immediate detection of unstable metabolites, something which is generally not possible with conventional well-plate systems.

12:00 pm Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Dino Di Carlo, University of California, Los Angeles, dicarlo@seas.ucla.edu

Co-Authors: Daniel Gossett, Henry Tse, Serena Lee, Amander Clark, University of California, Los Angeles

Deformability Cytometry: High-Throughput Label-Free Measurement of Cancer Cell Malignancy and Stem Cell Differentiation State

There is growing evidence that cell deformability (i.e. the ability to change shape under load) is a useful indicator of abnormal cytoskeletal changes and may provide a label-free biomarker for determining cell states or properties such as metastatic potential, cell cycle stage, degree of differentiation, and leukocyte activation. Clinically, a measure of metastatic potential could guide treatment decisions, or a measure of degree of differentiation could prevent transplantation of undifferentiated, tumorigenic stem cells in regenerative therapies. In order for deformability measurements to be clinically valuable, given the heterogeneity within a population of single cells, there exists a need for high-throughput automated assays of these mechanical properties. Here, we present a novel microfluidic device for the continuous deformation of cells in flow (~ 2000 cells/sec, throughputs comparable to traditional flow cytometry). A unique combination of inertial focusing and automated image analysis enables unprecedented mechanical measurements of cells. Using inertial focusing we have devised and fabricated a channel where opposing streams of cells are precisely delivered to the center of an extensional flow where the cells undergo controlled deformation and are imaged with high-speed microscopy. This technique enables us to perform approximately 2000 deformations per second, >3 orders of magnitude over the current state-of-the-art methods for measuring mechanical properties of cells. A custom algorithm tracks cells within the captured images and measures their deformation, circularity, and initial diameter. The code uses a polar mapping strategy to extract deformability information from images. We apply our system to effectively address two applications of extreme importance: measurement of metastatic potential of cancer cells and evaluating embryonic stem cell differentiation protocols for regenerative medicine. We demonstrated our ability to distinguish between populations of a normal cell line (MCF10A), a cancerous cell line (MCF7), and the same cancerous cell line modified to have increased motility or metastatic potential (modMCF7). We show the measurements of deformability clearly distinguish the three populations; the medians were determined to be statistically different with a confidence of $P<0.01$. We also compared the deformability of self-renewing mouse ESC to mouse ESC differentiated by two common protocols: adherent and embryoid body differentiation and found that both methods resulted in less deformable cells. We have also evaluated deformability of oil-in-water emulsions of known viscosities and elastomeric polymer microparticles with known stiffness and determined a physical basis for our deformability measures connected to viscoelastic properties of cells. Further, we have chemically disrupted the individual cytoskeletal components of cells identifying their role in large scale, whole cell deformation. The dramatic increase in throughput provided by deformability cytometry will make it a useful tool for answering biophysical questions as well as enable the use of single-cell mechanical properties as a viable clinical biomarker for diagnostics and regenerative medicine.

Micro- and Nanotechnologies—Track 2

3:00 pm Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Michelle Khine, University of California, Irvine, mkhine@uci.edu

Think Big...Then Shrink

In order for microfluidic technology to fulfill its potential of making a significant impact on fields such as stem cell technologies, systems biology, and point-of-care diagnostics the persistent chasm between academic prototyping and industry-standard devices must be bridged. While most academic labs prototype via soft lithography in polydimethylsiloxane (PDMS), industry is largely intolerant to the inherent material drawbacks of PDMS, including: swelling, non-selective absorption, and poor mechanical properties. Instead, industry relies on plastics, including polystyrene (PS) and polyolefins (PO). To create such fine features in plastics, however, typically requires either hot embossing or injection molding. Both of these approaches require substantial investments in expensive capital equipment and extensive processing time that largely precludes academic prototyping. We introduce a novel, rapid, and ultra-low-cost strategy to fabricate microsystems with integrated nanostructures using shrink-film technology. We pattern at the large scale, which is easy and inexpensive, and rely on the heat-induced relaxation of pre-stressed shape memory polymer sheets to achieve our desired structures. Our previous works with shrink films have focused on the applications of a polystyrene toy called “Shrinky-Dinks”. PS was shown to display a 60% reduction in area upon shrinkage and was used in conjunction with a laser printer to fabricate masters for the fabrication of PDMS microfluidic devices and micro wells for cell culture. Direct patterning of the sheets through etching or deposition was shown to create complete microfluidic devices, and was expanded upon to create a functional biochip that integrated complex microfluidic designs and proteins spots. Recently, we demonstrated that a polyolefin shrink thin film exhibits a 95% reduction in area for high-aspect templates for soft lithography. By combining with a low-cost digital craft cutter, we were able to also achieve relatively uniform and consistent complete high-aspect ratio microfluidic channels with lateral resolutions well beyond the tool used to cut them. The thermal bonding of the layers results in a strongly bonded chip, with leak proof channels, and homogenous surface and bulk properties. Complex microfluidic designs can be easily designed on the fly and protein assays also readily integrated into the device. We have also robustly integrated sharp metallic nanostructures into the microfluidic channels for high-sensitivity surface enhanced sensing. Using this approach, we have demonstrated that we can create fully functional and complete microfluidic devices with integrated nanostructures within minutes. These devices can be created for only pennies per chip and without any dedicated costly equipment. This enables researchers to make custom microsystems on demand for a range of applications from basic biology studies to stem cell research to point of care diagnostic devices to detect infectious diseases.

3:30 pm Tuesday, February 1

Location: Pasadena, Renaissance Palm Springs Hotel

Bruce M. Peterson, Douglas Scientific, bpeters@douglas-machine.com

The Drive to Low Ultra Low Cost Screening

How is it possible for a group of 4 people in their spare time (2,140 Hrs) with no experience in a new field build a HTSI that reduced the screening cost on an average of 70 percent for their customers. The Power of Collaboration.

Micro- and Nanotechnologies—Track 2

4:00 pm Tuesday, February 1

FINALIST SLAS INNOVATION™ AWARD

Location: Pasadena, Renaissance Palm Springs Hotel

Kamlesh Patel, Sandia National Laboratories, kdpatel@sandia.gov

Co-Authors: Hanyoup Kim, Michael S. Bartsch, and Ronald F. Renzi

Preparation of Nucleic Acid Libraries for Ultra High-Throughput Sequencing With a Digital Microfluidic Hub

While DNA sequencing technology is advancing at an unprecedented rate, sample preparation technology still relies primarily on manual bench-top processes, which are slow, labor-intensive, inefficient and often inconsistent. Automation of sample preparation using microfluidic techniques is well-suited to address these limitations. However, fabricating a single monolithic microfluidic device that replicates all the relevant benchtop processes can be prohibitively complicated and does not allow the flexibility to execute diverse protocols for processing different samples and sample volumes. We have designed, fabricated, and characterized a digital microfluidic (DMF) platform to function as a central hub for interfacing multiple lab-on-a-chip sample processing modules towards automating the preparation of clinically-derived DNA samples for ultrahigh throughput sequencing (UHTS). The automated molecular biology platform (AMB) is designed to interface directly with UHTS to detect unknown pathogens by enriching informative nucleic acids sequences (those derived from the pathogen) and suppressing background DNA (those from the host) to maximize the sensitivity of state-of-the-art UHTS. The AMB platform will be able to carry out a diverse series of benchtop-like steps at a scale adapted to handling small, but precious, samples for DNA manipulations, but with far greater speed and efficiency than at the benchtop. We will present our recent developments on the core architecture of the AMB platform, the DMF central hub, and demonstrate its flexibility in coupling droplet-based microfluidics with continuous-flow microchannel devices to prepare DNA samples for UHTS. The strength of combining these two different, but complementary, fluid processing methods enables the manipulation of nanograms to picograms of DNA with precise temporal and spatial control. Features of the platform include the plug-and-play installation of a two-plate DMF device, flexible connectivity for transferring samples between modules, and an intuitive programmable interface to control droplet/electrode actuations. Additionally, the hub platform uses transparent indium-tin oxide electrodes to allow complete top and bottom optical access to the droplets on the DMF array, providing additional flexibility for various detection schemes. We will discuss our results for collecting fractions of nanogram amounts of normalized DNA from pathogen-infected blood plasma in discrete 1-uL droplets on the DMF device. Additionally, we will also present the integration of capillary-based solid-phase extraction columns to clean-up and concentrate the DNA. Processed DNA is now ready for an in-droplet, enzyme-based fragmentation and ligation of appropriate Illumina sequence adapters and barcode tags. Fragmented DNA is analyzed in real-time with microchip-based gel electrophoresis separation interfaced to the sample droplet for the correct size distribution. The resulting amplifed DNA library can be directly transferred to the Illumina flowcell for cluster generation and high-throughput sequencing to discover the pathogen by its genomic sequence.

4:30 pm Tuesday, February 1

Location: Pasadena, Renaissance Palm Springs Hotel

Kai-Chun Lin, Arizona State University, kn314159@gmail.com

Co-Authors: Huang-Chiao Huan, B. L. Ramakrishna, Michael Goryll, Kaushal Rege, Shalini Prasad, Arizona State University

Transport of Ions, Polymers, and Gold Nanoparticles Through Nanopore-Based Device of Hierarchical Biogenic Silica Nanostructures

The technique known as coulter counting can be used to measure the size of the nanoscale analyte when it passes through the cylindrical nanopores. The biogenetic silica nanostructure offers the user the ability to detect and separate the nano-molecules. This sensor has been developed with the aim of creating a system for sensitive, rapid, and portable for multiple targets of interest. Biogenic silica nanostructures, derived from diatoms, possess highly ordered porous hierarchical nanostructures and afford flexibility in design in large part due to the availability of a great variety of shapes, sizes, and symmetries from the over 100,000 known species. These advantages have been exploited for study of transport phenomena of ions and molecules towards the goal of developing ultrasensitive and selective filters and biosensors. Controlled etching of the silica structure has been employed to tune both manipulate the dimensions of the nanopores as well as the hierarchy. These structures across a wide length scale regime have been characterized by scanning electron microscopy. Results from the study of the transport phenomena based on size and chemical considerations will be presented for ions, such as potassium and sodium, present in physiologically relevant isotonic buffers, polystyrene nanobeads and gold nanoparticles. We will further use this nanopore-based sensor with electrochemical signal transduction for the detection of biologically molecular targets, such as DNA and proteins. We expect to leverage the understanding of the transport phenomena through well-characterized hierarchical pore structures towards designing hybrid devices for applications in separation and sensor technologies.

Micro- and Nanotechnologies—Track 2

9:00 am Wednesday, February 2

Location: Pasadena, Renaissance Palm Springs Hotel

Sumita Pennathur, University of California, Santa Barbara, sumita@engineering.ucsb.edu

Understanding the Surface of Fused Silica Nanofluidic Channels Towards Efficient Biological Separations

Nanofluidic technologies provide revolutionary opportunities to separate, identify and analyze biomolecular species in a novel way. The coupled physics unique to nanofluidic systems allows for separations based on different particle properties, including charge, size, conformation, hydrophobicity, and mass. Coupled with optical or electronic detection technologies, such systems have the potential to revolutionize the field of biomolecular analysis, by providing separation, identification, and detection of biomolecules with superior speed, sensitivity, selectivity and quantitation, compared to any existing commercial device. One key physical parameter that distinguishes nanofluidics from conventional microfluidic separation systems is the influence of surface properties, and more specifically the solid-liquid interface properties, on separation behavior. Therefore, in order to fully utilize the power of nanofluidic separations, a comprehensive understanding of the surface is imperative. In this talk, I will present the result of two investigations we have performed in our lab, aimed at understanding the role and impact of fused silica surface properties in nanofluidic channels. The first study is a combined theoretical and experimental analysis of the solid-liquid interface of fused-silica nanofabricated channels, with and without a hydrophilic cyanosilane coating at high salt concentrations. The second is a nanochannel ion transport study at low salt concentrations, demonstrating the influence of atmospheric carbon dioxide on the behavior of the ions in solution. In both cases, our theoretical model consists of three parts: (1) a chemical equilibrium model of the wall, (2) a chemical equilibrium model of the bulk electrolyte, and (3) a self-consistent Gouy--Chapman--Stern triple-layer model of the electrochemical double layer coupling between (1) and (2). To validate our model at high concentrations, we use both pH-sensitive dye-based capillary filling experiments and electro-osmotic current-monitoring measurements. We show that the important fitting parameters are the inner Stern capacitance C1 and the surface reaction constant pK+. We also find that assuming a changing value for the outer Stern capacitance C2, depending on surface composition, results in more accurate fits of experimentally determined zeta potentials. Our new triple-layer model also successfully predicts that at low salt cocentrations, hydronium ions dominate nanofluidic ion flow under surface-charge regime. These hydronium ions arise from dissolution of ambient CO2 and are not treated in other theories. In addition, we identify a minimum conductance value before saturation, at a value independent of salt concentration, in the dilute limit. Our model self-consistently couples chemical equilibrium models of the silica wall and the electrolyte bulk, and is parameterized by only the surface reaction equilibrium constant for silica/hydronium reactions. We use experimental data with aqueous potassium chloride solutions in silica nanochannels to fit the value of this constant, finding a good match between data and model predictions. Model predictions are also found to agree well with measurements in bulk and in nanochannels with hydrochloric acid solutions. I will conclude by demonstrating that our new model works for realistic important applications, including miRNA and proteomics.

9:30 am Wednesday, February 2

Location: Pasadena, Renaissance Palm Springs Hotel

Jason L. Poulos, Librede, Inc., poulos@librede.com

Co-Author: Jacob J. Schmidt, University of California, Los Angeles

An Artificial Cell Membrane Platform for High-Throughput Cell-Free Electrophysiology

Ion channels are crucial physiologically, involved in nearly all biological processes. Their many roles make them important drug targets as well as targets to avoid, as for drug safety screening (e.g. the hERG channel). Patch clamp provides the highest quality measurements of ion channel function and is used by the pharmaceutical industry to test drug interactions with ion channels, but has poor cost and throughput performance. As an alternative ion channel measurement platform, artificial lipid bilayers are well established to provide a highly controllable environment capable of measurement at the single molecule level, although they also suffer from their own technological shortcomings. Here we present the development of an ion channel measurement platform based on artificial planar lipid bilayers. The technological shortcomings of lipid bilayer systems have been mitigated by utilizing a low-freezing point solvent. As a result of this solvent formulation, artificial bilayers and ion channels can be deposited in a 96-well plate format and shipped to end users where, upon warming, bilayer self-assembly and ion channel measurement occur spontaneously. The ease of measurement, low cost chip designs, and integrated ion channels have the potential to increase ion channel measurements by an order of magnitude while simultaneously reducing cost and the burden of cell culture. Librede will present the ongoing development of this low cost high throughput cell-free electrophysiology platform.

Micro- and Nanotechnologies—Track 2

10:00 am Wednesday, February 2

Location: Pasadena, Renaissance Palm Springs Hotel

Simon Ekstrom, University of Lund, simon.ekstrom@elmat.lth.se

Co-Authors: Belinda Adler, Björn Hammarström, Thomas Laurell, Johan Nilsson, György Marko-Varga

Solid-Phase Based Nanobiotechnology Platform for Label Free Screening in an Array Format Interfacing Mass Spectroscopy for Analysis Read-Out

A fully automated nanobiotechnology SPE sample preparation platform will be presented. The corner stone in this platform is the Integrated Selective Enrichment Target, ISET, sample preparation and presentation device manufactured in silicon, having 96 individual array positions. Each array position has an inlet (1 x 1 mm) and an array of nine outlets (15 x 15 micrometer), defining a volume that can be filled with capture medium, maximal capacity is 0.6 microL of beads. Each of these 96 perforated nanovial positions on an ISET chip can be filled with beads for bead based sample preparation prior to analysis with any MALDI MS instrument. The ISET platform has previously been successfully used in applications, such as reversed-phase SPE of proteomics samples, phosphopeptide capture using metal oxide affinity capture (TiO2 beads), antigen capture using antibody covered beads and on-bead digestion. The main strength of the ISET-platform is that any bead or solid-phase protocol can be accommodated on the ISET in a high-through put format at a very high performance level. The uncomplicated standard SBS 386 format (4.5 mm pitch) allows for interfacing to standard liquid handling robotics and robust characteristics makes the ISET an obvious candidate for applications in protein-ligand screening applications. Robust methods that monitor enzyme activity and inhibitor potency are crucial to understand and map molecular function and drug development. Over the past 20 years, mass spectrometric methods have increasingly been used to measure enzyme activity and kinetics. By using ISET sample preparation and MALDI MS for analysis read-out neither the proteins nor small molecules needs to be labelled, thus minimizing introduction of artefacts into the measurements. Here we will show that the ISET platform can be interfaced to any a SBS compliant liquid handling system. Two different systems have been used, a “low-end” set-up using a Beckman-Coulter Biomek 3000 and a “high-end” set-up using 8-channel solenoid dispensing (Seyonic SA) for sample delivery and preparation. In addition it will be demonstrated the ISET platform can be used in any commercial MALDI MS instrument (Waters, ABI/Sciex, Thermo Scientific, Bruker). Data covering previously mentioned applications will be shown, as well as unpublished data demonstrating the ISET platform for label free screening of enzyme inhibition (cytochrome P450 and PKA). The ISET platform together with high resolution MALDI Orbitrap MS and MS/MS performs just as good, as or better than traditional ESI MS approaches with an analysis time that is significantly faster.

10:30 am Wednesday, February 2

Location: Pasadena, Renaissance Palm Springs Hotel

Don W. Arnold, Eksigent Technologies, dwarnold@eksigent.com

Co-Authors: Nicole Hebert, Bryce Young, David Neyer, Remco van Soest

Novel Microfabricated HPLC Tools for Complex Chemical Analysis

Because of the prevalence of HPLC as a chemical analysis tool and the advantages of this liquid phase analysis method in micro and nanoflow mode of operation, we have focused on the development of microfluidic HPLC instrumentation with increased capability and improved opportunities for laboratory automation. HPLC analyses carried out in capillary format often suffers from poor analytical precision due to system design. This talk will focus on our development of microfabricated HPLC components and systems for micro- and nanoscale analyses that use the precision and accuracy of microfabrication to design systems optimized for micro- and nano-scale HPLC. By using microfabrication techniques to form the capillary channel and frit in a silica substrate, parts are made in a precisely controlled, scalable batch process. The result is higher performance and dramatically reduced column-to-column variability. We will introduce novel sample collection and preparation components, sample enrichment components and discuss an innovative microconnector system that enables quick and reliable workflow change. When combined with precise fluid delivery systems, microfabricated analytical chromatography columns and detector interfaces these components form unique microfabrication enabled chemical analysis tools. Several examples of analyses of complex chemical mixtures will be discussed with a focus on the analytical metrics and system precision. Samples ranging from complex protein digests used in proteomics applications to reaction mixtures in a typical synthetic reaction vessel will be discussed. The microfabricated systems address the root of the variability, delivering state-of-the-art precision for separations carried out at flow rates as low as 100 nL/min. Combined with a broad range of chip functionality, this technology allows rapid changes in workflow, greatly simplifying a broad range of biochemical analysis protocols and delivering a significantly improved degree of precision as compared to current protocols. Microfluidic tools address common challenges to nL-volume sample manipulation, such as evaporation, adsorption, precipitation, etc., and allow researchers to manipulate samples whose volumes are too small to manipulate easily with traditional laboratory equipment. These tools provide a solution for those analytical scientists working with smaller and smaller sample volumes.

High-Throughput Technologies—Track 3

10:30 am Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Malgorzata Borowiak, Harvard Stem Cell Institute, mborowiak@mcb.harvard.edu

Small Molecules in the Differentiation of ES Cells Toward Pancreatic Lineage

One of the goals of regenerative medicine is to generate mature cell types to replenish damaged or lost cells in the human body. Insulin-secreting beta cells are one of such cells in need as their loss or deficient leads to the diabetes. Pluripotent cell, including embryonic stem (ES) cells, offer an unique starting population for this purpose as they are unlimited due to their ability to renew and can differentiate in any cell type. There are several strategies that can be applied to direct the pluripotent to the desired cell type, including growth factors and co-culture with other cell types. Small molecules can be very potent in the directing the fate of ES cells and many of them are already tested on other systems or FDA approved drugs. Along with increasing knowledge of ES culture and differentiation with increasing number of accessible small molecules Results from high throughput screens to seek inducers of pancreatic fate from mouse and human ES cells will be presented.

11:00 am Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Andreas Vogt, University of Pittsburgh, avogt@pitt.edu

Automating Image Acquisition and Analysis of Multicellular Organisms

Fueled by advances in genomics research, combinatorial chemistry, and laboratory automation, high throughput screening (HTS) has become a key component of contemporary drug discovery. The ubiquitous use of HTS has, however, not resulted in a concomitant increase in the number of new drugs discovered. An increasingly popular sentiment is that better models are needed to improve the discovery of new drug candidates, and it has been proposed that multicellular organisms could provide such models. The zebrafish in particular is a promising model for in vivo drug screening. The 1-day old embryo is approximately 1mm in diameter and can be easily arrayed into 96-well plates. During the first few days of development, embryos are transparent with most of the major organs present, thus enabling visualization of tissue formation during embryogenesis. Several groups including our own have begun to exploit these features using high-content screening (HCS) methodology. A key challenge has been the automation of phenotypic analysis because existing image analysis methods fail to cope with the heterogeneity and complexity of a whole organism. Here I will present the development of automated image acquisition and analysis solutions for transgenic fluorescent zebrafish reporter lines, and discuss their application to the discovery of small molecule inhibitors of elusive drug discovery targets.

High-Throughput Technologies—Track 3

11:30 am Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

John K. Westwick, Odyssey Thera, Inc., jwestwick@obysseyphera.com

Co-Author: Jane E. Lamerdin

Contextual Drug Discovery and Development Via High-Content Analysis of Cellular Networks

The past decade has seen an enormous increase in our understanding of the individual components of signal transduction pathways and their connections to human disease. Development of high-throughput screens for suspected or validated targets is essential for the translation of this basic knowledge to clinically useful therapeutics. Recent experience has shown, however, that reductionist in vitro screening strategies often fail to identify active and safe therapeutics. We believe that addressing two central challenges can improve the success rate of preclinical discovery and development. First, assays that capture the activity of drug targets in their native cellular context should be employed. Second, drug candidate activity should be monitored not only on its purported target or with panels of in vitro assays, but as a function of the cellular system. To address these challenges, we have developed live cell, protein complex-based high-content signal transduction assays. We expanded these capabilities to create a large and diverse panel of signaling assays that are run in parallel arrays, enabling system-wide signaling analysis and definition of compound mechanisms, selectivity and safety. We also engineered an integrated platform comprised of semi-automated cell culture, automated liquid handling, high throughput automated confocal microscopy, and the requisite automated image analysis, IT infrastructure and data mining capabilities. The technology platform has been used extensively to expedite success rates of preclinical drug discovery and development programs. Here we describe the application of the technology platform to two highly validated but challenging therapeutic target classes - proteasome-related events and nuclear hormone receptors. Protein-fragment complementation assays (PCAs) were developed to enable direct visualization and measurement of ubiqutination and sumoylation of specific target proteins. Assays were also engineered to measure other specific events within ubiquitin/proteasome pathways, including ubiquitin ligase/protein substrate complexes in living human cells. We also developed assays that measure the existence and sub-cellular dynamics of a broad panel of nuclear receptors with multiple transcriptional co-regulators. Hormones acting on these receptors exert their effects via modulation of transcriptional complex formation, stability and localization; thus these assays can directly illuminate the central regulatory step for these therapeutically important targets. We will describe assay engineering for multiple examples of both target classes. These assays were used individually in drug screening campaigns to identify novel small molecules and nucleic acids (siRNAs, miRNAs) capable of modifying these pathways in living cells. In addition, the assays were used collectively to assess the mechanisms and selectivity of thousands of small molecules and nucleic acids, leading to the identification unexpected connections between signaling pathways.

12:00 pm Monday, January 31

FINALIST SLAS INNOVATION™ AWARD

Location: Catalina, Renaissance Palm Springs Hotel

David Nolte, Purdue University, nolte@physics.purdue.edu

Co-Authors: Kwan Jeong, Korean Military Academy; Ran An, John Turek, Purdue University; Dallas Morisette, Advanced BioImaging Systems

Motility Contrast Imaging in Three-Dimensional Tissue-Based Drug Screening

Motility contrast imaging (MCI) detects sub-cellular motion in living tissue as a fully endogenous label-free imaging contrast agent. Three-dimensional imaging assays of anti-mitotic, metabolic and cell-signaling pharmacological agents have extracted label-free functional signatures in tumor tissues for the first time. Motility contrast imaging is a digital holographic imaging technique that uses wide-field illumination to extract sub-cellular motion as deep as 1 mm inside tissue as a function of three-dimensional location. Motility contrast presents an unexpected imaging approach that is well matched to the problem of imaging the effects of broad classes of drugs Motility contrast allows us to explore how function defines motion, and how altered function is detected as changes in motion. To perform motility contrast imaging we use multicellular tumor spheroids that have a well-defined morphology with proliferating cells in an outer shell surrounding a necrotic core. The speckle images of the tumor spheroids shimmer due to cellular motility, and statistical properties of the dynamic speckle are obtained by capturing temporally fluctuating images at successive depths. The motility of the tissue in the proliferating shell and the necrotic core are quantified using spectrogram fingerprints from fluctuation spectra. The fluctuating speckle that is the basis of the motility contrast shows distinct frequency bands in the spectral power density that can be related to the response of the cells and tissue to external perturbations, such as actin depolymerization (responding to the anti-mitotic drug cytochalasin), thermal effects, changing osmolarity and glycolosys inhibition. These different perturbations on the cellular biochemistry show significantly different responses in the three frequency bands. These different responses represent distinct signatures that relate to the mode of action of the various perturbations. These signatures can be used to construct high throughput screens of drug candidates. We applied anti-mitotic drugs to tumor spheroids and observed the changes in the spectral signatures of the fluctuation spectra. Differential changes in the fluctuation spectra (measured through the dynamic speckle) as a function of time show distinct signatures that are specific to the modes of drug action. The label-free character of motion as a contrast agent, the sensitivity and specificity of motion to cellular function, and the three-dimensional access to tissue properties far from surface effects, make motility contrast imaging an attractive new approach for high throughput label-free screening of drug candidates.

High-Throughput Technologies—Track 3

3:00 pm Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Marcie A. Glicksman, Brigham & Womens Hospital and Harvard Medical School, mglicksman@rics.bwh.harvard.edu

Drug Discovery in Academics: What Have We Learned?

Neurodegenerative diseases are challenging from a drug discovery perspective with virtually no disease modifying agents available on the market. The Laboratory for Drug Discovery in Neurodegeneration (LDDN) at the Harvard NeuroDiscovery Center works with academic labs around the world in a collaborative model of drug discovery. The LDDN was established in 2001 as a model for how academic research can be applied to drug discovery. Our strategy complements the efforts in industry. The LDDN has an established track record of progressing projects along the drug discovery pathway, from assay development and high-throughput screening through medicinal chemistry on lead compounds and testing candidate drugs in animal models of disease. A retrospective analysis on lessons we have learned will be presented.

3:30 pm Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Michelle Palmer, Broad Institute of Harvard and MIT, mpalmer@broadinstitute.org

Probe Development in the Public Domain: Coupling Complex Biology With Novel Chemistry

The Broad Institute Probe Development Center, a comprehensive screening center in the NIH Molecular Libraries Probe Production Centers Network (MLPCN), is in a unique position to apply a series of complementary approaches to a large number of screening projects. In addition to the 320,000-member compound set used for MLPCN projects, the Broad Institute has created a novel diversity oriented synthesis (DOS) library with stereochemical diversity and complexity similar to naturally occurring small molecules. Screening of this DOS library has lead to the identification of chemical probes that act through novel mechanisms against targets in psychiatric disease and diabetes. This presentation will discuss our outreach mechanism to bring novel targets to chemical biology and the screening strategies we have used to address these challenging biological targets.

High-Throughput Technologies—Track 3

4:00 pm Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Larry A. Sklar, University of New Mexico Health Science Center, lsklar@salud.unm.edu

High-Throughput Flow Cytometry for Small Molecule Discovery in the NIH Molecular Libraries Initiative and Beyond

The University of New Mexico Center for Molecule Discovery (U54MH084690, http://screening.health.unm.edu/) identifies and implements novel applications of the HyperCyt flow cytometry platform for high throughput small molecule discovery. Flow cytometry is recognized for its unique ability to analyze complex and multiplexed target populations in cell and molecular screening where multi-parameter analysis is required. Recent applications include complex cells mixtures for immunological and oncological targets, pathway analysis, protein trafficking, and host-pathogen interactions as well as multiplexed bead-based molecular targets.

4:30 pm Monday, January 31

Location: Catalina, Renaissance Palm Springs Hotel

Lynn Rasmussen, Southern Research Institute, rasmussen@sri.org

Co-Author: Clinton Maddox, E. Lucile White

Infectious Agents and Drug Discovery: How to Conduct HTS Screening Campaigns Under BSL-2 and BSL-3 Level Containment

The need to develop new antimicrobial and antiviral therapeutics has never been more pressing. The development of antibiotic resistant organisms such as MRSA and MDR-TB, have created serious public health outbreaks with limited treatment options. The need for effective antiviral drugs was highlighted during the 2009 H1N1 flu pandemic. Vaccine production lagged far behind the rapidly spreading virus and quarantine was ineffective in controlling the spread of the virus, which was rapidly disseminated worldwide. In addition to naturally occurring pathogens, bioterrorism is a significant concern since many biological agents can be engineered for drug resistance. Southern Research Institute is actively working on drug discovery and development for a variety of infectious agents. A key part of this program was the development of a screening strategy to enable High Throughput Screening campaigns under BSL-2 and BSL-3 level containment. The results of the infectious agent screen program at SRI includes the screening of 6.5 million compounds on 30 different assays under BSL-2 containment and 2.6 million compounds on 7 different assays under BSL-3 containment, including select agents. Novel scaffolds have been identified and the process of developing new therapeutics is in progress for many of the agents screened. How the HTS Center has implemented high throughput screening with infectious agents will be discussed. This includes the overall screening strategy, safety issues associated with infectious agents, equipment selection and assay design.

High-Throughput Technologies—Track 3

10:30 am Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Paul Downey, UK BioBank, paul.downey@ukbiobank.ac.uk

Enabling Technologies Employed in Sample Management of the World's Largest Prospective Study

UK Biobank is a charitable organization, set up to facilitate efficient medical and scientific research. The project has recruited over 500,000 volunteer members of the UK public over the last three years. Via an extensive questionnaire and interview the volunteers have provided detailed data about their health and lifestyle and critically consented to anonymous access to their National Health Service (NHS) health care records. This combination of baseline data, retrospective exposures, treatment record and outcomes will form an incredibly valuable resource that can be rapidly accessed by research organizations. In order to deliver the project, a national network of recruitment clinics was established to receive and process the volunteer participants. A dedicated centralized biological specimen processing facility was constructed, including a 10 million sample capacity -800C automated biological specimen repository developed. The use and delivered benefits of management practices to design implement and operate a study of this sale will be discussed. The use of appropriate enabling technologies during the evolving phases of the project: the research and design phase, the operations development phase and the operational phase will be covered.

11:00 am Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Craig Mickanin, Novartis Institutes for BioMedical Research Inc., craig.mickanin@novartis.com

Accelerating the Drug Discovery Process Via Global Distribution of Protein- and Nucleic Acid-Based Reagents

A major bottleneck in the early stages of the drug discovery process is the generation of genetic reagents to support target identification and validation. These reagents include, but are not limited to, full-length cDNA clones, purified recombinant protein, DNA and RNA oligonucleotides, and short hairpin RNA. These reagents represent a valuable class of assets within a drug discovery organization but have traditionally been excluded from enterprise-wide collection and distribution practices. Moreover, the pharmaceutical industry has transitioned from a primary focus on low-molecular weight compounds to a diversified portfolio of biological (protein and antibody) and nucleic acid-based therapeutics. With this shift has come a need to define methods for registration of complex macromolecules based on reagent-class specific properties. This presentation will describe the efforts within our group to establish an enterprise-wide system for the collection, registration, and distribution of genetic reagents to support early target validation activities.

High-Throughput Technologies—Track 3

11:30 am Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Dale Larson, Charles Stark Draper Laboratory, dlarson@draper.com

Co-Authors: Jorge de Dios, CryoXtract Instruments, LLC; Steve Bellio, Linda Maloney, Charles Stark Draper Laboratory; Helena Judge Ellis, Duke University

A Novel Robotic System for Optimizing the Processing and Protecting the Value of Critical Frozen Biospecimens

Biological materials are invaluable to support translational research and advance molecular medicine, and continuous access by researchers to high-quality biospecimen collections over time is essential. Therefore, optimal processing, biopreservation and sampling are crucial to protect the biospecimens' biochemical composition and molecular integrity and promote ideal conditions for analysis. These vital materials typically are cryopreserved to prolong cell life during long- and short-term storage and must be thawed before sampling. Yet, repeated freeze/thaw cycles might degrade critical biological molecules (i.e. RNA, proteins), damage antibodies of interest, and affect the results of a particular study—often in unpredictable ways. Thus, current best practices recommend minimizing the frequency of sampling to avoid or reduce potential molecular damage from repeated freeze/thaw cycles. Biorepositories and researchers, then, must weigh ease of access to these stored biomaterials against possible data variation caused by specimen degradation over time. Their dilemma when building biospecimen inventories is whether to freeze specimens in multiple small volumes (consuming significant storage space and increasing costs) or in fewer large volumes (reducing initial processing time and storage space at the expense of freeze/thaw cycling later). We developed and tested an Automated Frozen Sample Aliquotter that eliminates the current trade-off. The novel robot enables hands-free extraction of multiple frozen aliquots from one single frozen biospecimen without exposing it to multiple freeze/thaw cycles, allowing specimens to be stored in higher volumes while protecting specimen and sample integrity. The Automated Frozen Sample Aliquotter will prolong utilization of frozen biological specimens, deliver high-quality samples, increase trust in research results, and enhance access to an the value of critical biological collections—while improving processing efficiencies, reducing cost and administrative burden, and bolstering research. Applications for this novel instrument might include serum, plasma and whole blood; frozen tissue; cells; and small molecule compounds in DMSO. Since presenting the concept for this robot at ALA in 2009 we have built proof-of-concept systems for serum and plasma sampling which completed 3rd-party testing. The independent tests successfully demonstrated that the Automated Frozen Sample Aliquotter can extract multiple uniformly-sized and homogeneous frozen 100 microliter aliquots in a hands-free environment from a single 1.8mL cryovial of frozen source biospecimen without exposing it to freeze/thaw cycling; that the cored samples give reproducible results with very low variability when analyzed for common analytes to assess the degree to which the frozen aliquots represented the original specimen; and that the robotic instrument maintains samples and specimens under cryogenic conditions pre-, during and post-coring. This presentation will describe the technology and current robot development and will present the independent test results that clearly demonstrate the proof-of-concept for the automated Frozen Sample Aliquotter.

12:00 pm Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Timo Cuntz, Fraunhofer IPA, timo.cuntz@ipa.fraunhofer.de

Co-Authors: Ilona Brändlin, R. Huchler, M. Fritsche, Fraunhofer Institute for Manufacturing Engineering and Automation IPA; A. Brandenburg, FhI for Physical Measurement Techniques IPM; D. Zühlke, FhI for Applied Information Technology

Flexible, Application-Depended HTS Automation Concept in Genomics and Proteomics

A flexible new concept of a HTS-automation center allows any application in the topics of genomics and proteomics. The fundament of this new concept are our fully automated cell culture systems, in which up to five hundred stem cell pools can be cultivated, sorted in MTPs or flasks per month. Based on this automated cell-culture handling the new HTS technique allows a modular set-up of different process steps. Starting with handling of human/animal cells in an incubator, the system is able to count and split the cells in MTPs or flasks. Liquid handling options allow fully automated and precise dosing of biopharmaceutical compounds to the cells in the MTPs. Process modules are including life-cell-imaging, where fluorescent marked cells are detected. This is interesting in the focus of protein localization or co-localization studies. In order to setup stable cell lines, a specific picking robot separates the positive and negative fluorescent cells in different containers. Afterwards they are processed in an integrated incubator. Possible applications in the field of proteomics are the analysis of recombinant expressed proteins after compounds incubation. If the protein won't be secreted, the cells are fully automated lysed and the supernatant analyzed in a specific screening centre or cellular assay like microarrays. As well functional protein-studies are possible e.g. RNAi. In the field of genomics the DNA/RNA can be separated and analyzed under the use of different assays. We realized this flexible application-depended HTS automation center in a version combining cell-culture handling, life-cell-imaging, compound handling, picking cells and separation between cells and supernatant. Operated via an easy to use graphical-user-interface (GUI) and controlled by efficient scheduling module, it's possible to handle the fully automated system in a flexible way to get validated results in HTS. The talk will cover the technological and application aspects of a fully automated system for pharmaceutical and biotechnological researches and will point out the innovative solutions.

High-Throughput Technologies—Track 3

3:00 pm Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Dave Weaver, Vanderbilt School of Medicine, david.weaver@vanderbilt.edu

Automation-Enabled Screening Approaches for Discovering and Characterizing Allosteric Modulators of Seven-Transmembrane Receptors

Seven-transmembrane receptors are one of the most productive families of drug targets and they are also among the most commonly screened target families. Over the past few years it has become increasingly apparent that 7-TMR modulation is substantially more complex that once thought. Concepts such as allosteric modulation, context dependence, and functional selectivity are changing the way we think about 7-TMR pharmacology, how these receptors are screened, and how they are pursued as therapeutic targets. In order to expand our understanding of and to harness the great richness of 7-TMR pharmacology we have developed and implemented a number of automation-enabled screening strategies to support the discovery and characterization of allosteric modulators of 7-TMRs. Our attention has been focused on the utilization of live-cell, real-time, kinetic imaging approaches for Gq, Gi/o, and Gs-coupled receptors combine with medium-throughput automated screening platforms to support hit identification, hits-to-leads, and lead development. Results, key learnings from our work, and our future directions in this area will be discussed.

3:30 pm Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Jonathan O'Connell, Bristol-Myers Squibb Company, jonathan.oconnell@bms.com

Let the Biology Drive: The Complexity of Enabling Automated Multiparameter GPCR Assays

In the majority of High-Throughput Screening (HTS) laboratories, GPCR assays constitute a significant proportion of the screens that are run each year. While very stable platforms have existed for a number of years that enable the quantification of GPCR signaling via fluorescent or luminescent detection, recent years have seen a significant increase in the number and complexity of readouts that are available. This has been driven by the demands of Drug Discovery, requiring not just agonists or antagonists but positive, neutral and silent allosteric modulators (PAMs, NAMs and SAMs). Configuring automation to be able to screen in multiple modes concurrently, in physiologically relevant models, and in an efficient, miniaturized manner is a significant challenge. This talk will present some of the challenges and solutions developed at Bristol-Myers Squibb.

High-Throughput Technologies—Track 3

4:00 pm Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Jeffrey Irelan, ACEA Biosciences, Inc., jirelan@gmail.com

Co-Authors: Ning Ke, Jing Zhang, Wen Zheng, Xiao Xu, Xiaobo Wang, Yama Abassi, ACEA Biosciences, Inc.

Label-Free, Dynamic Monitoring and Screening for Modulators of GPCR Function Using the xCELLigence RTCA High-Throughput System

The xCELLigence RTCA HT System is a label-free and real time system for dynamic monitoring of cellular responses. It allows for accurate determination of GPCR response kinetics, has the ability to use native receptors (especially in the context of disease-relevant cells), and the ability to capture biological responses involving multiple second messenger pathways. The HT system is flexible, allowing for integration into a user's existing liquid handling and lab automation platform. Up to four Stations, each having a small footprint, may be integrated onto the automation platform deck at a time. Each Station accommodates one Society for Biomolecular Sciences (SBS) compatible 384-well plate (E-Plate 384), which is maintained at physiological temperature by an integrated heating system. We present evidence that the xCELLigence HT System can be used to screen for modulators of GPCR activity in recombinant cell lines and with endogenously expressed receptors in native cells. These assays are highly sensitive and robust, and can be performed on a wide variety of GPCR families including all coupling classes. In a proof of concept screen, the HT System was used to identify true modulators of the angiotensin 1 receptor in an antagonist screening mode. The HT system was used in the primary screening step through hit reconfirmation and secondary screening, including the use of disease-relevant primary cells. These features should allow users to maximize hit quality and potentially minimize subsequent attrition rates in the drug discovery process.

4:30 pm Tuesday, February 1

Location: Catalina, Renaissance Palm Springs Hotel

Michael Finley, Merck, michael.finley@merck.com

Cross Assay Correlation in Ion Channel Screening

For ion channels and transporters, cell based high-throughput screening largely relies on fluorescence assays using dyes that detect permeant ions or changes in membrane potential. Whole-cell patch clamp of single cells remains the "gold-standard" approach for studying ion channel pharmacology, but its application to ion channel screening is limited due to extremely low throughput and technical difficulty. In the last decade, patch-clamp electrophysiology has become increasingly automated, bringing data from this technology earlier into the screening cascade. We describe our approach to incorporating automated electrophysiology into the ion channel screening workflow. We present data comparing high-throughput fluorescence and medium-throughput electrophysiology assays from several distinct ion channel targets and discuss strategies for identifying compounds most likely to have desirable activity in the "gold-standard" manual whole-cell patch-clamp assay.

High-Throughput Technologies—Track 3

9:00 am Wednesday, February 2

Location: Catalina, Renaissance Palm Springs Hotel

Bill Arthur, Merck & Co., Inc., William_Arthur@Merck.com

Identification of Oncology Biomarkers by Integrating HTS With Molecular Profiling

Biomarkers can be used to identify patients with the best chances of responding to a treatment, to monitor target engagement, and to report early indications of treatment efficacy. We are committed to using these three types of biomarkers to increase the success rate of novel therapeutics in clinical trials. However, biomarkers are often defined and validated retrospectively from the analysis of data from limited numbers of patients treated early in the clinical trial process. This approach is inefficient and does not allow for early decisions to forgo or modify a trial to best ensure success. Our studies aim to prospectively identify enrollment biomarkers for oncology drugs prior to heavy investment in clinical trials. We explored many characteristics of cell lines that may determine resistance or response to a given drug treatment. Viability responses to seventy compounds or combinations were determined in cancer type-specific panels of approximately one hundred cell lines. In parallel we collected baseline DNA, RNA, and protein samples to run mutational analysis, CNV arrays, cDNA microarrays, and phospho-protein profiling. Following the generation of the drug response and molecular profiling data, we drew correlations between the two data sets to formulate biomarker hypotheses and inform clinical trials.

9:30 am Wednesday, February 2

FINALIST SLAS INNOVATION™ AWARD

Location: Catalina, Renaissance Palm Springs Hotel

Yama Abassi, ACEA Biosciences, Inc., yabassi@aceabio.com

Co-Authors: Biao Xi, ACEA Biosciences, Inc.; Wei Ouyang, Nancy Li, YiGuo Sun, ACEA,Bio China; XiaoDan, Ni Wallson Xu, Jenny Zhu Min, Zhen Xiao Xu, ACEA Biosciences, Inc.; XiaoBo Wang

Real Time Beat to Beat Contraction Profiling Based Cardiotoxicity Screening Using the xCELLigence RTCA Cardio System

Preclinical cardio-safety is an important part of the drug discovery process. All new chemical entities (NCE) need to be subjected to extensive preclinical assessment for cardiac liability. The current thinking in the drug discovery arena is to initiate safety testing as early as possible in order to avoid the huge financial losses due to attrition at late stages. Most of the current assay systems in preclinical cardio-safety are designed to test surrogates of cardiotoxicity, such as arrhythmia, rather than arrhythmia itself. We have developed an innovative label free, real-time cardio analysis (RTCA) System, designed to measure cardiomyocytes contraction and relaxation based on impedance measurement. The impedance signals can be obtained as millisecond time resolution to resolve the beating profile of cardiomyocytes and can be adjusted to also assess longer term changes such as changes in morphology and viability. The assay is performed in 96 well Cardio Plates and provides sufficient throughput to test many compounds in dose-response manner. We have validated the system with mouse embryonic stem cell-derived cardiomyocytes, human induced-pluripotent stem cell-derived cardiomyocytes and rat neonatal primary cardiomyocytes by applying varieties of known mechanism chemical reagents and drugs. Our data shows that the time-resolution in the assay can provide important information about compound action. Furthermore, the impedance-based beating profile in response to compound treatment can provide mechanistic toxicity information regarding the target being modulated and maybe able to flag pro-arrhythmic compounds. We believe the real-time and kinetic aspect of this technology combined with beat to beat measurement of cardiomyocytes contraction would make it an important part of preclinical cardio-safety assessment.

High-Throughput Technologies—Track 3

10:00 am Wednesday, February 2

Location: Catalina, Renaissance Palm Springs Hotel

Jonathan A. Lee, Eli Lilly Research Labs, Jonathan_A_Lee@Lilly.com

Co-Authors: Chris Moxham, Dan Sall, Dirk Tomandl, Mark Uhlik, Eli Lilly

Phenotypic Screening: A Complementary Drug Discovery Paradigm

The Pharmaceutical industry is in the midst of the "perfect storm" with R&D efficiency hindered by Phase II and III attrition. Paul et al suggest that increased target validation (TV) will increase late stage p(TS), but these activities are difficult, time consuming and currently limits the utility of new targets discovered by genomic sequencing. Lilly Discovery is exploring the use of phenotypic assays to chemically interrogate complex biological systems composed of multiple or unknown biochemical components/pathways. Such Phenotypic Drug Discovery (PDD) approaches complement classical, gene specific, Target-directed Drug Discovery strategies and are expected to minimize risk due to poor TV by directly interrogating biologically relevant systems. Concerns with PDD include the design of appropriate compound libraries, the statistical robustness and throughput of assays, and whether cellular assays can provide compound structure-activity relationships. We have experimentally investigated these factors using a phenotypic angiogenesis assay composed of primary cells in co-culture. Our data indicate that phenotypic assays can efficiently support screening, hit expansion, and SAR efforts. Chemo-informatics analysis of the actives indicates that multiple molecular targets and pathways relevant to angiogenesis were simultaneously interrogated. Finally, the PDD approach identified compounds which inhibit angiogenesis by novel mechanisms. This presentation is a status report on the use of PDD approaches at Eli Lilly and Company.

10:30 am Wednesday, February 2

Location: Catalina, Renaissance Palm Springs Hotel

Eric Tang, AstraZeneca PLC, eric.tang@astrazeneca.com

Co-Author: John Vincent

High-Throughput In Vitro Combination Profiling—Frontier of Acoustic Reformatting Technology and Novel Imaging

Cancer is a multi-factorial disease and requires multiple therapeutic interventions to achieve significant clinical efficacy in patients. Most chemotherapy and targeted agents only exhibit limited clinical efficacy and better prediction of efficacy is required for combination therapies in the early clinical setting. Advances in the understanding of tumour biology and genetic profiling of cancer cell line panels provides a basis for high throughput combination profiling using in vitro cancer cell line panels. The throughput of such broad compound combination screening is made feasible by an amalgamation of enhanced acoustic reformatting technologies in conjunction with a high throughput whole cell imaging workflow and rapid data visualization approaches. The output of such profiling campaigns will help generate testable hypothesis and combination utilities to be evaluated in in vivo and/or clinical programmes.

Informatics—Track 4

10:30 am Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Marty R. Jacobson, St. Mary's Saccomanno Research Institute, marty.jacobson@stmarygj.org

Implementation of a Cloud-Based Biorepository Informatics Solution at a Community Hospital Research Facility

Saccomanno Research Institute will present on implementing a biorepository informatics solution on the Amazon EC2 cloud platform. Research overview, project requirements, approach, implementation, and lessons learned will be shared.

11:00 am Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Daniel Crichton, NASA Jet Propulsion Laboratory, dan.crichton@jpl.nasa.gov

Developing a National Computing Infrastructure to Support Early Cancer Detection For the NCI EDRN-Canary Foundation

NASA JPL will present on using federating technologies and semantic web, coupled with a cloud computing approach, to solve translational research data integration, analysis, and knowledge management challenges. Special focus will be given to the NCI EDRN - Canary Foundation Never Smokers Lung Cancer Program.

Informatics—Track 4

11:30 am Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Cliff McCollum, GenoLogics Life Sciences Software, cliff.mccollum@genologics.com

A Change in the Weather—Using the Cloud to Manage Business and Development Operations

In the past, any group that wanted to use IT capabilities needed to budget for expensive IT skills, hardware and software. With the recent surge of Cloud-based services, any organization can make use of extremely sophisticated IT with almost no in-house IT expertise. Learn how GenoLogics is making use of cloud-based services to grow and enhance their corporate and engineering operations, while reducing IT costs and challenges.

12:00 pm Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Mohammad Shaikh, Bristol-Myers Squibb Company, mohammad.shaikh@bms.com

Co-Authors: Marc Pfister, Raj Malatker

Synergizing Clinical Modeling Using Cloud Computing

Clinical modeling & trial simulations are iterative processes and require CPU intensive compute resources. This presentation focuses on use of cloud computing resources to provide parallel processing of simulations for PK/PD and exposure response analysis. Estimating optimal samples of biomarkers that provide information for exposure response analysis require indirect PK/PD models. The simulations are designed to access the impact of different sampling schemes on precision and bias of PD parameter estimates. Most simulations require multiple data sets and the batch programs processing these algorithms run over multiple days on compute clusters in data centers. High volumes of data and complex integrated data environments present unique challenges in providing a high performance compute environment. These large volumes of data are then analyzed via complex algorithms as part of the ongoing trials, enabling faster turn around times for early and informed decision making. Compute resources in cloud can be provisioned in minutes and with adequate parallelization & security measures simulation jobs can be run simultaneously over a large number of server instances. As individual components of the batch runs complete results can be cumulatively analyzed to estimate the results event before the completion of the entire runs. The paper will discuss our experiences in configuring clinical modeling environments on Cloud. Lessons learned can be applied to other applications of cloud resources as well. Operational Challenges: The initial operational hurdles posed were security concerns and compliance with the FDA - 21 CFR Part 11 for validated processes. Although provisioning servers in cloud was a well optimized process; moving large data sets, queuing jobs and parallelization of jobs were still major challenges for a complex simulation problem. Part of the solutions came from the industry like virtual private cloud, data encryptions in transit & at rest. A host of whitepapers and proposed standards offered a wide variety of options. Some of the solutions like queuing and parallelization techniques were developed internally. The paper will elaborate our combination of solutions and point out the limitations of these. Next generation of solutions that could further the maturity of some of the services in cloud will also be summarized. Results: Finally, we will discuss the results and ROI achieved in addressing some of the clinical modeling & simulations problems: Trial Simulations, Boot strapping & Covariate analysis using compute resources in cloud.

Informatics—Track 4

3:00 pm Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Matt Waldbusser, International Business Machines, mwaldbus@us.ibm.com

Cloud Computing: Driving Innovation Delivering New Efficiencies

This new IT delivery model can significantly reduce enterprise IT costs & complexities while improving workload optimization and service delivery. Cloud computing is massively scalable, provides a superior user experience, and is characterized by new, internet-driven economics. Information technology is changing rapidly, and now forms an invisible layer that increasingly touches every aspect of our lives. Power grids, traffic control, healthcare, water supplies, food and energy, along with most of the world's financial transactions, now depend on information technology. This session explores how Cloud computing addresses the increasing complexity of our world and how information technology is transforming the way we live.

3:30 pm Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Mick Gallagher, LST, Mick@lifescitech.com

Cost Analytics

In today technology world and with the current economics stakeholder in companies are more conscience than ever to the capital expenditures. Also with advent of Cloud Computing this field offers alternatives to the traditional ways companies have approached their expenditures. Conservation of capital expenditures is foremost in the minds of executives. With this said the industry is starved for innovative approaches to understanding the capital expenditures and having an analytical sound approach to where the capital is spent. The questions on most executive's minds are: 1.It is necessary to spend at the current rates? 2.Will these capital expenditures justify a significant market advantage? 3.Are these investments enhancing our business processes? 4.As a Stakeholder what is the benefits to our departments for these expenditures? 5.What is the impact of alternative practices and the impact it will have on our equity positions? In our session we will educate executives to analytical offering in the market place today that will help them cross the chasm between their expenditure and stakeholder understanding of where all the monies go. The results of this should be the following. A) helping executives in a concise approach to their stakeholders understanding "where the monies go" and their ROI from these types of expenditures. B) Knowledge of Industry Products that will help the executives rapidly deploy and concisely share an analytical pro-active approach to capital being spent. C) Justification of the costing around such expenditures. D) Uses of the Cloud as an alternative approach. E. Safe guards to a secure cloud environment.

Informatics—Track 4

4:00 pm Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Thomas Kent, Sciformatix Corporation, tkent@sciformatix.com

Lifting Lab Operations to the Cloud

Lab operations heavily depend on information technology, and a new age has dawned in the world of computing and information technology – the Cloud Computing Age. Cloud Computing is a transformational phenomenon, and has already positively impacted organizations and individuals around the world. Every lab operation stands to gain mightily from Cloud Computing if the organization understands Cloud Computing and how to reap the benefits that Cloud Computing offers. Conversely, a lab operation that ignores Cloud Computing will risk placing itself at an operational and competitive disadvantage. In this presentation, we'll show how your lab operation can strategically take advantage of Cloud Computing and reap its many benefits. Because Cloud Computing is still relatively new to many, the majority of people involved in lab activities are not sufficiently aware of the benefits and risks of Cloud Computing. Thus, it is difficult to make an informed decision about leveraging the advantages of Cloud Computing while avoiding its pitfalls. Some of the key areas that will be covered in this presentation include: 1) A definition of Cloud Computing. 2) What's driving the move to Cloud Computing? 3) Benefits of Cloud Computing for life science organizations, including lab operations. 4) Key players that deliver Cloud Computing solutions. 5) Pitfalls to avoid when adopting Cloud Computing. At the end of this presentation, attendees will come away with an understanding of Cloud Computing, the many benefits to be derived from Cloud Computing, and how to start putting Cloud Computing to work for their lab operations.

4:30 pm Monday, January 31

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

David Brown, Neudesic, david.brown@neudesic.com

Turning the Cloud Into Practical Reality

The "Cloud" is one of the hottest subjects in technology today, and a "bet the farm" play for a number of major technology companies. What is the Cloud? How can your organization leverage it? Who are the players? The Cloud offers large scale infrastructure and market cost savings opportunities, but more importantly represents the future of scaled computing and data storage. "Turning the Cloud Into Practical Reality" provides the attendee with a high level overview of this dramatic shift in technology, and a discussion of the practical, "right now" elements that your organization can benefit from.

Informatics—Track 4

10:30 am Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Chrissie Ongaco, Center for Inherited Disease Research, congaco@jhmi.edu

Co-Authors: Brian Craig, Marcia Adams-Carr, Michelle Zilka, Kurt Hetrick, Michael Barnhart, Yichun Sun, Oluyomi Osimokun, Beth Marosy, Dorian Leary, Sean Griffin, Ivy McMullen, Mary Jewell, Filipp Kistenev, Jane Romm, Janet Goldstein, Corinne Boehm, Lee Watkins, Kim Doheny

Informatic Sample Handling Processes: A High-Throughput Genotyping Facility's Workflow for Sample Information Tracking

The Center for Inherited Disease Research was established at Johns Hopkins University in 1996. CIDR provides high quality genotyping and sequencing services and statistical genetics consultation to investigators working to discover genes that contribute to common disease. The handling of large numbers of samples requires a mechanism for tracking detailed information throughout experimental processes. Progress can be recorded and results retrieved in a manner appropriate to the individual lab's custom procedures. CIDR processes approximately 100,000 samples each year, tracking this information through unique combinations of database types, Web, Java and Perl applications and integration with robotics for sample movements. Use of MySQL®, Oracle® and MS SQL® databases have allowed CIDR to create new and adapt legacy schemas to store changing information about samples for use in downstream processes and release to investigators. Creation of Java and Perl applications to integrate with databases provides the flexibility to implement and adapt custom validation frameworks. These methods are put into operation through unique sample map construction, verification of pedigree and other investigator supplied information, confirmation of sample receipt, recording history of sample movements, queuing samples into production workflows, tracking of problems and recording auxiliary information and quality control (QC) metrics. Development of custom laboratory information management systems (LIMS) for CIDR's, Illumina® Infinium™ and BeadXpress™ services using JavaScript, Java and the Google Web Toolkit allow for rapid changes to workflows and improved troubleshooting methods based on input from CIDR personnel or vendors. The use of liquid and tube handling robotics has allowed CIDR to streamline sample processing by re-arraying samples with investigator-supplied plating criteria to better mix sample phenotype information prior to processing. Samples can be removed, re-genotyped or replaced based on problems identified throughout CIDR's workflow, thereby optimizing processing time and reagent use. Automated processes perform genotype calling and QC analysis which are linked to the sample for reporting to CIDR personnel and investigators. The use of these resources, as well as the experience to adapt or engineer new ones as needed, permit CIDR to efficiently receive and process large sample studies at present and allow for continued growth into the future.

11:00 am Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Andrew Barry, Broad Institute Genome Sequencing Platform, barry@broadinstitute.org

Feeding The Beasts: High-Throughput Sample Preparation for Next Generation Sequencing Applications

Next generation sequencing platforms produce unparalleled quantities of data at a low cost. As these technologies mature, novel applications for sequence analysis include de novo assembly, variant detection, metagenomic, and epigenomic analyses. With falling costs, higher throughput, and a growing number of applications, the quantity of samples requiring sequence data has exponentially increased. In order to meet this demand, alternative methods to prepare, track, and interrogate samples prior to sequencing are rapidly being developed. My talk will focus on the methods developed at the Broad Institute to create a high-throughput sample preparation pipeline for use upstream of next generation sequencing.

Informatics—Track 4

11:30 am Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

William McGuire, Kaiser Permanente, william.b.mcguire@kp.org

An Information System for Supporting Large Scale DNA Extraction and Normalization

Kaiser Permanente Division of Research in Northern California in collaboration with the University of California at San Francisco has embarked on an NIH-funded project to genotype and measure telomere length of 100,000 DNA samples in 18 months. Over 100,000 saliva samples have been collected from consented KP patients as part of the Research Program on Genes, Environment and Health (RPGEH) study at the Division of Research. In order to accomplish this ambitious goal it was apparent that a highly streamlined, robust and absolutely accurate IT solution was needed. This IT solution had to support a wide range of lab operations including initial sample check-in, DNA extraction, quantification and normalization of DNA concentrations, storage of excess DNA in several formats and locations, shipping to the collaborating UCSF lab for genotyping, and return of the genotype results. Because of the compressed timeline and the complexity of the workflows, it was decided to custom build the system in-house and defer the acquisition of a commercial LIMS product. Technologies employed include C# and Microsoft dot Net framework, Windows XP, and SQL Server databases. Hardware includes Wintel PCs and servers. This custom solution had to interface with six laboratory robots in order to capture instrument data and control highly complex hit picking and normalization operations. To achieve the necessary high throughput automation was used to minimize lab staff's interaction with the robots wherever possible. Additional software functionality was built to automate storage and retrieval operations for three local and one remote location. The system also prepares shipping manifests and transfer reports for samples shipped for assays. Interfaces were developed to automate and capture quality control measurements and returned genotyping data. Several challenges needed to be overcome during this effort. Functional requirements tended to change with very little lead time as lab protocols were evaluated and refined to improve throughput and quality Exceptional situations such as broken and spilled plates, machine failures and other processing interruptions were common and needed to be accommodated by the information system To this end, flexibility was designed into both the software and data model.

12:00 pm Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Sean Kim, Illumina, Inc., skim@illumina.com

LIMS to LIMS Integration Using RESTful Web Services

Illumina provides an enterprise quality black box LIMS with its genotyping assay. The LIMS was designed to be an all inclusive system that includes Project Management, Sample Management, Assay Workflow and Analysis. However, larger customers with their own in house LIMS looked for seamless integration between systems. The solution was a middleware application using J2EE technology and RESTful Web Services to create a generic integration platform to the Illumina LIMS.

Informatics—Track 4

3:00 pm Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Thomas Wedehase, Xavo Systems AG, Thomas.Wedehase@xavo.com

Standards Within Laboratory Environments

Standardization and innovation are often seen as contrasting objectives. Especially within a laboratory, where innovation is the key driver, standardization is commonly perceived as a restriction, limiting scientific work and the introduction of new technologies. However, if standards are implemented correctly, they actually function as a basis for innovation. This talk will focus on two different areas within a laboratory to exemplify the advantages arising from standardization, as well as its limits. Device interface standards enable the rapid and future proven integration of laboratory equipment as we will show in an example of a storage network. Standards in data storage and transmission will help in dealing with the steadily growing amount of scientific data. We will demonstrate the progress of several standardization initiatives and outline their positive effect on daily laboratory work.

3:30 pm Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Jay Gill, Bristol-Myers Squibb Company, james.gill@bms.com

Co-Authors: Victor Cardenas, Thomas J. Curneal, David Dorsett, Bristol-Myers Squibb Company;
Eric Marshall, Scientific Knowledge Informatics, LLC; Kathleen Oday, Viral Vyas, Bristol-Myers Squibb Company

BIO: A Unified Informatics Strategy for Biological Data Management in a Mid-Sized Pharmaceutical Company

The variability in technologies, processes, precision and throughput used in the measurement of biological endpoints across modern drug discovery assays presents a major challenge to the development of informatics systems. When confronted with the need to overhaul our existing systems across the in-vitro screening and in-vivo space, we identified several prototypical workflows and after analysis, came to the conclusion that a single approach would not work. Subsequently, we deployed a suite of tools including an in house HTS system for support of basic dose response assays; IDBS BioBook for in-vivo pharmacological assays; and an Excel add-in to collect and store data from ubiquitous Excel spreadsheets throughout biology research. These three core tools are unified by a number of tightly integrated “helper apps”, including R statistics integration, a custom curve fitting application, a protocol definition system, and data publication links to our ELN and research data warehouse. To date these tools have been deployed to support ~1500 assays over the past year across biology from HTS through pre-clinical research. We are currently developing additional tools to facilitate long term support, including a RESTful service layer to make additional integration with analysis and visualization systems easier to build and maintain.

Informatics—Track 4

4:00 pm Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Serhiy Y. Hnatyshyn, Bristol-Myers Squibb Company, serhiy.hnatyshyn@bms.com

Co-Authors: Serhiy Hnatyshyn, Bristol-Myers Squibb Company, Petia Shipkova, Bristol-Myers Squibb Company Mark Sanders, Thermo Fisher Scientific, Michael Reily, Bristol-Myers Squibb Company

Computational Algorithms for Fully Automated Navigation Through Hyper-Complex High Resolution Accurate Mass LC-MS Datasets

High resolution accurate mass (HRAM) LC-MS measurements are routinely used to qualitatively and quantitatively study the make up of chemical and biological samples. Recent improvements in instrumentation, especially chromatographic advances, detector ruggedness and increased data acquisition rates, have resulted in better peak shapes, peak capacity, resolution and sensitivity. These improvements have enabled the collection of extremely information-rich data sets which cannot be fully exploited with the currently available data processing tools. In order to address this significant gap in the information processing of HRAM LCMS data, Component Elucidator (CE), a collection of data interpretation algorithms constituting a method for fully automated systematic analysis of high resolution data, was developed in house. Recently, BMS has formed an alliance with Thermo Fisher Scientific Inc. to package and commercialize it as a complete product. The algorithm collection addresses all major aspects of LC-MS data processing, signal annotation and statistical analysis. The data processing routines enable reduction of millions of data points to a few hundred real "components" by eliminating noise and combining information from isotopes, adducts, fragments and dimmers into a single data structure. Such computationally obtained data structure is called "component", and linked by accurate mass and retention time to a corresponding unique analyte in the sample. Annotation algorithms process components in each sample to perform the assignment of molecular identities using various knowledge bases and databases. Automatic alignment procedures enable comparison between different samples by generating an output table of annotated components aligned by mass and retention time along with relative abundance in each sample. Finally, the statistical routines apply both univariate and multivariate statistics across the samples in a study, to reveal changes and trends that can be correlated with various biological or analytical endpoints. Data from HRAM LC-MS analysis of in-vivo rat plasma samples will be presented to demonstrate the utility of developed algorithms for multiplexed detection and quantitation of xenobiotics and endogenous metabolites.

4:30 pm Tuesday, February 1

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Burkhard Schaefer, BSSN Software, b.schaefer@bssn-software.de

An Open Platform for Scientific Applications Based on the AnIML Standard

Today, many laboratory processes rely on sophisticated software tools to evaluate the data produced by experiments. Looking at typical laboratory software packages, we recognize that they have many things in common – no matter which discipline they are used for. A piece of domain-specific software, be it a Chromatography Data System or a Spectral Library Search Tool, needs to contain many common infrastructure components which are only peripherally important. This includes I/O, data management, user interface, navigation, reporting, and other features. Even though these components are not really helping solve the problem addressed by the application, they still need to be present so a user can interact with the application, connect it to instruments, integrate it with other systems, and so on. To a vendor, this infrastructure is tedious to maintain. It's like reinventing the wheel with every application. To an end user, it makes every application look and behave differently, leading to confusion and extra training for every application in use. Additionally, each software package is an island, and integration with 3rd-party systems is tedious and cost-intensive. This talk presents a new open software platform for the creation of sophisticated laboratory informatics solutions. The platform provides a set of core infrastructure services for tedious and repetitive tasks like data management, navigation, visualization, and interactive manipulation of scientific data out of the box. Using a plug-in architecture, the platform is extended with domain-specific scientific features. Third parties can contribute services (functionality) to the platform, while consuming the infrastructure already in place. This allows vendors to focus on those parts of the application that create actual value: instrument control, data mining, scientific algorithms, reporting, or integration with other systems like LIMS or ELN. With such an architecture, users can combine multiple components to put together the features they need to get the job done. All plug-ins can coexist peacefully within the platform, providing a seamlessly integrated experience to the end user. Plug-ins can either implement the functionality directly or communicate with an external system to render the services. This provides an elegant mechanism to integrate multiple different systems and tools into a single user interface. The platform is able to handle multiple different measurement techniques within the same workflow. For the actual data representation, we use the emerging Analytical Information Markup Language (AnIML) standard. This standard provides an XML-based format for results, method data, and sample information. Our implementation of an AnIML-based data container is based on a generic set of software components. These make it possible to handle data for arbitrary laboratory techniques within the same application. The talk presents an overview of the platform architecture and the infrastructure provided. It shows how we used AnIML as a native data format. Additionally, we demonstrate how the platform was used to implement a complete system, integrating multiple instruments (LC-MS), performing domain-specific scientific calculations (chromatographic and spectral processing), LIMS integration, and reporting. The talk concludes with a review of the performance and time savings achieved for the described system.

Informatics—Track 4

9:00 am Wednesday, February 2

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Steve Bolton, Labtronics Inc., sbolton@labtronics.com

Co-Author: Robert Pavlis, Labtronics Inc.

Can SAP be Integrated Into Lab Processes?

There is a growing interest in using SAP or other ERP systems in place of a LIMS. This presentation will review the pros and cons of both options. LIMS is the traditional solution for managing samples in a lab. As companies deploy their ERP more fully in the rest of the business, senior management is asking to also interface the lab to the ERP system. In the past the functionality in ERP was not sufficient but in recent years these systems have improved. SAP was investigated and evaluated as a LIMS substitute. This presentation will present these findings. It will also examine informatics tools that can be used to simplify the transition away from LIMS to ERP.

9:30 am Wednesday, February 2

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Mark Yuzuk, Bristol-Myers Squibb Company, mark.yuzuk@bms.com

Accelerating Adoption of Collaboration in R&D

Collaboration is a concept repeatedly discussed in organizations. Benefits are promising, yet collaboration is seldom practiced. So what is the problem? The lack of a shared definition is one barrier. Additionally, the complexity of collaboration and the skills required to facilitate the process are formidable. Emerging tools, new processes, and changing behaviors are all areas that need to be addressed. As more and more work is done outside of the organization with key partners, defining and executing on a collaboration strategy is becoming more important both to individuals and team effectiveness across the organization. Much of the literature on collaboration describes what it should look like as an outcome, but little is outlined on how to practically approach the process of enabling collaboration. This discussion will be focused at how to accelerate adoption of new collaboration capabilities, common challenges experienced, and approaches to practically leverage collaboration best practices in your teams and working with your partners.

Informatics—Track 4

10:00 am Wednesday, February 2

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

Erik Rubin, Bristol-Myers Squibb Company, erik.rubin@bms.com

Co-Author: Harold Weller, Bristol-Myers Squibb Company

Toward a Distributed Research Model for Effecting Productivity Improvements in R&D

Effecting technology-based productivity improvements in research labs has become increasingly challenging due to financial pressures to reduce capital investments, strategic corporate changes (including mergers and acquisitions) that make prediction of future workload and demand difficult, and introduction of disruptive technologies which fundamentally change the way research is performed. As a result of these pressures, a flexible work model will be required in the future to minimize long term investment (and thus risk), allow dynamic scaling and balancing of work output, and adapt quickly to strategic or technical changes. This prospective model may have many attributes of a typical marketplace as work, investment, and risk become distributed among marketplace partners. Conventional outsourcing models are now common and may represent the first step in a broader transformation to a true research marketplace. Some of the challenges presented by the distributed model include assessing and maintaining product quality, establishing intellectual property records and rights, maintaining key skills with a distributed staffing model, and maintaining coordination among marketplace providers with varying internal IT systems. We will describe our vision of this model, initial steps we have taken toward distributed work, and techniques used to address the challenges.

10:30 am Wednesday, February 2

Location: Mojave Learning Center, Renaissance Palm Springs Hotel

John McCarthy, Accelrys, john.mccarthy@symyx.com

Analytical Science Collaboration From Lab to Plant: Using an ELN to Streamline the Exchange of Analytical Methods Between the Laboratory and QA/QC Systems

From early R&D to manufacturing the analytical lab provides an essential service to biologists, chemists, (bio)process, and (bio)formulation scientists. This presentation will show how software suppliers and industry are working together to accelerate information and products from lab to plant through gains in process efficiency and reduced compliance burden. Accelrys, the provider of Symyx Notebook, has developed a highly collaborative Electronic Lab Notebook (ELN) designed to optimize the flow of materials and information between analytical scientists and dependant scientists within their network. Partnering with Velquest, Accelrys has developed a mechanism to transfer analytical methods utilizing industry standards such as S88, BatchML, and B2MML. The resulting electronic laboratory environment enables rapid method development/optimization, method validation, and transfer of methods into highly regulated, quality controlled method execution environments within pilot and manufacturing plants.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

10:30 am Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Nathan Hillson, Joint BioEnergy Institute, njhillson@lbl.gov

j5: Scar-Less Multi-Part DNA Assembly Design Automation

Standardized DNA assembly protocols (e.g. BioBricks) facilitate the cloning process and promote part reuse. However, these standardized methods often introduce undesirable scar sequences and limit the combinatorial complexity of the derived plasmid libraries. Complementary protocols, including scar-less multi-part SLIC/Gibson/CPEC and Golden-gate, do not suffer those limitations, but require time-consuming DNA oligo design and validation processes that safeguard against homologous repeat-induced incompatibilities. j5, a DNA assembly design tool, along with DeviceEditor, a Synthetic Biology drag-and-drop graphical design canvas, provide web-based automation of these scar-less multi-part assembly protocols. The forthcoming integration of j5 and DeviceEditor with repositories of biological parts (e.g. the JBEI Registry), as well as liquid-handling robotics and microfluidics platforms, promises to reduce the labor and error-prone tedium of the cloning process, allowing researchers to focus on the design and assay of biological devices, rather than on their construction.

11:00 am Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Yaeta Endo, Ehime University, yendo@eng.ehime-u.ac.jp

RIBOENGINE: Wheat Germ Cell-Free Protein Production System for Rapid Functional and Structural Genomics Screens and Scale-Up

Recent advances in the ability to generate genomic data has made selection of appropriate expression systems a critical bottleneck for post-genomic studies, functional validation and structural studies. Although a variety of cell-based expression systems have been widely used for a long time, all of them have significant inherent limitations in terms of their adaptability to be implemented in high-throughput screening and production system as well as the quality of proteins produced. Many of these limitations can be circumvented by the use of cell-free translation systems. Among them, the wheat germ based system due to its eukaryotic nature has significant advantage for producing functional multi-domain proteins. We have established two parallel processes for our highly productive wheat germ cell-free system, one for genome-wide small-scale protein production in order to biochemically annotate genetic information and the other for large-scale production for structural biology and biocatalysts. These protocols have been successfully incorporated into custom automation platforms: GenDecoder -genome-wide functional analysis of protein. This integrated platform has three robotic arms for fluid and lab-ware transfers, integrated incubator for transcription and translation, centrifugation for mRNA recovery and concentration and is capable of producing 384 individual proteins overnight. The scaled-up platform -ProtemistDTII is also capable of fully automated transcription, translation, and in addition performs protein purification for up to six proteins at large scale (200 µg). A follow-up model, ProtemistXE designed for even larger-scale is capable of continuous production through “Filter-and-Feed” and typically yields a gram of protein in 24 hours. I will discuss some our recent results and present a perspective on the future technology development and implementation.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

11:30 am Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Masood Hadi, Sandia National Labs, mzhadi@sandia.gov

Cellulosic BioFuels: Automation Challenges for Enzyme Test Bench

Climate concerns, finite supply of traditional fossil fuels and national security issues have been the driver for producing liquid transportation fuels from renewable sources. Lignocellulosic biomass is a potential renewable source of fermentable sugars that can be converted into biofuels and other high value biopolymers. A number of technologies have been developed over several decades to facilitate this deconstruction process but most of them are still in experimental scale and until now this process has been considered too expensive to compete with fossil fuels. Typically after harvest, biomass is reduced in size and then treated to allow access to lignin-cellulose. This pretreated solid suspension is then mixed with cellulolytic enzymes to release sugars that can be fermented into biofuels. Most of the cellulolytic enzymes discovery and engineering/ optimization efforts utilize artificial substrates and the resulting "improved" enzymes exhibit reduced performance on real world substrates. In order to engineer enzymes on real world substrates, automated high throughput technologies need to be deployed in the context of a research environment followed by validation in the application space. To this end we have developed an automation platform and increased throughput. We discuss some of our processes to date and present results. This work was part of the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U. S. of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000

12:00 pm Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Andy Thompson, Aurora Algae, athompson@aurorainc.com

Co-Author: Jon Marshall, Aurora Algae

An Automated Method of Preparing Algae Samples for Analysis of Fatty Acid Profile and Content

Determining the fatty acid profile and content of an algal strain is an important task for producers of biofuels or other lipids from algae. Fatty acid profile provides a basis for strain selection; it also aids evaluation of conventional aquaculture techniques and provides insight into lipid biosynthesis pathways. Traditional methods for preparing algae samples for fatty acid analysis using gas chromatography involve extracting lipids from biomass samples using solvent mixtures, then removing potential interferences using liquid-liquid extractions before derivatization and analysis. These methods are labor-intensive, and often are the bottleneck in an analysis pipeline. In addition, traditional methods typically require large amounts of material, and can use relatively large quantities of solvent. We present an automated method for preparation of algae samples for fatty acid analysis capable of preparing samples for GC analysis at a rate increased by almost an order of magnitude, while demonstrating recoveries comparable to those achieved by slower, more traditional methods and requiring a smaller amount of solvents. This method uses an automated liquid handler for direct sample derivatization, followed by extraction of the fatty acid methyl esters produced into a matrix suitable for injection into the GC. Coupled with fast gas chromatography, this method of automated sample preparation can be used to provide rapid, quantitative assessment of the fatty acid content and profile of an algal culture. It can also be used as a part of a higher-throughput screen used to identify cultivars with desirable fatty acid profiles or to support research into lipid biosynthetic pathways.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

3:00 pm Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Lei Tian, University of Illinois, lei-tian@illinois.edu

Near-Real-Time Field Sensing Systems for Precision Agriculture

The development of a tower mounted, near-real-time field monitoring and imaging system. This system is a high throughput and reliable remote sensing platform that can precisely and timely capture the signatures of different crop stresses. The geo-referenced images obtained from this system have the advantage of locating spatial variation in water stress, salinity, and nitrogen deficiency, and other growth conditions of corn, soybeans, maize and potential biofuel feedstocks. Another system is the application of an "Unmanned Aerial Vehicle (UAV) Image Acquisition Platform" for a large scale field sensing operations.

3:30 pm Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Catherine Piper, Syngenta, catherine.piper@syngenta.com

Formulating for the Future—High-Throughput Formulation Opportunities in Agrochemicals

Syngenta is a world leading agrochemical company with a broad portfolio of active ingredients (AIs) and a successful track record of inventing new ones. By leveraging both its formulation expertise and breadth of portfolio, Syngenta can offer growers multi-AI mixture products that are safe, convenient and cost-effective. To deliver these benefits, formulations have become increasingly sophisticated while at the same time there has been a drive towards faster delivery and lower product costs. We are at the limit of what we can deliver with conventional formulation technology but high-throughput formulation (HTF) screening offers a step-change in formulation capability to enable us to meet these new challenges. High-throughput formulation screening methodologies are not new, and in many industries have not delivered the promised benefits, however formulation – whether of agrochemicals, pharmaceuticals, paints or cosmetics – is ideally suited to this approach and does not suffer from the limitations that have restricted success in other areas such as high-throughput screening of new drug candidates. Formulations – independent of the industry- are multi-component mixtures of known AIs and other commercially available components such as dispersants, emulsifiers, stabilisers and biocides that interact non-linearly. By adopting HTF screening, hundreds of formulations can be made and tested each day using integrated, automated systems. Diversity (which is difficult to achieve in classical drug compound library screening) is inherently built into experiments through the vast array of formulation components on the market, their range of physical properties and their non-linear interactions. Similarly there is no dependence on the availability of parallel in-vivo testing capability – assessments can be made against key physical parameters that can be measured via automated technologies and which are directly applicable to end product quality, for example, shelf-life and user convenience. The benefits of HTF include opportunities for the development of more complex and more robust products through exploration of broader design spaces. Syngenta has a clear focus on development of new crop product products but HTF technology offers a fundamental enhancement in formulation capability that companies across diverse industries are now actively pursuing.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

4:00 pm Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Kevin Deppermann, Monsanto Company, kevin.l.deppermann@monsanto.com

Harvesting Innovation by Accelerating Ideas: Engineering and Scientific Solutions for Plant Breeding

Remaining on the forefront of developing new technologies and implementing high throughput, scalable solutions is critical when accelerating a new seeds and traits pipeline. By harvesting and creating a network of scientific and engineering innovation, revolutionary ideas can be realized which can lead to significant advancements in plant breeding. Supporting technology advancement in research and breeding requires the development of innovative lab instrumentation and field equipment for high throughput seed and plant analyses and processing. The ability to effectively screen individual seeds and plants for genetic and phenotypic information, understand and map each seed or plant's performance, while processing associated information throughout the breeding process at the same rapid pace, creates the cornerstone in developing new products that are valuable to agriculture. This presentation will focus on technological developments in new equipment and lab processes that increase process efficiencies, throughput capacities, and reduce safety issues, process costs, and labor required. Also covered will be new methods that expand detection technologies and processes in analytical practices as related to seeds and plants.

4:30 pm Monday, January 31

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Jason Downing, Illumina, Inc., jdowning@illumina.com

Integrating High-Throughput Systems Into Commercial Bovine Genomics

Cattle breeding is undergoing a paradigm shift from searching for animals to searching for genes and genetic loci responsible for valuable traits. Breeders are extracting more value from their herds by genetically testing and selecting for preferable traits. This paradigm shift is being brought on by revolutionary new tools that enable scientists, animal breeders and producers to screen large populations and assess genetic variation on a genome-wide basis. The transition from phenotypic observation to array based genotyping analysis in the commercial bovine market drives the need for control systems and the integration of automation, sample tracking, and management system to ensure data integrity.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

10:30 am Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Mike Luther, David H. Murdock Research Institute, mluther@dhmri.org

Co-Author: Mary Ann Lila, North Carolina State University

Biomarkers at the Intersection of Agriculture, Nutrition and Human Health

Biomarkers are the critical indicators required for objective evaluation of high-value traits and responses in plant, animal and human clinical research. They signal biological events, or provide measures of exposure, composition, response, or susceptibility. In the wake of new biotechnologies including genomics, epigenetics, transcriptomics, proteomics, metabolomics, glycomics, nutragenomics, imaging and meta-analysis, biomarkers provide evidence-based direction to navigate through evaluations of pharmaceutical or natural products that impact human health and wellness. Sensitive, cutting-edge technology platforms coupled with high performance computing and data analysis are now available to monitor, with an unprecedented degree of precision, biomarkers of potent therapeutically-active phytochemicals in plants, or of disease susceptibility, diagnosis, or treatment response in animal and human systems. By integrating our analyses of these complex and previously unintelligible or intractable biomarkers, and the wealth of insights acquired by probing genomic/epigenetic, proteomic and metabolomic status, science can now, for the first time, begin to interpret how functional foods and phytopharmaceuticals interface with human pathophysiology at the molecular level to protect or enhance health and metabolism. Many of the same approaches applied to pharmacogenetic and pharmacogenomic responses for drug therapies can now be adapted for the plant and food arenas. This talk focuses on the intersection of technologies and approaches for these trans-disciplinary studies with examples of ongoing work.

11:00 am Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

John Kotyk, Washington University, kotykj@mir.wustl.edu

Co-Authors: Randall Rader, Brad White, Monsanto, Kevin Depperman, Keith Cromack, Monsanto

Translation of Clinical Imaging Technologies Into Plant Sciences

An increasing need exists in agricultural sciences to deliver new and improved approaches that can rapidly measure biologic, genetic and phenotypic properties and traits in plants and seeds. Adaptation of clinical and pharmaceutical imaging methods can help address these needs and provide unique opportunities to obtain information on the development, structure, function and composition of plants and seeds, including such things as water and nutrient acquisition, stress resistance, and the study of pathology and disease. Translation of clinical imaging ideas and technologies into the agricultural domain brings with it many new advantages/challenges, not unlike those faced by the more traditional preclinical-to-clinical translation of imaging-based biomarker development and qualification in the pharmaceutical sciences. In this presentation, a connection between clinical imaging approaches and agricultural sciences will be presented. Existing high-throughput (~100 seeds/min) methods will be discussed that non-destructively measure oil content in intact seeds using a 1.5T magnetic resonance imaging (MRI) clinical MRI scanner. Recent collaborations between Monsanto and the Center for Clinical Imaging Research at Washington University also will be presented that demonstrate the use of new state-of-the-art clinical imaging modalities such as, spiral computed tomography (CT) and 3T MRI. Initial CT studies demonstrate the ability to visualize and quantify complex 3D root structure, extract metrics (length, depth, diameter and volume), monitor growth and measure water uptake in real time. Similarly, preliminary in vivo MRI studies of whole, intact plants provide measures of plant structure and map water properties at a microscopic levels. Integration of automated data management and image analyses practices used in the clinic also can significantly enhance and improve efficiencies in an agricultural science paradigm. The combination of clinical imaging equipment (a detector), the clinical imaging protocol (a standard operating procedure), and the clinical imaging data management/analyses tools (an infrastructure) offers many new, exciting and novel opportunities when applied in plant sciences. The ability to translate or to cross-fertilize between these two seemingly different disciplines provides new insights that enhance agricultural research and development and can lead to more rapid improvements in seed development, crop yield and, ultimately, positive impacts on animal/human nutrition and health.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

11:30 am Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Randall Pearson, Southern Illinois University Edwardsville, rapears@siue.edu

Applications of Remote Sensing and Automated Imaging for Field-Based Agriculture

Remote sensing and digital image processing are not new tools in agriculture. In fact, these high-tech tools have been experimented with and ultimately employed in agriculture since the 1960s. Even so, incredible advancement in the technologies themselves (computers, airborne and satellite-based sensors, image processing and information extraction algorithms, etc.) has given growers, field scouts, government agencies, and agricultural companies new methods with which to view, analyze, and understand this ever changing world of agriculture. This talk will address these advancements over the last half-century by looking back at "what was", taking a current look at "what is", and finally speculating a bit on what "may be". More specifically, this talk will discuss: 1) advancements in sensors and imaging systems (both airborne and space-based), 2) advancements in information extraction processes, and 3) actual uses of remote sensing and image processing technologies at various scales.

12:00 pm Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

David A. Somers, Monsanto Company, david.a.somers@monsanto.com

Co-Authors: Michael T. Mann, Douglas C. Boyes, T. Michael Spencer, Michael W. Petersen, Brian J. Martinell, Monsanto Company

Automating Plant Transformation

Crop plant transformation systems are comprised of the following requisite steps: 1) introducing DNA followed by integration into the genome of a plant cell, 2) selection of the transformed cell, and 3) regeneration of the selected cell into a whole plant. These steps are usually performed by personnel skilled in cell biology and often require precise hand manipulations. This presentation will cover strategies for and experiences with applying automation to transgenic plant pipelines with the aim of increasing system throughput and capacity while reducing labor and costs associated with production of transgenic plants.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

3:00 pm Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Daniel Lim, University of South Florida, Lim@usf.edu

Co-Authors: Elizabeth A. Kearns, Stephaney D. Leskinen, Joyce M. Stroot, Kelly M. Leach, Sonia Magaña, Sarah M. Schlemmer, Dawn M. Hunter, University of South Florida

Integrated Rapid Sample Processing/Detection of Waterborne and Foodborne Pathogens

Rapid identification of bacterial contaminants in food and water is important in preventing foodborne and waterborne illness. Various detection methods and instruments have been developed in recent years to identify microbial pathogens, but their detection limits typically require time-consuming sample processing, culture and/or enrichment of the target pathogens for detection. Sample processing remains the Achilles' heel for rapid detection. Our laboratory has developed a Portable Multi-use Automated Concentration System (PMACS) that rapidly concentrates microbial pathogens from large volumes of water such as potable water and produce wash for subsequent identification by conventional and emerging technologies. E. coli O157:H7, enterococci, Bacillus spores, Cryptosporidium parvum oocysts, MS2 bacteriophage, and other types of microorganisms can be concentrated at low levels from potable water, recreational water and produce wash, and identified by techniques such as electrochemiluminescence and ATP-bioluminescence in 3 to 4 hours. The PMACS protocol not only provides a larger, more representative sample for microbial contamination testing, but also rapidly concentrates pathogens to significantly reduce the time from sample collection to specific pathogen identification and quantification. Such rapid detection could significantly reduce morbidities and mortalities from foodborne and waterborne diseases and eliminate the need for extensive, expensive product recalls.

3:30 pm Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Pierre Varineau, Advanced Analytical Technologies, pvarineau@aati-us.com

Co-Authors: Steve Siembieda, Deepak Dibya; Advanced Analytical Technologies

Parallel Capillary Electrophoresis With Fluorescence Detection for Sensitive, Reproducible and Automated Analysis of DNA

Gel electrophoresis is a primary method for the analysis of DNA fragments, and is frequently used for the genotypic identification of genus or species of organisms using techniques such as AFLP, RAPD, and rep-PCR, as well as older techniques such as the RFLP based Southern Blot technique. AATI has developed new simplified, automated 96-channel parallel capillary electrophoresis instruments that are used for a wide variety of DNA fragment analysis techniques, including routine PCR validation, general fragment analysis, and species/genus/strain identification of organisms based on the pattern recognition techniques. These highly multiplexed capillary instruments offer significant advantages over gel-slab analysis in terms of data quality, ease of use, operability, and automation. In addition, AATI has developed a 12-channel capillary instrument for performing genus/species identification based on semi-sequence analysis of 100-500 base-pair fragments. This new method offers advantages over traditional Sanger sequencing such as ease of use, automation, and the ability to use multiplexed PCR amplification for identification. Results of a variety of DNA fragment analysis and species identification studies will be presented.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

4:00 pm Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

May Chiu, GeneFluidics, Inc., mchiu@genefluidics.com

Co-Authors: Scott Fall, Matthew Wu, Matthew Davis, Genaro Sepulveda, Vincent Gau, GeneFluidics, Inc.

Automated Pathogen Identification and Simultaneous Antimicrobial Susceptibility Testing in 2 Hours

Quickly identifying a pathogen and a suitable antimicrobial drug is essential in the effort to treat infection. Antimicrobial drugs are commonly administered before susceptibility is determined which can lead to life threatening complications in patients who are especially compromised. Currently antimicrobial susceptibility testing (AST) relies on overnight cultures, and a doctor may not have the results for 48 to 72 hours. The ability to rapidly identify a pathogen and determine its antimicrobial susceptibility would improve treatment and patient outcome greatly. A novel automated approach has been demonstrated to simultaneously identify pathogens genotypically and determine susceptibility to antibiotics phenotypically. The approach is based on electrochemical detection which makes the process of identification and susceptibility determination possible in as short as 2 hours. For pathogen identification a genotypic approach is advantageous, however for determining resistance or susceptibility to an antimicrobial drug a phenotypic approach is most accurate, since resistance can be built through multiple genetic pathways. The automated Proteus Molecular Analysis platform allows species-specific detection based on a panel of 16 electrochemical sensors treated to detect the concentration of specific 16S rRNA from a bacterial pathogen. This allows specific genotypic identification. To detect antibiotic susceptibility, our approach takes advantage of the platform's quantitative detection of genetic material along with the increase in 16S rRNA concentration as the pathogen population grows. The electrochemical sensor is able to distinguish susceptibility from resistance within 60 minutes in a culture of E. coli in Mueller Hinton broth. Two strains were grown in the presence of 32 µg/mL of ampicillin to determine susceptibility, pACYC184 (ampicillin susceptible) and pUC19 (ampicillin resistant). The inoculum concentration of each strain was 1x10^5 CFU/mL which resulted in electrochemical sensor signals of 8.0 nA and 8.4 nA, respectively. After 60 minutes of growth in culture the ampicillin susceptible strain showed 9.5 nA and the resistant strain showed 16 nA. After 90 minutes the signals were 8.9 nA and 71 nA respectively. Signal current has been shown to scale linearly with CFU count, so this shows non-growth in the susceptible strain and growth in the resistant strain. The method of electrochemical detection of genetic material benefits from high sensitivity and no purification requirements, and hence genetic material can be quantitatively detected directly from culture media without any target amplification or purification steps in a fully automated environment. During the culture incubation, sensors can be functionalized with specific capture probes for pathogen identification. At the conclusion of the culture incubation period, a genetic assay can be completed in another 60 minutes using the cultured samples for both AST and pathogen identification.

4:30 pm Tuesday, February 1

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

David A. Basiji, Amnis Corporation

Co-Authors: William Ortyn, Thaddeus George, Raymond Kong, Amnis Corporation

Imaging Flow Cytometry for High Content, High-Throughput Food Safety Assessment

The ImageStreamX imaging flow cytometer combines the speed and statistical power of conventional flow cytometry with the information content of fluorescence microscopy. The technology images cells and particulates directly in fluid suspension at speed exceeding 1000 cells per second and simultaneously produces up to 12 high resolution images per cell. The suspension format is a natural fit for the analysis of water, milk, and other liquids, as well as rinses of other foodstuffs. The imagery consists of a combination of brightfield, darkfield, and multiple fluorescent colors to provide a weath of morphologic, textural, and organism-specific fluorescent information from each cell in the sample. The speed of the technology allows it to analyze even rare species in the presence of overwhelming numbers of background organisms and debris. This talk will present several examples of bacterial analysis on the ImageStream platform of relevance to food safety testing.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

9:00 am Wednesday, February 2

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Nathan Lawrence, Pressure Biosciences

The Challenges of Automating Sample Preparation in the Proteomics Era

Sample preparation is particularly challenging in proteomics applications. Unlike DNA, which can be amplified, trace recovery of protein risks the exclusion of extremely low abundance proteins of biological significance from the analysis. The efficient release and recovery of the total protein constituencies of tissues and cells thus becomes a critically important initial step in most analytical processes, and is essential to reliable proteomic analyses. However, sample preparation is complicated by the intrinsic diversity of proteins, which can result in bias for or against specific protein subpopulations (e.g. membrane proteins) leading to an inaccurate representation of the proteome. The complexity of proteomes is further compounded by the broad concentration range over which proteins are expressed, as exemplified in human plasma where the mass of albumin is nearly 10 billion times greater than that of cell-signaling proteins like the interleukins. The disruption of cells and tissues can be accomplished by methods such as sonication, bead beating, homogenization and French press, all of which are disruptive but are capable of generating heat and denaturing the proteins. This is undesirable for downstream applications where protein conformation and biological activity need to be preserved (e.g. the disruption of epitopes required for antibody recognition). The use of intermittent cooling to offset heat during sample preparation may produce thermal fluctuations that, if not carefully controlled, may compromise experimental reproducibility and have deleterious effect. A pressure cycling technology (PCT) has been described that uses maximum pressure of 5,000 to 45,000 psi without imposing sheer forces. The rapid oscillation between two pressures has been shown to be more disruptive to cells than sustained high pressure. Moreover, pressure cycling exploits opposing adiabatic processes, since rapid decompression is an exothermic process which offsets temperature increases resulting from compression.

9:30 am Wednesday, February 2

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Reed Kelso, Bionex Solutions, reed@bionexsolutions.com

Co-Authors: Ben Shamah, Mark Sibenac, Dave Matsumoto, Tony Lima

A Revolutionary Automated Pipetting Paradigm

In order to keep pace with the demands of high throughput liquid handling operations we have conceived of a device which allows for random cherry pick operations with pick rates fewer than two seconds per transfer. Our combination of novel channel and plate movements allow the device to pick from random location and place into any plate mapping configuration (row, column, other) without any impact on the throughput of the device. The novel motion architectures combined with a flexible random access storage system combine to create an automation platform capable of processing over 40,000 randomly distributed samples per day. Additionally we have non-contact liquid level sensing technology to instantaneously detect liquid levels and determine if liquid transfers have successfully executed. The combination of these technologies has resulted in a flexible hit-picking platform that offers throughput rates that could previously only be accomplished by implementing multiple hit-picking systems.

Evolving Applications of Laboratory Automation, Featuring Agriculture and Food—Track 5

10:00 am Wednesday, February 2

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Tza-Huei "Jeff" Wang, Johns Hopkins University, thwang@jhu.edu

Co-Author: Yi Zhang, Johns Hopkins University

A Droplet Microfluidics Based Miniaturized Total Analysis System for Point-of-Care Molecular Diagnostics

Introduction Being able to perform molecular diagnostics at the point of care (POC) in a resource-scarce environment is crucial for making timely clinical decisions and therapeutic interventions, hence resulting in better clinical outcomes. However, total analysis from crude biosamples remains challenging because such a multi-step process is very resource demanding and limited to centralized laboratories. Therefore, a miniaturized total analysis system (μTAS) for POC that is able to operate with minimum resource requirement and has the sample-in-answer-out capability is highly desired. Methods We have developed a digital droplet microfluidics μTAS that integrates a droplet sample preparation and reaction chip, a magnet actuation stage, a temperature controller and a miniaturized fluorescence detector onto a single handheld platform. The microfluidic device is self contained, with all reagents being stored in droplets, thereby eliminating the external fluidic coupling. The platform incorporates unique surface topological features to facilitate magnetic droplet manipulation. Silica superparamagnetic particles (SSP) are employed for both magnetic actuation and solid phase extraction. The amplification reaction is performed in the droplet and monitored with the miniaturized fluorescence detector in real time. The fluorescence detector excites the sample with an LED and amplifies the photodiode detected signals with a lock-in amplifier, allowing the operation under ambient light. Results We have demonstrated the sample-in-answer-out capability of the proposed μTAS. Samples are dispensed onto the platform and the subsequent cell lysis, DNA extraction, amplification and detection are carried out in droplets with the assistance of SSP. The SSP provides the functional substrate for DNA adsorption and the actuation force for droplet movement, enabling the buffer exchange and material transfer by splitting and remerging the SSP with the droplets. Finally, the DNA is eluted in the reaction buffer droplet where the target sequence is amplified and detected in real time with the integrated fluorescence detector. We have shown the surface topology assisted droplet manipulation eases the SSP splitting, hence offering wider volume range and more flexible operation. As such, our platform allows the entire analysis to be done without external reagent supply or peripheral fluidic delivery apparatus, making it ideal for POC molecular diagnostics. We have successfully detected the remodeling and spacing factor (Rsf-1) gene, which is a promising biomarker for ovarian cancer, from crude whole blood samples using real time polymerase chain reaction (PCR) and helicase dependent amplification. Melting curve analysis has been performed to verify the amplification specificity in both cases. We have also successfully applied the μTAS to identify E.coli from the crude samples using a TaqMan based sequence specific real time PCR assay. Conclusion Combining the advantages of digital droplet microfluidics, magnetic actuation and integrated temperature controller and fluorescence detector, we have developed a μTAS ideal for POC applications.

10:30 am Wednesday, February 2

Location: Sierra/Ventura, Renaissance Palm Springs Hotel

Veit Bergendahl, Miltenyi Biotec GmbH, VeitB@miltenyibiotec.de

High-Throughput Applications for Magnetic Separations in Molecular Biology and Cell Culture

Recent advances in cell therapy and biopharmaceutical processing have called for new automated solutions for cell culture, cell separation and analysis of cells. MACS technology has filled a significant niche in the field of magnetic sample preparation even beyond cell separation and is continuing to add new technologies for the purpose of advancing biomedical research and cellular therapy. We will present automated solutions developed at Miltenyi and with customers, for multi-channel cell separation, enhanced biomolecule purification (e.g. mRNA separation with on-column cDNA synthesis) and new flow-cytometric HTS applications. We will describe how these solutions have been integrated into typical liquid handling and automation platforms and how the field can profit from these applications. Furthermore we will discuss the next challenges and propose new solutions in this growing field of biomedical research.

Industry-Sponsored Workshops

Monday, January 31, 12:30 – 2 pm

Agilent Technologies, Inc.—Booth 329—www.agilent.com/chem

Location: Mesquite AB, Palm Springs Convention Center

The Newest Tools Today for Tomorrow's Automated Laboratory
Agilent Technologies introduces two revolutionary new products: the Bravo for Protein Purification and the BenchBot robot for assay automation. The Agilent Bravo for protein purification leverages the AssayMAP micro-chromatography technology to enable highly parallel and precise antibody purification and titer. Come learn about micro-chromatography, hear real-world examples of the AssayMAP technology in action, and learn how the Bravo enables high-throughput bioprocess development. Learn how the Agilent BenchBot robot revolutionizes the set up and use of small to medium sized assay development workstations, offering unmatched flexibility, throughput, and ease of use.

Artel—Booth 551—www.artel-usa.com; Tecan—Booth 305—www.tecan.com

Location: Smoketree DE, Palm Springs Convention Center

New Validation and Optimization Guidance for the Tecan Freedom EVO Liquid Handling Platform Using ARTEL MVS® Multichannel Verification System
The Artel MVS Multichannel Verification System provides an automated, commercially available tool that significantly reduces the time needed to verify and optimize liquid handler performance. Together with Tecan US, Artel demonstrates the use of the MVS technology as a tool for validating the performance of Tecan Freedom EVO liquid handlers with a variety of equipment configurations (i.e. tubing sized, syringe sizes, fixed tips and disposable tips). In addition, detailed methods for testing of the Freedom EVO platforms are discussed. Workshop participants learn about the latest capabilities of the Artel MVS technology as well as the capabilities of the Freedom EVO Liquid Handling Platform including specific guidance for performance optimization and QC testing.

Beckman Coulter, Inc.—Booth 217—www.beckmancoulter.com

Location: Santa Rosa, Renaissance Palm Springs Hotel

Next Generation Sequencing Sample Prep Solutions
Beckman Coulter's Continuum of Solutions for Manual to High-Throughput NGS Sample Prep Processing. This workshop provides a preview of Beckman Coulter's products and services to simplify and automate your NGS workflow, and will provide data generated from currently available sequencing platforms. SPRI chemistry is proven for use in constructing quality fragment libraries for major NGS platforms. This chemistry, currently available in convenient prefilled cartridges for low to medium throughput, will also soon be available in a kit format that will provide flexibility for high-throughput automated or manual processing. Combined with Biomek automation, the new kits will enable the efficiency and reliability for high-throughput library construction that customers expect from Beckman Coulter.

BioTek Instruments, Inc.—Booth 151—www.biotek.com

Location: Mesquite E, Palm Springs Convention Center

Automation of Cell-Based Assays
This workshop demonstrates the automation of a number of cell-based assays suitable for non-primary screening applications. The automated liquid handing instruments demonstrated consist of an automated pipetting station that performs serial dilution of small molecule compounds necessary for generating dose-response curves and a combination washer dispenser that carries out cell dispensing, washing, media exchanges and bulk reagent dispensing. The footprint of each is small enough to fit in a standard biological safety cabinet. Applications demonstrated include intra-cellular protein kinase signaling, passive and active drug transport, metabolic inhibition and induction, and toxicity assays.

BMG Labtech, Inc.—Booth 351—www.bmglabtech.com; Hudson Robotics, Inc.—Booth 451—www.hudsonrobotics.com

Location: San Jacinto, Renaissance Palm Springs Hotel

Intelligent Screening Through a Unique Combination of Instrumentation and Software
Hudson Robotics and BMG Labtech, Inc. have created modular automation platforms for a wide range of applications. This workshop shows solutions for cost effective, single-assay platforms such as ELISA screens, or DNA and protein preparation and purification protocols. This workshop also shows how to design more advanced, multifunctional platforms which include detection methods such as TR-FRET and fluorescence polarization; cell-based assays and ADME-Tox studies.

CyBio AG—Booth 345—www.cybio-ag.com

Location Smoketree A, Palm Springs Convention Center

CyBio® Scheduler—Superior Biology Support by Smart Scheduling
Users present their experiences using various CyBio integrated platforms and the powerful features of CyBio's scheduling software. As an introduction the CyBio® Scheduler are presented, highlighting the dynamic abilities of the software, its ease of use and error handling options. CyBio AG has a 20 year history producing robust, reliable and high quality pipetting robots. CyBio has also been an innovator in the field of laboratory automation since the early 90's.

HighRes Biosolutions Inc.—Booth 105—www.highresbio.com

Location: Pueblo AB, Renaissance Palm Springs Hotel

A Modular High-Throughput Automated System for Cell Based and Biochemical Assays
Rigel Pharmaceuticals, a South San Francisco, California-based clinical-stage drug development company, recently added a dual arm NanoCell system from HighRes Biosolutions to their automation platform. This new system works in conjunction with the existing systems to enhance Rigel's capabilities in high-throughput screening of cellbased and biochemical assays against a large library of compounds. This system also includes two HighRes Ambistore high speed plate carousels with high speed barcode scanning capability. Angela Gray, West Coast Director of Sales and Support for HighRes Biosolutions, outlines how HighRes' highly flexible, expandable and modular integrated technology accelerates drug discovery and biorepository science. Christina Coquilla, Senior Manager of Rigel's HTS group, describes the flexibility and capabilities of this system. Ruby Daniel, VP of HTS at Rigel is available to answer any additional questions.

Labcyte, Inc.—Booth 205—www.labcyte.com

Location: Smoketree C, Palm Springs Convention Center

Labcyte Automation for the Echo® Liquid Handler—Introducing a Novel Bench Top Workstation to Easily Automate Any Echo® Liquid Handler Application
The Labcyte Access™ laboratory workstation maximizes the ability of the revolutionary Echo liquid handler to significantly save time and cost while moving life science applications to lower volumes with unmatched precision and accuracy. The operating software allows users to quickly create and load protocols from Echo® software applications then add key automation tasks to create a dynamic and optimize system workflow. Regardless of their experience with automation, researchers can walk-up and instantly use the Access system to automate any Echo liquid handler application on their bench top. Lunch is served.

Schaeffler Group Industrial—Booth 685—www.schaeffler.us

Location: Andreas, Renaissance Palm Springs Hotel

Integrated Motion Systems and Solutions for Laboratory Automation
Cost effective, integrated bearing and drive solutions. Please join us to learn about innovative approaches to the design and selection of cost-effective integrated motion systems with application in laboratory automation equipment. The Schaeffler Group shares with you our unique experience in medical and laboratory systems.

Topics include:

- When and how to select the proper bearing system BearinX® software for sizing calculations
- Mechatronic capabilities within the Schaeffler Group
- Application examples
- RoHS compliant products
- Lubrication selection
- Special materials options

Tecan—Booth 305—www.tecan.com

Location: Smoketree B, Palm Springs Convention Center

Speaker: Jason D. Meredith, Software Product Manager, Tecan

Interfacing with the Freedom EVOware API Using C#
Freedom EVOware's powerful API enables you to integrate EVOware with your LIMS or in-house software. Learn how to use the EVOware API to do the following tasks programmatically: starting EVOware and login, getting & setting variables, starting scripts, generating deck layouts (worktables) and scripts, installing custom error handlers, and hiding the EVOware GUI. Examples are provided using C# programming language. Lunch provided.

Thermo Scientific—Booth 505—www.thermo.com/automate

Location: Mesquite C, Palm Springs Convention Center

Thermo Fisher Scientific host a series of short talks by a number of esteemed guest speakers who presents their perspective on the science and performance of their Thermo Scientific automated systems. A panel discussion will follow.

Speakers:
- Hakim Djaballah—Director HTS Core Facility, Memorial Sloan Kettering Cancer Centre; Cancer Research
- Kyle Lapham—Research Associate, University of California, Blackburn Lab; Diseases of Aging
- Annes Lambert—Senior Experimental Officer, Centre of Excellence for Biopharmaceuticals, U. of Manchester; High Throughput Image Analysis
- Egor Zindy—Senior Experimental Officer, Centre of Excellence for Biopharmaceuticals, University of Manchester; High-Throughput Image Analysis
- Tom Lade—Automation Development Manager, Pioneer Hybrid; High-Throughput qPCR Studies

The Science of Lab Automation—Different Approaches for Different Challenges

Join us to discuss:

- Automating Science, automating workflows
- Design considerations for meeting performance goals
- Developments in Momentum™ automation software
- Examples of Thermo Scientific integrated solutions
- Perspectives on future needs

Join us for a complimentary lunch.

Tuesday, February 1, 7:30 – 9 am

Chemspeed Technologies AG—Booth 181—www.chemspeed.com

Location: Mesquite E, Palm Springs Convention Center

SMOLE©: The Next Step to Pre-Filled Chemicals

Since the early 20th century and despite all the innovation, Synthetic Organic Chemistry basically remained the same. Prior to the reaction, the synthesis has to be designed, the reaction parameter calculated, the solids weighed out, and solid and liquid substances added into the reaction vessel. It is obvious that mis-calculations, wrong dispenses or variation in purity especially with reactive chemicals may occur. After preparation, all tools such as spatulas, balance, transfer pipettes and funnels have to be cleaned. The used and now opened chemicals have to be stored in a specific way or have to be discarded at high costs. All of these is cumbersome, costly, and unproductive work, which bear a potential risk to health and environment but have been unavoidable until now. Chemspeed Technologies with its leading team of scientists and years of experience as pioneers in the field of automated parallel chemistry, developed a revolutionary product for chemical laboratories: SMOLE©. It consists of pre-filled chemicals (no matter what physical state) in a PIN format. The PINs are sealed glass vial sealed containing chemicals packaged in a range of stoichometric amounts (e.g. 0.05, 0.10, 0.20, 0.50, 1.00, 2.00 and 5.00 mmol. Chemicals are now easily available in the correct amount—without calculation, weighing, cleaning and waste management!

Tuesday, February 1, 12:30 – 2 pm

Agilent Technologies, Inc.—Booth 329—www.agilent.com/chem

Location: Mesquite AB, Palm Springs Convention Center

Automated Next Generation Sequencing Library Preparation and Target Enrichment With Agilent's SureSelectXT
Agilent Technologies presents an automated solution for Next Generation sequencing library preparation and target enrichment that provides high quality and high-throughput sample processing capability combined with maximum walk away time.

Beckman Coulter, Inc.—Booth 217—www.beckmancoulter.com

Location: Smoketree DE, Palm Springs Convention Center

Automated Cell Workflow Processes and Analysis by Flow Cytometry
The potential of stem cells in research and therapies is enormous. For example, the propensity of stem cells to differentiate into cell lineages is exploited for drug discovery and for therapeutic regimens. Cellular growth, maintenance and differentiation can benefit from automation and designed experiments to enhance development of specific cell lineages. Accurate analysis of stem cell pluripotency and differentiation is critical to the success of such experiments.

BMG Labtech, Inc.—Booth 351—www.bmglabtech.com

Location: Mesquite E, Palm Springs Convention Center

Quantitative Cell-Based Screening Assays Using the Next Generation HTS Microplate Reader
Many new cell-based HTS assays have emerged that measure receptor responses in endogenous signaling conditions. These fluorescent, luminescent, and time-resolved fluorescent assays require sensitive high resolution bottom reading. Expensive high-content screening instruments that use confocal microscopes are typically required, followed by complex software analysis. Now, quantitative high-resolution cell layer scanning can be easily done using a multifunctional microplate reader. Engineered with the precision of a microscope, the next generation PHERAstar FS has BMG LABTECH's proprietary Direct Optic Bottom Reading. Learn how this unique feature provides unmatched sensitivity in all cell based assays, including GFP/CFP/YFP, GeneBLAzer®, LanthaScreen™, HTRF® Tag-lite®, AlphaScreen®, and PathHunter™.

Eppendorf North America—Booth 251—www.eppendorfna.com

Location: Smoketree A, Palm Springs Convention Center

21 CFR Part II Software Compliance for Automated Liquid Handling Instruments
The objective of this seminar is to educate the participant on the requirements for 21 CFR Part II compliant software on automated liquid handling instruments. Topics includes: requirements for record keeping and subsequent review; legacy data when hardware is being upgraded or replaced; electronic signature requirements; username and password requirements; and company SO and training recommendations and the implementation of different controls used to demonstrate compliance among other discussion points.

Hamilton Robotics and Hamilton Storage Technologies—Booth 405 —www.hamiltoncompany.com

Location: Pueblo AB, Renaissance Palm Springs Hotel

Fitting the Pieces Together: Hamilton's Integrated Solutions for Biobanking Applications
As biobanking applications continue to evolve and radically advance life science research, so do the challenges associated with the latest trends. Join this informative Hamilton Robotics and Hamilton Storage Technologies workshop to learn how our innovative solutions for automated liquid handling and sample storage combine to resolve the puzzle of biobanking.

Labcyte, Inc.—Booth 205—www.labcyte.com

Location: Smoketree C, Palm Springs Convention Center

Redefining Assay Development Utilizing the Echo® Liquid Handler
Miniaturized assay development can be hindered by precise and accurate transfer requirements for solutions of different viscosities, which can be problematic for traditional tip-based liquid handlers. Acoustic transfer with the Echo liquid handler, and software capabilities to allow for any-well to any-well transfer, enables for more efficient and cost-effective assay development. Workflow changes to utilize low volume transfer are discussed for applications such as RNAi, cell based assays, qPCR, and other biochemical assays. Labcyte highlights the capabilities of the Echo liquid handler to assemble assays with volumes as low as 250 nL in 384- and 1536-well formats.

MaxCyte, Inc.—Booth 590—www.maxcyte.com

Location: Smoketree B, Palm Springs Convention Center

Using Scalable Transient Transfection to Improve the Productivity of Drug Screening
The MaxCyte® STX™ Scalable Transfection System enables the rapid development and production of (co)transfected primary cells, stem cells, and cell lines for screening ion channels, GPCRs, and other tough targets, with comparable assay results to stable cell lines and Seamless Scalability™ from small scale (5E5 cells/seconds) to HTS scale (1E10 cells/20-30 minutes).

In this workshop, participants learn the following:

- Ideal characteristics of a transient transfection system for drug screening
- The simple operation of the MaxCyte STX
- How to optimize an assay using the MaxCyte STX
- Case studies demonstrating the performance of the MaxCyte STX.

Tecan—Booth 305—www.tecan.com

Location: Mesquite C, Palm Springs Convention Center

Speaker: Kevin Moore

Emerging Technologies for Dose-Response Assays
This workshop demonstrates emerging technology for improved IC50 determination by bioassay scientists. Data demonstrating the utility in the area of bioassays will be presented showing how time-consuming serial dilution workflows can be simplified in order to improve the speed at which results for drug discovery are obtained. The audience who will be interested in this workshop includes: bioassay scientists and lab managers; fans of new technology, lean practices, and simplicity; and those who wish for alternative IC50 capabilities to improve their research. Lunch provided.

Speaker Index (with page numbers)

Notes