slas 2016

5th annual INTERNATIONAL CONFERENCE & EXHIBITION

JANUARY 23-27 | SAN DIEGO CONVENTION CENTER
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SHORT COURSES | JANUARY 23-24
CONFERENCE | JANUARY 25-27
EXHIBITION | JANUARY 25-27

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TABLE OF CONTENTS

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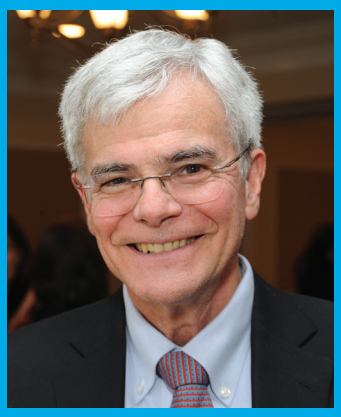
KEYNOTE SPEAKERS

Sponsored by: ThermoFisher SCIENTIFIC

Monday | January 25 | 9:00 am



Michael Gottesman

Chief, Laboratory of Cell Biology, National Cancer Institute

Analyzing the Complexity of Drug Resistance in Cancer

Drug resistance is the major impediment to successful chemotherapy of cancer, but the detailed study of mechanisms of drug resistance in cancer cells reveals the daunting complexity of resistance mechanisms. Our goal is to define as many mechanisms of drug resistance in cancer as we can, and then determine the clinical relevance of these mechanisms so that we can develop means to circumvent or target these resistance mechanisms. Mechanisms of resistance can be cell-based, or may be due to the interactions of cancer cells with their host. Our studies have emphasized cell-based mechanisms, including the expression of energy-dependent efflux pumps for anti-cancer drugs such as the ABC (ATP-binding cassette) transporters, ABCB1 (P-glycoprotein, or P-gp), ABCC1 (MRP), and ABCG2 (BCRP, MXR).

Wednesday | January 27 | 3:45 pm

Adam Diedrich Steltzner

Chief Landing Phase Engineer and Aeronautics Expert, NASA Jet Propulsion Laboratory

The Right Kind of Crazy: A True Story of Teamwork, Leadership and High Stakes Innovation

With a rich and varied background, Adam Steltzner had many of the needed skills to lead the landing team for the Curiosity rover. That said, his team would struggle for almost a decade with design challenges and setbacks.

How did he keep the team focused and on task? What makes a team gel and enables truly innovative thinking? How do team dynamics drive that process forward or inhibit it? And how can organizational culture create an environment for sustained performance? The challenges he and the team faced and the lessons learned from those struggles can help audiences understand how to better lead their high performing teams, manage innovation and drive towards excellence.

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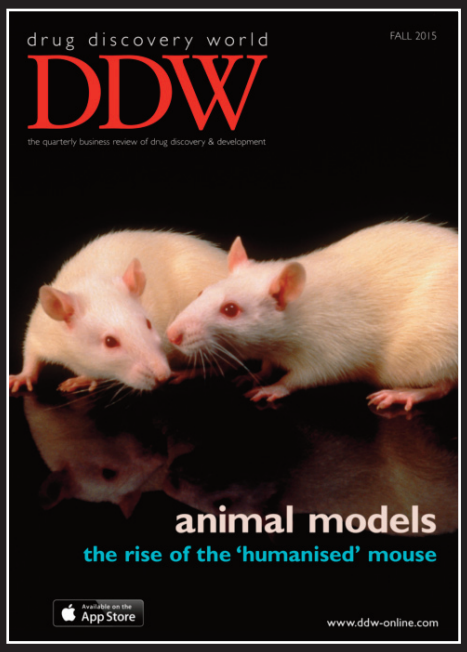

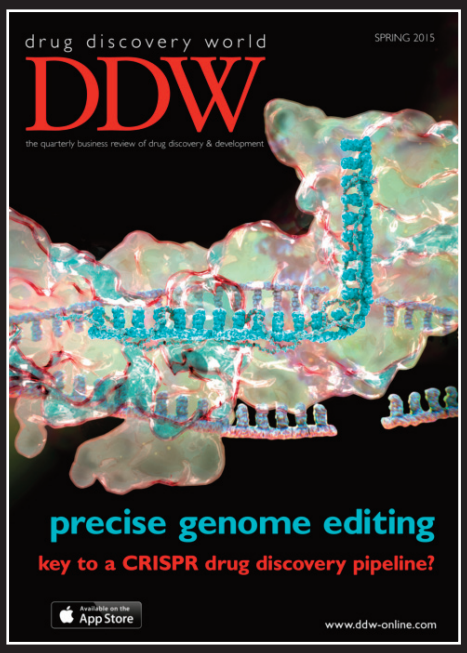

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CONFERENCE-AT-A-GLANCE (Schedule Subject to Change)

SATURDAY, JANUARY 23, 2016	
7:30 am - 5:00 pm	**Registration Open**
8:30 am - 4:30 pm	**Short Courses:** 3D Cell-Based Assays for Drug De-Risking; Introduction to Laboratory Automation; Lab-on-a-Chip: From Technology to Bioanalysis on Chip; Multiparametric Analysis of Scientific Image Data: Laptop Required (NEW) **Two-Day Course:** Getting Started with Excel & VBA in the Laboratory Laptop Required
SUNDAY, JANUARY 24, 2016	
7:30 am - 5:00 pm	**Registration Open**
8:30 am - Noon	**Half-Day Course:** Screening Strategies for Drug Discovery: Matching Tools with Solutions (NEW)
8:30 am - 4:30 pm	**Short Courses:** Affinity-Based, Biophysical Methods for Screening and Mechanistic Studies; Cross Functional Project Management for Technical Professionals; Data Management in the Age of Big Data, Mobile, and the Cloud; Derivation of iPS Cells and Maintenance Techniques of iPS-Derived Cells for Use in High-Throughput Screening and Disease Modeling; Digital Image Processing and Analysis for the Laboratory Scientist: Theory and Application Laptop Required; Establishing Cell-Based Assays for Screening; High-Content Screening: An Introduction to Instrumentation, Assay Development, Screening, Image and Data Analysis (NEW); Lab-on-a-Chip: Case Studies in Diagnostics and Screening (NEW); Liquid Handling Essentials (Interactive Course); Next Generation Sequencing Technology Fundamentals and Applications; Sample Management: Best Practice, Trends and Challenges; Study Design and Statistical Analysis for High-Throughput Screening (HTS) Experiments (NEW) **Two-Day Course:** Getting Started With Excel & VBA Laptop Required
1:00 - 4:30 pm	**Technology to Propel Science: GNF Automation Tour and Seminar**
1:00 - 4:30 pm	**Half-Day Course:** Gene Editing for Drug Discovery (NEW) **Sponsored by:** Advanced Analytical
5:30 - 7:00 pm	**SLAS Student and Early Career Professionals Networking Event at Tin Roof, San Diego**
MONDAY, JANUARY 25, 2016	
6:30 am	**2016 SLAS FUNd Run** **Sponsored by:** Agilent Technologies
7:00 - 8:00 am	**Laboratory Products Association (LPA) Special Session**
7:30 - 8:30 am	**Career Connections:** The Negotiation Process: Dan Eustace, University of Connecticut
7:30 am - 6:00 pm	**Registration Open**
8:00 - 8:30 am	**Mid-Morning Beverage Break**
8:30 - 9:00 am	**Keynote Session: Welcome and Opening Remarks**
9:00 - 10:00 am	**Keynote Speaker:** Michael Gottesman, Chief, Laboratory of Cell Biology, National Cancer Institute; Analyzing the Complexity of Drug Resistance in Cancer **Sponsored by:** ThermoFisher SCIENTIFIC
10:00 - 10:30 am	**Mid-Morning Beverage Break**
10:00 am - 6:30 pm	**Exhibition Open**
10:00 am - 6:30 pm	**Posters on Display**
10:00 am - 6:30 pm	**SLAS Member Center Open**

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Session One	Advances in Bioanalytics, Biomarkers and Diagnostics	Assay Development and Screening	Automation and High-Throughput Technologies	Informatics	Micro/Nano Technologies	
10:30 am - 12:30 pm	**Biomarkers and Translational Sciences** Session Chair: Peter Grandsard, *Amgen*	**Biochemical and Biophysical Screening Assays** Session Chair: Thomas Lundback, *Karolinska Institutet*	**Automating Phenotypic and Target Based Discovery Using Parallel Automated Approaches** Session Chair: Taosheng Chen, *St. Jude Children's Research Hospital*	**Machine Learning to Optimize Experiments** Session Chair: Alex Clark, *Molecular Materials Informatics*	**Digital and Droplet Microfludics** Session Chair: Sindy Tang, *Stanford University*	
10:30 am - 12:30 pm	**Career Connections:** Mentoring Sessions/One-on-One Career Counseling Sessions/Job Boards					
10:30 am - 2:00 pm	**2016 SLAS Leadership Forum (Invitation Only)**					
12:30 - 1:15 pm	**Exhibitor Tutorials:** Artel; BMG Labtech; Nanion Technologies; PerkinElmer; Thermo Fisher Scientific; TTP Labtech					
12:30 - 1:30 pm	**Lunch in Exhibition Hall**					
12:30 - 1:45 pm	**Exhibitor Tutorials:** Brooks Automation; IntelliCyt Corporation; Labcyte Inc.; Thermo Fisher Scientific					
12:30 - 2:30 pm	**Exhibitor Tutorial:** Beckman Coulter Life Sciences					
1:00 - 1:30 pm	**JALA & JBS VIP Meet & Greet:** JALA Editor-in-Chief Edward Kai-Hua Chow, National University of Singapore					
1:00 - 2:00 pm	**Career Connections Workshop:** Negotiating Work and Life; Joanne Kamens, Addgene					
1:00 - 3:00 pm	**Poster Presentations (Odd Numbered Posters)**					
1:30 - 2:00 pm	**JALA & JBS VIP Meet & Greet:** Development of a Highly Sensitive Cell-Based Assay for Detecting Botulinum Neurotoxin Type A Through Neural Culture Media Optimization; JBS Author: David Beebe, University of Wisconsin					
2:00 - 2:30 pm	**JALA & JBS VIP Meet & Greet:** Advancing Scientific Innovation with Acoustic Droplet Ejection; JALA Special Issue Guest Editor: Joe Olechno, Labcyte					
2:00 - 2:45 pm	**Exhibitor Tutorials:** Cellular Dynamics international; Eppendorf; Genedata, Inc.: InSphero, Inc.; IonField Systems; Molecular Devices; Multispan; SiLA Consortium; Universal Robots USA, Inc.					
2:00 - 5:00 pm	**Career Connections in SLAS:** Mentoring Sessions/One-on-One Career Counseling Sessions/Job Boards					
2:30 - 3:00 pm	**Afternoon Beverage Break**					
Session Two	**Advances in Bioanalytics, Biomarkers and Diagnostics**	**Assay Development and Screening**	**Automation and High-Throughput Technologies**	**Informatics**	**Micro/Nano Technologies**	**Special Sessions**
3:00 - 5:00 pm	**MS and Other Label-Free Bioanalytics for Screening** Session Chair: Wilson Shou, *Bristol-Myers Squibb*	**Cellular Biosensors and Genome Editing in Screening Assay Design** Session Chair: Jim Inglese, *National Institutes of Health*	**High-Content and High-Throughput Automation** Session Chair: Jason Matzen, *Genomics Novartis Institute of the Research Foundation*	**Data Visualization: Expert Data for Non-Experts** Session Chair: Christopher Lipinski, *Melio Discovery*	**Microphysiological Systems** Session Chair: Bobak Mosadegh, *Weill Cornell Medical College*	**Biobanking: Evolving from Managing Small Molecules to Biological Molecules** *Presented in Partnership with the International Society for Biological and Environmental Repositories* Session Chairs: Jonathan O'Connell, *Forma Therapeutics, Inc.* and Andy Zaayenga, *SmarteLabs*
5:00 - 5:15 pm	**Student Poster Award Winner Announcement**					
5:00 - 5:30 pm	**Live Podcast from The Lab Man:** Student Poster Competition Winners					
5:30 - 6:30 pm	**Reception Celebrating 2016 SLAS Journal Achievement Award Honorees**					
5:30 - 6:30 pm	**SLAS Student and Early Career Professionals Mixer**					
6:30 - 8:30 pm	**Late Night with LRIG - Rapid-Fire Innovation Session**					

TUESDAY, JANUARY 26, 2016						
7:15 - 9:00 am	**Analytical, Life Science and Diagnostic Association Special Session (ALDA)** (Invitation Only)					
7:30 - 8:30 am	**Career Connections Workshop:** Transitions; Joanne Kamens, Addgene					
7:45 - 8:00 am	**Morning Beverage Break**					
7:30 am - 6:00 pm	**Registration Open**					
8:00 - 9:15 am	**Special Interest Groups:** HCS/HCA Data and Informatics; Sample Management; Screen Design and Assay Technology; Stem Cells and 3D Microtissues **Sponsored by:** CORNING; Technology Transfer and CRO/CMO Project Management					
9:30 - 10:15 am	**Exhibitor Tutorials:** Formulatrix; Genedata, Inc.; Horizon Discovery Ltd; Lumigen, a Beckman Coulter Company; PerkinElmer; Thermo Fisher Scientific; Transcriptic					
9:30 am - 12:30 pm	**Career Connections in SLAS Member Center:** Mentoring Sessions/One-on-One Career Counseling Sessions/Job Boards					
9:30 am - 6:00 pm	**Exhibition Open**					
9:30 am - 6:00 pm	**Posters on Display**					
9:30 am - 6:00 pm	**SLAS Member Center Open**					
9:45 - 10:15 am	**Live Podcast from The Lab Man:** JBS Guest Editor, Jonathan Wingfield of AstraZeneca; JBS Special Issue on Advances in Mass Spectrometry Within Drug Discovery					
10:00 - 10:30 am	**Mid-Morning Beverage Break**					
Session Three	**Advances in Bioanalytics, Biomarkers and Diagnostics**	**Assay Development and Screening**	**Automation and High-Throughput Technologies**	**Cellular Technologies**	**Informatics**	**Micro/Nano Technologies**
10:30 am - 12:30 pm	**Advances in Biomarkers Discovery: Sample Preparation, Analysis and Data Processing** Session Chair: Dieter Drexler, *Bristol-Myers Squibb*	**Phenotypic, Model Organism and High Content Screening** Session Chair: Florian Fuchs, *Novartis Pharma AG*	**Emerging Techniques for Clinical Laboratory Automation** Session Chair: Omai Garner, *University of California, Los Angeles*	**Application of CRISPR and RNAi in Genetic Screens for Target Discovery and Validation** Session Chair: John Doench, *Broad Institute of MIT and Harvard*	**Data Wrangling** Session Chair: Matt Hahn, *3ds*	**Commercialization Perspectives for Micro and Nanofluidic Devices** Session Chair: Josh Molho, *Zephyrus Biosciences, Inc.*
12:30 - 1:15 pm	**Exhibitor Tutorials:** Artel, Biodesy; Festo Corporation; Hamilton Company; PerkinElmer; TTP Labtech					
12:30 - 1:30 pm	**Lunch in Exhibition Hall** **Sponsored by:** SelectScience® The Fastest Way to Expert Opinion					
12:30 - 1:45 pm	**Exhibitor Tutorials:** Brooks Automation; Labcyte, Inc.; Molecular Sensing; PerkinElmer; Promega Corporation					
12:30 - 2:30 pm	**Exhibitor Tutorials:** Agilent Technologies Inc.; Beckman Coulter Life Sciences					
1:00 - 1:30 pm	**JALA & JBS VIP Meet & Greet:** JBS Editor-in-Chief, Robert Campbell, Eli Lilly and Company					
1:00 - 2:00 pm	**Career Connections Workshop:** Mentoring; Joanne Kamens, Addgene					
1:00 - 3:00 pm	**Poster Presentation (Even Numbered Posters)**					
1:30 - 2:00 pm	**JALA & JBS VIP Meet & Greet:** Mass Spectrometry within Drug Discovery, JBS Special Issue Guest Editor Jonathan Wingfield of AstraZeneca					
2:00 - 2:30 pm	**JALA & JBS VIP Meet & Greet:** Assembly and Transformation of a Synthetic Yeast Artificial Chromosome with a Multigene Cassette that Enhances Xylose Utilization into Saccharomyces Cerevisiae, JALA Author Steve Riedmuller, Hudson Control Group					
2:00 - 2:45 pm	**Exhibitor Tutorials:** Axol Bioscience; Biosero; BMG Labtech, Inc.; Clontech Laboratories, Inc.; Helix Linear Technologies, Inc.; Icagen, Inc.; InSphero, Inc.; Labcyte, Inc.; Molecular Devices; Nexcelom Bioscience; Titian Software					
2:00 - 5:00 pm	**Career Connections:** Mentoring Sessions/One-on-One Career Counseling Sessions/Job Boards					
2:30 - 3:00 pm	**Afternoon Beverage Break**					
3:00 - 3:30 pm	**Live Podcast from The Lab Man:** JALA Guest Editor, Joe Olechno, Labcyte; JALA Special Issue on Advancing Scientific Innovation with Acoustic Droplet Ejection					

Session Four	Assay Development and Screening	Automation and High-Throughput Technologies	Cellular Technologies	Drug Target Strategies	Informatics	Micro/Nano Technologies
3:00 - 5:00 pm	**Compound Libraries and Medicinal Chemistry in Screening** Session Chair: Jonathon Baell, *Monash Institute of Pharmaceutical Sciences*	**Extreme Automation** Session Chair: Brian Rasnow, *Etaluma Inc. & CSUCI*	**Gene Editing for Disease Models** Session Chair: Edward Rebar, *Sangamo BioSciences*	**Physiologically Relevant Target Strategies** Session Chair: David Swinney, *Institutes for Rare and Neglected Dieases Drug Discovery*	**Collaboration: Drug Discovery in the Internet Era** Session Chair: Barry Bunin, *CDD*	**Diagnostics and Point-of-Care Microdevices** Session Chair: Michelle Khine, *University of California, Irvine*
5:00 - 6:00 pm	**Networking Reception**					
6:30 - 9:30 pm	**SLAS2016 Tuesday Night Celebration: USS Midway** Sponsored by: HAMILTON					

WEDNESDAY, JANUARY 27, 2016	
7:45 - 8:00 am	**Morning Beverage Break**
8:00 - 9:15 am	**Special Interest Groups: Academic Drug Discovery;** Automation Quality Control; Compound Combination; Drug Repurposing; Informatics; Labware Leachables; Women Professionals in Science and Technology
8:00 - 9:15 am	**JALA & JBS Author Workshop:** How to Get Your Work Published
8:00 am - 1:30 pm	**Registration Open**
9:00 - 9:30 am	**Mid-Morning Beverage Break**
9:00 - 11:45 am	**Career Connections:** Mentoring Sessions/One-on-One Career Counseling Sessions/Job Boards
9:00 am - 1:00 pm	**Exhibition Open**
9:00 am - 1:00 pm	**SLAS Member Center Open**
9:15 - 9:30 am	**New Product Award Announcement**

Session Five	Assay Development and Screening	Automation and High-Throughput Technologies	Cellular Technologies	Drug Target Strategies	Informatics	Micro/Nano Technologies
9:30 - 11:30 am	**Assay Platforms for Biologics** Session Chair: Robert Damoiseaux, *University of California, Los Angeles*	**Automating Novel Analytical Tools for PKA, Drug-Drug Combination and Synergy Assays, Drug Repurposing** Session Chair: Peter Chase, *Bristol-Myers Squibb*	**Scaling Challenging Cell Models for High-Quality High-Throughput Screens** Session Chair: Imran Rizvi, *Harvard*	**Phenotypic and Systems-Based Strategies for Novel Drug Targets** Session Chair: Marcos Milla, *Janssen Research & Development*	**Crowdsourcing Science** Session Chair: Ethan Perlstein, *Perlstein Labs PBC*	**Single Cell Analyses** Session Chair: Amy Herr, *University of California, Berkeley*
10:00 - 11:00 am	**Live Podcast from The LabMan:** New Product Award Winners					
11:30 am - Noon	**Lunch in Exhibition Hall**					
11:45 am - Noon	**Passport to Prizes Winners Announced**					
Noon - 1:15 pm	**Special Interest Groups:** ADMET; Automated Sample Preparation of Pharmaceutical Dosage Forms; Phenotypic Drug Discovery; Standards Initiatives					

Session Six	Assay Development and Screening	Automation and High-Throughput Technologies	Drug Target Strategies	Informatics	Micro/Nano Technologies	
1:30 - 3:30 pm	**Secondary Screens, ADME-Tox, Removing Artifacts and Compound Profiling Assays in High-Throughput Screening** Session Chair: Cathy Tralau-Stewart, *University of California, San Francisco*	**Screening Automation Modular Systems vs. Highly Integrated Systems** Session Chair: Sam Micheal, *National Institutes of Health*	**Successful Strategies for Difficult Targets** Session Chair: Chun-Wa Chung, *GlaxoSmithKline*	**Handling Complex Data: Phenotypic High-Content, Integrative** Session Chair: Anne Carpenter, *Broad Institute of Harvard and MIT*	**Emerging Micro and Nanosystems for Pathogen Detection** Session Chair: Welan Zhao, *University of California, Irvine*	
3:30 - 3:45 pm	**Afternoon Beverage Break**					
3:45 - 5:00 pm	**Closing Remarks, Keynote Presentation and Announcement of the SLAS Innovation Award Winner** Sponsored by: ThermoFisher SCIENTIFIC Adam Diedrich Steltzner, Chief Landing Phase Engineer and Aeronautics Expert, NASA Jet Propulsion Laboratory; From Leading a High Performance Team to Landing on Mars					

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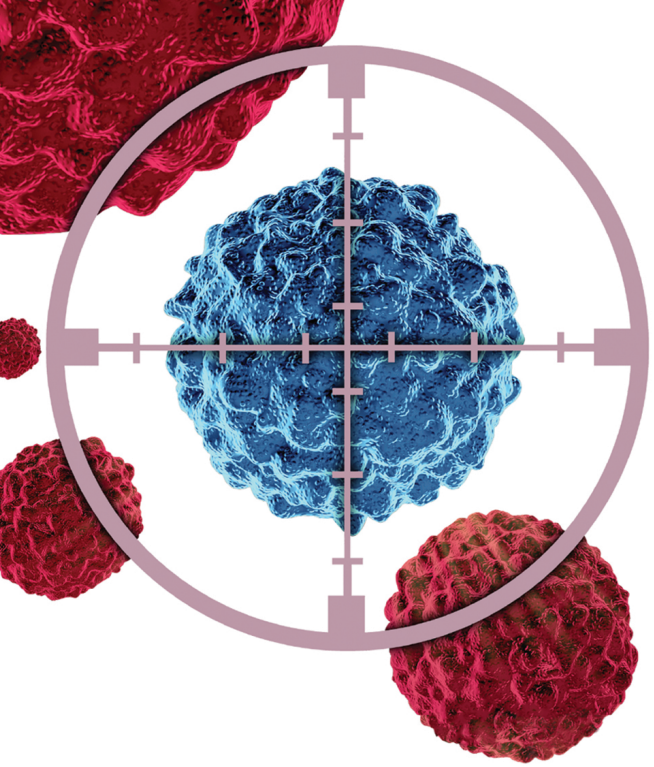

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The Society's highest honor, the SLAS Innovation Award recognizes the work behind one conference podium presentation that proves to be exceedingly innovative and contributes to the exploration of technologies in the laboratory. The Innovation Award winner is announced immediately following the Keynote by Adam Diedrich Steltzner during the closing session on Wednesday | January 27 | 3:45 pm.

The SLAS Innovation Award Finalists are:

Tiffany Chen, Cytobank and Stanford University
Identifying Druggable Cells: Automated Methods for High-Content Single-Cell Screening

Shana Kelley, University of Toronto
New Devices for the Detection and Classification of Antibiotic-Resistant Bacteria

Jonathan Lin, University of California, Los Angeles
A Frequency-Multiplexed Parallel Flow Cytometer for High-Throughput Screening and Drug Discovery

Elena Molokanova, NTBS
Graphene-Based Biocompatible Optoelectronic Platform for Light-Controlled Activation of Cardiomyocytes

Sumita Pennathur, University of California, Santa Barbara
Electrokinetic Micro- and Nanofluidic Technologies for Quantitative Detection of Viral Nucleic Acids

Anna Popova, Institute of Toxicology and Genetics (ITG), Karlsruhe Institute of Technology
Droplet-Microarray Sandwiching Chip as Miniaturized Pipetting Free Platform for High-Throughput Screenings of Live Cells Based on Superhydrophilic-Superhydrophobic Surface Patterning

Ivan Pushkarsky, University of California, Los Angeles
Elastomeric Sensor Surfaces for High-Throughput Phenotypic Screening of Cellular Force Generation

Glenn Smith, CryoXtract Instruments
The Application of Automated Frozen Aliquotting Technology to the Bioanalysis of Labile Compounds

Sindy Tang, Stanford University
Droplet Microfluidics: Amphiphilic Nanoparticles as Droplet Stabilizers for High-Fidelity and Ultrahigh-Throughput Droplet Assays

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— Professor Sir Ian Wilmut, OBE, FRS, FRSE, University of Edinburgh

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LABXMEDIA GROUP

ADVANCES IN BIOANALYTICS, BIOMARKERS AND DIAGNOSTICS

10:30 am | Monday | January 25

George Vasmatzis, Biomarker Discovery, Center for Individualized Medicine, Mayo Clinic

MPseq Is a Whole Genome Sequencing Platform That Has Been Used to Develop Biomarker Tests

Recent breakthroughs in genomics technologies provide a new view of the molecular alterations acquired by tumor cells. Unfortunately, these alterations are quite different from patient to patient demanding comprehensive diagnostic tools to delineate what is clinically relevant and actionable for each patient. We have developed a protocol and algorisms that can replace several cytogenetic tests and provide comprehensive diagnostic value with high specificity, sensitivity and cost-effectiveness. Most importantly our process can provide a detailed description of all DNA rearrangements at a resolution that can show how individual genes are disturbed thus providing necessary novel insight for correct clinical interpretation. The technologies and protocols developed allow for the genomic evaluation of small tissue samples. Our software package excels in several metrics with the most important being the elimination of false positive calling and the substantial reduction of false negatives. Our algorithm takes advantage of a context data set of approximately 1,300 tumors that have been analyzed. The extensive dataset was used as learning platform to increase the specificity and the sensitivity of the algorithm. Several filters and masks reduce false positive rates. BIMA, our home-grown mapping algorithm, is the fastest mapper of next gen sequences to the latest human genome (GRCh38) by a factor of three up to 20 times faster than any other existing algorithm. Furthermore, BIMA is the most accurate algorithm for mapping mate-pair sequences (95% accuracy). It also adapts to different pre-analytical capabilities to capture cells and extract DNA. Chromosomal rearrangement detection offers several benefits over point mutation analysis. For one, it allows coverage of the entire genome as opposed to narrowly focusing on selected genes, is far less prone to false positives, can potentially identify driving rearrangements/ fusions, and allows for the ability to identify rearrangements within pathways that may be therapeutically targetable. Due to tumor-specificity of the breakpoint-junctions, it can be used for defining clonal relationships and allows for designing individualized diagnostic tests for monitoring a patient's disease progression.

11:00 am | Monday | January 25

Harsha Gunawardena, Amgen Inc.

Enabling Deep Tissue Proteomics with Limited Prefractionation

Breast cancer (BC) is one of the leading causes of death in women with approximately 450,000 fatalities worldwide each year. Recently a comprehensive analysis was carried out by The Cancer Genome Atlas (TCGA) using six different technologies to delineate subtype-specific molecular changes in several cancers. The analytical platforms used in the TCGA study were mostly focused on genomics and transcriptomics sequencing methods with limited amount of proteomic performed via reverse phase protein arrays (RPPA). In scale with analyzing proteomes from tens to hundreds of TCGA samples in an unbiased manner, multiplexing with isobaric labeling technologies is the method of choice for high-throughput proteomics. This presentation demonstrates single-shot and limited pre-fractionation (5 fractions per sample) methods that enable faster LC-MS acquisition times for label-free quantitation (LFQ) as a viable alternative to multiplexing. A new unified LFQ method that we term QuantFusion gives us improved precision and coverage of tissue proteomes with shorter acquisition times. We demonstrate the identification of 22,000 site-specific phosphorylations with ~10,900 unified quantification estimates, and the assembly of ~11,000 distinct protein groups per tumor subtype in 2-3 days. We apply proteogenomic methodologies to the same data set to detect splicing events, novel coding regions and cancer mutations. In addition, we demonstrate an affinity-based approach to characterize regulation of trans-factors associated with the BC subtype-specific splicing machinery.

11:30 am | Monday | January 25

Armin Nourani, The University of Texas at Austin

Implementation of Commercial Glucometer in Detection of Melanoma Nucleic Acid Biomarkers

Melanoma is one of the deadliest forms of skin cancer, and has recently faced an uprising in both its incident and mortality rates. Early detection of the cancer can prove essential in reducing mortality rates by exposing patients to treatment options when most effective. Currently, however, methods of melanoma detection depend on either biopsy analysis or a visual inspection, significantly limiting the capabilities of determining early-stage presence and tumor metastasis. In efforts to improve the diagnostic framework of melanoma, a molecular approach could be implemented to increase the viability of treatment options. This can potentially be achieved by incorporating several recent developments in nucleic acid circuitry and electrochemical signal transduction of molecular targets. An initial source of melanoma biomarkers have already been identified, namely certain genes found in circulating nucleic acids (CNAs) present throughout bodily fluids (such as blood, saliva, and urine) in trace amounts. To make recognition of these targets more feasible for a diagnostic setting, the method of loop-mediate isothermal amplification (LAMP) was selected for these nucleic acids due to its selectivity and sensitivity for in vitro amplification. For simplistic detection, one-step toehold mediated strand displacement (OSD) probes have been implemented and further functionalized to release a thermo-stable invertase enzyme upon binding and recognizing specific melanoma LAMP amplicons. In doing so, the free-invertase can then catalyze the conversion of sucrose into glucose for monitoring by any commercially available glucometer. Currently, LAMP circuitry has been verified and progress has been made towards optimizing the invertase-based assays. Steps have also been made towards testing a point-of-care diagnostic device that compartmentalizes each part of the detection pathway into a compact system. Near future experiments will involve the testing of human samples in the optimized device for validation as a future diagnostic method. In implementation of this work, melanoma could be determined at a much earlier stage, strengthening the future effectiveness of treatments and survival outlooks.

12:00 pm | Monday | January 25

Alberto Bresciani, IRBM Science Park SpA
Co-Authors: Douglas Macdonald, CHDI Management/CHDI Foundation; Roberto Boggio, Andreas Weiss, IRBM Promidis; Sergio Altamura, Cristina Cariulo, Manuel Daldin, Valentina Fodale, IRBM Science Park; Holly Kordasiewicz, ISIS Pharmaceuticals

Development and Validation of an Assay to Detect Mutant Huntingtin Protein for the Assessment of Pharmacodynamic Effects in Human and Rodent Samples

Huntington's disease (HD) is a neurodegenerative disorder caused by a genetic mutation in Huntingtin gene (HTT) that leads to the expression of an expanded poly-glutamine protein (polyQ). HD has a broad impact on a person's functional abilities due to progressive motor dysfunction, cognitive decline, and psychiatric disturbance, probably caused by both neuronal dysfunction and neuronal cell death.New strategies for drug discovery and new therapeutic approaches are now aimed at slowing the progression of HD; one of these promising strategies consists in reducing the mutant protein level through its direct reduction (gene silencing) or increasing its clearance (compound treatment). In this framework the mutant huntingtin (mHTT) protein can be a biomarker for treatment efficacy, raising the necessity to develop highly sensitive immunoassays to quantify its level in tissues and biological fluids. Being that Huntington's disease a neurological disorder, the ideal HTT quantification target organ would be the brain. Nonetheless, there are obvious limitations on the accessibility of such tissues in patients. As a consequence, the ultimate goal of this pharmacodynamic assay would be to detect HTT levels in a surrogate tissue tightly brain related. The cerebrospinal fluid (CSF) is the most attractive fluid with the above mentioned features, but HTT was not previously detected in CSF and, if any, its concentration may be estimated in the pM range. The present work describes the use of the Single Molecule Counting (SMC™) technology, powered by Singulex©, for ultrasensitive measurement of HTT at levels previously undetectable in human CSF. The present immunoassay was developed using one N-terminus directed antibody and one antibody that recognizes the poly-Q domain of the protein. The assay was successfully applied to human and murine derived fluids and murine tissues.The presentation summarizes the assay validation which includes: the evaluation of the calibration curve performance, specificity, matrix effect (spike recovery, parallelism and dilution linearity), selectivity, accuracy and precision.In addition, selected cases in which the assay was successfully applied will be presented such as the evaluation of tissue specific mutant HTT levels in HD mouse models, the stratification of two patient cohorts by mutant HTT levels quantification in the CSF, and finally the application of the pharmacodynamic assay to an HTT lowering efficacy study in BAC HD rats.

ADVANCES IN BIOANALYTICS, BIOMARKERS AND DIAGNOSTICS

3:00 pm | Monday | January 25

ON DEMAND

Scott Busby, Novartis Institutes for Biomedical Research
Co-Authors: Dominick Casalena, W. Adam Hill, Novartis Institutes for Biomedical Research

HT-MALDI-MS as a Complete Label-Free Drug Discovery Platform: From Target Characterization Through High-Throughput Screening to Hit Follow Up

Label-free assay techniques have many advantages over standard fluorescence based assays in that they allow for rapid measurements of multiple reaction products simultaneously, are compatible with most enzymatic reactions and don't suffer from the prevalence of fluorescence based-assay artifacts that cost time and money in the drug discovery process. However, mass spectrometry based techniques have not been deployed in high-throughput screening campaigns due to limitations of throughput, cost of reagents and a need for expert resources to perform these assays. We believe our HT-MALDI-MS platform overcomes many of these previous limitations of mass-spectrometry based screening assays by: having superior throughput to LCMS and SPE coupled ESI assays in both assay development and screening times; significantly reducing assay costs by eliminating expensive solvents and antibody reagents used in other techniques; enabling new assays that are not possible with conventional TR-FRET assays. To illustrate these points, we will show a retrospective of the development and execution of a 100K MALDI-MS screen demonstrating the speed of this platform for assay development when coupled to our Design of Experiment (DOE) techniques. In addition, we will compare the results of the primary and dose-response validations from this screen to a TR-FRET readout to illustrate the enabling nature of the assay design unique to the HT-MALDI platform. Finally, we will illustrate the use of the HT-MALDI to characterize the mode of action of some of these hits from the screen using a new computational methodology developed at Novartis.

3:30 pm | Monday | January 25

Melanie Leveridge, GlaxoSmithKline
Co-Authors: Stuart Baddeley, Adrian Dunn, Neil Hardy, Carl Haslam, Peter Marshall, GlaxoSmithKline

The Evolution of MALDI-TOF Mass Spectrometry Towards Ultra High-Throughput Screening - 1536 Well Format and Beyond

Mass spectrometry (MS) offers a label free, direct detection method for screening of enzyme targets. The technique offers a number of advantages over indirect, fluorescent assay formats, such as reduced compound interference. However hit identification and compound screening by mass spectrometry has historically been limited by low throughput sample preparation, typically using HPLC. Over recent years solid phase extraction based techniques, such as the Agilent RapidFire platform have emerged, which are capable of analyzing samples in less than 10 seconds, and these platforms are now routinely applied to screening and compound profiling. However, whilst dramatically faster than liquid chromatography coupled MS, an analysis time of 8-10 seconds is still considered relatively slow for a full diversity ultra high-throughput screening (uHTS) of millions of compounds. Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF) offers an alternative for high-throughput MS detection. Historically, sample preparation and deposition onto the MALDI target has been considered a limitation for the use of MALDI for screening assays, but more recently incorporation of nL dispensing techniques into this workflow have enabled 1536 well assays to be performed with analysis times of around 1 second per sample. Here we describe the development and validation of assays for both small molecule and peptide analytes using MALDI-TOF coupled to nanolitre liquid handling. Robust assay validation data in 1536 well format will be shown, as well as correlation to 384 well RapidFire formats. We also describe work to further increase throughput via high density sample deposition in 6144 format. Overall, we demonstrate that this technology has potential to deliver an MS based readout at speeds and sample volumes compatible with uHTS.

ADVANCES IN BIOANALYTICS, BIOMARKERS AND DIAGNOSTICS

4:00 pm | Monday | January 25

Andrew Wagner, Bristol-Myers Squibb
Co-Authors: Kingsley Appiah, Jefferson Chin, David Harden, Shannon McCabe, Wilson Shou, Elizabeth Wood, Bristol-Myers Squibb

Continuing the Quest for a Mass Spectrometry-Based Plate Reader: Evaluating Laser Diode Thermal Desorption (LDTD) Coupled with Nanoliter Dispensing for HTS and HT-ADME Applications

Improvements in mass spectrometry (MS)-based analytical throughput have been achieved using laser desorption ionization (LDI) techniques and this has led to increased utility for this technology amongst research groups supporting early drug discovery efforts. In particular, lead discovery teams that perform high-throughput screening (HTS) assays that are intended to identify active compounds against therapeutic targets of interest have begun to adopt this label-free methodology as an orthogonal screening approach to well-established fluoresence-based assays. Furthermore, optimization of the "hits" identified through HTS requires additional in vitro studies to assess absorption, distribution, metabolism, and elimination (ADME) properties by lead profiling groups who prefer to use native, clinically-relevant substrates. Since HTS and HT-ADME groups can generate thousands of samples on a daily basis, it is necessary to have high-throughput analytical platforms capable of processing these demands. The move towards label-free screening in drug discovery has increased the demand for mass spectrometry-based analysis, and because of this, it is important to explore new technologies aimed at improving MS-based readout speeds in order to continue to support the increasing sample volume demands of these groups. Assays that utilize mass spectrometry for analysis are both sensitive and selective while offering the ability to provide label-free detection of physiologically relevant substrates and products. MS-based methodologies, however, have traditionally relied upon liquid chromatography (LC) or on-line solid phase extraction (SPE) as front-end sample delivery mechanisms. Because of this, they typically have slow cycle times that aren't amenable to high-throughput screening efforts. Using newer approaches that employ laser desorption techniques to directly introduce the contents of wells into the mass spectrometer, it is possible to achieve throughputs that approach those of plate-reader assays and meet the demands of early discovery screening applications. Here we investigated the approach of coupling nanoliter sample deposition with Laser Diode Thermal Desorption (LDTD) - tandem mass spectrometry (MS/MS) and evaluated its utility in providing an ultra high-throughput, label-free detection method for various applications in HTS and HT-ADME groups.

4:30 pm | Monday | January 25

Erika Rehnström, AstraZeneca
Co-Authors: Linda Fredlund, Jan Holmgren, Johan Hulthe, Anna Noven, Per-Erik Strömstedt, Siavash Tavakoli, Johan Wernevik, AstraZeneca

DMPK Wave 1 Screening – an Integral Part of Early Drug Discovery at AstraZeneca

Evaluation of drug physicochemical and metabolic properties is crucial in the early drug development process. Access to this information early during the design-make-test-analysis (DMTA) cycle enable identification of the best new chemical entities and efficient project progression. Five assays that are considered to give important information about compound properties have been included in a screening panel (DMPK Wave 1); lipophilicity, solubility, human plasma protein binding, and metabolic stability in rat hepatocytes and human liver microsomes. All assays have been set up in an automated 96-well format with a UPLC-MS/MS read-out. A streamlined overall workflow has been developed by optimizing all parts of the process, such as shipping of compounds from other sites, delivery of compound assay ready plates, use of fit-for purpose equipment and IS/IT systems for data requesting, analysis and reporting. As a result, lead times that well match project demand independent of compound synthesis site have been achieved, yet maintaining high quality, and today screening of a high number of compounds on a weekly basis is performed. This high-throughput screening strategy enable optimization of structure-ADME and structure-activity relationships in parallel and allow efficient and informed decision making in early chemistry.

ADVANCES IN BIOANALYTICS, BIOMARKERS AND DIAGNOSTICS

10:30 am | Tuesday | January 26

Hanno Steen, Harvard Medical School and Boston Children's Hospital

Urine Proteomics: From New Developments to New Insights

The urinary proteome has been studied since the early 2000s. Initially, mass spectrometry-based proteomics efforts resulted in the identification of only 124 proteins. Since then, the field has rapidly evolved, such that it is assumed that the urinary proteome comprises in total ~2,500 proteins. Because urine is an easily obtainable body fluid that features several additional advantages over other blood-derived body fluids, the urinary proteome has been extensively studied within the biomarker field. The ultimate aim of these efforts is to unleash the diagnostic and prognostic potential of urine in the context of diseases related and unrelated to the urogenital tract. For the former, urine functions as a proximal fluid, while urine is a systemic body fluid for the latter. To ensure the high throughput necessary for successful biomarker studies, robust sample processing and analysis strategies are needed. To meet these needs, the Steen Group has developed innovative approaches to efficiently process urine in a highly parallel fashion using 96-well plates with PVDF membranes for immobilizing and manipulating the urinary proteins. Similarly, data-independent acquisition (DIA)-based LC/MS methods were established that robustly identify and quantify 1,000+ proteins per sample using less than one hour of instrument time. These high-throughput processing and analysis strategies have the potential to shift the current paradigm of biomarker studies since the discovery and verification stages can easily be combined. Using this newly developed analysis pipeline, several urine proteomics-based biomarker discovery studies have been carried out and will be presented to highlight the potential of our urine proteomics pipeline for the discovery of diagnostic and prognostic biomarkers for a wide range of diseases.

11:00 am | Tuesday | January 26

Richard Yost, University of Florida
Co-Author: Timothy Garrett, University of Florida

Imaging Mass Spectrometry Applied to Biomarker Discovery

This presentation will address the potential for imaging mass spectrometry to help advance biomarker discovery, with a particular emphasis on metabolomics (small molecule biomarkers). Technology and methodologies to be explored include a variety of imaging mass spectrometry platforms, including MALDI and the ambient ionization methods DESI and in situ microextraction. The talk will examine the capability of these imaging techniques for biomarkers discovery directly in intact tissue, as well as integrated with traditional metabolomics platforms such as LC/MS/MS. The talk will cover applications to a wide variety of diseases and biomedical problems, including myocardial infarction, glaucoma, Parkinson's disease, melanoma, and aging, as well as response to treatment. Sample types including cardiac, ocular, brain, skeletal muscle, and skin tissues.

ADVANCES IN BIOANALYTICS, BIOMARKERS AND DIAGNOSTICS

11:30 am | Tuesday | January 26

Steven Piccoli, Bristol-Myers Squibb

Biomarker ELISA Assays to the Clinic: Same Old Gold Standard

Biomarker discovery and assay technology undergoes a massive evolution during phase progression in the protracted pharmaceutical pipeline. Different needs in target sample matrix, throughput, sensitivity and cost dictate the directed development into a feasible final format which must satisfy clinical needs. Integrated design, execution and analysis of experimental design may or may not employ parallel processes, or require integrated operation of multiple instruments, but in optimal embodiments may include multiple smaller enhancements leading to a vastly streamlined process. To solve a pressing need for a PD biomarker requirement in a therapeutic program directed against atopic dermatitis, we evaluated novel multiple assay technologies and applied them against more conventional technologies in conjunction with specific automation processes to deliver vastly improved sensitivity (from picograms/mL to femtograms/mL of the Interleukin-31 analyte), required robustness, and decreased execution time leading to an easily performed , straightforwardly transferrable assay capable of supporting a therapeutic program in full development.

12:00 pm | Tuesday | January 26

Ian Moore, SCIEX
Co-Authors: Tara Jones-Roe, Michael Kowalski, Beckman Coulter; Gary Impey, Hua-Fen Liu, Suma Ramagiri, Lei Xiong, SCIEX

Decoding a Complex Sample Extraction Process for Biotherapeutics Mass Spectrometry Analysis

Small molecule bioanalysis is a more mature application segment within the pharma/CRO industry and as a result, bioanalytical scientists have an extant framework to address some of the critical issues in this area. However, the surge in development of large molecule-based therapeutics presents new challenges for small molecule LC-MS experts. These scientists are increasingly tasked with the quantitation of large molecule drugs - i.e., biologics bioanalysis of monoclonal antibodies (mAb), antibody drug conjugates, fusion proteins, and PEGylated proteins in complex biological matrices – all with an evolving set of standards and protocols. The challenges are numerous but broadly can be classified in three buckets: standardization and robustness in sample preparation strategies, robust and reliable analytical instrumentation that allows scientist to reach requisite LLOQs and finally, efficient reporting modes that enable scientists to move from sample to answer faster. After testing several enrichment strategies, we have developed a streamlined method for the enrichment of IgGs, with the goal of developing a standardized approach. This standardized approach requires an automated procedure which can accommodate a very wide dynamic range without sacrificing sensitivity. A major application of this approach is the use of Fc-binding particles for the enrichment of human mAbs. Using this approach, several recombinant human mAbs were isolated from spiked animal sera and enriched using magnetic bead based capture, digested with trypsin and quantified using LC-MS/MS. We looked at multiple factors including various capture antibodies (species absorbed versus non-absorbed), the amount of capture antibody, as well as different elution buffers. Studies in multiple matrices were required so as to identify potential interfering peptides. For the digestion component, we focused on shortening the digestion step so as to make the process executable within one work day. This optimized workflow was then automated on a Biomek FXP Workstation with excellent reproducibility. This fully optimized and automated solution can provide a standardized approach to biologics bioanalysis.

10:30 am | Monday | January 25

Jarrod Walsh, AstraZeneca
Co-Author: Gareth Davies, AstraZeneca

Impact of Biophysical Approaches Immediately Post HTS in Aiding Hit Confirmation Activities

The past decade has witnessed an increasing use of biophysical methodologies within drug discovery. These applications vary from bespoke studies to intimately characterize a compound's interaction with a target, to simple confirmation of binding activities. As the discipline has matured more sensitive and higher throughput versions of the technologies have developed. This has enabled their potential application as a hit confirmation strategy for other hit finding approaches e.g. High-Throughput Screening (HTS). The assay technologies applied to perform biochemical HTS campaigns are prone to compound driven detection system artefacts that manifest as false positive results. The relative insensitivity of many biophysical methodologies to such artefacts coupled with their ability to physically detect compound/target interactions should make them an ideal orthogonal strategy for hit confirmation. Such approaches can additionally yield valuable insights into mechanism and specificity. These merits have resulted in a growing demand from AstraZeneca's medicinal chemistry community to apply the technologies earlier in the drug discovery process against increasing numbers of compounds. Within this presentation, we describe our efforts to meet this demand by creating an internal high throughput biophysical screening capability. Four commercially available systems that utilize three different methodologies (Surface Plasmon Resonance (SPR), Microscale Thermophoresis (MST) and Differential Scanning Fluorimetry (DSF)) were identified. Each approach had inherent strengths and weaknesses necessitating a thorough evaluation to assess their fitness for the intended purpose. Three diverse drug targets were selected and over 300 compounds, identified via HTS campaigns, were profiled against them using each technique. We will present our results and discuss the advantages and limitations encountered. Finally, we will explain our conclusions and recommendations for applying biophysical approaches for large scale HTS hit confirmation.

11:00 am | Monday | January 25

Charles Wartchow, Novartis Institutes for Biomedical Research
Co-Authors: Ben Moree, Joshua Salafsky, Biodesy Inc; Cornelia Bellamacina, Dirksen Bussiere, John Fuller, Andreas Lingel, Jacob Shaul, Wei Shu, Micah Steffek, Novartis Institutes for Biomedical Research

Fragment Screening with Biodesy's Second Harmonic Generation Platform for Detecting Ligand-Induced Changes in Protein Conformation

Many important therapeutic protein targets undergo conformational change as part of their functional cycle. Examples include mammalian kinases and GPCRs, and numerous proteins from infectious agents. Understanding conformational changes, and more importantly, having a robust, assay that probes conformational changes induced by low-molecular weight modulators, has great potential in the drug discovery process. Accordingly, detection of conformational changes in proteins with Biodesy's second harmonic generation (SHG) platform has been validated in model systems (see Salafsky, Phys Chem Chem Phys, 2007, 9, 5704-5711), and more recently with therapeutic targets. The SHG detection method is distinct from traditional detection methods involving compound binding, since a change in protein conformation is required to generate a signal. Thus compounds that bind, but do not cause conformational change are silent in the SHG assay. As a result, SHG studies are most effective for systems where a conformational change can be directly correlated to the biological function of the target. Our goal is to evaluate SHG methodology in Biodesy's Delta platform in a fragment screening campaign in an early-stage drug discovery program, and to further validate the technology by comparing SHG results in a blinded study with those obtained from orthogonal methods, including 2D NMR and X-ray crystallography. We performed pilot studies with fully validated tool compounds to verify that the target, labeled with an SHG-active dye, was active in SHG assays. Subsequently, we performed a fragment screen of ~1700 compounds in duplicate. Hit validation data from 2D NMR studies were available for a subset of the compounds, and results for SHG-active compounds were compared to results from the NMR assay. A follow-up 2D NMR study on SHG-active compounds that were not originally tested by NMR confirmed the hit rate observed in the full screening campaign. Based on these comparisons, we find that SHG has potential as a tool for screening fragments with therapeutic targets that undergo ligand-induced conformational change.

11:30 am | Monday | January 25

Peter Coombs, MRC Technology

Discovery and Development of Inhibitors of the De Novo Purine Biosynthetic Enzyme PAICS – From Fragments to Lead Compounds

Targeting the de novo purine biosynthetic pathway is a recognised strategy in the development of anti-cancer therapeutics. Recent data suggests that PAICS (phosphoribosylaminoimidazole carboxylase/phosphoribosylaminoimidazole succinocarboxamide synthetase), which catalyses two steps of the de novo purine biosynthesis pathway, is an unexploited key target in this pathway. In order to identify novel inhibitor chemistry to this unprecedented target class, we pursued a number of hit finding strategies, including a biochemical HTS of ~200k compounds and fragment screen, incorporating in silico modeling and substrate/product analogue guided chemistry. The fragment screen was conducted by SPR, in parallel with an orthogonal activity-based screen. This approach successfully led to the efficient identification of biochemically-active fragment hits with confirmation of target engagement. Further biochemical and biophysical characterization of these fragments showed that they were ATP-competitive with IC50 and KD values in the low micromolar range and were suitable for chemical optimization. A site-directed mutagenesis approach was used to mutate key residues in the ATP binding pocket, which together with in silico modeling, allowed us to further probe the binding mode. Using these approaches the initial hits have been progressed as part of an ongoing medicinal chemistry program, with recent compounds exhibiting low nanomolar potencies in in vitro assays and sub-200nM potencies in cell-based assays, as well as favorable ADMET properties.

12:00 pm | Monday | January 25

Thomas Lundbäck, Chemical Biology Consortium Sweden, Science for Life Laboratories, Karolinska Institutet
Co-Authors: Anna-Lena Gustavsson, Chemical Biology Consortium Sweden; Thomas Helleday, Evert Homan, Ann-Sofie Jemth, Karolinska Institutet; Fraser Glickman, Rockefeller University

Small Molecule Inhibition Amenability of Nucleotide Pyrophosphatases Involved in DNA Repair - From MTH1 to dUTPase

Successful identification of suitable starting points through high throughput screening is dependent on several factors. These include the biological relevance and chemical amenability of the chosen biological material, the development and validation of a suitable assay and a good match with the chosen screening library. Herein we present and compare the results from screening of a broad selection of nucleotide pyrophosphatases involved in various aspects of DNA repair. This was accomplished using the same screening library and assay concept, thus allowing a direct comparison between enzymes and efficient identification of promiscuous inhibitors with undesirable mechanisms. The applied coupled enzymatic assay is based on hydrolysis of the pyrophosphate product by inorganic pyrophosphatase, followed by phosphate quantification using the malachite green assay in 384-well format. The experimental outcome is compared with expectations based on analysis of the active site architectures using computational methods and also with the known substrate specificity of these enzymes. Studied enzymes include the human MutT homolog 1 (MTH1), for which identified and optimized inhibitors were recently published (Gad et al. Nature 2014), related proteins of the NUDIX family as well as other pyrophosphatases involved in managing the intracellular pool of damaged nucleotides.

3:00 pm | Monday | January 25

Mary Ellen Digan, Center for Proteomic Chemistry, Novartis Institutes for Biomedical Research, Inc.
Co-Authors: Gary Trakshel; Kara Herlihy, AstraZeneca; Huaping Tang, Merck & Co Inc.; Meir Glick, Merck Research Laboratories; Kamal Azzaoui, Frederic Berst, Jutta Blank, Johannes Ottl, Novartis Institutes for Biomedical Research, Inc., Basel Switzerland; Anette Huebner, Novartis Institutes for Biomedical Research, Inc.; Haiyan Zhang, Yucheng Ni, Novartis Institutes for Biomedical Research, Inc.; Huyen Nguyen, Novartis Institutes for Biomedical Research, Inc.; Douglas Auld, Sandra Cerruti, David Farley, Ali Farsidjani, Adam Hill, Tami Hood, Honglin Niu, Dale Porter, Xiaoling Xie, Novartis Institutes for Biomedical Research, Inc.; Richard Kim, TTP Labtech, Cambridge

Lessons Learned Using BRET2+ in High-Throughput Screening

The ability to disrupt therapeutically important intracellular protein:protein interactions (PPIs) is of interest for drug discovery. In the past several years, we have improved methods and vectors to allow the use of bioluminescence resonance energy transfer (BRET) assays for high throughput screening (HTS). We discuss these improvements, as well as the results of HTS campaigns and some of their downstream follow-up for two targets: MCL1/NOXA and PUMA/BCL2. These strengths and weaknesses of using intracellular BRET assays to identify hits affecting the PPI as well as to reveal alternative pathways of potential therapeutic utility are discussed.

3:30 pm | Monday | January 25

Melissa Mendez, Laboratory of Assay Development and Screening Technology (ADST), NCATS, NIH
Co-Author: James Inglese, NIH/NCATS

Genome Edited Gigaxonin-Knockout Cell Line Development and Primary Screening to Investigate Biological Mechanisms Underlying the Rare Disease Giant Axonal Neuropathy

Giant Axonal Neuropathy (GAN) is a rare, autosomal recessive peripheral neuropathy that manifests early in life and is caused by mutations in the gene encoding gigaxonin (also GAN). Mutated gigaxonin inhibits normal nerve physiology, presumptively by failing to appropriately mediate the ongoing degradation and turnover of intermediate filaments (IF); in turn, IF accumulate in large aggregates that eventually cause axonal swelling, myelin thinning, and conduction velocity loss. However, little is understood about why this occurs. In order to pharmacologically and molecularly (siRNA) dissect the mechanisms underlying GAN-mediated neurodegeneration, we used CRISPR/Cas9 to knockout and knockdown GAN, and obtained twelve cell lines: 6 homozygous and 6 heterozygous deletion mutants. The number of alleles per clone was confirmed by fluorescence in situ hybridization, and mutations confirmed by sequencing. Protein loss/knockdown was confirmed by immunodetection, and rescue of the phenotype confirmed by transient gigaxonin expression. Genome-edited GAN-null mutants exhibit characteristic clustering of IF proteins and mitochondria (Z' = 0.89, 0.79, respectively) similar to patient pathobiology and the phenotypes of cultured patient cells. Heterozygous mutants exhibit IF clustering to a lesser extent. An initial small screen designed to identify probes capable of inducing IF and/or mitochondrial clustering for the purpose of training automated phenotype detection used primary image analysis parameters that evaluated only those features. That initial screen revealed a number of potential positive control compounds. In order to gain insights into the genes and pathways involved in disease progression, we have initiated subsequent library screening that will enable comparisons of differential phenotypic responses between the normal and knockout cells. For this ongoing screening, we are evaluating the utility of applying a wide variety of parameters – parameters that evaluate intuitively and non-intuitively relevant phenotypic features – in order to capture unforeseen phenotypic differences in the responses of the two cell types. Screening is conducted in 7- or 11-point titration in 1,536-well format by immunofluorescence against vimentin (an IF) and MT (beta-tubulin), with chromatin (Hoechst) and mitochondrial (MitoTracker CMXRos) dyes. Validation screens in progress will determine the optimal number of features with which to compare responses of normal and GAN-null cells; subsequently, fluorescent sensor lines will be developed to abrogate the need for immunofluorescence staining.

4:00 pm | Monday | January 25

Frederick King, The Genomics Institute of the Novartis Research Foundation
Co-Authors: Edward Ainscow, Sheryll Espinola, Jacob Haling, The Genomics Institute of the Novartis Research Foundation

A Luciferase-Based Assay System to Monitor Protein-Protein Interactions in Live Cells

Therapeutics often are designed based upon their ability to affect the enzymatic activity of a target. However, enzymatic inhibition does not always translate into decreased pathway signaling. For example, certain inhibitors of RAF kinase promote "paradoxical activation" by inducing the formation of RAF-RAF dimers where the unliganded RAF molecule takes on an active conformation and promotes downstream signaling. This emphasizes that signal transduction relies on protein-protein interactions (PPI's) and the development of therapeutics can benefit by an assessment of how they impact the constituents of relevant protein complexes. Previously designed approaches to studying PPI's in cells have suffered from artifacts ascribed to large protein tags, complicated workflows, and overexpression of the targets. Consequently, we evaluated a protein complementation assay from Promega called "NanoBit™" that uses an Oplophorus gracilirostris luciferase with a very high quantum yield. The assay system was used to study the KRAS-BRAF and BRAF-CRAF interaction in a cellular context. Using proteins tagged with the luciferase subunits we were able replicate published data demonstrating increased dimerization upon exposure with certain kinase inhibitors, a hallmark of paradoxical activation. Furthermore, the sensitivity and signal to noise of the assay enabled workflows to be developed and validated that allowed statistically significant screening results using a 1536 well format. These results provided confidence that the assay system can be applied not only to the characterization of lead molecules that affect BRAF dimerization but also to the identification of novel PPI inhibitors through high throughput screening.

4:30 pm | Monday | January 25

Layton Smith, Sanford Burnham Prebys Medical Discovery Institute
Co-Authors: Jane Lamerdin, Daniel Bassoni, DiscoveRx; Humberto Avila, Becky Hood, Danielle McAnally, Camilo Morfa, Haleli Sharir, Andras Szabo, Stefan Vasile, Sanford Burnham Prebys Medical Discovery Institute

Ultra-High Throughput Screen to Identify Chemical Probes of the Orphan Receptor GPR20 Using a Pharmacochaperone Strategy

The GPCR superfamily is the largest and single most important family of cell surface receptors in the human body. GPCRs play central roles in many biological processes and are linked to a wide range of pathologies. It is therefore not surprising that more than 30% of currently FDA-approved medicines target members of this family. There are over 375 GPCRs encoded in the human genome, of which 150 are orphan, lacking any known ligand or probe compounds that could be used to investigate their function. Given the successful exploitation of GPCRs as drug targets, these orphan receptors represent an untapped resource of potential new targets for drug discovery. Before that promise can be fulfilled, it is necessary to identify chemical probes to characterize the function and pharmacology of these receptors and to validate them as therapeutic targets. Traditional approaches to receptor deorphanization are arduous and unreliable at best. The application of high-throughput screening systems and advanced reporter technologies can be applied to identify chemical probes of orphan receptors circumventing the need to identify the natural ligand a priori in order to investigate function and pharmacology. Here we report the use of a pharmacochaperone strategy to identify chemical probes of GPR20. Human GPR20 was cloned using a PCR-based approach. Sequence and phylogenetic analysis revealed that human GPR20 was closely related to the opioid receptor family, and to functional receptors for nucleotides or lipids, suggesting that GPR20 may recognize lipids or nucleotides as ligands, yet to date its endogenous ligand remains unclear. Like all proteins, GPCRs are subjected to a stringent quality control (QC) system in the endoplasmic reticulum (ER) which prevents misfolded proteins from being trafficked to the plasma membrane. A unique aspect of this QC system is that it discriminates mutated receptors from wild-type based on their structure. Thus, many of these mutants can be rescued by pharmacochaperones. These target-specific, small molecules induce the proper structure necessary to satisfy the ER QC system. To identify novel chemical probes of GPR20, we developed a pharmacochaperone approach that employed a mutant GPR20 and _-galactosidase enzyme fragment complementation (EFC) reporter technology. Using this approach, we screened ~128,000 compounds of the Sanford Burnham Prebys compound collection. The assay performed well yielding good Z'-factor >0.5, and a signal-to-noise ratio >2.0. Selectivity for GPR20 was determined using a secondary assay that applied the same pharmacochaperone approach to the _2-adrendergic receptor. Hits were validated using cells expressing wild-type GPR20 and a cAMP and cellular impedance assays. This work provides proof-of-concept data supporting the pharmacochaperone screening approach to identify chemical probes of orphan GPCRs. Moreover, compounds resulting from this effort will be useful tools to further investigate the physiological and cellular functions of GPR20.

10:30 am | Tuesday | January 26

Florian Fuchs, Novartis Pharma AG

High Content Screening is Made Alive

The Center of Proteomic Chemistry of the Novartis Institute for Biomedical Research is executing a variety of cell-based assays ranging from target based to phenotypic readouts. These assays, in many times, evolve from engineered cell lines towards more complex models utilizing iPS derived cells or primary cells and readouts close to physiology. The use of high content screening and more particularly live cell kinetic imaging is taking off with novel assay types, introducing new challenges not only in terms of execution but as well in image analysis, data management, IT infrastructure and processes. This increased complexity and the way our hit finding group is addressing it, will be illustrated with examples of novel imaging assays that were developed to screen for compound activity in drug discovery.

11:00 am | Tuesday | January 26

Wei Zheng, NCATS/NIH
Co-Authors: Francis Aguisanda, Bradley Class, Nasir Malik, Noel Southall, Menghang Xia, Jean Zhao, Wei Zheng, NIH; John McKew, NIH & aTyr Pharma; Mahendra Rao, NIH & The New York Stem Cell Foundation; Sonia Shah, NIH and Drexel University College of Medicine

Using Human Astrocyte-based Disease Models for Drug Discovery Targeting Neurological Diseases

It is becoming increasingly clear that astrocytes play key roles in proper neuronal function in the central nervous system. Astrocytes serve to maintain an extracellular environment conducive to neuronal signaling, in addition to supplying neurons with nutrients necessary for cell health and metabolic precursors that can be used for neurotransmitter synthesis. Recently, astrocytes have been implicated in the pathogenesis of a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS), and have thus become a new target for drug discovery. In order to identify potential therapeutics for these diseases, we have developed human astrocyte-based assays that can be used for high-throughput screening (HTS), and that can be used to screen astrocytes differentiated from induced pluripotent stem cells (iPSC) from neurodegenerative disease patients. Specifically, we have developed a 1536-well format, high-content screening assay to identify compounds that are cytoprotective of astrocytes faced with oxidative stress. Using this assay for a pilot screen of 4000 compounds, we identified 24 compounds that prevent apoptosis in human embryonic stem cell (hESC)-differentiated astrocytes. Some of these compounds were found to activate the antioxidant response element (ARE) and Nrf2 (nuclear factor erythroid 2 [NF-E2]-related factor 2) pathway, while other compounds have previously been shown to be neuroprotective. To our knowledge, this is the first study in which human astrocytes have been used for HTS. In addition, our results demonstrate the utility of using human astrocytes differentiated from stem cells for neurodegenerative disease-focused drug discovery.

11:30 am | Tuesday | January 26

Franck Madoux, The Scripps Research Institute
Co-Authors: Allison Tanner, Michelle Vessels, Lynsey Willetts, Corning Incorporated; Pierre Baillargeon, Louis Scampavia, Timothy Spicer, Scripps Florida

Enabling 3D Cell-Based Spheroid HTS Assays in 384 and 1,536-well Plate Formats

Miniaturized cell-based assays have played an important role in drug discovery for the past few decades. Two dimensional cell monolayers constitute powerful and relatively easy to implement tools that are routinely used to assess a wide variety of biological features such as cytotoxicity, gene and protein expression, target activation, morphology changes, wound healing, etc. Despite their widespread use, recent evidence indicates that 2D cell models are poorly predictive, partly because of their lack of complex 3D architecture that adequately mimics the functionality and complexity of intricate in vivo systems. While 3D cell models have gained popularity, their use is mainly limited to lower throughput, secondary assays such as DMPK-Tox and/or MOA determination studies. With an increased demand for more physiologically-relevant cell-based models for drug discovery, there is a need to further miniaturize 3D models to unlock the screening of large chemical libraries by ultra-high throughput approaches. Working with Corning, Inc. we have tested and developed miniaturized HTS-amenable 3D spheroid cell based assays. In this presentation, we will discuss the challenges and solutions associated with consistently producing and characterizing large quantities of 3D spheroids for uHTS of cancer targets in both 384- and 1,536-well plate formats that are fully compatible with automated systems.

12:00 pm | Tuesday | January 26

Andrew Rennekamp, Massachusetts General Hospital, Harvard Medical School, and The Broad Institute
Co-Authors: Randall Peterson, Massachusetts General Hospital, Harvard Medical School, and The Broad Institute; Xi-Ping Huang, Bryan Roth, University of North Carolina Chapel Hill

A Powerful New High-Throughput Zebrafish Behavioral Chemical Screen to Rapidly Identify and Optimize Novel Neurotherapeutic Drug Candidates in a Target-Agnostic Manner

Approximately 30% of adults in the United States suffer from some form of mental illness. The economic burden caused by healthcare expenses and loss of productivity is estimated to be hundreds of billions of dollars each year. Yet success rates for neuroactive small molecules in clinical trials are currently less than half those for non-CNS drugs. One reason for this low success rate is the industry's reliance on target-based approaches during the chemical discovery phase, which are likely too simplistic. Target-based approaches have the potential to miss systems-modulating compounds, which may be useful for the treatment of complex diseases like mental illnesses. Target-based approaches are also far less likely to yield polypharmacologic therapeutics. In contrast, phenotype-based discovery approaches for CNS drug discovery have been 7 times more likely to produce successful Ist-in-class compounds than target-based programs. An ideal phenotypic endpoint for a neurotherapeutic drug screen is behavior modification. Tiny zebrafish larvae can be used for whole-organism high-throughput discovery of behavior-modifying small molecules. Zebrafish and humans share the same neurotransmitters and many of the same neural substrates. Furthermore, drugs that affect human biology have similar effects in zebrafish and vice versa. To identify novel, complex neuroactive small molecules, we developed an innovative, inexpensive, 7-minute behavioral assay using zebrafish larvae arrayed in 96-well plates and characterized the effects of more than 10,000 compounds on behavior. We took advantage of an unpublished discovery that 7-day-old fish rapidly become hypoactive in response to strobe light. By screening libraries of known drugs, we found that strobe-induced hypoactivity can be disrupted by specific classes of neurotherapeutics, notably neuroprotectives and nootropics. We then screened for novel compounds and identified structural families of small molecules able to phenocopy the known drugs. Zebrafish larvae were then used to conduct inexpensive in vivo structure-activity relationship studies. We then took related compounds with varying in vivo potencies and screened them for binding at more than 40 candidate mammalian neuronal targets. Our results implicated sigma-1 receptor as an efficacy target with nanomolar affinity for our lead compounds. To confirm sigma-1's involvement, we created knockout zebrafish using CISPR-cas9 technology. We found that our lead compounds no longer worked in sigma-1 KO fish, but surprisingly this mutant phenotype was only observed when unmasked with an antimuscarinic (amnesic) agent. Similarly, antimuscarinics could only counteract our lead compounds in sigma-1 KO animals, not wild-type controls. This indicates polypharmacology, as these particular compounds are both sigmaergic and pro-muscarinic. We tested these compounds for both muscarinic receptor binding and acetylcholine esterase inhibition, but the results were negative. This suggests the muscarinic effects are indirect and could not have been discovered using a simple target-based screen. We are now testing these compounds in rodents to determine their translational potential.

3:00 pm | Tuesday | January 26

Jonathan Baell, Monash Institute of Pharmaceutical Sciences

Histone Acetyltransferase Inhibitors, from Screening to Optimization - A Tricky Track

Histone acetyltransferase inhibitors, from screening to optimization - a tricky trackThere is currently great interest in compounds that modulate epigenetics. With respect to some epigenetic targets, such as histone decacetylases (HDACs), many inhibitors have been successfully developed and are in clinical trials for a variety of indications. Similarly, bromodomains have been shown, somewhat unexpectedly by some, to be highly druggable. However, there is an elephant in the room, and that is the histone acetyltransferase (HAT) family, which is large but essentially "undrugged" and barely has any compounds that could be considered to be useful tools. Why is it so hard to find good tool compounds for these enzymes? Not so long ago we ran HTS against a MYST HAT [1] and eventually discovered a genuine hit that we have recently just optimized to nanomolar levels of inhibition. However, we encountered many problems en route. In this talk we will discuss such issues and how these could help explain why there are so few, if any, useful tool compounds for these enzymes.[1] Falk H, Connor T, Yang H, Loft KJ, Alcindor JL, Nikolakopoulos GE, Surjadi RN, Bentley JD, Hattarki MK, Dolezal O, Murphy JM, Monohan BJ, Peat TS, Thomas T, Baell JB, Parisot JP, Street IP. An efficient high-throughput screening method for MYST family acetyltransferases, a new class of epigenetic drug targets. J. Biomol. Screening 16 (2011) 1196-1205.

3:30 pm | Tuesday | January 26

Louis Cohen, Icahn School of Medicine at Mount Sinai

ON DEMAND

High Throughput Screening of Metagenomic DNA Libraries

The trillions of bacteria that make up the human microbiome are believed to encode functions that are important to human health; however, little is known about the specific effectors that commensal bacteria use to interact with the human host. Functional metagenomics provides a systematic means of surveying commensal DNA for genes that encode effector functions. Here we examine 3,000 megabases of metagenomic DNA cloned from three phenotypically distinct patients for effectors that activate NF-_B, a transcription factor known to play a central role in mediating responses to environmental stimuli. This screen led to the identification of 26 unique commensal bacteria effector genes (Cbegs) that are predicted to encode proteins with diverse catabolic, anabolic and ligand binding functions and most frequently interact with either glycans or lipids. Detailed analysis of one effector gene family (Cbeg12) recovered from all three patient libraries found that it encodes for the production of N-acyl-3-hydroxypalmitoyl-glycine (commendamide). This metabolite was also found in culture broth from the commensal bacterium Bacteroides vulgatus, which harbors a gene highly similar to Cbeg12. Commendamide resembles long-chain N-acyl-amides that function as mammalian signaling molecules through activation of GPCRs, which led us to the observation that commendamide activates the GPCR G2A/GPR132. G2A has been implicated in disease models of autoimmunity and atherosclerosis. This study shows the utility of functional metagenomics for identifying potential mechanisms used by commensal bacteria for host interactions and outlines a functional metagenomics-based pipeline for the systematic identification of diverse commensal bacteria effectors that impact host cellular functions.

4:00 pm | Tuesday | January 26

Kristian Birchall, MRC Technology

Key Considerations in Compound Collection Composition and Quality – Seeding Success in Hit Follow Up

The adage "garbage in, garbage out" is painfully true in HTS. Attrition at the stage of hit triage is a frustrating waste of resource, especially given the repeated deployment of a screening collection, the difficulty of removing troublesome compounds and the consequences of following up poor quality hits. Thankfully there is an increasing awareness of the factors associated with poor prognosis, including physicochemical property profiles (e.g. QED score) and sub-structural flags (e.g. PAINS). However, many more subtle combinations of factors such as the location and frequency of undesirable groups alongside synthetic tractability can also diminish the prospects of a compound in hit follow up. Whilst it can be difficult to develop a set of rules that cover all possible scenarios, undesirable compounds can easily be spotted by experienced medicinal chemists. We describe a "wisdom of crowds" approach used as the final step when building our compound collection. Other examples of strategies employed to improve the chance of success will be described including; the use of upfront experimental data to steer compound selection away from off-target liabilities, the use of target-focused sets to improve hit rates and the use of knowledge annotated sets to expedite hit follow up.

4:30 pm | Tuesday | January 26

Allan Beck Christensen, Vipergen APS
Co-Authors: Peter Blakskjær, Allan Christensen, Nils Hansen, Tara Heitner, Johan Holmkvist, Stine Jørgensen, Lars Petersen, Frank Sløk, Vipergen

Efficient Drug Discovery Using High Fidelity DNA-Encoded Small-Molecule Library Technologies

Screening based on technologies relying on DNA encoded libraries (DELs) of small molecules have in recent years become increasingly used, due to the ability to screen large libraries in a cost- and time-efficient way. Of importance in such screens is the quality of the library as well as the reliability of the technologies used for screening the library. The yoctoreactor (yR), a second generation technology for preparing DNA-encoded libraries was used to ensure that libraries of maximal fidelity could be generated for seamless drug discovery. In this technology, three dimensional DNA structures are formed through self-assembly of DNA oligonucleotides, each 3D structure serving as a single-molecule chemical reactor. The reactor allows efficient chemical reactions between chemical building blocks to take place in a cavity in the center, which comprises a volume in the order of a yoctoliter (10-24 L). The DNA is used both for enabling the synthesis of the library compounds, purification handle during the library synthesis, and as barcodes for the compounds selected in the subsequent process of screening. Drug hits/leads are identified from the yR library using a second generation screening technology, the Binder Trap Enrichment (BTE) which is a homogeneous (immobilization-free) screening assay. BTE employs a unique principle of trapping small molecule binders together with the protein target in miniscule droplets and enables direct identification of potent inhibitors with low false-positive rates. These second-generation technologies are shown to enable the identification of low nanomolar cellular active inhibitors of both classical small molecule targets such as kinases, and more challenging small molecule targets such as protein-protein interaction targets and epigenetic targets.

9:30 am | Wednesday | January 27

Ben Hoffstrom, Fred Hutch Cancer Research Center
Co-Author: Norman Boiani, Fred Hutch Cancer Research Center

High-Throughput Screening of Monoclonal Antibodies to Multiplexed Antigens

The Fred Hutch Antibody Technology core offers time and cost-efficient custom monoclonal antibody development for in-house and external clients. Since its inception in 1995, the lab has developed over 7500 hybridoma cell lines from mice, rats, and rabbits, several of which are commercially licensed reagents. We offer a high-throughput platform for simultaneous screening of monoclonal antibodies to 5 different antigens. The platform uses a two-stage workflow coupling a combination of target-based hybridoma colony selection and cytometric bead-based target deconvolution. The target identification assay is 25-50 times more sensitive than traditional ELISA-based screens, which allows for rapid ranking of several thousand antibodies based on their target affinity and murine isotype. We have validated the platform for modified peptide targets, small molecules, recombinant proteins, and cell surface receptors. A general overview of the workflow will be outlined and specific projects will be highlighted.

10:00 am | Wednesday | January 27

Elizabeth England, Antibody Discovery and Protein Engineering, MedImmune, UK

Screening and Characterisation Assays to Overcome Challenges in Biologics Discovery

Advances in the monoclonal antibody field, namely hybridoma and phage display technologies and the ability to produce humanized or fully human antibodies, have resulted in antibodies becoming an increasingly significant component of the therapeutic landscape. These molecules have utility across a range of therapeutic areas and their success has been driven by some of their unique properties, in particular their high specificity and target selectivity. Identification of potential lead antibodies in the drug discovery process requires the use of assays that not only measure binding of the antibody to the target molecule, but assess a wide range of other characteristics. These include affinity ranking, measurement of their ability to inhibit relevant protein-protein interactions, assessment of their selectivity for the target protein and determination of their species cross reactivity profiles to support in vivo studies. Often a potential lead antibody is identified that has almost all of the desired drug properties but requires optimization of a particular characteristic such as potency, affinity, species cross-reactivity or stability. Mutagenesis of the amino acid sequence of the antibody to optimize these qualities is a well validated process at MedImmune and screening strategies to identify optimized antibodies have been employed successfully. This presentation highlights some of the challenges in identifying optimized antibodies and describes how biochemical and cell-based assays have been employed at MedImmune, to effectively identify antibodies with all the desired drug properties.

10:30 am | Wednesday | January 27

Joel Blanchard, Scripps Research Institute
Co-Authors: Kristin Baldwin, Nadja El-Mecharrafie, Jia Xie, Richard Lerner, The Scripps Research Institute

Autocrine-Based Selection from Antibody Libraries Reveals New Pathways to Pluripotency and Cancer

Identification of biological targets that drive disease progression has been hampered by a limited ability to systematically perturb biochemical interactions in complex, heterogeneous cellular systems. Here we developed an antibody-based autocrine selection platform to identify rare biochemical mechanisms that regulate emergent biological properties in live cells. In this platform, each cell is a reaction compartment in which a single biochemical event is perturbed by the presence of an antibody. By genetically encoding the antibodies we link antibody genotype to phenotypic outcomes. This facilitates rapid and efficient screening of very large (>108) combinatorial antibody libraries in mammalian cells. The addition of signaling sequences can be used to sequester antibodies in discrete intracellular and extracellular signaling compartments where they reach much higher effective concentration than if added directly to the solution. The use of antibodies also permits easy and rapid target identification through standard biochemistry. As a proof of principle that antibody selection in mammalian cells can identify novel targets from complex, heterogeneous cellular systems, we asked whether perturbing events at the cell surface with antibodies can reverse development and reprogram a differentiated cell back to an embryonic state. After screening more than 100 million unique antibodies we captured multiple antibodies that can replace Sox2 or Oct4 during reprogramming. Induced pluripotent cells derived using these antibodies express appropriate pluripotency markers, can be directed to differentiate in vitro, contribute to the inner cell mass of a blastocyst in vivo yielding live chimeric offspring. Characterization of a single hit antibody revealed a novel intracellular signaling pathway that plays a fundamental role during reprogramming. We further show that this pathway is active and plays a physiological role in several cancers and also correlates with disease progression and drug resistance in patients. Collectively, this study demonstrates autocrine-selection of antibodies from large combinatorial libraries is a powerful technology for dissecting signal from noise capable of identifying unrecognized causal events that drive complex biological processes.

11:00 am | Wednesday | January 27

Mark Cameron, Beckman Coulter
Co-Authors: Douglas Astry, Kristen Pauly, Beckman Coulter

A Universal Pharmacokinetic Assay for Biologic IgG Drugs with Utility in Multiple Preclinical Species Using Spatial Proximity Analyte Reagent Capture Luminescence (SPARCL)

For quantification of human IgG drugs in preclinical species, we screened anti-human IgG antibodies for use in the development of a universal pharmacokinetic (UPK) assay using SPARCL, a novel homogeneous chemiluminescent detection technology. In a SPARCL assay, a chemiluminescent substrate (acridan) is brought into the proximity of an oxidative enzyme (horseradish peroxidase, HRP) through the specific antigen/antibody interaction. A flash of light proportional to the quantity of analyte present in the sample is generated upon addition of a trigger solution containing hydrogen peroxide. SPARCL assays are run on plate-based luminometers and require no specialized equipment beyond the luminometer with injectors. The primary objectives of this effort were; (i) to identify a single set of reagents for the quantification of human IgG1, IgG2, IgG3 and IgG4 drugs dosed in mouse, rat, canine and non-human primate (NHP), (ii) to compress assay development timelines, and (iii) to allow rapid throughput in the sample analysis phase of pharmacokinetic projects. Antibodies were labeled with acridan or HRP and screened for endogenous IgG against 10% serum from each species. Antibody candidates selected from matrix screening were evaluated for utility in detecting human IgG1, IgG2, IgG3 and IgG4 in mouse, rat, canine and NHP serum. Antibody candidates passing both matrix screening and human IgG detection screening were used to develop the SPARCL homogeneous immunoassay. The SPARCL assay delivers a wide dynamic range (3.125-1,000 ng/mL), short run time (30 minutes), requires no washing steps and is run in neat (undiluted) matrix. The intra assay total error in mouse serum was under 17.4% for all of the quality control (QC) samples (400, 40 and 10 ng/mL), under 14.9% for rat QC's, under 19.3% for canine QC's and under 17.1% for NHP QC's. The SPARCL format provides for rapid UPK assay development of IgG1, IgG2, IgG3 and IgG4 drugs in all species tested. The assay may become a valuable tool in the study of preclinical pharmacokinetics. This cost effective, resouce saving SPARCL technology may have utility in many other ligand binding assay formats in a variety of industries that use immunoassays for low, medium and high throughput applications.

1:30 pm | Wednesday | January 27

Clive Dilworth, Cyprotex

Predicting the in Vivo Phospholipidosis-Inducing Potential of Drugs by a Combined in Vitro High Content Screening and in Silico Modeling Approach

Drug induced phospholipidosis (PLD) is an adverse side effect which can affect registration of new drug entities. Phospholipids can accumulate in lysosomes, organelles essential in cellular biogenesis and if compromised can lead to cellular toxicity. Drug accumulation in lysosomes (lysosomotropism) is a known mechanism leading to PLD, however phospholipidosis can also occur indirectly by altering synthesis and processing of phospholipids.Drug induced PLD can be measured in vitro using high content screening (HCS) approaches, by either determining accumulation of phospholipids conjugated to dyes in cells or by determining accumulation of drugs within lysosomes measuring competitive loss of lysosomal dye uptake. In this presentation we will show the validation of two in vitro assays using HepG2 and H9c2 cells in conjunction with in silico models based on compound derived physico-chemical properties. We assessed a total of 56 compounds of which 28 were phospholipidogenic, 25 were non-phospholipidogenic and three were kidney specific and compared the in vitro data to the observed in vivo result. Using a combined HCS approach with in silico modelling in HepG2 cells the overall prediction of PLD in vivo with a sensitivity of 96%, specificity of 92% and an overall accuracy of 94%.We will demonstrate the applicability of in vitro and in silico approaches to understand the mechanisms underlying PLD and the utility of these approaches as a screening strategy in the pharmaceutical industry to select drug candidates with a low in vivo PLD liability.

2:00 pm | Wednesday | January 27

Barbara Saxty, MRC Technology

Development of a Screen Cascade to Validate ULK 1 Kinase Inhibitors

Autophagy is a membrane-driven lysosomal degradation pathway, whereby initially an isolation membrane sequesters a portion of the cytoplasm leading to autophagosome formation, which then fuses with lysosomes exposing the contents to hydolases. ULK1 (Unc-51-Like Autophagy activating Kinase 1) is a serine/threonine protein kinase which forms a complex with FIP200 and ATG13. This complex is essential for autophagosome formation, and activation of ULK1 is required for initiation of autophagy. Targeting ULK1 kinase activity may represent a means of attenuating both basal and induced autophagy. Basal autophagy plays a critical role in maintaining cellular homeostasis and genomic integrity by degrading aged or malfunctioning organelles and damaged misfolded proteins. Induction of excess autophagy during cellular stress, such as nutrient deprivation, hypoxia or chemotherapy has been proposed as a potential cell survival mechanism in cancer, and may lead to resistance. We have identified a panel of dual ULK1/ULK2 inhibitors from a biochemical screen using full length recombinant human ULK1. A panel of cell based reporter and phenotypic assays were developed to profile these inhibitors and I will present the outcome of a hit to lead program aimed at improving potency, selectivity and ADME properties.

2:30 pm | Wednesday | January 27

Remko van Vught, Mimetas
Co-Authors: Jos Joore, Henriëtte Lanz, Bas Trietsch, Paul Vulto, Nienke Wevers, Karlijn Wilschut, Mimetas

3D Networks of iPSC-Derived Neurons and Glia for High-Throughput Neurotoxicity Screening

The assessment of neurotoxicity remains a major scientific challenge due to the complexity of the central nervous system. Current strategies to evaluate toxicity of drugs and chemicals are predominantly based on ex vivo or in vivo animal studies. These models have limited predictability for neurotoxicity in humans and are not amenable to high-throughput testing. In order to overcome these limitations we are developing a neurotoxicity model based on iPSC-derived neurons in OrganoPlates™ (1, 2). This microfluidic platform enables high-throughput screening of miniaturized organ models. A mixed population of human iPSC-derived neurons consisting of GABAergic and glutamatergic neurons with supporting astrocytes was cultured in 3D, closely representing the physiology of the human brain. As a part of the validation, proper network formation was observed by neuron-specific immunostainings and neuronal electrophysiology was analyzed by a calcium sensitive dye indicating spontaneous neuronal firing. Additionally, we investigated the dose-response neurotoxic effects of methylmercury and endosulfan on neuronal viability. The OrganoPlate™ platform enables real time analysis of neurotoxic effects of compounds in high-throughput. This iPSC-derived neuronal model can be used to refine animal experiments and has the potential to better predict adverse effect in humans and hence to improve clinical development success.[1] Trietsch, S.J., Israels, G.D., Joore, J., Hankemeier, T., Vulto, P. , Microfluidic titer plate for stratified 3D cell culture, Lab Chip, 2013 vol. 13, no. 18, pp. 3548-54 [2] E. Lucumi Moreno, S. Hachi, K. Hemmer, S. J. Trietsch, A. S. Baumuratov, T. Hankemeier, P. Vulto, J. C. Schwamborn, R. M. T. Fleming, Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture, Lab Chip, 2015, DOI: 10.1039/C5LC00180C

3:00 pm | Wednesday | January 27

Gregory Luerman, Axiogenesis Inc.
Co-Authors: Ralf Kettenhofen, Axiogenesis AG; Hirofumi Horai, Hamamatsu Photonics, Hamamatsu, Japan; Thomas Niedereicholz, Hamamatsu Photonics, Herrsching, Germany; Jean Marc D'Angelo, Hamamatsu Photonics, Massy, France; Jia-Ren Lin, Peter Sorger, Huan Wang, Harvard Medical School

Human iPSC-Derived Cardiomyocytes: A Predictive, HTS-Scalable Tool for Comprehensive Cardiac Safety/Toxicity Assessment

Current assessment of the clinical potential of drug candidates to induce life threatening Torsade-des-Pointes arrhythmias (TdP) is primarily based on hERG block and clinical QT prolongation as surrogate markers for proarrhythmia. These methods largely eliminated new drugs with unanticipated proarrhythmic potential from entering the market but eventually led to a steep decline in the development of potentially valuable drug candidates. The FDA/HESI/CSRC-sponsored Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative proposes a paradigm shift in cardiac safety assessment relying on the comprehensive mechanistic understanding of TdP. However, this assessment paradigm represents only a fraction of a comprehensive cardiac safety/toxicity package necessary to advance preclinical molecules into lead candidates and beyond. Chronic dosing effects on (sometimes cardiac-specific) intracellular signaling pathways, such as seen with many tyrosine kinase inhibitors (TKI), as well as structural cardiotoxicity concerns are also of great mechanistic importance in selecting lead matter. In the studies presented we utilized hiPSC-derived Cor.4U cardiomyocytes to compare the effects of dofetilide, E-4031, flecainide, moxifloxacin, ranolazine, quinidine, nifedipine, verapamil, TTX, and ATX II reference compounds in either manual patch clamp and/or microelectrode array (MEA) recordings with HTS compatible 384 well fluorescent calcium and voltage sensitive dye assays. We also present high content imaging (using an Opera HCS Reader) and Hamamatsu FDSS7000 (Fluo-4 based calcium dye) results from cardiomyocytes treated with tyrosine and broad spectrum kinase inhibitors using a sub-acute dosing strategy for cardiotoxicity assessment.We have found human induced pluripotent stem cell (hIPS) derived cardiomyocytes provide a predictive, flexible, and HTS-scalable tool for comprehensive cardiac risk assessment.

10:30 am | Monday | January 25

Robin Felder, The University of Virginia

Fully Automated 3D Cell Culture Provides Standardized, Biologically Relevant, and High Production for Human Cells

The consistent and optimized production of living human cells for drug discovery and regenerative medicine faces many challenges including the need for cost effective large scale expansion, improved representation of in vivo cellular physiology, and the ability to achieve reproducible data and/or cellular products. In order to achieve these goals there has been an evolution in the methods used to culture cells involving the use of 3D approaches that include the growth of cells in and on biomimetic substrates, optimization of cell culture media, and exposing cells to shear forces and oxygen tension that more closely mimics the in vivo environment. In addition, in order to make these new 3D processes more cost effective there is an increasing interest to fully automate the cell culture process. However, many new in vitro 3D cell culture methods, which provide improved physiologically and biologically relevant cellular phenotypes neither lend themselves to automation nor allow the process to be scaled for large cell biomass production. We have designed and are building a fully automated 3D cell culture robotic system that allows for parallel or random access processing of many cell lines each sourced from unique individuals. This next generation cell culture robot will allow cell based assays on biologically diverse populations of cells in order to test lead compounds for their biodiverse effects (varying effective doses and toxicology). This presentation will discuss the current 3D cell culture systems and their suitability for automation. Data will be shown demonstrating the benefits of each automated 3D cell culture process in terms of cell morphology and function. Each system will be evaluated for its cost/benefit in terms of biological relevance, yield, and quality metrics. Modern 3D cell culture techniques will be objectively discussed in the context of creating improved standards for primary/stem cell production and screening.

11:00 am | Monday | January 25

Taosheng Chen, St. Jude Children's Research Hospital
Co-Author: Frederick Ausubel, Massachusetts General Hospital

Fully Automated and Integrated Approaches Identify Novel Modulators of Xenobiotic Receptors PXR and CAR

Xenobiotic receptors pregnane X receptor (PXR) and constitutive androstane receptor (CAR) regulate drug toxicity and resistance, which are the leading causes of treatment failure and for which no clinically safe and effective remedy is available. PXR and CAR play central roles in activating the expression of CYP3A4, a major enzyme responsible for metabolizing more than 50% of clinically prescribed drugs; ALAS1, a rate-limiting porphyrin biosynthesis enzyme that increases the levels of hepatotoxic protoporphyrin IX (PPIX); and MDR1, an efflux pump elevated in some cancer cells and associated with cancer drug resistance. Therefore activation of PXR and CAR might decrease drug efficacy, induce drug toxicity and resistance. While PXR is ligand-inducible, CAR is constitutively active. Negative modulators of PXR and CAR (i.e., antagonists of PXR and inverse agonists of CAR) may prevent drug-induced liver toxicity and overcome drug resistance. However, specific and non-toxic PXR antagonist effective in vivo is still lacking. In addition, known inhibitors of CAR (acting either as inverse agonists that reduce its constitutive activity or as antagonists in the presence of agonists) also activate PXR, rendering them mechanistically counterproductive in tissues where both PXR and CAR are present and active. Therefore, we sought to identify novel PXR antagonists and CAR inhibitors that do not activate PXR. The unique ligand binding domains of both PXR and CAR enable them to bind many structurally distinct compounds (referred to as "ligand promiscuity"), making a structure-based drug design approach to discover their modulators challenging. By using a large-scale and fully automated HTS approach, we have identified and optimized PXR antagonists. By focusing on the putative PXR antagonists identified from the HTS, we have also identified potent and novel inverse agonists of human CAR that do not activate human PXR. We have investigated the mechanism of action of these novel modulators of xenobiotic receptors by performing structural and functional analysis, and evaluated their in vivo activities by using humanized animal models. To establish structure-activity relationship, we used the novel chemical scaffolds of the lead compounds to design analogs and evaluated their activity, leading to more potent modulators. For example, the most potent analog of the lead CAR inverse agonist showed >50 fold enhancement in its inhibitory activity against human CAR. Our data indicate that it is feasible to use large-scale, automated and integrated approaches to effectively discover and develop modulators of xenobiotic receptors and use them to prevent drug-induced liver toxicity and overcome drug resistance.

11:30 am | Monday | January 25

Yueming Wang, High-Throughput Screening Center, Chemical Biology and Therapeutics, St. Jude Children's Research Hospital
Co-Authors: Taosheng Chen, Douglas Green, Fabien Llambi, St. Jude Children's Research Hospital

High-Throughput Screening Using BOK-mediated Apoptotic Assay Identifies Novel Proteasome Inhibitors Against Human Cancer

Promising preclinical and clinical studies on proteasome inhibitors have shown proteasome inhibition as an effective anticancer strategy. However, the current proteasome inhibitors exhibit severe toxic effects, decreased efficacy toward solid tumors and the occurrence of drug-resistance in patients because they target multiple pathways involving various cell functions; therefore, novel proteasome inhibitors with high selectivity on targeting cancer cell survival or proliferation pathways are warranted. Pro-apoptotic proteins can induce cell cancer apoptosis, and stabilizing such proteins and preventing their degradation by inhibiting proteasome activity may provide a promising approach to discover highly selective proteasome inhibitor against cancer cells. Our studies found that Bcl-2 related ovarian killer (BOK) is a proapoptotic protein in B-cell lymphoma-2 (BCL-2) family, and can be rapidly degraded through a proteasome-mediated pathway; inhibition of proteasome can stabilize BOK protein and increase its accumulation, which subsequently elicits apoptotic cell death. Using Bax-/-/Bak-/- double knockout mouse embryonic fibroblasts (MEFs), which display a pronounced resistance to apoptosis, we developed a doxycycline-inducible BOK overexpression cell line (BOK-dox/Bax-/-/Bak-/- MEFs). In this cell line, BOK can induce apoptosis only when BOK protein levels are stabilized. Using caspase-3/7 activation as a marker of BOK-mediated apoptosis, we performed a phenotypic high-throughput screening (HTS) to identify novel proteasome inhibitors that can stabilize BOK and induce BOK-mediated apoptosis. In the primary screen, 8,904 (4,282 unique) compounds with known biological activity from the St. Jude bioactive collection were tested at 12 _M using an automated HTS system. The Z'-factor in the primary screen was 0.88 ± 0.027 (n = 34), indicating a high degree of assay sensitivity and reproducibility for HTS. In the confirmation screen, the top 59 hits (> 15% activities of the positive control) from the primary screen were further tested for their dose responses (56 _M to 2.8 nM final compound concentration). Using secondary cell-based proteasome-Glo assays on the confirmed hits, we identified that two natural compounds, obtusaquinone and pomiferin, exhibited previously unreported proteasome inhibitory effects in the micromolar range and showed effectiveness against a number of human cancer cells through BOK-mediated apoptosis. This screen effectively exploited a parallel phenotypic- and target-based approach and successfully discovered novel proteasome inhibitors, suggesting that the current assay and screen method can be employed for automated HTS using large chemical libraries.

12:00 pm | Monday | January 25

Helen Plant, AstraZeneca Pharmaceuticals
Co-Authors: Paul Harper, Sinead Knight, Darren Plant, AstraZeneca Pharmaceuticals; Rebecca Dixon-Steele, University of Bristol

Addressing the Challenges of 1536 Cell Based Screening

Many phenotypic cell-based assays are limited to 384 well format due to the challenges of liquid handling, and the need for consistency during microplate washing and media change steps. Use of expensive assay formats in 384 format impacts the number of compounds that can be screened through such assays. The BlueCatBio centrifugal plate washer integrates centrifugal emptying with the individual addition of up to four separate solutions for complex phenotypic assays. The achievement of near zero residual volume by using centrifugation rather than aspiration is enabling for 1536 well formats. A typical phenotypic imaging assay previously used to screen the AstraZeneca (AZ) 1.8 million compound collection was limited to 384 well format due to the need for consistent washing steps after fixation and staining. To miniaturize this assay to 1536 well format the centrifugal plate washer was used to perform washing cycles. To assess the robustness and reproducibility of the assay in 1536, both Fluorescence Intensity (FI) and Imaging (2X channels) endpoints were employed. There was no disruption of the cell monolayer post-centrifugal washing cycles, and similar HTS quality metrics were obtained in 1536 format compared to that obtained in the original HTS using 384 format. The Corning® Epic® system is a label-free detection system that uses resonant waveguide grating to measure the drug-induced response of cells using dynamic mass redistribution (DMR). DMR is measured by the refraction of light from a biosensor integral to an Epic plate, but the cost of these high value plates has limited the use of this technology at scale. To enable its use in HTS we have developed a 1536 format Epic® assay, but many challenges have been encountered throughout this process. We have previously used commercially available 384 Epic® plates which are pre-coated with fibronectin. For 1536 plates we investigated whether we could apply this coating in house to reduce plate costs. We evaluated a range of different matrices for optimal cell adherence and use of the centrifugal plate washer was enabling allowing development of a standardized protocol for coating. We optimized the liquid handling capability for 1536 as the majority of equipment had been optimized only for 384 Epic® plates. Comparable EC50 data was generated in both 384 and 1536 well formats, allowing use of this miniaturized DMR assay to screen a library of 43K compounds over a 2 week period. Successful conversion of complex phenotypic assays to 1536 format resulted in up to a 25% reduction in cell number requirement and approximately a 4-fold reduction in time taken to perform a full HTS screening campaign. It has also allowed screening of expensive assay formats and cell types in high-content phenotypic HTS screens.

3:00 pm | Monday | January 25

Mitchell Hull, California Institute for Biomedical Research

Cell-Based Assay Development Through Design of Experiment and Automation

Once limited to large institutes, ultra-HTS systems are now more common and the necessary software and hardware is available more or less "off-the-shelf". The faster screening rate made possible by these systems has placed an increased demand for high quality assays against diverse targets. Because of this, assay development is a bottleneck at many facilities. Design of Experiment (DoE) is a process that allows many factors to be tested simultaneously to quickly cover a broad area of experimental space and find relationships between factors. Combining automation platforms with existing DoE software packages leads to rapid acceleration of the assay development process. This has gained popularity in biochemical assay development where testing a broad range of buffer constituents can be very advantageous. Its advantage in cell-based assay development is less apparent as fewer factors can be easily modified. However, even though the experimental space is more limited, DoE's elucidation of factor relationships is powerful by itself. It is here that DoE brings value to cell-based assay development where parameters are very likely to affect one another. By finding these interactions early, development is accelerated and unexpected interactions are less likely to derailed optimization. We will present our process, describe the tools and provide examples of how we use automation paired with DoE to improve cell-based assay optimization.

3:30 pm | Monday | January 25

Oliver Leven, Genedata AG, Switzerland
Co-Authors: Matthias Fassler, Stephan Heyse, Daniel Siegismund, Stephan Steigele, Genedata AG

A Flexible Workflow for Exploration and Prototyping of Analytical Solutions in High-Content Screening

Analysis of image-based high content screens mainly adhere to one simple pattern: Computer-assisted analysis of images generated on automated microscopes followed by processing of the numerical data on the object level and/or its higher level aggregates (well and plate level). Result integrity is ensured by appropriate QC procedures and comparability by data normalization. The final activity or potency of individual compounds is defined based on a single or a few readout(s) and hit lists are generated by simple filtering rules. Whereas such a procedure is suitable for many production screens with a well-defined biology, it is not readily applicable in assay development, for phenotypic screens, or for MOA studies. In these cases, possible experimental end points have yet to be defined or are simply not obtainable a priori. There are diverse strategies available to obtain the required knowledge, but these are difficult to realize as they require fundamental expert knowledge and often multiple solutions with conflicting results exist. Here, we describe a comprehensive HCS analysis workflow that allows us to explore complex analytical strategies. Every analysis step can be fully controlled, starting from image analysis up to multivariate analysis scenarios.
The modular design of the analysis workflow allows for flexible selection of the appropriate analytical strategy to address a certain target biology. The workflow includes image analysis, data pre-processing, feature reduction of data, and un-supervised and supervised (machine learning) methods. The individual applicability of particular methods can be evaluated by appropriate performance measures, e.g. by comparison against a known ground truth. State-of-the-art visualization techniques facilitate the review of data and classification results. Using benchmark datasets, we show the versatility and efficiency of this pipeline when exploring strategies for complex analytical questions.

4:00 pm | Monday | January 25

Alexander Schreiner, PerkinElmer
Co-Authors: Karin Boettcher, Angelika Foitzik, PerkinElmer

Analyzing Signal Transduction on a High Content Screening System Using Förster Resonance Energy Transfer Based Biosensors

Cells constantly receive signals of different kinds and origin. This could be e.g. signals originating from growth factor receptors, cytokine receptors, cell adhesion molecules or nuclear receptors. Hence, cell behavior is a result of a spatial-temporal integration of these different signals. Analyzing these signals in situ (in living ore fixed cells) can be quite challenging. One powerful way to do this is to use FRET based biosensors. We have used an ERK biosensor and present data, how such a biosensor can be used to study signaling pathways leading to altered ERK activity in live cells. The data has been generated on an optimized high content screening system. It shows, that FRET based biosensors can be used to set up robust high content assays that could enable one to study the effect of a large number of compounds on signal transduction. Furthermore, with the large number of biosensors that have been generated over the years it should be possible to analyze all kinds of different receptors, for example receptor tyrosine kinases or G protein coupled receptors.

4:30 pm | Monday | January 25

Ivan Pushkarsky, University of California, Los Angeles
Co-Authors: Robert Damoiseaux, Dino Di Carlo, University of California, Los Angeles

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Elastomeric Sensor Surfaces for High-Throughput Phenotypic Screening of Cellular Force Generation

Cell-generated forces play key roles in many physiological systems, such as regulating local vascular resistance, producing cardiac contractions, and driving intestinal motility. Additionally, these forces are necessary to critical processes at the cellular level such as phagocytosis of pathogens and remodeling of extracellular matrix (ECM) fibers. Since forces are intrinsic to function, a variety of disorders can be triggered directly as a result of faulty force generation, including stroke and asthma. Furthermore, cellular force may in some cases assist the progression of diseases like cancer. Thus, the force-generating phenotype should be a target for new therapies and it becomes important to screen for compounds and other agents that selectively modulate this phenotype across a range of cell types. We describe a novel high-throughput phenotypic screening platform to directly measure the forces generated by single-cells that is fully integrated with a standard 96-wellplate.The platform is composed of a glass-backed thin-film of soft PDMS containing arrays of uniformly shaped adhesive and fluorescently-labeled biomolecular patterns that are displaced by forces applied by adhered cells. The patterns are covalently coupled to the PDMS using a sacrificial process we developed which can be used to pattern virtually any ECM protein enabling us to uniquely simulate various physiological environments. Custom image analysis software measures the displacements of those patterns adhered to by single-cells relative to the non-displaced patterns (i.e. patterns not adhered to by cells) present in the same image set, which serve as internal controls, and displays a visual report of the population distribution of this phenotype. The planar substrate is mounted onto a bottom-less 96-wellplate to facilitate typical high-content screens using standard laboratory automation equipment. This approach greatly simplifies cell-generated force evaluation compared to the standard traction force microscopy (TFM) method. By micro-patterning adhesive biomolecules into specific shapes, our platform limits the degrees of freedom in cell orientation and force directionality. This allows for direct comparisons between pattern displacements without intensive computations to normalize complex cell behaviors as is required in TFM. Additionally, the predetermination of cell morphology defines the full range of possible substrate deformations (e.g. pattern displacement) making time-dependent analysis simple. Using the assay, we first characterized the myosin II inhibitor blebbistatin and calculated an IC50 matching previously reported values obtained using molecular rather than phenotypic assays. Next, we induced contraction in human bronchial smooth muscle cells with bronchoconstrictive agents histamine and acetylcholine against which we are screening compounds from the Prestwick Chemical Library® and LOPAC®1280 to identify new therapies for asthma. Finally, we demonstrated the compatibility of the platform with a wide range of cell types including both tissue and blood cells like macrophages, which will be important to use in counter-screens to prevent off-target effects on cell contractility.

10:30 am | Tuesday | January 26

Omai Garner, UCLA Department of Pathology and Laboratory Medicine

MALDI-TOF MS as a High-Throughput Identification System for Clinical Microbiology

The clinical microbiology laboratory finds itself on the precipice of lab automation. Taking innovations and techniques from clinical chemistry, new technologies are being developed and implemented to make clinical microbiology high-throughput in order to meet the increasing demand of health care. The implementation of Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) for the routine identification of clinical bacterial and yeast isolates has had a dramatic impact on patient care, turn-around time, and work-flow within the clinical microbiology laboratory. Classic bacterial identification revolved around biochemical techniques that could take days to process, were very labor intensive, and low throughput. In this study, we show that MALDI-TOF microbial identification is highly reproducible and can tolerate numerous variables, including differences in testing environments, instruments, operators, reagent lots, and sample positioning patterns. Data collected over the last 6 months shows a significant decrease in turn-around time to positive identification within multiple bacterial and yeast identification areas of the lab. A user evaluation was conducted to qualitatively assess technologist impressions of MALDI-TOF based on specific metrics related to suitability of the instruments in the current workflow. The median years of clinical laboratory experience of the technologists who responded was 7.5 years. MALDI-TOF identification rated highly with median scores of 4 to 5 for all usability domains including “walk away criteria” and work flow fit. We found that the Vitek MS (an FDA approved MALDI-TOF platform) can identify 48 isolates per hour and can hold up to four plates in a single run. This high throughput identification is ideal for laboratories that have multiple benches. Technologists at each bench can spot their own plate, enter isolate information at their own workspace, and transfer the information over to the analysis software. At designated times, all plates can be loaded into the instrument and analysed. Multiple studies and clinical practice have now revealed MALDI-TOF MS for high throughput bacterial and yeast identification as the new gold standard in clinical microbiology.

11:00 am | Tuesday | January 26

Christian Oste, Precision System Science Co., Ltd.
Co-Authors: Anders Holmberg, Morten Lukacs, Tetsuya Ueda, Precision System Science Co., Ltd.

Usable Nucleic Acids from Problematic Sources

The rapid advances made in personalized medicine are compelling the scientific and medical communities to gather an increasing volume of genetic/genomic information from an ever broadening diversity of sources.One of the major issues with this endeavor is the quality of the nucleic acids samples which can be obtained from the source material. Another one has to deal with how the purified nucleic acids are being conserved for future reference, which in itself would be the topic of another presentation. This presentation will focus on two particular cases, whose handling requires some precaution in order to safely obtain usable nucleic acids.The first case deals with the purification of sequence-able nucleic acids from paraffin-embedded tissues (FFPE). An automated protocol will be described, from starting sample to DNA ready to be sequenced.The second case addresses more specifically the purification of DNA samples from large volume, complex and heterogeneous samples, such as in the cases of microbiome and metagenome analysis. The automated protocols which are being developed for these applications will be described as well.

11:30 am | Tuesday | January 26

Michael Farnum, Covance Inc.

Improving the Design and Execution of Clinical Trials Through Big Data and Elegant Software

This presentation will outline a new technology platform designed to improve the monitoring of clinical trials, resulting in higher quality, faster timelines, and lower costs. Covance's Xcellerate® Monitoring is an industry-leading platform that uses advanced data integration, analytic, and visualization capabilities to ensure patient safety and data quality throughout the clinical development process. Xcellerate Monitoring provides unprecedented access to all clinical trial data and enables comprehensive assessment and mitigation of risk at the study, site, and patient level. Its powerful but intuitive user interfaces allow central monitoring staff to maintain site monitoring plans, identify and mitigate potential risks in the conduct of a clinical trial, and efficiently direct site monitors to the right locations with the right frequency to assure patient safety and data quality with greater insight, speed, and efficiency. The underlying methodology is fully aligned with FDA and EMA guidance and the TransCelerate principles, and is based on the three cornerstones of Risk Based Monitoring (RBM): quality by design, central monitoring, and triggered adaptive on-site and remote monitoring.

12:00 pm | Tuesday | January 26

Yong Zeng, University of Kansas
Co-Authors: Yuqin Shang, Peng Zhang, University of Kansas

Multi-Scale Integrated Lab-on-a-Chips: From Bioanalysis to Clinical Diagnostics

Lab-on-a-Chip (microfluidics) has evolved from initially a scale-dependent chemical analysis technology towards a versatile platform that opens up new opportunities to addressing the challenges in biology and medicine. In particular, it provides a powerful tool to promote quantitative and systems analysis of molecular and cellular events in complex biological systems, such as cancer and other diseases. This presentation will introduce our efforts on developing multi-scale integrated LOC systems for sensitive and quantitative molecular analysis and their applications to clinical diagnosis of cancer. The first part of the talk will be focused on microfluidic integration of bioaffinity assays on the pico- to femtoliter scales to substantially improve the quantitative detection and biochemical characterization of biomolecules. Their applications to detection and glycan profiling of proteins markers associated with tumors will be presented to demonstrate the potential use for cancer diagnosis. In the second part, we will be discuss developing new nano-bio interfaced LOC systems to investigate circulating exosomes in blood for non-invasive cancer diagnosis. Our results demonstrate that these new methods enables quantitative detection and molecular profiling of tumor-derived exosomes directly from minimally invasive amount of plasma samples with unmatched detection sensitivity and markedly improved speed. These studies would suggest the feasibility of translating these microfluidics-enabled biotechnologies into biomedical studies and clinical utilities.

3:00 pm | Tuesday | January 26

John Gregoire, California Institute of Technology

High-Throughput Discovery of Solar Fuels Materials for Operation Outdoors in Corrosive Electrolyte

The High-Throughput Experimentation (HTE) project of the Joint Center for Artificial Photosynthesis (JCAP, http://solarfuelshub.org/) performs accelerated discovery of new earth-abundant photoabsorbers and electrocatalysts to enable a scalable solar fuels technology. Solar fuels generators typically operate in highly acidic (pH 0) or caustic (pH 14) electrolyte, requiring the functional materials to endure these conditions under temperature and illumination variations of the outdoors. JCAP-HTE has designed and built high-throughput pipelines for the synthesis and characterization of photoelectrochemical materials, including performance screening in harsh environments. On the synthesis side, the pipelines employ a combination of complementary synthesis techniques: 1. ultra-high throughput printing of precursor libraries followed by reactive annealing and 2. direct synthesis of photoelectrochemical material libraries through physical vapor deposition. To establish composition-property-performance trends within these libraries, a suite of parallel and serial screening instruments have been developed, providing an unprecedented combination of experiment throughput and data quality. The pipelines also include automated implementations of standard material characterization techniques and custom data management infrastructure. This material is based upon work performed by the Joint Center for Artificial Photosynthesis, a DOE Energy Innovation Hub, supported through the Office of Science of the U.S. Department of Energy under Award Number DE-SC0004993.

3:30 pm | Tuesday | January 26

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Glenn Smith, CryoXtract Instruments
Co-Author: Joseph Fraone, CryoXtract Instruments

The Application of Automated Frozen Aliquotting Technology to the Bioanalysis of Labile Compounds

The regulated bioanalysis of many drug compounds can be a challenging proposition as such compounds are often subject to degradation and modification in typical biological matrices such as plasma and serum. A significant consequence of compound instability is its impact on incurred sample re-analysis pass rates. As such, validated bio-analytical protocols must establish reproducibility of drug concentrations from biological samples over multiple freeze-thaw cycles for the purpose of sample re-analysis during the regulated phases of a drug development campaign. Such efforts often require the development of upstream stabilization protocols that can add significant time to assay validation timelinesand introduce complexity to the overall bioanalytical protocol, which again can add risk to ISR. A novel technology and instrument, in which frozen cores are generated from frozen biospecimens for distribution and analysis in place of thawing and liquid aliquotting, was evaluated as a methodology for the in vivostabilization of drug compounds for bioanalysis. Among a number of automated features, the instrument can employ mechanical level sensing of the parent sample surface in order to generate quantitative aliquots of frozen biofluids (40 – 110uL) with an average accuracy of 2% and precision of 9% (results based on frozen EDTA plasma). LC-MS/MS analysis was used to study the uniformity of analyte distribution in frozen samples and to demonstrate the improved stabilization of labile compounds when (re)assayed using frozen aliquots. The application of the technology to the field of regulated drug analysis has great potential for simplifying and streamlining the development, validation, and execution of bioanalytical assays. Because the cold chain of the parent biospecimen is fully maintained during aliquotting procedures, pre-analytical variability associated with freeze-thaw and extreme temperature differentials is significantly decreased over the lifespan and utilization of the sample. Moreover, the ability to generate quantitative frozen aliquots of a specified volume enables streamlining of the sample preparation for bioanalysis, reducing analytical variability as a result of thawing procedures for compounds where bench stability may be a concern. Ultimately, both these advantages of frozen aliquotting may result in faster assay validation timelines, more robust clinical execution, and increased pass rates for ISR.

4:00 pm | Tuesday | January 26

Samantha Grist, The University of British Columbia
Co-Authors: Karen Cheung, Lukas Chrostowski, Meng-Chi (Andy) Liu, S. Soroush Nasseri, Jonathan Schmok, The University of British Columbia

A Microfluidic Platform for 3-D Cell-based Screening of Cancer Treatments Under Chronic and Transient Hypoxia

We present a microfluidic system for screening of cancer treatments using 3-D cell cultures under chronic and transient (cycling) hypoxia. Both chronic and transient hypoxia can affect tumour cell behavior and response to treatment, and the degree of hypoxia has also been found to affect tumour prognosis. Various degrees of hypoxia, oxygen gradients, and transient hypoxia at rates of a few cycles/h have been measured in tumours; however, traditional cell culture environments such as well plates are unable to reproduce these cycling rates due to their long diffusion distances.Microfluidic technology ideally addresses this problem, as the small size scale affords both precise control of the oxygen levels and fast switching times. We have developed a multi-layer microfluidic device fabricated using gas-permeable polydimethylsiloxane. The device contains a chamber with hydrodynamic trapping structures to immobilize 3-D cell cultures. This chamber permits perfusion of cell culture media, and cancer treatments can be added to this perfusion fluid for eventual drug screening applications. The chamber could accommodate either arrays of spheroids or cells in monolayer. The cell culture chamber is surrounded on three sides by gas-control channels, through which gases flow to control the oxygen levels, creating chronic and transient hypoxic profiles as well as oxygen gradients. Profiles are independent of the oxygen level outside the chip or that of the perfused media within the operating flow regime. Optical oxygen sensors measured using fluorescence microscopy are integrated into the bottom of the cell culture channel, permitting non-invasive, in situ monitoring of the oxygen levels in the device.We produce tumour spheroids of controlled size using microfluidically-generated alginate beads containing dispersed cells as well as collagen and Matrigel. Cells within the beads proliferate into spheroids within ~7 days. We demonstrate multi-day cell culture of 3-D tumour spheroids within the microfluidic device. We show that two-photon imaging can be used to monitor tumour spheroid proliferation on-chip with minimal photodamage as well as improved sample penetration due to the long-wavelength excitation, and demonstrate that on-chip spheroid proliferation closely follows proliferation within standard cell culture flasks.We present and compare both experimental and simulated oxygen profiles and switching times within the device at various input flow rates. We demonstrate switching times of < 10 minutes, permitting physiologically-relevant oxygen cycling rates, and demonstrate simulated oxygen control independent of oxygen levels outside the device and input fluid oxygenation down to 0.05% oxygen. We expose breast cancer tumour spheroids to cycling oxygen profiles and observe spheroid response to the cycling profiles, measuring good viability after multi-day on-chip culture with oxygen control. Our system, which combines 3-D cell culture with microenvironment control to reproduce physiologically-relevant conditions, could permit more predictive cell-based assays, potentially forming a highly powerful tool in future cancer research.

4:30 pm | Tuesday | January 26

Brian Rasnow, Etaluma Inc & CSUCI

Extreme Automation: From Hypoxic Chambers to the World's Oceans and 3 Billion Poor People in the Developing World

This talk will describe some example applications and tools for applying lab automation in extreme environments. Modern lab automation began with radioactive materials, and the keynote talk described the state of the art for extraterrestrial automation. Here I'll explore examples from 3 extreme domains: 1) deploying automated measuring devices at a global scale, to improve predictions of global warming models and validate nuclear treaty compliance. 2) Cell culture requires environments that replicate conditions inside living bodies. CO2 incubators provide necessary CO2, humidity, and temperature, but don't replicate in vivo O2 levels. Etaluma's compact fluorescence microscopes have revealed different cell behavior in hypoxic environments, which raise questions about the general applicability of many historical results. 3) What opportunities and challenges are there for lab automation in the developing world? How can the 3 billion people who live on less than $2.50/day better benefit from lab automation and related technologies? I'll describe a proposal by Cure Pharmaceuticals for automated fill and finish machines that could radically reduce the cost of delivering pharmaceuticals in poor countries, and highlight other examples of simple machines for processing food and fuel to potentially and dramatically improve millions of lives. Radical cost-cutting is also enabling in underfunded academic environments, and I'll present some example student projects from measuring levels in 500' deep domestic water wells to focusing microscopes using audio speakers. A common theme is that we rarely can directly measure the quantities we most care about. For example we can't measure "health" directly, but instead measure what we can, e.g., cholesterol and blood pressure, and use models to co-relate the measured quantities with the inferred ones. We commonly apply statistics to propagate uncertainty from measurements to calculated values, but too rarely do we explicitly address uncertainties in the models themselves. I will end with some ideas on how to address these often fundamental uncertainties so one can get closer to learning what we need to know from the limited parameters we are able to measure.

9:30 am | Wednesday | January 27

Olivier Déry, Nexcelom Bioscience
Co-Author: Scott Cribbes, Olivier Dery, Sarah Kessel, Dmitry Kuksin, Jean Qiu, Nexcelom Bioscience; Sue Eccles, Lisa Patterson, Maria Vinci, The Institute of Cancer Research, London

A Rapid High-Throughput 3D Tumor Spheroid Image Cytometry Screening Method for Drug Discovery

The current 2D methods for cancer drug discovery have had some difficulty in identifying potential drug candidates that can be used for clinical testing. To overcome this challenge, there has been an increase in research of 3D tissue culture that facilitated the development of new in-vitro tumor model assays. Traditional 2D and 3D spheroid analysis method relied heavily on visual observation using standard microscopy, which is time-consuming and has high operator variations. In the recent years, high-throughput image-based cytometers have demonstrated the ability to perform bright-field and fluorescence cell-based assays. This type of automated plate-based imaging cytometer can be employed to rapidly analyze and characterize 3D tumor spheroids, which can be used to generate both quantitative and qualitative results. In this work, we demonstrate a novel 3D tumor spheroid analysis method using the Celigo imaging cytometer for spheroid counting, size analysis, tumor migration and invasion, tumor viability, and dose response of drug induced/inhibited tumor growth. The plate-based imaging cytometer utilizes bright-field and three fluorescence channels (Blue, Green, and Red) for multi-channel analysis. By utilizing the F theta lens technology, uniform bright-field image is captured for more accurate cell counting and analysis of the entire well. In addition, Celigo analysis software is used to report numerous parameters allowing detailed spheroid characterization. In addition to direct spheroid counting in the well, the use of specific fluorescent dyes and probes allow the researcher to define viable and hypoxic areas within spheroids and monitor migration and invasion on or into supporting cells and/or tissues. The results showed that Celigo imaging cytometer can accurately count and measure spheroid sizes in response to drug induction. Furthermore, tumor migration and invasion were clearly observed and quantified in the captured images. By utilizing the 3D spheroid imaging cytometry method, researchers can quickly characterize and quantify tumor spheroids in a high-throughput format, which can improve the efficiency of identification of potential cancer drug candidates.

10:00 am | Wednesday | January 27

Giovanni Paternostro, Sanford Burnham Prebys Medical Discovery Institute

Automated and High-Throughput Studies of Drug Combinations Using Acoustic Liquid Handling in Multiple Biomedical Fields

Combination therapies are often needed for effective clinical outcomes in the management of complex diseases, but presently they are generally based on empirical clinical experience. New approaches for designing combinations arising from systems biology are needed, including the design of algorithms that identify optimal control parameters in cellular networks based on experimental measurements. The use of new technology for high-throughput measurements is key to these new approaches to combination therapy and essential for the implementation of the algorithms.The Echo acoustic liquid handler as been used to in large scale combinatorial experiments in multiple fields. We will describe the development of drug combination applied to oncology, using both cell lines and primary patient cells, to the pharmacology of hypoxic damage and to the study of antibacterial compounds.

10:30 am | Wednesday | January 27

Jason Cassaday, Merck
Co-Authors: Michael Finley, Jeff Hermes, Michelle Homsher, Brian Squadroni, Sylvie Sur, Vic Uebele, Paul Zuck, Merck; Bharti Gajera, Perkin Elmer

ON DEMAND

Development of a Platform to Enable Fully Automated Cross-Titration Experiments

For some targets, allosteric modulators may provide a better safety margin by tying enhancement or inhibition of receptor activity to the activity of the endogenous ligand. Improving the ability to measure allosteric interactions at earlier stages of hit finding and on larger compound sets may facilitate early selection of desirable chemical series. However, quantification of allostery is especially difficult for G-protein coupled receptors (GPCRs) given the coupling of ligand binding to down-stream effectors. Several mathematical models exist which can be employed to extract values that reflect allosteric potency (a) and efficacy (ß) as well as intrinsic activity (t) of agonist and modulator from a global fit of agonist shift data. Due to the nature of these experiments (cross-titrations of agonists and unknowns) the experiments grow exponentially in size compared to a standard dose titration. To enable implementation of these models across a large screening hit list, our Automation and Engineering Team has employed its multi-functional expertise in robotics, information technology and assay development to design an automation platform capable of performing agonist shift assays in a high-density (1536 well), high-throughput format that can test hundreds of compounds per day in cross-titration experiments. An internally developed automation control system (Telios) integrates compound handling, compound dispensing, sample tracking, assay read instrumentation, and data management. To handle the large data sets generated by agonist shift assays, an automated data analysis tool is employed. Here we will outline the implementation of specific automation to perform these complex, data rich experiments. Results will be presented from a Gq coupled Negative Allosteric Modulator (NAM) FLIPR calcium dye imaging assay and a Gq coupled Positive Allosteric Modulator (PAM) IP1 HTRF assay.

11:00 am | Wednesday | January 27

Timothy Spicer, Scripps Florida
Co-Authors: Peter Chase, Bristol-Myers Squibb; Imarhia Enogieru, Harvard Medical School; Jacob Beer, Franck Madoux, Louis Scampavia, Scripps Florida; Eric Bishop, University of Miami"

An Automated Miniaturized Method to Perform and Analyze Antimicrobial Drug Synergy Assays

In the light of emerging antibiotic resistance mechanisms found in bacteria throughout the world, discovery of drugs that potentiate the effect of currently available antibiotics remains an important aspect of pharmaceutical research in the 21st century. Good tests do exist to determine synergy in-vitro but, these involve low throughput experiments with non standard results analysis and interpretation that is poorly standardized. I will describe a miniaturized broth microdilution checkerboard assay and data analysis method in 384-well plate format that conforms to the Clinical Laboratory and Standards Institute (CLSI) methods. This method has been automated using liquid handlers and HTS readers allowing us to rapidly determine the synergism of current antibiotics with various beta-lactamase inhibitors emerging from our antimicrobial research efforts. This technique increases test throughput and integrity of results, and saves test compound and labor. We facilitated interpretation of the results with an automated analysis tool allowing us to rapidly qualify inter- and intra-plate robustness, determine efficacy of multiple antibiotics at the same time, and standardize the results of synergy interpretation. This procedure should enhance high throughput antimicrobial drug discovery and supersedes former techniques.

1:30 pm | Wednesday | January 27

Carleen Klumpp-Thomas, NCATS
Co-Author: Sam Michael, NCATS

Analysis of Automated Robotic Platforms Used to Accommodate Various Screening Processes and Peripheral Operations

Over the last 15 years automated high-throughput screening systems have been heavily utilized to examine interactions of hundreds of thousands of chemical compounds against a virus of interest for example or tens of thousands of gene targeted siRNA against a specific cancer cell line. When it comes to the collection of samples being tested there are three primary factors that must be regarded to help ensure that the compiled data is both meaningful and repeatable. First and foremost is the process that is to be run on the integrated robotic platform. Once the process has been defined, careful consideration must be taken to properly select the appropriate equipment to be integrated onto the automated screening system. Finally, in addition to the process and the robotic platform itself, it takes a highly skilled team of professionals to make this endeavor worthwhile; ranging from chemists, biologists, informaticians, software developers, compound management personnel to engineers. Flexibility and ability to evolve is necessary in order to continue producing relevant and quality data. The focus here will be on the variety of robotic high-throughput screening systems, the components involved in each and how each platform can accommodate specific processes if designed and operated appropriately. Considerations about scheduling software, modularity, liquid handlers, space footprint, library transfer techniques, cell addition, expected plastic and reagent consumption, storage needs, environmental requirements, throughput, anticipated readouts and future needs all play critical roles in the success of the customized installed system. If all factors are properly synchronized, the possibility of enabling patient focused disease treatment becomes more realistic. Spanning the past 10 years, NCATS has run thousands of screens and by focusing on the process, robotic system design and the proper team structure has been able to handle an ever changing scientific landscape to support the needs of a variety of researchers.

2:00 pm | Wednesday | January 27

Felix Lenk, Dresden University of Technology, Institute of Food Technology and Bioprocess Engineering, Chair of Bioprocess Engineering
Co-Authors: Thomas Bley, Elke Boschke, Patrick Oberthuer, Christoph Otto, TU Dresden

The Internet of Things and Bench-top Lab Automation Systems Bring You into the SmartLab of the Future - A Case Study of Ongoing Process Developments

Laboratory automation was, also due to devices sizes and technologies used, often unavailable to small and middle sized laboratories. However based on the constant development in automation engineering innovative components offer endless possibilities for an engineering of bench-top devices for laboratory automation. In order to improve the development of bioprocesses and routine tasks in microbiology labs the most current technologies are presented. The Internet of Things currently transforms our lifestyle and will soon also improve laboratory tasks. However until now no clear interfaces exist which enable user to interact with lab devices as they are used to it with their consumer electronics such as smartphones and tablets.Methods:A fully automated and IoT-based solution for nutrient medium preparation with a volume of up to 2 L is currently developed at the Chair of Bioprocess Engineering at the TU Dresden. The device is fully compliant to the guidelines for laboratory devices and produced batches can be tracked (GMP-compliant). With this solution for the automation of nutrient medium preparation the risk of human errors is consequently lowered and the quality of medium preparation is expedited. Users select the appropriate recipes for the nutrient medium from the data-base and after 7 minutes the medium preparation is completed. The PetriJet, also developed at the TU Dresden, aims at all processes associated with culture dishes and can handle more than 100 culture dishes automatically with a patented multi-functional mechanical gripper. The gripper allows to perform various tasks in conjunction with different processing stations such as imaging of cultures dishes using the PetriCam tripod, filling of empty culture dishes with nutrient medium or even transferring samples from on culture dish to another. A unique feature of PetriJet is a processing station that provides real-time stereo images using a stereo camera setup. The integrated software allows a reconstruction of the 3D-image and extensive image analysis for parameter extraction. The PetriJet is also fully IoT enabled.Conclusion:Many standard tasks in laboratory environments have the potential for an automated solution that fits on one standard work-bench. The authors present a detailed comparison between manual work and the respective prototype automation solution as well as commercial products already available to the market based on a field study finding that the presented lab automation solutions typically enable for a 4 times higher sample throughput while cutting down labor cost by about 75%.

2:30 pm | Wednesday | January 27

Nallakkan Arvindan, Five Prime Therapeutics
Co-Authors: Wyatt Eppinett, Kevin Lau, John Lin, Stephen Mehi, Benjamin Schudel, Five Prime Therapeutics

From Clone to Clinic: An Integrated Robotics and Informatics Platform for High-Throughput in Vivo and in Vitro Biologics Screening and Development

Traditional ways to discover new targets for protein therapeutics have relied on a slow approach studying a single or a small number of proteins at a time. At Five Prime, we have built a robotics and informatics platform for producing our proprietary library of 5,700 human extracellular proteins in its entirety, from transfection to purification. The Five Prime platform enables automated workflows for high-throughput cell based screens (including high content imaging, FACS, gene expression, and other techniques), biophysical screens (such as AlphaLISA, SPRi), and in vivo screening models using a rapid in vivo protein production system. The platform also supports drug discovery and development of our targets with automated workflows for screening monoclonal antibodies, identifying production cell lines, and scaling up cell culture. In addition, our informatics platform has been used in clinical trial monitoring. We have integrated these different data sources together (such as clinical trials, biomarker studies, PK/PD studies, toxicology studies, and research experiments) into one repository for visualizing information from clone to clinic.

3:00 pm | Wednesday | January 27

J. Colin Cox, Genentech, Inc.
Co-Authors: Carol Cain-Hom, Ryan Pabalate, Anna Pham, Rhonda Wiler, Genentech, Inc.

Ultra-High Throughput Genotyping: A Novel Platform That Curtails Cross-Contamination and Increases Data-Quality

Sophisticated models of human disease are derived by creating multiple and complex genetic modifications in a given animal model. Validation and production genotyping of these highly-modified, multi-allele animals requires increasingly complex genetic analyses. With a growing need for higher throughput and consistency in multiple-allele genotyping, traditional aspiration and dispense liquid-handling robots no longer have the necessary speed, quality, or reproducibility. We present an adaptation and installation of an acoustic droplet ejection (ADE) liquid handling system for ultra-high throughput screening of genetically engineered models. Our ADE system is fully integrated with a microplate sealer, peeler, centrifuge, and bulk reagent dispenser that is driven by a real-time sample management LIMS. A SCARA-compliant articulated arm transfers microplates containing DNA samples and prepared assay reagents to the ADE pipettor and peripherals. Such a configuration enables interrogation of highly complex genetic models in a variety of backgrounds using PCR/qPCR. Our findings demonstrate that a single ADE system replaces eight-to-ten traditional liquid handling robots, while simultaneously increasing quality and reproducibility. Our system can assemble 10,000 – 15,000 genotyping reactions with extensive cherry-picking, in a single work shift without operator attendance.With over one million (and counting) assembled reactions thus far, we demonstrate significant improvements by transitioning to a fully automated and integrated ADE device: • Extremely low cross-contamination in PCR/qPCR despite extensive thermal cycling• Nearly a 10X increase in speed compared to traditional pipetting robots • Hundreds of assays (comprised of thousands of oligos) are stored on a few 384-well plates• Greatly increased data reproducibility (large increases in data quality and Cq/Ct consistency)• Lowered reaction volumes for large reagent/cost-savings• Reduction of laboratory instrument count and reclamation of floor and bench spaceWe show several comparisons between traditional- and ADE-based pipetting for a PCR and qPCR-based workflow. Specifically, we discuss: upgrading from traditional robotics to an acoustic dispenser while maintaining current production needs, transitioning from assays stored in tubes to microplate-stored assays, previous and current workflows, and new research capabilities brought by an ADE liquid handler. With few identifiable downsides, we believe ADE-based technology will become commonplace in genetics and genomics laboratories.

10:30 am | Tuesday | January 26

John Doench, Broad Institute of MIT and Harvard

Genetic Screens with CRISPR-Cas9 Technology: A New Hope in Functional Genomics

The application of CRISPR-Cas9 technology to large scale genetic screens has started a new chapter in functional genomics. Here, we will presents experimental and computational results that examine the optimization of on-target activity and avoidance of off-target activity in the application of this system to human cells. We show that the use of rationally designed libraries leads to more robust screening results in both pooled and arrayed formats.

11:00 am | Tuesday | January 26

Madhu Lal-Nag, NCATS/NIH
Co-Authors: Ken Cheng, James Inglese, Scott Martin, NCATS; Eugen Buehler, Nadia Slepushkina, NCATS/NIH

A High-Throughput Functional Genomics Screening Approach to Identify Modulators of Nonsense-mediated mRNA Decay to Treat Mendelian Disorders

An estimated one-third of genetic disorders such as cystic fibrosis and βthalassemia can be attributed to a premature termination codon (PTC), many causing disease through haploinsufficiency. Cells have evolved a surveillance pathway called nonsense-mediated mRNA decay (NMD) to eliminate PTC-containing transcripts as a quality control mechanism in RNA biogenesis. NMD is rarely 100% efficient with 5-25% of PTC-containing mRNA escaping NMD, resulting in a small amount of truncated polypeptide produced, with low-frequency PTC bypass increasing with transcript abundance. Studies on nonsense codon-mediated diseases have demonstrated that up-regulating expression of a disease specific protein from < 1% to as little as 5% of normal levels may significantly reduce the severity of the disease phenotype. Recent translational research efforts have attempted to identify novel compounds that suppress nonsense codon recognition; however, the lack of an identified molecular target of these compounds has made mechanistic studies and medicinal chemistry optimization difficult and unreliable. Secondly, as nonsense suppressors do not appear to inhibit NMD, few transcripts remain for translation. Therefore, this suggests the intriguing possibility that modulating NMD directly may serve to circumvent some of the inherent deficiencies of PTC suppression. Some evidence suggests that patients with the same nonsense mutations exhibit phenotypes of varying severity because of the difference in NMD efficiency. As a result, any pathway that inhibits NMD will result in increasing levels of the PTC-containing mRNA, which may partially rescue the disease phenotypes (if the C-terminal truncated polypeptides still retain partial function); or may increase the percent of full-length protein that normally occurs by low-frequency PTC bypass. No factors or pathways acting upstream of NMD have yet been discovered. In addition, the role of NMD in regulating gene expression is still poorly understood. Identification of such regulatory effectors could permit development of therapies to manipulate the NMD machinery, potentially allowing more targeted interventions in patients with PTC-associated diseases. We performed a genome-wide RNAi screen by taking advantage of a luciferase reporter based assay. Silencing of critical NMD components by siRNA boosted the expression of the luciferase reporter. Screening clearly identified many of the known proteins and some novel ones as well. Since the efficiency of NMD is variable across patient phenotypes, we are working on corroborating the results of the top hits from the well vetted RNAi screen using complementary CRISPR based genome editing that we anticipate will help us compare the extent of pathological phenotypes for NMD between knock down and knock out approaches. Top actives from both screens will then been further validated in in vitro secondary biological assays to further prioritize potential targets for the treatment of PTC-associated diseases through pharmacological manipulation of NMD.

11:30 am | Tuesday | January 26

Ralph Garippa, Memorial Sloan-Kettering Cancer Center

Genetic Screens as Target Fishing Tournaments: Landing the Big One--Hook, Line and Sinker

Finding a new molecular target via a genetic screen is often the central goal of the investigator who initiates the study, be it shRNA or siRNA. The resulting conundrum which must be dealt with is providing sufficient validity and context for one or more poorly annotated protein targets. In an ideal situation, the screen will identify both an expected target, one which lies on a canonical pathway, along with a novel target(s) which will subsequently create unique investigative space in which to conduct additional experimental queries. We will present data regarding successful screening outcomes where such instances were encountered, allowing for the pursuit of novel molecular entities and translation into small molecule discovery.

12:00 pm | Tuesday | January 26

John Feder, Bristol-Myers Squibb
Co-Authors: Qi Guo, Donald Jackson, Burt Rose, Daniel Tenney, Bristol-Myers Squibb

A Pooled FACS Based Genome Scale RNAi and CRISPR Hit Follow up Loss of Function Screen Identifies Genes Involved in Maintaining HIV Latency in a Jurkat Cell Model of HIV Reactivation

Reactivation of latent HIV and its elimination with highly active retroviral therapy (HART) remains as an unmet medical need. In order to gain insights into what cellular factors are required to maintain HIV in its dormant latent state we have developed a Jurkat minimal HIV genome GFP luciferase reporter model of reactivation and have used it in a RNAi loss of function genetic screen. Cells were infected with a pooled 55000 shRNA lentiviral library representing 11000 genes and then subjected to fluorescence activated cell sorting to isolate the reactivated HIV promoter GFP tagged cells. Genomic DNA was isolated and the integrated shRNAs isolated and sequenced to identify those hairpins which were enriched in the sorted GFP + populations relative to the unsorted controls. A custom mini-pool of shRNA lentiviruses for the candidate primary hit genes was constructed and the infection and cell sorting repeated. After applying rigorous hit calling filters and the elimination of shRNAs with common 'seed' sequences, a high priority hit list was established and individual hairpins tested for their ability to reactivate the reporter as well as for their ability to knock down their intended mRNA target to establish an on-target knock down/phenotype correlation. The result of this work was the identification of two genes that function to strongly repressed HIV reactivation in our cell model, NCBP1 and SRRT, both members of the same protein complex, the nuclear cap-binding protein complex (CBC). However, a third member of the complex NCPB2, whose shRNAs were included in the pooled library did not score has a 'hit'. To investigate this further, and to determine if other potential high priority genes had been missed in the validation process, we took the top 20 RNAi hits, as well as NCBP2 and repeated the luciferase reporter assay with CRISPR gene disruption reagents. To do this we first transduced the Jurkat reporter clonal cell line with a Cas9 expressing virus and infected those cells with viruses containing gene specific guide RNAs (5-4/gene). Both NCBP1 and SRRT re-confirmed using CRISPRs as did NCBP2, the gene missed by RNAi. The CRISPR data was remarkable in both the consistency across the guide RNAs and in the extremely high signal to noise ratio which allowed for one gene, missed in the RNAi screen, to score as a hit even though it was a very weak re-activator. Our data clearly show the value of CRISPR based approaches for not only augmenting RNAi loss of function screens but for applying the technology directly to primary arrayed based genetic screens where 'true hit' identification would be expedited due to minimal off target activity. Other technical issues and solutions associated with genome scale pooled based FACS screens will also be presented.

3:00 pm | Tuesday | January 26

Edward Rebar, Sangamo BioSciences

ON DEMAND

Genome Engineering with Zinc Finger Nucleases

Proteins that can be designed to cleave user-chosen sites in a living genome provide powerful tools for engineering eukaryotic cells with new and useful properties. By provoking break repair of the targeted locus, such proteins can mediate highly efficient rates of gene disruption, gene editing or gene addition at levels that allow ready isolation of cells or organisms bearing a desired genetic change. These capabilities can enable diverse applications in research, medicine, and biotechnology, including the creation of customized cell and animal models for drug development. Realizing the full potential of these technologies, however, requires methods for generating sufficiently active nucleases for the widest range of sequence targets while minimizing off-target effects. This talk will describe strategies and recent work that use zinc finger nucleases to address these considerations. It will also summarize examples of genome engineering in animal and cellular models including iPSCs.

3:30 pm | Tuesday | January 26

Andrea Ventura, MSKCC

Modeling Human Cancer in the Mouse via Somatic Genome Editing

The CRISPR-Cas9 system in bacteria uses short non-coding RNAs as an adaptive defense mechanism against invading DNAs. This system has recently been adapted to introduce specific double strand DNA breaks in mammalian cells using a short RNA as a guide to determine site specificity. I will discuss novel applications of CRISPR-Cas9 for in vivo somatic genome editing. More specifically, I will present data showing that in vivo delivery of appropriately designed pair of guide RNAs can be used to generate a variety of cancer-promoting chromosomal rearrangements, enabling us to rapidly generate more faithful mouse models of human cancers. Lastly, I will discuss how we are applying this novel technology for in vivo and in vitro functional genomic screens aimed at identifying cancer associated long-non coding RNAs and of DNA regulatory elements.

4:00 pm | Tuesday | January 26

Lorenz Mayr, AstraZeneca

CRISPR/Cas9 Technology in Target Discovery, Hit Finding and Translational Studies

The recent discovery of the CRISPR/Cas9 technology (Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-associated protein 9) technology enables precise genome editing (PGE) in eukaryotic genomes at unprecedented ease, precision and short timelines. The technology has tremendous potential for drug discovery with its wide applications for target discovery & target validation, generation of in-vitro and in-vivo models of disease, bespoke models for safety & DMPK and its future applications in gene therapy as a therapeutic agent to treat disease at the molecular level with high efficacy and high safety.

AstraZeneca has built a strong CRISPR/Cas platform with internal activities and external partnerships (Welcome Trust - Sanger Centre; Whitehead Institute/M.I.T.; University of California at Berkeley & University of California at San Francisco; Life Technologies/ThermoFisher) to make full use of this revolutionary technology in its drug discovery processes across all therapeutic areas.

We will present case studies showing the impact of the technology in the following areas:

- Screening and compound testing in drug-resistant tumor cell lines by generation of PGE-derived isogenic cell lines (case study 1)
- Target validation in autoimmune diseases by the use of iPSC-derived human macrophages generated by PGE and cell reprogramming (case study 2)
- Target identification in oncology, cardiovascular, metabolic, autoimmune & respiratory diseases by the use of pooled and arrayed genome-wide libraries (CRISPR wildtype vs. CRISPRi technology) for the identification of novel drug targets (case study 3)
- Hit discovery & SAR compound profiling by the use of endogenous promoter tagging strategies for high-sensitivity phenotypic assay with promoters in their native context (case study 4)

4:30 pm | Tuesday | January 26

Jonathan Moore, Horizon Discovery Ltd.
Co-Authors: Benedict Cross, Jessica Hunt, Steffen Lawo, Horizon Discovery Ltd.

CRISPR-Cas9 KO Screening Identifies Pathway Regulators in Glucose Starvation

RNA-guided CRISPR-Cas9 gene editing can be deployed in functional genomic screening to provide whole-genome phenotypic analysis. CRISPR-Cas9 knock-out screening depletes gene expression by sequence-specific disruption at the genomic level, circumventing many of the problems encountered by RNA interference and providing new opportunities in both target identification and validation. In particular, complete ablation of gene expression allows the opportunity to identify targets that had no phenotype when depleted by RNA interference due to the partial knock-down achievable with this technology. We have adapted the infrastructure developed for traditional shRNA approaches for CRISPR-Cas9 KO screening, which is conducted with large-scale complex libraries using a pooled lentivirus transduction strategy. Next generation sequencing is then used to identify which sgRNAs are accumulating or being depleted from the population. As there are multiple sgRNAs per gene, we can rank genes for their likelihood of having a phenotype under the conditions used in the screen. Our proof-of-principle small molecule resistance and sensitivity screens using both whole-genome libraries and custom subset libraries have successfully identified previously published hits and also yielded novel hits. Horizon has also developed a fully haploid cell line (eHAP), which has the potential to provide additional clarity in genetic screening. To evaluate the power of CRISPR-Cas9 KO screening in haploid cells, we have used our screening platform to map the cellular response to glucose starvation. Whole-genome screening using the GeCKOv2 library identified sensitising factors to glucose starvation including core components of the mitochondrial NADH dehydrogenase complex I. These data are in concordance with the literature where multiple compounds identified in phenotypic screens as sensitizers to glucose starvation have proved to be electron transport chain inhibitors.

9:30 am | Wednesday | January 27

Imran Rizvi, Brigham and Women's Hospital and Massachusetts General Hospital, Harvard Medical School
Co-Authors: Umut Gurkan, Case Western Reserve University; Sriram Anbil, Howard Hughes Medical Institute; Emma Briars, Yujiro Tsujita, Massachusetts General Hospital; Anne-Laure Bulin, Tayyaba Hasan, Huang-Chiao Huang, Massachusetts General Hospital, Harvard Medical School; Utkan Demirci, Stanford University School of Medicine; Jonathan Celli, University of Massachusetts Boston

Integrating Bioengineering and Optical Imaging Approaches for Quantitative Assessment of Treatment Response in 3D Tumor Models

Cell-based research platforms that integrate key microenvironmental cues are emerging as increasingly important tools to improve the translational efficiency of new agents and combination regimens. Among the challenges associated with developing and scaling complex cell-based screening platforms is the need to integrate, and balance, biological relevance with appropriate, high-content imaging routines that provide meaningful quantitative readouts of therapeutic response. Here, 3D in vitro models that restore the architectural features, physical stress and heterocellular signaling experienced by tumors in vivo are described in the context of metastatic ovarian cancer, the leading cause of death among gynecologic malignancies. Ovarian cancer cells often spread under the influence of shear stress from ascitic currents, and communicate with the local microenvironment to initiate the peritoneal implants that form metastatic colonies at distant sites. A microfluidic model that supports 3D tumor growth under shear stress, and corresponding image analysis routines, were developed to characterize the effect of flow on the heterogeneity and aggressiveness of metastatic ovarian cancer. The motivation for this study stems from clinical observations that the most stubborn tumors are often found in regions such as the peritoneal gutter, a common site of resistance and recurrence, and also a region that is subjected to fluidic stress from ascites. Tumor nodules cultured under flow showed increased epithelial-mesenchymal transition compared to non-flow 3D cultures. Molecular and morphological changes consistent with epithelial-mesenchymal transition included a transcriptionally-regulated significant decrease in E-cadherin, a significant increase in vimentin, and significant decrease in fractal dimension, a metric adapted to quantify spindle-like morphology. A concomitant significant post-translational upregulation of epidermal growth factor receptor expression and activation was seen under flow. Collectively 3D nodules grown under flow showed morphological features as well as expression and activation of molecular markers associated with poor prognosis in ovarian cancer, relative to non-flow 3D cultures. The potential value, and challenges, associated with developing complex cell-based models that include physical (e.g. flow-based) stress and communication with stromal partners (e.g. tumor endothelial cells, which are emerging as dynamic regulators of treatment resistance), will be presented, with a particular focus on addressing resistance to conventional agents.

10:00 am | Wednesday | January 27

Christophe Antczak, Novartis Institutes for Biomedical Research

Toward Minimally Invasive Readouts for Unlocking the Potential of Complex Cell Models in Lead Discovery

Complex in vitro cell models derived from primary or iPS cells that recapitulate aspects of in vivo tissue organization and function are of great interest for lead discovery, due their potential as more physiologically relevant cell systems. However, their multi-cellular and three-dimensional nature presents challenges for developing miniaturized phenotypic assays amenable to high throughput screening. Minimally invasive approaches are needed to enable kinetic readouts able to distinguish the response of different cell types. Chemical sensors reporting on specific biological events in live, untransformed cells are considered a key tool that may unlock the full potential of complex cell models: to constitute a driver toward phenotypic assays more predictive of in vivo behavior.

10:30 am | Wednesday | January 27

Serena Silver, Novartis Institutes for BioMedical Research

Next Generation Assays for Next Generation Oncology Targets

Growth of cancer cells in 2D format has been a workhorse of the cancer research world, enabling high throughput biology endeavors to identify new targets and new drugs. However, it is clear that we are sampling only a subset of cancer complexity in these models, for example by comparison of genomic characterization between primary tumors, xenografts, and cell lines grown in 2D. Methods such as co-culture and high content imaging of cells grown in 3D systems may indeed "fill the gap" and advance oncology drug discovery, but must be examined critically to determine whether different sensitivities in these systems are truly more predictive. We have compared the results of compound profiling in 2D and 3D culture conditions with readouts of cell viability and cell signaling to examine the differential responses detected through both methods. These studies were enabled by the development of high throughput imaging assays for cell cycle as well as methods to assess the activity of key signaling nodes such as MAPK and AKT in a 3D spheroid growth setting. As shown previously, we observe differential sensitivity to a number of standard oncology drugs between the 2D and 3D settings when measured by typical cell viability assays. However we also observe differential efficacy for these same inhibitors in alternative assays such as apoptotic induction, cell cycle and pathway modulation. Through comparison to xenograft data, we will ask which methods in 2D or 3D are most predictive of in vivo efficacy and therefore enable more efficient drug discovery. We find that the specific target biology may dictate which assay is most impactful, and that often target centric high content assays may be as predictive as complex 3D assays.

11:00 am | Wednesday | January 27

Adam Zweifach, Department of Molecular and Cell Biology, University of Connecticut

Flow Cytometry Enables Multiplexed Measurements of Genetically-encoded Sensors Based on Intramolecular Förster Resonance Energy Transfer

Cell-based screening methods offer the ability to monitor effects of small molecules on targets of interest in their native context. A wide variety of genetically-encoded cellular activity sensors based on intramolecular Forster resonance energy transfer (FRET) between CFP and YFP have been developed and used extensively in cell biology research, but despite their suitability for use in cell-based screening, their use in screens has been limited. Flow cytometers with 405 nm lasers offer a means to measure CFP-YFP FRET in a rapid and easy-to-quantify way. Flow cytometry also offers the possibility of conducting multiplexed measurements from samples of cells expressing different FRET sensors. We directly compared signals measured with microscopy and cytometry from a FRET sensor for the MAP kinase ERK (EKAR) expressed transiently in TALL-104 human leukemic lymphocytes. The methods provide identical results, and allowed us to define the conditions needed for accurate cytometric intramolecular FRET measurements. We then created a series of stable K562 human cell lines expressing the EKAR and also sensors for JNK (JNKAR), protein kinase C (CKAR), protein kinase D (DKAR), diacylglycerol (DAGR) and intracellular calcium (D3CPV). We measured FRET signals from these cell lines, stimulating them with appropriate chemical agents, and obtained results that agree well with reports from the literature. We developed a protocol for bar-coding cells with orange and red dyes that can be excited by 488 nm and 640 nm lasers, respectively, so that cell lines expressing four different sensors can be labeled and mixed, with the identity of the sensor a given cell expresses determined by reading the bar-code when FRET is measured. We compared the responses of individual and multiplexed samples of the EKAR, JNKAR, CKAR and D3CPV to PMA, anisomycin and thapsigargin, and found that patterns of responses were the same. We also assessed the responses of multiplexed DAGR, CKAR, EKAR and DKAR samples as a function of time after stimulation with PMA and as a function of PMA concentration. Taken together, these results indicate that multiplexed FRET measurements perform similarly to individual measurements. To test whether changes in FRET ratio are robust enough for HTS, we developed a 96-well format for measuring 3-plexed signals from the DAGR, CKAR and DKAR cell lines. When plates contained unstimulated and PMA-stimulated samples, Z' for the DAGR and CKAR expressing cells was > 0.5, while for the DKAR, which gives smaller changes in FRET ratio, Z' was 0.35. We suggest that cyometry-based multiplexed FRET measurements might be used for a number of purposes, including a novel kind of high-content screening in which effects on multiple user-selected targets of interest are monitored simultaneously so as to assure that hits with desired activity are identified as early as possible in the screening process.

3:00 pm | Tuesday | January 26

Scott Baraban, University of California, San Francisco

High-Throughput Drug Discovery in Zebrafish Models for Dravet Syndrome

Dravet syndrome (DS) is a devastating genetic epileptic encephalopathy linked to more than 300 de novo mutations in a neuronal voltage-gated sodium channel (SCN). Children with DS are at a higher risk for sudden unexplained death in epilepsy (SUDEP) and episodes of uncontrolled status epilepticus. Delayed language development, motor and cognitive impairment, and disruption of autonomic function are also associated with this disease. Seizure management includes treatment with a ketogenic diet, benzodiazepines, valproate, and/or stiripentol. Despite these options, currently available antiepileptic drugs (AEDs) do not achieve adequate seizure control in most DS patients, making identification of new drugs a critical unmet need. Although high-throughput screening offers a powerful tool to identify new drug candidates for these patients, commonly available screening approaches rely on in vitro cell-based assays and do not recapitulate the complicated neural networks that generate seizures in vivo. Given the need for new treatments for these children, and the growing number of genetic epileptic encephalopathies that are medically intractable, we developed an alternative phenotype-based in vivo drug screening strategy. Zebrafish larvae with mutations in orthologs for SCN1A (scn1Lab and scn1Laa) were identified and characterized between 3 and 7 days post-fertilization (dpf). Both mutant lines exhibit prominent levels of gene expression in central nervous system structures, spontaneous unprovoked seizure-like behavior and frequent electrographic seizure events. Both mutant lines are unresponsive to 3 or more commonly available AEDs i.e., pharmaco-resistant. Using scn1Lab mutants we screened commercially available drug libraries (n > 1500 compounds). First-stage screening involved automated locomotion tracking in a 96-well format. Secondary screening was performed using in vivo electrophysiology recording measures on individual larvae. To date, we have identified 3 compounds effective in reducing swim behavior to control levels and suppressing electrographic seizure activity e.g., clemizole, dimethadione, and fenfluramine. Positive hits were confirmed in scn1Laa mutants and concentration-response studies. On-going locomotion-based screening is currently being performed at a rate of approximately 100 compounds per month. Integrated zebrafish multi-fluidic recording devices are being developed for secondary electrophysiology assays at a rate of 12 larve per chip. This device is compatible with continuous non-invasive monitoring of surface EEG and cross-over drug application protocols. Taken together, our overall program is designed as a rapid and sensitive method to identify lead compounds for genetic forms of epilepsy in a personalized medicine manner.

3:30 pm | Tuesday | January 26

Fred Ehlert

Quantifying Biased Ligands for G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) are ubiquitous targets for many of the most important drugs used in medicine. They activate both G protein and β arrestin signaling pathways, often mediating therapeutic effects through one pathway and unwanted side effects through the other. Better treatments for diseases may be achieved by identification of new agents (direct receptor agonists or allosteric modulators) that selectively activate or inhibit the appropriate intracellular signaling pathways (agonist bias). Such biased ligands are potentially useful in a number of conditions including asthma, hypertension, congestive heart failure, chronic pain syndromes and many others. The mechanism for selective pathway activation involves agonist induction of a unique active receptor state that engages one pathway over others. In typical screening assays, however, which usually measure downstream drug responses, it is challenging to measure the extent of such bias. This is because the observed selectivity of a candidate drug depends not only on the receptor state that it induces but also on downstream proteins and cofactors that influence the responsiveness of the output pathway. For example, the affinity, efficacy, potency and maximal response of an agonist for eliciting a specific signaling pathway, through a specific receptor state, can change, depending on the cell background and the level of expression of signaling proteins and receptor-associated proteins. A better way to analyze functional screening data is to use a receptor-state analysis that yields ligand-affinity estimates for active (Kact) and inactive (Kinact) receptor states involved in initiating signaling. Unlike observed affinity and efficacy, estimates of receptor-state affinity constants are unperturbed by G proteins, guanine nucleotides and other signaling proteins that interact with the receptor. Recent advances in the analysis of the functional responses of GPCRs have enabled the estimation of receptor-state affinity constants. These constants provide a more fundamental measure of drug-receptor interactions and are useful in analyzing structure-activity relationships and in quantifying allosterism, biased signaling and receptor-subtype selectivity.

4:00 pm | Tuesday | January 26

John Fuller, Johns Hopkins University School of Medicine
Co-Authors: Cynthia Berlinicke, Karl Wahlin, Donald Zack, Johns Hopkins School of Medicine; Patricia Dranchak, James Inglese, Ryan MacArthur, NIH NCATS; Dominic Helm, Bernhard Kuster, Technische Universitaet Muenchen; Matthew LaVail, Michael Matthes, University of California, San Francisco

Phenotypic Primary Cell Screening and Tissue-specific Chemical Proteomics Identifies a Novel Small Molecule That Enhances Photoreceptor Differentiation and Survival

The photoreceptor degenerative disorders are a heterologous group of diseases in which there is a progressive loss of photoreceptors and are the leading cause of blindness in the developed world. Recent advances in organotypic stem cell culture are leading to the increasingly realistic possibility for photoreceptor transplantation as a therapy for photoreceptor loss. However, the process for creating stem cell and iPS-derived photoreceptors is currently time consuming, inefficient, and laborious. We therefore developed primary cell-based assays utilizing a knock-in GFP reporter mouse to screen retinal precursor cells for small molecules that enhance photoreceptor differentiation. Upon screening a focused set of 107 small molecules in an 11-point concentration format, we found that phthalazinone pyrazole (PHPZ), originally identified as a potent Aurora-A kinase inhibitor, enhances the development of photoreceptors in a concentration-dependent format. We also developed an immunopanning-based photoreceptor survival assay and found that PHPZ significantly protects the photoreceptors from cell death in vitro as well as in a rat model for retinitis pigmentosa. In an effort to identify a potential molecular mechanism of action, we employed a chemical proteomics methodology (Kinobeads) to interrogate the retina kinome. We found that PHPZ interacts with several targets in the Wnt, Jnk, and Hippo pathways with sub-nanomolar affinity and may therefore enhance photoreceptor differentiation and viability by simultaneously inhibiting multiple kinases proposed to be involved in photoreceptor death and development. We believe that this work suggests that combining more physiologically relevant cell-based assays with in situ chemical proteomic profiling may lead to novel therapies for complex diseases.

4:30 pm | Tuesday | January 26

Joshua Salafsky, Biodesy, Inc.

Conformational Screening for Hard and Soft Targets

Proteins populate a landscape of conformations in solution with occupancy distributed over time. Changes in this landscape upon binding native ligands or drugs –conformational changes, in space, time or both – engender protein function. In this talk I describe a screening approach for challenging targets using an optical technique called second-harmonic generation (SHG). We have applied this technique, which is highly sensitive to the angular change of a probe attached to cysteines or lysines, to more than twenty proteins in collaborations with Pharma and academic scientists. Here I review SHG's capabilities and highlights from our work with targets across the gamut of current interest. The conformation seen in an X-ray crystal structure is typically the dominant one in a protein's landscape, and clearly many proteins have structurally homologous active sites. Therefore, to gain selectivity, it may be beneficial if drugs bind to conformations at least somewhat different from the dominant one. In other words, drugs that change conformation may be more selective. SHG offers a direct and sensitive means to identify and classify such compounds, whether orthosteric or allosteric, by measuring protein structure in real time and in solution. Moreover, as SHG reveals a range of conformations that a protein adopts when bound to different compounds, and these conformations are correlated with different functional outcomes in vivo, SHG offers the opportunity to precisely select conformation and function. We have used the Delta instrument system in our collaborations for primary and secondary screens, and for SAR work, in which conformational changes were classified by magnitude, direction of change, and kinetics, and correlated with functional data. Our results demonstrate that SHG can sensitively measure the conformational effect of compounds across an affinity range of nM – mM (Kd). Assay set-up is rapid, the workflow integrates well with existing streams, and the Delta system is capable of measuring 1000's of compounds per day. Importantly, an SHG assay requires functional protein as seen by an ability to change conformation, increasing confidence that a screen will identify the most relevant compounds, particularly for hard targets. SHG offers a unique approach to reveal and define the causal link between target conformation and function. Conformational modulation by compounds, akin to regulation in vivo, could lead to less attrition in the clinic.

9:30 am | Wednesday | January 27

Richard Lerner, The Scripps Research Institute

Fratricidin: An Agonist Antibody That Induces Human Malignant Cells to Kill One Another

An attractive, but as yet generally unrealized, approach to cancer therapy concerns discovering agents that change the state of differentiation of the cancer cells. Recently, we have discovered a phenomenon that we call “receptor pleiotropism” where agonist antibodies against known receptors induce cell fates that are very different from those induced by the natural agonist to the same receptor. Here, we show that one can take advantage of this phenomenon to convert acute myeloblastic leukemic cells into natural killer cells. Upon induction with the antibody, these cells enter into a differentiation cascade ending with cells that make large amounts of perforin, interferon _, and granzyme B. These cells attack and kill other members of the leukemic clone. Thus, it seems possible to use agonist antibodies to change the differentiation state of cancer into cells that attack and kill other members of the malignant clone from whence they originate.

10:00 am | Wednesday | January 27

Or Gozani, Stanford University

New Tools for the Study of Lysine Methylation Signaling in Epigenetic and Disease Regulation

The main focus of my group is to understand the molecular mechanisms by which protein lysine methylation regulates chromatin biology, epigenetics, and cellular signaling, and how disruption in these mechanisms contribute to cancer and other diseases. We study how lysine methylation events on histone and non-histone proteins are generated, sensed, and transduced, and how these chemical marks integrate with other modification and cellular signaling networks to govern diverse functions. We previously identified the PHD finger and the BAH domain as methyl lysine-binding “reader” domains and provided evidence that disrupting the read-out of histone modifications can cause inherited human diseases. Current research efforts are aimed at discovery and characterization of new methyl-sensitive reader domains functioning in both chromatin and non-chromatin pathways. Another major focus of the lab is to develop and apply proteomic strategies to uncover the catalytic and biological functions of the many orphan or poorly characterized protein lysine methyltransferases present in the human genome. I will discuss our most recent work in these areas.

10:30 am | Wednesday | January 27

Neha Garg, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego
Co-Authors: Amina Bouslimani, Douglas Conrad, Pieter Dorrestein, Embriette Hyde, Rob Knight, University of California, San Diego

Three Dimensional Spatial Mapping of Human Explant Lungs Associated with Cystic Fibrosis

Lungs of patients afflicted with cystic fibrosis represent an ecosystem that is affected by the chemical interchange between microbes, the host response, external factors such as antibiotics, and the altered physiology of the lung. We currently possess a poor understanding of this molecular environment and its heterogeneity inside the lung, and henceforth, there are no directed treatments to reverse the build-up of multi-microbial communities present in the lung. In order to better understand the ecology of the microbial biofilms within the lungs of cystic fibrosis patients, we have created molecular spatial maps of the microbial inhabitants and the human-host. This work comprises data from hundreds of sections of human lungs from cystic fibrosis patients and is able to demonstrate a variance between abundance of particular microbes and certain chemical signatures. Furthermore, our data show that the microbial secondary metabolites observed in human cystic fibrosis lungs differ greatly from those observed by growing microbial isolates from the lung under laboratory conditions, demonstrating the importance of investigating these complex systems in-vivo. Our spatial maps also suggest that differential penetration of antibiotics within the lung can affect the presence and abundance of microbes. Such spatial correlations between chemistry and microbial distributions will provide a better understanding of the molecular and pathogenic heterogeneity within the complex structure of the lung. The unique “real disease” and not petri dish model is a key for our understanding of the microbes in the lung environment and will assists in future clinical efforts directed towards microbial pathogen clearance and improved quality of life for cystic fibrosis patients.

11:00 am | Wednesday | January 27

Daniel Abankwa, Turku Centre for Biotechnology, Åbo Akademi University

Phenotypic Screening with Ras-FRET Biosensors Identifies Cancer Stem Cell Inhibitors

Cancer stem cells (CSC) are considered a major target in cancer therapy, due to their critical involvement in patient relapse after initial chemotherapy. Here we describe a phenotypic screen that is suitable to identify promising anti-CSC compounds. We used customized FRET-biosensors expressed in mammalian cell lines to screen for compounds that specifically affect the membrane organization of Ras. We identified a number of recently published CSC inhibitors, such as salinomycin, to affect K-ras4B (hereafter K-ras), but not H-ras. Cells with low caveolae levels have an increased dependence on K-ras signaling, due to their altered phosphatidylserine distribution. We show that such cells are particularly sensitive to CSC drugs. Based on this novel mechanistic insight, we defined a K-ras-associated and stem cell-derived gene expression signature that predicts the drug response of cancer cells to salinomycin. Consistent with therapy resistance of CSC, 8% of tumor samples in the TCGA-database with our signature were associated with a significantly higher mortality. Intriguingly, the CSC drug sensitivity-predicting signature was enriched in tumors of the female reproductive system, suggesting that these would particularly benefit from treatment with CSC drugs. Finally, using our FRET-based screening approach and an a posteriori enriched chemical library design, we discovered additional six novel anti-CSC candidate compounds, with two of them having an equal or higher anti-CSC potential than salinomycin.Our work establishes K-ras as the molecular target of CSC drugs, provides a straight forward screening rational to identify novel potential CSC drugs and clarifies that CSC drugs target indeed a stemness gene expression signature. This work may have profound implications for CSC- and Ras-drug discovery, as well as for stem cell reprogramming and therefore regenerative medicine.

1:30 pm | Wednesday | January 27

Peter Tonge, Stony Brook University

ON DEMAND

Co-Authors: Stewart Fisher, Broad Institute; Fereidoon Daryaee, Stony Brook University

Drug-Target Residence Time: Target Engagement, Target Vulnerability and Predictions of in Vivo Drug Activity

Predicting drug efficacy in humans remains a major barrier to the development of novel therapeutics. To improve the prediction of in vivo drug activity we propose that the kinetics of drug-target interactions, and in particular the life-time of the drug-target complex (residence time), should be integrated into predictive models since drug and target are not at equilibrium in vivo. In particular, drugs that dissociate slowly from their targets will have extended activity at low drug concentration thus mitigating a reduction in the frequency of dosing and hence an increase in therapeutic index. We have consequently developed a mechanistic PK/PD model that incorporates drug-target kinetics and have used this model to successfully predict efficacy in models of bacterial infection caused by Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus. We believe that our approach, which is relevant across all disease areas, will have a profound impact on the development of new drugs.

2:00 pm | Wednesday | January 27

Amaury Fernández-Montalván, Bayer Pharma AG, Lead Discovery Berlin
Co-Author: Felix Schiele, Boehringer Ingelheim Pharma GmbH & Co. KG

Non-Equilibrium Profiling of Drug Candidates with kPCA: A Universal Homogeneous Assay for High-Throughput Determination of Binding Kinetics

There is an increasing demand for assay technologies that enable accurate, cost-effective, and high-throughput measurements of drug–target association and dissociation rates. Here we introduce a universal homogeneous kinetic probe competition assay (kPCA) that meets these requirements. The time-resolved fluorescence energy transfer (TR–FRET) procedure combines the versatility of radioligand binding assays with the advantages of homogeneous nonradioactive techniques while approaching the time resolution of surface plasmon resonance (SPR) and related biosensors. We show application of kPCA for three important target classes: enzymes, protein–protein interactions, and G protein-coupled receptors (GPCRs). We further exemplify the use of the method for otherwise inaccessible targets and finally, we discuss the establishment of a binding kinetics selectivity panel for high throughput profiling of kinase inhibitors. Our results demonstrate that kPCA is capable of supporting early stages of drug discovery with large amounts of kinetic data. Information obtained with kPCA will increase our understanding on the role played by binding dynamics on the PK/PD, efficacy and safety profiles of both marketed medicines and pipeline assets.Reference: Schiele F., Ayaz P. and Fernández-Montalván A., “A universal homogeneous assay for high-throughput determination of binding kinetics,” Analytical Biochemistry, vol. 468, pp. 42-49, 2015.

2:30 pm | Wednesday | January 27

Steve Rees, AstraZeneca

Discovery of Novel Antagonists at the Protease Activates Receptor (PAR2) Receptor Through DNA Encoded Library Screening of a Thermostabilised Receptor

The G-protein coupled Protease Activated Receptor (PAR-2) is one of a small family of related receptors that is activated following protease cleavage of the N-terminus of the receptor to reveal a tethered ligand that acts as the agonist at the orthosteric binding site. Antagonist ligands for this receptor are thought to have potential therapeutic utility in a number of inflammatory disorders. While some ligands for this receptor have been identified through classical high throughput screening methods such ligands have not progressed into the clinic. To identify novel ligands with activity at this receptor we established a collaboration with Heptares Therapeutics to generate a thermostable PAR-2 receptor using the STAR technology. A thermostable receptor was successfully generated and used as the substrate in a DNA Encoded Library screen at X-Chem Pharmaceuticals. This screen successfully identified multiple novel antagonist lead series with low nM potency at this receptor. In this presentation I will describe the generation of the PAR-2 stabliised receptor, and the subsequent generation of a PAR-2 receptor structure, together with the first reported use of a stabilized GPCR to generate high affinity ligands through screening DNA Encoded chemical libraries. A pharmacological characterization of the ligands will be presented, as will data that demonstrates the binding sites of these ligands to the receptor.

3:00 pm | Wednesday | January 27

Christina Rau, Cellzome GmbH, a GSK Company
Co-Authors: Marcus Bantscheff, Gerard Drewes, Friedrich Reinhard, Elsa Salzer, Cellzome GmbH

Chemoproteomics Approaches for Target-Based Screening of Large Compound Libraries in Cell Extracts and Live Cells

In target-based drug discovery, high-throughput screening of compound libraries typically relies on biochemical assays with recombinant proteins or protein fragments.However, assay results often poorly correlate with cellular on-target activity which might at least in part be explained by the circumstance that recombinant proteins not fully reflect the proteoform active in relevant cellular systems, e.g. by displaying differences in primary sequence (splice isoforms, proteolytic processing), post-translational modifications and interactions with other cellular components.To address these limitations, we have developed chemoproteomics technologies that enable measuring compound-target interactions on endogenous target proteins in cell extracts and in live cells.Affinity enrichment-based chemoproteomics assays enable the determination of potency and selectivity of inhibitors for multiple targets in parallel. Target proteins are enriched from cell extracts using a compound-derivatized bead matrix. Test compounds spiked into cell extracts in excess concentrations compete for target binding to the bead matrix. After elution of affinity-captured proteins and quantification based on immunostaining of nanoliter dot blots, active compounds are identified by significantly reduced target protein signals. These assays are robust and have a low false-positive rate, with very few compounds interfering with the assay read-out. These characteristics make the assay eligible for screening compounds pools instead of individual compounds in single concentration screens, leading to a substantial increase of screening capacity. In a chemoproteomics screen for TBK1 we have demonstrated applicability of this approach by testing 3345 compounds individually or as pools of 10 per well. As assay quality was comparable in both approaches and neither false-positive, nor false-negative rates were increased, this approach was applied to a PI3K_, PI3K_, mTOR chemoproteomics screen. This screen resulted in good quality and enabled the identification of hits with an expected hit rate demonstrating applicability for high-throughput screening. To enable screening of compounds in live cells we have adopted the cellular shift assay (Molina et al., Science, 2013) into a format compatible with medium sized compound collections. This assay measures changes in protein thermal stability in presence of active binders. Cells are cultured in presence or absence of test compound and heated to different temperatures to induce protein denaturation. Remaining soluble proteins are extracted and multiple target proteins can be detected and quantified using antibody-based approaches. The stabilization relative to vehicle at a defined temperature correlates well with binding affinity, thus enabling affinity ranking of inhibitors at a single concentration. To rank binding affinities among multiple targets, stability profiles are determined across a range of compound concentrations. We have established for example cellular thermal shift assays for HDAC2 using antibody-based readout, which could be directly applied in a screening format. In the presentation, concepts and experimental workflows will be outlined and illustrated by recent examples.

10:30 am | Monday | January 25

Alex Clark, Molecular Materials Informatics
Co-Author: Sean Ekins, Collaborative Drug Discovery

Why Have One Model When You Could Have Thousands?

Building structure-activity models using currently available assay data to guide the selection of subsequent experiments is a routine activity in the pharmaceutical discovery process. Models are generally built on a per-project basis, i.e. the current target of interest, as well as for common ADME/Tox properties. Within large pharmaceutical companies these models are often able to draw from a large amount of relatively high quality data that has been created and validated in-house. Recent developments in the realm of publicly curated datasets such as ChEMBL mean that scientists no longer have to choose between quality and quantity, regardless of whether or not they have access to proprietary compound libraries. Reliable data for thousands of biological targets is available, with the number of corresponding structure measurements for each ranging from a handful to thousands. By creating a straightforward automated script to judiciously sort and merge this content, it is possible to obtain materials for thousands of subsets which are suitable for building structure-activity models for a particular target. For example, Bayesian models constructed from chemical structure fingerprints can be created automatically, after applying an algorithm to detect the optimal partitioning between active or inactive. This work follows from ECFP6 fingerprints and Bayesian models that were originally implemented for use in CDD Vault and subsequently made open source. Having thousands of models on hand requires the creation of new methods for visualisation of activity predictions, and this presentation will discuss recent developments in software tools for this purpose. New approaches to drug discovery are significantly aided by having access to an abundance of models and the techniques to use them, especially polypharmacology, repurposing of discarded drug candidates, and multiobjective optimisation with avoidance of unwanted side effects.

11:00 am | Monday | January 25

Mannix Aklian, Label Independent, Inc.
Co-Author: Lisa Minor, In Vitro Strategies

The Visual Assay™ Platform: A Novel Mobile Assay Workflow and Project Management Tool to Ensure Data Reproducibility - Using Mobile Devices in the Lab to Capture & Share Assay Data

The lack of data reproducibility has been a growing concern across the scientific community. This has led to false conclusions, lost time, and wasted money trying to reproduce these data. Therefore, ensuring data reproducibility and data integrity have become key initiatives of NIH, the pharmaceutical industry, and the scientific community at large. Factors leading to poor data reproducibility include inadequate personnel training, poor documentation of the experimental details including assay flow, reagents used and lot, timing of the experiment, inaccurately linking the data directly to the experiment performed as well as selectively choosing which experiments or which data to include in the notebook and ignoring those that didn't work thus losing potentially valuable information. Visual Assay® is software designed for tablets, mobile devices, and desktop computers. It is a non-browser based software application that continuously saves the assay details, data, and related information and synchronizes with all Visual Assay platforms that are sharing the assay making all experimental details transparent to the researchers and their collaborators. It allows real time joint development of assay protocols and assay data within a lab or across the globe with a gesture-driven, image-intensive interface minimizing the amount of writing necessary and using images to convey information alleviating language barriers. While performing an assay, it captures all data including who did each step of the experiment (with time stamps of each step) and seamlessly captures and links data files to the assay in real time. It enables the user to create and share plate maps which can be used to calculate the data results from within the program. It allows the scientist to capture observations that occurred during the experiment using either written, spoken, or photographic documentation. It enables tracking of experiments that worked and those that didn't work and enables the researchers to overlay those experiments to identify differences. It is auditable and can communicate with external chemical databases and electronic notebooks. It can also show the current progress of multiple assays enabling the manager to reallocate resources if necessary. With a continually evolving platform, Visual Assay should be a tool that can help improve data reproducibility by capturing work as it happens and making it shareable in real-time with colleagues and collaborators. We will present the application followed by a demonstration of the platform.

11:30 am | Monday | January 25

Michael Stadnisky, FlowJo, LLC
Co-Authors: Jay Almarode, Michael Golden, Seth Holstein, John Quinn, Shahid Siddiq, Maciej Simm, Clayton Simons, Matthew Swindle, Ian Taylor, FlowJo, LLC

From out That Shadow: Diagnosis, Discovery, and Data Integration in Single Cell Phenomics

The standardization, throughput, and content of single cell assays has brought these techniques into the mainstream. Despite this democratization, data analysis in flow cytometry and digital PCR has remained in the shadows, relying on expert supervision and manual analysis, and rarely if ever integrated into the life science data ecosystem. Herein, we implemented density estimation techniques to perform truly automated population clustering to identify rare populations of CD34+ hematopoietic progenitor cells, a process critical in cell therapy assessment. We show that this intuitive approach recapitulates, and in some cases performs better than, the population identification performed by a human expert. As no algorithm can determine what populations are of interest, this technique relies on a human to set the populations of interest on a control sample and creates a validated, reproducible, human-understandable, two-dimensional template of populations which can be shared in multisite trials. Furthermore, we show the development of an extensible platform which automates the comparison of any expert and algorithm in population identification, which can be used for any type of cluster comparison and validation. Diagnosis, however, in which all populations are 'known' is only part the problem. Working with a large cell phenotyping panel (2^18 possible phenotypes) examining peripheral blood mononuclear cells which contains a 'known' set of populations, we use stochastic neighbor embedding (tSNE) to reduce n-dimensional data to a two-dimensional representation while keeping event-level resolution. We show the rapid visualization of activation and phenotype markers facilitates discovery, and leverage boolean combinations to identify and separate unknown populations in SNE space. Furthermore, we show that open application architecture allowed us to plugin the open source flowCL package and phenotype unknown populations by querying an ontology database, which returns a score based on identifying markers and a cell hierarchy representation. Thus, we show the creation of a true discovery toolbox for single cell phenomics which allows for the rapid identification and naming of previously unknown populations. Finally, we show that these techniques can be applied to an analogous transcriptomic data streams from digital PCR and RNA-Seq, and show the integration of single cell phenotype and transcript data for population diagnosis and discovery using a data stream in which individual cells are sorted into individual well for further interrogation. We show that an intuitive analysis platform can democratize the diagnosis and discovery process in single cell assays, and significantly accelerate time to insight.

12:00 pm | Monday | January 25

Albert Gough, University of Pittsburgh
Co-Authors: Tong Ying Shun, University of Pittsburgh; Mark Schurdak, D. Taylor, UPitt Drug Discovery Institute

Mining Cellular Heterogeneity for Mechanistic Insights in Phenotypic Profiling and Drug Discovery

Heterogeneity is well recognized as a common property of cellular systems that impacts biomedical research and the development of therapeutics and diagnostics. Several studies have shown that analysis of heterogeneity gives insight into mechanisms of action of perturbagens, can be used to predict optimal combination therapies, and to quantify heterogeneity in tumors where heterogeneity is believed to be associated with adaptation and resistance. Cytometry methods including high content screening (HCS), high throughput microscopy, flow cytometry, mass spec imaging and digital pathology capture cell level data for populations of cells. However it is often assumed that the population response is normally distributed and therefore the population average adequately describes the result of the measurement. A deeper understanding of the biological mechanisms, and a more effective comparison of perturbagen effects, require analysis that takes into account the distribution of the cellular phenotypes. However, the reproducibility of heterogeneous data collected on different days, and in different plates has not previously been evaluated. Here we show that conventional assay quality metrics alone are not adequate for quality control of the heterogeneity in the cellular response. To enable routine analysis of heterogeneity in screening/profiling we have developed an effective means to normalize population distributions from plate-to-plate; a standard approach to quality control of heterogeneity in large-scale biology projects using the Kolmogorov Smirnov (KS) statistic; and demonstrate the use of a set of three heterogeneity indices that measure diversity, normality and outliers in the population to quantify, compare and review variations in heterogeneity in thousands of distributions resulting from treatment with perturbagens. We apply these methods in a retrospective analysis of heterogeneity in an SAR screen for inhibitors of STAT3 activation by IL-6. Results of this analysis show that there are: reproducible and compound dependent variations in the distribution of cellular responses; some compounds only inhibit STAT3 activation in a subpopulations of cells leading to a bimodal distribution of activity; heterogeneity in the inhibition of STAT3 may result in a misleadingly potent IC50 determination; and that compounds with different mechanisms of action exhibit differences in the distribution of cellular responses. These results indicate that quantifying and comparing heterogeneity may lead to a better understanding of compound mechanisms, help prioritize compounds for development and help guide an SAR on a particular mechanism, even when the target is not known. The metrics and methods are presented as a workflow for analysis of heterogeneity in large scale biology projects.

3:00 pm | Monday | January 25

John Overington, Stratified Medical

Bioassay Variability and Reliability in the Published and Patent Literature

There is recent folklore that the majority of pharmacology led target validation data cannot be trusted, as evidenced by recent meta-analyses of published data. To balance this though is the reality, that the reported (peer reviewed literature and patent disclosures) form the prior art on which new innovation and improvements are based, and consequently, on average must be reliable. Due to the crucial importance of target validation in drug discovery, especially that involving tool compounds or first-in-class clinical agents, we have analysed the variability of publicly available bioassay data across various classes of assay complexity - from minimal defined component biochemical assays through to in vivo assays. As expected variance increases (and reliability decreases) as the complexity of the assay, and the number of implicit variables increases, especially for those in vivo, fixed dose-response type assays. These data, on aggregate allow the development of some rules of thumb for expected assay result variance.

3:30 pm | Monday | January 25

Sean Ekins, Collaborative Drug Discovery
Co-Author: Alex Clark, Molecular Materials Informatics

Ensuring Chemical Structure, Biological Data and Computational Model Quality

Scientists want to believe that everything in the literature as well as in the databases they use are error free. This is not reality. Several projects over the last few years have opened our eyes to the magnitude of the problem. For example we have seen how public databases are established with clearly erroneous chemical structures [1, 2] and how NIH funded programs using small molecules neglect to include structures [3]. Projects that have had massive public investment have poorly considered how they make structures and data available [4, 5]. Instead we should actively be ensuring that chemistry structures in papers and databases are corrected when errors are found, rather than proliferating. Similarly, biological data deposited in databases like PubChem and ChEMBL also has errors [6-8] which are apparent when compared to the primary literature. This is in addition to the differences in data that may be observed when using something as mundane as dispensing technologies [9]. These issues may come to a head when one tries to build computational models with such data. Recent examples suggest that data quality can obviously influence the outcome of these models and send scientists in potentially the wrong direction. These issues are not only common with public data but it is also likely some are observed in the pharmaceutical industry and resulted in their limited productivity over the past decades despite massive investments in technologies and screening campaigns. So what can we do about it? It is our duty to point out such difficulties and develop approaches to improve the situation in the future. Some strategies will be described for cleaning up structures and biological data so that we can provide high quality computational models [10, 11]. It is also hoped that this will lead to a wider awareness of the issues amongst scientists, publishers and funding agencies. 1. Williams, A.J. and S. Ekins, Drug Disc Today, 2011. 16: p. 747-750.2. Williams, A.J., S. Ekins, and V. Tkachenko, Drug Disc Today, 2012. 17: p. 685-701.3. Southan, C., A.J. Williams, and S. Ekins, Drug Disc Today, 2013. 18: p. 58-70.4. Lipinski, C.A., et al., J Med Chem, 2015. 58: p. 2068-2076.5. Litterman, N.K., et al., C J Chem Inf Model, 2014. 54(10): p. 2996-3004.6. Kramer, C., et al., J Med Chem, 2012. 55(11): p. 5165-73.7. Lagarde, N., et al., J Med Chem, 2014. 57(7): p. 3117-25.8. Calhoun, B.T., et al., J Biomol Screen, 2012. 17(8): p. 1071-9.9. Ekins, S., J. Olechno, and A.J. Williams, PLoS One, 2013. 8(5): p. e62325.10. Clark, A.M. and S. Ekins, J Chem Inf Model, 2015. In Press.11. Clark, A.M., et al., J Chem Inf Model, 2015. In Press.

4:00 pm | Monday | January 25

Antony Williams, Environmental Protection Agency, National Center of Computational Toxicology
Co-Authors: Ann Richard, Environmental Protection Agency; Chris Grulke, Lockheed Martin-EPA

The Needs for Chemistry Standards, Database Tools and Data Curation at the Chemical-biology Interface

This presentation will highlight known challenges with the production of high quality chemical databases, and will outline recent efforts made to address these challenges. Specific examples will be provided illustrating these challenges within the U.S. Environmental Protection Agency (EPA) Computational Toxicology Program, including consolidating EPA's ACToR and DSSTox databases; augmenting computed properties and list search features, and introducing quality metrics to assess confidence in chemical structure assignments across hundreds of thousands of chemical substance records. The past decade has seen enormous investments in the generation and release of data from studies of chemicals and their toxicological effects. There is, however, commonly little concern given to provenance and, more generally, to the quality of the data. The presentation will emphasize the importance of rigorous data review procedures, progress in web-based public access to accurate chemical data sets for use in predictive modeling, and the benefits that these efforts will deliver to toxicologists to embrace the "Big Data" era.

4:30 pm | Monday | January 25

Moderator: Christopher Lipinski, Lipinski Consulting

Panel Discussion: Data Visualization: Expert Data for Non-Experts

Panel discussion focused on bio data for non-biologists and chem data for non-chemists featuring: Antony Williams, Environmental Protection Agency, National Center of Computational Toxicology Sean Ekins, Collaboration in Chemistry John Overington, Stratified Medical.

10:30 am | Tuesday | January 26

Matt Hahn

Addressing Today's Scientific Data Challenges Through Workflow Tools

There are many approaches to scientific 'data wrangling', wherein we need the ability to access, manipulate, clean and format data for convenient consumption. Some of the most flexible methods are based on workflow or data flow systems, where the definition of how to process the data is defined graphically through a set of connected nodes or components. This talk will discuss how these systems are ideally suited to deal with the ever increasing number of disparate scientific data sources that reside both in on-premise environments as well as in the cloud.

11:00 am | Tuesday | January 26

Andrew Anderson, ACD/Labs, Inc.
Co-Authors: Hans De Bie, Graham McGibbon, ACD/Labs, Inc.

Tackling Analytical Knowledge Transfer Obstacles in Externalized Environments

Across most chemical R&D industries today the trend towards outsourcing of both core and non-core scientific activities is growing dramatically. Current industry estimates suggest that >40% [1] of Pharmaceutical and Biotech R&D spending is now done beyond company boundaries and some of the largest employers of synthetic chemists are working for Contract Research Organizations (CRO) in China (Wuxi and ChemPartner). [2]As externalization and research virtualization continues to evolve into more specific and core business critical specific tasks for library synthesis, assay development services, process chemistry, core manufacturing, etc. the challenges surrounding data sharing and collaboration will need to be addressed. Today, the process of data sharing between a Sponsor Company and their respective CROs is not an optimized one. It involves sharing of data on demand via email or collaboration solutions like Microsoft Sharepoint. The most common media formats shared are in the form of excel spreadsheets or PDF files which limits their ability to be leveraged in a scientifically meaningful way in the future. This presentation will use analytical data sharing as a use case model to suggest a better way to share data and collaborate on an efficient level that ensures data is effectively managed in a way that enables scientific data mining for the purpose of identifying raw materials, impurities, metabolites, etc. It will highlight a new laboratory informatics externalization model that highlights:• A collaborative workspace to create an analytical knowledge package that can include 'live' analytical data, metadata, and chemistry information independent of the instrument source for seamless knowledge sharing. • The value and importance of fingertip access to this 'live' information and knowledge that can be searched, shared, re-processed, re-purposed, and re-analyzed. • A system that is easily accessed via web or mobile client interfaces and is amenable to integration and cloud-based deployments. In addition, this presentation will offer a view on emerging informatics strategies, integration and platforms with an emphasis on the applicability of technologies in the Unified Laboratory Intelligence (ULI) category for chemistry R&D sub-disciplines that feature a substantial variety and volume of analytical data in their workflows.[1] M.E. Elliot, The De-Evolution of Informatics, Scientific Computing, October 2012[2] D. Lowe, The Biggest Chemistry Employers- Guess, In the Pipeline. http://pipeline.corante.com/archives/2014/05/22/the_biggest_chemistry_employers_guess.php

11:30 am | Tuesday | January 26

Daniel Addison, AstraZeneca

Towards Automated Interpretation of LC-MS Data for Quality Assurance of a Screening Collection

High Performance Liquid Chromatography-Mass Spectrometry (LC-MS) is now the de facto technique for Quality Assurance of small molecule compound collections within the pharmaceutical industry. It is used by the AstraZeneca Compound Management (CM) group for structure elucidation and purity determination of the compound collection. These activities are conducted in a high-throughput environment where the rate-limiting step is the review and interpretation of analytical results, which is time-consuming and experience-dependent. Despite the development of a semi-automated review system capable of automatically flagging 33.8% of reviews as 'passed', the manual interpretation of results remains a bottleneck. An archive of over 700,000 analyses, many of which were annotated by LC-MS experts, was identified as an opportunity to apply a data mining approach to further automate the classification of LC-MS results. Whilst the application of data mining techniques to LC-MS data in the –omics cascade is well documented this is, to the best of our knowledge, the first time this approach has been applied to LC-MS data for quality assurance of small molecule compound libraries. A number of features described herein are novel, such as the classification of each individual integrated peak and the application of an overall confidence per analysis. Various classification models were generated using WEKA and Pipeline Pilot (Pipeline Pilot version 8.5.0.200, BIOVIA, San Diego, CA) including Naïve Bayes, Decision Tree induction, Random Forests, Support Vector Machines and Neural Nets. Each model's performance at making accurate predictions on the pass/fail outcome of analyses was assessed using a range of metrics including precision, recall and ROC area. Models were evaluated first as a cost-insensitive classifier and again using MetaCost to apply cost-sensitivity. The intention was to reduce the false negative rate whilst minimizing a rise in false positive rates. Various methods of pruning were also investigated. Variable importance analysis was performed using the chi-squared measure. All models showed high accuracy when predicting the outcomes of analyses from unseen data. Even very simple rules such as Decision Stump showed high accuracy at predicting outcomes. However, the more sophisticated algorithms capable of examining the subtleties of the data made the most accurate predictions. A 10 tree Random Forest using Pipeline Pilot was found to reduce the number of analyses requiring a manual review to 36.4% using a threshold of 90% confidence in predictions. This represents a 45% reduction in the number of manual reviews when compared to the previous system, or a reduction in time required for results analysis from 15 hours per week to 8.25 hours per week. In terms of AstraZeneca full time employees (FTE) this represents a reduction from 40% of one FTE to 22% with an annual saving of $45,000.

12:00 pm | Tuesday | January 26

Ji-Hu Zhang, Novartis

Surface Pattern Classification as a Novel Mode of Inhibition (MOI) Characterization for Inhibitors from High Throughput Screening

A central question in the characterization of enzyme inhibitors is determining the mode of inhibition (MOI). Classically this is done with a number of low-throughput methods where inhibition models are fitted to the data. The ability to rapidly characterize the MOI for inhibitors arising from high-throughput screening efforts where hundreds to thousands of primary inhibitors may need to be characterized would greatly help in lead selection efforts. Here we describe a method for determining the MOI of a compound without the need for curve fitting of the enzyme inhibition data. We describe the theoretical basis for determining the MOI by constructing a matrix of several different substrate and inhibitor concentrations and propose a simple method to analyze the response pattern in order to determine the MOI. This new method employs a non-parametric approach to determine or classify the MOI which improves on the previously described protocols based on IC50 shifts.

3:00 pm | Tuesday | January 26

Barry Bunin, Collaborative Drug Discovery (CDD)

CDD Vault: Co-Evolving Collaborative Drug Discovery 3 Ways

Drug Discovery Collaborations have been securely hosted in the CDD Vault since before "The Cloud" was part of our everyday vocabulary. Over a decade of experience provides unique insights to make individual drug discovery collaborations, and complex collaborations economically scale. Layering unique collaborative capabilities upon sophisticated drug discovery informatics functionality unlocks and amplifies synergy between biologists and chemists. In addition to intuitive registration, structure activity relationship tools, and secure collaboration tools, CDD has given back to the community Public data sets (in CDD Public and Pubchem), Open Source Descriptors and Models (in GitHub and CDK toolkits), and via free mobile applications (TB Mobile). Researchers need to have tools that balance individual needs for robust, intuitive registration and bioactivity analyses while at the same time facilitating collaborations with secure data partitioning, communication, and group engagement. CDD Vault is co-evolving with the scientific community in three ways:1) Simplified collaborative workflows to archive, mine, and securely collaborate around chemical and biological data (co-evolving with data)2) Advanced analytics and visualization and modeling software (co-evolving with software)3) Research informatics collaborations with networks of experimentalists (co-evolving with networks)Collaborative hypothesis generation and evaluation are emerging trends. Technical innovation facilitates network effects within and between organizations in traditionally non-collaborative fields (due to IP sensitivity). Technology matters by changing the natural workflows for collaborations around data and people. Whether working with existing or new scientific collaborations, web-based technologies fundamentally transform team dynamics.

3:30 pm | Tuesday | January 26

Yanli Wang, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health

The PubChem BioAssay Data Warehouse: A Showcase of Big Data Management

The PubChem BioAssay database (http://pubchem.ncbi.nlm.nih.gov) is a public repository for biological screening results for drugs, small molecules, and RNAi reagents. Hosted by the National Center for Biotechnology Information (NCBI), the PubChem project was initiated in 2004, which is becoming a widely used public archival system and information platform for drug development, chemical biology and medicinal chemistry study, as well as small molecule and RNAi screening research. PubChem BioAssay's data content is contributed by industrial, academic and government organizations, worldwide screening facilities, research laboratories, publishers, and literature based text mining curation projects. The database currently contains 30,000 protein and gene targets, 1,000,000 bioassay records, 3,000,000 tested substances, 200,000,000 million bioactivity outcomes, and 1,000,000,000 data-response data points. Warehousing such big data with big diversity and making it easily accessible to the public presents a big challenge to a public repository. PubChem strives to serve the community through steady efforts on improving all key elements of this powerful information platform, including: robust database system, powerful search engine, biomedical information integration, handy data analysis tools, user friendly data submission system, and carefully designed data embargo and private assay data sharing mechanisms. Data content of the PubChem BioAssay database may be searched and accessed through the NCBI information retrieval system at http://www.ncbi.nlm.nih.gov/pcassay/. BioAssay data may be downloaded at the FTP site: http://ftp.ncbi.nlm.nih.gov/pubchem/Bioassay/, and as well as via the various download services. PubChem welcomes contributions from the Laboratory Automation and Screening community by providing feedback for further enhancing PubChem's data repository functionality, and by sharing research findings. Data can be submitted using the PubChem Upload system at http://pubchem.ncbi.nlm.nih.gov/upload/#welcome.References:PubChem's BioAssay Database: http://www.ncbi.nlm.nih.gov/pubmed/22140110An overview of the PubChem BioAssay resource: http://www.ncbi.nlm.nih.gov/pubmed/19933261Examples of web services and URLs for accessing PubChem BioAssay data:Text search:http://www.ncbi.nlm.nih.gov/pcassay/BioAssay record: http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=1284Bioactivity data for a protein bioassay target, tyrosine protein phosphatase:http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?ocfilter=act&gi=90652853Bioactivity data for a gene target, EGFR:http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?ocfilter=act&geneid=1956Bioactivity data for a compound, aspirin: http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?cid=2244BioAssay FTP: ftp://ftp.ncbi.nlm.nih.gov/pubchem/Bioassay/"

4:00 pm | Tuesday | January 26, 2016

David Lee, UCB
Co-Authors: Didier Bastogne, Anthony Hammett, Didier Laureys, UCB

Outsourcing Routine Drug Discovery - Theory into Practice. UCBs Informatics Experience of Supporting Outsourced Discovery

Recently UCB, like many other members of the industry has outsourced many aspects of drug discovery, including some medicinal chemistry, reagent production and routine screening. This has allowed UCB scientists to focus on more complex and specialised aspects of drug discovery. We will report on the practical steps the Informatics Department has taken to address the challenges UCB has experienced integrating our research partners with our own corporate screening culture and systems. We will describe the different procedures and business rules we apply to the various classes of screening activities and the experiences we have had with leading off-the-shelf software. We will sum up with our findings after working with many CROs.

4:30 pm | Tuesday | January 26

Inge Moelans, Janssen Research & Development

Role of Compound Management in an Environment of Increasing (Contractual & Regulatory) Complexity

J&J's Corporate Compound Collection is continuously growing in an environment of increasing (contractual & regulatory) complexity. Many potential collaboration partners are interested in the use of our compound collection. Hence, our compound collection is increasingly viewed, by both the Therapeutic Areas and the Innovation Centres, as an asset in the attraction of external collaborations and the Janssen Corporate Compound Collection starts to take a central position in external collaboration dynamics. The associated need for increased flexibility and quality control to support internal and external projects has required a tremendous evolution in Compound Management. Many significant advancements and continuous improvements in hardware & software have been made over the past decade to accurately and efficiently manage our Corporate Library. New tools/compound flows ensuring compliance in the process of ordering and distribution of our compound samples have been implemented recently. Therefore, expanding the access to the compound library to IC-driven partnerships has been put forward as a Strategic Imperative for Discovery Sciences.

9:30 am | Wednesday | January 27

KT Pickard, StartCodon

Crowdfunding Personal Genomics

This talk will recap lessons learned from crowdfunding a DIY family trio project using whole genome sequence (WGS) information. KT will present recent findings that focused on genomic clues in autism using his family's WGS data, as well as the importance and benefits of sharing personal health information.

10:00 am | Wednesday | January 27

Ethan Perlstein, Perlstein Lab PBC

Co-Authors: Nina DiPrimio, Tom Hartl, Sangeetha Iyer, Alec Ludin, Tamy Rodriguez, Perlstein Lab PBC

Orphan Disease Drug Discovery Using Model Organisms

We develop high-throughput, low-cost and genetically targeted screening pipelines for orphan diseases by CRISPR engineering yeast, worms, flies, and fish to express patient mutations. These simple disease models are used for drug discovery, followed by optimization of orphan drug candidates in patient cells and mammals. In parallel, these same simple disease models can be used for target discovery in genetic modifier screens. Our pipeline includes 250 monogenic diseases caused by defects in genes that function in evolutionarily conserved cellular processes, egs, lysosomal storage disorders. As proof-of-concept, we completed a 50,000-compound, 3-species drug screen for Niemann-Pick Type C (NPC) in half a year. We identified hundreds of primary screening hits that rescue developmental delay and lethality in NPC worms and flies. Dozens of validated hits are being characterized for the ability to reverse disease phenotypes in NPC patient fibroblasts and NPC mice. Our first robust chemotype (Perl1) appears to work by a novel disease-modifying mechanism, demonstrating that a target-agnostic, organism-based screening approach yields promising novel molecular entities.

10:30 am | Wednesday | January 27

Michael Pollastri, Northeastern University

Crowdsourced Lead Discovery: Accelerated Neglected Tropical Disease Medicinal Chemistry Using a Distributed Model

Drug discovery for neglected tropical diseases receives a share of research and development investment that is disproportionately low compared to the overall health burden to society. As a result, streamlined approaches to identification of new lead compounds is needed. With an eye towards meeting the targeted product profiles for new antiparasitic agents, we have primarily focused on assessment and of classes of kinase inhibitors established for other indications (such as cancer and inflammation) and reoptimization for their antiparasitic activities. As with any lead discovery program, prosecution of these programs relies on broad integration of various disciplines; in the case of such a program for NTDs, these disciplines are most frequently spread across multiple institutions. Our progress in lead discovery for several NTDs will be reported, highlighting he medicinal chemistry and, importantly, the unique collaborative framework and mechanisms of funding that we have employed to accomplish this work, combining efforts between academia and industry. Finally, a model for wider collaboration will be presented, with an invitation for wider collaboration in this area.

11:00 am | Wednesday | January 27

Steven Corsello, Broad Institute
Co-Authors: John Athanasopoulos, Joshua Bittker, Todd Golub, Joshua Gould, Jodi Hirschman, Stephen Johnston, Zihan Liu, Patrick McCarren, Vinay Nathan, Aravind Subramanian, Nicola Tolliday, Anita Vrcic, Bang Wong, Broad Institute

The Repurposing Hub: A Next-generation Clinical Drug Library and Web Resource for Crowdsourced Drug Discovery

We have created a valuable screening resource, the Broad Repurposing Hub, that enables the systematic evaluation of drug function across a variety of information-rich genomic and proteomic high-throughput assays to generate repurposing hypotheses. Our effort resulted in two complementary components: 1) A best-in-class physical collection of 5,000 well-annotated compounds with more than 3,000 clinical drugs; and 2) A public web application to enable browsing of library contents (including extensive drug annotations) and results. The Hubenables active participation of the user community via drug library revisions and additions, review and annotation of connectivity reports, and deposition of new external assay results. Major challenges encountered in assembling the drug collection and the associated metadata included locating chemical structures, differentiating true differences in structure from trivial errors, and purchasing compounds in a cost-effective manner. Our ultimate solution integrates information from multiple public and proprietary drug development resources and utilizes a standardized process for chemical structure processing and matching. Following exhaustive searches of vendor catalogs, compounds were purchased from fifty different suppliers. Compounds were annotated with clinical development status by searching the FDA OrangeBook, clinicaltrials.gov, Pubmed, and other internet-accessible resources for clinical trial information. Compounds were mapped to entries in three publicly available drug databases: Drugbank, TTD, and IUPHAR. Using the source identifiers, gene target and mechanism ofaction information was located. Targets were mapped to the official HUGO gene symbol identifiers. Centralized annotations provide a complete blueprint to enable replication of this library by other groups. We anticipate that public input will improve the annotations over time resulting in a dataset more accurate and complete than what is available from the initial sources. Drug repurposing is of high importance given that the majority of drugs fail in clinical trials due to lack of efficacy for their original intended indication. These molecules are generally relegated to the metaphorical dustbin, despite the extensive pharmacologic and safety data collected at great cost. Ideally, all existing drugs should now be tested in systematic profiling assays such asperturbational gene expression profiling and cell viability analysis to uncover additional activities. While determining a comprehensive list of drugs tested in the clinic was surprisingly difficult, and no complete library of such drugs was readily available for laboratory use, we hope that sharing our experience will guide other researchers who wish to access or assemble similar collections. We also encourage pharmaceutical companies and other academic institutions to deposit clinical trial agents into the collection for profiling assays. The assembly and testing of parallel repurposing sets at different institutions will allow a crowdsourced approach to understanding the biology and potential therapeutic applications of these compounds, facilitated by the common informatics resources available in the Repurposing Hub.

1:30 pm | Wednesday | January 27

Tiffany Chen, Cytobank, Inc. and Stanford University
Co-Authors: Matthew Clutter, Center for Molecular Innovation and Drug Discovery; Nikesh Kotecha, Cytobank, Inc; Serafim Batzoglou, Wendy Fantl, Garry Nolan, Karen Sachs, Stanford University

ON DEMAND

SLAS INNOVATION AWARD FINALISTS

Identifying Druggable Cells: Automated Methods for High-content Single-cell Screening

Historically, cancer therapy screening has been rooted in the action of the drug itself, leveraging either the identification of new drug targets, or an overall measure of cancer cell death. However, drug resistance and cancer stem cell research has revealed that a critical set of cancer cells are extremely potent, wreaking havoc on patients even when the majority of proliferating cells have been eliminated. Identification and targeting of unique cell subtypes remains a critical challenge for the fight against cancer and immunotherapy. Single-cell technologies such as flow and mass cytometry allow for efficient sorting and profiling of millions of cells. The two primary challenges, however are scalability of both processing these cellular subsets as well as identification of all of the cellular subtypes from a patient in response to treatment. To this end, we have developed an automated pipeline leveraging robotics, as well as algorithmic and machine learning methods to process and computationally identify cancer cell subsets in response to therapeutic perturbations. From this pipeline, we have derived a universal measure for therapeutic similarity based on the systematic mechanism of a drug. Not only are we able to detect and identify cell subsets that respond to therapeutics as early as 1 hour, but the DNA damage response which defines these cells. Furthermore, we find that the mechanisms of cell cycle and cell signaling have a much stronger signal than cell death in stratifying cellular responses to therapy. In this talk I will discuss my initial applications of this workflow to cells treated with 89 therapeutics currently approved for cancer therapy, creating a new landscape for classification of cancer therapeutics, from canonical checkpoint to kinase inhibitors. In addition I will highlight newer applications of our pipeline to high-content mass cytometry analysis, as well as extensions of our machine learning methods for identification of rare and differential cell subsets.

2:00 pm | Wednesday | January 27

Eugene Lee, University of California, Irvine
Co-Authors: Michelle Khine, Lucy Zhang, University of California, Irvine; Ronald Li, Andy Wong, University of Hong Kong

Integration of Machine Learning and Biomimetic Substrate for High-Throughput Screening of Drug-Induced Cardiotoxicity in Human Cardiomyocytes

Current preclinical screening methods are ineffective at detecting cardiotoxicity: 30% of drug attritions are attributed drug-induced cardiotoxicity. While human pluripotent stem cells-derived cardiomyocytes (hPSC-CM) have been demonstrated as more physiologically relevant in vitro model of the myocardium, hPSC-CMs still exhibit embryonic-like phenotypes in terms of electrophysiological properties, contractility, and structural development. The alignment of hPSC-CMs has been shown to align sarcomere structures, produce stronger contractile forces, and cause anisotropic action potential (AP) propagation. We have previously demonstrated that biomimetic substrates with uniaxial and multi-scale wrinkles that recapitulate the anisotropic nature of the ECM of the native myocardium can be fabricated by using pre-stressed thermoplastic shrink film. When aligned on these substrates, hPSC-CMs exhibited a more sensitive response to drug compounds than their unaligned counterparts Another persistent challenge for developing a high-throughput drug-screening platform using human CMs is the need to develop a simple and reliable method to measure key electrophysiological and contractile parameters. We have demonstrated a brightfield detection technique that combines both machine learning and optical flow into a simple and robust tool that can automate the detection of drug effects on the CMs. Requiring only a brightfield microscope and camera, the optical flow method is inexpensive and non-invasive, and importantly, allows for longitudinal studies. The machine learning provides a singular quantitative index that summarizes the impact of multiple parameters, and thus simplifies the assessment of drug effects on hPSC-CMs. Through the evaluation of several cardioactive drugs with dissimilar effects, we determined that this optical flow and machine learning method was comparable – and even superior to – a fluorescence-based detection scheme common in commercially available systems. Now, by integrating both these technologies, biomimetic wrinkled substrates and the brightfield detection method, we create a platform that addresses current pitfalls of using hPSC-CM for the high-throughput screening of drug-induced cardiotoxicity. To obviate the need for such large volumes of these precious CMs, we deposit extracellular matrix proteins necessary for cellular adhesion in deterministic locations using a simple silanization treatment. This process yields multiple independent samples on each substrates, making it suitable for high-throughput screening. In addition to just reporting whether a compound has cardioactive effects, importantly, our machine learning algorithms determine what drug class (e.g. positive chronotropic agents) a compound belongs to. In addition, this platform is not limited to high-throughput screening of drug-induced cardiotoxicity. The platform can be tasked in evaluating maturation of hPSC-CMs that are subject to various physical and chemical stimulations.

2:30 pm | Wednesday | January 27

Bartek Rajwa, Purdue University
Co-Authors: Murat Dundar, IUPUI; Euiwon Bae, Valery Patsekin, J. Paul Robinson, Purdue University

Machine-learning Methods for Screening, Recognition and Classification of Bacterial Colonies on the Basis of Elastic Light Scattering Characteristics

The majority of tools for recognition and classification of bacteria are based on their physiological or genetic properties. However, there is also an enormous interest in devising label-free and reagentless methods that would operate utilizing the biophysical signatures of microbial samples without the need for labeling and reporting biochemistry. MALDI-TOF is a known recent example of such an approach. Elastic light scattering (ELS) – one of the most fundamental optical processes whereby electromagnetic waves are forced to deviate from a straight trajectory by non-uniformities in the medium that they traverse – can be employed to provide a much less expansive, yet equally accurate implementation of the label-free phenotypic classification concept. The major difficulty posed by ELS-based detection lies in deciphering the highly complicated ELS patterns formed by bacterial colonies irradiated with laser light. Even though the well-developed light-scattering theory and accompanying computational tools such as dipole-dipole approximation could be used for modeling and subsequent interpretation of the raw ELS signals, such a rigorous approach to the inverse-scattering problem remains extremely difficult and computationally expensive. The presented work demonstrates a robust and rapid methodology for colony screening and selection, taking advantage of machine-learning (ML) and computer-vision tools for classification of ELS patterns formed by interaction between laser light and colony structures. The constructed ML classifiers allow for rapid recognition of scatter patterns produced by the colonies without the need to use any specific model of light scattering on biological material. The described classification algorithms do not operate on raw ELS patterns, but utilize complex moments that are calculated in the polar coordinate space of the patterns using complex polynomials. The pseudo-Zernike, Fourier-Mellin, or Chebyshev-Fourier moment invariants are subsequently used to train various ML classifiers (e.g., SVM, NN, etc.) in order to recognize the samples. The results demonstrate the use of the method to classify colonies of E.coli, Listeria, and Salmonella with accuracy above 95%, indicating that the demonstrated technology can be implemented in automated screening devices for sterility testing, colony identification, and colony picking. Owing to reproducible morphological differences in internal colony organization, the method can robustly classify the samples on the genus, species, or even serotype level. We also discuss limitation of the traditional supervised approach, namely the reliance on a priori selection of features. We will conclude by briefly introducing possible future solutions such as unsupervised feature learning (including Deep Learning), which provides a framework for dynamic construction of ELS-derived features describing bacterial scatter patterns without any assumptions regarding the quality and information content of these features.

3:00 pm | Wednesday | January 27

Ali Zarrinpar, University of California, Los Angeles
Co-Authors: Nakul Datta, Chih-Ming Ho, Dean Ho, Theodore Kee, Dong-Keun Lee, Aleidy Silva, University of California, Los Angeles

Patient-Specific Drug Interaction Mapping in Personalized Immunosuppression

After liver transplantation, patients require a large number of drugs including tacrolimus, anti-inflammatories, antibiotics, antivirals, and additional immunosuppressants. These regimens vary highly among patients, and each patient's regimen is constantly altered by changes in drug dosages and formulations (e.g. intravenous to oral administration). The trough blood level of tacrolimus is a gold standard for treatment efficacy and is greatly influenced by changes to the treatment regimen. Each patient is assigned a target tacrolimus trough blood level according to their clinical scenario. Prior studies have sought to personalize tacrolimus dosing using population pharmacokinetics and other predictive modeling approaches. However, it is difficult to simultaneously account for the substantial degree of inter-patient and intra-patient variability in treatment regimens. This study demonstrated the use of a powerful phenotypic personalized medicine (PPM) platform that individualized tacrolimus dosing based on empirical phenotypic clinical data. A novel phenotypic mapping approach was implemented with no patient exclusion criteria, and personalized tacrolimus treatment was clinically validated. This strategy represented a paradigm-shifting pathway towards optimized new drug development as well as patient-specific therapy.Patients were enrolled at the Ronald Reagan UCLA Medical under an IRB approved protocol. Daily patient trough blood levels were measured and sent to the PPM team for dosing recommendations that were provided the same day for evening tacrolimus administration. Trough levels were utilized to construct drug interaction maps that correlated drug dose with trough levels and provided patient-specific drug synergism and antagonism information. This data was used to determine the impact of drug interactions on determining trough levels. Three-dimensional drug interaction plots were constructed to assess the synergistic or antagonistic relationships between compounds (Figure 1). This patient was prescribed a target tacrolimus trough range of 9-11 ng/ml. The drug interaction plot provided a clear range of tacrolimus and mycophenolate dosages that would result in favorable treatment conditions was provided. The phenotypic mapping process accurately regulated patient trough levels within desired ranges on a consistent basis. Changes to patient treatment regimens and subsequent changes in tacrolimus trough levels were effectively regulated by re-adjusting the tacrolimus dosages based upon the PPM platform. This process mediated the re-convergence of trough levels back into the patient-specific target ranges. Drug interaction mapping provided individualized antagonism and synergism information, substantiating the need for personalized treatment regimens. In addition, personalized tacrolimus dosing was demonstrated i n the clinical setting, representing a promising and broadly applicable PPM platform that can be expanded to other indications.

10:30 am | Monday | January 25

Sindy Tang, Stanford University
Co-Authors: Minkyu Kim, Ming Pan, Stanford University

ON DEMAND

SLAS INNOVATION AWARD FINALISTS

Droplet Microfluidics: Amphiphilic Nanoparticles as Droplet Stabilizers for High-Fidelity and Ultrahigh-Throughput Droplet Assays

Droplet microfluidics, in which nanoliter- to picoliter-sized drops are used to encapsulate and compartmentalize molecules or cells, has enabled a wide range of biochemical applications. Examples include digital PCR and directed evolution of enzymes. The first part of the talk will focus on the design and synthesis of amphiphilic silica nanoparticles for the stabilization of aqueous drops in fluorinated oils for applications in droplet microfluidics. The success of droplet microfluidics has thus far relied on one type of surfactant for the stabilization of drops. However, surfactants are known to cause interdrop transport of small, hydrophobic molecules. Such transport leads to the cross-contamination of droplet contents. The use of nanoparticles mitigates this transport as particles are irreversibly adsorbed to the liquid–liquid interface. They do not form micelles as surfactants do, and thus, a major pathway for interdrop transport is eliminated. The second part of the talk will describe a high-throughput optofluidic droplet interrogation device capable of counting fluorescent drops at a throughput of 254,000 drops per second. The device consists of 16 parallel microfluidic channels bonded directly to a filter-coated two-dimensional Complementary Metal-Oxide-Semiconductor (CMOS) sensor array. Fluorescence signals emitted from the drops are collected by the sensor that forms the bottom of the channel. The proximity of the drops to the sensor facilitates efficient collection of fluorescence emission from the drops, and overcomes the trade-off between light collection efficiency and field of view in conventional microscopy. The interrogation rate of the device is currently limited by the acquisition speed of CMOS sensor, and is expected to increase further as high-speed sensors become increasingly available.

11:00 am | Monday | January 25

Alexander Jönsson, University of Copenhagen
Co-Authors: Nanna Bøgelund, Jörg Kutter, Josiane Lafleur, Kasper Rand, University of Copenhagen

Thiol-ene Based Microreactors for Protein Digestion and Peptide Mapping

We report the fabrication and use of novel pepsin-based microreactors for protein digestion and peptide mapping. The microreactors are based on off-stoichiometry thiol-ene (OSTE) chemistry and allow the rapid synthesis of and anchoring of high surface area monoliths inside thiol-ene microfluidic channel as well as easy post-functionalization with pepsin for protein digestion. The development of rapid and efficient methods to map the binding sites of ligands on protein receptors is essential to the understanding of pharmaceutical properties and function. The conventional protein digestion by enzymes in solution is time consuming; therefore it is important to develop novel methods to achieve highly efficient alternatives. Typically, protein biochips are prepared by immobilizing proteins/enzymes on chemically activated glass substrates. However, the usefulness of glass as a substrate is restricted by its non-specific adsorption of proteins and its high processing cost. Alternative materials, which natively feature surface functional groups for biomolecule immobilization, such as thiol-ene polymers are attractive alternatives. An additional advantage of using thiol-ene as a material is that it can be made into porous materials (monoliths). The novel rapid and simple fabrication of highly tailorable thiol-ene monoliths and their potential for use as enzymatic microreactors for a galactose assay and the deglycosylation of ribonuclease B was recently reported. Here, we use related technology to prepare microreactors for highly efficient protein digestion. Emulsions were created by vigorously stirring thiol-ene/methanol mixtures using a magnetic stirrer prior to injection in thiol-ene microfluidic channel and UV exposure. Polymerization occurred in the dispersed monomeric phase, resulting in the formation of a monolith of highly regular interconnected thiol-ene beads with diameters around 1 βm. The covalent linkage of pepsin to the thiol-ene monolith was achieved via free primary amino groups on the pepsin surface by means of thiol–ene click chemistry and L-ascorbic acid linkage. The pepsin microreactor has been used for the digestion of myoglobin and hemoglobin prior to ESI-MS and compared to results obtained by performing the digestion on a commercially available chitosan-based pepsin packed column. Hemoglobin and myoglobin were digested on the prepared microreactors and the positive ion ESI mass spectra of the protein digests were acquired. The digestion of hemoglobin using the pepsin microreactor gives comparable results to that obtained with a commercially available packed column, surpassing it in terms of sequence coverage (99.3% for the on-chip digestion versus 83.1-87.8% for the commercially available column, respectively) and coverage redundancy. The microfluidic set-up allows operating with a two orders of magnitude lower flow-rate and thus minimal sample consumption. Very good performance is also shown for the digestion of myoglobin. These devices show promise in a wide array of bottom-up proteomics workflows involving rapid enzymatic proteolysis of proteins followed by LC and/or MS detection.

11:30 am | Monday | January 25

Dennis Eastburn, Mission Bio, Inc.
Co-Authors: Joshua Mast, Maurizio Pellegrino, Adam Sciambi, Charlie Silver, Jamie Yates, Mission Bio, Inc.

RNA-Seq Following PCR-activated Cell Sorting (PACS) Reveals Rare Cell Transcriptional Signatures

PCR-activated cell sorting (PACS) is a genomic cytometry method that holds great promise for the rapid enumeration and molecular characterization of rare cells within mixed cell populations. PACS is analogous to Fluorescence-Activated Cell Sorting (FACS) in throughput and the ability to analyze single cells; however, unlike conventional flow cytometric immunophenotyping, PACS uses extremely sensitive microfluidic droplet-based TaqMan PCR reactions to identify and, if needed, sort cells of interest for additional downstream molecular characterization. This unique approach enables PACS to identify and isolate cells based on the presence of transcripts, genomic DNA sequences, mRNA splice variants, SNPs, non-coding RNAs, and other biomarkers not amenable to antibody-based probe detection. The PACS approach is well suited for applications in oncology, such as circulating tumor cell analysis, where the presence of rare and/or heterogeneous cancer cells can have a profound effect on the progression and treatment of the disease. For PACS to be most useful in these applications it must be both highly sensitive and specific in targeting cancer cells of interest. Consequently, we sought to investigate the performance parameters of PACS when detecting and sorting cancer cells identified with a single-cell multiplex TaqMan PCR assay. To do this, we performed experiments where varying numbers of DU145 or PC3 prostate cancer cells were spiked into “background” populations of ~10,000 B-lymphocytes and the heterogeneous suspensions were run on the PACS platform for cancer cell enumeration and isolation. These experiments demonstrated that the PACS prototype platform can efficiently identify and recover, on average, ~80% of the cancer cells from the heterogeneous spike in samples. Additionally, downstream qRT-PCR analysis on the sorted cell lysate showed that PACS was able to specifically enrich for transcripts associated with prostate cancer cells. These findings motivated us to develop a strategy for obtaining high content gene expression information from PACS sorted transcriptomes. To accomplish this, we implemented a heavily modified RNA-Seq protocol that is capable of generating sequencing libraries from degraded RNA samples. With this method, we performed next-generation sequencing on RNA purified from the heterogeneous spike in cell samples prior to PACS sorting or from prostate cancer cells identified and isolated with PACS. Transcriptome libraries prepared from the sorted population were highly enriched for genes characteristic of epithelial cancer cells, while the heterogeneous cell population showed a prevalence of immune-related transcripts from the much more prevalent lymphocyte cells. Collectively, our results confirm the potential of PACS to rapidly profile clinical cancer samples and enable a detailed molecular characterization of the disease.

12:00 pm | Monday | January 25

Alan Lyons, ARL Designs LLC
Co-Authors: Xiaoxiao Chen, QianFeng Xu, ARL Designs LLC; Alan Lyons, ARL Designs LLC and College of Staten Island, City University of New York; Yuri Bushkin, Richard Pine, Public Health Research Institute, New Jersey Medical School - Rutgers, The State University of New Jersey

Nano-Droplet Array Plates (nDAPs): Detection of IFN_-expressing Cells in Circulating PBMCs Immobilized in Nano-gel Arrays

The generation and manipulation of large numbers of nano/picoliter droplets containing cells is highly desirable for high throughput/content screening. This is especially true when working with peripheral blood mononuclear cells (PBMCs) obtained from immunocompromised individuals, cancer patients and infants, whose blood samples may provide fewer cells or who cannot tolerate larger blood draws required for typical diagnostics. Conventional dispensing techniques do not offer sufficient precision for dispensing droplets in the nanoliter range and well-plates are too large to handle such small volumes. Microfluidic and nano-well devices attempt to address these challenges, but these devices are expensive, are not compatible with 3D gel environments and the ability to track cells as a function of time is limited. In this presentation, we describe a novel nano-Droplet Array Plate (nDAP) dispensing system that enables the precise dispensing of arrays of nano/picoliter droplets on a specially engineered surface. The nDAP surface is composed of arrays of polydimethylsiloxane (PDMS) posts formed on a glass microscope slide. The morphology and chemistry of the nDAP surface defines the dispense volume and so relaxes the mechanical alignment tolerances required for dispensing compared to conventional dispensing techniques. Thus the unique surface structure enables the use of low-cost robotics. Droplets ranging from 500 nL to as small as 0.1 nL are dispensed with We illustrate the capabilities of the nDAP system by demonstrating the detection of activated lymphocytes using single molecule fluorescent in situ hybridization (smFISH) measurements. The nDAP substrate is used to dispense human PBMC previously stimulated with phorbol myristate acetate and ionomycin within a reversible and biocompatible alginate hydrogel. This 3D scaffold is used to lock and unlock cells in addressable positions on the nDAP device. The immobilized cell arrays (2x104 cells/500 nL droplet) undergo standard treatments such as permeabilization, hybridization with interferon (IFN) _-specific mRNA probes conjugated to Cy5 fluorophore, and numerous washing steps eliminating the need to centrifuge after each step, thereby minimizing cell loss and volumes of expensive reagents. Labeled cells are collected and the frequencies of T cells expressing IFN_ mRNAs were detected by conventional flow cytometric analysis. Prospects for using nDAP technology to detect rare Ag-specific T cells will also be discussed.

3:00 pm | Monday | January 25

Dmitry Markov, Vanderbilt University
Co-Authors: Frank Block, Jacquelyn Brown, Lisa Mcawley, John Wikswo, Vanderbilt University

Compact Mechanatronic System for the Perfusion, Control, Analysis, and Maintenance of Organ-on-chip Microbioreactors

Recent advances and successes in microfabricated organs-on-chips and human organ constructs have made it possible to design scaled and interconnected organ systems that may significantly augment the current drug development pipeline and lead to advances in toxicology and systems biology. Physiologically realistic, live microHuman (_Hu) and milliHuman (mHu) systems will have the fluid budgets of 5 µL and 5 mL, respectively, based on the ~5 L volume of blood in an adult human. Each organ in a multi-organ system is expected to operate for weeks to months. It is critical to interrogate non-invasively the physiological state of each organ over time and in response to drug or toxin challenges. These requirements represent exciting and important engineering challenges. In particular, the volume of circulating blood surrogate must match organ size to avoid non-physiological dilution of metabolites, hormones, and paracrine signals, lest each organ operate independent of the other organs. The requirement for such small volumes for mHu and _Hu in the context of drug development, toxicology and systems biology places severe constraints on many system components. In high-throughput screening (HTS) systems, a centralized fluid-handling robot and pipettes are used to deliver small quantities of media, drugs, and toxins to cells growing in well plates. While this technology can be integrated into organ-on-chip and human organ construct systems, the pipetting robot presents serious limitations with regard to size, cost, system bandwidth in serial rather than parallel operation, media evaporation, fluid retention, transients in media composition associated with intermittent media changes, system dead volumes, and the need to provide repeated organ-organ fluid transfer. The Vanderbilt-developed microfluidic, sensor, and control technologies are addressing these shortcomings and can readily be scaled to a 1 to 10 _Hu size, consistent with many of the organs under development. We are combining our Rotary Planar Peristaltic Micropumps (RPPMs), Rotary Planar Valves (RPVs), MicroFormulator (µF), and MicroClinical Analyzer (µCA) into a fully programmable, customizable, automated perfusion and analysis system that can operate continuously for several weeks, deliver predetermined concentrations of media or drug treatments, and measure metabolic responses of individual organs. An individual RPPM or RPV consists of a polydimethylsiloxane insert containing microfluidic channels that are actuated by a small stepper-motor controlled by a microprocessor, resulting in very low-cost, compact pumps and valves whose fluidics can be considered disposable. The overall system integration and component synchronization are achieved through our Automated Multi-Pump Experiment Running Environment (AMPERE) software. The system has been used successfully to develop, maintain, and interrogate in vitro a physiologically functioning neurovascular unit bioreactor that recapitulates the human blood-brain barrier, and a mammary gland bioreactor. Ongoing efforts are directed towards using these two systems to study breast-to-brain metastasis.

3:30 pm | Monday | January 25

Joseph Siegel, University of Kansas
Co-Authors: Susan Lunte, Kelci Schilly, Manjula Wijesinghe, University of Kansas; Dulan Gunasekara, University of North Carolina

Development of Microchip-Based Methods for Monitoring Reactive Nitrogen Species in Macrophage Cells

Nitric oxide (NO) is naturally produced by the body during the immune response to eliminate foreign species. Large amounts of NO can be produced in immune cells, such as macrophages, during chronic inflammation or over-stimulation. This overproduction can result in the formation of dangerous other reactive nitrogen species (RNS) such as peroxynitrite that are known to cause nitrosative stress in cells by reacting with important biomolecules, which can ultimately result in neurodegenerative and cardiovascular diseases. Therefore, a method to detect and monitor RNS in vitro and in vivo is of the utmost importance. However, these molecules are highly reactive and unstable under physiological conditions and therefore extremely difficult to accurately quantitate. Microchip electrophoresis (ME) provides sub-minute separation times, making it possible to separate multiple RNS, such as NO and peroxynitrite, in a single analysis before significant degradation occurs. Fortunately, these molecules are electroactive, and can therefore be detected with electrochemical detection (EC). The research presented here describes the development of a ME-EC method to monitor the nitrosative stress in bulk and single macrophage cells. We have successfully separated nitrite (the primary degradation product of NO) from cellular interferences in bulk macrophage cell lysate samples. The ME-EC method was used to measure and compare the relative concentration changes of nitrite and glutathione (endogenous antioxidant) in native (non-stimulated) macrophages and macrophages stimulated with lipopolysaccharide (LPS). The current method, however, suffers from high LODs and NO is detected indirectly as nitrite. To improve the LODs, a platinum working electrode was modified with platinum black, which enhances the electrochemical signal for RNS. Platinum black was deposited using a constant current density. To investigate the signal enhancement for specific RNS, NO and peroxynitrite were generated using the PROLI/NONOate and SIN-1 system, respectively. A greater than 3-fold signal enhancement was observed for both species. In addition to improving the LOD, conditions were modified to acheive a baseline separation of nitrite, NO, and peroxynitrite. This method was then applied to the analysis of bulk macrophage cells stimulated with LPS and interferon-_ to further induce NO production. This method provides a means of estimating the average RNS production in a single cell, which is crucial in moving forward into the single cell analysis (SCA) format. A SCA system with EC is currently under development and is initially being tested using ascorbic acid as a model analyte. Non-adherent THP-1 cells will be loaded with ascorbic acid and the ascorbic acid released after cell lysis will be measured using the system. In the future, the bulk cell analysis method will be transferred to SCA and the nitrosative stress due to other stimulates such as beta amyloid will be analyzed to obtain a better understanding of neurodegenerative diseases.

4:00 pm | Monday | January 25

Elena Molokanova, NTBS
Co-Authors: Wesley McKeithan, Christine Wahlquist, Ke Wei, Sanford Burnham Prebys Medical Discovery Institute; Pilar Ruiz-Lozano, Stanford University; Darren Lipomi, Mark Mercola, Alex Savtchenko, Aliaksandr Zaretski, UCSD

SLAS INNOVATION AWARD FINALISTS

Graphene-Based Biocompatible Optoelectronic Platform for Light-Controlled Activation of Cardiomyocytes

Changes in the cell membrane potential play a key role in controlling functional activity of cells, including excitability, ion homeostasis, posttranslational modifications, regulation of protein expression, and cell proliferation. Innovative methods that will enable non-invasive manipulation of the membrane potential are expected to dramatically increase the predictability value of live-cell optical assays during both pharmacologic profiling and cardiotoxicity screening. Here we present a novel nanotechnology-based external platform that utilizes a light signal to provide fast, reversible, and non-invasive control of cellular activity. The central component of this optical stimulation platform is graphene and reduced graphene oxide (rGO). Considered "the wonder material of the 21st century", graphene has a unique combination of optoelectronic properties, making it very attractive for the proposed application. Here we pioneer a ground-breaking approach to utilize external stimuli (e.g., light) to actively change physico-chemical properties of graphene-based materials, and subsequently to change the functional state of cells interacting with these materials. To implement our idea in its simplest form, we produced various planar substrates (i.e., glass coverslips, or microtiter plates) coated with graphene-based materials. We used our graphene-based biointerfaces (G-biointerfaces) to culture human stem cell-derived cardiomyocytes (CMs) and murine neonatal CMs. Fluorescent and scanning electron microscopy experiments revealed exceptional biocompatibility of G-biointerfaces with long-term cell culture by comparing morphology, density, and membrane integrity of cells cultured on substrates with and without graphene-based coatings. We evaluated the effects of light illumination of G-biointerfaces on behavior of CMs in a label-free mode by monitoring their spontaneous contractions using bright-field microscopy. We demonstrated that light can trigger the dramatic increase of the contraction frequency of CMs in an extremely fast and reversible manner. To quantify these effects, we directly monitored the membrane potential of cells cultured on G-biointerfaces using electrophysiology. We determined that light did not affect CMs on "naked" substrates, but did trigger membrane depolarization in CMs on rGO-coated substrates, leading to the launch of the train of action potentials or the increase in frequency of action potential generation.To test G-biointerfaces in an all-optical assay, we used a brief pulse of high-intensity light at 525nm to activate CMs on a G-biointerface, and continuous illumination with low-intensity light at 480 nm to excite Fluo-4, a calcium indicator. We successfully detected the changes in intracellular calcium concentrations that occur during light-triggered action potentials, and confirmed feasibility of all-optical assays using G-biointerfaces. In summary, we present a novel graphene-based optoelectrical platform for activation of cells. We demonstrate its exceptional biocompatibility, the ability to depolarize cells, trigger action potentials, and induce changes in CM contractions. Moreover, due to its transparency, the G-biointerface is compatible with optical detection methods, thus enabling all-optical assays for truly non-invasive probing of cellular activity and pharmacological profiling.

4:30 pm | Monday | January 25

Bobak Mosadegh, Weill Cornell Medical College

Paper-Based 3D Culture for the Study of Cancer Cells in Vitro

This work reports the development of stackable and reconfigurable layers of paper as scaffolds for 3D cell culture of cancer cells. Specifically, tissue-like constructs are formed in vitro to recapitulate aspects of the tumor microenvironment, particularly gradients of oxygen. Previously our group showed that cancer cells grown in this novel 3D cell culture system (CiGiP) will form necrotic cores, due to the depletion of oxygen, similar to spheroid models. The CiGiP system has the advantage that cells can be assembled in a layer-by-layer fashion to form tissues with predefined thickness and composition. Furthermore, subpopulations of cells at discrete positions within the tissue can be isolated and evaluated without any fixation. We recently demonstrated two new applications of this system: i) a novel 3D migration assay and ii) a radiosensitivity study. In both of these systems, monotonic gradients of oxygen are formed due to the metabolism of cancer cells seeded within the system. We show that lung cancer cell lines of known differences in their metastatic potential in vivo, respond differently in this system in both their migration behavior and sensitivity to radiation. For the invasion assay, cells are seeded in a 40 μm thick layer of paper and then sandwiched in the middle of 8 layers of paper containing only hydrogel. Since the layers are in conformal contact, cells are able to migrate from the seeded layer into adjacent layers. We show that the distribution of cells is indicative of a population responding to oxygen as a chemoattractant. Furthermore, we show that a subpopulation of cells migrate rapidly towards the source of oxygen, and that cell lines, which are known to be more metastatic, have a greater bias of migration towards the source of oxygen. We also demonstrate that this system allows for the isolation of the fast moving subpopulation to be retested in the assay, but that the phenotype is not enriched. We believe this result suggests that the rapid migration is due to a population response and not a stable phenotype within the subpopulation. For the radiation study, we seeded cells in every layer of the stack and used thicker sheets of paper to induce steeper gradients of oxygen. We demonstrate that cells in the oxygen-rich regions show higher sensitivity to radiation treatment than those in oxygen-poor regions. Furthermore, we show that cells with a higher metastatic potential in vivo are able to recover from the radiation treatment faster than less metastatic lines. This result is seen only within our stacked culture setting and not in 2D culture or within single layers of paper. We, therefore, believe that our stacked system enhances effects from autocrine factors, potentially providing a more predictive response of cancer cells to radiation treatment.

10:30 am | Tuesday | January 26

Luke Linz, Douglas Scientific

A New Integrated Platform for High Quality and Low Cost Gene Expression Analysis

The breadth of technology offerings for quantitative PCR (qPCR) analysis has continued to grow in the past several years. The introduction of instrument features such as multiplexing, microfluidics, and integration with automation have been successfully leveraged to increase throughput, drive down operating costs, and increase testing flexibility for laboratories performing qPCR. For researchers and pharma institutions performing probe-based expression analysis of tumor cells or other gene expression testing, sensitivity and assay performance has remained a key consideration when evaluating the use of these new technologies. The IntelliQube from Douglas Scientific is a new integrated qPCR platform that seeks to provide researchers the economic benefits of miniaturized reaction volumes, without compromising the performance or sensitivity of gene expression assays. In partnership with leading scientists conducting tumor cell profiling research, Douglas Scientific will present study findings from gene expression profiling performed on the IntelliQube platform. For each of the studies presented, we will review some of the key pre-analytical considerations of tumor cell profiling experiments including RNA extraction, target enrichment, reverse-transcription, and pre-amplification. Using expression profiling data from these research studies, we will assess and discuss the analytical performance of these assays on the IntelliQube and address the advantages and broader application of this integrated platform as a tool for conducting high-quality, low-cost gene expression analysis.

11:00 am | Tuesday | January 26

Rajiv Bharadwaj, 10X Genomics

Advanced Genomic Analyses Using Massively Parallel Molecular Barcoding

Much of the genome remains inaccessible due to the inherent limitations of current tools, with essential long-range information largely absent. At 10X, we have developed a Platform for molecular barcoding and analysis suite that delivers structural variants, haplotypes, and other valuable long-range information while leveraging existing short read sequencers. In this talk, we'll provide a brief overview of the technology. In addition, the author will provide a personal account of working in a fast paced startup and lessons for successful product development.

11:30 am | Tuesday | January 26

Kathi Williams, Genentech, Inc.
Co-Authors: Rich Erickson, Sally Fischer, Amy Noyes, Genentech, Inc.

Overcoming Matrix Effects from Disease Populations Using Microfluidic Technology: Implementation in Phase III Clinical Study

A humanized mAb that has been through Phase I and II programs is being evaluated in a new disease state in Phase III. Upon evaluation of the existing pharmacokinetic (PK) ELISA-based assay poor matrix tolerance was observed. Attempts to optimize assay conditions to eliminate this matrix effect were not successful, necessitating a speedy assay re-development using an alternative technology. The Gyros immunoassay platform was chosen for the assay re-development due to its high matrix tolerance. The high matrix tolerance of this technology is attributed to its flow-through, microfluidic technology, which minimizes incubation time of the serum with the capture reagents, leading to minimal matrix interference. The Gyros technology allowed for a quick assay re-development and qualification, which was completed within three weeks of initiation. This presentation will highlight our challenges with the ELISA format, our attempts to optimize and overcome the matrix interference in the disease sera and our final assay format and results using the Gyros platform.

12:00 pm | Tuesday | January 26

John Herich, V&P SCIENTIFIC
Co-Authors: John Chavez, Patrick Cleveland, Robert Dafau, Kristi Myers, V&P SCIENTIFIC

A Powerful New Pin Tool Method for Precisely Delivering Nanoliter Samples to Dry Microplates

Transferring volumes less than a microliter remains a challenging task for many biological applications. A common trend observed in life science testing over the past several years has been the ever shrinking test volume. Increasing costs of chemical compounds and commonly used solvents has pushed high throughput screening labs towards lower working volumes, specifically in the low nanoliter range. Another factor driving current testing in compound screening and drug discovery labs to the nanoliter volume range is the desire to directly dispense from the source plates versus serially diluted test plates, thereby avoiding compounding dilution errors and reducing consumable costs. At test volumes in the nanoliter range, concentrations of chemical compounds are often kept very high and used essentially undiluted. Nearly all of these types of assays are dosage-dependent, thus knowing the exact volume transferred is critical for data interpretation and confidence in the final assay results. Hence, the ability to controllably dispense “known” nanoliter aliquots of samples is desired, which can readily and inexpensively be achieved using Pin Tools. Pin Tools pick up and transfer liquids using a combination of the liquid’s surface tension, the physical and chemical properties of the pin’s surface, and capillary action of slots or other features in the pin. This technology has been developed and used for over a decade to controllably dispense volumes in the nanoliter range to liquid filled “wet” destination plates, which has resulted in wide adoption of Pin Tools for low volume assays such as high-throughput screens. In this study we demonstrate that Pin Tools can be used for precision delivery (CVs less than 10%) dispensing volumes in the 1 to 200 nanoliter range to empty “dry” destination plates. The ability to dispense into dry microplates greatly simplifies the Pin Tool transfer process, and allows for the prepping of multiple plates with samples to be screened at a later time. This study examined both aqueous (RNA Stable and DNA Stable –BiomatricaTM) and DMSO solvents, a variety of treated and non-treated microplates as well as a range of pins of various diameters, both non-slotted and various slot sizes.

3:00 pm | Tuesday | January 26

Sumita Pennathur, UCSB

SLAS INNOVATION™ AWARD FINALISTS

Electrokinetic Micro- and Nanofluidic Technologies for Quantitative Detection of Viral Nucleic Acids

Rapidly evolving acute respiratory infectious diseases (for example, Influenza, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), and West Nile Flavivirus (WNF)) now have significantly deleterious impacts on human health and economic productivity worldwide. Due to their highly contagious nature, and rapid negative impact on human health and economies, these diseases require developing a simple, high throughput, and immediate (within 30 minutes) screening methodology that can affordably and accurately determine virus diagnosis, so that treatments can be administered in a timely fashion. Furthermore, the expense of anti-virals now prohibits broad distribution even in developed countries. The diagnostic approaches that we are developing in the Pennathur lab enables rapid regionally based deployment of medications to stymie the spread of viruses. These approaches include (1) the development of a nanofluidic conductivity sensor for general nucleic acid detection, (2) fluorescent silver nanocluster DNA probes (AgNC-DNA) combined with microfluidic capillary electrophoresis (mCE), to detect and identify DNA sequences from HepA, HepB and HepC viruses, and (3) microfluidic tangential flow filtration (_TFF) of blood and serum for efficient on-chip sample preparation. Specifically, we have developed a novel nanofluidic-based platform for the efficient detection of nucleic acids. The transduction method is label-free, inducing the formation DNA complexes that result in changes in flow velocity and current in a nanofluidic channel. This innovation takes into account the changes in surface and bulk conductivity in a nanochannel due to the concentration of ions in the bulk. Furthermore, we have developed a method for modifying a low cost, molecular beacon-like AgNC-DNA probe so that multiple DNA sequences can be detected and identified simultaneously and rapidly using microfluidic capillary electrophoresis. As a demonstration, we used this technique to design probes for nucleic acid targets of Hepatitis A, B and C virus. Finally, to truly make this work translational, we have developed a microfluidic based method for biological sample filtration. Such a method allows for facile integration with the above diagnostic sensors, and uses tangential flow filtration methods to effectively isolate targets of interest.

3:30 pm | Tuesday | January 26

Soojung Hur, Rowland Institute at Harvard University
Co-Authors: Jung Lee, Massachusetts General Hospital; Chris Choi, Winfield Hill, Mengxing Ouyang, Rowland Institute at Harvard University

Integrated Microfluidic Electroporator for Multimolecular Delivery into Purified Target Cells

The technique capable of efficient multi-molecule delivery into a small population of fragile target cells would expedite the clinical adoption of gene and cellular therapies. We developed an integrated microfluidic electroporator array that is parallelizable and capable of delivering a wide range of molecules, commonly used in biology, into cells purified from heterogeneous cell populations. The system can deliver multiple molecules with precisely and independently controlled dosages in a sequential manner. The types of molecules that the system has been demonstrated for include chemotherapeutic drugs, siRNA, DNA plasmids, miRNA, and intact proteins. Various cells, including HEK293T, MECF7, MDA-MB-231, HCC827, and patient derived melanoma cells spiked into healthy whole blood, were electroporated by applying short-pulsed square waves via micropatterned Au electrodes. Target cells were purified from buffers or diluted whole blood using microscale cell trapping vortices (i.e. Vortex chip) prior to electroporation. Gentle, yet effective, cell trapping mechanism utilized in the current system not only enables to create cell populations with a uniform size distribution, enhancing the electroporation efficiency, but also promotes a uniform cytosolic distribution of transferred molecules. Moreover, the system provides the real-time monitoring capability for visualization of delivery process of the fluorescent molecules, allowing prompt cell-specific parameter optimizations. Furthermore, superior electroporation efficiency and viability to those of conventional counterparts were achieved when smaller amounts of molecules of interest were administered. Ongoing investigations focus on evaluating the system's feasibility to perform biological assays on clinical samples. The system's improved performance suggests a great potential to expand the horizons of research and clinical applications where on-chip electroporation techniques can be incorporated.

4:00 pm | Tuesday | January 26

Jaekyung Koh, Bioengineering, University of California, Los Angeles
Co-Authors: Dino Di Carlo, Westbrook Weaver, University of California, Los Angeles

Microfluidically-Fabricated Flowable Microporous Hydrogel Scaffolds for 3D Tissue Culture

A 3D culture microenvironment in which cells have significant cell-cell and cell-ECM contacts within a large volume has been shown to better mimic physiology when trying to develop tissues in vitro for drug discovery or therapeutic development. The most common method of creating a 3D environment for cell growth is through seeding of cells within a hydrogel matrix in which cells can grow, invade, and degrade the matrix to develop a 3D tissue. Key factors such as stiffness, porosity and degradation kinetics, play a major role in regulating cell behavior and the rate of the tissue formation. However, low initial porosity for hydrogels with sufficient stiffness to support cell invasion often hinders initial cell contact, growth, and development of tissues. That is, there is a fundamental trade-off between porosity and mechanical strength. We have developed a bottom-up synthesized microporous hydrogel scaffold for three-dimensional tissue culture which is formed from aggregates of microparticle building blocks that are covalently linked together. Scaffold building blocks are uniformly segmented by a microfluidic water-in-oil emulsion method. Following production, the microparticles are mixed with cells and annealed to one another enzymatically to form a contiguous scaffold with cells embedded within pores formed in the void spaces between linked spherical particles. Specifically, the building blocks are composed of a synthetic hydrogel mesh of multi-armed poly(ethylene) glycol-vinyl sulphone (PEG-VS) backbone decorated with cell-adhesive peptide (RGD) and two transglutaminase peptide substrates (K and Q), and assembled to form microporous annealed particle (MAP) scaffold of arbitrary shape dynamically through K and Q crosslinking by thrombin-activated Factor XIII, an enzyme responsible for blood clots. The microporosity of the scaffold was modulated by the size of building blocks, which is precisely controlled by flow rate and geometry of a microfluidic device. By varying PEG weight percentages and crosslinker stoichiometries, the building block moduli spans the stiffness regime necessary for mammalian soft tissue mimetics. The human breast cancer cell line, MDA-MB-231, was cultured in the MAP scaffold modulated to have properties of soft tissue regime. The cell line grew four times faster than in 2D culture or in the non-porous gel of identical chemical and mechanical properties. Moreover, the cell line in the MAP scaffold could better represent a cancer model, as it formed large-scale connective cell growth throughout the MAP gel, while the cell line showed separate small clustered structures in the non-porous gel. The microporosity of the gel enhanced transport and allowed immediate cell-cell and cell-ECM contacts on the surface of the linked microgel particles, accelerating growth into a physiologically-relevant tumor structure. This new paradigm of bottom-up assembly of 3D cell culture scaffolds should be able to accelerate the development of anatomically and physiologically relevant tissue ex vivo and organ-on-chip technologies.

4:30 pm | Tuesday | January 26

Marinella Sandros, HORIBA Scientific
Co-Authors: Stephen Vance, Effat Zeidan, UNCG

Ultrasensitive Detection of Serum Biomarkers Using Surface Plasmon Resonance Imaging (SPRi)

Surface Plasmon Resonance imaging (SPRi) is a label free surface-sensitive optical detection method for biomolecular interactions in real time with high throughput. Serum biomarkers for neurological disorders, cardiovascular diseases, and cancer are often in low abundance in bodily fluids presenting many challenges for their detection. The availability of an ultrasensitive detection platform that can profile multiple biomarkers simultaneously is a potentially powerful method for the diagnosis of diseases and monitoring of subsequent therapeutic treatments. In the present work, a technology platform is introduced that integrates SPRi and aptamer technology with nanomaterials and microwave-assisted surface functionalization. This unique combination and integration makes it possible for the SPRi biosensor to detect C-reactive protein (biomarker) in spiked human serum at ultrasensitive level (fg/ml or attomolar). The preliminary results are encouraging and show promise in extending the platform to detect an array of biomarkers in complex biological fluids.

9:30 am | Wednesday | January 27

Somin Eunice Lee, University of Michigan, Department of Electrical Engineering & Computer Science

Plasmonics-Enabled Single-Molecule and Temperature Detection

Intracellular temperatures dynamically change during cell activities such as division, gene expression and protein activity. We present a plasmonics approach for the detection of single molecules in conjunction with temperature. Plasmon rulers, consisting of pairs of gold nanoparticles, have recently enabled dynamic detection of single molecule activity without photobleaching or blinking. Here, we present a reversible plasmon ruler - comprised of coupled gold nanoparticles linked by a DNA aptamer - capable of binding individual target matrix metalloproteinase molecules with high specificity. We show in vitro and in cultures of mammary epithelia that the binding of the DNA aptamer to target matrix metalloproteinases is characterized by single-molecule sensitivity, high specificity and reversibility, allowing for the parallel detection of temperatures and secreted single molecules in their native microenvironment.

10:00 am | Wednesday | January 27

SLAS INNOVATION™ AWARD FINALISTS

Jonathan Lin, University of California, Los Angeles
Co-Authors: Dale Capewell, Omega Biosystems; Eric Diebold, Omega Biosystems; Dino Di Carlo, Keegan Owsley, University of California, Los Angeles

A Frequency-Multiplexed Parallel Flow Cytometer for High-Throughput Screening and Drug Discovery

Conventional fluorescence flow cytometry is an invaluable and ubiquitous tool in biology and drug screening, allowing multi-parameter, single-cell-level measurements of biochemical markers. Though conventional flow cytometers can be capable of high throughputs (~10,000 cells/second), their sample throughput is low due to the need for serial processing. In this work, we demonstrate a parallel flow cytometer system that is capable of simultaneous interrogation of multiple samples through combination of Fluorescence Imaging using Radiofrequency-tagged Emission (FIRE) and inertial microfluidic focusing. FIRE, a high-speed optical technique, enables the use of a single photomultiplier tube to measure the fluorescence and scatter from multiple points in space using radiofrequency-domain multiplexing of a single laser. FIRE is used to interrogate parallel streams of cells that are focused using inertial flow field shaping, removing the need for a complex sheathing system. Inertial focusing also allows for high cell densities by decreasing the occurrence of multi-cell events. By significantly improving the sample throughput of flow cytometry, we dramatically improve the utility of flow cytometry for applications such as drug discovery, which rely on screening large libraries of compounds (>100,000 samples/day). In doing so, we bring a powerful biological research tool to the high sample throughput screening world, allowing for single-cell-level measurements which better represent population drug-cell interactions compared to traditional aggregate readout techniques.

10:30 am | Wednesday | January 27

Anna Popova, Institute of Toxicology and Genetics (ITG), Karlsruhe Institute of Technology
Co-Authors: Claire Depew, Karlruhe Institute of Technology (KIT); Konstatntin Demir, Pavel Levkin, Katya Manuella, Karlsruhe Institute of Technology (KIT)

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Droplet-Microarray Sandwiching Chip as Miniaturized Pipetting Free Platform for High-Throughput Screenings of Live Cells Based on Superhydrophilic-Superhydrophobic Surface Patterning

High throughput screening (HTS) of live cells is important technology which is widely used in fundamental research and industry in the fields of stem cell biology, cancer research and diagnostics, drug discovery and toxicology. Due to rapid development of molecular biology, bioinformatics and chemical tools the throughput of experiments is continuously increasing. Considering that most of HTS in our days are performed in microtiter plates, cell-based HTS are associated with high consumption of reagent, cells, resources and time. In addition, the screenings of primary and stem cells are difficult or impossible to perform in microtiter plates due to restricted amount of cell material. To address this problem we developed a miniaturized pipetting free platform for HTS of live cells called Droplet-Microarray Sandwiching Chip. Droplet-Microarray has dimensions of standard glass slide and consists of an array of superhydrophilic spots divided by superhydrophobic borders. Depending on size of superhydrophilic spots, which can be easily controlled, one Droplet-Microarray can contain from 600 to 4500 spots per slide. Due to the extreme contrast in wettability of superhydrophilic and superhydrophobic areas suspension of cells applied on such a surface will spontaneously form an array of separated homogeneous droplets containing cells. This technique enables one step pipetting free seeding of cells on the whole Droplet-Microarray Chip. In order to enable manipulation with each individual droplet we developed Droplet-Microarray Sandwiching Chip, a methodology that allows parallel addition of substances into each individual droplet by sandwiching Droplet-Microarray containing cells with glass slide containing pre printed library of interest (drugs, chemicals, transfection mitures). This technology enables parallel addition of substances into individual droplets without cross contamination and simultaneous initiation of screening on the whole Droplet-Microarray chip. Overall Droplet-Microarray Sandwiching Chip allows for: (1) minute reagent and cell consumption; (2) high throughput; (3) separated compartments; (5) compatibility with any type of cells, including cells of semi- and non-adherent nature; (5) one-step pipetting free seeding of cells; (6) simultaneous initiation of screening; (7) compatibility with standard screening equipment. Currently we optimized cell culturing conditions in individual droplets of different sizes for several adherent and non adherent cell lines; and developed protocols for drug treatment and transfection of pDNA and siRNA. We evaluated Droplet-Microarray Sandwich Chip by performing combinatorial drug screening on T cell leukemia Jurkat cell line. Taking in account all the advantages of Droplet-Microarray Sandwich Chip it carries a great potential to be utilized for routine cell-based HTS as well as for diagnostic applications in the field of personalized medicine.

11:00 am | Wednesday | January 27

Jing Chen, BMS
Co-Authors: Mary Ellen Cvijic, Larnie Myers, BMS; Daniel Appledorn, Lindy Oclair, Courtney Shin, Essen Bioscience

Leveraging the IncuCyte Technology for High-Throughput Chemotaxis Assays

Chemotaxis is the directional movement of cells in response to a chemical stimulus and is vital for many physiologically processes including immune responses, tumor metastasis, wound healing and blood vessel formation. Therefore, modulation of chemotaxis is likely to be of therapeutic benefit. Hence, a high-throughput means to conduct chemotaxis assays is essential for lead evaluation and optimization. Historically, low-throughput Boyden chambers have been utilized to measure chemotaxis. In this study, we have developed high-throughput, label-free, image-based IncuCyte chemotaxis assays encompassing various cell types. These assays enables us to visualize chemotactic cell migration in real time and perform cell motility studies in an automated platform, thereby allowing us to incorporate the quantitative studies of cell migration behavior into a routine drug discovery screening cascade.

1:30 pm | Wednesday | January 27

Sung-Hoon Kwon, Dept. of Electrical and Computer Engineering, Seoul National University
Co-Authors: Jungil Choi, Yong-Gyun Jung, Eun-Geun Kim, Jungheon Yoo, QuantaMatrix Inc.

A Rapid Antimicrobial Susceptibility Test Based on Single-cell Morphological Analysis

A rapid antibiotic susceptibility test (AST) is desperately needed in clinical settings for fast and appropriate antibiotic administration. Traditional ASTs, which rely on cell culture, are not suitable for urgent cases of bacterial infection and antibiotic resistance owing to their relatively long test times. Here, we describe a novel AST called single-cell morphological analysis (SCMA) that can determine antimicrobial susceptibility by automatically analyzing and categorizing morphological changes in single bacterial cells under various antimicrobial conditions. The SCMA was tested with four Clinical and Laboratory Standards Institute standard bacterial strains and 189 clinical samples from hospitals. The results were compared with the gold standard broth microdilution test. The SCMA results were obtained in less than 4 hours with 91.5% categorical agreement and 6.51% minor, 2.56% major, and 1.49% very major discrepancies. Thus, SCMA provides rapid and accurate antimicrobial susceptibility data.

2:00 pm | Wednesday | January 27

Shana Kelley, University of Toronto

SLAS INNOVATION™ AWARD FINALISTS

New Devices for the Detection and Classification of Antibiotic-Resistant Bacteria

Rapid identification and classification of antibiotic-resistant bacteria is an important need for infectious disease management. We are developing new electrochemical tools for direct molecular analysis of bacteria, in addition to devices that allow phenotypic profiling of bacteria for rapid susceptibility analysis using electrochemical readout. In the former approach, we take advantage of nanostructured micro electrodes (1-6) that possess larger surface areas and hierarchical structures that promote the display of probe molecules with active conformations. Using these structures, we have demonstrated that it is possible to detect clinically-relevant levels of bacteria in less than 30 minutes. To perform antibiotic susceptibility analyses using intact bacteria, we have developed a device that concentrates and monitors viability of bacteria to allow resistance profiles to be identified in under 2 hours. (7) This presentation will summarize the design and development of these approaches and will highlight potential clinical applications. 1. L. Soleymani, Z. Fang, E.H. Sargent, S.O. Kelley, Nature Nanotechnology, 2009, 4, 844.2. B. Lam, R.D. Holmes, L. Live, A. Sage, E. H. Sargent, S.O. Kelley, Nature Communications, 2013, 4, 2001.3. J. Das, K.B. Cederquist, P. Lee, E.H. Sargent, S.O. Kelley, Nature Chemistry, 2012, 4, 642.4. L. Soleymani, Z. Fang, B. Lam, X. Bin, E. Vasilyeva, A. Ross, E.H. Sargent, S.O. Kelley, ACS Nano, 2011, 5, 3360.5. J. Das & S.O. Kelley, Analytical Chemistry, 2011, 83, 1167.6. B. Lam, Z. Fang, E.H. Sargent, S.O. Kelley, Analytical Chemistry, 2012, 84, 217. J.D. Besant, E.H. Sargent, S.O. Kelley, Lab Chip, 2015, 15, 2799-2807.

2:30 pm | Wednesday | January 27

Dong-Ku Kang, University of California Irvine
Co-Authors: Enrico Gratton, Monsur Ali, Michelle Digman, Ellena Peterson Gratton, Weian Zhao, University of California Irvine

Rapid Detection of Antibiotic-resistant Bacteria by Droplet Digital Detection

Antimicrobial resistance is a growing health problem in the United States and worldwide. According to the Centers for Disease Control and Prevention (CDC), more than two million people are infected annually with antibiotic-resistant bacteria, resulting in >23,000 deaths. Aggressive bacterial infections associated with antimicrobial resistance are often managed within intensive care units (ICUs) with high associated costs, which impose significant healthcare, economic, and social burdens. However, the lack of rapid diagnostics results in either the overuse of unnecessarily broad empiric antibiotics, or a delay of several days in administering the appropriate antibiotic(s).Rapid diagnostics are particularly needed for pathogens such as E. coli, which are common, virulent, and have acquired ESBLs.1 Furthermore, diagnostic tests that can confirm the presence of ESBLs regardless of the species would be exceedingly valuable in directing early therapy and enabling better antimicrobial stewardship for those not infected with antibiotic-resistant pathogens. Unfortunately, existing bacterial detection methods are limited by their inability to rapidly detect and identify pathogens that typically occur at low concentrations in blood (1 to 100 colony-forming unit (CFU)/mL), as is commonly found in adult blood stream infections (BSIs). Conventional bacterial blood cultures coupled with susceptibility testing (automated methods or disk diffusion) require days to obtain a result. This lag in time to identify a patient with a culture-positive BSI, identification of the isolate, and establishing the antimicrobial susceptibility of the isolate contribute to the high mortality rate observed. In this study, we have developed an assay that can detect beta-lactamase producing bacteria at single-cell sensitivity within a few hours. Our Integrated Comprehensive Droplet Digital Detection (IC 3D) can analyze mLs of samples containing beta-lactamase producing bacteria. Our system integrates bacterium-detecting sensors specific for drug resistance using a fluorogenic substrate with droplet microfluidics for sensitive analysis. Central hypothesis is that the confinement of actively growing, beta-lactamase producing bacteria in droplets significantly increases the effective concentration of the released beta-lactamase, which is then detected via cleavage of a fluorogenic substrate in a rapid, real-time fashion. Our novel approach of integrating real-time beta-lactamase sensors with droplet microfluidics bypasses many challenges faced by current techniques (e.g., blood culture). This rapid detection and early intervention tool can provide a means to significantly improve the chances of treating blood stream infections and reduce mortality."

3:00 pm | Wednesday | January 27

Rishikesh Pandey, Massachusetts Institute of Technology
Co-Authors: Tulio Valdez, Connecticut Children's Medical Center; Ramachandra Dasari, Jeonwoong Kang, Nicolas Spegazzini, Massachusetts Institute of Technology

Shining Light on Middle Ear Diseases: Raman Spectral Signatures of Infection

Middle ear infections -also known as otitis media- is the most common childhood illness; and accounts one third of all pediatric visits in the United States. Diagnosis of middle ear infection remains challenging in the clinical setting and suffers from significant observer variability and accuracy of physician diagnosis with currently available methods has been shown to be lacking with accurate diagnosis rates for otitis media ranging between 40 and 80%. Exploiting the endogenous molecular contrast may provide sufficient information to aid in obtaining objective and reproducible disease diagnoses. Here we present first technique that has the potential to non-invasively identify different types of otitis media by providing biochemical signatures that can reveal latent information on pathological conditions. Identification of the spectral markers offer much-desired quantifiable data to enable early detection and longitudinal monitoring of middle ear infection that not only improves patient management but also serves as a basis for the molecular-level understanding of the infection. Additionally, this study unravels the first demonstration of a real-time approach to differentiate the types of infection namely bacterial and viral non-invasively. Our findings represent the first step toward the deployment of vibrational spectroscopy as a non-invasive, label-free and molecular specific probe for the identification of different stages and types of ear infections.

Presented in partnership with ISBER (the International Society for Biological and Environmental Repositories).

3:00 pm | Monday | January 25

Clive Green, AstraZeneca

From Collection to Utilization: The Critical Role of Technology Innovation in Delivering Scientific Advances with Biological Samples

The banking of human biological samples (HBS) for potential use in exploratory scientific research is a rising global phenomenon, with a report by the company Vision Gain, published in September 2014, predicting that "the biobank world market will reach $22.7 billion in 2018". Whilst the collection of samples is set to maintain an upward trajectory, the true value of the samples in discovering medicines that impact patients lives can only be realized when they are utilized in scientific research to support target validation, biomarker identification, translational science and agile clinical trials. A continual increase in banked samples has been observed in AstraZeneca. In 2014, 210,000 samples were banked, whilst utilization lagged significantly behind at 60,000 samples. By comparison, the mature sister discipline for small molecule compounds (SMC) in AstraZeneca banked 45,000 samples in 2014, with utilization vastly exceeding this at 1,150,000 samples. These crude indicators serve to identify problems with 1) inappropriate retention and/or 2) under utilization of HBS for exploratory research. In addressing the challenges in HBS utilization for the medium-term we reasoned that 1) transparency to samples annotated with critical scientifically-relevant data was essential to meet the needs of researchers, and 2) current levels of sample utilization fell below that required to trigger investment in fully automated, discrete vessel-level, low temperature retrieval technology. In surveying the IT marketplace, we failed to identify an off-the-shelf solution capable of integrating patient and sample data, and opted instead to customize a commercially-available application to meet our requirements. The lack of discrete vessel-level retrieval technology led us, in collaboration with our external partner, to implement semi-automated, container-level storage and retrieval. This presentation will address the major issues underpinning the negative utilization of HBS. We will discuss the challenges associated with developing an IT application with complex data integration in a validated environment, and provide an early indication of the value of data warehousing and advanced searching for our scientific colleagues and projects. In addition, the current status and impact of automated storage and retrieval technology will be assessed. Comparisons will be made with the SMC environment to identify learning from over a decade of advanced technology evolution, and how further technology innovations in HBS can impact science and deliver value for patients.

3:30 pm | Monday | January 25

Karen Billeci

Lab Automation to Support a Biologics Repository: Lessons Learned

The application of automation to the management and processing of large collections of high-value samples is already very common in the field of small molecule sample management. The centralization and thus automation is becoming increasingly important for the management of biologics, as high through assay technologies evolve to support antibody discovery, identify protein:protein interactions, and perform gene knock down studies via RNAi technologies such as CRISPR, and siRNA. Here we describe the development and implementation automation integrated to informatics to support the management of nucleic acid based reagents libraries and proteins archives. Our strategy was to implement highly flexible, modular automation to manage traditional liquid handling and storage, but also to perform basic molecular biology techniques such as transformation, inoculations, colony picking and plasmid purification. Through the implementation of automation we learned ways to manage biologics reagents in a high throughput environment that deviates from an artisan approach more suitable to low through put labs. Finally, we have developed in house or have integrated 3rd party software to allow for the search, request, inventory management and registration of biologics.

Presented in partnership with ISBER (the International Society for Biological and Environmental Repositories).

4:00 pm | Monday | January 25

Marcie Glicksman, Orig3n, Inc.

Creating an IPSC Biobank as a Service and for Developing Novel Therapeutics

A great deal of phenotypic and genotypic diversity can be found among both disease and non-disease populations. This is being captured in a program at ORIG3N that is called Life Capsule™. Life Capsule is a direct to people blood and induced pluripotent stem cell (iPSC) banking service that will be one of the largest repositories for disease and non-disease cells. By using the clinically diverse population of inherited and idiopathic disease patients represented in Life Capsule, we will address differences in disease phenotypes in both patient populations. To this end we use differentiation of iPSCs as a route to access neural, hepatic and cardiac cells with specific genetic backgrounds. The logistics and challenges of creating a biobank and the great potential it has will be discussed.

4:30 pm | Monday | January 25

Oliver Peter

The Actelion Research Biobank: Experiences from Launching Biobanking Operations Within R&D of a Medium-sized Pharmaceutical Company

Exploratory biomarkers are increasingly important at all stages of Drug Discovery and Development. Several years ago, Actelion reacted by introducing formal biomarker strategies for all projects. At the same time it became obvious that a system was needed to enable efficient exploratory research with clinical samples. Based on the previous positive experience of introducing state-of-the-art compound logistics, the Actelion Research Biobank was initiated by the same team in a bottom-up effort about three years ago. In 2015, important cornerstones of this function are operational: A collaborative software system to manage specimens, related data, and results obtained, in the context of studies; manual and automated -80°C storage; adaptive automated BSL2 liquid handling; and a comprehensive quality management system on the way to completion. I will present our experiences in setting up this biobank in a medium-sized pharmaceutical company, covering both technical and organizational challenges. I will also suggest some development points that might be taken up by the research community or commercial equipment providers to improve biobanking operations in the future.

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