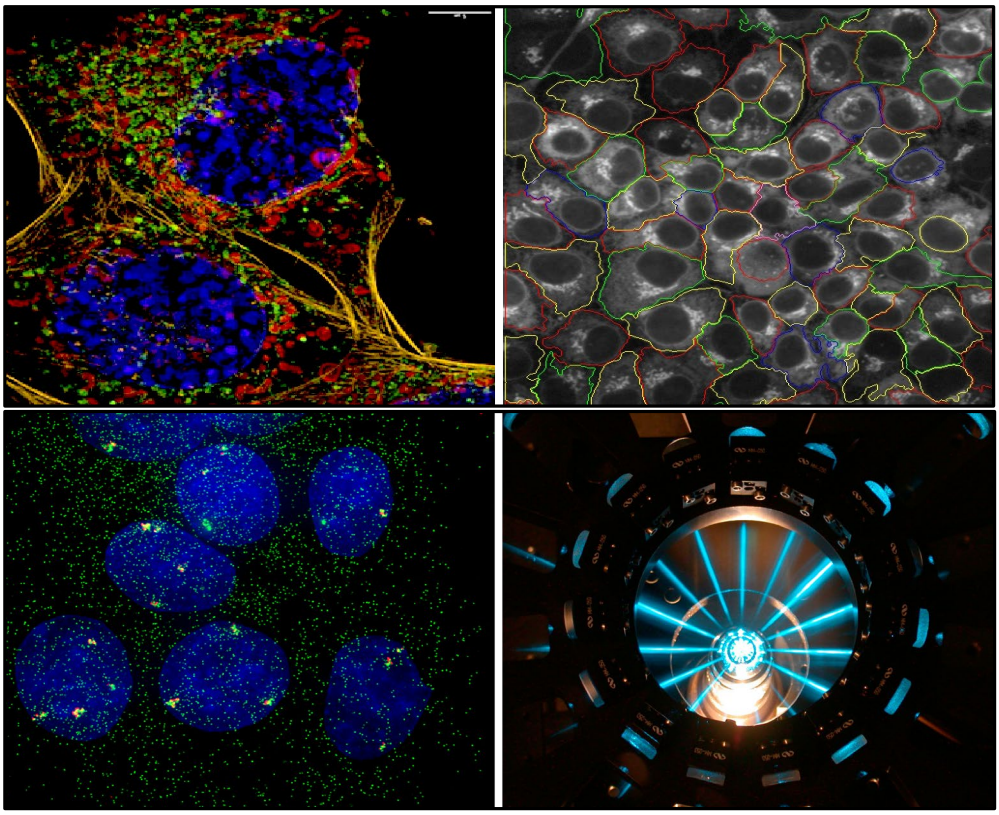
Imaging-based Single Cell Analytics: Applications for Cancer Cell Biology and Therapeutics

February 25-27, 2019

BioScience Research Collaborative
6500 Main St.
Houston, Texas



Co-sponsors:

GCC Combinatorial Drug Discovery Program
GCC Center for Advanced Microscopy and Image Informatics
Society of Biomolecular Imaging and Informatics
Texas Screening Alliance for Cancer Therapeutics

Organizers:

Michael Mancini, Baylor College of Medicine
David Andrews, University of Toronto
Peter Davies, Institute of BioSciences, Texas A&M Health Science Center
Fabio Stossi, Baylor College of Medicine

Gulf Coast Consortia QUANTITATIVE BIOMEDICAL SCIENCES | INSTITUTE OF BIOSCIENCES AND TECHNOLOGY TEXAS A&M UNIVERSITY | Baylor College of Medicine | DAN L DUNCAN COMPREHENSIVE CANCER CENTER | SBI² SOCIETY OF BIOMOLECULAR IMAGING AND INFORMATICS | CPRIT CANCER PREVENTION & RESEARCH INSTITUTE OF TEXAS

Thank you to our industry supporters:

Platinum Plus

Akoyabio  Pg. 47

Greiner  Pg. 48

Platinum

Molecular Devices  Pg. 49

Gold

GE Healthcare  Pg. 50

Silver

Ibidi  Pg.51

Olympus  Pg. 50

Perkin Elmer  Pg.51

Fluidigm  Pg. 51

Optical Biosystems  Pg. 51

Leica 

Bronze

EXTBIO 

Zeiss 

Bruker 

Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on **Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Neuroengineering and Pharmacological Sciences.** GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include **Antimicrobial Resistance, Nanox, Mental Health, Chemical Genomics, Translational Pain Research, Theoretical and Computational Neuroscience, Alcohol and Addiction Research, Regenerative Medicine, Translational Imaging and Cellular and Molecular Biophysics**. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

Gulfcoastconsortia.org

Agenda

Day 1 - Monday, February 25, 2019

8:00 Light Breakfast and Registration

8:50 Welcoming Remarks/Convener David Andrews

Education Sessions 1:

9:00 *Imaging Mass Cytometry: High-dimension Imaging, Segmentation and Analysis of Spatially Resolved Single Cells*
Hartland Jackson, Institute for Molecular Life Sciences, University of Zürich

9:45 *Multiplexed Imaging for Multi-protein Analysis at the Single Cell Level in Fixed Tissue (MultiOmyx™ and Cell DIVE™)*
Fiona Ginty, Biosciences Technology Manager & Principal Investigator at GE Global Research

10:30 *3D Models for High Content Analysis*
Judi Wardwell, InSphero

11:15 *3D Models for Cancer Biology*
Shannon Mumenthaler, University of Southern California

12:00-1:30 Lunch – Installation of Cell Profiler and dataset – bring your laptop.

Education Session 2:
Convener: Mike Mancini

1:30 *Rapid Quantification of Protein-protein Interactions in Live Cells*
David Andrews, University of Toronto

2:15 *Statiscial Analysis of High Content Screening Data*
Bartek Rajwa, Purdue University

3:15 Break

3:30-5:00 *Cell Profiler Workshop*
Santosh Hariharan, Pfizer
Bring your own laptop with Cell Profiler installed.

Day 2 – Tuesday, February 26, 2019

8:30 Light Breakfast and Registration

9:00 **Welcoming Remarks**
Mike Mancini, Baylor College of Medicine David Andrews, University of Toronto

Session 1: Nuclear Genomics and Chromatin Organization
Convener: Mike Mancini, Baylor College of Medicine

9:15 **Keynote Presentation:**
Spatial Genomics: Nascent Transcriptome Profiling by intron seqFISH
Long Cai, California Institute for Technology

10:00 *3D HCS Imaging Challenges and Solutions for Complex Cell Model Systems*
Erez Leiberman, Baylor College of Medicine

10:30 Break

10:45 *Microscopic Imaging of Epigenetic Landscape in Single Cells*
Alexey Terkskikh, Ph.D., Sanford Burnham Prebys Research Institute

11:15 *Nuclear Speckles as a Transcriptional Hub and Amplifier: a Combined Genomic and Live-cell Imaging Approach*
Andrew Belmont, University of Illinois at Urbana-Champagne

11:45 **Selected Abstract:**

Imaging Freeze-frame Proteins Identifies Cancer-Protein Functions and Protein Networks of Endogenous DNA Damage
Jun Xia, Baylor College of Medicine

12:00 Lunch and poster session

12:45 Presenters at posters

Session 2: Advanced Cellular Models
Convener: Fabio Stossi, Baylor College of Medicine

1:30 *Non-opsin Based Optogenetics to Illuminate Physiology*
Yubin Zhou, Institute of Biosciences and Technology, Texas A&M Health Science Center

2:00 *The Single Cell Pathology Landscape of Breast Cancer: Tumour Cells and Their Microenvironments*
Hartland Jackson, Institute for Molecular Life Sciences, University of Zürich

2:30 *Multiplex Imaging for Single Cell Mechanistic Analysis of Estrogen Receptor Functions*
Mancini/Stossi, Baylor College of Medicine

3:00 Break

3:15 *Expansion Microscopy: A Novel Tool for Single Cell Analysis in Intact Biological Systems*
Mahander Dewal, Expansion Technologies, Inc.

3:45 **Selected Abstract:**

A 3D in vitro Platform for High-Throughput Screening of Diverse Prostate Cancer Patient-Derived Xenograft Models
Lindsey Sablatura, Rice University

Day 3 Wednesday, February 27, 2019

8:00 Light Breakfast and Registration

Session 3: Image Informatics
Convener: David Andrews, University of Toronto

9:00 **Keynote Presentation:** Integrating Information from Diverse Microscope Images: Learning and Using Generative Models of Cell Organization
Bob Murphy, Carnegie-Mellon University

9:45 *Phindr3D: Data-driven Segmentation-free Phenotyping of 3D Microscopy Data*
Santosh Hariharan, Pfizer

10:15 *Spheroid Imaging and Sequencing*
Christian Conrad, Berlin Institute of Health, Germany

10:45 Break

11:00 *OME's Bio-Formats, OMERO, & IDR: Open Tools for Accessing, Integrating, Mining and Publishing Image Data @ Scale*
Jason Swedlow, University of Dundee

11:30 *Magnetic 3D Bioprinting, from Generating Spheroids to Fingerprinting Cell-Types*
Glauco Souza, Greiner Bio One

12:00 *Bridging the Phenotypic-Genomic Continuum: Case Studies Linking Histology and Genomics*
Arvind Rao, University of Michigan

12:30 Lunch and Posters

Session 4: Image-Based Assays for Cancer Research
Convener: Peter Davies, Institute of Biosciences and Technology, Texas A&M Health Science Center

2:00 *Image-based Assays Reveal Fibroblast-Mediated Drug Resistance in Colorectal Cancer*
Shannon Mumenthaler, University of Southern California

2:30 *High Content Chemoresponse Assays for Personalized Management of Cancer*
David Andrews, University of Toronto

3:00 **Selected Abstract:**

3D Modeling of Chromosomes Territories in Normal and Aneuploid Nuclei
Fatima Merchant, University of Houston

3:15 *3D HCS Imaging Challenges and Solutions for Complex Cell Model Systems*
Joe Trask, Perkin Elmer

3:45 Break

4:00 *Fluorescent multiplex IHC: An Integrated Approach for High Throughput Panel-driven and Ultra-high-plex Multiplexing Tissue with Single-Cell Resolution*
Grady Carlson, Akoya Biosciences

4:30 *Protein Marker Multiplexing and Quantitative Image Analysis for Disease Characterization*
Alison Cheung, Sunnybrook Research Institute

Speaker Abstracts
(In alphabetical order)

David Andrews, Director and Senior Scientist
Biological Sciences, Sunnybrook Research Institute
Professor, Biochemistry and Medical Biophysics, University of Toronto

Dr. David Andrews is Director of and senior scientist in Biological Sciences at Sunnybrook Research Institute, Professor of Biochemistry and Medical Biophysics at University of Toronto and a Tier 1 Canada Research Chair in Membrane Biogenesis. His research includes, the molecular mechanisms by which Bcl-2 family proteins regulate apoptosis at mitochondria, mechanisms of protein-protein interactions, the assembly of proteins into membranes, high-content screening and development of new microscopes for fluorescence lifetime imaging microscopy. Dr. Andrews uses fluorescence spectroscopy, fluorescence lifetime imaging and automated fluorescence microscopy to study protein-protein interactions in live cells and in membranes, protein localization in cells and the effects of drugs on cells. At Sunnybrook Research Institute he established a facility for image-based high-content cellular analysis that includes instrumentation for automated imaging and analysis of cells in monolayer and 3D cultures, genome scale gene knockdown and screening of libraries of small molecules. His lab has discovered and characterized small molecules that accelerate and inhibit Bcl-2 family protein mediated mitochondrial outer membrane permeabilization that have applications to cancer and regenerative medicine.
Dr. Andrews is active in the public and private sector. In recent years he was president of the Society for Biomolecular Imaging and Informatics. He is a member of the editorial board of Cell Death and Differentiation. He participated in the start-up of two companies, Fermentas and Isogenica and consults for several other companies and academic centers. His group performs collaborative and contract research for a variety of biotech companies including ABBVIE, Eli Lilly, Johnson and Johnson, Novartis and Celgene. He holds licensed patents in areas such as translational regulation, in vitro evolution, peptide display technologies and optical microscopy.

Day 1 Workshop
Rapid Quantification of Protein-protein Interactions in Live Cells

Day 3 Lecture
High Content Chemoresponse Assays for Personalized Management of Cancer
Abstract: Until recently, the prognosis for CLL patients who relapsed after first-line cytotoxic chemotherapy was often only a few years. The Bruton's tyrosine kinase (BTK) inhibitor Ibrutinib has dramatically altered this situation but is not curative and outcomes for patients who develop progressive disease on Ibrutinib are poor. Another recently successful approach to the treatment of CLL is via inhibition of the apoptosis inhibitory protein Bcl-2 using the highly specific inhibitor Venetoclax. An advantage of Venetoclax in the treatment of cancer is that a positive response results in cell killing as opposed to cells entering a quiescent state. However, clinical responses to Ibrutinib and Venetoclax are highly variable, likely as a consequence of the heterogeneity of the disease. For any one patient there is no clear way to decide which drug would be most effective.
We use in vitro models of the microenvironment and High Content Screening (HCS) to make image based assessments of cellular responses to drugs (Chemoresponse assays). We have found that much of the biology of the microenvironment that supports survival of CLL during treatment can be captured by culturing circulating CLL cells with IL2 to represent T cell activity and the TLR7-agonist Resiquimod. In response to these representative microenvironmental signals, CLL cells proliferate and make cytokines, and become profoundly drug resistant. We use HCS with this model to provide chemoresponse data for drugs including standard chemotherapy, Ibrutinib, Venetoclax and other

targeted therapies. Our hypothesis is that using HCS and an appropriate model of the microenvironment we can determine how CLL cells in patients will respond to therapies. In order to test this hypothesis, we evaluate in primary samples from newly diagnosed, high risk and relapsed/refractory CLL patients, conventional and proposed novel therapies as single agents and in combinations. Our data suggest that microenvironmental models and HCS can be used to predict patient responses providing a biomarker for potential personalization of therapies.

Andrew Belmont, Professor, Cell & Developmental Biology
and Biophysics and Quantitative Biology, University of Illinois at Urbana-Champagne

Nuclear Speckles as a Transcriptional Hub and Amplifier: A Combined Genomic and Live-cell Imaging Approach

Dr. Belmont received his AB from Princeton University in Physics. MD with PhD in Physiology and Biophysics from Temple University. Postdoctoral work with David Agard and John Sedat at UCSF focused on applying EM tomography, in its early days, to mitotic and interphase chromosomes. 1983 to present at the University of Illinois, Urbana, transitioning from Assistant to full Professor with a 6 year stint as Department head. As an independent investigator, his primary interest has been in understanding the highest levels of chromatin organization. As an Assistant Professor, his laboratory developed the lac operator / repressor system for labeling chromosome loci. Much of his research focus has been devoted to demonstrating that large-scale chromatin domains exist in living cells and represent the actual chromatin template for both transcription and DNA replication in typical somatic nuclei. Surprisingly, they also found that different large-scale chromatin states can be tightly correlated with differential positioning within the nucleus. More surprisingly, they have observed directed, long-range interphase chromosome movements occurring through an apparent force-generating and actin-dependent mechanism. Most recently, his research program is transitioning from analysis of engineered chromosome regions to endogenous loci, combining light microscopy methods with developing new genomic approaches to study differential chromosome positioning and compaction.

Abstract: Striking variations in transcriptional activity have been correlated with nuclear compartmentalization. Previously a small number of active genes were shown to localize near nuclear speckles, leading to the proposal that nuclear speckles serve as a transcriptional hub for a subset of genes. This contrasts with the textbook picture of nuclear speckles primarily as storage sites for RNA processing factors. Two key questions emerge from these previous, older studies: (1) How prevalent is this observation of active genes associating with nuclear speckles; (2) Does transcription actually change with nuclear speckle association?
To address the first question, we developed TSA-Seq to probe genome organization. Tyramide Signal Amplification (TSA) enables genome-wide measurement of relative chromosome loci distances to particular nuclear compartments. Combining Lamina and Speckle TSA-Seq, provides new insights into nuclear architecture: Inter-LADs vary in how far they protrude into the nuclear interior, with centers of transcriptional “hot-zones” mapping to the most interior location of an inter-LAD chromosome trajectory. Transcription hot-zones mapping closest to nuclear speckles correspond to the A1 Hi-C subcompartment, and are enriched in super-enhancers, the most highly expressed genes, and genes with low transcriptional pausing. Comparing different cell lines, we have identified chromatin domains with Mbps-size that shift variable distances along this Nuclear Speckle- Lamina axis, with accompanying changes in transcriptional activity.
To address the second question, we examined both endogenous and BAC transgene Hsp70 genes. Using smRNA FISH, both Hsp70 BAC transgenes and endogenous genes turn on 2-4 mins after heat shock irrespective of their distance to nuclear speckles. However, we observe 12-56-fold and 3-7-fold higher transcription levels for speckle-associated Hsp70 transgenes and endogenous genes, respectively, after 1-2 hrs heat shock. Several fold higher transcription levels for several genes flanking

the Hsp70 locus also correlate with speckle-association at 37 oC. Live-cell imaging reveals this modulation of Hsp70 transcription temporally correlates with speckle association/disassociation. Stable, increased production of nascent transcripts follows typically 0-2 minutes after speckle contact. Rare, transient increases in nascent transcripts are seen without speckle association but do not persist more than 1-2 minutes.
Together our results suggest that nuclear speckles represent a major active chromosomal nuclear compartment which may amplify the expression of large numbers of genes.

Long Cai, Professor
Biology and Biological Engineering, California Institute for Technology
Spatial Genomics: Nascent Transcriptome Profiling by intron seqFISH

Long Cai is a Professor of Biology and Biological Engineering at Caltech. His lab pioneered the field of spatial genomics by developing a method that allows the simultaneous imaging of over 10,000 genes in single cells within their native spatial context. This technology has opened a new way to directly visualize the genome in situ with microscopy, with many applications in neuroscience, stem cell biology, developmental biology and precision medicine. For this work, Dr. Cai has received the NIH New Innovator Award, Transformative Award, Paul G. Allen Frontiers Foundation Distinguished Investigator Award. Dr. Cai received his AB/AM in Physics and Chemistry at Harvard College, under the supervision of Dudley Herschbach, and his PhD in Chemistry at Harvard with Sunney Xie. He trained as a Beckman Institute Postdoctoral Fellow with Michael Elowitz at Caltech.

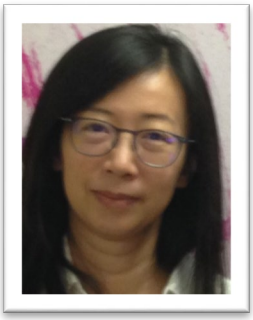
Abstract: Visualization of the transcriptome and the nuclear organization in situ in individual cell is the holy grail of single cell analysis. Here, we demonstrate a multiplexed single molecule in situ method, intron seqFISH, that allows imaging of 10,421 genes at their nascent transcription active sites in single cells, followed by mRNA and lncRNA seqFISH and immunofluorescence. This nascent transcriptome profiling method can identify different cell types and states with mouse embryonic stem cells and fibroblasts. The nascent sites of RNA synthesis tend to be localized on the surfaces of chromosome territories and their organization in individual cells is highly variable. Surprisingly, the global nascent transcription oscillated asynchronously in individual cells with a period of 2 hours in mouse embryonic stem cells as well as in fibroblasts. Together, spatial genomics of the nascent transcriptome by intron seqFISH reveals nuclear organizational principles and fast dynamics in single cells that are otherwise obscured.

Grady Carlson, Technical Applications Scientist
Akoya Biosciences
Fluorescent Multiplex IHC: An Integrated Approach for High Throughput Panel- Driven and Ultra-High-Plex Multiplexing Tissue with Single-Cell Resolution

Dr. Carlson is a technical applications scientist with experience in basic and translational pathology-based research. Throughout his career Grady has utilized flow-based and multispectral imaging techniques for detection and characterization of biomarkers in tissue. Grady joined Akoya Biosciences with the PerkinElmer quantitative pathology team.

Abstract: Advancements in immunotherapy have brought about a paradigm shift for the treatment of cancer yet are effective for a limited population of cancer patients. Purported response factors include

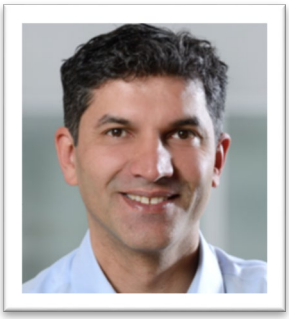
presentation of targetable antigens, e.g., PDL1, tumor immunogenicity, and immunosuppressive cells in the tumor microenvironment. In effort to increase the percentage of patients that respond to immunotherapy, physicians and scientists utilize multiplex immunohistochemistry (mIHC) techniques, such as spectral unmixing and serial staining, to increase the accuracy of antigen quantification and identify immune cell populations defined by complex molecular signatures. Studies using mIHC demonstrate that enumeration of effector immune cells in the tumor allows for limited stratification of responsive patients. Notably, greater stratification is achieved by evaluating both the density of immune cells as well as, spatial relationships between tumor and immune cell subsets in the tumor microenvironment, marking spatial relationships as an important metric revealed by mIHC. In this review, I will discuss methods for high dimensional analysis enabling detection of 50 markers in tissue, high-throughput mIHC for visualization of immune cell subsets identified by complex molecular signatures, and spatial profiling with single-cell resolution.



Alison Cheung, Research Associate
Biomarker Imaging Laboratory, Sunnybrook Research Institute
Protein Marker Multiplexing and Quantitative Image Analysis for Disease Characterization

Dr. Alison Cheung completed her PhD training at the Department of Medical Biophysics, University of Toronto, studying the role of breast cancer gene BRCA2 in cancer development. After her PhD, she continued her research work at Sunnybrook Research Institute and Princess Margaret Hospital in Toronto, focusing on monitoring cancer progression and therapeutic response in preclinical models using imaging. Dr. Cheung is currently a research associate at the Biomarker Imaging Research Laboratory led by Dr. Martin Yaffe at the Sunnybrook Research Institute. Her research interests are quantitative analysis of high-dimensional data in protein multiplexing to examine cellular and spatial heterogeneity in cancer, and the integration of radiological imaging features and molecular signatures to improve disease characterization.

Abstract: The effectiveness of targeted cancer regimen or immunotherapy could be limited by the heterogeneous spatial distributions of cells in the cancer lesion and tumor microenvironment. In our approach to quantify the extent of intra-tumoral heterogeneity using an in situ immunofluorescent protein marker multiplexing system (MxIF, developed by GE Global Research Centre), we have identified distinct sub-populations of cancer cells and tumor-infiltrating lymphocytes (TILs) in breast and ovarian cancer. The MxIF protein multiplexing approach is based on the sequential-stain (image)-bleach method which allows dozens of protein markers to be labelled and imaged on a single tissue section from formalin-fixed paraffin-embedded specimen. Single cells were segmented and the signal intensity of each labelled protein marker was quantified. Thresholding and Kmeans clustering methods were applied to define sub-populations. We have built tools to visualize their distributions and localizations and quantified their associations based on co-expression patterns and their proximity in cellular organization. In addition to phenotyping cells in the cancer epithelium, TILs were also studied. I will discuss our work studying TILs in ovarian cancer and their co-expressions with proteins in the PD1-PDL1 immune checkpoint pathway.



Christian Conrad, Intelligent Imaging Group
Berlin Institute of Health, Germany
Spheroid Imaging and Sequencing

Christian Conrad, a trained biologist from University Heidelberg and Freiburg, has long-standing record in automated high content screening applied to cell and molecular biology using machine learning algorithms. As bioinformatician, he recently entered the field of single cell and single cancer spheroid sequencing with the aim of correlating cellular phenotypes and genomic profiles. This correlative analysis and classification open new avenues in understanding biological heterogeneity, cancer progression and diagnostic monitoring. His scientific strength is the interdisciplinary background and work in personalized oncology, imaging and systems biology, at the newly formed Berlin Institute of Health Digital Center, Charité Berlin.

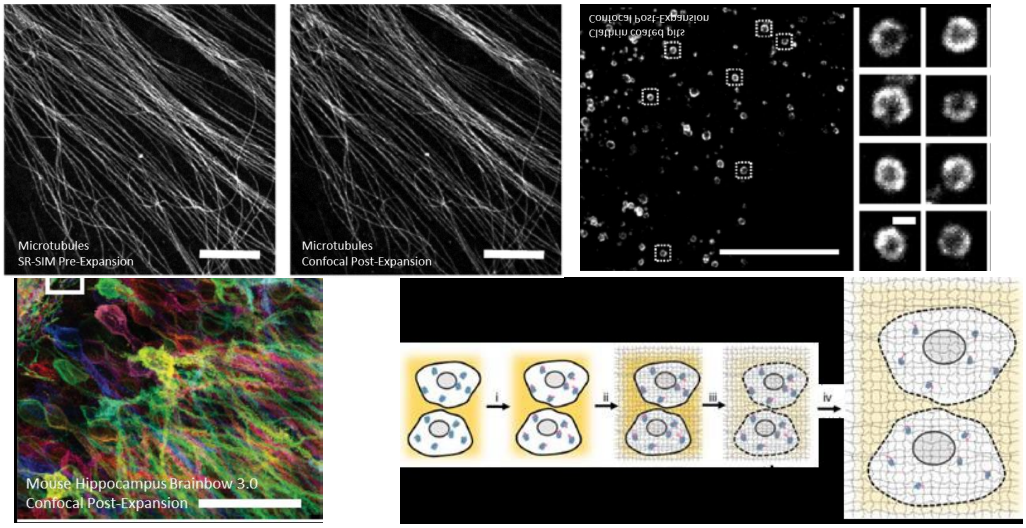
Abstract: The correlative genomic analysis and classification of single cells allows to understanding biological heterogeneity and cancer disease progression in preclinical research. We have developed workflows to isolate and classify micro tissues for single cell or tissue genomics to more accurately correlate 3D phenotypes with multicellular gene expression profiles. By combining image-based classification, drug sensitivities or genomic profiling, we can link specific gene expression programs to heterogeneous breast, pancreas, and colon cancer 3D cell culture. Moreover, we identify specific drug sensitive pathways and their associated morphologies of patient-derived cultures which potentially facilitate tumor therapy monitoring by in vitro assays.

Mahander Dewal, Principal Scientist, Founding Member, Expansion Technologies, Inc.
Expansion Microscopy: A novel tool for Single Cell Analysis in Intact Biological Systems

Mahender Dewal, completed bachelor's and Master's in Chemistry from Osmania University, Hyderabad, India. Then moved United States and completed Ph. D. in Organic chemistry from University of South Carolina, Columbia. Then briefly worked as postdoctoral fellow at Wayne State University in Department of Pharmaceutical Sciences and joined Department of Chemistry at Massachusetts Institute of Technology as research fellow. In, 2015 joined as a founding scientist in Expansion Technologies Inc. (EXT) a biotech startup founded by Prof. Ed Boyden of MIT based on his recent invention Expansion Microscopy. Presently, as Principal Scientist/Team leader at EXT leading Product and R&D teams working on early stage diagnostic methods for various cancers.

Abstract: Single cell analysis methods play vital role in the identification and localization of different kinds of biomolecules throughout cells and intact tissues, thereby allowing characterization and classification of individual cells and their relationships to each other within intact systems. However, phenotyping the cellular organization and configuration in cancer tissue remain challenging due to the large population of cells and their diverse properties. Current methods of tumor analysis rely on bulk sequencing of the specimen and do not retain spatial information which is crucial for determining the most effective therapeutic option. The scarcity of spatial information about the tumor heterogeneity within cancer tissue can be attributed to a lack of adequate technology that can map relevant biomarkers three-dimensionally with high resolution. Expansion microscopy (ExM) is a rapidly emerging technology that allows super resolution imaging of specimens with conventional diffraction limited microscopes. ExM is well-positioned to integrate molecular content, cellular morphology and heterogeneity, with the spatial precision sufficient to resolve individual biological building blocks, and the scale and accessibility required to deploy over extended 3-D objects like

tissues and organs. ExM's method is to physically expand biological samples in isotropic way within a polymer gel allowing super resolution microscopy to be performed using readily accessible wide-field and confocal microscopes to obtain multicolor, 3-D images of cells and tissue at nano-scale resolution. Compared to existing super resolution techniques, ExM is inexpensive, faster, and less technically demanding. Additionally, ExM is scalable and capable of multiplexed imaging and quantification of a large number of protein and nucleic acid biomarkers within a single sample. Expansion Technologies Inc. (www.extbio.com) is pioneering and deploying this radically new ExM technology. We take this opportunity to discuss the ExM technology development and its applications in the biology and medicine.



Fiona Ginty, Biosciences Technology Manager & Principal Investigator at GE Global Research

Multiplexed Imaging for Multi-protein Analysis at the Single Cell Level in Fixed Tissue (MultiOmyx™ and Cell DIVE™)

As Biosciences Technology Manager at GE Research, Fiona leads a group of scientists who are inventing new applications in cellular and molecular biology including cell therapy, forensics, synthetic biology diagnostics and imaging. Over the last 10+ years, she has also led a multidisciplinary program to develop a highly multiplexed imaging method and technology platform (Cell DIVE™ and MultiOmyx™), which is providing deep new insights into tumor spatial biology, heterogeneity and immune response. She is also Principal Investigator on a NIH RO1, which is investigating systems modeling of cellular apoptosis and heterogeneity in colorectal cancer, and co-PI on a Provocative Question RO1 award with Indiana University, which is investigating cellular differences and immune response in DCIS in different ethnic groups. She previously worked as senior scientist with the Medical Research Council, Cambridge, UK and post-doc at Nestle Research Center, Switzerland. Fiona graduated from the National University of Ireland, Galway with a B.S. in Microbiology and Ph.D. from University College Cork in Nutritional Science.

Abstract: The prognostic and therapeutic significance tumor heterogeneity is slowly being unraveled using a variety of -omic technologies including single cell transcriptomics, DNA sequencing and multiplexed imaging and single cell analysis in situ. Over the last 10 years at GE Research, we have been developing and validating a tissue multiplexing platform (Cell DIVE™ and MultiOmyx™) that provides automated registered images on up to 60 proteins in a single FFPE tissue section. Single cell segmentation conserves the spatial coordinates and provides potentially millions of cell biomarker

datapoints in a single tissue section. The application of this high-dimensional imaging approach is revealing new insights into the tumor biology and cell behavior and interactions, including immune response at the earliest stages of breast cancer (ductal carcinoma in situ (DCIS)), tumor heterogeneity, and correlation with outcomes in colorectal and breast cancer. Massive data integration, translation to meaningful mechanistic insights and new types of tissue-based diagnostics are amongst the challenges ahead in this very exciting new field.

Santosh Hariharan, Senior Scientist
Pfizer

Phindr3D: Data-driven Segmentation-free Phenotyping of 3D Microscopy Data

Dr. Santosh Hariharan is a Senior Scientist at Pfizer Inc. His research interests comprise of application of image based single cell profiling to understand mechanism of action of small molecules, developing image analysis solutions for quantifying three-dimensional cell culture system and application of machine learning to understand and quantify protein trafficking pathways. He completed his PhD in medical physics from University of Toronto under the supervision of Dr. David Andrews.

Abstract: Three-dimensional (3D) are now routinely used in image-based screens. This is due to the ability of 3D culture system to better recapitulate the underlying physiology. Using automated confocal microscopy, it is now easy to capture large number of images from 3D cultures in a short time. While there are tools that can segment single cells in 3D, analysis of large number of 3D multi stack images is not feasible owing to the amount of time and computational resources necessary to perform such large-scale analysis. Further, 3D segmentation is a challenging task for cases such as dense neuronal cultures. An alternate solution is to analyze multi-stack images by flattening the stacks to a 2D plane and then using standard 2D image segmentation along with subsequent hand-crafted feature extraction for downstream analysis. However, by flattening the stack critical information from the third dimension is lost.
To overcome the above limitations, we developed Phindr3D, a data driven segmentation free method for automated profiling of 3D phenotypes. Phindr3D aids in making decisions along with advanced visualization to display results. We applied this method to quantify response of primary neuronal cultures to anti-apoptotic protein inhibitors. We analyzed 218700 images across 3 plates and used automated clustering from Phindr3D to estimate the number of phenotypic groups in the data. The unsupervised clustering revealed phenotypic similarities between inhibitors of the same anti-apoptotic protein (Bcl-XL) at the same time revealing phenotypic differences between compounds that inhibit different anti-apoptotic proteins. Compound that inhibited multiple anti-apoptotic proteins formed its own cluster in multi-parametric space. Thus, Phindr3D was able to capture the underlying biology without the need for explicit segmentation or hand

Hartland Jackson, Research Assistant
Institute for Molecular Life Sciences, University of Zürich

Hartland Jackson studied biochemistry at Queen's University and obtained his PhD for his work on mammary stem cells and breast cancer in the laboratory of Rama Khokha at Princess Margaret Cancer Center and the University of Toronto. In 2014 he joined the laboratory of Bernd Bodenmiller at the University of Zürich. There, as an EMBO Fellow, he has focused on the use of mass cytometry for highly multiplexed imaging of tumor tissues while developing methods to use spatially resolved single cell data. Currently, his work is focused on

the stratification of patient outcome based on single cell phenotyping and multi-cell interactions in intact tissues from large clinical cohorts.

Day 1 Workshop
Imaging Mass Cytometry: High-dimension Imaging, Segmentation and Analysis of Spatially Resolved Single Cells

Abstract: Single cell analysis has revealed extensive cancer heterogeneity, but histologic stratification of tumors is still the foundation of most clinical decisions. Breast cancer diagnosis based on cellular organization and the expression of hormone receptors, and amplified HER2 has led to subtype targeted therapies and improved patient outcomes, but intra-tumor cellular heterogeneity and the existence of many more molecular subtypes may be responsible for therapeutic resistance and relapse. To extend the histology classification of breast cancer to the single cell level, we applied Imaging Mass Cytometry (IMC) with a 40-parameter antibody panel to create high-dimensional immunohistochemistry pathology images. Single cell segmentation and analysis quantified tumor and stromal single cell phenotypes, their interactions, and spatial heterogeneity in 281 breast cancers. Classification at the cellular level identifies the single cell phenotypes present in hormone receptor positive, HER2 amplified, and triple negative breast cancers as well as the spatial organization of specific tumor microenvironments. Further, classification of breast cancers based on their single cell phenotypes identifies novel subtypes with distinct clinical outcomes and responses to targeted therapies. Here, single cell pathology provides spatially resolved, multi-cell definitions of breast cancer and identifies cellular targets for future patient-specific therapeutic interventions.

Day 2 Lecture
The Single Cell Pathology Landscape of Breast Cancer: Tumour Cells and Their Microenvironments
Abstract: This education session will cover all aspects of imaging mass cytometry from antibody panel design to data analysis. This will include an overview of high-dimension imaging using mass-tag staining, image acquisition using a Hyperion mass-spec based system, and required data processing. This will be paired with methodologies for spatially resolved single cell analysis from any high-dimension imaging platform using a flexible computational pipeline based on open source tools.

Erez Leiberman, Assistant Professor, Molecular and Human Genetics,
Baylor College of Medicine
Assistant Professor, Computer Science, Rice University
Tracking Genome Architecture in Space, in Time, and in Single Cells

I am a mathematician and a molecular biologist. My laboratory's primary emphasis is on exploring the three dimensional structure of the human genome. Much of the lab's work centers on the experimental interrogation of genome folding and structure via molecular biology and microscopy. I conceived of the Hi-C protocol, built the team that created the first 3D map of the human genome, and led all aspects of the resulting data analysis. Hi-C couples DNA proximity ligation to sequencing in order to comprehensively map long-range genomic interactions. My lab has since developed in situ Hi-C, in which proximity ligation is performed in intact nuclei. We used this method to publish the first kilobase- resolution contact maps of the human genome and the first reliable genome-wide annotation of chromatin loops. This work led to our proposal that loops in the human genome form by a process of extrusion. We also published the first reliable methods for assembling Human Genome Project quality de novo genome assemblies created only from short Illumina reads, enabling the generation of mammalian genomes from scratch for under $1,000.

In addition, my lab has created extremely powerful computational and visualization tools for enabling the interpretation of such datasets, including reliable loop mapping and de novo genome assembly. Our tools are publicly available and have been used at over 1000 institutions worldwide. My laboratory occupies a fully renovated, 3300 square foot facility with extensive wet lab and dry lab space at the Baylor College of Medicine.

Abstract: The human genome is over 2 meters long, yet it folds to fit inside a cell nucleus that is only a few microns wide. In this talk, I describe our work, along with many collaborators, developing 'Hi-C' (Lieberman-Aiden et al., Science, 2009; Aiden, Science, 2011) and 'in-situ Hi-C' (Rao & Huntley et al., Cell, 2014) to map the 3D architecture of the genome. Our data has revealed the existence of a sequence-based code governing nuclear architecture, in which loops form by a process of extrusion between CTCF-bound sites in the convergent orientation (Sanborn & Rao et al., PNAS, 2015; Vian et al., Cell, 2018). I will describe our efforts to track loop formation over time (Rao et al., Cell, 2017) and to explicitly trace chromosomes in single cells (Nir et al., PLOS Genetics, 2018). Finally, I will discuss applications of these methods to genome assembly (Dudchenko et al., Science, 2017; Dudchenko et al., Biorxiv, 2018; Matthews, Dudchenko, & Kingan, Nature, 2018).

Michael Mancini, Professor
Molecular & Cell Biology, Baylor College of Medicine
Multiplex Imaging for Single Cell Mechanistic Analysis of Estrogen Receptor Functions

The Mancini lab has been developing/pioneering image-based approaches for >20 years to better understand transcription within a cellular context, and at a single cell level, including taking this approach all the way to high throughput (HT) screening. These approaches have necessitated the need for a robust shared microscopy resource, the college-wide accessible Integrated Microscopy Core (IMC), directed by Dr. Mancini, and the new Center for Advanced Microscopy and Image Informatics (CAMII), a CPRIT-funded joint BCM/Texas A&M imaging/analysis Center. The IMC and CAMII are staffed with experienced personnel, hardware and software to carry out these types of studies, with >100 laboratories utilizing the facilities each year. The IMC/CAMII houses routine and advanced light and electron microscopy, high throughput confocal and wide-field microscopy, long term live cell imaging, super-resolution microscopy (STORM, SIM, STED), robotics and software platforms obtained from several NIH/NSF equipment grants with excellent and cooperative vendor support for new platforms (including prototype development and beta testing).
The main projects from the Mancini lab are highly multiplexed and HT amenable, centering on quantitative imaging of transcription using approaches that *simultaneously* assay mRNA synthesis of defined target genes as well as antibody labeling. mRNA FISH and antibody labeling are used in conjunction with robust quantitative software for cell-by-cell analyses, at HT microscopy-based speeds. Historically, the primary Mancini lab projects have centered upon single cell analysis of ER and AR in the context of chromatin and nuclear structure in model cell culture systems. The lab is particularly interested in transcriptional regulation in response to known or novel ligands and, especially during its past NIEHS-funded projects for the past ~10 years, including a recent NIEHS Superfund grant to investigate EDC's present in the Galveston Bay/Ship Channel region. to generate quantitative, HT acquisition of endogenous gene expression.

Abstract: Our lab has a longstanding interest in single cell analysis-based transcription studies. We have developed novel mechanistic and phenotypic approaches to study transcription within a cellular context, but with sensitive, high throughput approaches. Our main platform allows us to quantify

transcription using high throughput microscopy and image analytics that are designed to link, at the single cell level, mRNA synthesis to DNA binding and promoter occupancy of nuclear receptors (NRs) and coregulators, histone modifications and large-scale chromatin modeling. A growing list of endocrine disrupting chemicals (EDC) from the environment have been shown to target NRs, including estrogen and androgen receptors (ER, AR), with large scale efforts to develop and test environmental samples for EDC activities. Our multiplex assays with engineered cells or tumor cell lines endogenously-expressing ER/AR are currently being used to assess individual or mixtures of known hormones and EDCs via machine learning approaches. These studies are currently being applied to environmental samples obtained Galveston Bay and the Houston Ship Channel via funding from the NIEHS Superfund Research Program.

Shannon Mumenthaler, Assistant Professor
Medicine, University of Southern California

Shannon Mumenthaler, PhD, is an Assistant Professor of Medicine at the University of Southern California. She is also the Director of the Stephenson Family Personalized Medicine Center of USC. Dr. Mumenthaler completed her Bachelors of Science in Genetics at UC Davis. She then went on to earn a doctoral degree in Cellular and Molecular Pathology at UCLA and carried out her postdoctoral training at Cedars-Sinai Medical Center and USC. Shannon has over 10 years of experience working with various tumor models and cell biology techniques. Her research interests involve a multidisciplinary approach to examine the impact of the tumor microenvironment on treatment response. She is developing more physiologically-relevant model systems that capture the microenvironmental context of a tumor and coupling these preclinical models with microscopy-based imaging pipelines to provide deeper insights into the evolving tumor.

Day 1 Workshop
3D Models for Cancer Biology

Day 3 Lecture
Image-based Assays Reveal Fibroblast-Mediated Drug Resistance in Colorectal Cancer
Abstract: Cancer is a complex adaptive system orchestrated by the interactions between tumor cells and their microenvironment. In particular, cancer-associated fibroblasts (CAFs), the dominant cellular component of the tumor stroma, are often associated with a poor prognosis and play an important role in tumor progression for a number of cancers. While significant literature has highlighted the influence of CAFs on cancer cell phenotypes including tumor cell proliferation and invasion, the role of CAF heterogeneity on treatment response remains largely understudied. Additionally, preclinical treatment studies often focus on drug-induced changes to tumor cells with little investigation into the impact on surrounding stromal cells. To advance our biological understanding of cancer and improve treatment efficacy, we are utilizing quantitative high-content imaging coupled with more physiologically-relevant patient-derived model systems to illuminate the dynamic interactions between cancer cells and their microenvironment. These studies are aimed at increasing our understanding of the functional and therapeutic utility of CAFs by leveraging expertise across disciplines. Our lab has developed several imaging-based workflows, combined with machine learning and other image analysis techniques, to rapidly and accurately classify cell types and cell behaviors within heterocellular populations. Using these approaches, we have identified cancer-associated fibroblasts as a source of environment mediated drug resistance in colorectal cancer. Specifically we discovered a novel mechanism by which drug treated CAFs render adjacent tumor cells resistant to anti-EGFR therapy.

Robert F. Murphy, Carnegie-Mellon University
Biological Sciences, Biomedical Engineering and Machine Learning, Carnegie Mellon University
Honorary Professor of Biology, Albert Ludwig University of Freiburg
Senior Fellow, Allen Institute for Cell Science
Integrating Information from Diverse Microscope Images: Learning and Using Generative Models of Cell Organization

Dr. Murphy is the Ray and Stephanie Lane Professor of Computational Biology and Head of the Computational Biology Department in the School of Computer Science at Carnegie Mellon University. He also is Professor of Biological Sciences, Biomedical Engineering, and Machine Learning, Honorary Professor of Biology at the University of Freiburg, Germany, and Senior Fellow of the Allen Institute for Cell Science. He is a Fellow of the American Institute for Medical and Biological Engineering and received the Distinguished Service Award from the International Society for Advancement of Cytometry; he served as President of the Society from 2008-2010. He was a member of the National Advisory General Medical Sciences Council and the National Institutes of Health Council of Councils, and is a Senior Member of the IEEE and ISCB. He is an Associate Editor for Bioinformatics, and was Associate Editor for BMC Bioinformatics and Software Editor for PLoS Computational Biology. He is also a co-founder of Quantitative Medicine, LLC.
Dr. Murphy's career has centered on combining fluorescence-based cell measurements with quantitative and computational methods. His group at Carnegie Mellon pioneered the application of machine learning methods to fluorescence microscope images depicting subcellular location patterns, and was the first to demonstrate superior machine performance in interpreting diverse patterns in biological images compared to human interpretation. His current research interests include machine learning of image-derived models of cell organization, automated detection of protein location changes during oncogenesis, and active machine learning approaches to experimental biology.

Abstract: Systems biology efforts require accurate, cell-type specific information about the shape and distributions of subcellular structures and the distributions of proteins and other macromolecules in order to be able to capture and simulate cellular spatiotemporal dynamics. We have developed tools to build generative models of cell organization directly from microscope images of many cells. Our open source system, CellOrganizer (http://CellOrganizer.org), contains components that can build probabilistic generative models of cell, nucleus, and organelle shape as well as organelle and protein distributions. The models also capture heterogeneity within cell populations. We have recently addressed two critical challenges in constructing these models. The first is to choose appropriate methods for generating cell, nucleus or organelle shapes. We have recently evaluated a number of generative methods with respect to their reconstruction accuracy: how well they can capture/represent a complicated shape. The results show that spherical harmonic methods provide the best reconstructions and most reasonable synthetic shapes. The second challenge is to be able to learn the dependence of component distributions upon each other, i.e., the spatial relationships between different components. As these relationships are learned, generative models can be created from images of different proteins and organelles and then combined to create synthetic cells having many more components than can be imaged together. We have explored approaches for inferring spatial relationships between components without directly measuring them. An important use for the resulting models of cell organization is to generate synthetic cell shapes and organelle distributions that can be used as geometries for cell simulations. This permits a structured exploration of the dependencies of cellular biochemistry upon cell morphology and organization.

Bartek Rajwa, Research Associate Professor
Computational Life Sciences, Purdue University
Statistical Analysis of High Content Screening Data

Dr. Bartek Rajwa is a Research Associate Professor of Computational Life Sciences in the Bindley Bioscience Center at Purdue University where he conducts studies on the technology of high-throughput cytometry, high-content imaging, biological image analysis, biological pattern recognition, and applications of statistical machine learning in cell biology, neuroscience and agriculture. Bartek is an Associate Editor of Cytometry Part A (the official journal of ISAC), and a member of the Board of Directors of the Society of Biomolecular Imaging and Informatics (SBI2).

Abstract: This tutorial will briefly review the statistical approaches used to analyze, visualize and interpret the HT/HC screening data, and formulate conclusion regarding the screening results. We will discuss the measures of effect sizes (Cohen's d and its multivariate generalization), the dedicated metrics of assay quality such as Z' (Z-prime) and Sw (assay window), and demonstrate the relationship between them. The presentation will explain the conceptual origins of the common HT/HC assay quality indices, the logic behind the formulas, as well as their applicability, implicit assumptions, and limitations. The talk will address the relationship between the traditional measures used in screening, and classification performance measures employed in machine-learning (sensitivity, specificity, predictive values, F1 score, and AUC). The tutorial will also touch upon other essential concepts of data analysis in phenotypic screening: the notions of significance, replication, statistical power, fixed and random effects, and meta-analysis, and link those ideas to the everyday praxis of assay design, optimization, and execution. The intended audience includes the screening practitioners working with all the types of HT or HC screens (bulk assays, image-based system, and flow cytometry instruments).

Arvind Rao, Associate Professor
Department of Computational Medicine and Bioinformatics, University of Michigan
Bridging the Phenotypic-Genomic Continuum: Case Studies Linking Histology and Genomics

Arvind Rao is an Associate Professor in the Departments of Computational Medicine and Bioinformatics , as well as Radiation Oncology at the University of Michigan. His group uses image analysis and machine learning methods to link image-derived phenotypes with genetic data, across biological scale (i.e. single cell, tissue and radiology data). Such methods have found application in radiogenomics and drug repurposing based on phenotypic screens. Arvind received his PhD in Electrical Engineering and Bioinformatics from the University of Michigan, specializing in transcriptional genomics, and was a Lane Postdoctoral Fellow at Carnegie Mellon University, specializing in bioimage informatics.

Abstract: With the accomplishment of large scale cancer profiling studies such as the TCGA and CPTAC, there is now a large amount of information interlinked across multiple modalities (multi-omics, imaging etc). This talk will discuss some of our recent ongoing work in examining the relationships between spatially-derived readouts of tumor architecture and underlying genomics measurements.

Glauco R. Souza, Director of Global Business Development and Innovation, 3D Cell Culture, Greiner Bio-One
Adjunct Assistant Professor, UT Health Science Center at Houston
Magnetic 3D Bioprinting, from Generating Spheroids to Fingerprinting Cell-Types

Dr. Glauco R. Souza is the Director of Global Business Development and Innovation, 3D Cell Culture at Greiner Bio-One and former President and co-founder of Nano3D Biosciences, Inc. (n3D). He is one of the co-inventors and inventor of all eleven patents related to magnetic 3D cell, including magnetic 3D bioprinting and levitation technologies. Dr. Souza is also Adjunct Assistant Professor at University of Texas Health Science Center at Houston. Prior to co-founding n3D, Dr. Souza was an Odyssey Scholar at UT MD Anderson Cancer Center. During his work at MD Anderson, he applied a multidisciplinary approach to developing nanotechnology tools for tissue targeted imaging, gene delivery, and tissue engineering. Towards the end of his Odyssey Scholarship tenure at MD Anderson, in collaboration with Rice University, the genesis of 3D cell culturing by magnetic levitation and magnetic 3D bioprinting took place. Results using these groundbreaking technologies have been reported in various high-impact scientific journals, including Nature Nanotechnology, Proceedings of the National Academy of Sciences, Nature Protocols, Biomaterials, and Nature Reviews Cancer. Now, Glauco's mission is to advance magnetic 3D cell culture into a routine laboratory tool that will measurably improve the drug discovery process, cancer research, personalized medicine, and regenerative medicine. Recently, his work was selected for the Short List of The 2017 Lush Prize for outstanding research producing an effective non-animal safety test. He was also an invited speaker at TEDx Houston. Dr. Souza's research has been funded by grants from National Science Foundation (NSF), National Institute of Health (NIH), Department of Defense (DOD), Center for Advancement of Science in Space (CASIS), and Texas Emerging Technology Fund (ETF). Glauco received B.S. in Chemistry and M.S. and Ph.D. in Physical Chemistry from The George Washington University in Washington DC (GWU).

Abstract: The growing push for 3D cell culture models is limited by technical challenges in handling, processing, and scalability to high-throughput applications. To meet these challenges, we first use our platform, magnetic 3D bioprinting, in which cells are individually magnetized and assembled with magnetic forces. In magnetizing cells, not only do we make routine cell culture and experiments feasible and scalable, but we also gain fine spatial control in the formation of spheroids and more complex structures and insight into the dynamic process of 3D cell culture assembly. This presentation will focus on recent developments using this platform, particularly high-throughput applications in cancer biology, toxicology, and immunology. Especially, we will present a new approach for probing biology using real-time high-throughput imaging to phenotypically profile cell-cell and cell-drug interactions. This new method combines magnetic 3D bioprinting, real-time imaging, and Principle Component Analysis (PCA) to characterize the dynamic process of three-dimensional cell assembly. This label-free method allows for multiplexing with other assay endpoints for high-content screening. Overall, we use magnetic 3D bioprinting to create functionally and structurally representative tissue for high-throughput screening.

Fabio Stossi, Assistant Professor
Molecular & Cell Biology, Baylor College of Medicine
Multiplex Imaging for Single Cell Mechanistic Analysis of Estrogen Receptor Functions

Dr Fabio Stossi, a native of Milan, Italy, completed his studies at Universita' degli Studi di Milano, in Pharmaceutical Chemistry and Technology, and in Endocrinology and Metabolism. He then moved to the US as a postdoc in Dr. Benita S. Katzenellenbogen's laboratory at University of Illinois at Urbana-Champaign, where he became interested in gene transcription and its modulation by steroid receptors, particularly focusing on estrogen receptor in breast cancer. More recently, he joined Dr. Michael A. Mancini's group in the Department of Molecular and Cellular Biology, Baylor College of Medicine, as an Assistant Professor. He is currently Technical Director of the Integrated Microscopy Core and group leader for imaging in the GCC Center for Advanced Microscopy and Image Informatics. His interests are imaging and analysis of single cell gene transcription and assay development in environmental toxicology.

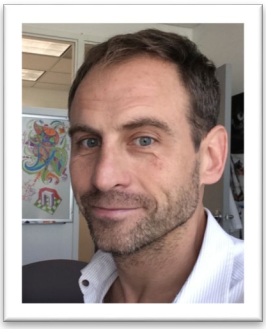
Abstract: Our lab has a longstanding interest in single cell analysis-based transcription studies. We have developed novel mechanistic and phenotypic approaches to study transcription within a cellular context, but with sensitive, high throughput approaches. Our main platform allows us to quantify transcription using high throughput microscopy and image analytics that are designed to link, at the single cell level, mRNA synthesis to DNA binding and promoter occupancy of nuclear receptors (NRs) and coregulators, histone modifications and large-scale chromatin modeling. A growing list of endocrine disrupting chemicals (EDC) from the environment have been shown to target NRs, including estrogen and androgen receptors (ER, AR), with large scale efforts to develop and test environmental samples for EDC activities. Our multiplex assays with engineered cells or tumor cell lines endogenously-expressing ER/AR are currently being used to assess individual or mixtures of known hormones and EDCs via machine learning approaches. These studies are currently being applied to environmental samples obtained Galveston Bay and the Houston Ship Channel via funding from the NIEHS Superfund Research Program.

Jason Swedlow, Professor
School of Life Sciences, University of Dundee
OME's Bio-Formats, OMERO, & IDR: Open Tools for Accessing, Integrating, Mining and Publishing Image Data @ Scale

Jason Swedlow earned a BA in Chemistry from Brandeis University in 1982 and PhD in Biophysics from UCSF in 1994. After a postdoctoral fellowship with Dr T. J. Mitchison at UCSF and then Harvard Medical School, Dr Swedlow established his own laboratory in 1998 at the Wellcome Trust Biocentre, University of Dundee, as a Wellcome Trust Career Development Fellow. He was awarded a Wellcome Trust Senior Research Fellowship in 2002 and named Professor of Quantitative Cell Biology in 2007. His lab focuses on studies of mitotic chromosome structure and dynamics and has published numerous leading papers in the field. He is co-founder of the Open Microscopy Environment (OME), a community-led open source software project that develops specifications and tools for biological imaging. In 2005, he founded Glencoe Software, Inc., a commercial start-up that provides commercial licenses and customization for OME software. In 2011, Prof Swedlow and the OME Consortium were named BBSRC's Social Innovator of the Year and Overall Innovator of the Year. In 2012, he was named Fellow of the Royal Society of Edinburgh. Prof Swedlow has organized or directed several courses in quantitative microscopy at the Marine Biological

Laboratory, Woods Hole, USA, Cold Spring Harbor Laboratory, USA and the National Centre for Biological Science, Bangalore. India.

Abstract: Despite significant advances in biological imaging and analysis, major informatics challenges remain unsolved: file formats are proprietary, storage and analysis facilities are lacking, as are standards for sharing image data and results. The Open Microscopy Environment (OME;http://openmicroscopy.org) is an open-source software framework developed to address these challenges. OME releases specifications and software for managing image datasets and integrating them with other scientific data. OME's Bio-Formats and OMERO are used in 1000's of labs worldwide to enable discovery with imaging.
We have used Bio-Formats and OMERO to build solutions for sharing and publishing imaging data. The Image Data Resource (IDR; https://idr.openmicroscopy.org) includes image data linked to >40 independent studies from genetic, RNAi, chemical, localisation and geographic high content screens, super-resolution microscopy, and digital pathology. Datasets range from several GBs to tens of TBs. Wherever possible, we have integrated image data with all relevant experimental, imaging and analytic metadata. With this metadata integration, we have run queries across studies to identify gene networks that link to cellular phenotypes. We have also built cloud-based analysis tools portals to catalyse the re-use and re-analysis of published imaging data.
To catalyse commercial access to OME's tools, we formed Glencoe Software in 2005 as a commercial arm of OME. Glencoe provides commercial licenses of OME software, along with guaranteed support and customisation. Glencoe has built PathViewer (http://www.glencoesoftware.com/products/pathviewer/), a web-based WSI visualisation and annotation tool that is used in several top 10 pharmaceutical companies and for digital pathology data sharing, analysis and also for e-learning in medical education.
In our latest work, OME and Glencoe have collaborated to build Parade, a web-based data mining application that uses data analytics stored in OMERO to query, select and analyse large collection of images in drug discovery and digital pathology.



Alexey Terkskikh, Associate Professor
Sanford Burnham Prebys Research Institute
Microscopic Imaging of Epigenetic Landscape in Single Cells

1991-1997, PhD in molecular immunology from University of Lausanne.
1997-2002, postdoc with Irv Weissman, Stanford, molecular genetics of hematopoietic stem cell.
2002-20012 Swiss Polytechnic School, Lausanne, Switzerland and Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, Assistant Professor.
2012-Present, SBP Medical Discovery Institute, La Jolla, CA, USA. Associate Professor

Abstract: We have developed a unique phenotypic high throughput screening platform (MIEL), which interrogates the epigenetic landscape at both population and single cell level using image derived features and machine learning. We have demonstrated the utility of such approach to identify epigenetic signature of differentiated primary glioblastoma cells, which enables high content drug screening for differentiation-inducing compounds (Farhy et al., biorxiv 2018). More recently, we applied MIEL to identify epigenetically toxic environmental compounds and epigenetic differences associated with aging. To get insight into the dynamic properties of epigenome, we have developed Genetically Engineered Epigenetic Probes (GEEPs) enabling, for the first time, a real-time imaging of epigenetic alterations in live cells.

Joe Trask, Senior Application Scientist
Perkin Elmer
3D HCS imaging challenges and solutions for complex cell model systems

Joe Trask is a Senior Application Scientist in the Cellular Imaging and Analysis group at PerkinElmer. He is instrumental in strategic customer support through teaching, training, and collaborations. He brings over 20 years of experience in high content screening technology from academia (Duke University and The Ohio State University), pharmaceutical industry (Abbott Laboratories and Eli Lilly & Company), and biotechnology (The Hamner Institute for Health Sciences and ScitoVation LLC). Joe has extensive experience in cell based technologies from flow cytometry, confocal microscopy and computer-assisted automated microscopy studying cancer, immunology, neurodegeneration, and toxicology. Joe is an author and associate scientific editor of the NIH/NCATS Assay Guidance Manual. And he was a co-founder and first President of Society for Biomolecular Imaging and Informatics (SBI2).

Abstract: The recent emergence of more relevant advance and complex in vitro cell models (organoids, spheroids, microtissue, and organ-on-a-chip) to recapitulate an in vivo response requires new approaches to interrogate cells using existing HCS technologies. These advances require researchers to adapt to these novel platform systems that may include imaging non-complaint ANSI/SLAS microplate standard formats. In this talk, I will discuss the HCS imaging process of simple 3D cell modes to more complex organ-on-a-chip models and provide guidelines to overcome challenging barriers to develop reproducible robust assays.

Judi Wardwell
Senior Applications Scientist, InSphero, Inc.
Image-Based Single Cell Analytics

Judi Wardwell-Swanson is a Senior Application Scientist at InSphero, Inc., a 3D microtissue company. She received a B.S. in Biology from Bucknell University and completed her post-graduate studies in Molecular Biology at the University of Connecticut Health Center before starting her career in the pharmaceutical industry. Prior to joining InSphero, Judi spent 15 years at Bristol-Myers Squibb where she led a Chemi-Genomics team aimed at identifying new drug targets using phenotypic screens. As a Senior Application Scientist at InSphero, Judi utilizes her previous experience with complex cellular models to assist scientists in industry and academia implement physiologically relevant 3D models to bridge the gap between their in vitro and preclinical models. She is currently working with InSphero scientists and industry experts to develop multicellular 3D models for a variety of Toxicology and Drug Discovery Applications.

Abstract: High attrition rates continue to be a major hurdle for the pharmaceutical industry, despite the recent focus on targeted drug treatments and personalized medicine. One potential opportunity for improving preclinical-clinical translation is the utilization of more predictive in vitro and ex vivo models. However, modelling complex biology for more physiologically relevant assays remains a significant challenge across all fields of biological research. Three-dimensional (3D) in vitro cellular models are a step towards these more predictive and relevant culture systems. This presentation will highlight the application of 3D cell models to real-world workflows as well as some of the advantages and pitfalls of incorporating these models into your research. Topics to be covered include an overview of 3D platforms currently in use and the challenges of 3D assay development with an emphasis on deploying complex 3D models such as patient-derived tumour organoids, primary cell-based systems,

co-cultures, and higher order biological systems such as Organ-on-chip. Issues regarding compound diffusion and the efficacy of reagents and dyes designed for traditional monolayer culture models will also be addressed. Finally, a focus on 3D compatible endpoints including high content image-based approaches and analysis techniques required for single cell quantification will be discussed.

Yubin Zhou, Associate Professor
Translational Cancer Research, Institute of Biosciences and Technology,
Texas A&M Health Science Center

Remote Control of Inter-Organellar Communication in Single Cells with Optogenetic Tools

Dr Yubin Zhou is currently an associate professor in the Center for Translational Cancer Research, Institute of Biosciences and Technology at Texas A&M University. Dr. Yubin Zhou received his medical training from Zhejiang University 0School of Medicine during 1998 to 2003, obtained his Ph.D. in Chemistry from Georgia State University in 2008, and did his postdoctoral work at Harvard Medical School and La Jolla Institute for Allergy and Immunology (2008-2012). Dr. Zhou was the recipient of the Leukemia & Lymphoma Society Fellow Award and Special Fellow Award, the American Cancer Soiciety Research Scholar Award, and the Texas A&M Reseach Excellence Award. He serves as an Associate Editor for Current Molecular Medicine and sits in the editorial board of Molecular and Cellular Biology, Scientific Reports, and Frontiers in Molecular Biosciences. Dr. Zhou's research interests focus on elucidating the role of calcium signaling and epigenetic modifications in health and disease, and developing novel anti-cancer therapeutics and optogenetic platforms for disease intervention. He holds four patents and has published over 90 peer-reviewed journal articles, reviews and book chapters. Dr. Zhou's recent work in calcium channels, optogenetics and immunoengineering was highlighted by multiple media outlets. His ongoing research is supported by multiple grants from NIH, CPRIT, the Welch Foundation, the John S. Dunn Foundation, and the American Cancer Society.

Abstract: Membrane contact sites (MCSs) are specialized subcellular compartments formed by closely apposed membranes from two organelles, with a gap distance ranging from 10 nm to 35 nm without membrane fusion. MCSs are typically maintained through dynamic protein-protein and protein-lipid interactions. These contact sites constitute important intracellular signaling hubs to mediate a variety of cell physiological functions, including calcium homeostasis, lipid metabolism, membrane biogenesis and organelle remodeling. Over the recent years, a series of genetically-encoded probes and chemogenetic or optogenetic actuators have been invented to aid the visualization and interrogation of MCSs in both fixed and living cells. We present herein the development of optogenetic tools for inducible assembly of ER-PM contact sites, as well as remote control of inter-organellar communication (e.g., ER-mitochondria and ER-endosome tethering) in single cells.

Selected Abstracts

(In alphabetical order)

Fatima Merchant, Associate Professor
University of Houston

3D Modeling of Chromosomes Territories in Normal and Aneuploid Nuclei

FATIMAA.MERCHANT (M'97 SM'07) received the B.E. degree in biomedical engineering from the University of Mumbai, India, in 1989, and the M.S. and Ph.D. degrees in biomedical engineering from The University of Texas at Austin. She is currently an Associate Professor with the Department of Engineering Technology, University of Houston, and holds joint appointments with the Departments of Electrical and Computer Engineering, and Computer Science and Biomedical Engineering. She has co-edited a book on Microscope Image Analysis, and authored numerous papers in the area of imaging, and tissue engineering. She directs research at the Computational Biology and Medicine Laboratory. Her current research focuses on multidimensional imaging and image analysis, and tissue engineering. She is on the Editorial Board of the journal Computers in Biology and Medicine, and Associate Editor of Biomedical Engineering Online.

Abstract: Aneuploid chromosomes (gain or loss of chromosomes) are a frequently observed trait in cancers. One of the prevailing method for detecting chromosomal abnormalities such as an increase or decrease in the number of chromosomes is using chromosome specific DNA probes via multi-color fluorescence in situ hybridization (FISH). Previous studies have investigated the characteristics of positioning of genomic components in terms of (1) radial positioning that derives from a ratio between the center and border of the nucleus, and (2) relative positioning with respect to other chromosomes, or to landmarks such as the nuclear envelope or nucleolus. Importantly, the lack of incontrovertible structural landmarks in nuclei, makes it impossible to define absolute genomic region positioning, and, most studies present probability distribution maps for the spatial organization of genomes.

We present a new approach based on spherical harmonics (SPHARM) to model entire chromosome territories from FISH samples. We test the hypothesis that alterations in CT position are associated with chromosomal aneuploidy by visualizing CTs via 3D FISH with whole chromosome panting (WCP), and reconstructing the CTs and nuclei surface in 3D using SPHARM, which provides a detailed description of shapes. Modeling was assessed using the human embryonic (hES) cell line WA09 that acquires an extra copy of chromosome 12 in culture with increasing passages. Both diploid and aneuploid nuclei are analyzed to quantitate the differences in the localization of CTs for chromosome 12 as it transitions from euploidy to aneuploidy. The CTs are detected with chromosome specific DNA probes via multi-color FISH in conjunction with confocal microscopy. SPHARM surface modeling is used to generate a well-defined 3D surface for both the nuclei and enclosed CTs, thereby allowing precise quantification of their size and shape. The estimated models are compared across multiple cells by aligning the nuclei to a well-defined shape based template followed by determining CT position with respect to a local landmark. The results present evidence of statistically significant changes in the relative positioning of CTs in trisomy-12 cells when compared to diploid cells from the same population. The computational framework presented can be used to compare the spatial distribution of CTs across multiple nuclei from a given population and/or between populations. Our data also

suggests that computational modeling of chromosome territories may be useful in distinguishing normal and tumor cells.

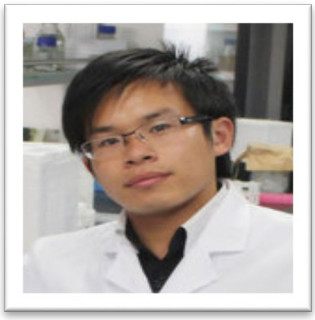
Lindsey Sablatura, Graduate Student
Rice University

A 3D in vitro Platform for High-Throughput Screening of Diverse Prostate Cancer Patient-Derived Xenograft Models

Lindsey Sablatura is a senior graduate student in the BioSciences department at Rice University. In her research, she creates 3-dimensional co-culture models of the tumor microenvironment using synthetic hydrogels. She led the research efforts of an academic-industry partnership to implement in vitro tri-cultures of patient-derived xenografts, stromal fibroblasts, and endothelial cells in a microfluidics-based drug screening model.

Abstract: The prostate cancer (PCa) incidence and mortality rates for African American men are double those of any other race/ethnicity in the United States. Conversely, incidence and mortality in Asian American and Native American men are much lower than in the population as a whole. Socioeconomic and sociocultural factors are thought to incompletely explain this disparity. Sufficiently diverse pre-clinical models of PCa are necessary to elucidate how differentially distributed biological factors impact cancer progression and drug response. PCa patient-derived xenograft (PDX) models provide a means to preserve individual tumor characteristics but mouse models are low-throughput, time consuming, and expensive. PCa PDX cells are non-adherent and ultimately non-viable in standard 2D in vitro culture, necessitating a culture method that more accurately recreates the 3D tumor microenvironment (TME). Our goal is to establish a platform that supports 1) high-throughput and high-content screening, 2) the culture of clinically relevant and diverse PCa PDX cells, and 3) complex cultures with other cell types (stroma and endothelial) recapitulating the PCa TME.

We employed MIMETAS' OrganoPlate®, a high-throughput microfluidic culture platform, for the ex vivo culture of a number of PCa PDXs with diverse racial/ethnic origin. PCa PDX cells from the enzymatic digestion of fresh tissue or from frozen stocks were allowed to form multicellular clusters in suspension culture for 48 hours. Cell clusters were then suspended in hyaluronic acid-based hydrogel (HyStem®) precursor solution and seeded into the OrganoPlate®. PCa PDX cell viability over 7 days was confirmed with automated fluorescent imaging of confocal z-stacks and 3D image analysis using the Imaris software package. To enable cell-level numeric viability assessment within a reasonable timeframe, automated imaging was optimized using a Nikon A1R confocal microscope to retain suitable image quality for quantification of 140 µm z-stacks and across 96-chip plates. Immunofluorescent staining verified the presence of key phenotypic markers—human nuclear antigen (HNA), prostate-specific antigen (PSA), and androgen receptor (AR)—over the culture period. Finally, PCa PDX cultures were treated with a small panel of clinically-relevant chemotherapeutic compounds and response was monitored for comparison with known treatment histories. This platform will provide new tools for the prediction of patient response and for the elucidation of biological factors underlying prostate cancer health disparity.



Jun Xia, Postdoc
Baylor College of Medicine
Imaging Freeze-frame Proteins Identifies Cancer-Protein Functions and Protein Networks of Endogenous DNA Damage

Jun Xia received his PhD from the Integrative Program in Molecular and Biomedical Sciences from Baylor College of Medicine in 2017 under the supervision of Dr. Susan Rosenberg. He is an unconventional cell biologist and geneticist that combines microbial and human genetics, genomics, high-throughput imaging with a special research interest on the origins of endogenous DNA damage in cancer.

Abstract: DNA damage provokes mutations and cancer, and results from external carcinogens or endogenous cellular processes. Yet, the intrinsic instigators of endogenous DNA damage are poorly understood. Here we identify proteins that promote endogenous DNA damage when overproduced: the DNA “damage-up” proteins (DDPs). We discover a large network of DDPs in Escherichia coli and deconvolute them into six function clusters, demonstrating DDP mechanisms in three: reactive-oxygen increase by transmembrane transporters, chromosome loss by replisome binding, and replication stalling by transcription factors. Their 284 human homologs are over-represented among known cancer drivers, and their RNAs in tumors predict heavy mutagenesis and poor prognosis. Half of tested human homologs promote DNA damage and mutation when overproduced in human cells, with DNA-damage-elevating mechanisms like those in E. coli. Together, our work identifies networks of DDPs that provoke endogenous DNA damage and may reveal DNA-damage-associated functions of many human known and newly implicated cancer-promoting proteins (Xia et al., 2019 Cell).

First Name	Last Name	Institution	Title	Poster #
Nicholas	Albrecht	BCM	STORM Imaging Methods for Characterizing Retinal Synapses	1
Candler Paige	AND Andi Wangshou	UT Dallas	A Cell-Type Specific Interactome to Identify Novel Targets for Cancer Pain Therapeutics	9
Jessica	Di Re	UTMB	Role of the Glycogen Synthase Kinase 3 Pathway in the Pathophysiology of Schizophrenia	2
Chenlin	Hu	UH	Application of advanced imaging flow cytometry to at single-cell level characterize the mode of cellular death in human fungal pathogen *Candida glabrata*	3
Frank (Lei)	Huang	HMRI	Deep Learning Automated Analysis of High-Content Screening Images of Epithelial-Mesenchymal Transition	4
Ji	Jing	IBT	Photoswitchable visualization and interrogation of inter-organellar membrane contact sites	5
Rahul	Kadakia	UT Austin	Bimodal 19F Fluorescent MR Probes	6
Demetrio	Labate	UH	Automated Sorting of Neuronal Trees in Fluorescent Images of Neuronal Networks using NeuroTreeTracer	7
Jose Felix	Moruno Manchon	UTHSC	The G-quadruplex DNA Stabilizing Drug Pyridostatin Promotes DNA Damage and Downregulates Transcription of Brca1 in Neurons	8
Reid	Powell	IBT	Multiplexed analysis of patient-derived xenograft models aimed at elucidating mechanisms of resistance for triple-negative breast cancer	15
Pankaj	Singh	IBT	Spatial Organization and Molecular Association of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images	14
Adam	Szafran	BCM	Intercellular Pro-survival Pathway Heterogeneity in Bladder Cancer Cells is Predictive of Therapeutic Resistance in *In Vitro* Cultures	10

Tomasz	Zal	MDACC	Relating Individual T Cell Motility to Local Oxygen Partial Pressures in Solid and Hematological Cancers by FaST-PLIM	11
Ana Maria	Zaske	UTHSC	Sensitivity of Detection by Biological AFM for Gold-Labeled Liposomes on Human Coronary Arterial Endothelial Cell Membranes	12
Ying	Zhu	MHMRI	An Image Informatics Pipeline for Imaging Mass Cytometry and its Application to Characterize and Visualize the Immune Landscape in Epithelial Ovarian Cancer Patients with Long-Term and Short-Term Survival Periods	13

Poster #1

STORM Imaging Methods for Characterizing Retinal Synapses

Nicholas E. Albrecht[1,2,3], Carl G. Ebeling[4], Robert Hobson[4], and Melanie A. Samuel[1,2,3]
[1]Department of Neuroscience, Baylor College of Medicine, Houston, TX
[2]Huffington Center on Aging, Baylor College of Medicine, Houston, TX
[3]Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX
[4]Bruker Nano Surfaces Division, Salt Lake City, UT 84108

Objectives:
Proper neural function depends on the correct arrangement and development of synapses, the primary site of communication between neurons. However, the molecular mechanisms that underlie synapse formation and specificity are resolved to a limited degree in part because the internal landscape of synapse protein arrangement remains largely unknown. Two challenges have limited progress towards these goals: 1) synapses are beyond the diffraction limit of light, limiting our ability to resolve them, and 2) nanoscopic approaches to visualize synapses are often time-consuming and limited in their ability to see multiple proteins. To overcome these limitations, we present a rapid and optimized application of Stochastic Optical Reconstruction Microscopy (STORM) for neural tissue that allows us to quickly screen for synaptic protein content and structure from relevant *in vivo* samples.

Methods:
In our workflow, we have tested the histological preparation of murine retina and optimized it for a) the speed of processing, b) final image clarity, and c) the ability to accurately resolve synaptic structures. Towards this end, we have tested the timing and concentration of basic tissue fixatives, the inclusion of quenching methods post-fixation, adjustments to the working buffers used in preparing the tissue, and rigorous testing of the antibody staining process.

Results:
With this workflow, we have successfully prepared tissue for STORM imaging using optimized cryosectioning techniques. We have subsequently imaged synapses and neurons at up to 10µm of depth and have achieved resolutions in tissue of 20-40 nanometers. Our final protocol gives us a workflow that takes 48 hours from mouse to image, allowing us to rapidly screen and test synaptic proteins.

Conclusions:
This rapid approach enables high-throughput super-resolution imaging and screening of neural tissue for the discovery of nanoscale synaptic structures. This represents a vast improvement over more traditional preparations that can take up to a week prior to imaging and allows us to image multiple proteins per sample at a high resolution, all in a relevant biological context. We are now applying these methods to synapse development and disease to uncover the molecular regulators of synapse decline.

Funding Sources:
This work was supported by the NIH (R00AG044444 and DP2EY02798 to M.A.S), the Ted Nash Long Life Foundation, and the Brain Research Foundation. N.E.A is supported by the NIH and the National Institute of General Medical Sciences under award T32GM088129.

Corresponding author: Nicholas E. Albrecht, Department of Neuroscience, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX, E-mail: nalbrech@bcm.edu

Poster #2

Role of the Glycogen Synthase Kinase 3 Pathway in the Pathophysiology of Schizophrenia

Jessica Di Re[1,2], Jim Hsu[2,3,4], Laura Stertz[5], Kamil Khanipov[2], Gonzalo Botello-Lins[6], Yuriy Fofanov[2], Henriette Raventos[7], Consuelo Walss-Bass[5] and Fernanda Laezza[2]
1 Neuroscience Graduate Program, University of Texas Medical Branch, Galveston, TX, USA
2 Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA
3. Biochemistry and Molecular Biology Graduate Program, University of Texas Medical Branch, Galveston, TX USA.
4. MD/PhD Program, University of Texas Medical Branch, Galveston, TX, USA
5. Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, TX, USA.
6. Clear Falls ISD Biotechnology Practicum
7. Department of Cellular and Molecular Biology, University of Costa Rica, San Jose, Costa Rica

Corresponding Author: Fernanda Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX, Email: felaezza@utmb.edu

The mechanisms underlying schizophrenia (SZ), one of the most severe and debilitating mental health disorders, are not well understood. Studies and clinical evidence suggest that multiple environmental and genomic risk factors contribute to the risk of developing SZ. As such, preclinical animal models do not fully recapitulate the complexity of the disease and can be only used to characterize specific endophenotypes associated with disease presentation. Integrative translational approaches that include *in vitro* models and fine-tuned human genetic studies are therefore necessary to elucidate the contribution of these genomic risk factors to endophenotypes of SZ. Emerging evidence indicates that dysregulation of the protein kinase B (AKT)/glycogen synthase kinase 3β (GSK3β) pathway is a risk factor for SZ. As such, there is a need to understand the molecular targets of this pathway that directly affect neuronal excitability. We have previously shown that GSK3β regulates the complex assembly and protein:protein interactions (PPI) within the voltage-gated sodium (NaV) channel complex at the axon initial segment (AIS), the molecular determinant of neuronal excitability. Based on this premise we hypothesized that dysfunction of the GSK3/AKT pathway could lead to disruption of PPI of the AIS and excitability that could recapitulate molecular endophenotypes of SZ. Using the split-luciferase in-cell assay we have reconstituted the PPI complex between neurofascin, an important AIS cell adhesion molecule, and voltage-gated sodium (Nav) channels and found that this interaction is increased by increasing the level of active GSK3. By integrating genomic and functional studies neurons differentiated from induced pluripotent stem cells (iPSCs) from a small, homogeneous population with SZ we have also found a decrease in the mRNA level of GSK3β in SZ patients ($p<.05$, $n=11$, T-test with Welch's Corrections) compared to controls. We also identified a missense mutation in the NFASC protein associated with the disease in a combined cohort including patients from the NIMH Human Brain Collection Core ($p<.01$, $n=424$, 1-sample test of proportions). We are currently evaluating whether changes associated with SZ and GSK3β distribution and intensity of neurofascin and Nav channels could be identified in neurons derived from patient iPSCs compared to unaffected relatives, which may underlie changes in intrinsic excitability that have previously been linked to SZ. Overall, these studies might help elucidate new endophenotypes associated with SZ due to a dysregulation in the GSK3 pathway that could lead to a biological based classification of the disease and future targeted therapeutics.

This research was funded by NIMH R01 MH095995, UT System BRAIN Initiative and UTMB Jeane B. Kempner Predoctoral Fellowship.

Poster #3

Application of advanced imaging flow cytometry to at single-cell level characterize the mode of cellular death in human fungal pathogen *Candida glabrata*

Chenlin Hu[1], Dimitrios P. Kontoyiannis[2], Nicholas D. Beyda 1, 3*

[1]College of Pharmacy, University of Houston, Houston, Texas
[2] Department of Infectious Diseases Infection Control and Employee Health, University of Texas MD Anderson Cancer Center, Houston, Texas
[3]Baylor St. Luke's Medical Center, Houston, Texas
*Corresponding author: Nicholas D. Beyda (ndbeyda@uh.edu)

Objectives: *Candida glabrata* is a leading cause of fungal infections in hospitalized patients. Echinocandins are the first line treatment for *C. glabrata* and work by inhibiting synthesis of β-glucan, an integral cell wall component. While their mechanism of action is well described, physiological mechanisms of echinocandin-induced cell death in *C. glabrata* remain uncharacterized but are important for guiding new strategies to enhance their activity. The objective of this study was to characterize caspofungin-induced cell death pathways in *C. glabrata*.

Methods: A pair of clinical bloodstream isolates of *C. glabrata* consisting of an index-wildtype (WT) and persistent-FKS mutant (S663P) were chosen for experimental materials. *C. glabrata* isolates were grown for 24 h in a shaking incubator at 37 °C in Erlenmeyer flasks containing RPMI growth medium alone or in the presence of caspofungin. The WT and FKS strains were exposed to sub-MIC of caspofungin at 0.008 and 16 μg/mL, respectively. Cellular necrosis and programmed cell death (apoptosis) was determined simultaneously at the single-cell level utilizing a dual staining assay of propidium iodide and Annexin V-FITC in combination with imaging flow cytometry.

Results: Two different cell death pathways (apoptosis and necrosis) co-occurred in both WT and FKS strains of *C. glabrata* when grown in the presence of inhibitory concentrations of caspofungin. Specifically, in caspofungin-treated WT culture, the viable, early apoptotic, late apoptotic/necrotic, and indirect necrotic cells accounted for 72%, 4%, 11%, and 13%, respectively, and in capofungin-treated FKS culture, the viable, early apoptotic, late apoptotic/necrotic, and direct necrotic cells accounted for 68%, 8%, 11%, and 9%, respectively. In both cultures, cell survival was correlated with an increase in cell biovolume. Moreover, variability in the predominant mechanism of cell death was observed between sub-populations of large and small cells, with early apoptosis occurring more frequently in the large cell population. Differences were also observed between mother cells and their attached buds. Mother cells were more likely to be viable when compared to buds, viability rates were higher among mother cells compared to buds, and a higher proportion of early apoptotic cells were also observed among these nonviable mother cells.

Conclusions: Imaging flow cytometry is a useful tool to study the mode of cell death in yeast pathogen. Both necrosis and apoptosis occurred in *C. glabrata* when exposed to inhibitory concentrations of caspofungin. The high degree of heterogeneity in cell death mechanisms was observed to be associated with cell size and cell-cycle stage. This study provides new insights into caspofungin-induced cell death pathways in *C. glabrata.* Further investigation into the mechanism by which *C. glabrata* undergoes apoptosis is warranted to identify strategies which could enhance echinocandin activity.

Acknowledgement: This work was supported in part by an investigator-initiated grant from Astellas Inc. to NDB.

Poster #4

Deep Learning Automated Analysis of High-Content Screening Images of Epithelial-Mesenchymal Transition

Lei Huang[1*], Yunjie He[1*], Xin Wang[1], Tian Cheng He[1], Vivek Mittal[2], Ding Cheng Gao[2], and Stephen T. C. Wong[1,#]

1. Department of Systems Medicine and Bioengineering, Houston Methodist Cancer Center and Houston Methodist Research Institute, Weill Cornell Medcine, Houston, TX
2. Department of Cardiothoracic Surgery, Department of Cell and Developmental Biology, and Neuberger Berman Lung Cancer Center, Weill Cornell Medicine, New York, New York, USA.

Epithelial-mesenchymal transition (EMT) tumor cells exhibit survival advantages under chemotherapeutic stress and significantly contributed to the formation of chemoresistant cancer metastasis. Development of chemoresistance remains the major therapeutic barrier in the treatment of metastatic breast cancer. To identify more efficient therapeutic regiments for chemoresistant breast tumor, we have performed an EMT-cell image-based high-content screening (HCS) of thousands of drugs or bioactive compounds to identify effective agents specifically targeting EMT tumor cells. Cost-effective analysis of large amounts of image-based phenotypic screening data always pose significant challenges however. To fill the gap, we have adopted artificial intelligence (AI) strategies in analyzing HCS data. In specific, we transformed deep learning methods, including Inception-v3, Inception-v4 and Capsule Network for automatic analysis of 99,480 HCS images. Conventional ways for quantitative analysis of HCS based image data requires extensive image preprocessing and feature extraction before applying statistical or machine learning analysis. This study demonstrates the capability of AI deep learning algorithms to automatically characterize and classify large-scale HCS based EMT images with high accuracy and cost-efficiently. In addition, we were able to rank drug candidates for targeting breast cancer EMT cells enabled by applying non-parametric analysis on the image features that extracted by the deep learning classifiers. Experimentally testing all the drugs belong to the effective groups is expensive, we thus propose to rank the effective drugs using the probability obtained from the capsule network model. As shown in the appendix, we demonstrated those top 5-ranked drug candidates for targeting breast cancer EMT cells. The techniques demonstrated in this study has the potential to quickly provide insights for the screening hits for in-depth functional studies and preclinical studies.

Co-first authors: Lei Huang, Ph.D. and Yunjie He, M.Sc.

Corresponding authors: Stephen T.C. Wong, Ph.D., P.E, 6670 Bertner Ave, R6-211, Department of Systems Medicine and Bioengineering, Houston Methodist Cancer Center and Houston Methodist Research Institute, Houston, TX, 77030. Email:stwong@houstonmethodist.org

Acknowledgements:

The research is supported by NCI U01CA188388, W81XWH-17-1-0146, W81XWH-16-1-0038, DOD OC160042, John S Dunn Research Foundation and TT & WF Chao Foundation Endowment Fund

Appendix:

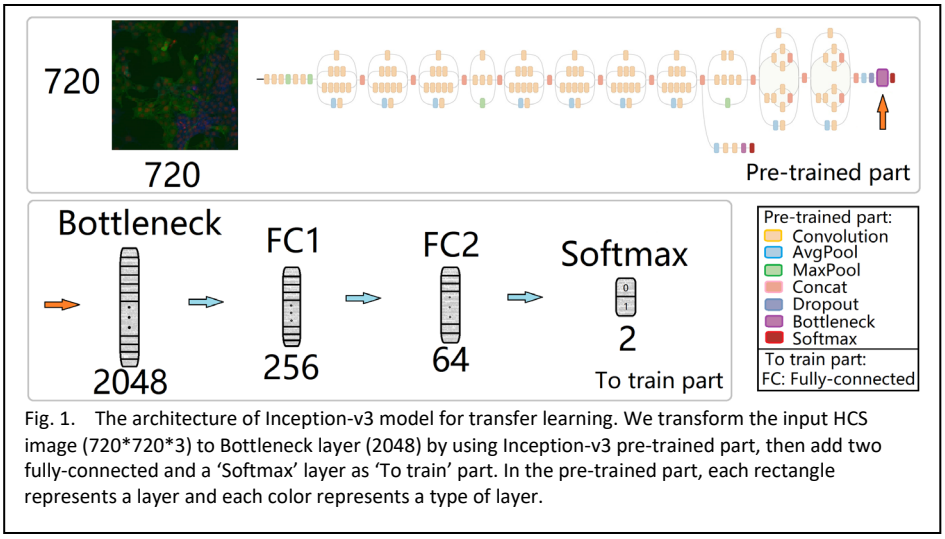


Fig. 1. The architecture of Inception-v3 model for transfer learning. We transform the input HCS image (720*720*3) to Bottleneck layer (2048) by using Inception-v3 pre-trained part, then add two fully-connected and a 'Softmax' layer as 'To train' part. In the pre-trained part, each rectangle represents a layer and each color represents a type of layer.

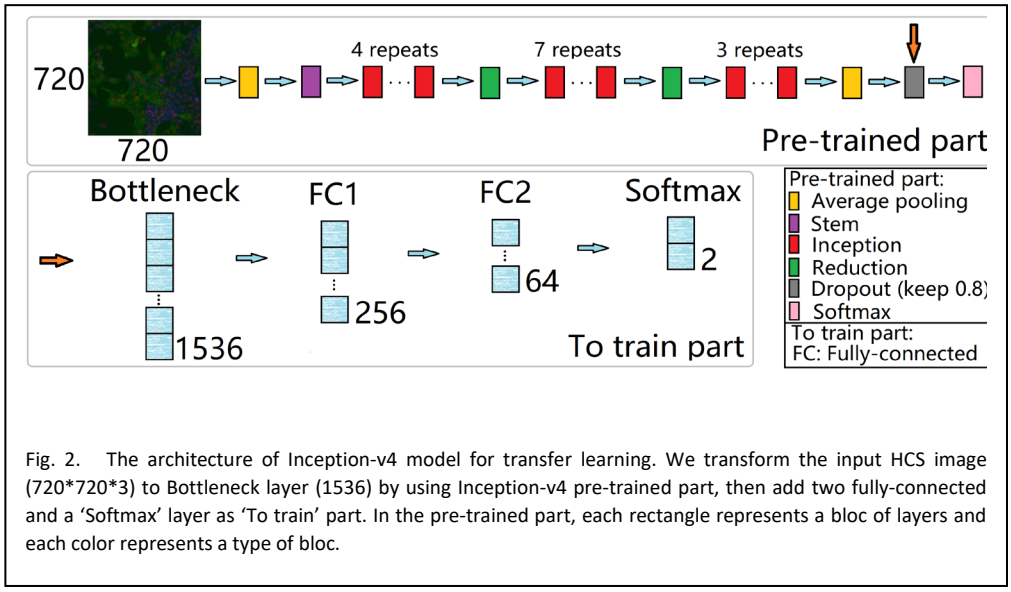


Fig. 2. The architecture of Inception-v4 model for transfer learning. We transform the input HCS image (720*720*3) to Bottleneck layer (1536) by using Inception-v4 pre-trained part, then add two fully-connected and a 'Softmax' layer as 'To train' part. In the pre-trained part, each rectangle represents a bloc of layers and each color represents a type of bloc.

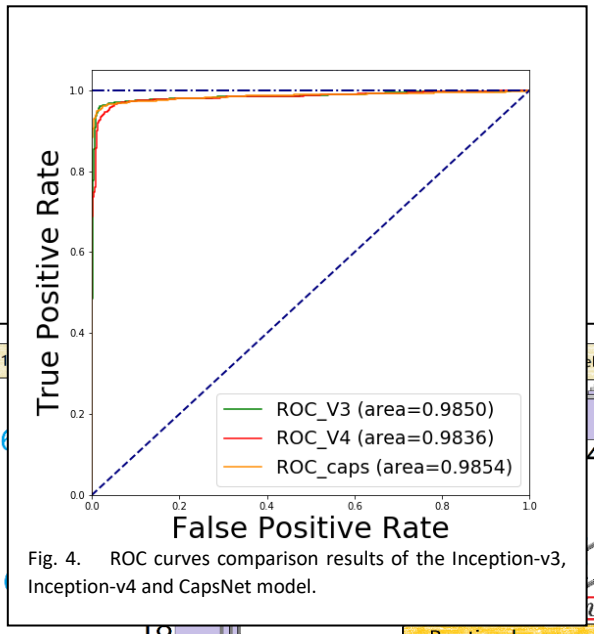


Fig. 4. ROC curves comparison results of the Inception-v3, Inception-v4 and CapsNet model.

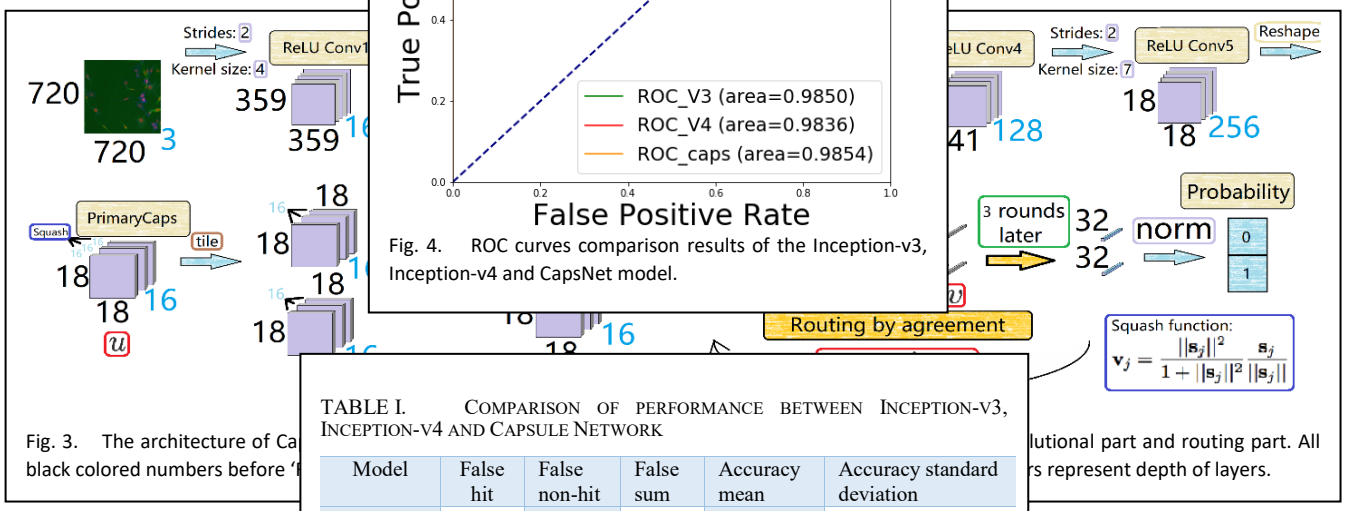


Fig. 3. The architecture of Ca lutional part and routing part. All black colored numbers before ' rs represent depth of layers.

TABLE I. COMPARISON OF PERFORMANCE BETWEEN INCEPTION-V3, INCEPTION-V4 AND CAPSULE NETWORK

Model	False hit	False non-hit	False sum	Accuracy mean	Accuracy standard deviation
Inception-v3	17	43	60	0.96	0.01745
Inception-v4	56	33	89	0.9453	0.02060
CapsNet	10	65	75	0.9635	0.01453

Drug name	IC50	Efficiency	Class	Rank
Emetine dihydrochloride hydrate	16-80nM	100%	Apoptosis	1
Idarubicin	16-80nM	100%	DNA Metabolism	2
PD-166285 hydrate	200nM	100%	Kinase/Phosphatase	3
Thapsigargin	0.4-2uM	100%	Intracellular Calcium	4
PD-161570	1uM	90%	Tyrosine kinase	5

Fig. 5. Top 5-ranked drugs for breast cancer EMT cells.

Poster #5

Photoswitchable visualization and interrogation of inter-organellar membrane contact sites

Ji Jing[1, #], Lian He[1, #], Guolin Ma[1], Nhung T. Nguyen[1], Yun Huang[1], and Yubin Zhou[1, 2, *]

[1] Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M University, Houston, TX 77030, USA

[2] Department of Medical Physiology, College of Medicine, Texas A&M University, Temple, Texas 76504, USA.

*E-mail: yzhou@ibt.tamhsc.edu; [#] These authors contributed equally

Proper localization of organelles to the right place at the right time is critical for its functions. One way to facilitate correct organelle localization and organelle communications is through membrane contact sites (MCSs), areas of interorganellar tethering (usually 10-40 nm distance) that are bridged by protein/lipid complexes without membrane fusion. MCS plays critical roles in controlling lipid metabolism, calcium homeostasis, and cell signaling. Although fluorescence microscopy and electron microscopy are two most powerful techniques currently available to study MCSs, systematic molecular dissection of these specialized subcellular compartments remains challenging due to the lack of appropriate methods and convenient tools to enable rapid and reversible control of MCSs assembly and remodeling with high spatiotemporal resolution without disrupting the integrity of MCSs in living cells. Here, we extended optogenetic engineering approaches to study MCSs. We created a set of novel optogenetic tools (LiMETER or OptoPBer) to label and manipulate MCSs in situ at real time with high precision while minimizing perturbations to the host physiology. Our tools can be used as scaffolds for grafting lipid-binding domains to dissect molecular determinants that govern protein-phospholipids interactions. In addition, photostimulation can be applied to reversibly control the tethering between two different organellar membranes. These optogenetic tools open new opportunities for i) dynamically examining lipid-protein interactions with a simple grafting approach, ii) delivering proteins of interest to this specialized structure, iii) manipulating signaling at MCSs between different organelles, iv) screening protein and chemical modulators that are involved in maintaining and remodeling MCSs, and v) reversibly photo-tuning the gap distances at nanometer scales between two organellar membranes at MCSs. Our study represents a solid step forward towards the goal of mechanistically dissecting the elusive MCSs in situ with tailored function. The similar optogenetic engineering approach can be further extended to control of organellar localization and interorganellar communications (ER-mitochondria or ER-endosome/lysosome MCSs) that are crucial for cell signaling.

Poster #6

Bimodal ^{19}F Fluorescent MR Probes for Detecting Cellular Hypoxia

Rahul T. Kadakia, Da Xie, Daniel Martinez, Meng Yu, Emily L. Que*

Department of Chemistry, UT Austin, 105 E 24th St Stop A5300, Austin, TX 78712

Magnetic resonance imaging (MRI) is a well-established imaging modality that has been used for *in vivo* human diagnostics for over 30 years. ^{19}F MRI, as an emerging technique, allows non-invasive imaging of whole organisms with negligible background signal. In a biological context, there is minimal endogenous fluorine MR signal in the body, since all fluorine is present in solid ionic form in bones and teeth. This makes the modality especially promising for sensing applications as all the observed signal will come from exogenous imaging agents. Paramagnetic Cu^{2+} can be used to modulate the NMR relaxation rates of interacting fluorines via Paramagnetic Relaxation Enhancement (PRE). Moreover, this metal is known to quench the fluorescence signal due to electron transfer from the excited fluorophore to the metal center. My research focuses on exploiting both metal redox chemistry and coordination changes to design ^{19}F MR-based sensors for detecting cellular hypoxia. The general design relies on a switch from a paramagnetic ("off") state to a diamagnetic ("on") state, increasing the ^{19}F MR signal, and allowing for hotspot imaging of oxygen deficient cells. Moreover, appending a fluorophore to the imaging agent allows us to determine intracellular localization of the bimodal probe via fluorescence. The quenched initial fluorescence increases upon reduction of the probe in hypoxic environments. Studies have demonstrated a fourteen-fold increase in fluorescence in DMF, a two-fold increase in aqueous buffer, and a thirty-seven-fold MRI SNR increase upon reduction. Moreover, we are also able to see a sharp ^{19}F NMR peak in hypoxic HeLa ovarian cancer cells, whereas there is minimal ^{19}F signal in normoxic cells. Therefore, the one electron reduction of Cu^{2+} can be used to tune the fluorescence and ^{19}F NMR/MRI signal for potential *in vivo* applications.

This work was supported by start-up funds from the University of Texas at Austin and the Welch Foundation (F-1883).

Poster #7

Automated Sorting of Neuronal Trees in Fluorescent Images of Neuronal Networks using NeuroTreeTracer

Kayasandik C[1], Papadakis M[1], Laezza F[2], Labate D[1]

1. Department of Mathematics, University of Houston
2. Department of Pharmacology and Toxicology, University of Texas Medical Branch

Corresponding author: Demetrio Labate, Department of Mathematics, University of Houston, 651 Phillip G Hoffman, Houston, TX 77204-3008, USA, E-mail: dlabate@math.uh.edu.

Objectives: Fluorescence confocal microscopy has become increasingly more important in neuroscience due to its applications in image-based screening and profiling of neurons. Multispectral confocal imaging is useful to simultaneously probe for distribution of multiple analytes over networks of neurons. However, current automated image analysis algorithms are not designed to extract single-neuron arbors in images where neurons are not separated, hampering the ability map fluorescence signals at the single cell level. To overcome this limitation, we introduce a novel image processing framework, called NeuroTreeTracer, aimed at automatically extracting and sorting single-neuron traces in fluorescent images of multicellular neuronal networks.

Methods: NeuroTreeTracer includes a SVM-based image segmentation routine and applies directional multiscale filters for automated soma detection. This image processing pipeline also includes a novel tracing routine that has the ability to separate neuronal trees in the image by resolving network connectivity even when neurites appear to intersect. As a result, the software outputs individually labeled neuronal trees and makes it possible to measure local morphological properties as well as local fluorescent intensity expression for each neuron relative to a local reference system, e.g., the soma location of the neuron.

Results: We demonstrate the features of NeuroTreeTracer by tracing representative confocal images of neuronal cultures containing multiple neurons. The software automatically separates individual neuronal traces in images where neurons are not separated and their neurites may appear to overlap. We apply the output of NeuroTreeTracer to automatically quantify the spatial distribution of analytes of interest in the subcellular compartments of each individual neuron and generate features for cell classification. This software is released open-source and freely available.

Conclusions: Our new image processing pipeline aims at addressing current limitations of single-neuron image processing software that are unable to separate individual neuron arborizations when cells are not separated. This automated platform will facilitate applications in neuron screening and profiling.

Funding: NSF DMS 1720487 (DL), NSF DMS 1720487 (DL,MP), GEAR 113491 (DL); R01MH095995 (FL); R01MH111107 (FL).

Poster #8

The G-quadruplex DNA Stabilizing Drug Pyridostatin Promotes DNA Damage and Downregulates Transcription of Brca1 in Neurons

Jose F. Moruno-Manchon[1], Edward C. Koellhoffer[2], Shashank Hambarde[3], Nayun Kim[3], Louise D. McCullough[2] and Andrey S. Tsvetkov[1,4]

[1]Department of Neurobiology and Anatomy, the University of Texas McGovern Medical School at Houston, TX
[2]Department of Neurology, the University of Texas McGovern Medical School at Houston, TX
[3]Department of Microbiology and Molecular Genetics, the University of Texas McGovern Medical School at Houston, TX
[4]The University of Texas Graduate School of Biomedical Sciences, Houston, TX

Abstract

The G-quadruplex is a non-canonical DNA secondary structure formed by four DNA strands containing multiple runs of guanines. The G-quadruplexes play an important role in DNA recombination, replication, telomere maintenance, and regulation of transcription. Small molecules that stabilize the G-quadruplexes alter gene expression in cancer cells. Here, we hypothesized that the G-quadruplexes regulate transcription in neurons. We discovered that pyridostatin, a small molecule that specifically stabilizes G-quadruplex DNA complexes, induced neurotoxicity and promoted the formation of DNA double–strand breaks (DSBs) in cultured neurons. We also found that pyridostatin downregulated transcription of the *Brca1* gene, a gene that is critical for DSB repair. Importantly, in an *in vitro* gel shift assay, we discovered that an antibody specific to the G-quadruplex structure binds to a synthetic oligonucleotide, which corresponds to the first putative G-quadruplex in the *Brca1* gene promoter. Together, our results suggest that the G-quadruplex complexes may regulate transcription in neurons. Studying the G-quadruplexes could represent a new avenue for neurodegeneration and brain aging research.

Correspondence to: Andrey S. Tsvetkov, Department of Neurobiology and Anatomy, University of Texas McGovern Medical School at Houston, 6431 Fannin Street, Houston, TX, E-mail: andrey.s.tsvetkov@uth.tmc.edu

Poster #15

Multiplexed analysis of patient-derived xenograft models aimed at elucidating mechanisms of resistance for triple-negative breast cancer

Reid Powell[1], Abena Redwood[2], Shirong Cai[2], Yizheng Tu[2], Yan Jiang[2], Xinhui Zhou[2], Lie Guo[2], Clifford Stephan[1], Jeffery Chang[3], Helen Piwnica-Worms[2], W. Fraser Symmons[4], and Peter Davies[1]

1) *Center for translational Cancer Research, Texas A&M University, Houston, TX*
2) *Department of Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX*
3) *Department of Integrative Biology and Pharmacology, UT Health Science Center, Houston, TX*
4) *Department of Pathology, MD Anderson Cancer Center, Houston, TX*

Triple-negative breast cancer (TNBC) makes up approximately 10–20% of all breast cancers and is characterized by a lack of targeted therapeutic options accompanied with frequent development of resistance to front-line chemotherapies. Likewise, there is a concerted two-fold effort to understand critical drivers of this disease in addition to the mechanistic underpinnings of resistance. To address this, our collaborative network of clinicians and basic researchers have established a pipeline to generate patient-derived xenograft (PDX) mouse models that are longitudinally sampled over the development of chemoresistance. Through this technique, a relatively small tumor biopsy is expanded *in vivo* and can serve as starting material to perform multiplexed analyses. These include chemical profiling of PDX cell lines against a library of 638 mechanistically annotated and FDA approved therapeutic agents, in addition to collecting genomic and transcriptomic information. At present, this platform has been used to test relatively few samples but has begun to show consolidated mechanistic profiles of targets associated with TNBC. Additionally, it has demonstrated a robust ability to describe how the reliance of these targets vary at a personalized level over the course of developing chemoresistance. Likewise, clonal analysis derived from genomic sequencing is beginning to show alterations in clonal architecture of the tumor and transcriptomics are revealing similar changes in molecular signaling, which can collectively account for differential activity observed in the screening data. Ultimately, we hope these high-dimensional analyses will provide a translationally relevant data set in which machine learning or other techniques may be employed to further elucidate the mechanisms of chemoresistance and provide insights into rational drug combinations used for the management of patients who have developed chemoresistance.

The above stated research was conducted using the following funds: CPRIT RP160710 awarded to Dr. W. Fraser Symmons; CPRIT RP110532 and RP150578 awarded to Dr. Peter Davies; The Susan G. Koman (R) Organization PDF16377811 awarded to Dr. Aben Redwood; and Institutional funding from MD Anderson Breast Cancer Moon Shot

Poster #14

Spatial Organization and Molecular Association of Tumor-Infiltrating Lymphocytes Using Deep Learning on Pathology Images

Pankaj Singh,[1] Joel Saltz,[3] Rajarsi Gupta,[3,4] Le Hou,[2] Tahsin Kurc,[1] Vu Nguyen,[2] Dimitris Samaras,[2] Kenneth R. Shroyer,[4] Tianhao Zhao,[4] Rebecca Batiste,[4] John Van Arnam,[5] The Cancer Genome Atlas Research Network, Ilya Shmulevich,[6] Arvind U.K. Rao,[7, 10] Alexander J. Lazar,[8] Ashish Sharma,[9] and Vésteinn Thorsson[6]

[1]Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX; [3]Department of Biomedical Informatics, Stony Brook Medicine, Stony Brook, NY; [2]Department of Computer Science, Stony Brook University, Stony Brook, NY; [4]Department of Pathology, Stony Brook Medicine, Stony Brook, NY; [5]Department of Pathology and Laboratory Medicine, Perelman School at the University of Pennsylvania, Philadelphia, PA; [6]Institute for Systems Biology, Seattle, WA; [7] Department of Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, MI; [8]Departments of Pathology, Genomic Medicine, and Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX; [9]Department of Biomedical Informatics, Emory University, Atlanta, GA; [10]Department of Radiation Oncology, University of Michigan, Ann Arbor, MI.

Beyond sample curation and basic pathologic characterization, the digitized H&E-stained images of TCGA samples remain underutilized. To highlight this resource, we present mappings of tumor-infiltrating lymphocytes (TILs) based on H&E images from 13 TCGA tumor types. Using a convolutional neural network (CNN), we classify fixed size patches of the images. These TIL maps are obtained as the tiles of patches classified as patches with TILs or without TILs. Next, to identify TIL patterns, we first used affinity propagation to find spatially connected and coherent clusters of TIL image patches. The resulting cluster pattern was characterized using measures for simple count and extent statistics but also by clustering indices, which assess more complex characteristics such as cluster shape. Interestingly, the various indices were significant across different tumor types. In Skin Cutaneous Melanoma (SKCM), increased Banfield Raftery-index associates with superior survival, while in Breast invasive carcinoma (BRCA) increased Ball-Hall index associates with inferior survival, both adjusted for overall TIL density.

Acknowledgments:

We are grateful to all the patients and families who contributed to this study. Funding from the Cancer Research Institute is gratefully acknowledged, as is support from National Cancer Institute (NCI) through U54 HG003273, U54 HG003067, U54 HG003079, U24 CA143799, U24 CA143835, U24 CA143840, U24 CA143843, U24 CA143845,U24 CA143848, U24 CA143858, U24 CA143866, U24 CA143867, U24 CA143882, U24 CA143883, U24 CA144025, P30 CA016672, U24CA180924, U24CA210950, U24CA215109, NCI Contract HHSN261201400007C, and Leidos Biomedical Contract 14X138. A.U.K.R. and P.S were supported by CCSG Bioinformatics Shared Resource P30 CA01667, ITCR U24 Supplement 1U24CA199461-01, a gift from Agilent technologies, CPRIT RP150578, and a Research Scholar Grant from the American Cancer Society (RSG-16-005-01). This work used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation

XSEDE Science Gateways program under grant ACI-1548562 allocation TG-ASC130023. The authors would like to thank Stony Brook Research Computing and Cyberinfrastructure and the Institute for Advanced Computational Science at Stony Brook University for access to the high-performance LIred and SeaWulf computing systems, the latter of which was supported by National Science Foundation grant (#1531492).

Poster #10

Intercellular Pro-survival Pathway Heterogeneity in Bladder Cancer Cells is Predictive of Therapeutic Resistance in *In Vitro* Cultures

Szafran, A[1]; Mancini, MG[1]; Mancini, MA[1]

[1]Department of Molecular and Cellular Biology and Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine

Corresponding Author: Adam Szafran, Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; adam.szafran@bcm.edu

Background: Significant effort has been made to generate a molecular and genetic profile of advanced stage bladder cancer, resulting in the identification of multiple clusters/types that exhibit alterations in signaling pathways frequently targeted by small molecule inhibitors in clinical use or development. Early trials using targeted therapies have shown some promising results but have not shown a significant survival benefit compared to standard therapy. A potential cause is an increased heterogeneity within the tumor, resulting in a diverse collection of cell with differential levels of sensitivity to inhibitors. However, the relationship between bladder cancer pathway heterogeneity and targeted therapy resistance is yet to be fully explored.

Material and Methods: An image-based High Content Analysis (HCA) approach was used to characterize 20 pro-survival pathway activation markers at the single cell level in nine urothelial transitional cell cancer (TCC) cell lines at both the single cell and population levels. Pathway features were then compared with the effects of small molecule inhibitors targeting pro-survival pathways determined using continuous monitoring kinetic viability assays.

Results: As expected based on previous studies, significant differences in the average level of pro-survival pathway activation was observed between TCC cell lines, however, these levels were generally lower than a reference primary non-cancerous bladder epithelial cell line. In contrast, subcellular localization of activated pathway components in 4 TCC cell lines differed significantly compared to other TCC cells and primary cells. Cell-to-cell heterogeneity did not correlate with expression or localization changes, suggesting an independent mechanism involved. When cells were treated with inhibitors targeting cell surface receptors (RTK's), PI3K-Akt, p38 MAPK, and p42 MAPK signaling pathways and examined in kinetic viability assays, heterogeneity in pathway activation marker levels and localization were more predictive of treatment resistance than the population average itself.

Conclusion: These results suggest that cell-to-cell heterogeneity may be an important contributor to resistance to targeted therapy in bladder cancer and warrants further study in *in vivo* models and patient samples

Funding: Work was funded by a pilot grant from the Partnership for Bladder Cancer Research at Baylor College of Medicine.

Poster #9

A Cell-Type Specific Interactome to Identify Novel Targets for Cancer Pain Therapeutics

Wangzhou A[1], Ray P[1], Paige CA[1], Dussor G[1], Price TJ[1]
1. Department of Neuroscience, School of Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, TX.

While pain researchers have been searching for novel drug targets, few new cancer pain therapeutics have been developed in the past decade. This is due, in part, to the fact that new targets have been slow to be identified. Here we present a novel technique for single-cell RNA sequencing data that analyzes ligand and receptor interactions between sensory neurons and other cell types. Known protein-protein interactions between cell types associated with neuropathic pain and sensory neurons are evaluated. The resulting pairs are then validated using molecular and behavioral assays. Using this technique to analyze single cell sequencing data allows for a higher resolution of cell-type specific pathways underlying the development and maintenance of pain plasticity leading to chronic pain. This resulting interactome is a new resource for analysis of genomic data from patients with chronic cancer pain. We are confident that this technique coupled with in situ single cell sequencing of DRGs from patients with cancer will be able to identify cell-type specific pathways and drug targets for cancer pain.

Poster #11

Relating Individual T Cell Motility to Local Oxygen Partial Pressures in Solid and Hematological Cancers by FaST-PLIM

Rytelewski M[1], Haryutyunan K[2], Nwajei F[1], Shanmugasundaram M[1], Wspanialy P[3], Zal MA[1], Chen CH[1], Khatib ME[4], Plunkett S[4], Vinogradov SA[4], Konopleva M[2], Zal T[1].

1. Department of Immunology, University of Texas MD Anderson Cancer Center, Houston, TX, USA
2. Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX, USA
3. School of Engineering, University of Guelph, Canada
4. Departments of Biochemistry and Biophysics and of Chemistry, University of Pennsylvania, Philadelphia, PA, USA

Corresponding author: Tomasz Zal, Department of Immunology, University of Texas MD Anderson Cancer Center, 7455 Fannin St, Houston, TX, USA, E-mail: tzal@mdanderson.org

Active migration of lymphocytes within tumors is pre-requisite to immune therapies. Both solid tumors as well as bone marrow malignancies develop regions of low oxygen partial pressures, i.e. below 5 mmHg, known as tumor hypoxia. Tumor hypoxia plays multiple roles in tumor immune suppression including tissue expression of checkpoint molecules, decreased antigen presentation and deactivation of various tumor-infiltrating lymphocytes (TILs). However, the relationship of intratumoral oxygen distribution to the motility of TILs remains undefined, largely owing to the lack of a suitable method for contextual imaging of oxygen gradients and cell dynamics in vivo.

Using phosphorescent oxygen probe, we developed a regimen of intravital 2-photon microscopy that combines TIL motility recording with oxygen imaging based on phosphorescence lifetimes. We applied this method, termed Fast Scanning Two Photon Phosphorescence Lifetime Imaging Microscopy (FaST-PLIM) to relate the dynamic behavior of T cells to the local oxygen gradients that develop inside solid lung tumors and leukemic bone marrow in pre-clinical mouse models.

We found that tumor infiltrating T-lymphocytes traversed regions of varying oxygen concentrations, including regions of hypoxia that developed within the solid tumor cores and in bone marrow with advanced-stage B-cell acute lymphocytic leukemia (B-ALL). T cell motilities were markedly decreased in hypoxic regions compared to the neighboring normoxia and many of the TILs appeared stalled. Remarkably, breathing 100% oxygen, which alleviated tumor hypoxia, rapidly increased the migratory behavior of stalled T cells. Our results reveal reinvigoration of TIL motility as a new mechanistic benefit of tumor hypoxia countermeasures in overcoming tumor immune suppression.

Funding sources: This work was supported by grants NCI R01CA155056 and CPRIT MIRA 160683 to TZ and MK, and grants EB018464 and NS092986 from the National Institutes of Health (USA) to SAV. MR was the recipient of a post-doctoral fellowship from the Canadian Institutes of Health Research (CIHR).

Poster #12

Sensitivity of Detection by Biological AFM for Gold-Labeled Liposomes on Human Coronary Arterial Endothelial Cell Membranes

Ana María Zaske[1], Delia Danila[1], Eva Golunski[1], David Mc. Pherson[1]
[1]Internal Medicine, Cardiology Division, UT- Health Science Center at Houston

Corresponding author: Ana María Zaske, Internal Medicine, University of Texas Health Science Center at Houston, 1881 East Rd, 3SCRB6.3728, Houston, TX 77054, E-mail: Ana.M.Zaske@uth.tmc.edu

A B S T R A C T

Engineering of non-toxic nanoparticles for tissue-specific targeting has gained widespread attention. Liposomes were one of the earliest classes of engineered nanoparticles to be utilized for the purpose of delivering drugs within the human body. The ability to pegylate liposomes for the purpose of mitigating a systemic immune response, targeting specific tissues, and/or enhance prolonged vascular circulation has made these particles attractive nano-vectors to increase the efficacy of anti-cancer drugs.

While many have successfully designed and tested such "nanovectors" *in-vivo*, little is known about how these particles dynamically interact with cell membranes. Atomic Force Microscopy (AFM) is a surface analytical technique that can generate nano-scale topographic images under physiological conditions. Therefore, AFM results in an attractive tool for interrogating nanoparticle:cell membrane interactions and may afford the opportunity to image biological processes *in-vitro*.

The properties of colloidal gold have provided excellent detection capabilities for single-molecule tracking. The main aim of this project was to standardize the use of 90 nm nano-gold particles as a non-toxic means to detect immuno-liposomes on the membrane of living cells to elucidate internalization processes and binding efficiency. We were able to visualize the uptake of gold-coupled liposomes on endothelial cells, by using AFM. We found that the gold-liposomes attached to the HCAEC membrane during the first 15–30 min of incubation, liposome cell internalization occurred from 30 to 60 min (Fig 1), and most of the gold-labelled liposomes had invaginated after 2 hr of incubation. Liposomal uptake took place most commonly at the periphery of the nuclear zone. We successfully established a suitable and systematic method to study the endocytosis mechanism, binding sites, and distribution of gold labeled liposomes as a novel delivery system in HCAEC's. The 90 nm colloidal gold nanoparticles resulted in a non-invasive contrast agent that efficiently improved visual enhancement when using AFM to study biological nano-processes.

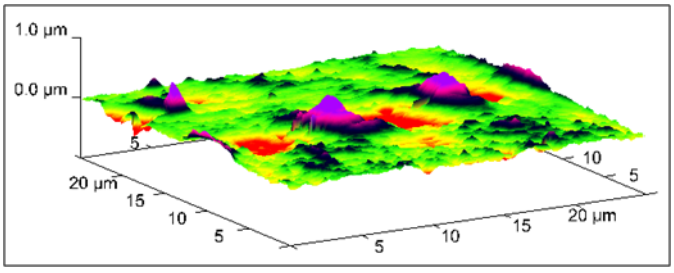


Figure 1.- AFM 3D image showing gold-coupled liposomes on human coronary arterial endothelial cells at the moment of membrane internalization (60 minutes incubation).

Poster #13

An Image Informatics Pipeline for Imaging Mass Cytometry and its Application to Characterize and Visualize the Immune Landscape in Epithelial Ovarian Cancer Patients with Long-Term and Short-Term Survival Periods

Zhu Y[1], Yeung TZ[2], Sheng J[1], Mok SC[2,*], Wong STC[1*]

1. Department of Systems Medicine and Bioengineering, Houston Methodist Cancer Center, Weill Cornell Medcine, Houston, TX
2. Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX

Imaging mass cytometry (IMC) integrates immunocytochemical and immunohistochemical methods to visualize over thirty protein markers simultaneously at subcellular resolution in the spatial context of the tissue microenvironment, enabling comprehensive analysis of cellular phenotypes and their interrelationships. There is, however, a lack of robust data processing pipelines for analyzing and modeling complex IMC data. To fill this gap, we developed an image informatics pipeline to analyze the immune landscape and spatial interactions between different cell types of the tumor tissues of advanced epithelial ovarian cancer (EOC) patients. In this study, we presented a data processing workflow of IMC and applied it to analyze and model cancer patient tissue specimen, from cell segmentation and clustering for identifying cell types to spatial information extraction, mapping and analysis. We compared tissue samples from EOC patients with short-term vs. long-term survivals. Our results showed that IMC reveals the immune cell diversity of the EOC tumor ecosystem. We found that the numbers of a macrophage subtype and B7H4 positive tumor cells were significantly lower in long-term survivors than short-term survivor did. We also developed algorithms to visualize the overall proximity and spatial correlation between any two cell types in the patient tissue specimen.

Corresponding authors:
*Samuel C. Mok, Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX. scmok@mdanderson.org
*Stephen T. C. Wong, Department of Systems Medicine and Bioengineering, Houston Methodist Cancer Center, Weill Cornell Medicine, Houston, TX. stwong@houstonmethodist.org

Acknowledgement:
The research is supported by NCI U01CA188388, W81XWH-17-1-0126, W81XWH-17-1-0146, W81XWH-16-1-0038, DOD OC160042, CPRIT RP100094, John S Dunn Research Foundation, TT & WF Chao Foundation, and the Sister Institution Network Fund from the University of Texas MD Anderson Cancer Center.

AKOYA

BIOSCIENCES®

akoyabio.com

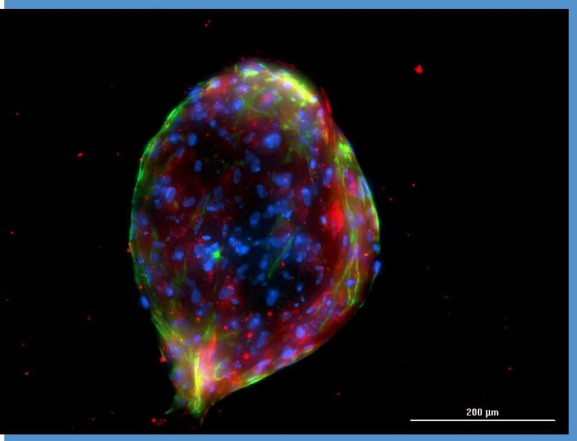
Your Power for Health

greiner bio-one

Watch our movie about 3D bioprinting!

Magnetic 3D Cell Culture

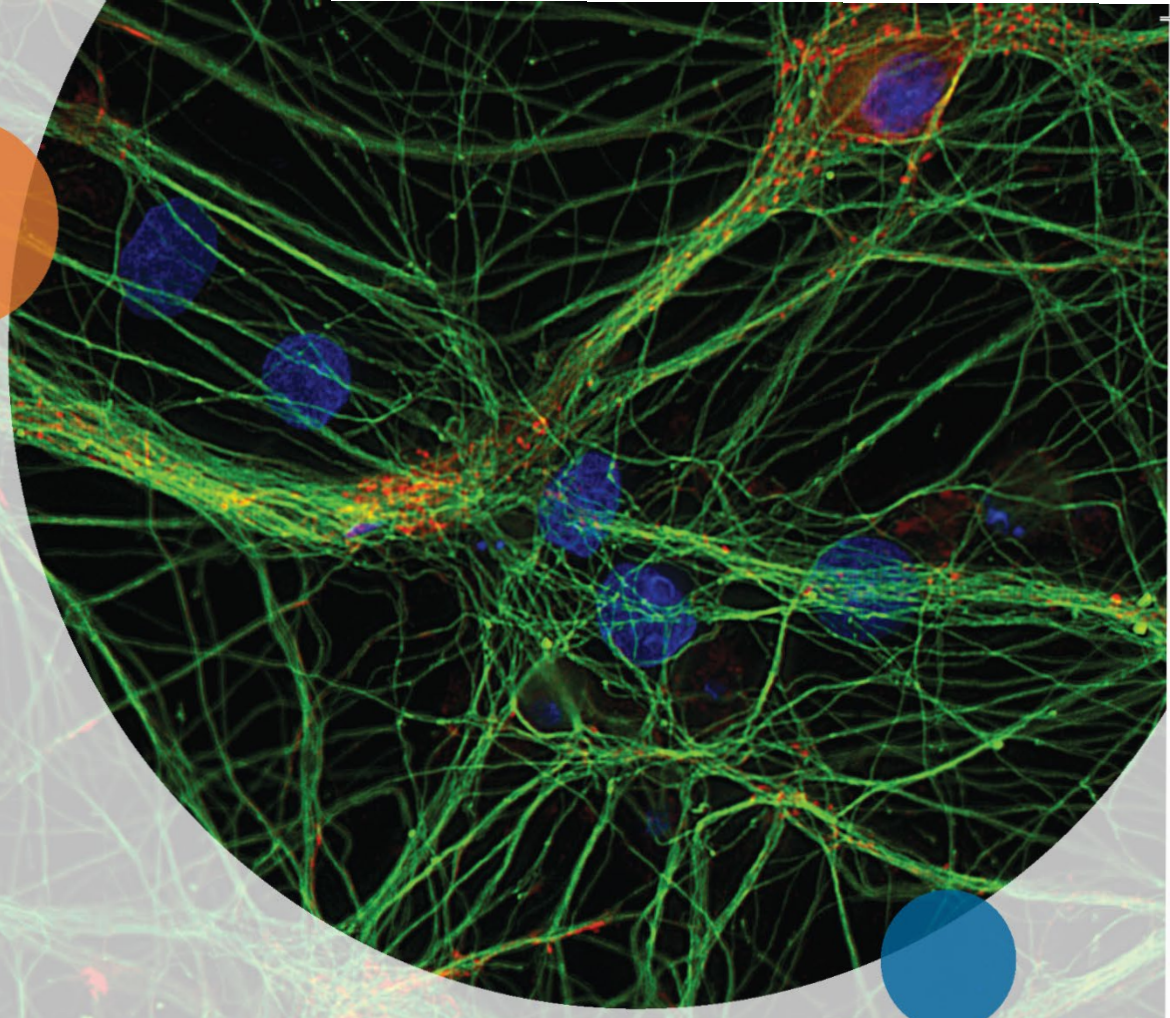
3D in a 2D workflow



- Fast 3D tissue assembly
- Reproducible - one spheroid per well
- Scalable – single well to 1536 well
- High-throughput Screening (HTS) with flat bottom for optical imaging
- Easy to handle / no sample loss
- Ready for automation

Scan to access the new m3D microsite

Greiner Bio-One
Phone: 1.800.884.4703
Fax: 1.800.726.0052
e-mail: office@us-gbo.com
http://www.gbo.com



Imaging solutions to answer your biological questions

Stunning Images | Fast Throughput | Powerful Analysis

moleculardevices.com | 800.635.5577

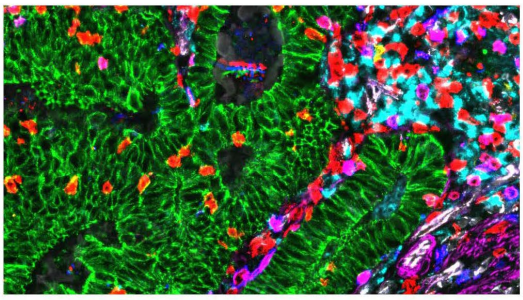
MOLECULAR DEVICES

See more with Cell DIVE™ Multiplexed Imaging

Get 1000s of spatial cellular data in **just one tissue section**. Achieved through an iterative tissue staining, imaging and destaining process, automated image registration and cell biomarker intensity quantification.

How:

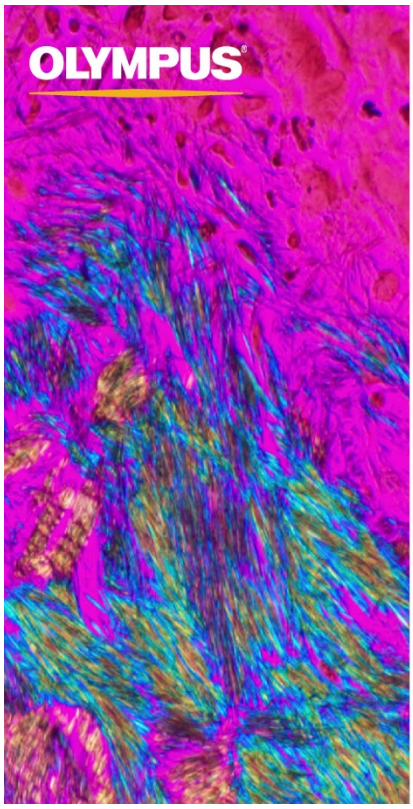
- Standard Fluorescence Microscopy: typically 4-5 markers
- Multispectral imaging: typically 6-8 markers
- **GE Cell DIVE Multiplexed Immunofluorescence (MxIF): up to 60 markers on one sample**



See change with Cell DIVE: immune cell response and infiltration in colorectal cancer. Gerdes *et al.* (2013) PNAS 110(29): 11982.

AI image analysis, bioinformatics and image visualization are enabling new discoveries in tumor cell biology and spatial interactions. Understanding cell types, heterogeneity and immune response will help researchers and oncologists match the right therapies with the patient tumor profiles.

Learn more from **Speaker Fiona Ginty**, Biosciences Technology Manager and Principal Investigator at GE Global Research on **Monday Feb 25 at 9:45am** or email **Cell.DIVE@ge.com**



Partner in Discovery

At Olympus, we understand the value of your bioscience research.

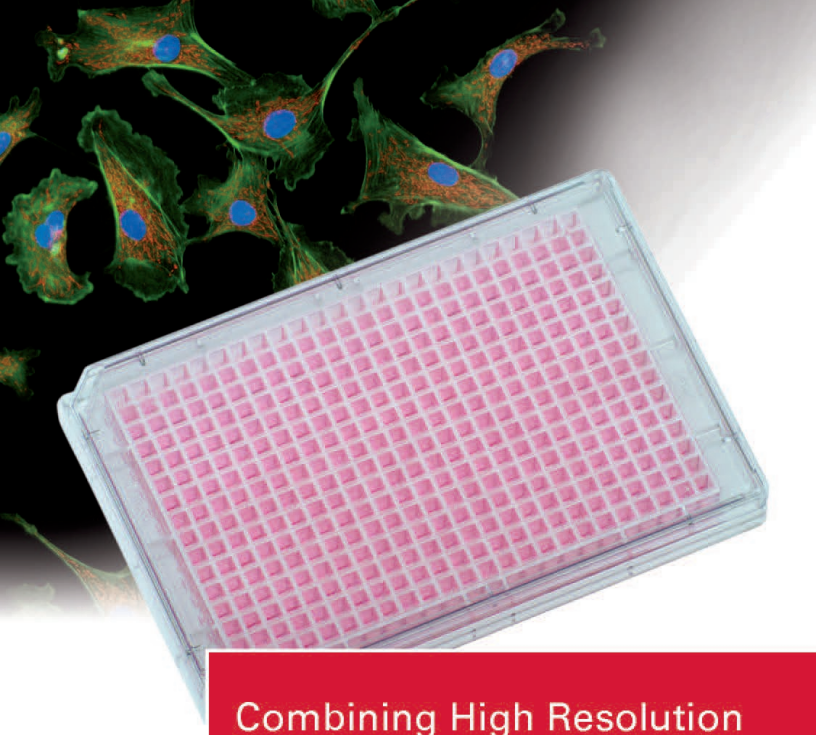
We focus on developing and supplying quality resources to meet the needs of your laboratory. Our line of imaging solutions is built with quality, clarity, and value in mind.

Let us work with you to create your ideal environment for discovery.

Your Science Matters™

For further information, please visit:
www.olympus-lifescience.com
or stop by the Olympus booth today.

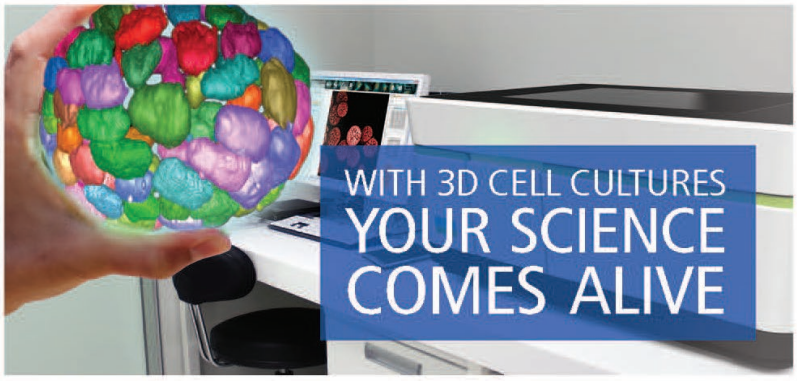
Uric acid crystal captured on the Olympus BX53 microscope



Combining High Resolution
With High Throughput

New at ibidi: µ-Plate 384 Well

ibidi USA, Inc. | 844.276.6363 | ibidiusa@ibidi.com ibidi.com

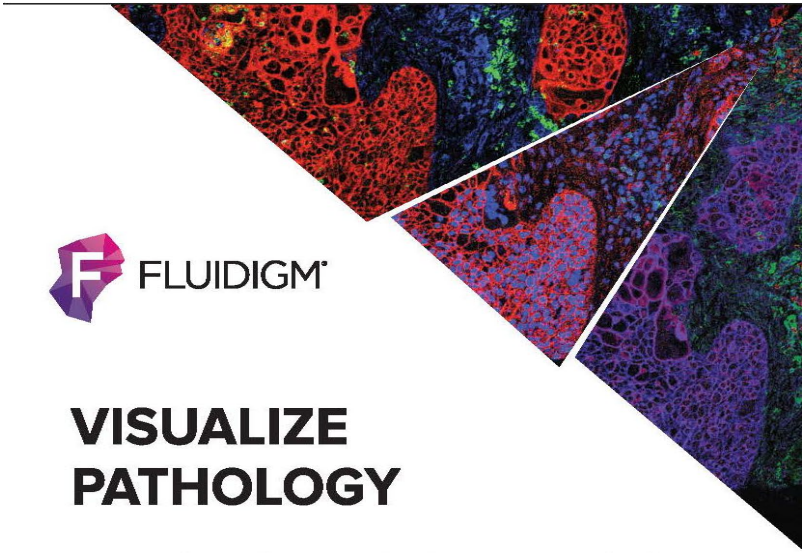


Challenged to develop more predictive cellular assays?

3D cell cultures bridge the gap between 2D cell cultures and animal models, and more closely mimic the biological processes that occur *in vivo*. Now you can address the challenges of growing, detecting, and analyzing these advanced models with our state-of-the-art solutions for 3D cell cultures, including high-content imaging systems, microplates, and reagents. And with Harmony® software, you can visualize and analyze your samples in 3D for more relevant information and new insights.

Learn more at
www.perkinelmer.com/3DCellSolutions

PerkinElmer®
For the Better



FLUIDIGM®

VISUALIZE PATHOLOGY

Imaging Mass Cytometry™ using the Hyperion™ Imaging System lets you gain a comprehensive understanding of complex cellular phenotypes in spatial context of the tissue or tumor microenvironment. Learn how you can image up to 37 markers at subcellular resolution in a single scan.

Explore the possibilities at **fluidigm.com/imc**

For Research Use Only.
Not for use in diagnostic procedures.

StellarVision Next-Gen Fluorescent Microscopy

Wishing you could image 1000's of cells with the same XY spatial resolution without time-consuming Z-stack?

Quantify single RNA or DNA molecules, fluorescent proteins, immunofluorescence in each of these 1000's cells? We use Synthetic Aperture Optics (SAO) to attain 100x oil resolutions with an AIR lens in high throughput. Visit the website or contact me for more information – mpbeal@opticalbiosystems.com