2013 NIH Common Fund Single Cell Analysis Investigators Meeting

Where: Residence Inn, Bethesda, Maryland

When: April 15-16, 2013

The NIH Common Fund held a Single Cell Analysis Investigators Meeting on April 15-16, 2013 in Bethesda, Maryland. This year's meeting objectives are to:

- Disseminate current research findings in single cell analysis.
- Convene the funded SCAP investigative teams to update the community on their research progress, problems, and new opportunities in single cell analysis.
- Identify roadblocks and emerging challenges in the understanding of single cell level heterogeneity.
- Consider current conceptual, technical, and/or methodological challenges in single cell analysis and brainstorm possible solutions.
- Discuss major themes in basic and/or clinical research for which additional focus on single cell analysis may provide the most significant and broadest impact.
- Determine major biomedical research opportunities that can be addressed by single cell analysis.
- Discuss how relevant groundbreaking technologies and approaches in SCA can be disseminated to the research community.
- Facilitate new research collaborations between funded grantees and others interested in entering the Single Cell Analysis field.
- Build synergy between the Single Cell Analysis Program and other projects funded by NIH.

The meeting incorporated presentations by 5 invited keynote speakers and 15 funded investigators, 28 poster presentations, and 13 lightning talks, and yielded opportunities to review progress, collaborate, and brainstorm novel ideas. Over 160 participants attended this year's meeting, with representatives in academia (55%), government (30%), industry (10%), and nonprofits (5%). Almost one-third of attendees were funded investigators and collaborators, while 21% were postdoctoral fellows and graduate students. Five scientific sessions were held. A general description and summary of each session follows.

Session 1 – Phenotypic characterization of an individual cell: Defining the "normal cell" parameters

- Single Cell Sequencing: Cell-based Parameters, Mahendra Rao, NIH Center for Regenerative Medicine
- Evaluation of Cellular Heterogeneity Using Patchclamp and RNA-Seq of Single Cells, James Knowles, University of Southern California
- Mapping pH on the Surface of Cancer Cells, Yana Reshetnyak, University of Rhode Island

- In Situ Single Cell Laser Lysis and Downstream qRT-PCR Profiling, Deirdre Meldrum, Arizona State University

The keynote speaker, Dr. Mahendra Rao, highlighted the challenges in single cell analyses for generating relevant data, reproducing, cross-validating, and interpreting this data to solve a biological problem. Dr. James Knowles discussed his and his team's efforts to use patch clamp for acquiring single cells in placenta, olfactory epithelium, temporal cortex, and cerebellum, with the aim of generating transcriptome dataset, addressing variation due to technical noise or biological noise, and uncovering transcriptional events underlying long term potentiation and long term differentiation. Dr. Yana Reshetnyak describes her group's effort to measure pH in vivo on the cell's surface using a pH low insertion peptide (pHLIP), and plans to evaluate differences in pH between metastatic and non-metastatic tumors. Dr. Deirdre Meldrum explored the ability to study clonal evolution in cancer with its spatial information preserved using precise *in situ* laser lysis and qRT-PCR profiling, with the aim of optimizing single cell PCR with minimum cell-cell contamination and determining cell heterogeneity in Barrett's esophagus

Session 2 – What are the current technical limits to identifying and characterizing a cell? Innovations in sensitivity and specificity

- Microengineered Devices for Advancing Preclinical and Clinical Research, Nancy Allbritton, University of North Carolina
- Turning Single Cell into Microarrays by Super-Resolution Barcoding, Long Cai, California Institute of Technology
- WGS of Single Cells Reveals Extensive Clonal Diversity in Breast Cancer, Nicholas Navin, University of Texas MD Anderson Cancer Center
- Molecular Analysis of Live Single Cells and Subcellular Regions: Insights and Surprises, James Eberwine, University of Pennsylvania

Dr. Nancy Allbritton discussed several applications for signaling at the single cell level, including fine need biopsy, loading fluorescent reports/substrates into cells, single cell enzyme assays, moderate throughput capillary based separation system, and new raft arrays for cell separation. The goal of Dr. Long Cai's project is to probe many RNAs at a time without dissecting the cell; using single molecular FISH, he is able to efficient encode the entire transcriptome using photo-switchable fluorophores as a barcode for each transcript. Dr. Nicholas Navin shared insights to his studies on copy number evolution with single nucleus sequencing, which have shown a significant improvement in single cell genomic coverage. Dr. James Eberwine and team are focused on a genomic analysis of the central nervous system and wish to examine neuron-glia interactions using linear amplification schemes, TIVA-tags for in vivo analyses.

Session 3— From prototype to practice: Accelerating the translation and commercialization of promising technologies

- The Road to Commercialization of Tools for Single Cell Genomics, Gajus Worthington, Fluidigm Corporation

- Massively Parallel Polymerase Cloning and Sequencing of Single Cells, Kun Zhang, University of California San Diego
- Technologies for Integrative Analysis of Cell Types, Edward Boyden, Massachusetts Institute of Technology
- A High Throughput Approach to Develop FRET Biosensors, Yingxiao Wang, University of Illinois Urbana-Champaign

Mr. Gajus Worthington started the third session by describing his journey in founding Fluidigm and provided advice in making business decisions, particularly in commercializing technology and breaking down the problem of advancing commercialization of technology. Dr. Kun Zhang focused on the development of a high-resolution three dimensional map of transcriptional activities in single cells of the human cortex using MIDAS (Microwell, displacement amplification system) and its potential to detect increases in copy number in post-mortem brains. Dr. Edward Boyden discussed the development of an automatic patch clamping system that would enable integrative analysis of brain cells, including their electrophysiological properties, as well as gene expression and morphology. Dr. Yingxiao Wang elaborated on the development of a high-throughput screening method based on directed evolution to develop a FRET biosensor for Fyn kinase that can be eventually applied to any kinase of interest, allowing characterization of micro-domains in which different neighbors can be identified.

Session 4 – Promoting single cell analysis using multiscale models and integrated, quantitative measurements

- Spatiotemporal Considerations in Analyzing and Computationally Modeling Single Cells, Ronald Germain, NIAID, NIH
- Live Single Cell Functional Phenotyping in Droplet Micro-Reactors, Tania Konry, Massachusetts General Hospital
- Genetic Tagging of Active Neural Circuits, Mark Mayford, Scripps Research Institute
- An Integrated System to Monitor Complex Tissues at Single-Cell Resolution, Zhirong Bao, Memorial Sloan-Kettering Cancer Center

Dr. Ronald Germain emphasized the importance of looking at individual cells over time and to consider the intracellular and extracellular spatial considerations of a cell when doing single cell analysis; he described the challenges associated with simulations of the early signaling events in T-cell activation, the Simmune software package which allows biologists to do multiscale mnodeling with a graphic interface, and histocytochemistry methods to quantify cell populations and map cell locations in tissue, thus providing FACS-like quantitation and spatial information for up to ten different probes. Dr. Tania Konry discussed the application of miniaturized nanoliter reaction droplets to single cell analysis, with applications for cell culture and drug screening studies. Dr. Mark Mayford is interested in identifying where cell changes in the brain occur in response to learning; he has developed an inducible binary expression system under doxycycline control (tet-based active circuit tagging) to induce reporter gene expression in cells where activity-dependent cfos expression occurs, allowing him to generate and compare 2-point neural activity maps of animals with and without learning experiences. Dr. Zhirong Bao discussed a collaborative effort to monitor single cells in tissues; this approach (diSPIM and

StarryNite) combines the ability to perform rapid 4D visualization of thick specimens with advanced software to track many individual cells simultaneously.

Session 5 – Challenges in correlating function and phenotype in single cell measurements

- Single-Cell Genomics: Life at the Single Molecule Level, Sunney Xie, Harvard University
- A Phenotypic Signature for Pancreatic Cancer Metastasis, Denis Wirtz, Johns Hopkins University
- Single-Cell Multiomyx Analysis of Normal and Cancerous Colon Tissues, Michael Gerdes, General Electric Global Research Center
- A Nanowell-Based Integrated Single Cell Analytical Technology for Ex Vivo Characterization of Clinical Samples, J. Christopher Love, Massachusetts Institute of Technology

Dr. Sunney Xie described the need for single-cell genome sequencing, human health applications for the technique that he and his lab developed, Multiple Annealing and Looping Based Amplification Cycles (MALBAC), including detection of circulating tumor cells and recombination rates in sperm cells. Dr. Denis Wirtz developed a comprehensive morphological analysis to classify irregular cellular and nuclear shapes using a limited number of common shape modes through eigenshape decomposition and clustering approaches to investigate the possibility that pancreatic cancer cells that had successfully metastasized to the liver have distinct physical properties from those in the primary tumor. Dr. Michael Gerdes described a sequential staining method, referred to as MultiOmyx that allows for quantitative, sub-cellular measurement of multiple proteins in paraffin-embedded, formalin-fixed tissues and cells, with the goal of determining expression patterns for established colon stem cell markers and validation of the single cell algorithms. Dr. J. Christopher Love developed a modular analytical platform for characterizing functions, phenotypes, genotypes with single-cell resolution and presented advances in this technology.

AGENDA

MONDAY, APRIL 15, 2013

7:30 a.m. **Registration Check-In**
Poster Set-Up in Calvert I and II

8:00 a.m. **Welcome**
Thomas Insel, Director of National Institute of Mental Health
Roderic Pettigrew, Director of National Institute of Biomedical Imaging and Bioengineering
James Anderson, Director of Division of Program Coordination, Planning and Strategic Initiatives

8:15 a.m. **Overview of Single Cell Analysis Program**
Andrea Beckel-Mitchener, Program Coordinator, NIMH, NIH
Richard Conroy, Program Coordinator, NIBIB, NIH

8:30 a.m. **Session 1 – Phenotypic characterization of an individual cell: Defining the "normal cell" parameters**

8:30 a.m. ***Keynote Address***
Single Cell Sequencing: Cell-based Parameters
Mahendra Rao, NIH Center for Regenerative Medicine

9:00 a.m. **Evaluation of Cellular Heterogeneity Using Patchclamp and RNA-Seq of Single Cells**
James Knowles, University of Southern California

9:20 a.m. **Mapping pH on the Surface of Cancer Cells**
Yana Reshetnyak, University of Rhode Island

9:40 a.m. **In Situ Single Cell Laser Lysis and Downstream qRT-PCR Profiling**
Deirdre Meldrum, Arizona State University

10:00 a.m. **BREAK**

10:30 a.m. **Session 2 – What are the current technical limits to identifying and characterizing a cell? Innovations in sensitivity and specificity**

10:30 a.m. ***Keynote Address***
Microengineered Devices for Advancing Preclinical and Clinical Research
Nancy Allbritton, University of North Carolina

11:00 a.m. **Turning Single Cell into Microarrays by Super-Resolution Barcoding**
Long Cai, California Institute of Technology

11:20 a.m. **WGS of Single Cells Reveals Extensive Clonal Diversity in Breast Cancer**
Nicolas Navin, University of Texas MD Anderson Cancer Center

11:40 a.m. **Molecular Analysis of Live Single Cells and Subcellular Regions: Insights and Surprises**
James Eberwine, University of Pennsylvania

12:00 p.m. Lunch on your own and Poster Viewing

1:00 p.m. Session 3— From prototype to practice: Accelerating the translation and commercialization of promising technologies

1:00 p.m. ***Keynote Address***
The Road to Commercialization of Tools for Single Cell Genomics
Gajus Worthington, Fluidigm Corporation

1:30 p.m. **Massively Parallel Polymerase Cloning and Sequencing of Single Cells**
Kun Zhang, University of California San Diego

1:50 p.m. **Technologies for Integrative Analysis of Cell Types**
Edward Boyden, Massachusetts Institute of Technology

2:10 p.m. **A High Throughput Approach to Develop FRET Biosensors**
Yingxiao Wang, University of Illinois Urbana-Champaign

2:30 p.m. BREAK

3:00 p.m. Lightning Talks

5 minutes and 1 slide each

Nanoscale Laser Ablation Capture Mass Spectrometry for Single Cell Proteomics
Kermit Murray, Louisiana State University

High Throughput Single Cell Analysis and Cell-by-Cell Imaging by LAESI Mass Spectrometry
Akos Vertes, George Washington University

Invariance and Variability in an Endogenous Negative-Feedback Circuit
Markus Covert, Stanford University

Single Cell Spectroscopy Using Combined AFM-NMR Microcoils
Corey Neu, Purdue University West Lafayette

A Microfluidic and Sequencing-Based Method for Profiling mRNA and Protein Levels in Single Cells
Ilke Akartuna, Harvard Medical School

Microwell Arrays for Massively Parallel Transcriptome and Proteomics with Single Molecule Sensitivity
Peter Sims, Columbia University Health Sciences

Real-Time Visualization of Neural Stem Cell Transcriptome
Claudia Mizutani, Case Western Reserve University

Single Cell Analysis of Epigenetic States at Selected Loci
Oleg Denisenko, University of Washington

Histo Mosaic: A Novel Diagnostic Technique to Detect Genetic Mutations in Tissue Slides
Cheng-Chung Lee, University of Southern California

Quantitative Single-Cell Functional Characterization of CD19-Specific Chimeric Antigen Receptor T-Cells
Navin Varadarajan, University of Houston

Fluorescent Probes for Quantitation of Secretory Protein Levels in Single Cells
Matthew Levy, Albert Einstein College of Medicine

Rapid Analysis of Single T-Cell Immunity Signatures in Tuberculosis
Yuri Bushkin, University of Medicine and Dentistry of New Jersey

Bifurcation Analysis of Single-Cell Gene Expression Data Reveals Epigenetic Landscape
Eugenio Marco, Harvard University

4:30 p.m. Poster Session

Calvert I and II – cash bar available

7:00 p.m. Dinner on your own

TUESDAY, APRIL 16, 2013

7:30 a.m. Registration Check-in

8:00 a.m. Session 4 – Promoting single cell analysis using multiscale models and integrated, quantitative measurements

8:00 a.m. ***Keynote Address***
Spatiotemporal Considerations in Analyzing and Computationally Modeling Single Cells
Ronald Germain, NIAID, NIH

8:30 a.m. **Live Single Cell Functional Phenotyping in Droplet Micro-Reactors**
Tania Konry, Massachusetts General Hospital

8:50 a.m. **Genetic Tagging of Active Neural Circuits**
Mark Mayford, Scripps Research Institute

9:10 a.m. **An Integrated System to Monitor Complex Tissues at Single-Cell Resolution**
Zhirong Bao, Memorial Sloan-Kettering Cancer Center

9:30 a.m. BREAK

9:45 a.m. Session 5 – Challenges in correlating function and phenotype in single cell measurements

9:45 a.m. ***Keynote Address***
Single-Cell Genomics: Life at the Single Molecule Level
Sunney Xie, Harvard University

10:15 a.m. **A Phenotypic Signature for Pancreatic Cancer Metastasis**
Denis Wirtz, Johns Hopkins University

10:35 a.m. **Single-Cell Multiomyx Analysis of Normal and Cancerous Colon Tissues**
Michael Gerdes, General Electric Global Research Center

10:55 a.m. **A Nanowell-Based Integrated Single Cell Analytical Technology for Ex Vivo Characterization of Clinical Samples**
J. Christopher Love, Massachusetts Institute of Technology

11:15 a.m. Overview of Additional Common Fund Efforts

11:15 a.m. **The Genotype-Tissue Expression (GTEx) Project, an Atlas of Human Gene Expression and Regulation**
Simona Volpi, NHGRI, NIH

11:30 a.m. **Organs on Chips: The Future of Translational Research**
Kristin Fabre, NCATS, NIH

11:45 a.m. Meeting Wrap-Up

12:00 p.m. Investigators Meeting Ends
Reminder to check out of hotel and take down posters

1:00 p.m. U01 SCAP-Transcriptome Steering Group Meeting Begins
U01 project teams, Steering Group members, and NIH staff only

5:00 p.m. Meeting Adjourned

SPEAKER ABSTRACTS

1. Single Cell Sequencing: Cell-based Parameters

Mahendra Rao

NIH Center for Regenerative Medicine, Bethesda, Maryland

New technologies and the ability to sort single cells and a reduction in the cost of sequencing all have made it possible to sequence the genome of a single cell. While there are clearly advantages to the ability to miniaturize experiments one also introduces new variables simply from the inherent variability of cells or from the limitations of technologies. I will discuss cell based variability issues briefly. Variability when one is sampling from a single cells can be due to the history of the cell chosen, the tissue culture conditions which may have micro environments in a homogenous platform or the state of cell cycle of the cell. Further since cell of ten make stochastic choices based on an aggregate sampling of the incoming signals and have more than one choice any cell we pick will show differences from other cells in the same environment. Distinguishing between differences that are relevant to the biological question one wishes to address and the irrelevant differences is difficult as the number of key relevant genes may be small. One must carefully design the experiment to take into account such variables in any single cell analysis.

2. Evaluation of Cellular Heterogeneity Using Patchclamp and RNA-Seq of Single Cells

James Knowles, Robert Chow, Oleg Evgrafov, Jae Mun Kim

University of Southern California, Keck School of Medicine, Los Angeles, California

Our overall aim is to assess the technical and biological noise in measured RNA levels in single cells in a number of human tissue types, and to develop analytical tools to address the complexity observed at the single-cell level. Understanding the sources and relative sizes of technical and biological noise has become essential, as the lower detection limit of RNA-Seq is now in the range of 10 picograms of total RNA -- i.e. the amount of RNA in single cells. Technical noise can come from several different sources that we will attempt to evaluate separately. These include: 1) sample procurement and RNA retrieval, 2) sequencing library preparation, 3) sequencing methodology, 4) batch effects in sequencing experiments, 5) bioinformatics approaches for data analysis, 6) gene-gene variability.

Assessing the relative magnitude of technical noise from different sources will inform how to reduce that noise in future experiments, and thereby reduce interference with studies of meaningful biological variations or noise. Biological noise, or inter-cell differences arise from differences in cellular history or fate, stages of cell cycle, connections to neighboring cells, and true functional differences of ostensibly identical cells (e.g., different olfactory receptors among olfactory neurons).

We are studying three different cellular systems that we expect to have different levels of inter-cell variation (biological noise); first, syncytiotrophoblast cells from placenta, which are expected to have relatively low inter-cell variation; second, olfactory neurons from nasal neuroepithelium, each of which is expected to express a different olfactory receptor, providing a positive control for differences in the RNA-Seq data; and third, individual Purkinje neurons from the cerebellum and layer three cortical neurons, which may have larger inter-cell variation. The method to extract cytoplasm from individual cells -- patch clamp pipette extraction -- does not require fully disrupting the tissue or dispersing the cells. We have already used patch clamp to determine the transcriptomes of multiple individual neurons in the mouse brain, using the cytoplasm extracted from single cells on which we had already performed patch-clamp electrophysiology recordings, followed by RNA-Seq.

For each of the cellular systems chosen we are making single-cell transcriptome datasets, using patch clamp to extract cell contents and RNA-Seq. We will present an overview of our project and our recent progress in phenotyping the different types of cells and improving the sensitivity and quality of low-input RNA-seq.

3. Mapping pH on the Surface of Cancer Cells

Yana K. Reshetnyak, Michael Anderson, Oleg A. Andreev

University of Rhode Island, Physics Department, Kingston, Rhode Island

The acidity is associated with development of various pathological states such as solid tumors, ischemic stroke, neurotrauma, epileptic seizure, inflammation, infection, wounds, cystic fibrosis and others. Normal cell could be distinguished from highly glycolytic cell (for example, metastatic cancer cell) by transmembrane pH gradient and value of pH at surface of plasma membrane. We are developing novel tool to map pH at the extracellular and intracellular surfaces of individual cell in highly heterogeneous environment of cells *in vivo*. The tool would allow opening an opportunity to contribute in understanding of diseases progression and development of approaches of pH-based image-guided intervention. Our strategy is based on use of peptides of pHLIP® (pH Low Insertion Peptide) family. pHLIPs are water-soluble membrane peptides, which insert and fold in lipid bilayer of membrane only at slightly acidic conditions. Since the equilibrium is strongly shifted toward membrane inserted form at low pH, pHLIP injected into blood, circulates in body and accumulates in acidic tissue of tumors, site of inflammatory arthritis and ischemic regions. At 24 h after i.p. or i.v. administration of pHLIP, it is washed out completely from the blood and stays in plasma membrane of cells with low extracellular pH. pHLIP labeled with optical, PET or SPECT probes is considered to be first acidity markers, which are currently under development for clinical uses. We have developed scheme of conjugation of pHLIP peptides with pH-sensitive fluorophore, SNARF and activation of it to transfer to its fluorescent form. The main goal of using pHLIPs is to deliver and tether SNARF to the outer or inner leaflet of bilayer of plasma membrane. The probe will be attached to the N- or C-terminus of pHLIPs. In first case, SNARF-1 will stay in the extracellular space being tethered to the cell surface. On the other hand, when SNARF-1 would be conjugated with the peptide inserting end (C-terminus), pHLIP would “flip” SNARF-1 across the bilayer and expose it to the intracellular space, while keeping it close to the inner leaflet of membrane. Thus, we propose to measure pH from the outer and inner leaflets of plasma membrane and identify transmembrane pH gradient. Experiments on cultures cells are underway.

4. In Situ Single Cell Laser Lysis and Downstream qRT-PCR Profiling

Deirdre R. Meldrum, Shui-Hui Chao, Thai Tran, Laimonas Kelbauskas, Weimin Gao

Center for Biosignatures Discovery Automation, the Biodesign Institute, Arizona State University, Tempe, Arizona

We are developing an innovative integration of nanometer-resolution laser lysis and microfluidic tools to analyze single-cell heterogeneity in situ using qRT-PCR. In its ultimate implementation, users can simply load a 3D tissue slice in the device, select the target cells based on their location and morphology, and then the system delivers mRNA expression of dozens of genes of individual cells in one hour. The system will correlate the mRNA expression levels of individual cells and their in situ coordinates within the tissue to reconstruct a 3D map of gene expression at the single-cell level. We will present the preliminary results and validation on using laser lysis to harvest and quantify mRNA transcripts from cell lines that were seeded in our newly designed microfluidic channels. Specifically, we have 1) fine-tuned the 2-photon laser cell lysis system (2PLL) to precisely lyse target cells, 2) optimized conditions for harvesting and detecting mRNA transcripts from 2PLL lysed cells, and 3) minimized mRNA contamination cells that are serially lysed in the same compartment. In conclusion, we have established conditions for analyzing single-cells that are lysed by a 2PLL system at the cell line level and our future studies will focus on examining RNA samples that are serially obtained from intact tissues.

5. Microengineered Devices for Advancing Preclinical and Clinical Research

Nancy L. Allbritton

UNC/NCSU Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Much of the basic biological phenomena underlying the complex behaviors of living cells remain unknown largely due to the technical challenges of measurements at the single cell level. Thus development of new analytical tools to assess signal processing pathways in large numbers of single cells would enable a better understanding of the complex signaling circuitry within the single cell. The goal of my lab is to utilize principles from the engineering and the physical sciences to develop novel methods to tackle the demands of single-cell analyses with improved throughput. Much of my research is focused on the development and testing of bioanalytical platforms to assess single cell information processing. We are currently developing integrated microanalytical platforms in both array and microfluidic formats to address critical preclinical and clinical needs in accurately monitoring drug action and identifying the patient who will respond to new therapies aimed at modulating signal transduction pathways. In order to manipulate individual cells, the lab has also pioneered the development of novel microfabricated devices to enable the analysis and isolation of cells while they remain in culture. These technologies possess a number of advantages over current cell separation methods as cells can be monitored over time and selected based on a wide range of characteristics. The devices are made in almost any size for use with small samples obtained from needle biopsies to large-scale arrays with millions of sites for high-throughput screens and rare cell isolation. Numerous applications of these microfabricated devices are being pursued, including efficient cloning of mouse stem cells, purification of cancer stem cells from patient samples and isolation of tumor-targeted lymphocytes for cancer immunotherapy.

6. Turning Single Cell into Microarrays by Super-Resolution Barcoding

Long Cai and Eric Lubeck

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California

Fluorescence microscopy is a powerful quantitative tool for exploring regulatory networks in single cells. However, the number of molecular species that can be measured simultaneously is limited by the spectral separability of fluorophores. Here we demonstrate a simple but general strategy to drastically increase the capacity for multiplex detection of molecules in single cells by using optical super-resolution microscopy (SRM) and combinatorial labeling. The basis for this new approach are the following: given the 10 nanometers resolution of a super-resolution microscope and a typical cell a size of (10um)^3, individual cells contains effectively 10^9 super-resolution pixels or bits of information. Most eukaryotic cells have 10^4 genes and cellular abundances of 10-100 copies per transcript. Thus, under a super-resolution microscope, an individual cell has 1000 times more pixel volume or information capacities than is needed to encode all transcripts within that cell. As a proof of principle, we labeled mRNAs with unique combinations of fluorophores using Fluorescence in situ Hybridization (FISH), and resolved the sequences and combinations of fluorophores with SRM. We measured the mRNA levels of 32 genes simultaneously in single yeast cells. These experiments demonstrate that combinatorial labeling and super-resolution imaging of single cells provides a natural approach to bring systems level analysis into single cells.

7. Whole-Genome Sequencing of Single Cells Reveals Extensive Clonal Diversity in Breast Cancer

Nicholas Navin, Yong Wang, Marco Leung, Jill Waters, Ken Chen, Paul Scheet, Selina Vattathil, Han Liang, Asha Multani, Amy Zhang, Funda Meric-Bernstam

MD Anderson Cancer Center, Houston, Texas

Sequencing of luminal A breast tumor cells en masse has revealed few somatic mutations and limited genetic heterogeneity. Here we investigated the clonal diversity and evolution of these tumors by whole-genome, single-cell sequencing. We developed a method called Nuc-Seq to sequence the genomes of single human cells at high-coverage breadth (73-89%) and depth (35-66X). We sequenced an ER+/PR+/Her2- breast tumor en masse and detected only 39 coding mutations. We also measured copy number in 50 single cells and found that they share highly similar genomic rearrangements. We then performed Nuc-Seq on four single tumor cells, which identified thousands of additional rare and de novo somatic mutations. Targeted deep-sequencing showed that most of these mutations occur at low frequencies (< 1%) in the tumor. Our data suggests that copy number rearrangements occurred early in tumor progression and remained highly stable as the mass expanded, while point mutations and indels evolved gradually, generating extensive clonal diversity.

8. Molecular Analysis of Live Single Cells and Subcellular Regions: Insights and Surprises

James Eberwine[1], Jai-Yoon Sul[1], Hannah Dueck[2], Mugda Khaadkar[2], Jennifer Spaethling[1], Ditte Lovatt[1], Brittani Ruble[3], Tae Kyung Kim[1], Ivan Dmochowski[3], Junhyong Kim[2]

[1]Department of Pharmacology, [2]Department of Biology, [3]Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania

The first description of a cell by Robert Hooke in 1665 laid the foundation for the development of the Modern Cell Theory, which has been the fundamental driver of biological discovery for the last 450 yrs. In the field of neuroscience Ramon-y-Cajal was the first to demonstrate and understand that the myriad of morphologically different cell types in the central nervous system is critical to its function. Over the last century, cell types have been enumerated and characterized through morphometric approaches. More modern approaches have utilized proteomic, metabolomic and molecular characterization of cells to expand the catalog of cell types and enhance our understanding of their generation and maintenance.

A singular characteristic of the brain is that its function involves systems-level integration of inherently heterogeneous actions of its components. The diversity and heterogeneity of component actions is manifest at all scales: from large-scale anatomical structures to tissues to individual cells, and even to sub-cellular components. Yet, the degree of individual variation, the generation of such variation, and the functional importance of such variation at the whole genome-scale is still poorly understood, especially at the level of individual cells, dendrites, axons, and synapses. It is useful to conceptualize each cell type as having reached a distinct molecular steady state through an incompletely understood genome dynamics involving gene regulation, epigenomic modifications and molecular physiology. This suggests that cellular phenotype can be thought of as a continuum of states, and particular cell types are the locally stable states that are reached during cell differentiation in a particular microenvironment.

We will present data highlighting similarities and variation in gene expression across cell types, describe some of the unique aspects of the dendritic subcellular domain of neurons and how it is regulated and finally highlight our view on how gene expression variation contributes to cellular phenotype. We will highlight our experimental approaches for quantifying this. It is hoped that these studies will help to define the genomic logic that separates different phenotypic states, both normal and diseased. The ability to selectively and rationally create cellular phenotypes promises to provide important insights into the fundamental mechanisms underlying cellular polarity, functioning and phenotype stability. In presenting these data particular attention will be paid to the absolute need to perform such studies on cells in their natural microenviroment as natural systems influences are critically important in maintaining and modulating normal cellular functioning.

9. The Road to Commercialization of Tools for Single Cell Genomics

Gajus V. Worthington

Fluidigm Corporation

Fluidigm's thirteen year journey to develop and commercialize tools for single cell analysis offers insight into the more general challenge of technology development. Today, Fluidigm is the leading provider of life science tools for single cell analysis, including systems for gene expression profiling, handling and library preparation for NGS. Over 65 publications in this emerging field of single-cell genomics have already been published using Fluidigm's technologies. However, the road from concept to fully commercialized product was far from straightforward. The multitude of challenges faced by the company, its leadership and its collaborators in bringing innovative life science tools to market offer valuable lessons for anyone contemplating a similar path.

10. Massively Parallel Polymerase Cloning and Sequencing of Single Cells

Kun Zhang

Department of Bioengineering, University of California at San Diego, San Diego, California

Genome sequencing of single cells has a variety of applications including characterizing difficult-to-culture microorganisms in various environments and human body sites, and identification of somatic mutations in single tumor cells. A key step is to generate clonal copies of DNA molecules within a single cell by DNA polymerase, a process that we called polymerase cloning. Polymerase cloning has been implemented through a number of amplification strategies, including multiple displacement amplification (MDA), hyper-branched rolling circle amplification (hRCA) and PCR based approaches. A common limitation is the highly biased representation of the template genome after amplification, which increases the sequencing cost, limits the genome coverage and compromises the sensitivity of detecting mutations. Additional difficulties include frequent contamination, high cost, and low throughput.

To tackle these challenges, we have developed a massively parallel polymerase cloning method using microwell arrays. In this method, hundreds to thousands of single cells are randomly deposited into a PDMS microwell array fabricated on a standard microscope slide. Polymerase cloning of all the individual cells is performed in parallel in a nanoliter volume, and the amplification is monitored in real-time with an epifluorescent microscope. Positive polymerase clones are extracted with a micromanipulator, and converted into multiplexed sequencing libraries for Illumina sequencing. This method is inherently scalable, low-cost and yields an unbiased representation of the genome from single cells. Results on *de novo* genome sequencing of single microbial genomes and copy number analysis of single mammalian cells will be presented.

11. Technologies for Integrative Analysis of Cell Types

Edward S. Boyden[1], *Hongkui Zeng*[2], *Craig R. Forest*[3]

[1]*Biological Engineering and Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge, Massachusetts,* [2]*Allen Institute for Brain Science, Seattle, Washington,* [3]*Georgia Institute of Technology, Atlanta, Georgia*

The cells of the brain exhibit a diversity of expressed genes, morphologies, and electrophysiological properties, and have come to be grouped into “cell types” that are distinguished by one or more of these characteristics. However, there is no one-to-one correspondence between cell type-defining expressed genes, morphological characteristics, and electrophysiological properties, and no unified taxonomy of brain cells. Furthermore, cells routinely change their expressed genes, morphologies, and electrophysiological properties, as a result of development, plasticity, or disease, raising the question of how to categorize cell types as they change their states as a result of experience. Accordingly, we propose to develop a powerful, easy-to-use tool that enables the integrative phenotyping of cells of the brain – namely, a robot that can acquire simultaneously the gene expression patterns, morphologies, and electrophysiological properties of single cells in brain tissue, in an automated fashion. To achieve this, we note that whole-cell patch clamp cellular recording in vivo enables high-quality electrophysiological characterization, dye infusion for morphological visualization, and extraction of cell contents for transcriptomic analysis, but is a difficult technique for humans to perform. Recently, two of our labs (Boyden lab at MIT, Forest lab at Georgia Tech) developed a prototype “autopatching” robot (published in 2012 in Nature Methods) that enables automated whole-cell patch clamp recording of neurons in living mouse brain, building off our discovery that blind in vivo whole-cell patching could be reduced to a reliable and stereotyped algorithm. With this device we can electrophysiologically profile neurons in living brain. In a multidisciplinary team with the Allen Institute for Brain Science (Zeng lab), we propose to augment this robot with transcriptomic and morphological analysis capabilities, yielding a platform for the integrative characterization of single cells in intact tissues. In this talk we will discuss our progress on developing image-guided autopatching methods, transcriptomic and morphologic evaluation, scaling up of the autopatcher, and performing integrative cell type analysis.

12. A High Throughput Approach to Develop FRET Biosensors

Yingxiao Wang, Jin Liang, Pengzhi Wang, Yi Wang, Huimin Zhao

University of California at San Diego, San Diego, California

Genetically-encoded biosensors based on fluorescence proteins (FPs) and fluorescence resonance energy transfer (FRET) have enabled the specific targeting and visualization of signaling events in live cells with high spatiotemporal resolutions. Single-molecule FRET biosensors, consisting of a donor and an acceptor FP fused together with two molecular domains capable of interacting with each other, have been successfully developed to monitor the activity of a variety of signaling molecules, e.g. Ca2+, proteases, phosphor-lipids, small GTPases, and tyrosine/serine/threonine kinases. However, several weaknesses of these biosensors have hindered their broader application: (1) the dynamic range of these biosensors is generally limited; (2) the biosensors may interfere with and perturbed by endogenous signaling molecules at certain subcellular locations. At the current stage, the optimization of these FP-based biosensors is rather semi-rational and labor intensive. We have a developed a general high-throughput screening (HTS) method based on directed evolution to develop sensitive and specific FRET biosensors. We have first developed a yeast library and screened for a mutated binding domain for phosphorylated peptide sequence. When this mutated binding domain and the peptide sequence are connected by a linker and then concatenated in between a pair of FRET FPs, a 20-50 fold increase in sensitivity can be achieved. Further work is conducted to improve the specificity of the mutated biosensor via directed evolution. While the results provide the proof-of-concept for this systematic approach of engineering biosensors, this HTS method should be generalized for the development of, in principle, any FP-based biosensor to detect posttranslational modifications. We believe that this will advance the development of genetically-encoded biosensors and impact significantly on live cell imaging and cell biology in general.

13. Spatiotemporal Considerations in Analyzing and Computationally Modeling Single Cells

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In thinking about the value and use of single cell data, whether at the biochemical, transcriptomic, epigenomic, proteomic , or metabolomic levels, key issues are the relationship of a cell's state at one moment to its possible state at some future time, the issue of intracellular location on the activity of catalogued components, and the influence of positioning in tissue space on cell state and behavior. In this overview, I will summarize data on intraclonal variation in protein expression and how this affects signaling upon ligand exposure, discuss evidence for time-dependent state-space traverse of individual cells, highlight a computational approach for modeling the behavior of many individual cells with variations in ground state, describe our new methods for multiplex histo-cytometry for dense phenotyping of individual cells in intact tissues, and suggest how this technology can be combined with multiplex transcript analysis for in situ evaluation of single cell states in complex tissue environments.

14. Live Single Cell Functional Phenotyping in Droplet Micro-Reactors

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The cellular phenotype is a conglomeration of multiple cellular processes, representing varying expression levels of genes and proteins that determine the cell's particular function in activities such as cellular communication, adhesion, or metabolism. Average measurements of such molecular markers in cell populations obscure inherent cell-cell heterogeneity and restrict the ability to distinguish between the responses of individual cells within a sample. While flow cytometry has traditionally been used to determine single cell phenotypes, it cannot provide continuous measurements of proteins in the same individual cells over time. In the presented work, we describe a novel technology that allows stimulation and dynamic characterization of live single cells for surface and secreted protein expressions. This technology should have a broad impact on diverse biological systems for the study of cell surface and secretion proteins as potential biomarkers and targets for diagnostics and therapeutics as well as cell-cell interactions imaging.

Our work provides a platform technology to stimulate specific cellular processes, such as dendritic cell (DC) maturation and Tcell/DC interactions, and to measure the effects of these cellular processes, such as CD-86 and IL-6 expression, in a time dependent manner for single live cells in population. In the future, this technology could be applied to characterize functional phenotypes in various heterogeneous populations at a single cell level. Furthermore, the developed approach allows to dynamically monitoring the interaction events that transpire during cell-cell communication in the immune system on a single cell level to avoid ambiguities that arise due to heterogeneous responses in cell populations.

15. Genetic Tagging of Active Neural Circuits

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Information encoding in the brain is thought to be reflected in the pattern of activation of excitatory neurons in response to a given stimulus. This suggests that, in essence, a neural cell type is defined by the various stimuli and conditions that recruit its electrical activity. Alterations in activity in specific brain regions are associated with a variety of neurological and psychiatric diseases and the pharmacological interventions to treat these diseases alter activity in specific circuits. The cellular and molecular changes that underlie complex cognitive functions such as learning and memory are likely to occur at critical specific points in the circuits activated by the relevant stimuli. A great deal of effort in neuroscience is focused on defining these activated circuits however, currently available techniques are limited to discrete brain areas, lack cellular specificity, or provide a record of activity at only a single time point preventing the identification of consistent patterns of network activation from noise or the identification of network changes over time in response to intervention. I will discuss an approach that uses a single florescent marker to identify neural activity patterns at two independent time points. This provides a number of advantages over existing technology including, the ability to analyze the brain using high throughput automated imaging techniques to identify specific cell populations in brain slices based on their activation patterns in the whole animal for electrophysiological, morphological, or molecular studies.

16. An integrated system to monitor complex tissues at single-cell resolution

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We have recently developed a prototype system for long-term monitoring of single-cell behaviors in complex tissues. Our system comes from the synergistic integration and optimization of two technologies in microscopy and computational cell tracking: the inverted selective plane illumination microscope (iSPIM) and the StarryNite software package. Since joining the SCAP, we have developed iSPIM into dual-view imaging (diSPIM). By acquiring and fusing two orthogonal views, diSPIM compensates for signal loss in thick samples and achieves isotropic resolution of 300 nm, while maintaining 30 to 100 fold improvement over competing 4D imaging technologies, such as spinning disk confocal microscopy, in terms of imaging speed, photo-bleaching and cumulative phototoxicity for long-term imaging. As a result, we have been able to track microtubule tips in 3D in a single cell over extended periods. We also achieved in toto imaging of C. elegans embryogenesis at 1 volume/second for the entire 14 hours and acquire clear images for complete lineaging even after the embryo starts to move. On the software side, we developed methods for systematic measurements of single cell behaviors based on cell tracking in various model organisms. These methods allow rule-based inference of in vivo developmental landscape from images, which includes computationally defined cellular states of differentiation, paths traversing the states, as well as predicted genetic pathways and cell-to-cell signaling events that regulate path choices. We continue our efforts to integrate and package the system and bring it into the hands of the research community as a powerful and versatile tool for single-cell studies in complex, differentiating cell populations.

17. Single-Cell Genomics: Life at the Single Molecule Level

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Isogenic cells lead different genomes because of dynamic changes in DNA, single-nucleotide variation (SNV) and copy number variation (CNV). Single-cell genome sequencing is needed to characterize these variations but has been hindered by whole-genome amplification bias, which results in low genome coverage. We have developed a new amplification method—multiple annealing and looping-based amplification cycles (MALBAC)—that offers high uniformity across the genome. Sequencing MALBAC-amplified DNA achieves 93% genome coverage1x for a single human cell at 25x mean sequencing depth. We detected quantized CNVs of a single cancer cell [1]. By sequencing three kindred cells, we were able to identify individual SNVs with no false positives [1]. We directly measured the genome-wide mutation rate of a cancer cell line and found that purine-pyrimidine exchanges occurred unusually frequently among the newly acquired SNVs [1].

Applying MALBAC to cancer research and detection, we demonstrated the feasibility of whole genome sequencing of circulating tumor cells (CTC). We found that CTCs of the same patient exhibit reproducible CNV gain and loss patterns, which are similar to the pattern of the metastatic site. The combination of CNV and SNV detection of CTCs provides prospect for noninvasive cancer diagnostics and prognostics.

Meiotic recombination creates genetic diversity and ensures segregation of homologous chromosomes. Previous population analyses yielded results averaged among individuals and affected by evolutionary pressures. Using MALBAC, we sequenced 99 sperm from an Asian male and created a map of recombination events at high resolution, which are nonuniformly distributed across the genome in the absence of selection pressure [2]. We phased the personal genome of the donor from the single sperm sequences [2]. Recently, we were able to carry out whole genome sequencing of individual polar bodies, the female counterpart of sperm, phase the genomes of female donors, and identify chromosome abnormality. We demonstrated the proof of principle of preimplantation genomic screen for selecting ovum in *in vitro* fertilization in order to avoid miscarriage and genetic diseases associated with chromosome abnormality.

[1] Zong et al. "Genome-Wide Detection of Single-Nucleotide and Copy-Number Variations of a Single Human Cell," *Science* **338**, 1622 (2012).
[2] Lu et al. "Probing Meiotic Recombination and Aneuploidy of Single Sperm Cells by Whole-Genome Sequencing," *Science* **338**, 1627 (2012).

18. A Phenotypic Signature for Pancreatic Cancer Metastasis

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Pancreatic cancer is one of the most lethal cancers: at the time of diagnosis, > 50% of patients present with metastatic disease typically to the liver and their 5-year survival rate is <3%. Genomic analysis of pancreatic cancer suggests no consensus molecular signatures specific to metastases. Here, we investigated the possibility that pancreatic cancer cells that had successfully metastasized to the liver displayed distinct physical properties from those in the primary tumor. We developed a comprehensive morphological analysis to classify irregular cellular and nuclear shapes using a limited number of common shape modes through eigenshape decomposition and clustering approaches. Using an automated high-throughput microscopy assay, approximately 39,000 cells were analyzed from 13 previously sequenced patient-derived cell lines. Our results show that the lack of cell/nuclear morphological heterogeneity is a highly predictive feature of metastatic pancreatic cancer cells and suggest that metastasis is associated with a selection process for biophysical features.

19. Single-Cell Multiomyx Analysis of Normal and Cancerous Colon Tissues

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Phenotypic characterization of normal and disease cells frequently requires the use of multiple biomarkers to define individual cell populations and to localize specific biochemical activities associated with different cells. However, multiple target detection and quantification from the same biological sample typically requires the loss of valuable morphological and sub-cellular information which confounds analysis of complex phenotypes. We have developed a sequential staining method, referred to as MultiOmyx which circumvents this limitation. MO allows for quantitative, sub-cellular measurement of multiple proteins in paraffin-embedded, formalin-fixed tissues and cells. The process is enabled via mild chemical inactivation of the fluorophore after each imaging step, allowing a finite set of 3-4 fluorophores to be used repeatedly with direct conjugated antibodies. After acquisition of images for all markers, images are precisely registered and compartmentalized to different tissue components, including epithelium, stroma, membrane, nucleus, and cytoplasm using segmentation maps to quantify marker expression at the sub-cellular level for all cells identified in the image. Studies examining the phenotypic heterogeneity in colon cancer and colon stem cell biology are currently under analysis by our group. First, we used MxIF to study 63 targets in individual cells of 747 colorectal cancer (CRC) patients. We performed queries of clinically relevant functional readouts of mTOR (phospho-RPS6 and phospho-4E-BP1) and MAPK (phospho-ERK1/2) in individual CRC cells. Cluster analysis of single cell staining patterns reveals divergent signaling through S6K1 and 4E-BP1 downstream of mTOR and MAPK. Surprisingly, we found that cells exhibiting RPS6 phosphorylation were distinct from the vast majority of cells with mTORC1-mediated 4E-BP1 phosphorylation and ERK1/2 activation loop phosphorylation. In a second study, MO is being applied to analyze changes in a novel marker for epithelial-to-mesehchymal transformation (EMT), PLAC8, during colon cancer progression and acquisition of the EMT phenotype at invasive borders. PLAC8 intracellular distribution is found to be (not a quantitative finding) altered from the usual apical localization of PLAC8 seen in normal colonic epithelium, in tumor cells co-expressing vimentin, cytokeratin, ZEB1, and exhibiting altered expression of cadherins. Finally, a novel mouse model for colon stem cells has been developed. Initial analysis of the model indicates that stem cell-specific expression is obtained in the colon using a promoter to the Lrig1 gene, which has previously been shown to be preferentially expressed in largely non-cycling colon stem cells. This model will be to determine expression patterns for established colon stem cell markers and validation of the single cell algorithms.

20. A Nanowell-Based Integrated Single Cell Analytical Technology for Ex Vivo Characterization of Clinical Samples

J. Christopher Love

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Many immune-mediated diseases—infectious diseases like HIV and autoimmune diseases like multiple sclerosis or diabetes—mediate pathology in specific tissues, yet most of our knowledge about them has resulted from studying cells circulating in blood. Increasing evidence suggests, however, that the biology of diseases in affected tissues can vary substantially from that in the blood, and understanding these differences may be critical to improve patient care. The significant heterogeneities among cells resident in tissues necessitates characterizing such samples with single-cell resolution, but existing technologies routinely employed by clinical immunologists (flow cytometry, ELISpot) require an excess of cells to use for analysis. Their inefficiencies can hinder the ability to pursue science understanding the human biology of diseases and treatments in tissues because biopsies yield very few cells.

We have developed a modular analytical platform for characterizing functions, phenotypes, and in some instances, genotypes with single-cell resolution. This approach emphasizes the conservation of individual cells available from clinical samples, and uses dense arrays of subnanoliter wells (nanowells) to allow multiple measurements for each cell. The widespread adoption of this technology in the clinical research community, however, requires addressing certain critical technical elements identified by end-users. Some of these elements include the throughput of unique clinical samples, breadth of data, data extraction and simple analysis/visualization, and cell recovery for clonal expansion or gene expression analysis).

In this talk, we will present advances on 1) the ability to examine multiple samples concurrently on a single array of nanowells using combinations of physical partitioning and spectral labeling, 2) the breadth of surface-expressed phenotyping on single cells using automated image-based cytometry to a range competitive with conventional flow cytometry (up to 16 unique markers per cell), and 3) a new package of software tools designed to facilitate user-driven exploration of data for selecting rare cells for cell recovery and to reduce the time and expertise required by end users to extract raw data from measurements. Importantly, this set of software establishes an analytical pipeline for integrating multiple datasets generated from a set of single cells. The implications of these advances will be discussed for the deployment of this technology platform, and range of applications, in core facilities using this platform as an alternative to the conventional methods when numbers of cells are sparse or limited, opening up new biology in all areas of human cellular disease and treatments.

21. The Genotype-Tissue Expression (GTEx) Project, an Atlas of Human Gene Expression and Regulation

Simona Volpi, on behalf of The GTEx Consortium

National Institutes of Health, Bethesda, Maryland

The Genotype-Tissue Expression (GTEx) project is an NIH Common initiative to develop an atlas of human gene expression and regulation. The primary goas is to establish a resource database and associated tissue bank in which to study the relationship between genetic variation and gene expression and other molecular phenotypes in multiple reference tissues and to make this resource broadly available for furthering research. This will aid in the interpretation of genome-wide association (GWAS) and other genomic study findings, many of which do not correlate with protein-coding changes, but instead point to regulatory regions of the genome. The GTEx project involves the rapid collection of multiple tissue types from deceased donors. Currently, approximately 30 tissue types are collected from each donor, with the exact number dependent on sex and availability of each organ. When authorized, the cerebral cortex and cerebellum are sampled immediately, and the remaining whole brain is shipped to a brain bank, where 9 additional regions are sampled. Post-mortem donors of any racial and ethnic group and sex who are age 21-70 in whom biospecimen collection can start within 24 hours of death are eligible. Donors are expected to have a typical prevalence of chronic diseases because there are only a few exclusionary criteria, such as human immunodeficiency virus (HIV), viral hepatitis, and metastatic cancer. Many organs will be free of major pathology and serve as a reference set of well-characterized human tissues. Donor enrollment, sample collection and analysis are ongoing, with approximately 200 donors enrolled in a pilot period by the fall of 2012, toward a goal of 900 post-mortem donors by the end of 2015. All donors are characterized for germline genetic variation through dense genotyping of a blood sample and all tissues of sufficient quality undergo gene expression analysis using deep mRNA sequencing. The GTEx resource will be a powerful tool to help unravel the complex patterns of genetic variation and gene regulation across a wide range of human tissues, a critical next step in understanding genomic associations and their translational potential.

22. Organs on Chips: The Future of Translational Research

Kristin Fabre, on behalf of the Microphysiological Systems Consortium

National Institutes of Health, Bethesda, Maryland

Less than 0.1% of the thousands of drug leads will make it to clinical trials, and one may make it to FDA review. This labor and resource intensive process can take up to 15 years and cost billions of dollars. The National Center for Advancing Translational Sciences (NCATS) was established to address this problem and fosters novel methods, technologies and collaborations that enhance success in translational research. Traditional 2-D cell cultures and assays do not address the intricacies of multiple cellular networks. Animal models are useful to an extent, but they do not accurately represent human physiology. The Microphysiological Systems (MPS, or Organs on Chips) Project confronts these challenges by developing platforms to mimic human physiology and will recapitulate the complex environment for human multi-cellular tissues to be studied. Within five years, major organs systems will be developed and applied to the assessment of biomarkers, bioavailability, efficacy, and toxicity of therapeutic agents prior to clinical trials.

MPS will deliver a valid alternative to standard methodologies and will 1) produce human-physiologically relevant findings, 2) significantly reduce the use of animal experimentation, and 3) improve translational research efficacy. Enhancement of induced pluripotent stem cells, biosensor cells, toxicology databases and nutrient delivery systems will also arise from the Organs on Chips project

The NCATS, in conjunction with the NIH Common Fund, is supporting the MPS collaborative agreement and exemplifies the NCATS mission by working closely with investigators from 25+ universities, 15+ NIH Institutes/Centers, the FDA, pharmaceutical companies and DARPA on this pivotal, complex project.

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1. Nanoscale Laser Ablation Capture Mass Spectrometry for Single Cell Proteomics

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Mass spectrometry is a key analysis technique for cell biochemistry, but there are technological barriers in sampling scale that must be overcome for it to be used to its full potential with single cells. The goal of this research is to construct and test a system for nanometer scale laser ablation sampling of single cells and tissue coupled with electrospray ionization mass spectrometry. This system uses apertureless near-field laser ablation to transfer peptides, proteins and other biomolecules to a microdroplet that is used for ultra high sensitivity electrospray ionization. The first part of the project, now underway, is aimed at system setup, validation, and testing with biomolecule standards, which will establish detection limit and spatial resolution benchmarks. Following that, proof of concept testing with cells and tissue will establish a working limit of detection and spatial resolution as well as provide a benchmark sample complexity determination that will aid in designing follow-on experiments. Nanoscale laser ablation sampling developed in this project will have applications not only in mass spectrometry but also in microfluidics. Laser ablation sampling with droplet capture will provide a new method for spatially resolved sampling into a microfluidic device. It will also allow a separation step to be added to mass spectrometry imaging that will enable the imaging of minor biomolecule components of tissue.

2. High Throughput Single Cell Analysis and Cell-by-Cell Imaging by LAESI Mass Spectrometry

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We are developing two new approaches to automatically find and analyze the molecular composition of single cells thin layers and tissues. Images of the cellular structure acquired by optical microscopy are processed by an algorithm to identify individual cells and locate their centroids. The calculated cell coordinates are used for sequential targeting of the cells for mid-IR laser ablation. In the first approach a sharpened optical fiber is used to ablate the cells or tissue resting on a substrate from the front side. The ablation plume is intercepted by an electrospray projecting toward the inlet of a mass spectrometer. In the laser ablation electrospray ionization (LAESI) technique, the charged droplets of the spray are seeded by ablated material and the produced ions are detected by the mass spectrometer. In the second approach the sample is resting on a thin transparent substrate and the laser pulse is focused on the cells by a high numerical aperture aspherical lens from the back side. Ionization of the forward directed ablation plume is imparted by the electrospray. We demonstrate the capabilities of this automated system on thin layers of tissue-embedded cells.

3. Invariance and Variability in an Endogenous Negative-Feedback Circuit

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The importance of a particular element in a biological system is often highlighted by its recurrence. However, most experimental studies of signal transduction have focused on the differences among responses to stimuli. To identify invariant features in the NF-κB signaling network, we treated cells with ligands encompassing a broad range of receptors and concentrations. We then quantified the NF-κB activation of thousands of single cells for up to twenty hours. Most properties of the dynamics varied with either ligand or concentration, but one property had an invariant distribution across all stimuli and concentrations: the period of oscillation. Contrary to existing theory of NF-κB dynamics, this invariance is only observed in the population, while individual cells vary significantly. Comparative analysis of daughter-cell pairs and isogenic populations indicated that the dynamics of the NF-κB response were heritable, but diverged over multiple divisions, on the time scale of a few weeks. Our results point to a paradox: that the invariance we observed in the population depends on variability created as single cells diverge over time.

4. Single Cell Spectroscopy Using Combined AFM-NMR Microcoils

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We present the conjunction of atomic force microscopy (AFM) and nuclear magnetic resonance (NMR) technologies to enable topographical, mechanical, and chemical profiling of biological samples. Here, we fabricate and perform proof-of-concept testing of radiofrequency planar microcoils on commercial AFM cantilevers. The sensitive region of the coil was estimated to cover an approximate volume of 19.4×10^3 μm^3 (19.4 pl). Functionality of the spectroscopic module of the prototype device is illustrated through the detection of 1H resonance in deionized water. The acquired spectra depict combined NMR capability with AFM that can ultimately enable biophysical and biochemical studies at the single cell level.

5. A Microfluidic and Sequencing-Based Method for Profiling mRNA and Protein Levels in Single Cells

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One of the central goals of single cell analysis is the identification of rare and transient cell states. For this goal, a system must satisfy four requirements: it should be scalable to a large number of cells; it should capture multiple measurements per cell such as mRNA, protein levels and protein modifications; it should provide sensitivity and accuracy in these measurements; and it should be performed in tissues, or with cells obtained from tissues for immediate analysis. Today there is a trade-off between analyzing many cells (i.e. scale), and many cellular components per cell (e.g. "-omics"). Methods that analyze many cells, for example FACS, are restricted to a limited number of components whereas "-omics" approaches are too costly and labor-intensive to apply to statistically significant numbers of cells, and for these methods sensitivity remains a problem. A few methods address the accessible "middle ground" of assaying up to 100 cells for a wide but targeted panel of components, but this scale is not sufficient for many of the goals of single cell analysis.

We describe our progress in developing tools for profiling the expression levels of 100-200 mRNA transcripts in at least 1,000 cells per run, with the aim of simultaneously profiling the concentration of tens of proteins at a next stage of method development. Our focus is on a modular, scalable method for immediate use without requiring specialized facilities. The method makes use of reverse-transcription, multiplex PCR and immuno-PCR for a very small number of cycles in 20pL to 200nL volumes in either emulsions or isolated droplets. The resulting 1000-plex libraries are analyzed by Next-Generation Sequencing (NGS). The method cost and accuracy are currently set by NGS alone, ensuring that they will continue to improve. The method is made possible by combinatorial barcoding of samples (combPCR-Seq) that allows combining an arbitrarily large number of samples per sequencing run using a limited number of barcode sequences. Our presentation reviews technical innovations, assays of accuracy and sensitivity, and developmental milestones we have achieved to date.

6. Microwell Arrays for Massively Parallel Transcriptome and Proteomics with Single Molecule Sensitivity

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We are developing new tools for RNA and protein expression profiling that combine microfluidics and single molecule fluorescence microscopy to facilitate sensitive, direct detection of individual RNA transcripts and proteins. Direct detection is highly advantageous for RNA expression analysis of individual cells, because amplification bias confounds accurate quantification, particularly for low input samples. Because proteins cannot be amplified, direct detection is essentially the only option. Both of our technologies take advantage of reversibly sealable microwell arrays fabricated in polydimethylsiloxane (PDMS), which can be chemically functionalized to capture proteins and nucleic acids. For transcriptomics, we use the microwells for highly parallel "printing" of mRNA from individual cells on a glass surface for quantification using single molecule fluorescence detection. For proteomics, we are developing a new approach to protein identification that uses the microwells to capture tryptic peptides on a glass surface for identification using amino acid-specific labels and endopeptidases. This technique will be sensitive enough to identify and detect individual protein molecules without relying on antibodies.

7. Real-Time Visualization of Neural Stem Cell Transcriptome

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Our goal is to develop the necessary tools to analyze the transcriptional activity of single neural stem cells (NSCs) during self-renewal, proliferation and differentiation in vivo. Currently the main challenges of this type of analysis are: 1) the determination of individual cell lineages, which are normally obscured by several cell populations; 2) the assessment of the relevant transcriptome in these processes; 3) the visualization and quantification of gene expression in living tissues in real-time. To circumvent these challenges, we are combining the G-Trace clonal analysis tool, a method that tags individual cell lineages with fluorescent colors according to cell birth order, with Multiplex in situ hybridization, which allows the use a spectral bar coding to visualize the expression of large numbers of genes. Our preliminary results indicate that G-tracing effectively detects individual cell lineages in the central and peripheral nervous system. In addition, we successfully obtained images of individual nuclei contours using antibodies directed to the nuclear Lamin protein. These data reveal that NSCs have large and relatively flat nuclei and are usually adjacent to the much smaller Ganglion Mother Cells and neurons. To identify nuclear shapes and segment these images for quantification, we are developing mathematical algorithms and visualization tools. These tools now allow the generation of 2D segmentations that can be reconstructed as 3D images of individual nuclei using the simple and regular shapes of embryonic nuclei as a training set. Like in the experimental set individual nuclei of the training set were outlined by anti-Lamin. Additionally, to automate the spectral bar coding system to detect multiple nascent transcripts, we used probes labeled with two different fluors targeting a single gene, plus a probe targeting a second gene labeled with only one of the two fluors. These experiments show that the current tools effectively segment individual nuclei and identify co-localizing transcriptional dots. The results obtained so far with the embryonic training set and actual experimental brains are encouraging, since they suggest that with some adjustments these tools should allow the counting cells and sort NSCs from their descendants by size, and monitor transcriptional activity within these cells. The next challenge to be resolved is the complexity of the experimental set, in which the nuclei are ruffled and packed across deep sections within the brain tissue. To overcome these difficulties, we intend to use a mounting media that increases sample translucence and employ multi-layer segmentation to outline these nuclei.

8. Single Cell Analysis of Epigenetic States at Selected Loci

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Epigenetic processes, including transitions in chromatin structure and histone/DNA modifications, orchestrate patterns of gene expression that determine distinct cell phenotypes/functions within muticellular organisms. Aberrant changes in epigenetic states at certain genes contribute to developmental anomalies, aging and multiple diseases. As such changes may occur in single/several cells, particular cell types or an entire organ, the ability to detect epigenetic alterations at individual genes with single cell resolution is important for understanding cell function in vivo. Yet currently available approaches for chromatin analysis, such as widely used chromatin immunoprecipitation (ChIP) assay, yield averaged gene states for cells present in a sample, therefore have limited application to study tissues composed of multiple cell types. New approaches to map epigenetic changes in individual cells will facilitate many areas of epigenetic research and advance understanding of cell function within the heterogeneous environment in vivo. We set out to develop novel method for epigenetic analysis of individual genes in a single cell. Protein recruitment to a gene could be detected by using two-component proximity assay, where component A is tethered to the gene (via DNA probe), and component B is bound to the protein (through antibody). In close proximity, interaction between A and B produces fluorescent signal visible under microscope, and signal intensity is expected to be proportional to the density of protein of interest at the locus. Feasibility of this idea was tested in an application where component A is fluorogenic substrate fluorescein diacetate (FDA) that we attached to streptavidin, and component B is corresponding enzyme, porcine liver esterase that we conjugated to anti-rabbit IgGs. Esterase converts FDA to fluorescein. These reagents were tested in budding yeast model system. First, with biotinylated probes to different loci, we show that FDA-streptavidin produces no detectable signal, however, after reaction with esterase added to solution, bright green foci were observed. Second, we show that esterase, recruited to Sir3 protein, reacts with FDA-streptavidin at a silenced locus and produces detectable fluorescent signal. To quantify signals, Texas Red labeled antibodies that recognize both, FDA and fluorescein were used. Green signal (fluorescein) was normalized to Red signal (FDA + fluorescein), providing an estimate of Sir3p occupancy. For method validation, conventional ChIP assay was used. These data support feasibility of the proposed approach, and provide reagents and protocol for mammalian cell analysis. Method challenges and alternative routes that are currently tested will be also discussed.

9. Histo Mosaic: A Novel Diagnostic Technique to Detect Genetic Mutations in Tissue Slides

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Current cancer diagnostic methods for tissue samples have high throughput but low sensitivity (immunohistochemistry (IHC), in-situ hybridization (ISH), bulk PCR), or high sensitivity but very low throughput (laser capture microdissection (LCM)). We have developed a novel technique for tissue analysis, called HistoMosaic that uniquely combines high throughput and high sensitivity. It leverages semiconductor technology to build millions of in-situ wells on tissue slides, and then runs PCR in each well in parallel. The result is a potentially high-resolution genomic map of the tissue down to single cell resolution depending on the size of the wells. The compartmentalization of tissue directly on glass slide assures that rare mutations are not drowned out by wild-types and spatial information remains intact. We have demonstrated the ability to perform PCR on wild-type KRAS template directly from tissue on top of formalin fixed paraffin embedded slide and are developing assay to detect specific mutations of KRAS gene. This technique could be extended to reverse-transcriptase PCR to create map of gene expression as well. Because the technique uses FFPE tissue slides, we can perform retroactive studies using patient histories and access archival sample to understand mutation influence on outcomes.

10. Quantitative Single-Cell Functional Characterization of CD19-Specific Chimeric Antigen Receptor T-Cells

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Adoptive cell therapy (ACT) utilizing chimeric antigen receptor (CAR) T cells rendered specific for CD19 have demonstrated significant anti-tumor effects in patients with CD19+ chronic lymphocytic leukemia (CLL). In spite of the clinical promise of ACT in achieving complete responses, their efficacy remains unpredictable and new approaches are needed to a priori define the therapeutic potential of T-cell based therapies. In our current work, we characterize the in vitro functionality of CD19-specific (CD19RCD28) CAR+ T cells propagated using artificial antigen presenting cells, by employing a novel methodology single-cell nanowell screening (SNS).We show that CAR+ T cells exert specific cytotoxicity against NALM6 cells (31 ± 8 %) when co-incubated at a 1:1 ratio in nanowell containers. Furthermore, single CAR+ T cells were capable of engaging and killing multiple targets; 17 ± 8% of T cells killed two target cells within the 6 hour window of observation. Hierarchical clustering indicated that interferon-gamma (IFNγ) secretion is not correlated to cytotoxicity of T cells. CAR+ T cells that secreted IFNγ upon target ligation did not undergo activation-induced cell death (AICD) whereas T cells that engaged in repeated killing showed an increased propensity to undergo AICD ($p = 0.04$). In summary, our SNS based methodology allows the deep functional characterization of clinical grade CAR+ T cells and can be used to determine in vitro functions of CAR+ T cells.

11. Fluorescent Probes for Quantitation of Secretory Protein Levels in Single Cells

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Secretory proteins are often robust markers of changes in disease-relevant cellular states including ER stress and metastasis. The absence of technologies for detecting specific luminal secretory proteins in live cells represents a major gap in cell imaging tools. Our goal is to develop and deliver highly sensitive reporters that can detect differences in the expression of diagnostic secretory proteins within a population of live cells. To do this, we will combine three existing technologies to create a new class of imaging tools, STABs (**S**ecretory **T**argeting **A**ptamer **B**eacons). As proof of concept we will focus on developing reagents which when internalized by cells signal the presence of vascular endothelial growth factor (VEGF). Successful results with this secreted protein will be extended to the UPR-induced endoplasmic reticulum proteins Ero1 and ERdj4. Theoretically, up to four distinct fluorescent dyes can be paired with unique aptamers to report on the expression of four different proteins. We envision the probe technology will have utility for basic research and rapid clinical analysis of tissue samples.

12. Rapid Analysis of Single T-Cell Immunity Signatures in Tuberculosis

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We intend to develop a next-generation, host-based diagnostic assay platform. Our platform measures T-cell responses to ex vivo antigen stimulation using fluorescence *in situ* hybridization (FISH) to detect induced expression of T-cell activation markers and flow cytometry to obtain single-cell, multi-parameter readouts. In developing this FISH-Flow platform, we are applying it to the stage-specific diagnosis of the respiratory infection caused by *Mycobacterium tuberculosis*. For this infectious pathology, our assay platform is expected to be far more dynamic and sensitive, and faster (<2 h vs. 24 h) than the IFNγ release assays currently in clinical use. The benefits of this integrated diagnostic platform may extend far beyond tuberculosis, since the methodology developed in the present program applies to detection of receptor-mediated responses in many other infectious and non-infectious pathologies, and will enable timely implementation of medical or public health intervention.

In our initial studies on technical aspects of platform development, we measured expression of key activation markers in peripheral blood mononuclear cells (PBMC) stimulated globally with PMA and ionomycin. Kinetics of IL-2, IFNγ, TNFα and MIP-1α/CCL3 expression in PBMC was monitored by FISH-Flow and conventional immunostaining with mAb followed by flow cytometry. Moreover, the expression of cytokines was assessed by FISH-Flow and microscopy in parallel. Our results demonstrated differences in kinetics showing superior profile discrimination with FISH-Flow and greatly improved sensitivity for detection of activation. Additionally, the FISH-Flow potential for multiplexing and detecting various mRNA targets was demonstrated by two-color flow cytometry and microscopy. To fully define cell functional signatures, knowing expression of all other mRNAs in activated cells vs. non-activated (non-responding) cells may be important. Detailed analysis of activated cells will be presented.

13. Bifurcation Analysis of Single-Cell Gene Expression Data Reveals Epigenetic Landscape

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Waddington's epigenetic landscape has been widely accepted as a fundamental concept to describe the development process in complex systems. However, the molecular underpinnings of the epigenetic landscape remain poorly characterized. Here we present a novel mathematical approach to reconstruct the epigenetic landscape from single-cell gene expression data, combining dynamic clustering and bifurcation analysis. By analyzing two different datasets, obtained from mouse early embryos and bone marrow, respectively, we provide strong evidence that dynamic changes in gene expression during cell differentiation are highly organized and can be well described by the bifurcation theory. Characterization of the epigenetic landscape identified candidate regulators driving the initial events during cell differentiation, without relying on prior knowledge about the underlying gene regulatory network. Furthermore, we predicted the effect of perturbations of transcription factor expression levels on cell differentiation. Our molecular characterization of the epigenetic landscape may serve as a useful guide for future mechanistic studies of developmental regulation.

14. Studying Tumor Initiating Cell (TIC) Heterogeneity and Treatment Resistance by Single-Cell Microfluidics Technology

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We hereby present the implementation of single cell microfluidics proteome analysis to study breast tumor initiating cells (TICs). There is increasing evidence using in vitro and in vivo animal models that CD44+/CD24-/low human breast TICs are resistant to chemotherapy and radiation therapy. We completed a neoadjuvant breast cancer trial that provided strong clinical evidence for the resistance of breast TICs to conventional chemotherapy. These findings indicate that this subset of tumor cells, not affected by standard therapies, may be responsible for tumor initiation and self-renewal; thereby, forming the basis of our hypothesis that these residual cells bearing stem cell-like properties may be responsible for treatment resistance, leading to relapse and metastases. Research work from our group and others has elevated the significance of TIC targeted breast cancer therapy.

However, TICs have a few intrinsic properties that lead to challenges in molecular targeting and inhibition, particularly for personalized treatment. TICs are 1) heterogeneous, 2) dynamically changing states, 3) often rare and 4) lack robust biomarkers/biomarker combinations, hence challenging conventional bulk in vitro experimental tools and complicating the prediction and explanation of animal results. It is clear that high throughput, in situ, and cell-by-cell measurements on TICs are urgently need to fully decipher the TIC molecular signature and signaling pathways. We believe such measurements can lead to 1) revolutionary implementation of TIC models; 2) identification of new therapeutic targets; and 3) reduction of breast cancer recurrence and metastasis. Therefore, we will utilize our Single Cell Barcode Chip (SCBC) platform to characterize protein expression from individual breast TIC. The approach is based on a high throughput microfluidics technology to measure in situ protein production from enriched TICs at single cell sensitivity. Our investigation may lead to discovery of new signaling mechanisms of TIC renewal and survival, and identify new drug targets to eliminate TICs.

15. Integrated Measurements of Individual Natural Killer Cell-Target Cell Interactions

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Natural killer (NK) cells are lymphocytes classically defined by their ability to rapidly recognize and kill virally infected or tumor cells. They can also secrete cytokines that promote the induction of a robust immune response. The efficacy of the NK cell-mediated immune response is determined by the cytolytic and secretory decisions that each NK cell makes after interacting with a target cell. To elucidate how these interactions modulate the functional responses of primary human NK cells, we used arrays of subnanoliter wells (nanowells) to isolate and monitor thousands of individual NK cell-target cell interactions in parallel, and then quantified the resulting cytolytic and secretory responses. We found that short-term secretory responses arising from individual NK cells are correlated with the dynamics of NK cell-target cell interactions but not cytolysis. These results illustrate new properties by which NK cells perform immune surveillance and mediate the elimination of target cells.

16. Multiscale Stochastic Model of Heterogeneous Nanog Expression in Population of Self-Renewing Human Embryonic Stem Cells

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Heterogeneity is an intrinsic attribute of stem cell populations that is commonly unappreciated. Yet, heterogeneous ensembles of stem cells exhibit responses that vary among individuals in the population when presented with stimuli for self-renewal or differentiation. Sources contributing to the diversity of stem cells within populations include gene regulatory network (GRN) noise and structure, stochastic partitioning during mitosis as well as the kinetics of protein synthesis and degradation. Better understanding of the effects of these sources on the inhomogeneity of stem cell populations and the capacity for multilineage differentiation necessitates the coupling of rationally designed experiments with the development of multiscale computational frameworks[1].

We investigated how partitioning and allelic regulation impacts the expression profile of the pluripotency marker NANOG in ensembles of human embryonic stem cells (hESCs). In conjunction with experiments on hESC cultures, a multiscale cell population balance equation model was constructed accounting for gene expression noise and stochastic partitioning at division as sources of NANOG heterogeneity in the hESC population[2]. Data on the physiological state vector variables -cell size and NANOG- of single cells were acquired through flow cytometry. Elimination of transcriptional noise led to greater changes in the dispersion of the NANOG distribution. Blocking hESC division by treatment with nocodazole or colcemid caused a shift in NANOG distribution with over 68% of growth-arrested hESCs exhibiting higher NANOG expression than the mean level of untreated cells. Model predictions corroborated these findings revealing for the first time that stochastic partitioning accounted for 17% of the total noise in the NANOG profile of self-renewing hESCs.

In addition to mitotic partitioning and transcriptional noise, allelic control of Nanog expression was also considered. The multiscale model predicted three distinct states for the distribution of Nanog collapsing to a bimodal profile upon increased transcriptional noise in line with experimental observations. Allelic transcription of Nanog also explains the significant divergence in the native protein profiles and reporter (e.g. GFP) in stem cell lines featuring a reporter transgene knocked in the locus of a target gene.

This computational framework will aid in gaining a deeper insights on how pluripotent stem/progenitor cells orchestrate processes (e.g. gene expression, proliferation) for maintenance of pluripotency or differentiation along particular lineages. Extension of our approach by incorporating additional GRNs involved in fate decisions will greatly enhance our ability to predict and control differentiation and reprogramming outcomes in cell populations under different conditions.

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17. An Excitable Signal Integrator Couples to an Idling Cytoskeletal Oscillator to Drive Cell Migration

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Cell migration is mediated by the stochastic appearance of protrusions. While Scar/Wave and actin binding proteins as well as Ras, PI3K, and Rac activation appear on these protrusions, the roles of these events are unclear. Here we show these activities fall into distinct cytoskeletal and signal transduction networks. The cytoskeletal network alone displays rapid oscillations that drive small amplitude undulations at convex regions of cell boundary. In contrast, the signal transduction network is excitable, activating stochastically every few minutes even when the cytoskeleton is blocked. Protrusions coincide with the active phase of the signal transduction network but expand with short bursts of cytoskeletal activity. We propose that protrusions result from coupling of “pacemaker” signal transduction and “idling motor” cytoskeletal networks. Consistently, uniform inhibition of multiple signal transduction pathways eliminates the large protrusions while a gradient of inhibition repels cells. Simulations of coupled slow and fast networks generate realistic migratory behavior.

18. Functional Interplay Between Cell Cycle and Cell Phenotypes

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Cell cycle distribution of adherent cells is typically assessed using flow cytometry, which precludes the measurements of many cell properties and their cycle phase in the same environment. Here we develop and validate a microscopy system to quantitatively analyze the cell-cycle phase of thousands of adherent cells and their associated cell properties simultaneously. This assay demonstrates that population-averaged cell phenotypes can be written as a linear combination of cell-cycle fractions and phase-dependent phenotypes. By perturbing cell cycle through inhibition of cell-cycle regulators or changing nuclear morphology by depletion of structural proteins, our results reveal that cell cycle regulators and structural proteins can significantly interfere with each other's *prima facie* functions. This study introduces a high-throughput method to simultaneously measure cell cycle and phenotypes at single-cell resolution, which reveals a complex functional interplay between cell cycle and cell phenotypes.

19. Selective Capture and On-Demand Local Release of Rare Cells in Stimuli-Responsive Microfluidic Channels

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Microfluidic label-free selective capture and on-demand local release of cells has broad applications in rare cell isolation, stem cell purification for regenerative medicine, proteomic/genomic analyses, cancer research, and clonal studies, with down to single cell resolution and sensitivity. Selective capture of cells using microfluidics has been a rapidly growing field with clinical applications in medical diagnostics. Although capturing cells in microchannels has been achieved, spatiotemporal control of capture and on-demand local release of cells still remains as a challenge in microfluidics. The advances in stimuli responsive smart interface materials have enabled new functionalities in controlling the material-cell interactions. Here, we present a microfluidic system integrated with stimuli responsive smart interface material (poly(N-isopropylacrylamide)) and thermoelectric local temperature control, enabling both spatial and temporal control over selective capture and on-demand release of selective rare cells in microchannels from unprocessed whole blood. We developed biotin-binding protein and biotinylated antibody based surface chemistry on stimuli-responsive microfluidic channels for highly specific separation of individual cell populations from blood based on cell surface antigen expression. This platform allows not only detection of rare cells in a highly heterogeneous population (*e.g.,* $CD34^+$ endothelial progenitor cells in blood), but also label-free isolation and retrieval with significantly enhanced sensitivity and specificity.

20. Differential Expression of Neuron-Glial Antigen 2 (NG2) and Melanoma Cell Adhesion Molecule (CD146) in Heterogeneous Cultures of Mesenchymal Stem Cells

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Cellular heterogeneity of mesenchymal stem cells (MSCs) impedes their use in regenerative medicine. The objective of this research is to identify potential biomarkers for the enrichment of progenitors from heterogeneous MSC cultures. To this end, the present study examines variation in expression of neuron-glial antigen 2 (NG2) and melanoma cell adhesion molecule (CD146) on the surface of MSCs derived from human bone marrow in response to culture conditions and among cell populations. Multipotent cells isolated from heterogeneous MSC cultures exhibit a >3-fold increase in surface expression for NG2 and >2-fold increase for CD146 as compared with parental and lineage-committed MSCs. For both antigens, surface expression is downregulated by ≥6-fold when MSCs become confluent. During serial passage, maximum surface expression of NG2 and CD146 is associated with minimum doubling time. Upregulation of NG2 and CD146 during loss of adipogenic potential at early passage suggests some limits to their utility as potency markers. A potential relationship between proliferation and antigen expression was explored by sorting heterogeneous MSCs into rapidly and slowly dividing groups. Fluorescence-activated cell sorting revealed that rapidly dividing MSCs display lower scatter and 50% higher NG2 surface expression than slowly dividing cells, but CD146 expression is comparable in both groups. Heterogeneous MSCs were sorted based on scatter properties and surface expression of NG2 and CD146 into high (HI) and low (LO) groups. $Sc^{LO}NG2^{HI}$ and $Sc^{LO}NG2^{HI}CD146^{HI}$ MSCs have the highest proliferative potential of the sorted groups, with colony-forming efficiencies that are 1.5-2.2 times the value for the parental controls. The Sc^{LO} gate enriches for rapidly dividing cells. Addition of the $NG2^{HI}$ gate selects for enhanced cell survival. Further addition of the $CD146^{HI}$ gate does not significantly improve cell division or survival. The combination of low scatter and high NG2 surface expression is a promising selection criterion to enrich a proliferative phenotype from heterogeneous MSCs in subconfluent cultures. There are numerous basic research and clinical applications for this sort strategy.

21. A Phenotypic Signature for Pancreatic Cancer Metastasis

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Ninety percent of all cancer-related deaths are caused by metastatic disease, i.e. the spreading of a subset of cells from a primary tumor in an organ to distal sites in other organs. Understanding this progression from localized to metastatic disease is essential for further developing effective therapeutic and treatment strategies. However, despite research efforts, no distinct genetic, epigenetic, or proteomic signature of cancer metastasis has been identified so far. Metastasis is a physical event: through invasion and migration through the dense, tortuous stromal matrix, intravasation, shear forces of blood flow, successful re-attachment to blood vessel walls, migration, the colonization of a distal site, and, finally, reactivation following dormancy, metastatic cells may share precise physical properties. Cell morphology is the most direct physical property that can be measured. In this work, we develop a high throughput cell phenotyping process and investigate the morphological signature of primary tumor cells and liver metastatic pancreatic cancer cells.

22. Single Copy/Cell Analysis of Rare Mutations Using High-Throughput Microfluidic Digital PCR

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Single cell analysis technology enables resolving the rare biological events masked by the ensemble average, thus providing a promising tool for the investigation of early-stage biomarkers of carcinogenesis. We have developed a microfluidic digital PCR technology as a core platform for high-throughput single cell/copy genetic analysis (SCGA) of rare mutations. Using this approach, we first demonstrated the feasibility of SCGA for pre-clinical diagnosis of blood cancer by quantitative detection of t(14;18), a chromosome translocation associated with lymphoma, with a detection limit of one copy in 10 million genomes. This single molecule resolution approach confers the ability to quantify and sequence unique genetic variants that occur in individual patients, which led to the discovery of new clonal forms of t(14;18) undetectable for conventional quantitative PCR. Furthermore, we have adapted the microfluidic digital PCR technology to multi-locus detection and sequencing of genes in single cells for high-throughput analysis of mutation co-localization. Thus our SCGA technology would open new opportunities for the study of early-stage carcinogenesis and rare tumor cells important for metastasis (i.e., circulating tumor cells).

23. From Cellular Characteristics to Disease Diagnosis

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Cell heterogeneity and the inherent complexity due to the interplay of multiple molecular processes within the cell pose difficult challenges for current single-cell biology. Here, we introduce a novel approach that identifies a disease phenotype from multiparameter single-cell measurements, which is based on a cell-averaging procedure combined with a standard machine learning classification scheme. We are able to assess the optimal tradeoff between the number of single cells averaged and the number of measurements needed to capture phenotypic differences between healthy and diseased patients, as well as between different diseases that are difficult to diagnose otherwise. We validate our approach on two kinds of single-cell datasets, addressing the diagnosis of a premature aging disorder using images of cell nuclei, as well as the phenotypes of two non-infectious uveitides (the ocular manifestations of Behçet's disease and sarcoidosis) based on multicolor flow cytometry. Beyond these specific examples, the approach proposed here is applicable to datasets generated by other kinds of current, state-of-the-art and forthcoming single-cell technologies, such as multidimensional mass cytometry, single-cell gene expression, and single-cell full genome sequencing techniques.

24. A Dual-function Nanopore Biomimetic Device Quantitatively Detects Single Breast Cancer Cells; a Contour Map of Multiple Variable Correlation Method Assesses the Heat Released by Cancer Cells

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We report a portable dual-functioning low abundance single cancer cell detection device that has the capability to detect the breast cancer MDA-MB-231 line in a single cell concentration quantitatively using the unique land marker ratio of “Action Potential” vs. “Resting Potential” (RAPRP) values that distinguish from the normal breast cells under antibody-free and labeling-free conditions. The device is based on a nanopore biomimetic cross linked polymer membrane fabricated on gold chips. The real time *in vitro* “cell potential” was recorded in 4 seconds using a double step chronopotentiometry (DSCPO) under a fixed current in cell culture media without prior sample preparation. The first order rate constant of the device to detect live cancer cells is 530 mV/cell (mL-1) over 1-50 cell/mL range after 24 hrs incubation (the device acts as an energy storage device). A Chronoamperometric method (CA) (the device acts as a sensor) further confirmed the quantitation over a linear range of 1-100 cell/mL with a sensitivity of 80 µA/cell.mL-1. Factors of cancer cell concentration and current affect on the DSCPO profiles are reported. The heat released by the cancer cells is an order of magnitude higher in calories compared with a normal live breast cell assessed by a Contour Map of Multiple Variable Correlation method (CMMVC).

A Cyclic Voltammetry (CV) method was conducted to characterize the potential utility at the clinical settings of the device in situ. NIST human standard sera SRM 965A with a certified known blood glucose concentration was used for spiked cancer cells. The preliminary results revealed the cancer cell signaling were blocked by 53 and 100% after the second and third pulses at 100 µA.

25. Methods for isolation and analysis of single circulating tumor cells (CTCs)

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The concept of 'liquid biopsy' refers to analysis of rare cells in the blood such as circulating tumor cells (CTC) and represents a powerful tool for molecular characterization of tumors for which biopsies are not available. To improve prognosis and better guide treatment, an urgent need exits for improved technologies for detection (isolation) and molecular characterization of CTCs at the single cell level. We are developing methods for both isolation and analysis of single CTCs.

For cell isolation, we have developed an instrument capable of rapid and accurate detection of rare cells in circulation utilizing fiber-optic array scanning technology (FAST). The FAST cytometer can locate immunofluorescently-labeled rare cells on glass substrates at scan rates 500 times faster than conventional automated digital microscopy. Key advantages of the FAST method are that no single-protein enrichment strategy is required and the cells are localized on a fixed substrate where subsequent imaging and analysis can be performed on individual cells. All nucleated cells are retained and immunofluorescently stained with monoclonal antibodies targeting cytokeratin (CK), an intermediate filament found exclusively in epithelial cells, a pan leukocyte specific antibody targeting CD45, and a nuclear stain, DAPI. Following identification by FAST, CTCs are imaged in multiple fluorescent channels to produce high quality and high resolution digital images that retain fine cytologic details of nuclear contour and cytoplasmic distribution .This enrichment-free strategy results in high sensitivity and high specificity, while adding high resolution cytomorphology enables detailed morphologic characterization of a CTC population known to be heterogeneous.

For cell analysis, we are developing a single cell multiplexed immunophenotyping and genomic analysis assay for characterization of important cancer markers in CTCs. By applying nanodroplet volumes of reagents to a FAST substrate, we can perform polymerase chain reaction (PCR) in situ for rapid multiplexed analysis of DNA or RNA of CTCs using PCR and reverse transcription PCR (RT-PCR). Laser heating is used to perform PCR directly on the substrate without heating neighboring cells. In situ analysis minimizes loss and contamination possible when removing cells from the substrate.

We are expanding the integration of the single cell analysis platform with other compatible single-cell-analysis platforms (SCAP) to allow genomic, metabolomic, proteomic and mutation analysis at the individual cell level. Ultimately this technology has the potential to serve as a clinically useful diagnostic and a prognostic tool for cancer clinicians.

26. Method for Detection of Secreted Proteins in Single Cell Assays

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A major goal of biological research is to provide a greater understanding of human physiology as it relates to pathological process involved in disease. Because cytokines play an important role during inflammation and disease, they are the best tool to measure the activation of immune cells. Measurements of cytokines are not without problems; their detection is hampered by their biological properties, e.g. local secretion, rapid uptake and utilization, and short half-life. These properties have led to the development of limited techniques commonly used to measure cytokines based on single cell level, such as flow cytometry, intracellular cytokine staining (ICCS), and enzyme-linked immunospot (ELISPOT) assays. These methods for single cell assays require complex procedures, cell treatment, and inconvenient enzymatic or chemical signal amplification. Moreover, none of above methods has real-time detection capability and potential direct quantification of secretion of cytokines.

Our goal is to develop the method for single cell assays that is simpler in biochemical procedure, provides new capabilities such as real–time monitoring of secretion and direct quantification of secreted proteins. The method called MEFspot relies on the metal-enhanced fluorescence (MEF) using plasmonic substrates. We developed plasmonic substrates resulting in signal enhancement up to 200-fold allowing detection and monitoring of secreted proteins from single cells.

Here we present our approach for nanofabrication of plasmonic substrates and their performance for optical signal amplification. Next we demonstrate their use and methodology for single cell assays; sensitivity in the range of single pg/ml, real-time monitoring of secretion, capability for direct quantification of secretion, and capability for visual analysis of secretion.

We envision that MEFspot method can be an excellent alternative to other methods for single cell assays providing additional valuable features as described above.

27. Robust Synthetic Aperture Phase Microscopy For Label-Free Single Cell Analysis

Remy Tumbar

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I present quantitative phase images of phase calibration targets and fixed cells obtained with a robust quantitative microscope for amplitude, phase and polarization (qMAPP) in a synthetic aperture (SA) modality. This is, to my knowledge, the first demonstration of SA microscopy with a robust system using common video microscopy hardware. This method may allow reliable cell state (proliferative, differentiated, quiescent, senescent or apoptotic) or cell type identification, without the use of specific fluorescent markers.

Quantitative phase microscopy (QPM) has been shown to reveal the structure of single cells without the use of labels/stains. Unfortunately, its practical application is severely hampered by the difficulty of accurately measuring phase, while dealing with speckle noise due to source coherence and phase noise due to insufficient shielding in typical bio-lab conditions. In addition, background phase bias is difficult to remove with high accuracy when the field of view is essentially covered by the target.

To address these issues, the qMAPP system uses a novel robust (insensitive to vibrations and misalignment) yet sensitive interferometric phase and polarization sensor, the sampling field sensor (SFS). Interferometric data is acquired at different illumination angles in transmission. The image is computationally generated from the coherent synthesis of individual frames and is background and speckle-noise-free, despite using a high-coherence laser source.

The SFS robustness allows simple background removal using a calibration frame acquired in a clear region of the target. It generates intrinsically phase coherent data, thus eliminating the need for “phasing” the individual images. Quantitative phase calibration is demonstrated in images of five-micron-diameter polystyrene microspheres in microscope immersion oil. Comparison with conventional (non SA) QPM shows the latter underestimating the phase under moderate to high NA illumination conditions.

Keratinocytes were plated in chambered coverslips and fixed in formalin after one day incubation. Z-stacked images demonstrate simple optical sectioning. The major cellular structure (cytoplasmic body and organelles, nucleus and nucleoli) is clearly visible. The consistent background rejection capability permits clear visualization, via “virtual staining” using the phase alone (non tomographic), of structures of similar refractive index. For example, comparison with typical images of fluorescently labeled cells indicates that keratin fiber structures are visible and morphologically different in differentiated and undifferentiated cells, which may be useful for simple cell state identification. Work is underway to develop optical tomography algorithms using this method, which will provide the full 3D refractive index map of single cells, thus presenting a label-free “virtually stained” image.

This work was supported in part by NIH Grant Number R21RR023117.

28. Isoforms are transcribed in a developmental stage-dependent manner in the early embryogenesis in C. elegans

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In order to understand the developmental genetic programs and determine the signature genes of each cell during the C. elegans embryogenesis, we applied the high-throughput sequencing to precisely staged embryos at single-cell resolution. After collecting single blastomeres at 4-cell, 5-cell and 16-cell stage embryos, RNAs-seq libraries were prepared and sequenced on an Illumina HiSeq platform. Transcription abundance was quantified in RPKM for each transcript of all annotated protein coding genes. In each cell, around 20% to 40% of the annotated genes were detected, and the cells in earlier stages transcribe more genes than later stages. Our data also showed that nearly half of the genes were not transcribed in all stages analyzed, around 10% genes were unique to a certain cell, and 15% genes were ubiquitously transcribed in all the cells. The Pearson's correlation coefficient of the transcription level was on average higher between cells in the same stage (0.70) than that between different stages (0.52). Besides, PCA analysis of the transcription level showed that the cells from the same stage were likely to be clustered together. However, the correlation coefficient could not always separate cells successfully by stages, as in some cases the transcription level was highly varying among cells from the same stage and in some cases it was also similar between different stages. As isoforms are known to be associated with cell types, we compared the transcription levels of isoforms among cells. A total of 11,729 isoforms from 4,163 genes that were detected at least in one cell were included in our study. We found that more than one isoforms of a gene could be transcribed in a single cell, although in most cases one isoform was dominantly transcribed. Interestingly, the dominant isoform was specific to developmental stages. Specifically, cells from the same stage could be clustered together by just using the information of each isoform is dominantly transcribed or not for each gene. Besides, the percentage of the contribution from either the dominant or the non-dominant isoforms to a gene's total transcription level was also cell stage specific. These results suggest that the alternative splicing mechanism might be similar in cells from the same developmental stage. It will be very interesting to understand why the cells maintain a certain ratio between the transcription levels of isoforms of a gene and how it is regulated in a stage-dependent manner.

SPEAKER BIOSKETCHES

Nancy Allbritton, M.D., Ph.D.

Dr. Allbritton obtained her B.S. in physics from Louisiana State University, her Ph.D. in Medical Physics/Medical Engineering from the Massachusetts Institute of Technology, and her M.D. from the Johns Hopkins University. After a postdoctoral fellowship in cell biology at Stanford University, she joined the faculty of the University of California at Irvine in 1994 where she held joint appointments in the Departments of Physiology and Biophysics, Biomedical Engineering, Chemistry, and Chemical Engineering & Materials Science. She has received multiple awards including a Beckman Young Investigator Award, and a Searle Scholar Award. She joined the University of North Carolina at Chapel Hill (UNC) as the Debreczeny Distinguished Professor in the Department of Chemistry in July, 2007. In 2009, she was appointed Professor and Chair of the Department of Biomedical Engineering, a joint department between the School of Medicine at UNC and the College of Engineering at North Carolina State University. Dr. Allbritton's research studies are directed at the development of new technologies by bringing to bear methods from engineering, chemistry, physics and biology to address biomedical problems. Dr. Allbritton is the scientific founder of two companies, Protein Simple and Cell Microsystems, and has 8 issued patents with over 20 more pending.

Zhirong Bao, Ph.D.

Dr. Bao received his PhD in Genetics from Washington University and completed his postdoc training at the University of Washington. During his postdoc, he established live imaging techniques for C. elegans embryogenesis and image analysis software for systematic cell tracking and lineage tracing. His group at the Sloan-Kettering Institute continues to develop technologies for systematic cell tracking in complex tissues and automated analysis of complex in vivo phenotypes at single-cell resolution.

Edward Boyden, Ph.D.

Dr. Boyden is Associate Professor of Biological Engineering and Brain and Cognitive Sciences, at the MIT Media Lab and the MIT McGovern Institute. He leads the Synthetic Neurobiology Group, which develops tools for analyzing and engineering the circuits of the brain. These technologies, created often in interdisciplinary collaborations, include 'optogenetic' tools, which enable the activation and silencing of neural circuit elements with light, 3-D microfabricated neural interfaces that enable control and readout of neural activity, and robotic methods for automatically performing single-cell analyses in living brain. He was named to the "Top 35 Innovators Under the Age of 35" by Technology Review and has received the NIH Director's New Innovator Award, the Society for Neuroscience Research Award for Innovation in Neuroscience, the Paul Allen Distinguished Investigator Award, the New York Stem Cell Foundation-Robertson Investigator Award, the Perl/UNC prize, the IET Harvey Prize, and other recognitions, including having his work featured in 2010 as the "Method of the Year" by Nature Methods. Ed received his Ph.D. in neurosciences from Stanford University as a Hertz Fellow, and before that received three degrees in electrical engineering and physics from MIT.

Long Cai, Ph.D.

Dr. Cai received his Ph.D. at Harvard with Sunney Xie in single molecule detection of gene expression in single cells. He then did his postdoctoral work at Caltech as a Beckman Institute fellow in the laboratory of Michael Elowitz in 2006, where he worked on signaling in single yeast cells and discovered that many pathways operated in a frequency modulated fashion in response to extracellular signals. Long started his laboratory in the division of Chemistry and Chemical Engineering at Caltech in July 2010.

James Eberwine, Ph.D.

Dr. Eberwine is the Elmer Holmes Bobst Professor of Pharmacology at PENN where he also serves as Co-Director of the PENN Genome Frontiers Institute. Dr. Eberwine coined the phrase "expression profile" to describe the relative abundances of RNAs. Further he and his colleagues were the first to identify the large complexity of RNAs that are targeted to neuronal dendrites; directly demonstrate protein synthesis in dendrites; prove that membrane proteins could be synthesized in dendrites and inserted into the membrane to form functional receptors; identify the largest verified lists of in vivo RNA cargoes for specific RNA binding proteins; to demonstrate the capacity for and functional requirement for dendritic splicing of RNAs and to show that RNA expression profiles could be transferred between cells carrying their phenotypic memory with them. These research accomplishments were possible because of technology developed by Dr. Eberwine including: single cell PCR, the aRNA procedure, the phototransfection methodology, the PAIR procedure and the functional genomics TIPeR methodology as well as other single cell analysis technologies. Dr. Eberwine originated the Cold Spring Harbor Summer Course entitled "Advanced Techniques in Neuroscience" in 1988. In 2012, he developed and Co-Directed the first Cold Spring Harbor Course on "Single Cell Techniques".

Michael Gerdes, Ph.D.

After starting his current role at GE-GR Dr. Gerdes was tasked with developing a way in which fixed tissues could be probed with 10 or more antibodies. The result is a novel platform for iteratively probing paraffin sections with dye-conjugated antibodies, with an inactivation of the dyes following image acquisition that allows re-probing with a different dye-conjugated antibody. Much of his work has been histological/immunohistochemical in nature, with an emphasis on the characterization of altered epidermal biology. His expertise in cancer biology is in mechanisms for cell differentiation and the role of the tumor microenvironment in cancer progression. Dr. Gerdes recently concluded the coordination of efforts by GE in an NIH ARRA project with Dr. Larsen of SUNY-Albany to produce a developmental atlas of glandular morphogenesis (manuscript in preparation) that includes mapping of salivary progenitor cells in ducts and acinar structures over time. As the technology has advanced significantly over 6 years, he is now applying it to numerous biomarker discovery studies in breast cancer, colon cancer, and lung cancer. Dr. Gerdes is currently leading an NIH-RO1 for the Single Cell Analysis Program that utilizes the multiplex methodologies and image analysis routines.

Ronald Germain, M.D., Ph.D.

Dr. Germain received his M.D. and Ph.D. from Harvard University, the latter for research with B. Benacerraf, recipient of the 1980 Nobel Prize in Physiology and Medicine. Since that time, he has investigated basic T-cell immunobiology, first on the faculty of Harvard Medical School and, since 1982, as the Chief, Lymphocyte Biology Section in the Laboratory of Immunology and now

as Chief of the new Laboratory of Systems Biology at the NIAID, NIH. His team's early work contributed to understanding of Major Histocompatibility Complex (MHC) class II molecule structure–function relationships, the cell biology of antigen processing, and the molecular basis of T cell recognition. More recently, his lab has focused immune tissue organization and dynamic control of immunity utilizing *in situ* microscopic live animal and multiplex histochemical imaging methods that we helped pioneer. The laboratory also conducts studies involving computer models of immune system function and uses the tools of systems biology to explore host-pathogen interactions. Dr. Germain is an Associate (foreign) member of EMBO (2008), received the Landsteiner Medal of the Austrian Society for Allerology and Immunology (2008), was elected honorary member of the Scandinavian Society for Immunology, designated an NIH Distinguished Investigator, and elected fellow of the AAAS. He serves as an associate or advisory editor of the J Exp Med, Immunity, Current Biology, Mol Systems Biol, Int Immunol, BMC Biology, and Nature Scientific Reports and am an Associate Director for the trans-NIH Center for Human Immunology.

James Knowles, M.D., Ph.D.

Dr. Knowles is Professor and the Associate Chair for research at the Department of Psychiatry and the Behavioral Sciences at the Keck School of Medicine and a member of the Zilkha Neurogenetics Institute (ZNI). He is both a board-certified psychiatrist and a well-established psychiatric geneticist with years of experience in large-scale collaborations. He has cloned the genes for 2 Mendelian disorders, Retinitis Pigmentosa and Primary Pulmonary Hypertension (PPH) and studies the genetics of multiple complex disorders (Panic Disorder, Nicotine Addiction, Opiate Addiction, Early-Onset Depression and Obsessive-Compulsive Disorder). His laboratory also is facile with the techniques for determining the function of disease genes (transgenic mice models, cell culture, yeast two- hybrid screening, in situ hybridization, Confocal microscopy, etc.). He is also an expert in Next-Generation Sequencing (NGS) and was one of the PIs of the BrainSpan project (BrainSpan.org). His laboratory is also a leader in the analysis of NGS data, and he is one of the co-PIs of an award by NHGRI to make and distribute software for NGS analysis.

Tania Konry, Ph.D.

Dr. Konry is currently an Instructor at Harvard Medical School and an Assistant in Bioengineering and co-leader of Nano- and Microsystems Bioengineering group at The Center for Engineering in Medicine at Massachusetts General Hospital (MGH). The goal of her research program is focused towards merging of microspheres and microfluidic technologies for the development of "lab-on-a-chip" platforms with relation to bianalytical and cellular analysis. Such a technology would have a big impact on understanding biological processes, which are inherently dynamic. In particular, her work is focused on development of a microfluidic droplet approach for dynamic monitoring of the biochemical events that transpire during cell-cell communication in the immune system on a single cell level to avoid ambiguities that arise due to heterogeneous responses in cell populations. Once the complex interactions and signaling outcomes at cell-cell interactions are defined, it should be possible to build multiple co-stimulatory signals and block the inhibitory pathways into vaccines in an appropriate combinatorial fashion. She was awarded with a competitive CIMIT Innovation Grant to support her collaborative research projects on bio-assay and devices, procedures and diagnosis for improving patient care as well as R21/NIH/NCI grant as principal investigator to study the biochemical event at single cell level.

J. Christopher Love, Ph.D.

Dr. Love is an associate professor in chemical engineering and member of the Koch Institute for Integrative Cancer Research at MIT. In addition, he is an associate member at the Eli and Edythe L. Broad Institute, and at the Ragon Institute of MGH, MIT, and Harvard. Dr. Love received his Ph.D. in 2004 in physical chemistry at Harvard University. He extended his research into immunology at Harvard Medical School from 2004-2005, and at the Immune Disease Institute from 2005-2007. His research centers on using simple microsystems to measure and correlate multiple phenotypic and functional characteristics of individual lymphocytes, and from these single-cell data, generate quantitative, system-wide profiles of immune responses. Current areas of research in the lab include i) multiplexed, functional profiling of lymphocytes from HIV+ patients, ii) clonal analysis of autoreactive T cells and B cells in type 1 diabetes and multiple sclerosis, and iii) development of quantitative, cell-based diagnostics for allergy testing. In addition, his lab is understanding how to engineer microbial strains for manufacturing therapeutic antibodies. Dr. Love was named a Dana Scholar for Human Immunology and a Keck Distinguished Young Scholar in Medical Research in 2009, as well as one of Popular Science's Brilliant 10 in 2010. Dr. Love is also a Camille Dreyfus Teacher-Scholar.

Mark Mayford, Ph.D.

Dr. Mayford received his PhD in Molecular Biology from the University of Wisconsin-Madison. His thesis focused on the translational regulation of prokaryotic gene expression. He did post-doctoral work with Dr. Eric Kandel at Columbia University where he developed mice that allowed inducible expression of transgenes in the brain and used this approach to show that Ca2+/calmodulin kinase was critical for learning in the adult brain independent of its role in development. He moved to University of California San Diego as an Assistant Professor in Neuroscience in 1997 and to the Scripps Research Institute in 2000 where he is Associate Professor in the Department of Molecular and Cellular Neuroscience. He has continued to use mouse genetics to try to understand the cellular and molecular mechanisms of memory. His recent work has focused on genetic manipulation of neural circuits that are activated specifically during the learning process.

Deirdre Meldrum, Ph.D.

Dr. Meldrum received B.S. Civil Engineering from University of Washington (UW), 1983; M.S. Electrical Engineering from Rensselaer Polytechnic Institute, 1985; Ph.D. Electrical Engineering from Stanford University, 1993; and completed the Stanford Executive Program, 2009. At the NASA Johnson Space Center, 1980-1981, she was an instructor for the astronauts on the Shuttle Mission Simulator. From 1985-1987, at the Jet Propulsion Laboratory, she worked on the Galileo spacecraft, large flexible space structures, and robotics. At UW, 1992-2006, she was Professor of Electrical Engineering and Director of the Genomation Laboratory. As Dean of the Ira A. Fulton Schools of Engineering, Arizona State University, 2007-2010, Meldrum transformed the engineering school into an organization fully aligned with the U.S. grand challenges. She is currently ASU Senior Scientist, Director of the Biosignature Initiative, Director of the Center for Biosignatures Discovery Automation in the Biodesign Institute, and Professor of Electrical Engineering. She is PI/Director of the NIH Center of Excellence in Genomic Science: Microscale Life Sciences Center, 2001-2013. Her research interests include single-cell analyses, genome automation, microscale systems, biosignatures, ecogenomics, and robotics. Her honors include: NIH Special Emphasis Research Career Award, 1993; Presidential Early Career Award for Scientists and Engineers, 1996; AAAS Fellow, 2003; IEEE Fellow, 2004;

member of the National Advisory Council for Human Genome Research, 2006-2008, 2011-present; member of the advisory board for external research for Microsoft Research Connections, 2007-present.

Nicholas Navin, Ph.D.

Dr. Navin is currently a tenure-track assistant professor at the M.D. Anderson Cancer Center where he holds a dual appointment in the Department of Genetics and the Department of Bioinformatics. He conducted his postdoctoral training at the Cold Spring Harbor Laboratory and graduate training at Stony Brook University. The Navin laboratory is interested in understanding clonal diversity and genome evolution in human tumors, with a strong focus on breast cancer. Intratumor heterogeneity is a major problem that confounds the clinical diagnosis and basic research of human cancers, because bulk genomic methods are limited to reporting an average signal from a complex population of cells. To address this problem the Navin laboratory is actively developing single-cell sequencing tools to identify mutations that evolve in individual cancer cells. Dr. Navin has been the recipient of numerous awards including the King & Miller scholarship, Abraham's Award, Lindsay-Goldberg fellowship, NCI T32 fellowship and the Nadia's Gift Foundation Damon-Runyon Innovator Award. Dr. Navin also received the 5th Annual Young Investigator Award by Genome Technology Magazine. Research from the Navin lab has been highlighted in many news features including Nature, Science, Nature Medicine Nature Reviews Cancer, Nature Methods and the MIT Technology Review.

Mahendra Rao, M.D., Ph.D.

Dr. Rao is internationally renowned for his research involving human embryonic stem cells (hESCs) and other somatic stem cells. He has worked in the stem cell field for more than 20 years, with stints in academia, government and regulatory affairs and industry. He received his M.D. from Bombay University in India and his Ph.D. in developmental neurobiology from the California Institute of Technology. Following postdoctoral training at Case Western Reserve University, he established his research laboratory in neural development at the University of Utah. He next joined the National Institute on Aging as chief of the Neurosciences Section, where he studied neural progenitor cells and continued to explore his longstanding interest in their clinical potential. Most recently, he spent six years as the vice president of Regenerative Medicine at Life Technologies in Carlsbad, California. He co-founded Q Therapeutics, a neural stem cell company based in Salt Lake City, Utah. He also served internationally on advisory boards for companies involved in stem cell processing and therapy; on committees, including as the U.S. Food and Drug Administration's Cellular Tissue and Gene Therapies Advisory Committee chair; and as the California Institute of Regenerative Medicine and International Society for Stem Cell Research liaison to the International Society for Cellular Therapy.

Yana K. Reshetnyak, Ph.D.

Dr. Reshetnyak is a Professor of Physics at the University of Rhode Island, who got her MS and Ph.D. in physics at the Saint-Petersburg State University and the Institute of Theoretical and Experimental Biophysics, Russian Academy of Science, Puschino, Russia, respectively. Her expertise is in membrane biophysics, drug delivery, fluorescence spectroscopy, microscopy and *in vivo* fluorescence imaging. She is one of the co-inventors of pHLIP® technology. Family of pH (Low) Insertion Peptides (pHLIPs) represents novel approach in targeting of acidic diseased tissue, which is based on the energy of pH-dependent membrane-associated folding. Currently,

pHLIPs are considered as novel pH-sensitive delivery agents. Also, for many years she has been engaged in development of fluorescence spectroscopy for the study of protein function and structure. Her lab has developed mathematical approaches for the analysis of fluorescence spectra and protein structure, and established correlations between protein fluorescence and structural properties. Lately, they have created a web-based toolkit PFAST: Protein Fluorescence And Structural Toolkit: http://pfast.phys.uri.edu/.

Yingxiao (Peter) Wang, Ph.D.

Dr. Wang obtained his bachelor's and master's degrees in Mechanics and Fluid Mechanics from Peking University, Beijing, P.R. China, in 1992 and 1996, respectively. He received his Ph.D. degree in Bioengineering from the University of California, San Diego Jacobs School of Engineering in 2002 and continued his postdoctoral work at UC San Diego working under Bioengineering Professor Shu Chien and Professor Roger Y. Tsien in the Department of Pharmacology. Before joining the UC San Diego faculty in 2012, he was an associate professor at the University of Illinois, Urbana-Champaign (UIUC), Department of Bioengineering and a full-time faculty member in the Beckman Institute for Advanced Science and Technology at the University of Illinois. He was also affiliated with the Department of Molecular and Integrative Physiology, Neuroscience Program, the Center for Biophysics and Computational Biology, and Institute of Genomic Biology at UIUC. Dr. Wang is the recipient of the Wallace H. Coulter Early Career Award (both Phase I and Phase II), the National Science Foundation CAREER Award, and National Institutes of Health Independent Scientist Award. His research is supported by the National Institutes of Health, National Science Foundation, and private foundations. Dr. Wang teaches undergraduate and graduate courses on molecular engineering, live cell imaging, and mechanobiology.

Denis Wirtz, Ph.D.

Dr. Wirtz's research area is Nanotechnology for Cancer Research and has been funded by the NIH, NSF, and the American Heart Association. He is co-Director of the Johns Hopkins Institute for NanoaBioTechnology (INBT), Director of the HHMI graduate training program, Director of the NCI-funded postdoctoral training program in nanotechnology for cancer medicine, and Director of the new NCI-funded Engineering in Oncology Center. Wirtz is author and co-author of 135 peer-reviewed articles published in journals such as Science, Nature, Nature Cell Biology, Nature Methods, Nature Materials, Nature Protocols, PNAS, Nature Communications, and Nature Reviews Cancer. His work at Hopkins has been cited >6,700 times and has an h-index of 48. Wirtz received the NSF Career award in 1995, was named Theophilius H. Smoot Professor of Engineering and Science in 2009, fellow of the Institute for Medical and Biological Engineering in 2007, fellow of the American Association for the Advancement of Science (AAAS) in 2009, and fellow of the American Physical Society in 2010. Wirtz received a physics engineering degree from the Free University of Brussels in 1988, and MSc and PhD in Chemical Engineering from Stanford University in 1993.

Gajus Worthington

Mr. Worthington is a co-founder of Fluidigm Corporation, and has been its President and CEO since its inception in May of 1999. He has a BS in Physics and an MS in Electrical Engineering from Stanford University. Gajus has been driven by a desire to help build an enduring and innovative company since age 19, and this obsession has shaped his career and interests ever since. Fluidigm was born out of this drive and from the technological breakthroughs made in the

lab of his college friend Steve Quake. Gajus has had an enduring interest in single-cell analysis since Fluidigm's early days, and has been a champion for single cell gene expression technology for nearly a decade.

Xiaoliang Sunney Xie, Ph.D.

Dr. Xie received his B.S., Peking University, 1984; Ph.D., University of California, San Diego, 1990; followed by postdoctoral experience at the University of Chicago. In 1992, Dr. Xie joined Pacific Northwest National Laboratory, where he later became a Chief Scientist. In 1999, he received tenure at Harvard and later became Mallinckrodt Professor of Chemistry and Chemical Biology, and Cheung Kong Visiting Professor at Peking University. He is a member of the National Academy of Sciences. Dr. Xie has made major contributions to the emergence of the field of single-molecule biophysical chemistry and its application to biology, in particular, single molecule enzymology, and gene expression and regulation. His team also pioneered the development of coherent anti-Stokes Raman scattering microscopy and stimulated Raman scattering microscopy, which allowed highly sensitive label-free imaging of cells, organisms, and human tissues with vibrational spectroscopy. Recently, his group made new advances in single cell whole genome sequencing.

Kun Zhang, Ph.D.

Dr. Zhang is an Associate Professor of Bioengineering at UCSD. His current group is developing genome technologies based on single-molecule sequencing, single-cell manipulation/amplification, and chip-based synthesis and manipulation of complex DNA libraries. They are applying these novel technologies to stem cell research and personalized medicine. Dr. Zhang is also leading one of the three single cell genomics centers under the NIH Roadmap program.