2014 NIH Common Fund Single Cell Analysis Investigators Meeting

Executive Summary

Where: Neuroscience Center, 6001 Executive Blvd, Rockville, Maryland

When: April 20-21, 2014

The NIH Common Fund held its 2nd Annual Single Cell Analysis Investigators Meeting on April 20-21, 2014 in Rockville, Maryland. This year's meeting objectives are to:

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

The meeting incorporated presentations by 4 invited keynote speakers and 18 funded investigators, 42 poster presentations, and 15 lightning talks, and yielded opportunities to review progress, collaborate, and brainstorm novel ideas. Over 180 participants attended this year's meeting, with representatives in academia (55%), government (25%), industry (15%), and nonprofits (5%). Almost 30% of attendees were funded investigators and collaborators, while 20% were postdoctoral fellows and graduate students. Five scientific sessions were held. A general description and summary of each session follows. The complete meeting report includes this executive summary, the meeting agenda, presentation and poster abstracts, and biosketches of our keynote speakers.

Session 1 – Single cell analysis in a complex dynamic environment

- **Keynote Address: How the Nanomechanics by Which Macrophages Pick-Up Their Prey is Adversely Affected by Few Common Drugs,** Viola Vogel, ETH Zurich Swiss Federal Institute of Technology, Switzerland
- **Novel Tools for Monitoring the Innate Immune Signaling Network at Single Cell Level,** Sergi Regot, Covert Lab, Stanford University

- **Near-Field Laser Ablation Sampling for Single Cell Biomolecule Mass Spectrometry,** Kermit Murray, Louisiana State University
- **Microwell Arrays for High Throughput Transcriptomics and Proteomics,** Peter Sims, Columbia University
- **Single Cell in situ RNA Profiling by Sequential Hybridization,** Long Cai, California Institute of Technology

The keynote speaker, Dr. Viola Vogel, described the study of cellular biomechanics and highlighted how understanding mechanical properties can provide insights into cell function and potentially lead to novel therapies that exploit changing mechanical forces. Dr. Sergi Regot presented recent work on the group's efforts to develop biosensors for live cells. Using fluorescent tags, it is possible to visualize changes in response to activation of kinase-dependent signaling pathways. Dr. Kermit Murray is attempting to bring mass spectrometry (MS) sampling to the single cell level. His group uses and AFM tip to position a laser on the cell surface. Upon ablation, the cell contents are released and captured for analysis. Dr. Peter Sims is combining microfluidics and microscopy to create "microreactors, small chambers for single cell analysis. The technology could be broadly useful in analyzing many individual cells simultaneously for a number of cell-specific phenotypes. Dr. Long Cai is using FISH barcoding technology to detect many unique transcripts. This technique can be applied to thicker specimens and tissue sections making it quite usable for a number of labs. For practical purposes, the approach may allow for detection of up to 100 genes but more are theoretically possible making this quite powerful.

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

- **Keynote Address: Spatially-Resolved Proteomic Mapping of Living Cells Using Engineered Peroxidase Reporters,** Alice Ting, Massachusetts Institute of Technology
- **Temporal and Spatial Analyses of the of Neural Stem Cells Transcriptome in situ,** Rui Sousa-Neves, Case Western Reserve University
- **Fluorescent Probes for Quantitation of Secretory Protein Levels in Single Live Cells,** Erik Snapp, Albert Einstein College of Medicine
- **Monitoring T Cell Activation by Single Molecule Fish and Flow Cytometry,** Sanjay Tyagi, Rutgers University
- **The Development of a Fyn FRET Biosensor by Directed Evolution for Single Cell Imaging,** Yingxiao Peter Wang, University of California San Diego

The keynote for this session was delivered by Dr. Alice Ting, who described a method that combines imaging and mass spectrometry to generate spatially resolved proteomic data in live cells. Dr. Rui Sousa-Neves presented work on new technology that exploits the drosophila model and provides the ability to multiplex measures of lineage decisions in time and space. Dr. Erik Snapp described progress developing RNA aptamers as smaller alternatives to labeled antibodies. Optimization continues to improve targeting specificity, signal-to-noise ratios, and the ability to label live or fixed cells. Dr. Sanjay Tyagi is focused on developing a sensitive assay to detect cell-based responses to pathogens using TB as a proof of concept. The key is improving sensitivity so low level signals are detectable. Dr. Yingxiao Peter Wang reported on progress developing molecular biosensors to detect dynamic changes in cell states. The approach uses directed evolution to improve sensitivity and speed the design and synthesis of novel biosensors with high specificity and better dynamic range.

Session 3 – Phenotypic & Genotypic characterizations of complex populations of individual cells

- **Highly Multiplexed Subcellular RNA Sequencing in situ,** Je Hyuk Lee, Wyss Institute and Harvard University
- **Individual CAR+ T Cells Recycle Effector Functions by Conjugating to Multiple Tumor Cells,** Navin Varadarajan, University of Houston
- **Whole Exome Sequencing of Circulating Tumor Cells Provides a Window into Metastatic Cancer,** Viktor Adalsteinsson, J. Chris Love Lab, Koch Institute at MIT & Broad Institute
- **Barcoding Thousands of Single Cells in a Single Tube by Droplet Microfluidics**, Allon Klein, Kirschner Lab, Harvard University

This session's keynote talk was delivered by Dr. Je Hyuk Lee, who described significant advances using FISH in single cells using methods that reduce noise, improve stability, and allow detection of molecules in a 3D matrix. The methods are sensitive and will allow for functional and spatial analysis in numerous cell types. Dr. Navin Varadarajan presented work using time-lapse imaging microscopy in nanowell grids examine modified T-cells to examine the function and behavior of individual cells in recognizing targets. The hope is that T-cell immunotherapy will tip the balance in favor of the patient as more is learned about this important cell type. Dr. Viktor Adalsteinsson has demonstrated the feasibility of sequencing circulating tumor cells (CTCs) as a non-invasive tool to evaluate and monitor the genetic state of certain metastatic cancers. This technology could be a major step toward precision medicine and targeted therapies. Dr. Allon Klein is focused on developing a high capacity barcoding approach to be used to target many thousands of cells. The group uses droplet microfluidics and a number of technical challenges have been identified and are being resolved using ES differentiation as a model system for proof of concept.

Session 4 – Using multi-scale models and integrated, quantitative measurements to study function and disease

- **Keynote Address: Single Cell RNA-Seq Dissection of Kidney Development,** Steve Potter, University of Cincinnati
- **Instrumentation Development for the Simultaneous Atomic Force Microscopy, Nuclear Magnetic Resonance Spectroscopy, and Fluorescence Microscopy of Single Cells,** Charilaos Mousoulis, Neu Lab, Purdue University
- **Transcriptome Analysis of Cells in their Natural Microenvironment Reveals the Constraints on Generation and Regulation of Functional Plasticity,** James Eberwine, University of Pennsylvania
- **Histo Mosaic: A Novel Diagnostic Technique to Detect Genetic Mutations in Tissue Slices,** Frank Cheng-Chung Lee, Kartalov Lab, University of Southern California

Dr. Steve Potter delivered the keynote for this session's keynote talk. He described a variety of techniques used to identify and characterize single cell transcriptomes in renal progenitor cells. The data suggests a paradigm shift in the mechanism of terminal differential from activation of specification genes to repression of specific lineages. Dr. Charilaos Mousoulis presented progress in the proof of concept to combine AFM and NMR to measure cellular composition. Fabrication and testing of a hybrid probe is ongoing. Dr. James Eberwine updated the group on progress related to the TIVA-tag methodology. Using this technique, his group has shown rich transcript diversity in individual hippocampal cells sampled in situ. Dr. Frank Cheng-Chung Lee presented progress in

developing a tissue-slide method to simultaneously sample multiple quadrants of a tissue to look at DNA changes. The group intends to develop a 3-D grid that can be laid over the tissues to establish a virtual multi well plate.

Session 5 – Challenges in longitudinal single cell analysis during development

- **Keynote Address: Induced Pluripotent Stem Cells and the Impact of Genomic Variation on Psychiatric Disorders,** Flora Vaccarino, Yale University
- **Assembly and Use of Dual-View Inverted Plane Illumination Microscopy for Rapid, Spatially Isotropic Four-Dimensional Imaging,** Hari Shroff, Section on High Resolution Optical Imaging, NIBIB/NIH
- **Integration of Single Cell Surface Phenotype, Function, and Transcriptome to Study the Proliferative and Homing Capacity of Human Mucosal T-Cells,** Todd Gierahn, J. Chris Love Lab, Koch Institute at MIT
- **Common Analysis of Reference RNA at Single Cell Levels,** Hannah Dueck, Kim Lab, University of Pennsylvania

Dr. Flora Vaccarino delivered the keynote talk where she described current work on iPS cell lines derived from normal skin cells in order to examine low frequency somatic mutations. The analysis shows that there is significant genomic variation in normal skin fibroblasts. Dr. Hari Shroff presented advances in dual view inverted selective plane illumination microscopy (diSPIM). This DIY microscope improves Z-resolution and image capturing speed as well as reducing phototoxicity. Dr. Todd Gierahn described a nonowell array that is being used for single cell analysis of T-cells from a human mucosal sample. Multiple assays can be conducted and a more longitudinal study of metabolic states is planned. Dr. Hannah Dueck presented recent work on a comparative analysis of two reference RNAs for single cell transcriptome data generated from three centers (U.Penn, UCSD, and USC). The data indicate that three protocols yield similar sensitivity, precision, and accuracy in assays of 10 or 100 pg RNA samples, detecting ~30-50% of genes in the reference libraries. More work to refine the analysis and understand lessons learned is planned.