2015 NIH Common Fund Single Cell Analysis Investigators Meeting

Executive Summary

Where: Natcher Conference Center, Bethesda, Maryland

When: April 20-21, 2015

The NIH Common Fund held its 3rd Annual Single Cell Analysis Investigators Meeting on April 20-21, 2015 on the NIH Campus in Bethesda, Maryland. This year's meeting objectives were to:

- Convene the funded SCAP investigative teams to update the community on their research and consider current conceptual, technical, and methodological challenges in single cell analysis.
- Determine major biomedical research opportunities that can be addressed by the Common Fund rather than individual NIH Institutes or Centers.
- Discuss how relevant groundbreaking technologies and approaches in SCA can be disseminated to the research community effectively in the near future.
- Disseminate current research findings in single cell analysis (SCA).

The meeting incorporated presentations by 5 invited keynote speakers and 12 funded investigators, 68 poster presentations, and 22 lightning talks, and yielded opportunities to review progress, collaborate, and brainstorm novel ideas. Over 210 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 in addition to the poster session and a breakout session. A summary of each scientific session follows. The complete meeting report includes this executive summary, the meeting agenda, presentation and poster abstracts.

Session 1

- **Keynote Address: A new focus on the cell: The Allen Institute for Cell Science,** Alan "Rick" Horwitz, Ph.D., Allen Institute for Cell Science
- **Expansion microscopy: Towards imaging at arbitrary resolution, scale, and multiplexing,** Edward Boyden, Ph.D., MIT Media Lab and McGovern Institute, MIT
- **Towards the living connectome: Imaging of individual neurons in intact, developing embryos,** Daniel Colón-Ramos, Ph.D., Department of Cell Biology, Yale University School of Medicine

The keynote speaker, Dr. Rick Horwitz, described the establishment of the new Allen Institute for Cell Science and its focus on integrated, predictive models of cell behavior. Dr. Horwitz cited his

own work on Src signaling, noting the spatiotemporal and molecular complexity involved in cell migration. Dr. Ed Boyden presented recent work from his group on using sodium polyacrylate to "swell" tissue in 3D by 4.5 times with very low distortion. The degree of swelling can be controlled and enables the use of standard resolution microscopy for many samples instead of super-resolution microscopy. Dr. Daniel Colón-Ramos described the development of a DiSPIM microscope and its use for studying the C. elegans connectome as part of a success collaboration with Hari Schroff and Zhirong Bao. The partnership has created an open source microscope design, software for tracking cells in 4D, and data access integration as part of their dissemination efforts.

Session 2

- **Keynote Address: Probing stem cell biology with single-cell gene expression,** Stuart Orkin, M.D., Dana Farber Cancer Institute, Boston Children's Hospital, Harvard Medical School
- **Single-cell dissection of transcription factor expression heterogeneity in a lymphocyte developmental gene network",** Ellen Rothenberg, Ph.D., Division of Biology & Biological Engineering, California Institute of Technology
- **Detection of cell-type specific effects of pathway manipulation in neural cells,** Tracy Young-Pearse, Ph.D., Brigham and Women's Hospital; Harvard Medical School

The keynote for this session was delivered by Dr. Stuart Orkin, who described how highly multiplexed, quantitative PCR can be used in an unbiased manner to study stem cell differentiation pathways. His group has studied 1500 single cells in the mouse hematopoietic system to study lineage progression, and in particular to identify cellular hierarchies in acute myeloid leukemia. Dr. Ellen Rothenberg described the analysis of the transcription factor PU.1 which influences T-cell precursor development. Her group's work has focused on populations of cells (40+) but is now working with Long Cai through a SCAP supplement to study how removal or antagonism of PU.1 influences heterogeneity in T-cell differentiation. Dr. Young-Pearse reported on progress towards understanding the heterogeneity underlying the London familial variation of Alzheimer's disease (AD), where beta-secretase can cleave at different sites. Her group is conduction drug screens at the single cell level to assess how inhibitors may affect cleavage patterns and impact the accumulation of amyloid tangles downstream.

Session 3

- **Keynote Address: Dynamic functional heterogeneity of primary immune cells revealed by single-cell transcriptomics,** Hongkun Park, Ph.D., Department of Chemistry and Chemical Biology, Harvard University
- **Single cell transcriptomics analysis of neurons and cardiomyocytes from live human tissue,** Mugdha Khaladkar, Ph.D., and Jennifer Singh, Ph.D., Perelman School of Medicine, University of Pennsylvania
- **Dynamic single-cell analysis and interactions in cancer, allergy and senescence,** Tania Konry, Ph.D., Department of Pharmaceutical Sciences, Northeastern University
- **Cross-scale integrin regulation organizes ECM and tissue topology**, Scott Holley, Ph.D., Department of Molecular, Cellular and Developmental Biology, Yale University

This session's keynote talk was delivered by Dr. Hongkun Park, who described recent work in his group using nano-bio interfaces to interrogate living cells and cell networks. These tools involve nanowares which are capable of intracellular sampling and can be used for recording and stimulating. Drs. Singh and Khaladkar presented recent work on the transcriptional analysis of neurons and cardiomyocytes from live human samples. Dr. Tania Konry discussed her work on functional phenotyping of individual cells and secretion analysis which can be used for studying interactions in cancern allergic responses and senescence. Dr. Scott Holley described the work on his group in studying pattern formation and morphogenesis during zebrafish development and how integrin regulation at the single cell level is involved in tissue topology.

Session 4

- **Keynote Address: Targeted proteomics with single-cell and sub-cellular resolution,** Amy Herr, Ph.D., Department of Bioengineering, University of California, Berkeley
- **Single-cell metabolic imaging using molecular fingerprinting,** Ji-Xin Cheng, Ph.D., Weldon School of Biomedical Engineering, Purdue University
- **Single-cell microsampling mass spectrometry for elucidating cell heterogeneity in the developing embryo,** Peter Nemes, Ph.D., Department of Chemistry, George Washington University

Dr. Amy Herr delivered the keynote for this session, describing her group's work on generating "disruptive precision tools" has allowed them design highly specific single cell multiplexed proteomic assays. Dr. Herr spoke about recent advances in microfluidics technology and highlighted the lag in progress with respect to multi-stage separations. During her talk, she presented data to establish performance and operational gains, including quantitation capability, total assay automation, and minimization of dispersion to maximize information content. She concluded her presentation by re-emphasizing the need for Precision targeted proteomics for protein multiplexing in single cells. In his introduction, Dr. Cheng drew the audience's attention to the importance of Metabolic imaging in single cell analysis. His group has developed tools and techniques based on Raman spectroscopy, and Dr. Cheng showed images of how such a technique may be used to image membrane potential in living neurons to measuring dynamics of Liquid Droplets (LDs) in MiaPaCa2 cells by single color SRS imaging. He concluded by noting that pushing the limits of detection sensitivity, or microsecond-scale acquisition of SRS spectra, spectral profiling will go far in monitoring and mapping single tumor cells. He closed with a brief description of ongoing work on a hand-held stimulated Raman spectroscopic imaging pen which he expects will enable real-time in vivo tumor imaging under ambient light. Dr. Nemes described the mass spectrometry technologies being developed in his laboratory that make it possible to measure small molecules (metabolites, small peptides) in single embryonic cells (blastomeres) with exceptional analytical sensitivity for detection and quantitation (50 nM lower limit of detection/quantitation). The group is now extending this single-cell mass spectrometer to in situ operation and to smaller cells by combining it with microcapillary sampling. Dr. Nemes concluded by summarizing that it is now technologically feasible to use mass spectrometry for single-cell analysis with sufficient sensitivity to perform actual measurements on the metabolome and suggested that based on their work, similar techniques may be adapted for various types of cells to complement transcriptomic and proteomic measurements, ranging from muscle cells, cells of the heart, to neurons.

Session 5

- **Keynote Address: Towards a human cell atlas,** Aviv Regev, Ph.D., Department of Biology, MIT; Broad Institute of MIT & Harvard
- **Single-cell SNP FISH reveals epigenetic mosaicism in genomic imprinting mutants,** Arjun Raj, Ph.D., Department of Bioengineering, University of Pennsylvania
- **Characterizing neuronal subtypes in human adult cortex with single-nucleus transcriptome sequencing,** Kun Zhang, Ph.D., Bioengineering Department, University of California, San Diego
- **Transcription profiling in situ by seqFISH,** Long Cai, Ph.D., Division of Biology and Biological Engineering, California Institute of Technology

Dr. Aviv Regev delivered the keynote talk where she outlined her laboratory's goal of building a Human Cell Atlas by using Single Cell genomics. The Atlas itself is defined by cell types, states, locations, transitions (Salient features) and lineages. Dr. Regev's laboratory uses high-throughput single-cell transcriptomics, single-cell RNA-seq libraries in conjunction with a computation method that they have developed called SEURAT, to build a spatial reference map in order to understand how individual cells in a tissue network are organized. Using a test case, her group has applied SEURAT to data from 851 dissociated single cells from zebrafish early embryos and confirmed the method's accuracy. In conclusion, Dr. Regev pointed out that given available technologies, completing a comprehensive Human Cell Atlas is within reach.

Dr. Raj described his group's efforts to visualize gene expression in single cells, and focused his talk on a techniques his group has developed for RNA FISH that enables them to detect individual RNA molecules. He acknowledged support from SCAP for an extension of this method that allows them to detect single base differences on these individual RNA molecules. They have called this method SNV FISH or SNP FISH. He concluded by briefly summarizing how the group has gone on to applying SNP FISH to mitochondria and chromosomes. He ended his talk by suggesting that based on their observations, it would be possible to use SNP-FISH as a diagnostic tool.

Dr. Zhang described his group's goal of generating 10,000 single-cell transcriptome of the human adult cortex, and providing spatial information within the 5-year project period. He explained that due to the difficulty of dissociating human post-mortem cortical sections into single cells, they opted to sequencing single neuronal nuclei, which contain ~1/10 of the RNA in a cell. He presented data to show that they have established a pipeline for single nucleus transriptome sequencing, and have amplified >13,000 single neuronal nuclei, among which >5,000 have been sequenced by 2015.

Dr. Cai briefly explained the basis for seqFISH and how the technique allows quantitative profiling of mRNAs in single cells. He discussed various applications of the techniques as applied to various tissues. He showed examples of how his group has developed multiplex analysis by barcoding FISH (Spatial barcodes; Spectral barcodes; Temporal barcodes). Dr. Cai concluded his presentation by suggesting that based on workflow (RNA seq > 100 gene seqFISH > GFP+ movies) and data presented, 100 genes at 100 copies per cell can be detected at >90% efficiency. He made the prediction that 100 genes in the entire mouse brain $(10mm)^3$ can be imaged in about 1 month.

Breakout Sessions

Five breakout sessions met on Monday evening to discuss a wide range of topics. Below is a brief summary of some of the concepts discussed.

Complete Single Cell 'omics – Alex Shalek (Chair)

There was wide appreciation among the group discussants on the rapid progress made recently in the development and use of ‘omics technologies. The main points of discussion were: 1) why do we need to measure more than one variable class? (confidence, compare with other approaches / results, bridge scales, identify relationships) Which ‘omics? (which define a cell, is there bias?) What variables do we need to measure? (time, space, perturbations, scale). The group also discussed quality of results, what assays should be used more commonly and how to apply assays clinically.

Intracellular, Intercellular and Multi-scale Correlations – Ellen Rothenberg (Chair)

This group discussed whether it is possible to fractionate the components of a cell for analytical purposes and study intra and inter cellular correlations. Major consideration identified by this group included: 1) optimizing single-cell biology in the content of the biological question of interest (one size does not fit all), 2) collaboration helps drive technology develop biological investigation and analytical and predictive modeling, 3) dissemination of technologies at an early stage is important, though this is often too early for commercialization, 4) challenges should be biology-driven not technology-driven.

Stochasticity at the Single Cell Level - Jerilyn Ann Timlin (Chair)

This breakout group discussed why we should care about stochasticity in biological systems. The group discussed why randomness is important, how it can be used as a biomarker, whether it can be exploited and what tools might be needed. The group felt that too much emphasis was put on measures of central tendency which is a barrier to wider acceptance and consideration of stochastic statistics. The group identified a need for tools to be able to manipulate noise, cross-train biologists and statisticians, have tools to identify intermediate states in cells, bring time resolution to genomic assays and understand the relationship between intracellular, intercellular and extracellular variability in disease processes to identify important variables.

Is there a Periodic Table of Cell Types?

This breakout session discussed what is a cell type and what is a cell state? Can we describe transitions between states? Do they fall into discreet classes; how do we capture dynamic state? Evidence so far suggests that we can and the hematopoietic system is an example. How is cell state (molecular characterization) different from cell type (functional, morphological etc.)? The group felt we need to look at multiple points and systems to understand dynamical differences – though is there / must there be some intrinsic signal in each cell that is deterministic?

The Future of the Single Cell Analysis Community – Claudia Mizutani & John Parant (Chairs)

This breakout session was attended by 15-20 people. The group felt that the short term impact of the program could be assessed by publications, but that dissemination, clinical translation and companies adopting technologies into products are the long term measures. The majority of single cell work happens outside the U.S. but within the U.S. the NIH funds the majority of single cell work. Some of the significant challenges in the field include: technology is expensive / difficult to use, no support for collaborations with technology developers, access to software, data acquisition and reproducibility, how to do multi-omics experiments.