Summary of the Common Fund Single Cell Analysis Workshop

The Single Cell Analysis Workgroup organized a workshop on single cell analysis on April 28 – April 29, 2011 and was held in Rockville, MD. The goal of the meeting was to identify gaps and opportunities in single cell analysis. Despite the short timeframe in which this workshop was organized, twenty-one eminent scientists participated. They represented a diverse set of perspectives, including academic, commercial, and NIH intramural; domestic and international; technologists and biologists; and experimentalists and informaticists. To further broaden community input, approximately 80 people across the nation participated in the meeting through the videocast and a social media website.

The meeting was divided into four sessions under the following four topics:

1. What is the most useful information needed for single cell analysis?
2. What are the driving biomedical problems that will be addressed by understanding the heterogeneity of single cells?
3. What are the major technical/methodological hurdles we currently face in analyzing single cells?
4. What will be the basic/translational/clinical impact of advances in single cell analysis?

The summaries of the sessions submitted by the respective session chairs are provided in on the following pages, followed by the agenda and participant list. Several underlying themes emerged from the discussions across the various sessions. Namely,

1. Understanding and eventually manipulating single cell behavior in the context of surrounding tissues requires that new biological paradigms be proposed and novel approaches tested.
2. A deep understanding of single cell behavior requires multimodal analysis that integrates disparate –omics datasets with spatial and temporal measures, which in turn requires development of exceptionally innovative technologies.
3. Technologies currently exist that provide insight into single cell behavior; however, these need to be validated, standardized, and be made more user-friendly in order for the broader biological and clinical communities to adopt them.

Session 1: What is the most useful information needed for single cell analysis?

Discussion Leader: James H. Eberwine (University of Pennsylvania)

Initial discussants: Sunney Xie (Harvard University), Paul Soloway (Cornell University), Cynthia McMurray (Lawrence Berkeley Laboratories), Jennifer Lippincott-Schwartz (NICHD) and Susan Janicki (Wistar Institute).

The session started with a general discussion (with many participants) of how to define the phenotype of a cell. There was general recognition that phenotype should be defined by multiple parameters among which might be included, transcriptome, epigenetic markers, function, what stimuli do the cells respond to (behavior of the cell), morphology, nearest neighbor cells, cells to which it is connected. The question of phenotypic outliers and whether they are good or bad was discussed with the consensus that such variation underlies the ability of the cell to adapt to different environments and hence is important. The question of stochasticity versus determinism was also discussed with no clear resolution to the importance of one versus the other. The need to define phenotype measurements based upon the question being addressed was emphasized.

Sunney Xie emphasized the need for technical development especially related to single cell genome sequencing, transcriptomics, proteomics and the metabalome. The combination of imaging with sequencing may be particularly useful in analyzing single cells. In particular the utility of current super resolution microscopy and envisioned enhancements should make some of these measures routine.

Jennifer Lippincott-Schwartz emphasized the need to study and understand single cell metabolism, in particular mitochondria or chloroplasts. The dynamism of mitochondria is regulated by cellular dynamics. Studying organelles is justified as they connect to other regulatory systems within cells. Understanding the architectural framework of a cell is necessary to understanding cellular metabolism. Techniques to do this include vital imaging and mass spectrometry.

Paul Soloway commented on the need to understand the nutrient status of a cell to properly understand phenotype. This involves analysis of intracellular chemical gradients, local gas concentrations the environmental context of the analyzed cell and the orientation of the cell in the environment. Analysis of the single cell epigenome and methylated states of DNA and proteins is necessary to provide an understanding of the phenotypic plasticity that exists in every cell.

Cynthia McMurray discussed the idea of noise within single cell measurements and thought that attempts to amplify the signal and the linear range of sensitivity of probes would help to minimize noise. This is particularly important in the resolving single cell metabolism. Further progress is needed in generating an integrated approach to analysis of multiple phenotypic parameters in live native tissue (rather than dispersed cells). Additionally the study of same cell over time would provide a necessary analysis of cellular responsiveness over time. This is particularly important given the seemingly large heterogeneity in selected phenotypic measurements in different cells.

Susan Janicki pushed the idea of enhancing single cell transcriptomics by also directly assessing gene regulation at the level of chromatin. This requires enhancement of CHiP-seq technologies as well other live cell and biochemical analyses. She also questioned how to define particular genes, as there are many noncoding RNAs that are expressed and need to be analyzed in the single cell context. The need for temporal resolution and quantitative measures of single cell biology was further emphasized.

The take home messages of this session were:
- Phenotypic analysis of a cell in its natural environment is necessary and should be encouraged.
- Methods for analysis of the same cell over time should be developed.
- Transcriptomics, metabolomics and proteomics datasets at the single cell level in a quantitative manner are desirable.
- Analysis of subcellular compartments and organelles and their impact on overall cell functioning will provide a single cell systems analysis of that cells functioning.
- Defining the phenotype to be measured prior to the start of experiments is important with regard to interpretation of results.
- Methods for quantitative analysis of the architectural framework of the cell need to be developed.
- The dynamism of cellular metabolism needs to be quantified.
- Better probes are necessary to quantify aspects of single cell phenotype including fluorescent probes that activate and emit at multiple wavelength, more two photon probes and cages, more sensitive and reliable markers of activity (e.g. voltage sensitive dyes) and other non-fluorescent probes that enable quantitative measurements to be taken.
- Live cell molecular analysis and phenotyping should be encouraged.
- Cultured cell studies are justified if one wants to assess a cells capacity to respond in a well-controlled environment. This will entail nanofabrication and micro-patterning capacity.
- The ability to perform any and all of these single cell measurements in a high-throughput manner will facilitate an understanding of individual cell variation within a defined phenotypic cell class.
- The best approach to single cell studies involves a multimodal analysis of phenotype, where more than one measure is quantified. Combinatorial measure phenotyping will yield the most useful information.

Session 2: What are the driving biomedical problems that will be addressed by understanding the heterogeneity of single cells?

Discussion Leader: Sherman M. Weissman (Yale University)

Initial discussants: Edward Callaway (Salk Institute), David Yule (University of Rochester), Leslie Loew (University of Connecticut), Lani Wu (University of Texas) and Paola Arlotta (Harvard University).

- Two areas where technical developments would be important to implement:
    a) The need for good techniques to isolate single cells from tissues directly or with minimal disturbance and with as much in situ characterization as possible (morphology, histologic patterns, nature of neighboring cells, electrophysiologic properties, etc.
    b) The combination of microfluidic approaches with "omics" technologies such as full length RNA sequencing, microRNA sequencing, markers of chromatin structure, potentially analysis of nascent RNAs or ongoing transcription, DNA methylation analysis on a genomic scale, possibly analysis of translation rates, etc. It is recognized that some approaches that require statistical sampling will be impossible, but the limits of single cell analysis need to be explored.
- Analysis of single cell properties and heterogeneity would be invaluable for
    a) characterizing nature and variability of empirically defined stem cells from various normal tissues and malignancies,
    b) Understanding of the sequence of events in de-differentiation or reprogramming of cells, for understanding some of the complexity of immunological responses,
    c) Understanding the extent and distribution of escape of genes from X inactivation, the variability in allele specific patterns of gene expression, and for
    d) Understanding molecular and potentially functional heterogeneity underlying organization in the nervous system.
- Biomedical issues that would benefit from single cell analyses would include:
    a) Antenatal diagnoses, most dramatically in diagnoses from examination of single cells isolated from 2-8 cell blastocysts,
    b) Potentially in characterizing circulating cancer cells, or minor populations of cells from various malignancies. In addition,
    c) The ability to perform a wider range of analyses on single cells might make it feasible to perform "micro-biopsies" on a wider range of tissues, and to analyze disease progression in terms of heterogeneity of disease processes in individual cells in an organ.
- Learning at the single cell level how different cell types interact in diseased tissues would be important for a number of disease processes and could even point the way towards more refined therapies. For example:
    a) Analysis of single immunocytes such as T cells that are in proximity to cancer cells, coupled with analysis of the adjacent malignant cell could more specifically disclose T cell receptor structures that are involved in killing of tumor cells.
    b) Similarly in autoimmune disorders, more insights could be obtained about relevant reactive immunocytes, their cell surface receptors and their targets.

- Differences in each ES or IPS cell lines were recognized as potential limits to using these cells for studying disease processes and therapeutic responses in vitro, but the ability to economically characterize in an unbiased and genomic scale the behavior of IPS cells derived from patients could give new insights into the significance of this heterogeneity.

Session 3: What are major the technical/methodological hurdles we currently face in analyzing single cells?

Discussion Leader: Deirdre Meldrum (Arizona State University)

Initial discussants: Enrico Gratton (University of California, Irvine), Hideki Kambara (Hitachi Central Research Laboratory), Scott Fraser (California Institute of Technology), Saju Nettikadan (NanoInk, Inc.) and Thierry Emonet (Yale University).

- How does a single cell work and how do cells interact? What technologies do we need to fully characterize a live cell – "normal" and disease-specific?
- There is a need for more probes and ways to measure more parameters in the single cell to measure spatio-temporal events including molecular flow in the cell as well as both intracellular and extracellular: fluorescent probes, nanoparticles, genetically expressed probes, labels, reagents; include biomimetic sensors.
- Imaging – need to be able to excite spatial areas and penetrate more deeply to image cells in tissue; need to be able to image faster to capture fast events (e.g. millisecond events). Need technologies to understand the cell but also how the cell works in the context of a tissue.
- Fully characterize a live "normal" cell and identify the biosignatures that are indicative of disease. To do this we need technology to be able to simultaneously measure multiple parameters in live single cells to be able to map out the spatiotemporal events in cells – the dynamics. This can be done by measuring intracellular and extracellular parameters while performing stimulus-response experiments and starting to truly characterize live cells. Over time, measure physiological and morphological parameters, metabolism, including forces, then take snapshots in time to get the state of the cell by looking at the genomics, transcriptomics, proteomics of single cells and integrate/correlate all of this information to map out all the processes and fully characterize the dynamics of the live cell. It was mentioned the challenge of amplification for transcriptomics. Also approaches mentioned to get the protein signature with mass spec or to use high content microscopy. Need to do this in a very well-controlled environment (in vitro) that simulates "normal environment" as closely as possible. Also need to develop the capability to do this on cells in their truly natural environment.
- Perhaps we want to be able to collect this data to characterize a "normal" healthy cell and then move on to different perturbations – chemical inputs, infection, mutations, etc. – and characterize the changes in the cell due to these perturbations.
- Datasets and modeling are necessary elements of single cell analysis. Quantitative modeling and experimentation need to go hand-in-hand in an iterative fashion to inform each. We need to think about the cell as a nonlinear dynamical system and take into account the range of parameters required to characterize the cell and the range of time scales required to take into account the variety of cellular processes. We need to be able to incorporate data for single cell as well as cell-cell and be able to compare between them. This all needs to incorporated into a model of the cell that starts with coarse resolution information and is built upon to be more and more detailed and as we are able to measure more. These are very high combinatorial

complex datasets so will require methods to be developed to keep pace with the experimental data.

- Other technologies we would like to have:
    - o More high-throughput microscopy methods
    - o Better caging groups (get more details from Jim Eberwine?)
    - o Ability to measure more parameters simultaneously
    - o Integration of technologies (e.g. flow cytometry with microscopy, etc.)

Session 4: What will be the basic/translational/clinical impact of advances in single cell analysis?

Discussion Leader: Daniel T. Chiu (University of Washington)

Initial discussants: Jan Vijg (Albert Einstein College of Medicine), Deirdre Meldrum (Arizona State University), James Inglese (NHGRI), Sherman Weissman (Yale University), Ulf Landegren (Uppsala University) and James Eberwine (University of Pennsylvania).

- There is already a bunch of single-cell diagnostics, such as fetal cells, flow cytometry, etc.
- In many cases, there is no option but to do single cell, such as CTCs and fetal cells
- There has been quite a bit of discussion about educating pathologist/clinicians; some think it is not an issue, while others disagree.
- Standardization and having sufficient statistical, informatics, and validation tools for single cell data analysis
- Some discussion about the possibility of classifying all cell types and determine the natural variability
- There has been discussion about high-throughput tools for single cell studies, stem cells for personalized medicine.
- Also discussion of integration of single-cell technology with pathology
- single cell transcriptomics leading to druggable targets
- Understand effects of single cell and the importance of single cell; for example does it matter or how might it affect the tissue/organism if a perturbed cell is introduced, such as knock down or knock in studies.
- Notion of single-cell disease (perhaps cancer is a good example).
- Single nucleotide polymorphism and single cells
- Single cell biochemistry and signature studies
- Susceptibility and functional changes as a result of changes in a single cell
- High affinity reagents are needed
- Use of DNA technology to study proteins, because DNA technologies are better developed
- Notion of error propagation and error introduced in handling of single cells was discussed
- Quantify disease states with single cell resolution
- Technology for clinical application needs to be robust, cheap, etc.

April 28–29, 2011
SINGLE CELL ANALYSIS
Workshop
5635 FISHERS LANE, ROCKVILLE, MD

NIH Common Fund Workshop on Single Cell Analysis
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AGENDA

<u>**Thursday, April 28**</u>

3:30 – 4:00 pm	**Registration**

4:00 – 4:30 pm　　**Welcome, Opening Remarks and Introductions**
Roderic I. Pettigrew, M.D., Ph.D. (NIBIB Director)
James H. Eberwine, Ph.D. (University of Pennsylvania)

4:30 – 6:30 pm　　**Session 1: What is the most useful information needed for single cell analysis?**
Discussion Leader: James H. Eberwine, Ph.D. (University of Pennsylvania)

Broad topics for consideration:
- What set of measurements is required to unambiguously characterize the state of a living cell?
- What is the relationship between phenotypic information, genotypic information, environmental conditions and cell behavior?
- How do cells and researchers deal with environmental and intracellular "noise" in determining cell state?
- How significant are the physical characteristics of the cell and distribution of sub-cellular components and biomolecules in analyzing a cell?
- What information would be required to spatio-temporally map and understand signaling pathways, cell lineage and differentiation pathways, cell-cell coupling and the impact of perturbations at the single cell level?

<u>**Friday, April 29**</u>

8:00 – 9:45 am　　**Session 2: What are the driving biomedical problems that will be addressed by understanding the heterogeneity of single cells?**
Discussion Leader: Sherman M. Weissman, M.D. (Yale University)

Broad topics for consideration:
- How does heterogeneity of individual cells correlate with the function of large tissue structures and distributed networks? Are patterns of single cell function linked to tissue level (dys)function?
- How do abnormal cells and tissues interact with surrounding cells?
- Can disease patterns be predicted from single cell phenomena?
- How does the state of the cell influence cell communication, migration, invasion and susceptibility to pathogens and drugs?
- What are the limits to manipulating cells into different states?

9:45 – 10:00 am   **Break – Light Refreshments**

10:00 – 11:45 am   **Session 3: What are major the technical/methodological hurdles we currently face in analyzing single cells?**
Discussion Leader: Deirdre Meldrum, Ph.D. (Arizona State University)

Broad topics for consideration:
- What are the challenges in being able to non-destructively image any arbitrary cell in a living organism with sub-cellular resolution and minimum perturbation in a physiologically relevant timeframe? What are the challenges with respect to specific cell types?
- How can a human or pathogenic cell of unknown origin be characterized be analyzed quickly and accurately?
- How can large numbers of diverse, live cells under physiologically relevant conditions be characterized initially and then monitored simultaneously for long periods of time to understand interactions, development or response to perturbation?
- How can single cells be identified, processed, characterized and preserved with the minimum of perturbation, and data collected in an unbiased and high fidelity way?
- What tools and methods need to be applied to multi-dimensional datasets to represent, analyze and build models of single cell characteristics?

11:45 – 12:30 pm   **Lunch**

12:30 – 2:00 pm   **Session 4: What will be the basic/translational/clinical impact of advances in single cell analysis?**
Discussion Leader: Daniel T. Chiu, Ph.D. (University of Washington)

Broad topics for consideration:
- Under what circumstances would a diagnosis or treatment regime be modified by understanding either the state of a single cell of interest or the heterogeneity of a tissue sample? What are the challenges in single cell diagnostic tests and single cell therapies?
- What resources and standards would support the basic science community and augment translation of methods and technology?
- What technologies, resources or tools need to be integrated, made more turn-key, or have higher throughput in order to be widely used by the basic science community in addressing single cell biomedical problems? Where would an order on magnitude improvement in sensitivity / specificity / throughput / parameters measured have the greatest impact?
- What technologies, resources or tools do not yet exist, are at a conceptual stage or have significant barriers to use but which are likely to play a pivotal role in our ability to analyze single cells?
- In a decade, which biomedical questions should we have an answer to?

2:00 – 2:30 pm   **Meeting wrap-up**
Discussion Leaders of all sessions

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