# Breakout Sessions
## April 20, 2015 • 5:00-7:00 p.m.

### Purpose
The goals of the annual investigators meeting are to:

- Disseminate current research findings in single cell analysis
- Discuss and overcome roadblocks to gain a deeper understanding of the significance of cell level heterogeneity
- Identify new, emerging challenges in single cell analysis and potential technological solutions
- Build synergy between initiative and non-initiative projects

These meetings serve as opportunities to foster communication and build networks among multidisciplinary investigators. At the annual meeting each year, we explore different ways of meeting these goals and use participant feedback to identify what was successful and what was not. Breakout sessions are included in this year's meeting with two purposes:

- Stimulate discussion among meeting attendees to foster future collaborations
- Collect information for future development of the single cell analysis program

The current phase of the Single Cell Analysis Program will end in October 2016. We are currently evaluating the need for a second phase of the program and are identifying unique opportunities and challenges remaining in the single cell analysis field. The breakout discussions will be a valuable contribution to that process.

With the final program year approaching, we would like research projects to continue moving forward outside the program. We hope these breakout sessions will foster fruitful discussions and collaborations leading to new grant applications or supplement requests. There are no plans to release additional funding opportunities or supplements and no allocated monies; however, requests are possible under the parent administrative supplement mechanism (PA-14-077).

The five most popular breakout sessions were identified during the meeting registration process. You are welcome to attend any breakout session and move freely between them. Each of the breakout sessions have an assigned moderator to help stimulate discussions. Please come with your own questions, thoughts on the theme, examples of current research in that area, and thoughts on what the challenges and opportunities are moving forward. Each breakout session will give a report on the second day of the meeting. We look forward to hearing your thoughts!

## 1. Complete Single Cell 'omics

**Location: Room E1/E2**
Moderator: Alex Shalek
Note-taker: Yong Yao

Over the past five years, we have seen an explosion of techniques, interest, and understanding in the genomic and transcriptomic profiles of individual cells. Nucleic acids have the advantage of amplification, raising the possibility of being able to detect single biomolecules from a cell's milieu. This program has funded a number of projects to broaden our grasp of single cell proteomics and metabolomics. However, we are far from having complete information about a cell. We believe there are of order 100,000 distinct endogenous biomolecules in a cell, varying from 1 to $10^9$ copies, and across a wide range of molecular weights. The theme of this session is to discuss the challenges and opportunities for measuring and analyzing all the 'omics at the single cell level.
Questions for the breakout session to consider include:

- What biological insight would we gain from a single cell epigenome / proteome / metabolome / lipidome / interactome? What advances in technology would we need to let us see past the most abundant molecules in each class?
- Where are potential dividends for a better understanding cell biology? Are they in carrying out a detailed inventory of biomolecules present, understanding the interaction of different classes of biomolecules within a cell, tracking the spatiotemporal dynamics of a subset of biomolecules?
- The focus of the program has been on multicellular eukaryotic cells in a complex environment. How important is context (spatial and temporal) and how can it be better accounted for in reporting results?

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## 2. Intracellular, Intercellular and Multi-Scale Correlations

**Location: Room A**
Moderator: Ellen Rothenberg
Note-taker: Grace Shen

The theme for this session is to discuss challenges and opportunities in measuring and analyzing correlations within cells, between cells and over different length and time scales. Techniques are emerging to analyze different classes of biomolecules (e.g. RNAs & proteins) from a single cell. This program has focused on understanding heterogeneity at the single cell level primarily focused on molecular analysis. In several areas there has also been progress to try and link physical / morphological measures, molecular analysis or functional characteristics across different length and time scales.

Questions for this breakout session to consider include:

- Can we fractionate the components of a single cell with high efficiency?
- Is a single cell transcriptome representative of the proteome? Over what time scales?
- How well does an analysis of the epigenome predict the phenotype of an individual cell?
- Do correlations exist within populations of cells? Over what length and time scales do correlations exist?
- How can we better link molecular / physical analysis at the single cell level with tissue and organ function?

## 3. Stochasticity at the Single Cell Level

**Location: Room G1/G2**

Moderator: Jerilyn Ann Timlin

Note-taker: Richard Conroy

The production and destruction of biomolecules is driven by regulated and random factors. The theme for this breakout session is how do cells tolerate or utilize fluctuations, “noise” and heterogeneity. We often describe unwanted variance without realized biological significance as noise and quantify it using simple statistical notations. In other areas of science there is a deeper understanding of how to model noise to tease out factors such as extrinsic influences and future volatility. As part of this program we have seen significant variation in data from cells that are nominally the same – some of this variation is driven by technical uncertainties, some by biological stochasticity. For sensitive measurements at the level of a single copy of a biomolecule, can we reliably detect and understand the effects of cellular noise?
Questions for this breakout session to consider include:

- What are the design principles that allow cells to function and evolve in a stochastic environment?
- What role does stochasticity play in cell fate, disease progression and development processes?
- How to differentiate, measure, and model technical, intrinsic and extrinsic sources of noise in single cell analysis?
- How does stocasticity vary among prokaryotic cells, unicellular eurkaryotic organisms, and multicellular organisms and what insights can we gain from comparing across different cell types and environments?

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## 4. Is there a Periodic Table of Cell Types?

**Location: Room F1/F2**

Moderator: Long Cai

Note-taker: Andrea Beckel-Mitchener

Historically our definition of cell types has relied on morphological information, presence or absence of a small number of cell surface markers or knowledge of where the cells were isolated from. Recently several efforts to develop more comprehensive cell catalogs have started. The theme for this session is to discuss challenges and opportunities in defining cell types in the age of ‘omics technologies, how we account for dynamic cells states, perturbation, environment and lineage in these databases. This session can also consider whether information in these catalogs can be used to provide insights into higher level organization of cell types and what would be required to reliably engineer cell phenotypes.
Questions for this breakout session to consider include:

- What kind of framework (standards, ontology, processes etc.) do we need to promote validation of cell types in research to promote reproducibility of results?
- Can we readily identify the original and type of a single cell of unknown origin?
- Can cell types be uniquely and robustly described solely by molecular characteristics?
- What tools or techniques are required to reliably engineer a population of cells into any arbitrary pattern of known cell types?
- What new insights would a systematic survey of cells at the level of level of an organ or mammal provide?
- How do we map out and extract basic organizing principles of cells in complex environments?

## 5. The Future of the Single Cell Analysis Community

**Location: Room C1/C2**

Moderator: Claudia Mizutani

Note-taker: Liz Stansell

At the beginning of this program we chose not to build a tight consortium of a small number of larger projects but to let as many flowers as possible bloom given the rapidly growing and multidisciplinary nature of the field. The theme for this breakout session is what has been the impact of single cell analysis so far on the wider biological and biomedical research community, where will the field be in a decade and what resources are would significantly help the community.

Questions for this breakout session to consider include:

- What indicators are there of the impact that single cell analysis has had on the research community?
- What work is going on supported by private foundations, other government agencies and across the world that is having a dramatic impact on the field of single cell analysis?
- Is wider access to community resources (e.g. technologies, model systems, databases etc.) a significant barrier to the development or adoption of single cell analysis techniques? If so, what resources are needed and why would they make a difference?
- What will be the level of performance of technologies and what level of understanding of heterogeneity at the single cell level could we reasonably expect to have by 2020? What new insights will we have from being able to comprehensively track cell phenotypes? For example, could we expect to be able to engineer synthetic multicellular organisms or re-engineer cells in vivo to halt or reverse disease progression?