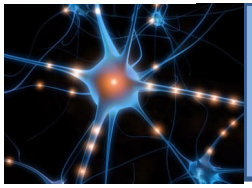


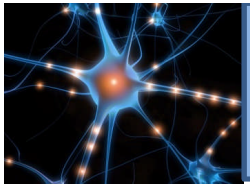
SINGLE CELL ANALYSIS

Proposal for Common Fund Program

Tom Insel (NIMH), Roderic Pettigrew (NIBIB)

Andrea Beckel-Mitchener (NIMH), Richard Conroy (NIBIB)

June 29, 2011

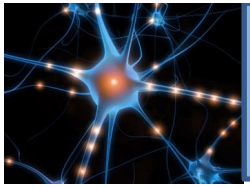


Key Biological Questions to be Addressed

1. How does one characterize a heterogeneous population of cells?

2. How can one measure and interpret biological “noise”?

3. How can the detection and treatment of diseases be improved using single cell approaches?



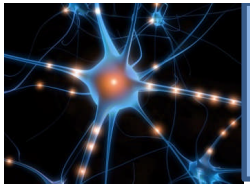
Goals of this Initiative

1. Accelerate the discovery of new approaches

2. Improve our understanding of cell heterogeneity

3. Accelerate the validation and translation of new technologies

4. Engage multidisciplinary teams to confront defined challenges

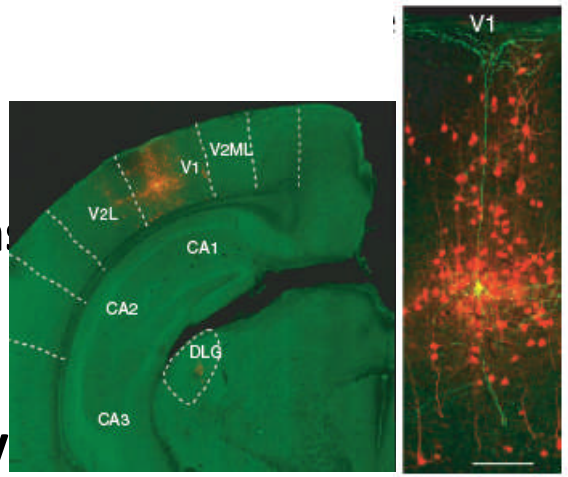


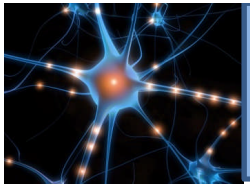
Why study single cells?

- **Most studies look at ensemble averages – obscures underlying heterogeneity, rare species, dynamic states, emergent properties & direct measurement of individual components in a complex system**

What are the challenges?

- **Systematically describing cell "state"**
- **Distinguishing functional variation from "noise"**
- **Environment is complex & sensitive to perturbation**
- **Linking cell responses to tissue/network function**
- **Sensitivity & specificity of measurement systems**
- **Complex tools have limited applicability/availability**



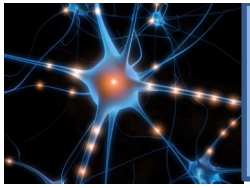


Why a Common Fund Initiative?

- **Accelerate the discovery & translation of next-generation technologies & approaches which are applicable across all cell types & organ systems**
- **22 Institutes already active in this emerging field**

Why now?

- **In last 2 years there has been a convergence:**
  - **-omic techniques reaching single cell sensitivity**
  - **Single cell techniques increasing dimensions & throughput**
  - **Rapidly improving micro-environment control**
- **Increasing clinical interest in cell-level heterogeneity**

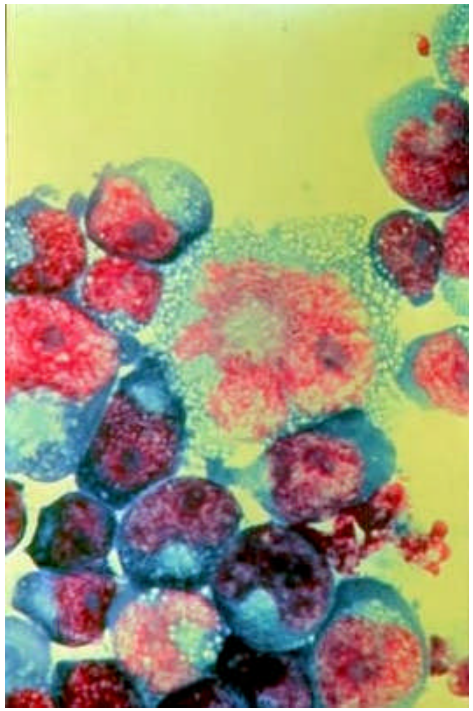


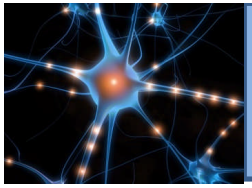
Ideally, what would a successful initiative lead to?

- **A >10x improvement in detection of [x]**
- **Prototype of first-in-class clinical diagnostic tool**
- **A guide to characterizing individual cells**

What do we want to avoid?

- **Funding projects solely focused on:**
  - **Conventional cell and molecular biology …**
  - **Standard electrophysiology, imaging …**
  - **In vitro, < 4D analysis, 1 cell type…**
  - **Incremental advances in transcriptomics, genomics …**
- **Funding work without broad applicability**



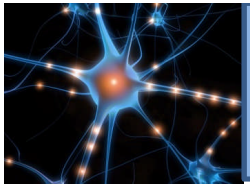


Steps in Developing this Proposal:

July 2010	**"The Big Think" -- Early conceptual discussion**
Fall/Winter 2010	**Trans-NIH Workgroup assembled/convened**
March-April 2011	**Portfolio Analysis**
February 2011	**RFI published, 75 responses including IRP**
April 2011	**Single Cell Analysis Workshop**
May 2011	**Innovation Brainstorm meeting**

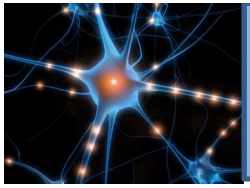
The Big Think:

- A distinguished panel of experts gathered to provide input to NIH on the most pressing issues facing healthcare today
- The panel identified “single cell analysis” as a cross-cutting theme, which, if developed further, would help define and resolve biological phenotypes as well as disease processes at a level not yet fully realized



Single Cell Analysis Workgroup:

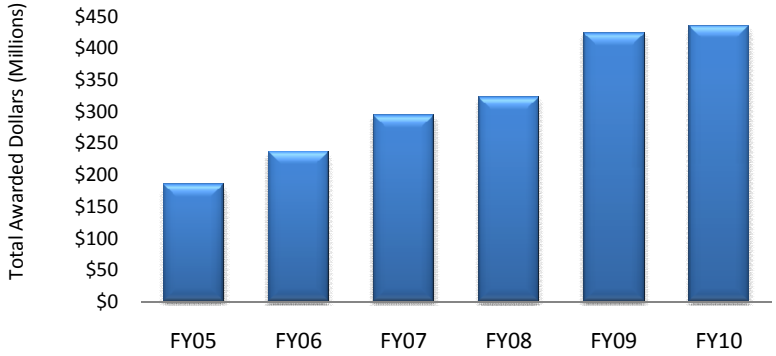
- Ravi Basavappa (OD)*
- Andrea Beckel-Mitchener (NIMH)*
- Olivier Blondel (NIDDK)
- Joseph Breen (NIAID)
- Lynda Chiodetti (NIAID
- Richard Conroy (NIBIB)*
- Jennifer Couch (NCI)
- James Deatherage (NIGMS)
- Paula Fearon (OD)
- John R Knowlton (NCI)
- Brenda Korte (NIBIB)*
- Roger Little (NIMH)
- Su-Yau Mao (NIAMS)
- Alan Michelson (NHLBI)
- Oleg Mirochnitchenko (NCRR)
- David Owens (NINDS)
- Carol Pontzer (NCCAM)
- Karl Salzwedel (NIAID)
- Jeff Schloss (NHGRI)
- Grace Shen (NEI)
- Lillian Shum (NIDCR)
- Susan Taymans (NICHD)
- Jose Velazquez (NIA)
- Da-Yu Wu (NIDA)

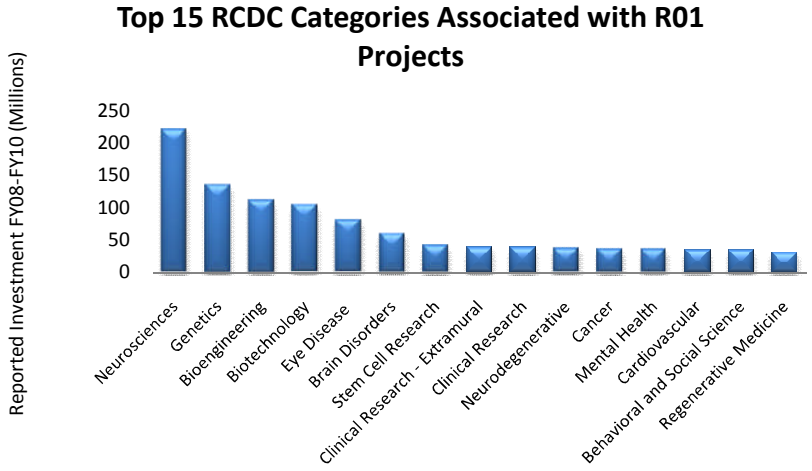


SINGLE CELL ANALYSIS

Portfolio Analysis I (Spring 2011)

Analysis of NIH Portfolio:

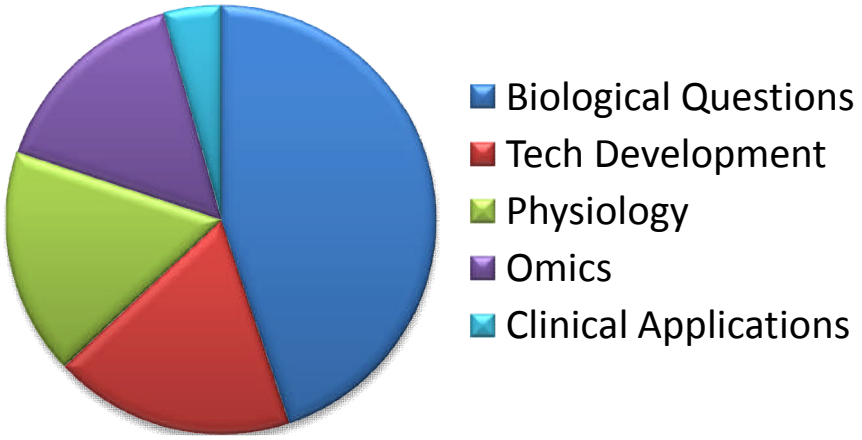


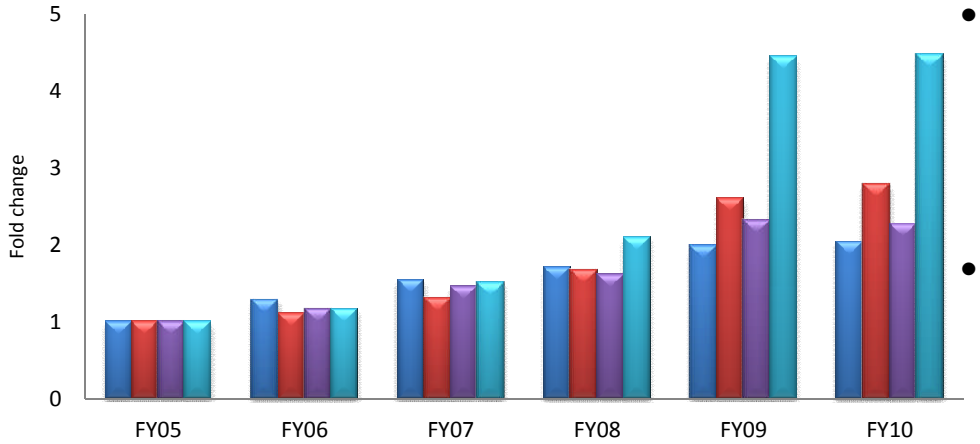


- **Broad definition used**
- **Manual assessment of hits by Workgroup**
- **There has been a significant (>200%), but uncoordinated, increase in NIH investment**
- **No previous NIH initiatives**
- **Very broad reaching and multidisciplinary field – 22 institutes have grants in this field**
- **Majority of individual awards focus on standard biology using conventional approaches**

Workgroup relied heavily on outside experts to define gaps and opportunities.

Analysis of NIH Portfolio:

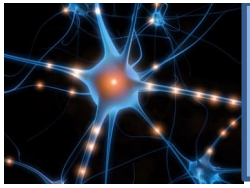




- **Major disease areas: brain disorders, vision disorders, cancer and cardiovascular disease**
- **Major non-disease categories: neuroscience, genetics, biotechnology and bioengineering**
- **The fastest growing segments of the portfolio are technology development and clinical applications**
- **Less than 5% of the NIH has been in high-risk, high-reward discovery projects**

Analysis of National and International Activities:

- From a literature analysis:
  - 36% of "single cell analysis" papers are from U.S. institutions
  - 14% of papers attributing funding support acknowledge NIH
- In 2011 a £5M (~$8M) multidisciplinary Single Cell Proteomics and Lipidomics initiative was funded by the U.K. EPSRC
- 4 workshops within the next 6 months
- 2011 supplementary issue of *Nature Methods* on single cell analysis



SINGLE CELL ANALYSIS

RFI (Spring 2011)

Request for Information:

1….which additional focus on single cell analysis may provide the most significant and broadest impact?

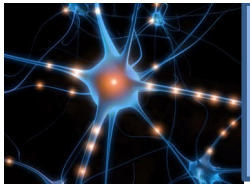
2.Current conceptual, technical, and/or methodological challenges in single cell analysis.

3.Major biomedical research opportunities that can be addressed by single cell analysis.

4.The 5 highest priority tools…

Major themes from 75 responses:

- **Map and understand cell state, importance of cell variability at tissue / system level, clinical impact, interpreting "noise"**
- **Imaging technologies, models & data analysis, sample handling, -omic analysis**
- **Single cell profiling, linking cells to tissue / system phenomena, cell development, cell-cell interactions, clinical screening**
- **Imaging technologies, community resources, sample manipulation, sensitive & quantitative -omics, measurement technologies**

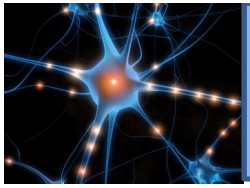


SINGLE CELL ANALYSIS

SCA Workshop (April 2011)

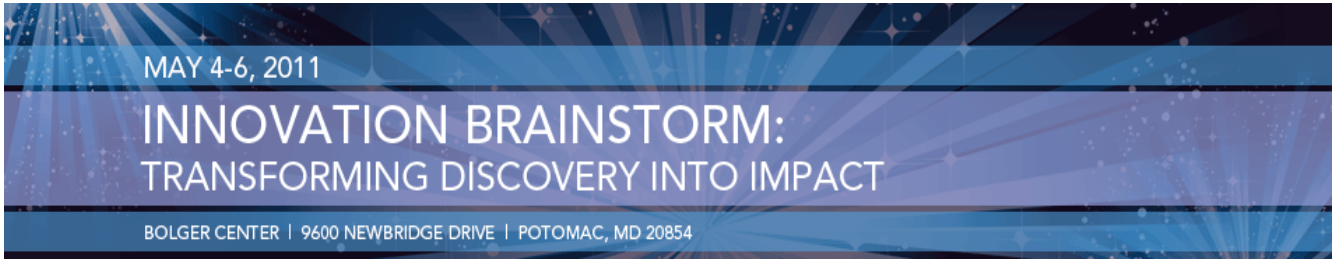
From the four sessions, a number of underlying themes emerged:

1. Understanding and manipulating single cells *in situ* requires new biological paradigms and novel approaches.
2. A deep understanding of single cell function requires multimodal analysis that integrates disparate –omics datasets with sub-cellular spatial and temporal measures. This requires a new toolkit.
3. Technologies currently exist that provide insight into single cell function; however, these need to be validated and be made available to the broader biological and clinical communities.



SINGLE CELL ANALYSIS

Innovative Brainstorm (May 2011)

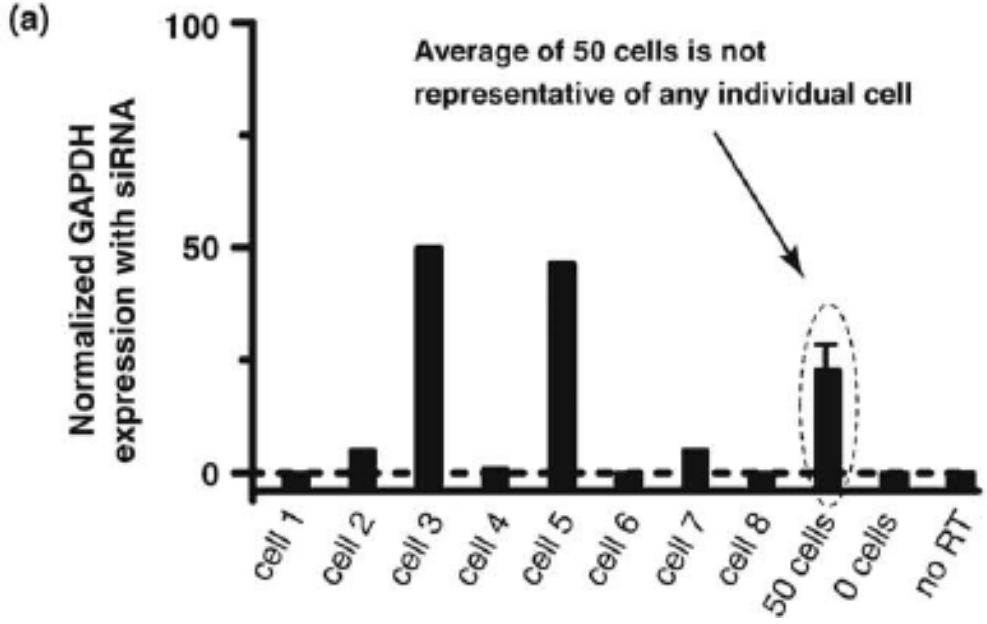


After discussing recent papers on the significance of heterogeneity at the single cell level several principles were identified by participants:

1. Complex populations require complex analyses
2. Single cell analysis requires different tools versus ensemble analysis
3. Cell characteristics are dynamic in multiple ways
4. Understanding the environment is critical

Opportunities identified included:

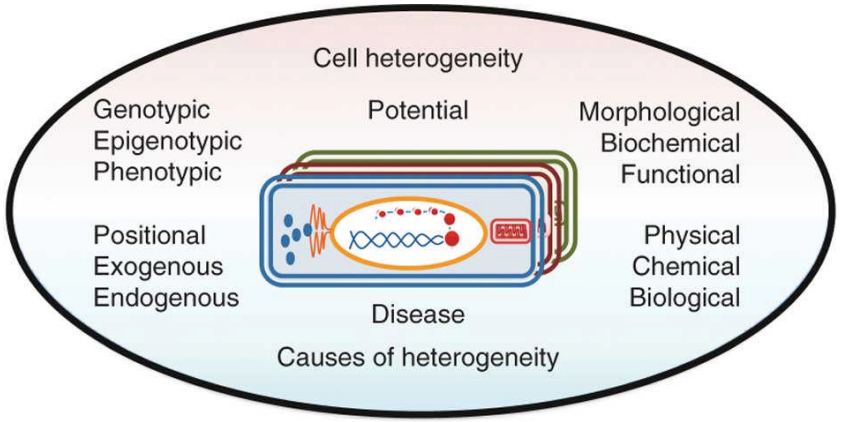
1. Improved imaging & –omics tools and analysis; “tissues-on-a-chip”
2. Innovative forums to bring together researchers from diverse fields
3. Challenges for the field for both development and clinical translation



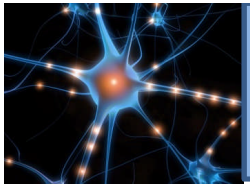
Trends in Biotechnology 28 (2010) 281–290

Ensemble averaging obscures what is happening with individual cells

Cells and their environment are complex and dynamic and require multidimensional analysis to characterize

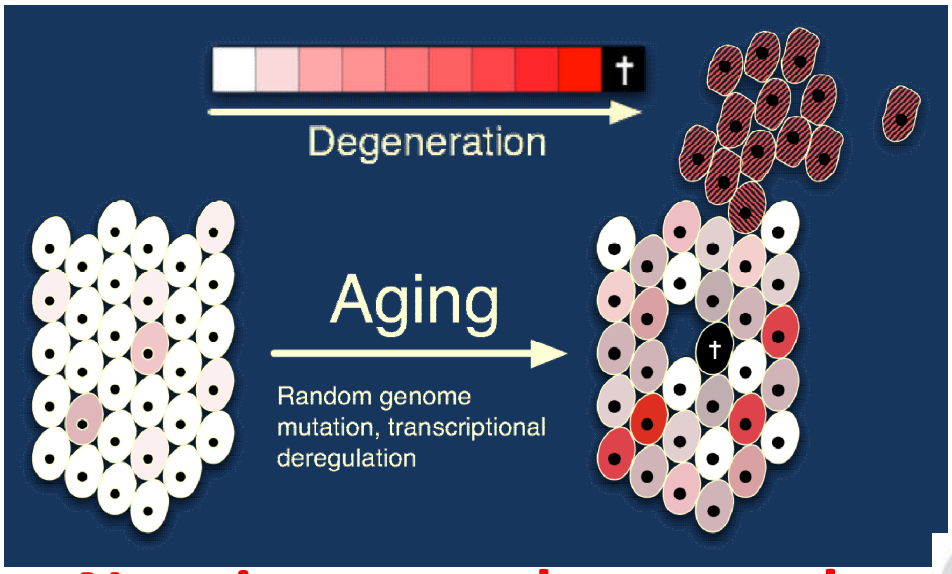


Nature Methods 8, S20–S29 (2011)



SINGLE CELL ANALYSIS

Key Concepts II

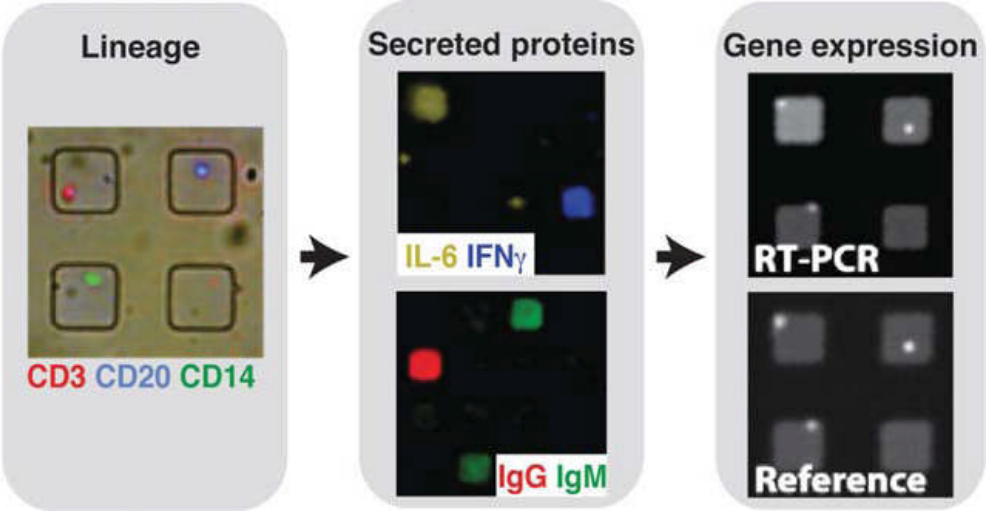


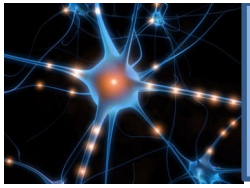
Courtesy of Jan Vijg, Ph.D.

Understanding the link between cell heterogeneity, tissue function and emergent characteristics is important

New integrated approaches to bioanalytic operations are needed to analyze heterogeneous populations of single cells efficiently

AIChE Journal October 2010 Vol. 56, No. 10, 2499





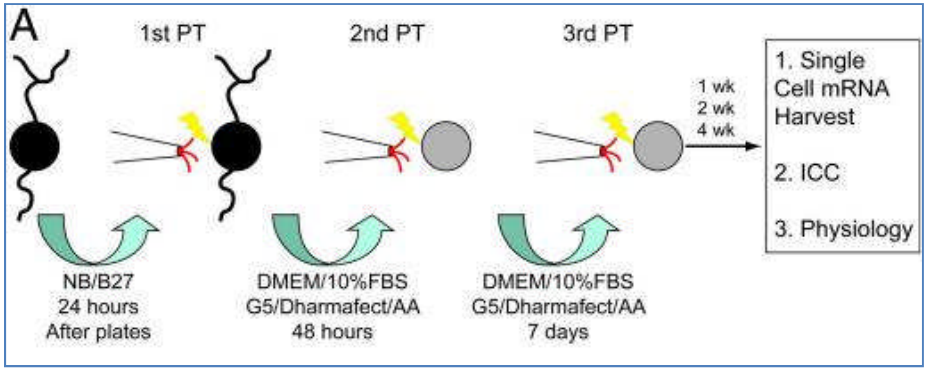
SINGLE CELL ANALYSIS

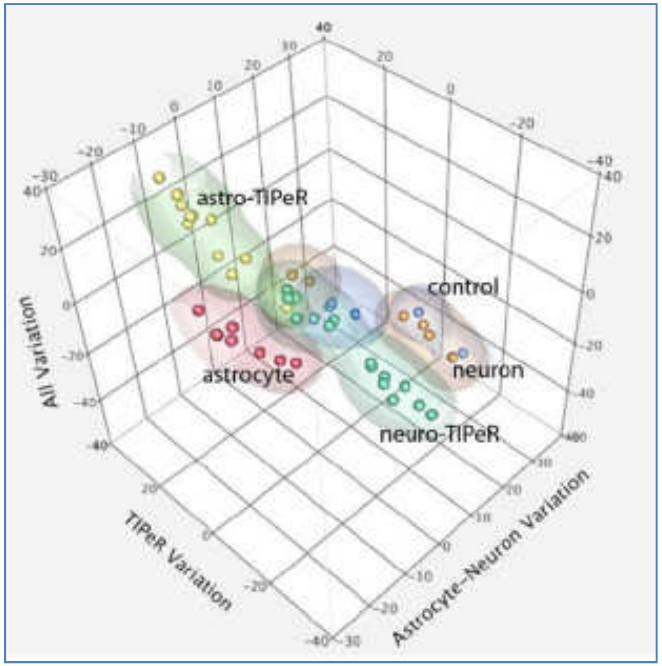
Recent Example I

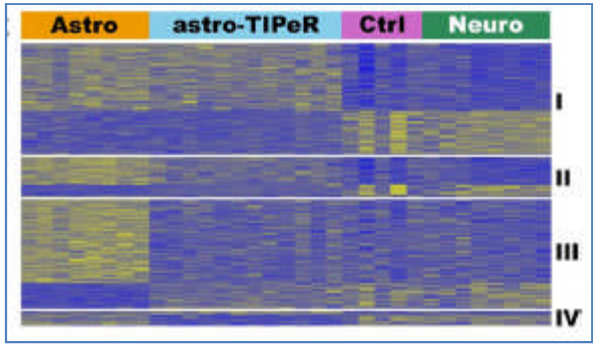
Transcriptome transfer produces a predictable cellular phenotype

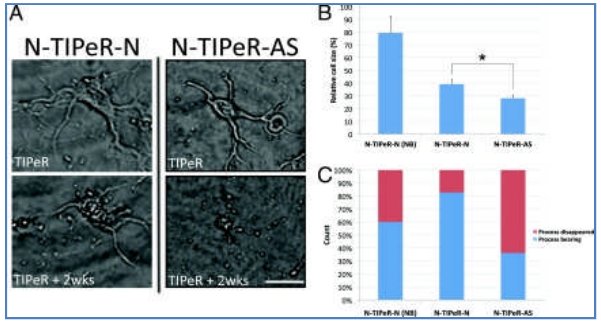
Jai-Yoon Sul et al.

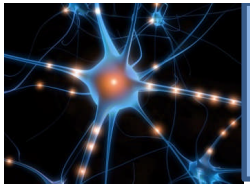
Proc Natl Acad Sci U S A. 2009 May 5; 106(18): 7624–7629











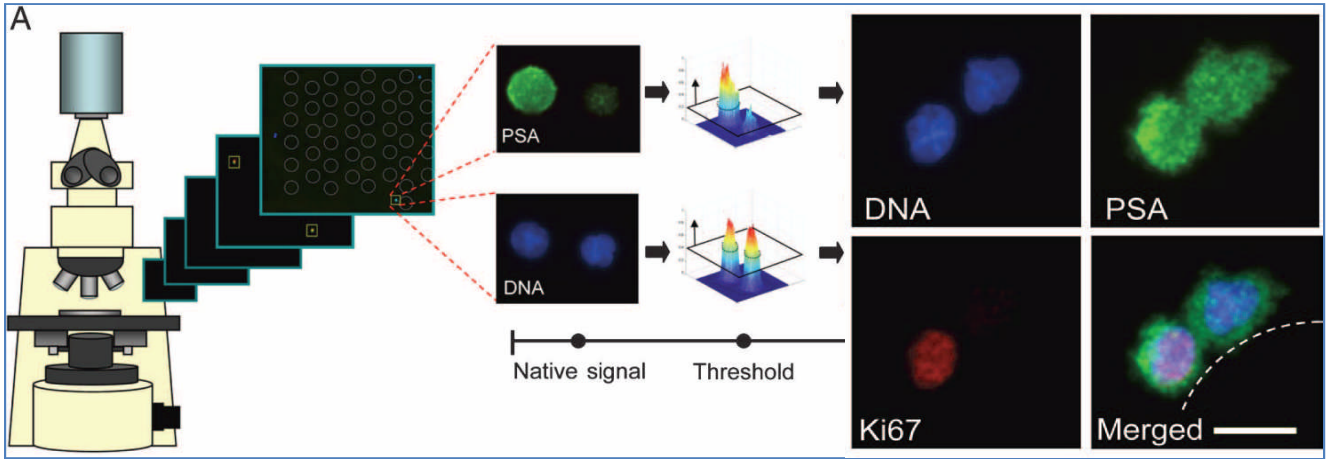
SINGLE CELL ANALYSIS

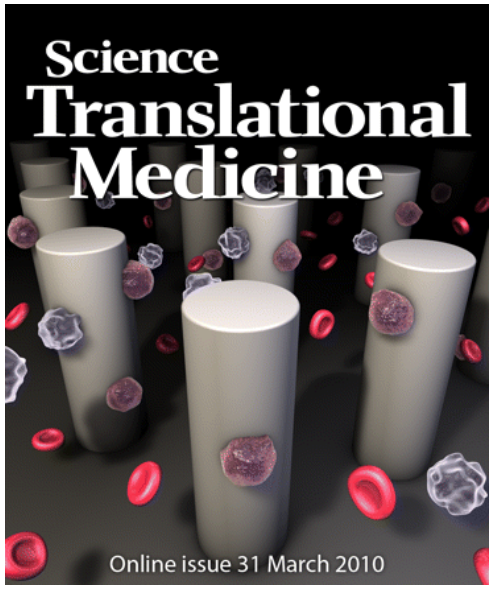
Recent Example II

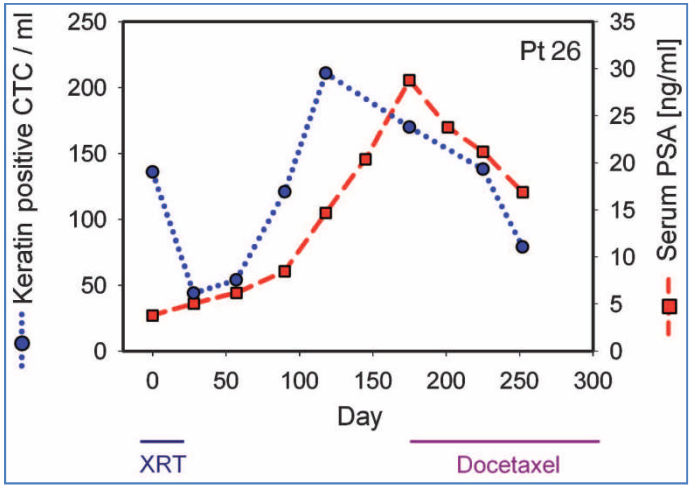
Isolation and Characterization of Circulating Tumor Cells from Patients with Localized and Metastatic Prostate Cancer

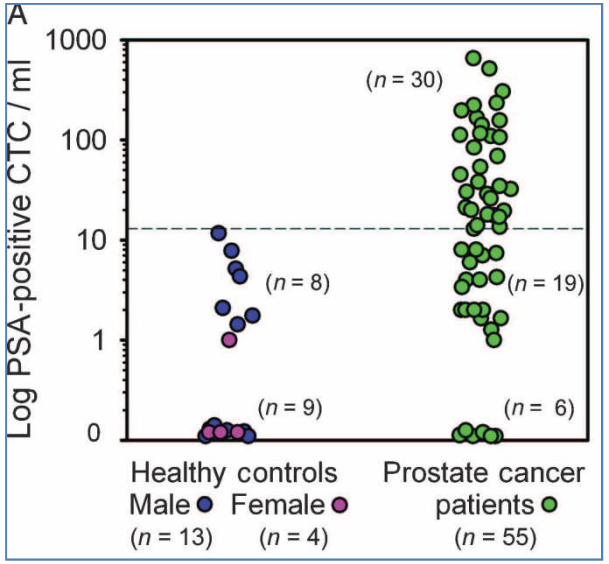
Shannon L. Stott et al.

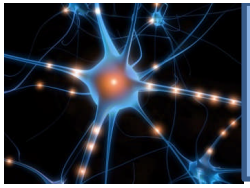
Sci. Transl. Med. 2, 25ra23 (2010)











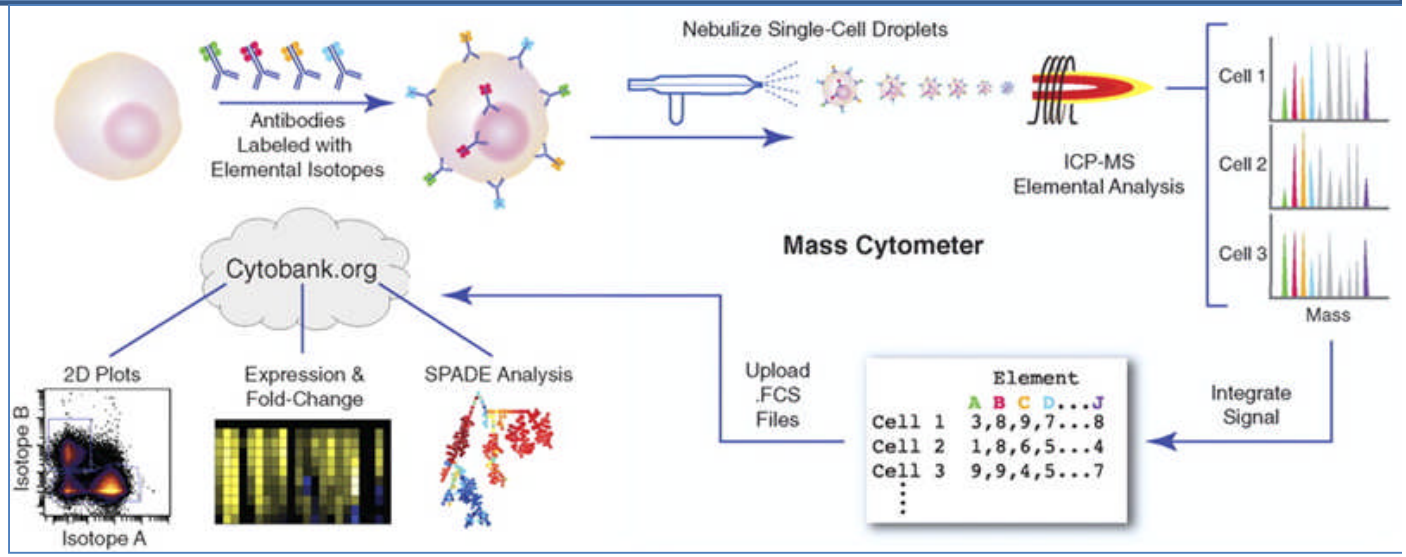
SINGLE CELL ANALYSIS

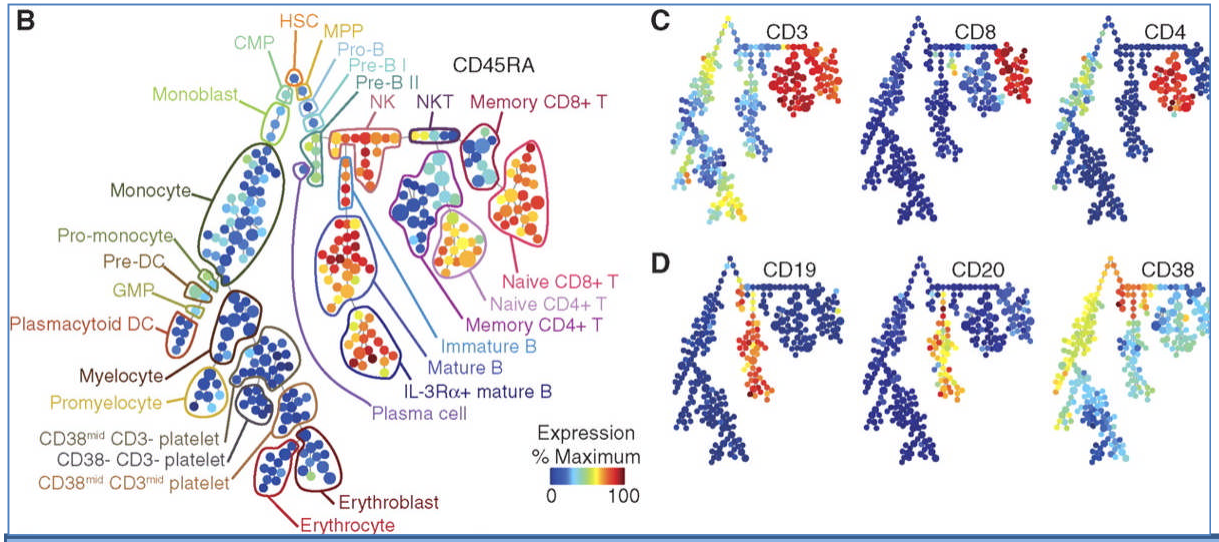
Recent Example III

Single-Cell Mass Cytometry of Differential Immune and Drug Responses Across a Human Hematopoietic Continuum

Sean C. Bendall et al.

SCIENCE VOL 332 6 MAY 2011 687

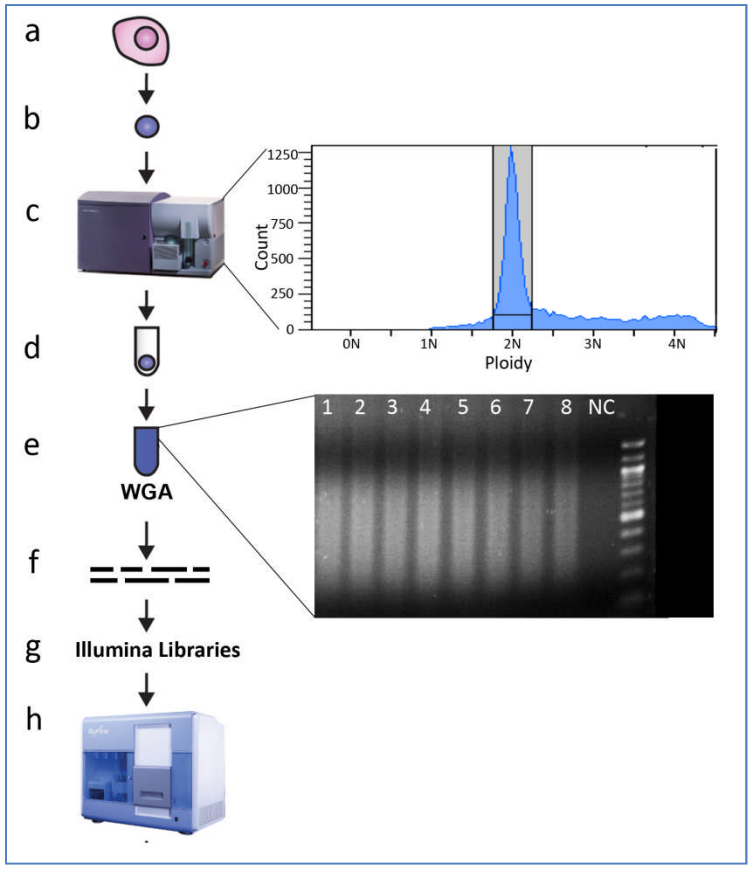


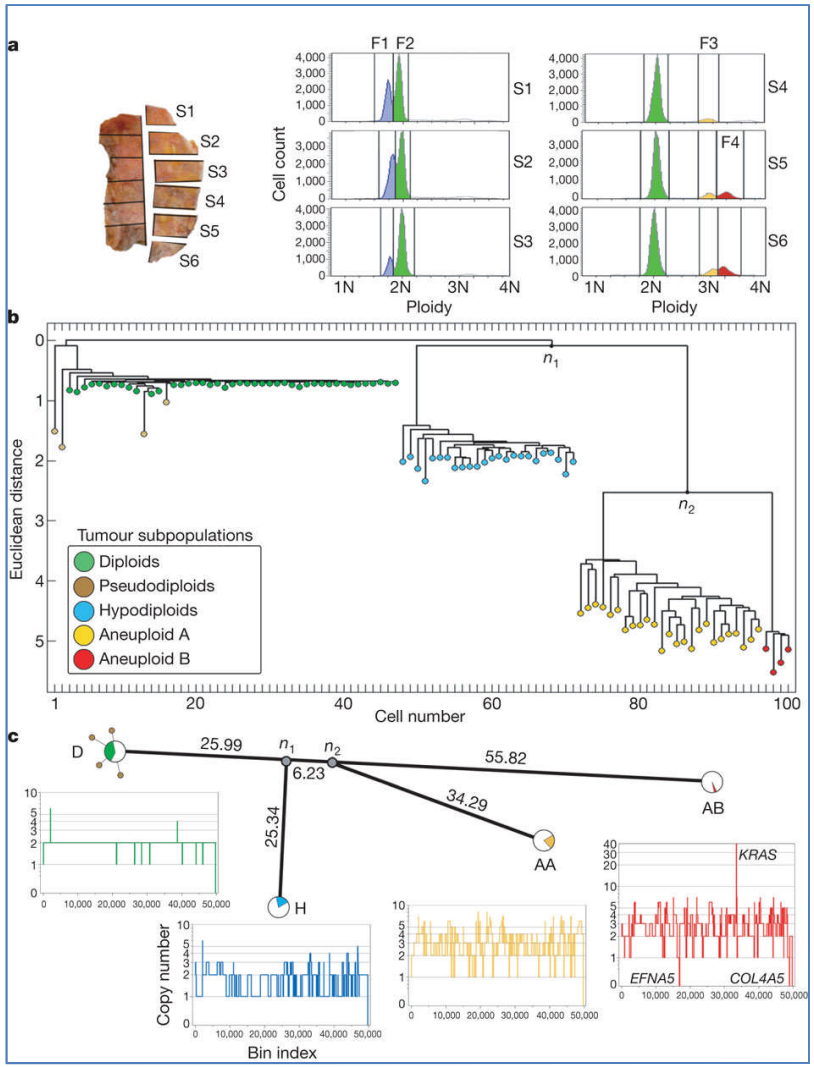


Tumour evolution inferred by single-cell sequencing

Nicholas Navin et al.

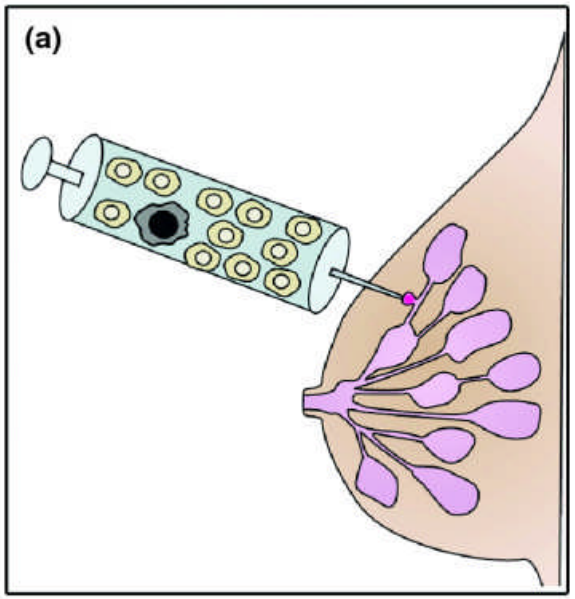
Nature VOL 472 6 MAY 2011 90



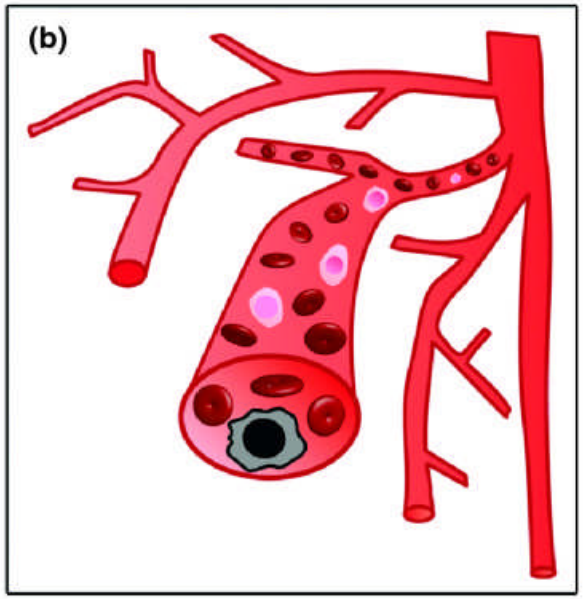


Where will Single Cell Analysis make a difference?

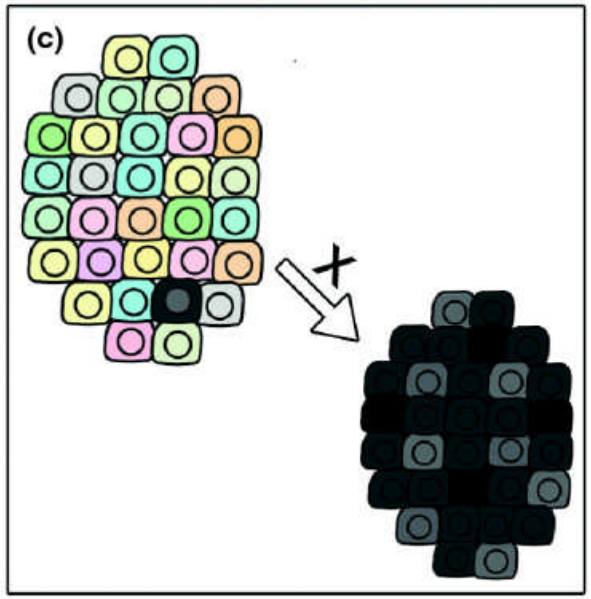
- **Detecting rare cells / states / events**
- **Understanding heterogeneous populations & their response to therapy**
- **Many aspects of stem cell biology**
- **Pathological analysis**
- **Compound screening**



Scarce clinical samples

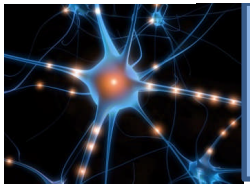


Circulating tumour cells



Rare chemo resistant cells

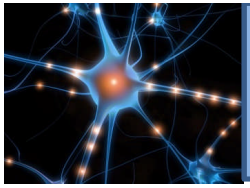
Navin and Hicks, *Genome Medicine* 2011 **3**:31



SINGLE CELL ANALYSIS

Draft Milestones & Timeline

	FY12	FY13	FY14	FY15	FY16
1. Accelerate discovery of new approaches	•Solicit Projects		•Re-assess needs and opportunities	•Solicit Projects	
2.Understand cell heterogeneity	•Solicit Projects	• Solicit Projects			
3. Accelerate validation and translation of single cell technologies	•Clinical needs assessment •Solicit Projects	•Solicit Projects			•Significant steps towards wider adoption
4. Multidisciplinary Team Challenges	•Workshop	•Workshop	•Workshop •Define Community Challenge	•Workshop	•Workshop recognize solutions to Challenge
Additional Goals and Milestones	•Set up a Special Interest Group to increase awareness within NIH	•Use experience from FY13 to tailor focus of RFAs in FY14	•Assess if the initial gaps and opportunities have been addressed and identify new ones	•Build interest and support participants in the Single Cell Challenge	•Explore outcomes and transition opportunities for funded projects •Evaluate Concept



Is this initiative transformative?

- **This initiative is focused on discovery and translation of multiplexed, *in situ* approaches for the spatiotemporal analysis of a heterogeneous population of cells**

Will this initiative advance the individual missions of NIH ICs?

- **Cells are at the heart of biomedical research - this initiative will build synergy between the Institutes and Centers already active in this rapidly emerging field**

Does this initiative require participation of NIH as a whole?

- **Understanding the link between cell states and tissue-level function is central to understanding the emergence & progression of many diseases and disorders**

Is this initiative something that no other entity is likely or able to do?

- **A coordinated effort by the NIH will promote scientific advancement and ensures essential biomedical needs are addressed**

QUESTIONS?