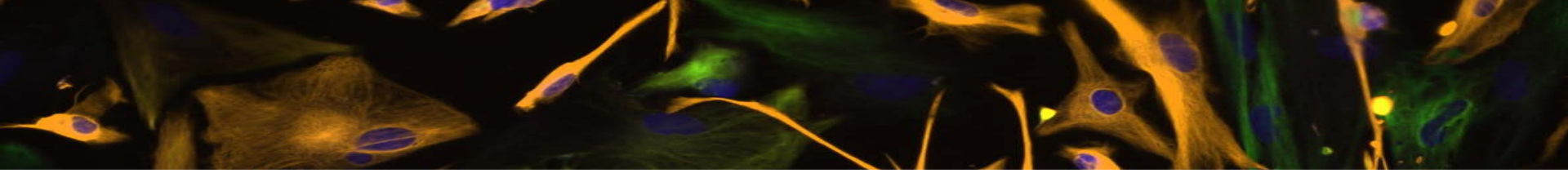


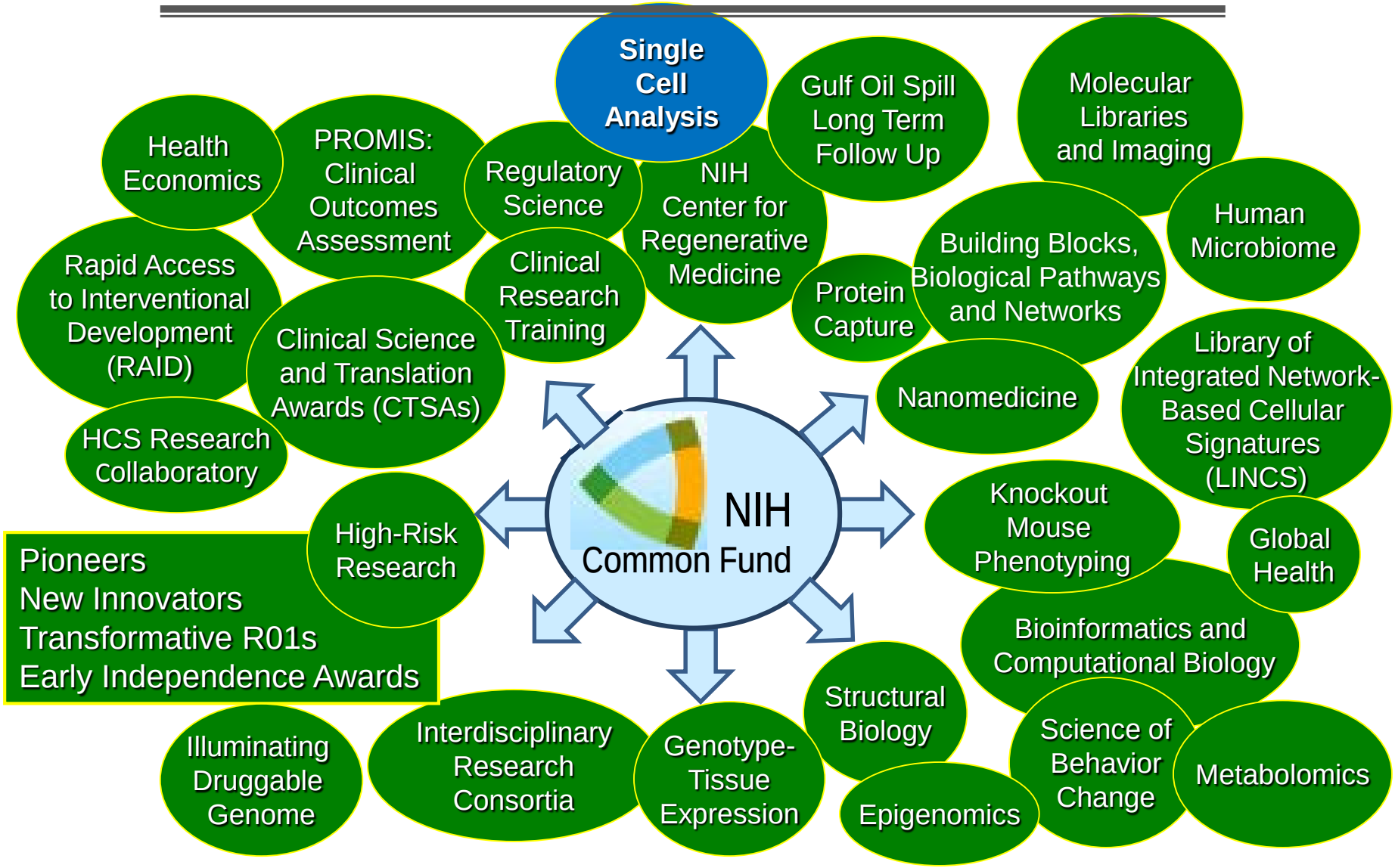
SINGLE CELL ANALYSIS

NIH Common Fund Program

May 4, 2016

Yong Yao,
National Institute of Mental Health

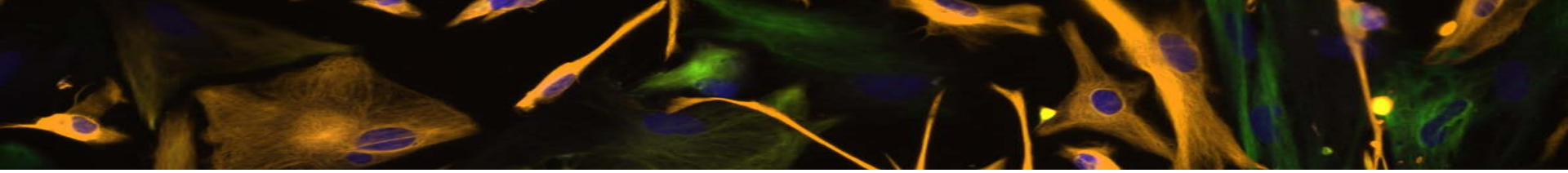
NIH Common Fund Programs



See updates @ http://commonfund.nih.gov/initiativeslist

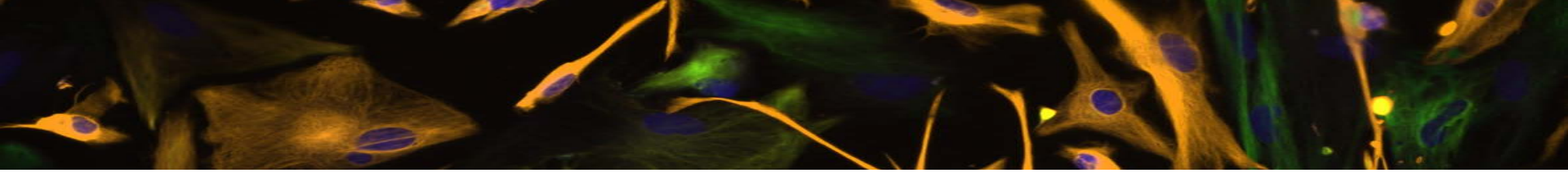
Criteria for Common Fund Programs

- **Transformative:** Must have high potential to dramatically affect biomedical and/or behavioral research over the next decade
- **Synergistic:** Outcomes must synergistically promote and advance individual missions of NIH Institutes and Centers to benefit health
- **Catalytic**: Must achieve a defined set of high impact goals within 5-10 years
- **Cross-cutting:** Program areas must cut across missions of multiple NIH Institutes and Centers, be relevant to multiple diseases or conditions, and be sufficiently complex to require a coordinated, trans-NIH approach
- **Unique:** Must be something no other entity is likely or able to do



Single Cell Analysis Workgroup:

- David Balasundaram (CSR)
- Ravi Basavappa (OD)+
- Andrea Beckel-Mitchener (NIMH)
- Olivier Blondel (NIDDK)
- Joseph Breen (NIAID)
- Jill Carrington (NIDDK)
- Lynda Chiodetti (NIAID)
- Richard Conroy (NIBIB)*
- Jennifer Couch (NCI)
- Paula Fearon (OD)
- Michelle Freund (NIMH)
- Joe Gindhart (NIGMS)
- Deborah Hoshizaki (NIDDK)
- J. Randy Knowlton (NCI)
- Jennie Larkin (NHLBI)
- Sara Lin (NHLBI)
- Roger Little (NIMH)
- Su-Yau Mao (NIAMS)
- Alan Michelson (NHLBI)
- Oleg Mirochnitchenko (OD)
- Cathy Ng (NIMH)
- David Owens (NINDS)
- David Panchision (NIMH)
- Ajay Pillai (NHGRI)
- Carol Pontzer (NCCAM)
- Erin Shannon (NIMH)
- Grace Shen (NEI)
- Lillian Shum (NIDCR)
- Elizabeth Stansell (NIAID)
- Susan Taymans (NICHD)
- Jose Velazquez (NIA)
- Da-Yu Wu (NIDA)
- Yong Yao (NIMH)*



Goals of the NIH Single Cell Analysis Program:

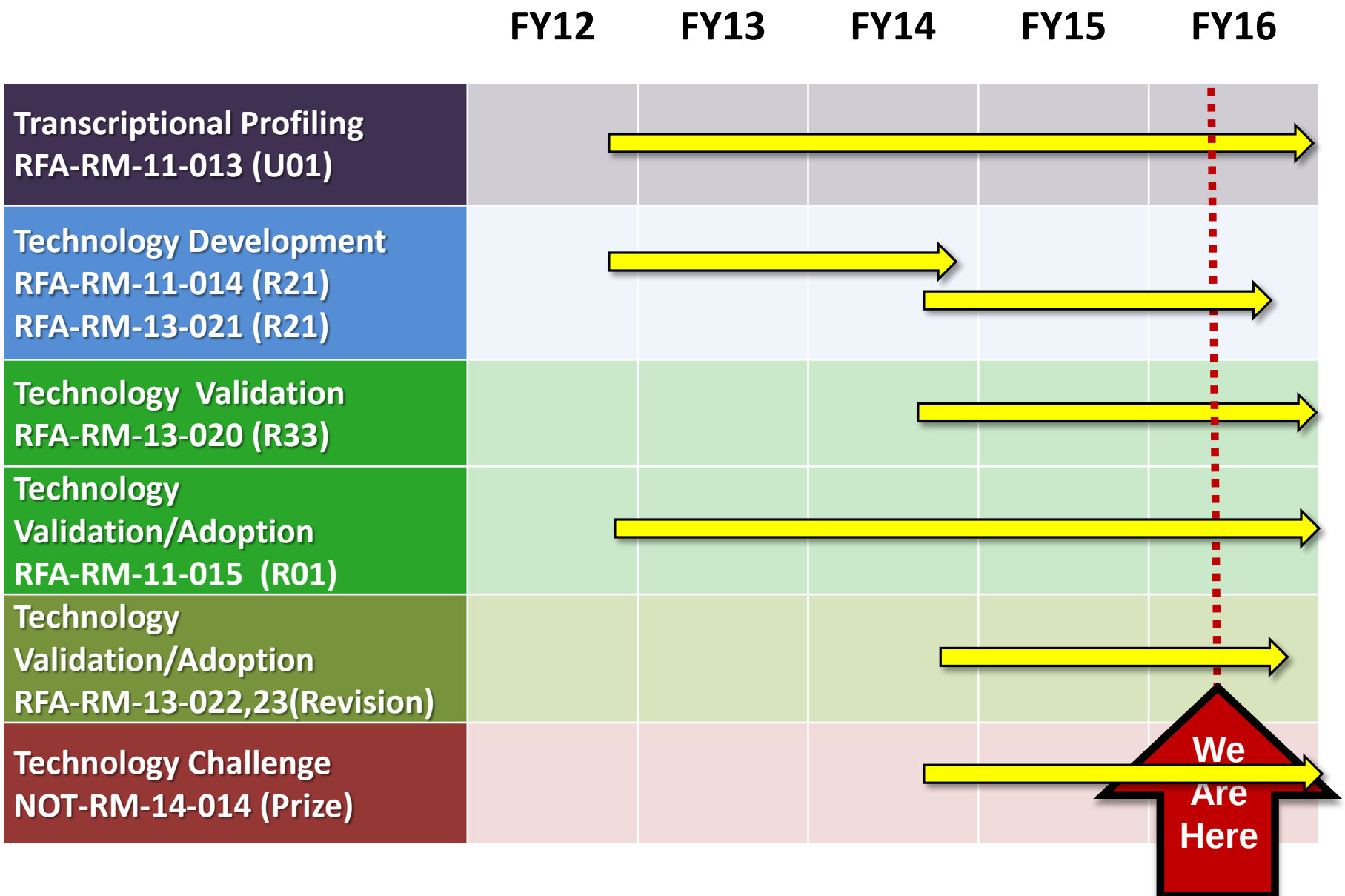
1. Improve our understanding of cell heterogeneity, including defining cell types and dynamic cell states

2. Accelerate the development of new tools and approaches

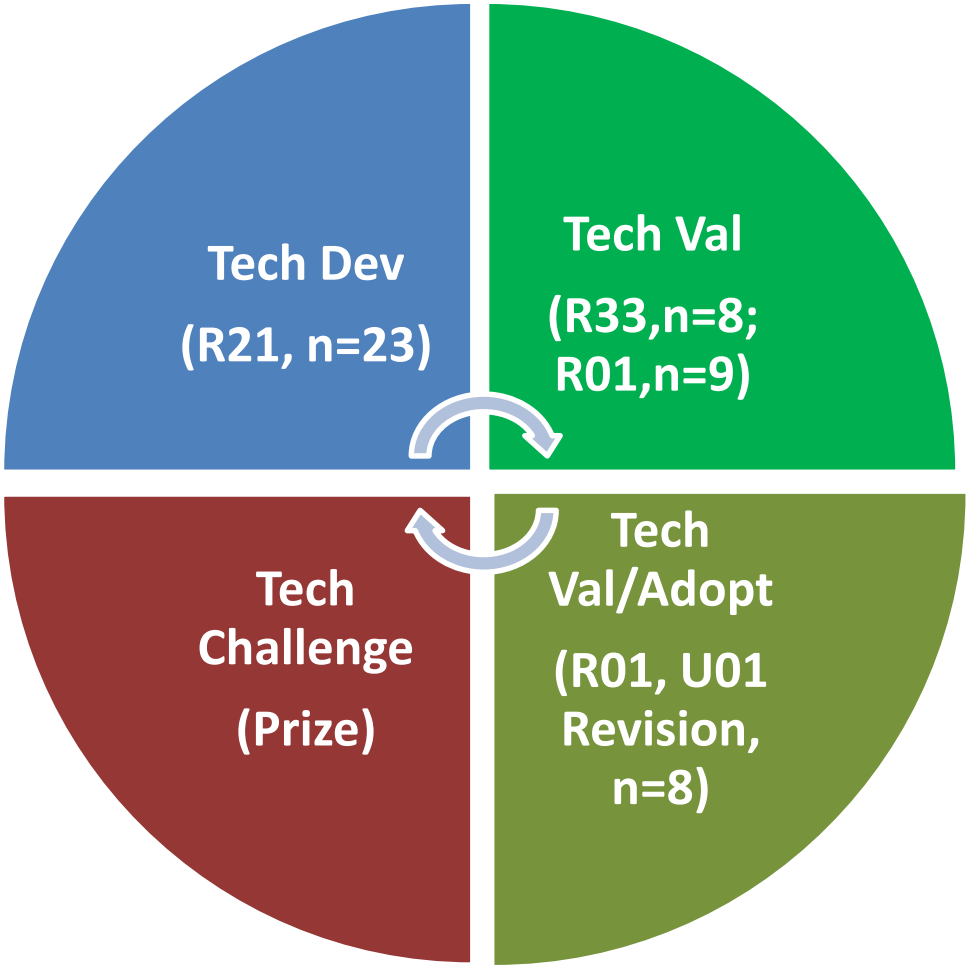
3. Accelerate the validation, translation, and adoption of new technologies

4. Engage multidisciplinary teams to confront defined challenges

Timeline of the Funded Projects

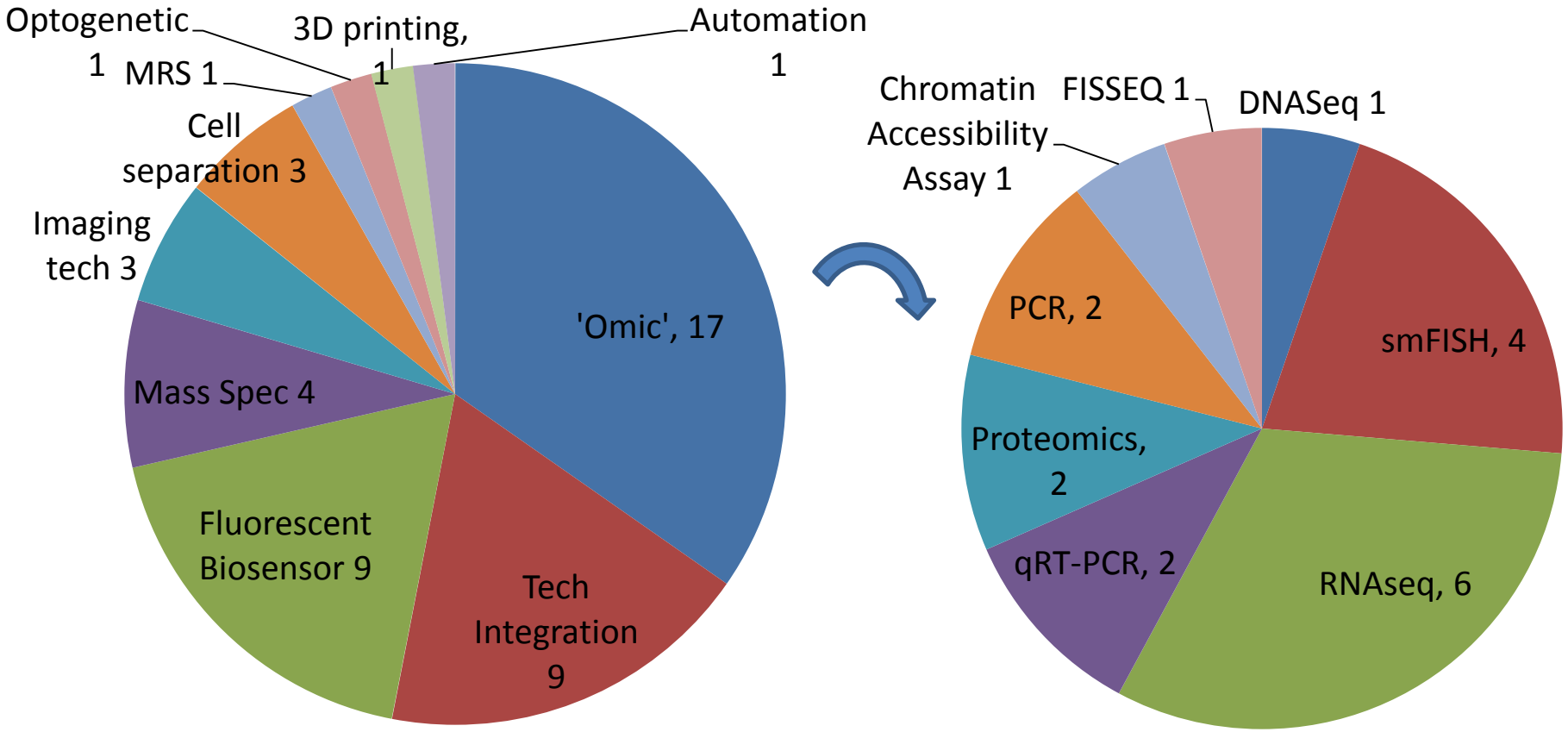


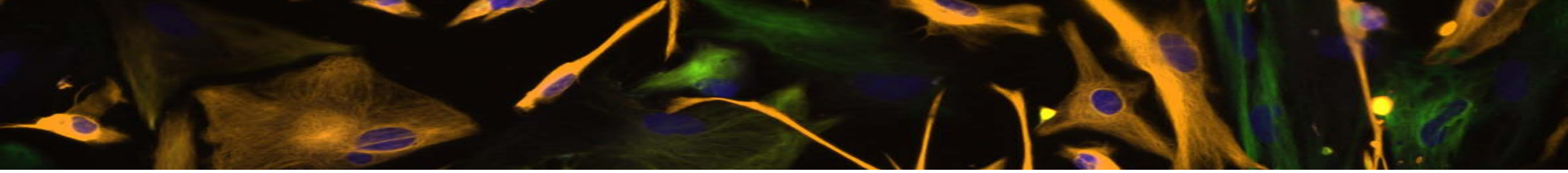
Portfolio of SCAP Awards



Transcriptional Profiling Centers (U01, n=3)

Current SCAP Technology Portfolio





Highlights from the SCAP Portfolio

Nat Methods. 2014 Feb;11(2):190-6. **Transcriptome in vivo analysis (TIVA) of spatially defined single cells in live tissue.**

Lovatt D[1], Ruble BK[2], Lee J[3], Dueck H[4], Kim TK[3], Fisher S[4], Francis C[4], Spaethling JM[3], Wolf JA[5], Grady MS[5], Ulyanova AV[5], Yeldell SB[6], Griepenburg JC[6], Buckley PT[3], Kim J[7], Sul JY[3], Dmochowski IJ[2], Eberwine J[8].

Science. 2014 Mar 21;343(6177):1360-3. **Highly multiplexed subcellular RNA sequencing in situ.**

Lee JH[1], Daugharthy ER, Scheiman J, Kalhor R, Yang JL, Ferrante TC, Terry R, Jeanty SS, Li C, Amamoto R, Peters DT, Turczyk BM, Marblestone AH, Inverso SA, Bernard A, Mali P, Rios X, Aach J, Church GM.

Nat Methods. 2014 Apr;11(4):360-1. **Single-cell in situ RNA profiling by sequential hybridization.**

Lubeck E[1], Coskun AF[1], Zhiyentayev T[2], Ahmad M[2], Cai L[2].

Cell. 2014 Jun 19;157(7):1724-34. **High-sensitivity measurements of multiple kinase activities in live single cells.**

Regot S[1], Hughey JJ[2], Bajar BT[2], Carrasco S[2], Covert MW[3].

Nature. 2014 Aug 14;512(7513):155-60. **Clonal evolution in breast cancer revealed by single nucleus genome sequencing.**

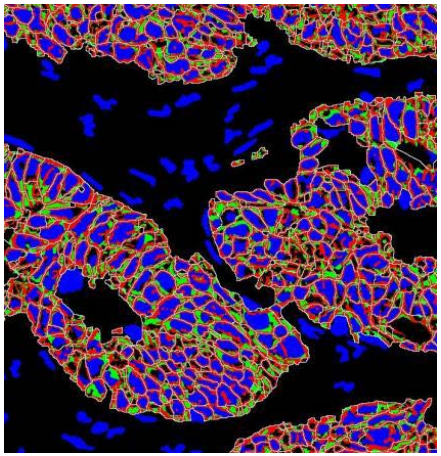
Wang Y[1], Waters J[1], Leung ML[2], Unruh A[1], Roh W[1], Shi X[1], Chen K[3], Scheet P[4], Vattathil S[4], Liang H[3], Multani A[1], Zhang H[5], Zhao R[6], Michor F[6], Meric-Bernstam F[7], Navin NE[8].

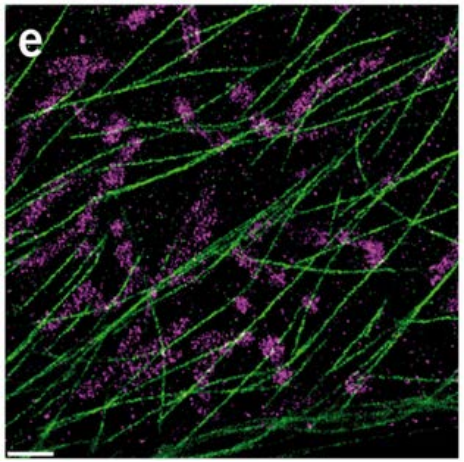
Cell. 2015 May 21;161(5):1187-201. **Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells.**

Klein AM[1], Mazutis L[2], Akartuna I[3], Tallapragada N[1], Veres A[4], Li V[1], Peshkin L[1], Weitz DA[5], Kirschner MW[6].

Technology Innovation in Single-Cell Proteomics and Metabolomics

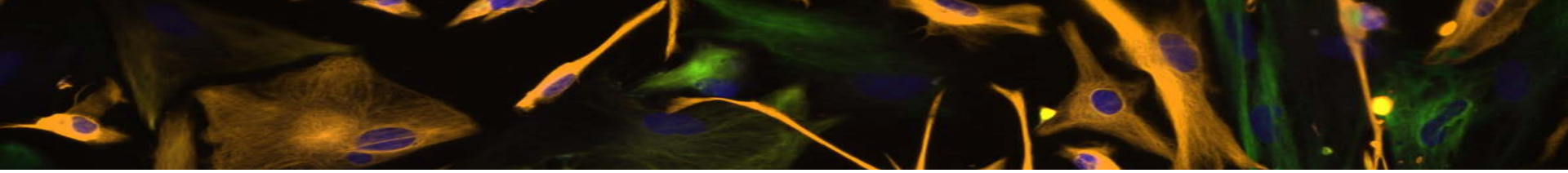
>60 Proteins then DNA FISH
Gerdes, GE Global Research
SCAP Project: R01CA173377



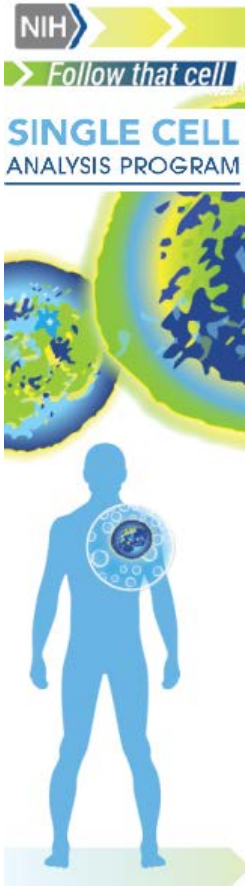


Multiplexed cellular super-resolution imaging using DNA-PAINT and Exchange-PAINT, Yin et al., Nat Methods. 2014 Mar;11(3):313-8.

Imaging lipid metabolism in live C. elegans using stimulated Raman scattering imaging. Cheng et al. SCAP Project: R21 GM114853



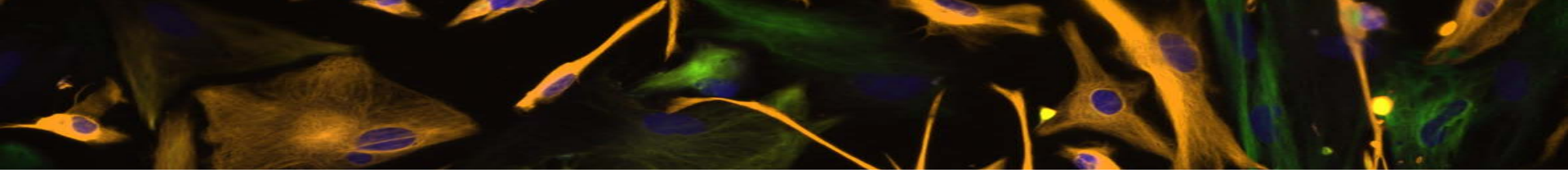
“Follow that Cell” Challenge



- <u>GOAL</u>: To identify novel robust methods for analysis of individual cells that can detect and assess dynamic changes in cell behavior and function over time

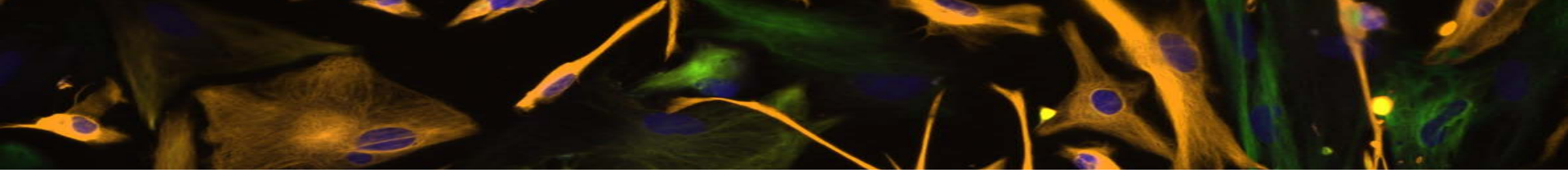
- Total prize amount of $500,000
  - Awards to U.S. individuals/teams/entities
  - Foreign solvers may only compete in a U.S. team

- Structured in TWO linked phases:
  - Phase 1 is Theoretical
  - Phase 2 is Reduction to Practice of Phase 1 Solution
  - Phase 2 submission: March 30, 2017



Other Recent Technology Breakthroughs in Single-Cell Analysis

- DNA seq, DNA methylation, chromosome conformation, and chromatin state can now be analyzed in single cells
- Multiple single-cell RNA-seq protocols have been established recently, including microdroplet- and Fluidigm microchip-based high throughput protocols
- 100 to 1000 unique RNA species can be imaged in hundreds of individual cells at single copy sensitivity (MERFISH, CLARITY)
- ~40 unique protein species can be assayed simultaneously in high throughput manner (mass cytometry, CyTOF, CLARITY)

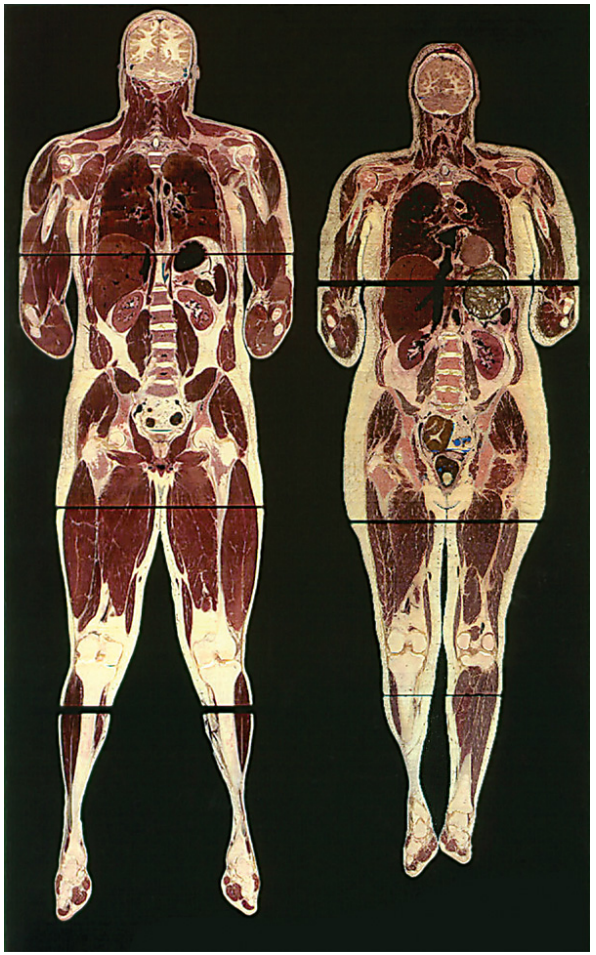


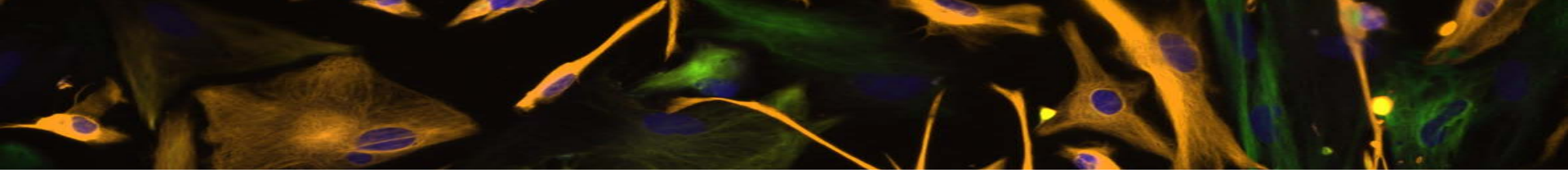
What About A Human Cell Taxonomy Project?

- A human has 40 trillion cells (4x 10^{13}), and as many as 100,000 distinct biomolecules per cell
- We can now measure ~25,000 distinct biomolecules at single cell level, and can measure 10-100's of molecules / 1000 cells / s.

- It would take ~1000 years to characterize every cell at this rate. Can increase throughput and distribute effort focus on key organs first.

- 20 years ago the Visible Human Project achieved 0.3mm resolution

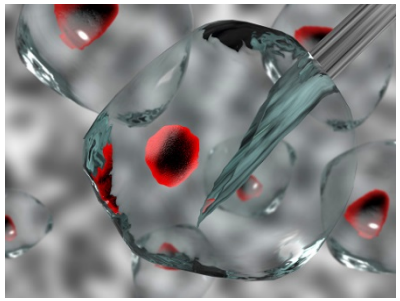




A Human Cell Atlas in Dawn

- Three concepts were cleared for a potential launch in FY 2018 or beyond (pending availability of funds)

- **Human Cell Atlas**: The goals of the Human Cell Atlas would be to a) use newly emerging technologies and methods to create an atlas that describes cells in human tissues at a single cell level, b) to establish a data resource, and c) to continue the development of tools and methods that support this endeavor.



FY 2018 Phase 2 Strategic Planning Activities

NIH and the BRAIN Initiative

Brain Research through Advancing Innovative Neurotechnologies

Andrea Beckel-Mitchener
NIMH
May 4, 2016

“The Next Great American Project”

“So there is this enormous mystery waiting to be unlocked, and the BRAIN Initiative will change that by **giving scientists the tools they need to get a dynamic picture of the brain in action** and better understand how we think and how we learn and how we remember. And that knowledge could be – will be – transformative.”

~President Obama, April 2, 2013

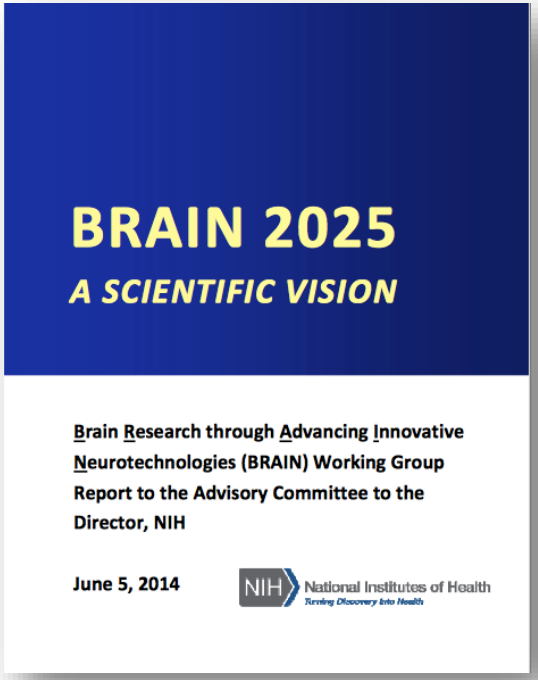
BRAIN Initiative

“a public and private effort”

+

Private Investments

The NIH BRAIN Scientific Plan



***Focus on circuits and networks**

FIRST FIVE YEARS → **SECOND FIVE YEARS**

Emphasize technology development

Emphasize discovery driven science

www.braininitiative.nih.gov

Seven High Priority Research Areas

1. **Discovering diversity:** Identify and provide experimental access to the different brain cell types to determine their roles in health and disease.

2. **Maps at multiple scales:** Generate circuit diagrams that vary in resolution from synapses to the whole brain.

3. **The brain in action:** Produce a dynamic picture of the functioning brain by developing and applying improved methods for large-scale monitoring of neural activity.

4. **Demonstrating causality:** Link brain activity to behavior with precise interventional tools that change neural circuit dynamics.

5. **Identifying fundamental principles:** Produce conceptual foundations for understanding the biological basis of mental processes through development of new theoretical and data analysis tools.

6. **Advancing human neuroscience:** Develop innovative technologies to understand the human brain and treat its disorders; create and support integrated human brain research networks.

7. **From BRAIN Initiative to the brain**: Integrate new technological and conceptual approaches produced in goals #1-6 to discover how dynamic patterns of neural activity are transformed into cognition, emotion, perception, and action in health and disease.

Original FY14 RFA	Title
MH-14-215 **(3 yr project period)**	**Transformative Approaches for Cell-Type Classification in the Brain**

Funded Cell Census Projects

Pilot Studies for Cell Classification

- 10 Funded Projects, ~$12 million per year for three years
- Pilot classification strategies for a systematic inventory/census of cell types
- *Species*: zebrafish, mouse, rat, macaque, human
- *Brain Regions*: cortex, basal ganglia, retina, habenula, hypothalamus, amygdala, whole brain
- *Approaches*: **single cell gene expression**, anatomical projections, electrophysiology, developmental lineage, DNA methylation patterns
- Operate as a consortium, identifying best practices and gaps in understanding

Exciting New Discoveries

Cell

Resource

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Evan Z. Macosko,[1,2,3,*] Anindita Basu,[4,5] Rahul Satija,[4,6,7] James Nemesh,[1,2,3] Karthik Shekh
Itay Tirosh,[4] Allison R. Bialas,[8] Nolan Kamitaki,[1,2,3] Emily M. Martersteck,[8] John J. Trombett
Joshua R. Sanes,[9] Alex K. Shalek,[4,11,12] Aviv Regev,[4,13,14] and Steven A. McCarroll[1,2,3,*]
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA

CellPress

Resource

Mapping Social Behavior-Induced Brain Activation at Cellular Resolution in the Mouse

Yongsoo Kim,[1] Kannan Umadevi Venkataraju,[1] Kith Pradhan,[1] Carolin Mende,[1] Julian Taranda,[1] Srinivas C. Turaga,[2] Ignacio Arganda-Carreras,[2] Lydia Ng,[3] Michael J. Hawrylycz,[3] Kathleen S. Rockland,[1,4] H. Sebastian Seung,[2] and Pavel Osten[1,*]

Neuron

NeuroResource

Epigenomic Signatures of Neuronal Diversity in the Mammalian Brain

Alisa Mo,[1,2,11] Eran A. Mukamel,[3,4,11] Fred P. Davis,[5,11] Chongyuan Luo,[6,11] Gilbert L. Henry,[5] Serge Picard,[5] Mark A. Urich,[6] Joseph R. Nery,[6] Terrence J. Sejnowski,[3,7,8] Ryan Lister,[6,9] Sean R. Eddy,[5] Joseph R. Ecker,[6,8,*] and Jeremy Nathans[1,2,10,*]

Cell

Molecular Identity of Human Outer Radial Glia during Cortical Development

Alex A. Pollen,[1,2,5,*] Tomasz J. Nowakowski,[1,2,5] Jiadong Chen,[1,2] Hanna Retallack,[1,2] Carmen Sandoval-Espinosa,[1,2] Cory R. Nicholas,[1,6] Joe Shuga,[3] Siyuan J. Liu,[1,2] Michael C. Oldham,[1] Aaron Diaz,[1,4] Daniel A. Lim,[1,4] Anne A. Leyrat,[3] Jay A. West,[3] and Arnold R. Kriegstein[1,2,*]

nature neuroscience

Adult mouse cortical cell taxonomy revealed by single cell transcriptomics

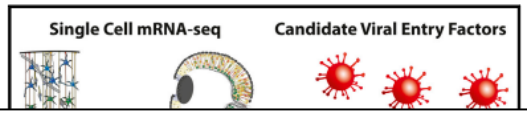
Bosiljka Tasic[1,2], Vilas Menon[1,2], Thuc Nghi Nguyen[1], Tae Kyung Kim[1], Tim Jarsky[1], Zizhen Yao[1], Boaz Lucas T Gray[1], Staci A Sorensen[1], Tim Dolbeare[1], Darren Bertagnolli[1], Jeff Goldy[1], Nadiya Shapovalov Sheana Parry[1], Changkyu Lee[1], Kimberly Smith[1], Amy Bernard[1], Linda Madisen[1], Susan M Sunkin[1], Michael Hawrylycz[1], Christof Koch[1] & Hongkui Zeng[1]

Brief Report

Cell Stem Cell

Expression Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells

Graphical Abstract



Authors

Tomasz J. Nowakowski, Alex A. Pollen, Elizabeth Di Lullo, Carmen Sandoval-Espinosa, Marina Bershteyn, Arnold R. Kriegstein

Conceptual Framework

Ongoing discussions on cell classification

- **Morphological considerations**
- **Molecular signatures**
- **Quantifiable functional measures**
- **Increasing spatial knowledge of cellular environment and interactions (or connections)**
- **Temporal considerations**
- **Current technologies**
- **Data driven classifications**
- **Data harmonization and data sharing**

Defining Cellular Phenotype

Workshop held April 22-23, 2015

http://www.braininitiative.nih.gov/meetings/DCPSummary.htm

Next steps

"Production phase"

Large-scale Cell Census Project(s)

- Single cell analysis likely to be prominent
- Measure multiple endpoints to understand/define cellular phenotype
- Focus on mouse; include human and non-human primate models
- Support novel tool development
- Plan for data management, harmonization and sharing

Stay tuned for Funding Opportunities!

Acknowledgements

NIH Team Members

Michelle Freund (NIMH)
Cathy Ng (NIMH)
Wen Chen (NCCIH)
James Coulombe (NICHD)
Fred Friedman (NIMH)
Lindsey Grandison (NIAAA)
Thomas Greenwell (NEI)
Debbie Henken (NICHD)
Megan Kinnane (NIMH)
John Kusiak (NIDCR)
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Dave Owens (NINDS)
Vinay Pai (NIBIB)
Jonathan Pollock (NIDA)
Brad Wise (NIA)
Da Yu Wu (NIDA)
Yong Yao (NIMH)
Steve Zalcman (NIMH)

www.braininitiative.nih.gov

Challenges and Opportunities for Single Cell Analysis at NCI

NIH NATIONAL CANCER INSTITUTE

May 4, 2016

Examples of Single Analysis in Cancer Research

- 1. Peter Sims; 1R33CA202827-01(IMAT) Large-Scale Integration of Single Cell RNA-Seq and High-Content Imaging for Analyzing Drug Response in Cancer: Leveraging two components of SCAP to address a pressing clinical problem.
- 2. Lidong Qin; 1R21CA191179-01A1 (IMAT Sample Prep) High Throughput Single-Cell Phenotype Isolation by Protrusion Analysis Chip (PAC): Method to capture solid tumor cells with a defined phenotype (metastatic) for downstream analysis.
- 3. Kevin Janes; 1R01CA194470-01 (PQB4) Stochastic Profiling of Functional Single-Cell States Within Solid Tumors: Link single-cell regulatory states to clinical parameters.

A Few Topics of Interest to NCI (biased and not inclusive)

- How clonal expansion of a single cell can lead to tumor phenotype and the corollary of lineage tracing back to the progenitor cell
- More reproducible and sensitive omics technologies
- How a single cell molds it's environment and vis-versa. What dominates the interactions at what time points and how does this drive phenotype
- Live cell in situ single cell analysis that maintains the spatial and temporal fidelity of the tissue.

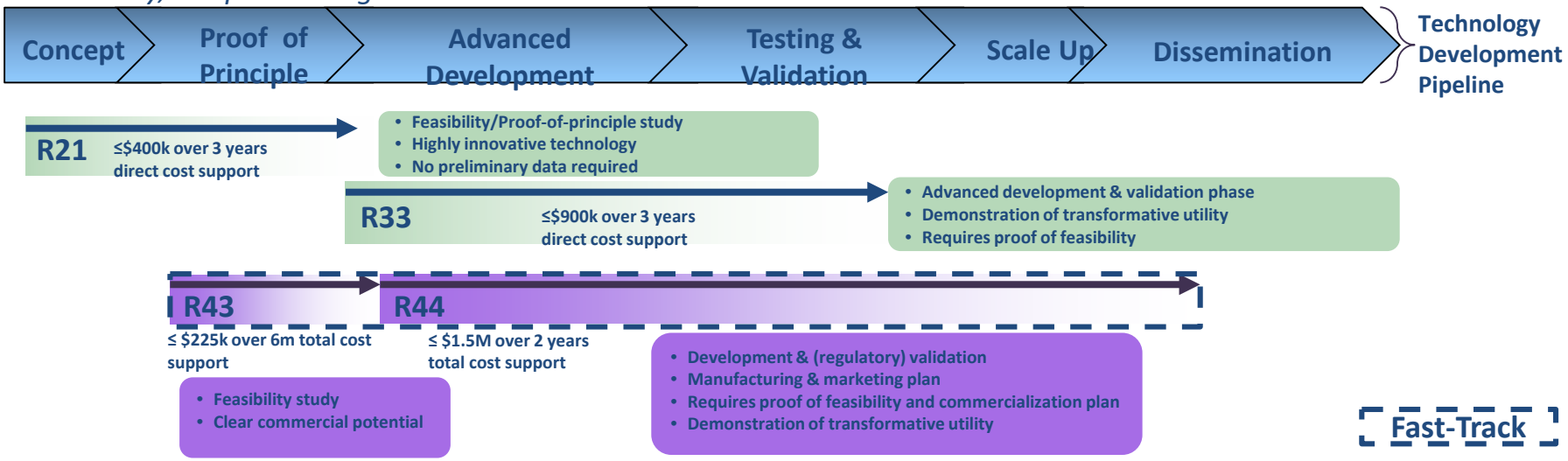
Current Funding Mechanisms for SCA Technology Development and Hypothesis Driven Research

- Innovative Molecular Analysis Technologies (IMAT); http://innovation.cancer.gov/
- Enabling Bioengineering Research Grants (EBRG; R21): http://grants.nih.gov/grants/guide/pa-files/PA-16-040.html
- Bioengineering Research Grants (BRG; R01): (to be reissued soon) http://grants.nih.gov/grants/guide/pa-files/PAR-13-137.html
- Bioengineering Research Partnership (BRP; U01): http://grants.nih.gov/grants/guide/pa-files/PAR-16-116.html
- Provocative Questions (PQ's; R01's and R21's): http://provocativequestions.nci.nih.gov/rfa/mainquestions_listview

Innovative Molecular Analysis Technologies (IMAT) Program

Program Mission:

To support the development, maturation, and dissemination of novel and potentially transformative next-generation technologies through an approach of balanced but targeted innovation in support of clinical, laboratory, or epidemiological research on cancer.



Open Funding Opportunities for Innovative Technology Development

INNOVATIVE MOLECULAR ANALYSIS TECHNOLOGIES

Molecular/Cellular Analysis Tools

R21 –
- **Proof-of-Concept**
- **no preliminary data required**

R33 –
- **Optimization/ scaling**
- **Validation**

Sample Quality Assessment & Control Tools

R21 –
- **Proof-of-Concept**
- **no preliminary data required**

R33 –
- **Optimization/ scaling**
- **Validation**

RFA-CA16-001: Early-Stage Innovative Molecular Analysis Technology Development for Cancer Research • Budget: **$400k/3yrs (direct cost cap)**
RFA-CA16-002: Advanced Development and Validation of Emerging Molecular Analysis Technologies for Cancer Research • Budget: **$900k/3yrs (direct cost cap)**
RFA-CA16-003: Innovative Technologies for Cancer-Relevant Biospecimen Science • Budget: **$400k/3yrs (direct cost cap)**
RFA-CA16-004: Advanced Development and Validation of Emerging Technologies for Cancer-Relevant Biospecimen Science • Budget: **$900k/3yrs (direct cost cap)**

Application deadlines: May 26 and September 26, 2016

Innovative Molecular Analysis Technologies (IMAT)

1. Reviewed by NCI Special Emphasis Panel (SEP).
2. Technology development and validation only. Applications that have a hypothesis or address a biological question are not accepted.
3. Must propose a novel technology or a novel combination of existing technologies that will enable new research capabilities or make existing capabilities significantly better (specificity, reliability, repeatability), faster, and/or cheaper.
4. R21 is proof of principle and requires milestones.
5. R33 is demonstration of how it can be used as a research, translational, or clinical tool.

Bioengineering Research Grants

1. NIH wide with specific institutes signed on to participate in each
2. CSR review with some going to standard review panels and some going to special emphasis panels (SEPS)
3. More biology driven with the engineering portion usually couched in terms of answering a specific biological question
4. The BRP is like a mini program project. Larger budget with each SA like an extremely focused R01 with the whole program centered around a theme.

NCI Provocative Questions (PQ's)

1. R01's and R21's; The questions change about every 2 years
2. Each question is unique and may be for tech dev or a biological question
3. Reviewed by NCI SEPS

Questions

Contact Information:

Randy Knowlton
jk339o@nih.gov
240 276 6193

www.cancer.gov

www.cancer.gov/espanol