2012 Single Cell Analysis Workshop

Where: Hyatt Regency, Bethesda MD

When: April 17-18, 2012

Co-chairs: Dr. Gary Nolan (Stanford University) and Dr. Nancy Allbritton (University of North Carolina)

The NIH Common Fund held a Single Cell Analysis meeting on April 17-18, 2012 in Bethesda, MD. The objective was to provide a forum to bring together a multidisciplinary group of investigators and federal staff to discuss the latest technological advances and transformative discoveries in the area of single cell analysis. The focus was on approaches that further our understanding of cell heterogeneity or variation, including identifying unique cell types and cell "states". The goal was to share knowledge and accelerate the dissemination of technological advances within the relevant research community. The format incorporated short talks, poster presentations, and panel discussions providing the group with opportunities to review recent progress and consider the major challenges facing the field over the next decade. There were over 90 registered participants from academia as well as the public and private sectors with 20 attendees presenting posters during two day meeting. Four presentation/discussion sessions were held. A general description and summary of each follows.

Session 1—Profiling Individual Cell State: Genomics

Roger Lasken, Craig Venter Institute
Barbara Wold, California Institute of Technology
Robert Singer, Albert Einstein College of Medicine
Paul Soloway, Cornell University
Junhyong Kim, University of Pennsylvania

Presenters discussed sequencing whole genomes at the level of a single cell or single nuclei; measuring and visualizing RNA expression and localization including technical challenges associated with multiplexed analyses; advances in single cell epigenomic analysis; and bioinformatics and computational questions using genomic/transcriptomic data from single cell experiments.

The panel agreed that the challenges are significant and there is a need for improved tools on multiple fronts including computational approaches. The ability to integrate data, especially with regard to dynamic measures, is critical. Further, there are various applications where kinetic measures in live cells in "native" contexts would be advantageous, since knowledge of a cell's microenvironment may be

critical to understanding biological outcomes. The panel noted that RNA half-life is variable and can differ from protein half-life, which should be considered when function is evaluated. What may end up being most useful is not the absolute concentration of biomolecules, but rather the stoichiometric relationships observed. Ultimately, discussants noted the importance of understanding normal, variable cell states, which will impact detection and interpretation of disease processes as well as responses or potential treatment outcomes at the single cell level. The group discussed the use and definition of the term "noise", which can have multiple meanings and may sometimes be misleading if not appropriately defined. Perceived "noise" may simply be normal variation in cell states and/or may actually be necessary and interesting from a biological perspective.

Session 2—Profiling Individual Cell State: Proteomics and Metabolomics

Daniel Chiu, University of Washington
Garry Nolan, Stanford University
Cynthia McMurray, Lawrence Berkeley National Laboratory
Anup Singh, Sandia National Laboratories
Jonathan Sweedler, University of Illinois at Urbana-Champaign
Deirdre Meldrum, Arizona State University

Speakers in this session discussed topics related to the measurement of biochemical and biophysical properties in single cells. Tools for isolating and studying single cells and subcellular compartments are currently in development, but while the technology is moving quickly, adapting these systems for many biological contexts and promoting broader use will be challenging. Most of the techniques measure a single parameter in an isolated cellular unit and it was generally agreed that developing approaches that integrate multiple measures will be valuable. There was a discussion on distinguishing steady-state readouts from measures associated with perturbations. Prior knowledge of normal cell processes is essential for interpretation.

Again, the importance of microenvironments was raised, especially with regard to ultimately understanding signatures associated with disease progression. Concern about how many cells will need to be analyzed for any given endpoint was expressed; much will depend on the tissue/cell type and the endpoints examined. Integration of datasets from the same cells may help define cell states better thereby reducing the numbers needed. Participants discussed a number of additional points including the importance of addressing bias in both data collection and analysis; steady state vs. perturbation measurements; the challenges associated with using different techniques on the same cell; and the difficulties of comparing and sharing data because there are no existing standards.

Session 3—Analyzing Dynamic Cell States

Hong Qian, University of Washington
Klaus Hahn, University of North Carolina at Chapel

Nancy Allbritton, University of North Carolina at Chapel Hill:
Mark Mercola, Sanford Burnham Medical Research Institute
Andre Levchenko, Johns Hopkins University
Rustem Ismagilov, California Institute of Technology

A significant challenge in single cell analysis is devising ways to examine and define biologically relevant cell states and to understand transitions from one state to another. Approaches discussed include attempts to computationally model state changes, high resolution visualization of cell motility and signaling pathways, and methods to isolate cells and establish measures that can be higher throughput and/or miniaturized retaining the required precision and accuracy.

With regard to understanding dynamic states, participants felt that advances in probe sensitivity, instrumentation, and computation will be necessary to achieve the full promise of the approaches discussed. A number of approaches and methods were discussed. Understanding spatial and temporal components in defining cell state is essential. There was discussion in the group regarding the merits of devising and applying standards in order to have some mechanism to validate measures. Better "tissue in a dish" and/or "disease in a dish" models that capture complexity would be very useful. There was high enthusiasm for multiple groups using a single model system to develop techniques and to integrate and cross-validate results. An agreed-upon model could be a specific tissue or perhaps mouse ES cells, but should be something that the greater scientific community would embrace and find interesting. One way to accelerate progress is to encourage collaborative projects that leverage existing efforts while simultaneously stimulating exciting new avenues. It is also important to recognize the importance of getting new technology to end users.

Session 4—Linking Single Cell Measurements to Complex Biological Functions

Steven Altschuler, University of Texas Southwestern Medical Center
Scott Manalis, Massachusetts Institute of Technology
Norm Dovichi, University of Notre Dame
Scott Fraser, California Institute of Technology
Steven DeVries, Northwestern University
Ed Boyden, Massachusetts Institute of Technology

The final meeting session covered topics related to phenotypic measures in single cells that relate to specific functional outcomes in complex populations. Presenters discussed novel approaches to image and analyze single cells within a population with the potential to track changes over time. Other endpoints include physical properties such as cell size, deformability and surface charge, which may relate to metastatic potential and would therefore have significant clinical relevance. Other phenotypes that can be measured include buoyant density, lipid/membrane composition, and electrical properties of the cell.

The panel considered the challenges involved with analyzing single cells in a complex environment. Improvements to existing technology are needed such as new probes, better dynamic range detectability, and improved throughput, but novel approaches are also needed (for example, analytical methods for three-dimensional super-resolution imaging and the ability to track samples over time.) In general, there is a need to study developmental and aging effects; to have better optical tools and probes for monitoring live animals, and faster ways to image organs *in vivo*. Several participants expressed a desire to better detect and monitor macromolecular complexes and others noted the importance of developing informatics to help analyze the complex datasets generated through integrated approaches.

THE NIH COMMON FUND SINGLE CELL ANALYSIS WORKSHOP

APRIL 17–18, 2012 • HYATT REGENCY BETHESDA • BETHESDA, MD

Co-chairs: Garry Nolan and Nancy Allbritton

AGENDA

APRIL 17, 2012

7:30 a.m. Registration Check-in

8:00 a.m. NIH Staff: Welcome

James Anderson, Director of Division of Program Coordination, Planning, and Strategic Initiatives, NIH

8:15 a.m. Session 1—Profiling Individual Cell State: Genomics

8:15 a.m. Roger Lasken, Craig Venter Institute: *Single Cell Genomic DNA and cDNA Sequencing*

8:45 a.m. Barbara Wold, California Institute of Technology: *Single Cell Transcriptomes: Technological and Conceptual Challenges*

9:15 a.m. Robert Singer, Albert Einstein College of Medicine: *RNA Expression and Movement in Single Cells*

9:45 a.m. Paul Soloway, Cornell University: *Single Cell Epigenomics*

BREAK

10:30 a.m. Junhyong Kim, University of Pennsylvania: *Whence single cell variation: case studies from mammalian neurons*

11:00 a.m. Session 1 Grand Challenges

11:45 a.m. Lunch

1:00 p.m. Session 2—Profiling Individual Cell State: Proteomics and Metabolomics

1:00 p.m. Daniel Chiu, University of Washington: *Tools for studying single circulating tumor cells and subcellular compartments (cancer)*

1:30 p.m. Garry Nolan, Stanford University: *Single Cell Phosphoproteomics*

2:00 p.m. Cynthia McMurray, Lawrence Berkeley National Laboratory: *Single Cell Metabolomics*

2:30 p.m. Anup Singh, Sandia National Laboratories: *Integrated biophysical and biochemical measurements in single cells*

BREAK

3:15 p.m. Jonathan Sweedler, University of Illinois at Urbana-Champaign: *Single neuron peptidomics and metabolomics: from discovery of novel molecules to understanding their functions*

3:45 p.m. Deirdre Meldrum, Arizona State University: *Single Cell Biosignatures*

4:15 p.m. Session 2 Grand Challenges

5:00 p.m. Dinner on your own

AGENDA

APRIL 18, 2012

7:30 a.m. Registration Check-in

8:00 a.m. Session 3—Analyzing Dynamic Cell States

8:00 a.m. Hong Qian, University of Washington: *Mathematical Modeling of Cell Dynamics*

8:30 a.m. Klaus Hahn, The University of North Carolina at Chapel Hill: *Peeking and poking at rapid networks in living cells*

9:00 a.m. Nancy Allbritton, The University of North Carolina at Chapel Hill: *Protein Activity in Single Cell Signaling Networks*

BREAK

9:45 a.m. Mark Mercola, Sanford Burnham Medical Research Institute: *High performance image cytometry to discover drugs for cardiac contractility and arrhythmogenicity*

10:15 a.m. Andre Levchenko, Johns Hopkins University: *Modeling Signal Transduction & Cell-Cell Communication*

10:45 a.m. Rustem Ismagilov, California Institute of Technology: *Dynamics of Cell Networks in Space and Time*

11:15 a.m. Session 3 Grand Challenges

Noon Lunch

1:00 p.m. Session 4—Linking Single Cell Measurements to Complex Biological Functions

1:00 p.m. Steven Altschuler, University of Texas Southwestern Medical Center: *Cellular Heterogeneity in Multicellular Organisms*

1:30 p.m. Scott Manalis, Massachusetts Institute of Technology: *Measuring physical properties of single cells*

2:00 p.m. Norm Dovichi, University of Notre Dame: *Metabolic cytometry: monitoring sphingolipid metabolism in single primary neurons*

BREAK

2:45 p.m. Scott Fraser, California Institute of Technology: *Imaging Cell Lineage & Interactions in Developing Embryos*

3:15 p.m. Steven DeVries, Northwestern University: *Interneuron diversity and parallel processing in the Retina*

3:45 p.m. Ed Boyden, Massachusetts Institute of Technology: *Controlling cell functions with light, and robotically analyzing cell phenotypes*

4:15 p.m. Session 4 Grand Challenges

5:00 p.m. Meeting Adjourned

Alexandra Ainsztein, Ph.D.
Program Director
Cell Biology and Biophysics Division
National Institute of General Medical Sciences
National Institutes of Health
45 Center Drive, Room 2As-19K
Bethesda, MD 20892
Phone: 301-594-0828
Email: alexandra.ainsztein@nih.gov

Nancy Allbritton, M.D., Ph.D.
Professor, Department Chair
The University of North Carolina at Chapel Hill
Chapman Hall, Mail Code 3216
Chapel Hill, NC 27599
Phone: 919-966-2291
Email: nlallbri@unc.edu

Steven Altschuler, M.D., Ph.D.
Associate Professor
The University of Texas
Southwestern Medical Center
6001 Forest Park Road
Dallas, TX 75390
Phone: 214-645-6181
Email: steven.altschuler@utsouthwestern.edu

James Anderson, M.D., Ph.D.
Director
Division of Program Coordination,
Planning and Strategic Initiatives
National Institutes of Health
1 Center Drive, Building 1, Room 260
Bethesda, MD 20892
Phone: 301-496-9852
Email: andersonjm@mail.nih.gov

Richard Baird, Ph.D.
Director, Division of Interdisciplinary Training
National Institute of Biomedical
Imaging and Bioengineering
National Institutes of Health
6707 Democracy Boulevard, Suite 200
Bethesda, MD 20892
Phone: 301-496-7671
Email: bairdri@mail.nih.gov

David Balasundaram, Ph.D.
Scientific Review Officer
Center for Scientific Review
National Institutes of Health
6701 Rockledge Drive
Room 5136, MSC 7840
Bethesda, MD 20892
Phone: 301-435-1022
Email: balasundaramd@csr.nih.gov

Susan Banks-Schlegel, Ph.D.
Program Director, Asthma and Cystic Fibrosis
National Heart, Lung, and Blood Institute
National Institutes of Health
Two Rockledge Center
6701 Rockledge Drive
Suite 10042, MSC 7952
Bethesda, MD 20892
Phone: 301-435-0204
Email: schleges@nih.gov

Ravi Basavappa, Ph.D.
Program Director, Office of Strategic Coordination
Division of Program Coordination,
Planning, and Strategic Initiatives
National Institutes of Health
Building 1, Room 201B
Bethesda, MD 20892
Phone: 301-435-7204
Email: basavapr@od.nih.gov

Andrea Beckel-Mitchener, Ph.D.
Chief, Functional Neurogenomics
Neuroscience Center
National Institute of Mental Health
National Institutes of Health
6001 Executive Boulevard, Room 7187
Bethesda, MD 20892
Phone: 301-443-5288
Email: amitchen@mail.nih.gov

Olivier Blondel, Ph.D.
Program Director, Division of Diabetes, Endocrinology, and Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Democracy 2, Room 796
Bethesda, MD 20892
Phone: 301-451-7334
Email: blondelol@niddk.nih.gov

Edward Boyden, Ph.D.
Associate Professor
Massachusetts Institute of Technology
20 Ames Street, E15-421
Cambridge, MA 02139
Phone: 650-468-5625
Email: esb@media.mit.edu

Long Cai, Ph.D.
Assistant Professor of Chemistry
California Institute of Technology
1200 East California Boulevard, MC127-72
Pasadena, CA 91125
Phone: 626-395-6570
Email: lcai@caltech.edu

Julian Candia, Ph.D.
Research Scientist
University of Maryland, College Park
College Park, MD 20740
Phone: 574-386-1896
Email: juliancandia@gmail.com

Lynda Chiodetti, Ph.D.
Section Chief, Innate Immunity Section
Basic Immunology Branch
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
National Insitutes of Health
6610 Rockledge Drive, Room 6411
Bethesda, MD 20892
Phone: 301-451-3119
Email: lchiodetti@niaid.nih.gov

Daniel Chiu, Ph.D.
Professor
Department of Chemistry
University of Washington
204 Bagley Hall
Seattle, WA 98195
Phone: 206-543-1655
Email: chiu@chem.washington.edu

John Collins, Ph.D.
Director
Biopico Systems
23 Pembroke
Irvine, CA 92618
Phone: 949-651-8650
Email: collins@biopico.com

John Collins, Ph.D.
Senior Applications Scientist
NanoInk, Inc.
8025 Lamon Avenue
Skokie, IL 60077
Phone: 847-745-3641
Email: jcollins@nanoink.net

Richard Conroy, Ph.D.
Program Director
National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health
6707 Democracy Boulevard
Bethesda, MD 20892
Phone: 301-402-1486
Email: richard.conroy@nih.gov

Antione Coulon, Ph.D.
Postdoctoral Fellow
Laboratory of Biological Modeling
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Building12A, Room 1003
9000 Rockville Pike
Bethesda, MD 20892
Phone: 301-451-7808
Email: antione.coulon@nih.gov

Steven DeVries, M.D., Ph.D.
Associate Professor
Northwestern University
303 East Chicago Avenue, Tarry 5-715
Chicago, IL 60611
Phone: 312-503-9845
Email: s-devries@northwestern.edu

Norman Dovichi, Ph.D.
Professor
Department of Chemistry and Biochemistry
University of Notre Dame
433 Stepan Hall
Notre Dame, IN 46556
Phone: 574-631-2778
Email: ndovichi@nd.edu

Rong Fan, Ph.D.
Assistant Professor, Biomedical Engineering
Yale University
55 Prospect Street, MEC 213
New Haven, CT 06511
Phone: 203-432-9905
Email: rong.fan@yale.edu

Matthew Ferguson, Ph.D.
CRTA Postdoctoral
Laboratory of Receptor Biology and Gene Expression, National Cancer Institute
National Institutes of Health
41 Library Drive
Bethesda, MD 20892
Phone: 301-451-8120
Email: matthew.ferguson@nih.gov

Scott Fraser, Ph.D.
Biological Imaging Center, Beckman Institute
California Institute of Technology
Pasadena, CA 91125
Email: sefraser@caltech.edu

Michael Gerdes, Ph.D.
Project Leader
Cell Biology Lab
GE Global Research
1 Research Circle, K1-5D39
Niskayuna, NY 12209
Phone: 518-387-7825
Email: gerdes@research.ge.com

Ronald Germain, M.D., Ph.D.
Chief, Laboratory of Systems Biology
National Institute of Allergy and Infectious Disease
National Institutes of Health
Building 10, Room 11N311
10 Center Drive, Mail Stop -1892
Bethesda, MD 20892
Phone: 301-496-1904
Email: rngermain@comcast.net

Klaus Hahn, Ph.D.
Professor
Department of Pharmacology
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: 919-843-2775
Email: khahn@med.unc.edu

Jongyoon Han, Ph.D.
Associate Professor
Massachusetts Institute of Technology
77 Massachusetts Avenue, 36-841
Cambridge, MA 02139
Phone: 617-253-2290
Email: jyhan@mit.edu

Chun-Nan Hsu, Ph.D.
Computer Scientist
Information Sciences Institute
University of Southern California
4676 Admiralty Way, Suite 1001
Marina del Rey, CA 90292
Phone: 310-822-1511
Email: chunnan@isi.edu

Rosemarie Hunziker, Ph.D.
Program Director
National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health
6707 Democracy Boulevard
Room 234, Mail Stop 5477
Bethesda, MD 20892
Phone: 301-451-1609
Email: hunziker@mail.nigh.gov

Thomas Insel, M.D.
Director
National Institute of Mental Health
National Institutes of Health
Building 15K, Room 107, Mail Stop 9669
Bethesda, MD
Phone: 301-443-3673
Email: ti4g@nih.gov

Rustem Ismagilov, Ph.D.
John W. and Herberta M. Miles Professor of Chemistry and Chemical Engineering
California Institute of Technology
1200 East California Boulevard, MC 210-41
Pasadena, CA 91125
Phone: 626-395-8130
Email: rustem.admin@caltech.edu

James Johnson, Ph.D.
Associate Professor
University of British Columbia
5358 Life Science Building
2350 Health Sciences Mall
Vancouver, BC Canada V6T 1Z8
Phone: 604-822-7187
Email: jimjohn@mail.ubc.ca

Robert Jones
Executive Vice President, Research and Development
Fluidigm Corporation
7000 Shoreline Court, Suite 100
San Francisco, CA 94080
Phone: 650-266-6105
Email: bob.jones@fluidigm.com

Junhyong Kim, Ph.D.
Professor
Department of Biology
University of Pennsylvania
415 South University Avenue
Philadelphia, PA 19104
Phone: 215-746-5187
Email: junhyong@sas.upenn.edu

John Knowlton, Ph.D.
Program Director
National Cancer Institute
National Institutes of Health
6130 Executive Boulevard, Suite 5001
Bethesda, MD 20852
Phone: 301-435-5226
Email: jk339o@nih.gov

Jennie Larkin, Ph.D.
Program Director
National Heart, Lung, and Blood Institute
National Institutes of Health
Rockledge 2, 6701 Rockledge Drive, Room 8200
Bethesda, MD 20892
Phone: 301-435-0513
Email: larkinj2@nhlbi.nih.gov

Daniel Larson, Ph. D.
Investigator
National Cancer Institute
National Institutes of Health
41 Library Drive, Building 41
Bethesda, MD 20892
Phone: 301-496-0986
Email: dan.larson@nih.gov

Roger Lasken, Ph.D.
Professor
J. Craig Venter Institute
10355 Science Center Drive
San Diego, CA 92121
Phone: 858-200-1823
Email: rlasken@jcvi.org

David Lawrence, Ph.D.
Professor
Wadsworth Center
David Axelrod Institute
120 New Scotland Avenue
Albany, NY 12208
Phone: 518-474-8285
Email: david.lawrence@wadsworth.org

Andre Levchenko, Ph.D.
Professor
Johns Hopkins University
208C Clark Hall
3400 North Charles Street
Baltimore, MD 21218
Phone: 410-516-5584
Email: alev@jhu.edu

Wei Li, M.D., Ph.D.
Investigator
National Eye Institute
National Institutes of Health
35 Convent Drive, 2A-108
Bethesda, MD 20892
Phone: 301-496-6669
Email: liwei2@nei.nih.gov

Sara Lin
Program Director
National Heart, Lung, and Blood Institute
National Institutes of Health
6701 Rockledge Drive, Suite 10042
Bethesda, MD 20892
Phone: 301-435-0222
Email: sara.lin@nih.gov

Wolfgang Losert, Ph.D.
Associate Professor
University of Maryland
IREAP, Paint Branch Drive, Building 223
College Park, MD 20742
Phone: 301-405-0629
Email: wlosert@umd.edu

Michael Lynes, Ph.D.
Professor
University of Connecticut
91 North Eagleville Road, U-3125
Storrs, CT 06269
Phone: 860-486-4350
Email: michael.lynes@uconn.edu

Patrick Ma, M.D.
Director, Aerodigestive Oncology
Translational Research
Cleveland Clinic Taussig Cancer Institute
9500 Euclid Avenue, R40
Cleveland, OH 44195
Phone: 216-445-5545
Email: map@ccf.org

Raghu Machiraju, Ph.D.
Associate Professor
Ohio State
395 Dreese Laboratories
2015 Neil Avenue Mall
Columbus, OH 43210
Phone: 614-292-6730
Email: machiraju.1@osu.edu

Scott Manalis, Ph.D.
Professor
Massachusetts Institute of Technology
77 Massachusetts Avenue, 76-261
Cambridge, MA 02139
Phone: 617-253-0632
Email: scottm@media.mit.edu

Andrew Martins, Ph.D.
Visiting Fellow
National Institute of Allergy and Infectious
Disease
National Institutes of Health
Building 4, Room 211, 4 Memorial Drive
Bethesda, MD 20814
Phone: 301-496-4000
Email: martinsaj@niaid.nih.gov

Andrew May, D.Phil.
Director, Research and Development
Fluidigm Corporation
7000 Shoreline Court, Suite 100
San Francisco, CA 94080
Phone: 650-266-6131
Email: andy.may@fluidigm.com

John McCord
Senior Account Executive
NanoFabrication Systems Division
NanoInk, Inc.
8025 Lamon Avenue
Skokie, IL 60077
Phone: 609-702-5598
Email: jmccord@nanoink.net

Cynthia McMurray, Ph.D.
Senior Scientist
Lawrence Berkeley National Laboratory
1 Cyclotron Road, Mailstop 83R0101
Berkeley, CA 94720
Phone: 510-486-6526
Email: ctmcmurray@lbl.gov

Deirdre Meldrum, Ph.D.
Senior Scientist and Professor
Arizona State University
1001 South McAllister Avenue, Box 877101
Tempe, AZ 85287
Phone: 480-522-4120
Email: deirdre.meldrum@asu.edu

Simon Melov, Ph.D.
Associate Professor
Buck Institute for Research on Aging
8001 Redwood Boulevard
Novato, CA 94945
Phone: 415-209-2068
Email: smelov@buckinstitute.org

Mark Mercola, Ph.D.
Professor and Director
Sanford-Burnham Medical Research Institute
10901 North Torrey Pines Road
La Jolla, CA 92037
Phone: 858-795-5242
Email: mmercola@sanfordburnham.org

William Mohler, Ph.D.
Associate Professor
University of Connecticut Health Center
263 Farmington Avenue, MC-6403
Farmington, CT 06030
Phone: 860-679-8350
Email: wmohler@neuron.uchc.edu

Peter Nemes, Ph.D.
Division of Chemistry and Material Science
Office of Science and Engineering Laboratories
Center for Devices and Radiological Health
U.S. Food and Drug Administration
Silver Spring, MD 20993
Phone: 301-796-3366
Email: peter.nemes@fda.hhs.gov

Garry Nolan, Ph.D.
Professor
Stanford University School of Medicine
291 Campus Drive, East
Stanford, CA 94305
Email: gnolan@stanford.edu

David Owens, Ph.D.
Program Director
National Institute of Neurological
Disorders and Stroke
National Institutes of Health
6001 Executive Boulevard, Room 2204
Bethesda, MD 20852
Phone: 301-496-1447
Email: do47h@nih.gov

Murali Palangat, Ph.D.
Research Biologist, Laboratory of Receptor Biology and Gene Expression
National Cancer Institute
National Institutes of Health
41 Library Drive
Bethesda, MD 20892
Phone: 301-594-0691
Email: murali.palangat@nih.gov

Xinghua Pan, M.D., Ph.D.
Associate Research Scientist
Department of Genetics
Yale School of Medicine
300 Cedar Street, TAC-S320
New Haven, CT 06519
Phone: 203-737-2616
Email: xinghua.pan@yale.edu

David Panchision, Ph.D.
Chief, Developmental Neurobiology Program
Division of Neuroscience and Basic Behavioral Science
National Institute of Mental Health
National Institutes of Health
6001 Executive Boulevard
Room 7186, Mail Stop Code 9641
Bethesda, MD 20892
Phone: 301-443-5288
Email: panchisiond@mail.nih.gov

Jonathan Pollock, Ph.D.
Chief, Genetics & Molecular Neurobiology Research Branch
National Institute on Drug Abuse
National Institutes of Health
6001 Executive Boulevard
Bethesda, MD 20892
Phone: 301-435-1309
Email: jpollock@mail.nig.gov

Steve Potter, Ph.D.
Professor
Cincinnati Children's Medical Center
3333 Burnet Avenue, S3.234
Cincinnati, OH 45229
Phone: 513-636-4850
Email: steven.potter@cchmc.org

Hong Qian, Ph.D.
Professor
Applied Mathematics, Guggenheim Hall 451E
University of Washington
Seattle, WA 98195
Phone: 206-543-2584
Email: qian@amath.washington.edu

Lidong Qin, Ph.D.
Assistant Professor
The Methodist Hospital Research Institute
6670 Bertner Avenue, R7-121
Houston, TX 77030
Phone: 713-441-7328
Email: lqin@tmhs.org

Paul Robinson, Ph.D.
School of Veterinary Medicine
Professor of Cytomics
Professor of Biomedical Engineering Director
Cytometry Laboratories, Bindley Bioscience Center
Purdue University
1203 West State Street
West Lafayette, IN 47907
Phone: 765-494 0757
Email: jpr@flowcyt.cyto.purdue.edu

Stanislav Rubakhin, Ph.D.
Senior Research Scientist
Beckman Institute
405 North Mathews Avenue, MC-251
Urbana, IL 61802
Phone: 217-333-7446
Email: roubakhi@illinois.edu

Belinda Seto, Ph.D.
Deputy Director
National Institute of Biomedical Imaging and Bioengineering
National Institutes of Health
6707 Democracy Boulevard, Suite 202
Bethesda, MD 20892
Email: setob@mail.nih.gov

Grace Shen, Ph.D.
Group Leader and Director, Retinal Diseases Program
National Eye Institute
National Institutes of Health
5635 Fishers Lane
Suite 1300, MSC 9300
Bethesda, MD 20892
Phone: 301-451-2020
Email: sheng@mail.nih.gov

Sergey Shevkoplyas, Ph.D.
Assistant Professor
Biomedical Engineering
Tulane University
Lindy Boggs Building, Suite 500
New Orleans, LA 70118
Phone: 504-865-5897
Email: shevkop@tulane.edu

Leming Shi, Ph.D.
Associate Director of Research
Center for Bioinformatics
National Center for Toxicological Research
U.S. Food and Drug Administration
3900 National Center for Toxicological Research Road
Jefferson, AK 72079
Phone: 870-543-7387
Email: leming.shi@fda.hhs.gov

Tieliu Shi
University of Louisville
Room 907 HSC-A Building
500 South Preston Street
Louisville, KY 40202
Phone: 502-852-7502
Email: tieliushi01@gmail.com

Lillian Shum, Ph.D.
Chief, Integrative Biology and Infectious Diseases Branch
Division of Extramural Research, National Institute of Dental and Craniofacial Research
National Institutes of Health
6701 Democracy Boulevard, Room 624
Bethesda, MD 20892
Phone: 301-594-0618
Email: shuml@mail.nih.gov

Robert Singer, Ph.D.
Professor, Co-Chairman
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, NY 10461
Phone: 718-430-8646
Email: robert.singer@einstein.yu.edu

Anup Singh, Ph.D.
Manager, Biotechnology and Bioengineering
Sandia National Laboratories
7011 East Avenue, MS 9291
Livermore, CA 94551
Phone: 925-294-1260
Email: aksingh@sandia.gov

Paul Soloway, Ph.D.
Professor
Cornell University
211 Weill Hall
Ithaca, NY 14853
Phone: 607-254-6444
Email: soloway@cornell.edu

Oleg Suslov, Ph.D.
Research Scientist
Neurosurgery Department
University of Florida
McKnight Brain Institute
100 South Newell Drive, Room L2-100
Gainesville, FL 32610
Phone: 352-392-6754
Email: osuslov@yahoo.com

Jonathan Sweedler, Ph.D.
Professor
University of Illinois
600 South Mathews Avenue, 63-5
Urbana, IL 61801
Phone: 217-333-1987
Email: jsweedle@illinois.edu

Henryk Szmacinski, Ph.D.
Associate Professor
University of Maryland School of Medicine
725 West Lombard Street
Baltimore, MD 21201
Phone: 410-706-2116
Email: hszmacinski@umaryland.edu

Jian Tajbakhsh, Ph.D.
Assistant Professor
Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048
Phone: 310-423-0543
Email: jian.tajbakhsh@cshs.org

Jianyong Tang, Ph.D.
Staff Scientist
National Institute of Allergy and Infectious Disease
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20878
Phone: 301-496-1355
Email: jianyong.tang@nih.gov

John Tsang
Investigator
National Institute of Allergy and Infectious Disease
National Institutes of Health
4 Memorial Drive, 4-201
Bethesda, MD 20892
Phone: 617-913-0412
Email: john.tsang@nih.gov

Jose Velazquez, Ph.D.
Program Director
National Institute on Aging
National Institutes of Health
7201 Wisconsin Avenue
Bethesda, MD 20892
Phone: 301-496-6428
Email: juelazqu@mail.nih.gov

Akos Vertes, Ph.D.
Professor
Department of Chemistry
The George Washington University
725 21st Street, NW
Washington, DC 20052
Phone: 202-994-2717
Email: vertes@gwu.edu

Charles Wang, M.D., Ph.D., M.P.H.
Director and Associate Research Professor
City of Hope Comprehensive Cancer Center
1500 East Duarte Road
Duarte, CA 91010
Phone: 626-256-4673
Email: cwang@coh.org

Barbara Wold, Ph.D.
Director, Center for Cancer Genomics
National Cancer Institute
National Institutes of Health
Building 31A, 31 Center Drive, 11th Floor
Bethesda, MD 20892
Phone: 301-496-6613
Email: sobalvarroam@mail.nih.gov

Euisik Yoon, Ph.D.
Professor
University of Michigan
1301 Beal Avenue
Ann Arbor, MI 48109
Phone: 734-615-4469
Email: esyoon@umich.edu

Kun Zhang, Ph.D.
Assistant Professor
Department of Bioengineering
University of California at San Diego
9500 Gilman Drive, MC0412, PFBH402
La Jolla, CA 92093
Phone: 858-822-7876
Email: kzhang@bioeng.ucsd.edu

Peter Znamenskiy
Ph.D. Student
Cold Spring Harbor Laboratory
One Bungtown Road
Cold Spring Harbor, NY 11724
Email: peter.cshl@gmail.com

Nancy L. Allbritton, M.D., Ph.D.
Professor, Department Chair
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Dr. Nancy L. Allbritton obtained her B.S. in physics from Louisiana State University, her Ph.D. in medical physics and medical engineering from the Massachusetts Institute of Technology, and her M.D. from Johns Hopkins University. Upon completion of a postdoctoral fellowship in cell biology at Stanford University in 1994, she joined the faculty of the University of California at Irvine, where she held appointments in the departments of Physiology and Biophysics, Biomedical Engineering, and Chemistry. In July 2007, she joined the University of North Carolina at Chapel Hill (UNC) as the Debreczeny Distinguished Professor in the Department of Chemistry. In 2009, she was appointed professor and chair of the Department of Biomedical Engineering, a joint department between the School of Medicine at UNC and the College of Engineering at North Carolina State University. Dr. Allbritton's research studies are directed at the development of new technologies by bringing to bear methods from engineering, chemistry, physics, and biology to address biomedical problems.

Abstract Title: Protein Activity in Single Cell Signaling Networks

Microelectrophoretic methods can make direct measurements of the activity of normal and oncogenic kinases in single tumor cells. Issues of heterogeneity within cell populations, both in terms of cell type and biochemical behavior, often mandate single-cell studies for accurate determination of kinase activity in tumor cells. Chemical cytometry, the application of high-sensitivity chemical separations to characterize the contents of single or small numbers of cells, is emerging as an important approach for such studies. For these studies, fluorescent substrates for the enzyme(s) of interest are introduced into cytoplasm of intact living cells. The cells are brought to the entry way of a separation channel and then sequentially lysed in microseconds, terminating cellular reactions. The contents of each cell are then introduced as a discrete packet into a separation channel so that the fluorescent substrates and any products formed are separated and detected with high sensitivity. Automated capillary-based and microfluidic devices for the continuous separation of the contents of single cells possess a current throughput of ~30 cells/min. To date, these devices can operate in a stable manner over a 2 h-time span for the analysis of 600 cells. Development of robust enzyme substrates that can be loaded into the cytoplasm of primary or cultured cells is a necessary component and assay development to measure the activity of lipid and protein kinases, including phosphoinositide 3-kinase, sphingosine kinase, Bcr-Abl, Akt, epidermal growth factor receptor, and others are under way.

The ability to manipulate and analyze single cells is crucial to understanding the molecular processes controlling cell behavior, and these microengineered platforms and assays provide the experimentalist with unprecedented opportunities for cell manipulation and analysis on a single-cell basis.

Steven Altschuler, Ph.D.
Associate Professor
The University of Texas Southwestern Medical Center
Dallas, Texas

Dr. Altschuler combines his backgrounds in biology, mathematics, and engineering to understanding cell polarity and signaling heterogeneity in cancer and metabolism. Prior to arriving at the UT Southwestern, Dr. Altschuler worked at the Bauer Center for Genomics Research at Harvard University, Rosetta Informatics, Microsoft, and Princeton University.

Abstract Title: PhenoRipper: A wickedly fast approach for analyzing cell-level heterogeneity

The ability to perform single-cell resolution analyses enables previously missed subpopulations to be discovered within heterogeneous populations. We will describe two new image-based approaches for interrogating the cellular signaling that push the envelope of marker limitations, deep profiling, and speed of analysis—PhenoRipper—for existing approaches. The discovery of new adipogeneic states in differentiating adipocytes will be used to showcase these approaches.

Edward S. Boyden, Ph.D.

Associate Professor
Massachusetts Institute of Technology
Cambridge, Massachusetts

Dr. Edward S. Boyden is the Benesse Career Development Professor and associate professor of Biological Engineering and Brain and Cognitive Sciences at the Massachusetts Institute of Technology (MIT) Media Lab and the MIT McGovern Institute. He leads the Synthetic Neurobiology Group, which develops tools for controlling and observing the dynamic circuits of the brain and uses these neurotechnologies to understand how cognition and emotion arise from brain network operation, as well as to enable systematic repair of intractable brain disorders, such as epilepsy, Parkinson's disease, post- traumatic stress disorder, and chronic pain. The tools his group has invented include a suite of “optogenetic” tools that are now in use by hundreds of groups around the world for activating and silencing neurons with light. He has received the National Institutes of Health (NIH) Director's New Innovator Award, the Society for Neuroscience Research Award for Innovation in Neuroscience, the National Science Foundation (NSF) CAREER Award, and the Paul Allen Distinguished Investigator Award. He has contributed to over 250 peer-reviewed papers, current or pending patents, and articles and has given more than 160 invited talks on his work.

Abstract Title: Controlling cell functions with light, and robotically analyzing cell phenotypes

Understanding how single cells within neural circuits work together to implement brain functions and how these computations go awry in brain disorders is a top priority. Over the last several years, we have developed a rapidly-expanding suite of genetically-encoded reagents that, when expressed in specific cells in the nervous system, enable their electrical activities to be powerfully and precisely activated and silenced in response to pulses of light. These tools are in widespread use for analyzing the causal role of defined cell types in normal and pathological brain functions. In this talk, I will briefly give an overview of the field, and then I will discuss a number of new tools for neural activation and silencing that we are developing, including new molecules with augmented amplitudes, improved safety profiles, novel color and light-sensitivity capabilities, and unique new capabilities. We have begun to develop hardware to enable complex and distributed neural circuits to be precisely controlled and for the network-wide impact of a neural control event to be measured. We have also developed robots that can perform automated, robotic, intracellular, neural recording and systematic single-cell analysis in the living brain. We explore how these tools can be used to enable systematic analysis of neural circuit functions in the fields of emotion, sensation, and movement, and in neurological and psychiatric disorders.

Daniel T. Chiu, Ph.D.
Professor
University of Washington
Seattle, Washington

Dr. Daniel T. Chiu is the A. Bruce Montgomery Professor of Chemistry, endowed professor of Analytical Chemistry, and professor of Bioengineering at the University of Washington, Seattle. He obtained a B.A. in neurobiology and a B.S. in chemistry from the University of California at Berkeley in 1993, then a Ph.D. in chemistry from Stanford University in 1998. After completing postdoctoral research at Harvard University, he started in the fall of 2000 as an assistant professor of Chemistry at the University of Washington. He moved through the ranks from assistant professor to associate professor, then to professor in 2006. He is currently a member of the Center for Nanotechnology and the Neurobiology and Behavior Program at the University of Washington, as well as a member of the Cancer Consortium at the Fred Hutchinson Cancer Research Center.

Abstract Title: Tools for studying single circulating tumor cells and subcellular compartments

This presentation will describe an approach called ensemble decision aliquot ranking (eDAR) for isolating rare cells from peripheral blood. eDAR is well suited for the enrichment of rare cells, such as circulating tumor cells (CTCs), because it can rapidly and efficiently reduce sample complexity by over a million fold. We applied eDAR in isolating CTCs from breast-cancer patient samples and carried out a side-by-side comparison between eDAR and CellSearch, the current FDA approved instrument for isolating CTCs. We were also able to detect cancer stem cells, as determined by CD44+/CD24-/low, within the isolated CTCs from these patients. Importantly, eDAR isolated the CTCs within a small field-of-view (<1 mm2), and provided direct easy access to individual live CTCs for downstream single-cell manipulations and analyses.

Steven H. DeVries, M.D., Ph.D.
Associate Professor
Northwestern University
Chicago, Illinois

Dr. Steven H. DeVries attended Tulane University as an undergraduate, where he majored in chemistry and conducted laboratory research on the mechanisms of catalytic hydrogenation by organometallic compounds. His background in kinetics led to an interest in membrane channels and then neuroscience, which he pursued in both graduate and medical school at the University of Chicago. For his graduate work, Dr. DeVries used patch clamp recording to characterize how dopamine, which acts as a day-night switch in the retina, changes the strength of lateral inhibition by modulating gap junction channels. His interest in how neuromodulators change processing in the retina then led his to a postdoctoral in the Department of Neurobiology at Stanford University, where he used a newly developed planar multielectrode array to study how ganglion cell receptive fields are organized to mediate spatial vision. Finally, in his own lab—first at the The University of Texas Health Science Center at Houston and subsequently at Northwestern University—Dr. DeVries' work has used various approaches to study how visual signals are transformed as the flow from synapse to synapse across the retina.

Abstract Title: Interneuron diversity and parallel processing in the retina

Nervous systems obtain their computational power and efficiency through parallel processing, which starts in the visual system when cone photoreceptors signal to 12 or more types of bipolar cells that consequently carry different representations of the visual scene. Studying the functional properties of these different types can be challenging for three reasons: all bipolar cells share a common morphological plan, the processes and somata of different types are intermixed, and the distinctions between types can be subtle. Indeed, these challenges are not unique to bipolar cells but apply generally to CNS interneurons. For the different bipolar cell types, more specifically, we would like to understand how they arise both developmentally and evolutionarily; independently tile the retinal surface; make spatially and numerically distinct contacts with cone photoreceptors; make selective contacts with subsets of the 30+ amacrine and 15+ ganglion cell types; express distinctive constellations of membrane channels and transporters; and individually adapt and respond to photoreceptor dysfunction and death. The bulk of our functional information about bipolar cells comes from labor-intensive whole cell voltage clamp recording. However, recent technological advances hold great promise for targeting specific bipolar cell types for study and making their study more efficient. These new approaches, which have both advantages and drawbacks, include single-cell transcriptomics, bipolar cell-type specific cre lines, viral transduction and circuit tracing with AAV and rabies in the mouse and other mammals, optogenetics, 2–3 color 3D super-resolution microscopy, and serial and block face EM sectioning and reconstruction.

Norman Dovichi, Ph.D.
Professor
University of Notre Dame
Notre Dame, IN

Dr. Norman Dovichi currently holds the Grace-Rupley Professorship at the University of Notre Dame. Dovichi has graduated 57 Ph.D. students, has published over 200 papers, holds 2 U.S. patents, and has given over 350 invited talks. He now serves as associate editor for Analytical Chemistry.

Dr. Dovichi has received a number of honors for his work. These include the Chemical Instrumentation Award and the Spectrochemical Analysis Award from the American Chemical Society. The Canadian Institute of Chemistry recognized him with the McBryde, Noranda, and Fisher awards. He received the Heinrich Emanuel Merck Award for Analytical Chemistry. He has also been named as an honorary professor of the Chinese Academy of Sciences. Finally, the journal *Science* included Dr. Dovichi as the only chemist among their list of a dozen Unsung Heroes of the Human Genome Project.

Abstract Title: Metabolic cytometry: monitoring sphingolipid metabolism in single primary neurons

Authors: Norman J. Dovichi, Richard Keithley, David Essaka, Ole HIndsgaul, Monica Palcic, Ronald Schnaar, Jillian Prendergast, Yayoi Yoshimura
University of Notre Dame, Carlsberg Institute, and Johns Hopkins University

Cell-to-cell heterogeneity in ganglioside catabolism was determined by profiling fluorescent tetramethylrhodamine-labeled GM1 (TMR-GM1) breakdown in individual primary neurons and glia from the rat cerebellum. Cells isolated from 5–6 day old rat cerebella were cultured for 7 days, and then incubated for 14 h with TMR-GM1. Intact cells were recovered from cultures by mild proteolysis, paraformaldehyde fixed, and subjected to single-cell analysis. Individual cells were captured in a capillary, lysed, and the released single-cell contents analyzed by capillary electrophoresis with quantitative laser-induced fluorescent detection of metabolites. Non-neuronal cells, on average, took up much more exogenous TMR-GM1 than neuronal cells, and catabolized it more extensively. After 14 h of incubation, non-neuronal cells retained only 14% of the TMR products as GM1 and GM2, compared to >50% for neurons. On average, non-neuronal cells contained 74% of TMR-labeled product as TMR-ceramide, compared to only 42% for neurons. Non-neuronal cells retained 7 times as much TMR-GM3 (7%) compared to neuronal cells (1%). To confirm the observed single-cell metabolomics, we lysed and compared TMR-GM1 catabolic profiles from mixed neuron/glial cell cultures and from cultures depleted of non-neuronal cells by treatment with the antimitotic agent cytosine arabinoside. The lysed culture catabolic profiles were consistent with the average profiles of single neurons and glia. We conclude that the ultrasensitive analytic methods described accurately reflect single-cell ganglioside catabolism in different cell populations from the brain.

Scott E. Fraser, Ph.D.

Biological Imaging Center, Beckman Institute
Pasadena, California

Professor Scott E. Fraser has a longstanding interest in the imaging and molecular analysis of intact biological systems. After training in physics (B.S., Harvey Mudd College, 1976) and biophysics (Ph.D., Johns Hopkins University, 1979), he joined the faculty at University of California, Irvine, and rose through the ranks to become chair of the Department of Physiology and Biophysics. He is the director of the Biological Imaging Center and helped found the Caltech Brain Imaging Center and the Kavli Institute of Nanoscience. He now serves as the director of the Rosen Center for Biological Engineering and is active in interdisciplinary training and in translational research.

Abstract Title: Imaging cell lineage and interactions in developing embryos

Intravital imaging offers unprecedented opportunities for studying the cell lineages, cell interactions, and intercellular signaling during embryogenesis and organogenesis. We are assisted in our imaging by our recent FlipTrap screens, which generated a large set of functional fusions of native gene products with fluorescent proteins. The FlipTrap offers a simple and direct way to perform molecular imaging in complex settings and provides a means for conditional mutagenesis in zebrafish, permitting a new class of functional tests during embryogenesis and organogenesis.

Imaging techniques are challenged by major trade-offs between spatial resolution, temporal resolution, and the limited photon budget. We are attempting to advance this trade-off by constructing faster and more efficient microscopes that maintain subcellular resolution. This combination of speed and resolution is required as intravital imaging can only generate accurate data on cell lineages and cell migration if it can re-acquire the 3-dimensional image of the entire specimen before any of the cells can move half of the distance separating them from their neighbors. Failing this, imaging tools can only give information on the averaged behaviors of cells—such as optical flow or PIV—which is often mistakenly taken as revealing cellular mechanism.

We have developed a new microscope, combining the deep penetration of two-photon microscopy and the speed of light sheet microscopy to generate images with more than 10-fold improved imaging speed and sensitivity. As with other light-sheet technologies, the collection of an entire 2-D optical section in parallel offers dramatically faster acquisitions rates. This two-photon SPIM is far less subject to light scattering, permitting subcellular resolution to be maintained far better than conventional light-sheet microscopes.

Combined, these molecular and imaging tools define a new compromise between spatial resolution, temporal resolution, and the limited photon budget, combining needed resolution, speed, and sensitivity to follow complex events within single cells—down to the single molecule level—over the prolonged periods needed to study events in a longitudinal fashion.

Klaus Hahn, Ph.D.
Professor
The University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Dr. Klaus Hahn earned his Ph.D. in organic chemistry from the University of Virginia, followed by postdoctoral work at Carnegie Mellon and Scripps. He was an associate professor at Scripps and is now Thurman Professor of Pharmacology at The University of North Carolina at Chapel Hill. His lab focuses on two different but synergistic areas: Developing novel molecules that can report and manipulate signaling in vivo and understanding the signaling networks that govern motility and polarization, with an emphasis on the cytoskeleton and adhesion complexes. The Hahn Laboratory has explored different biosensor designs capable of visualizing the conformational changes of endogenous proteins, or studying multiple proteins in the same cell. Using novel environment-sensing organic dyes, they image protein activities with minimal perturbation of cell behavior. They are also focused on genetically encoded approaches to manipulate signaling with light or engineering artificial protein folds to confer allosteric regulation on signaling proteins.

Abstract Title: Peeking and poking at GTPase signaling circuits in living cells

Cell motility requires the orchestration of many dynamic cellular systems, including the cytoskeleton, adhesion complexes, and vesicle trafficking. Understanding the spatio-temporal coordination of molecules mediating motility requires quantitation of protein activity in living cells. This talk will describe new tools to visualize and manipulate rapid Rho family signaling in vivo. Computational multiplexing will be used to examine the spatio-temporal coordination of three or more GTPases during cell protrusion. The role of specific GTPases and kinases will be dissected through development of novel methods to activate and inactivate proteins at specific subcellular locations with light or through engineered allosteric activation. The talk will also describe studies using new biosensors to elucidate the roles of RhoG in polarized motility.

Rustem Ismagilov, Ph.D.

John W. and Herberta M. Miles Professor of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California

Dr. Rustem Ismagilov was born in Ufa, Russia. He graduated from the Higher Chemical College of the Russian Academy of Sciences, Moscow in 1994, before coming to the United States to complete his Ph.D. in physical organic chemistry at the University of Wisconsin, Madison in 1998. Dr. Ismagilov conducted his postdoctoral work at Harvard University and began his independent research career in 2001 as an assistant professor in the Department of Chemistry at the University of Chicago. In 2011, he joined the Division of Chemistry and Chemical Engineering at the California Institute of Technology as the John W. and Herberta M. Miles Professor of Chemistry and Chemical Engineering. His research group's work has been recognized by a number of awards, most recently the Cozzarelli Prize from the National Academy of Sciences (2007), the National Institutes of Health Director's Pioneer Award (2007), the American Chemical Society Award in Pure Chemistry (2008), and election as a Fellow of the American Academy for the Advancement of Science (2010).

Abstract Title: Dynamics of Cell Networks in Space and Time

Recent developments in microtechnologies enable multistep parallel manipulations of small volumes, small enough that these manipulations can be carried out at single-cell and single-molecule level. Such technologies are compatible with imaging, and enable five capabilities relevant to single-cell analysis:

- Quantification and identification of nucleic acids and proteins down to single molecules
- Stimulation/response profiling of individual cells in a population
- Understanding of autocrine signaling in single cells
- Understanding of paracrine signaling among single cells
- Performing the above four measurements in the context of controlled density of the single cells (in cell/volume) and controlled chemical microenvironment

Junhyong Kim, Ph.D.
Professor
University of Pennsylvania
Philadelphia, Pennsylvania

Dr. Junhyong Kim is the Edmund J. and Louise W. Kahn Term Endowed Professor of Biology and the Co-Director of the Penn Genome Frontiers Institute. Dr. Kim received his Ph.D. in ecology and evolution from The State University of New York (SUNY) at Stony Brook in 1992. From 1994 to 2002, he was an assistant and associate professor in the Department of Biology and the Department of Ecology and Evolutionary Biology at Yale University. In 2002, he moved to the University of Pennsylvania, where he carries out research in genomics and computational biology, with a focus on evolution and neuroscience. His current projects include: Understanding the nature of single-cell transcriptome variability, evolutionary diversification of single-neuron function, mechanisms of RNA localization in neurons, systems architecture of temporal regulatory control, and theory of complex evolutionary dynamics. Dr. Kim has received numerous awards, including a Guggenheim Fellowship, the Ellison Foundation Senior Scholar Award, and the Sloan Foundation Young Investigator Award.

Abstract Title: Whence single cell variation: case studies from mammalian neurons

Recent studies have shown significant variability in the transcriptome of single cells, even within cells of putatively same phenotype. In this talk, I discuss some of the single-cell transcriptome data generated in our labs and consider the significance of the single-cell variation in light of five hypotheses about gene regulation in differentiated cells. Our main hypothesis is that there is a natural many-to-one relationship between the transcriptome state and a cell's phenotype. We call this the equi-phenotype support of a transcriptome. I postulate that the equi-phenotype support is modulated by a cell's external conditions, such as processes of development, aging, and therapeutic regimes. This conceptual model explains heterogeneity in a cell's response to changing conditions, and I outline our research program to address the role of single-cell variability in cellular responses.

Roger Lasken, Ph.D.
Professor
J. Craig Venter Institute
San Diego, California

Dr. Roger Lasken has studied DNA replication and DNA amplification techniques for over 30 years. His graduate work was with Myron Goodman at the University of Southern California, investigating DNA polymerase enzymology and fidelity. Dr. Lasken's postdoctoral work was with Arthur Kornberg at Stanford University, studying E. coli replication proteins. During 6 years at Life Technologies, he focused on DNA amplification and sequencing methods. After moving to New Haven, Connecticut, to become the director of Genomics for Molecular Staging, Inc., he began to work on whole genome amplification methods. The team developed the multiple displacement amplification method (MDA), which was licensed to Qiagen (Repli-g kit) and GE Healthcare (TempliPhi and GenomiPhi). In 2001, Molecular Staging was funded by the U.S. Department of Energy (DOE) to develop a method for sequencing DNA from a single cell, which was introduced in 2005. The following year, Dr. Lasken became a professor at the J. Craig Venter Institute, where he works to integrate single-cell genomic methods into a high-throughput sequencing center.

Abstract Title: Sequencing of uncultured bacteria from single cells for the Human Microbiome Project

Authors: Mary-Jane Lombardo, Joyclyn L. Yee-Greenbaum, Mark Novotny, Jonathan H. Badger, Jeffrey S. McLean, Maria Kim, Barbara Methe, Karen Nelson, and Roger S. Lasken

Uncultured bacteria comprise the majority of complex microbial communities, including the human microbiome. Single-cell genomic methods allow extensive sequencing of these otherwise inaccessible organisms. Acquisition of single-cell genomes relies on the isolation of single bacterial cells, robust genome amplification by multiple displacement amplification (MDA), deep sequencing of amplified DNA, and de novo assembly. We report on a program for acquisition of MDA-amplified single-cell bacterial genomes from the human microbiome for genome sequencing, including high-throughput methods for sorting of single cells by flow cytometry, MDA, and 16S PCR for taxonomic identification1. A quality-control assay was developed, based on shallow 454 Titanium sequencing of bar-coded MDA reactions. The QC assay was tested on a set of MDA-amplified genomes of known quality. It was then used to assess genome representation and purity of 95 GI tract single-cell amplified genomes prior to deciding on which should proceed to deep sequencing and de novo assembly. Amplified DNAs are currently being distributed to the HMP sequencing centers for sequencing of reference genomes.

A second project in the lab focuses on gene-expression analysis from single eukaryotic cells. New methods have been published for synthesis of double-stranded cDNA from a single cell for use in DNA sequencing. Gene expression is derived from the number of reads mapping to each gene, and mutations and mRNA splicing variants are revealed by the sequences. We have tried one of the published protocols and find that it is very effective for use in global transcriptomic analysis. Some the characteristics of the method will be discussed.

The Human Microbiome Project work has been funded in whole or part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, and the U.S. Department of Health & Human Services under contract number HHSN272200900007C. MDA method development and single-cell transcriptomic work were supported by grants from the National Human Genome Research Institute.

Andre Levchenko, Ph.D.
Professor
Johns Hopkins University
Baltimore, Maryland

Dr. Andre Levchenko is a professor of Biomedical Engineering at Johns Hopkins University. He received his B.S. and M.S. degrees at Moscow Institute of Physics and Technology and his second M.S. and Ph.D. degrees at Columbia University. He did his postdoctoral work in biology and computer science at Caltech. His major interests revolve around analysis of cell decisionmaking, using novel experimental and theoretical tools.

Abstract Title: Analysis of information capacity of signaling networks

Authors: Raymond Cheong, Ilya Nemenman, Alex Rhee, Joanne Wang, and Andre Levchenko
Johns Hopkins University and Emory University

Molecular noise restricts the ability of an individual cell to resolve input signals of different strengths and gather information about the external environment. Transmitting information through complex signaling networks with redundancies can overcome this limitation. We developed an integrative theoretical and experimental framework, based on the formalism of information theory, to quantitatively predict and measure the amount of information transduced by molecular and cellular networks. Analyzing tumor necrosis factor (TNF) signaling revealed that individual TNF signaling pathways transduce information sufficient for accurate binary decisions, and an upstream bottleneck limits the information gained via multiple integrated pathways. Negative feedback to this bottleneck could both alleviate and enhance its limiting effect, despite decreasing noise. Bottlenecks likewise constrain information attained by networks signaling through multiple genes or cells.

Scott Manalis, Ph.D.
Professor
Massachusetts Institute of Technology
Cambridge, Massachusetts

Dr. Scott Manalis is a professor of Biological and Mechanical Engineering at the Massachusetts Institute of Technology (MIT) and has been a faculty member at MIT since 1999. He was the recipient of the Presidential Early Career Award for Scientists and Engineers (PECASE) from the U.S. Department of Defense (DOD). He has also been selected by *Technology Review* magazine as one of the 100 innovators under the age of 35. Dr. Manalis received his B.S. degree in physics from the University of California at Santa Barbara in 1994 and his Ph.D. degree in applied physics from Stanford University in 1998.

Abstract Title: Measuring physical properties of single cells

While much is known about the genetic and cellular defects that cause cancer, comparatively little is known about the progress of the disease in individual cells. Approaches for quantifying the molecular properties of single cells are routinely used and rapidly advancing, but techniques for measuring physical properties have largely remained limited to conventional microscopy, light scattering and the Coulter Principle, which measure only a single property of cells: their volume. This talk will focus on microfluidic approaches for measuring the physical properties of single cells with particular focus on high-precision measurement of cell mass, growth, density, and deformability. Ultimately, the ability to combine multiparameter physical with molecular measurements at the single-cell level could not only be used to further understanding of important cellular processes, such as malignant transformation, but may also be used to increase the predictive power of clinical diagnostics.

Cynthia McMurray, Ph.D.

Senior Scientist
Lawrence Berkeley National Laboratory
Berkeley, California

Dr. Cynthia McMurray is a senior scientist at Lawrence Berkeley National Laboratory and holds appointments as a professor at the Mayo Clinic, where she has been recognized as Distinguished Investigator.

Dr. McMurray has a longstanding interest in DNA instability, genome folding, and neuropathology, and seeks to understand human neurodegenerative disease from molecules to man, with over 100 publications integrating protein biochemistry, mouse genetics, and imaging, including special prominence in the first edition of *ASBMB* magazine. She is internationally renowned for her work on the mutational mechanism called DNA expansion, which is the underlying cause for a number of progressive neurodegenerative diseases. She organizes the Annual West Coast Chromatin Meeting, and is organizing the 4th International US-EU Genome Dynamics and Neurological Disease Meeting in Oslo. Dr. McMurray is a regular speaker at national and international meetings. She serves on the Scientific Advisory Board of the National Institutes of Environmental Health, as well as on several planning boards.

Abstract Title: Resolving regional and cell-type specific differences metabolism in the brain

Regional toxicity remains the most puzzling and poorly understood feature of neurodegenerative disease. AD, PD, and HD display region-specific cell death, and even within those regions, only select cell types are targeted. In HD, for example, the toxic disease-causing protein is ubiquitously expressed. However, only the medium spiny neurons in the striatum are initially targeted for death. Regional specificity, therefore, implies that cellular dysfunction develops in response to the changing cellular metabolism in only specific cells with age and disease progression. Understanding the functional basis for region-specific toxicity holds the key to understanding why some cells live and others die. Due to system heterogeneity, however, resolution of the cell and region-specific changes in the brain are unknown. High-resolution spatial methods are needed to dissect the integrated dynamics among cells. To overcome these barriers, we have used Nanostructure-initiator Mass Spectrometry (NIMS) to spatially resolve the metabolite distribution in an intact tissue slice without disruption of the *in situ* tissue architecture. NIMS works by absorbing metabolites onto a vacuum compatible “initiator” liquid in the pores of a nanostructured surface. The surface, when irradiated with a laser, vaporizes the pore-trapped initiator and triggers desorption/ionization of surface absorbed analytes in the tissue. By aligning each pixel in a two-dimensional, x-y coordinate position, we have functionally imaged the brain, and identified metabolic differences that characterize four regions. NIMS creates a molecular “signature” that uniquely distinguishes distinct and even adjacent cell-types within the native architecture of the brain. The intensity of any ion within each cell can be used to image metabolites across the entire tissue and approaching single-cell resolution. NIMS profiling forms the basis for defining functional changes associated with region-specific toxicity in the brain.

Deidre R. Meldrum, Ph.D.

Senior Scientist and Professor
Arizona State University
Tempe, Arizona

Dr. Deirdre R. Meldrum is a senior scientist at Arizona State University (ASU), director of the Biosignature Initiative, director of the Center for Biosignature Discovery Automation in the Biodesign Institute, professor of Electrical Engineering and PI of the National Institutes of Health (NIH) Center of Excellence in Genomic Sciences: Microscale Life Sciences Center. As dean of the ASU Ira A. Fulton Schools of Engineering from 2007 to 2010, Meldrum transformed the engineering school into an organization fully aligned with the United States' grand challenges. She was professor of Electrical Engineering at the University of Washington from 1992 to 2006 and received her Ph.D. in electrical engineering from Stanford University. Dr. Meldrum is a member of the National Advisory Council for Human Genome Research and is a member of the Microsoft Research Advisory Board. She is a Fellow of the American Association for the Advancement of Science and a Fellow of the Institute of Electrical and Electronics Engineers. In 1996, she was awarded a Presidential Early Career Award for Scientists and Engineers.

Abstract Title: Single Cell Biosignatures

Everyone has the goal of living a long, healthy life. Our society aspires to chronic health. Biosignatures would enable a health care system that focuses on keeping healthy people healthy and reducing the time sick people spend in hospitals and in clinical care. The goal is to enable the prediction of disease risk and the prevention of disease, with pre-symptomatic diagnosis and interventional therapeutic treatment of individuals, based upon their personal biosignature of all the information about an individual—genomic, proteomic, cellomic, imaging, behavior, and other information—that enables prediction of disease predisposition and future health status. This presentation will focus on single-cell biosignatures, including the quantification of physiological and morphological manifestation of underlying gene and protein alterations with disease. Examples will be provided with lung cancer, esophageal cancer, and breast cancer. Successful implementation of biosignatures in a nationwide health care program will require high-throughput automation for biosignature discovery, clinical validation, standardization, and qualification for use in pre-symptomatic diagnoses, drug development research, commercialization, and patient management for healthy patient outcomes.

Mark Mercola, Ph.D.

Professor and Director
Sanford-Burnham Medical Research Institute
La Jolla, California

Dr. Mark Mercola is professor and director of the Muscle Development and Regeneration Program at the Sanford-Burnham Medical Research Institute (SBMRI) and holds adjunct professorships in the departments of Pathology and Pediatrics at the University of California, San Diego.

Dr. Mercola is known for discovering signaling pathways that control heart formation during embryonic development. These discoveries have provided a mechanistic understanding of how primitive cells in the embryo form heart muscle and are the basis of his current work to regenerate heart muscle cells from human embryonic stem cells and adult cardiac stem cells. He established and directed assay development and screening for the Conrad Prebys Center for Chemical Genomics at Sanford-Burnham. Currently, he directs a multidisciplinary team of engineers, chemists, and stem cell biologists to develop automated, high-throughput techniques to discover small drug-like molecules that direct stem cells to form heart muscle cells that could lead to new classes of drugs to stimulate regeneration of damaged heart muscle. In addition, his lab uses transgenic and surgical models of heart disease to evaluate candidate drug targets and genes involved in stem cell-based creation of new muscle tissue, as well as preservation of heart muscle and function post-injury. Dr. Mercola's research is supported by grants from the Heart, Lung, and Blood Institute of the National Institutes of Health (NIH) and the California Institute for Regenerative Medicine. Dr. Mercola also directs the Stem Cell Biology Training Program at SBMRI and is a co-founder of ChemRegen, Inc. He serves on the advisory boards of Vala Sciences (San Diego), Cardiostem (London, UK) and the Beta Cell Biology Consortium (NIH, USA), as well as for numerous scholarly journals.

Abstract Title: High performance image cytometry to discovery drugs for cardiac contractility and arrhythmogenicity

Current methods to measure physiological properties of cardiomyocytes and predict fatal arrhythmias that can cause sudden death, such as Torsade de Pointes, lack either the automation and throughput needed for early-stage drug discovery and/or have poor predictive value, as demonstrated by a doubling of the cost of related drug failures to an estimated $2.55 billion annually over the last decade. To increase throughput and predictive power of in vitro assays, we developed kinetic imaging cytometry (KIC) for automated cell-by-cell analyses via intracellular fluorescence Ca2+ indicators. The KIC instrument simultaneously records and analyzes intracellular calcium concentration [Ca2+]i at 30-ms resolution from hundreds of individual cells/well of 96-well plates in seconds, providing kinetic details not previously possible with well averaging technologies, such as plate readers. Analyses of human embryonic stem cell and induced pluripotent stem cell-derived cardiomyocytes validated the ability of kinetic image cytometry to detect arrhythmogenic drugs. As an example of this technology, we have used the instrument to discover microRNAs involved in the control of contractility and to develop anti-miRs that can be administered in vivo and shown to preserve cardiac output in the face of heart failure. This technology should accelerate elucidation of pathogenic mechanisms of heart disease associated with drugs treatment and/or genetic background.

Garry P. Nolan, Ph.D.

Professor
Stanford University School of Medicine
Stanford, CA

Dr. Garry P. Nolan is the Rachford and Carlota A. Harris Professor in the Department of Microbiology and Immunology at Stanford University School of Medicine. He trained with Leonard Herzenberg for his Ph.D. and with Dr. David Baltimore for postdoctoral work for the first cloning/characterization of NF-κB p65/RelA and the development of rapid retroviral production systems. Dr. Nolan has published over 160 research articles, is the holder of 17 U.S. patents, and has been honored as one of the top 25 inventors at Stanford University. He is a recent recipient of the Teal Innovator Award from the Department of Defense and received an award for "Outstanding Research Achievement in 2011" from the Nature Publishing Group.

Dr. Nolan is the founder of Rigel, Inc., as well as Nodality, Inc., a diagnostics development company. He serves on the boards of directors of several companies and consults for other biotechnology companies. He is an outspoken proponent of translating public investment in basic research to serve public welfare.

His areas of research include hematopoiesis, cancer and leukemia, autoimmunity and inflammation, and computational approaches for network and systems immunology. Dr. Nolan's most recent efforts are focused on a single-cell analysis advance using a mass spectrometry-flow cytometry hybrid device, the so-called "CyTOF". The approach uses an advanced ion plasma source to determine the levels of tagged reagents bound to cells, enabling a vast increase in the number of parameters that can be measured per cell. His laboratory has already begun a large-scale mapping of the hematopoietic hierarchy in healthy human bone marrow at an unprecedented level of detail. Dr. Nolan's efforts are to enable a deeper understanding not only of normal immune function but also detailed substructures of leukemias and solid cancers, which will enable wholly new understandings that will allow better management of disease and clinical outcomes.

Abstract Title: A Definable "Structure" for the Immune System and Cancers at the Single Cell

It is insufficient to state that cancer is "heterogeneous" in nature. This is akin to stating the problem without suggesting a solution. We focus on the development of intracellular assays of signaling that correlate subsets of cells in complex populations with functional signaling and clinical states. Such correlations allow for documentation and ordering of the inherent heterogeneity in leukemias and other cancers into understandable progressions. Using a next-generation single-cell "mass cytometry" platform, we quantify surface and cytokine or drug responsive indices of kinase target with 45 or more parameter analysis (e.g. 45 antibodies, viability, nucleic acid content, and relative cell size). We have recently extended this parameterization to mRNA with the capability to measure down to five molecules per cell, in combination with any other set of previously created markers.

I will present evidence of deep internal order in immune functionality that demonstrates that differentiation and immune activities have evolved with a definable "shape". A hierarchy of functional transcellular modules is observable that can be used for mechanistic and clinical insights, including manners by which these modules become transposed during cancer progression. I will focus upon AML and ovarian cancer in the presentation and demonstrate the apparent existence of reproducible ordering of cellular substates that define a limited boundary condition of "what is" a given cancer.

Hong Qian, Ph.D.
Professor
University of Washington
Seattle, Washington

Dr. Hong Qian holds a B.A. in astrophysics from Peking University and worked on fluorescence correlation spectroscopy and single-particle tracking for his Ph.D. in biochemistry, earned at Washington University in St. Louis. He studied biophysical chemistry and mathematical biology—specifically protein folding and fluctuations—as a postdoctoral fellow at the University of Oregon and at the California Institute of Technology. From 1994 to 1997, Dr. Qian was with the Department of Biomathematics at the University of California, Los Angeles (UCLA) and worked on the theory of motor proteins and single-molecule biophysics. He joined the University of Washington in Seattle in 1997 and is now professor of Applied Mathematics. He is a fellow of the American Physical Society and serves on the editorial boards of journals on computational and systems biology. Dr. Qian's current research is in the fundamental theory of stochastic physics and its applications to cellular systems. He sees single-cell level cancer carcinogenesis as a unique opportunity to integrate cellular biochemistry with Darwin's evolution theory. With D.A. Beard, he published "Chemical Biophysics: Quantitative Analysis of Cellular Systems."

Abstract Title: Cellular Biology in Terms of Fluctuating Biochemical Network Dynamics: Isogenetic States of a Single Cell and a Possible Second Stochastic Origin of Cancer

Based on a stochastic, nonlinear, open iochemical reaction system perspective, we present an analytical theory for cellular biochemical processes. We apply this theory, and related computational methods, to both self-regulating gene networks and phosphorylation-dephosphorylation signaling, as well as GTPase modules with feedbacks. Chemical bistability, as a form of epigenetic state, is illustrated in mesoscopic biochemical systems. We argue that the thermal fluctuations inherent in molecular processes do not disappear in mesoscopic cell-sized nonlinear systems; rather they manifest themselves as isogenetic variations on a different time scale. Isogenetic biochemical variations in terms of the stochastic attractors can have an extremely long lifetime. Robustness against both internal and external perturbations is a natural consequence of such an attractor. Transitions among discrete stochastic attractors spend most of the time in "waiting," exhibiting punctuated equilibria. It can be naturally passed to "daughter cells" via a simple growth and division process. A novel view of biochemical "mutation" will be discussed.

Robert H. Singer, Ph.D.

Professor and Co-Chairman
Albert Einstein College of Medicine
Bronx, New York

Dr. Robert H. Singer received his Ph.D. in developmental biology from Brandeis University and performed postdoctoral work at the Massachusetts Institute of Technology (MIT) and the Weizmann Institute of Science in Rehovot, Israel. He focused his career on the cell biology of RNA, its isolation, detection, expression, and translation. A patented in situ hybridization technique his lab developed for detecting RNA in morphologically preserved cells revealed that messenger RNA can localize in specific cellular compartments. This work—enhanced by Dr. Singer and his colleagues' development of RNA reporters—gave rise to the field of RNA transport and localization. His lab has shown the dynamics of RNA transcription interrogated by live cell imaging, as well as by FISH using multiplexed fluorescent probes. His lab has developed rapid and sensitive microscopy to study single molecules of RNA in living cells and has devised methods to track RNA from their site of synthesis to the sites of their function. These technologies have implications for understanding of the role of RNA in diseases such as cancer metastasis and mental retardation. Dr. Singer holds 12 patents on his work.

Abstract Title: Following Single mRNAs from Birth to Death

Authors: Robert H. Singer, Ph.D.; Timothée Lionnet, Ph.D.; Hye Yoon Park, Ph.D.; Bin Wu, Ph.D.; Tatjana Trcek, Ph.D.; Sami Hocine, M.S.; and Daniel Larson, Ph.D.
Department of Anatomy and Structural Biology and Gruss Lipper Biophotonics Center, Albert Einstein College of Medicine

Imaging has been instrumental in analyzing the dynamic properties of RNA. New technologies in optical microscopy and fluorescent probe development have been pushing the envelope of our analysis capabilities. We have been dedicated to developing and implementing these technologies to further the understanding of single mRNA dynamics in cells and organisms. In addition to FISH, we have utilized computational approaches to analyze real-time transcription activities of endogenous genes from yeast to human cells. We have employed a plethora of imaging methods, ranging from confocal and multiphoton microscopy; long-term cell imaging; high-speed, real-time, wide-field microscopy; single molecule tracking; and we have developed super-registration microscopy and fluorescence fluctuation analysis. We have investigated key processes of RNA synthesis: initiation, elongation, termination, as well as nuclear pore export, cytoplasmic trafficking, localization, and decay. Mathematical modeling allowed us to extract quantitative kinetic parameters that precisely describe these processes in living cells.

Supported by NIH GMS Grants to R.H. Singer

Anup Singh, Ph.D.

Manager
Biotechnology and Bioengineering
Sandia National Laboratories
Livermore, California

Dr. Anup Singh is the manager of the Biotechnology and Bioengineering Department at Sandia National Laboratories and senior director of Analytical Technologies at the Joint BioEnergy Institute. He also serves as the director of Microfluidics Core in the National Institute of General Medical Sciences (NIGMS)-funded Systems Biology Center (STMC) at the University of New Mexico, Albuquerque. Dr. Singh supervises more than 30 scientists, including technical staff, postdoctoral researchers, and technologists involved in biodefense, bioenergy, and microfluidics research. The department has research projects of over $8M per year funded by multiple agencies, including the National Institutes of Health (NIH), the U.S. Department of Energy (DOE), the U.S. Department of Defense (DoD), and the Sandia Laboratory Directed Research and Development (LDRD) program. Dr. Singh has published over 100 works (over 60 in peer-reviewed journals), delivered over 150 presentations at national and international conferences, and his inventions have led to over 25 patents and patent applications. Major areas of research in his group include: Analysis of genome, metagenome, transcriptome, and proteome of cells at single-cell resolution; development of point-of-care devices; and high-throughput screening for biofuel applications.

Abstract Title: Integrated biophysical and biochemical measurements in single cells

I will present advances made in my laboratory in analysis of single-cell proteome and genome as described below.

1. Interrogating Cell Signaling with Single Cell Resolution: Cell signaling experiments currently are done using a large number of cells and hence, provide population-averaged data that in many instances may be misleading. We are developing a one-stop shop platform that integrates cell handling and analysis with high-resolution imaging and flow cytometry to provide spatio-temporal measurement of signaling pathways with single-cell resolution. The platform is capable of imaging single cells to obtain dynamic translocation data, as well as high-throughput acquisition of quantitative protein expression and phosphorylation information of selected cell populations. The platform consists of multiple modules, such as single-cell array, cell sorter, and phosphoflow chips to provide confocal imaging, cell sorting, and flow cytometry-based phosphorylation assays. The various modules have been integrated into a portable package that can be mounted on a typical inverted microscope.

2. Single-Cell Genomic Analysis: Current metagenomic techniques (e.g., microarray or 16s rRNA sequencing) relying on pooled nucleic acids from lysed bacteria can independently measure metabolic activity and the species present but cannot link the activity deterministically to the species. We are developing high-throughput tools for studying bacteria one cell at a time, allowing us to unravel the complicated dynamics of population, gene expression, and metabolic function in mixed microbial communities (e.g., human microbiome). Our approach includes FISH-based identification of desired species, enrichment by cell sorting, followed by single-cell encapsulation, whole genome amplification, and sequencing. Encapsulation of bacteria in pico-liter plugs, in particular, allows us to scale down conventional (microliter-volume) assays, such as WGA, into much smaller reaction volumes better suited to the size of an individual microbe.

Paul Soloway, Ph.D.
Professor
Cornell University
Ithica, New York

Dr. Paul Soloway earned a B.A. in biochemistry at Cornell University, a Ph.D. in molecular genetics from Princeton University, and did postdoctoral studies at the Massachusetts Institute of Technology (MIT) and the Whitehead Institute for Biomedical Research. He worked as a research scientist at Roswell Park Cancer Institute before joining the Cornell University faculty, where he is professor of Nutritional Sciences in the College of Agriculture and Life Sciences. Dr. Soloway studies genomic imprinting, the mechanisms regulating placement of epigenetic marks in the genome and is developing single molecule and single-cell approaches for multiplexed epigenomic analysis.

Abstract Title: Single Molecule and Single Cell Epigenomic Methods

Authors: P.J. Murphy, B.R. Cipriany, J.Topolancik, C.B. Wallin, H.G. Craighead, and P.D. Soloway

Epigenomic data come primarily from ChIP and bisulfite sequencing. Though powerful, these methods have limitations. First, they are cumbersome for studies seeking to determine only the abundance of a given epigenetic feature. Second, because epigenomic features are typically characterized one at a time, it is not possible to detect directly when multiple features are present on the same DNA molecule. Re-ChIP can solve this problem, but generally, data from single feature experiments are superimposed and coincidence of features is only surmised. Third, for ChIP, substantial amounts of chromatin are usually needed for analysis, limiting the capacity to query epigenomic states in rare cells. Here we describe three sets of studies, using micro and nanofluidic devices for manipulating single cells and single chromatin molecules that overcome these limitations.

First, we used high-throughput analytical nanofluidic devices to quantify the abundance of various epigenetic on chromatin molecules from embryonic stem cells and to detect coincidence between various pairs of features. Second, we modified the analytical device to enable separation of individual molecules bearing DNA methylation from mixtures that included methylated and unmethylated DNAs with 95% efficiency. Finally, we developed a microfluidic device capable of efficiently extracting DNA from single cells and directing them to the analytical nanoscale device. Data from each of these three approaches will be described, which, in combination, can provide tools for next generation, multiplexed, epigenomic analysis from small inputs cells.

Jonathan V. Sweedler, Ph.D.
Professor
University of Illinois
Urbana, Illinois

Dr. Jonathan V. Sweedler holds the James R. Eiszner Family Chair in Chemistry at the University of Illinois, is the director of the University of Illinois Carver Biotechnology Center, and has appointments in the Neuroscience Program in the Department of Physiology and Bioengineering. His research interests are in bioanalytical chemistry, and his focus is on new metabolomic, peptidomic, and imaging technologies for assaying small volume samples (often at the single-cell level). These technologies include enhancements to capillary electrophoresis separation methods, MALDI sampling techniques, nanoliter volume NMR, and micro/nanofluidic sampling. He applies these approaches to study novel neurochemistry occurring in selected cells. Dr. Sweedler has received numerous awards, including the Merck Prize, the Instrumentation Award from the Analytical Division of the American Chemical Society (ACS), and the Gill Prize. He is the editor-in-chief of *Analytical Chemistry*.

Abstract Title: Metabolomics at the single cell level: cell by cell chemical characterization

Authors: Jonathan V. Sweedler and Stanislav S. Rubakhin
Department of Chemistry and the Beckman Institute, University of Illinois

In the postgenomic era, one expects the suite of chemical players in a brain region to be known and their functions uncovered. Significant challenges in the characterization of intercellular signaling molecules arise in part from their large chemical diversity and broad range of concentrations. The enormous biochemical complexity of the nervous system, where even adjacent cells often have very different and dynamic metabolic profiles, necessitates development and application of technologies capable of characterization of the neurometabolome on the individual cell and even the subcellular level. Here, we present a suite of bioanalytical approaches that are starting to allow the investigation of individual neurons and small brain regions. These approaches include capillary electrophoresis with laser-induced fluorescence and mass spectrometric detection and direct mass spectrometric-based profiling and imaging, as well as their combination with spectroscopic imaging. Several applications of single-cell microanalysis are highlighted, including novel indolamine neurochemistry and characterizing the peptides in single cells. Specifically, new serotonin-related compounds and literally hundreds of new neuropeptides have been characterized in well-defined neuronal networks, and in several cases, the functional roles of these molecules described. Discovery of new neurochemical pathways often relies not only on structural information provided by traditional mass spectrometry but also requires knowledge on the spatial and temporal dynamics of these signaling molecules in the brain. Imaging mass spectrometry and dynamic sampling of the extracellular environment are used for elucidating novel cell-to-cell signaling molecules in a range of neuronal model systems. Current technology efforts involve extending the depth of metabolome coverage and adapting these analytical approaches to higher throughput single-cell assays. Our overarching goal is to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

Barbara Wold, Ph.D.
Director
Center for Cancer Genomics
National Cancer Institute
Bethesda, Maryland

Barbara Wold is the Bren Professor of Molecular Biology at Caltech. In 2011-2012 she is on leave at the National Cancer Institute to establish its new Cancer Genomics Center. She began working on genome structure and gene regulation during embryo development for her PhD thesis with Eric Davidson, and then developed ways to dissect regulatory DNA elements during postdoctoral work at Columbia with Richard Axel. She returned to Caltech where she and her students and collaborators study genome function in development. Recent foci have been on developing and then applying new ways to map the inputs and outputs of gene networks in a genomewide manner using "next generation" DNA sequencing (ChIP-Seq, RNA-Seq, single-cell RNA-Seq etc), and applying these methods to comparative studies of gene networks that govern mouse and worm myogenesis, mouse T-cell development, neuronal identity.

Abstract Title: Single cell transcriptomes: technological and conceptual challenges

RNA-Seq is usually applied to bulk tissue and cell cultures, which forces averaging over populations of cells that represent multiple states (cell cycle phases, stress states, dynamic signaling differences),and mixed cell types that typify complex tissues. Recent progress is allowing us to move RNA-Seq methods to the single cell level. This talk will report on single cell RNA-Seq studies in three distinct mammalian cell types. Issues associated with gene detection limits, RNA integrity and coverage, RNA-Seq informatics, technical and biological stochasticity will be discussed.

Abstract Title: Single cell microenvironment arrays fabricated via tip-based lithography

Authors: John M. Collins, Ruby T.S. Lam and Saju Nettikadan
NanoInk, Inc. NanoFabrication Systems Division, Skokie, IL

It is well known that the microenvironmental niche provides complex chemical and structural cues to cells. Here, we illustrate a flexible methodology, tip-based lithography, to construct patterns of sub-cellular features using a wide range of materials. The ability to pattern features at this scale allows for precise control and patterning of cues that mimic the cellular microenvironment. Tip-based lithography is a direct write process in which multiple materials can be deposited simultaneously at scales which affect and manipulate single cells (<1-10 μm). This technique can be utilized to rapidly prototype cellular scaffolds with multiple components on the same substrate, useful for single cell assays. Polymers, proteins and other bio-molecules can be patterned with nanoscale precision in biocompatible conditions. Applications such as cell polarization, targeted delivery and precision co-culture at single cell levels have been demonstrated.

Substrates were fabricated using either the NLP2000 or DPN5000 systems (NanoInk, Inc., Skokie, IL). We have demonstrated the ability to control cell morphology by patterning fibronectin and other extracellular matrix proteins at feature sizes between 5-8 μm. Specific cell attachment and spreading were observed over the patterned areas with greater than 80% of patterns have cell attachment. With time, cell morphology takes on the shape of the patterned proteins enabling study of actin dynamics at single cell levels. Further experiments demonstrate that 500 nm features of the RGD cell-binding domain patterned in various conformations of differing pitch controls cell polarity and direction of cell division. We have also demonstrated the ability to deliver different materials to arrays of single cells patterned within 10's of microns of each other. Finally, because of fibroblast's preferential binding to fibronectin compared to laminin, NIH 3T3 fibroblasts were cultured with C2C12 myoblasts demonstrating precision co-culture of multiple cell types.

The ability to precisely control the cellular microenvironment could allow for a more complete understanding of the cues impacting cell function. Just as genomic and proteomic arrays have been useful for identification of biomarkers of various biological functions or diseases, the technique described here enables a next-step in the characterization of biological function. Tip based lithography is a unique and powerful way to fabricate complex scaffolds for investigating cell behavior at single cell levels.

Abstract Title: Single cell microfluidics for systems oncology

Author: Rong Fan, Department of Biomedical Engineering, Yale University, New Haven, CT and Yale Comprehensive Cancer Center, New Haven, CT

The singular term "cancer" is never one kind of disease, but deceivingly encompasses a large number of heterogeneous disease states, requiring systems approaches to patient stratification and personalized treatment. We developed a single cell microfluidics chip that can measure a panel of proteins associated with inter-cellular signaling network in tumor microenvironment. Hundreds of single cancer cells were analyzed in parallel. We conducted highly multiplexed profiling of single-cell secreted proteins including tumor-immune signaling molecules from both cancer cells and immune cells, and observed profound cellular heterogeneity with all functional phenotypes quantitatively identified. Correlation analysis further indicates the existence of an intercellular cytokine feedback loop in which TNFα-induced secondary signaling cascades further increased functional cellular diversity. Upon further development, this platform may potentially become a clinical tool for patient stratification based upon single-cell proteomic signature, inter-cellular signaling network and aid the design of new anti-cancer therapy by targeting microenvironmental components.

Abstract Title: Microfluidic multiplexed single cell lysis and kinase activity assay interface

Author: Jongyoon Han, Massachusetts Institute of Technology, Cambridge, MA

In order to gain mechanistic understanding of cell signalling pathways, it is necessary to monitor activities of several key cellular kinases, preferably with single cell resolution. In this poster, we demonstrate a microfluidic cell lysis interface which is used to lyse individual cells in adherent culture and reliably sample the lysates. Collected single cell lysates are then divided for measuring activities of several kinases simultaneously. Mobility shift assay can be used to enhance the sensitivity of measurements further, providing toolsets for various single cell signaling measurements to address the question Of cell heterogeneity.

Abstract Title: Mitochondria are important subcellular organelles in most eukaryotic cells

Author: Chun-Nan Hsu, Information Sciences Institute, University of Southern California, Marina del Rey, CA

Mitochondrial fusion and fission dynamics plays an important role in maintaining a population of healthy mitochondria in human cells. Unbalanced mitochondrial dynamics has been linked to aging, neurodegenerative diseases and a wide variety of mitochondrial disorders. Because of the central role of mitochondrial dynamics in cell physiology, there is increasing demand to evaluate the morphological distribution of mitochondria in single cells by fluorescence microscopy. Most commonly, the morphology of mitochondria is roughly scored in single cells by whether they are fragmented or tubular, but most manual scoring criteria are subject to user bias. Moreover, manual scoring methods are tedious and can be applied to only limited numbers of cells. There is therefore a need to develop automated and unbiased methods to evaluate mitochondrial morphology in cells. In addition, recent evidence shows that mitochondria may perform not just end-to-end fusion but also end-to-side fusion that results in branched or even grid-like configurations. They may bend to form twisted tubules and degrade through mitophagy. Manual scoring methods do not accommodate these less standard morphologies. Accurate quantification of mitochondrial morphology requires the ability to comprehensively tabulate the complex morphological subtypes present in cells. In this project, we propose to develop cell imaging and analysis tools that allow this quantification. These tools will help researchers in many areas of biomedical science and will be useful in high-throughput assays that require analysis of mitochondrial dynamics. Our software tools will include a component that can automatically recognize mitochondrial objects in cell images/movies and classify them into well-defined morphological subtypes, thereby providing a multiplex readout that quantifies the spatial-temporal composition of mitochondria in a single cell. Another component will detect and quantify heterogeneity of single cells in terms of mitochondrial morphological compositions. We have developed a knockin mouse model where we can label mitochondria in any cell type. With the new software tools and the mouse model, it will be feasible to analyze single cells in tissues with abnormal mitochondrial morphology within a heterogeneous population, systematically associate mutations with the degrees of mitochondrial morphological defects, and identify cell subpopulations in mixed populations of wildtype and mutant cells.

Abstract Title: Single cell, multiplexed, high-content analysis of pancreatic beta-cell differentiation and survival: New clues about islet cell heterogeneity

Authors: James D. Johnson, Carol Y.H. Yang, Marta Szabat, Department of Cellular and Physiological Sciences, Department of Surgery, University of British Columbia, British Columbia, Canada

Programmed cell death and dedifferentiation of pancreatic beta-cells contributes to the progression of type 1 diabetes and type 2 diabetes, and hinders clinical islet transplantation. Our overall goal is to discover factors that significantly increase the survival and differentiation of human beta-cells, initially in cultures of islets destined for transplantation and eventually in the bodies of those that are at risk for type 1 diabetes. Here, we employ a multi-parameter, high-content, high-throughput, imaging platforms to perform simultaneous live-cell imaging of beta-cell fate and function. We present the first evidence of single-cell differentiation in beta-cells, and define distinct maturation stages that individual beta-cells pass through. Dual reporter lentiviruses were used simultaneously track the promoter activities of Insulin1 and Pdx1, together with cell death tracking via the combination of a eBFP2-DEVD-eGFP caspase-3 sensor, propidium iodide, annexin V and nuclear morphology. We image reporter-expressing dispersed pancreatic islet cells using robotic, high-content imaging systems for 3-5 days in basal conditions and conditions relevant to type 1 diabetes, such as a pro-apoptotic cytokine cocktail, hyperglycemia, or nutrient deprivation. The results of these kinetic studies illustrate, for the first time in beta-cells, the temporal relationship between the distinct cell death stages and reveal the incidence on non-apoptotic cell death in beta-cells. The results of small molecule library screening will also be presented. Simultaneous efforts to characterize the effects of 296 locally secreted or acting islet growth factors will also be discussed. These studies represent unbiased attempts to compare, discover and validate islet growth factors that inhibit apoptosis and promote the mature function of human and rodent islets. Novel survival factors represent excellent therapeutic targets for future diabetes treatments. To date, factors that can increase the survival and function of β-cells have been discovered mostly by a process of trial and error. We believe that more potent growth factors capable of controlling beta-cell apoptosis and maintaining beta-cell functional differentiation remain to be discovered using unbiased, high-throughput, single cell analysis.

Abstract Title: From molecules to cells to organisms: understanding health and disease with multidimensional single-cell methods

Authors: Julian Candia(1), Wei Lai(2), Robert Nussenblatt(2), Amos Maritan (3), Jayanth Banavar(1), Wolfgang Losert(1)
(1)Department of Physics, University of Maryland, College Park, MD (2) NEI, NIH, Bethesda, MD (3) INFM, University of Padua, Italy

The multidimensional nature of many single cell measurements (e.g. multiple markers, measured simultaneously using FACS) offers unprecedented opportunities to unravel emergent phenomena that are governed by cooperative action of multiple elements across different scales, from molecules and proteins to cells and organisms. We developed an integrated analysis framework to investigate multicolor FACS data from different perspectives: Singular Value Decomposition to achieve an effective dimensional reduction in the data representation, machine learning techniques to separate different patient classes and improve diagnosis, and a novel cell-similarity network analysis method to identify cell subpopulations in an unbiased manner. Besides FACS data, our framework is versatile: We demonstrate an application to mechanical analysis of single cells with optical stretchers - a new high-throughput approach to measure mechanical properties of single cells.

Abstract Title: Streamlined analysis of heterogeneity in cell populations using single-cell gene expression profiling

Authors: Andrew May, Ronald Lebofsky, Anne Leyrat, Brian Fowler, Joseph Shuga, Peilin Chen, Jing Wang, Dominique Toppani, Myo Thu, Michael Wong, Darnell Kemp, Barry Clerkson, Michael Norris, Jay West, Marc Unger, Robert Jones, Fluidigm Corporation San Francisco, CA

Single-cell gene expression profiling has recently been used to characterize emergent properties in cell populations that drive lineage choice and specificity in reprogrammed cells; resolution of cell fate decisions in very early embryonic development, and for identification of cancer stem cell biomarkers in tumour biopsies. We have developed a simple, modular workflow for streamlined analysis of cell populations down to the single-cell level. The workflow is centred on two key components: cell isolation and cDNA preparation, and the Biomark HD™ system for highly parallel gene expression analysis. Cells are processed to prepare high-quality targeted cDNA for gene expression analysis. The targeted cDNA samples are then loaded and analyzed in parallel with up to 96 gene expression assays in parallel on Dynamic Array™ IFCs using the Biomark HD system. This workflow has been used to identify and analyze underlying heterogeneity in a variety of cell types and provides a general method for detailed analysis of stem cell cultures, neuronal development, tumour heterogeneity and identification of biomarkers for subsequent isolation of cellular subsets.

Abstract Title: Single cell approaches to complex disease

Author: Simon Melov, Buck Institute for Research on Aging, Novato, CA

Many diseases are inherently complex, due to both specific and non-specific interactions between different cell types within tissues.

Age related heart disease is among the leading causes of death in the developed world. A better understanding of the molecular etiology of heart disease becomes increasingly important as the proportion of aged individuals in the population grows. While many studies examine changes within heart tissue in association with cardiac dysfunction, such an approach may prevent a basic understanding of the inherent variation between different cell types in such a complex tissue. We routinely isolate pure populations of viable cardiomyocytes though perfusion of mouse heart, and subsequently assay single cells for both gene expression as well as functional deficits.

Similarly, the study of the age-related bone disorders (such as osteoporosis) has been hampered due to an inability to study specific cell types implicated in bone disease. Isolation of specific cell types (such as the osteoblast) from bone is inherently difficult, and frequently has a low yield impairing many techniques which require large amounts of biological material in order to generate robust results. We have developed single cell approaches to study the genomics of cells isolated directly from intact bone, and have begun to characterize gene expression within specific cell types of bone.

Overall, our approach allows for the examination of a number of distinct cellular physiologies at the single cell level which has not been previously possible. It allows us to ask novel questions such as addressing the variability of gene expression within specific cell types of a given tissue. This may uncover new insights into cell physiology which are not possible when analyzing mean values from heterogeneous cell populations derived from bulk tissue.

Abstract Title: MRNA transcriptome and cpg methylome analysis for single cells and low quantities of cells

Authors: Xinghua Pan[1], Haiying Zhu[1], Yumei Li[1], Sadie Marjani[1], Chao Ma[2], Yoshiaki Tanaka[3], Lin Han[4], Xiaoyuan Zi[1,4], Robert LaMotte[2], InHyun Park[3], Rong Fan[4], Michael Snyder[5], and Sherman Weissman[1]

[1]Dept Genetics, [2]Anesthesiology, [3]Yale Stem Cell Center, School of Medicine; [4]Biomedical Engineering, School of Engineering & Applied Science, Yale University, New Haven, CT

[5]Department of Genetics, Stanford University School of Medicine, Stanford, CA

We previously established a procedure called whole DNA pool amplification (WPA) for highly sensitive and specific amplification of a complex DNA mixture [Pan, PNAS 105:15499, 2008]. Basing on WPA, we developed a method for amplifying the whole mRNA transcriptome (WMA), and a method of analyzing the CpG methylion pattern (CpGMaps) in single cells or a small number of cells, beyond other methods. We have begun to apply them to analyze hematopoietic precursors, ESCs, iPSCs, and neurons. For high-throughput performance, we are adapting a microfiuidic platform previously used for multiplexed analysis of single cell proteins [Fan, Nat. Med. 17:738, 2011; Shi & Fan, PNAS 109:419,2012] now for WMA and CpGMaps.

We initially generate single or double strand cDNAs using a poly-dT primer. The cDNA is then intra-molecularly circularized and WPA amplified. The single strand protocol was applied to a panel of single neurons, and the amplicons were analyzed with an Illumina bead array. Hierarchical clustering of the gene expression patterns of 5 single neurons identified in vivo by electro-physiology matched the relationship of the neurons based on phenotypic properties. A pair of amplicons from the double strand protocol was sequenced on Illumina HiSeq, detecting both new and expected patterns. With 100 or more smaller non-neuron cells, the procedure also provides a near compete representation of expressed genes. WMA demonstrates unique features: covering the full length of almost any size of transcripts, up to 23 kb, and retaining the strandedness message.

The CpGMaps method takes advantage of unique characteristics of the WPA, combined with methylation sensitive restriction endonucleases and specially designed intube purification. It selectively recovers highly methylated CpG-rich sequences for sequencing library generation. As a proof of concept, this method worked nicely with 10-cells and single cells for a limited number of loci, but more robustly on 100-cells or more, covering CpG islands genome-wide.

Abstract Title: Informative cancer diagnostics based on microfluidics devices

Author: Lidong Qin, The Methodist Hospital Research Institute, Houston, TX

Integrated microfluidic devices, with ultra-miniaturized antibody arrays coated under micro-channels, are developed for the detection of cancer biomarkers from blood. Despite changes of plasma protein profiles reflect physiological or pathological conditions associated with many human diseases, only a few plasma proteins are routinely used in clinical tests. Reasons for this include the intrinsic complexity of the plasma proteome, the heterogeneity of human diseases and the rapid degradation of proteins in sampled blood. Our integrated blood barcode chip can sensitively sample a large panel of protein biomarkers over broad concentration ranges and within 10 minutes of sample collection.

Based on the same microfluidic technique, we have also developed an approach that integrates on-chip cell handling and in situ protein secretion profiling. The platform enables us to assess the functional heterogeneity of single cells, with extensions to small cell colonies. We have measured a dozen proteins secreted from human prostate cancer cell lines and correlation analysis showed the unusual protein regulation network for single cells, in comparison to traditional measurement on many cells system. Our platform may lead to discoveries in cancer cell molecular pathways and microenvironment regulating mechanism.

Abstract Title: Very high content single cell assays: an interactive analytical solution

Authors: J. Paul Robinson, V. J. Davisson, B. Rajwa, V. Patsekin, Purdue University Cytometry Laboratories, Basic Medical Science, Weldon School of Biomedical Engineering, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN

The process of quantitatively describing complex cellular populations at the single-cell level characterized using multiple biomarkers of function, and measured within context of various signaling pathways is a difficult, but tangible task. Among available technologies, modern high-throughput (HT) and very high content flow cytometry (VHC) offers the capability to provide descriptors of function on a single-cell level comprehensively characterizing heterogeneous cell populations.

The leading-edge VHC flow cytometry technologies allow simultaneous collection of over 30 variables measured on as many as 100 million cells in a single assay. This enormous progress in data acquisition opens a new era in cytomics that will enable rapid hypothesis testing in systems biology domain. These advances also enable complex analysis of cell states across a broad range of populations. However, despite the innovation in the HT flow cytometry hardware, the advancements in analytical tools have lagged behind.

Herein we present a new approach to managing HTHC-FC datasets, and we demonstrate its usability and practical utility for drug screening. The described system can analyze automatically multiplexed cell lines, multiple kinase inhibitors and multiple drug candidates. One of the most important aspects of developed approach is interactivity of data processing pipeline which is maintained during every step of analysis.

This allows the assay designer, to directly control the complete dataset, regardless of the size or complexity. To facilitate this functionality, we implemented a “logic map” concept that integrates the analytical pathway with a variety of complex statistical processes all of which operate in real time. The results are automatically visualized using an easy-to-understand convention such as does-response curves, IC50s values, interactive heatmaps, etc. We will demonstrate simultaneous analysis of as many as 14 populations of cells, with 14 phosphorylation states and 12 activators at 8 concentrations each in real time for all 96 wells of a complex assay.

Abstract Title: Quantitative and qualitative single cell matrix assisted laser desorption/ionization mass spectrometry

Authors: Stanislav S. Rubakhin, Jonathan S. Sweedler, Department of Chemistry and the Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana−Champaign, IL

Single cell matrix assisted laser desorption/ionization mass spectrometry (scMALDI-MS) is an approach that allows the characterization of multiple metabolites and peptides in individual vertebrate and invertebrate cells. Attomole detection limits, characterization of large number of compounds and high throughput cell profiling allows the detailed measurement of the neurochemistry within hundreds of cells in a single experiment. We are creating and enhancing scMALDI-MS methods for detecting metabolites and peptides in individual invertebrate neurons, vertebrate endocrine cells and a variety of rat and mouse neurons including dorsal root ganglia cells. scMALDI-MS has two modes — single cell profiling and MS imaging; profiling involves characterizing the content of individually isolated cells and MS imaging probes cells in tissue sections and those spread out on a glass slide. Here we demonstrate utility for studying cell to cell chemical heterogeneity from complex samples. One example obtains cellular peptide profiles and genome-linked analyte identification from the invertebrate neurobiological model *Aplysia californica*. We combine this with absolute quantitative peptide measurements using both standard addition using internal standards and isotope labeling, as well as identify analytes via tandem MS. scMALDI-MS profiling of mammalian dorsal root ganglion neurons and their neurites surrounded by Schwann cells allows a unique glimpse into mammalian cellular heterogeneity and how to identify the rare cells whose chemical profiles change based on specific exposure to nociceptive signals. The scMALDI-MS approach can be scaled and automated, and thus adapted to characterizing a larger number of cells and cell types. We envision the use of this technology by laboratories and research groups that engage in clinical diagnostic measurements, fundamental scientific investigations, and pharmaceutical discovery.

Abstract Title: Utilizing 3D nuclear DNA methylation patterns in cell-based Assays for Epigenetic Drug Screening

Authors: Arkadiusz Gertych, Daniel L. Farkas, and Jian Tajbakhsh,
Cedars-Sinai Medical Center, Los Angeles, CA

The reversibility of epigenetic modifications, such as cytosine methylation, has gained much attraction in cancer therapy, with an emphasis on the development of anti-cancer drugs with demethylating potential to primarily reestablish tumor-suppressor gene activity. The interest in the ***development of novel epigenetic drugs has increased the demand for cell-based assays to evaluate drug performance,*** including genotoxicity in the important pre-clinical phases of drug screening. Ideally a demethylating drug should reduce hypermethylation of tumor-suppressor genes for their reactivation, without creating unwanted effects: i.e. the hypomethylation of heterochromatin, of which a large percentage occurs in repetitive elements and can lead to genome instability. We have recently developed ***3D quantitative DNA Methylation Imaging (3D-qDMI)***, a non-invasive imagecytometrical approach, that can measure two important parameters in DNA methylation changes (i) the nuclear methylcytosine (MeC) load, and (ii) the spatial nuclear co-distributions of methylated cytosine (MeC) and global DNA (gDNA) ***in a high-throughput and cell-by-cell fashion in situ*** to verify unwanted effects such as increased heterochromatin hypomethylation and decondensation that involve large-scale chromatin reorganization visualized by high-resolution confocal microscopy. Our novel approach succeeded in tracking differential changes of higher-order DNA organization due to drug-induced demethylation on a genomic scale, in human and mouse cancer cells. Our study showed that the differential distribution patterns of these two classes of DNA have the potential to be utilized as novel signatures — ***surrogate pharmacodynamic biomarkers of drug action*** — in the characterization of different demethylating agents such as 5-azacytidine and zebularine.

Abstract Title: Nuclear DNA methylation patterns in early in vitro differentiation of mouse embryonic stem cells towards definitive endoderm

Authors: Jian Tajbakhsh, Arkadiusz Gertych, W. Samuel Fagg, Seigo Hatada, Jeffrey H. Fair, Cedars-Sinai Medical Center, Los Angeles, CA

The genome organization in pluripotent cells is highly relevant to the cell reprogramming process during early differentiation. Therefore relevant chromatin texture patterns that identify cells at the very early stage of lineage commitment could serve as valuable tools in the selection of optimal cell phenotypes for regenerative medicine applications. We used high-resolution 3D imaging and complementary topological cell-by-cell analysis with a novel in-house developed image-cytometrical approach ***— 3D quantitative DNA Methylation Imaging (3D-qDMI) —*** to identify *in situ* global nuclear DNA methylation patterns in early endodermal differentiation of mouse ES cells and the correlations of these patterns with a set of putative markers for pluripotency, endodermal commitment, and the epithelial and mesenchymal character of cells. ***Our cell-by-cell analysis approach allowed for the flexible characterization of single cells, cell clusters and larger groups of clusters across all imaged cells.*** We found: differentiating cell populations display an increasing number of cells with a gain in DNA methylation load — first within their euchromatin then extending into heterochromatic areas of the nucleus, also resulting in significant changes of methylcytosine distribution patterns. However no correlations between the concomitant stochastic marker expression and methylcytosine loads/distribution patterns could be found. We conclude that progression of global DNA methylation is not correlated with standard transcription factors associated with endodermal development. Further studies will determine whether the progression of global methylation could represent a robust signature of cellular differentiation.

Abstract Title: The microarray and sequencing quality control project

Authors: Leming Shi[1], Charles Wang[2], Jean & Danielle Thierry-Mieg[3], On behalf of the SEQC Consortium[1]National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, [2]Beckman Research Institute, City of Hope Comprehensive Cancer Center, Duarte, CA[3]National Center for Biotechnology Information, National Library of Medicine, NIH, Bethesda, MD

Microarrays and next-generation sequencing (NGS) technologies are widely used for transcriptomic analysis in pharmacogenomics, toxicogenomics, biomedical research and personalized medicine. However, before these technologies can be reliably used in clinical practice and regulatory decision-making, their technical consistency and quality measures must be evaluated in order to develop certain guidelines. The MicroArray and Sequencing Quality Control (MAQC/SEQC) project (http://www.fda.gov/MicroArrayQC/) is an FDA-led community-wide effort involving experts from over 100 organizations around the world, aimed at reaching consensus on the best practices for the generation, analysis, and application of microarray and RNA-Seq data in the discovery, development, and review of FDA-regulated products. The MAQC-I evaluated the technical performance of various microarray gene-expression platforms and assessed the advantages and limitations of competing data analysis methods for identifying differentially expressed genes (www.nature.com/nbt/focus/maqc/). The MAQC-II evaluated methodologies for developing and validating classification models based on high-dimensional microarray transcriptomic data to predict clinical and toxicological endpoints (www.nature.com/focus/maqc2/). Currently, the on-going MAQC-III, the SEQC project aims to assess the technical performance of NGS platforms by generating large benchmark RNA-Seq transcriptomic datasets with reference samples and evaluating the advantages and limitations of various bioinformatic strategies in transcriptomic analysis. A central component of the SEQC is cross-site and cross-platform comparisons using RNA-Seq data sets generated on well-characterized reference RNA samples established by MAQC-I with spike-in external RNA controls of known sequences and relative concentrations. These data sets will allow us to objectively evaluate the performance of RNA-Seq and data analysis methods in terms of base-call accuracy, absolute expression levels, differential gene-expression levels, and lists of differentially expressed genes, and to assess intra-site repeatability, inter-site reproducibility, and cross-platform comparability. A second component of the SEQC is to expand reference annotations for human and rat transcriptomes by generating and integrating large RNA-Seq data sets from multiple tissue types. This poster will present the rationale, study design, and preliminary results of the SEQC project. With broad participation from academia, industry, and government sectors, the MAQC/SEQC project is helping to realize personalized medicine by ensuring the reliability of patient-specific genomic information and its appropriate analysis and interpretation. The experience gained from the MAQC/SEQC project may be helpful for large-scale single-cell transcriptomic studies. *Views expressed in this presentation are those of the presenter and not necessarily those of the US FDA.*

Abstract Title: Single cell assay chips for cancer drug screening and pairwise cell-to-cell interactions

Author: Euisik Yoon, University of Michigan, Ann Arbor, MI

Studying the heterogeneity of cancer cells is an area of importance as malignant sub-phenotypes such as cancer stem cells are believed to be responsible for drug resistance and cancer relapse. We present a prototype device that allows us to track the progeny of each single cell for differences in proliferation and morphology, thus identify different sub-phenotypes and their different drug responses. The second prototype device is developed for studying cell to cell interactions for the comprehensive and high-throughput analysis of contact and secretion based interactions occurring between cell pairs at single cell resolution.