Multi-scale Fabrication Technologies for Single Cell and Subcellular Measurements on Living Cells

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Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.
Novel Experimental Biology with Microsystem Technologies

- Single cell manipulation and measurement – with potential for automation
- Laminar flow (fluidic isolation, precise delivery of reagents)
- Built in controls (arrays of “experiment units” for biological or technical replicates)
- Environmental controls are improved

Nam et al. Biomed Microdev 2005
L. Lee, 2005
Schneegab et al. Lab Chip 2001
Microscale Immune Studies Laboratory (MISL) Grand Challenge

To create an integrated single-cell manipulation and interrogation platform and predictive models to provide molecular- and cellular-level understanding of innate immunity signaling pathways with unprecedented speed, resolution, sensitivity, and multiplexing

• The benefits will be:
  ✓ Key discoveries in the understanding and application of innate immunity to anticipate, detect and counter biothreats
  ✓ An enabling tool for high-throughput biological pathway studies – also applicable to cancer, asthma, cell differentiation, and microbial communities
Overview of the Approach

Hypothesis: TLR4 network response varies with LPS chemotype

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Develop an experimental plan to test the hypothesis

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Develop an integrated experimental platform to conduct experiments with single cells, develop measurement techniques

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Use bioinformatics and rate calculations to create a dynamic model of signaling pathways

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Use predictive modeling to generate further hypotheses

Biology Team

Microsystem & Imaging Team

Computation Team
MISL: A Multi-Disciplinary Team

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MISL Microsystem Platform Components

Single cell capture and imaging:
- time-course
- dynamics
- protein-protein interactions

Population measurements
- sample preparation
- higher throughput cytometry
- cell sorting

Electrokinetic immunoassays
- cytokine profiling
- multiplexed detection
- high sensitivity
Microfluidic Cell Sorting

Pre-processing of cells is required for procuring “high value” cells
- optical force based sorting
- scatter and fluorescence signals
- AOM-based scanning to deflect cells

Hydrodynamic focusing

Scattering and LIF

Sort

NO

YES

Raster IR laser

Binning

T. Perroud, K. Patel

Frame rate slowed 13X
Single Cell Capture

Mechanical capture of single cells
-100 traps in parallel with fluidic isolation
-cell assessment: reporter, nucleus, live/dead

Viability: max length is 5 hrs, with 10/13 cells surviving
LPS Challenge Induced RelA Translocation

- Cell response is similar to that in bulk measurements

- 1 μM smooth E. Coli LPS injected at 3 min; translocation seen after 22 minutes

### Graph
- Produce the dose-response curve of challenged cells in the SCA – Computational Core
Single Cell Isolation

• Goal: primary vs. secondary immune response:

• Method: fluidically isolated single cells

LPS

cytokines (~4hrs)

\[ |v| (\mu m/s) \]

0

2.5

5
Enhanced Performance of Engineered Neural Networks using Nanostructured Probes and Predictive Computational Modeling

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Deciphering Neural Tissue Circuitry

All methods are lacking in regards to understanding core processes involved in network architecture and function, and specifically in regard to strategies to enhance network performance (processing speed, robustness to noise, etc.)

Reconstruct dissociated cells into networks using microfabrication techniques - engineered networks can be user-defined, replicated, and readily interrogated (optically and electrically) at single cell and subcellular levels for long terms (>1 year)
- Generate falsifiable hypotheses about network structure and function
- First step towards modifying network structure of tissue slices
Fully engineered networks of neural cells require controlled:

- cell body positioning
- outgrowth and branching of neurites (axons and dendrites)
- polarity of neurites (axons vs dendrites)
- formation of synapses

Oliva et al., Neurochem Res 2003

Withers et al., J Neurobio 2006
Network Activity Detection with Microfabricated Electrode Arrays

Non-invasive, long-term extracellular stimulation and recording from cell networks.

Engineered network on an electrode array

Burst Rate = 13 Hz

Electrochemical Detector Array for Neurotransmitters

Electrochemical detector array for amperometry studies:
- detect exocytosis of adrenaline/noradrenaline

M. Lindau, Cornell U.
Advanced Electrode Array Technology

• Goal: high-density, sub-μm electrodes
  - multiple recording sites within a cell network, and within a single cell
  - multiple recording sites within single synapses

• Method: chemical synthesis and sub-μm lithography

Interferometric lithography:

H. Fan, 2007
Conclusions/Final Thoughts

• Microsystems technology offers experimental biology capabilities at all scales…
  – unprecedented measurements (throughput, variable control, etc.) of dynamic changes in living host cells during an infection
  – evaluate the correlation between cytoarchitecture and function, simultaneous orthogonal measurements on living neurons