SAND2007-6143P

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

Conrad James, Ph.D.
Advanced Sensor Technologies
Sandia National Laboratories

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.

Sandia Facilities

Highly collaborative research

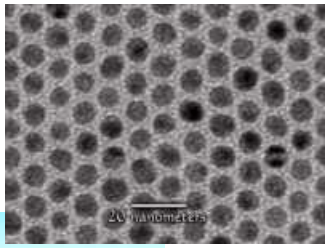
- microsystems engineering (1700), biological science (8300), nanomaterials synthesis (1800), cognitive science (6300)

Microsystems and Engineering Sciences Applications Project (MESA)

-Microsystems technology development;
-Computational and engineering sciences and analysis;

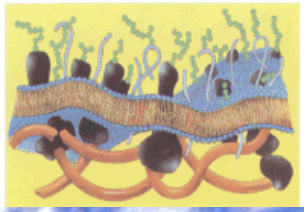
Advanced Materials Lab (AML); Center for Integrated Nanotechnologies (CINT)

-nanomaterials synthesis and analysis facilities;



Processing and Environmental Technology Lab (PETL); Integrated Materials Research Lab (IMRL)

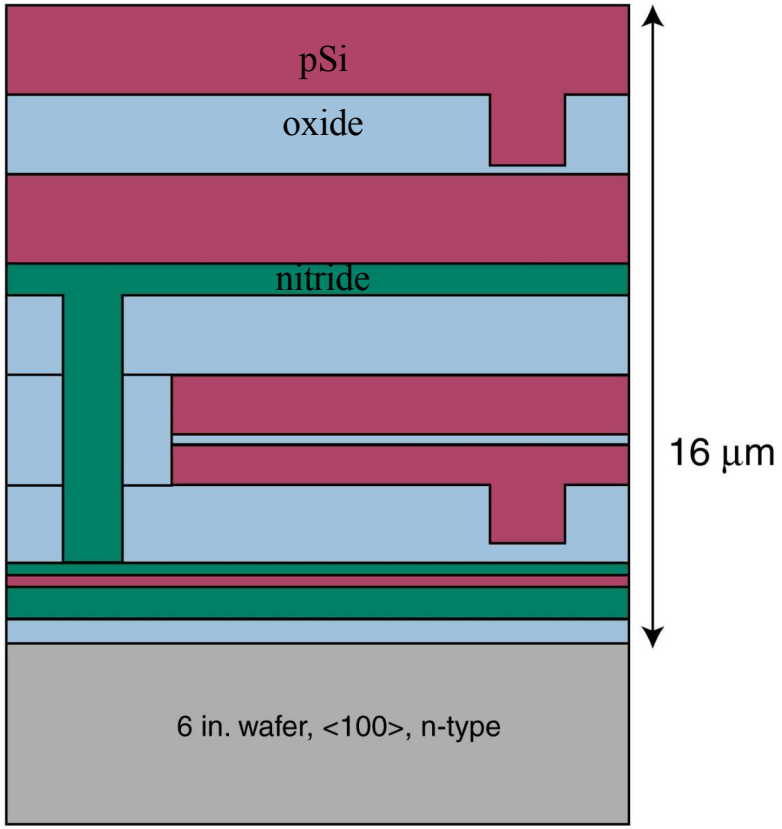
-materials and biological science facilities



Surface Micromachining Technology

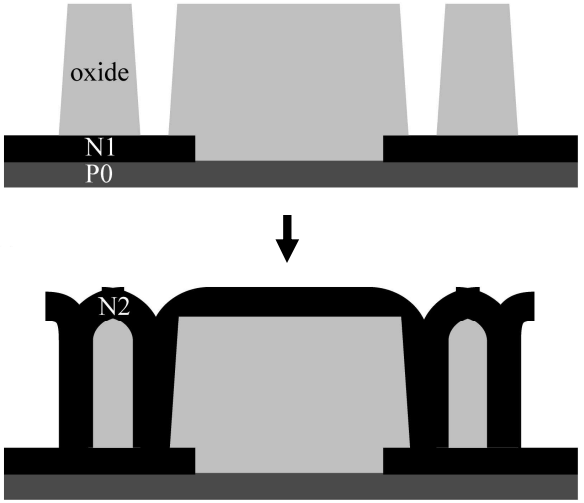
SwIFT ™ (Surface Micromachining with Integrated Fluidic Technology)

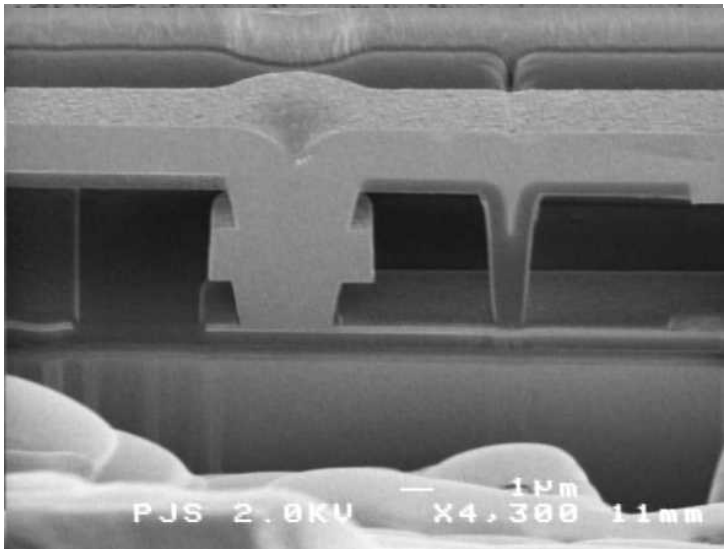
sacrificial layers of SiO_2, five layers of doped pSi, ~ 200 nm resolution, three layers of Si_xN_y



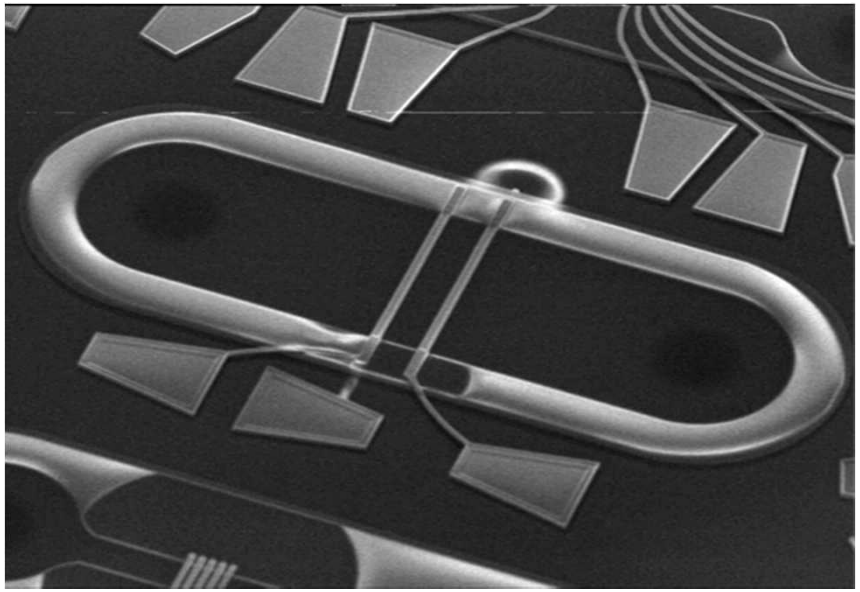
sized for nanoscale fluidic applications

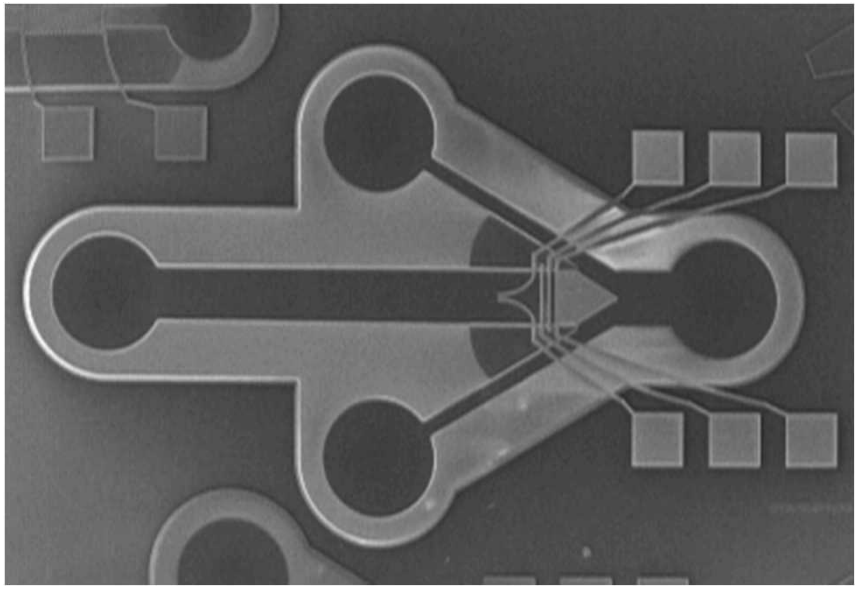
Fluidic channel fabrication:

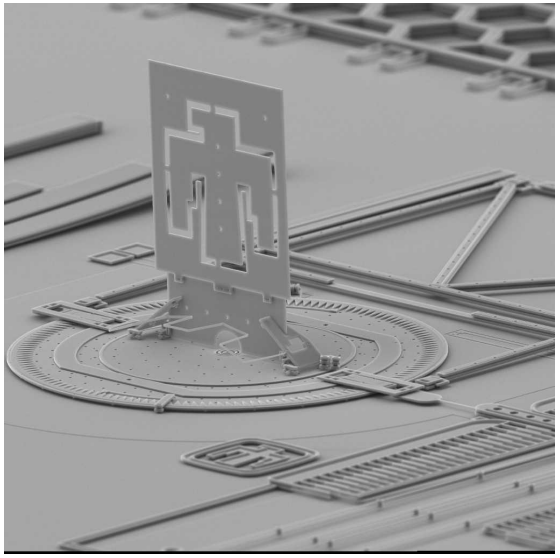


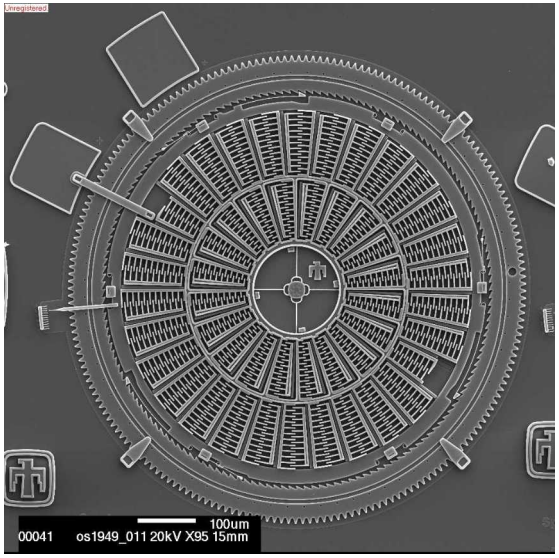


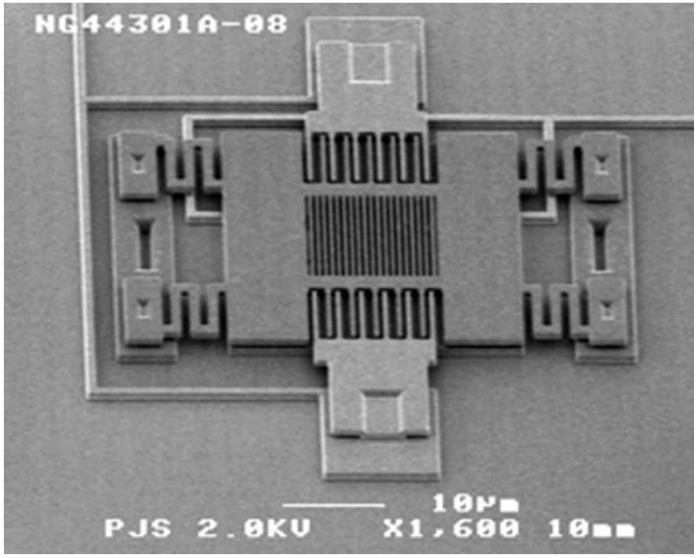
Surface Micromachining Technology





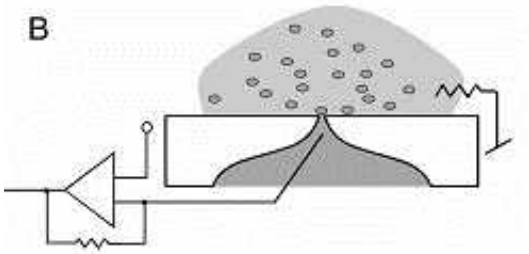




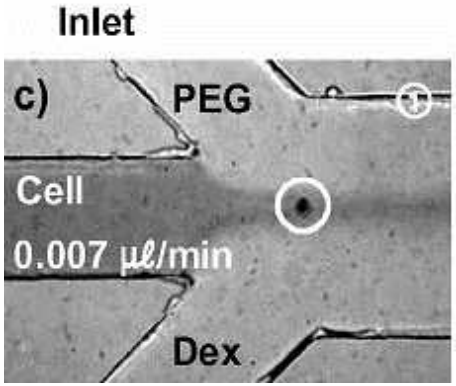


Novel Experimental Biology with Microsystem Technologies

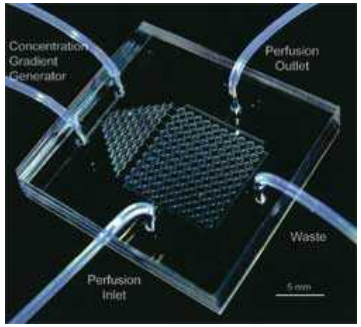
- Single cell manipulation and measurement – with potential for automation
- Laminar flow (fluidic isolation, precise delivery of reagents)
- Built in controls (arrays of “experiment units” for biological or technical replicates)
- Environmental controls are improved



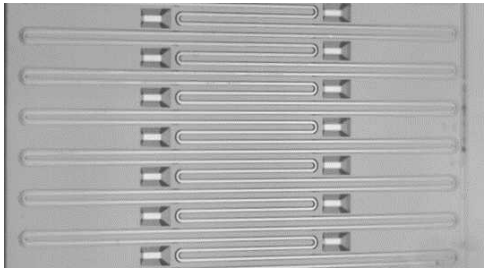
nanion.de



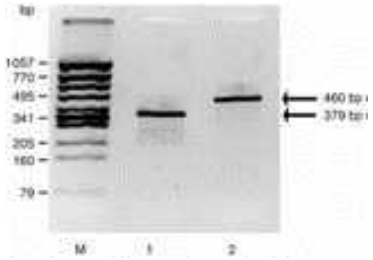
Nam et al. Biomed Microdev 2005



L. Lee, 2005



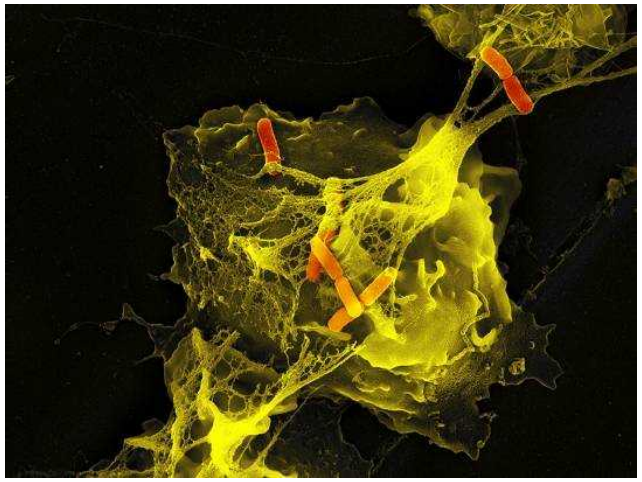
Schneegab et al. Lab Chip 2001



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**



MISL: A Multi-Disciplinary Team

PI: Anup Singh

PM: Glenn Kubiak

Biology Core Team:

Tony Martino – Coordinator
Steve Branda
Cathy Branda
Bryan Carson
Todd Lane
Jens Poschet
Roberto Rebeil
Bryce Ricken
Meiye Wu
Zhaoduo Zhang

UTMB
Allan Brasier

UCSF
William Seaman

Platform and Detection Systems Core Team:

Anup Singh – Coordinator
Jim Brennan
Susan Brozik
David Haaland
Amy Herr
Conrad James
Howland Jones
Ron Manginell
Matt Moorman
Kamlesh Patel
Thomas Perroud
Ron Renzi
Mike Sinclair
Nimisha Srivastava
Dan Throckmorton

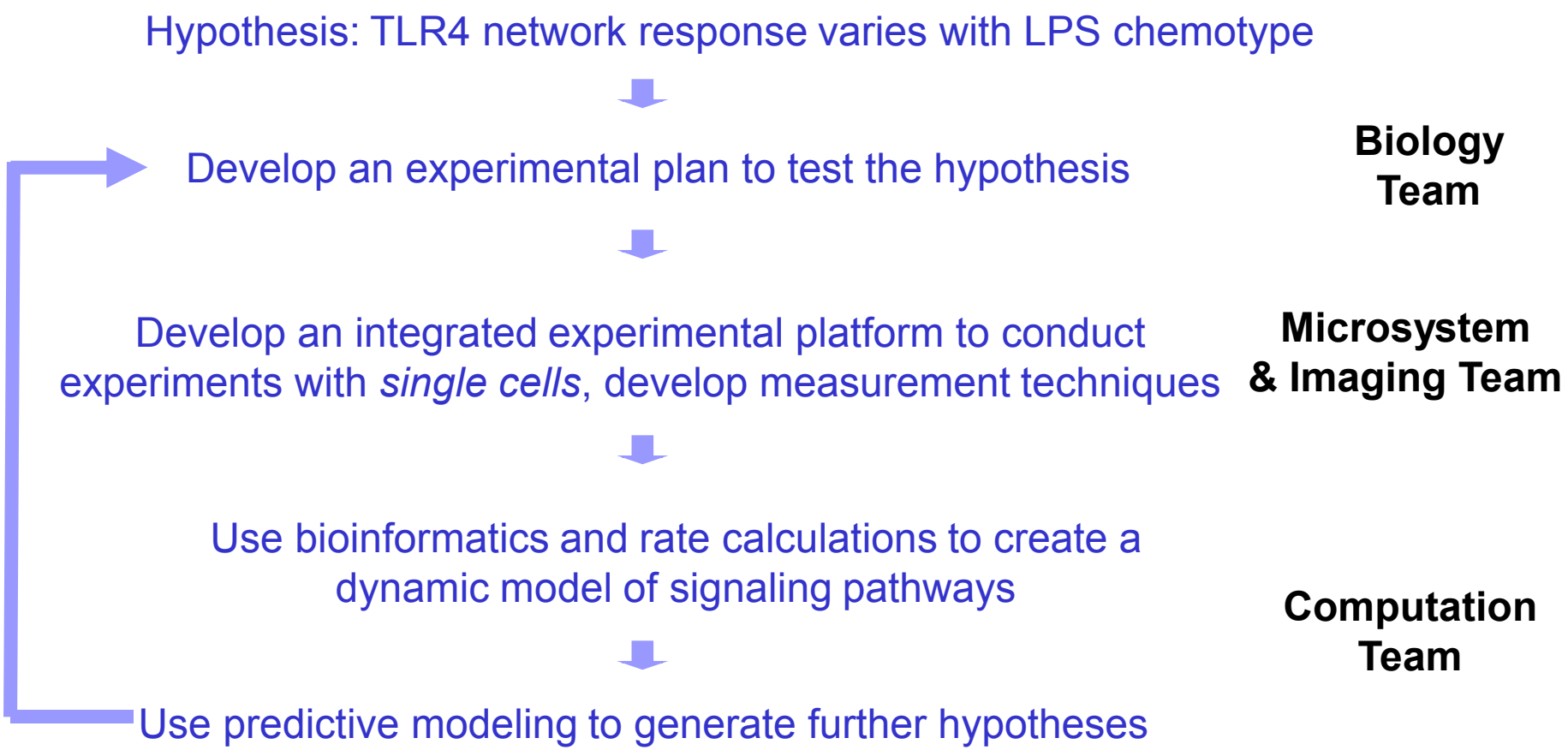
East Carolina University
Paul Gemperline

Computational Biology Core Team:

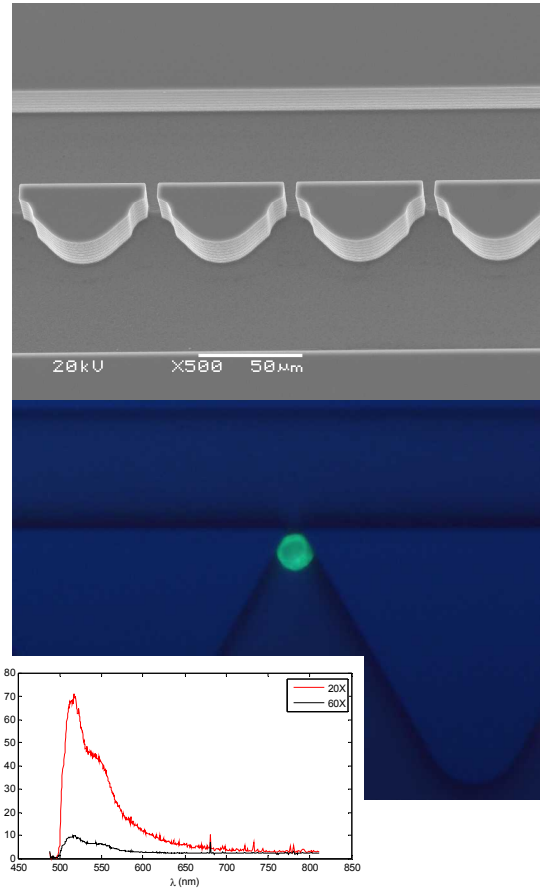
Jean-Loup Faulon – Coordinator
Jaewook Joo
Shawn Martin
Steve Plimpton
Susan Rempe
Ken Sale

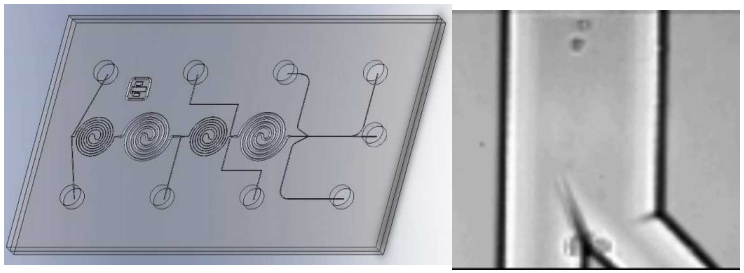
Funding: Sandia LDRD Program

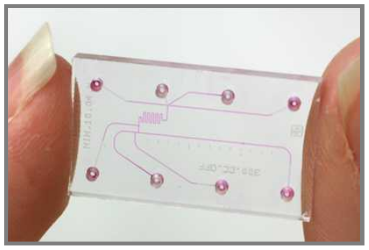
Overview of the Approach

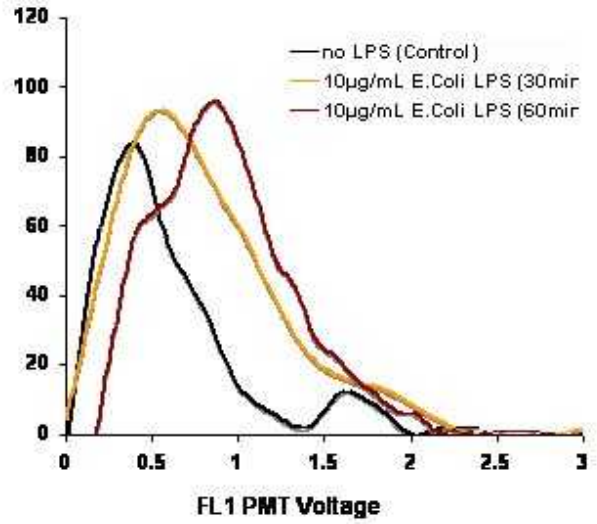


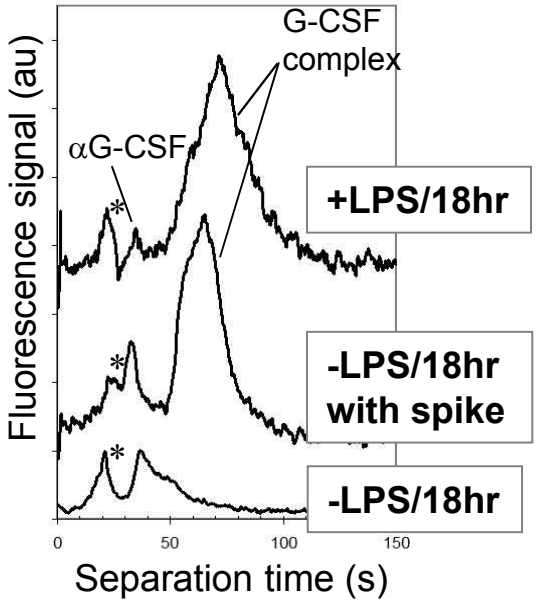
MISL Microsystem Platform Components











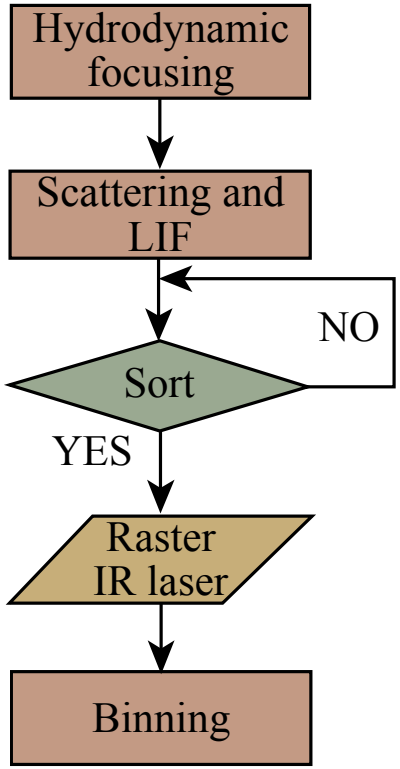
Single cell capture and imaging:
- time-course
- dynamics
- protein-protein interactions

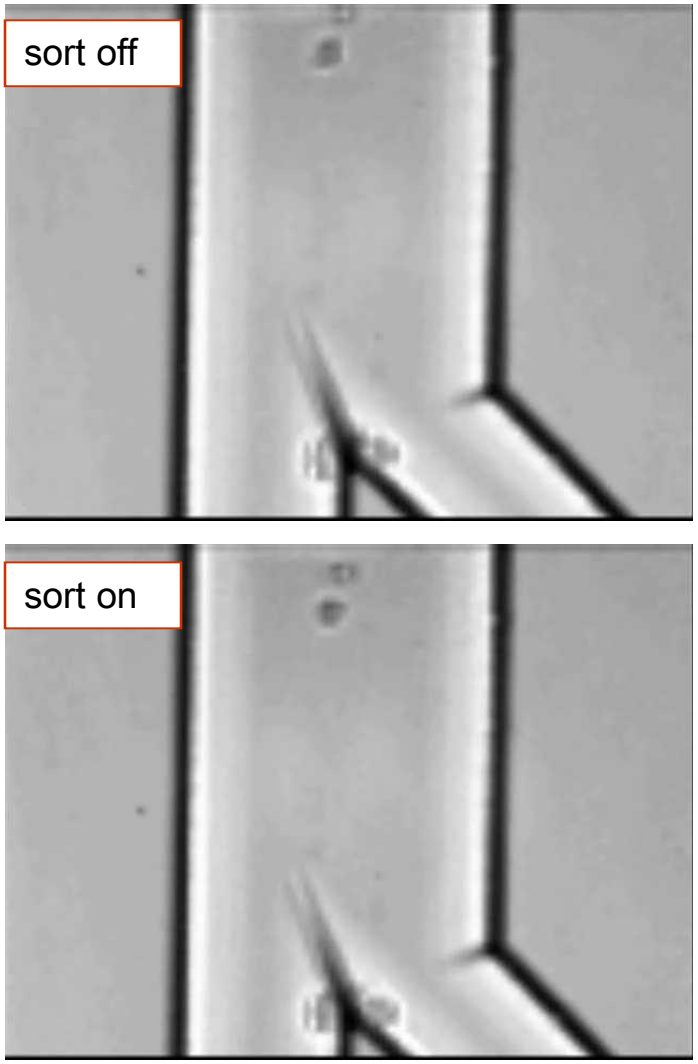
Population measurements
- sample preparation
- higher throughput cytometry
- cell sorting

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



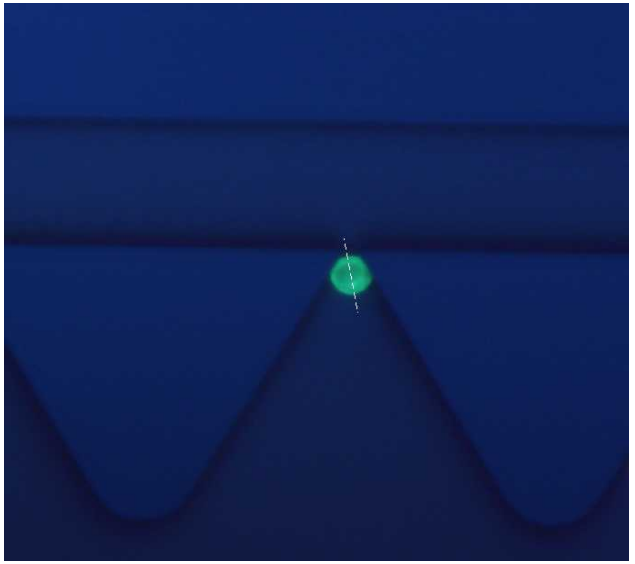


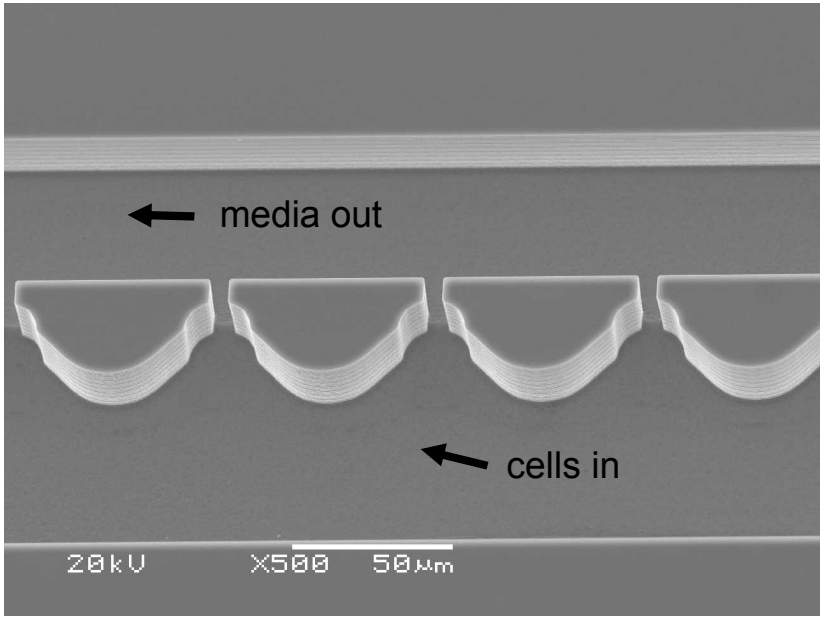
Frame rate slowed 13X

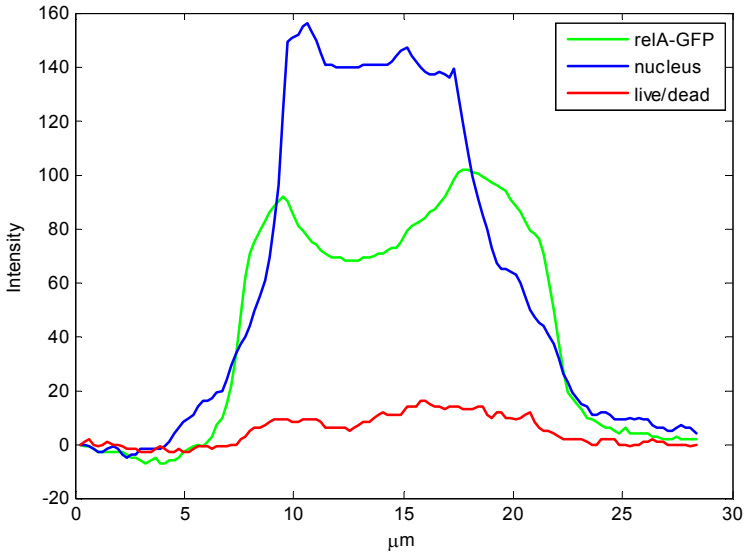
T. Perroud, K. Patel

Single Cell Capture

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

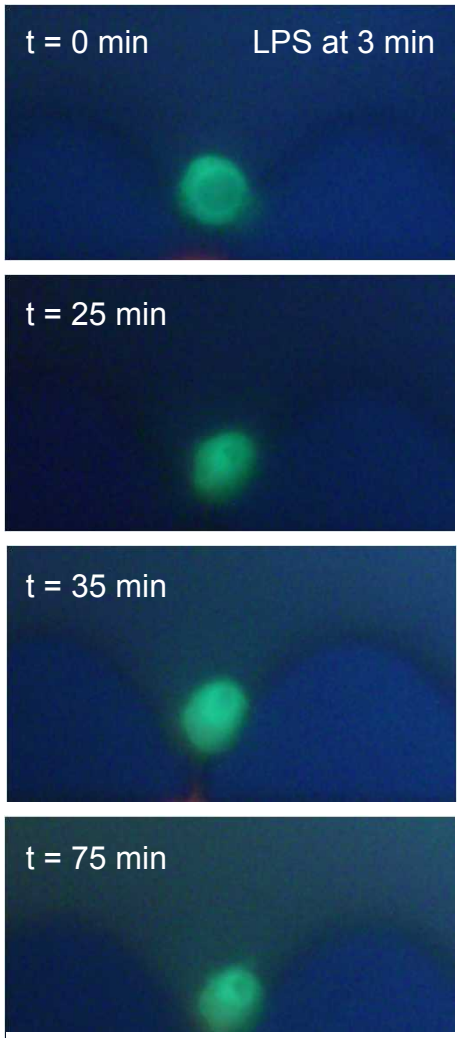


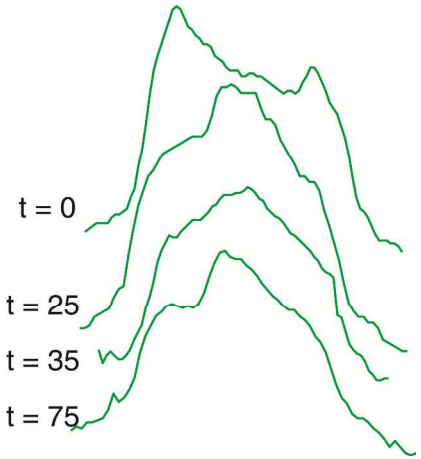




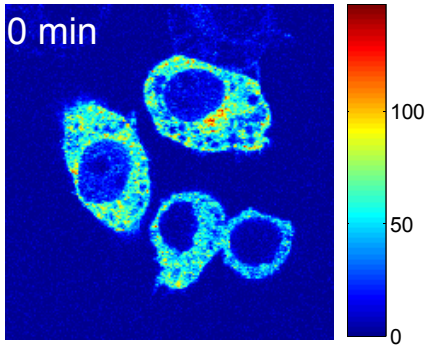
Viability: max length is 5 hrs, with 10/13 cells surviving

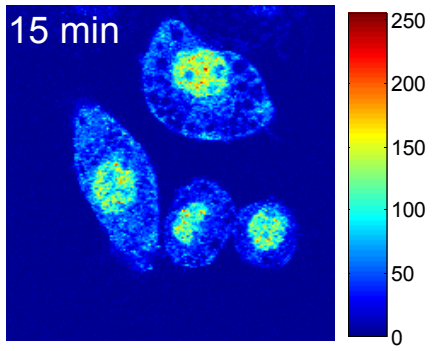
LPS Challenge Induced RelA Translocation

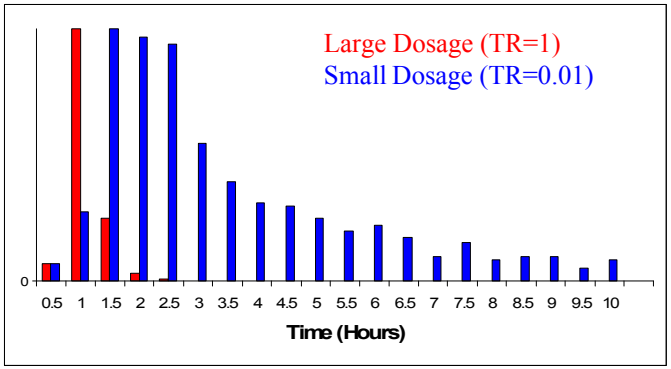




- Cell response is similar to that in bulk measurements





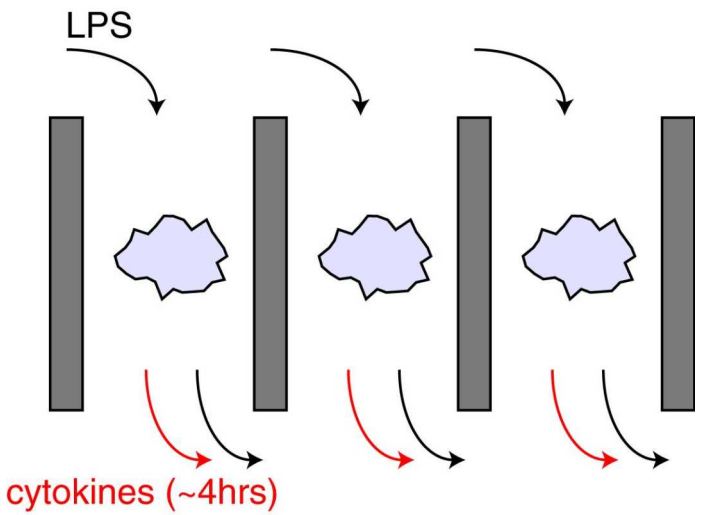


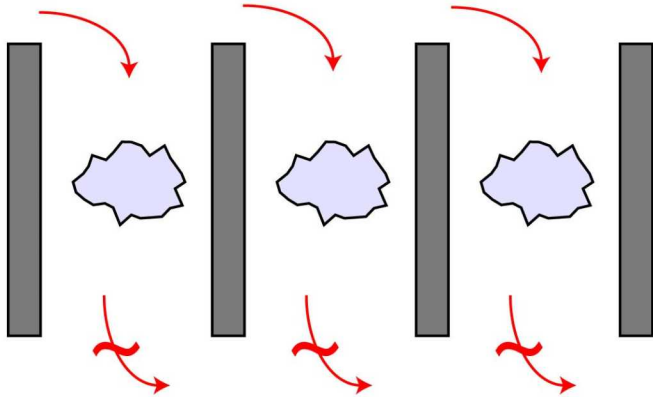
• 1 μM smooth E. Coli LPS injected at 3 min; translocation seen after 22 minutes

Produce the dose-response curve of challenged cells in the SCA – Computational Core

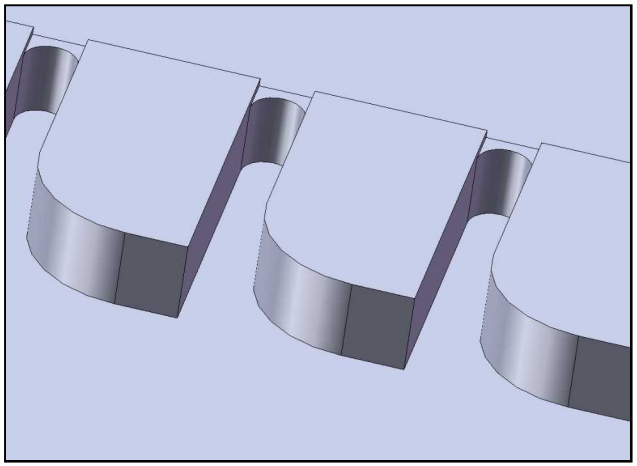
Single Cell Isolation

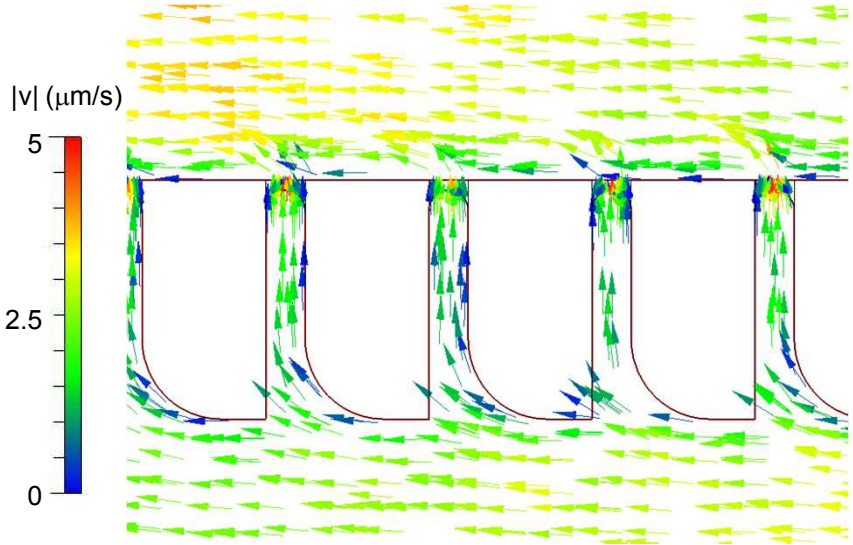
•Goal: primary vs. secondary immune response:





•Method: fluidically isolated single cells





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

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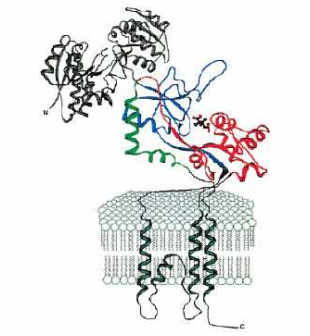
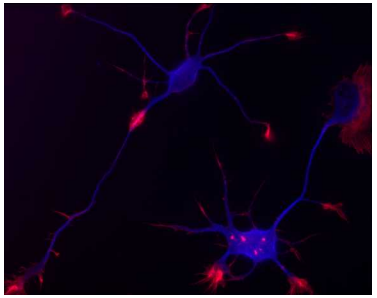
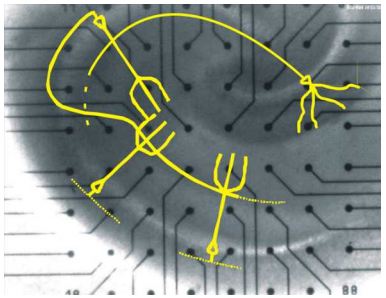
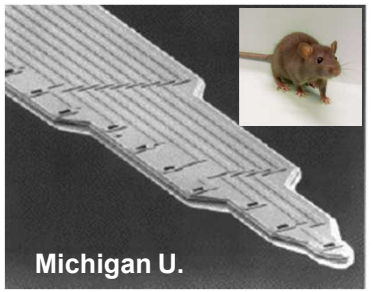
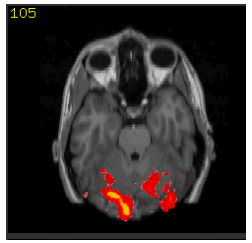
PM- Katherine Andrews

Manager, Computational Systems Biology 8333

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.)



Project cognition

genes/proteins → subcellular compartments → cells/ cell networks → tissue networks → animal

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 **sub-cellular** levels for long terms (>1 year)

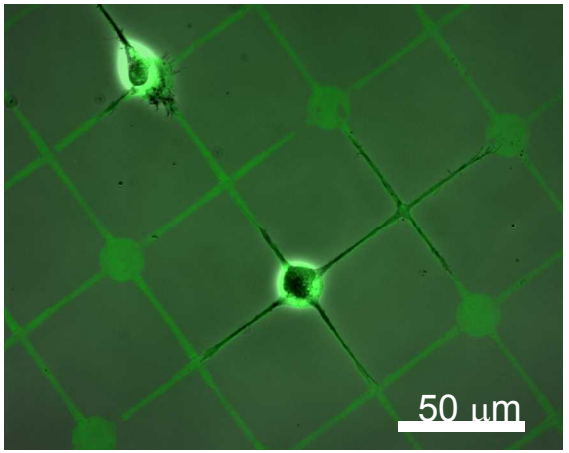
-Generate falsifiable hypotheses about network structure and function

-First step towards modifying network structure of tissue slices

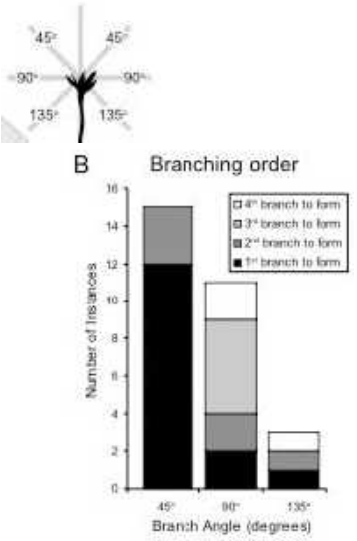
Engineered Cell Networks using Microfabrication Techniques

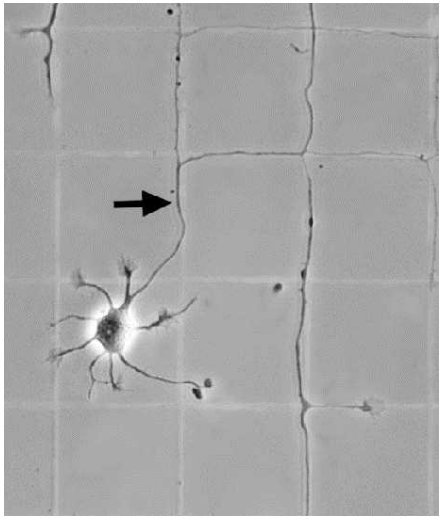
Fully engineered networks of neural cells require controlled:

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

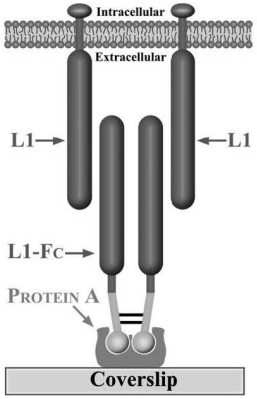


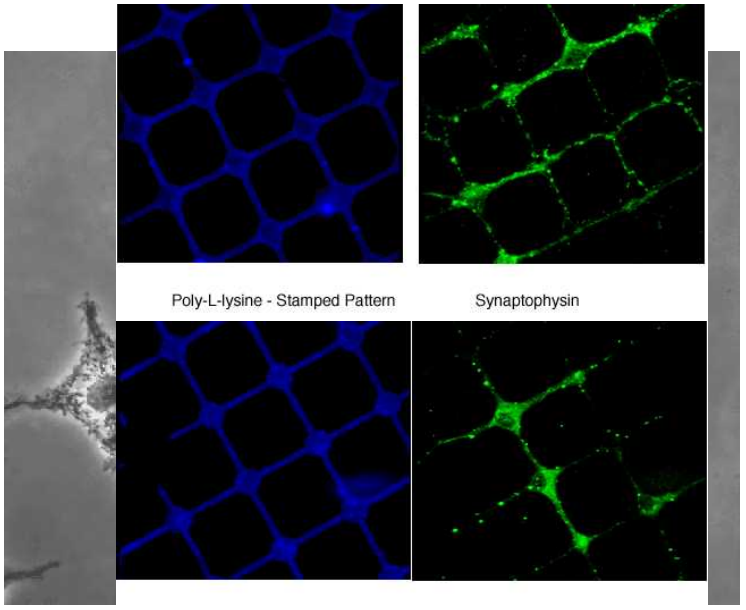
Withers et al., J Neurobio 2006





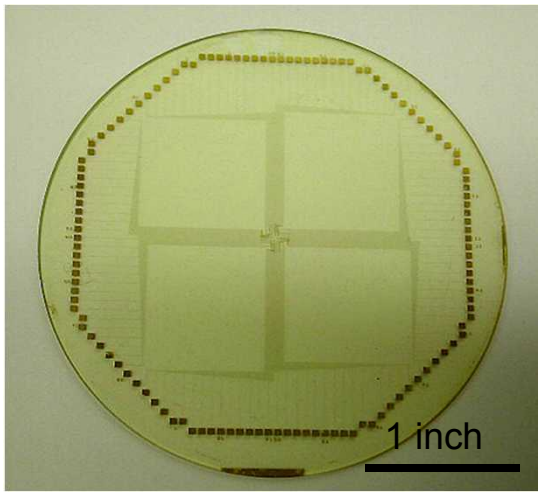
Oliva et al., Neurochem Res 2003

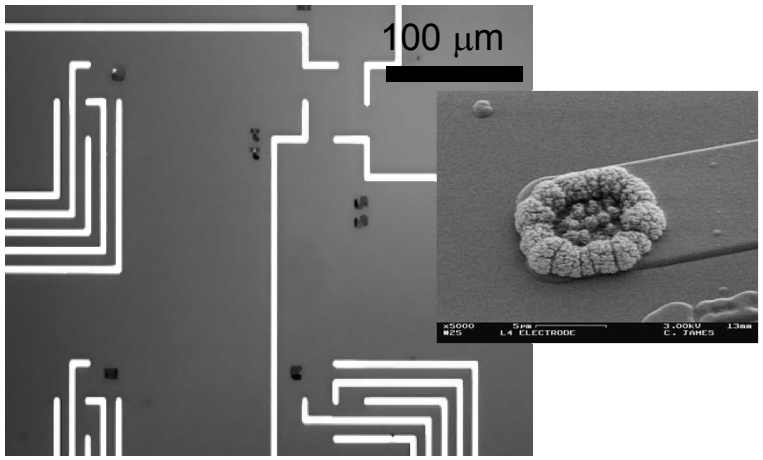


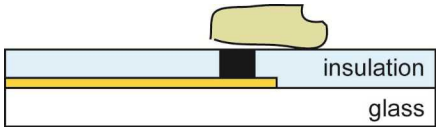


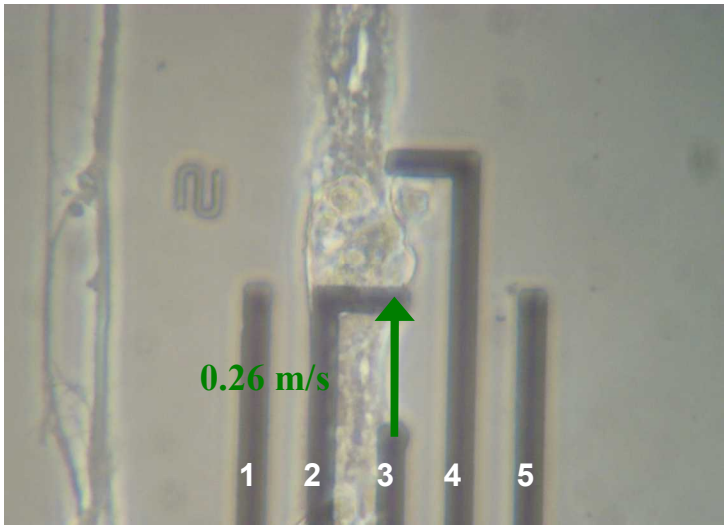
Network Activity Detection with Microfabricated Electrode Arrays

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

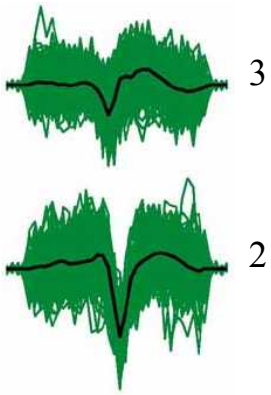


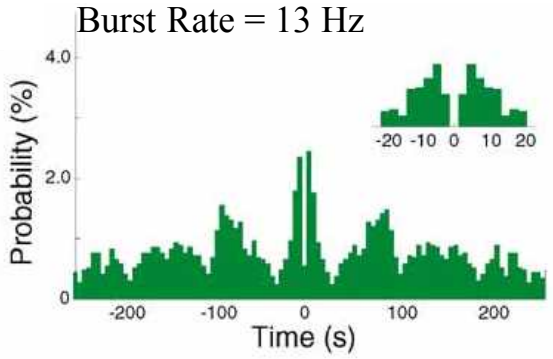






Engineered network on an electrode array



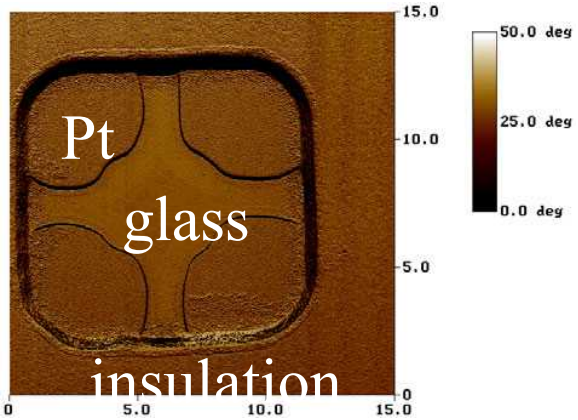


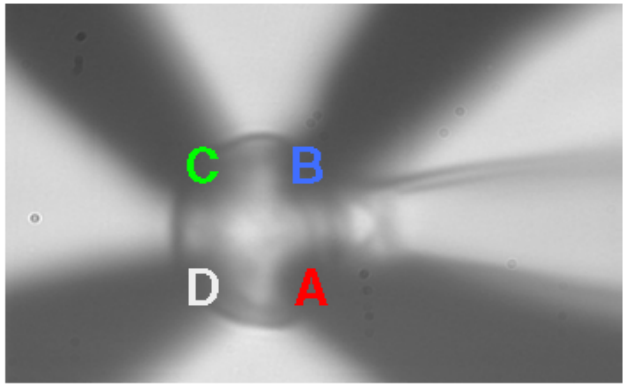
C.D. James, et al., IEEE Trans. Biomed. Eng. 2000, 2004

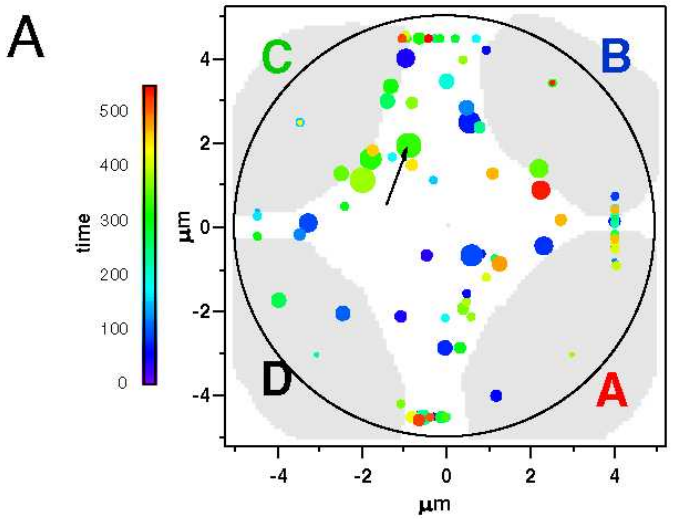
Electrochemical Detector Array for Neurotransmitters

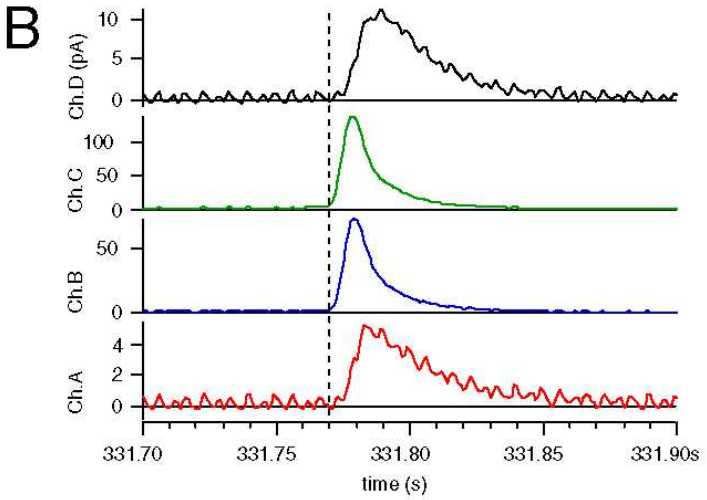
Electrochemical detector array for amperometry studies:

- detect exocytosis of adrenaline/noradrenaline









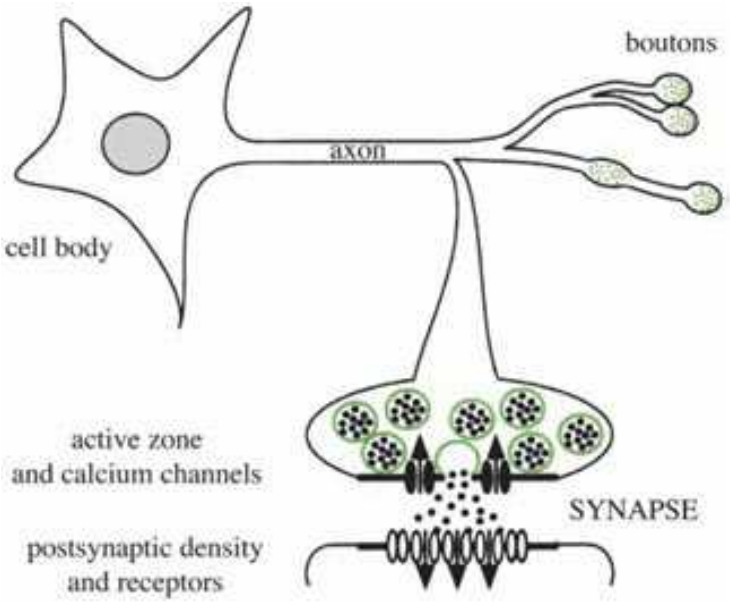
Dias et al., 2002, Nanotechnology, **13**, pg. 285.
M. Lindau, Cornell U.

Advanced Electrode Array Technology

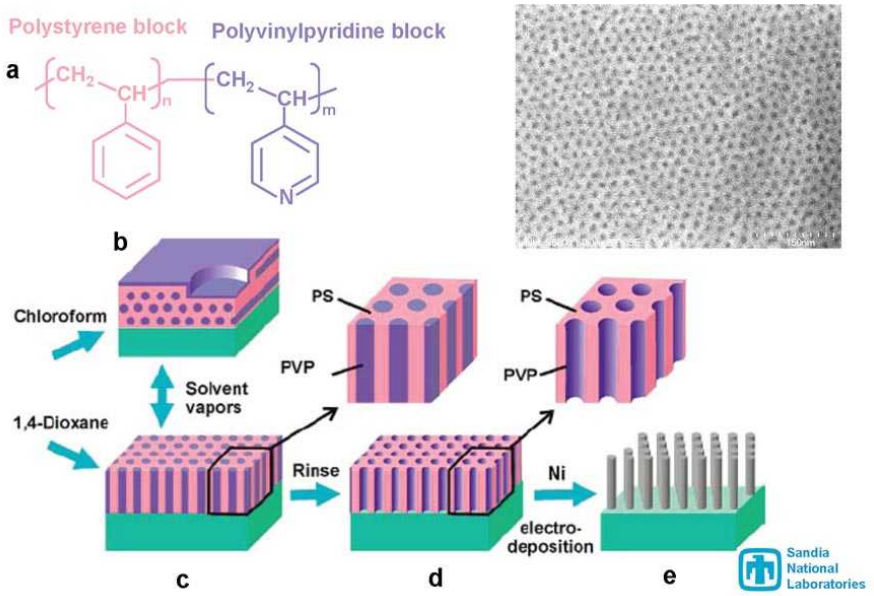
•Goal: high-density, sub-μm electrodes

-multiple recording sites within a cell network, and within a single cell

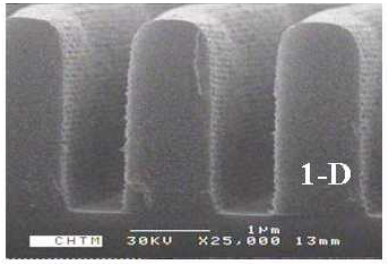
-**multiple recording sites within single synapses**

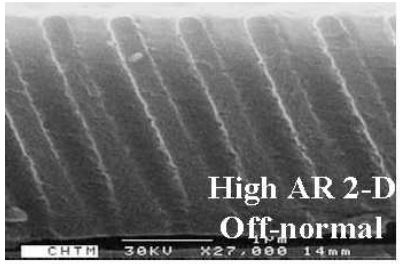


•Method: chemical synthesis and sub-μm lithography



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