

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

Conrad James, Ph.D.

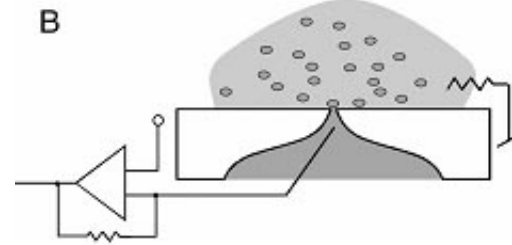
Advanced Sensor Technologies

Sandia National Laboratories

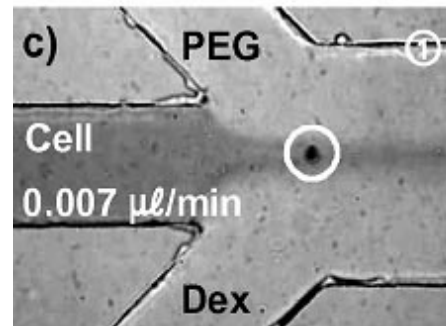
Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

# Novel Experimental Biology with Microsystem Technologies

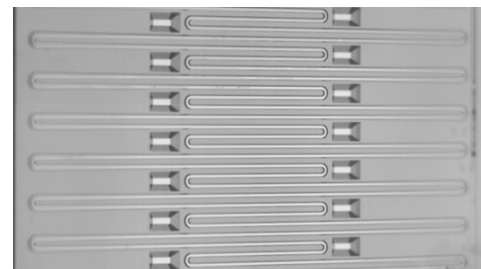
- Single cell manipulation and measurement – with potential for automation
- Laminar flow (fluidic isolation, precise delivery of reagents)
- Built in controls (arrays of “experiment units” for biological or technical replicates)
- Environmental controls are improved



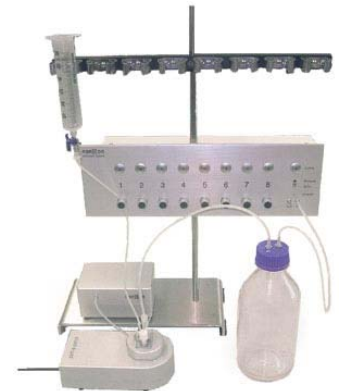
Inlet



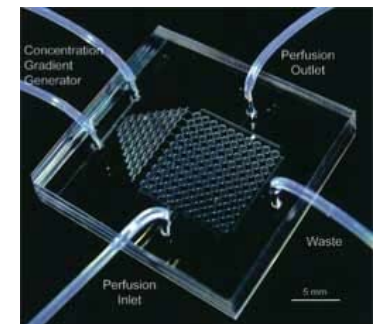
Nam et al. *Biomed Microdev* 2005



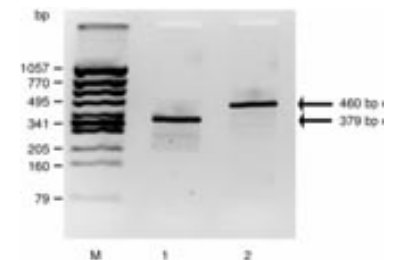
Schneegab et al. *Lab Chip* 2001



nanion.de



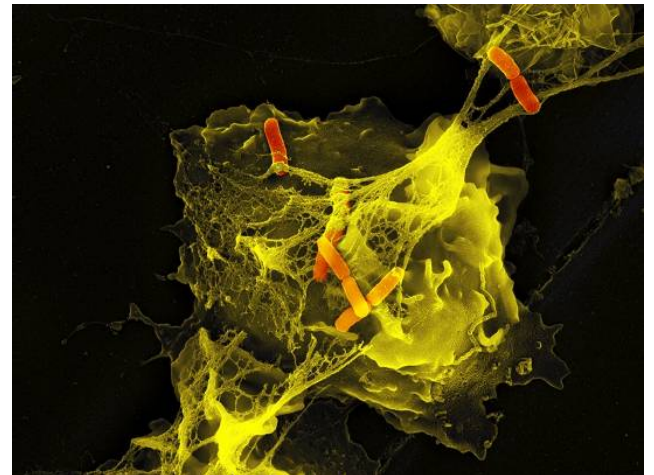
L. Lee, 2005



# 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



Develop an experimental plan to test the hypothesis

**Biology  
Team**



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

**Microsystem  
& Imaging Team**



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

**Computation  
Team**



Use predictive modeling to generate further hypotheses



# 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



## **Computational Biology Core Team:**

Jean-Loup Faulon – Coordinator

Jaewook Joo

Shawn Martin

Steve Plimpton

Susan Rempe

Ken Sale

## **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

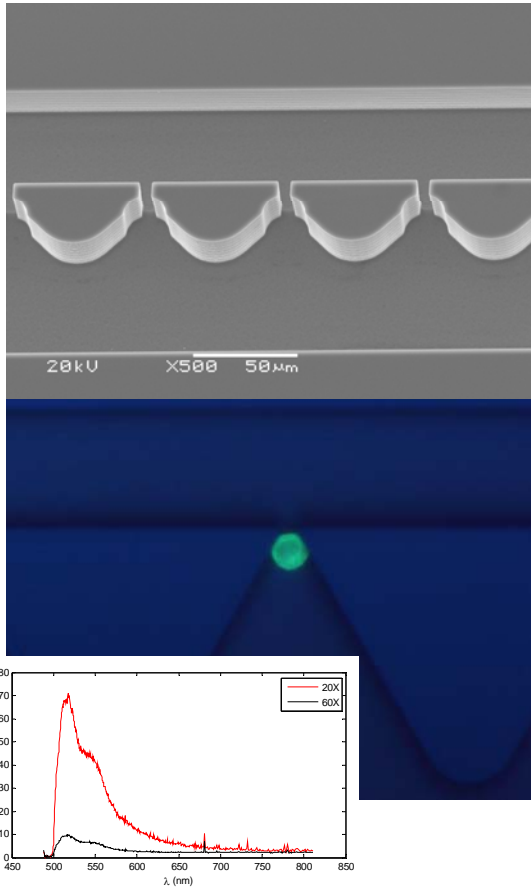
Nimisha Srivastava

Dan Throckmorton

East Carolina University  
Paul Gemperline

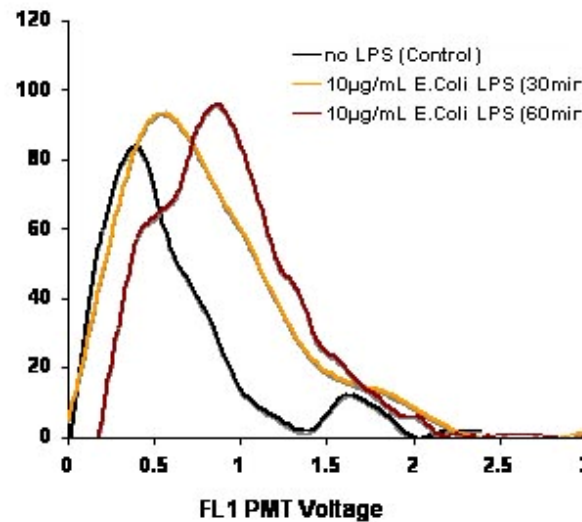
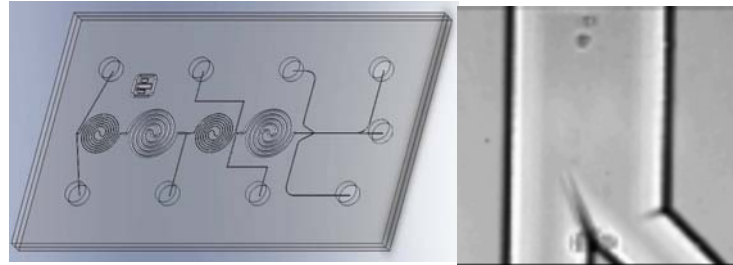
Funding: Sandia LDRD Program

# MISL Microsystem Platform Components



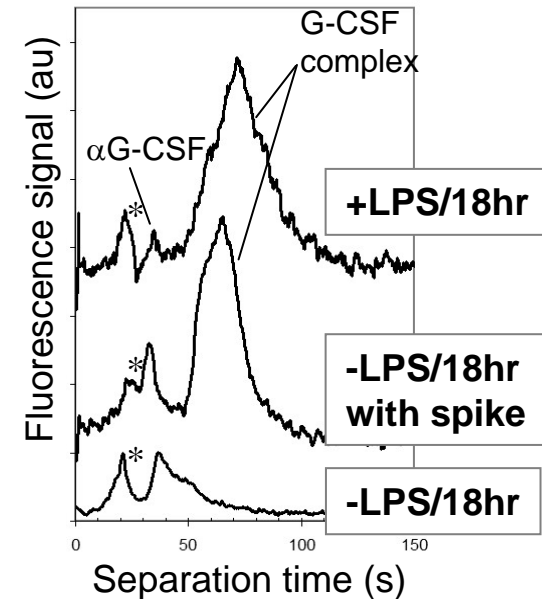
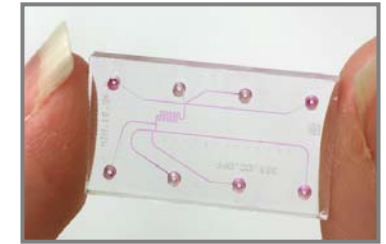
Single cell capture and imaging:

- time-course
- dynamics
- protein-protein interactions



Population measurements

- sample preparation
- higher throughput cytometry
- cell sorting



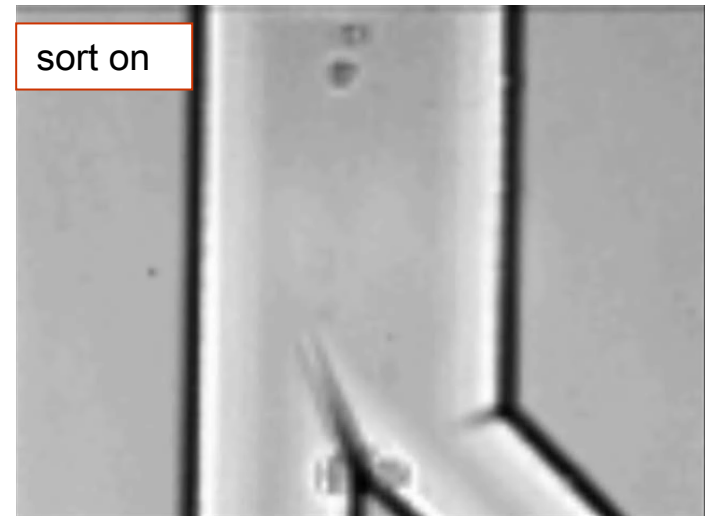
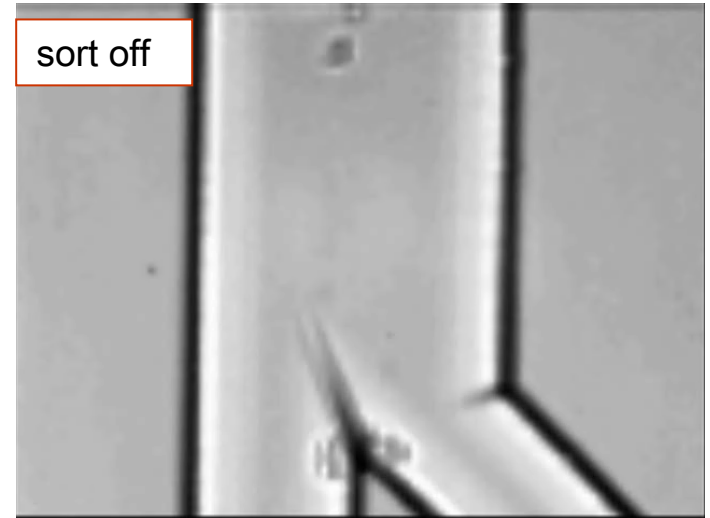
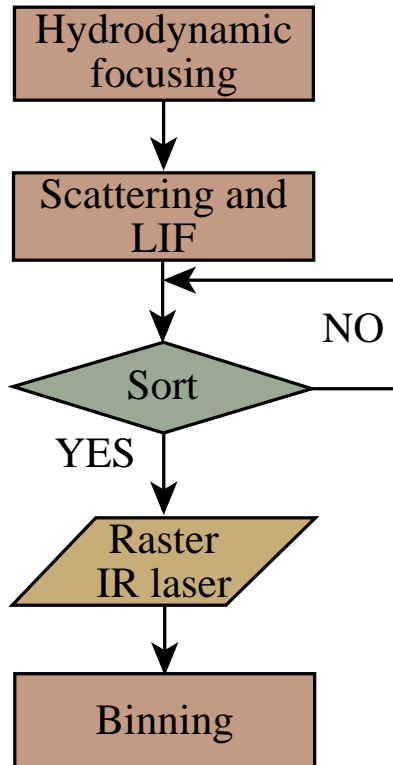
Electrokinetic immunoassays

- 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

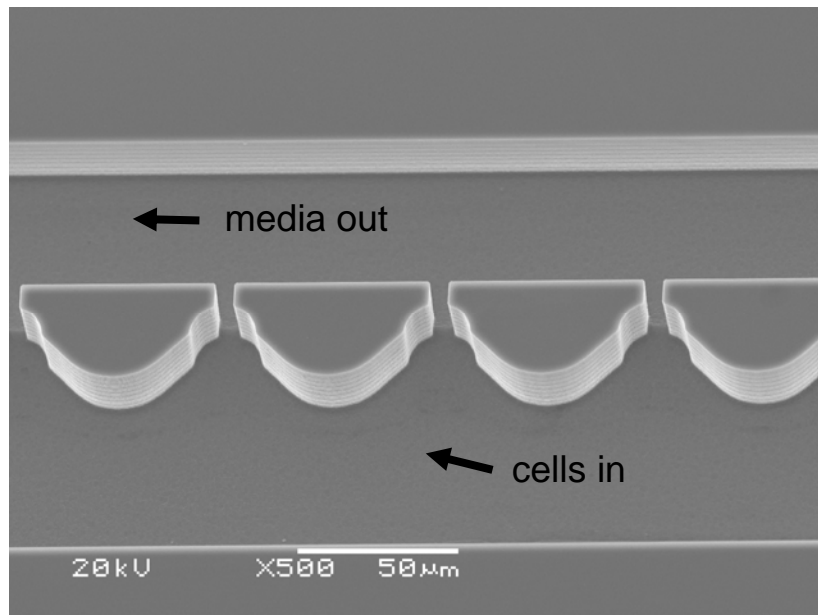


Frame rate slowed 13X

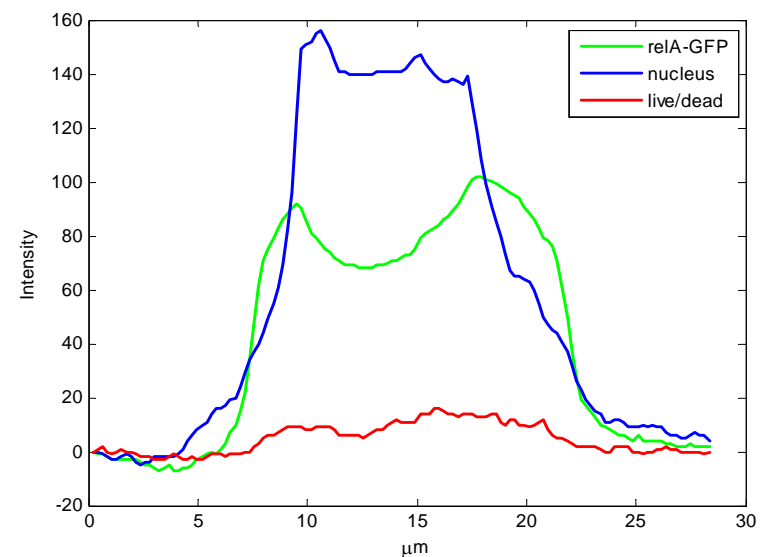
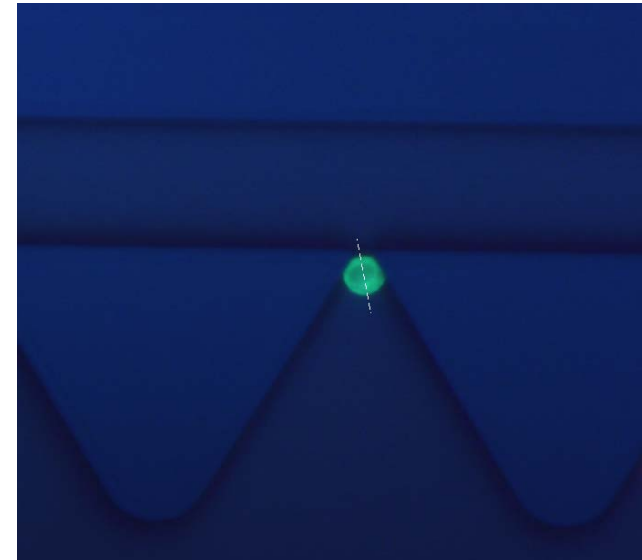
# Single Cell Capture

## Mechanical capture of single cells

- 100 traps in parallel with fluidic isolation
- cell assessment: reporter, nucleus, live/dead

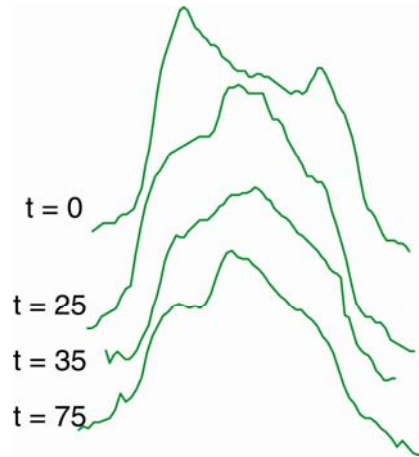
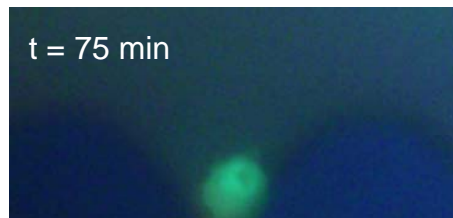
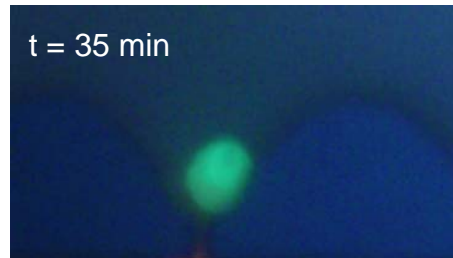
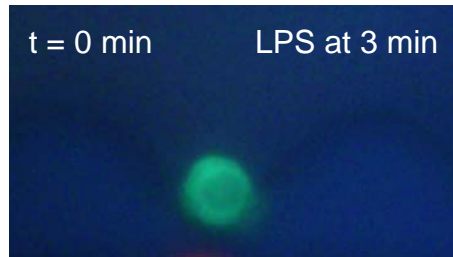


Viability: max length is 5 hrs,  
with 10/13 cells surviving

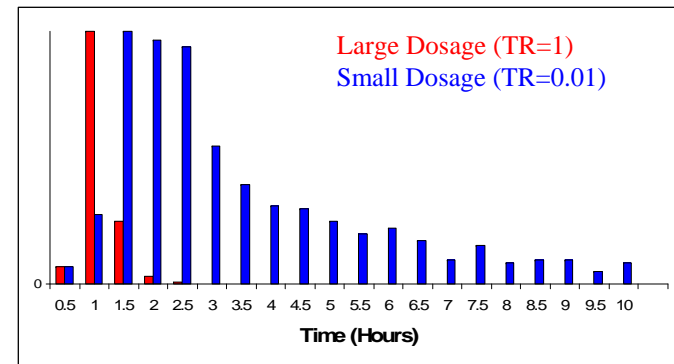
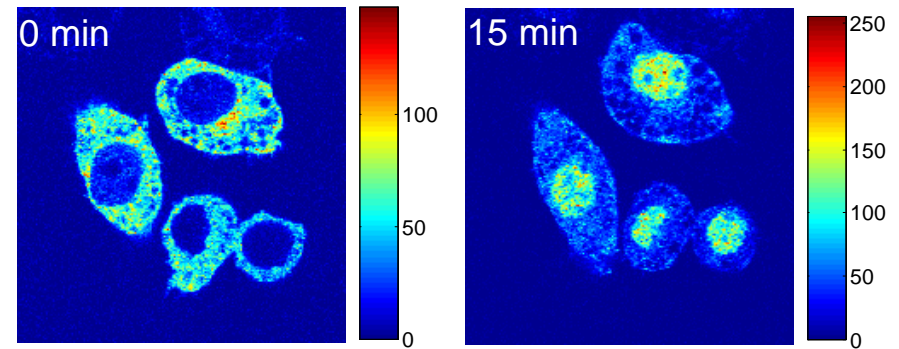




# LPS Challenge Induced RelA Translocation



- Cell response is similar to that in bulk measurements



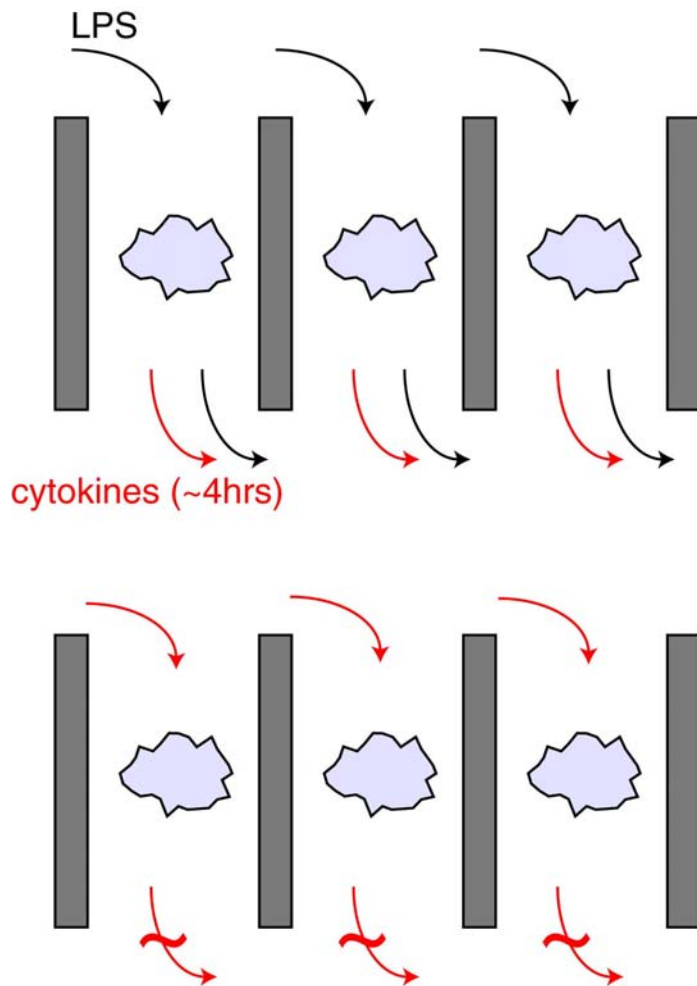
- 1  $\mu$ M smooth E. Coli LPS injected at 3 min; translocation seen after 22 minutes



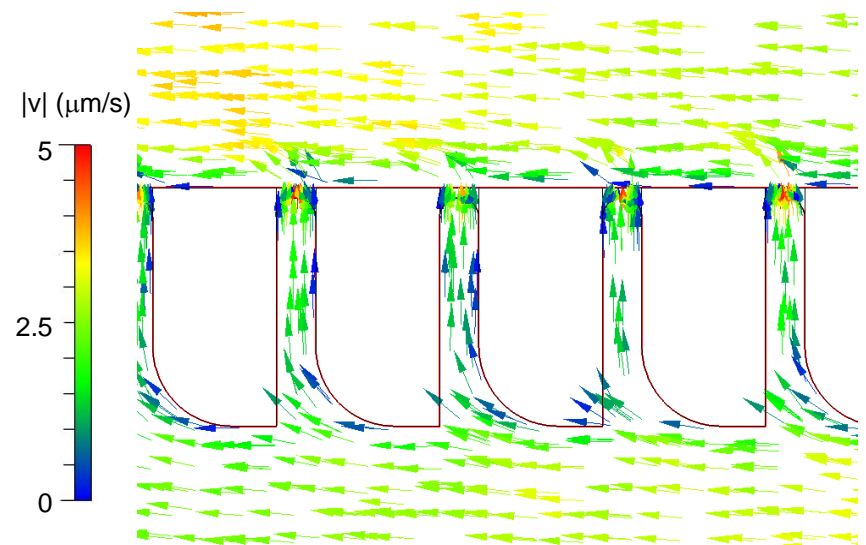
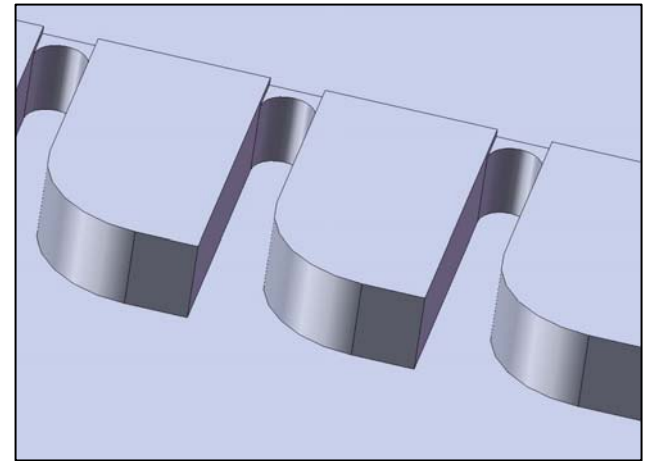
Produce the dose-response curve of challenged cells in the SCA – Computational Core

# 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

PI - Conrad James

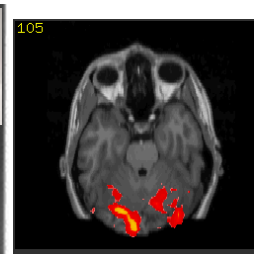
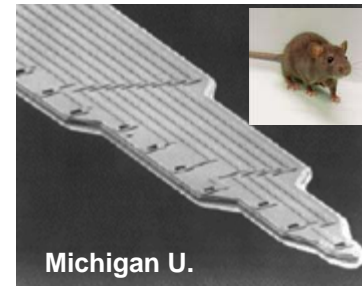
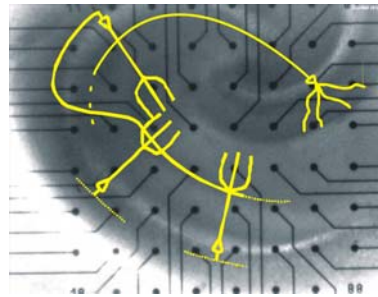
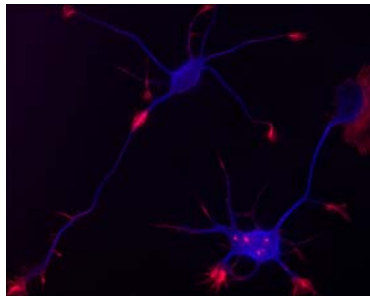
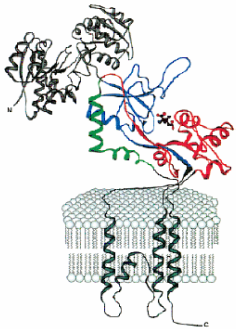
Principal Member of Technical Staff, Advanced Sensor Technologies 1744

PM- Katherine Andrews

Manager, Computational Systems Biology 8333

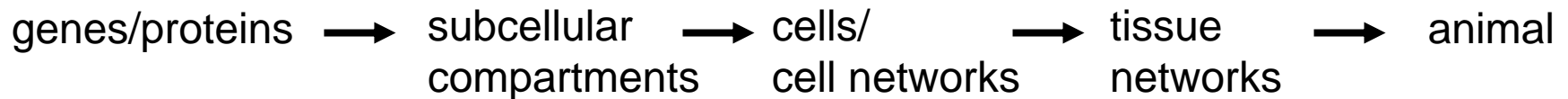
# Deciphering Neural Tissue Circuitry

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



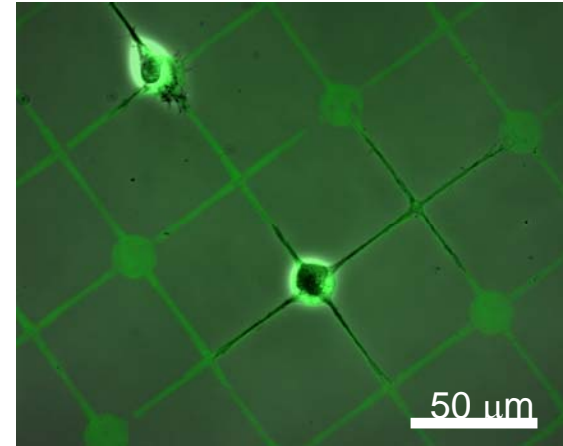
Reconstruct dissociated cells into networks using microfabrication techniques - engineered networks can be user-defined, **replicated**, and readily interrogated (optically and electrically) at **single cell** and **sub-cellular** levels for long terms (>1 year)

- Generate falsifiable hypotheses about network structure and function
- First step towards modifying network structure of tissue slices

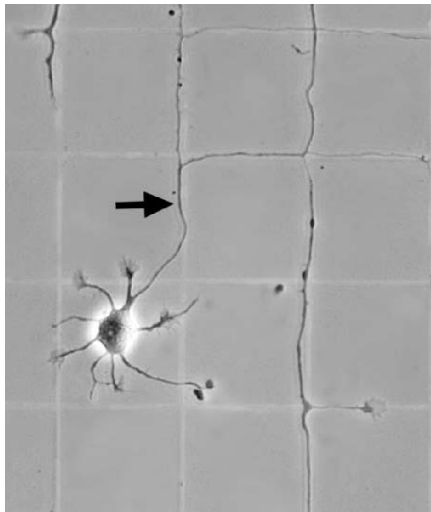
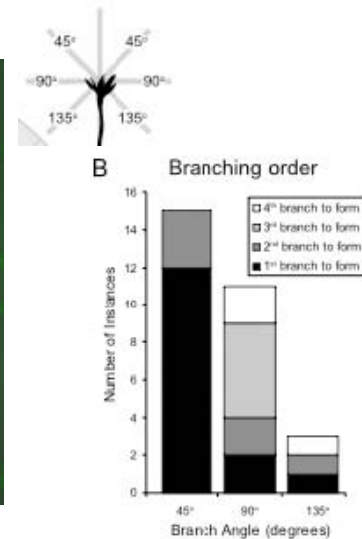
# Engineered Cell Networks using Microfabrication Techniques

Fully engineered networks of neural cells require controlled:

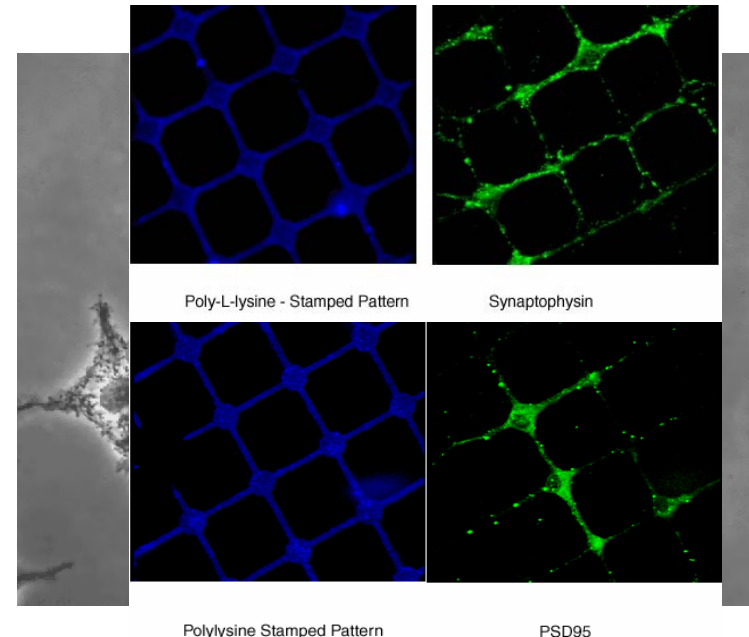
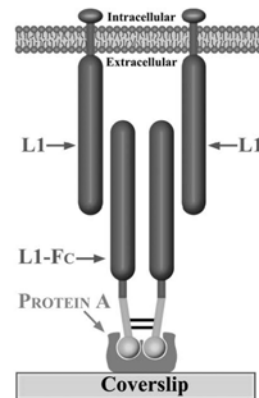
- cell body positioning
- outgrowth and branching of neurites (axons and dendrites)
- polarity of neurites (axons vs dendrites)**
- formation of synapses**



Withers et al., J Neurobio 2006

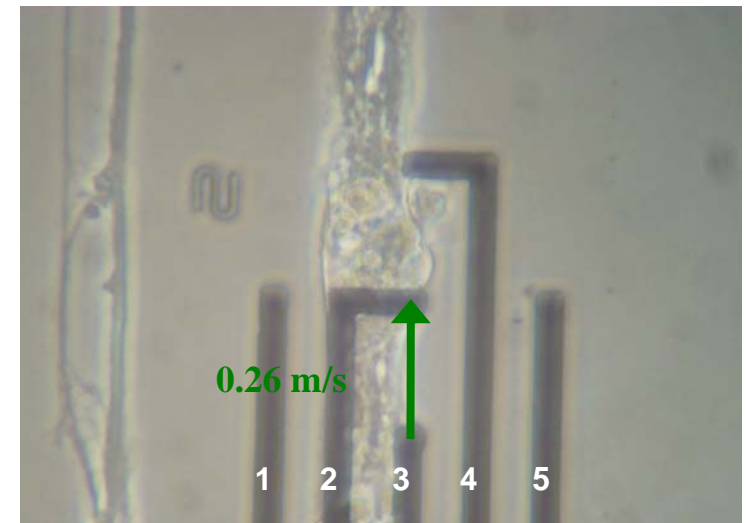
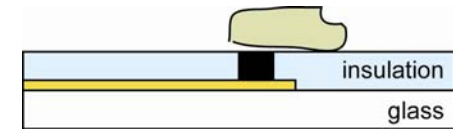
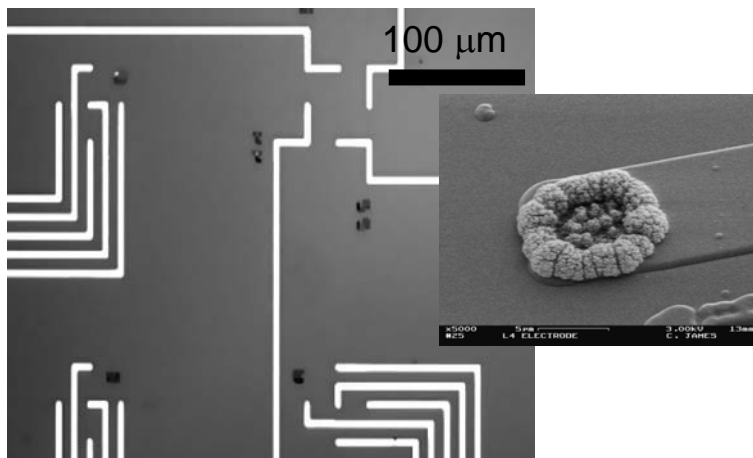
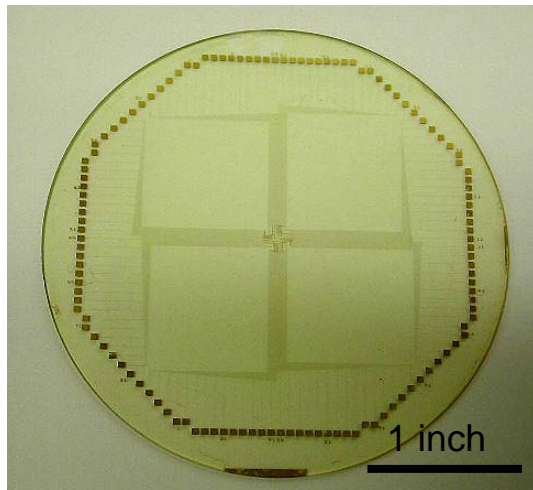


Oliva et al., Neurochem Res 2003

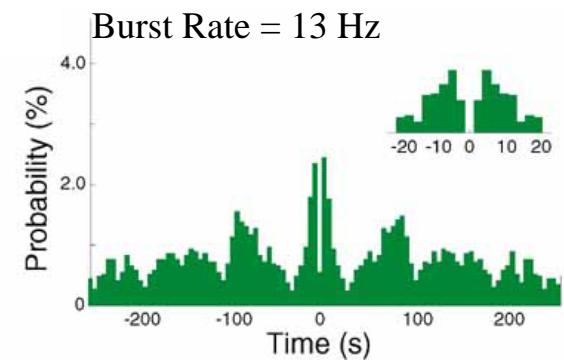
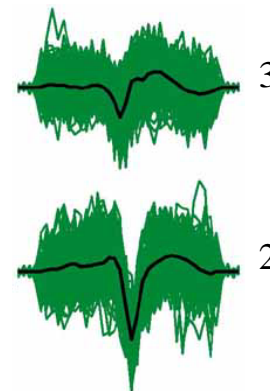


# Network Activity Detection with Microfabricated Electrode Arrays

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



Engineered network on an electrode array

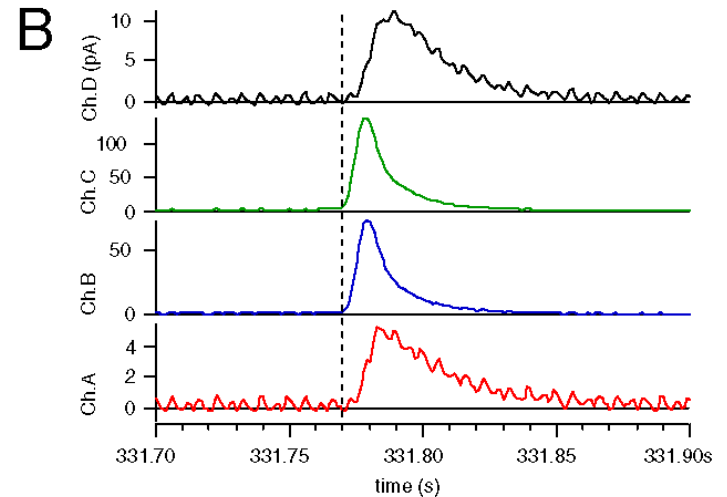
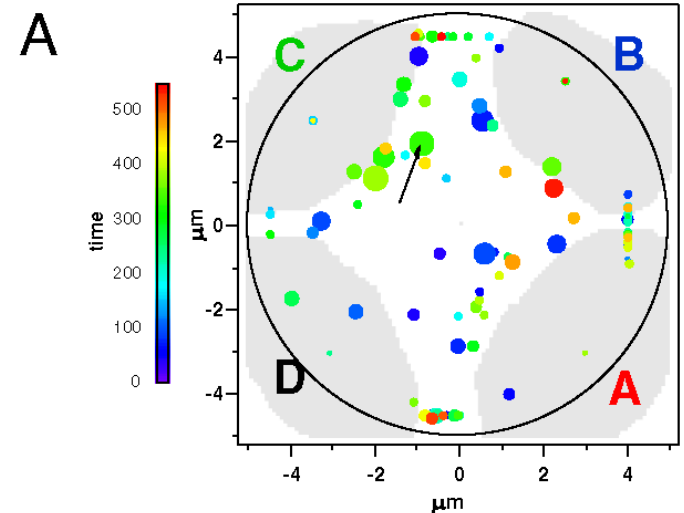
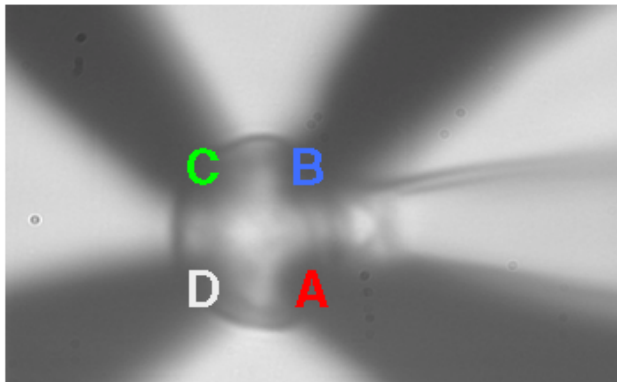
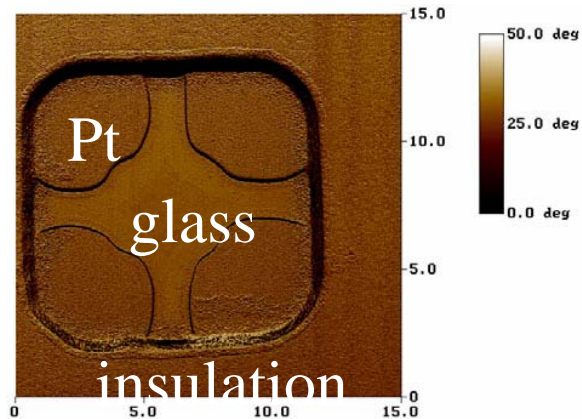




# Electrochemical Detector Array for Neurotransmitters

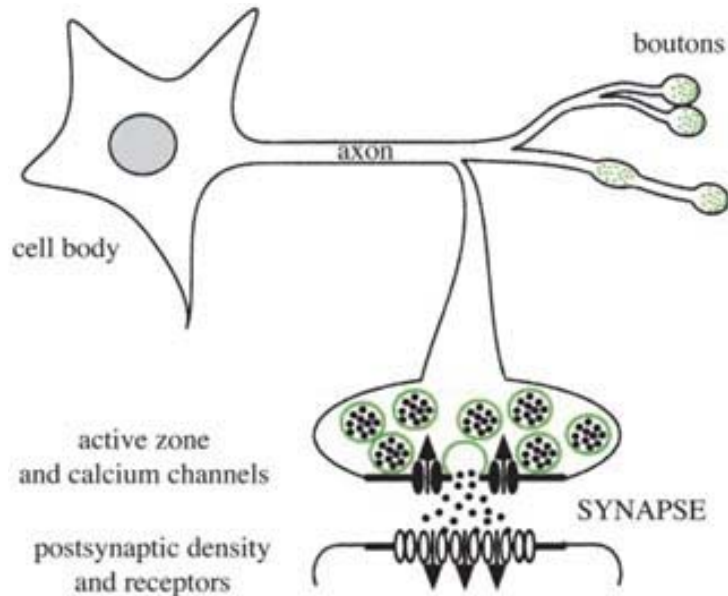
Electrochemical detector array  
for amperometry studies:

- detect exocytosis of adrenaline/noradrenaline

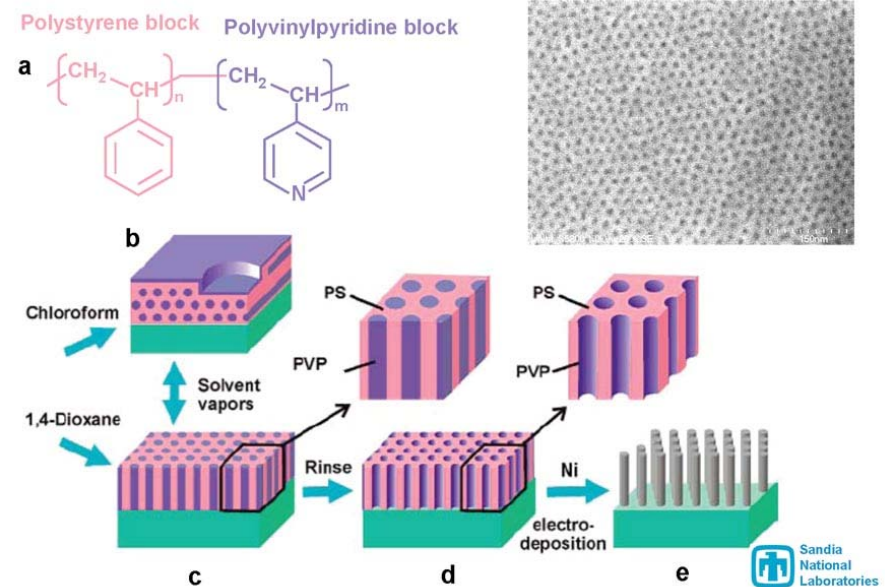


# Advanced Electrode Array Technology

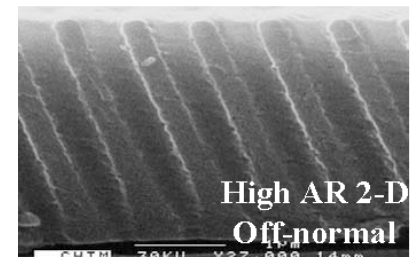
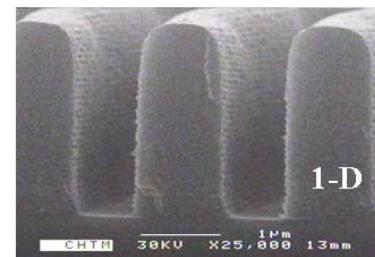
- Goal: high-density, sub- $\mu\text{m}$  electrodes
- multiple recording sites within a cell network, and within a single cell
- multiple recording sites within single synapses



- Method: chemical synthesis and sub- $\mu\text{m}$  lithography



Interferometric lithography:





# 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