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Real-time imaging of pathogenic immune response in single macrophages in a microfluidic device

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Advanced Sensor Technologies
Sandia National Laboratories

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MISL: A Multi-Disciplinary Team

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Funding: Sandia LDRD Program

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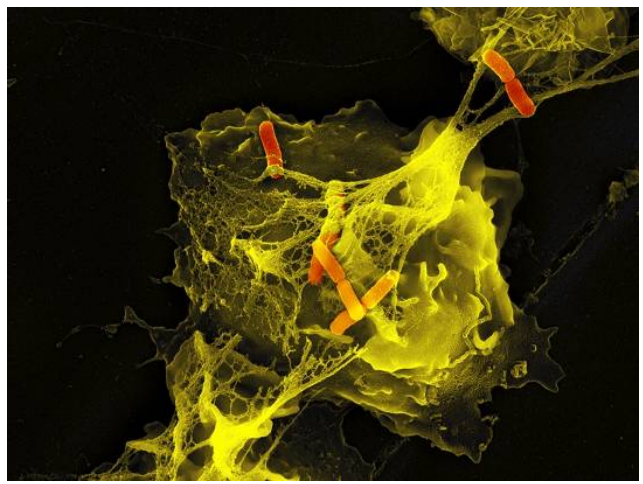
Microscale Immune Studies Laboratory (MISL) Grand Challenge

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**



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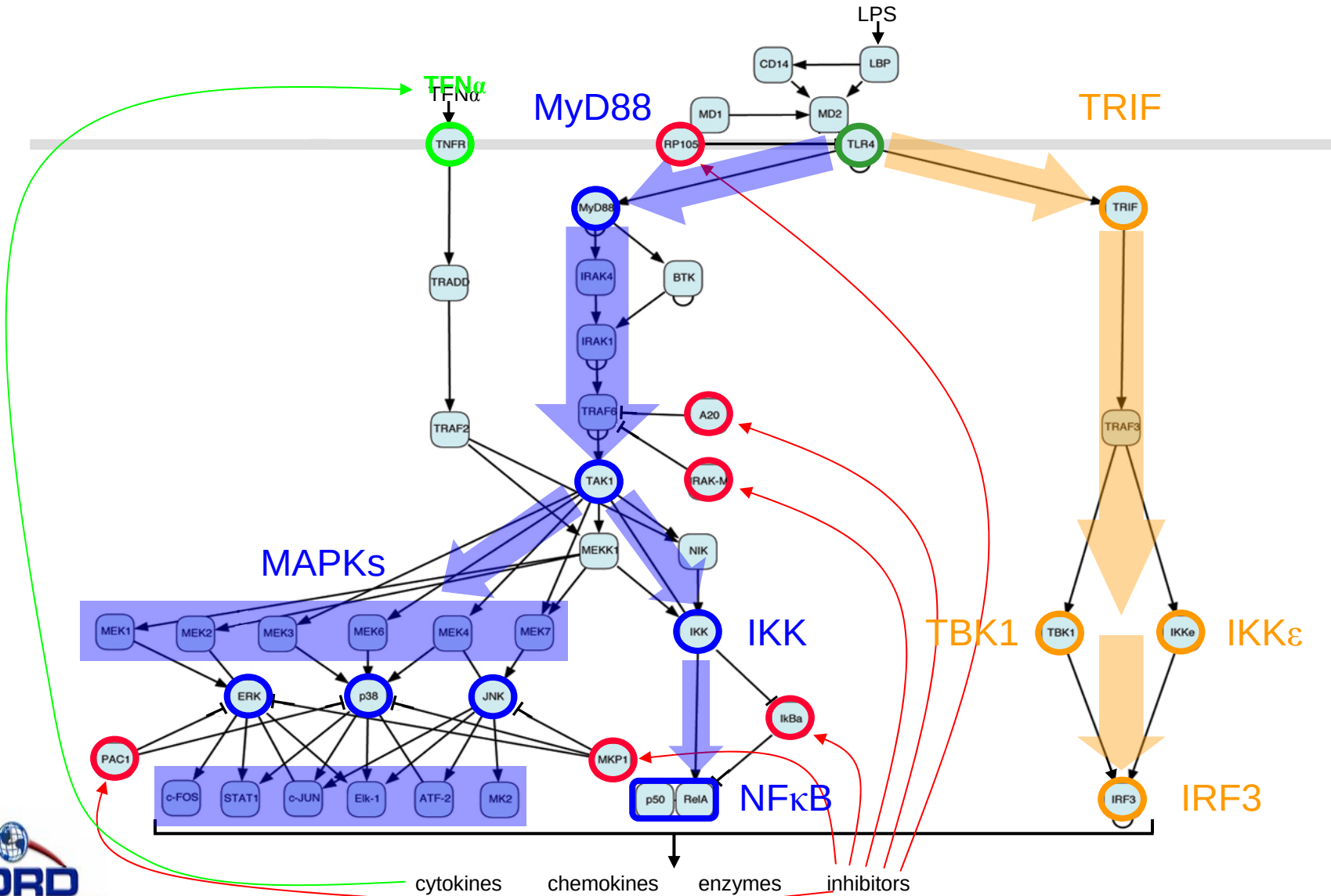
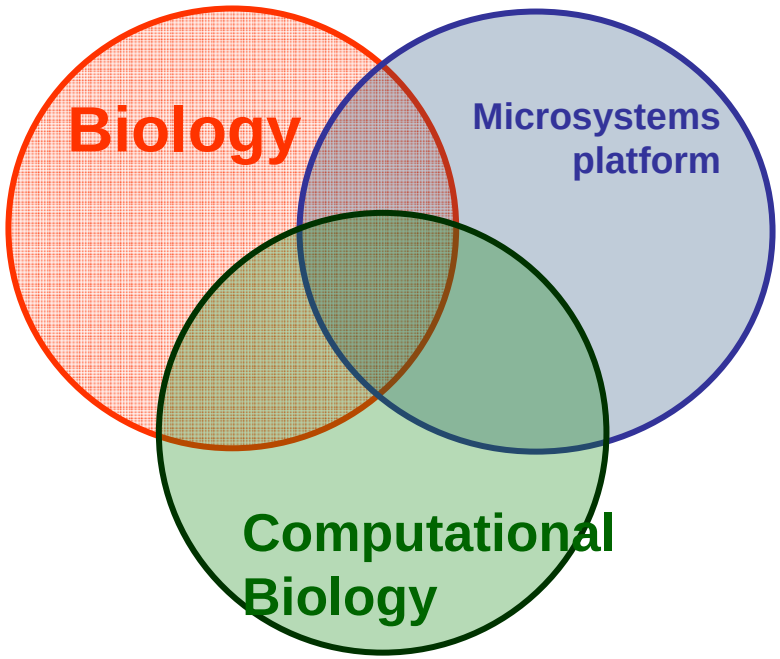
Global dynamics of the TLR4 signaling network

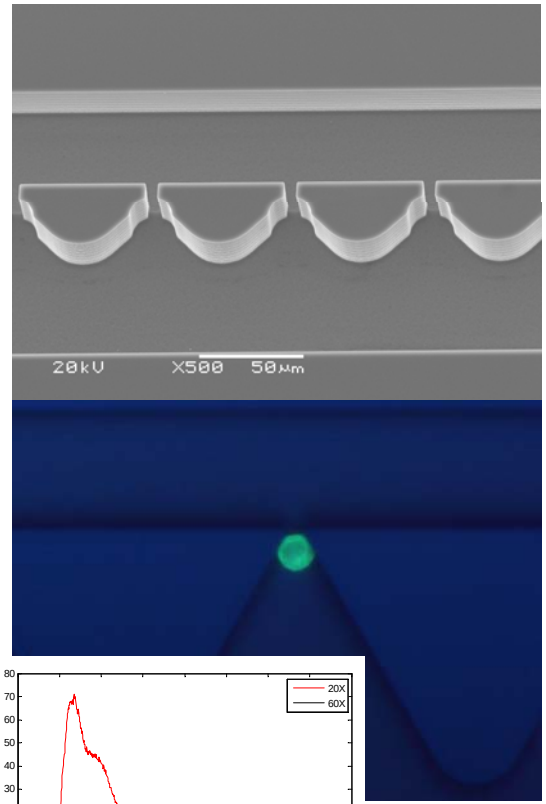


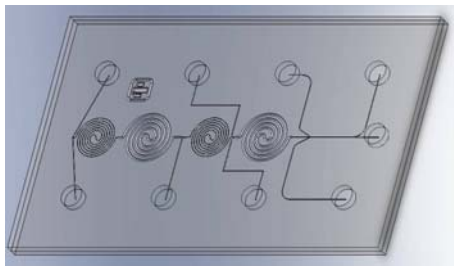
Integrated Goal

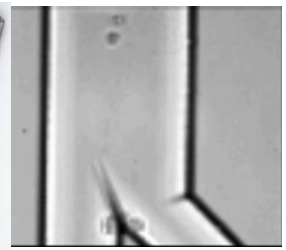
Test and validate the microsystems platform to measure the desired variables in macrophages and HeLa cells challenged with LPS, cytokines or pathogens and use the data to refine TLR network models

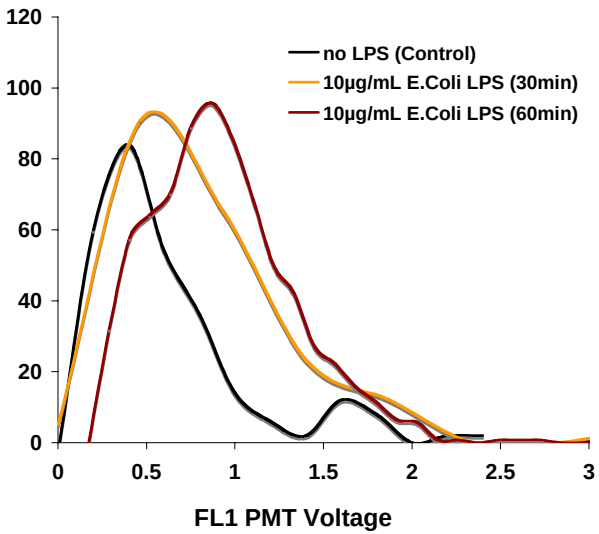


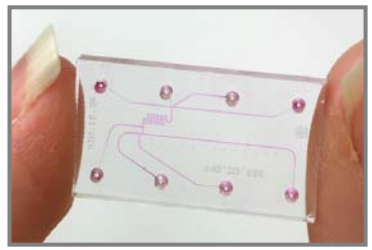
MISL Microsystem Platform Components

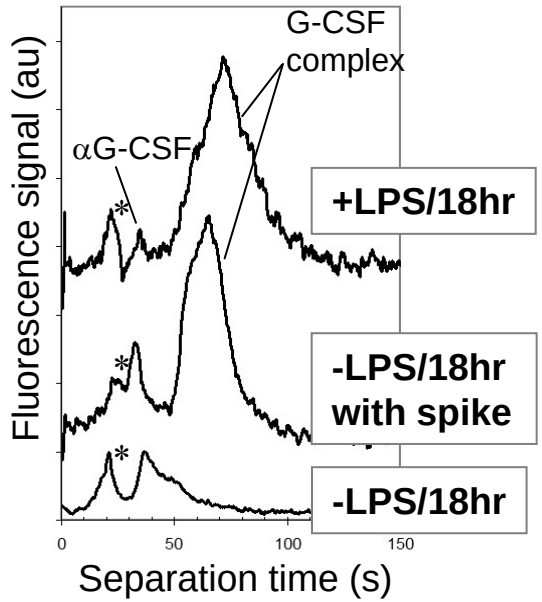












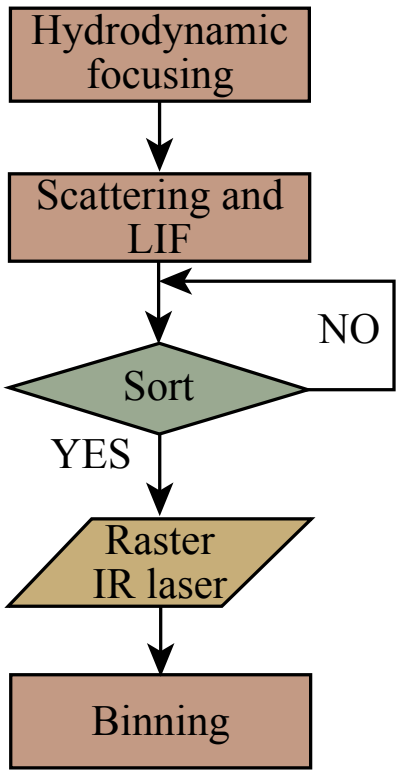
Single cell capture and imaging:
- time-course
- dynamics
- protein-protein interactions

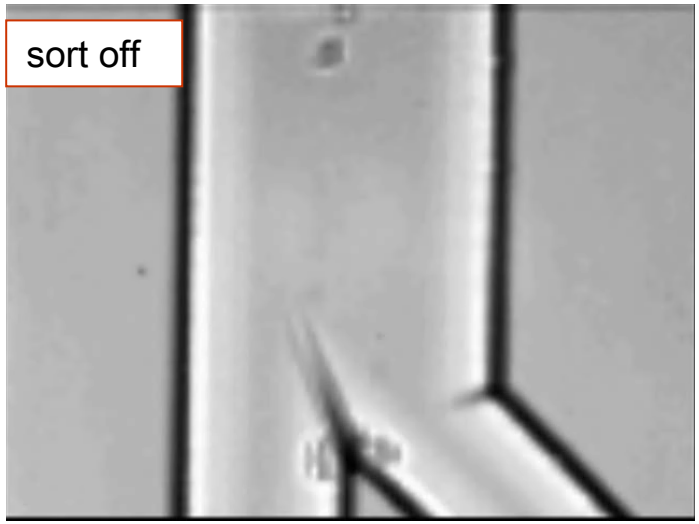
Population measurements
- sample preparation
- higher throughput cytometry
- cell sorting

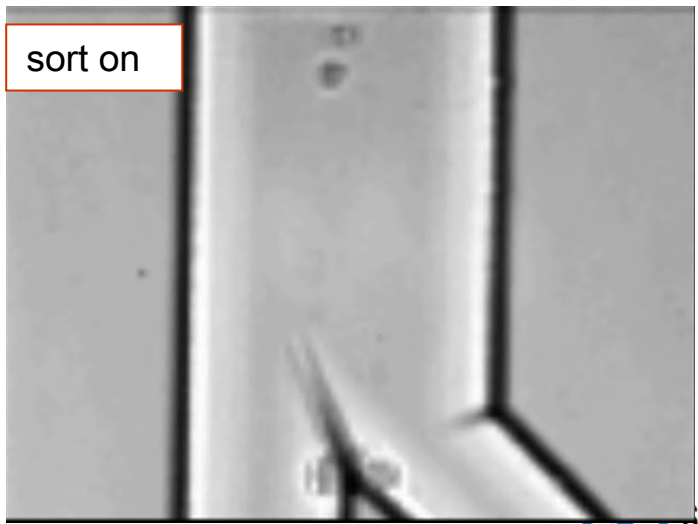
Electrokinetic immunoassays
- cytokine profiling
- multiplexed detection
- high sensitivity

Microfluidic Cell Sorting

Pre-processing of cells is required for procuring “high value” cells
- optical force based sorting
- scatter and fluorescence signals
- not integrated with single cell imaging chip yet





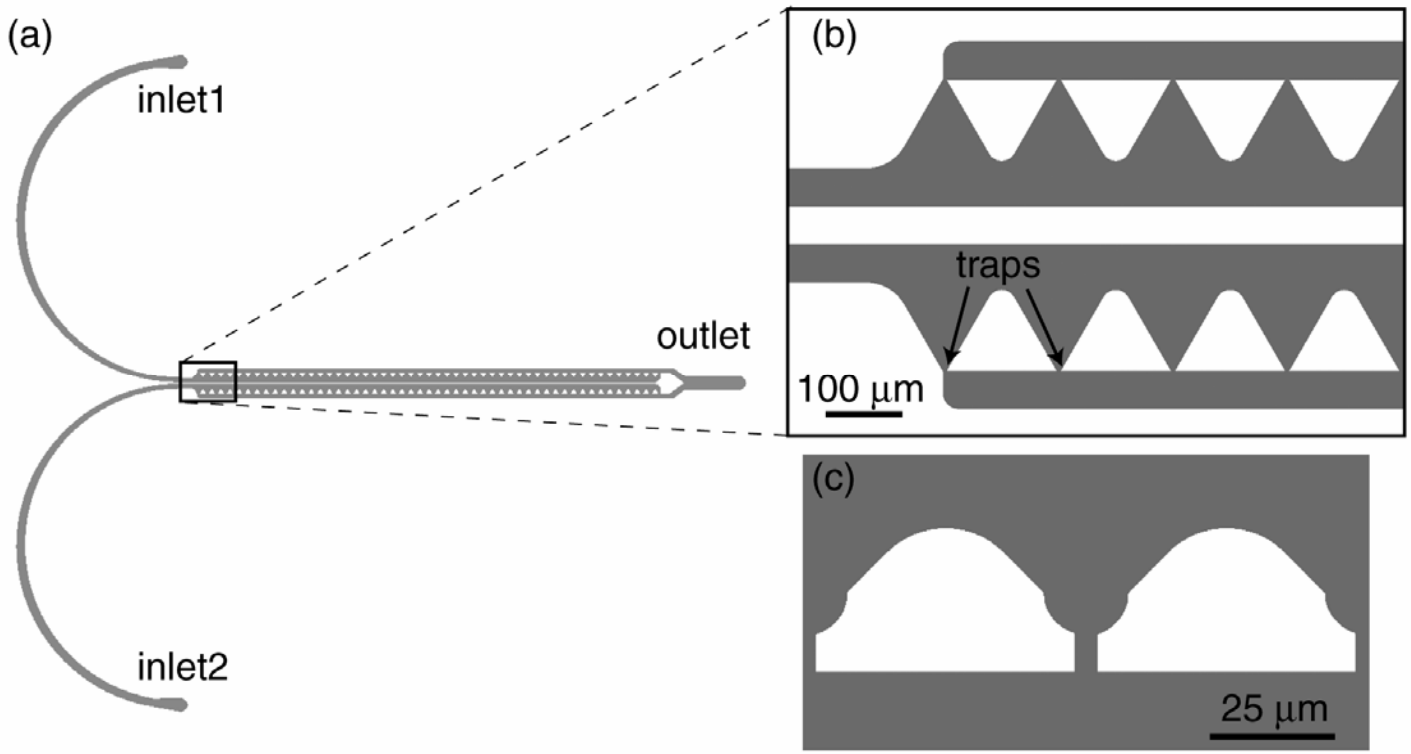


Frame rate slowed 13X

Single Cell Array Chip Design

Mechanical capture of single cells – version 1

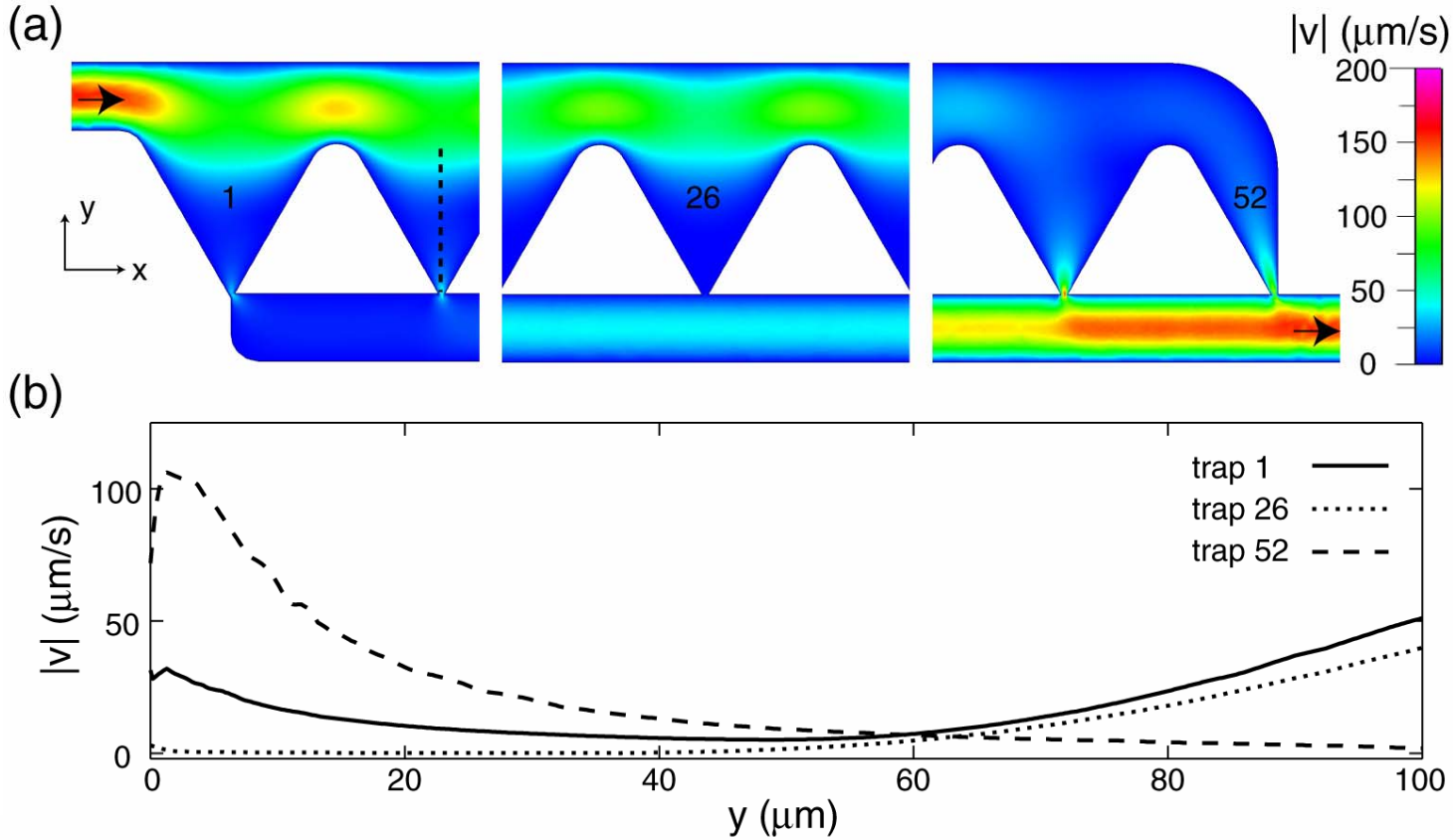
- multiple isolated trap regions
- 50-100 traps in parallel to a common outlet
- different cell interface designs



CFD Simulations of SCA Chip

Analyze flow and pressure gradients

- flowrates 10-1000 nL/min
- different flow characteristics in different locations



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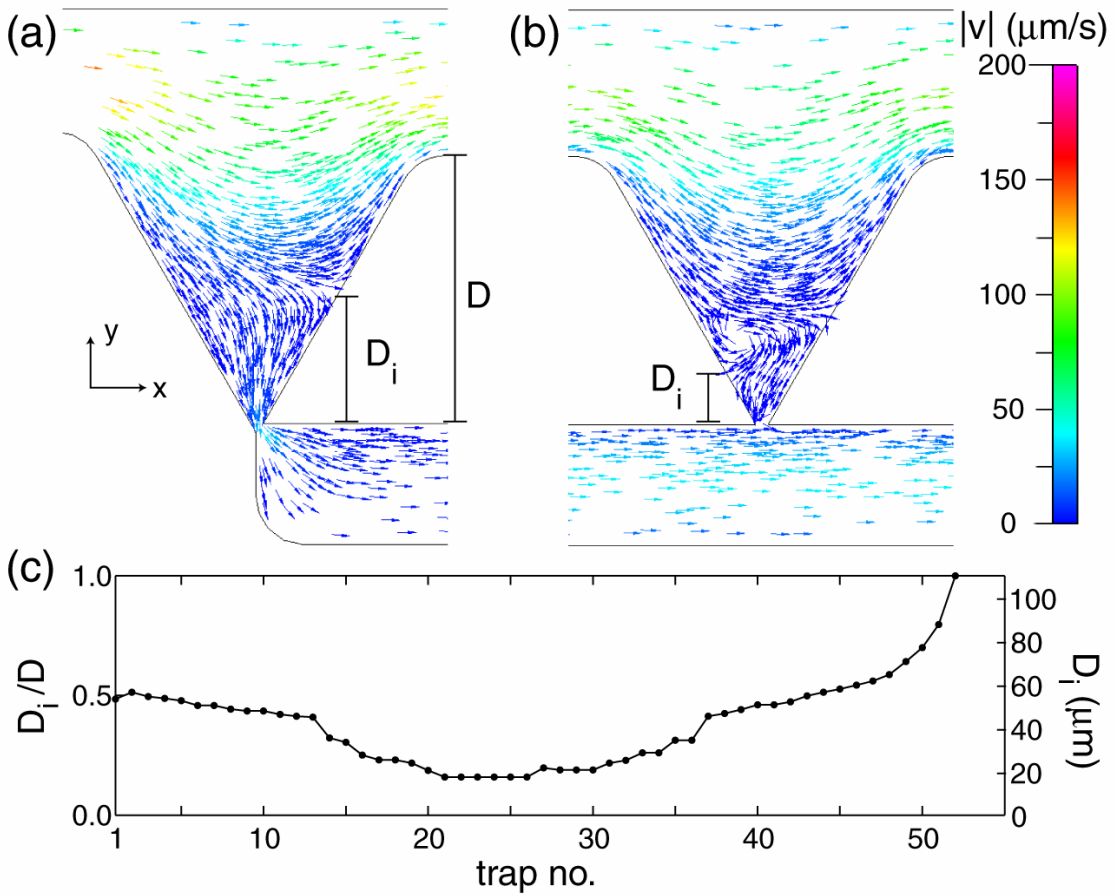
CFD Simulations of SCA Chip

Fluidic isolation within trap inlets

- trap depth (D) and the isolation depth (D_i) within the device
- convection vs. diffusion



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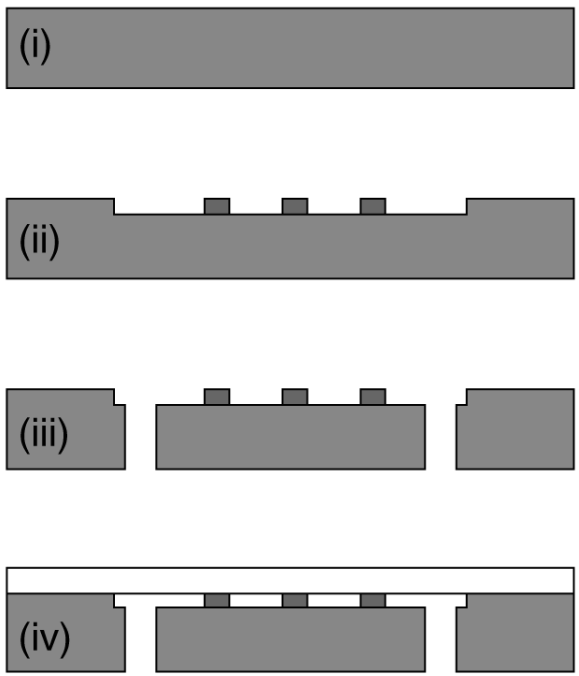
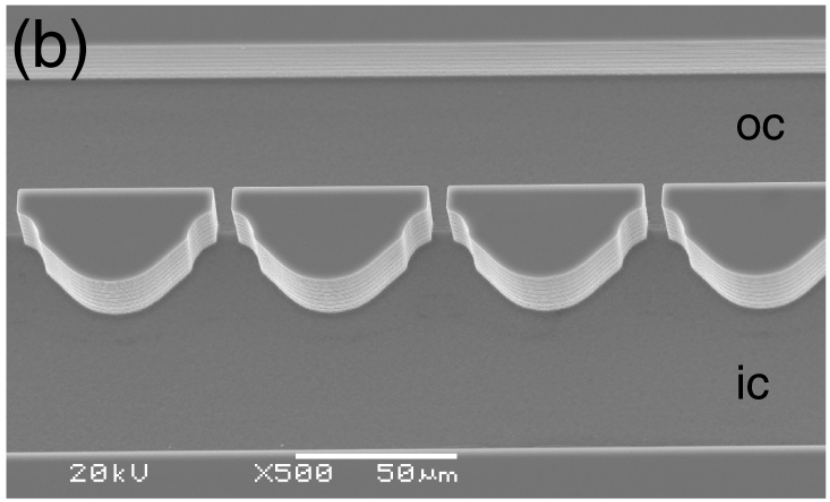
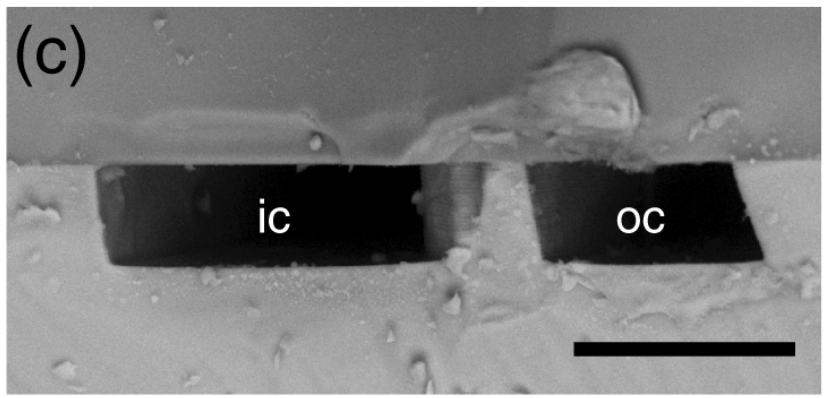
SCA Chip Fabrication

Silicon substrate processing
- one front-side, one back-side etch
- anodically bonded coverslip
- commercial fluidic interface fittings







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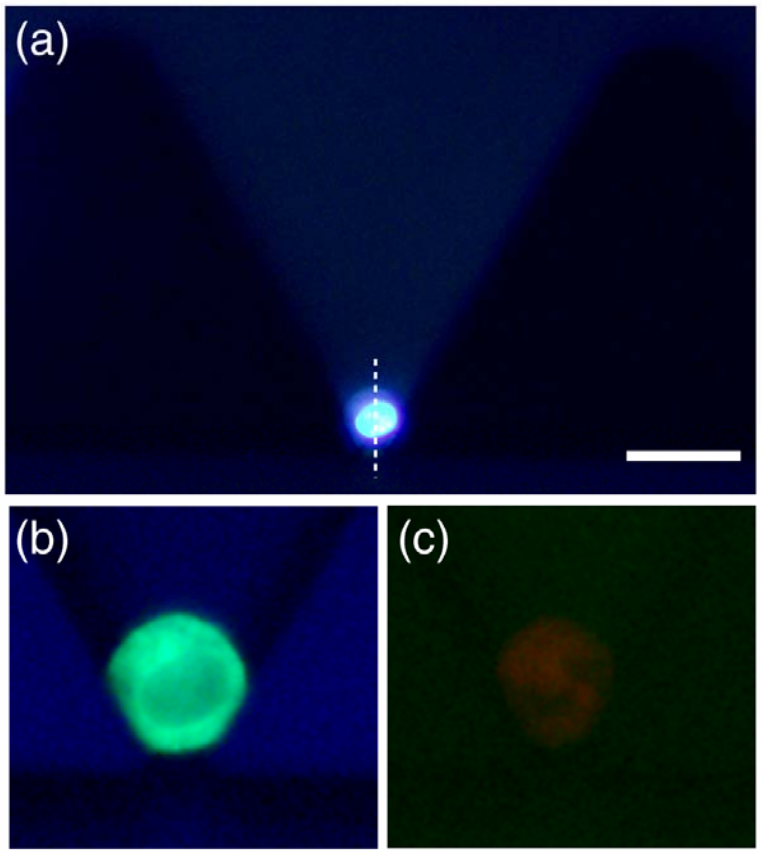
Live Cell Imaging of Immune Response

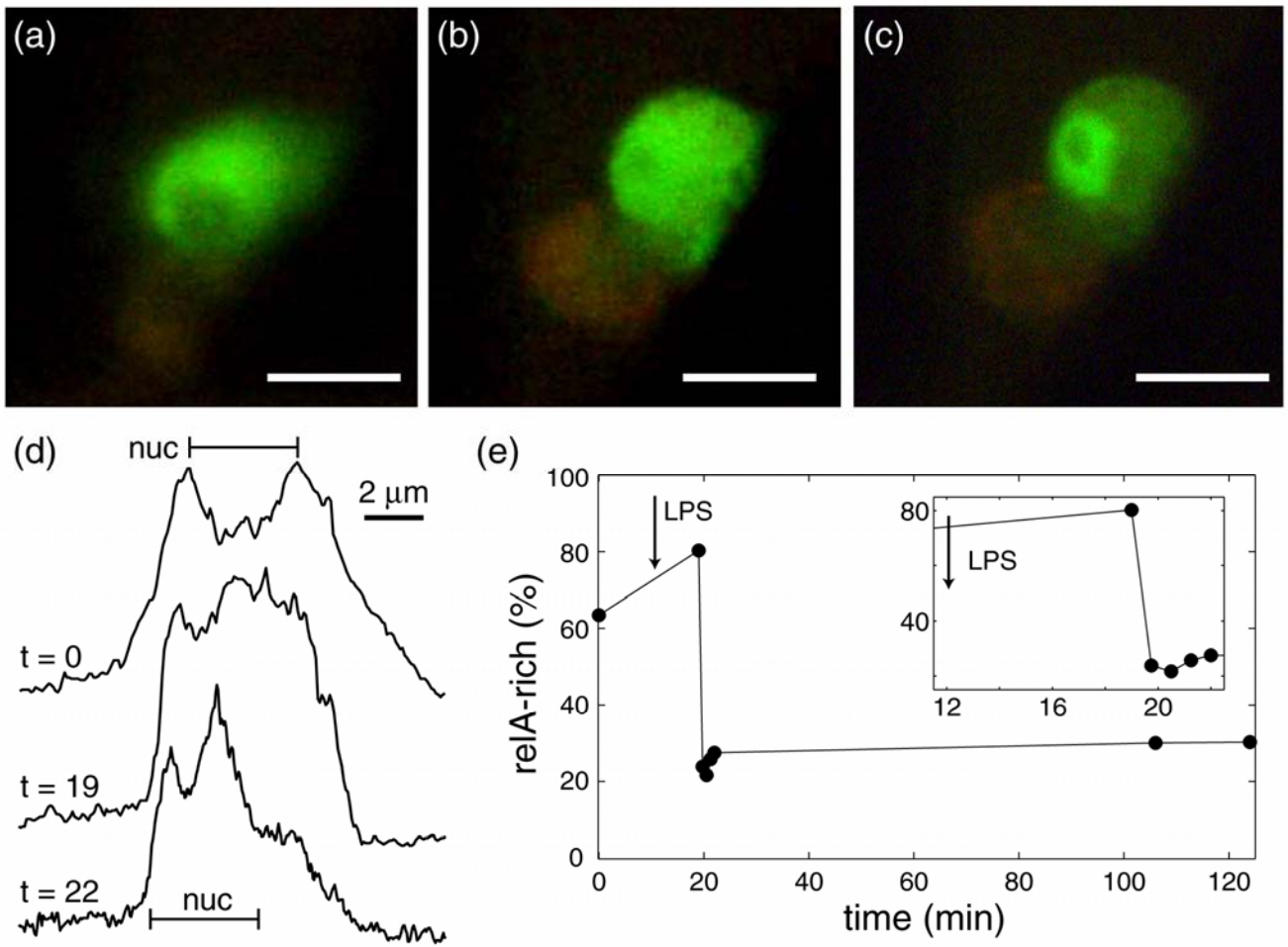
Real-time, long-term imaging of macrophages during pathogen challenges

- immune response: relA-GFP construct (NFκB)
- nucleus, live/dead



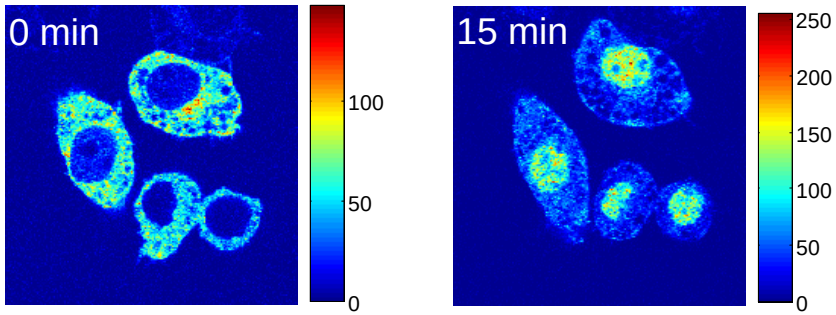
Translocation of relA after LPS challenge

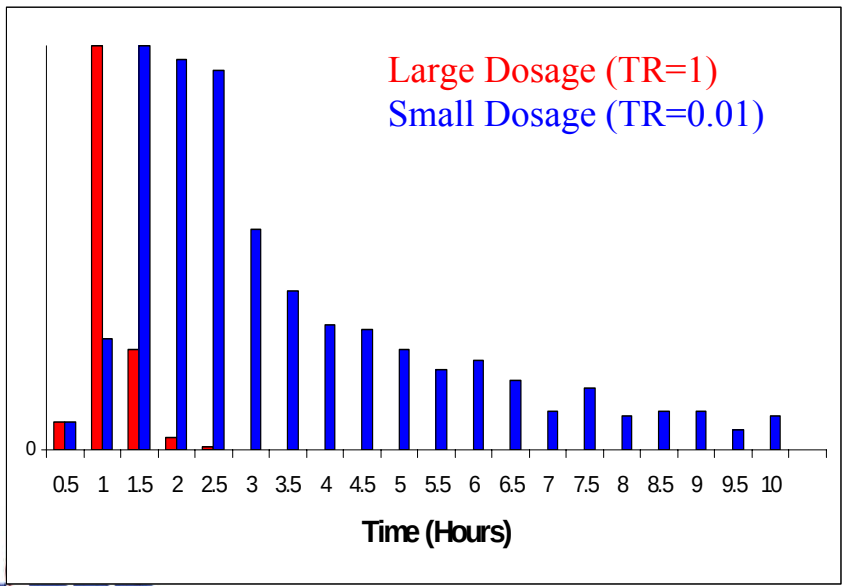
- after activation, relA translocates from the cytoplasm to the nucleus
- response is concentration and chemotype dependent



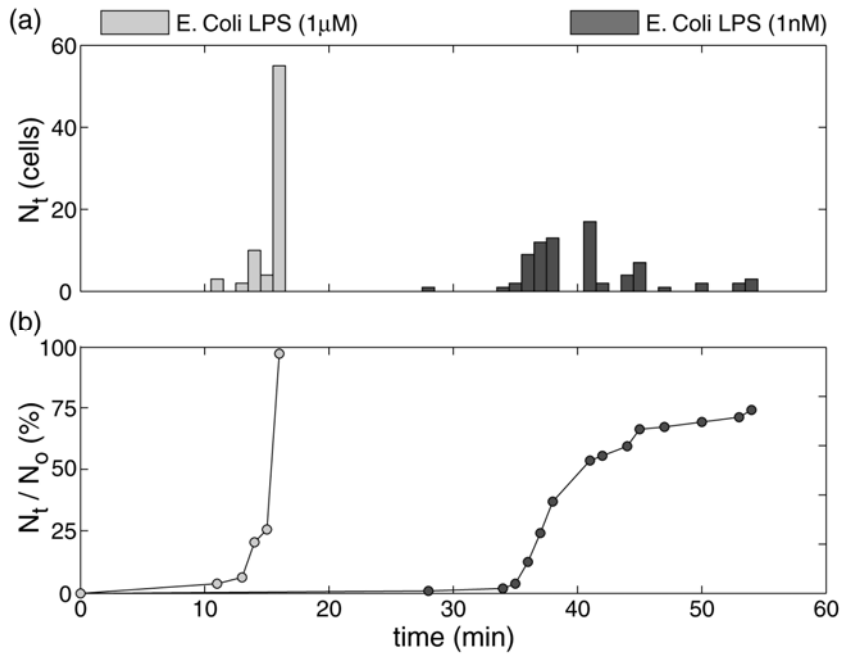
Experimental Data and Computational Predictions

- Bulk measurements on macrophages
- Computational predictions





- Experimental data from the SCA chip
  - E. Coli LPS: 1 nM, 1 μM



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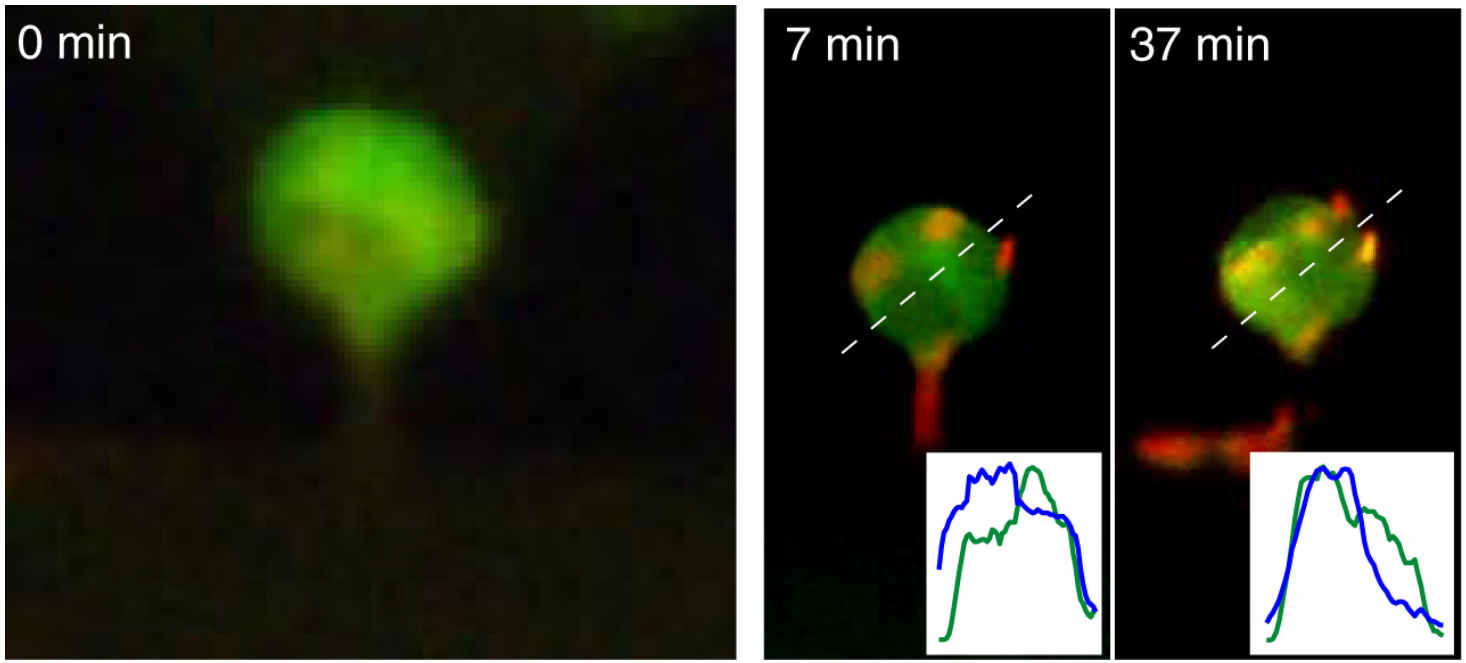
Live Pathogen Challenges

- model pathogens: E. Coli, Y. Pestis, F. Tularensis
- after activation, relA translocates from the cytoplasm to the nucleus
- monitor response as a function of multiplicity of infection



Conclusions

- Generate dose and chemotype response curves for macrophages
- Refine computational models with experimental data points
- Produce additional reporter constructs, antibodies, for assessing nodes in the TLR4 network
- Hyperspectral Imaging with SCA v2

Extra Slides

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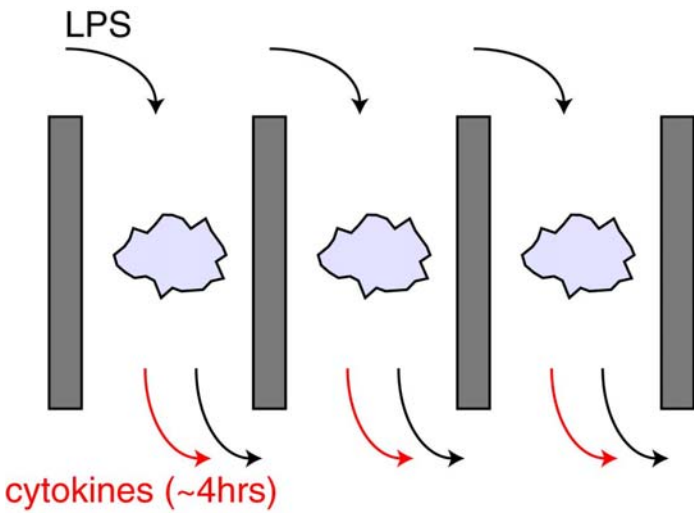
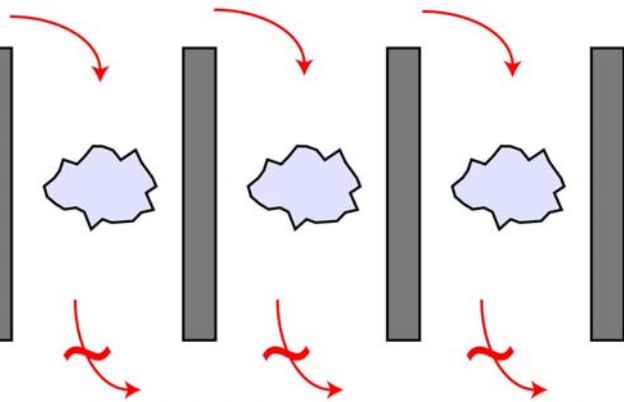
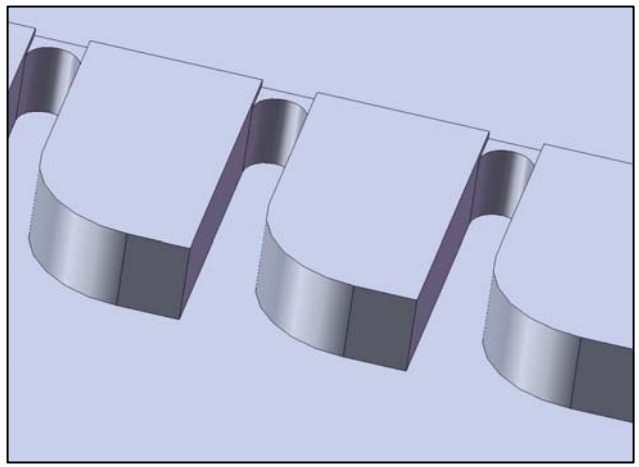
Single Cell Isolation

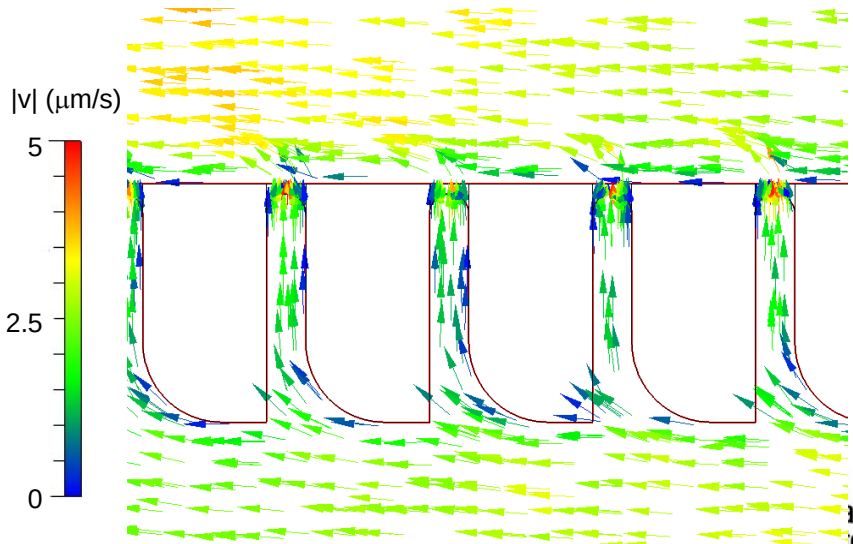
primary vs. secondary immune response:





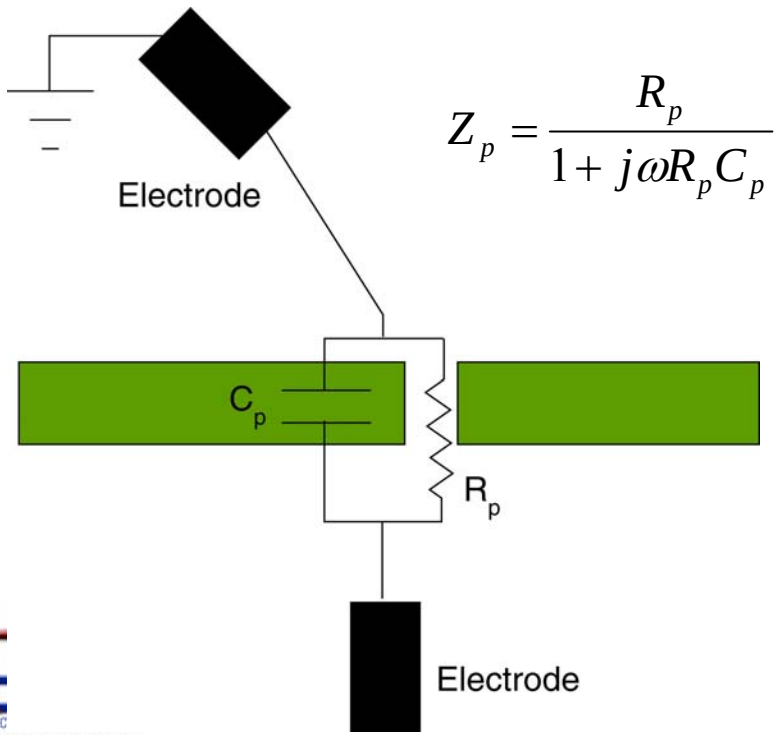
- Fluidically isolated single cells



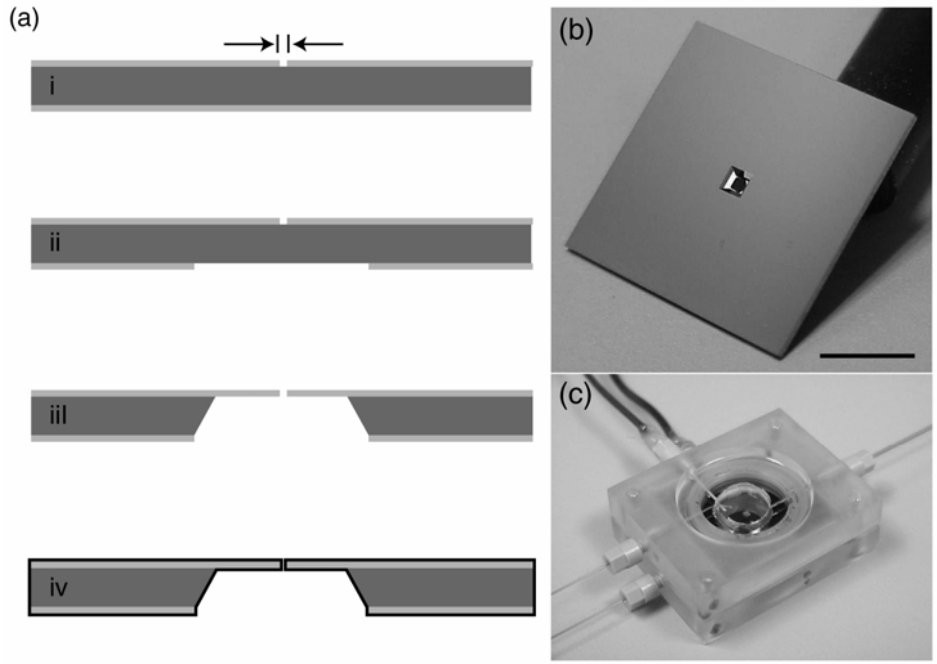


Impedimetric Assessment of Macrophages

- A microhole to which negative pressure is applied is used to reversibly capture single cells
- Captured cells are then probed with low amplitude AC voltages
- Changes in cell membrane properties (ion conductance, membrane capacitance) can be monitored during exposure to chemicals

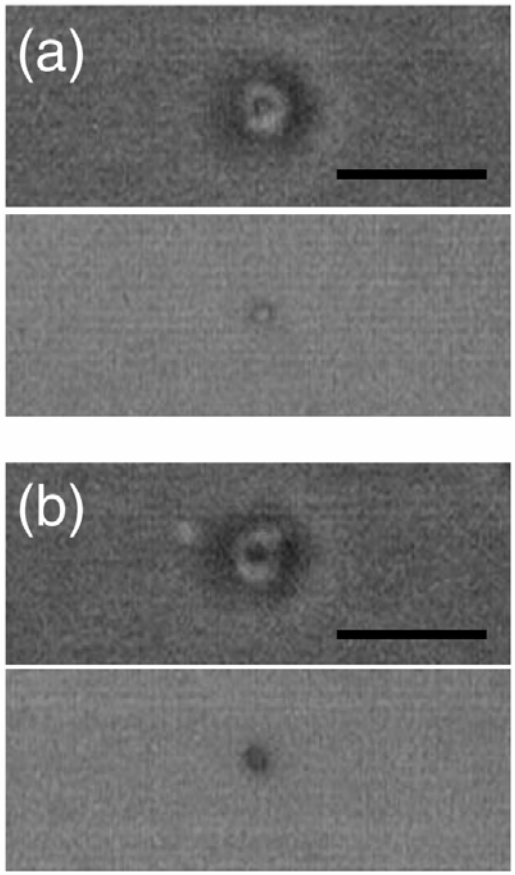


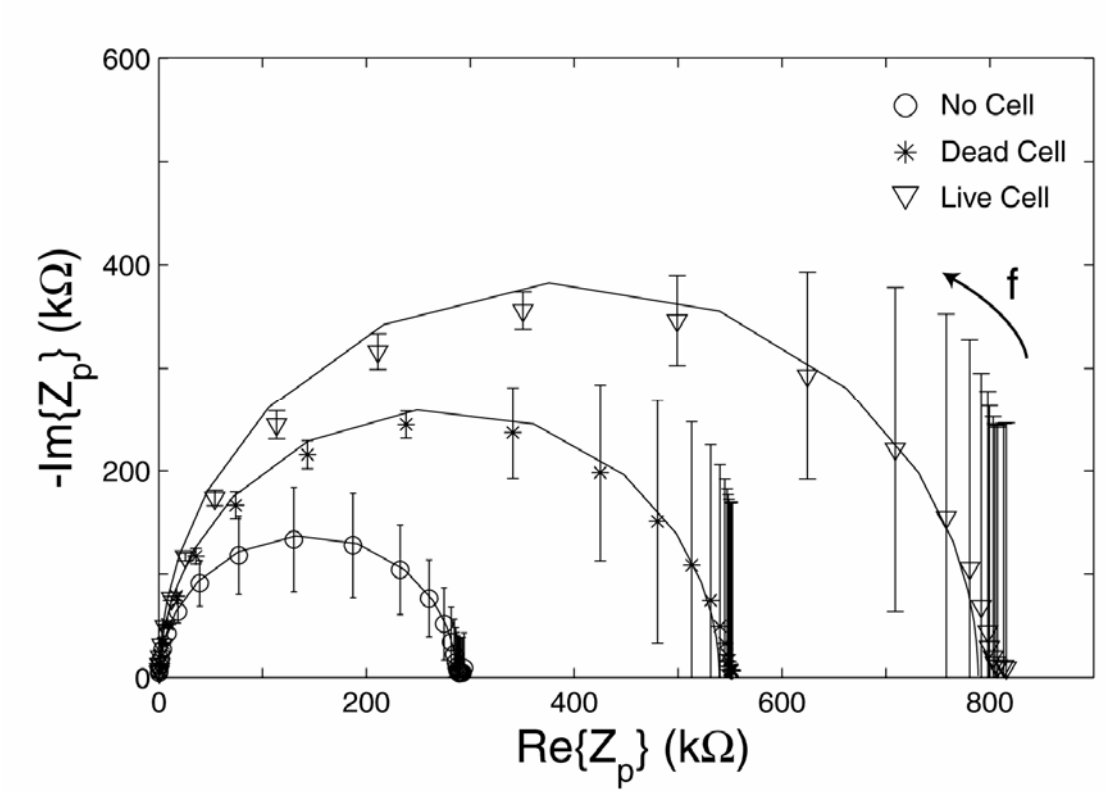
$$Z_p = \frac{R_p}{1 + j\omega R_p C_p}$$



C.D. James, et al., Biosens. Bioelec. 2001

Impedimetric Assessment of Cell Viability

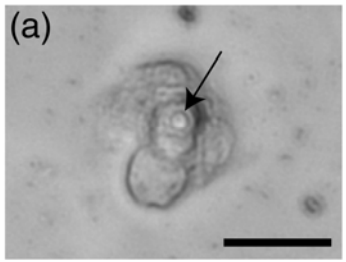


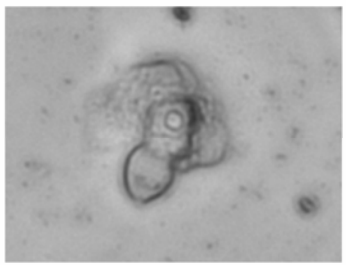


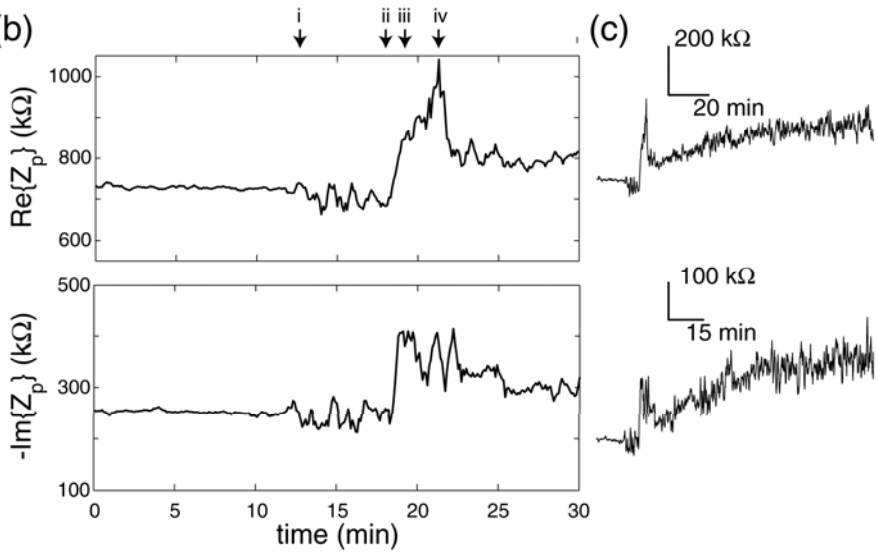
Live/dead assay using changes in the impedance spectrum

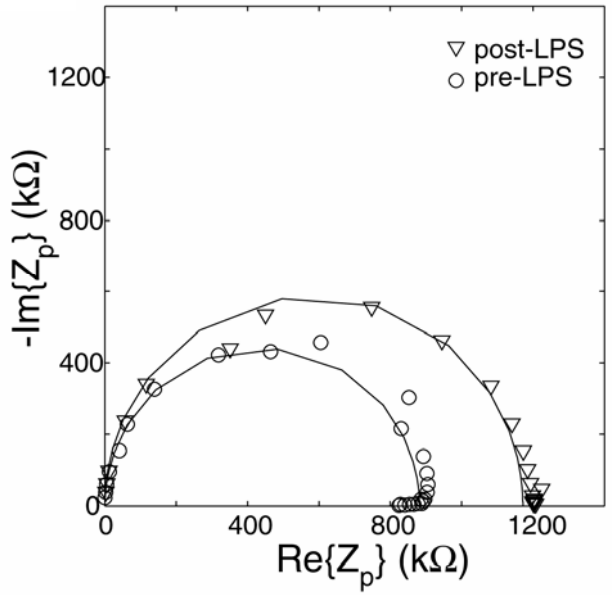
C.D. James, et al., Biosens. Bioelec. 2001

Impedimetric Assessment of LPS-challenged Macrophages

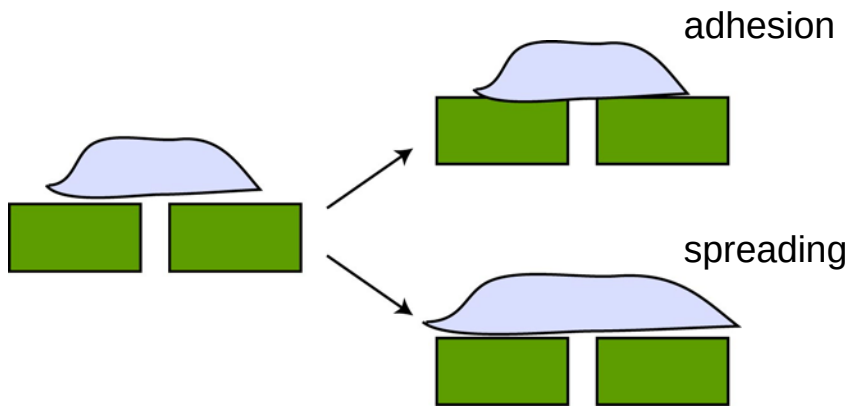








Two mechanisms for an increase in impedance across the microhole: increased spreading (longer path of resistance) and increased adhesion (narrower path of resistance)



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C.D. James, et al., Biosens. Bioelec. 2005