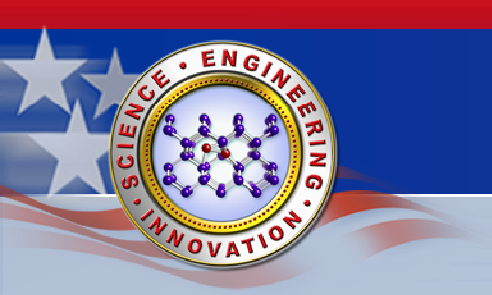
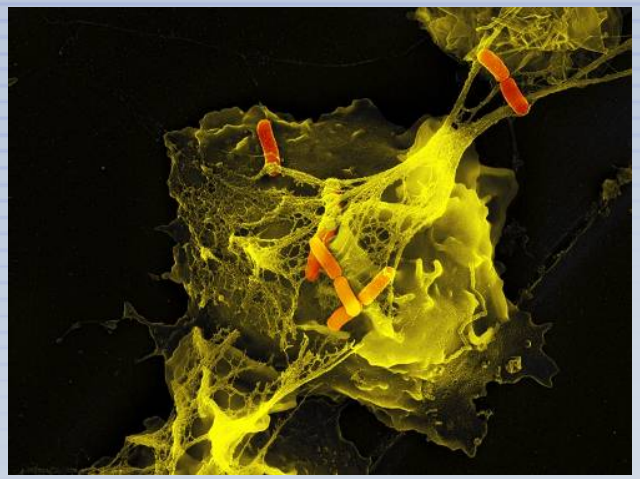


Microscale Immune Studies Laboratory: Studying Cell Signaling with Single-Cell Resoluton

Anup K Singh

Our Overarching Goal is Elucidation of Signaling Pathways in Innate Immunity

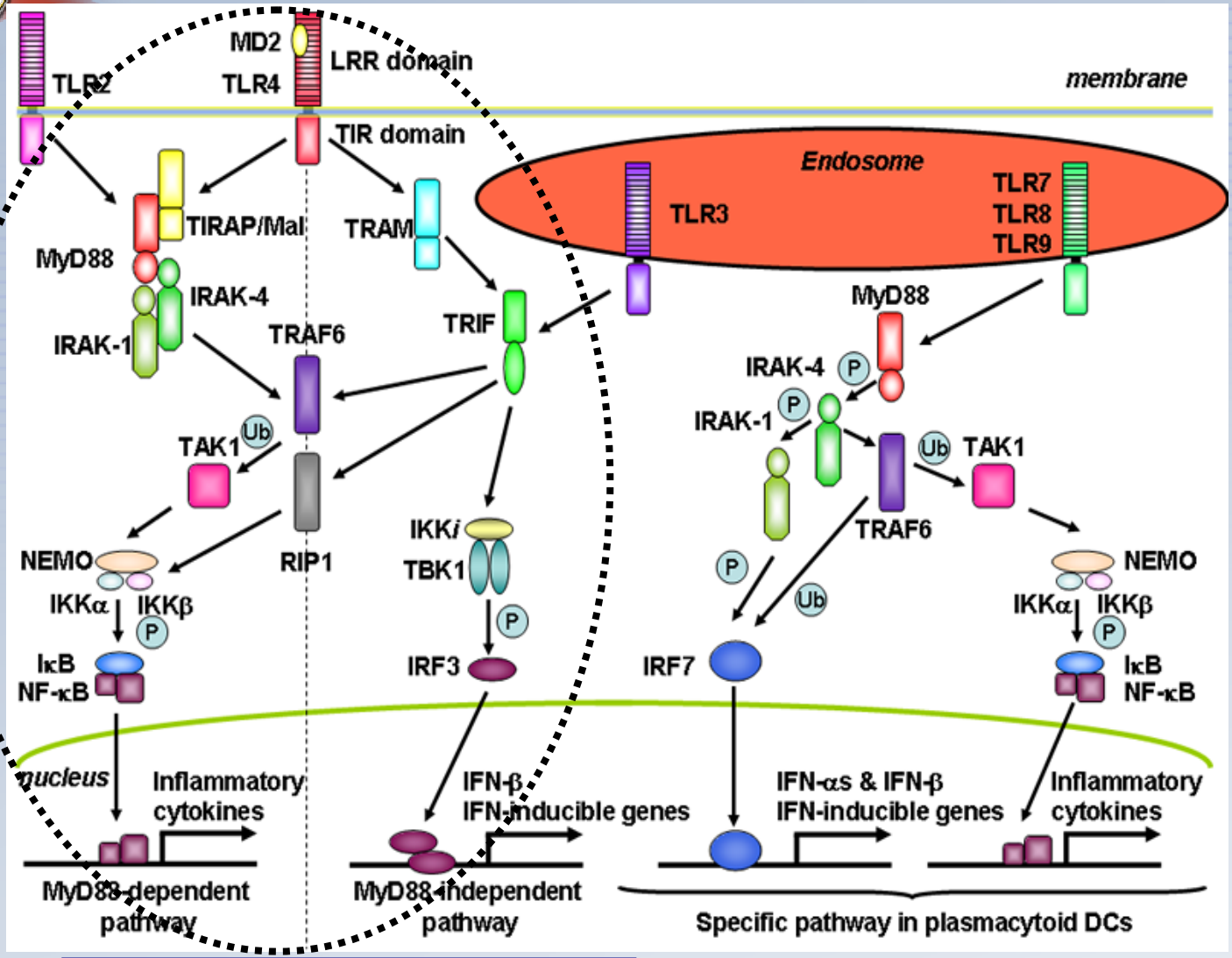
Goal: **To elucidate molecular mechanisms of the innate immune response in host cells to pathogens such as bacteria and viruses.**



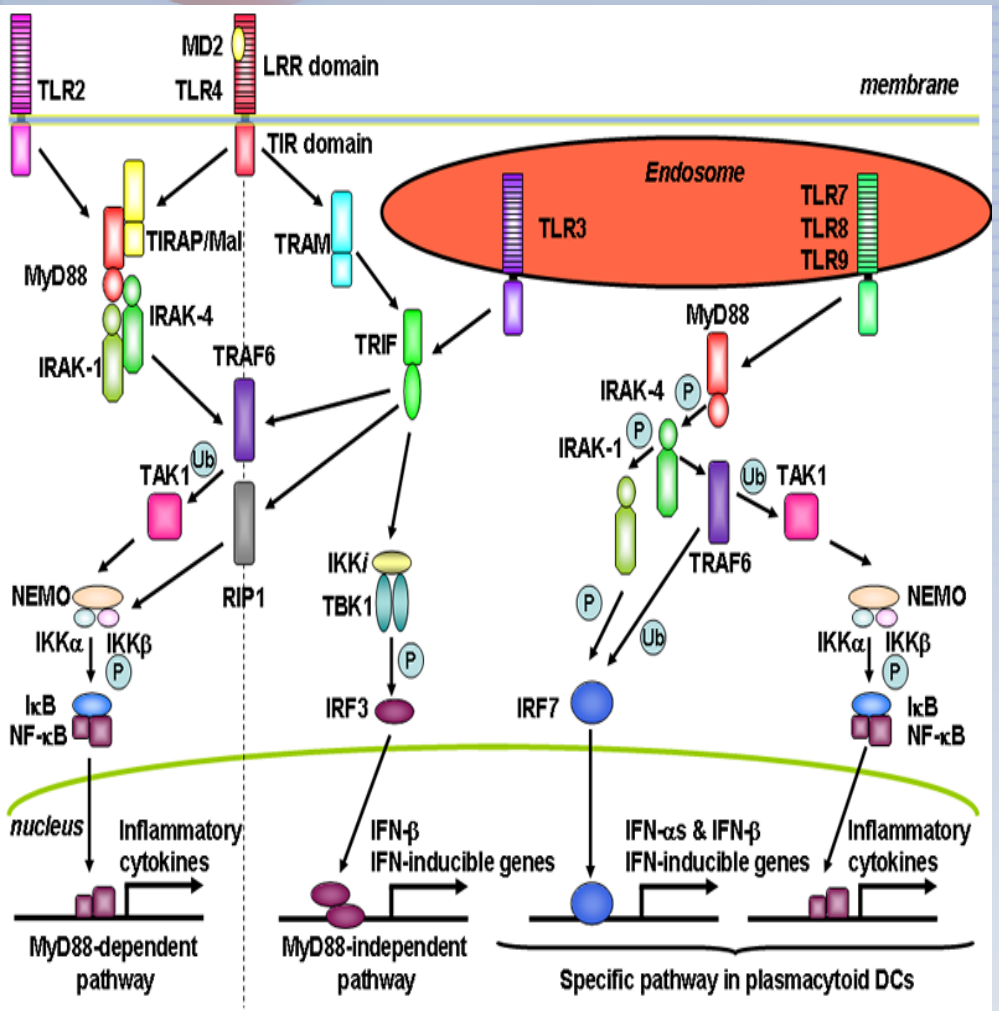
Why: Innate immunity is our first line of response to a bacterial or viral invasion. Subversion of innate immunity is the common denominator used by many pathogens, especially the emerging ones. Molecular-level understanding is key to improvements in:

Diagnostics & Therapeutics

Signaling Pathways in Innate Immunity



Critical gaps exist in our understanding of Signaling Pathways



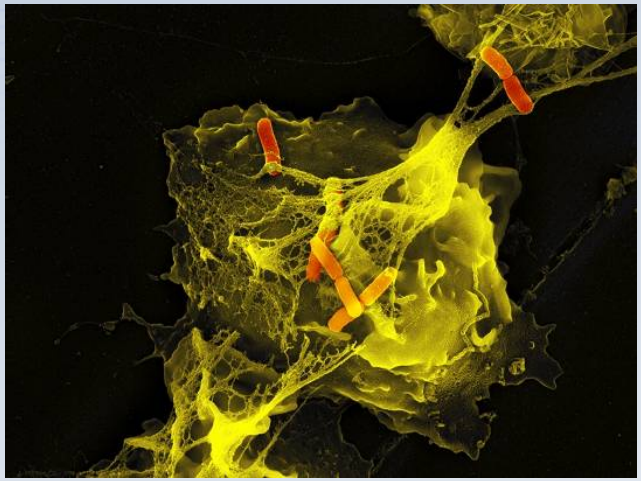
While much is known, a lot remains unknown about signaling pathways-

- Network dynamics is poorly understood
- Existing pathway models lack quantitative data
- Current methods do not provide a systems-level view
- Current methods lack single-cell resolution

The 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.*

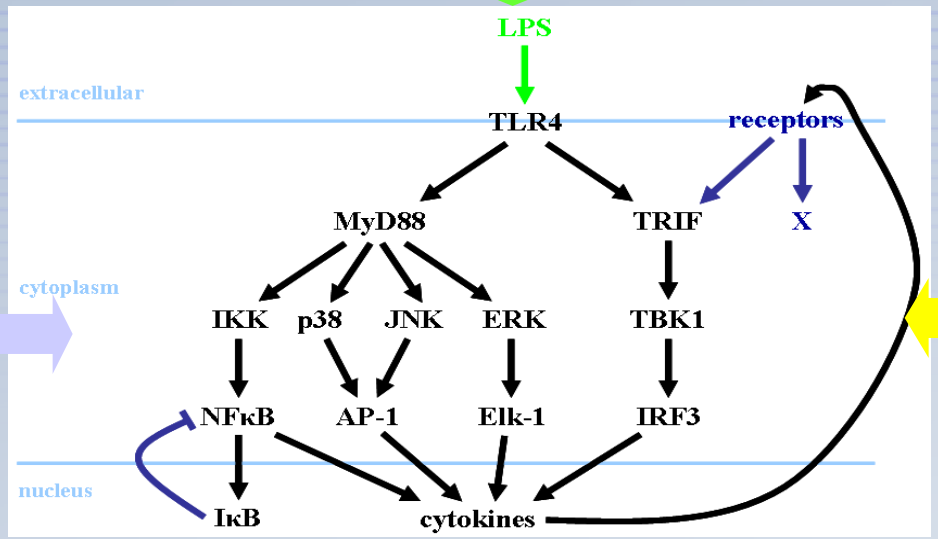
- **The benefits will be:**
  - ✓ **Key discoveries in the understanding and application of innate immunity to anticipate, detect and counter biothreats**
  - ✓ **An enabling tool for high-throughput biological pathway studies – also applicable to cancer, asthma, cell differentiation, and microbial communities**



We are using a Systems Biology Approach to Interrogate Signaling Pathways

Hyperspectral Confocal Imaging

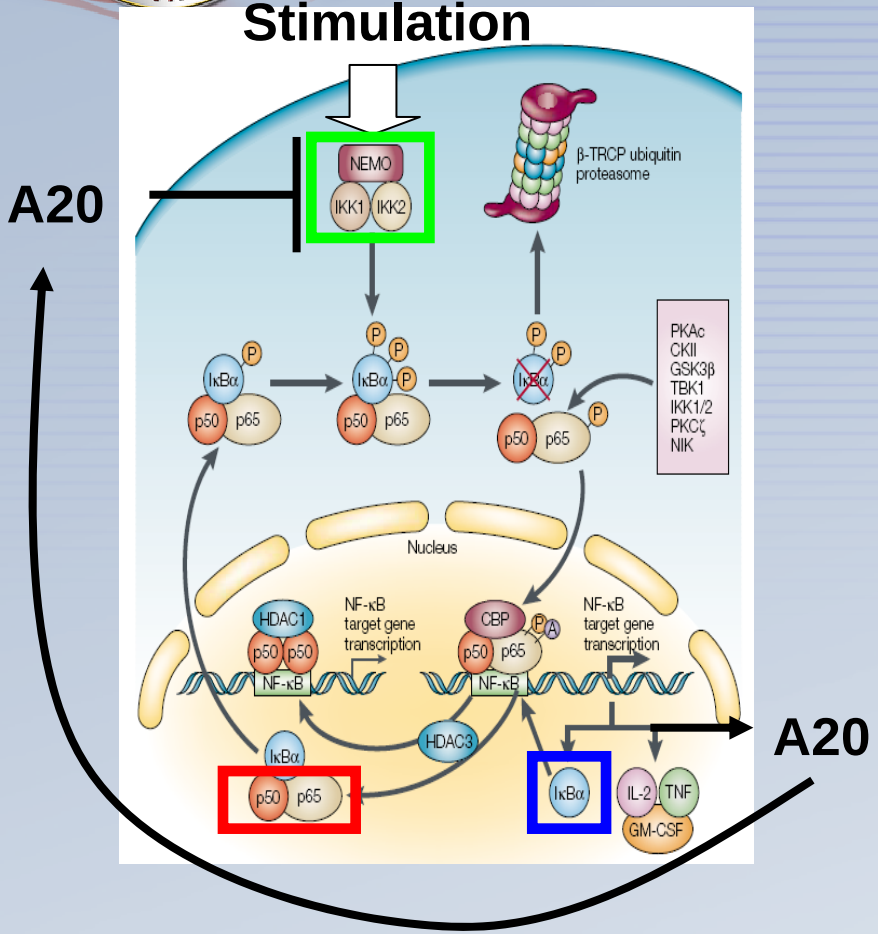
Microfluidic Platform for Proteomic Measurements



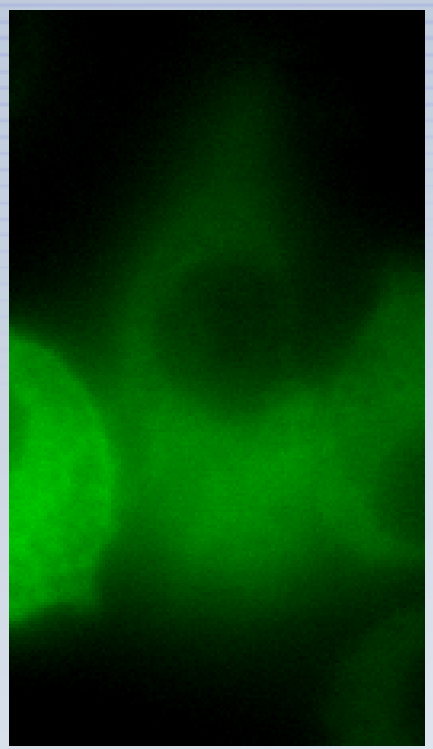
Biological Reagents & Assays

Bioinformatics & Computational Modeling

Oscillations in NF-kB localization observed in macrophages for the first time

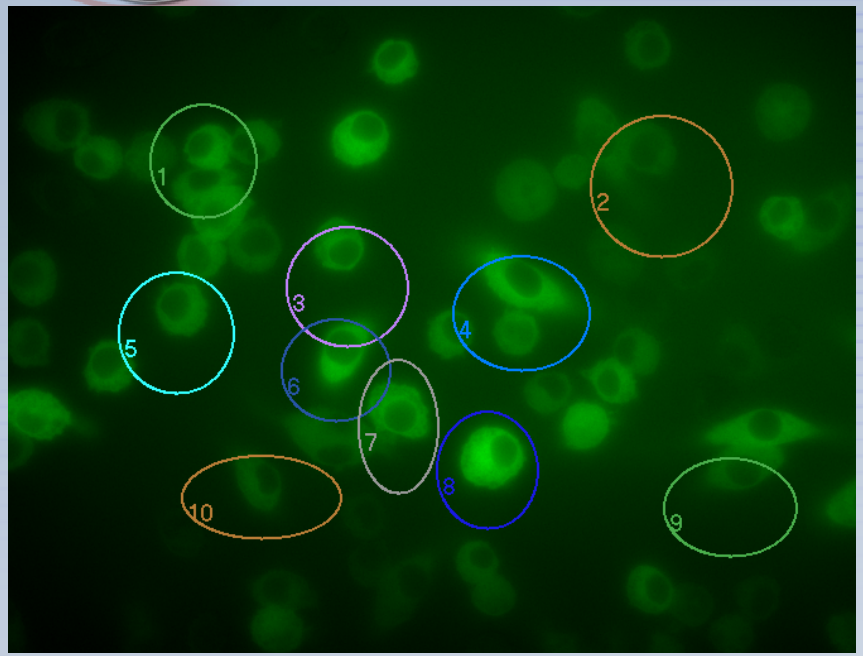


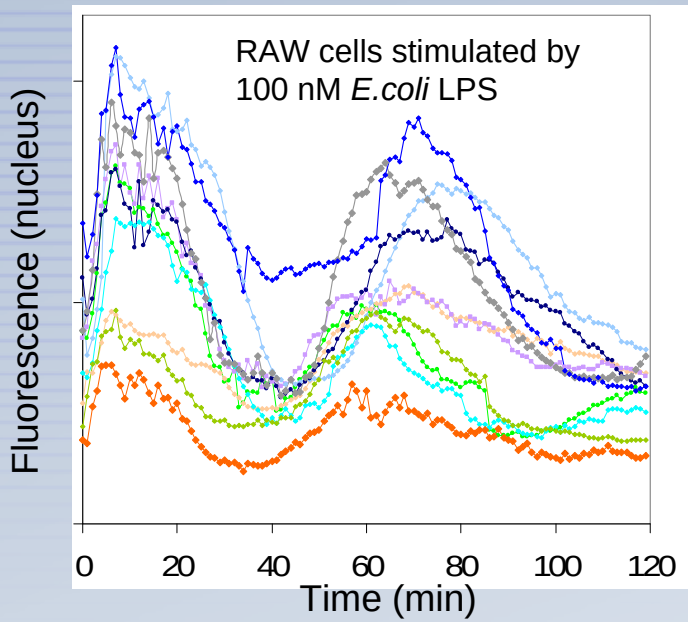
Macrophages challenged with 100 nM *E. coli* LPS



Nature Rev. Immunol. 2:725 (2002)

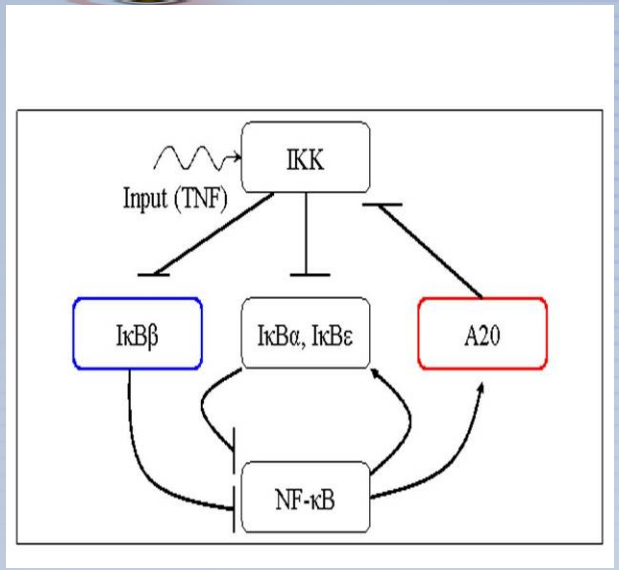
NF-kB translocation behavior is heterogeneous



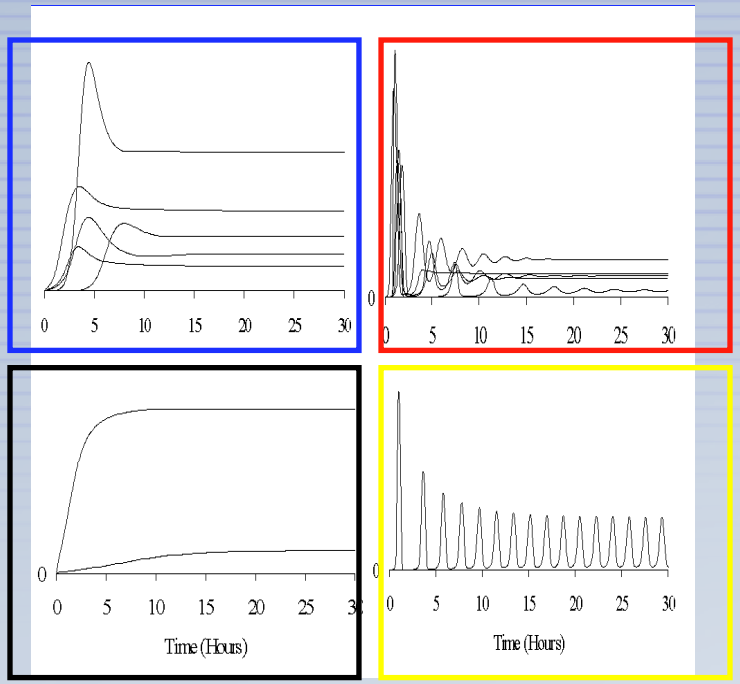


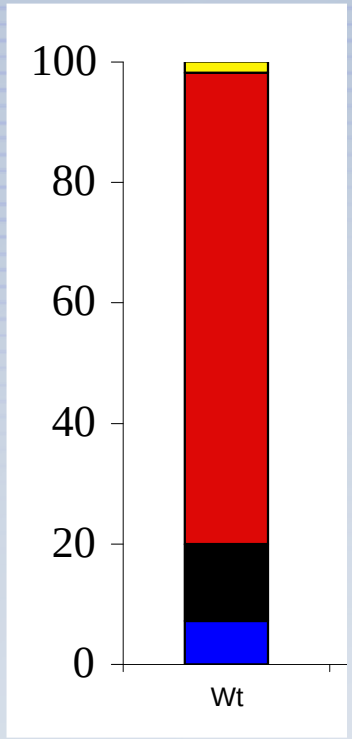
- Multiple oscillations of NFkB observed in macrophages challenged with LPS
- Oscillation behavior is heterogeneous across the population of cells
- Modeling predicted the oscillatory behavior, as well as the heterogeneity in this behavior

Computational modeling predicts the heterogeneity in oscillatory behavior



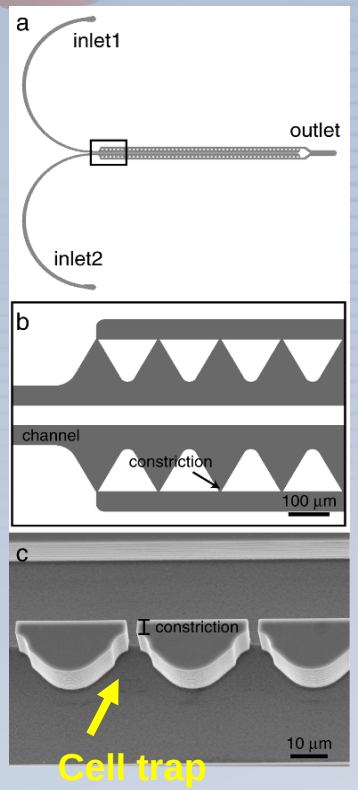
NF-kB model: 28 species, 70 reactions. Oscillatory pattern induced by negative regulators (A20, IKB)





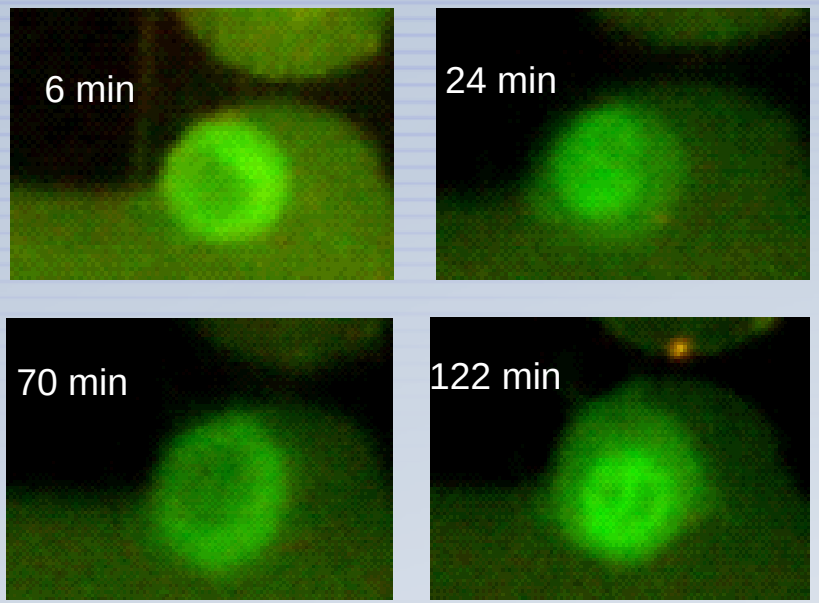
- ***Modeling predicts, and experiments confirm, four possible dynamics responses in translocation behavior of NF-kB in macrophages challenged with LPS***
- ***Quantifying cell-to-cell variability requires experimental platform with single-cell resolution***

Single-cell array chip greatly improves study of NF-kB oscillation in macrophages



- Cells trapped at constrictions using hydrodynamic forces
- 100 traps per device
- Cell viability maintained for ~18 hrs

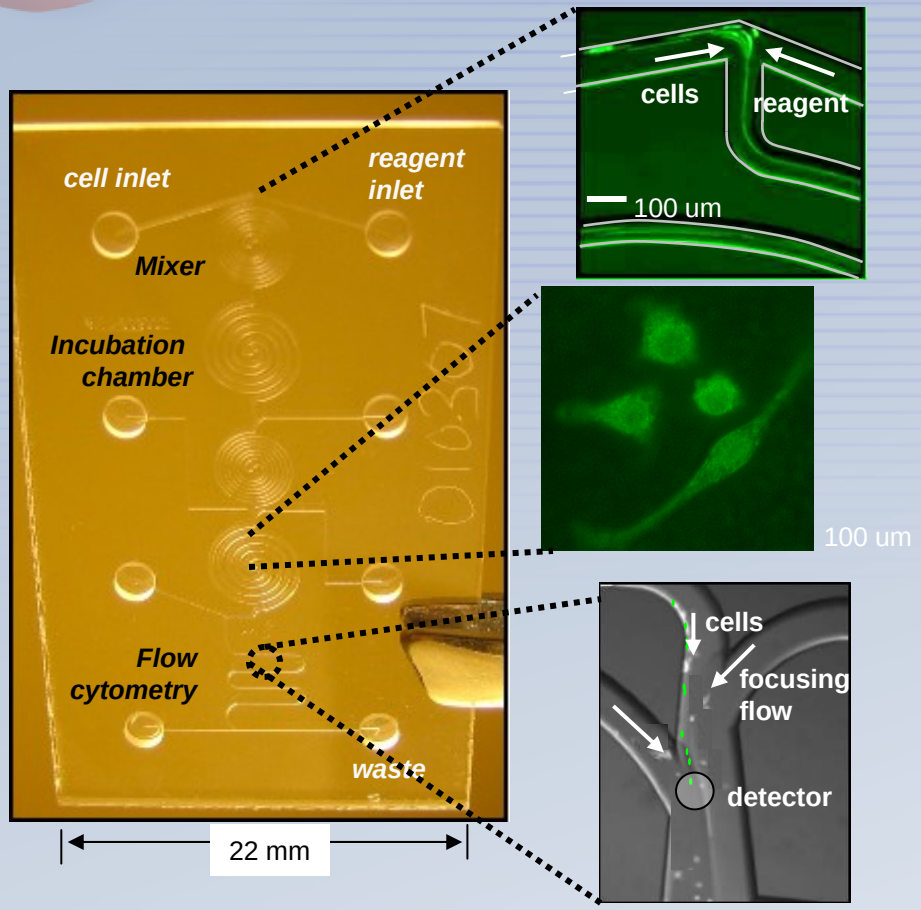
RAW 264.7 cells display NF-kB oscillation upon challenge by 1 nM *E. coli* LPS



Single-Cell Array provides:

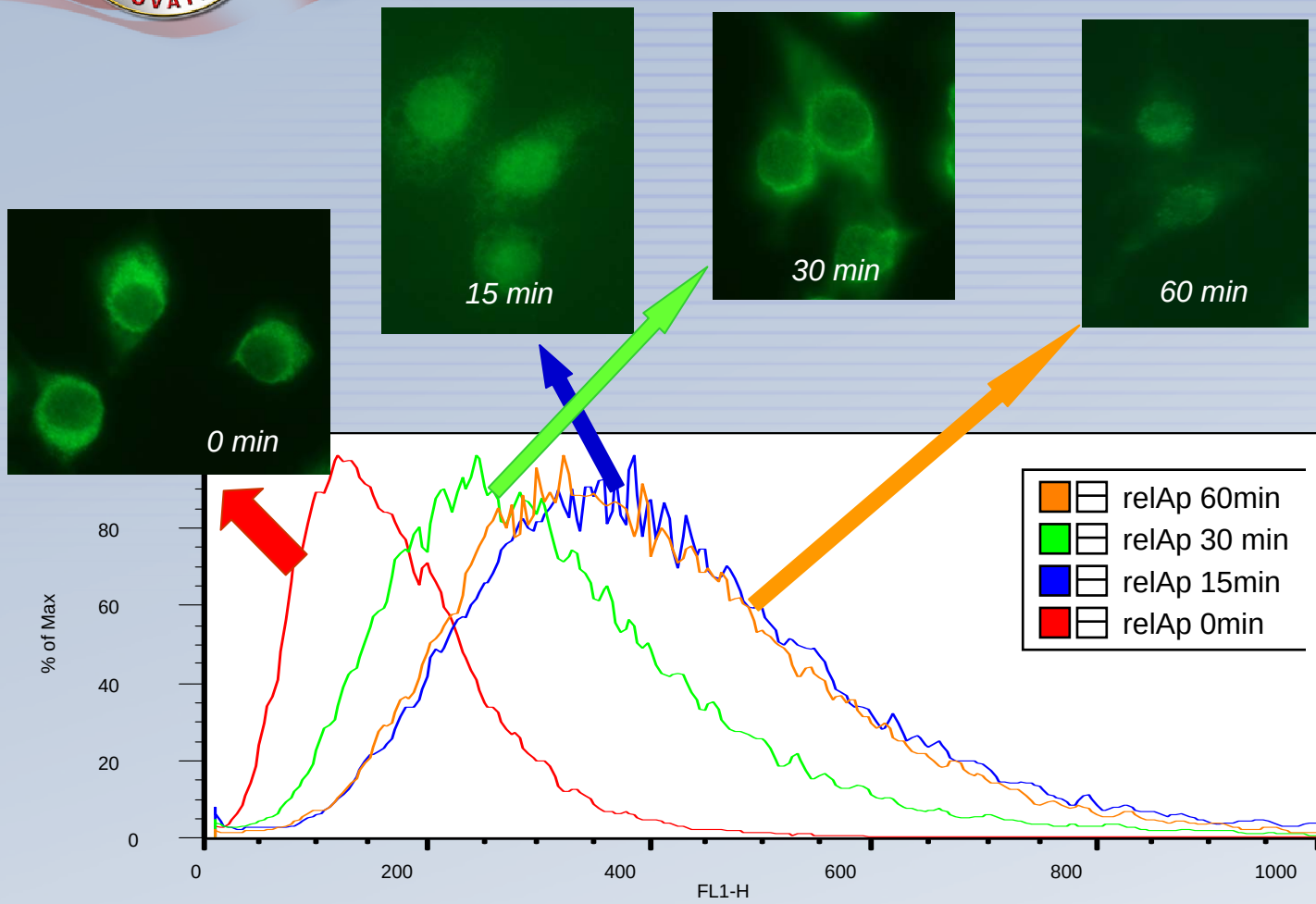
- precise localization of cells
- individual addressing of cells
- facile interfacing with commercial or custom microscopes

Imaging and Phosphoassays Performed in the Same Population of Cells in a Single Chip



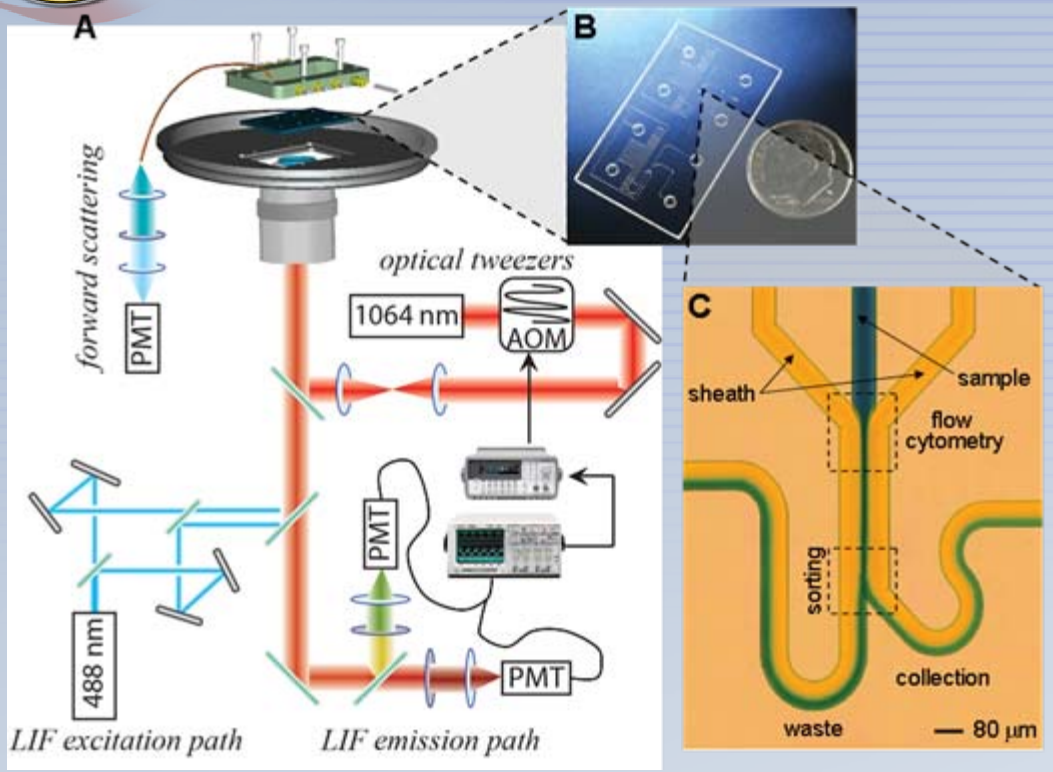
Integrated flow cytometry and imaging has allowed, for the first time, measurement of kinase activity and their translocation in the same population of cells

Kinase Activity Correlated with NFkB Translocation in the Same Population of Cells



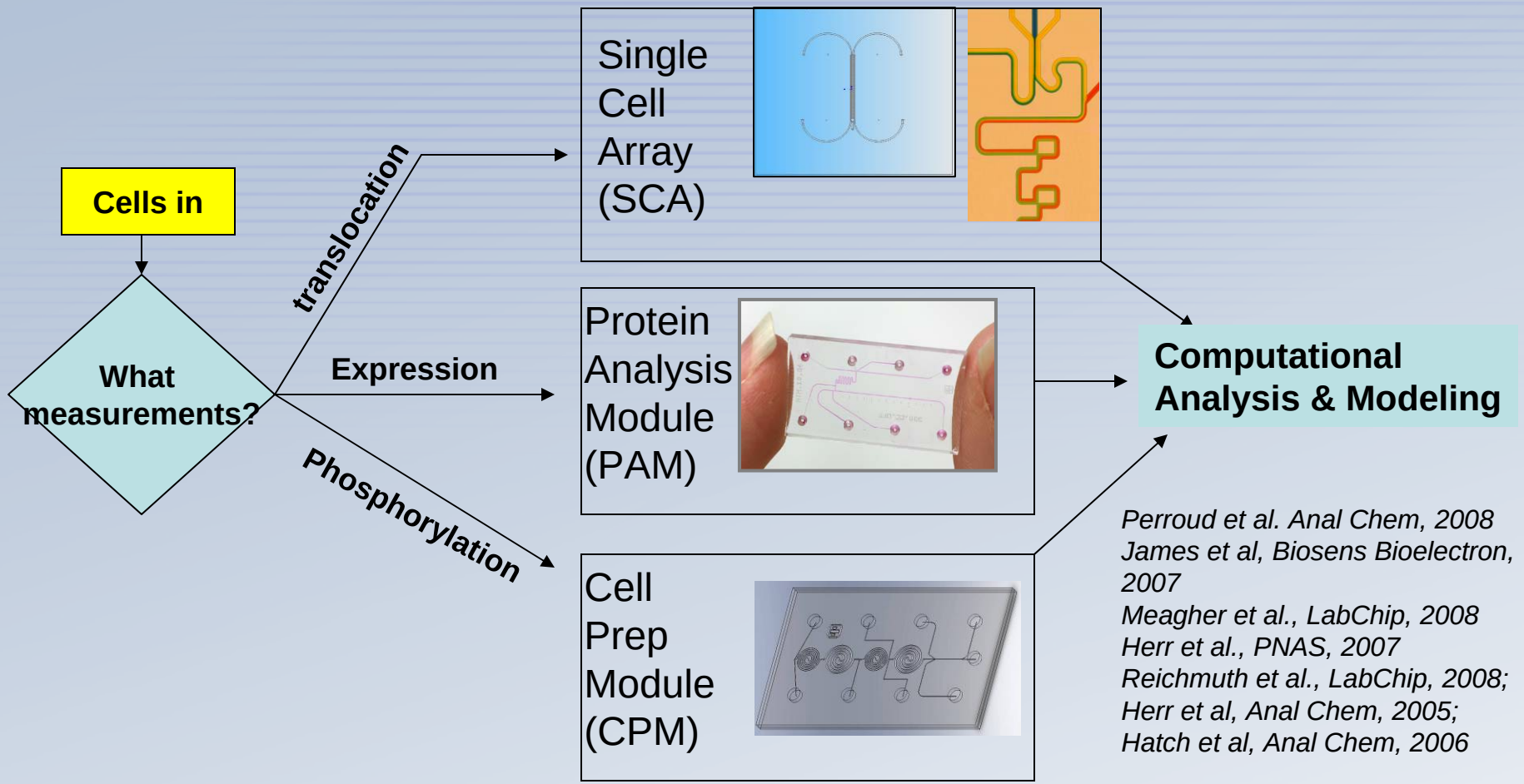
Correlated imaging and phosphoassay can not be done with conventional instruments!

Integrating cell sorting with arraying for on-demand imaging of desired cells



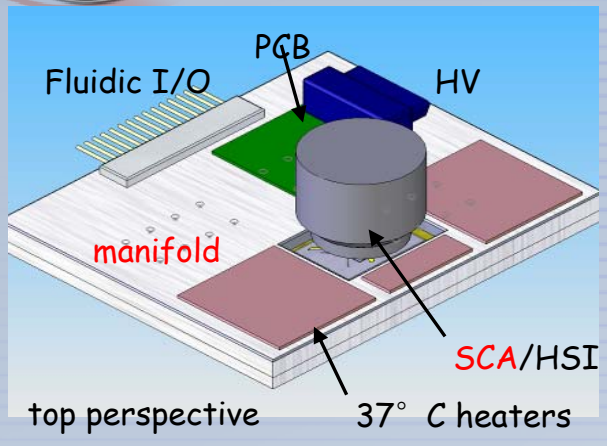
Unique chip-design has enabled integration of flow cytomtery, optical tweezer-bases sorting, and subsequent arraying of sorted cells for imaging

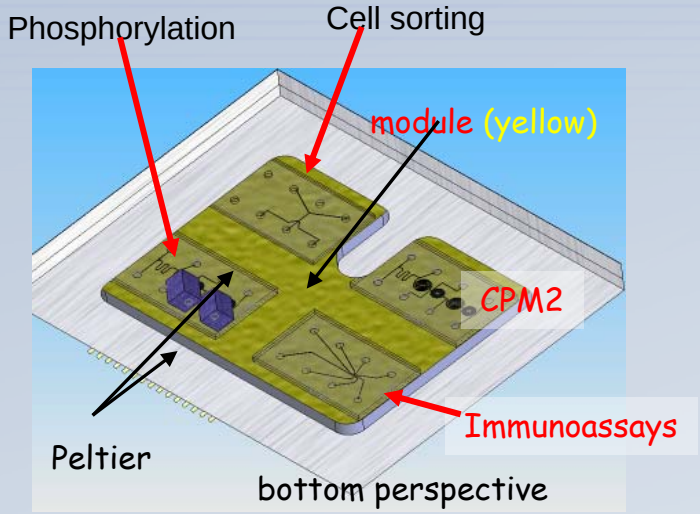
MISL Integrated Platform



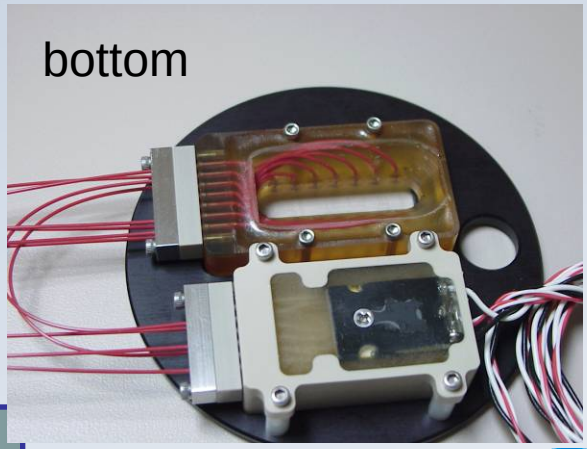
Perroud et al. Anal Chem, 2008
James et al, Biosens Bioelectron, 2007
Meagher et al., LabChip, 2008
Herr et al., PNAS, 2007
Reichmuth et al., LabChip, 2008;
Herr et al, Anal Chem, 2005;
Hatch et al, Anal Chem, 2006

MISL Integrated Platform

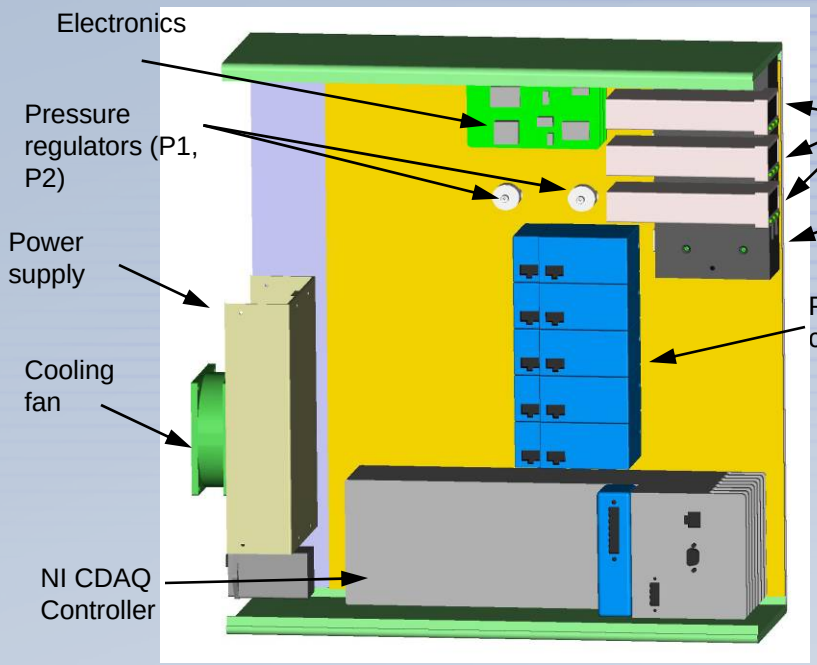




Single-cell array and cell sorter mounted in a microscope plate



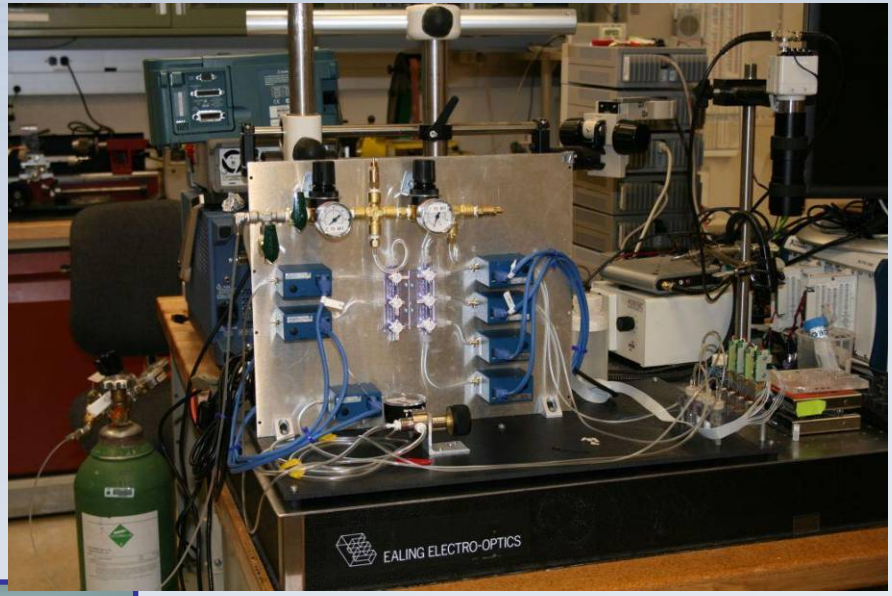
MISL has created a self-contained, automated platform for studying cell signaling



Approximate size: 14x15x5.5 inches

Schematic of the electronic and fluidic controller for MISL platform

- Automated operation
- Can be interfaced with hyperspectral confocal microscope or a commercial epifluorescence microscope
- Small foot-print will permit usage in BSL-3 & 4 environments



How MISL has changed the landscape: an example

Goal: study crosstalk between TLR4 and TNFa pathways

Experimental design: monitor activation of 10 TLR4 pathway kinases and 3 TNFa pathway kinases over 10 time-points, in response to mixed challenges of 5 LPS concentrations and 5 TNFa concentrations

Represents ~150 Western blots

Conventional Approach

Western: 6-8 hours
1 FTE, 5 gels a day, 1 month
Additional 2-4 weeks for data analysis

MISL Approach

With automated controller and 5 CPM chips running simultaneously, and with a 4-color detector, will take ~3 days

Slow, labor-intensive → ***Fast, automated***

MISL approach will reduce time by a factor of ~20 and automate measurements

How MISL has changed the landscape: an example

MISL approach allows measurements *not possible* with conventional assays (e.g., Western analysis)

Conventional Approach		***MISL Approach***
Semi-quantitative	→	Quantitative
Sensitivity: nM;	→	As low as ~50 fM
No single-cell resolution	→	Single-cell resolution
Poor time-resolution (~2 min; miss early events)	→	Time-resolution of ~sec
Need large number of cells (10^6 cells per assay)	→	1000 cells per assay
Hence, impractical for primary cell assays	→	HT expts with primary cells
Reagent cost high	→	1000-fold lower reagent consumption

Moreover, MISL platform allows correlated imaging and proteomic measurements

A Multi-Disciplinary Team

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PM: Glenn Kubiak

Biology Core Team:

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Steve Branda
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Amanda Carroll-Portillo
Bryan Carson
Julie Kaiser
Todd Lane
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Bryce Ricken
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Susan Rempe
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Paul Gemperline

Diane Lidke, UNM

Funding: Sandia LDRD Program