SAND2008-0591C

Bioanalysis Approaches Applied to Biosecurity Problems

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

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Biosciences play a *key role* in many National Security challenges

Homeland Security and Defense

- Pathogen Detection

- Decontamination

- Early Detection of Infectious Outbreak

Energy, Resources, and Nonproliferation

- Biofuels

- Water

- BW proliferation

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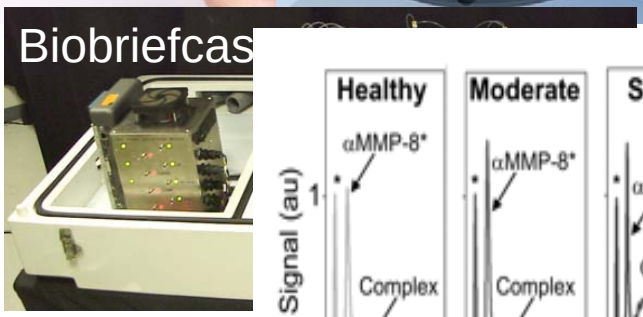
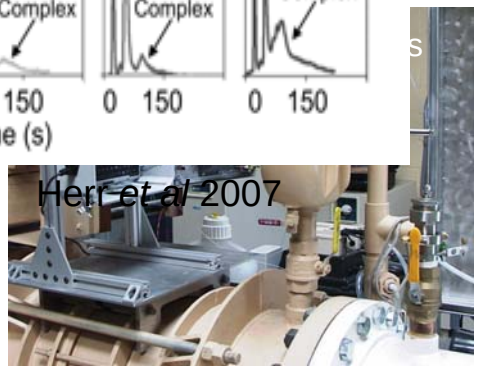
Sandia develops detection, analysis, and diagnostic technology with dual use

- Environmental monitoring
  - **Facilities/infrastructure**
  - **First responders**
- Biomedical Surveillance
  - **Presymptomatic detection**
  - **Early infection detection**





Solutions to the most challenging biological analysis problems where failure to meet these challenges threatens our national security .

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Two on-going areas of research in bioanalysis for biosecurity at Sandia

Nanofluidic Chromatography for Biomolecule Detection

D. Huber, M. Bartsch, M. Markel, M. McCrink, S. Pennathur and K. Patel

Rapid Fluorescence-Activated Cell Sorting using Photonic Forces in a Microfluidic device

T. Perroud, T. Lane, J. Kaiser, J Sy, A. Singh, and K. Patel

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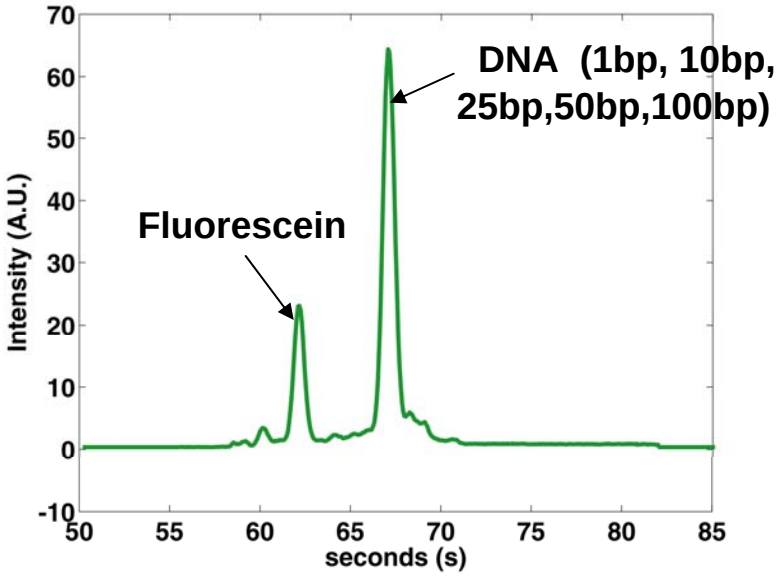
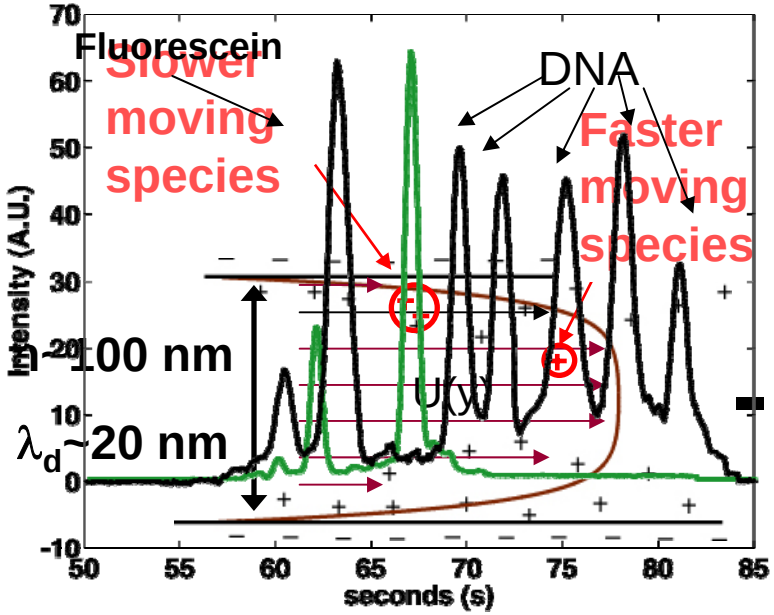
Develop a bioassay separation based on nanofluidic channels for bioagent detection

- **Inherent properties of a nanoscale dimension**
  - Double layer interactions on the order of channel dimensions
  - Steric Interactions
  - Small dimensions enhances reaction kinetics



Microchannel



Nanochannel

Separation occurs due to a transverse electric field and nonuniform velocity profile

Pennathur et al, *Anal. Chem* **2007**

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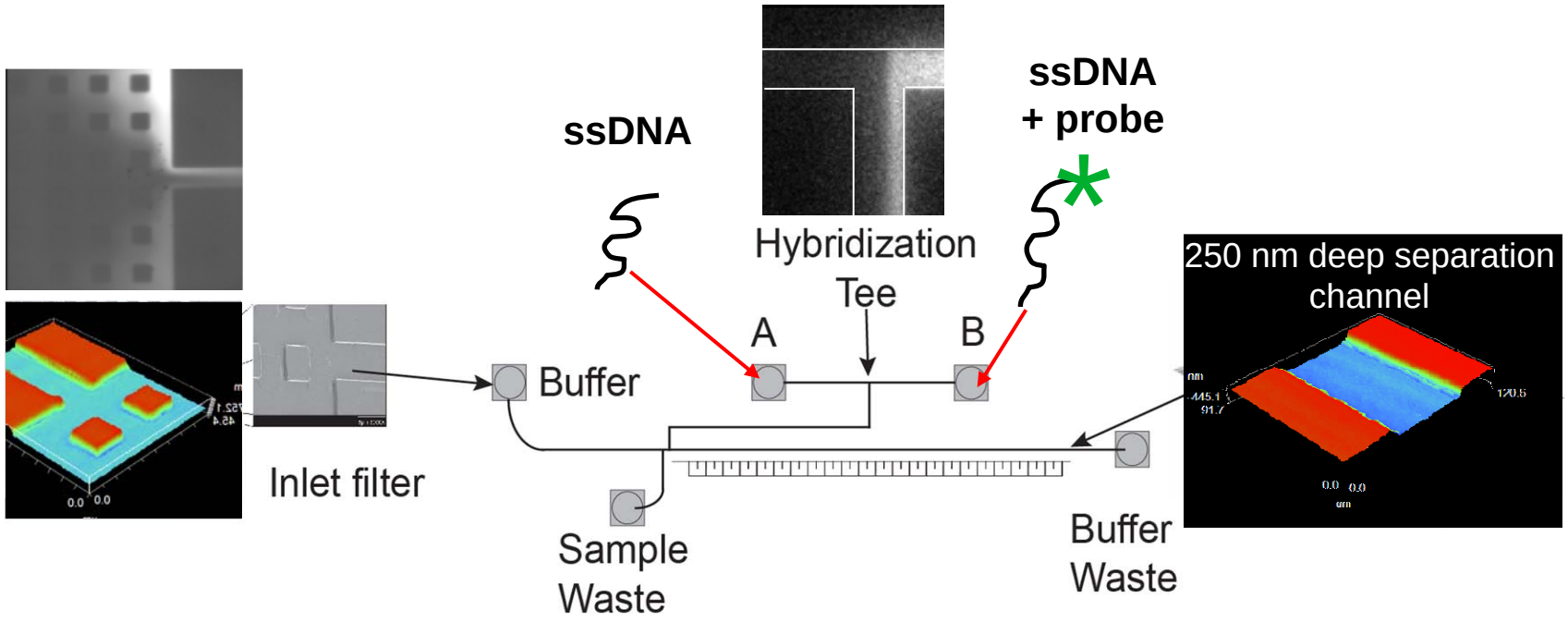
Nanofluidic channels can separate species with same mobility but different charge in free-solution

- No gel or sieving matrix need to separate DNA
- Integrate on-chip hybridization and separation of oligonucleotides (double stranded from single stranded)



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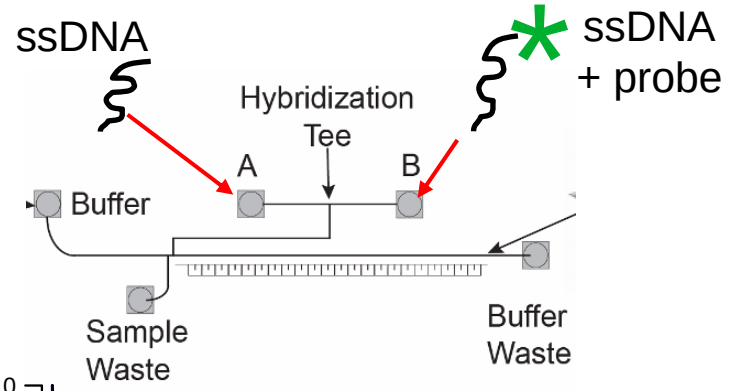
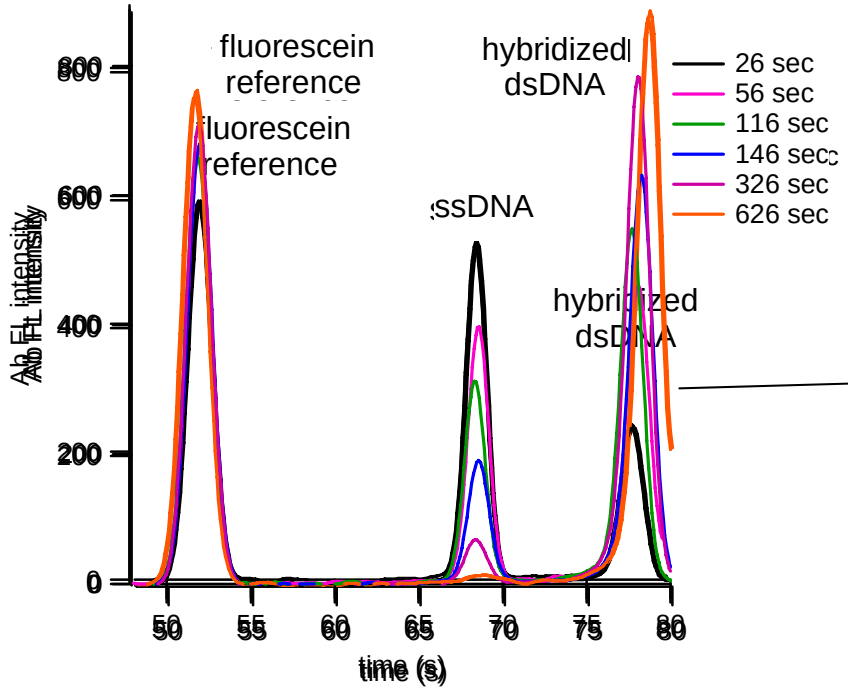
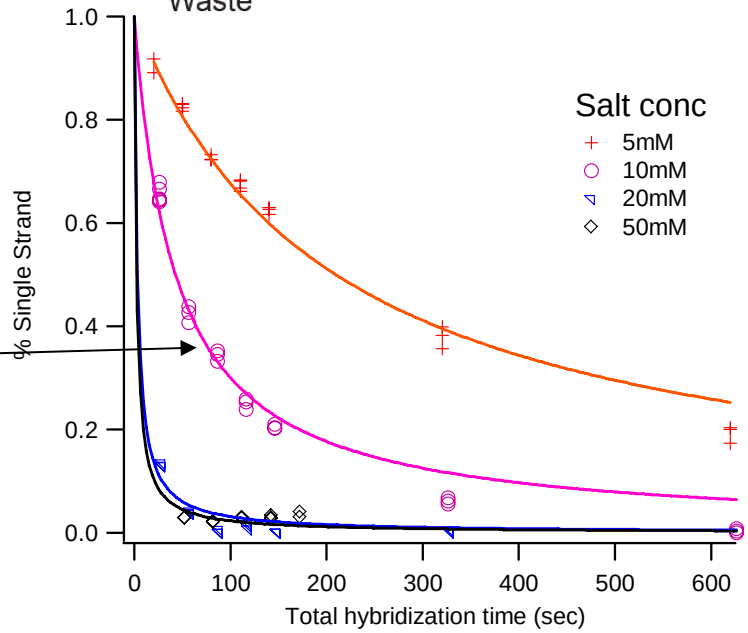
Separation of ssDNA from dsDNA in a nanofluidic device integrated with a hybridization tee

- Hybridization and separation of 22-mer oligonucleotide pair
- 250 nm nanochannel
- Residence time in Tee corresponds to hybridization time







Hybridization rate increases with salt concentration

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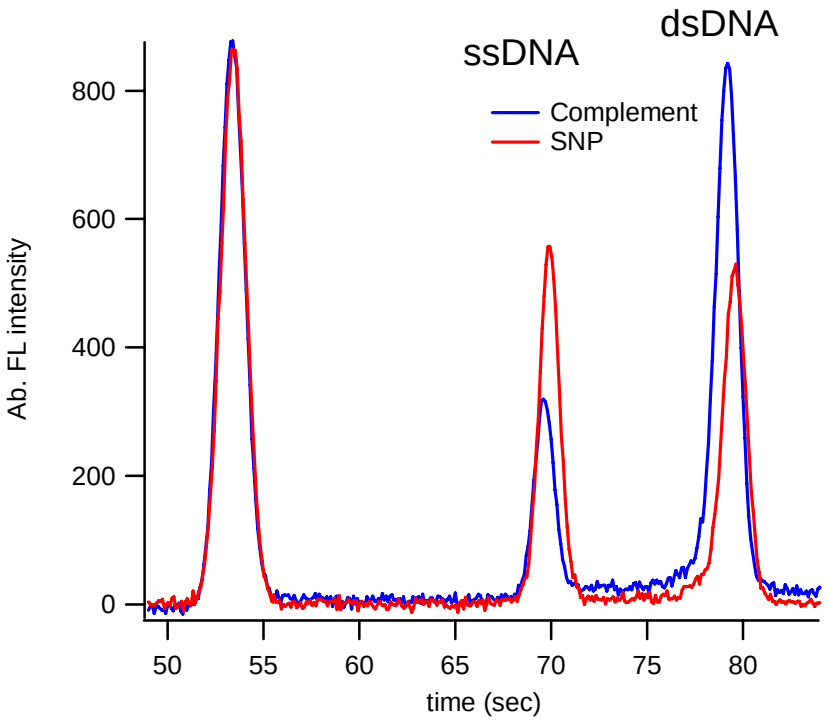
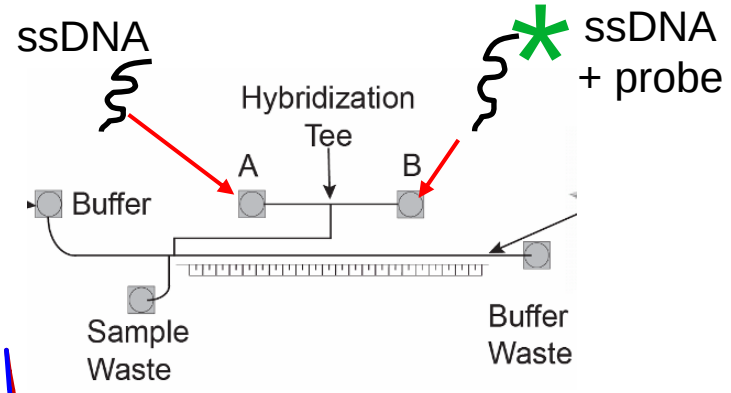
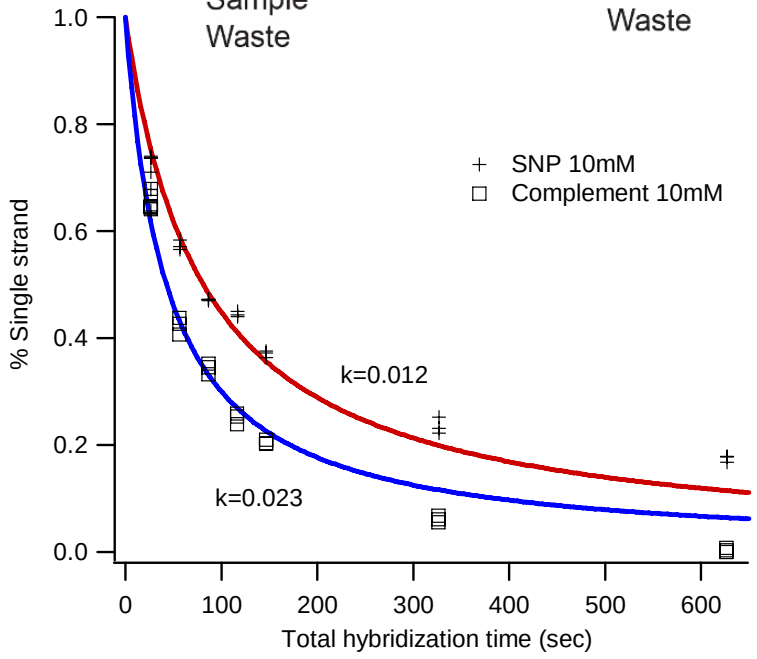
Detection of single nucleotide polymorphism with on-chip hybridization and separation

- **Single Nucleotide Polymorphism**
  - One base pair mismatch
  - Very difficult to detect







SNP shows a slower hybridization rate constant

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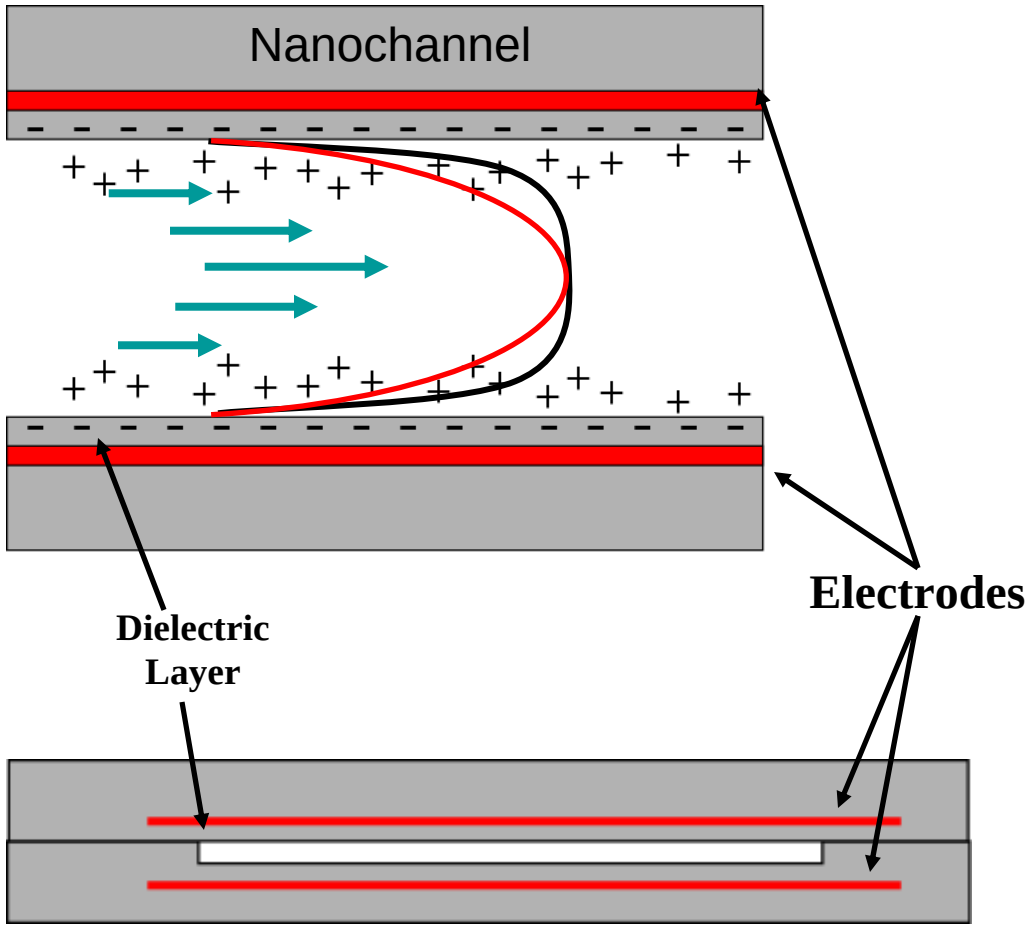
Actively control electrical double layer within a nanochannel to enhance bioseparations

Explore the coupling of transverse and axial applied electrical fields in a nanochannel

- **Distinct Advantages**
  - •Control and optimize separations
  - •Minimize surface adsorption
  - •Switchable preconcentration
  - •Make microchannels behave look like nanochannels



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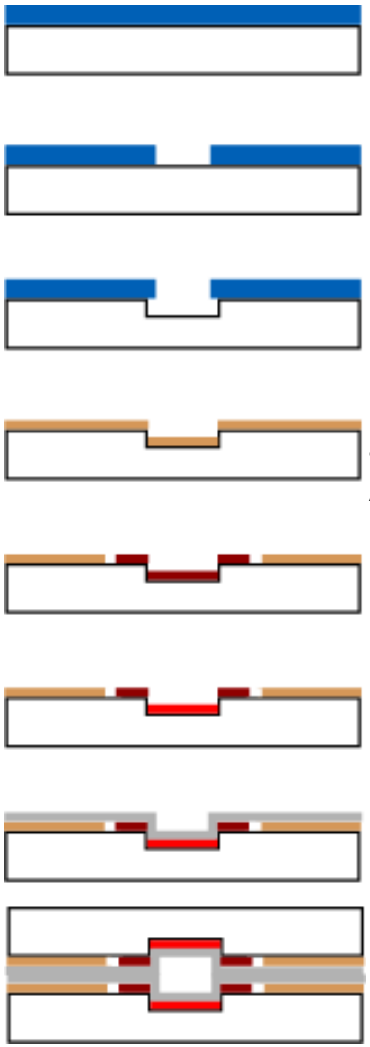
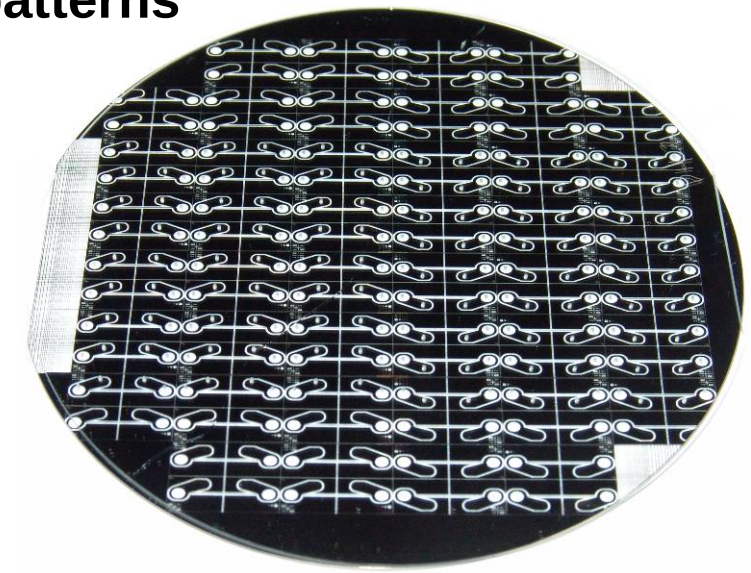
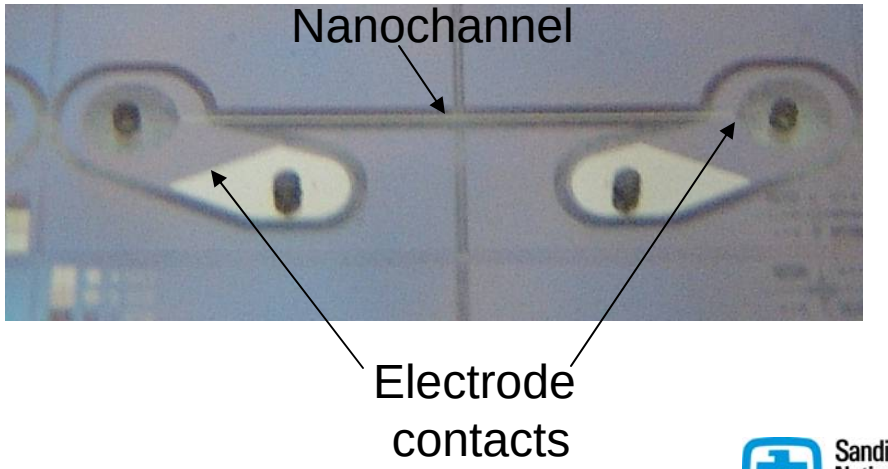
Buried electrodes integrated with nanochannels

- **18 step process requiring 4 mask patterns**







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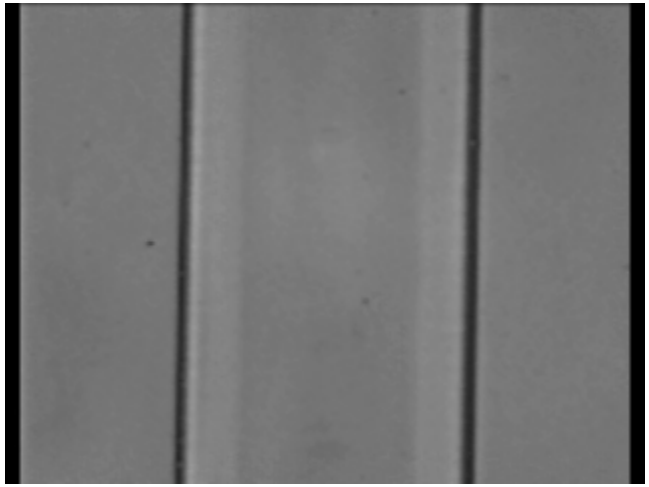
Rapid Fluorescence-activated Cell Sorting using Photonic Forces in a Microfluidic device

T. Perroud, T. Lane, J Sy, J. Kaiser, A. Singh, and K. Patel



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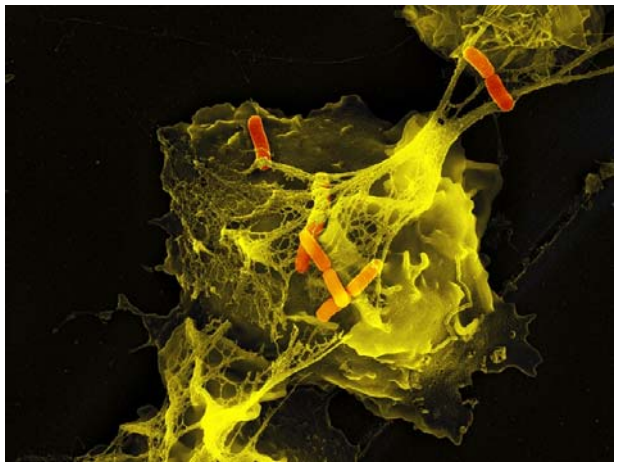
MISL—Microsystems Immune Studies Laboratory

Our overarching goal is elucidation of molecular mechanisms of host-pathogen interaction



Innate immunity is our first line of defense against microbial invasion.

Subversion of innate immunity is a virulence strategy used by a number of pathogens.

Molecular-level understanding is key to improvements in: Diagnostics Prophylaxis and Therapeutics

Significant impact in Biodefense and Infectious Disease research

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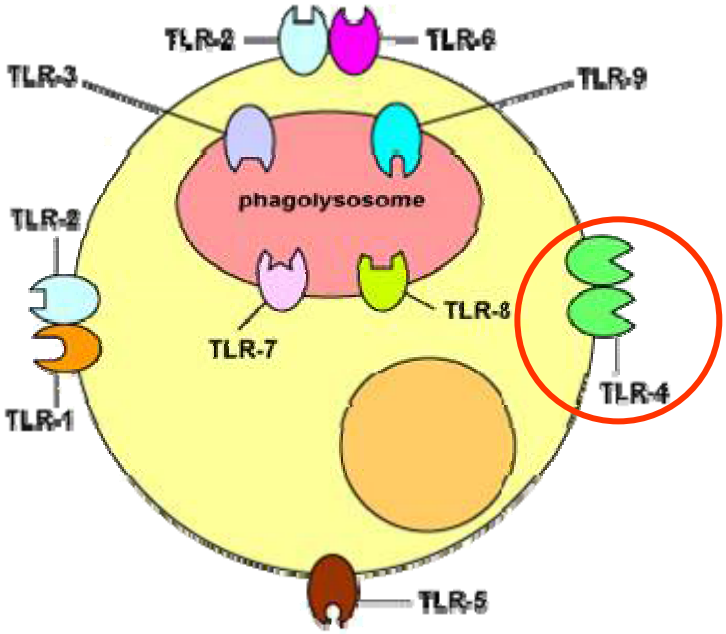
Toll-like receptors (TLRs) in macrophage recognize microbial pathogens

Model host: Macrophage

Key sentry cells of innate immune system

- Recognize, ingest, & kill microbes
- Have key recognition molecules called TLRs that recognize pathogen associated molecular patterns
- Activate neighboring cells, recruit them to site of infection



Lipopolysaccharide (LPS) to TLR4 receptor - ligand binding initiates signaling pathway that leads to macrophage activation

F. tularensis and *Y. pestis* virulence factors play direct roles in subverting TLR4-mediated signaling.

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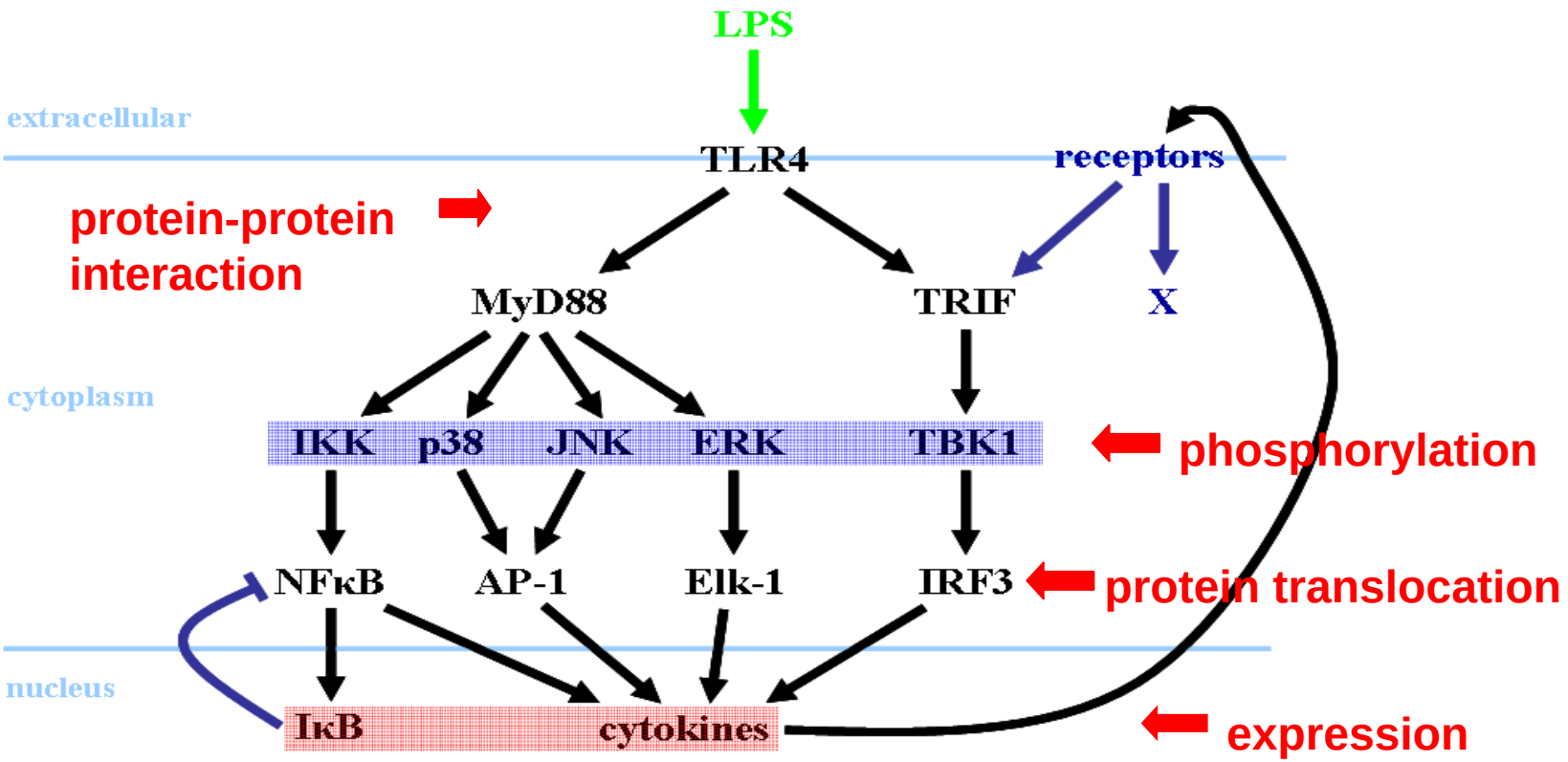
TLR4 signaling pathway upon LPS recognition



Our approach integrates biology, microsystems platform & computational modeling

Biological Goal

Elucidate TLR4 signaling pathway in the innate immune response of host cell to a pathogen

Variable to be measured

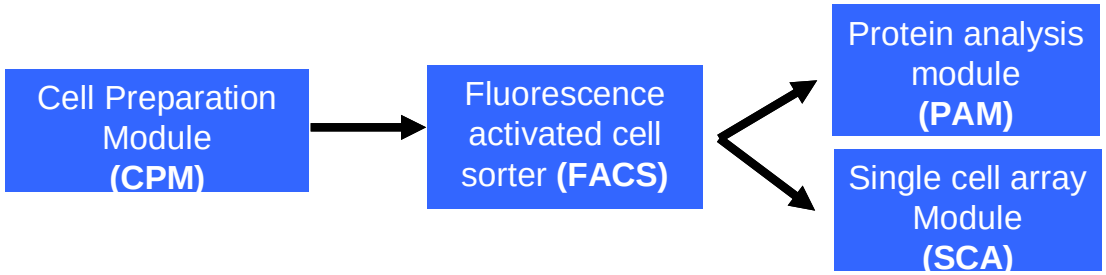
- **Phosphorylation**
- **Protein translocation**
- **Protein concentration**
- **Protein-protein interaction**

Host: Mouse Macrophage

Pathogen: *Francisella tularensis, Yersinia Pestis*

Reporters: Fluorescent antibodies, fluorescent fusion proteins

An integrated platform capable of quantitative and high-throughput proteomic measurements at a single cell level



Create a predictive model for TLR4 signaling pathway, generate hypotheses for specific variables to be measured

Biology Core

Platform Core

Computational Core

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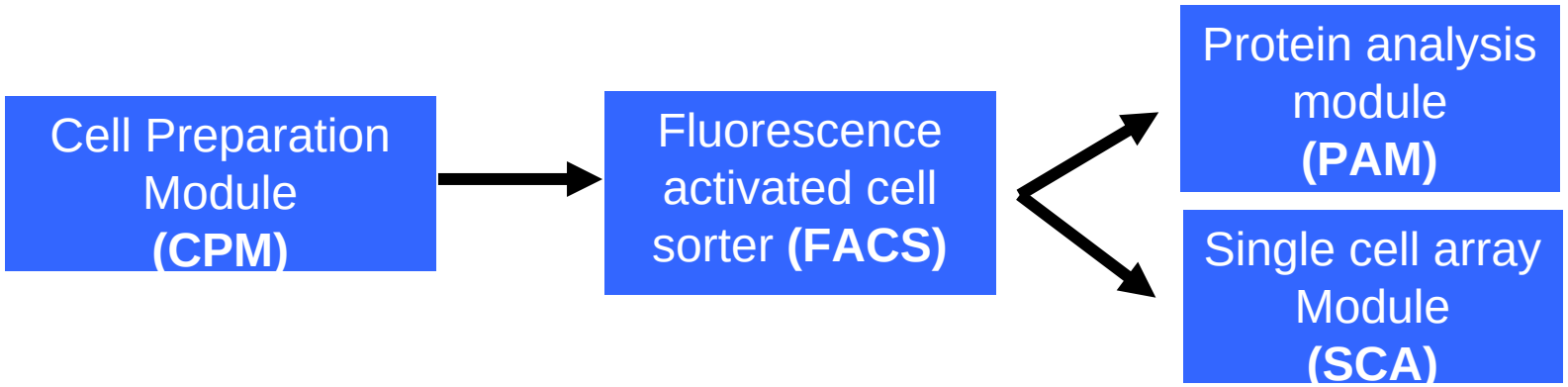
Cell sorting is central for connecting flow cytometry output to downstream analysis modules



High-throughput sorting requirements

- Selective for single-cell array module
- Rapid sorting mechanism (up to 50 cell/s)
- Simple chip fabrication
- High duty cycle and rare sorting capabilities

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Cell sorting technique for the platform is photonic-force deflection

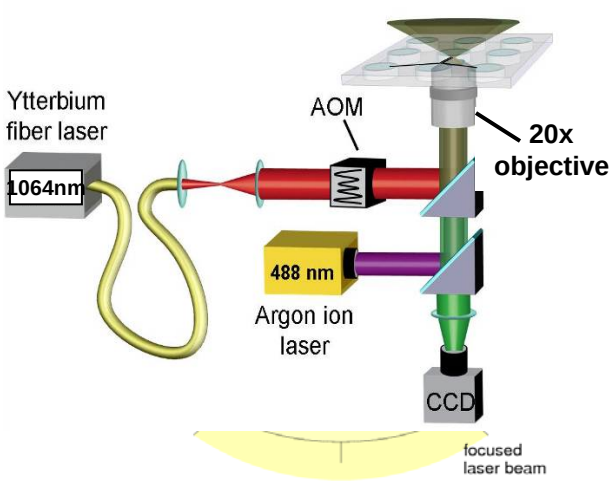
Photonic-force deflection

- Technique based on laser tweezers
  - Adapted from Wang[1] and McDonald[2]
- Use low NA objectives
- Use a high-power near-IR laser

1. Wang *et al. Nat. Biotech*, 2005
2. McDonald et al. *Nature,* 2003

Laser Tweezers

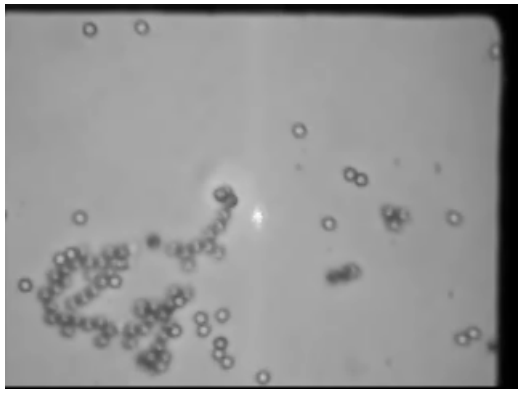
- Focused laser-beam to "trap" objects



http://physics.nyu.edu/grierlab/hot.html

Advantage for microfluidic systems

- Fast, selective, and easy to integrate on a chip
- Noninvasive: sterile, safe
- Location independent technique
- Potential for different sorting schemes



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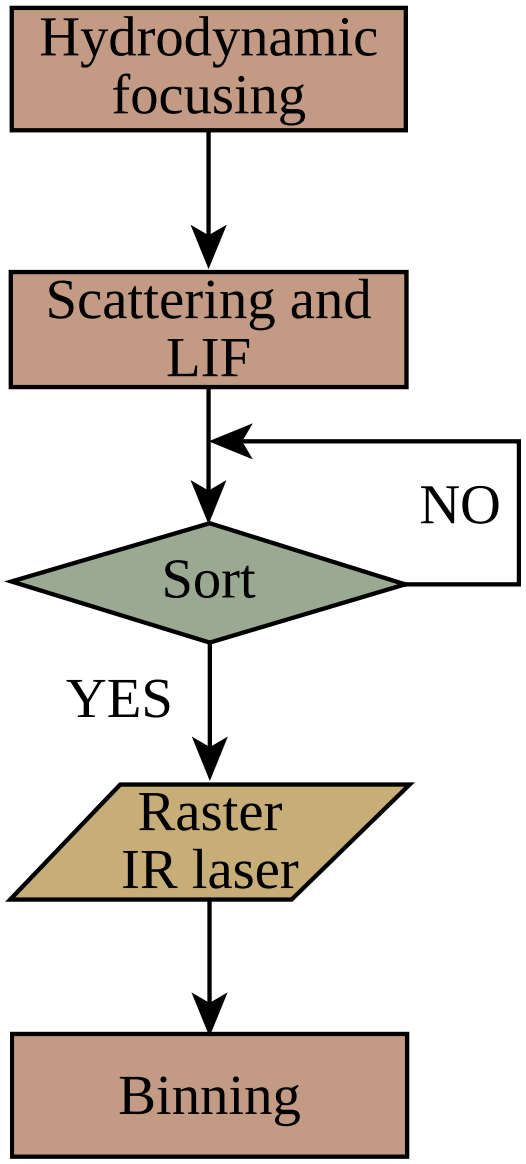

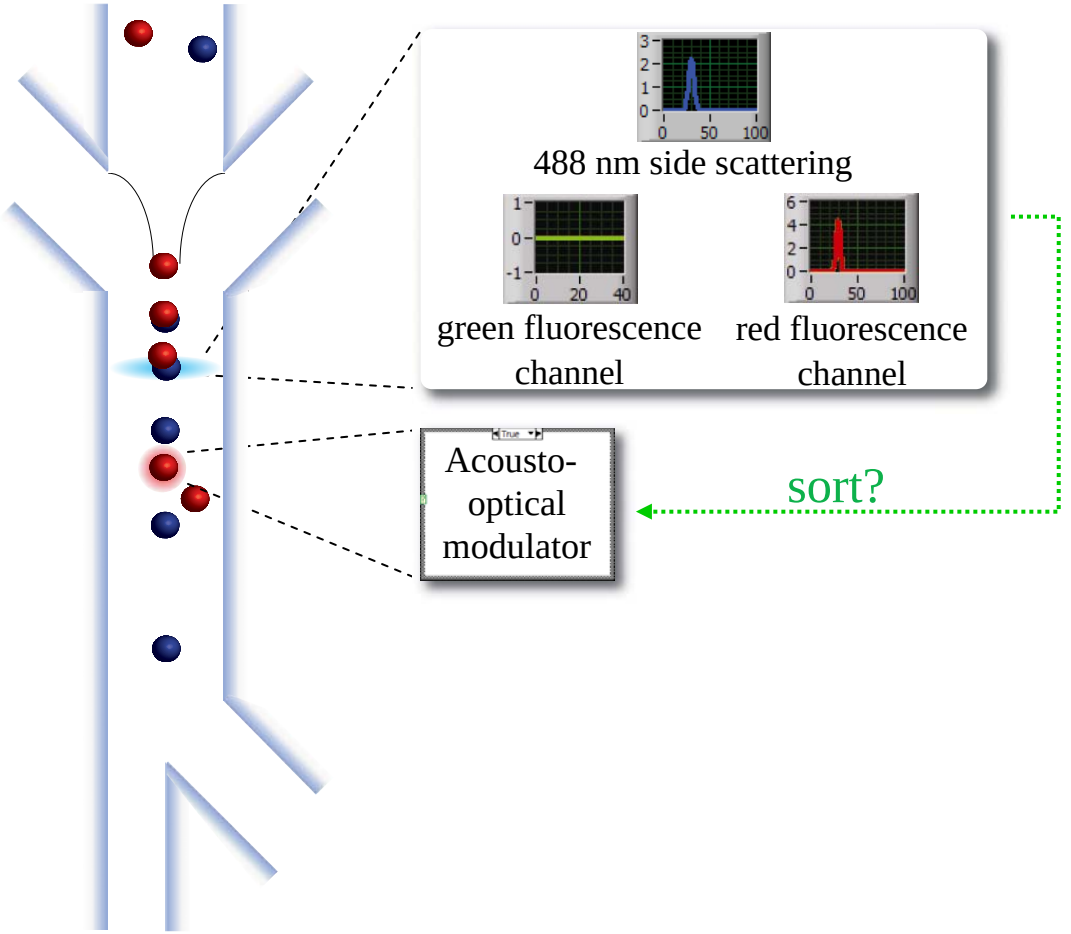

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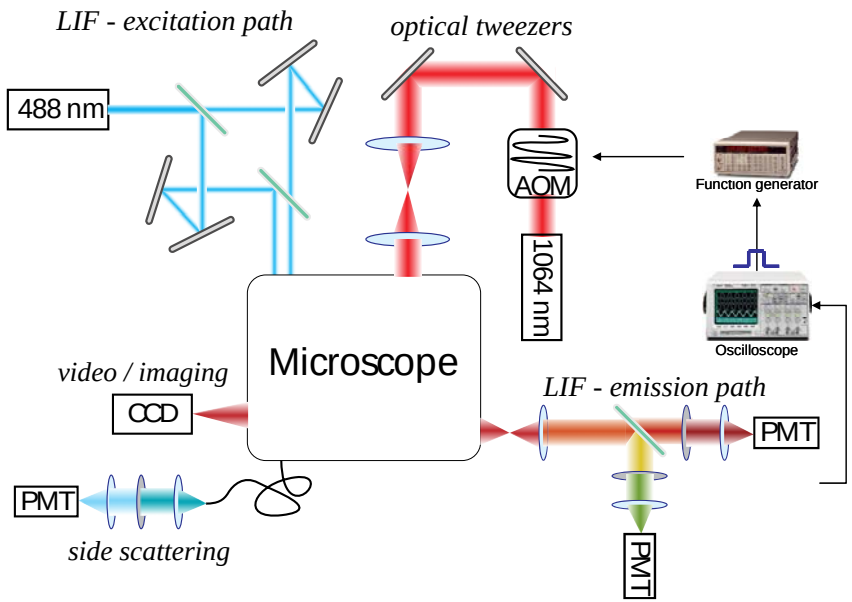
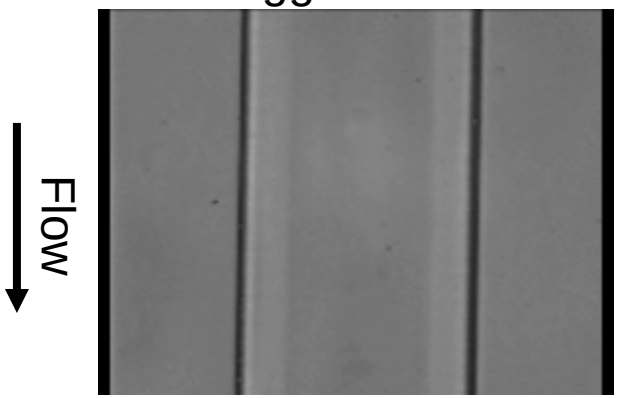
Schematic of μFACS with optical deflection

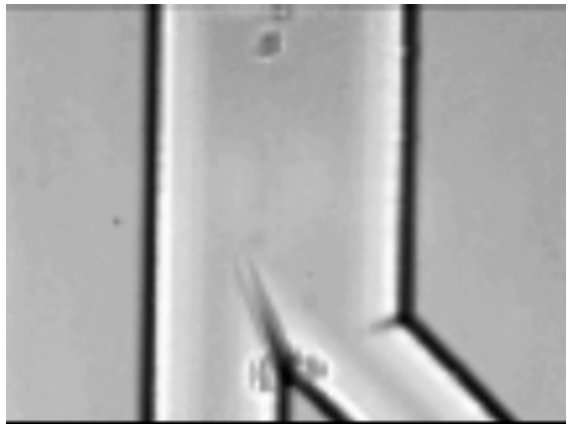


AOM trigger



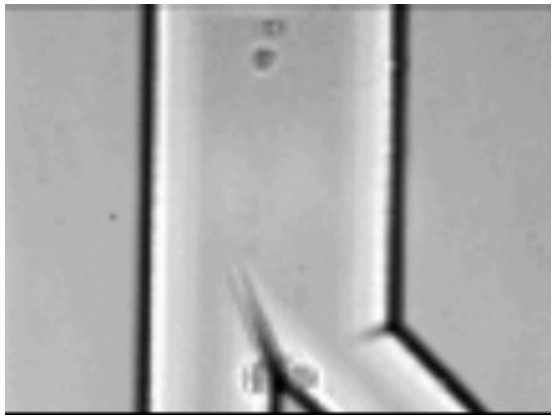
Slowed down 13X from 400 fps

Laser off—no sort



Waste Collection

Laser on— 100% sort



Waste Collection

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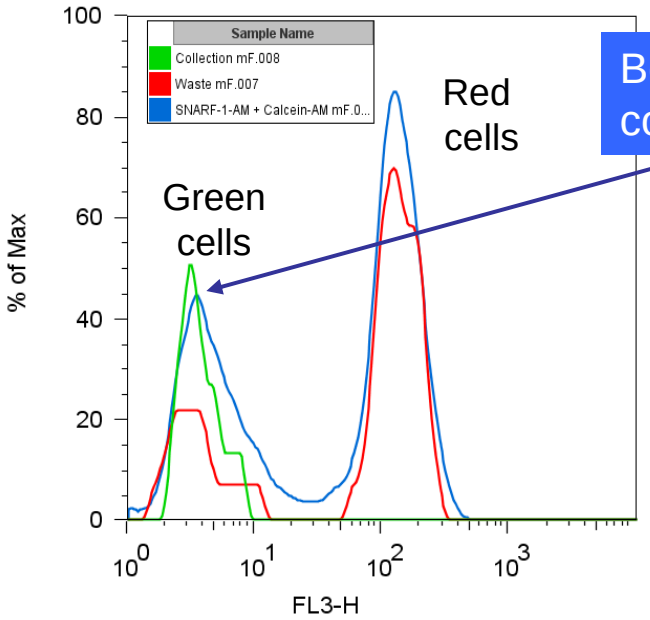
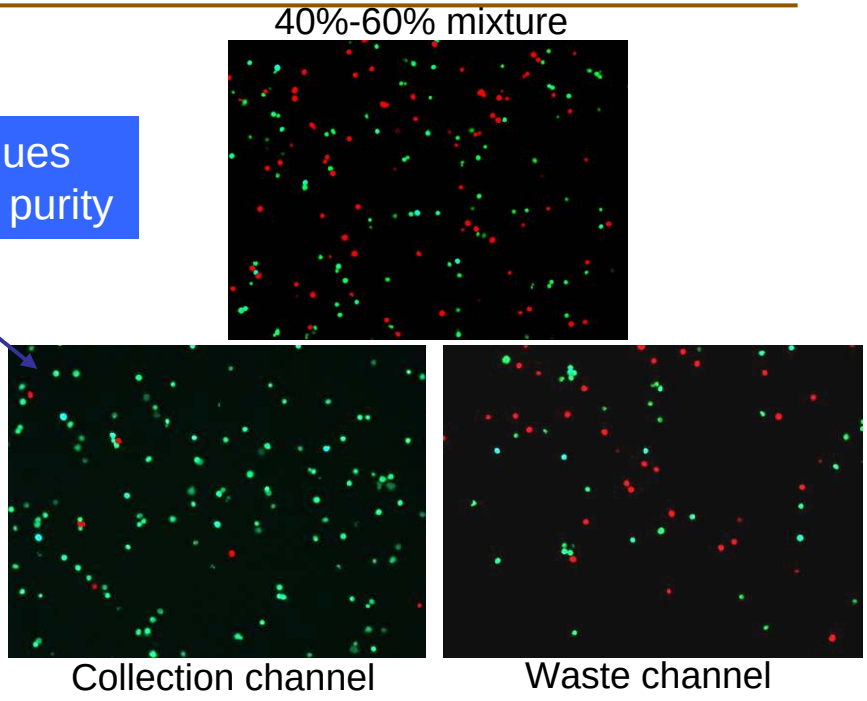
Optical deflection sorting performance



FACScan benchmark

Both techniques confirm high purity



Fluorescence microscopy

Green cell / Red Cells	Throughput	Recovery	Purity	Cells sorted
90/10 (High-duty cycle)	**16 cells/s**	**55 ± 5**	**97 ± 3**	**21,698**
40/60 (Medium-duty cycle)	22 cells/s	60 ± 10	93 ± 3	38,877
10/90 (Rare-cell sort)	**14 cells/s**	**63 ± 9**	**75 ± 10**	**24,847**

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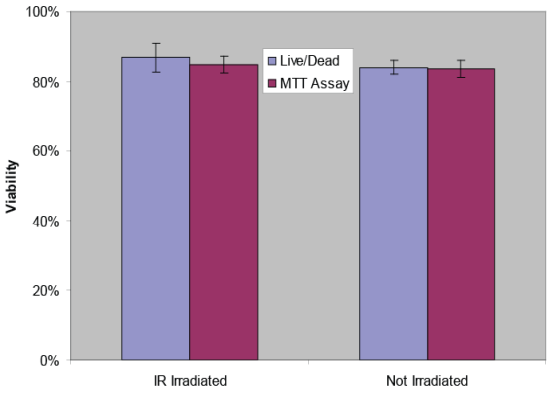
Effect of the near-IR laser on macrophages

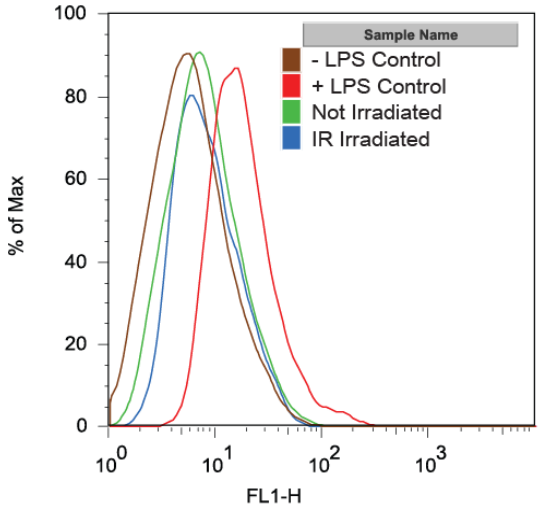
- 9.6 Watts of 1064nm at sample
- Short 2-4 msec interaction time

Viability/Proliferation



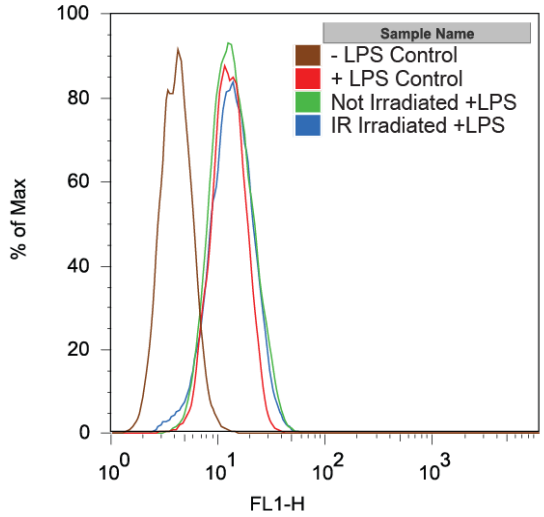
Live-dead stain asses short-term viability,
MTT assay measures mitochondrial activity

Activation



ERK phosphorylation assay confirms IR laser does not activate macrophages

Functionality



IR laser irradiated cells challenged with LPS show ERK phosphorylaton

Results confirm that the laser is valid technique to sort macrophage cells

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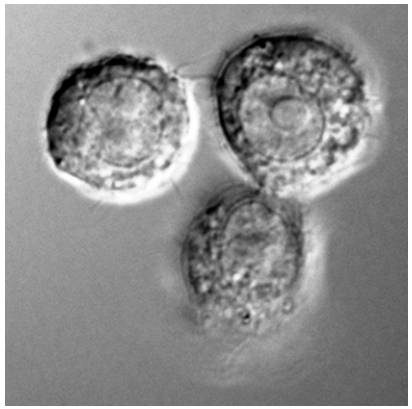
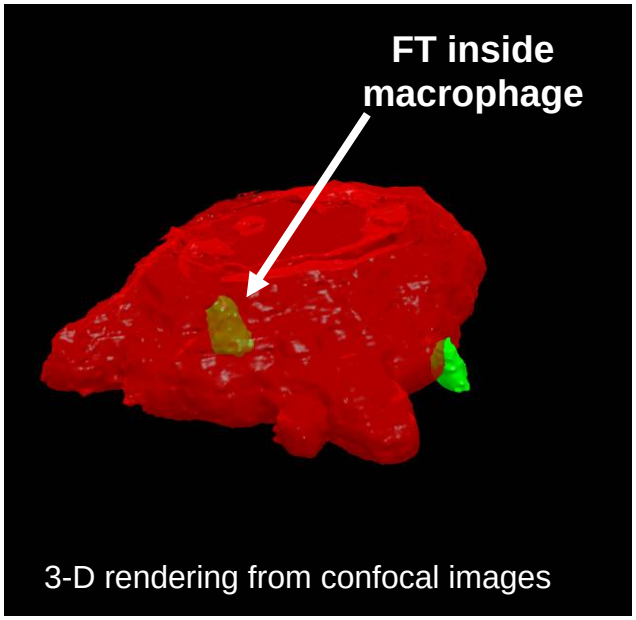
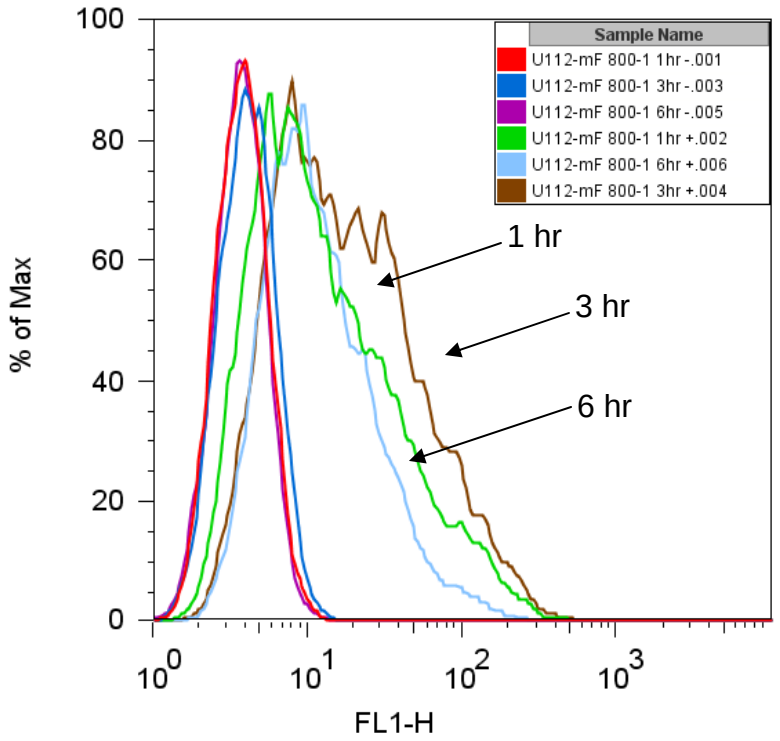
Enrichment of subpopulations of macrophages infected with the pathogen Francisella tularensis







Flow Cytometry results confirms FT infection

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Acknowledgements

Nanofluidic Chromatography

David Huber
Marci Markel
Mike Bartsch
Dave Heredia
Sumita Pennathur
Matt McCrink

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

MISL-multi disciplinary team

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PM: Glenn Kubiak

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Cathy Branda
Todd Lane
Julie Kisiar
Jens Poschet
Roberto Rebeil
Zhaoduo Zhang
Bryan Carson
Meiye Wu
Julia Kaiser

UTMB
Allan Brasier Group

UCSF
William Seaman Group

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Kamlesh Patel
Thomas Perroud
Ron Renzi
Mike Sinclair
Nimisha Srivastava
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Questions

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