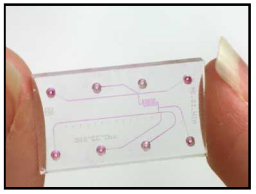
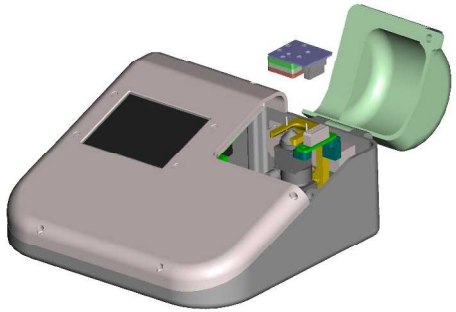
Rapid Automated Portable Infection/exposure/disease *Dx*

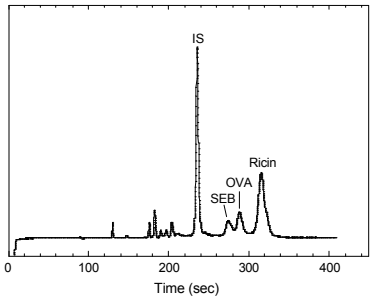
SAND2008-5963P

RAPI*Dx*

Greg J. Sommer
Biosystems Research Dept.







Better, Faster, Cheaper, Smaller

Sandia is a multi-program laboratory operated by Sandia Corp., a Lockheed Martin Co., for the United States Department of Energy under Contract DE-AC04-94AL85000.

Acknowledgements

Funding Sources:

NIDCR (Saliva Diagnostics)

NIAID (Toxin Diagnostics)

LDRD (Biomarker Verification, Deployable Dx Pathogen Exposure)

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Ron Renzi
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Gabriela Chirica
Bal Ram Singh (UMASS Dartmouth)
Easwaran Ravichandran (UMASS Dartmouth)
Will Giannobile (U Michigan)
Steve Binder (Bio-Rad)

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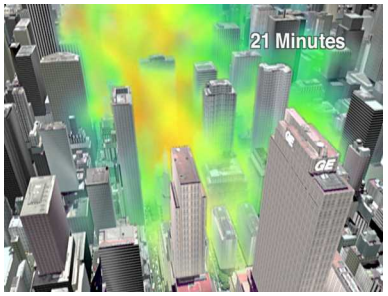
Versatile Platform

Exposure

City



Crowd

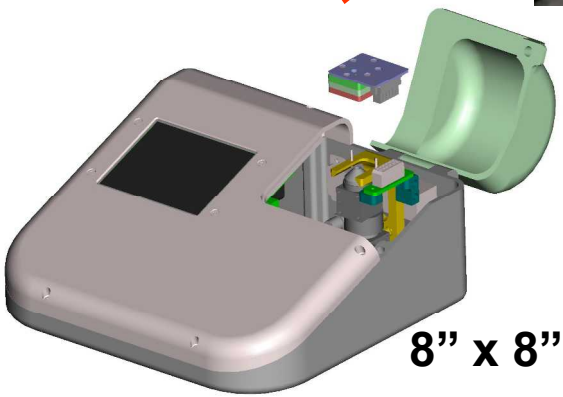
Field

Portable, Rapid Triage

Low Cost, Non-invasive, Convenient and Easy to use

Screening

High Risk Traffic



8” x 8” x 4”

Routine Clinic Visit

BSL Compatible

Research

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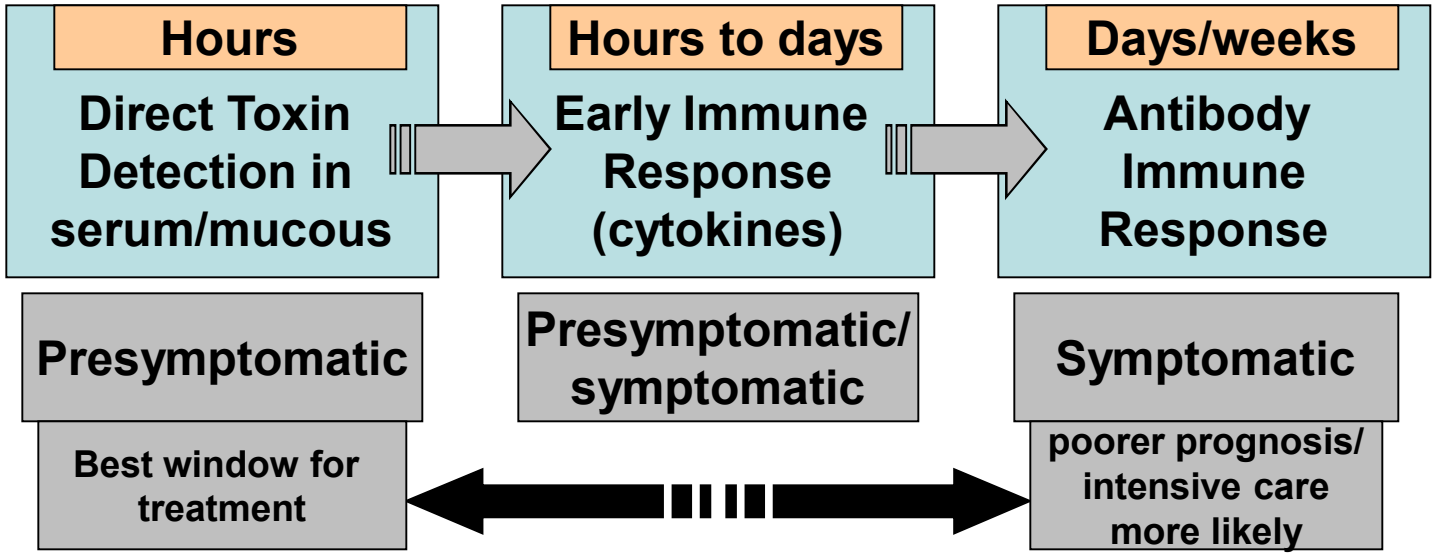
Biomarkers of Toxin Exposure

Detection and treatment windows following exposure



BoNT A,B, E
Ricin
SEB
Shiga I,II
Epsilon toxin

IL-6
IL-2
TNFa
IFNg

IgM
IgG

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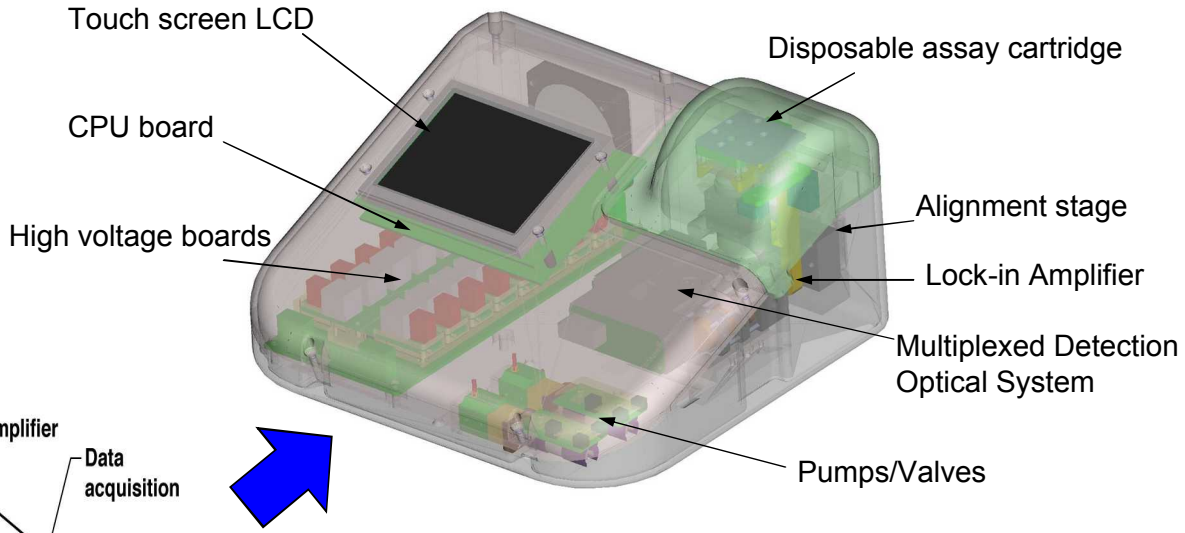
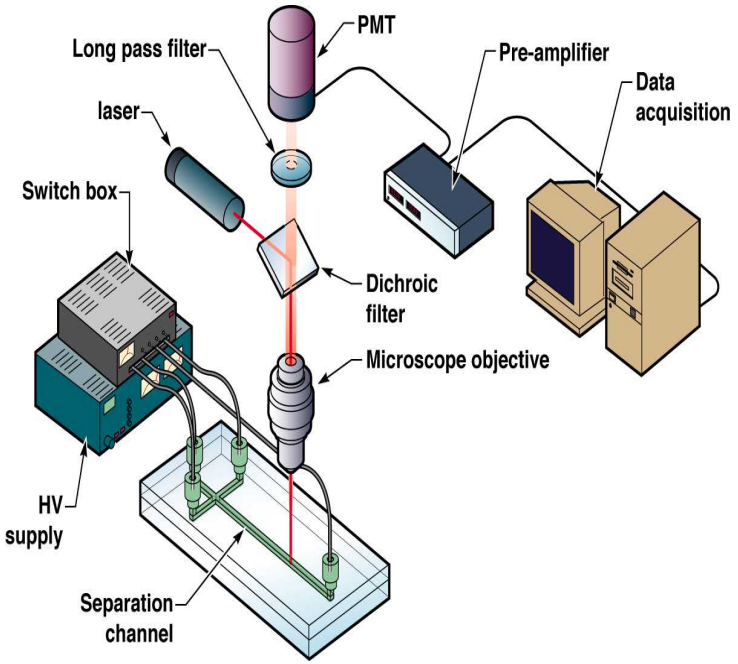
Deployable System

Powerful & Adaptable





Salient Features

- Microfluidic platform: gel shift assays
- Self-contained
- Easy to use
- Microliters of blood or saliva (non-invasive)
- Rapid (<10 min sample to result)
- Multiplexed (up to 32 assays simultaneously)
- Low cost per assay
- Low risk to operator
- Sensitive (0.1 pg/ml, 10 fM, Detection limit)

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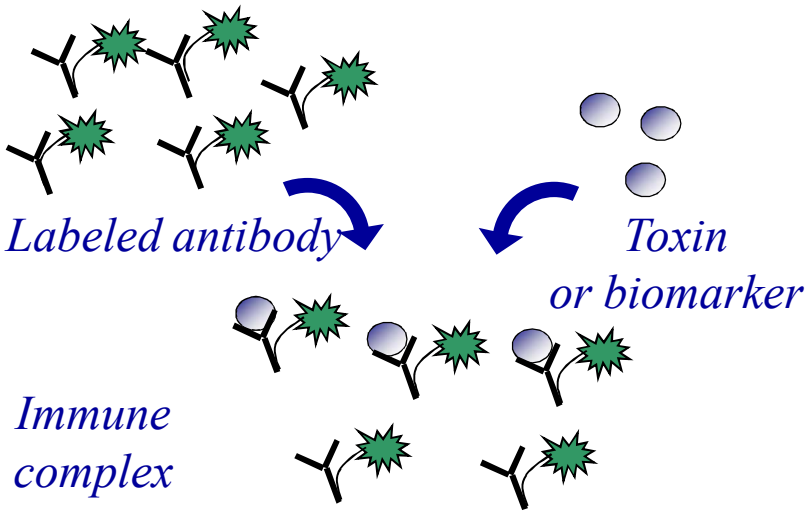
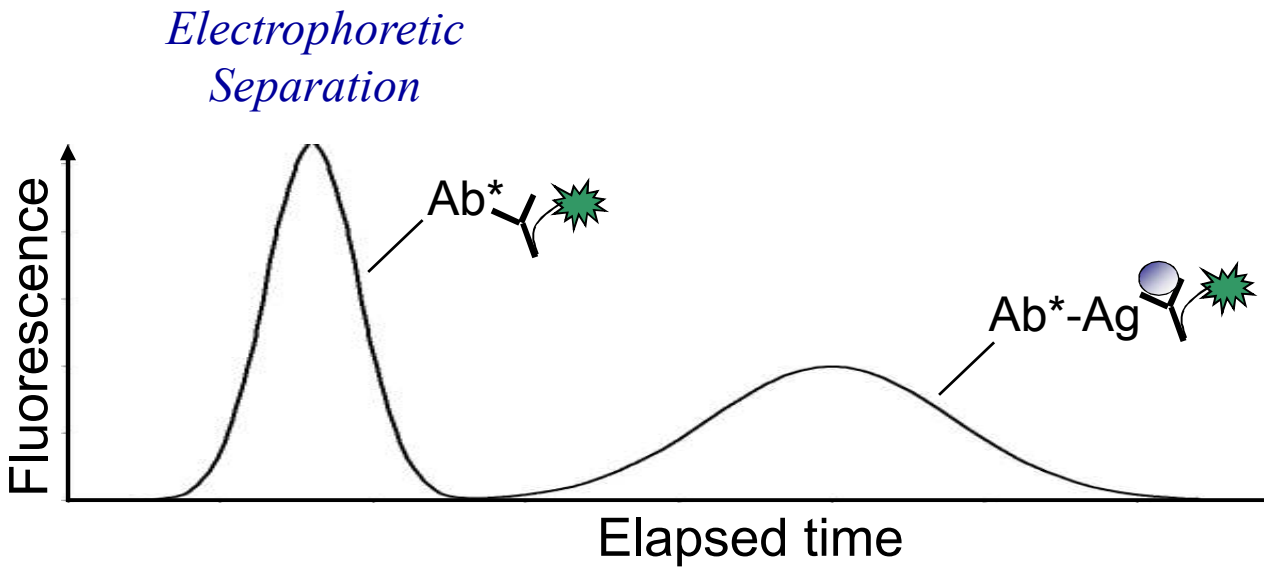
Microfluidic Immunoassays





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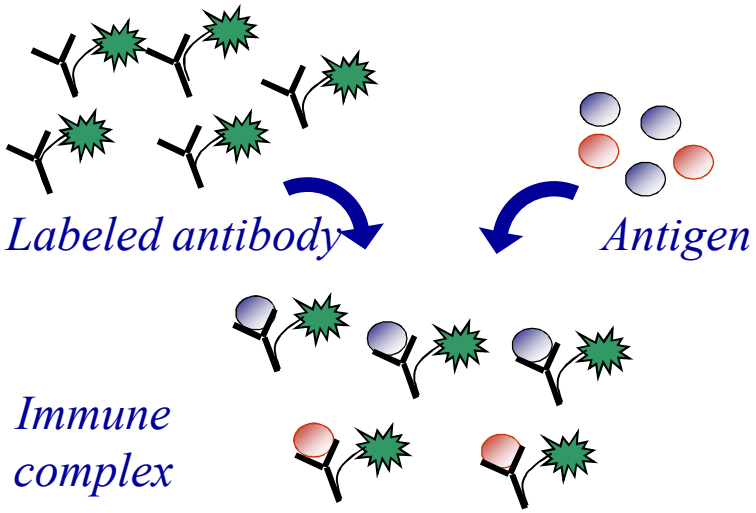
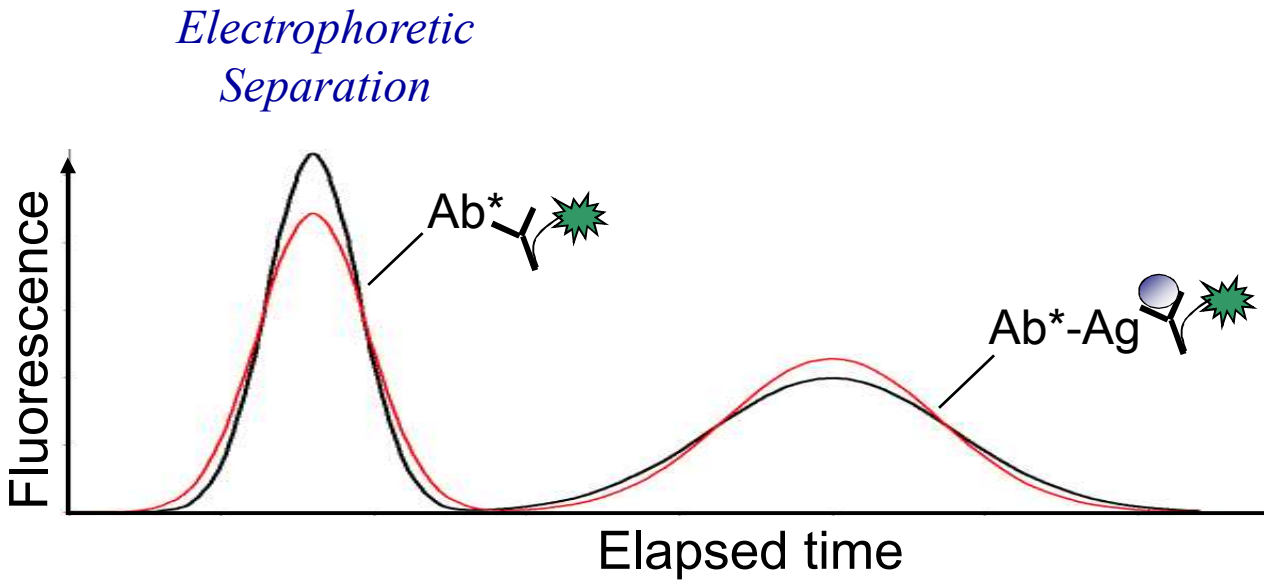
Microfluidic Immunoassays





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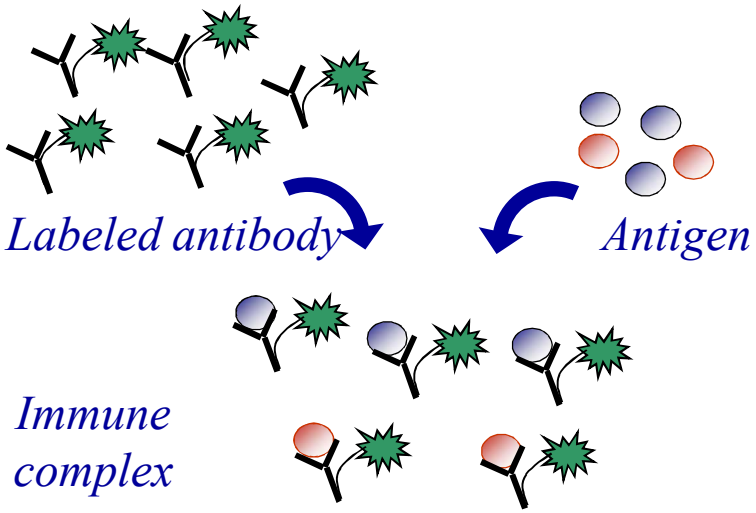
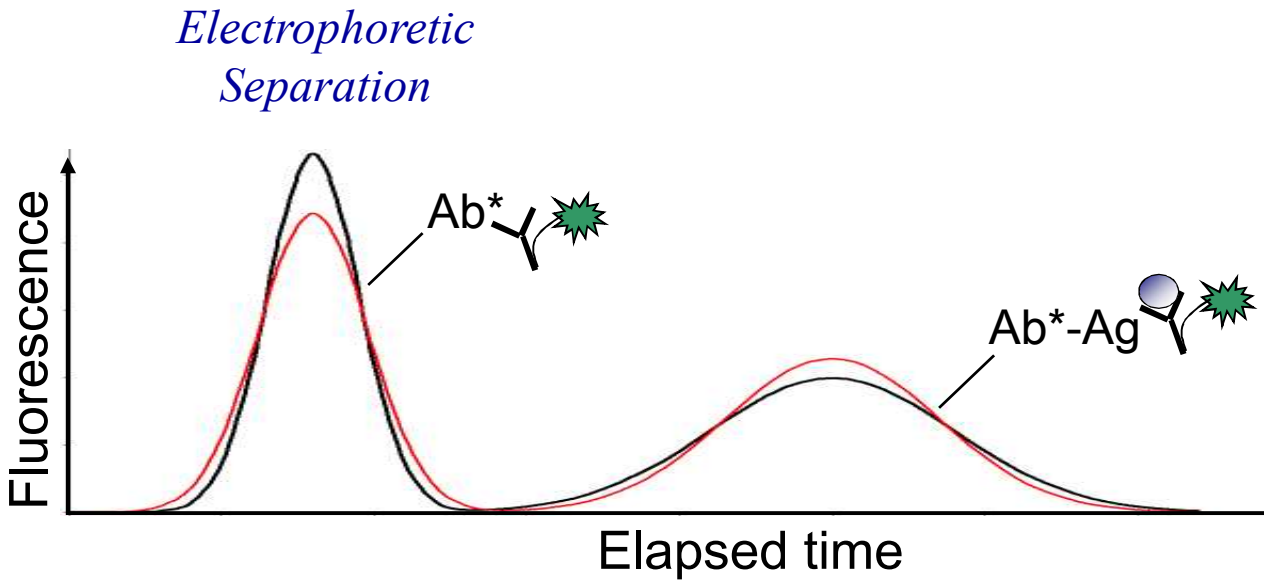
Microfluidic Immunoassays



- Solution phase technique
- No antibody immobilization
- No secondary antibody required
- Rapid, reproducible binding
- Simple electrokinetic manipulations on-chip
- Only specific Ab probes are labeled
- Clean baselines and easily interpreted electropherograms



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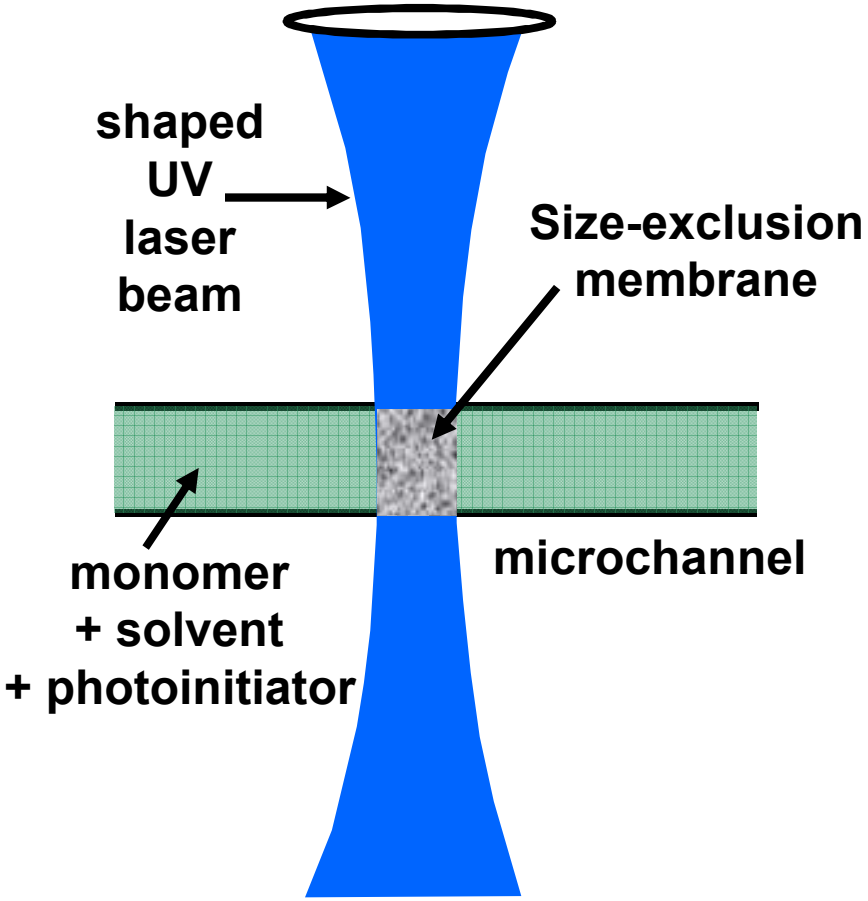
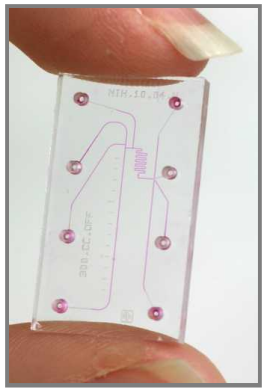
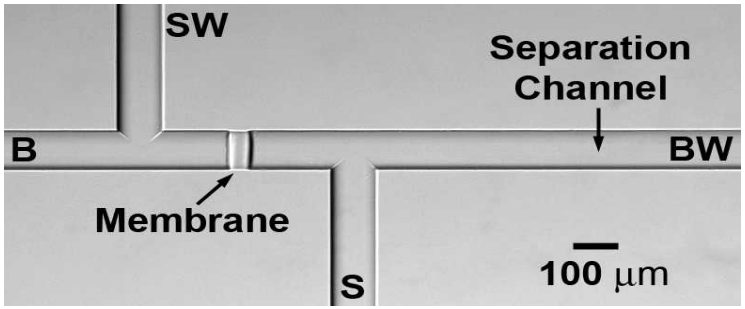
Gel/Membrane Fabrication







Hatch et al., *Analytical Chemistry*, 2006

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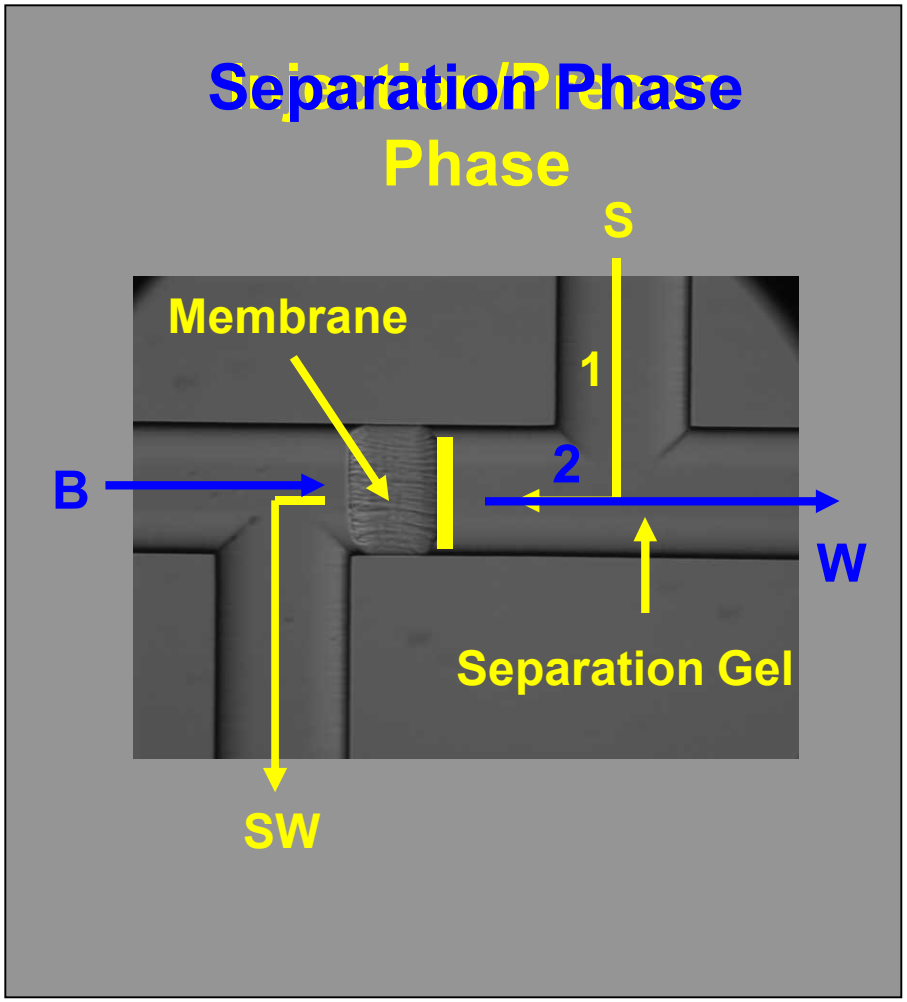
Meeting Needs for Protein Analysis

- Low abundance markers: need sub-pM detection
- Direct detection limit of LIF system is ~100 pM
- Solution preconcentration
  - Also simplifies on-chip mixing
  - Favorable kinetics for 10^{-9} or lower affinity constants

Hatch et al., *Analytical Chemistry*, 78, 4976-4984, 2006



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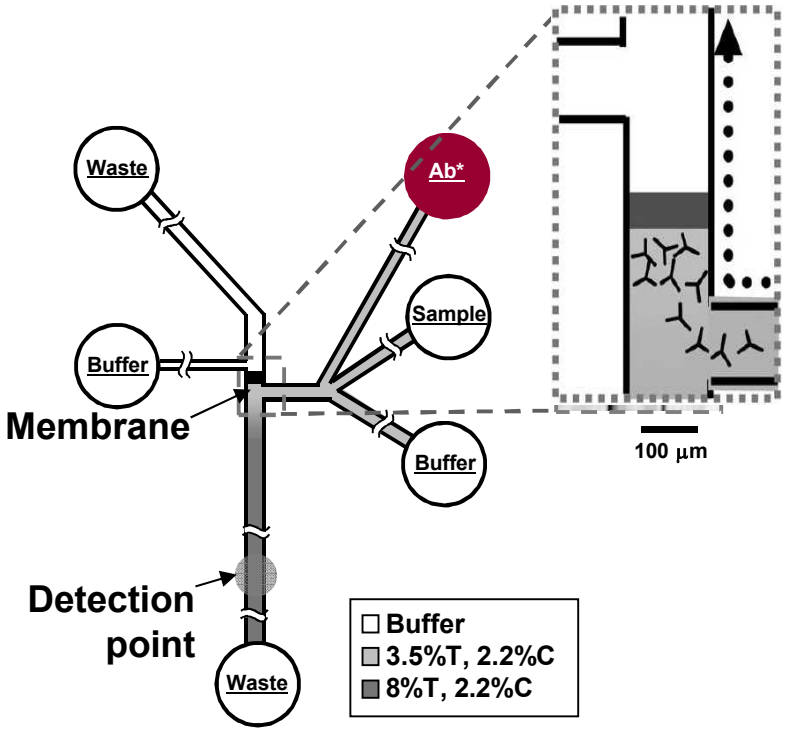
Microfluidic Immunoassays

Load Specific Antibody (labeled)

- *Specific to toxin or biomarker*



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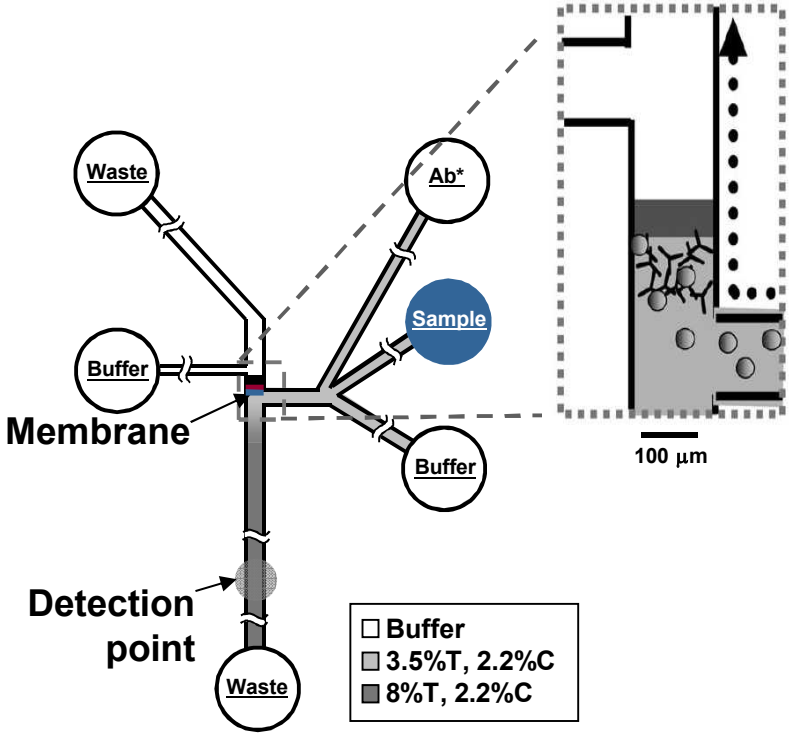
Microfluidic Immunoassays

Load Specific Antibody (labeled)

- *Specific to toxin or biomarker*

Load and Mix Sample

- *Toxin concentrated at size exclusion membrane*
- *Binds to antibody (solution phase)*



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Microfluidic Immunoassays

Load Specific Antibody (labeled)

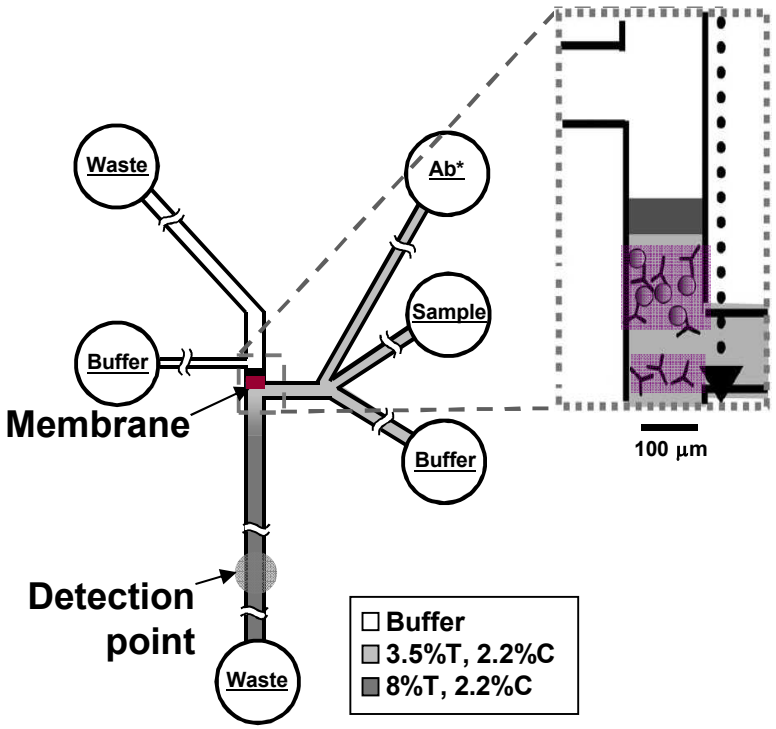
- *Specific to toxin or biomarker*

Load and Mix Sample

- *Toxin concentrated at size exclusion membrane*
- *Binds to antibody (solution phase)*

Electrophoretic Separation

- *Detect level bound*



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Microfluidic Immunoassays

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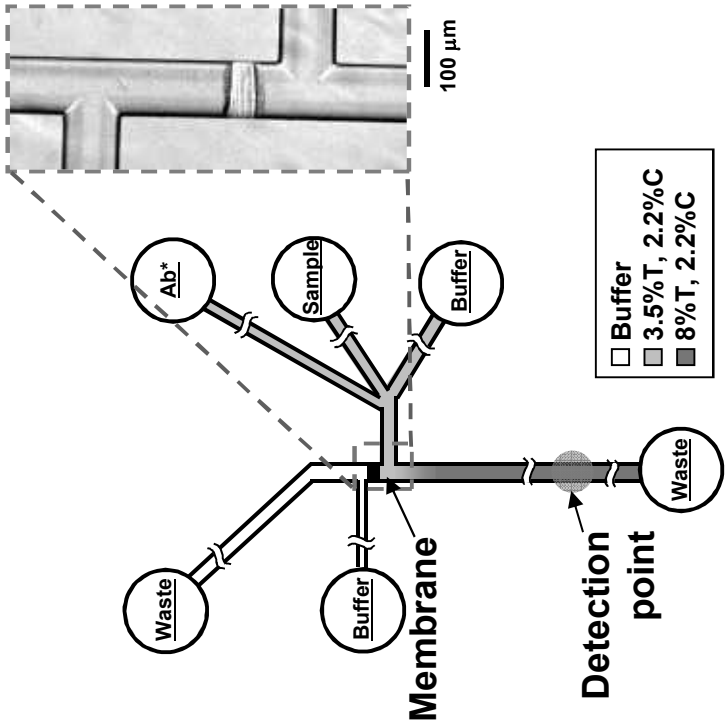
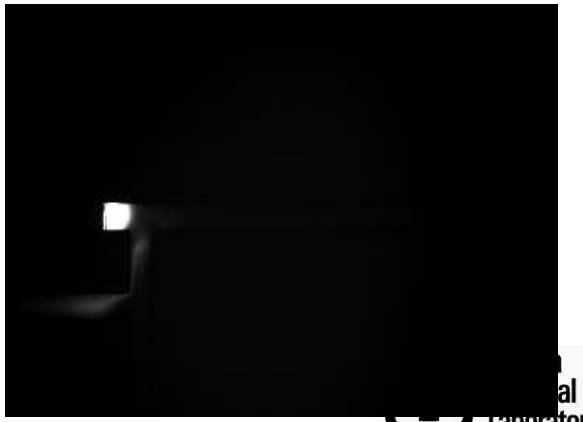
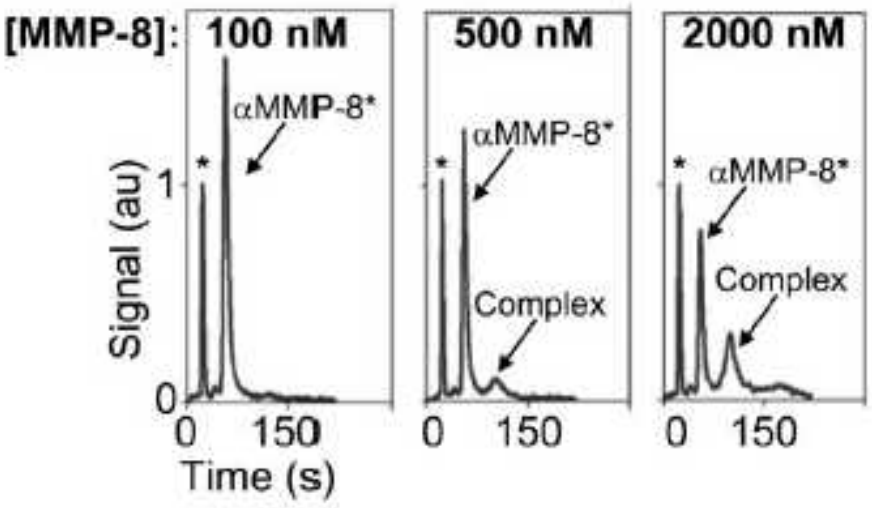




Biomarkers of Disease



- Herr et al, *PNAS* 2007
- Non-invasive saliva sampling
- Stastically validated MMP-8 as biomarker of periodontal disease
- Presymptomatic diagnosis, higher treatment efficacy
  - Traditional diagnosis based on pocket depth, bone loss

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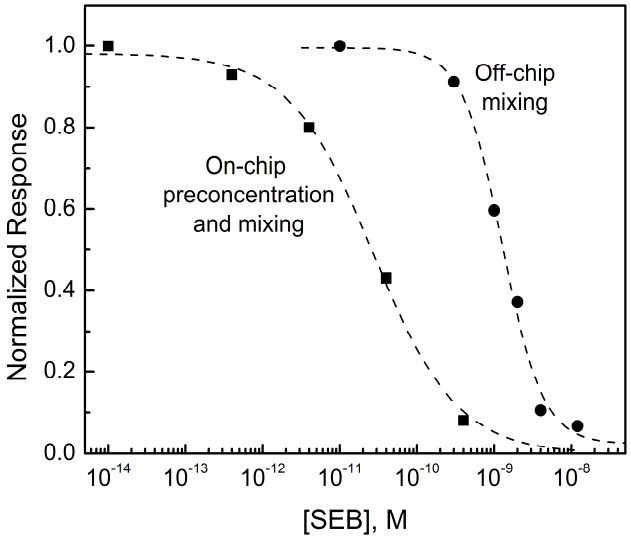
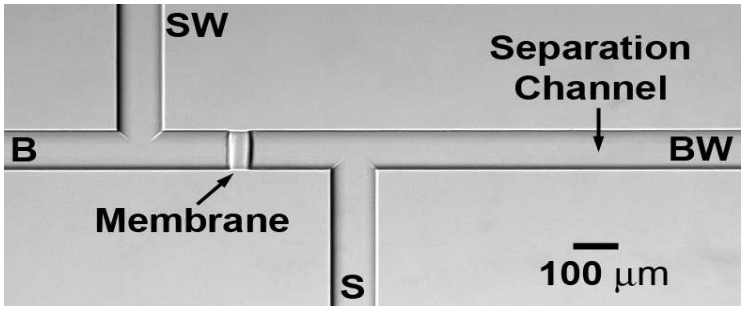
Diagnostics of Exposure

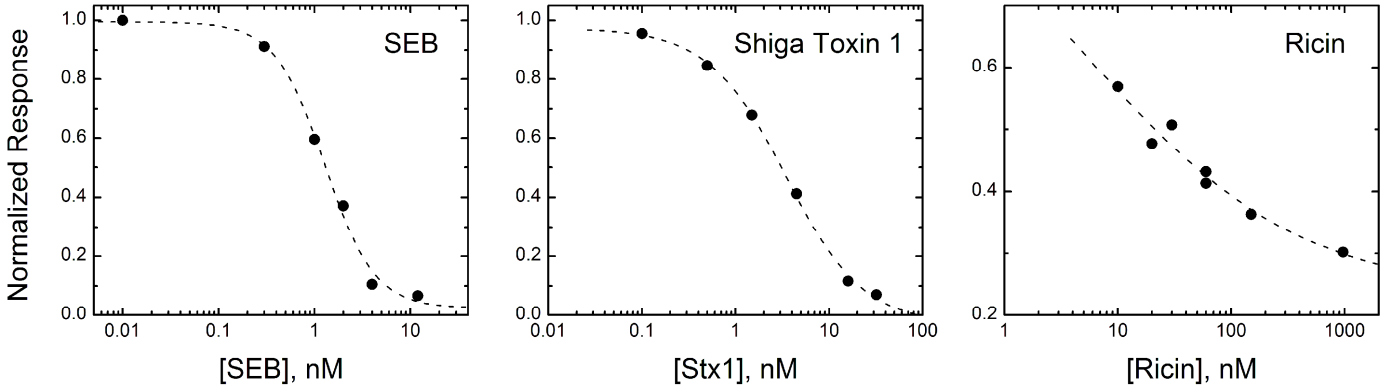
Preconcentration for fM detection





Hatch et al., *Analytical Chemistry*, 2006

Multiplexed Panel of Toxins, Host Response Markers – NIAID Funded U01



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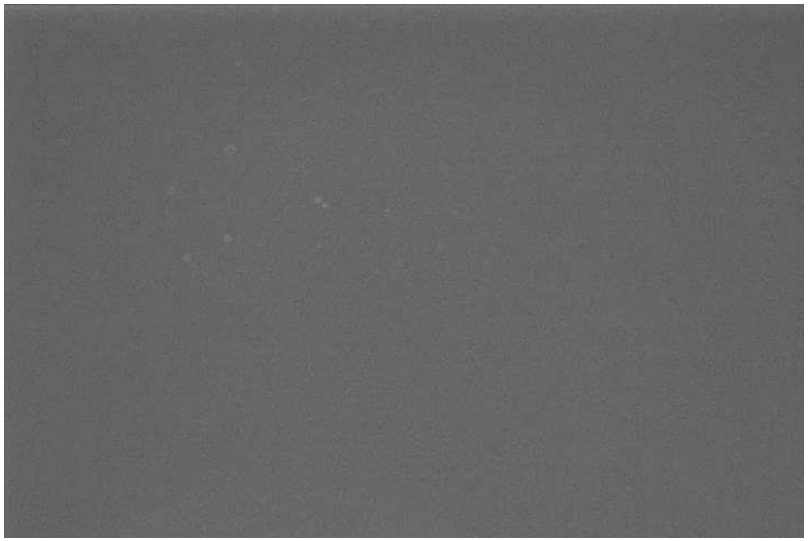
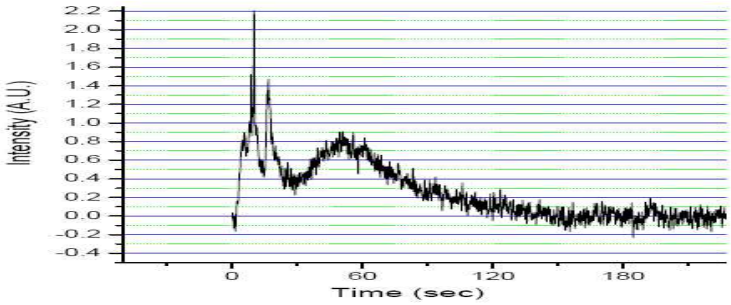
Meeting Needs for Protein Analysis

- Wide Channel Preconcentration:
  - Direct laser writing of membrane in channel
  - Seamless integration with separation channel
- Detection of 10 fM analyte (direct labeled)
  - >4 orders of magnitude preconcentration
  - <5 minute step
  - S/N ratio of 28





Wang et al., *microTAS 2008*

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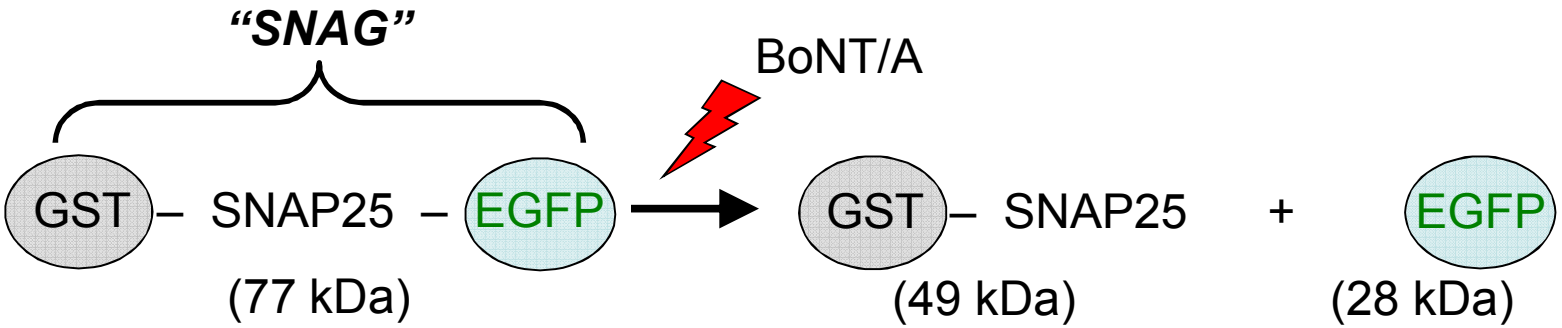
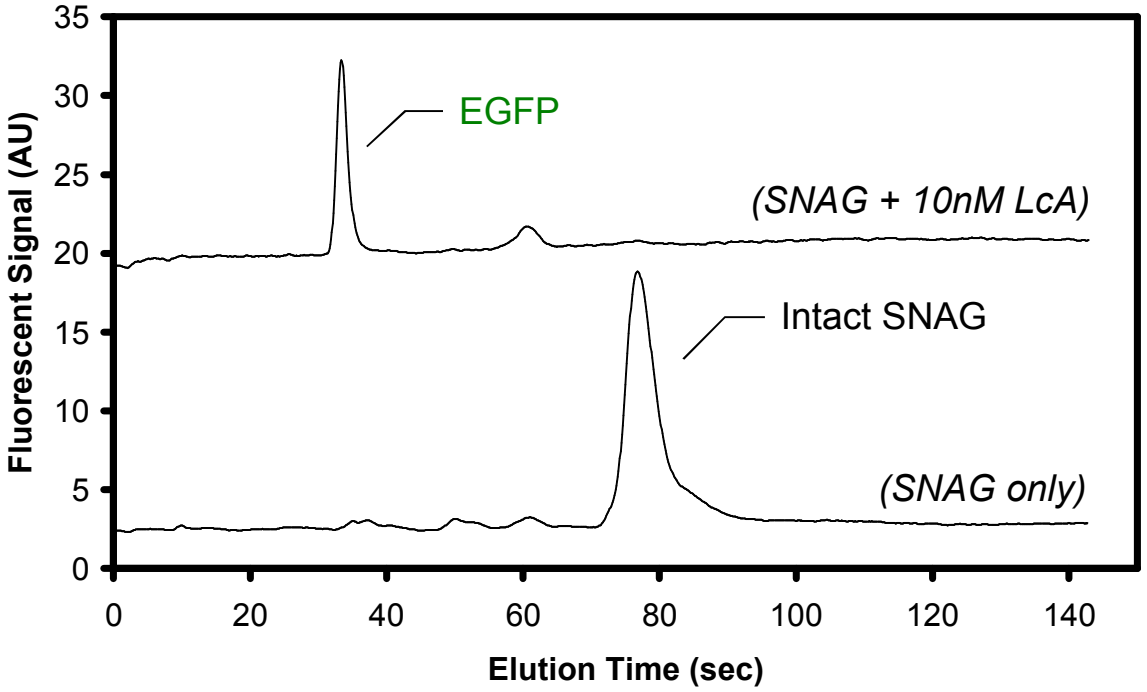
Botulinum Neurotoxin Enzyme Activity Assay





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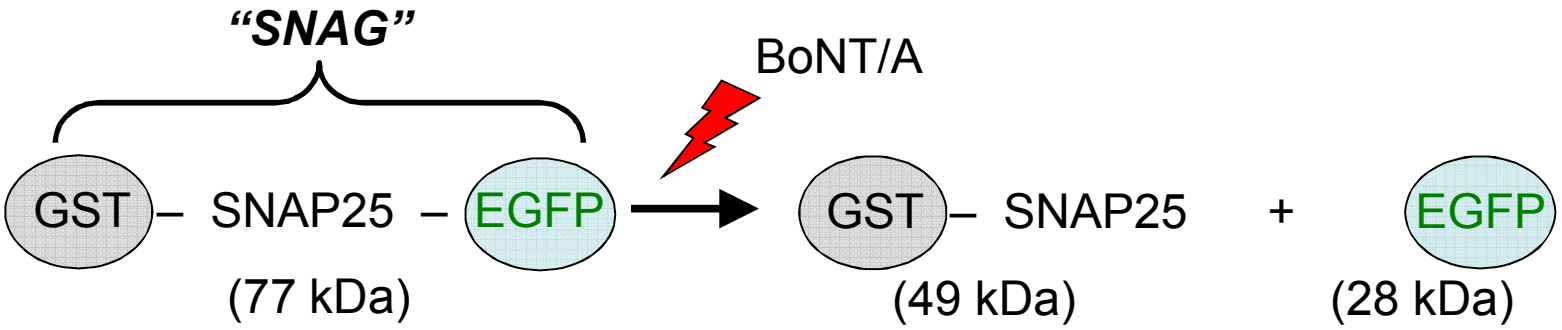
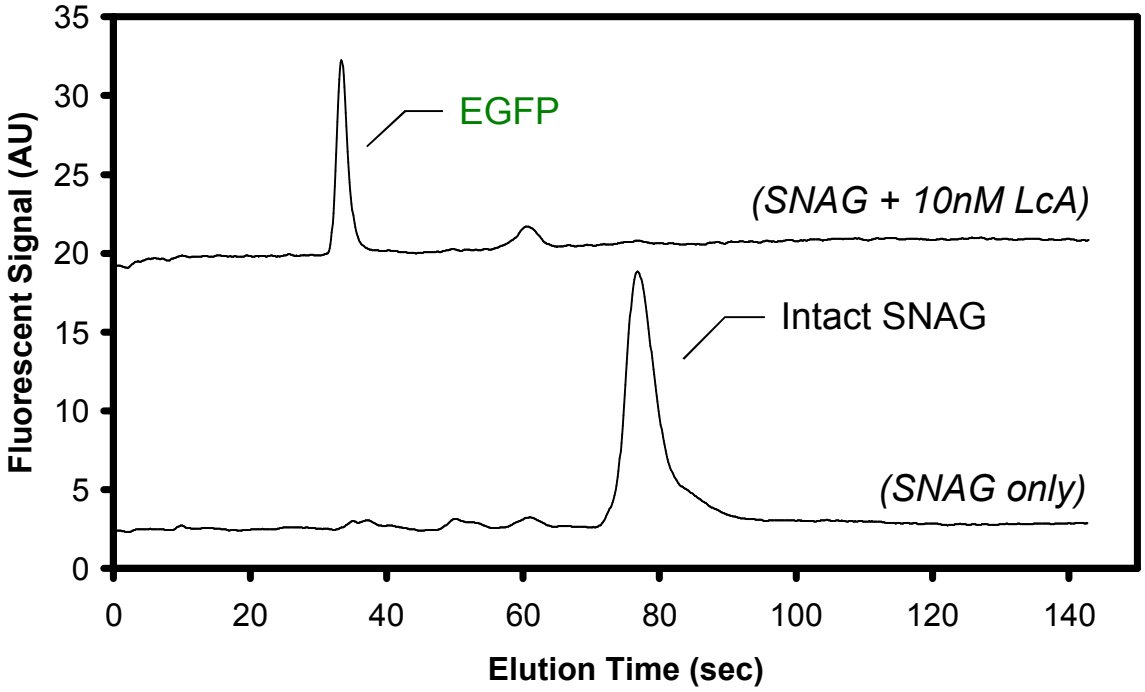
Botulinum Neurotoxin Enzyme Activity Assay





On-Chip Process

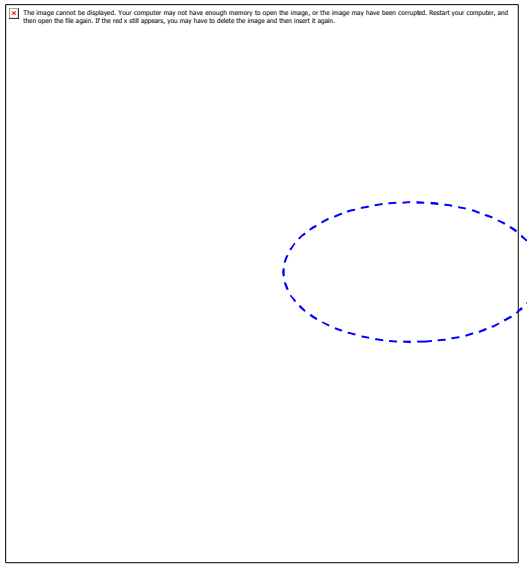
- Preconcentrate BoNT/A, mix probe, react, separate

Specific to active toxin

Signal amplified vs. direct-labeled immunoassay

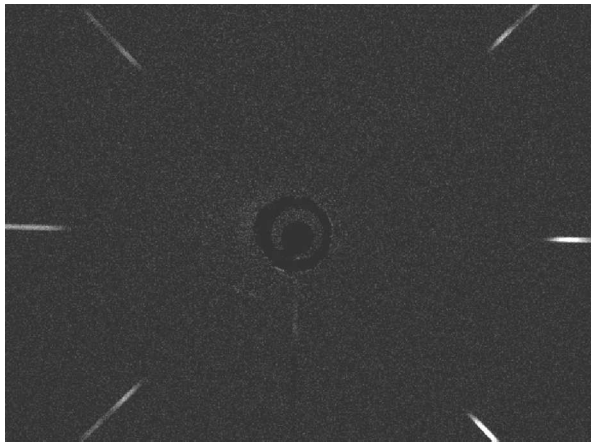
- Detection limit ??

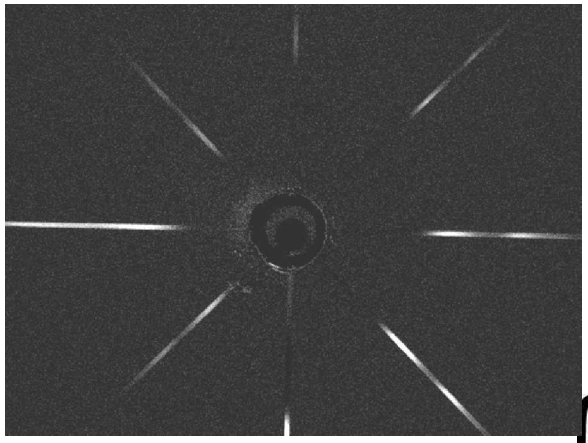
Spatial Multiplexing



1” Square Chip

Simultaneous separations with all 8 modules x 4 analytes per





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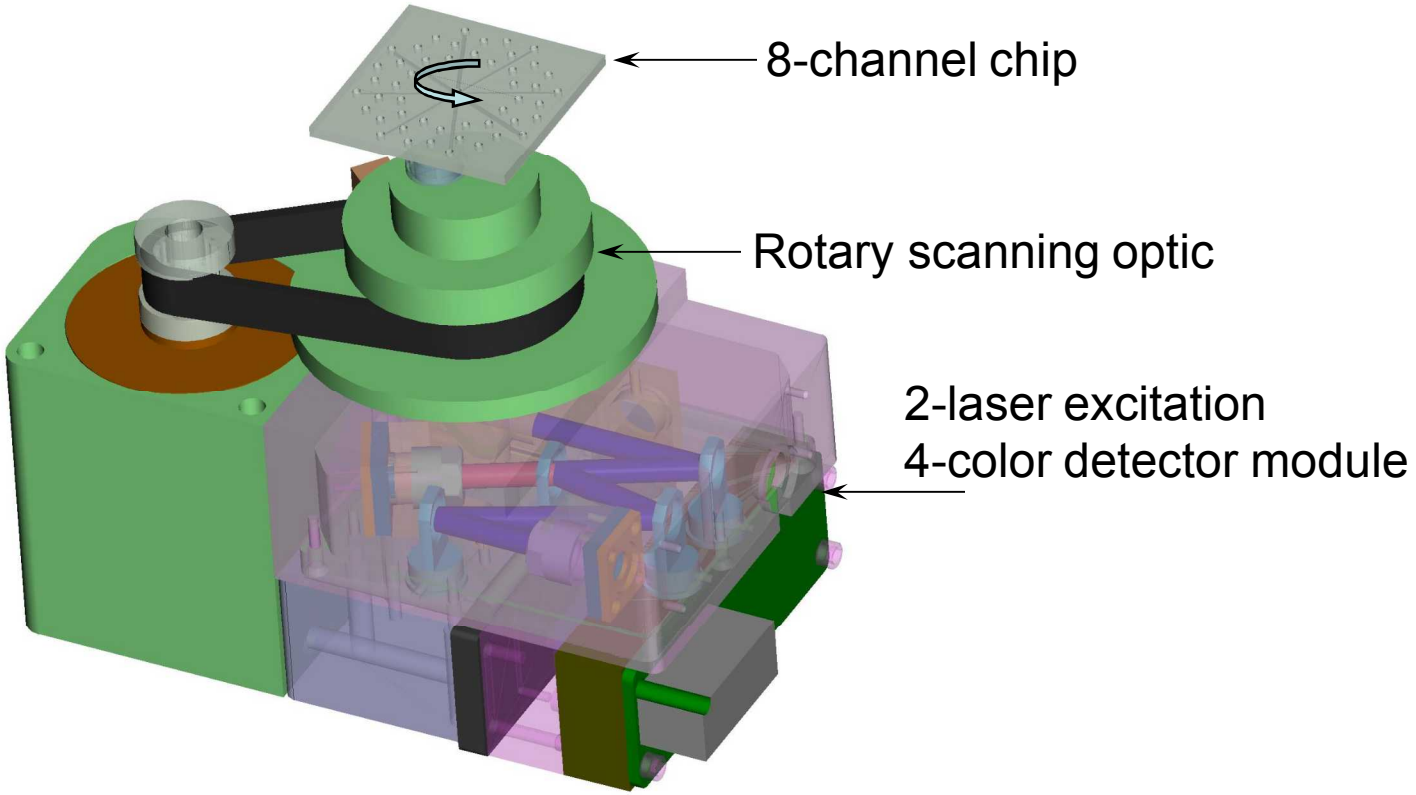
Spatial Multiplexing: Rotary Optical Scanner



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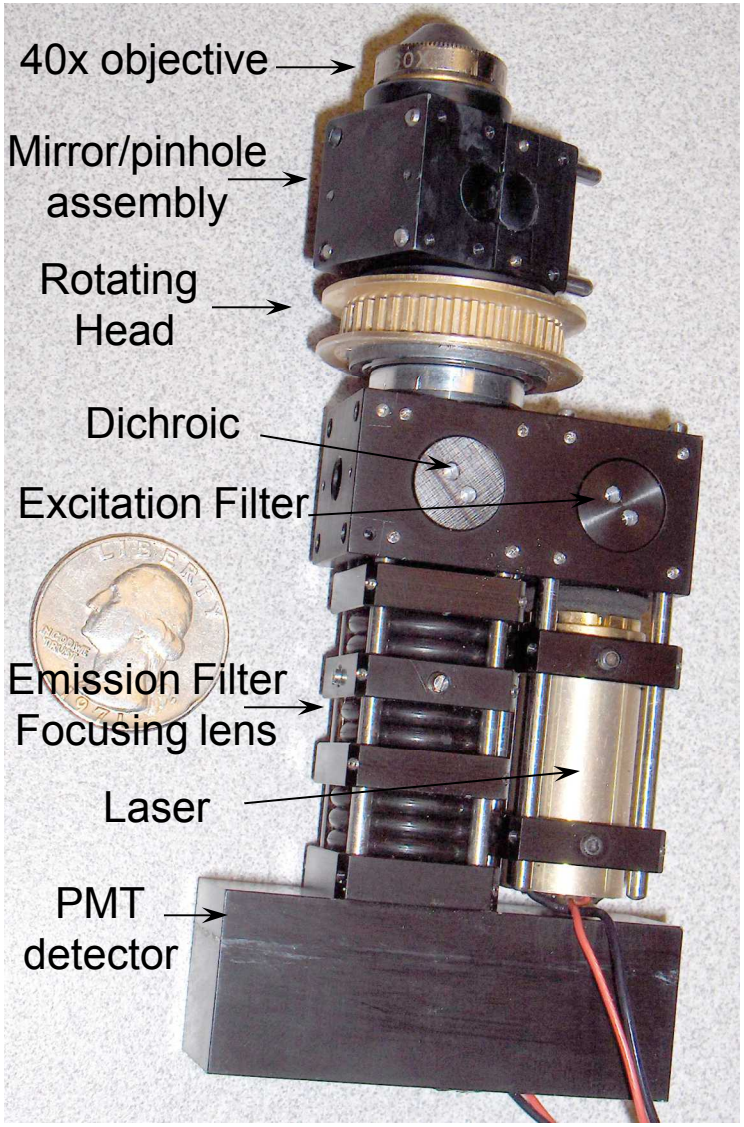
Rotary Optical Scanner



Brennan et al.

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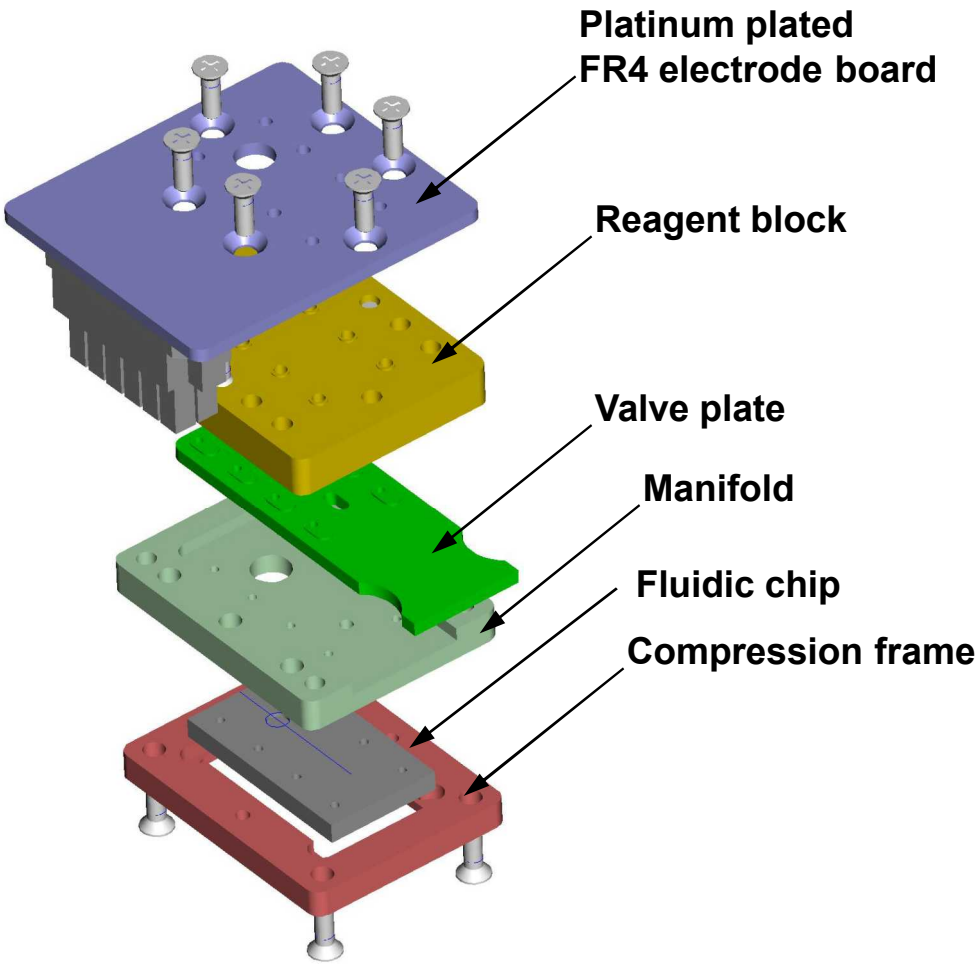
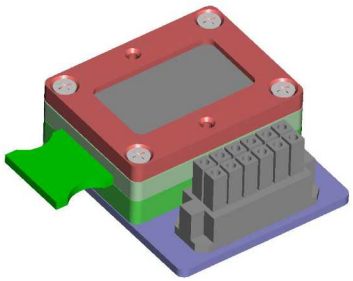
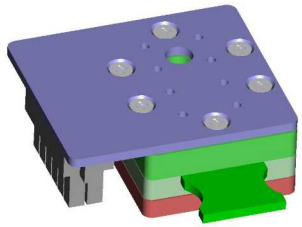
Disposable Assay Cartridge





Bottom view



Top View

- isolated reagent storage
- valve-sealed gels on-chip until use
- simple loading of cartridge and sample
- concept amenable to low-cost mass fabrication

Approximate dimensions: 3 x 3 x 1.5 cm

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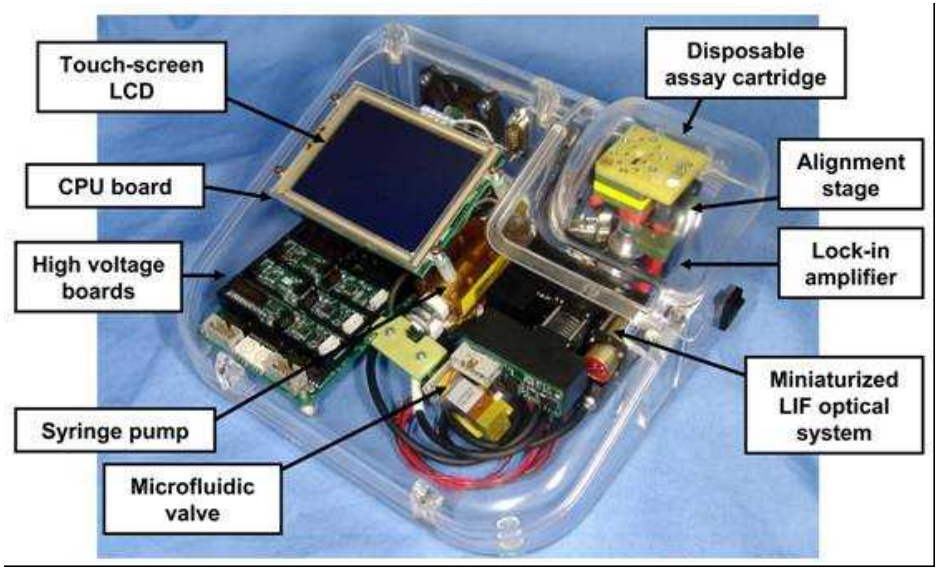
Current Status



- TRL4 Device ~ 1.5 years
- On-going development of toxin and host response marker assays
- On-chip and off-chip sample pretreatment for complex samples
- Disposable cartridge engineering

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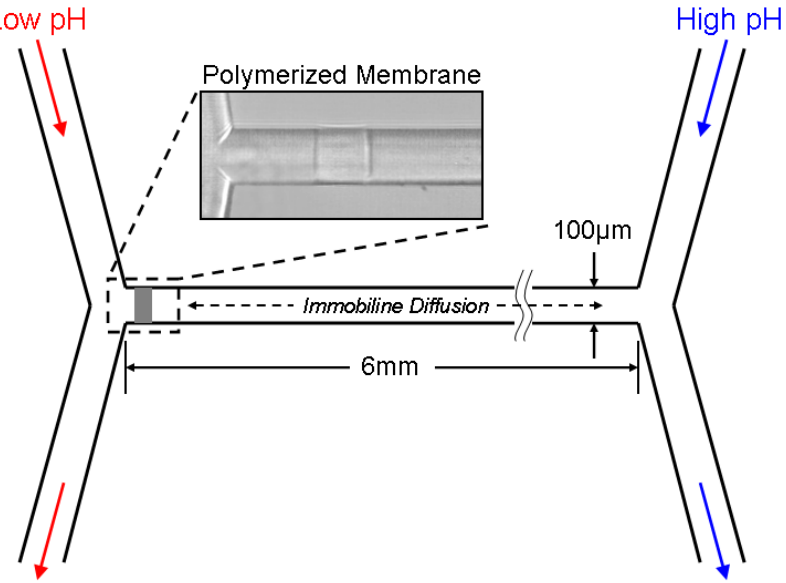
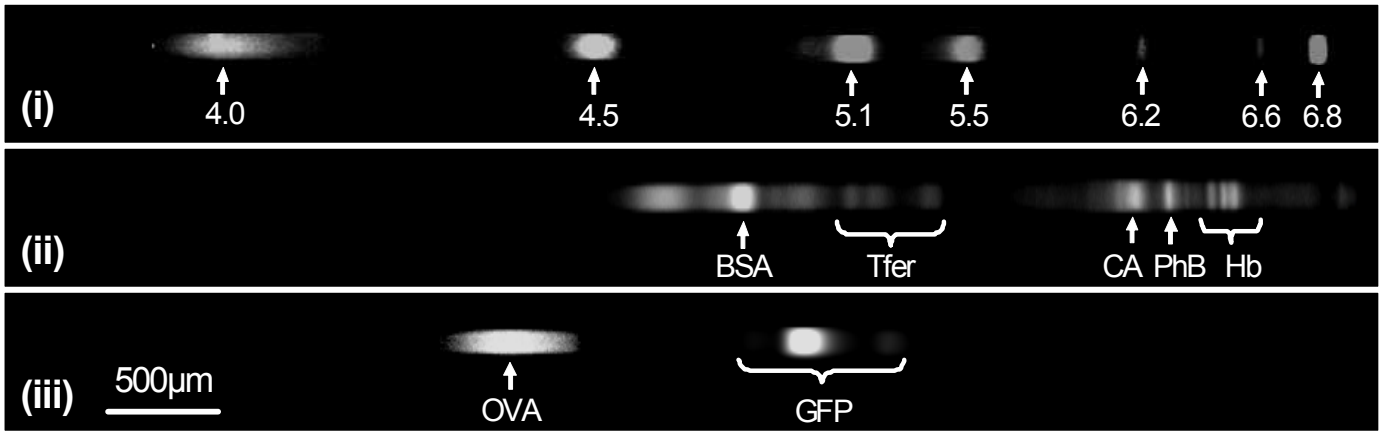
Microscale Immobilized pH Gradients (μIPG's)

Diffusion-controlled pH gradient immobilized within polyacrylamide matrix on-chip





p*I* Markers

AF647 Proteins

AF488 Proteins

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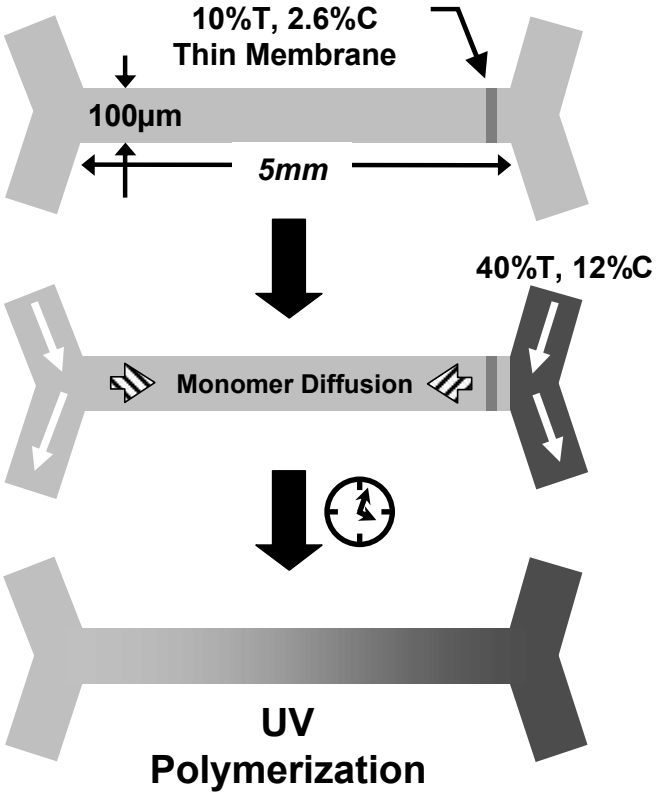
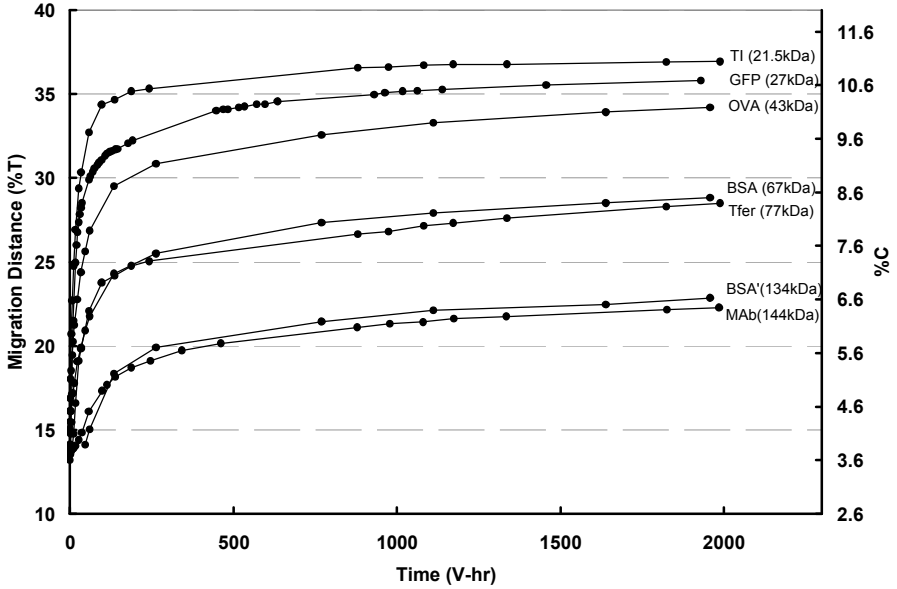
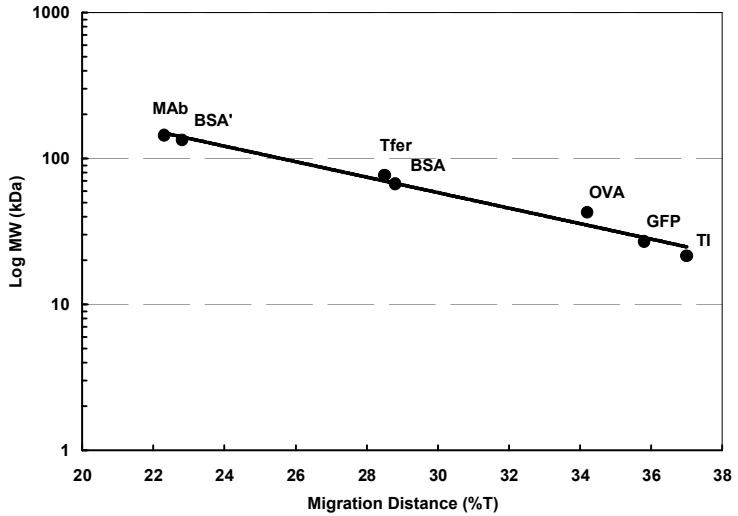
Pore Limit Electrophoresis







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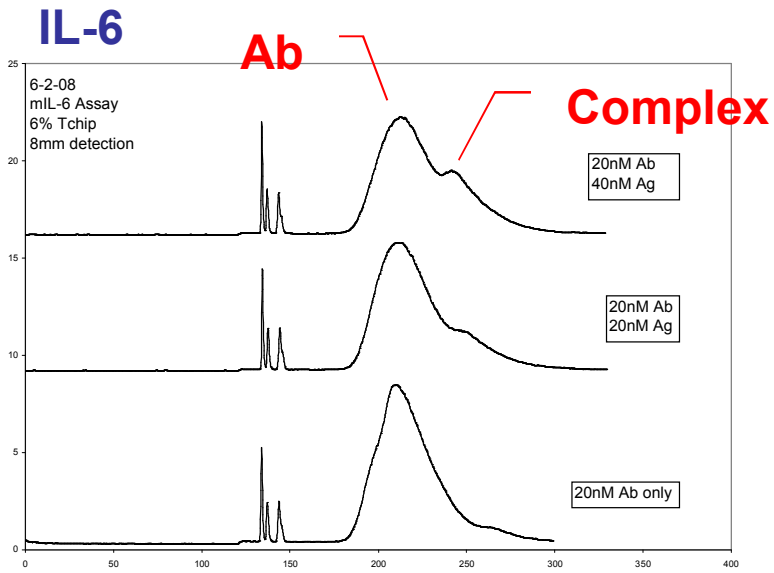
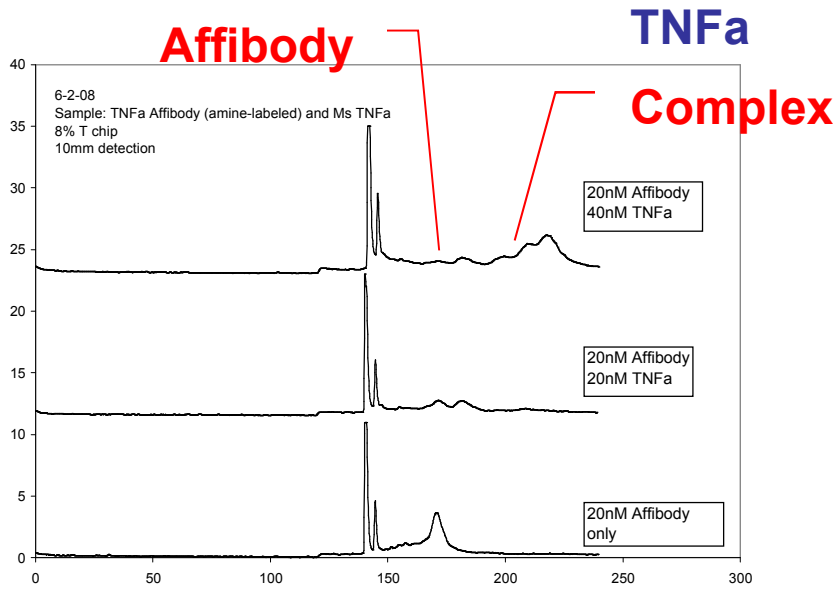
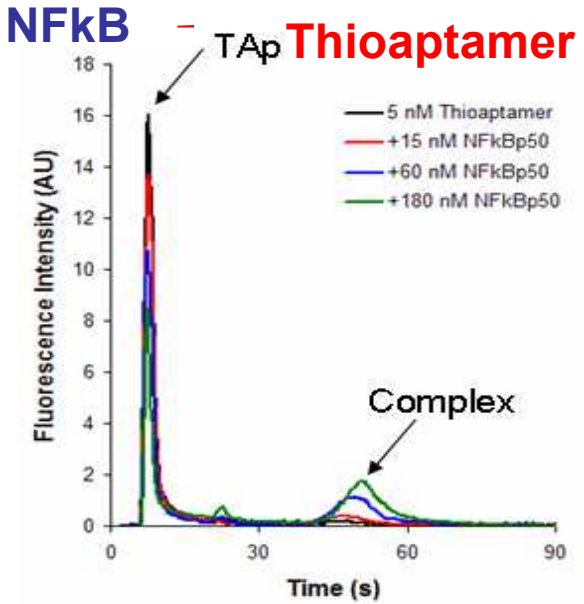
Alternative Affinity Reagents







- Cytokines at size threshold for immuno-gel-shift assay
- Much improved with smaller affinity reagent such as affibody, aptamer
- Advantages in uniformity, production

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