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Timely multi-threat biological, chemical, and nuclide detection in large volume water samples.

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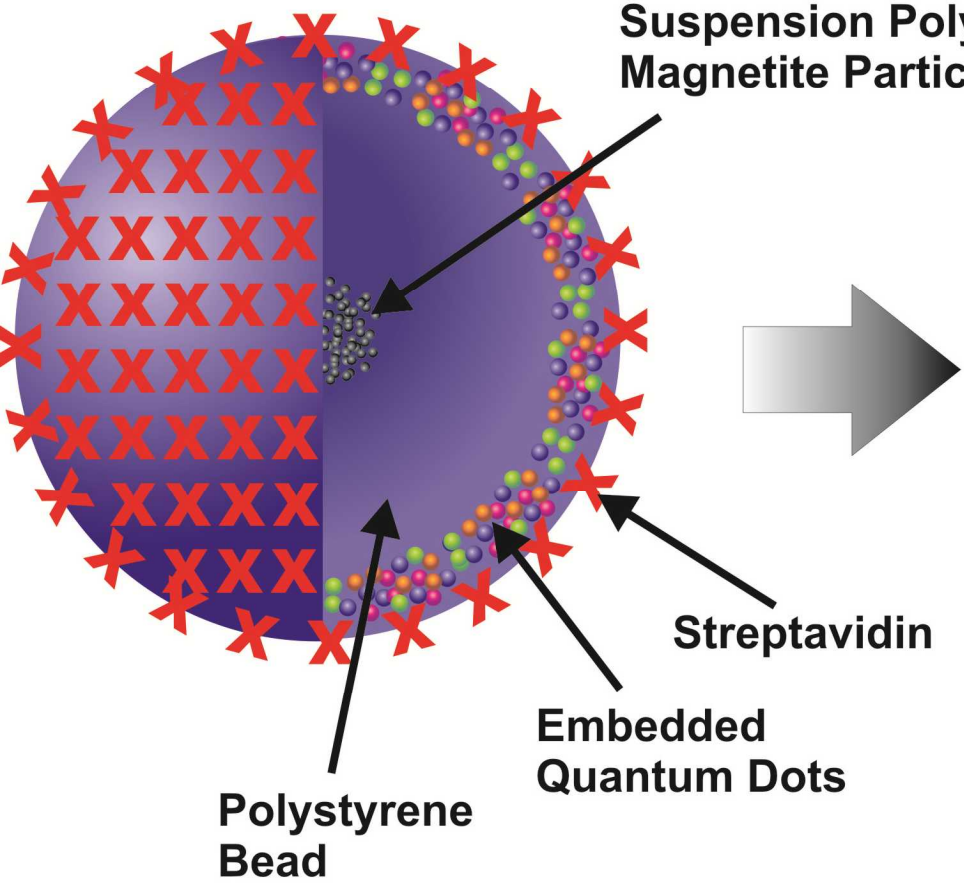
Introduction

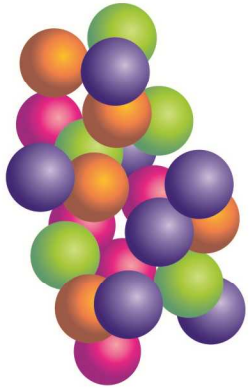
- Problem: Need to detect multiple dangerous agents (CBNE – Chem/Bio/Nuclear/Explosive) in various dirty samples at high levels of sensitivity and specificity (low false negatives and low false positives).
- Solution under development: Bead based multiplexed detection of many agents in the same solution with raw sample handling and cleanup.
- Can we apply this solution to detection of dangerous microorganisms in water?

Outline

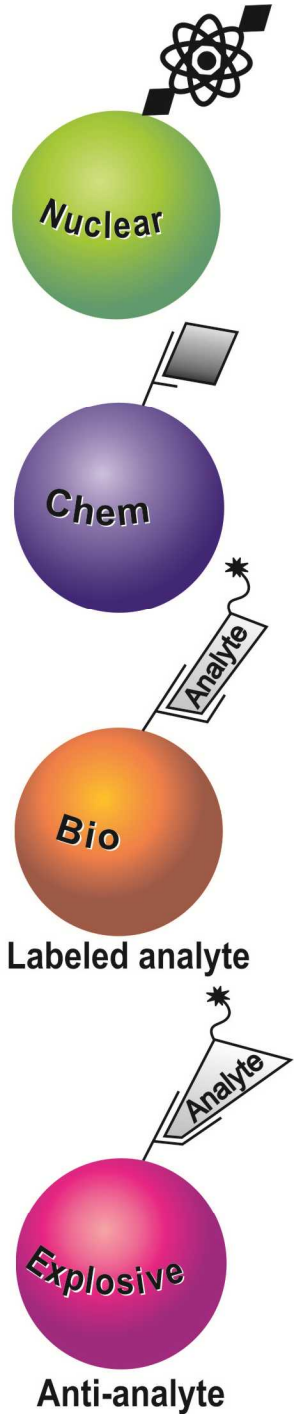
- Two key enablers – bead and concentrator
- Airplane – problem and modeled system solution
- Milk – problem and modeled system solution
- Milk system testing and future developments
- Strawman water system discussion

Multi-Spectral Magnetic Bead



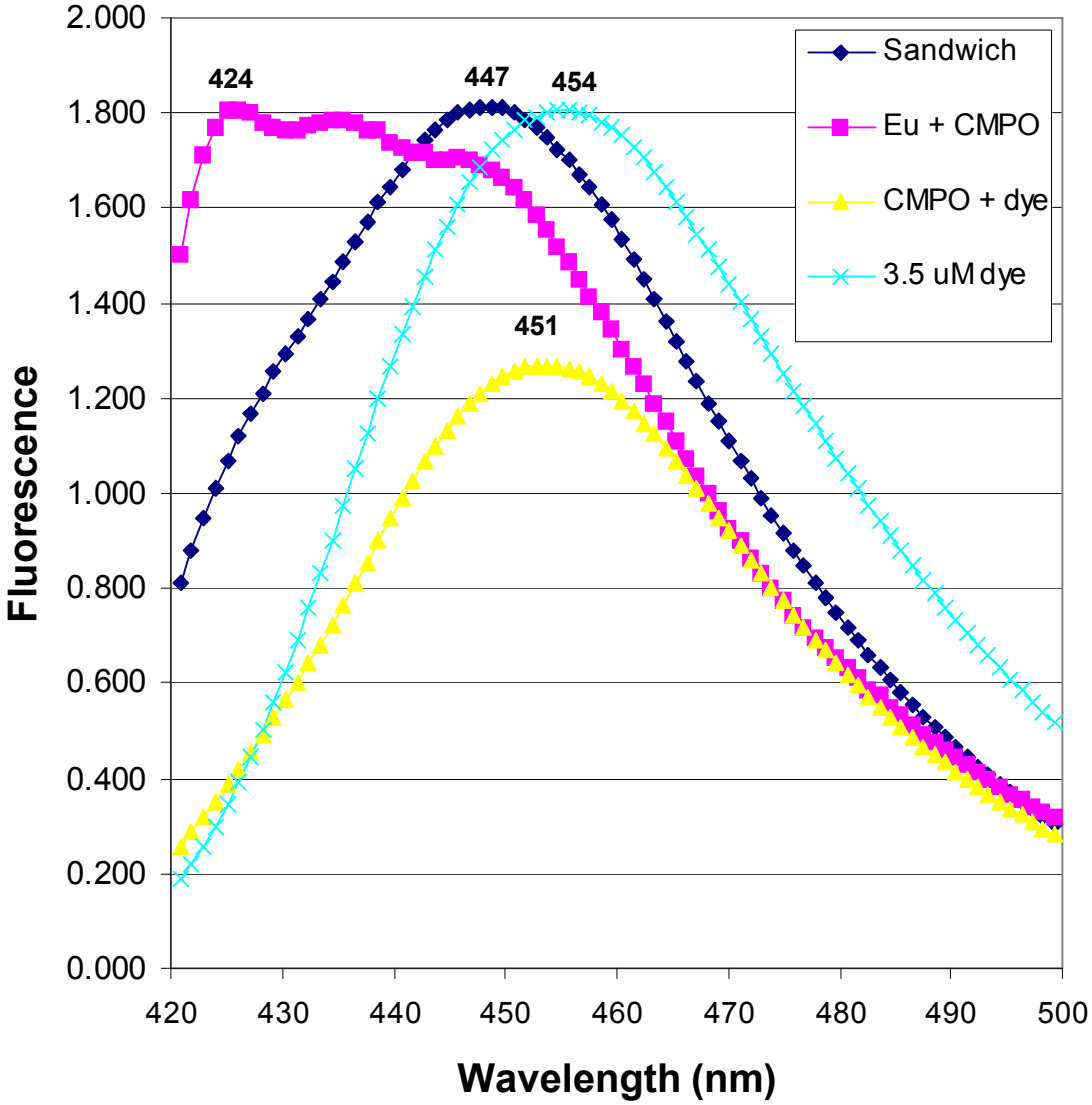


Multi-Spectral
Magnetic
Bead in Solution

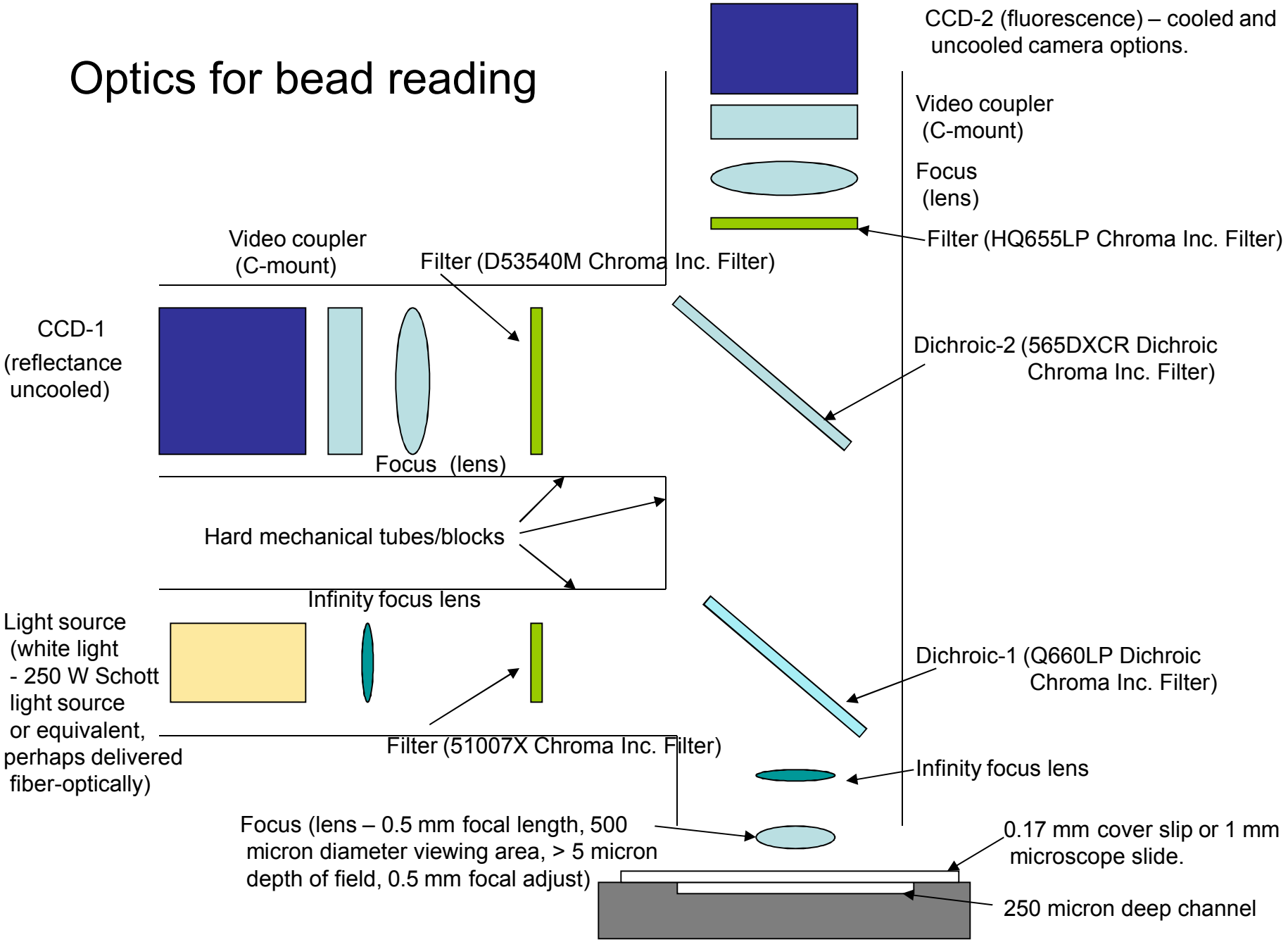


Key Enabler – Bar-coded, surface functionalized, magnetic bead

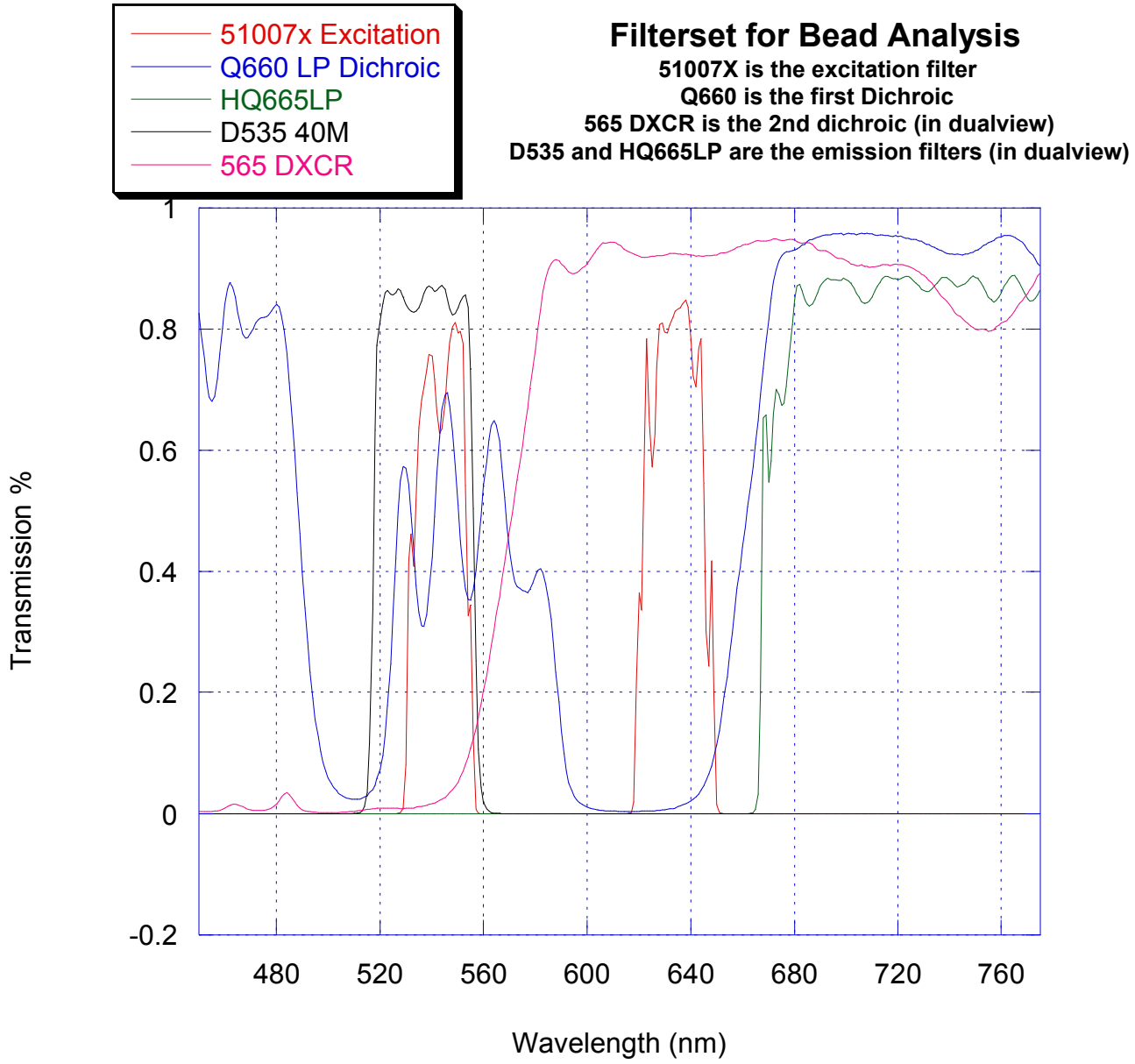
Radio-nuclide detection



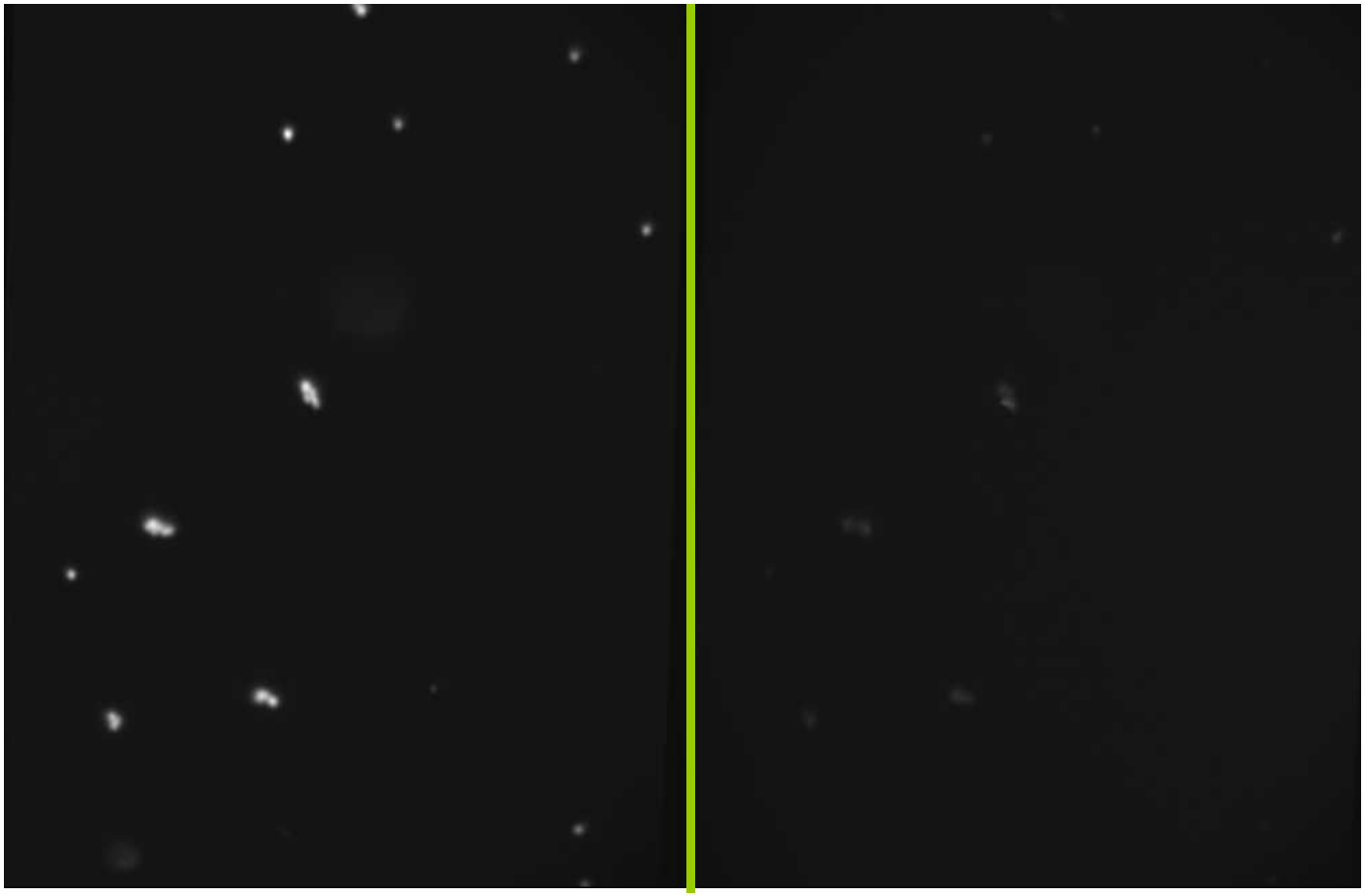
Optics for bead reading



Spectral windows allow concurrent bar-code and tag reading



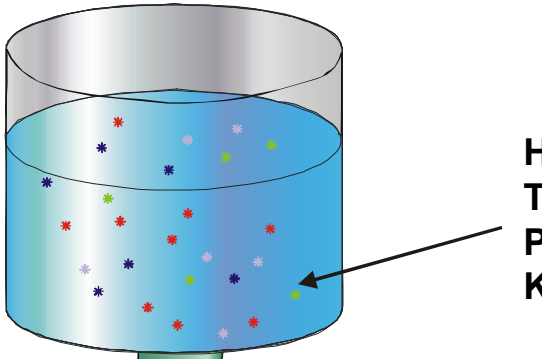
Biodetection – Botulinum toxin substitute in milk



Two-signal detection.

Bead identification on left; captured antibody with fluorescent label on right. Software uses information from both images to identify bead location, type and capture of target antibody.

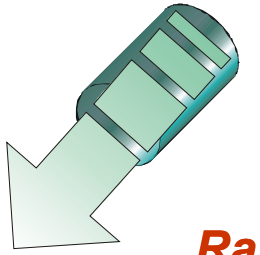
Key Enabler: High Volume Trace Sampling - Concentrator



Hybridize
Trace
Pathogen:
Kinetics Limited

flow

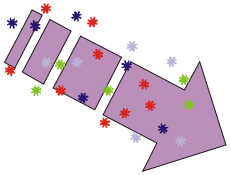
Volume Gain
Bead trap, wash,
S/N

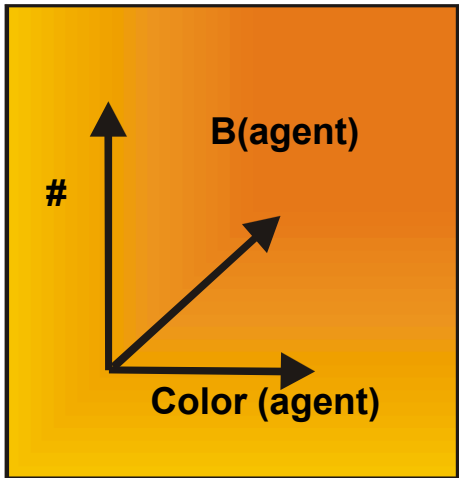


Bulk Fluid

Solve the volume impedance mismatch

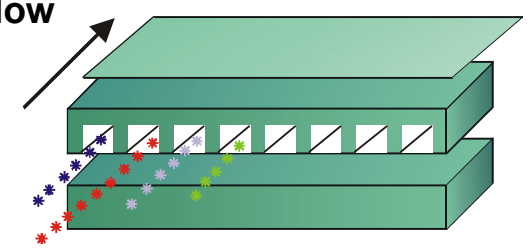
Washed beads ~ 1 L





Optic/CCD

flow



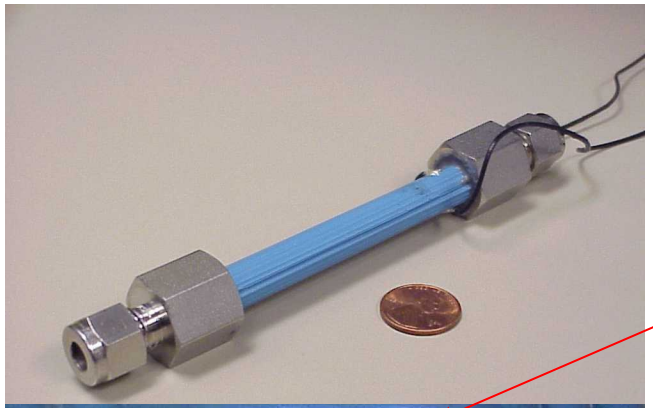
Plastic

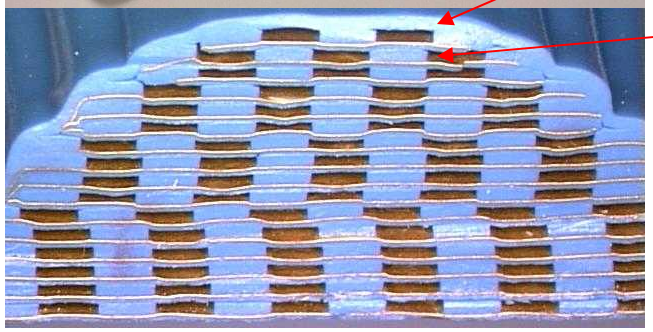
2D sort
and
sense

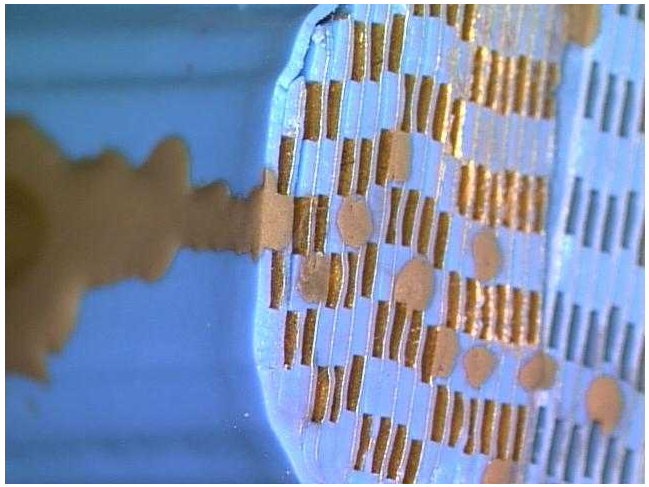
Rapidly identify primary and confirm, large volume and small

MEMS/LIGA/Surface or bulk (structures (valves, channels)), light sources, etc.

Meso-scale Trap Prototype using LTCC technology







Each row has electrodes in parallel within each fluid channel (top row has two channels, with two electrodes in parallel)

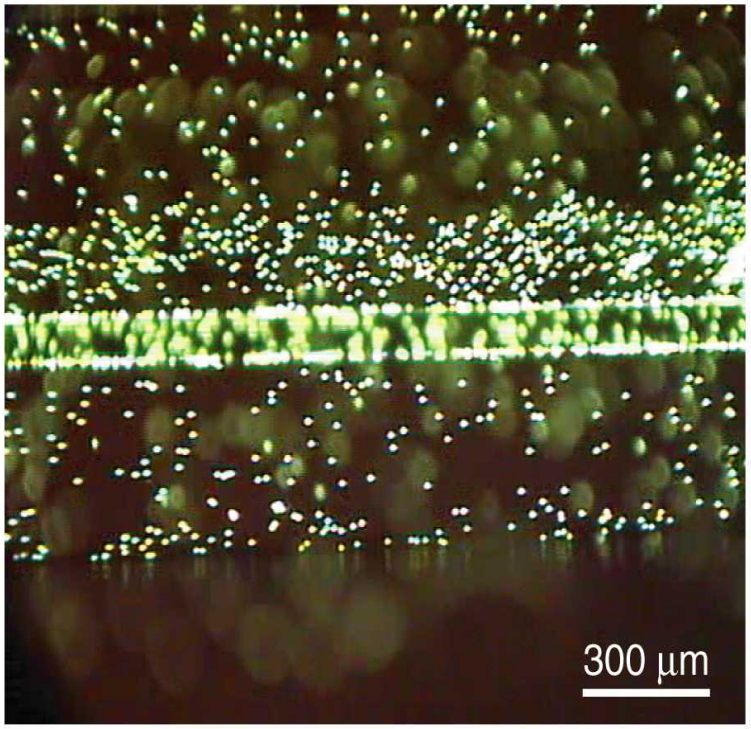
Rows of electrodes are connected to each other in series (the top row, which has two electrodes in parallel, has its electrodes connected in series to electrodes in row 2, which has three channels and three electrodes in parallel)

Thus if we pass a total of 1 A of current through the entire device (total resistance of 20 ohms, which will require 20V), the top row will have 0.5 A going through each of the two conductors in the two channels). The rows in the center of the device (where there are 6 channels and 6 electrodes) will have 0.17 A passing through each wire.

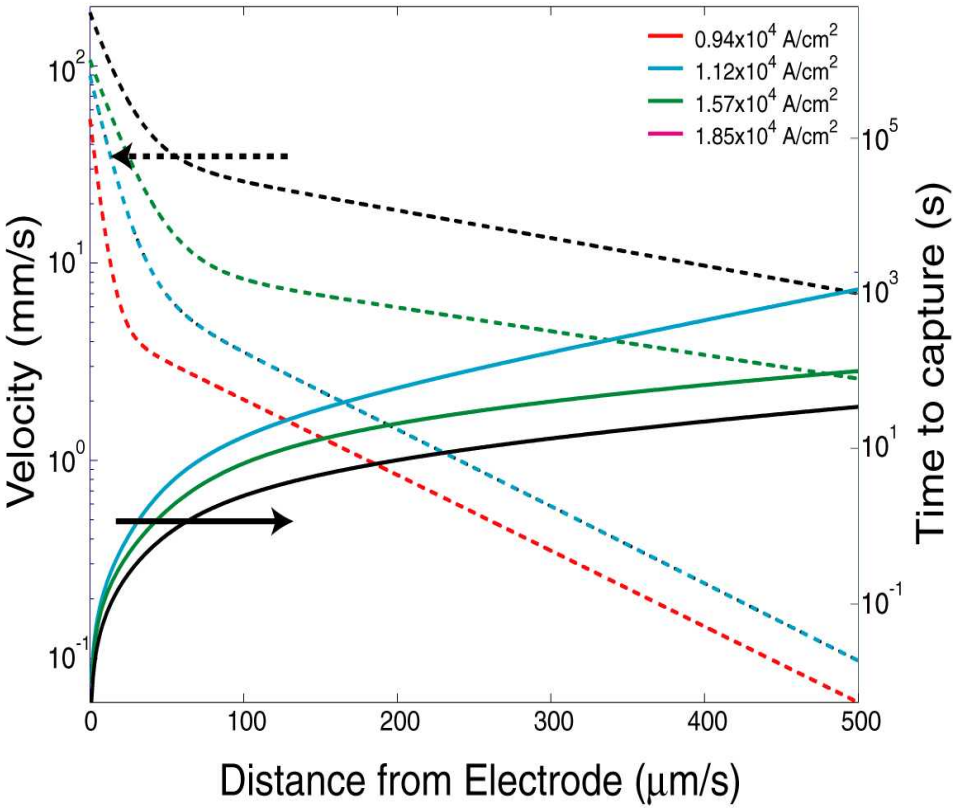
Testing will involve passing a sample of magnetic beads through the trap, and observing the signal intensity measured downstream of the trap with a spectrometer.

Experiments with single wire demonstrate electromagnetic capture of magnetic beads for sample cleanup.

(a)



(b)



cation: Pathogen on a plane

Model description

- TTI (Time-To-Identify) = $t_{collection} + t_{mixing} + t_{trapping} + t_{sensing} + t_{transport}$

$t_{collection}$ = f(flowrate, target size, pipe size, viscosity)

t_{mixing}= f(Volume, # beads, #targets, target size, probability of capture)

$t_{trapping}$ = f(flowrate, bead size, pipe size, viscosity)

$t_{sensing}$ = f(SNR,Quantum eff, dark current, signal stgth, wavelength)

$t_{transport}$ = f(flowrate, scale)

Calculations pertaining to airplane from model

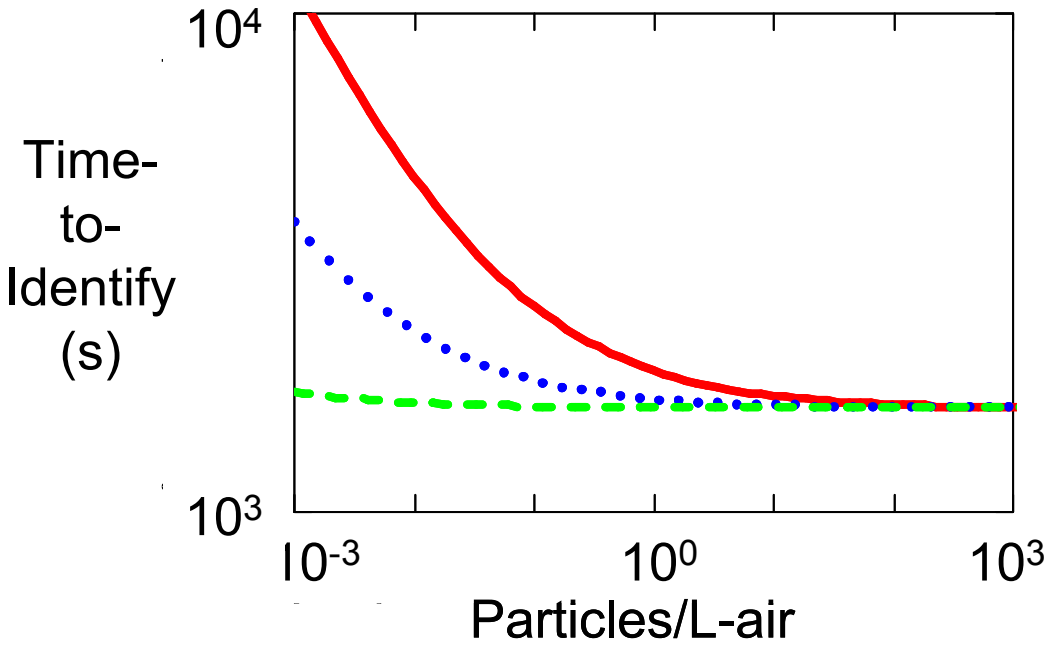


Figure 8. Estimated sensitivity for analyte detection in high-volume air collection system. The solid dark line, dotted line and the dashed line are for 2 mm (bacterial) sized particles, 100nm (viral), 0.3 nm (molecular detection), respectively, for 20,000 beads and 103 beads/s in 10 parallel counting channels. The nominal 1500 second limit is generated primarily by the concentration time and that can be performed in parallel with other processing. It was not necessary to reduce TTI further in this CONOP.

Milk problem

- Milk supply is vulnerable to contamination (perhaps on purpose) between the cow and the grocery shelf (reference PNAS paper on milk vulnerability)

Calculations pertaining to milk from model

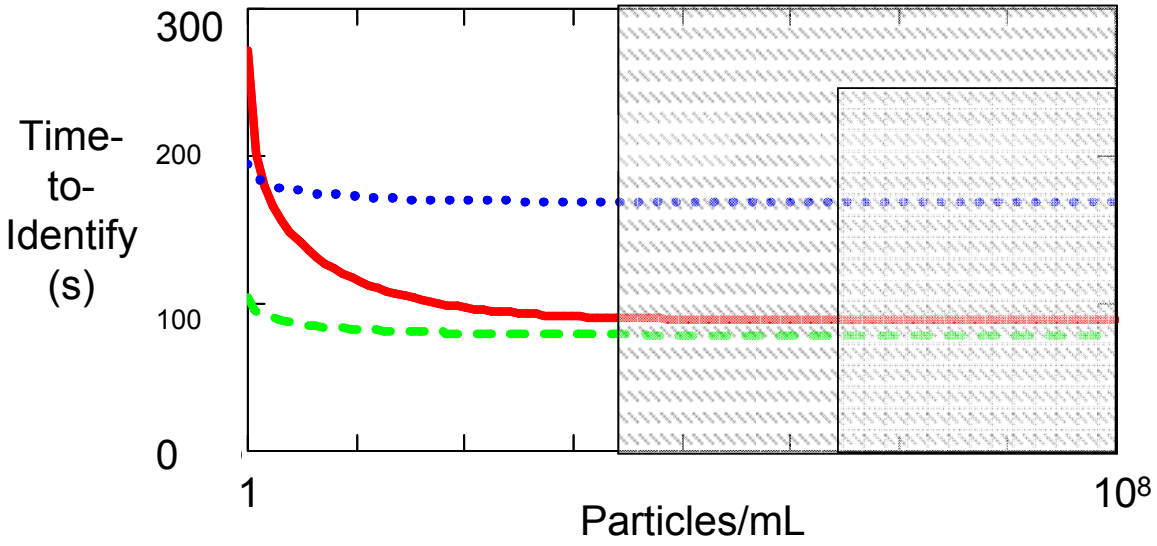
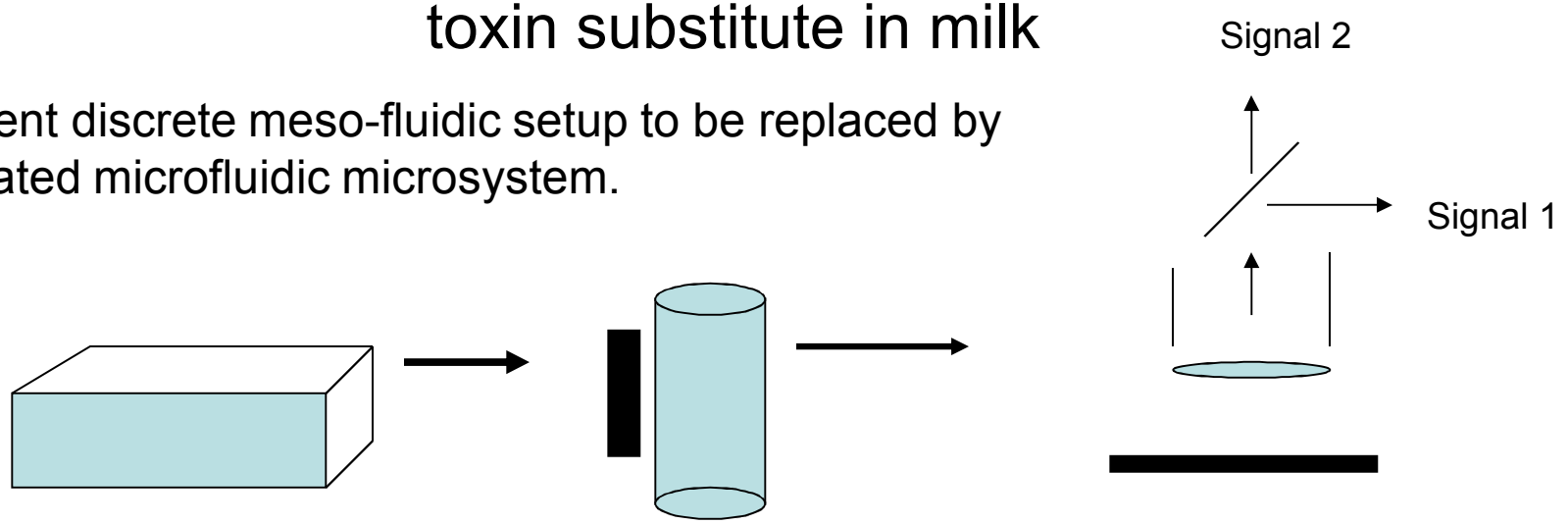


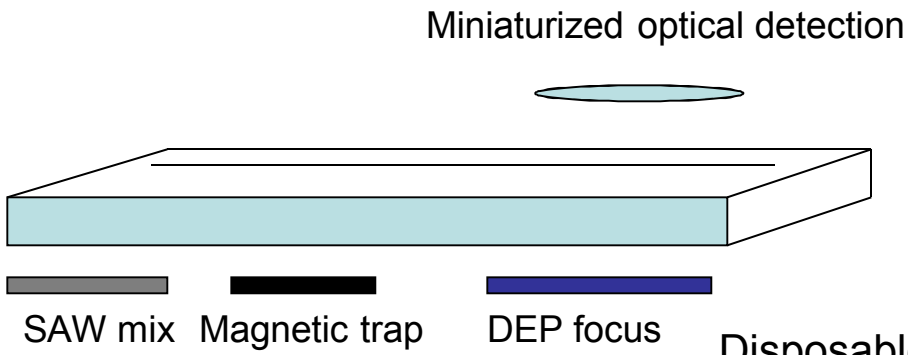
Figure 6. Variation in system performance for small analytes (e.g. *botulinum* toxin) with respect to the number of beads and bead count rate in 1ml samples. This figure represents system performance envelopes expected in raw milk samples. The dashed curve, solid curve, and dotted curve curves are for (2x104 beads,103/s count rate), (106 beads,104/s count rate) and (106 beads,105/s count rate), respectively.

Rapid and accurate bead-based identification botulinum toxin substitute in milk

- Current discrete meso-fluidic setup to be replaced by integrated microfluidic microsystem.

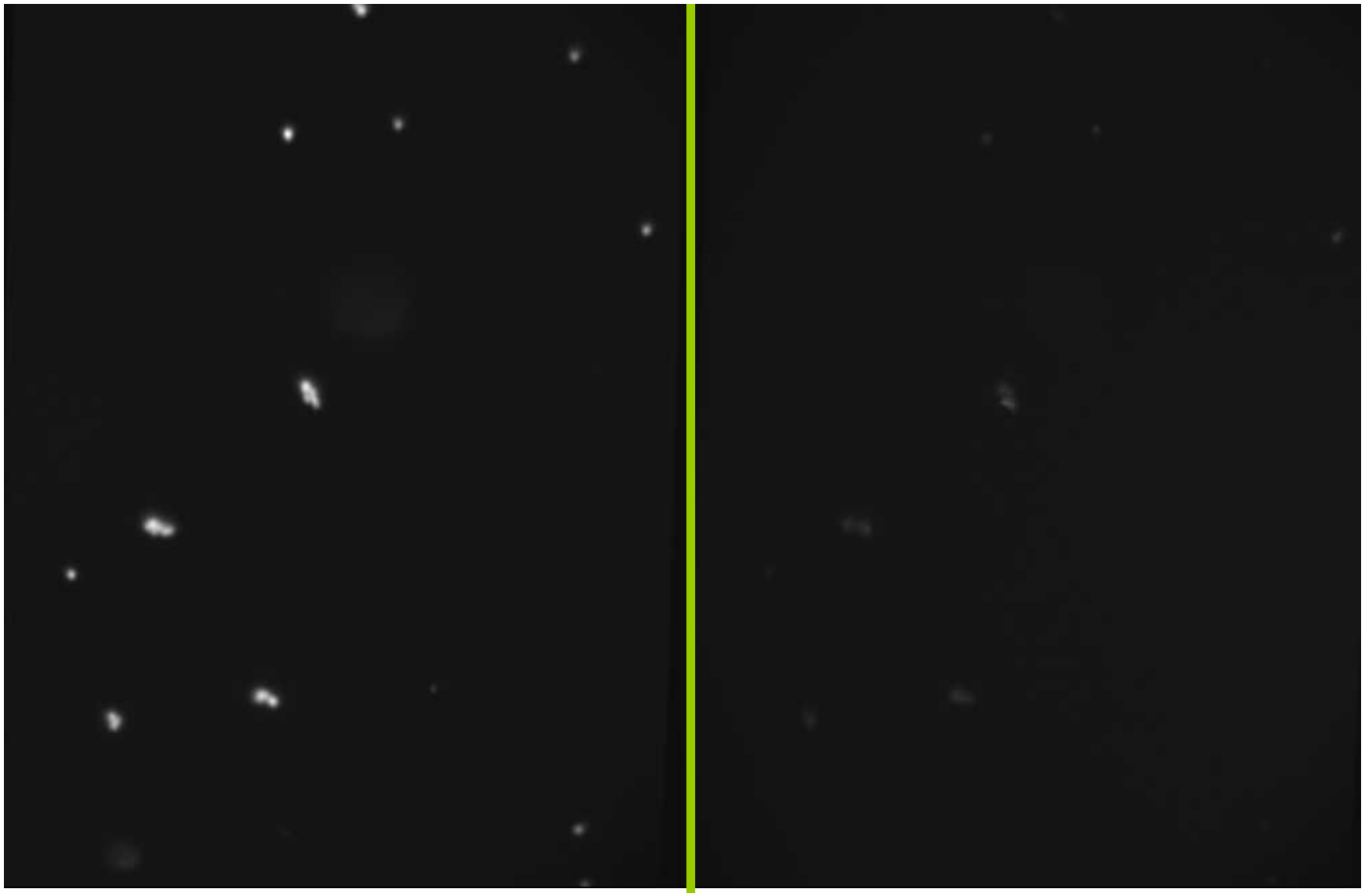


Current discrete system



Disposable microfluidic cartridge system.

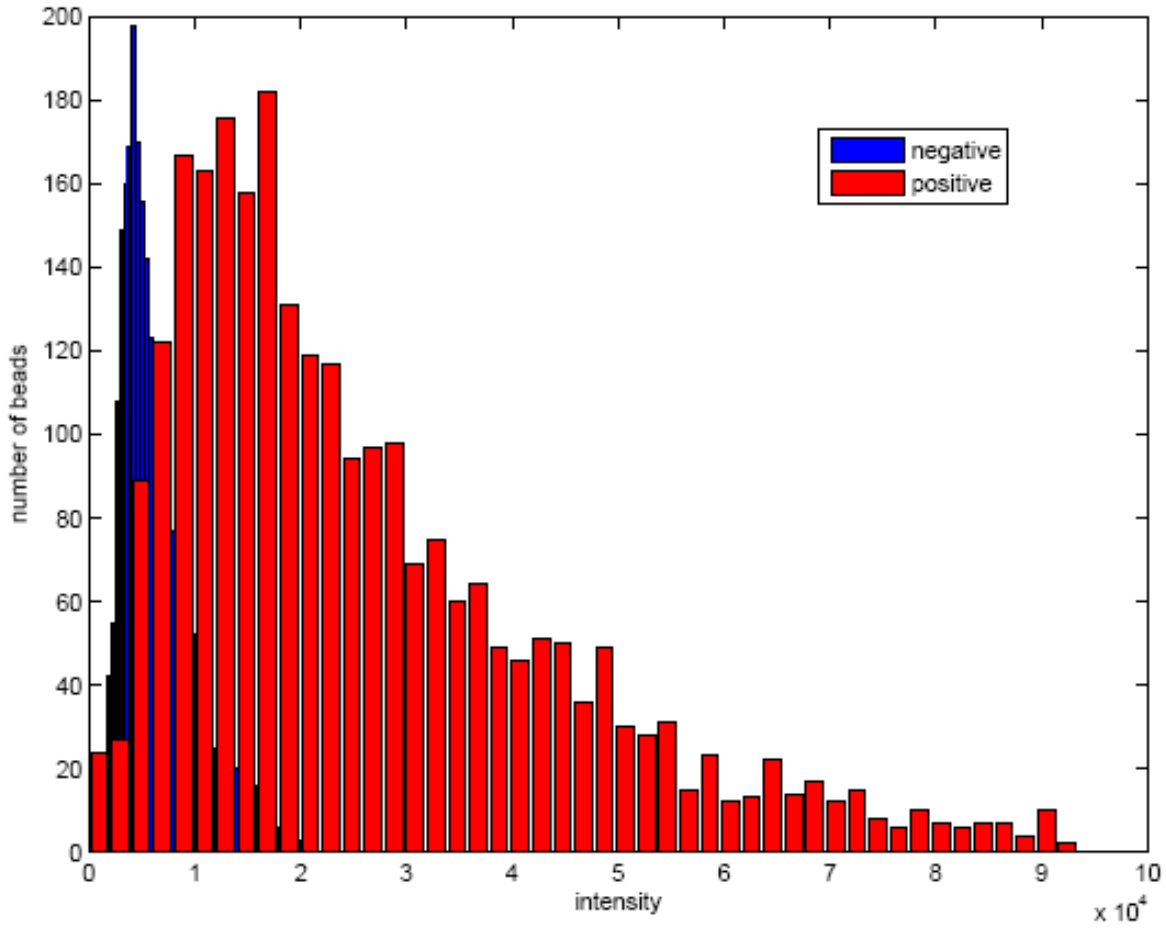
Biodetection – Botulinum toxin substitute in milk



Two-signal detection.

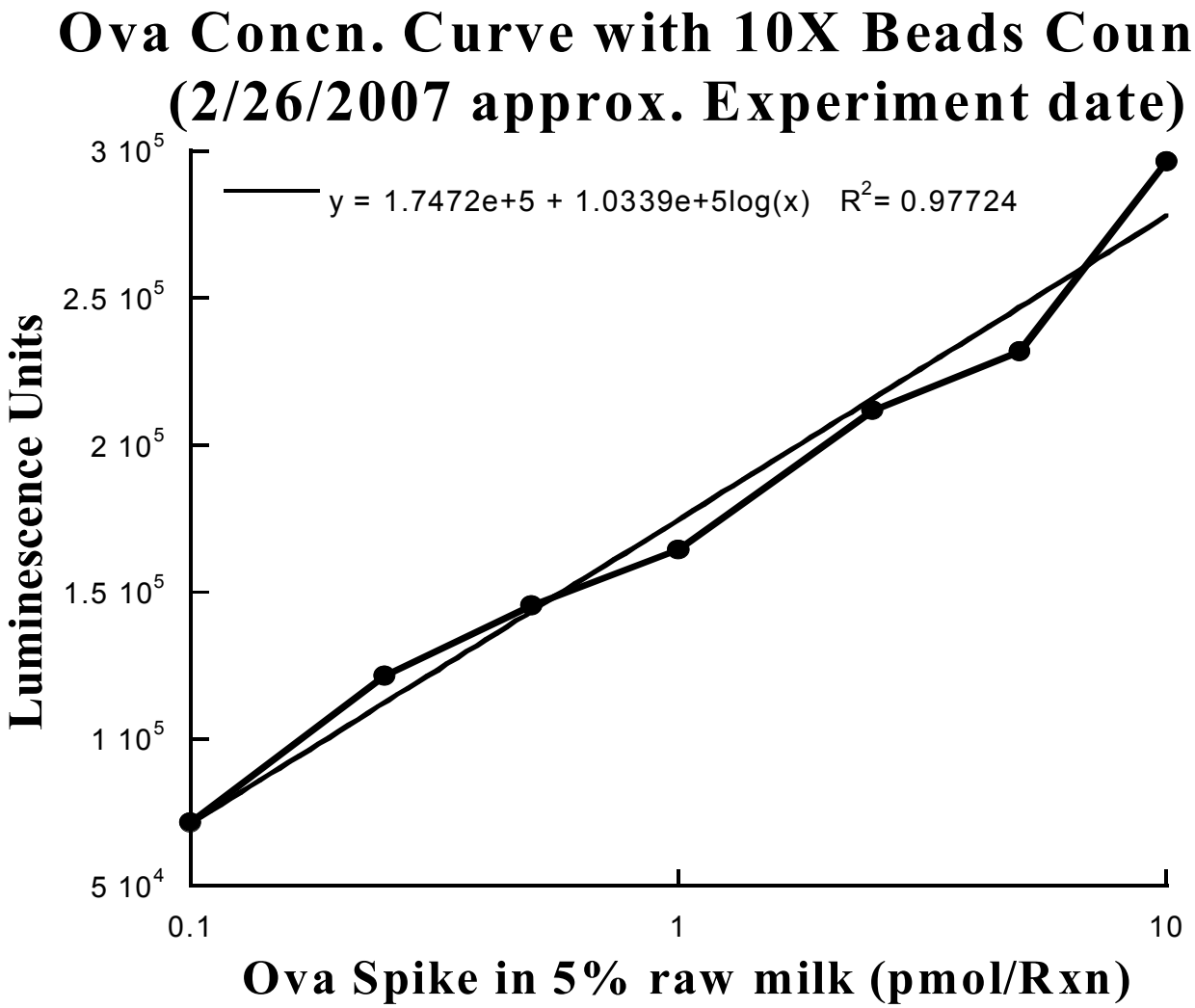
Bead identification on left; captured antibody with fluorescent label on right. Software uses information from both images to identify bead location, type and capture of target antibody.

Test Results: Identification of Botulinum substitute (Ovalbumin) in Milk



Shift in number of beads at higher intensity at wavelength of antibody label indicates positive capture of target (Ovalbumin – Botox substitute).

Sensitivity curve – Ova in milk



Time-to-Identify (TTI) Experimental

- **14 samples identified correctly in one day.**

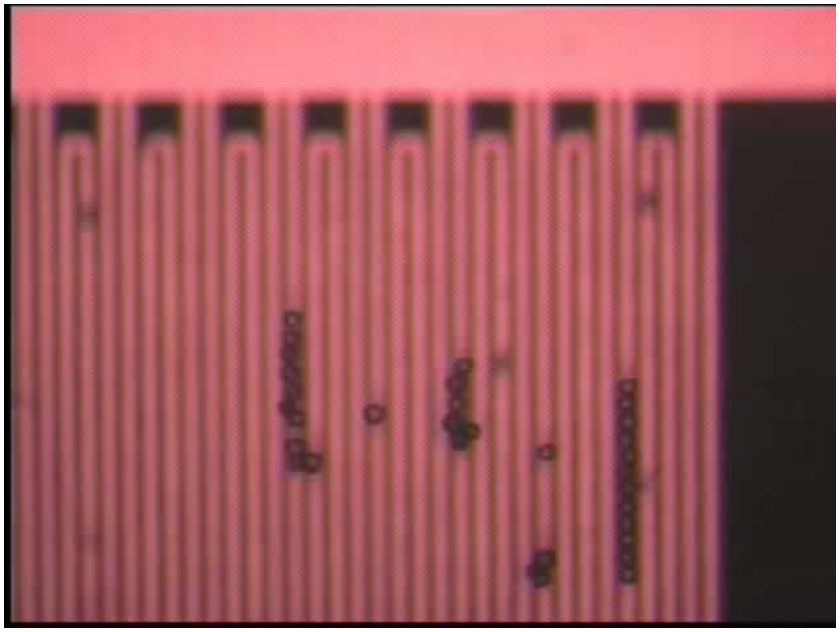
Sample Timeline

Add all reagents to tube- 1 minute
Mix (on SAW chip)- 15 minutes
Clean SAW chip and pipette mixture into clean tube- 2 minutes
Pellet beads- 10 minutes
Clean pellet and re-suspend pellet in buffer- 2 minutes
Centrifuge pellet and pipette sample onto glass slide- 3 minutes
Move sample to scope and check focus on sample- 3 minutes
Data collection (180 frames)- 15 minutes
Save data and start code: 1 minute
Code analysis-10 minutes
Data output with positive or negative ID- 3 minutes
Time Sum: 65 minutes

***Note*:** Because the next sample can be prepared and mixed while the previous sample is being optically analyzed, we are obtaining positive or negative ID's approximately every 30-35 minutes.

- **Modifications to hardware and procedure to reduce time and increase sensitivity are on-going in preparation for field test in July.**

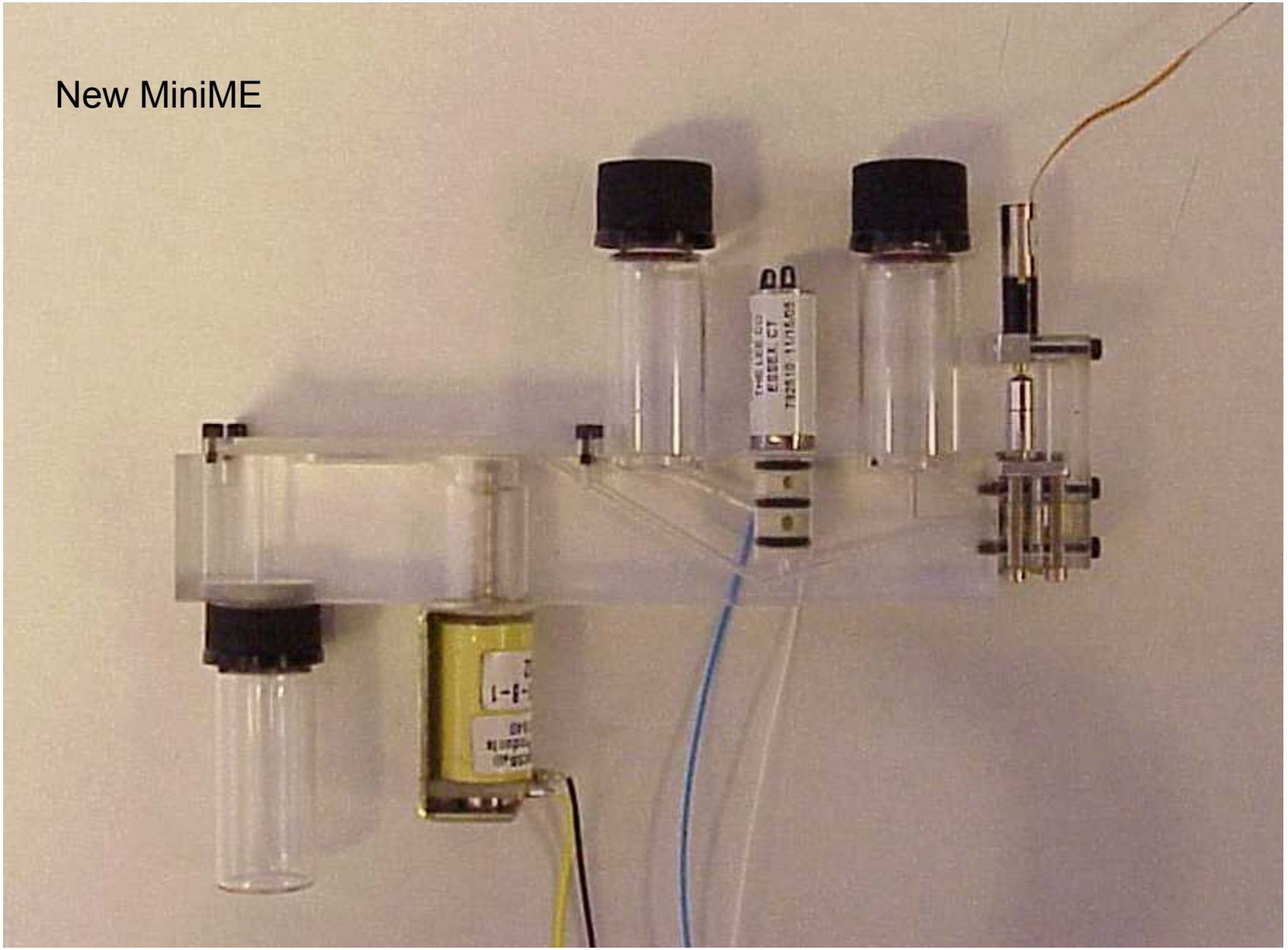
DEP (dielectrophoretic) focus will allow flow-through continuous bead reading.



9 micron diameter Spherotech Nile Red Fluorescent Magnetic beads at 5V, 1MHz (5 V and 0V signals). Switched to 15 MHz at the end of the video- disperses the beads

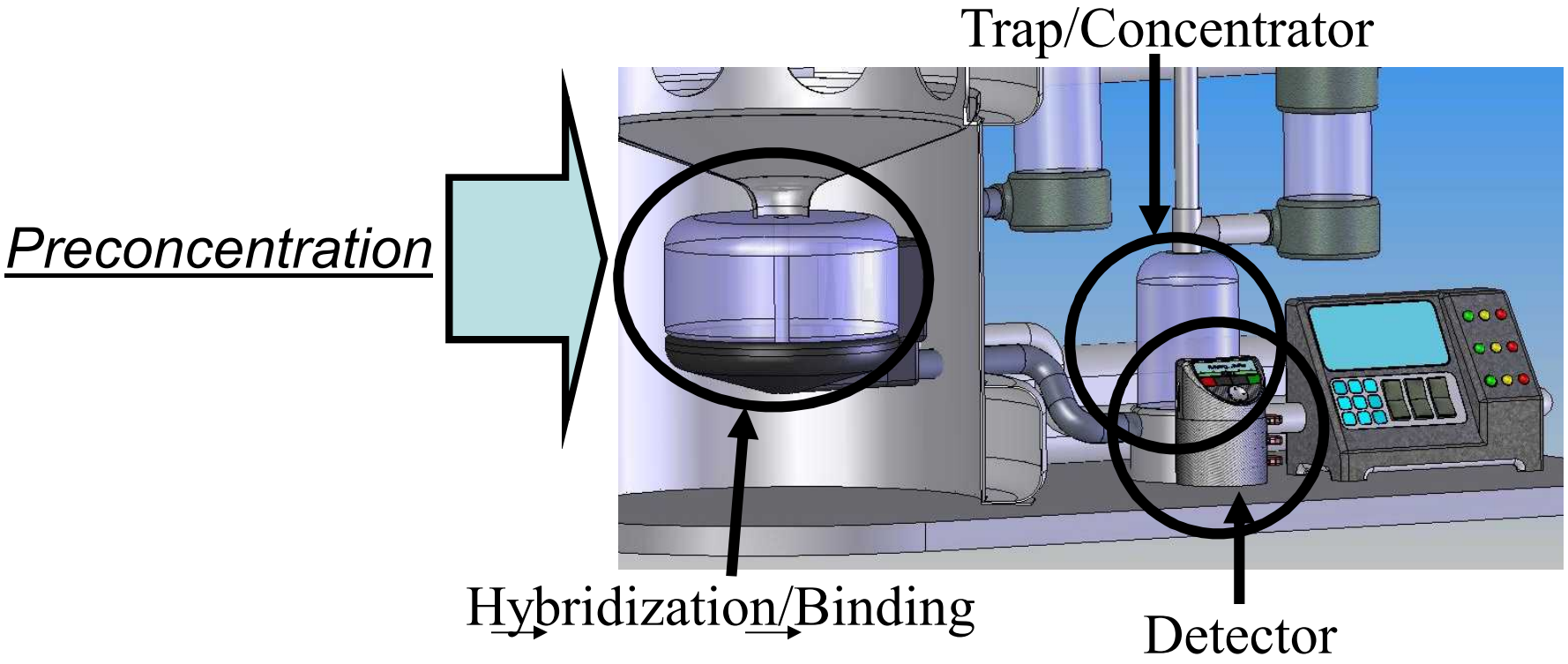
Bottom Line: we can create 2D null points where the latex magnetic beads will migrate. By modifying the dimensions + spacings of the electrodes, we can ensure that single beads are on an axis (above right movie). Questions to be answered are the frequency effects (which freq to use?) and interparticle interactions that will cause beads to pearl-chain as in the above right movie. We could design the electrodes to define 3D nulls that would separate individual beads, but translational motion would be difficult (would need shifting traps to get translation of the trapped beads).

Envisioned Flow-Through Prototype hand-held system



Strawman system – water

- Replace air-water collection with large volume of water to small volume of bead solution with preconcentrator.



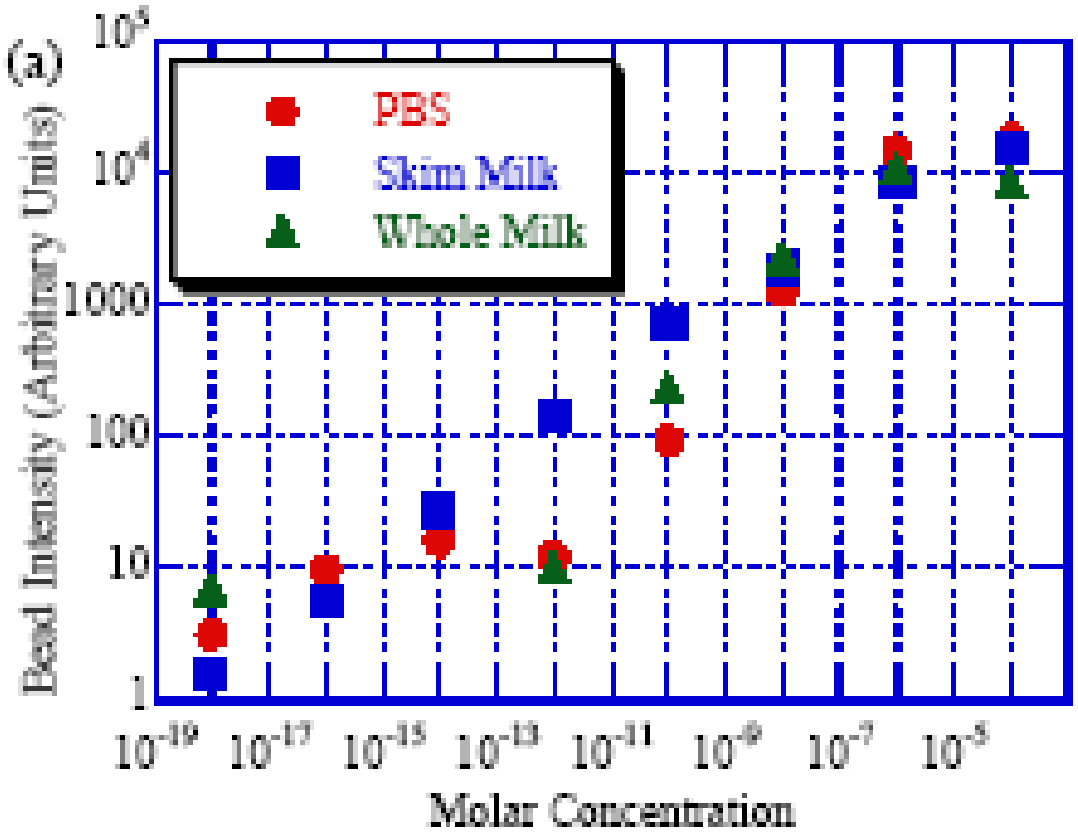
I invite your input – especially on pre-concentration.

Conclusions

- Bead based detection of botulinum substitute in milk indicates utility of concept for liquid based dangerous agent detection in dirty liquids.
- System concept adaptable to many problem scales – spanning macro to nano scales.
- Opportunity to adapt partially developed bead-based detection systems to high throughput sensors for micro-organism detection in water.

Acknowledgements

Extras



- Measurements with confocal microscope show sensitivity advantage
- Raw milk causes problems – being worked
- We need this kind of curve in the new MiniME

  Note: Use later sensitivity curve from Achyuthan

- DEP video, trapping video

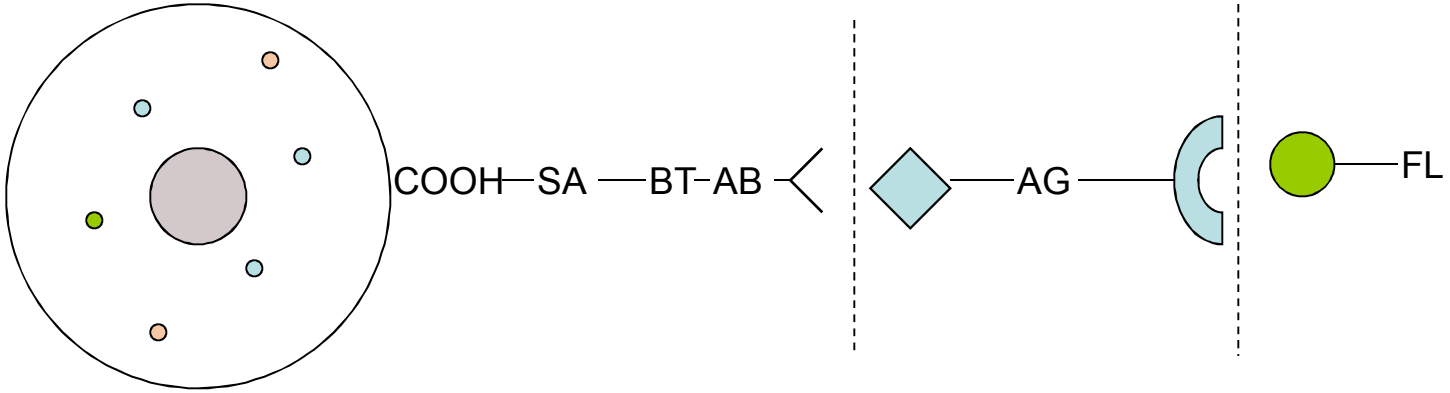
FL – Fluorescent label

AB – Surrogate antibody

AG– Surrogate antigen

SA - Streptavidin

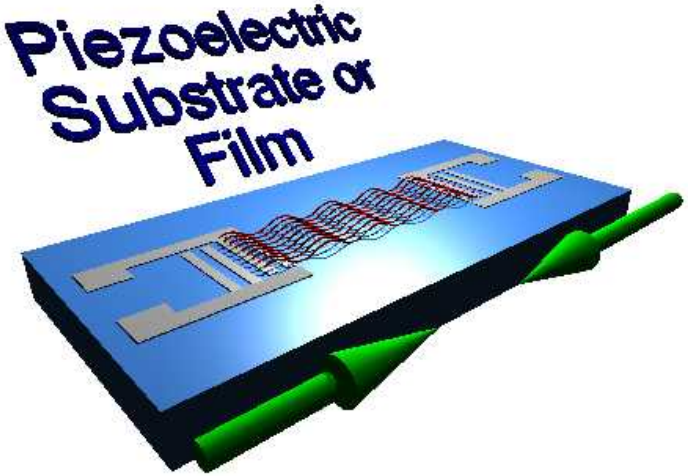
BT - Biotin

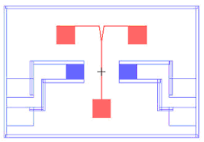


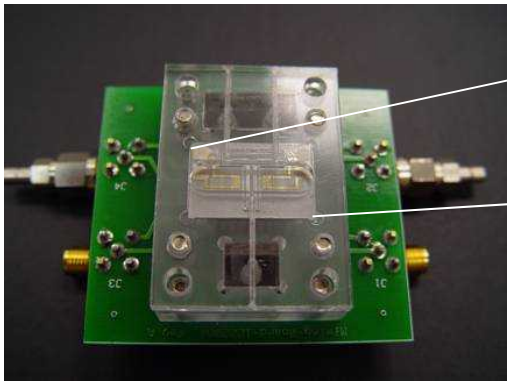
Surrogate Sandwich Assay

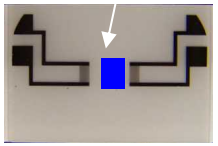
Summary: Acoustic Technology

- Robust & Simple = Reliable & Low cost
- Small active region (<1 mm^2)
- No mechanical failure
- Rapid (seconds to minutes)
- Thermal Shift less than 1°C
- Low power Surface Acoustic

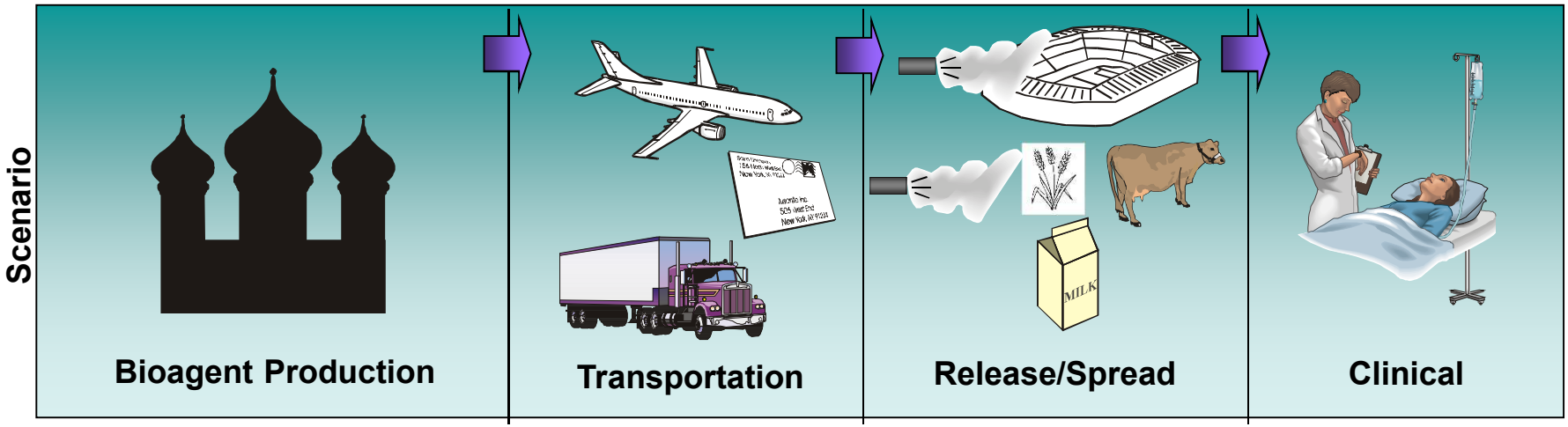








Malicious Disease Outbreak



- DNA analysis backup slide