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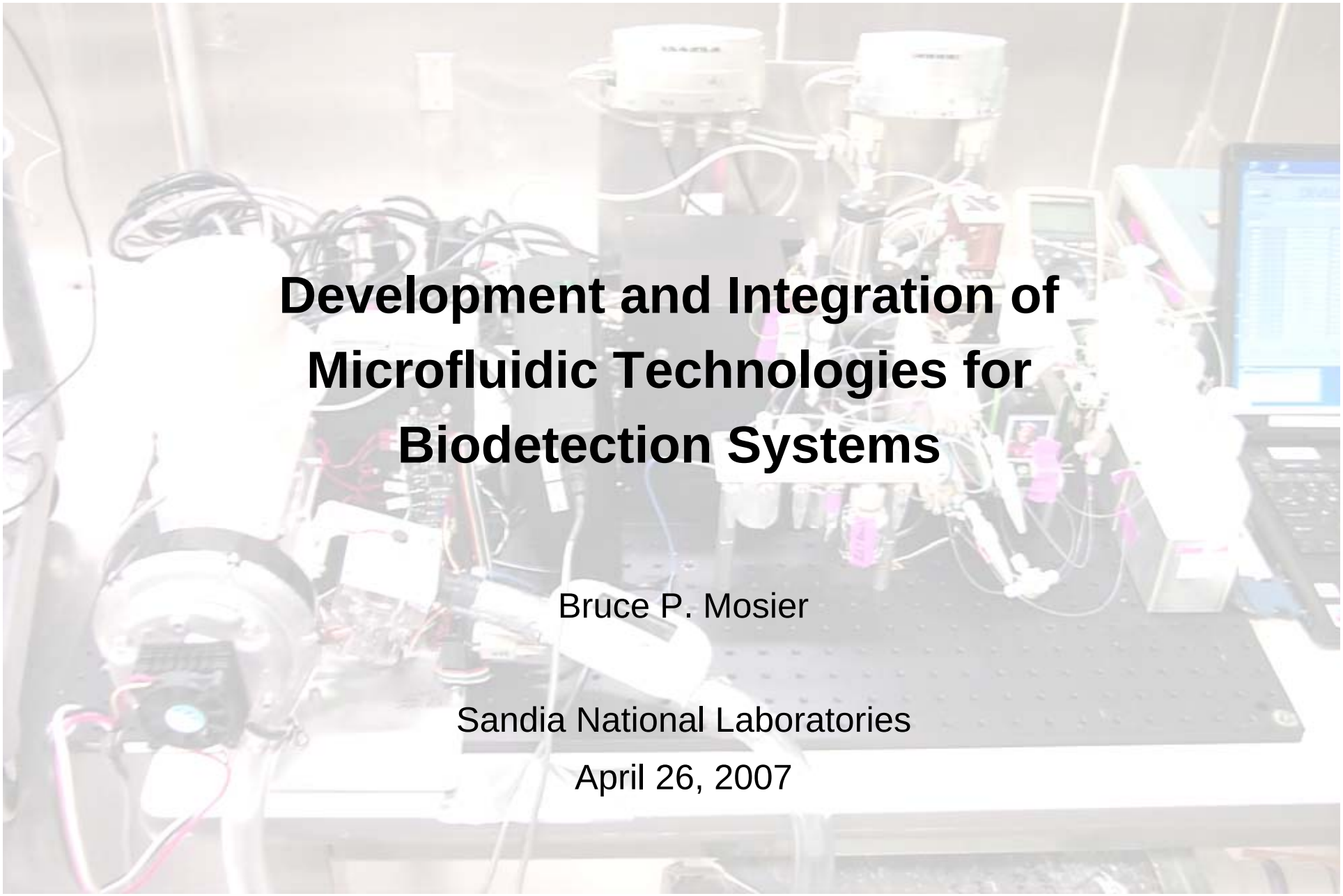

Development and Integration of Microfluidic Technologies for Biodetection Systems

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Sandia National Laboratories

April 26, 2007

Presentation Outline

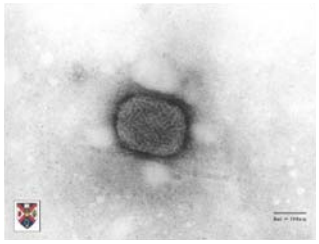
- Overview of MicroChemLab (µChemLab)
- Improvements to µChemLab
- Development of Electrokinetic (EK) Pumps and Flow Sensors
- Automated Sample Preparation
  - EK Pumps
  - Stepper Motor / Valve Arrays
  - Flow Injection Analysis (FIA)
- Electronics and Software for System Automation and Control
- Tenix/CH2M HILL Unattended Water Monitoring System
- DoD µChemLab Spore Detection System
- BioBriefcase System, Field Test Results from UNLV and ECBC
- Summary

µChemLab: Handheld Biodetection System

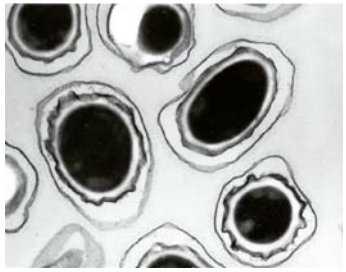
- Applications
  - First responders
  - Facilities monitoring
  - Pathogen detection in water
- Detect full suite of bioagents
  - Biotoxins, viruses, spores, bacteria
- Requirements
  - Rapid detection for detect-to-warn
  - Low power for field use
  - Low false-alarm rate
  - Little or no consumables
  - Adaptable to new threat agents

Bioagents Pose Diverse Sample Processing Challenges

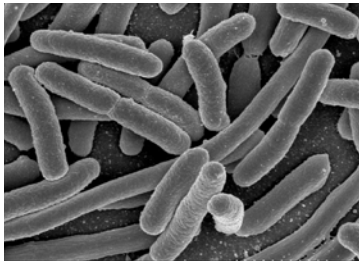
Toxins
- 1-10 nm
- Protein or small molecule
- May have variants



Viruses
- 50-200 nm
- 1-50 proteins
- May have host specific proteins



Spores
- 1 μm
- 50+ proteins
- Vary in copy number



Bacteria
- 1-3 μm
- 2000-5000 proteins
- Protein content dependent on growth conditions

- Sensitivity: 500 pM
- Analysis time: 10 min
- Power consumption: 3.5 W (8 hours of continous battery-powered operation)
- Demonstrated signatures for:
  - Ricin
  - Staphylococcal enterotoxin A, B
  - Botulinum toxin surrogate
  - Cholera and Tetanus toxins
  - Viruses: including A. encephalitis, V. stomatitis, Vaccinia
  - B. Anthracis spores
  - E. Coli

Overview of Detection Process

Manual Sample Preparation for Viruses, Spores, and Bacteria

Collect Sample

Thermal Lysis

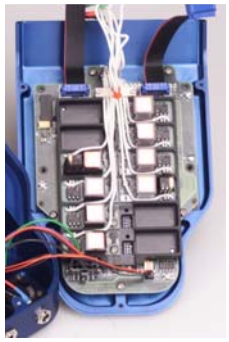
Concentrate

Label

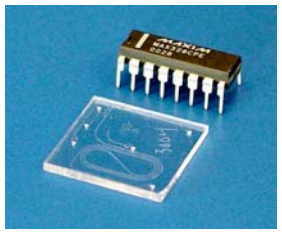
Load Chip

Automate Sample Prep for Unattended Operation and Portability

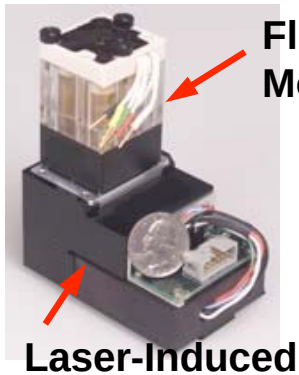
Automated Separation



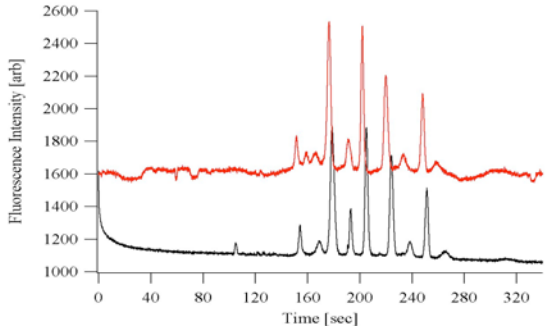
High-Voltage Power Supplies



Microchip

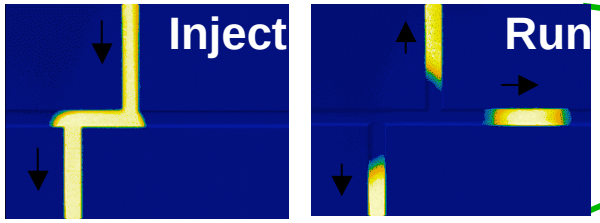


Laser-Induced Fluorescence Optics Module



µChemLab On-Chip Separations

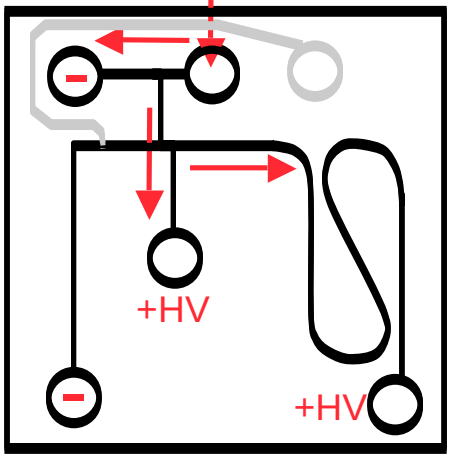
Offset “Tee” Defines Injection



Sample plug

Electrophoretic Pressure Injection (<5 psi)

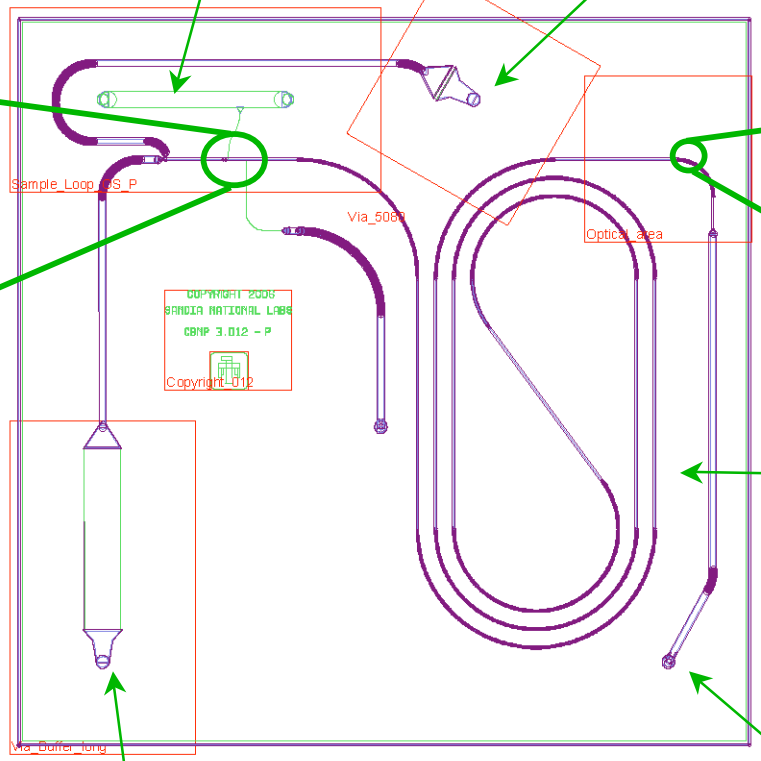
Electrophoretic Separation (5 kV)



Sample Loop

Flush port

UV-LIF Detection Region



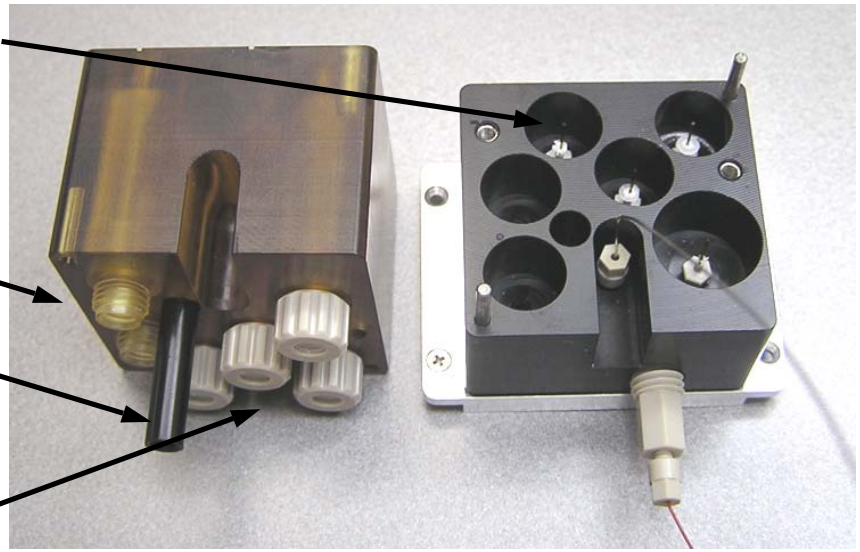
Separation column, 10 cm length, 500 V/cm

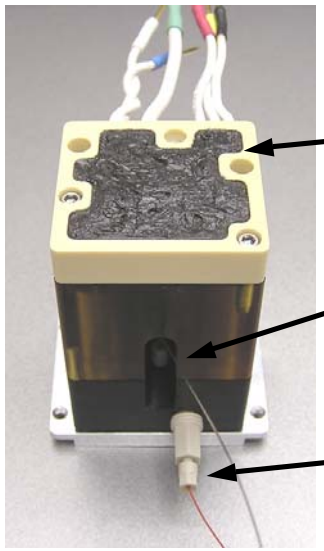
Overall 20 x 20 mm

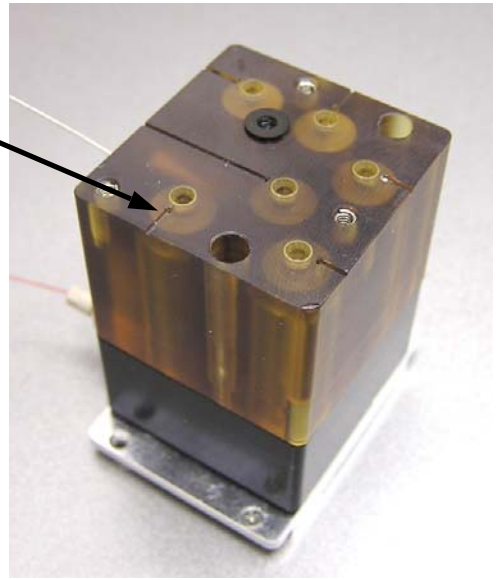
Column Waste

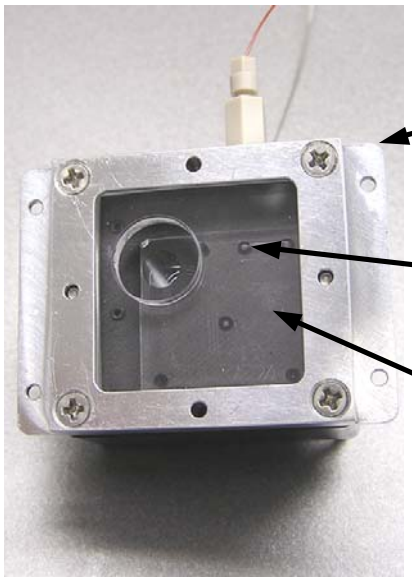
Column Head

Original (2001-2006) μChemLab Fluid Module



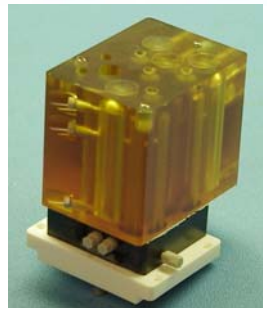


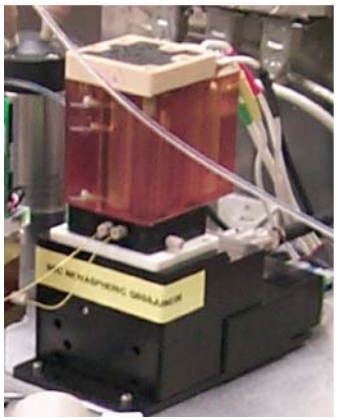


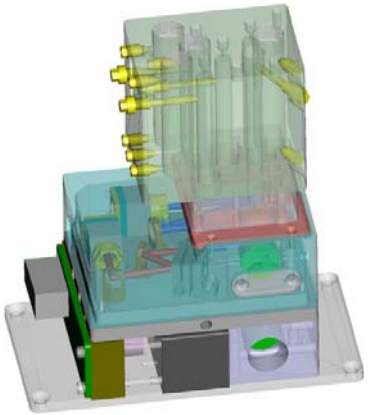


μChemLab Fluids Module: Reliability and Ease-of-Use Improvements

- **Improved Chip Design:**
  - **Lower Pressure and More Balanced Gel Flushing with 10 μm Shallow Etch Depth (vs 4 μm)**
  - **Less Carrover with Narrower Sample Channel**
- **New Fluids Module:**
  - **Fewer parts: press-fit manifold/cartridge and single-piece compression frame eliminate glass capillaries, septa**
  - **Replaced large syringe port with low dead volume nanotight port**
  - **Added individual drains to each reservoir**
  - **Replaced air vents with nanotight ports to permit sealing for transport**
  - **Added high-relief o-ring captures**
  - **More uniform compression with symmetric compression frame**
  - **Added gel spillway to automatically maintain same gel level for more consistent separations**

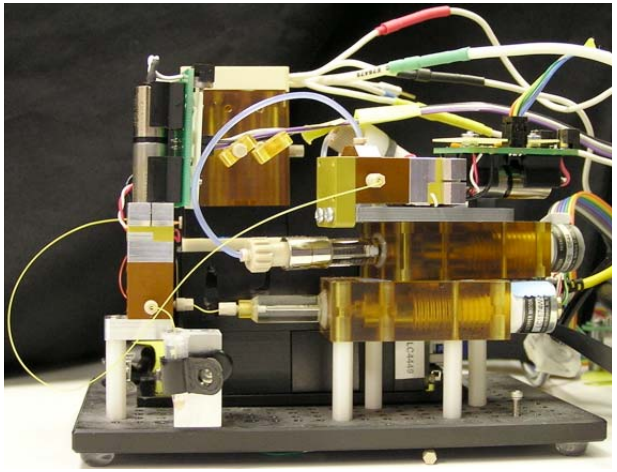


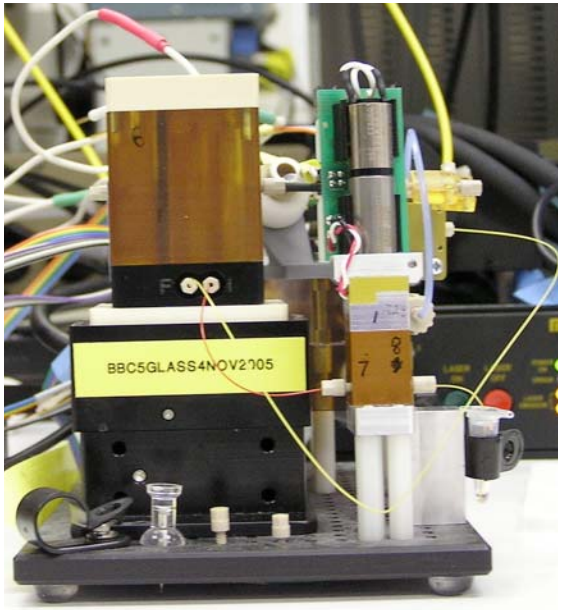


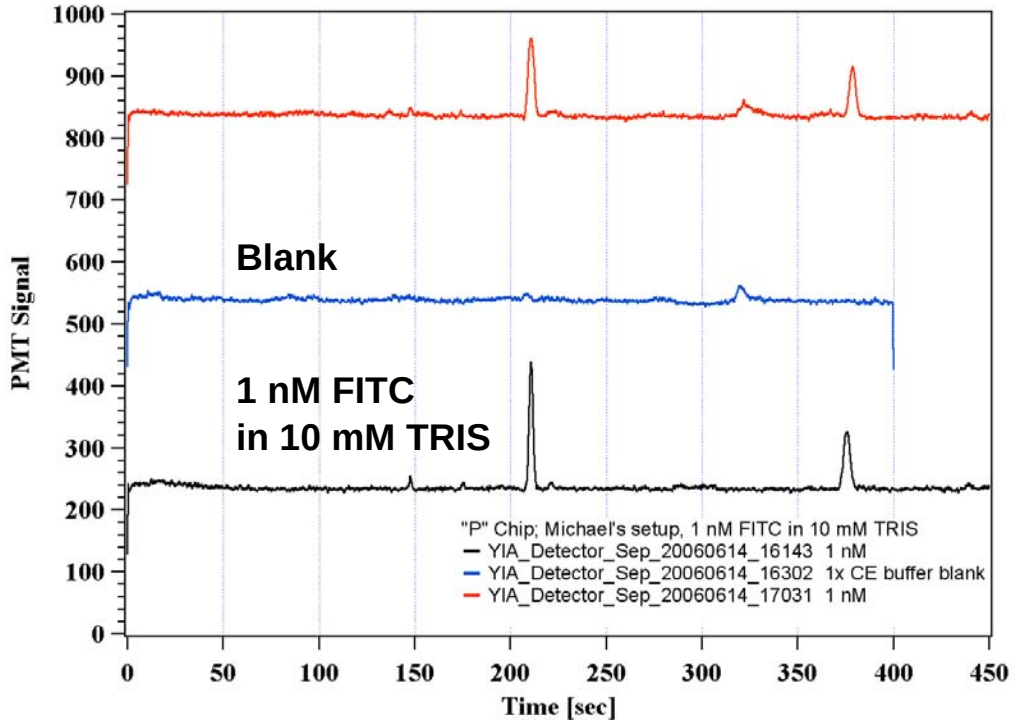


Automated μChemLab

- Easy operation -- place centrifuge tube on holder, start software routine
- Used to demonstrate >600 separations on one chip
- No maintenance necessary other than refreshing gel reservoirs
- Glass or plastic chips may be used



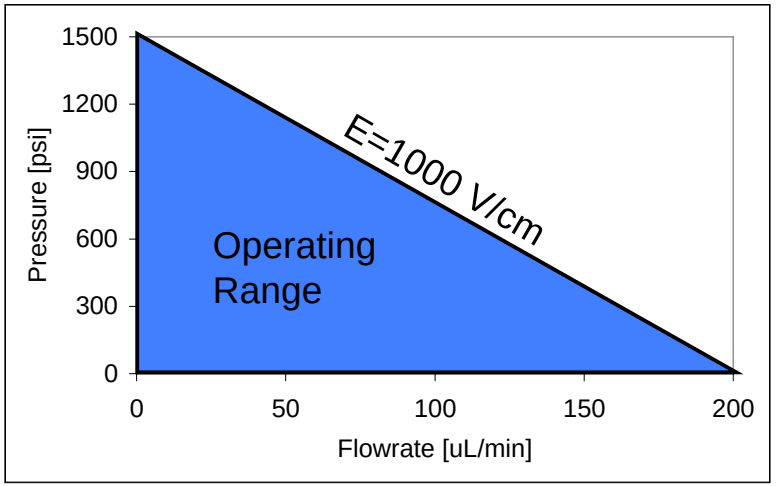




Electrokinetic Pump Performance Summary

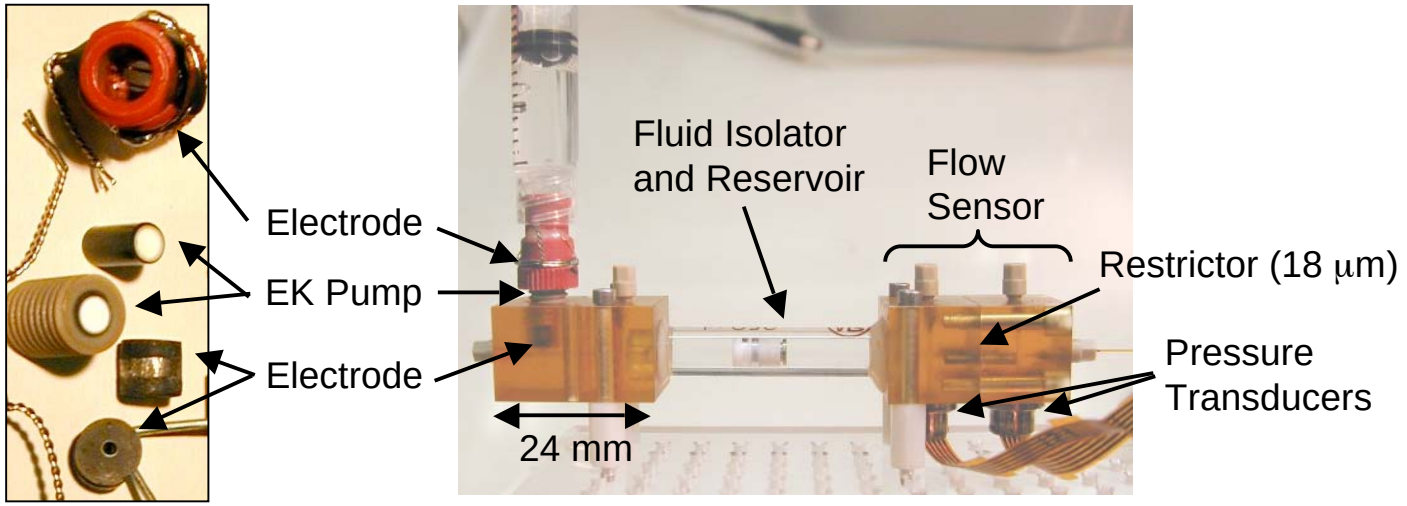
Operating Summary (for 1 cm-long, 2.5-mm-diameter core)

- Flowrates up to 200 uL/min
- Pressures up to 1500 psi (10 MPa)
- Operating Voltage up to 1500 V (drawing 2 mA)
- 1% Electric-to-Hydraulic Conversion Efficiency



Why use EK pumps for fluid metering?

- Compact Electrodes and Pump, Scalable to Chip
- High-Pressure Capabilility
- Pulseless Flow, No Moving Parts
- Fast Transient Response



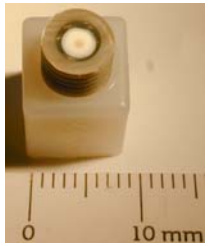
Fabrication of Polymer & Silica Monoliths

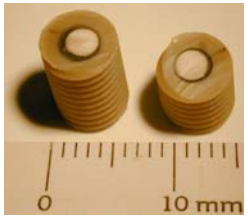
Polymer:

Prep & Mix Monomers → UV or Thermal Cure → Supercritical CO_2 Extraction → Bond in PEEK, Thermal Cure → Grind to Shape

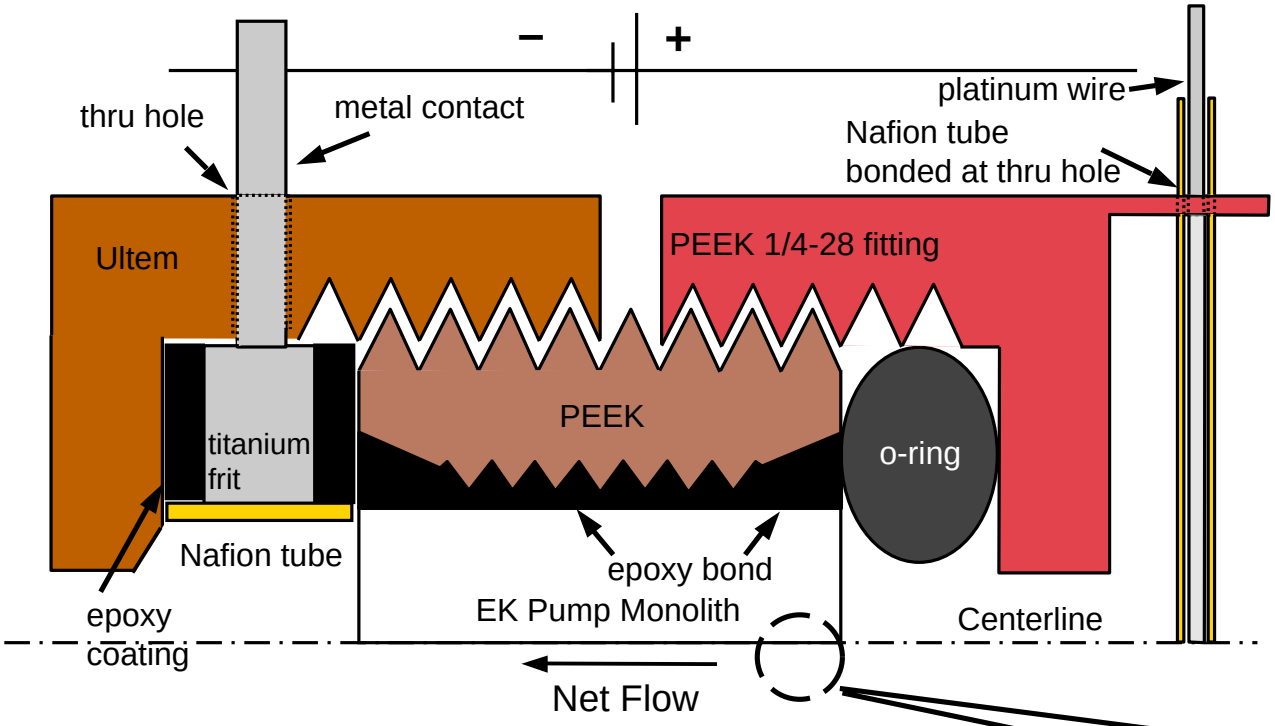
Silica:

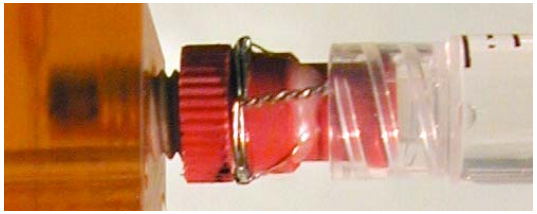
Stober Process → Mix Slurry → Flush & Fill Packing Tube → Pack up to 30 ksi → Flush at 40 ksi → Dry 3 hrs @ 90 C → Sinter 90 min @ 1050 C → Hydroxylate 5 min @ 60 C → Bond in PEEK, Thermal Cure → Grind to Shape





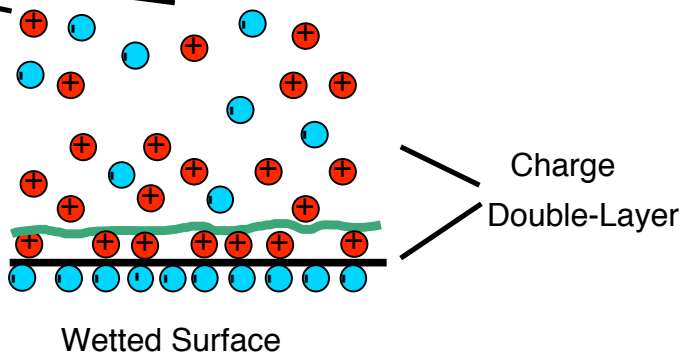
EK Pump Cross-Section



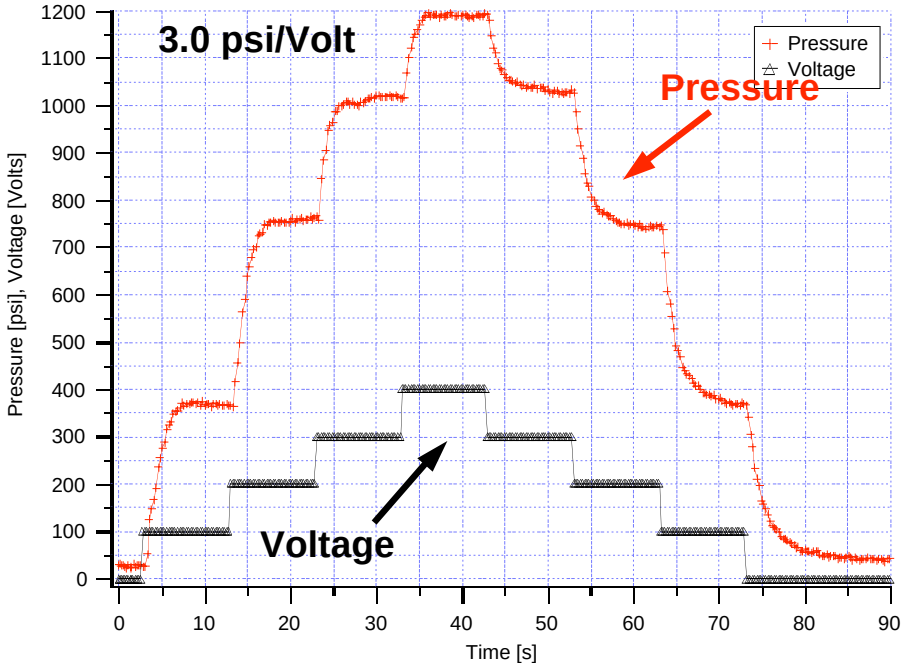


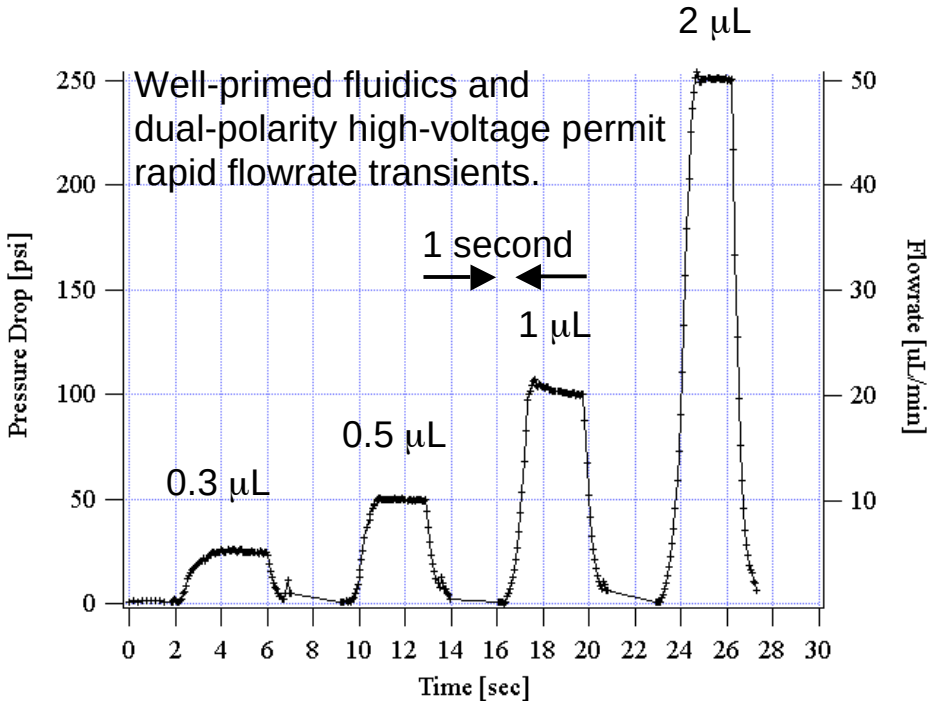
- O-ring and Face Seals
- Modular Design
- Easy to Fabricate

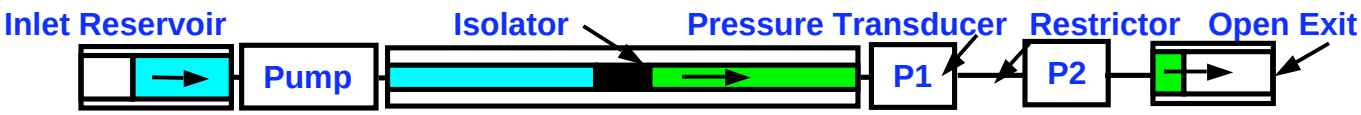
- Net Charge in Electric Double Layer (EDL)
- Electric Field Drives EDL
- Viscous Forces Drive Bulk Fluid
- Pressure is Obtained by Restricting Outlet Flow



EK Pump Pressure Transients and Volume Metering

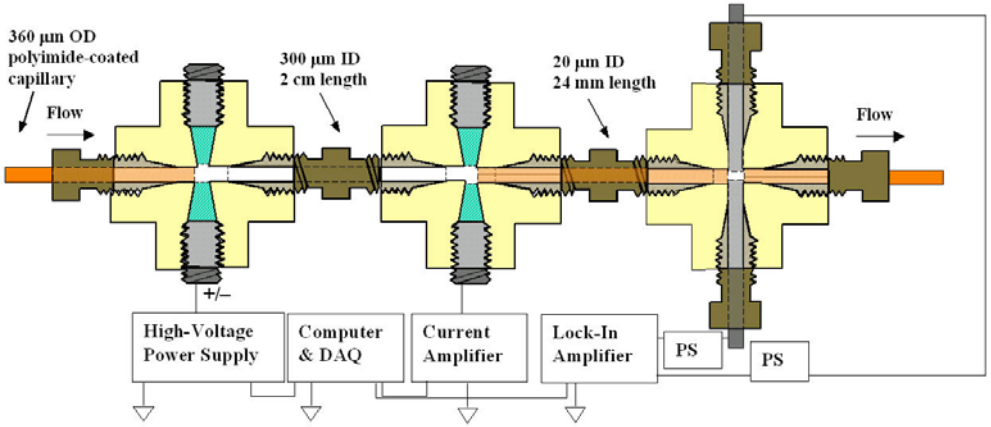


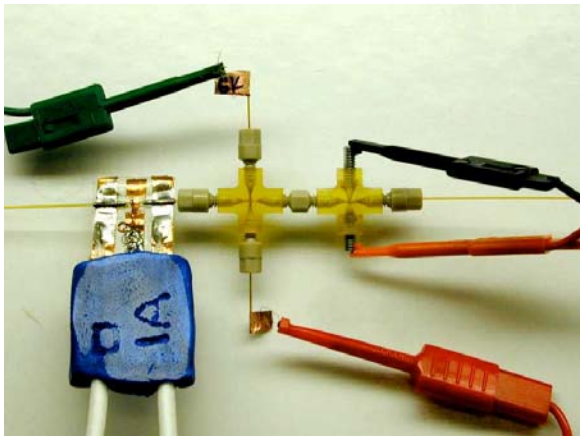


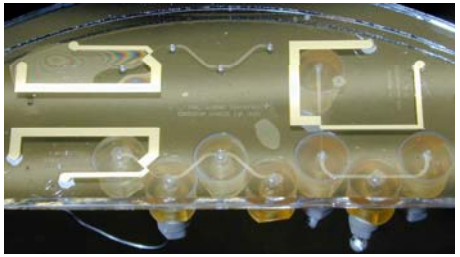


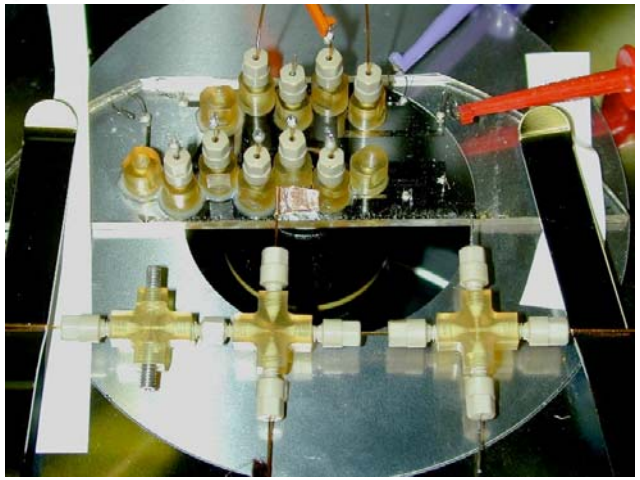
Waters CRADA: Flow Sensors

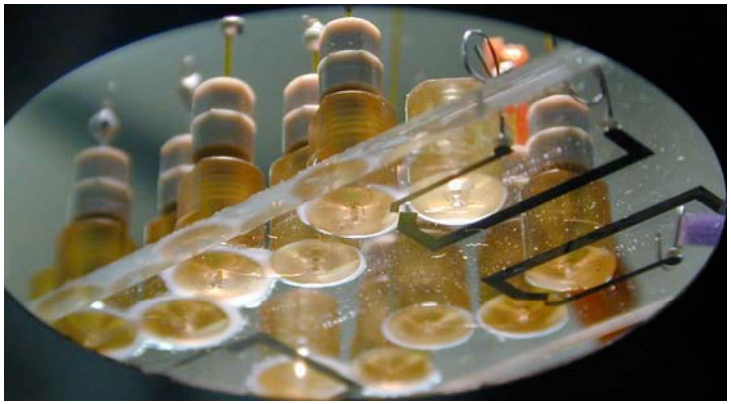
- **Direct or contactless flight time measurement of conductivity pulse**



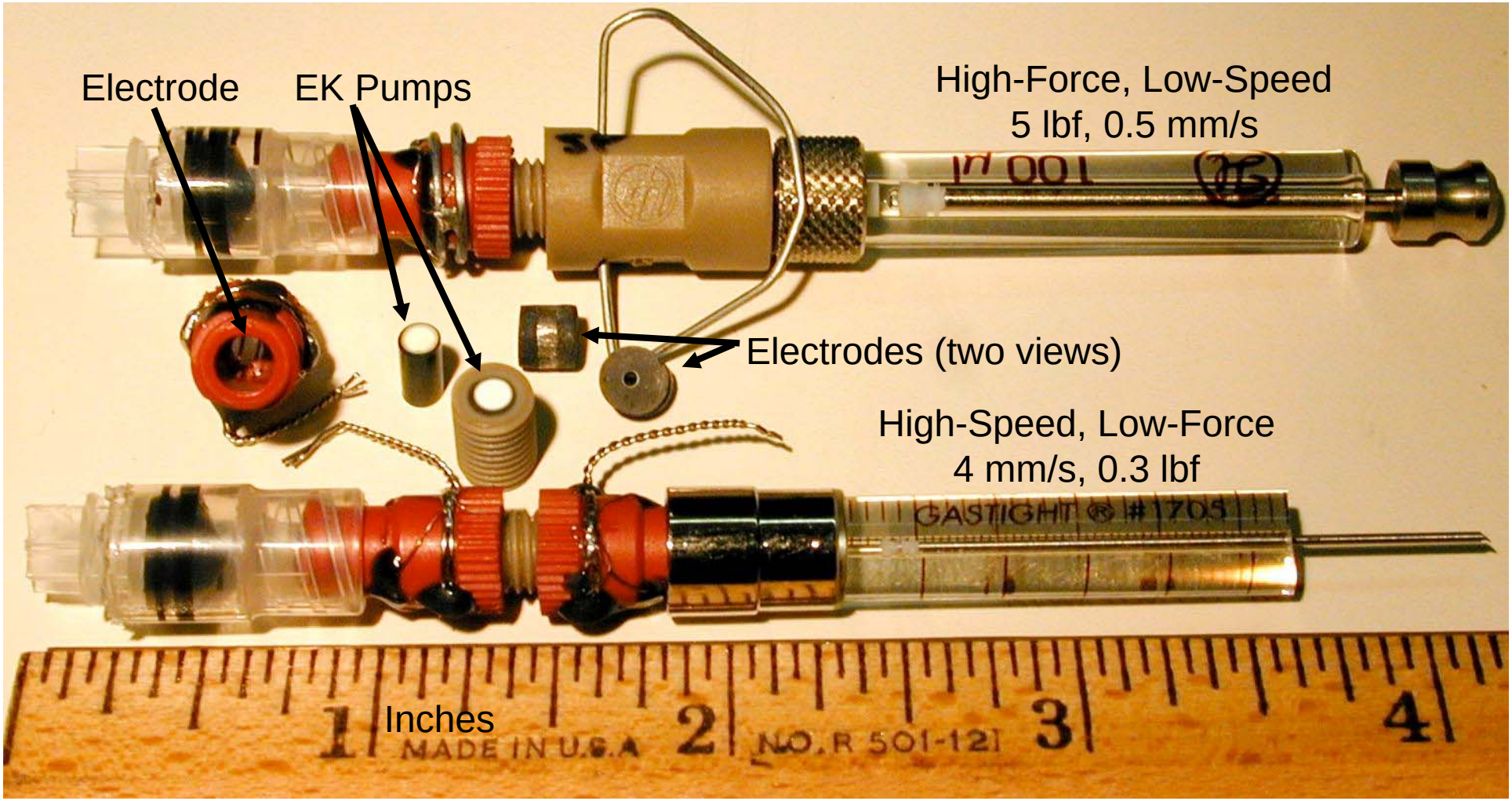


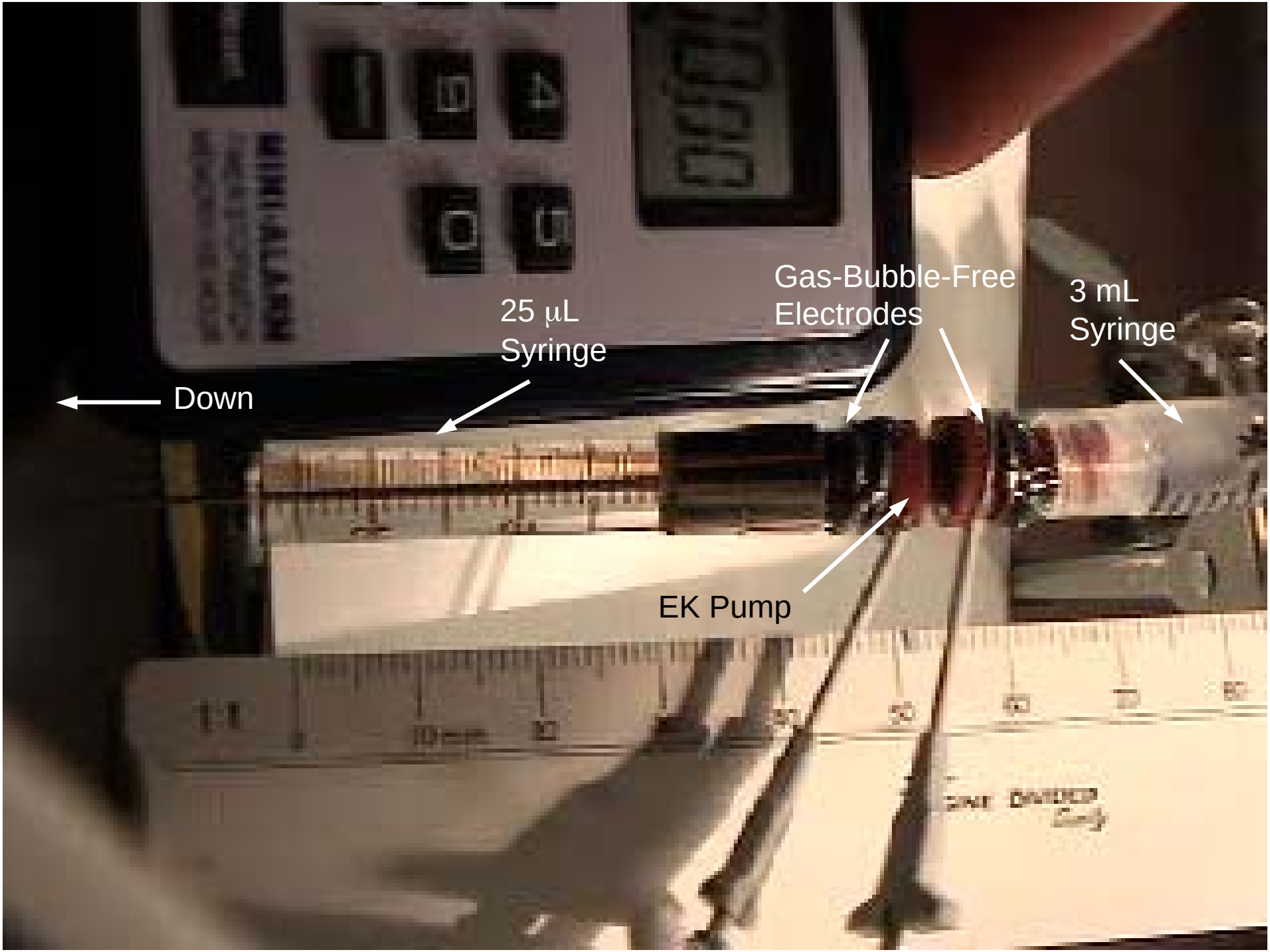






Actuators and Components




Down
25 μL
Syringe
Gas-Bubble-Free
Electrodes
3 mL
Syringe
EK Pump

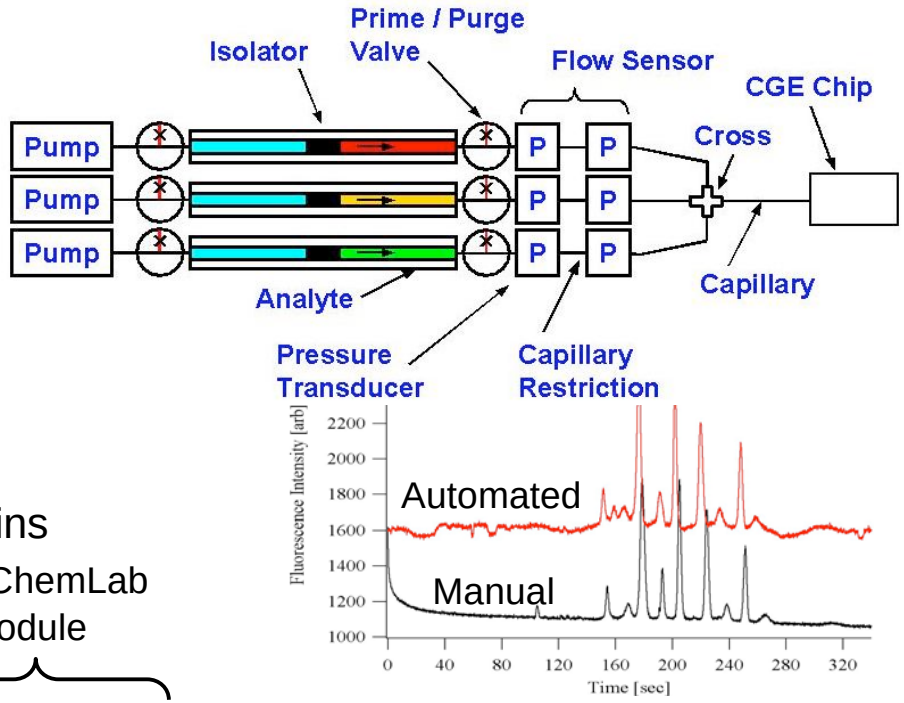

0.5-mL Syringe
1 x 3 x 0.038”
Glass Slide
Pressure [psi]

Automated Sample Labeling: Electrokinetic Pumps

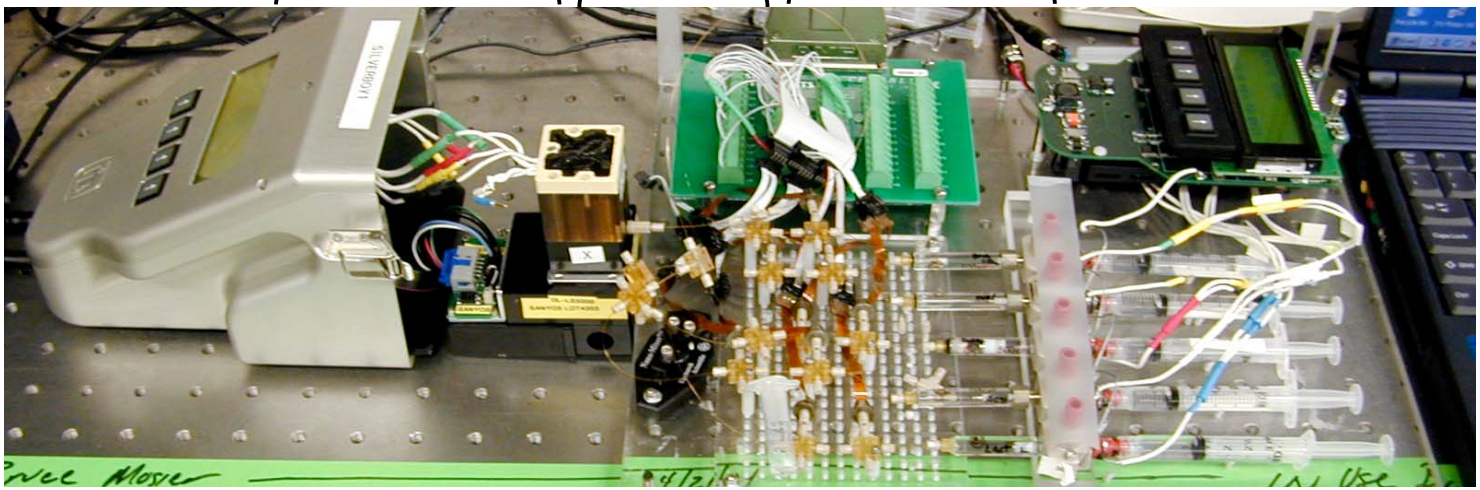
Automated In-Capillary Labeling
- Meter dye and sample at 1:9 flowrate
- Stop flow and wait 1 minute for labeling
- Meter labeled sample into μChemLab

Automated Gel Metering:
- Reduces wait time
- Improved run-to-run reproducibility

Demonstrated Labeling:
- Bacteria (GK anthracis), peptides, and proteins

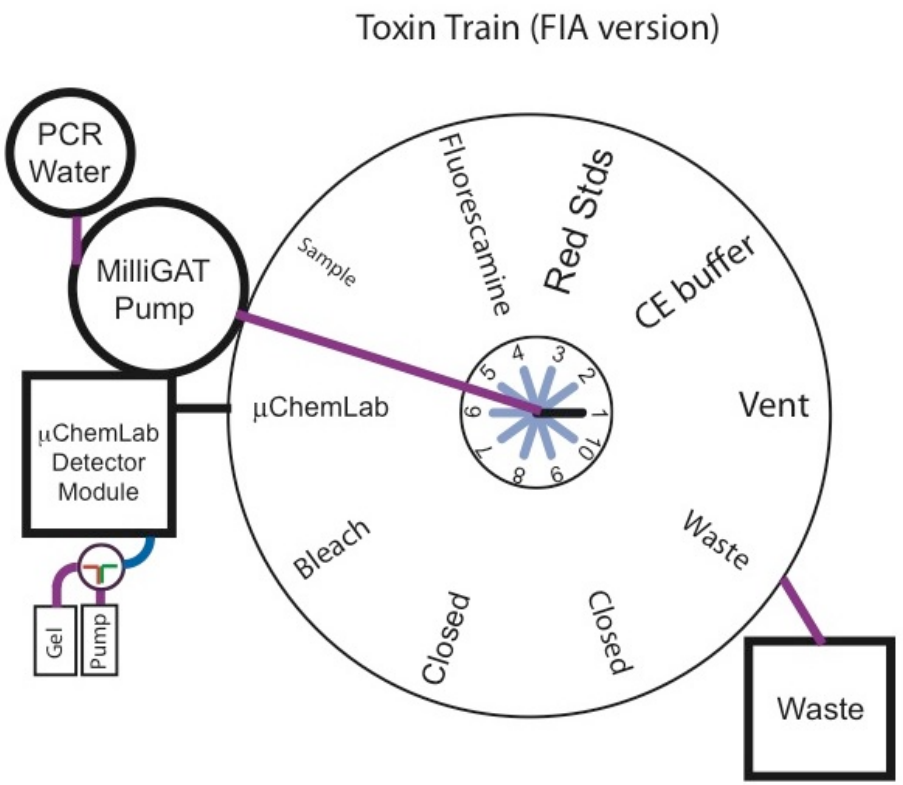


EK Pumps | Flow Sensors, Capillary Labeling | μChemLab Module



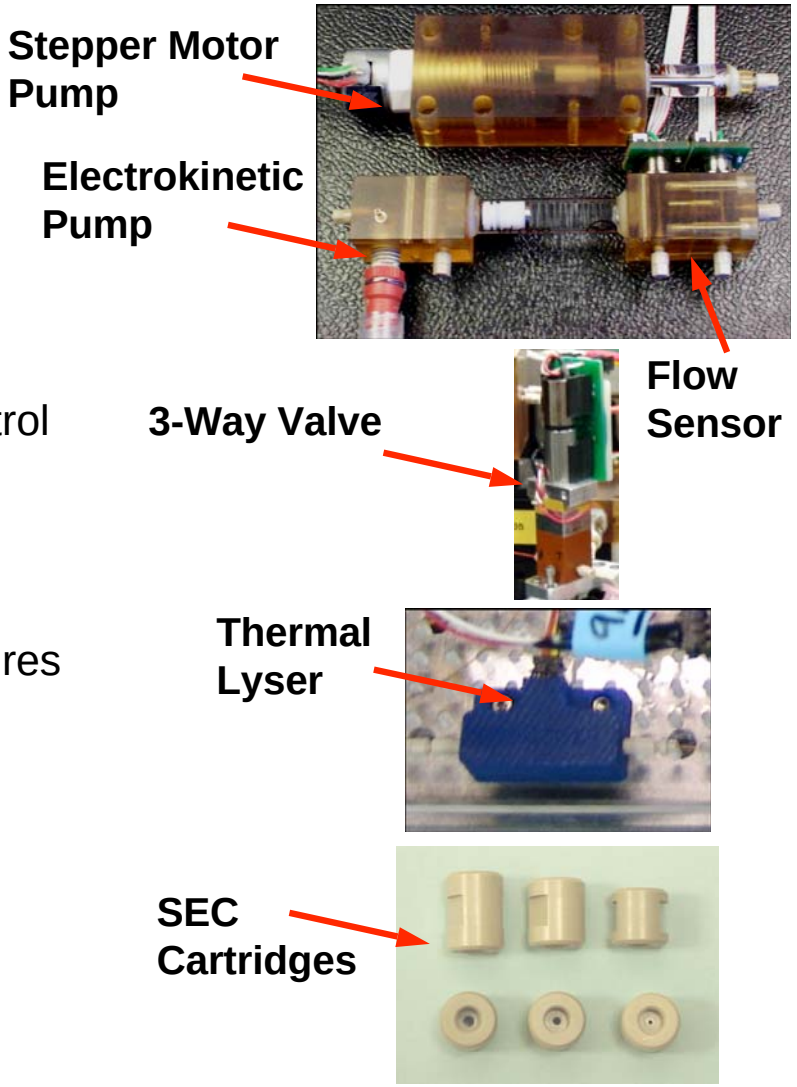
Flow Injection Analysis Toxin Train

- Simple Design
- Fewer Parts than Stepper-Motor & Valve Version
- Less Prone to Clogging
- Lower Reagent Consumption



Sample Prep Components and Actuators

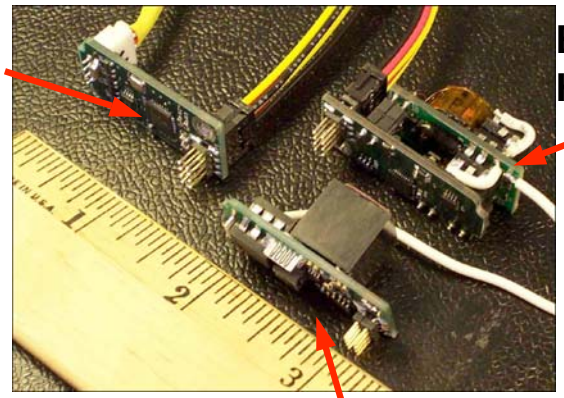
- Flow Sensors
- Electrokinetic Pumps
  - Compact design; flexible reservoir volume
  - Pulse-less flow; high-pressure capability
  - Scalable to chip
  - Built-in flowrate and pressure sensors
  - Fast transient response with closed-loop control
- Stepper Motor Pumps
  - Capable of suction for automated loading
  - Fast transient response
  - Open-loop possible for sufficiently low pressures
- Valves
- Thermal Lyser
- Solid Phase Extraction Cartridges



Electronic Controls

Three types of control boards:

- EK pump HV power supply
- High-speed switch
- 1/5/10 kV separation supplies

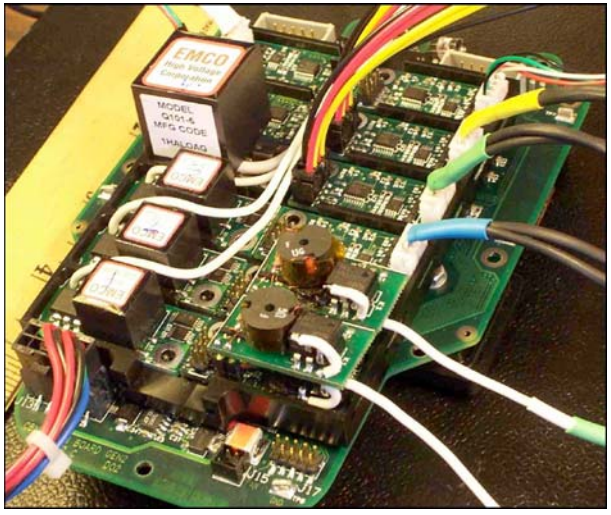


High-Speed Switch Boards:

- Stepper motor, variable voltage (PWM), on/off, and pulse
- High current capacity (5 Amps)

EK Pump High-Voltage Boards:

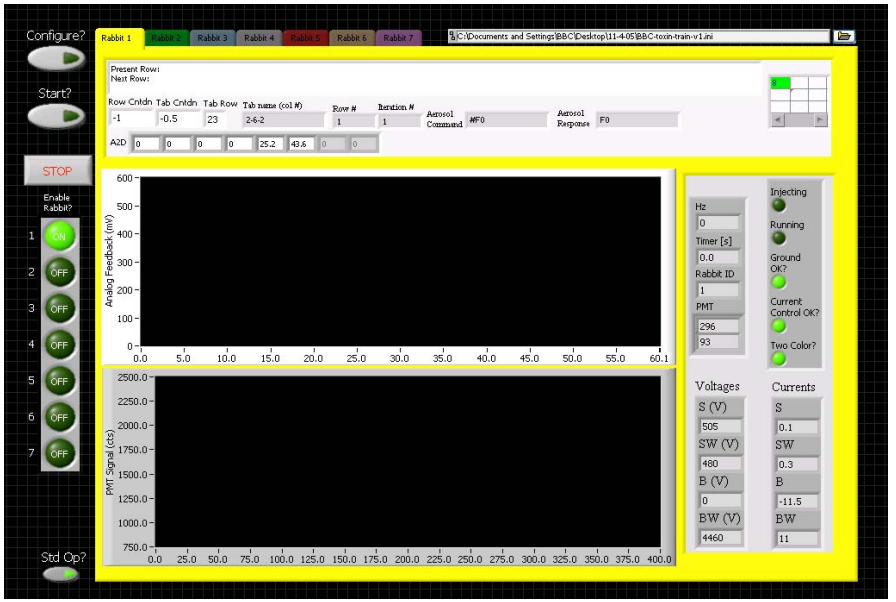
- ±1500 Volts, 2 mA, 500 Hz
- Constant flowrate and volume metering



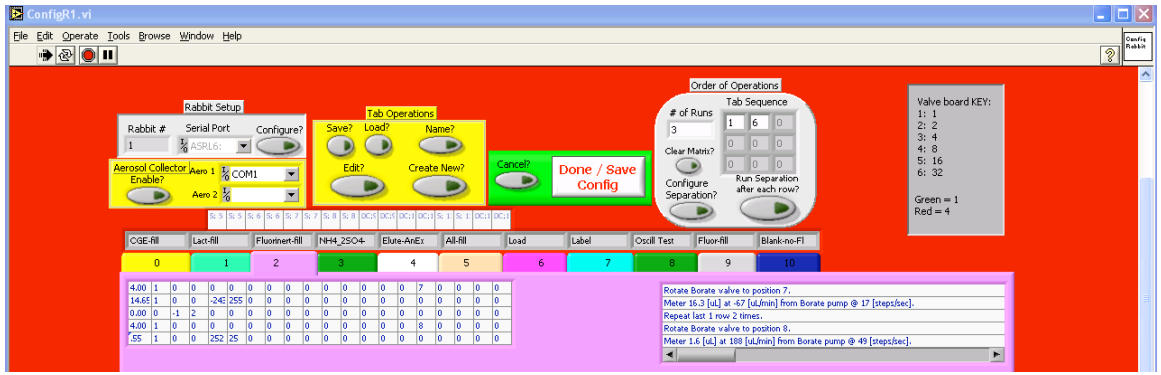
Rabbit Microprocessor Board with HV supplies and Switch Boards

“MasterMod” LabVIEW Controls Software

- Front Panel:
  - Start / Stop and Monitors A2D feedback and PMT signals



- Configuration Panel
  - User enters numbers that correspond to particular flowrates, valve positions, etc.
  - Interpreter describes functions as numbers are entered
  - 11 different “tabs” can be programmed and called in any order
  - Up to 7 Independent Rabbit controllers may be run simultaneously

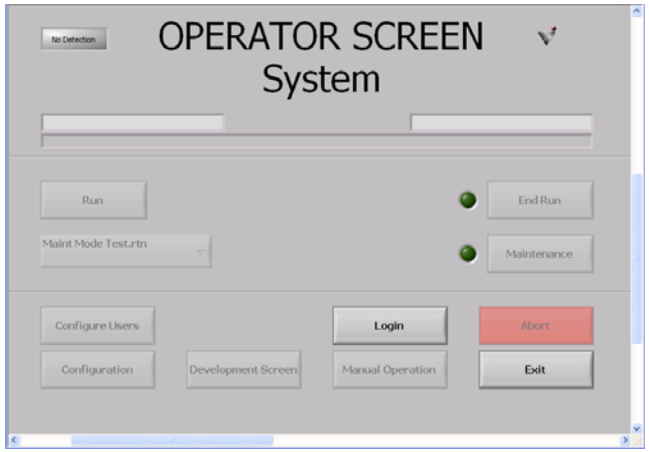


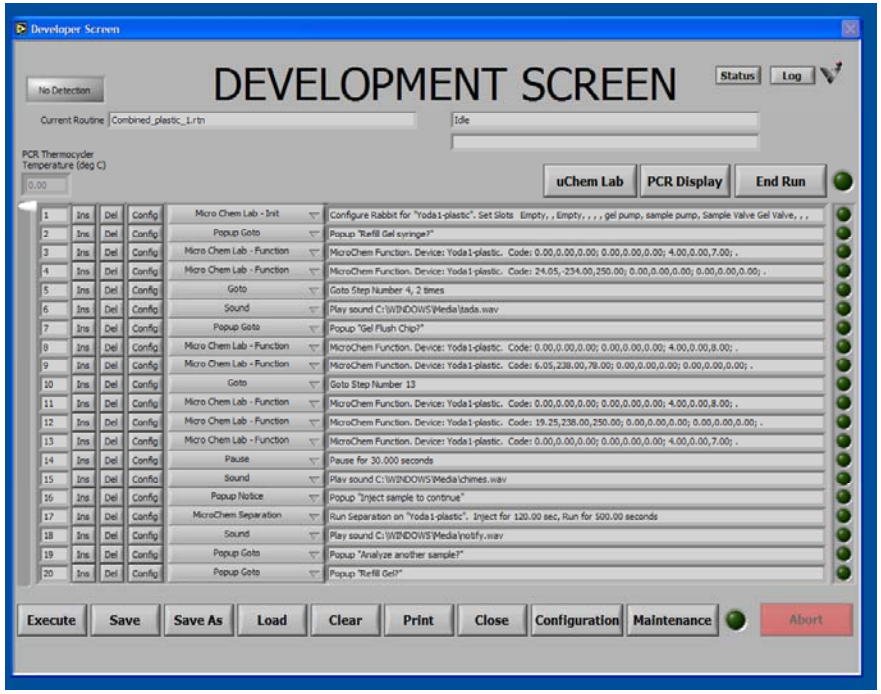
LLNL's "ISDAT" Controls Software

- Originally Developed for Autonomous Pathogen Detection System (APDS)

- Operator / Login Screen:
  - Logon Screen -- different users have different access / operational privileges
  - Built-in Network Capabilities for Remote Control and Monitoring

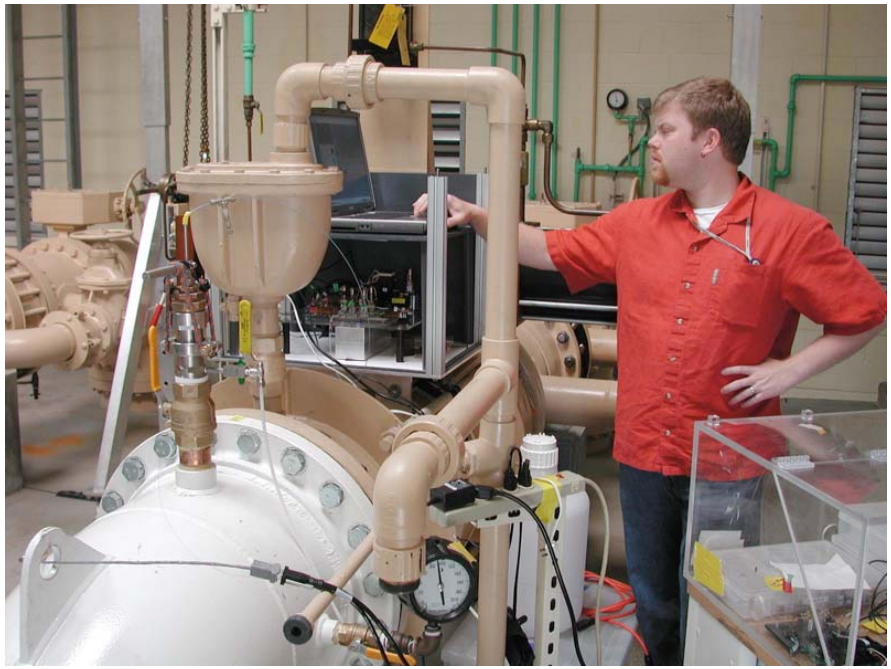
- Development Screen:
  - Flexible Operation -- Goto, Subroutines, User Query
  - User Programs Steps for Each Device Type
  - Text Summary Describes Function

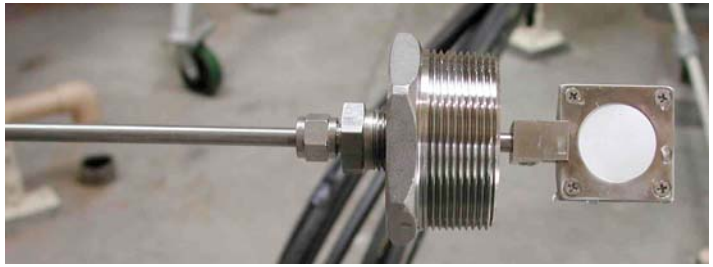




Tenix / CH2M HILL CRADA Overview

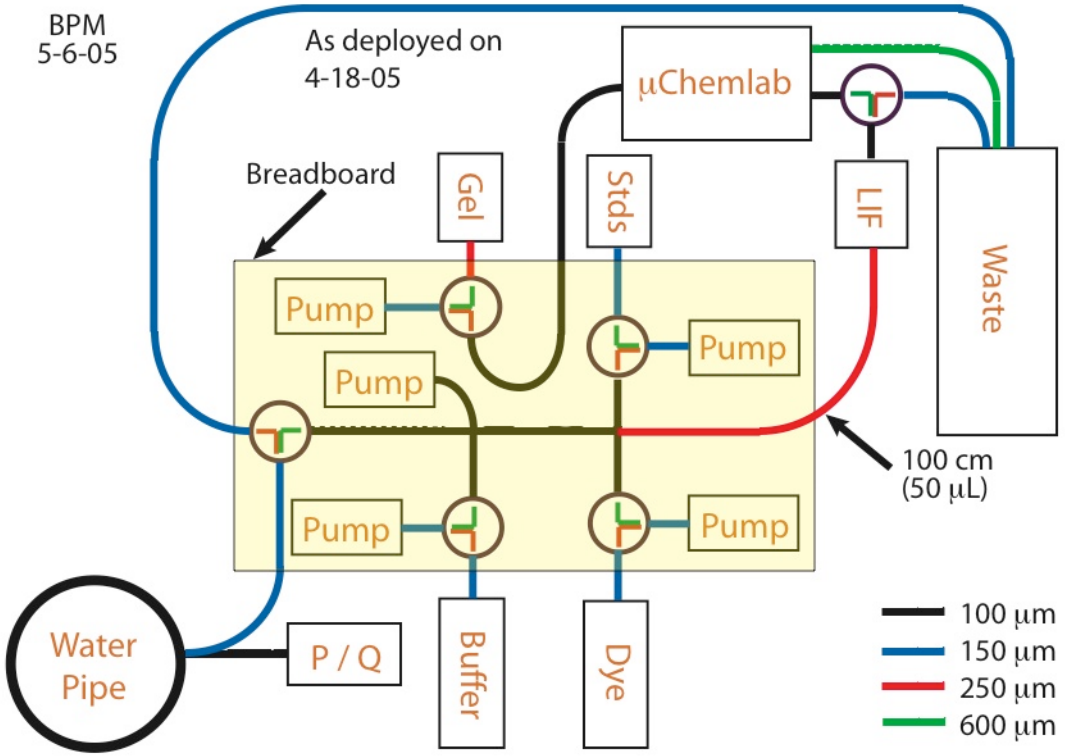
- Leverage μChemLab module
- Leverage Autonomous Sample Prep
- Build Unattended Water Monitoring System (UWS) and deploy at Willow Pass pump station in Concord (part of Contra Costa Water District)





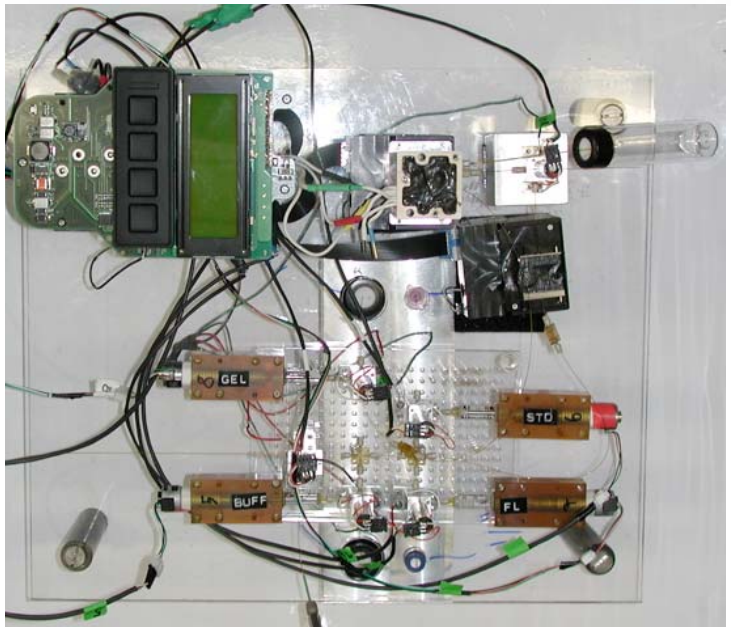
Tenix System Architecture

- Simple layout to maximize reliability
- Capillary sample prep and chip-based CGE separation
- System state of health included for reduced false positives
- Labeling in capillary
- Automatic gel flushing

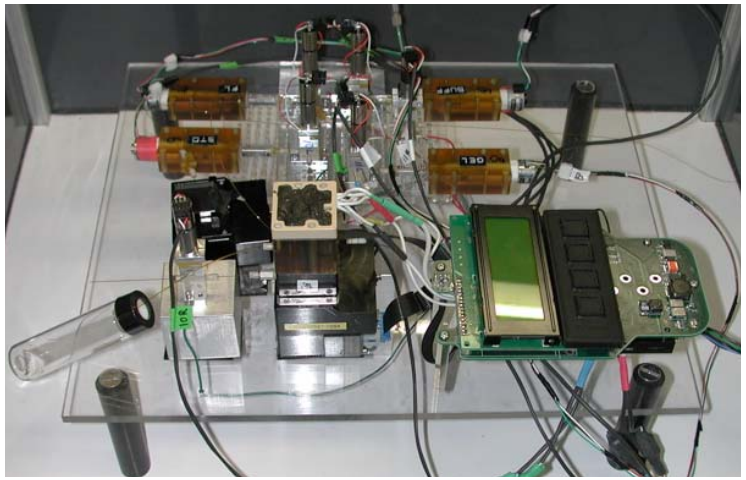


Original (2005) and Recent (2007) UWS Systems

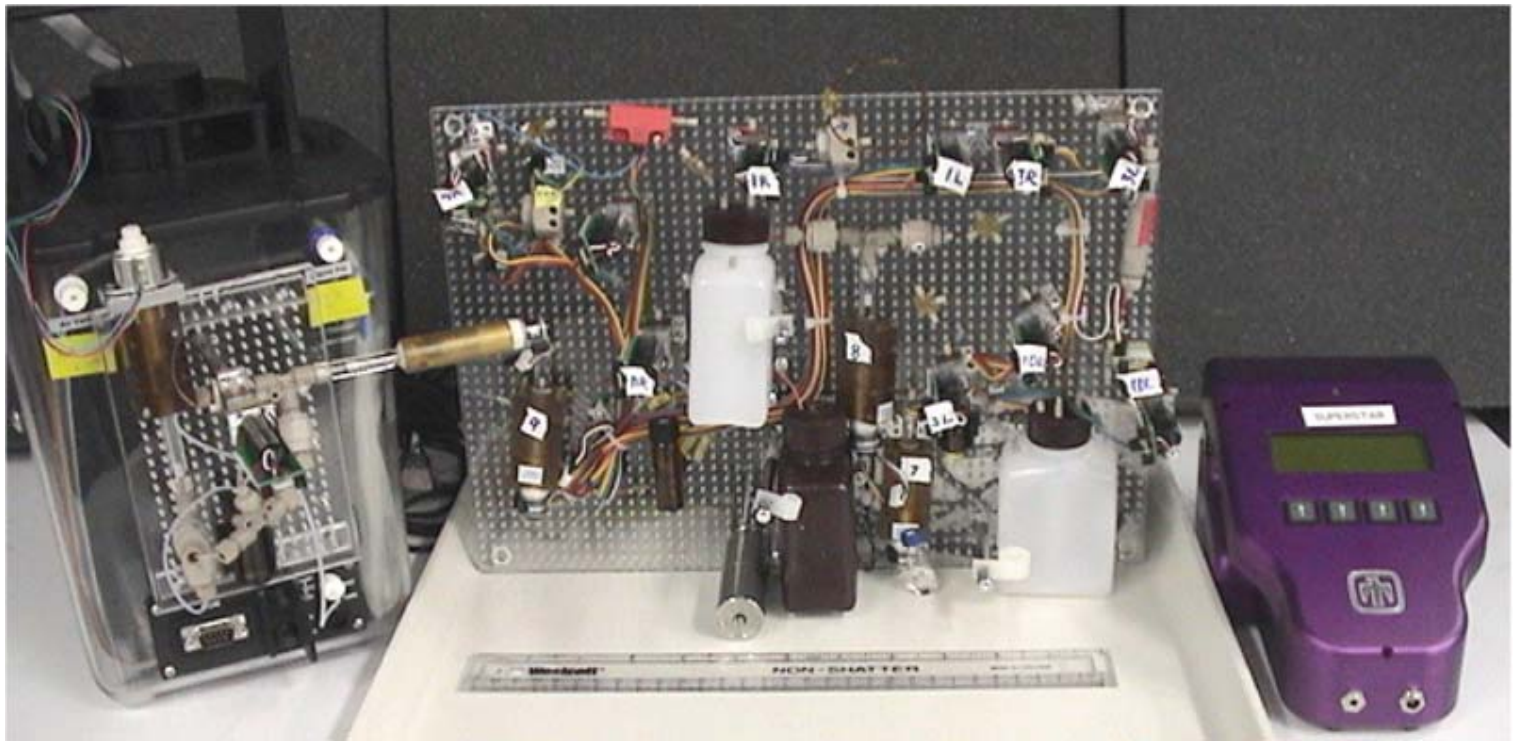
Original UWS Prototype



UWS Version 4



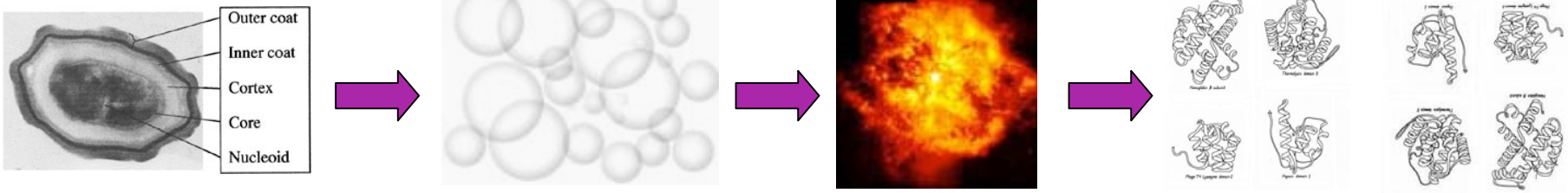
DoD μChemLab: Spore Detection System



- **Demonstrated sensitivity down to 10ACPLA (simulated) for *Bacillus subtilis* spores.**

Lysing Spores in µLiter Volumes

- Mechanical methods (ultrasonic, bead mill) – can kill spores and release DNA, but do not solubilize proteins
- Chemical – solubilizes proteins, but requires clean-up

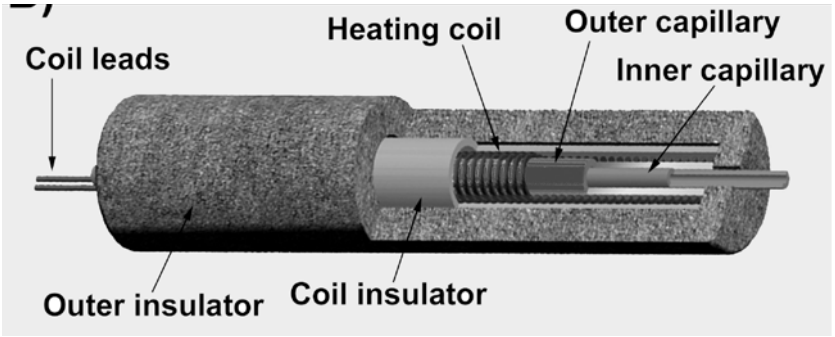


***Bacillus* spore slurry**

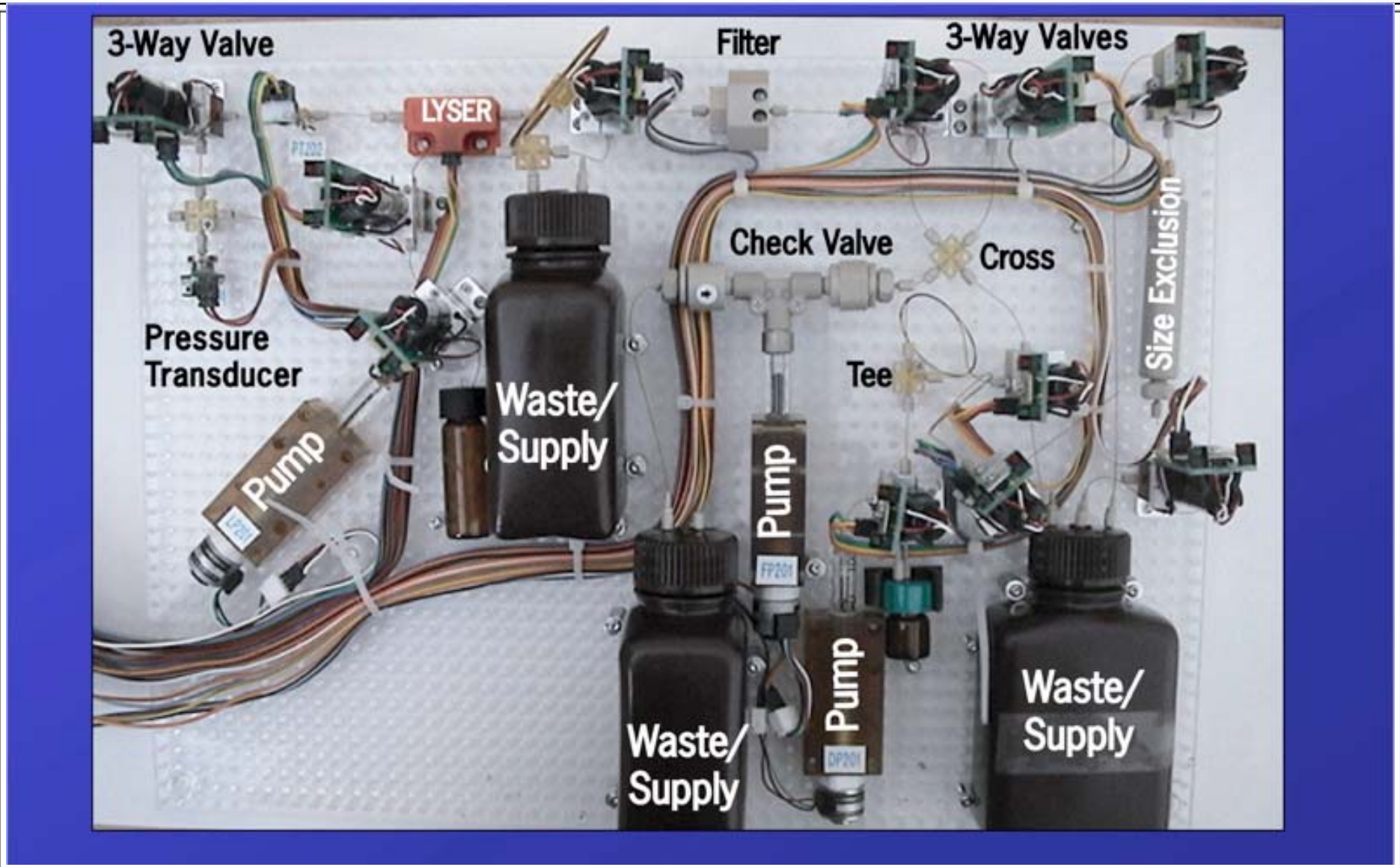
add lysis buffer
+ detergent
+ reducing agent

Heat
80 – 180 °C

protein lysate



DoD μChemLab Sample Prep



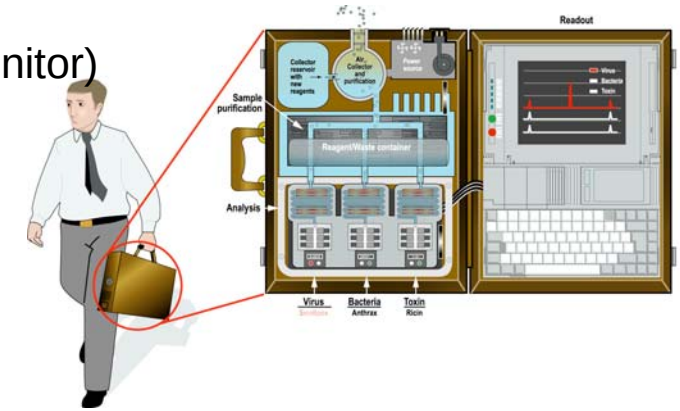
DHS-Sponsored BioBriefcase Project

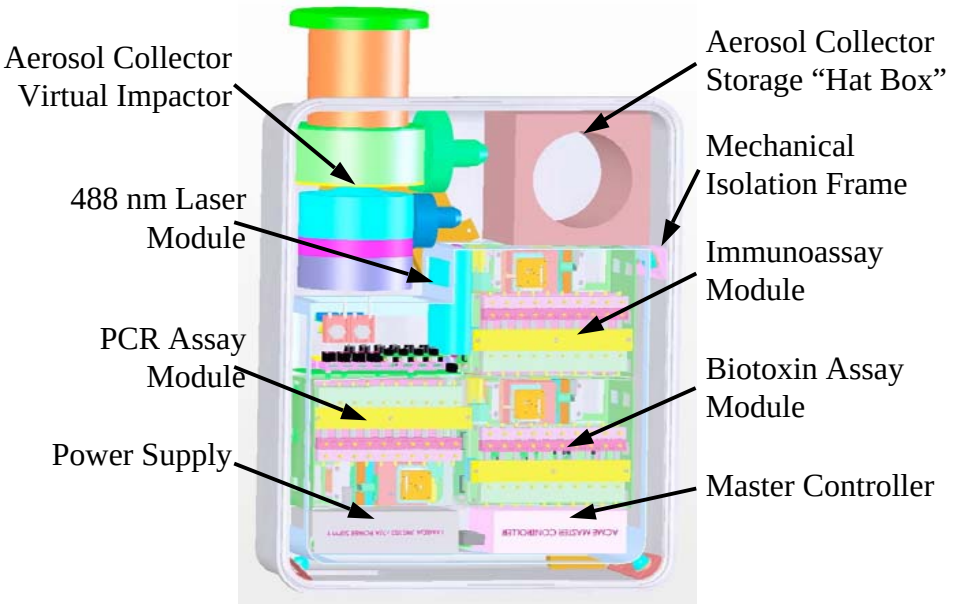
- Miniaturized, multiplex, broad spectrum detection system that includes the latest advances of LLNL and Sandia.

1. Autonomous collection and detection (Environmental Monitor)
   - Long term (public venues) or short term (special events)
   - Easily deployed and networked
   - Low reagent consumption
   - Cheaper to operate
   - Modular design allows for purpose optimization

2. Portable laboratory
   - Rapidly deployed
   - Minimal user training
   - Broad spectrum of agents
   - Short time between samples and action

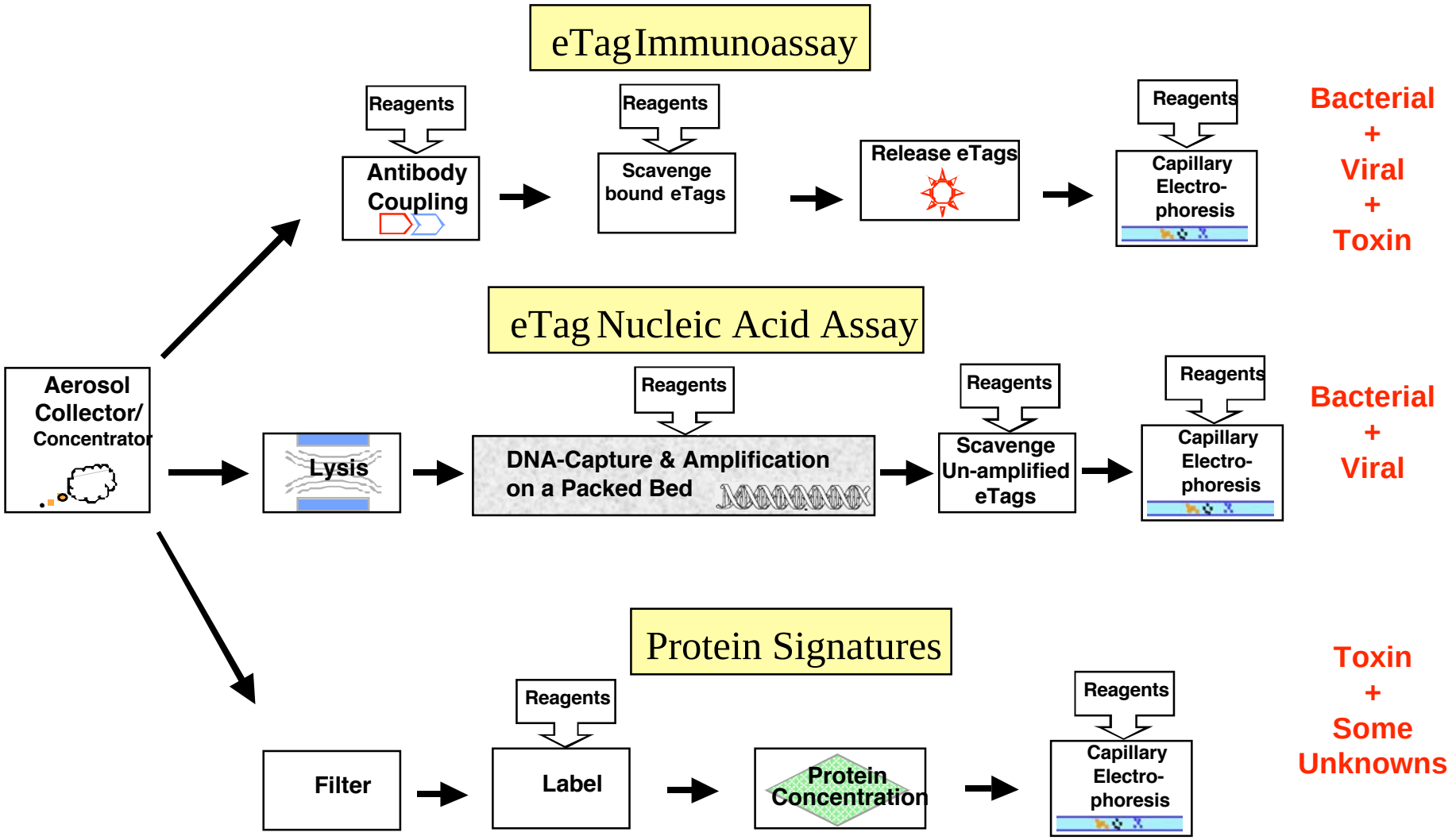
3. Fits into existing surveillance schemes



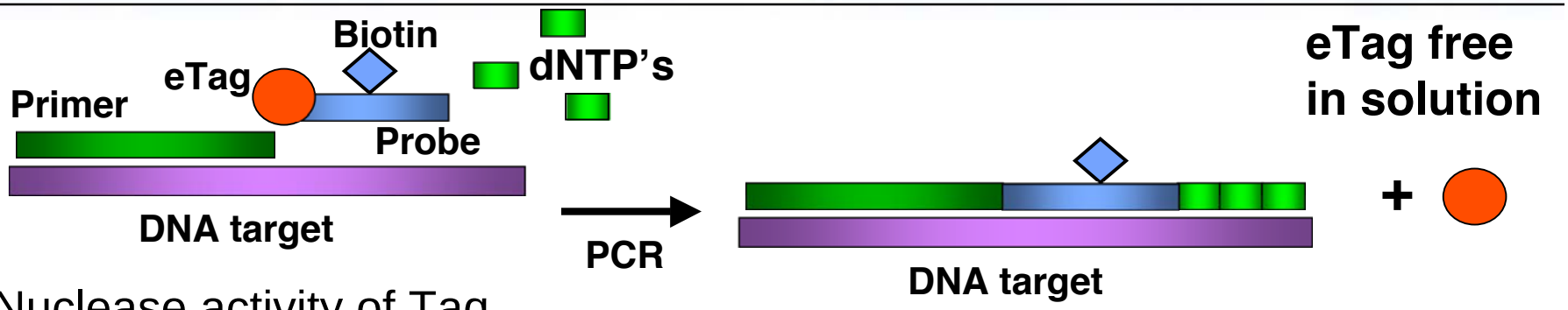


Orthogonal Assays for Low False Alarm Rate

3 BioBriefcase Analysis Trains to detect threats:

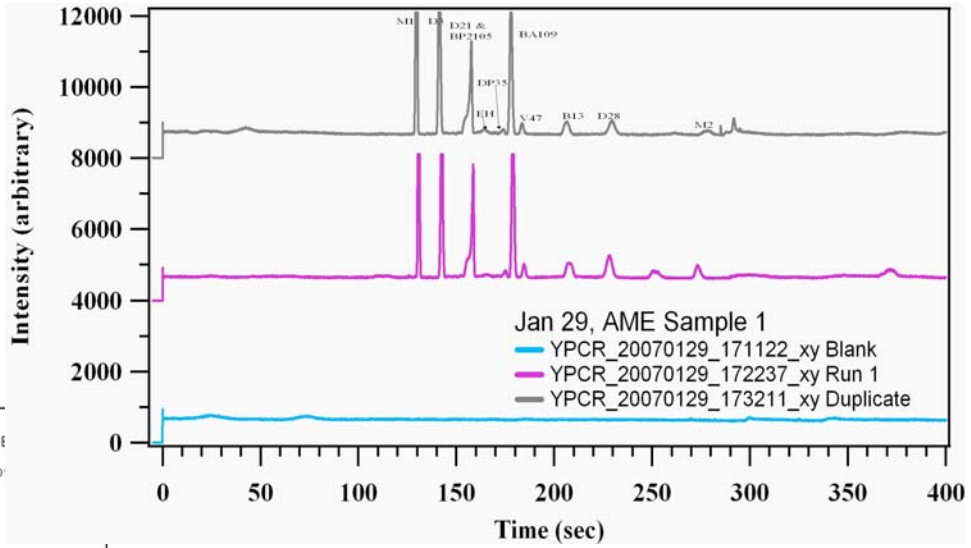


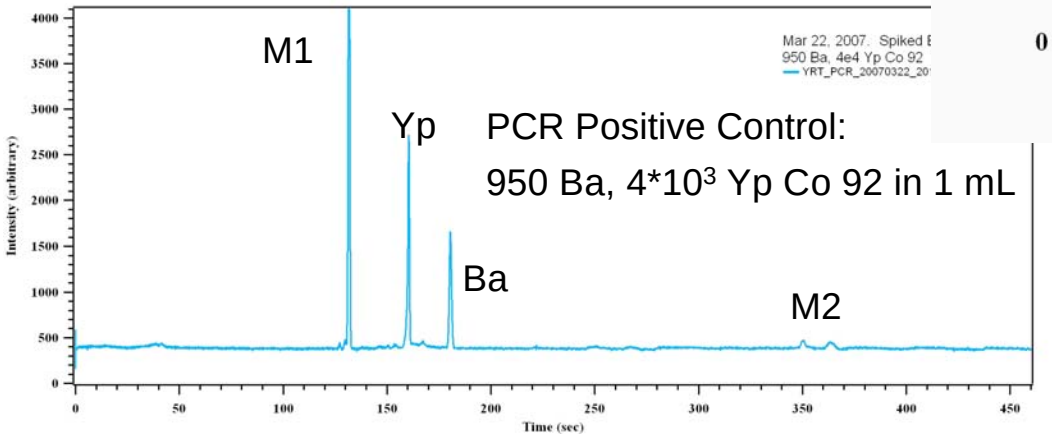
Monogram eTag Assay for Nucleic Acids (PCR)



5'-Nuclease activity of Taq polymerase (enzyme) cleaves eTag during PCR amplification

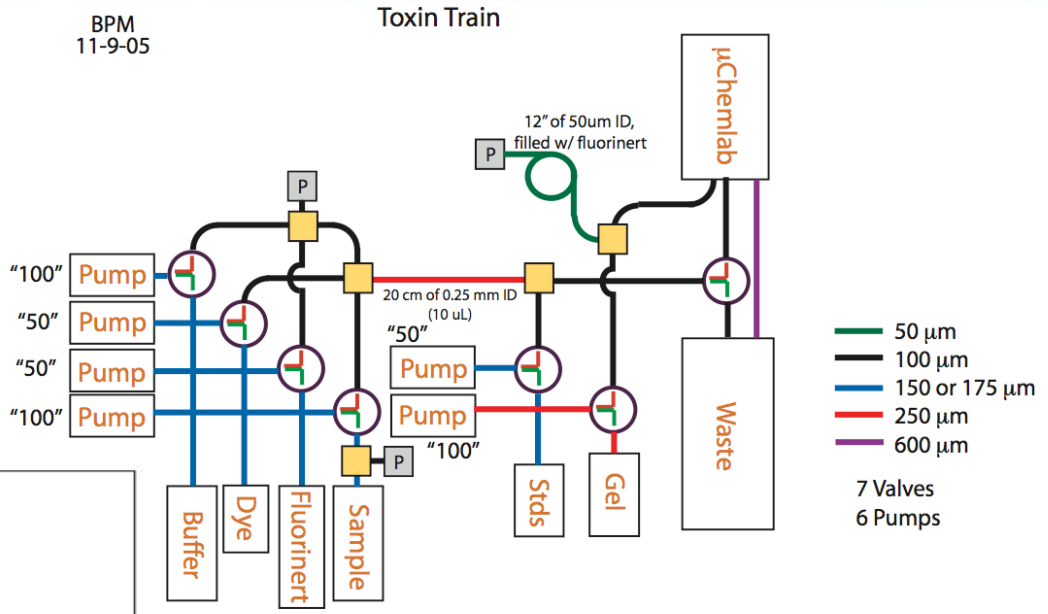
1. Free eTags during amplification
2. Use CE to distinguish free eTags

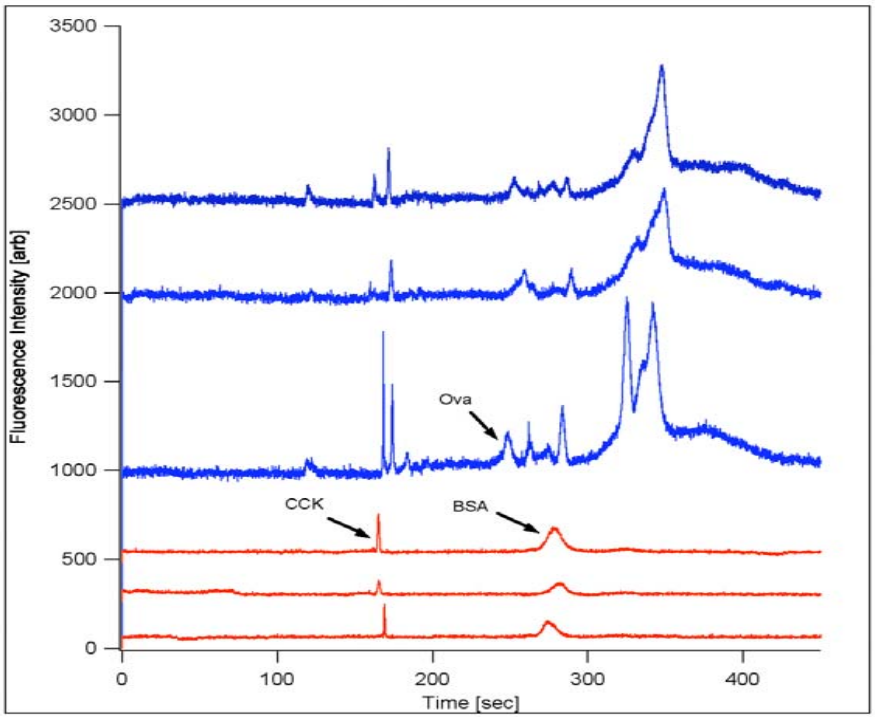


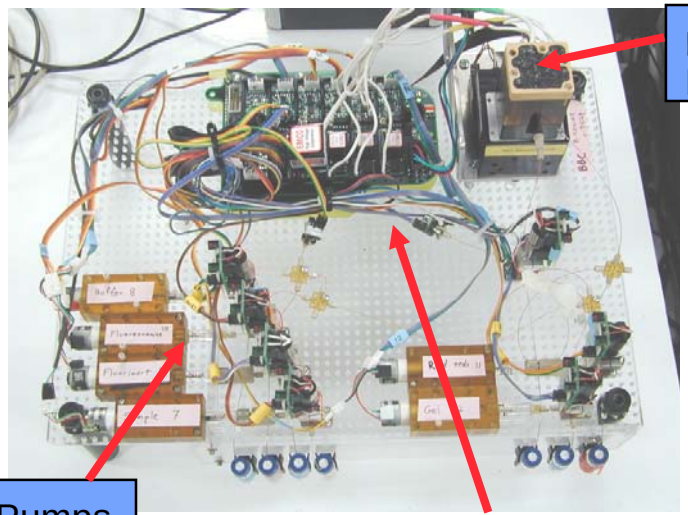


BioBriefcase Toxin Train

- Capillary sample prep and chip-based CGE separation
- Labeling in capillary
- Automatic gel replenishment



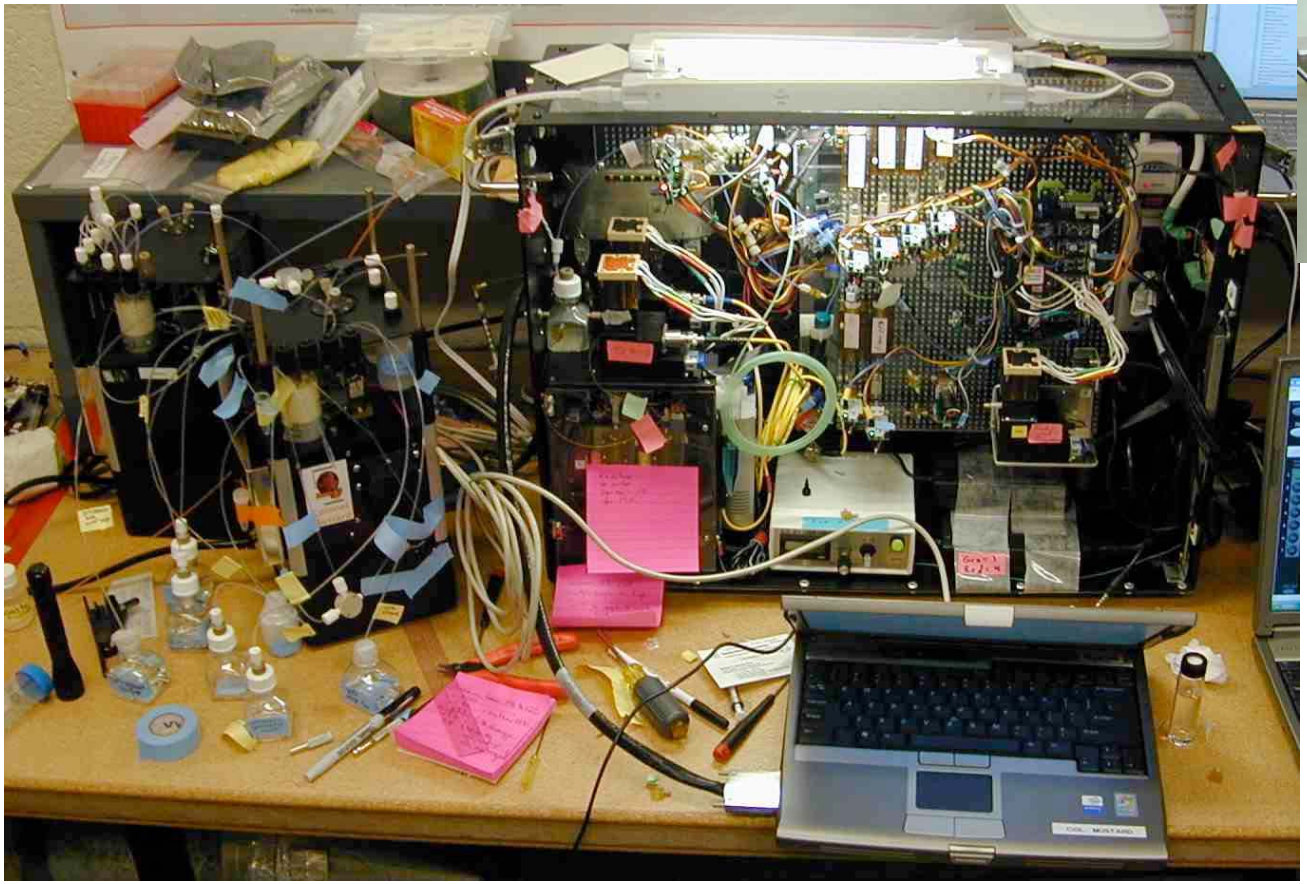




BioBriefcase Integrated System Version 1

Aerosol Collector inside
UNLV Aerosol Chamber

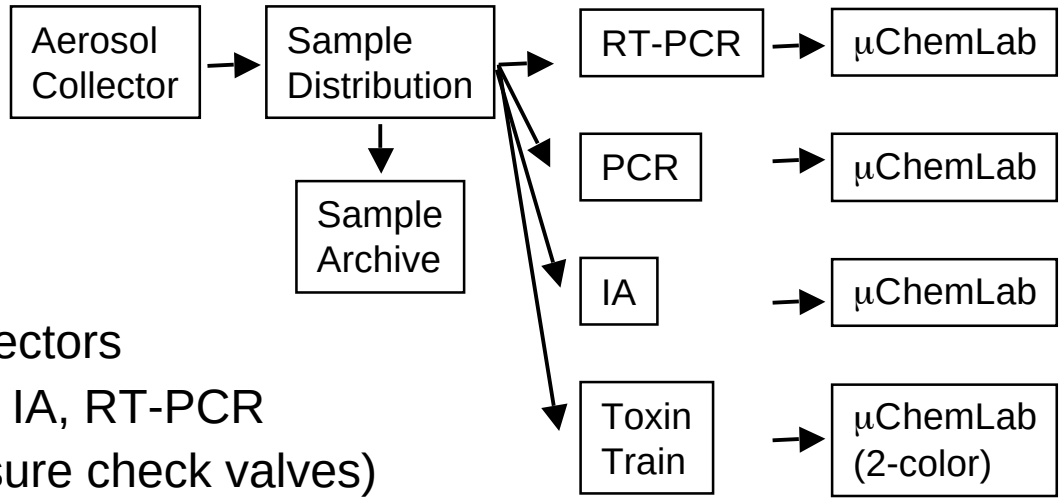
“R2D2” outside UNLV Aerosol Chamber



“R2D2”

2 Cu Ft Package Goal

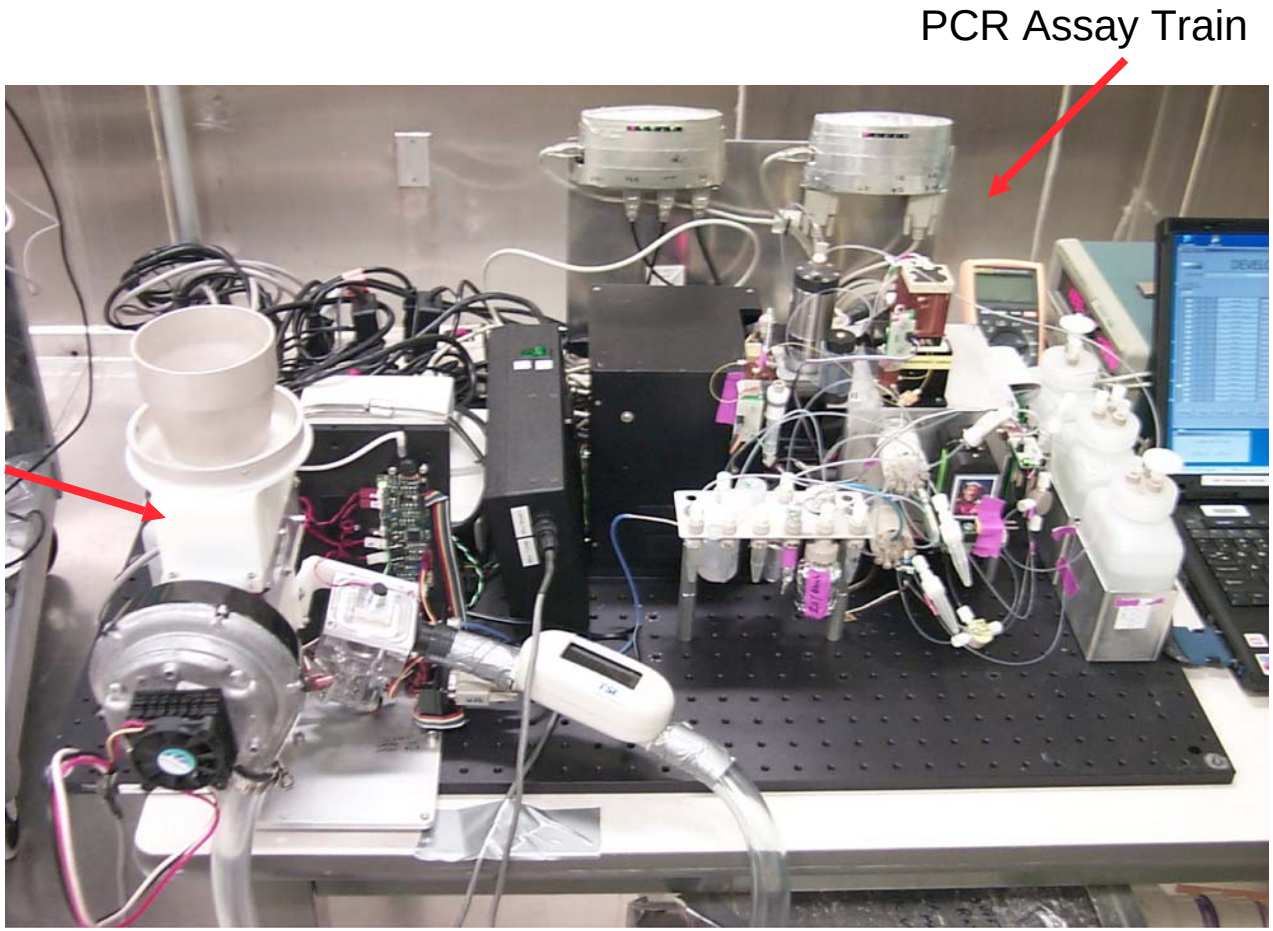
Sample Handling and Modes of Operation



- Startup
  - Gel flush on μChemLab detectors
  - Prime reagent lines in PCR, IA, RT-PCR
  - Check state of health (pressure check valves)
- Run
  - All assays pull their sample from sample distribution
  - Assays run simultaneously; TT, IA finish prior to PCR
- Decon
  - Bleach and rinse all fluidics in contact with sample
- Autosampler
  - Run overnight using standards to test reliability

BioBriefcase Field Testing at ECBC

- Three Months at Edgewood Chemical Biological Center
- Gamma-Killed Bacillus Anthrasis nebulized at entrance to aerosol collector



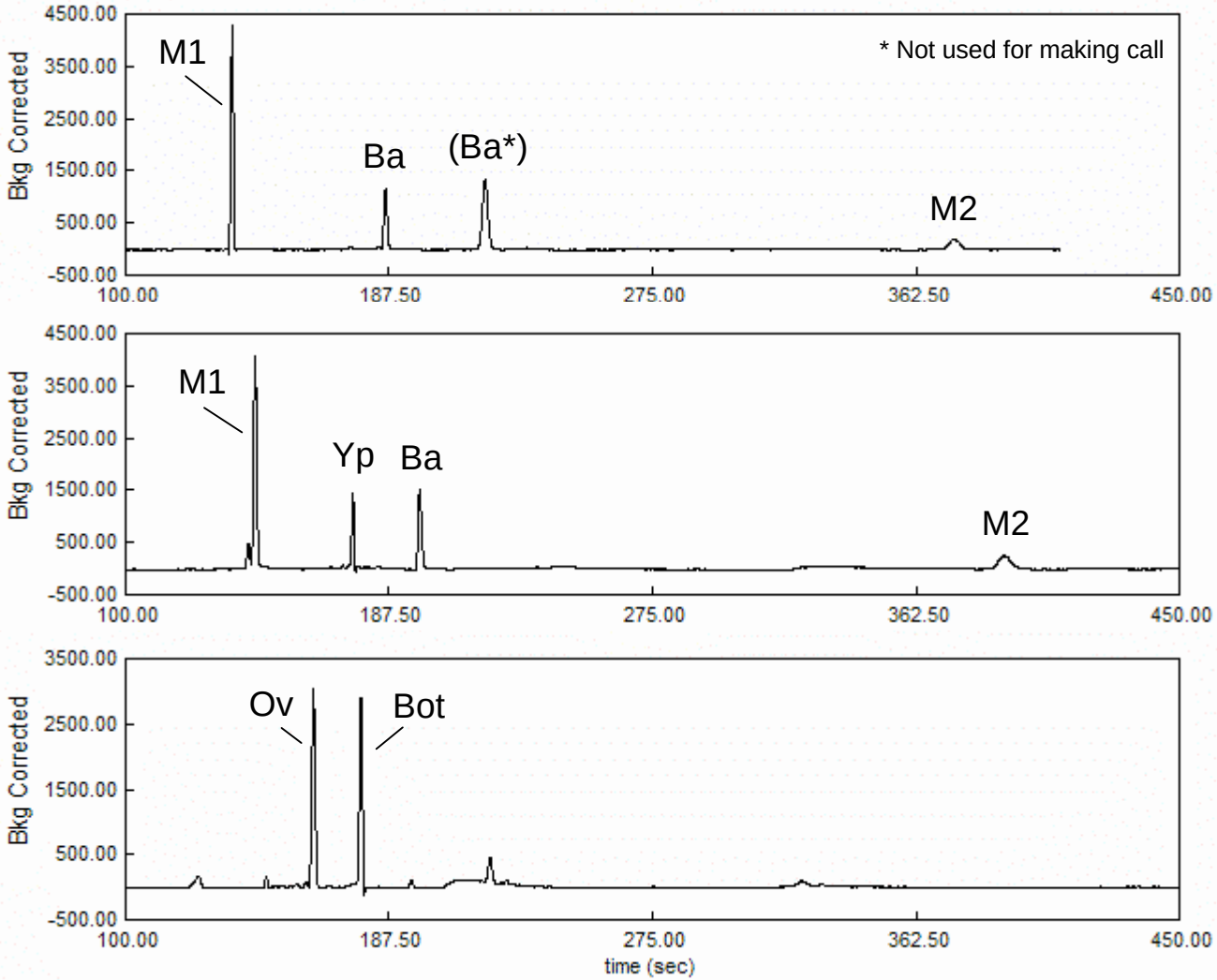
"Falcon"

Databases used for BAND testing

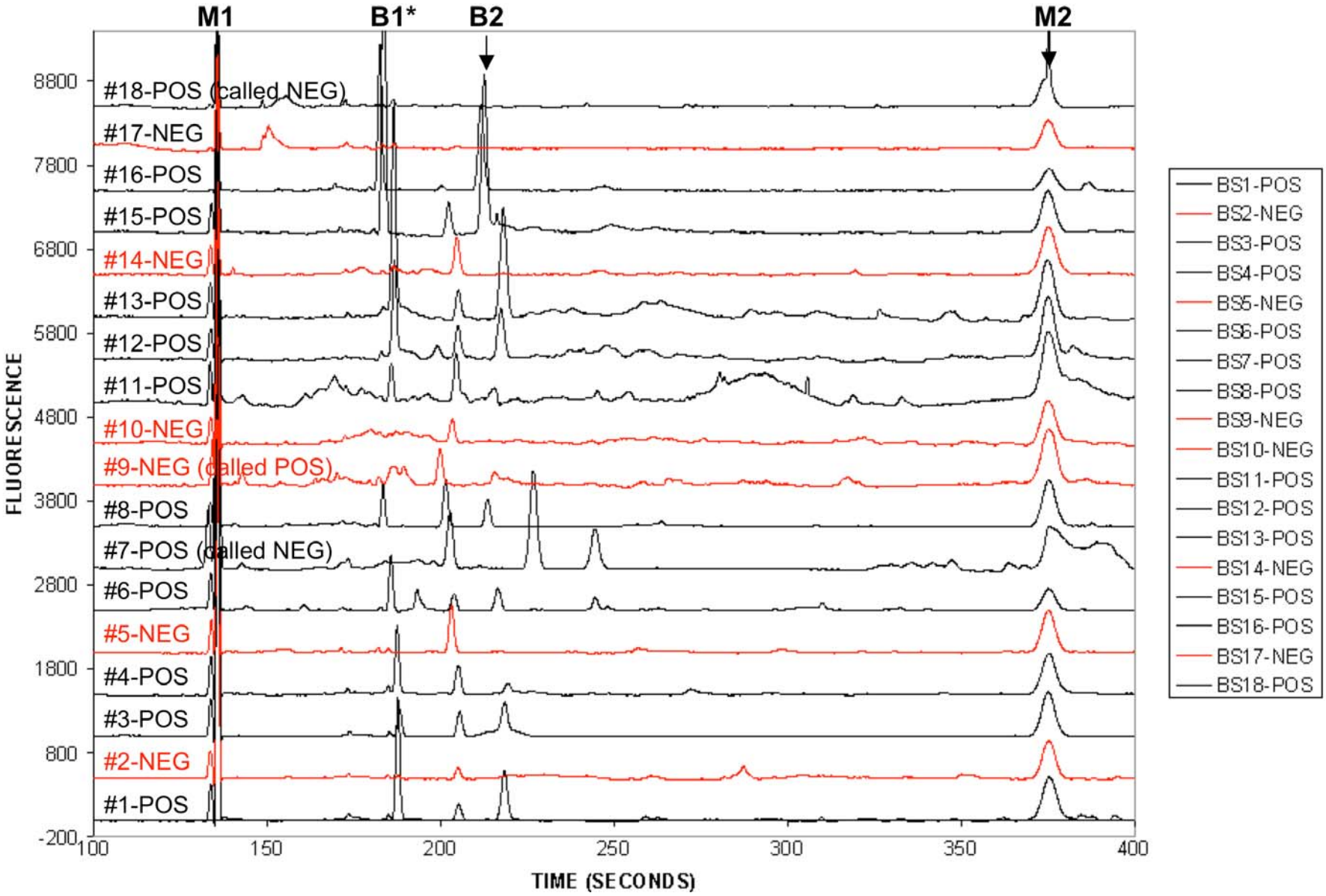
Tests 3 and 4

Test 5 – PCR

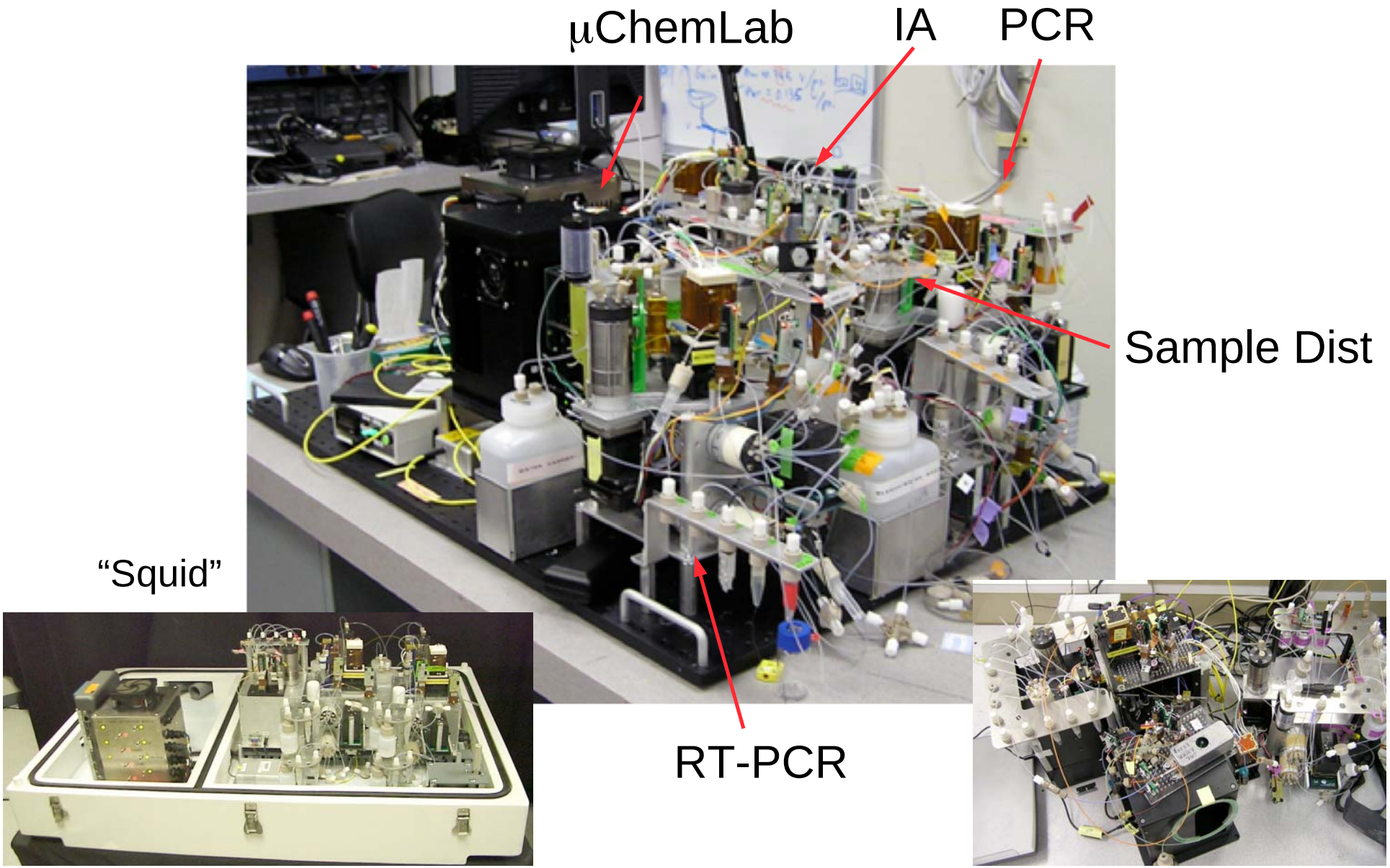
Test 5 – IA



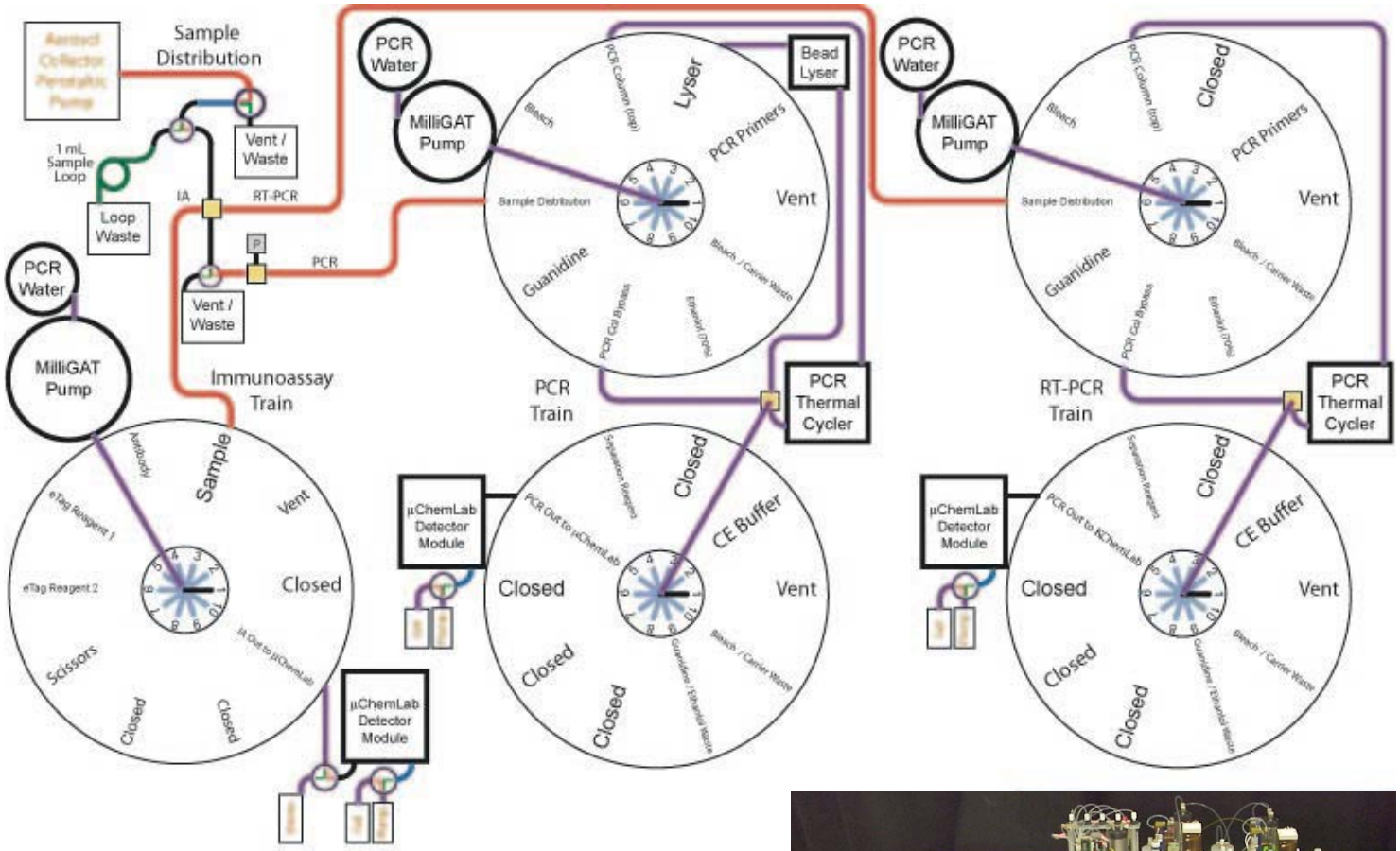
Summary of “Test 4” Results



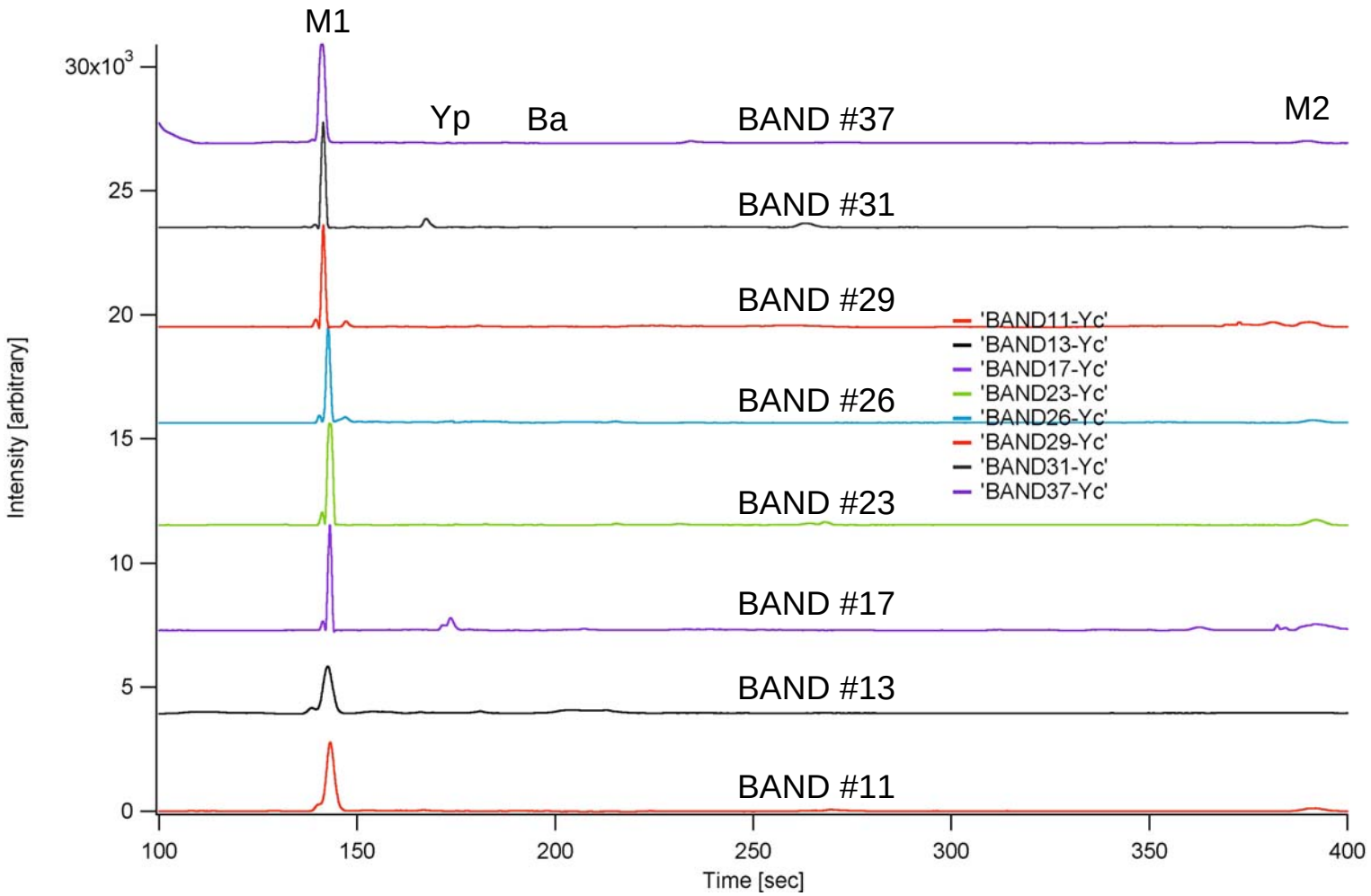
BioBriefcase “Test 5” at LLNL



Integrated Fluidics Architecture

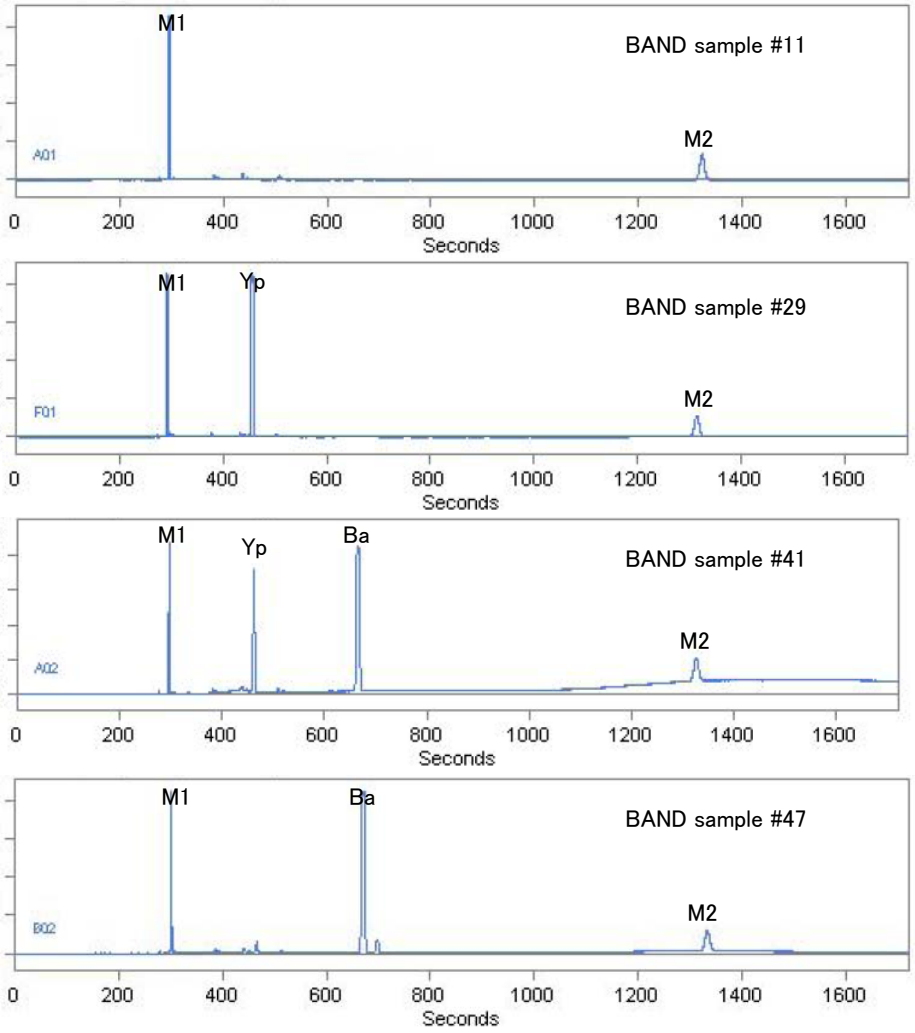


Test 5: Blind ECBC Samples



Bench-Top eTag Analyses of Test 5 Samples

Sample ID	*B. anthracis*	*Y. pestis*
BAND #11	**Neg**	**Neg**
BAND #13	**Neg**	**Pos**
BAND #17	**Neg**	**Pos (weak)**
BAND #23	**Neg**	**Neg**
BAND #26	**Neg**	**Neg**
BAND #29	**Neg**	**Pos**
BAND #31	**Neg**	**Neg**
BAND #37	**Pos (weak)**	**Pos**
BAND #41	**Pos**	**Pos**
BAND #47	**Pos**	**Neg**
BAND #53	**Pos**	**Pos**
BAND #57	**Neg**	**Neg**
BAND #61	**Pos (weak)**	**Pos (weak)**
BAND #71	**Neg**	**Neg**
BAND #79	**Pos**	**Neg**
BAND #97	**Pos**	**Pos (weak)**
475 cfu *B. anthracis* control	**Pos**	**Neg**
2000 cfu *Y. pestis* control	**Neg**	**Pos**
950 cfu *B.a.*, 4000 cfu *Y.p.*	**Pos**	**Pos**
No template control	**Neg**	**Neg**



BioBriefcase Summary

- ECBC Field Tests Successfully Demonstrated System Reliability and Limit of Detection
- 4-agent Testing Demonstrated at LLNL -- Higher Multiplexing Possible, but yet to be Demonstrated on Integrated System (Benchtop Only)
- System Integration Facilitated by Flexible Software Platform
- μChemLab Improvements Significantly Improved Ease of Use and Reliability
- 3-Hour Cycle Time Could be Cut in Half with Additional Testing
- Test 5 ECBC Samples did not capture on packed bed in same manner as spores from Tests 3 and 4
- Seeking to Leverage Technology for DoD

Acknowledgements

UNLV Field Test: Ron Renzi, Michael Bartsch, Bob Crocker, Shane Sickafoose, Dan Yee, Jim Van de Vreudge, Tom Raber, Tim Shepodd, Chris Bailey, Elizabeth Wheeler, Christine Hara, Kirsten Johnson, Anne-Marie Ehler, Perry Bell, Ladona Willis, Tom Hindley, Bruce Henderer

DoD uChemLab: Brent Haroldsen, Ron Renzi, Victoria Vandernoot, Jeanne Stachowiak, Erin Shugard, Tom Raber, Dan Yee, Jim Van de Vreudge,

Tenix CRADA: Brent Haroldsen, Ron Renzi, Victoria Vandernoot, Jay West, Kyle Hukari, Gary Hux, Tom Raber, Dan Yee, Jim Van de Vreudge

ECBC Field Test: Perry Bell, Chris Bailey, Elizabeth Wheeler, Paul Butler, Shanavaz Nasarabadi, Christine Hara, Sean McNary, Joe Wassi, Michael Nguyen, Tom Raber, Ron Renzi, Julie Fruetel

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.