SAND2008-2526C

Development of a Portable and Automated Electrophoresis Based System for in-field Toxin Detection

"*HABLab*"

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Sandia National Laboratories

Livermore, CA

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy. This work was sponsored by the U.S. DOE under contract DE-AC04-94AL85000.

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μChemLab Detection Technology

Hand portable, multi-channel electrophoretic separations platform

- Integrated liquid and detection modules
- Modular design for versatility
- Fieldable

Chip based separations for characterization purposes:

- initial demonstrations of select agent protein toxins (in collaboration w/ DSTL in Porton Down, UK)
- amenable to a variety of applications (proteins, organisms, nucleic acids, immunoassay)

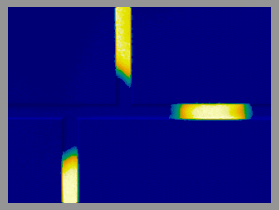
Ronald F. Renzi, James Stamps, Brent A. Horn, Scott Ferko, Victoria A. VanderNoot, Jay A.A. West, Robert Crocker, Boyd Wiedenman, Daniel Yee and Julia A. Fruetel, (2004) *Analytical Chemistry*, **77**, 435-441.

Universal Protein and Biochemical Analysis Platform

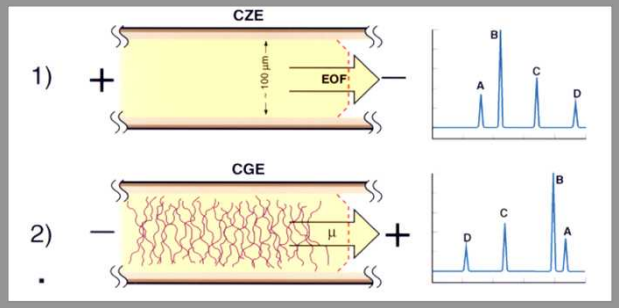
Collect sample

Fluorescently label

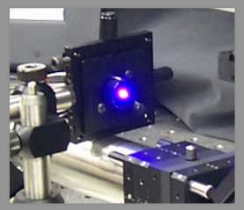
Liquid Analysis Module



Microfluidic Sample Delivery

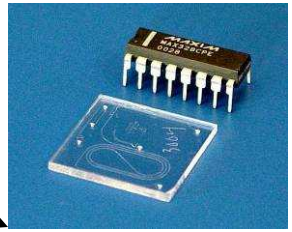


Dual Channel Microchip Separations



Laser-Induced Fluorescence Detection

Chip 20 x 20 mm



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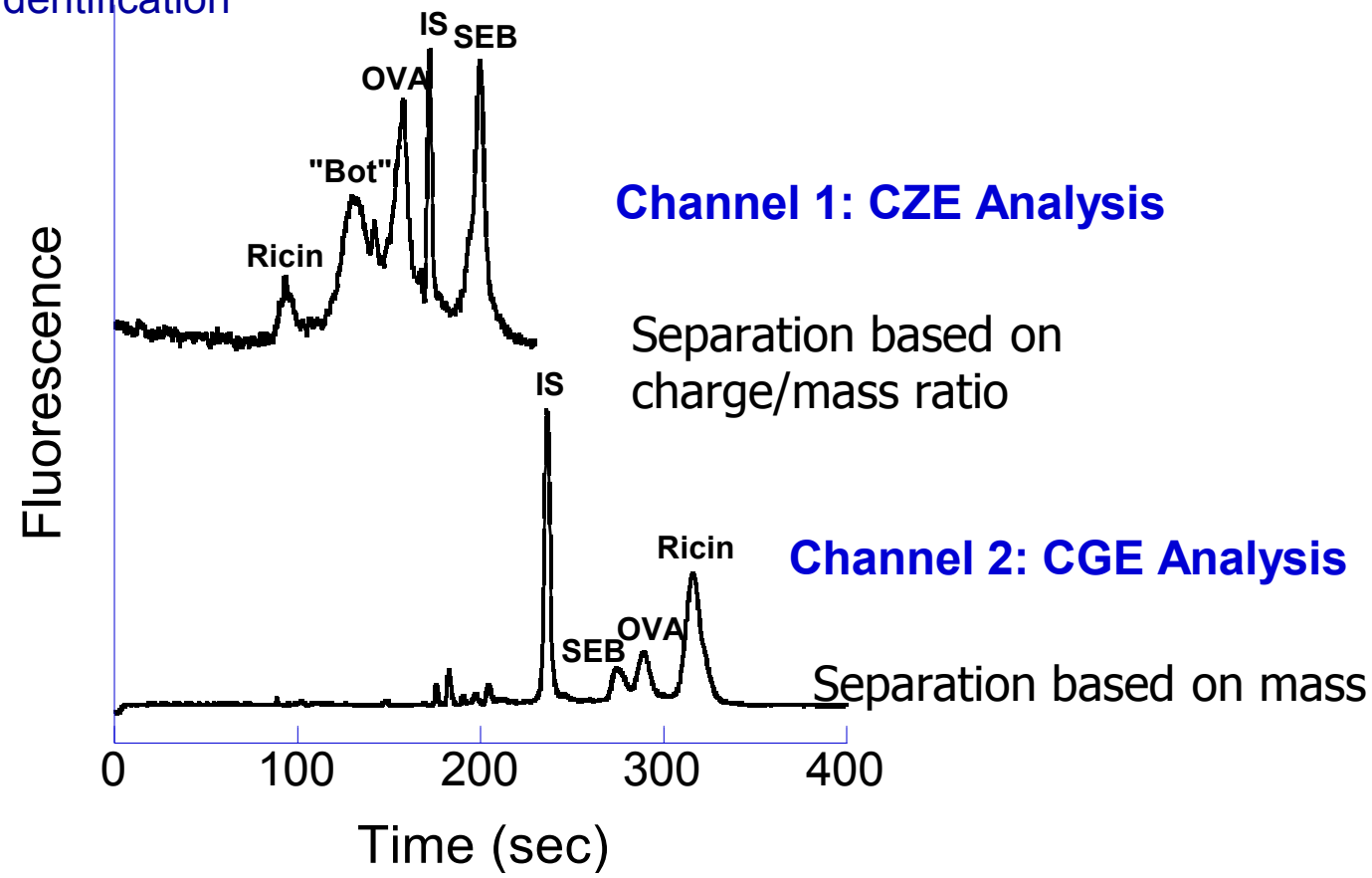
µChemLab Protein Toxin Analysis

- Multiple Separation Methods Yield Characteristic Migration Times Leading to Identification



Julia A. Fruetel, Ronald F. Renzi, Victoria A. VanderNoot, James Stamps, Brent A. Horn, Jay A. A. West, Scott Ferko, Robert Crocker, Christopher G. Bailey, Don Arnold, Boyd Wiedenman, Wen-Yee Choi, Daniel Yee, Isaac Shokair, Ernest Hasselbrink, Philip Paul, David Rakestraw, and Debbie Padgen, (2005) *Electrophoresis*, **26,** 1144 – 1154.

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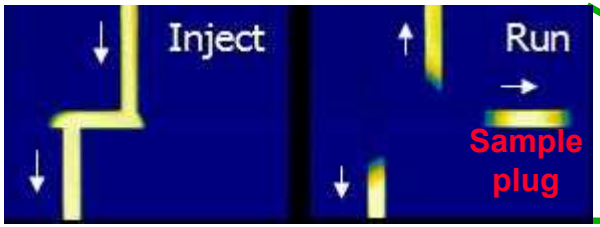
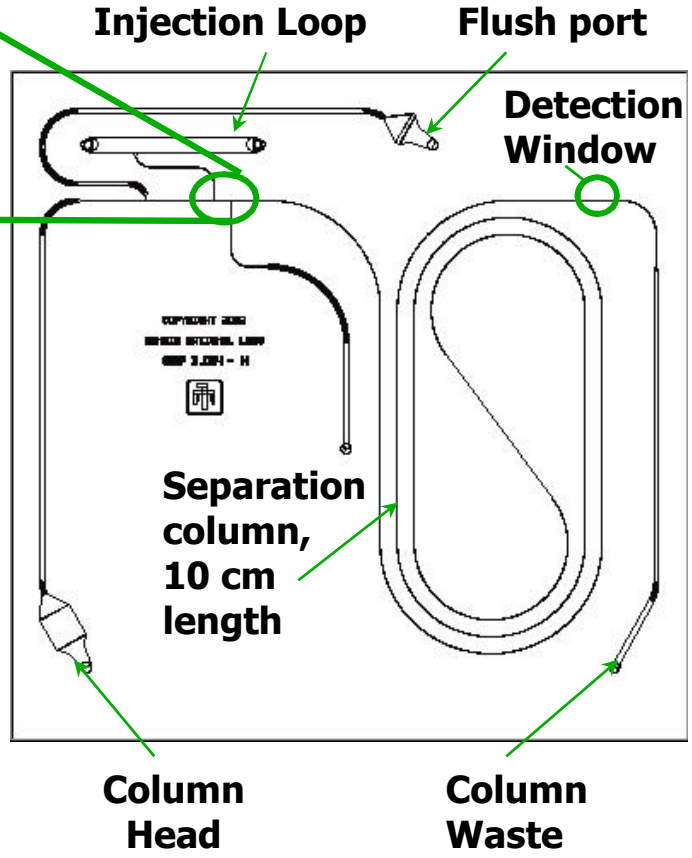
Chip-Based Microseparations

Electrokinetic injection at offset "T"





Chip-based Microseparations Provide Benefits and Flexibility

- **Assay time dramatically reduced**
- **High resolution**
- **Minimal reagent volumes**
- **Parallel/sequential separations facilitated**
  - Provide differential selectivity
  - Improve detection reliability/Lower false alarm rates
- **Chip-based methods**
  - Capillary zone electrophoresis
  - Capillary gel electrophoresis
  - IsoElectric Focusing
  - CEC
  - MEKC

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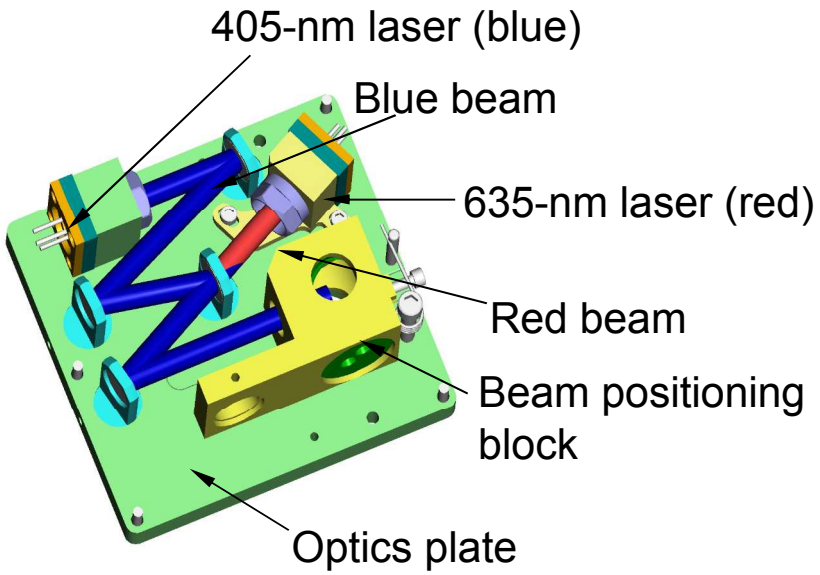
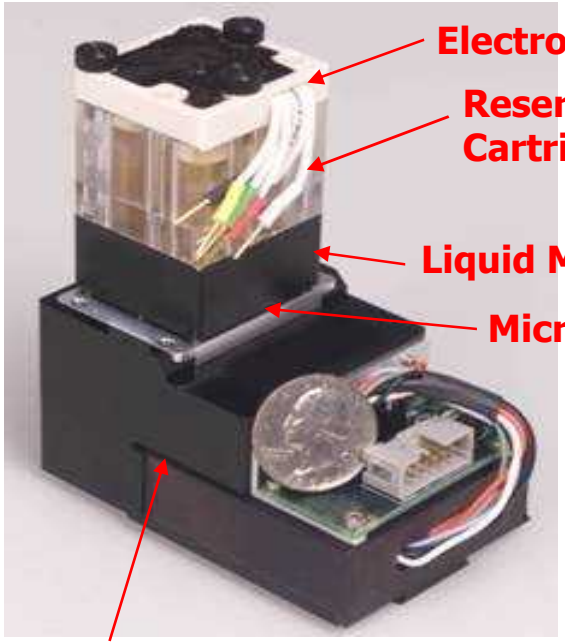
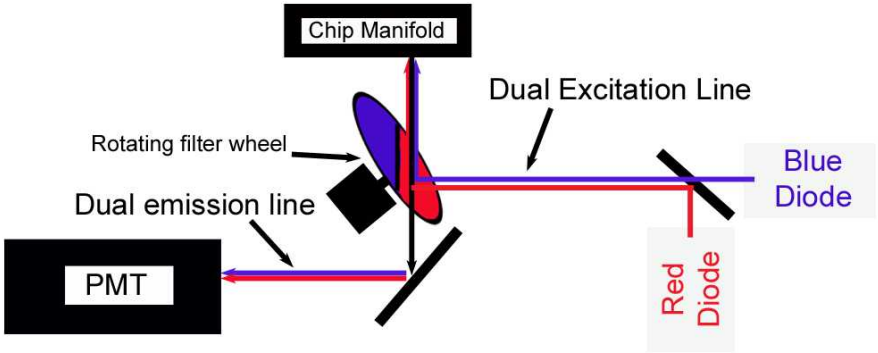
Enabling Technology: High-Sensitivity, Two-Color Optical Detection*







*Internal Standards allows correction of minor variations in migration times and use of 2 color means that internal standards do not mask analytical signals

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Integrated Biodetection Platforms

Multiple Applications:

Automated Microfluidic Protein Profiling System (AMPPS)

* *continuous aerosol collection*
* *automated sample preparation and analysis*
* *toxins, viruses, spores and bacteria*

The Unattended Water Sensor (UWS)

**Cooperative Research and Development Agreement with Tenix (Australia) and CH2M Hill (US)*

* *Initial demonstration aimed at protein biotoxins*
* *Future interest in expanding capabilities*
  - *live agents (viruses, bacteria)*

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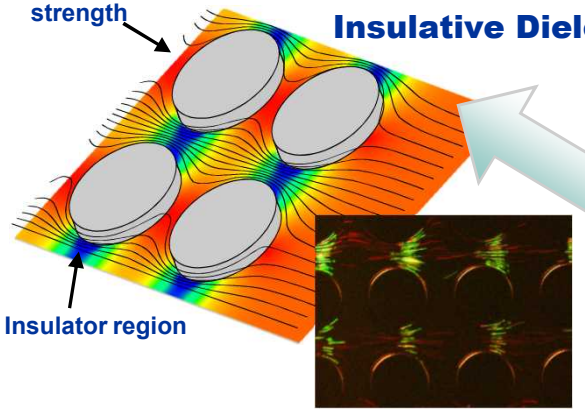
Integrated Biodetection Platforms – Automated Sample Preparation Train

Higher field strength

Selective Concentration via Insulative Dielectrophoresis



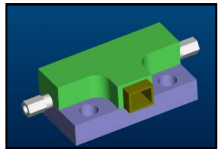
Insulator region

Concentration
- iDEP system or filter
- 5mL to 10-20uL
- 1mL/min flowrate

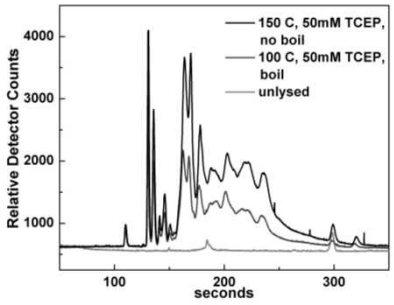
Lysis
- Thermo-Chemical protocol
- 150 C, 300psia, 30seconds, 50mM TCEP

Filtration
- 0.2um to remove unlysed species

Lysing/solubilizing spores using reducing agent and detergent



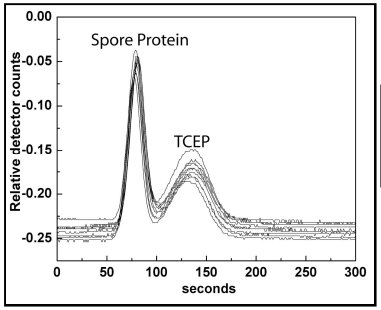
In-line Lyser



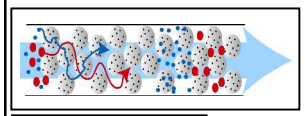
Size Exclusion Chromatography
- To remove TCEP, which interferes with labeling.
- About 1 minute
- Optimizatio of collection fraction

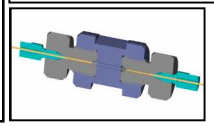
Labeling
- 10:1 ratio, sample to dye
- 10mM fluorescamine in dry acetonitrile.
- Mixing by interleaved plugs

Injection
- 5uL injections



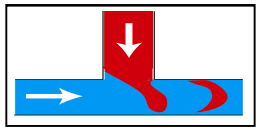
Separation Based on Molecular Size

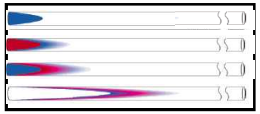


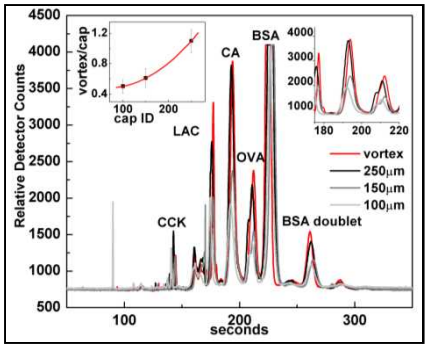


In-line Cartridge

Microfluidic Reagent Mixing







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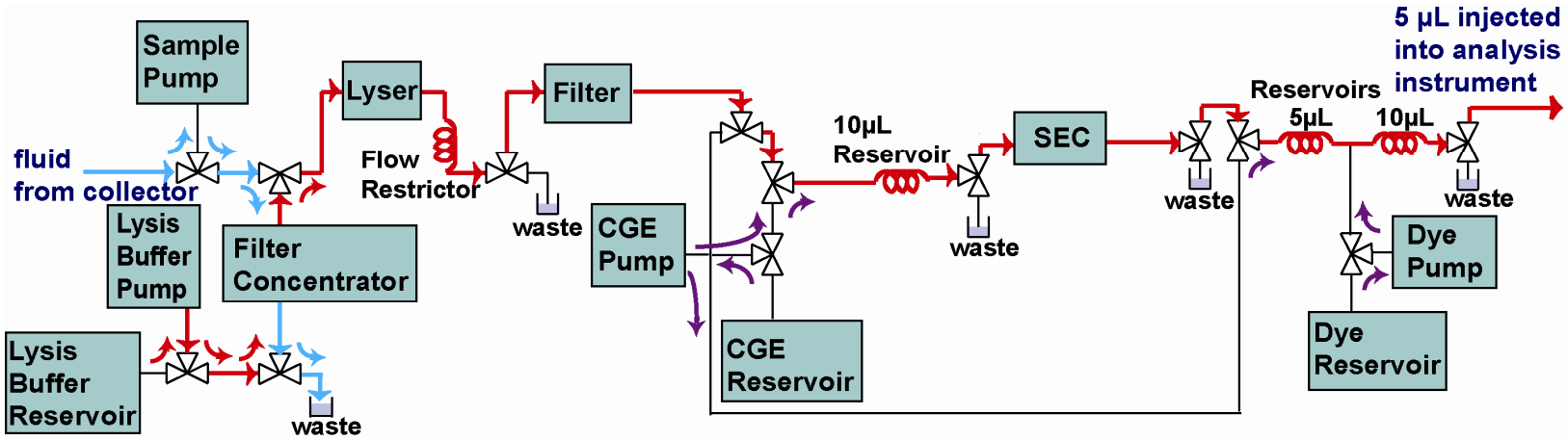
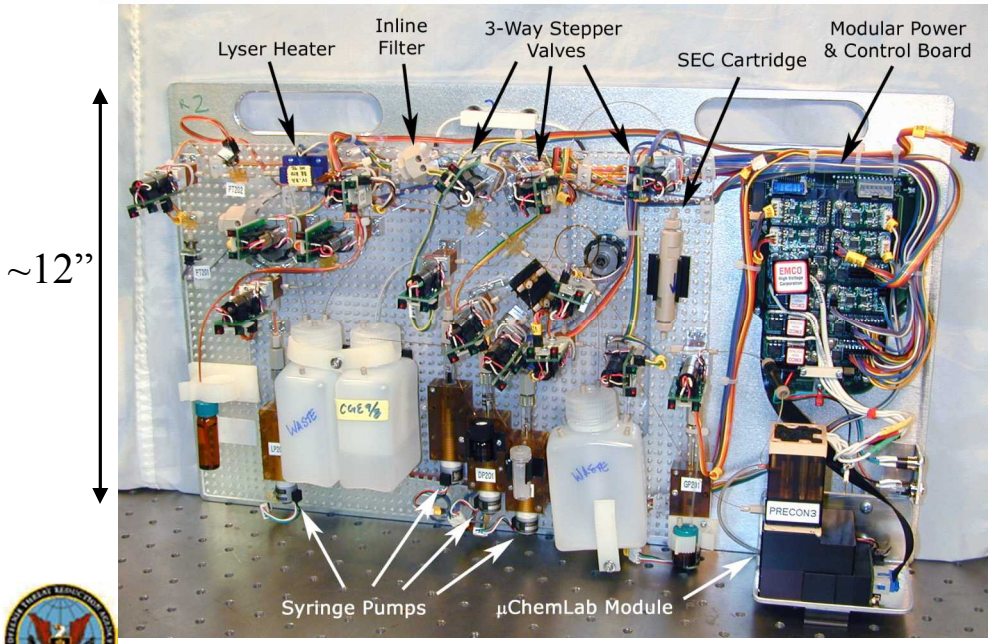
Integrated Biodetection Platforms – AMPPS





Point Detection Platform Developed for JCBPDS

- Continuous, autonomous operation
- Toxins, viruses, spores, and vegetative cells
- Portable
- Rapid response (~20 minutes)
- Minimal reagents

S. Pizarro, et. al, (2007) *Electrophoresis,* **28**, 4697–4704.
J. Stachowiak, et. al, (2007) *Analytical Chemistry,* **79**, 5763 -5770.

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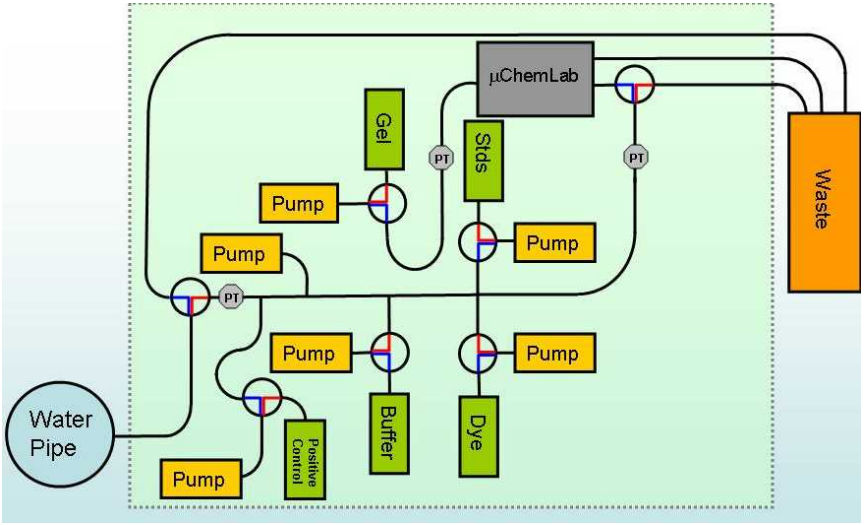
Integrated Biodetection Platforms – UWS

Continuous water monitoring

- 30 day unattended operation
- Analysis every 30 minutes
- Detect biotoxins; future expansion to live agents



V. VanderNoot, et. al, *Environmental & Water Resources Institute Currents,* (2008) **9**, 6-7.

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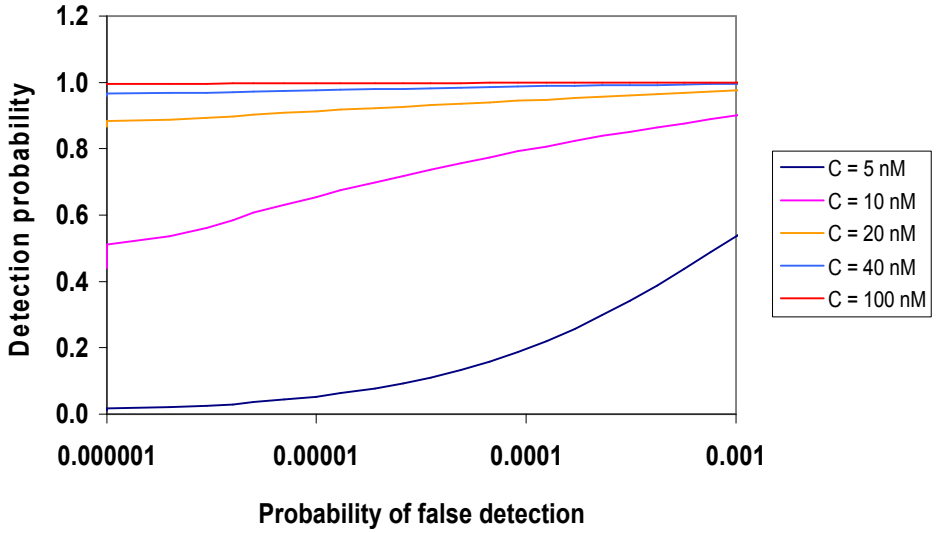
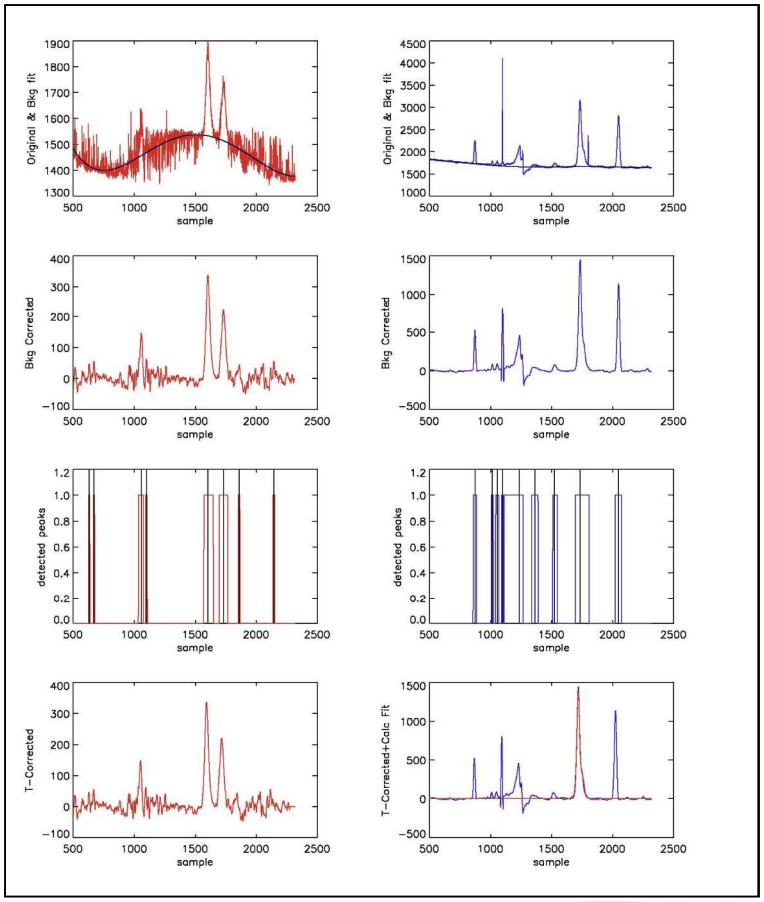
System Performance Data



Preliminary UWS Receiver Operating Characteristic Curves for Ricin

Automated Analysis of Two-color UWS data



Ricin concentration of 369 nM

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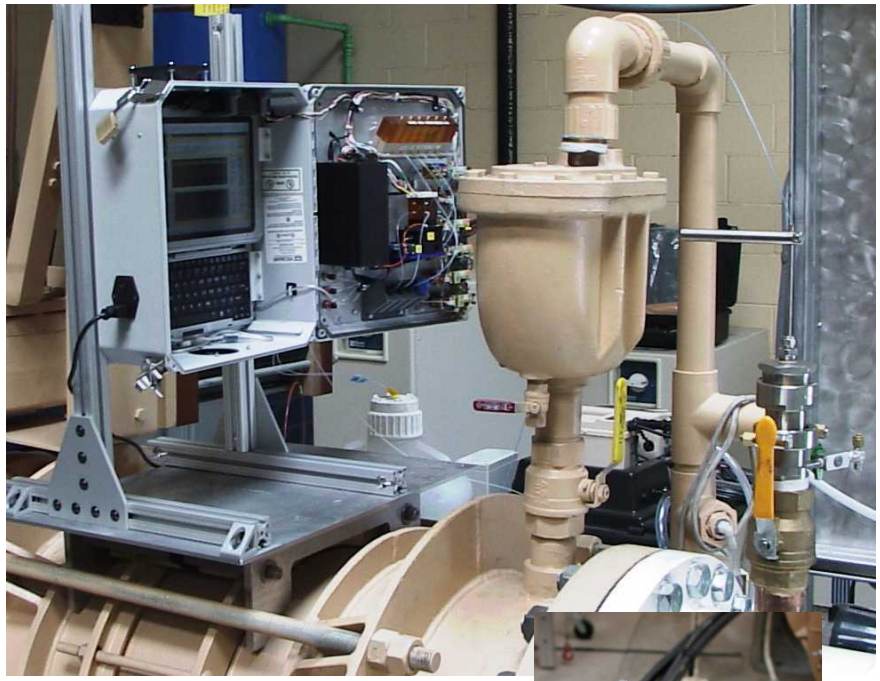
Field Testing at Local Northern California Water District and in Arizona

Current Generation UWS

System Upgrades

- More Streamlined Design for reliability
- Enhance maintainability
  - Modular design for fluids and components to ease change out and system replenishment
- Incorporation of system diagnostics
  - Pressure transducers
  - Positive controls
- Incorporation of data transmission and alarm capabilities



Local Utility Pump Station (CCWD)

Sampling Probe

In Field Testing:
•Contra Costa Water – 2005 through 2007
•Glendale, AR – Feb through June 2007

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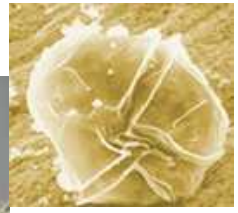
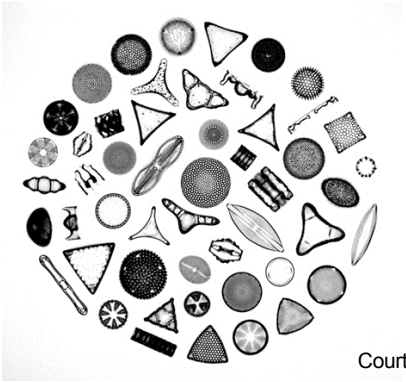
Expanding Capabilities to Analyze Marine Samples

- **Incorporate additional sample preparation steps**
  - Tissue homogenization (shellfish)
  - Filtration/concentration (phytoplankton)
  - Selective concentration and/or sorting
  - Lysis and solubilization
  - Solid Phase extraction (SPE)
  - Sample clean-up (SEC)
- **Methods Development**
  - Multiple separation methods
  - Fluorescent labeling strategies



Courtesy NWSFC/NOAA*

Existing UWS, much common hardware



Courtesy NBII**

*http://www.nwfsc.noaa.gov/hab/habs_toxins/index.html
**http://images.nbii.gov/microflora.php

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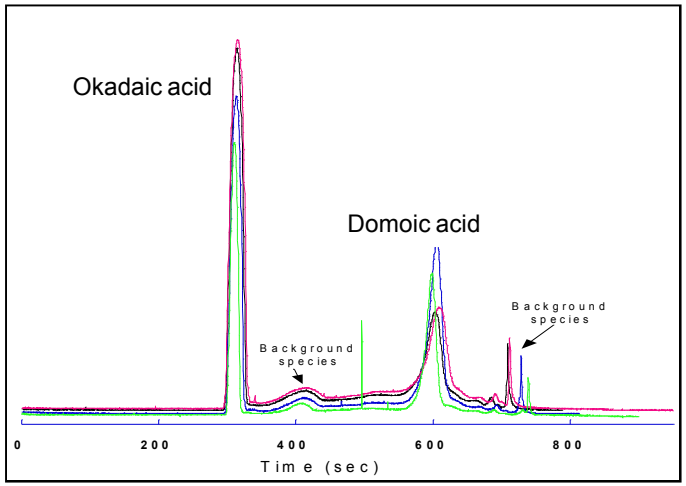
Capillary Electrophoresis with UV Detection of Marine Toxins

1) S. J. Locke and P. Thlbault' Analytical Chemistry, Vol, 66, No. 20, October 15, 1994
   - Successfully resolved saxitoxin an neosaxitoxin and GTX2 & GTX3

2) Youyi Wu, Alvin Yam, Tat Ho, Pei-Yuan Qian, Kelvin Sze-Yin Leung, Zongwei Cai, Jin-Ming Lin, J. Sep. Sci. 2006, 29, 399 – 404
   - Successfully resolved seven saxitoxins and gonyautoxins

Singh et al, Sandia National Labs 2006



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Technical Approach

Small scouting project (CICEET)

- Capillary with UV detection for scouting separation methods (transition to ChemLab)
  - Saxitoxins (focus on saxitoxin/neosaxtoxin)
  - Domoic acid
  - Examine a variety of CE methods
    - MEKC, CZE, buffer additives
    - isotachophoresis for increased detection sensitivity
- **Labeling (significantly better sensitivity than UV detection)**
  - Fluorogenic amine reactive dyes (fluorescamine, NBD-F)
  - Non-fluorogenic hydrazide dyes for –COOH functional groups — “Universal”
  - Indirect fluorescence detection — “Universal”
- **Samples from collaborator at Woods Hole Oceanographic Institution, (Donald Anderson)**
  - Establishing sample preparation methods that will be compatible with subsequent automation, labeling and electrophoresis

CICEET

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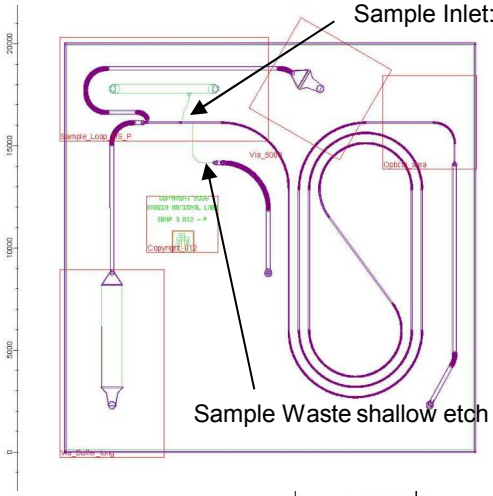
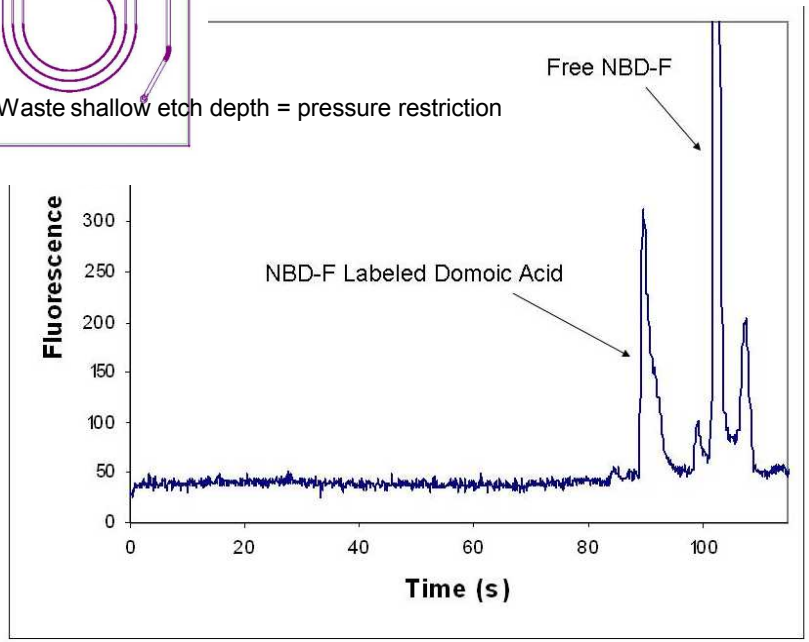
Fluorescent Labeling: Domoic Acid





Chip based separation
- CZE chip
- Normal polarity
- Running buffer: pH 7.4 phosphate with HEC (low EOF buffer)

LIF detection 488 nm detector
- 5 nM Detection limit (no preconcentration or extensive optimization)

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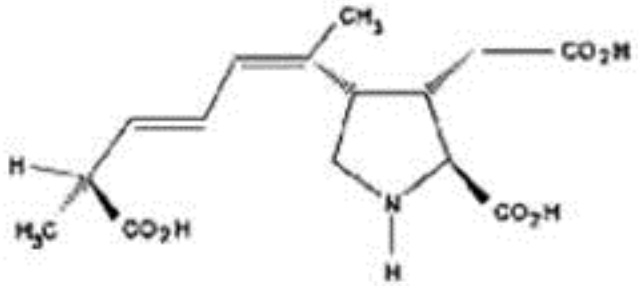
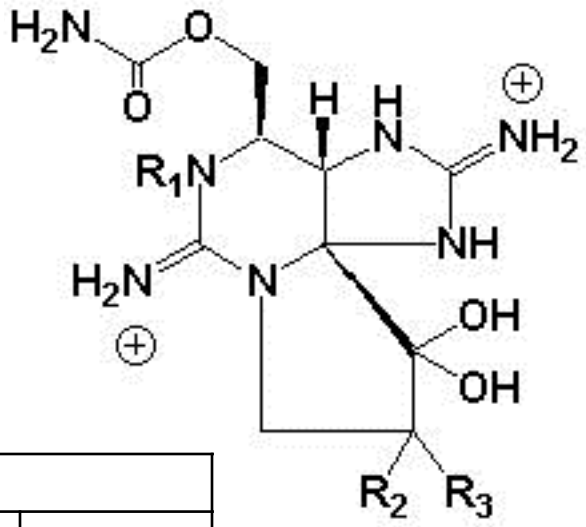
Toxins and their Approximate Charges at a Range of pH Values

Domoic Acid



Saxitoxins



Toxin	Approximate Molecular Charge					
	pH 3.0	pH 4.5	pH 6.0	pH 7.4	pH 9.5	pH 12+
Saxitoxin (pKa 11.5, 8.5)	**+2**	**+2**	**+2**	**+2**	**+1**	**0**
GTX II & III*	+1	+1	+1	+1	0	-1
Neo-Saxitoxin (pKa 6.75, 8.65)	**+2**	**+2**	**+2**	**+1**	**0**	**0**
GTX I & IV*	+1	+1	+1	0	-1	-1
Domoic acid (pKa 2.1, 3.7, 5.0 & 9.8)	**-1**	**-2**	**-3**	**-3**	**-3**	**-4**

* Estimates based on similarities to either Saxitoxin or neosaxitoxin

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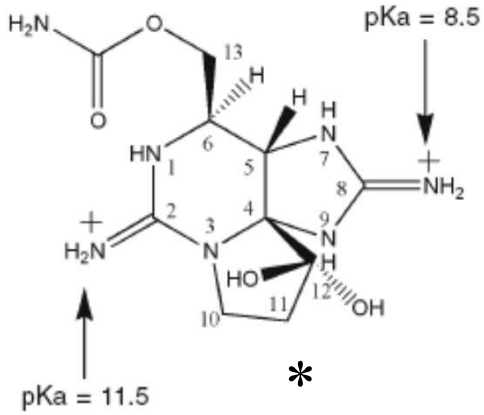
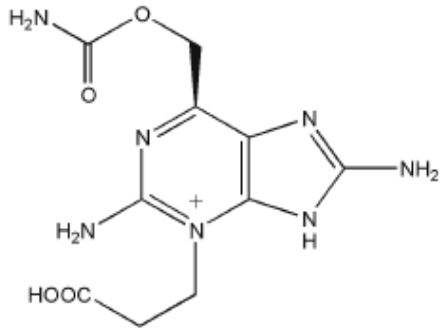
Fluorescent Labeling: Saxitoxins

Fluorescent derivatization methods used for LC based separations of saxitoxins not suitable for ChemLab detection platform (wavelength and/or brightness)



H_2O_2 or periodate



R-COOH

Oxidized saxitoxin

-$CONHNH_2$

AlexaFluor Hydrazide

EDC

R-CONHNHCO-

Alexa-saxitoxin

*Bhakuni, D.S., and D.S. Rawat. 2006. Chapter 7—bioactive marine toxins. Pp. 151–207 in *Bioactive Marine Natural Products*. The Netherlands: Springer.

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Accomplishments-To-Date

So far:

- Capillary resolution of domoic acid, saxitoxin and neosaxitoxin
  - @ pH 7.4 DA is negative while saxitoxin/neosaxitoxin is positive
  - In the absence of EOF they migrate opposite directions (verified experimentally)
  - @ pH 7.4, saxitoxin and neosaxitoxin separate (verified experimentally)
- Demonstrated fluorescent labeling of domoic acid via two different strategies
- Demonstrated on chip detection of domoic acid (with NBD-F)
- Eliminated two strategies for labeling saxitoxins
  - Successful labeling of saxitoxin/neosaxitoxin via periodic acid followed by reaction with hydrazide dye derivatives

What's Next:

- Optimize separation of labeled saxitoxin/neosaxitoxin and determine detection limits
- Determine detection limits
- Detect toxins in "real" samples (Alexandrium extracts from Don Anderson @ WHOI)
  - In storage awaiting optimized separation and labeling method

Questions?

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