Integration of Chip-based Sample Preconcentration into the µChemLab™/CB Analysis Platform

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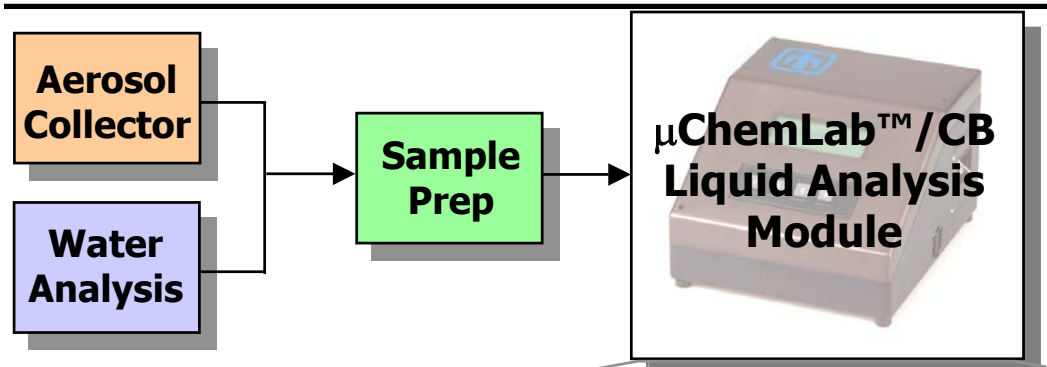
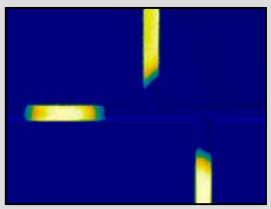
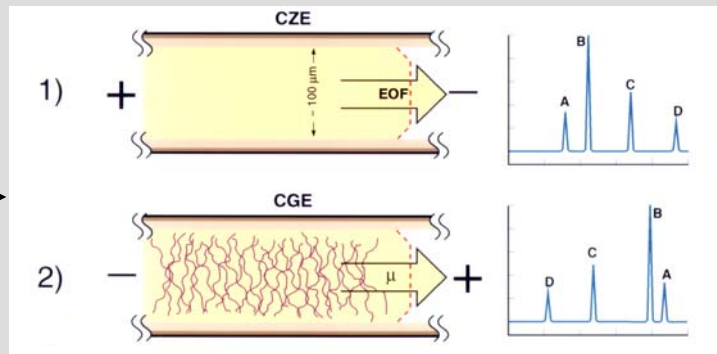
µChemLab™ Separation Platform



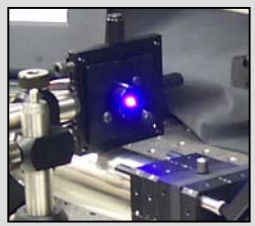


Microfluidic Delivery

•Application of electric fields moves fluids



Dual Channel Microchip Separations



Laser Induced Fluorescence Detection

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Beckman 14-200 SDS Gel

Reversed polarity

Capillary Zone Electrophoresis (CZE) Channel

10 mM phytic acid, pH 9.5 containing 2 mM DAPS (zwitterionic detergent)

Normal polarity

Fluorescamine labeling

Fluorogenic dye

Fast-reacting, amine specific

Ex/Em 390 nm/480nm

Detection limits

nM for CGE

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Approaches to Preconcentration

- **Solid Phase Extraction** (on-going efforts for the miniaturization of SPE)

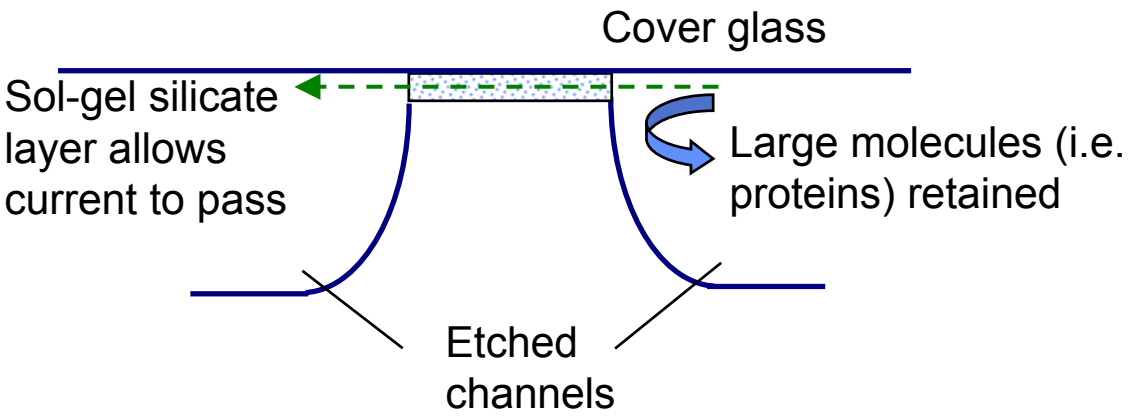
- **Electrokinetic Trapping** (**P411-T:** Novel Miniaturized Protein Preconcentrator Based On Electrokinetic Trapping, Anup K. Singh; Daniel J. Throckmorton; Brian J. Kirby)

- **Salt-Bridge**

[1] J. Khandurina, T.E. McKnight, S.C. Jacobson, L.C. Waters, R.S. Foote, J.M. Ramsey, Analytical Chemistry 72 (2000) 2995-3000
[2] R. S. Foote, J. Khandurina, S. C. Jacobson, J. M. Ramsey, Preconcentration of Proteins on Microchips for Enhanced Detection, Poster presentation at HPCE 2001

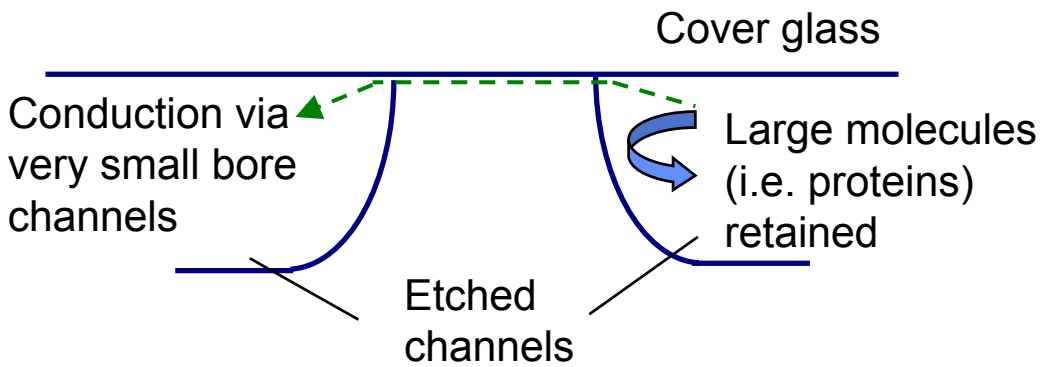
Integration into the μChemLab™/CB

Silicate layer Approach:



Alternative Approach:

- Eliminate silicate layer
- Makes use of narrow gap and natural surface roughness



▪ Jean Pierre Alaire, Stephen C. Jacobson, B. Scott Broyles, Timothy E. McKnight, Christopher T. Culbertson and J. Michael Ramsey, Electroosmotically Induced Hydraulic Pumping on Microchips. *μTAS 2001*. J. Michael Ramsey and A. van den Berg. ed.s, (Kluwer Academic, Amsterdam), (2001), pp. 127-128.

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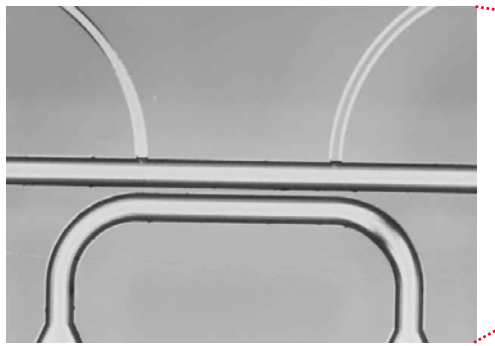
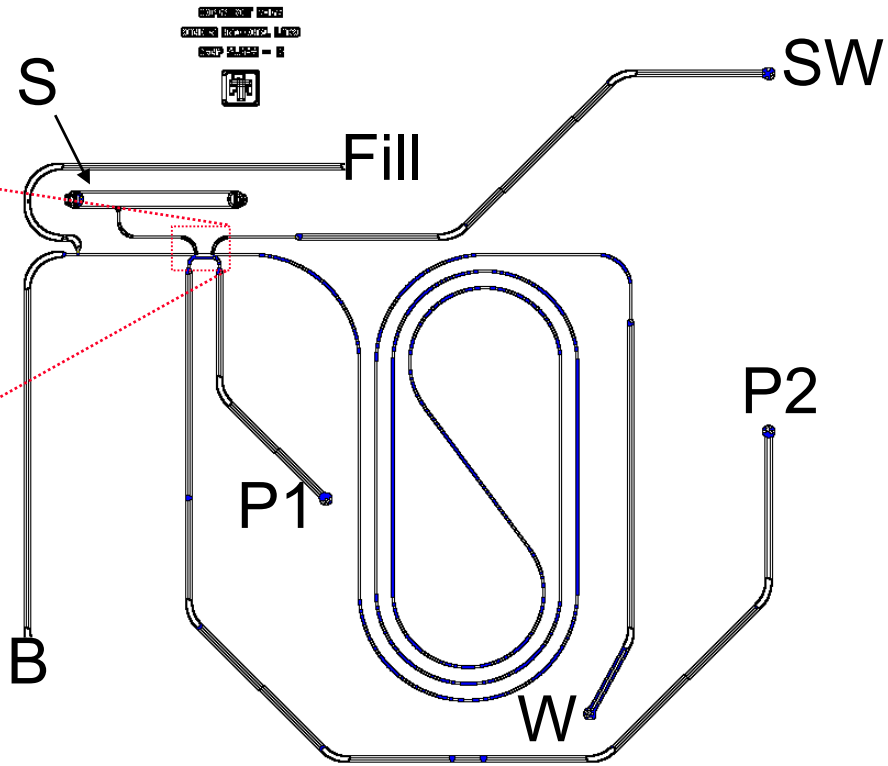
Chip Layout



Close-up of the Preconcentrator Region



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Detection limits

- nM for CGE

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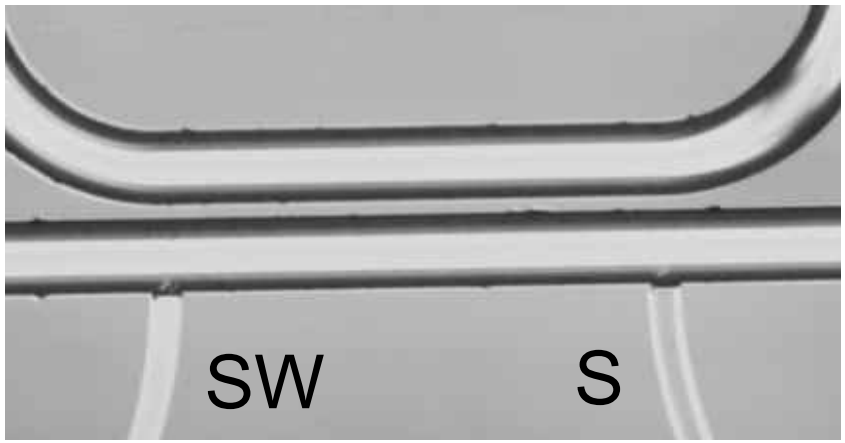
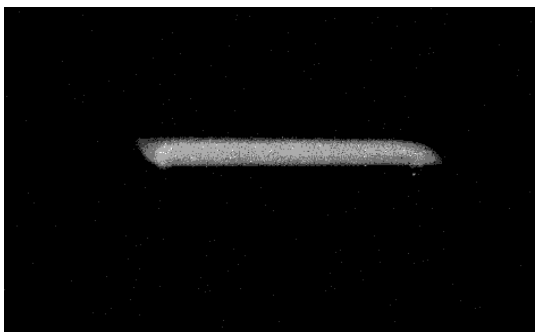
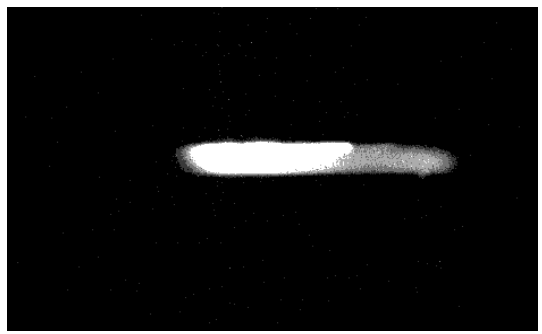
Imaging Normal and Preconcentrated Injections in CGE





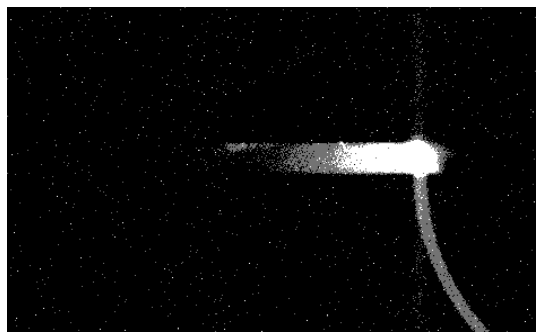
Normal Mode

S → SW



P1 Preconcentration Mode

S → P1



P2 Preconcentration Mode

S → P2

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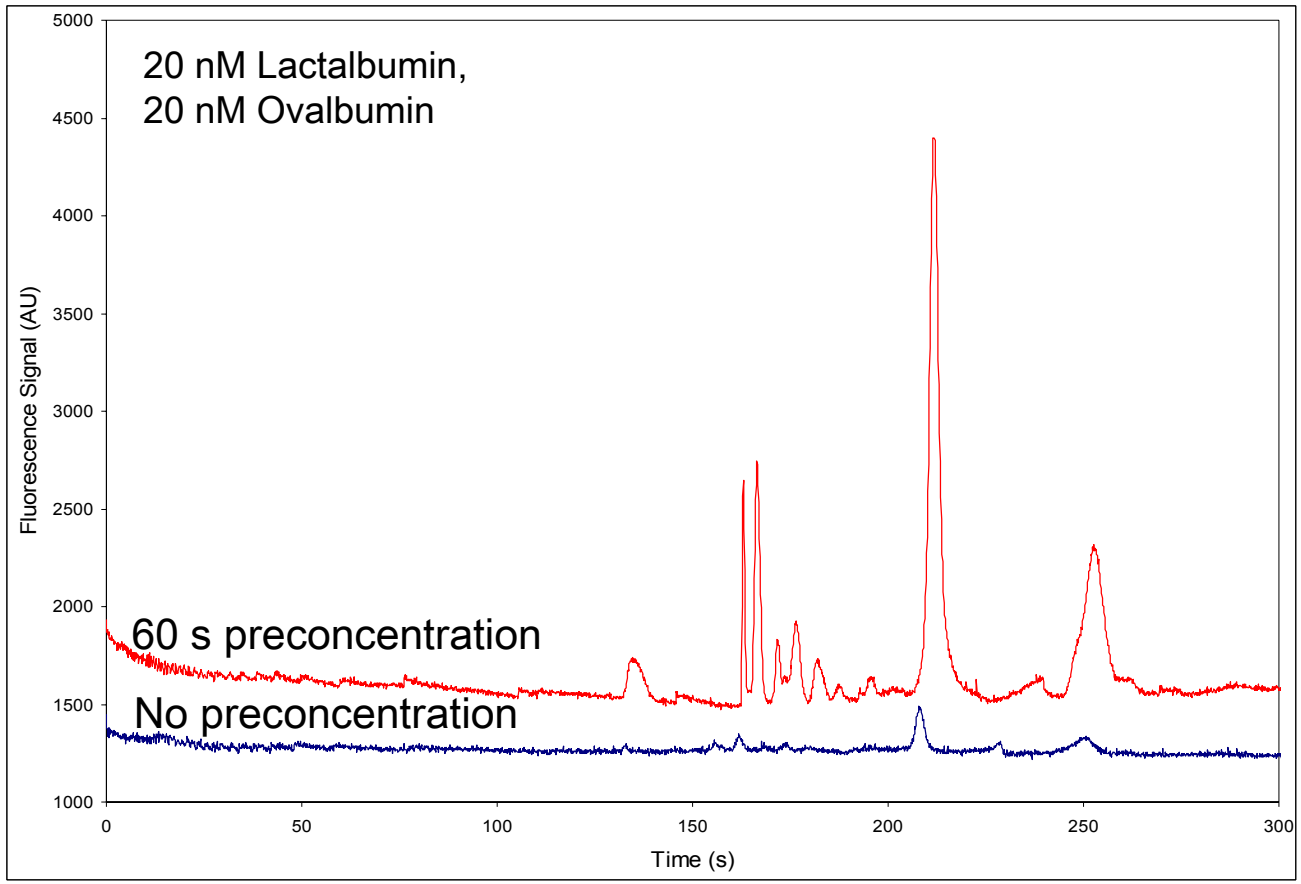
CGE Separations Using Normal and Preconcentrated Injections



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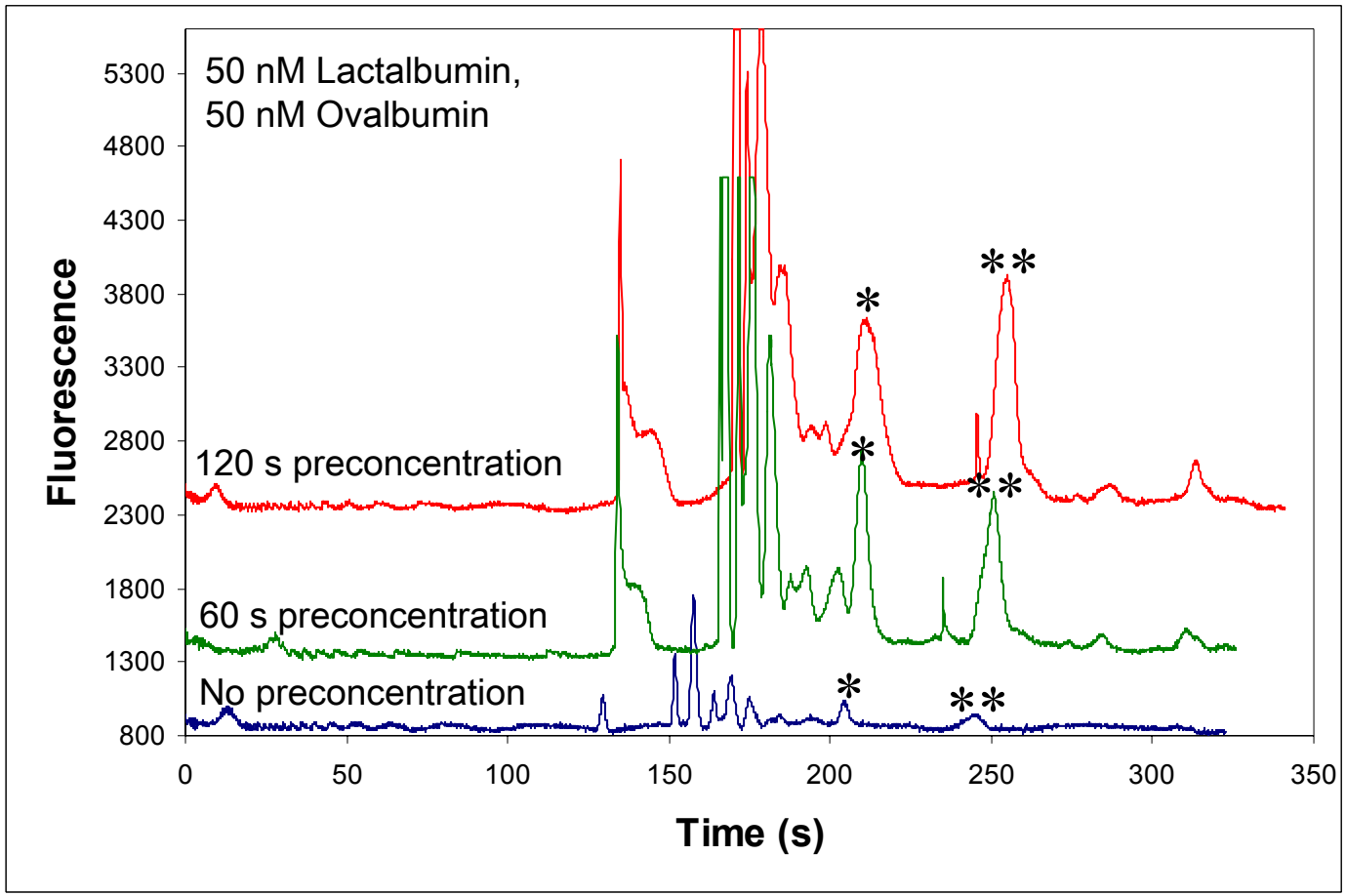
Peak Areas in CGE Separations Increase as a Function of Preconcentration Duration



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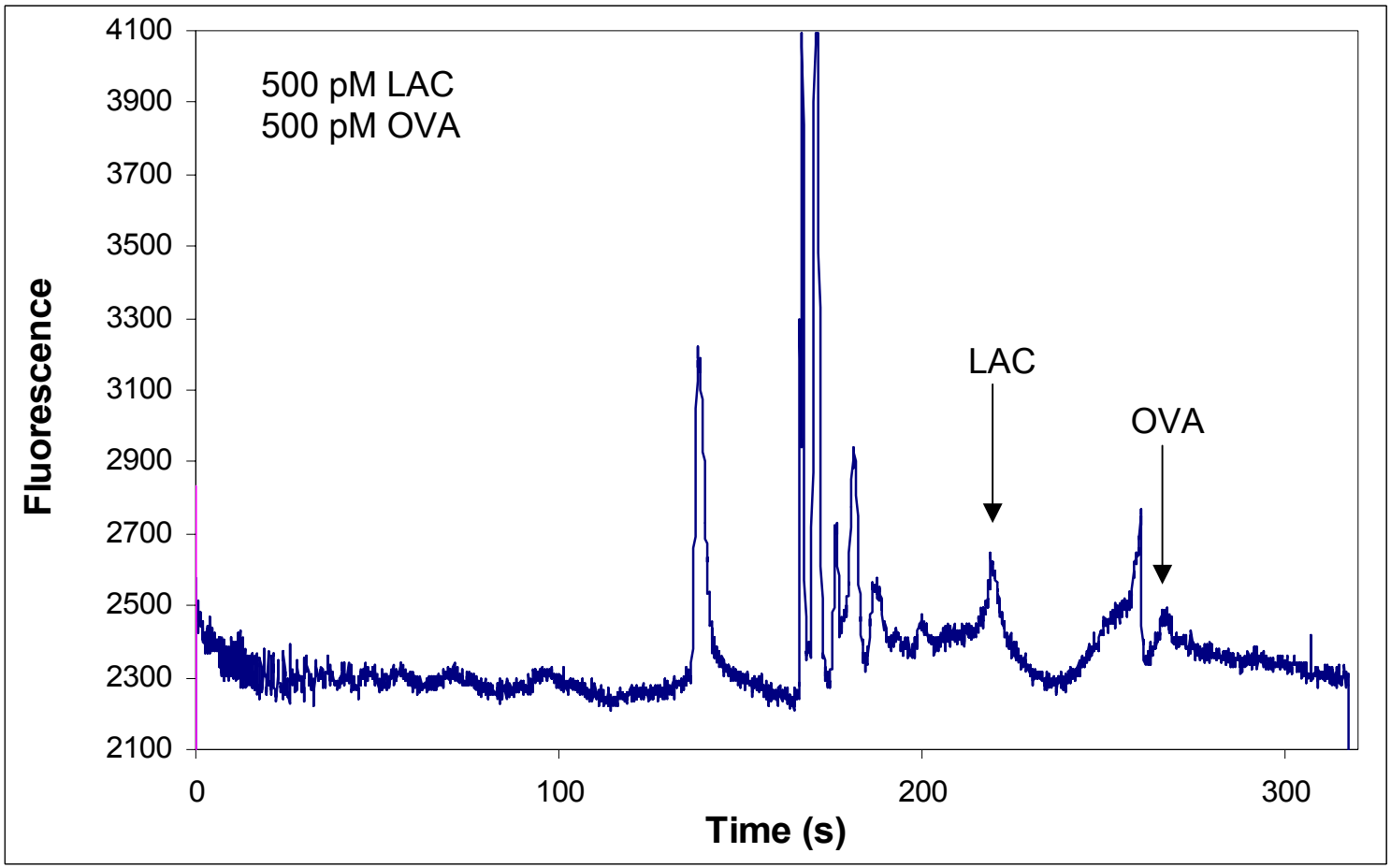
Sub-Nanomolar Detection Limits Using CGE Separations and Preconcentration



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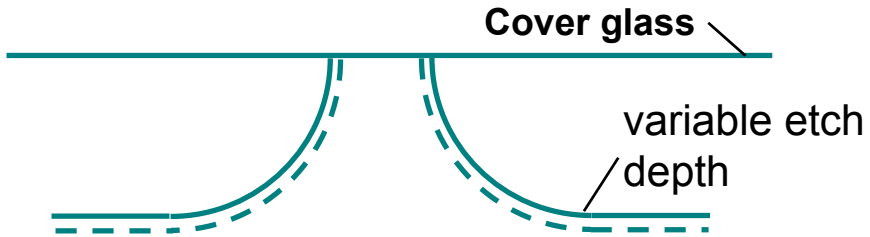
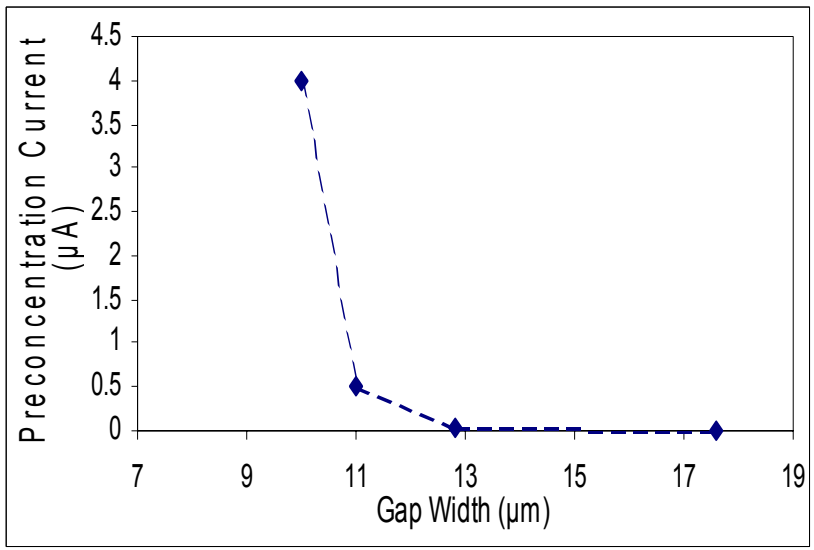
Characterization of Performance as a Function of Gap Width

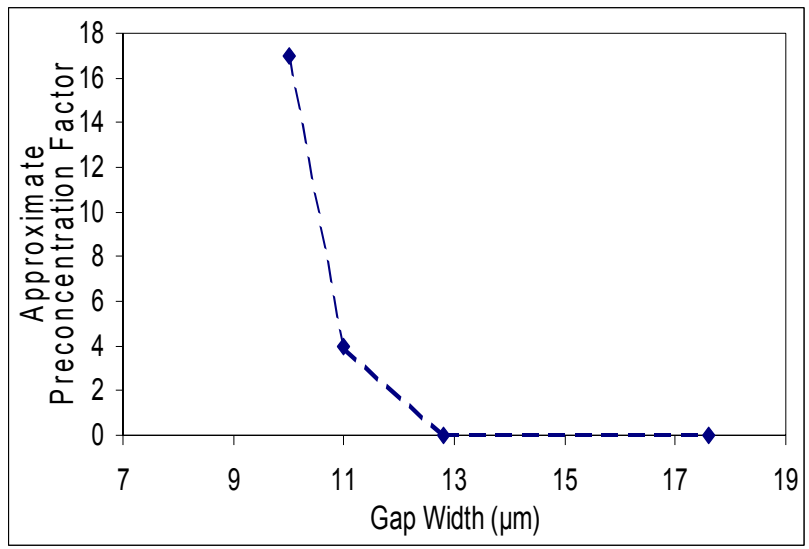
Gap width

- Isotropic etch depth used to generate gaps of different widths
- For comparison purposes, all preconcentrations were held to 60s





*Single Batch Data



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Preconcentration Using CZE

Elimination of EOF is required

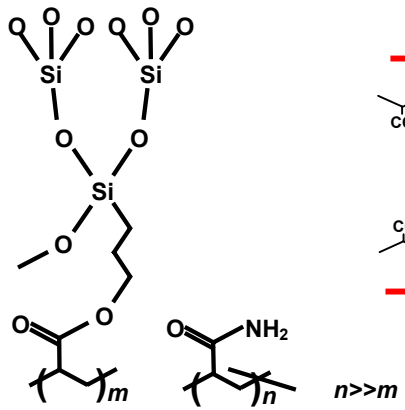
- no bulk flow of fluid through the gap

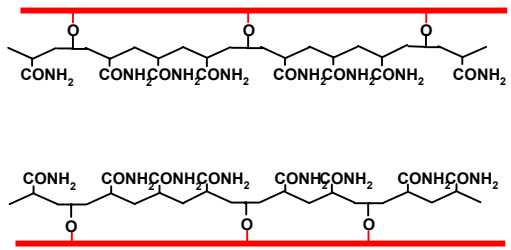
  ∴ residual EOF could lead to pressure generation

Coating with linear polyacrylamide

SAM application

UV light polymerization





•Julie Fruetel, Victoria VanderNoot, Jay West, Brian Kirby, Ernest Hasselbrink and Timothy Shepodd, Laser-polymerized thin-film coating for protein analysis by CGE in a microchip, HPCE 2002

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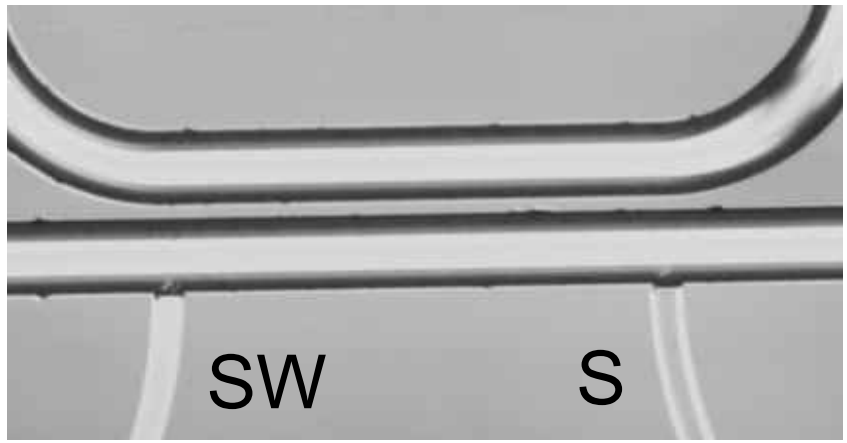
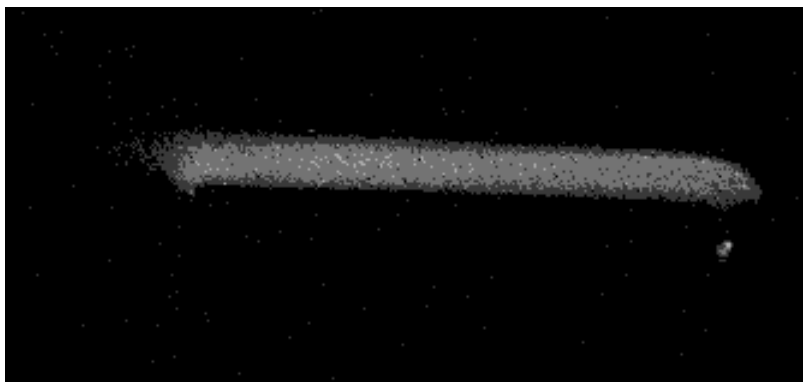
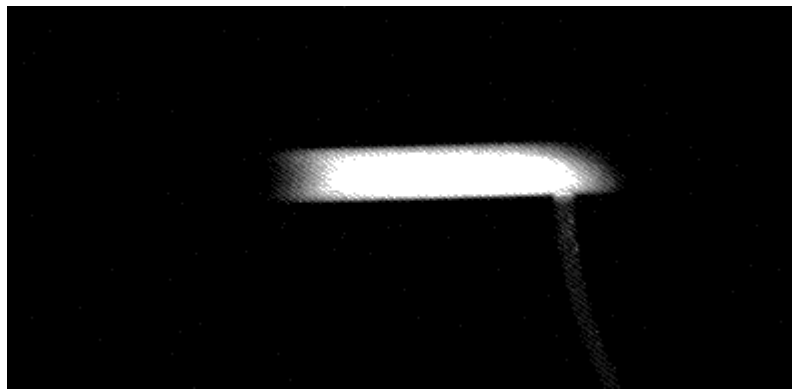
Imaging Normal and Preconcentrated Injections in CZE





Normal Mode

S → SW



P1 Preconcentration Mode

S → P1

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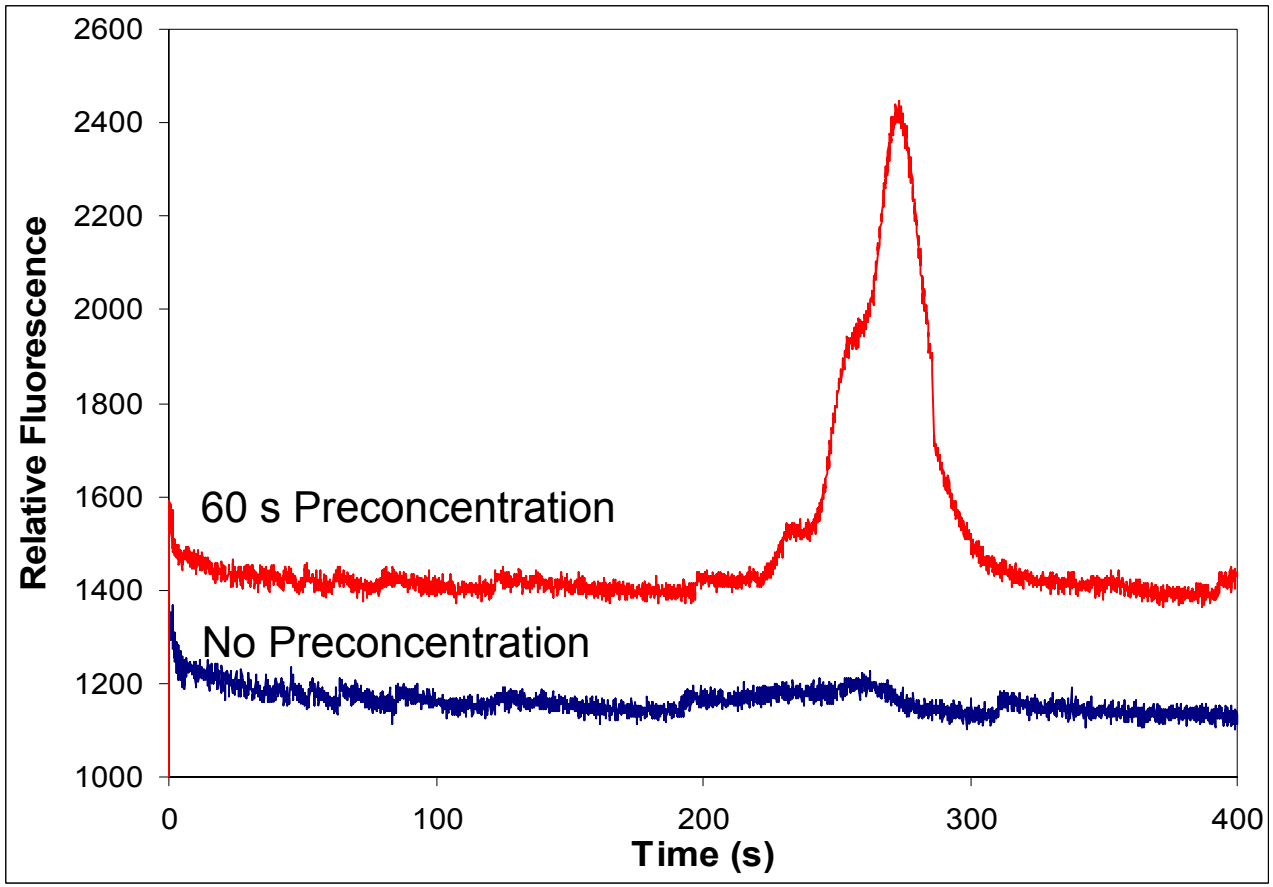
CZE Separations Using Normal and Preconcentrated Injections



Running buffer: 5 mM phosphate, pH 8 containing 5 mM CAS-U zwitterionic detergent
Reverse polarity

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Summary

- Preconcentration has been incorporated in the chip design with no additional processing steps during chip fabrication

- Successfully demonstrated with both CGE and CZE
  - Preconcentration factors of 10-20x are routinely achievable in only 60s
  - Coating and/or elimination of EOF is essential for CZE separations
  - Buffer conditions in CZE will need to be optimized to achieve both good separation efficiency and the elimination of EOF

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Robert S. Foote and J. Michael Ramsey at Oak Ridge National Labs for their valuable insights
&
Nicole Baryla and Charles A. Lucy for supplying us with a sample of CAS-U zwitterionic detergent

The μChemLab Technical Team

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