Stanford Microfluidics Lab

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microfluidics.stanford.edu

Activities:

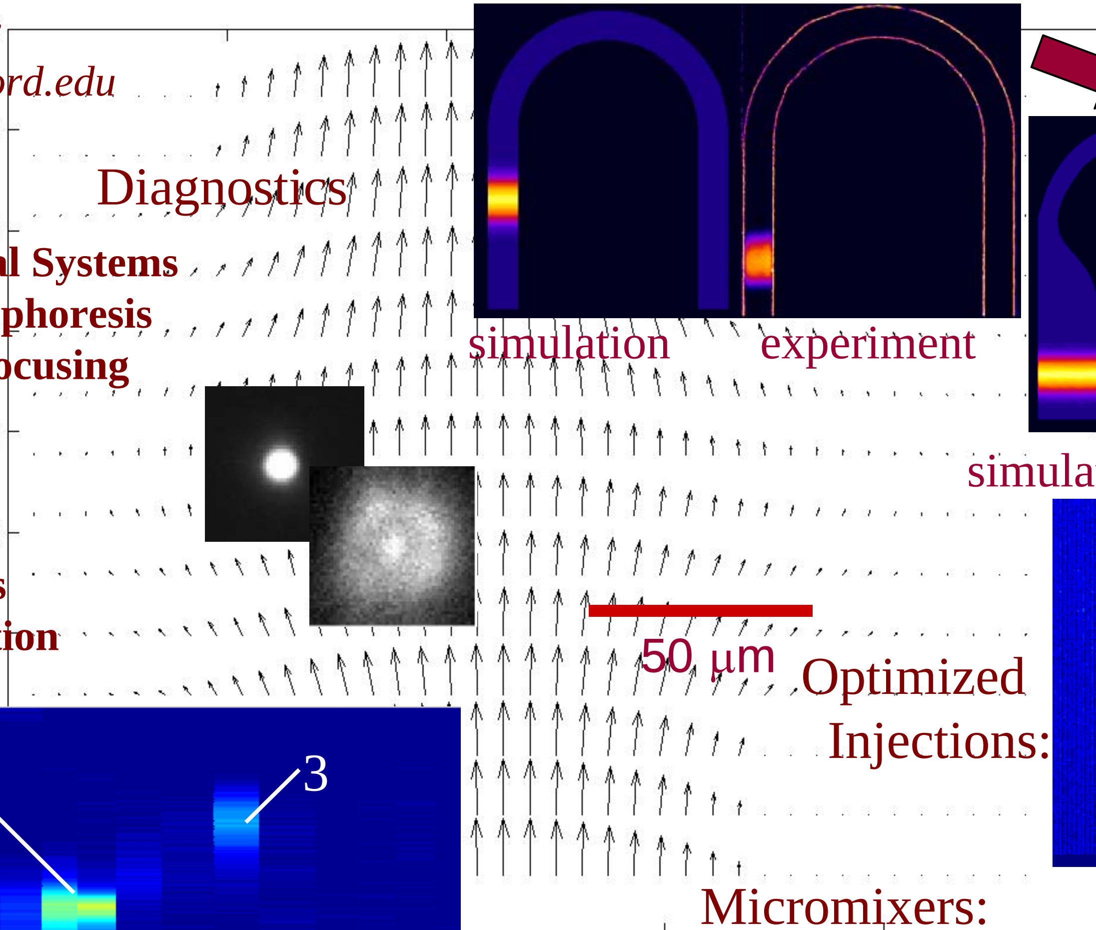
Miniature Bioanalytical Systems
- **Capillary zone electrophoresis**
- **Capillary isoelectric focusing**
- **CE Binding Assays**

Microflow Devices
- **Micromixers**
- **Electroosmotic pumps**
- **Sample preconcentration**
- **On-chip 2D assays**

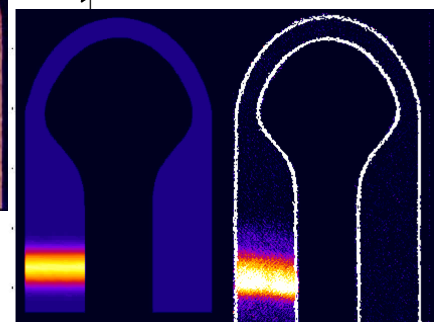
Applications
- **Drug discovery**
- **Genetic studies**
- **Proteomics**
- **BW detection**
- **Electronics cooling**

Research Examples:

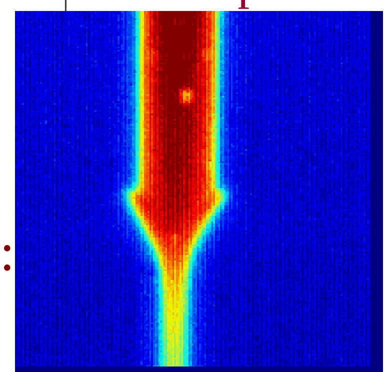
Diagnostics

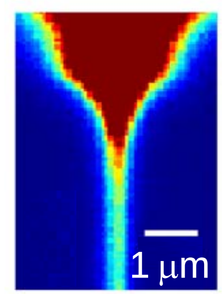


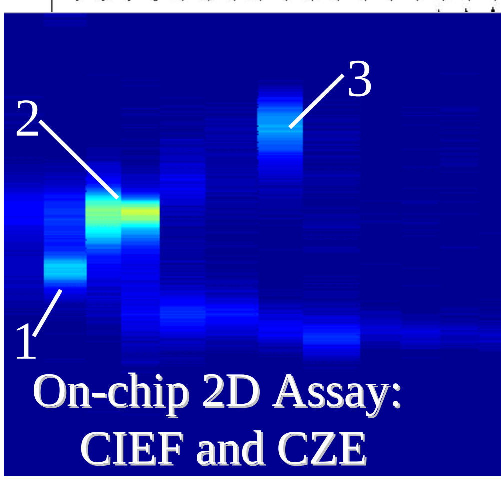
Optimized Geometries:



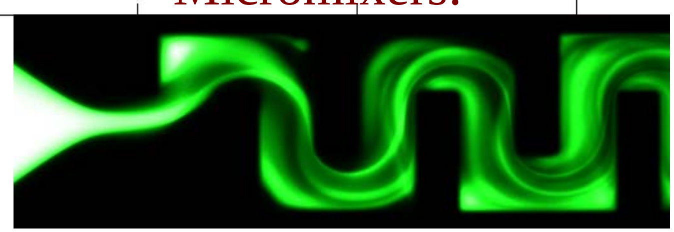
Optimized Injections:



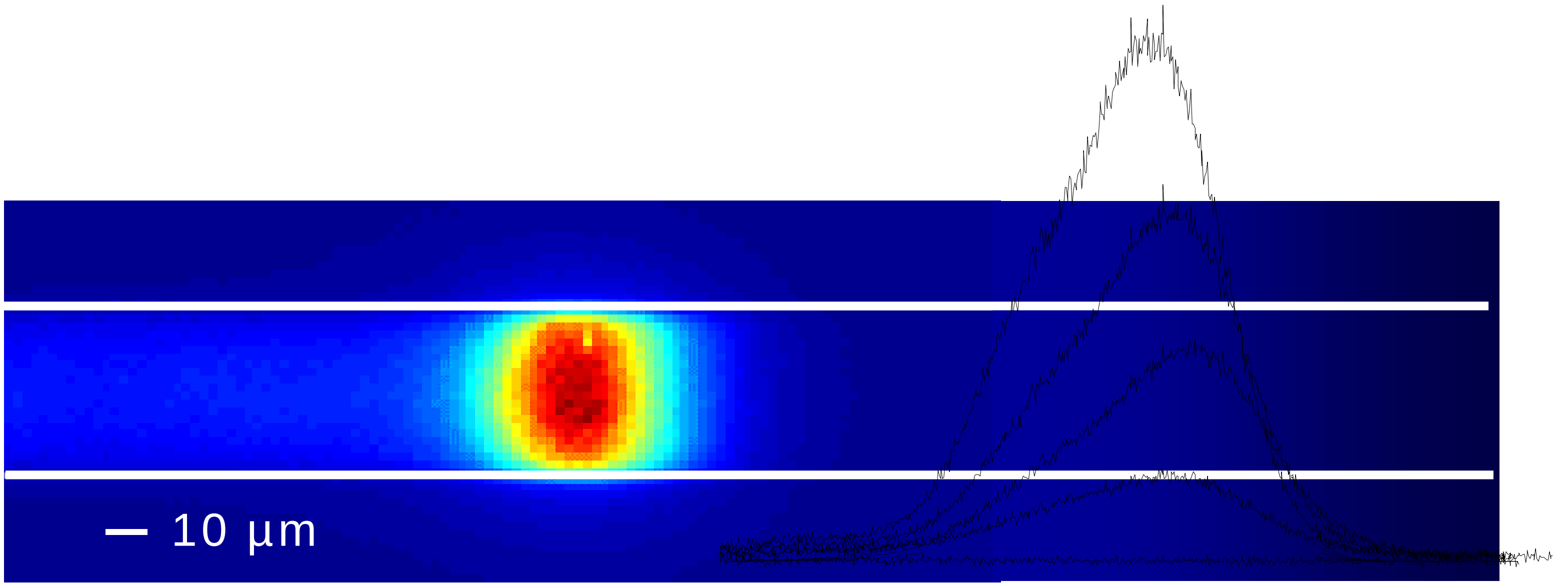




Micromixers:



Electrokinetics Microfluidics at Extreme Scales



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Outline

(time, concentration, length)

- Introduction
  - Microfluidics
  - Electrokinetic (EK) flows
- Electrokinetic instabilities (time)
  - Mechanism and model
  - Transition to chaos
- ITP (concentration)
  - Sensitivity and practice
  - Extreme concentration scales
- Nanochannel electrophoresis (length)
  - Small ion separations
  - DNA sample separation
  - Near-Future Work

Microfluidics

- Applications
  - Point-of-care medical diagnostics
  - Bio-weapon detection
  - Pharmaceuticals/drug discovery
  - Environmental monitoring
- Challenges and Advantages
  - Reduced reagent use
  - Specificity, robustness
  - Portability vs. sensitivity
  - Integration and automation
  - Potential for parallel analyses

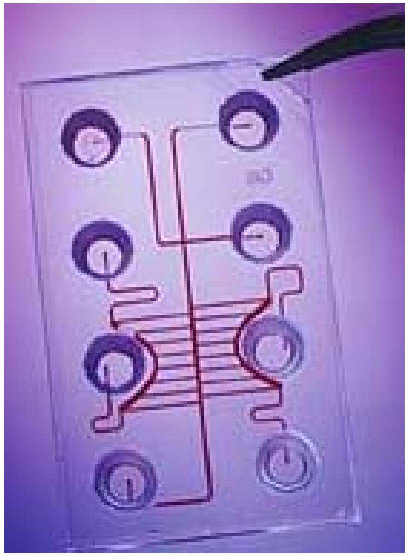
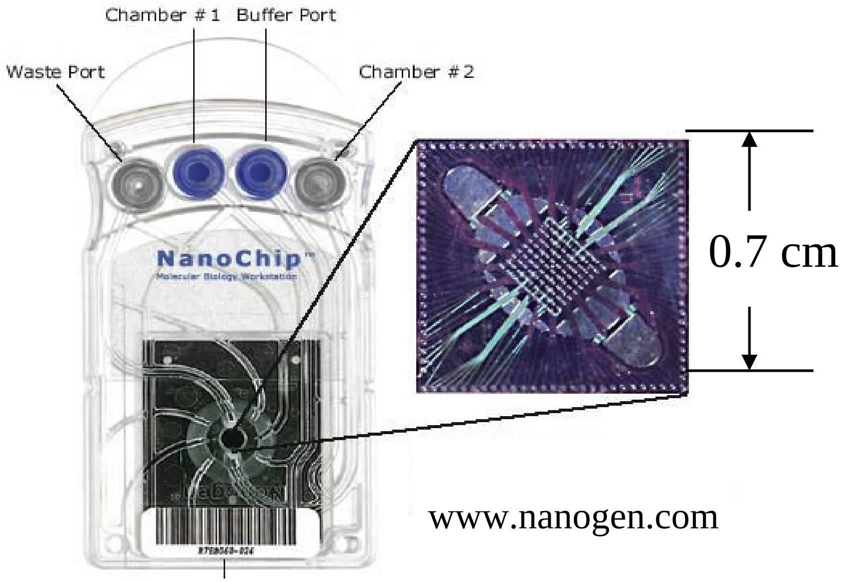
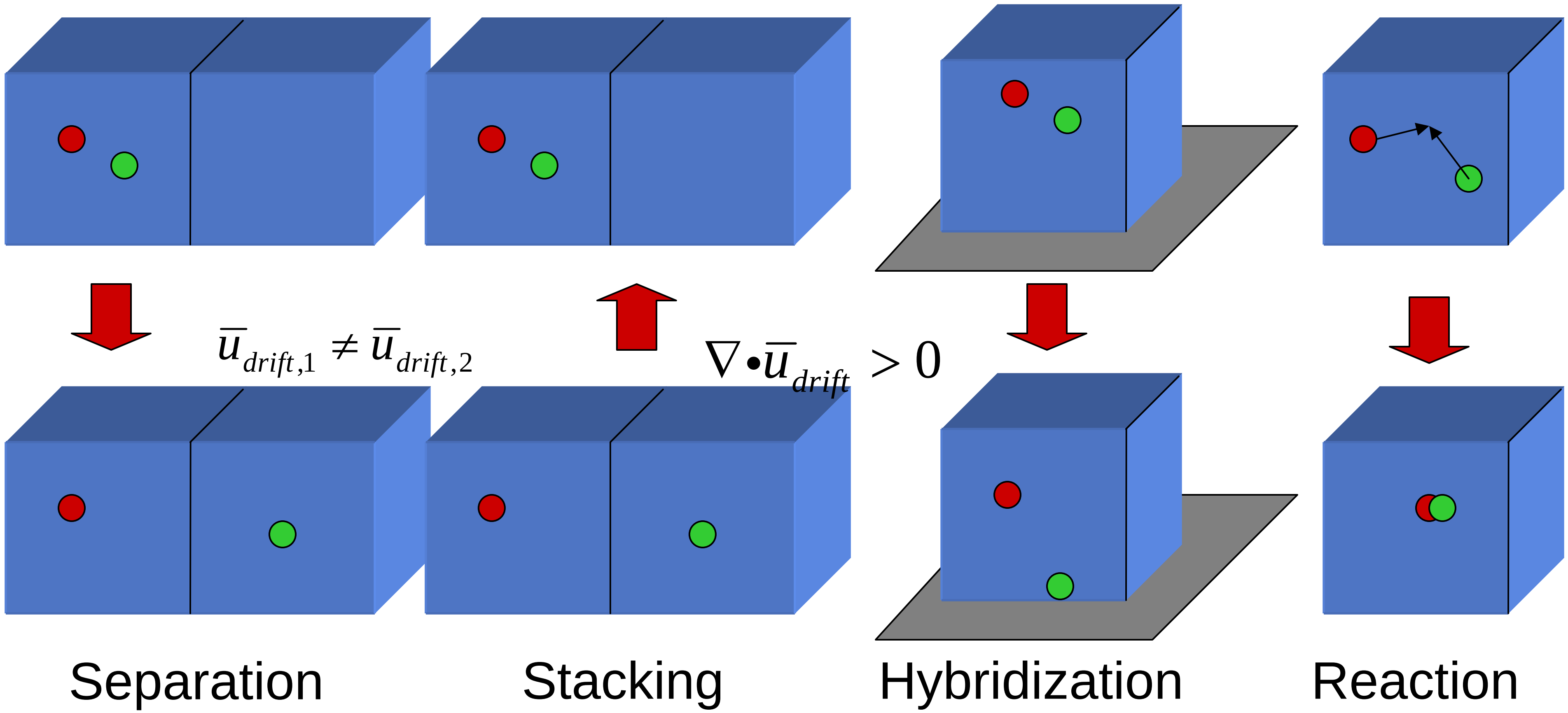


Image courtesy www.calipertech.com

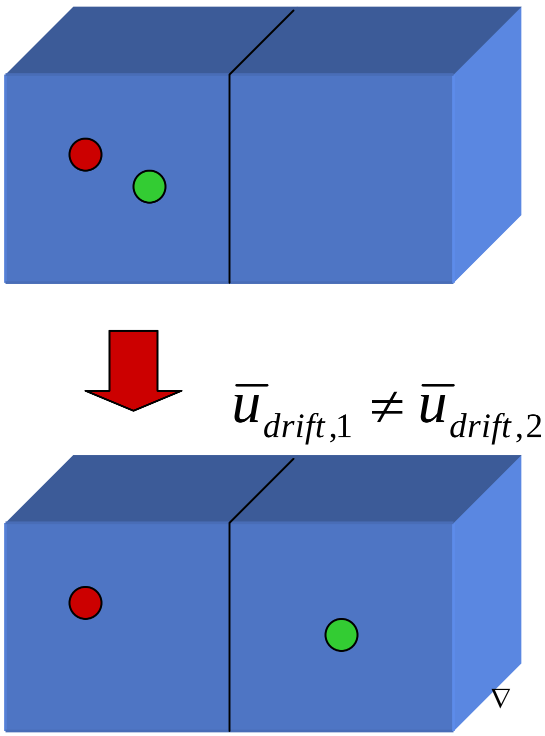


www.nanogen.com

Processes in microfluidics

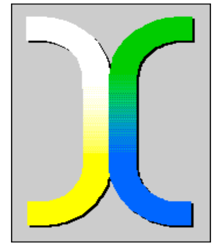


Separation in uFluidics



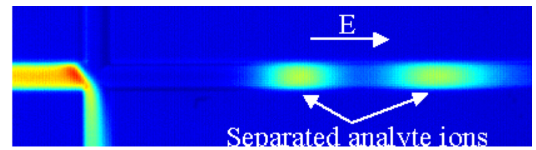
$$\overline{u}_{drift,1} \neq \overline{u}_{drift,2}$$

- Δdiffusivity



H-filter

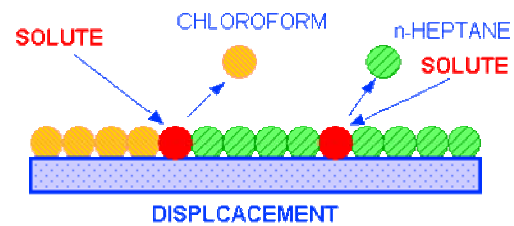
- Δmobility



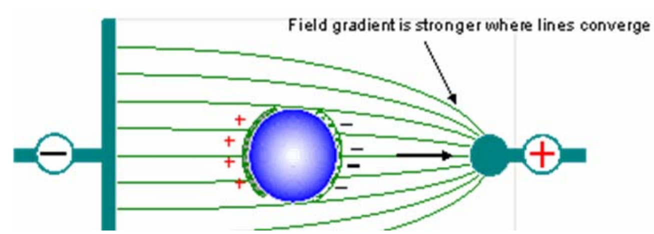
On-chip CE

Nanochannel electrophoresis

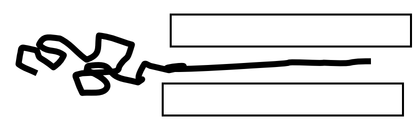
- Δvalence
- Δaffinity



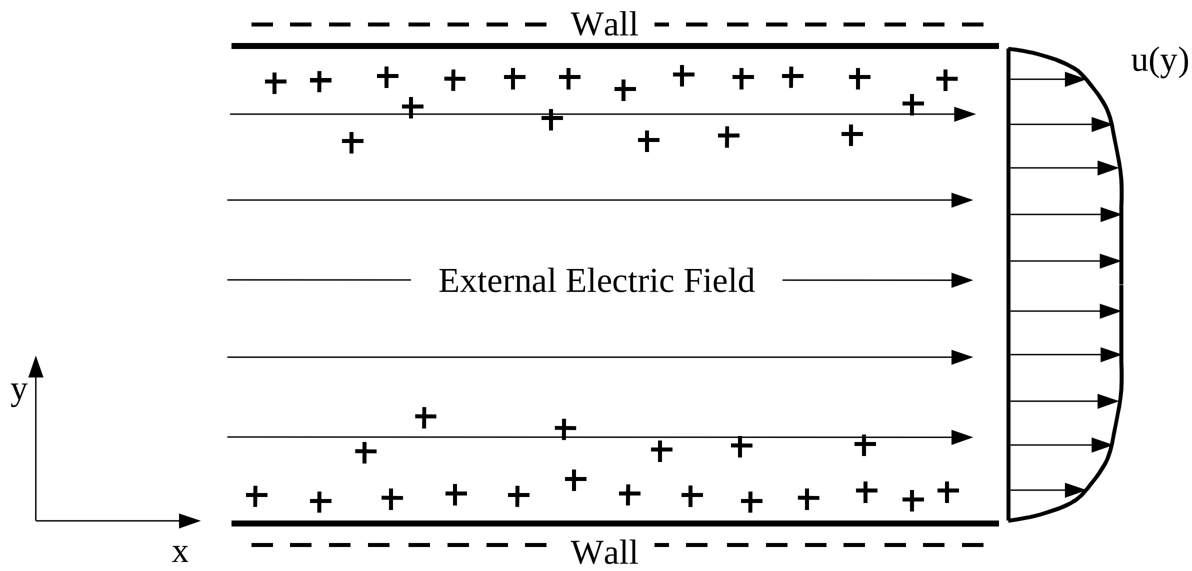
- Δpolarizability



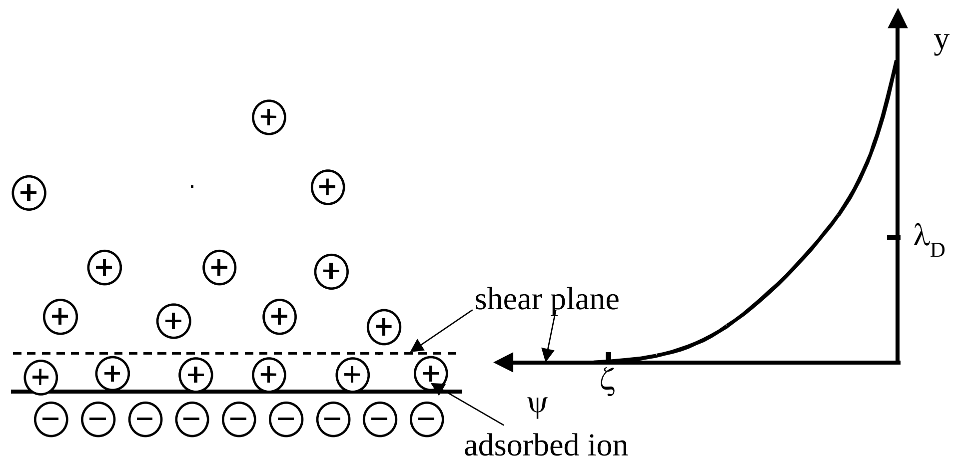
- Δsize/steric force



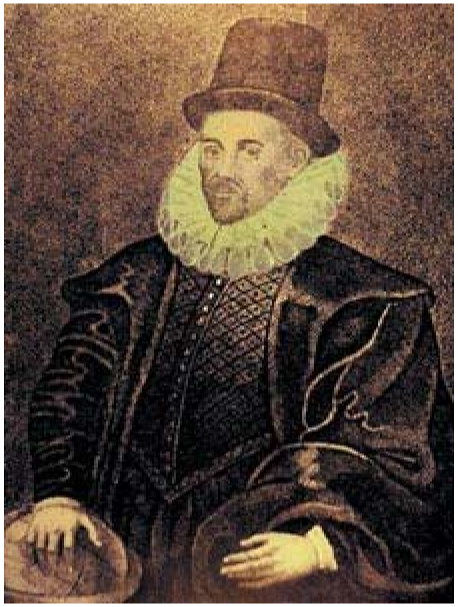
Electroosmotic flow



- Zeta vs. chemistry?
- EDL overlap?
- Condensation?



Electrohydrodynamics and Electrokinetics History

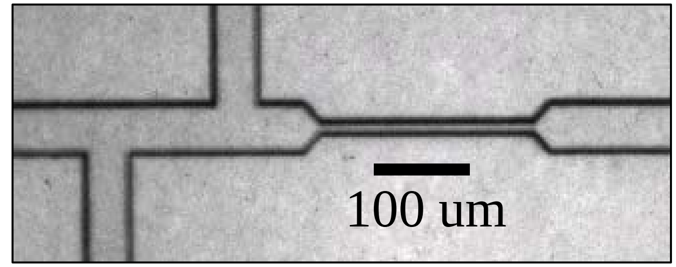


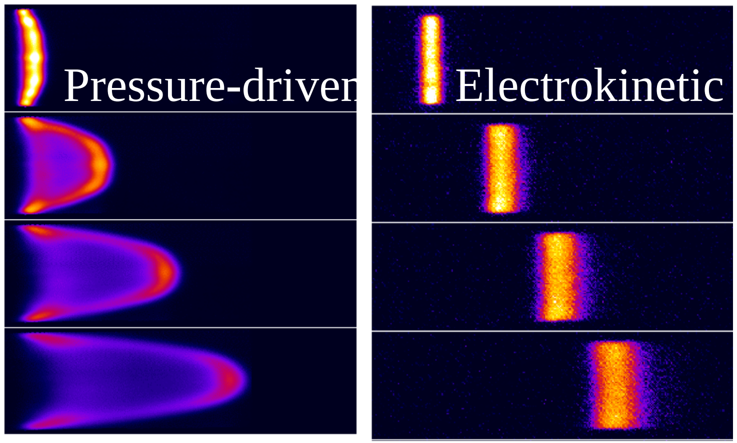
Gilbert, ~1580s

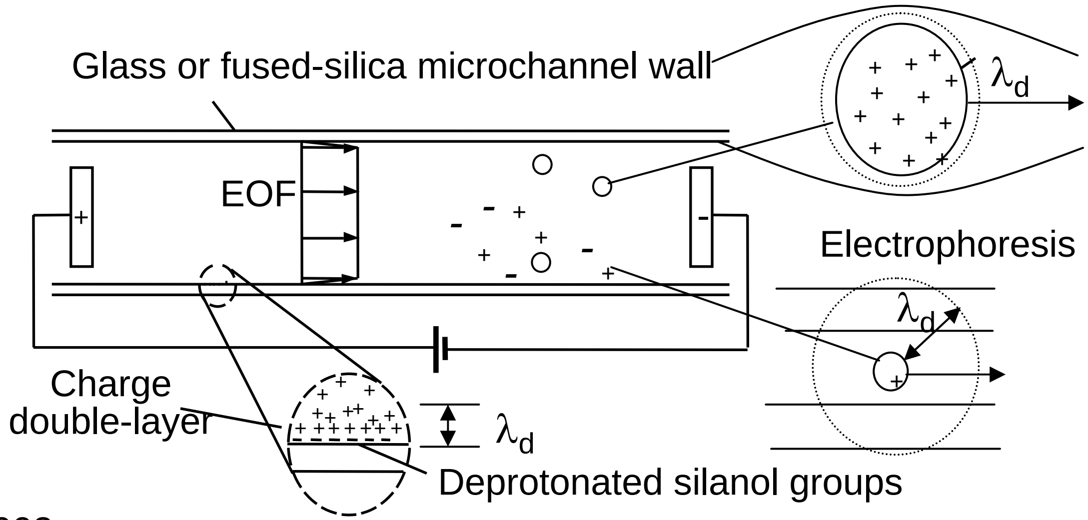
Of the attraction exerted by amber

Reuss, F.F. 1809. Memoires de ls Societe Imperial des Naturalistes de Moscow. 2:327.

Electrokinetic Microfluidics







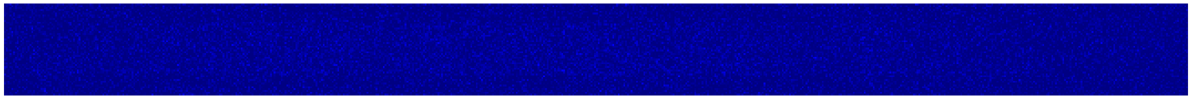
Devasenathipathy S. and J. Santiago, *Micro- and nano-scale diagnostics*, Springer-Verlag, 2003

- Electric control (no moving parts)
- Switching, valving
- Low dispersion
- Integrated w/ separation techniques

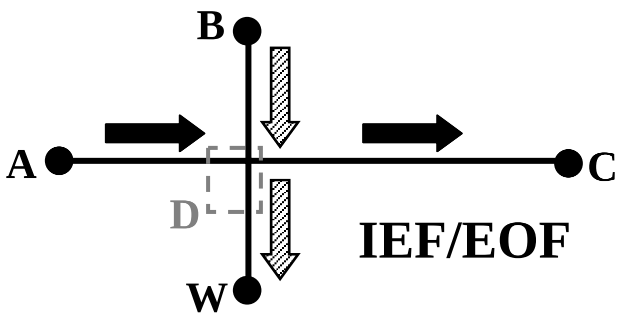
Electrokinetic Instabilities

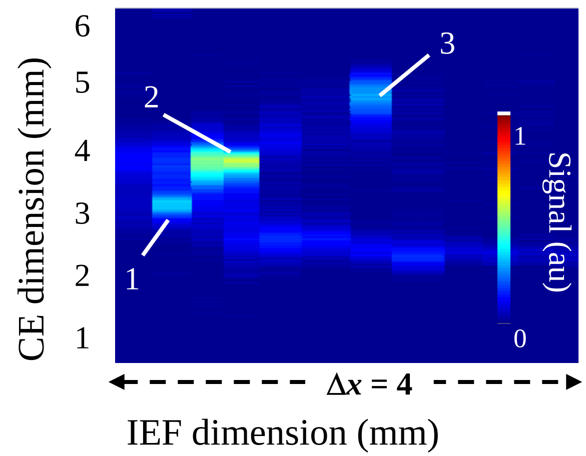
Complex electrokinetics

- Sample preconcentration methods
  - Thermal gradient focusing
  - Field amplified sample stacking
  - Isotachophoresis



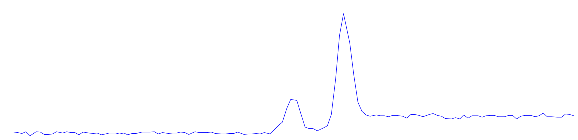
- On-chip two-dimensional assays





Herr, A.E. et al. *Analytical Chemistry*, Vol. 75, No. 5, pp. 1180-1187, 2003

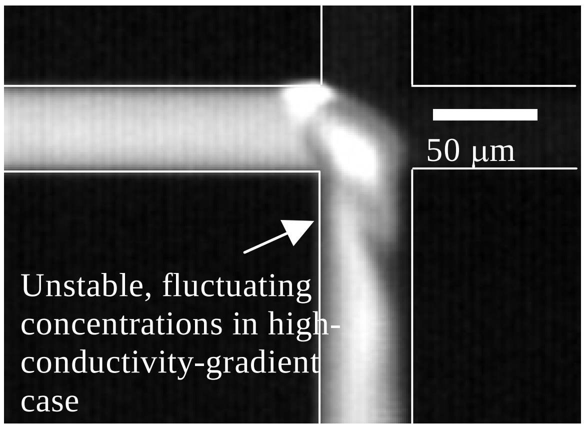
- On-chip CE with unknown or poorly controlled sample chemistry

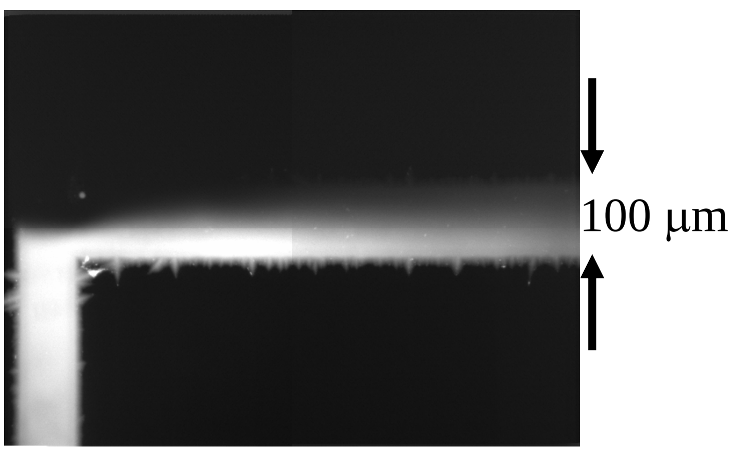


- On-chip mixing and buffer exchange

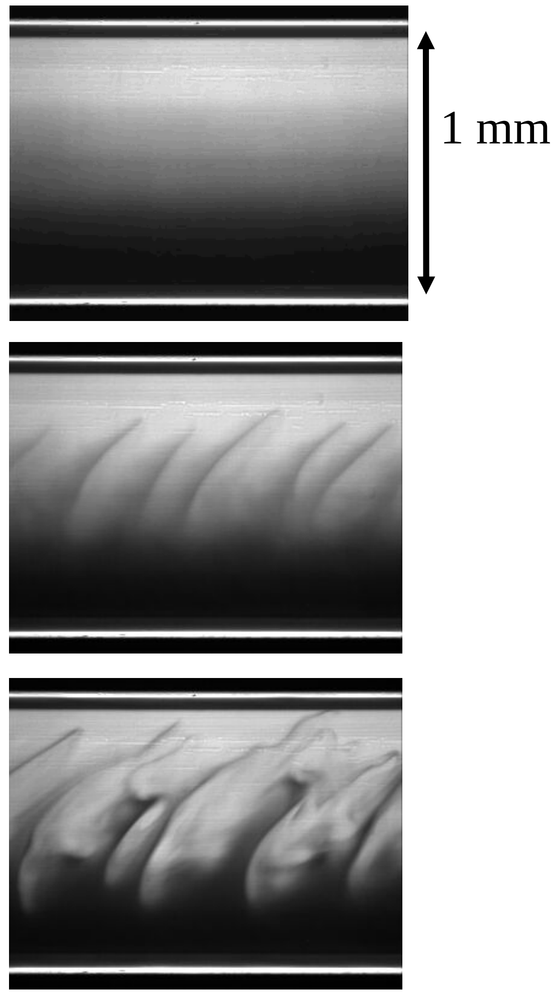
Major Challenge in Heterogenous EK Systems: Instabilities

Flows at Intersections:

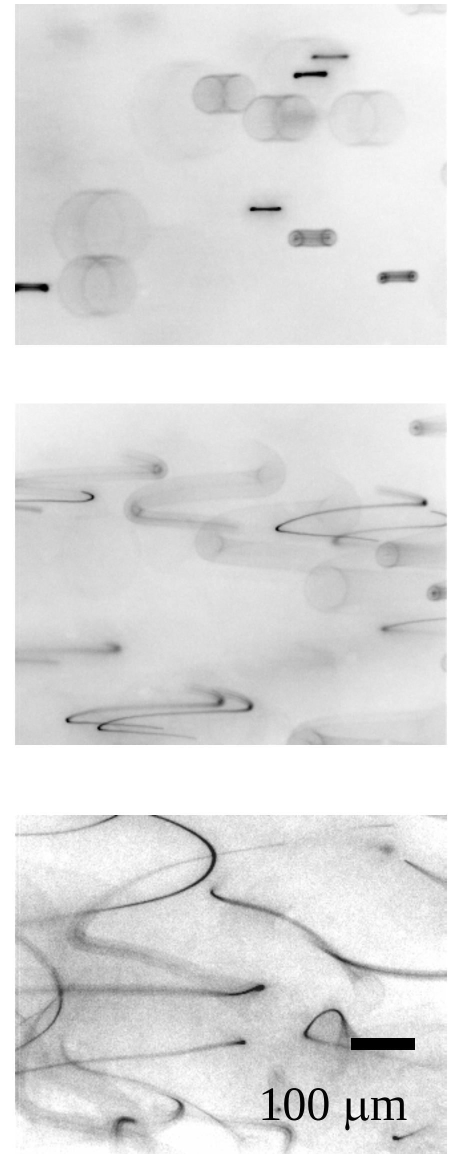




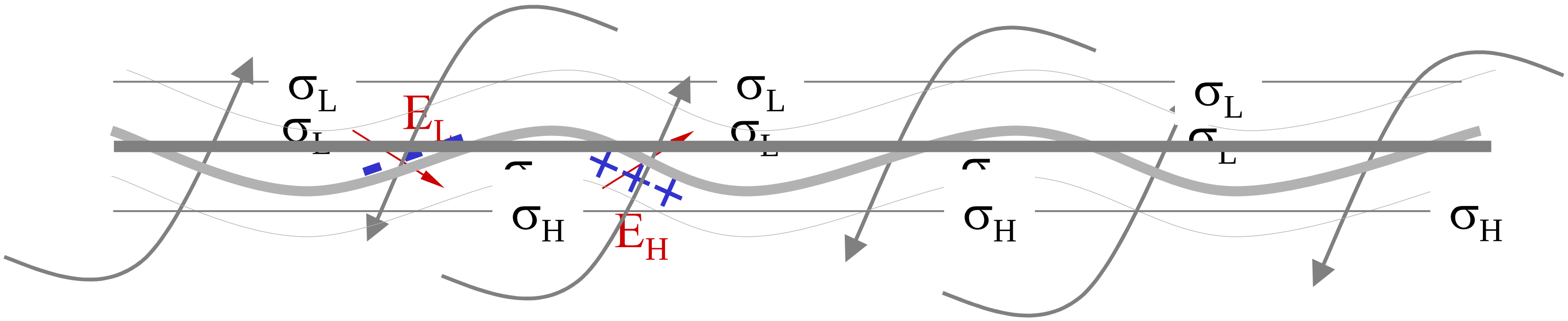
Axial interface:

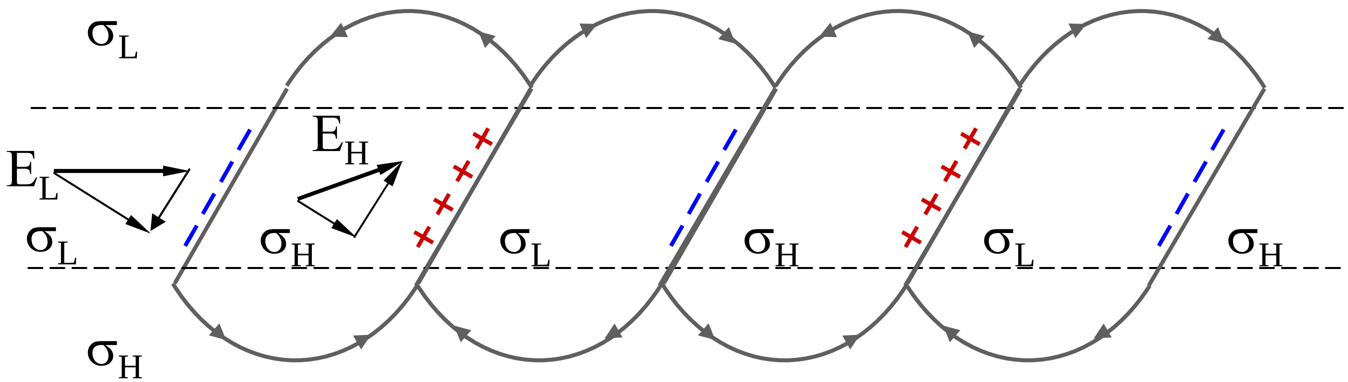


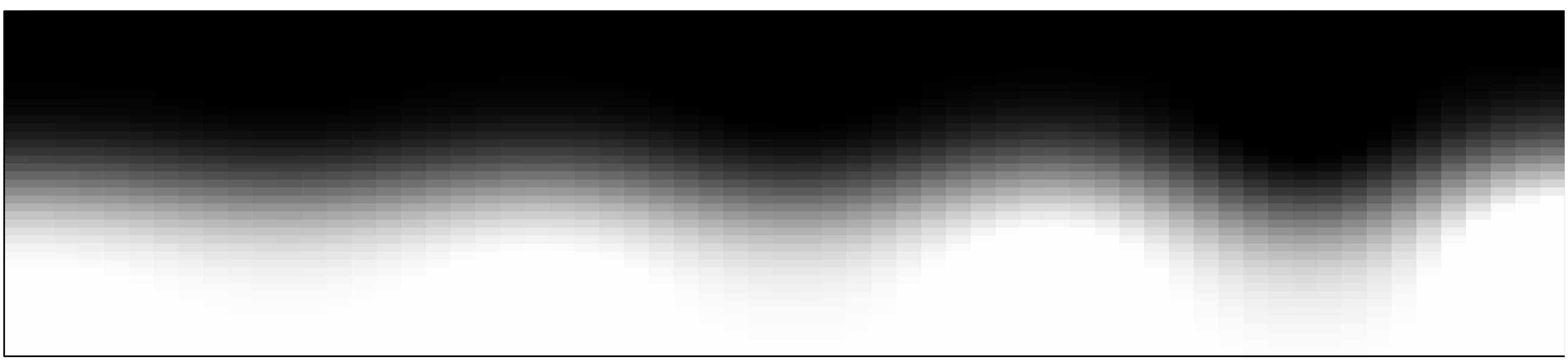
Particle visualization



EK Instability Mechanism

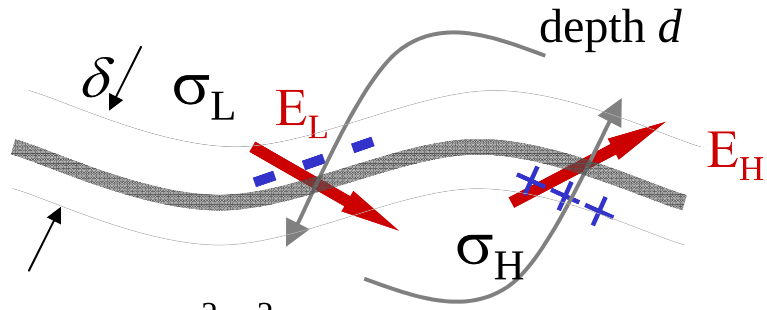




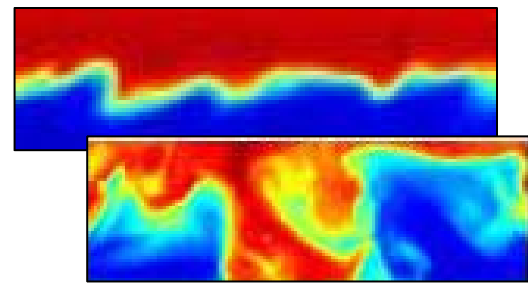


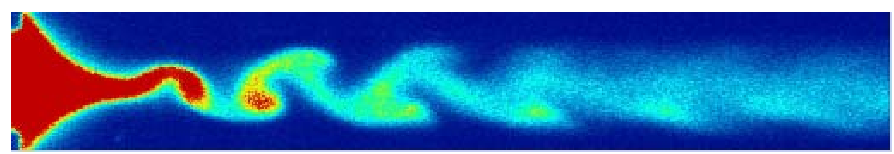
Electrokinetic Flow Instabilities

- Generated by net charge in bulk
  - $\rho_E \cong \varepsilon \bar{E} \cdot \nabla \sigma / \sigma \sim \Delta C$
  - Velocity scale: $U_{EV} = \dfrac{\varepsilon E_L E_a H}{\mu}$
  - Controlling parameter: $Ra_{e,\ell} \equiv \dfrac{U_{ev} d}{D} = \dfrac{\varepsilon E_0^2 d^2}{D\mu} \dfrac{\gamma - 1}{\gamma} \nabla^* \sigma^* \Big|_{\max}$



- ΔC_0 scales as $\varepsilon E_0 (\gamma - 1) / F \gamma \delta \; \sim 10^{-5} - 10^{-6}$
- EOF coupling produces convective instab.
- Multiple ion mobilities have severe impact





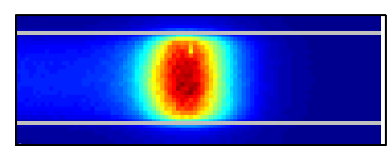
$$\frac{D\sigma}{Dt} = \overbrace{\sum_j \frac{1}{Pe_j} \nabla^2 c_j}^{\text{Diffusion}} + \overbrace{\sum_j \frac{\chi_j}{Pe_j} \nabla \cdot (c_j \nabla \phi)}^{\text{Electromigration}}$$

Oddy and Santiago, in press, *Physics of Fluids*, 2005.

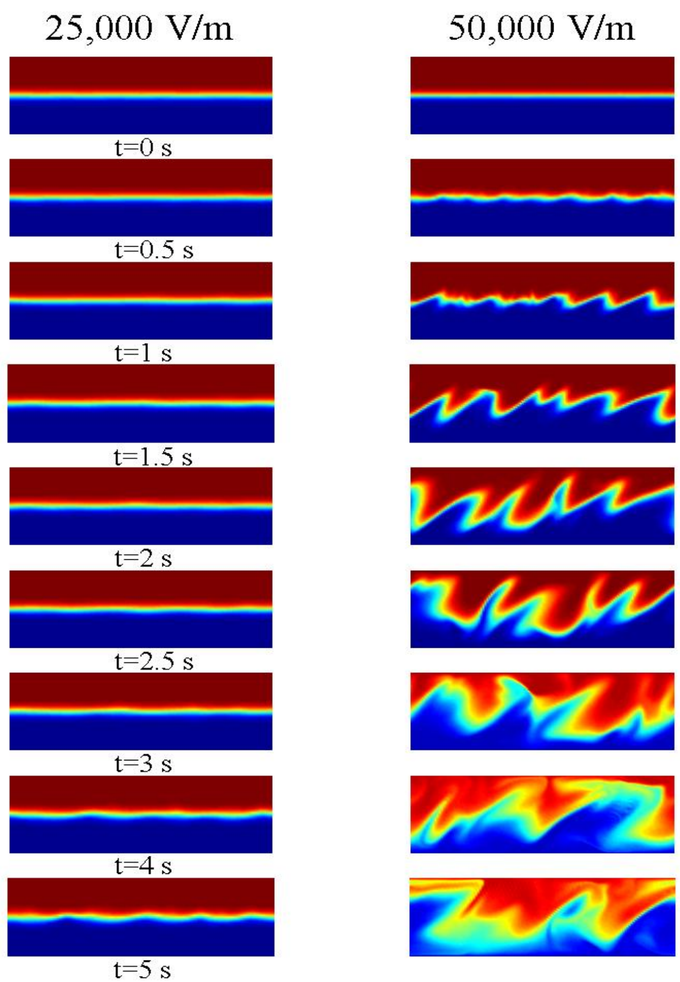
Posner and Santiago, *J. of Fluid Mechanics*, pp. 1-42, 2006.

Chen, C.-H. et al., *J. of Fluid Mechanics*, 524, pp. 263 – 303, 2005.

Lin, H. et al., Vol. 16, No. 6 *Physics of Fluids*, p.1922-1935, 2004.



Electrokinetic instabilities



t = 0.0 s

t = 0.5 s

t = 1.0 s

t = 1.5 s

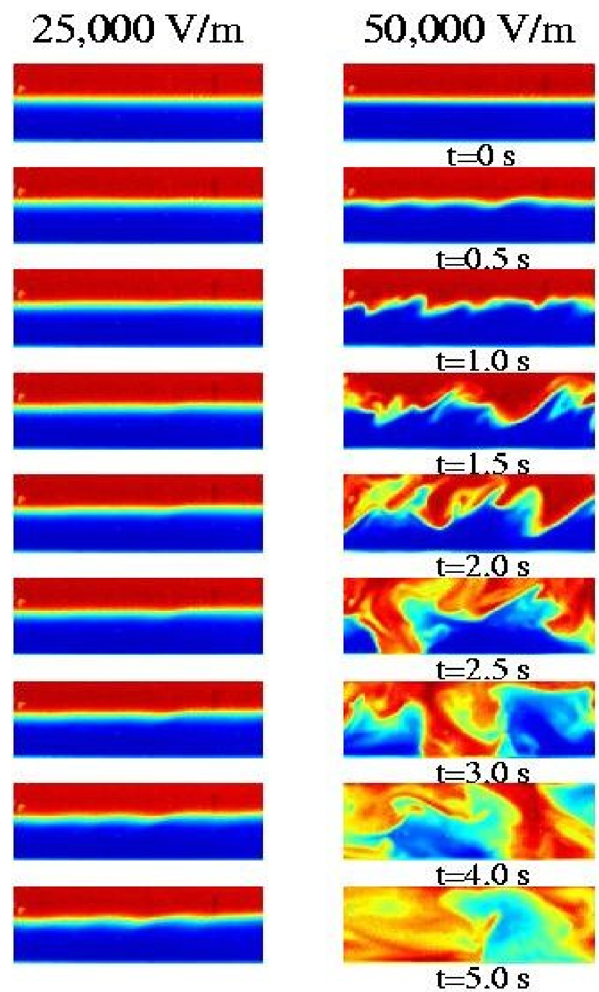
t = 2.0 s

t = 2.5 s

t = 3.0 s

t = 4.0 s

t = 5.0 s

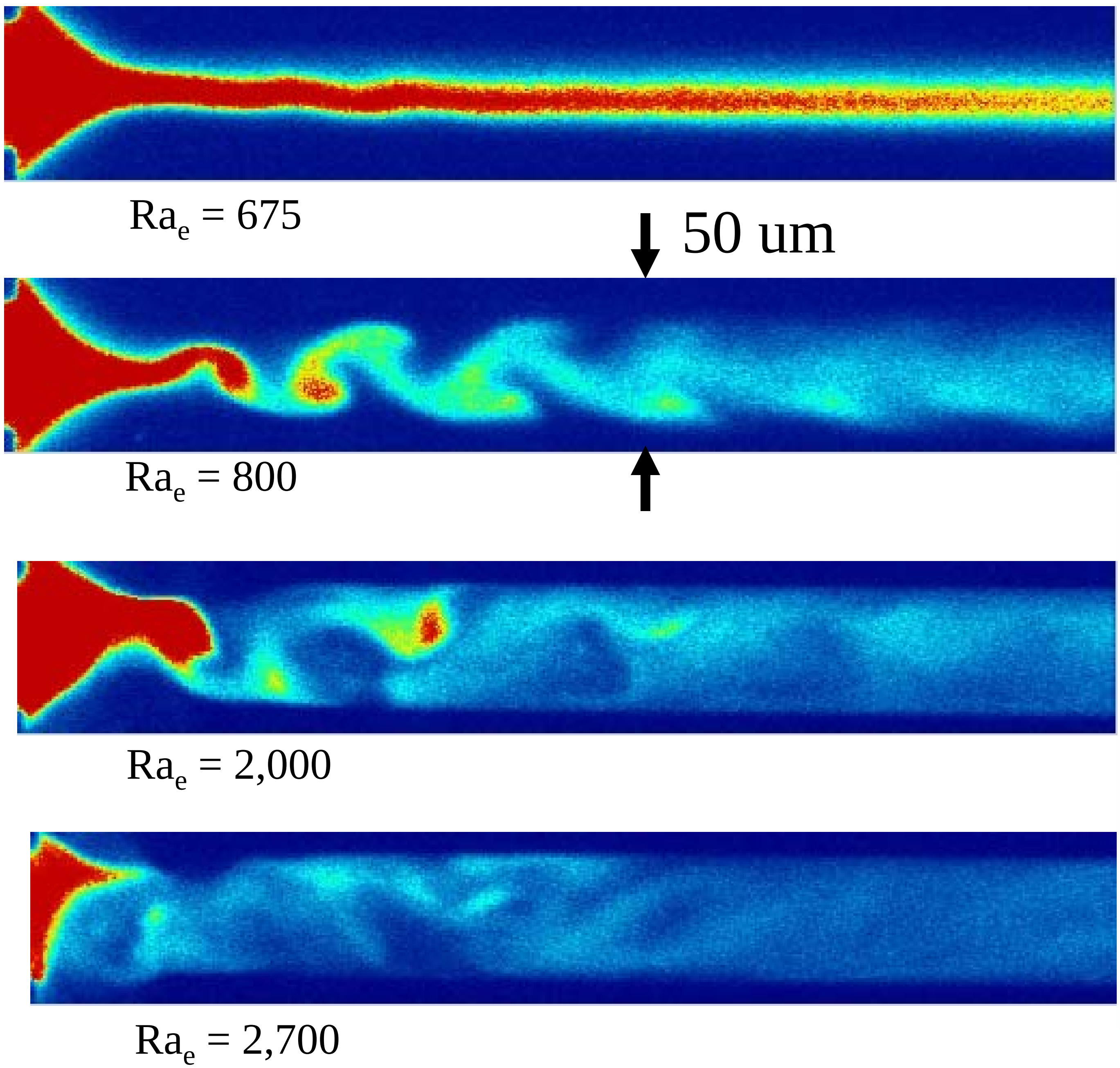


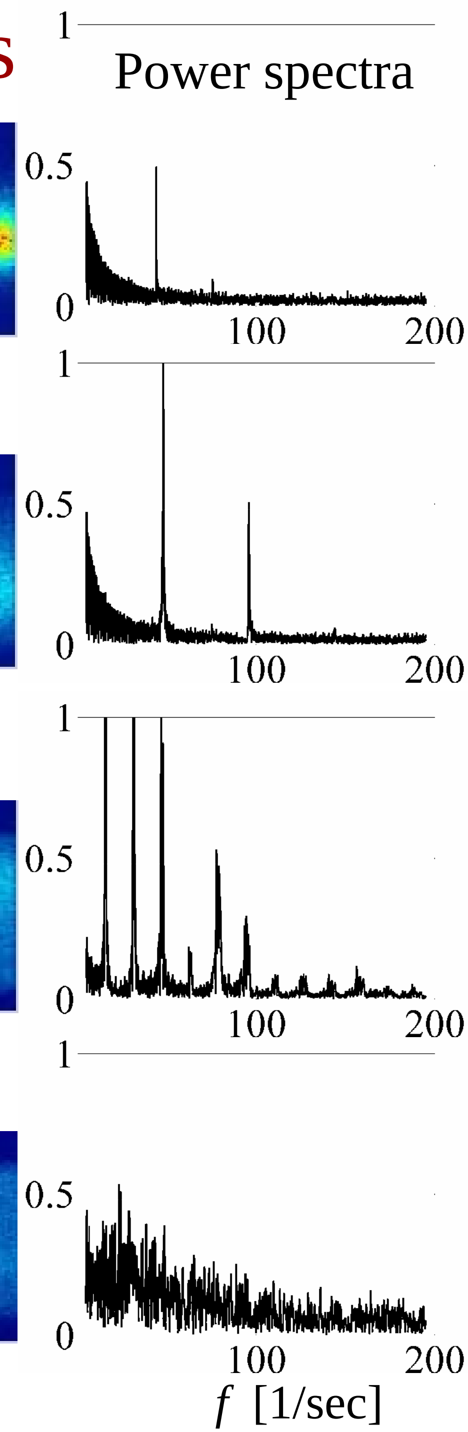
Model

Experiment

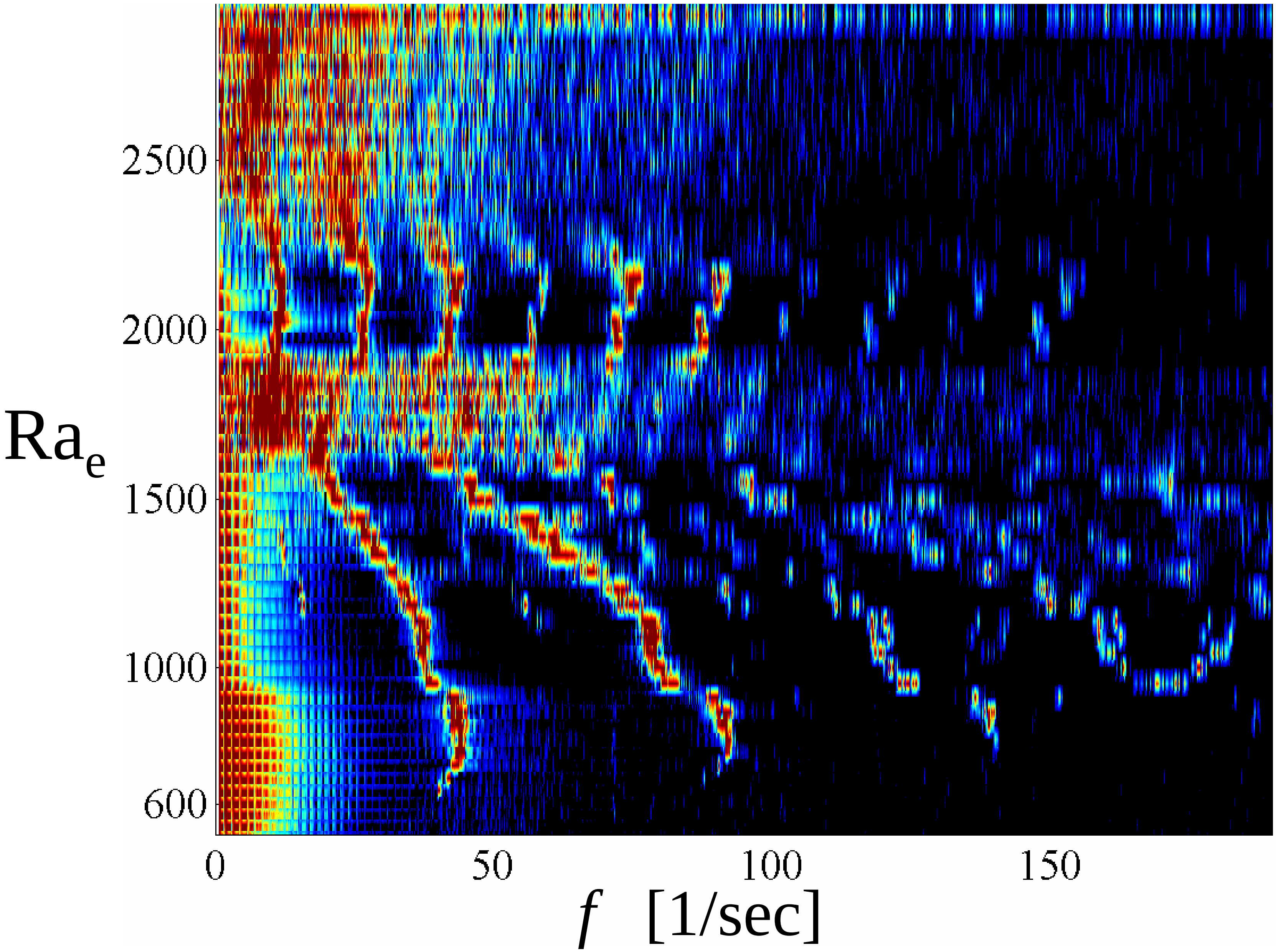
Storey, B.D. et al. *Physics of Fluids*, Vol. 16, No. 6, p.1922-1935, 2004.
Lin et al., submitted to *J. Fluid Mechanics*, 2005.

EKI in a cross intersection: Experiments

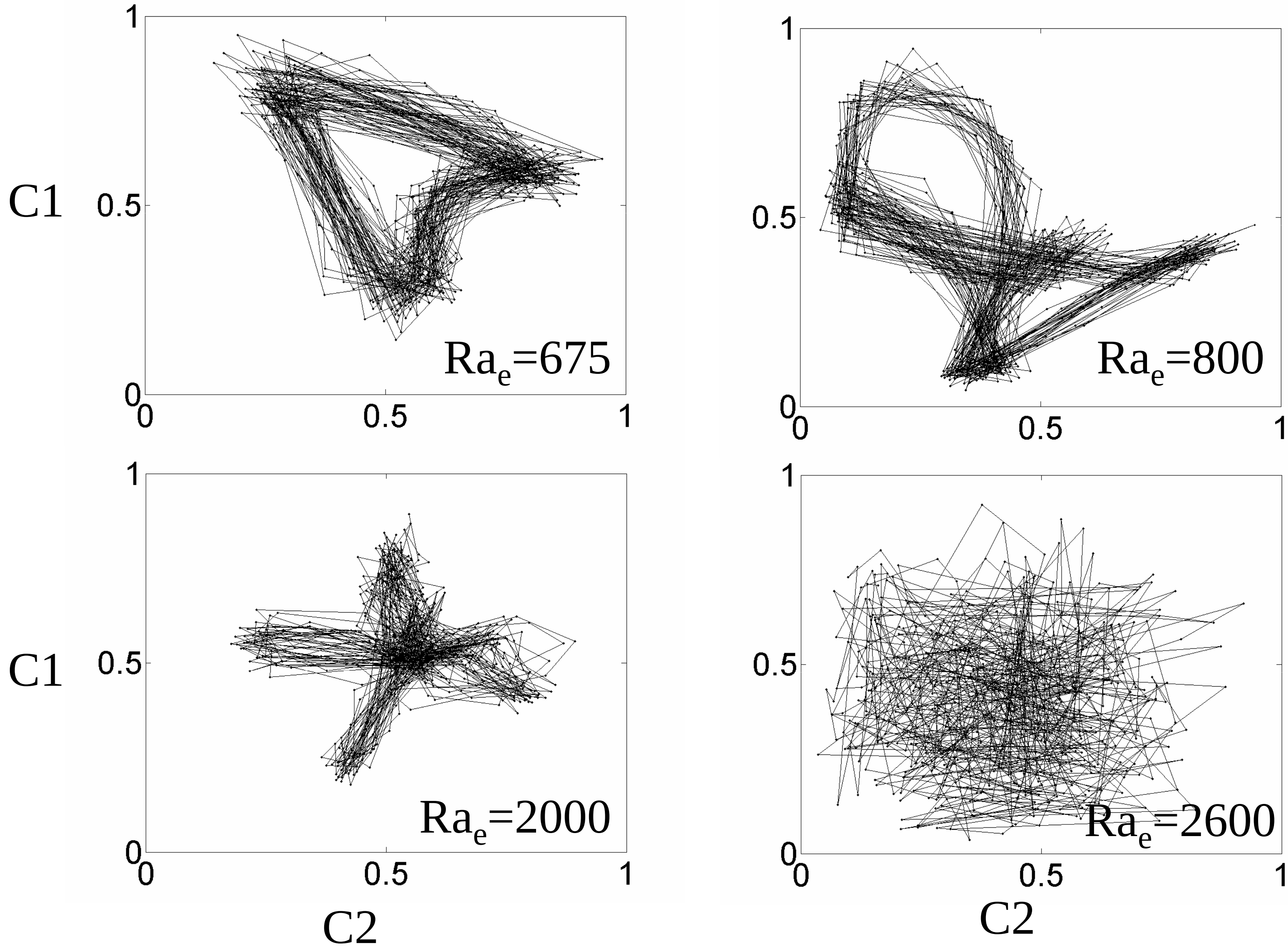




Temporal Power Spectrum



Correlation plots



Isotachophoresis

ITP History

•**Kohlrausch**: KRF function in 1897.

•**Tiselius**: Moving boudnary electrophoresis, 1930

•**Longsworth**: Performed moving boundary electrophoresis in 1939.

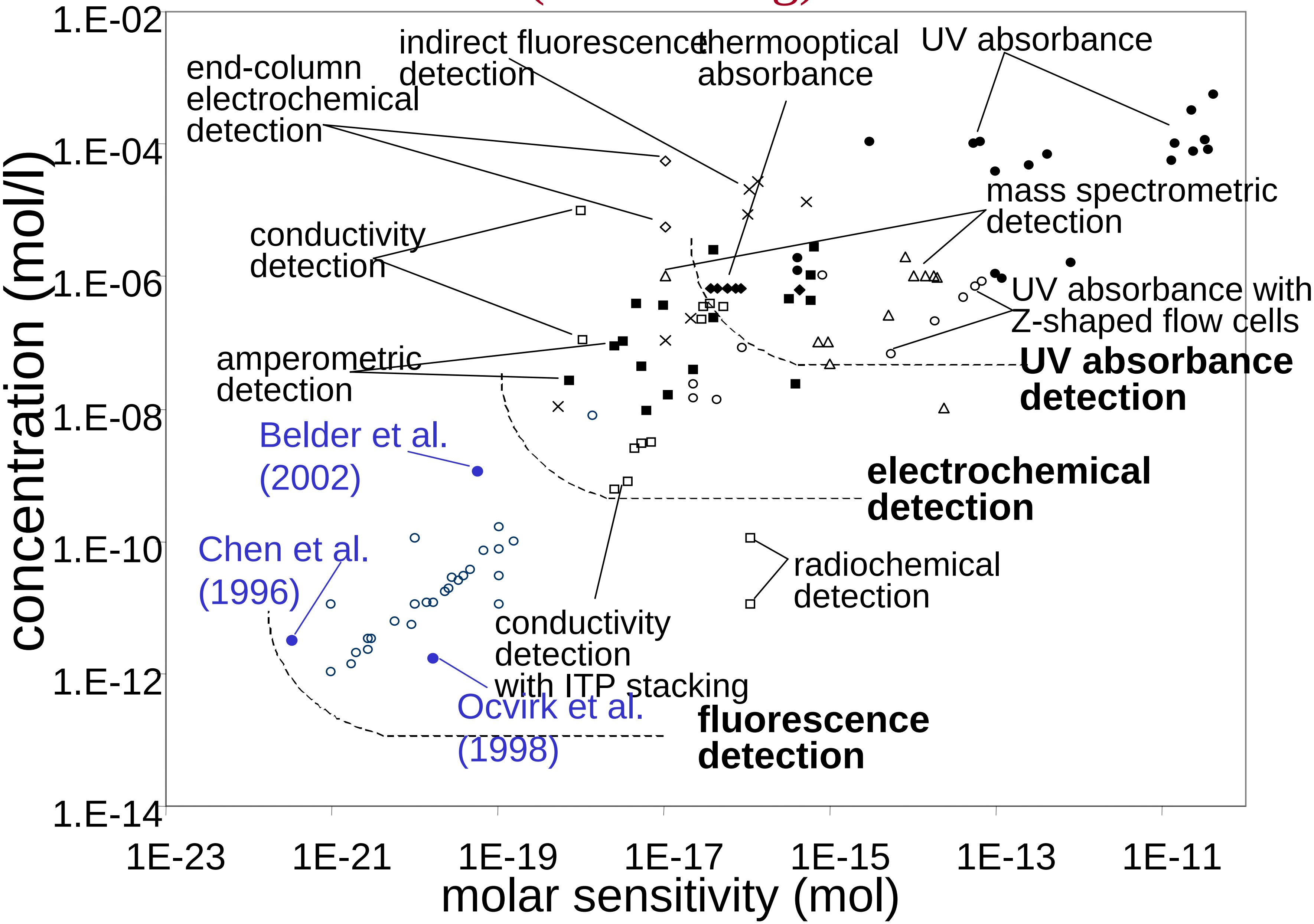
•**Martin AJP:** Displacement electrophoresis (also called ITP) in 1942 for cation.

•**Everaerts and Martin:** First to perform ITP in thin capillaries (200 to 500 micron) in 1963. Used HEC to suppress EOF.

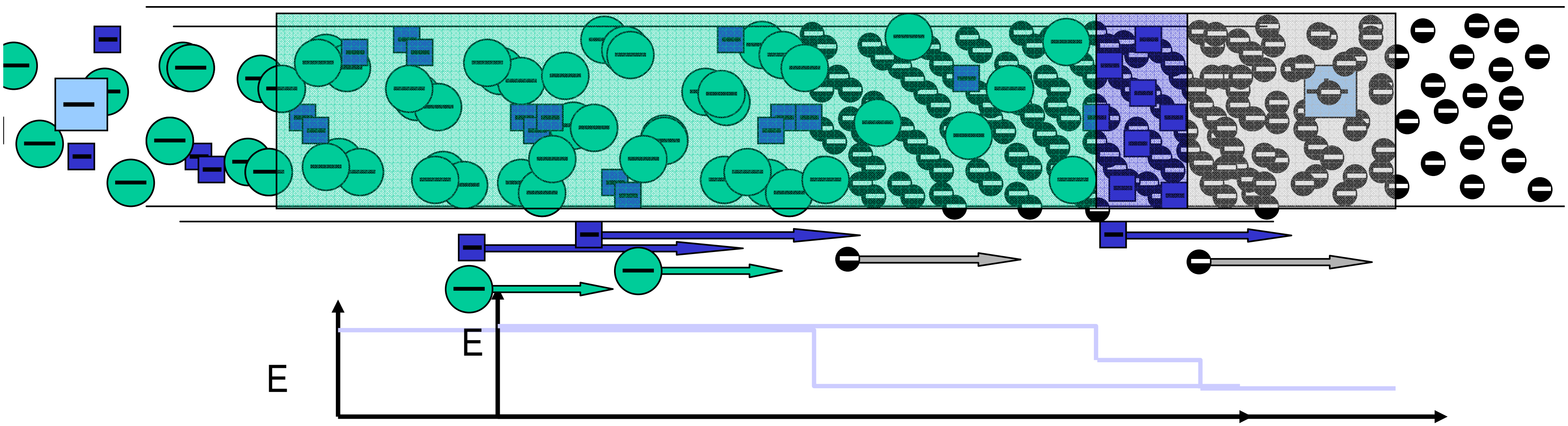
Method	**Detection Mode**	**Microchip/ capillary**	**Sample**	**Electrolyte**	**SE**	**Comments**	**Ref.**
FASS	Fluore-scence	Microchip	didansyl-lysine	Sodium tetraborate (0.5 mM and 500 mM)	13.8	First on-chip FASS Run-time: 20 sec	1995
FASS	Fluore-scence	Microchip	FITC-arginine	Carbonate (200 μM and 32 mM)	65	Six-channel geometry Run-time: 2 min	2001
FASS	Fluore-scence	Microchip	Fluorescein sodium salt	HEPES (0.1 mM and 100 mM), NaCl (0.2 mM and 200 mM)	100	Five-channel geometry. Run-time: 1 min.	2003
FASS	Fluore-scence	Microchip	Fluorescein disodium salt	175 mM Phosphate, DI water	80	Narrow sample channel Run-time: 2 min	2003
ITP	Fluore-scence	Microchip	eTags	LE: 25 mM imidazole, 20 mM HCl, TE: 160 mM imidazole, 40 mM HEPES	530	Run-time: 1-2 min	2002
ITP	Fluore-scence	Microchip	Fluorescein	LE: 250 mM NaCl, TE: 95 mM TAPS, 73 mM TEA	500	Run-time: 2 min	2005
LVSS	UV	Capillary	PTH-aspartic acid, PTH-glutamine acid	100 mM MES and 100 mM histidine, DI water	-	First LVSS Run-time: 6-10 min L [d)] = 65 cm	1992
FASS	UV	Capillary	Dese, Amino	45 mM NaH_2PO_4 and 15 mM Na_2HPO_4, 60% v/v 1-propanol	1000	Run-time: 4-6 min L = 24.6 cm	1996
LVSS	UV	Capillary	Maleic, fumaric acids, bromide, nitrate	1 mM phosphoric acid, 40 mM potassium dihydrogen phosphate	300	Run-time: 5-10 min L = 61 cm	1999
FASI	UV	Capillary	Bromide, nitrate, bromate	75 mM phosphate, DI water	1000	Run-time: 15-17 min L = 25 cm	1999
FASI	UV	Capillary	1-naphthylamine, laudanosine	50 mM phosphoric acid with 20 % acetonitrile, DI water	200	Run-time: 10-13 min L = 64 cm	2000
ITP	UV	Capillary	NXX-066	LE: 10 mM NaOH titrated with H_3PO_4, TE: 6.13 mM THeACl titrated with H_3PO_4	5500	Highest ITP stacking Run-time: 6-10 min L = 53.5 cm	1998

Sensitivity in Capillary Electrophoresis

(no stacking)



Single Interface Isotachophoresis



Order of mobility

ν > ν > ν

Leading Ion (LE)

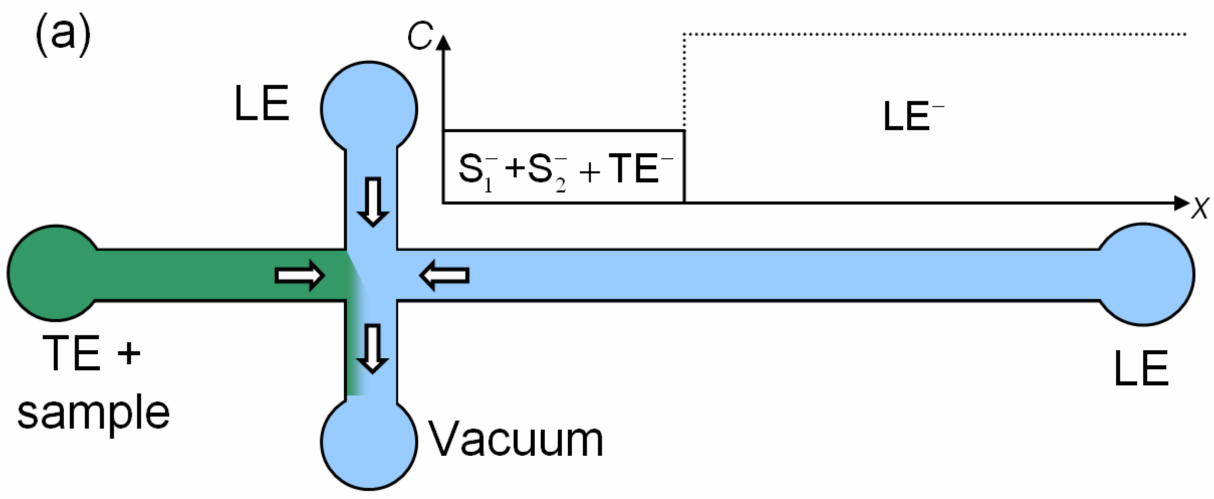
Sample Ion

Trailing Ion (TE)

Counterion not shown

- Characteristics
  - Sample zone grows with time.
  - Stable concentration boundaries
  - Final Sample Concentration depends on Leading ion concentration

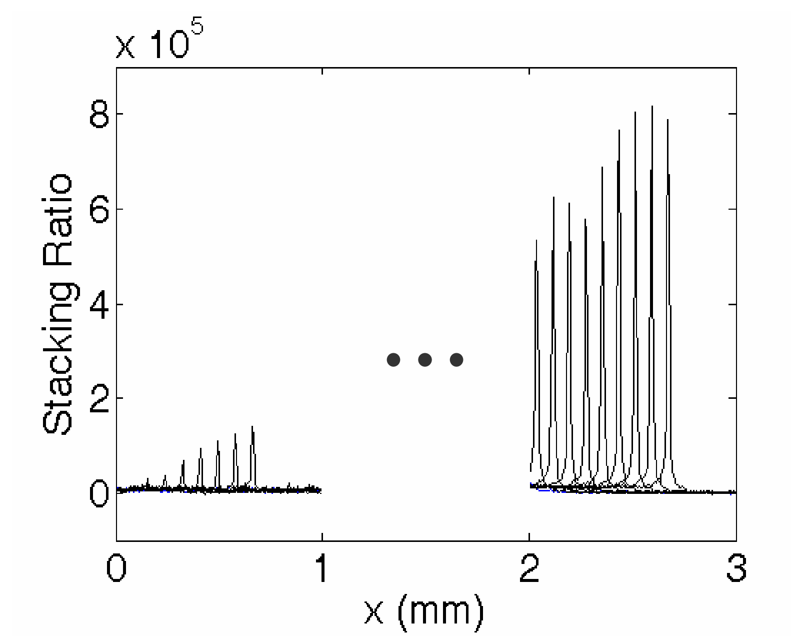
ITP



(S⁻) Alexa fluor (1 µM)
HEPES (5mM)(TE)

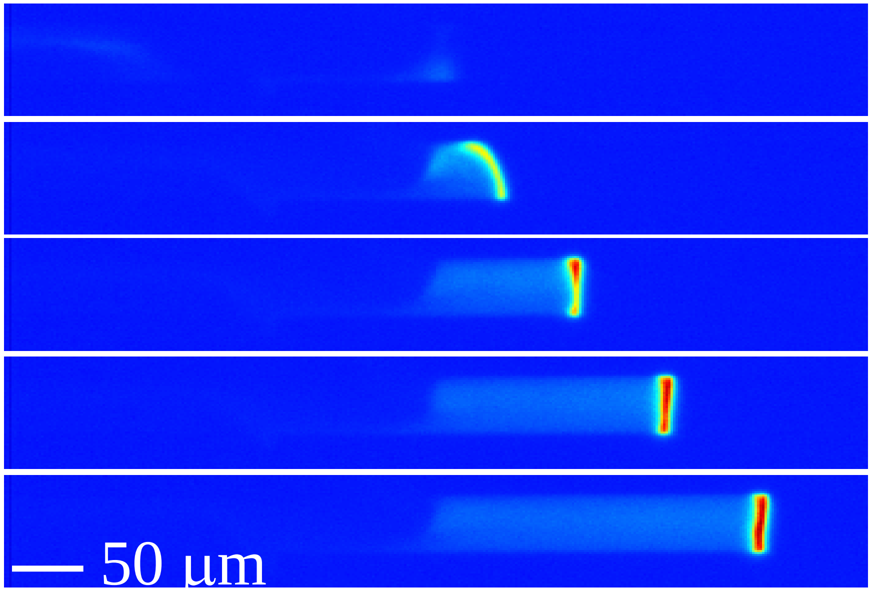
HEPES (5mM)
NaCl (large excess) (LE)

50 µm

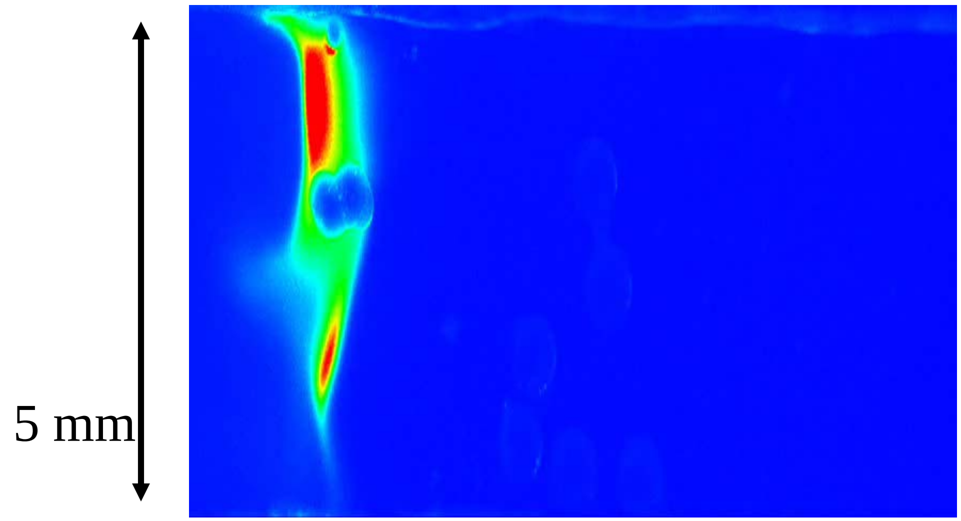


- Concentration enhancement greater than γ
- Buffer selections allow for both ITP and FASS
- ITP-type stacking with CE separation

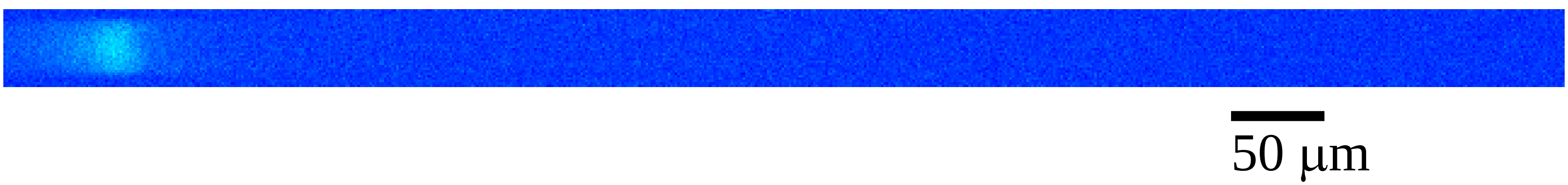
Stable over 1000+ diameters



Stable across flow geometries



Stability under large disturbances

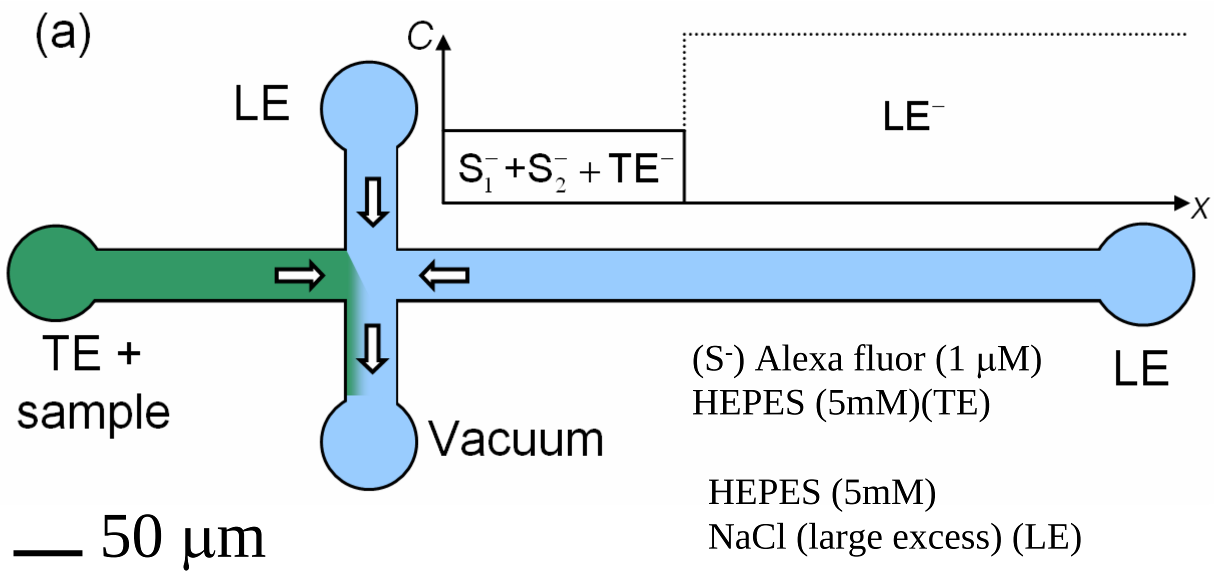


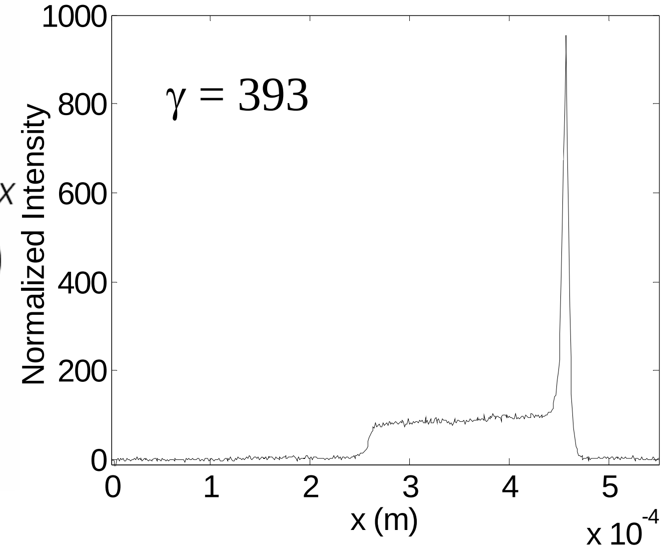
ITP Optimization

- Surface Chemistry: Suppressed EOF to minimize advective dispersions.
- High gradients: High LE concentration and very low initial sample concentration (100 fM vs. 1 M or 10^{15} ratio)
- Flow control: Injection protocol has
  - High TE concentration (maximizes local electric Peclet number)
  - Requires no manual buffer exchange step (fast ITP to CZE transition w/ minimal dispersion)
  - Large effective sample width
  - LE introduced within TE region, not end of capillary (this reduces time to overtake ITP zone)
- Achieved 1E6 stacking in < 2 minutes

Jung, B., Bharadwaj, R., Santiago, J.G., in press, *Analytical Chemistry*, 2005.

ITP and CE

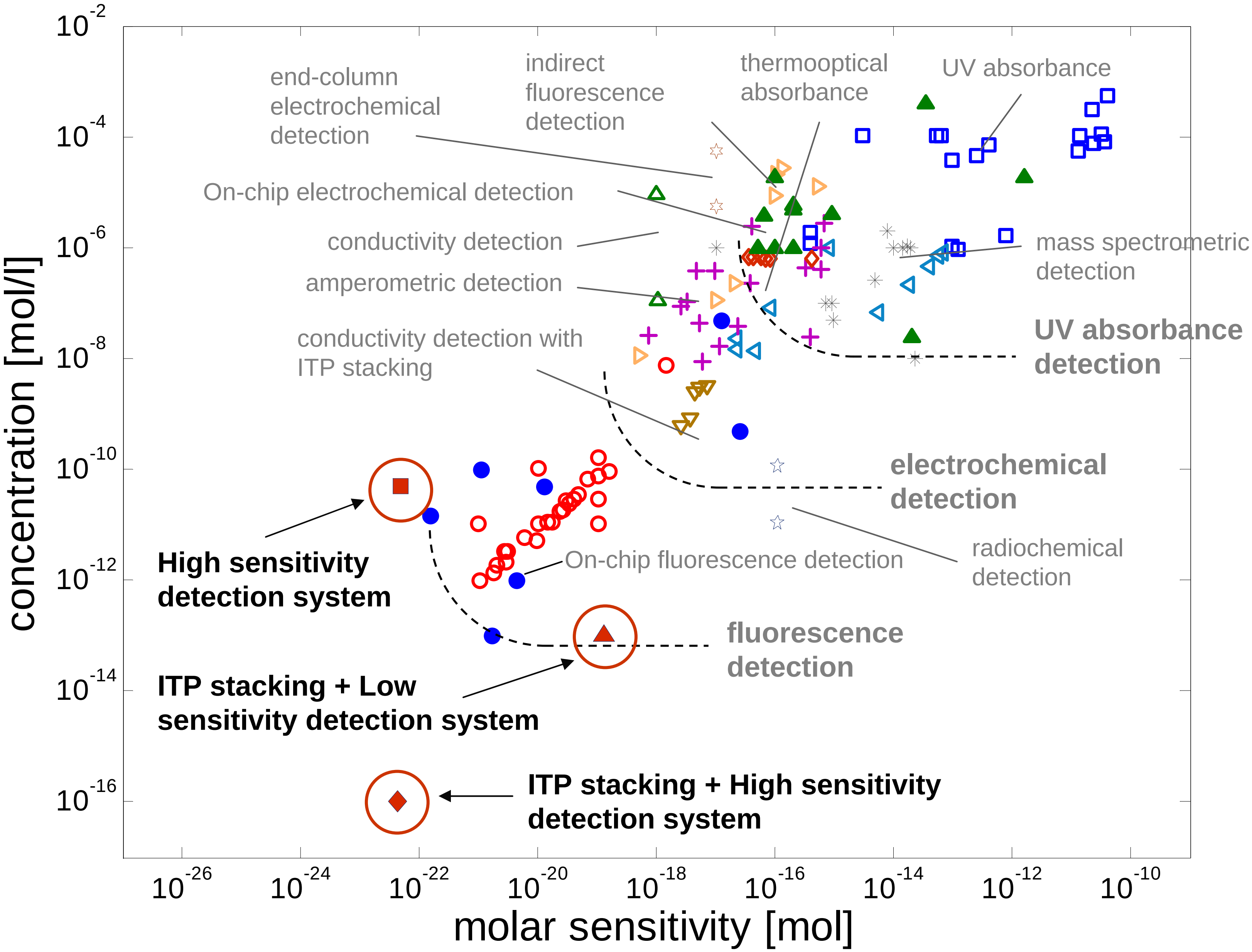




Demonstrated >1e6-fold stacking on chip

Stacking achieved in less than 120 sec and < 1 cm

CE Sensitivity revisited

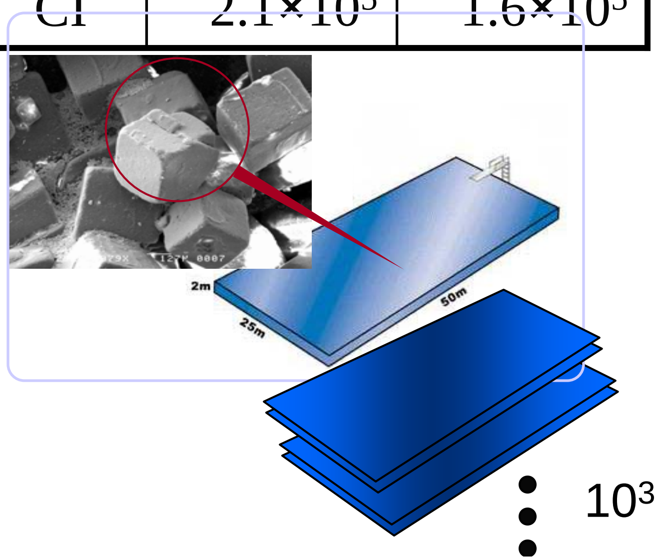


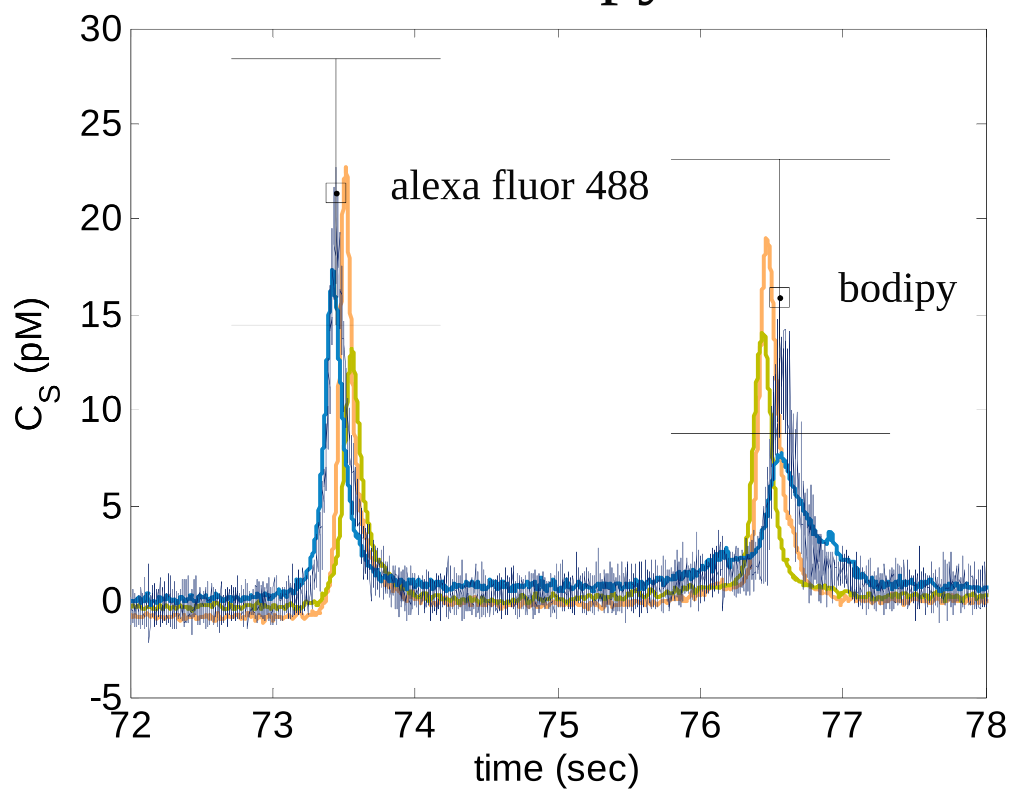
ITP/CE: LIF high sensitivity detection

- ITP stacking + High sensitivity CE detection system
  - Optimized LIF w/ 60x objective (NA = 0.9) objective and PMT
  - Brief ITP stacking (~40s) followed by CE mode

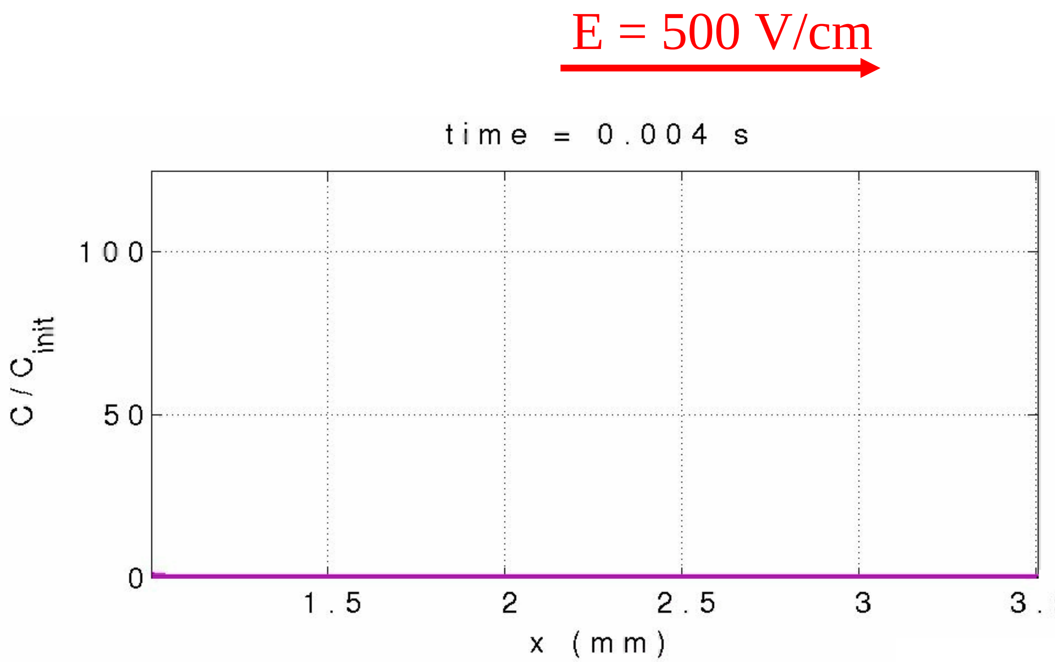
exa fluor 488 and bodipy

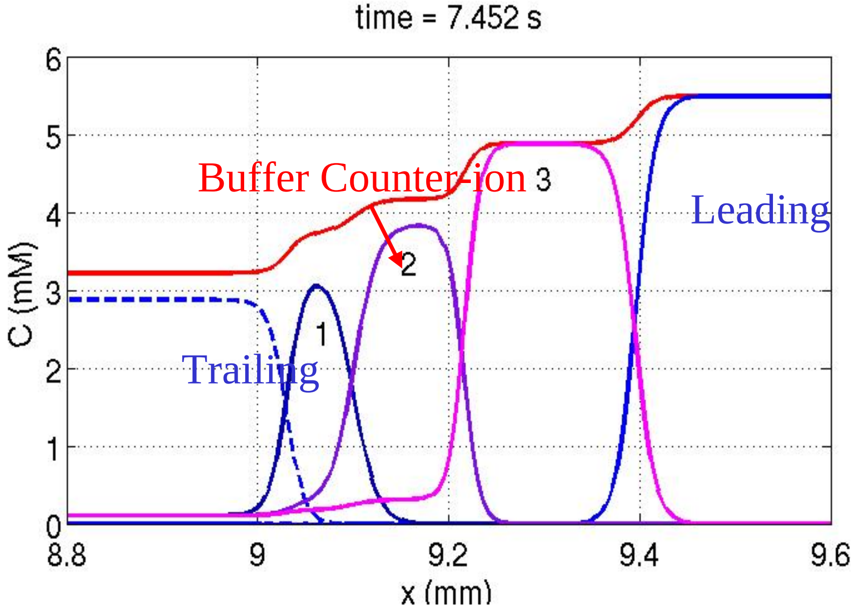
	alexa fluor	bodipy
$C_{S,initial}$	100 aM	100 aM
$C_{S,final}$	21.4 pM	15.9 pM
CI	2.1×10^5	1.6×10^5





ITP Simulations



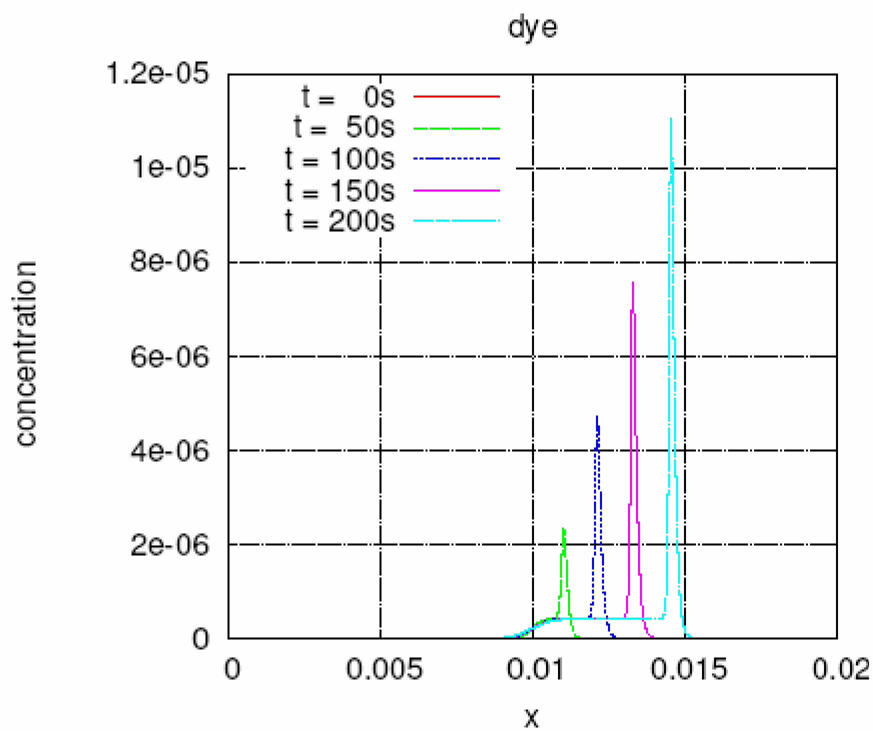


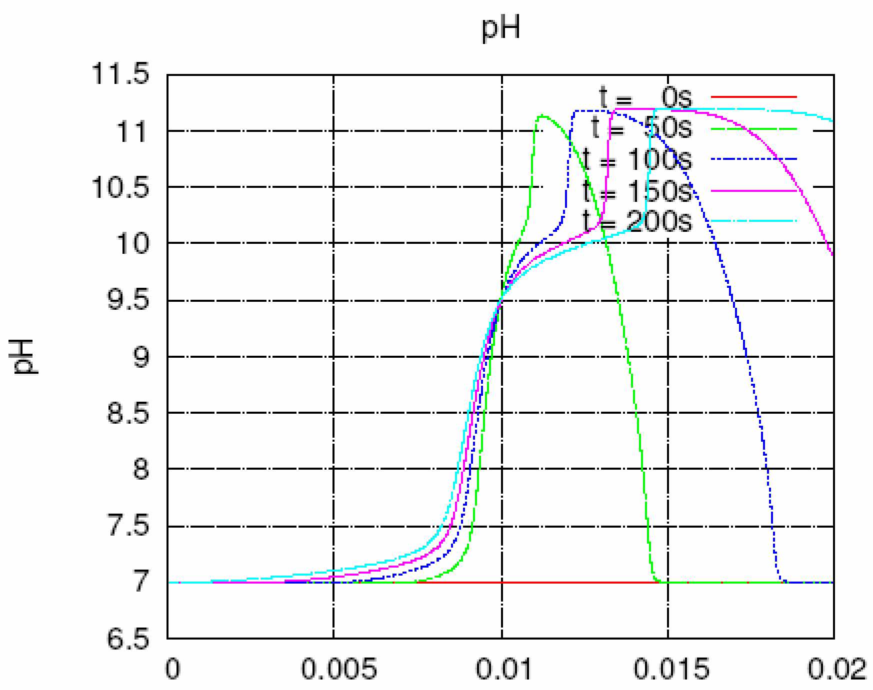
Collaboration with
Bijan Mohammadhi,
Montpellier University, France

Lin, Storey, Santiago, submitted to *Journal of Fluid Mechanics*, 2006.
Baldessari et al., under preparation, 2006

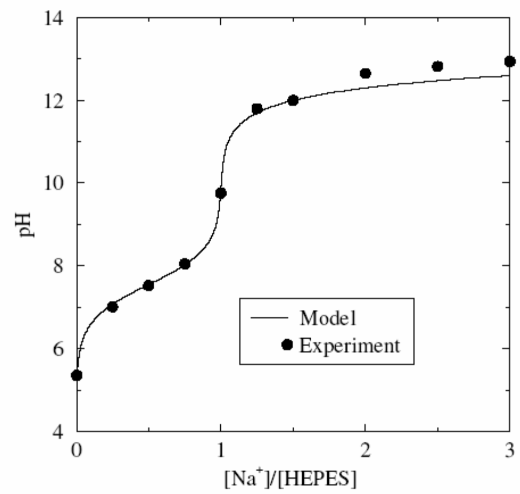
ITP Simulations, cont.

Modeling by H. Najm, B. Debussere of Sandia Natl. Labs





- Multi-species transport coupled with full Poisson equation formula
- Detailed buffer electrolyte reactions as wsell as ampholytic sample
- Electrostatic body force term in Navier-Stokes equations
- Electroosmotic flow boundary conditions on the channel walls



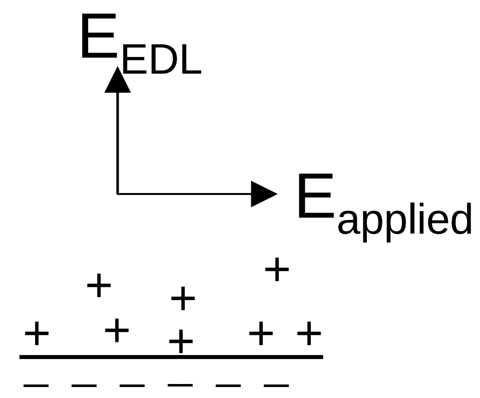
Nanochannel electrophoresis

Prior Work

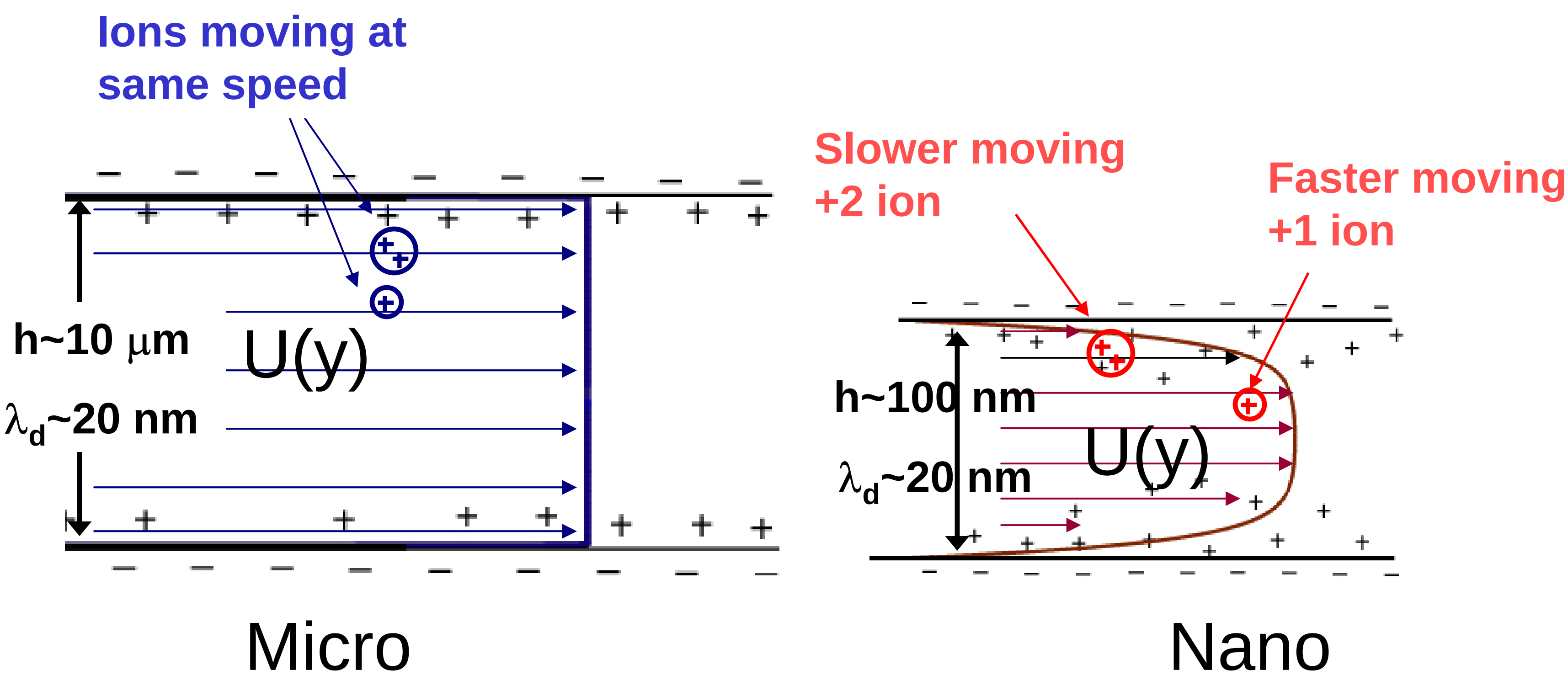
Theoretical	Experimental
Analytical studies of flow Burgeen/Nakache, Rice/Whitehead	**Conductance measurements** Stein/Kruithof/Dekker
Transport and dispersion of neutral species Griffiths/Nilson, Dutta/Kotamurthi	**Ion depletion** Pu/Yun/Datta/Gangopadhyay/ Temkin/Liu
Molecular dynamics simulations Qiao/Aluru	**Separation of DNA** Petersen/Alarie/Jacobson/ Ramsey

No work on charged species transport	**No work separations or ion dispersion dynamics**

Nanoscale Electrokinetics for small ions



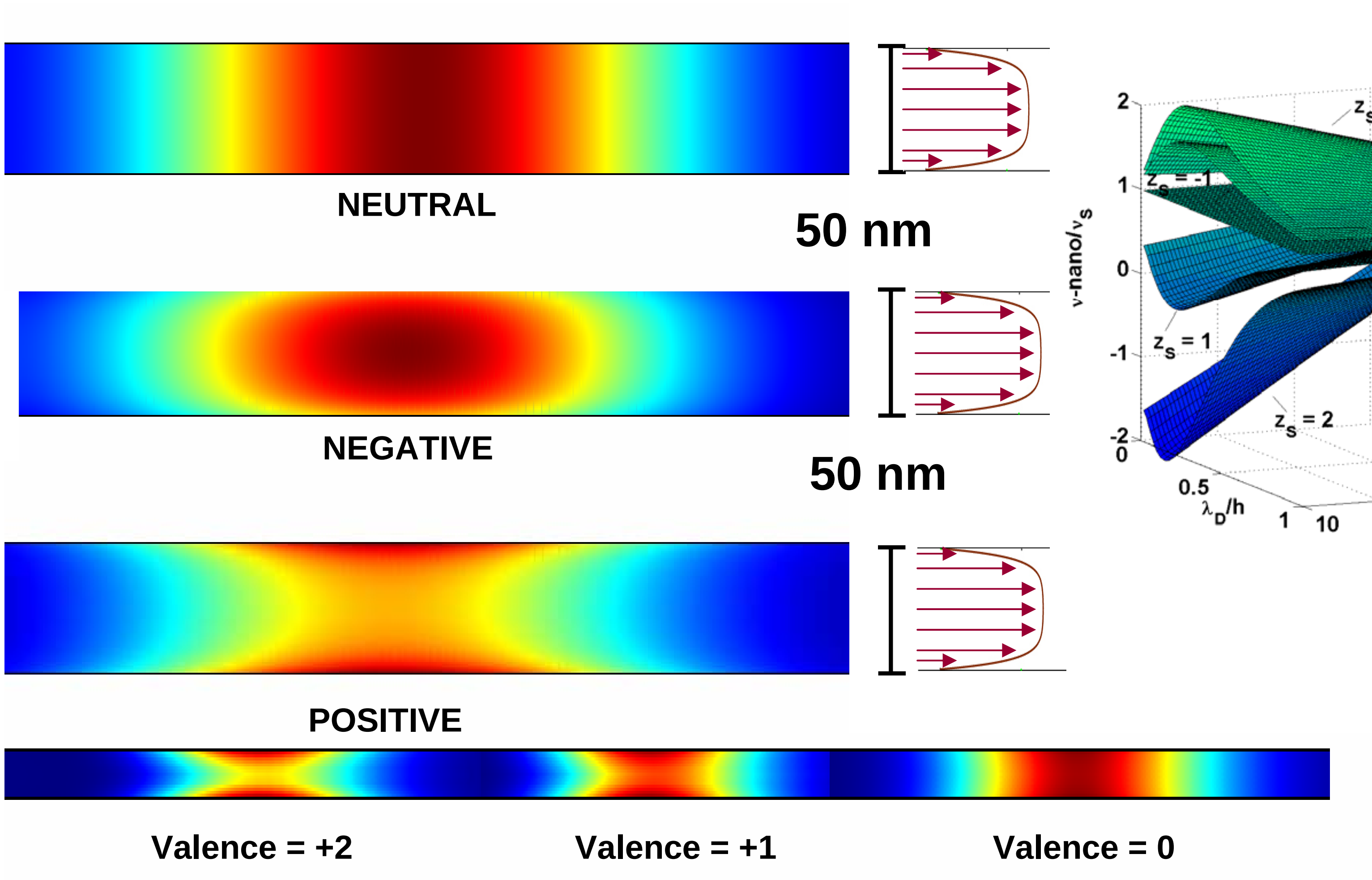
- Nanofluidics → Thick EDL
  - ➢ Enables new functionality
  - ➢ Method to determine both valence and mobility

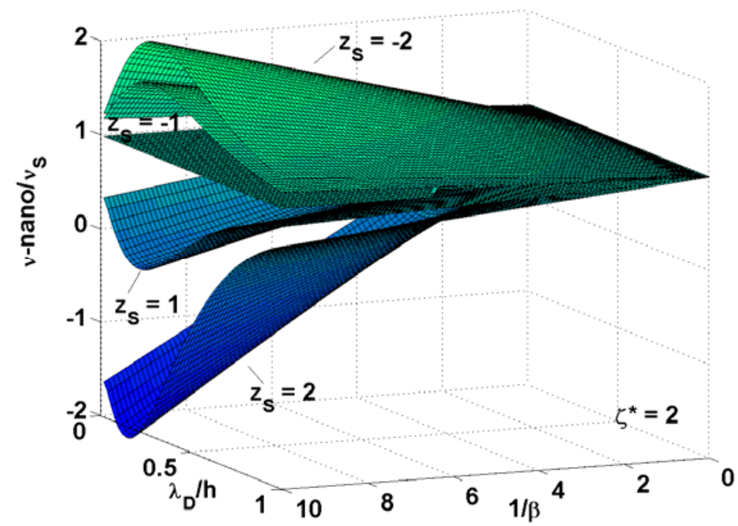


Concentration Profiles

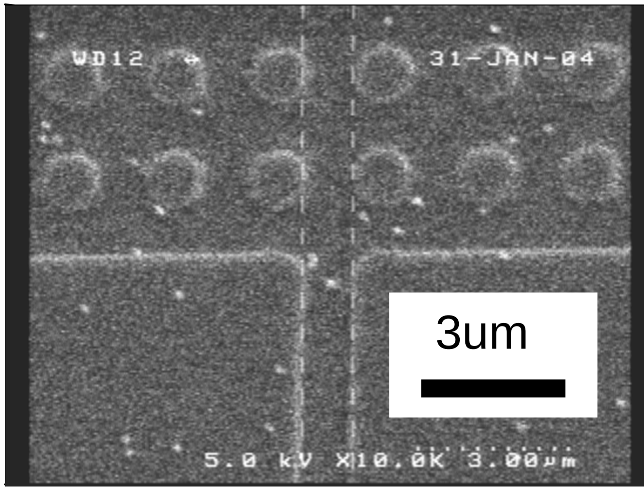
Species is in quasi-steady transverse equilibrium

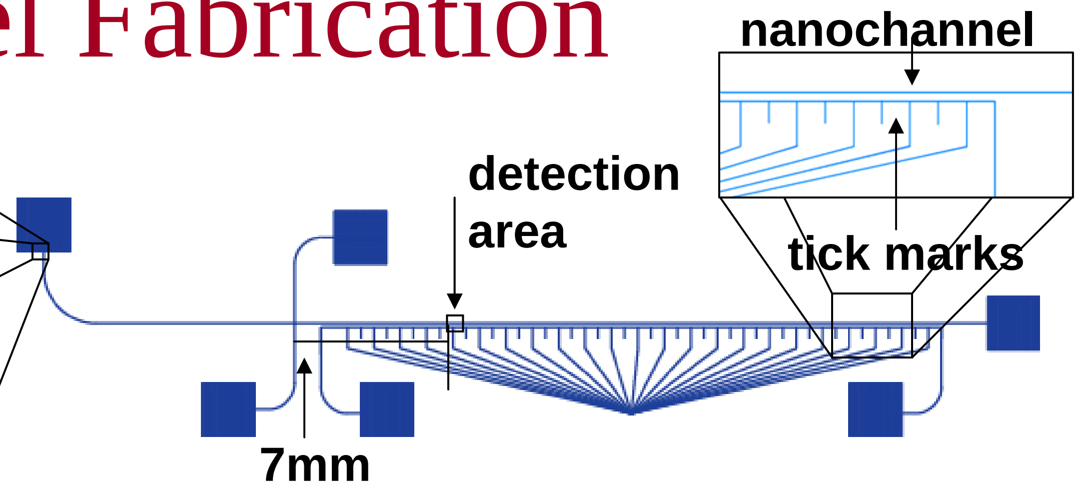
$$\langle u_{charged} \rangle = v_S z_S F E_x - \frac{\varepsilon E_x \zeta}{\mu G} \left\langle \exp\left(\frac{-z_S e(\psi(y) - \psi_c)}{kT} \right) \left(1 - \frac{\psi(y)}{\zeta} \right) \right\rangle$$

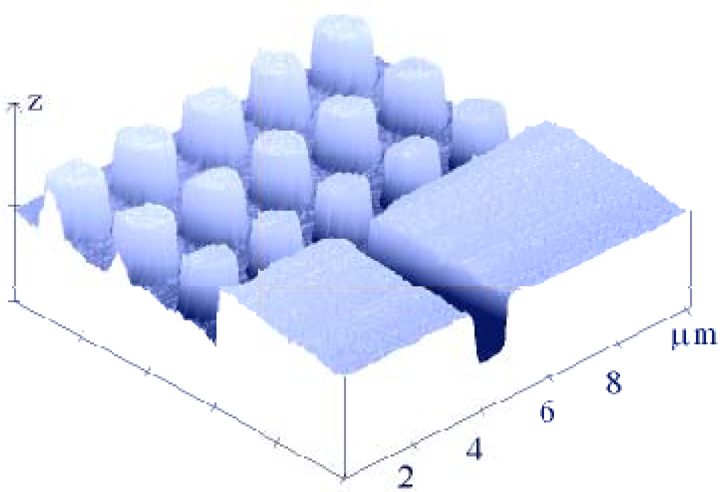




Nanochannel Fabrication

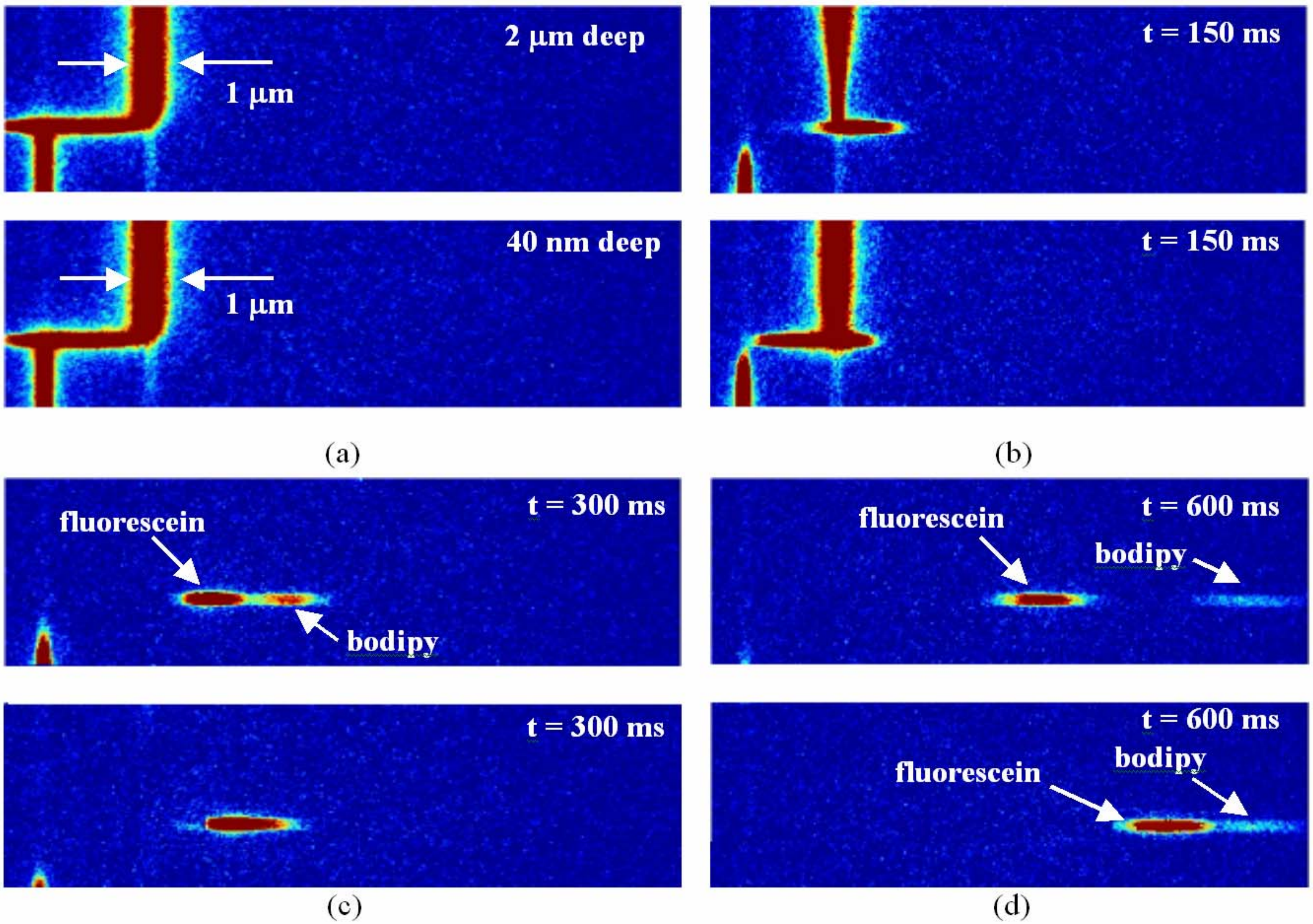




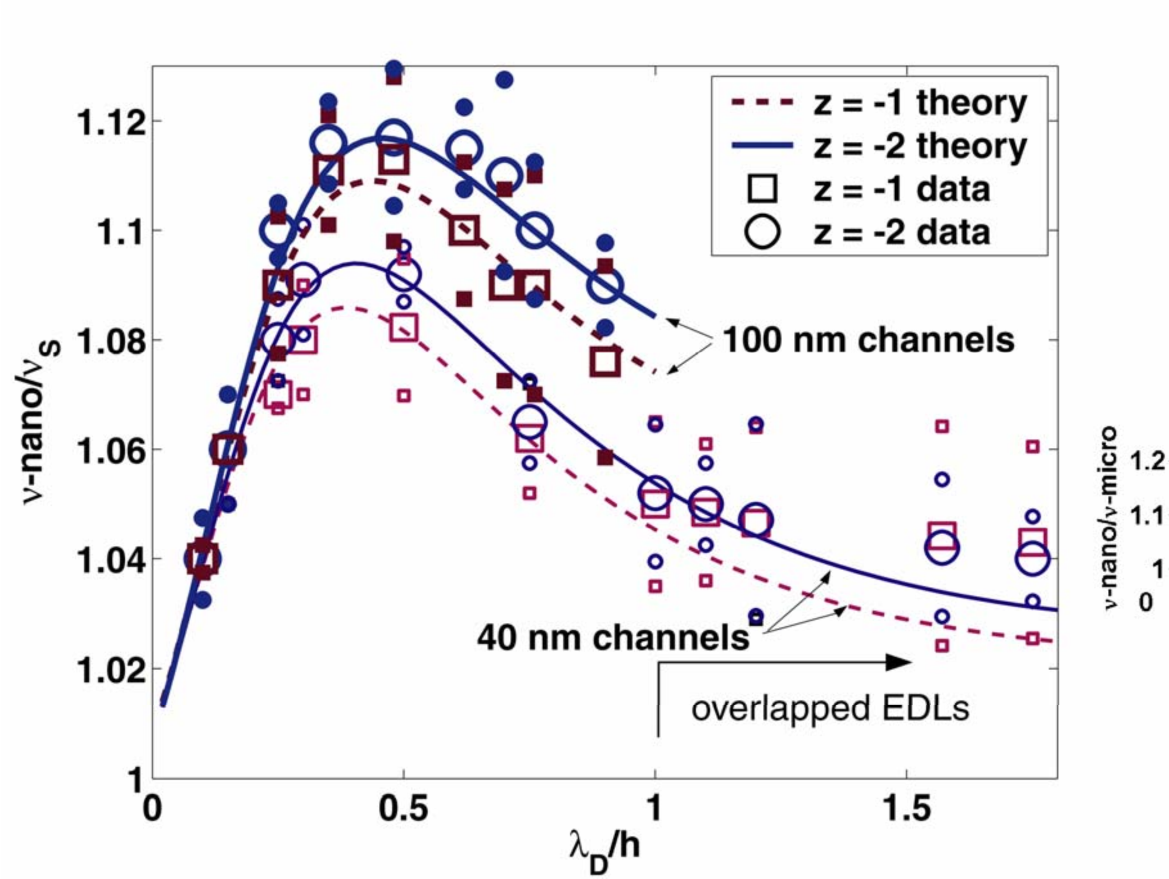


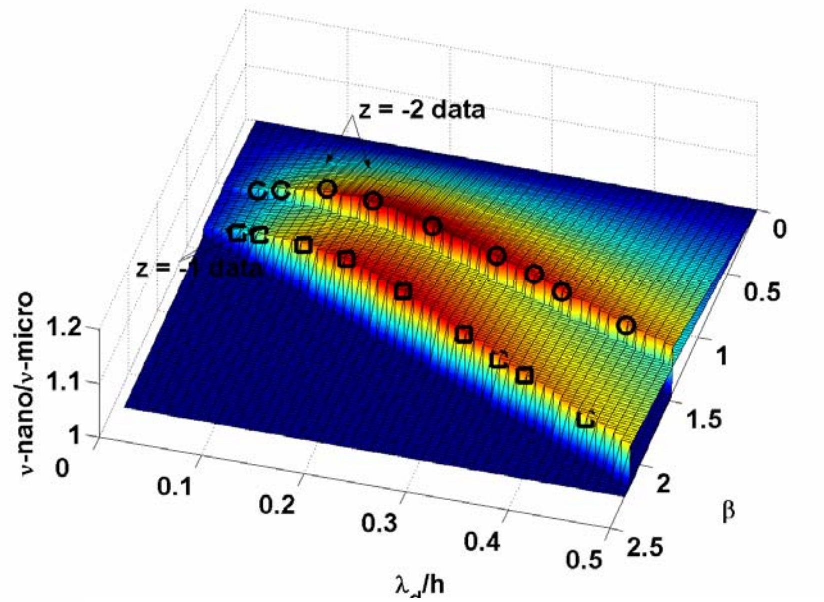
- Techniques:
  - E-beam Lith < 500 nm
  - Photolith > 500 nm
  - Dry Plasma Etching
  - Fusion Bonding

Species Transport



Experimental Results



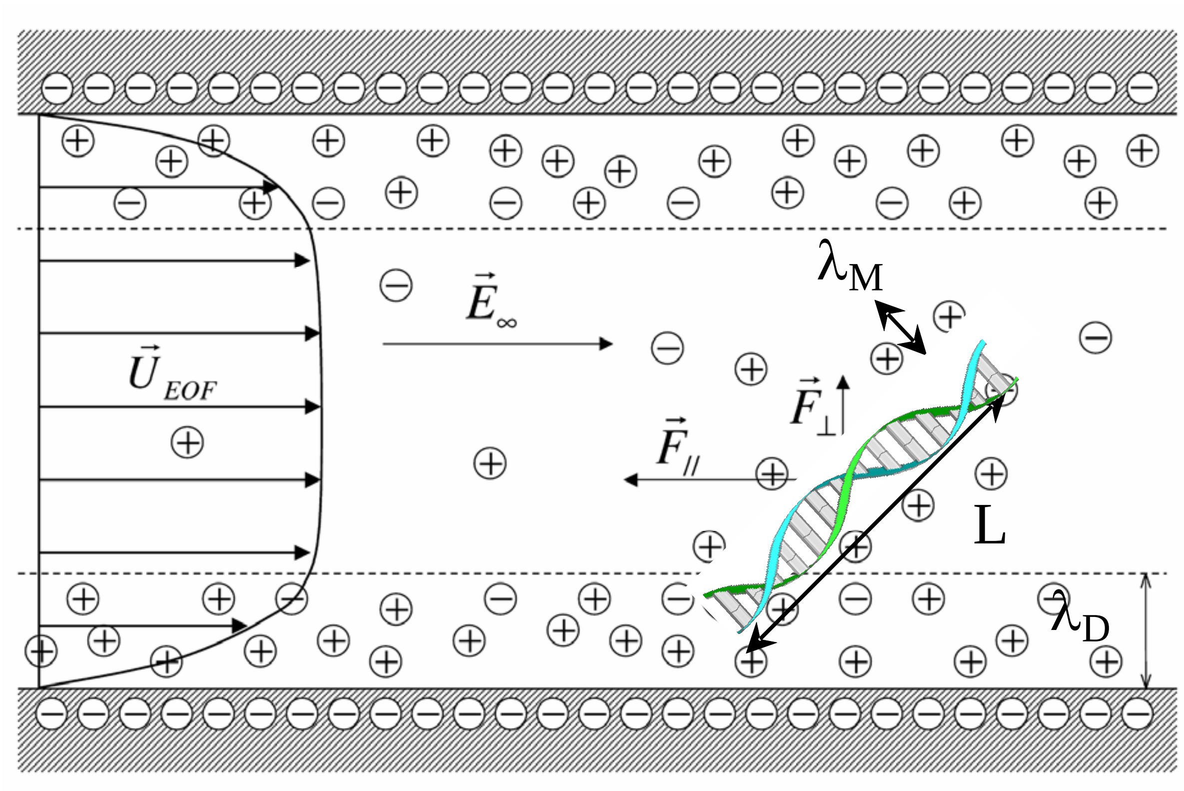


Pennathur and Santiago. "Electrokinetic transport in nanochannels: 1. Theory," Vol. 77, No. 21, *Analytical Chemistry*, pp. 6772-6781, 2005.
Pennathur and Santiago "Electrokinetic transport in nanochannels: 2. Experiments," Vol. 77, No. 21, *Analytical Chemistry*, pp. 6782-6789, 2005.

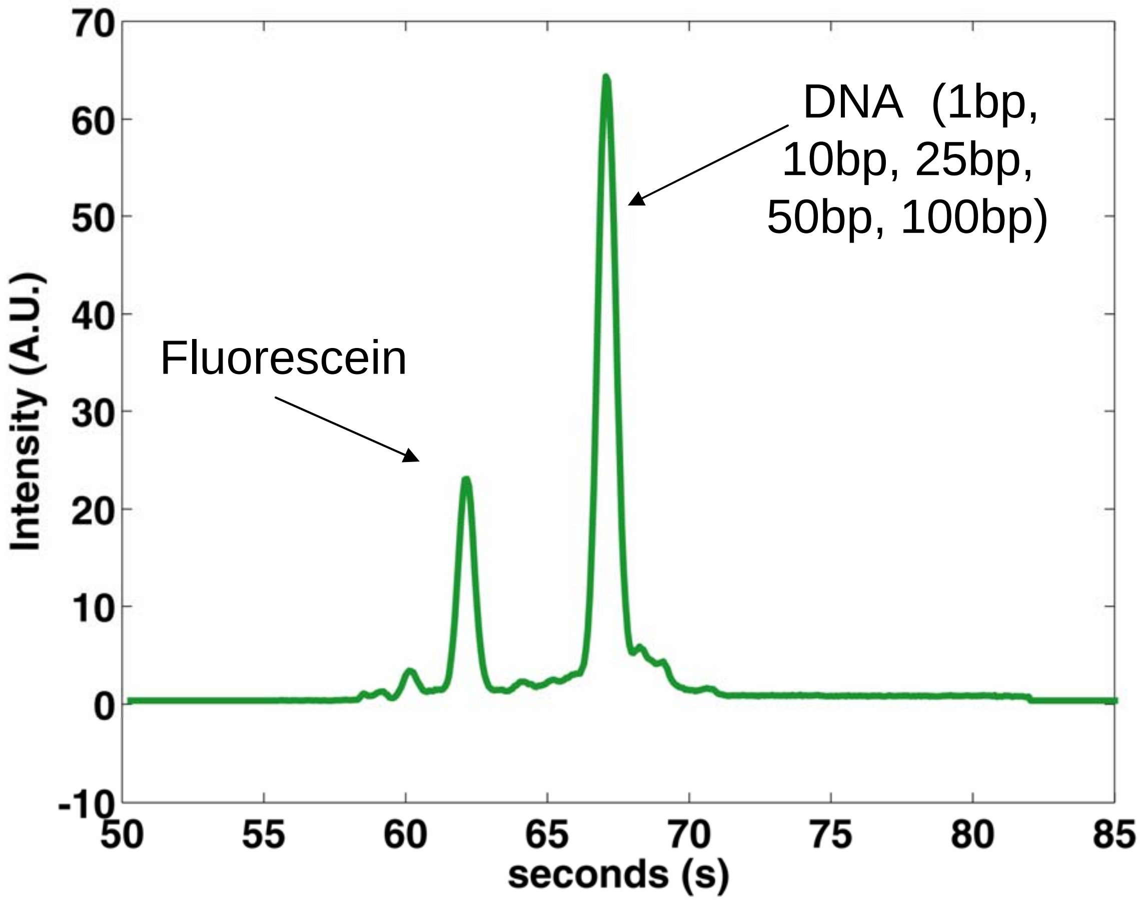
Oligonucleotide Separation

- Length scales on same order
  - Nanochannel height
  - EDL thickness
  - DNA length

3 Length Scales:



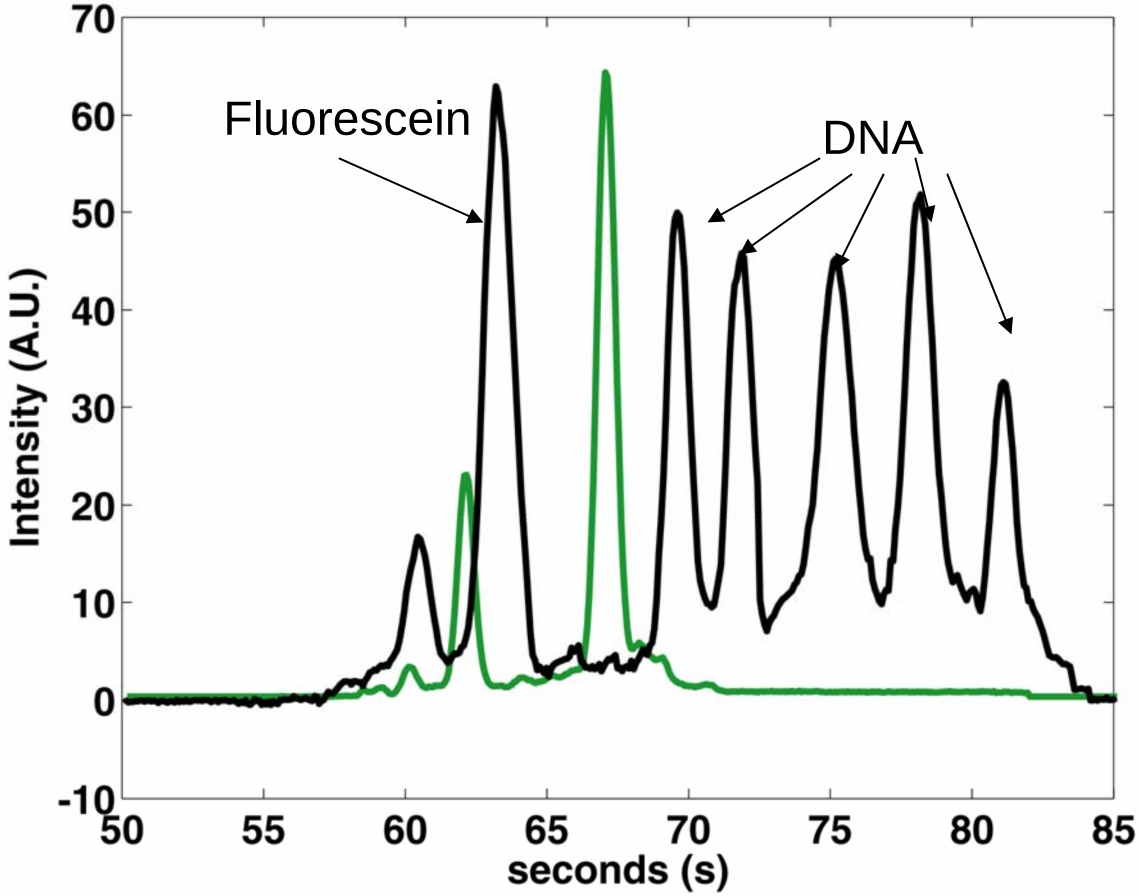
Microchannel DNA Separation



Conditions:

- 10 mM Borate Buffer
- 1 nm EDL thickness
- DNA lengths: 1 - 34 nm
- 25 mm separation length

Nanochannel DNA Separation

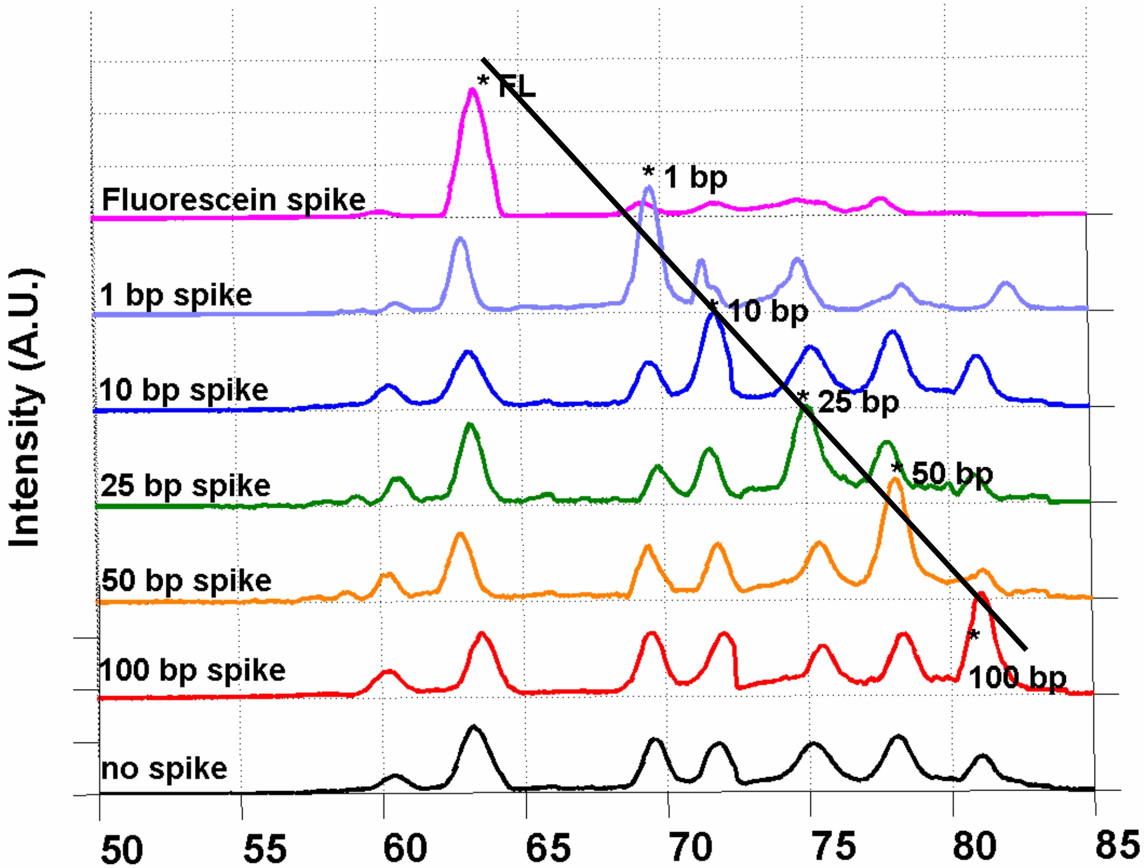


Conditions:

- 10 mM Borate Buffer
- 1 nm EDL thickness
- DNA lengths: 1 - 34 nm
- 25 mm separation length

Nanochannel DNA Separation

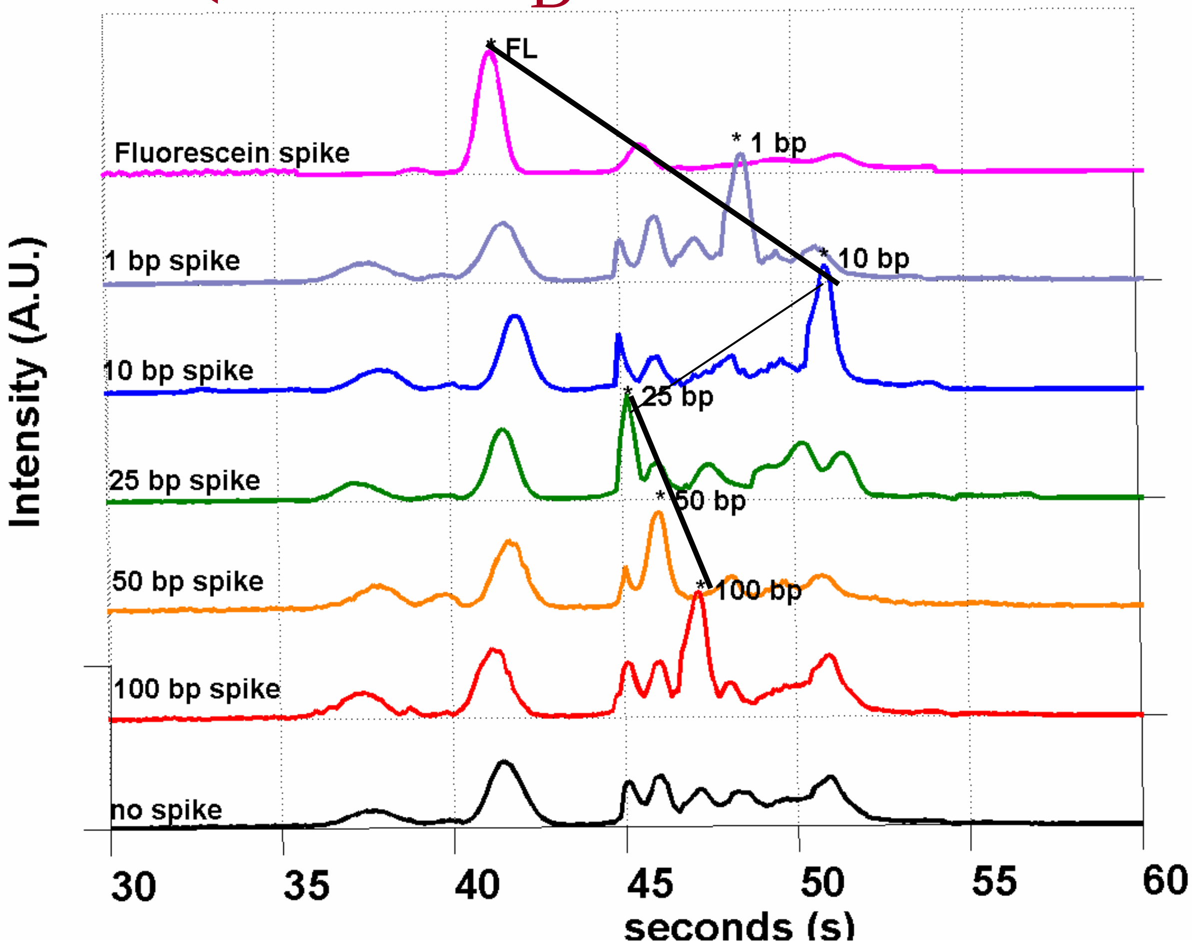
(10 mM λ_D= 3 nm in 100 nm channel)



Experiments:

- 5 buffer concentrations
- 6 bp lengths
- 3+ realizations per exp.
- ~135 exp.

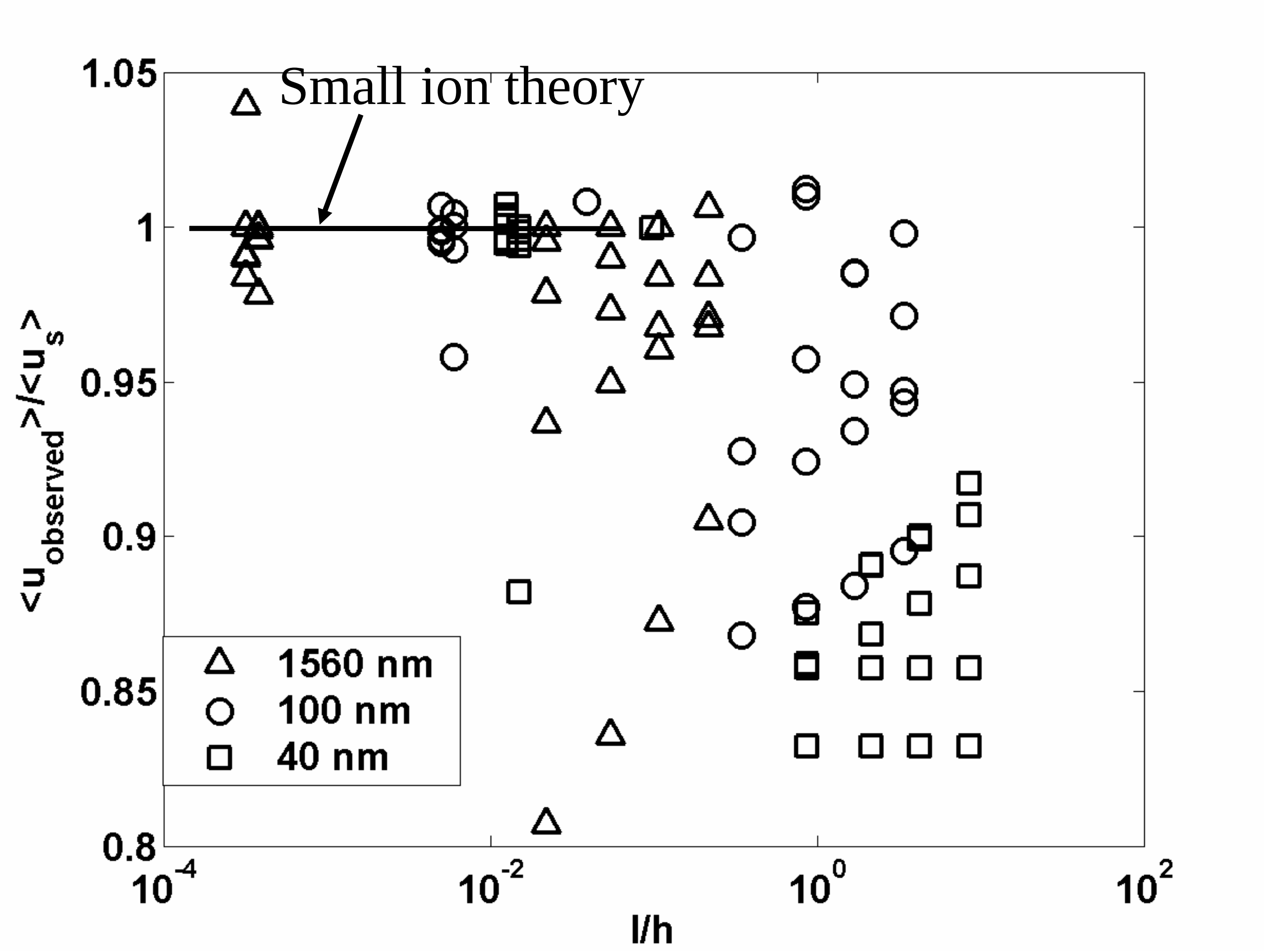
Nanochannel DNA Separation
(1 mM λ_D= 10 nm in 100 nm channel)



Conditions:

- 1 mM Borate Buffer
- 10 nm EDL thickness
- DNA lengths: 1 - 34 nm
- 25 mm separation length

DNA separation in nanochannels

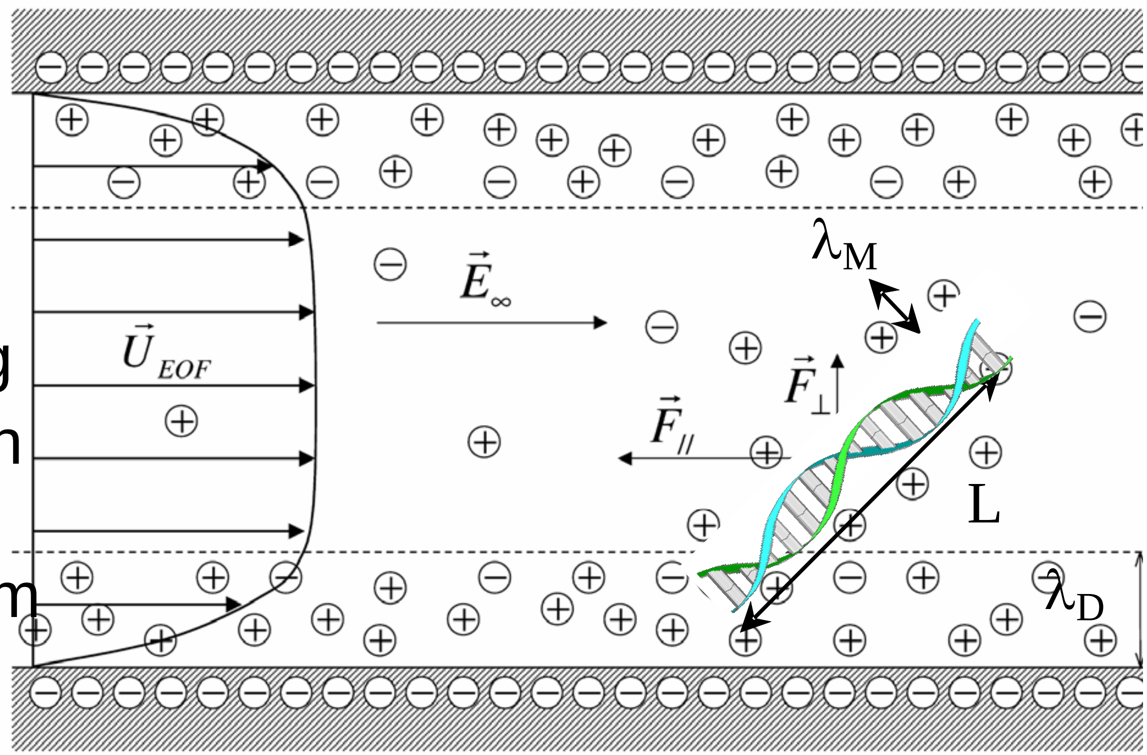


Oligonucleotide Separation

- Length scales on same order
  - Nanochannel height
  - EDL thickness
  - DNA length

3 Length Scales:

- Interaction between
  - Transverse electromigration
  - Non-uniform velocity field/diffusion coupling
  - Steric interactions with wall
  - Polarization @10^5V/cm



Near-Future Work

- 3D electrokinetic instability modeling
- ITP modeling
  - Analytical models for shock width and concentration increase
  - Numerical models including
    - N species
    - Reaction kinetics of buffer
    - pH gradients
- DNA separation in nanochannels
  - Single bp resolution DNA separation in nanochannels
    - Roughly 1/3rd of human genome costs was reagents
    - Ultra high sensitivity
  - Molecular dynamics modeling (w/ Eric Shaqfeh)
- Combine ITP and nanochannel electrophoresis

Acknowledgements

Microfluidics Lab Members:

- EK instabilities and micromixing
  - Dr. Michael Oddy*
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  - Dr. Jonathan Posner *
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  - Rajiv Bharadwaj *
  - Byoungsok Jung
  - David Huber
  - Alexandre Persat
- Particle tracking & control
  - Klint Rose
  - (Shankar Devasenathipathy) *
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  - Dr. Fabio Baldessari
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