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Injection Molded Microfluidic Devices for Homeland Security Applications

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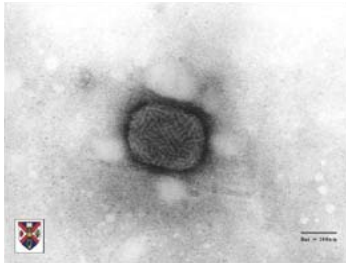
Diversity of Bioagents Requires Flexible Biodetection Scheme

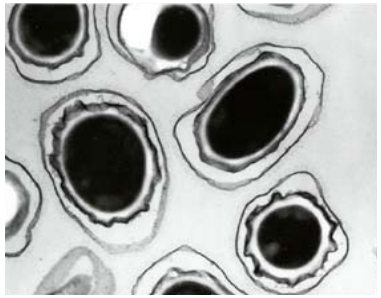
Toxins

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



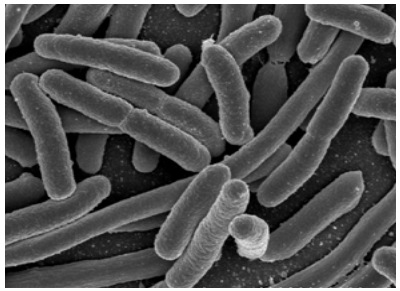
Viruses

- 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

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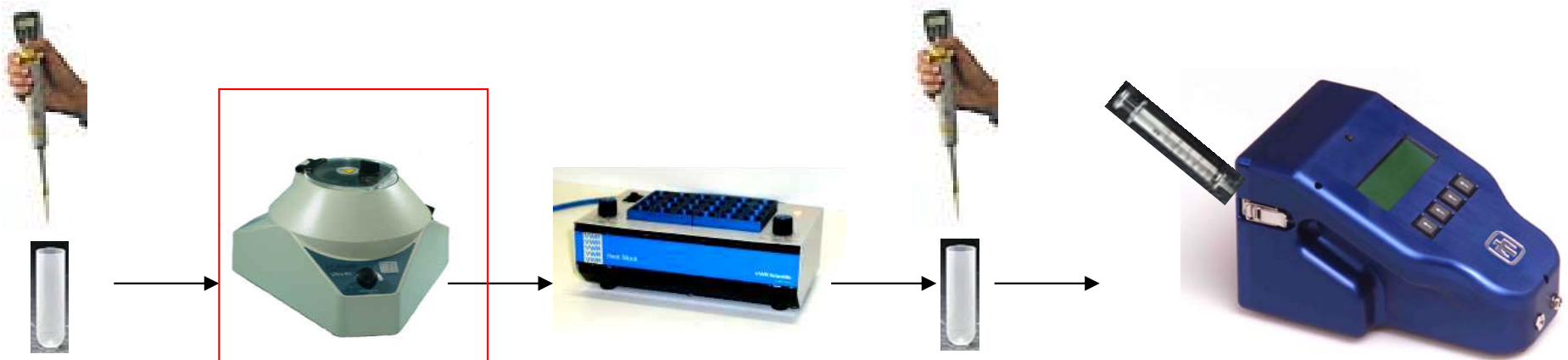
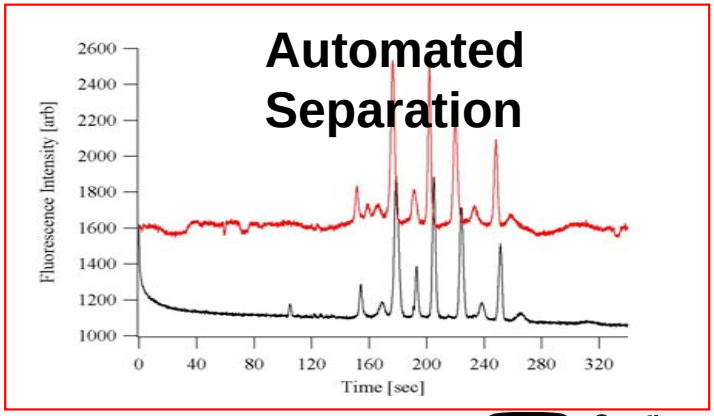
Well Developed Bench Top Assays Must Be Miniaturized and Automated

Manual sample preparation for toxins, viruses, spores, and bacteria



Objective: Economical, automated, miniature system that collects, concentrates, lyses, labels, and hands sample off for analysis.



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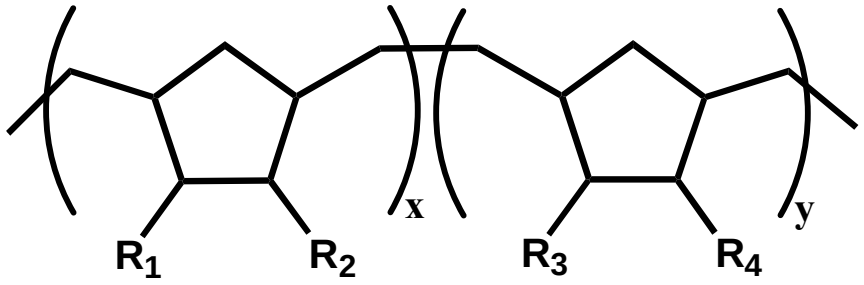
Zeonor Has Best Combination of Materials Properties



Chemical, optical, and mechanical properties

- **Cyclic olefin copolymer. R groups depend on resin grade.**
- **Impervious to water, alcohols, acetonitrile.**
- **Negligible UV absorbance and fluorescence (450 to 600 nm).**
- **Amorphous solid. Negligible elastic scattering of light.**
- **Available in variety of resin grades. Zeonor currently in use softens at 100 ˚C, appropriate for BioBriefcase and easy to work with.**

The Use of Polymer Instead of Glass Significantly Reduces Cost

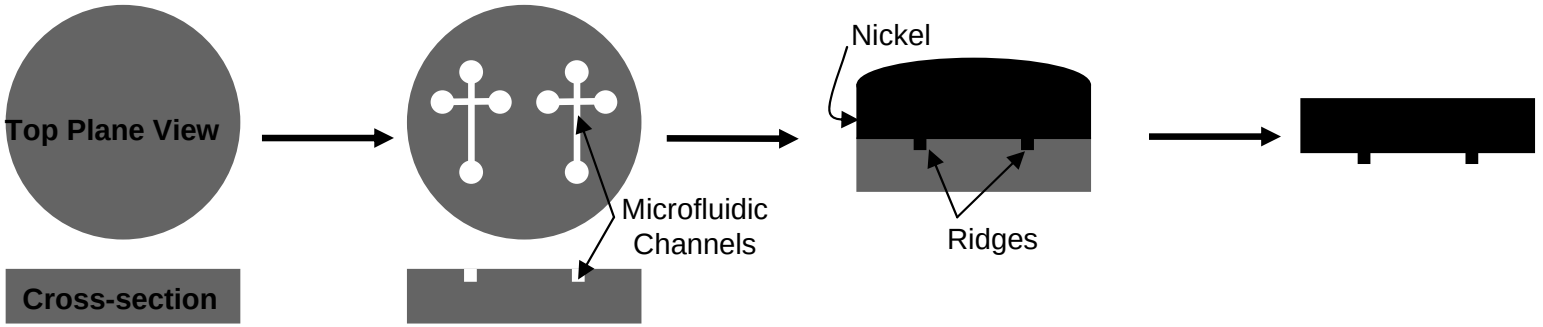
Glass

- $10,000 per lot
- 6 wafers per lot
- 9 dies per wafer
- 50% yield
- $370 per glass chip
- Possible economies of scale
- Cost to operate impact

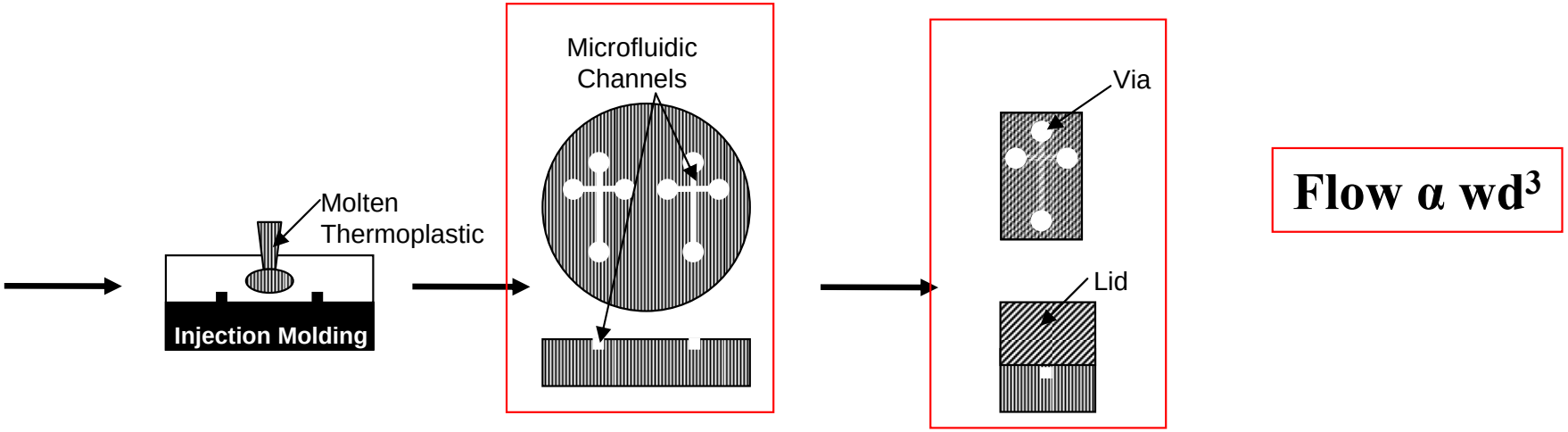
Plastic

- $1670 per master
- $1000 for Nickel stamp. Zeonor cost is negligible ($13 per pound, 30 wafer replicates per pound).
- Injection molding, bonding, and dicing labor: $10 per wafer
- 9 dies per wafer
- 50% yield
- 1000 replicates
- <$3 per plastic chip
- Possible economies of scale
- Cost to operate impact

Polymer Chip Fabrication Steps Are Now Well Developed



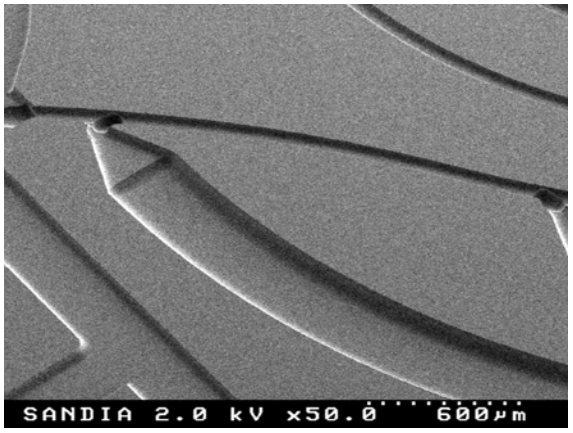
(a) Glass substrate (b) Etched microfluidic master (c) Nickel electroforming (d) Stamp machining



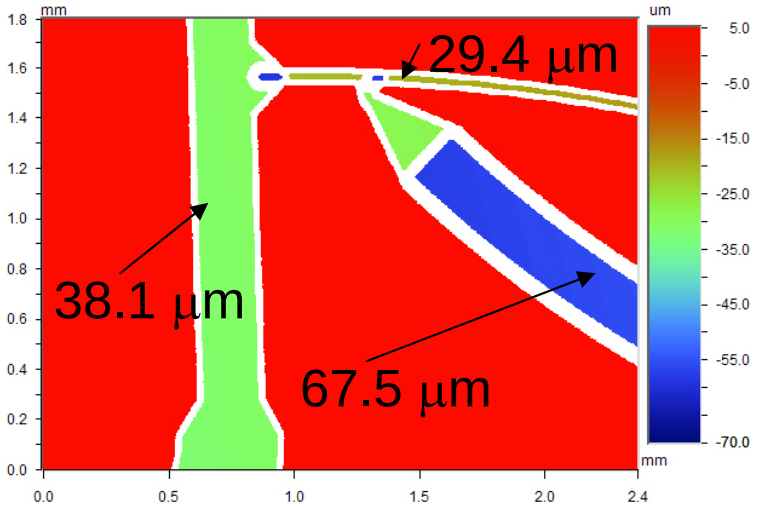
(e) Polymer molding (f) Bonding and dicing

$$\text{Flow } \alpha \text{ } wd^3$$

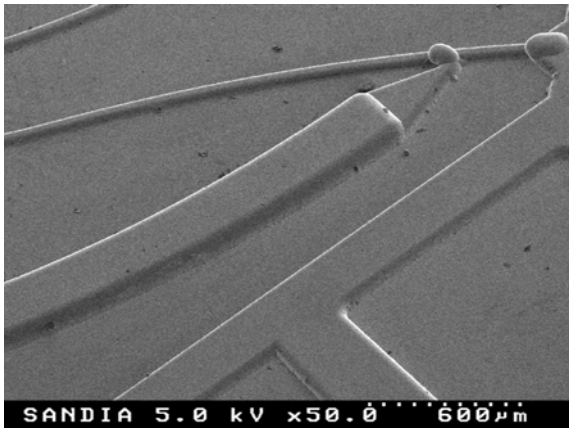
Polymer Chips Can Be Produced in Large Lots in a Few Weeks



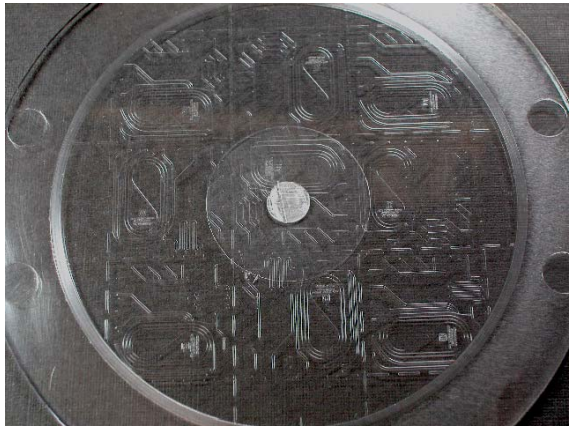
Glass Master

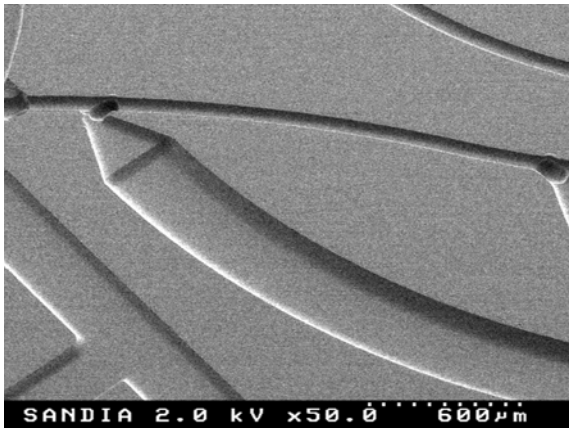


Interferometric Depth Analysis



Nickel Stamp





Injection Molded Zeonor 1060 Replicate

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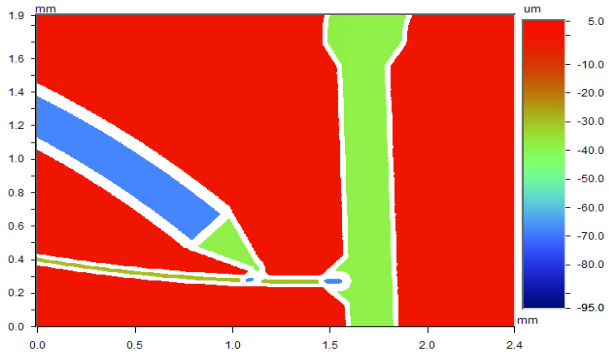
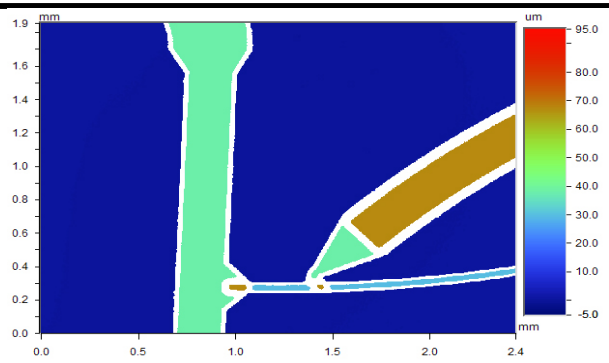
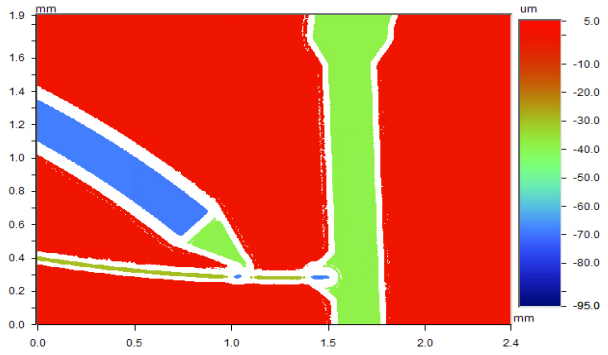
Injection Molding Replication Fidelity of the Order of One Micron or Better



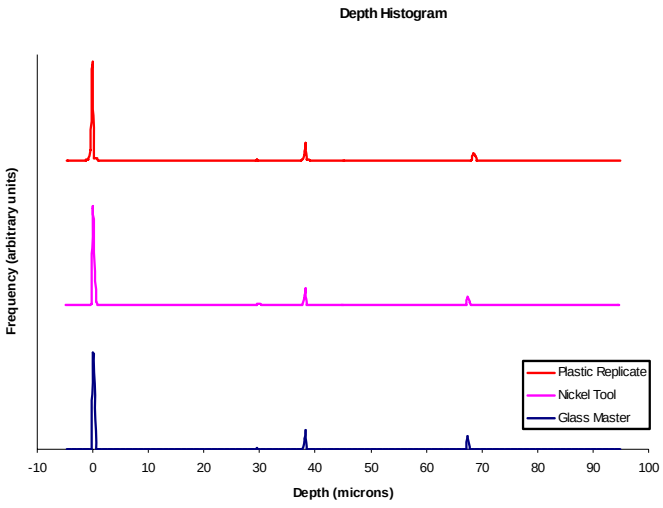
Glass Master



Nickel Stamp



Zeonor Replicate



Comparison of peak positions and widths (in microns) from Gaussian fits to depth histograms

Sample	Surface	Shallow Etch	Deep Etch	Additive Etch
Glass Master	0.00 ± 0.25	29.50 ± 0.25	38.00 ± 0.25	67.00 ± 0.50
Nickel Stamp	0.00 ± 0.25	29.50 ± 0.25	38.00 ± 0.25	67.25 ± 0.25
Injection Molded Zeonor® Wafers	0.00 ± 0.25	29.50 ± 0.25	38.25 ± 0.25	68.50 ± 0.25

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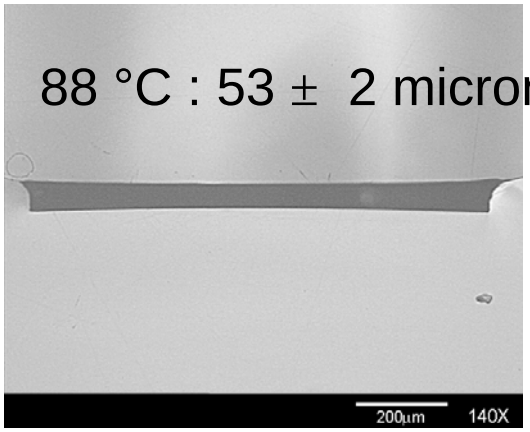
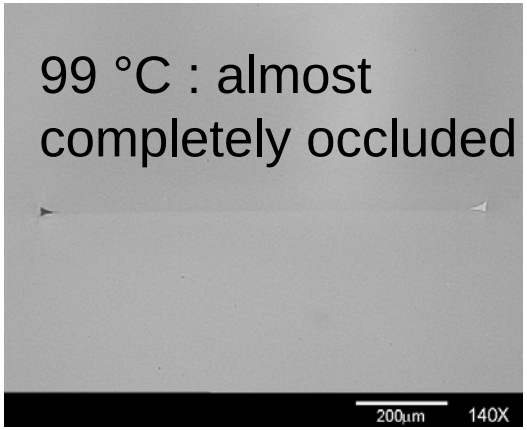
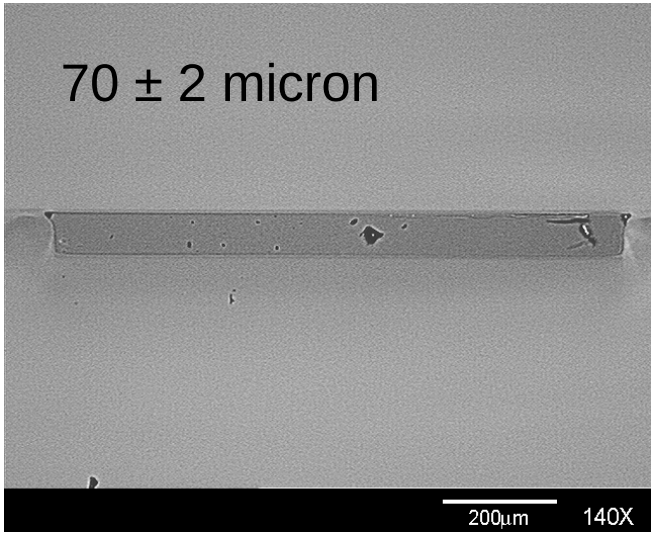
Novel Polymer Chip Bonding Method Developed





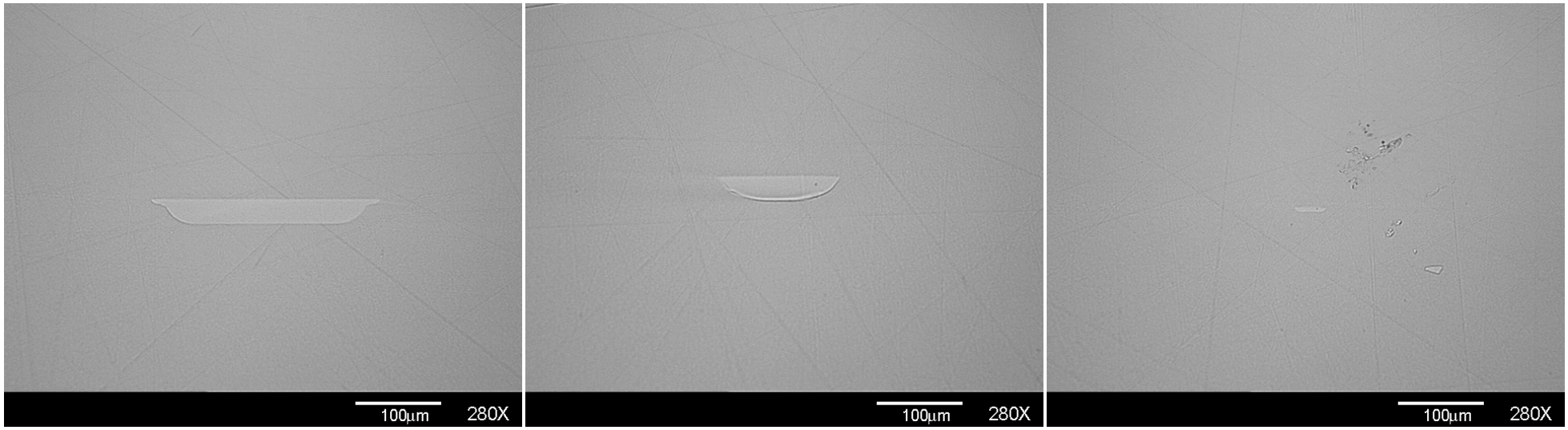
1 mm x 70 micron thermally bonded channels.

1 mm x 70 micron channel bonded using case II diffusion solvent assistance

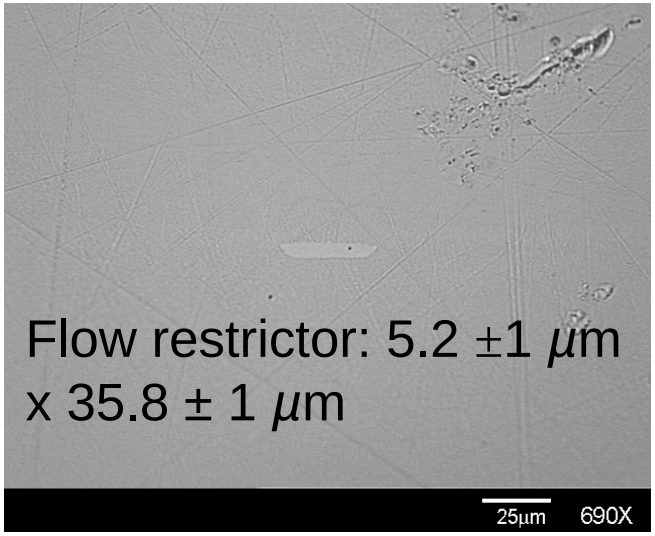


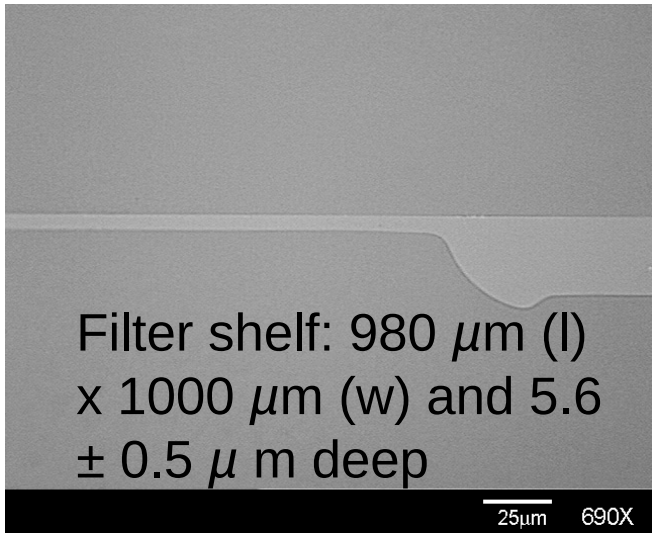
Failure levels of bonded channels under hydrostatic pressure.		
Bonding Method	**Failure Pressure (psi)**	**Failure Mechanism**
TDB, 88 °C	**930-1000**	**Adhesive**
TDB, 99 °C	**600-1000**	**Adhesive**
Solvent, 60 °C	**1900-2340**	**Cohesive**

Case II Solvent Bonding Yields High Fidelity Chips



Three different size channels resolved in bonded chip. Scale bars are 100 µm.





Scale bars are 25 µm

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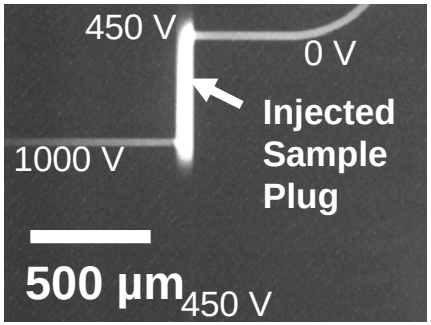
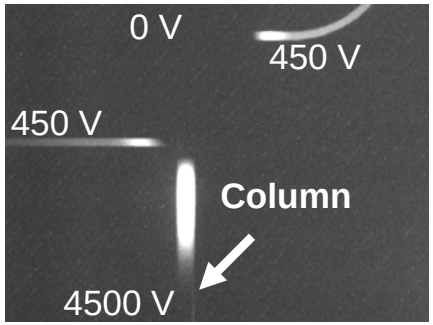
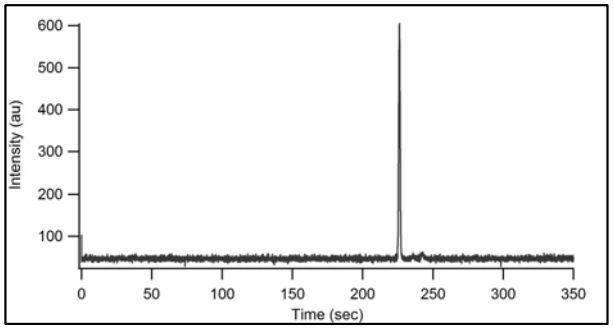
Zeonor CE Polymer Chips Comparable to Glass Chips







FITC

From 55 Zeonor experiments,

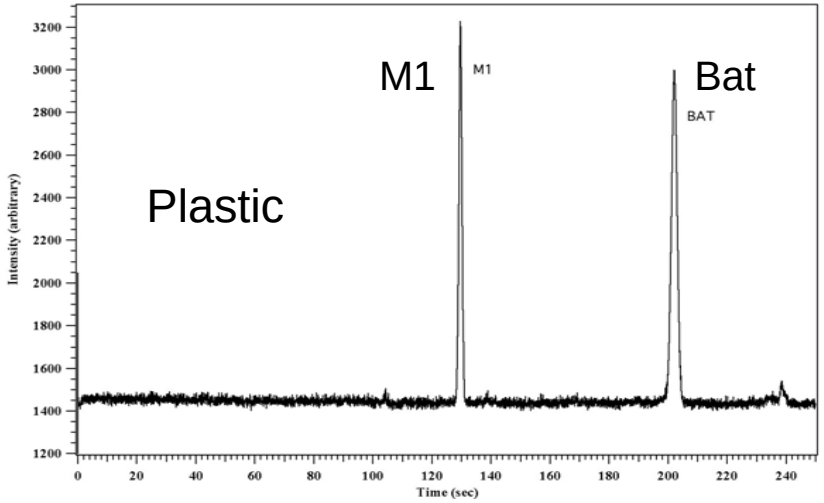
- T = 225.95 ± 1.57 sec

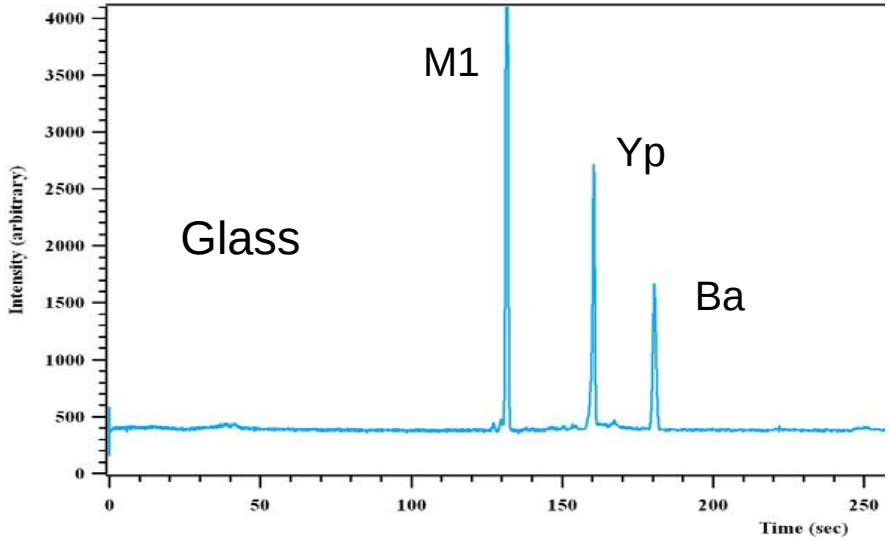
Average Gaussian Width = 0.54 sec

(Glass: T = 184.07 ± 3.11 sec, AGW = 0.63 sec)

- Number of Theoretical Plates = 175,080/cm

(Glass: 85,660/cm)





PCR produced eTags

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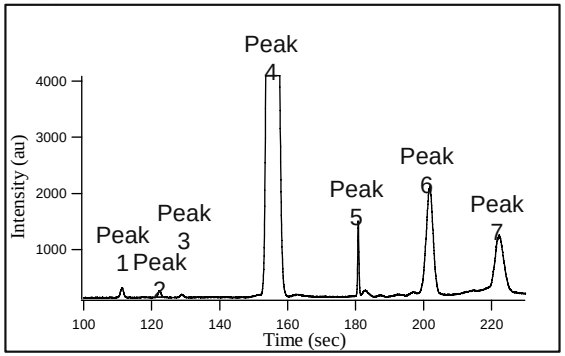
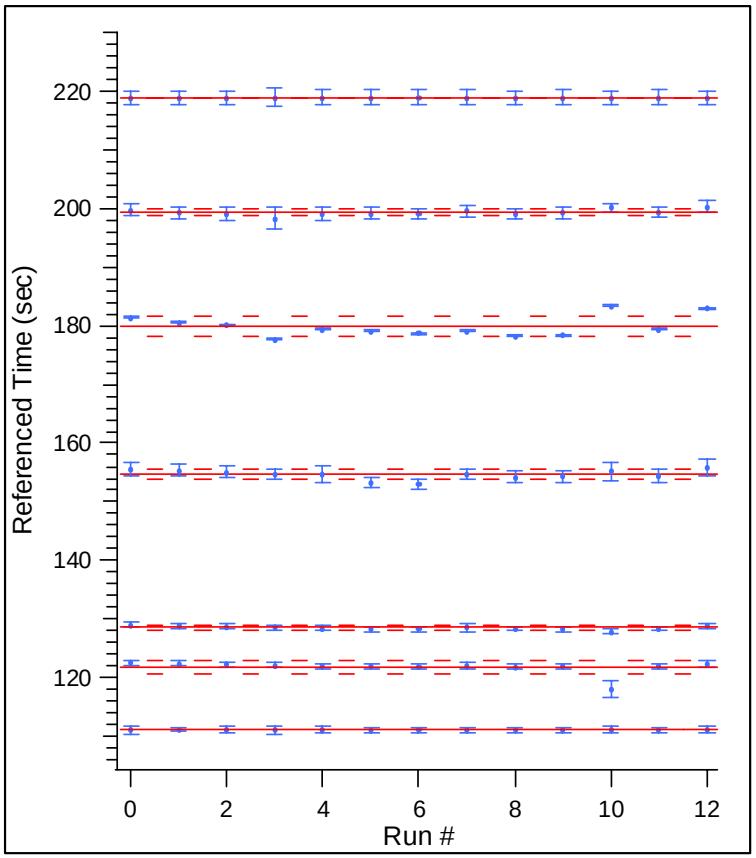
Time Referenced Protein Separation Demonstrated in Zeonor Chips





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CE statistics of molecular weight marker protein peaks including FITC. Time referenced values shown in parenthesis.

Peak #	Protein/Molecule Name	Molecular Weight (kDa)	Avg Peak Position (sec)	Avg Peak Position Std Dev (sec)	Avg Gaussian Fit Std Dev (sec)	Avg Number of Theoretical Plates/cm
1	Trypsin Inhibitor, soybean	20.1	111.16	0.83	0.50 (0.50)	56000
2	Carbonic Anhydrase, bovine erythrocyte	29	121.81 (121.82)	1.28 (1.14)	0.51 (0.51)	72000
3	Alcohol Dehydrogenase, equine liver	39	128.50 (128.51)	1.10 (0.32)	0.42 (0.43)	123000
4	Albumin, bovine serum	66	154.62 (154.64)	1.63 (0.85)	1.10 (1.10)	22000
5	Fluorescein isothiocyanate (FITC)	Molecular Marker	179.87 (179.91)	2.36 (1.78)	0.15 (0.15)	1628000
6	β-Galactosidase, *E. Coli*	116	199.34 (199.35)	3.30 (0.52)	1.05 (1.05)	40000
7	Myosin, rabbit muscle	205	218.96	4.16	1.23 (1.23)	33000

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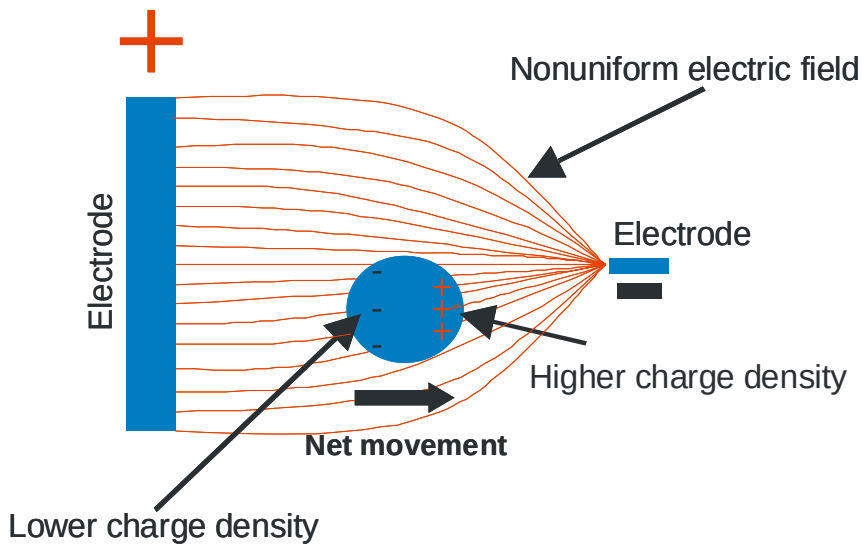
Dielectrophoresis can be Used to Trap Particulate Matter

- **Dielectrophoresis (DEP) is the movement of matter caused by induced dipole polarization effects within particles present in a nonuniform electric field.**

- **In the presence of a nonuniform electric field, one side of the dipole will be in a region with a lower field intensity. This will produce an uneven charge alignment in the particle, inducing it to move toward the regions of greater field strength.**

- **DC or AC electric fields.**

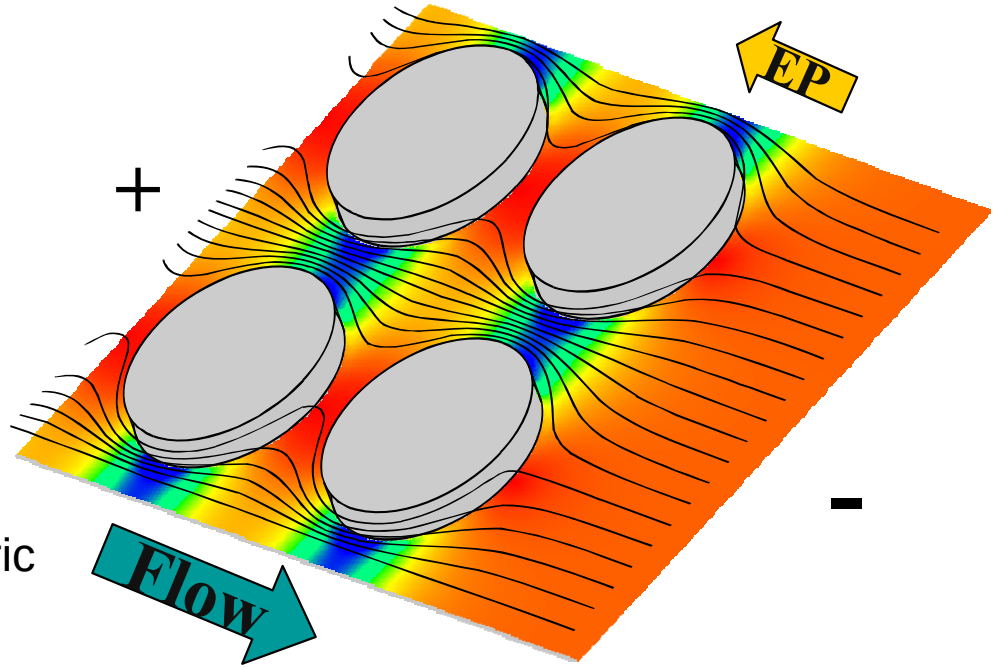


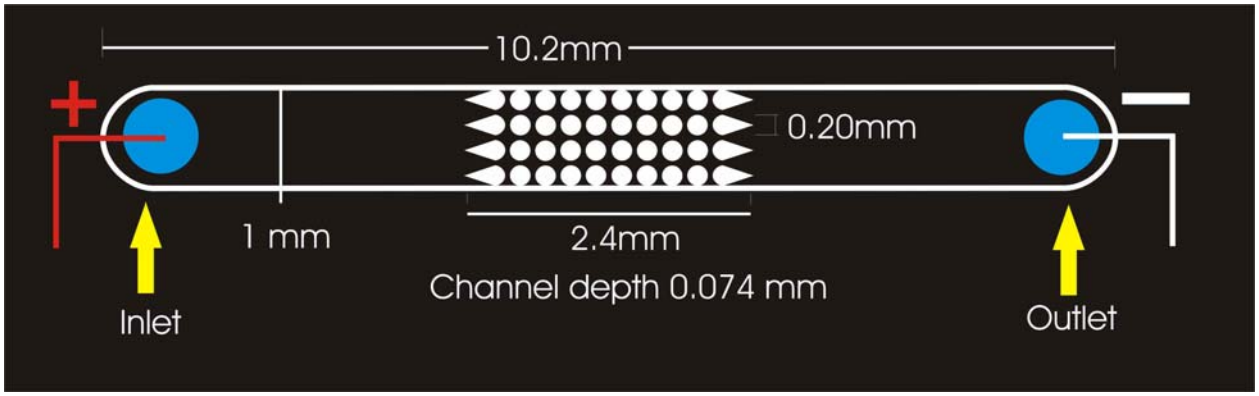
$$F_{DEP} = 2\pi \in_0 \in_m r^3 \mathrm{Re}\left[f\left(\sigma_p^*, \sigma_m^*\right)\right]\nabla E^2$$

$$f\left(\sigma_p^*, \sigma_m^*\right) = \left[\frac{\sigma_p^* - \sigma_m^*}{\sigma_p^* + 2\sigma_m^*}\right]$$

Insulating Polymer Chips Used to Created Required Electric Field Gradient

- Must have a non-uniform electric field
  - Arrays of electrodes
  - Arrays of insulators
- Arrays of electrodes have certain limitations
  - Microfabrication techniques can be expensive and time-consuming
  - Sample volume
- Arrays of insulators can be utilized to create integral structures that create nonuniform electric fields
- Polymers are essential to produce high throughput, high aspect ratio insulating structures.





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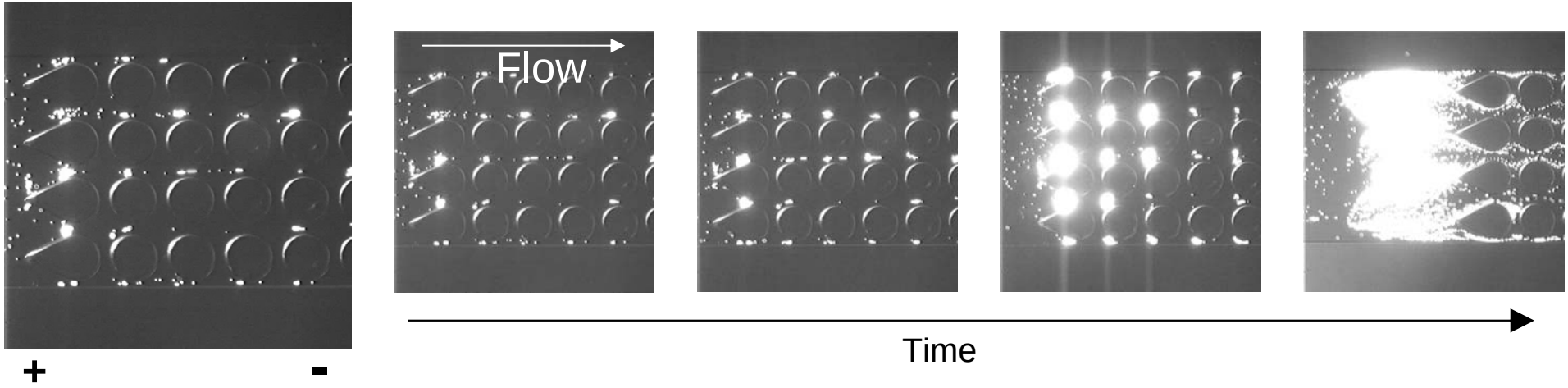
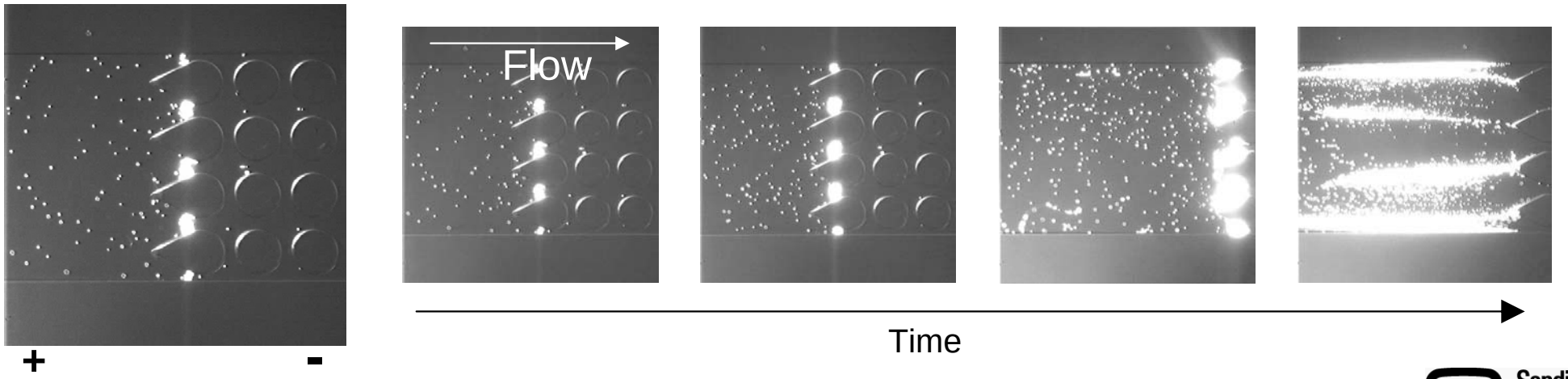
iDEP Trapping Depends on Field Strength

300 V_{DC} (29.41 V/mm)



400 V_{DC} (39.22 V/mm)



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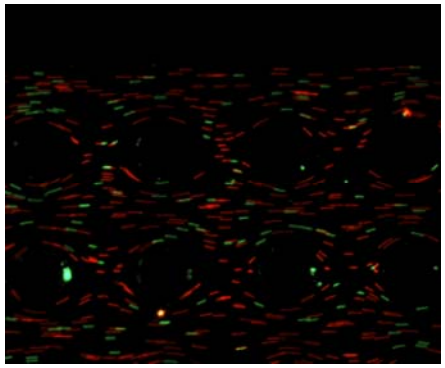
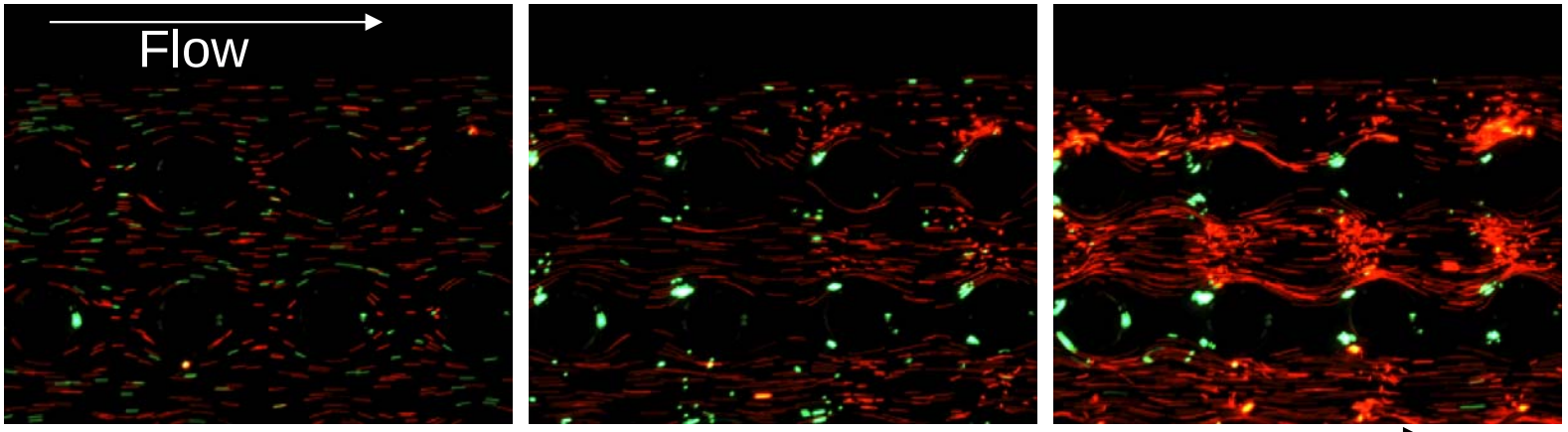
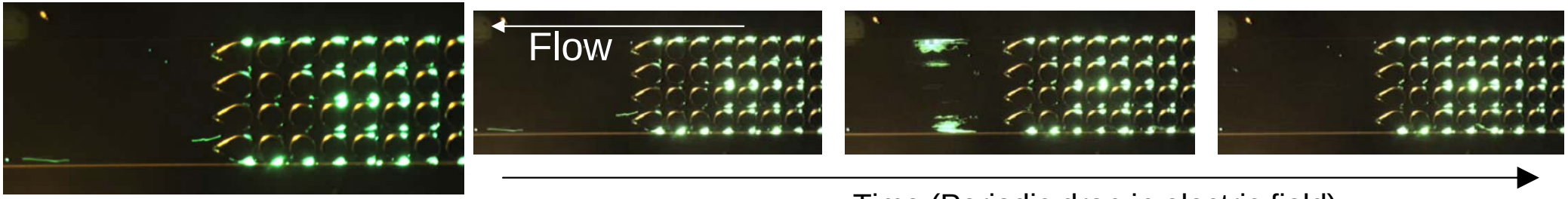
Particles can be Manipulated Based on Type and Size



+ **Separation by size:** -
2 micron (green) and 1 micron (red)



Time **(0 → 500VDC → 750VDC → 1000VDC)**



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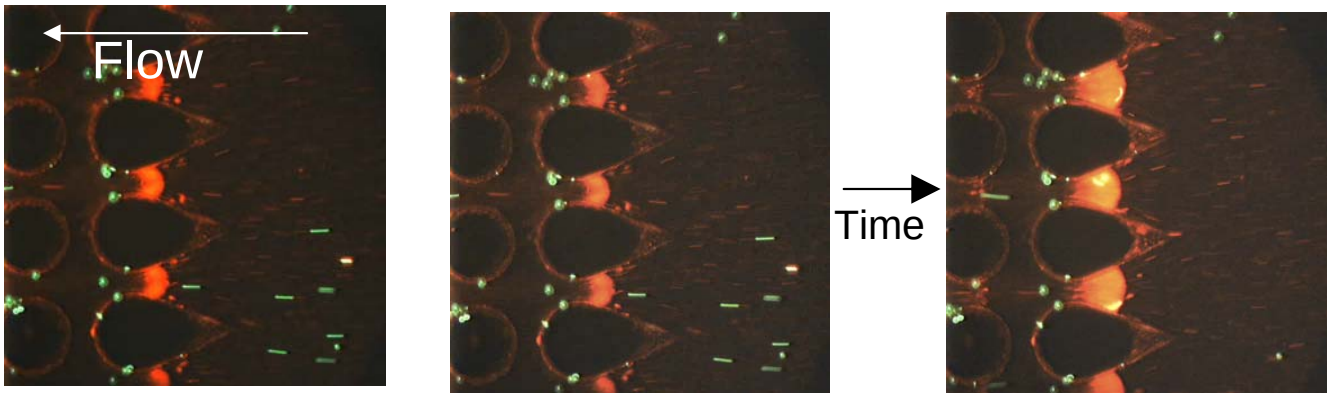
Time (Periodic drop in electric field)

Bacillus subtilis **spores (red) are trapped.**
1 micron polystyrene beads (green) pass.



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Summary

- Developed and documented robust, high fidelity injection molding process and novel bonding method.
- Savings in cost to operate by using plastic chips.
- Demonstrated the potential of polymer-based microfluidics
  - » Capillary Electrophoresis
  - » Insulator-based Dielectrophoresis
- Technology available for licensing from Sandia.

Acknowledgement

- **Chip Fabrication: John Hachman, Karen Krafcik, Brian Holliday, Renee Shediac, Kevin MacDonald, Linda Domeier, Eric Goods, Devin Hoffman, Sita Mani, Dorrance MacLean, Jerry Fordham.**
- **Chip Characterization: Brian Kirby, Petra Mela, Jeffrey Chames, Kevin MacDonald, Joe Ceremuga.**
- **Chip Testing: Gregory McGraw, Blanca Lapizco-Encinas, Rafael Davalos, Yolanda Fintschenko, Eric Cummings, Greg Fiechtner, Andrew Skulan, Louise Barrett, Karen Krafcik, Shane Sickafoose, Cindy Harnett, Bruce Mosier, Mike Kanouff, Marion Hunter, Karen Krafcik, Jeff Campbell, Tom Wallow.**
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