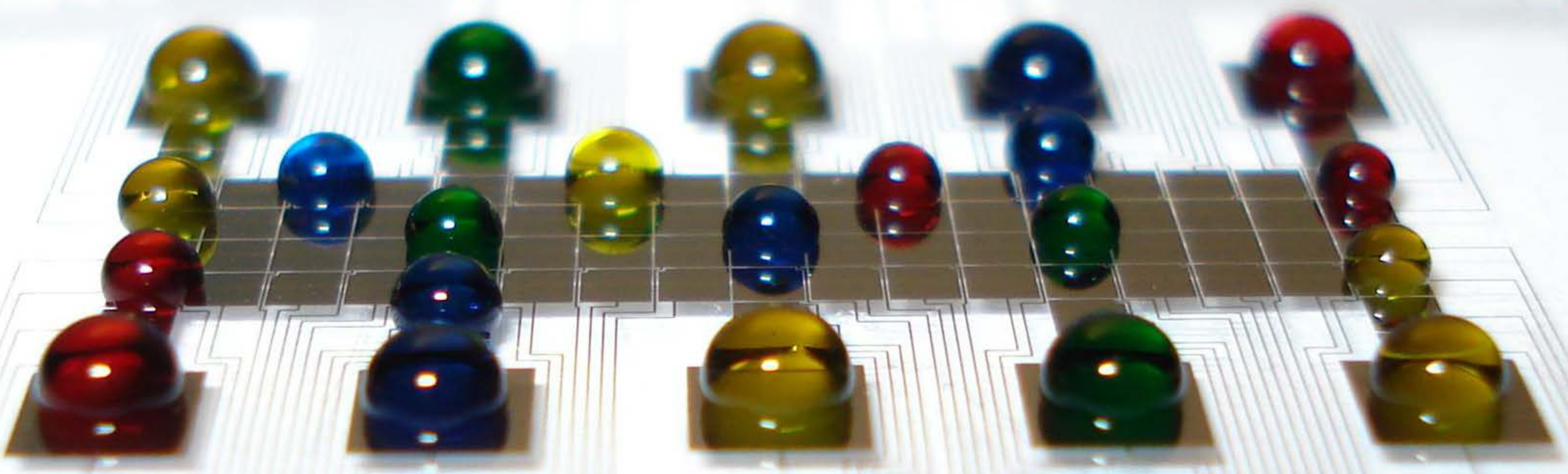


Digital Microfluidics

Aaron Wheeler
University of Toronto

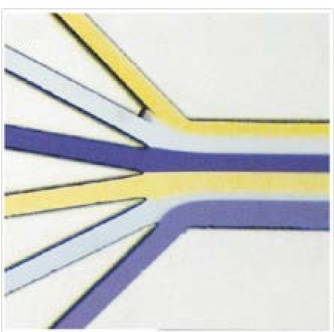
Digital Microfluidics - Outline

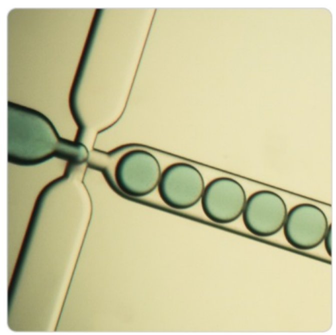
- DMF: Introduction and Theory
  - *DMF vs. Microchannels, DMF vs. Droplets-In-Channels, General Mechanism, Two-Plate vs. One-Plate, Electrowetting-on-Dielectric, Electromechanical Model*
- DMF: Alternate Mechanisms
  - *Wiring Complications, Optical Forces, Magnetic Forces, Thermocapillary Forces, Acoustic Forces*
- DMF: Integration and Sensors
  - *Integrated Optics, Modular Optics, SPR, Electrochemistry, Mass Spectrometry, Sample Processing and Separations*
- DMF: Applications
  - *Synthesis, Genomics, Proteomics, Diagnostics, Cell Culture*
- Final Notes
  - *Accessibility, A Look to the Future*

Digital Microfluidics

- Digital microfluidics (DMF) is one of a group of techniques used to miniaturize fluid-handling for “lab on a chip” applications

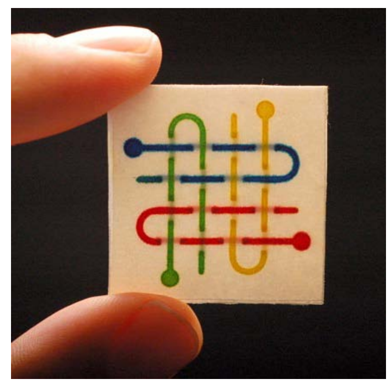
Microchannels (continuous)



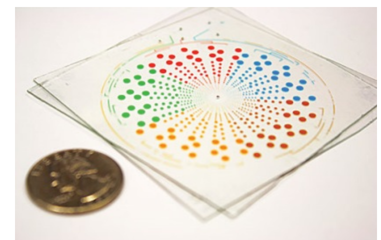


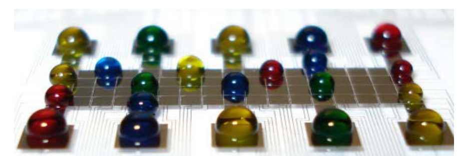
Microchannels (droplets)

Paper Microfluidics



Slip Chips

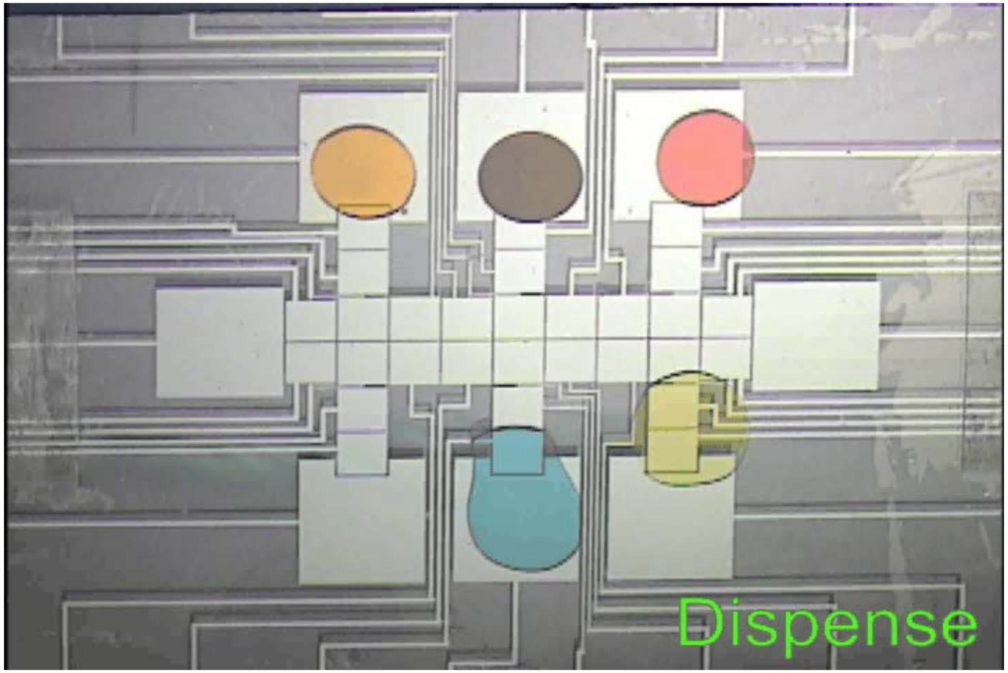




Digital Microfluidics

Digital Microfluidics

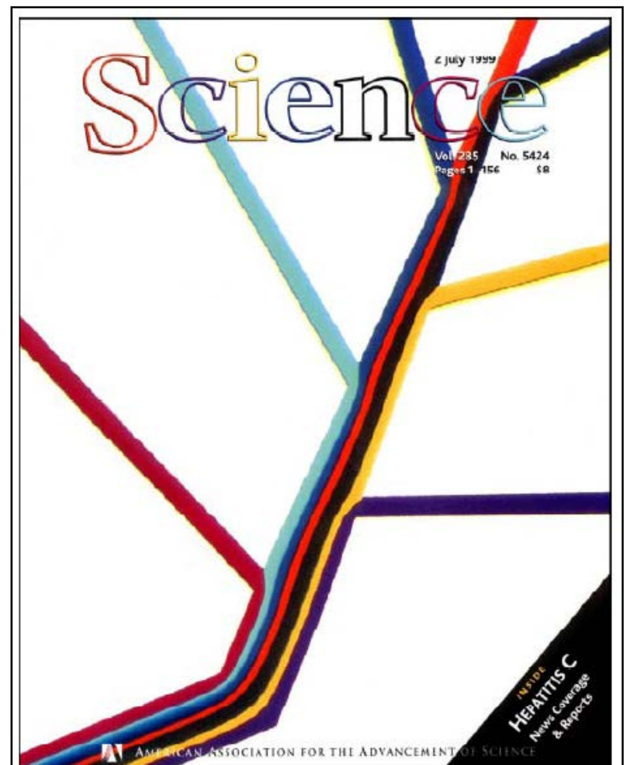
- Digital microfluidics (DMF) is one of a group of techniques used to miniaturize fluid-handling for “lab on a chip” applications
- A unique feature of DMF: samples are manipulated as droplets on an open surface



Typical operations include dispense (precisions of ~2-3% CV), move, merge, mix, and split

DMF vs. Microchannels

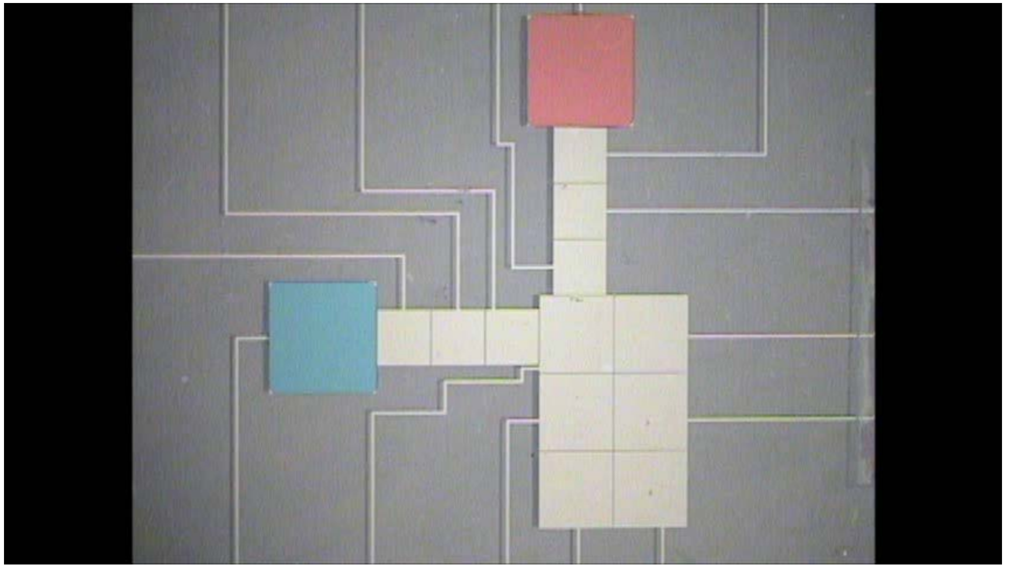
- DMF and microchannels share many characteristics, including laminar flow (i.e., no turbulence)
- This is useful for some applications (e.g., the generation of chemical gradients), but makes things challenging for others, such as reagent mixing



Kenis et al., *Science*, 1999 *285* 83-85

DMF vs. Microchannels

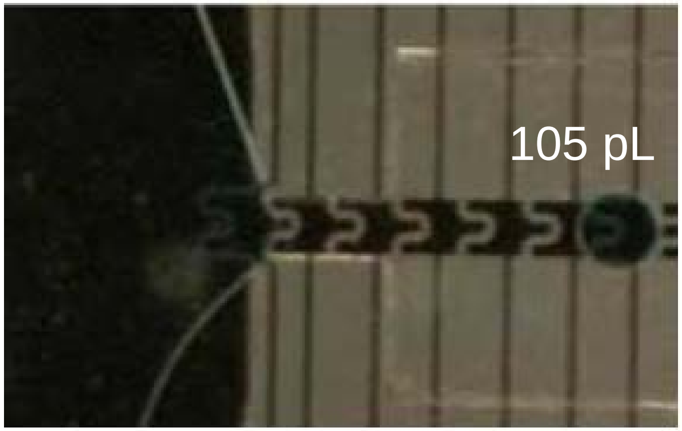
- DMF and microchannels share many characteristics, including laminar flow (i.e., no turbulence)
- Thankfully, the act of DMF droplet translation brings droplet constituents together **10-100x** faster than diffusion



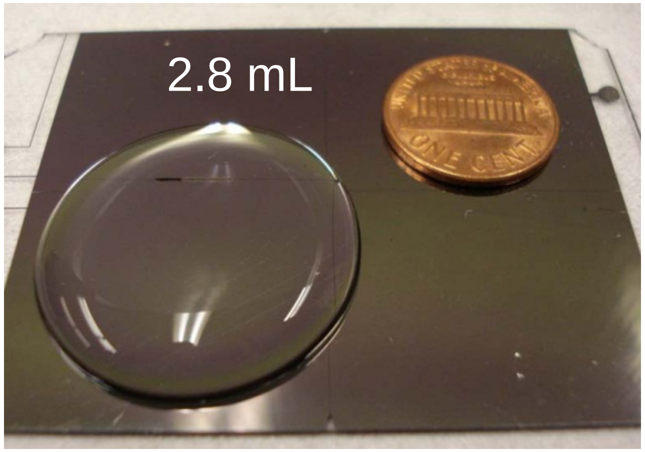
for more, see: Paik, et al. *Lab Chip* 2003, 3, 28-33, Paik, et al. *Lab Chip* 2003, 3, 253-259, Lu, et al. *Lab Chip* 2008, 8, 456-461

DMF vs. Microchannels

- The DMF format offers a number of advantages, including freedom from moving parts, fittings, and connectors, and compatibility with a wide range of sample sizes



Song et al. *Microfluid. Nanofluid*. 2009*, 7*, 75



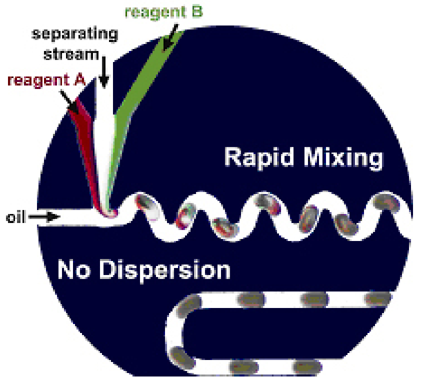
Abdelgawad et al. *Lab Chip*, 2008, *8*, 672

DMF vs. Microchannels

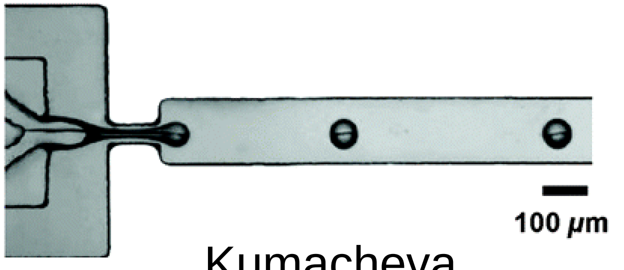
- The DMF format offers a number of advantages, including freedom from moving parts, fittings, and connectors, and compatibility with a wide range of sample sizes
- But of course, DMF is not appropriate for many applications that are well-suited for microchannels (e.g., chromatography, shear flow experiments, long linear arrays, mazes, etc.)
- I view the two techniques as being **complementary** – depending on the application, either DMF or microchannels (or both) may be useful

DMF vs. Droplets-In-Channels

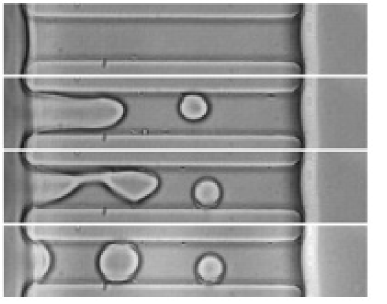
- Note that DMF (as defined here) is distinct from alternative methods in which droplets are manipulated in channels…



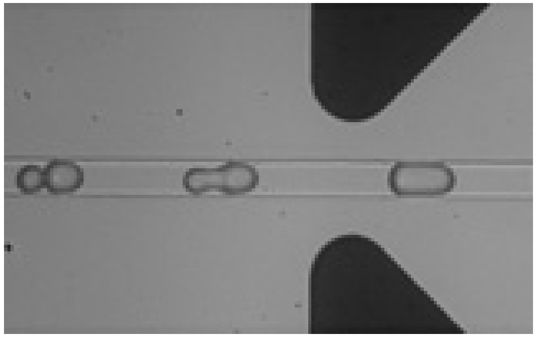
Ismagilov, Cal Tech



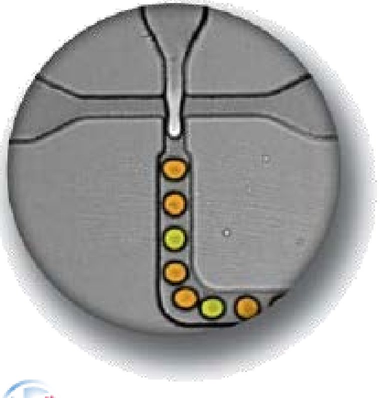
Kumacheva, Toronto



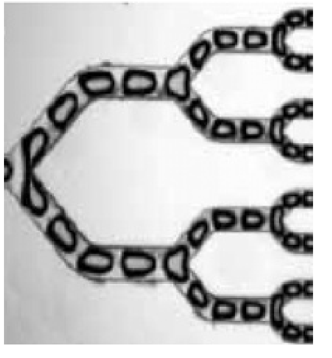
Chiu, Washington



Weitz, Harvard



RainDance Technologies



Lee, Irvine

DMF vs. Droplets-In-Channels

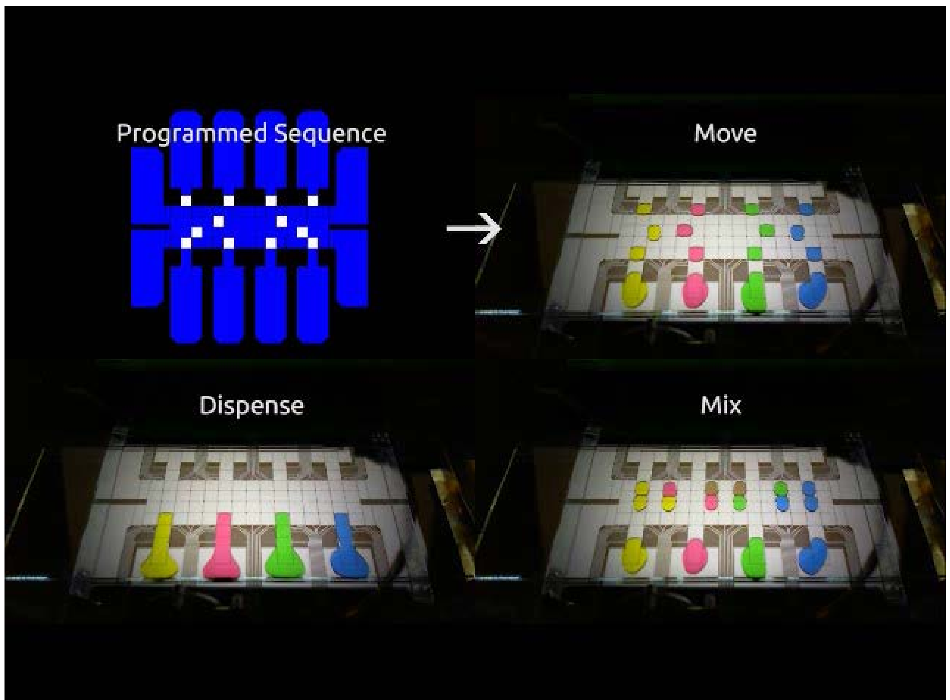
- Note that DMF (as defined here) is distinct from alternative methods in which droplets are manipulated in channels…
- The key distinction:
  - In DMF, each droplet is independently addressable (in parallel), but often with modest throughput
  - In droplets-in-channels, droplets are manipulated in series, but often with very high throughput

DMF vs. Droplets-In-Channels

- Another key distinction –DMF enables a "programming" approach to sample processing, allowing the user to build, combine, and execute "functions" in parallel

Programmed function for "Move"

Droplets excecuting "Dispense"

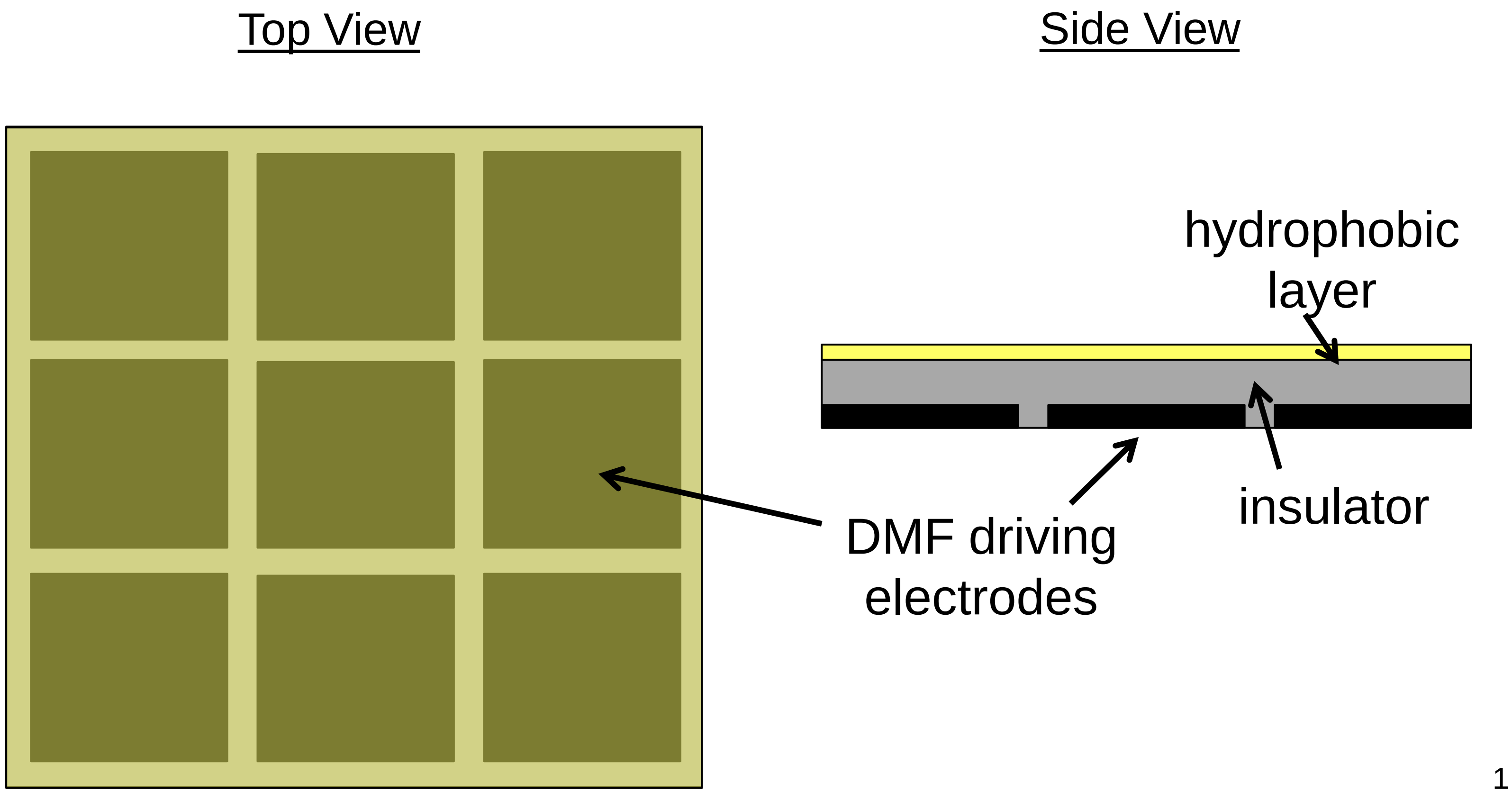


Droplets executing "Move"

Droplets executing "Mix"

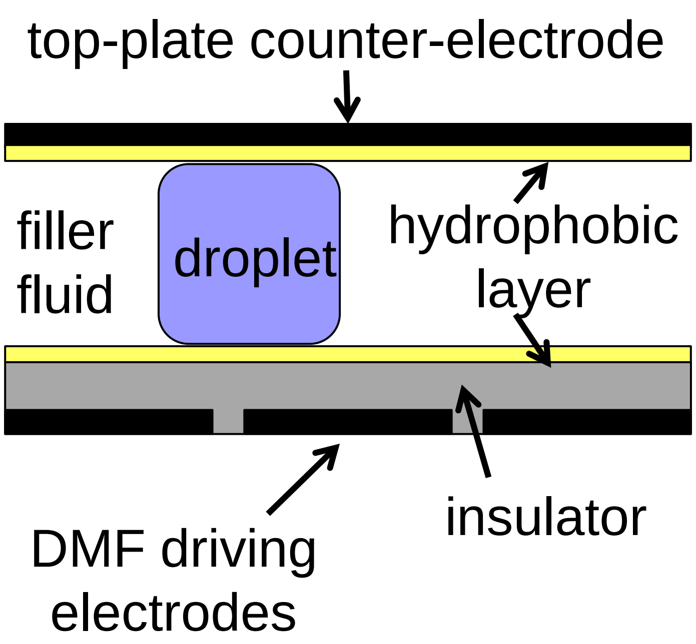
DMF – General Mechanism

- In the most common implementation of DMF, the device includes an array of driving electrodes coated by an insulator and a hydrophobic layer



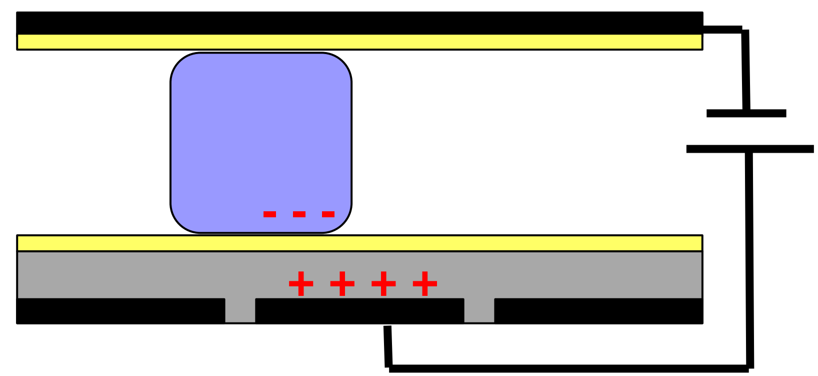
DMF – General Mechanism

- In the most common implementation of DMF, the device includes an array of driving electrodes coated by an insulator and a hydrophobic layer
- Driving electrodes are typically referenced to a "top-plate" counter-electrode (often transparent)
- Droplets are surrounded by a filler fluid (often air or oil)



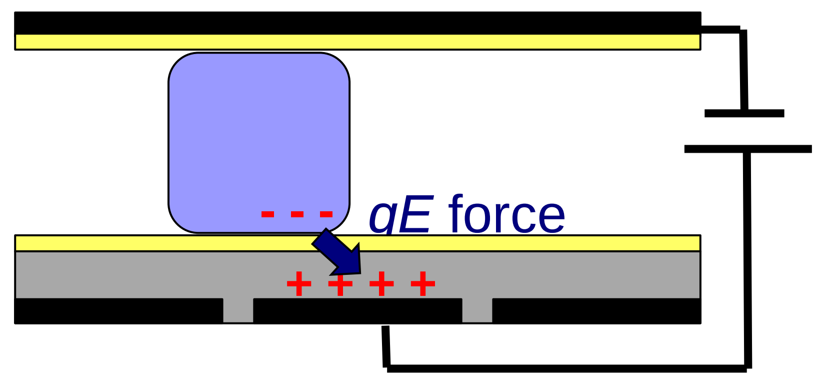
DMF – General Mechanism

- In the most common implementation of DMF, the device includes an array of driving electrodes coated by an insulator and a hydrophobic layer
- When an electrical potential (AC or DC) is applied between a driving electrode and the counter-electrode, charges accumulate at the insulator



DMF – General Mechanism

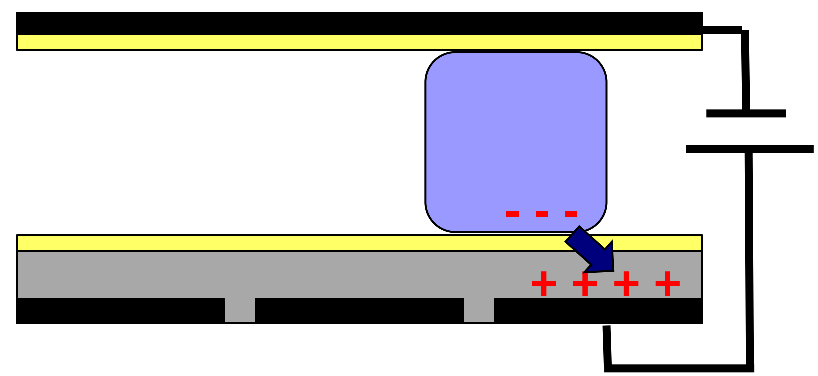
- In the most common implementation of DMF, the device includes an array of driving electrodes coated by an insulator and a hydrophobic layer
- An electrostatic force *pulls* the droplet toward the charged electrode



This is analogous to the common "water bending" demonstration with a charged comb

DMF – General Mechanism

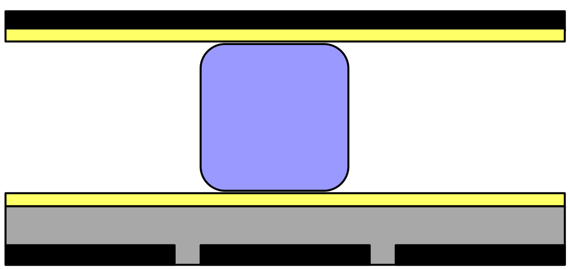
- In the most common implementation of DMF, the device includes an array of driving electrodes coated by an insulator and a hydrophobic layer
- An electrostatic force *pulls* the droplet toward the charged electrode
- By switching the field to successive electrodes, droplets can be positioned anywhere on the array



Two-Plate vs. One-Plate DMF

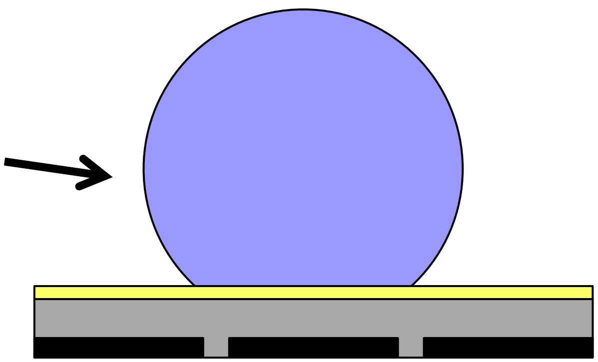
- “Two-plate” DMF is most common, but a “one-plate” format is also used

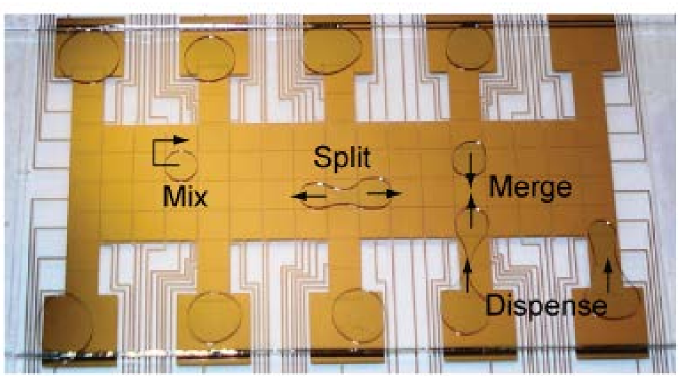
Two-Pate



side view

One-Pate





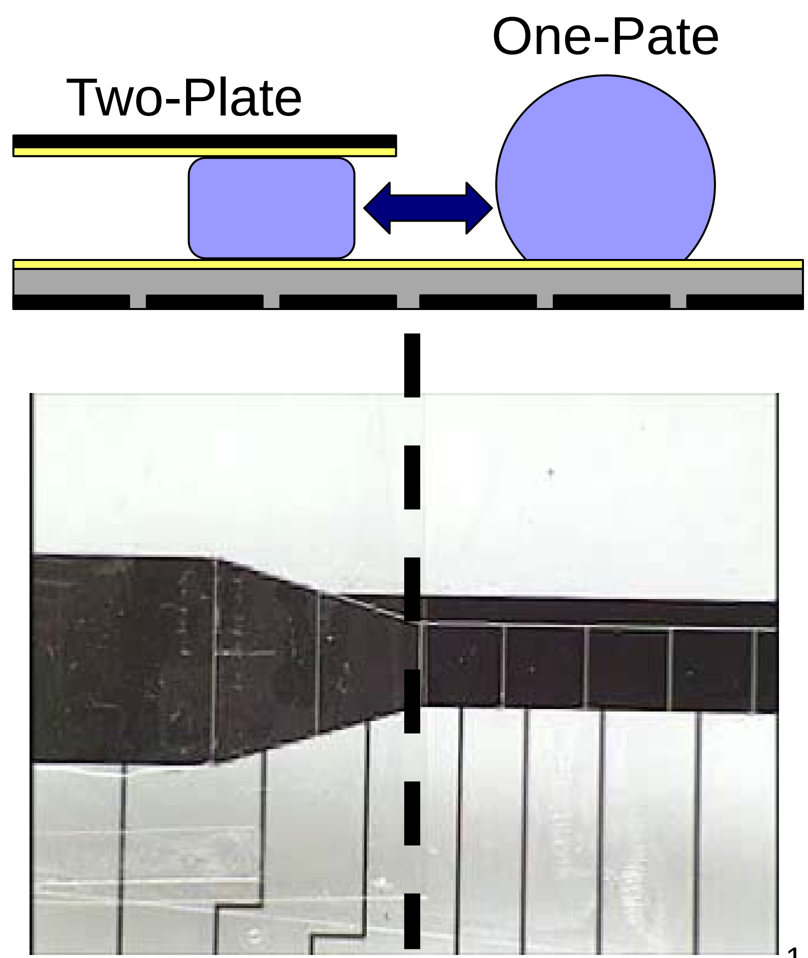
top view

Compatible with all operations (dispensing, merging, mixing, splitting)

Not compatible with dispensing or splitting, but easy access to samples

Two-Plate vs. One-Plate DMF

- “Two-plate” DMF is most common, but a “one-plate” format is also used
- The two formats were recently shown to be integratable on a single device, allowing droplets to transit back and forth between the two regimes

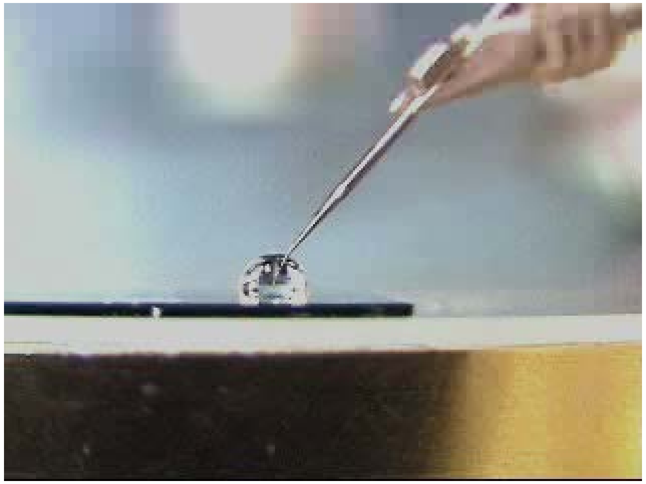


Kirby and Wheeler, *Lab Chip,* 2013, *13*, 2533-2540

Electrowetting on Dielectric

- Digital microfluidics is related to a phenomenon known as “electrowetting on dielectric” (EWOD), in which droplet shape is observed to change upon application of an E-Field
- This behaviour can be approximated by the Young-Lippman equ’n:

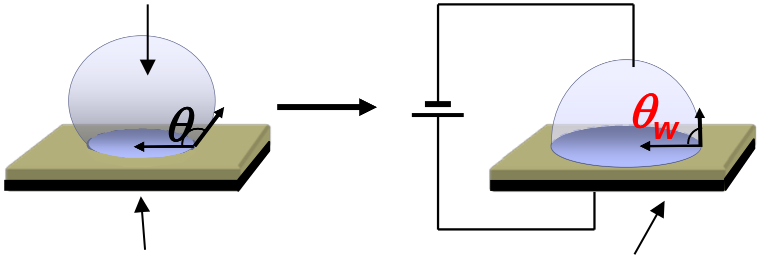
$$\cos\theta_w = \cos\theta + \frac{\varepsilon_o \varepsilon V^2}{2\gamma_{LG} t}$$



Cho and Moon, Kim Lab, UCLA

droplet with contact angle θ and liquid-gas surface tension γ_{LG}

wetted contact angle $\theta_w < \theta$



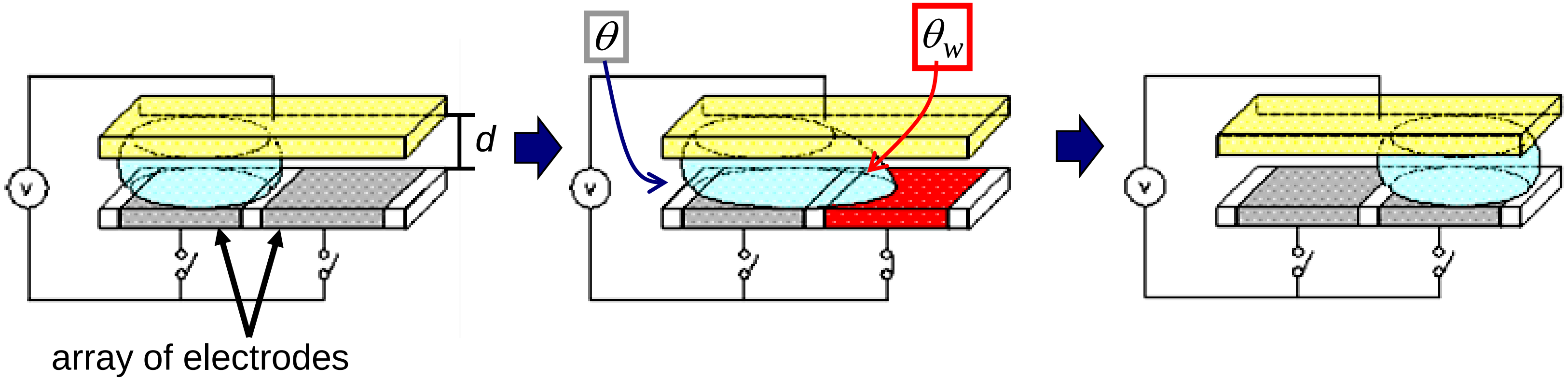
electrode coated by hydrophobic insulator with thickness t and permittivity ε

Electrowetting on Dielectric

- In addition to describing droplet shape change, EWOD is sometimes invoked to describe droplet movement (as a function of Laplace pressure, Δp) in DMF

$$\Delta p = \frac{\gamma_{LG}}{d}\left(\cos\boxed{\theta_w} - \cos\boxed{\theta}\right)$$

Lee, *et al. Sens. and Act. A* 2002, *95,* 259-268

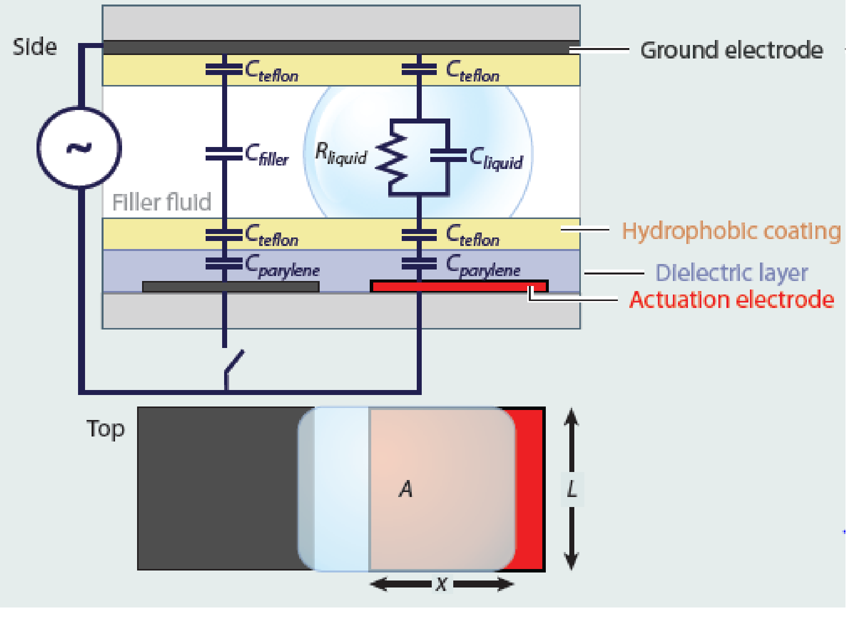


Electrowetting on Dielectric

- In addition to describing droplet shape change, EWOD is sometimes invoked to describe droplet movement (as a function of Laplace pressure, Δp) in DMF
- The EWOD approximation is useful for modeling movement of aqueous droplets with high surface tension (and large $\Delta\theta$ in response to electric fields), but is limited in that it:
  - requires empirical measurements of θ for each new combination of liquid/hydr.surface/filler
  - may not be suitable for describing movement of droplets with low surface tension

Electromechanical Model

- A more general description of DMF driving force can be made using electromechanics and a lumped-sum model



- Electromechanical energy in the system is:

$$E(f,x)=\frac{L}{2}\left(x\sum_{i}\frac{\varepsilon_0\varepsilon_{\mathrm{r.i,liquid}}V^2{}_{\mathrm{i,liquid}}(j2\pi f)}{d_{\mathrm{i}}}+(L-x)\sum_{i}\frac{\varepsilon_0\varepsilon_{\mathrm{r.i,filler}}V^2{}_{\mathrm{i,filler}}(j2\pi f)}{d_{\mathrm{i}}}\right)$$

$\varepsilon_{r,i,liquid}$, $V_{i,liquid}$, and $\varepsilon_{r,i,filler}$, $V_{i,filler}$ are the relative permittivity and voltage drop for the liquid and filler fluid portion of the electrode respectively, and d_i is the thickness of layer i

Choi et al. *Annu. Rev. Anal. Chem.* 2012, *5*, 413–440

Electromechanical Model

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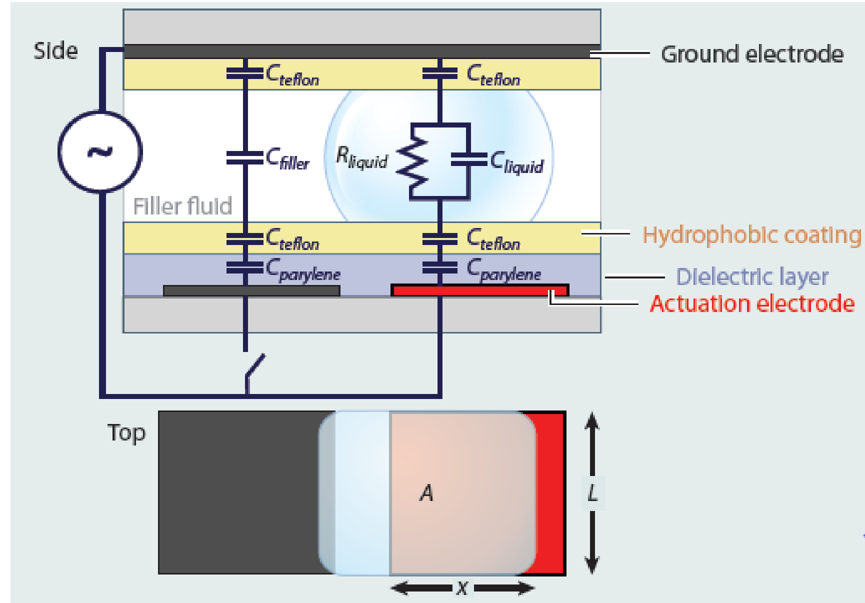

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$$E(f,x)=\frac{L}{2}\left(x\sum_i \frac{\varepsilon_0\varepsilon_{\mathrm{r.i,liquid}}V^2{}_{\mathrm{i,liquid}}(j2\pi f)}{d_\mathrm{i}}+(L-x)\sum_i \frac{\varepsilon_0\varepsilon_{\mathrm{r.i,filler}}V^2{}_{\mathrm{i,filler}}(j2\pi f)}{d_\mathrm{i}}\right)$$

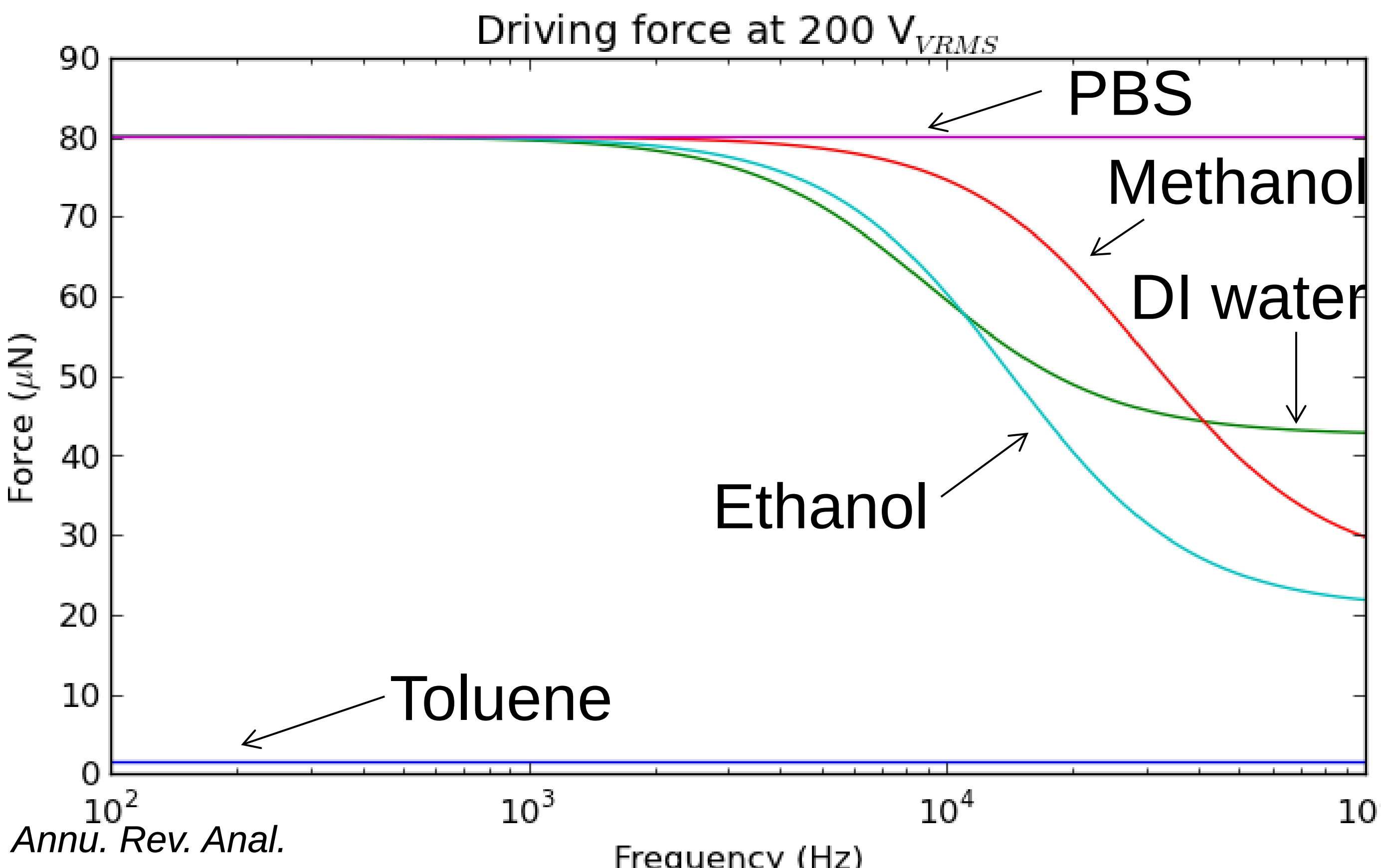
- and driving force (by differentiating) is:

$$F(f)=\frac{\partial E(f,x)}{\partial x}=\frac{L}{2}\left(\sum_i \frac{\varepsilon_0\varepsilon_{\mathrm{r.i,liquid}}V^2{}_{\mathrm{i,liquid}}(j2\pi f)}{d_\mathrm{i}}-\sum_i \frac{\varepsilon_0\varepsilon_{\mathrm{r.i,filler}}V^2{}_{\mathrm{i,filler}}(j2\pi f)}{d_\mathrm{i}}\right)$$

Choi et al. *Annu. Rev. Anal. Chem.* 2012, *5*, 413–440

Electromechanical Model

- The electromechanical model allows for convenient estimation of forces generated on different reagents at different frequencies



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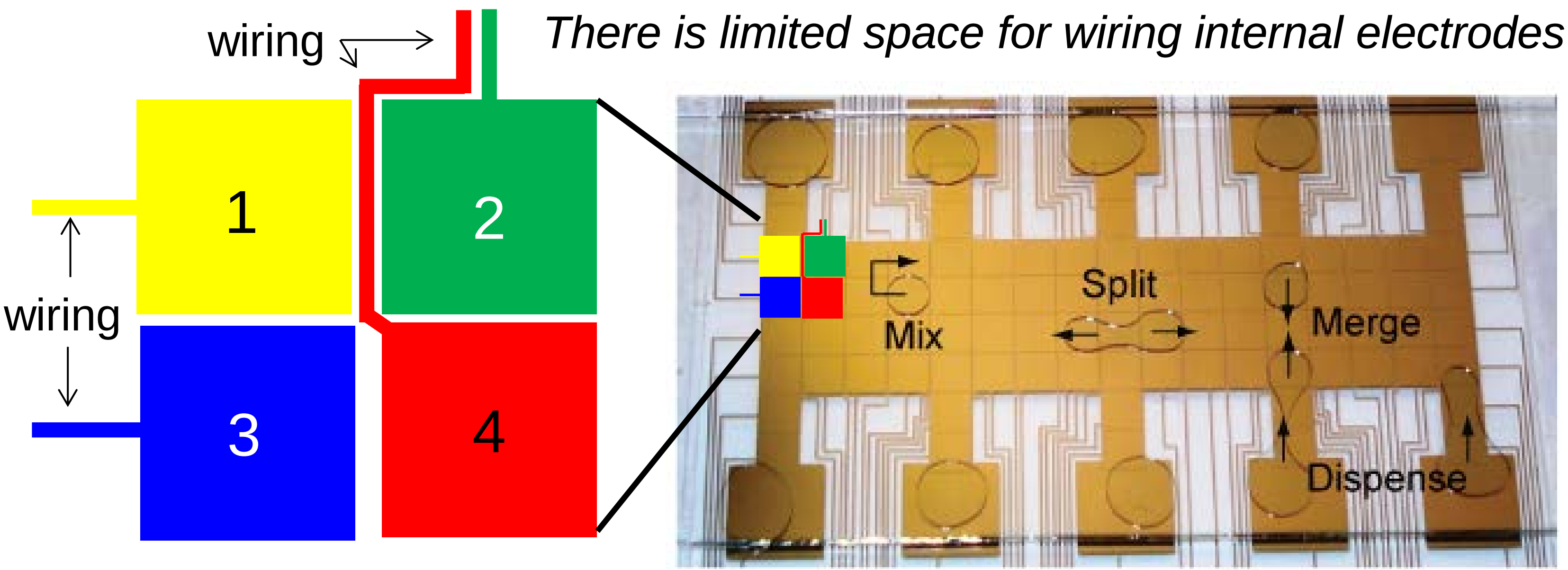
Alternate Mechanisms

- The most common actuation mechanism used in DMF relies on electrostatic forces (as above)
- But wiring complications represent a major challenge for electrostatic actuation

There is limited space for wiring internal electrodes



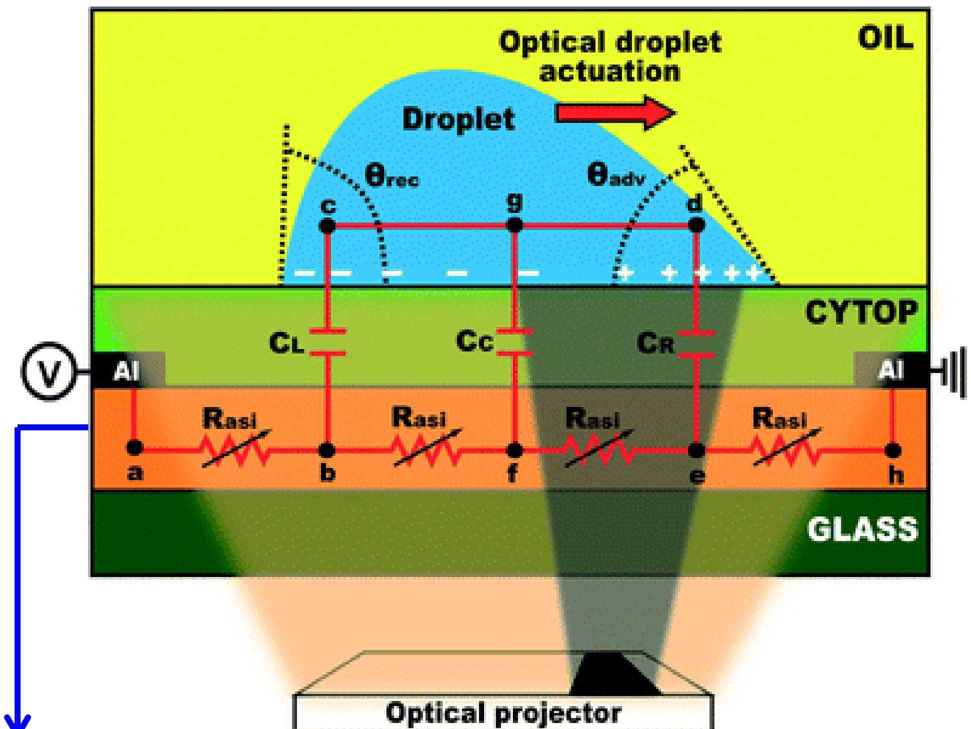
Note: one solution is to use printed circuit board (PCB) fab or other multilayer addressing methods

Alternate Mechanisms

- The most common actuation mechanism used in DMF relies on electrostatic forces (as above)
- But wiring complications represent a major challenge for electrostatic actuation
- Alternate mechanisms provide some relief from this problem, including:
  - Optical forces
  - Magnetic forces
  - Thermocap. forces
  - Acoustic forces

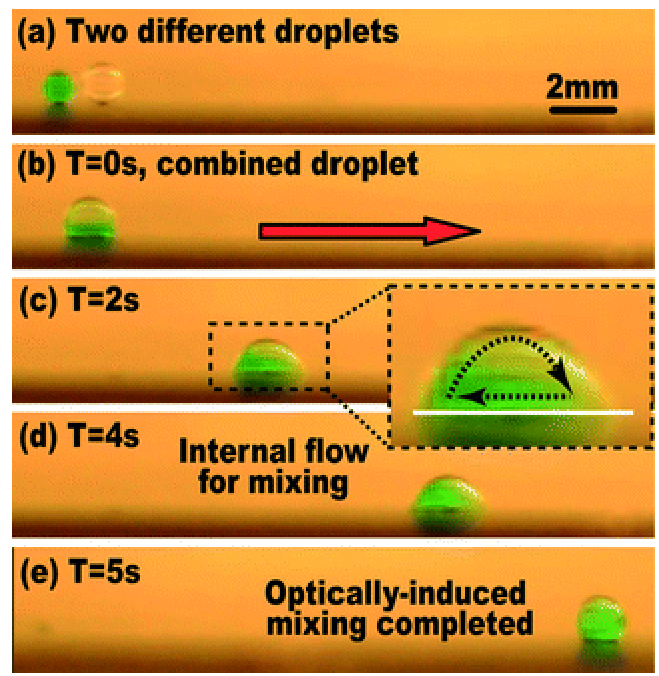
Optical DMF

- Device formed from photoconductor – when exposed to a pattern of light, the impedance of the exposed area is reduced (forming a virtual electrode)



Continuous photoconductive layer

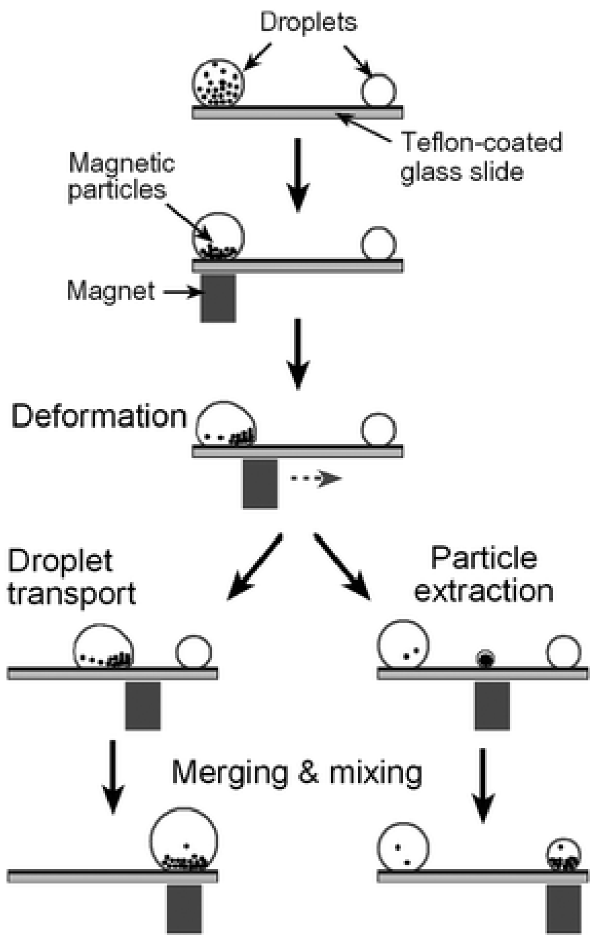
Droplet merging and mixing



Park et al. *Lab Chip*, 2010, *10*, 1655–1661

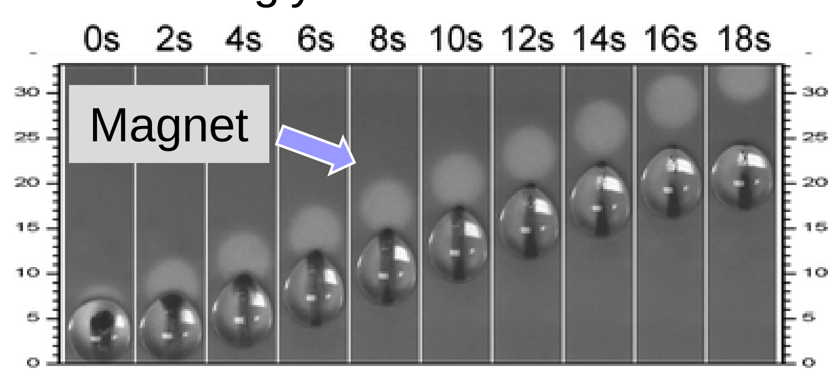
Magnetic DMF

- Droplets containing magnetic particles are actuated through the movement of magnets underneath a flat substrate



Droplet motion and particle extraction can be switched by changing magnet velocities and magnetic particle concentrations

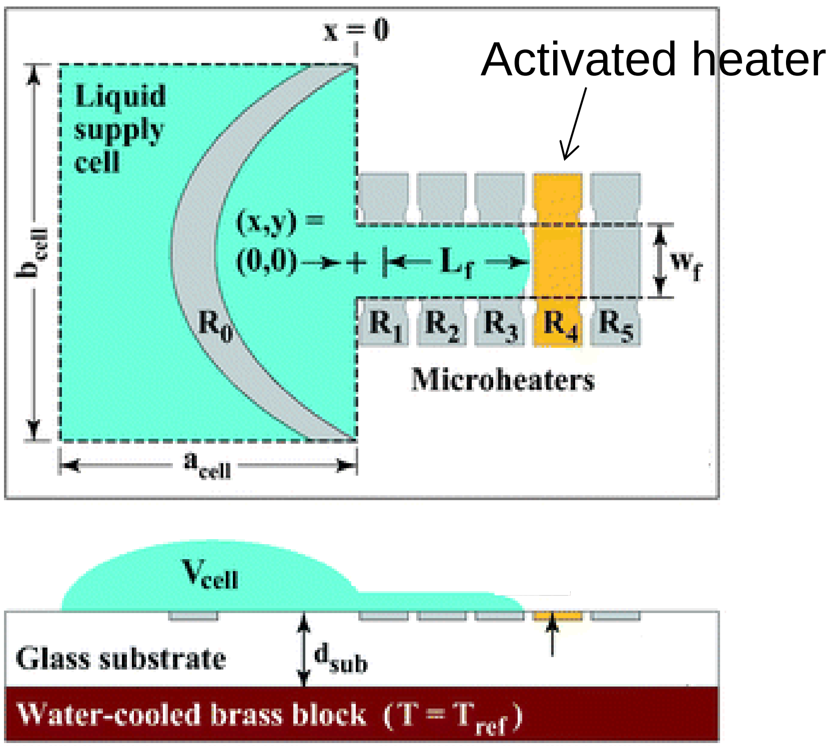
Actuation of droplet containing 50% glycerin in water



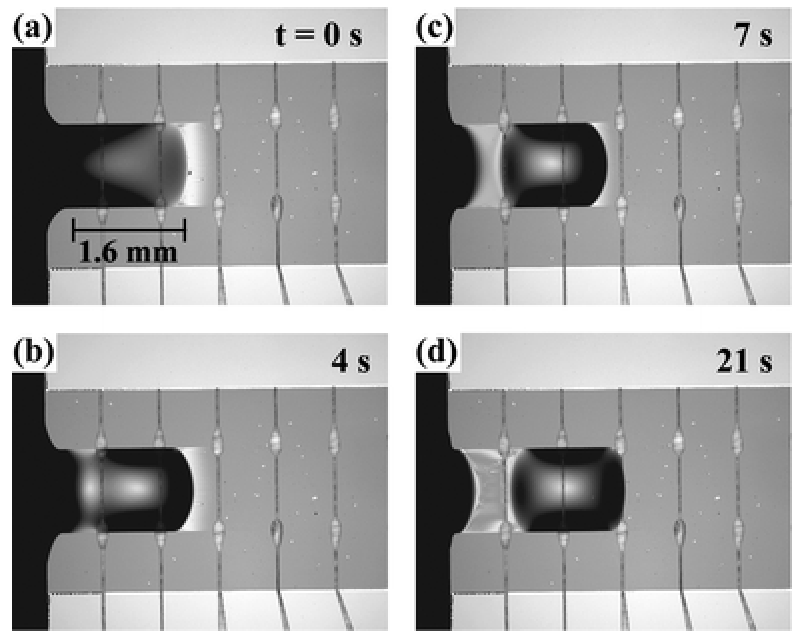
Long et al. *Lab Chip*, 2009, *9*, 1567–1575

Thermocapillary DMF

- Droplets are actuated by the variation of a liquid's surface tension with temperature, which is used to *pull* liquid toward cooler regions of the supporting substrate



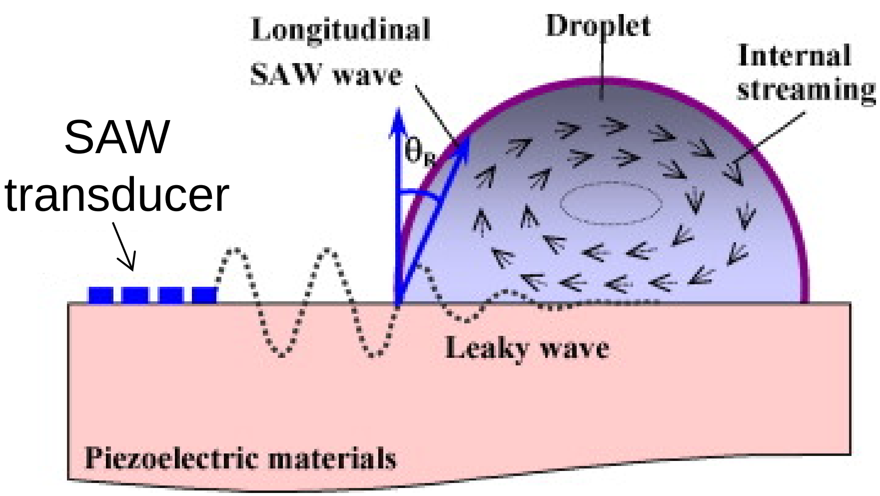
Droplet dispensing by activation of resistor

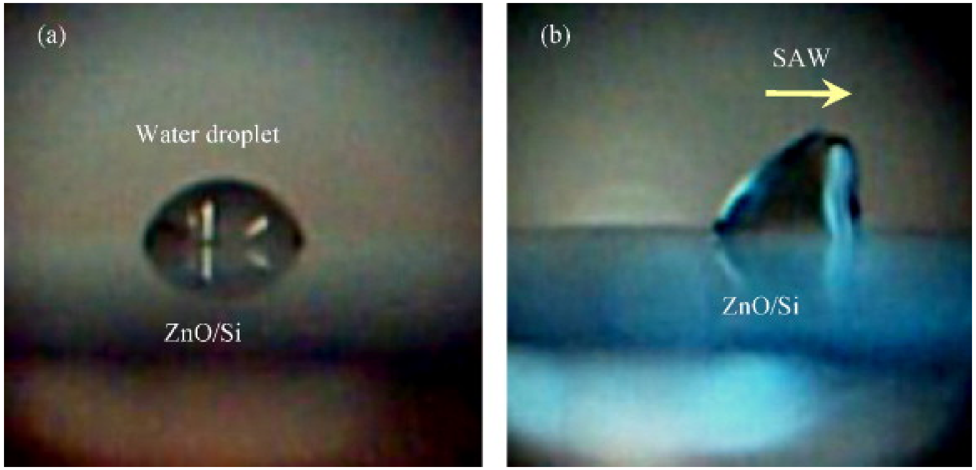


Darhuber et al. *Lab Chip,* 2010, *10,* 1061–1071

Acoustic DMF

- Surface acoustic waves (SAWs) generated by a high-frequency power source is propagated onto a droplet, which *pushes* the droplet along the surface





Internal agitation enhances the mixing and reaction rate

Fu et al. *Sens. Actuators. B*, 2010, *143*, 606-619

Alternate Mechanisms

- The most common actuation mechanism used in DMF relies on electrostatic forces (as above)
- But wiring complications represent a major challenge for electrostatic actuation
- Alternate mechanisms provide some relief from this problem, including:

	Advantage	Disadvantage
❑ Optical forces	No wiring problems	Requires ph.conductor and tunable imaging
❑ Magnetic forces	No wiring problems	Requires movable magnet and particles
❑ Thermocap. forces	Wiring less of a problem	Thermal gradients complicate fine control
❑ Acoustic forces	Wiring less of a problem	“Push” forces complicate fine control

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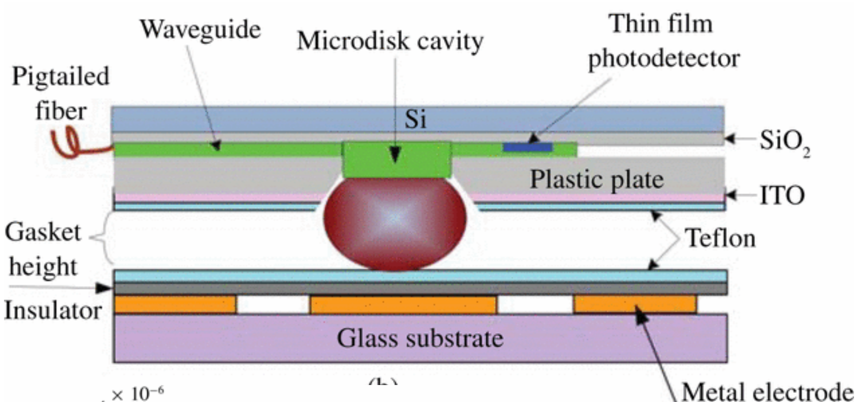
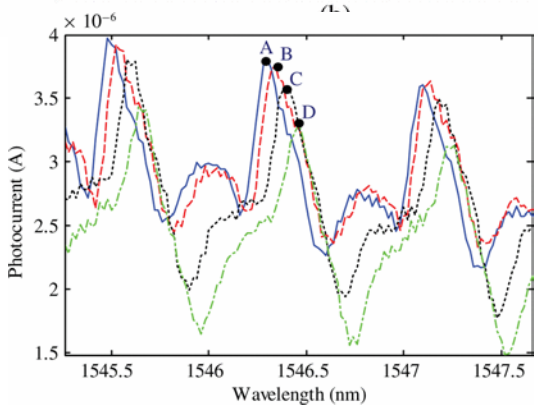
DMF: Integration and Sensors

- Lab-on-a-chip systems require more than just fluid-handling – they must be compatible with integration with sensors and other analytical techniques
- DMF is well-suited for integration and coupling with sensors, including:
  - Integrated optics
  - Modular optics
  - Surface plasmon resonance (SPR)
  - Electrochemistry
  - Sample Processing and Separations

Integrated Optical Techniques

- **Thin film photodetectors** with thin film polymer microresonator sensor was integrated

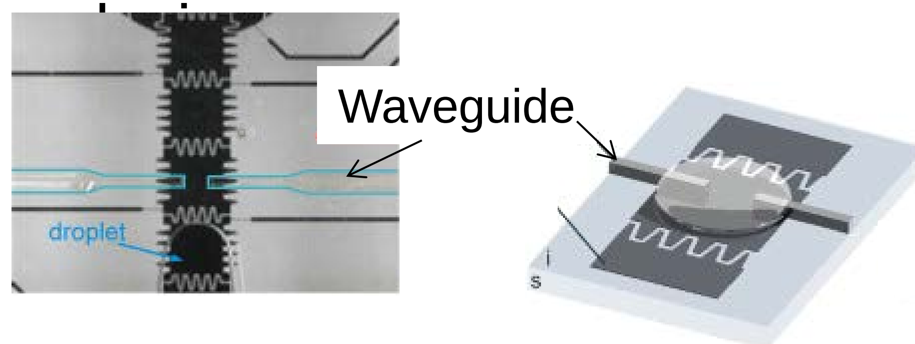




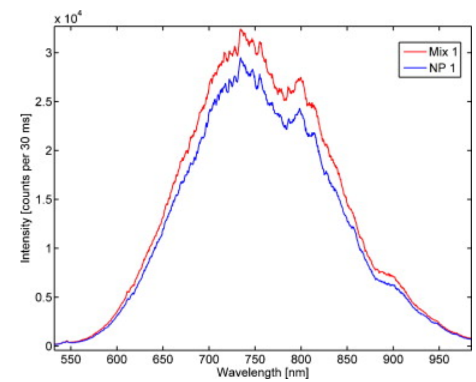
Spectra of glucose solutions

Luan et al. *IEEE Sens. J.* 2012, *12*, 1794-1800

- **Optical waveguides** was integrated on top of DMF



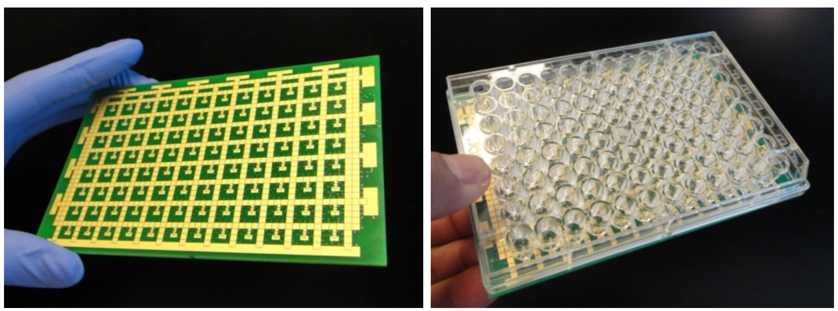
Transmission spectrum for a 1 μL droplet of a gold nanoparticle dispersion



Ceyssens et al. *Sens. Actuators B*. 2013, *181*, 166-171

Modular Optical Techniques

- Integrated sensors are useful, but modular instruments are also powerful – the device can be used and discarded, while the detector be used again and again



DMF device in mutliwell plate format

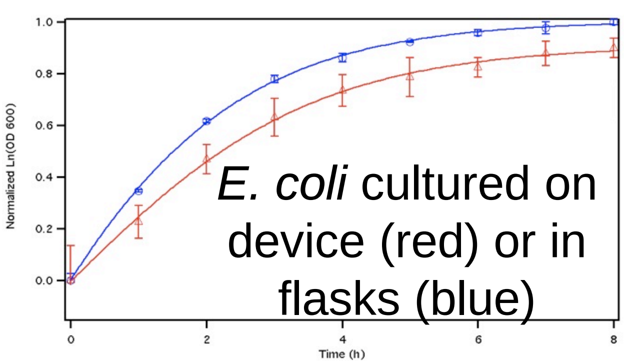
Plate Reader

- The ability to use off-the shelf detectors (such as a plate reader) is an advantage for translation of DMF methods to end users

Modular Optical Techniques

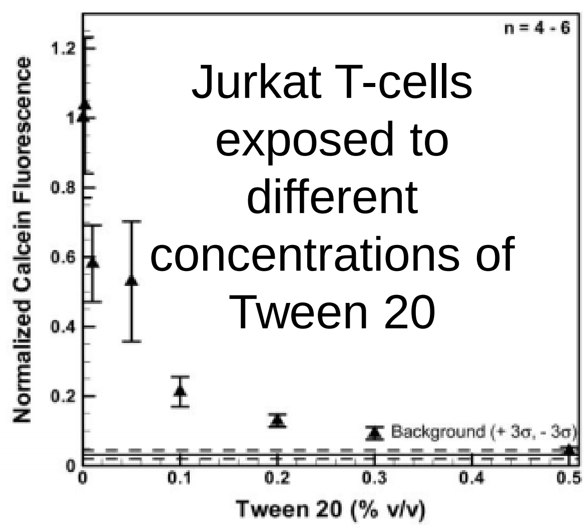
- The modular approach for optical detection (combining DMF and a plate reader) has been used widely in varying modes, including:

Absorbance
for bacterial O.D. measurements



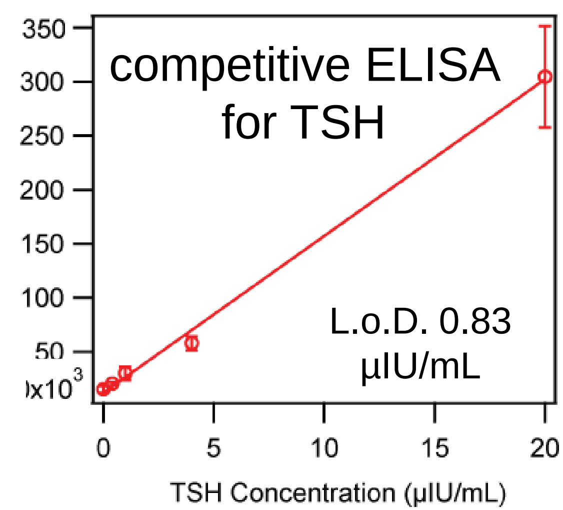
Au, Shih, Wheeler, *Biomed. Midcrodev.* 2011, *13*, 41-50

Fluorescence
for mammalian cell live/dead assays



Barbulovic-Nad et al, *Lab Chip,* 2008, *8*, 519-526

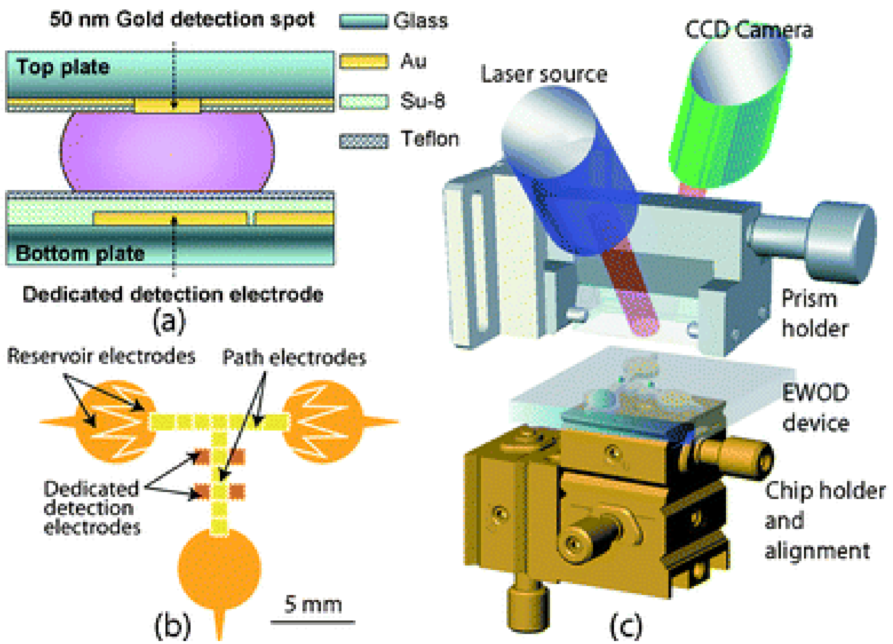
Chemiluminescence
for immunoassays

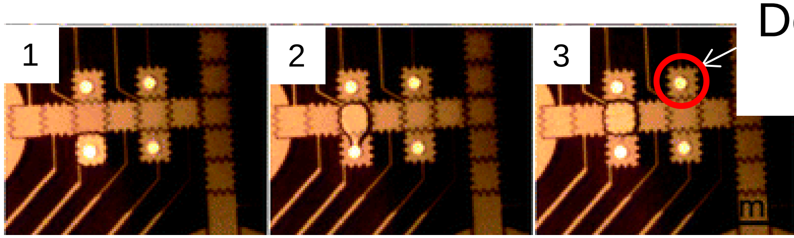


Ng et al. *Anal. Chem.,* 2012, *84*, 8805-8812

Surface Plasmon Resonance

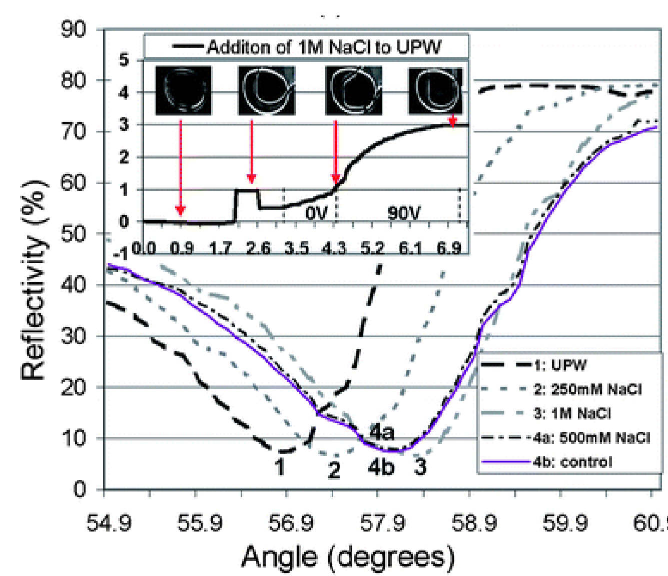
- SPR coupled with DMF enables real-time, label-free analysis on array of detection points





Detection spot

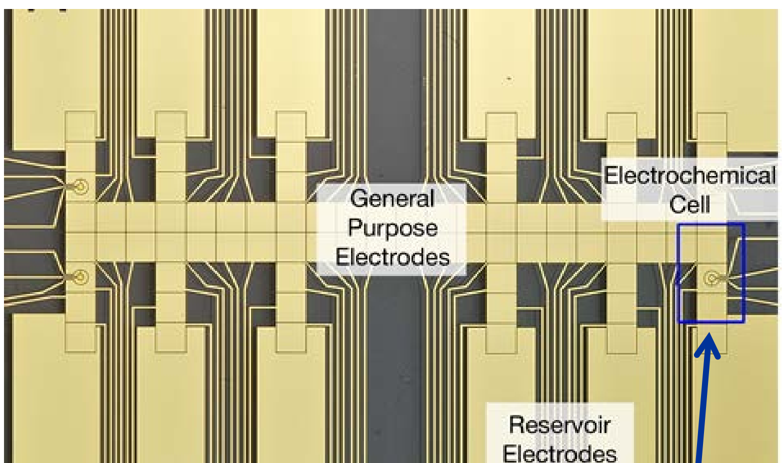
SPR curves for each sample prior to and following the merging of ultra-pure water with 1 M NaCl droplet.



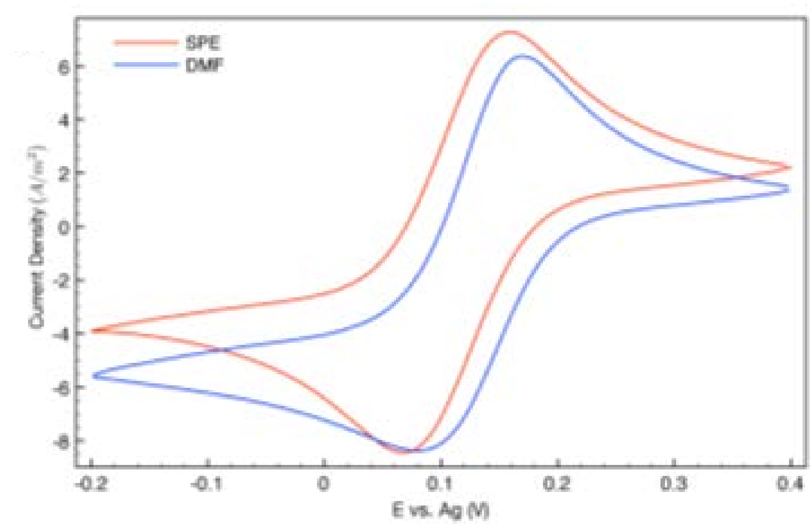
Malic et al. *Lab Chip,* 2009, *9,* 473-475

Electrochemistry

- Electrochemistry is an obvious match for DMF, but (strangely) there are only a few reports of this combination – an example:



Cyclic voltammograms of potassium hexacyanoferrate on the DMF system (blue) and a commercial screen printed electrode (SPE) (red)

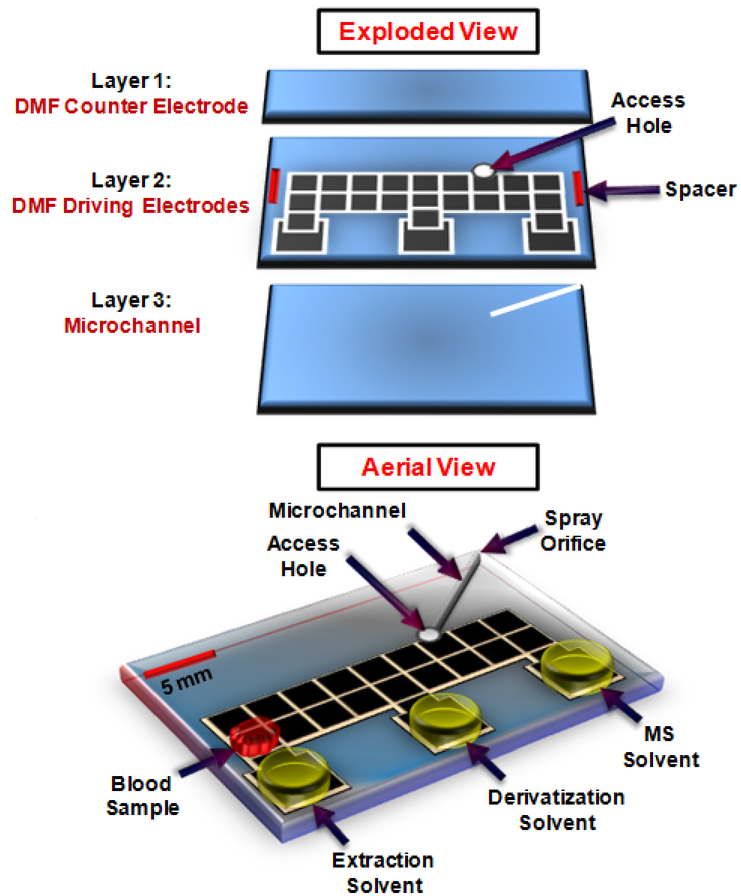


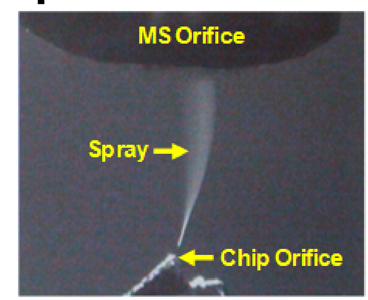
working, common, and reference electrodes integrated into the bottom plate

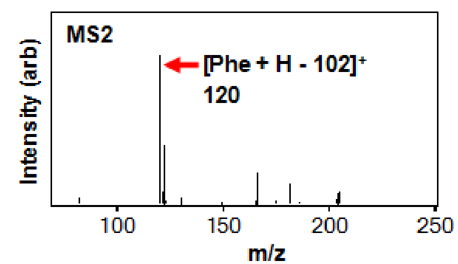
Dryden et al., *Anal. Chem.* 2013, *85*, 8809-8816

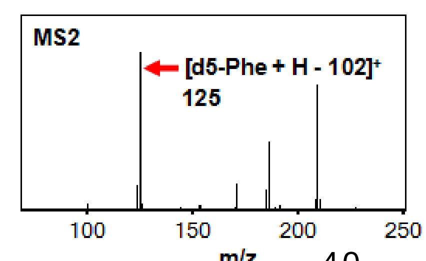
Mass Spectrometry

- Mass spectrometry (MS) is not an obvious instrumental match, but the tedious sample prep. required for MS has made DMF-MS popular
- Example 1: an integrated nESI emitter







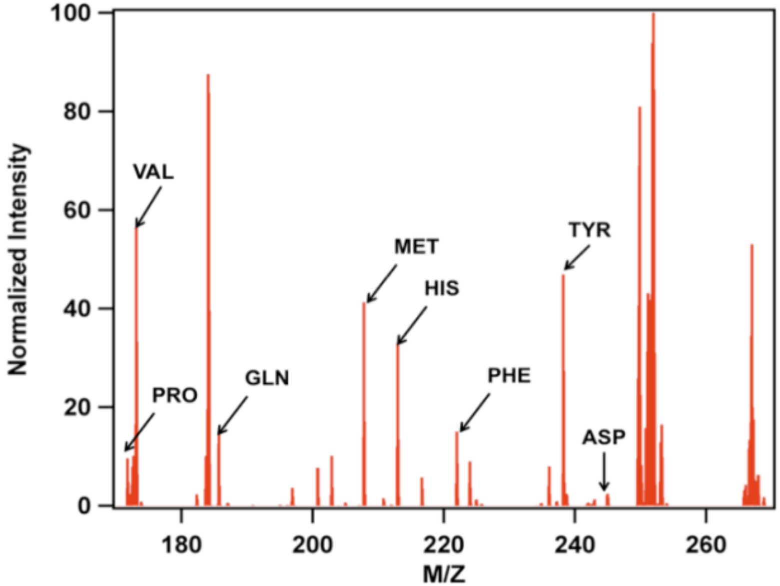


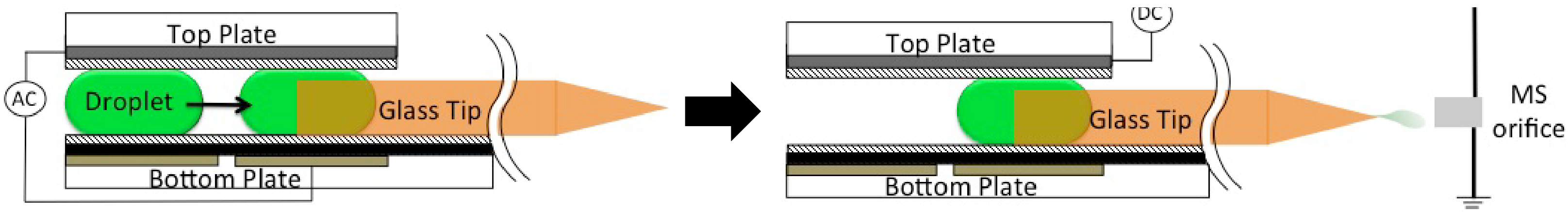
Jebrail and Yang et al. *Lab on a Chip* 2011, 1: 3218-3224

Mass Spectrometry

- Mass spectrometry (MS) is not an obvious instrumental match, but the tedious sample prep. required for MS has made DMF-MS popular
- Example 2: pulled-glass capillary emitter

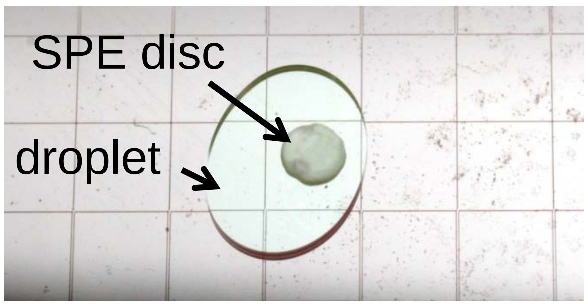
Shih and Yang et al., *Anal. Chem.* 2012, *84*, 3731−3738



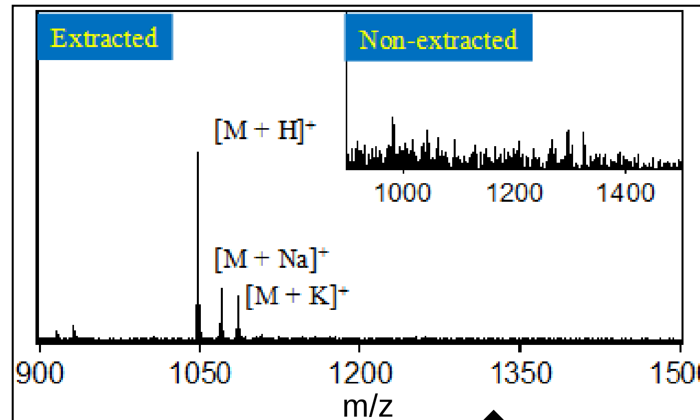


Sample Proc. and Separations

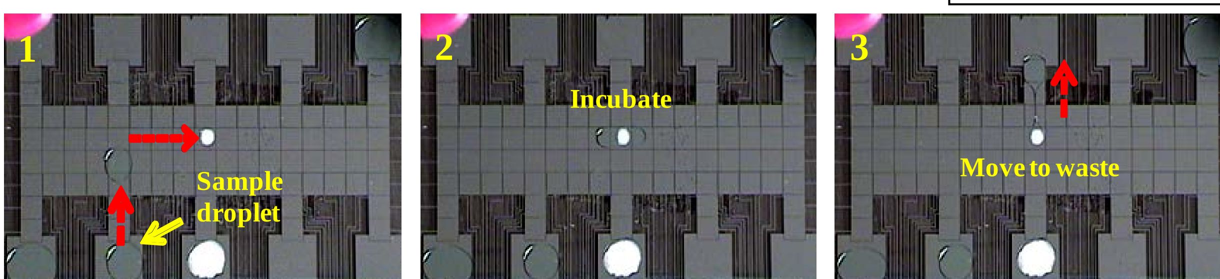
- DMF is useful for a wide range of sample processing steps, including solid phase extraction (SPE):



Yang and Mudrik et al. *Anal. Chem.* 2011, 83, 3824-3830

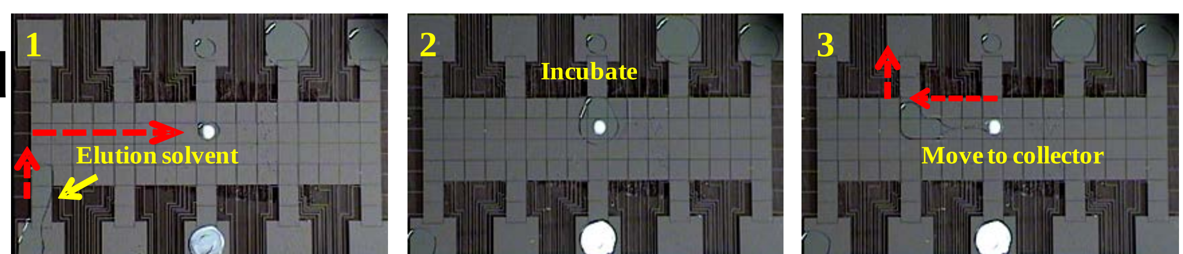


ESI-MS spectra of angiotensin in 100 mM NaCl



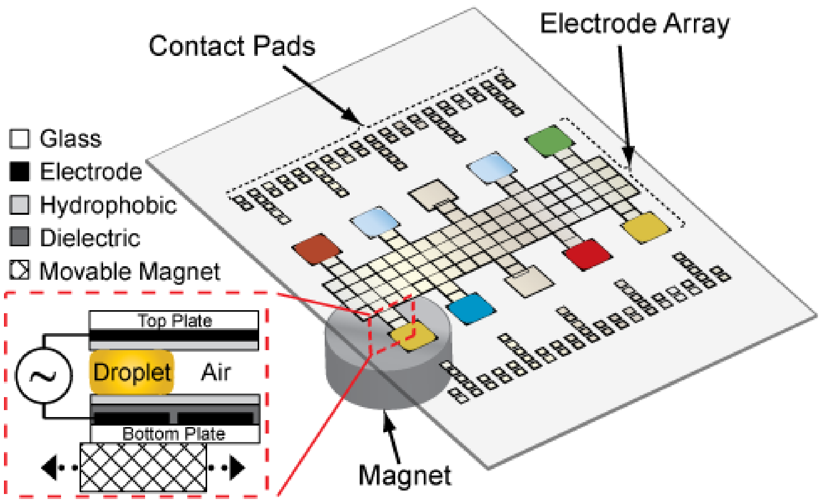
Sample load

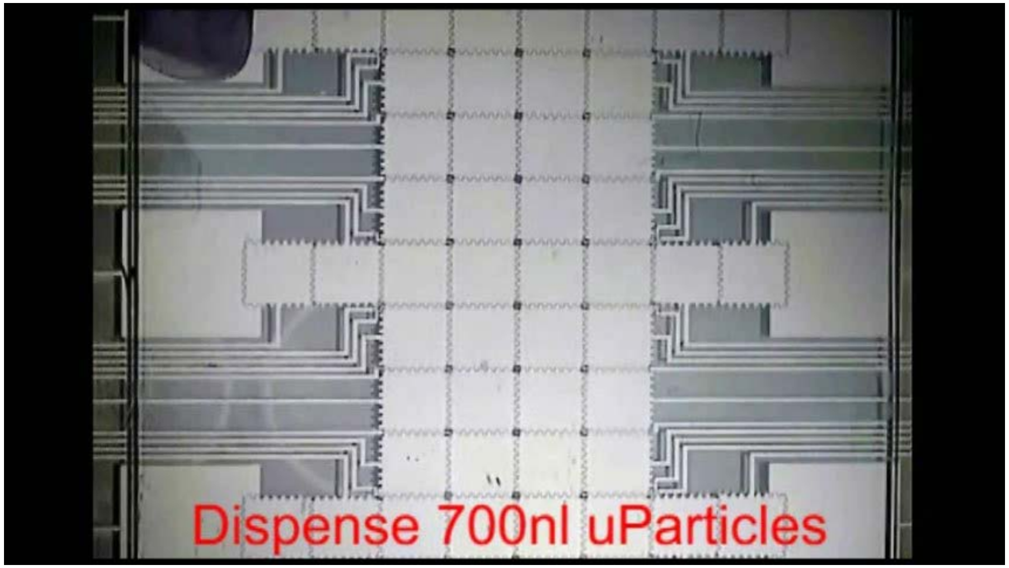
Sample elute



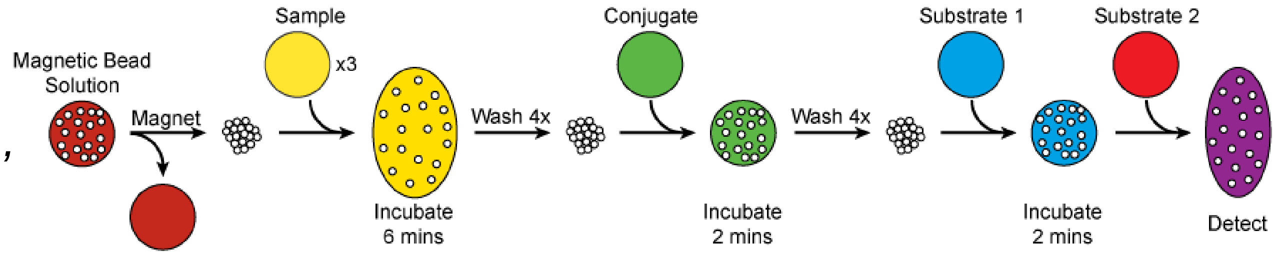
Sample Proc. and Separations

- DMF is useful for a wide range of sample processing steps, including solid phase extraction (SPE), magnetic bead pulldown:





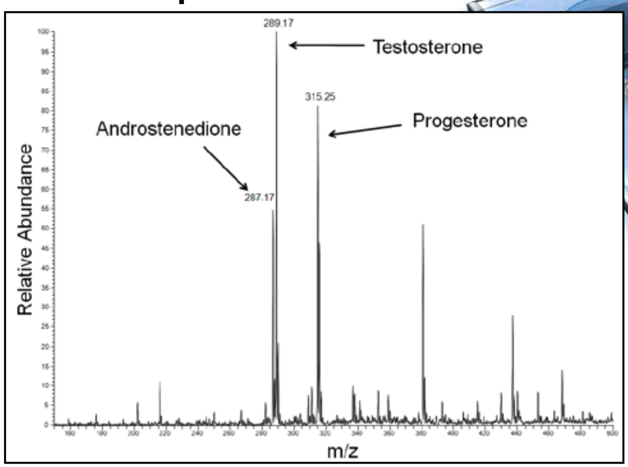
Ng et al. *Anal. Chem.*, 2012, *84*, 8805-8812

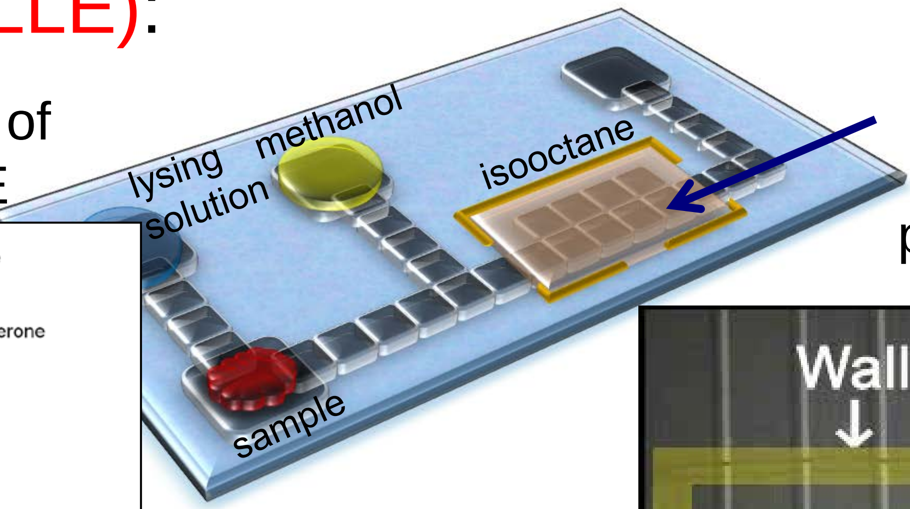


Sample Proc. and Separations

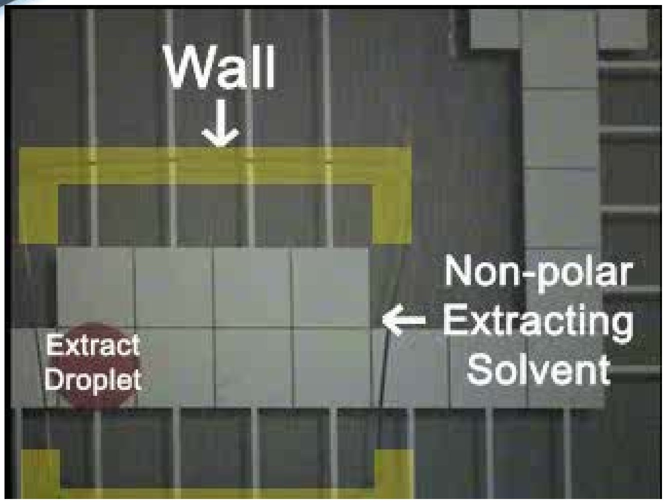
- DMF is useful for a wide range of sample processing steps, including solid phase extraction (SPE), magnetic bead pulldown, and liquid-liquid extraction (LLE):

ESI-MS spectrum of droplet after LLE





isooctane pool corralled in a photoresist wall

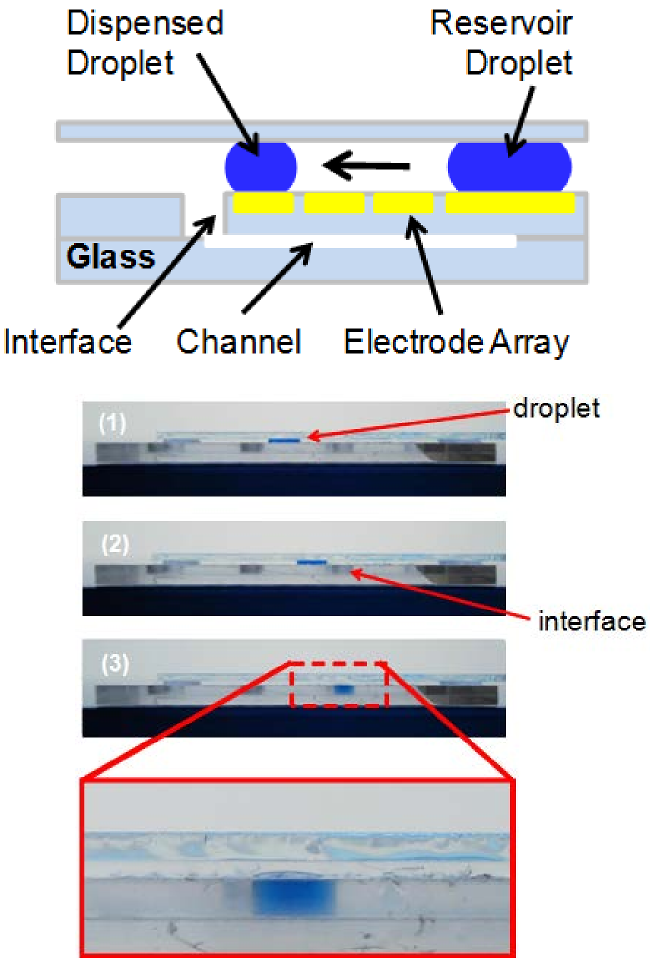


methanolic droplet translated through a pool of isooctane to extract unwanted non-polar constituents

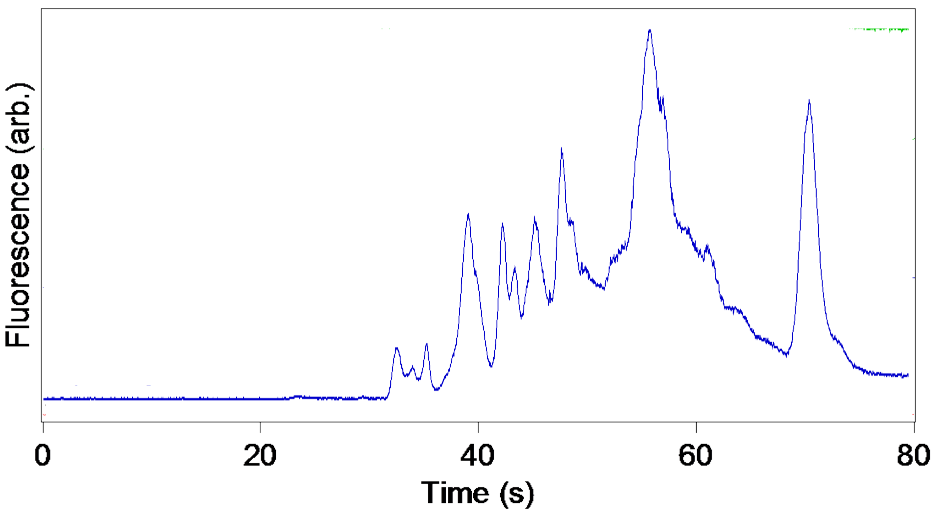
Mousa, Jebrail, et al. *Sci. Trans. Med,* 2009, *1*, 1ra2

Sample Proc. and Separations

- For chemical separations, one can interface DMF with microchannels…



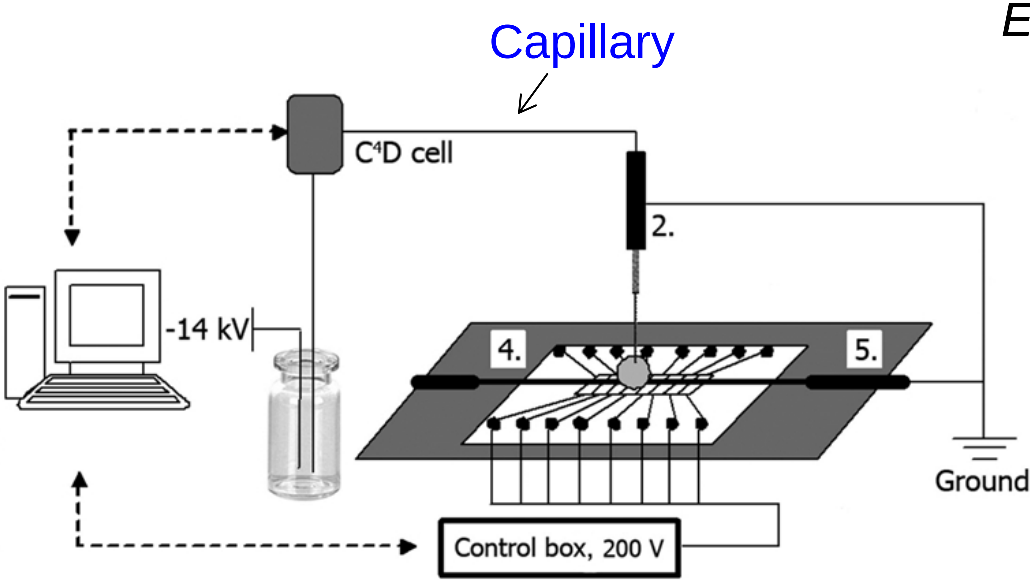
Electropherogram of protein sample digested on-chip



Watson et al. *Anal. Chem.*, 2010, *82*, 6680-6686

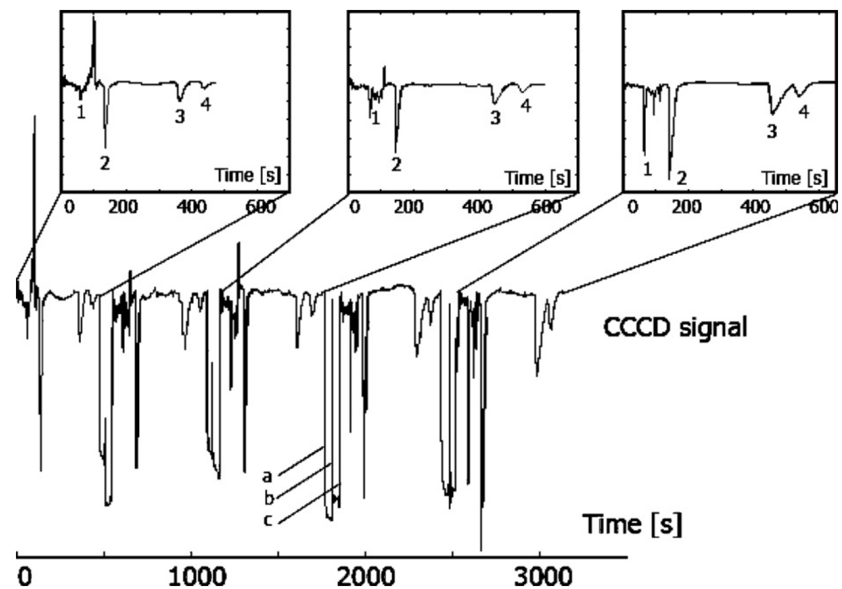
Sample Proc. and Separations

- For chemical separations, one can interface DMF with microchannels or with a capillary electrophoresis system…



C^{4}D: contactless conductivity detection

Electropherograms of vitamins mixture



Gorbatsova et al. *Anal. Chem.*, 2009, *81*, 8590-8595

Digital Microfluidics - Outline

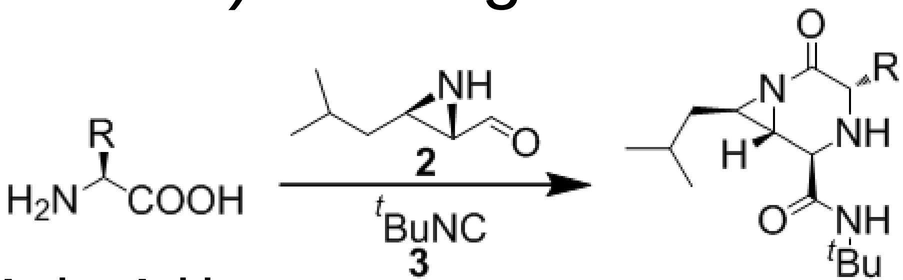
- DMF: Introduction and Theory
  - *DMF vs. Microchannels, DMF vs. Droplets-In-Channels, General Mechanism, Two-Plate vs. One-Plate, Electrowetting-on-Dielectric, Electromechanical Model*
- DMF: Alternate Mechanisms
  - *Wiring Complications, Optical Forces, Magnetic Forces, Thermocapillary Forces, Acoustic Forces*
- DMF: Integration and Sensors
  - *Integrated Optics, Modular Optics, SPR, Electrochemistry, Mass Spectrometry, Sample Processing and Separations*
- DMF: Applications
  - *Synthesis, Genomics, Proteomics, Diagnostics, Cell Culture*
- Final Notes
  - *Accessibility, A Look to the Future*

DMF: Integration and Sensors

- The unique properties of digital microfluidics make the technology useful for a wide range of applications
- Examples include:
  - Chemical Synthesis
  - Genomics
  - Proteomics
  - Diagnostics
  - Cell Culture

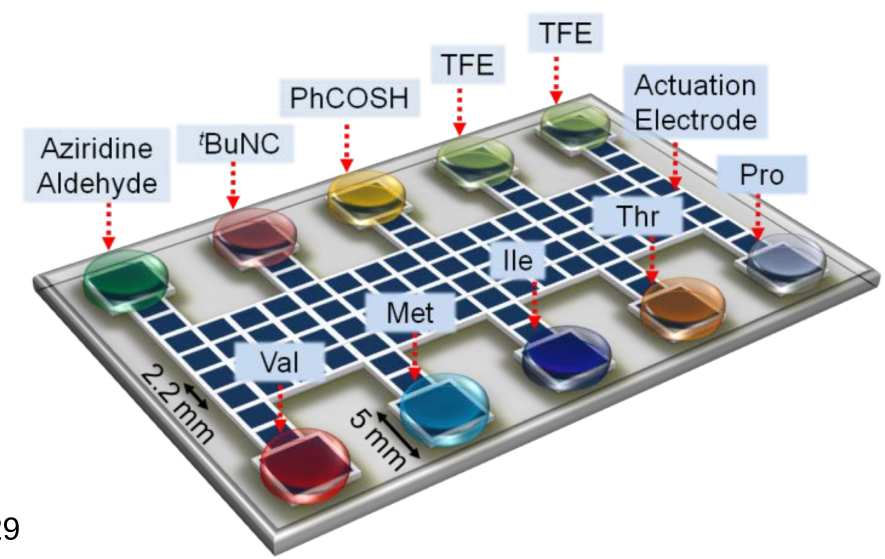
DMF-Synthesis Example One

- There is great interest in the development of libraries of peptide macrocycles (which resist digestion *in vivo*) as drug candidates



Amino Acid **Peptide Macrocycle**

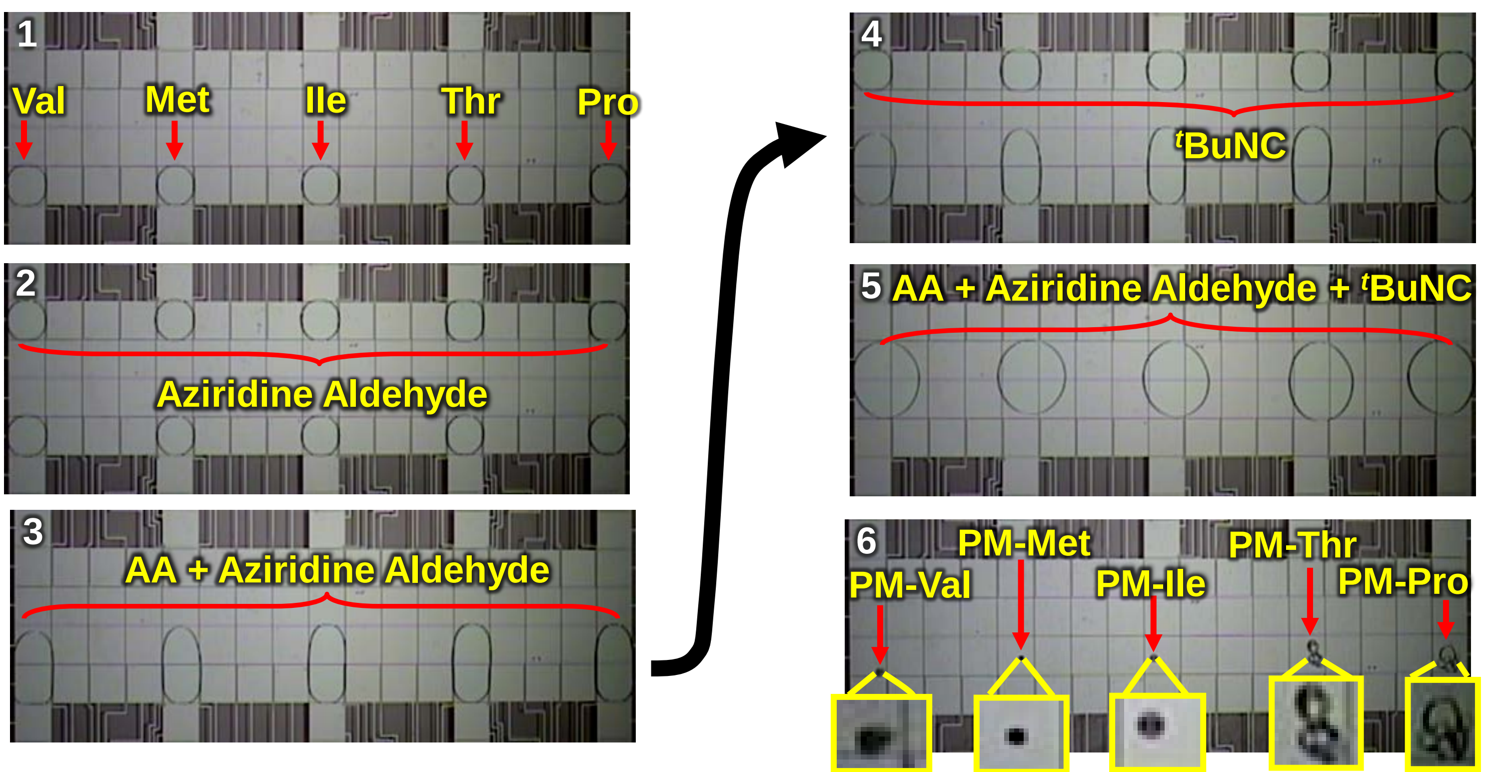
device designed for synchronized synthesis of five peptide macrocyles



Jebrail, et al. *Angew. Chemie,* 2010, *49*, 8625-8629

DMF-Synthesis Example One

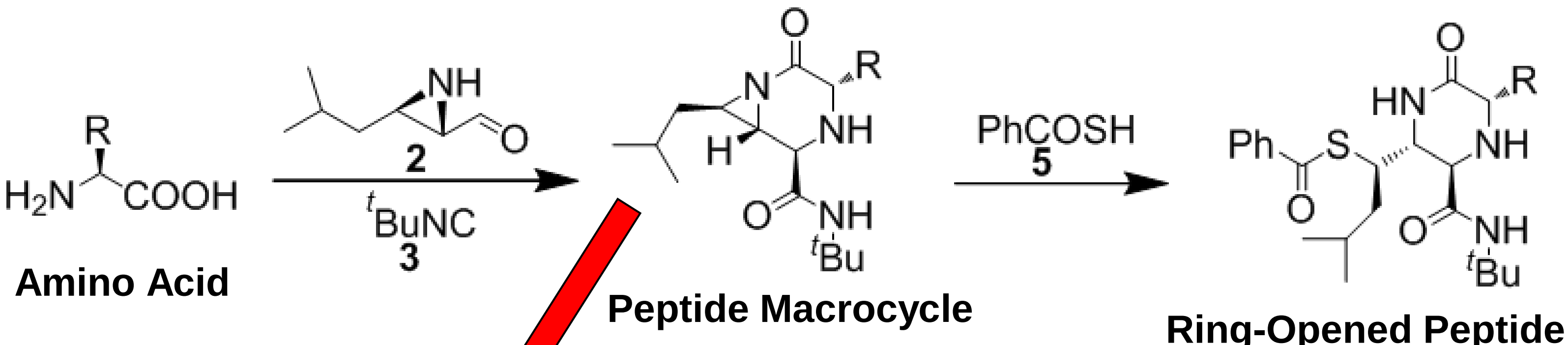
- There is great interest in developing libraries of peptide macrocycles (which resist digestion *in vivo*) as drug candidates

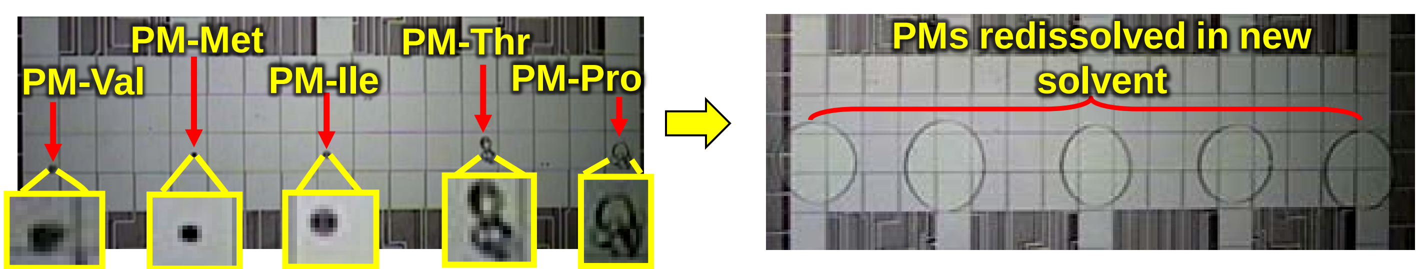


Jebrail, et al. *Angew. Chemie,* 2010, *49*, 8625-8629

DMF-Synthesis Example One

- DMF is particularly useful for solvent exchange, in which the old solvent is removed or evaporated, followed by dissolution in new solvent (challenging for microchannels)

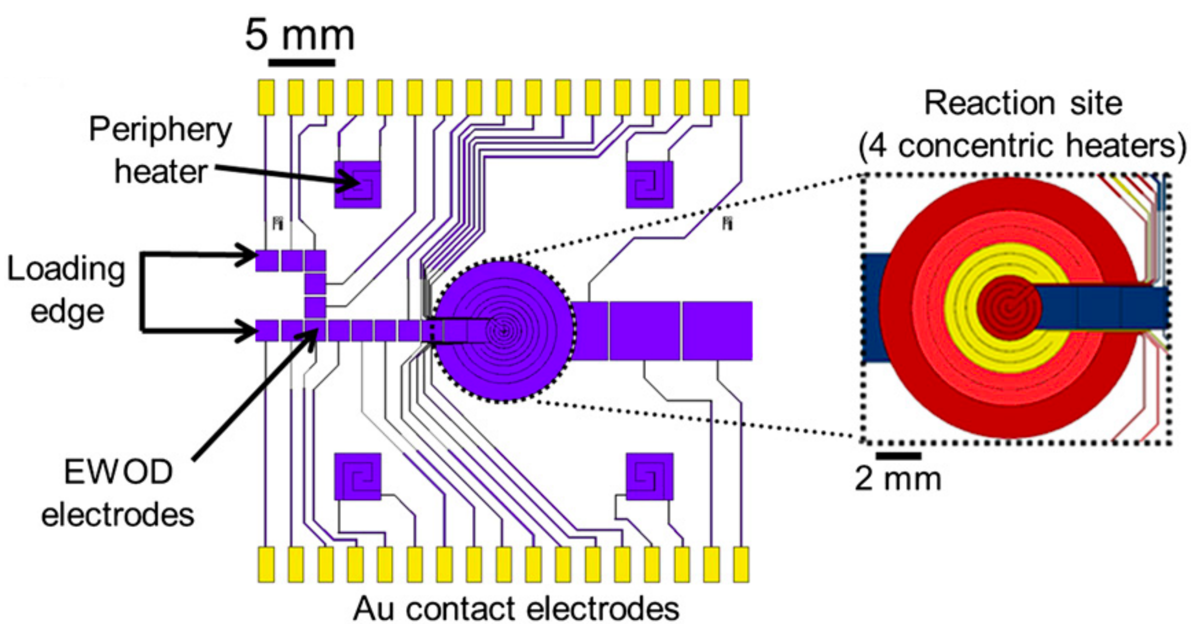




Jebrail, et al. *Angew. Chemie,* 2010, *49*, 8625-8629

DMF-Synthesis Example Two

- It would be useful to be able to generate radio-tracers (used for imaging) in hospitals, as they have short half-lives (and must be used quickly)



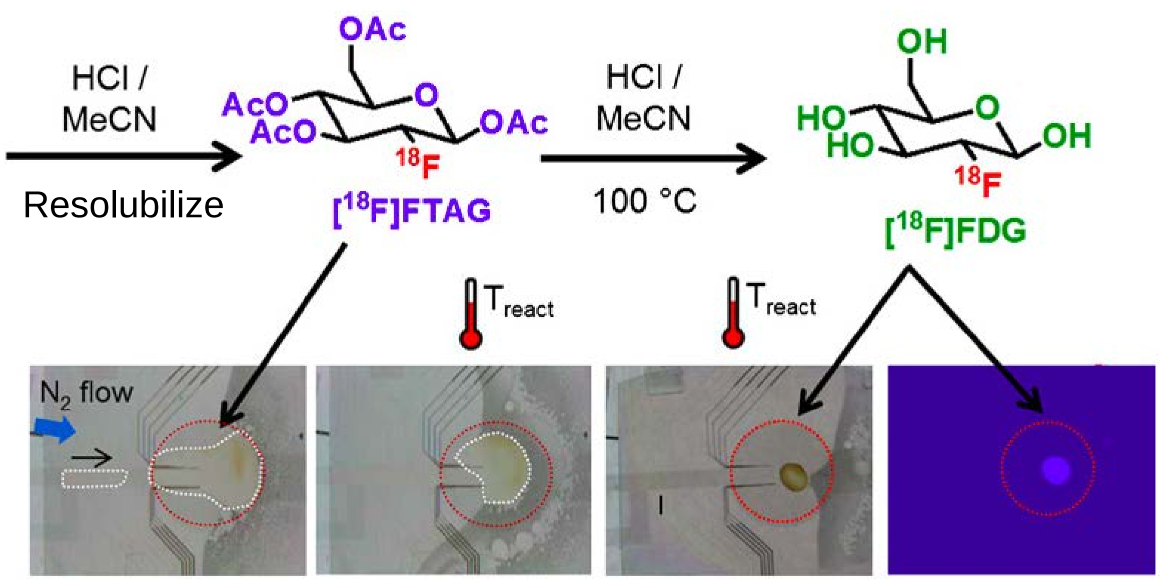
Multifunctional reaction site:

1) *Resistive Heater*
2) *Temperature Sensor w/ Feedback control*
3) *Droplet transport*

Keng, et al. *PNAS*, 2012*, 109,* 690-695

DMF-Synthesis Example Two

- It would be useful to be able to generate radio-tracers (used for imaging) in hospitals, as they have short half-lives (and must be used quickly)
- Synthesis of 2-[18F]fluoro-2-deoxy-D-glucose ([18F]-FDG) requires 4 solvent exchange steps



Keng, et al. *PNAS*, 2012, *109,* 690-695

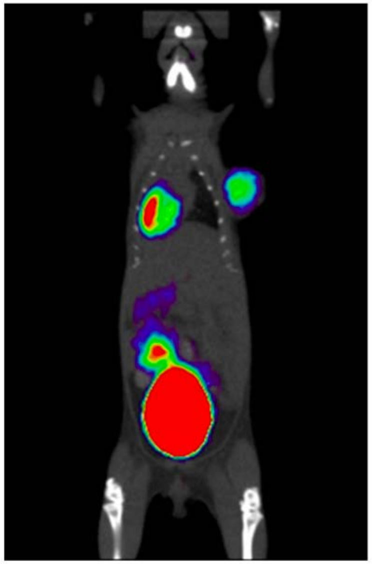
DMF-Synthesis Example Two

- It would be useful to be able to generate radio-tracers (used for imaging) in hospitals, as they have short half-lives (and must be used quickly)
- *In vivo* biodistribution of tracer in mouse with a tumor xenograft on shoulder

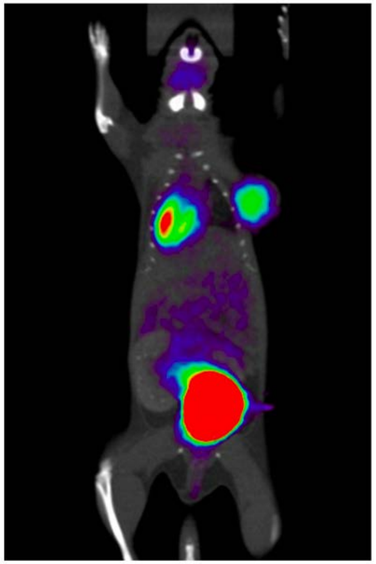
Organ	% Uptake (EWOD FDG)	% Uptake (cyclotron facility FDG)
Whole body	100	100
Heart	1.6	1.5
Tumor	2.8	4.6
Left kidney	1.2	1.2
Right kidney	1.2	1.3
Bladder	43.4	43.5

Tracer synthesized on-chip was comparable to ones synthesized in macroscale

Microscale



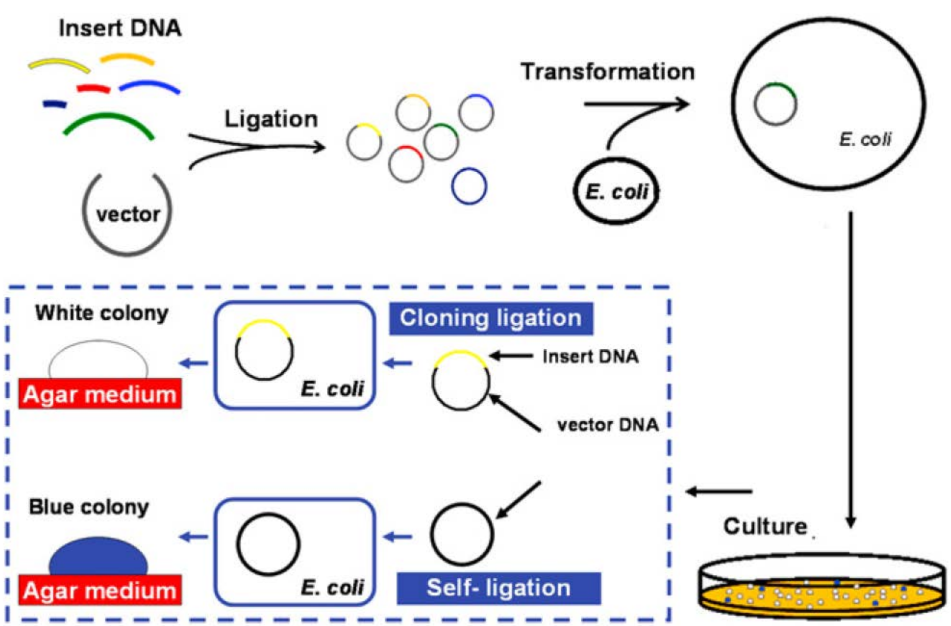
Macroscale

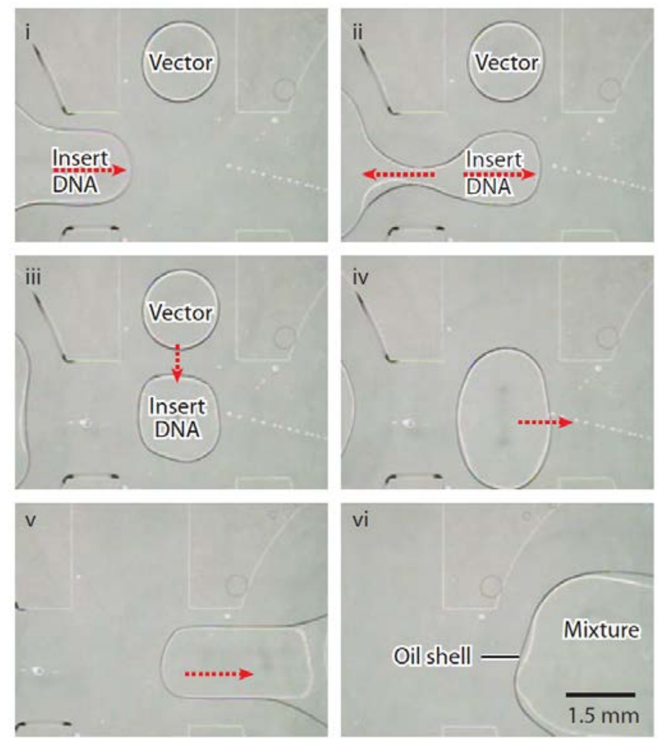


Keng, et al. *PNAS*, 2012*, 109,* 690-695

DMF-Genomics Example One

- DNA ligation to generate recombinant DNA are critical steps in cloning
- These tedious steps can be automated by DMF

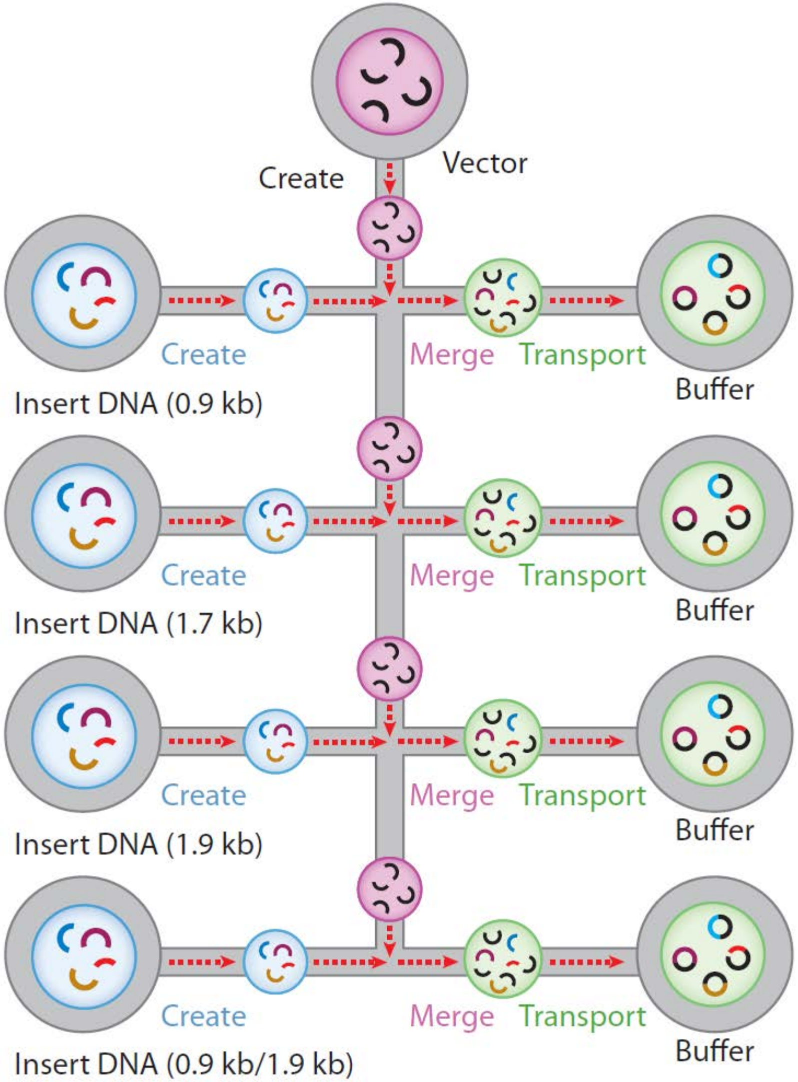




Lin et al. *J. Assoc. Lab. Autom*. 2010, *15*, 210-215

DMF-Genomics Example One

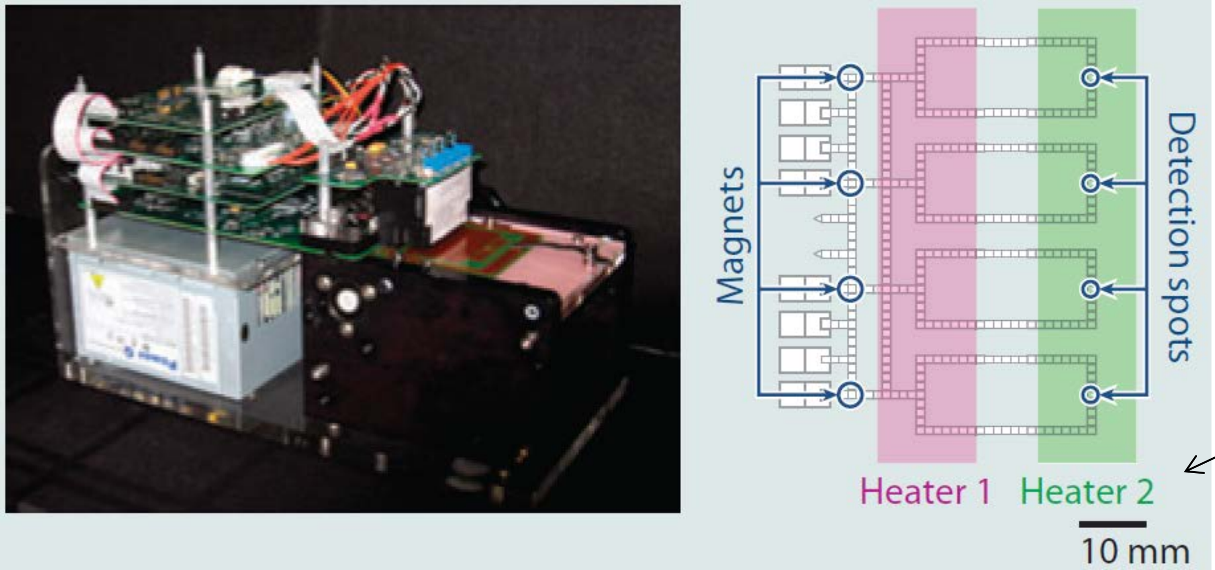
- The DMF device enables:
  - 4 parallel reactions
  - Reagent volume reduced 7-fold
  - Optimized and reduced total reaction time to just 5 minutes



Lin et al. *J. Assoc. Lab. Autom*. 2010, *15*, 210-215

DMF-Genomics Example Two

- There is great need for qPCR-based tools to identify infectious microorganisms like MRSA, *Mycoplasma pneumoniae*, and *Andida albicans*
- A DMF-enabled instrument makes this convenient (perhaps useful for POC analysis)

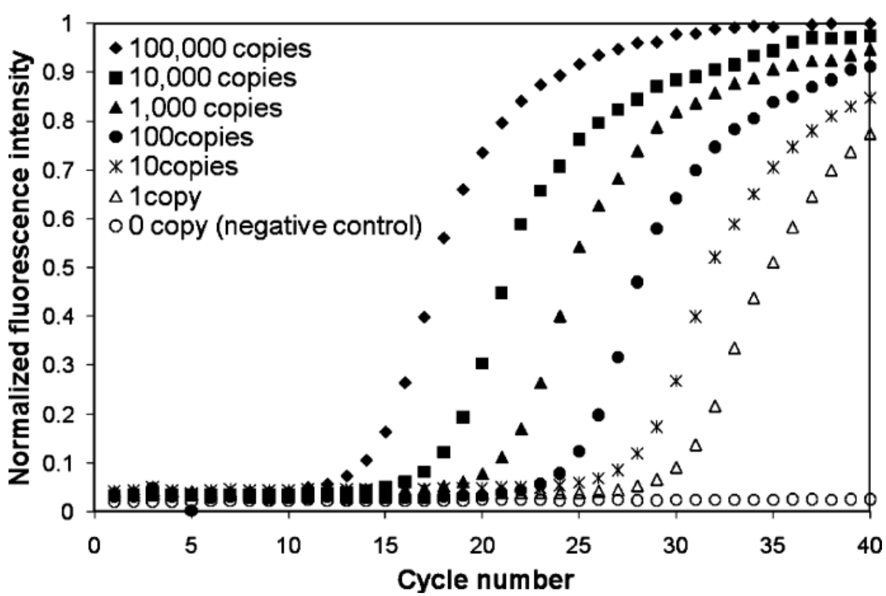


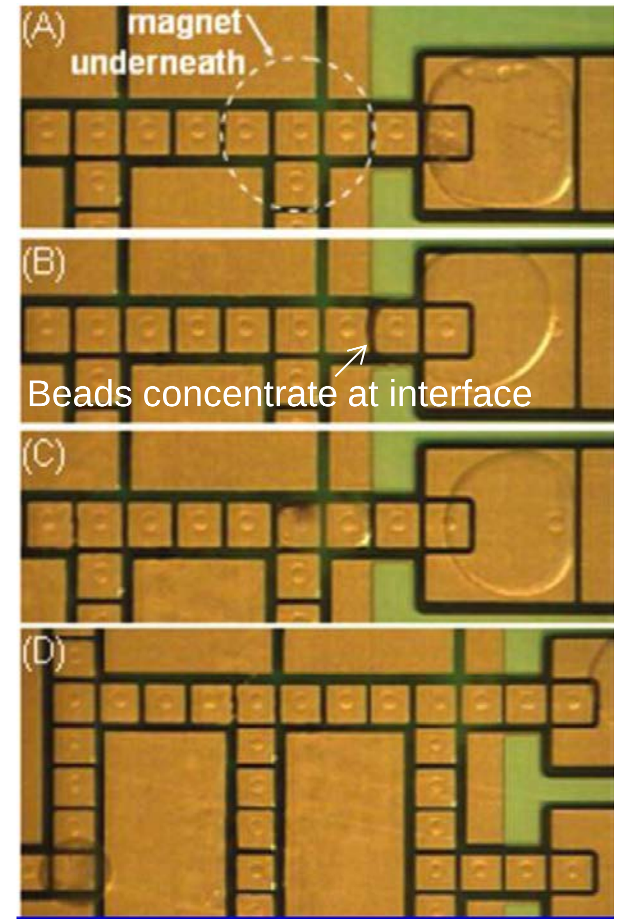
Drops shuttled between different temperatures for PCR amplification

Hua et al. *Anal. Chem.* 2010, *82*, 2310-2316

DMF-Genomics Example Two

- DNA analytes are extracted using magnetic bead-based isolation…
- …and an amplification efficiency of 94.7% allows for the detection of (the equivalent of) a single MRSA bacterium

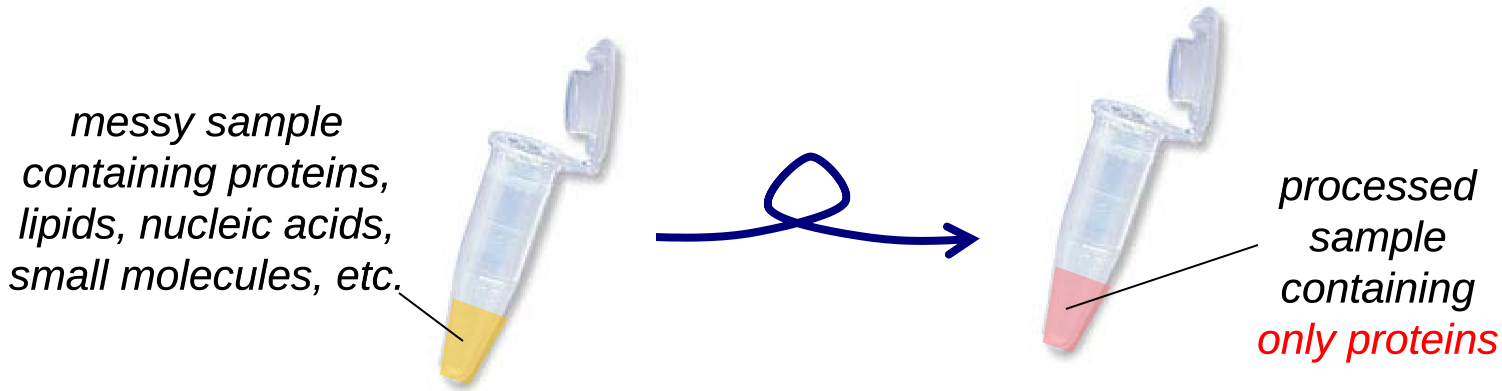




Hua et al. *Anal. Chem.* 2010, *82,* 2310-2316

DMF-Proteomics Example One

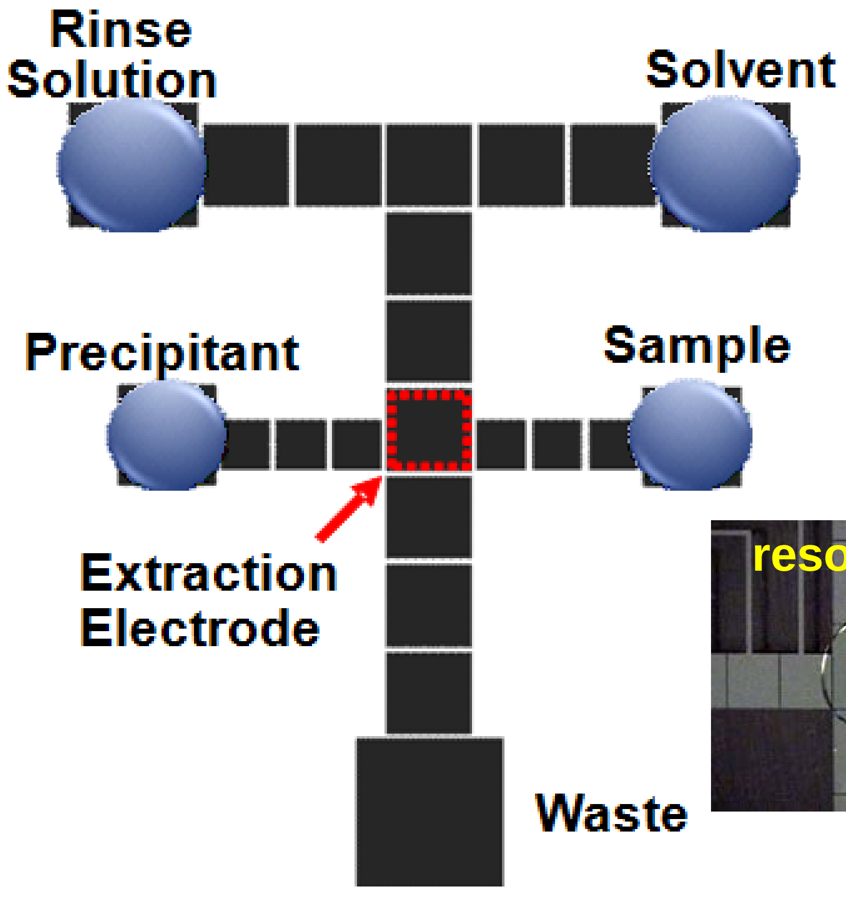
- The first step in proteomic analysis of “real” samples is sample purification

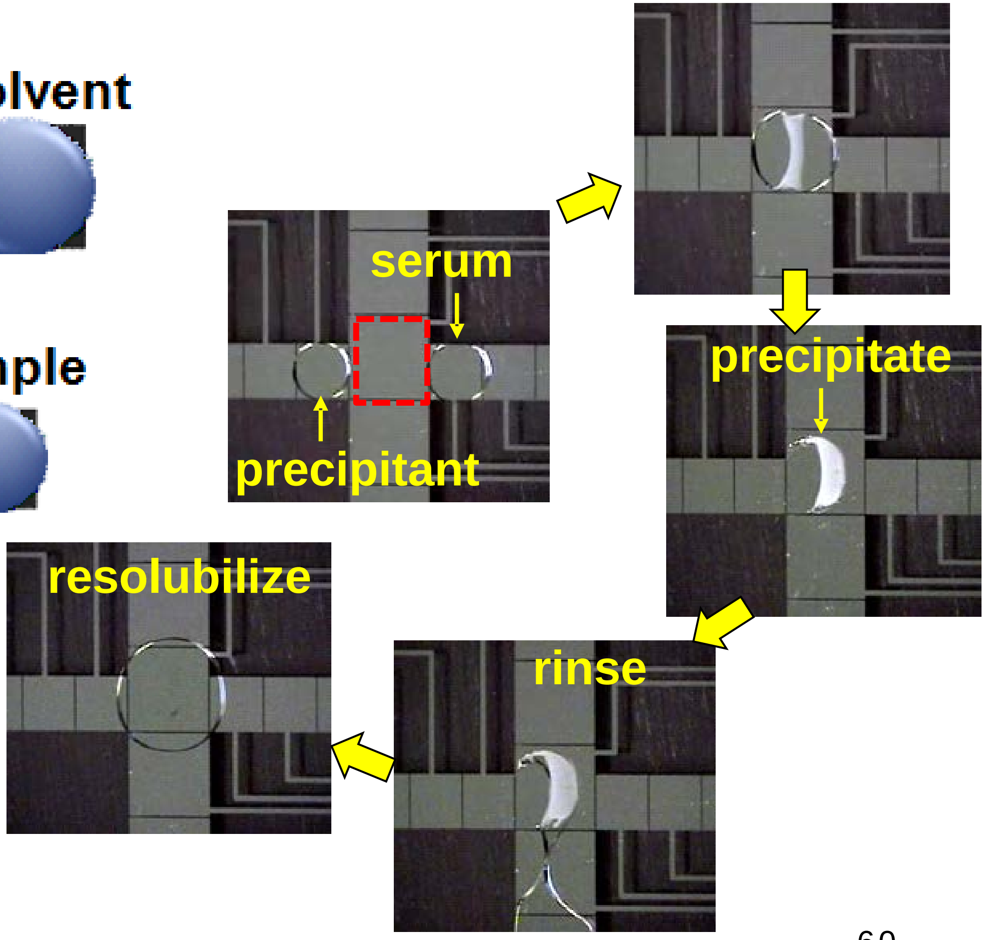


- Classically, this is achieved by precipitating proteins, disposing soluble constituents, and resolubilizing the proteins. This is fairly arduous (multiple centrifugation and drying steps), etc.

DMF-Proteomics Example One

- An automated DMF-enabled method achieves the same goal (with no centrifuge required!)

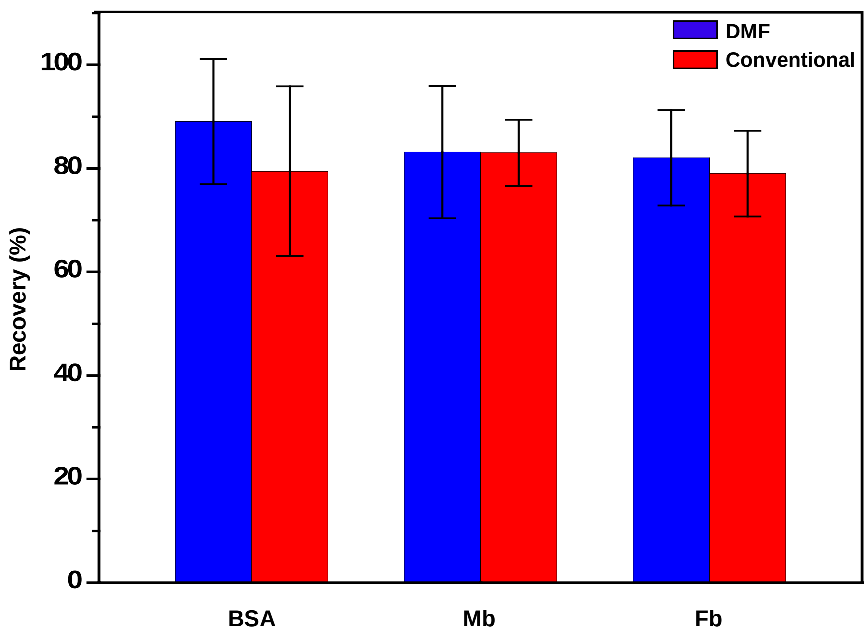




Jebrail and Wheeler *Anal. Chem.*, 2009, 81, 330-335

DMF-Proteomics Example One

- An automated DMF-enabled method achieves the same goal (with no centrifuge required!)
- % recovery by the DMF method (blue) is indistinguishable from that of conventional techniques (red)

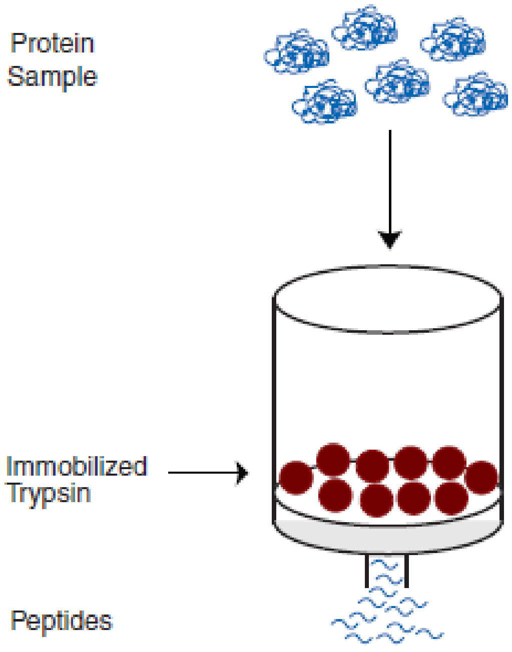


- Similar results for extraction from serum (84%) and cell lysate (82%)

Jebrail and Wheeler *Anal. Chem.*, 2009, 81, 330-335

DMF-Proteomics Example Two

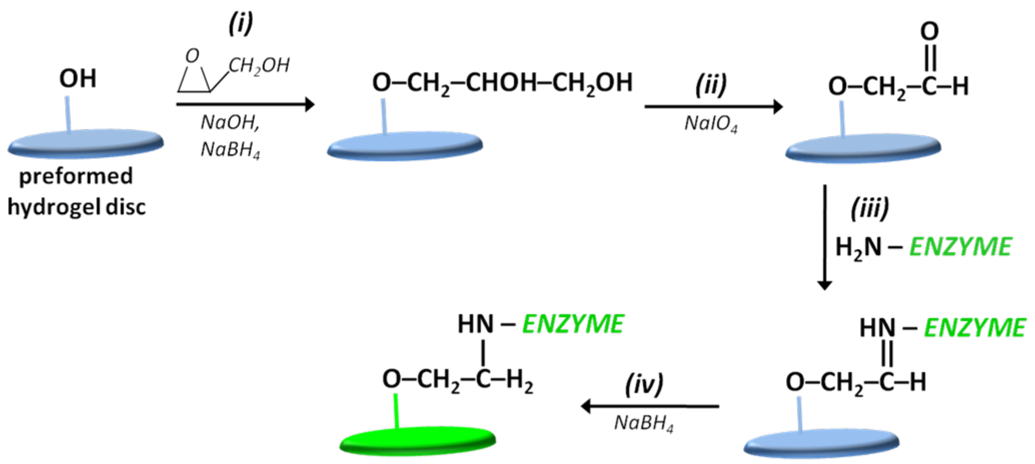
- There is great interest in immobilized enzymatic micro-reactors for proteomics



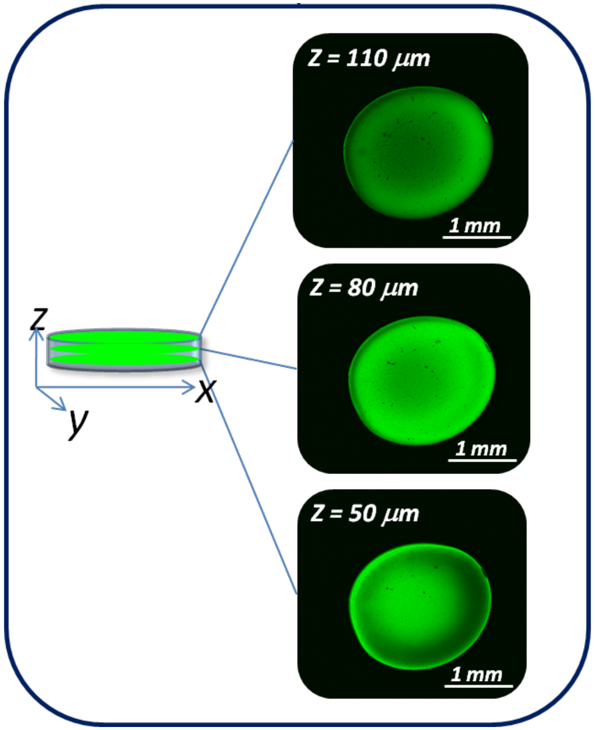
Digesting protein samples into peptides is a time-consuming step in many proteomic analysis protocols

DMF-Proteomics Example Two

- There is great interest in immobilized enzymatic micro-reactors for proteomics
- Enzymes can be covalently attached to gel discs for use with DMF



confocal images of disc bearing dye-labeled trypsin molecules



Luk and Fiddes et al., *Proteomics*, 2012, 12, 1310-1318

DMF-Proteomics Example Two

- There is great interest in immobilized enzymatic micro-reactors for proteomics
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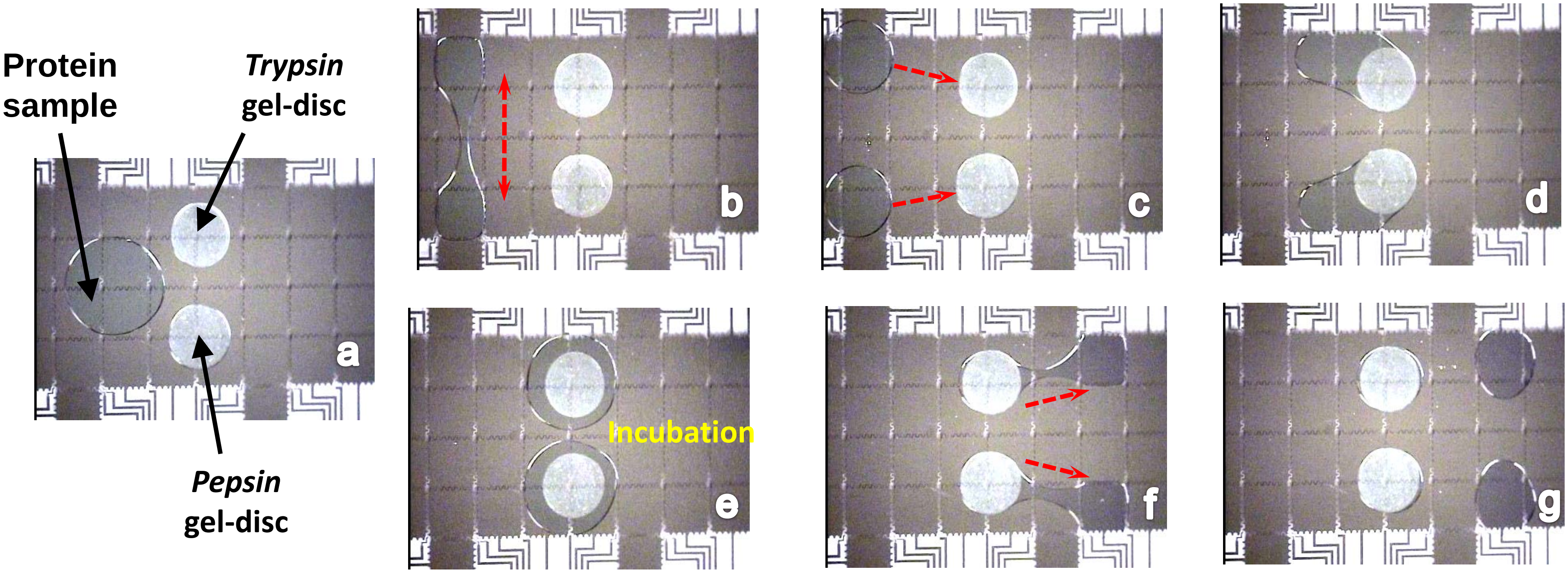

Luk and Fiddes et al., *Proteomics*, 2012, 12, 1310-1318

DMF-Proteomics Example Two

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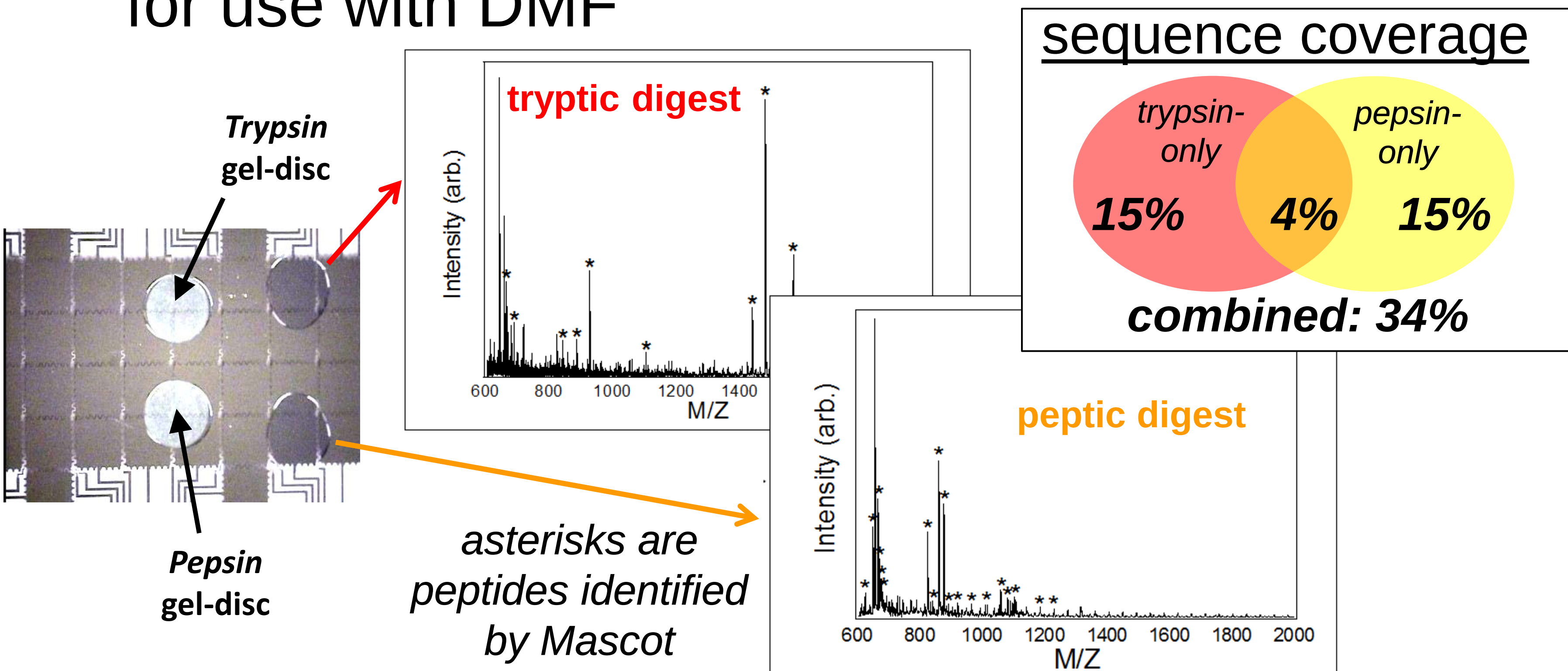
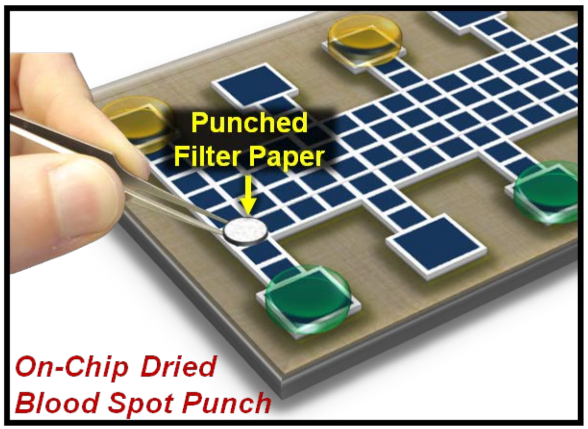
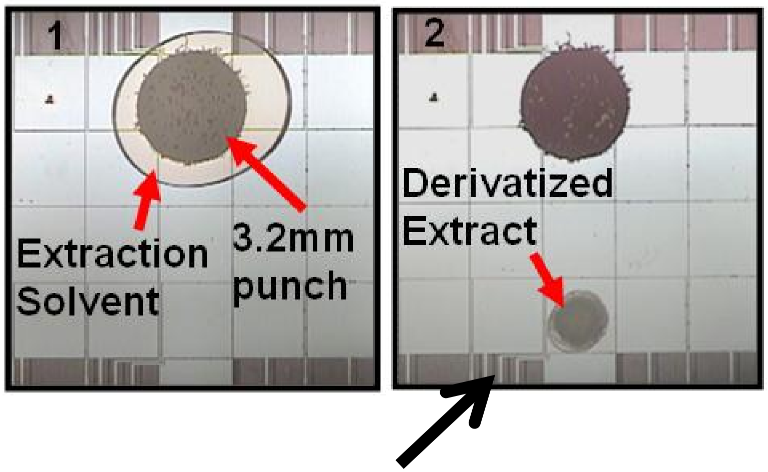


Luk and Fiddes et al., *Proteomics*, 2012, 12, 1310-1318

DMF-Diagnostics Example One

- Dried blood spot (DBS) samples from newborn patients are routinely screened for genetic disorders using semi-automated, tedious methods
- A DMF method allows for rapid, automated analysis



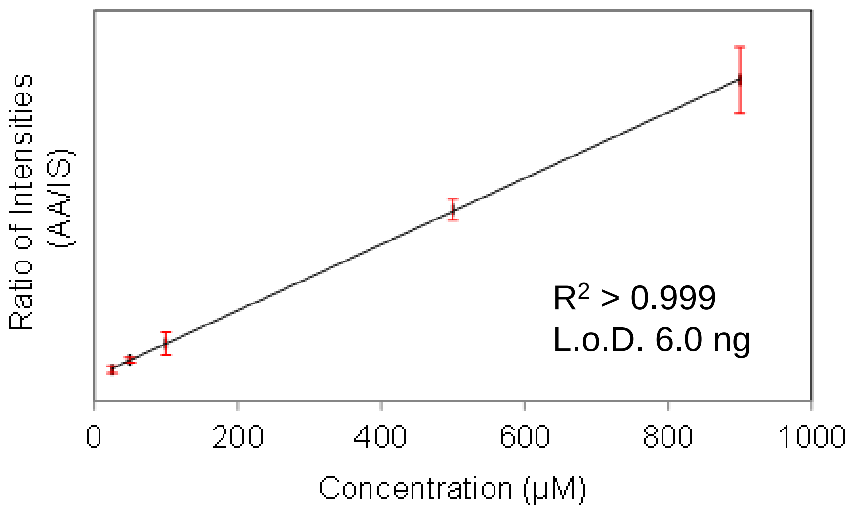


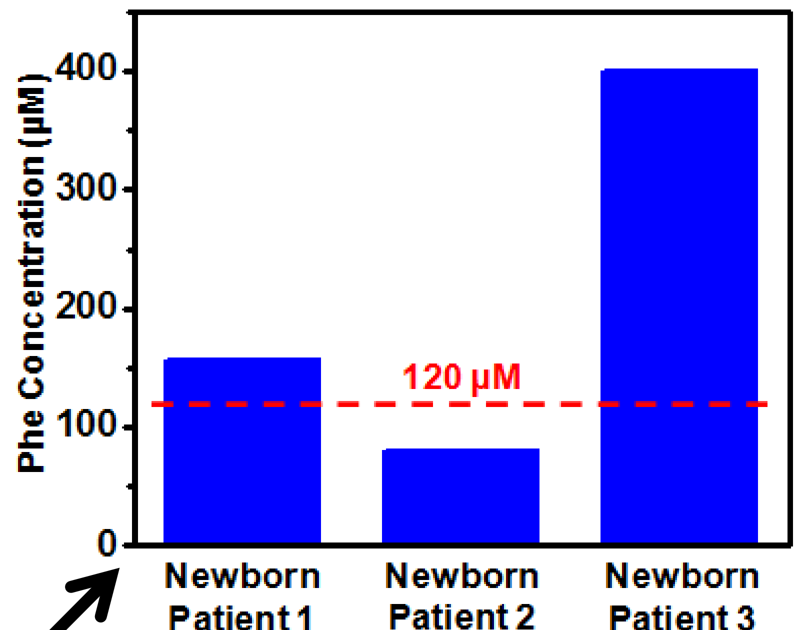
- Extract analytes (80-90% extraction in minutes)
- Mix with standards and derivatize
- Exchange solvents for analysis by MS

Jebrail and Yang et al., *Lab on a Chip* 2011, 11, 3218-3224

DMF-Diagnostics Example One

- DMF-enabled quantitation of Phe in DBS samples from newborns is rapid, fully automated, and allows for identification of newborns with PKU





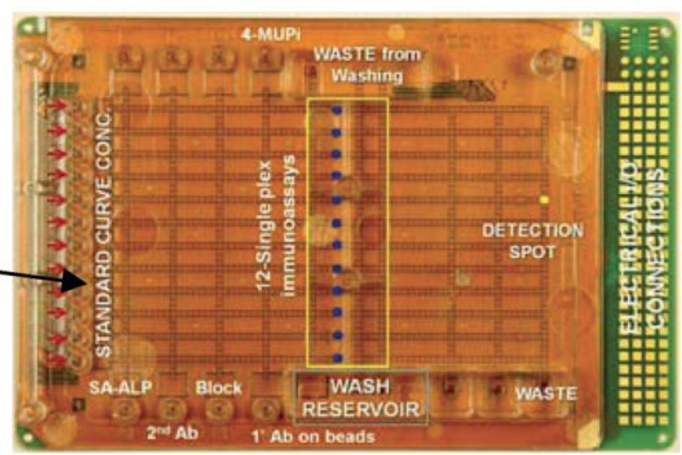
Data generated using the new method from DBS samples from newborn patients

Jebrail and Yang et al., *Lab on a Chip* 2011, 11, 3218-3224

DMF-Diagnostics Example Two

- Observation of hypercoagulability is critical to identify patients at risk of thrombosis and stroke
- A DMF-enabled device allows for portable (potentially POC) analysis

Portable DMF platform with integrated fluorescent detection

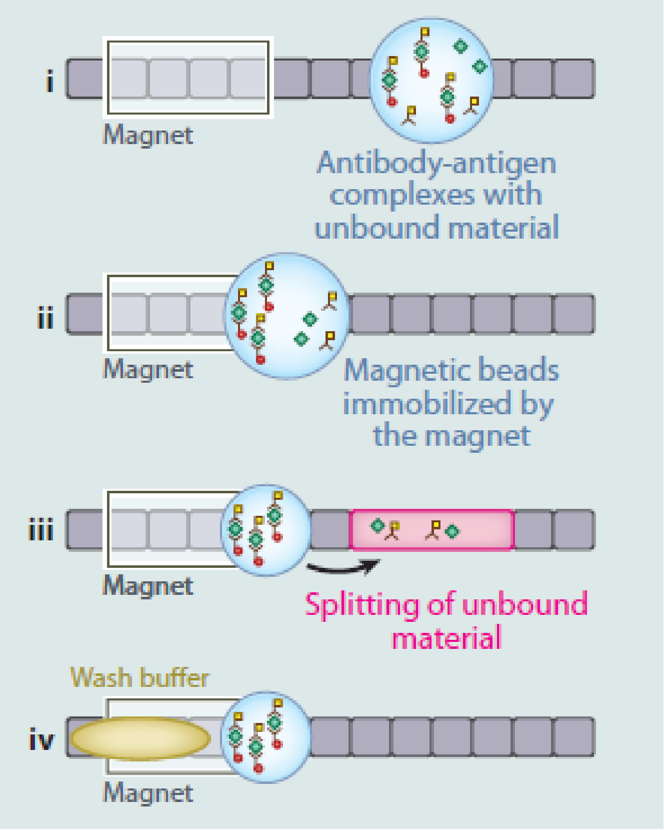


PCB-based DMF device with magnets and reservoirs for reagents

Emani et al., Blood Coagul. Fibrinolysis, 2012*, 23,* 760-768

DMF-Diagnostics Example Two

- Analytes are identified by magnetic-bead enabled immunoassays…
- …with significant reductions in process time, reagents, and sample volume



	On-chip assay	Bench-top assay
Volume of reagents 10 assays, µl		
Sample	3.3	100
Primary antibody coated beads per assay	6.6	100
Secondary biotinylated antibody per assay	6.6	100
Streptavidin alkaline phosphatase	6.6	100
4-Methylumbelliferyl phosphate	6.6	100
Wash buffer used for the whole assay	750	12 000
Incubation time, min		
Samples	4	120
Secondary biotinylated antibody	4	90
Streptavidin alkaline phosphatase	4	60
Washes	30	30
Droplet movement (on-chip)	30	–
Total time for processing 10 samples	72	300

Emani et al., Blood Coagul. Fibrinolysis, 2012, *23,* 760-768

DMF-Cell Culture Example

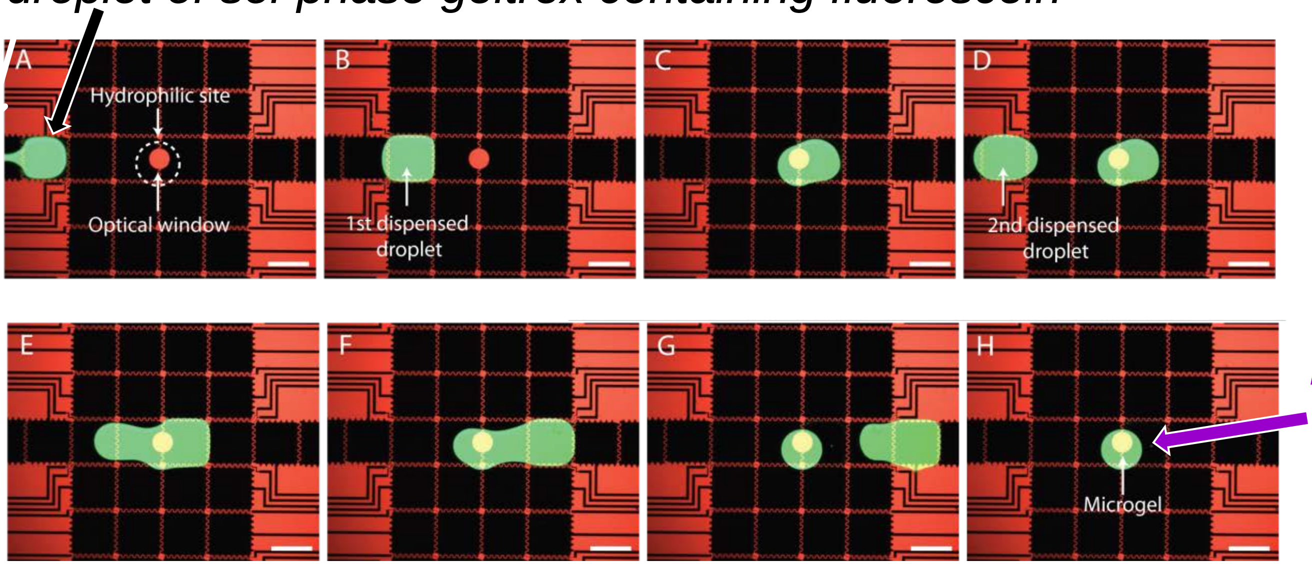
- Three-dimensional (3D) cell culture is universally recognized as being desirable,* but it remains relatively rare in day-to-day laboratory work…
- …primarily because reagents are expensive, techniques are tricky, and the 3D matrices required for culture (typically hydrogels) are soft and degrade rapidly

* "flat and hard plastic or glass substrates that are commonly used for cell culture are not representative of the cellular environment found in organisms" - *Nat. Rev. Mol. Cell Biol.* 2007, *8*, 839-845

DMF-Cell Culture Example

- A DMF method has been developed for 3D cell culture, in which sol-phase droplets are cross-linked to form **microgels**

droplet of sol-phase geltrex containing fluorescein

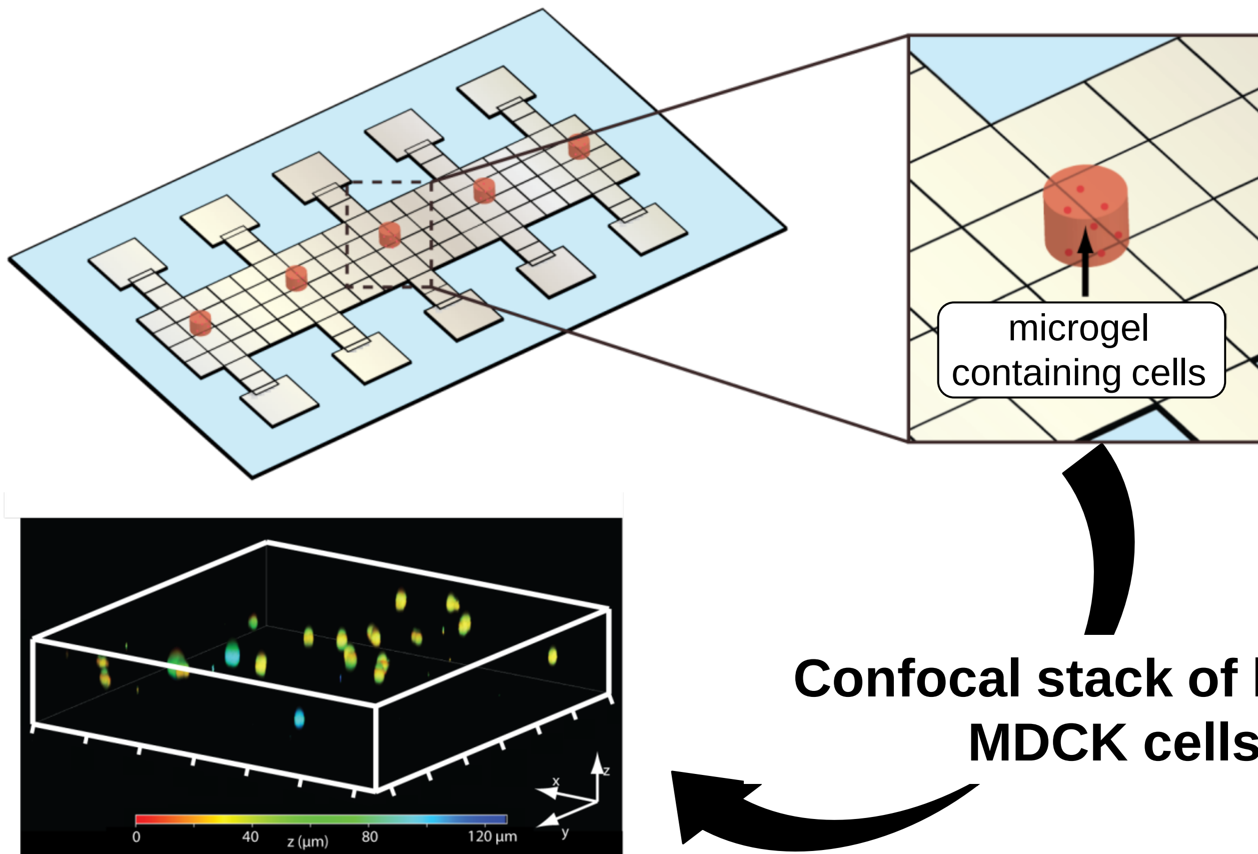


~500 nL ***microgel*** *after cross-linking*

Eydelnant et al. *Nature Comm.* 2014, *5*, 3355

DMF-Cell Culture Example

- Cells seeded in microgels in DMF devices are distributed through all axes, and can sustained for days with regular delivery of media droplets

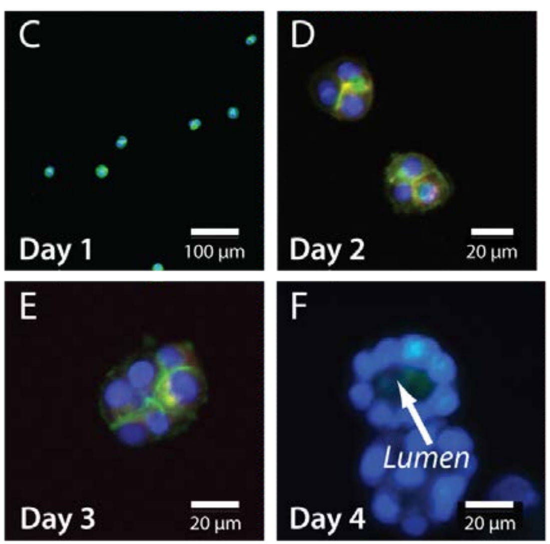


Confocal stack of labeled MDCK cells

Eydelnant et al. *Nature Comm.* 2014, *5*, 3355

DMF-Cell Culture Example

- Cells seeded in microgels in DMF devices are distributed through all axes, and can sustained for days with regular delivery of media droplets
- Cells in microgels on DMF devices form spheroids comparable to those formed using conventional methods

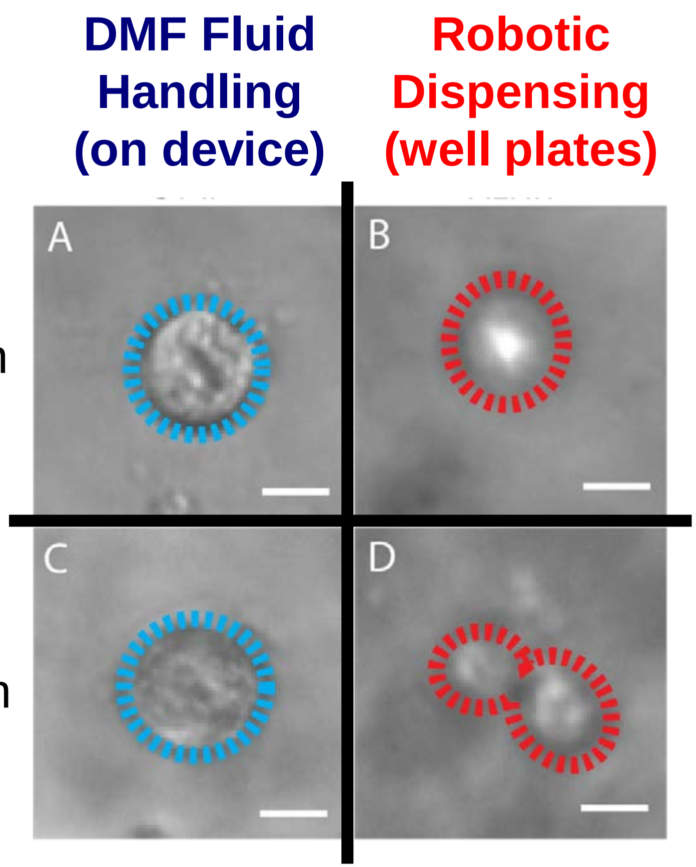


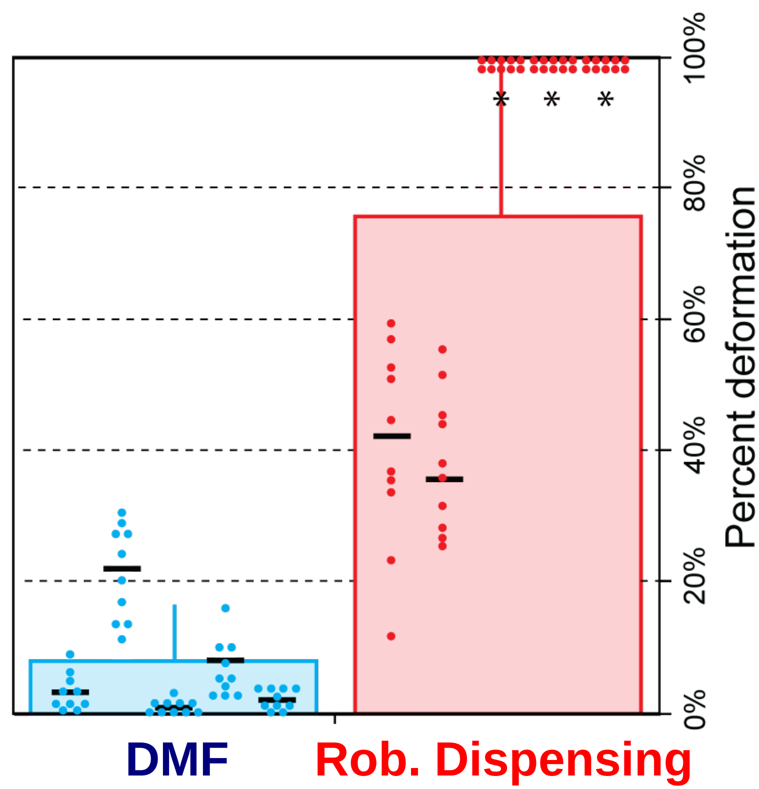
MDCK spheroids formed and imaged in microgels on DMF devices (all seeding, culture, staining and fixation steps driven by DMF droplet manipulation)

Eydelnant et al. *Nature Comm.* 2014, *5*, 3355

DMF-Cell Culture Example

- Importantly, DMF manipulation is gentle, making it **much simpler to keep these delicate structures alive**





Eydelnant et al. *Nature Comm.* 2014, *5*, 3355

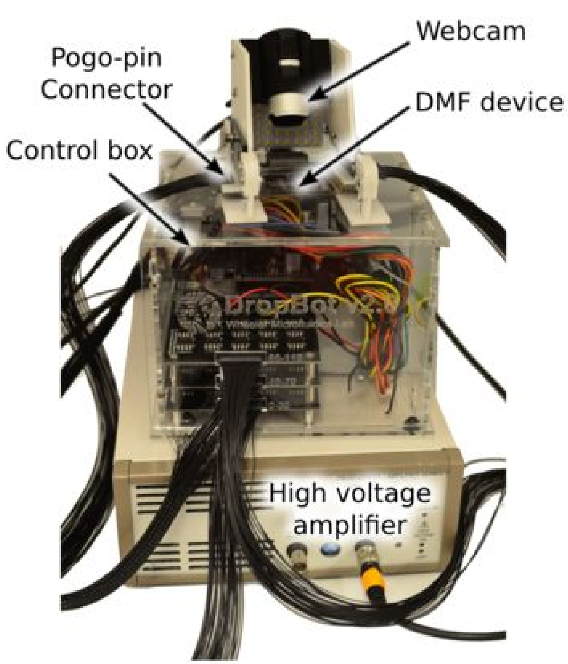
Digital Microfluidics - Outline

- DMF: Introduction and Theory
  - *DMF vs. Microchannels, DMF vs. Droplets-In-Channels, General Mechanism, Two-Plate vs. One-Plate, Electrowetting-on-Dielectric, Electromechanical Model*
- DMF: Alternate Mechanisms
  - *Wiring Complications, Optical Forces, Magnetic Forces, Thermocapillary Forces, Acoustic Forces*
- DMF: Integration and Sensors
  - *Integrated Optics, Modular Optics, SPR, Electrochemistry, Mass Spectrometry, Sample Processing and Separations*
- DMF: Applications
  - *Synthesis, Genomics, Proteomics, Diagnostics, Cell Culture*
- Final Notes
  - *Accessibility, A Look to the Future*

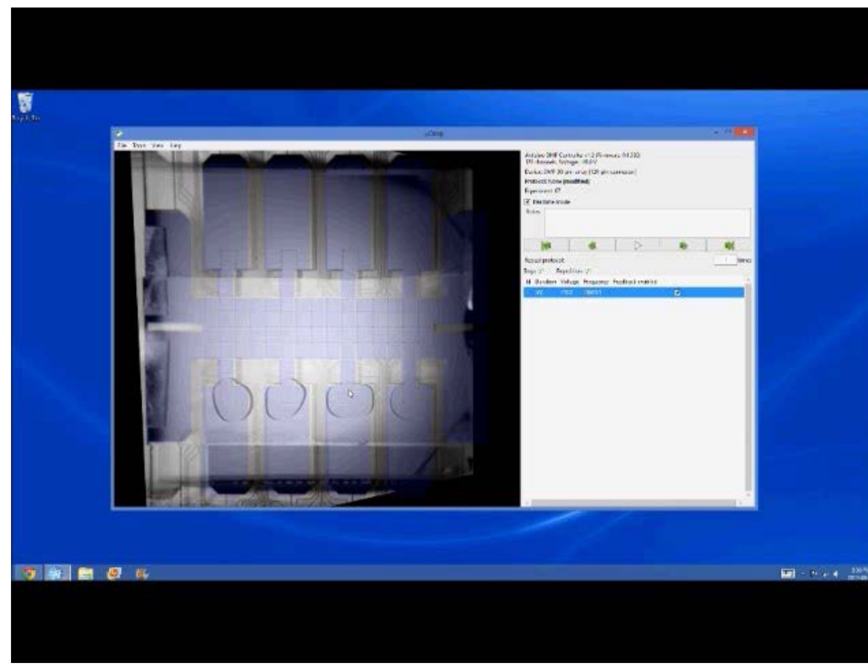
DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
- To make control accessible, we developed a system known affectionately as "DropBot"

Picture of DropBot



Video of DropBot



Fobel et al., *Appl. Phys. Lett.*. 2013, *102*, 193513

DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
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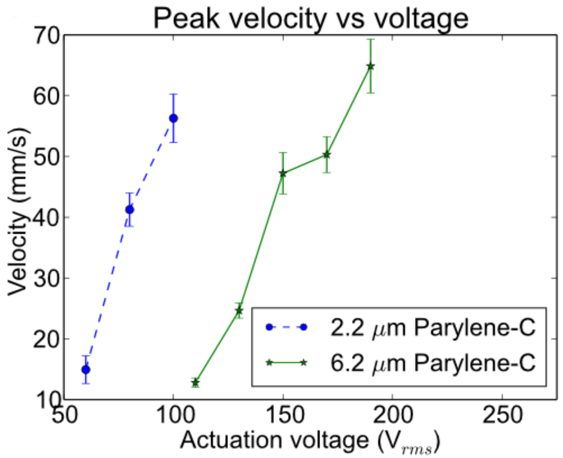
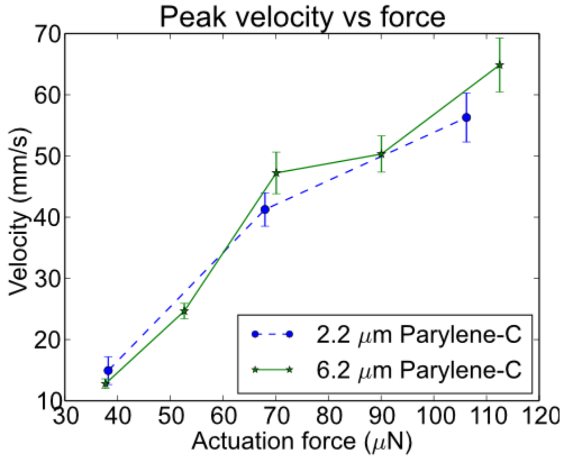
- DropBot is:
  - loaded with powerful features, including real-time force calculation to compensate for geom. differences





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DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
- To make control accessible, we developed a system known affectionately as "DropBot"
- DropBot is:
  - loaded with powerful features, including real-time force calculation to compensate for geom. differences
  - Inexpensive: ~$5k to build
  - open-source: schematics, assembly instructions, software, and more available (for free!) at www.microfluidics.utoronto.ca/dropbot

Fobel et al., *Appl. Phys. Lett.*. 2013, *102*, 193513

DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
- To make fabrication accessible, we have developed means to print devices on paper

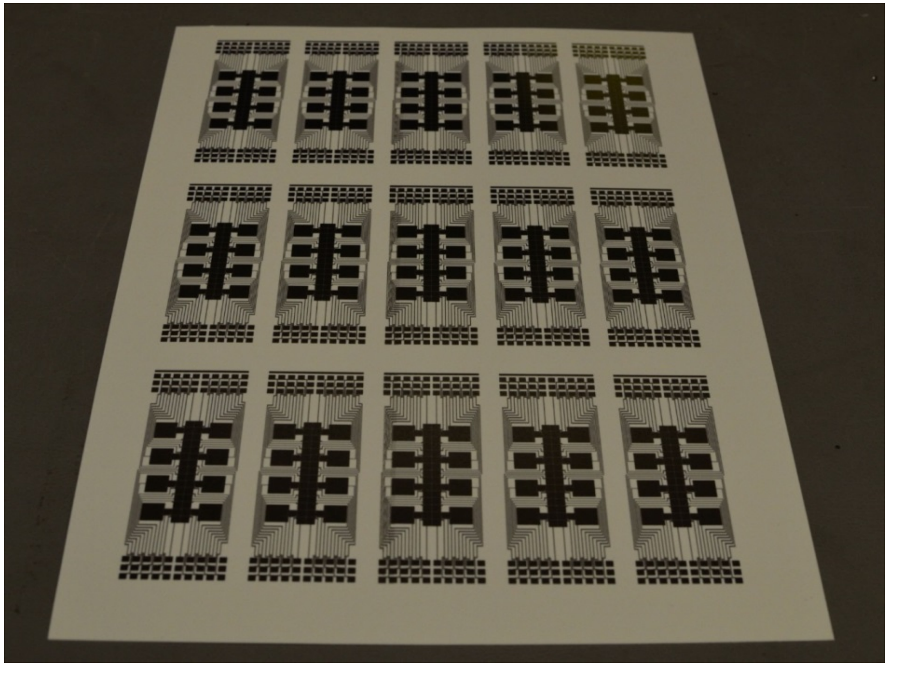
Silver Ink →  → **Paper DMF Devices**

Inkjet Printer

Fobel et al. *Adv. Mater.,* 2014, 26, 2838-2843

DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
- To make fabrication accessible, we have developed means to print devices on paper
- Paper devices are inexpensive (<$0.05), they do not require cleanroom fabrication, and they enable rapid prototyping of new device designs

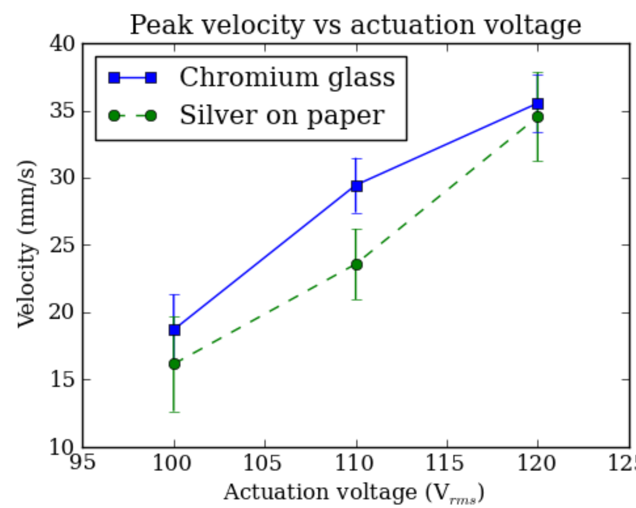


Fobel et al. *Adv. Mater.,* 2014, 26, 2838-2843

DMF – Is it for everyone?

- Impediments include **control** and **fabrication**
- To make fabrication accessible, we have developed means to print devices on paper
- Importantly, paper devices do not sacrifice performance – droplet movement is nearly identical to that in conventional devices

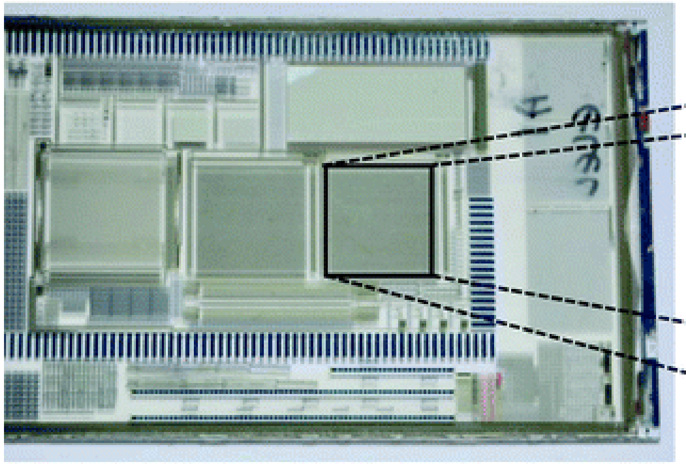
droplet moving on a paper DMF device

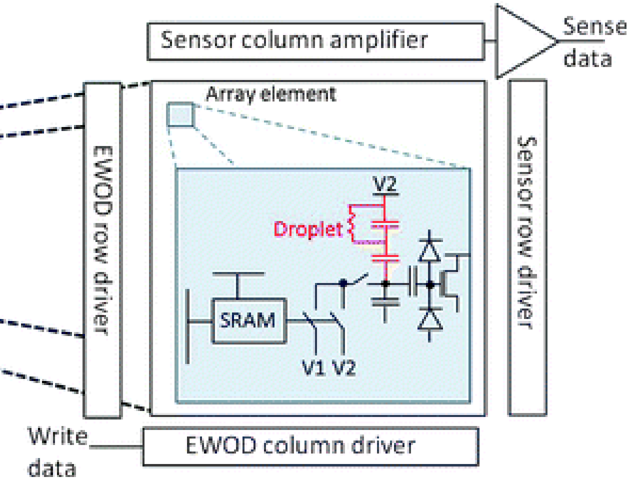


Fobel et al. *Adv. Mater.,* 2014, 26, 2838-2843

A Look to the Future…

- Most device examples I have presented have been fairly simple – in the future, will it be possible to use large arrays of electrodes for high levels of multiplexing?
- Yes! Scientists at Sharp are developing thin film transistor (TFT) DMF arrays with >4,000 individually addressable elements

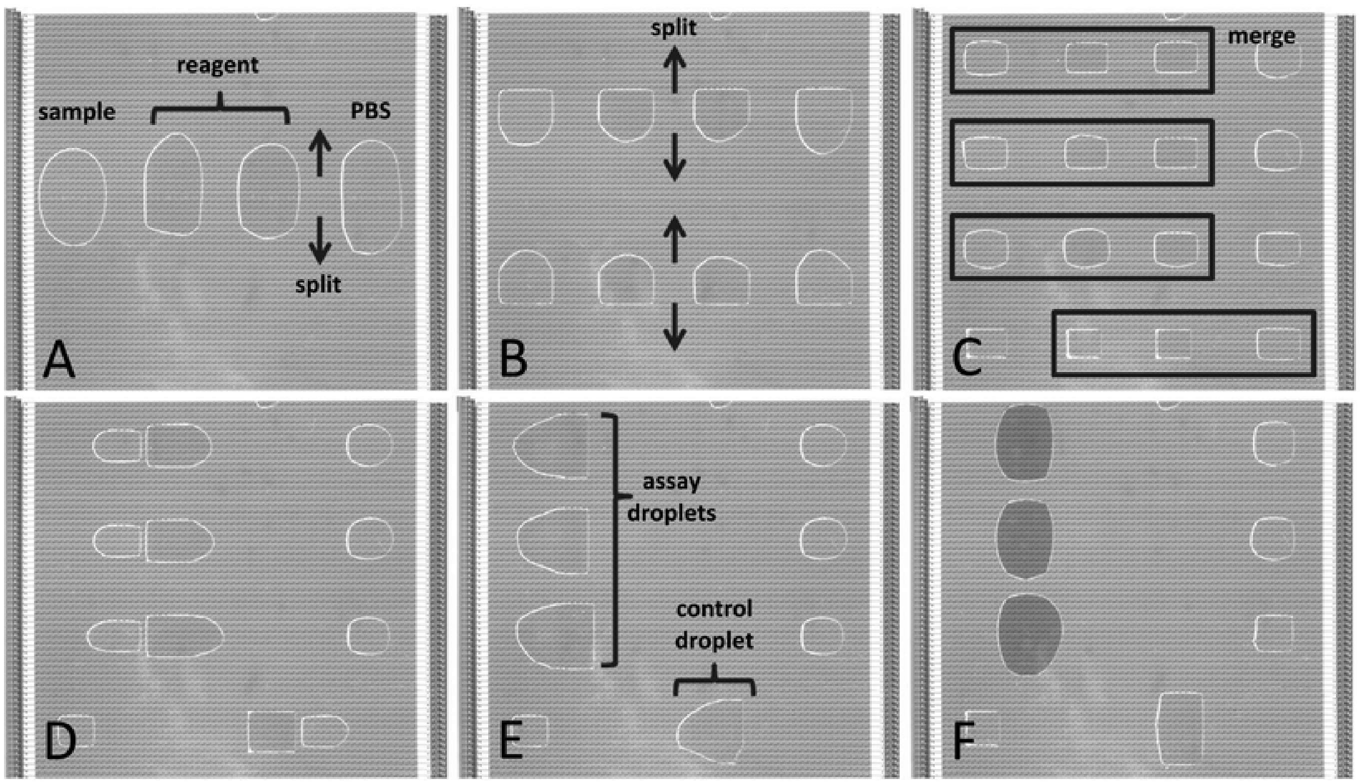




Hadwen et al., *Lab Chip* 2012, *12*, 3305-3313

A Look to the Future…

- There are (of course) bugs to be worked out, but this development is likely to be an exciting game-changer for DMF, going forward



Hadwen et al., *Lab Chip* 2012, *12*, 3305-3313

Acknowledgments

- Many of the examples described herein can be found in a comprehensive review article: Choi et al. *Annu. Rev. Anal. Chem.* 2012, *5*, 413–440
- This lecture was prepared by me, Kihwan Choi, Alphonsus Ng, and Ryan Fobel

Ki Hwan Choi

Alphonsus Ng

Ryan Fobel