



BioSensing BioActuation BioNanotechnology Summer Institute 2012

University of Illinois at Urbana-Champaign
July 30 – August 10, 2012

MICROFLUIDICS AND ENABLING TECHNOLOGY

LAB MODULE 1: Fabrication and Application of Electrochemical Sensing Devices

Location: BioNano Lab, 3119 Micro and Nanotechnology Laboratory (MNTL)

Instructors: Austin Hsiao, Bioengineering; Te-Wei Chang, Electrical and Computer Engineering; Manas Gartia, Nuclear, Plasma, and Radiological Engineering; and Logan Liu, Electrical and Computer Engineering

NO SHORTS OR SANDLES/FLIP FLOPS: Shoes and Pants are required!

Purpose and Expected Outcome:

The purpose of this module is to demonstrate fabrication of electrochemical sensors using a materials printer and the use of these sensors for detecting nitrate solutions. For demonstration, we will fabricate electrodes by printing, using a commercial inkjet printer, NanoSilver ink on a plastic substrate in a predefined pattern. Then, we will use the fabricated electrodes to sense different concentrations of nitrate solutions. Understanding how much nitrate in our water resource is important because around 70% to 80% of agricultural greenhouse emissions come from the production and use of nitrogen fertilizers. Real-time, in-field, reliable and low cost way to monitor nitrate concentration could help us to achieve optimized fertilization and electrochemical sensing is one of the promising solutions to this problem. Besides that, infants below six months who drink water that contains nitrate in excess of the maximum contaminant level (MCL) could become seriously ill and, if untreated, may die. Symptoms include shortness of breath and blue baby syndrome.

Overview:

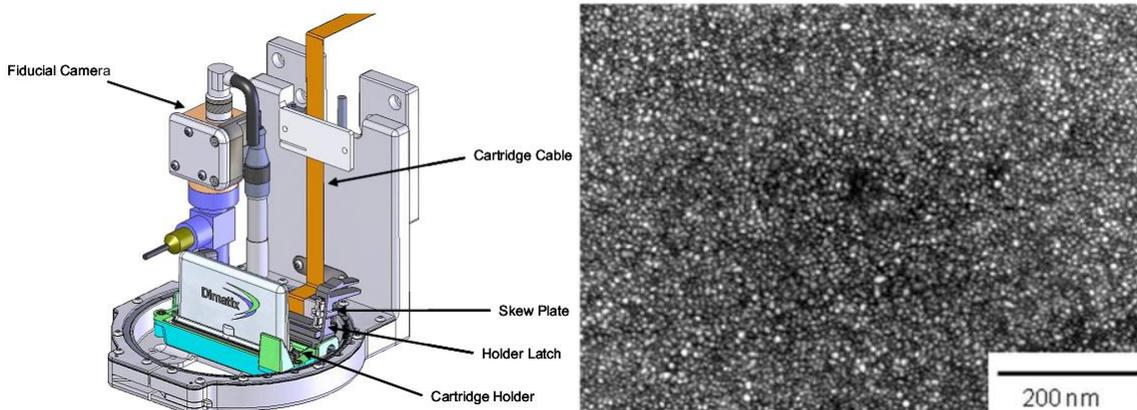


Figure 1. Dimatix Materials Printer Head (left) and NanoSilver Ink (right)

We will use a materials printer, Dimatix, to fabricate electrode patterns on a high-temperature-resistant polyethylene terephthalate (PET) substrate. The ink we will use is composed of sub-10 nm silver particles (50 wt. %) that are dispersed in a solvent; after curing, the dried ink exhibits low electrical resistivity. This type of ink is ideal for printing because of its low curing temperatures, high printing resolution ($< 250 \mu\text{m}$), and minimal particle settling. (Nano Mas, Product Data Sheet, NanoSilver Conductive Ink) This process eliminates the need for a clean room environment and techniques (e.g. photolithography, dry and wet chemical etching, thin film evaporation) previously required to fabricate micro-scale metallic features (e.g. electrodes).

The printed electrodes will be used in electrochemical sensing of different concentrations of nitrate solutions. The equipment we use for electrochemical sensing is 600D series electrochemical analyzer/workstation using the cyclic voltammetry method to determine the nitrate concentration. In cyclic voltammetry method, electrodes are placed inside the target solution, which are working, reference and counter electrodes (figure 2a). We apply a complete voltage sweeping cycle between reference and working electrodes, the counter electrode will provide a current depending on the applied voltage and target concentration accordingly. A typical current-voltage diagram of cyclic voltammetry is shown in figure 2b. There will be reduction/oxidation peaks and the magnitude of current depends on the concentration of the nitrate in the solution. According to the magnitude of those peaks we can determine the concentration of the nitrate molecules inside the solution.

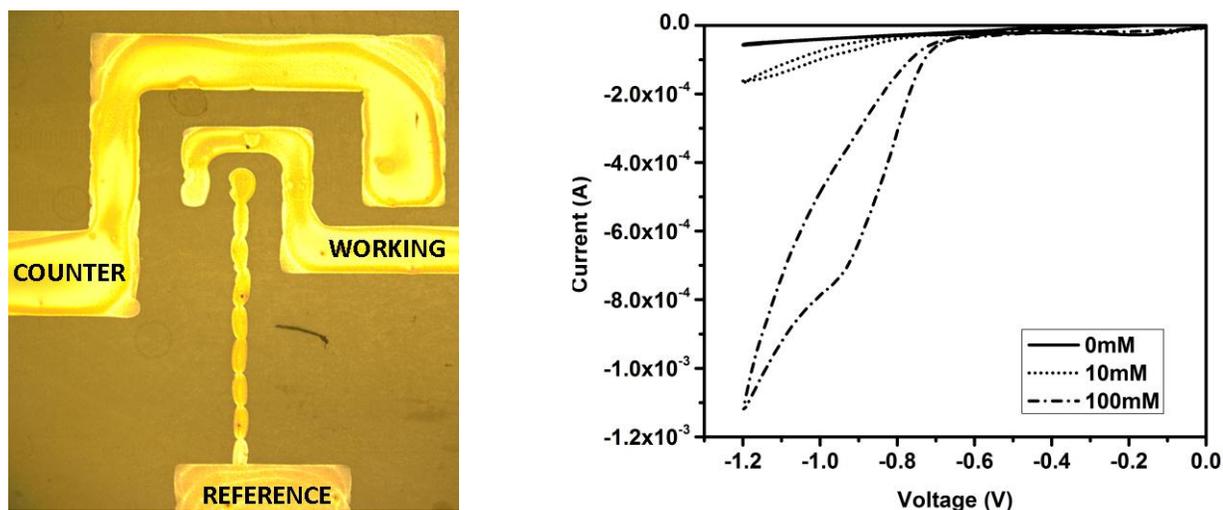


Figure 2. (a) Schematic of three-electrode electrochemical sensing setup with counter, working, and reference electrodes. (b) Typical current- voltage diagram of cyclic voltammetry with nitrate concentrations of 0 mM, 10 mM, and 100 mM.

Equipment, Materials, and Supplies:

- Lab coats, gloves, safety glasses
- Syringe
- 5 mL glass vial
- Printer cartridge

- NanoSilver ink
- (High temperature resistant) PET sheets
- Ultrasonic bath
- Hot plate
- Dimatix printer
- Oven or hot plate
- Copper anode
- Copper sulfate solution
- Beaker

Module Outline and Workflow:

Fabrication of electrodes

1. Preparation of NanoSilver ink and printer cartridge
 - 1.1. Put on a clean pair of gloves, lab coat, and eye protection
 - 1.2. Draw 1.5 mL of NanoSilver ink from the stock bottle using a 1 mL syringe and dispense into a glass vial
 - 1.3. Place the glass vial with NanoSilver ink in the Ultrasonic bath for 15 minutes
 - 1.4. Fill the cartridge with 1.5 mL of the sonicated NanoSilver ink (or the recommendation volume for the cartridge)
 - 1.5. Load the cartridge in the printer and let the ink settle for at least 30 minutes (this duration is important to have high-quality ink jetting)
2. Preparation of the PET substrate
 - 2.1. Clean the PET substrate by rinsing with isopropanol and drying with the Nitrogen gas gun
 - 2.2. Place the cleaned PET substrate in the printing surface against the upper left-hand corner (**Note: cover all the vacuum holes not covered the PET substrate with tape.**)

3. Dimatix Printer Software (Dimatix Drop Manager)
 - 3.1. Select the pattern that will be printed
 - 3.2. Set the thickness of the substrate: 120 μm for PET substrate
 - 3.3. Set the temperature of the substrate: 50°C
 - 3.4. Turn on the vacuum (**Note: make sure all the vacuum holes are covered either by the substrate or by tape.**)
 - 3.5. Set the height of the cartridge head at 300 μm .
4. Create a pattern or load a drawn pattern from printing
 - 4.1. The Pattern Block Array values are used to create an array of the pattern. Set the values for the pattern size X start, Y start, X Width, Y Width, and set the gap between each element of the array using X Pitch, Y Pitch. (**Note: New blocks can be created by pressing shift and drawing a rectangle using the mouse.**)
 - 4.2. Edit existing blocks by click once it (the color of the block changes to red) and modify the parameters from the pattern editor screen
 - 4.3. The Pattern Block Drop Positions values are used to modify each block by itself, and add new blocks. You can change 4 values for each block, (X start, Y start, X Width, Y Width)
 - 4.4. Click on “Preview Drops” to see the entire pattern and make sure to zoom in to check the coverage in the generated pattern
 - 4.5. Enter an approximate value for the expected drop size. This value varies depending on the choice of print substrate and the ink solvent. (For NanoSilver ink on PET substrate, the drop diameter is 78 μm , and on glass, the drop size is about 40 μm .) ***(Note: A test pattern with large drop spacing was printed to measure the drop size of the ink on different substrates. This method is recommended for the most accurate way of finding the drop size.)**
5. Calibration of the print cartridge
 - 5.1. Select five consecutive nozzles to begin the ejection of ink droplets.
 - 5.1.1. Good nozzles have straight ink droplets with the right velocity
 - 5.1.2. The tradeoff here is the more nozzles you use the faster the printing is; however, there may not be a large consecutive number of usable nozzles.
 - 5.2. Click on “Drop Watcher” to view a video feed of the ejection of ink from the nozzles
 - 5.3. Click on “Video Mode” to view a continuous feed of the ejection of ink from the individual nozzles. Click on “Image Mode” to view a snapshot for the nozzles by setting a time delay

- 5.4. Set the delay to 100 μs and measure the distance traveled in that duration. The goal is to maintain an ejection velocity of 7 m/s.
- 5.5. Increase the firing voltage for each nozzle until the ejection velocity reaches 7 m/s. (**Note: the nozzles may require a cleaning cycle if the drops are not ejecting straight or they are blurred.**)
6. Printing the pattern on PET substrate
 - 6.1. Check to make sure the correct pattern is selected and the position of the pattern is correctly aligned.
 - 6.2. Click “Print” to begin the printing process.
7. Inspection of the printed pattern
 - 7.1. Open “Fiducial Camera” for measurements and alignments and for the inspection of the printed pattern
 - 7.2. Scan around the printed patterns to look for breakage or flooding or incorrect alignment.
8. Annealing
 - 8.1. Place the printed pattern in the oven set at 125 °C for at least 2 hours to improve the adhesion and the conductivity of the silver patterns.

9. Electroplating of the electrodes

(Note: In the electrochemical sensing, we will apply negative voltage on one of the electrodes and positive voltage on the other electrode. We need to electroplate the positive electrode because silver gets oxidized when applied a positive voltage.)

- 9.1. Prepare the electrolyte solution; it is a Copper Sulfate solution with a concentration of at least 0.16 M.
- 9.2. Clean the copper anode with IPA, and connect it to the positive side of the voltage source, and put in the solution.
- 9.3. Connect your printed electrode with the negative side using the alligator clip.
- 9.4. Set the voltage source on around 0.7 V.
- 9.5. Dip the electrode in the solution and observe the current, change the voltage and try to keep the current around 15-20 mA, this current represent a quite slow, however high quality plating. (Faster plating causes the copper to oxidize and that increases its resistivity)
- 9.6. Keep looking at the electrode; you will easily notice the color difference.
- 9.7. When you are done, dip it in water few times, and be gentle dealing with it, because the electroplating process could affect the adhesion.
- 9.8. Put it on the hot plate (at 130° C), for at least 20 minutes to make sure it is totally dry.

10. Wire bonding: in order to connect sensor to electrochemical analyzer, we need to make connections
 - 10.1. Cut three wires with appropriate length.
 - 10.2. Fix wires on all three electrodes with scotch tape.
 - 10.3. Mix silver conductive epoxy part A and part B with the ratio 1:1.
 - 10.4. Place silver conductive epoxy on the contact point between wires and electrodes.
 - 10.5. Put the sensor into oven (65° C) for 10 minutes.

Measurement of Nitrate Solution

1. Hardware test

- 1.1. Open the power on the front of electrochemical analyzer.
- 1.2. Make sure the USB wire is connected to computer.
- 1.3. Open the shortcut “chi 600D shortcut” on the desktop.
- 1.4. Click **setup > hardware test**
- 1.5. The machine will start a short testing procedure automatically and after that there will be a report window.

2. Cyclic voltammetry sensing

- 2.1. Identify the connection wires with labels “working”, “reference” and “counter”.
- 2.2. Connect two silver electrodes with working and reference wires and copper electrode with counter electrode respectively.
- 2.3. Click **setup > technique > choose cyclic voltammetry.**
- 2.4. Click **setup > parameters** to adjust the parameters we want:
 - Init E (V): initial voltage value we apply. Set to **0.**
 - High E (V): highest voltage value we apply. Set to **0.**
 - Low E (V): lowest voltage value we apply. Set to **-1.4.**
 - Final E (V): the end voltage we want. Set to **0.**
 - Initial scan polarity: the direction for the initial sweeping, set to **negative.**
 - Scan rate (V/s): set to **0.05.**
 - Sample segments: set to **2.**
 - Sample intervals (s): set to **0.001.**

Quiet time (s): set to **2**.

Sensitivity: choose **1e-003**.

Choose **auto sense**

2.5. Click play button on the toolbar, it will start sensing automatically.

2.6. Click **file > save as** to save your result

References:

NanoMas, NanoSilver Conductive Ink, NTS5IJ06, Endicott, NY, USA

ADDITIONAL INFORMATION:

Considerations for the choice of ink for your research:

- Low evaporation rate (addition of humectants such as glycol to lower evaporation rate)
- Allowable range of viscosity: 10-12 cPs at room temperature (lower viscosity liquid can be used by heating the print head)
- Allowable range of surface tension: 28-33 dynes/cm (a surfactant can be added to lower the viscosity)
- Minimal particle aggregation or settling
- Maximum particle or aggregate size: 0.2 μm
- Degassable by sonication to remove dissolved gas



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MICROFLUIDICS AND ENABLING TECHNOLOGY

LAB MODULE 2: Fabrication of PDMS-based Microfluidics

Location: Room 3302 Micro and Nanotechnology Laboratory (MNTL)

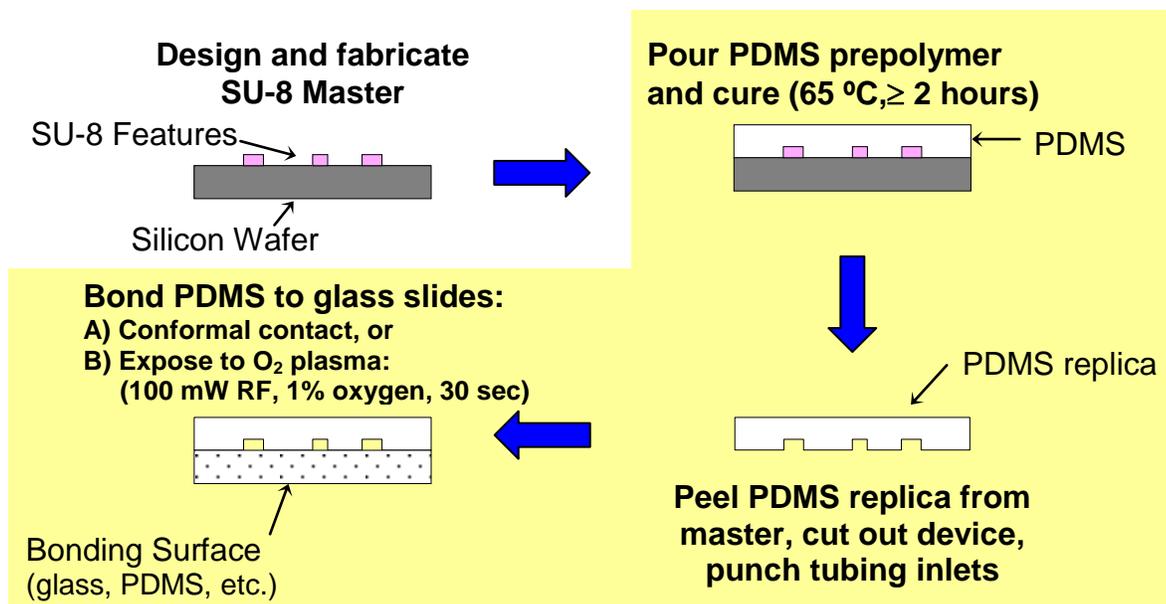
Instructors: Larry Millet MNTL, Electrical and Computer Engineering and Rashid Bashir, Electrical and Computer Engineering, and Bioengineering

Purpose and Expected Outcome:

The purpose of this laboratory module is to provide an introduction and hands-on demonstrations of both the micro-fabrication of PDMS devices and the methods of controlling fluid flow within the device. We will start with a SU-8 master and fabricate devices in PDMS, assemble the chip, and modulate flow while monitoring polystyrene beads and/or food dyes within the device.

Overview of Polydimethyl Siloxane (PDMS) Device Fabrication:

PDMS device fabrication is one of the easiest methods for the rapid prototyping of microfluidic devices. The main steps in the fabrication process are sketched in Scheme 1 below.

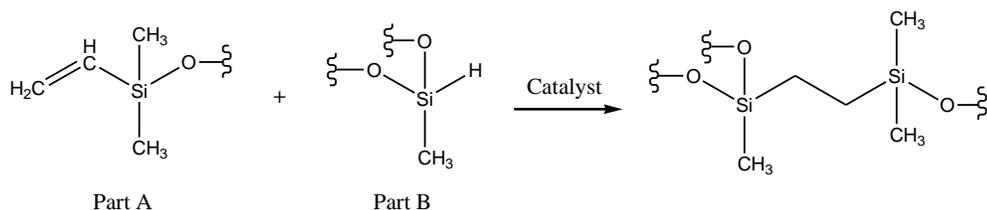


Scheme 1. Schematic overview of PDMS Device Fabrication.

In this experimental module, we will perform the steps outlined above in yellow. The general principles of microfabrication are important considerations for the production of ideal masters. This includes the design and manufacture of positive resists and matching resists to the proper reticles or transparencies. The bonding of the elastomer device will be discussed, a process that is accomplished with an UV-ozone source. Post-bonding surface modifications of PDMS are additional surface chemistry alterations that

enable the functionalization of PDMS and channels. Such surface modifications are advantageous for functionalizing microchannels for studies investigating the interaction of cells and microdomains.

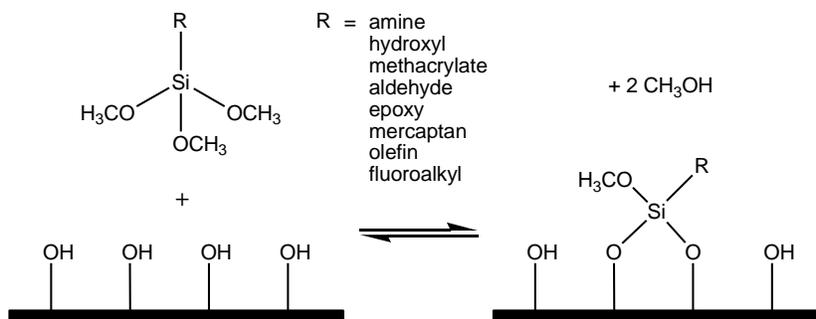
PDMS is a flexible elastomeric polymer that is an excellent material for microfluidic device fabrication.¹ In this lab module, we will use one of the most common PDMS elastomers, Sylgard® 184 from Dow Corning®. Sylgard is a two part resin system containing vinyl groups (part A) and hydrosiloxane groups (part B) shown in Scheme 2 below. Mixing the two resin components together leads to a cross-linked network of dimethyl siloxane groups. Because this material is flexible, it can be unmolded (peeled) from the SU-8 master, leaving the master intact and ready to produce another device.



Scheme 2. PDMS Crosslinking

Once the device is peeled from the mold, it is prepared for assembly into a final device. Devices are cut to size with standard surgical steel blades and access holes are punched to the desired size for tubing or fluidic reservoirs. For tubing inputs, a needle hole is drilled slightly smaller than the outer diameter of the tubing being used. This provides adequate sealing for typical fluidic pressures. For fluidic reservoirs for use without micropumps, wells can be punched (using dermal biopsy punches) into the PDMS to sustain fluidic flow depending on the required duration of the selected application.

Another useful property of PDMS is that its surface can be chemically modified in order to obtain the interfacial properties of interest.² The most reliable method to covalently functionalize PDMS is to expose it to an oxygen plasma, whereby surface Si-CH₃ groups along the PDMS backbone are transformed into Si-OH groups by the reactive oxygen species in the plasma. These silanol surfaces are easily transformed with alkoxy silanes to yield many different chemistries as shown below in Scheme 3.^{3,4}



Scheme 3. Silanization of plasma-exposed PDMS.

Equipment, Materials, and Supplies:

- Lab coats, gloves, safety glasses
- SU-8 Silicon wafer masters
- PDMS Resin - Dow Corning Sylgard 184 Part A, Part B
- Scale

- Weigh boats
- Stirring bars
- Vacuum jar (desiccator) with vacuum pump (or house vacuum)
- Surgical knives with blades
- Cutting surface (Petri dish lid)
- Sharpened blunt needles to punch holes (See chart at end of document)
- Plasma (oxygen, water, air) source for non-reversible bonding

Module Outline and Workflow:

In this lab module, participants will get hands-on experience casting (pouring) PDMS over a silicon master device. Because PDMS takes > 2 hours to cure, another set of devices will be prepared ahead of time for cutting.

Protocol

1. PDMS pouring
 - 1.1. Put on a clean pair of gloves, lab coat, and eye glasses and a face mask.
 - 1.2. Place the clean master mold in a Petri dish. The master can be cleaned of dust or debris that may have accumulated by blowing it with the nitrogen gun.
 - 1.3. On the scale, weigh out and mix PDMS (1:10 ratio) into a weigh boat. Do this by first weighing out 15 g of polymer base, and then add 1.5 g of curing agent, for 16.5 g.
 - 1.4. Please dispose of any extra pre-cured PDMS into a 50 mL conical tube for reuse. PDMS can be stored for overnight at 4°C or for weeks at -20°C without noticeable loss of performance.
 - 1.5. Mix the pre-cured PDMS with a stir bar. Be sure to both swirl and fold the mixture to ensure that the curing agent is evenly distributed.
 - 1.6. Pour the PDMS into the SU-8 master mold placed in a Petri dish.
 - 1.7. Degas the PDMS by placing the mixed pre-cured PDMS in the vacuum desiccator and evacuating the chamber. Bubbles will appear, rise to the surface of the mixture and pop. Degas the mixture for a minimum of 2.0 min. This step may be repeated to completely remove bubbles. Degassing is complete when there are no longer bubbles visible in the mixture. Once all bubbles have been removed, cover the Petri dish and place in an oven at 65-80 °C for 2-6 hrs to cure the PDMS.
2. PDMS release
 - 2.1. Remove the PDMS casting from the oven and place on a clean bench top.
 - 2.2. Using an X-acto knife with a new blade, make straight cuts about 0.5-1 cm from the edge of the master mold. To make each cut, sink the point of the knife vertically into the PDMS until it reaches the polystyrene Petri dish. Keep the knife perpendicular to the master and follow the outline of the master. Make sure to maintain pressure on the knife such that the tip is always in contact with the plastic dish substrate. Continue cutting until the PDMS-master device can be released from the Petri dish with forceps.
 - 2.3. Once all the edges have been liberated, lift the mold up and out of the Petri dish as demonstrated by the instructor. Then carefully peel away the remaining portions of the cured PDMS from the underside (the side without resist features) of the master. Discard this and any excess PDMS.
 - 2.4. Place the released PDMS layer in the lid of your Petri dish with the channel features up.
 - 2.5. With a straight-edge razor, block off the edges of the PDMS to produce a flat PDMS structure that can be bonded to the microscope slide as demonstrated by the instructor.

3. Fluidic port punching
 - 3.1. Align a blunt needle or clean dermal biopsy punch with the first port you will punch.
 - 3.2. Adjust the needle or puncher so that it is as vertical as possible. Push the puncher through the PDMS until you touch the plastic Petri dish. Remove the puncher.
 - 3.3. Push the yellow stick into punched hole and the puncher to drive out the cored section of PDMS.
 - 3.4. Retrieve and discard the cored section from the under side of the device using a pair of forceps. Repeat steps 3.1 to 3.4 for each port.
 - 3.5. Place the punched PDMS device onto a Petri dish with feature side UP.

Once the devices have been poured, cut, and punched, you can *reversibly* bond the PDMS to microscope slides through conformal contact, or irreversibly through plasma treatment or UV-ozone. Equipment varies between labs for cleaning and activating PDMS replicas and glass slides for covalent bonding. Recommended conditions for cleaned PDMS in an oxygen plasma chamber or alternatively with a UV-ozone system that includes (100 mW, 2% oxygen, 35 s) in a PX-250 plasma chamber (March Instruments, Concord, MA). After plasma or UV-ozone treatment, immediately place the oxidized PDMS in contact with the glass to irreversibly bond the surfaces. Chambers are then baked at 70 °C for 10-30 min following bonding.

4. Device bonding (with UV-ozone or oxygen plasma surface treatment)
 - 4.1. Follow protocols specified by the local equipment owner for the oxygen plasma treatment.
 - 4.2. Using forceps or tweezers, place the PDMS device with the feature side facing upwards to be exposed to UV-ozone or oxygen plasma.
 - 4.3. Using forceps or tweezers, place clean glass slides next to the device to be bonded.
 - 4.4. If there is any visible dust particles on the PDMS or the device or slide to be bonded to the PDMS, wipe with a clean lint-free kim wipe or clean room cloth soaked in IPA.
 - 4.5. Place cover on the UV-ozone or oxygen plasma source. For UV-ozone, ensure that the device is approximately 3-5 mm from the UV lamp, which is housed in the cover. Distance requirements are not necessary for oxygen plasma treatments.
 - 4.6. Expose device to oxygen plasma or UV-ozone for 3-5 minutes.
 - 4.7. Remove cover and retrieve PDMS channels using forceps or tweezers, grasp PDMS slab from its side and flip device over onto the glass side so that the features are bonded against the glass.
 - 4.8. Place the devices on a hotplate at 70 °C for 5-10 minutes.

The reactive silanol bonds at the surface of the PDMS will slowly diffuse back into the bulk of the PDMS elastomer. For longer PDMS surface activation and quicker PDMS-substrate bonding, solvent extracted PDMS⁵ can be used. Therefore, chemical modification of the PDMS surface should immediately follow the oxygen plasma/ozone bonding for optimal results.

5. Actuating flow control in microfluidic devices can be achieved through a number of methods, during this lab course you will have the opportunity to observe a few of these approaches. They include passive pumping, gravity flow, and syringe pumping.
 - 5.1. Demonstration of passive pumping
 - 5.2. Demonstration of gravity flow
 - 5.3. Demonstration of syringe pumping
 - 5.4. Student group discussions: discuss the advantages of each method of flow control.

References:

1. McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H.; Schueller, O. J.; Whitesides, G. M., Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* **2000**, 21, (1), 27-40.
2. Makamba, H.; Kim, J. H.; Lim, K.; Park, N.; Hahn, J. H., Surface modification of poly(dimethylsiloxane) microchannels. *Electrophoresis* **2003**, 24, (21), 3607-19.
3. *Silicon Compounds: Silanes and Silicones*. Gelest, Inc.: Morrisville, PA, 2004; p 560.
4. Hermanson, G. T.; Mallia, A. K.; Smith, P. K., *Immobilized Affinity Ligand Techniques*. Academic Press: San Diego, CA, 1992; p 454.
5. Millet LJ, Stewart ME, Sweedler JV, Nuzzo RG, Gillette MU., Microfluidic devices for culturing primary mammalian neurons at low densities. *Lab Chip*. **2007** Aug, 7, (8):987-94.

Dermal biopsy punches:

Integra* Miltex* Standard Biopsy Punches

Assorted sizes, 2mm, 3mm, 4mm, 5mm, and 6mm; 10 each

Fisher Scientific Cat#: 12-460-414

MILTEX INC product no.: 33-38

Sources for tubing and blunt end needles:

Small Parts, Inc.

13980 N.W. 58th Court

P.O. Box 4650

Miami Lakes, FL 33014-0650

<http://www.smallparts.com>

Recommended parts descriptions:

Part Description	Usage	Inner Diameter	Outer Diameter	Small Parts Part #
20G x 1/2" Stainless Steel Blunt Needles	Needles for cutting holes	0.023"	0.036"	NE-201PL-C
22G x 1/2" Stainless Steel Blunt Needles	Needles for direct injecting	0.016"	0.028"	NE-221PL-C
30G x 1/2" Stainless Steel Blunt Needles	Needles for tubing	0.006"	0.012"	NE-301PL-C
Tygon Tubing	Connect needles to device	0.01"	0.03"	TGY-010-5C

PDMS – [Dow Corning Sylgard 184](#)

Available from Ellsworth Adhesives: Part#: 182 SIL ELAST KIT .5KG

UV-Ozone source – PSD-UV, Novascan Technologies



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MICROFLUIDICS AND ENABLING TECHNOLOGY

LAB MODULE 3: Dielectrophoresis

Location: Room 3302 Micro and Nanotechnology Laboratory (MNTL)

Instructors: Kidong Park, MNTL, Larry Millet MNTL, Electrical and Computer Engineering, and Rashid Bashir, Electrical and Computer Engineering, and Bioengineering

Purpose and Expected Outcome:

The purpose of this laboratory module is to provide an introduction and a hands-on demonstration of microfluidic dielectrophoresis (DEP). The DEP devices are electrodes patterned on a printed circuit board (PCB) that are brought into contact with a very thin glass coverslip that is attached to PDMS microfluidic channels. This assembly will be used to demonstrate trapping and concentration of micro-particles. The students will be able to vary DEP waveform characteristics and related experimental parameters to examine the interactions between the particles and the electric fields generated by the inter-digitated electrodes patterned on the chip. The expected outcome is for the students to gain a basic understanding of dielectrophoresis and its potential applications for biology and medicine.

Overview of Dielectrophoresis:

Dielectrophoresis is the electrokinetic movement of electrically polarizable particles in non-uniform electric fields. The non-uniform electric field exerts a force to each end of the polarized particle, with the difference in the magnitude of the two forces determining the direction of particle mobility. DEP occurs for charge-neutral particles and for both DC and AC excitation. Forces in the direction of increasing electric field strength (positive DEP) occur when the permittivity of the particle (ϵ_p) exceeds that of the medium (ϵ_m), whereas particles are pushed towards lower levels of electric field strength when $\epsilon_p < \epsilon_m$ (Fig. 1), making the polarity of the applied field irrelevant.

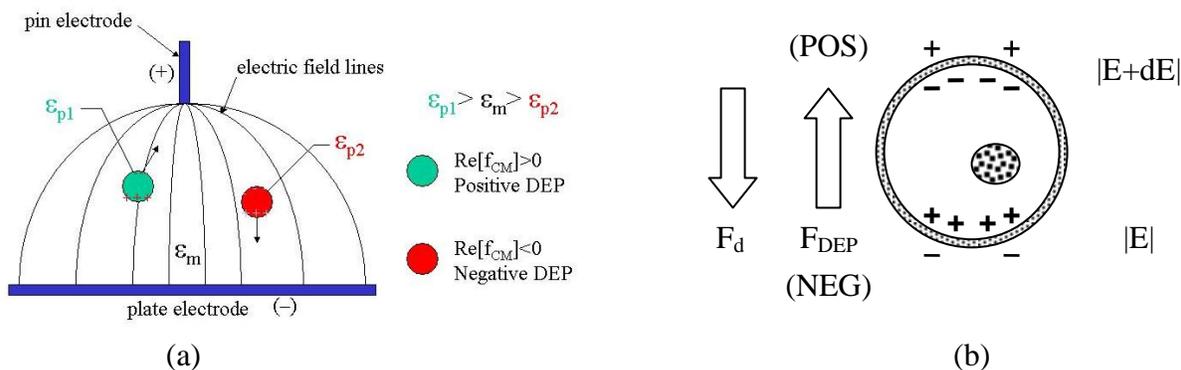


Fig. 1: (a) Schematic illustration of dielectrophoresis and (b) representative p-DEP on a cell.

The DEP force on a homogeneous and lossless ($\sigma/\omega\epsilon \ll 1$) sphere of radius (r) is:

$$F_{\text{DEP}} = 2\pi r^3 \epsilon_m \operatorname{Re} \left\{ \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \right\} \nabla |\mathbf{E}|^2,$$

where complex permittivity (ϵ^*) is equal to $\epsilon + \sigma / j\omega$ and $\operatorname{Re}\{\dots\}$ is the *Clausius-Mossotti* factor. Note that this force scales with V^2 and r^3 . Viscous drag on a spherical particle is described by Stokes flow as $F_d = 6\pi r \eta u$, where η and u are the fluid viscosity and velocity, respectively.

Many biologically important particles are polarizable. For example, cells can be described by a shell model, in which the particle is assumed to be composed of a thin membrane surrounding the core, with specified conductivity and permittivity, allowing the DEP force to be estimated.

The implementation of DEP requires patterning of conductive electrodes for the application of non-uniform electric fields, and preferably a layer of insulator on the electrodes to prevent electro-thermal induced reactions at the electrode interface. Alternatively, the electrodes can be integrated within a microfluidic channel for improved functionality depending on the application of interest. In this experiment, we take advantage of a simple and effective microfabrication process that eliminates direct exposure of the target particles to the electrodes (Fig. 2(a)).

The PCB is widely used in the electronics industry to provide mechanical support for electrical connections within the electronic devices. Due to broad use of PCBs, they can be made-to-order from PCB manufacturers. Furthermore, a biochip composed of a PDMS microfluidic channel and a microscope coverslip (Fig. 2(b)) isolates the sample from the electrodes and reduces the risk of cross-contamination between experiments performed on the same PCB.

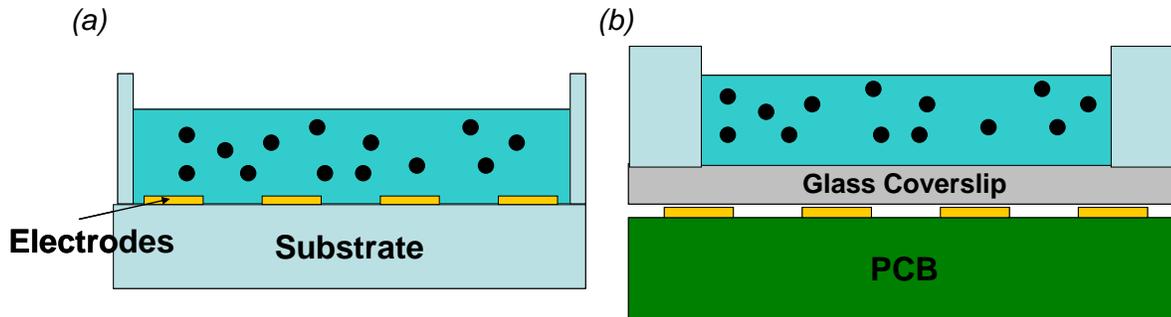


Fig. 2. (a) A schematic diagram of the conventional approach in implementing DEP. Electrodes and substrate should be disposed of after each experiment. (b) A schematic diagram of the PCB-based DEP implementation. DEP electrodes are easily fabricated on a PCB and can be reused again and again since the electrodes are not in direct contact with the sample.

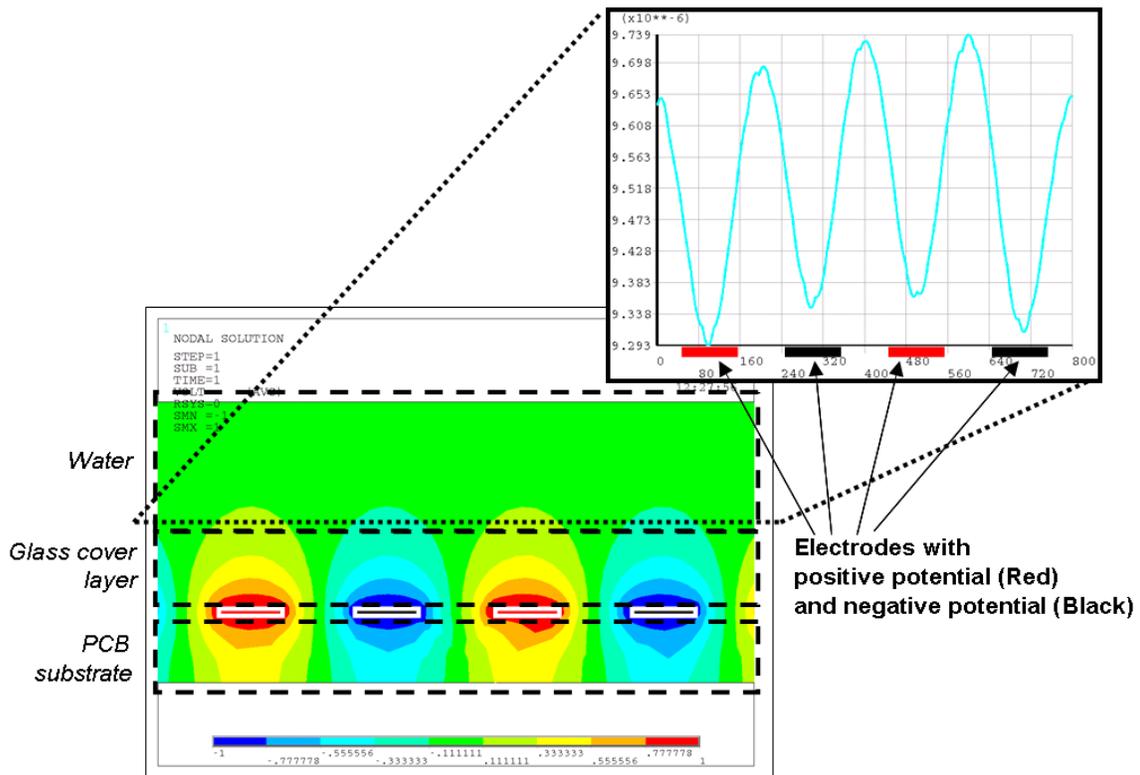


Fig. 3: Numerical analysis of the electric potential generated by the PCB electrodes. Electrodes in red and in black are set to +1V and -1V respectively. *Inset:* Square of the electric field magnitude, $|E|^2$, on the dotted line 10um above the glass cover layer. Black and red bar shows the position of the electrodes. The electric field intensity is weakest at the center of the electrode and strongest between the electrodes.

Equipment, Materials, and Supplies:

- Printed Circuit Board (PCB) with electrode patterns fabricated on the surface
- Polydimethylsiloxane (PDMS) microfluidic channels and *No.0* thickness ($\sim 100 \mu\text{m}$) coverslips
- Oxygen plasma system
- Function generator, oscilloscope, BNC T-junction and connectors
- AC voltage amplifier
- Upright Optical Fluorescence Microscope with 10-20X objective, with CCD camera.
- Cells or Poly-styrene beads
- Mineral oil
- Double-sided tape
- Soldering iron and solder
- Dermal tissue biopsy hole puncher.

Module Outline and Workflow:

The students are expected to gain an understanding of dielectrophoretic (DEP) phenomena during an experiment that involves device assembly, microfluidic connection and variation of the applied voltage waveform necessary for DEP manipulation of beads and/or cells. The following are steps the student will take to investigate DEP.

1. Enable connection of PCB electrodes to BNC connectors by soldering two wires onto ends of PCB board. One wire is for positive polarity, the other is for negative polarity.
2. Create PDMS/glass microfluidics chip
 - a. Punch inlet/outlet ports on both ends of channel with PDMS hole puncher by gentle pressure and twisting (punch into channel side first)
 - b. If channel side of PDMS is noticeably dirty, spray off debris with ethanol and dry with nitrogen (PDMS needs to be clean for glass bonding) or remove any debris using adhesive tape
 - c. Lightly press PDMS (channel side down) onto the coverslip to create a bond.
 - d. Align the pipette's tip to the punched inlet and inject $\sim 20 \mu\text{L}$ of bead suspension into one of the chip's ports to load the channel.
3. Perform DEP on the injected beads/cells and optimize the input voltages and frequencies.
 - a. Mount PCB board and microfluidic chip assembly on microscope stage with double-sided tape and connect the PCB to the amplifier output using mini-grabber BNC connectors (with signal generator and AC voltage amplifier turned off).
 - b. Vary the input voltage and frequency (1 to 12 MHz) to optimize the focusing ability of the device.

NOTE: do not allow the AC voltage amplifier's power output to go above 200 Watts! This may cause the PCB to melt or to damage the amplifier's internal components. Also, do not go below 1 MHz to ensure the aforementioned problem does not happen.

 - c. Measure the time it takes for the particles to align to the pattern during DEP. From this data, estimate particle velocities and calculate the hydrodynamic drag force ($F_{drag} = 6\pi\eta Rv$, where η = fluid viscosity = 1mPa·s for DI, R = radius of the particle = 7.5 μm (for polystyrene beads), and v = velocity of the particle motion), which is equal to the DEP force exerted on the particles. This will help in gaining an intuitive understanding of dielectrophoretic manipulation of particles within microfluidic devices (Fig. 4 and 5).

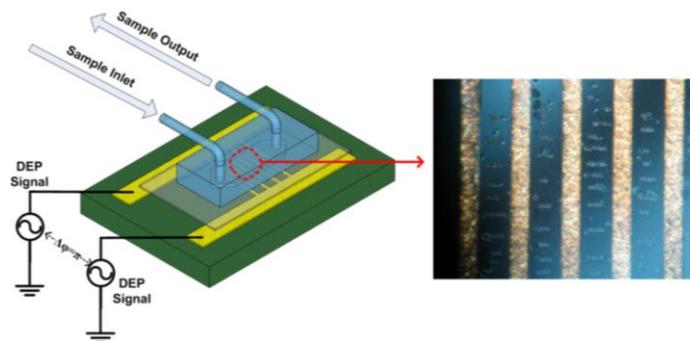


Fig. 4: The device is made from a PCB and a thin glass coverslip. The glass coverslip serves as an insulator between the solution and the electrode pattern.

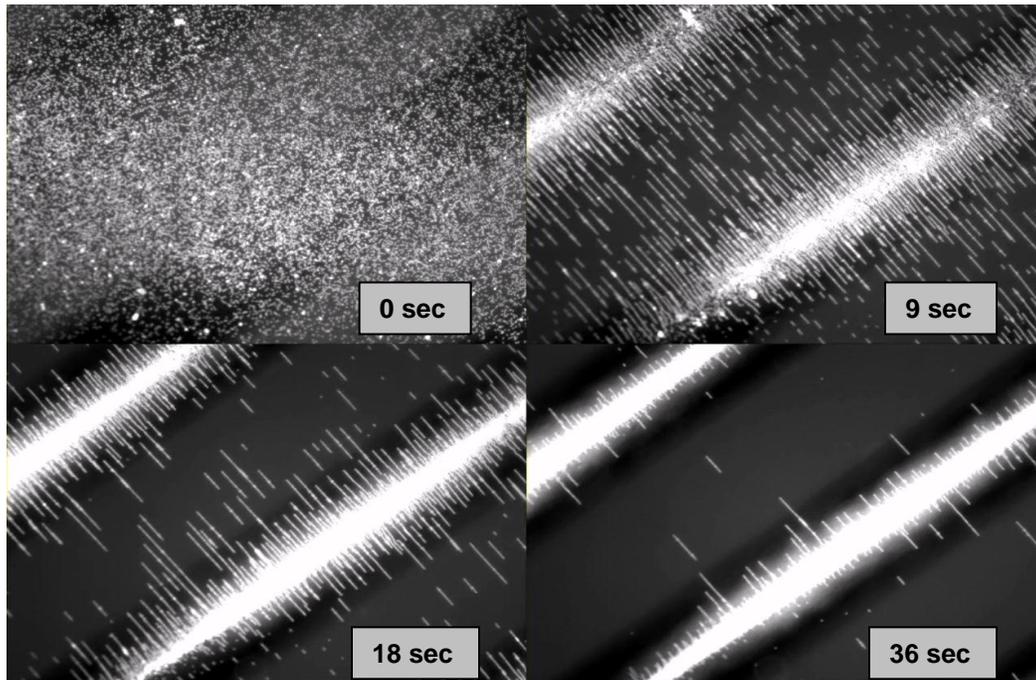


Fig. 5. Dark field images of the fluorescently labeled 3µm polystyrene beads during DEP. The trajectories of the polystyrene beads are visible as short lines, indicating the traveled distance during exposure time of the camera. After applying the AC voltages for 36 seconds, most of the beads become focused on top of the electrodes by negative DEP force

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