BIONANOTECHNOLOGY SUMMER INSTITUTE 2011
University of Illinois at Urbana-Champaign
July 25 - August 5, 2011

MICROFLUIDICS AND ENABLING TECHNOLOGY LAB MODULE:
Fabrication of PDMS-based Microfluidics

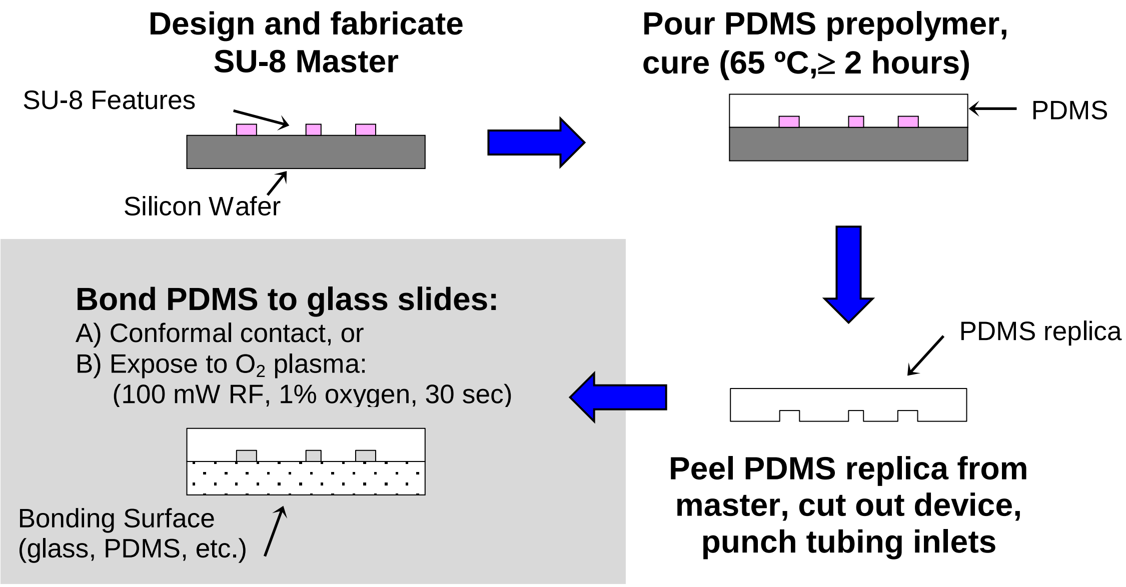
Location: BioNano Lab, 3119 Micro and Nanotechnology Laboratory (MNTL)
Instructors: Larry Millet, MNTL, Electrical and Computer Engineering
Mitchell Collens, MNTL, Bioengineering

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 Poly(dimethylsiloxane) (PDMS) Device Fabrication:

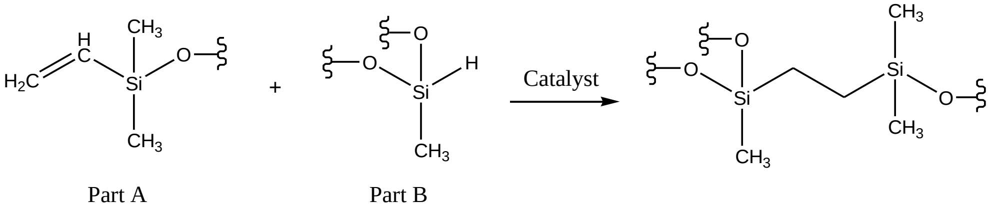
PDMS device fabrication is one of the easiest methods for the rapid prototyping of microfluidic devices. The main steps in the fabrication process are sketched in Scheme 1 below.



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.

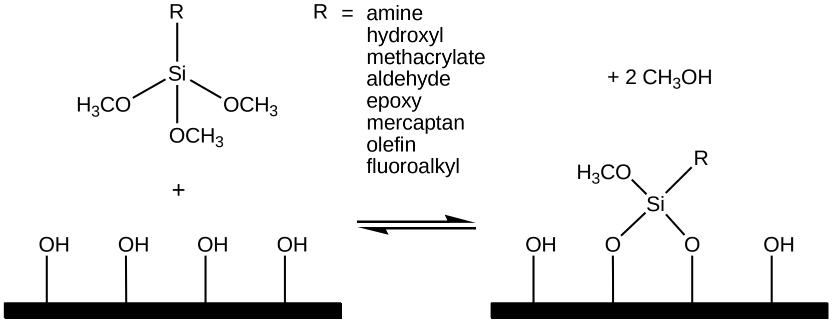
PDMS is a flexible elastomeric polymer that is an excellent material for microfluidic device fabrication.[1] In this lab module, we will use one of the most common PDMS elastomers, Sylgard® 184 from Dow Corning®. Sylgard is a two part resin system containing vinyl groups (part A) and hydrosiloxane groups (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, wells can be punched 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-CH_3 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 alkoxysilanes 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.

<u>Protocol</u>

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 <u>15 g</u> of polymer base, and then add <u>1.5 g</u> of curing agent, for <u>16.5 g</u>.
    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^{o}C or for weeks at -20^{o}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 clean dermal biopsy punch with the first port you will punch.
    - 3.2. Adjust the 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 procotols 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 wetted with 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 PDMS[5] 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.
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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 ½” Stainless Steel Blunt Needles	Needles for cutting holes	0.023”	0.036”	NE-201PL-C
22G x ½” Stainless Steel Blunt Needles	Needles for direct injecting	0.016”	0.028”	NE-221PL-C
30G x ½” 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
UV-Ozone source – PSD-UV, Novascan Technologies

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MICROFLUIDICS AND ENABLING TECHNOLOGY LAB MODULE: Dielectrophoresis

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

Instructors: Larry Millet, MNTL, Electrical and Computer Engineering
Elise Corbin, Mechanical Science and Engineering
Mitchell Collens, MNTL, 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 $\varepsilon_p < \varepsilon_m$ (Fig. 1), making the polarity of the applied field irrelevant.

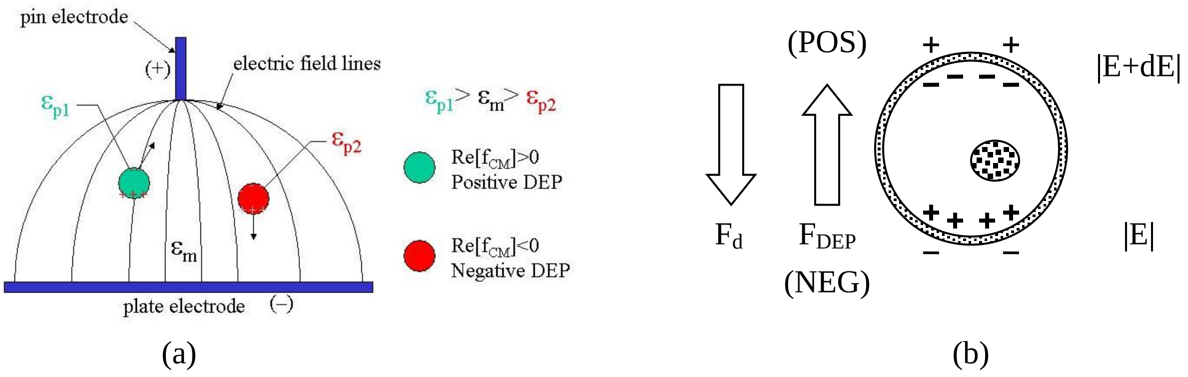

Fig. 1: **(a)** Schematic illustration of dielectrophoresis and **(b)** representative p-DEP on a cell.

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

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

where complex permittivity (ε^*) is equal to $\varepsilon + \sigma / j\omega$ and Re{…} is the *Clausius-Mossoti* 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.

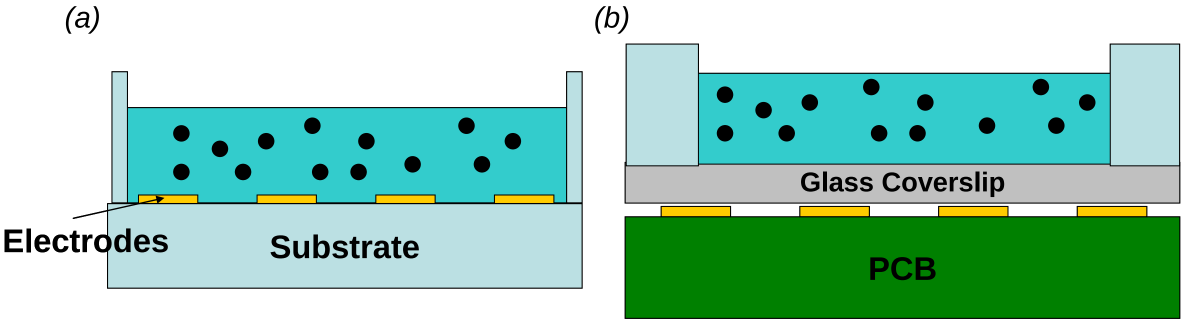

Fig. 2. (a) A schematic diagram of the conventional approach in implementing DEP. Electrodes and substrate should be disposed of after each experiment. (b) A schematic diagram of the PCB-based DEP implementation. DEP electrodes are easily fabricated on a PCB and can be reused again and again since the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

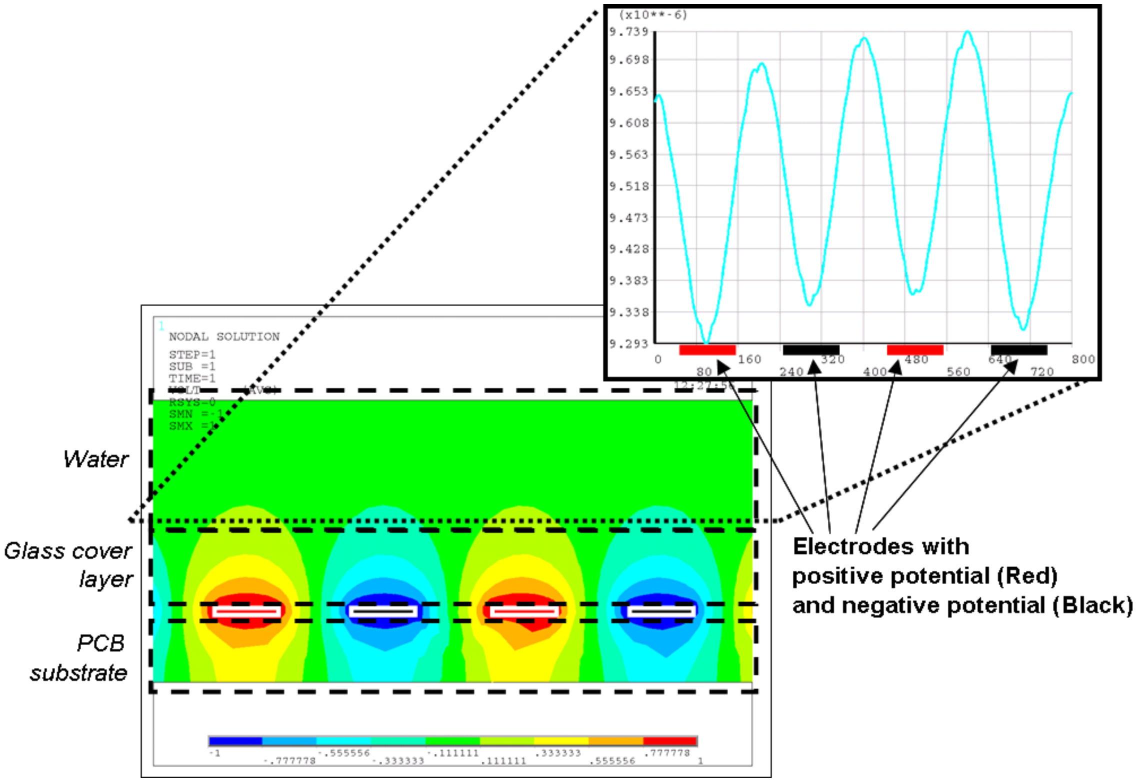

Fig. 3: Numerical analysis of the electric potential generated by the PCB electrodes. Electrodes in red and in black are set to +1V and -1V respectively. *Inset:* Square of the electric field magnitude, $|E|^2$, on the dotted line 10um above the glass cover layer. Black and red bar shows the position 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 (~100 µm) 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 15 µm diameter FITC fluorescent 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)
    c. Place glass coverslip and PDMS chip (channel side up) into the oxygen plasma chamber. The coverslip and PDMS surfaces will be activated for covalent bonding by this process. Once plasma oxidation is finished, lightly press PDMS (channel side down) onto the coverslip to create a permanent bond.
    d. Immediately pipette ~20 μL of bead/cell solution into one of the chip's ports to load the channel. The PDMS is normally hydrophobic, but becomes temporarily hydrophilic after plasma oxidation, allowing the aqueous bead solution to easily fill the channel. Plasma activation lasts ~10 minutes. The pressure gradient between the two ports will create a bead drift velocity which will help in the visualization of DEP capture. To adjust the flow velocity, PBS can be pipetted to the other port to create a smaller pressure gradient; the larger the gradient, the greater the bead/cell velocity.
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 um (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).

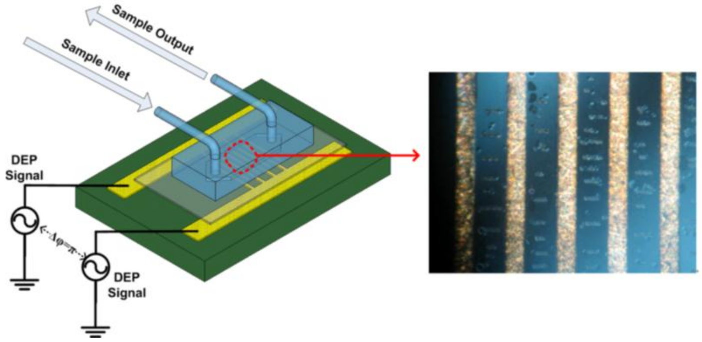

Fig. 4: The device is made from a PCB and a thin glass coverslip. The glass coverslip serves as an insulator between the solution and the electrode pattern.

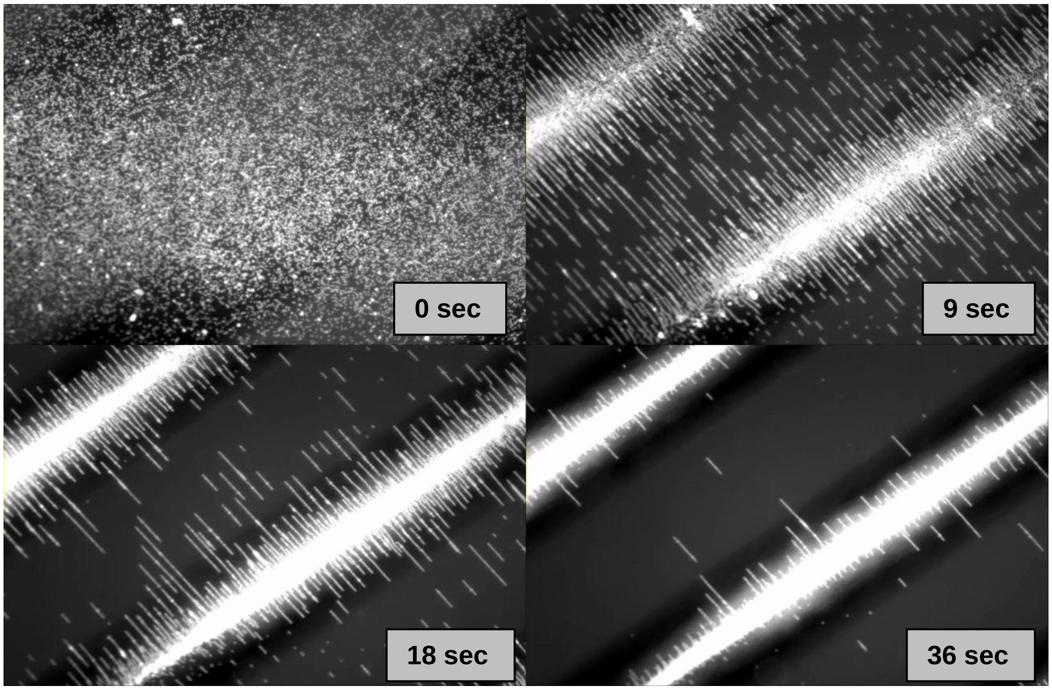

Fig. 5. Dark field images of the fluorescently labeled 3μm polystyrene beads during DEP. The trajectories of the polystyrene beads are visible as short lines, indicating the traveled distance during exposure time of the camera. After applying the AC voltages for 36 seconds, most of the beads become focused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

Related References:

*www.**jove**.com/details.php?id=2545*

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MICROFLUIDICS AND ENABLING TECHNOLOGY LAB MODULE:
Photonic Crystal Biosensing

Location: BioNano Lab, 3119 Micro and Nanotechnology Laboratory (MNTL)
Instructors: Erich Lidstone, Bioengineering
Larry Millet, MNTL, Electrical and Computer Engineering
Mitchell Collens, MNTL, Bioengineering

Purpose and Expected Outcome:

The purpose of this laboratory module is to provide an introduction and a hands-on demonstration of biosensing using photonic crystals (PC). The students will be able to prepare a biochemical assay for examining the changes in the biochemical interactions at the sensor surface. The expected outcome is for the students to gain a basic understanding of PC biosensors and the potential applications for biosensing in biology and medicine.

Overview of Photonic Crystals:

Label-free photonic crystal optical biosensors have recently been demonstrated as a highly sensitive method for performing a wide variety of biochemical and cell-based assays [1-3]. The device structure is designed to reflect only a narrow band of wavelengths when illuminated with white light at normal incidence [1]. Reflected light is collected through a detection fiber, guided into a spectrometer and can be collected over a period of hours to generate kinetic and endpoint plots of biomolecular binding events.

More specifically, optical PC-based biosensors consist of a periodic arrangement of materials with a differing dielectric permittivity along one or more dimensions. By choosing the materials and period carefully, it is possible to specify a resonant wavelength for the device. Using two materials with different refractive indexes (low and high), the electromagnetic field is localized near the surface to selectively alter the reflection or transmittance of specific wavelengths of light. The devices are illuminated from the bottom at a normal angle using a fiber optic cable and broadband white light source. Because only certain wavelengths of light can effectively couple with the photonic crystal structure, only the resonant wavelength is reflected at a normal angle.
The reflected light is collected from the illumination probe using a beam-splitter, and the wavelength is read with a spectrometer.

Events that change the dielectric permittivity (alter the refractive index) of the region near the sensor surface alter the electric field, and result in a shift of the reflected peak wavelength value (PWV). Changes in PWV are measured in nanometers, and can be measured with great precision, accuracy, and reproducibility. Events capable of producing a PWV shift include protein-protein binding events, nucleic acid-protein interactions, protein-small molecule interactions, and cell attachment and adhesion. Thus, PC biosensors can be used effectively for studying a range of biological processes, including: protein-protein, protein-cell and small molecule-protein interactions, cytotoxicity. In this exercise, PC biosensors will be characterized and used to demonstrate protein-protein binding interactions.

Module Outline and Workflow:

The students are expected to gain an understanding of PC biosensors and their biological applications through a three-part lab. One component of the lab will demonstrate sensor construction and sample preparation. In another component, students will select samples to measure on the SRU Biosystems BIND reader using a PC microplate array. The remaning lab component will demonstrate one application of PC biosensors - determining enzyme kinetics using a concentration series of two putative protein binding partners. Each of these three components will be setup into individual stations, where all students will have the opportunity to learn each lab component by rotating through each station once.

Equipment, Materials, and Supplies:

SRU Biosystems BIND plate reader.
SRU Biosystems blank 96-well TiO2 plate.
Phosphate buffered saline (PBS)
Dimethyl Sulfoxide (DMSO)
deionized water (DI)
Protein/biochemical samples for sample measurement.

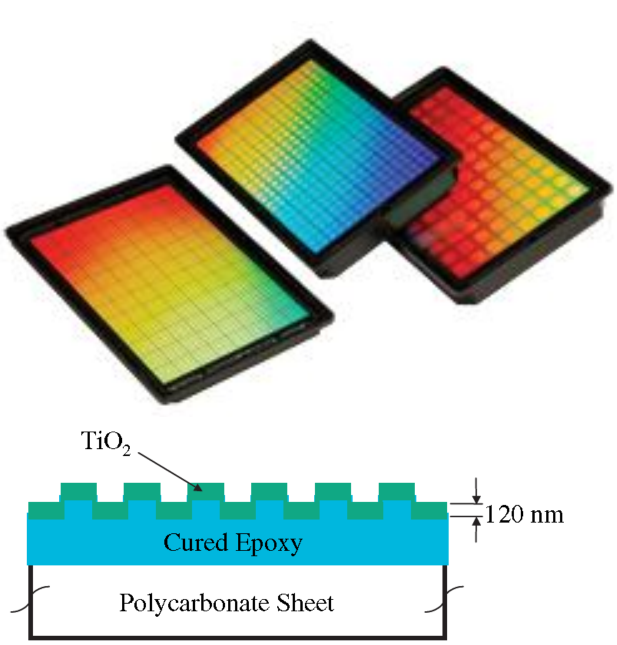

Fig. 1. (a) Photonic crystal plates 1536-, 384- and 96-well plates. (b) Schematic structure of Photonic Crystal biosensor displays periodic arrangement and nano-scale topography. Changes in the refractive index of the two material components and structural characteristics enable the detection of small molecule interactions.

Station 1: Experimental Setup:

Preparation

Premix 7 different concentrations of Dimethyl Sulfoxide (DMSO) at 120 µL, ranging from 100 to 1.56% DMSO with DI in the centrifuge tubes

Lay out 7 centrifuge tubes in the tube rack and label each tube with its appropriate concentration.

Fill each tube with 120 µL of deionized water (DI) except 100% tube.
Pipette 240 µL of 100% DMSO into the 100% tube, then pipette out 120 µL from that tube to dilute by a factor of 2 in the 50% DMSO tube.
Repeat this process until all the concentrations are made (100, 50, 25, 12.5, 6.25, 3.125, 1.56)

Station 2: Sensor Characterization and the Protein-Protein Binding Assay

Part A: Characterize the Sensor Noise, Uniformity, and Bulk Sensitivity

Before measuring samples of interest, the illumination/reflection behavior of a photonic crystal biosensor must be measured to characterize the uniformity, the resolution limits, and thermal drift. This is easily accomplished by measuring the "bulk" sensitivity to changes in the refractive index of the sample induced by mixtures of water and DMSO at a range of concentrations. To perform the calibration, a range of Dimethyl Sulfoxide (DMSO) concentrations in sample buffer are prepared.

Bulk shift air to DI → Illumination/reflection → DMSO concentrations.

Step 1. Bulk Shift Measurement of Air to DI: This part of the lab will demonstrate that the device structure responds to changes in refractive index and medium, and that the device is configured to work best in aqueous media, rather than in air.
Select the wells to be scanned (A1-A4)
Place the microplate in the holder and press collect
Record the PWV (nm) and FWHM (nm) for each of 4 empty wells
Press the Stop/Cancel button, and add 40 µL DI water to each well

Step 2. Illumination/Reflection Behavior: In this portion of the lab, we'll characterize the PC biosensor and determine the resonant wavelength. We'll also measure the peak width by determining the full width at half maximum (FWHM, nm) for 4 wells filled with water.
Add 40 µl of DI water to wells A1-D1
Place the microplate in the holder and press collect
Record the PWV (nm) and FWHM (nm) for each of 4 wells filled with water

WELL A1: PWV (nm):_____ FWHM (nm):______
WELL A2: PWV (nm):_____ FWHM (nm):______
WELL A3: PWV (nm):_____ FWHM (nm):______
WELL A4: PWV (nm):_____ FWHM (nm):______

Step 3. Bulk Shift Measurement of Various DMSO Concentrations: In this section of the lab, we'll examine how PC biosensors respond to changes in bulk refractive index. To do so, we'll look at the PWV shifts created by introducing a solution of differing refractive index to each well.
For wells A1-A4, add 40 uL of one of the following to each well: DI water, 100% DMSO, 50% DMSO, 25% DMSO.
Place the microplate in the holder and press collect
Measure the change in PWV as a result of the refractive index change of the bulk solution.

0.00% DMSO PWV Shift (nm):________
12.5% DMSO PWV Shift (nm):________
25.0% DMSO PWV Shift (nm):________
50.0% DMSO PWV Shift (nm):________

Part B: Protein-Protein Binding Assay

Goal: Attach a protein (Protein A) to the sensor by simple adsorption, and rinse the wells with water. Expose the protein to sheep IgG and pig IgG at an intermediate concentration, and determine which IgG has a higher affinity for Protein A.

Preparation

Retrieve the microplate from the BIND Reader
Prepare 0.1 mg/ml of Pig and Sheep IgGs by mixing 0.2 mg of each IgG with 2 mL of PBS buffer in a centrifuge tube
Aspirate the Protein A/PBS solutions from wells A5-A8. The protein was adsorbed to the sensor surface before the lab to save time.
Add 40 uL PBS to wells A5-A6. Well A5 is a negative control, and so has not been treated with protein of any kind. Well A6 has been treated with Protein A, along with Wells A7 and A8.
Add 40 uL of 0.1 mg/ml Pig IgG to well A7 and 0.1 mg/mL Sheep IgG to well A8.
Place the microplate into the BIND Reader and click Collect to obtain the binding information.

PBS PWV Shift (nm):________
Protein A PWV Shift (nm):_________
Protein A + Pig IgG PWV Shift (nm): _________
Protein A + Sheep IgG PWV Shift (nm): _________

Station 3: Protein-Protein Binding Assay

Obtaining quantitative binding information with the BIND System

Goal: Given a set of BIND PWV Shift data, determine the Kd, or dissociation constant, for a protein-protein interaction.

The dissociation constant Kd for a given biomolecular interaction (such as two proteins binding one another) may be defined as the concentration of analyte at which half of the mass of analyte in the solution is bound by the binding partner. Similar to the Protein A-IgG binding experiment conducted in Module 2, the PC biosensors may be functionalized with a target protein to measure the binding affinity of another protein for the target protein. If the sensor surface is saturated with the target protein, a variety of concentrations of analyte can be incubated on the sensor, and the resulting PWV shift value can be measured. The concentration of analyte at which the Reader registers 50% of the saturated sensor may thus be set as the Kd.

Please determine the Kd of these two antibodies with respect to a sensor coated with Protein A:

IgG Concentration (mM)	Pig IgG PWV Shift (nm)	Sheep IgG PWV Shift (nm)
0.001	0.01	0.00
0.005	0.02	0.03
0.01	0.03	0.32
0.05	0.21	0.54
0.1	0.52	0.63
0.5	0.83	0.62
1.0	0.91	0.63
5.0	0.88	0.61

Summary: Photonic crystal-based biosensors take advantage of the change in refractive indexes (low and high) of different materials to localize the electromagnetic field near the sensor surface to selectively alter the reflection or transmittance of specific wavelengths of light. By measuring nanometer-scale changes of the reflected peak wavelength value, biochemical interactions can be measured with accuracy, and reproducibility. These include protein-protein binding events, nucleic acid-protein interactions, protein-small molecule interactions, and cell attachment and adhesion. In conclusion, improved biosensors are advancing the ability to detect, diagnose and characterize biochemical processes in research, medicine, and beyond.

Related References:

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