MICROFLUIDICS HANDS-ON WORKSHOP GUIDE

Professor Todd Squires * and
Dr. Yolanda Fintschenko†

* squires@engineering.ucsb.edu, University of California, Santa Barbara, Chemical Engineering Department, Mail Code 5080, Santa Barbara, CA 93106-5080 USA† yfintschenko@labsmith.com, LabSmith, Inc., 6111 Southfront Road, Ste. E, Livermore, CA 94551 USA
COURSE OBJECTIVES

- Introduce experimental parameters that are important for successful microfluidic experiments
- Provide practical experience constructing microfluidic circuits using tubing and microfluidic chips.
- Demonstrate controlling and imaging fluid movement in microfluidic circuits using manual and automated solution delivery.

WORKSHOP AGENDA

- Lecture – 9 – 11 am.
  *Dr. Todd Squires (UCSB)* [http://squerver.chemengr.ucsb.edu/Welcome.html](http://squerver.chemengr.ucsb.edu/Welcome.html) Topics will include:
  - Linear electrokinetic phenomena and how to think about them.
  - Nonlinear electrokinetic phenomena.
  - Effects of surface conduction and ion conservation.
  - Convection vs. diffusion, Peclet number for separations, filtration and mixing.
  - Convection, diffusion, and reactions for sensor applications.
  - Live demo experiments with LabSmith equipment.

- Demonstration and Hands-On Experiments: 11 am - 12 pm.
  Attendees will be divided into small groups in order for the class to perform an experiment and see a demo.

TABLE OF CONTENTS

1 DEMO: ELECTROKINETIC FLOW: ELECTROKINESIS, ELECTROOSMOSIS AND ELECTROPHORESIS ......................3
2 DEMO: PARTICLE IMAGE VELOCIMETRY .................................................................5
3 DEMO: PROGRAMMING AN ELECTROKINETIC INJECTION ............................................7
4 DEMO: DIELECTROPHORESIS ....................................................................................10
5 DEMO: HYDRODYNAMIC FOCUSING ........................................................................13
6 HANDS ON: BUILDING A MICROFLUIDIC CIRCUIT FOR MANUAL OPERATION USING CAPILLARY TUBING ......15
7 HANDS-ON: AUTOMATING FILLING AND DISPENSING OF A SOLUTION .................................18
1 DEMO: ELECTROKINETIC FLOW: ELECTROKINESIS, ELECTROOSMOSIS AND ELECTROPHORESIS

GOAL: Understand and control the effects of applied voltage, suspending medium properties (pH and conductivity), particle charge and size, and zeta potential of the microchannel on flow due to electroosmosis and electrophoresis.

In this activity you will learn:

1. How to fill a microfluidic channel on a chip by capillary action
2. How to prepare solutions for microfluidic chips
3. How to image an electrokinetic flow using micro Particle Image Velocimetry (microPIV)

Supplies:

- Chip with straight channel and mini luer connector (microfluidic ChipShop)
- Male mini luer adapter (microfluidicChipShop)
- 1 um fluorescent beads (Life Technologies)
- Buffer
- Syringe and flexible tubing
- 0.2 um diameter Nylon syringe filter (Millipore)

LabSmith Equipment:

- SVM340 synchronized video microscope and uScope™ software
  - EPI –BLUE camera
  - 10X Objective
- HVS448 eight channel high voltage sequencer and Sequence™ software
  - 2 high voltage cables
  - 2 Pt electrodes
- integrated Breadboard (iBB), chip clips, electrode clips

PREPARE THE CHIP

Remove particles from your solution and preserve solution from bacterial growth:

1. Fill 1 ml plastic syringe with buffer.
2. Attach Millipore syringe filter to Luer connector on syringe.
3. Using syringe plunger, push solution through the filter.
4. Collect filtered solution in vial or small cup

**Filling the microfluidic channel with solution:**

Add buffer to ONE well. Observe the fluid filling the channel by eye by holding the chip at an angle and observing the difference in refractive index. Use slight pressure from syringe (filled with filtered solution) if necessary. Add solution with beads to opposite Luer well (the one that is mostly dry).

**Chip set up and conditions:**

Place bead loaded microchannel chip on the SVM340 platform. Using the uScope™ software, examine the microchannel. Bead density should look like static on TV and microchannel should be bubble and particle free. If bubbles or particles are present, attempt to remove by withdrawing solution or flushing using syringe. Place the electrodes labeled A and B in each reservoir at each end of the channel. There should be only one electrode per well. Turn HVS on and open Sequence software. Using Manual settings apply an electric field by setting Electrode A to 500 V and Electrode B to -500 V. The microchannel surface is negatively charged therefore the electroosmotic flow (EOF) should be going to Electrode B. Using the SVM340 observe the direction the beads move. As you switch polarity of the electrode, note the change in direction.
2 DEMO: PARTICLE IMAGE VELOCIMETRY

GOAL: Using the same chip for examining Electroosmosis, you will use the PIV probe function of the uScope™ software to create a real time PIV probe so you can view the velocity in um/sec in real time and record the data to a file.

Create a PIV Velocity Probe:
LabSmith uScope™ software makes it easy to create probes to monitor flow characteristics. Select a square probe with a size that is large enough to encompass a large number of particles, but not too large so the mean velocity varies over the probe area.

To create a probe:
1. Left click on the Velocity Probe toolbar button to highlight the button.
2. With the mouse curser over the image, right click on the mouse and select New Probe. Bring the mouse to the center point for the new probe and left click the mouse to place the probe. A probe such as the one below (Figure 2) will appear.

![Figure 2. New probe showing correlation field, vector arrow and real time velocity as text.](image)

3. Repeat step 2 to create as many probes as desired.
4. Once created, the probes can be moved by clicking and holding the mouse button over the probe and dragging it to the desired location.
5. To remove a probe, right click on it and choose Delete.

Velocity Probe Properties
1. Double-click on left mouse button on a probe to open the PIV Probe Properties dialog box (Figure 3). You can also right-click on the probe and choose Properties to open the dialog box.
2. Select the Width and Height of the probe window, the area over which statistics will be calculated.

Probe Window Size Guidelines
Probes should typically be the same size in both x and y directions. Smaller probes require less processing power, so use smaller window sizes to run more probes simultaneously. Increasing the probe size will improve the signal-to-noise ratio; decreasing the size will increase spatial resolution. For fast flow, the probe size must be large enough that the correlation does not fall beyond the

![Figure 3. PIV Probe Properties dialog box.](image)
window. The Cross Correlation field (see Figure 3) can be an aid in setting the size.

**Recording Probe Data**

Data can be recorded simultaneously from all probes. To record data:

1. Choose File > Measurement File Saving to select how the recorded data will be saved (Figure 4):
   a. If you choose Do not auto-name, uScope™ will prompt you for a file name and location for each new recording.
   b. Choose Auto-name files to automatically name each recording. Check Append the date, Append the time, and/or Append counter to add these values to the new file names.

2. To begin recording choose File > Record, or click the Start/Stop Record Data toolbar button ▶. If Auto-naming is selected, recording will begin immediately. Otherwise, recording will begin after you name the file and click OK.

3. To end recording, click the Start/Stop button again.

The PIV output file will include four columns for each probe: the X and Y locations of its centroid, measured from the upper left of the window, and the X and Y velocity at each point in time. The X/Y location columns will only have entries in the first row.

It is possible to use the probes on recorded data as well. In uScope select Video > Playback/Process Saved Video, then create probes and record data as described above.

**Observations**

Note the velocity. Raise the voltage and vary the polarity on one or both electrodes - you should observe the velocity changing in real time. Save the data if you wish.

Using the same chip, take a snapshot in uScope™. Note that by varying the strobe time it is possible to create a “flash” setting. Observe the effect of averaging. Now record a movie. Save the AVI to your LabSmith USB. If you have time in class or at a later time you can open the movie and apply the PIV and intensity probes.
3 DEMO: PROGRAMMING AN ELECTROKINETIC INJECTION

GOAL: For electrokinetic separations, it is important to consider the effects of channel geometry, timing and electric field distribution and control on the injection plug defined in a microfluidic channel.

In this activity you will learn:

1. How to use Sequence software to program an eight channel high voltage power supply
2. How to image electrokinetic phenomena using fluorescent dye
3. How to make an on-chip electrokinetic injection
   a. Gated
   b. Pinched

Supplies:

- Chip with cross channel and CapTite™ bonded port connectors and reservoirs
- Oregon Green in 10 mM Tris, pH 8
- Buffer - 10 mM Tris, pH 8
- 1 ml syringe
- 0.2 um diameter Millipore Nylon syringe filter

LabSmith Equipment:

- SVM340 synchronized video microscope and uScope™ software
  - EPI-BLUE camera
  - 10X Objective
- HVS448 eight channel high voltage sequencer and Sequence™ software
  - 4 high voltage cables (2)
  - 4 Pt electrodes
- Integrated Breadboard (iBB) + chip clips and electrode clips + on-chip reservoirs

PREPARE THE CHIP

Remove particles from your solution and preserve solution from bacterial growth:
Refer to Figure 5 for chip diagram. Withdraw 10 mM Tris buffer with 1 ml syringe. Attach syringe filter to Luer connector. Using syringe plunger, push solution through the filter.

Add buffer to Buffer Waste (East reservoir). Observe the fluid filling the channel by eye by holding the chip at an angle and observing the difference in refractive index. Use slight pressure from syringe (filled with filtered solution) if necessary. Add Oregon Green in 10 mM Tris to Sample reservoir (North).

**Positioning chip on SVM340.** Using uScope™, check channels for bubbles/particles. If OK, position cross over objective. Position the electrodes in each well with electrode A at North proceeding clockwise to electrode D at West. Then, open Sequence software and use the Simple Sequence wizard to program the voltages in Table 1 below. Use Table 2 to note any changes you made in the program.

**Table 1. Suggested voltage programs for injection and separation**

<table>
<thead>
<tr>
<th>Step</th>
<th>( V_{\text{North}} )</th>
<th>( V_{\text{East}} )</th>
<th>( V_{\text{South}} )</th>
<th>( V_{\text{West}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading</td>
<td>828</td>
<td>1500</td>
<td>0</td>
<td>752</td>
</tr>
<tr>
<td>Gating</td>
<td>1000</td>
<td>-3000</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>Pinching</td>
<td>-600</td>
<td>-1500</td>
<td>402</td>
<td>-792</td>
</tr>
<tr>
<td>Pinched Injection/separation</td>
<td>-61</td>
<td>-100</td>
<td>-11</td>
<td>-1500</td>
</tr>
<tr>
<td>Gated Injection/separation</td>
<td>280</td>
<td>0</td>
<td>280</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Table 2. Adjusted voltage programs for injection and separation**

<table>
<thead>
<tr>
<th>Step</th>
<th>( V_{\text{North}} )</th>
<th>( V_{\text{East}} )</th>
<th>( V_{\text{South}} )</th>
<th>( V_{\text{West}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading</td>
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<tr>
<td>Gating</td>
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<td>Pinching</td>
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<tr>
<td>Pinched Injection/separation</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gated Injection/separation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Enhanced visualization for microfluidics applications,** modular bottom-up viewing and fluorescent illumination for compact microfluidics microscope.

Using the same chip, take a snapshot. Note that by varying the strobe time it is possible to create a “flash” setting. Observe the effect of averaging. Now take a movie. Open the movie and apply the PIV
and intensity probes. Save your movie on your LabSmith thumb drive. It will open as an AVI on your computer. You have now made electrokinetic injections of dye, imaged the injection, and recorded the images.
4 DEMO: DIELECTROPHORESIS

Focus on insulator based dielectrophoresis, streaming and trapping regimes. Applications for the concentration and sorting of particles in a single device.

In this activity you will learn:

1. How to fill a microchannel
2. How to introduce a sample of particles
3. How to image an array of insulating structures
4. How to manipulate particles with insulator-based Dielectrophoresis

Supplies:

- Chip with microchannel with insulating posts
- 1 um beads suspended in buffer solution
- Filtered buffer solution
- SVM340
- HVX488
- EPI–Blue camera
- 4X objective
- Micropipette

PREPARE THE CHIP

A diagram of the microchannel is shown in Figure 6 for your reference.

Preparing the microchannel:

Similar to the process used with PIV, fill the microchannel with filtered buffer solution. Revise the microchannel with the microscope to make sure that there are not significant air bubbles, since air bubbles also act as insulating structures. Try to eliminate air bubbles by pushing more solution into the channel. Once the channel is filled with the buffer, introduce...
50 uL of bead solution to the inlet (just pick one reservoir) by using a micropipette. Then place the electrodes in the reservoirs, one electrode in the inlet and one electrode in the outlet reservoir. You are now ready to start Dielectrophoresis!

**Insulator-based Dielectrophoresis (iDEP)**, streaming and trapping.

**Fundamentals:** Before applying the electric field the particle should like static, as shown in Figure 7a. As mentioned in the theory part of this workshop, Dielectrophoresis is negligible at low applied electric fields, for a system like the one we are using, at an applied potential of 200-300 V you will see streaming Dielectrophoresis. This a regime where Dielectrophoresis and linear electrokinetics (mainly electroosmotic flow) have similar magnitudes and particle will move in streamlines, as shown in Figure 7b. Both, electrokinetics and Dielectrophoresis are influencing particle movement. At higher applied electric fields, Dielectrophoresis overcomes electrokinetics, and this is when particles can be immobilized or “trapped”. An example of particles being dominated by Dielectrophoresis is shown in Figure 7c, where you can observe that a potential of 350 V, particles are mainly being trapped, only few particles “escape” the dielectrophoretic traps and flow through the post array. By increasing the applied potential to 600V, Dielectrophoresis becomes the dominant mechanism and 100% of the particles are immobilized as shown in Figure 7d.

For more examples and videos of insulator based DEP visit [http://microbioseplab.org/videos.html](http://microbioseplab.org/videos.html)
**PROCEDURE for iDEP**, streaming and trapping:

1. Place bead loaded iDEP microchannel chip on the SVM340 platform; make sure you are using the 4X microscope objective. Focus the microchannel and observe the beads, you will be able to see the beads as tiny particles. You will see only a small part of the microchannel as shown in Figure 6; try to focus four constrictions so you can observe four different regions of dielectrophoretic trapping.

2. Turn HVS on and open Sequence software. Using Manual settings apply an electric field by setting Electrode A (inlet reservoir) to 200 V and Electrode B (outlet reservoir) to 0 V. You should see the beads moving towards the outlet reservoir, due to electroosmotic flow. If the particles are moving in streamlines, it means that you already reached “streaming” iDEP, snap a picture or make a short video (3-5 seconds) of the particles flowing. Name your video iDEP-200V.avi. Save your movie on your LabSmith thumb drive. It will open as an AVI on your computer.

3. Increase your voltage to 350 V, you should see a combination of streaming and trapping iDEP, Snap a picture or make a short video (3-5 seconds) of the particles flowing. Name your video iDEP-350V.avi. Save your movie on your LabSmith thumb drive. It will open as an AVI on your computer.

4. Increase your voltage to 600 V, you should see dominant trapping iDEP, snap a picture or make a short video (3-5 seconds) of the particles flowing. Name your video iDEP-600V.avi. Save your movie on your LabSmith thumb drive. It will open as an AVI on your computer.

You have now successfully completed a set of insulator-based DEP experiments, Congratulations!

For more examples and videos of insulator based DEP visit [http://microbioseplab.org/videos.html](http://microbioseplab.org/videos.html)
5 DEMO: HYDRODYNAMIC FOCUSING

GOAL: Using pressure driven flow for microfluidic applications

In this activity you will learn:

1. How to build a hydrodynamic focusing fluid circuit
2. How to control hydrodynamic focusing on a chip using uProcess software
3. How to take a snapshot and movie using uScope software on the SVM340

Supplies:

- Cross channel through-hole chip with LabSmith bonded port connectors (C360-400) bonded to each port
- 10 mM Tris buffer, pH 8
- Oregon Green in 10 mM Tris buffer
- 0.2 um syringe filters (Millipore)
- 1 ml syringes (2)
- CapTite direct-connect syringe

LabSmith Equipment:

- uProcess software
- 3 SPSO1 syringe pumps
- 3 AV201-360 automated valves
- 1 Electronic Interface Board (EIB)
- 1 4VM01 valve manifold
- 1 iBB
- ~25 C360-100 one-piece fittings
- 10 C360-101 plugs
- 3 chip clips
- ~1 m 150 um i.d./360 um o.d. PEEK
- 6 Breadboard Reservoirs (1.1 ml volume)
- SVM340 + EPI-BLUE camera+10X objective & uScope software

PREPARE THE CHIP

![Diagram of basic cross microfluidic chip and fluid circuit for hydrodynamic focusing. Pump 1=dye; Pumps 2,3 =buffer; = valve; = breadboard reservoir, Reservoir A=Dye; B=Buffer; C= Buffer Outlet; D=Buffer.](image)
If time permits, perform the following, if not skip to Controlling Hydrodynamic Focusing below:

*Remove particles from your solution and preserve solution from bacterial growth:*

1. Fill 1 ml syringe with buffer.
2. Attach syringe filter to Luer connector.
3. Using syringe plunger, push solution through the filter and into cup or vial

*Filling the microfluidic channel with solution:*

Using CapTite syringe, fill chip from buffer outlet well with filtered solution buffer. Examine channels using SVM340 and uScope™ software to ensure channel is bubble and particle free. Hydrodynamic circuit is already assembled. Using uScope™, make sure the microscope is positioned on the channel cross intersection.

*Controlling Hydrodynamic Focusing:*

Open uProcess™ to control the syringe pumps and valves. Load Hydrodynamic Focusing file. Run program. Use the uProcess software to change flows and observe the effect on focusing the dye stream. If you wish, use the uScope™ software to take a snap shot and record a movie on your LabSmith USB for later viewing. In the device control window found on the top right of your screen (Figure 9) make the necessary adjustments to syringe pump flow rate to change the focusing. You have performed, imaged, and recorded hydrodynamic focusing on–a-chip.

![Device Control Overview Window](image)

*Figure 9. Device Control Overview Window.*
6 HANDS-ON: BUILDING A MICROFLUIDIC CIRCUIT FOR MANUAL OPERATION USING CAPILLARY TUBING

GOAL: Understand and control the movement of fluid to miniaturize, automate, and perform useful laboratory tasks such as making injections, performing reactions, and centrifugation using simple tubing and microconnector kits.

In this activity you will learn:

1. How to design and build a simple fluid circuit using tubing.
2. How to test circuit for leak-tight connection
3. How to trouble shoot and fix leaks

Supplies:

- Water
- Food coloring (optional)
- 1 cc Luer Syringe (3)
- 3 cc Luer-Lock Syringe (2)
- 0.2 um diameter Nylon syringe filter (Millipore) (3)

LabSmith Equipment:

- One-piece fittings to connect tubing to CapTite components
- One-piece plugs to leak test fluid circuit
- 360 um o.d./150 μm i.d. PEEK tubing
- 360 um o.d./100 μm i.d. fused silica tubing
- Manual Valve (3 port, 2-position)
- Luer-Lock Adapters for connection of Luer-Lock syringe to tubing
- Luer adapters for connection of luer syringe to CapTite female port
- Filter holder for reaction/filtration/extraction membrane
- Union
- Cross
- Breadboard
- Tubing cutter
- ¼” Torx head screws for mounting valves
- ½” Torx head screws for mounting crosses and unions
- Stand-offs
- Torx wrench
- Microfluidic chip
PREPARE THE FLUID CIRCUITS

Remove particles from your solution and preserve solution from bacterial growth:

1. Withdraw buffer with 1 ml plastic syringe.
2. Attach syringe filter to Luer connector on syringe.
3. Using syringe plunger, push solution through the filter
4. Collect filtered solution in vial or small cup

Construct and test the fluid circuit

Select a fluid circuit to construct (A, B, or C above). Gather all parts required. If using a valve, select a breadboard with the manual valve mounted on the breadboard. Lay out fluid circuit without cutting tubing so tubing length can be estimated. Construct the fluid circuit as follows:

1) Cut tubing to length
2) Pass one end of tubing through a one-piece fitting, thread into corresponding component, and finger tighten.
3) Tug gently on tubing to ensure it is secure.
4) If tubing comes loose, remove the one-piece fitting and repeat steps 2 and 3. If the tubing cannot be secured in the component one of the following problems may have occurred:
   - Tubing end does not have a clean cut. This is common for fused silica, which can easily break and cause jagged ends. PEEK tubing cut at an angle can cause the same problem.
   - Component port has trapped debris. If possible, flush the port with water or solvent to remove debris. Inspection under a microscope is useful for detecting debris.
5) Connect next leg of tubing using steps 2-4 above.
6) Test that the connections are leak free by slightly pressurizing circuit while blocking component outlet(s) either using plugs or by turning the valve to the closed position. Any leaking ports should be re-secured using steps 2-3 above.

Once successful with water, you may wish to try the following:

1. Replace water in one or both syringe(s) with food coloring

Figure 10. Examples of tubing circuits to construct A. Injector B. Reactor C. Centrifuge.
2. Connect circuit to a microfluidic chip. See if you can observe solution by eye.

3. If time permits, place chip connected to circuit on SVM microscope and observe operation.
7 HANDS-ON: AUTOMATING FILLING AND DISPENSING OF A SOLUTION

GOAL: Learn to automate the withdrawal of solution into a syringe pump and delivery of the solution into a fluid circuit.

In this activity you will learn:

1. How to build a simple automated circuit for filling and refilling a syringe pump that delivers solution to a microfluidic channel
2. Test and troubleshoot connections for leak free connection
3. Fill automated syringe pump without air bubbles

Supplies:

- Laptop or computer
- Water
- Food coloring (optional)
- 0.2 um diameter Nylon syringe filter (Millipore)
- Luer syringe

LabSmith Equipment:

**Microfluidic Circuit**

- Breadboard reservoir
- One-piece fittings for 360 um o.d. tubing
- 360 um o.d./150 um i.d. PEEK tubing
- 360 um o.d./100 um i.d. fused silica tubing
- Manual Valve (3 port, 2-position). Note: an automated valve (LabSmith AV201) could also be used for this experiment.
- Tubing cutter
- ¼” Torx head screws for mounting valves
- ½” Torx head screws for mounting tees, crosses, unions
- Torx wrench
- Microfluidic chip
- One-piece plugs
- CapTite direct-connect syringe

**Automation components**

- uPB breadboard for 5 uProcess uDevices
- EIB electronic interface board
- 10 “ cable
- Power supply
- RS232 Cable
PREPARE THE FLUID CIRCUITS

Remove particles from your solution and preserve solution from bacterial growth:

1. Withdraw buffer with 1 ml plastic syringe.
2. Attach syringe filter to Luer connector on syringe.
3. Using syringe plunger, push solution through the filter
4. Collect filtered solution in vial or small cup

Construct and test the fluid circuit

Figure 11 shows the layout of the fluid circuit for automated solution withdrawal and dispensing using an automated syringe pump and manual valve. Using techniques described in Section 1, create fluid circuit shown above except do not connect tubing to syringe glass. Use plugs to block unused breadboard reservoir ports.

Connect the uPB5 breadboard to the EIB via the flat ribbon cable. Then connect the EIB to the ribbon cable, power, and computer via the RS232 connector. Open the uProcess software and select USB port to communicate.

Removing air bubbles from your fluid circuit

Upon initializing a fluid circuit it is important to remove air bubbles from the system.

1. Use the uProcess software to move the syringe to the full out position (position 0).
2. Fill a direct-connect syringe with water or the dispensing liquid. Alternately, a Luer-lock syringe with a short section of tubing can be used instead of the direct-connect syringe.
3. Connect the direct-connect syringe to the SPS01 syringe tip.
4. While placing slight pressure on the plunger of the direct-connect syringe, use the uProcess software to fill the SPS01 syringe at moderate speed (~50-100 ul/min).
5. Disconnect the direct-connect syringe from the SPS01 syringe tip and connect the capillary tubing connected to the valve inlet, as shown in Figure 11.
6. Ensure the manual valve is set to flow between the SPS01 syringe and the breadboard reservoir.
7. Use the uProcess software to push the syringe fluid into the reservoir.
8. Repeat this process as necessary until the breadboard reservoir begins to fill with fluid. For larger syringe sizes one iteration will probably be sufficient.
9. Fill the breadboard reservoir with the dispensing fluid.

Your system is now ready for use. Alternate filling the syringe with the valve open in the syringe-reservoir direction, and then dispense the fluid with the valve open to the syringe-downstream position.

Once successful with water, you may wish to try to connect the circuit to a microfluidic chip. See if you can observe solution by eye.

If time permits, connect the pump circuit to chip and place on SVM microscope and view.