

**MIE 439 – Introductory Biomechanics – Micropipette Aspiration Lab**  
**Department of Mechanical & Industrial Engineering**  
**University of Toronto**

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**Objectives:**

1. To determine the Young's modulus of a single suspended cell using a modified micropipette aspiration technique.
2. To quantify the degree of variation within a supposedly homogenous cell population.
3. To experience hands-on experimental research in the field of cellular biomechanics
4. To critically analyze and discuss the applicability, advantages and disadvantages of the current experimental setup as compared to a traditional micropipette aspiration setup.
5. To design improvements to device and experimental methods, based on theoretical knowledge and practical experience

## **1. Background Information**

Being able to obtain data about the mechanical properties of individual cells can provide us with a better understanding of how cells change in response to their environment – and this could be important in understanding disease progression and pathology. Cells have been shown to have a wide range of mechanical properties based on their type, function, environment and environmental history. Several techniques have been developed to determine the material properties of a single cell, including measurement using atomic force microscopy, optical tweezers, micropipette aspiration and magnetic bead microrheometry. In this lab, the cellular modulus will be determined using a modified version of micropipette aspiration.

### **1.1 *Micropipette Aspiration (MA)***

Section 2.5.4 of the textbook “Introductory Biomechanics” by Ethier & Simmons provides an excellent description of the function and capabilities of this technique. Figure 2.26 provides a schematic overview of the system. The following discussion assumes you have read this section.

An image of a typical micropipette aspiration setup is shown in Figure 1. Briefly, a micropipette with an internal diameter of 1-10 $\mu$ m (smaller than the diameter of a cell) is mounted on a micromanipulator. The manipulator is capable of moving objects in three-dimensions with micron-scale precision. While observing the micropipette tip in a microscope viewfield, the tip

is brought in contact with a floating cell (expanded view shown in Figure 2). A pressure head is then generated by adjusting the height of a liquid reservoir, as represented by Equation 1.

$$\Delta P = \rho g h \quad (\text{Equation 1})$$

where:  $\Delta P$  = change in pressure;  $h$  = height of liquid column  
 $\rho$  = density of liquid (1g/mL for water);  $g$  = standard gravity (9.8m/s<sup>2</sup>)

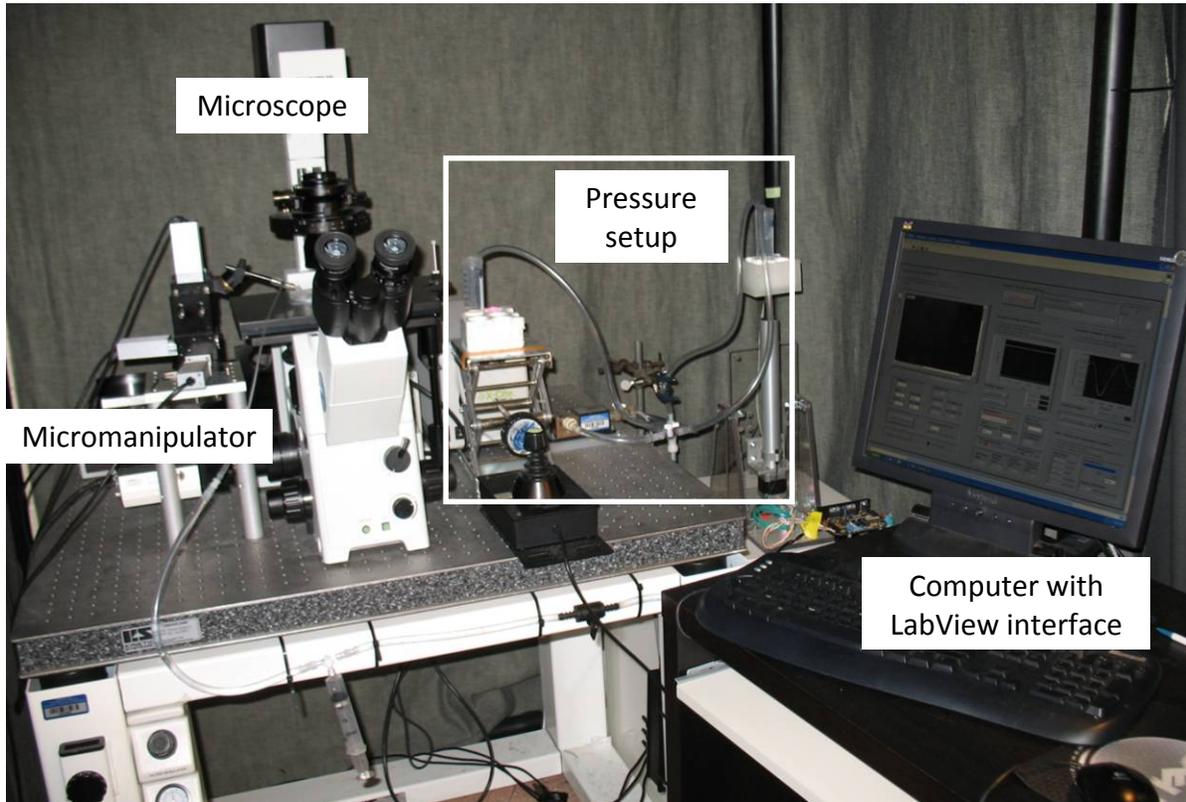


Figure 1 - Typical micropipette aspiration system (image courtesy Kristine Wyss, University of Toronto Cellular Mechanobiology lab)

The pressure differential caused by the height of the liquid column aspirates the floating cell into the micropipette, as shown in Figure 3. The aspiration length ( $L_A$ ) in conjunction with the applied pressure, can then be used to determine cellular stiffness. While there are a number of analytical models to extract stiffness information from these results, the half-space model is the simplest and is summarized in Equation 2.

$$E = \Phi \times \left( \frac{3r}{2\pi} \right) \times \left( \frac{\Delta P}{L_A} \right) \quad (\text{Equation 2})$$

where:  $r$  = pipette radius;  $L_A$  = aspiration length;  
 $\Delta P$  = change in pressure;  $\Phi$  = dimensionless constant

$\Phi$  is a dimensionless constant which depends on a number of factors. For the purposes of this lab, a value of  $\Phi = 2.1$  will suffice.

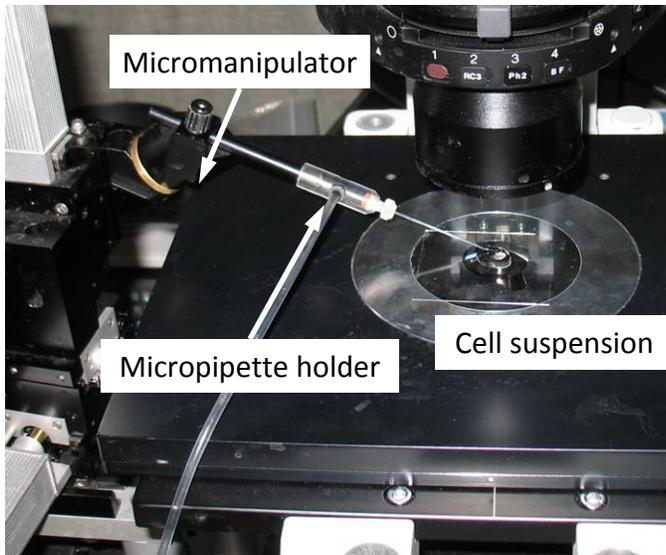


Figure 2: Micromanipulator setup

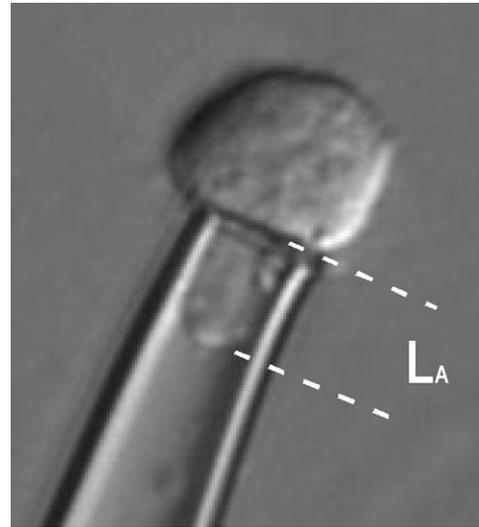


Figure 3: Aspiration length

Micropipette aspiration is an advantageous technique for mechanical characterization of cells because it is simple, relatively inexpensive and can provide force resolutions as low as picoNewtons. The primary costs associated with this system is in the microscopy station and in the micromanipulator. While most labs equipped for biomedical research will have a microscopy station, the micromanipulator is a specialized piece of equipment, costing approximately CAD \$4,000 each. In order to make this experimental technique easily available to a larger number of labs, we have developed a ‘soft’ microdevice capable of performing micropipette aspiration, while eliminating the micromanipulator component of the system.

## 1.2 Soft Lithography

Soft lithography refers to the microdevice fabrication technique in which a single microfabricated template is prepared using physical and chemical techniques under cleanroom conditions. This ‘master’ serves as a negative relief mold to produce a large number of polymeric devices.

In this lab, a Y-shaped channel is fabricated by casting polydimethylsiloxane (PDMS) onto a master, peeling it away and bonding it to a glass slide (Figure 4). The resulting channels are  $\sim 40\mu\text{m}$  in height.

## 1.3 Integrated MA Chip

A micropipette was then carefully placed and fixed into the base of the Y-shaped channel. Standard fluidic port connectors were used to connect the Integrated Micropipette Aspiration chip (iMAchip; Figure 5) to the fluid pressure setup. In this way, the microchannels can be used to deliver cells to the pipette, without the use of a micromanipulator during the experiment.

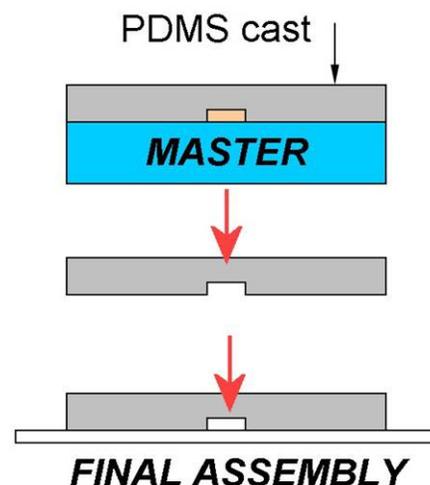


Figure 4: Soft lithography fabrication process

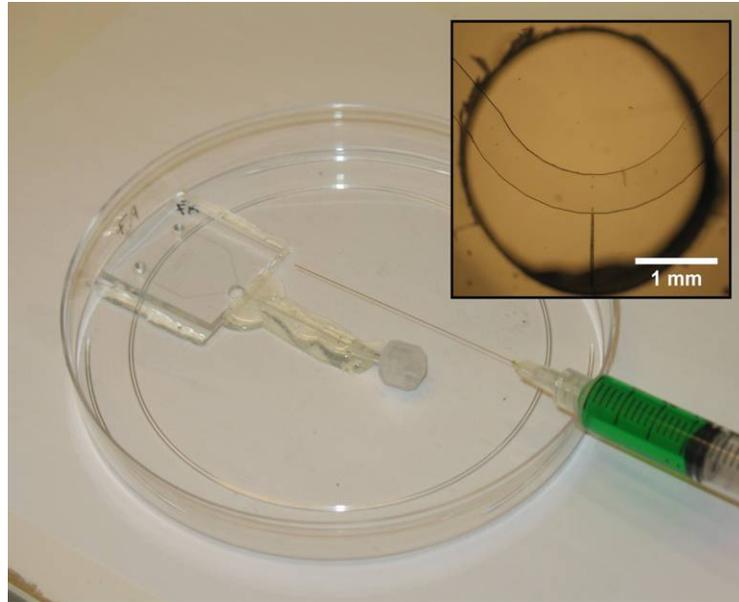


Figure 5: integrated Micropipette Aspiration chip (iMAchip)

## 2. Materials & Experimental Setup

### 2.1 Material list

- 0.5-1mL PAVIC cells, [1 million cells / mL]
- Pipetting bulb
- iMAchip microdevice
- Deionized water for washing the channels
- Pipetting equipment
- Fluid pressure setup:
  - PVC tubing with adapters
  - 1 luer valve (3way)
  - 60mL reservoir
  - 1x 10mL syringe
  - ruler, retort stand, clamp

### 2.2 Experimental Basics

#### 2.2.1 FLUID PRESSURE SETUP

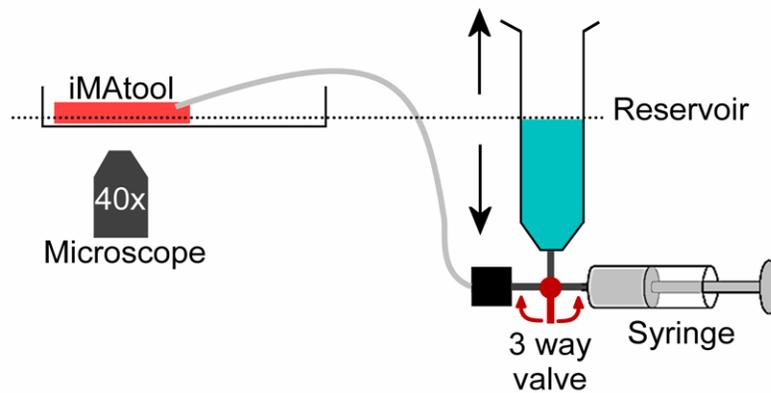


Figure 7: Schematic outline of iMAchip and pressure delivery setup

Prior to the lab, the teaching assistant will have set up this equipment. The fluid pressure setup consists of a height-adjustable reservoir, connected to a 3-way valve, a syringe and the iMAchip (Figure 7). The selector valve can be used to apply two ranges of pressure to the iMAchip.

- When the valve isolates the syringe from the rest of the system, liquid can be added to or removed from the reservoir to raise and lower liquid levels, thereby applying pressures to cells being aspirated. This pressure head system is typically used to observe cell deformation, once a cell has been held by the pipette.
- When the valve isolates the reservoir from the rest of the system, the syringe can be used to apply large suction or positive pressures to move cells within the microchannel. This system is typically used to attract a floating cell to the micropipette tip. If necessary, it can also be used to flush a cell away from the micropipette.

### 2.2.2 CONTROLLING ASPIRATION PRESSURES

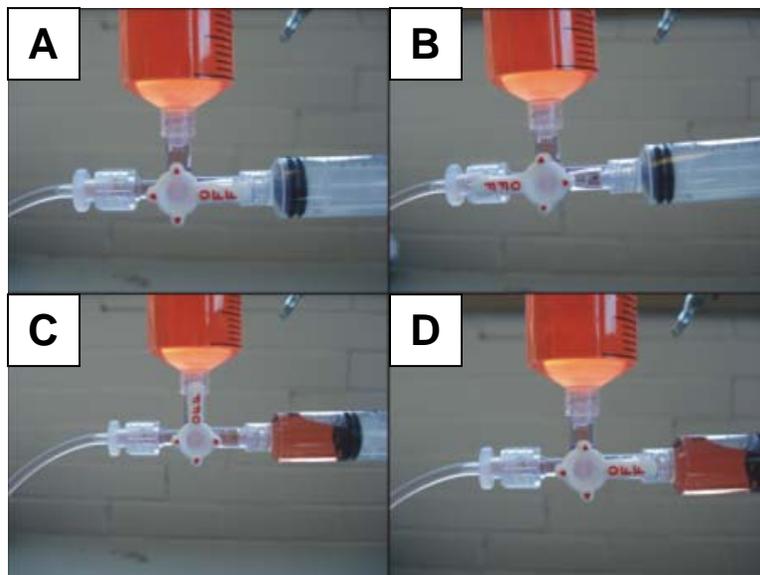


Figure 8: Controlling the pressures applied to the iMAchip

**A, D:** Luer valve stopcock isolates the syringe from the reservoir and iMAchip. Raising and lowering the height of liquid in the reservoir applies positive and negative pressures to the cell.

**B:** Luer valve stopcock isolates the iMAchip from the reservoir and syringe. This position can be used to fill the syringe half-way. Pumping the syringe here will eliminate any bubbles that may have formed.

**C:** The reservoir is isolated from the system, allowing the syringe to be used to attract and repulse cells within the microchannel.

In position A, the applied pressure depends on the height of the reservoir in relation to the microdevice. When the liquid level of the reservoir is at the same height as the iMAchip, the system is in equilibrium and zero pressure is applied.

### SAMPLE CALCULATION:

Reducing liquid height in the syringe by 5cm applies an aspiration pressure of

$$\Delta P = \rho g h \quad (1\text{g/mL} \times 9.8 \text{ m/s}^2 \times 5\text{cm} = \sim 0.5\text{kPa})$$

### 2.2.3 MANIPULATING FLUID WITHIN THE MICRODEVICE

Cell suspensions and washing solutions are introduced into the microchannel using an applied pressure head. To flush a cell suspension into the channel:

1. Mix the cell suspension thoroughly by gently pipetting up and down
2. Pipette 10 $\mu\text{L}$  of cell suspension into one of the ports in the arms of the Y-channel
3. Place the bulb securely around the port and gently press down to maintain a seal
4. Gently squeeze the bulb to apply the pressure head and drive fluid through the channels
5. Remove the bulb

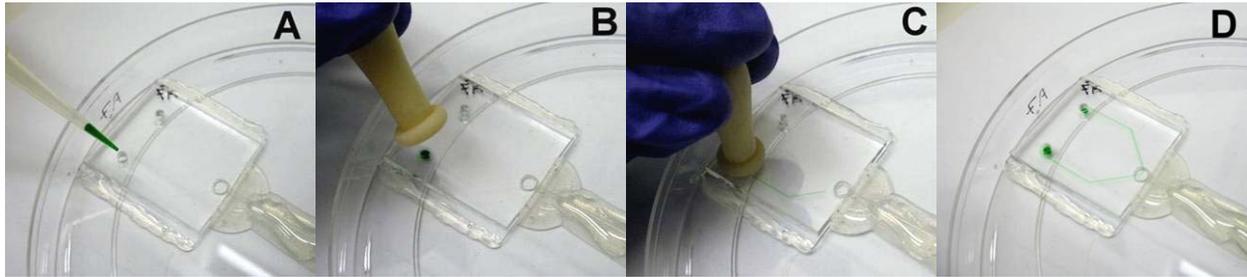


Figure 9: Using a pipette bulb to drive fluid flow in microchannels

To wash the channel, pipette 10 $\mu\text{L}$  of deionized water into a port, and apply the pressure head. Use a kimwipe to wick away the fluid from the other port.

*Note: be sure to evacuate the channel of all liquid before flushing in a new cell suspension. This is to prevent mixing the cell suspension with water, which will change the osmolarity of the fluid. To do this, force air through the channel and wick away the excess with a Kimwipe.*

## 3. Experimental Procedure

### Step 1: Calculate pipette radius

Play with the camera settings to obtain a clear image of the pipette at 40 $\times$  objective. Using ImageJ (covered in an appendix to this lab manual), and a calibration image (supplied on Blackboard), the diameter of the pipette can be measured. Record this value.

## Step 2: Initial setup of the pressure system

- Isolate the iMAchip from the reservoir and syringe (Figure 8B).
- Fill the syringe half-way. Pump syringe to remove air bubbles if necessary.
- Set the reservoir water level to 2-3mm lower than the microscope stage.
  - This is to apply an initial tare pressure to the cells to seal the cell to the pipette.
  - Be approximate with the positioning, but record the exact height difference.
  - You may need to play with this initial tare pressure to find one which works for you.
- Isolate the reservoir from the syringe and iMAchip (Figure 8C).

## Step 3: Micropipette Aspiration

- Using the pipette bulb apply a pressure head to flush the cell suspension into the channels
  - Do this *\*slowly\** to prevent trapping of air bubbles
- Add a small quantity of cell suspension to the opposite reservoir to balance the ports and more quickly reach flow equilibrium
- Using the syringe to apply negative and positive pressures try and draw a cell close to the micropipette tip. This will take some practice. Try and avoid dead cells and cells which exhibit ‘bubbling’ of the membrane (colloquially, ‘blebbing’; see Figure 10).

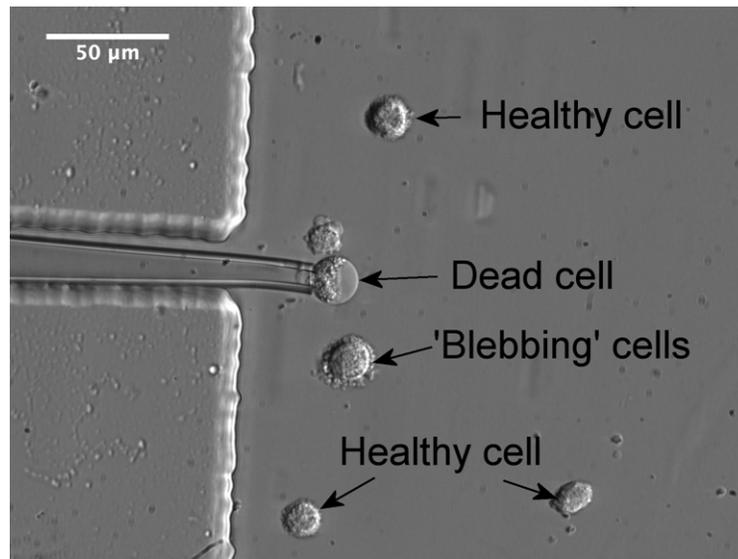
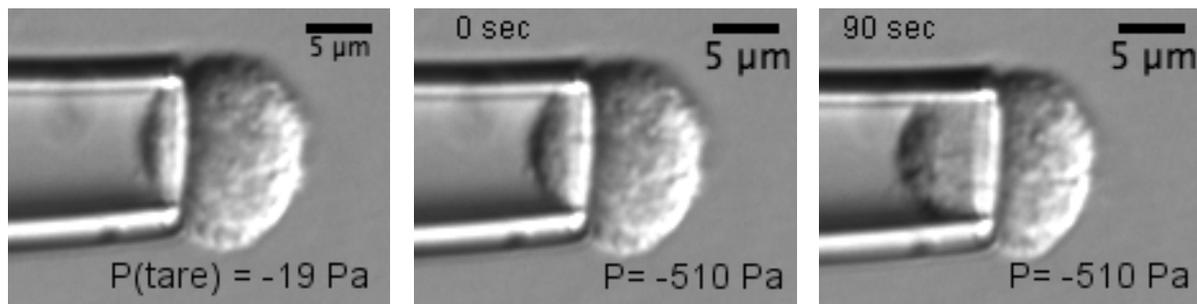


Figure 10: Different types of cells

- Bring a cell in contact with the pipette tip
- As quickly as possible, switch the pressure setup from the syringe to the reservoir (Fig 8D).
- The cell will equilibrate to the tare pressure in about 30 - 60 seconds. Watch the cell deformation, and record an image when the cell does not appear to deform any further.
- Apply a negative pressure by removing liquid (using a pipette) from the reservoir, lowering the liquid column height by ~5cm.
- Record the exact height to which the reservoir is lowered.

- Over the next 90-120 seconds, the cell will deform into the pipette.
  - Because the cell remodels itself in response to these mechanical forces, it will continue to extend very slowly into the pipette, even after 90 seconds. Record an image when the rate of deformation has become very low (ie, cell has reached equilibrium with new pressure).
- Switch the pressure setup from the reservoir to the syringe.
- Blow the cell away from the pipette tip, and attempt to attract another.
- Reset the reservoir height to a new tare pressure, and repeat the procedure for a different cell. Try and obtain data for at least 5 cells.

The following represents typical images obtained using this procedure:



30-60 seconds after cell is trapped by tare pressure.

Immediately on applying aspiration pressure.

>90 seconds after applying aspiration pressure

Information obtained:

*Tare pressure*  
*Cell radius*  
*Tare aspiration length*

Information obtained:

Aspiration length can be used to measure elastic modulus in more complex analytical models

Information obtained:

*Aspiration pressure*  
*Aspiration length*

Figure 11: Sample images of cell being aspirated

## 4. Analysis

Due to time constraints with this lab, data analysis must be performed outside of lab hours. Download the open-source program ImageJ from: <http://rsbweb.nih.gov/ij/>, and install. ImageJ is an open-source java-based image analysis program developed by the NIH, and frequently used by researchers in cell and tissue biology. The software can be used to take measurements of images. Please refer to the ImageJ appendix to get detailed instructions on making calibrated measurements of images.

To analyze the resulting data, using the half-space model: 
$$E = \Phi \times \left( \frac{3r}{2\pi} \right) \times \left( \frac{\Delta P}{L_A} \right)$$

$\Phi$  = dimensionless constant; 2.1

r = cell radius at tare pressure

$\Delta P$  = (Aspiration Pressure applied – Tare pressure)

$L_A$  = (Aspiration Length – Tare aspiration length)

## 5. Discussion Questions

What range of material properties are observed from cells of the same population? Why is this the case?

What sources of error are present in the current iMAchip experimental setup? What is the relative impact of these sources on the results for cellular stiffness?

How would you design this system to reduce those errors? What changes would you make to the device, setup or methodology to improve the accuracy and/or simplicity of the system?

If cells are flowing in the microchannels at the time of aspiration, the pressure within the microfluidic device channels are not at atmosphere. What is this pressure, and how much will it affect the aspiration length? What can be done to reduce or eliminate this?

# Appendix I: Using ImageJ to obtain measurements on images

## Background

ImageJ is an open-source image analysis program written in Java and maintained by the National Institute of Health (NIH). It is the software tool that is most often used in biology-related research, because of its availability, price and customizability. With basic knowledge of Java, automated image analysis algorithms can be written to batch process large quantities of images, which saves considerable time and effort in analyzing data. A wealth of 3<sup>rd</sup> party plugins is also freely available, which are specialized for specific image analysis functions. In this lab we will be using basic functionality of ImageJ to measure pipette diameters and cell aspiration lengths.

## Program

Download the program at <http://rsbweb.nih.gov/ij/>, and install.

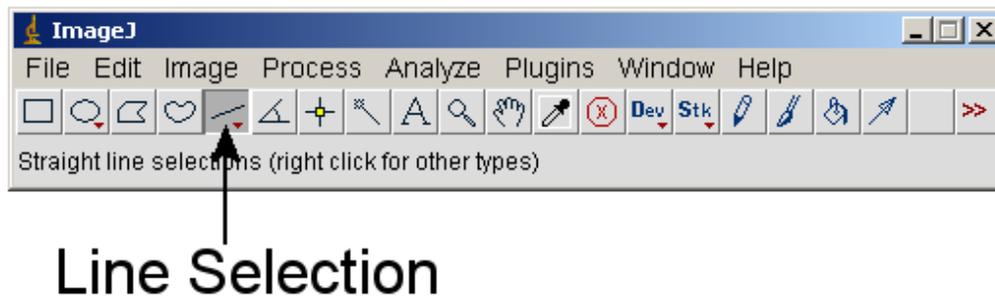


Figure 1: The Line selection tool is the one most often used in this exercise.

## Measurement Procedure

### Step 1: Calibration

In order to provide measurements in meaningful units, ImageJ needs to be 'told' how many pixels correspond to a specific length. In order to do this:

- Download the calibration image from Blackboard
- Open the calibration image in ImageJ (File >> Open)
  - The calibration image is of a hemacytometer – each of the squares in the image is  $50\mu\text{m} \times 50\mu\text{m}$
- Use the line selection tool to draw a line across 2 squares in the hemacytometer

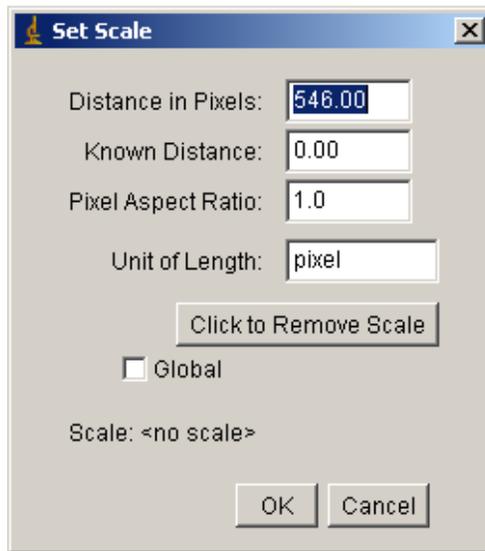


Figure 2: Set Scale Option Dialog

- Set the calibration length (Analyze >> Set Scale)
  - The “Distance in pixels” field is already filled in with the pixel length of the line
  - Fill in the “Known Distance” (100 $\mu$ m for two squares)
  - Change the unit of length to “ $\mu$ m”
  - Check the ‘Global’ box – this makes the calibration apply to all images
  - Click OK

#### Step 2: Measuring distances in images

- Open the image under analysis (File >> Open)
- Use the line selector tool to select the distance to be measured
- Use the measure function (Ctrl+M or Analyze >> Measure)
- The measurement results window appears (look for the ‘length’ measurement)