MIE 439 – Introductory Biomechanics – Fahraeus-Lindqvist Lab
Department of Mechanical & Industrial Engineering
University of Toronto

Contact: Craig A. Simmons, Ph.D., P.Eng.
Assistant Professor and Canada Research Chair in Mechanobiology
Mechanical & Industrial Engineering, Dentistry, and
Institute of Biomaterials and Biomedical Engineering
University of Toronto
5 King's College Road
Toronto, ON
Canada M5S 3G8

Tel: 416-946-0548
Fax: 416-978-7753
E-mail: c.simmons@utoronto.ca

Objectives:

1. To observe the effect of microchannel (or capillary) dimensions on the viscosity and cell density (i.e. hematocrit) of a given cell suspension flown through the microchannel
2. To measure effective viscosity as a function of microchannel width
3. To measure microchannel relative cell density (i.e. relative tube hematocrit) as a function of microchannel width
4. To discuss sources of error of the current setup compared to the original Fahraeus-Lindqvist setup
5. To evaluate the applicability of the current experimental setup as a suitable modified version of the original Fahraeus-Lindqvist setup

1.0 Introduction

In this lab, we will attempt to reproduce the interesting results obtained by Fahraeus and Lindqvist in their experiments on blood flow in small diameter capillaries. In these experiments, they observed two somewhat surprising phenomena:

1. Effective viscosity of blood decreased with decreasing capillary radius.
2. The tube hematocrit was always less than the hematocrit in the feed reservoir.

Can we reproduce these observations using a modified setup*?*

NOTE: You should read Section 3.3 of "Introductory Biomechanics" (Ethier and Simmons) for a more detailed explanation before performing the lab.[1]

1.1 Synopsis

Using a fabrication technique called soft lithography, four microchannels of rectangular cross section were designed and assembled, each having different widths and heights. These microchannels will serve as alternative conduits to the capillaries (of circular cross section) used by Fahraeus and Lindqvist. Cells in suspension will be forced to flow through the microchannels to mimic blood flow through fine

capillaries. Images will be taken using a CCD camera mounted on a light microscope. These images will be used to determine cell density in the microchannels by cell counting, and flow rate of the suspension by particle streak velocimetry (described below).

2.0 Background

2.1 Flow in Rectangular Channels

Fahraeus and Lindqvist used fine glass capillaries with circular cross sections in their experiments. To calculate the effective viscosity, μ_{eff}, in their conduits, they used the classical Poiseuille law:

$$Q = \frac{\pi R^4}{8\mu_{eff}} \frac{\Delta P}{L} \tag{2.1}$$

where Q is the flow rate, ΔP is the pressure drop across the capillary, L is the capillary length, and R is the capillary radius. However, for channels of rectangular cross section, like those used in this lab, the equation takes on a slightly different form to account for the change in geometry. The accepted form of the equation for rectangular ducts is:

$$u_m = \frac{2}{\beta} \frac{D_h^2}{\mu_{eff}} \frac{\Delta P}{L} \tag{2.2}$$

In this equation, $u_m = Q/A$ is the mean velocity in the channel for cross-sectional area A (A is width w × height h for a rectangular channel). The capillary radius of Eq. (2.1) has been replaced by the hydraulic diameter defined as $D_h = 4A/\mathsf{P}$, where P is the wetted perimeter (i.e. the total length of the four sides of the rectangle, $2(w + h)$). Finally, β is a constant relating the friction factor f of the channel and the Reynolds number Re, and is given by the following empirical relationship[2] for channel aspect ratio $\alpha = h/w$:

$$\beta = f \cdot \mathrm{Re} = 96\left[1 - 1.3553\alpha + 1.9467\alpha^2 - 1.7012\alpha^3 + 0.9564\alpha^4 - 0.2537\alpha^5\right] \tag{2.3}$$

For gravity driven flow, the pressure drop across the channel can be predicted by $\Delta P = \rho g H$, where H is the height difference from inlet to outlet reservoir. Figure 1 illustrates some of these definitions.

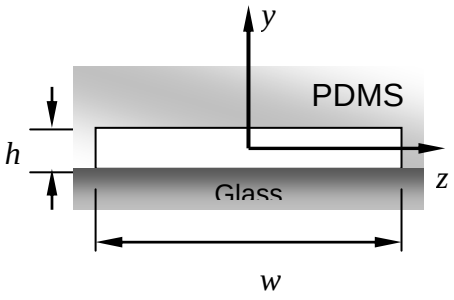


Figure 1. Cross section of a typical rectangular channel of width w and height h. The channels used in this lab are constructed from a PDMS cast bonded to a glass slide.

For laminar flow in rectangular channels, a theoretical solution is available that requires Fourier series expansions. For convenience, however, a much simpler formula was proposed by Purday.[2] For a microchannel of half-width $a = w/2$, and half-height $b = h/2$, the laminar velocity profile through a rectangular cross-section can be approximated by:

$$\frac{u}{u_m} = \left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right)\left[1-\left(\frac{y}{b}\right)^n\right]\left[1-\left(\frac{z}{a}\right)^m\right] \tag{2.4}$$

and

$$\frac{u_{\max}}{u_m} = \left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right) \tag{2.5}$$

where u, and u_{max} are the local axial and maximum velocities, respectively, and m and n are empirical parameters governed by:

$$m = 1.7 + 0.5\alpha^{-1.4} \tag{2.6}$$

$$n = \begin{cases} 2 & \alpha \le 1/3 \\ 2 + 0.3(\alpha - 1/3) & \alpha > 1/3 \end{cases} \tag{2.7}$$

Figure 2 illustrates the velocity profile of Eq. (2.4). The profile is parabolic in the y-direction. The maximum velocity occurs at the midplane at $y = 0$ (see Figure 1). This maximum velocity is fairly constant throughout the midplane, except near the side walls.

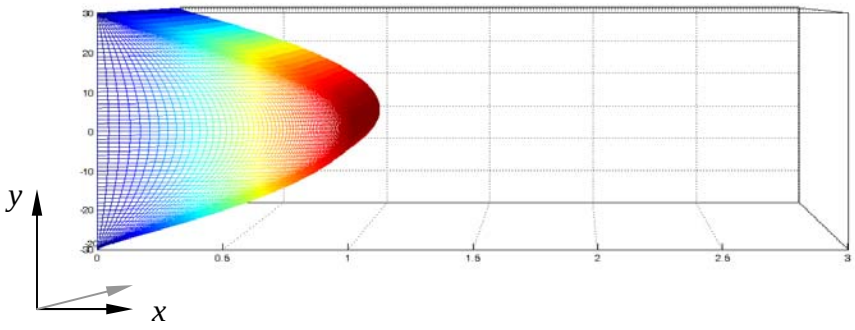


Figure 2. Parabolic velocity profile in a rectangular channel, representing Eq. (2.4).

2.2 Cell Density

In this lab, you will be asked to estimate the cell density in the microchannels. For red blood cells (RBCs) in normal mammalian blood, the cell density, or hematocrit, is ~ 45%. This value can be easily verified by a simple calculation using some known information:

5×10^6 RBCs/mm^3 = 5×10^6 RBCs/μL = 5×10^9 RBCs/mL (Guyton & Hall)

Volume of one RBC = 98 μm^3 (Ethier & Simmons)

Volume of RBCs/mL = 5×10^9 RBCs/mL x 98 μm^3 = 0.49 mL/mL

0.49 mL/mL = 49% hematocrit (in approximate agreement with 45% actual value)

Similar calculations can be performed for any cell suspension. For this lab, you will be using L929 mouse fibroblasts. You can assume that these cells are spherical while in suspension, and have a diameter d = 16.5 μm.

2.3 Particle Streak Velocimetry

To determine effective viscosity, you are required to know the velocity in the microchannel. For the purposes of this lab, the most convenient way to measure the velocity is by particle streak velocimetry. Briefly, seeded particles traveling at a steady velocity U generate a streakline in flow of length l over time t. Measuring the lengths of streaklines for an image taken with a given exposure time then yields velocity $U = l/t$. Since particles can reside on different streamlines of flow, streaklines may have different lengths depending on the particle's location. However, if one takes care to focus on the midplane of the channel during experimentation, and to take measurements of the longest streaklines near the centre of the channel, you can obtain a reasonably accurate measurement of the maximum velocity in the channel. Then using the velocity profile of Eq. (2.4) and the approximate solution to u_{max} in Eq. (2.5), one can determine the mean velocity u_m, and ultimately μ_{eff}. Figure 3 shows a typical particle streakline image, as obtained using fluorescent microbeads seeded into a rectangular microchannel.

Figure 3. Particle streak image for 500 μm microchannel, using 200 ms exposure time.

3.0 Materials and Setup

The following materials are needed to operate this lab:

1. Four microchannels of nearly constant height, but of varying widths: 50, 100, 200, and 500 um. Fabricated from poly(dimethylsiloxane) and glass (Figure 4). Table 1 lists the dimensions for the microchannels used in this lab.

2. Cells in suspension, ~ 1 mL of suspension with density ~ 20 million cells/mL. Cell type used in this lab is a mouse fibroblast cell line (L929) of ~16.5 um diameter. Details of L929 cell line can be found at the following link:
   http://www.atcc.org/common/catalog/numSearch/numResults.cfm?atccNum=CCL-1

3. Light microscope with mounted CCD camera and imaging software
4. 1 mL syringe, 21-gauge needle, and microfluidic tubing
5. Micropipettor

Table 1. Measured microchannel dimensions.

Channel	*Height (um)*	*Width (um)*	*Cross Sectional Area (10^3 sq. um)*	*Hydraulic Diameter (um)*
1	33.2	66		
2	35.5	116		
3	37.7	176		
4	36	465		

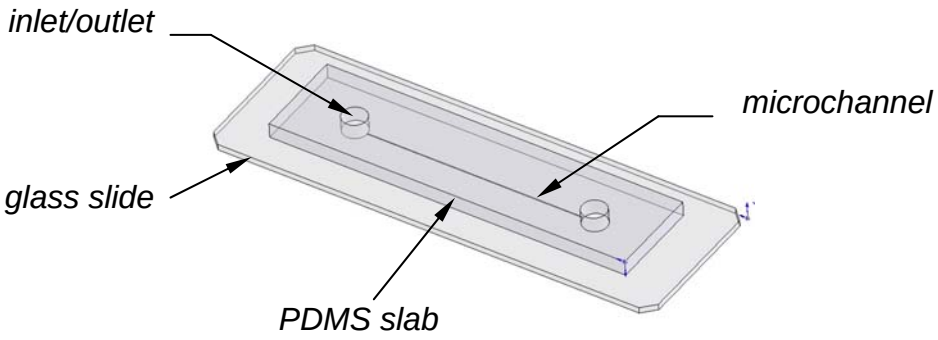


Figure 4. Illustration of a typical microchannel device used in this lab.

Here is an image of the experimental setup:

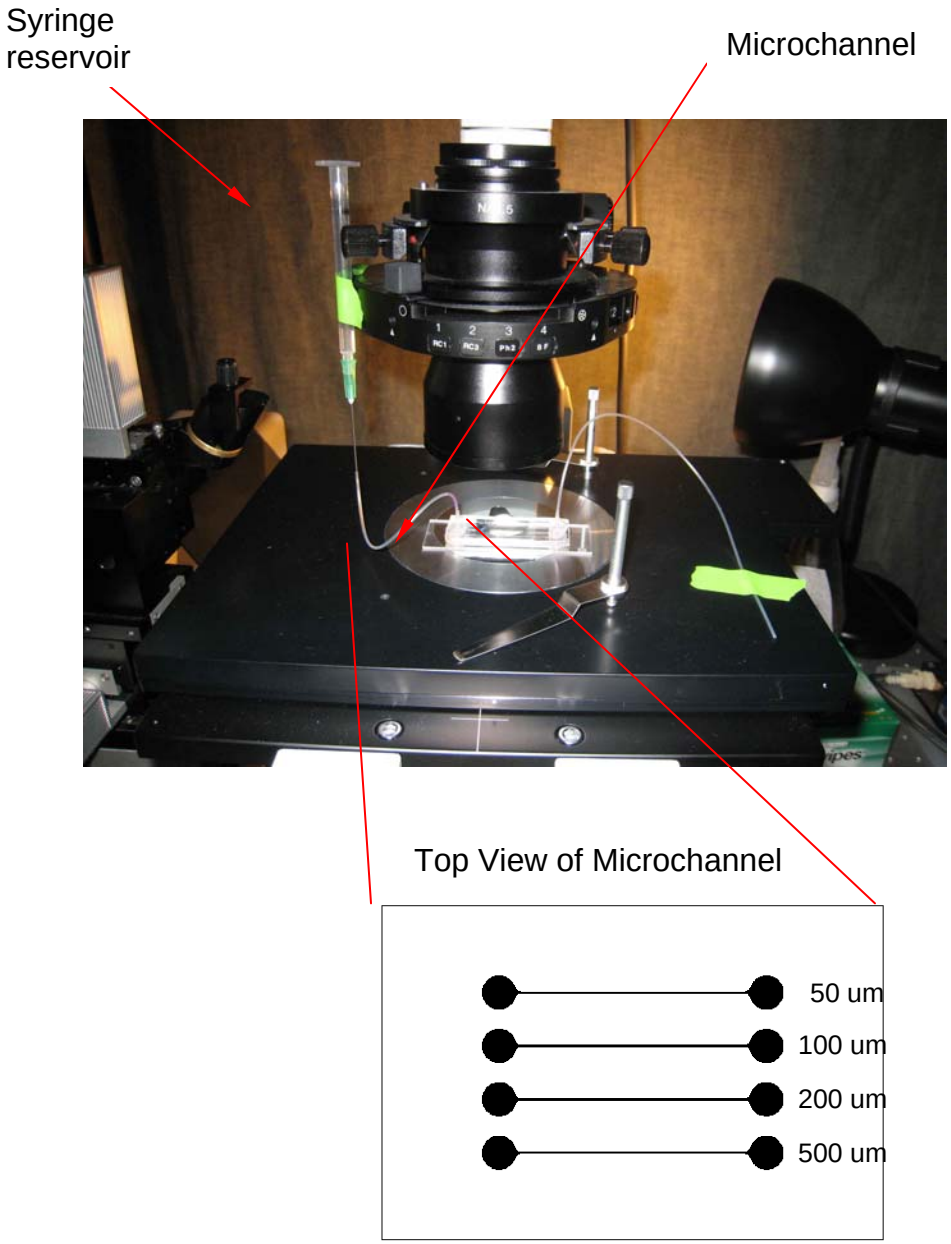


Figure 5. Experimental setup. Reservoir height H is measured from the top of the cell suspension to the bottom of the microchannel, and determines the flow rate in the microchannel.

4.0 Procedure

1. Secure the microchannel slide onto the microscope stage and focus the objective lens on the channel of interest.

2. Set up the camera software:
    a. START -> IBBME Software -> Spot Insight -> Spot Insight Camera
    b. Obtain a live feed by clicking on the LIVE icon. Balance the light on the screen by clicking on the BALANCE icon.
    c. Adjust the intensity and the focus of the microscope to obtain a clear image **on the computer screen**. Note that when the image is focused on screen, it is likely no longer in focus through the microscope eyepiece.
    d. Go to Image Settings and go into Manual mode.
    e. To take a photograph, click on the Camera icon.

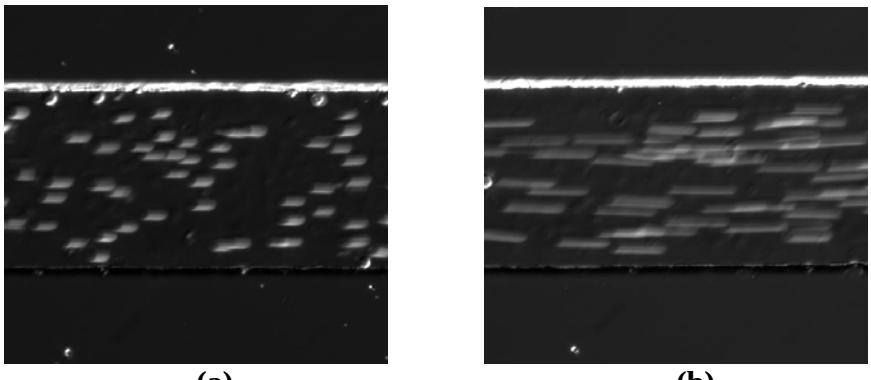
3. Using a micropipettor, load the syringe barrel that has been secured to the microscope stand with the supplied cell suspension. Gently reattach the plunger from the syringe and apply a little force to force the cell suspension into the microchannel. Once the channel is loaded with cells, remove the plunger quickly.

Image Acquisition – Cell Density

4. Take 5 to 10 images with short exposure time so that cells appear spherical. These images will be used to count the number of cells in the image to determine cell density within the channel (Figure 6a).

Image Acquisition – Channel Velocity, *U*

5. Take 5 to 10 images with longer exposure time so that cells appear as streaks. These images will be used to estimate the velocity and flow rate within the microchannel. Note that for convenience, we use the cells themselves as the streakline particles instead of fluorescent beads (Figure 6b).



(a) **(b)**

Figure 6. L929 at 20 million cells/mL in a 200 um channel, (a) 3 ms exposure time, and (b) 10 ms exposure time. Note that the streaklines in (b) are a suitable length for determining velocity.

5.0 Post-lab Questions

1. Using the images obtained during the lab and the equations in the background section, calculate the estimated relative "tube hematocrit", or cell density, and the effective viscosity of the cell suspension for each of the four microchannels. For direct comparison with Fahraeus and Lindqvist, plot your results on two separate graphs:
    a. Relative cell density vs. feed reservoir cell density (see Figure 7), and
    b. Effective viscosity vs. hydraulic diameter (see Figure 8).

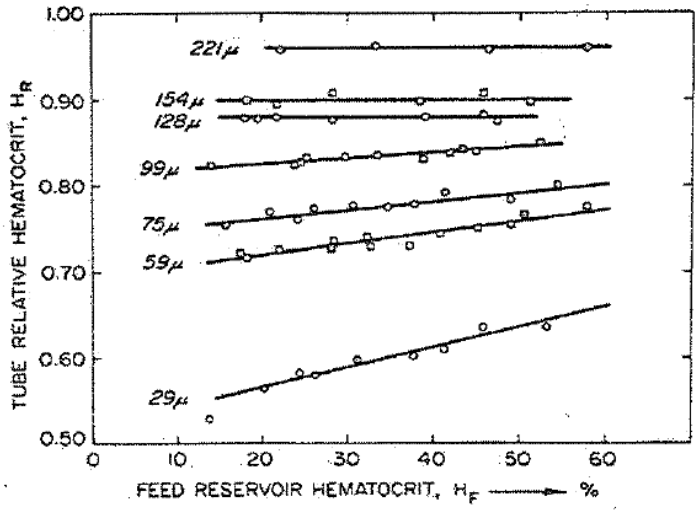


Figure 7. Tube relative hematocrit for capillaries of different sizes (reproduced from Figure 3-16 of Ethier and Simmons)

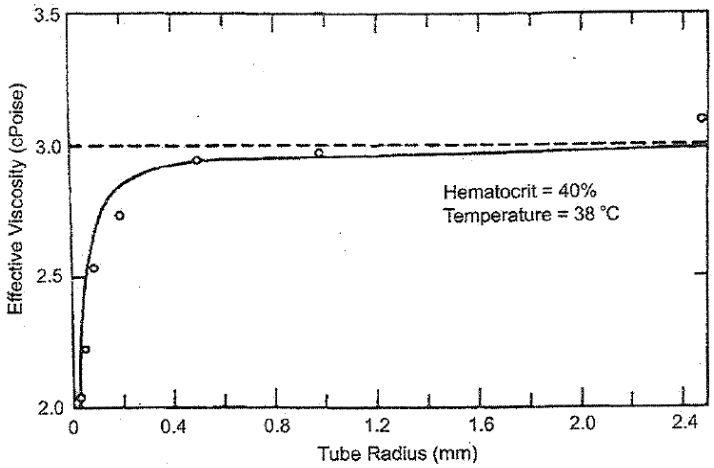


Figure 8. Plot of effective viscosity versus capillary tube radius (reproduced from Figure 3-15 of Ethier and Simmons)

2. Do your results follow the same trends as those observed by Fahraeus and Lindqvist? Why is it difficult to do a direct comparison between your results and those from Figures 7 and 8?

3. What are the major differences between the experimental setup in this lab and the original setup of Fahraeus and Lindqvist? Discuss three of these differences in detail, and suggest why these differences may contribute to the discrepancies found in the results.

References

1. C.R. Ethier and C.A. Simmons, Introductory Biomechanics, Cambridge University Press, New York, 2007.

2. R. K. Shah and A. L. London, *Laminar Flow Forced Convection in Ducts,* Academic Press, New York, 1978.