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Quantitative Physiology: Cells and Tissues
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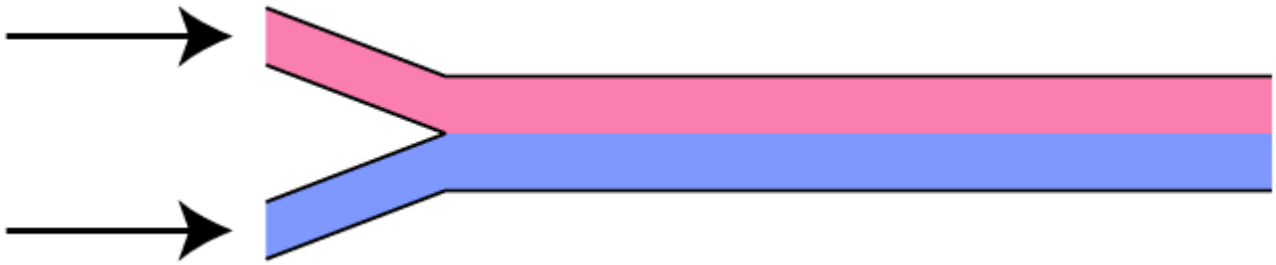
Fall, 2003

MicroFluidics Project Laboratory



Introduction

This page contains helpful information about the proposal, experimentation, analysis, and report-writing stages of this laboratory. You should read through it at each stage to make sure you understand what is required.

Overview

This laboratory project is intended to provide an opportunity to learn about

1. designing an experiment,
2. acquiring, processing, and interpreting experimental data, and
3. communicating the results to others.

This laboratory project is also intended to introduce the emerging field of microfluidics. Microfluidics refers to the use of devices in which fluid flows are restricted to channels with micrometer dimensions. Such devices are interesting for at least 3 reasons:

1. Microfluidic devices can be manufactured using photolithographic techniques that allow many devices to be constructed simultaneously (just as modern electronic devices are manufactured). Bulk manufacturing reduces the cost per device.

2. Since they are small, many devices can be fit into a small volume, leading to the idea of total "labs-on-a-chip" replacing labs that occupy benches or even whole rooms today.
3. Flows in microscopic chambers can exhibit behaviors that are difficult or impossible to produce in macroscopic chambers. These flow regimes can be used to simplify measurements that are difficult or impossible to make macroscopically.

This laboratory project is intended to take advantage of microfluidics to measure the properties of molecular transport by diffusion.

Microfluidics

More than any other single factor, bulk fabrication has made possible today's vast array of powerful and inexpensive electronic devices. The millions of components in a modern computer are fabricated in parallel, making the manufacture of such integrated circuits little more costly than the manufacture of circuits that contain only tens of components. Similar bulk fabrication techniques are currently being developed for fluidic devices, and the resulting microfluidic devices hold promise to similarly revolutionize chemical and biochemical analysis systems. For example, the integration of all of the components needed for the sequencing of DNA (i.e., valves to control flows, incubation chambers, mixing chambers, heating/cooling chambers) may soon allow "labs-on-a-chip" to replace whole biochemistry labs.

Microfluidic devices are smaller than conventional macrofluidic components, and their smaller size facilitates many kinds of analysis. For example, when two fluids come into contact, they mix by a variety of mechanisms. The most familiar mechanism from our macroscopic experiences is convection: i.e., the mixing of fluids caused by motions of fluid, as in stirring. When fluids are constrained to small volumes, convection is constrained and mixing results almost entirely by diffusion (i.e., by random collisions between molecules). As a result, it is possible to design microfluidic systems in which dissimilar fluids flow along side each other over long distances without significant mixing. Such "laminar-flow" profiles are difficult or impossible to produce macroscopically and provide unique opportunities to study molecular transport by diffusion.

Methods

This section describes basic experimental methods used in microfluidic systems. The descriptions are not complete: you are encouraged to devise more detailed procedures where appropriate. You may also substitute your own novel methods. However, if your novel methods require the use of additional equipment, you are responsible for obtaining that equipment. The staff is only responsible for supplying the equipment described in this section.

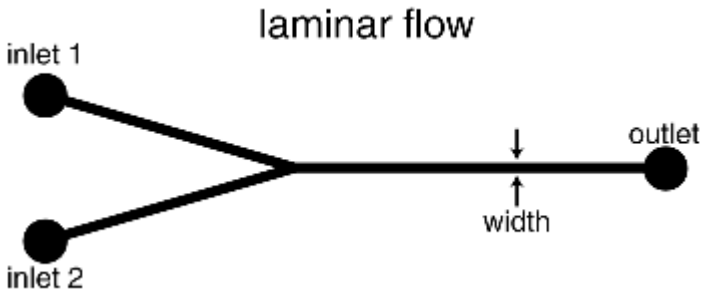
In this experiment, you will use dyes and other chemicals that could stain or irritate your skin. Wash your hands thoroughly immediately after exposure to any chemicals. Clean up minor chemical spills immediately. Report major spills to the staff. Rubber gloves will be available.

No foods or beverages will be allowed in the laboratory.

Chambers

Our experiment chambers are 2-dimensional microscopic channels formed on the surface of a transparent silicone rubber. The silicone rubber is then bonded to glass slide. Fabrication methods can be found in the literature (Whitesides-1998).

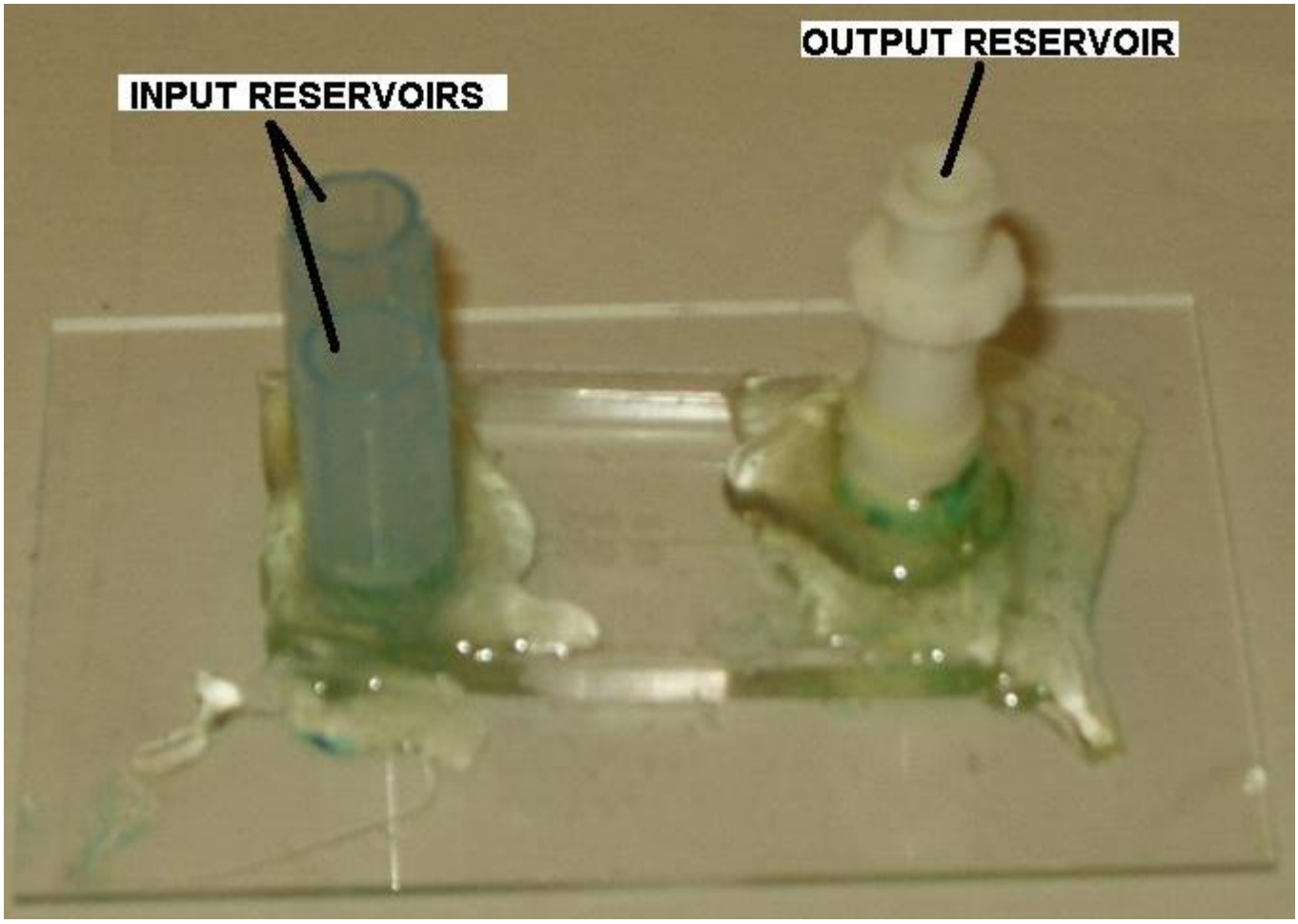
The following figure shows the topology. The channels are 500 &mum wide and approximately 100 &mum deep. The inlet channels are approximately 25 mm long. The outlet channel is approximately 35 mm long.



Establishing Fluid Flow

A variety of methods have been used to pump fluids through microchambers. Some investigators use macroscopic pumps (e.g., syringe pumps, peristaltic pumps, etc.). Others use electrically driven flows (e.g., electro-osmosis or electrophoresis). In this lab, we recommend that you use gravity flow. With our channel designs, a few millimeter difference in fluid level is sufficient to drive flow rates on the order of micrometers per second, which are satisfactory for most experiment designs.

Input and output ports connect the microfluidic channels to macroscopic fluid reservoirs, as shown in the following photograph.



While gravity feed is simple to implement, it is also very sensitive to external disturbances, such as table vibrations. Be aware that even atmospheric disturbances (caused by breathing) can affect your results.

Gravity feed can also be difficult to start. We recommend the following procedure for filling your chamber with fluid.

- Fill the input reservoir(s) approximately 2/3's full with the desired liquids, taking care not to introduce any foreign matter, and not to break the reservoirs off of the slide.
- Carefully attach the provided syringe to the output reservoir, and use the syringe to gently pull liquid through the channel. Watch the channel carefully to see if liquid has been pulled through. Because of the small width of the channel, you may not build up a noticeable amount of liquid in the output reservoir, but if the channel is full, the experiment will work fine.
- Now fill the output chamber with liquid until the liquid reaches approximately the same height as the input chambers. You are now ready to begin observation of your channel.

Please take care when using these chambers. They are easily broken. Always flush your chamber with deionized water after use. If saline solutions are allowed to evaporate, the salts will clog the channels and solids are not easily removed.

Microscope

Our microscopes have been customized for this microfluidics laboratory, as shown below.

The microfluidics chambers can be clamped to the stage of the microscope using a slide holder that has two position control knobs. The microscope has 3 objectives: 4X, 10X, and 20X. These objectives focus an image of the chamber onto a video camera. Illumination is provided by LEDs and can come from the top (epi-illumination) or bottom (trans-illumination). Epi-illumination works best for fluorescent imaging and trans-illumination works best for brightfield illumination. The microscope has a focus control knob that allows focusing with &mum resolution.

Please avoid touching the optical components. They are sensitive, and easily scratched. Even fingerprints can badly degrade the images you will get.

Measuring Flow Rates

Quantitative interpretation of data from microfluidic systems often requires knowledge of the flow rates. Although flow rates can in principle be determined by measuring the time it takes for some known volume of fluid to flow through system, it is often more convenient to measure flow rates by tracking microscopic particles that are suspended in the fluid. Microscopic polystyrene beads with diameters on the order of 1 &mum are available for this purpose. Bead solutions can be used in separate experiments to calibrate flow rates. Alternatively, beads can be added to test solutions to directly measure the flow rates of test solutions. If the concentration of beads is dilute and if the flow rates are slow, then individual beads can be tracked across frames that are recorded at a 10-30 frame/second rate (depending on the speed of the computer). If the concentration of beads is dense or if the flow rates are high, then the LED can be pulsed to produce a stroboscopic illumination with multiple strobes per frame. Then multiple images of each bead on a single frame can be analyzed to determine the flow rate.

Computer

A computer running Linux is attached to the video camera via FireWire. A program called `camscope` can be used to view and record images and brightness profiles.

Activating the Software

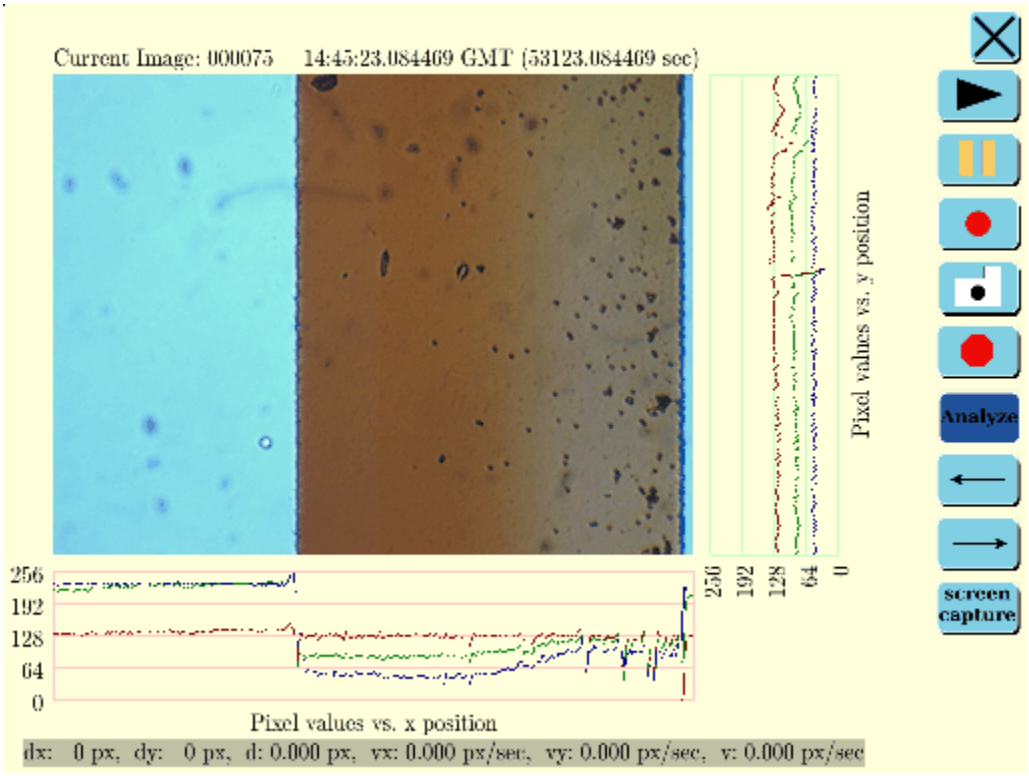
The main program for acquiring and processing data is called `camscope`. Source code for camscope is available at http://www.sourceforge.net/projects/camscope.. This program stores results and temporary files in the current working directory. At the login prompt, log in with the username *knoppix* and the password *knoppix*. To avoid confusing your data with that of another group, each laboratory group should store results in a separate directory. Please use the following naming convention. If you are in laboratory group B2, then type the Linux commands

```
> cd
> mkdir B2
> cd B2
```

to make a new directory named `B2` and to make that directory the current working directory. Then type

```
> camscope
```

to activate the main program. The `camscope` program provides facilities to view and record images and to view and record simple brightness profiles. All of these facilities are directly accessible from a single main control screen illustrated in the following figure.



Buttons on the right provide the following functions (top to bottom, with keyboard shortcuts in parentheses):

- **EXIT** (ctrl-q): terminate `camscope` and return to Linux prompt.
- **PLAY** (ctrl-p): display live images from video camera, or loop through previously recorded images if the program is in Analyze mode.
- **PAUSE** (ctrl-z): stop displaying new images and hold last one displayed.
- **RECORD** (ctrl-r): start recording images and brightness traces. Records ten images and then stops (your TA can change this behavior if needed, but it is your responsibility to make sure your measurements fit on a CD). Note: pressing the record button takes you out of Analyze mode.
- **SNAPSHOT** (ctrl-s): record a singe image and associated brightness traces.
- **STOP** (ctrl-b): stop recording.
- **Analyze** (ctrl-a): toggles Analyze mode, which displays previously recorded images.
- **BACK** (ctrl-left_arrow): display previous recorded image (only works in Analyze mode).
- **NEXT** (ctrl-right_arrow): display next recorded image (only works in Analyze mode).
- **Screen Capture** (ctrl-d): save image of screen (screendump).

Using Multiple Data Sets to Organize Your Measurements

In addition to these buttons, the upper left corner of the screen has + and - buttons (with keyboard shortcuts ctrl-. (period) and ctrl-, (comma) respectively). These buttons control which data set is currently being recorded or analyzed. All of camscope's other features

(record, analyze, playback, etc) operate on only the current data set (which can be changed on the fly). Although you can do the entire lab without using multiple data sets, they are extremely useful for organizing your data. You should use a separate data set for each type of measurement, and for each measurement location. Be sure to make a note in your protocol book about which data set corresponds to which measurement!

Manual Gain Control

By default, the camera uses automatic gain control to set the brightness and exposure time to levels that give a good image. However, in some cases you may wish to manually set these values in order to quantitatively compare brightnesses under different lighting conditions (for example, comparing brightnesses with two different dye concentrations in the chamber). Although there are no buttons to adjust these values, you can use the following keyboard shortcuts:

- **Ctrl-1**: turn down the brightness by 3 points (brightness varies from 0 to 511).
- **Ctrl-2**: turn up the brightness by 3 points.
- **Ctrl-3**: turn on auto-brightness (Ctrl-1 and Ctrl-2 both turn it off).
- **Ctrl-5**: turn down the exposure by 3 points (exposure varies from 0 to 511).
- **Ctrl-6**: turn up the exposure by 3 points.
- **Ctrl-7**: turn on auto-exposure (Ctrl-1 and Ctrl-2 both turn it off).
- **Ctrl-9**: turn down the color saturation by 3 points (saturation varies from 0 to 255, and has no auto mode).
- **Ctrl-0**: turn up the saturation by 3 points. When any of these keys is pressed, the current values of these parameters are printed to the console.

Taking Measurements

Camscope allows you to make quantitative measurements both in real-time and from previously recorded images, by switching to/from Analyze mode. Several types of measurements can be made:

1. **Pixel Values**: When the cursor is moved into the image, the position of the cursor and the RGB values of the pixel at that position are displayed in the lower right.
2. **Line Plots**: When the cursor is moved into the image, its vertical (and horizontal) position selects a line (and column) whose brightness is displayed below (and to the side of) the image. When images are being recorded, the red, green, and blue intensities in these brightness contours are also saved as text files (the Ctrl-r and Ctrl-s keyboard shortcuts are handy for saving files while the cursor is in the image).
3. **Average Brightness**: When a box is drawn with the right mouse button, the average RGB values within the box are displayed in the lower right corner of the screen.
4. **Distance/Velocity**: When a line is drawn by holding the left mouse button and dragging the mouse, the x, y, and total distance of the line is displayed in pixels at the bottom of the screen. In addition, the velocity is computed automatically by

subtracting the time at which the line was started from the current time (in Analyze mode, the times at which the images were acquired are used instead). To measure the velocity of a bead in Analyze mode, for example, you would click and hold the mouse button over a bead, press Ctrl-right_arrow to switch to the next image (or to skip through several images), and move the pointer to the new bead position. The velocity of the bead will be reported at the bottom of the screen, next to the distance.

File Formats

Images are recorded sequentially as `.BMP` files. The first image recorded into an empty directory is named `image.000.000000.bmp`, the second is named `image.000.000001.bmp`, and so on. Changing the data set changes the three-digit number near the beginning of the file, and resets the six-digit counter to zero. If the cursor is in the image at the time of recording, a text file is also created that contains the red, green, and blue intensities that were displayed below and to the right of the image. The text file associated with `image.000.000000.bmp` is named `info.000.000000.txt`. Screen dumps are saved in a similar sequential fashion, with the first one named `print.000.000000.bmp`.

Saving Your Results

When you are ready to leave the lab, you should save your data. We cannot guarantee that your data will be preserved on the lab computer, so copy everything before you leave. The easiest way to save your data is to record it on a CD. Put a blank CD into the CD burner and type

```
cdcp .
```

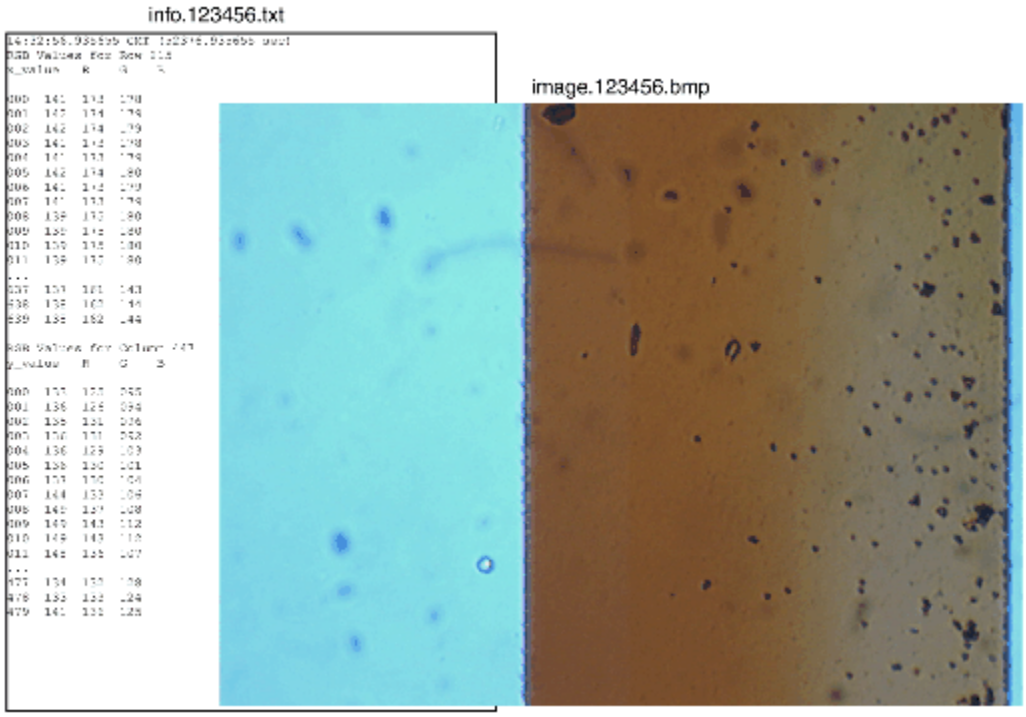
at the linux prompt. This command will record a CD that contains all of the files the working directory (i.e., ".") and its subdirectories. If your directory is larger than the size of one CD (about 700 MB), you will need to split your data into multiple subdirectories and copy each onto a separate CD. Your TA can help you do this.

Analyzing Results

The `camscope` program is intended to provide sufficient information for you to monitor how your experiment is proceeding while you are in the lab. This is important so that you can discover problems and fix the problems before taking your final data. It is also important so that you have a good idea that your experiment was successful or not before you leave the lab. If you discover problems with your data, it is generally easier to take new data now than it is to come back at a later time and start again.

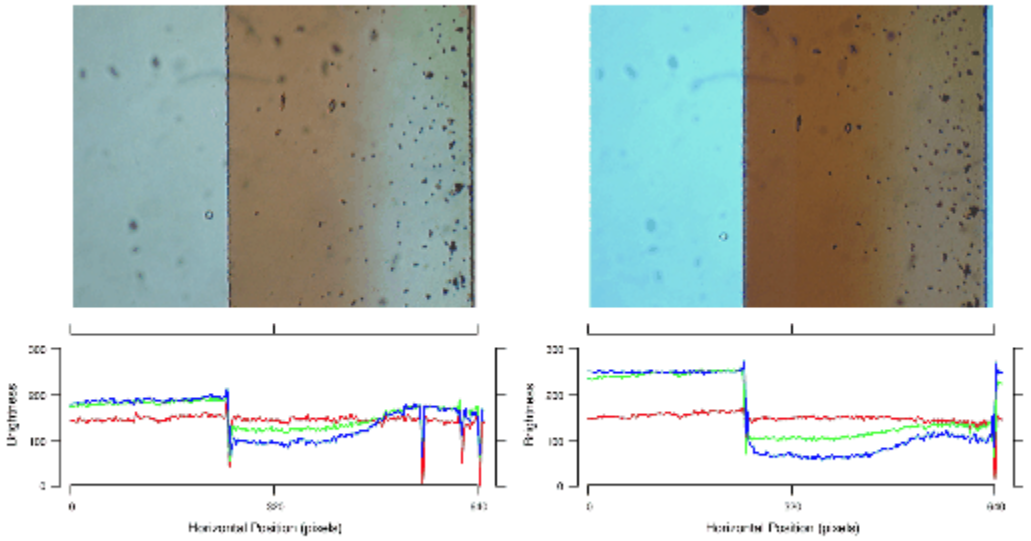
Results from `camscope` can also be directly incorporated into your final report. However, quantitative statistics to test specific hypotheses generally require additional analyses, based on images and brightness profiles saved by the `camscope` program. Every time

camscope takes a picture, it saves the image as a .BMP file. When the cursor marks a "line-of-interest" and a "column-of-interest" at the time that an image is recorded, the red, green, and blue intensitives of those lines and columns of interest are also recorded as "info" files (see "File Formats" above). The following figure shows a graphical representation of these two files.



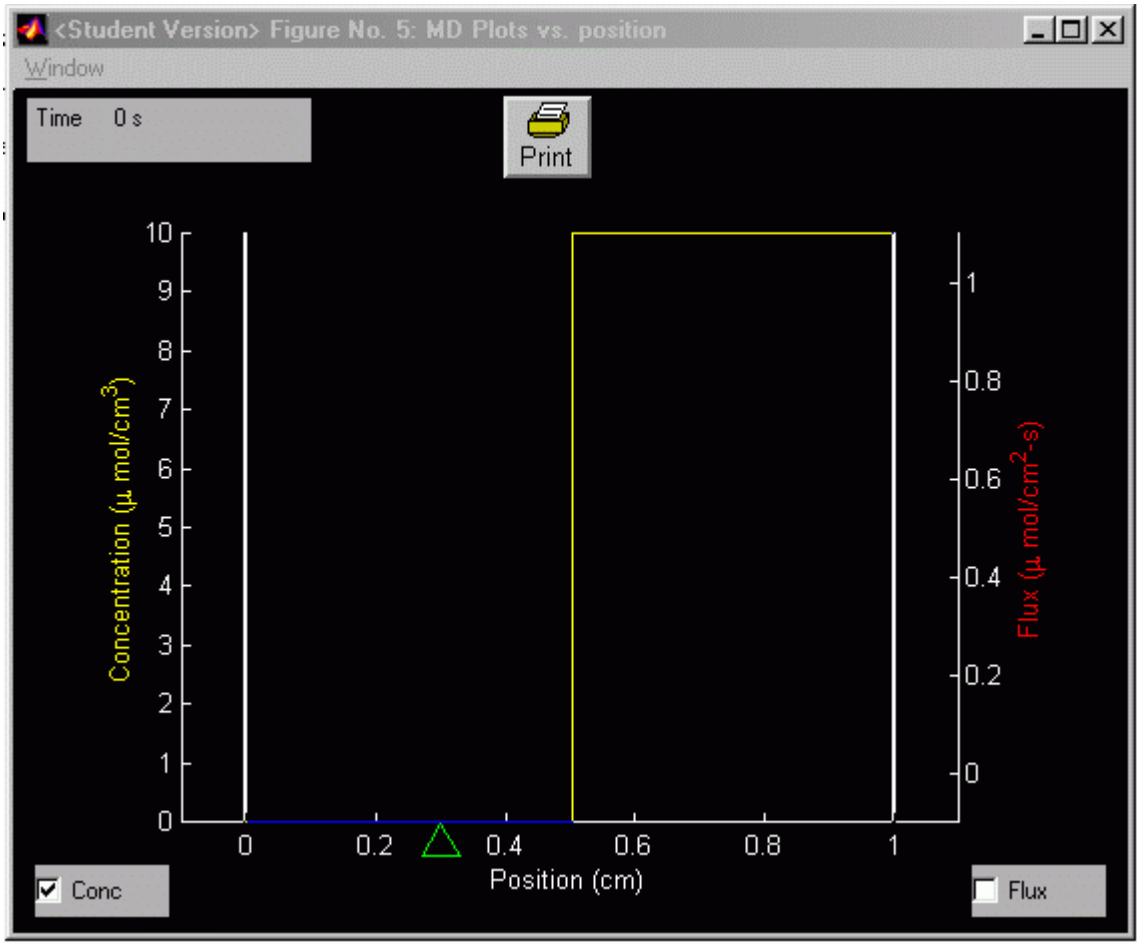
Intensity Profiles

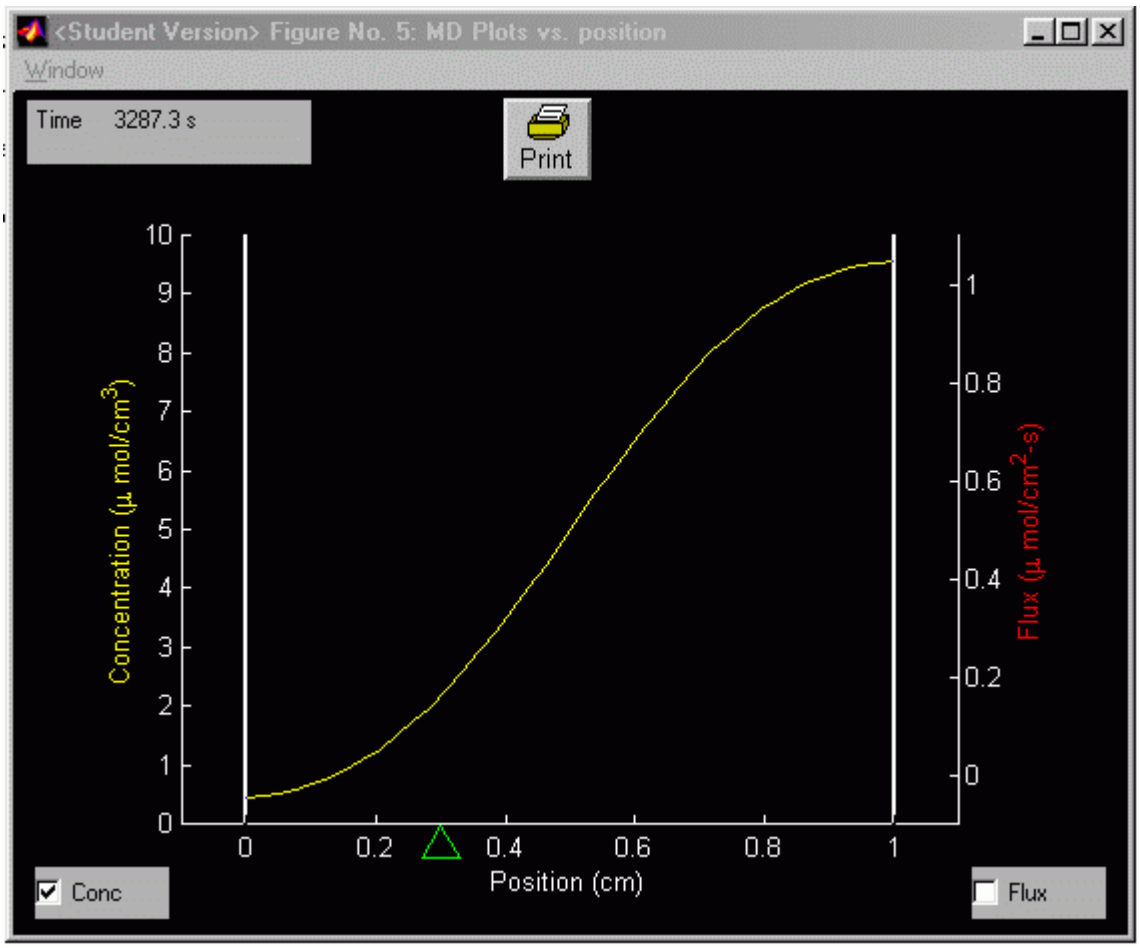
The numbers in the info files can also be read by text processing / spreadsheet / plotting programs and used to generate plots. The following figure shows the red, green, and blue traces obtained from the center lines of two images saved at different times by camscope.

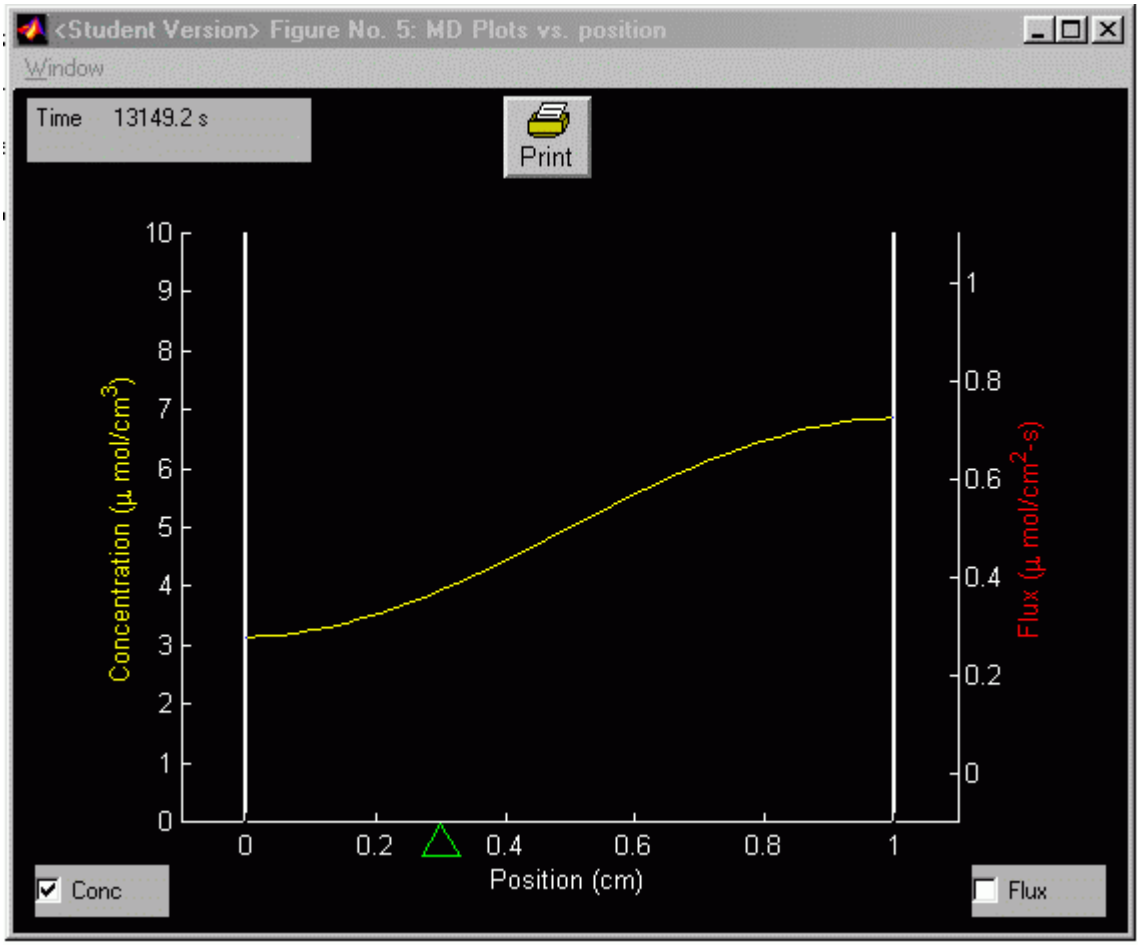


Some trends are easy to see just from the traces. However, the traces are noisy, and averaging can give more reliable statistics. Brightnesses can be averaged in the horizontal or vertical direction from the line-of-interest and column-of-interest numbers contained in a single info file. Brightnesses can be averaged across time by combining information contained in many info files. Arbitrary analyses are possible by calculating directly from the images. Many software packages, including Matlab, provides functions that access brightness values in .bmp image files.

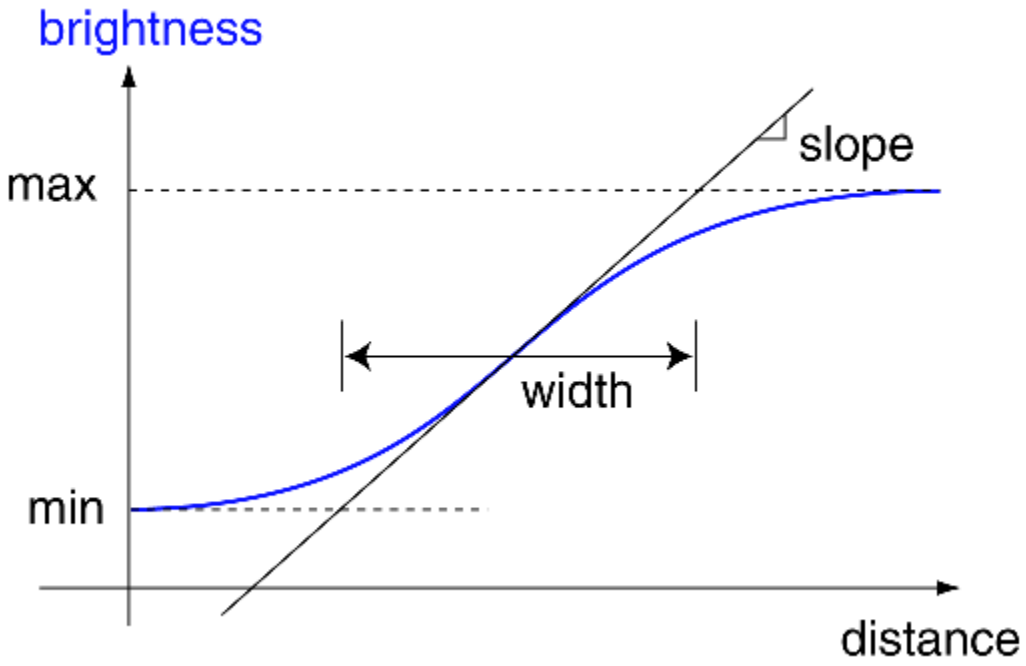
More meaningful statistics can be generated from analytic theories and/or simulations. The course software (available on the MIT server) can be used to calculate solutions to the diffusion equation. The following plots show calculated concentration profiles for solute confined in a 1D box for 3 successive times starting from an initial step concentration.


<Student Version> Figure No. 5: MD Plots vs. position
Window
Time 0 s
Print
Concentration (μ mol/cm3)
Flux (μ mol/cm2-s)
Position (cm)
0
0.2
0.4
0.6
0.8
1
Conc
Flux


<Student Version> Figure No. 5: MD Plots vs. position
Window
Time 3287.3 s
Print
Concentration (μ mol/cm3)
Flux (μ mol/cm2-s)
Position (cm)
0
0.2
0.4
0.6
0.8
1
Conc
Flux



These plots illustrate that as time proceeds, the concentration profile flattens. Based on this observation, one might measure the slope of the brightness contour measured in the center of the laminar flow chamber and compare that slope the one measured in the simulation. Alternatively, one might measure the minimum and maximum brightnesses and compare those statistics to the simulation. The following figure illustrate some potentially meaningful statistics to extract from brightness contours. You may be able to suggest better statistics for your particular study.



You could also analyze your data by comparing it to an analytic theory. Such a theory is developed in the course text (volume 1, Figure 3.16). Based on that theory, we expect the concentration profiles to be integrals of the gauss function (i.e., the error function, erf). One could find the erf function that best fits the measured brightness contours as a way to estimate how much diffusion has occured.

Each of these analysis methods has advantages and disadvantages. Fitting the data with a known function takes advantage of ALL the brightness data, and may therefore be good for reducing the effects of measurement noise. However, the erf analysis does not take the walls of the laminar flow chamber into account. Numerical analysis, using the course software or some user written code, can take the walls into account, and thereby be more accurate. However, numerical analysis may be more difficult to implement.

There is not single best way to analyse your data. Simple methods are fine so long as their limitations are taken into account. Part of your project should be developing an analysis method that makes sense for the question that you are addressing.

Experiments

Basic Observations: Laminar Flow and Diffusion

Before beginning your project, you should perform a vew basic observations to familiarize yourself with the equipment.

Draw about 1 mL of deionized water into an empty syringe. Add 100 &muL of microspheres to the syringe. Draw a few mL of air into the syringe and shake to disperse the microspheres. Fill one input reservoir of a laminar flow chamber with this fluid. Repeat the procedure, substituting food coloring for deionized water, and fill the second input reservoir.

Filling the input reservoirs will not necessarily start the flow. To start the flow, carefully attach the provided syringe to the output reservoir, and use the syringe to gently pull liquid through the channel. Watch the channel carefully to see if liquid has been pulled through. Because of the small cross-sectional area of the channel, you may not be able to see fluid in the output reservoir even after the flow has started.

Move the chamber to the video microscope. Start the `camscope` program. Position the stage so that you see the microchannel. Positioning can be tricky: you must get the microchannel centered under the objective AND in focus in order to see any image at all!

When properly focused, you should see microspheres streaking across the image. Add or remove liquid to the input and output reservoirs to adjust the flow rate. Measure the flow rate by recording a sequence of images and measuring the distance traved by one microsphere per frame. Are all of the beads moving at the same velocity? Should they move at the same velocity?

You should see laminar flow. Estimate the maximum spatial gradient of brightness. is the maximum centered in the microchannel? Should it be centered? Can you make it more off center?

Projects

Your project is the main educational experience of this laboratory. You and your partner should plan your project well in advance of going to the laboratory. This section lists several topics that you could explore as your project. You may choose one of these or you can construct an entirely new one. The descriptions given here are incomplete. Your plan should be well-defined. Keep it simple so that you can complete the work, including some preliminary plotting of results and thinking about interpretation within a several-hour lab session. You may also want time to check your interpretation by taking additional measurements.

Linearity of Fick's Law

Motivation: Fick's law states that the diffusive flux of a solute should be proportional to the gradient of concentration of that solute. Design an experiment to test if this is true.

Hypothesis: The flux of a solute is proportional to the gradient of the concentration of that solute and is independent of the absolute concentration of solute.

Method: Perform laminar flow experiments with pairs of solutions with concentrations X and $X+Y$ of dye. Vary X keeping Y constant and test whether the diffusive flux depends on X.

Diffusion of one Species in the Presence of Others

Motivation: Fick's law states that the diffusive flux of a solute should be proportional to the gradient of concentration of that solute and independent of the presense of other solutes. Design an experiment to test if this is true.

Hypothesis: The flux of a solute is proportional to the gradient of the concentration of that solute and is independent of the presence of other solutes.

Method: Perform laminar flow experiments with pairs of solutions with fixed differences in the concentration of dye but variable amounts of added glucose.

Effect of Solvent on Diffusivity

Motivation: According to the random walk model, solutes diffuse because they collide with water molecules. Replacing water by a different solvent might change the number or magnitude of these collisions.

Hypothesis: The diffusivity of a dye in water is greater than that of the same dye in oil.

Method: Perform laminar flow experiments with food coloring in one input reservoir and either water or oil in the other.

Analysis of Microfluidic Data

Motivation: A typical laminar flow experiment can generate many megabytes of data. How should one analyze this data to get the most information about diffusivity?

Hypothesis: Tracking the slope of concentration near the center of a laminar flow provides more information about diffusivity that does tracking the width of the mixed region.

Method: Determine transverse concentration profiles across a laminar flow at multiple locations along the channel. Determine the spatial derivative of these concentrations across that channel. According to the simple theory developed in lecture, these derivative curves should have gaussian shapes. Compare estimates of diffusivity based on the heights and widths of these gaussian shapes.

Microfluidic Mixing

Motivation: Microscale flows are dominated by viscosity. Therefore, mixing is primarily by diffusion. But can we actually ignore other mechanisms?

Hypothesis: Diffusion is not the only important mixing mechanism in laminar flow.

Method: Measure mixing of two fluids that contain particles that are so large that they should not diffuse during the experiment (e.g., microspheres). Any mixing that occurs could not be due to diffusion.

Practical Considerations in Choosing a Topic

Projects can involve any of the topics discussed above or a topic of your own creation. A well thought-out project, whether based on one of the ones above or not, will usually promote better data collection, better report writing, and ultimately a better grade. If you create a novel project, you must obtain any supplies or equipment that is not part of the standard laboratory setup described in Methods. For example, studying the diffusivity of some large biological molecule like a drug molecule might be an interesting project. However, you are responsible for obtaining the biological molecule and determining a method to measure its concentration.

When you choose your topic, remember that your experiment should take only a few hours to complete. Think through how many measurements you will need to make and how long it will take to make each measurement.

Formulate a specific and testable hypothesis, and center your project on that hypothesis. Avoid vague hypotheses, such as: "I would like to understand how concentration affects the diffusivity of dyes." Instead choose a more narrrowly focused hypothesis, such as: "Increasing the concentration of dye to near saturation values will decrease the diffusivity of the dye." This more-focused hypothesis is testable: it can be true or false. If you form a clear hypothesis, you will be able to plan a logical set of measurements to test the hypothesis, and you will be able to come to a clear conclusion when you write your report.

When you do your experiment, you may get unexpected results. For example, you may have planned to measure effects of glucose on the diffusivity of a dye. However, you may find that the added glucose affects the velocity of flow in the chambers. You should explore unexpected results and try to understand their bases. Your aim should be not simply to reject or accept your hypothesis, but to develop insight into the phenomena. For example, perhaps changes in velocity result from changes in viscosity and changes in viscosity of the medium affect your estimate of diffusivity. If so, the unanticipated changes in velocity could be key to understanding the measured changes in diffusivity.

Keep in mind that this is an **experimental** project. Your goal is to characterize **what** happens, not **why** it is happening. Theoretical analyses may support your experimental findings. However, your grade will be primarily based on the reliability of your data.

Control Observations

Control observations are important parts of the design of any experiment. The purpose of a control observation is to determine whether the variable that is directly manipulated by the experimenter is the one that *controls* the change in response. Two common kinds of control experiments test *repeatability* (i.e., if you make the same measurement several times, do you get the same answer) and *reversibility* (i.e., if you make a change and then undo it, does the response go back to what it was before the change), although other control observations are often used. Suppose, for example, that you wish to determine the effect of adding glucose to the diffusion of a dye. You could measure the time-space evolution of dye concentration with no glucose, repeat the measurement with glucose, and compare. If the results are different, can you conclude that the reason is the glucose? What if you inadvertently filled the supply reservoirs differently in the two experiments? Perhaps the difference is because the flow rates were different, not because glucose was added. Control observations are intended to assess the extent to which factors that are not part of the experimental design are contributing to response patterns. Simply repeating the experiment with no glucose is a good way to assess many sources of error. Repeated sequences of measurements can be even better.

Proposals, Reports, and Logistics

Scheduling a Laboratory Session

Students should schedule a time slot for their laboratory session by submitting an electronic schedule form. At that time, you may also request a partner. The laboratory accomodates 10 students in each session. We will work out the laboratory schedule in lecture 6. You have the best chance to get your most desired time slot by submitting your schedule form by lecture 5. After initial laboratory assignments have been made, you will be permitted to change your laboratory session only if you request the change more than 24 hours before your assigned slot and only if there is an open slot in the schedule at your desired time.

Proposal

You and your partner should meet to plan your project and to write a proposal. The proposal should contain a brief statement of the hypothesis you propose to test and the method that you will use to test it. Include a list of the experiments you will perform and the measurements you plan to make in each experiment. Indicate how the measurements will be used to help you test your hypothesis. The proposal should fit on a single sheet of paper. A sample proposal is shown in following figure. The proposal should be submitted by lecture 5. You can submit your proposal electronically. The staff will review the proposals and make suggestions. Acceptable proposals will be **APPROVED**; flawed proposals will be **REJECTED**. Proposals that are not approved may be revised and resubmitted.

Proposal: Linearity of Mixing in Microchambers

Partner Names (E-mail):} Partner One (pone@email) and Partner Two (ptwo@email)

Hypothesis: The process by which fluids mix as they flow through microchambers is linear.

Background: The primary mechanism by which fluids mix when they flow through microchambers is diffusion. Since diffusion is a linear process (i.e., the relation between flux and concentration gradient is linear), we expect that the fluid mixing in microchambers will be linear.

Procedure: A laminar flow chamber with two inputs will be used to assess fluid mixing in microchambers. The concentration of dye in the two chambers will be investigated in 3 conditions:

	input 1	input 2
condition 1	0%	50%
condition 2	50%	50%
condition 3	50%	100%

Mixing will be assessed by analyzing video images of the flow at a point 3 mm downstream from the point where the laminar flow begins. Under the assumption that mixing is linear, the results for condition 3 should be the sum of the results for conditions 1 and 2.

To increase the confidence in these results, images will also be obtained at 2 additional locations. The entire experiment will then be repeated to assess effects of uncontrolled variables, such as flow rate.

Laboratory Notebook

The written record of your experiment is the original record of your work and the basis for everything that you conclude. Such records should be kept in a laboratory notebook, which is typically a special notebook with sewn binding (not a loose-leaf notebook), whose pages were numbered when the notebook was printed. Records should be written in ink. The laboratory notebook is the permanent record of your experiment.

Laboratory notes should be sufficiently detailed so that (1) the experiment could be repeated at some later time, (2) results from different experiments can be compared, and (3) so that your procedures can be reconstructed at a later time without relying on your memory. The first page for each experiment should give the date and time, as well as a brief description of the purpose of the experiment. All relevant procedures and observations should be entered in the protocol book and the time should be indicated regularly.

Nothing should ever be erased. If an error is detected in some procedure or some reading, that should be noted in the book. The original observation should under no circumstances be obliterated so that it cannot be read. Perhaps you will subsequently find that the original observation was correct and that the correction was in error. The general rule is to write down everything that is done that may later become relevant. Scientists are rarely sorry that they wrote too much in the protocol book -- only that they wrote too little.

You do not have to purchase a special laboratory notebook for this project. However, we do ask that you take laboratory notes during your experiment, as a step toward learning about effective practices in experimental work. You will be asked to attach your notes to your laboratory report, and we will assess these notes as part of the grading procedure.

Project Report

The project report should be concise. Do not repeat material that is easily referenced. For example, there is no need to reproduce any figure from this laboratory manual or from the course text: simply refer to it. Technical writing is necessarily directed at some particular intended audience. Write the project report as though it were to be published in a journal that is read primarily by students who have taken this subject. Thus, you can assume some working knowledge about the subject. The project report should contain the following sections.

- **Cover Page.** On the cover page include the title of the laboratory session, the authors' names, your laboratory subsection, the dates of the laboratory session(s), and the name of your partner (if not a co-author).
- **Abstract.** The abstract is a one paragraph (<100 words) summary of the report including the question investigated, the methods used, and the principal results and conclusions. Your intended audience should be able to understand the abstract without having to read any of the report. This section should be written last.
- **Introduction.** The introduction is a brief section (about 1 page) designed to motivate the reader. Include background information on the problem, hypotheses to be tested, significance of the work, etc. You may give citations to material in this laboratory manual or elsewhere. The introduction should be directly relevant to your report; broad discussions of microfabrication or biology are not necessary or desireable.
- **Methods.** Briefly describe any special methods that you used that are not in this laboratory description. Give details of calculations used to obtain the results. Do not repeat material that is in this description; just refer to it.
- **Results.** Describe the measurements (whether or not they fit with expectations) in the results section. Generally, results can be communicated more efficiently and accurately with pictures and graphs than with words alone. Describe those aspects that are important to your interpretation, but omit interpretations from this section. A collection of printed graphs without a written description of their relevance is unacceptable as a results section. Students frequently err on the side of including a large number of graphs and little description of their relevance. You need only include results that are relevant to your conclusions. Most of us have trouble

leaving things out. Ask yourself why a particular result is a necessary part of your story; omit those that aren't necessary.

- **Discussion.** In the discussion section, assess the results for dependability and accuracy, and interpret results in the light of other knowledge. The discussion section can include relevant speculations and ideas for improving the experiment to test the hypotheses more rigorously.
- **Appendix.** The appendix should include a copy of the notes taken in your lab notebook during the laboratory session.

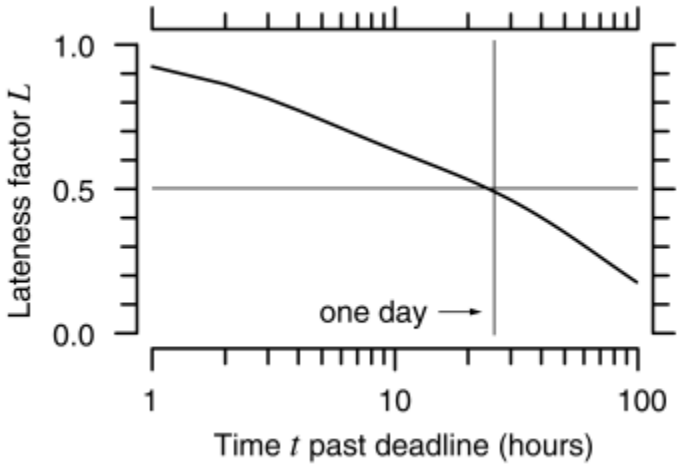
Reports that are written haphazardly and without planning are usually unintelligible and receive poor grades. We strongly recommend that you start by writing an outline to structure the logic of the report. Revise your outline until the flow of your argument makes sense (ask yourself, "If this were someone else's report, would I believe the conclusion?"). At that point, you can usually write the report by fleshing out the outline.

Grade

The grade for the experimental project will be based on the proposal and on the project report using the following considerations:

- **First draft & Critique (15%).** Your first draft and your critique of a colleague's first draft will be graded primarily for completeness.
- **Report structure (15%).** The sections of the report should be coherent.
- **Clarity/Conciseness (20%).** A good report is easy to read. The content of each paragraph and each graph should be clear. Everything included in the report should be there for a reason. Points will be deducted for extraneous material. Reports should be less than 10 pages long, unless there are good reasons for additional pages.
- **Conceptual correctness (20%).** Is the report free of obvious errors, exaggerations, and omissions? Are the prominent features of the data described accurately? Are the results (which follow directly from the measurements) clearly separated from the interpretations (which rely on information other than what was observed)? Can the conclusions be derived from the results?
- **Insightfulness (30%).** Insightfulness can be demonstrated by (1) proposing an experimental method that can resolve some scientific issue, (2) carrying out experiments and/or analyses that lead to clear conclusions, (3) preparing a report that demonstrates a clear understanding of the strengths and weaknesses of your results and analyses. Simply performing one of the standard experiments and showing unmotivated measurements will receive 0 points. Clever design of an experiment or imaginative analysis of the results will receive 30 points. Demonstrating a clear understanding of your experiment, your analyses, and what can be concluded is sufficient for 15 points.

DUE DATES ARE FIRM, AND THERE IS A SEVERE LATENESS PENALTY. The grade for a late report will be multiplied by a lateness factor

$L = 0.3 \exp(-t/4) + 0.7 \exp(-t/72)$

where t is the number of hours late. The lateness factor is plotted below. Notice that the maximum grade for a report that is more than ONE DAY LATE is less than 50%.