E90 Proposal

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Abstract

Using a microsphere and water solution to simulated blood, converging blood flow will be modeled. 2-D Fluorescing microscopy and, eventually 3-D confocal microscopy will be used to examine the separation boundary at a range of relative volumetric flow rates. The project is estimated to last 16 weeks, excluding winter break and spring break. One hundred and seventy three hours of effort will be expended to complete the project.

Introduction

The study of blood flow is one of great importance for bioengineers. By gaining a better understanding how blood flows, better drug delivery systems can be designed and malfunctions can be better understood and treated. This project is an attempt to create a basic simulation of blood flow through small vessels as blood returns to the heart. Microspheres in water solution are used to simulate blood. In this project, the separation surface of these beaded solutions is examined using both 2D and 3D microscopy under a range of laminar conditions (established by varying flow rates).

This proposal will consist of further explanation of the physical properties of blood, the nature of fluorescence and the microscopy associated with fluorescing, and the experimental methods that will be used to conduct this project. It will also consist of further details explaining exactly what will be involved in completing this project in a satisfactory manner, including a detailed activities list and timeline.

Background

Blood

Blood is made up of a mixture of red blood cells, white blood cells, platelets, proteins, and salts in water. When constructing a model of blood, the component that is most often considered is red blood cells, since they make up about 40% of human blood volume (this value is known as the hemocrit). Red blood cells (RBC) are normally shaped like biconcave, flexible discs that are capable of severe deformation without damage as they squeeze through small capillaries. There are about 5 million RBC per mm^3 of blood. The specific gravity of RBC is about 1.10.(Purves et al. 2001)

In isotonic solutions (where the concentration of water outside the cell equals the concentration of water inside the cell), the RBC is 7.65 micrometers in diameter and has a volume 97.91 microns cubed. In hypertonic solutions (where the concentration of water outside the cell is less than the concentration of water inside the cell), the cell will shrink and wrinkle. In hypotonic solutions, where the concentration of water outside the cell is more than the concentration of water inside of the cell, the cell swells into a sphere (diameter 6.78 microns, volume 164 microns cubed) and eventually bursts. For the sake of this model, we will be focusing on modeling blood in isotonic solutions. (Fung 1993) Blood plasma behaves like a Newtonian fluid with a coefficient of viscosity of about 1.2 cP. However, whole blood is a non-Newtonian fluid. The viscosity of blood varies with the hemocrit and the temperature. For a hemocrit of about 45%, the viscosity varies between 10 and 100 cP. Blood in larger vessels (much larger diameter than the diameter of RBC) is considered a homogenous mixture of plasma and cells. In vessels with

diameters that are less than or equal to the diameter of RBC (ranging from 4 microns to 10 microns), also known as capillaries, the RBC must flow through the capillary in single file. Because of this, it makes more sense to consider blood in capillaries as nonhomogenous. In our model, we will consider blood to be a homogenous solution. As a homogenous solution, we can assume that blood is an isotropic and incompressible fluid. Blood is considered isotropic because when the shear stress and shear strain rate are zero, the RBC have no preferred orientation. Incompressibility is assumed because under the pressures considered in physiology, and given the mass densities of plasma and RBC, blood as a whole should not be affected by pressure. (Fung 1993)

For this project, we are attempting to model RBC's as rigid spheres. By attempting to model RBC's, which tend to behave more like encased droplets, as rigid spheres, we compromise our ability to most accurately model blood flow. For example, the hemocrit (particle volume fraction) of blood in normal mammals is about 40%. At this particle volume fraction, the relative viscosity of an RBC is about 3 cP. For a rigid sphere, the relative viscosity is about 10 cP. It should be noted that at a particle volume fraction of 40%, there is no blood substitute that serves as a good model. Rigid spheres most closely resemble RBC's at a much lower particle volume fraction. At a particle volume fraction of about 10%, both rigid spheres and RBC have a relative viscosity of about 1.5 cP. (Fung 1993)

Fluorescence

Substances that fluoresce absorb and remit light at a different wavelength than non-fluorescing substances. These substances are commonly used as markers and dyes in a laboratory setting. When light at a particular wavelength is shone on a molecule that can fluoresce, an electron is promoted from it's highest occupied molecular orbital (HOMO) to it's higher energy, lowest unoccupied molecular orbital (LUMO). The energy difference between the HOMO and LUMO is inversely proportional to the wavelength of light needed to excite the electron. The greater the energy difference, the shorter the wavelength of light needed. After being excited into the LUMO, the electron will eventually back to the HOMO. Due to some intermediate energy loss, the final energy drop is less than the energy that was originally inputted. So as the electron falls, it will give off light at a higher wavelength (and lower energy) than the excitation wavelength. Each fluorescing compound has a characteristic excitation wavelength (peak of absorbance range) and emission wavelength (peak of fluorescence range) Since different substances emit at different wavelengths, dyes with distinct fluorescing colors can be used to distinguish between two materials. For this project, two fluorescent dyes will be used, FITC and resorufin.(Christensen 2003)flirt

FITC

Fluorescenin isothicyante, also known as FITC, is a green, water soluble fluorescent dye that has an excitation wavelength of 488nm and emission wavelength of 520nm.

Resorufin

Resorufin is a red, water soluble dye with an excitation wavelength of 577nm and an emission wavelength of 620nm.

2D Fluorescence Microscopy

Fluorescence microscopy uses broadband fluorescing to excite dyes at different wavelengths and, using specialized filters, observe their emission spectra. The view is a two dimensional, overhead view. Although all dyes are simultaneously excited, by the use of the specialized filters, it is possible to choose which one to image and observe. Multiple dyes can be imaged sequentially by switching the filters. The microscope is typically connected to a camera and a computer to aid in imaging. (Christensen 2003)

3D Confocal Microscope

Confocal microscopy takes 2D images at carefully controlled depths and stacks the images on top of each other, developing a 3D image. A confocal microscope uses laser to excite specific fluorescing materials at their characteristic excitation wavelength. This added specificity leads to crisper images than those you would get with 2D fluorescence microscopy. Since a laser is used in order to excite the fluorescing dyes, viewing the image directly is not feasible. Rather, a confocal camera attached to a computer is used to in imaging. Therefore, except for initial setup controlled under standard 2D microscopy conditions, all imaging is controlled through a computer interface. Through the computer, it is possible to define how the images will be taken, including but not limited to the starting plane where the first image will be taken and the spacing between successive 2D images. The user also must specify the emission wavelength of the material that is being imaged. Other choices involve determining whether to take sequential or simultaneous images and whether to take direct images or to use a Kalman filter to average several images at once, generally producing a crisper final image. It should be noted that in order of a confocal microscope to be useful, steady state conditions must be maintained. To complete imaging takes several minutes, which will wash away most dynamic phenomenon. (Christensen 2003)

Volume Reconstruction

To reconstruct the images from the confocal microscope, the software, Slicer will be used. Currently the program Slicer can only be used in the University of Vermont. Hopefully by the time we start confocal imaging, Slicer will be available at Swarthmore. If not, results will have to be sent to University of Vermont to be analyzed there. One common problem that occurs during volume reconstruction is lost data and optical aberrations. In order to address this, correction factors for “apparent stretch” and careful planning for slice thickness must be implemented.(Christensen 2003)

Experimental Methods

Fluids

Two fluids will be used during the course of the project. One will simply be deionized water. The other will be a 10% particle volume fraction bead solution in water. Glycerol must be added in order to match the fluids specific gravity to that of the beads. Small amounts of surfactant (Tween) must also be added in order to prevent bead clumping. Appropriate fluorescent dyes will be added to each fluid to enable detection by the 2D fluorescent microscope and the confocal microscope.

Equipment setup

Module

The flow module was precision milled into acrylic substrate so that it consists of a daughter microchannels converging into a main microchannel at a 90 degree angle. The microchannels are 127 microns long per side. The microchannels converge 1.25 inches along the 3 inch long module. Tape has been used to close the channels off on their fourth side. Plastic fitting have been screwed into the ends of the microchannels to facilitate fluid delivery. Sealant is added to the threads to prevent leakage. Water flows from the daughter channel to the main channel, which if filled with the beaded solution.

Fluid Delivery

Tubes topped with syringes are added to the entry fittings. A drainage tube is fitted to the exit fitting. Tube-to-tube connections are added (to reduce fluid inventory) via epoxy. The syringe used is a 1mL tuberculin syringe.(Christensen 2003)

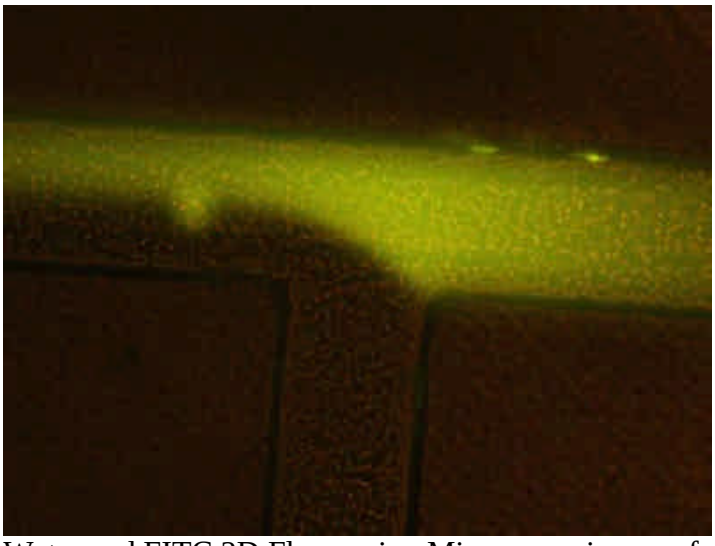
Project Details

Background tasks

These tasks must be completed prior to Dec 15

Familiarization with instrumentation

The flow module will be used with FITC in water and distilled water flowing through the channels. 2D Microscope images will be rendered. The purpose of this task is to ensure that the Flow Module is in working condition (no addition equipment needs to be purchased) as well as to ensure that I learn how to operate the 2D microscope well enough to take clear images of the flow module.



Water and FITC 2D Fluorescing Microscope image of module with 5 microliters/minute flows through each channel. Taken on 11/26/04.

Defining Experimental Model

The most important part of defining the experimental model is determining what bead size is appropriate for modeling blood. Beyond that, a method must be established to construct a bead solution that most resembles blood. To do this, we must determine what the particle volume fraction is of the stock solution of beads is and match the bead solution so that it best simulates the red blood cell particle volume fraction in blood. We must also determine what ranges of flow rates we would like to use in the flow modules. Research must be done to determine what range of flow rates are found in blood vessels.

Ordering materials

The most important material that must be ordered is the beads. We must determine how far we are willing to deviate from the actual size of a red blood cell in our beads in order to meet cost requirements. We must also determine what type of beads will be best suited for this project. Beads can be manufactured to fluoresce, made with different chemicals including polystyrene, PMMA and others. Other equipment to be ordered will include replacement parts for the flow module if we find that it is not functioning adequately.

Tasks

These tasks will be completed after Winter Break. They must be completed in order to complete the project.

Preparation of Bead Solution

The bead solution must be prepared so that it has a similar particle volume fraction as blood.

Test Flow Module with FITC and water

This is just to ensure, once again, that the flow module is functioning and no repairs need to be done prior to experimentation.

2D Microscopy

Once the Bead Solution is prepared, we must image the flow module with the FITC/bead solution and water.

Confocal Microscopy

Once we are convinced that we can get reliable data and images from the 2D microscope, the confocal microscope must be used image the flow module with the FITC/bead solution and water. The confocal microscope is located at the University of Pennsylvania and we must arrange to rent the space when necessary. Also, the confocal microscope is a complicated piece of equipment so prior to imaging the bead solution in the flow module, time should be devoted to imaging FITC and water in the flow module on the confocal microscope in order to learn how to operate the confocal microscope best.

CPM

Activities List

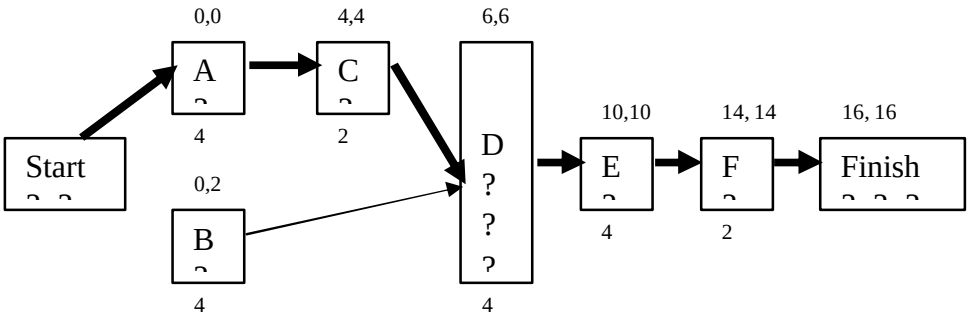
Activity	Action
A	Order Beads
B	2D Microscope Test with FITC and Water
C	Prepare Bead Solution
D	2D Microscope Test with Bead Solution
E	3D Microscope Test with Bead Solution
F	Write Final Report

Needs/Feeds Chart with Duration and Effort Estimates

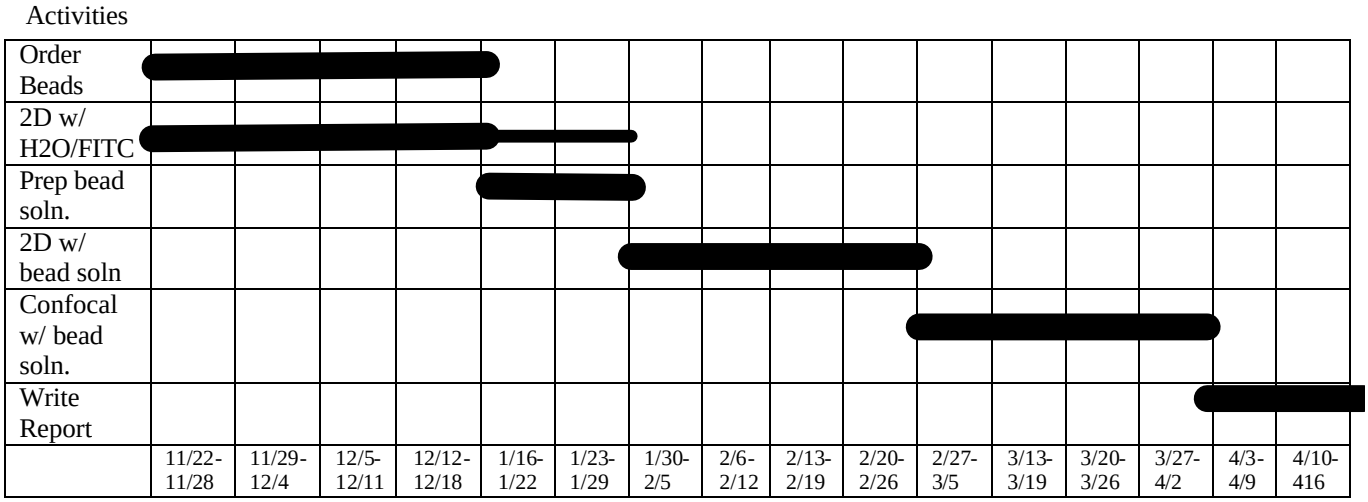
Activities	Needs	Feeds	Duration	Effort	Action
A*	--------	C	4 weeks	3 hrs	Order Beads
B*	--------	D	4 weeks	20 hrs	2D FITC and H2O
C	A	D	2 weeks	10 hrs	Prep bead soln.
D	B, C	E	4 weeks	40 hrs	2D Bead soln.
E	D	F	4 weeks	60 hrs	3D Bead soln.
F	E	---------	2 weeks	40 hrs	Write report

* Will be completed before Dec 15

CPM diagram



GANNT diagram



The project will take 16 weeks to complete, excluding breaks. 173 effort hours will be expended to complete the project**.**

Materials List

Microspheres from Bangs Laboratory (already ordered and have arrived)
Plumbing (specifics)
Rental time on the confocal microscope (located at Upenn)

References

1. **Purves WK, Sadava D, Orians GH and Heller HC**. *Life: The Science of Biology.* Sunderland, MA: W.H. Freeman, 2001.
2. **Fung YC**. *Biomechanics: Mechanical Properties of Living Tissues*. New York, New York: Springer, 1993.
3. **Christensen S**. *Convergent Blood Flow Simulation*. Swarthmore, PA: Swarthmore College, 2003.