

Converging Flow of Microsphere Solution
Through Microchannels:
Development of a Model for Blood Flow

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Table of Contents

I. ACKNOWLEDGMENTS	3
II. ABSTRACT.....	4
III. INTRODUCTION.....	5
1. BACKGROUND.....	5
A. <i>Properties of Blood</i>	5
a. Components of Blood.....	5
b. Viscosity of Blood.....	7
c. Blood Vessels.....	8
d. Our Model.....	9
IV. EXPERIMENTAL METHODS.....	12
1. FLUIDS.....	12
2. FLUORESCENT DYES.....	12
3. EQUIPMENT SETUP.....	13
A. <i>Flow Module</i>	13
B. <i>Delivery System</i>	14
C. <i>Syringe Pumps</i>	15
4. IMAGING.....	15
A. <i>Fluorescence</i>	15
a. 2D Fluorescence Microscopy.....	16
b. 3D Confocal Microscopy.....	16
c. Volume Reconstruction.....	17
B. <i>Our Experiments</i>	17
a. Fluorescent Microscopy.....	17
b. Confocal Microscopy.....	18
c. Interface Positions.....	19
V. EXPERIMENTAL RESULTS.....	20
1. DETERMINATION OF REYNOLDS NUMBER.....	20
2. 2D EXPERIMENTAL RESULTS.....	21
A. <i>Experiments with Water in both Channels</i>	21
B. <i>Experiments with 2% Beaded Solution in Both Channels</i>	22
C. <i>Experiments with 4% Beaded Solution in Both Channels</i>	24
3. 3D EXPERIMENTAL RESULTS.....	28
VI. CONCLUSIONS AND FUTURE WORK.....	29
VII. REFERENCES.....	31
VII. APPENDIX	32
1. BEADED SOLUTION.....	32
2. FLUORESCENT DYE.....	32
3. DELIVERY SYSTEM.....	33
A. <i>Syringes</i>	33
B. <i>Tubing</i>	33
C. <i>Small Parts</i>	33
D. <i>Sealant/Epoxies</i>	34
4. CONFOCAL DATA.....	35
5. CONFOCAL ANALYSIS.....	50

Table of Figures, Graphs and Tables

Figure 1: The Components of Blood	6
Figure 2: Red Blood Cells	6
Figure 3: Graph of Shear stress – shear rate plot for whole blood	7
Figure 4: Graph of variation of viscosity of whole blood with shear rate and hemocrit ...	8
Figure 5: The whole cardiovascular system	9
Figure 6: Order of blood flow through the cardiovascular system	9
Figure 7: Graph of particle fraction vs relative viscosity for various red blood cell model substitutes.....	11
Figure 8: Flasks of 2% beaded solution.....	12
Figure 9: Flask of 0.002% FITC dye	13
Figure 10: The experimental setup	13
Figure 11: The flow module	14
Figure 12: The delivery system tubing	14
Figure 13: To the left is the Cole-Parmer Syringe Pump. To the right is the Model 11 Syringe Pump:.....	15
Figure 14: 1mL syringe.....	33
Figure 15: Thick (left) and Thin (right) tubing for the delivery system	33
Figure 16: Small Parts: Barbed-to-male	34
Figure 17: Small Parts: Female Luer	34
Figure 18: Small Parts: Fittings	34
Figure 19: Dow Corning Sealant	34
Figure 20: Epoxy.....	34
Graph 1: Effect of relative flow on surface location for water experimental data	21
Graph 2: Effect of relative flow on surface location for all 2% beaded solution experimental data	23
Graph 3: Effect of relative flow on surface location for average 2% beaded solution experimental data	24
Graph 4: Effect of relative flow on surface location for all 4% beaded solution experimental data	25
Graph 5: Effect of relative flow on surface location for average 4% beaded solution experimental data	27
Graph 6: Effect of relative flow on surface location for average 2% bead solution and 4% bead solution experimental data.....	27
Table 1: Experimental Data for Water Trial	21
Table 2: Experimental Data for 2% beaded solution Trial	22
Table 3: Experimental Data for 4% beaded solution Trial	24

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Other individuals have also provided assistance and materials. Professor Kathy Siwicki and the Department of Biology at Swarthmore College provided the use of their 2D fluorescent microscope. Gladys Gray Board, of the University of Pennsylvania Department of Bioengineering, provided assistance and invaluable guidance in the use of the confocal microscope.

II. Abstract

We investigated the flow of water-microsphere mixtures in converging microchannels. The two- and three-dimensional steady-state separation surface formed by the 90 degree microchannel convergence of the microsphere solutions was characterized using fluorescence and confocal microscopy techniques. Steady state convergence was achieved for a range of main channel to daughter channel flow ratios, with a constant combined flow Reynold's number of approximately one. For 2% microsphere solutions, the separation surfaces deviated only slightly from those reported for pure water. For 4% beaded solution, the data were less repeatable but indicated that again, the separation surfaces deviated to some extent from those reported for pure water, to a larger degree than the deviation found in the 2% case.

III. Introduction

Converging flow of microsphere (or beaded) solutions through microchannels were studied. The Reynolds number of the microsphere solutions was set to be similar to that of blood and the size of the microspheres was approximately the same size as red blood cells. The concentration of the microspheres was altered to determine if the non-Newtonian effects seen in whole blood could be reproduced. We will begin this report by discussing the properties of blood and how those properties were accounted for in our model. We will then discuss the experimental methods used for this project, including the equipment setup and the imaging techniques used. Finally, we will discuss what we discovered from our series of experiments.

1. Background

A. Properties of Blood

a. Components of Blood

Blood is made up of a mixture of red blood cells, white blood cells, platelets, proteins, and salts in water. (Figure 1)

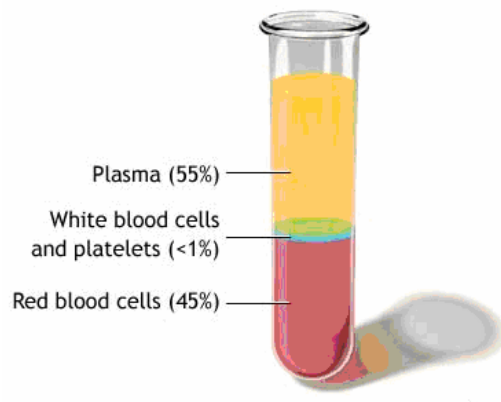


Figure 1: The Components of Blood [1]

When constructing a model of blood, the red blood cells and blood plasma are primarily considered. Blood plasma makes up about 60% of human blood volume. Blood plasma itself is considered the liquid matrix of blood, consisting mainly of dissolved gases, ions and small blood proteins like albumin. Red blood cells make up about 40% of human blood volume (this value is known as the hemocrit), a much larger portion than of human blood volume than white blood cells and platelets, the other large components in blood. Red blood cells (RBC) consist of a fluid interior surrounded by a flexible membrane that resists area changes but bends and shears rather easily. (Figure 2) RBCs 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.[2]



Figure 2: Red Blood Cells [3]

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. Both the

hypertonic and the hypotonic cases occur infrequently in biological systems. Thus, for the sake of this model, we will be focusing on modeling blood in isotonic solutions. [4]

b. Viscosity of Blood

Blood plasma behaves like a Newtonian fluid with a coefficient of viscosity of about 1.2 cP. [5] However, whole blood (which includes red blood cells) is a non-Newtonian fluid. The blood plasma alone is incompressible but the addition of cellular components gives blood its non-Newtonian characteristics.

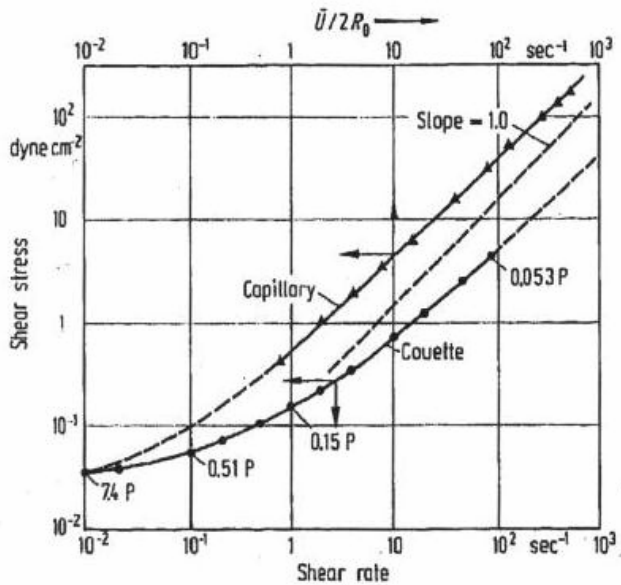


Figure 3: Graph of Shear stress – shear rate plot for whole blood [6]

Figure 3 illustrates the variation of the shear stress of blood with the shear rate.

The capillary and Couette lines are the results of two different methods used to obtain these plots. The Couette results in the figure above were obtained by running blood through a Couette-flow viscometer whose diameter is much greater than the diameter of a red blood cell. The numbers referred to on the Couette line (i.e. 7.4 P, 0.51 P...) refer to the tangential slope at specific points on the Couette curve. Shear rate is the rate of change of velocity at which one layer of fluid passes over an adjacent layer. In a

Newtonian fluid, a linear relationship exists between shear stress and shear rate. The shear rate in the Couette experiment is defined as the relative velocity of the blood at the walls divided by the diameter of the tube the blood was being sent through. In whole blood, the viscosity varies with the hemocrit, the temperature and disease state, a non-Newtonian effect. Figure 4 illustrates the variation in the viscosity with shear rate and hemocrit. For a hemocrit of about 45%, the viscosity varies between 10 and 100 cP.

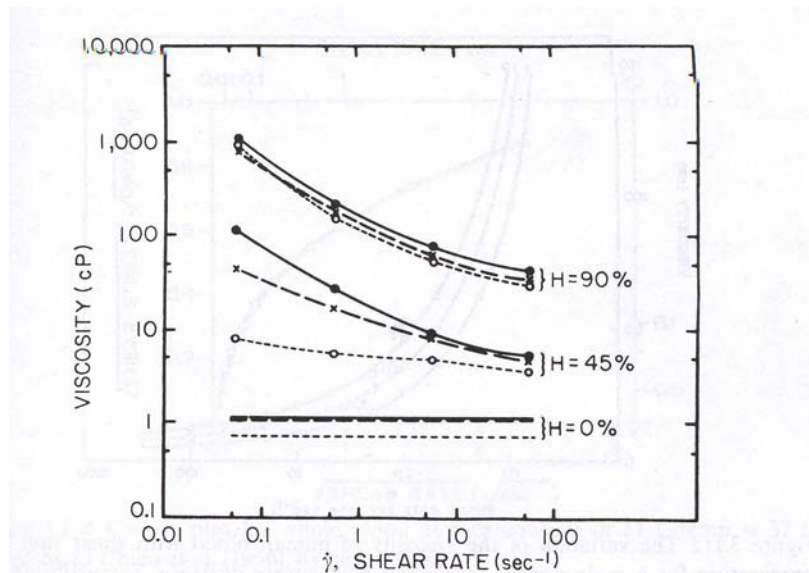


Figure 4: Graph of variation of viscosity of whole blood with shear rate and hemocrit [4]

c. Blood Vessels

The vessels in the body are separated into two categories, arterial and ventral vessels. Arterial vessels carry oxygenated blood from the lungs to the tissues. Ventral vessels carry deoxygenated blood from the tissues back to the lungs. See Figure 5 and 6. The size of arterial and ventral vessels can vary greatly. Arteries and veins can be as large as a few millimeters. Arterioles and venules are between 10 and 150 microns. Capillaries, the smallest blood vessels, where oxygen is transported to the tissues from blood are between 4 and 8 microns in diameter.

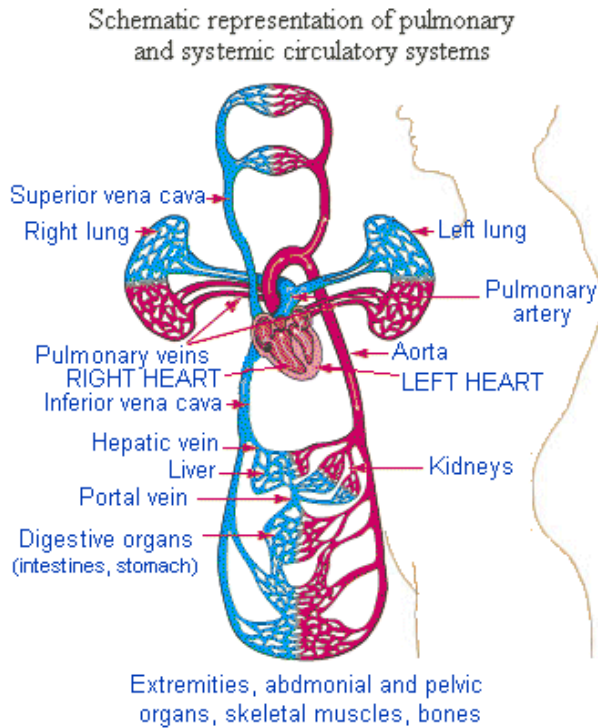


Figure 5: The whole cardiovascular system [7]

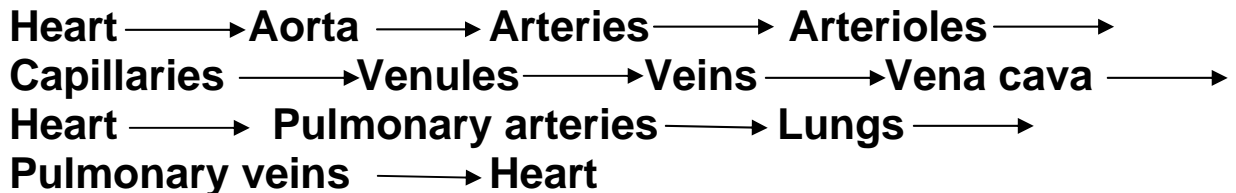


Figure 6: Order of blood flow through the cardiovascular system

The 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.

d. Our Model

As a homogenous solution, we can assume that blood is an incompressible fluid with isotropic flow. To have isotropic flow, blood plasma flowing over the red blood

cells must flow the same irregardless of the orientation of the red blood cell or the way the plasma hits the cell. Blood is considered isotropic because when the shear stress and shear strain rate are zero along the RBC, the RBCs have no preferred orientation.

Incompressibility is assumed since under the pressures considered in physiology, and considering the mass densities of plasma and RBC, blood pressure remains constant. [4]

RBCs flowing through larger microvessels, like those examined in this project, travel away from the wall in a core of cells, creating a different viscosity along the center of the microvessel than along the cell-depleted walls. In addition, viscosity effects from cell aggregation are greater in microvessels than in larger vessels. In this experiment however, steps were taken to prevent aggregation and clumping. By preventing clump formation, it is assumed that the viscosity in the experiment is constant throughout the channel.

For this project, we attempt to model RBC's as rigid microspheres. 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%. (See Figure 7) At this particle volume fraction, the relative viscosity of a RBC is about 3. Relative viscosity refers to the apparent viscosity of whole blood compared to the viscosity of blood plasma (which is about the same as water). It is the ratio of the apparent viscosity over the viscosity of blood plasma and is thus dimensionless. For a rigid sphere, the relative viscosity is about 10 at a 40% particle volume fraction. 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. The

concentrations of rigid sphere solutions used in these experiments were 2% by volume and 4% by volume, well below the 10% limit where rigid spheres begin to act differently than red blood cells. [4]

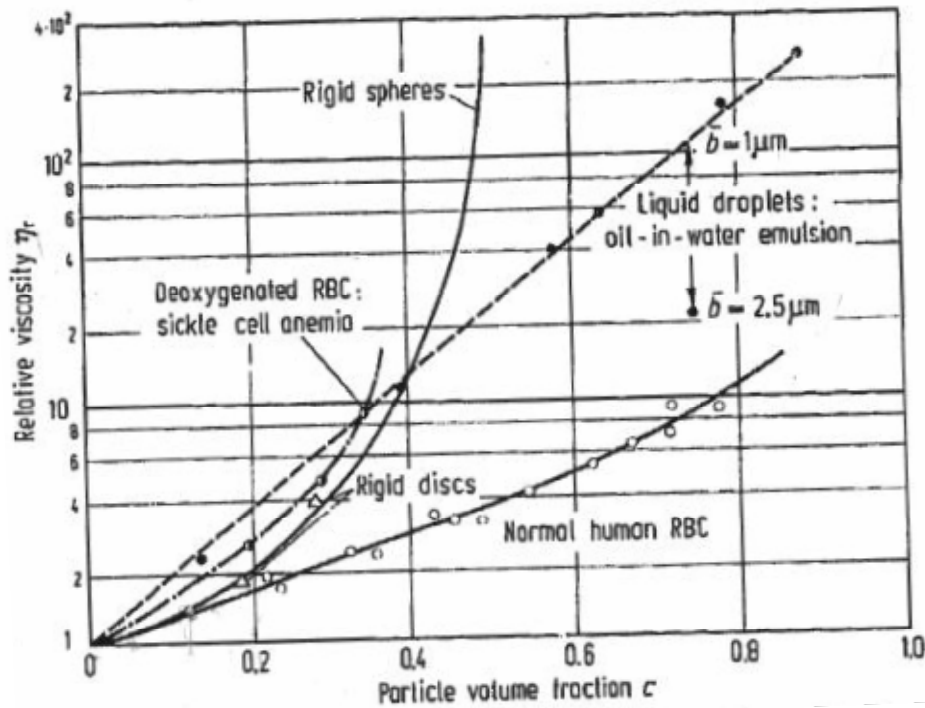


Figure 7: Graph of particle fraction vs. relative viscosity for various red blood cell model substitutes [6]

The Reynolds number of blood in arterioles and venules is about unity. [5] In our experiment the Reynolds numbers were approximately one. The Reynolds number (see equation below) is a quantity that takes into account the diameter of the channel (D), the absolute viscosity of the liquid (μ), the density of the liquid (ρ) and the velocity (V) the liquid is flowing through the channel.

$$R_e = \frac{\rho V D}{\mu}$$

IV. Experimental Methods

1. Fluids

Three different fluid solutions were used. The first was simply deionized water, used for “shake down” tests, and comparison purposes. The second solution was a 2% by weight bead solution in water, with glycerol to match the fluid’s specific gravity to that of the suspended beads. (See Figure 8) The third was a 4% by weight bead solution in water with glycerol. Additionally, a small amount of the surfactant Tween was added to the solution to prevent bead clumping. Appropriate fluorescent dyes were included in both fluids (deionized water solution and beaded solutions) to enable detection with the 2D fluorescence microscopy and the 3D confocal microscopy. See Appendix for more explicit mixing instructions.

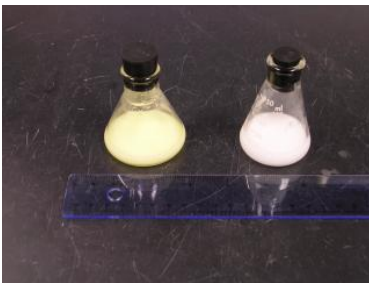


Figure 8: Flasks of 2% beaded solution

2. Fluorescent Dyes

A fluorescent dye, FITC (which fluoresces yellow-green) is used in one of the converging fluids to facilitate interface identification and location since color distinction

can emphasize the boundary between two liquids. The FITC solution was prepared as a 0.002% solution. See Figure 9. See Appendix for more explicit mixing instructions.



Figure 9: Flask of 0.002% FITC dye

3. Equipment Setup

The testing system used was developed previously by D. L. Hitt at University of Vermont and later modified by Dr. Nelson Macken and Susan Christensen, Class of 2003 at Swarthmore College. The system consisted of a specially developed flow module in which the convergence of two fluids occurred and a delivery system to appropriately route the fluids. See Figure 10.

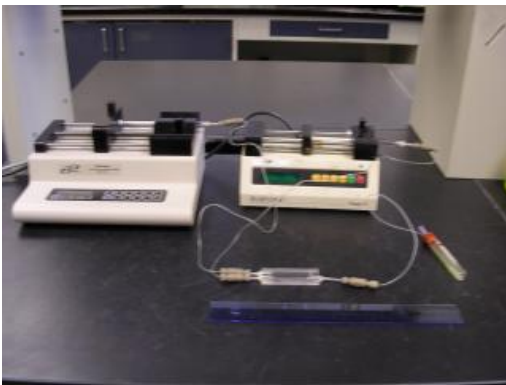


Figure 10: The experimental setup

A. Flow 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 is used to close the channels off on their fourth side. Plastic fittings have been screwed into the ends of the microchannels to facilitate fluid delivery. Sealant is added to the threads to prevent leakage. See Figure 11.

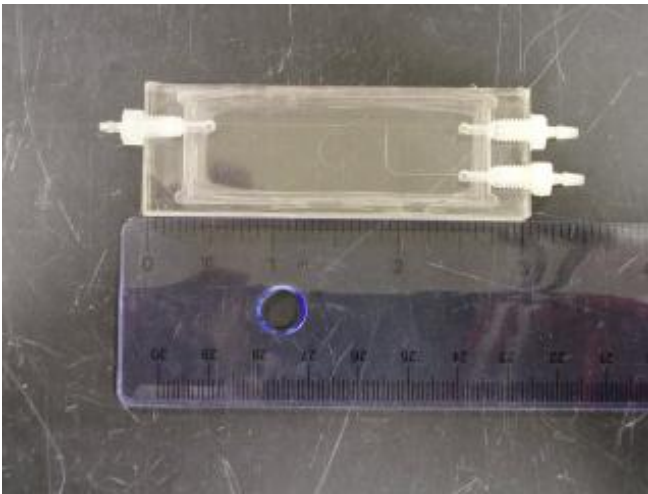


Figure 11: The flow module

B. Delivery System

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. See Figure 12. For more specifics on the construction of and parts included in the Delivery System, see Appendix.



Figure 12: The delivery system tubing

C. Syringe Pumps

Two syringe pumps were used to control the flow through the module. The two syringe pumps were a Cole-Parmer 74900 Series Multichannel Syringe Pump, provided by Dr. Carr Everbach of Swarthmore College and a Harvard Apparatus “Model 11” Single Syringe Pump from the University of Vermont. See Figure 13 The Cole-Parmer Syringe Pump has an accuracy of $\pm 0.5\%$. The accuracy information is unavailable for the Harvard Model 11 Syringe Pump.



Figure 13: To the left is the Cole-Parmer Syringe Pump. To the right is the Model 11 Syringe Pump:

4. Imaging

A. Fluorescence

Substances that fluoresce absorb and reemit light at a different wavelength, unlike 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 its highest occupied molecular orbital (HOMO) to its 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 added. 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, the fluorescent dye FITC was used.[8] Fluorescein isothiocyanate, also known as FITC, is a green, water soluble fluorescent dye that has an excitation wavelength of 488nm and emission wavelength of 520nm.

a. 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, top-down 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. [8]

b. 3D Confocal Microscopy

A confocal microscope uses a laser to excite individual fluorescent molecules at their characteristic wavelengths. This greater specificity results in crisper images with more well defined boundaries. Because a laser is used to excite the fluorescent materials, a confocal camera connected to the computer must be used to look at the resultant image, since the image cannot be viewed directly by human eyes. Thus, except for initial placement and focusing of the specimen, accomplished under standard 2D fluorescent

microscope conditions, all image taking is controlled through a computer interface. The software used for confocal imaging is called BioRad. The computer allows the user to carefully define how all the images are to be taken. The user must determine the starting plane where the first picture is taken and the ending plane where the last picture will be taken. The user must also determine the spacing between neighboring images. These three factors determine the total number of 2D images that will be taken, referred to as slices. The user also must specify the excitation and emission wavelengths of the material to be imaged. Other decisions include whether sequential or simultaneous images are used, whether to take direct images or to use a Kalman filter algorithm to average multiple images, which generally produces a crisper image. [4]

c. Volume Reconstruction

After spending a session on the confocal microscope, the entire image data collected is contained in a BioRad file. This file can be extended out into a montage of the individual slices using the software Confocal Assistant developed for D. L. Hitt of the University of Vermont. Once these sequential images are obtained, they can be rendered to give a 3D image using the software Slicer developed by Fortner Research, Inc. For D. L. Hitt (University of Vermont).

B. Our Experiments

a. Fluorescent Microscopy

The fluid flow was examined using a traditional two-dimensional Zeiss fluorescent microscope from Dr. Kathy Siwicki's lab in the Swarthmore Biology Department. Two-dimensional imaging is useful for establishing reasonable flow rates in ranges in which the convergence system would remain at steady state.

For example, the FITC-dyed beaded solution at 7 μ L/min (white in Figure) approaches the intersection from the right while the non-dyed solution at 3 μ L/min approaches the intersection from the bottom. Both fluids exit to the left. This picture was taken using SpotAdvanced imaging software.

b. Confocal Microscopy

To use the confocal microscope, the module had to be inverted upon the microscope stage since the laser was located beneath the stage. This module orientation allowed the light to travel the shortest distance to reach the fluorescing fluids. The objective selected was 20X. The picture size was set to 512x512 pixels. Through trial and error, a good color resolution was obtained for the FITC by adjusting the setting for the iris, gain and offset. For the case where water was in both channels, the gain was set to 12.3, the Iris was set to 3.0 and the offset was set to -29.8. The laser was set to work at a speed of 500lps. For the case where the 2% beaded solution was in both channels, the gain was set to 15.8, the Iris set to 5.0 and the offset was at -19.3. The laser speed was set to 166lps.

The Kalman value was set to 5 in the water case, indicating that five pictures would be averaged together to produce a single saved picture. The 127 μ m-deep channel was divided into 35 slices with images taken in order from highest slice (at the acrylic channel bottom) to the lowest slice (at the tape cover). In the 2% beaded solution case, the Kalman value was set to 3. The 127 μ m-deep channel was divided into 40 slices taken in order from highest slice to the lowest slice, as in the water case.

c. Interface Positions

Images taken from the Fluorescent Microscope were rotated so that main channel was exactly horizontal and the daughter channel was exactly vertical. Then, in the SpotAdvanced program, the difference between the bottom wall and the interface and the difference between the top wall and the bottom wall were measured (about one lateral channel width from the more downstream convergent corner) in pixels and recorded in a spreadsheet for all flow rates. The accuracy for interface measurements in pixels is $\pm 2.0\%$. The theoretical water-water curve is based on analytical calculations published by Harris et al. (2002) for equal viscosity fluids.

V. Experimental Results

The interface position was measured at different flow ratios, with the setup illustrated above.

1. Determination of Reynolds Number

The flows in the water, 2% beaded solution and the 4% beaded solution were all set to make Reynolds numbers on the order of one in each case. The viscosity of water is 1 cp. The density of water is 1g/mL. For the 2% beaded solution, the Reynolds numbers was calculated based on the known density (1.062 g/mL) and an estimate of 1.44 cP for the viscosity at room temperature. The viscosity of the 4% solution was estimated to be 1.54 cP at room temperature.

To find the relative viscosity of the beaded solution, a modified Sabalt test was conducted. Water was placed in a syringe with a 1/8" outer diameter tube with a fitting to fit flush within the syringe to prevent leakage. The time it took for the water to flow out of the syringe was measured. Next, the beaded solution was placed in the syringe and the time it took for the beaded solution to flow out was measured. These two times were compared and it was found that the 2% beaded solution was 1.5 time more viscous than water, so a relative viscosity was determined to calculate the Reynold's number. The 4% beaded solution was 1.6 times more viscous than water.

2. 2D Experimental Results

A. Experiments with Water in both Channels

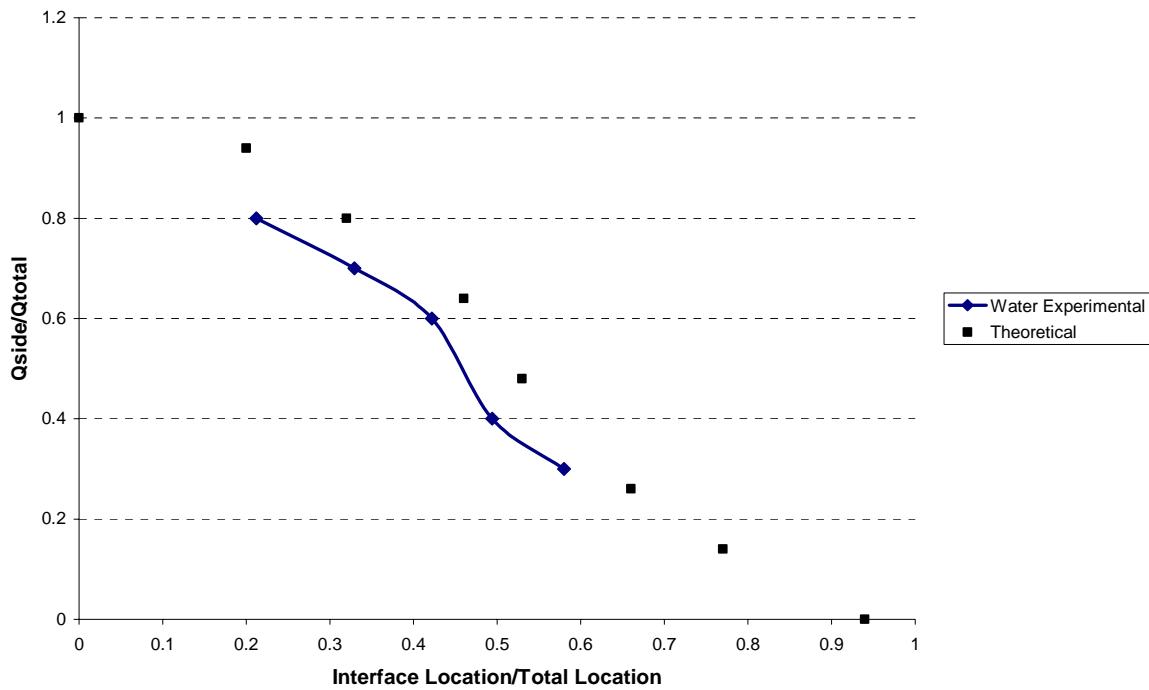
With water entering through both the main and the daughter channel, steady state convergence was achieved for a range of relative volumetric flow rates. The flow ratios extended from 2:8 uL/min and from 7:3 uL/min.

The data sets collected are in Table 1. These water/water trials were used as a shake-down test to test the microscope and the testing system as well a comparison for other steady state convergence tests.

Table 1: Experimental Data for Water Trial

Horizontal Channel Flow (Water)	Vertical Channel Flow (Water)	Ratio of Interface:Total Channel Size	Qside/Qtotal	Reynold's Number
7	3	.58	.3	1.37
6	4	.49	.4	1.37
4	6	.42	.6	1.37
3	7	.33	.7	1.37
2	8	.21	.8	1.37

Experimental Measurement for Water in both Channels



Graph 1: Effect of relative flow on surface location for water experimental data

On first glance, the experimental water measurements do not seem to match up very well with the theoretical model. (See Graph 1) However, our experimental results match up well with the experimental results compared against this model in the literature. The deviation may come from the developed theoretical model and not necessarily from the experimental results. The theoretical model does not take into account 3-D effects and only considers fully developed laminar flow. The results of this experiment with water flowing in both the main and the daughter channel will be used as a benchmark for comparison to the cases where the 2% and the 4% beaded solutions were flowing in both the main and the daughter channel.

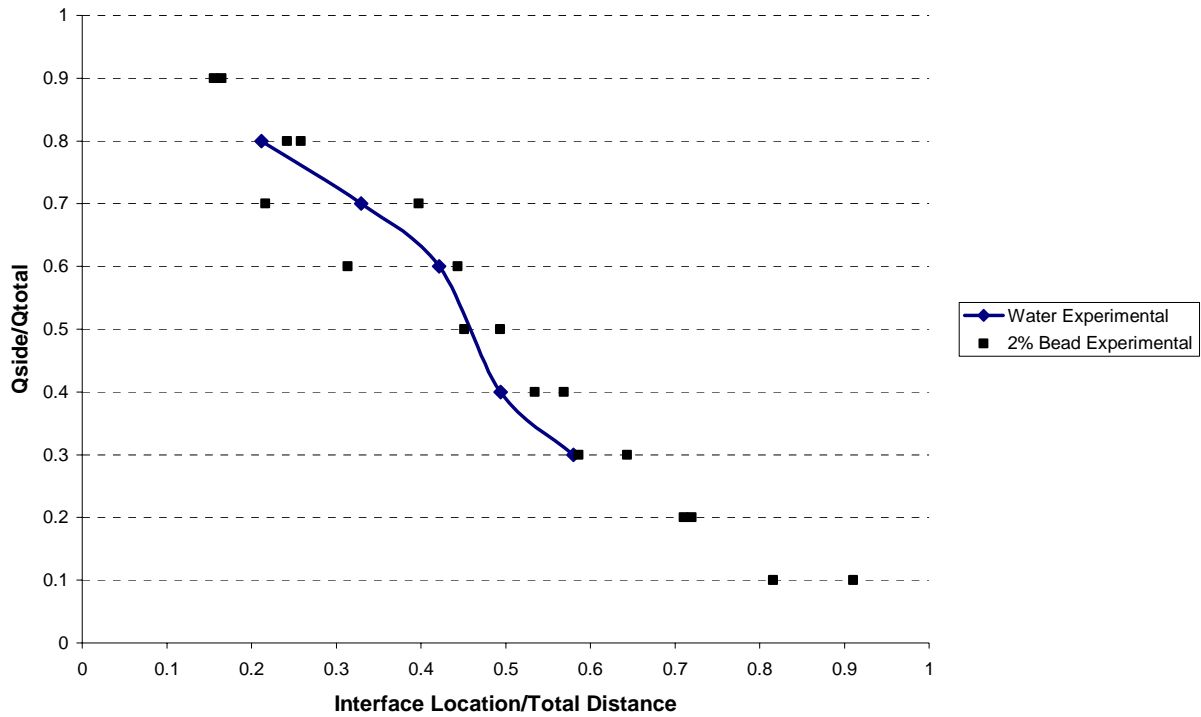
B. Experiments with 2% Beaded Solution in Both Channels

With 2% beaded solution entering both the main and daughter channel, steady state convergence was achieved for a range of relative volumetric flow rates, ranging from 1:9 uL/min to 9:1 uL/min. The data sets collected are in Table 2. These tests were compared to other steady state convergence tests.

Table 2: Experimental Data for 2% beaded solution Trial

Horizontal Channel Flow (2% Solution)	Vertical Channel Flow (2% Solution)	Ratio of Interface:Total Channel Size	Qside/Qtotal	Reynold's Number
1	9	0.16	.9	0.96
2	8	0.26	.8	0.96
3	7	0.40	.7	0.96
4	6	0.44	.6	0.96
5	5	0.49	.5	0.96
6	4	0.57	.4	0.96
7	3	0.64	.3	0.96
8	2	0.72	.2	0.96
9	1	0.82	.1	0.96
1	9	0.15	.9	0.96
2	8	0.24	.8	0.96
3	7	0.22	.7	0.96
4	6	0.31	.6	0.96
5	5	0.45	.5	0.96
6	4	0.53	.4	0.96
7	3	0.59	.3	0.96
8	2	0.71	.2	0.96
9	1	0.91	.1	0.96

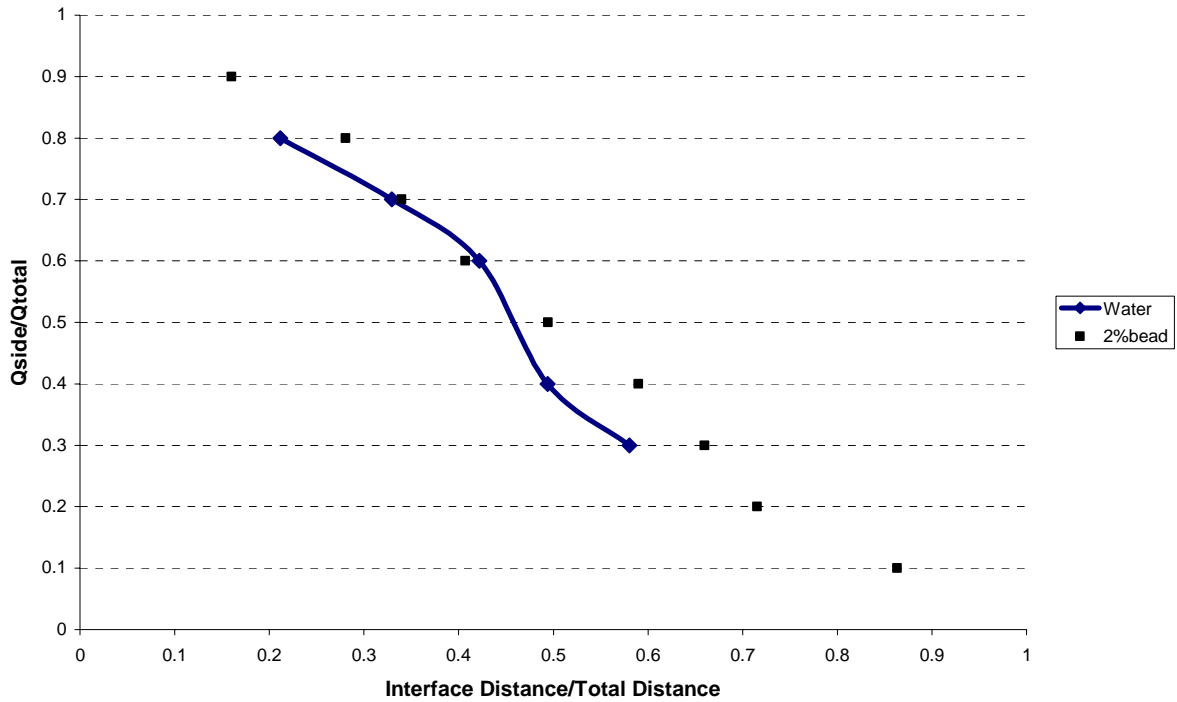
All Experimental Measurements for 2% bead Solution in Both Channels

**Graph 2: Effect of relative flow on surface location for all 2% beaded solution experimental data**

The multiple points at a fixed flow ratio represent a different experimental run.

We tried to take data for the complete complement of flow ratios during each experimental run. See Graph 2. Some scatter from the water experimental data is evident in the 2% beaded solution experimental data, but the variance is not large. This is more evident in Graph 3, which looks at the averaged 2% beaded solution experimental data in comparison to the water experimental data. The beads do not seem to have a noticeable effect on the interface.

Average Experimental Measurements for 2% Bead Solution in both Channels



Graph 3: Effect of relative flow on surface location for average 2% beaded solution experimental data

C. Experiments with 4% Beaded Solution in Both Channels

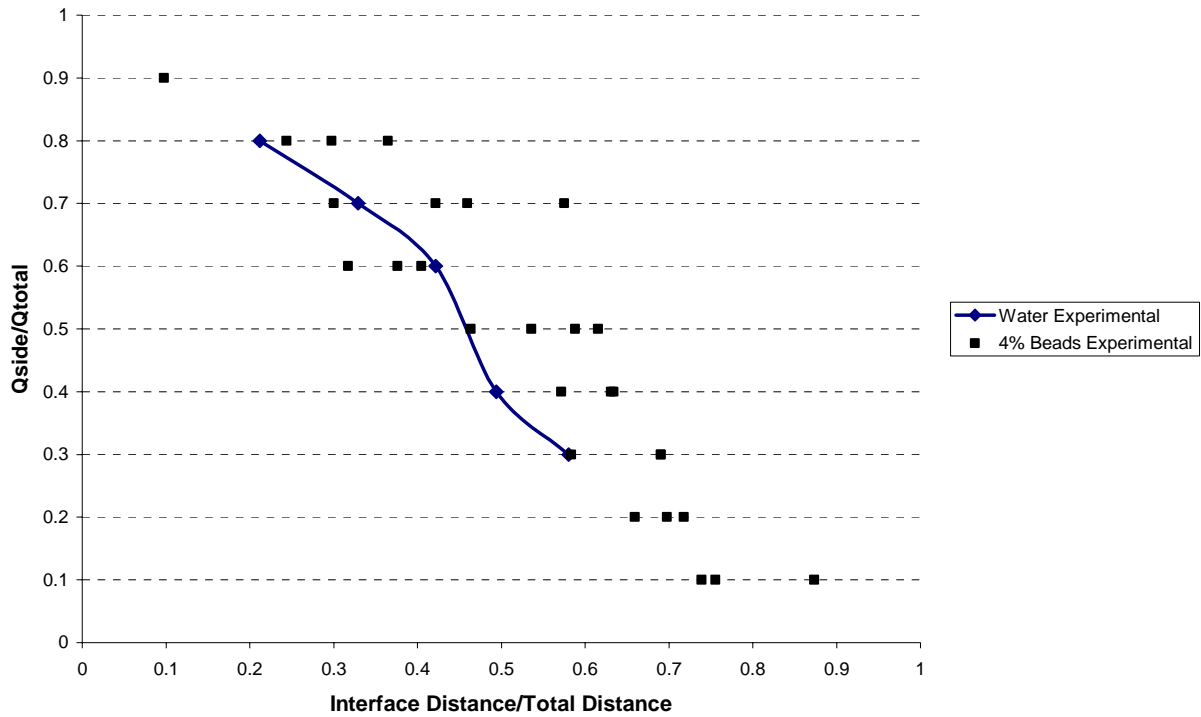
With 4% beaded solution entering both the main and daughter channel, steady state convergence was achieved for a range of relative volumetric flow rates, ranging from 1:9 uL/min to 9:1 uL/min. The data sets collected are in Table 3. These tests were compared to other steady state convergence tests.

Table 3: Experimental Data for 4% beaded solution Trial

Horizontal Channel Flow (4% solution)	Vertical Channel Flow (4% solution)	Ratio of Interface:Total Channel Size	Qside/Qtotal	Reynolds Number
1	9	0.10	0.9	0.90
2	8	0.24	0.8	0.90
3	7	0.30	0.7	0.90
4	6	0.32	0.6	0.90
5	5	0.61	0.5	0.90
6	4	0.63	0.4	0.90

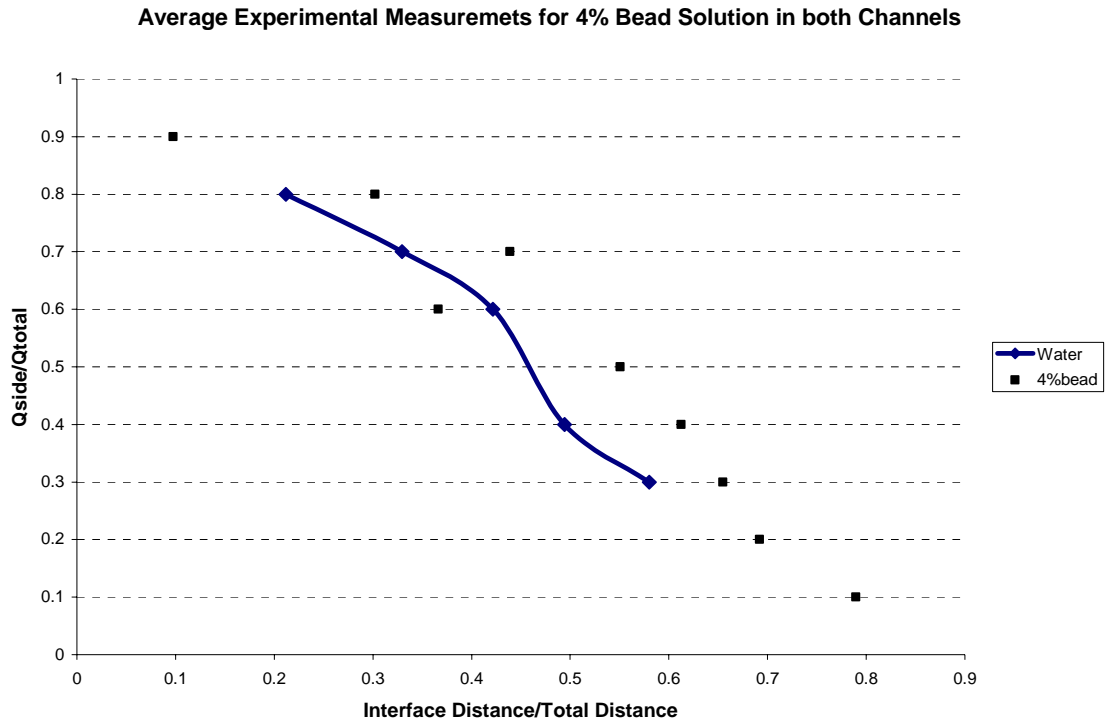
7	3	0.69	0.3	0.90
8	2	0.72	0.2	0.90
9	1	0.75	0.1	0.90
3	7	0.57	0.7	0.90
5	5	0.46	0.5	0.90
2	8	0.36	0.8	0.90
3	7	0.46	0.7	0.90
4	6	0.38	0.6	0.90
5	5	0.59	0.5	0.90
6	4	0.63	0.4	0.90
7	3	0.69	0.3	0.90
8	2	0.70	0.2	0.90
9	1	0.74	0.1	0.90
2	8	0.30	0.8	0.90
3	7	0.42	0.7	0.90
4	6	0.40	0.6	0.90
5	5	0.53	0.5	0.90
6	4	0.57	0.4	0.90
7	3	0.58	0.3	0.90
8	2	0.66	0.2	0.90
9	1	0.87	0.1	0.90

All Experimental Measurements for 4% Bead Solution in both Channels

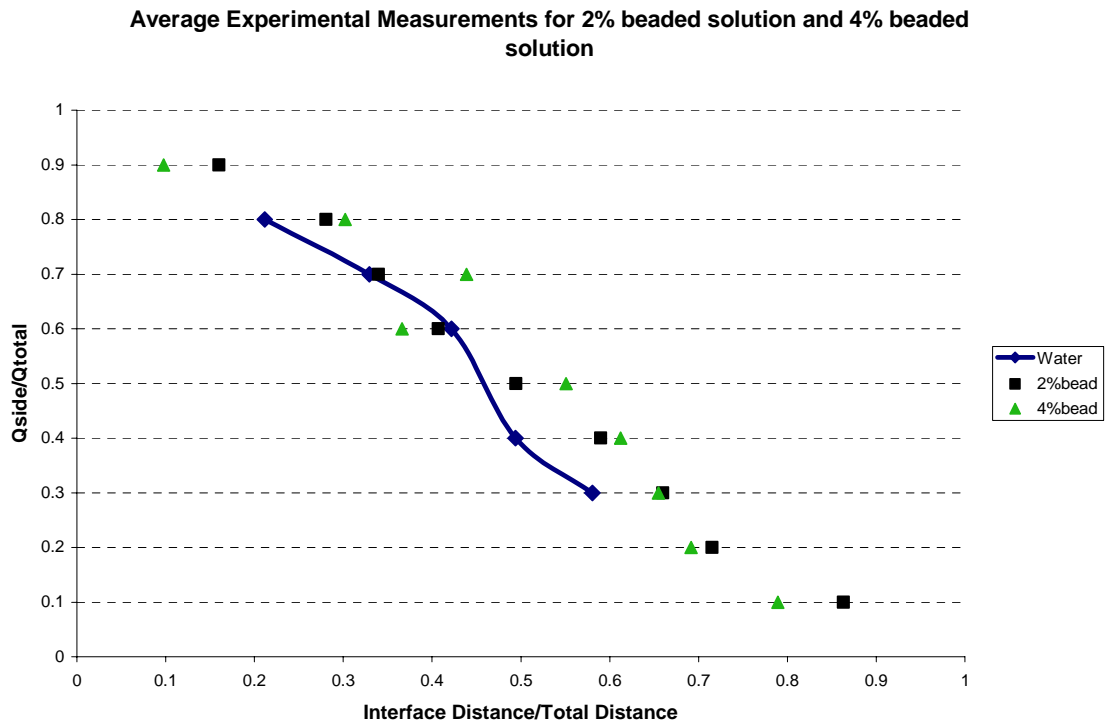


Graph 4: Effect of relative flow on surface location for all 4% beaded solution experimental data

More scatter is evident with the 4% beaded solution experimental data. The 4% beaded solution had a lot more variation from the typical water-water flows with a Reynold's number of 1. Investigation under the 2D microscope with the fluorescence turned off revealed little plagues of beads would form along the corners of the junction between the main and the daughter channel, piling up to a maximum point and then suddenly dispersing, affecting the flow and the position of the interface. Thus, as the concentration of beads increased, they began to have a larger effect on the interface. A look at Graph 5, a comparison of the average 4% bead experimental data to the water experimental data reveals that there is a some more deviation from the water experimental data, but not enough deviation to claim the 4% beaded solution had an extreme effect on the interface. Graph 6 shows a comparison of the water experimental data, the 2% bead experimental data and the 4% bead experimental data. It is evident from this graph that up to 4% concentrated bead solution, the two phase effects are not large enough to greatly effect the solution flow.



Graph 5: Effect of relative flow on surface location for average 4% beaded solution experimental data



Graph 6: Effect of relative flow on surface location for average 2% bead solution and 4% bead solution experimental data

3. 3D Experimental Results

Preliminary experimentation has been completed with the confocal microscope, however the data was in such a state, that further investigation could not be completed.

The data and initial attempts at analysis can be viewed in the Appendix

VI. Conclusions and Future Work

This project examined the microchannel convergence of water, a 2% beaded solution and a 4% beaded solution simulating blood. The comparative flow rates were manipulated within a range which maintained a total Reynold's number of approximately 1. Investigation and characterization of the resultant boundary surfaces was then conducted. We successfully completed 2D experimental work with water, 2% beaded solution and 4% beaded solution. In addition, some preliminary 3D experimental work was started but not completed.

The 2% beaded solution was fairly typical in comparison to water-water flows (Reynold's number = 1). The beads did not seem to have a noticeable effect on the interface. The 4% beaded solution had a lot more variation from the typical water-water flows with a Reynold's number of 1. Little plaques of beads would form along the corners of the junctions, piling up to a maximum point and then suddenly dispersing, affecting the flow and the position of the interface and resulting in a lack of repeatability in the interface location. Thus, as the concentration of beads increased, they began to have a larger effect on the interface. Perhaps in whole blood, red blood cells have the same effect, plaques of red blood cells forming along junctions, affecting the flow. It would be interesting to see if this trend continues as the bead solution is concentrated even more.

Beyond increasing the bead concentration, perhaps it would be interesting to compare how using different solutions, like rigid discs and liquid droplet emulsions, rather than rigid spheres affects the flow. It would also be interesting to see the effect

allowing the rigid spheres to clump together would have on the solution flow. This would complicate our experimental model even further, perhaps ultimately leading to a better understanding of convergent blood flow. Also, the angle of convergence should be varied. A more realistic convergence angle could increase the accuracy of the model.

VII. References

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3. http://whyfiles.org/090doping_sport/images/redblood.jpg.
4. Fung, Y.C., *Biomechanics: Mechanical Properties of Living Tissues*. 2 ed. 1993, New York, New York: Springer.
5. Goldsmith, H.L. *The microrheology of human erythrocyte suspensions*. in *Theoretical and applied mechanics; proceedings of the Thirteenth International Congress of Theoretical and Applied Mechanics*. 1973. Moscow University.
6. Goldsmith, H.L. "The microrheology of human erythrocyte suspensions,". in *Theoretical and applied mechanics Thirteenth International Congress of Theoretical and Applied Mechanics*. 1973. Moscow University.
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VII. Appendix

1. Beaded Solution

In order to make a 20mL 2% beaded solution with a specific gravity of 1.05g/cc (the specific gravity of the 10% by weight beaded solution purchased by Bang's Lab(URL: www.bangslabs.com phone: 1- 800-387-0672), 4.2 g of the 10% by weight beaded solution was added to 16.8 grams of a water/glycerol mixture made to match the specific gravity of the 10% beaded solution. In order to match the specific gravity of the 10% beaded solution, the water/glycerol mixture was made with 20.6% glycerol (3.5 g glycerol and 13.3 g water).

To make a 7.5mL 4% beaded solution with a specific gravity of 1.05 g/cc, 3.15 g of the 10% by weight beaded solution was added to 4.725g of a water/glycerol mixture made to match the specific gravity of the 10% beaded solution. In order to match the specific gravity of the 10% beaded solution, the water/glycerol mixture was made with 20.6% glycerol (1.0 g glycerol and 3.8 g water).

2. Fluorescent Dye

To prepare the solution, 2.5 mg of FITC were weighed out and placed in an Erlenmeyer flask. 125mL of a 20.6% glycerol/water mixture is added to the flask. A stirring rod is added to the flask and the flask is placed on a stirring plate turned on medium speed for 3 minutes.

3. Delivery System

A. Syringes

1 mL Tuberculin Syringes from Becton-Dickinson (1-888-237-2762) were used

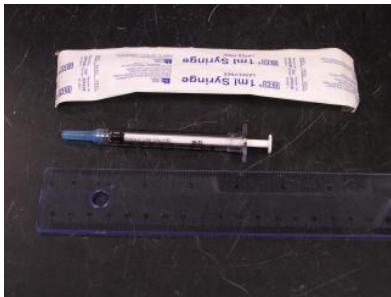


Figure 14: 1mL syringe

B. Tubing

The majority of the inventory consists of 1/16" outer diameter tubing. At the ends of the system, where the flow module connected with the delivery system, 1/8" outer diameter tubing was used. Tubing was PTFE scavenged from another lab.

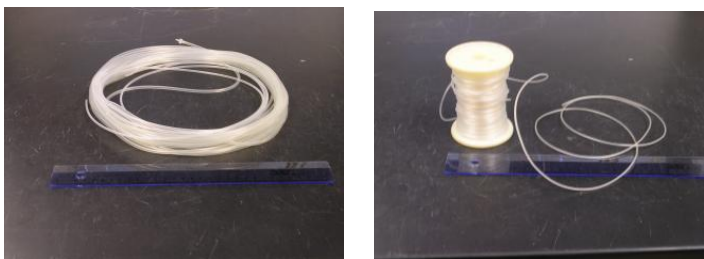


Figure 15: Thick (left) and Thin (right) tubing for the delivery system

C. Small Parts

To connect up the delivery system from syringe to flow module, 1/16" Female Luers (B-1005106) and Barbed (B-1004110) small parts were used, purchased from Small Parts (1-800-220-4242).



Figure 16: Small Parts: Barbed-to-male

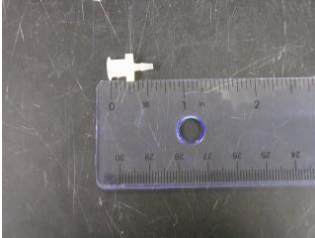


Figure 17: Small Parts: Female Luer

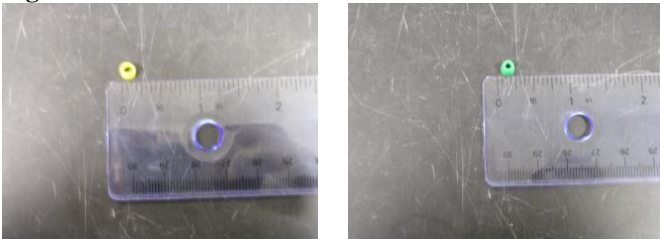


Figure 18: Small Parts: Fittings

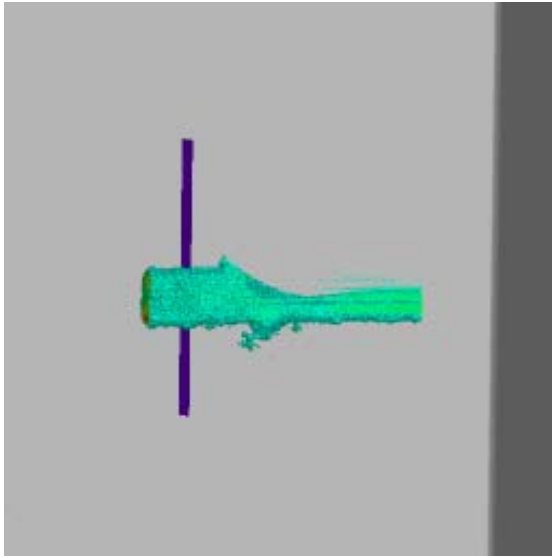
D. Sealant/Epoxies

In order to attach the small parts to the tubing to create the delivery system, Dow Corning sealant (purchased from McMaster-Carr, 1-723-329-3200, Item 75825A1) and Hardman Extra-Fast Setting Non-Sag epoxy 2 (purchased from Harcros Chemicals Inc., Item 04008) was used.

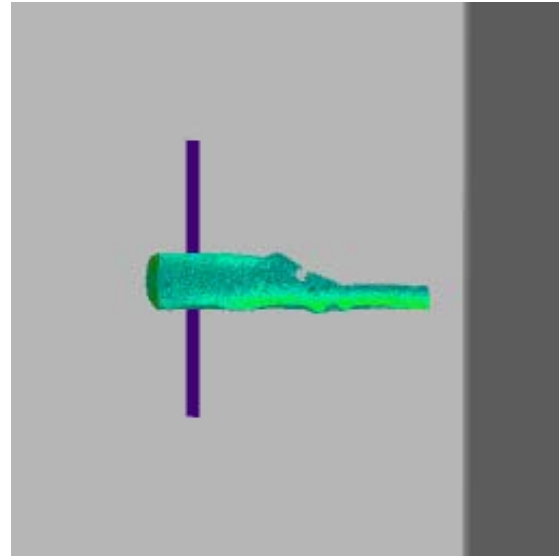


Figure 19: Dow Corning Sealant **Figure 20: Epoxy**

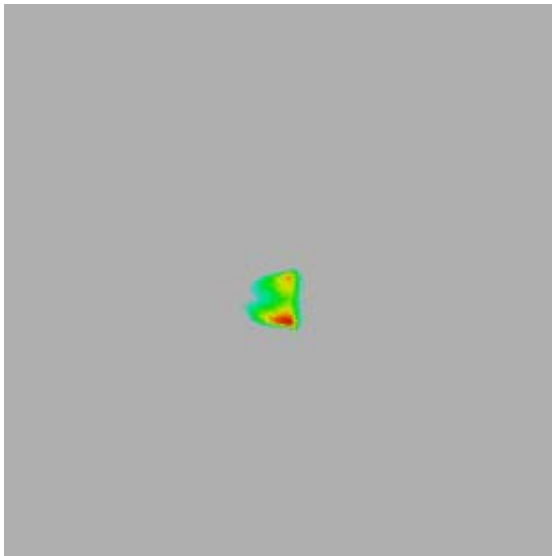
4. Confocal Data



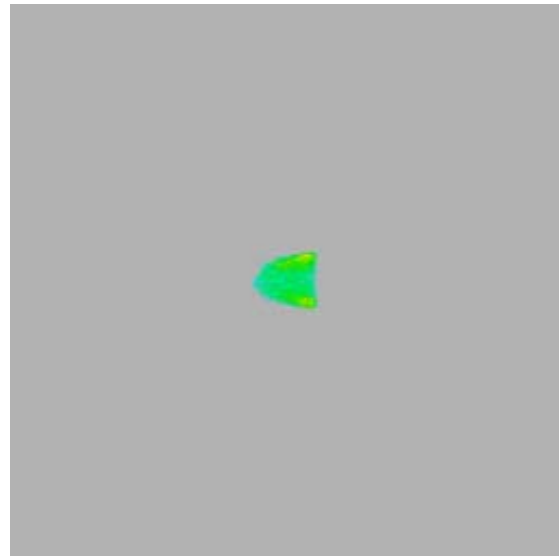
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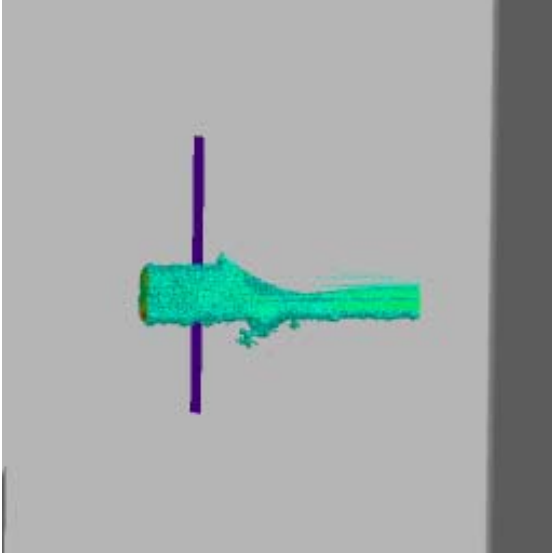
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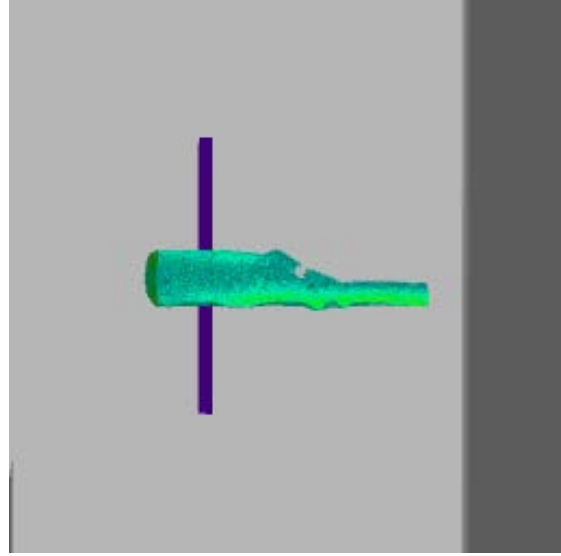
Bead slide



Water slice

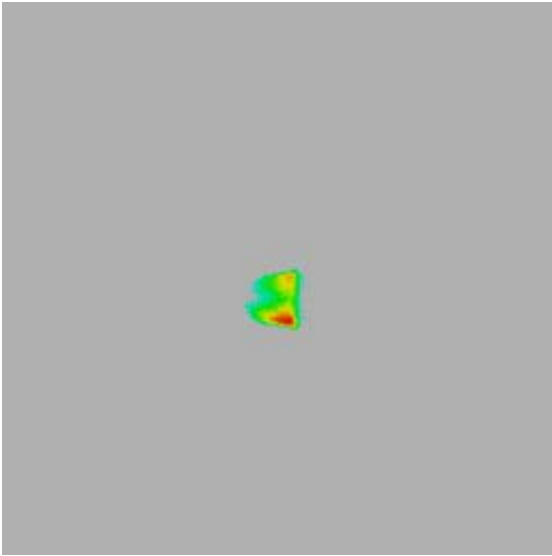


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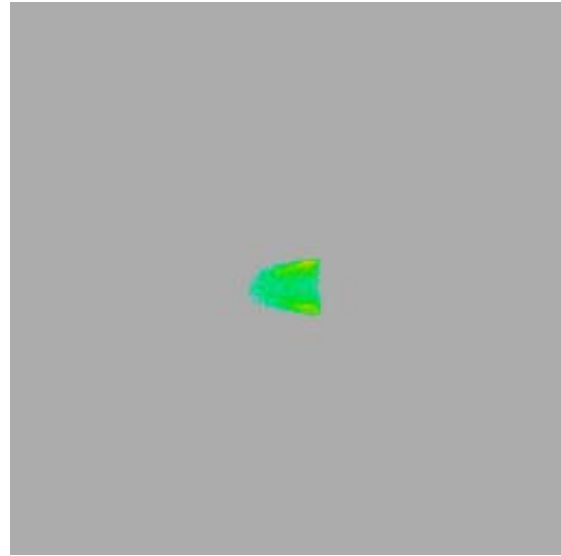


Water

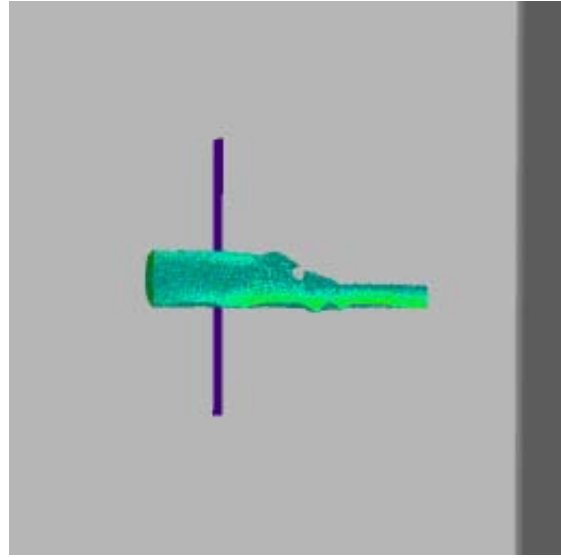
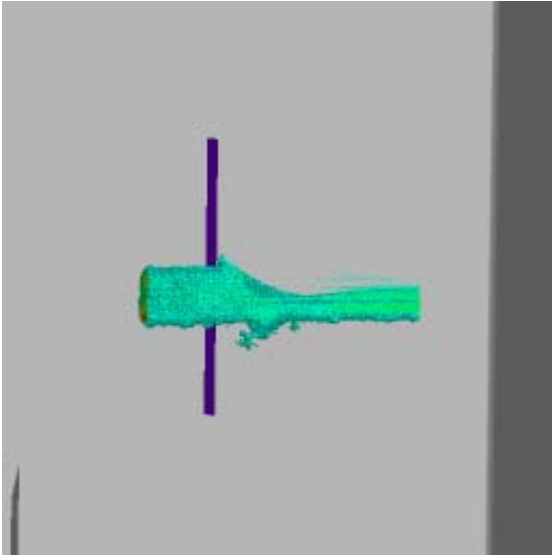
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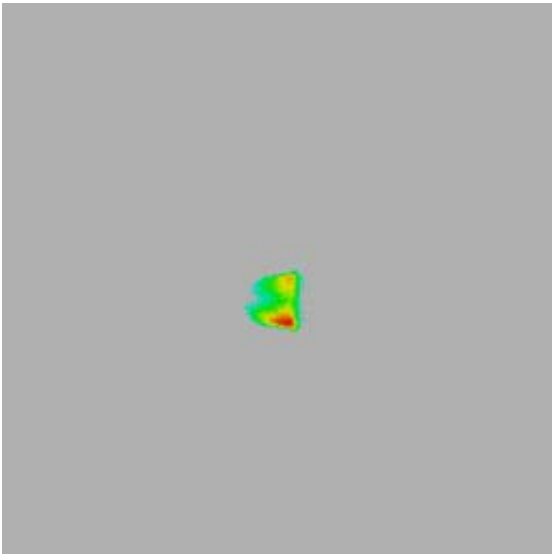
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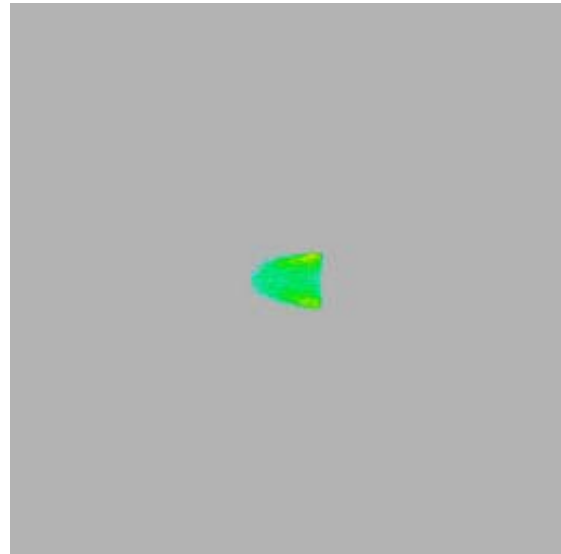
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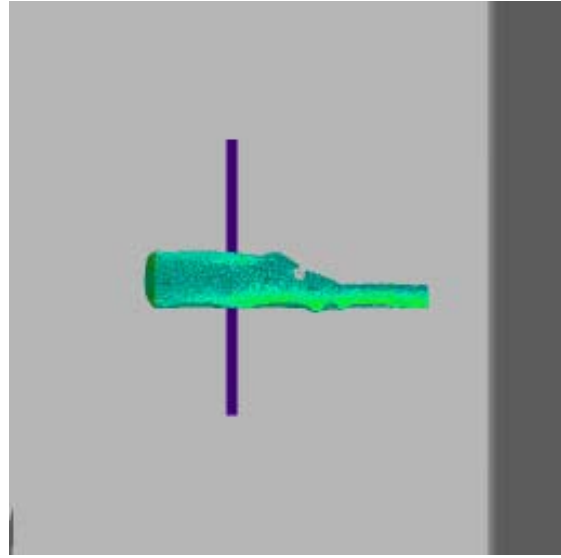
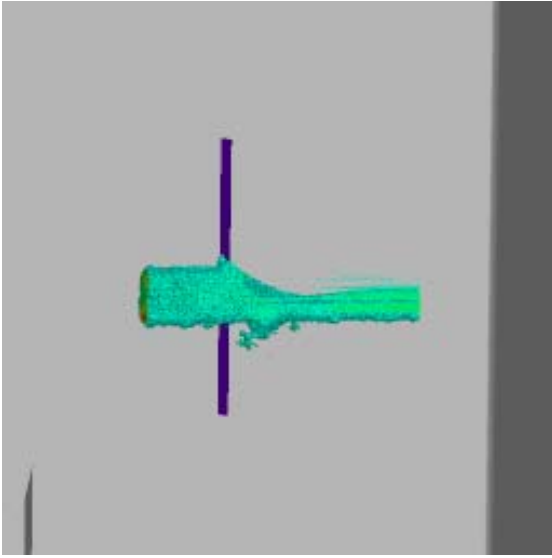
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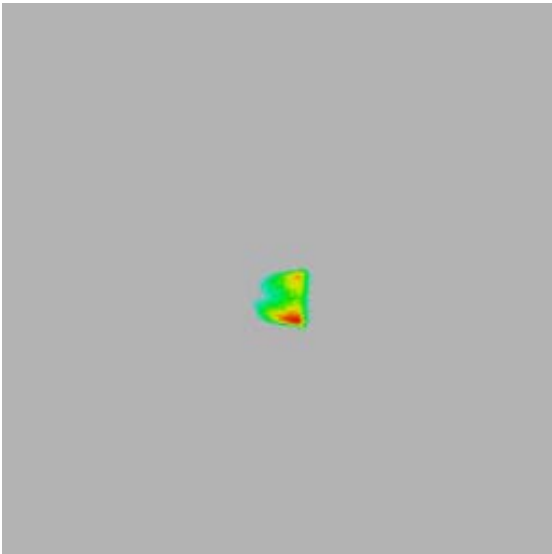
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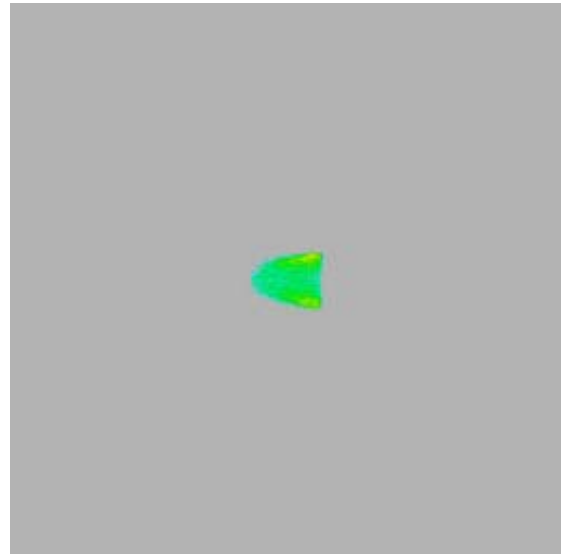
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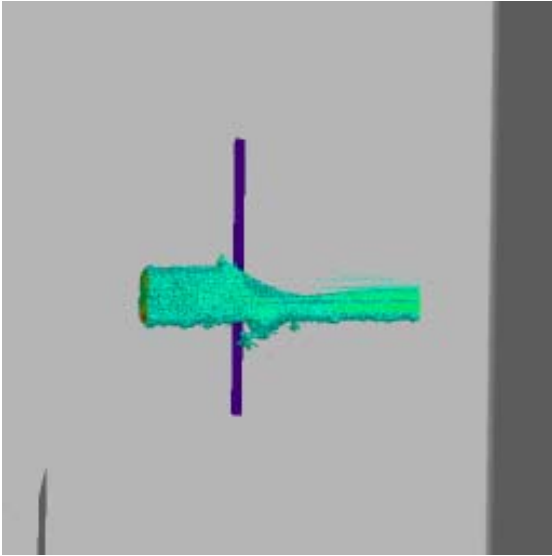
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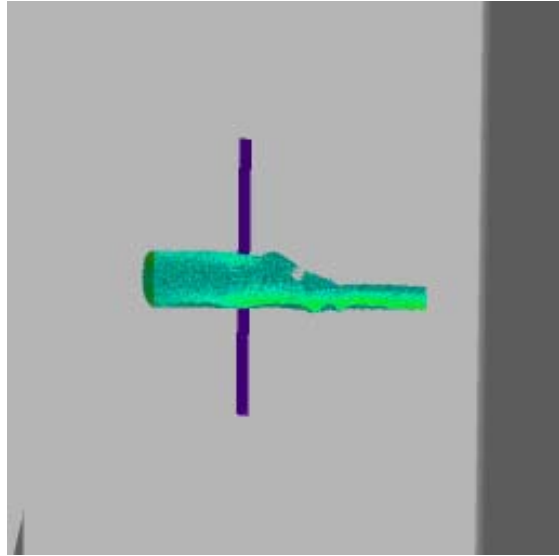
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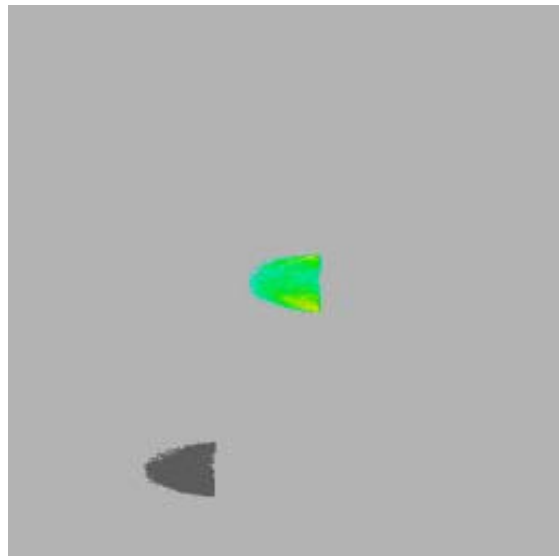
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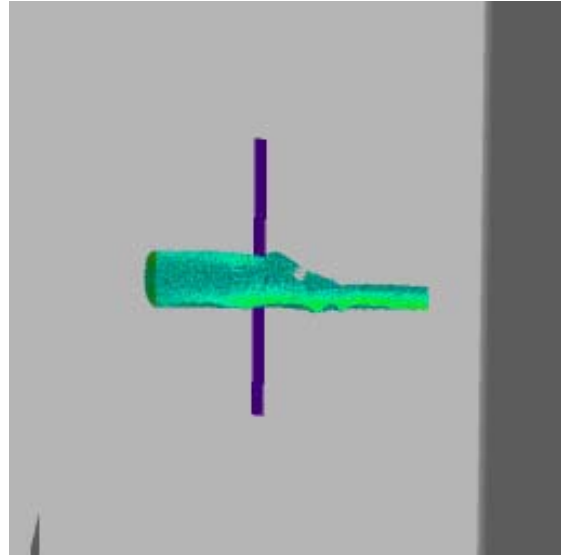
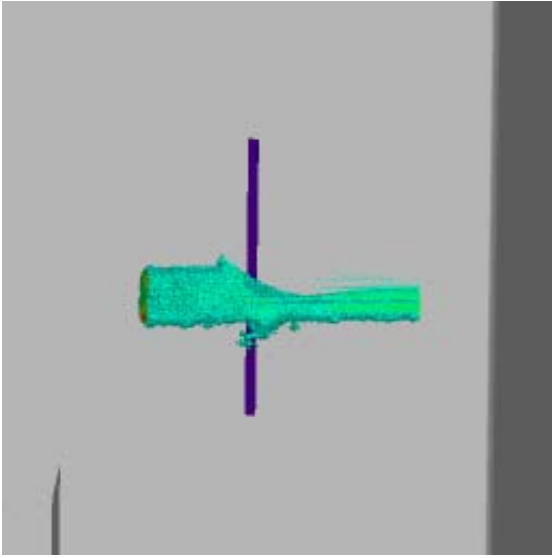
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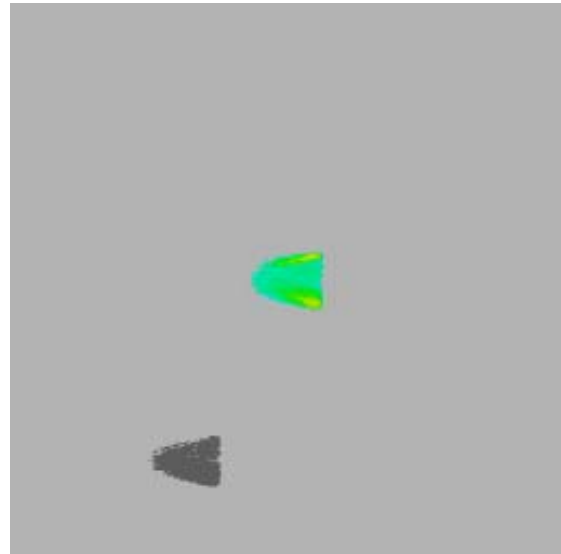
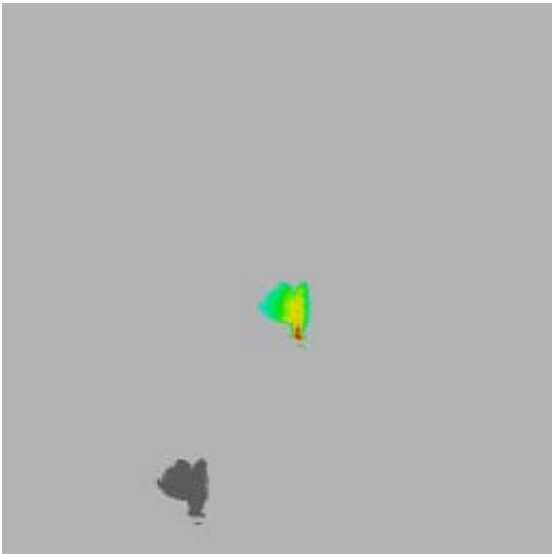
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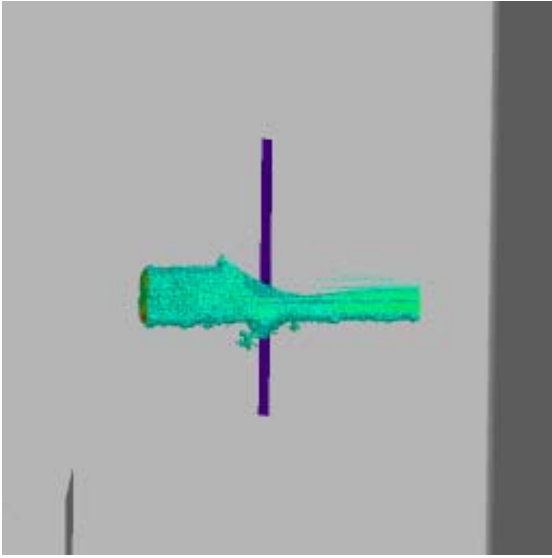


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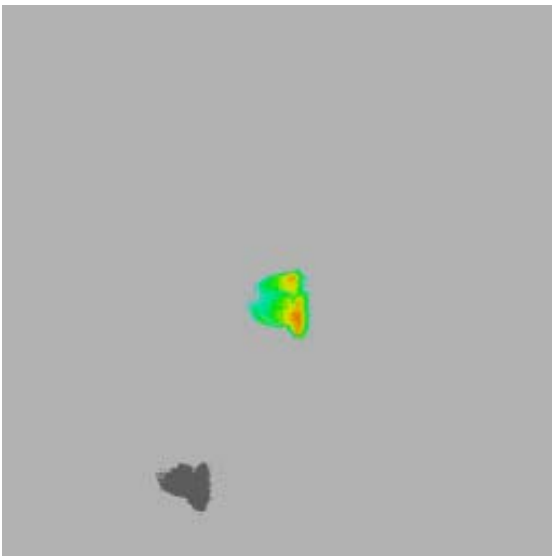
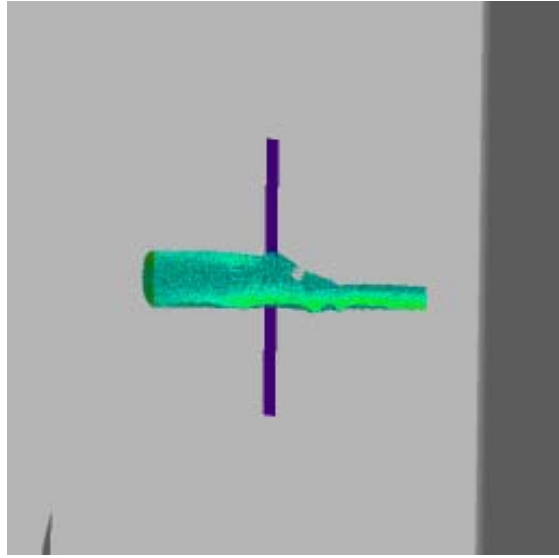


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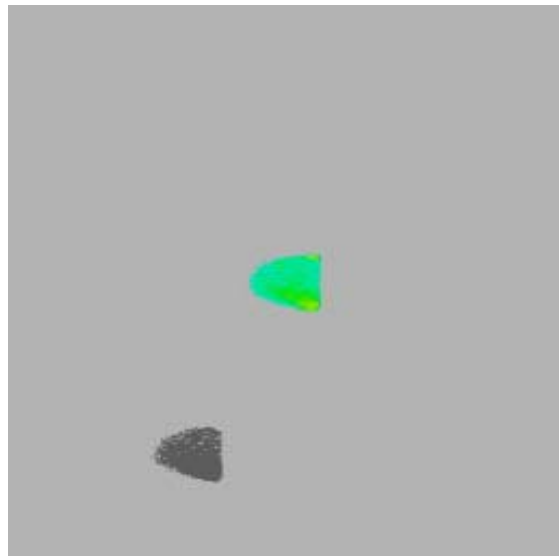
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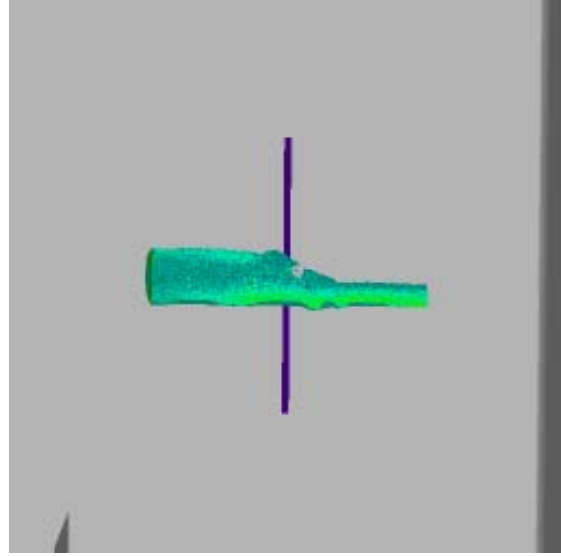
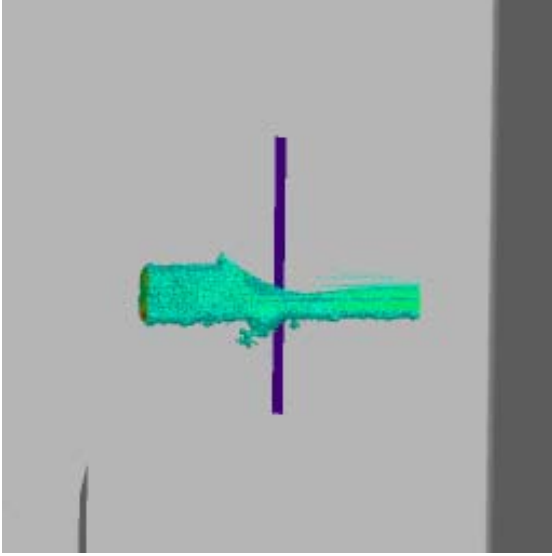
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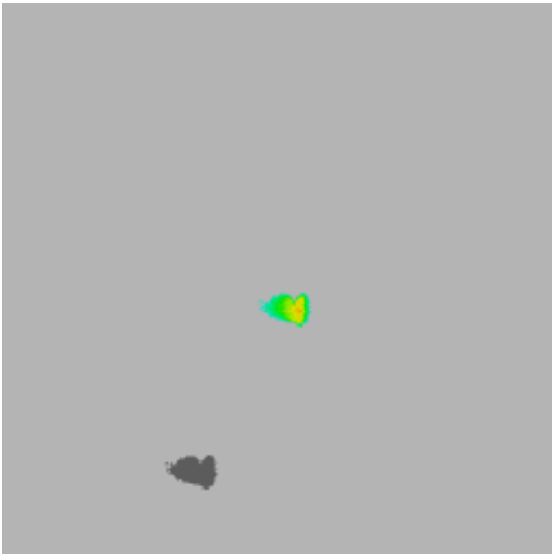
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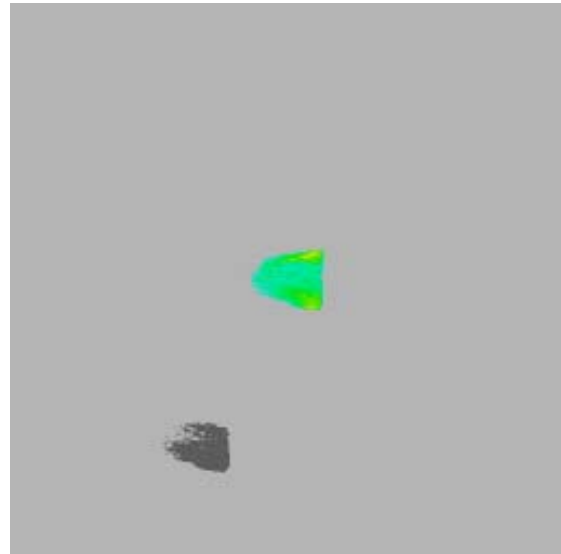
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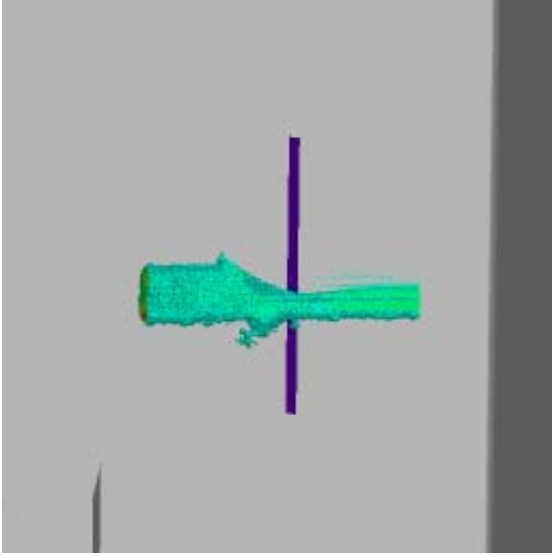
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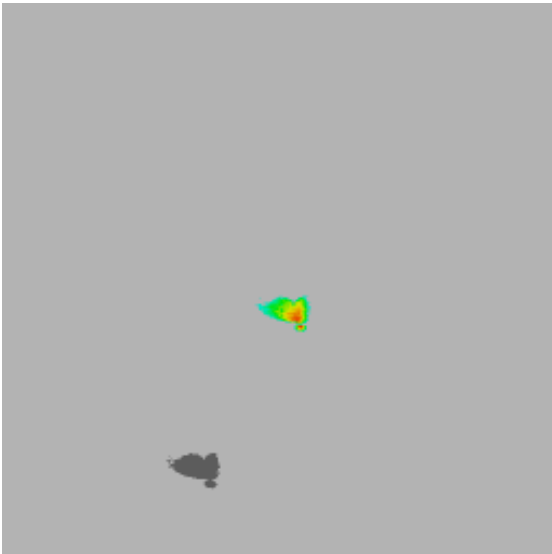
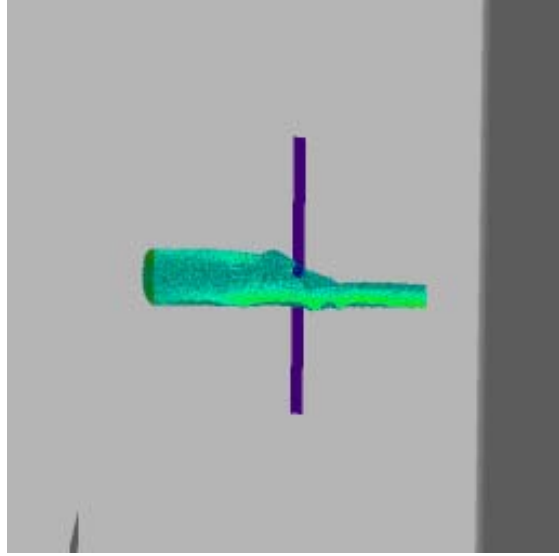
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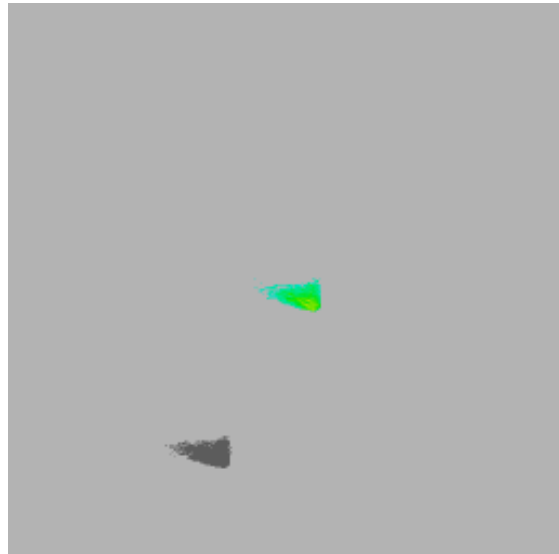
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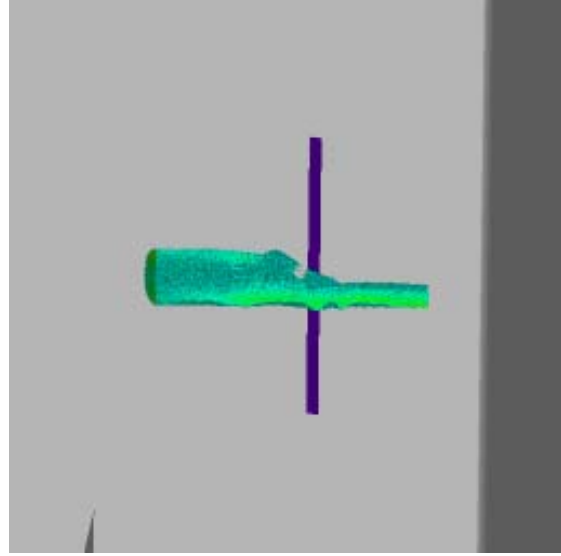
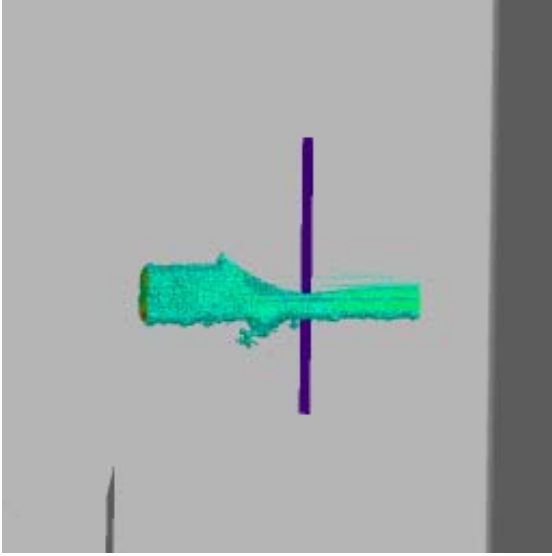
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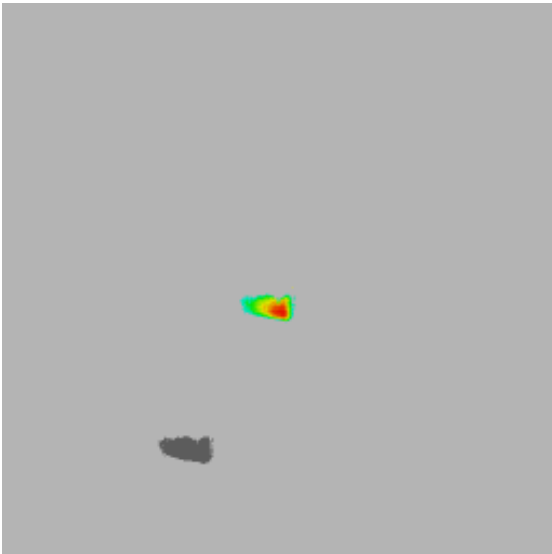
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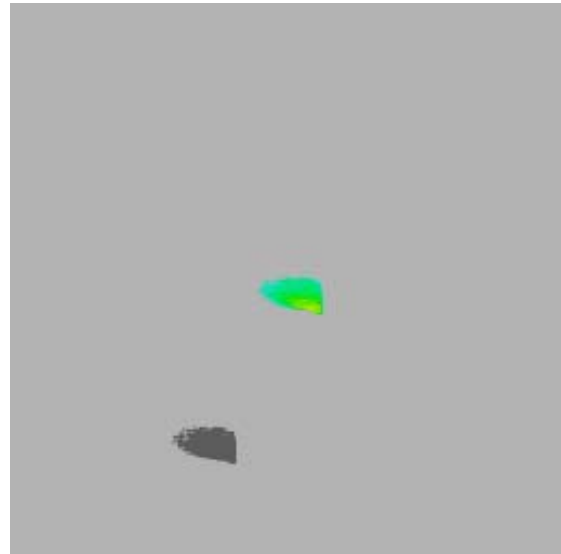
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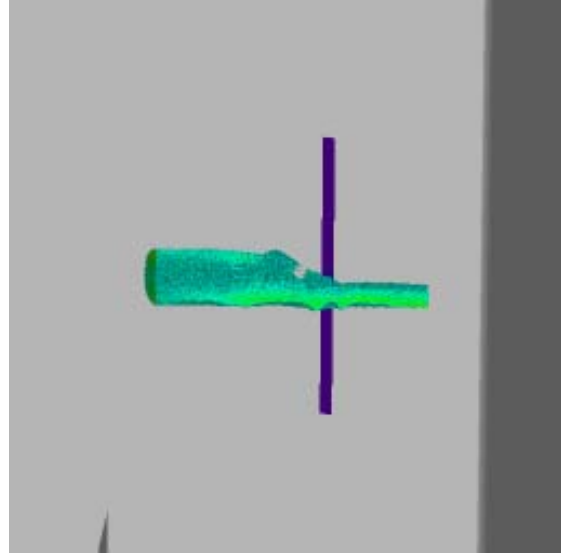
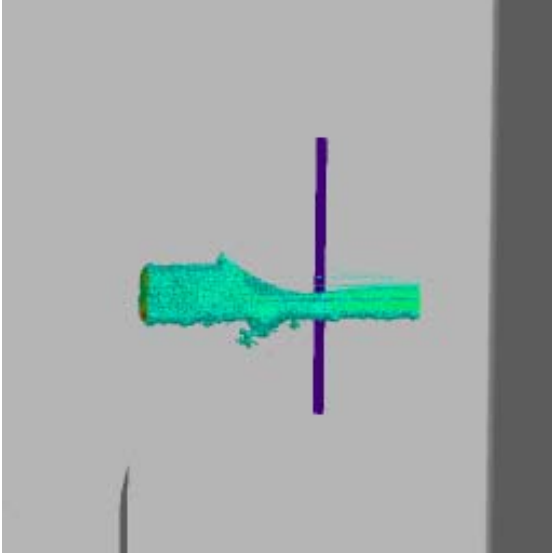
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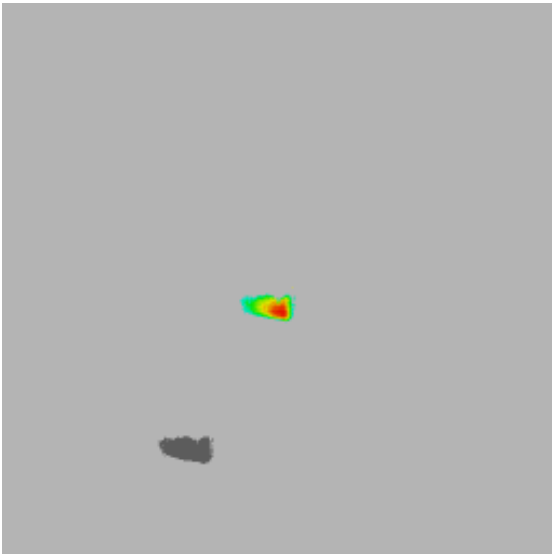
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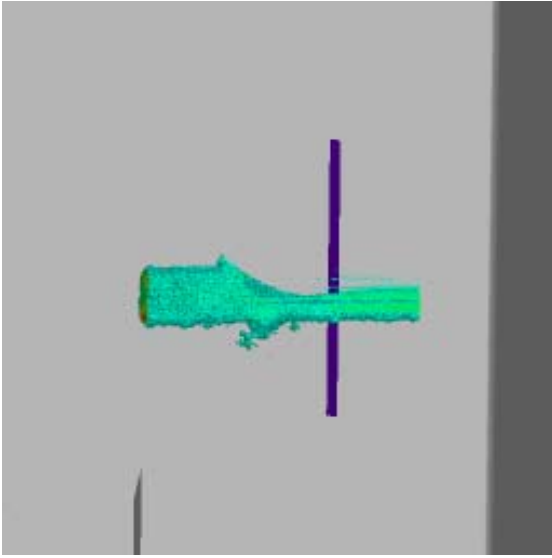
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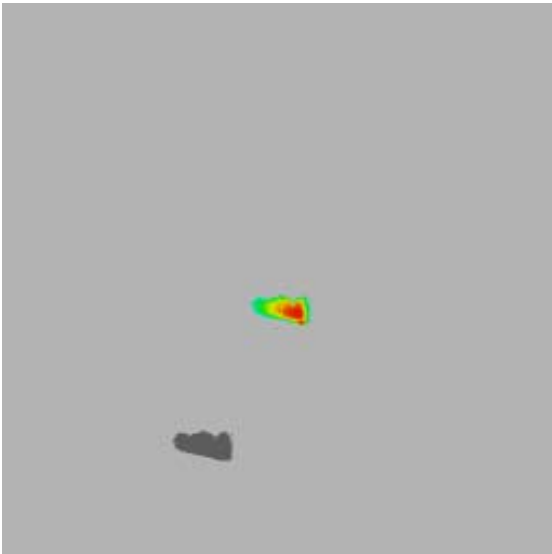
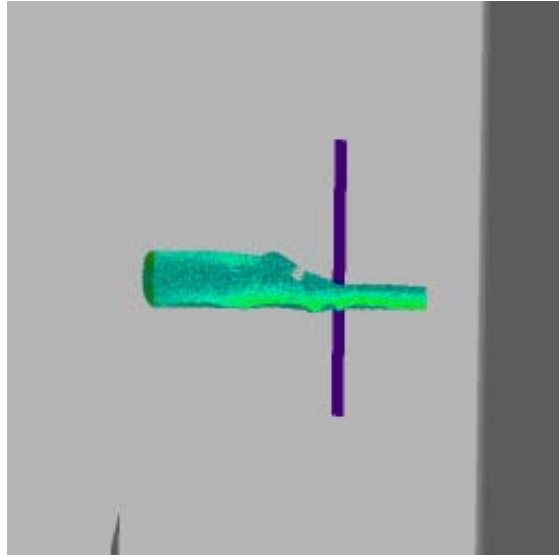
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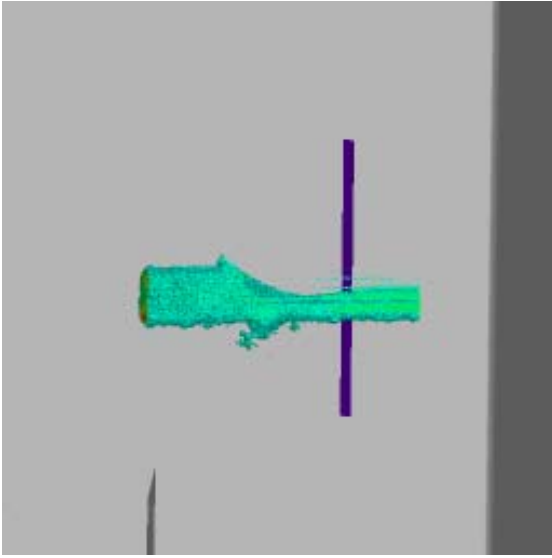
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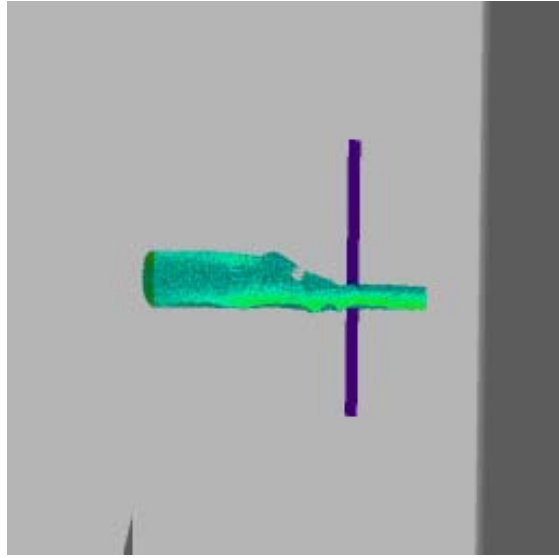
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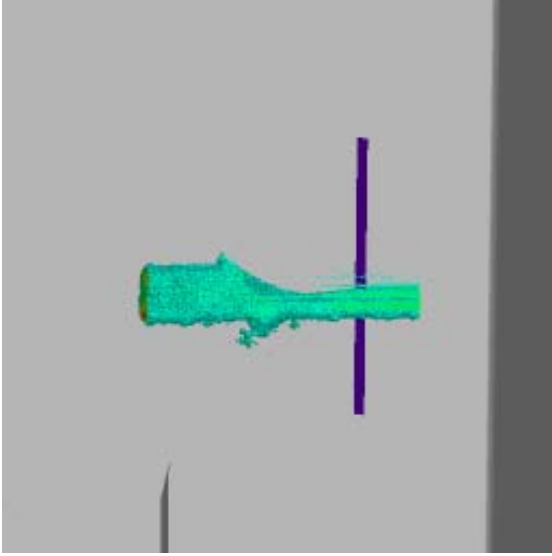
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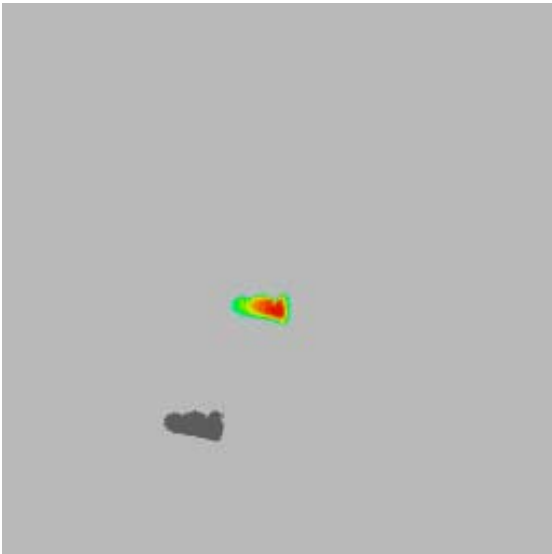
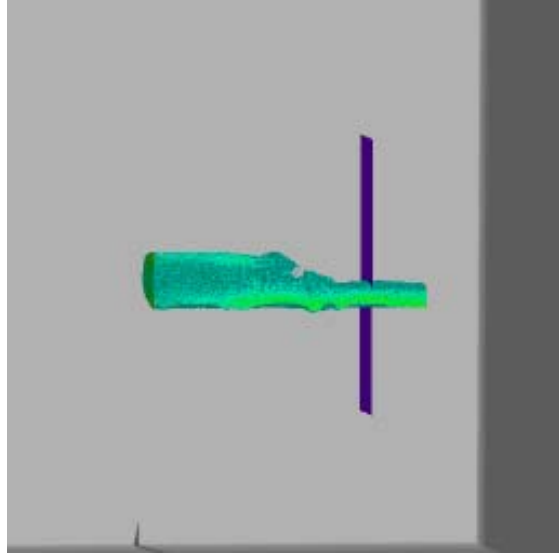
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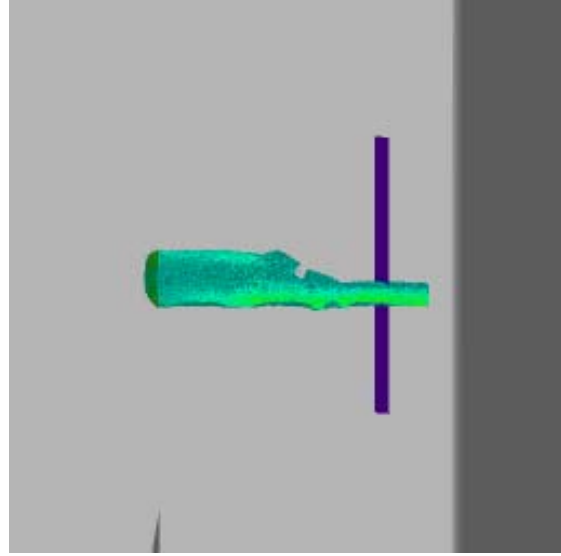
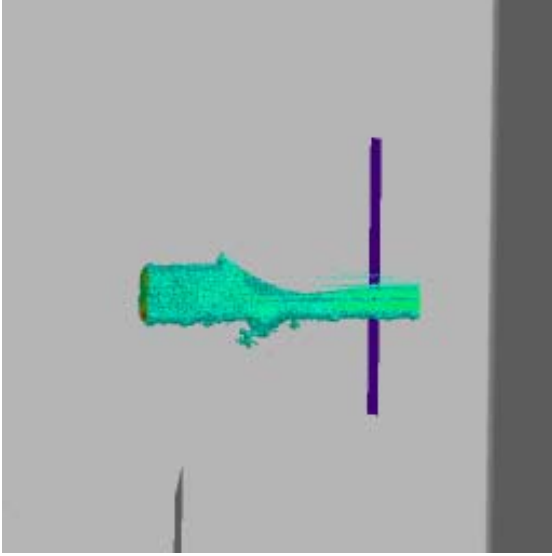
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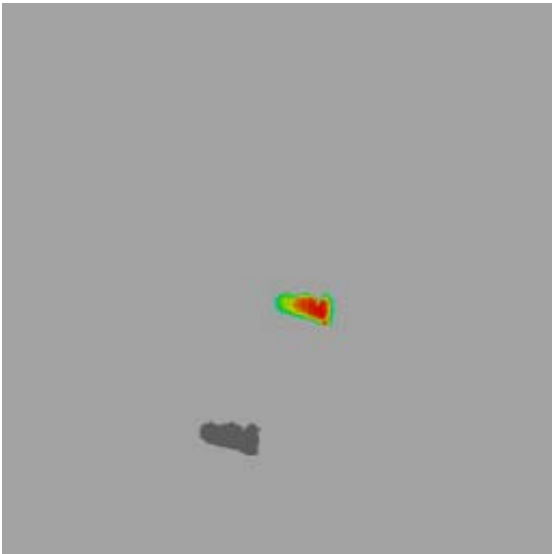
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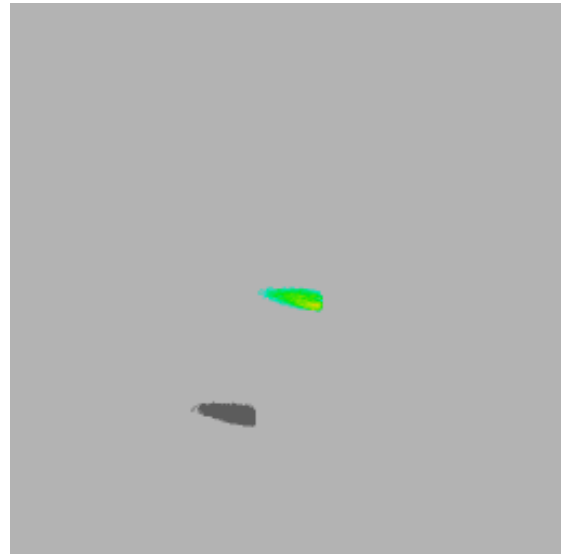
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Slice 15: Offset 0.7



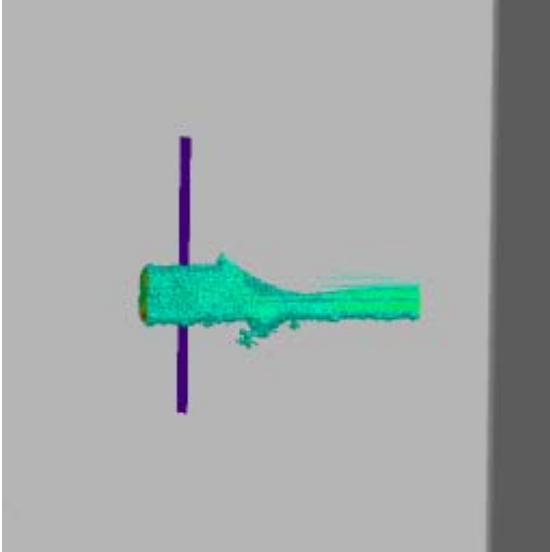
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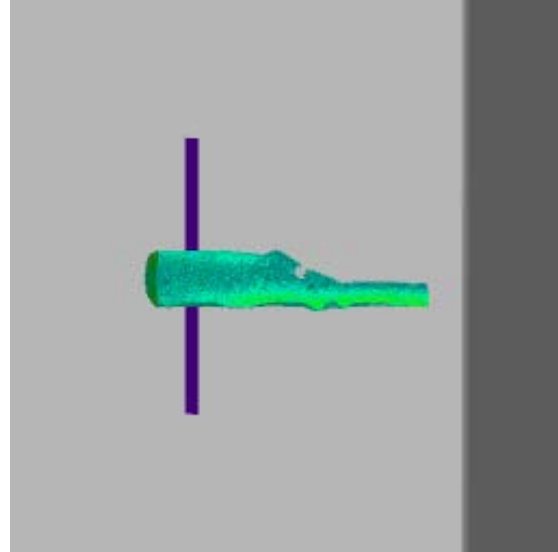
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5. Confocal Analysis

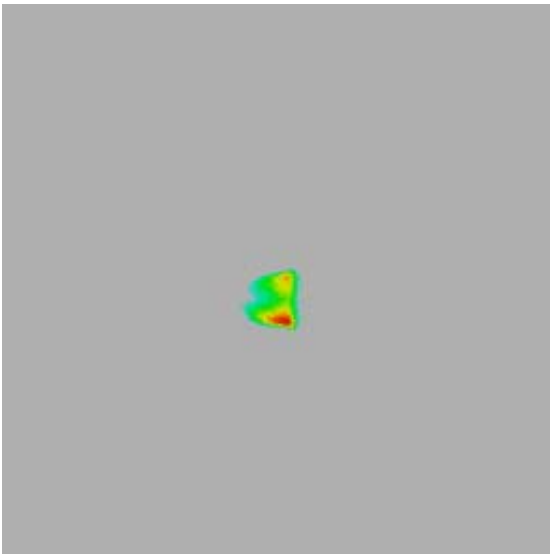
Before convergence



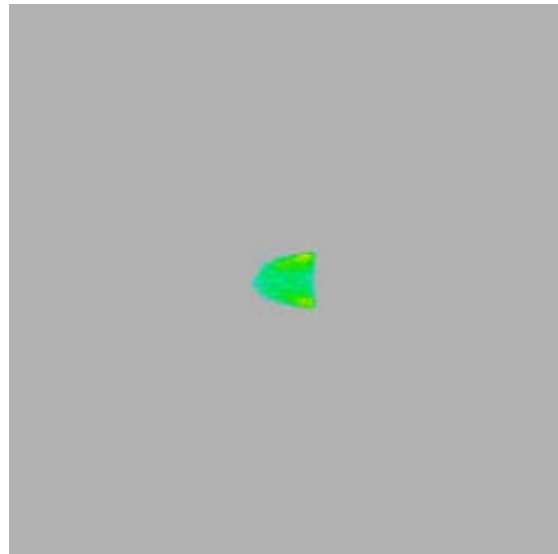
Bead
Slice 1: Offset= -0.7



Water
Slice 1: Offset -0.7

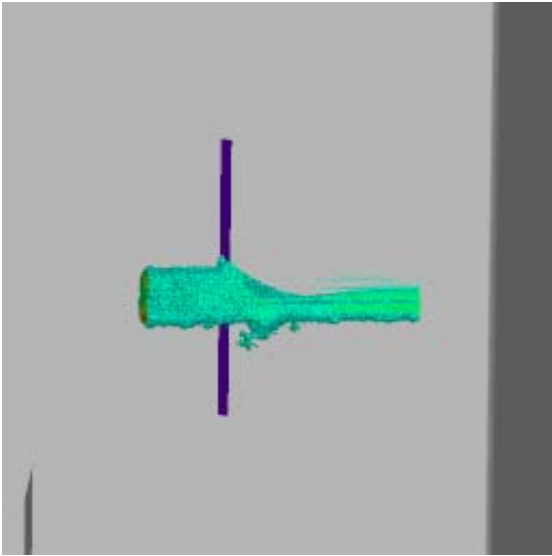


Bead slide

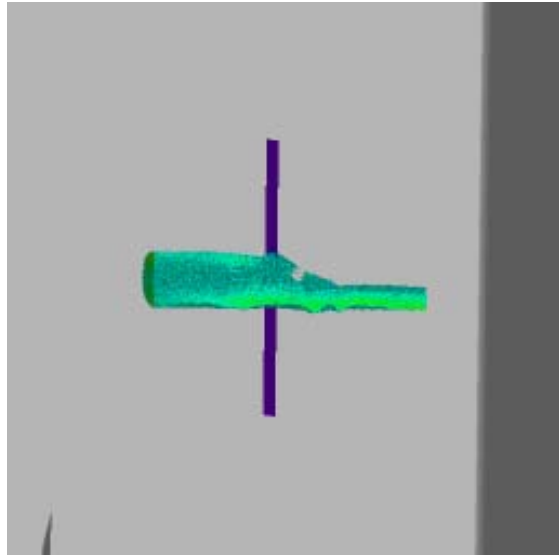


Water slice

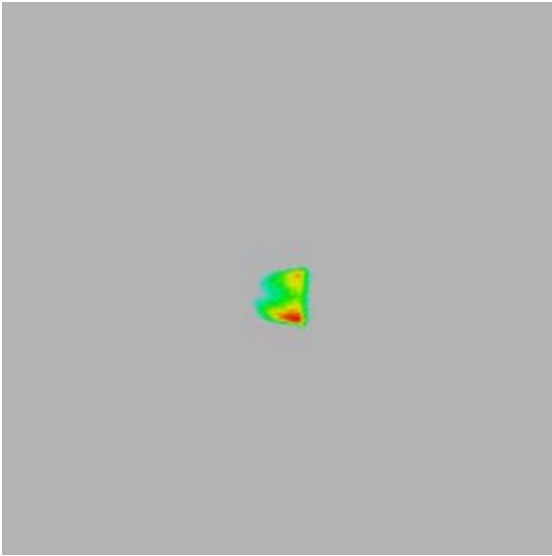
At edge of Convergence



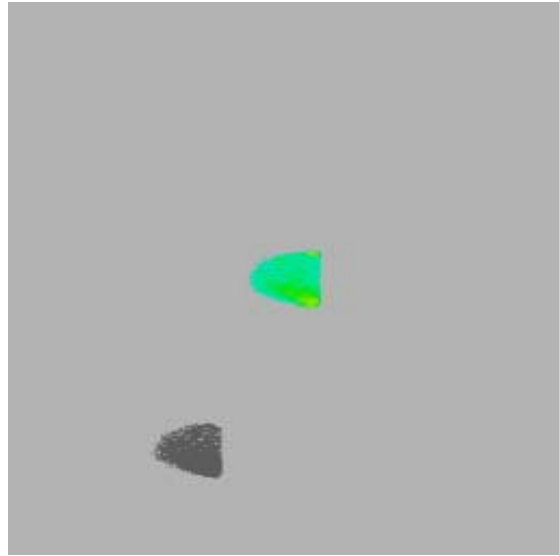
Bead Slice 4 Offset -0.4



Water Slice Offset -0.1

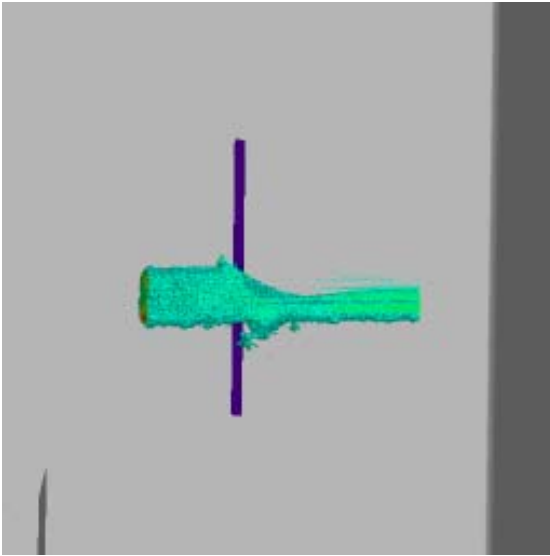


Bead

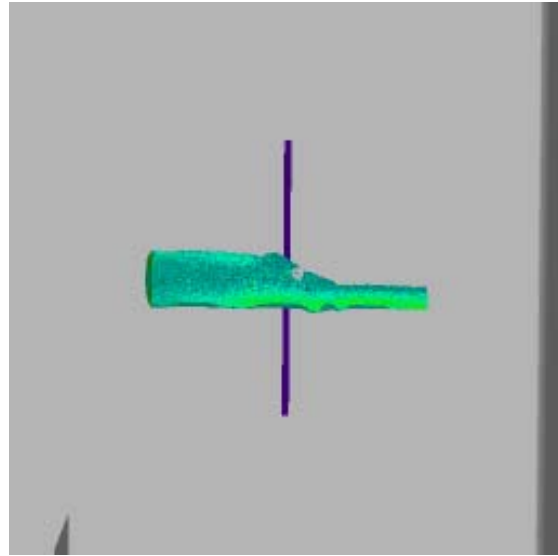


Water

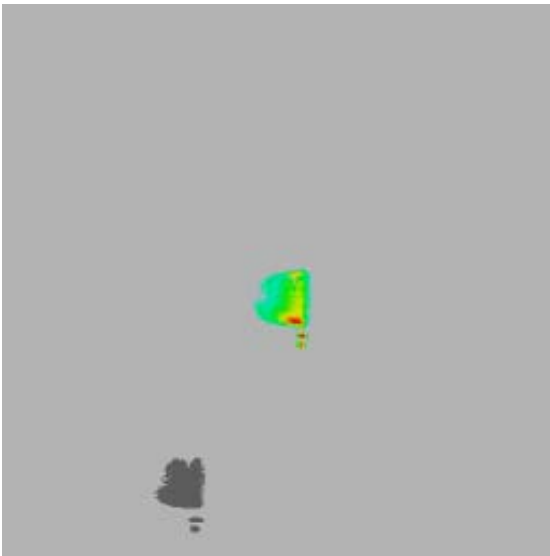
One Slice into convergence



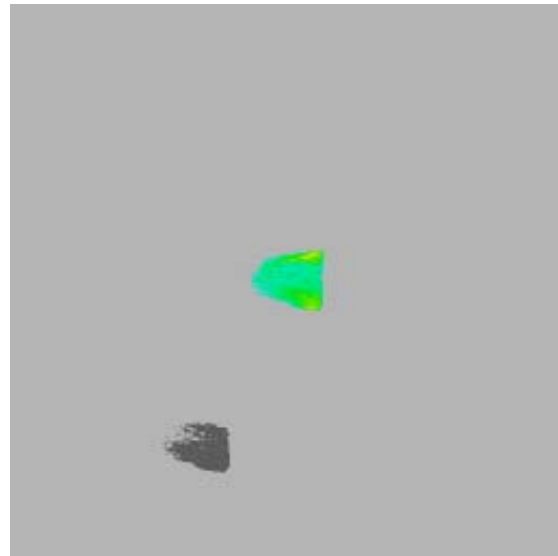
Bead Slide 5 Offset -0.3



Water Slice Offset 0.0

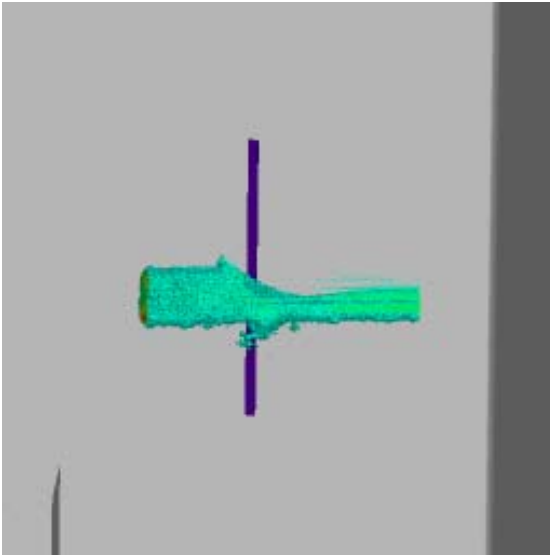


Bead

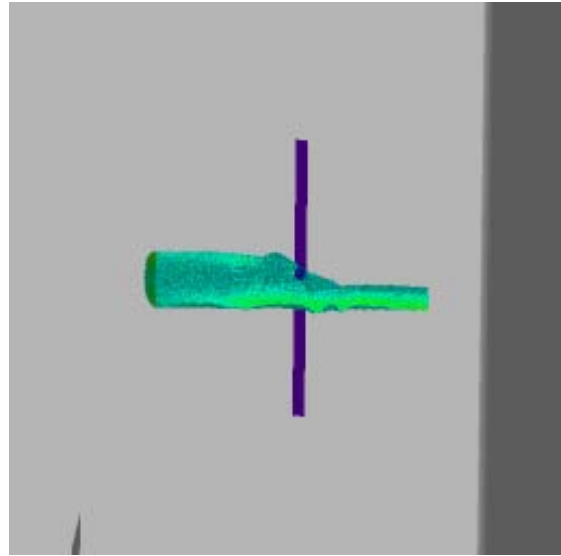


Water

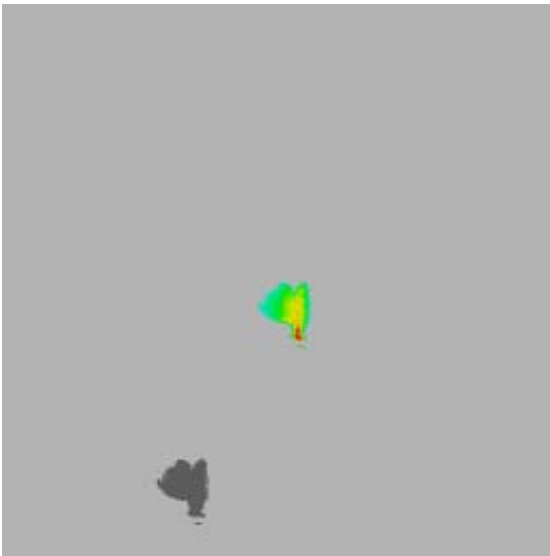
Two slices into convergence



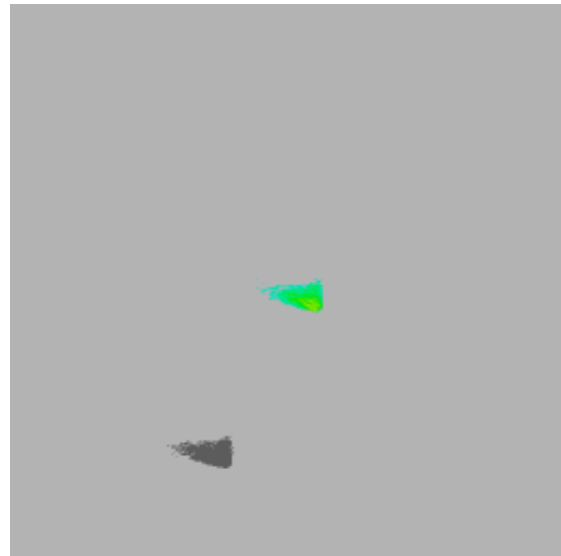
Bead Slice 6 Offset -0.2



Water Slide 9 Offset 0.1

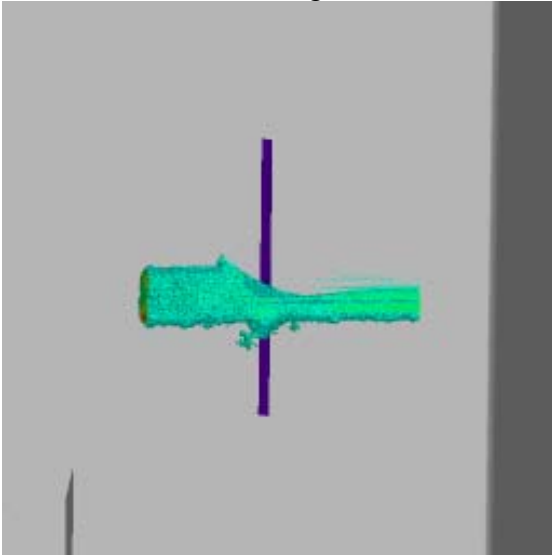


Bead

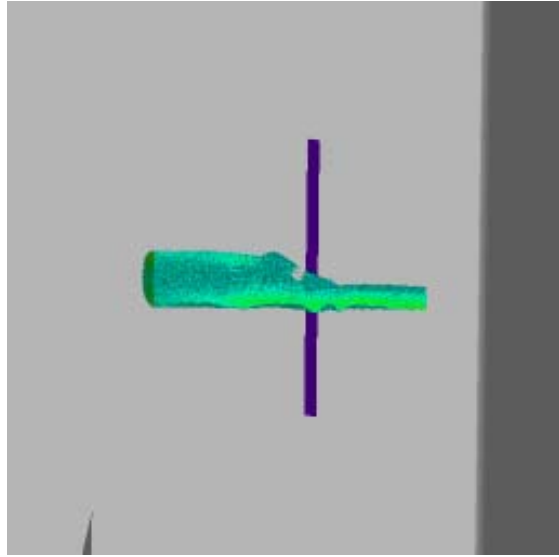


Water

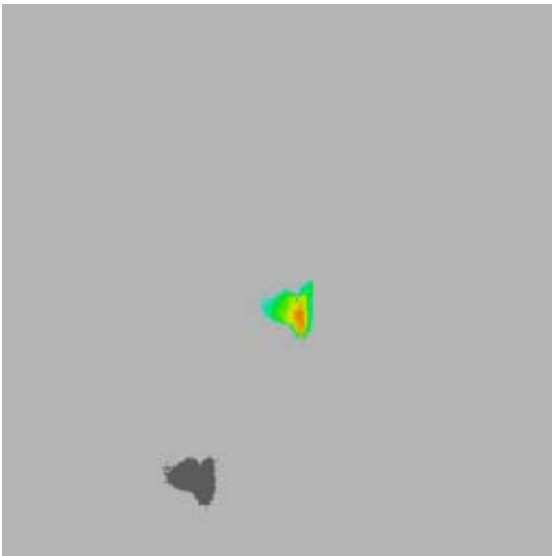
Three slices into convergence



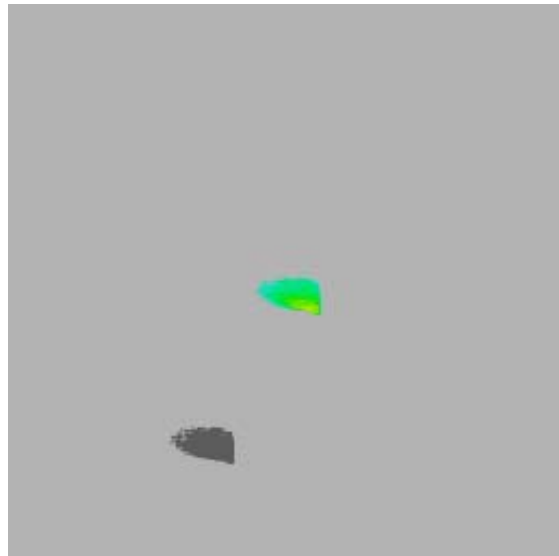
Bead Slice 7 Offset -0.1



Water Slice 10 Offset 0.2

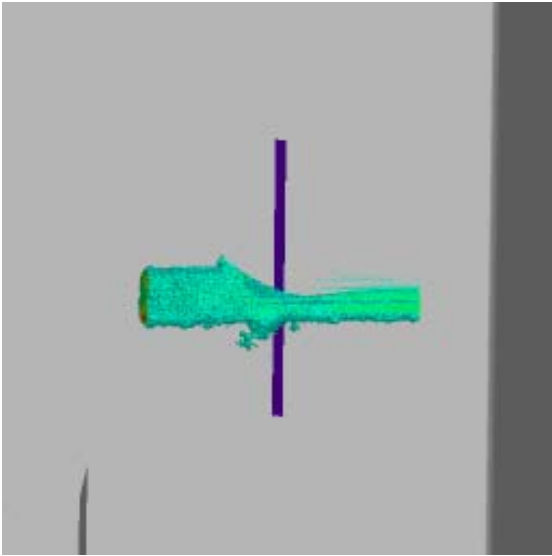


Bead

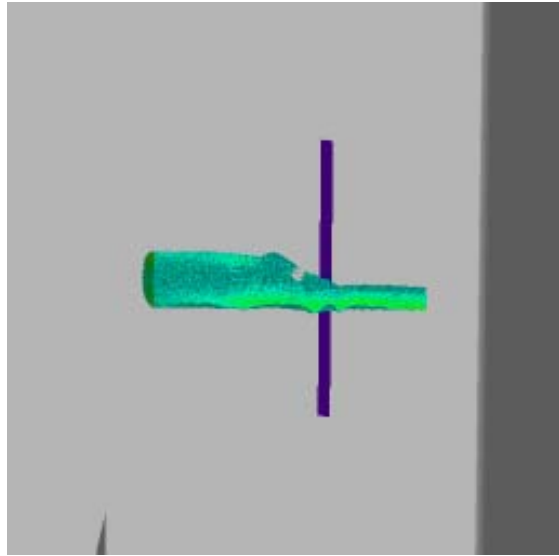


Water

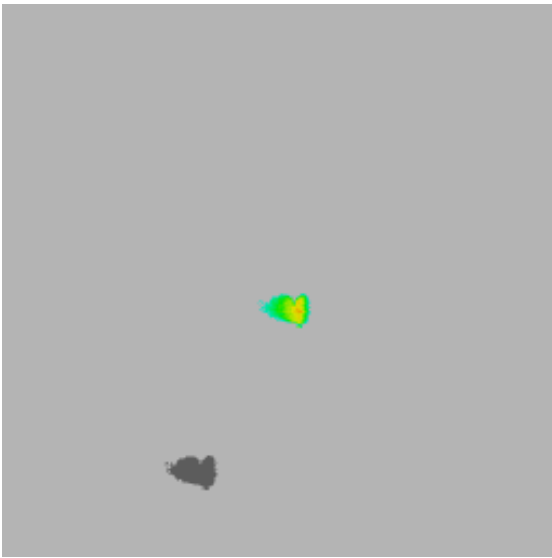
Four slices in



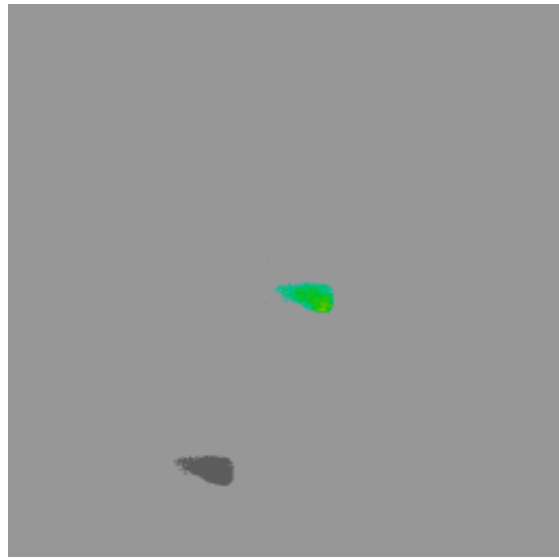
Bead Slide 8 Offset 0.0



Water Slide 11 Offset 0.3

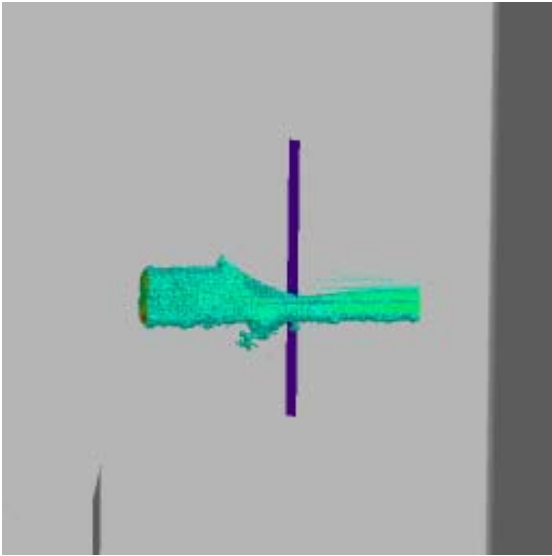


Bead

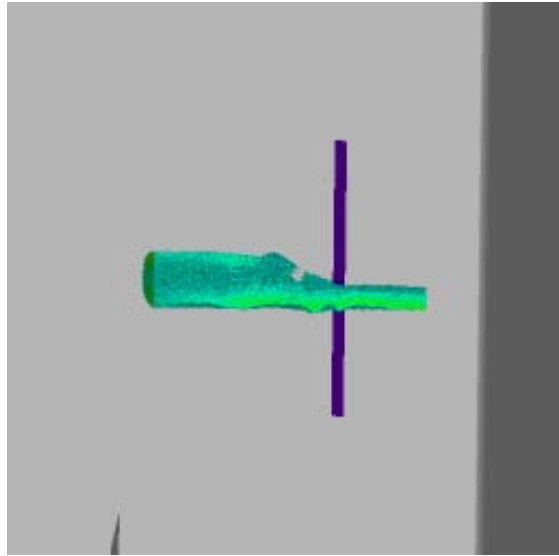


Water

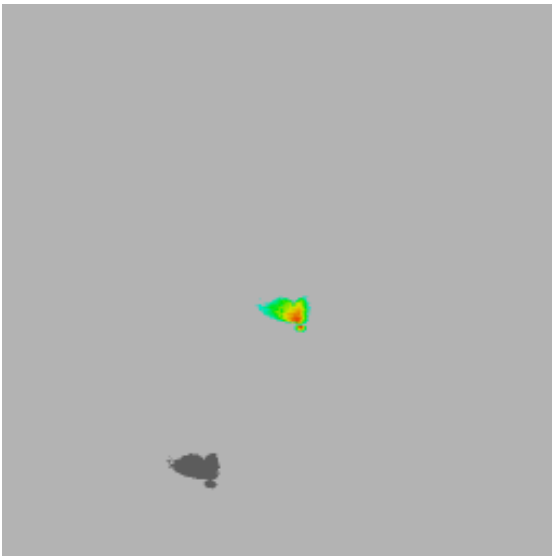
Five Slides in



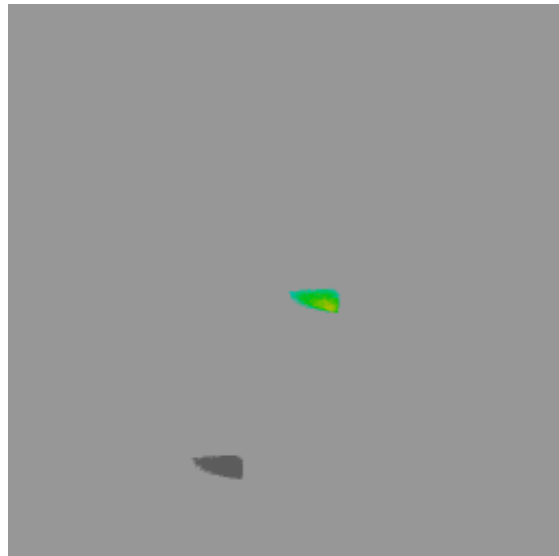
Bead Slide 9 Offset 0.1



Water Slide 12 Offset 0.4

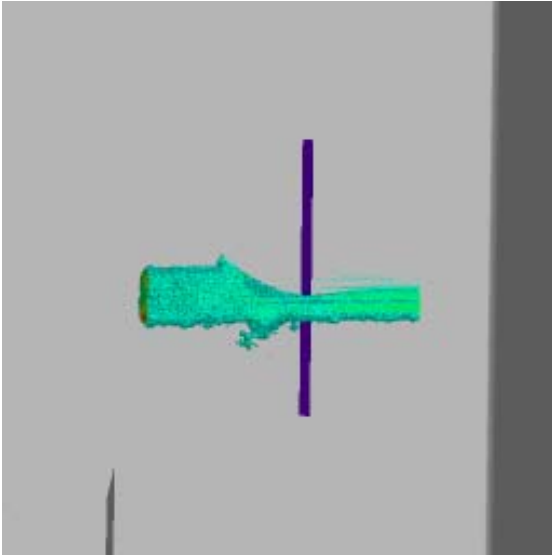


Bead

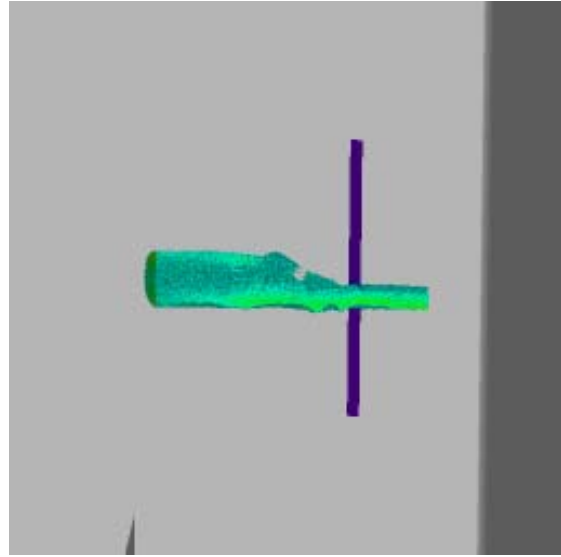


Water

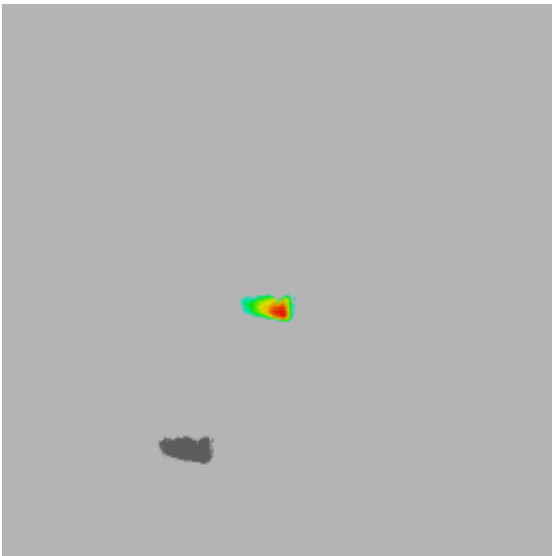
Six slides in



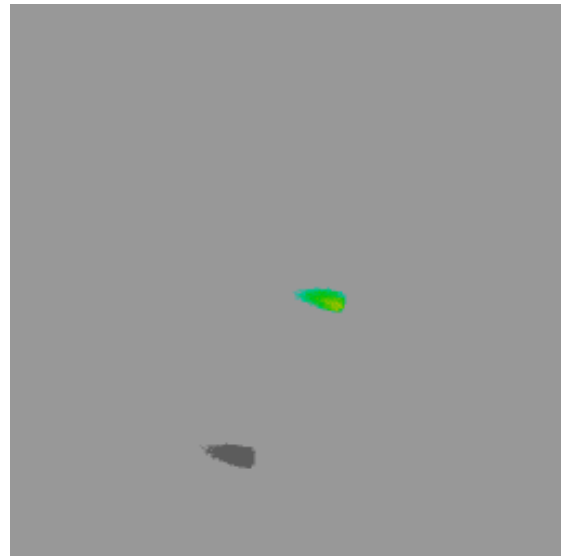
Bead Slide 10 Offset 0.2



Water Slide 14 Offset 0.5

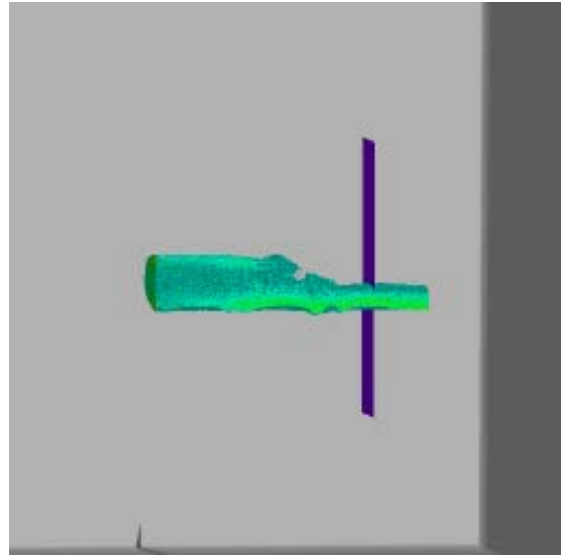
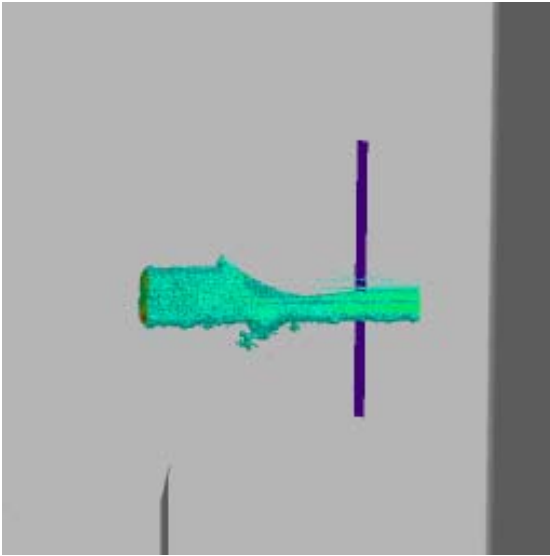


Bead

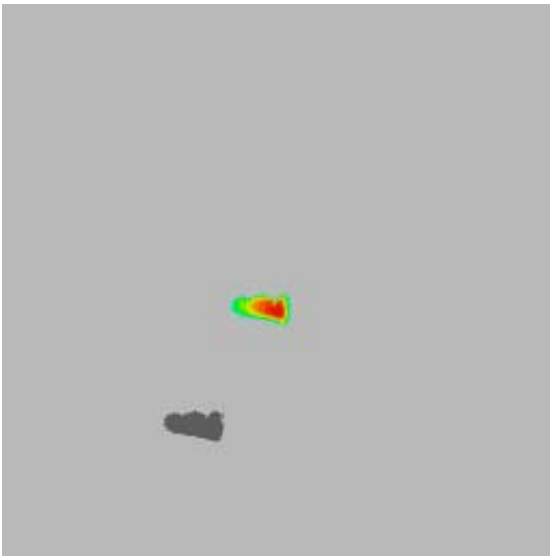


Water

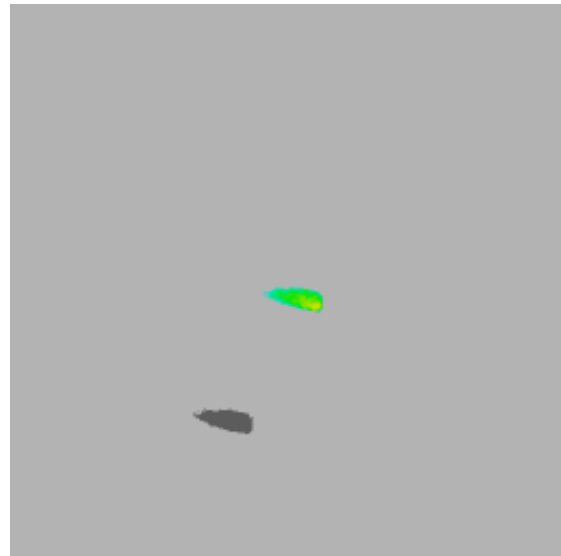
Outside of Converging Field on other end



Slice 14: Offset 0.6



Bead



Water