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Development and Evaluation of a Mass Conservation Lab Module in a Microfluidics Environment

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Development and Evaluation of a Mass Conservation Lab Module in a Microfluidics Environment

by

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Dedication

In loving memory of Bobby Abercrombie.

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Abstract

Development and Evaluation of a Mass Conservation Lab Module in a Microfluidics Environment

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The University of Texas at Austin, 2013

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This thesis presents a mass conservation laboratory module developed for use in the undergraduate mechanical engineering curriculum. The module investigates mass conservation fundamentals in a simple T-junction microfluidic device and exposes students to the rapidly developing fields of microfluidics and optical diagnostics. The module is a novel application of microfluidics-based instruction, is highly repeatable, and can be conducted at relatively low cost. A benchmark study was also conducted to evaluate the laboratory module's efficacy as a teaching tool. This was accomplished through utilizing knowledge assessments before and after student exposure to the laboratory module.

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Chapter 1: Introduction

Laboratory-based learning is hugely important for effective education in Science, Technology, Engineering and Mathematics (STEM) disciplines (Elliott, Stewart, & Lagowski, 2008; Fintschenko, 2011; Jewell, 2008). Moreover, education in engineering disciplines is nearly impossible without the laboratory setting (Krivickas, 2007). The experimental process affords students skills in comprehension, critical thinking, statistical knowledge, and technical writing, all of which are necessary to consider "real world" engineering challenges in both the professional setting and in academia (Fintschenko, 2011).

The primary benefit of utilizing module-based learning in a laboratory setting stems from its amenability to the "active learning" methodology. As per Felder et. al, active learning allows students the opportunity to see the application of methods through an instructor *and* apply the methods themselves, while also being given an opportunity to reflect on outcomes at the end of the application (Felder, Brent, & Prince, 2011). This use of action and reflection is often referred to as *praxis* and is becoming widely recognized in learner-centric education techniques. Praxis is utilized in a laboratory setting through the use of pre-lab instruction, experimentation with teaching assistant supervision, and reflection through the use of write-ups and lab deliverables. The use of module-based instruction also lends itself to the growing "Millennial" generational theory (Wilson & Gerber, 2008). Modules help to break educational topics into manageable sizes, as many students in the undergraduate curriculum today are admittedly highly stressed. Team-orienting module goals help to mitigate student stress levels as well.

In 2000, the Mechanical Engineering department at The University of Texas at Austin developed a "department-wide curriculum reform effort, with the objective of

more closely tying all elements of the ME undergraduate experience to real-world engineering" (Schmidt, n.d.). In the past 13 years, PROCEED has funded a wide spectrum of curriculum and laboratory development projects. In 2012, ME 130L: Experimental Fluid Mechanics was selected for PROCEED funding to continue development of a "module-based" educational program in laboratory-oriented education for a mass conservation laboratory module. This funding was granted, in no small part, due to previous work in integrating module-based education with wind energy technology in ME 130L (Sheble, Bickle, & Hidrovo, 2013).

In 2012, the National Science Foundation granted The University of Texas at Austin a CAREER grant for research in "Inertial Two-Phase Gas-Liquid Droplet Microflows". The educational potential of utilizing two-phase liquid-liquid microflows in mass conservation education was realized as a consequence of the CAREER grant requirement that the research must feature an educational outreach component. The research funded by the CAREER grant produced two primary publications (Carroll & Hidrovo, 2012, 2013) that provided a substantial framework for continuing research in droplet generation in microfluidic devices. The research has helped shape the justification for using microfluidics to teach mass conservation fundamentals.

The research presented in this thesis aims to both develop and evaluate a novel fluid mechanics laboratory module for use in the undergraduate mechanical engineering curriculum. This is accomplished through using a simple microfluidic device, in conjunction with optical diagnostic tools and droplet detection computer algorithms, to demonstrate the ubiquity of mass conservation laws at the micro-scale.

Chapter 2: Background

Reynold's Transport Theorem postulates that "for a system and a fixed, non-deforming control volume" (Munson, Young, & Okiishi, 2006), there exists a governing conservation equation that quantifies fluid parameters (whether they be mass, momentum, energy, etc.) entering and leaving the control volume. Eq. 1 forms the fundamental basis of the continuity equation.

$$\frac{Dm_{sys}}{Dt} = \frac{\partial}{\partial t}\int_{cv} \rho * d\hat{V} + \int_{cv} \rho v \cdot \hat{n}\, dA = 0 \qquad \text{Eq. 1}$$

Eq. 1 can be simplified, and has been for the purposes of the lab module, to discount transient effects (i.e. steady-state). The time rate of change of mass in the control volume is set equal to zero, thus yielding

$$\int_{cs} \rho v \cdot \hat{n} dA = \sum \dot{m}_{out} - \sum \dot{m}_{in} \qquad \text{Eq. 2}$$

where

$$\dot{m} = \rho Q \qquad \text{Eq. 3}$$

As shown in Eq. 2, any constituent mass input is equal to the product of the fluid density and it's respective flow rate. In essence, if the mass of the defined control volume is steady in time, all mass flow rates (both into and out of the system) must sum to zero. This fundamental conclusion is present in many thermal/fluid system courses throughout

the undergraduate mechanical engineering curriculum and is hugely important to the understanding of fluid thermodynamics (Munson et al., 2006). As such, it is paramount that undergraduates have a firm understanding of the implications of this natural law.

The power of Eq. 2 lies in its ubiquity. All systems must adhere to the principles dictated by this natural law. Although it is a simple equation, it is applicable across a wide spectrum of length scales. Given this wide range of applicability, the author has chosen to investigate the field of microfluidics as an avenue for mass conservation education. The goal of this research is to demonstrate the principles of mass conservation in a simple microfluidics environment, making it the first application of its kind. No other existing research to date outlines a methodology to utilize microfluidics to teach mass conservation fundamentals.

Using microfluidics in education is not a novel application. In fact, it is widely documented and often used in both chemical sciences and engineering. It has been used as a tool for investigating chemical titration (Greener, Tumarkin, Debono, Dicks, & Kumacheva, 2012), tissue rheology (Young & Simmons, n.d.), crystallization phenomena (Chia, Sweeney, States, & Odom, 2011), and has substantial potential for fluid mechanics education (Fintschenko, 2011). As per Fintschenko, microfluidics is defined as "the controlled transport and manipulation of liquid solutions, suspensions, or microscopic objects in a volume regime of about 1 femtolitre to microliters with the dimension of the channels containing the liquid in the one micrometre to hundred micrometre range" (Fintschenko, 2011). This research utilizes microfluidic "plug flow" as the primary teaching mechanism, since the physics behind this regime offers unique insight into the laws of continuity.

In plug flow, two immiscible phases (often oil and water) are injected into a microchannel device to form alternating "plugs" of fluid. Aptly named, these plugs span

the length of the microchannel and prevent any flow past the plug. This behavior forces the two phases to translate at the same velocity while inside the microchannel. The characteristic plug velocity is a result of the input mass flow rates, as discussed in Eq. 2, and can be predicted based on the laws of continuity. Validating an experimental plug flow system against a theoretical model derived from Eq. 2 will prove or disprove the system's adherence to the laws of continuity. However, it is necessary to choose a type of microfluidic system to consistently generate the plugs shown in Figure 1, as the system will dictate the application of the conservation laws.

Figure 1: Oil and water plug flow in a 300 μm microchannel

Microfluidic droplet generation systems come in a variety of architectures, the choice of which is mainly driven by cost and application. Specific architectures, including electro-motive systems (Link et al., 2006; Pollack, Shenderov, & Fair, 2002) and acoustic generation of droplets (Elrod et al., 1989) are among these. Flow-focusing devices (Anna, Bontoux, & Stone, 2003) are renowned for having high-throughput capabilities and consistent generation of droplets for oil-in-water or water-in-oil emulsifications. However, T-junction architectures offer similar capabilities (Garstecki,

Fuerstman, Stone, & Whitesides, 2006) while being less complex than the flow-focusing alternative. In addition, the photolithography and soft lithography processes required to fabricate multiple "lab-on-a-chip" devices (Fujii, 2002) are simplified with basic channel designs. Given the need for having many microfluidic devices for students to run fresh experiments, T-junction architectures have been chosen for this experiment.

Figure 2, shown below, features a microfluidic plug flow generator that utilizes the T-junction architecture. The schematic shown in Figure 2 features the orientation of the inlet and outlet ports for the device. A SolidWorks model is used to generate a microchannel photo-mask for positive photolithography. An SU-8 photoresist is used on a silicon wafer to generate a positive mold. Soft lithography with polydimethylsiloxane (PDMS) is then used to fabricate the microchannels. Each microchannel is bonded to a PDMS-coated glass microscope slide, thus creating the microfluidic device shown in Figure 3. The device features microchannels 300 μm wide by 100 μm tall, generating a channel cross sectional area of 0.03 mm^2.

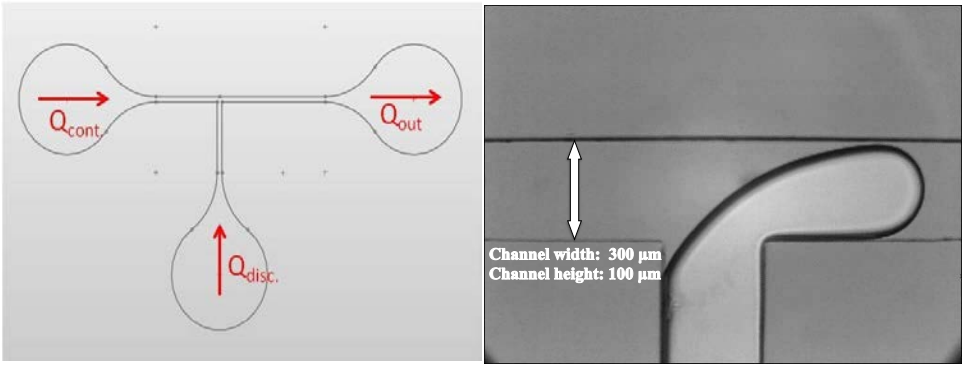


Figure 2: Schematic (left) and T-junction (right) of the microfluidic device.

Figure 3: Slide-mounted 200 μm microfluidic T-junction device

To form plugs, water and oil are supplied at appropriate rates via syringe pumps. At steady state, the device will generate oil and water plugs in a regularly cyclic flow, i.e. the system will exhibit a flow frequency as defined by the syringe pump set points. The left-most port shown in Figure 2 is used to supply the continuous phase fluid, which wets the walls of the microchannel and "pinches" the dispersed phase at the T-junction. A solution of low-density mineral oil (SG = 0.86) and 1 wt% of sorbitan monolaurate is used for this purpose. Sorbitan monolaurate, commercially known as Span® 20, is an oil-soluble surfactant that decreases the surface energy of the mineral oil and helps generate consistently sized plugs downstream of the T-junction. The perpendicular port is used to supply the dispersed phase fluid. A solution of de-ionized water and Fluorescein-548, a non-toxic fluorochrome, is used for this purpose.

Introduction of the fluorochrome into the water allows the experimentalist to optically distinguish the presence of oil or water inside the microchannel. This is accomplished through utilizing wide-field fluorescence microscopy. As outlined in the *Handbook of Optical Filters for Fluorescence Microscopy* (Reichman, 2010), special optical filtration techniques allow the experimentalist to illuminate regions containing the photo-responsive fluorochrome. The images shown in Figure 4 demonstrate a region of

interest downstream of the microfluidic T-junction imaged in both bright field and epi-fluoresence. Notably, epi-fluorescence generates a light signal only when water is present in the microscope's field of view. This method is non-invasive and will not disrupt the plug flow behavior inside the microfluidic device. The microscope will probe a single phase at a given time in a specific region of interest (ROI). This Eulerian approach allows the experimentalist to probe the regularly cyclic flow generated by the device.

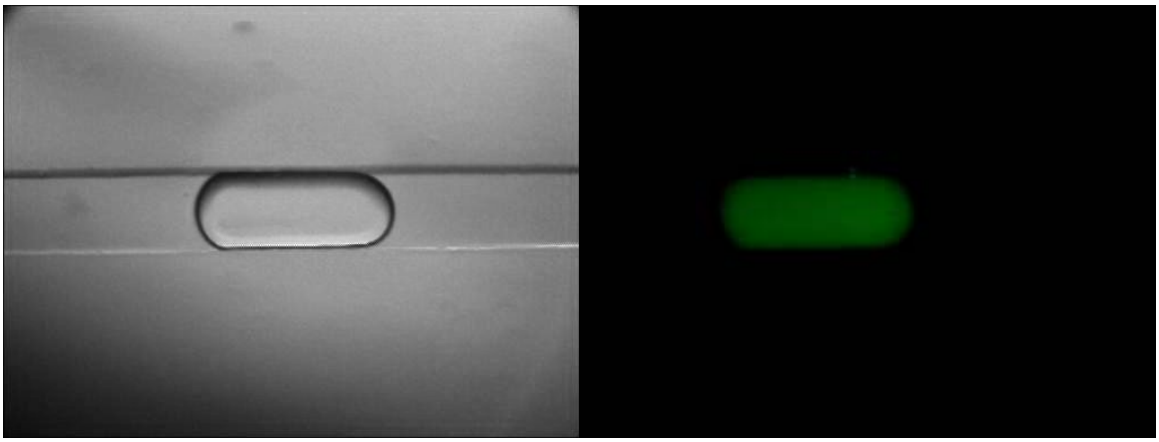


Figure 4: Imaging in bright field (left) and epi-fluorescence (right)

Chapter 3: Experimental Lab Module Setup

The mass conservation lab module is intended for 3-4 students per session and is divided into two stations: operation of the microscope equipment and pumps, and operation of the video acquisition software and MATLAB processing scripts. Table 1 outlines the equipment needed to conduct the lab module along with cost estimates for each item. A cost estimate for the microfluidic T-junction device has been neglected as the photolithography and soft lithography processes required to fabricate the device will vary. The laboratory procedure used by the students, along with the pre-lab document, is available in the Appendix.

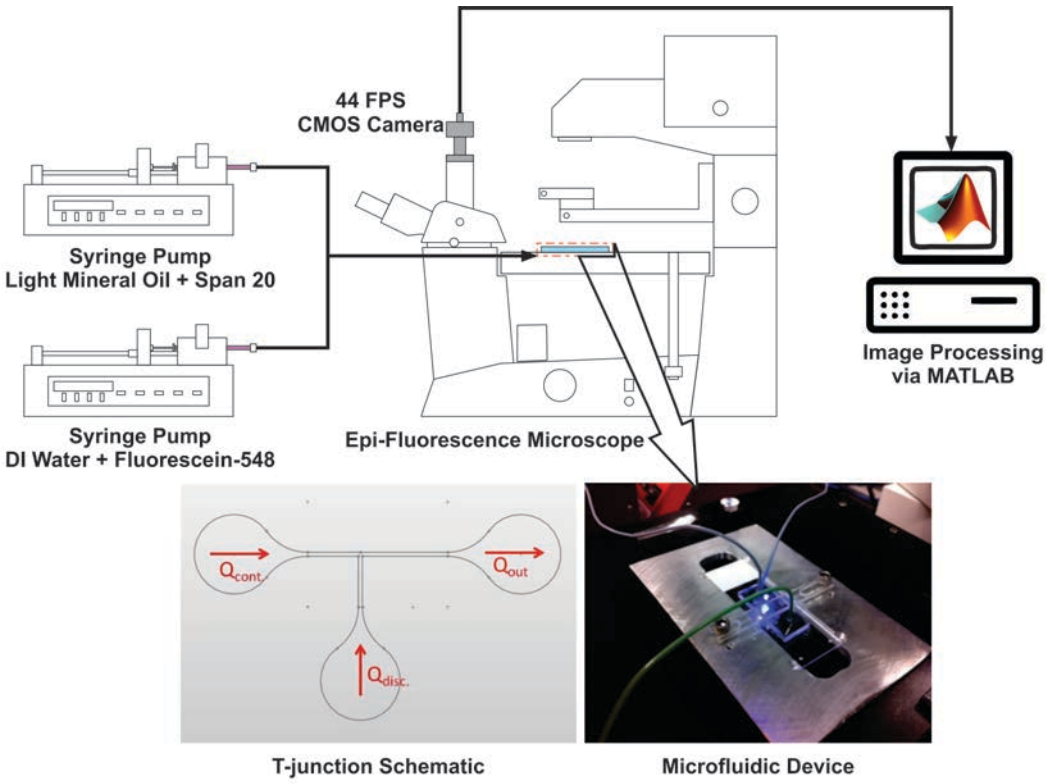


Figure 5: Schematic of the mass conservation lab module system

Table 1: List of materials for mass conservation lab module

Item	Quantity	Description	Estimated Cost
100 μL Syringe	2	Hamilton Syringe, 1710 Luer Lock Tip, ID 1.457 mm	$140
Syringe Pump	2	New Era Pump Systems, NE-300	$550
Luer Lock Fittings	2	Compression fittings, 1/16" OD	$70
PEEK Tubing	1	Blue 1/16" OD, 0.01" ID, 5 ft	$20
Mineral Oil	1	5 L, SG = 0.86	$50
Span® 20	1	Oil-soluble surfactant, 250 g	$30
Fluorescein-548	1	Water-soluble fluorescent dye, 1 g	$70
Microfluidic T-junction device	1	PDMS-based microchannel mounted on slide glass	---
Epi-Fluorescence Microscope w/ USB Camera	1	SpecialtyMicroscopes.com. 3.2M pixels, ½" chip w/ 3.2 μm/pix	$6,000
Consumables (Gloves, glass slides, paper towels, IPA for cleaning)	1	1 box of gloves will supply ~100 students	$50
		Total	~$7050

As shown in Figure 5, the microfluidic device is mounted on the epi-fluorescence microscope stage. Syringe pumps are connected to the device, which supply the oil and water used for two-phase flow. When exposed with filtered light from the dichroic filter, water plugs inside the microchannel device will emit fluorescence for detection by the microscope camera. Image-processing algorithms in MATLAB can then generate a fluorescence signal for further analysis. The setup is relatively simple, as it only requires the two syringe pumps, an epi-fluorescence microscope and camera, and image acquisition/processing software.

The advantage for utilizing microfluidic plug flow for mass conservation analysis stems from two sources: (a) the plug velocity of the two immiscible phases are interlinked and therefore equal, and (b) a regularly cyclic flow pattern can be observed with a Eulerian frame of reference. This cyclic flow pattern, as discussed in Chapter 4, allows the experimentalist to measure two key parameters of interest: (a) the plug velocity, and (b) the flow fraction of water vs. oil (to be later identified as the "duty cycle"). The goal of this experiment is to allow students to measure both the plug velocity and flow fraction for a constant mass flow rate experiment. The students will then investigate the relationship between the duty cycle and the plug velocity and compare it to a theoretical model they derive through the laws of mass conservation.

Students are to use a clean microfluidic device for their experiment. Clean devices are purged with a series of soapy water, methanol, and DI water, followed by low pressure drying in a desiccator. This ensures that all chips are dry and clear of debris. Prior to experimentation, a lab assistant will ensure that both syringes are appropriately filled (i.e. clear of any gas bubbles) and the lines purged of air. Introduction of gas into the microfluidic device will cause errors in the plug flow measurements.

Students are to turn on both the bright field and epi-fluorescence bulbs. The epi-fluorescence bulb requires 15 minutes to warm up and should not be turned off during this time. Students may mount the device on the microscope stage and connect the lines from the syringes to the device during this time. A purge line must also be connected to route the oil and water out of the chip.

Once the bulbs are warm, students will use the microscope camera (or eyepiece) to image the device. This is done to ensure that no debris is present inside the device. At this point, students are to switch on the syringe pumps and set the pump diameter setting equal to the ID of the syringes (1.457 mm). Oil and water are initially flowed through the device at a rate of 5 μL/min. This purges air out of the microchannel and fills the device with oil and water. Flow rates are reduced to 1 μL/min after all air is purged from the device. At this point, students should visualize the plug formation at the T-junction. Modification of the oil surface tension should ensure that the water plugs consistently "break" at the 90° junction, as shown in Figure 6.

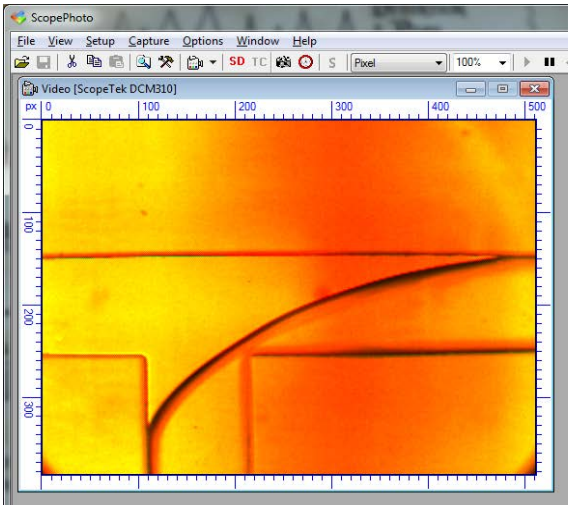


Figure 6: Visualization of T-junction plug flow

Once confirming proper plug formation, the microscope stage is repositioned to image the microchannel downstream of the T-junction. Students will ensure that the microchannel is centered in the middle of the camera's FOV. In addition, the camera must be aligned such that the channel appears straight. This is necessary for proper image processing and can be accomplished by using the tic marks in the imaging software. Students must also ensure the microscope is appropriately focused. At this point, the microscope stage should not be moved. The droplets are very sensitive to changes in inertia, and will require a few moments to come back to steady-state once the stage has been moved.

This lab module will have students investigate microchannel plug flow at a constant mass flow rate of 2 mg/min. This is accomplished by varying the inlet volumetric flow rates of oil and water such that the output mass flow rate remains fixed. Oil and water densities have been determined to be equal to 0.861 and 0.999 mg/μL, respectively, with relative uncertainties no larger than 1%. Calculation of the oil and water density, along with their uncertainties, is outlined in Chapter 5. Table 2 outlines the flow rates used in this experiment.

Table 2: Syringe pump set points for 2 mg/min plug flow

Water Flow Rate (uL/min)	Oil Flow Rate (uL/min)
0.200	2.091
0.400	1.856
0.600	1.627
0.800	1.394
1.000	1.162

Students will take 30-second *.WMV video recordings at each set point. MATLAB scripts, found in the Appendix, are used to convert the *.WMV files into *.TIF stacks for the image processing outlined in Chapter 4. The MATLAB scripts will output information about the plug velocity, the duty cycle, and the raw signal. Students are to use this information to answer questions about the lab, found at the end of the lab procedure document.

Chapter 4: Signal Generation and Processing Algorithms

Light signals from the microfluidic device can be easily captured through the use of a simple microscope camera. The lab module utilizes the DCM310 3.2 mega-pixel ½" CMOS chip camera from Oplenic. The camera views pixels at a size of 3.2 micrometers per pixel and runs at a nominal frame rate of 44 FPS when imaged in a 512 x 384 video mode. The camera records video in 24-bit RGB; however, image processing is run in grayscale and therefore utilizes only 8-bit. This yields a total of 256 possible values between black (i.e. 0) and white (i.e. 255).

The camera is used to image a static region of interest while the syringe pumps are running. Due to the plug flow phenomena, the light intensity inside the camera's field of view (FOV) will vary cyclically. As shown in Figure 7, oil and water plugs will alternate within the camera's FOV, keeping in mind that these plugs will share the same velocity.

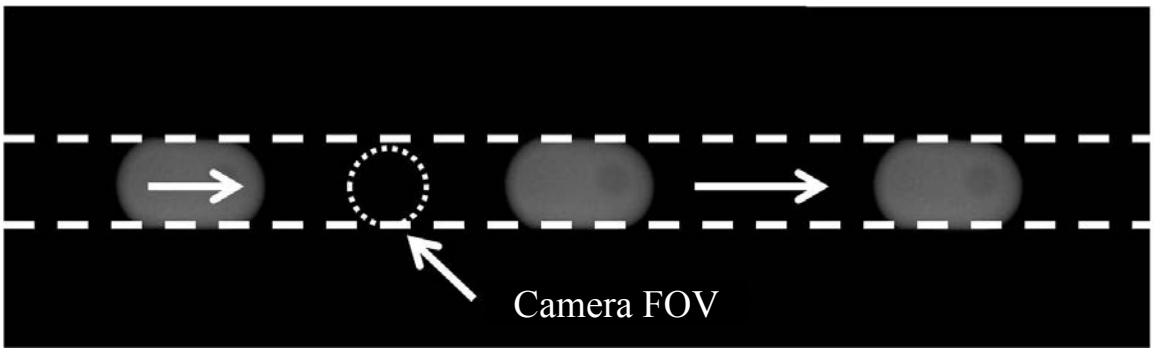


Figure 7: Cyclic light signal due to plug flow

SIGNAL GENERATION ALGORITHM

Figure 8: Signal generation though the use of a photo-mask

The MATLAB-based signal generation is mainly accomplished through the MATLAB script *Oil_Water_Slug*.m, as shown in the Appendix. The plug flow behavior can easily be used to generate a photo-signal. This is accomplished through using simple image multiplication in MATLAB. The microscope camera is used to generate a *.TIF stack with each frame separated by $1/44^{th}$ of a second. This is based on the camera's frame rate. From here, each image in the *.TIF stack is multiplied against a static detection mask, shown as an overlay in Figure 8 (left). The plug represents one frame out of the *.TIF stack recording, while the white rectangle marks the detection region of the detection mask. Each pixel is assigned a corresponding numeric value based on the image multiplication, after which the values are floored to either 0 or 1 based on MATLAB's *im2bw()* function. The dark current for the microscope camera was determined to be no larger than 10/255. Therefore, 10/255 was selected as the threshold value for the *im2bw()* function. All values above this threshold are assigned a value of 1 (white) while all values below this threshold are assigned a value of 0 (black). This process returns the image matrix shown in Figure 8 (right). From here, a signal value is generated for each frame by determining the length of the vector (i.e. a single numeric value) returned by MATLAB's

find() function. This function returns the pixel location of all non-zero values in the image shown in Figure 8 (right). This process is repeated in a *for()* loop across the total length of the video recording, resulting in a signal value for every 1/44th of a second.

SIGNAL PROCESSING ALGORITHM

The signal processing algorithm is mainly accomplished through the MATLAB script *mr_imagesort.m*. The plug flow signal gives the experimentalist key insight into two factors: (a) the downstream velocity of the oil and water plugs and (b) the relative fractions of oil and water. The plot shown in Figure 9 (left) is generated when the signal vector is plotted against time. As seen in Figure 9 (right), the rise time is non-instantaneous. This is a consequence of the water plug front moving across the detector boundary. If (a) the dimensions of the detector boundary are known (in pixels) and (b) the pixel size is known (3.2 μm/pix), it is possible to calculate the plug velocity of both the oil and water. This is accomplished through Eq. 4, where L_{det} represents the detector size (in pixels), R represents the pixel size, and T represents the time required to transition from a fully "OFF" signal to a fully "ON" signal.

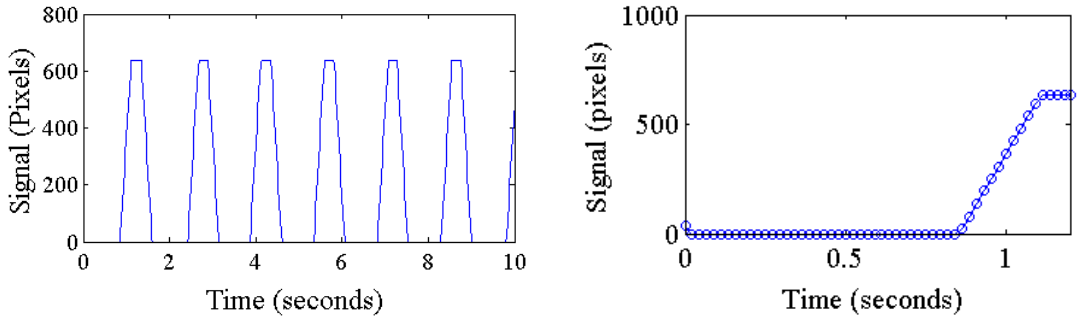


Figure 9: Droplet signal from the MATLAB-based signal generator

$$V_{plug} = \frac{RL_{\det}}{T} \qquad \text{Eq. 4}$$

The *mr_imagesort.m* script is used to demarcate the plug flow signal for computation of both the plug velocity shown in Eq. 4 and the relative presence of oil and water. As shown in Figure 10, the signal is marked with five "triggers". The rise time T shown in Eq. 4 is equal to the number of frames between Trigger 2 and Trigger 1, divided by the camera's frame rate.

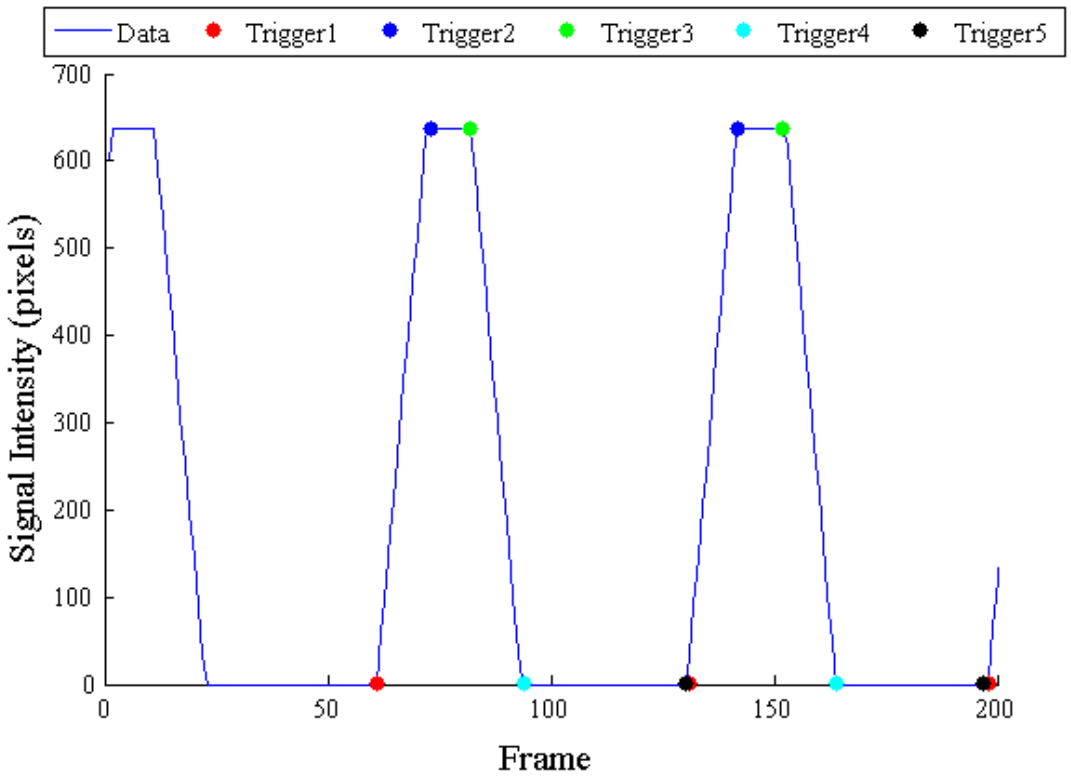


Figure 10: Plug flow signal with trigger points

The lab module utilizes a signal parameter known as the "duty cycle", D, shown in Eq. 5. It is analogous to the duty cycle definition in periodic electronic systems, formed by the ratio of time spent in an active state (τ) vs. the total time in the cyclic period (T). This parameter serves as a tool to gauge the fluorescence fraction of the plug flow and is analogous to the flow rate fraction of water to oil. The duty cycle will tend towards a value of 1 as the flow rate fraction of water-to-oil increases. As opposed to the two-phase analogue found in liquid-gas microfluidics (Carroll & Hidrovo, 2012), droplet regimes in liquid-liquid flows are easier to control. Modifying the Capillary number ($\mu V/\gamma$) through the introduction of surfactants allows the experimentalist better control over the plug flow regime and the repeatability of duty cycle measurements.

$$D = \frac{\tau}{T} = \frac{trig_3 - trig_1}{trig_5 - trig_1} \qquad \text{Eq. 5}$$

Given the duty cycle parameter and the downstream plug velocity, one can infer mass flow rates of both the water and oil based on Eq. 6 and Eq. 7, respectively. These are inferred from the fluorescence signal.

$$\dot{m}_w = \rho_w A V_{plug} * D \qquad \text{Eq. 6}$$

$$\dot{m}_o = \rho_o A V_{plug} * (1 - D) \qquad \text{Eq. 7}$$

Based on the conservation law shown in Eq. 2, all mass flow rates out the microfluidic device must equal the mass flow rates into the device. Eq. 8 reflects this conclusion. In

addition, the mass flow rates into the device will strictly depend on the volumetric flow rate set points of the syringe pumps and the respective fluid density, as shown in Eq. 9.

$$\dot{m}_{out} = \dot{m}_o + \dot{m}_w \qquad \text{Eq. 8}$$

$$\dot{m}_{in} = \rho_o Q_o + \rho_w Q_w \qquad \text{Eq. 9}$$

Eq. 2, Eq. 6, Eq. 7, Eq. 8, and Eq. 9 can be used to derive the theoretical model shown in Eq. 10. This model is used in the lab module to benchmark and verify the experimental findings of the MATLAB-based plug flow detector.

$$V_{plug} = \frac{1}{A}\frac{\dot{m}_{in}}{\left[\rho_w - \rho_o\right]D + \rho_o} \qquad \text{Eq. 10}$$

Chapter 5: Experimental System Limitations

Syringe Pump Calibration

A flow rate calibration was performed on New Era's *NE-300* syringe pump, as shown in Figure 11, to determine the level of flow rate fluctuation within the device. This was accomplished through purging a 100-µL syringe into a collection beaker and measuring the beaker's time-dependent change in mass. An AND GH-232 mass balance was connected to LabVIEW (via RS-232 data cable) to make these measurements.

Figure 11: The NE-300 syringe pump

A 100-µL syringe was filled with the oil solution specified in Figure 5 and purged into the collection beaker. A Luer-Lock compression fitting and flexible PEEK tubing was used to route the purged fluid into the collection beaker. Prior to measurement, the collection beaker was filled with a calibration mineral oil. The end of the PEEK tubing was routed to discharge beneath the surface of the calibration oil, which enabled discrete measurement of mass change in time.

Table 3: Calibration range of the NE-300 pump

Setpoint No.	Setpoint Flowrate (μL/min)
1	0.250
2	0.500
3	0.750
4	1.000
5	1.250
6	1.500
7	1.750
8	2.000
9	2.250

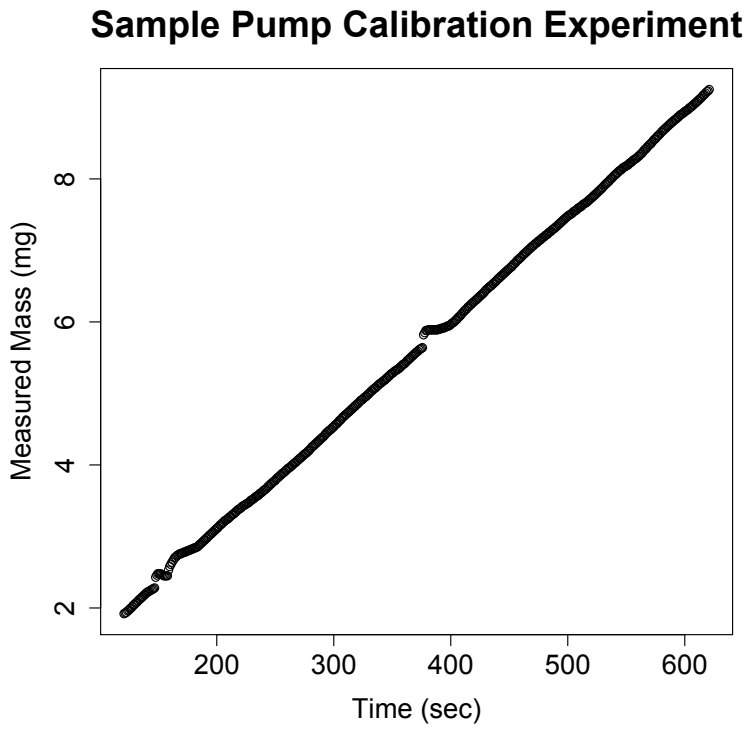


Figure 12: Sample result of the NE-300 flow rate calibration.

Table 3 features a total of 9 calibration points used in the syringe pump calibration. For each flow rate listed, the syringe pump was run for a 2 minute "settling" period (to eliminate transient effects) followed by a 600 second measurement period. The measurement period was used to generate the level of uncertainty for each flow rate. This was accomplished by breaking the 600-second period into ten equal parts of 60 seconds. This generated a sample of 10 mass flow rates (i.e. slopes) from each measurement via linear regression in MATLAB.

$$Q = \frac{\dot{m}}{\rho} \qquad \text{Eq. 11}$$

In order to calculate a statistical uncertainty of volumetric flow rate, as shown in Eq. 11, the uncertainty of the oil solution's density must also be known. This was accomplished by taking a series of 10 mass measurements of 500 μL of the oil solution. The uncertainty of the 500 μL syringe was assumed to be 2% FS. These measurements yield a density of 0.861 ± 0.01 mg/μL.

Table 4: Evaluation of the density bias limit

Measured Mass (mg)
430.2
431.5
429.7
430.5
430.7
431.1
430.1
429.8
431.2
430.6
430.2

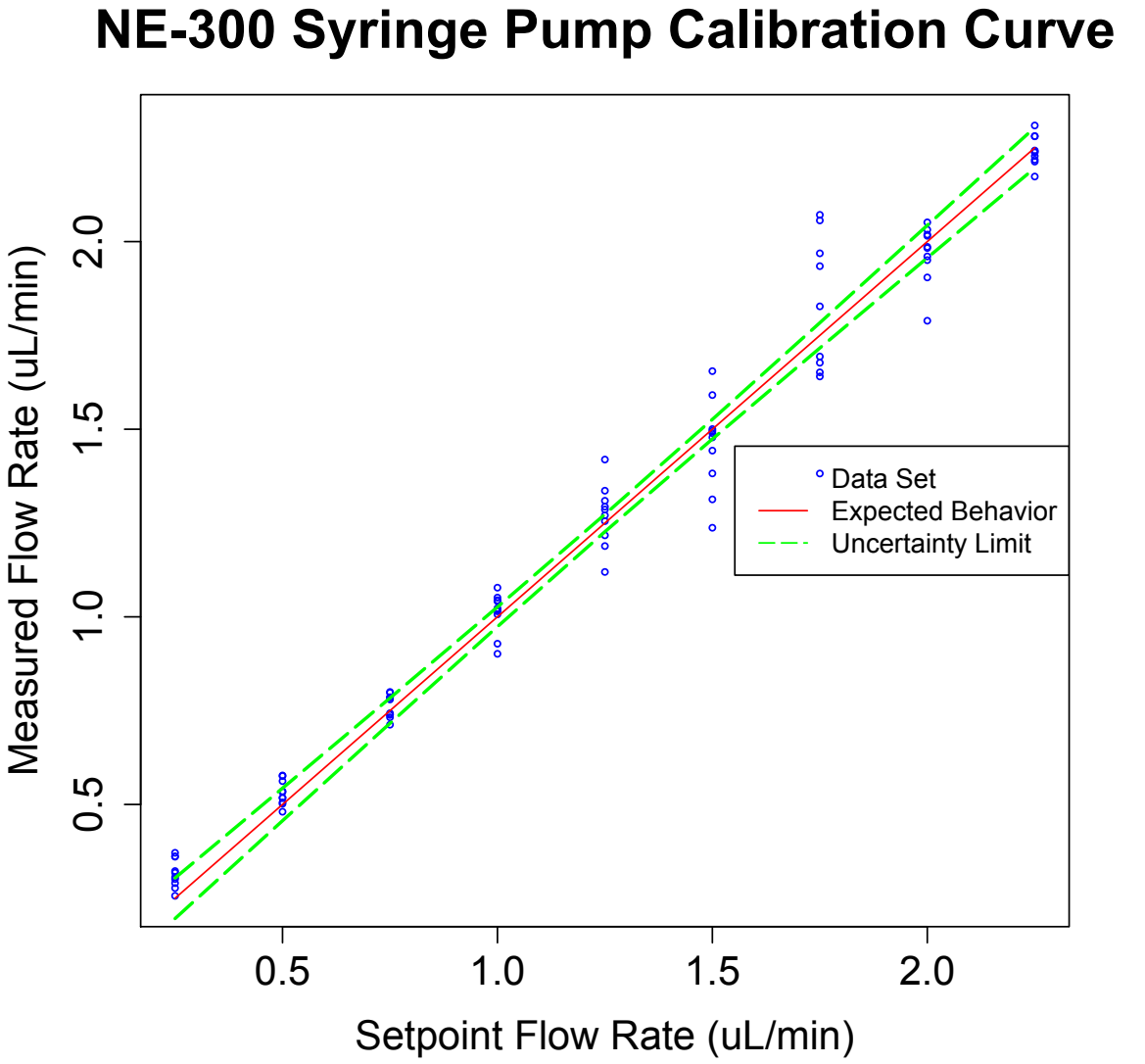


Figure 13: Syringe pump uncertainty interval.

The NE-300 pump features a maximum uncertainty of ± 0.053 µL/min and a minimum uncertainty of ± 0.020 µL/min across the calibration range shown in Table 3.

Table 5, below, features the mass flow rates generated for each calibration experiment shown in Figure 13.

Table 5: Calibration of the NE-300 syringe pump with light mineral oil

Measured Mass Flow Rate (mg/min)	Flow Rate Set Point (µL/min)									
	Trial No.	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.25**
	1	0.264	0.497	0.640	0.799	1.114	1.288	1.771	1.749	1.910
	2	0.311	0.414	0.636	0.928	1.048	1.370	1.574	1.710	1.927
	3	0.250	0.432	0.676	0.897	1.222	1.283	1.695	1.541	1.964
	4	0.274	0.459	0.637	0.776	1.107	1.066	1.458	1.680	1.871
	5	0.239	0.484	0.631	0.881	0.964	1.425	1.422	1.735	1.989
	6	0.311	0.446	0.689	0.867	1.094	1.273	1.666	1.738	1.918
	7	0.320	0.435	0.676	0.905	1.024	1.292	1.784	1.766	1.964
	8	0.278	0.461	0.687	0.874	1.151	1.131	1.444	1.688	1.931
	9	0.221	0.446	0.614	0.899	1.081	1.190	1.413	1.707	1.929
	10	0.259	0.496	0.671	0.877	1.127	1.243	1.190	1.640	1.906

The total uncertainty shown in Figure 13 is a combination of precision and bias uncertainty, as shown in Eq. 12. This assumes a confidence interval of 95% for precision uncertainty. Bias uncertainty, in this case, is generated through the uncertainty of the oil density.

$$u_T = \sqrt{b^2 + p^2} \qquad \text{Eq. 12}$$

The precision uncertainty for the given flow rate set points were generated through equations available by A.F. Mills (Mills, Angeles, Chang, & Korea, 2004). The precision uncertainty interval for a linear curve fit is

$$P_{\hat{Y}} = 2\left\{S_Y^2\left[\frac{1}{N} + \frac{(x-\bar{x})^2}{S_{xx}}\right]\right\}^{1/2} \qquad \text{Eq. 13}$$

given that S_Y is equal to the standard error of the estimate

$$S_Y = \left[\frac{1}{N-2} \sum D_i^2 \right]^{1/2} \quad \text{Eq. 14}$$

and S_{XX} is

$$S_{xx} = \sum x_i^2 - \left(\frac{1}{N} \right) \left(\sum x_i \right)^2 . \quad \text{Eq. 15}$$

Rather than using a least-squares curve fit to generate the expected behavior shown in Figure 13, a 1:1 linear regression is used. For a given flow rate set point entered into the syringe pump, ideal behavior would yield exactly the same flow rate at measurement. Residuals are calculated based on this line.

THEORETICAL UNCERTAINTY VS. EXPERIMENTAL MEASUREMENTS

The results of the syringe pump calibration allow for an interval of uncertainty to be calculated for the theoretical plug velocity model, shown in Eq. 10. This is accomplished, initially, by fitting a quadratic regression to the results of the calibration, which are shown in Table 6.

Table 6: Uncertainty range of the NE-300 pump

Setpoint Flowrate (µL/min)	**Setpoint Uncertainty (µL/min)**
0.250	0.054
0.500	0.043
0.750	0.034
1.000	0.026
1.250	0.023
1.500	0.026
1.750	0.034
2.000	0.043
2.250	0.054

Table 7: Flow rate uncertainty curve, parabolic fit Ax2 + Bx + C

A	B	C
0.0302	-0.0754	0.0721

The results of the quadratic curve fit, shown in Table 7, are used to generate uncertainties for the experimental set points used in the laboratory experiment.

Table 8: Associated uncertainties for 2 mg/min experiment

Setpoint No.	Water Flow Rate (uL/min)	Uncertainy (uL/min)	Oil Flow Rate (uL/min)	Uncertainty (uL/min)
1	0.200	0.058	2.143	0.046
2	0.400	0.047	1.905	0.036
3	0.600	0.038	1.667	0.029
4	0.800	0.031	1.430	0.026
5	1.000	0.027	1.192	0.025

Although the input mass flow rate remains constant (2 mg/min) throughout the laboratory experiment, each experimental set point (for both oil and water) has an associated uncertainty. As shown in Table 8, these uncertainties decrease as the set point approaches the mean calibration set point of 1.25 uL/min. Therefore, any experiments operating near the bounds of the calibration region are expected to have the largest uncertainty. These uncertainties are easily propagated into input mass flow rate, as shown in Eq. 16. Given a calculated oil density uncertainty of 1% and an assumed water density uncertainty of 1%, the results of this error propagation are shown in Table 9.

$$\frac{\delta \dot{m}_{in}}{\dot{m}_{in}} = \sqrt{\left(\frac{\delta \rho_o}{\rho_o}\right)^2 + \left(\frac{\delta \rho_w}{\rho_w}\right)^2 + \left(\frac{\delta Q_o}{Q_o}\right)^2 + \left(\frac{\delta Q_w}{Q_w}\right)^2} \qquad \text{Eq. 16}$$

Table 9: Mass flow rate uncertainties per set point

Setpoint No.	**Water Flow Rate (uL/min)**	**Oil Flow Rate (uL/min)**	**Mass Flow Rate (mg/min)**	**Mass Flow Uncertainty (%)**
1	0.200	2.143	2.000	29.2
2	0.400	1.905	2.000	11.9
3	0.600	1.667	2.000	6.7
4	0.800	1.430	2.000	4.5
5	1.000	1.192	2.000	3.7

This process is easily re-iterated to calculate the theoretical uncertainty of the plug velocity, shown in Eq. 17. Oil and water densities have been neglected in this case, given their relatively small error and previous inclusion in mass flow rate uncertainty. The uncertainty of the area of the microchannel, A, has been found to be approximately 1% as well.

$$\frac{\delta V_{plug}}{V_{plug}} \cong \sqrt{\left(\frac{\delta A}{A}\right)^2 + \left(\frac{\delta \dot{m}_{in}}{\dot{m}_{in}}\right)^2} \qquad \text{Eq. 17}$$

Figure 14, shown below, compares the theoretical and the experimental results of the duty cycle vs. plug velocity. At small duty cycles the theoretical trend (shown in red) features large error bars, as based on Eq. 17. This theoretical error drastically improves as the duty cycle increases. This is due, in large part, to the water's relative uncertainty at

low flow rates. At Set Point 1, for example, the water's relative uncertainty nears ± 29%. As the water's flow rate increases to Set Point 5, however, the relative error drops to nearly ± 2.7%. This results in a total velocity uncertainty of ± 3.9% Ideally the flow rate calibration range shown in Table 3 would greatly exceed the experimental range shown in Table 2. However, the limited speed of the microscope camera prevents accurate velocity detection beyond 2 mg/min. In addition, the syringe pumps (which are driven by stepper motors) become highly inaccurate for flow rates below 0.2 uL/min. These effectively behave as upper and lower limits for experimental operation.

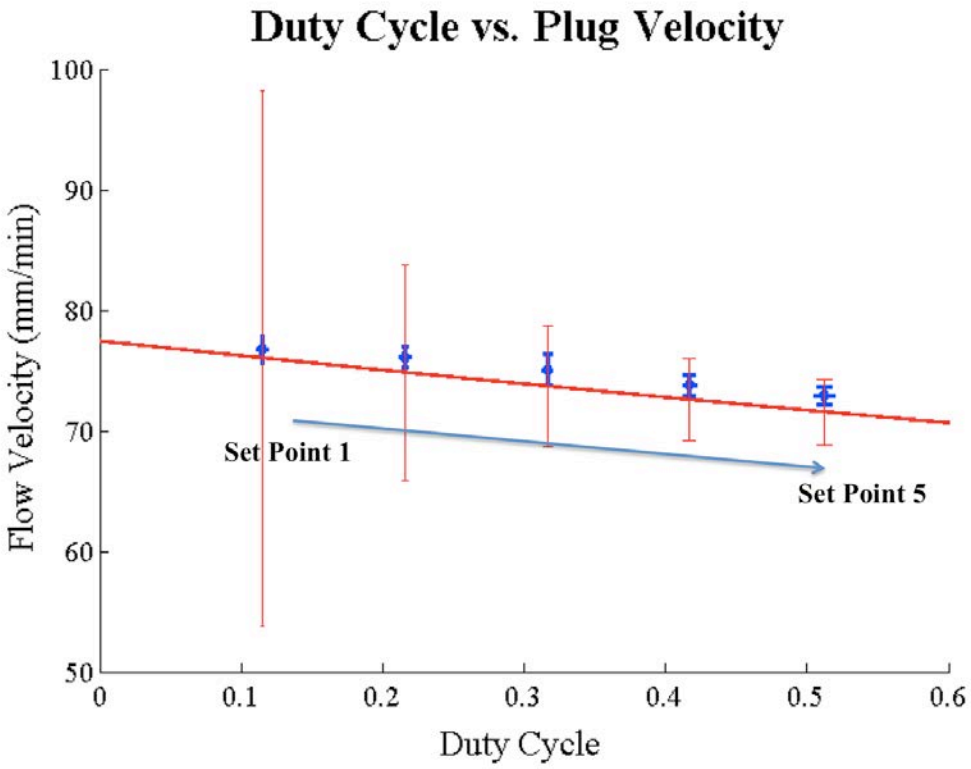


Figure 14: Theoretical calibration (red) vs. experimental results (blue)

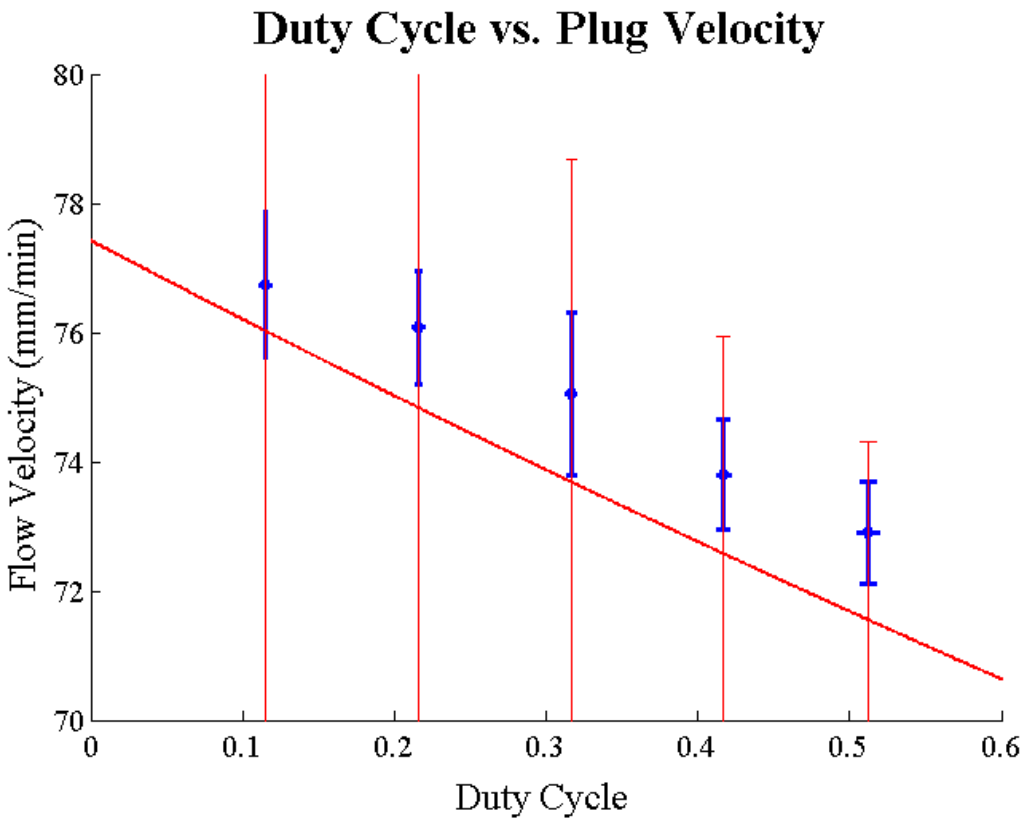


Figure 15: Zoomed theoretical calibration (red) vs. experimental results (blue)

One notable difference found between the theoretical syringe pump calibration and the experimental MATLAB results is the marked offset between the two sets. This bias is consistent, and very likely due to the frame rate set point used in the MATLAB calculations. The software used to control the USB camera equipment displays a frame rate near 44 FPS at the recording resolution mentioned above. If the camera happens to record, instead, at 44.5 FPS, the MATLAB code will over-represent the velocity of the plugs. This is reflected in Figure 15.

Chapter 6: Benchmark Evaluation Results

Evaluation of the mass conservation lab module was conducted though pre- and post-evaluation benchmarking. A sample of 36 student volunteers from the Spring 2013 ME 130L course were selected and gauged prior to receiving laboratory instruction and after conducting the mass conservation experiment. In addition to completing these knowledge benchmarks, students were also asked to submit questionnaires regarding the lab module and a group lab write-up. Each team group consisted of 3-4 team members. The pre- and post- evaluation, both based on the same test, consisted of 24 evaluation questions that tested the student's knowledge of the fundamental principles listed in Table 10. The test questions, grouped by category, can be found in the Appendix. Results from the student questionnaire, which gauged 22 students in total, can also be found in the Appendix. Pre- and post-evaluation results, averaged for each question, can also be found in the Appendix. The benchmarks tests were administered through the Blackboard academic service, which allowed students to answer test questions at home on their own time. Although over half of the questions developed for the evaluation were intended to test on mass conservation principles, additional questions were developed to query the remaining groups listed in Table 10. In experimental fluid mechanics, 10 samples form the ideal minimum for a multiple sample experiment, as to minimize the Student's critical-t value used to determine the interval of precision uncertainty for that measurement (Mills et al., 2004), as reflected in Eq. 18. This equation assumes each measurement is a "statistically independent" measurement, i.e. measurements do not influence subsequent measurements. If this same logic is applied to consider each question in a Group category as a "measurement" of student understanding for that particular Group, statistics argue that 10 questions form the ideal minimum for the

question size for each Group. As such, the significance of the lab module's evaluation should be weighted on Group 1, plug flow physics with a total of 8 questions; and Group 4, mass conservation with a total of 14 questions.

$$u_p = \frac{t\sigma_{\bar{X}}}{\sqrt{N}} \qquad \text{Eq. 18}$$

Table 10: Evaluation test groups and group sizes

Group	Fundamental Principle	Question No.	Group Size
1	Non-dimensional numbers, flow regimes, and plug flow physics	1, 2, 3, 4, 5, 6, 16, 19	8
2	Microscopy and micro-fabrication	7, 8, 9, 17, 18	5
3	Uncertainty analysis	10, 11, 12, 13	4
4	Mass conservation	10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	14
5	Signal generation and analysis	19, 22, 23, 24	4

Results from each question group are presented in the boxplots found below. The solid band inside each box represents the data median, while the box represents the interquartile region (IQR) between Q1 and Q3. The whiskers represent the highest and lowest datum within 1.5 times the IQR for the upper and lower quartiles, respectively. Any outliers beyond this region are represented as points. Each set of results is based on a sample size of 36 students.

A paired, two-tailed Student's t-test was conducted in R in order to determine the statistical significance of each test group. Results of this test are shown in Table 11. This requires the sample Groups to be normally distributed, as is shown for the total evaluation in Figure 16. Since the evaluation used a total of 36 students, the t-test was conducted under 35 degrees of freedom (df). The pre- and post-evaluation results for the total assessment, along with Groups 1, 2, and 4 were found to be statistically significant (i.e. the probability of the null hypothesis is less than 0.05). Results for Group 3 and 5 were not found to be statistically significant in the pre- and post-evaluation results. This is due to a combination of factors, as discussed in the Group's respective sections.

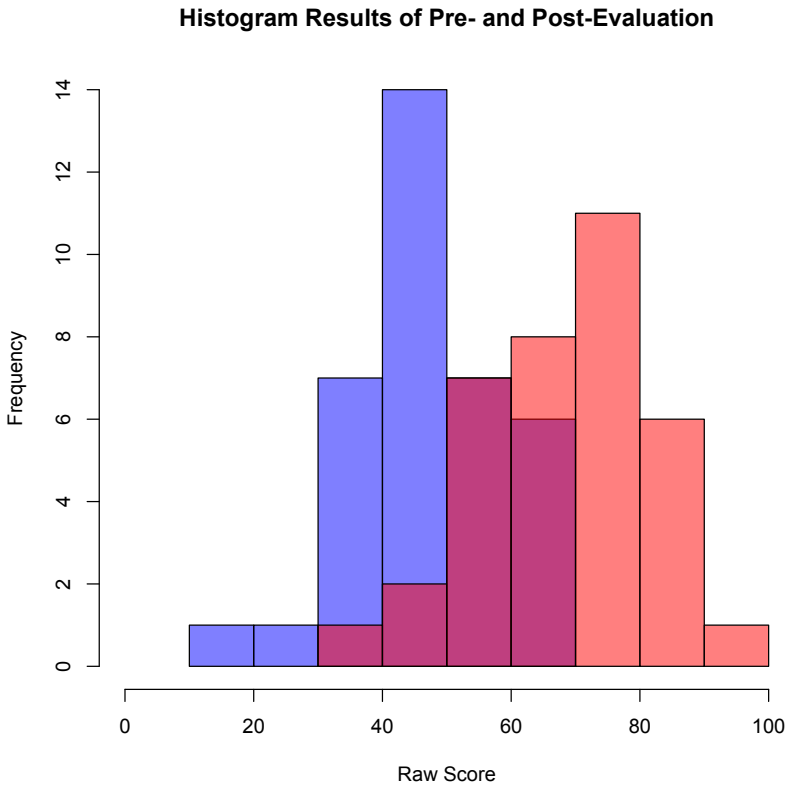


Figure 16: Histogram results of the pre (blue) and post (red) evaluation

Table 11: Results of the Student's t-test for statistical significance

	T-value	Degrees of freedom (df)	$p < 0.05$	Mean of the differences
Total Assessment	-8.26	35	YES	-21.24
Group 1	-11.14	35	YES	-37.33
Group 2	-3.45	35	YES	-14.44
Group 3	-1.23	35	NO	-7.64
Group 4	-4.56	35	YES	-15.97
Group 5	-1.54	35	NO	-8.68

TOTAL ASSESSMENT

Results of the student evaluation benchmark indicate an increase in median test score from less than 50% to almost 70%. The IQR remained nominally the same size for pre- and post-evaluation. It is important to note that most students knew very little about microfluidic systems or their possible applications in signal generation and mass conservation. The results from the total assessment bear similar to the results found in Group 4, which specifically analyzes mass conservation. The evaluation benchmark suggests that the student sample, overall, retained concepts listed in Table 10 after conducting the laboratory experiment. Over 90% of student responses in the questionnaire agreed or strongly agreed that they practiced teamwork, communication, and organizational skills during the process of the lab. Student impressions of the lab module were positive as well, with claims that the module was "[an] interesting subject [that] will make an amazing addition to the fluids curriculum".

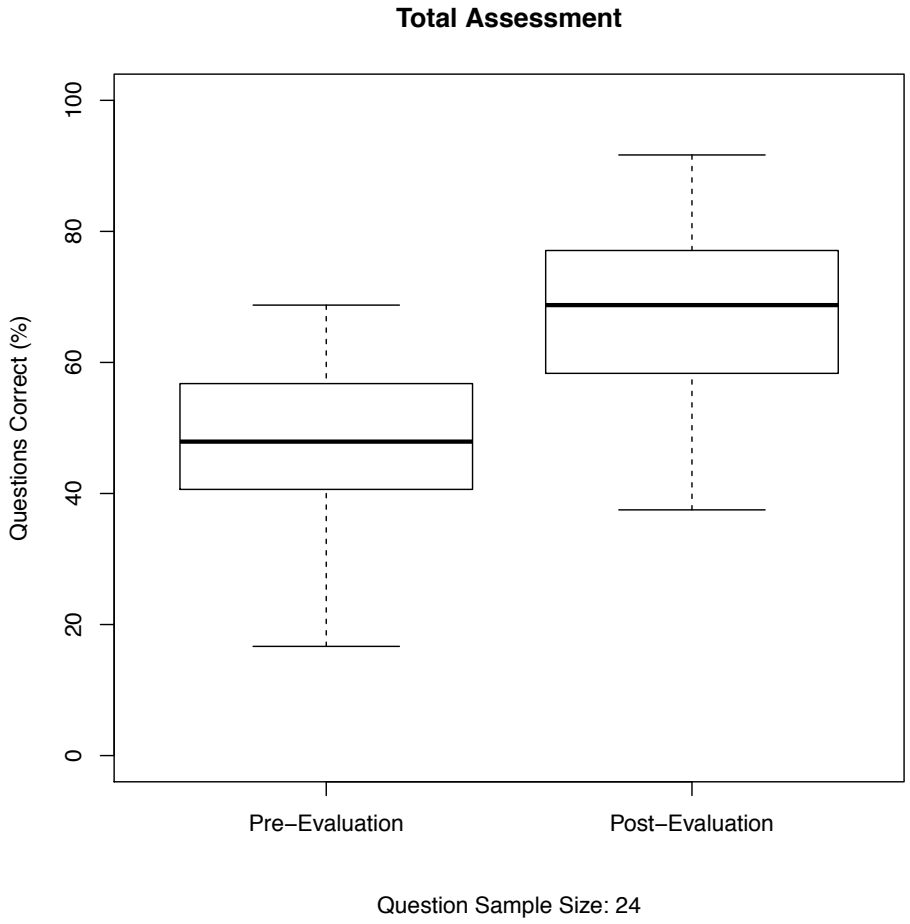


Figure 17: Evaluation results of the total assessment

GROUP 1 ASSESSMENT: PLUG FLOW PHYSICS

The Group 1 assessment was intended to evaluate the student's understanding of microfluidics-related non-dimensional numbers, flow regimes and regime transitions, and basic plug flow physics. The primary goal in this assessment was to help students see the relationship between oil plug velocity and water plug velocity, i.e. they are equal. This conclusion is important, as it directly links the signal generated by the microfluidic device to the aforementioned conservation laws. Without this criterion, students would be

unable to infer both the oil and water velocity from the light signal alone and could not prove mass conservation in the device.

Students were asked to categorize microflows based on the Reynold's number (<0.1) and the Capillary number (<0.001). In addition, students gained basic exposure to flow regime shifts, namely the transition between the plug flow regime and the "bubble flow" regime shown in Figure 18. The implications of this shift concern the relative velocities of oil and water downstream of the T-junction. As the water plugs shift to droplets in crossflow, mass conservation can no longer be inferred from the light signal generated by the water droplets.

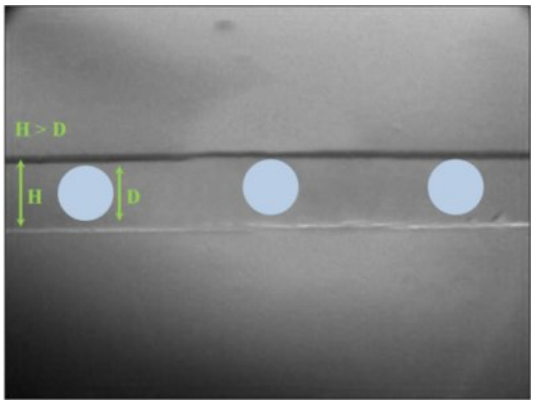


Figure 18: The bubble flow regime

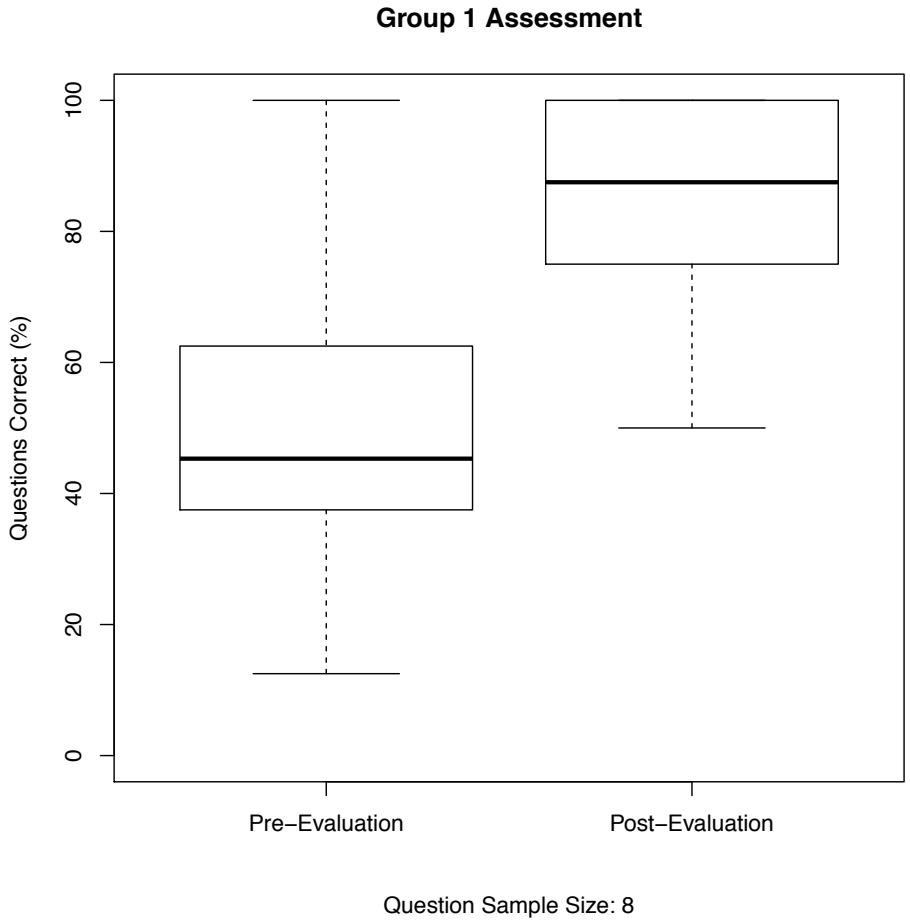


Figure 19: Evaluation results of the Group 1 assessment

Almost all questions outlined in Group 1 featured post-evaluation averages over 85%. However, the post-evaluation average on Question 19 scored no higher than 75%. This is likely due to the question's presentation on Blackboard, which may have been confusing to students based on the student survey feedback. The Group 1 assessment demonstrated a median increase of over 40 points, with consistent IQR sizes between pre- and post-evaluation. Lower quartile whiskers remained relatively the same size,

suggesting that the lab module increased student understanding about plug flow physics across the board.

GROUP 2 ASSESSMENT: MICROSCOPY AND MICRO-FABRICATION

The Group 2 assessment sought to assess the student's exposure to both fluorescence microscopy and to micro-fabrication techniques like photolithography and soft lithography, which were used to fabricate the microchannels. Student understanding of these concepts was poor in both pre- and post-evaluation, with no average scores reaching higher than 56%. Although the principles found in this group are not crucial to the operation of the lab experiment, many students expressed their interest in microfluidics, stating, "it was very interesting to see fluid mechanics applied on a micro scale". Over 90% of students surveyed agreed or strongly agreed that their interest in microfluidic devices and their fabrication increased as a result of the lab module. In addition, over 86% of the students surveyed agreed or strongly agreed that their skill with optical measurement tools like epi-fluorescence microscopes and cameras increased as a result of the lab module. This result seems to conflict with the results found in Figure 20, and suggest that more testing is necessary to accurately gauge this metric. The median scores of the 36 students questioned increased by about 20 points, while the IQR doubled in size. Lower and upper whiskers remained nearly equal in magnitude for pre- and post-evaluation.

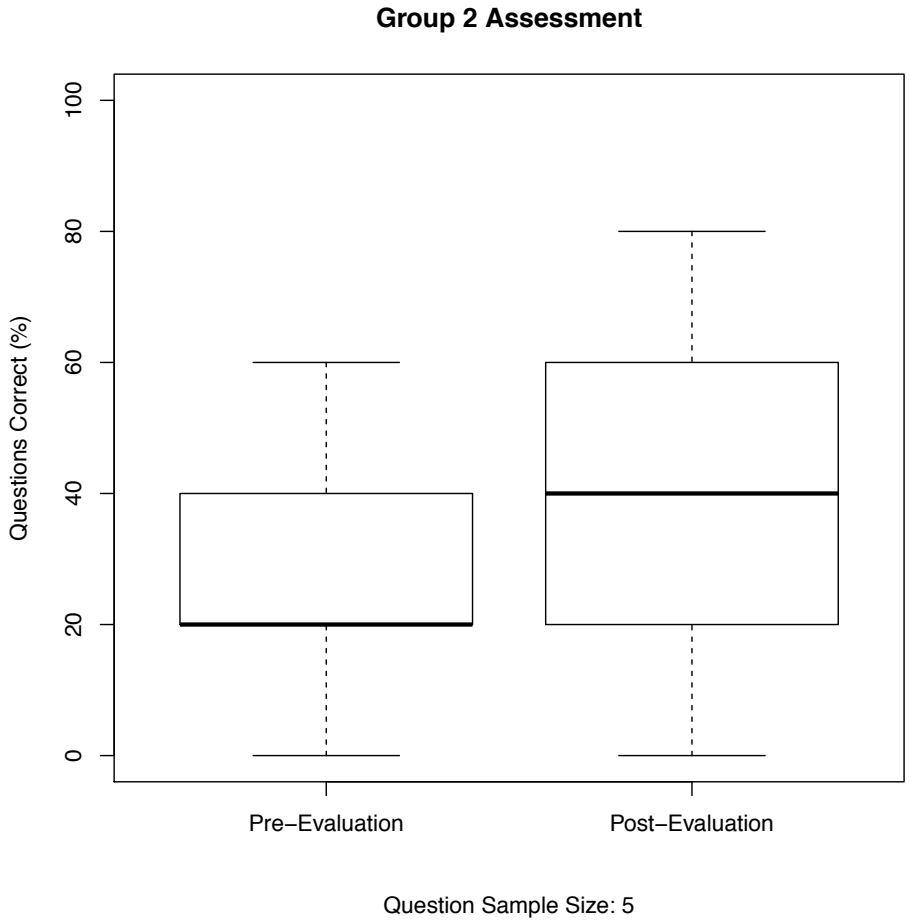


Figure 20: Evaluation results of the Group 2 assessment

GROUP 3 ASSESSMENT: UNCERTAINTY ANALYSIS

The Group 3 assessment was used to categorize questions related to uncertainty analysis. Questions were crafted to gauge student understanding of both precision and bias errors, and required students to propagate uncertainty with either sequential perturbation or the standard partial-derivative method. By design, the MATLAB-based droplet detector returned both a duty cycle and plug velocity for each oil-water plug

couple. This returned anywhere from 30-50 droplet detections per video recording, allowing students to generate a precision uncertainty for both the duty cycle and the plug velocity. Group 3 assessment showed no appreciable increase in the sample median between pre- and post-evaluation. In addition, Question 13 showed no increase from the pre-evaluation average of 81%. More than 70% of students quizzed post-evaluation understood the difference between precision and bias error, but less than 50% were able to successfully conduct a sample error analysis with sequential perturbation or partial derivatives on Question 10. Scores in the lower quartile increased over 20 points, while the upper quartile increased over 15 points.

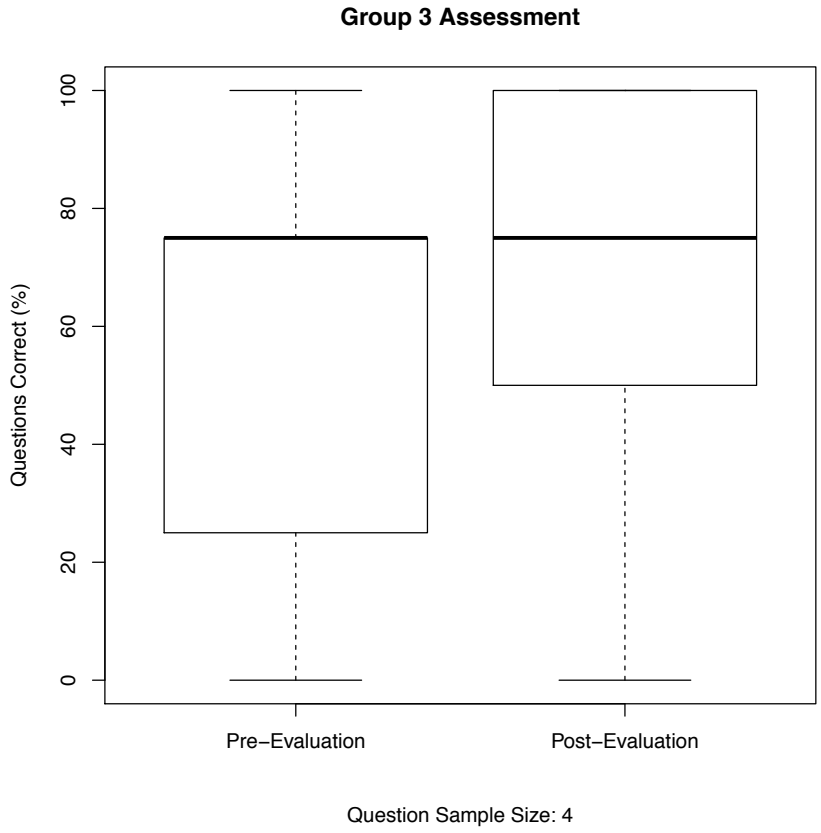


Figure 21: Evaluation results of the Group 3 assessment

GROUP 4 ASSESSMENT: MASS CONSERVATION

The Group 4 assessment constitutes the largest component of the student evaluation at 14 questions. This assessment was intended to evaluate student learning of mass conservation principles in the microfluidics environment. The results shown in Figure 21 suggest that the lab module does indeed convey mass conservation fundamentals to the volunteer student group. The sample median increased over 15% from pre- to post-evaluation, along with increases in both the upper and lower quartiles. Boxplot whiskers remain approximately the same for pre- and post-evaluation.

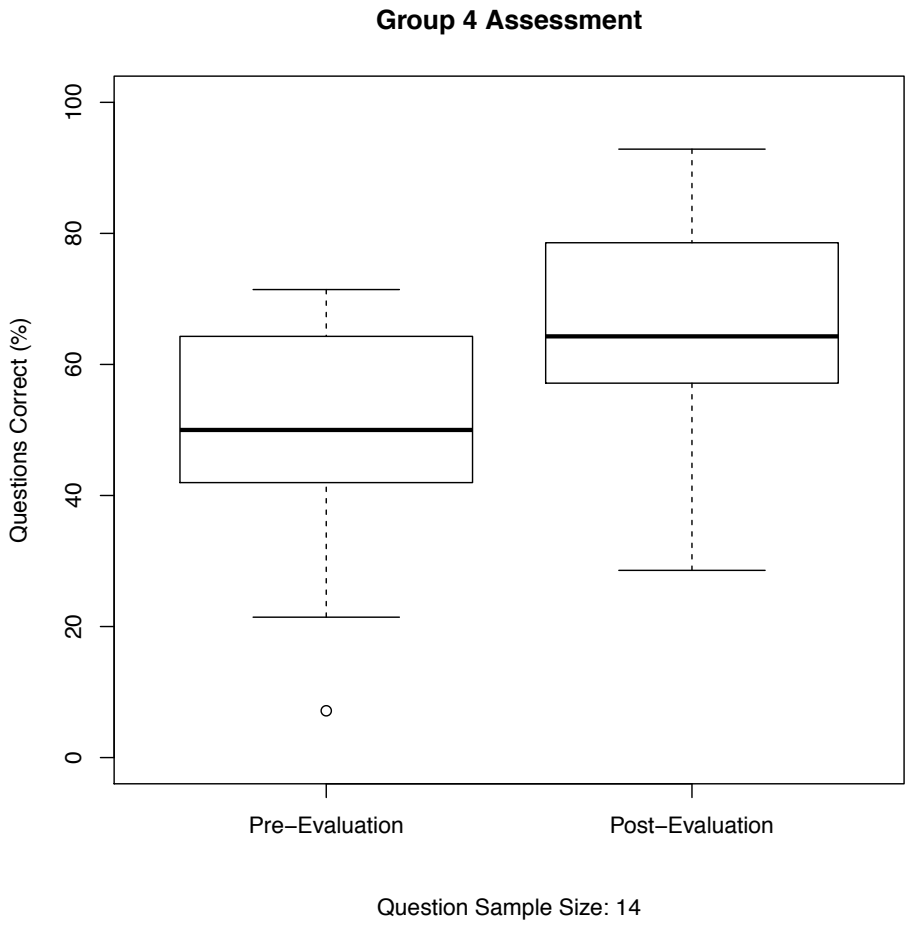


Figure 22: Evaluation results of the Group 4 assessment

Students were required to understand the relationship between the syringe pump set points and the plug velocities. Since the water solution (SG = 0.99) is denser than the oil solution (SG = 0.86), increases to the water-to-oil volumetric flow rate fraction will cause a net decrease in the plug velocity. This a direct effect of the conservation of mass, and will always be the case provided that the plug velocity of the water is always equal to the plug velocity of the oil.

In addition, students were also asked to develop the theoretical mass conservation equation for plug flow, as shown in Eq. 10. This equation depends on both the total mass input rate and the difference in density between the continuous oil phase and the dispersed water phase. As shown in Figure 23, utilizing a low-density mineral oil (SG = 0.86), results in a more drastic change in velocity (i.e. more non-linear) with changes in the duty cycle (which is analogous to the water-to-oil volumetric flow rate ratio). However, Group 4 assessment featured a decrease from 39 points to 28 points from pre- to post-evaluation averages on Question 21, and therefore did not appropriately convey this conclusion to students. More work is needed to illustrate this point, possibly through experiments utilizing both light mineral oil and silicone oil (SG = 0.95) as the continuous phase.

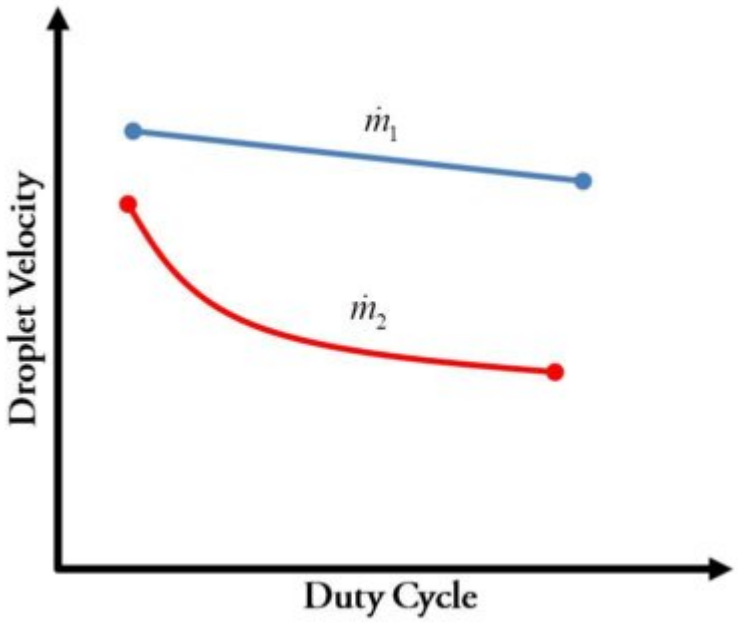


Figure 23: High density (m_1) and low density (m_2) velocity dependence on the continuous phase fluid

As shown in Figure 23, increasing (or decreasing) the total input mass flow rate will cause the trend to translate up (or down). Case 1 features a higher total input mass flow rate than Case 2. Increasing the difference between the densities of the continuous and dispersed phase, shown in Eq. 10, will cause higher velocity sensitivity to changes in the duty cycle. Case 2 features a larger difference between continuous and dispersed phase density than Case 1.

Students did well to understand the connection between the signal rise-time to the plug velocity, as shown in Figure 24. As the rise-time decreases (i.e. as the slope increases), the plug velocity increases. Average score improved from pre- to post-evaluation by 14 points for both Question 22 and 23. These results have implications on Group 5, signal generation and analysis.

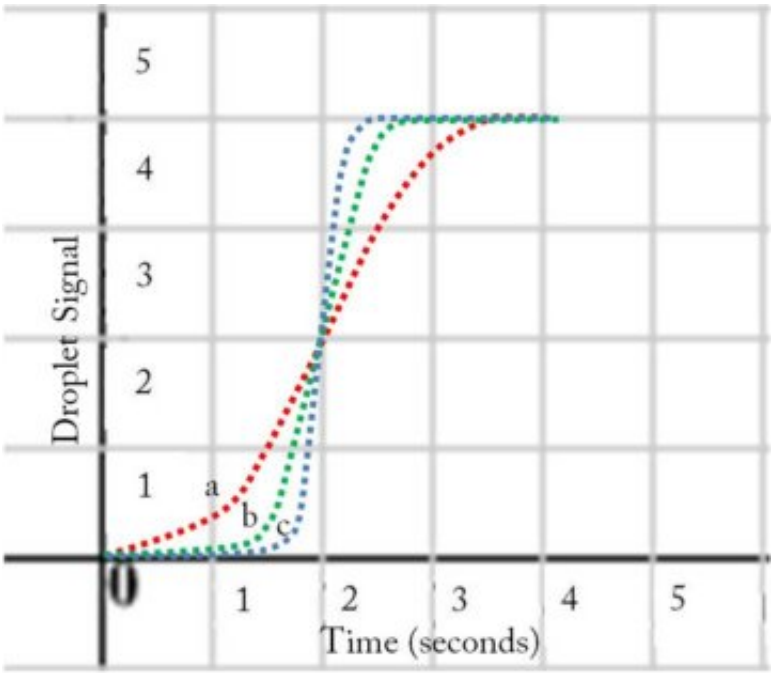


Figure 24: Signal rise-time for slow (a), medium (b), and fast (c) plug speeds

GROUP 5 ASSESSMENT: SIGNAL GENERATION AND PROCESSING

Group 5 assessment questions were based on signal generation and signal processing. It featured a sample of 4 questions in total. The questions were designed to gauge student understanding of the MATLAB-based droplet detector. Students were asked to match signal profiles to water plug size and spacing images. As mentioned in Group 4, students well understood the relationship between signal rise-time and plug velocity featured in Question 22 and 23. Difficulties with Question 19's interface in the Blackboard system yields questionable results, as students indicated the problem was difficult to interpret. Over 90% of students surveyed either agreed or strongly agreed that their competency in conducting signal analysis increased as a result of the lab. Although the sample median shown in Figure 25 remains the same between pre- and post-evaluation, lower quartile whiskers are seen to dramatically decrease to within the IQR of the pre-evaluation.

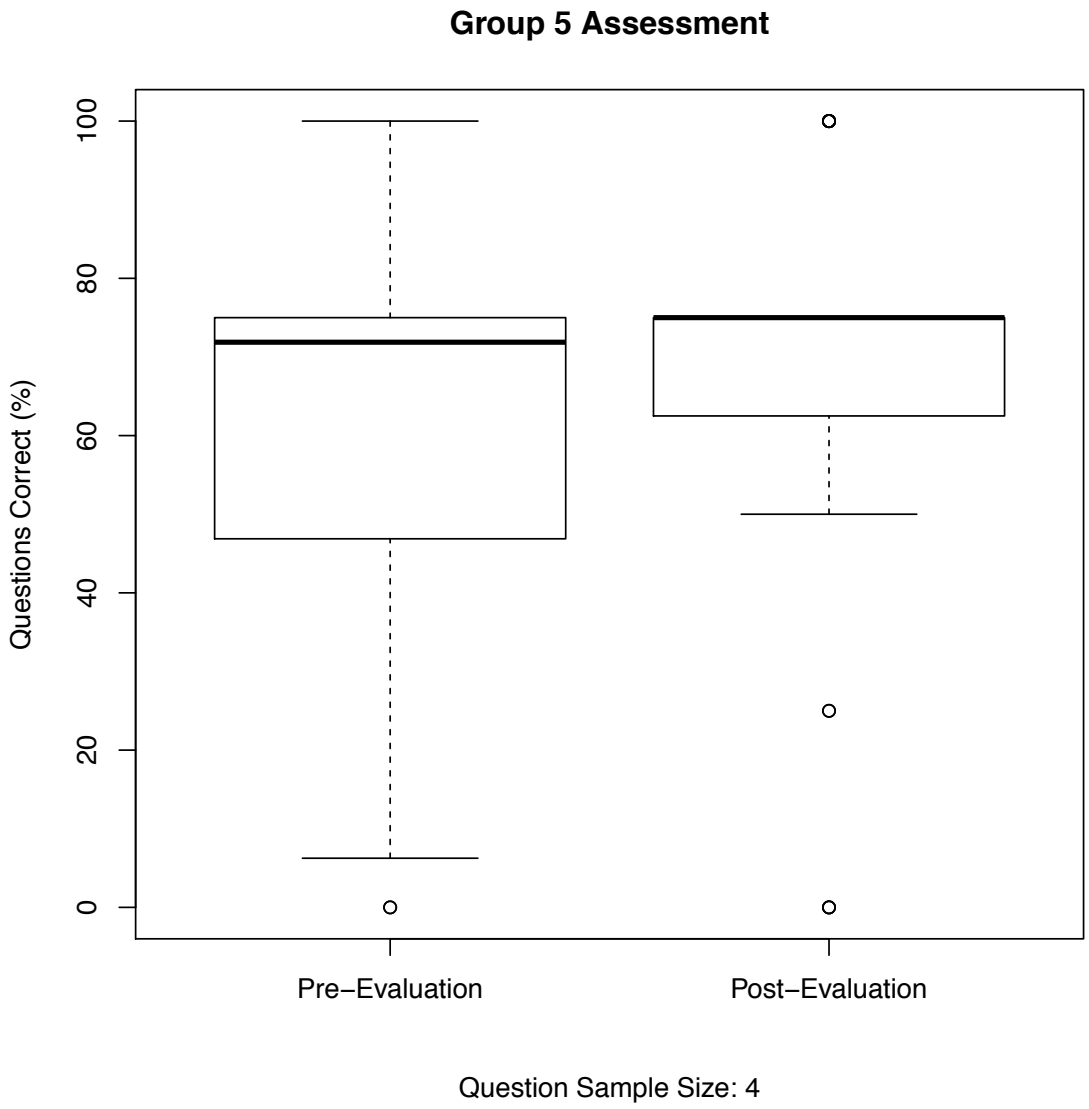


Figure 25: Evaluation results of the Group 5 assessment

Chapter 7: Conclusion

This thesis features the development and evaluation of a mass conservation laboratory module in a microfluidics environment for application in the undergraduate mechanical engineering curriculum. It offers insight into how the laws of continuity can be applied to a microfluidic T-junction device operated under two-phase liquid-liquid plug flow, and offers recommendations on how to implement this experiment into an undergraduate mechanical engineering curriculum. In addition, it provides a preliminary evaluation of the module's efficacy at teaching mass conservation fundamentals to a sample of thirty-six undergraduate mechanical engineering students at The University of Texas at Austin.

System Calibration

The theoretical plug flow model shown in Eq. 10 proves to be an accurate model for plug velocity when compared to the experimental results of the MATLAB-based droplet detector outlined in Chapter 3. As in Figure 15, the experimental results (shown in blue) track the trend predicted by the theoretical model (shown as the red line) almost exactly. A bias error exists in the experimental results, likely due to the prediction of the camera's frame rate at 44 FPS. Deviation from this value will cause experimental results to shift up or down, as shown.

The true accomplishment for the laboratory module outlined in this thesis is its ability to generate near research-grade experimental results at minimal cost. The epi-fluorescence microscope and syringe pump equipment, nominally the two most expensive pieces of equipment in microfluidic systems, were obtained at a fraction of the

price offered for “high-end” manufacturers like Nikon and Harvard Apparatus, with no real sacrifice to experimental results.

The precision error demonstrated by the experimental MATLAB-based detector is significantly smaller than the uncertainty determined through the theoretical model shown in Eq. 10. This is due, in no small part, to the introduction of surfactant into the continuous phase fluid. Controlling the Capillary number by manipulating the oil-water interface surface tension allows the experimentalist much finer control over the droplet formation process without needing to drastically change plug velocities. This action effectively minimized uncertainty in the duty cycle measurement.

The range of error of this theoretical model is significantly larger than the precision error returned by the droplet detector. This is largely due to the calibration range selected. Ideally, the range of calibration shown in Table 3 should be substantially larger than the range of experimentation shown in Table 2. Doing so will force the non-linear (i.e. parabolic) precision error prediction shown in Eq. 13 to appear linear, as the experimentation range will lie closer to the mean of the calibration range. Equipment limitations prevented such action. Stepper motors drive the NE-300 syringe pump, whose actuation at each micro-step causes “pulsing” when dispensing fluid. This pulsing introduces error into the mass calibration and establishes a lower limit at which the syringe pumps can be run. The frame rate of the camera establishes the upper limit of the calibration range, as the experiment cannot be run over 2 mg/min. Faster flow rates exceed the camera’s ability to generate a statistically acceptable (ten or more) number of points for plug velocity calculation.

BENCHMARK EVALUATION

Results from the pre- and post-evaluations for student learning suggest, with statistical significance, that the lab module had a notably positive impact on student learning of plug flow physics, mass conservation, and microscopy/micro-fabrication topics. The significance of said impacts are listed in decreasing order. Based on these results, it is reasonable to claim that this novel laboratory module succeeds in conveying mass conservation fundamentals to undergraduate mechanical engineering students and has the added benefit of introducing students to a field of fluid mechanics that is beyond the current mechanical engineering curriculum. Given the rate at which the field of microfluidics is growing, this stands to place students instructed with this module at an occupational competitive advantage in micro- and nanofabrication industries post-graduation. With improvements, this laboratory module has the potential to become a powerful mass conservation teaching-tool that requires a relatively small financial investment.

No statistically significant improvement was witnessed in either uncertainty analysis or signal generation/processing was. Given this, the laboratory module would benefit from improvements in a few areas, namely the instruction of the aforementioned topics and the implementation of the student evaluation.

First, more laboratory time may be required to allow the students to gain more exposure to the droplet detection system. Adapting the signal detection MATLAB scripts to detect droplets in real time, as opposed to the current method that processes recorded videos, would give students direct exposure the variation of plug velocity and duty cycle. This could be accomplished in conjunction with the pre-lab materials, along with a more in-depth teaching assistant lecture over statistics and uncertainty analysis fundamentals.

Second, improving the number of evaluation questions may return more realistic evaluation results. Groups 1, 2, 3, and 5 all feature fewer than 10 questions. This may influence the statistical findings shown in Table 11, as per discussion of Eq. 18. The current model utilizes the same evaluation questions in pre- and post-evaluation, which may introduce undesired learning effects into the statistical evaluation. Ideally, the pre- and post-evaluation would have similar but different evaluation questions. This might eliminate the tendency of students remembering or memorizing answers to already-seen questions. However, evaluation feedback was not provided to the students, so they had no indication of their performance on the test beyond their total score. This limitation may provide ample, but further investigation is required.

Integration of the Felder-Soloman Learning Styles Inventory (Heywood, 2005) would provide a more specific categorization of student learning results. This learning inventory, influenced by Kolb, Myers-Briggs Type Indicator (MBTI), and Witkin theories, would categorize the students among 32 learning styles. Categorizing the students involved in the module evaluation would provide the educator insight into which learning styles (i.e. active/reflective, sensing/intuitive, visual/verbal, and sequential/global) benefit the most from the laboratory module. This would also allow the educator to make changes to the module to maximize the benefit for each learning type.

Appendices

MATLAB Scripts

Oil_Water_Slug.m

```
function [  ] = Oil_Water_Slug( direc )
    tic

    %Constants
    FPS = 44;
    dt  = 1/FPS;
    Obj_mag = 10;
    Camera_mag = 1/10;
    um_per_pix = 3.2;
    Resolution = Obj_mag*Camera_mag*um_per_pix;
    Chan_dep = 0.100; %In milimeters
    Chan_wid = 0.300; %In milimeters
    Chan_area = Chan_dep*Chan_wid;

    %File Structure
    wd = direc;
    wd = [wd,'/'];
    fn = dir([wd,'*.tif']);

    %Delete Detector_Area.tif from File Structure
    for k = 1:length(fn)
        bool = strcmp(fn(k).name,'Detector_Area.tif');
        if bool == 1
            fn(k) = [];
        end
    end


    %Signal structure
        SigStruc.Signal = [];
        SigStruc.trigger_data_A = [];
        SigStruc.trigger_data_B = [];
        SigStruc.trigger_data_C = [];
        SigStruc.trigger_data_D = [];
        SigStruc.trigger_data_E = [];

    %Iterate Oil_Water_Slug for all files in directory

    for iter = 1:length(fn)

        %File Name
        mfn = [wd,fn(iter).name];
```

```
        %Detectors
        start = 1;
        info = imfinfo(mfn);
        frames = numel(info);
        threshold = 10/255;
        Detector = im2double(imread([wd,'Detector_Area.tif']));
        Detector = im2bw(Detector,threshold);
        Detector_Width = Detector_Sizer(Detector);

        % Image Data
        inc=1;
        n = 0;
        for k=start:inc:frames
            n = n+1;
            Mix_Image = im2double(imread(mfn,k)); %Read current frame
            Mix_Image = im2bw(Mix_Image,threshold); %Convert to binary
            Signal(n) = length(find(Mix_Image.*Detector)); %Find
remaing pixels that are unity
            clear Mix_Image New_Image count;
        end

        %Calculate 1st Difference Vector
        diff_stor = diff(Signal);
        dydt = zeros(1,length(Signal));
            for k=start:inc:frames-1
                dydt(k+1)=diff_stor(k);
            end

        %Calculate 2nd Difference Vector
        diff_stor = diff(dydt);
        sec_dydt = zeros(1,length(Signal));
            for k=start:inc:frames-1
                sec_dydt(k+1)=diff_stor(k);
            end

        %Data
        time = 0:dt:(n-1)*dt;
        x = transpose(time);
        y = transpose(Signal);
        z = transpose(dydt);
        sec_z = transpose(sec_dydt);



        %Export Sorted Data
        [mr_matrix,trigger_vec,Vel_vec,Duty_vec] =
mr_imagesort(Signal,dt,Detector_Width);
        Flow_out = Vel_vec*Resolution*60/1000; %In mm/min
        Data = [x,y,z,sec_z,trigger_vec];

        SigStruc(iter).Signal         = Signal;
        SigStruc(iter).trigger_data_A =
```

```
trigger_vec(1:1:length(trigger_vec),2);
        SigStruc(iter).trigger_data_B =
trigger_vec(1:1:length(trigger_vec),3);
        SigStruc(iter).trigger_data_C =
trigger_vec(1:1:length(trigger_vec),4);
        SigStruc(iter).trigger_data_D =
trigger_vec(1:1:length(trigger_vec),5);
        SigStruc(iter).trigger_data_E =
trigger_vec(1:1:length(trigger_vec),6);

        FlowData = [Vel_vec,Flow_out];
        dlmwrite([wd,'\Data\Signal
Data\',fn(iter).name,'_SignalData.txt'],Data);
        dlmwrite([wd,'\Data\Flow
Data\',fn(iter).name,'_FlowData.txt'],FlowData);
        dlmwrite([wd,'\Data\Duty
Data\',fn(iter).name,'_DutyData.txt'],Duty_vec);

        clear Signal
    end

    %Plot Data from Signal Structure
    for iter=1:length(fn)
        figure()
        hold on
        plot(SigStruc(iter).Signal)

plot(find(SigStruc(iter).trigger_data_A),SigStruc(iter).trigger_data_A(
find(SigStruc(iter).trigger_data_A)),'ro');

plot(find(SigStruc(iter).trigger_data_B),SigStruc(iter).trigger_data_B(
find(SigStruc(iter).trigger_data_B)),'bo');

plot(find(SigStruc(iter).trigger_data_C),SigStruc(iter).trigger_data_C(
find(SigStruc(iter).trigger_data_C)),'go');

plot(find(SigStruc(iter).trigger_data_D),SigStruc(iter).trigger_data_D(
find(SigStruc(iter).trigger_data_D)),'co');

plot(find(SigStruc(iter).trigger_data_E),SigStruc(iter).trigger_data_E(
find(SigStruc(iter).trigger_data_E)),'ko');

legend('Data','Trigger1','Trigger2','Trigger3','Trigger4','Trigger5',..
.
               'Location','NorthOutside','Orientation','horizontal')
        xlabel('Frame')
        ylabel('Signal Intensity (pixels)')
        title(fn(iter).name)
    end

    toc
end
```

Mr_Imagesort.m

```
function [ mr_matrix, trigger_vec, Vel_vec, Duty_vec] = mr_imagesort(
Signal, dt, D_Width)
%mr_imagesort The purpose of this function is to detect, demarcate, and
%sort dye/water droplets as they pass thru the FOV of the camera. The
first
%column of the returned matrix
%is the frame index. This column steps from the first frame to the
%last frame in the TIF file. Subsequent columns demarcate individual
%droplets. Data in these columns indicate fluid volume (in pixels).

%In addition, the final two rows in the matrix will contain data about
%droplet velocity. The upper row will contain maximum velocity detected
%for the given droplet, while the lower row will contain the frame in
which
%maximim velocity was detected.

% The inputs to this function are:
%
% FPS: Scalar. The frame rate of the camera. An estimate for the number
of
% datapoints per droplet
%
% tif_length: Scalar. Total # of frames in the tif file. FPS*tif_length
is an
% estimated
%
% Signal: Vector. Signal value returned from the script
Oil_Water_Slug.m
%
% dydt: Vector. Measures the point-to-point change of the vector
Signal.
% Effectively a numeric derivative of Signal wrt time.

%Trigger_vec return a matrix that spans the length of the number of
% %non-zero elements in the Signal. The first column indiciates the
frame
% %number, the second indicates the size (the mean of scope_dat) of
change in
% signal from zero to rise, the third indicates the size
% the mean of scope_dat) of the change in signal from rise to steady-
state
% i.e. full droplet exposure.

mr_matrix  = zeros(length(Signal)+1,round(length(Signal)/80));
Detector_width = D_Width;
start = 1;
inc = 1;
n   = 1;
p   = 1;
q   = 1;
```

```
m   = 1;
r   = 1;
d   = 1;

%Signal Detection Variables
det_cond_1 = 0;
det_cond_2 = 0;
det_cond_3 = 0;

Duty_On = 0;
Duty_Off = 0;

%Mr_Matrix Variables
mr_matrix(1:length(Signal),1) = 1:length(Signal);
vector = find(Signal);
frames = length(Signal);
det_fm_1 = 0;
det_fm_2 = 0;
det_fm_3 = 0;
det_fm_4 = 0;
det_fm_5 = 0;
max_Sig = max(Signal);

q = q+1;

%Calculate 1st Difference Vector
diff_stor = diff(Signal);
dydt = zeros(1,length(Signal));
for k=start:inc:frames-1
    dydt(k+1)=diff_stor(k);
end

%Calculate 2st Difference Vector
diff_stor = diff(dydt);
sec_dydt = zeros(1,length(Signal));
for k=start:inc:frames-1
    sec_dydt(k+1)=diff_stor(k);
end

%Populate Matrix Data

trigger_vec = zeros(length(vector),6);
trigger_vec(1:length(Signal),1) = 1:numel(Signal);
diff_vec = diff(vector);

Drop_count = 1;
for k = start:inc:(length(find(Signal))-1)

    if (diff_vec(n) == 1)
        mr_matrix(p,q) = Signal(vector(n));

        p = p+1;
```

```
    else
        mr_matrix(p,q) = Signal(vector(n));
        Drop_count = Drop_count + 1;

        if vector(n) == vector(length(vector))
            p = p;
            q = q;
        else
            p = vector(n+1);
            q = q+1;
        end
    end

    n = n+1;
end

Vel_vec  = zeros(Drop_count,4);
Duty_vec = zeros(Drop_count,1);

for k = start:inc:length(Signal)
    if (Signal(m) == 0)
        Drop_frame = 0;
    else
        Drop_frame = 1;
    end
    if(m ~= length(Signal))

        if (Drop_frame == 1 && m ~=1 )

            if(sec_dydt(m) > 0 && Signal(m-1)==0)
                det_fm_1 = m-1;
                det_cond_1 = 1;
                trigger_vec(m,2) = 1;
            end

            if(det_cond_1 == 1)

                if(dydt(m) == 0 || Signal(m) == max_Sig)
                    det_fm_2 = m;
                    det_cond_1 = 0;
                    det_cond_2 = 1;
                    trigger_vec(m,3) = Signal(m);
                end
            end

            if(det_cond_2 == 1)
                if(dydt(m) == 0 && dydt(m+1) < 0)
                    det_fm_3= m;
                    det_cond_2 = 0;
                    det_cond_3 = 1;
                    trigger_vec(m,4) = Signal(m);
```

```
            end
        end
    end

    if (Drop_frame == 0 && m ~=1)
        if (det_cond_3 == 1 && m ~= length(Signal))
            if (Signal(m) == 0 && Signal(m-1) ~=0)
                det_fm_4 = m;
                trigger_vec(m,5) = 1;
            end
            if (Signal(m) == 0 && Signal(m+1) ~=0)
                det_fm_5 = m;
                det_cond_3 = 0;
                trigger_vec(m,6) = 1;
            end
        end
    end

    if (det_fm_3 ~= 0 && det_fm_5 ~=0)
       Duty_On  = (det_fm_3 - det_fm_1)*dt;
       Duty_Length = (det_fm_5 - det_fm_1)*dt;
       Duty_vec(r) = Duty_On/Duty_Length;

       Transit_time = (det_fm_2 - det_fm_1)*dt;
       Transit_chk  = (det_fm_4 - det_fm_3)*dt;
       t_chksm      = abs(Transit_time-Transit_chk)/dt;
       Drop_vel     = Detector_width/Transit_time;
       Drop_chk     = Detector_width/Transit_chk;
       Vel_vec(r,1) = mean([Drop_vel,Drop_chk]); %Pixels/second
       Vel_vec(r,2) = Drop_vel;
       Vel_vec(r,3) = Drop_chk;
       Vel_vec(r,4) = t_chksm;

       %Use of t_chksm assumes that each velocity is accurate
within
       %1 frame, i.e. error can be equal to +-1 OR 0 frames. Since
       %t_chksm compares two variables, the effect is additive,
i.e.
       %t_chksm can equal 0, 1, or 2. No difference in velocity if
this
       %criteria holds true.

       r = r+1;

       d = d+1;
       det_fm_1 = 0;
       det_fm_2 = 0;
       det_fm_3 = 0;
       det_fm_4 = 0;
       det_fm_5 = 0;
    end
     m = m+1;
end
```

PumpCalibration.m

```
function [TrimStruc] = PumpCalibration (direc)

%File Structure
    wd = direc;
    wd = [wd,'/'];
    fn = dir([wd,'*.txt']);

%Read .txt files
for iter = 1:length(fn)
    PumpStruc(iter).name = fn(iter).name;
    PumpStruc(iter).data = dlmread([wd,fn(iter).name]);
end

% %Visualize Raw Data
% for iter = 1:length(PumpStruc)
%     figure()
%     hold on
%     plot(PumpStruc(iter).data(:,1),PumpStruc(iter).data(:,2))
%     title(PumpStruc(iter).name)
% end

%Trim/Package Data
framestart = 200;
framestop  = 800;
cutvec     = zeros(length(fn),2);

for n = 1:length(fn)
    for iter = 1:length(PumpStruc(n).data)
        cutstor  = PumpStruc(n).data(iter,1);
        if cutstor < framestart
            cutstart = iter;
        end
        if cutstor < framestop
            cutstop = iter;
        end
    cutvec(n,:) = [cutstart,cutstop];
    end
end

for iter = 1:length(fn)
    TrimStruc(iter).name = PumpStruc(iter).name;
    TrimStruc(iter).data =
PumpStruc(iter).data(cutvec(iter,1):cutvec(iter,2),:);
end

%Visualized Trimmed Data
for iter = 1:length(TrimStruc)
    figure()
    hold on
```

```
    plot(TrimStruc(iter).data(:,1),TrimStruc(iter).data(:,2))
    title(TrimStruc(iter).name)
end

%Polyfit to TrimStruc data
%Split each dataset into 10 parts to generate 10 slopes

a = 0;
b = 1;
SamSize   = 10;
index     = 60;
rho       = 1;  %in mg/uL 0.870 for Oil, 1.005 for Water
poly([1:length(TrimStruc)]) =
struct('name',[],'data',zeros(SamSize,2));

for iter = 1:length(TrimStruc)
    for k = 1:SamSize
        poly(iter).name = TrimStruc(iter).name;
        poly(iter).data(k,:) =
polyfit(TrimStruc(iter).data((a*index+1):b*index,1),TrimStruc(iter).dat
a((a*index+1):b*index,2),1);
        a = a+1;
        b = b+1;
    end
    a = 0;
    b = 1;
end

%mg/sec to uL/min
for iter = 1:length(TrimStruc)
    TrimStruc(iter).slopes = poly(iter).data(:,1)*60/rho; %to uL/min
end
```

Detector_Sizer.m

```
function [ Detector_Width ] = Detector_Sizer( Detector )
%Detector_Sizer: Used to detect the width of the detector window
%Passes detector size to mr_imagesort
%Function "bwboundaries" is a function from the image processing
toolbox
%See help file for specifics, perimeter of detected shapes is output
%clockwise

B = bwboundaries(Detector);
Window = B{1,1};

n = 1;
while Window(n,1) == Window(1,1)
    n = n+1;
    Detector_Width = n;
end
```

R SCRIPTS

Residuals Plotgen

```
##Set Number Formatting##
options(digits=4)

##Get Calibration Data, Exported in *.csv##

setwd('~/Dropbox/MTFL/Data/Flowrate Calibration/Chip
 Calibration/100 uL syringe/Oil')

 calib.data <- read.csv('RDump.csv')
 as.data.frame(calib.data)

 mass.data <- calib.data[-1,]

 dens.data <- read.csv('OilDensity.csv')
 as.data.frame(dens.data)



#Assign Variables for Computation, from Calibration Data#
 setpoint   <- calib.data[1,] ##In units of uL/min
 setpoint   <- as.numeric(setpoint)

#Assign Variables for Computation, from Density Data#
 meas.mass  <- dens.data$mass
 volume     <- dens.data$volume[1]
 per.volume <- dens.data$v_unc_percent[1]
 del.volume <- per.volume/100*volume

#Calculate Density Data Statistics#
 critical.t <- qt(1-0.05/2,length(meas.mass))
 mass.sd    <- sd(meas.mass)
 mass.avg   <- mean(meas.mass)
 del.mass   <- critical.t*mass.sd/sqrt(length(meas.mass))

 partial.mass   <- (del.mass/volume)
 partial.volume <- (mass.avg*del.volume/volume ^ 2)
```

```
density      <- mass.avg/volume
del.density  <- sqrt(partial.mass^2 + partial.volume^2)

del.rho      <-
sqrt((del.mass/mass.avg)^2+(del.volume/volume)^2)

#Calculate Calibration Statistics#
flowrate   <- mass.data/density
dims       <- dim(flowrate)
samsize    <- dims[1]*dims[2]

#Calculate Residuals#
    resid    <- array(0,c(dim(flowrate)))
    for(i in 1:length(setpoint)){
        resid[,i] <- flowrate[,i] - setpoint[i]
    }
    resid.sq <- resid*resid
    sum.sq.d <- sum(resid.sq)

#Calculate Standard Error
    s.y2      <- (1/(samsize-2))*sum.sq.d

#Calculate Sxx and Precision Uncertainty
    x.bar    <- mean(setpoint)
    s.xx     <- sum(setpoint^2) - 1/samsize*(sum(setpoint))^2
    p.y      <- 2*sqrt(s.y2*((1/samsize)+((setpoint-
x.bar)^2/(s.xx))))
    u.y      <- sqrt(del.rho^2 + p.y^2)
    u.yplus  <- setpoint + u.y
    u.yminus <- setpoint - u.y

#Calculate Uncertainty from Bias and Precision

#Export Data for MATLAB

# matlab.frame <- data.frame(Setpoint.uL.min=setpoint,
                         # Uncertainty.uL.min=u.y,
                         # del.rho=del.rho)
# write.csv(matlab.frame,file='Export for Matlab.csv')
#Generate Plots
```

```
flow.points <- as.vector(as.matrix(flowrate))
set.points  <- rep(c(setpoint),each=10)

 #Scatter Plot
     plot(set.points,flow.points,
     type="p",
     col="blue",
     cex=0.5,
     main = "NE-300 Syringe Pump Calibration Curve",
     xlab = "Setpoint Flow Rate (uL/min)",
     ylab = "Measured Flow Rate (uL/min)",
     cex.lab=1.5,
     cex.axis=1.5,
     cex.main=2
     )
     par(new=T)

 #Expected Behavior
     lines(setpoint,setpoint,
     col="red"
     )

 #Upper and Lower Precision Limits
     lines(setpoint,u.yplus,
     col="green",
     lty = 5,
     lwd=2
     )

     lines(setpoint,u.yminus,
     col="green",
     lty = 5,
     lwd = 2
     )

 #Legend
     legend("right",
     c("Data Set", "Expected Behavior", "Uncertainty Limit"),
     lty = c(0,1,5),
     col = c("blue","red","green"))
```

Evaluation Long

```
setwd('/Users/ANDREW_WK/Dropbox/MTFL/Data/Pilot Lab Data')

#Import Data

assessment_data.pre   <- read.csv("Pilot Lab Pre-Assesment
 Quiz.downloadlong.csv")
assessment_data.post  <- read.csv("Pilot Lab Post-Assesment
 Quiz.downloadlong.csv")
question_data         <- read.csv("assessment_data.csv")

#Assign Variables

data.length      <- max(dim(assessment_data.pre))
repeats.pre      <- duplicated(assessment_data.pre$Username)
repeats.post     <- duplicated(assessment_data.post$Username)
repeats          <- which(repeats.pre == F)
index            <- repeats - 1
index[1]         <- 1
samsize          <- length(repeats)-1
scores           <- data.frame()
q.number         <- 24
datamatrix.pre   <- matrix(0,ncol=q.number,nrow=samsize)
datamatrix.post  <- matrix(0,ncol=q.number,nrow=samsize)

#Populate Data Matricies -> Data Frame

for (i in 1:samsize){
 q.id                 <-
 assessment_data.pre$Question.ID[repeats[i]:index[i+1]]
 pre.score            <-
 assessment_data.pre$Auto.Score[repeats[i]: index[i+1]]
 post.score           <-
 assessment_data.post$Auto.Score[repeats[i]: index[i+1]]
 datamatrix.pre[i,]   <- pre.score
 datamatrix.post[i,]  <- post.score

}
```

```
dataframe.pre            <- data.frame(datamatrix.pre)
dataframe.post           <- data.frame(datamatrix.post)
colnames(dataframe.pre)  <- q.id
colnames(dataframe.post) <- q.id

#Sort Data by Question Category

#Group 1: Non-dimensional analysis / Flow Regimes
#Group 2: Plug flow physics
#Group 3: Microscopy and Micro-Processing
#Group 4: Uncertainty Analysis
#Group 5: Conservation of Mass
#Group 6: Signal Generation and Analysis

 #Groups
    g1 <- which(question_data$Cat.1 == 1)
    g2 <- which(question_data$Cat.2 == 1)
    g3 <- which(question_data$Cat.3 == 1)
    g4 <- which(question_data$Cat.4 == 1)
    g5 <- which(question_data$Cat.5 == 1)
    g6 <- which(question_data$Cat.6 == 1)

    g12 <- unique(c(g1,g2))
    gtot <- c(1:q.number)


 #Data by Pre-Group
    g1.pre <- dataframe.pre[g1]
    g2.pre <- dataframe.pre[g2]
    g3.pre <- dataframe.pre[g3]
    g4.pre <- dataframe.pre[g4]
    g5.pre <- dataframe.pre[g5]
    g6.pre <- dataframe.pre[g6]
    g12.pre <- dataframe.pre[g12]
    gtot.pre <- dataframe.pre

    g1.pre.totals   <- rowSums(g1.pre)
    g1.pre.percent <- g1.pre.totals/length(g1)*100

    g2.pre.totals   <- rowSums(g2.pre)
    g2.pre.percent <- g2.pre.totals/length(g2)*100
```

```
    g3.pre.totals   <- rowSums(g3.pre)
    g3.pre.percent <- g3.pre.totals/length(g3)*100

    g4.pre.totals   <- rowSums(g4.pre)
    g4.pre.percent <- g4.pre.totals/length(g4)*100

    g5.pre.totals   <- rowSums(g5.pre)
    g5.pre.percent <- g5.pre.totals/length(g5)*100

    g6.pre.totals   <- rowSums(g6.pre)
    g6.pre.percent <- g6.pre.totals/length(g6)*100

    g12.pre.totals <-rowSums(g12.pre)
    g12.pre.percent <- g12.pre.totals/length(g12)*100

    gtot.pre.totals <-rowSums(gtot.pre)
    gtot.pre.percent <-gtot.pre.totals/q.number*100

#Data by Post-Group
    g1.post <- dataframe.post[g1]
    g2.post <- dataframe.post[g2]
    g3.post <- dataframe.post[g3]
    g4.post <- dataframe.post[g4]
    g5.post <- dataframe.post[g5]
    g6.post <- dataframe.post[g6]
    g12.post <- dataframe.post[g12]
    gtot.post <- dataframe.post

    g1.post.totals   <- rowSums(g1.post)
    g1.post.percent <- g1.post.totals/length(g1)*100

    g2.post.totals   <- rowSums(g2.post)
    g2.post.percent <- g2.post.totals/length(g2)*100

    g3.post.totals   <- rowSums(g3.post)
    g3.post.percent <- g3.post.totals/length(g3)*100

    g4.post.totals   <- rowSums(g4.post)
    g4.post.percent <- g4.post.totals/length(g4)*100
```

```
    g5.post.totals   <- rowSums(g5.post)
    g5.post.percent <- g5.post.totals/length(g5)*100

    g6.post.totals   <- rowSums(g6.post)
    g6.post.percent <- g6.post.totals/length(g6)*100

    g12.post.totals <- rowSums(g12.post)
    g12.post.percent <- g12.post.totals/length(g12)*100

    gtot.post.totals <-rowSums(gtot.post)
    gtot.post.percent <-gtot.post.totals/q.number*100

# ## Plot Survey Results ##

##Note Titles and grouping are offset, due to combining 1 and 2
 for the journal#

y.limit <- c(0,100)

 #Groups 1 and 2#
    pdf('Group 1 and 2 Results.pdf')

    vec.pre  <- rep('Pre-
 Evaluation',each=length(g12.pre.percent))
    vec.post <- rep('Post-
 Evaluation',each=length(g12.post.percent))

    g12.vec.pre   <- data.frame(Cat=vec.pre, Percent =
 g12.pre.percent)
    g12.vec.post <- data.frame(Cat=vec.post, Percent =
 g12.post.percent)
    g12.boxplot  <- rbind(g12.vec.pre,g12.vec.post)

    boxplot(g12.boxplot$Percent ~ g12.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Group 1 Assessment',
          sub=paste('Question Sample Size:',length(g12)))
    dev.off()

 #Group 3#
    pdf('Group 2 Results.pdf')
```

```
    vec.pre  <- rep('Pre-Evaluation',each=length(g3.pre.percent))
    vec.post <- rep('Post-
Evaluation',each=length(g3.post.percent))

    g3.vec.pre    <- data.frame(Cat=vec.pre, Percent =
g3.pre.percent)
    g3.vec.post <- data.frame(Cat=vec.post, Percent =
g3.post.percent)
    g3.boxplot  <- rbind(g3.vec.pre,g3.vec.post)

    boxplot(g3.boxplot$Percent ~ g3.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Group 2 Assessment',
          sub=paste('Question Sample Size:',length(g3)))
    dev.off()

#Group 4#
    pdf('Group 3 Results.pdf')

    vec.pre  <- rep('Pre-Evaluation',each=length(g4.pre.percent))
    vec.post <- rep('Post-
Evaluation',each=length(g4.post.percent))

    g4.vec.pre    <- data.frame(Cat=vec.pre, Percent =
g4.pre.percent)
    g4.vec.post <- data.frame(Cat=vec.post, Percent =
g4.post.percent)
    g4.boxplot  <- rbind(g4.vec.pre,g4.vec.post)

    boxplot(g4.boxplot$Percent ~ g4.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Group 3 Assessment',
          sub=paste('Question Sample Size:',length(g4)))
    dev.off()

#Group 5#
    pdf('Group 4 Results.pdf')

    vec.pre  <- rep('Pre-Evaluation',each=length(g5.pre.percent))
    vec.post <- rep('Post-
```

```
Evaluation',each=length(g5.post.percent))

    g5.vec.pre    <- data.frame(Cat=vec.pre, Percent =
g5.pre.percent)
    g5.vec.post <- data.frame(Cat=vec.post, Percent =
g5.post.percent)
    g5.boxplot  <- rbind(g5.vec.pre,g5.vec.post)

    boxplot(g5.boxplot$Percent ~ g5.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Group 4 Assessment',
          sub=paste('Question Sample Size:',length(g5)))
    dev.off()

#Group 6#
    pdf('Group 5 Results.pdf')

    vec.pre  <- rep('Pre-Evaluation',each=length(g6.pre.percent))
    vec.post <- rep('Post-
Evaluation',each=length(g6.post.percent))

    g6.vec.pre    <- data.frame(Cat=vec.pre, Percent =
g6.pre.percent)
    g6.vec.post <- data.frame(Cat=vec.post, Percent =
g6.post.percent)
    g6.boxplot  <- rbind(g6.vec.pre,g6.vec.post)

    boxplot(g6.boxplot$Percent ~ g6.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Group 5 Assessment',
          sub=paste('Question Sample Size:',length(g6)))
    dev.off()

#Total Test Evaluation#
    pdf('Evaluation Results.pdf')

    vec.pre  <- rep('Pre-
Evaluation',each=length(gtot.pre.percent))
    vec.post <- rep('Post-
Evaluation',each=length(gtot.post.percent))
```

```
    gtot.vec.pre  <- data.frame(Cat=vec.pre, Percent =
gtot.pre.percent)
    gtot.vec.post <- data.frame(Cat=vec.post, Percent =
gtot.post.percent)
    gtot.boxplot  <- rbind(gtot.vec.pre,gtot.vec.post)

    boxplot(gtot.boxplot$Percent ~ gtot.boxplot$Cat,
            ylim=y.limit,ylab='Questions Correct (%)')
    title(main='Total Assessment',
          sub=paste('Question Sample Size:',length(gtot)))

          dev.off()
```

ME 130L
Experimental Fluid Mechanics
Background and Pre-Lab #3 - The Conservation of Mass in Microfluidics

I. Background

This lab investigates the fundamentals of the continuity equation, i.e. the conservation of mass. In addition, this lab will introduce students to the basics of fluorescence microscopy and image processing as tools for analysis on fluid systems. This is accomplished through the use of polydimethylsiloxane (PDMS) micro-channels mounted on microscope slides.

II. The Conservation of Mass

Reynold's Transport Theorem (RTT) postulates that "for a system and a fixed, non-deforming control volume" (Mills et al., 2004), there exists a governing conservation equation that quantifies all fluid parameters (whether they be mass, momentum, energy, etc.) entering and leaving the control volume. By choosing mass as the parameter of interest, it can be shown that

$$\frac{Dm_{sys}}{Dt} = \frac{\partial}{\partial t}\int_{CV} \rho \, dV + \int_{CS} \rho \vec{v} \cdot \hat{n} \, dA = 0 \tag{1}$$

Equation 1 forms the fundamental basis of the ***continuity equation***, and can be simplified further when the system is considered to be constant in time (i.e. steady-state). In this instance, the time rate of change of mass in the control volume is set equal to zero, and Equation 1 is reduced to

$$\int_{CS} \rho \vec{v} \cdot \hat{n} \, dA = \sum \dot{m}_{out} - \sum \dot{m}_{in} = 0 \tag{2}$$

In this lab, you will use these governing principles to analyze fluid flows at the sub-millimeter length scale.

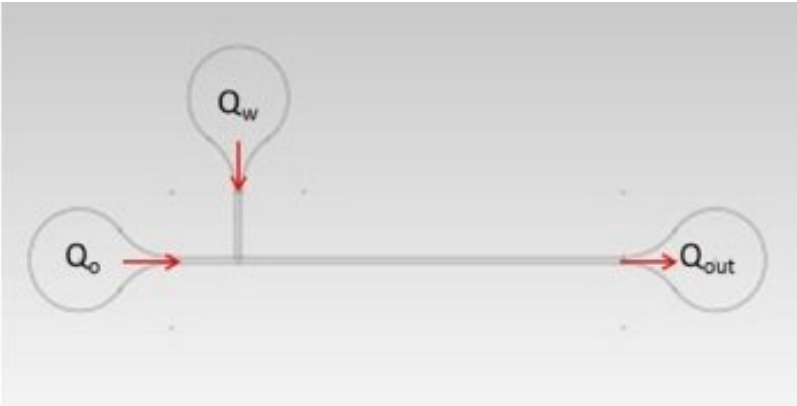


Fig. 1. Schematic of the PDMS T-junction

Consider the schematic shown in Figure 1. This graphical representation of the T-junctions to be used in the pilot lab show all inlet and exit ports available to the system. As such, it becomes fairly establish a conservation of mass relationship based on the inlet flows. The system is assumed to be lossless, i.e. all mass entering the system leaves through the exit port. Therefore, the conservation of mass relation in Equation 3 defines the system.

$$\dot{m}_{in} = \rho_{\text{water}} Q_{\text{water}} + \rho_{\text{oil}} Q_{\text{oil}} = \dot{m}_{out} \tag{3}$$

Since the oil and water are immiscible, the water will form droplets downstream of the T-junction. The oil acts as the carrier fluid, called the continuous media, while the water forms droplets inside the oil, called the dispersed media. You will use specialized optical equipment and MATLAB scripts to analyze both the downstream droplet spacing and the droplet velocity.

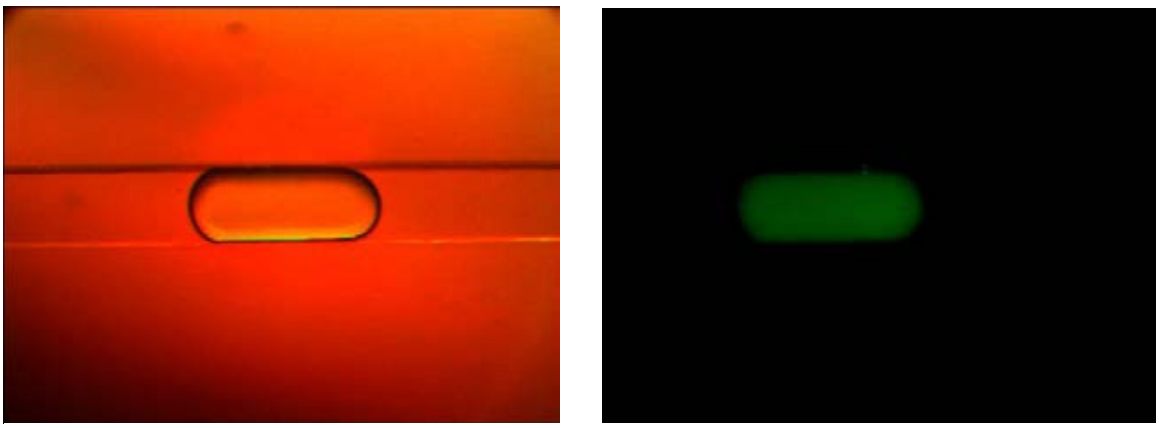


Fig 2: Water droplet plug flow visualized in brightfield (left) and epi-fluorescence (right).

The flow rate of water, Q_w, and the flow rate of oil, Q_o, are both controlled using a syringe pump setup shown in Figure 3. If a control volume is defined such that all inlet and exit flows are normal to the control volume boundary (see Equation 2), then Equation 4 may be assumed. Fluid density, represented by ϱ, is defined by the fluid. Channel geometry, A, is fixed for the microchannel. Fluid velocity, V, is a function of the syringe pump setpoint.

$$\dot{m} = \rho * A * V \tag{4}$$

Fig. 3. The syringe pump operates by advancing the plunger block at a certain velocity. Since the inner diameter of the syringe is known, this dispenses the syringe at a certain flow rate.

Syringe pumps are often calibrated to investigate their accuracy. Small fluctuations in the plunger block velocity (which stem from the type of motor used to drive the block) can cause downstream droplets to "pulse", which may be undesirable. This means that the droplets will exhibit a range of downstream velocities as opposed to one steady velocity. In order to ensure that these pulses fall within an expected range, statistical analysis must be performed on both the droplet size, spacing, and their downstream velocity.

Your goal for this lab is to investigate the relationship between the downstream droplet size-to-spacing ratio and the droplet velocity to the upstream setpoints of the oil and water syringe pumps. Given that the conservation of mass is a natural law (i.e. ubiquitous), we can combine equations based on the conservation of mass with assumptions made on the downstream droplet signal. Figure 4 illustrates a sample signal generated through MATLAB.

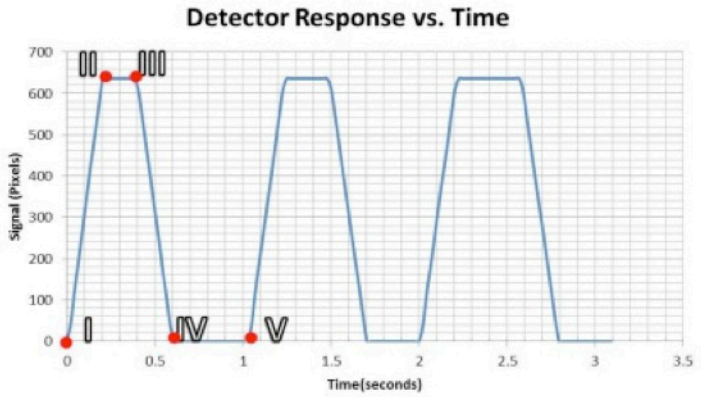


Fig. 4. MATLAB scripts are used to generate a signal based on downstream water droplets. The slope of the signal rise/fall is directly correlated to droplet velocity.

This lab defines a parameter known as the ***duty cycle***, which is simply a ratio of the time the droplet detector spends "ON" to the total period of the droplet detector. The detector operates by comparing a droplet's size to a known reference length. Since the camera speed is known, it allows MATLAB to calculate both the duty cycle and the droplet velocity fairly easily.

$$D = \frac{\tau}{T} = \frac{Time_{active}}{Time_{total}} = \frac{III - I}{V - I} \tag{5}$$

We know that this signal must be a function only of the mass flow rate of the water and the mass flow rate of the oil, so we may use the signal to generate equations for both of these rates.

$$\dot{m}_{out} = \dot{m}_{water} + \dot{m}_{oil} \tag{6}$$

$$\dot{m}_{water} = \rho_w * A * V_{drop} * D \tag{7}$$

$$\dot{m}_{oil} = \rho_o * A * V_{drop} * (1 - D) \tag{8}$$

III. Pre-lab Questions

1. What is plug flow? How does it differ from viscous flow in a household pipe? How does plug flow apply to this experiment?

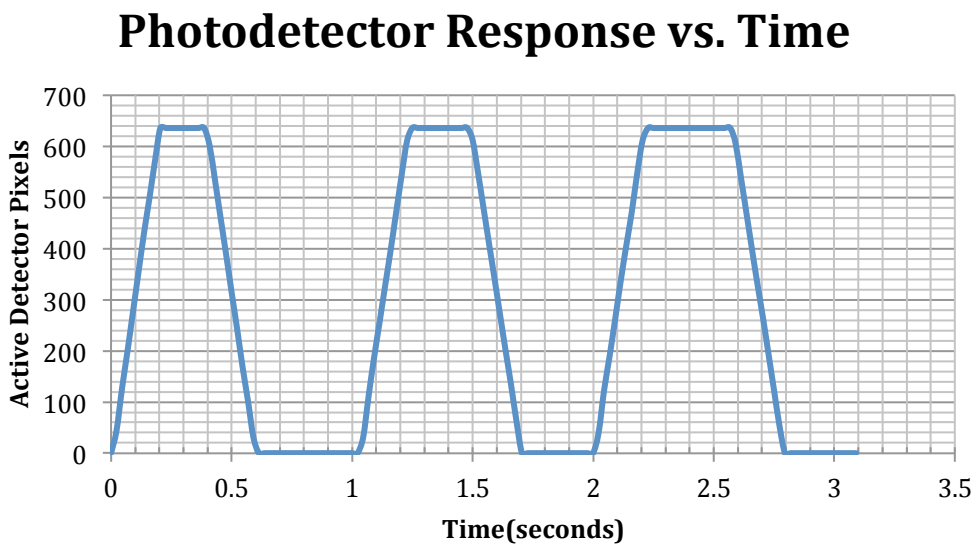
2. Why is the Capillary number used in microfluidics, as opposed to just using the Reynold's number? What methods can be used to manipulate the Capillary number?

3. Given the table below, determine what oil flow rates must be used in order to maintain a constant mass flow rate of 5 mg/min for the T-junction system described above. You may assume: $\varrho_{water} = 0.999$ mg/uL, $\varrho_{oil} = 0.84$ mg/uL

Q_{water} (uL/min)	Q_{oil} (uL/min)
0.200	
0.400	
0.600	
0.800	
1.000	

4. The plot shown below is a sample detector response for an oil-water T-junction microchannel system, downstream of the T-junction. For this system, you may assume rectangular geometry, with channel width and height of 300 μm and 100 μm, respectively. You may also assume that the droplet detector has a characteristic length of 100 pixels and that the camera detects square pixels at 3.2 μm per pixel. Given this information, determine:

- $V_{channel}$, the average downstream channel velocity
- D, the average duty cycle
- Q_{water}, the flowrate setpoint for the water syringe
- Q_{oil}, the flowrate setpoint for the oil syringe



5. It will become necessary to plot your measured data against a theoretical model in order to validate the model. Given Equations 3, 6, 7 and 8, derive a relationship for the droplet velocity V_{drop} in terms of:

- Inlet mass flow rate, $\dot{m}_{in}$
- Fluid densities, ρ_{oil} and ρ_{water}
- The duty cycle, D
- Channel geometry, A

ME 130L
Experimental Fluid Mechanics
Pilot Lab #6 - The Conservation of Mass in Microfluidics

IV. Microfluidics and Fluorescence Microscopy

The field of microfluidics considers fluid flows at very small length scales, generally on the order of tens to hundreds of micrometers. Fluids are generally constrained to microchannels molded out of ***polydimethylsiloxane*** (PDMS), a non-reactive and optically clear organo-silicon polymer. PDMS microchannels are manufactured through a photolithography and soft lithography (i.e. mold casting) process shown in Figure 2. For this lab, PDMS samples are bonded to glass microscope slides spin-coated with PDMS. This allows visualization of liquid-liquid or liquid-gas interactions at the micro-scale. Microfluidics has gained notoriety in recent years because of its wide-reaching applications not only in micro chemical reactors, DNA synthesis/analysis, and fuel cell research (to name just a few), but is also making great strides in developing next generation Lab-on-a-Chip (LOC) and micro-Total Analysis Systems (μTAS). These systems can be used to run laboratory tests at very high reagent usage efficiency and may be implemented in circumstances where full-scale laboratory equipment is cost prohibitive.

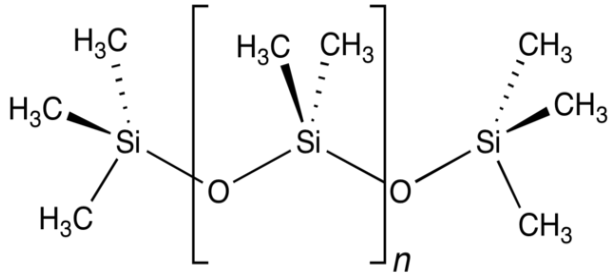


Fig. 1: Polydimethylsiloxane (PDMS) polymer structure

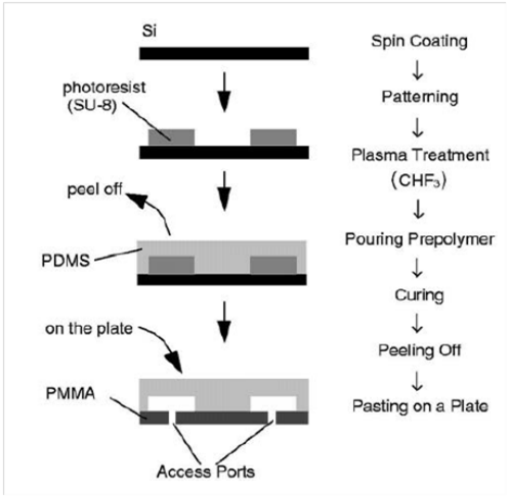


Fig. 2: Construction of the PDMS microchannel (Fujii, 2002)

In this lab you will be using the conservation of mass to analyze T-junction micro-channels, as shown in Figure 3. The left-most port is used to supply the continuous phase fluid, which wets the walls of the microchannel and forces fluid transport to the exit port. Light (i.e. low density) mineral oil will be used as the continuous phase. The top port is used to supply the dispersed phase fluid, which forms droplets inside the continuous phase.

The field of microfludics is very diverse, and it is possible to have many different flow "regimes", or patterns of fluid behavior. Flow regimes are most often controlled by varying the continuous or dispersed phase (either liquid or gas), and by varying the flow rates of the continuous or dispersed phase. In this lab, you will investigate a flow regime known as ***plug flow (or slug flow)***. In this regime, water and oil are supplied at rates such that the water "droplets" span the entire length of the microchannel to form plugs. The main difference between plug flow and a regime referred to as ***bubble flow***, where the dispersed phase forms drops or bubbles smaller than the channel width, is that **the downstream velocity of oil and water are equal to each other**.

Flow regimes are usually categorized with non-dimensional numbers. Although the Reynolds number is normally used as a non-dimensional measure of fluid velocity, it does not take surface tension effects into account, which become much more important at small length scales. The ***Capillary number***, as shown below, is a much better tool for categorizing fluid velocity. It forms the ratio of viscous fluid forces to surface tension forces. In this lab, plug flow is categorized in a region with Ca < 0.001.

$$Ca = \frac{\mu V}{\gamma}$$

Eq. 1

Deionized water mixed with a fluorescent dye (***Fluorescein-548***) will be used as the dispersed phase. The continuous phase and dispersed phase flow rates will each be controlled via syringe pumps and connected to the PDMS chip via flexible PEEK tubing. You will set various flow rates for both the continuous phase and dispersed phase and visualize the fluid behavior inside the microchannel.

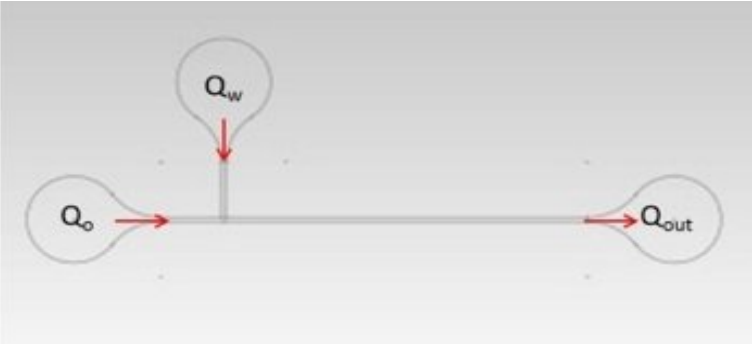


Fig. 3: This T-junction schematic was designed in SolidWorks. The continuous phase forces fluid flow towards the exit port, while the dispersed phase is injected into the continuous.

Microfluidic experiments are inherently difficult to investigate with traditional fluid measurement devices like thermocouples and Pitot-static probes. Insertion of any measurement device is likely to disrupt the droplet flow; so non-invasive means must be used. Optical techniques like fluorescence imaging are becoming more popular, especially with recent improvements of high-speed camera technology. In order to take advantage of fluorescence imaging, microscopists often utilize photo-reactive dyes called ***fluorochromes***. These dyes absorb light at a specific wavelength and almost instantaneously re-emit light of a slightly longer wavelength, i.e. lower energy. As opposed to phosphoresence, which occurs over much longer time scales, the fluorescence excitation-to-emission happens on the order of nanoseconds (Reichman, 2010). ***Fluorescein-548***, as its name would suggest, has a peak emission wavelength at about 548 nm, essentially in the middle of the green light spectrum. Therefore, blue light is required to illuminate specimens dyed with ***Fluorescein-548***.

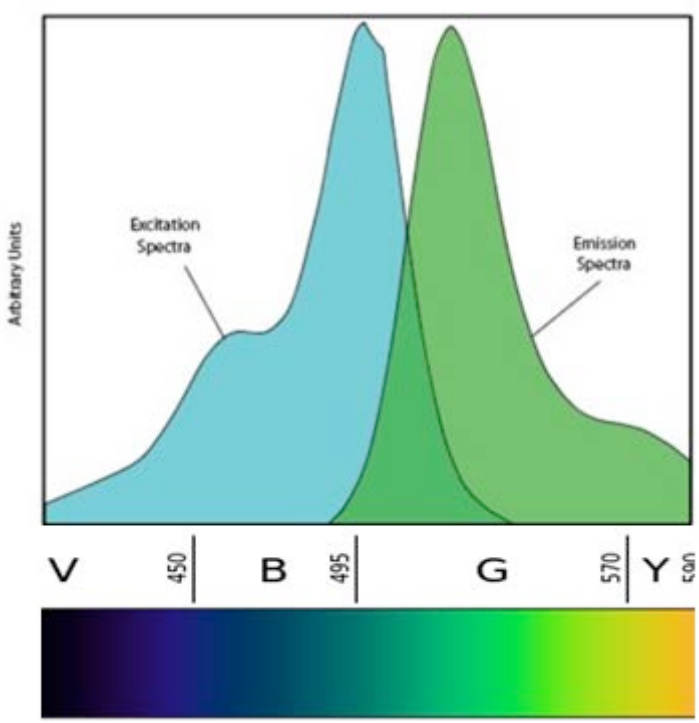


Fig. 4: The difference between the peak excitation and emission wavelength of a fluorochrome is known as the ***Stokes shift***, and is based on the quantum properties of the fluorochrome (Reichman, 2010).

In order to visualize the interaction between the oil and water inside the microchannel, a specially designed microscope known as an ***epi-fluorescence microscope*** will be used. A schematic of this microscope design is shown in Figure 6. Epi-fluoresence microscopes feature a special optical filter known as a fluorescence filter cube, or dichroic filter, that only permits light of a certain wavelength band (i.e. color) to illuminate the specimen. This color is paired with the fluorescent dye of interest. Once illuminated, the dye will emit light that can be detected through the microscope's objective. Since the water is the only fluid that contains the fluorescent dye, the water will appear fluorescent green as it passes through the microscope's field of view. The oil, on the other hand, will appear optically dark, as shown in Figure 5. Since these two fluids are immiscible, the intensity of the emitted light in a specific region of interest will vary cyclically. In this lab, we refer to this cycle as a duty cycle. We define the duty cycle in the same way as it is in electric devices, as shown in Equation 2. It is a ratio of the active signal time in a period over the total time in that period. Your goal for this lab is to find the relationship between the duty cycle (which is analogous to the water droplet size) and the downstream droplet velocity. You should find that your results match well with theory, i.e. equations derived from the conservation of mass.

$$D = \frac{\tau}{T} = \frac{Time_{active}}{Time_{total}}$$

Eq. 2

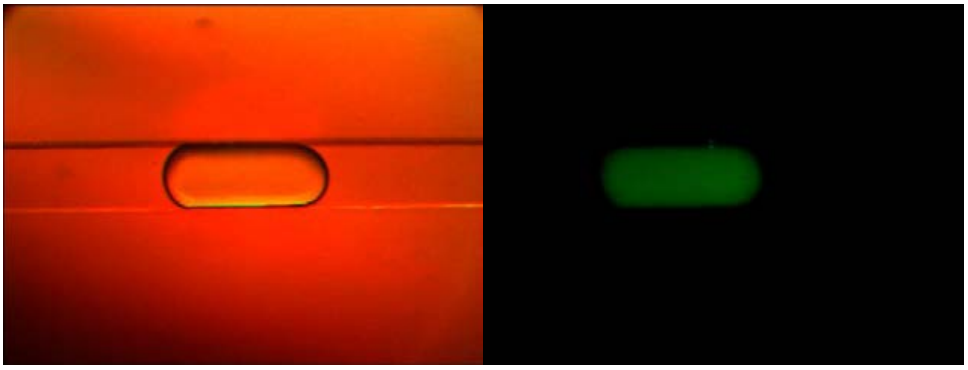


Fig. 5: Water droplet plug flow visualized in brightfield (left) and epi-fluorescence (right).

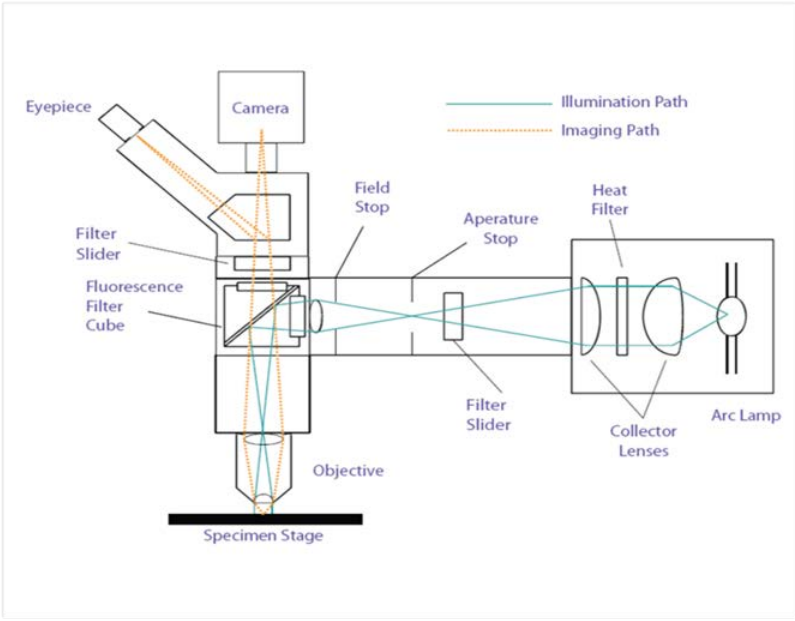


Fig. 6: Water droplet plug flow visualized in brightfield (left) and epi-fluorescence (right)

In this lab, you will use an epi-fluorescence microscope and a series of MATLAB scripts to determine a characteristic duty cycle based on input flow rates to the syringe pumps. These scripts will determine both the duty cycle and a characteristic channel velocity.

$$Q_{water} = D * ab * V_{drop}$$

Eq. 3

$$Q_{oil} = (1 - D) * ab * V_{drop}$$

Eq. 4

V. A Brief Tutorial on Statistical Uncertainty

Experimental work in fluid mechanics (or any engineering/scientific field, really) is inherently uncertain. Most often, it is the job of the engineer to both conduct experiments to gather data and conduct uncertainty analysis to ***confidently*** claim what the data suggests. For the purposes of this lab, we will be using a ***confidence interval*** of 95%, or an assurance that any measurements taken will fall within an expected range 95% of the time. This interval is based on the Gaussian (normal) distribution, as shown below.

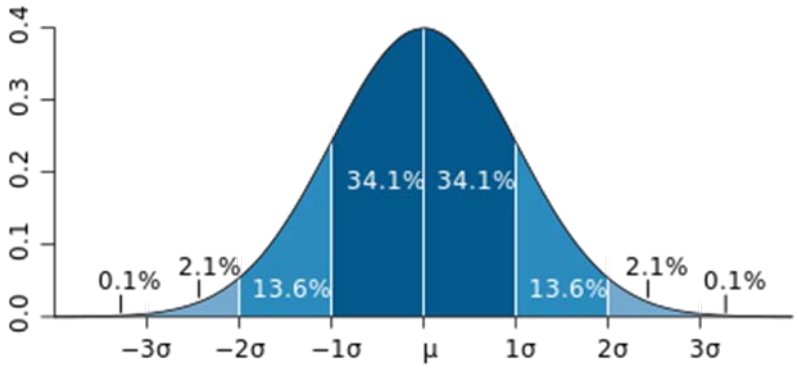


Fig. 7: A 95% confidence interval suggests that all data will fall within ±2σ, or two standard deviations away from the mean value

A. Sample Mean and Standard Deviation of Multiple Measurements

When experiments are conducted multiple times (in respect to this lab, this means measuring multiple droplets at the same flow rate), we can obtain a sample of values.

The two main statistics used to describe a scatter of data are the ***sample mean*** and the ***sample standard deviation***. Sample mean, shown below, illustrates the average value from n number of samples. Sample standard deviation illustrates the scatter of the measured data AROUND the sample mean.

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

Eq. 5

$$S_x = \sqrt{\left[\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2\right]}$$

Eq. 6

The standard deviation found in Equation X is a powerful tool for measuring uncertainty when coupled with the ***standard deviation of the mean***, found in Equation X. The standard deviation of the mean is a measure of how the **sample mean** can be expected to vary over n number of samples. As such, the accuracy of the standard deviation of the mean will increase with the number of measurements taken. This will be used to determine your interval of uncertainty, shown in Equation 5.

$$S_{\bar{x}} = \frac{S_x}{\sqrt{N}}$$

Eq. 7

$$Uncertainty = U = \pm 2S_{\bar{x}}$$

Eq. 8

These Equations will be useful in determining your uncertainty intervals for both the duty cycle and the droplet velocity. In order to generate an uncertainty interval for syringe pump calibration, refer to Section B.

B. Residuals, Linear Fits, Precision Uncertainty and Equipment Calibration

The job of an experimentalist is to ask two main questions. First, "If I change X, how does that impact Y?". For example, if I change the flow rate set point of a syringe pump, how does that change my measurement of flow rate? Second, "What is the relationship between X and Y?", i.e. can I make an equation that gives me Y based on X? In respect to this lab, one would hope for a direct, linear relationship between the syringe pump set point and the resulting measured rate.

When given a scatter of data for calibration, the most simple option is to make a linear curve fit to that data. For this lab, we expect a syringe pump setting to linearly correspond to a measured flow rate, even if that flow rate seems to fluctuate a bit.

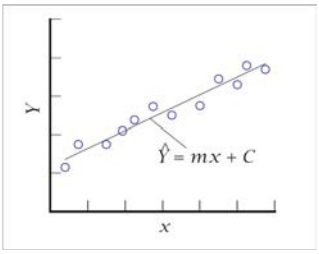


Fig. 8: Excel and MATLAB are both powerful tools for creating linear regressions. This manual will assume you are capable of generating these regressions on your data

$$\hat{Y} = mx + C$$

Eq. 9

Equation 6 is an idealized relationship between your independent variable (flow

rate set point) and your dependent variable (measured flow rate). Each data point in the data set will likely differ from the idealized regression by a little bit. This small difference between the measured data and the regression is called a residual, for obvious reasons. Residuals are used as a measure of how far off the regression is from representing your data set as a whole. They can be summed up and squared to generate a single metric, as shown in Equation 7. The residuals can then be used to generate the standard error of the estimate, also shown below. The standard error of the estimate is analogous to the standard deviation of a dataset.

$$\sum {R_i}^2 = \sum (Y_i - \hat{Y}_i)^2 \qquad \text{Eq. 10}$$

$$S_Y = \left[\frac{1}{N-2}\sum R_i^2\right]^{1/2} \qquad \text{Eq. 11}$$

VI. Laboratory Procedure and Guidelines

All students using the microscope and syringe pumps **MUST** wear gloves and a lab coat!

DO NOT ATTEMPT TO REFILL THE SYRINGES YOURSELF, THE TA WILL DO THIS. Take care to always run the pumps with fluid in the syringe. If the syringes go below 10 uL in volume, **STOP THE PUMPS AND INFORM YOUR TA SO THEY CAN REFILL THE SYRINGE. DO NOT DO THIS YOURSELF**.

The syringes are very delicate and will break if not handled properly. There are no syringe replacements. **EVERYONE IN YOUR LAB GROUP WILL RECEIVE A ZERO IF YOU BREAK THE SYRINGES.**

Swing the brightfield focusing lens out of the way. Mount a CLEAN T-junction chip on the microscope. Use the plexiglass slide holder and screws to hold the slide in place. Do not overtighten the slide holder. It's made of plexiglass and can break. After the slide is in place, swing the focusing lens back into place over the slide.

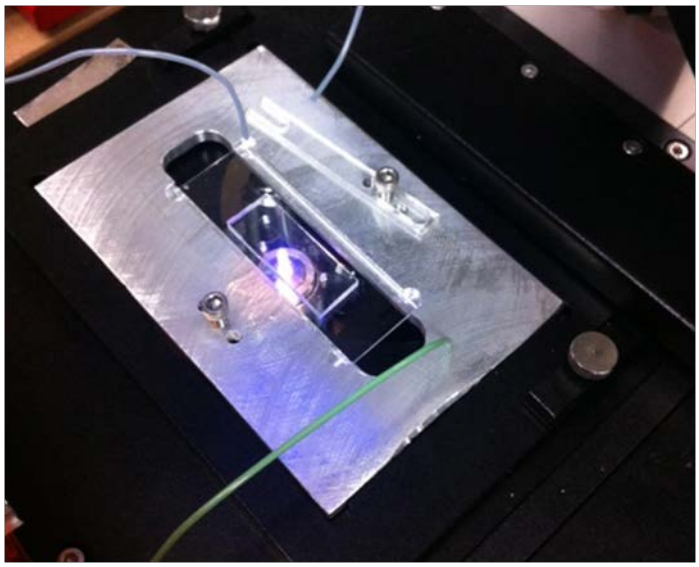


Fig. 9: T-junction must be oriented with the water leg facing the user. Take care to not overtighten the slide holder.

Switch on the power for both the bright field lighting (green switch on the right side of the microscope) and for the epi-fluoresence (tan box marked "Power Supply"). Allow the epi-fluorescence a few moments to come up to power. You should see blue light coming up thru the objectives, and the power supply will draw about 3.5 amps when fully warm. **ALLOW AT LEAST 15 MINUTES OF USE BEFORE POWERING OFF THE POWER SUPPLY**. Cycling power to the bulb can damage it, and will cause it to burn out.

Although power to the epi-fluorescence bulb cannot be controlled, the knob to the right of the bright field switch controls the bright field intensity. Be careful using this, as the bulb can be very bright if turned up too high and can hurt your eyes.

Once the bulbs have come up to power, you may attach the tubing from the syringes to the microfludic chip. Oil will be attached on the left-most branch, water will be attached on either the top or bottom-most branch (depending on the slide orientation). You must also attach the green purge tubing to the long leg of the chip.

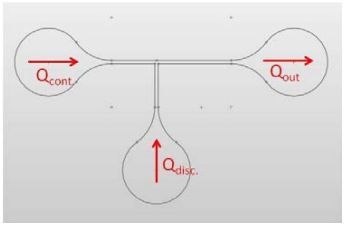


Fig. 10: Ensure connections to the T-junction are correct. Oil is connected as the continuous phase, water is connected as the discrete phate. The purge tubing is attached on the far right.

Once all tubing is in place, make sure the USB camera is connected to the computer. The trinocular control arm is used to redirect light from the eyepiece to the camera. If it is pushed in, you can visualize the flow through the eyepiece. If it is pulled out, light is redirected to the camera.

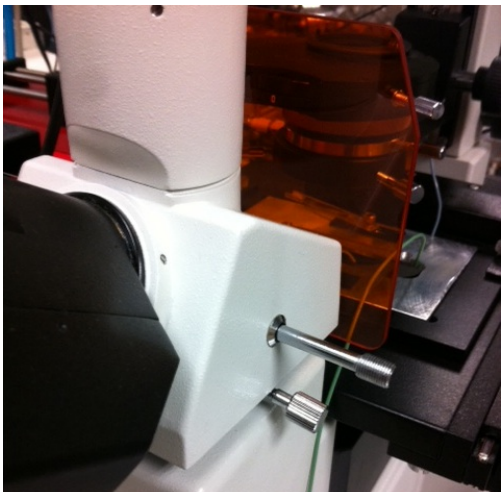


Fig. 11: The trinocular arm control directs light to the eyepiece (if pushed) or to the camera (if pulled).

After everything is in place, turn on the power to the syringe pumps by the switch located on the back of each pump. Ensure the syringe diameter is set to **1.457 mm**. This is accomplished by pressing the "Diameter" button and using the arrows located underneath the display to change values. Pressing two arrows at the same time relocates the position of the decimal point. Syringe pump flow rate is controlled using the "Rate" button.

Fig. 12: The syringe pump is turned on/off using the Start/Stop button. Press once to run, and once to stop the pump.

Ensure that you select the "Rate" button before running the pump. Don't accidentally leave the pump on the diameter setting and try to change the flow rate this way. You will get erroneous measurements and run the risk of rupturing the PDMS bond.

If not already logged into the student account, click on the "Student" icon. Input "studentpass" as the password, case sensitive.

On the Desktop, copy the folder labeled "Student Dummy Folder". Open "ME 130L Student Data" and paste a copy of the dummy folder. Rename the folder to something that UNIQUELY identifies your group.

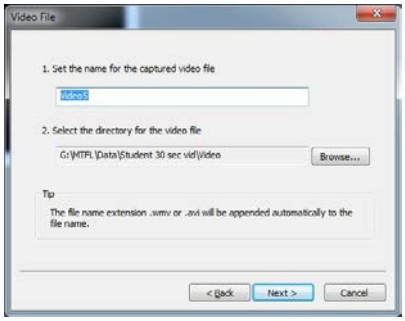


Fig. 13: The naming convention is required for both the MATLAB scripts to work properly.

Open the program "ScopePhoto" located on the Desktop. A blank window will open. Select the "ScopeTek DCM310" from the "Live Capture" dropdown menu on the toolbar. A window will pop up displaying the camera image. Pull the trinocular control arm out to calibrate the camera.

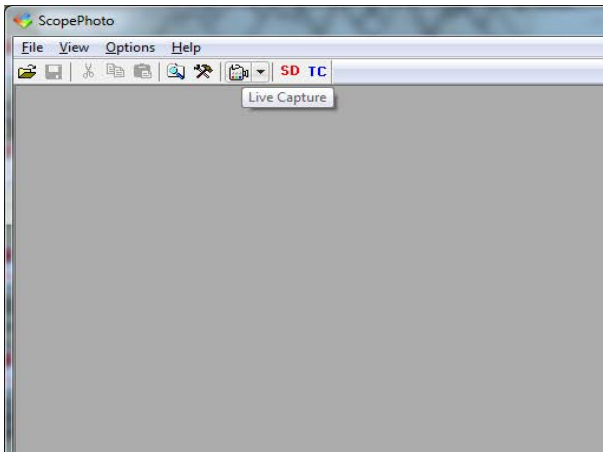


Fig. 14: If an error message pops up, unplug the USB camera, close ScopePhoto, re-plug the camera, and restart ScopePhoto. Ensure you are seeing live video feed before proceeding.

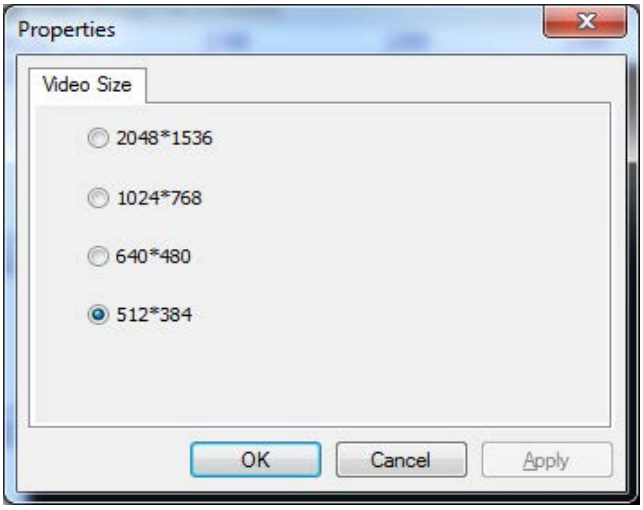


Fig. 15: Image resolution.

Once the camera is collecting live feed, press "Setup" and select "Video Stream Format". Choose 512 x 384 as your image resolution. Press OK.

After making all connections and ensuring all settings are correct, set both the oil and the water syringe pumps to 5 uL/min and start the pumps. The chips will begin to fill with fluid and come to steady state after a few moments.
After about a minute or two, ramp the syringe pumps down to 1 uL/min. Take this time to visualize what is going on inside the microchannel. Anomalies may occur due to air being trapped inside the channels. Just allow the chip to come up to steady state. You should also visualize the T-junction to ensure that the droplets are "breaking" at the 90 degree bend at the junction. Inform your TA if this is not the case.

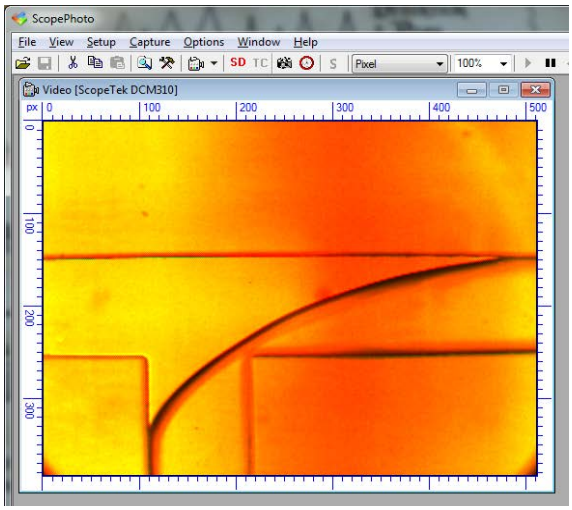


Fig. 16: Surface tension properties of the oil have been modified slightly to ensure droplets will break at the 90 degree junction. Inform your TA if droplets are not forming in this manner.

At this point, you should move the stage downstream so you can visualize the droplets moving down the microchannel. Reposition the stage such that the microchannel is CENTERED IN THE MIDDLE OF THE FIELD OF VIEW. It is imperative that this is done correctly, as the MATLAB scripts will miscalculate duty cycle and droplet velocity if this is done incorrectly. You will also need to ensure that the camera is adjusted so that the channels appear straight, and not pitched up or down. Gently twist the camera body to adjust the image rotation so that the channel is as parallel as possible.

Make sure to use the focusing knob on the right of the microscope to finely focus your plug image. You want to have as clear an image of the plugs as possible.

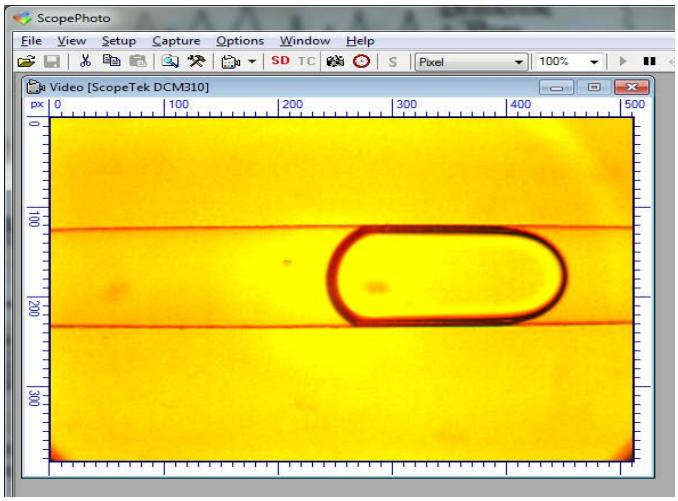


Fig. 17: Align the channel so that it appears straight. Use the pixel markers on the left and right side of the window to accomplish this. This is most easily done with the brightfield on.

Once you have centered the channel and aligned it properly, **DO NOT MOVE THE STAGE**. The droplets are sensitive to movement, and it takes a little time for the channel to come back to steady state after movement. You may also switch off the brightfield lamp, as you will not need it for imaging.

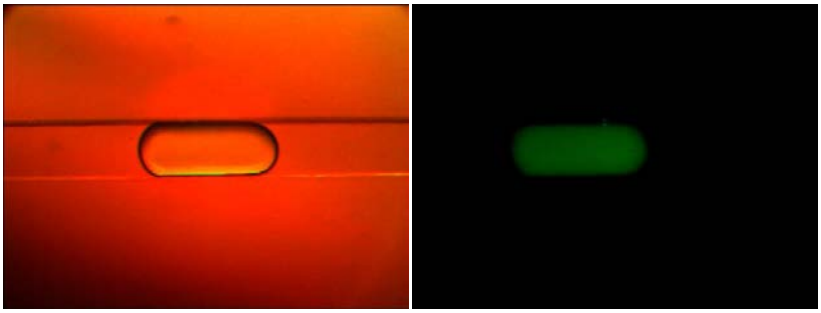


Fig. 18: Image processing can only be accomplished in fluorescence imaging. Don't forget to switch off bright field when you're ready to start!

Now that your droplets are at steady state and the microscope/camera is lined up, you may begin to take video recording.
Select "Capture" -> "Start Capture Video". Select wmv/asf as your video format. Your file naming convention is as follows:
File naming convention:
"MMDDYYYY"_"TIME"_300_um_"OilFlowRate"_oil_"WaterFlowRate"_water
i.e.
03192013_555_300_um_2_143_oil_0_200_water
When selecting your video directory, choose browse and locate your unique folder in the "ME 130L Student Data" folder. Make sure to save your videos in the "Video" folder.

Each video recording should only be 30 seconds each. Use your phone or a clock to time each video. Videos can be stopped by clicking "Capture" -> "Stop Video Capture".

Your videos will investigate oil/water plug flow at a constant mass flow rate of 2 mg/min. Each video recording must use the naming convention above, as your data will be amassed with other students. This will be used to illustrate the effect sample size has on statistically uncertain measurements.

Water Flow Rate (uL/min)	Oil Flow Rate (uL/min)
0.200	2.143
0.400	1.905
0.600	1.667
0.800	1.430
1.000	1.192

When changing your flow rate settings, allow a minute to pass to allow the chip to come to steady state. **Keep in mind that you must use gloves and a lab coat to handle any of the microscope, pump, or microfluidic device equipment.**
Once all 5 videos are recorded, turn off the pumps, disconnect the tubing from the device, and loosen the slide holder. Each experiment requires a "fresh" chip, so remove the device and place it in the "USED" petri dish.
Open MATLAB, located on the Desktop. The "Current Folder" directory should be set to "**C:\Users\student\Documents\MATLAB\ME 130L COM Scripts**". Select "DO NOT EDIT" and right click. Highlight "Add to Path" and select "Selected Folders and Subfolders". If it is not already clear from the naming scheme, **DO NOT EDIT THE MATLAB SCRIPTS**. Your TA can provide you a copy of the script if you have interest in learning more about the droplet detector algorithm.
NOTE: Make sure to close any open folders that display your video files before running MATLAB scripts. Windows 7 returns folder permission errors if you try to run processes with the folder open.
Type "VidSwapGUI" into the MATLAB command line and press enter. A prompt will ask you to locate your UNIQUE video files. Choose the folder where your files are located and click OK.
VidSwapGUI may take as long as 20 minutes to run. The program transcodes the .WMV video files into a stack of .TIF image files that MATLAB will use for image processing.
After VidSwapGUI finishes running, locate your transcoded files in the same directory as your videos. Select the image files and use Ctrl-X to cut them. Locate the "Tif Files"

folder in your directory and paste the images here. They will share the folder with a folder named "Data", and another image called "Detector_Area".
Now run the command "DropletGUI". Computation time should take about minute. MATLAB will open figures based on the data from your 5 videos, as shown below. In addition, it will export data (raw signal data, droplet velocity data, and duty cycle data) into the "Data" folder. Feel free to save the figures for your own use, or just recreate them in MATLAB. They will be marked with "Trigger" points to show where the code parses the signal into duty cycle and velocity calculations
And that's it! Make sure to save a copy of your data to a flash drive. You should leave the lab with:

- 5 video recordings of 2 mg/min plug flow at various oil/water ratios
- 5 transcoded .TIF files that MATLAB uses to run calculation
- Data files in each of the "Data" subdirectories:
    - Flow Data
    - Duty Data
    - Signal Data
    - Note: Raw Data will be empty

VII. Deliverable Requirements

<u>Objective</u>: You are to determine the relationship between upstream flow settings at the syringe pump to the downstream signal generated by the fluorescent water plugs. After conducting signal analysis, you should be able to claim (with certainty) if your experimental findings agree with theoretical models derived from the conservation of mass.

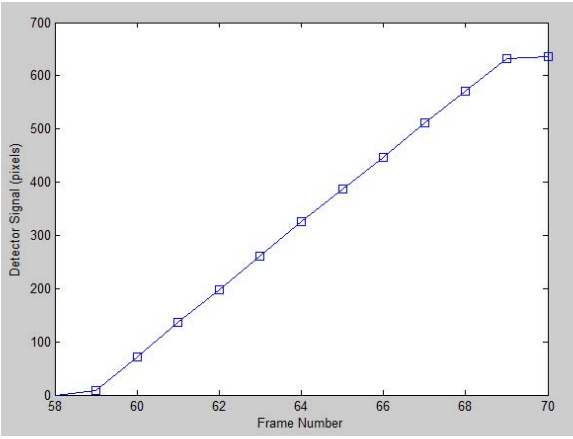
1. Given the information in Section II about measurement uncertainty, determine the known range of accuracy of the syringe pump for both oil and water experiments. This data should be made available to you by your TA. You will need to:

A. Generate a linear regression based on the mean setpoints. This regression acts as a comparison of the input setpoint to the measured flow rate.
B. Calculate the residuals from the mean (i.e. the difference between the measured data and the regression) for each setpoint.
C. Calculate the Standard Error of the Estimate (SEE), which is used as a measure of the total uncertainty of the regression.
D. Use the SEE as your uncertainty in the syringe pump.

2. Use your data found in the "Signal Data" *.csv files to generate a single plot of Frame Number vs. Signal Response. Overlay signals from all five syringe pump set points on one plot. Normalize the signals such that all plots start at signal equal to zero at Frame 1, and go to the signal's first maximum value (max value will be the same for all experiments). An example is shown below. You can use this to compare

the slopes (i.e. droplet velocity) of each experiment. Your plot will have 5 curves. Please include data points and a legend.

A. Thought question: How does the droplet velocity change as the ratio of water flow rate to oil flow rate ($^{Q_w}/_{Q_o}$) changes? How does this make sense, intuitively?
B. The information given by the signal alone is not enough to determine droplet velocity. What additional information will you need to manually calculate droplet velocity?



3. Create a plot of average duty cycle vs. average droplet velocity for all five flow settings. Use the standard error method to generate uncertainty in both the duty cycle (X) and droplet velocity (Y) directions. Plot your data with error bars. If you plan on using MATLAB, consider downloading the *herrorbar.m* file from MatlabCentral, as MATLAB does not plot horizontal error bars natively.

A. Comment on the size of your error bars. How does the uncertainty of your measurements change as duty cycle changes?
B. Suppose you're running this experiment for an employer. How might you change the accuracy of your results if: You had a large spending budget to work with? If you had a very tight budget to work with?

4. The pre-lab document asks for you to derive a relationship for droplet velocity as a function of duty cycle, input mass flow rate, geometry, and fluid properties. Use the following constants to generate a plot of theoretical droplet velocity vs. duty cycle from D = 0 to 0.6. Overlay this plot on your data.

- $\dot{m}_{in} = \rho_{oil}Q_{oil} + \rho_{water}Q_{water}$ = 2 mg/min
- A = (0.100 mm X 0.300 mm) = 0.030 mm^2
- $\rho_{oil} = 0.84\ mg/uL$ AND $\rho_{water} = 0.99\ mg/uL$
- Note: 1 uL = 1 mm^3

A. Remark on the comparison between your experimental data and the curve generated by theory. Do all experimental points follow theory within reasonable error? Explain any anomalies you find.

5. Now generate curves of droplet velocity based on the standard error of the estimate calculated in Equation 1. This will give you a lower bound and upper bound estimate of your velocity vs. duty cycle curve, and helps illustrate the effects of the uncertainty of the syringe pump equipment. Overlay these plots on your data.

And that's it! Make sure to take the post-evaluation exam after thoroughly answering these questions, and don't forget to give feedback. Thanks so much for electing to help in this lab development program!

ASSESSMENT QUIZ

This lab investigates the fundamentals of the continuity equation, i.e. mass conservation. In addition, this lab will introduce students to the basics of fluorescence microscopy and image processing as tools for analysis on fluid systems. This is accomplished through the use of polydimethylsiloxane (PDMS) micro-channels mounted on microscope slides. Figure 1, shown below, features a schematic for a rectangular T-junction micro-channel used for fluid flow. The geometry of the channel is known. Oil is supplied to the left branch at a flow rate Q_o. Water is supplied at the top branch at a flow rate Q_w. Fluid densities are such that $\varrho_{oil} < \varrho_{water}$. The two liquids can be assumed immiscible. Once downstream of the T-junction, oil and water will flow towards the exit at a flow rate Q_{out}.

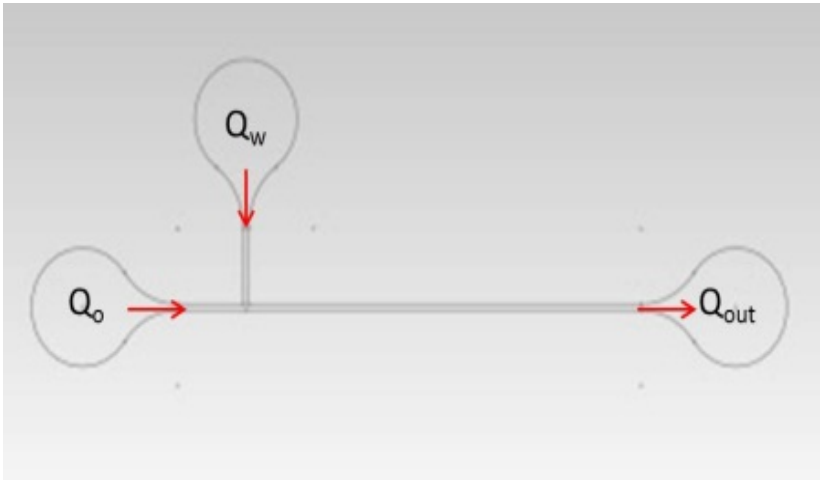
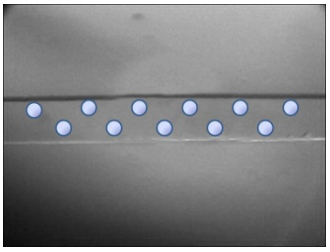
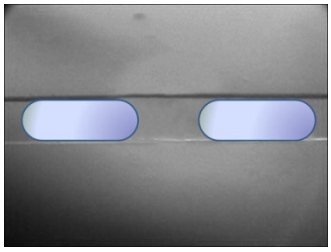
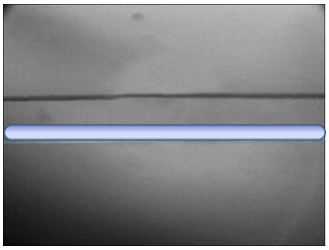
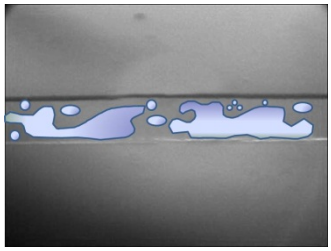


Figure 1: T-Junction schematic

1. What two fluid forces does the Capillary (*Ca*) number compare?
    a. Inertial forces to viscous forces
    b. Inertial forces to surface tension forces
    c. Viscous forces to surface tension forces
    d. Pressure forces to inertial forces

2. Assuming two-phase flow with oil and water, which flow regime best features **small** (<0.1) Reynolds and Capillary numbers?

a.		b.	
c.		d.	

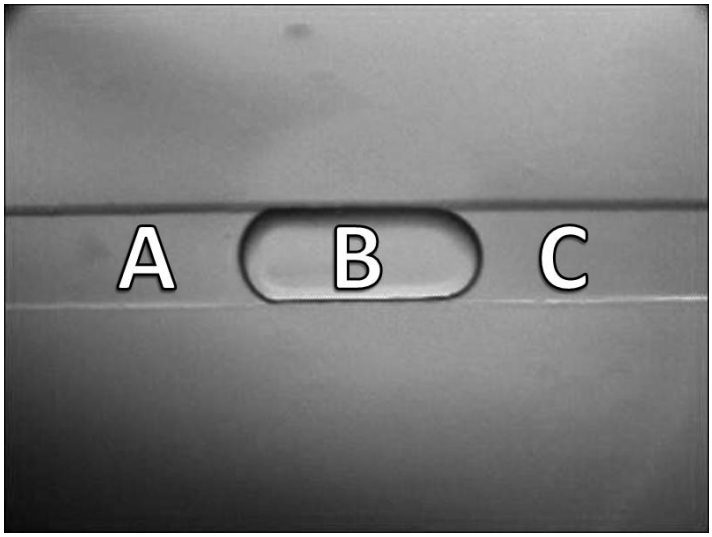


Figure 2: Microscope image of oil and water in steady-state plug flow, with sections A, B, and C labeled. The plugs will translate to the right.

3. For the plug flow shown in Figure 2, which sections (A, B, or C) represent oil?
    a. Section A
    b. Section B
    c. Section C

d. A and C
e. B and C

4. For the plug flow shown in Figure 2, which sections (A, B, or C) represent water?
    a. Section A
    b. Section B
    c. Section C
    d. A and C
    e. B and C

5. For the plug flow shown in Figure 2, Section A has a higher velocity than Section C.
    a. True
    b. False

6. For the plug flow shown in Figure 2, Section A has a lower velocity than Section B.
    a. True
    b. False

7. A dyed specimen is to be imaged with an epi-fluoresence microscope using a blue light source. Which of the following colors CANNOT be emitted by the dyed specimen?
    a. Blue
    b. Yellow
    c. Red
    d. A and B only
    e. A and C only
    f. None of the above

8. An elastomer is mixed with a curing agent and poured over a microchannel mold. This process is an example of:
    a. Soft lithography
    b. Nanoimprint lithography

c. Photolithography
d. Macrolithography

9. PDMS is most often used in microfluidic applications because:
    a. It is naturally hydrophillic
    b. It is easy to bond
    c. It's ability to mold fine microstructures
    d. A and B only
    e. B and C only
    f. A, B, and C

10. Mass flow rate is a function of fluid density, bulk fluid velocity, and cross-sectional area. Given the following information, what is the uncertainty of the mass flow rate? Density: 0.998 +/- 0.001 mg/uL, Cross-section: 1.667 +/- 0.001 mm^2, Velocity: 1.000 +/- 0.080 mm/min. NoteL 1mm^3 = 1 uL

11. Fluid is supplied to a microchannel through a syringe pump driven by a stepper motor. This induces small pressure fluctuations in the supplied fluid, What type of error will this introduce into downstream velocity measurements?
    a. Precision errors
    b. Bias errors

12. The cross-sectional area of the microchannel is slightly smaller than expected. What type of error will this introduce into downstream velocity measurements?
    a. Precision errors
    b. Bias errors

13. During lab prep, the TA accidentally warmed the water sample to body temperature by over-handling the sample. What type of error will this introduce into downstream velocity measurements?
    a. Precision errors
    b. Bias errors

14. Given the T-Junction system described in Figure 1, which of the following equations are true?

a. $Q_{out} = Q_{oil} + Q_{water}$

b. $\dot{m}_{in} = \dot{m}_{out}$

c. $\rho_{total} = \rho_{oil} + \rho_{water}$

d. $\dot{m}_{out} = \rho_{oil}Q_{oil} + \rho_{water}Q_{water}$

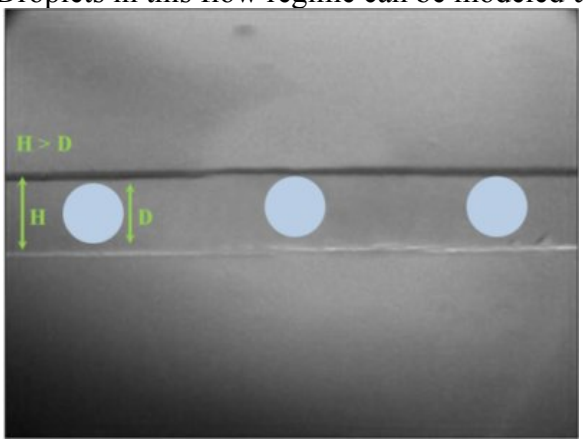
e. A and C only

f. B and D only

g. A, B, and D only

15. Provided all other variables remain constant, which of the following will increase droplet velocity?
    a. Increasing cross sectional area
    b. Decreasing total mass flow rate
    c. Increasing camera magnification
    d. A and B only
    e. None of the above

16. Droplets in this flow regime can be modeled using the plug flow assumption



    a. True
    b. False

A steady-state T-junction flow of oil and fluorescent water is recorded on camera. An imaging algorithm with an active detection region of known length is used mid-channel to generate the signal vs. time plot shown below

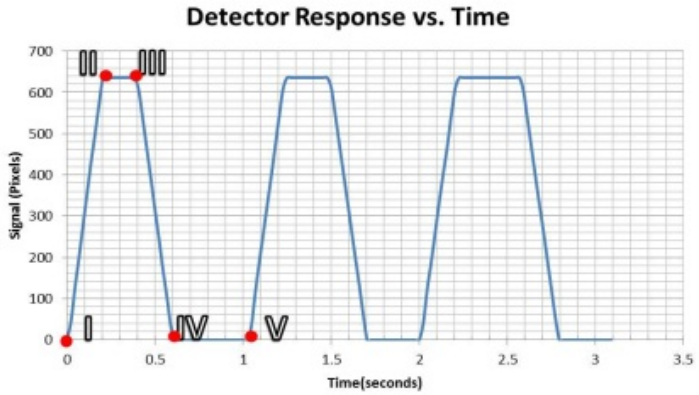
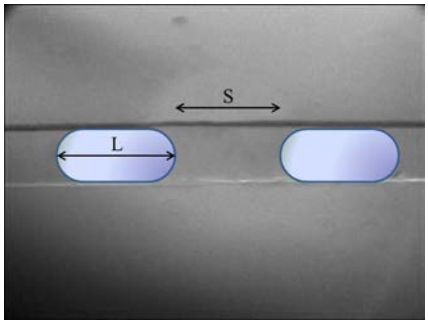


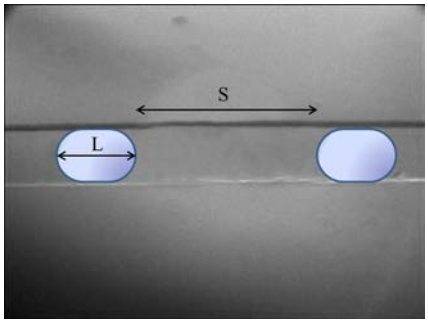
Figure 3: Signal generated after image processing

17. Channel velocity can be determined by using points
    a. III and IV
    b. II and III
    c. I and II
    d. A and C only
    e. B and C only

18. Use Figure 3 as reference. Droplet length is measured using L. Droplet spacing is measured using S. Droplet period, i.e. front-to-front droplet distance, is equal to L + S. Which of the following answers illustrates the ratio of droplet length, L, to droplet period, L + S
    a. From II to III and from IV to V
    b. From I to II and from III to IV
    c. From I to III and from III to V
    d. A and B only
    e. None of the above

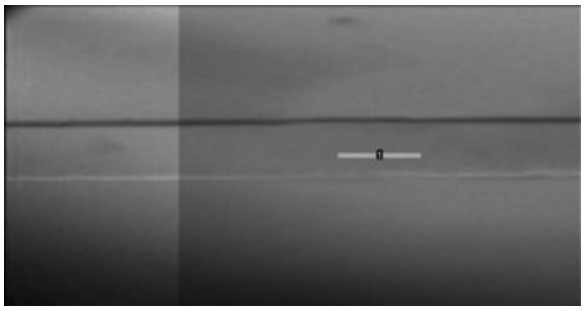


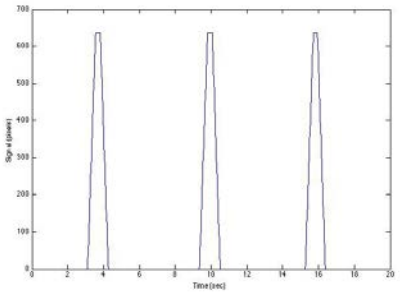
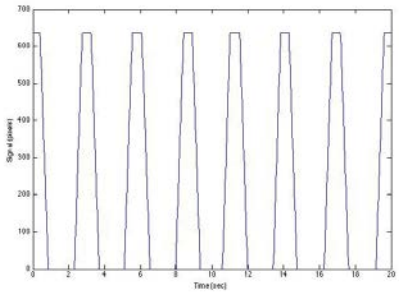
Larger droplets, smaller spacing

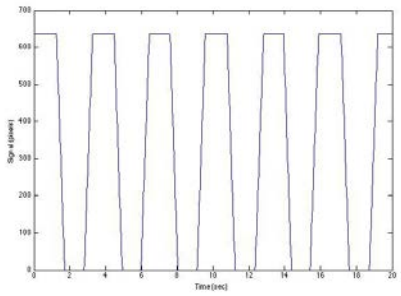


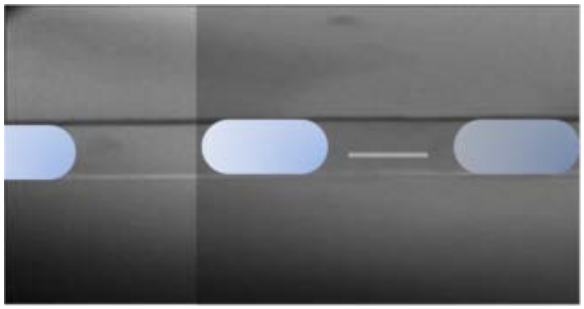
Smaller droplets, larger spacing

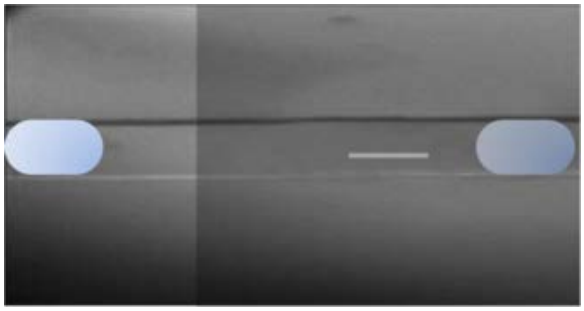
19. The picture below shows an image of a microchannel with a droplet detector overlay. The droplet detector is on the right-hand side of the image. The droplet detector generates signal as droplets pass thru the field of view, shown by the white rectangular slit. If all answer choices move with the same droplet velocity, match the droplet visualization to the generated signal

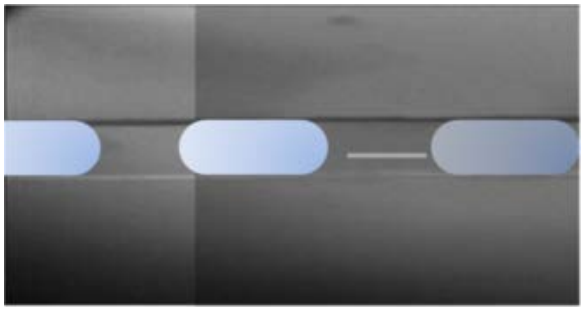


Signal		Signal
		



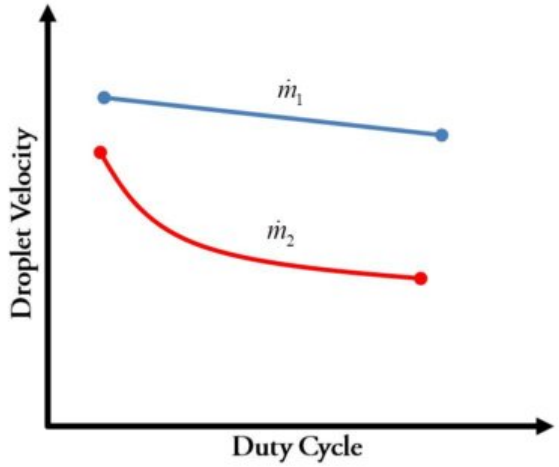
a. 

b. 

c. 

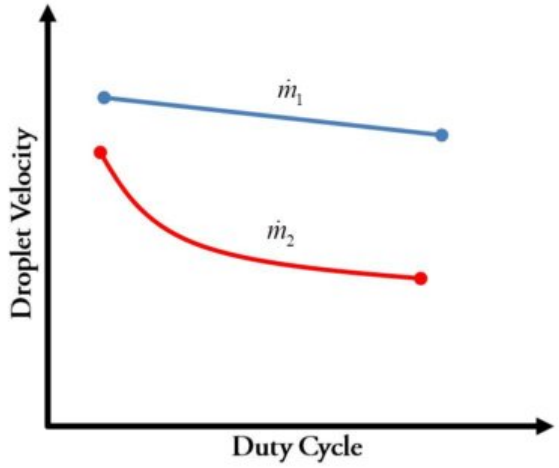
20. A duty cycle is defined as a ratio between a periodic signal's active state vs. it's total cyclic period. Refer to Figure 3. Based on the plot below, which curve has a

higher total mass flow rate? Assume the droplets behave under plug flow regime.



a. m_dot1
b. m_dot2
c. Neither
d. Cannot be determined

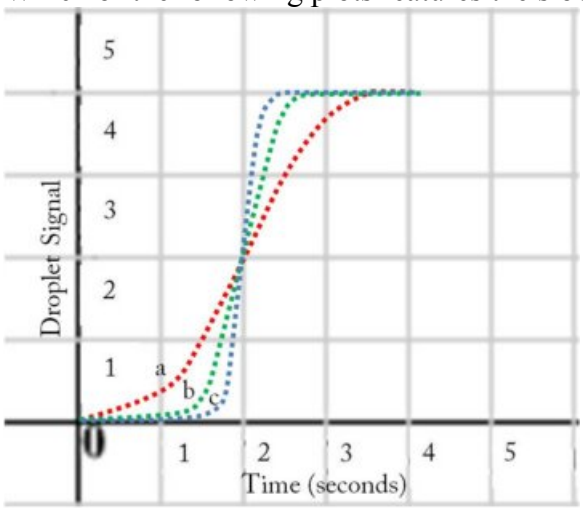
21. A duty cycle is defined as a ratio between a periodic signal's active state vs. it's total cyclic period. Refer to Figure 3. Based on the plot below, which of the following is true? Assume the droplets behave under a plug flow regime. Note: Subscripts o and w denote oil and water, respectively.



a. $\left(\rho_w - \rho_o\right)_1 > \left(\rho_w - \rho_o\right)_2$
b. $\left(\rho_w - \rho_o\right)_1 < \left(\rho_w - \rho_o\right)_2$
c. Neither

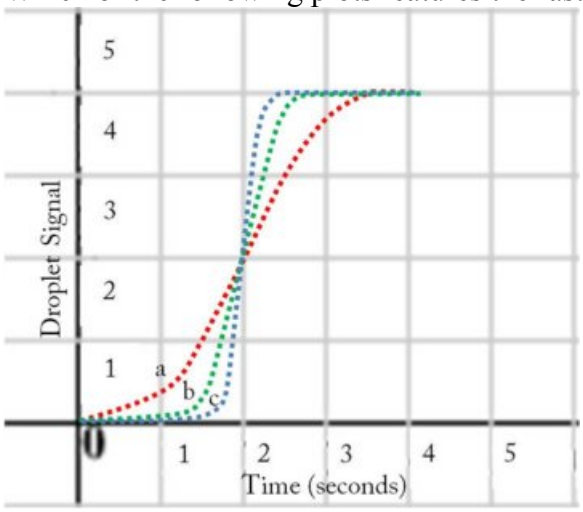
d. Cannot be determined

22. Which of the following plots features the slowest droplet velocity?



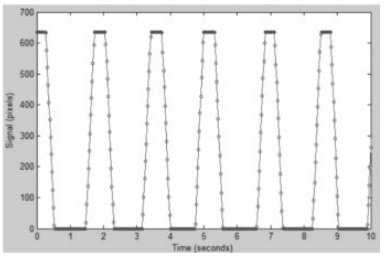
a. Curve a
b. Curve b
c. Curve c
d. None of the above

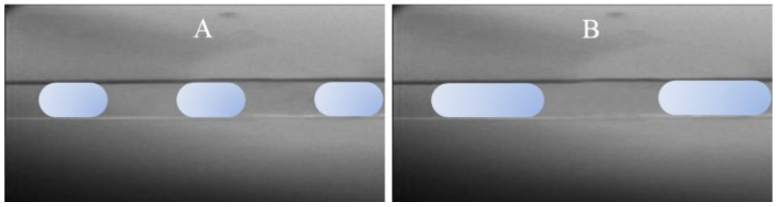
23. Which of the following plots features the fastest droplet velocity?



a. Curve a
b. Curve b
c. Curve c
d. None of the above

24. Compare the microflows between “A” and “B”. Both microflows use the same water and the same oil. Assume droplet size is equal to droplet spacing for both cases. True or false: It is not possible for both “A” and “B” to generate the same signal points shown below.





a. True
b. False

EVALUATION AVERAGE SCORES

Question Number	Pre Score Average	Post Score Average
1	36.1%	86.1%
2	27.8%	88.9%
3	36.1%	91.7%
4	36.1%	91.7%
5	72.2%	97.2%
6	75.0%	86.1%
7	50.0%	47.2%
8	25.0%	30.6%
9	11.1%	36.1%
10	38.9%	44.5%
11	77.8%	72.2%
12	58.3%	88.9%
13	80.6%	80.6%
14	16.7%	38.9%
15	47.2%	88.9%
16	52.8%	94.4%
17	33.3%	47.2%
18	25.0%	55.6%
19	66.7%	75.0%
20	61.1%	80.6%
21	38.9%	27.8%
22	72.2%	86.1%
23	72.2%	86.1%
24	38.9%	47.2%

STUDENT SURVEY RESULTS

	Not Applicable	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
My understanding of scientific uncertainty has improved as a result of Lab #6	0%	4.545%	13.636%	36.364%	36.364%	9.091%
My ability to conduct error analysis has improved as a result of Lab #6	4.545%	4.545%	18.182%	22.727%	40.909%	9.091%
My competency in using MATLAB has improved as a result of Lab #6	9.090%	4.545%	4.545%	40.909%	40.909%	4.545%
My competency in using Microsoft Excel has improved as a result of Lab #6	4.545%	4.545%	4.545%	31.818%	36.364$	18.182%
My understanding of the conservation of mass has increased as a result of Lab #6	0%	0%	0%	18.182%	63.636%	18.182%
My competency in conducting signal analysis has increased as a result of Lab #6	0%	0%	0%	9.091%	45.455%	45.455%

My understanding of plug flow regime physics has increased as a result of Lab #6	4.545%	0%	4.545%	18.182%	18.182%	54.545%
My understanding of the non-dimensional parameter Ca, the capillary number, has increased as a result of Lab #6	0%	0%	9.091%	18.182%	50%	22.727%
My interest in microfluidic devices and their fabrication has increased as a result of Lab #6	0%	0%	0%	9.091%	63.636%	27.273%
My skill with optical measurement tools (microscopes, CMOS cameras) has increased as a result of Lab #6	0%	0%	0%	13.636%	54.545%	31.818%
I practiced teamwork, communication, and/or organizational skills during my completion of Lab #6	0%	0%	0%	9.091%	68.182%	22.727%

Student Responses:

Perhaps there could be some more emphasis on the Capillary number in the analysis - maybe plotting things vs Ca to get a better physical understanding of how Ca affects things.

Fun lab

This lab was very interesting, and it was cool to see fluid mechanics applied on a micro scale

Interesting Lab. It would be cool to have this as a standard in the curriculum of the future.

It was a very good lab that would be a great addition to the ME 130L curriculum as long as it has the theory lectures to go along with it.

Some clarity in question 5 of the report and a little more in all those questions would be nice, but other than that it was a great lab! VERY interesting subject and it will make an amazing addition to the curriculum of fluids. As far as the actual data capture and lab performance portion it was perfect. Good length, just the right amount of challenge and good exposure.

It would have been nice knowing that we needed to wear pants to the lab experiment.

I really thought this was interesting and gained a lot from being introduced to microfluidics.

The lab manual is very long compared to other ones. Students will not read unnecessary background information (what surfactant you used, etc...). I would be more likely to fully read a 7 page lab manual than skim a 17 page one. Running the lab was very self explanatory though!

The lab manual was not very informative on what we were expected to do for the analysis. The concepts investigated were somewhat new and thus we needed some form of lecture to introduce us to it properly. It was hard to understand duty cycle, velocity and other terms used in the lab manual

The lab was really interesting but I felt it didn't apply to the fluids syllabus covered in ME 330. The analysis was really difficult because the calculations were difficult to understand and complete. The lab focuses on capillary number and this was never mentioned in any fluids lectures so a lack of prior knowledge on the basics of the material wasn't even there. To improve the lab, guidance should be offered for completing the calculations so the analysis can be completed efficiently.

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