AIRWAY ON A CHIP: DATA PROCESSING OF OCCLUDED PULMONARY AIRWAY REOPENING AT BIFURCATIONS

AN ABSTRACT

SUBMITTED ON THE FIRST DAY OF MAY 2013

TO THE DEPARTMENT OF BIOMEDICAL ENGINEERING

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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BY

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ABSTRACT

In the reopening of fluid occluded airways, the pressure gradient due to the propagation of an air bubble causes extensive epithelial cell damage. The mechanism of cell necrosis and biotransport may be further understood by characterizing the flow fields near the tip of a semi-infinite bubble propagating through a fluid-filled bifurcation. A symmetric microfluidic pulmonary bifurcation model was fabricated for optical diagnostics with an instantaneous μ-PIV/ shadowgraphy microscopy system. Data handling and processing techniques were developed to calculate interfacial characteristics of multiphase flow from the microscopy system and accuracy was quantified through varying the apparatus set up. Differences in the interfacial geometric characteristics were quantified for changes in static and dynamic surface tension in comparisons of water, SDS, and Infasurf that may reflect changes in the mechanical stress that stimulate, and potentially damage, epithelial cells that line the airways. From these results, the asymmetrical tendencies of opening a symmetric pulmonary bifurcation model were quantified. It was found that pulmonary surfactant stabilized symmetric bifurcations that opened asymmetrically without the aid of surfactant.
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INTRODUCTION

The lungs are composed of an extensive system of bifurcations, alveoli, and conductive zones that facilitate oxygen and carbon dioxide transport. Without a healthy lung, effective respiration does not occur and health complications may result. Mechanical ventilation is required for airway reopening and the associated ventilation may cause trauma. In the reopening of fluid occluded airways, the pressure gradient due to the propagation of an air bubble may cause extensive epithelial cell damage. By characterizing the flow fields near the tip of a semi-infinite bubble propagating through a fluid-filled bifurcation, the stimulus responsible for cell necrosis and biotransport may be further understood. This knowledge may be applied for more effective therapies and techniques to prevent the further damage of the lung. To study these fluid flows in the neighborhood of the propagating meniscus, microchannels were fabricated through soft lithography techniques, and analyzed by particle image velocimetry and shadowgraphy techniques. The specific goals of this manuscript were to:

1. Successfully design and implement a microfluidic bifurcating airway model that is biofidelic and compatible with the instantaneous μ-PIV/shadowgraph microscopy system;

2. Develop methods to mitigate the inherent image noise from the microscopy system and quantify noise reduction through adaptation of the microscopy system; and

3. Examine the changes in the instantaneous interfacial shape of multiphase flow from varying surface tension and velocity.
CHAPTER 1. BACKGROUND

1.1. RESPIRATORY ANATOMY AND PHYSIOLOGY

Respiratory Anatomy

The lungs, found in the thoracic cavity, are responsible for gas transport and respiration in the human body. The distal airways of both zones branch dichotomously. Each branch is referred to as a generation. The respiratory zone airways contain alveolar ducts, which are composed of a central air channel surrounded by alveoli. These alveolar ducts terminate into alveolar sacs after about 23 generations (Weibel 1962). The terminal lung unit (TLU), or acinus, is composed of the assemblage of alveoli which share a common air space. A connective tissue network located in the interstitial space forms the suprastructure of the TLUs and is responsible for the elastic properties of the tissue. The vascular and lymphatic network is also found in the interstitium. Each alveoli is a single layer of epithelial cells. Included in the epithelial cells are type I pneumocytes, which occupy over 90 percent of the structural area, and type II pneumocytes, which synthesize and secrete pulmonary surfactant (Scarpelli 1998). Weibel and Gomez calculated that human lungs contain approximately 300 million alveoli and 14 million alveolar ducts and sacs. Their average diameter was found to be on the order of 250 to 290 µm in adults (Weibel 1962). Figure 1 demonstrates the complexities of the lung through the creation of a vinyl cast. It illustrates that the lung is composed of numerous bifurcations.
Figure 1  Vinyl cast of an adult human bronchial tree (Weibel and Gomez 1962). The airways branch dichotomously starting proximally at the bronchial tube and moving distally away. The surface area increases with each generation.

Respiratory Physiology

It is evident that the structure and anatomy of the lungs closely parallel their physiology. The lungs consist of both conduction and respiratory zones, where the alveoli in the respiratory zones are the location of gas exchange. The respiratory zone comprises approximately 90 percent of the lung volume, while the remaining 10 percent is composed of the conductive zone. The vast network of alveoli creates a gas-tissue interface with a surface area which is around 25-fold greater than that of the body (Harding 2006). A pulmonary capillary network lined with a single layer of endothelial cells sheaths the alveoli for effective gas exchange and diffusion. Figure 2 is a resin cast of the acinus, which shows alveoli at the distal ends of the respiratory zone.
Figure 2 Resin cast of an acinus (Horsfield and Cumming 1968). The conductive zone proximal to the bronchiole is seen at the bottom of the figure. It branches into two respiratory regions, composed of alveolar ducts surrounded by alveoli.

1.2. PULMONARY SURFACTANT

The alveolar surface is coated with the alveolar lining layer, which is a thin fluid continuum consisting of an aqueous hypophase and a film of pulmonary surfactant. Surfactant is composed of approximately 90% lipids and 10% proteins, by weight (Postle 2001). It is clear that surfactant significantly contributes to respiration mechanics by lowering the surface tension of the alveolar surface (Goerke 1998). Compliance, the ratio of volume change to distending pressure, is increased through reducing surface tension. This is significant because the energy required for inspiration and expiration is reduced. Through varying surfactant concentration in respect to alveoli size, the alveoli are stabilized during expiration, reducing the chance of alveolar collapse (Zuo 2008). Surfactant also has many other functions, which are displayed in Table 1.
Table 1 Physiological functions of pulmonary surfactant (Zuo 2008)

<table>
<thead>
<tr>
<th>Surface tension related</th>
<th>Non-surface tension related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintaining a large gas transfer area</td>
<td>Specific and non-specific host defense</td>
</tr>
<tr>
<td>Increasing lung compliance on inspiration</td>
<td>Pathogen barrier</td>
</tr>
<tr>
<td>Stabilizing alveoli on expiration</td>
<td>Antibacterial/antiviral activity</td>
</tr>
<tr>
<td>Airway stabilization</td>
<td>Smooth muscle relaxation</td>
</tr>
<tr>
<td>Anti-edema effects</td>
<td>Anti-adhesion agent</td>
</tr>
<tr>
<td>Protecting epithelial cells in airway reopening</td>
<td></td>
</tr>
<tr>
<td>Facilitating mucociliary transport</td>
<td></td>
</tr>
<tr>
<td>Fluid dispersal</td>
<td></td>
</tr>
<tr>
<td>Particle removal</td>
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</tr>
</tbody>
</table>

Von Neergaard first demonstrated the relationship between surface tension and Boyle’s Law in the lung (Von Neergaard 1929). This can be represented by the Young-Laplace relationship

\[ \Delta P = \frac{2\gamma}{R} \]  

where \( \Delta P \) is the pressure drop across a spherical membrane, \( \gamma \) is the surface tension, and \( R \) is the radius. Using the spherical membrane as a simplistic alveoli model, it can be shown that surface tension, which directly correlates to surfactant surface concentration, and the radius of the alveoli can play a role in the stability of the lungs. When surfactant concentration remains constant in alveoli with varying radii a pressure difference exists, which leads to the smaller alveoli to deflate. This situation is unstable because it would lead to all alveoli deflating into one large alveolus. It has been shown that a dynamic surface tension can stabilize this system and is essential for the structural integrity of the
lungs. A simple lung analogue of two different sized alveoli is shown in Figure 3. In this example, both alveoli have the same surface tension. Recalling the law of Laplace, (Equation (1)), it is clear that the pressure in the smaller alveolus will have a larger magnitude than the pressure in the large alveolus. Therefore, the airflow will travel with the pressure gradient, decreasing the size of the smaller alveolus while increasing the size of the larger one. This pressure difference forms a positive loop: the air flux will increase the pressure gradient until the smaller alveoli collapses.

Figure 3 Airflow in a simplified lung model with a constant surface tension (Gaver 2005). As airflow is driven from alveolus 2 to alveolus 1, the pressure gradient increases, driving a larger flux of air in that direction.

It is clear that pulmonary surfactant’s kinetics are integral for its functionality due to its physiochemical properties (Kruger 2000). Marangoni stresses govern the transport properties of surfactant on air-liquid interfaces, where regions of high surface tension pull
on regions of low surface tension. Lung surfactant populates the interface through adsorption and respreading of the surfactant monolayer. Upon compression of the surface, the surfactant monolayer present on the surface will increase in concentration and therefore lower in surface tension until a critical concentration is reached. Then, the monolayer will collapse into a multilayer complex in the subphase, drastically lowering the surface tension (Lipp 1998, Takamoto 2001). **Figure 4** illustrates this process.

![Surfactant kinetics under surface compression](image)

**Figure 4** Surfactant kinetics under surface compression (Gaver 2005). Super low surface tensions are achieved during surface compression, where the monolayer concentration of surfactant, $\Gamma$, reaches a critical concentration, $\Gamma_{\text{max}}$, and buckles into a multilayer in the subphase.

Extending this to concept to both expansion and contraction of the surface, the multilayer subphase is available to repopulate the surface upon expansion (Morris 2001). Experiments using Infasurf, a lung surfactant analogue, were conducted by Krueger in which the surface area was cyclically oscillated and the dynamic surface tension was analyzed. (Krueger 1999). **Figure 5** offers a theoretical model to explain the results. The line intersecting point A is equivalent to the static surface tension associated with
sorption equilibrium, where a maximum interfacial packing state exists when $\Gamma = \Gamma_\infty$. Following the hysteresis loop in a clockwise direction from Point A, the contracting surface area increases monolayer surfactant concentration while decreasing surface tension. From point A to B, the surfactant monolayer buckles and collapses into the multilayer formation, where the surface tension reaches an ultra-low minimum. After reaching the multilayer’s maximum concentration $\Gamma_{\text{max}}$ at B, the surface tension does not change substantially until expansion of the interface at C. Upon reaching point D, the surface tension is at a maximum with the surfactant concentration at a minimum. The multilayer in the subphase repopulates the surface, decreasing surface tension with the continued expansion of the surface area. Upon contraction, the surface area decreases, along with surface tension drastically decreasing.
Figure 5 Dynamic surface tension of pulmonary surfactant in relation to an air-liquid area hysteresis loop. (Krueger 2000). (A) Without cyclic oscillations, the pulmonary surfactant will undergo static adsorption to a surface tension of 22 dyn/cm with a surface concentration of $\Gamma_\infty$. (B) Following the hysteresis loop in a clockwise motion, compression of the interface will lower the surface tension until the collapse of the surface surfactant into a multilayer formation. (C) The surface tension cannot reach a lower value after multilayer collapse, even with decreasing the surface area. (D) Expansion of the interface increases surface tension until respreading of the multilayer onto the surface decreases surface tension.
1.3. **AIRWAY MECHANICS**

*Inspiration and Expiration*

During inspiration, the lungs increase in surface area. As the surface area increases, so does the surface tension inside the lung. During expiration, the lung contracts, decreasing in surface area. The area hysteresis loop in **Figure 5** is an excellent analogue to the effects of expansion and contraction of surface area and surface tension in the presence of surfactant.

*Airway Closure*

Under pathological conditions, airways may become obstructed by airway lining fluid. There are two types of airway closure: meniscus occlusion and compliant collapse. During meniscus occlusion, air flow instabilities occur which lead to the formation of a fluid plug in the airway. In compliant collapse, the airway walls adhere to one another, and buckle with the airway walls in a ribbon-like configuration.
Figure 6 Types of airway closure (Gaver 2005). (A) Meniscus occlusion. (B) Compliant collapse.

Airway Opening

Reintroducing air to the occluded airway has the potential to damage the epithelial lining as the interface propagates through the obstruction. Bilek et al and Kay et al demonstrated that the mechanical stimulus is associated with the normal-stress gradient that sweeps across the cell surface (Bilek 2003, Kay 2004). The cells undergo different pressure gradients depending on their orientation in respect to the propagating air-liquid interface. Referring to Figure 7, cells far downstream of the air bubble (1) see no significant force. However, the approaching air bubble changes the force seen on the epithelium. Cells near the bubble surface (2) see a shear stress pointing towards the bubble, while cells directly at the interface see a large shear stress and sudden pressure jump. Cells upstream (1) of the interface see a uniform outward normal force from the air interface. It has been shown that slower air-liquid interfaces cause more damage than
faster interfaces (Kay 2004). In particular, the damage events due to interfacial velocity are seen at the cells in positions 2 and 3 that are not seen in single-phase flow conditions.

Figure 7  Schematic of stresses on airway epithelium from the steady propagation of a bubble in an occluded airway. As the bubble approaches the cell, the cell undergoes differentiating forces (Gaver 1996).

1.4. LUNG DEFICIENCIES

By studying respiratory fluid mechanics, more effective treatments may be produced to mitigate the damages seen in these lung deficiencies and diseases.

Understanding the damage mechanisms of lung ventilation and biotransport of surfactant will offer insight on improving current therapies.

Acute Respiratory Distress Syndrome
Acute respiratory distress syndrome (ARDS) affects 225,000 individuals in the United States annually, with a 40% mortality rate. During ARDS, fluid, inflammatory cells, and proteins aggregate throughout the lung. This addition of fluid exudate from the vascular system into the airspaces harmfully affects gas exchange by decreasing compliance and stability. Surfactant is deactivated by the proteins in the exudate, leading to surfactant deficiency and causing trauma. This disorder occurs after damage to the endothelial and epithelial cell layers, which stimulates the inflammatory response and edema (Fowler 1983). The mechanical damage, coupled with the responses, decreases the permeability of the blood-gas barrier. Plasma proteins in the edema fluid inactivate the SP-B and SP-C surfactant proteins. The diminished number of functioning type II alveolar cells may not have the capacity to produce enough surfactant for the respiratory system due to the initial mechanical trauma.

With the decreased surfactant concentration, the surface tension of the pulmonary fluids inhibiting respiration will increase, which will also increase the pressure required to open those airways (Gaver 2006). Somerson has shown that the disruption of the pulmonary surfactant system will make ventilation of infected lungs more difficult than healthy lungs (Somerson 1971). Alveoli become unstable with the increased surface tension, and may collapse as a result. With such abnormalities, the pulmonary, especially the epithelial, tissue is more prone to mechanical trauma. Oftentimes, this leads to an increased risk in mortality. Although treatments for these conditions have improved significantly to the point of recurrent case reports, the numbers in mortality are still high (Fowler 1983, Bice 2011).

**Infantile Respiratory Distress Syndrome**
Infant respiratory distress syndrome (IRDS) affects approximately 1 out of 100 newborn infants, and is the leading cause of death in preterm neonates (Rodriguez 2002). During IRDS, the lungs are developmentally deficient in pulmonary surfactant, which stabilizes the terminal air-spaces during normal respiration by lowering the pressure gradient required to propagate air through the lung. The increased interfacial stresses due to the larger pressure gradient cause extensive epithelial cell damage and inflammatory responses. The addition of fluid exudate due to cell necrosis and the inflammatory cells deactivate the present surfactant, leading to more damage.

**Ventilator Induced Lung Injury**

Current treatments for pulmonary irregularities include mechanical ventilation, which, although invaluable, may further propagate the damages caused by the conditions (Marini 1994). Known as ventilator induced lung injury (VILI), it may significantly contribute to the high mortality rates. The distribution of the mechanically ventilated air, in the presence of collapsed alveoli, has the potential to excessively distend the remaining alveoli. This pressure adds additional shear and mechanical stress on the already damage prone tissue, further injuring the lungs. Recruitment of the collapsed alveoli, without overdistention of the patent areas of the lung, is a challenge. It has been postulated that cyclic collapsing and recruitment of alveoli is detrimental to the delicate tissue through the large stresses it creates (Mead 1970). It is evident through recent studies that mortality rates can decrease through using different ventilation strategies (Laffey, 2000; Amato 1998).

Implementation of positive end-expiratory pressure (PEEP) can minimize injury from mechanical ventilation (Pavone 2007).
Figure 8  Pressure-volume relationship of the lung (Gaver 2005). Current protective ventilation strategies remain within the lower and upper inflection points to mitigate damage.

PEEP may protect the lungs by decreasing repeated recruitment/derecruitment of alveoli, which causes damage to the epithelium (Suh 2002, Halter 2007). The shear stresses exerted on alveoli during unchecked mechanical ventilation induces cellular responses, which further perpetuates the VILI. The sloughing of the epithelium, necrosis, and desquamation further triggers the inflammatory response. Pavone has shown on healthy rat lungs that large changes in tidal volume will provoke acute lung injury (ALI). He suggests that the tidal volume change deactivates surfactant. This deactivation leads to alveolar instability through direct inhibition, increased vascular permeability resulting in edema-induced surfactant deactivation, and removal of hyperactive type II cells from the alveolar surface due to increased surfactant production (Pavone 2007).
1.5. **Summary**

Studying interfacial flows is important to quantify the mechanisms responsible for cell death in the airway reopening of disease-state lungs. Herein, we will discuss how we approached quantifying the flow characteristics in biofidelic lung models through the developed image processing and model fitting techniques.
CHAPTER 2. MICROFLUIDIC RESPIRATORY MODELS

2.1. BACKGROUND

Simple geometric analogues of the lung are required to gain insight on the fluid mechanics of airway reopening before the complex geometries may be studied. Prior art includes single straight tubes, with various modalities for studying the flow, as well as more complicated geometric configurations.

*Straight Tube and Channel Models*

Straight, flexible-walled channels were first used as respiratory ventilation models (Gaver 1990, 1996). In these, a propagating semi-infinite finger of air penetrated fluid obstructions in the channel to study the prevalent mechanical stresses in the system. Epithelial cells were introduced in follow up *in vitro* experiments to determine the flow characteristics responsible for cell death (Bilek 2003, Gaver 1996). These conclusions were subsequently followed with experiments aimed at mitigating necrosis through taking advantage of the physiochemical properties of surfactant (Smith 2008, Glindmeyer 2011, Zimmer 2005).

*Bifurcation Models*

Although the straight tube prior art contained interesting results and data, the lungs anatomically are not straight tubes. Previous work on bifurcations focuses single phase flow characteristics in the upper bronchioles (Comer 2000, Fresconi 2003). Therefore, microfluidic bifurcation models were developed to address the effects of airway reopening on the distal airways.
2.2. **SOFT LITHOGRAPHY**

Currently, it is impossible to characterize and quantify fluid flow mechanics in pulmonary airways in vivo. Therefore, we chose to idealize the characteristics of a bifurcating network to simulate the lungs. Existing technologies allow precise microfabrication techniques that may accurately replicate branching networks. Soft lithography uses non-photolithographic techniques based on self-assembly and replica molding to fabricate micro- and nanodevices (Xia and Whitesides, 1998). The procedure ultimately outputs elastomeric molds of microchannels through the steps outlined below:

1. The creation of a mask,
2. The fabrication of a master wafer,
3. The molding of a replica, and
4. The final fabrication of the device.

**Mask Creation**

A mask is a 2-D microchannel blueprint used to transfer the design to a master wafer for channel production. The desired patterns are printed on the mask with a scale size of 1:1. The pattern may either be a positive or negative image of the final device, depending on the photoresist chosen.

Transparency plastic is typically used in mask production. Specifically, it is a Mylar sheet plotted with a high resolution laser printer, which may attain a feature resolution of approximately 5-10 µm.

**Master Fabrication**
Functionally, the master wafer transfers the channel pattern to the elastomeric molds from the mask. There are two types of masters, bas-relief and sunken-relief. In the former, bas-relief, the background is flat, with the pattern as protuberant shallow features. Sunken-relief is the opposite; the pattern is etched into a flat surface.

The master wafer is a rigid silicon substrate, which is evenly coated with photoresist through spin coating. Different heights of photoresist may be achieved by altering the time the wafer is spun and the angular velocity. After spinning the wafer, the edge may have beading, which is an aggregation of photoresist near the edge. This may be removed to improve resolution of the channel by allowing the mask to be placed in direct contact with the photoresist. The thickness of the photoresist, and ultimately the height of the microdevice, is given by:

\[ h = h_0 \times \left[1 + \frac{16\pi^2 f^2}{3v}h_0^2 t\right]^{-1/2}, \]

where \( h \) is the height, \( h_0 \) is the initial height, \( v \) is the viscosity, \( f \) is the angular velocity, and \( t \) is the time (Meyerhofer 1978).

**Figure 9** Spin coating of a master wafer (Shevkoplyas 2011). The photoresist is poured onto the center of the master wafer. The spinning parameters are chosen for the desired height of uniform thickness.

After spin coating, the wafer is soft baked to evaporate the solvent out of the photoresist before exposure. It should be performed on a level hot plate to ensure
uniform thickness. There should be no convection currents above the wafer; this produces a skin on the top of the photoresist. Depending on the photoresist used and the desired output, the time and temperatures will vary. The parameters of the soft baking process are integral for precise fabrication of a device. The duration of soft baking and the temperature both affect the master wafer. If the solvent is over-baked, the photosensitivity of the photoresist will decrease through destruction of the sensitizer or reduced developer solubility. This will reduce the resolution of the channels through both refraction of the exposure, as well as diffusion of the acid produced from exposure. If the solvent is under baked, light will not reach the sensitizer and will result in incomplete or no exposure.

The mask is aligned with the wafer in order to transfer the pattern to the master wafer, which selectively filters out UV light through the printed pattern. The exposed photoresist produces acid upon exposure to UV light, catalyzing the photoresist epoxy groups to cross-link. The cross-linked photoresist is chemically inert, stable, and binds irreversibly to the wafer.

The post bake immediately follows the exposure. It allows the photoresist to continue cross-linking. The wafer is then developed, which flushes the unexposed photoresist off of the wafer. Isopropanol is used to rinse the remaining developer off of the wafer.

Most wafers are subsequently treated to reduce the surface tension, allowing for ease of mold removal. For polydimethylsiloxane molds, trichlorosilane is used as treatment for the master wafer.
Figure 10  Master fabrication overview (Shevkoplyas 2011). The mask is aligned with the wafer spin coated with photoresist. The master wafer is then exposed to UV light to treat the photoresist. After removing the undeveloped photoresist with a solution, the master wafer is coated with trichlorosilane to reduce surface tension.

**Replica Molding**

A replica of the master is produced by pouring an elastomer over the master wafer then curing it to solidify. After the elastomer is cured, it is peeled off the wafer to have a negative of the photoresist patterns. Polydimethylsiloxane (PDMS) is the most commonly used elastomer (refer to the following polydimethylsiloxane section for its advantageous properties). It takes at least 3 hours of curing at 65°C for PDMS to solidify.
Figure 11  Replica molding (Shevkoplyas 2011). An uncured elastomer is poured over the master wafer and solidifies through a curing agent and heat. The hardened elastomer is then cut out and peeled off the master wafer to produce a replica.

**Device Fabrication**

Input and output holes are then punched into the replica mold. For PDMS channels, a glass slide is spin coated with a thin film of PDMS and cured. Both the glass slide and the PDMS mold are then treated with oxygen plasma. Oxygen plasma treatment uses ionized oxygen particles to change the surface activity of the subjects. The slide and mold are both placed inside a vacuum chamber, which is then evacuated of atmospheric air. The chamber is then refilled with low pressure oxygen and energized with an electrical current, microwaves, or radio frequency. The plasma ions bombard the exposed surfaces, cleaving off the –Me groups on PDMS and creating an active surface. The surface-active PDMS slide and mold are then pressed together to form a strong cohesive bond. More surface modification procedures may be done on the assembled device by flooding the microdevice with the desired treatments, then flushing it out.
Figure 12  Plasma cleaning (Shevkoplyas 2011). The glass slide and the PDMS mold undergo oxygen plasma treatment to volatize surface contaminants and promote adhesion.

**Polydimethylsiloxane**

Polydimethylsiloxane (PDMS) is a common substrate used as an elastomeric mold. It is a useful material for microfluidic device prototyping because:

a. It is chemically inert. Therefore, the molecules which are either in the device or flushed through the channels with have minimal interactions with the PDMS.

b. It is non-hygroscopic, meaning that it does not swell in water or in humidity. This is important because the device will not change shape due to the presence of water. Aqueous solutions in the device will not be affected either.

c. It is permeable to gases and water vapor, allowing for easy removal of gases. This removal allows for easy optical studies because gases would otherwise blemish the view. It also relieves the high pressures that may occur in the device through gas diffusion.
d. It is optically transparent to wavelengths over 300nm, which allows for easy optical testing.

e. It has a high yield stress, which makes the devices durable.

f. Its surface properties are easy to modify. Depending on the desired usage of the device, it may easily be manipulated for the properties required. These properties include hydrophobicity and cell interactions.

g. It has a modifiable Young’s Modulus. Changing the ratio of the elastomer base and the curing agent will produce different Moduli.

h. It is a vulcanizing rubber at room temperature. It enables ease of device fabrication due to its ability to mold to objects before solidifying.

i. It is relatively inexpensive. Many devices may be produced for a small cost.

**Surface Modification**

Although PDMS is an attractive substrate for soft lithography, there are still potential shortcomings. Due to other inherent properties, such as hydrophobicity, there has been extensive research on surface modification for more desirable attributes (Kim 2010, Efimenko 2002, Sharma 2007). Therefore, surface modification was employed to induce hydrophilicity, which is integral for microfluidic experiments and procedures. Below, we will discuss the surface modification necessary for our studies.

### 2.3. METHODS AND MATERIALS

In this section, the design parameters and specific fabrication methods to construct a biofidelic model are described.
2.3.1. Microdevice Fabrication

Mask: Bifurcation Geometry

The bifurcations were designed using anatomically and physiologically relevant data. The ratios and parameters were based on parameters both seen and derived from various researchers (Murray 1926, Raabe 1976, Hansen 1975, Heistracher 1995, Horsfield 1967, Horsfield 1971, Parker 1971, Weibel 1962, Weibel 2005). Figure 13 summarizes the model parameters with anatomical restraints.

Figure 13 Bifurcation model, where $D_p$ is the diameter of the parent channel, $D_d$ is the diameter of the daughter channel, $R$ is the daughter channel radius of curvature, $r_c$ is the carinal radius, and $\theta$ is the daughter channel angulation. The carinal radius is the “bluntness” of the bifurcation tip.
In particular, the following relationships are reported and used in the design parameters:

- $\mathcal{A}$, the ratio of the cross sectional area between the daughter channels and parent channel, is

  $$\mathcal{A} = \frac{\sum \text{Cross Sectional Area of Daughter Channels}}{\text{Cross Sectional Area of Parent Channel}} = 1.26. \quad (3)$$

  It has been shown that this area ratio corresponds to the area ratio for minimal work in a bifurcating pulmonary system (Murray 1926).

- The daughter radius of curvature, $R$, is 6 times as large as the diameter of the daughter channel, $D_d$: $\frac{R}{D_d} = 6$.

- The carina tip radius, $r_c$, is one tenth the size of the $D_d$: $\frac{r_c}{D_d} = \frac{1}{10}$

**Table 2 Bifurcation model parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_p$</td>
<td>Diameter of the parent channel</td>
<td>250 µm</td>
</tr>
<tr>
<td>$D_d$</td>
<td>Diameter of the daughter channel</td>
<td>150 µm</td>
</tr>
<tr>
<td>$R$</td>
<td>Daughter channel radius of curvature</td>
<td>525 µm</td>
</tr>
<tr>
<td>$r_c$</td>
<td>Carinal radius of curvature</td>
<td>15 µm</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Branching angle</td>
<td>21°</td>
</tr>
<tr>
<td>$h$</td>
<td>Height</td>
<td>150 µm</td>
</tr>
</tbody>
</table>

Masks were created in AutoCAD prepared by Fineline-Imaging (Colorado Springs). A sample mask file is shown in **Figure 14**. Additional channels with different geometries were included on the mask for potential experimentation.
Figure 14  Wafer mask. Each channel on the mask has different parameters, such as: asymmetry, different radii of curvature, and a multigenerational model.

Master Fabrication

Initially, the photoresist spin coating performed in the laboratory had craters and dimpling due to the heterogeneous contamination of the wafer substrate. To ensure that the surface of the 3” silicon wafer (University Wafer, South Boston, MA) was clean, a piranha etching cleaning procedure was adapted for small scale production. A 35% weight concentration hydrogen peroxide in water (Sigma-Aldrich) was carefully mixed with 98% concentrated sulfuric acid (Sigma-Aldrich) in a 1:3 ratio on a hot plate at 100°C to make the piranha solution, which is very corrosive and a strong oxidizer. Equation (4) summarizes the reaction below:

$$H_2SO_4 + H_2O_2 \rightarrow H_3O^+ + HSO_4^- + O$$  (4)
The wafer was slowly lowered into the solution with our designed Teflon PTFE (McMaster-Carr) wafer carrier, shown in Figure 15.

![Teflon wafer carrier](image)

Figure 15 Teflon wafer carrier. The carrier is shown holding a master wafer, with the counterweight resting on top of the wafer.

With continual stirring, the wafer remained in the piranha solution for 10 minutes. A PTFE Teflon counterweight was used to keep the wafer from floating to the surface of the solution. The wafer was then removed and immersed in a rinse breaker filled with ultra-filtered water (~18 MOhm) for five minutes, followed by another rinse beaker for five minutes. The wafer, upon removal, was sprayed with ultra-filtered water one last time before air dried.

The 3” silicon wafer was then spin coated with a 150 µm-thick layer of SU8-2100 negative photoresist (MicroChem Corp, Newton, MA). The spin coating occurred first for 30 seconds at a rotation velocity at 500 RPM, then for 30 seconds at 1800 RPM. The soft bake started at 65°C and was slowly ramped to 95°C. It was left overnight at 95°C.
It was exposed to UV light (ETI/6/350/NUV/DCCD/M mask aligner, Evergreen Technology Inc., San Jose, CA) through a Mylar transparency photomask (FineLine Imaging, Colorado Springs, CO) for a total of 80 seconds. The post bake procedure ramped from 65°C to 95°C, and was left overnight. The master wafer was then developed for 15 minutes, before silanizing the surface for 3 hours in a vacuum chamber with (1,1,2,2 tetrahydrooctyl) triclorosilane (CAS #78560-45-9, Gelest Inc., Morrisville, PA).

**Replica Molding**

Replicas were molded with PDMS (Sylgard 184, Dow Corning Corp, Midland, MI). The PDMS was prepared by mixing the 184 silicone elastomer base (Sylgard 184, Dow Corning Corp, Midland, MI) with 184 silicone elastomer curing agent (Sylgard 184, Dow Corning Corp, Midland, MI) in a 10:1 ratio. The mixture was then desiccated to remove the bubbles for approximately an hour in a vacuum chamber. Next, the PDMS mixture was poured over the master wafer and baked overnight on a hot plate at 65°C. A 23mm x 75 mm glass slide was spin-coated with PDMS and baked overnight to produce an ultra-thin film.

**Prefabrication Procedures**

First, the PDMS mold was cut out of the petri dish with a scalpel to a size of approximately 25mm x 75mm. Using a modified 16 gauge hypodermic tube (Small Parts) as described by Christensen et al, two holes were punched into the PDMS mold as ports (Christensen 2005). As seen in Figure 16, the tube was sanded at the tip to sharpen the edges. The 16 gauge needle was chosen because it was slightly smaller than the
1/16” tubing (PEEK) used to deliver the fluid into the device. The bored holes were smaller than the tubing so that as the tubing was pressed into the channel, the PDMS would apply a compressive force and directly form a tight seal around the tubing.

**Figure 16** Interconnect construction (Christenson 2005). The tip of the hypodermic tube is sanded into a point, then used to bore a hole into the PDMS. An interconnect with a smaller gauge (which corresponds to a larger diameter) is then inserted into the PDMS, using a compression fit to form a tight seal.

A glass microscope slide (Fisher Scientific, PA) was then spin-coated at 550 RPM for 18 seconds and 1600 RPM for 60 seconds with PDMS to produce a thin film. It was then baked overnight at 65°C.

**Plasma Bonding**

The PDMS mold and the PDMS-coated slide were then treated in an oxygen plasma cleaner (Harrick Scientific Corporation, model PDC-3XG) for 100 seconds. Upon removal, the treated surfaces were pressed together, forming a strong cohesive bond. The device was left overnight for any air to permeate out from between the surfaces. A completed microdevice is shown in **Figure 17**.
2.3.2. Surface Modification

Surface properties of PDMS with various modifications and treatments were studied for choosing a feasible procedure. The temporal hydrophobic recoveries of treatments reported in previous publications were examined in a series of experiments (Sharma 2007, Efimenko 2002, Kim 2010, Xia 2007). The surface modifications tested were oxygen plasma, polyvinylpyrrolidone (PVP), and poly(ethylene glycol) (PEG) silane.

The PDMS was prepared as described above in Replica Molding. Next, the PDMS mixture was poured over glass 25x75 glass microscope slides and then baked overnight on a hot plate at 65°C. Afterwards, the PDMS was cut into the same dimensions as the glass microscope slides. These PDMS slabs received either no treatment, oxygen plasma exposure, oxygen plasma exposure and PVP, or oxygen plasma exposure and PEG.
Hydrophobic recovery was quantified through contact angle analysis at different times, ranging from 30 minutes to two weeks after the treatment. Water, 2 mM sodium dodecyl sulfate (SDS) (Fisher), 6 mM SDS, Diethylene glycol (Sigma-Aldrich), and ethanol were used as test liquids due to their varying surface tensions. A contact angle goniometer (Tantec, IL) was used to measure the sessile drops. Figure 18 outlines the measurement procedure.

![Figure 18](image)

**Figure 18** Contact angle measurement (Zisman 1964). The surface tension of the solid in respect to the gas may be calculated through Young’s Equation, which is defined as the sum of mechanical equilibriums on the sessile drop:

$$\gamma_{sg} = \gamma_{sl} + \gamma_{lg} \cos(\theta)$$  \(5\)

Where $\theta$ is the contact angle and $\gamma_{sg}$, $\gamma_{sl}$, and $\gamma_{lg}$ are the interfacial tensions of the solid-gas, solid-liquid, and liquid-gas, respectively. Zisman defines the solid-gas surface energy as the highest surface-liquid energy that completely wets the solid. The theoretical surface energy may be extrapolated by taking multiple probe liquids of differing surface tensions and plotting their $\cos(\theta)$ over their surface tension.

### 2.4. RESULTS

*Microdevice Fabrication*

After device fabrication, one of the devices was sectioned to determine uniform channel thickness in respect to radial position on the wafer. The heights were measured
by taking a photograph of the cross sectioned area and correlating the pixel density to microns. It was found that for channels with a large height requirement that there is an acceptable region to place the channel in order to maintain uniform height. Figure 19 shows the height measurements conducted on the channel depth. This is due to the beading that occurs during the spin coating procedure. Therefore, the channels used for experimentation all were produced in the region bounded by a radius equal to half of the radius of the wafer.

**Figure 19** Depth analysis of the channels in respect to radial position. The channel maintains uniform height when the radial position is less than 0.5, but increases considerably after 0.5.

**Surface Modification**

A sample contact angle graph is shown in **Figure 20**. The contact angle of water in respect to the different surface modification treatments were graphed on a semi-logarithmic scale. The contact angle increased with time for the samples that underwent
surface modification. The PEG silane treatment was chosen because of its stable hydrophobic recovery, as well as its ease of implementation. The contact angle of PEG silane rapidly stabilized to $76.4^\circ \pm 4.6^\circ$ within the appropriate timescale of our experiments.

![Graph showing contact angle vs. time (Water)](image)

**Figure 20** Water contact angle on different surface treatments over time. Hydrophobic recovery was quantified over time for different surface modification treatments of PDMS by measuring contact angle. It is seen that PEG silane maintains a low contact angle over time.

From these results, a combined oxygen plasma and PEG treatment was chosen for surface modification of the channels in situ, and was implemented by the method described by Sui *et al* (Sui 2006). After the oxygen plasma treatment and assembly of the device, the channels were injected with neat 2-[methoxy(polyethylene)propyl]-trimethoxysilane (Gelast Inc., Tullytown, PA) to perform silanization reactions for 30 minutes at room
temperature. The unreacted silane was then flushed out of the microchannel with deionized water to leave PEG-grafted microfluidic channels.

**Figure 21** Schematic of PEG-silane treatment (Sui 2006). Untreated PDMS (1) is treated with oxygen plasma to produce (2). (2) was treated with a PEG-silane for thirty minutes to produce (4)

### 2.5. Summary

The soft lithography methods were successful in the fabrication of a biofidelic symmetric bifurcation. Anatomically relevant microdevices with uniform height were successfully reproduced with tailored surface modifications.

The oxygen plasma and PEG silane treatment is effective for reducing hydrophobicity of PDMS in comparison to other treatments. It maintains stable hydrophilic properties for an acceptable timeframe to conduct microfluidic experiments. In conjunction with the adapted piranha solution procedure, soft lithography can successfully fabricate anatomically relevant bifurcating models for fluid-occluded airway reopening.
CHAPTER 3. FLOW AND INTERFACE VISUALIZATION SYSTEM

3.1. BACKGROUND

The imaging modalities and their apparatuses are described below, with the subsequent data processing techniques to gather information from the captured images.

3.1.1. Microscopy

*Microscale Particle Image Velocimetry (µ-PIV)*

µ-PIV is employed to determine flow characteristics of a fluid. Conceptually, it is a simple process; although in reality measurements are complex. The velocity vectors of a fluid are visualized through tracking the displacement of particles in the fluid. For experimentation, tracing particles are seeded into the fluid, and their displacement over time is tracked to statistically correlate the velocity of local flow fields. The tracers are fluorescent for optical inspection while maintaining a small diameter to minimize their effect on the flow characteristics. An example of PIV flow obtained from our apparatus is shown in Figure 22.

The PIV apparatus is composed of a pulsed laser, the tracers that make up the light sheet illuminating particles, and either a video recorder or a camera to capture the images. Interrogation of the images is done through correlation analysis. A typical monoscopic PIV system is shown in Figure 23. As the physical experiment is conducted, the pulsed light source excites the tracers to emit light. This emission is captured through the image recorder, which is then digitized and processed for the flow statistics.
Figure 22 μ-PIV multiphase flow in a bifurcation. (a) The μ-PIV quantifies liquid velocities downstream of the air phase, where red is the maximum velocity and blue is the minimum. (b) The image on the right is a magnified perspective of the flow fields when the bubble is at the bifurcation point.

Santiago et al introduced μ-PIV, which is the extension of PIV to microfluidic applications (Santiago 1998). μ-PIV is advantageous because (Lindken 2009):

1. The vector field output is intuitive and allows for direct comparison to computational fluid dynamics (CFD) data.
2. The data are obtained optically, with illumination and image acquisition performed through the same microscope lens. Therefore, no changes in the microdevice are required.
3. Fluid flow is not altered through measurement because optical interrogation is non-intrusive and adds negligible energy to the system.
Figure 23  Standard monoscopic macro-PIV system (Adrian 2005). The pulsed light source creates a light sheet in the interrogation window to excite the tracers. The excitation wavelengths images are recorded and digitized for image processing. Displacement of the particles is tracked to correlate into local flow statistics.

**Shadowgraphy**

Although $\mu$-PIV is excellent for studying complex single phase flow, there are difficulties with $\mu$-PIV techniques in multiphase flow. The interfaces and boundary shapes in multiphase flow have poor resolution in $\mu$-PIV, which makes data acquisition near the geometries invalid. Shadowgraphy addresses the poor resolution by subjecting the test apparatus to back illumination. The back illumination casts an accurate shadow of the interfacial boundaries, which an image recorder captures for processing. Relevant
statistics concerning flow phenomena may be determined from the interfacial shape, such as the instantaneous pressure gradient at the boundary.

**Dual µ-PIV/Shadowgraphy System**

Combining the two imaging modalities for a microscale application has inherent difficulties. Excessive noise in the shadowgraph images from the illumination techniques is prevalent in the microscale systems, whereas larger µ-PIV/shadowgraph systems are not adversely affected (Lindken 2002). This noise is chiefly due to speckle noise, which is a product of the limited laser bandwidth required to separate the two modalities. Although white light averages out the speckle noise for the shadowgraphy measurements, it substantially degrades the µ-PIV image quality. A larger bandwidth, at the sacrifice of µ-PIV quality, may increase the shadowgraph image quality. Regardless, processing techniques are required to overcome the noise complications. Previous studies applied a phase averaging technique to obtain the interfacial geometry by repositioning the frame of reference (Yamaguchi 2012). Unlike in simple geometric models, this noise cannot be averaged out through superposition of the interface in more complex geometries. Instead, image processing and model fitting techniques are necessary to determine instantaneous interfacial geometries.

**3.1.2. Image Processing**

Digital images are numerical representations of pictorial data. Pictorial images are expressed as a continuous function $f(x, y)$ in an $xy$-plane. During digitization, pictorial data is sampled into a $m \times n$ grid of picture elements referred to as pixels. Each pixel is assigned an intensity value through quantization, which defines an integer
describing the brightness or darkness to each sample interval at the corresponding pixel location.

Images have gray-scale resolution, which is the number of gray levels per unit of measure for image amplitude. Bits refer to the number of gray levels per unit of measure of image amplitude. As the number of bits increases, so does the gray-scale resolution of the image. In each case, black is the minimum value ($I_{\text{min}} = 0$), while white is the maximum value ($I_{\text{max}} = 2^K - 1$, where $K$ is the number of bits). For instance, binary images are stored at 1-bit bytes, while 8-bit images have 256 grayscale intensity bytes.

There are numerous powerful image processing techniques that can resolve issues associated with the shadowgraph images due to the dual microscopy system. These techniques reduce the high magnitude and frequency of noise while maintaining the interfacial geometry in order to reduce oversegmentation of the images. For our shadowgraph images, it is assumed that the bubble pixels have low intensity values with minimal variance, while the noise pixels have a larger variance of intensity values. Therefore, the techniques were aimed at smoothing the image into a bimodal spread of intensity values, where the bubble is the relative peak of lower intensities and the background is the relative peak of higher intensities.

**Median Filtering**

Median filtering is a nonlinear neighborhood operation that employs order-statistics to filter out unwanted noise. Neighborhood operations define pixel outputs by analyzing the nearby pixels in its defined interrogation window. Order-statistics rank the elements of the neighborhood, as opposed to computations, to determine the output. In median filters, pixels are ranked in the order of their gray levels and the midvalue of the
group is stored as the output pixel. Median filtering is effective at removing objects less than half the size of the neighborhood while preserving edges. The gray level output for an N-by-N filter, where N is odd, is the gray level corresponding to the input pixel that is greater or equal to \((N^2 - 1)/2\) of the neighborhood pixels and less than or equal to \((N^2 - 1)/2\) of them. The median filter was chosen because it preserves edges more effectively while reducing random noise than other linear low pass filters (Castleman textbook). A 1-dimensional example is shown in Figure 24.

**Figure 24** 1-D Comparison of an average filter against a median filter (Wang 2012). Each filter has a neighborhood size of 5. The input signal is the first column, in blue, where the second column is an average filter and the third column is a median filter. The median filter preserves sharp edges while the size of the noise is less than half of the filter size, while an average filter blurs edges.

Applying a 2-D median filter to the image reduces the smaller “salt and pepper” noise in the image, while maintaining the sharp edges of the interface. We chose to use a median filter because it can reduce high frequency, small area noise without blurring the interfaces.
**Morphological Operations**

Morphological operations probe an input image with a predefined structuring element and determine an output based off of how the probe fits into the shapes of the input. The structuring element’s size, shape, and output operation are tailored for the application. For each pixel, the structuring element performs the specified logical operation on the underlying image, and the result is the output image. We chose to use image opening and closing by reconstruction, which smooth out pixel variation while maintaining similar area size for the image objects. Both opening and closing use a combination of erosion and dilation operations to determine the output for each pixel, and are explained in detail below.

Erosion eliminates all boundary points from an object by using the structuring element. Erosion removes objects that are too small to be of interest from a segmented image. Mathematically, general erosion for a binary image is defined by:

\[
E = B \ominus S = \{x, y | S_{xy} \subseteq B\},
\]

where \(E\) is the output image, \(B\) is the input binary image, and \(S\) is the structuring element. Image \(E\) is the set of points resulting from eroding \(B\) by \(S\) such that if \(S\) is translated so that its origin is located at \((x, y)\), all of \(S\) is contained in \(B\). Dilation, on the other hand, incorporates the background points that are in proximity to the object. It fills holes in segmented objects. General dilation for a binary image is defined by:

\[
D = B \oplus S = \{x, y | S_{xy} \cap B \neq \emptyset\},
\]

where \(D\) is the resulting image from dilating \(B\) by the structuring element \(S\), such that if the origin of \(S\) is translated to \((x, y)\), then its intersection with \(B\) is not empty.

Examples of erosion and dilation are shown in **Figure 25**.
Figure 25 The erosion (a) and dilation (b) of a square by a disk (Keshet 2008). (a) Erosion: the input is the dark blue square, with a disk as the structuring element. Each pixel in the input image is analyzed by the disk. If the entire structuring element, centered on the pixel, fits inside the dark blue square, that pixel is marked ON for the output. The erosion of this dark blue square by the disk produces the smaller light blue square. (b) Dilation: the input is the dark blue square, which is dilated by the disk as the structuring element. The output pixel is the center of the disk. If any portion of the structuring element lies inside the input image, the output pixel is marked ON. Therefore, dilation of the dark blue square by a disk produces the larger light blue square with rounded edges.

The erosion and dilation of binary images is an excellent analogue for the grayscale images. The binary operation is extended to grayscale. If we denote the grayscale image as $G$, erosion outputs the minimum value of $G$ from all values of $G$ in the region of $G$ coincident with $S$. Grayscale erosion is denoted by:

$$E = G \otimes S = \min\{x, y|S_{xy} \subseteq G\}. \quad (8)$$

Dilation outputs the maximum value of $G$ from all values of $G$ in the region of $G$ coincident with $S$. Grayscale dilation is denoted by:

$$D = G \oplus S = \max\{x, y|S_{xy} \cap G \neq \emptyset\}. \quad (9)$$
Using erosion and dilation in conjunction with one another has numerous applications. Opening is applying erosion, followed by dilation of the resulting image. This eliminates the small and thin objects, breaks apart objects with thin bridges, and smooths boundaries without significantly altering their area. Mathematically, opening is:

\[ G \circ S = (G \otimes S) \oplus S. \]  

Closing is the dilation, followed by erosion of an image. This fills small holes, connects nearby objects, and smooths out boundaries without significantly altering their area. Mathematically, closing is:

\[ G \bullet S = (G \oplus S) \otimes S. \]  

Examples of binary opening and closing are found in Figure 26.

**Figure 26** The opening (a) and closing (b) of squares by a disk (Keshet 2008). (a) Opening is the erosion followed by the dilation of the input. The dark blue square is the input while the disk is the structuring element. The output of opening an image results in the light blue square of similar area with rounded corners. (b) Closing is the dilation followed by the erosion of an input. The dark blue union of two squares is the input image while the disk is the structuring element. The output image includes both the dark blue input, as well as the light blue rounded inside corners.
Morphological reconstruction is the operation which uses two images and the structuring element. The first image, the marker, is the image produced by either erosion or dilation from the structuring element. The second image, or the mask, is the original, which constrains the operation.

Image opening by reconstruction restores the remaining images after erosion to their original shapes. For grayscale images, this removes the large variation in intensity and instead only leaves the large pixel areas of similar intensity. Therefore, small areas of similar intensity, or noise, are suppressed out of the image. For image closing by reconstruction, the areas that have small areas of intensity difference, or holes, that are filled are preserved in the initial geometry (Tcheslavski 2010).

**Image Segmentation**

Edge detection requires image segmentation, where the objects in the image are isolated. After isolation, features of the object such as the interfacial shape may be extracted. Image thresholding is an invaluable tool for segmentation of solid objects with a contrasting background. Objects that differ from the background due to a property that is not gray level (e.g. texture) may use an operation that takes advantage of the property to convert it to a gray level before thresholding.

In image thresholding, a gray level value is chosen as the threshold to compare against each pixel. Pixel intensities equal to or higher than the threshold are assigned to the object, while the pixels lower than the threshold are assigned to the background. The threshold value may be determined through evaluating the histogram. Objects with a contrasting background have bimodal gray level histograms, which contain two distinct peaks.
Otsu’s method is an effective histogram-based image segmentation technique for bimodal gray level histograms which automatically selects an optimal threshold value (Zhang 2008, Otsu 1979, O.D. Trier 1995). In this method, the threshold is selected by minimizing the within-class variance of the background and the object. Within-class variance is defined as:

$$\sigma_w^2(T) = \omega_o(T)\sigma_o^2(T) + \omega_b(T)\sigma_b^2,$$

where $\sigma_w^2$, $\sigma_o^2$, and $\sigma_b^2$ are the within-class, object, and background variances; $\omega_o$ and $\omega_b$ are the object and background weights; and $T$ is the threshold.

### 3.2. METHODS AND MATERIALS

#### 3.2.1. Data Acquisition

**µ-PIV/Shadowgraphy Microscopy Setup**

The simultaneous µ-PIV/Shadowgraphy data acquisition system is shown in Figure 27, following the method in Yamaguchi (2013). The system is based off a microscope, µ-PIV components, shadowgraphy components, and a pulse synchronizer. An inverted microscope (Nikon Eclipse TE2000-U, Nikon Corporation, Japan) with a 10x objective lens (NA=0.30 Plan Flour, Nikon Corporation, Japan), has two CCD cameras (12 bit, 4MP, TSI POWERVIEW Plus, TSI Incorporated, MN) attached to the side optical port through a double port adapter (Y-QT, Nikon Corporation, Japan). Two dichroic prisms are used as a low-pass filter at 625 nm (625DCLOP, Chroma Technology Corp, VT) with a cleaning band filter of 595 nm/±40 nm (d595/40x, Chroma Technology Corp, VT) to separate the µ-PIV and shadowgraph images to different cameras. This set
up provides a 1523x1523 µm observation area with a theoretical pixel resolution of 0.744 µm /pixel.

The µ-PIV system employed a dual pulse green Nd:YAG laser (New Wave Laser Pulse Solo Mini, New Wave Research, CA) for volumetric illumination. Fiber optic cables directed the laser pulses to an optical port on the microscope. The epi-fluorescent prism/filter cube refracted and guided the pulses through the objective lens to volumetrically illuminate the test section. The liquid phase of the samples were seeded with $d_p = 1$ µm fluorescent particles (Nile Red FluoSpheres; Invitrogen Corporation, CA) with excitation and emission peaks at 535 nm and 575 nm, respectively. The returning emissions passed through the first dichroic filter ($\lambda > 550$ nm). The second epi-fluorescent prism/filter cube guided the fluorescent particle image of the test section to CCD Camera A.
Figure 27  µ-PIV /Shadowgraph Set Up. The 532 nm Nd:YAG laser excites the fluorescent particles, which creates an image that is captured by CCD camera A. The shadowgraph images are captured by the LED red laser, which back illuminates the interfacial flow at a wavelength higher than the fluorescent particles by a narrow bandwidth.

The shadowgraph used an LED pulsed red laser (MPL-III-660, Opto Engine LLC, UT) for backlight illumination. Fiber optics directed the laser pulses to back illuminate the microdevice from above through a collimator (NA=0.25, f=36.01 mm, F810FC-70,
ThorLabs, NJ), which provided a 1.0 cm diameter observation window of uniform illumination. The laser pulses, after passing through the microdevice, passed through the same two dichroic filters used by the µ-PIV to capture the shadowgraphy interfacial information to camera B. The motivation for each laser is summarized in Figure 28. Filters take advantage of the limited bandwidth between the two lasers to isolate the shadowgraph and PIV information.

**Table 3 Laser properties**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Power</th>
<th>Pulse Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIV</td>
<td>532 nm</td>
<td>15 mJ/pulse</td>
</tr>
<tr>
<td>Shadowgraph</td>
<td>660 nm</td>
<td>2 nJ/pulse</td>
</tr>
</tbody>
</table>
Figure 28  The instantaneous µ-PIV/Shadowgraphy system used lasers separated by a limited bandwidth to gather information from the experiments. The µ-PIV tracer, Nile Red, is excited by the ND:Yag laser for µ-PIV data. The shadowgraph is illuminated by a RD Red laser. The bandwidth between each laser is employed to separate the experimental information from one another.

A laser pulse synchronizer (Model 610035, TSI Inc.) controlled the timing of the lasers and cameras to capture simultaneous µ-PIV and shadowgraph images. To track displacement of the µ-PIV particles using cross-correlation analysis, camera A captured two fluorescence images separated by a very short time (dT=200-700 ns) for every shadowgraph image. The first fluorescence image is correlated with the shadowgraph image. Insight 3G (TSI Inc., MN) controlled system management, image display, and data acquisition.

The data acquisition frame rate was set to 7.5 images/sec, or 15 pulses/sec for the ND:YAG laser to determine the µ-PIV vector fields. The shadowgraph CCD red laser was correlated to capture 7.5 images/sec as well.
Experiments were run using both the µ-PIV/shadowgraph simultaneous microscopy system, as well as only using the shadowgraph camera with white light for comparison of the shadowgraph image quality.

3.2.2. Experimental Flow Control

A 25 µL syringe (Gastight Syringe 1707; Hamilton Company, NV) was used to drive multiphase flow through the microdevice through applying a negative pressure downstream. This syringe, attached to a glass capillary tube (Flexible Fused Silica Capillary Tubing; ID=550 µm, Polymicro Technologies, AZ), was chosen to reduce dead space volume in the test chamber while providing acceptable accuracy (~5% of error) while in conjunction with the syringe pump (Yamaguchi 2009). Negative pressure to drive the flow applies a uniform pressure gradient for the duration of the experiment. This design was implemented because if flow was induced through a positive pressure, the gaseous portion of the flow may act like a dashpot due to its compressibility. The syringe pump was calibrated to output $3.0 \mu L \cdot \mu m/min$ as the fast velocity and $0.5 \mu L \cdot \mu m/min$ for the slow velocity.

3.2.3. Sample Preparation

Pure water (18.2 MOhm Millipore Direct-Q) was mixed with Nile Red as the µ-PIV marker. Red Nile has excitation/emission peaks at $\lambda=535/575$ nm, which is acceptable for the chosen filters to capture both µ-PIV and shadowgraph images simultaneously with the current set up. The fluorescent particle diameter is 1 µm (Nile Red FluoSpheres, Invitrogen Corporation, CA), with a 0.04% concentration by weight in pure water.
3.2.4. Microfluidic Device

The bifurcation described previously in Section 2.3.1, Microdevice Fabrication, was used for the flow chamber. Figure 29 shows the bifurcation model blueprint with the interrogation window in (a), with sample output images for µPIV and shadowgraphy in (b).

![Microfluidic device model blueprint](image)

**Figure 29** Microfluidic device model blueprint. (a) The interfacial flow travels from left to right through the bifurcation, with the interrogation window on the opening bifurcation branch. (b) The output images for the µ-PIV and shadowgraphy modalities are shown. The sample shadowgraph image is taken with white light, and thus has no speckle noise.

3.2.5. Data Handling

The shadowgraph images then underwent the processing steps listed below. Figure 30 shows a sample shadowgraph image from the dual µ-PIV/shadowgraph microscopy system undergoing the image processing steps.
Image Processing

1. Image Contrasting

Since the shadowgraph camera captures images in 12 bit, but the computer stores them as 16 bit images, the images needed to undergo contrasting in order to take advantage of the full range of intensities. The intensity values in the image were mapped so that 1% of the pixels are saturated at low and high intensities of the image. For digital viewing, the images were then converted to 8-bit bytes. The contrasted image is displayed in Frame 1 of Figure 30.

2. Median Filtering

After image contrasting, a 15x15 pixel mask was used for median filtering. Although this vastly improves the image by suppressing the “salt and pepper” noise, the larger noise patterns still lead to oversegmentation when attempting to outline the interface. The median filtered image is displayed in Frame 2 of Figure 30.

3. Image Opening by Reconstruction, Closing by Reconstruction

A disk with a 25 pixel radius was used as the structuring element for both operations. Image opening by reconstruction was followed by closing by reconstruction to remove the larger noise patterns and further reduce the oversegmentation through decreasing intensity variance in objects. These operations were chosen because they maintained the bubble area without blurring the bubble boundaries. Image opening and closing by reconstruction are shown in Frames 3 and 4 of Figure 30.

4. Image Segmentation

After the smoothing operations, Otsu’s method was used to threshold the image to determine the edge of the bubble domain. The bubble may be segmented from the
background due to the similar variance of its pixel intensities (Sahoo 1984). A mask of the bifurcation was applied to remove traced bubble points that were not on the interface (i.e. the bubble points on the channel wall). The thresholded image is displayed in Frame 5, while the interface points taken from the gray thresholded image are superimposed back onto the original image in Frame 6 of Figure 30.
**Figure 30** Image processing overview. The speckle noise and inter-object pixel variance is reduced in order to segment out the interfacial boundaries. The operations occur in the specified numerical order of the frames. The structuring elements used in Frames 2, 3, and 4 are superimposed in the bottom right corners of their respective frames.
**Model Fitting and Outlier Removal (Pruning)**

Although the image processing removed the oversegmentation errors and successfully traced the air-liquid interface, noise diffractions patterns of similar intensity that are connected by pixels to the interface are erroneously included in the interface points. A model is iteratively fit to the surface to remove or “prune” the noisy data from the interface (Motulsky 2006). The statistical analysis for outlier removal is outlined in **Figure 31** and described in detail in the text.

---

**Figure 31** Flowchart overview of fitting a model to the interface.

1. **Fitting the Model Choice:** An air-liquid interface model was chosen for a nonlinear regression fit. Nonlinear regression was chosen, as opposed to linear regression, because outliers affect the fit less. The model equation was (REINELT 1984):
\[ y(x) = \frac{1}{k} \log \left\{ \left[ 1 - \left( \frac{x}{\beta} \right)^2 \right] \left[ 1 + \left( \frac{x}{\beta} \right)^2 \sum_{j=0}^{m} c_j T_{2j} \left( \frac{x}{\beta} \right) \right] \right\}, \]  

(13)

where \( x \) is the domain of the data points, \( \beta \) is the thickness of the bubble, \( c_j \) and \( k \) are the parameters that define the shape of the interface, and \( T_{2j} \) are the even Tchebychev polynomials of the first kind. It is assumed that \( \beta=1 \), or that the lubrication region is minimal, because the surface tension of PDMS is higher than the surface tension of glass. Tchebychev polynomials were chosen because they converge rapidly in the sum series expansion, where Tchebychev polynomials are determined recursively:

\[
T_0 \left( \frac{x}{\beta} \right) = 1 \\
T_1 \left( \frac{x}{\beta} \right) = \frac{x}{\beta} \\
T_{j+1} \left( \frac{x}{\beta} \right) = 2x T_j \left( \frac{x}{\beta} \right) - T_{j-1} \left( \frac{x}{\beta} \right).
\]

(14)

For a good fit, as \( j \) increases in the sum, \( c_j \) decreases. The summation may be summed to \( m \), as opposed to infinity, because the decreasing \( c_j \) values rapidly converge the Tchebychev polynomial sum series expansion. For this study, \( m = 12 \) was sufficiently large to define the model.

It is important to note a few important characteristics of the equation. The equation expansion is constructed such that the interfacial tip is located at the origin, and that the expansion is symmetric about the y-axis (i.e. \((-x) = y(x)\) ). The equation exhibits asymptotic behavior. As \( y \to -\infty \),

\[ x \sim \beta - A \exp(ky), \]

(15)

where \( A \) is assumed to equal 1. Rearranging, the equation becomes
\[ y \sim \frac{1}{k} \log \left( \frac{\beta}{A} \left( 1 - \frac{x}{\beta} \right) \right). \quad (16) \]

2. **Interfacial Data Orientation:** The data were oriented appropriately in order to fit the model. The interfacial data are indexed such that \( i = 1 \) is the first point on the interface, where \( i = 1, 2, \ldots, N \). \( N \) is the number of data points on the traced interface. The interfacial data were translated such that \( X' = \begin{bmatrix} y' \\ x' \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix} \). The translation for the entire interface was:

\[ X' = X - X_1. \quad (17) \]

Next, a counterclockwise rotation tensor was used to position the last interfacial point, \( X_N \), on the x-axis. The angle of rotation, \( \theta \), was determined by

\[ \theta = \arctan \left( \frac{y_N}{x_N} \right). \quad (18) \]

The computed value of \( \theta \) was used as such below:

\[ X' = \begin{bmatrix} y' \\ x' \end{bmatrix} = \begin{bmatrix} \cos(\theta) & -\sin(\theta) \\ \sin(\theta) & \cos(\theta) \end{bmatrix} \begin{bmatrix} y \\ x \end{bmatrix}. \quad (19) \]

Afterwards, the maximum of \( X \) was found to normalize the data. If either maximum of \( X \) equated 0, the interface was inverted in order to find the maximum.

Finally, \( X \) was translated such that the theoretical tip point was on the origin.

\[ X' = X - \begin{bmatrix} 1 \\ 0.5 \end{bmatrix}. \quad (20) \]

The model assumes a symmetrical bubble shape, as shown in **Figure 32**.

However, the bubble is not symmetric about the centerline due to deformation.
Theoretical bubble orientation. The model assumes symmetry about the x-axis. Experimentally, however, this is not the case. The deformation of the model may be broken down into its rotation and shear tensors, as in equation (24). Since the rotation angle is small, we assumed that the shear component was equal to the identity matrix, and therefore had no stretch components. The model was oriented by rotating the axes to $X'$, which is defined by Equation (19). (a) depicts calculating the bubble shape as a shear deformation, while (b) depicts rotating the axis to maintain symmetry about the $x'$ axis. Experimentally, (b) was found to be an accurate assumption.

The deformation gradient may be broken down into its rotation and shear tensors:

$$F_{ij} = R_{ik}U_{kj},$$  \hspace{1cm} (21)

where $R_{ik}$ and $U_{kj}$ are the rotation and shear components, respectively. Since rotation is small, $U_{kj}$ is assumed to equal the identity matrix, $I$, to reduce the deformation.
gradient to only its rotation tensor. Under our flow conditions, this assumption remained valid. It maintained symmetry about the newly defined axis from the rotation tensor, $X'$, while accurately fitting the bubble shape.

3. Curve Fitting and Outlier Removal: The interfacial points of the PIV/shadowgraphy experiments contained noise due to image degradation. A method based on robust nonlinear regression and false discovery rate was used in order to appropriately fit a model to the interfacial points (Motulsky 2006). Although robust methods do not report reliable standard errors or confident intervals, they still report best-fit values for the parameters. Nonlinear regression was chosen over least squares regression because the output is not substantially affected by outliers. The steps are outlined below:

i. An estimate of the model parameters in Equation (13) were chosen and used for an initial approximation of the interface. These parameters $k$ and $c_j$ are hereafter referred to as $a_j$. Initially the values are assumed to equal 1. The initial guess of 1 for the first iteration was sufficiently close because the data sets were normalized. Looking at Equation (13), the maximum data point is $X_0$, which is equal to 1 with the first iteration initial conditions. For a traced interface, the theoretical maximum is at the bubble tip, which normalized is also equal to 1. The nonlinear regression assumed a Cauchy distribution of the residuals, and the Levenberg-Marquardt algorithm was used to minimize the merit function in order to determine the best-fit parameters, $\hat{a}_j$. This was computed using MATLAB

60
function `nlinfit`. This uses a sum-of-squares calculation for the merit function and seeks to minimize that value to identify the best fit.

ii. Next, the absolute value of the residuals were computed for the data, \( y \), and the model, \( \hat{y} \). These were then ranked in ascending order to calculate a P value using a modified two tailed-t test. The P value is the probability of measuring a residual at least as large as the one observed while holding the null hypothesis true. If the residual is large, the P value will be small. A varied significance threshold is defined using the parameters described herein to determine which residuals are outliers. A robust standard of residuals was then computed by:

\[
RSDR = P68 \frac{N}{N-K},
\]

(22)

where \( P68 \) refers to the residual value that is 68.27% of the maximum residual value. This value is chosen because 68.27% of the values lie within one standard deviation of the mean in a Gaussian distribution. \( N \) is the number of data points in the analysis, and \( K \) is the number of parameters fit by nonlinear regression. Motulsky showed that RSDR is more beneficial than just the P68 value because it is much closer to the standard error of fit, \( S_e \).

iii. The \( \alpha \) parameters for a modified two tailed t-test were then computed by:

\[
\alpha_i = \frac{Q(N-(i-1))}{N},
\]

(23)

where \( Q \) is set as the proportion of the rejected null hypotheses which are mistakenly rejected. It is used to control the false discovery rate (FDR) (Benjamani and Hochberg 1994). The lower \( Q \) is set, fewer of the ‘statistically significant’ findings will be considered false positives. For instance, if \( Q \) is chosen to be 1%, fewer than 1% of the discoveries will be considered false
positives, while the remaining values are real. Since the interfacial shape had
significant noise, an aggressive value of 35% was chosen for Q.

The two-tailed P values for each data point were then computed for the t
distribution divided by the RSDR for the degrees of freedom:

\[ t_i = \frac{|\text{residual}_i|}{\text{RSDR}}. \]  

(24)

Next, \( P_i \) was compared to \( \alpha_i \). The P value tests whether each point is an outlier by
testing the null hypothesis that the point came from a Gaussian distribution with a
mean of zero, a standard deviation of RSDR, and N-K degrees of freedom. If \( P_i \)
was less than \( \alpha_i \), the data point \( i \) was considered an outlier and deleted. Another
least-squares regression was conducted on the remaining points after outlier
removal using \( \tilde{a}_j \) as the input parameters.

iv. Steps ii. and iii. were repeated, using the least-squares parameters from the
previous iteration as the new guess for the next fit. The loop stopped when no
more outliers were detected, which then outputted the best model approximation
for the interface. The final output are the \( k \) and \( c_j \) values for \( j = 1, 2, \ldots, 12 \).

3.3. Results

The processed images and interfacial data was compared to determine feasibility
of using the described methods as an effective way to study multiphase flow.
Qualitatively looking at Figure 33, it can be seen that the interfacial data before model
fitting can be determined through the image processing techniques. However, as seen in
the first row, the interfacial data in shadowgraph images with laser illumination still has
significant noise, and thus requires further smoothing to determine the interfacial
geometry. On the other hand, the interfacial data from shadowgraph images using white light illumination is not populated with significant noise. The same interfacial model from the laser illuminated images is used for the white light images to output the blue model fit on the final image. In both cases, the model fits the interfacial shape extremely well.
Figure 33  Comparison of the traced interface and fit model for shadowgraph images from the laser illuminated dual μ-PIV/Shadowgraph images and the white light illuminated shadowgraph images. The traced interfaces are seen in the first column, in red, while the fit models are seen in the second column, in blue.

3.3.1. Accuracy of Outlier Removal and Model Fitting

The outlier removal algorithm was first validated with synthetic data generated from the model, as defined in Equation (13). Artificial noise was added to the model
and ranked according to the outlier removal protocol. The addition of Gaussian-distributed residuals from the model did not produce outliers, as expected. This is because the modified two-tailed t test is based off a null hypothesis that the residual came from Gaussian distribution with a mean of zero. Adding non-Gaussian displacements to the model produced residuals that were ranked as outliers and were appropriately removed.

The modified two-tailed t test is an effective way to detect the noise as outliers on the interface and subsequently remove it for a closer model fit. Since the outlier removal is completed in a defined ranking system, as opposed to in an ad hoc manner, it is acceptable to remove the points determined as noise from the interface. Figure 34 shows the model fitting and subsequent outlier removal process for the example μ-PIV/shadowgraph image. The method was repeated for three iterations to attain the best fit model, which was determined when no more outliers were found on the model for the fit calculated by nonlinear regression. For each iteration, the residuals were determined and their P values were ranked accordingly. If the P value of the fit was lower than the FDR threshold set by Q, that point was considered an outlier and removed from the data before the next nonlinear regression iteration.

Contrasting Figure 34 to Figure 35, which is the model fitting algorithm for the shadowgraph-only experiments. No outliers are found at all, and the area between the fit and the interface is negligible. To quantify the accuracy of the interfacial tracing and the model fitting, the normalized area between the interfacial data and the fit was determined for images on the complete experimental domain and the parent domain for both experiments. The parent domain is defined as the area of the channel that is
upstream the carina tip. In reference to the images, it is channel to the left of the carina. The results are shown in Figure 36. The normalized area between the interfacial trace and the model fit is substantially larger for the images from the dual µ-PIV/shadowgraph system than for images captured from only a shadowgraph.
Figure 34 Example of the outlier removal method for a shadowgraph image from the dual µ-PIV/shadowgraph system. These interfacial points are from the parent channel. It took three iterations to find the best fit. The interfacial points (in red), the outliers (in yellow), and the fit (in blue) are on the left side while the associated P values of the residuals are on the right. The P values less than the FDR threshold are considered ‘statistically significant’, and those outliers are removed from the interface.
Figure 35  Example of the outlier removal method for a shadowgraph image from white light illumination, as opposed to the dual µ-PIV/shadowgraph system. Without speckle noise, the model fitting only took one iteration. No outliers were determined.
Figure 36 Image quality improves model accuracy. Shadowgraph images degraded from PIV were compared with shadowgraph images captured only using white light for water occluded bifurcations. Accuracy of the model fit to the interface was determined by calculating the area between the two curves and dividing it by the area underneath the model. Interfaces from the entire experimental domain are compared in the first set of columns while interfaces only populating the parent domain are compared in the second.

3.4. DISCUSSION

3.4.1. Image Processing

The image processing steps were effective at reducing the speckle noise in the system through smoothing techniques. Each technique smoothed out the variance in the background and foreground without vastly changing the area of each. Otsu’s method successfully separated the background and foreground of the image because the
smoothing techniques produced a bimodal histogram, where the bubble was one set of pixel intensities and the background was another. Figure 37 shows the histogram of each operation with its respective image, which shows the effect each filter has on boundary detection.

It is clear from figure Figure 37 (d) that the smoothing techniques create a bimodal distribution of pixel densities, which is integral for Otsu’s method in determining the interfacial boundary. As stated in 3.1.2, the lower intensity mode is assumed to correlate to the bubble while the higher intensity mode is the noise and background pixels. These intensities are successfully used in Otsu’s Method to threshold the image into the bubble and erase the noise. While each of these smoothing methods loses “details” of the image, we are not concerned with preserving the pixel variations of the objects. Instead, we only focus on the geometrical shapes and interfaces, which are not affected by the smoothing operations.

The corresponding histograms for an image captured with white light, or a “clean” image, are shown in Figure 38. The white light illumination does not produce speckle noise, which degrades the bimodal histograms in Figure 37. Therefore, the two grayscale modes in the clean image are easier to separate into the bubble and background points.
Figure 37  Histograms of image processing a shadowgraph image with speckle noise. Each process reduces the variance of pixels in the image, creating a bimodal histogram at the end which may be used to threshold the image to find the boundary position. (d) The threshold divides the pixels into two regions. The NYM orange corresponds to the bubble pixels, while the NYM blue corresponds to the background. The bubble pixels and the background pixels were converted to black and white, respectively. The large number of pixels with an intensity of 0 corresponds to the border pixels of the image, which are included with the bubble pixels upon thresholding.
Figure 38  Histograms of image processing a shadowgraph image with no speckle noise. The clean image has two distinct pixel regions, where the concentration of pixels with an intensity around 30 corresponds to the bulk of the bubble interface. Comparing to Figure 37, it is clear that the bubble and background modes are separated by a substantially larger amount of gray scale values.

3.4.2. Model Fitting

Referring to Figure 36, the model accuracy improves with image quality. Table 4 summarizes the normalized area averages and standard deviations for the full experimental and parent channel domains. The interfacial the lower normalized area in the parent channel domain in comparison to the full experimental domain may be attributed to the size of the interfaces that are seen in the full experimental domain. The
daughter channels have a smaller diameter, so noise from the µ-PIV/shadowgraph laser bandwidth will have a larger effect on the normalized area calculations than on the interfaces found in the parent channel, which have a larger arc length.

Table 4 The average normalized area between the interfacial data and the model fit for µ-PIV/shadowgraph images and shadowgraph-only images and the standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Full Experimental Domain</th>
<th>Parent Channel Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µ-PIV/Shadowgraph</td>
<td>Shadowgraph</td>
</tr>
<tr>
<td>Average of Normalized Area</td>
<td>9.9398</td>
<td>0.5826</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>5.5595</td>
<td>0.5425</td>
</tr>
</tbody>
</table>

3.5. SUMMARY

The described image processing techniques used to segment the interface of the multiphase flow, in conjunction with the robust outlier removal and model fitting method, is an effective way to determine the shape of the flow. As image quality improves, as seen with the comparison of interrogation techniques, so does the quality of the method.
CHAPTER 4. INTERFACIAL FLOW CHARACTERISTICS

4.1. INTRODUCTION

The interfacial flow characteristics of airway reopening in a bifurcation may finally be studied with the bifurcation microdevice, shadowgraph system, and image processing techniques in place. Different surfactant analogues were introduced to the system and the differences were quantified.

4.2. METHODS AND MATERIALS

4.2.1. Data Acquisition

The previously described shadowgraph apparatus in Section 3.2.1 was used in conjunction with white light to capture images. No µ-PIV was employed for these experiments.

4.2.2. Experimental Flow Control

Experimental flow was controlled by the same configuration as previously described in Section 513.2.2. Flow rates of 0.5 µL · µm/min and 3.0 µL · µm/min were used.

4.2.3. Sample Preparation

SDS (Fisher) and Infasurf (calfactant) (35 mg/ml concentration, ONY Inc, NY) were prepared with varying concentrations. SDS samples were prepared in concentrations of 6.0 mM, 3.0 mM, and 0.6 mM in pure water (18.2 MOhm Millipore...
Direct-Q). These concentrations were chosen because they span the surface tension of SDS, including the critical micelle concentration (CMC), which is the minimum concentration required for micelle formation (Hernainz-Bermudez de Castro 1998). A 1.0 mg/mL Infasurf sample was prepared through dilution with phosphate buffered solution 1x (PBS) (Invitrogen, CA). Ultrapure water (18.2 MOhm Millipore Direct-Q) was used as the control.

4.2.4. Microfluidic Device

The previously described symmetric bifurcation with the specified parameters in Section 2.3.1 was used for experimentation. It was connected to the syringe pump by the same methods as before.

4.2.5. Data Handling

The same image processing and model fitting techniques as described in Section 3.2.5 were employed to determine the interface model. The threshold value of $Q = 0.35$ was used for each image.

**Radius of Curvature Calculation**

The radius of curvature for the interface was calculated as the radius of the circle that fits at the tip point of the interface, which has been defined as $X_0$. The best radius approximation was calculated by incrementally increasing the radius until a positive radial residual value was found, where

$$\Delta R = R_{Circle} - R_{Model}$$

$$R_{Circle} = \sqrt{(x_{circle} - x_c)^2 + (y_{circle} - y_c)^2} \quad (25)$$
\[ R_{\text{Curve}} = \sqrt{(x_{\text{model}} - x_c)^2 + (y_{\text{model}} - y_c)^2}, \]

with \( x_{\text{circle}} \) and \( y_{\text{circle}} \) as the x and y circle values, \( x_c \) and \( y_c \) as the center of the circle, and \( x_{\text{model}} \) and \( y_{\text{model}} \) as the x and y model values. By specifying the point to fit a circle, the radius of curvature found is unique. The tip point was chosen for the radius of curvature measurement because it is a good approximation for the dynamics of the system. Figure 39 depicts the calculated circle fit to the interfacial model.

**Figure 39** Radius of curvature approximation. (a) The bubble tip point \( X_0 \) is fit with a circle, and the radius of the circle is assumed to be an accurate approximation of the radius of curvature at the tip point. (b) For reference, the circle is superimposed on the original image.

**Domain Definitions**

The experimental domain was divided into Channel 0, Channel 1, and Channel 2 for data handling, where they correspond to the parent, top daughter, and bottom channels, respectively. The coordinate system for each channel corresponded to the centerline, where the top half of the channel above the centerline is considered positive.
and the bottom negative. Pictorial representations are shown in Figure 40 and Figure 41. The zero coordinate for Channel 0 is the carina tip. For Channel 1 and Channel 2, the coordinate system is defined as:

\[
x' = \begin{bmatrix} y' \\ x' \end{bmatrix} = \begin{bmatrix} \cos(\phi) & -\sin(\phi) \\ \sin(\phi) & \cos(\phi) \end{bmatrix} \begin{bmatrix} y \\ x \end{bmatrix} - \begin{bmatrix} R \\ 0 \end{bmatrix}
\] (26)

where \( \phi \) is the rotation necessary for the \( y' \) coordinate to be perpendicular to the channel walls, and \( R \) is the half-width of Channels 1 and 2. The coordinate frame changes from Channel 0 to Channels 1 and 2 when the air-liquid interface reaches the carina tip.

**Figure 40** Defined experimental domains. Channel 0, Channel 1, and Channel 2 correspond to the parent, top daughter, and bottom channels, respectively. In Channel 0, the centerline corresponds to the carina tip, with \((0,0)\) at the carina tip. The coordinate system changes to Channels 1 and 2 at the carina tip.
Figure 41 Coordinate systems in the bifurcation. (a) The original for Channel 0 is at the carina tip, with the y axis perpendicular to the bifurcation wall. (b) At the carina, the frame of reference switches to maintain a perpendicular axis to the bifurcation wall.

4.3. RESULTS

The radius, distance of the bubble tip to the carina, and the distance of the bubble tip to the centerline of the domain were calculated and compared in respect to constant flow rate and surface tension. The measurements were left in pixels for accuracy, although the theoretical pixel resolution was 0.744 µm/pixel. Figure 42 and Figure 43 show that the radius of curvature is seen to decrease with the reduction in surface tension.
The results for the slow and fast flow rate experiments are summarized in Table 5 and Table 6, respectively. The radii of curvature in the parent and daughter channels are compared against the different test fluids, as well as their entrance lengths. The radii of curvature did not vary substantially in respect to flow rate. Water had the highest radius of curvature, with \( R_{\text{Parent}} = 280 \) pixels and \( R_{\text{Daughter}} = 210 \) pixels. 0.6 mM SDS had reduced measurements of \( R_{\text{Parent}} = 270 \) pixels and \( R_{\text{Daughter}} = 200 \) pixels. The 6.0 mM SDS sample, where 6.0 mM is higher than the CMC, had even lower measurements of \( R_{\text{Parent}} = 245 \) pixels and \( R_{\text{Daughter}} = 170 \) pixels. The 1 mg/mL Infasurf solution had R values that were equivalent to the 6.0 mM SDS sample. This observation suggests that the radius of curvature does not vary due to the dynamic surface tension tendencies of Infasurf, but instead is due to the static surface tension. In each trial, the radius of curvature increases in Channel 0 as the interface reaches the bifurcation tip because the channel walls begin to diverge as they reach the bifurcation to form Channels 1 and 2.

Decreasing surface tension by increasing surfactant concentration is shown to increase bubble stability and experimental precision, as shown in the following figures. The trials have noticeably less variation, or “scatter”, overall as surface tension is decreased. Therefore, water had the highest amount of scatter, while 6.0 mM SDS and Infasurf had the least. The faster flow rate also decreased scatter in comparison to the slow flow rate. Therefore, the faster flow rate experiments have greater precision than the slow flow rate experiments.

The entrance length \( L \) may be approximated as the distance from the carina tip (0,0) to the region Channel 2 reached an asymptotic radius of curvature. The entrance length increased with decreasing surface tension for both flow rates of 0.5 \( \mu \text{L} \cdot \mu \text{m/min} \).
and 3.0 µL · µm/min, with similar entrance lengths in each. Water had the smallest entrance length of 300 pixels, while Infasurf had the largest entrance length of 500 pixels. Sharp increases in Channel 1’s radius of curvature after the initial decrease were attributed to the stagnation and subsequent regression of the interface in Channel 1.

**Table 5** Summary of results for the flow rate of \( Q = 0.5 \frac{\mu L \cdot \mu m}{min} \). The radius of curvature decreased with surface tension. The entrance length, quantified by the asymptotic radius of curvature behavior in the post bifurcation domains, increased with decreasing surface tension. Scatter decreased with decreased surface tension, suggesting a stabilization due to SDS and Infasurf.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>( R_{Parent} )</th>
<th>( R_{Daughter} )</th>
<th>( \frac{R_{Parent}}{R_{Daughter}} )</th>
<th>( L_{0.5 \frac{\mu L \cdot \mu m}{min}} )</th>
<th>Scatter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>280</td>
<td>210</td>
<td>1.33</td>
<td>300</td>
<td>High</td>
</tr>
<tr>
<td>0.6 mM SDS</td>
<td>260</td>
<td>200</td>
<td>1.30</td>
<td>320</td>
<td>Medium</td>
</tr>
<tr>
<td>6.0 mM SDS</td>
<td>245</td>
<td>170</td>
<td>1.44</td>
<td>440</td>
<td>Low</td>
</tr>
<tr>
<td>Infasurf</td>
<td>245</td>
<td>170</td>
<td>1.44</td>
<td>500</td>
<td>Low</td>
</tr>
</tbody>
</table>

**Table 6** Summary of results for the flow rate of \( Q = 3.0 \frac{\mu L \cdot \mu m}{min} \). The radii of curvature had magnitudes similar to \( Q = 0.5 \frac{\mu L \cdot \mu m}{min} \). The entrance lengths were similar as well. Scatter was reduced in the faster flow rate experiments.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>( R_{Parent} )</th>
<th>( R_{Daughter} )</th>
<th>( \frac{R_{Parent}}{R_{Daughter}} )</th>
<th>( L_{3.0 \frac{\mu L \cdot \mu m}{min}} )</th>
<th>Scatter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>280</td>
<td>210</td>
<td>1.33</td>
<td>300</td>
<td>Medium</td>
</tr>
<tr>
<td>0.6 mM SDS</td>
<td>270</td>
<td>200</td>
<td>1.59</td>
<td>320</td>
<td>Medium</td>
</tr>
<tr>
<td>6.0 mM SDS</td>
<td>245</td>
<td>170</td>
<td>1.44</td>
<td>480</td>
<td>Low</td>
</tr>
<tr>
<td>Infasurf</td>
<td>245</td>
<td>170</td>
<td>1.44</td>
<td>500</td>
<td>Low</td>
</tr>
</tbody>
</table>
Figure 42 The radius of curvature for different surface tensions during the 0.5 μL·μm/min flow rate. The radius of curvature for the stabilized domains is seen to decrease from (a) to (d). This may be attributed to the decrease in surface tension from the addition of SDS and Infasurf. The entrance length, which is approximated as the distance from the carina tip until Channel 2 reaches a horizontal asymptote, is seen to increase from (a) to (d). The scatter of the data is reduced with the addition of SDS and Infasurf. (c) and (d) have similar trends because the concentrations of SDS and Infasurf in each are higher than the critical micelle concentration.
The radius of curvature for different surface tensions during the 3.0 \( \mu L \cdot \mu m/min \) flow rate. The scatter in the faster flow rate is smaller than in the slower flow rate. The entrance lengths in the faster flow rate are similar to their corresponding entrance lengths in the slower flow rate.

The bubble tip point \( X_0 \) in Channel 0 is above the centerline before the splitting point in each experiment. Recall from Figure 32 that \( X_0 \) is not defined as the interface point on the centerline, but rather the interface point on the interfacial axis of symmetry.

Figure 44 and Figure 45 plot the centerline distance with respect to carina distance. The deformation of the bubble may be quantified as the distance from the centerline, which is an indirect measurement of the angle of rotation used to fit the model. As the rotation angle decreases, so does the distance to the centerline. For all experiments, the
centerline distance in Channel 0 was approximately +15 pixels. For both flow rates, Channel 2 initially had a large distance of +20 pixels from the centerline, but it approached zero as the interface progressed through the domain. Channel 1 did not undergo a large deformation at either flow rate, where the centerline distances fluctuated around -4 pixels. The finger of air in Channel 1 for the slow flow rate stagnated sooner than in the faster flow rate, which is evident in the concentrated stagnation Channel 1 data points.

Figure 44 The distance of the bubble tip to the centerline as a function of location to the carina tip for $Q = 0.5 \mu L \cdot \mu m/min$. Channel 2 had an initial deformation when Channel 0 split at the bifurcation. Channel 1 did not undergo a large deformation and stagnated quickly. Channel 2, on the other hand, propagated completely through the channel for all experiments.
Figure 45  The distance of the bubble tip to the centerline as a function of location to the carina tip for $Q = 3.0 \, \mu L \cdot \mu m/min$. Unlike the slower flow rate, the faster flow rate has a more symmetrical opening. Both channels underwent similar deformation characteristics as they do with the slow flow rate, but Channel 1 propagated further than it did previously. In Infasurf, Channel 1 is completely opened by the air bubble.

4.4. DISCUSSION

The radius of curvature measurements are larger than the channel radii, but they are still accurate. This may be attributed to the high contact angle of the interface with the channel wall, which is defined by the material properties of PDMS with its surface treatment. The minimal visualization of the lubrication region also contributes to the
large radius of curvature measurements. The scattering effect of the results was also due to the same reasons.

The trends in the radius of curvature are reasonable. Rearranging the Law of Laplace (Equation (1))

\[ R = \frac{2\gamma}{\Delta P}, \]

the radius is a function of the surface tension and pressure drop. For a constant pressure drop, the radius decreases with surface tension. This corresponds to our measured radii of curvature against the decreasing surface tensions of the test liquids. Figure 46 summarizes the radius of curvature trend. Infasurf and the 6.0 mM SDS samples produced the same low radius of curvature. Therefore, the radius of curvature is not dependent on the dynamic surface tension properties of surfactant, or the \( \beta U_i \) term in \( (P_{cap})_i \).
The radius of curvature increases with static surface tension. These measurements were taken from Channel 0. It is important to note that Infasurf and 6.0 mM SDS had the same radius of curvature, implying that the radius is independent of dynamic surface tension.

The entrance length decreased with surface tension, which is summarized in Figure 47. In this observation, however, Infasurf had a larger entrance length than 6.0 mM SDS. Therefore, the entrance length is dependent on the dynamic surface tension.
Figure 47  The entrance length decreases with surface tension. Unlike the radius of curvature, the entrance length for Infasurf and 6.0 mM SDS are different. This difference may be due to the dynamic surface tension of Infasurf, which increased the overall entrance length but stabilized the bifurcation reopening.

For each experiment, the bubble tip in Channel 0 was above the centerline, which may be seen in the results of Figure 44 and Figure 45. This implies that the symmetric interface in Channel 0 is rotated towards Channel 1, where more of the surface area of the bubble is entering Channel 1. It is important to note that surfactant deposits onto the interface into a stagnation point at the bubble tip, which was experimentally determined by Yamaguchi and diagrammed in Figure 48 (Yamaguchi 2009). A high concentration persists at the bubble tip due to the convection currents in the liquid phase. This stagnation point is critical in the reopening dynamics of the system. Depending on the position of the bubble tip upon contact with the carina tip, one daughter channel will receive substantially more surfactant than the other.
Figure 48  Bulk phase surfactant deposits into the stagnation point corresponding to the bubble tip (Yamaguchi 2009). The convection patterns in the liquid phase converge to the bubble tip.

Therefore, in the experiments with surfactant, Channel 1 had more surfactant on its interface in comparison to Channel 2. The extra surfactant correlated to a smaller surface tension on Channel 1’s interface, which was responsible for the symmetrical opening events. Channel 2, which would contain less surfactant upon reaching the bifurcation point, had the higher surface tension, and therefore had a larger pressure drop over the air-liquid interface. The interface required more deformation to propagate further, which was seen as a large displacement of the bubble tip from the centerline at $t = 0$ reducing as the interface propagates downstream. This correlated to the interface rotation from a large angle, as defined in Figure 32 (b), to a smaller orientation rotation angle. Comparing the 6.0 mM SDS sample with water, the initial asymmetrical reopening characteristics are reversed, which are diagrammed in Figure 49. The high concentration of SDS on the bubble tip entered Channel 1, which lowered the surface tension and capillary pressure seen in Channel 1. This decrease in pressure drop was responsible for the reversal of the asymmetrical tendency of the water experiments.
6.0 mM SDS changes the asymmetrical reopening characteristics in respect to water experiments. The bubble tip, containing a large concentration of SDS, enters Channel 1 at $t = 0$, spreading a much larger dose of SDS on the interface in Channel 1 and lowers the surface tension drastically.

Infasurf, unlike the other trials, split symmetrically. This symmetrical reopening event was due to the dynamic surface tension seen with pulmonary surfactant, which had a large value for $\beta$. The bubble tip entered Channel 1, which delivered a high concentration of Infasurf at $t = 0$. Figure 50 diagrams the Infasurf experiments. The interfaces in the daughter channels opened symmetrically in the channel with asymmetric opening characteristics without surfactant.
4.5. SUMMARY

The microdevice fabrication technique, in conjunction with the developed image processing and robust fitting method, was sensitive enough to detect changes in the interfacial geometries from altering velocity and surface tension through the addition of surfactants. The airway reopening was contingent upon surfactant concentration and deformation of the bubble in Channel 0 immediately before the bifurcation tip, where the surfactant aggregations affect bifurcation reopening. While the control had an asymmetrical reopening for Channel 2, the 6.0 mM SDS has an opposite asymmetrical reopening initially. Infasurf had a symmetrical reopening due to the dynamic surface tension, which stabilized the interfaces in the daughter channels.
CHAPTER 5. CONCLUSION

We have shown the feasibility of using soft lithography and a dual μ-PIV/shadowgraphy microscopy system in conjunction with rigorous image processing and model fitting to determine the instantaneous interfacial geometries of occluded bifurcation reopening events. The methods are sensitive enough to determine the differences in geometrical interfaces for different chemical compositions and velocities.

Biofidelic models were successfully fabricated using soft lithography that are interrogated optically. An effective surface modification treatment was chosen after a time-dependent surface tension study to reduce the pressure required to fill the microdevices with fluid and multiphase flow. The master wafer feature height was analyzed as a function of radial distance to ensure production quality of the microchannels.

Speckle noise in the shadowgraph images from the instantaneous dual μ-PIV/shadowgraph visualization system was overcome using numerous image processing techniques. The processed images still had jagged air-liquid interfaces, so a novel model fitting and noise-pruning method was successfully implemented to smooth out the interface and find a satisfactory geometrical approximation.

Finally, occluded airway reopening events were simulated using different surfactants and concentrations. Asymmetrical reopening events were successfully interrogated with methods that were sensitive enough to calculate differences in interfacial deformation and geometries.
5.1. **Experimental Limitations**

The experiments had a few design limitations. First and foremost, the microfluidic devices were rigid, square channels. Lung airways are flexible circular cross sectional areas. However, this approximation is justified because Low et al described collapsed airways as having ribbon-like configurations, which more closely correlate to a square geometry than circular (Low 1997). Follow up studies may offer systems that more closely mimic pulmonary airway morphology.

The Capillary number, \( Ca = \frac{\mu V}{\gamma} \), for these experiments are orders of magnitude lower than the related computational models. The disparity in Capillary number creates difficulty in comparing computational and experimental results. Experiments that have Capillary numbers on the same order of computational models would be invaluable for validation of results, as well as producing new theories.

5.2. **Future Work**

The work on the shadowgraph image processing techniques offers insight on improvements for the instantaneous \( \mu \)-PIV/shadowgraphy system. Experiments to optimize the illumination conditions by varying the laser bandwidth need to be performed to increase shadowgraph image quality while minimizing \( \mu \)-PIV image degradation. More accurate experiments on the flow characteristics of reopening occluded bifurcations need to occur after illumination optimization. So far, experiments utilizing both have produced either shadowgraph or \( \mu \)-PIV images with too much image degradation to analyze for data analysis.
The radius of curvature measurements need to be validated through another means of calculation, such as through determining it through a function of arc length. Although the characteristic radius of curvature may be approximated by the radius of curvature at the bubble tip, determining the varied radius of curvature in respect to location on the interface may produce interesting results.
BIOGRAPHY

Matthew Van Houten was born and raised in Stamford, CT, where his close proximity to New York City grew his love for music and the saxophone. This enthusiasm for music blossomed at Tulane University in New Orleans, where Matthew studied the unique heritage of jazz, funk, and soul in conjunction with his biomedical engineering coursework. He regularly gigs around the city and Louisiana with various groups, notably, Doombalaya; the 9-piece infectious blend of New Orleans music that brings you “Funk for the Rapture”. He can be found performing with Doombalaya at the Howlin’ Wolf every Wednesday night, and sitting in during the weekends on Frenchmen Street. Matthew performs at the Annual New Orleans Jazz and Heritage Festival with titans such as Delfeayo Marsalis, Rex Gregory, James Westfall, and Jesse McBride, to name a few.
REFERENCES


