Rheological and velocity profile measurements of blood in microflow using micro-particle image velocimetry

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Abstract

Microhemodynamics is the study of blood flow in small vessels, usually on the order of 50 to 100 $\mu$m. The in vitro study of blood flow in small channels is analogous to the in vivo study of the microcirculation. At this scale the Reynolds and Womersly numbers are significantly less than 1 and the viscous stress and pressure gradient are the main determinant of flow. Blood is a non-homogeneous, non-Newtonian fluid and this complex composition and behavior has a greater impact at the microscale. A key parameter is the shear stress at the wall, which is involved in many processes such as platelet activation, gas exchange, embryogenesis and angiogenesis. In order to measure the shear rate in these blood flows the velocity profile must be measured. The measured profile can be characterized by the maximum velocity, the flow rate, the shear rate at the wall, or a shape parameter reflecting the bluntness of the velocity profile.

The technique of micro-particle image velocimetry ($\mu$PIV) was investigated to measure the velocity profiles of blood microflows. The material of the channel, the type of tracer particles, the camera used, and the choice in data processing were all validated to improve the overall accuracy of $\mu$PIV as a blood microflow measurement method. The knowledge gained through these experiments is of immediate interest to applications such as the design of lab-on-a-chip components for blood analysis, analysis of blood flow behavior, understanding the shear stress on blood in the microcirculation and blood substitute analysis.

Polymer channels were fabricated from polydimethylsiloxane (PDMS) by soft lithography in a clean room. PDMS was chosen for ease of fabrication and biocompatibility. The contacting properties of saline, water, and blood with various polymer channel materials was measured. As PDMS is naturally hydrophilic, surface treatment options were explored. Oxygenated plasma treatment was found to be less beneficial for blood than
for water.

The choice of camera and tracer particles were validated. Generally, for \textit{in vivo} studies, red blood cells (RBCs) are used as tracer particles for the $\mu$PIV method, while for \textit{in vitro} studies, artificial fluorescent micro particles are added to the blood. It is demonstrated here that the use of RBCs as tracer particles creates a large depth of correlation (DOC), which can approach the size of vessel itself and decreases the accuracy of the method. Next, the accuracy of each method is compared directly. Pulsed images used in conjunction with fluoresecing tracer particles are shown to give results closest to theoretical approximations. The effect of the various post-processing methods currently available were compared for accuracy and computation time. It was shown that changing the amount of overlap in the post-processing parameters affects the results by nearly 10%. Using the greatest amount of correlation window overlap with elongated windows aligned with the flow was shown to give the best results when coupled with a image pre-processing method previously published for microflows of water.

Finally the developed method was applied to a relevant biomedical engineering problem: the evaluation of blood substitutes and blood viscosity modifiers. Alginate is a frequently used viscosity modifier which has many uses in industry, including biomedical applications. Here the effect of alginate on the blood rheology, i.e., the shape of the velocity profiles and the maximum velocity of blood flow in microchannels, was investigated. Alginate was found to blunt the shape of the velocity profile while also decreasing the shear rate at the wall.

Overall, the accuracy of $\mu$PIV measurements of blood flows has been improved by this thesis. The work presented here has extended the known methods and accuracy issues of blood flow measurements in $\mu$PIV, improved the understanding of the blood velocity profile behavior, and applied that knowledge and methods to interesting, relevant
problems in biomedical and biofluids engineering.
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Chapter 1

Introduction

The medical and fluid mechanics communities have become increasingly interested in disturbances of blood viscosity and microcirculation. The study of blood flow in small vessels, microhemodynamics, is on the scale of 50 to 100 µm. *In vitro* microhemodynamic studies are analogous to the *in vivo* studies of the microcirculation. At this scale the Reynolds and Womersly numbers are significantly less than 1 and the viscous stress and pressure gradient are the main determinations of flow. Blood is a non-homogeneous, non-Newtonian fluid, composed of both plasma and particles and this complex composition and behavior are more apparent and important at the micro scale. The understanding of the non-Newtonian nature of whole blood at the macro level has been much studied (Thurston 1972, 1979; Bureau 1978, 1979, 1980; Merrill 1963a, 1969). The behavior at the micro level is significantly more complex (Merrill 1963b; Fung 1993, 1996; Pries 1990, 1995, 1997, 2003, 2011). In the body, the microcirculation is the site of exchange of all nutrients including oxygen. In order to fully understand the flow of blood in these small vessels, we must know the shape of the velocity profile. This shape is essential in understanding the shear behavior at the walls of the vessel, in addition to predicting the viscosity parameters. Much of the nutrient exchange in the microcirculation is shear-
dependent, especially the exchange of oxygen (Cabrales et al. 2006a; Chin et al. 2011; Chiu and Chen 2011; Ulker et al. 2011). The extent of perfusion of the microvessels can be affected by blood behavior, which in turn can affect drug uptake into the system (Cabrales et al. 2006b). In addition, recognizing the characteristics of blood flow in the microcirculation is essential to understanding the mechanical interactions between the blood and the vessel (shear stress) consequently giving insight into the causes of cardiovascular disease, embryogenesis or angiogenesis. Examples of application areas include developmental biology, the mechanics of platelet activation and tumor perfusion studies. There are many other applications of micro level velocity profile behaviors such as blood disease analysis, lab-on-a-chip fabrication and analysis, and other pharmaceutical applications motivating the need to investigate rheological properties at the micro level. In order to measure or calculate the shear rate or shear stress in these blood flows the velocity profile must be measured. The measured profile can be characterized by the maximum velocity, the flow rate, the shear rate at the wall, or a calculated shape parameter reflecting the bluntness of the velocity profile.

Micro-particle image velocimetry (µPIV) is a relatively new technique that is coupled with cross-correlation image processing to determine the velocity field and consequently the velocity profile of blood flow through microchannels. This technique has become the most widely used tool in vivo as well as in vitro. In this technique, a camera is used to record the displacement of tracer particles in a sequence of images. However the lack of accuracy inherent in this technique when applied to blood microflow has slowed scientific advancement in the microhemodynamics field. This technique is commonly applied with several parameters such as the type of tracer particles, the camera used, and data processing method. Each of these factors effect the accuracy of the final measurement. Results from different methods are usually directly compared in biological studies and
also used as input for numerical flow simulations.

This thesis is focused on velocity profile measurement of blood microflows using micro-particle image velocimetry (µPIV) to improve the accuracy of this technology when blood is the working fluid in order to then apply the technique to relevant biomedical problems like blood viscosity modification. The knowledge gained through these experiments will greatly assist in the development of applications such as the design of lab-on-a-chip components for blood analysis, analysis of blood flow behavior, understanding the shear stress on blood in the microcirculation and blood substitute analysis.

1.1 Blood composition

As a working fluid, blood has a unique set of properties. It has long been understood that blood viscosity behaves in a non-Newtonian manner (Thurston 1979). Much of this behavior is related to the particulate nature of blood as it is composed of a Newtonian base (plasma) with suspended formed elements (red blood cells, white blood cells, platelets, etc). It is generally accepted that the behavior of blood depends on the percentage of red blood cells (RBC) present, referred to as hematocrit, as well as the size of the measured channel (Pries 1992).

Blood is about 40 to 45% solids and 55 to 60% plasma, which is a clear liquid with viscosity close to that of water (Waite 2006). Of the solids in blood, 95% are red blood cells (Chandran et al. 2007). On its own, plasma is a Newtonian fluid (Fung 1993). The average adult has 4.5 to 6 liters of blood in their system, constituting about 6 to 8% of their body weight (Waite 2006). The composition of blood greatly affects its properties and flow, and one of the major solid components in blood is erythrocytes (red blood cells). Red blood cells are biconcave discs. These cells bind the oxygen and distribute it to the various parts of the body, and have a typical life span of about 125 days (Waite
The pH of blood is usually between 7.35 and 7.45 (Waite 2006).

An individual red blood cell is about 7 microns in diameter and 2 microns in thickness. Red blood cells under static or low shear stress tend to clump together and then move apart. This phenomenon is defined as red blood cell aggregation. These aggregations form and disaggregate under shear stress. The aggregating properties of blood were first documented by John Hunter in 1786. This is a healthy part of blood function, and can be viewed as a natural defense function preserving a more constant set of properties in the body (Popel and Johnston 2005). Bishop, et. al. (2001) suggest that 60% of all venous vascular resistance in resting muscles is due to red blood cell aggregation and in small vessels, this effect is more pronounced and can have much greater effect on the blood flow. Aggregation in small vessels has been suggested to be the cause of the increased resistance to flow at low flow rates (Bishop and Johnson 2005). The function of aggregation determines the shear-thinning properties of the bulk fluid and the forces in the local fluid (Bishop and Johnson 2005).

Aggregation characteristics vary within athletic mammals. In addition to aggregation, the blood of some large athletic mammals form into regular stacks called rouleaux. The forces between the adjacent blood cells are the greatest when they are oriented in rouleaux. These stacks are not static, and constantly move and break apart the same as the non-uniform aggregates. Human, equestrian, and porcine blood form rouleaux, while bovine and sheep blood do not (Weng et al. 1996). Porcine blood aggregation kinetics have been demonstrated to be the closest to human blood (Weng et al. 1996). If there were no aggregation whatsoever, and blood cells did not deform, then laminar flow of blood could be described using the Poiseuille flow model. Aggregation changes the flow profile of the blood (Bishop et al. 2001). Frequently, during in vitro studies, blood cells are washed in saline to remove platelets and fibrin and then tested as saline suspensions.
This removes the aggregating properties of the blood.

1.2 Microcirculation

The properties of blood are increasingly important as the scale of the blood vessel decreases. Microcirculation is the range of blood vessels where the Reynolds and Wormersly numbers are significantly less than 1 and the viscous stress and pressure gradient are the main determinations of flow (Fung 1996). The microvascular system is made up of various veins, arteries, channels and capillaries, some of which are embedded within or around the internal organs (Popel and Johnson 2005). These vessels are generally defined as microcirculation in any vessel less than 50 to 100 micrometers in diameter. The behavior of the blood in the vessel depends on the size of the vessel in addition to the composition of the blood. The Fåhræus and Fåhræus-Lindqvist effects further describe such phenomena: the former describing the dependence of the apparent viscosity of the blood on capillary diameter and the latter describing the apparent decrease in RBC concentration with decreasing tube diameter (Fåhræus 1929, Fåhræus and Lindqvist 1931, Goldsmith et al. 1989). Also apparent at this scale is the cell-free layer, a RBC depleted layer near the wall of the vessel or microchannel. The cell-free layer is caused in part by the higher viscosity of the RBCs in comparison with the bulk flow (Popel and Johnson 2005). In the presence of aggregation, the cell-free layer is more pronounced as entire aggregates move to the center of the channel (Bishop et al. 2001).

These small vessels are the primary site of exchange between the tissues of the body and the blood itself, and 80% of the pressure drop in the vascular system occurs in micro-scaled vessels (Popel and Johnson 2005). Oxygen exchange is an important factor in these beds. Shear stress on the endothelial cells at the wall of the arterioles has been shown to affect both the oxygen uptake of the endothelial cells and the viscosity
of the blood (Cabales 2006). Chiu and Chien present a review of the current research on endothelial cells from a clinical perspective (2011), but an analytical study in vitro including validation of the post-processing methods used in μPIV is lacking. The shear stress at the endothelial cell walls and the velocity profile are key factors in extending clinical understanding (Popel and Johnson 2005).

1.3 Micro-particle image velocimetry

In order to measure velocity profile characteristics in the microflow, an accurate flow measurement method must be employed. While macro scale flows can be imaged using a variety of methods such as ultrasound doppler, or particle tracking software, these measurements are instantaneous or average measurements of flow. They cannot directly give velocity profile characteristics, just a measurement of flow or a series of individual particles’ movements. One of the only methods that can be used for velocity profile measurements is particle image velocimetry (PIV) when coupled with cross-correlation. In vitro μPIV is advantageous over in vivo or intravital microscopy in that it can be coupled with an accurate flow-regulating device such as a microsyringe pump and does not depend on live animals or organs.

PIV is the process of taking two images of a flow at very short time intervals and calculating the movement of the fluid in that time. This is accomplished by using cross-correlation to calculate the movement of tracer particles. The overall procedure is shown in Figure 1.1, taken from the video portion of Chapter 3. In macro scale PIV, focused laser light is used to highlight exact planes within the flow. μPIV is a micro-scale version of macro PIV but as with micro scale fluid dynamics the physics involved becomes increasingly more complex as the scale is reduced. At the micro scale volume illumination is required which can create complications with seeding particles and correlation


$\mu$PIV was first developed by Santiago et al. and has been applied to blood since Sugii et. al. first used the technique to measure blood flow in 100 $\mu$m round glass tubes in 2001, and then again in 2002 used the technique in vivo to measure the velocity profile developed in a rat’s mesentery arteriole (Santiago et. al., 1998; Sugii et. al., 2002a and b). There are many challenges associated with using $\mu$PIV, which are compounded by the complexity of blood as a working fluid as summarized previously. In macro PIV a sheet of light, generally supplied by a laser, is used to select a plane of measurement. In $\mu$PIV volume illumination must be used due to the scale. The choice of tracking particles...
to be used and method of applying them can be difficult. RBCs can be tracked as can fluorescing microparticles. Individual RBCs could be fluorescently labeled, or dyed in their entirety, but this requires significant chemical interaction with the RBCs which could alter their behavior. The choice of tracers affects the camera choice as well.

There are three general concepts to using $\mu$PIV: approach, accuracy, and application. The approach chosen depends on the subject. *In vivo* research frequently requires use of high speed cameras while *in vitro* research can used pulsed cameras and laser fluorescent microparticles, which can be toxic to live subjects. Using smaller particles can decrease the error in the measurement. Once the camera is chosen, it needs to be affixed to a microscope. *In vivo* work generally uses an upright microscope with light from above and the lens touching the tissue (subject), while *in vitro* research can use an inverted microscopes with the laser light directed up, and the fluorescence captured below as chips are generally optically clear. These options are summarized in Figure 1.2. Another option is the confocal microscope, which scans optical slices of an image giving three-dimensional images. The method was introduced by Park *et al.* in 2004 and then applied to blood by Lima *et al.* in 2006.

Important metrics in high speed and pulsed camera data are the depth of focus and the depth of correlation (DOF and DOC). The DOF is a measurement of the accuracy of the placement of the focal plane in a microscopy image. Kloosterman *et al.* have shown that the focal length in biological applications of $\mu$PIV affects the final results (2010). The DOC is a microscopy concept that describes how far one particle, above or below the focal plane, affects image-processing algorithms. It can be interpreted as a measurement of spatial resolution in the $z$ dimension. The DOC is depicted in Figure 1.3, taken from Chapter 4. Once the images are acquired, the accuracy of the data processing technique must be determined. Data is generally given as velocity vectors, and this data can be
pre-processed, processed and post-processed in a variety of techniques. The technique applied can affect the accuracy of the final results. Finally, in presenting the data, either averages or snapshots of data can be shown. As micro scale flows are not always stable, an average across a length of channel or vessel for a set amount of time is commonly used.

Once the camera, microscope and data processing method are chosen, data can finally be acquired. Evaluating the resulting velocity profiles can be done numerically by fitting curves to the data and finding the coefficient of a power-law model for the curve, or expanding the 2D profile into a 3D flow rate through projection. More complex models than the power-law can be fitted, and other calculations can be made, such as the shear rate at the wall, which can be used to compare the shear rate values between samples. However, each choice depends on the application. When measuring velocity profiles of
unique solutions (i.e. modifying blood viscosity for medical uses), the specific properties of those fluids must be accounted for. Accuracy becomes increasingly important when \( \mu \)PIV data is used for calculations and any error will propagate.

### 1.4 Microchips

In order to use micro-particle image velocimetry effectively, the channels must be designed with the system in mind. Glass is the standard microfluidic chip material, but glass is not biocompatible as it can cause the red blood cells (RBCs) to change shape.
(echinocytosis) and, in whole blood, causes platelets to adhere to the sides of the channel. Red blood cells adhere to glass walls even if the fibrin and white blood cells are removed. Complicated channel geometries may be cut from silicon wafers, but silicon is not optically clear. In microfluidics, usually, the experiment needs to be visible. For these reasons polymers are frequently used for blood microchannel studies. Acrylics such as poly(methyl methacrylate) (PMMA) and silicones such as polydimethylsiloxane (PDMS) are often chosen for their biocompatibility. PDMS has become the microfluidic chip material of choice in most labs due to its ease of use, optical properties, and low price point. The one major drawback to using PDMS is the inherent high hydrophobicity. Much research is dedicated to defeating that drawback (Zou et al. 2010). In order to overcome the hydrophobic nature of PDMS, oxygen plasma treatment is generally used. Plasma treatment of PDMS can be used to create a permanent chemical bond to glass slides when the glass slides were also treated by oxygen plasma. The treated surface of the slide and the treated surface of the channel are contacted firmly for a few seconds to achieve the chemical bond. The full PDMS channel fabrication procedure can be found in Appendix B. Various channel materials were compared in a contact angle study with water, plasma, phosphate buffered saline, and varying concentrations of red blood cells in plasma and phosphate buffered saline. The hydrophilic-inducing properties of plasma treatment were not found to be as significant on the higher concentrations of blood, nor on the native plasma.

1.5 Gap in the current knowledge and novelty

µPIV is a relatively young technique; since it has only been applied to blood since 2001 outstanding questions remain. Frequently studies are done using glass, yet the contacting properties of blood on more biocompatible channel materials which are optically clear
for use in a μPIV have not been reported. There has also not been a direct comparison of the two μPIV methods used: tracing fluorescent micro particles or tracing the RBCs directly. A method for determining when high speed video is acceptable and when fluorescent tracers must be used is also needed. Additionally, it is understood that data processing affects the final results (Kloosterman 2010). However, direct comparisons of all available methods when applied directly to blood suspensions have not been published. These necessary operational validations will increase the impact of velocity profile characterizations done with this method. Once the method has been validated for blood flows, it can be applied to more complicated configurations, such as viscosity-modified blood. It has been demonstrated that the shear stress on endothelial cells at the vessel wall control the uptake of nutrients and oxygen (Chiu and Chen 2011). Using the velocity profile changes to calculate the measurable effect of the additives on the shear rate were demonstrated for the first time. Additionally, using the velocity profiles measured, a more accurate measure of blood viscosity at the micro scale can be achieved. This increase in accuracy will be very useful for studies into shear-dependent drug delivery in the microcirculation, oxygenation studies, perfusion and hemorrhagic shock studies, and complex rheological characterizations using μPIV.

1.6 Thesis structure and author contributions

Chapter 1 presents an overview and introduction to the various aspects of the thesis: blood as a working fluid, the microcirculation as a framework, μPIV as a measurement method, and the fabrication of test channels. A general glossary of all terms can be found in Appendix A. Specific terms for chapters are identified at the beginning of each chapter. Chapter 2 is explanation of the microchip fabrication materials selection process based on the contact angle of blood on the surfaces of common microchip materials.
This chapter was published in the Journal of the Mechanical Behavior of Biomedical Materials. A literature review and protocol for biofluidic microchip fabrication using soft lithography can be found in Appendix B. Chapter 3 is a μPIV operation protocol for using the microchips in a μPIV system to measure microblood flow velocity profiles. This section forms the written portion of an invited article in press in the Journal of Visualized Experiments. Appendix C provides a detailed explanation of flow characterization calculations that were done with the measured velocity profiles.

Chapters 4 through 6 detail the accuracy of the μPIV method when applied to blood. Chapter 4, using a high speed camera to compare experimental data with computer simulations, was published in Physiological Measurement. Chapter 5 is an analytical comparison of the high speed camera method and the pulsed camera method, forming a journal article submitted for publication. Chapter 6 is an analysis of the data processing of the pulsed camera μPIV data using fluorescent tracers and has been published in Measurement Science and Technology. Appendix D presents a paper of an invited keynote presentation of the work summarized in Chapters 4 through 6, presented at the 2012 ASME International Conference on Nanochannels, Microchannels and Minichannels in Puerto Rico, USA.

Chapter 7 is an application of the tested μPIV method to relevant biofluid engineering problems where the method is used to measure the effect of sodium alginate, a viscosity modifier, on the shape of the velocity profiles of blood microflows. This modifier has potential applications in increasing perfusion of delivered drugs intravenously and in cases of hemorrhagic shock. Chapter 7 has also been submitted for publication. Finally, in Chapter 8 general conclusions and recommendations are made based on the entirety of the thesis. It also presents future works which extend the method of velocity profile measurement to optical micro rheomeotry, where the velocity profiles are used as an in
situ verification of pressure drop measurement. This project was designed by the author and then constructed by CMC (Canadian Microelectronics Corporation) as the winner of a design competition.

Katie Pitts was the first and main author and performed the main experimental investigations in all of the publications and chapters except for Chapter 4, which was a collaboration with Boris Chayer of the Laboratoire de biorhéologie et d’ultrasonographie médicale (LBUM) at the Centre Hospitalier de l’Université de Montréal (CHUM). He is the first author. The third author of Chapter 4, Guy Cloutier, is Boris Chayer’s supervisor and also provided the lab space and equipment for the experimental portion of the work. In this work Katie Pitts collaborated in the experimental investigation and participated in the editing of the manuscript for publication. In Chapter 2 the second author, Sura Abu-Malloh, was an undergraduate student under Katie Pitts’ supervision who assisted with data collection. In Chapter 6 the second author, Rym Mehri, was a Master’s of Science student who obtained some of the data. The third author in Chapter 6, Catherine Mavriplis, is Rym Mehri’s co-supervisor with Marianne Fenech. Authorization for collection of bovine and porcine blood was obtained by OMAFRA (Ontario Ministry of Food, Agriculture and Rural Affairs) and can be found in Appendix E.

1.7 References


Bureau M, Healy JC, Bourgoi D, Joly M. Etude expérimentale in vitro du comporte-


Chapter 2

Contact angle study of blood dilutions on common microchip materials

This chapter was published as: Pitts KL, Abu-Mallouh S, Fenech M. Contact angle study of blood dilutions on common microchip materials, Journal of the Mechanical Behavior of Biomedical Materials, 2013; 17:333-336. DOI: 10.1016/j.jmbbm.2012.07.007

Biocompatible polymers are commonly used to fabricate microfluidic channels for the study of biological flows, such as blood microflows. The most common of these materials is polydimethylsiloxane (PDMS), which is very hydrophobic. Oxygenated plasma is advocated to treat the PDMS with reported decreases in contact angle i.e increase the hydrophilicity of the material in order to make the liquid to flow easily. All contact angle studies have been reported with water. Here the contact angles of blood suspensions, in
saline and native plasma, are compared to each other and water on common microfluidic chip materials. The hydrophilic effect of plasma-treatment on PDMS is not found to be as significant with blood suspensions as it is with water. Red blood cells suspended in native plasma are found to have a greater contact angle than those suspended in saline.

2.1 Introduction

Microfluidic chips are frequently fabricated in order to study biological flows such as blood. These microchips are scaled to mimic the microcirculation in the body, where vessels are on the order of 100 µm in diameter (Popel, Johnson 2005), or for blood tests in lab-on-a-chip devices like immunoassays. For the lab-on-a-chip devices the hydrophilicity or “wettablity” of the material is of great importance as the blood products are usually driven by capillary effect. Regarding the \textit{in vitro} study of the microcirculation, the hydrophilic behavior of the material will modify important physiological parameters such as the shear rate at the wall. The surface interactions required to generate blood oxygenation in a microchip environment also depend on the “wettability” of surface (Atencia and Beebe 2005). Contact angle is used here as a measure of “wettability.” The blood products used in these studies could be plasma, whole blood, suspensions of red blood cells (RBCs) in native plasma, or in artificial plasma such as saline. These suspensions avoid the clotting that occurs with platelets and whole blood. Glass is generally avoided as a medium for these chips due to its tendency to cause echinocytosis in red blood cells (RBC). Commonly used biocompatible materials are polydimethylsiloxane (PDMS) and poly(methyl methacrylate) (PMMA). For biological applications of PDMS microchips, fabrication is generally done through soft lithography (Cheung \textit{et al.} 2009, Sia and Whitesides 2003, McDonald and Whitesides 2002). Bélanger and Marois reviewed the research into the biocompatibility and hemocompatibility of PDMS as a biomaterial.
Their review indicates that PDMS has good hemocompatibility and other research indicate porcine blood samples are a good indicator of human blood, at least in the case of platelet interaction (Bélanger and Marois 2001). In addition to biocompatibility tests, there has been significant research in the surface modification of PDMS such as oxygen plasma treatment, to change the inherent hydrophobic nature of the polymer (Zhou et al. 2010). While not used as widely, PMMA has been put forward as an alternative to PDMS as a channel material, as reviewed by Chen, Zhang and Chen in part because it was thought to be the most hydrophilic of the biocompatible polymer options (Chen et al. 2008). Another thermoplastic previously suggested for use in blood research was polypropylene (PP) (Kawamoto et al. 1998). The problems associated with using thermoplastics like PMMA or PP is the high processing temperatures and difficulty creating inexpensive, small-volume runs of a microchip, which is why PDMS is so widely used in research.

When fabricating channels out of PDMS, oxygen plasma treatment is a way to change its naturally hydrophobic surface properties to a more hydrophilic surface. This is supported by contact angle studies done by Bhattacharya et al. (Bhattacharya et al. 2005). While oxygen plasma treatment is documented to work for water or water-based suspensions, the increase in hydrophilicity has not been demonstrated for blood solutions. This paper aims to test the effect of various common channel materials on the contact angle with changing the blood products composition and to tabulate this data to be used as a reference for the microfluidics community.

2.2 Materials and methods

For this study six samples of fresh, healthy porcine blood were obtained from an accredited slaughterhouse. All samples were centrifuged for ten minutes, three times at 3000
RPM. After each round of centrifugation all the plasma was separated from the sample, and held (each samples plasma was held individually). After the removal of the plasma, all white blood cells, and platelets were removed, leaving only the RBCs. For the second and third round of centrifugation, some native plasma was returned to the samples, and they were gently mixed before centrifugation. After the third round, RBCs of each sample were obtained. These RBC were suspended in either PBS or their own native plasma at hematocrits (H) of 10%, 20% and 30%. Additional plasma from each sample was held for separate testing. Surfaces tested were glass (clean glass microscope slides), PDMS in a 10:1 ratio of polymer to curing agent (Sylgard-184, Dow, USA), PDMS in a 10:1 ratio exposed to oxygen plasma for 45 seconds (Anatech, LTD, USA) and clean PMMA. The oxygen-plasma treated PDMS portions were cut from the same sample as the untreated PDMS.

All of the testing was done using the VCA-Optima (AST Products, Inc, USA) contact angle set-up. For each measurement, at least three different samples were tested, and each test was repeated 3 times. Thus the contact angles reported are the average of the left and right contact angle for 9 different images, resulting in 18 measurements. Exceptions are water, which was 12 measurements each surface; PBS 6 measurements each surface; H = 20 in PBS 12 measurements on PMMA, H = 30 in plasma 14 measurements on plasma-treated PDMS, native plasma 12 measurements on PDMS and 14 on plasma-treated PDMS.

### 2.3 Results and discussion

Results of the contact angle studied are summarized in Table 2.1. A direct comparison on the base fluids (native plasma or PBS) with distilled water is shown in Figure 2.1. Error bars in the figure are the standard deviations listed in Table 2.1. Figures 2.2 and
2.3 highlight the results of increasing the hematocrit on the contact angle of the blood on the various surfaces.

![Figure 2.1: Contact angles of suspending fluids for the blood solutions, as compared to distilled water, on various microchip substrate materials.](image)

<table>
<thead>
<tr>
<th></th>
<th>glass</th>
<th>PDMS</th>
<th>p-PDMS</th>
<th>PMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>9.1</td>
<td>111.1</td>
<td>31.1</td>
<td>91.3</td>
</tr>
<tr>
<td>PBS</td>
<td>49.1</td>
<td>119.7</td>
<td>80.7</td>
<td>81.8</td>
</tr>
<tr>
<td>plasma</td>
<td>27.0</td>
<td>109.6</td>
<td>8.6</td>
<td>72.1</td>
</tr>
<tr>
<td>H10, PBS</td>
<td>32.9</td>
<td>108.2</td>
<td>85.6</td>
<td>80.4</td>
</tr>
<tr>
<td>H10, plasma</td>
<td>41.7</td>
<td>80.3</td>
<td>88.1</td>
<td>72.6</td>
</tr>
<tr>
<td>H20, PBS</td>
<td>23.6</td>
<td>99.6</td>
<td>71.6</td>
<td>80.1</td>
</tr>
<tr>
<td>H20, plasma</td>
<td>41.0</td>
<td>98.0</td>
<td>102.4</td>
<td>67.5</td>
</tr>
<tr>
<td>H30, PBS</td>
<td>32.5</td>
<td>8.4</td>
<td>74.9</td>
<td>74.5</td>
</tr>
<tr>
<td>H30, plasma</td>
<td>35.6</td>
<td>91.6</td>
<td>92.8</td>
<td>60.4</td>
</tr>
</tbody>
</table>

Table 2.1: Contact angle data collected.
Using artificial suspending fluids like PBS removes the native proteins and associated molecules in the plasma. Biological samples inherently have greater variance than artificial solutions created in a controlled environment as protein content can vary from sample to sample within the same species. RBCs suspended in PBS solution therefore have less variation between samples since the suspending solution is identical for all the samples. This clearly has an effect on the contact angle of the fluid on certain surfaces, especially the plasma treated PDMS. It is especially interesting to see the increase in contact angle of the native plasma on its own, which is 109° on plasma treated PDMS, while the water usually used to report the success of the plasma treatment has a contact angle...
Figure 2.3: Contact angles of blood dilutions suspended in native plasma, at hematocrit 10%, 20% and 30%, on various microchip substrate materials.

angle of only 49°. There is almost no impact on the contact angle of the native plasma by the oxygen plasma treatment. The difference in contact angle fails a two-tailed t-test for unequal means in all concentrations of RBC at a confidence level of 99%. With the actual blood suspensions shown in Figures 2-2 and 2-3, the PBS suspensions have a more pronounced reduction in contact angle with the oxygen plasma treatment than those suspended in native plasma ($P \leq 0.01$) for all concentrations of RBC. PMMA seems to be an a great advantage in Figures 2-1 and 2-3, as the contact angle is lower than for PDMS even when treated with plasma. This could be a viable option for hydrophilic microchips using blood suspensions in native plasma. In comparing the plasma treated
PDMS with PMMA, at low hematocrit the difference between the contact angles on the two surfaces is not significant when suspended in saline, while it is significant when hematocrit reaches 30% ($P \leq 0.01$). For RBCs suspended in plasma, the difference in contact angles between plasma treated PDMS and PMMA is not statistically significant at $H=10$, but it is for plasma alone and for $H=20$ and $30$ ($P \leq 0.01$). Future research of blood flow in biocompatible microchips needs to consider the impact of these protein or molecular interactions with their surfaces since the surface-fluid interaction could affect the final flow.

2.4 Conclusions

In this work we have tested the contact angle of suspensions of RBCs in saline versus native plasma on common microchip fabrication materials. It was demonstrated that the expected increase in hydrophobicity (measured by decreased contact angle) of PDMS treated with oxygen-plasma is not present when using blood. It was also demonstrated that the contact angle of the plasma and saline alone are significantly different on oxygen-plasma treated PDMS. Finally, it was shown that the contact angle of suspensions in RBCs in plasma have a higher contact angle on the treated PDMS than suspensions of RBCs in PBS.

2.5 References


Bélanger M, Marois Y. Hemocompatibility, biocompatibility, inflammatory and in vivo studies of primary reference materials low-density polyethylene and polydimethyl-
Contact angle study of blood dilutions on common microchip materials


Chapter 3

Micro-particle image velocimetry operation protocol

This chapter forms the written portion of an invited article in press: Pitts KL, Fenech M. Micro-particle image velocimetry for velocity profile measurements of micro blood flows, Journal of Visualized Experiments, 2013; e50314, DOI:10.3791/50314.

Micro-particle image velocimetry (μPIV) is used to visualize paired images of micro particles seeded in blood flows. The images are cross-correlated to give an accurate velocity profile. A protocol is presented for μPIV measurements of blood flows in microchannels. At the scale of the microcirculation, blood cannot be considered a homogeneous fluid, as it is a suspension of flexible particles suspended in plasma, a Newtonian fluid. Shear rate, maximum velocity, velocity profile shape, and flow rate can be derived from these measurements. Several key parameters such as focal depth, particle concentration, and system compliance, are presented in order to ensure accurate, useful data along with
examples and representative results for various hematocrits and flow conditions.

3.1 Introduction

The human body contains numerous vessels with diameters less than 50 µm, which are the main exchange site between blood and tissues. The study of blood flow in these vessels represents a considerable challenge due to both the scale of the measurements and the fluid properties of blood. These measurements, including the pressure gradient, the shear at the wall, and velocity profiles in arterioles and venules, are key factors linked with physiological responses. There are now unprecedented opportunities to resolve these measurement challenges, thanks to new experimental techniques at the micro scale to study the microcirculation and solve this multiscale problem.

Micro-particle image velocimetry (µPIV) is a particle based flow visualization technique that is used to evaluate velocity profiles of blood flow in microchannels via cross correlation. µPIV, first developed by Santiago et al, has been used with hemorheology studies since Sugii et al in 2001 used the technique to measure blood flow in 100 µm round glass tubes (Santiago et al. 1998, Sugii et al. 2001). Different approaches to µPIV exist. High speed cameras can be used to correlate the movement of RBC, and pulsed images can be used to correlate the movement of tracer particles. Either of these options can be coupled with an upright or inverted microscope, depending on the application. In both cases, the result is a 2D velocity profile. Another approach is to use a confocal microscope to achieve 2D and 3D profiles. This method has been applied to blood (Park et al. 2004, King et al. 2004, Lima et al. 2006).

Micro scale PIV has several complications when compared with macro PIV. In macro PIV the data can be limited to a single plane through sheets of light, but at the micro scale volume illumination is necessary. Volume illumination is a greater problem for the
imaging of micro blood flows, as the RBCs themselves are large in comparison to the channels, and using the RBCs as the tracer particles leads to a depth of correlation (DOC) which can significantly decrease the accuracy of the cross-correlation results (Wereley et al. 1998, Meinhart et al. 2000, Chayer et al. 2012). Following Wereley et al. (1998) the DOC for a 40 µm tall channel with RBC as the tracers is 8.8 µm, while with a 1 µm tracer particle the DOC is 6.7 µm. This difference becomes more pronounced when changing channel height and magnification. Additionally, RBCs are opaque, and increasing the density of the RBCs in the flow cause imaging difficulties. Fluorescing tracer particles, first used by Santiago et al. (1998), have been advocated as a tool to decrease the influence of out-of-focus particles, when using the smallest particles possible. Using 1 µm diameter fluorescing microparticles coupled with a laser is one approach that may decrease the depth of focus problem in micro blood flow imaging (Olsen and Adrian 2000). There are several current reviews of the state of PIV technology, each of which highlights the importance of PIV to blood flow studies (Wereley and Meinhart 2010, Williams et al. 2010). Several important considerations must be taken into account when using µPIV for blood. At the micro level, the scale of the microcirculation, blood cannot be considered a homogeneous fluid, as it is a suspension of flexible red blood cells (RBCs), large white blood cells, platelets and other proteins suspended in a Newtonian fluid (plasma).

The velocity profiles measured here can be used to measure certain characteristics of the micro blood flows. The important factors in microhemorheology are the flow rate of the blood, the shape of the velocity profile, and the shear stress at the wall of the vessel. This information has clinical implications, as the microcirculation is the site for nutrient exchange in the body, and this exchange is shear-dependant. There are several current review studies on the state of research in the microcirculation as well (Popel and
Presented here is a protocol for $\mu$PIV measurements of blood flows in poly(dimethylsiloxane) (PDMS) microchannels. PDMS channels were fabricated in-house following the sources in section 1 of the protocol. Porcine blood samples were obtained from an accredited slaughterhouse and cleaned following section 2 of the protocol. All data was obtained using the LaVision MITAS $\mu$PIV system, as described in section 3 of the protocol. The set-up consists of a Nd:YAG laser (New Wave Research, USA) and CCD camera (Image Intense, Lavision) controlled by a programmable triggering unite, a fluorescent microscope coupled with a stage moving in 3 axes, and a computer, in addition to a high speed camera (Dalsa 1M150, Netherlands) was added for visualization of the RBCs themselves. Both cameras are connected to a 2 port optic box (Custom by Zeiss, Germany). In typical in vivo measurements of blood flow, an upright microscope is used to track the RBCs themselves, while in typical in vitro applications an inverted microscope is used to track the tracer particles. In this unique dual set-up, the optics box allows both tracers to be imaged using the inverted microscope. Blood was introduced into the microchips via high precision syringe pump (Nexus3000, Chemyx Inc., USA). A diagram of the system is shown in Figure 3.1, where the top portion of the figure represents the 140 m by 40 $\mu$m rectangular channels fabricated of PDMS, and the bottom portion represents the entire system including both cameras, the laser, the syringe pump and the microscope.

Current $\mu$PIV set-ups available, usually with proprietary software, include TSI, Dantec Dynamics, and LaVision. Standard cross-correlation algorithms can be achieved through numerous software options, including the MATLAB. The software is not the key, understanding what the dialogue boxes correspond to mathematically will serve the user much better. In this protocol DaVis, LaVisions proprietary software or MATLAB are utilized. The protocol is not software specific, but the menu options might be in
Figure 3.1: Diagram of the µPIV set-up where (a) represents the 140 µm by 40 µm rectangular channels fabricated of PDMS and (b) represents the entire system.

different places in different software packages.

3.2 Protocol

1. Microchip fabrication

The first step is to create or purchase your microchannel. There are many options for microchip material. One of the most common materials chosen is poly(dimethylsiloxane) (PDMS). There are many publications on directions for PDMS fabrication through
soft lithography (Xia and Whitesides 1998, Whitesides and Stroock 2001, Sia and Whitesides, 2003). Once the PDMS channel is fabricated, there are several surface treatments available to reverse its natural hydrophobicity. Oxygenated plasma treatment is a common option. Zhou, Ellis, and Voelcker (2009) give a review of surface treatments and how they affect PDMS. These results are for water however, and blood has different properties. A study on what that surface treatment means for blood has been done by Pitts et al. (2012a). For the results presented here, microchip surfaces and the glass slides they were bonded to were both exposed to oxygenated plasma for 45 seconds and then pressed together firmly resulting in a permanent bond.

2. Blood preparation

Collect blood samples from an accredited facility. For example porcine blood can be used with EDTA (EDTA) as an anticoagulant. Add 1 g EDTA with 4 mL of water, and then add the solution to 1 L of whole blood, mixing gently 10 times. When picking up blood samples, allow them to cool slowly to room temperature. Blood samples should be fresh, and ideally used the day they are collected to conserve the rheological properties of the blood. Samples may be refrigerated once at room temperature; however whole blood without anticoagulant will be unusable after refrigeration. Depending on the anticoagulant, RBCs can be kept refrigerated for up to 42 days, and whole blood can be kept refrigerated for 35 days, but contamination would be possible in a non-medical facility (Hovav et al. 1999). Additionally, be careful of the collection method with bovine or porcine samples, and avoid contamination by bone marrow.

Centrifuge the blood samples 3 times at 3,000 RPM for 10 minutes each time. After the first centrifugation, remove the plasma and buffy coat, discard. It is
generally easiest to remove the plasma first, and then discard or keep for future use, then to remove the buffy coat alone. Introduce the pipette slowly, so as to not mix the buffy coat back into the blood. After removing the buffy coat, add about 20 mL of phosphate buffered saline (PBS) and mix gently, recentrifuge. Repeat last step. After the third centrifugation, remove the PBS and remaining buffy coat, discard. It is also possible to use the native plasma instead of PBS if you wish to keep the aggregating properties of the blood. Make sure to not mix any buffy coat or white blood cells into the plasma.

Take cleaned red blood cells (RBCs) and suspend in PBS or plasma at desired concentrations (hematocrits). Check hematocrits with a microcentrifuge (CritSpin FisherSci, USA). Blood at 10 to 20% hematocrit should be easier to visualize than 40 or 50% (physiological hematocrit). In the microcirculation, the blood is generally half the hematocrit of the macro circulation, so H=20 is adequate (Popel and Johnson 2005).

Add fluorescing tracer particles at the desired concentration to the blood samples. A general rule is to have 10 particles in a correlation window, which can be calculated ahead of time. For example, 30 µL of particles are added to 1 mL of blood solution for the set-up depicted in the video (www.jove.com). Alternatively, the RBCs themselves could be fluorescently tagged.

Additionally, make a calibration solution with water and fluorescing particles. It will be necessary to calibrate the system with a Newtonian solution. For example, when using a 10X objective with a channel on the scale of 100 μm, a suitable concentration is 300 µL of particles mixed with 15 mL of distilled water. Another option is to use glycerol diluted with distilled water to a viscosity of 3 cP, which is closer to blood’s macro viscosity.
3. $\mu$PIV measurements

Laser safety: Check temperature and humidity. Wear appropriate laser safety glasses. Close the laser curtain or turn on the laser sign on the lab door on to prevent people not protected from being exposed.

Procedure with Davis software is shown in the video (www.jove.com). For more details refer to the user manual of a specific system.

Before taking data, the camera scale needs to be calibrated using a micrometer.

To reduce the compliance of the system, use the shortest amount of tubing and the smallest rigid syringe possible, such as a 15 or 50 $\mu$L Hamilton Gastight syringe. To reduce leakage of blood or air intake into the system, seal the tubing to the syringe and to the chip. This can be done with glue, more PDMS or Vaseline (being careful not to contaminate the blood). To fill the micro-syringe with water laced with tracer particles use backflow method because the volume of the micro-syringe is usually much smaller than the volume to fill. Our backflow method consists to fill the micro-syringe and channel together via the output of the channel using a secondary plastic syringe. For that, first remove the micro-syringe plunger, then push the liquid using the classical plastic syringe attached to the outlet of the channel, let the liquid flow out the micro-syringe until all microbubbles are out of the micro-syringe, tubing or chip, finally put the plunger back, and unplug the plastic syringe. The presence or absence of microbubbles can be verified with a microscope.

Level the syringe pump to achieve horizontal tubing. Program the syringe pump to desired flow rate. Be aware of the possible transitory effects, like the bottleneck effect, for rigid system or the compliance of the system that modify the actual flow rate. Characteristics times of a system can be estimated as a function
of the materials and dimensions of the system. (Chapter 2, pp. 77-81, Tabeling 2005)

Place microchip on microscope stage and start the pump. Use the water with particles to calibrate the system to the middle velocity profile and calibrate the dT (the time between pulsed images). The particles should move between 5 and 10 pixels between frames for a good correlation (Wereley and Meinhart 2010). When calibrating, be careful to measure in the middle of the channel. The focus plane in the middle has the highest velocity (Chayer et al. 2012). If using a round channel, be careful of shadowing effects. A sample image is shown in Figure 3.2, using a pulsed camera, whereas the top image is the first pulse and the bottom image is the second pulse. It can be seen in the figure that the brightest points (fluorescing particles) are the most in-focus.

OPTIONAL: By taking data at different heights a 3D velocity profile can be reconstructed by adding the different vectors into a single image. The video presents a routine that was developed for the automation of the process (www.jove.com).

When taking data with the blood, fill syringe with desired quantity of blood in the same method as the water. Program the syringe pump to the desired flow rate, and take desired data. An example of raw images obtained is presented Figure 3.2. Be careful of RBC sedimentation as well. Refilling the syringe every run or every other run will reduce the settling of the RBCs.

After acquiring images, cross correlation is performed on the image pairs to obtain velocity vector fields between images. It is important to have good images to have a good correlation. Before correlating, pre-processing can be done to remove background noise. Cross correlation is done between windows in the adjacent images, which can be varied in size and shape to affect the correlation. After cross
correlation, image post processing can be done to remove erroneous vectors or outliers. A detailed description of the different available options and their accuracies can be found in Pitts et al. (2012b), where the best processing for blood was found to be the method of “image overlapping” with windows extended in length and aligned with the flow. These operations can be done manually in a program like MATLAB, or within the software package with your system. The software is not important, the math behind the operations is more important and each operation can affect the accuracy of the final velocity profile. The resulting vectors can be averaged in space across the channel to obtain instantaneous velocity profiles, and then averaged again in time to obtain an average, representative velocity profile. Kloosterman et al. (2011) give an explanation of the error in µPIV measurements where the depth of field is large. Discussions on the error of the data processing presented here can be found in Pitts et al. (2012b) for the pulsed measurements and Chayer et al. (2012) for the high speed measurements. Examples of velocity profiles are presented in Figure 3.3 and 3.4. Figure 3.3 presents a single velocity profile for the centerline of a 10 µL/hr flow of RBC at H=10 in a 140 µm wide channel. This single profile is achieved by averaging the correlated vectors across the channel to get a velocity profile, and then averaged in time to get a representative measurement. In this case, 100 pairs were averaged in time across the field of view. An example of the 3D reconstructed profile taken for a water calibration flow in a 100 µm square channel with a programmed flow rate of 30 µL/hr is found in Figure 3.4. In Figure 3.4, the profiles are measured at 1 µm intervals.

OPTIONAL: In the depicted set-up high speed images can be taken at the same conditions by switching cameras (and data acquisition systems). This can be useful for visualizing the cell-free layer in the channel, and for quantifying aggregation.
The data analysis is slightly different (Chayer et al. 2012). Examples of white light images with and without RBC aggregation are presented in Figure 3.5 at H=20. The top images shows H=20 in PBS, which removes the ability of the RBC to aggregate, at 1 µL/hr. The bottom image is H=20 in native plasma at 0.5 µL/hr. In the bottom image, aggregation is visible.

Shut down the system in a safe manner when measurements have been completed. Use proper laser safety procedures until laser power source is completely shut down.

3.3 Representative results

In all figures, flow is left to right in raw images, and upwards in calculated velocity profiles. An example of the raw data obtained with blood at hematocrit H=10 flowing at 10 µL/hr is shown in Figure 3-2. Raw data may be cross-correlated without any data processing to achieve velocity profiles. The impact of pre-processing and data processing methods is discussed by Pitts, et al. (2012b). An example of a resultant velocity profiles from data similar to Figure 3.2 at hematorcrt H=10 and a flow rate of 10 µL/hr is shown in Figure 3.3, a 3D profile from water calibration is shown Figure 3-4. Figure 3-3 included standard data processing. These velocity profiles can be used to make measurements such as the maximum velocity at the center of the channel, the flow rate in the channel, and the shear rate at the wall for various flow conditions, channel configurations, and physiologies. Figure 3.4 gives an example of a high speed image with and without aggregation of the RBC. High speed images such as those in Figure 3.4 can also be used to calculate velocity profiles using cross correlation.
Figure 3.2: Resulting raw pulsed image data from the $\mu$PIV system with $H=10$ in PBS at a programmed flow rate of 10 $\mu$L/hr (flowing left to right). Top image is first pulse.

### 3.4 Discussion

Using $\mu$PIV for blood flow measurements at the scale of the microcirculation can give insight into a great number of relevant biomedical, mechanical and chemical engineering
Some of the key factors to account for are the density of the RBC themselves, the aggregation and deformability of the RBC, aggregation or movement of the fluorescing micro particles, and the settling of the RBC in the channels. All of these can be accounted for if the general guidelines laid out above are followed. There is a basic checklist to keep in mind for good measurements using this system. First of all, determine how much compliance is in the system or proposed system. To minimize the compliance use the shortest amount of tubing possible and the smallest syringe possible. Rigid systems have less compliance, but can lead to bottlenecking. Despite the small scale of the measurements, gravity will affect the RBC. Keeping the syringe, tubing and
Figure 3.4: Example of a reconstructed 3D velocity profile of water calibration in a 100 \( \mu m \) square channel with multiple profiles taken 1 \( \mu m \) apart. Profile is rotated to show flow upward.

channel at the same height is ideal. Secondly, determine the concentrations of the suspensions. Ensure there are enough fluorescing tracer particles in the solution and not too high of a concentration of RBC. Increasing the density of tracer particles of a sample will not overcome the inherent opacity of the RBCs. The amount of tracer particles will affect the correlation, but having too many RBCs will make the fluid opaque and the tracer particles difficult to image. The overall hematocrit of the sample must be kept low enough that the middle plane of the channel can be imaged clearly. This is a trade off between hematocrit and channel size. At an increased depth, the amount of RBC between the lens and the plane of measurement grows, even at low hematocrit. In order to image higher hematocrit samples or larger channels, the ghost cells method can be utilized whereas the opaque interior of RBC is replaced with clear fluid for a portion of
Micro-particle image velocimetry operation protocol

Figure 3.5: Example of high speed images where (a) is $H=20$ in PBS at $1 \mu L/hr$ (no aggregation) and (b) is $H=20$ in native plasma at $0.5 \mu L/hr$ (aggregation). In both images flow is left to right.

the RBC (Goldsmith and Skalak, 1975). Thirdly, ensure that the microscope is focusing on the middle plane of the channel. Knowing the exact height of the channel is vital to finding the middle plane. Keep in mind the size of the particles and the size of the
channel, and calculate the depth of correlation (DOC) in your system (Wereley, et a., 1998). The DOC can greatly affect the accuracy necessary to achieve in finding the middle plane. A difference of one micron can adversely affect the accuracy. Once the amount of particles, the size of the channel, and the DOC are found, the difference in time between pulsed images (dT) needs to be determined. Ensure the 5 to 10 pixels of movement of the particles between windows. The movement of the particles must fit into the correlation windows outlined in section 3.9. Finally, decide on the method of pre-processing, processing or post-processing of vector data. Many methods are available in literature, but some are more successful than others when applied to blood. The method of “image overlapping” is advised for blood flows (Pitts, et a., 2012b).

Another factor to keep in mind is the “cell-free” layer, which is well documented in microhemodynamics. This is the lack of RBCs at the wall of the channel or vessel. In following the fluorescing tracer particles, the user is actually following the movement of the plasma. At the center of the channel or vessel, the velocity is at a maximum and both will be moving at the same speed. At the wall, the plasma will still have movement while there will be few or no RBCs present. At the scale presented here with 10X magnification, the cell-free layer is not measureable but at higher magnifications it must be accounted for. High speed video would allow for a measurement of the thickness of the cell-free layer.

3.5 Conclusions

µPIV has been used for hemorheology and biomedical studies since shortly after its development. When applied to blood flows at the scale of the microcirculation, the complex behavior of the blood must be accounted for. A protocol for µPIV measurements of blood flows in microchannels was presented here along with tips for dealing with the
complex nature of blood. Several imaging options were outlined, including pulsed images, high speed images and a 3D option as well as data processing information. In using \( \mu \text{PIV} \) for blood microflow research, the effect of medications, diseases and therapeutic treatments on the shape of the velocity profile can be analyzed. These measurements useful to modern medicine and engineering since the uptake of nutrients and medications is shear dependent.

### 3.6 Acknowledgements

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3.7 Materials

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3.8 References


Olsen MG, Adrian RJ. Out-of-focus effects on particle image visibility and correlation in microscopic particle image velocimetry. Experiments in Fluids 2000; 29(0):S166-74.


Chapter 4

Velocity measurement accuracy in optical microhemodynamics: experiment and simulation

This chapter was published as: Chayer B, Pitts KL, Cloutier G, Fenech M. Velocity measurement accuracy in optical microhemodynamics: experiment and simulation, Physiological Measurement, 2012; 33:1585-1602. DOI:10.1088/0967-3334/33/10/1585

Micro particle image velocimetry (µPIV) is a common method to assess flow behavior in blood microvessels in vitro as well as in vivo. The use of red blood cells (RBCs) as tracer particles, as generally considered in vivo, creates a large depth of correlation (DOC), even as large as the vessel itself, which decreases the accuracy of the method. The limitations of µPIV for blood flow measurements based on RBC tracking still have to be evaluated. In this study, in vitro and in silico models were used to understand the effect
of the DOC on blood flow measurements using PIV RBC tracer particles. We therefore employed a µPIV technique to assess blood flow in a 15 µm radius glass tube with a high-speed CMOS camera. The tube was perfused with a sample of 40% hematocrit blood. The flow measured by a cross-correlating speckle tracking technique was compared to the flow rate of the pump. In addition, a three-dimensional mechanical RBC-flow model was used to simulate optical moving speckle at 20% and 40% hematocrits, in 15 and 20 µm radius circular tubes, at different focus planes, flow rates, and for various velocity profile shapes. The velocity profiles extracted from the simulated pictures were compared with good agreement with the corresponding velocity profiles implemented in the mechanical model. The flow rates from both the in vitro flow phantom and the mathematical model were accurately measured with less than 10% errors. Simulation results demonstrated that the hematocrit (paired t-tests, $P = 0.5$) and the tube radius ($P = 0.1$) are not influencing the precision of the measured flow rate, whereas the shape of the velocity profile ($P < 0.001$) and the location of the focus plane ($P < 0.001$) do, as indicated by measured errors ranging from 3% to 97%. In conclusion, the use of RBCs as tracer particles makes a large DOC and affects the image processing required to estimate the flow velocities. We found that current µPIV method is acceptable to estimate the flow rate on the condition that the measurement takes place at the equatorial plane of the vessel. Otherwise, it is not an appropriate method to estimate the shape of the velocity profile.

4.1 Glossary

µPIV microscopic particle image velocimetry

2D two-dimensional
3D three-dimensional

\( a \) radius of a particle

\( CC \) cross-correlation

\( DOC \) depth of correlation

\( D_a \) lens aperture diameter

\( d_e \) effective diameter of an out-of-focus RBC (due to shadowing)

\( d_{ij} \) distance between two centers of mass of RBCs \( i \) and \( j \)

\( d_p \) particle diameter

\( H \) hematocrit (volume fraction of RBCs in blood)

\( f^h_i \) hydrodynamic force acting on particle \( i \)

\( f^e_{ij} \) force of the particle \( j \) acting on particle \( i \)

\( f^e_{iw} \) the force induced by the wall \( W \) on \( i \)

\( J_p \) flux of light from the particle

\( k \) velocity profile shape coefficient

\( K_E \) elasticity coefficient

\( M \) magnification

\( m_i \) mass of the particle \( i \)

\( MVP \) temporal mean velocity of a particle
Velocity measurement accuracy in optical microhemodynamics

$n$ index of refraction of the immersion medium between the microfluid and the lens

$NA$ the numerical aperture of the lens

$n_{ij}$ the normalized vector pointing from RBC $j$ to $i$.

$p$ p-value from paired t test

$Q$ volume flow rate

$R$ vessel radius

$RBC$ red blood cell

$\text{i}ve$ used for estimation from the in vitro experiment

$\text{o}se$ used for estimation from the optical simulation

$\text{ms}$ used for mechanical simulations

$p$ used for pump

$v_i$ velocity of the particle $i$

$V_{\text{max}}$ maximum velocity

$\beta$ parameter of the Gaussian distribution of the light

$\lambda$ wavelength of light

$\mu$ dynamic viscosity of the fluid

$\epsilon$ distance from the object plane beyond which the contribution becomes insignificant
4.2 Introduction

The medical and fluid mechanics communities have become increasingly interested in properties of blood flow in the microcirculation. The microcirculation, which is composed of numerous vessels with diameters less than 50 $\mu$m typically, is the main exchange site between blood and tissues. Because blood behaviour in the microcirculation cannot be considered as a liquid flow, but rather as a collection of interacting particles in suspension, the study of blood flow in the microcirculation represents a considerable challenge. The better understanding of the characteristics of blood flow in the microcirculation is of interest to understand mechanical interactions between blood components and the vessel wall, consequently giving insight into the causes of cardiovascular diseases, cardiogenesis and angiogenesis.

Micro-particle image velocimetry ($\mu$PIV) has been recently used to determine the blood velocity field and consequently the instantaneous velocity profiles of blood flowing through micro-channels. Generally, for in vivo studies (i.e., for measuring the blood velocity profile in a microcirculation bed on living subject) red blood cells (RBCs) are used as tracer particles for the $\mu$PIV method (Bitsch et al. 2005, Kikuchi and Mochizuki 2011, Koutsiaris and Pogiatzi 2004, Lee et al. 2007, Sugii et al. 2002, Tsukada et al. 2000); while for in vitro studies, artificial micro fluorescent particles are usually added to blood (Lima et al. 2006, Lima et al. 2007, Lima et al. 2008, Sugii et al. 2005). Note that few studies showed the possibility of using artificial tracer particles in living subjects; see for examples results in an embryonic heart (Poelma et al. 2010, Vennemann et al. 2006) or in the chicken vitelline network (Poelma et al. 2008). The use of RBCs has the advantage of not requiring particle injection and more importantly, it avoids confounding interpretation attributed to the modification of the system under study. Thus, it is a method of high interest for dynamic video microscopy of the human microcirculatory
flow. The use of RBCs as imaging tracers has nevertheless some disadvantages because its diameter is approximately 7 µm, while the diameter of an artificial fluorescent particle is typically on the order of 1 µm. The higher RBC particle size and its density are responsible for the decrease in accuracy of the measurement method (Nguyen et al. 2010, Kloosterman et al. 2011).

The goal of this study was thus to assess the intrinsic error of the measurement method when using RBCs as tracer particles. In this study, in vitro and in silico models were used to assess the accuracy of the flow rate and velocity profile estimations. The in vitro model allowed for a highly controlled pump flow that could be compared to the µPIV video microscopy measurements. In order to further validate the instantaneous blood flow velocity profiles obtained with the imaging technique, a computational model of the microscopy image formation was developed. The synthetic images of moving RBCs were built based on a mechanical model mimicking three-dimensional (3D) flow in a circular channel.

4.3 Theoretical background

The cross-correlation (CC) between two consecutive optical images is a standard method used to calculate particle velocities. An interrogation window in the first image is compared within an interrogation window grid in the second image to find the most look-alike windows. The distance between the center of both windows can be divided by the time elapsed between each frame to calculate the mean velocity of particles for this position. Even if the CC is accepted as a flexible and robust method (Cheezum et al. 2001), this algorithm needs to be validated for applications dealing with flowing cells, as in the case of microcirculatory blood flow. More specifically, it is important to understand the effect of out-of-focus particles and resulting image blurring on the estimation of velocities.
The depth of correlation (DOC) is a concept in microscopy that takes into account the manner in which the image is built and used (Meinhart et al. 2000). It describes how far one particle, over or under the focal plane, affects image-processing algorithms and can be interpreted as a measurement of the spatial resolution in the Z dimension.

To measure velocities only of particles in the focal plane, as in PIV with seeding particles at a low volume concentration, some recommendations were made. Indeed, the particle volume concentration should be less than 0.1% and the particle diameter should be below 1 μm (Meinhart et al. 2000, Olsen and Adrian 2000). These guidelines are not applicable to blood flow, because the largest RBC diameter is approximately 7 μm and the hematocrit (volume concentration) is between 20% (in the microcirculation) and 45% (in the systemic circulation), approximately. Consequently, all RBCs typically present in a microvessel with a 15 μm radius may affect image processing algorithms; measured velocities could then be biased because they correspond to a weighted average of cell velocities both in and out the focal plane. To describe this phenomenon, the DOC was defined as the minimal distance that separates a particle from the image plane without affecting the image processing method (Wereley et al. 1998):

\[
DOC = \frac{1}{2} \left[ \frac{(1 - \sqrt{\epsilon})}{\sqrt{\epsilon}} \left( \frac{n^2}{NA^2} - 1 \right) \left( d_p^2 + \frac{1.49(M + 1)^2 \lambda^2}{M^2 NA^2} \right) \right]^{1/2}
\]  

where \(\epsilon\) is the minimal intensity of an away particle affecting the CC (we can empirically set \(\epsilon\) to a value of 10% of the intensity of an in-focus particle) (Meinhart et al. 2000), \(n\) is the index of refraction of the immersion medium between the microfluid and the lens (\(n = 1.33\) for water), \(\lambda = 0.6\) μm is the wavelength of light, \(NA\) is the numerical aperture of the lens, \(d_p\) is the particle diameter, and \(M\) is the magnification.

Equation 4.1 gives a DOC of 14 μm for a RBC with \(d_p = 7\) μm, when it is observed with a lens characterized by \(M = 40X\) and \(NA = 0.8\). This confirms that all RBCs
present in a typical microvessel of 15 μm radius are visible and can affect the assessment of velocity profiles. Figure 4.1 shows the theoretical values for the DOC relative to the 15 μm radius capillary for different sizes of tracer particles. As shown later, the effect of using RBCs as tracer particles was highlighted both experimentally and numerically by forming synthetic images of blood flow in the microcirculation.

Figure 4.1: Theoretical values for the depth-of-correlation (DOC) relative to 15 μm radius capillary for different size of tracer particle.
4.4 Materials and method

4.4.1 Experimental model

In vitro model

An in vitro model of the microcirculation was built with a fused silica tubing (no. 062801, SGE Inc, Austin, TX, USA) having a radius of 15 µm and an outer diameter of 363 µm, glued with Epoxy in 1 mm inner diameter glass capillaries at both ends (Figure 4.2). The geometry (round channel) and scale (15 µm radius) used here position this in vitro study as the closest to the physiological measurements currently being made in intravital microscopy. A microsyringe connected to a 32-gauge needle (no. 80016, Hamilton, Reno, NV, USA) was glued at one end of the model to allow perfusion of the microtube with minimal dead volume between it and the needle. The syringe was fixed on a computer-controlled syringe pump to inject blood into the microcirculation model. Blood flow images were acquired with an Axiotech Vario microscope (Zeiss, Oberkochen, Germany) equipped with a 40X water immersion lens (NA = 0.8) in transmission light mode. A Zeiss, KL 1500 LCD cold light source connected to flexible optic fiber was used to improve the illumination of in vitro model. In addition, a cold filter cutting the red component of the lighting and increasing the contrast between the RBC and the background was added. A 0.63X C-mount adaptor allowed the recording of image sequences via a high-speed digital camera (no. 1M150, Dalsa, Waterloo, Ontario, Canada). The camera connected to a digital frame grabber (Hélios, Matrox, Montreal, Quebec, Canada) recorded 50 frames at a frame rate of 2,000 s\(^{-1}\) at a resolution of 120 by 170 pixels with a calibrated isotropic pixel width of 0.42 µm. Image sequences were post-processed with a 2D CC algorithm implemented in Matlab (ver. 7 R14, Natick, MA, USA).
Figure 4.2: Schematic of the microcirculation *in vitro* set-up with glass tubing having a 15 µm radius.

**Experiments**

Whole porcine blood was used. Blood samples were collected from a slaughterhouse and mixed with 3 gm of EDTA per liter of blood. The blood was centrifuged and RBCs were washed twice with saline to remove the white blood cells and platelets, and RBCs were then re-suspended at 40% hematocrit in the native plasma. Platelet and white blood cells were removed as it was assumed they do not affect the image formation as their concentrations are negligible regarding the concentration of RBC and thus their participation in the image formation are negligible. The RBCs were re-suspended in saline.
to avoid aggregation in order to eliminate this confounding factor. First, aggregation increases the variability between samples. Second, aggregation is well known to make the velocity profile flatter at low shear increasing the viscosity and the non-Newtonian behaviour. The interpretation of the shape of the velocity profile would be then difficult to attribute either to aggregating characteristic of the sample or to the measurement method. Note that the mechanical model takes into account possible flat shape of the velocity profile. Flow measure series varied from 1 to $3 \mu L/h$ (using a $5 \mu L$ syringe) or at $8 \mu L/h$ (using a $10 \mu L$ syringe) (Tuma et al. 2008) at a fixed focus plane in the middle of the tube. These flow rates are based on physiological velocities in the microcirculation from $0.2 \text{mm/s}$ to $2.4 \text{mm/s}$ in venules with a diameter from $18 \mu m$ to $72 \mu m$ (Popel and Johnson, 2005). Forty-five seconds were allowed to elapse between each flow step to obtain a steady flow in the model. A $40 \mu m$ range of focus, including the tube center plane, was investigated by changing the focal plane in $2 \mu m$ steps above and below the central plane. The effect of changing the focus plane was evaluated at constant flow rates of $1.5 \mu L/h$ and at $8 \mu L/h$. Each measurement for different flows and focus planes was repeated nine times and averaged; each of them was realized with: three blood samples from different pigs and repeated three times each. The center of the tube was defined as the location where the velocity is maximum, as done by Kloosterman et al. (2011).

### 4.4.2 Synthetic image of blood

A computational model of the microscopy image formation was developed. This section presents the mechanical model that was used to mimic three-dimensional (3D) RBC movements in a tubular parabolic flow, the strategy adopted to produce synthetic images, and the CC algorithm used to measure velocity profiles. Figure 4.3 summarizes these different steps.
Figure 4.3: (a) 3D RBC flow from the mechanical model, (b) particle light and size depending on position, (c) image formation (d) a synthetic image obtained and (e) dots representing the MVP of RBCs from a time series of synthetic images with the CC algorithm. The full line in (e) represents the true velocity profile of RBCs imposed in the mechanical modeling of (a).

**Mechanical modeling of 3D RBC motions**

Blood was considered as a collection of spheres interacting with each other and with the vascular wall. The mechanical 2D modeling in Fenech et al. (2009) was extended to 3D to mimic real RBC movements in a tubular parabolic flow. The acceleration of a particle $i$ over time $t$ was given by:
where \( m_i \) is the mass of the particle \( i \), \( v_i \) is its velocity, \( f_{ij}^e \) represents the force of the particle \( j \) acting on particle \( i \), \( f_{iw}^e \) is the force induced by the vascular wall \( W \) on \( i \), and \( f_i^h \) is the hydrodynamic force. The hydrodynamic force \( f_i^h \) induced by the Stokes drag on a sphere, assuming spherical isolated solid particles in a Newtonian fluid (here the plasma), with very small Reynolds and Stokes numbers (respectively \(<1\) and \(<0.005\) ), was given by:

\[
f_i^h = 6\pi \mu a (v_i^0 e_i^0 - v_i)
\]  

(4.3)

where \( \mu \) is the dynamic viscosity of the fluid, \( a \) is the radius of the particle, \( v_i \) is the particle velocity at a given time, and \( v_i^0 e_i^0 \) denotes the velocity of the fluid in the absence of particles, which drives the particle \( i \) to flow along the direction \( e_i^0 \) at a speed of \( v_i^0 \). To simulate a parabolic or a flat velocity profile, the drive velocity was defined as follows:

\[
v_i^0 e_i^0 = V_{\text{max}} \left[ 1 - \left( \frac{r}{R} \right)^k \right] x
\]  

(4.4)

where \( x \) is the flow direction, \( r \) is the radial coordinate, \( R \) is the radius of the vessel, and \( V_{\text{max}} \) is the maximum centerline velocity. Note that \( k = 2 \) defines a parabolic profile and \( k > 2 \) a blunted profile. The elastic force was to exempt overlapping of RBCs. This force was inspired by a granular interaction model (Duran 1997) and is given by:
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\[ f_{ij}^e = \begin{cases} K_E(2R - d_{ij})^{3/2}n_{ij} & \text{if } d_{ij} < 2a \\ 0 & \text{otherwise} \end{cases} \quad (4.5) \]

where \( K_E \) is the elasticity coefficient, \( d_{ij} \) represents the distance between centers of mass of RBCs \( i \) and \( j \), and \( n_{ij} \) is the normalized vector pointing from RBC \( j \) to \( i \). Equation 4.5 shows that this force tends to repel RBCs when the distance between their centers of mass is smaller than the cell diameter. In the same way, the force between particle \( i \) and the wall was calculated as:

\[ f_{iw}^e = \begin{cases} K_E(R - d_{iw})^{3/2}n_{ij} & \text{if } d_{iw} < a \\ 0 & \text{otherwise} \end{cases} \quad (4.6) \]

RBCs were modeled as spheres of radius \( a = 2.8 \, \mu m \) to mimic the true volume of an erythrocyte, and their mass \( m \) was set to \( 9.8 \times 10^{-14} \, kg \). Particles were initially positioned randomly without overlap in the 3D space. With the use of a finite difference first order scheme, the velocities and positions of particles at time \( t + dt \) were deduced from the acceleration at instant \( t \) according to Equation 4.2. The time step was adapted at each iteration to obtain a maximal displacement of \( 0.05 \, \mu m \) for all RBCs. This method offered a good compromise between the stability of the numerical results and the computation time.

**Synthetic images**

Mechanical simulations gave the positions of RBCs as a function of time. From these positions at each time-step (2000 images per second as in the experimental part), we simulated a 2D magnified image using microscopy image formation equations that consider
shadowing and illumination effects (Fenech et al. 2008).

**Shadowing effect**

The effective diameter \(d_e\) of an out-of-focus RBC (due to shadowing) shifted a distance \(z\) from the object plane was approximated by (Olsen and Adrian 2000):

\[
d_e(z) = \left[ M^2 d_p^2 \frac{1.49(M + 1)^2 \lambda^2}{M^2 NA^2} + \frac{M^2 D_a^2 \beta^2}{(s_0 + z)^2} \right]
\]

(4.7)

where the magnification \(M = 40\) X, the numerical aperture of the lens \(NA = 0.8\), the wavelength of light emitted by the particles is \(\lambda = 0.6\ \mu m\) (red), the particle diameter \(d_p = 5.6\ \mu m\), the working distance \(so = 3.6\ \mu m\), and the lens aperture diameter \(D_a = s_0/(2 \times NA)\).

**Illumination effect**

The total light flux from a single voxel was modeled as 0 if it is in an empty zone and as \(I(z)/N_v\) if the voxel is located in an RBC. \(N_v\) is the number of voxels that discretized the RBC and \(I(z)\) is given by Equation 4.8 (Olsen and Adrian 2000):

\[
I(z) = \frac{J_p D_a^2 \beta^2}{4\pi d_e^2 (s_0 + z)^2} \exp \left( \frac{-4\beta^2 a^2}{d_e^2} \right)
\]

(4.8)

where \(J_p\) is the flux of light from the particle that was normalized to 1, \(\beta = \sqrt{3.67}\) is the parameter of the Gaussian distribution of the light intensity (Olsen and Adrian 2000), and \(a = d_p/2\) is the RBC radius. The 3D space was discretized in voxels of \(0.42 \times 0.42 \times 0.42\ \mu m^3\) to fit images obtained with the experimental set-up. The 2D image was obtained by summing up the weights of all voxels following the direction \(z\). Finally, the matrix was normalized by applying a linear transformation to have the
same maximum intensity as in the experimental pictures, and, as CMOS sensor noise can blur the contrast information in the picture, a noise level equivalent to that of the actual digital camera was added by superposition of a real “white” picture taken with our camera.

**Simulation parameters**

In the following, the velocities obtained from mechanical simulations based on Equations 4.2 to 4.6 are designated with the superscript $^{ms}$ (for mechanical simulations) and velocities estimated with the CC method and computed from simulated optical images (Equations 4.7 to 4.8) are labeled with the superscript $^{ose}$ (for estimation from optical simulations). RBC displacements were simulated for hematocrits (H) of 20% and 40%, in vessels of 15 $\mu$m and 20 $\mu$m radii (R). To consider a range of flowing conditions, three shapes of velocity profile were implemented from sharp to flat as shown by Bishop and al.(Bishop et al. 2001b) who found k from 1.5 to 4 as a function of the aggregation level of moving RBCs. Therefore, a sharp velocity profile ($k = 1.5$), a parabolic profile ($k = 2$) and a blunted profile ($k = 3$) were implemented. For each case, the maximum velocity $V_{max}$ was incremented, using 20 steps of 0.1 mm/s, from 0.1 mm/s to 2 mm/s, which correspond to about 0.1 to 2.5 $\mu$L/h (it could vary depending of the shape of the velocity profile and the tube diameter). Note that velocities above 1 mm/s could not be reached for the 20 $\mu$m radius with a 40% hematocrit on account of processor capability. The synthetic images were made for seven focus planes, labeled F = 0 to F = 30, where F indicates the distance in the z direction between the focus plane and the center of the vessel. The different combinations of these parameters generated 1428 movies. Velocity profiles were fitted using Equation 4.4 to determine maximum velocities $V_{max}^{ms}$ and $V_{max}^{ose}$ and shape parameters $k^{ms}$ and $k^{ose}$. Note that $V_{max}^{ms}$ and $k^{ms}$ could be slightly different.
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(less than 5%) from $V_{\text{max}}$ and $k$ entered in Equation 4.4 since the mechanical model accounts for RBC interactions due to collisions between neighboring cells. The flow rates $Q_{\text{ms}}$ and $Q_{\text{ose}}$ were computed from $V_{\text{max}}^{\text{ms}}$, $k_{\text{ms}}$ or $k_{\text{ose}}$, $V_{\text{max}}^{\text{ose}}$ using Equation 4.10.

### 4.4.3 Computation of velocity profiles with the cross-correlation method

The same algorithm was used to extract the velocity for both experimental and synthetic images. Due to the very low RBC speed with regard to the camera simulated frame rate, the CC was computed on frames 1 and 4, 2 and 5, and so on, in manner that increases the time between images to 0.002 second. RBCs at a physiological hematocrit are not distinguishable on microscopic experimental images; thus, the speckle size was used to ascertain the dimension of correlation windows. That size was set by an empirical method that allowed the determination of particle displacements for the range of flow rates considered in this study. Square windows were chosen instead of rectangular ones to facilitate the eventual application of the algorithm on tortuous real vessels, therefore it will be not necessary to rotate the images or the windows in regard of the vessel direction. The size of the first correlation window was set to 15 by 15 pixels (6.3 by 6.3 µm), the size of the second interrogation window to 65 by 65 pixels (27.3 by 27.3 µm). An overlap of 100% minus one pixel was chosen to get a high density of velocity vectors (71 speed vectors for the width of the glass microvessel) because the often-used sampling with 50% overlap would only provide 11 speed vectors. Oversampling was helpful in this study, as it allowed narrow-band filtering to decimate spurious vectors created by noisy images. A median filter was employed to reject all vectors with a module or angle different from the median value plus one standard deviation computed from all vectors on the same streamline. In addition, a mean filter was used on 50 successive images based on the hypothesis
that for a given point in an image, steady velocity rates were achieved by the use of
the high precision syringe pump (having a resolution of 0.082 µm/step). By using both
filtering strategies (median filter and time mean filter), a mean velocity profile (MVP)
was determined for each video sequence, for a flow rate of 1.5 µL/h, a hematocrit of 40%,
and acquisitions at the center of the tube (Kloosterman et al. 2011).

4.4.4 Estimation of blood flow

Experimentally

To validate the precision of the CC algorithm and to assess its sensitivity to out-of-focus
particles, estimated flows were compared with flows of the pump $Q^p$ for a wide range of
blood flows and focal planes within the tube. The MVP represents the temporal mean
speed of particles, and with Equation 4.9, it allowed the estimation of the flow rate $Q^{ive}$
(for estimation from in vitro).

$$Q^{ive} = \pi \int_{-R}^{R} MVP \, dr$$ (4.9)

As the MVP extended out of the microtube because of the relatively large correlation
windows of our microscopic images, velocity profiles were limited to the dimension of the
tube to reduce errors in flow estimation during the integration.

From simulations

Both parameters $V_{max}$ and $k$ obtained from the best fit of Equation 4.4 were used to
compute the flow rate by integrating the velocity profile as:
\[ Q = V_{\text{max}} \pi R^2 \frac{k}{k + 2} \]  

(4.10)

4.5 Results

In this section, the experimental and synthetic results are compared, the parameters that generate an error in the estimation of the flow are determined, and estimation errors of the flow rate are quantified. Finally, estimated shapes of velocity profiles are evaluated.

4.5.1 Flow rate estimation with the CC method

In silico and in vitro flow rate comparison

Figure 4.4 presents in vitro flow rate estimations with the CC method versus those of the syringe pump for a 15 \( \mu \)m radius glass tube at H = 40\% for the focus in the middle of the vessel (F = 0). The linear relation between the estimated flow and the pump flow can be seen by the linear regression (long dashed line) \( Q^{\text{ive}} = 0.91Q^p \) with R-squared equal to 0.95. The error bars show the standard deviations for nine experimentations. The standard deviations are quite large because blood samples from different pigs behaved differently. Figure 4.4 also shows in silico data for different hematocrits (H20\% and H40\%) and k (1.5, 2 and 3) in the 15 \( \mu \)m radius vessel. The in silico data linear regression (small dashed line) corresponds to \( Q^{\text{ose}} = 0.90Q^{ms} \) with R-squared equal to 0.91. in vitro and in silico linear regressions are well matching. Both experimental and simulated flow rate assessments show an underestimation with respect to the true flow rate of less than 10\%.
Figure 4.4: Estimated flow rate by CC analysis from the *in vitro* and *in silico* data as a function of the flow rate of the pump or implemented in the mechanical 3D model for a 15 $\mu$m radius vessel with an hematocrit of 40% and 20% and a velocity profile with $k = 1.5, 2, \text{ and } 3$.

*In silico* estimated flow rate with the CC method versus the flow rate of the mechanical simulation model

For the 1428 *in silico* sequences, corresponding to the parameters (R, H, k, F, V), the flow was calculated by the CC method. Then for each combination (R, H, k, F), the estimated CC flow was plotted as a function of the flow imposed in the mechanical model. Regarding the synthetic data, for each of the 84 combinations (R, H, k, F), a linear relationship was obtained, where R-squared was found always higher than 0.9 except for
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(R15, H20, k3, F5) where R-squared was 0.81. Figure 4.5 presents examples of these linear relationships for different positions of the focus in a 15 \( \mu m \) radius vessel at a 40% hematocrit and an imposed parabolic profile \((k = 2)\). In this figure, \((1 - Q^{ose}/Q^{ms}) \times 100\) (with \(Q^{ose}/Q^{ms}\) the estimated flow rate divided by the gold standard flow rate \(Q^{ms}\) of the mechanical model) quantifies the error of the CC method with regard to the theoretical blood flow. The relative errors were found between 3% and 97% depending mostly on the parameters \(k\) and \(F\) as stated in the paragraph 4.4.2. Table 4.1 presents the ratio \(Q^{ose}/Q^{ms}\) for the focus at 0 (i.e., at the center of the simulated tube). At this position, the minimum and maximum errors were 3 and 35%, respectively.

Paired t tests were used to determine which variables among \((R, H, k, F)\) were influential on the ratio \(Q^{ose}/Q^{ms}\). The influence of \(R, H,\) and \(k\) was studied for \(F = 0\), which means that the focal plane was in the middle of the tube, where the velocity profile can be used to estimate the flow rate. The analysis led to the following results:

- When \(Q^{ose}/Q^{ms}\) for a 15 \( \mu m \) radius vessel (R15) is compared with that of a 20 \( \mu m \) radius vessel (R20), all other factors being equal, the paired t test showed no significant difference \((P = 0.1)\).

- Moreover, no significant difference was found between the flow rate ratio obtained with a hematocrit of 20% and that at a hematocrit of 40% \((P = 0.5)\).

- Similarly, no significant difference \((P = 0.3)\) was obtained between the flow rate ratio obtained with a parabolic velocity profile \((k = 2)\) and that with a blunted profile \((k = 3)\). As against, a significant difference \((P < 0.001)\) was found between the sharp velocity profile \((k = 1.5)\) and the parabolic profile \((k = 2)\), meaning that the estimated flow rate was less precise when the profile was sharp, as shown in Table 4.1. For example, for the 15 \( \mu m \) vessel with a hematocrit of 20%, \((Q^{ose}/Q^{ms})\)
Figure 4.5: Estimated flow rate by CC analysis from the synthetic data as a function of the flow rate implemented in the mechanical 3D model for a 15 \( \mu \text{m} \) radius vessel with a hematocrit of 40\% and a parabolic profile (\( k = 2 \)). Results for different positions of the focus plane (numbers represent the distance of the focus plane from the center of the tube).

\( k = 1.5 \) was found to be equal to 0.88 for \( k = 1.5 \), whereas \( (Q^{ose}/Q^{ms}) k = 2 \) was found to be equal to 0.92 for \( k = 2 \).

Regarding the distance of the focal plane from the center of the tube, as expected paired t tests showed very clearly the influence of this parameter on the ratio \( Q^{ose}/Q^{ms} \). This parameter had the greatest influence, as shown in the detailed study that follows.
4.5.2 Focus effect on the flow estimation

It is important to quantify the estimation error as a function of the position of the focal plane because in the case of intravital in vivo study it may be difficult to place the focal plane at the center of vessels, especially because the vessel could move during the breath cycle. Even with rigid tubes in vitro, the precise position of the focal plane may be difficult to adjust.

Focus effect on the in vitro flow estimation

Figure 4.6 presents the curve $Q^{ive}/Q^p$ (in vitro estimated flow divided by the pump flow) as a function of the focus plane. For these experiments, a 15 µm radius glass tube was perfused with H40% blood with constant flow rates of 1.5 µL/h and 8 µL/h. Each point is the mean of nine flow estimations and the error bar is the standard deviation of these measurements. The negative axis represents the position of the focus plane above the middle of the tube, whereas the positive axis is under the middle of the tube. We note that the standard deviation is smaller for the 8 µL/h experiment.

Focus effect on the in silico flow estimation

Figure 4.6 and 4.7 show the in silico ratio $Q^{ose}/Q^{ms}$ as a function of the focal plane position in simulated vessels with radii of 15 µm and 20 µm, respectively. The results from synthetic images are consistent with the experimental results in Figure 4.6 for the 15 m radius glass tube. We notice a shift of the in vitro curve under the middle of the tube that could be due to the high opacity of the blood at 40% hematocrit, which was not taken into account in the mechanical model. It is clear that when the focal plane was far from the center of the tube, the flow rate was highly underestimated. When the focal plane was located at the center of the simulated tube, the flow rate calculated by
Figure 4.6: *In vitro* and *in silico* estimated flow rates by CC analysis normalized by the
gold standard flow (flow rate of the mechanical model or pump flow rate) as a function
of the focus plane. For both, tube radii were equal to 15 µm. For the experiments, the
glass tube was perfused with H40% blood at a constant flow rate of 1.5 µL/h and 8 µL/h.
For *in silico*, note that each dot is an average for flow rates from 0 to 2 µL/h.

the CC method reflected at least 90% of the gold standard flow rate; the CC flow was
underestimated by 20% when the focal plane was located 5 µm from the center of the
tube and by 60% when the focal plane was located 15 µm from the center of the tube. At
30 µm from the center, the flow rate error was around 90%.
Figure 4.7: *In silico* estimated flow rates by CC analysis normalized by the gold standard flow (flow rate of the mechanical model) as a function of the focus plane. Tube radii equal to 20 µm. Note that each dot is an average for flow rates from 0 to 2 µL/h.

4.5.3 Shape of the velocity profile

The shape of the velocity profile under physiological conditions, which impacts the wall shear rate, is known to depend on the RBC aggregation level and hemodynamic conditions, such as vessel curvature, entrance length, etc (Bishop *et al.* 2001b, Bishop *et al.* 2001a, Bishop *et al.* 2001c). Only data with focus in the middle of the tube are considered here (F = 0). Figure 4.8 (experimental) and Figures 4.9 and 4.10 (*in silico*) show examples of velocity profiles obtained using the CC method. Experimental velocity profiles are compared with a non-Newtonian blunt profile (k = 3) computed based of
the pump rate. In silico velocity profiles are compared with the corresponding velocity profile imposed in the mechanical model. We note clearly that CC velocity profiles are blunted due to the averaging effect of the method in the 15 µm radius vessel. It is also clear from an observation of these profiles that, even if we can have a reasonable estimation of the flow rate with the CC method (if the focus plane is centered), the shape of the velocity profile for the radius of 15 µm does not reflect the true profile. However, the prediction of the velocity shape is much better for the vessel with a radius of 20 µm. Table 4.2, which shows the relation between the shape parameter k obtained from the CC profile and the gold standard coming from the mechanical model, confirms these observations.

4.6 Discussion

According to both in vitro and in silico studies, the CC method applied on whole blood optical pictures, in the middle of the tube, gave an estimation of the flow rate with a small underestimation of about ±10% (for in vitro measurements). As expected, the in silico studies showed smaller underestimations when the velocity profiles are flatten. However, when the focus is not in the middle of the vessel, the underestimation increased drastically with increasing distance from the middle of the tube. For example, the error on the CC flow estimation was two times bigger, when the focal plane was located at only 5 µm from the center of the vessel. On the other hand, the result in the region nearest to the center, at 2 µm, showed a stable precision of around 10%. As earlier mentioned, it is difficult to place the focal plane exactly at the center of the vessel, which is why we think that this observation is helpful because the center plane of the tube can more easily be reached with a precision of ±2µm. Thus, we recommend, as done by Kloosterman et al. (2011), to scan the velocity across the tube and to define the center of the tube as the
location where the velocity is maximum.

The CC method on whole blood should not be used to deduce fluid velocity profile shapes for vessels with a radius of 15 µm or less, as shown in experimental and in silico data analyses. Despite the theoretical value of the DOC (14 µm) computed for the current experimental conditions, in silico data suggest that for the 20 µm radius tube, the CC method could provide a better estimation of the velocity profile shape than for the 15 µm vessel.

Note that we were able to see a lack of RBC near the wall, a well know phenomena
in blood microcirculation, called the “cell free layer.” This phenomenon is due to the spinning of the RBC making them move toward the center of the tube. We can see it change in the videos as Q changes, but it would be difficult to measure at the magnification we used. In the numerical model, we also observe a less density of cell close the wall due to the elastic interactions forces between the wall and the RBCs. It is here also difficult to quantify the thickness of this layer. This lack of particle close the wall may affect the accuracy of the cross correlation method close the wall.

We showed in this study that the use of RBCs as tracer particles in $\mu$PIV applica-

Figure 4.9: Typical velocity profiles obtained by the CC method in the *in silico* model ($V^{ose}$) for a radius of 15 $\mu$m, $H = 20$, $k = 3$. The continuous line is the 3D reference velocity profile of the RBCs in the mechanical model ($V^{ms}$). x-axis: distance from the middle of the tube in m; y-axis: velocity in mm/s.
tions gave biased velocity profiles for vessel diameters corresponding to microcirculatory flow. To circumvent this limitation, several techniques have been adopted to overcome the optical DOC limitation, principally for vessel radii of 50 µm and above. Bitsch et al. (2005) proposed to use a so-called base-clipping technique. A specific grayscale threshold level was chosen to remove out-of-focus particles and grayscale values below this level were discarded when processing moving speckle in optical images. This approach seems attractive because it is possible to work under physiological conditions with whole blood; its performance, however, has not yet been assessed. Another proposed technique is to label a given proportion of RBCs with florescence to attenuate the effect of concentrated
cells but this technique does not totally eliminate the impact of the DOC. The solution is to sort particles to keep only those in the focal plane by considering the size of the fluorescent spot as done by Bishop et al. (2001b) or that of the emitted light intensity as done by Suggi and al. (2005). Finaly, a promising technique is to add small artificial fluorescent particles, such as proposed by Sugii (2005) who computed velocity profiles by adding spherical particles of 1 µm diameter using a laser to fluoresce particles in the PIV system. Lima et al. (2006, 2007, 2008) also adding 1 µm-diameter fluorescent microparticles to RBCs, obtained successfully 3D velocities of blood flow in square microchannels. However, to be able to get accurate velocity profiles, the blood had to be diluted to non-physiological hematocrits in order to see fluorescent microparticles. Indeed, the reliability of the velocity profile decreases with the increase of the hematocrit (Lima et al. 2007).

We had shown in this study that µPIV using RBCs as tracer particles gave flattened velocity profiles than expected ones due to the large size of RBCs with respect to the size of the vessel. In agreement with our conclusion, velocity profiles based on RBC tracking were always flattened when compared to detecting RBCs with added fluorescent microparticles. Figure 4.11 shows together normalized velocity profiles extracted from different studies. Even if those profiles were not computed under the same conditions (i.e., vessel diameters, flow rates and hematocrits) the general appearance is specific to the post processing method. Also, we should be careful in such comparisons because results obtained by using artificial fluorescent particles gives the velocity of the plasma (or of the surrounding fluid), while methods tracking RBCs focus on the cell velocity. The comparison of the flow methods deserves to be done in the same experimental conditions.

It should be noted that optical theories on particle image formation have been formulated for the measurement of small, weakly concentrated particles. The in silico model
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Figure 4.11: Normalized velocity profiles of microbloodflow extracted from different studies using additional fluorescent particles or not. Continuous lines are used when fluorescent particles are used as tracer particles, dashed line when RBCs are used as tracer particles. Maximum velocities and vessel diameters are indicated in brackets. (a) Lima 2006 (Lima et al. 2006) blood cell suspension, at 10% Hct in vitro + fluorescent particle 1 µm (0.55mm/s, 100 µm) (b) Polema 2010 (Poelma et al. 2010), embryonic chicken, Blood + fluorescent particle 1 µm (40 mm/s, 300 µm) (c) Sugii 2002 (Sugii et al. 2002), intravital, embryonic chicken (2.2 5m/s, 25 µm) (d) Chayer 2008 (Chayer et al. 2008), in vitro circular, blood cell suspension at 20% Hct, in vitro (11 mm/s, 30 µm) (e) Sourice 2005 (Sourice et al. 2005), intravital (0.07 m/s, 40 µm).

Presented in this study can certainly be improved with a more accurate description of the image formation by considering theories on light ray propagation in matter. Moreover, future advancements in the domain of mechanical modeling of flowing RBCs should be profitable, especially to take into account the RBC biconcave shape, RBC deformation
and RBC aggregation in the microcirculation.

4.7 Conclusion

We evaluated a tool to quantify velocity profiles measured by optical microscopy when the use of artificial fluorescent particles is not feasible. We have proposed several recommendations for the use of this method. To summarize, the quantification of the flow rate and velocity profile obtained by the CC method using whole blood has to be interpreted by taking into account the following issues:

- the diameter of the vessel;
- the shape of the velocity profile; and
- the large dispersion of results because of intra and inter blood measurement variabilities. The original model used to generate synthetic images of optical PIV will support the calibration of post processing methods and the interpretation of \textit{in vitro} and \textit{in vivo} data. The database of the 1400 movies can be used by the community on request. The method is also presented in complete detail to be easily adapted for all vessel shapes, sizes, velocity profiles and concentrations of red blood cells.

4.8 Acknowledgments

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Chapter 5

High speed versus pulsed images for micro-particle image velocimetry: A direct comparison of red blood cells versus fluorescing tracers as tracking particles

This chapter has been submitted for publication

High speed photography in micro-particle image velocimetry (µPIV) using red blood cells (RBCs) as tracer particles and the use of fluorescing tracer particles (in conjunction with pulsed images) are directly compared by using both methods simultaneously.
Measurements are taken on the same blood sample in the same microchip using both methods. This work directly and statistically compares the two methods of µPIV measurement in a controlled *in vitro* environment for the first time in literature. The pulsed method using fluorescing tracer particles is found to decrease the depth of correlation (DOC) as expected, and to better represent the shape of the velocity profile. Two methods of velocity characterization are used (single and double parameter) and the pulsed images provide better shape representation in both cases.

### 5.1 Introduction

Micro-particle image velocimetry (µPIV) has evolved as method for measuring the velocity profiles of micron-scaled flows. It differs from traditional velocimetry by its requirement of volume illumination due to the scale (Santiago *et al.* 1998, Meinhart *et al.* 2000). The method of micro-particle image velocimetry has been particularly embraced by the hemorheological and microcirculatory research communities, both of which have applied the method to measure blood flow profiles *in vivo* and *in vitro* to better understand microcirculation (Sugii *et al.* 2001a, 2001b, 2002, 2005, Bitsch *et al.* 2005, Lima *et al.* 2006, 2007, 2008, 2009, Nakano *et al.* 2005). *In vivo* versus *in vitro* applications of micro-particle image velocimetry requires different choices in equipment and execution. Traditionally, *in vivo* applications require high-speed photography using the red blood cells (RBCs) themselves as tracers while *in vitro* applications use pulsed images and fluorescent tracer particles. The resulting images from the two approaches are different as seen in Figure 5.1. Pulsed images using fluorescing tracer particles is shown on the left, and the high speed camera images using red blood cells themselves as tracers shown on the right.

There have been significant contributions to the µPIV literature to combat the loss of
accuracy that comes with volume illumination using alternate microscopy methods like using confocal systems to image in 3D and advances in data analysis and data processing of both 2D and 3D images using different imaging systems. Several reviews of the current status of the \( \mu \)PIV literature have been published (Williams et al. 2010, Wereley and Meinhart 2010). Despite these advances, the basic problems remain that the effect of the relatively large size of RBC as tracer particles to the diameter of the channel is described as the depth of focus (DOF) and its effect on the cross-correlation measurement of the movement of the tracers called the depth of correlation (DOC) when using the RBC as tracers. While the fluorescing tracer particles are a solution, they are not applicable in all cases due to non-biocompatibility in vivo and significant expense in the required equipment (laser).

Due to biocompatibility, researchers use high speed video PIV for in vivo measurements. This method can also be applied to in vitro measurements, as it is here. Some researchers reject high speed PIV and move to pulsed images using laser fluoresces and assuming a superior measurement. There are advantages to both methods. High speed PIV can be more financially feasible. The data from both styles of measurement are compared in literature, but no direct comparison has been calculated. The goal of this paper is to analytically determine what is the quantitative outcome of using high speed
photography in µPIV compared to using micron-scale tracer particles (in conjunction with pulsed images), with experimental results to ensure validity. This direct comparison, initially done *in vitro* by Pitts and Fenech (2012) has been since repeated *in vivo* by Poelma et al. (2013). The only way to directly compare the two measurement styles is to do them simultaneously on the same blood sample in the same microchip, which has been achieved here. *In vitro* experimentation removes the inherent uncertainties in biological vessels.

5.2 Materials and methods

5.2.1 Experimental set-up

The dual camera set-up is depicted in Figure 5.3. The system employs a MITAS microscope (LaVision, Gmbh, GERMANY) using a 10x objective (Zeiss, GERMANY). Blood is introduced to the microchips via a Nexus3000 high-precision micro syringe pump (Chemyx Inc., USA) at programmed flow rates of 5 and 10 µL/hr. The cameras employed are an Imager Intense pulsed camera (LaVision, Gmbh, GERMANY) and high speed camera (Dalsa, CANADA). The time between frames for the pulsed camera was 30 ms for the 5 µL/hr and 10 ms for the 10 µL/hr programmed flow rate. The high speed camera frame rate was resulted in 7 ms between frames for both programmed flow rates. The rectangular microchannels are fabricated in house of polydimethylsiloxane (PDMS) in a 10:1 ratio of polymer to curing agent (Sylgard-184, Dow Chemical, USA) in cross section of 40 x 110 µm in a cleanroom following established soft lithography techniques (Whitesides and Stroock 2001, Sia and Whitesides 2003, Cheung *et al.* 2009). Channels were bonded to unused, standard glass microscope slides using oxygen plasma. Prior to plasma bonding, slides were soaked in acetone, rinsed in isopropanol then rinsed in methanol to ensure
they were free from contamination. Slides were dried using compressed nitrogen. PDMS channels were cleaned with scotch tape to ensure they were free from dust. As fully developed flow was desired, the entrance length was calculated using an experimentally verified technique for microfluidic channels (Ahmad and Hassan 2010). Entrance lengths for the channel were found to be on the order of the hydrodynamic radii of the channels. The data were taken at least 100 times the entrance lengths distance from the inlet of flow to ensure avoidance of entrance effects.

Figure 5.2: Diagram of the PIV set-up where (a) represents the rectangular PDMS channel and (b) represents the entire dual µPIV system.
5.2.2 Blood samples

Fresh, healthy porcine blood samples were obtained from an accredited slaughterhouse, with 1 g of ethylenediaminetetraacetic acid (EDTA) per 1 L of blood as an anticoagulant added. The samples were centrifuged for 10 minutes at 3,000 RPMs, three separate times. RBCs were then suspended in phosphate buffered saline (PBS), (Sigma, CANADA) in concentrations of 10% hematocrit (H). Fluorescent tracer particles of 1 µm diameter (Microgenics, USA) were added to the blood solutions at a concentration of 30 µL per 1 mL blood solution. All measurements were done with fluorescing tracers added, even when they were not used to image the flow, so that the effect of the tracers is accounted for in both styles of measurement. For the high speed measurements, the results presented are an average value from 100 frames each of two separate porcine samples. For the pulsed results presented here, the measurements are an average value from 6 sets of measurements of 100 image pairs, 3 sets from each of the porcine samples.

5.2.3 Data processing

For the pulsed image data, the velocity profiles were obtained using cross-correlation of the µPIV data to obtain velocity vectors, which were averaged across the channel to give a single velocity profile. Standard cross correlation was done on both styles of images using 64x64 pixel square interrogation windows followed by 32x32 square interrogation windows with a 50% overlap in subsequent interrogation windows. Identical standard processing was used on both styles of measurement in order to only compare the profiles, not the effect of the data processing. Flow rate calculations were obtained by taking a ratio of the experimental velocity profile to the theoretical Newtonian profile. The theoretical profile was calculated as:
High speed versus pulsed images for \( \mu PIV \)

\[
\begin{align*}
  u(y, z) &= \frac{4G}{\mu w} \left[ \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{\beta^3} \left( 1 - \frac{\cosh \beta z}{\cosh \frac{3b}{2}} \right) \cos(\beta y) \right] \\
  &\quad \text{(5.1)}
\end{align*}
\]

where \( G \) is the pressure drop, \( \mu \) is the viscosity, \( \beta \) is the \( (2n-1)(\pi/w), b \) (z-direction) is the height, and \( w \) (y-direction) is the width (Tabeling 2006). The ratio of the experimental profile to the theoretical Newtonian flow profile was then extended into three dimensions and calculated a projected, experimental flow rate, where:

\[
F(y, z) = u(y, z) \frac{V(z)}{u(\text{middle}, z)}
\quad \text{(5.2)}
\]

The high speed data was collected using custom software described elsewhere (Chayer et al. 2012). These images were then translated into uncompressed 16-bit TIFF image stacks using ImageJ (NIH, USA). These image stacks are then imported into DaVis software, and the results are calculated the same as above.

In order to characterize the velocity profiles, there needs to be a quantifiable method of comparison. One such way is the shear rate at the wall. This is calculated by

\[
\dot{\gamma} = \max \left( \frac{\partial u}{\partial y} \right)
\quad \text{(5.3)}
\]

Another method of velocity profile characterization is using the shape factor \( K \). Here, \( K \) is a measure of the parabolic nature of the profile.

\[
V(z) = V_{\text{max}} \left[ 1 - \left( \frac{|y|}{(w/2)} \right)^K \right]
\quad \text{(5.4)}
\]
where $K = 2$ giving parabolic profile (Newtonian), $K < 2$ giving a blunted profile. In microhemodynamics this description has been suggested as not specific enough. The shape of the profile can be described in a more detailed manner where the bluntness at the wall and at the center are calculated separately. Koutsiaris (2004) gives a two-factor model for a round channel which is adapted here for a rectangular channel where

$$
V(z) = V_{\text{max}} \left[ 1 - K_1 \left( \frac{|y|}{(w/2)} \right)^2 \right] \left[ 1 - \left( \frac{|y|}{(w/2)} \right)^{K_2} \right]
$$

with $K_1$ giving the bluntness near the axis and $K_2$ giving the bluntness near the wall.

### 5.3 Depth of correlation

A calculation required for analysis of the two methods is the depth of correlation (DOC), which can be described as the minimal distance that separates a particle from the image plane without affecting the image processing method (Wereley et al. 1998).

$$
DOC = \frac{1}{2} \left[ \frac{1}{\sqrt{\epsilon}} \left( \frac{n^2}{N A^2} - 1 \right) \left( d_p^2 + \frac{1.49(M + 1)^2 \lambda^2}{M^2 N A^2} \right) \right]^{1/2}
$$

where $\epsilon$ is the minimal intensity of an away particle affecting the CC (we can empirically set $\epsilon$ to a value of 10% of the intensity of an in-focus particle) (Meinhart et al. 2000), $n$ is the index of refraction of the immersion medium between the microfluid and the lens, $\lambda = 0.65\mu m$ is the wavelength of light (red) when using a dichroic filter, $N A$ is the numerical aperture of the lens, $d_p$ is the particle diameter, and $M$ is the magnification.
The DOC does not take into account the hematocrit or the size of the channel. However, as the channel size decreases, the DOC can approach the size of the channel, which is a significant decrease in accuracy. The magnification and particle size are the largest contributors for the given system, as all else is held constant. Contained in Tables 5.1 and 5.2 are the DOC calculated using Equation 5 for air (generally what would be used for an inverted microscope used with a pulsed camera) and for water immersion (generally what would be used for high magnification or highspeed video). In both Tables 5.1 and 5.2 DOC-p refers to the depth of correlation of a particle (1 $\mu$m diameter), DOC-RBC refers to the DOC of a human RBC (7 $\mu$m diameter), and DOC-pRBC refers to the DOC of a porcine RBC (6 $\mu$m diameter). These are example calculations for specific microscope objectives (A-plan and W N-Achroplan, Zeiss, GERMANY). The working distance on the objectives are necessarily different, as the application and lens type dictates.

Table 5.1: Depth of correlation for various magnifications of Zeiss A-plan microscope objectives (Zeiss, GERMANY) using air (n = 1), where M is magnification, NA is numerical aperture, DOC-p is the depth of correlation for a 1 $\mu$m particle, DOC-RBC is the depth of correlation for a human 7 $\mu$m RBC, and DOC-pRBC is the depth of correlation for a porcine 6 $\mu$m RBC. The set-up in this study is M = 10, and the calculations are for red light $\lambda$ = 0.65.

<table>
<thead>
<tr>
<th>M</th>
<th>NA</th>
<th>DOC-p ($\mu$m)</th>
<th>DOC-RBC ($\mu$m)</th>
<th>DOC-pRBC ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.25</td>
<td>10.3</td>
<td>12.5</td>
<td>12.1</td>
</tr>
<tr>
<td>20</td>
<td>0.45</td>
<td>3.07</td>
<td>4.71</td>
<td>4.48</td>
</tr>
<tr>
<td>40</td>
<td>0.65</td>
<td>1.38</td>
<td>2.52</td>
<td>2.36</td>
</tr>
</tbody>
</table>

Using the fluorescing particles in the set-up depicted here, the wavelength of light seen by the camera is $\lambda$ = 0.65$nm$ (red light) for both air and water immersion. However, if the microscope and camera set-up were altered to use white light making the violet and blue spectrum visible, the DOC could be calculated with $\lambda$ = 0.45$nm$. This alters the values as shown in the following table, shown for air immersion.
Table 5.2: Depth of correlation for various magnifications of Zeiss W N-Achroplan microscope objectives (Zeiss, GERMANY) using water immersion (n = 1.333), where M is magnification, NA is numerical aperture, DOC-p is the depth of correlation for a 1 µm particle, DOC-RBC is the depth of correlation for a human 7 µm RBC, and DOC-pRBC is the depth of correlation for a porcine 6 µm RBC. The calculations are for red light $\lambda = 0.65nm$.

<table>
<thead>
<tr>
<th>M</th>
<th>NA</th>
<th>DOC-p (µm)</th>
<th>DOC-RBC (µm)</th>
<th>DOC-pRBC (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.3</td>
<td>9.79</td>
<td>12.5</td>
<td>12.1</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>3.53</td>
<td>5.68</td>
<td>5.38</td>
</tr>
<tr>
<td>40</td>
<td>0.75</td>
<td>1.59</td>
<td>3.09</td>
<td>2.89</td>
</tr>
</tbody>
</table>

Table 5.3: Depth of correlation for various magnifications of Zeiss A-plan microscope objectives (Zeiss, GERMANY) using air (n = 1), where M is magnification, NA is numerical aperture, DOC-p is the depth of correlation for a 1 µm particle, DOC-RBC is the depth of correlation for a human 7 µm RBC, and DOC-pRBC is the depth of correlation for a porcine 6 µm RBC. The set-up in this study is M = 10, and the calculations are for violet/blue light $\lambda = 0.45$.

<table>
<thead>
<tr>
<th>M</th>
<th>NA</th>
<th>DOC-p (µm)</th>
<th>DOC-RBC (µm)</th>
<th>DOC-pRBC (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.25</td>
<td>7.45</td>
<td>10.2</td>
<td>9.80</td>
</tr>
<tr>
<td>20</td>
<td>0.45</td>
<td>2.37</td>
<td>4.28</td>
<td>4.03</td>
</tr>
<tr>
<td>40</td>
<td>0.65</td>
<td>1.14</td>
<td>2.39</td>
<td>2.23</td>
</tr>
</tbody>
</table>

5.4 Experimental Results

The effect of the DOC and style of measurement can be seen in the resulting data profiles, flow rates and profile shape factors. The maximum velocity, shear rate at the wall and the estimated flow rate are listed in Table 5.4. Standard deviations are listed for all three parameters. The results of shape factor calculations are shown in Tables 5.5 and 5.6. Table 5.5 lists the single shape factor results for blood solutions H=10 at to flow rates. Standard deviations are listed for both $Q_{est}$ and $K$. Also found in Table 5.4 are the estimated flow rates for each measurement using Equations 5.1 and 5.2. Table 5.5 lists the two-parameter shape factor results. Standard deviations are listed for both $K_1$.
and $K_2$.

Table 5.4: Flow rate and velocity profile results for both measurement methods, where 
 H stands for Hematocrit (% RBC), $Q_{\text{prog}}$ represents the flow rate programmed into the 
syringe pump, $V_{\text{max}}$ represents the maximum velocity, $\dot{\gamma}$ is the maximum shear rate at 
the wall, and SD represents the standard deviation. Pulsed results are an average of 6 
measurements, 3 each from two separate blood samples. High speed measurements are 
an average of two measurements, one each from two separate blood samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>H</th>
<th>$V_{\text{max}}$ (mm/s)</th>
<th>SD</th>
<th>$\dot{\gamma}$ (1/s)</th>
<th>SD</th>
<th>$Q_{\text{prog}}$ ($\mu$l/hr)</th>
<th>$Q_{\text{est}}$ ($\mu$l/hr)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>0.371</td>
<td>0.026</td>
<td>52.3</td>
<td>11.1</td>
<td>5</td>
<td>2.63</td>
<td>0.25</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>0.256</td>
<td>0.053</td>
<td>35.4</td>
<td>5.63</td>
<td>5</td>
<td>1.79</td>
<td>0.45</td>
</tr>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>0.913</td>
<td>0.040</td>
<td>110.9</td>
<td>8.70</td>
<td>10</td>
<td>6.67</td>
<td>0.11</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>0.695</td>
<td>0.063</td>
<td>59.2</td>
<td>6.21</td>
<td>10</td>
<td>5.28</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table 5.5: Shape parameter results for both measurement methods, where 
 H stands for Hematocrit (% RBC), $Q_{\text{prog}}$ represents the flow rate programmed into the syringe pump, 
K represents the profile parameter, and SD represents the standard deviation. Pulsed 
results are an average of 6 measurements, 3 each from two separate blood samples. High 
speed measurements are an average of two measurements, one each from two separate 
blood samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>H</th>
<th>$Q_{\text{prog}}$ ($\mu$l/hr)</th>
<th>K</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>5</td>
<td>1.80</td>
<td>0.13</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>5</td>
<td>1.48</td>
<td>0.22</td>
</tr>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>10</td>
<td>1.99</td>
<td>0.29</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>10</td>
<td>2.38</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Representative results can be found in Figures 5.3 and 5.4, where the two methods 
of measurement are plotted on the same axes. In both cases, the plot has been rotated 
and the flow is upwards, the x-axes in both images is the width of the channel in meters 
and the y-axes in both images is the velocity in m/s.
Table 5.6: Two-parameter shape factor results for both measurement methods, where H stands for Hematocrit (% RBC), $Q_{\text{prog}}$ represents the flow rate programmed into the syringe pump, $K_2$ represents the shape parameter at the wall and $K_1$ represents the shape parameter at the center. SD is standard deviation of each measurement. Pulsed results are an average of 6 measurements, 3 each from two separate blood samples. High speed measurements are an average of two measurements, one each from two separate blood samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>H</th>
<th>$Q_{\text{prog}}$ ($\mu$l/hr)</th>
<th>$K_1$</th>
<th>SD</th>
<th>$K_2$</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>5</td>
<td>0.0991</td>
<td>0.15</td>
<td>1.8482</td>
<td>0.28</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>5</td>
<td>0.950</td>
<td>0.18</td>
<td>3.6645</td>
<td>0.05</td>
</tr>
<tr>
<td>Pulsed</td>
<td>10</td>
<td>10</td>
<td>0.2984</td>
<td>0.27</td>
<td>2.7137</td>
<td>1.06</td>
</tr>
<tr>
<td>Highspeed</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0.59</td>
<td>1.7431</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Figure 5.3: Example experimental results for 10% hematocrit at a programmed flow rate of 5 $\mu$l/hr from the first blood sample. High speed $\mu$PIV using RBC as tracer particles are '●' symbols, while pulsed $\mu$PIV using micron-scale fluorescing tracer particles are '○' symbols.
High speed versus pulsed images for $\mu$PIV

Figure 5.4: Example experimental results for 10% hematocrit at a programmed flow rate of 10 $\mu$l/hr from the first blood sample. High speed $\mu$PIV using RBC as tracer particles are ‘•’ symbols, while pulsed $\mu$PIV using micron-scale fluorescing tracer particles are ‘○’ symbols.

5.5 Discussion

High speed and pulsed camera velocity profile data for blood microflows are frequently compared. The validity of comparing in vivo and in vitro data is not addressed here. Rather, this work directly compares the two camera methods in vitro. The only difference in the measurements shown here is the camera and tracer particle combination. Tracer particles are present in both flows despite only being used to image the pulsed flows.

The scale and hematocrit are the two main factors when considering the accuracy of the measurement, with the former being more directly related to the DOC. As the DOC is reduced with tracer particles compared to the RBCs, it also becomes easier
to determine the center plane of the flow accurately. The choice in microscopy affects the DOC, as can be seen in Tables 5.1 and 5.2. As magnification increases, the DOC is reduced. Using water immersion leads to a lower DOC at the same magnification, but the results presented here do not use immersion for a direct comparison of the two imaging methods. Also altering the DOC is the wavelength of the light used. Again, here the same wavelength is used for both cases for direct comparison.

The low hematocrit is used here for ease of imaging. In literature hematocrit of 20 to 60 have been applied, with channels sizes varying from 5 to 28 $\mu$m (Sugii et al. 2002, Bitsch et al. 2005, Nakano et al. 2005, Chayer et al. 2012, Koutsiairis and Pogiatzi 2004). Hematocrits are not reported for the in vivo works. However, it is known that the microcirculation generally has a hematocrit of half of the macro circulation (Popel and Johnson 2005). Assuming a hematocrit of 40 to 50 at the macro scale, then H=20 is a good approximation for the microcirculation. At low hematocrits to get the flow rate the method is not critical (Chayer et al. 2012).

In terms of Tables 5.4, 5.5 and 5.6 it is interesting to note the differences in the profile characteristics. This differences are also visible in Figures 5.3 and 5.4. The high speed data gives a more rounded velocity profile while the pulsed data gives a sharper profile. The pulsed images give a higher maximum velocity and a higher shear rate at the wall. For the single parameter $K$ value, the standard deviation is greater for the high speed images. Conversely for the dual-parameter $K$ model, the standard deviation of the $K$’s is lower for all but one $K$ (the $K_1$ value for the 5 $\mu$l/hr flow), in which it is nearly even. Looking solely at standard deviation, it could be concluded that the dual-parameter $K$ model is a better descriptor for the high speed data, and the single parameter $K$ value is a better descriptor for the pulsed data. However, using a Welch-Satterthwaite version of Student’s t-test for unequal variances in samples of equal sizes, there is no statistical
difference between the single parameter $K$ values ($P < 0.0001$). Using the Newtonian solution for the velocity profile, the theoretical $K$ value is just above 3 for our channel configuration.

Fundamentally, the two measurement methods should give different measurements at the wall, so the differences in $K$ values and shear rates is expected. High speed measurements track RBCs which are not at the wall due to the well-documented cell free layer, while the pulsed camera tracks the tracer particles suspended in the plasma, which is at the wall, this more accurate for wall shear of the plasma but maybe not the RBCs. This is a choice in the measurement methods for the applications of the measurements. It was previously concluded that the high speed data has acceptable accuracy when looking for the value of the flow rate, but not when looking for the specific shape of the velocity profile (Chayer et al. 2012). The results here support this conclusion.

In both measurement methods there is a discrepancy between the programmed flow rate and the resultant flow rate calculated with the profile ratio. Some degree of decrease in flow rate found is expected due to the compliance of the system. The flexible nature of PDMS which makes it a good model for the vascular network also means a channel will deform under flow (Hardy et al. 2009). However, this does not fully explain the discrepancy. It has been shown that the processing of pulsed $\mu$PIV blood flow data will affect the shape of the final profile with the method of “image-overlapping” being the ideal case (Pitts et al. 2012, Pitts and Fenech 2012). This increases the predicted $V_{max}$ and the calculated flow rate (Pitts et al. 2012). The “image-overlapping” procedure is pulsed camera data specific. A comparison of optimized processing of the pulsed camera data and the standard processing of the pulsed camera data and the high speed data are shown in Table 5.7. Figure 5.5 provides an example profile at H=10 and a programemd flow rate of 10 $\mu$l/hr for all cases shown in Table 5.7.
Table 5.7: Comparing standard processing of pulsed and high speed data with optimized data processing of pulsed data at a hematocrit of 10 and a flow rate of 10 µL/hr.

<table>
<thead>
<tr>
<th>Data Processing Method</th>
<th>H</th>
<th>(Q_{\text{prog}}) (µL/hr)</th>
<th>(V_{\text{max}}) (mm/s)</th>
<th>SD</th>
<th>(\dot{\gamma}) (1/s)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Pulsed</td>
<td>10</td>
<td>10</td>
<td>0.913</td>
<td>0.040</td>
<td>110.9</td>
<td>8.70</td>
</tr>
<tr>
<td>Standard Highspeed</td>
<td>10</td>
<td>10</td>
<td>0.695</td>
<td>0.064</td>
<td>59.2</td>
<td>6.21</td>
</tr>
<tr>
<td>Optimized Pulsed</td>
<td>10</td>
<td>10</td>
<td>1.044</td>
<td>0.034</td>
<td>88.4</td>
<td>6.61</td>
</tr>
</tbody>
</table>

Figure 5.5: Example experimental results for 10% hematocrit at a programmed flow rate of 10 µl/hr from the first blood sample. Standard processing of high speed µPIV using RBC as tracer particles are ‘•’ symbols, Standard processing of pulsed µPIV using micron-scale fluorescing tracer particles are ‘o’ symbols, Optimized processing of pulsed µPIV using micron-scale fluorescing tracer particles are ‘+’ symbols.

Standard processing of the high speed data gives a different result for maximum velocity, flow rate and shear rate. Optimizing the data processing of the high speed data is beyond the scope of this work and not all µPIV researchers are applying optimal processing to blood microflows. It can be seen in Table 5.7 that the optimized data
processing the pulsed data provides the closest approximation of the theoretical velocity profile (1.200 mm/s) and the theoretical shear rate (83.5 1/sec) (Pitts et al. 2012). The standard processing of the pulsed data comes much closer to these theoretical values than the standard processing of the high speed data. If standard data processing is to be used, tracer particles give a closer measurement to theory.

5.6 Conclusions

In this work the methods of high speed camera $\mu$PIV using RBC themselves as tracer particles and pulsed camera $\mu$PIV with fluorescing micron-scale tracer particles are directly compared for the first time in a dual-$\mu$PIV set-up. The pulsed camera data using fluorescing particles gives a higher shear rate and a higher maximum velocity. For wall or boundary layer calculations the pulsed camera data is more accurate (Pitts et al. 2012). When employing high speed camera measurements using RBC as the tracers with hematocrits over 20, the diameter of the channel should be less than 25 $\mu$m based on literature review (Sugii et al. 2002, Bitsch et al. 2005, Nakano et al. 2005, Chayer et al. 2012, Koutsiaris and Pogiatzi 2004). For diameters up to 40 or 50 $\mu$m, the hematocrit should be on the order of 10, as shown here. When not within these constraints, pulsed images using laser fluorescent tracer particles are a more accurate method. Laser fluorescent tagging of RBC themselves as tracers is another option, but the DOC reduction inherent in small-scale particles is not sustained. In using the shape parameter models to describe the data, the dual parameter model is more consistent with the high speed data while the single parameter model is more consistent when used with pulsed data. In all cases, there is a difference in measured velocity profile shape between the high speed and pulsed camera imaging methods.
5.7 References


Lima R, Wada S, Takeda M, Tsubota K, Yamaguchi T. in vitro confocal micro-PIV


Chapter 6

Micro-particle image velocimetry measurement of blood flow: validation and analysis of data pre-processing and processing methods

This chapter was published as: Pitts KL, Mehri R, Mavriplis C, Fenech M. Micro-particle image velocimetry measurement of blood flow: validation and analysis of data pre-processing and processing methods, Measurement Science and Technology, 2012; 23(10):5302-5311. DOI: 10.1088/0957-0233/23/10/105302
The intent of this paper is to investigate the application of a pre-processing method previously validated on glycerol to blood flows in microchannels and to compare the accuracy of results obtained when applied to a non-homogeneous fluid such as blood with results from previously applied processing methods for blood data. Comparisons of common processing methods are desired for a clear measure of accuracy in order to make recommendations for various flows. It is hypothesized that increasing the correlation window overlap improves the profile prediction. The amount of correlation window overlap and window shape in the processing of data have a significant effect on the results. Image pre-processing is explored to improve the correlation using the “image overlapping” which is extended to the case of blood and the blood-specific pre-processing “base-clipping” or “thresholding” technique currently applied to blood. Both pre-processing methods are tested with multiple processing methods for two channel geometries: a straight rectangular channel and a Y-channel resulting in a controlled shear flow. The resulting profiles and calculations demonstrate that “image-overlapping” is found to achieve a profile closer to the predicted theoretical profile than current blood pre-processing methods when both are applied to the same set of data and both are superior to conventional cross-correlation on its own. In all cases, pre-processing decreases the smoothness of the predicted profile. The use of “image-overlapping” is shown to have greater accuracy when calculating the shear rate at the wall of the channel as well.

6.1 Background

Micro-particle image velocimetry (µPIV) is a particle based flow visualization technique that can be used to evaluate two-dimensional or three-dimensional velocity profiles of blood flow in microchannels via cross correlation. There are several approaches to the practical application of micro-particle image velocimetry (µPIV), such as confocal mi-
microscopy or classical microscopy coupled with laser-induced fluorescence. Confocal and standard microscopy can be coupled with high speed cameras, or fluorescent microscopy can be coupled with pulsed charge coupled device (CCD) cameras. While the approach may vary, the resulting data is always a set of velocity vectors representing the flow of the fluid. These velocity profiles can be used to calculate the flow rate or the shear at the wall of the channel. $\mu$PIV was first developed by Santiago et al. and has been used in hemorheology studies since Sugii et al. in 2001 used the technique to measure blood flow in 100$\mu$m round glass tubes, and then again in 2002 in vivo to measure the velocity field in a rat’s mesentery arteriole (Santiago et al. 1998, Sugii et al. 2002a and b). The confocal microscopy approach for three-dimensional images, introduced by Park et al. in 2004, was first applied to blood by Lima et al. in 2006. Kloosterman et al. have shown that the focal length in biological applications of $\mu$PIV affects the final results (2010).

The important factors in microhemorheology are the flow rate of the blood, the shape of the velocity profile, and the shear stress at the wall of the vessel. Popel and Johnshon (2005) provide an excellent review of the research in microcirculation. At the microlevel, blood cannot be considered a homogeneous fluid, as it is composed of many flexible particles in a fluid medium. Blood is a suspension of flexible red blood cells (RBCs), large white blood cells (WBC), platelets and other proteins suspended in a Newtonian fluid (plasma). Due to its inhomogeneous composition, blood presents unique challenges in imaging and cross correlation accuracy. RBCs are large relative to the scale of the measurement and channel in these studies. In macro PIV the data can be limited to a single plane through sheets of light, but at the micro scale volume illumination is necessary. Volume illumination is a greater problem for the imaging of micro blood flows, as the RBCs themselves are large in comparison to the 40$\mu$m tall channel and using the RBCs as the tracer particles leads to a depth of correlation (DOC) that can be
as large as the channel (Meinhart et al. 2000). This large DOC can significantly decrease the accuracy of the cross-correlation result (Fenech et al. 2008). Using 1\(\mu\)m diameter fluorescing microparticles coupled with a laser is one approach that may decrease the depth of focus problem in micro blood flow imaging (Olsen and Adrian 2000). The Fahraeus and Fahraeus-Lindqvist effects are further complications where the quantity of RBC in the vessel decreases, resulting in lowered viscosity, and the viscosity of the blood changes with the diameter of the capillary. Fluorescing tracer particles, first used by Santiago et al. (1998), have been advocated as a tool to decrease the influence of out of focus particles, when using the smallest particles possible. Here the tracer particles used were 1 \(\mu\)m in diameter, in contrast to the 7\(\mu\)m scale of the RBCs.

Once a method of measurement has been chosen and data is collected there are various ways to pre-process, process and post-process the resulting velocity vectors with varying degrees of accuracy and success. For homogeneous fluids in microflow (such as water) Meinhart et al.’s processing method uses a 50% overlap in correlation windows, and the post-processing method generates an ensemble average of the results, (2000). Nguyen et al. test multiple methods of post-processing in a study with a glycerol solution and advocate a method of “image-overlapping” (2010). In blood specific literature Bitsch and colleagues employ an ensemble correlation with a 25% overlap of the \(\mu\)PIV correlation windows (2005).

The method of Nguyen et al. is especially formulated to remove the influence of out-of-focus particles, which can decrease the accuracy of the correlation (2010). Fenech et al. have shown this to be significant in microflow of blood (2008). Here Nguyen et al.’s method is extended to blood in order to validate its use with a non-homogeneous working fluid and to obtain a direct comparison with the standard pre- and post-processing methods used in blood velocity profile validation. Additionally, the amount of corre-
lation window overlap and shape of the correlation window required in the processing are evaluated for blood. Figure 6.1 depicts the data processing methods used in this study. Blood is tested in two flow configurations: pressure driven flow in a rectangular microchannel and Y-channel arrangement causing a controlled shear flow. The paper is organized as follows: first the experimental materials and methods of the data collection are described, then the data pre-processing and processing methods are outlined. The best resulting velocity profiles obtained using all methods are presented, compared, and discussed. Finally conclusions and recommendations are made for the data processing of blood microflow velocity profile measurements made with $\mu$PIV.

Figure 6.1: Diagram of available micro-particle image velocimetry data processing methods.
6.2 Materials and Methods

6.2.1 µPIV set-up

µPIV data is obtained with a double pulsed camera as a sequence of image pairs. The pairs of images can be used to determine the movement of particles from the first image in the pair to the second image using cross-correlation. The pairs of images give velocity vectors; the average of 100 sets of those vectors image frame gives a velocity field, which can be averaged in space across the region of interest (ROI) to give an average velocity profile or used to generate instantaneous velocity profiles at various locations in the channel. LaVision’s MITAS µPIV was used to collect the vector fields. The system contains a New Wave Solo-II Nd:YAG laser (New Wave Research, USA) and an Imager Intense CCD camera (LaVision GmbH, Germany) controlled by a programmable triggering unit, as well as a MITAS fluorescent microscope (LaVision Gmhb, Germany) using a 10X objective, coupled with a stage moving in three directions, all controlled by a computer. Flows were induced in Polydimethylsiloxane (PDMS) channels fabricated of Slygard-184 (Dow-Corning, USA) in a cleanroom following established soft lithography techniques with SU-8 photoresist spun onto silicon wafers to create molds for the PDMS (Cheung et al. 2009). Glass slides were bonded to the PDMS channels as a base using oxygenated plasma exposure for 45 seconds. PDMS was chosen for its recognized biocompatibility and good optical properties. Geometries considered are a rectangular channel in a straight line and a rectangular Y-channel configuration with two inlets resulting in a controlled shear flow. The width to height ratio of the channels is 2.5:1 and 3:1 (straight and Y-channel configurations, respectively). The height of the channel mold on the wafer was verified by an Ambios XP200 profilometer (Ambios Technology, USA) and the width of the final product was verified using a 0.01 mm micrometer (Fisher Sci-
Blood is introduced to the microchips via high precision syringe pumps (Nexus3000, Chemyx Inc., USA and PicoPlus, Harvard Apparatus, USA). The syringe used was a 50 µL Hamilton gas-tight (Hamilton, USA). It was refilled every few measurements and held level with the channel to avoid excessive settling of RBC in the tubing. A diagram of the set-up and chip geometries can be found in Figure 6.2.

Figure 6.2: Diagram of experimental μPIV set-up and channel configurations where (a) represents the straight rectangular channel, (b) represents the Y-channel configuration, and (c) represents the entire system.
6.2.2 Sample preparation

Healthy porcine blood samples were collected locally at an accredited slaughterhouse, and treated with 1 g ethylenediaminetetraacetic acid (EDTA) solids dissolved in 4 mL distilled water for each liter of blood. Blood samples were then centrifuged three times at 3000 rpm at 21°C for 10 minutes each round. After each round all but the RBC were removed. Phosphate buffered saline (PBS) was added after the first and second round. Desired hematocrit was created with the RBC left after the final round of centrifugation with PBS. Fluorescent tracer particles of 1 µm diameter (Microgenics, USA) were seeded at 30 µL per 1 mL of resulting test suspensions of RBCs and test suspension hematocrit was verified by Zipocrit microcentrifuge (LW Scientific, USA). For both channel configurations, blood was tested at 10% and 20% hematocrit. While multiple samples were tested, a single sample for each case is presented.

6.2.3 Channel configurations

The straight channels are nominally 40 × 110µm. Blood is introduced to the channel via the syringe pump at 10 µL/hr. The velocity profile for water (or any Newtonian fluid, such as PBS) under these conditions is parabolic. At low hematocrits, the velocity profile is expected to behave in a near-Newtonian manner. In all cases, the highest velocity is expected at the center of the channel. In order to evaluate and validate the success of the processing in the rectangular channel, the theoretical Newtonian velocity profile was calculated using Poiseuille’s Law for rectangular channels (Tabeling 2005) according to the input flow rate of the syringe pump. The velocity profile equation is:

\[
u(x, y) = \frac{4G}{\mu w} \left[ \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{\beta^3} \left( 1 - \frac{\cosh \beta y}{\cosh \beta b/2} \right) \cos(\beta x) \right]
\] (6.1)
where \( G \) is the pressure drop, \( \mu \) is the viscosity, \( \beta \) is \((2n - 1)(\pi/w)\), \( b \) (x-direction) is the height, and \( w \) (y-direction) is the width.

The data and plots of the velocity as a function of the position in the channel (y values) are extracted from the processed data obtained from the various methods applied. The projected flow rates are calculated by taking a ratio of the two-dimensional experimental profiles to the two-dimensional theoretical (Newtonian) profiles. This ratio is then applied to a three-dimensional theoretical profile, resulting in a predicted three-dimensional experimental profile, as shown by the following equation:

\[
F(x, y) = u(x, y) \frac{V(y)}{u(\text{middle}, y)}
\]

where \( F(x,y) \) is the predicted experimental profile, \( u(x,y) \) the theoretical profile (from Equation 1 for the straight channel), and \( u(\text{middle},y) \) the middle profile of the theoretical profile. The flow rate is calculated integrating the predicted experimental profile. Shear rate was calculated at the wall using the experimental profile. The projected flow rate can be compared to the theoretical flow rate programmed into the syringe pump, and the shear rate at the wall from the experimental profile can be compared to the shear rate at the wall for the theoretical profile; both as a measure of accuracy.

The Y-channel consists of two fluids with different properties flowing within a microchannel nominally 150\(\mu\)m wide and 50\(\mu\)m deep for the width and depth respectively. The two fluids enter from the two different branches of the microchannel at the same flow rate, \( Q_{\text{entrance}} = 5 \frac{\mu L}{hr} \), so that the average flow rate in the channel is about 10 \( \frac{\mu L}{hr} \), corresponding to the single fluid experiments. Blood enters from the top branch and is assumed to have a kinematic viscosity of about \( \nu = 3 \frac{mm^2}{s} \), whereas a diluted solution
of PBS (having a viscosity of about $\nu = 1 \text{mm}^2\text{s}^{-1}$) enters from the lower branch of the channel to entrain the blood. Both fluids contain fluorescing particles. The PBS was chosen as the entraining fluid in order to keep the equilibrium concentration between both fluids, to avoid ion and RBC diffusion as well as to avoid hemolysis of RBC. The velocity profile across the channel is expected to have the highest velocity within the layer of the less viscous fluid (PBS). Since the two fluids enter and flow with the same velocity and the blood has a higher viscosity, it is expected that the blood layer will expand and fill slightly more than half of the channel as shown by numerical simulation (Mehri and Mavriplis 2012). The theoretical profile from the simulation is used to create the ratio to the experimental profile in order to produce a projected three-dimensional experimental profile and then flow rate, in the same manner as the single fluid straight channel described above. Shear rate is found in the same manner as with the straight channel.

### 6.2.4 Data pre-processing

Due to the presence of RBCs, the microparticles are inclined to migrate to the wall which increases the number of fluorescent particles stuck to the wall. To extract background information from double-frame images a simple image pre-processing scheme can be applied. Any component that stays stagnant in the image (e.g., image background and light reflections from stationary objects) is assumed to be a source of disturbance and is removed by subtracting a reference image. This reference image is an average of all the images $I_{\text{ref}}$:

$$I_{\text{ref}}(i,j) = N_1 \frac{1}{N_{\text{pairs}}} \sum_{k=i}^{N_{\text{pairs}}} I_k(i,j)$$  \hspace{1cm} (6.3)

where $N$ is the number of images, $N_1$ means only one pair, $N_{\text{pairs}}$ is the total number
of pairs, $I_1$ is the first image and $I_k$ is some arbitrary image K. Figure 6.3 depicts an example of image averaging where (a) $N_1 = 1$, (b) $I_{\text{ref}}, N_{\text{pairs}} = 100$, and (c) $I_1 - I_{\text{ref}}$.

Figure 6.3: An example of image averaging where (a) $N_1 = 1$, (b) $I_{\text{ref}}, N_{\text{pairs}} = 100$, and (c) $I_1 - I_{\text{ref}}$.

To control the focal depth, i.e. the depth of measurement, specifically for blood
Bitsch et al. employs a technique called “base-clipping” (2005). This image thresholding technique cuts the intensity of the out-of-focus particles that is lower than that of the in-focus particles. The image thresholding is performed as:

\[
I_{\text{thresh}}(i,j) = \begin{cases} 
0 & \text{if } I(i,j) \leq \text{threshold} \\
I(i,j) - \text{threshold} & \text{if } I(i,j) > \text{threshold}
\end{cases}
\]  

(6.4)

where \(I_{\text{thresh}}\) is the resulting “thresholded” image. Figure 6.4 gives an example of image “base-clipping” where (a) threshold = 0 and (b) threshold is 75% of the max intensity.

Figure 6.4: An example of image “base-clipping” where (a) threshold = 0 and (b) threshold is 75% of the max intensity.

Image overlapping is a method proposed by Wereley et al. for classical PIV and
applied to μPIV by Nguyen et al. (Wereley et al. 2002, Nguyen et al. 2010). It works by taking the maximum pixel intensity at each pixel location, $I_{\text{max}}$:

$$I_{\text{max}}(i, j) = \max(I_k(i, j)), k = 1, 2, 3, ..., N_{\text{pairs}}.$$  \hspace{1cm} (6.5)

The correlation is then applied to the unique pair of images obtained, as seen in Figure 6.5, an example of “image overlapping” with (a) $N_{\text{pairs}} = 1$ and (b) $N_{\text{pairs}} = 100$.

Figure 6.5: An example of “image overlapping” with (a) $N_{\text{pairs}} = 1$ and (b) $N_{\text{pairs}} = 100$. 
6.2.5 Data processing: cross-correlation

Cross correlation is a measurement of the difference between two waveforms or vectors. Wereley and Meinhart (2010) give the equation for cross-correlation as:

\[ \Phi_{CC}(m, n) = \sum_{x=1}^{P} \sum_{y=1}^{Q} f(x, y)g(x + m, y + n). \]  

(6.6)

where P and Q are interrogation window sizes in the respective x and y directions, f and g are the image intensity functions, and m and n are the respective displacements. \( \Phi_{CC} \) is the resulting spatially averaged two-dimensional correlation. In this paper LaVision’s DaVis software was used to implement the cross-correlation method. A mask is first drawn in order to define the ROI then stored for further analysis. The ROI includes sufficient pixels on either side of the channel to ensure that no relevant data is cut out. Once the mask is defined, shape and size of the correlation window must be decided upon. There are two options: a square correlation window, or an elongated window (in this case we use an oval). A standard correlation window is a 32 pixel by 32 pixel square (Wereley and Meinhart, 2010). The window size chosen depends on the programmed flow rate of the experiment. Here, the both channel configurations used successive window sizes of 64 × 64 pixels followed by 32 × 32 pixels. The window sizes were held constant, but the shape of the windows was varied to a square or an oval (4:1 ratio of length to width) aligned with the flow. Aligning an oval window with the flow is theorized to improve the correlation, since the motion of the blood should be largely in the lateral direction.
6.2.6 Data post-processing

There are multiple post-processing options available as well. The technique of vector averaging employs vectors at a given location averaged over a period of time. This was demonstrated by Meinhart, Wereley and colleagues (Meinhart, Wereley, Gray, 2000; Wereley, Gui, Meinhart, 2002). An average of the correlation, or “ensemble correlation” was first advocated by Meinhart and Wereley (Meinhart et al. 2000; Wereley et al. 2002). This is a common method but does not solve the problem of out of focus particles which is especially problematic here since blood is inhomogeneous and contains RBC which are relatively large particles. While these post-processing methods are mentioned here for completeness, in this paper the post-processing was not varied so as to ensure a clear comparison between pre-processing and processing techniques.

6.3 Results

Blood with a hematocrit of 10% and 20% RBCs (H=10, 20) was first tested in the $40 \times 110 \mu m$ rectangular PDMS microchannel at a programmed flow rate of 10 $\mu$L/hr and then tested in the Y-channel configuration. The goal is to understand if changing the flow configuration has an impact on the choice of processing. When choosing the best method, it is important to consider the impact of each portion of the processing methodology in addition to the computational time. For each configuration and hematocrit, the preprocessing options were applied (thresholding, image overlapping, or no pre-processing) followed by cross-correlation. Table 6.11 provides numerical results for the theoretical flow rate, then the flow rate calculated with standard cross-correlation with a 50% overlap between successive correlation windows, an 87% overlap between successive correlation windows, both presented for square and oval correlation windows.
The best case is reprocessed with the “image-overlapping” and the “thresholding” methods. The same is repeated for the Y-channel configuration in Table 6.2. The best-case standard correlation without pre-precessing are plotted against the pre-processing are shown in Figure 6.6 for straight channel configuration, and Figure 6.7 for the Y-channel configuration.

Table 6.1: Results for the straight channel configuration, where H stands for Hematocrit (% RBC), $V_{\text{max}}$ is the maximum velocity achieved, Diff represents the difference from theoretical values, $Q$ represents the flow rate, $\dot{\gamma}$ represents the shear rate at the wall, 50 and 87 % refer to the amount of overlap between subsequent correlation windows, square and oval refer to the shape of the correlation window, and Image overlap and Threshold are the pre-processing methods of Nguyen et al. and Bitsch et al. respectively.

<table>
<thead>
<tr>
<th>Method</th>
<th>H</th>
<th>$V_{\text{max}}$ (m/s)</th>
<th>Diff (%)</th>
<th>Q (µl/hr)</th>
<th>Diff (%)</th>
<th>$\dot{\gamma}$ (1/s)</th>
<th>Diff (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical</td>
<td>0</td>
<td>0.001200</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>83.5</td>
<td>0</td>
</tr>
<tr>
<td>50% square</td>
<td>10</td>
<td>0.000954</td>
<td>20.5</td>
<td>6.4004</td>
<td>36.0</td>
<td>27.0567</td>
<td>67.6</td>
</tr>
<tr>
<td>50% square</td>
<td>20</td>
<td>0.000739</td>
<td>38.4</td>
<td>5.1552</td>
<td>48.4</td>
<td>20.6412</td>
<td>75.3</td>
</tr>
<tr>
<td>50% oval</td>
<td>10</td>
<td>0.001000</td>
<td>16.7</td>
<td>6.1564</td>
<td>38.4</td>
<td>47.8900</td>
<td>42.6</td>
</tr>
<tr>
<td>50% oval</td>
<td>20</td>
<td>0.000831</td>
<td>30.8</td>
<td>5.4134</td>
<td>45.9</td>
<td>28.1094</td>
<td>66.3</td>
</tr>
<tr>
<td>87% square</td>
<td>10</td>
<td>0.000933</td>
<td>22.3</td>
<td>6.1564</td>
<td>38.4</td>
<td>47.8900</td>
<td>42.6</td>
</tr>
<tr>
<td>87% square</td>
<td>20</td>
<td>0.000729</td>
<td>39.3</td>
<td>4.5025</td>
<td>55.0</td>
<td>17.7465</td>
<td>78.7</td>
</tr>
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<td>87% oval</td>
<td>10</td>
<td>0.000985</td>
<td>17.9</td>
<td>6.7676</td>
<td>32.3</td>
<td>37.1065</td>
<td>55.6</td>
</tr>
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<td>87% oval</td>
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<td>0.000785</td>
<td>34.6</td>
<td>5.4054</td>
<td>45.9</td>
<td>29.6644</td>
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<td>Image-overlap</td>
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<td>8.3</td>
<td>6.5034</td>
<td>35.0</td>
<td>83.2168</td>
<td>0.3</td>
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<td>0.000890</td>
<td>25.8</td>
<td>4.9274</td>
<td>50.7</td>
<td>56.6752</td>
<td>32.1</td>
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<tr>
<td>Threshold</td>
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<td>57.2</td>
<td>19.8262</td>
<td>76.3</td>
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</table>

6.3.1 No pre-processing method

For blood tested at H=10 in the straight rectangular channel, it can be seen that the method that best represents the flow condition is the profile obtained with the higher percentage of overlapping and the more highly weighted correlation windows. This con-
Table 6.2: Results for the Y-channel configuration, where H stands for Hematocrit(% RBC), Q represents the flow rate, Diff represents the difference from theoretical values, \( \dot{\gamma} \) represents the shear rate at the wall, 50 and 87 % refer to the amount of overlap between subsequent correlation windws, square and oval refer to the shape of the correlation window, and Image overlap and Threshold are the pre-processing methods of Nguyen et al. and Bitsch et al. respectively.

<table>
<thead>
<tr>
<th>Method</th>
<th>H</th>
<th>Q (ml/hr)</th>
<th>Diff (%)</th>
<th>( \dot{\gamma} ) (1/s)</th>
<th>Diff (%)</th>
</tr>
</thead>
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<tr>
<td>Theoretical</td>
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<td>8.78090</td>
<td>0</td>
<td>8.14544</td>
<td>0</td>
</tr>
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<td>8.70771</td>
<td>0.8</td>
<td>31.3000</td>
<td>284.2</td>
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<td>8.55443</td>
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<td>12.5000</td>
<td>53.5</td>
</tr>
<tr>
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<td>8.71791</td>
<td>0.7</td>
<td>22.0000</td>
<td>22.8</td>
</tr>
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<td>8.68769</td>
<td>1.1</td>
<td>15.4000</td>
<td>89.1</td>
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<td>87% overlap, square</td>
<td>10</td>
<td>8.67153</td>
<td>1.2</td>
<td>15.1000</td>
<td>85.4</td>
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<td>8.47813</td>
<td>3.4</td>
<td>11.5000</td>
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<td>8.63630</td>
<td>1.4</td>
<td>15.7100</td>
<td>92.9</td>
</tr>
</tbody>
</table>

Conclusion is based on the assumption that blood at H=10 acts a quasi-Newtonian fluid and therefore is expected to reach a maximum velocity of approximately 1 mm/s. It can be seen that the flow rate achieved is considerably lower than with the blood tested at H=10. This is a reasonable result since the blood tested at H=20 has a higher viscosity which affects the flow rate within the channel. It can be concluded from the first 9 entries in Table 1, that the best curve representative of the flow is the one obtained with the highest percentage of overlap and the oval shape aligned with the flow.

The velocity profile for the two fluid flow model is expected to first increase and reach a maximum value in the less viscous fluid layer (left part of the profile), then experience a plateau at the interface between the two fluids to decrease in the viscous fluid layer (right part of the profile) and reach zero velocity at the channel wall. For this specific
Figure 6.6: Best-case processed velocity profiles for straight channel configuration at H=10 on left (a) and H=20 on right (b) where 1 is the image overlapping technique with oval windows and 87% overlap between subsequent correlation windows, 2 is the thresholding technique with square windows with a 50% overlap between subsequent correlation windows, 3 is no pre-processing with oval windows and 87% overlap between subsequent correlation windows, and 4 is the theoretical solution for a Newtonian fluid case, the interface between the two fluids is located in the lower half of the channel since the fluid entering from the top branch is more viscous and the two fluids flow at the

Figure 6.7: Best-case processed velocity profiles for the Y-channel configuration at H=10 on left (a) and H=20 on right (b) where 1 is the image overlapping technique with oval windows and 87% overlap between subsequent correlation windows of, 2 is the thresholding technique with oval windows with a 87% overlap between subsequent correlation windows of, 3 is no pre-processing with oval windows and 87% overlap between subsequent correlation windows of, and 4 is the theoretical solution for a Newtonian fluid case, the interface between the two fluids is located in the lower half of the channel since the fluid entering from the top branch is more viscous and the two fluids flow at the
same flow rate. It is expected that the viscous fluid will expand faster in the channel. The expected maximum velocity for this specific flow rate is about 1 mm/s, which is reached approximately with the data processed using the higher percentage of overlap (87% overlap). For Y-channel configuration, the square correlation window does not provide a good approximation of the flow since the velocity profile does not follow the predicted shape. This phenomenon might be due to the reduced number of particles in the blood layer due to the forced migration of the particles caused by the mixing of two different particle sizes (RBC and fluorescent particles).

6.3.2 Pre-processing method

For the straight single-fluid channel, the best velocity profile seems be that processed with the highest overlap percentage and weighted window sizes for H=10 and H=20. The maximum velocity seems higher with “image overlapping” than when there is no pre-processing. For the Y-channel configuration, the best representative velocity profile is again the one obtained using the highest overlap percentage and the oval correlation window size. However, notice that the maximum velocity provided using the Nguyen et al.’s method appears to be higher than without pre-processing. For both cases, the “thresholding” method gives good agreement to the no-pre-processing method, but the “image-overlapping” method is closer to the theoretical solution.

6.4 Discussion

For this study the image size was 2752x1040 pixels, and the time step between images was 10,000 µs. The image size and flow rate determine the necessary time-step. For blood, a movement of 8 pixels between images has been demonstrated to be adequate
for a decent image (Wereley and Meinhart 2010). The size of the correlation window is determined by particle density, whereas Wereley and Meinhart give the standard window size to be $32 \times 32$ pixels (2010). Within those general guidelines, the results shown here are applicable to any data, not simply the set-up outlined here.

Another important consideration is that a reduced quantity of RBCs near the wall is observed in Figures 5-3 through 5-5, which is a well known phenomenon in blood microcirculation called the cell free layer. This is due to the spinning of the RBCs making them move toward the center of the tube. While the width of the cell free layer is known to change with flow rate, this was not the focus of this study, especially since at the magnification used here, this small layer would be hard to measure accurately.

### 6.4.1 Effect of pre-processing

Nguyen et al.’s method “overlaps” the experimental images, by combining all of the first images for a sequence, then combining all of the second images for the same sequence, then performing cross-correlation between the two resulting images. As a processing procedure, this is more of an image compilation than image overlapping. The post-processing correlation is actually an average of all of the images. Here, the method is extended to blood by applying it to the sequence of 100 image pairs for the case of our data, which provides the profiles shown. This method greatly decreases processing time from up to four hours per profile to around 4 minutes. The thresholding method proves nearly as effective as the method of Nguyen et al., but without the decrease in computational time. It can be said that Nguyen et al.’s method provides a better accuracy since the maximum velocity is higher in all cases and thus closer to the expected values. It is important to note that the image overlapping is not only the closest to the expected maximum value, it is the only method that is able to predict the shape of the
profile at the edge of the channel. This shape is important when considering that the shear rate is determined by the angle of the flow at that interface, and image overlapping would give the closest prediction of the shear rate. Looking at the two fluid flow model results, the curve is not as smooth as those obtained by the threshold method and those obtained without any pre-processing method. This might be due to the unsteady nature of the fluid interface when blood is tested in the Y-channel configuration. The issue with the two fluid flow model is that blood contains RBC and fluorescent particles, and the fluorescent particles are entrained by the PBS that also contains particles. However, the mixture of large and small diameter particles engenders the migration of fluorescent particles to the PBS layer, due to the dynamics of the RBC. This phenomenon can cause a lack of fluorescent particles in the blood layer resulting in a non-smooth velocity profile in the blood layer as can be seen through Figure 6.7.

6.4.2 Effect of processing

Changing the window shape, from a square shape to an oval shape window was thought to give more accurate results since the oval window is able to capture more data (higher maximum velocity) than the square window due to the window aspect ratios. Decreasing the window size was thought to result in more accurate profiles since the groups of particles are correlated in smaller batches. Increasing the degree of correlation window overlap was thought to give a more complete set of data and therefore more accurate results of the velocity field.

In all cases, the oval shape gives a higher calculated flow rate than its corresponding square counterpart, and the 87% overlap gives smoother profiles than its 50% overlap counterpart. A smoother profile is found with the larger degree of overlap. Comparing the four processing methods, it is reasonable to conclude that the more accurate method
would be that where the window shape is elongated with the flow and the overlapping percentage is the highest. Larger window sizes were found to work better for the oval shaped windows and smaller window sizes worked better with the square windows. For the two fluid flow model, larger windows were more accurate.

6.4.3 Effect of post-processing

As mentioned, the post-processing was not varied. For both the straight rectangular channel with a single fluid and the Y-channel with the two fluids, an average of each 100 pairs was taken. This approach follows current averaging approaches used for blood (Wereley and Meinhart, 2010). By taking the average after the processing is completed, the error inherent in averaging is not propagated into as many further calculations. At the microscale, the effect of Brownian motion can be significant, so a time-averaged profile gives a more indicative representation of the flow behavior in the channels than single profiles along certain sections. In this paper a time average was taken for all data sets, so as to remove the effect of Brownian motion.

6.5 Conclusions

When processing glycerol $\mu$PIV data, Nguyen et al. advocate the use of an “overlap” method that is possibly better described as an image compilation, extended in this paper to blood. Other published blood data uses a correlation average and window overlapping method, with an overlap that is set at 25% or a thresholding method (Bitsch et al. 2005). A higher degree of correlation window overlap was hypothesized to improve the results. A larger overlap of the correlation windows in the post-processing gives in theory a better prediction of the maximum velocity and the shape of the resulting velocity profile.
However, it has been shown that the degree of overlap is not the only factor in the post processing, and that changing the size and shape of the window is extremely influential on the final results.

Given the research presented here, the following general guidelines can be ascribed: For unidirectional flows, oval windows aligned with the known flow direction prove the most accurate when combined with the smallest windows possible for a given optical set-up combined with the greatest degree of overlap. Pre-processing using the “image overlapping” method of Nguyen et al. was found to have better accuracy compared to the “thresholding” method and to significantly decrease the computational cost, while reducing the impact of the out-of-focus particles (Nguyen et al. 2010). The application of this method both increases the accuracy of the profile prediction and decreases the computational time for blood flows in microchannels. As blood is a biological flow, a decreased processing time is advantageous to gathering more data. Finally, Nguyen et al.’s method is also the best predictor of shear rate at the wall of the channel, which is of primary importance to physiological processes. Shear stress in the circulation affects vasodilation and vascular remodeling, including endothelial cell morphology (Popel and Johnson 2005).

6.6 Acknowledgments

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Chapter 7

An analytic study on the effect of alginate on the velocity profiles of blood in rectangular microchannels using microparticle image velocimetry

This chapter has been submitted for publication

It is desired to understand the effect of alginic acid sodium salt from brown algae (alginate) as a viscosity modifier on the behavior of blood in vitro, using a micro-particle image velocimetry (µPIV) system. The effect of alginate on the shape of the velocity
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profile, the flow rate and the maximum velocity achieved in rectangular microchannels channels. The channels were constructed of polydimethylsiloxane (PDMS), a biocompatible silicone. Porcine blood cells suspended in saline was used as the working fluid at twenty percent hematocrit (H = 20). While alginate was only found to have minimal effect on the maximum velocity and the flow rate achieved, it was found to significantly affect the shear rate at the wall by between eight to a hundred percent.

7.1 Introduction

Viscosity modification of blood flows is of importance to biomedical research for many reasons. In a clinical setting, modifying the viscosity of blood can be desired in order to reverse non-standard physiology, or to incorporate a medical benefit such as drug delivery (Baskurt et al. 2012). While blood may be considered a homogeneous fluid for some macroscale studies, at the micro scale blood must be considered a suspension of flexible formed elements such as red blood cells (RBCs), white blood cells (WBCs) in a Newtonian matrix of plasma. Blood viscosity modification is frequently done by introducing other flexible elements in the form of polymers such as dextran, polyethylene glycol (PEG), polyethylene oxide (PEO), warfarin, heparin, and pluronics (Baskurt et al. 2012). Often the goal of these polymers is to thin the blood, avoid thrombosis or to decrease aggregation. Dextran, PEO and PEG can induce the aggregation of RBC if the correct molecular weight or concentration. In general, long-chain polymers induce aggregation and short-chain polymers inhibit aggregation or have no effect (Baskurt et al. 2012). Alginic acid, alginate, is a straight-chain polyuronic acid with hydrophilic properties. Alginate is a polymeric viscosity modifier that has many uses in industry including biomedical applications such as tissue engineering and drug encapsulation (Zhao et al. 2010). Alginate, a derivative of brown algae cells, is biocompatible and biomimetic
(Domachuck et al. 2012). Additionally alginate has been suggested to have applications within in vivo or in vitro blood research (Launiere et al. 2011).

There have been conflicting reports on the behavior of alginate when combined with blood. The addition of sodium alginate has been found to greatly increases the rate of sedimentation of human and horse red blood cells (RBCs) and can be lethal for cats, rabbits and mice when injected in vivo (Solandt 1942). Other research found that long-chain polymers of alginate could cause renal failure, while short-chain polymers of alginate are thought to not cause these effects (Zhao et al. 2010). Alginate has also been found to affect the aggregation of RBCs in a dose-dependent manner (Zhao et al. 2010). Significant research has gone into using alginate and other plasma expanders to change the viscosity of the blood during extreme hemodilution, to increase perfusion, maintenance of blood pressure, and in the avoidance of hemorrhagic shock (Cabrales et al. 2004a and b, 2005, 2006, Martini et al. 2006, Tsai and Ingletta 2001). Research has also suggested in vitro application of alginate to decrease large cell settling (Launiere et al. 2011). Alginate cannot both decrease settling and increase aggregation, but it is possible that settling effects are dose or chain length dependent as well, which could explain differences in results. The benefits of alginate as a plasma expander and viscosity modifier have been demonstrated but the flow profiles of blood modified by alginate are necessary to fully describe the phenomenon present. To that end, the actual effect of the alginate on the velocity profile must be measured in a repeatable manner.

The purpose of this paper is to demonstrate the effect of alginate on in vitro microblood flows, as demonstrated by the resulting flow rate, maximum centerline velocity, and shear rate developed at the wall of the channel. Additionally, it is desired to quantitatively compare change in the shape of the velocity profile at the micro scale and to compare findings with conflicting reports in the literature. It was hypothesized that the
sodium alginate will affect the velocity profiles measured. Flow rate and alginate addition were studied at 20% hematocrit in polydimethylsiloxane (PDMS) microchannels. PDMS channels have previously been shown to be effective in the study of blood (Lima et al. 2008, Zeng and Zhao 2009). PDMS is a biocompatible polymer and currently is one of the primary reference materials for the evaluation of other biomaterials (Bélanger and Marois 2001).

7.2 Experimental method

7.2.1 Channel design and fabrication

Rectangular (140 \( \mu m \) wide by 40 \( \mu m \) tall) PDMS channels were fabricated of Slygard-184 (Dow-Corning, USA) in a cleanroom following established soft lithography techniques (Whitesides and Stroock 2001, Sia and Whitesides 2003, Cheung et al. 2009). PDMS is an excellent choice to mimic the behavior of the vascular network, which includes its deformability. The tubing is necessarily flexible in order to integrate the microchip into the \( \mu \)PIV system. Both of these factors lead to a compliance in the system. The PDMS channels were bonded to unused, standard glass microscope slides using oxygen plasma. Prior to plasma bonding, slides were soaked in acetone, rinsed in isopropanol then rinsed in methanol to ensure they were free from contamination. Slides were dried using compressed nitrogen. PDMS channels were cleaned with scotch tape to ensure they were free from dust. As fully developed flow was desired, the entrance length was calculated using an experimentally verified technique for microfluidic channels (Ahmad and Hassan 2010). Entrance lengths for the channel were found to be on the order of the hydrodynamic radii of the channels. The data were taken at least 100 times the entrance lengths distance from the inlet of flow to ensure avoidance of entrance effects.
7.2.2 Sample preparation

A healthy porcine blood sample was obtained from an accredited facility. Porcine blood was chosen since RBCs in porcine blood aggregate in the same manner as human blood and the shear force required to break up aggregates is the closest to humans (Weng et al. 1996). The whole blood sample was treated with an addition of 1 g of ethylenediaminetetraacetic acid EDTA) per 1 L of blood as an anticoagulant. In order to obtain the RBCs the whole blood was centrifuged 3 times for 10 minutes each at 3000 RPM to separate the RBCs from the plasma and any other particles. After each centrifugation the plasma and remaining particles were removed. Small amounts of phosphate buffered saline (PBS) were added after the first and second centrifugation to the remaining RBC. After the third centrifugation the RBCs were suspended in either PBS or alginate solutions in PBS obtained by seeding low viscosity alginic acid sodium salt powder (Sigma-Aldrich, Canada) into PBS in concentrations of 4 mg of alginate per 1 mL (Launiere et al. 2011). Alginate solutions were fully dissolved in PBS before addition of RBCs. Fluorescent tracer particles (Microgenics, USA) were seeded into the resulting blood fluids at 27 µL of particles per 1 mL of solution.

The viscosity of the alginate in saline and in saline with RBC were tested in shear using a microviscometer (RheoSense, USA). A range of shear rates from 100 1/s to 1000 1/s were used for all samples. The 4 mg of alginate per 1 mL of PBS yielded viscosity measurements of 24.45 mPa-s to 17.29 mPa-s. This is a slight shear-thinning effect. The addition of RBCs increased the viscosity to 38.34 mPa-s to 19.83 mPa-s. A viscosity of 3.3 cP (3.3 mPa-s) is often reported for whole blood, while for plasma alone a viscosity of 1.1 to 1.6 cP (Chandran et al. 2006).
7.2.3 Design of experiment

In order to test the effect of the presence of alginate at various flow rates, RBC suspended in PBS at 20% hematocrit \((H = 20)\) were tested at three flow rates, \(Q = 10, 25, 40 \mu L/hr\). The tests were repeated at the same flow rates for \(H = 20\) with the alginate-seeded PBS. For all data sets triplicates of each data point were taken. The average value of the three measurements for the maximum velocity, flow rate, and the maximum shear rate at the wall are reported in Table 7.1.

7.2.4 Velocity profile measurement

Accurate measurements of the velocity profile and maximum centerline velocity of blood flow in microfluidic channels can be made using a \(\mu\)PIV system. The central plane is found by measuring the velocity profile across the height of the channel and finding the maximum. Due to the large relative size of RBC to the channel dimensions in microflow, there is a large depth of correlation (DOC) in using the RBC themselves as the tracer particles for the cross-correlation, the mathematical method in which the velocity vectors of the flow are calculated. This DOC can be as large as the channel itself (Meinhart et al. 2000). The out of focus particles have been demonstrated to be the greatest problem with the DOC (Fenech et al. 2008). To minimize the DOC problem, seeding with micron-scale fluorescing tracer particles have been described (Wereley and Meinhart 2010, Wereley et al. 2002) and this approach was used here.

Data was obtained using the LaVision MITAS \(\mu\)PIV system which consists of an Nd:YAG laser and CCD camera controlled by a programmable triggering unit, a fluorescent microscope coupled with a stage moving in three axes, and a computer. A pulsed camera (Imager Intense, LaVision GmbH, Germany) was used for the cross-correlation and vector. The pulsed camera is coupled with the laser to excite the particles. A di-
agram of the system is shown in Figure 7.1. Blood was introduced to the microchips via high precision micro-syringe pump (Nexus3000, Chemyx Inc., USA). Data processing was done following the image overlapping technique of Nguyen et al. as applied to blood by Pitts et al. (Nguyen et al. 2010, Pitts et al. 2012).

Figure 7.1: Diagram of experimental μPIV set-up and channel configurations where (a) represents the rectangular channel and (b) represents the entire system.

### 7.2.5 Calculations

The velocity profiles were obtained using cross-correlation of the μPIV data to obtain velocity vectors, which were averaged across the channel to give a single velocity profile as described in Pitts, et al. (2012). Shear rates given are the maximum shear rate calculated at the wall. Flow rate was calculated with a projected three-dimensional flow rate using a ratio of the experimental velocity profile to an existing theoretical, Newtonian velocity profile. The theoretical profile was calculated using Poiseuille’s Law for rectangular microchannels (Tabeling 2006). The input flow rate of the syringe pump is used to back-calculate the pressure contribution. The width to height ratio is not great enough to constitute slit flow.
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\[ u(y, z) = \frac{4G}{\mu w} \sum_{n=1}^{\infty} \frac{(-1)^n+1}{\beta^n} \left( 1 - \frac{\cosh\beta z}{\cosh\frac{b}{2}} \right) \cos(\beta y) \] (7.1)

Where \( G \) is the pressure drop, \( \mu \) is the viscosity, \( \beta \) is the \((2n-1)(\pi/w)\), \( b \) (z-direction) is the height, and \( w \) (y-direction) is the width (Tabeling 2006). The ratio of the experimental profile to the theoretical Newtonian flow profile was then extended into three dimensions and calculated a projected, experimental flow rate, where:

\[ F(y, z) = u(y, z) \frac{V(z)}{u(middle, z)} \] (7.2)

In order to quantify the effect on the velocity profile, a velocity profile factor is fitted to the middle profile of each data. In flows of blood through microvessels the velocity profile can be fitted with a power law model.

\[ V(z) = V_{\text{max}} \left[ 1 - \left( \frac{|y|}{(w/2)} \right)^K \right] \] (7.3)

where \( K = 2 \) giving parabolic profile (Newtonian), \( K > 2 \) giving a blunted profile. In microhemodynamics this description is sometimes not specific enough. Koutsiaris gives a two-factor model where

\[ V(z) = V_{\text{max}} \left[ 1 - K_1 \left( \frac{|y|}{(w/2)} \right)^2 \right] \left[ 1 - \left( \frac{|y|}{(w/2)} \right)^{K_2} \right] \] (7.4)

with \( K_1 \) giving the bluntness near the axis and \( K_2 \) giving the bluntness near the
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wall (Koutsiaris 2009). This equation is fitted to all of the data. While Koutsiaris used circular channels and this work presents rectangular channels the central profile of the channel can be characterized by shape parameters regardless.

Additionally, the effect of the alginate on the shear rate at the wall is measured by calculating the shear rate for each profile.

\[ \dot{\gamma} = \max \left( \frac{\partial u}{\partial y} \right) \] (7.5)

7.3 Results and discussion

Table 7.1 provides the calculated flow rate, shear rate and maximum velocity. There was almost no effect found on the maximum centerline velocity in the microchannel with the addition of alginate, at any of the programmed flow rates tested. In addition, there was little effect on the flow rate with the addition of alginate. The standard deviation of the calculated flow rates for both alginate and non-alginate samples are listed. There is a discrepancy between the calculated flow rate and the programmed flow rate of the pump. There is necessarily some compliance in the system due to the flexible nature of the PDMS and the tubing. This accounts for a portion of the discrepancy. Additionally, it is known that the PDMS deforms while flow is occurring (Hardy et al. 2009). It can be seen that in most cases (except the highest flow rate) the presence of alginate increases the calculated flow rate.

The shear rate is found to be significantly lowered by the presence of alginate at all flow rates, which can be seen visually in Figure 7.2, and numerically in Table 7.1. The standard deviations of the shear rate measurements for the alginate and non-alginate
samples are listed. The shear rate can be seen to reduce from 8% at the flow rate of 10 µL/hr to over 100% reduction at 25 µL/hr programmed flow rate. This reduction of shear rate has not been documented in literature, but has several possible implications on future research. The shear rates of the blood on the endothelial cell walls of the microcirculation regulate the uptake of nutrients and oxygen. Chiu and Chen provide an excellent overview of the current research of this nutrient uptake and shear rate behavior (Chiu and Chen 2011). The addition of alginate could be used to tune the shear rate of blood solutions or blood substitutes in future research.

Table 7.1: Results of the various test conditions for flow rate, shear rate and maximum centerline velocity; microchannels are PDMS, 40 µm x 140 µm.

<table>
<thead>
<tr>
<th>Test (fluid)</th>
<th>H (%)</th>
<th>( Q_{\text{prog}} ) (µL/hr)</th>
<th>( Q_{\text{calc}} ) (µL/hr)</th>
<th>SD</th>
<th>( \dot{\gamma} ) (1/s)</th>
<th>SD</th>
<th>( V_{\text{max}} ) (mm/s)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>20</td>
<td>10</td>
<td>3.71</td>
<td>0.11</td>
<td>56</td>
<td>7.0</td>
<td>0.4</td>
<td>0.000012</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>10</td>
<td>4.38</td>
<td>0.11</td>
<td>52</td>
<td>5.6</td>
<td>0.5</td>
<td>0.000043</td>
</tr>
<tr>
<td>blood</td>
<td>20</td>
<td>25</td>
<td>9.19</td>
<td>0.35</td>
<td>230</td>
<td>70.5</td>
<td>0.9</td>
<td>0.000012</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>25</td>
<td>9.72</td>
<td>0.54</td>
<td>100</td>
<td>24.0</td>
<td>0.9</td>
<td>0.000018</td>
</tr>
<tr>
<td>blood</td>
<td>20</td>
<td>40</td>
<td>13.99</td>
<td>0.20</td>
<td>224</td>
<td>34.3</td>
<td>1.5</td>
<td>0.000061</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>40</td>
<td>13.21</td>
<td>1.34</td>
<td>202</td>
<td>128.5</td>
<td>1.3</td>
<td>0.000101</td>
</tr>
</tbody>
</table>

Table 7.2: Velocity profile characterization results of the various test conditions using two-parameter K model.

<table>
<thead>
<tr>
<th>Test (fluid)</th>
<th>H (%)</th>
<th>( Q_{\text{prog}} ) (µL/hr)</th>
<th>( V_{\text{max, calc}} ) (mm/s)</th>
<th>( K_1 )</th>
<th>SD</th>
<th>( K_2 )</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>20</td>
<td>10</td>
<td>0.4</td>
<td>0.34</td>
<td>0.24</td>
<td>4.07</td>
<td>0.65</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>10</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
<td>4.15</td>
<td>0.67</td>
</tr>
<tr>
<td>blood</td>
<td>20</td>
<td>25</td>
<td>0.9</td>
<td>0.74</td>
<td>0.12</td>
<td>5.11</td>
<td>0.26</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>25</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>3.40</td>
<td>0.85</td>
</tr>
<tr>
<td>blood</td>
<td>20</td>
<td>40</td>
<td>1.5</td>
<td>0.92</td>
<td>0.12</td>
<td>7.06</td>
<td>2.07</td>
</tr>
<tr>
<td>with alginate</td>
<td>20</td>
<td>40</td>
<td>1.2</td>
<td>0</td>
<td>0</td>
<td>2.05</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 7.2 gives the results of the K-fitting parameters, with the values and placed in
Figure 7.2: Comparison of the effect of alginate on the velocity profiles of blood with 20% hematocrit at various flow rates in a 140 µm wide, 40 µm tall PDMS channel. Black line is data and red line is fitted two-parameter K-model.

representative profiles of Figure 7.2 for easier comparison between profiles. It is important to note that Figure 7.2 provides the middle profile of the various programmed flow rates in the syringe pump. Samples without alginate are in the top row, and samples with alginate are in the bottom row. It can be seen that for all of the cases of alginate, the $K_1$ parameter goes to zero, and the $K_2$ parameter decreases as the flow rate increases. $K_1$ is the bluntness at the wall, and when alginate is added the flow is essentially plug-flow, and the correlation gives a valid prediction of the shape of the profile. At the case of the highest flow rate, the profile is fitted to nearly $K = 2$, whereas looking at the profile in Figure 7.2 its apparent this is not the case. As the flow rate increases, while alginate is added to the sample, the profile becomes increasingly non-uniform in all three runs and
the correlation no longer gives meaningful results. This can also be seen by the severe increase in standard deviate for that sample. It more than doubles.

Another interesting result is the effect of alginate on the aggregation of the blood. Using PBS as a medium instead of native plasma removes the aggregation ability of the blood. Microscopy images have shown how the presence of alginate affects the aggregation of the RBC (Zhao et al. 2010), however all of their test suspensions contained at least some native plasma. In order to isolate the effect of the alginate from the effect of the presence of the proteins in the native plasma, here only saline was used. Research demonstrates that the RBCs deformability is not affected by the alginate and that alginate might be a useful aggregation-inducing additive to the blood (Zhao et al. 2010). Here it is demonstrated that the alginate also significantly reduces the shear rate at the wall of the channel, and alginate might also be a useful additive in that regard, and this requires further research directly comparing blood samples with and without alginate that are based on saline versus native plasma.

It is interesting to note that the flow rate calculated in the channel in Table 1 is less that the flow rate programmed into the pump, also listed in Table 1. It is known that bottlenecks occurs in rigid microfluidic channels and that systems also display a residence time (Tabeling 2006). Tabeling (2006) gives a relation were the residence time of a piston-driven rigid system (much like a microsyringe) will have a residence time that varies as \((1/b)^3\), \(b\) being the height of the channel. If we assume a viscosity of blood to be 3.3 cP and our system to be rigid, than the time constant for our system is on the order of many hours. However, RBCs settle out of saline solutions in a matter of minutes, thus the syringe is reloaded every 2 minutes to avoid RBC settling and steady state is never reached, thus the programmed flow rate is never achieved. We acknowledge that PDMS is not entirely rigid, and has been known to deform (Hardy 2009). This deformation is
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minor relative to the residence time issue.

There have been reported changes in the width of the cell-free layer with the addition of polymer additives to blood (Baskurt et al. 2012). In blood flows at this scale, the cell-free layer should be apparent, as well as the Fåhræus and Fåhræus-Lindqvist effects (Goldsmith et al. 1989). At the level of magnification used for this study, it is difficult to make an accurate measurement of the cell-free layer. Current work on this project is to increase magnification and work with the high-speed photography capabilities of the system in order to draw clearer conclusions on the effect of the alginate on the cell-free layer. Qualitatively, settling is not eliminated in the tubing and therefore the channel but was unable to be measured using this experimental configuration.

7.4 Conclusions

Alginate has been shown to have minimal effect on the maximum velocity achieved in microflows of blood, as measured by PIV. A significant reduction in the shear rate at the wall of the channel was found with the addition of alginate to the PBS. This could be due to the alginate-induced aggregation of the RBC as studied by Zhao et al. (2010). The addition of alginate could be used in future research to adjust the flow rate and shear properties of blood solutions or blood substitutes. Alginate gives a blunted velocity profile at all flow rates, so much so the edge K value goes to zero when describing the velocity profile with a two-parameter shape equation. In conclusion, the behavior of alginate in micro blood flows shows great promise for future research and applications in blood viscosity modification and as a blood substitute when added to saline with RBCs.
7.5 Acknowledgements

The authors would like to thank Sura Abu-Mallouh for the viscosity measurements of the alginate in saline, and The Natural Sciences and Engineering Research Council of Canada (NSERC) for funding.

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Effect of alginate on the velocity profiles of blood using μPIV


Nguyen CV, Fouras A, Carberry J. Improvement of measurement accuracy in micro PIV by image overlapping. Experiments in Fluids 2010; 49(3):701-12.


Chapter 8

Conclusions

The purpose of this thesis was to study velocity profile measurement of blood microflows using micro-particle image velocimetry (µPIV) to understand how accurately and in what manner this technology can be applied to blood in order to then apply the technique to relevant biomedical problems like blood viscosity modification. Chapter 2 experimentally determined the materials and methods of applying µPIV to blood microflows. The material of which the microchannels were to be constructed of had to be tested. The surface chemistry of the various materials was compared through contact angle testing. PDMS is significantly more hydrophobic than the other possible options and plasma treatment, the gold standard for increasing hydrophilic behavior, was found to not be as effective with suspensions of blood cells in native plasma. Chapters 3 and 4 investigated the accuracy of the application method, through the type of camera. The choice of camera was determined to be application specific. High speed cameras were used in Chapter 3 in conjunction with round channels. The method was found to accurately find the flow rate, but not to accurately describe the shape of the velocity profile. Chapter 4 used both methods simultaneously on rectangular channels. The shape of the velocity profile was found to be more blunted in the center with the high speed camera, to an
Conclusions

overall lower maximum velocity in comparison with that found using pulsed data. This was exacerbated by the data processing. Once the application was determined, the method of data processing needed to be determined. Chapter 5 investigated the the current methods and hypothesis of micro flow measurement, especially velocity profile measurement, in micro-particle image velocimetry and then to extend those techniques to blood. This was done through applying the data pre-processing method of “image-overlapping” to blood measurements and comparing the results with existing blood data pre-processing methods. Finally, Chapter 6 applied the validated method to relevant biomedical and biofluid problem of blood viscosity modification. The viscosity of blood was modified, which was done through the addition of sodium alginate, after extensive literature review. Sodium alginate was found to significantly impact the shape of the velocity profile. Understanding the modification of the viscosity through its effect on the shear rate can assist in medical research into increasing perfusion for drug delivery and in cases of extreme hemorrhagic shock.

8.1 Future works

The next step is to extend the validated method of $\mu$PIV to micro scale rheometry of biofluids like blood. There are several methods for measuring rheological properties. Many texts are devoted to this subject, including those by Walters (1975, 1980) and Schramm (1994). Three types of rheometers (sometimes called viscometers) are used in measuring the viscosity of blood: capillary, coaxial cylinder, and cone and plate. Capillary rheometry relies on the pressure driven properties of Poiseuille flow, coaxial cylinder rheometry relies on the Couette flow conditions, and cone and plate rheometry relies on a small angle of the cone to create a constant shear (Chandran et al. 2007). Each type of rheometry has its own advantages and disadvantages. The pressure driven
flow of capillary vicometry is the most applicable to microflow, since the shear rates are quite low in both capillary viscometers and in the microcirculation of the human circulatory system.

There are many issues with conventional viscometry and rheometry, including but not limited to: errors occur in zeroing the gap and as the gap narrows the error increases, proper calibration is key and often difficult, large sample quantities are often required, and the sample is difficult to contain. In microheometry these issues can be circumvented or avoided. Accuracy is obtained by using the 2-D Weissenberg Rabinowitsch correction to find wall shear rate for fluid with shear dependent viscosity (Macosko 1994). There are some inherent micro level behaviors to keep in mind however, including the bottleneck effect, residence time (Tabling 2006) and the entrance flow effects (Ahmad 2010).

The current, non-blood research and methods include: Srivastava and Burns (2007), who used water to obtain visual results via trapped air compression in capillary flow; Guillot et al. (2006) who used an optical viscometer via fluid-fluid interface, a MEMS pressure sensor developed by Baek and Magda (2003) and tested in high shear micro flows by Pipe et al. (2008, 2009) where they also compared the results to traditional parallel plate and cone and plate rheometry. Han and Zheng (2009) used vacuum driven visual flow with power law fluids including a blood analog fluid. Current blood-based research and methods include Kang et al. (2010) using continuous flow in 100 indicating channels with commercial viscometer comparison.

In order to apply the method of this thesis to rheometry, an optically clear pressure drop measuring viscometer is developed for use with the $\mu$PIV system. As a first step, the method of Srivastava and Burns design had been redone for blood to measure the pressure drop when a constant flow rate is introduced. By calculating the compression of the air chambers, a pressure is calculated. This system is currently in testing with water.
and blood substitutes, and will subsequently be tested with blood. The viscometer developed for use with the $\mu$PIV system uses a straight channel with micro pressure sensors to measure the pressure change via data acquisition system. This system was designed with assistance by CMC and has been constructed by CMC after being awarded a grant. The system is currently being installed and validated.

### 8.2 Novelty

There are several novel concepts to this thesis. The measured comparison contact angle of blood on common microchip materials has never been documented previously. Additionally, the accuracy discussions and applications presented in Chapters 3 through 5 are all before undocumented. Especially relevant is the first published account of a dual $\mu$PIV system (in Chapter 4 and Appendix D) with the first published *in vitro* direct comparison of measurement methods. In Chapter 5, the application of Nguyen *et al.*’s method (2010) to blood microflows is done for the first time in literature. The data processing methods of velocity profiles for blood microflows was optimized by this improvement. Additionally, these improvements to application technique and data processing accuracy will be of immediate use to the blood microflow community. Finally, the visualization of the effect of alginate on the shape of the velocity profile of the blood microflows has never been published in literature before. In conclusion, this thesis has extended the known methods and accuracy issues of blood flow measurements in $\mu$PIV. It has improved the understanding of the blood velocity profile’s behavior and applied that knowledge and methods to interesting, relevant problems in biomedical and biofluids engineering. Overall, the accuracy of $\mu$PIV measurements of blood flows has been improved.


8.3 References


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Appendix A

Glossary of Terms

µPIV  microscopic particle image velocimetry

2D  two-dimensional

3D  three-dimensional

a  radius of a particle

β  parameter of the Gaussian distribution of the light

CC  cross-correlation

DOC  depth of correlation

DOF  depth of focus

Da  lens aperture diameter

dp  particle diameter

ε  distance from the object plane beyond which the contribution becomes insignificant

EDTA  ethylenediaminetetraacetic acid
Glossary of Terms

$H$  hematocrit (volume fraction of RBCs in blood)

$K$  velocity profile shape coefficient

$\lambda$  wavelength of light

$M$  magnification

**MEMS**  microelectromechanical systems

$\mu$  dynamic viscosity of the fluid

$n$  index of refraction of the immersion medium between the microfluid and the lens

$NA$  the numerical aperture of the lens

$n_{ij}$  the normalized vector pointing from RBC j to i.

$P$  p-value from paired t test

**PBS**  phosphate buffered saline

**PDMS**  polydimethylsiloxane

**PIV**  particle image velocimetry

**PMMA**  poly(methyl methacrylate)

**p-PDMS**  oxygen plasma treated PDMS

**PPE**  personal protective equipment

$Q$  volume flow rate

$R$  vessel radius
Glossary of Terms

**RBC** red blood cell

\(\dot{\gamma}\) shear rate

**SU-8** photosensitive polymer

**TMSM** 3-(trimethoxysilyl) propyl methacrylate

\(V_{\text{max}}\) maximum velocity

**WBC** white blood cells
Appendix B

PDMS microchip fabrication

Soft lithography was developed 2001 (Whitesides and Stroock 2001, Sia and Whitesides 2004). This technique has become nearly standardized with entire textbooks dedicated to soft lithographic methods. For this work, PDMS channels were manufactured using standard soft photolithography techniques. Those requiring greater accuracy were manufactured at the MIAM facility at McGill University in Montreal, Quebec. For the larger scale channels, the cleanroom in the Pilot Plant at the University of Ottawas department of Chemical Engineering was used. Masks were designed using “LayoutEditor” software. Completed designs were sent to commercial printers. For features greater than 10 µm, a transparency printed on a digital printer sufficed. For accuracy less than 1 µm, like that required for features less than 10 µm, a glass mask printed with chrome emulsion was necessary.

B.1 Manufacture of microfluidic device protocol

In order to accomplish photolithography in the lab several items of personal protective equipment (PPE) are required. These include safety glasses, a labcoat, long pants, closed
toe shoes and nitrile or latex surgical gloves. The chemicals required for the complete procedure include a negative photoresist (i.e. SU-8 10 from Microchem), isopropyl alcohol (IPA), a negative photoresist developer (organic solvent), silicone polymer (such as Sylgard 184 elastomer kit from Dow Corning, purchasable from Ellsworth Adhesive), a curing agent for silicone (included in kit), 98% 3-(trimethoxysilyl) propyl methacrylate (TMSM), and pure (99%) ethanol. The required equipment includes a software package to design mask (i.e. CleWin or LayoutEditor), a clean silicon wafer (new or used), a sonicator or ultrasonic cleaner, a spin coater a source of compressed nitrogen and a nozzle to compress the gas, a heating element (i.e. hot plate or oven), a mask aligner, an ultraviolet (UV) light source (i.e. mercury lamp), an analytic balance, a vacuum pump, tweezers or forceps, a scalpel, a specimen punch (i.e. 18G 1/2 needle # 305196 from BD Sciences or 0.75 Harris Uni-core punch from Ted Pella, Inc), adhesive tape (i.e. Scotch tape).

The first step in the procedure is to create the mask, which can be drawn in the computer packages mentioned or any standard CAD package. The mask can be sent to a printer to be printed on either glass or a transparency. Once the mask is finished, the mold (master) needs to be created. To start, clean the silicon wafer (if it is a used wafer): acetone ultrasound bath and dry, then alcohol ultrasound bath and dry. (do under fume hood) New wafers do not need to be cleaned but can be rinsed and dried with nitrogen. Dry the cleaned wafer in oven or on a hot plate for 30 min at 200°C to evaporate any solvents. Then spin coat on the negative photoresist. The ramp speed and final spin speed are dependant on the resist used and the desired thickness. Depending on the resist used, soft bake in oven to remove solvents from resist. Next, align mask onto wafer and expose to the UV light in order to cure through the entire thickness of the polymer resist. Again, this depends on the choice of resist. Afterwards, post bake
in oven to set the polymer. Wait at least 2 minutes after post baking for the wafer to reach room temperature. Then dip the coated wafer into developer and agitate while developing (under fume hood). Rinse time depends on thickness. Once developed, rinse with IPA (under fume hood) and dry with compressed nitrogen. To set the final master mold, bake mold in oven at 200°C for at least 2 hours for mold stability.

After the mold is created, it can be used indefinitely until it fouls or breaks down to create PDMS replicas. In order to create the PDMS replicas, which will become the channels, first weigh the silicone in a 10:1 ratio of elastomer to curing agent. Mix for 5 minutes (under fume hood) then pour mixture onto wafer. Next, de-gas for 30 minutes in a desiccator or until no bubbles are releasing. The mixture can be de-gassed before pouring on the wafer as well. Make sure the PDMS is thick enough to not rip when peeled from the wafer. After de-gassing, cure the PDMS replica in oven at 60°C for 1.5 hours. Then carefully peel replica from mold using tweezers.

These PDMS replicas are used to make the microfluidic devices. To make a chip, first punch an inlet and outlet and then cut out desired portion of mold with scalpel. Use adhesive tape to remove any dust or slivers of PDMS from the channel surface. Treat new/clean glass microscope slides with TMSM (in order to attach free acrylate groups onto glass surface) by soaking slide in 98% TMSM for 1 hour (under fume hood). Next, wash slide (several times) with pure ethanol (under fume hood) and then bake slide at 200°C for 1 hour. Affix slide to open portion of mold to create sealed chip (chip can later be peeled off when using this attachment method). Alternatively, the glass slide base and the channel interior can be exposed to oxygenated plasma for 45 seconds to 1 minute, and then the pressed together. This bond is more robust and permanent. Oxygenated plasma was used for all of the studies in this thesis.

Glass slides can be cleaned with piranha solution, TMSM, or acetone bath. Acetone
was chosen for its reduced toxicity compared to piranha, and better performance when compared directly with TMSM in the lab. In order to clean the slides with acetone, first soak new, glass microscope slides in acetone for 10 minutes. Next, rinse several times with IPA and then rinse several times with methanol. Finally, dry fully with compressed nitrogen.

B.2 References


Appendix C

Data analysis of the velocity profile

Following the protocol in the preceding section, velocity profile measurements can be taken in with either a high speed camera or a pulsed camera. In the high speed camera set-up, the RBCs themselves are the tracers. The applications and limitations of this method are addressed in Chapter 4. If the pulsed camera set-up is used, fluorescing particles are used as the tracers. A direct comparison of these two methods is presented in Chapter 5. In either case, the resulting data is a set of velocity vectors. These vectors can be averaged across the channel at an instantaneous point in the measurement to create a velocity profile. This profile can then be averaged across the length of time of the measurements to create an average velocity profile. In order to compare profiles at different configurations of channel size, hematocrit, or data processing (as with Chapter 6, in which the accuracy of data processing of the vector data are compared) the following methods are described in detail.
C.1 Flow rate calculations

First and foremost, the Newtonian solution is sought as a benchmark. At very low concentrations of RBCs, the Newtonian solution can be used for comparison. Fluid mechanics differ at the micro scale from the macro scale, and a simple flow in a rectangular duct cannot be applied to these flows. Tableing (2006) gives the center-line velocity profile in a microchannel as:

\[ u(y, z) = \frac{4G}{\mu w} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{\beta^n} \left( 1 - \frac{\cosh \beta z}{\cosh \frac{\beta b}{2}} \right) \cos(\beta y) \]  

(C.1)

Where \( G \) is the pressure drop, \( \mu \) is the viscosity, \( \beta \) is the \( (2n-1)(\pi/w) \), \( b \) (x-direction) is the height, and \( w \) (y-direction) is the width. In the protocol, the method for finding the middle profile experimentally is described. This should be the maximum velocity profile across the channel. The flow rate can be computed for the Newtonian solution geometrically. This is done using MATLAB. A 3D plot can be generated of the Newtonian solution. Shown in Figure C-1 is the Newtonian 3D solution to Equation C-1 for a 30 \( \mu \)L/hr flow in a channel with a cross section of 40 by 140 \( \mu \)m.

For the experimental profile, an estimated flow rate can be found by taking the ratio of the experimental profile to the calculated center-line Newtonian profile.

\[ F(y, z) = u(y, z) \frac{V(z)}{u(middle, z)} \]  

(C.2)

A sample of this calculation is shown in Figure C-2 for the same channel as Figure C-1, but with an actual experimental flow with \( H = 10 \). This allows calculation of an estimate of the actual flow rate in the channel, which generally differs from the flow rate
Figure C.1: Newtonian 3D solution to Equation C.1 for a 30 \( \mu \text{L/hr} \) flow in a channel with a cross section of 40 by 140 \( \mu \text{m} \).

programmed into the syringe pump. The difference can be ascribed to the compliance of the system. As the tubing, PDMS channels and plastic connections are all flexible. The flexibility in these components creates significant compliance. It is possible to reduce compliance but not to eliminate it completely.

The ratio depicted in Figure C.2 is extended to the 3D solution plotted in Figure C.1. This results in an expected experimental 3D profile and flow rate calculation. This 3D ratio results in the experimental 3D profile shown in Figure C.3.
Data analysis of the velocity profile

Figure C.2: Centerline Newtonian solution to Equation C.1 for a 30 µL/hr flow in a channel with a cross section of 40 by 140 µm plotted with the experimental data for a sample of RBC at H=10 suspended in PBS.

C.2 Velocity profile characteristics calculation

Computing the $K$ or $K_1$ and $K_2$ parameters from the velocity profile.

$$V(z) = V_{max} \left[ 1 - \left( \frac{|y|}{(w/2)} \right)^K \right]$$  \hspace{1cm} (C.3)

where $K = 2$ giving parabolic profile (Newtonian), $K > 2$ giving a blunted profile.

This approach is used in chapters 3 and 5 to describe the shape of the velocity profile. The velocity profile is matched to a fitted velocity profile in MATLAB to calculate a
Data analysis of the velocity profile

Figure C.3: Experimental 3D solution from the ratio in Equation C.2 for a 30 µL/hr flow in a channel with a cross section of 40 by 140 µm.

$K$ value by minimizing the difference between the two curves. The fitted curves are smooth, while the velocity profile data is jagged, as its is experimental results of an averaged velocity profile. Figure C.4 depicts a fitted curve, for the case of $H = 20$ at a flow rate of 10 µL/hr, with the addition of alginate, as discussed in Chapter 7.

In microhemodynamics this description is sometimes not specific enough. The shape of the profile can be described in a more detailed manner where the bluntness at the wall and at the center are calculated separately. Koutsiaris (2009) gives a two-factor model for a round channel which is adapted for this thesis to a rectangular channel where
Figure C.4: Fitted velocity profile overlaid onto experimental velocity profile to determine $K$ for the case of $H = 20$ at a flow rate of $10 \, \mu\text{L}/\text{hr}$, with the addition of alginate, per Chapter 7, channel is $140 \, \mu\text{m}$ wide and $40 \, \mu\text{m}$ tall.

$$V(z) = V_{max} \left[ 1 - K_1 \left( \frac{|y|}{w/2} \right)^2 \right] \left[ 1 - \left( \frac{|y|}{w/2} \right)^{K_2} \right]$$  \hspace{1cm} (C.4)

with $K_1$ giving the bluntness near the axis and $K_2$ giving the bluntness near the wall. This equation is fitted to the data in the same manner as the previous equation. This method is used in Chapters 5 and 7. Figure C.5 depicts a fitted two-parameter curve, for the case of $H = 20$ at a flow rate of $25 \, \mu\text{L}/\text{hr}$. 
Data analysis of the velocity profile

Figure C.5: Fitted two-parameter velocity profile overlaid onto experimental velocity profile to determine $K_1, K_2$ for the case of $H = 20$ at a flow rate of 25 $\mu$L/hr, channel is 140 $\mu$m wide and 40 $\mu$m tall.

C.3 References


Appendix D

ICNMM Keynote - Blood velocity profile measurements in microchannels using micro-particle image velocimetry

This appendix was published in the Proceedings of the American Society of Mechanical Engineers 10th International Conference on Nanochannels, Microchannels and Minichannels (ICNMM-2012), and was presented as an invited keynote at that conference.

Experimental studies of blood microflows in rectangular biocompatible polymer microchannels measured using micro-particle image velocimetry are reported. The data processing methods, data collection methods, and choice of channel material are demonstrated to impact the velocity profile measurements obtained. Results show that the use of red blood cells as tracer particles creates a large depth of correlation which can approach the size of the vessel itself and decrease the accuracy of the method. It is
shown that changing the amount of overlap in the post-processing parameters affects the results by nearly 10%. The velocity profile is studied as a function of the flow rate of the blood, the hematocrit, or percentage of red blood cells, the shape of the channel, and the channel material. The results highlighted here show that the best processing options include pre-processing, the use of fluorescent tracer particles instead of the red blood cells themselves as tracers give a more accurate prediction of the profile, and the use of silicone as the channel material more closely mimics the behavior of physiology. Acrylic biocompatible polymer channels are shown to give a more parabolic profile at lower levels of hematocrit, while silicone biocompatible polymer channels give a velocity profile that looks more like in vivo flow studies.

D.1 Introduction

Microcirculation is the range of blood vessels, generally around 100 m or less, where the Reynolds and Womersley numbers are significantly less than unity, and the viscous stress and pressure gradient are the main determinations of flow. Blood is a non-homogeneous fluid, and this complex composition and environment makes characterization of the blood at the micro level difficult. As lab-on-a-chip and medicine move to the micro level, this characterization becomes important, especially velocity profile characterization. First, we will discuss the accuracy of measurement method before discussing the physical parameters influencing the velocity profile. Blood flow measurements in microflow, such as maximum velocity and the shape of the velocity profile, can be achieved through micro-particle image velocimetry (µPIV). Generally, for in vivo studies red blood cells (RBCs) are used as tracer particles for the µPIV method, while for in vitro studies, artificial fluorescent micro particles are added to the blood.

Microcirculation is of current basic science and clinical interest. Microcirculation
regulates the exchange between blood and tissues via vascular beds both in and around organs (Popel and Johnson 2005). Recognizing the characteristics of blood flow in microcirculation is essential in order to understand the mechanical interactions between the blood and the vessel consequently giving insight to the causes of cardiovascular disease, cardiogenesis and angiogenesis. The shear stress at the endothelial cell walls, the velocity profile and the flow rate are key factors in extending clinical understanding (Popel and Johnson 2005).

The velocity profiles of blood in micro channels are desired. The shape of the velocity profile gives insight into the shear stress developed at the sidewall of the channel. From this insight, inference can be made of the shear stress on endothelial cell walls in microcirculation. Chiu and Chien present a review of the current research on endothelial cells from a clinical perspective (Chiu and Chen 2011). However, the experimental study of blood flow in microcirculation represents a considerable challenge, and a complete picture of the accuracy of velocity measurement methods is lacking. A full understanding of the behavior of the velocity profile \textit{in vitro} would be useful for clinical and \textit{in vivo} studies as a benchmark comparison.

One of the key ways to measure blood velocity profiles on the micro level is through PIV. Some of these studies include research into the addition of fluorescing micro particles to increase the accuracy of the measurement at high levels of hematocrit. These included 35 $\mu$m x 300 $\mu$m rectangular channels and 100 m square channels (Lima \textit{et al.} 2007, 2008). Some work has been done with 100 $\mu$m round channels and fluorescent tracer particles (Sugii \textit{et al.} 2005). Kloosterman has focused on how different magnifications affect the final results (Kloosterman \textit{et al.} 2011). A method of comparing and validating not only the experimental set-up but also the post-processing of $\mu$PIV as applied to blood flow in straight microchannels is needed. In this paper we will highlight important parameters
influencing the accuracy of the measurement of the velocity profile. This will be done by first explaining the $\mu$PIV approaches in use and testing them, then examining the accuracy of these measurements, and finally applying the methods, and assessing future applications of accurate measurements.

Originally developed by Santiago, $\mu$PIV was further developed by Meinhart and colleagues (Santiago et al. 1998, Meinhart et al. 2000a and b). There are two main debates in blood $\mu$PIV: upright versus inverted microscopy, and high-speed versus pulsed camera data. Upright microscopy is generally done with in vivo studies, and inverse microscopy is generally done with in vitro studies. In blood it is further complicated by the choice of using the RBC themselves as the tracers, or adding fluorescing microparticles to the blood. Santiagos initial research used pulsed images from a CCD, coupled with an oil-immersion epi-fluorescent microscope using fluorescing microparticles as tracers (Santiago et al. 1998). Sugii used high speed video to accomplish $\mu$PIV using conventional microscopy in vivo using the RBC as the tracers (Sugii et al. 2002).

It is desired to show that the original method of Santiago, using pulsed images, gives more detailed results than the use of high speed video, and that fluorescent tracer particles give better resolution than RBCs as tracer particles. This is to be demonstrated through direct comparisons of the methods. Before accepting the results of such a comparison, the accuracy of the data processing methods must first be examined through analytical comparisons of the various processing methods applied to the same data. The choice of channel material is also verified.

**D.2 Nomenclature**

**DOC** depth of correlation
**DOF**  depth of focus

**EDTA**  ethylenediaminetetraacetic acid

**µPIV**  micro-particle image velocimetry

**PBS**  phosphate buffered saline

**PIV**  particle image velocimetry

**PDMS**  polydimethylsiloxane

**PMMA**  poly(methyl methacrylate)

**RBC**  red blood cell

### D.3 Materials and methods

#### D.3.1 Micro-PIV set-up

The µPIV set-up used consisted of an Imager Intense (LaVision GmbH, Germany) double pulsed camera mounted in conjunction with a high speed camera (Dalsa 1M150, Canada), recording images as both a sequence of image pairs and a high speed video. The double pulse camera was used to record images of exited fluorescent particles. The high speed camera was used to record continuous image sequences using red blood cells as tracer particles under white light. The images are used to determine the movement of the particles from the first image in the pair to the second image or from one frame of a video to the next frame. Both approaches use cross-correlation to determine velocity vectors which can be averaged or used instantaneously to give a velocity profile. Lighting was done with an Nd:YAG laser controlled by a programmable triggering unit and an overhead
fiber optic light source for the high speed video. Both cameras and light sources interfaced with a fluorescent microscope coupled with a 3 axis stage controlled by a computer. Flows were induced into 40 x 110 $\mu$m or 40 x 140 $\mu$m rectangular polydimethylsiloxane (PDMS) channels, a type of silicone, fabricated of Slygard-184 (Dow-Corning, USA) in a cleanroom following established soft lithography techniques (Cheung et al. 2009), or into 100 $\mu$m square poly(methyl methacrylate) (PMMA) channels, a type of acrylic, (Microfluidic ChipShop Gmbh, Germany). A high precision syringe pump was used to introduce the blood into the microfluidic chips (Nexus3000, Chemyx Inc., USA). Figure D.1 depicts the entire set-up.

Figure D.1: Diagram of the PIV set-up where (a) represents the rectangular or square channels fabricated of PDMS or PMMA and (b) represents the entire system.
D.3.2 Sample preparation

Porcine blood samples were collected from healthy animals slaughtered at an accredited facility. Each sample was treated with 1 g ethylenediaminetetraacetic acid (EDTA) solids dissolved in 4 mL distilled water for each liter of blood. Blood samples were then centrifuged at 3000 rpm at 20degC for 10 minutes 3 times. Everything except the RBCs was removed following each centrifugation. Phosphate buffered saline (PBS) was added after the first and second centrifugations. Desired hematocrit was created with the RBC left after the final round of centrifugation with PBS. A Zipocrit microcentrifuge (LW Scientific, USA) was used to verify hematocrits. Fluorescent tracer particles of 1 \( \mu \)m diameter (Microgenics, USA) were seeded at 30 \( \mu \)L per 1 mL of resulting test suspensions of RBCs.

D.3.3 Experimental approach

The experiments in this paper aim to assess the accuracy of \( \mu \)PIV. Accuracy can be broken down in to two main components: the data processing and the method. In order to compare the methods (approaches) it is necessary first to know if the processing of the data is accurate. Once the ideal processing parameters are decided, then the method of data collection is compared. The two methods compared are the use of high speed video with the RBC themselves as tracer particles and the use of pulsed camera data with fluorescing microparticles used as tracers. Finally, a comparison of two common biocompatible polymer channel materials is presented. General conclusions are then drawn about the accuracy and application of \( \mu \)PIV to blood flow in microchannels.
D.4 Results and discussion

D.4.1 Data processing

Data processing in blood $\mu$PIV is usually done by cross correlation between two images in order to obtain a correlation vector. Wereley and Meinhart give an excellent explanation of cross-correlation in their recent review (Wereley and Meinhart 2010). The data can be preprocessed before the cross-correlation (i.e. removal of background image, cropping, etc), the way the correlation is implemented can be adjusted (i.e. changing the size, shape or amount of overlap of the correlation windows) or it can be post-processed (i.e. averaging the resulting vectors, discarding outliers, etc).

The pre-processing consists of modifying the picture itself before using the cross-correlation method. While not blood specific, Nguyen et al worked with glycerol and advocate the use of pulsed data, which is used to generate a maximum intensity image for each of the pulses. The correlation is performed between these two maximum intensity images, instead of the entire sequence. This is referred to as image-overlapping, and has since been applied to blood (Nguyen 2010, Pitts et al. 2012). Bitsch and colleagues employ a thresholding method which they call a base-clipping technique that removes the lower intensity particles below some threshold (Bitsch et al. 2005). Figure D-2 depicts the methods of Nguyen and colleagues, standard cross-correlation without pre-processing and the method of Bitsch and colleagues. All of these methods are applied to the same data set of pulsed images using fluorescing tracer particles. Nguyens method requires the least amount of computation time since after pre-processing it requires a single image pair (Pitts et al. 2012).

After pre-processing, the cross-correlation is applied. Meinharts original method was to use a 50% overlap of correlation windows followed by ensemble averaging the results
Figure D.2: Pre-processing can change the achieved velocity profile. Channel is $40 \times 110 \mu m$. Blood sample at 10% hematocrit with a programmed flow rate of $10 \mu L/hr$. Y-axis position in the measurement is given on the x axis in millimeters; velocity is on the y axis in meters per second.

(Meinhart et al. 2000a and b). Bitsch and colleagues use an ensemble averaged correlation with a 25% overlap in the correlation windows (Bitsch et al. 2005). Adjusting the size, shape and overlap amount between subsequent correlation windows was hypothesized to improve the results. Using both LaVisions DaVis software and MATLAB, the most promising processing methods have been implemented (Pitts et al. 2012), to be compared with the standard correlation in use for blood, that is square correlation windows with a 50% overlap. The processing parameters changed were the shape of the correlation window, the size of the correlation window, and the post-processing. Corre-
lation window options used were square versus oval windows aligned in the direction of the flow, smaller sized windows, and 50% overlap or 87% overlap of the windows. It was found that changing solely the overlap of large square interrogation windows can affect the results by nearly 10%, and having the wrong size correlation windows has an equally large effect on the profile, as can be seen in Figure D.3. The exact same dataset was used for all of the processing cases, with hematocrit of 20% RBC, and a programmed flow rate of 10 $\mu$L/hr.

![Figure D.3: Change in overlap of the correlation windows affects the final results. Channel is 40 × 110 $\mu$m. Blood sample at 20% hematocrit with a programmed flow rate of 10 $\mu$L/hr. Y-axis position in the measurement is given on the x axis in millimeters; velocity is on the y axis in meters per second.](image-url)
Given appropriate seeding and window size, using an 87% overlap an oval window shape of direction of flow gives best results, i.e. the closest flow rate (5.41 µL/hr), maximum velocity (0.000785 m/s) and wall shear rate (29.66 l/s) to theoretical values (10 µl/hr, 0.0012 m/s and 83.5 l/s, respectively) but doing so is computationally expensive (four hours compared to about seven minutes). The pre-processing method of Nguyen et al. was found to increase the accuracy of the predictions of maximum velocity to 0.00089 m/s and shear rate to 56.66 l/s for H = 20, while also decreasing the computation time from four hours to four minutes. For H = 10, the pre-processing was found to increase the accuracy of the prediction even more for shear rate (83.2 l/s for pre-processing compared to 37.1 l/s for no pre-processing) and maximum velocity (0.0011 m/s compared to 0.000985 m/s). In all cases, the pre-processing slightly decreased the flow rate prediction. For H = 20, the flow rate with pre-processing is found to be 4.93 l/hr compared to 5.41 l/hr without pre-processing, while for H = 10 the flow rates were found to be 6.50 µL/hr versus 6.77 µL/hr (Pitts et al. 2012).

D.4.2 Data collection methods

Once the processing is set, it is required to determine the accuracy of the method. Here blood gives unique problems compared to conventional microfluidics. In macro PIV, the flow is illuminated by a sheet optic, while micro scale PIV requires volume illumination. Some work has gone into quantifying the depth of correlation (DOC) and depth of focus (DOF) requirements (Kloosterman et al. 2011, Wereley and Meinhart 2010, Fenech et al. 2008). In addition blood aggregates if, after centrifugation to remove the platelets, the RBC are suspended in native plasma instead of PBS, the RBC will still aggregate at low flow rates. When suspended in PBS, the RBC do not aggregate, which makes imaging easier.
With the construction of the dual \( \mu \)PIV system, high speed data using RBC and pulsed camera data, using microparticles in the same rectangular or square channel, could be evaluated and directly compared. Results can be seen in Figure D.4. Data were taken with the same blood, same set-up, and same channel (switching between cameras). High speed data were evaluated sequentially (100 images), while the pulsed data was evaluated in pairs (100 pairs). The high speed data used the RBC themselves as tracers, while pulsed used the fluorescing particles. The same blood sample was used for both images and contained particles, so the effect of the particles on the RBC is not considered. For the \( H=10\% \) profiles presented in Figure D.6, the pulsed data velocity profiles are close in shape to the high speed data velocity profiles, with the pulsed images having a smaller standard deviation of the calculated flow rate than the high speed images. The high speed data gives a slightly flattened velocity profile with a decreased shear rate at the wall. Overall this leads to a slightly larger prediction of flow rate (8.5 \( \mu \)L/hr compared to 6.9 \( \mu \)L/hr for the pulsed images). The high speed images seem to give a more accurate match to the programmed flow rate of 10 L/hr, however the shape of the profile and shear rate are better predicted by the pulsed data. Increasing the hematocrit was found to complicate the image processing of the high speed data, due to the density of the RBC causing imaging issues. Future work involves evaluating the image processing of the high speed data in a similar approach the previously outlined pulsed images processing methods, comparing the effect of hematocrit on the difference between the two methods and examining aggregation effects of the RBC when suspended in native plasma.
Figure D.4: The type of µPIV used changes the results for even the same samples in the same channels (40 x 140 µm). Blood sample at 10% hematocrit with a programmed flow rate of 10 µL/hr. Y-axis position in the measurement is given on the x axis in meters; velocity is on the y axis in meters per second.

D.4.3 Channel material

Figures D.2 through D.4 depict studies using PDMS channels fabricated in-house. However, it has been suggested that PMMA channels are good for biological applications (Chen et al. 2008). It was desired to see if that extended to blood, since PMMA has good optical properties. Additionally, PMMA is rigid, as is glass, whereas PDMS is a flexible silicone polymer. For these tests the 40 µm tall and 140 µm wide PDMS channels and 100 µm square PMMA channels were tested. The flow rates achieved were calculated
by taking a ratio of the velocity profile to the profile at that flow rate for a Newtonian fluid and then extending that ratio into the three-dimensional Newtonian solution, all of which are summarized below in Table D.1. For all case (H = 10, 20, 30), the programmed flow rate in the pump was 30 \( \mu \text{L/hr} \) which led to a Newtonian flow rate prediction of 30.0397 \( \mu \text{L/hr} \) for the PDMS and 30.0275 \( \mu \text{L/hr} \) for the PMMA channels. It was found that the PMMA channels are consistently measured to have lower flow rates and maximum velocities at higher hematocrits (H = 20, 40), despite the same input flow rate. At low hematocrit (H = 10) the PMMA channels achieved a higher flow rate. There has been some research into the effect of the flexibility of the PDMS on the velocity profile and pressure drop. Compared to rigid channel simulations, PDMS was found by Hardy et al. to have a decreased pressure drop (Hardy et al. 2009), which would lead to a higher flow rate for the PDMS. These experimental results validate the theoretical comparison of Hardy et al. Additionally, the shear rate at the wall is higher for the PDMS than for the PMMA, as can be seen in Figure D.5 with a direct comparison of the two channels center-plane velocity profile.

Table D.1: Results of PMMA versus PDMS channel data for various hematocrits at a programmed rate of 30 \( \mu \text{L/hr} \). Average and standard deviation is of 3 identical samples.

<table>
<thead>
<tr>
<th>H %</th>
<th>( Q_{PMMA} ) ( \mu \text{L/hr} )</th>
<th>( Q_{PDMS} ) ( \mu \text{L/hr} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>12.879 ± 0.342</td>
<td>10.482 ± 0.086</td>
</tr>
<tr>
<td>20</td>
<td>7.445 ± 0.071</td>
<td>8.823 ± 0.268</td>
</tr>
<tr>
<td>40</td>
<td>4.400 ± 0.093</td>
<td>5.124 ± 0.558</td>
</tr>
</tbody>
</table>

Finally, it was also found, that the shape of the velocity profile in PDMS more closely resembled published \textit{in vivo} data, as seen in Figure D.6 with hematocrits of 10 and 20. In Figure D.5 data was processed using standard processing parameters of a 64 \( \times \) 64 correlation window followed by a 32 \( \times \) 32 correlation window with a 50\% overlap.
Figure D.5: Velocity profiles from PDMS channels are shaped differently than those taken in PMMA channels. Blood sample at 20% hematocrit with programmed flow rate of 40 \( \mu \text{L/hr} \), respectively. Y-axis position is normalized by half the width since PDMS channel is \( 40 \times 140 \ \mu \text{m} \) and PMMA channel is 100 \( \mu \text{m} \) square. Velocity is relative to each data set's own maximum velocity. Comparison is for shape.

between the two correlation windows. It is desired in \textit{in vitro} studies to better understand physiology without doing \textit{in vivo} studies, so the PDMS channels are a better indicator of physiological response.

D.4.4 Effect of hematocrit

It can be seen looking at Figures D.2 through D.6 that changing the hematocrit or flow rate can significantly change the shape of the profile. This is more easily seen in three
Figure D.6: Velocity profiles from PDMS channels are shaped similar to published *in vivo* data (Sugii et al. 2005). Blood sample at 10 and 20% hematocrit with programmed flow rates of 30 and 40 µL/hr, respectively. Y-axis position is normalized by half the width; velocity is relative to each data set's own maximum velocity. Comparison is for shape.

dimensions. Since fluorescent µPIV only takes two-dimensional profiles, the only way to get a three-dimensional image is to take many profiles at various heights in the channel, or by taking the central two-dimensional profiles found previously and projecting them.
into three dimensions. This projects a square channel, but the general shape can be seen. A projected 3D profile in PDMS (of the H=20, Q=40 $\mu$L/hr from Figure D.6) is shown in Figure D.7, generated by mirroring the two dimensional profile cross-wise and matching the flow in three dimensions. Figure D.8 depicts the same flow rate at 40% hematocrit. As with changing the channel material, increasing the hematocrit changes the shear rate at the wall and the flow rate. A higher hematocrit will have an increased shear rate and decreased flow rate. For Figure D.7 and Figure D.8 the programmed flow rate is 40 $\mu$L/hr, but the calculated flow rate is only 5.739 $\mu$L/hr for Figure D.7 and 1.027 $\mu$L/hr for Figure D.8. The measurement is then assumed to be more accurate at the lower hematocrit. Future works involve quantifying this change in accuracy with increased hematocrit.

Figure D.7: Projected 3D velocity profile, from the same 20% hematocrit 40 $\mu$L/hr programmed flow rate sample as Figure D.5 in PDMS.
Figure D.8: Projected 3D velocity profile, at 40% hematocrit and the same 40 μL/hr programmed flow rate sample as Figure D.5 in PDMS.

D.5 Conclusions

A better understanding of the accuracy of the PIV processing and method is necessary to be applied to real world situations. The desired application of μPIV here is to use it to measure the shape of velocity profile, which can be used to determine shear rate at the wall. As mentioned previously, shear rate at the wall affects uptake of nutrients and oxygen. It is necessary to understand these mechanics in order to take advantage of these chemical processes. In order to accurately and appropriately employ μPIV techniques for blood microcirculation investigations, it is necessary to understand the unique challenges associated with blood flow in microchannels, and with the application of a macro technique like PIV at the micro level.

In the works reviewed here, several guidelines can be surmised. When processing the data taken in with any technique, appropriately sized correlation windows (shaped in the
direction of the flow) or an increased overlap to 87% between the subsequent windows is necessary for accuracy of the post-processing. For hematocrits greater than 10%, the use of fluorescent microparticles as tracers improves the accuracy of the velocity profile measurement and predictions made from that measurement. Additionally, using PDMS polymer channels in vitro gives more realistic predictions for in vivo velocity profiles. All of these parameters need to be kept in mind when applying PIV to applications to investigate the velocity profiles of blood in microcirculation when using viscosity modifiers, therapeutic treatments for blood disorders, or any number of biomicroflow issues. Several future works were also highlighted such as the further investigation on the influence of hematocrit on the type of imaging and the accuracy of the imaging techniques. Possible applications of this knowledge of PIV in microflow of blood includes: investigations of RBC aggregation which has clinical implications as the aggregating properties of blood can be used as a diagnostic tool, treatment and diagnosis of disorders of the blood such as sickle cell anemia, blood viscosity modifiers such as artificial blood substitutes, and modeling of microcirculation networks.

D.6 Acknowledgments

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D.7 References


Nguyen CV, Fouras A, Carberry J. Improvement of measurement accuracy in micro PIV by image overlapping. Experiments in Fluids 2010; 49(3):701-12.


Appendix E

Protocol for collecting material for research purposes at Tom Henderson Custom Meat Cutting, plant 202

This protocol is intended to be used in combination with The Food Safety and Quality Act 2001 as well as O.Reg. 31/05 s. 22, s. 85 to s. 88, s. 90, s. 126 and s. 129: as well as MPG S9.09.02.03.

E.1 General procedures

Summary of key regulatory requirements.

1. Research materials will be collected only from approved carcasses that have received post mortem inspection at the plant.

2. No condemned material will be collected for Research

3. The identity of corresponding carcass will be maintained throughout the collection
of materials for Research

4. Containers used for collection of research materials shall be clearly labelled RESEARCH MATERIALS ONLY and if made of plastic shall be uniquely color coded. The color used is described below in Plant Specific Procedures. O.Reg 31/05 s85

5. Research materials will be denatured if it is likely to be mistaken for a meat product for human consumption. O. Reg. 31/05 s. 88(2)

6. If processing, packaging or labelling the material for Research is likely to contaminate meat products intended for human consumption, then Research materials will only be processed, packaged, and labelled in a separate room in the plant as approved by the Regional Veterinarian. O. Reg. 31/05 s. 22(3), s90(2).

7. Packaging material must be durable, free of contaminants and not be re-used unless it is corrosion-resistant, cleaned and sanitized after each use and is capable of withstanding repeated cleaning. Re-usable boxes may be used if the boxes are lined, in good repair, free from contaminants and marked or labelled according to O.Reg. 31/05, section 126 O. Reg. 31/05 s. 129

8. After processing, packaging and labelling, material intended for use as Research materials may enter a room or area of a slaughter plant where meat products are handled or stored for the purposes of refrigeration, freezing, storage or shipping if it has been packaged in a manner that will protect meat products at the plant from contamination O.Reg. 31/05 s 87(4)

9. The operator of the plant shall ensure that Research materials, before they are shipped have a label on the exterior of the container that complies with O. Reg.
31/05 s. 126. The information needed on the shipping label includes:

Species from which the material is collected as well as the type of material collected (eg. Bovine livers)

The statement Research Materials Not For Human Consumption in letters at least 1.9cm high

Net quantity in weight or volume

Plant Number (stated as plant 123 for example)

Name and address of the plant where the material was produced, or the words Prepared for followed by name and address of the person the research material was collected for

Storage instructions keep refrigerated or frozen

No inspection legend is to be applied under any circumstances

10. Records will be kept for 12 months of all Research materials shipped from the plant. The records will include: Type of product, Weight of product, Name and address of the receiver/destination, Date of shipment or pickup, Initials of collector of delivery person.

E.2 Plant specific procedures

1. Request is from Dr Fenech and Katie Pitts at 161 Louis Pastuer Private (Her office is in room A328, and mine is in D218), Ottawa, Ontario, Canada, K1N 6N5. Dr. Fenech’s phone number is 613-562-5800 x1924, and I can be reached at x8852. The additional students involved in the research are Nona Ahmadi, Omemah Gilah, and John Morse.
2. Request for porcine blood.

3. Collected into medical grade containers provided by researcher.

4. Disposal as per university of Ottawa approved protocol.

5. The researchers may delegate the pick up and transportation of the blood to another party.

E.3 Authorization

Signed by regional veterinarian Dr. Richard D. Smith on February 3, 2012 Previous authorization signed by regional veterinarian Dr. Steven Palmer on May 27, 2010