

MICRO PIV AND NUMERICAL INVESTIGATION OF A MICRO-COUETTE BLOOD FLOW

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by

Rym Mehri, B.A.Sc

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Ottawa-Carleton Institute for Biomedical Engineering
University of Ottawa
Ottawa, Canada

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Abstract

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The purpose of this thesis is to design a physical microchannel model for micro-Couette blood flow that provides constant and controlled conditions to study and analyze Red Blood Cell (RBC) aggregation. The innovation of this work is that the Couette blood flow is created by the motion of a second fluid with different properties, thereby entraining the blood. The experimental work is coupled with three-dimensional numerical simulations performed using a research Computational Fluid Dynamic (CFD) Solver, *Nek5000*, based on the spectral element method, while the experiments are conducted using a micro Particle Image Velocimetry (μ PIV) system with a double frame CCD camera and an inverted laser imaging microscope. The design of the channel ($150 \times 33 \mu m$ and $170 \times 64 \mu m$ microchannels) is based on several parameters determined numerically, such as the velocity and viscosity ratios and the degree of miscibility between the fluids, and the resulting configurations are fabricated in the laboratory using standard photolithography methods.

The microchannel designed numerically is then tested experimentally, first, with a Newtonian fluid (glycerol), then with RBC suspensions to be compared to the simulations results. It was found that, numerically, using a velocity ratio of 4 between the two fluids, a third of the channel thickness corresponds to the blood layer. Within that range, it can be concluded, that the velocity profile of the blood layer is approximately linear as confirmed by experimental tests, resulting in the desired profile to study RBC aggregation in controlled conditions. The effect of several parameters, such as the hematocrit and the shear rate, on the RBC aggregates and the velocity profile is investigated, through experiments on the RBC suspensions.

The final goal of this research is to ensure the compatibility of the results between the experiments and the Newtonian numerical model for several ranges of shear rate with the future intention of finding an accurate method to be able to quantitatively analyze aggregates and determine the number of RBC in each aggregate depending on the flow conditions (the shear rate).

Keywords:

micro-Couette, red blood cell aggregation, blood, microchannel.

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List of Symbols

a	fluid consistency index
amp	amplitude
$A_{projected} [m^2]$	projected area of one aggregate
AI	aggregation index
$Area_A [m^2]$	area of light reflectance
$Area_B [m^2]$	area of light transmittance
ASI	aggregation size index
ASP	aggregation shape parameter
b	non-Newtonian behaviour index
$c [Pa.s]$	plastic viscosity
C, C_3, C_4	constants of the integration
$C_p [J/K]$	heat capacity
$C(x, y)$	cross-correlation function
$d_{particle} [\mu m]$	diameter of the fluorescent particle
$depth [\mu m]$	channel depth in 3D
$D [mm]$	half thickness of the channel
$D(c) [\frac{cm^2}{s}]$	diffusion coefficient
$Depth_{50} [\mu m]$	depth of the supposedly $50\mu m$ depth channel
$Depth_{100} [\mu m]$	depth of the supposedly $100\mu m$ depth channel
E	element number
$f [N]$	forcing function
$g_x [\frac{m}{s^2}]$	acceleration in x-direction
$g_y [\frac{m}{s^2}]$	acceleration in y-direction
$g_z [\frac{m}{s^2}]$	acceleration in z-direction
$h_y^E [\mu m]$	size of element E in y-direction
$h_z^E [\mu m]$	size of element E in z-direction

$I(x, y)$	image intensity of frame 1
$I'(x, y)$	image intensity of frame 2
k	Casson model constant
kt	total elements in cross-sectional area
K, L [<i>pixels</i>]	size of interrogation window
L_{branch} [μm]	branch length
$L_{channel}$ [μm]	channel length
L_n	n^{th} order Legendre polynomial
MAI	microscopic aggregation index
n_{image}	total number of recorded images
N	highest order of polynomial
$pixel_{im}$	the intensity of any pixel in image im
P	pressure
$Peri$ [m]	perimeter of one aggregate
$q^{(E)}(r)$	generic variable (e.g. Velocity, pressure or viscosity) to express as a polynomial expansion in <i>Nek5000</i>
Q [$\frac{\mu l}{hr}$]	average flow rate in the channel
Q_{bottom} [$\frac{\mu l}{hr}$]	pump flow rate for the fluid in the bottom branch
Q_{top} [$\frac{\mu l}{hr}$]	pump flow rate for the fluid in the top branch
$\dot{Q}_A$ [$\frac{\mu l}{hr \times m}$]	flow rate per unit length for fluid A
$\dot{Q}_B$ [$\frac{\mu l}{hr \times m}$]	flow rate per unit length for fluid B
r [m]	local spatial coordinate
T [K]	temperature
u [$\frac{mm}{s}$]	velocity of fluid in x-direction
u_A [$\frac{mm}{s}$]	velocity expression in x-direction
u_B [$\frac{mm}{s}$]	velocity expression in x-direction
$u_{interfaceA}$ [$\frac{mm}{s}$]	x-direction velocity at the interface of fluid A
$u_{interfaceB}$ [$\frac{mm}{s}$]	x-direction velocity at the interface of fluid B
v [$\frac{mm}{s}$]	velocity of fluid in y-direction
$V_{averageA}$ [$\frac{mm}{s}$]	uniform entrance velocity of fluid A
$V_{averageB}$ [$\frac{mm}{s}$]	uniform entrance velocity of fluid B

$V_{interface} [\frac{mm}{s}]$	velocity at the interface
$V_{bottom} [\frac{mm}{s}]$	uniform velocity for the fluid in the bottom branch
V_{ratio}	velocity ratio between the branches
$V_{top} [\frac{mm}{s}]$	uniform velocity for the fluid in the top branch
$V_{wall} [\frac{mm}{s}]$	velocity at the wall
$w [\frac{mm}{s}]$	velocity of fluid in z-direction
w_i	Gauss-quadrature weight for position i
w_j	Gauss-quadrature weight for position j
$width_{entranceA} [\mu m]$	width to calculate the uniform velocity of fluid A
$width_{entranceB} [\mu m]$	width to calculate the uniform velocity of fluid B
$W [\mu m]$	width of microchannel
x	dimensionless position along the channel
$x_{diff} [mm]$	diffusion length
y	position across the channel
$y_{interface} [\mu m]$	location of interface across the channel
$y_{wall} [\mu m]$	location of wall across the channel
$\alpha [\frac{m^2}{s}]$	thermal diffusivity
$\dot{\gamma} [s^{-1}]$	shear rate
$\delta [mm]$	location of the interface from the middle of the channel
ε	error variable in diffusion equation
$\lambda [nm]$	laser wavelength
$\lambda_{abs} [nm]$	absorption wavelength of fluorescent particle
$\lambda_{emission} [nm]$	emission wavelength of fluorescent particle
$\mu_{app} [Pa.s]$	apparent viscosity of blood
$\mu_A [Pa.s]$	dynamic viscosity of fluid A
$\mu_B [Pa.s]$	dynamic viscosity of fluid B
$\mu_H [Pa.s]$	viscosity of blood at high shear rate
$\mu_L [Pa.s]$	viscosity of blood at low shear rate
$\nu_A [\frac{mm^2}{s}]$	kinematic viscosity of fluid A

$\nu_{bottom} \left[\frac{mm^2}{s} \right]$	kinematic viscosity of the fluid in the bottom branch
$\nu_B \left[\frac{mm^2}{s} \right]$	kinematic viscosity of fluid B
ν_{ratio}	viscosity ratio between the branches
$\nu_{top} \left[\frac{mm^2}{s} \right]$	kinematic viscosity of the fluid in the top branch
$\rho_A \left[\frac{Kg}{m^3} \right]$	density of fluid A
$\rho_B \left[\frac{Kg}{m^3} \right]$	density of fluid B
$\tau \left[Pa \right]$	shear stress
$\tau_{interfaceA} \left[Pa \right]$	shear stress at the interface of fluid A
$\tau_{interfaceB} \left[Pa \right]$	shear stress at the interface of fluid B
$\tau_y \left[Pa \right]$	yield stress
ϕ	passive scalar
$\Phi \left[\% \right]$	glycerol mass fraction

List of Acronyms

ALE	Arbitrary Lagrangian-Eulerian
BGK	Bhatnagar-Gross-Krook
CFD	Computational Fluid Dynamics
CFL	Cell Free Layer
DP	Double pulse
E	Experiments in plots
ESR	Erythrocyte Sedimentation Rate
GL	Gauss-Legendre
GLL	Gauss-Lobatto-Legendre
H	Hematocrit
IBM	Immersed Boundary Method
IFEM	Immersed Finite Element
I/O	Inlet and Outlet boundary conditions
LBM	Lattice Boltzmann Method
LR	Light Reflection
LT	Light Transmission
MAS	Mean Aggregate Size
N-S	Navier-Stokes
PBS	Phosphate Buffered Saline
PDMS	Poly-Di-Methyl-Siloxane
PS	Passive Scalar
RBC	Red Blood Cells
RKPM	Reproducing Kernel Particle Method
RMS	Root Mean Square
S	Simulation in plots
SEM	Spectral Element Method

WBC	White Blood Cell
2D	Two-dimensional
3D	Three-dimensional
μ PIV	Micro Particle Image Velocimetry

Chapter 1

INTRODUCTION

1.1 MOTIVATION

Red Blood cells (erythrocytes or RBC) play an important role in determining the rheological properties of blood due to their unique characteristics. The understanding of RBC behaviour is of great interest to the field of hemorheology, the study of blood flow properties. RBC properties are almost singularly responsible for the particular characteristics of blood in *in vitro* testing. Due to their mechanical properties, RBC are able to deform and bridge together in order to form three-dimensional structures called aggregates or one-dimensional structures called rouleaux [1], which cause the variation in blood viscosity and the non-Newtonian behaviour of blood. However, the mechanism of RBC aggregation has not been completely understood. There are two theories, nowadays, that model the aggregation of RBC: the bridging of cells theory due to the cross-linking of the macromolecules [2] and the force attraction theory caused by depletion of the molecules due to the osmotic gradient [3]. An example of these entities is shown in Fig. 1.1.

Viscometric studies [4–8] that use rotational viscometers to examine the rheological behaviour of blood show that these aggregates usually form at low shear rates, which are, approximately under 10 s^{-1} for human blood [8]. Above this range, RBC tend to disaggregate and return to their equilibrium state. The effect of aggregates on blood viscosity is detailed in Sec. 1.2.1.2. From these studies, an inverse relationship is demonstrated between the shear rate and aggregation size. Therefore, aggregation size is an important factor in understanding the microrheological behaviour of blood. However, measurement of RBC aggregates presents a challenge in the hemorheology field due to the lack of consistency between the tests performed and the blood samples used. In fact, to our knowledge, there is no standard method to provide an estimation of aggregate size for prescribed flow conditions. The existing methods, detailed in

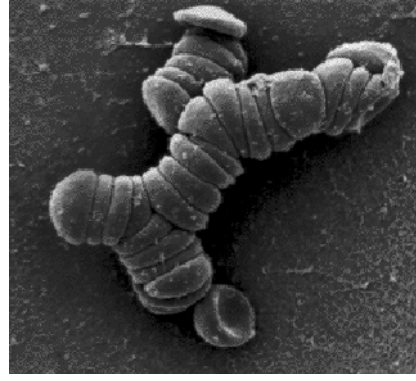


Figure 1.1: Red Blood Cell One Dimensional Rouleaux

Sec. 1.2.1.2, such as erythrocyte sedimentation rate, microscopic aggregation index and syllectometry method are only able to provide several aggregation indices to estimate the degree of aggregation in blood, which does not provide enough information to understand microrheological behaviour of blood. Numerical studies have been a great tool for investigation and modelling of RBC deformability and aggregation to understand the behaviour of RBC under physiological and pathological conditions [9–15]. These previous studies were also able to demonstrate an existing relationship between the RBC aggregates and the shear stress within the flow. However, since blood is non-Newtonian with a variable viscosity, it has not been possible to directly relate the aggregate size to the shear rate applied.

A physical model with constant flow conditions would be useful to visualize and estimate RBC aggregates under several controlled conditions. With this model, the defined relationship between aggregate size and shear rate could be implemented in the numerical studies to better model RBC behaviour especially in microcirculation. This model, studying RBC aggregation dynamically and instantaneously, could be used to better understand the non-Newtonian behaviour of blood. For such a model to provide controlled conditions, a constant shear rate is needed. Existing models [4–8] consisting of a Couette flow system have been widely used to understand blood behaviour. However, as implemented, these models did not allow for RBC aggregates to be visualized directly. Therefore, channels fabricated with transparent materials, such as glass, Poly-methyl-methacrylate or poly-di-methyl-siloxane have been

introduced in order to look at the flow and aggregates directly under the microscope [16–19]. Even with transparent materials, it is not possible to visualize the flow within the system since the velocity gradient is in the same plane as the microscope’s field of view. A possible alternative, is to use a confocal microscope to focus perpendicularly to the flow, which could reduce the accuracy of the results due to cell concentration within the system.

In the next section (Sec. 1.2), we review recent experiments and numerical studies performed on RBC aggregation to better understand their mechanism and their behaviour in microcirculation, emphasizing the need for a controlled experimental model.

1.2 LITERATURE REVIEW

1.2.1 BLOOD PROPERTIES

Human blood is a biofluid composed of several elements suspended in a Newtonian fluid known as plasma. The elements consist of White Blood Cells (WBC) or leukocytes, platelets or thrombocytes and Red Blood Cells or erythrocytes. This study only focuses on RBC as well as their properties and aggregation.

1.2.1.1 GEOMETRY AND PROPERTIES OF RBC

In a healthy individual, RBC represent biconcave discs with an approximate diameter of $8\ \mu\text{m}$ and a thickness of $2\ \mu\text{m}$ [20], as shown in Fig. 1.2. RBC have a volume of about $90\ \mu\text{m}^3$ for a surface area of about $140\ \mu\text{m}^2$, which represents a large surface area to volume ratio, about 40% larger than the ratio for a sphere. In fact this large surface to volume ratio, combined with membrane flexibility provides RBC with the ability to adapt to any vessel geometry in the body. Under normal conditions, in the human body, 5×10^6 RBC are contained in blood to occupy 40 to 45% of the blood volume. This measure of RBC is called hematocrit (the percentage by volume of RBC in blood). Low hematocrit is usually a symptom of anemia, while an excessive

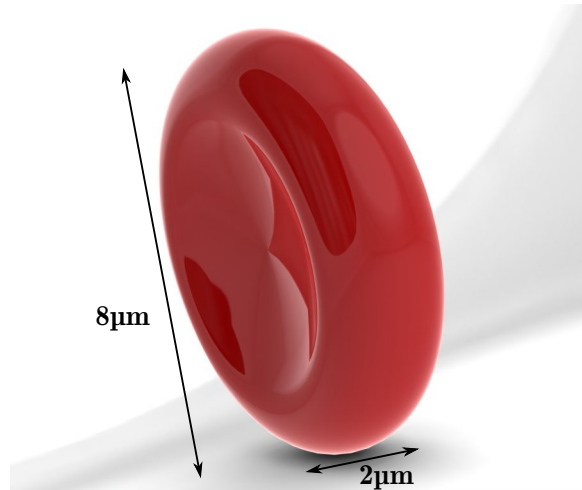


Figure 1.2: Geometry of a single Red Blood Cell

blood hematocrit is an indication of polycythemia which is unusual increase of the RBC number [21].

1.2.1.2 AGGREGATION OF RBC

Another major characteristic of RBC is their ability to aggregate at low appropriate shear rates as described in Sec. 1.1. Blood aggregation was first observed by Fåhræus in 1921 and described in [22], where the relationship between the aggregate formation and the sedimentation speed was investigated. From his research, the structure of aggregates in healthy and non-healthy individuals was clearly differentiated. However, the research of Fåhræus only included observations of stationary blood and RBC aggregates contained within two microscope slides. Therefore, *in vivo* experiments were carried out in order to visualize blood behaviour in vessels [23–26]. These studies focused on the visualization of the *bulbar conjunctiva* in anaesthetized healthy and non-healthy humans and rats. They provide evidence that, in the capillary vessels, RBC do not aggregate but flow as individual cells. They also claim that, depending on the pathological conditions, blood will behave differently.

The mechanism of RBC aggregation has not been completely understood. There are two theories, nowadays, that model the aggregation of RBC: The bridging of cells theory due to the cross-linking of the macromolecules [2] and the force attraction

theory caused by depletion of the molecules due to the osmotic gradient [3]. The first theory states that this mechanism is due to the bridging of the cells by the cross-linking of the macromolecules [27] and that aggregation occurs when the bridging force is greater than the forces due to electrostatic repulsion, membrane strain and mechanical shearing [3]. The second theory affirms that the aggregation mechanism is due to the osmotic gradient that is caused by the relative depletion of molecules near the cell surface with respect to the suspending fluid [3]. This osmotic gradient engenders an attraction force between the cells that causes the aggregation. Kim *et al.* investigated the effect of RBC collision rate on the rouleaux formation and claim that this collision rate plays an important role in aggregation initiation [28, 29].

EFFECT OF PLASMA PROTEIN ON AGGREGATION Due to the complexity of blood flow, further studies were performed in order to investigate and understand the rouleaux formation. Studies such as Merrill *et al.*'s investigated the effect of fibrinogen on RBC aggregation [30] by removing elements from blood plasma and observing the rheological properties of blood. They showed that the protein, such as the fibrinogen and the globulin, plays an important role in RBC aggregation. However, rouleaux formation is not only induced by fibrinogen. Chien *et al.* investigated the aggregation ability of several proteins and molecules in plasma and showed that globulin also plays a role in RBC aggregation, while albumin has no effect on aggregate formation [31]. When replacing blood plasma with Ringer's solution, such as Phosphate Buffered Saline (PBS), where no proteins or macromolecules are contained within the suspending fluid, no RBC aggregation was observed [32]. Rouleaux formation is greatly affected by the protein and macromolecules present in plasma, however, it has been shown that RBC aggregation can be affected by non-natural molecules present in plasma. Chien and Jan, in [27], present a study of erythrocytes suspended in Ringer's solution where polyglucose dextran, at different molecular weights, is added. They explore the relationship between the spaces between RBC aggregates and the dextran molecular weight. They observe that dextran, having a molecular weight above 40 kDa (kilodalton), induces RBC aggregation, while below this range,

the solution actually inhibits aggregation and has no effect on RBC aggregation without Ringer's solution. In fact, dextran affects the electronegativity of erythrocytes which has a great effect on rouleaux formation.

EFFECT OF SHEAR RATE ON AGGREGATION Several other factors greatly affect erythrocyte aggregation. Shear rate represents one of the most important factors influencing rouleaux formation. In fact, viscometric studies [4–8] show that shear rate or aggregation force is inversely related to aggregate size but also that such aggregates are usually only seen for shear rates below 10 s^{-1} . However, the aggregation force is not completely defined, in that there is no predetermined factor to provide sufficient information to describe the RBC aggregates. On the other hand, the disaggregation force is easily defined [33]. Under normal conditions, rouleaux disaggregate when subjected to shear rates ranging from 20 to 40 s^{-1} causing the RBC to flow separately.

EFFECT OF HEMATOCRIT ON AGGREGATION RBC aggregation is also hematocrit dependent. Referring to the study on Kim *et al.* that investigated the link between the collision rate of erythrocytes and the degree of aggregation, the number of RBC present within the medium greatly affect the collision rate since the fewer the number of cells, the lower the probability of the RBC to encounter, collide and initiate rouleaux formation, thereby decreasing aggregation [28, 29]. Experimental studies, conducted on healthy individuals with blood samples at different hematocrit subjected to different flow rates, showed that hematocrit has a significant effect on viscosity at high shear rate (above 10 s^{-1}), but that this effect is more significant for very low shear rates (below 1 s^{-1}) [34]. Moreover, the biconcave discoid shape of the RBC as well as the membrane elasticity can also affect the aggregability of RBC. In fact, the shape and elasticity of the cell provide the required geometry and freedom to adapt to the adjacent cell and increase aggregation probability. A

small variation in shape, caused by a change in osmotic pressure [35], or an increase in the cell rigidity [36], could decrease RBC aggregation. Temperature is also an important factor that influences RBC aggregation. Viscosity is shown to decrease with temperature but the effect of temperature increases with decreasing shear rate [37].

EFFECT OF AGGREGATION ON BLOOD VISCOSITY These different RBC aggregation behaviours greatly affect the behaviour of whole blood. One effect that can be visualized and clearly noted is blood sedimentation. Due to the difference in density between cells and plasma, RBC will settle in the suspended medium. However the rate of sedimentation depends on the collision rate of RBC and therefore rouleaux formation. In fact, sedimentation rate has been used as a tool to estimate aggregation properties but is also an indication of the nature of the sample (healthy or pathogenic), as will be detailed in the next paragraph. Another important consequence of RBC aggregation is the variable viscosity and hence the non-Newtonian behaviour of blood. As mentioned previously, the shear rate and hematocrit, having opposite effect on RBC aggregation, have been shown to greatly affect the viscosity of blood [34].

MEASUREMENT OF RBC AGGREGATION RBC aggregation is found to be a major factor of the unique rheological properties of blood. Therefore, it is important to quantify these aggregates in order to understand their behaviour under physiological and most importantly under pathological conditions. Rouleaux formation has been shown to be altered by several factors such as inflammatory or infectious conditions, cardiovascular diseases, genetic disorders and chronic diseases [33]. The major factors considered in several studies in order to qualitatively and quantitatively describe RBC aggregation are the rate of rouleaux formation, the number of RBC per aggregates and the force required to hold the entity together. The different methods presented in this section focused on determining several aspects of RBC aggregation.

ERYTHROCYTE SEDIMENTATION RATE The erythrocyte sedimentation rate (ESR) method consists of evaluating blood phase separation. This method

was first introduced by Biernacki in 1897 who noted that the phase separation rate increased under pathological conditions [38]. The method was further developed by Fåhræus [22] and is currently used as an index of inflammation and a screening method. Sedimentation of RBC in plasma occurs due to the difference in density of plasma and cells under the influence of gravity [39]. The mechanism behind this method is based on Stokes' law where the rate of sedimentation of a particle is proportional to the surface area and thus the square of the particle's radius. If the particles are dispersed in plasma, the ESR would be very low, however an increase in particle diameter (RBC aggregation) increases the sedimentation rate. The techniques used clinically include the Witrobe and Westergren methods. In general, these methods consist of measuring the plasma in the tube after a settling period of 60 minutes. The difference between the two methods mentioned is the tube used for the experiment as well as the coagulant used for blood. Experimentation performed on healthy individuals between the ages of 17 to 50 years provided an ESR of $10 \frac{mm}{hr}$ for men and $12 \frac{mm}{hr}$ for women [40]. Potron *et al.* explored the link between the ESR and RBC aggregation [41] and determined an existing relationship by way of correlations between these two factors [42]. However, a direct link between these two factors could not be determined.

LOW SHEAR VISCOSITY The low shear viscosity method is based on the rheology of blood at very low shear rates, below $1 s^{-1}$ [40]. It uses the technique of viscometry with a viscometer capable of measuring viscosity of blood at low shear rates (rotational viscometer and scanning capillary viscometer [43]). Using this method, the aggregation index, AI , is defined using Eq. (1.1):

$$AI = \frac{\mu_L - \mu_H}{\mu_H} \quad (1.1)$$

where μ_L represents the viscosity at low shear rates and μ_H the viscosity at high shear rates [33]. The low shear viscometry method also provides an index of aggregation and an indirect link between the aggregate size and the shear rate applied. However, this approach may produce altered results of aggregation indices due to RBC deformation since erythrocyte deformability is related to their ability to aggregate.

MICROSCOPIC AGGREGATION INDEX The microscopic aggregation index method consists of directly visualizing blood under the microscope to deduce the degree of RBC aggregation, more specifically, to determine the number of RBC per aggregate. The methodology is as follows: first dilute RBC suspensions of about 1% hematocrit in aggregating and non-aggregating media are placed into hemocytometer chambers and left to settle for 10-15 minutes. The entities in both the aggregating ($Entities_{count}$) and non-aggregating (RBC_{count}) media, including single RBC and rouleaux, are visually counted within the microscopic area [27]. The microscopic aggregation index (MAI) is defined using Eq. 1.2:

$$MAI = \frac{RBC_{count}}{Entities_{count}}. \quad (1.2)$$

The MAI increases with the degree of RBC aggregation. This method presents a good indication of the RBC aggregation, however, it does not provide information of the aggregation rate. Another main disadvantage is the non-automated and manual counting of RBC (visually), which can be time consuming [33].

IMAGE ANALYSIS TECHNIQUE In order to improve the microscopic aggregation index method, image analysis techniques are used in order to estimate RBC and entities numbers in each medium in an automated manner. Using these techniques, it is possible to quantitatively study RBC aggregation at stasis (at rest) and under several shear stresses in order to better mimic aggregation behaviour *in vivo*. Since RBC can be clearly visualized and captured with the digitized system, aggregate shapes can also be investigated along with aggregate sizes. According to Foresto *et al.* an aggregation shape parameter (ASP) can be defined using Eq. 1.3 [44]:

$$ASP = 4\pi \frac{A_{projected}}{Peri^2} \quad (1.3)$$

where $A_{projected}$ represents the projected area of one aggregate and $Peri$ the perimeter of one aggregate. For a perfect sphere aggregate, which is rarely seen, the ASP is at its maximum and will decrease for cylindrically shaped aggregate structures. Rotstein *et al.* used a different method based on digitized image analysis technique in order to

estimate the aggregate size of blood at stasis [45]. They affirm that the space between adjacent RBC aggregates, known as the vacuum radius, is a direct indicator of RBC aggregate size. Chen *et al.* used a computerized image analysis technique in order to provide RBC aggregate size distribution for different shear stresses by varying the flow rate of the suspensions in the flow chambers while monitoring the pressure drop across these chambers [46]. From this study, the average aggregation size, the peak aggregation size, the aggregation kinetics and the disaggregation force were related and described in terms of the shear stress applied.

Development of imaging analysis techniques provide a useful tool to quantify RBC aggregates. In their study, Kavitha and Ramakrishnan applied a two-dimensional wavelets transform to study RBC aggregates. They defined the Aggregation Size Index (ASI) to be the standard deviation of the coefficients generated by the wavelet functions, since it was shown to be directly proportional to the aggregation size [47].

SYLLECTOMETRY METHOD The syllectometry method consists of two similar approaches to quantify RBC aggregates: light transmission [48] and light reflection [49] methods. The light transmission method (LT) is based on the intensification of light when transmitted through RBC aggregates, while the light reflection method (LR) is based on the diminishing of light intensity when reflected from RBC aggregates. The higher the degree of aggregation, the larger the spaces forming between the rouleaux in the medium. Consequently, the higher the degree of RBC aggregation, the less light is allowed to pass within the gaps separating aggregates and thus the more light is being reflected. Using the syllectometry method, two major aggregation aspects can be investigated: information on aggregation size, shape and the start time of the mechanism can be obtained based on light intensity as well as the time required for this aggregation process, a major factor in diseases diagnosis. In fact, the difference between light intensities, known as amplitude (*amp*), determined at stasis and low shear rates, provides information on the aggregation morphology and size but not on the rate of the aggregation process. However, parameters such as the aggregation index provide information on the aggregation rate independently from

the aggregation morphology. For the LT method, AI can be expressed as follows:

$$AI = \frac{Area_B}{Area_A + Area_B} \times 100 \quad (1.4)$$

whereas, for LR method AI is written as follows:

$$AI = \frac{Area_A}{Area_A + Area_B} \times 100 \quad (1.5)$$

where $Area_A$ represents the area for light reflectance and $Area_B$ represents the area for light transmittance. Another major index of RBC aggregation that can be extracted from this method is the disaggregation maximum shear rate value that represents the value of shear rate at the maximum LT or minimum LR [50].

ULTRASOUND BACKSCATTERING The ultrasound backscattering is based on Rayleigh scattering theory where scattering is dependent on the ultrasound signal [51]. Cloutier states that RBC or RBC aggregates flowing in plasma will act as ultrasound scattering entities where the backscattering is proportional to the volume of the cell or aggregate [52]. Therefore, the backscattering coefficient increases with increasing RBC aggregability and thus decreases for high shear rates.

Several other methods exist such as the zeta sedimentation rate method, which is based on the zeta potential of the RBC (due to the negative charges on the RBC membrane), and provides an index of one of the aggregation aspects. These studies and methods presented above provide relevant information (several indices) on the aspects of aggregation but no standard model exists to quantify these rouleaux.

1.2.1.3 NON-NEWTONIAN BEHAVIOUR

The density of blood is about $1060 \frac{kg}{m^3}$ and its viscosity, at high shear rate (larger than $10 s^{-1}$), is approximately $3 cP$. However the viscosity of blood is not constant in time or space, but greatly depends on several factors, which leads to the non-Newtonian behaviour of blood. Blood viscosity, *in vivo*, depends on the shear rate, the plasma viscosity, the hematocrit, as well as the geometry and diameter of the

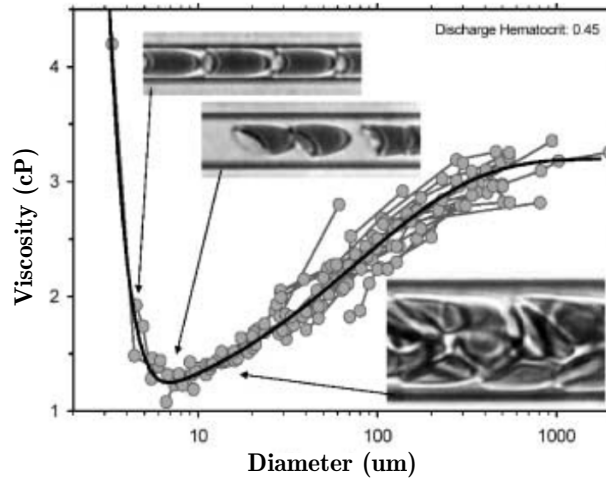


Figure 1.3: Blood viscosity in a function of the vessel diameter [54]

vessels [5, 53]. It is known that for large vessels, such as arteries and veins with diameters larger than $300 \mu\text{m}$, the apparent viscosity of blood is almost constant at approximately $\mu_{app} = 3 \text{ cP}$ [54]. Therefore, at this macroscale blood can be considered as a Newtonian fluid and as a homogeneous mixture. With decreasing vessel diameter, at the venules and arterioles level ($10\text{--}300 \mu\text{m}$), the apparent viscosity decreases to reach the minimum value of approximately 1.3 cP [5]. At the capillaries level ($4\text{--}10 \mu\text{m}$), the apparent viscosity of blood increases linearly. The smallest capillary diameter in which erythrocytes can flow with a maximum deformation is approximately $2.7 \mu\text{m}$. This phenomenon is due to the cell free layer (CFL) near the vessel wall caused by the migration of the cells towards the center of the vessel [1, 9, 10, 54–56]. This phenomenon is emphasized in microcirculation since the single file flow of the RBC in the capillaries allows a larger CFL [9]. At this scale, blood is considered to be a two phase flow that is composed of a fluid and solid phase [11].

Aggregation, which considerably affects the viscosity of whole blood, is the major factor for the non-Newtonian behaviour of blood. Therefore, factors affecting rouleaux formation affect blood viscosity. Factors such as the composition of the suspension medium, the elasticity of RBC membranes, hematocrit of blood and the shear rate applied greatly change the viscosity of blood [34] as detailed in Sec. 1.2.1.2. However, blood viscosity, *in vivo*, not only depends on these factors listed above but is also

affected by the geometry and diameter of the vessels [53]. This phenomenon is known as the Fåhræus-Lindqvist effect which is explained above. Another phenomenon observed in microcirculation is the decrease of hematocrit as the vessel diameter decreases for the same range of vessel diameter (10 to 300 μm) known as the Fåhræus effect [5], which influences the Fåhræus-Lindqvist effect [57, 58]. At this microscale, blood is considered to be a two phase flow that is composed of a fluid and solid phase [11] which behaves as a Newtonian fluid.

Several non-Newtonian models have been developed for blood flow and blood aggregates.

POWER LAW One of the most known and used non-Newtonian models for blood is the power law or Ostwald-de Waele power law. Based on this model the shear stress τ can be calculated as:

$$\tau = a \left(\frac{du}{dy} \right)^b \quad (1.6)$$

where a represents the fluid consistency index, b the non-Newtonian behaviour index, u the streamwise velocity of the fluid and y the coordinate normal to the vessel wall. When a increases, the viscosity of the fluid increases. However, when $b = 1$, the equation describes the behaviour of a Newtonian fluid having a constant viscosity and where the shear stress-shear rate relationship is linear. If $b < 1$, the resultant is a shear-thinning or pseudoplastic fluid where the viscosity decreases with increasing the shear rate. If $b > 1$, the resultant is a shear-thickening or dilatant fluid where the viscosity increases with increasing the shear rate. However, as can be clearly noted, this model is not capable of predicting the behaviour and viscosity of fluids at very high or very low shear rates due to the limitation of the power law [59–61]. At low or high shear rate, the viscosity, estimated with the power law, tends to infinity rather than reaching a constant value as observed experimentally [54]. However, this model is still widely used for its simplicity.

BINGHAM PLASTIC MODEL The non-Newtonian Bingham plastic model describes the behaviour of a plastic or viscoplastic material that exhibits yield stress

[59, 62]. A Bingham plastic is a viscoplastic material that behaves as a rigid body at low stresses but flows as a viscous fluid at high stress [59]. Based on this model the shear stress τ can be calculated as:

$$\tau = c \left(\frac{du}{dy} \right) + \tau_y \quad \text{if } \tau \geq \tau_y \quad (1.7)$$

$$\frac{du}{dy} = 0 \quad \text{if } \tau \leq \tau_y \quad (1.8)$$

where c represents the plastic viscosity and τ_y represents the yield stress of the material. The advantage of this model is the consideration of the yield stress in the equation, however it presents a major disadvantage in that the viscosity of the fluid is independent of the shear rate which cannot describe the behaviour of a shear thinning fluid (e.g. the behaviour of blood).

CASSON MODEL This non-Newtonian model, first introduced by Casson in 1959, is based on the behaviour of the liquid-solid interaction in a two phase flow [63]. The shear stress is expressed as:

$$\sqrt{\tau} = \sqrt{\tau_y} + \sqrt{k} \sqrt{\frac{du}{dy}} \quad \text{if } \tau \geq \tau_y \quad (1.9)$$

$$\frac{du}{dy} = 0 \quad \text{if } \tau \leq \tau_y \quad (1.10)$$

where k represents the Casson model constant. The advantage of this model is its ability to describe a non-Newtonian fluid exhibiting shear-thinning and yield stress simultaneously [64]. Therefore it can better describe the behaviour of blood. An illustration of the shear stress in terms of the shear rate for all the models described is given in Fig. 1.4. Several other models mimicking blood behaviour exist such as the Carreau model [64], which provides a constant shear at high shear rate (as opposed to the power law), the Walburn-Schneck model [65] that considers hematocrit, and the Hershel-Bulkley model which is a combination of the Bingham model and the power law [66]. Although it has been shown through experimental studies that these models can predict the behaviour of blood to some level of accuracy, none of these consider the RBC aggregates which greatly influence the micro-rheological behaviour of blood.

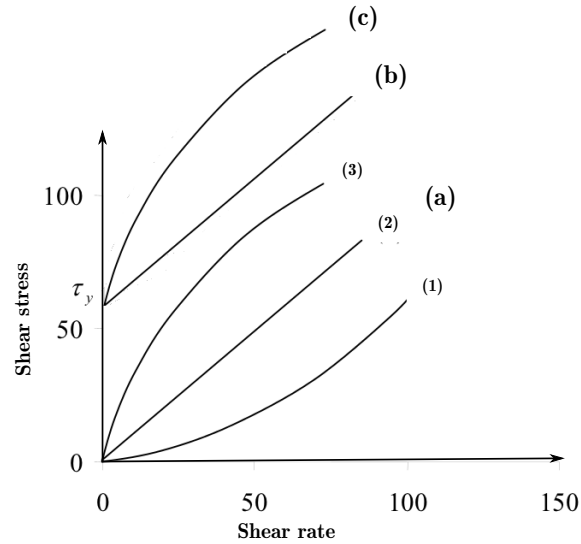


Figure 1.4: Illustration of shear stress in terms of shear rate for (a) The power law when (1) $b < 1$, (2) $b = 1$ and (3) $b > 1$, (b) the Bingham plastic model and (c) the Casson model [60]

Bureau *et al.* presented a dynamical study of blood to investigate the rheological behaviour of blood in transient flow regimes at low shear rates [67]. Several experiments were performed to determine the stress variation in time when the system is subjected to an "échelon" of rectangular and triangular shear rates (hysteresis cycles). They discovered that, dynamically, blood exhibits a viscoelastic, shear-thinning and thixotropic (shear-thinning without viscoelasticity; the fluid requires an infinite time to have a constant viscosity when subjected to a step shear rate) behaviour simultaneously as shown as (4) in Fig. 1.5.

Owens developed a new constitutive equation describing whole blood behaviour, based on the linear spring law and the multi-mode generalized Maxwell equation considering RBC aggregation (as a parameter) in the model. The model was compared to the Bureau *et al.* study [67] and showed similar results when subjected to an "échelon" of triangular shear rate.

The existing models described in this section, apart from those of Bureau *et al.* and Owens, do not take the dynamic factor into consideration in that blood samples are studied over a period of time and thus, the instantaneous effect of aggregation on the viscosity of blood is not accounted for. Moreover, all of these models only

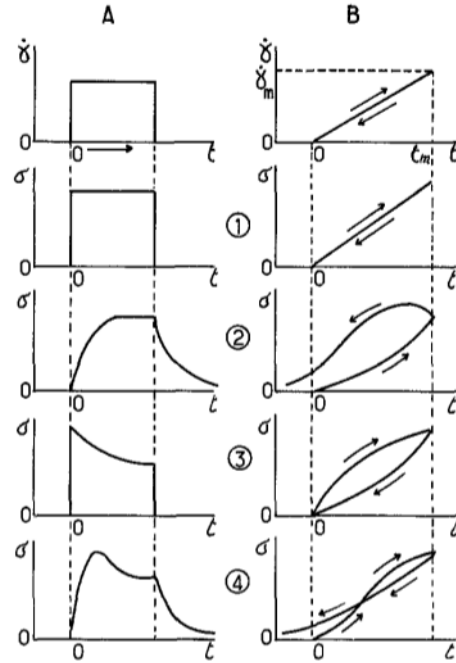


Figure 1.5: Illustration of shear stress time dependency for rectangular shear rate (A) and triangular shear rate (B) for a Newtonian fluid (1), a viscoelastic fluid (2), a thixotropic fluid (3) and viscoelastic-thixotropic fluid (4) [67]

consider the macroscopic behaviour of blood. To our knowledge, there is no model that provides enough information on the non-Newtonian behaviour of blood at the microscale.

1.2.2 NUMERICAL SIMULATIONS

Numerical simulations present a useful tool to better investigate and understand RBC behaviour in microcirculation. Several methods of Computational Fluid Dynamics (CFD) and numerical models were developed to especially mimic RBC aggregation observed in *in vitro* and *in vivo* experimental studies.

Numerous studies have investigated the motion of RBC at the capillary level [68]. However, early models did not take into consideration the deformability and elasticity of the cell membrane that is a major factor of RBC aggregation. The major considerations when modelling the behaviour of one or multiple RBC flowing in the vessel are the fluid-solid interaction between the RBC and the suspending medium and

more importantly the cell-cell interaction caused by the depletion of the molecules due to the osmotic gradient as explained in the depletion mediated aggregation theory. This factor combined with the deformability and elasticity of the cell membrane presents a significant challenge in the field of computational hemodynamics. Several methods have been developed to address this challenge.

Pozrikidis implemented a boundary-element method to simulate, in two dimensions, behaviour of a single RBC in simple shear flow, considering the RBC membrane stiffness [69]. This method is based on the boundary-integral formulation which provides an integral equation describing the localized velocity at a determined point as well as another integral equation describing the velocity on the cell membrane. The contour velocity integral equation is then solved engendering the membrane contour to travel in space by time integration [70]. For his study, Pozrikidis investigated the effect of the viscosity ratio between the cell inner fluid and the medium, the effect of the initial inclination of the cell in the tube as well as the effect of the membrane bending stiffness. His observations seemed to agree with experimental studies.

The Immersed Boundary Method (IBM) is the most extensively used method to numerically describe the behaviour of RBC in microcirculation. It was first introduced and used by Peskin in 1977 [71] to study the flow in the cardiovascular system accounting for the fluid-solid interaction (blood-vessel). For this method, the medium (plasma) and the solid structure (RBC) are treated as two separate entities interacting. The fluid domain is represented by uniform Eulerian grids, whereas the solid submerged structure is represented by a Lagrangian fibre network. The interaction between the fluid and the structure is accomplished by interpolating the velocities between the Eulerian and Lagrangian grids with a smoothed approximation of the Dirac delta function [72]. However, the IBM uses finite differences to solve for the structural domain. Eggleton and Popel combined the IBM with the finite element method allowing the submerged structure to be solved three-dimensionally and more accurately when subjected to very large deformation [73]. Bagchi extended the IBM to combine it with the finite element to model and simulate a group of non-interacting RBC flowing in vessels of 20 to 300 μm diameter. The front tracking method is used

to allow for a deformable interface [74]. The RBC are not considered as rigid structures but modelled as liquid capsules (viscous liquid drops enveloped by a thin elastic membrane). The particularity of this method is its ability to simulate a large number of deformable RBC flowing in a vessel [75]. However, these particles do not interact so aggregation cannot be studied with this method.

The lattice Boltzmann method (LBM) was first used by Sun and Munn to simulate two dimensional RBC, where the deformation of the cell was not considered [76]. In 2008, Sun and Munn extended their model to "examine the effect of one cell on macroscopic blood rheology" accounting for cell-cell and cell-wall interaction to study the RBC-WBC forces. The LBM is a particular computational fluid dynamics simulation method. Rather than solving the Navier-Stokes (N-S) equations, the discrete Boltzmann equation simulates a fluid with particle collision models such as the Bhatnagar-Gross-Krook (BGK) model [77].

The above overview provides pertinent information on RBC behaviour in micro-circulation specifically. Several aspects, shown experimentally, have been validated numerically using these studies such as the Fåhræus-Lindqvist and the Fåhræus effect. However, these studies do not include RBC aggregation. In fact, the consideration of aggregation is a major challenge in the computational field due to the complexity and the lack of a precise definition of the existing mechanism (only theories). The numerical studies, in fact, aim to better understand the exact mechanism of RBC interaction and to better quantify the rouleaux. Bagchi *et al.* adapted the IBM approach to incorporate cell-cell interaction, based on the Ligand-receptor binding model [11], in 2D, and to study a two-cell system behaviour in shear flow including the elasticity of the cell membrane. They focused on estimating the rate of bond breakage between the two cells when subjected to several ranges of shear rates and with different membrane elasticity.

Liu and Liu used the Immersed Finite Element Method (IFEM) to provide a three-dimensional model of RBC aggregation, study its behaviour under several conditions (orientation and shear rate) and investigate its effect on blood rheology. For this purpose, the IFEM, which consists of a modified IBM, is coupled with a cell-cell in-

teraction model and the Mooney-Rivlin strain energy function (for RBC deformation) [9]. This IFEM is, indeed, a combination of the IBM, the finite element method and the mesh-free Reproducing Kernel Particle Method (RKPM). The advantage of this specific mesh free method is its independence from the material to avoid any mesh distortion but also its ability to handle very large deformation since the connectivity among nodes is generated as part of the computation and can change with time [72]. Since the fluid-structure interaction is based on a smoothed approximation of the Dirac delta function, the higher order smoothness of the equation leads to a higher accuracy for the interaction. From their study, Liu and Liu were able to validate the dependency of blood mechanical properties on the cell-cell interaction force, the flow shear rate, the cell deformability and the vessel geometry. They also investigated the adhesion energy and the hematocrit shear rate dependence.

Zhang *et al.* combined the IBM (for the fluid-structure interaction) with the LBM (for the complex boundary conditions) using the Morse potential (for the cell-cell interaction) [10]. RBC are modelled as deformable fluid capsules considering the RBC membrane elasticity (neo-Hookean viscoelastic material) and the cytoplasm viscosity. The advantage of this study is that it considers most of the major aspects influencing RBC aggregation for a more accurate study.

Fenech *et al.* proposed a two-dimensional particle model to investigate RBC aggregation and explore its effect on the rheological blood behaviour. The particularity of this model is that the molecular mass and the concentration of neutral polymers affecting aggregates can be mimicked [12] so that a large group of RBC interacting can be investigated. This study couples a particle model of RBC with an aggregation and elasticity model. The authors based their study on the depletion mediated theory where the Morse potential model is used as the cell-cell interaction method. Several factors were investigated: among them, the effect of the aggregation force intensity as well as the aggregation kinetics and the shear rate dependence. These parameters are explored by estimating the Mean Aggregate Size (MAS) according to the aggregation rate. The observations from this study seem to agree with experimental *in vivo* studies performed under similar conditions.

Despite the development of these new approaches, the methods have only been applied to a small group of RBC aggregating, which does not reflect the real *in vivo* behaviour of blood. Further investigations are required in this field in order to develop a numerical model capable of predicting RBC aggregation with realistic hematocrit and RBC deformability.

1.2.3 EXPERIMENTS *IN VITRO*

As discussed previously, due to the complexity of blood flow in microcirculation, several *in vitro* studies have focused on estimating RBC suspension and RBC velocities in microchannels to better understand and estimate blood behaviour in arterioles, venules and capillaries. Several methods have been used for velocity measurement including image analysis of high speed motion pictures [78], double slit photometry [79, 80], laser Doppler anemometry [81, 82] and particle imaging techniques. These methods are detailed in Sec. 1.2.3.1 [83–87].

The microchannels used for blood velocity measurements require specific properties to mimic the properties of vessels in the human body. Glass microchannels have been widely used for their optic properties (transparent material) and their well known fabrication procedure. However, due to rigidity and fabrication limitations, numerous recent studies have started to investigate the use of polymer based microchannels such as poly-ethylenetere-phthalate glycerol (PETG), in poly-di-methyl-siloxane (PDMS) and poly-methyl-methacrylate (PMMA) [16, 88]. The properties and benefits of these microchannel materials are discussed in Sec. 1.2.3.2.

1.2.3.1 VELOCITY MEASUREMENT METHODS

IMAGE ANALYSIS OF HIGH SPEED MOTION PICTURES In 1962, Bugliarello *et al.* used an imaging technique to estimate the velocity of RBC flow in a tube [78]. This technique consists of controlling the motion of individual cells

between each frame by evaluating the distance travelled by the cell and dividing it by the time between these frames. The velocity profiles obtained in tubes of 40, 70 and 83 μm diameters with hematocrit up to 40% were found to be blunt but with a parabolic trend [80]. The simplicity of the method leads to several limitations.

The main disadvantage of this imaging technique is its time consuming frame correlation, since every frame requires analysis. Numerous others limitations including the hematocrit (difficulty of detecting single cells), tube diameter (larger diameters increase the number of RBC seen under the microscope which makes a single cell detection difficult) and the time between the frames (the smaller the rate of frames recorded, the more difficult it is to visualize a single cell due to RBC aggregation) leads to difficulties in velocity measurement. Because of these disadvantages, this technique is limited in accuracy but is simple to implement for estimating velocity of blood with RBC aggregation.

DOUBLE SLIT PHOTOMETRY The double slit photometry method was introduced in 1967 by Wayland and Johnson [89]. It is based on the passage of RBC in single file flow within two successive slits connected to two photomultipliers causing successive signal outputs (modulations) of the two photomultipliers. The time delay between the two modulations provides an estimation of RBC velocity. Although the method is efficient, it cannot be applied to larger vessels where RBC do not flow in a single file flow due to the inconsistency of this flow. This method is used for *in vivo* experiments. In 1970, Gaehtgens *et al.* extended this method to estimate the velocity of blood in vessels ranging from 25 μm to 130 μm in diameter. Although a velocity profile was obtained, several limitations of this method were revealed when applied to vessels larger than capillaries [79]. Due to the large amount of RBC in the channel, the difference in contrast between the cells and the suspension media was not sharp enough to provide a clear output of the photomultiplier, since the modulation of the system is based on the intracellular space between the cells. Also, the rotation and migration of RBC in the larger channel diameters result in two different signal

outputs preventing an exact estimation of the velocity profile in the tube [80]. Due to these limitations, RBC aggregation cannot be studied accurately with the double slit photometry method.

MICRO PARTICLE IMAGE VELOCIMETRY (μ PIV) The most widely used method nowadays for blood and RBC velocity measurement is the μ PIV method. This technique is a particle based flow visualization that is able to determine 2D velocity fields using the cross-correlation method. As discussed in Chapter 2, several distinctions can be found between the extensively used PIV method and its application to microfluidics system.

Micron-resolution Particle Image Velocimetry (μ PIV) was first introduced by Santiago *et al.* in 1998 [84] to study pressure driven micro flows. Since then, it was used by several researchers to determine the velocity of Newtonian flows, among them Kim *et al.* [85], where the mixing of two fluids with different refraction indices within a Y-channel configuration was studied. For their study Newtonian glycerol solutions with different mass fractions were used. The results of this study will be compared to our Newtonian model. Investigation of velocity profiles of RBC suspensions and RBC *in vitro* have been widely explored using the μ PIV method. Since this method is a particle based technique, tracer particles (emitting at a prescribed wavelength) are required. These tracer particles can either be RBC (dyed or not) flowing in the tube or fluorescent particles seeded in the suspension media. One of the first applications of μ PIV to hemorheology was performed by Sugii *et al.*, in 2001. They were able to estimate the velocity of blood flow within a 100 μm diameter round glass tube. From this study, the velocities near the tube wall were found to be smaller than the theoretical velocities based on Poiseuille flow due to particle interaction with the walls.

Bitsch *et al.* used μ PIV to measure the velocity of "blood plug flow in a flat micro capillary" [90], in order to determine the effect of the focal depth on the velocity. RBC flowing in an aqueous solution are used as tracer particles. In their study, the velocity profile obtained from RBC suspension is compared to suspension of beads

in water [91, 92]. In order to control the focal depth, the "base clipping" technique, discussed in Chapter 6, is implemented.

However due to the complexity of blood flow in microcirculation, the RBC velocity does not directly reflect the bulk velocity of blood. For this purpose, Sugii *et al.* separately studied the velocity of RBC flowing in physiological saline and the velocity of the suspension media (also physiological saline for this test) flowing in a round glass tube of 100 μm diameter. RBC, labelled with DiC_{16} (fluorescent dye), were used as tracer particles for the RBC velocity measurement while fluorescent particles were used as tracer particles for saline velocity measurement. The authors found the results of both experiments to be in agreement and to provide very similar velocity profiles, although the profile obtained from the RBC velocity measurement presents some fluctuations [93].

Lima *et al.*, in 2006, used the μPIV system to estimate the velocity of RBC suspended in physiological saline within a 100 μm glass square channel using fluorescent as tracer particles. The results were compared to tests with pure water flowing in the same channel. By combining conventional PIV with a spinning confocal microscope, they were able to obtain a fully detailed three dimensional velocity profile in a Newtonian and a non-Newtonian fluid in the square channel [94]. Using their previous findings, Lima *et al.* were able to study the effect of hematocrit on the 3D velocity profile of RBC suspended in physiological saline in a 100 μm glass channel using the confocal μPIV technique. They found that hematocrit has an important effect on the shape of the velocity profile but also that the root mean square error values increases with hematocrit (up to 17%) due to RBC motion and collision [83].

Several other techniques are used to improve the PIV measurements such as imaging techniques [91, 95], digital holography [96], stereo imaging [97] and other particle based techniques such as particle tracking velocimetry. With the development of these different methods, to our knowledge, μPIV has not been employed to measure the velocity of blood suspended in plasma and therefore aggregation effects have not been considered.

1.2.3.2 MICROCHANNEL MATERIAL

The foregoing studies (discussed in Sec. 1.2.3.1) use glass microchannels for *in vitro* blood velocity measurement. However, for *in vivo* comparisons, glass does not represent an ideal material to mimic vessel behaviour due primarily to its fabrication limitations [16]. In fact, glass microchannel fabrication is limited in depth and size because of the limited durability of the metal mask against the etchant (hydrofluoric acid), which limits the application of the microfluidic system. Also, the glass to glass bonding is difficult to accomplish due to the surface characteristics while the indirect bonding of the surface may block the channels with adhesive. But most importantly, glass does not show the same physiological properties as blood vessels such as elasticity and the presence of living tissue [17]. Due to all these limitations, researchers have started investigating the possibility of using polymer based materials such as PMMA and PDMS. The most extensively used material for biomedical applications *in vitro* such as blood velocity measurements is PDMS. It is an optically transparent, non-toxic silicon based polymer. This material presents several advantages for biological applications: the material fabrication is inexpensive (mixing of silicon based elastomer and curing agent) and the material bonding to any surface can be achieved easily since PDMS consists of polymer chains [18]. Another advantage is its permeability to cells but not to gases. The main advantage of PDMS is its flexibility and deformation based on the pressure drop in the channel, thus mimicking the elasticity of blood vessels [19].

Lima *et al.* used a 300 μm wide and 45 μm deep rectangular PDMS microchannel to estimate the 3D velocity profile of RBC suspension in physiological saline (at 20% hematocrit) and compare this velocity profile to the one obtained for pure physiological saline using the confocal μPIV system. Both samples are seeded with fluorescent particles acting as tracer particles ($\sim 0.9 \mu m$ diameter). A blunt velocity profile was obtained experimentally and was well aligned with the theoretical profile for the same rectangular channel dimensions [17]. Because of its unique properties PDMS is widely incorporated in microfluidics devices not only for blood flow measurement but

also for analytes (the presence of a substance) detection using immunoassays [98], for DNA and protein separation [99, 100] or sorting of cells [18, 101].

Although PDMS has been used in microfluidics devices, no reported studies have used this material to investigate velocity of blood flow with the presence of RBC aggregates.

1.2.4 EXPERIMENTS *IN VIVO*

Experimental studies of RBC aggregates *in vitro* detailed in Sec. 1.2.1.2 investigated the microscopic rheological properties of blood. However, the viscosity of blood, termed as the apparent viscosity, is dependent on several conditions and therefore behaves differently *in vivo*. For this purpose, numerous studies have analyzed blood flow in living blood vessels. The concept of *in vivo* studies was introduced by Whittaker and Winton, in 1933, who looked at the apparent viscosity in an isolated-perfused dog hind limb and compared their finding to their *in vitro* study of blood viscosity in a glass tube [102]. They reached the conclusion that a significant difference exists between the *in vitro* and *in vivo* results especially at high hematocrit, which encouraged researchers to investigate *in vivo* blood properties under several conditions [103–106].

Unfortunately, aggregation was not considered as an alteration factor of blood apparent viscosity in these studies. Cabel *et al.* presented a study relating the flow resistance, depending on RBC aggregation, to the flow rate [107]. Bishop *et al.* investigated the effect of RBC aggregates on the velocity of the flow for different perfusion pressures, by controlling the aggregates formation using dextran 500 [108, 109]. They demonstrated that, under slow flows [109], a blunted velocity profile is obtained which leads to a reduction of the perfusion pressure. Their findings agree with Cabel *et al.*'s observations thus providing confirmation of the dependence of the flow resistance on RBC aggregates. In [110], they provided a measure of velocity profile as well as a curve-fit based on the linear least squares regression to estimate the parabolic nature of the velocity for aggregating and non-aggregating blood. They have validated that, for non-aggregating blood, the velocity profiles are parabolic as

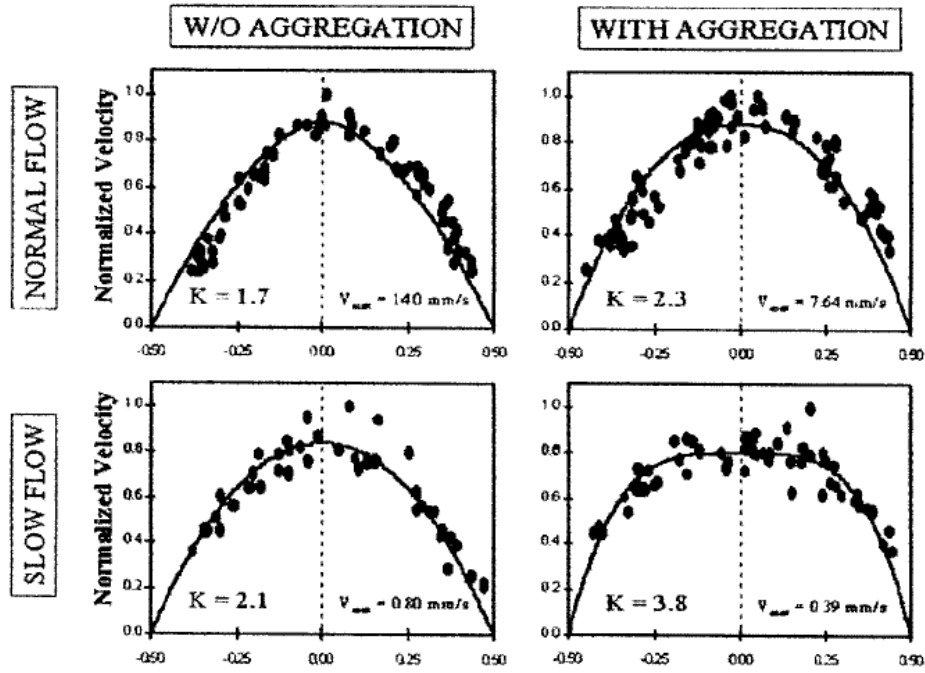


Figure 1.6: Velocity profile for aggregating and non-aggregating blood at normal and slow flow rates [4].

expected for Newtonian fluids. However, when RBC aggregate, the velocity profile is greatly affected especially for slow flows (blunt profile as seen in Fig. 1.6).

These studies used intravital microscopy, consisting of an inverted microscope with the appropriate illuminating system, a recording camera, image analysis system (computer), pressure monitors and suitable perfusion equipment [33], in order to visualize the living venules. The RBC are usually labelled to be fluorescent under the appropriate illumination. Combining a high speed camera to the intravital microscopy system, Bishop *et al.* were able to show that RBC aggregate sizes were inversely proportional to the shear rate applied [111].

These studies are of a great interest for understanding blood behaviour. However, even with the *in vivo* studies, no model has been developed to estimate the aggregation size according to flow condition (shear stress and shear rate applied), which demonstrates the importance of our current research.

1.2.5 SUMMARY

In Sec. 1.2, we have looked at the investigation of several studies dealing with blood rheology and aggregation. Since Fåhræus' observation of RBC aggregation in 1921 numerous studies have focused on understanding the mechanism behind these entities that could lead to a better understanding of blood behaviour under pathological conditions. Non-Newtonian models have been developed with the intention of mimicking blood behaviour. However, these models were only developed to study blood at the macroscale and therefore did not consider the effect of aggregation in the microcirculation. Other studies have focused on measuring these aggregates *in vitro* and *in vivo* in order to relate the size of these aggregates to cases seen under pathological conditions. However, these studies were only able to provide indices of aggregation which are not sufficient to describe the behaviour of RBC aggregation at the microscale. Numerical studies also attempted to model RBC behaviour and aggregation by considering RBC deformation of a single or a few RBC. However, due to numerical limitations, the methods developed cannot model blood behaviour with a realistic hematocrit (20% to 40% hematocrit). Therefore, a controlled model for RBC aggregation size estimation is required to determine the relationship between the aggregate size and flow conditions. The subsequent findings can be implemented in the previously mentioned models to improve the understanding of blood behaviour in microcirculation.

1.3 THESIS STATEMENT

The purpose of this project is to design a microchannel configuration in order to obtain a micro-Couette blood flow with the blood layer ranging from 30 to 100 μm . A Couette flow is a phenomenon created by the passage of a fluid between two parallel plates, one stationary and the second one moving at a constant velocity U as shown in Fig. 1.7. Since the shear rate $\dot{\gamma}$ is the gradient of velocity, $\dot{\gamma} = \frac{\partial u}{\partial y}$, Couette flow provides constant and controlled shear conditions, which represent the ideal environment to simultaneously visualize aggregates and determine the shear rate value. Here, we

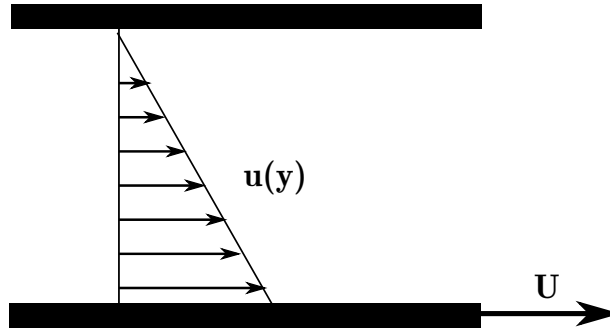


Figure 1.7: Illustration of the Couette flow with a moving and stationary wall

choose to create Couette flow by the motion of a second fluid that entrains blood. The advantage of using a second fluid as a moving wall is the ability to directly visualize the flow motion using an inverted microscope, but also to avoid any restriction of aggregates formation as a wall would impose. The results from this research could be used for further research to relate aggregate size to controlled flow conditions. A major goal of this study is to ensure the compatibility of the results between the experiments and simulations for several values of shear rates. For this purpose, the numerical simulation of the Newtonian flow in a Y-microchannel configuration is presented to be compared to the experimental model of the microchannel under the exact same flow conditions. Once the velocity profile is ensured to be linear, the same experimental model is used to test blood under several conditions.

The thesis outline is as follows: the present first chapter introduces the subject and explains the objective and relevance of the research. Chapter 2 describes the equipment and methods used to perform the numerical and experimental studies. We first describe the CFD solver used to perform the numerical simulation and then detail the numerical setup for our specific application. Then the equipment used experimentally is presented followed by the experimental setup in the laboratory. Chapter 3 includes a summary of the several tests performed with various setup and microchannel geometries to arrive at an ideal design of microchannel used to obtain a micro-Couette blood flow where the constant shear rate is within the range of RBC aggregation. In Chapter 4, an analytical solution is developed to be compared to simple test cases performed numerically to ensure the validity of the numerical velocity

profile and interface location between the two fluids. It is only then, in Chapter 5, that the ideal design of the microchannel is tested numerically and experimentally with a Newtonian fluid (glycerol) under the exact same flow conditions for comparison purposes. Once the model is verified with a Newtonian fluid, in Chapter 6, the experiments are performed on the ideal microchannel configuration using blood to study the aggregate formation and interpret the results found. Chapter 7 presents a concise but relevant discussion of the results found as compared to the results in the literature; the discussion is followed by a conclusion. Appendix A presents detailed information on the microchannel design process including the many tests performed numerically in order to obtain the proper geometry used experimentally.

1.4 OBJECTIVES AND RELEVANCE

The main objectives of this thesis are as follows:

- Create a microchannel geometry in order to obtain a micro-Couette blood flow.
- Entrain blood using a second fluid, with different properties, to create the Couette flow (two fluid flow system).
- Design a microchannel based on numerical simulations to ensure the linear velocity profile within the blood range.
- Study RBC aggregates under several flow conditions by varying the shear rate, hematocrit and the thickness of the blood layer.
- Provide preliminary quantitative analysis of RBC aggregates to verify the future possibility of relating them to the flow conditions.

This research is innovative in that a two fluid flow system is used instead of a moving wall, allowing for a controlled shear rate as well as an observable and quantifiable RBC aggregation testing apparatus. Under normal conditions, RBC aggregation, which increases blood viscosity, is rarely seen at the capillary level, but is rather an indication of pathological conditions such as diabetes, as detailed in [112],

and obesity which could lead to severe consequences. Other pathological conditions that could change RBC aggregation include inflammatory or infectious conditions, cardiovascular diseases such as hypertension or atherosclerosis, genetic disorders and chronic diseases [33]. Moreover, rouleaux can be altered not only under pathological conditions but also under physiological conditions. Factors affecting RBC aggregation include gender, age and exercise level. Therefore, it is important to quantify these rouleaux in order to understand the condition of their formation. To our knowledge, no standard method exists that is capable of predicting RBC aggregation behaviour or assessing these rouleaux. With an available controlled model providing a range of aggregate sizes according to the controlled shear rate, the flow conditions could be systematically related to the rouleaux size under pathological conditions and several pertinent physiological conditions.

Chapter 2

METHODOLOGY

The techniques used to perform this research consist of two distinct parts, the numerical simulations and the laboratory experiments. For both setups, velocity profiles were determined from which shear rate and flow rate were calculated.

2.1 NUMERICAL SIMULATIONS

2.1.1 CFD CODE OVERVIEW

The numerical simulations are conducted using the computational fluid dynamics research program *Nek5000* [113], based on the spectral element method. This is a direct simulation solver for laminar, transitional and turbulent incompressible low Reynolds number flows with heat transfer or transport phenomenon [113] solving for the unsteady incompressible two dimensional or three dimensional Navier-Stokes governing equations:

$$\nabla \cdot \mathbf{u} = 0 \quad (2.1)$$

$$\rho \left(\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) = -\nabla P + \nabla \cdot \mu \left(\nabla \mathbf{u} + (\nabla \mathbf{u})^T \right) + f \quad (2.2)$$

where the velocity, $\mathbf{u} = (u, v, w)$ and the pressure P are the solutions sought by the solver. $f = (f(x, t), f(y, t), f(z, t))$ represents a user defined forcing function. For constant viscosity μ , the second term of the viscous term is zero by the incompressibility condition, yielding what is known as the Laplacian formulation. However, for the majority of the simulations performed in this project, the stress formulation, consisting of considering the full stress tensor, is used. This formulation is more suitable for variable properties such as a two fluid flow. If the flow includes heat transfer, the energy equation is also solved with forced or natural convection heat transfer:

$$\rho C_p \left(\frac{\partial T}{\partial t} + \mathbf{u} \cdot \nabla T \right) = \nabla \cdot k \nabla T + q \quad (2.3)$$

where $q = (q(x, t), q(y, t), q(z, t))$ represents a user defined source term and the temperature T represents the temperature. In *Nek5000*, an option is provided to specify a passive scalar ϕ solved by:

$$\rho C_p \left(\frac{\partial \phi}{\partial t} + u \cdot \nabla \phi \right) = \nabla \cdot k \nabla \phi + q. \quad (2.4)$$

The Spectral Element Method (SEM), first introduced by Patera [114], combines the high accuracy of the Spectral Method and the geometric flexibility of the Finite Element Method [115]. For this method, the continuous domain is discretized into elements. Within each element the solution is approximated using tensor products of N th order Legendre polynomial Lagrangian interpolants. For the velocity space the allocation of points in each element is based on Gauss-Lobatto-Legendre (GLL) quadrature points, whereas for the pressure space, the allocation of points is based on Gauss-Legendre (GL) quadrature points as described in [116] as the $P_N - P_{N-2}$ formulation. For a 1D problem, the solution in each element is approximated as follows:

$$q^{(E)}(r) = \sum_{n=0}^N q_n^{(E)} L_n(r) \quad (2.5)$$

where $q^{(E)}(r)$ represents the variable to be approximated, r is the local spatial coordinate of the element E , L_n represents the n^{th} order polynomial whereas N represents the highest order of polynomials. Therefore the location of the GLL points in each element is based on Legendre polynomials and more specifically, N , the order of the highest order of polynomial. Fig. 2.1 represents an element with 6×6 points, where $N = 5$ as shown in Fig. 2.2. The incompressible Navier-Stokes equations are thus solved with this method allowing for a variable viscosity [116]. The advantage of using this numerical method for smooth solutions such as incompressible Navier-Stokes equations is the rapid convergence of the solution with the order of the Legendre polynomial N . The coarser the grid, the higher the order of the Legendre polynomial can be, however, higher polynomial order requires longer computational time. A balance between the number of elements and the order of the polynomial yields a reasonable efficient solution scheme.

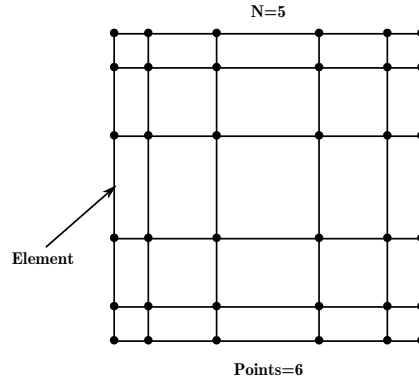


Figure 2.1: Example of a two dimensional element in the x-y plane with the highest order of polynomial $N = 5$ with 6×6 points

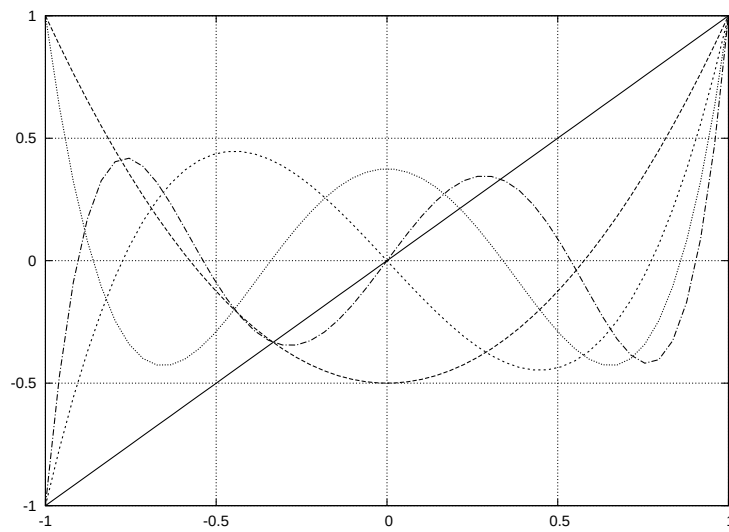


Figure 2.2: Illustration of the five Legendre polynomials used to approximate the solution

In order to solve for the velocity pressure and temperature variables, several files are required for the program compilation. The chart presented in Fig. 2.3 provides a clear overview of the tools required for our solver. First the mesh is generated with *prenek* in order to define the elements, their location and dimensions. A ".rea" file is generated containing first, the dimensional number information, second, several parameters such as viscosity and density, but also several logical switches such as heat transfer, stress formulation and variable properties. The ".rea" file also contains the element numbers and coordinates and the boundary conditions for the sides of each element. These boundary conditions for velocity and temperature are usually entered as a FORTRAN function in the ".usr" file. This file contains all the subroutines re-

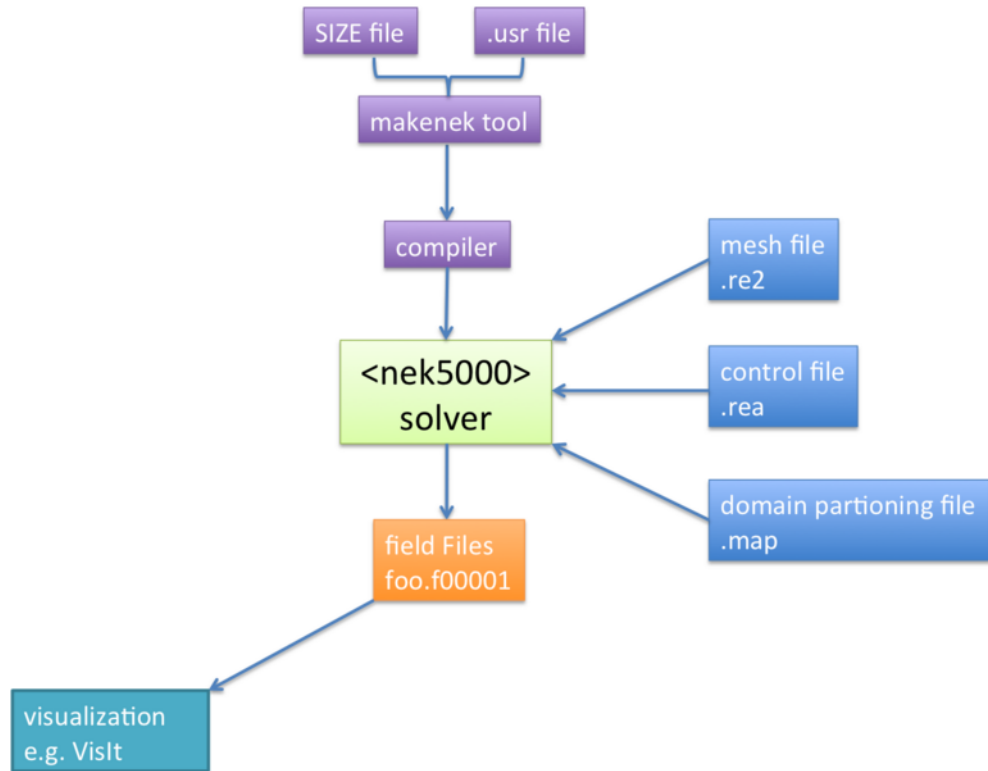


Figure 2.3: Nek5000 partitioning

quired such as the forcing function f , the heat source term q , the pressure gradient $\frac{dp}{dx}$, the definition for the variable properties such as viscosity, density, the heat capacity C_p and the thermal diffusivity α and the geometry reinitialization. The number of points in each element is prescribed in the "SIZE" file as well as the choice of $P_N - P_N$ or $P_N - P_{N-2}$ formulation, the number of processors used and the maximum number of elements to be solved for each processor. The last file required is the ".map" file which contains the domain partitioning according to the mesh definition in the ".rea" file. The system is then compiled in order solve for the variables solutions iteratively. The output of the simulation is saved in an ASCII (".fld") or a binary (".f0000*") file that is used to visualize the results using "visit", a visualization tool developed by the US Department of Energy (DOE) Advanced Simulation and Computing Initiative (ASCI) [117].

2.1.2 NUMERICAL SET UP

The purpose of the project, as mentioned in Chapter 1, is to design a microchannel model in order to obtain a micro-Couette blood flow. For this purpose, a second fluid is used to entrain the blood layer, where the linear profile is expected. Therefore, a numerical model is used to simulate the motion and behaviour of two fluids with different properties flowing in a Y-microchannel configuration.

For our project, several tests, described in Chapter 3, were performed in order to determine the ideal parameters for the microchannel in order to obtain a linear velocity profile in one of the micro scaled fluids layers. For the majority of the tests performed, the $P_N - P_{N-2}$ formulation was used to solve the variables in a semi-implicit temporal scheme (implicit for diffusion and explicit for convection). The stress formulation was also used to allow for variable viscosity. Variable viscosity is specified in the variable properties subroutine in the ".usr" file. However, if the viscosity is set as a function in the subroutine, the solution for viscosity will not change in time and the interface will remain constant between the two fluids. Therefore, the viscosity is treated here as a passive scalar in order to be solved separately and then incorporated, for the next time step, in the solution of the velocity and pressure. For a few tests, the free-surface hydrodynamic formulation is used. With this condition, the mesh is allowed to deform according to the flow. Therefore, not only the interface between the two fluids accommodates to the flow conditions but the mesh follows the same pattern. This formulation was implemented in *Nek5000* by Ho [118]. It is based on the Arbitrary Lagrangian-Eulerian (ALE) formulation where the geometric deformation is defined by an arbitrary mesh velocity that satisfy the kinematic condition described in [118]. In order to use this option, an internal fluid boundary condition needs to be prescribed where the interfacial tension between the two fluids is specified in the user ".usr" file as σ .

The final design of the microchannel model is presented in Fig. 2.4, in terms of the three dimensional computational mesh of the Y-channel configuration. Also, as mentioned in Chapter 3, the model counts 344 elements; 172 and 2 elements in the

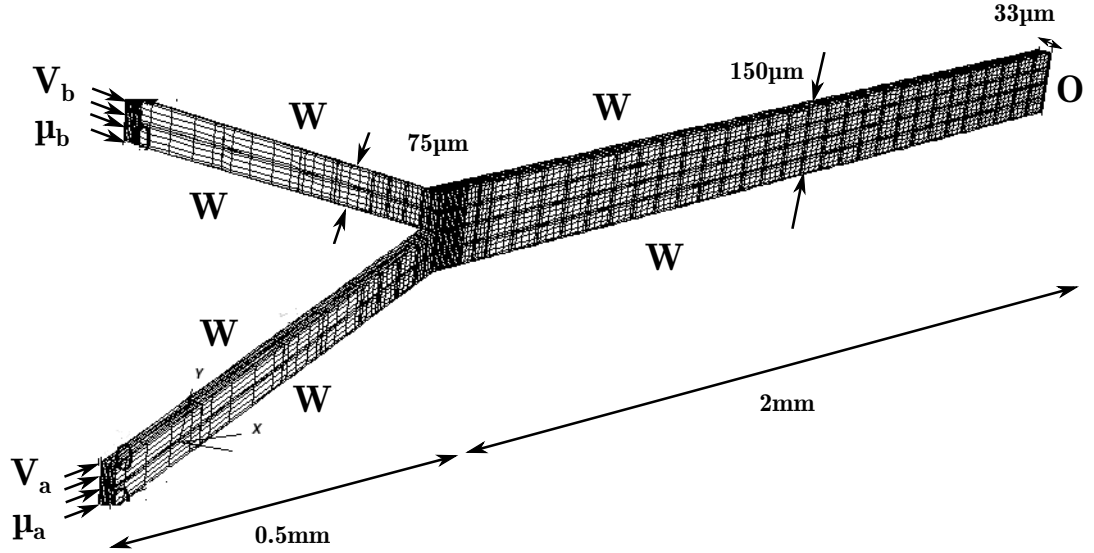


Figure 2.4: Boundary conditions and mesh distribution of the microchannel final design using the spectral element method

x-y and x-z planes respectively. However the distribution of the grid is not uniform. Elements are concentrated at the bifurcation due to the complexity of the solution at that location where the two fluids enter into contact. The order of the highest Legendre polynomial N was chosen to be $N = 5$ for the velocity and therefore $N = 3$ for the pressure in order to lower the cost of the simulation optimizing the accuracy of the solution.

The two fluids used for the simulations are assumed to be incompressible and the flow is expected to reach a steady and fully developed state. The boundary conditions used for this case are also shown in Fig. 2.4, where the twelve walls of the microchannel present a no-slip boundary condition. The outlet of the channel has a null pressure boundary condition, while a uniform velocity is prescribed as boundary condition at each of the entrances of the Y portion of the channel. The two fluids A and B with different viscosities and densities (described in Table 2.1, where T represents the type of the fluid for each case and ν the corresponding kinematic viscosity) entering from the bottom and top branches respectively, flow with different velocities. Fluid B represents the viscous fluid and is assumed to have the same viscosity as blood at high shear rates ($\nu_B = 3.10^{-6} Pa.s$). Fluid A represents the less viscous fluid and is

Table 2.1: Fluids A and B kinematic viscosities for the simulation, the Newtonian and non-Newtonian blood experiments. T represents the fluid type and ν represents the kinematic viscosity.

Fluid	Simulation		Newtonian		Blood	
	T	ν (<i>Pa.s</i>)	T	ν (<i>Pa.s</i>)	T	ν (<i>Pa.s</i>)
A	-	1.10^{-6}	Water	$\simeq 1.10^{-6}$	PBS	$\geq 1.10^{-6}$
B	-	3.10^{-6}	Glycerol	$\simeq 3.10^{-6}$	Blood	$\geq 3.10^{-6}$

assumed to have the same viscosity as water ($\nu_A = 1.10^{-6}$ *Pa.s*). Changing the ratio of velocities affects the location of the interface between the two fluids as well as the Reynolds number. The two fluids used for this project are in reality miscible, however at the micro scale, fluids are assumed to be immiscible due to the capillary effect in the microchannel and the low Reynolds number. On the other hand, at the interface between the two fluids, a thin diffusion layer will form and expand further along the channel. In order to simulate this phenomenon, the degree of diffusion between the fluids was set in the computational program. Three ratios of velocities, $V_{ratio} = 1, 2$ and 4 , using the same ratio of kinematic viscosity, $\nu_{ratio} = 3$, are tested for this research in order to determine the interface location and ensure the velocity profile linearity of fluid B. Therefore the flow will accommodate to the boundary conditions set at the entrances of the channel and then settle to an interface location based on the flow conditions. When the steady state is reached, the velocity profile is extracted from the channel at a specific location along the channel to then determine the shear rate according to the thickness of the fluid B layer.

2.2 LABORATORY EXPERIMENTATION

2.2.1 EQUIPMENT OVERVIEW

2.2.1.1 VELOCITY MEASUREMENTS: MICRO-PIV

The numerical simulations are compared to laboratory experiments in order to verify their validity and their capability of predicting blood flow. In order to perform the

laboratory experiments and determine the velocity within the microchannel, micro Particle Image Velocimetry (μ PIV) is used. This technique is a particle based flow visualization that is able to determine 2D velocity fields using the cross-correlation method. The PIV method is well documented and has been used extensively for macro fluid flow [119, 120].

The PIV system usually consists of a CCD camera that is able to capture sets of double frames at high speed separated by a dt set by the user. The dt chosen is usually on the order of hundreds to thousands of milliseconds depending on the volumetric flow rate to allow five to ten pixel displacement of one particle between the two frames. The advantage of using a CCD camera over a normal camera that is only able to capture single frames, is the accuracy of the results since the cross-correlation method is used. Otherwise, the auto-correlation method would be used for the set of single frames resulting in less accuracy. The pulsed or continuous laser serving to illuminate the region of interest is synchronized with the CCD camera so that the set of two frames captured displays the particles flowing with the medium. These particles are particular in the way that they illuminate when exposed to a certain wavelength depending on the size of the particles. The size of the particles for macro flow are, usually, on the order of 10 to 30 μm in diameter [121]. Therefore, the particles are seeded within the fluid to be analyzed and the motion of these particles will determine the velocity field. The set of double frames (usually, on the order of 100 frames) are post-processed using the appropriate software to be cross-correlated. For the cross-correlation process, the double frames are discretized into interrogation windows with a certain size set by the user. The choice of the interrogation windows, should be proportional to the displacement of the particles so that the full velocity distribution can be considered. For each correlation window, a correlation map will be plotted so that the velocity vector can be determined by relating the highest point of this correlation map displayed in Fig. 2.5 which illustrates the concept of the cross-correlation.

The theory behind the cross-correlation concept is explained in [120], where the displacement using the cross correlation method is found as follows:

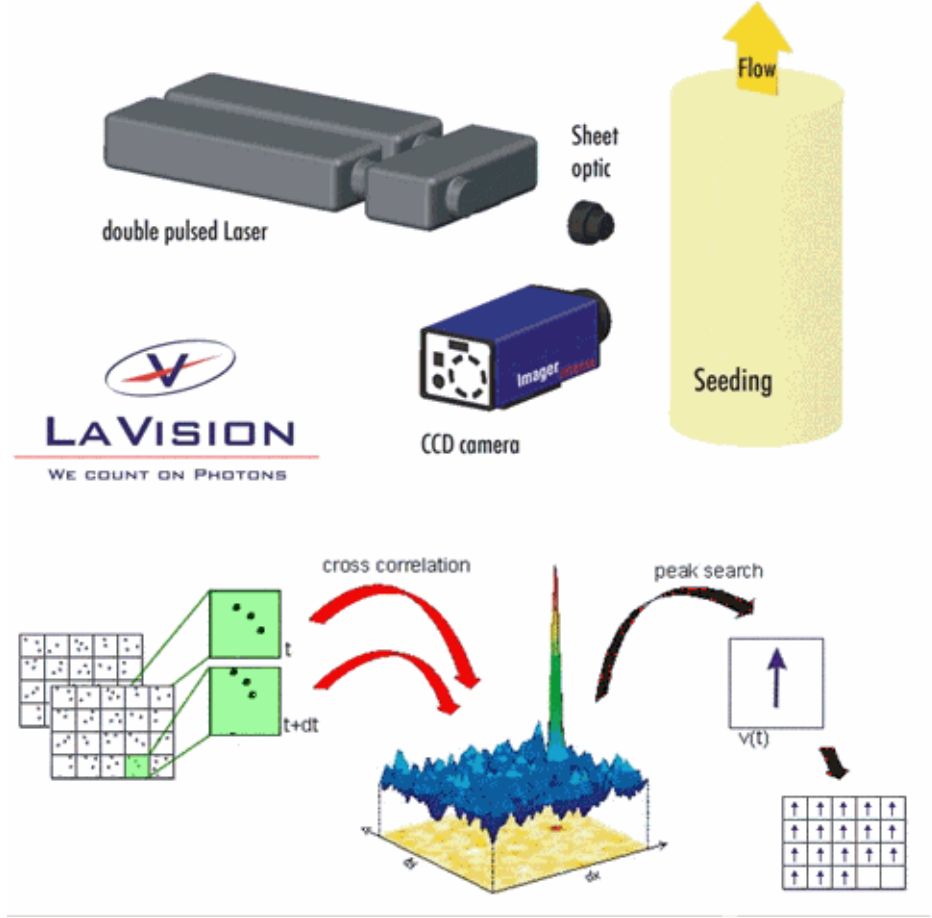


Figure 2.5: Illustration of the cross-correlation technique [122]

$$C(x, y) = \sum_{i=-K}^K \sum_{j=-L}^L I(i, j) I'(i + x, j + y) \quad (2.6)$$

where $C(x, y)$ represents the cross-correlation function, $I(x, y)$ and $I'(x, y)$ are the image intensity values of the two frames, K and L represent the size of the interrogation windows. When calculating $C(x, y)$ a cross-correlation plane is formed where the largest value represents the corresponding displacement of the particles in the window [123]. Although the system and post-processing are the same, there is a considerable difference between standard PIV and μ PIV. As mentioned in [87], there are three main differences: First, the particles for microfluidics devices are on the order of 1 to 5 μm since the particles have to be small enough to not interfere with the flow. The

problem is that the particles become too small compared to the wavelength of the green light (used for the laser). The solution was to label the particles so that they become fluorescent with the light source. Second, because of the small particle size, the Brownian motion, which is the random movement of the particles suspended in the medium, may have a great effect on the velocity values. However, the averaging of the set of frames captured by the camera can drastically reduce this effect. Finally, the illumination technique varies from the standard PIV. It is not possible to create a laser sheet illumination in μ PIV due to the restricted size of the domain that can cause a diffraction of the laser sheet. As a consequence, the volume illumination technique is required. This technique is mostly used with an optical system that has a depth of focus smaller than the flow depth. This method is explained in more detail in [86].

For this project, the following μ PIV system is used: an inverted imaging laser microscope from LaVision is used to visualize the flow and velocity field of a two fluid flow system in a Y-channel configuration as described in the previous section. It comprises a CCD camera (LaVision's Imager Intense camera), a laser (double pulsed Nd:YAG) having a wavelength of $\lambda = 532 \text{ nm}$ and a 10x lens. The software used for the cross-correlation is a DAVIS program. For blood tests, the LaVision devices are coupled with a high speed camera (Dalstar) in order to visualize the RBC flowing in the microchannel. A diagram of the set up is shown in Fig. 2.6 while the actual experimental set up in the laboratory is shown in Fig. 2.7. Fluorescent particles are introduced within the fluids to be illuminated and seen under the camera. The two pumps push the fluids with the flowing particles in the microchannel. Once the laser is turned on, the laser beam encounters the filter to be refracted in the direction of the microchannel observed using the inverted microscope. This laser beam will excite the fluorescent particles that in turn emit a wavelength that is refracted in the direction of the CCD double pulse camera to then be analyzed by the computer (record and cross-correlate). If the camera dual port is set to the high speed camera, the flow is visualized using a white light source that is refracted with the mirror to reach the high speed camera.

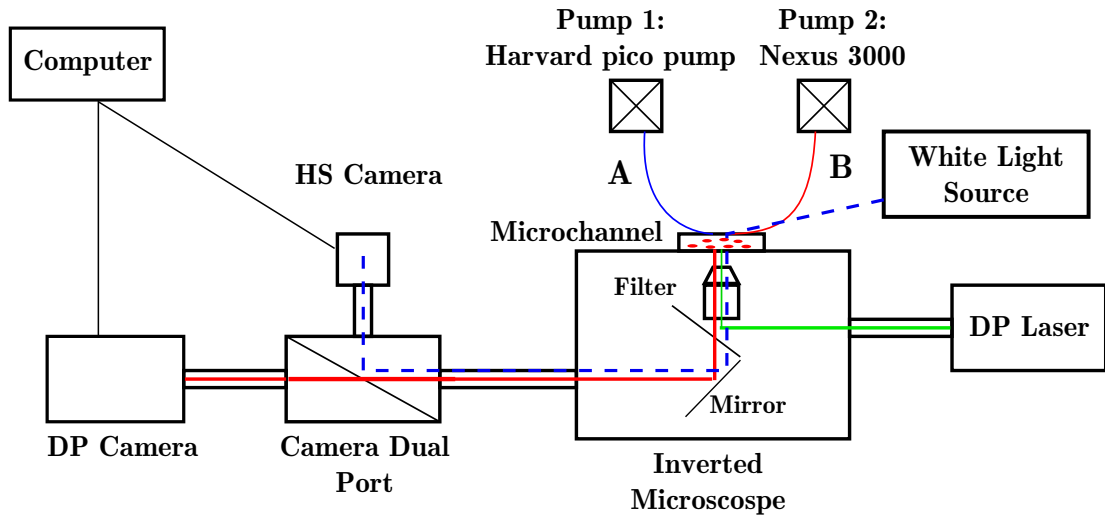


Figure 2.6: Micro PIV set up and light path within the system

2.2.1.2 MICROCHANNEL FABRICATION

The preliminary tests are performed with the commercial Ibidi μ slide III³ⁱⁿ¹ channel, while the microchannels used for the tests performed with the Newtonian (glycerol) and non-Newtonian (blood) fluid to be compared to the simulation results are fabricated in the biomedical laboratory of Dr. Fenech at the University of Ottawa. These microchannels are fabricated using standard photolithography methods as shown in Fig. 2.8. First, after deciding on the microchannel dimensions, a transparency photo-mask is created containing the desired configuration as seen in Fig. 2.8 (a). This geometry is then engraved into a silicon wafer to create the mold (Fig. 2.8 (b)). For this purpose, the SU-8-50 epoxy-based negative photo-resist is spread and spin coated at the appropriate speed to create the depth desired according to the manufacturer instructions. The silicon piece is then heated for few minutes to harden the photo-resist so that the photo-mask can be applied and exposed for few seconds to the UV-light delivering $20 \frac{mW}{cm^2}$. These manufacturer specifications including the spin coating speed and the heating and exposure time are shown in Table 2.2 [124]. Once the mold is fabricated, poly-di-methyl-siloxane (PDMS) made of a mixture of silicon base elastomer and a curing agent (Fig. 2.8 (c)) at a ratio of 10:1 to obtain the appropriate mixture viscosity suitable for our application, is then poured into the

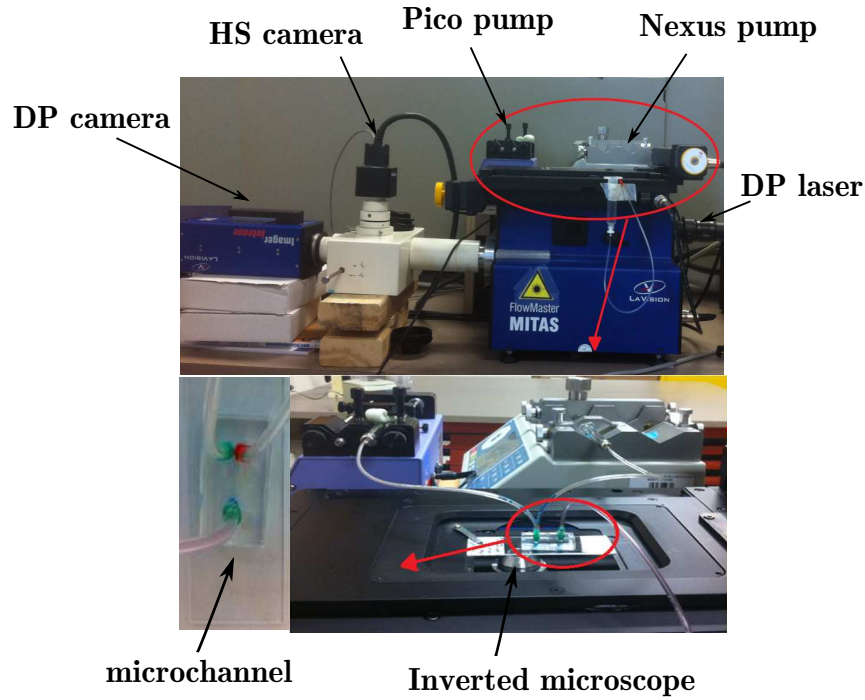


Figure 2.7: Laboratory set up

mold to create the microchannel. The PDMS is then heated for 90 minutes at 65°C to obtain a flexible but solid structure that is cut to separate the single channels as seen in Fig. 2.8 (d). The single channels are then punched at the channel inlets, as shown in Fig. 2.8 (e), to insert the connectors of 2.5 mm diameter. Finally the punched channels are then bonded to a microscope glass slide. The bonding process utilizes the oxygen plasma bonding method using the SP100 ANATECH, LTD-plasma series. When the two surfaces to be bonded are exposed to the oxygen plasma, the surface layers with the lowest molecular weight are removed while the uppermost atomic layer of the polymer is oxidized. The advantage of using this method is the non-reversible bonding created that comes with clean surfaces to avoid blockage of the channel. However, the clean surfaces engender a hydrophilic behaviour of the PDMS [100], which is not desired for these experiments where we wish to obtain a linear velocity profile. This hydrophilic behaviour is, however, reversible in time. For this purpose, the microchannels are used one week after the bonding process, to minimize the slip on the channel walls, hence reducing the hydrophobic behaviour of the PDMS.

Table 2.2: SU-8 photo-resist specifications including the spin coating speed and the heating and exposure time. This exposure time can be calculated based on the exposure dose and the delivered power of the UV light. P.E. represents the post exposure baking time.

SU-8 photo-resist					
Thickness (μm)	Spin speed (rpm)	Soft bake time (min)	Exposure dose ($\frac{mJ}{cm^2}$)	Exposure time (s)	P. E. baking time (min)
40	3000	20	250-300	12.5-15	6
50	2000	26	400-500	20-25	7
100	1000	40	500-650	25-32.5	13

2.2.2 FLUIDS

In order to replicate the simulation and verify the numerical model, a Newtonian model is first tested experimentally. For this purpose, the viscous fluid (fluid B) is represented by a diluted solution of glycerol with a mass fraction of $\Phi = 0.36$. The advantage of glycerol is its property to dissolve in water so that the viscosity of the solution can be controlled and so that the interfacial tension between the two fluids is minimized to reduce the risks of flow unsteadiness. The viscosity of the solution obtained was verified with an LV type viscometer (Brookfield viscometer) where it was found that the mixture has an average viscosity of $\mu_B = 3 \text{ cP}$. This viscometer consists of a motor synchronized with the spindle immersed in the fluid to be analyzed. When the spindle rotates at a constant speed, the force required to overcome the resistance of the fluid is directly proportional to the scale reading (using a pointer) which provide the estimation of the fluid viscosity [125]. This type of viscometer is dependent on the stability of the system to avoid the rotating spindle to be in contact with the outer stable cylinder. A solution of 3% of fluorescent particles ($d_{particle} = 0.86 \mu m$, $\lambda_{abs} = 542 \text{ nm}$ and $\lambda_{emission} = 612 \text{ nm}$), diluted at 1% in water, is added to the glycerol mixture as tracer particles for the PIV system. Concerning the less viscous fluid (fluid A), it is, in this case, represented by distilled water. As a first test, particles are introduced solely in the glycerol layer in order to visualize the interface location. Then, 3% of the particle solution is added to the distilled water to determine the complete velocity profile within the channel. The two fluids

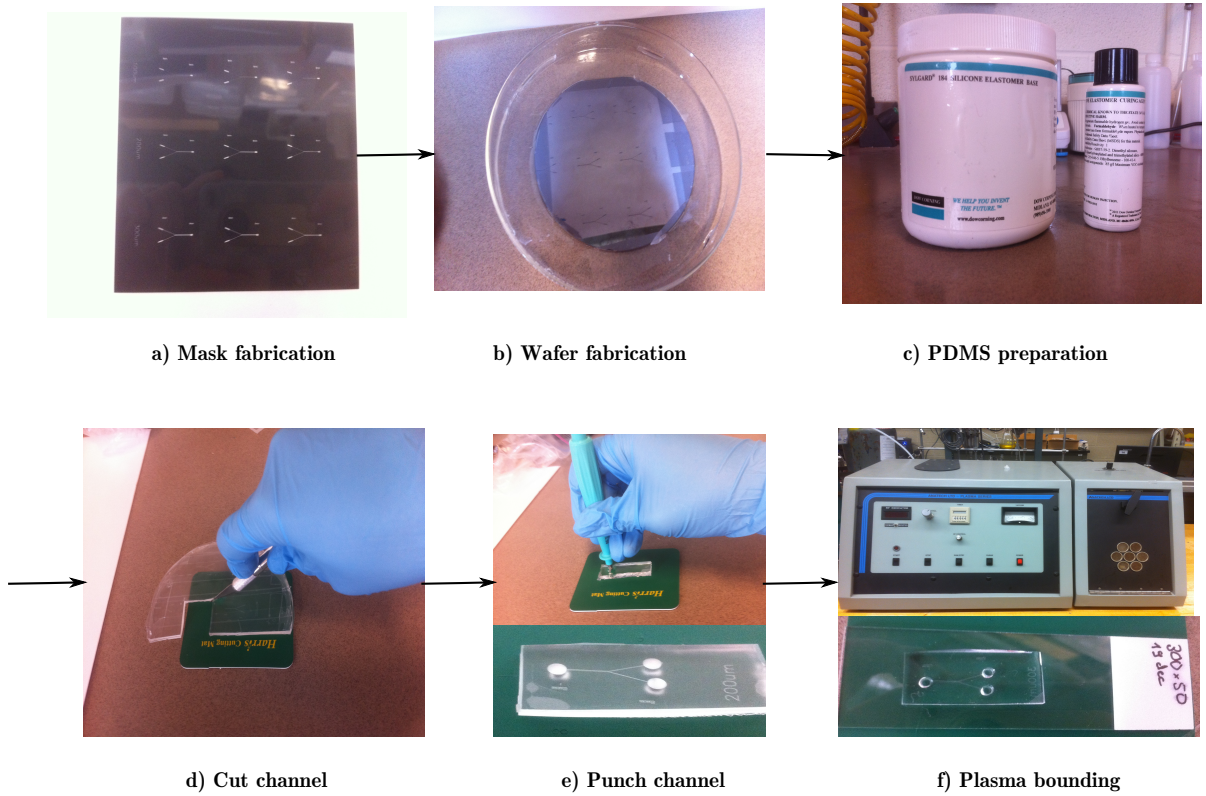


Figure 2.8: Fabrication process of the microchannel using the photolithography method

are injected into the channel using two glass syringes (Hamilton, 50 μm volume), controlled by two pumps (Harvard Apparatus Pico Pump and Nexus 3000) to set two different volumetric flow rates.

Blood is then tested to verify the linearity of the velocity profile. The less viscous fluid used to entrain the blood is Phosphate buffered saline (PBS) to keep the equilibrium concentration between the two fluids. It is important for the concentration to remain in equilibrium in order to avoid the migration of ions and RBC and hemolysis (RBC rupture). The blood used for these experiments is extracted from pigs. This blood is then treated and centrifuged to separate the blood components and remove the white blood cells and platelets. RBC are then resuspended in their plasma to allow the formation of aggregates. Several cases were performed with 10 and 20 % hematocrit to test the effect of these aggregates on the velocity profile. 3% of the particle solution is added to blood and PBS for the concentration to remain in

equilibrium to avoid the diffusion of the fluorescent particles.

2.3 INTERFACE DETERMINATION

Numerically, the interface is located using the calculated values of viscosity. $V_{interface}$ and $y_{interface}$ represent the value of velocity and position in the channel where the viscosity value decreases from the value set for blood viscosity (the top layer of the channel).

However, experimentally, the interface between the two fluids is located visually by the fluorescent particles flowing in the channel. Before introducing the particles within the two fluids, they are first only introduced within the fluid where the linear velocity profile is expected in order to capture this interface. Fig. 5.24 presents the experimental (a) and the numerical (b) fluids distribution in the channel where the interface can be located.

2.4 SHEAR RATE CALCULATIONS

Since we design for a micro-Couette flow where the velocity would be linear, the shear rate, representing the slope of the velocity profile, is calculated as follows:

$$\dot{\gamma} = \frac{du}{dy} = \frac{V_{interface} - V_{wall}}{y_{interface} - y_{wall}} = constant \quad (2.7)$$

where $\dot{\gamma}$ represents the shear rate, $V_{interface}$ the velocity at the interface $y_{interface}$ the position across the channel at the interface, V_{wall} the velocity at the wall in contact with the blood and y_{wall} the position across the channel at the wall facing blood. The numerical and experimental shear rates are calculated using the exact same Eq. 2.7. The difference is the interface location between the two fluids for both cases.

2.5 FLOW RATE CALCULATIONS

The total three dimensional flow rate in the channel is also calculated numerically and experimentally to be compared to the flow rate set by the two pumps. The three

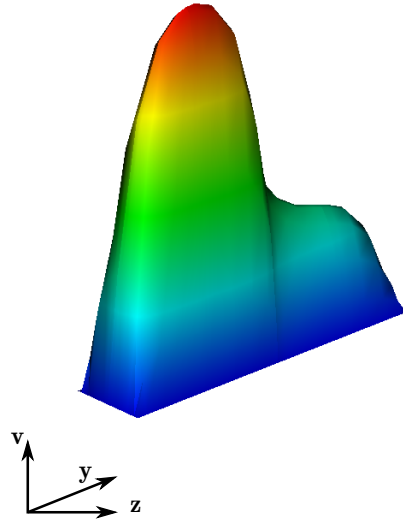


Figure 2.9: Three dimensional velocity profile extracted from cross-section at $x = 28$

dimensional velocity profile in the channel at the cross-section $x = 28$ (Fig. 5.1) is shown in Fig. 2.9.

Numerically, the flow rate is calculated using the spectral element solution by numerical quadrature:

$$Q_{3D} = \int \int u dy dz = \sum_{E=0}^{kt} \sum_{i=0}^{N+1} \sum_{j=0}^{N+1} \frac{h_y^E h_z^E}{4} w_i w_j u_{ij}^E \quad (2.8)$$

where kt represents the total number of elements in the cross sectional area, N the highest order of polynomials, i the point number in the y -direction, j the point number in the z -direction, w_i the Gauss quadrature weight for the position i , w_j the Gauss-quadrature weight for the position j , h_y^E the size of the element E in the y -direction, h_z^E the size of the element E in the z -direction and u_{ij}^E the value of the velocity for each (i, j) point for element E . The weights for each point are properties of the Legendre polynomial Gauss-quadrature.

Since the μ PIV only provides a two-dimensional profile from the middle depth of the channel, the three-dimensional experimental profile is approximated using the numerical data. The differences between the experimental and numerical values are estimated and used to scale the experimental values to fit the numerical ones, yielding

a full three-dimensional experimental profile estimate.

Chapter 3

MICROCHANNEL DESIGN

In order to design the actual microchannel used to test blood and visualize RBC aggregation, several tests were performed to obtain the best design parameters in order to ensure the linearity of the velocity profile within the "blood layer". Since the purpose of this project is to design a model Couette flow in a microchannel using a two fluid flow system, it was intuitive to choose a Y-channel configuration to allow the two fluids to enter from separate compartments and be easily controlled. Therefore the design of the microchannel is based on the configuration of a prefabricated Ibidi large channel of 3 *mm* thickness and 1 *mm* depth shown in Fig. 3.1. The purpose of conducting several tests is to determine and control the shear rate for the future "blood layer", while determining the ideal parameters. These tests are fully reported in Appendix A and the present chapter reports the principal steps in the design process. References are made to certain figures in Appendix A such as A.1.

3.1 PREFABRICATED IBIDI CHANNEL

The channel used for preliminary tests is the μ slide III³ⁱⁿ¹ model fabricated by Ibidi. It is manufactured from microscopy plastic that is treated or uncoated depending on the application. The dimensions and configuration of the channel are shown in Fig. 3.2. As can be seen, the channel consists of three entry branches of 1 *mm* thickness. Three fluids can then be pushed into the channel to be tested. However, for this specific project, only two entry branches are needed and therefore, the numerical simulation is performed with only two branches to study the two fluid flow eliminating the middle branch shown in Fig. 3.2. The flow rate in the channel is based on the flow rate in human body microcirculation. In capillaries with 5 μ m diameter, the flow rate varies between 1 to 3 $\frac{\mu\text{l}}{\text{hr}}$ [1].

The tests performed with the large Ibidi μ slide III³ⁱⁿ¹ consist of eight distinct

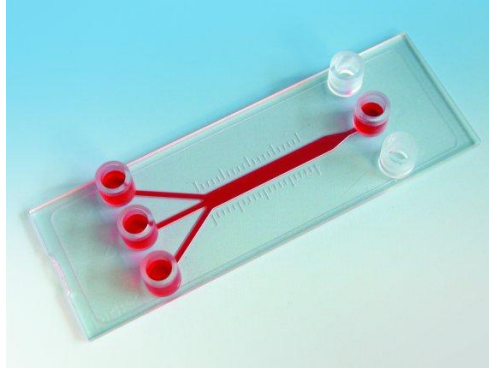


Figure 3.1: Ibidi μ slide III³ⁱⁿ¹ channel [126]

numerical trials, varying the viscosities, the velocities and numerical formulation, in order to obtain a linear velocity profile within blood that would provide a small "blood layer" compared to the channel size. These tests are presented in detail in Appendix A and summarized in Table 3.1.

It was found, for $\nu_{ratio} = 3$ and $V_{ratio} = 3$, that the velocity profile, as desired, is almost linear and the supposed "blood layer" covers approximately 38% of the channel thickness as seen in Fig. A.24 in Appendix A. Once the set up was validated, a first microchannel prototype was used to validate the findings with the Ibidi channel.

3.2 FIRST MICROCHANNEL PROTOTYPE

Two tests were performed on a microchannel of $100 \times 15 \mu m$ based on the the Ibidi channel configuration (three entry branches). The dimensions of this channel used in the numerical simulation are displayed in Fig. 3.3 where the third branch has not been considered since it is blocked in the experiments.

It was found that, using $\nu_{ratio} = 3$ and $V_{ratio} = 4$, the velocity profile seen in Fig. A.34 is almost linear within the "blood layer" that represents approximately 30% of the channel thickness. These results provide an effective model to study blood aggregation in controlled flow conditions. However, the velocity field obtained experimentally shows that the vectors are very small and does not correspond to the numerical solution performed for the same case as seen in A.36. In fact when looking at the intersection of the Y-channel, it was found that the viscous fluid flows

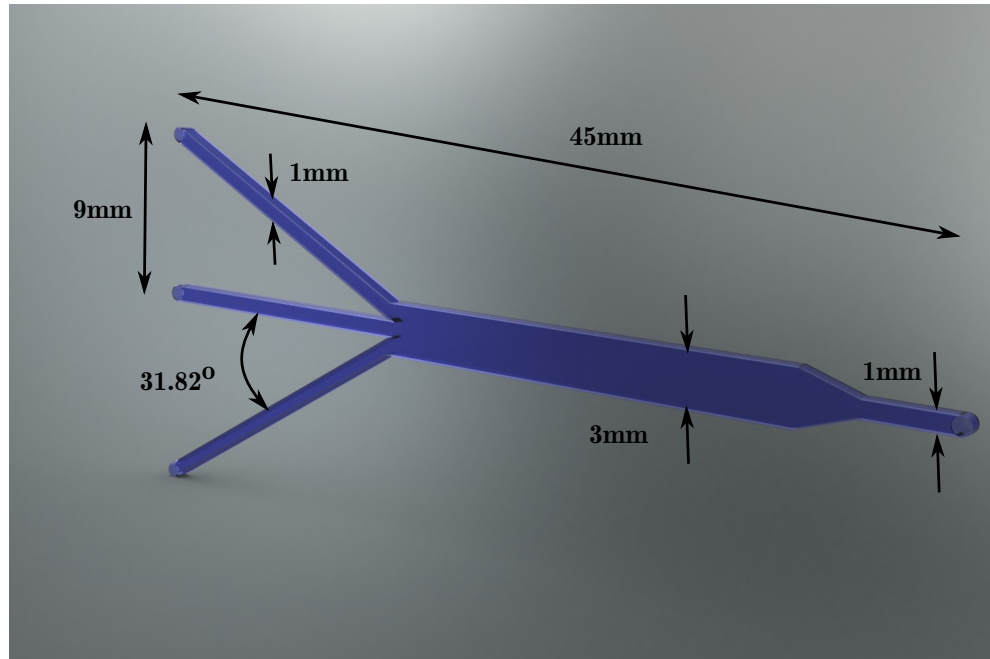


Figure 3.2: Ibidi channel dimensions and configuration

backwards to the blocked middle branch. Therefore, a new design of a microchannel is needed to avoid complications with the third branch. For more details about the microchannel tests to obtain the ideal parameters for an almost linear velocity profile, one can refer to Appendix A.

3.3 NEW DESIGNED SMALL CHANNEL

In order to design the microchannel, several factors have to be taken into consideration.

3.3.1 ANGLE BETWEEN BRANCHES

First of all, the angle between the two branches, for the previous designs was about 60° which is too large for these micro geometries. The interference possibly created from the large angle between the branches can affect the flow within the channel. Therefore, the angle between the two entry branches is reduced to 40° so that the sharp wall will not disturb the flow.

Table 3.1: Summary of numerical tests for the two different channels (Ibidi and small channel). N° test refers to the test number in the text, $N^\circ D$ to the number of dimensions, N° pts to the number of points in one direction in each elements, ν_{topB} to the viscosity of fluid B, V_{topB} to the velocity of fluid B, $\nu_{bottomA}$ to the viscosity of fluid A, $V_{bottomA}$ to the velocity of fluid A and finally Exp indicates if the test was performed experimentally or not. PS in the formulation refers to Passive scalar

Channel type	N° Test	$N^\circ D$	$N^\circ E$	N° pts	Formulation	ν_{topB} ($\frac{mm^2}{s}$)	V_{topB} ($\frac{mm}{s}$)	$\nu_{bottomA}$ ($\frac{mm^2}{s}$)	$V_{bottomA}$ ($\frac{mm}{s}$)	Exp	Conclusion
Ibidi III ³ⁱⁿ¹	1	2D	15	8	$P_N - P_N$	1	0.85	1	0.59	No	-Not easy to visualize velocity -Parabolic profile
Ibidi III ³ⁱⁿ¹	2	2D	40	8	$P_N - P_N$	1	0.85	1	0.59	No	-No outlet part -Parabolic profile
Ibidi III ³ⁱⁿ¹	3	2D	40	8	$P_N - P_N$	53.41	0.85	1	0.59	No	-profile agrees with expectation -Not able to determine interface location
Ibidi III ³ⁱⁿ¹	4	2D	40	8	$P_N - P_{N-2}$	53.41	0.85	1	0.59	No	-Same result as previous test -No motion of the interface
Ibidi III ³ⁱⁿ¹	5	2D	40	8	$P_N - P_{N-2}$ ν as PS	53.41	2.25	1	0.59	Yes	-Still no motion of the interface -Experiments: bubble formation -Immiscible fluids are not required at low Re
Ibidi III ³ⁱⁿ¹	6	2D	40	8	$P_N - P_{N-2}$ ν as PS Free-surface hydrodynamic	3	0.7	1	0.7	No	-Interface located at 1.3mm -Mesh distortion
Ibidi III ³ⁱⁿ¹	7	2D	40	8	$P_N - P_{N-2}$ ν as PS	3	0.7	1	0.7	Yes	-Interface located at 1.3mm too -No mesh distortion -Velocity profile not linear -Channel too large for the lens
Ibidi III ³ⁱⁿ¹	8	2D	40	8	$P_N - P_{N-2}$ ν as PS	3	0.7	1	2.1	Yes	-Small "blood layer" of 38% W -Interface located at 1.85mm -Velocity profile almost linear -Experiments: channel also too large for the lens
Small channel	1	2D	468	8	$P_N - P_{N-2}$ ν as PS	3	2.1	1	0.7	Yes	-Large "blood layer" of 76% W -The velocity in the blood range is not linear -Experiments: the velocity is too small due to the third branch
Small channel	2	2D	468	8	$P_N - P_{N-2}$ ν as PS	3	0.175	1	0.7	No	-small "blood layer" of 30% W -The "blood layer" is about 30 μm -The velocity in the blood range is almost completely linear

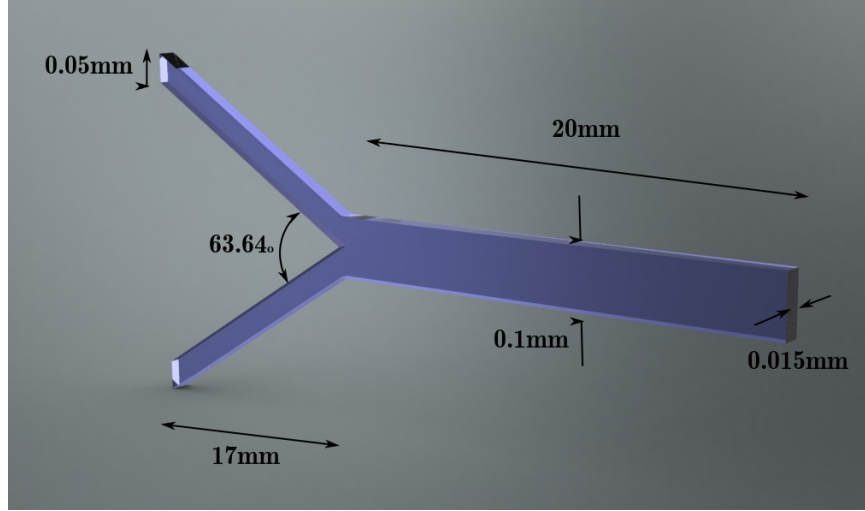


Figure 3.3: Dimensions of the first microchannel prototype based on the Ibidi channel configuration

3.3.2 MICROCHANNEL THICKNESS

The width of the microchannel is chosen based on the "blood layer" required: varying from $30\ \mu m$ to $100\ \mu m$. The tests on the previous microchannel of $100\ \mu m$ thickness, showed the "blood layer" taking up about one third of the channel thickness as expected.

3.3.3 MICROCHANNEL LENGTH

The length of the microchannel is determined based on the diffusion of glycerol into water in order to minimize the diffusion layer in the microchannel. In [127], the diffusion coefficient of glycerol is presented in terms of glycerol solution concentration in percentage of weight. Considering the frictional coefficient of the diffusing particles and the hydrodynamic factor, a solution of a mass fraction of $\Phi = 36\%$ has a diffusion coefficient $D(c) = 10^{-5} \frac{cm^2}{s}$. Assuming that the flow is steady, it is possible to find the time necessary for complete mixing of the two fluids in the microchannel, τ_{tot} , using Eq. (3.1) detailed in [128]:

$$\tau_{tot} = \frac{x}{U} = \left(\frac{(1 - \frac{2}{\pi})^2}{8} - \frac{\ln(\varepsilon)}{\pi^2} \right) \frac{W^2}{D(c)}. \quad (3.1)$$

where W represents the width of the microchannel, U denotes the average velocity in the channel and ε represents the error variable defined as:

$$\varepsilon = 2|c - \frac{1}{2}|_{max}. \quad (3.2)$$

Setting the error term to $\varepsilon = 0.01$ [127], for a microchannel width $W = 100 \mu m$ and for a diffusion coefficient $D(c) = 1.10^{-5} \frac{cm^2}{s}$, the time required for the two fluids to mix is about $\tau_{tot} = 4.85 s$. Therefore we can deduce the corresponding channel length based on the average velocity (about $U = 0.8 \frac{mm}{s}$) based on the the diffusion length using Eq. (3.3):

$$x_{diff} = U \times \tau_{tot} = 3.88 mm. \quad (3.3)$$

Therefore, in order to avoid complete mixture of both fluids, the channel length is set to be about $L_{channel} = 2 mm$.

3.3.4 LENGTH OF BRANCHES

The length of the branches is chosen so that the flow becomes fully developed but also short enough to minimize friction loss. Therefore the horizontal length of the branches is set to be four times less than the length of the channel so that $L_{branch} = 0.5 mm$.

3.3.5 MICROCHANNEL DEPTH

In order to determine the right depth of channel required for this application, several parameters have to be taken into consideration. Since the goal of this research is to design the channel so that the shear rate within the "blood layer" is within the range of shear rate where RBC can aggregate ($1 s^{-1}$ to $10 s^{-1}$), the depth of the channel is an important factor that can increase or decrease this shear rate. Therefore, the channel has to be deep enough so that the shear rate is minimized. However, if the channel is too deep, the aggregates will form larger 3D entities that would be hard to visualize with an inverted microscope. In this case, a confocal camera would be required to visualize the flow in depth and determine the aggregate size. A compromise is

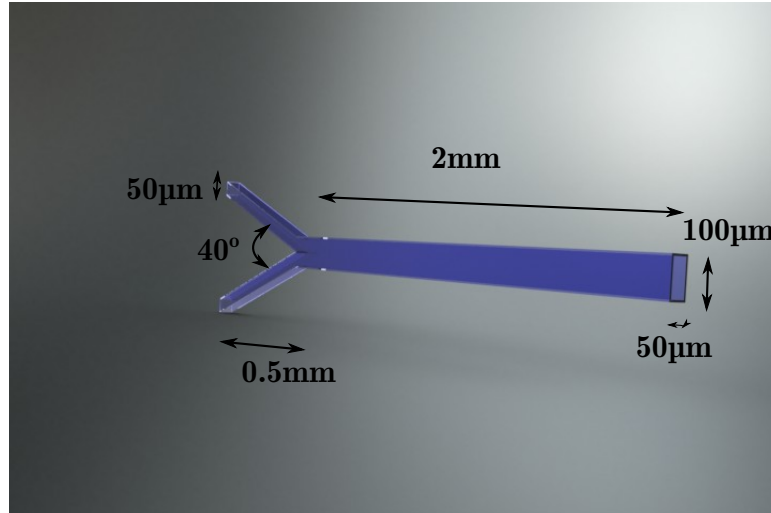


Figure 3.4: Geometry and dimensions of the new designed microchannel

required to define the appropriate depth for this application. Therefore we choose to design microchannels, one with a square cross section of $100 \times 100 \mu m$ and one with a rectangular cross section of $100 \times 50 \mu m$, and then compare the differences between the two cases in order to reach a final decision.

3.4 DIMENSION DETERMINATION

Taking into consideration all the previously mentioned factors, the geometry and dimensions of the microchannel are determined as shown in the Fig. 3.4. The model is tested numerically using *Nek5000*. However, when fabricating the model the vertical distance between the two branches is found to be too small to insert the inlet of the tubes related to the syringes. As mentioned in Chapter 2, the experimental set up consists of two pumps that push the syringes containing the two different fluids to be inserted in the microchannel. The syringes are connected to the microchannel via small tubes of 0.8 mm internal diameter. Since the tubes are not the same size as the microchannel entrance, connectors are required. The available connectors in the laboratory have a diameter of 2.5 mm . Therefore, two holes of 2.5 mm need to be punched at the entrance of the branches to allow the passage of the fluid. However, the vertical distance between the two branches (with an horizontal length of $L_{branch} = 0.5 \text{ mm}$) does not allow punching of two holes of 2.5 mm . Two choices are available to

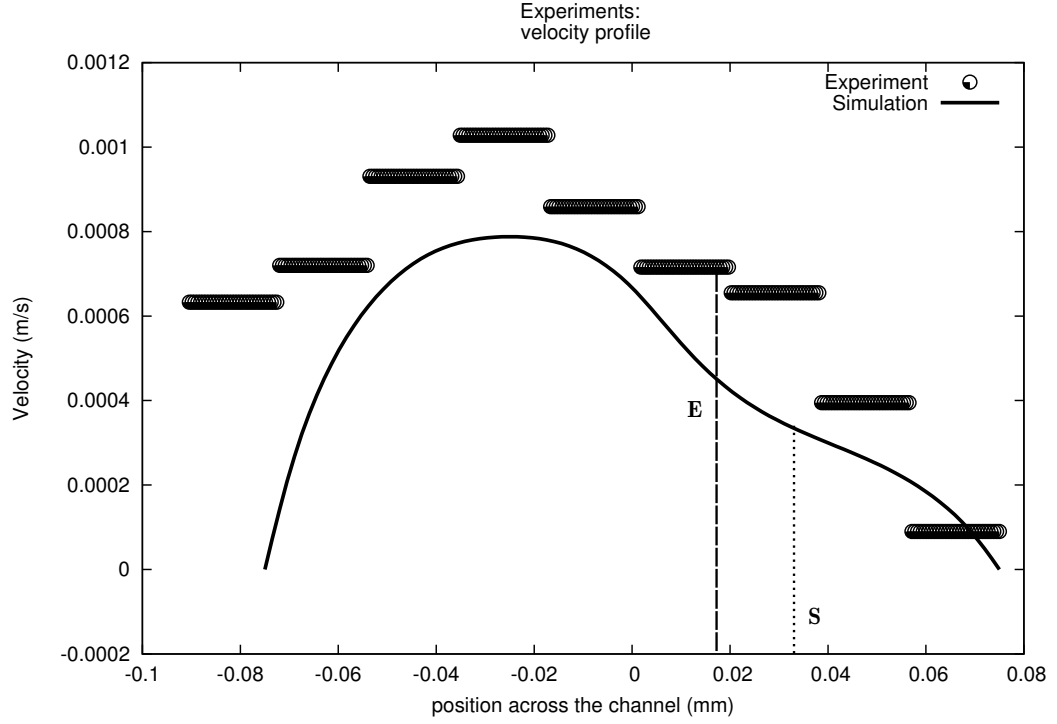


Figure 3.5: Experimental Newtonian results with a ratio of velocity of 4 and simulation results using dimensions of $150 \times 50 \mu m$

solve this issue. Either the angle between the branches can be increased to leave enough space for the insertion of the connectors or the branches could be elongated without changing the angle between them. In the second case, the length of the channel would also be increased so that the length of the branches does not exceed the channel length. The second solution was chosen over the first one in order to minimize the sharp transition to the main channel. With a branch length of $L_{branch} = 5 \text{ mm}$, it is possible to punch the two entry holes with a 1.5 mm space between them. For this reason the length of the channel was increased from $L_{channel} = 2 \text{ mm}$ to $L_{channel} = 6 \text{ mm}$.

When performing the tests under the microscope, that was calibrated using a microscale, it was found that the fabricated channels have different dimensions than designed. The $100 \mu m$ width actually measures $150 \mu m$, while a second channel from the same wafer is only $125 \mu m$ wide. Therefore, the simulations performed with the microchannel dimensions of " $100 \times 50 \mu m$ " are redone with the new dimensions of $150 \times 50 \mu m$. A Newtonian fluid having the same viscosity as blood is tested experi-



Figure 3.6: Profilometer Ambios XP-200

mentally with a $V_{ratio} = 1$, $V_{ratio} = 2$ and $V_{ratio} = 4$ to first ensure the linearity of the profile within the "blood layer". The results are then compared to the simulation performed with the channel of $150 \times 50 \mu m$ dimensions for the same corresponding ratios of velocity and viscosity. Fig. 3.5 represents an example of the results obtained for $V_{ratio} = 4$: the experimental velocity profile is unexpectedly higher than the numerical one. Similar trends exist for $V_{ratio} = 1$ and $V_{ratio} = 2$. The numerical simulations are assumed to represent a theoretical model of this two fluid flow, while several factors can interfere with the experimental results such as leakage in the microchannel from the fabrication process. We therefore expect to obtain more accurate and then higher velocities from the simulation results than the experimental one. We suspected that this variation of the velocity profile might be due to the difference in the dimensions used numerically and experimentally. We therefore decided to carefully measure the depth of the channel to ensure the right dimensions of the channels. For this purpose the Ambios XP200 Profilometer (McGill University laboratory, physics department) is used. This instrument, presented in Fig. 3.6, is a computerized, high sensitivity surface profiler that is able to scan different surfaces to provide accurate measurement. It can measure precision step heights from 10 angstroms to 1.2 millimeters. It consists of a CCD camera that is used to visualize the sample, a needle to follow the surface of the sample and a rotational stage to ensure the good positioning of the sample. The measurements are programmed and controlled using a computer to move the stage in all directions.

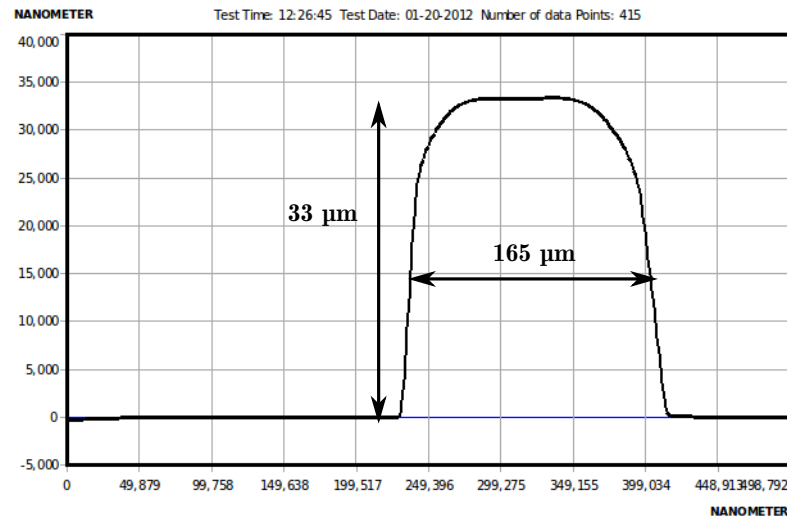


Figure 3.7: Example of the profile obtained with the Ambios XP-200Profilometer for a " $100 \times 50 \mu m$ " designed channel

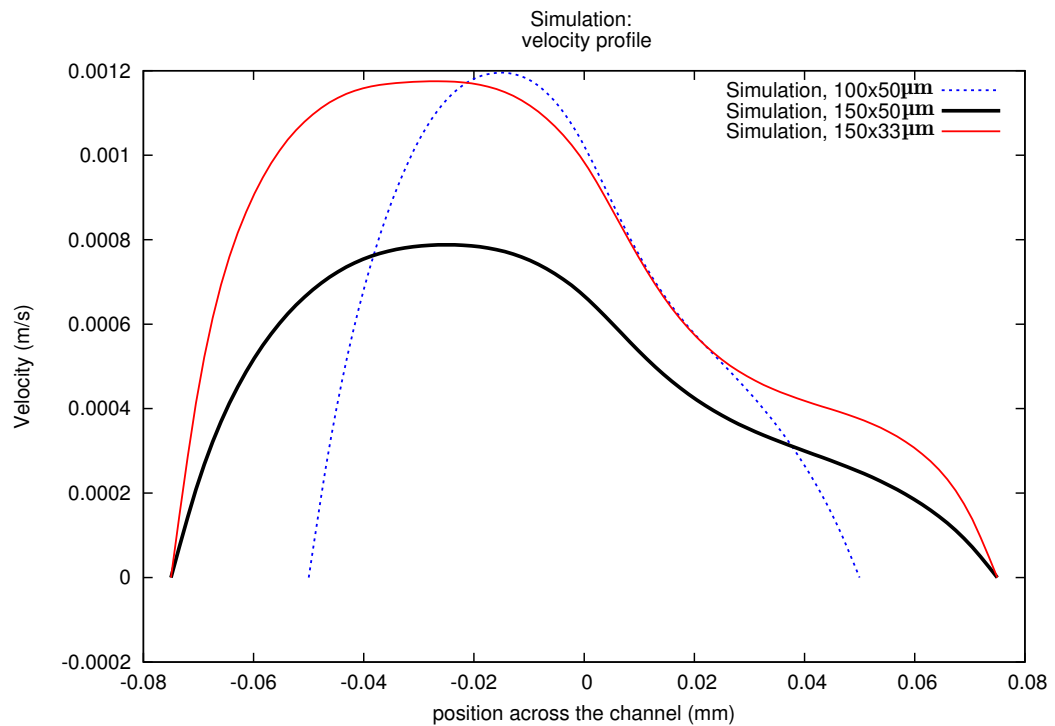


Figure 3.8: The simulation performed with three different channel geometries: $100 \times 50 \mu m$, $150 \times 50 \mu m$ and $150 \times 33 \mu m$

The measurements obtained from the samples are accurate in height but not in width due to the needle geometry. The needle used to take the measurement has a triangular head that cannot accurately capture the wall of the channel. Therefore, the

width of the channel is measured with the inverted microscope while the depth of the channel is based on the measurement taken with the profilometer. An example of the measurements taken for a designed " $100 \times 50 \mu m$ " channel is shown in Fig. 3.7 illustrating the poor accuracy of the width measurement.

The designed $50 \mu m$ depths are found to be $Depth_{50} = 33 \mu m$ and the $100 \mu m$ ones are found to have $Depth_{100} = 64 \mu m$. Fig. 3.8 shows the different velocity profiles obtained from the three cases tested with three different geometries, where the variance of the linearity of the velocity profile can be clearly seen. This will be discussed in more detail in Chapter 5.

3.5 CONCLUSION

In this chapter, several numerical and experimental tests were performed with different channel geometries, such as Ibidi $3 \times 1 mm$ channel, the first microchannel prototype ($100 \times 15 \mu m$ microchannel), the $100 \times 50 \mu m$, the $150 \times 33 \mu m$ and the $170 \times 64 \mu m$ microchannels in order to choose the appropriate channel dimensions based on several factors such as the thickness of the "blood layer" and the linearity of the velocity profile in this small "blood layer". Table 3.1 summarizes all the test performed numerically and experimentally with the Ibidi channel and the first microchannel prototype in order to select an actual geometry. For our application we settled on the $150 \times 33 \mu m$ and $170 \times 64 \mu m$ microchannels (the actual dimensions of the channels).

ANALYTICAL AND NUMERICAL INVESTIGATIONS: NUMERICAL SIMULATION VS. EXACT SOLUTION

In order to verify the results obtained with the computational simulation program, several numerical test cases were performed and compared to an exact solution. The purpose of this chapter is to demonstrate and verify the simulation results for the specific case of two fluid flows.

4.1 EXACT SOLUTION

The exact solution used for this case is based on the simultaneous motion of two fluids A (the less viscous fluid) and B (the more viscous fluid) with different properties μ_A , ρ_A and μ_B , ρ_B respectively through a channel with infinite parallel plates as illustrated in Fig. 4.1 below. It is assumed that both fluids have the same density. It is also assumed that both fluids are Newtonian immiscible fluids and that the flow is fully developed and reaches steady state.

The governing Navier-Stokes equations are expressed as follows:

$$\frac{\partial \rho}{\partial t} + \left(\frac{\partial \rho u}{\partial x} + \frac{\partial \rho v}{\partial y} + \frac{\partial \rho w}{\partial z} \right) = 0 \quad (4.1)$$

$$\frac{\partial \rho u}{\partial t} + u \frac{\partial \rho u}{\partial x} + v \frac{\partial \rho u}{\partial y} + w \frac{\partial \rho u}{\partial z} = -\frac{\partial P}{\partial x} + \rho g_x + \mu \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right) \quad (4.2)$$

$$\frac{\partial \rho v}{\partial t} + u \frac{\partial \rho v}{\partial x} + v \frac{\partial \rho v}{\partial y} + w \frac{\partial \rho v}{\partial z} = -\frac{\partial P}{\partial y} + \rho g_y + \mu \left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2} \right) \quad (4.3)$$

$$\frac{\partial \rho w}{\partial t} + u \frac{\partial \rho w}{\partial x} + v \frac{\partial \rho w}{\partial y} + w \frac{\partial \rho w}{\partial z} = -\frac{\partial P}{\partial z} + \rho g_z + \mu \left(\frac{\partial^2 w}{\partial x^2} + \frac{\partial^2 w}{\partial y^2} + \frac{\partial^2 w}{\partial z^2} \right) \quad (4.4)$$

where ρ represents the density of the fluid, μ the viscosity of the fluid, u the velocity in the x direction, v the velocity in the y direction, w the velocity in the z direction,

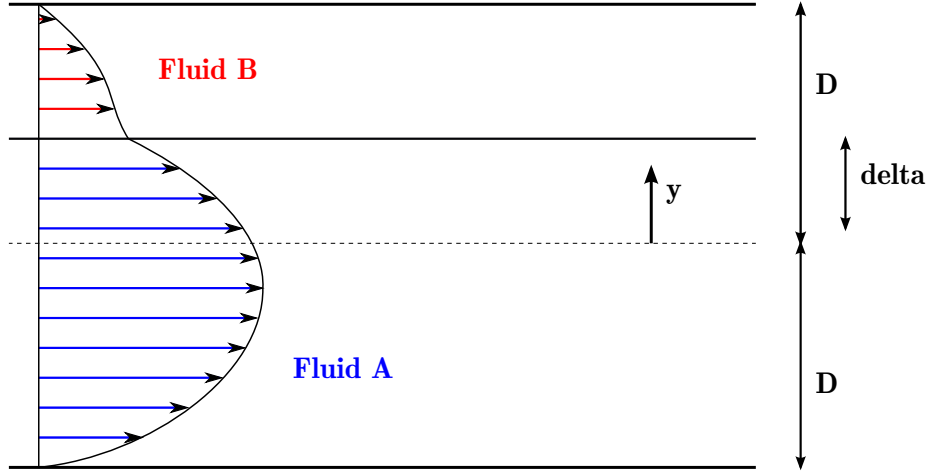


Figure 4.1: Two fluids in an infinite parallel plate channel

P the pressure in the channel and g_x , g_y and g_z the acceleration in the x, y and z directions respectively.

Simplifying the above equations by assuming an incompressible, two-dimensional (2D), steady state, fully developed flow, the equation set is reduced to:

$$\rho \frac{\partial v}{\partial y} = 0 \quad (4.5)$$

$$v \frac{\partial \rho u}{\partial y} = -\frac{\partial P}{\partial x} + \mu \frac{\partial^2 u}{\partial y^2} \quad (4.6)$$

$$v \frac{\partial \rho v}{\partial y} = \rho g_y + \mu \frac{\partial^2 v}{\partial y^2}. \quad (4.7)$$

By setting no slip boundary conditions on walls of the channel ($v = 0$ at $y = -D$), the pressure gradient equation can then be found using:

$$\frac{\partial P}{\partial x} = \mu \frac{\partial^2 u}{\partial y^2} = \text{constant}. \quad (4.8)$$

As seen in Eq. (4.8), the left hand side of the equation depends only on x , while the right hand side of the same equation depends only on y , resulting in a constant value.

Since the fluids are Newtonian, the shear stress expression can be written as follows:

$$\tau = \mu \frac{du}{dy}. \quad (4.9)$$

By substituting Eq. (4.9) into Eq. (4.8), the pressure gradient can be expressed using the following equations for both fluids A and B:

$$\frac{\partial P}{\partial x} = \left(\frac{\partial \tau}{\partial y} \right)_A = \left(\frac{\partial \tau}{\partial y} \right)_B. \quad (4.10)$$

By integrating Eq. (4.10) with respect to y the shear stress for both fluids A and B can be found:

$$\tau_A = \frac{\partial P}{\partial x} y + C_1 \quad (4.11)$$

$$\tau_B = \frac{\partial P}{\partial x} y + C_2. \quad (4.12)$$

Since the shear stress gradient is uniform across the channel, its expression at the interface between the two fluids is the same for fluid A and B, $\tau_{interfaceA} = \tau_{interfaceB}$. Therefore, we obtain:

$$C_1 = C_2 = C$$

Taking Eq. (4.8) for both fluids A and B, integrating it twice in terms of y , we obtain:

$$\begin{aligned} u_A &= \frac{1}{2\mu_A} y^2 \frac{\partial P}{\partial x} + C \frac{1}{\mu_A} y + C_3 \\ u_B &= \frac{1}{2\mu_B} y^2 \frac{\partial P}{\partial x} + C \frac{1}{\mu_B} y + C_4. \end{aligned} \quad (4.13)$$

It is reasonable to set as boundary condition that the velocity at the interface is the same for both fluids since the velocity gradient is uniform across the channel, $u_{interfaceA} = u_{interfaceB}$. Therefore we obtain from Eq. set (4.13), above, that:

$$\frac{1}{2} \delta^2 \frac{\partial P}{\partial x} \left(\frac{1}{\mu_A} - \frac{1}{\mu_B} \right) + C \delta \left(\frac{1}{\mu_A} - \frac{1}{\mu_B} \right) + C_3 - C_4 = 0 \quad (4.14)$$

where δ represents the location of the interface measured from the middle of the channel. Because of the no slip boundary condition set at the channel walls, it is safe to state that at $y = -D$, $u_A = 0$ and at $y = D$, $u_B = 0$. Therefore Eq. set (4.13) can be rewritten as follows:

$$\begin{aligned} u_A &= \frac{1}{2\mu_A} D^2 \frac{\partial P}{\partial x} - C \frac{1}{\mu_A} D + C_3 = 0 \\ u_B &= \frac{1}{2\mu_B} D^2 \frac{\partial P}{\partial x} + C \frac{1}{\mu_B} D + C_4 = 0 \end{aligned} \quad (4.15)$$

where D represents the half thickness of the channel. By manipulating Eqs. (4.14) and (4.15), we determine expressions for the three unknowns C , C_3 and C_4 as follows:

$$\begin{aligned} C &= \frac{1}{2} \frac{\partial P}{\partial x} \frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \\ C_3 &= \frac{-1}{2\mu_A} \frac{\partial P}{\partial x} + \frac{D}{2\mu_A} D^2 \frac{\partial P}{\partial x} \frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \\ C_4 &= \frac{-1}{2\mu_B} \frac{\partial P}{\partial x} - \frac{D}{2\mu_B} D^2 \frac{\partial P}{\partial x} \frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)}. \end{aligned}$$

Therefore, we deduce the expressions for the velocity profiles of both fluids A and B respectively:

$$u_A = \frac{1}{2\mu_A} \frac{\partial P}{\partial x} \times \left(y^2 + \left(\frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \right) y - D^2 + D \left(\frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \right) \right) \quad (4.16)$$

$$u_B = \frac{1}{2\mu_B} \frac{\partial P}{\partial x} \times \left(y^2 + \left(\frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \right) y - D^2 - D \left(\frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \right) \right) \quad (4.17)$$

Table 4.1: Parameters of the exact solution and simulation

Parameter	Units	Symbol	Value
pressure gradient	$\frac{Pa}{m}$	$\frac{\partial P}{\partial x}$	10^{-3}
Viscosity of Fluid A	cP	μ_A	1.0
Viscosity of Fluid B	cP	μ_B	3.0
Thickness of half the channel	mm	D	1.0
Location of the interface	mm	δ	0.3

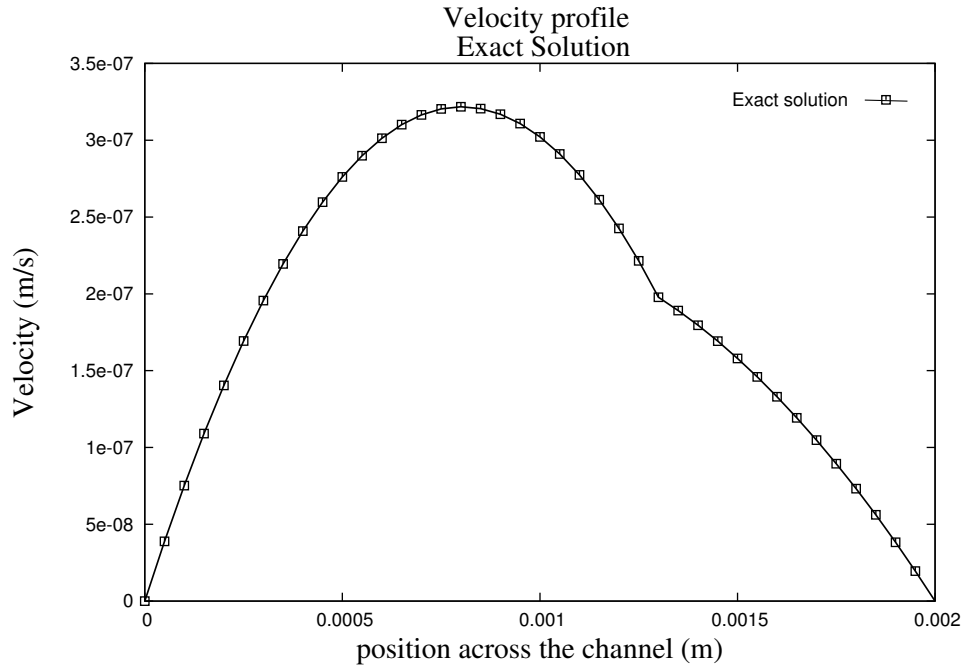


Figure 4.2: Velocity profile for the exact solution of two co-flowing fluids of viscosities $1.0 \times 10^{-3} Pa.s$ (left) and $3.0 \times 10^{-3} Pa.s$ (right)

By replacing Eq. (4.16) into (4.11), the shear stress can be expressed as follows:

$$\tau_A = \tau_B = \frac{1}{2} \frac{\partial P}{\partial x} \left(2y + \frac{(D^2 - \delta^2)(\mu_B - \mu_A)}{D(\mu_B + \mu_A) + \delta(\mu_B - \mu_A)} \right). \quad (4.18)$$

The parameters presented in Table 4.1 are used to calculate the results for the exact solution to be compared to the numerical simulations. The resultant velocity profile is shown in Fig. 4.2.

4.2 INPUT BOUNDARY CONDITIONS DETERMINATION

In order to compare with the exact solution, the same flow rate is used in the numerical simulation. For this purpose, the flow rate is deduced from the velocity profile determined previously. The uniform velocity calculated can then be used in the numerical simulation as input boundary condition.

The expression of the flow rate per unit length is written as follows:

$$\dot{Q} = \int u(y) dy.$$

Therefore, the flow rate per unit length, $\dot{Q}$, for both fluids A and B can be determined as follows:

$$\dot{Q}_A = \int_{-D}^{\delta} u_A(y) dy \quad (4.19)$$

$$\dot{Q}_B = \int_{\delta}^D u_B(y) dy. \quad (4.20)$$

By integrating for both fluids A and B we can obtain the expression of the flow rate per unit length as follows:

$$\dot{Q}_A = \frac{1}{2\mu_A} \frac{\partial P}{\partial x} \left(\frac{1}{3} (\delta^3 - 2D^3) + \frac{C'}{2} (\delta^2 - D^2) + DC' (\delta + D) - \delta D^2 \right) \quad (4.21)$$

$$\dot{Q}_B = \frac{1}{2\mu_B} \frac{\partial P}{\partial x} \left(\frac{-1}{3} (\delta^3 + 2D^3) + \frac{C'}{2} (D^2 - \delta^2) + DC' (\delta - D) + \delta D^2 \right) \quad (4.22)$$

where

$$C' = \frac{(D^2 - \delta^2) (\mu_B - \mu_A)}{D (\mu_B + \mu_A) + \delta (\mu_B - \mu_A)}.$$

The uniform velocity at the entrance for fluid A and B can then be determined using

the following equation:

$$V_{averageA} = \frac{\dot{Q}_A}{Area_{entranceA}} = \frac{Q_A}{width_{entranceA}} \quad (4.23)$$

$$V_{averageB} = \frac{Q_B}{Area_{entranceB}} = \frac{Q_B}{width_{entranceB}}. \quad (4.24)$$

4.3 SIMULATION

4.3.1 CASE 1

4.3.1.1 PROBLEM STATEMENT

The first case used to compare to the exact solution is a box with 3x2 elements: 2 vertically and 3 horizontally. In order to replicate the exact solution, the length of each element is set to be 1 *mm*. The boundary conditions set in the program and displayed in Fig. 4.3 for this case are as follows: periodic boundary conditions are set as inlet and outlet for the right and left faces of the box and the no slip boundary conditions (wall) are set for the top and bottom faces of the box. In order to drive the flow along the channel, a pressure gradient is fixed in the x direction. For compatibility, the same pressure gradient as the exact solution is used. However, the interface between the two fluids is set at a fixed location in the computational program. In fact, the viscosity is set as a function of the position across the channel. It is expressed as a step function with the step being located at the interface. Fig. 4.3 shows the mesh distribution in the channel, where one element is clearly defined. Each element is subdivided into a grid of five by five quadratic points, related to the highest polynomial ($N = 4$) order within each element. This low number of points is chosen based on the simplicity of the solution.

4.3.1.2 RESULT AND DISCUSSION

A steady state velocity distribution is displayed in the Fig. 4.4 and the velocity profile extracted from the channel at $x = 2.8$ *mm* is displayed in Fig. 4.5(a).

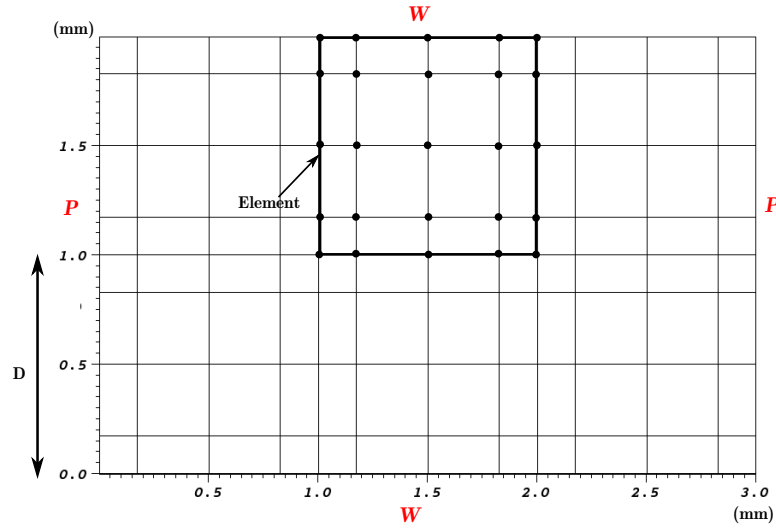


Figure 4.3: Mesh distribution within the channel and boundary conditions

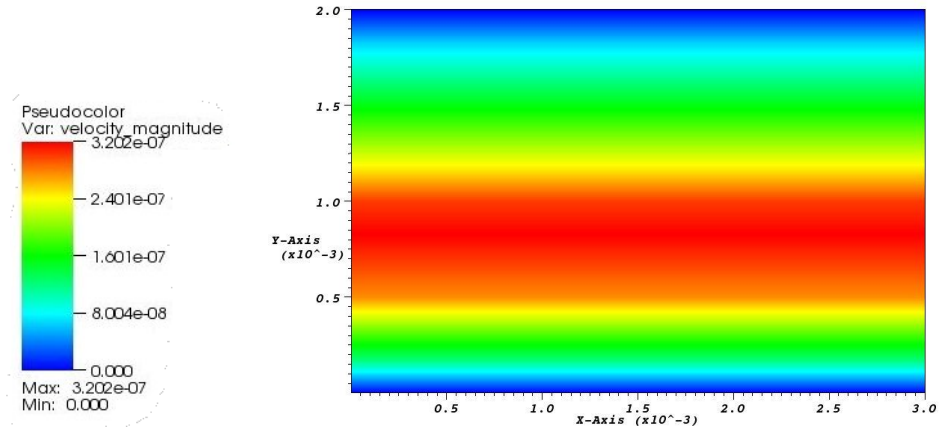
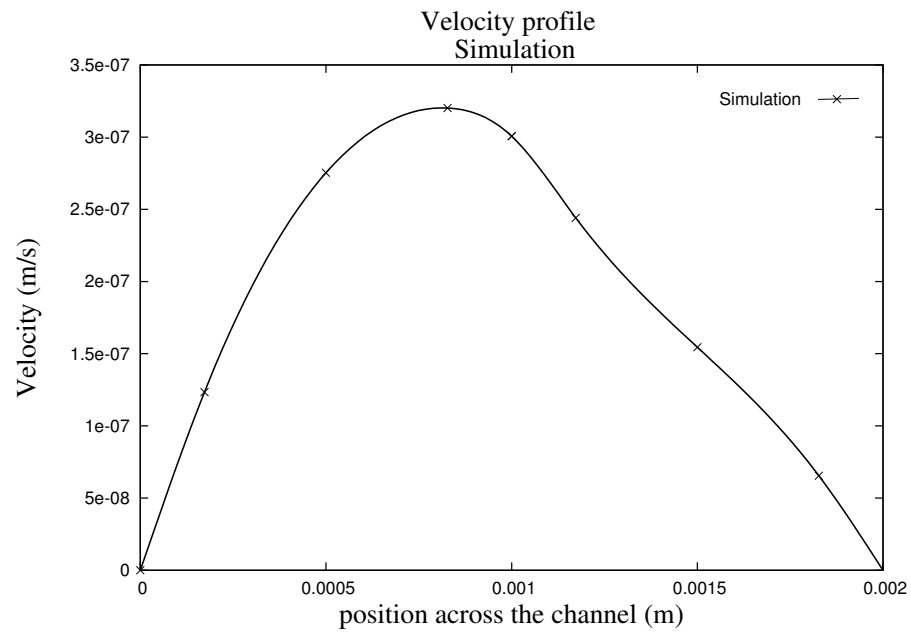
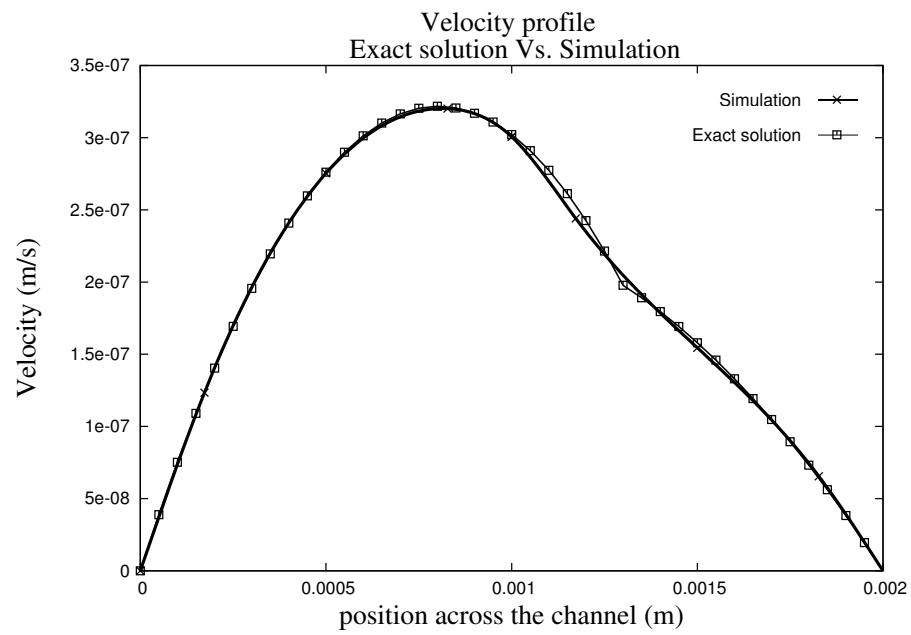


Figure 4.4: Simulated velocity distribution in the channel for Case 1

From Fig. 4.4, we see that the velocity reaches its maximum value in fluid A layer, as predicted by the exact solution. Since fluid A is less viscous than fluid B and the velocity entrance of fluid A layer is higher than for fluid B, it is expected that fluid A will entrain the viscous fluid. By comparing the two profiles in Fig. 4.5(b), we notice that they both share the same characteristics, where the velocity reaches its maximum in the less viscous fluid layer (left part of the figure), experiencing a transition that indicates the location of the interface, then decreasing in an almost linear fashion. However there is a slight difference between the exact and the simulation solutions. In fact the percentage of error varies from 1 to about 5%. This difference is probably



(a) Simulated velocity profile for Case 1



(b) Velocity profile: simulation vs. exact solution for Case 1

Figure 4.5: Velocity profiles comparison for Case 1

due to the interface condition imposed in the simulation (viscosity being expressed as a step function). In fact, the interface seen analytically does not reflect the reality due to the diffusion between both fluids.

4.3.2 CASE 2

4.3.2.1 PROBLEM STATEMENT

The second case is similar to the first case, except boundary conditions for the left and right faces of the channel are not periodic but inlet and outlet (I/O) boundary conditions, respectively. The flow rates for both fluids, detailed in the Sec. 4.2, are used to calculate the uniform velocity on each section of the channel to be set as input boundary condition. For this case, the widths to be used to calculate the average velocities for fluids A and B, $width_{entranceA}$ and $width_{entranceB}$, are $D + \delta$ and $D - \delta$ respectively since the interface location, δ , is still a fixed value in the program. The corresponding uniform velocities are $V_{averageA} = 2.4 \times 10^{-7} \frac{m}{s}$ and $V_{averageB} = 1.125 \times 10^{-7} \frac{m}{s}$ for the bottom and top branches respectively. At the outlet, the pressure is set to zero. The velocity profiles extracted from this case are compared to the previous case (Fig. 4.5(a) in Sec. 4.3.1) and the exact solution.

4.3.2.2 RESULT AND DISCUSSION

The velocity distribution in the channel is presented in Fig. 4.6, while Fig. 4.7 shows the viscosity distribution in the channel, determining the location of the interface between fluid A and B. The velocity profile seen in Fig. 4.8 is extracted from the channel at a location of $x = 2.8 \text{ mm}$. Comparing the results obtained from Case 1 and Case 2, there is a slight difference between the two profiles. The reason is that the first case is assumed to be a fully developed steady solution since it is driven by a pressure gradient, whereas the second case is assumed to be a steady but not completely fully developed, since flow inlet and outlet conditions were set. Therefore the highest order of polynomials was increased for Case 2 to obtain better results as seen through Fig. 4.9(b).

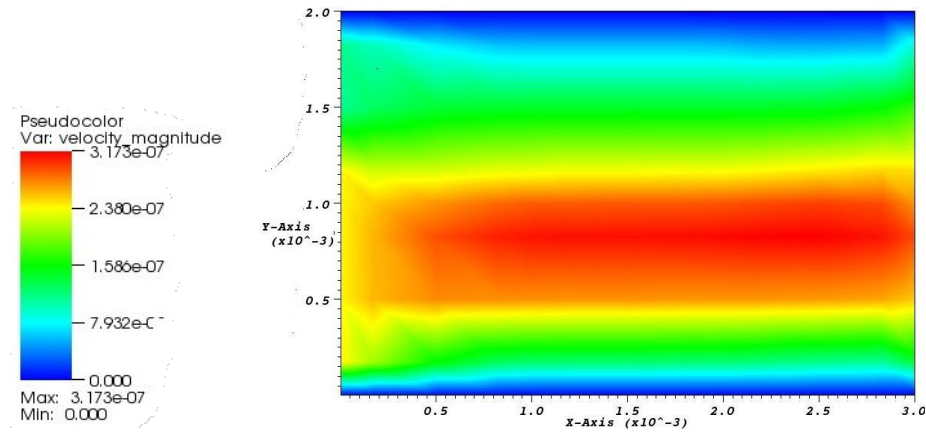


Figure 4.6: Simulated velocity distribution in the channel for Case 2

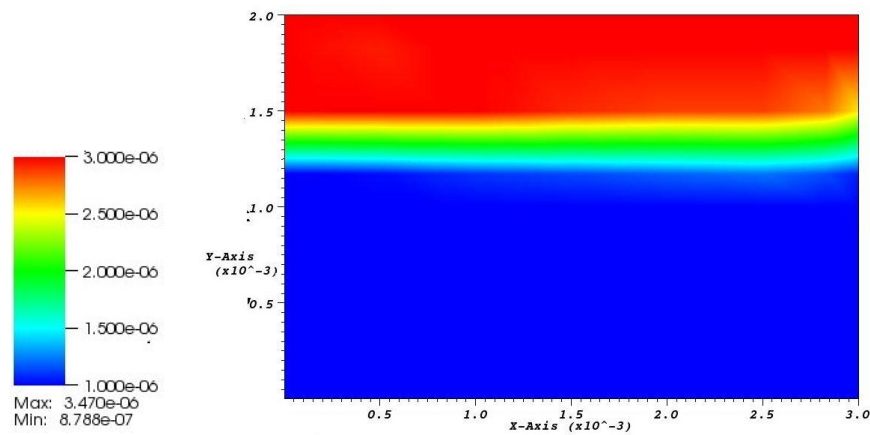


Figure 4.7: Simulated viscosity distribution in the channel for Case 2

Comparing the results obtained from Case 2 with the exact solution (Fig. 4.9(a)), it is clear that the two velocity profiles coincide with a small difference at the interface. In fact the percentage of error varies from 0.5% to about 10% at the interface.

4.3.3 CASE 3

4.3.3.1 PROBLEM STATEMENT

The third case describes the same situation as in the second and first cases of a two fluid flow. However, the channel represents, now, a box with 6x2 elements; 2 vertically and 6 horizontally, representing a channel of 6 mm length and 2mm height i.e. twice as long as in Case 2. The same boundary conditions (no-slip) on the upper and lower

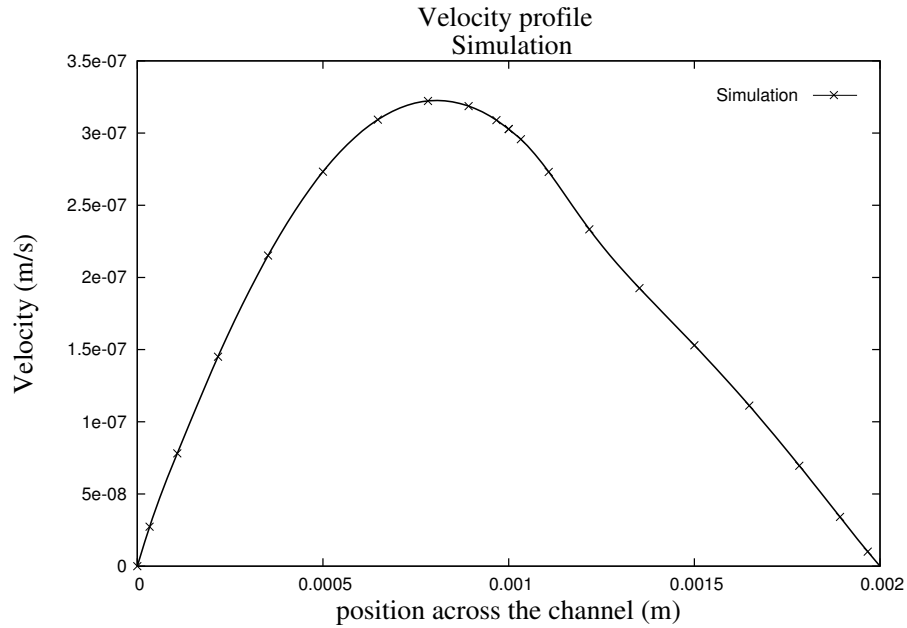
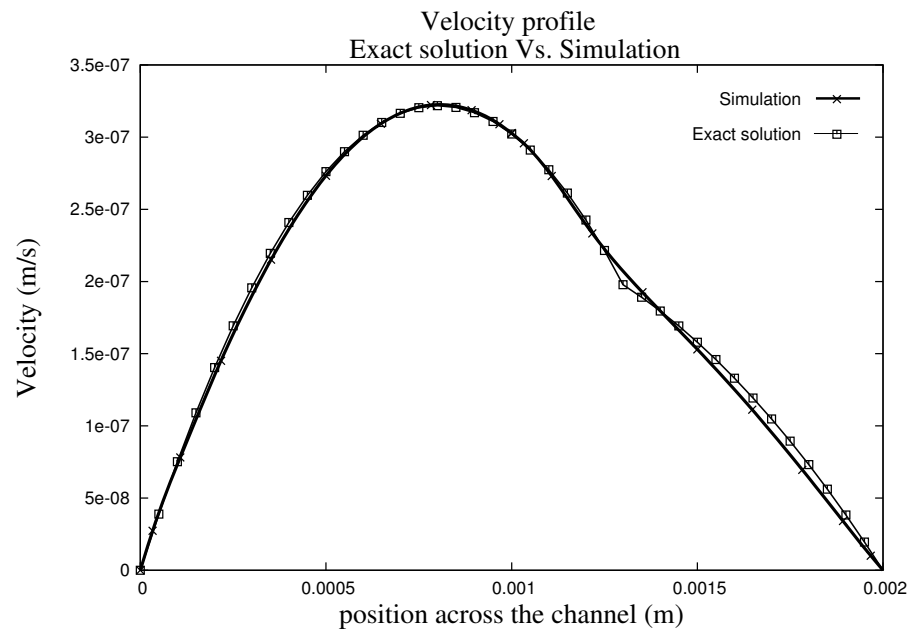
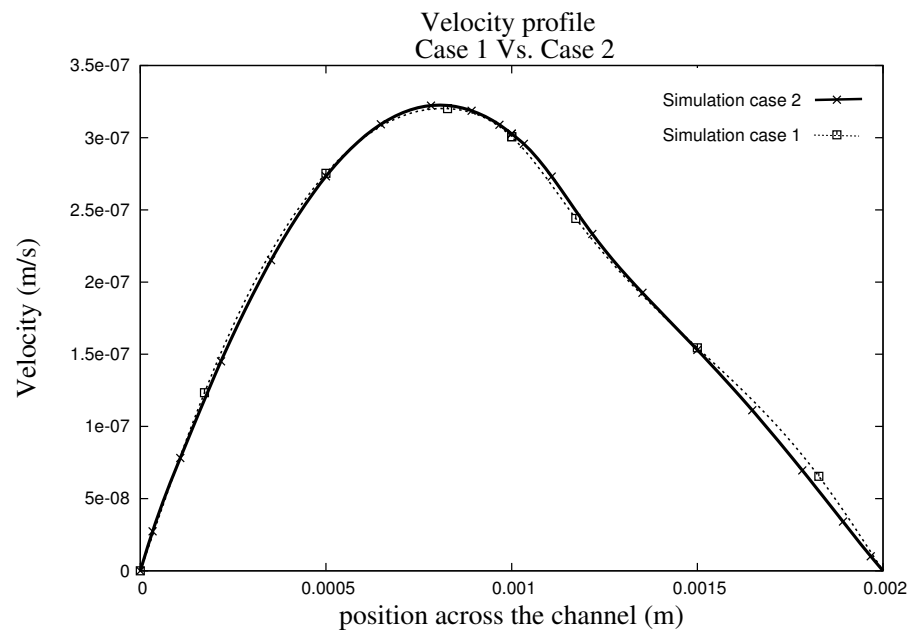


Figure 4.8: Simulated velocity profile for Case 2

walls of the channel are used as well as the outlet boundary conditions, however the inlet boundary conditions are changed to accommodate the flow conditions. In fact, the interface is not a fixed value in the CFD program anymore, however it is now determined according to the flow conditions as explained in Chapters 2 and 3. Therefore the longer the channel the more accurate the interface location, and then the more accurate the results. The average velocities for both branches of the channel are calculated using the flow rate determined in Sec. 4.3 as previously mentioned. The difference is that the width used to calculate the uniform velocity, $width_{entranceA}$, which is equal to $width_{entranceB}$ for this case, is the half width of the channel (D). The velocity profiles extracted from this case are compared to the previous case (Sec. 4.3.2) (a steady state quasi-fully developed case) and to the results obtained from the exact solution. Therefore, the velocities imposed at inflow in the program are $V_{averageA} = 3.12 \times 10^{-7} \frac{m}{s}$ and $V_{averageB} = 7.88 \times 10^{-8} \frac{m}{s}$ for the bottom and top branches respectively.



(a) Velocity profile: simulation vs. exact solution for Case 2



(b) Simulated velocity profiles: Case 2 vs. Case 1

Figure 4.9: Velocity profiles comparison for Case 2

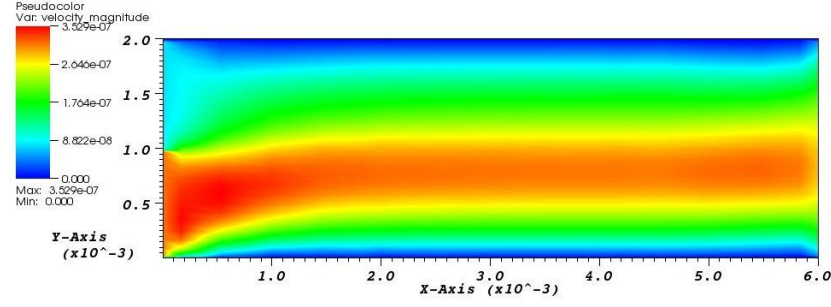


Figure 4.10: Simulated velocity distribution in the channel for Case 3

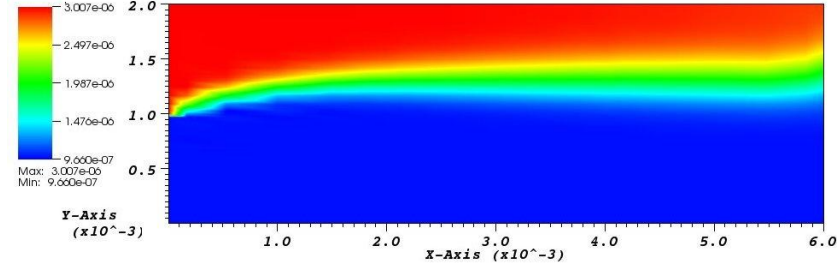


Figure 4.11: Simulated viscosity distribution in the channel for Case 3

4.3.3.2 RESULT AND DISCUSSION

The velocity distribution and the viscosity distribution in the channel are presented in Figs. 4.10 and 4.11, respectively. The velocity profile shown in Fig. 4.12 is extracted from the channel at a location of $x = 5.0 \text{ mm}$. The viscosity distribution in the channel displayed in Fig. 4.11 provides information about the location of the interface. Because of the diffusion layer, it is not easy to determine the exact location of the interface, however, at steady state, we see that the interface reaches $y = 1.3 \text{ mm}$ approximately (measured from the bottom of the channel), which results in δ to be equal to the value set for the exact solution ($\delta = 0.3 \text{ mm}$). This result confirms the validity of the numerical model concerning the interface determination according to the two fluid flow. Looking at Fig. 4.13(b), a small difference can be noticed between the velocity profiles obtained from Case 2 and Case 3. This difference can be explained by the fact that the flow is not completely fully developed in the channel, for Case 3, despite the elongation of the domain. The same remark can be made looking at Fig. 4.13(a), where the velocity profile resulting from Case 3 is compared to the results from the exact solution. In addition, looking at the interface location in

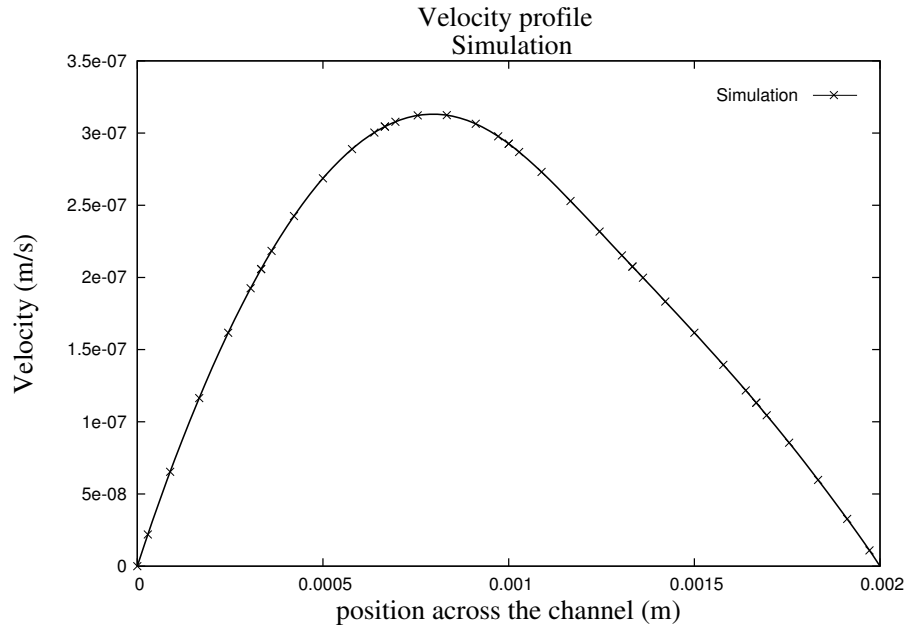


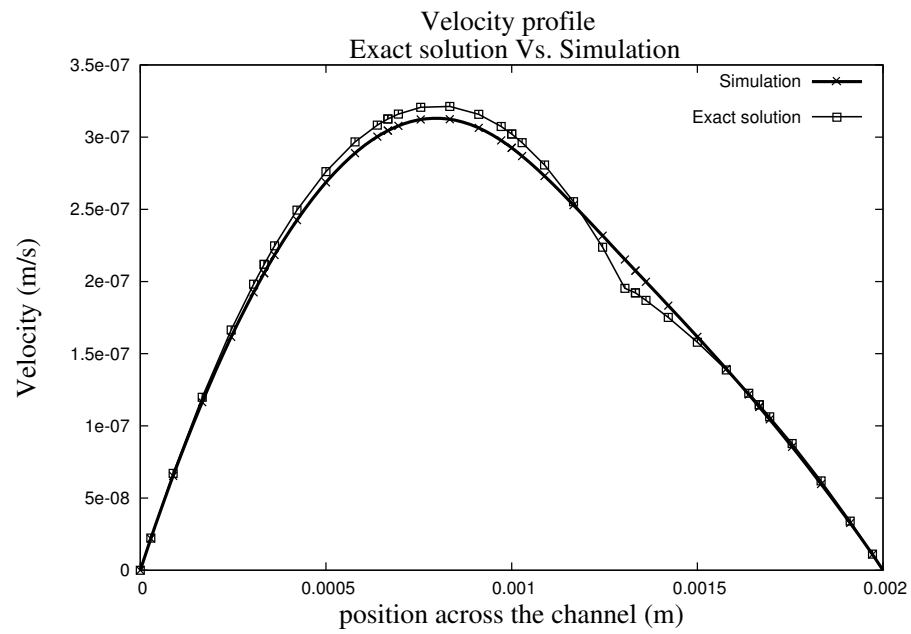
Figure 4.12: Simulated velocity profile for Case 3

Fig. 4.13(a), the two velocity profiles do not coincide. The error for this case varies from 0.5% to 8%.

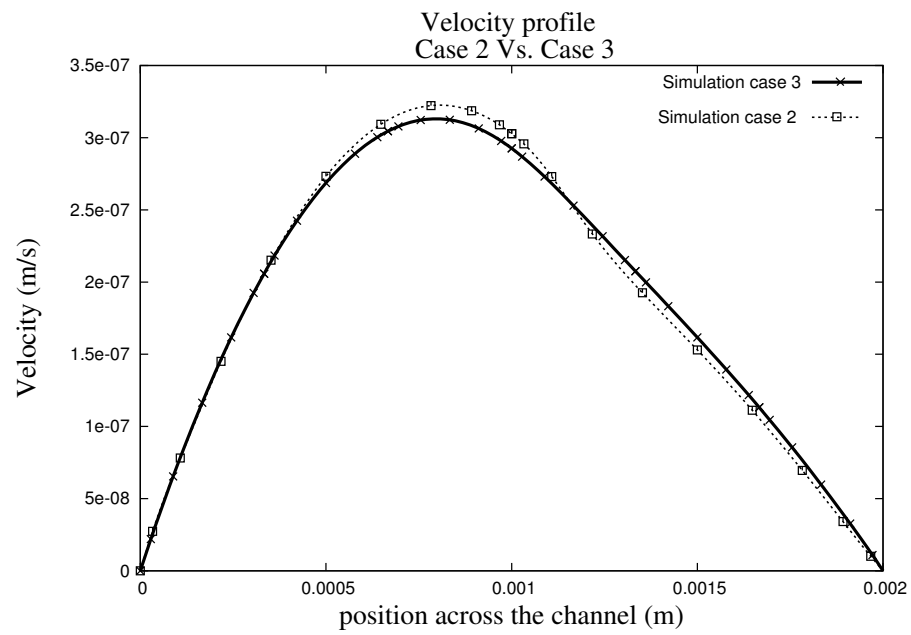
4.3.4 CASE 4

4.3.4.1 PROBLEM STATEMENT

The previous cases, Cases 1 to 3, verify the simulation program when comparing it to the exact solution. However, the channel geometry used for these cases does not reflect the actual channel geometry (Y-channel configuration). Therefore, for this present case, Case 4, the geometry is changed to accommodate the real channel configuration, where two branches are added at the entrance. Each fluid enters from a branch to meet at the intersection. Adding the branches allows the velocity distribution in the channel to be more uniform since at the intersection, the two fluids enter with a parabolic velocity profile, as opposed to the previous cases where the two fluids enter with an uniform velocities. The branch thicknesses are considered to be half the channel thickness, so that $width_{entranceA}$ and $width_{entranceB}$ are equal to D . Therefore the same boundary conditions as in Case 3 are used for this case, where



(a) Velocity profile: simulation vs. exact solution for Case 3



(b) Simulated velocity profiles: Case 3 vs. Case 2

Figure 4.13: Velocity profiles comparison for Case 3

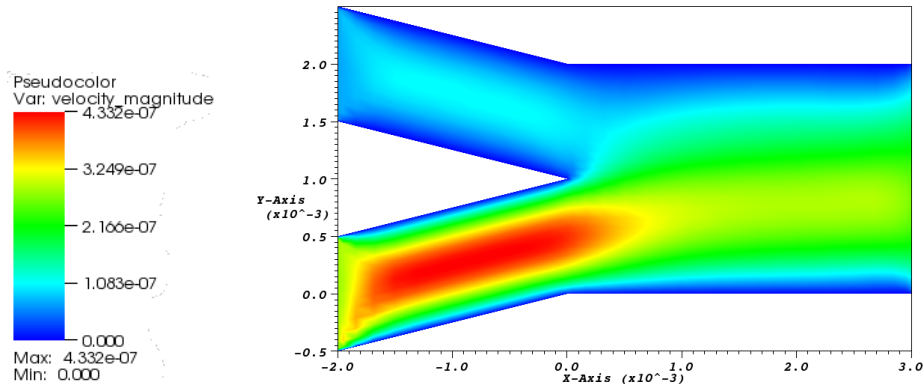


Figure 4.14: Simulated velocity distribution in the channel for Case 4

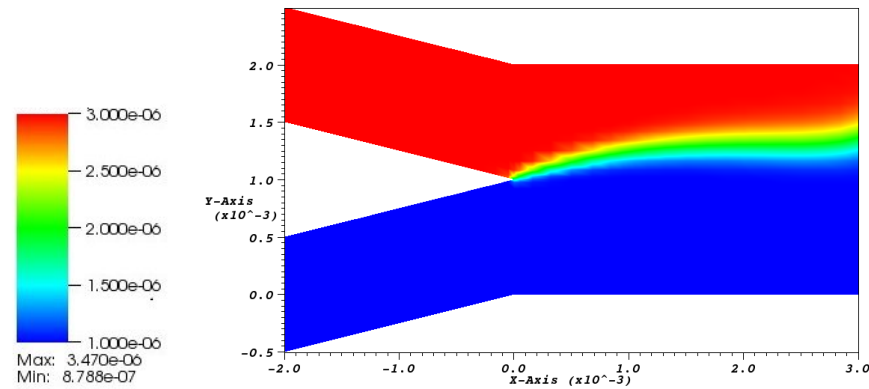


Figure 4.15: Simulated viscosity distribution in the channel for Case 4

the no slip boundary condition is set for the bottom and top walls of the channel, the null pressure is set at the outlet of the channel and a uniform velocity is set at the branches. These uniform velocities applied for this case are the same as in Case 3: $V_{averageA} = 3.12 \times 10^{-7} \frac{m}{s}$ and $V_{averageB} = 7.88 \times 10^{-8} \frac{m}{s}$ for the bottom and top branches respectively.

4.3.4.2 RESULT AND DISCUSSION

The velocity distribution and the viscosity distribution in the channel are presented in Fig. 4.14 and 4.15, respectively.

The resultant velocity profile extracted from the channel at a location of $x = 2.8 \text{ mm}$ is presented in Fig. 4.16. Changing the geometry of the channel by adding the branches at the entrance does not affect the location of the interface ($y = 1.3 \text{ mm}$

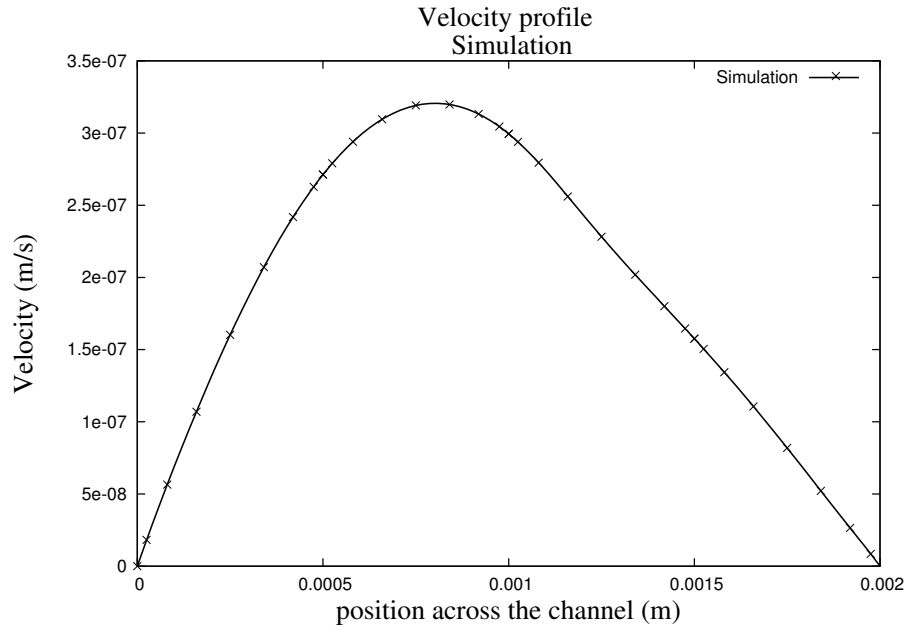


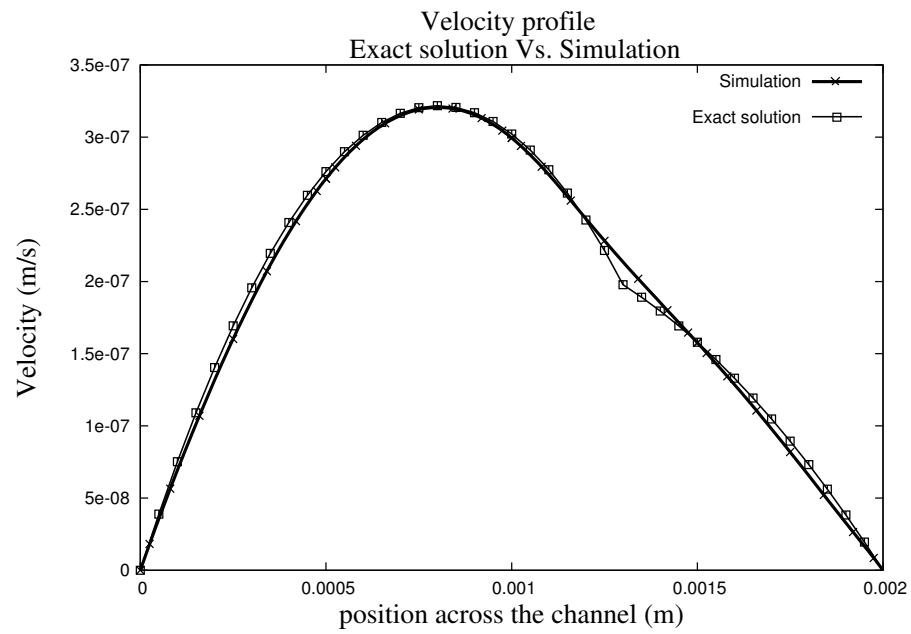
Figure 4.16: Simulated velocity profile for Case 4

measured from the bottom of the channel) as seen in Fig. 4.15. However, at the intersection of the Y-channel, the flow is slightly irregular. This phenomenon is due to the unsteady nature of the flow at that specific location where the two fluids enter into contact. Fig. 4.17(a), comparing the simulation velocity profile to the exact solution, shows that the two curves coincide with only a very small variation at the interface. The percentage error varies from 1% to 9%. We conclude that adding branches to insert the fluids results in more accurate and realistic velocity profiles than using the geometry described in the other cases, as seen in Fig. 4.17(b).

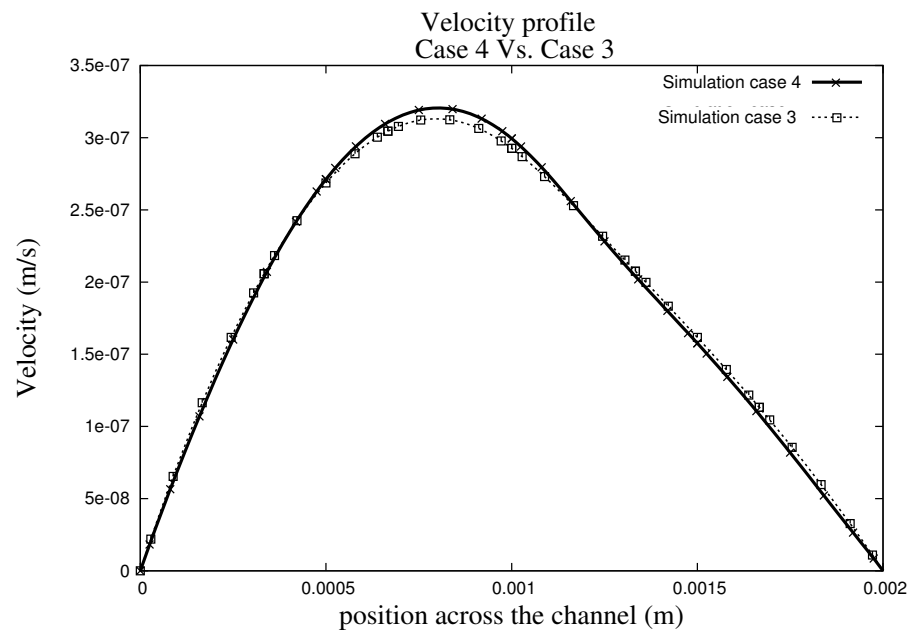
4.3.5 CASE 5: 3D CASE

4.3.5.1 PROBLEM STATEMENT

The previous cases provide a good approximation of reality, however, they are only in 2D. In order to obtain more accurate and realistic results, a 3D simulation describing the same situation as in Case 4 with a fixed depth of $depth = 1 \text{ mm}$, is presented in this section. The advantage is that the variation in depth affects the shear rate and stress, as well as the velocity of the fluids. Fig. 4.18 presents the mesh distribution



(a) Velocity profile: simulation vs. exact solution for Case 4



(b) Simulated Velocity profiles: Case 4 vs. Case 3

Figure 4.17: Velocity profiles comparison for Case 4

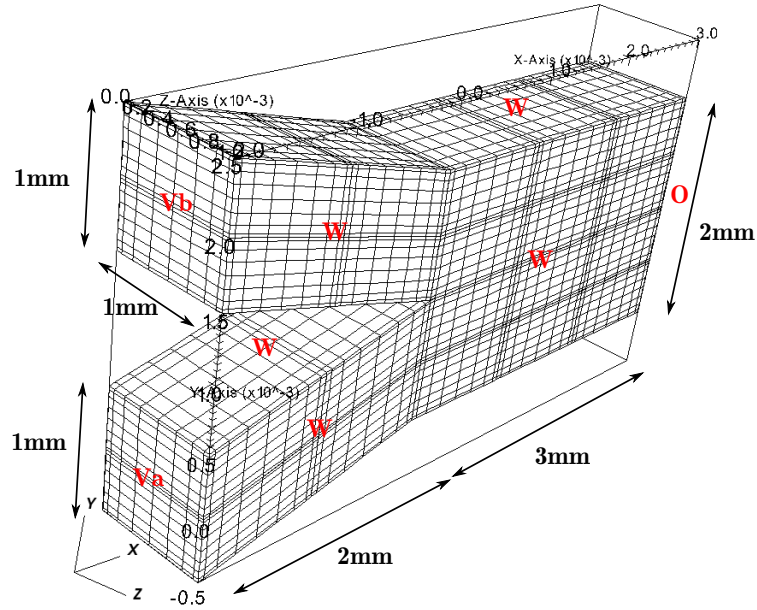


Figure 4.18: Mesh distribution within the channel and boundary conditions

in the 3D Y-channel configuration, its dimensions and the boundary conditions set for this specific case.

4.3.5.2 RESULT AND DISCUSSION

The velocity and viscosity distribution are shown in Figs. 4.19 and 4.20, respectively.

Fig. 4.21(a) provides the velocity profile from the middle depth of the channel and along the channel at 2.8 mm . Comparing the resulting profile to the previous 2D results in Case 4, it is clear that the velocity increases with the 3D case, as expected since changing the depth of the channel greatly affects the shear stress and hence the velocity. It can also be noted, for the 3D case, that the interface location can be determined through the velocity profile within the channel whereas it is not possible for the 2D case. Therefore, the simulations for the microchannel will be performed in 3D in order to obtain better and more accurate results to be compared to the experiments.

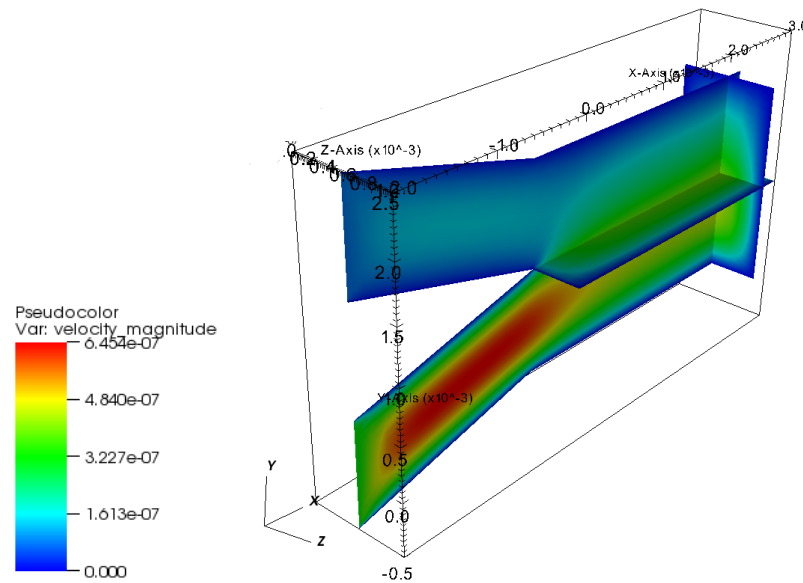


Figure 4.19: Simulated velocity distribution in the channel for Case 5

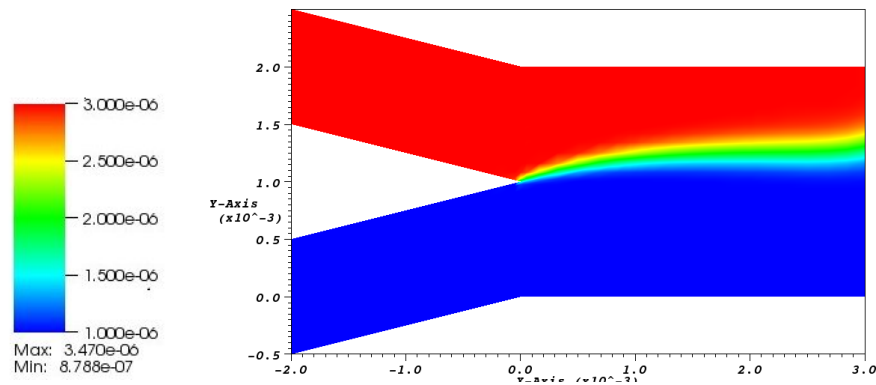
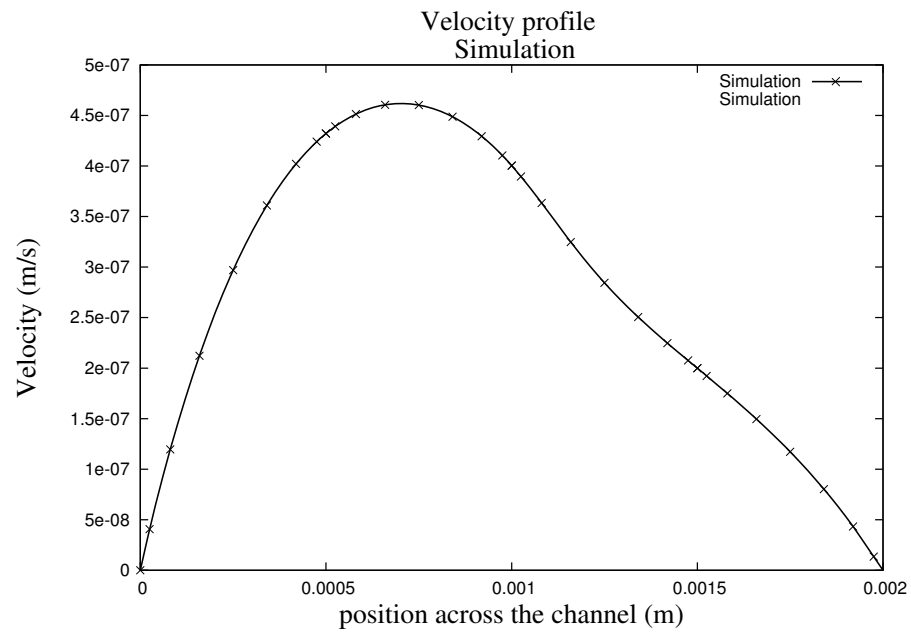


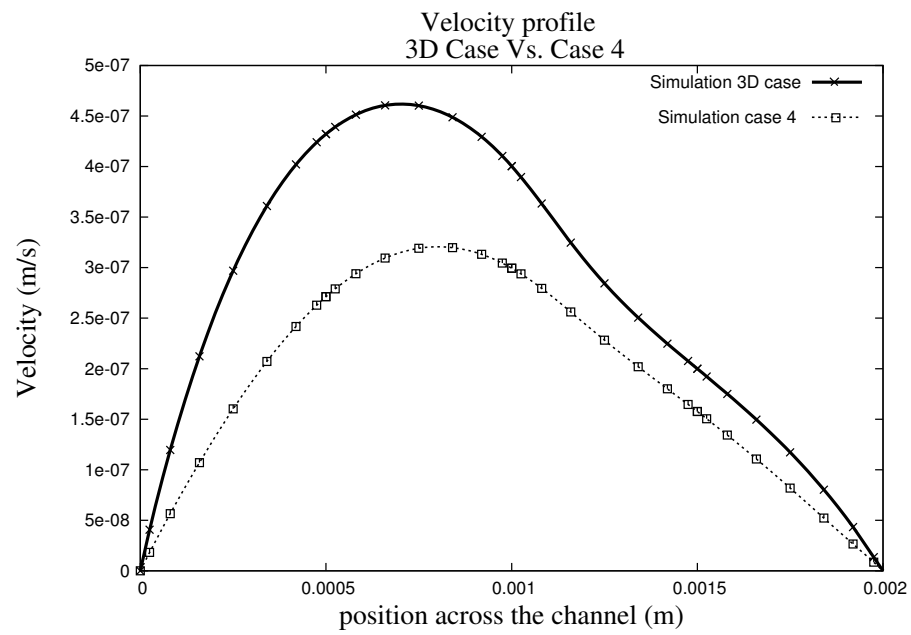
Figure 4.20: Simulated viscosity distribution in the channel for Case 5

4.4 CONCLUSION

In this chapter, we verified our numerical model by comparing the simulations to an analytical solution where the interface between the two fluids A and B is fixed. The results obtained from the analytical solution were then used as input boundary condition in our solver to numerically locate the interface and compare the findings to the value set. Several numerical tests, changing boundary conditions from periodic to I/O, varying the geometry of the channel and the dimension number, were performed in order to obtain the appropriate interface location and velocity profile that would agree with the analytical results. It was found that using the two branches as input



(a) Simulated velocity profile for Case 5



(b) Simulated velocity profiles: Case 4 vs. Case 5

Figure 4.21: Velocity profiles for Case 5

geometry, the numerical results agreed well with the analytical results. However, the three dimensional simulations provided a smoother curve that clearly indicates the location of the interface.

NEWTONIAN FLUIDS: SIMULATIONS VS. EXPERIMENTS

The numerical model, developed in order to obtain a linear velocity profile in the viscous fluid layer (referred as "blood layer" in this chapter), is compared to experimental tests performed with two Newtonian fluids (glycerol + water).

5.1 TWO-DIMENSIONAL NUMERICAL SIMULATIONS

In order to first understand the behaviour of the two fluid flow according to the boundary conditions set, 2D simulations are performed with the $100 \times 50 \mu m$ microchannel where, several velocity ratios between the two branches are tested while the flow rate remains constant in the channel. The flow rate used is based on the flow in microcirculation and was decided to be $Q = 10 \frac{\mu l}{hr}$. Therefore the velocity ratio $V_{ratio} = \frac{V_A}{V_B}$ was increased from 1 to 8 in order to visualize the two fluid flow in the channel and their corresponding velocity profile. For all cases, fluid B simulating blood, having a kinematic viscosity of $\nu_{top} = 3 \frac{mm^2}{s}$, enters from the top branch with a uniform velocity, while fluid A, having a viscosity $\nu_{top} = 1 \frac{mm^2}{s}$, enters from the bottom branch with a uniform velocity depending on the velocity ratio. The velocity profiles obtained from these cases, increasing the velocity ratio from 1 to 8, are shown in Fig. 5.2. We notice from this figure that the velocity profile is almost completely linear for $V_{ratio} \geq 4$. However when looking at the viscosity distribution for these cases and locating the interface between the two fluids (Table 5.1), we can see that the "blood layer" is very small for $V_{ratio} > 4$. In fact, this "blood layer" varies from 15 to $20 \mu m$ for these velocity ratios higher than 4, which is a thickness too small to study the blood aggregates.

With the 2D simulations, it is difficult to control the shear rate values since it is assumed that the channel is infinitely deep, which is not the case experimentally.

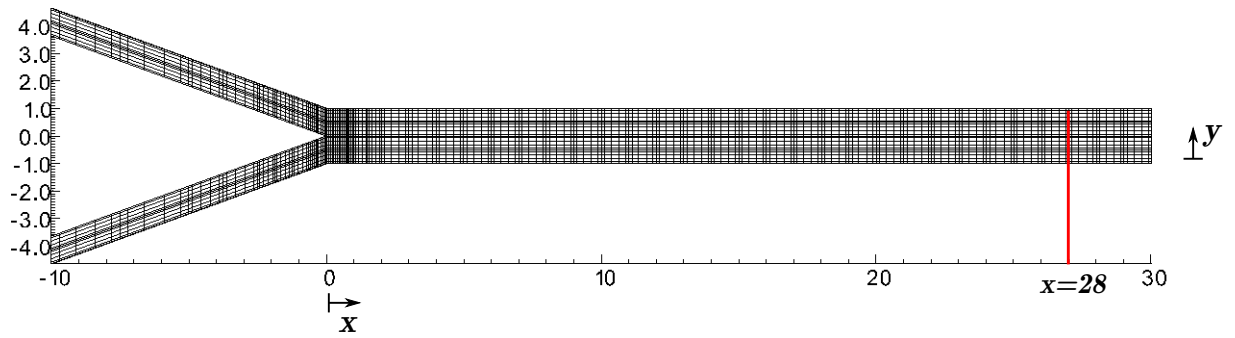


Figure 5.1: Dimensionless channel geometry used for the numerical simulations.

Table 5.1: 2D Numerical shear rate and interface location for $100 \times 50 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ for velocities ratio from 1 to 8. (Re: Reynolds number based on the average velocity of the bottom layer of the channel). The interface is measured from the bottom of the channel as shown in Fig. 5.1.

Ratio	Shear (s^{-1})	Interface (μm)	Re (10^{-2})
1	14.85	51.60	2.77
2	16.58	65.11	3.70
3	17.27	73.39	4.16
4	16.87	75.00	4.44
5	17.17	80.10	4.62
6	16.71	80.10	4.76
8	16.90	84.88	4.93

Therefore three-dimensional (3D) simulations are required.

5.2 THREE-DIMENSIONAL NUMERICAL SIMULATIONS

After several tests performed in order to determine the ideal parameters during the design process, verification of the linearity of the profile within the "blood layer" is still needed. For this purpose, several microchannel dimensions were tested with different velocity ratios keeping the same viscosity ratio of 3. In order to determine the shear rate accurately, three dimensional simulations were performed to control and vary the range of shear according to the channel dimensions. The $100 \times 50 \mu m$, $100 \times 100 \mu m$, $150 \times 33 \mu m$ and $170 \times 64 \mu m$ microchannels are employed for this

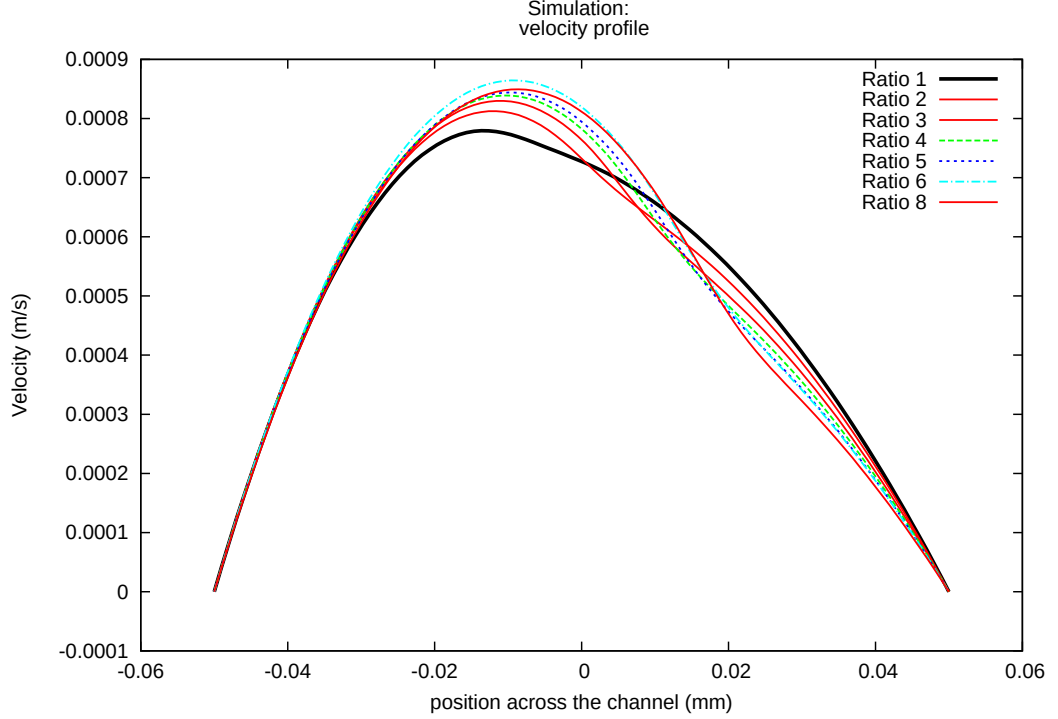


Figure 5.2: Velocity profiles for velocity ratios of 1 to 8 extracted from the 2D simulation performed with the $100 \times 50 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

investigation with flow rates of $Q = 10 \frac{\mu l}{hr}$ and $Q = 5 \frac{\mu l}{hr}$.

5.2.1 $100 \times 50 \mu m$ AND $100 \times 100 \mu m$ MICROCHANNELS WITH A FLOW RATE OF $10 \frac{\mu l}{hr}$

As mentioned in Sec. 3.4, the tests were first performed with the $100 \times 50 \mu m$ microchannel to ensure the linearity of the velocity profile within the "blood layer". Three different velocity ratios were tested: $V_{ratio} = \frac{V_A}{V_B} = \frac{V_{bottom}}{V_{top}} = 1, 2$ and 4 , keeping the same flow rate of $Q = 10 \frac{\mu l}{hr}$. The fluid that simulates blood (fluid B) enters from the top branch and is assumed to have a viscosity of $\nu_{top} = 3 \frac{mm^2}{s}$, while fluid A enters from the bottom branch and is assumed to have a viscosity of $\nu_{top} = 1 \frac{mm^2}{s}$. Comparing the velocity profiles obtained for the three cases in Fig. 5.3, we see that for $V_{ratio} = 4$ the velocity profile is almost linear within the layer of fluid B, which represents 35% of the channel thickness ($35 \mu m$). This "blood layer" represents the ideal thickness to study RBC aggregation since it is not too small

Table 5.2: 3D Numerical shear rate for $100 \times 50 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ (Re: Reynolds number based on the average velocity of the bottom layer of the channel)

Ratio	Shear (s^{-1})	Velocity ($\frac{mm}{s}$)	Interface (μm)	Re (10^{-2})
1	15.72	0.832	47.06	2.77
2	17.22	0.707	58.93	3.70
4	19.61	0.548	72.06	4.44

to restrict the aggregation formation but also not too large to obtain a non-linear velocity profile. The shear rate values obtained from this case, shown in Table 5.2, were calculated based on the interface location and the velocity at the interface. For the simulation results, the interface between the two fluids was found without considering the diffusion layer. Shear rate values for the $100 \times 50 \mu m$ microchannel, vary from about $15 s^{-1}$ to about $20 s^{-1}$ when increasing the ratio of velocity from 1 to 4. This range of shear rate is not within that where RBC can aggregate between (1 to $10 s^{-1}$). Therefore, the depth of the channel was increased in order to decrease the values of shear rate the microchannel of $100 \times 100 \mu m$. It was found that the shear rate values decreases by half the previous values found with the $100 \times 50 \mu m$ microchannel, however, since the depth was increased it would be difficult to visualize the RBC aggregation. After verification of the dimensions, as mentioned in Sec. 3.4 it was found that channels supposedly $100 \times 50 \mu m$ and $100 \times 100 \mu m$ are in reality $150 \times 33 \mu m$ and $170 \times 64 \mu m$ microchannels respectively which affect the velocity profile in the channel as well as the shear rate values for the "blood layer".

The 3D simulations were then performed for the right dimensions of the channels of $150 \times 33 \mu m$ and $170 \times 64 \mu m$.

5.2.2 $150 \times 33 \mu m$ MICROCHANNEL WITH A FLOW RATE OF $10 \frac{\mu l}{hr}$

The numerical set up for this case is shown in Fig. 5.4. As previously determined in Chapter 3, the fluid simulating blood enters with a kinematic viscosity of $\nu_{top} = 3 \frac{mm^2}{s}$, while the entraining fluid enters form the bottom branch with a kinematic

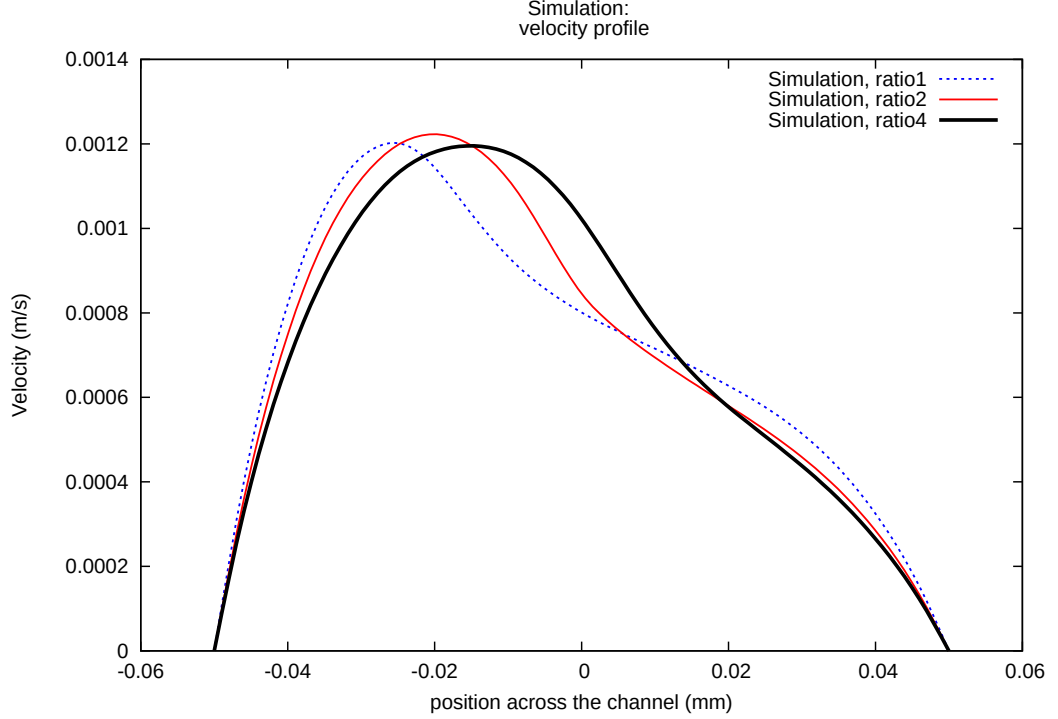


Figure 5.3: Velocity profiles for a velocity ratio of 1, 2 and 3 extracted from the $100 \times 50 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

viscosity of $\nu_{bottom} = 1 \frac{mm^2}{s}$ such as water. Three different cases are studied for this microchannel. As a first test, the two fluids enter into the channel with the same velocity. The ratio of velocity between the two branches V_{ratio} is increased from 1 to 2 for the second test and to 4 for the third test. Therefore, in all cases the velocity of fluid A on the bottom is either equal to or higher than the velocity of fluid B simulating blood, keeping the same flow rate in the channel for all cases.

5.2.2.1 VELOCITY RATIO OF 1

As mentioned, this first test consists of the two different fluids A and B entering the $150 \times 33 \mu m$ channel with the same velocity of $V_{top} = V_{bottom} = 0.56 \frac{mm}{s}$ in order to obtain a flow rate of $Q = 10 \frac{\mu l}{hr}$. The velocity and viscosity distributions for $V_{ratio} = 1$ are shown in Figs. 5.5 and 5.6 respectively. We can deduce, looking at Fig. 5.6, that the "blood layer" occupies about 70% of the channel thickness as expected since fluid B is three times more viscous than fluid A so it expands faster in the channel.

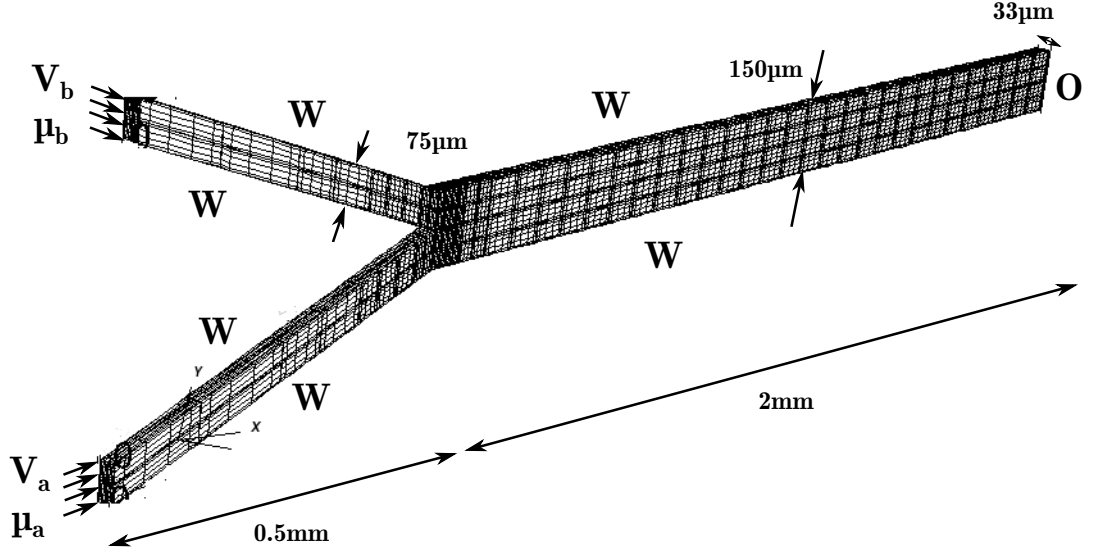


Figure 5.4: Numerical set up for the microchannel of $150 \times 33 \mu\text{m}$

Extracting the velocity profile at a location of $x = 28$ (where x represents the dimensionless position along the channel as shown in Fig 5.1) along the channel, Fig. 5.7 shows that the velocity profile within this layer of fluid B is not linear. In fact, we see that, within the layer of fluid A, the velocity increases and reaches its maximum then decreases and form a large plateau where the interface between the two fluids is located. The velocity then decreases when reaching the wall facing fluid B. Due to the nonlinearity, this case is not suitable for our application to obtain a constant shear rate.

5.2.2.2 VELOCITY RATIO OF 2

For this second test, fluid B, assumed to simulate blood, enters from the top branch with a uniform velocity of $V_{top} = 0.374 \frac{\text{mm}}{\text{s}}$, while fluid A enters from the lower branch at a velocity of $V_{bottom} = 0.748 \frac{\text{mm}}{\text{s}}$ to obtain $V_{ratio} = 2$ keeping the average flow rate in the channel of $Q = 10 \frac{\mu\text{l}}{\text{hr}}$. The viscosity distribution in the channel shown in Fig. 5.9 shows that the thickness of fluid B layer (top layer of the channel) covers about 55% of the channel width. The velocity profile corresponding to the $V_{ratio} = 2$ exhibits a nonlinear velocity profile within the layer of fluid B as shown in Fig. 5.10. However, when comparing Figs. 5.7 and 5.10, we see that both curves follow the same

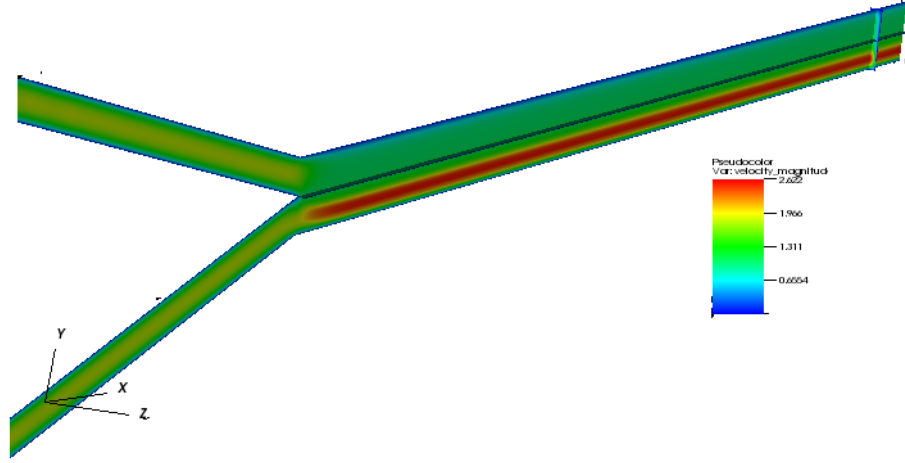


Figure 5.5: Velocity distribution for a velocity ratio of 1 for the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

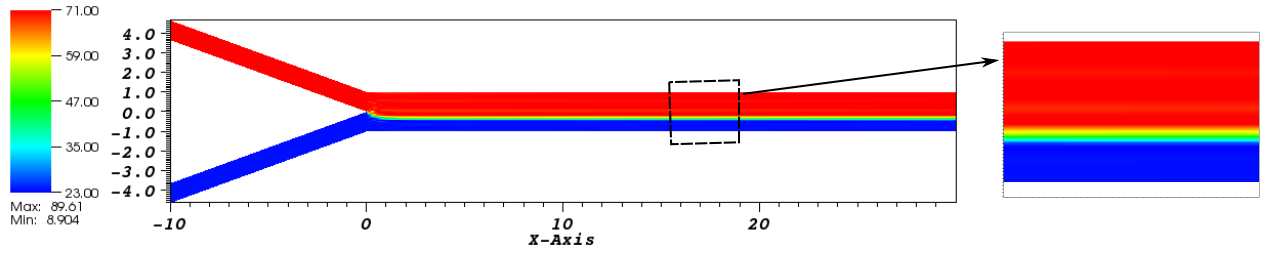


Figure 5.6: Viscosity distribution for a velocity ratio of 1 for the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

shape, where the maximum velocity is located within fluid A layer (bottom part of the channel) and the plateau indicates the location of the interface. This case also is not suitable for our application to obtain a constant shear rate

5.2.2.3 VELOCITY RATIO OF 4

Keeping the same average flow rate of $Q = 10 \frac{\mu l}{hr}$ in the channel as the previous tests, the velocity ratio is increased so that the velocity of fluid B entering from the top branch becomes $V_{top} = 0.224 \frac{mm}{s}$ and the velocity of fluid A entering from the bottom branch becomes $V_{bottom} = 0.897 \frac{mm}{s}$.

The results of the velocity and viscosity distributions obtained from this case are shown in Figs. 5.11 and 5.12. Looking closely at the viscosity distribution, we see that the fluid simulating blood covers 35% of the channel thickness: approximately

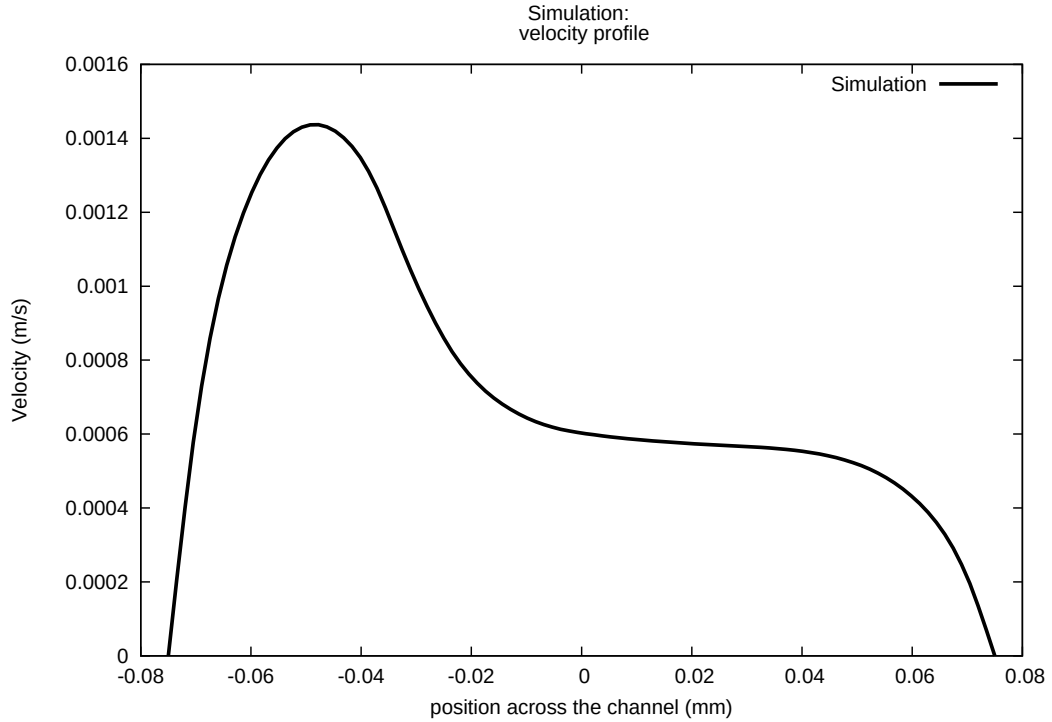


Figure 5.7: Velocity profile for a velocity ratio of 1 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

$52 \mu m$. However, through Fig. 5.13 representing the velocity profile extracted from the channel at the location of $x = 28$ along the channel, we note that the velocity within this specific layer of fluid B is not completely linear, while for the $100 \times 50 \mu m$ microchannel the velocity profile in the "blood layer" is almost completely linear as shown in Fig. 5.3.

The experimental results are compared to the results obtained in the simulation for the $150 \times 33 \mu m$ microchannel despite the non completely linear profile since the experiments were performed based on the results obtained with the $100 \times 50 \mu m$ microchannel, where it was found that the velocity profile is quasi-linear for a ratio of velocity of 4 before the dimension verification.

The shear rate values obtained from the tests performed on the $150 \times 33 \mu m$ microchannel with the three different ratios are presented in Table 5.5 where they are compared to the values obtained experimentally. Increasing the velocity ratio from 1 to 4, the shear rate varies from 7 to $11 s^{-1}$. However, for the case to be analyzed,

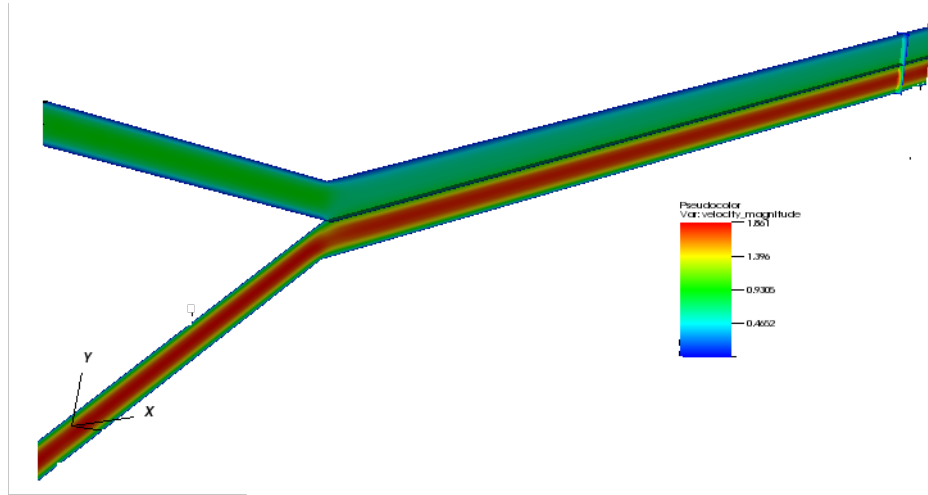


Figure 5.8: Velocity distribution for a velocity ratio of 2 for the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

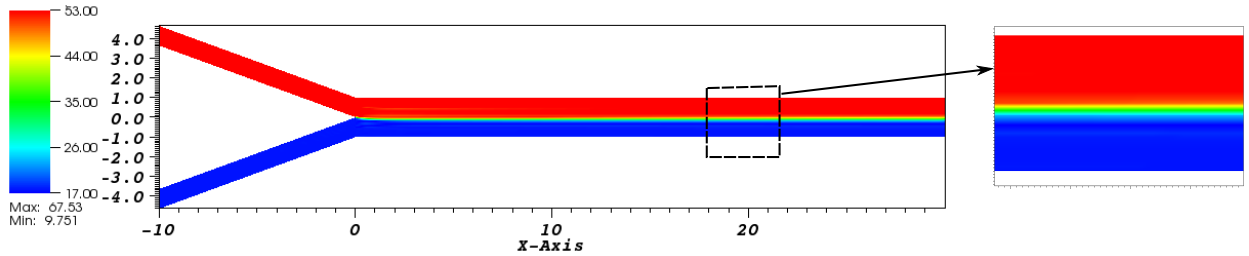


Figure 5.9: Viscosity distribution for a velocity ratio of 2 for the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

$V_{ratio} = 4$, the shear rate is at the limit of the RBC aggregation range. Therefore, the shear rate can be reduced by decreasing the flow rate keeping the same channel geometry.

5.2.3 $150 \times 33 \mu m$ MICROCHANNEL WITH A FLOW RATE OF $5 \frac{\mu l}{hr}$

In order to keep the same geometry but lower the shear rate values, the flow rate within the channel is decreased to $Q = 5 \frac{\mu l}{hr}$, so that fluid B, assumed to have a kinematic viscosity of $\nu = 3 \frac{mm^2}{s}$, enters from the top branch with a uniform velocity $V_{top} = 0.112 \frac{mm}{s}$ while fluid A, having a kinematic viscosity of $\nu = 1 \frac{mm^2}{s}$, enters the channel from the bottom branch with a uniform velocity $V_{top} = 0.448 \frac{mm}{s}$. Figs. 5.14, 5.15 and 5.16 show similar velocity distribution, viscosity distribution and velocity

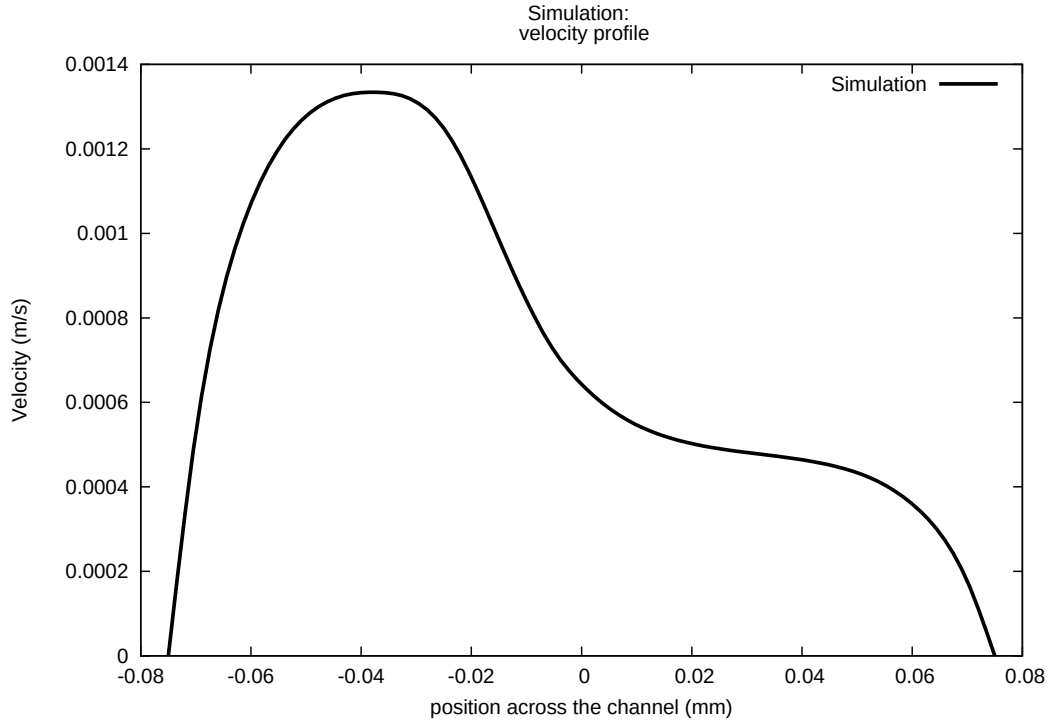


Figure 5.10: Velocity profile for a velocity ratio of 2 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

Table 5.3: 3D Numerical shear rate for $150 \times 33 \mu m$ microchannel with $Q = 5 \frac{\mu l}{hr}$ (Re: Reynolds number based on the average velocity of the bottom layer of the channel)

Ratio	Shear (s^{-1})	Velocity ($\frac{mm}{s}$)	Interface (μm)	Re (10^{-2})
4	5.40	0.22	108.00	3.74

profile as in the $Q = 10 \frac{\mu l}{hr}$ cases but with different magnitudes. In fact, we see that the velocity profile is also not completely linear within the "blood layer" which is also about 35% of the channel thickness, however, the values of velocity are about half the values found with $Q = 10 \frac{\mu l}{hr}$. Additionally the shear rate value, shown in Table 5.3, is also about half the value found previously, as expected for a lowered flow rate.

5.2.4 $170 \times 64 \mu m$ MICROCHANNEL WITH A FLOW RATE OF $10 \frac{\mu l}{hr}$

Similar tests performed previously for the $150 \times 33 \mu m$ microchannel are repeated for the $170 \times 64 \mu m$ microchannel in order to quantitatively measure the change of

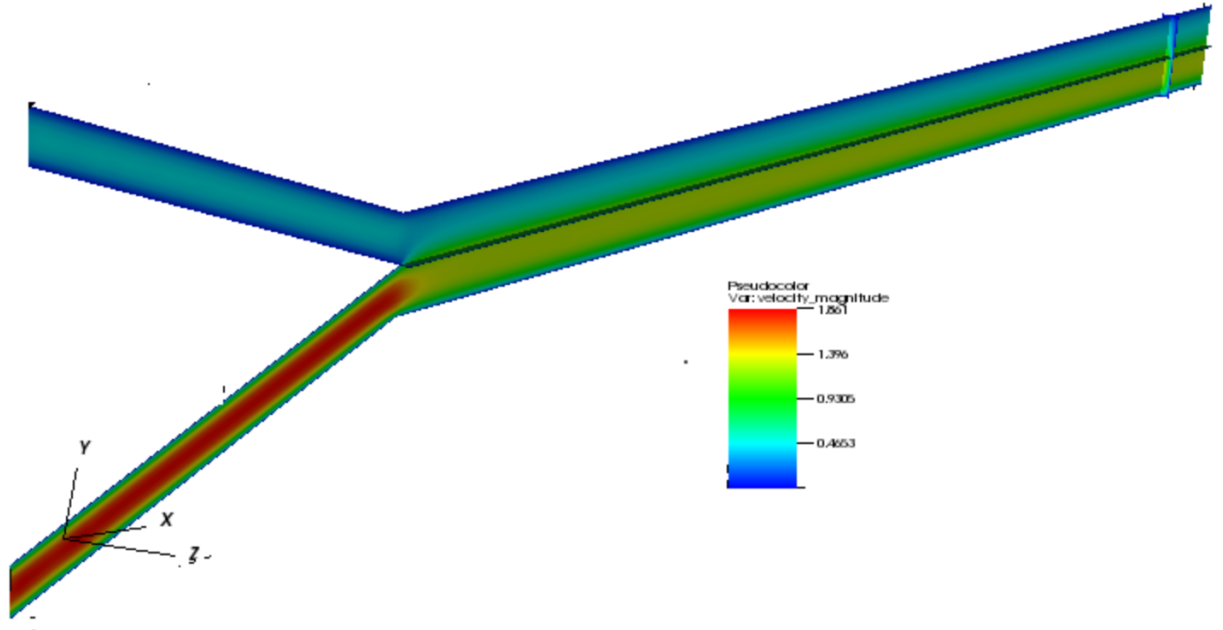


Figure 5.11: Velocity distribution for a velocity ratio of 4 for the $150 \times 33 \mu\text{m}$ microchannel with $Q = 10 \frac{\mu\text{l}}{\text{hr}}$

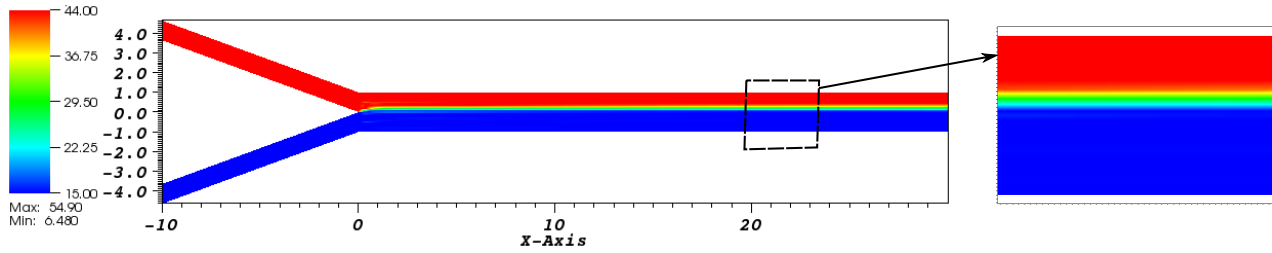


Figure 5.12: Viscosity distribution for a velocity ratio of 4 for the $150 \times 33 \mu\text{m}$ microchannel with $Q = 10 \frac{\mu\text{l}}{\text{hr}}$

the shear rate values. The same flow rate of $Q = 10 \frac{\mu\text{l}}{\text{hr}}$ was tested for $V_{ratio} = 4$ and $\nu_{ratio} = 4$, so that fluid B, assumed to have a kinematic viscosity of $\nu_{top} = 3 \frac{\text{mm}^2}{\text{s}}$, enters from the top branch with a uniform velocity of $V_{top} = 0.102 \frac{\text{mm}}{\text{s}}$, while fluid A enters from the bottom branch with a uniform velocity of $V_{bottom} = 0.408 \frac{\text{mm}}{\text{s}}$. The velocity and viscosity distributions in the microchannel are shown in Figs. 5.17 and 5.18 respectively. The "blood layer" in the channel fills approximately 35% of the channel thickness, as before. Looking at the velocity profile within this range, (Fig. 5.19) we see that within this range the velocity is almost completely linear as desired. Also the numerical shear rate value for this case shown in Table 5.4 is actually

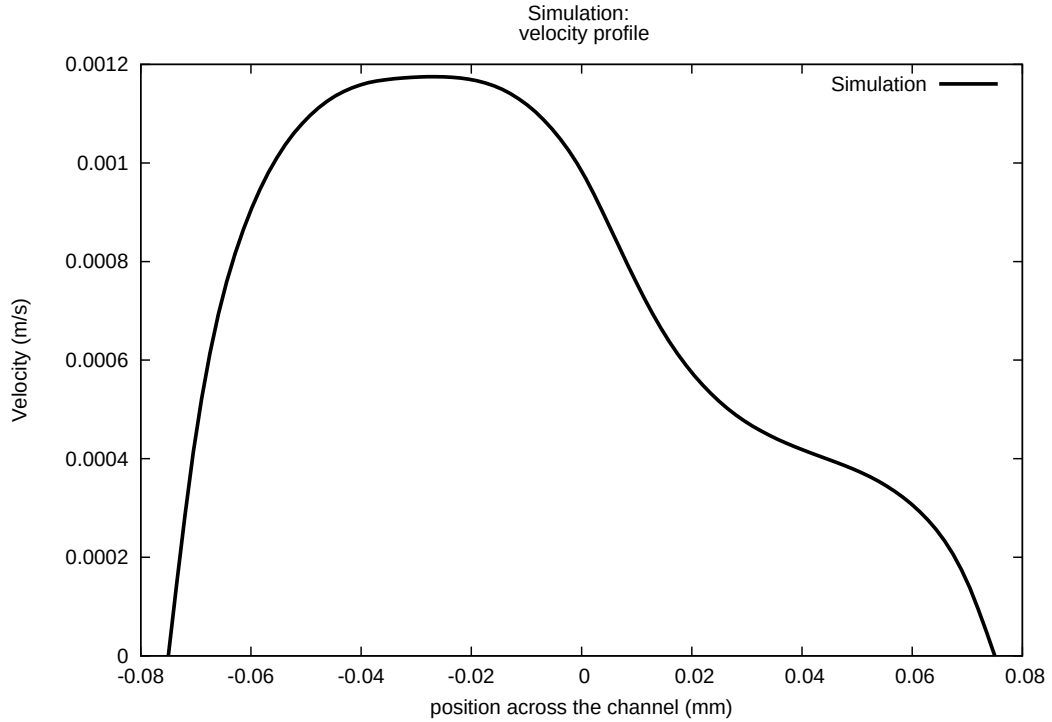


Figure 5.13: Velocity profile for a velocity ratio of 4 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

Table 5.4: Numerical shear rate for $170 \times 64 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ (Re: Reynolds number based on the average velocity of the bottom layer of the channel)

Ratio	Shear (s^{-1})	Velocity ($\frac{mm}{s}$)	Interface (μm)	Re (10^{-2})
4	5.00	0.237	122.50	3.45

within the range of RBC aggregation.

5.2.5 SUMMARY

Two-dimensional and three-dimensional numerical simulations were performed with different channel dimensions in order to determine the ideal channel geometry to obtain shear rate values within the RBC aggregation range. Using the $150 \times 33 \mu m$ and $170 \times 64 \mu m$ geometries, it was found that the "blood layer" thickness was ideal for $V_{ratio} = 4$ where the velocity profile is almost linear. With this velocity ratio, the shear rate values were found to be within the RBC aggregation range (1 to $10 s^{-1}$).

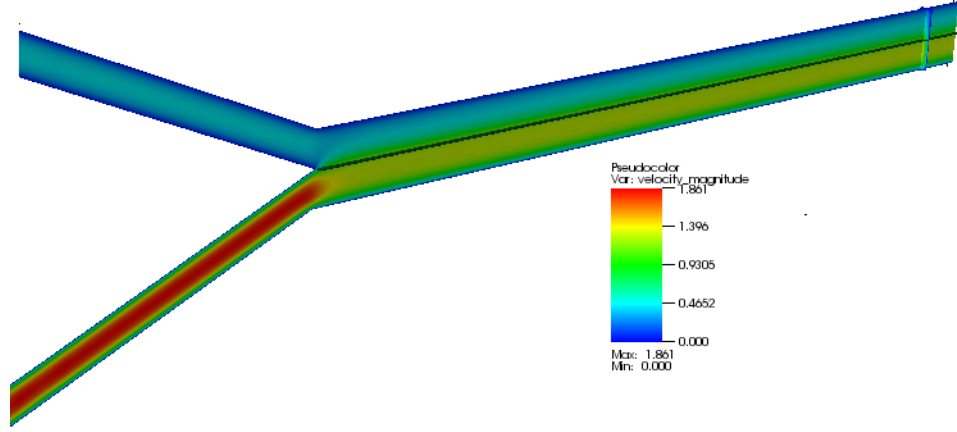


Figure 5.14: Velocity distribution for a velocity ratio of 4 for the $150 \times 33 \mu m$ microchannel with $Q = 5 \frac{\mu l}{hr}$

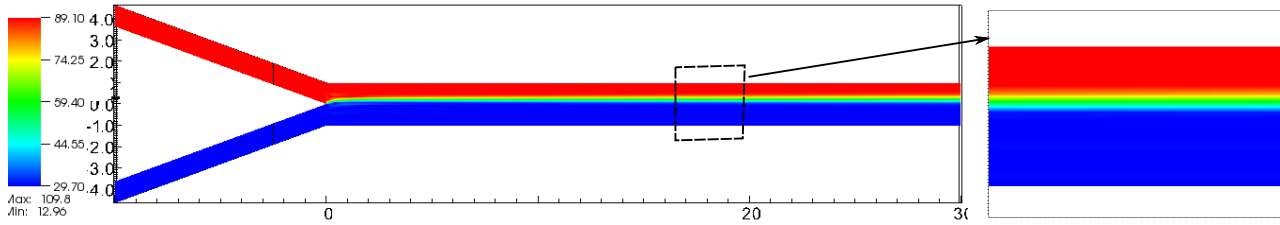


Figure 5.15: Viscosity distribution for a velocity ratio of 4 for the $150 \times 33 \mu m$ microchannel with $Q = 5 \frac{\mu l}{hr}$

The same model is tested experimentally for comparison with the numerical results.

5.3 EXPERIMENTAL VS. NUMERICAL NEWTONIAN RESULTS

In order to validate our model, several experimental tests are performed, first, with a Newtonian fluid in the laboratory using the μPIV system are then compared to the numerical simulations described in the previous Sec. 5.2. For all of the following experiments, the fluid simulating blood is a solution of glycerol diluted so that the dynamic viscosity of the mixture is approximately $\mu_{glycerol} = \mu_{topB} = 3 \text{ cP}$. The advantage of glycerol is its property to dissolve in water so that the viscosity of the solution can be controlled and so that the interfacial tension between the two fluids is minimized to reduce the risks of flow unsteadiness. The mass percentage of the glycerol solution is based on the values provided in [129]. In order to obtain the desired value of viscosity, the mass fraction required is found to be about $\Phi = 36\%$,

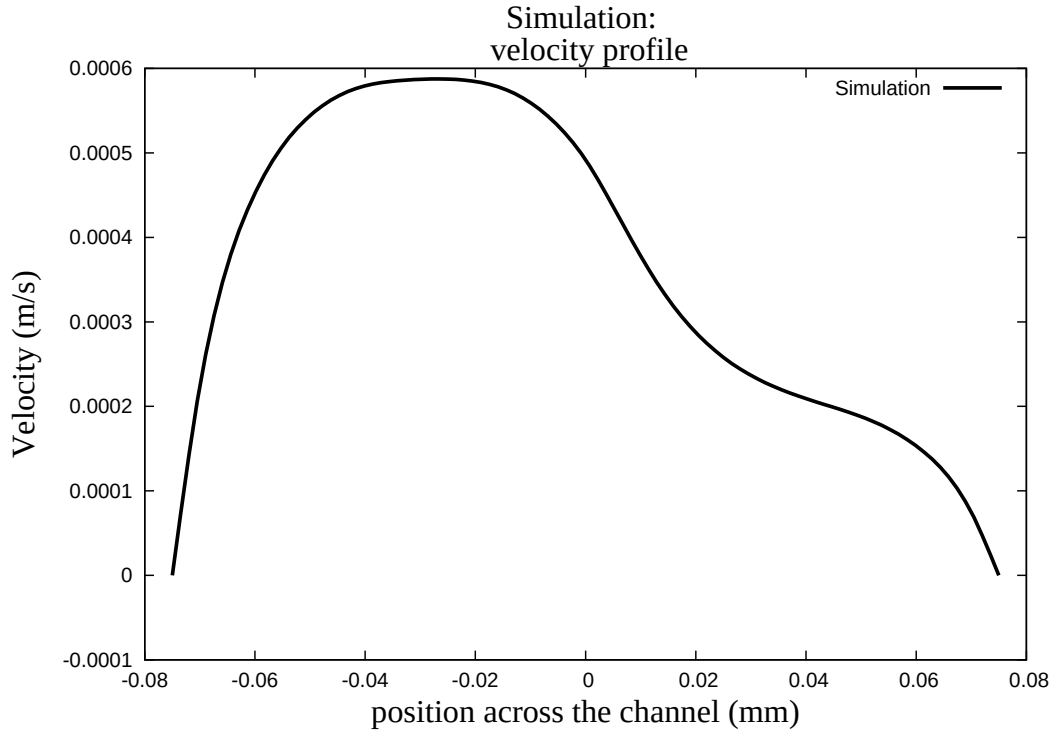


Figure 5.16: Velocity profile for a velocity ratio of 4 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 5 \frac{\mu l}{hr}$

considering the tracer particles solutions incorporated with the fluid.

As mentioned previously, the experimental tests are performed using the $150 \times 33 \mu m$ and $170 \times 50 \mu m$ microchannels, the actual dimensions of the channels.

5.3.1 $150 \times 33 \mu m$ MICROCHANNEL WITH A FLOW RATE OF $10 \frac{\mu l}{hr}$

Experimentally, we reproduce the conditions simulated numerically, where fluids A and B enter from the two different branches to obtain an average flow rate in the channel of $Q = 10 \frac{\mu l}{hr}$. As described in Chapter 2, the microchannel to be tested is connected to the μPIV system via small connectors glued to the tubes that are attached to the syringes containing the two fluids. The syringes are pushed by means of two different pumps to allow the difference in velocity between the two fluids. The second fluid used to entrain the glycerol solution is distilled water. As a first test, the tracer particles are only introduced within the glycerol layer in order to capture the location of the interface between the two fluids. Then the tracer particles are

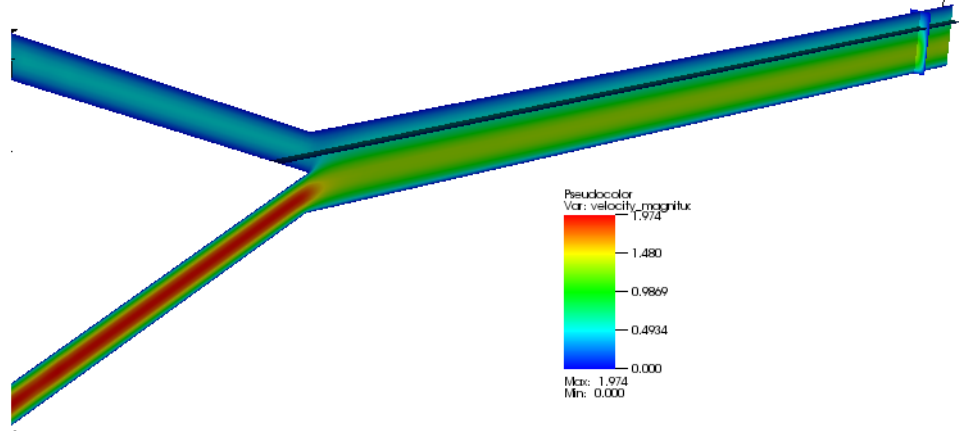


Figure 5.17: Velocity distribution for a velocity ratio of 4 for the $170 \times 64 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

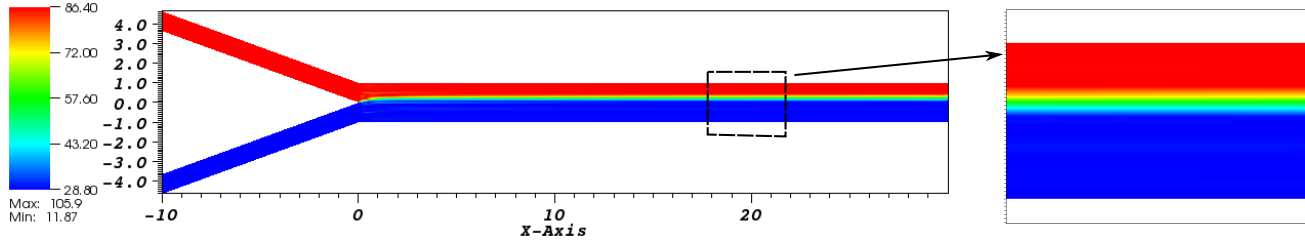


Figure 5.18: Viscosity distribution for a velocity ratio of 4 for the $170 \times 64 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$

introduced within the two fluids in order to obtain the whole velocity profile in the channel. Three different velocity ratios between the two branches are also tested experimentally to be compared to the simulations.

5.3.1.1 VELOCITY RATIO OF 1

As a first test, the two fluids enter from both branches with the same flow rate of $Q_{bottom} = Q_{top} = 5 \frac{\mu l}{hr}$ with a viscosity ratio of $\nu_{ratio} = 3$ between the two fluids. Fig. 5.20 shows the interface location between the two fluids obtained when the tracer particles are illuminated with the laser beam. Comparing to the viscosity distribution in the channel obtained numerically, we see that the experimental and numerical results actually coincide and that the glycerol layer occupies about 70% of the channel thickness, where the velocity profile is not linear as seen in Fig. 5.21. However, the

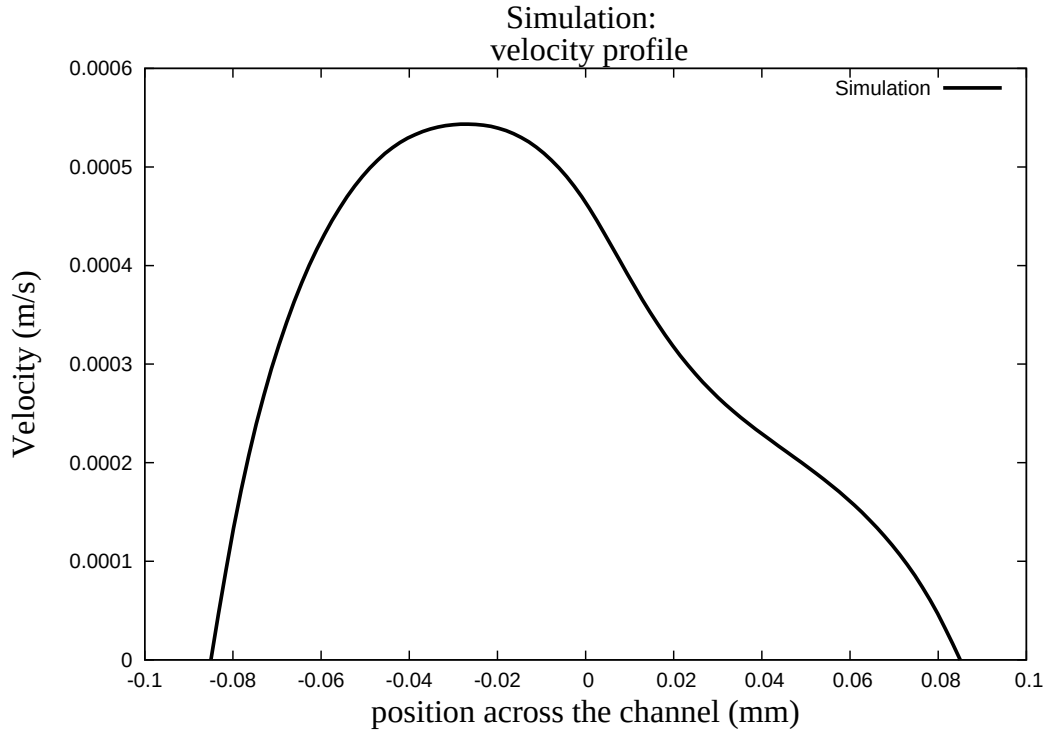


Figure 5.19: Velocity profile for a velocity ratio of 4 extracted from the $170 \times 64 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

similarity between the two experimental and numerical curves is clear where the velocity increases and reaches its maximum value in the distilled water layer (left hand side of the plot) then decreases to reach a plateau, indicating the location of the interface to finally decrease in an almost linear manner. The experimental curve presents several points that have the same velocity forming a step curve. These steps represent the size of the correlation window used to cross correlate the sets of double picture obtained from the experiment. As mentioned in Chapter 2, the size of the correlation window is chosen depending on the time interval between the two pictures and the flow rate used.

5.3.1.2 VELOCITY RATIO OF 2

For this experimental test, glycerol enters from the top branch at a flow rate of $Q_{top} = 3.33 \frac{\mu l}{hr}$, while the distilled water entraining the glycerol enters from the bottom branch with a flow rate of $Q_{bottom} = 6.66 \frac{\mu l}{hr}$. As a first trial, the fluorescent

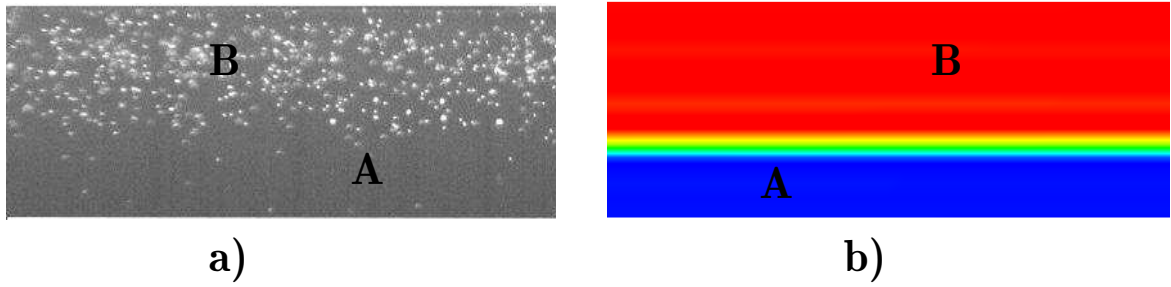


Figure 5.20: Experimental results of the fluid distribution in the the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ for a velocity ratio of 1, where the tracer particles are illuminated with the laser to capture the interface location (a) compared to the numerical viscosity distribution (b)

particles only introduced in the glycerol solution are illuminated with the laser as shown in Fig. 5.22. Comparing to the viscosity distribution in the channel obtained numerically, we see that the experimental results agree with the numerical ones, where the glycerol layer occupies about 55% of the channel thickness. Within this range, the velocity profile, shown in Fig. 5.23, is not linear either. However, the similarity between the two experimental and numerical curves is clear as in the previous tests.

5.3.1.3 VELOCITY RATIO OF 4

Now, the flow rate ratio between the two branches is changed again in order to obtain a velocity ratio of 4 between the two fluids. For this purpose, glycerol entering from the top branch at a flow rate of $Q_{top} = 2 \frac{\mu l}{hr}$ is entrained by the distilled water entering from the bottom branch with a flow rate of $Q_{bottom} = 8 \frac{\mu l}{hr}$. As a first trial, the fluorescent particles only introduced in the glycerol solution are also illuminated with the laser as shown in Fig. 5.24, where the results obtained are compared to the viscosity distribution obtained numerically for the same case. The experimental results show very good agreement with the numerical results and the glycerol layer is about 30% of the channel thickness. From the velocity profiles displayed in Fig. 5.25, we see that the experimental and numerical curves coincide and follow the same trend. It should also be noted that, within the glycerol layer, the experimental velocity profile is almost linear, despite the non continuity of the curve due to the correlation window step.

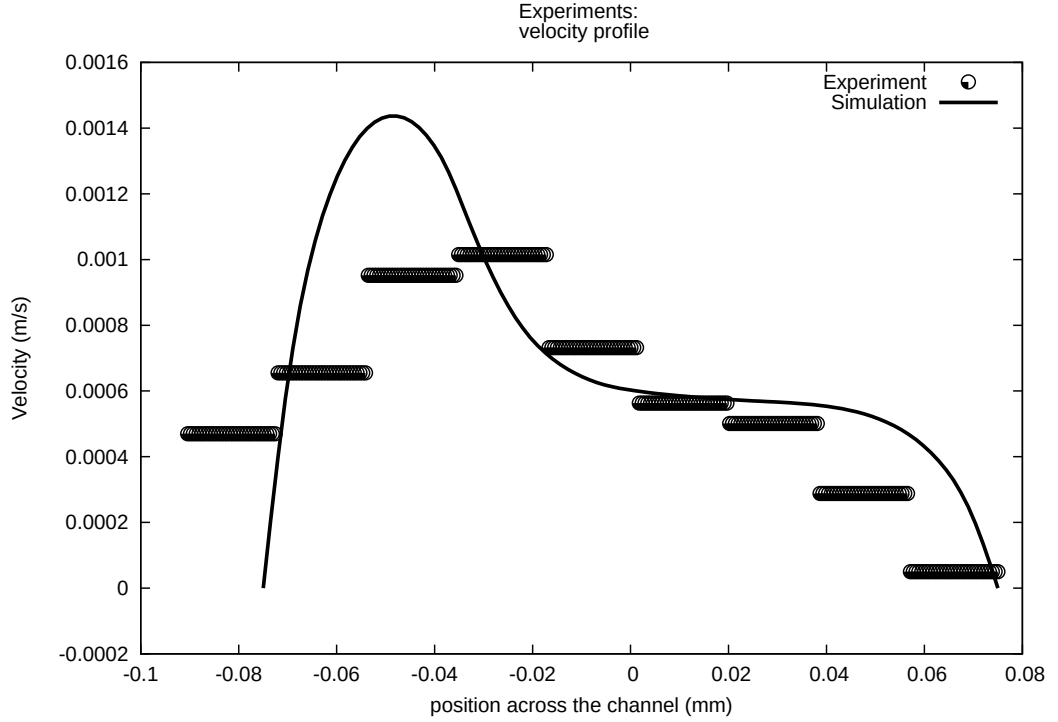


Figure 5.21: Velocity profiles comparison between the experimental and numerical results for a velocity ratio of 1 extracted from the $150 \times 33 \mu\text{m}$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu\text{l}}{\text{hr}}$. The bars in the experimental velocity profile represent the size of the correlation window.

The red curve shown within this figure represents the PIV error magnitude from this experiment. In fact, due to the averaging of the recorded images in time and space included in the cross-correlation method, the velocity presented in Fig. 5.25 does not reflect the local velocity measure but will vary significantly. The error, shown in Fig. 5.25 (red curve), termed as the Root Mean Square (RMS) estimation is calculated using the averaging of the instantaneous velocity measurement for n_{image} . It is determined using the following equation:

$$RMS = \sqrt{\frac{n_{\text{image}} \sum pixel_{im}^2 - (\sum pixel_{im})^2}{n_{\text{image}} (n_{\text{image}} - 1)}} \quad (5.1)$$

where n_{image} represents the total number of images, $pixel_{im}$ the intensity of any pixel in image im . The results obtained are in terms of pixels which is then scaled to the units of velocity ($\frac{\text{mm}}{\text{s}}$) using the timing between the frames dt and the scale factor set when calibrating the DP camera. We see that the error is larger at the interface location probably due to the flow unsteadiness at that specific location. The large

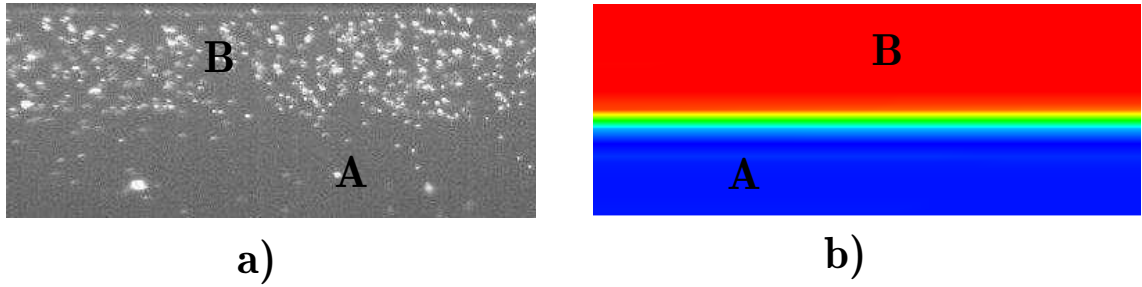


Figure 5.22: Experimental results of the fluid distribution in the the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ for a velocity ratio of 2, where the tracer particles are illuminated with the laser to capture the interface location (a) compared to the numerical viscosity distribution (b)

error magnitude of the PIV error can also be noted within the "blood layer". In fact, the timing between the two frames, dt , was optimized for the entraining fluid layer, which is not suitable for the "blood layer", since the glycerol flows four times slower than water in the microchannel.

We notice a difference in the maximum velocity value between the experimental and numerical profile when looking at Figs. 5.21, 5.23 and 5.25. This difference might be due to the processing of the recorded set of images with the μ PIV system. In fact, the velocity profile obtained experimentally greatly depends on the interval between the double frames (dt). However, since the velocity ratio between the two branches varies the dt set by the user might not correspond to both fluids simultaneously which could affect the processing.

In the same figures (Figs. 5.21, 5.23 and 5.25) we also notice that slip conditions appear at the channel wall facing fluid A (water), whereas the velocity at the channel wall facing fluid B (glycerol) is almost null. This phenomenon is probably due to the different interactions of the two fluids with the PDMS. It is possible that the molecules contained in the glycerol solution are more likely to attach to the PDMS than water increasing the surface tension between the fluid and solid structure.

The shear rate values obtained from these cases with the $150 \times 33 \mu m$ microchannel, for velocity ratios ranging from 1 to 4 are summarized in Table 5.5. The experimental and numerical shear rate increases with increasing the velocity ratio. For $V_{ratio} = 4$, the experimental and numerical values of shear rate are within the same range with a

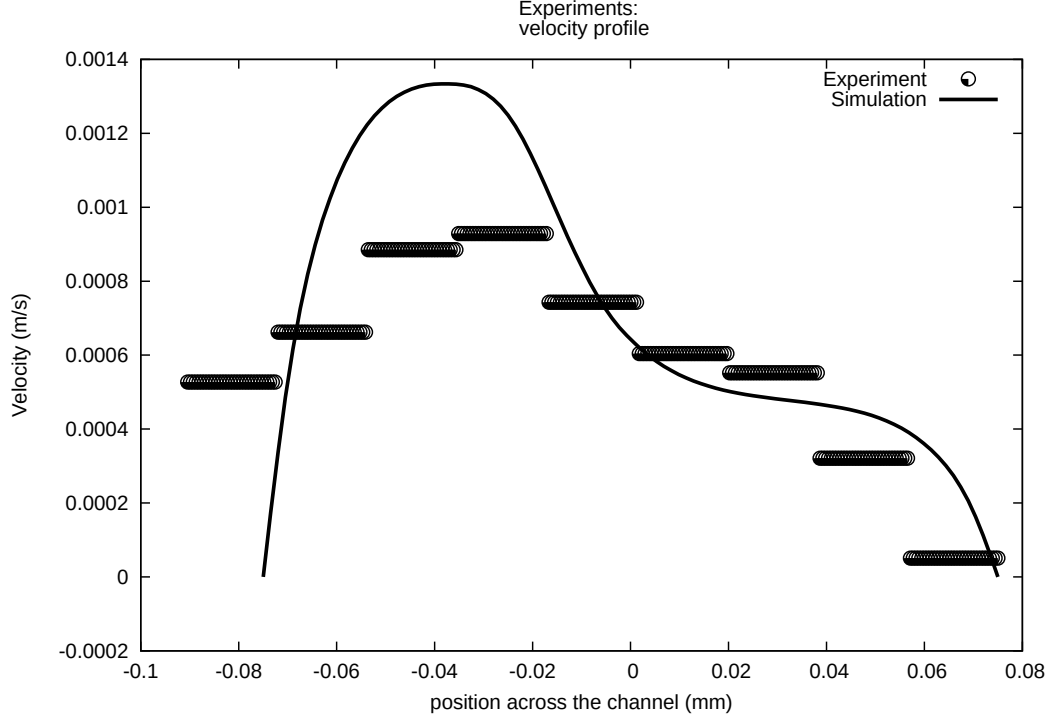


Figure 5.23: Velocity profiles comparison between the experimental and numerical results for a velocity ratio of 2 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

small difference probably due to the consideration of the diffusion layer experimentally when locating the interface between the two fluids but not numerically. However, we notice that the values of shear rate are close to the limit of the range of RBC aggregation.

5.3.2 $170 \times 64 \mu m$ MICROCHANNEL WITH A FLOW RATE OF $10 \frac{\mu l}{hr}$

Our model of the $150 \times 33 \mu m$ microchannel was tested experimentally with a Newtonian fluid flowing at a flow rate of $Q = 10 \frac{\mu l}{hr}$, where it was found that the ideal "blood layer" thickness to be analyzed is about 35% of the blood thickness where the velocity profile is almost linear. However, the shear rate values found were close to the limit of RBC aggregation. Therefore, a $170 \times 64 \mu m$ microchannel is also tested experimentally in order to verify the "blood layer" thickness, the velocity profile and the shear rate values. For this case, only the velocity ratio of 4 between the two branches is tested since it was demonstrated to provide the ideal parameters to study

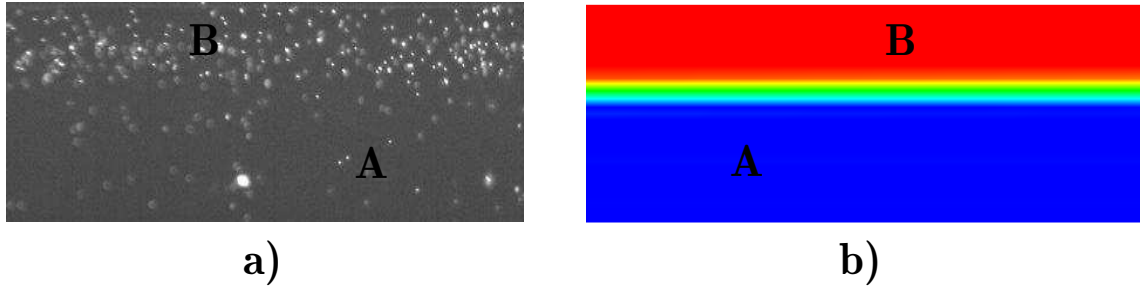


Figure 5.24: Experimental results of the fluid distribution in the the $150 \times 33 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ for a velocity ratio of 4, where the tracer particles are illuminated with the laser to capture the interface location (a) compared to the numerical viscosity distribution (b)

Table 5.5: Experimental (E) and numerical (S) shear rate comparison for $150 \times 33 \mu m$ microchannel (Re: Reynolds number based on the average velocity of the bottom layer of the channel).

Ratio	Shear (s^{-1})		Velocity ($\frac{mm}{s}$)		Interface (μm)		Re (10^{-2})
	S	E	S	E	S	E	
1	7.59	8.58	0.671	0.732	61.59	64.7	4.20
2	8.56	8.50	0.527	0.604	88.40	79.0	5.61
4	10.81	12.42	0.453	0.716	108.0	92.40	6.73

this "blood layer". Therefore, the glycerol enters from the top branch with a uniform velocity of $V_{top} = 0.102 \frac{mm}{s}$, while distilled water enters from the bottom branch with a uniform velocity of $V_{bottom} = 0.408 \frac{mm}{s}$. The tracer particles are only introduced with the glycerol layer to capture the location of the interface between the fluids. Looking at the distribution of the fluids in the channel experimentally and comparing it to the viscosity distribution found numerically, shown in Fig. 5.26, we see that both results coincide and that for this case also, the glycerol layer occupies about 35% of the channel thickness. However, looking at the comparison of experimental and numerical velocity profiles in Fig. 5.27, we see that the experimental values are significantly lower than the numerical ones. In fact, this difference might be due to the difficulty of the lens to focus in the mid-channel depth. The shear rate values obtained for this case, shown in Table 5.6, are still within the same range but are significantly reduced to almost half of their previous values.

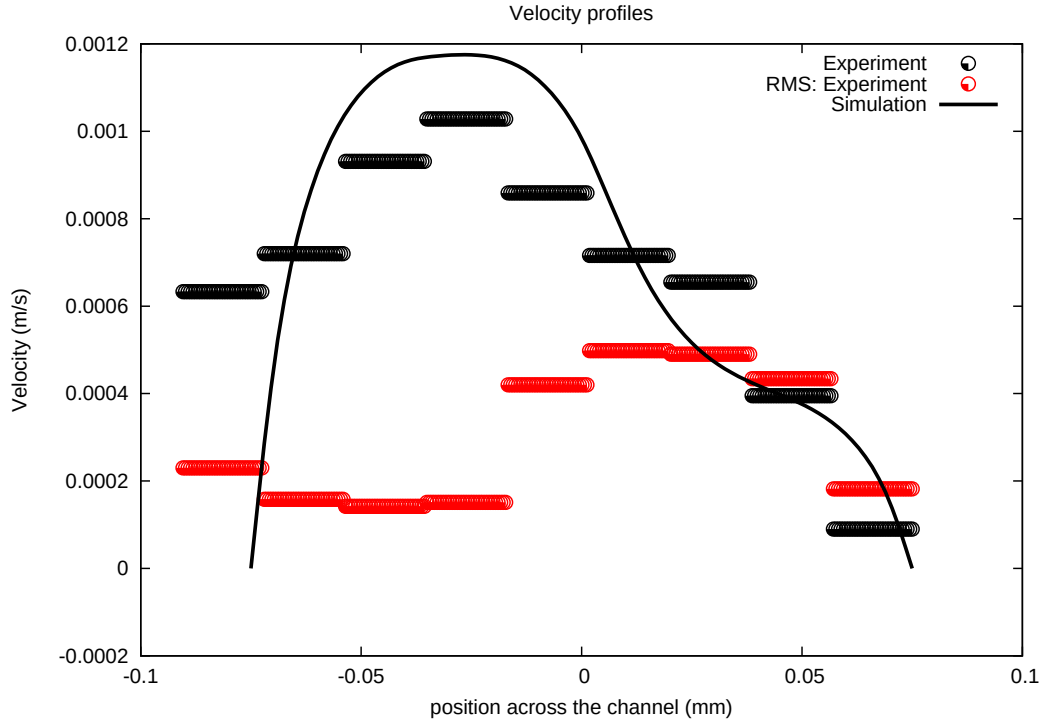


Figure 5.25: Velocity profiles comparison between the experimental and numerical results for a velocity ratio of 4 extracted from the $150 \times 33 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$. The red symbol represent the RMS velocity error obtained from the μPIV measurement for the present test.

5.4 CONCLUSION

In this chapter, we have investigated the numerical and experimental behaviour of the two fluid flow in our designed microchannel with different flow rates and velocity ratios between the two branches. First, our model was tested numerically with two-dimensional simulations in order to estimate the shear rate for eight different values of velocity ratios between the branches keeping a constant flow rate in the channel. It was found that the thickness of fluid B was ideal for a $V_{ratio} = 4$ and that the velocity profile within this range was almost linear. Therefore three-dimensional simulation for $150 \times 33 \mu m$ and $170 \times 64 \mu m$ microchannels were performed to accurately measure the shear rate values and compare the results obtained numerically, including the thickness of fluid B layer, the velocity profile within this layer and the shear rate calculated, to the results obtained experimentally for the same tests. It was found also that the ideal conditions were for the velocity ratio of 4. This model is then

Table 5.6: Experimental (E) and numerical (S) shear rate comparison for $170 \times 64 \mu m$ microchannel (Re: Reynolds number based on the average velocity of the bottom layer of the channel).

Ratio	Shear (s^{-1})		Velocity ($\frac{mm}{s}$)		Interface (μm)		Re (10^{-2})
	S	E	S	E	S	E	
4	5.00	3.16	0.237	0.171	122.5	116.0	3.45

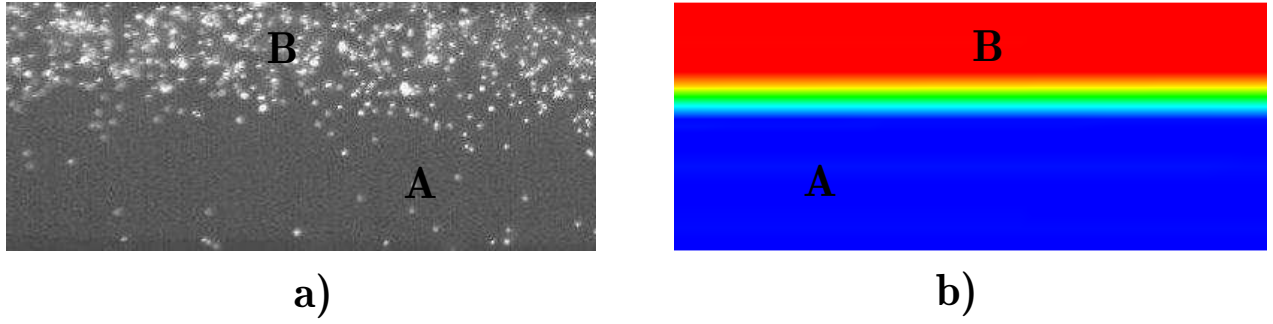


Figure 5.26: Experimental results of the fluid distribution in the the $170 \times 64 \mu m$ microchannel with $Q = 10 \frac{\mu l}{hr}$ for a velocity ratio of 4, where the tracer particles are illuminated with the laser to capture the interface location (a) compared to the numerical viscosity distribution (b)

tested with the RBC flowing in the channel as described in the next chapter.

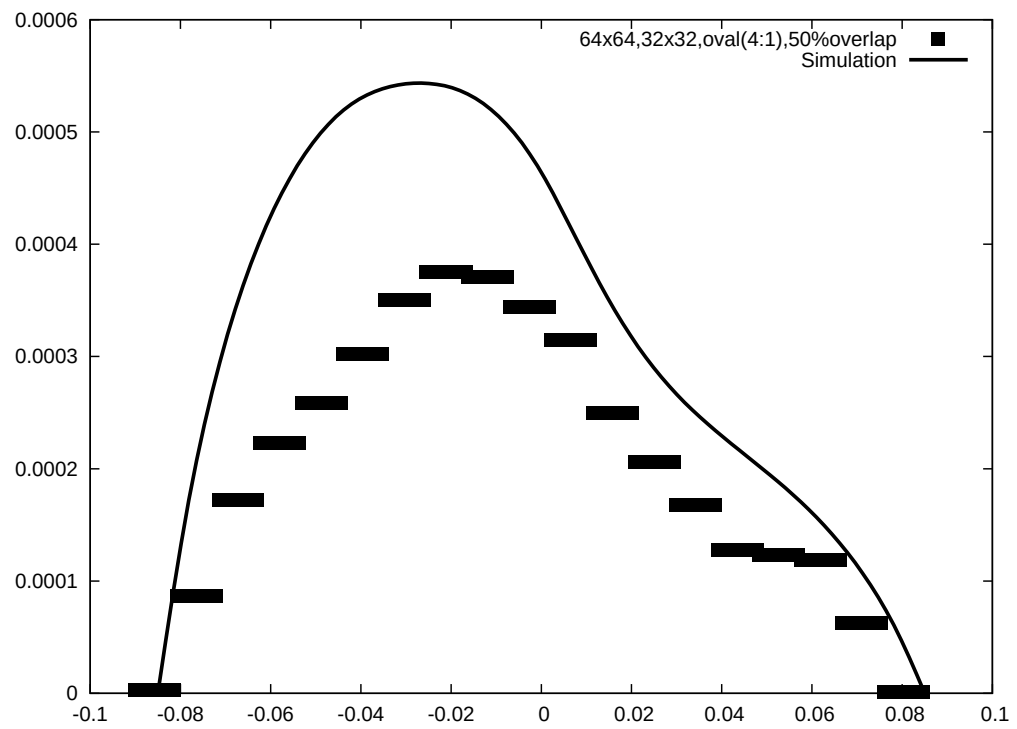


Figure 5.27: Velocity profiles comparison between the experimental and numerical results for a velocity ratio of 4 extracted from the $170 \times 64 \mu m$ microchannel at the location of $x = 28$ along the channel with $Q = 10 \frac{\mu l}{hr}$

Chapter 6

BLOOD EXPERIMENTS AND NUMERICAL RESULTS COMPARISON

6.1 BLOOD EXPERIMENTS OVERVIEW

As mentioned in Chapter 2, the samples of blood used for the following experiments are collected from pigs, cleaned and centrifuged to separate the RBC from the other constituents. These RBC are then resuspended in their own plasma or in Phosphate Buffered Saline (PBS) depending on the application. The RBC suspended in plasma are able to aggregate because of the fluid constituents, however no aggregation is seen in RBC-PBS suspensions. In this chapter both suspensions (RBC-plasma and RBC-PBS suspensions) are used and compared to one another. First, the RBC-PBS suspensions are used in order to evaluate the effect of several image processing pre-processing methods on the experimental velocity profile as described in Sec. 6.2. Then, RBC-plasma suspensions flowing at different hematocrit (10% and 20%) are tested with different flow rates (5 and 10 $\frac{\mu l}{hr}$) and compared to the non-aggregating suspensions.

6.2 IMAGE PROCESSING

Since blood is a non-Newtonian fluid, for which properties change according to the flow conditions, it is necessary to investigate the appropriate processing method, previously applied to Newtonian fluids, to be used with blood. For this purpose, RBC suspended in PBS at 10% and 20% hematocrit (H) flow in two different channel geometries to be tested and processed with the μ PIV system to obtain the velocity profile within the channels. Several processing methods are tested, varying the window size, window shape and the percentage of overlapping of the correlation windows, and are then compared to several pre-processing methods to be cross-correlated [130]. These pre-processing methods include the "base clipping" method introduced

by Bitsch *et. al.* in [91] tested on blood, which consists of screening the pictures obtained with the DP camera and removing the intensities of the out-of-focus particles that are lower than that of the in-focus particles [130]. The second pre-processing method, introduced by Wereley *et. al.* in [131] for PIV and applied to μ PIV by Nguyen *et. al.* in [95], consists of locating the maximum pixel intensity in all the double pictures captured by the camera to only cross correlate the pair of pictures having the highest intensities. This image overlapping method presents a great advantage in that it greatly decreases the processing time since only two pictures are correlated. The two channels used to test the different processing and pre-processing methods applied to RBC flowing in PBS consist of a straight rectangular channel of $140 \times 40 \mu m$ width and depth and our Y-configuration channel of $150 \times 33 \mu m$ width and depth as described in Chapter 3. For these tests the flowrate for the RBC flowing in the channels is $Q = 10 \frac{\mu l}{hr}$ for both cases. The velocity ratio between the two branches for the Y-configuration channel is $V_{ratio} = 1$ so that the flow rate in both branches is $Q_{top} = Q_{bottom} = 5 \frac{\mu l}{hr}$. It is expected to obtain an almost parabolic velocity profile within the straight channel due to the low H (hematocrit), while, for the Y-channel, it is expected to see the same profile of the two fluid flow found for the numerical simulations and Newtonian experiments in Sec. 5. It was found for both channel configurations that, using a correlation window of $64 \times 64 \mu m$ pixels, with 87% overlapping and the oval window shape weighted with a ratio of 4 to 1 in the x direction, we obtain the most sensible results since not only the profile is smoother than for the other cases but the velocity values are closer to the theoretical values. Fig. 6.1 shows the difference in velocity profile for the Y-channel configuration when varying the window size window shape and the overlapping percentage. The results mentioned in this section are included in [130]. Comparing this best case with no-processing to the "base clipping" pre-processing method, the image overlapping pre-processing method and the simulation results, it was found that the velocity profile was smoother for the case where no pre-processing method is applied, however, the velocity profile obtained using the image overlapping method seems to be the closest to the numerical results for both channel configurations. This method proves

to be the best method for this application also considering its lower computation cost. However, the results showed a better accuracy with the straight channel [130] due to the instability of the interface between the layers for the Y-channel because of the RBC in the flow. Therefore, the experiments performed with aggregation are processed using the cross-correlation without previous pre-processing.

6.3 RESULTS

The previous tests were performed with the RBC suspended in PBS in the designed Y-microchannel model. It was clear that the RBC could not aggregate for this case. However, since the purpose of this project is to study RBC aggregates formation, it is important to visualize these aggregates. Therefore, several tests are performed with different blood samples. For all of the following and previous experiments performed with blood, the second fluid used to entrain the RBC is PBS in order to keep the equilibrium in concentration between the two fluids.

6.3.1 COMPARISON OF RBC IN PLASMA AND RBC IN PBS AT 10% H WITH $Q = 10$

$$\frac{\mu l}{hr} \text{ AND } V_{ratio} = 4$$

First, RBC suspended in plasma at 10% H are tested in the Y-microchannel and compared to the same tests performed with RBC suspended in PBS also at 10% H. Both samples of blood are tested in the $150 \times 33 \mu m$ channel with a flow rate of $Q = 10 \frac{\mu l}{hr}$ using a velocity ratio of 4. In order to visualize the RBC flowing in the channel and obtain the velocity profile, the μPIV system is coupled with the high speed camera. The results are presented for two different samples extracted from two different pigs termed S and T. The results for each sample, consisting of both experiments of RBC in PBS and RBC in plasma, are shown in Figs. 6.3 and 6.4 respectively for sample S and 6.6 and 6.7 respectively for sample T. As mentioned above, Figs. 6.3 and 6.6 clearly show that the RBC cannot aggregate in PBS, while RBC suspended in plasma can form rouleaux in the channel as visualized in Figs. 6.4 and 6.7 for both samples of blood. These aggregates greatly change the blood properties. In fact, the velocity

Table 6.1: Experimental(E) and numerical (S) shear rate comparison for $150 \times 33 \mu m$ microchannel for blood samples S and T when RBC are suspended in PBS and plasma at 10% H and flowing at $Q = 10 \frac{\mu l}{hr}$. The interface between the two fluids is measured from the bottom of the channel as shown in Fig. 5.1.

ratio 4	Shear	Velocity	Interface	Re
Type of blood	(s^{-1})	$(\frac{mm}{s})$	(μm)	10^{-2}
Blood S (plasma)	7.35	0.40	94.67	-
Blood S (PBS)	13.05	0.66	99.00	-
Blood T (plasma)	6.55	0.34	96.00	-
Blood T (PBS)	13.56	0.72	97.00	-
Simulation	10.81	0.334	108.0	6.73

profiles comparison for RBC in plasma and RBC in PBS for blood sample S, shown in Fig. 6.2, show that within the blood layer, the velocity profile is almost linear for the two experimental curves, as desired. Also, we note that, within the blood layer, the velocity magnitude of RBC suspended in PBS is higher than for RBC in plasma. Moreover, the blood layer when the cells are suspended in plasma is slightly thicker than the blood layer resultant from RBC suspended in PBS. These factors could be explained by the aggregation of RBC in plasma which leads to a more viscous mixture than the blood obtained from the RBC suspended in PBS. The same conclusions hold for sample T. The velocity of RBC in PBS is slightly higher than the velocity of RBC in plasma within the blood layer, which is thicker for the RBC in plasma. Therefore, we can conclude that the aggregation of RBC affects the viscosity of blood as was demonstrated in [4, 8, 132].

Since the profile obtained is almost linear, we can deduce the shear rate values from the experiments and compare them to the simulation results. When comparing the experimental shear rate values to the simulation results in Table 6.1, we see that they are within the same range, around $10s^{-1}$. However we see that, for both blood samples S and T, the shear rate of RBC in plasma is lower than the other values and that it is within the range of shear rates where RBC can aggregate (1 to $10 s^{-1}$).

6.3.2 RBC IN PLASMA AT 10% H WITH $Q = 5 \frac{\mu l}{hr}$ AND $V_{ratio} = 4$

The aggregation of RBC was clearly visualized under the high speed camera for both samples S and T for flow rate $Q = 10 \frac{\mu l}{hr}$, however only small aggregates could form due to the high value of shear rate (about $7 s^{-1}$) close to the limit of the required range. We therefore analyze the effect of lowering the shear rate on the RBC aggregates in this section. In order to lower the shear rate value without changing the channel geometry, the flow rate was decreased to half of its previous value. These tests then consist of RBC in plasma flowing at $Q = 5 \frac{\mu l}{hr}$ at 10% H entrained by the PBS with $V_{ratio} = 4$ between the branches.

The RBC aggregation can be clearly seen in Fig. 6.8. In fact, we note that the aggregates are actually larger than those seen in the previous case with double the flow rate and that the blood layer is thicker for this case and covers almost half of the channel thickness. Figs. 6.9 and 6.11 show that the experimental velocity measurements agree very well with the numerical results since they follow the same curve, where the maximum velocity is reached on the PBS side of the channel (left side of the profile) and decreases in an almost linear manner on the blood side (right side of the profile) after forming a small plateau indicating the interface location.

The shear rate values presented in Table 6.2 show that the experimental and numerical results are in good agreement since all the values approach $5 s^{-1}$. Comparing with the values obtained numerically in the previous case, we see that the shear rate values for $Q = 5 \frac{\mu l}{hr}$ are one half of those for $Q = 10 \frac{\mu l}{hr}$, as expected. However, a small difference in the position of interface (measured from the bottom of the channel) between the two samples of blood can be noted through Table 6.2. Since the two samples come from two different pigs, the plasma composition might be different, which affects the RBC aggregation and in turn the viscosity of blood and the thickness of the blood layer in the channel.

Table 6.2: Experimental and numerical shear rate comparison for $150 \times 33 \mu m$ microchannel for blood samples S and T when RBC are suspended plasma at 10% H $Q = 5 \frac{\mu l}{hr}$. The interface between the two fluids is measured from the bottom of the channel as shown in Fig. 5.1.

ratio 4	Shear	Velocity	Interface	Re
Type of blood	(s^{-1})	$(\frac{mm}{s})$	(μm)	10^{-2}
Blood S ($Q = 5 \frac{\mu l}{hr}$)	5.18	0.337	85.00	-
Blood T ($Q = 5 \frac{\mu l}{hr}$)	5.21	0.391	75.00	-
Simulation ($Q = 5 \frac{\mu l}{hr}$)	5.40	0.22	108.0	3.74
Simulation ($Q = 10 \frac{\mu l}{hr}$)	10.81	0.334	108.0	6.73

6.3.3 RBC IN PLASMA 20% H WITH $Q = 10 \frac{\mu l}{hr}$ AND $V_{ratio} = 4$

The blood samples tested previously consist of RBC suspended in plasma or PBS, depending on the application, at 10% H. With this low value of hematocrit, it is expected that the fluid would behave as a Newtonian fluid especially when no aggregation is seen. This value of hematocrit is not completely realistic since the *in vivo* percentage of RBC in blood in microcirculation is about 20% [1]. Therefore it is important to visualize and understand the effect of hematocrit on the flow. For this purpose, the same tests performed previously are repeated with RBC suspended in plasma at 20% H. As a first test, RBC suspended in plasma at 20% H flowing at $Q = 10 \frac{\mu l}{hr}$ in a $150 \times 33 \mu m$ microchannel is entrained by PBS with $V_{ratio} = 4$ between the branches. The results obtained are then compared to those obtained from the same test performed with RBC suspended at 10% H.

Fig. 6.12 shows the RBC flowing in the channel visualized by the high speed camera. RBC aggregation is not as clearly visualized as in the 10% H suspension. It is more difficult to focus in the mid-plane of the channel depth due to the large number of RBC flowing in the top layer of the channel. The blood layer can still be measured in order to locate the interface between the two fluids. The velocity profile obtained from this present test, shown in Fig. 6.13, seems to also follow the same numerical and experimental (10% H) curves with differences in the interface location. The smallest blood layer is obtained numerically, as verified in Chapter 5, and is about 35% of the channel thickness. However, the thickest blood layer is obtained

Table 6.3: Experimental and numerical shear rate comparison for $150 \times 33 \mu m$ microchannel for blood samples S when RBC are suspended plasma at 20% H $Q = 10 \frac{\mu l}{hr}$. The interface between the two fluids is measured from the bottom of the channel as shown in Fig. 5.1.

ratio 4	Shear	Velocity	Interface	Re
Type of blood	(s^{-1})	$(\frac{mm}{s})$	(μm)	10^{-2}
Blood S: 20% H	12.70	0.95	75.00	-
Blood S: 10% H	7.35	0.40	94.67	-
Simulation	10.81	0.334	108.0	6.73

when the RBC are suspended in plasma at 20 % H, which is an indication of the largest blood viscosity. Since blood would be more viscous at 20% H, it is expected to expand faster in the channel.

Table 6.3 shows that the shear rate values obtained from the two experiments are within the same range when compared to the simulation. However, for this case especially, the shear rate values are only an approximation of the actual value since the velocity profile within the blood layer, when RBC are suspended in plasma at 20% H, is not linear due to the large blood layer and the shear rate is calculated based on a linear velocity profile.

6.3.4 RBC IN PLASMA AT 20% H WITH $Q = 5 \frac{\mu l}{hr}$ AND $V_{ratio} = 4$

In order to visualize and measure the effect of shear rate on the RBC aggregation when the particles are suspended in plasma at 20% H, the same test performed in Sec. 6.3.3 is repeated with a flow rate reduced to half of its previous value, thereby reducing the shear rate without changing the channel geometry. RBC suspended in plasma at 20% H are entrained by PBS and flowing in the $150 \times 33 \mu m$ with $Q = 5 \frac{\mu l}{hr}$.

Fig. 6.14 shows the RBC flowing in the channel visualized under the high speed camera. For this case also, it is difficult to focus on the middle plane of the channel depth due to the large number of RBC. However the thickness of the blood layer can still be measured to locate the interface between the two fluids. Looking at the velocity profile provided in Fig. 6.15, both curves follow the same numerical profile, where

Table 6.4: Experimental and numerical shear rate comparison for $150 \times 33 \mu m$ microchannel for blood samples S when RBC are suspended plasma at 20% H $Q = 5 \frac{\mu l}{hr}$. The interface between the two fluids is measured from the bottom of the channel as shown in Fig. 5.1.

ratio 4	Shear	Velocity	Interface	Re
Type of blood	(s^{-1})	$(\frac{mm}{s})$	(μm)	10^{-2}
Blood S: 20% H	5.125	0.41	80.00	-
Blood S: 10% H	5.18	0.337	85.00	-
Simulation	5.4	0.22	108.0	3.74

the velocity within the blood layer is almost linear. However, for RBC suspended at 10 and 20% H, a small variation in the location of the interface (measured from the bottom of the channel) is seen. This difference (about $5 \mu m$) is not significant when comparing to the difference between the two samples found with $Q = 10 \frac{\mu l}{hr}$ (about $20 \mu m$).

This interfaces as well as the shear rates extracted from these cases are presented in Table 6.4 and compared to the numerical shear rate. The shear rate for the three cases are seen to be very similar, about $5 s^{-1}$, and the interface location found experimentally is also within the same range. This observation might be due to the approximated shear rate calculations because of the large blood layer and the non-complete linearity of the profile within this layer. Therefore, we can deduce that the shear rate seems to have a greater effect on the viscosity of blood than the hematocrit.

6.4 CONCLUSION

In this chapter, we reported several samples of blood tested experimentally in our microchannel model with RBC suspensions. First, RBC suspended in PBS and plasma at 10% H are entrained with PBS and tested in the $150 \times 33 \mu m$ with $V_{ratio} = 4$ to be compared to the simulations. The results show good agreement with the numerical results when comparing the velocity profiles and the blood layers. It was found that, due to the aggregation ability of the RBC flowing in plasma, the blood sample was more viscous than for the RBC suspended in PBS. Then, for the same set up, the flow rate was decreased in order to reduce the shear rate value to investigate the effect on

aggregates. It was shown that the aggregates were much larger for this case. Since the hematocrit of blood plays an important role in RBC aggregation, this factor was also considered in our study. For this reason, the same first test was repeated but with RBC suspended in plasma at 20% H for this case. The velocity profile obtained was then compared to the first case with blood at 10% and the simulation results. The deviation from the linear velocity profile increases for the 20% H blood, since the apparent viscosity of the suspension is larger than 3 cP which is the value set in our simulations. The value of shear rates found are within the same range of the numerical shear rate values with large differences in the interface location. Decreasing the flow rate in order to reduce the shear rate for RBC at 20% H results in shear rates within the same range of the 10% H suspension and the simulation results. As mentioned previously, since the shear rates are approximated due to the large blood layer and the non-complete linearity of the profile within this layer, the values might vary.

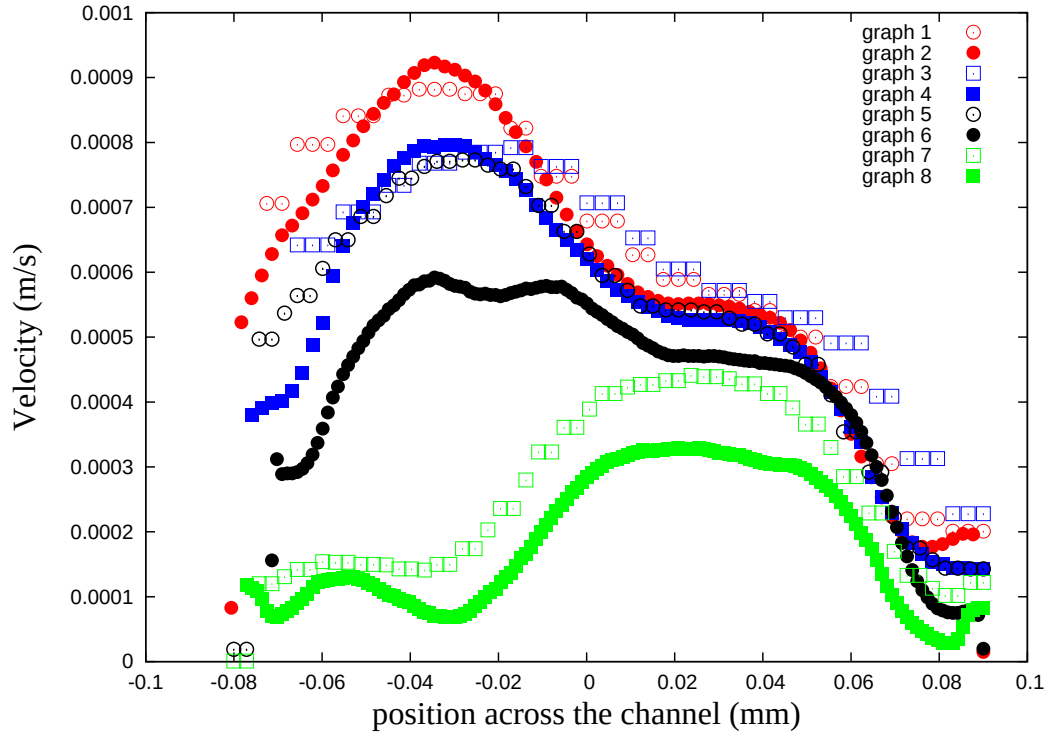


Figure 6.1: Velocity profile of a RBC-plasma suspension flowing in the $150 \times 33 \mu\text{m}$ microchannel at 10% H with a flow rate of $Q = 10 \frac{\mu\text{l}}{\text{hr}}$ varying the window size, window shape and the overlapping percentage. Graph 1 represents the velocity profile for a correlation window of 64×64 pixels, with 50% overlapping and the oval window shape weighted with a ratio of 4 to 1. Graph 2 represents the velocity profile for a correlation window of 64×64 pixels, with 87% overlapping and the oval window shape weighted with a ratio of 4 to 1. Graph 3 represents the velocity profile for a correlation window of 64×64 pixels, with 50% overlapping and the square window shape. Graph 4 represents the velocity profile for a correlation window of 64×64 pixels, with 87% overlapping and the square window shape. Graph 5 represents the velocity profile for a correlation window of 32×32 pixels, with 50% overlapping and the oval window shape weighted with a ratio of 4 to 1. Graph 6 represents the velocity profile for a correlation window of 32×32 pixels, with 87% overlapping and the oval window shape weighted with a ratio of 4 to 1. Graph 7 represents the velocity profile for a correlation window of 32×32 pixels, with 50% overlapping and the square window shape. Graph 8 represents the velocity profile for a correlation window of 32×32 pixels, with 87% overlapping and the square window shape.

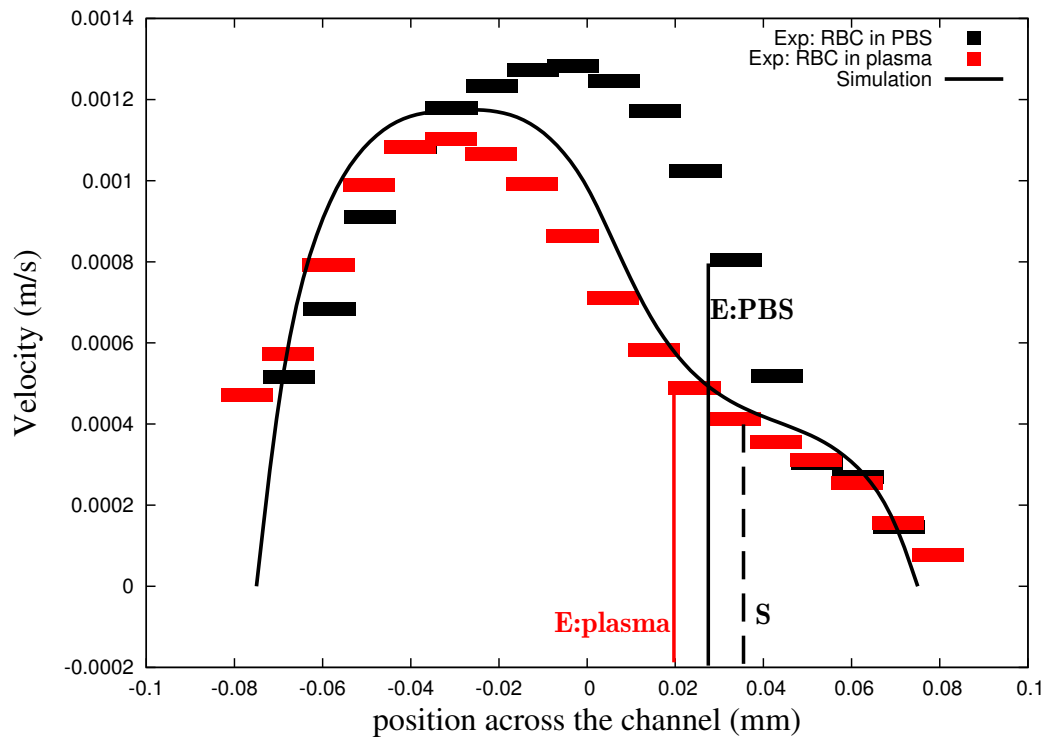


Figure 6.2: Velocity profile comparison of RBC in PBS (black curve) and RBC in plasma (red curve) from sample S at 10% H with simulation for the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$. The interface location is denoted by S for the simulation E for the experiments.

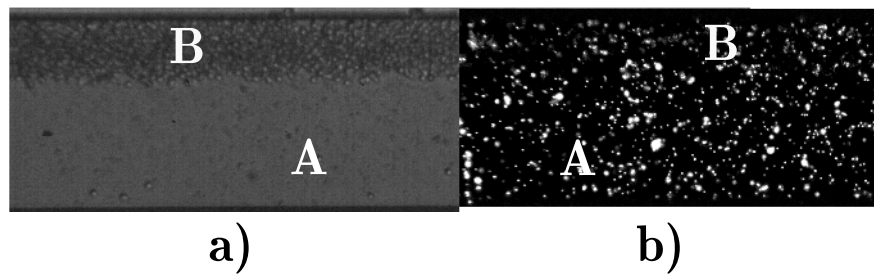


Figure 6.3: RBC from sample S suspended in PBS at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$ visualized with the high speed camera (a) and the μPIV system (b)

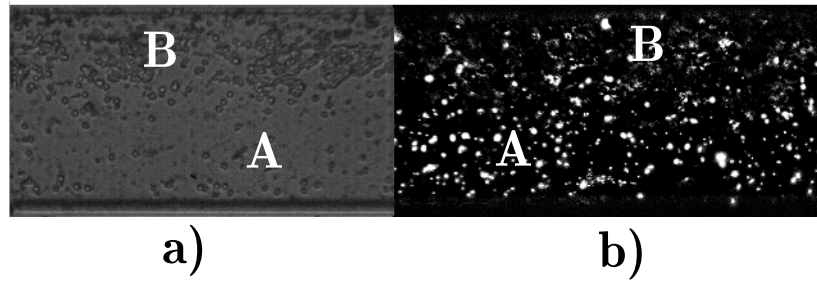


Figure 6.4: RBC from sample S suspended in plasma at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$ visualized with the high speed camera (a) and the μ PIV system (b)

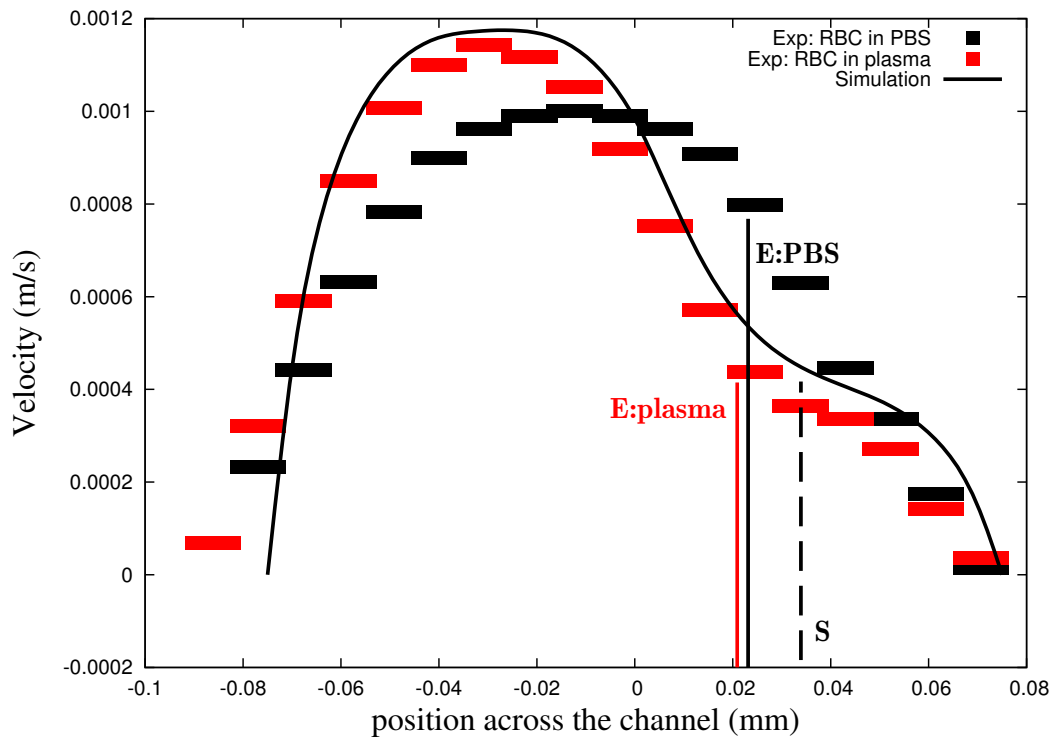


Figure 6.5: Velocity profile comparison of RBC in PBS (black curve) and RBC in plasma (red curve) from sample T at 10% H with simulation for the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$. The interface location is denoted by S for the simulation E for the experiments.

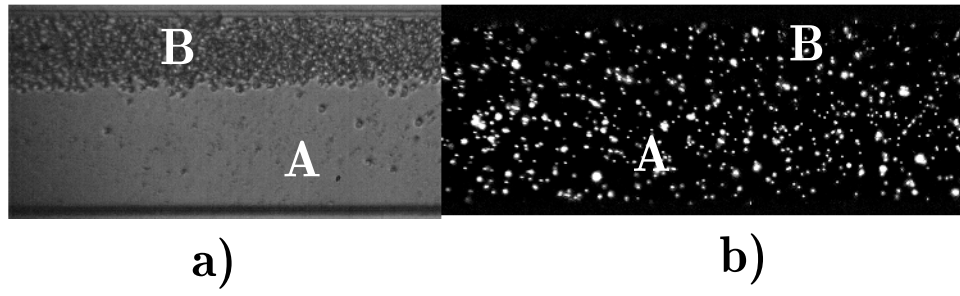


Figure 6.6: RBC from sample T suspended in PBS at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$ visualized with the high speed camera (a) and the μ PIV system (b)

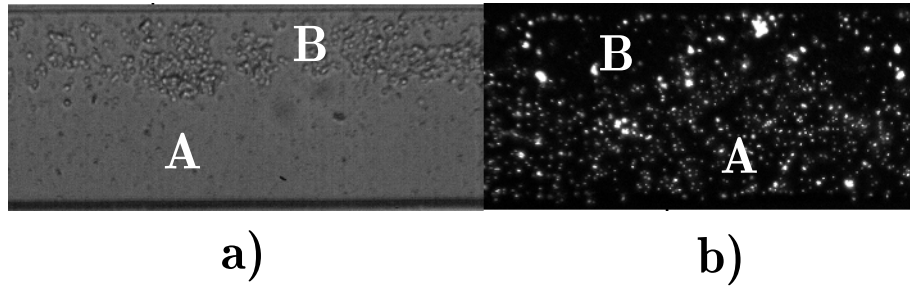


Figure 6.7: RBC from sample T suspended in plasma at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$ visualized with the high speed camera (a) and the μ PIV system (b)

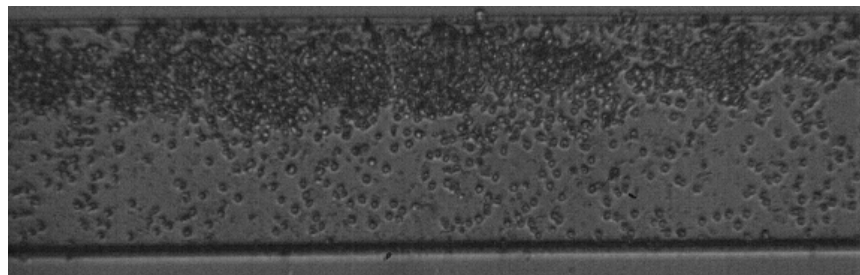


Figure 6.8: RBC from sample S suspended in plasma at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 5 \frac{\mu l}{hr}$ visualized with the high speed camera

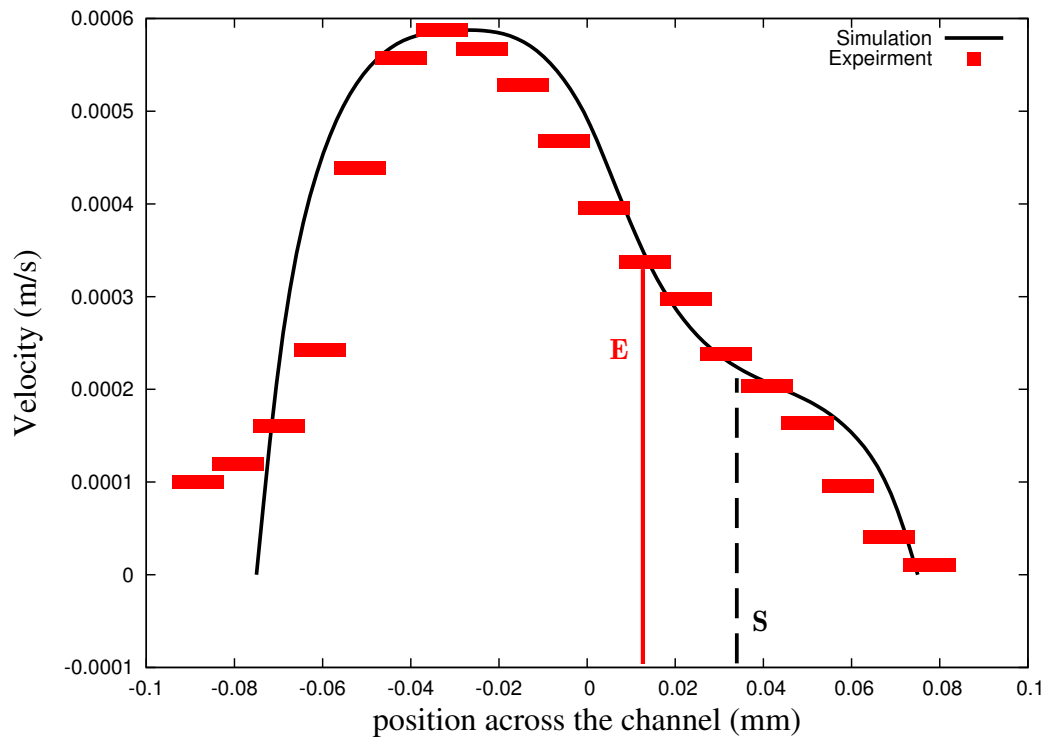


Figure 6.9: Velocity profile comparison of RBC in plasma from sample S at 10% H with simulation for the $150 \times 33 \mu m$ microchannel with a $Q = 5 \frac{\mu l}{hr}$. The interface location is denoted by S for the simulation E for the experiments.

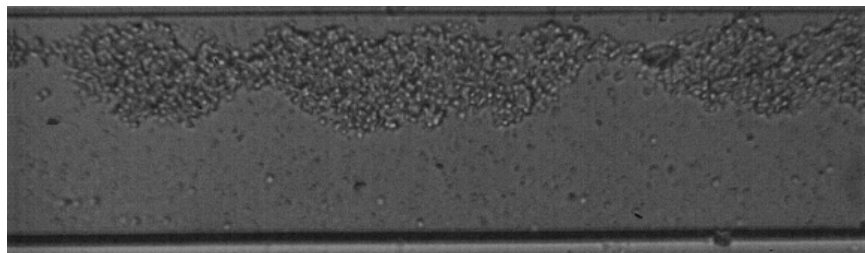


Figure 6.10: RBC from sample T suspended in plasma at 10% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 5 \frac{\mu l}{hr}$ visualized with the high speed camera

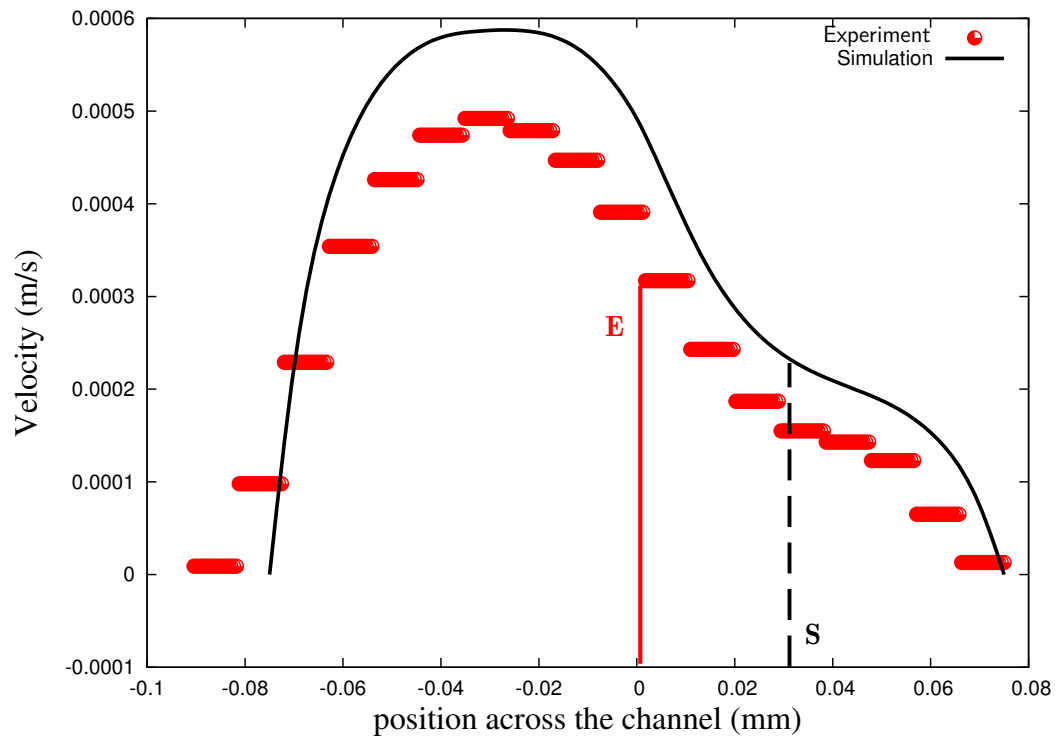


Figure 6.11: Velocity profile comparison of RBC in plasma from sample T at 10% H with simulation for the $150 \times 33 \mu m$ microchannel with a $Q = 5 \frac{\mu l}{hr}$. The interface location is denoted by S for the simulation E for the experiments.

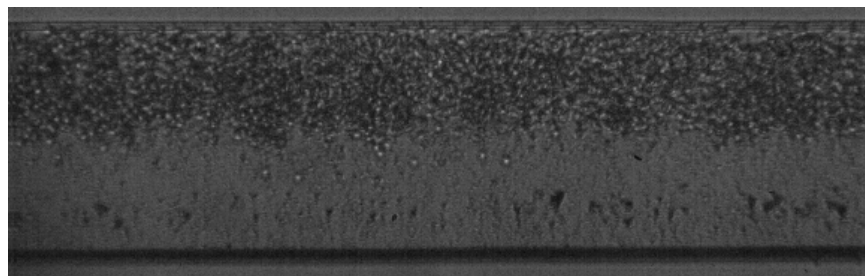


Figure 6.12: RBC from sample S suspended in plasma at 20% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$ visualized with the high speed camera

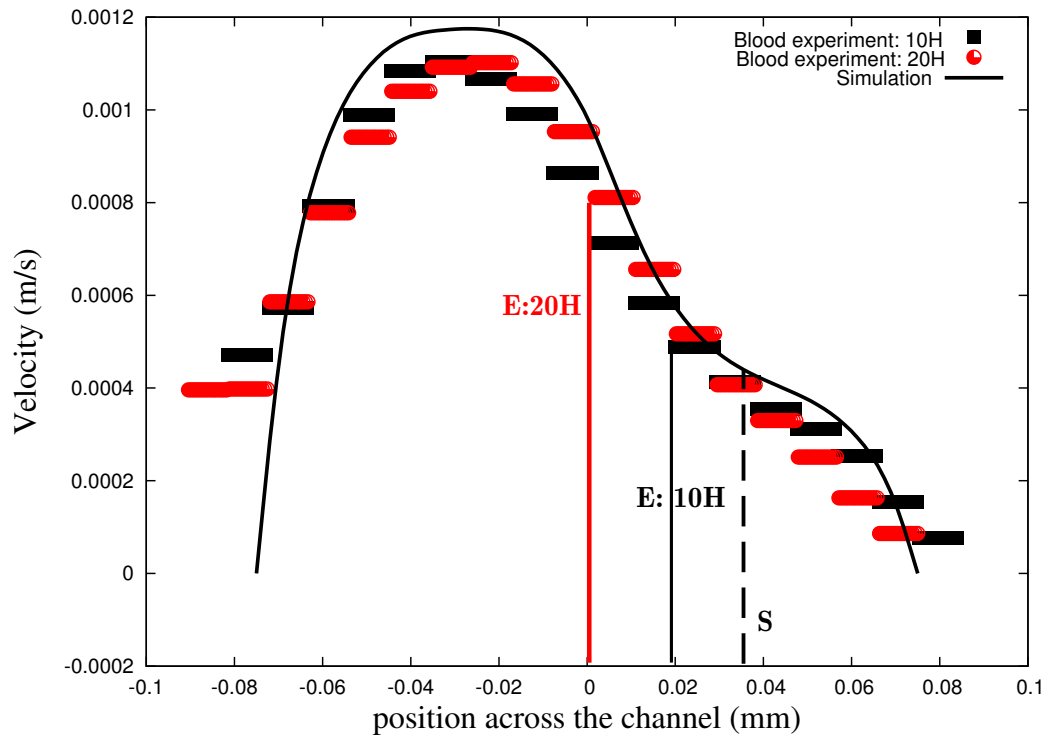


Figure 6.13: Velocity profile comparison of RBC in plasma from sample S at 20% H (red) with RBC in plasma 10% H (black) and simulation (solid line) for the $150 \times 33 \mu m$ microchannel with a $Q = 10 \frac{\mu l}{hr}$. The interface location is denoted by S for the simulation E for the experiments.

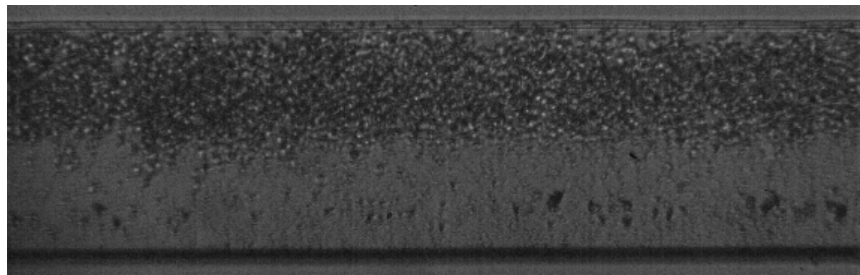


Figure 6.14: RBC from sample S suspended in plasma at 20% H flowing in the $150 \times 33 \mu m$ microchannel with a $Q = 5 \frac{\mu l}{hr}$ visualized with the high speed camera

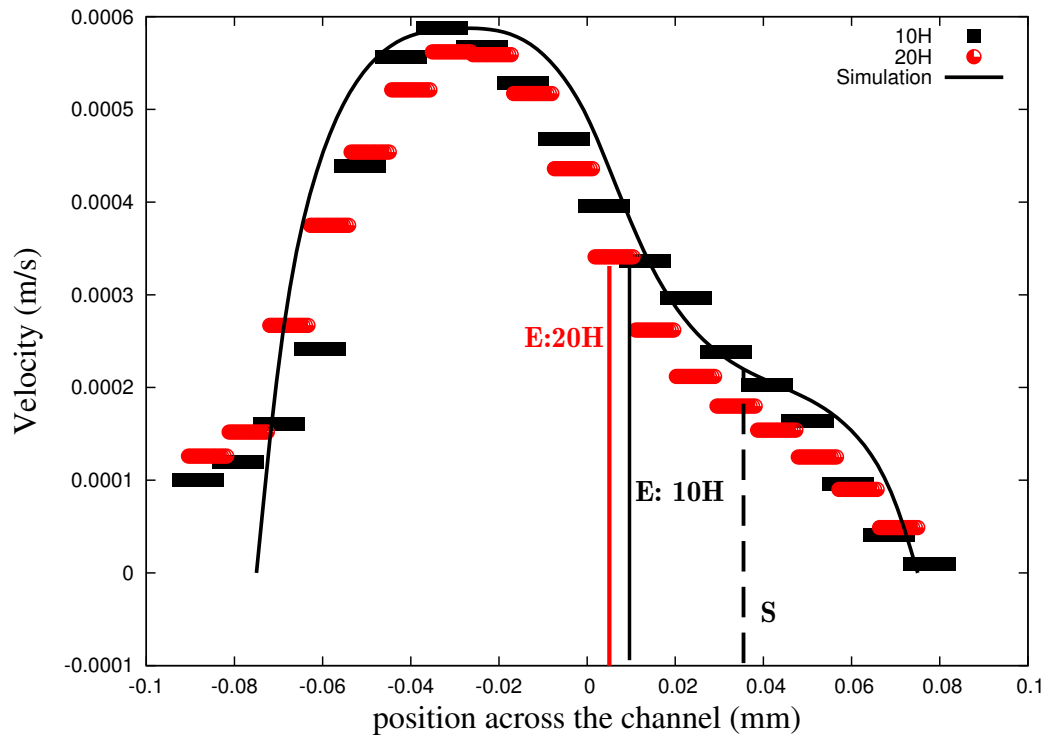


Figure 6.15: Velocity profile comparison of RBC in plasma from sample S at 20% H (Red) with RBC in plasma 10% H (black) and simulation (solid line) for the $150 \times 33 \mu\text{m}$ microchannel with a $Q = 5 \frac{\mu\text{l}}{\text{hr}}$. The interface location is denoted by S for the simulation E for the experiments.

Chapter 7

DISCUSSION

A physical microchannel model has been designed to create a micro-Couette blood flow apparatus using a two fluid flow system. The linear velocity profile obtained has been used to estimate the consequent constant shear rate that is found to be inversely proportional to the RBC aggregation size. The design process was first performed numerically using different channel geometries, such as the Ibidi $3 \times 1 \text{ mm}$ channel, the first microchannel prototype of $100 \times 15 \text{ }\mu\text{m}$, the $100 \times 50 \text{ }\mu\text{m}$, the $150 \times 33 \text{ }\mu\text{m}$ and the $170 \times 64 \text{ }\mu\text{m}$ microchannels to ensure the linearity of the profile within the small layer of the viscous fluid. The $150 \times 33 \text{ }\mu\text{m}$ microchannel was used for experimental testing.

Once the model was verified numerically, the validity of the numerical model was investigated and compared to an analytical solution of a two fluid flow in order to verify and compare the interface location and the velocity profile. Setting an interface location analytically, the velocity profile was compared to the simulated results where the interface was located and finally compared to the value set.

Then, the microchannel was tested experimentally first with a Newtonian fluid (glycerol) mimicking blood behaviour. The thickness of the blood layer as well as the velocity profile within this layer were also investigated by varying the velocity ratio between the two branches of the microchannel. For a velocity ratio of 4, the ideal thickness is obtained (one third of the channel thickness) with an almost linear velocity profile.

This model was then tested with the RBC flowing in the channel. Several RBC suspensions were tested:

- RBC suspended in PBS at 10% H at $Q = 10 \frac{\mu\text{l}}{\text{hr}}$
- RBC suspended in plasma at 10% H at $Q = 10 \frac{\mu\text{l}}{\text{hr}}$
- RBC suspended in plasma at 10% H at $Q = 5 \frac{\mu\text{l}}{\text{hr}}$

- RBC suspended in plasma at 20% H at $Q = 10 \frac{\mu l}{hr}$
- RBC suspended in plasma at 20% H at $Q = 5 \frac{\mu l}{hr}$.

The shear rates resulting from the linear velocity profiles were calculated and qualitatively related to the aggregate size visualized with the high speed camera.

Several important factors are considered in this study such as the linearity of the velocity profile and the effects of the RBC aggregates, shear rate and hematocrit of blood on the velocity profile.

7.1 LINEARITY OF VELOCITY PROFILE

Linearity of the velocity profile is required for our application in order to deduce constant shear rates that act as indices of aggregation size. When designing the microchannel, as discussed in Chapter 3, the ideal dimensions to provide a small thickness of the blood layer with a linear velocity profile were found to be a thickness of $100 \mu m$ and a depth of $50 \mu m$. However, after the fabrication process, the $100 \times 50 \mu m$ microchannel was found to measure $150 \times 33 \mu m$, which affects the linearity of the velocity profile. Although complete linearity of the velocity profile in the blood layer is not expected due to the ambient pressure gradient, the thickness of the blood layer is expected to be small enough to obtain a linear velocity profile and large enough to allow the formation of RBC aggregates. It was demonstrated that for the $100 \times 50 \mu m$ microchannel, the profile was almost completely linear as seen in Fig. 3.8, while for the $150 \times 33 \mu m$, we obtained a less linear velocity profile, which can be still be approximated as almost linear. Therefore, the values of shear rates obtained for the Newtonian fluid and the different RBC suspensions are good approximations of the flow conditions.

7.2 EFFECT OF SUSPENSION FLUID

When RBC suspended in plasma are compared to RBC suspended in PBS at a flow rate of $10 \frac{\mu l}{hr}$, the effect of RBC aggregation on the velocity profile can be visualized

and deduced. In fact, we showed that the velocity magnitude is higher in the case of the RBC-PBS suspension and that the shear rate is lower in the case of RBC-plasma suspension (Figs. 6.5 and 6.2). It can be then deduced that the viscosity of the RBC-plasma suspension was more viscous than the sample where RBC are suspended in PBS. These results seem to coincide with the *in vivo* observation in [110]. It was also demonstrated that lowering the shear rate increases aggregates size, which supports the finding of an inverse relationship between the shear rate and the aggregation size as stated in [111]. The results found, including the blood layer thickness and the velocity profile in the channel, showed good agreement with the numerical results, probably due to the low hematocrit of the blood samples.

7.3 EFFECT OF SHEAR RATE

The effect of the shear rate on the velocity profile was also investigated in our study. We demonstrated that lowering the shear rate by varying the pump flow rate and keeping the same velocity ratio between the branches affect the velocity values but not the blood layer thickness. Lowering the shear rate, by lowering the flow rate from $Q = 10 \frac{\mu l}{hr}$ to $Q = 5 \frac{\mu l}{hr}$, affects the velocity profile within the blood layer. It was found that the velocity at the interface was also reduced to about half of its previous value which, as shown, affects the size of RBC aggregates.

7.4 EFFECT OF HEMATOCRIT

The hematocrit is a major factor to consider. Several studies focused on demonstrating the hematocrit effect on the blood viscosity [28, 29, 34, 83]. In our study, RBC suspended in plasma at 10 and 20 % H were tested at two different flow rates and compared to the numerical results. We were able to explore the effect of hematocrit by showing the velocity and shear rate differences between the two samples. We noticed that the effect of hematocrit could not be clearly seen through the velocity results obtained for higher shear rates (for a flow rate of $Q = 10 \frac{\mu l}{hr}$). However, at a lower shear rate, a clear difference could be seen through the velocity profiles between the

two blood samples (10 and 20% H), where the velocity of blood at 10% H was higher than the one for blood at 20% H. Therefore it can be concluded that the viscosity of blood is proportional to the hematocrit, supporting the research of Kim *et. al.* [28, 29].

7.5 POSSIBLE ARTIFACTS

Experiments are usually subject to numerous artifacts that could interfere with the results, thus creating possible errors that need to be considered. Such artifacts include internal (blood sample and microchannel) and external (equipment precision) factors.

7.5.1 DIFFERENCES BETWEEN NUMERICAL SIMULATIONS AND EXPERIMENTS

Several factors can explain the small deviations between the numerical and experimental results.

The main difference is the nature of the fluids considered in both methods. The simulations are conducted using two Newtonian fluids flowing in the channel, while, experimentally, the RBC suspensions are non-Newtonian, entrained by a Newtonian fluid. Although the simulations are restricted to Newtonian fluids presently, they provide theoretical cases which do not entirely represent the actual blood flow behaviour. Due to this major difference, the viscosity of blood at low shear rate and high hematocrit is not expected to be the same as the value of viscosity set for fluid B simulating blood ($\mu_B = 3 \text{ cP}$), hence the difference in blood layer thickness when the velocity ratio remain constant.

Even with the glycerol experiments, which is a Newtonian fluid, the viscosity of fluid used for the simulations might not reflect the actual viscosity of the mixture. In fact, the viscosity of the glycerol mixture was calculated to obtain a viscosity of 3 *cP* considering the particle solution. It was then verified with a LV type viscometer that indicated an average viscosity of 3 *cP*. However, the equipment used was not very stable and kept fluctuating. The whole system is sensitive to the alignment of the equipment, which was very difficult to accomplish.

In addition, the interface is measured differently numerically and experimentally as discussed in Chapter 2, which can influence the shear rate values obtained.

Moreover, the experimental flow rates differ slightly from those expected by the numerical simulations as shown in [130]. Several explanations for this variation include the use of two different pumps, with different precision, to insert the two different fluids in the channel, but also include experimental factors not considered in the ideal numerical case such as surface tension between the material and the fluid, different pressure gradient (different dimensions due to fabrication limitations) and effect of the large holes for fluid insertion.

The difference between the numerical and experimental velocity profiles could also be due to the image processing method using the μ PIV system. An estimation of the RMS velocity error is provided in Fig. 5.25 where the significantly high error magnitude could be seen at the interface location. This error is probably caused by the averaging of the recorded data in time and space for cross-correlation purposes. In fact, the unsteady nature of the interface between the two fluids might greatly affect cross-correlation process accuracy.

7.5.2 EFFECT OF PROCESSING

Errors might also occur when processing the data recorded. The timing between the double frames dt in the cross-correlation calculation was optimized so that the displacement of one particle results in nine or ten pixels (based on the blood layer) according to Kim *et al.* [85]. However, the flow rate used for this experiment is ten times slower than in [85], therefore the dt chosen might not be large enough for slower flow rates which can result in loss of information when cross-correlating the set of frames. In the same paper Kim *et al.* suggest to decrease the window size for better results, however for this research decreasing the window size lowers the values of the velocity and changes the shape of the profile as seen in Fig. 6.1. A method that might improve the results is to use a stronger lens (40x) to ensure no loss of information as suggested in [85]. Another factor influencing the processed data is the diffusion of the tracer particle in and beyond the interface, which is not considered

in the CFD model. At the interface between the two fluids, some of the fluorescent particles used for velocity tracking diffuse to the second layer where no particles are flowing (due to the difference in concentration) as seen in Fig. 5.24. This diffusion might affect the experimental velocity values found at the interface where precision is required to calculate the shear rate.

7.5.3 EFFECT OF PARTICLE SIZE

When blood is tested experimentally to be visualized with the high speed camera, the fluorescent particles are inserted with the RBC suspension to capture the velocity of the flow, leading to a mixture of two different particle sizes. The RBC, five to six times larger than the fluorescent particles, hence push the tracer particles to the PBS layer. Therefore, fewer tracer particles flow within the RBC layer, which can affect the cross-correlation results, since they rely on the particle's intensity. In addition, due to the large RBC size mixed with smaller tracer particles, the small particles tend to segregate forming a large fluorescent entity that could affect the processing of the data recorded. In order to solve the segregation problem of the small particles, several studies tend to label the RBC to appear fluorescent under the laser pulse [91–93], which might not provide accurate results in our study due to the aggregation of these RBC, forming very large entities. Moreover, RBC suspended in plasma settle in the tube and syringes causing a paucity of RBC in the channel, which might lower the expected hematocrit and affect the flow. A possible solution would be to use an ultrasonic vibrating device on the tubes to avoid the settlements of these particles. However it might be difficult to accomplish without causing the vibration of the whole system and hence interfering with the motion of the particles in the microchannel.

7.5.4 EFFECT OF SLIP CONDITIONS

For the majority of the results, a slight difference on the left part of the velocity profile (fluid A, the entraining fluid) can be noticed between the numerical and experimental results. In fact, numerically, the no-slip boundary condition is set at the channel walls.

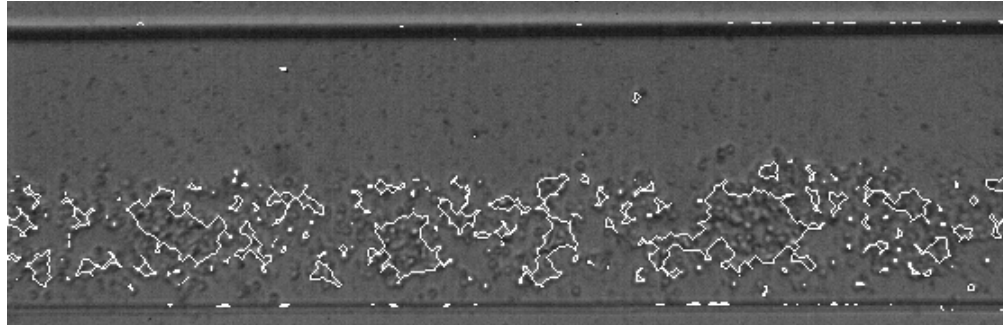


Figure 7.1: Image processing of recorded data of RBC in plasma flowing with a flow rate of $Q = 10 \frac{\mu l}{hr}$ at 10% H.

Experimentally, the microchannel was used a full week after the plasma bonding process accounting for the slippery walls and minimizing their effect. However, for most of the velocity profiles obtained, slip conditions appear at the channel wall facing fluid A (water or PBS) only, whereas the velocity at the channel wall facing fluid B (glycerol or blood) is almost null. This phenomenon might be due to the different interaction of the fluids with the PDMS. In fact, the surface tension between blood and PDMS or glycerol and PDMS was shown to be higher than the surface tension between water and PDMS or PBS and PDMS [133].

7.6 RBC AGGREGATE SIZE ESTIMATION

Different methods exist to estimate RBC aggregation size based on aggregation indices, however no method is capable of giving a precise estimation of the aggregation size. Our study did not focus on the aggregation size determination but rather on the design of the system (microchannel and two fluid flow model). However, we still attempted to obtain a range of aggregation size using image processing methods. A MATLAB program outlining the RBC aggregation is used. Once the RBC aggregates are clearly determined it becomes easier to estimate their size. By automating the

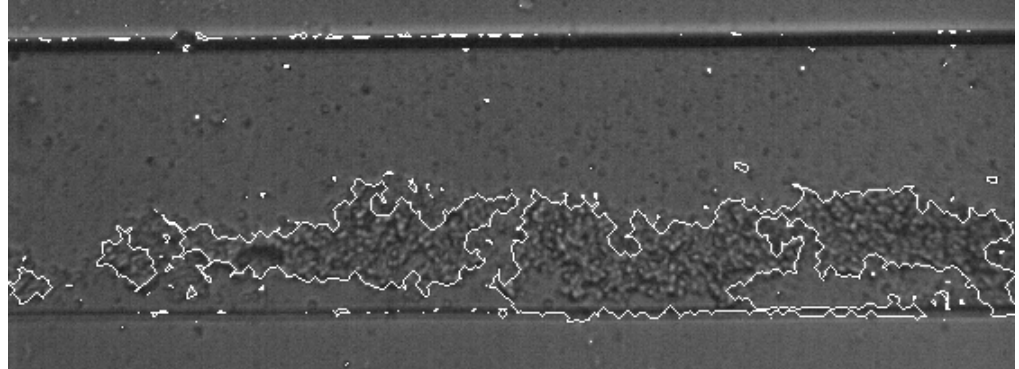


Figure 7.2: Image processing of recorded data of RBC in plasma flowing with a flow rate of $Q = 5 \frac{\mu l}{hr}$ at 10% H.

process, an average aggregate size can be related to the constant shear rate applied. The MATLAB program consists of several steps: first, the image to be analyzed is opened and read. The cells are then detected based on the difference in contrast between the segmented object and the background image. The segmentation is performed by calculating the gradient of the image, creating a binary mask delimitation by a threshold calculated using the edge and the "Sobel" operator. The outline around the segmented cell is created by the "Bwperim" function. Figures 7.1 and 7.2, show the difference in the outlined aggregation size for the samples of RBC in plasma at 10% H flowing at $Q = 10 \frac{\mu l}{hr}$ and $Q = 5 \frac{\mu l}{hr}$ respectively. We notice the increase in aggregate size with the shear rate diminution as stated previously in Sec. 7.2 and demonstrated *in vivo* in [111].

Chapter 8

CONCLUSION

A physical microchannel model providing a micro-Couette blood flow apparatus based on a two fluid flow system has been designed. The design process was first guided by numerical simulation of different channel geometries, such as the Ibidi $3 \times 1 \text{ mm}$ channel, the first microchannel prototype of $100 \times 15 \text{ }\mu\text{m}$, the $100 \times 50 \text{ }\mu\text{m}$, the $150 \times 33 \text{ }\mu\text{m}$ and the $170 \times 64 \text{ }\mu\text{m}$ microchannels to ensure the linearity of the profile within the small layer of the viscous fluid.

The final model consisted of a $150 \times 33 \text{ }\mu\text{m}$ microchannel where two fluids, with different properties and entering from the two different branches of the Y-configuration with two different flow rates, flow in the microchannel with different layer thicknesses. The model obtained numerically was then compared to a simple analytical solution to ensure the right position of the interface. The microchannel was then used for experimental testing with Newtonian fluids (glycerol entrained with water) and blood (RBC suspensions entrained with PBS). The thickness of the blood layer as well as the velocity profile within this layer were investigated by varying the flow rate and velocity ratio between the two branches of the microchannel.

The constant shear rate values were estimated based on the quasi-linear velocity profile. The experimental results showed good agreement with the numerical solutions. It was found that the ideal thickness of the viscous fluid to be analyzed was about one third of the channel thickness. The tests performed experimentally with the RBC suspensions took into consideration the hematocrit and shear rate effects on the aggregate size. The blood experiments also seemed to agree with the numerical model. Blood aggregates could be clearly visualized, using the high speed camera at 10% especially when flowing with a flow rate of $Q = 5 \frac{\mu\text{l}}{\text{hr}}$, where the shear rate was within the appropriate range, which was not clear for the RBC suspended at 20% H due to the large number of particles.

This study provided pertinent inputs for understanding blood behaviour by designing a controlled blood flow system that could be coupled to recent studies for RBC aggregation size estimation according to the flow conditions. Several points still require further investigation. The labelling of the RBC in the flow to avoid the segregation of the fluorescent particles could be more developed. For example, the tracer particles could be attached to the RBC so that each RBC in the aggregates could be seen under the microscope without forming a large fluorescent entity. Future work would include further RBC aggregate size analysis. A standard method could be developed to obtain an estimation of the rouleaux size under several conditions. This advanced method would help better understand blood behaviour in microcirculation under pathological conditions or even help find remedies to the problem of clogged arteries due to RBC aggregation under these pathological conditions.

This work has been and will be presented on several occasions and was multiply awarded for its relevance to the hemorheology field and innovation. We presented this study as a poster presentation in the special rheology workshop of the Canadian Society of Rheology that took place in June 2011. This research was also presented at the University of Ottawa Faculty of Engineering Graduate Research Day and won the third prize poster presentation award and the second prize poster presentation for two consecutive years in February 2011 and 2012. This work was also accepted to be published and presented in the ASME 2012 10th International Conference on Nanochannels, Microchannels and Minichannels (ICNMM 2012) in July 2012. A second paper, focusing on the image processing methods, discussed in Chapter 6, was submitted to *Measurement Science and Technology*. And finally the content of this research will be presented in the 9th International Conference on Spectral and High Order Methods (Icosahom 2012) that will take place in June 2012.

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Appendix A

TESTS PERFORMED ON THE LARGE IBIDI AND FIRST MICROCHANNEL PROTOTYPE

A.1 PREFABRICATED IBIDI CHANNEL

A.1.1 TWO DIMENSIONAL SIMULATIONS

This section presents several tests performed on the prefabricated Ibidi channel to determine the ideal parameters to create a micro-Couette flow model that can be used experimentally.

A.1.1.1 FIRST TEST

For the first test, the geometry of the μ slide III³ⁱⁿ¹ channel is used with only two entries. As a first trial, the domain is discretized into 15 elements, where the order of the highest Legendre polynomial N was chosen to be $N = 8$. For the velocity and pressure spaces the allocation of points in each element is based on Gauss-Lobatto-Legendre (GLL) quadrature points known as the $P_N - P_N$ formulation [116]. The same fluid, assumed to have the same kinematic viscosity as water of about $\nu = 1 \frac{mm^2}{s}$, enters from both branches with two different velocities. The entrance flow rate of the bottom branch was chosen to be $Q_{bottom} = 2 \frac{\mu l}{hr}$ based on the flow rate in capillaries as mentioned previously in 3.1. Therefore the entrance velocity of the bottom branch can be found using:

$$V_{bottom} = Q_{bottom} \times A_{branch} = 0.59 \frac{mm}{s} \quad (A.1)$$

where the cross sectional area of the branches was calculated to be $A_{branch} = 1mm^2$ based on the depth of the prefabricated channel. During the performance of these tests, only one pump (the Nexus 3000 pump) was available to test the channel in the laboratory and push both fluids in the channel. Therefore, two BD plastic syringes

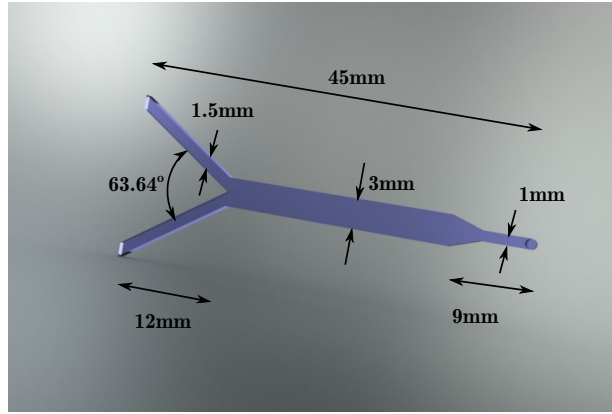


Figure A.1: Microchannel geometry for the first test

with different diameters are pushed with the pump to create two different flow rates. For this reason, the flow rate depends on the diameter ratio of the two syringes and cannot be easily controlled. The first syringe used on the top branch is the $5mL$ plastic BD syringe that has a diameter of $d = 12.07mm$, where as the second syringe, used for the bottom branch is the $10mL$ plastic BD syringe with a diameter of $D = 14.5mm$. The resultant flow rate from the second syringe Q_{top} and the entrance velocity in the top branch can be found using Equations (A.2) and (A.3) respectively:

$$Q_{top} = \left(\frac{d}{D}\right)^2 Q_{bottom} \quad (A.2)$$

$$V_{top} = Q_{top} \times A_{branch} = 0.85 \frac{mm}{s}. \quad (A.3)$$

The velocity distribution in the channel is shown in Fig. A.2 where it can be noted that the velocity is uniform along the channel except at the outlet since the channel decreases in width. However, it is not evident to visualize the velocity distribution within the channel since the highest velocity (shown in red) is concentrated at the end of the channel. Fig. A.3 shows that the velocity profile, extracted from the channel at the position $x = 23mm$ along the channel, is parabolic as expected.

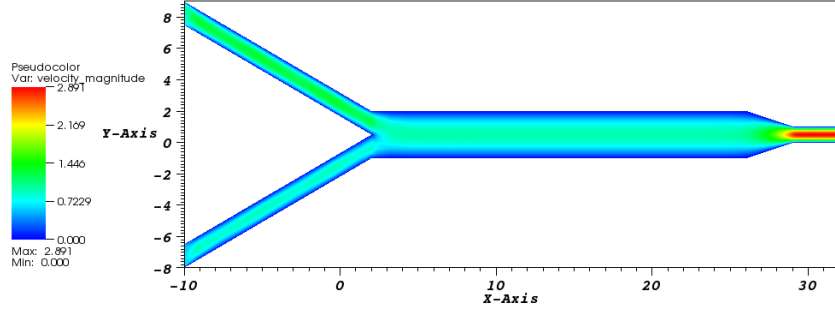
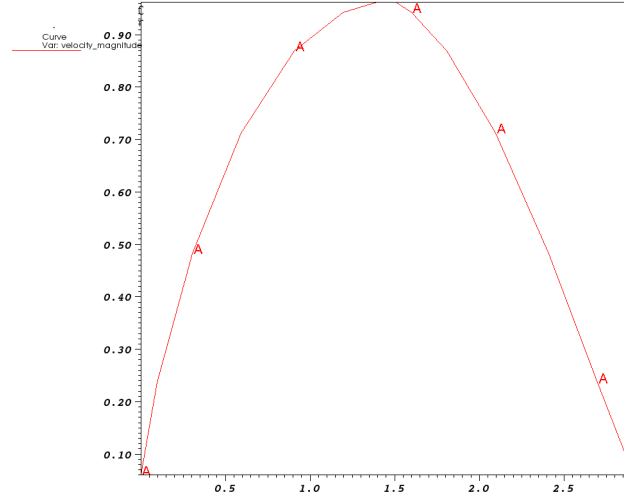


Figure A.2: Velocity distribution in the channel for the first test

Figure A.3: Velocity profile extracted from the channel at $x = 23$ mm for the first test

A.1.1.2 SECOND TEST

Since the velocity distribution was difficult to visualize for the previous test, the converging outlet of the channel is removed from the simulation for this second test. All other dimensions and parameters remained unchanged. However for this case more elements were added to the channel. A total of 40 elements are used to discretize the continuous domain, where the order of the highest Legendre polynomial N was kept as $N = 8$. Also the allocation of points within the elements is still based on the $P_N - P_N$ formulation. The same fluid with a kinematic viscosity of $\nu = 1 \frac{mm^2}{s}$ is pushed in the channel at a velocity of $V_{bottom} = 0.59 \frac{mm}{s}$ and $V_{top} = 0.85 \frac{mm}{s}$ from the bottom and top branches respectively.

From Fig. A.4, which represents the velocity distribution within the channel,

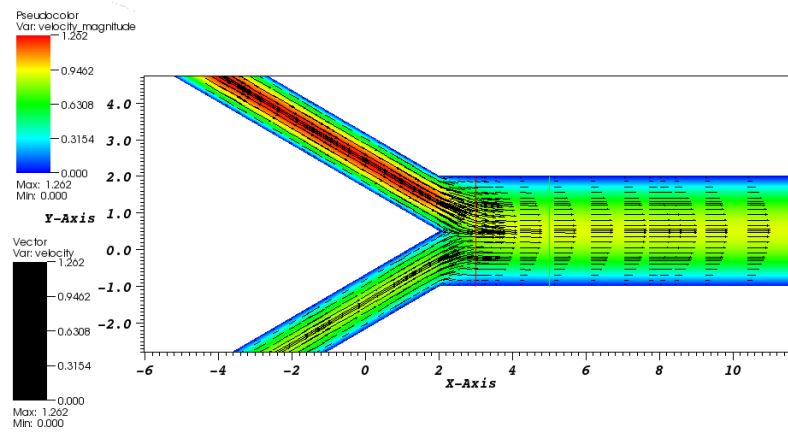


Figure A.4: Velocity distribution in the channel for the second test. Close up at the confluence of the two branches

it can be clearly seen that the velocity is uniform along the channel that shows a quasi-parabolic profile (Fig. A.5). As in the previous case of section A.1.1.1, the quasi-parabolic profile is expected since the same fluid is used in both branches.

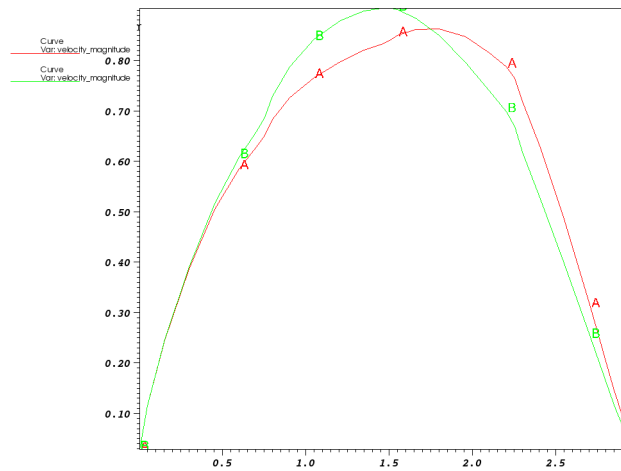


Figure A.5: Velocity profile extracted from the channel at $x = 23$ mm for the second test

A.1.1.3 THIRD TEST

The two previous tests were performed only one fluid, assumed to have the same viscosity as distilled water, flowing in the channel. However, in order to achieve the goal of this research i.e. to design a controlled environment to study RBC aggregation, the system requires the motion of two different fluids with different viscosities. The choice of the fluid that is responsible for entraining the RBC layer is an important

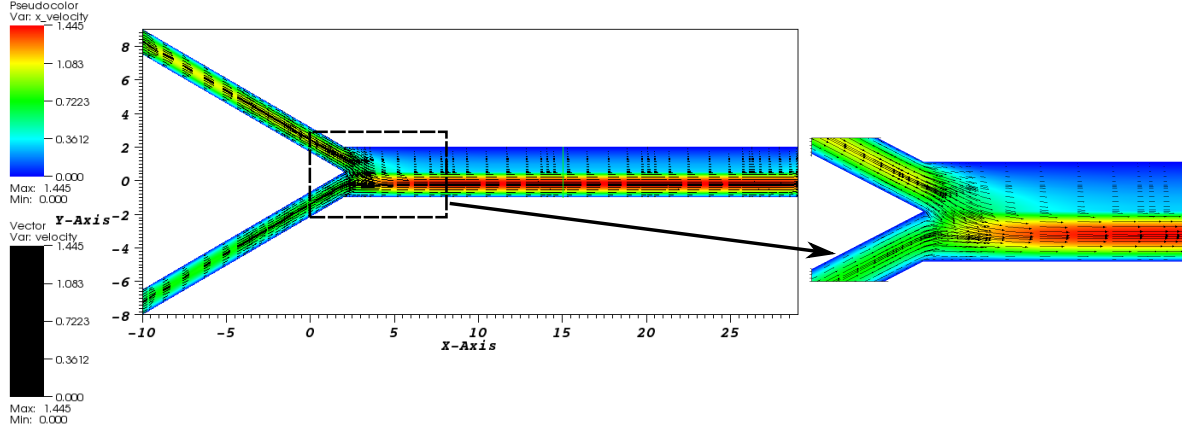


Figure A.6: Velocity distribution in the channel for the third test

factor to determine. As a first step, oil (unrefined sunflower oil) was tested as the intraining fluid due to its immiscible property with other fluids such as water solutions. This unrefined sunflower oil has a density of $920 \frac{kg}{m^3}$ and a dynamic viscosity of $49.14 cP$ [134]. Therefore its kinematic viscosity that will be used in the simulation is $53.41 \frac{mm^2}{s}$. The exact same geometry as in Sec. A.1.1.3 is used for this test. The difference is the two fluids, with different properties, entering from both branches. Oil enters from the top branch at a velocity of $V_{top} = 0.85 \frac{mm}{s}$, whereas water enters from the bottom branch with a uniform velocity of $V_{top} = 0.59 \frac{mm}{s}$.

The velocity profile extracted from the end of the channel, describing this specific case, is shown in Fig. A.7 which was extracted from the channel as shown in Fig. A.6. We see that the velocity reaches a maximum value on the lower half of the channel (left part of the curve), where the less viscous fluid is flowing, while the velocity seems to be linear within the right part of the plot. However, it is not possible to determine the thickness of each fluid layer, which is an important determinant of the shear rate. Therefore, it is important to locate the interface between the two fluids.

A.1.1.4 FOURTH TEST

For this test, the third test is replicated with some variations. The stress formulation $P_N - P_{N-2}$ is used for this case since we are dealing with variable viscosities in time and space. The difference is the allocation of points for velocity and pressure

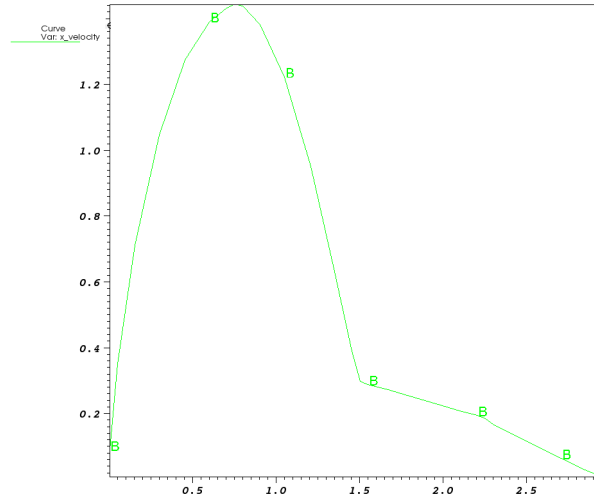


Figure A.7: Velocity profile extracted from the channel at $x = 23$ mm for the third test

spaces in each element which is now based on Gauss-Lobatto-Legendre (GLL) and the Gauss-Legendre quadrature points respectively. The advantage of this method is that the stress variable is treated implicitly and therefore more suitable for variable viscosities. Therefore, for this case, oil enters from the top branch at a velocity of $V_{top} = 0.85 \frac{mm}{s}$, whereas water enters from the bottom branch with a uniform velocity of $V_{top} = 0.59 \frac{mm}{s}$.

Figs. A.8 and A.9 compare well with the corresponding figs. A.6 and A.7 in section A.1.1.3, as expected since no parameters were changed between the two cases. Fig. A.10 shows the distribution of the viscosity in the channel, we notice that the interface between the two fluids remains stationary. However, we expect to obtain different thicknesses for the two fluid layers as we attempt to control the shear rate within the channel.

A.1.1.5 FIFTH TEST

This case is a repeat of the third test with some variation. Oil enters from the top branch with a different uniform velocity of $V_{top} = 2.25 \frac{mm}{s}$, whereas water still enters from the bottom branch with a velocity of $V_{top} = 0.59 \frac{mm}{s}$. Therefore, the velocity of the viscous fluid (oil in this case) is four times higher than the water solution in order to ensure the location of the interface depending on the flow conditions. For

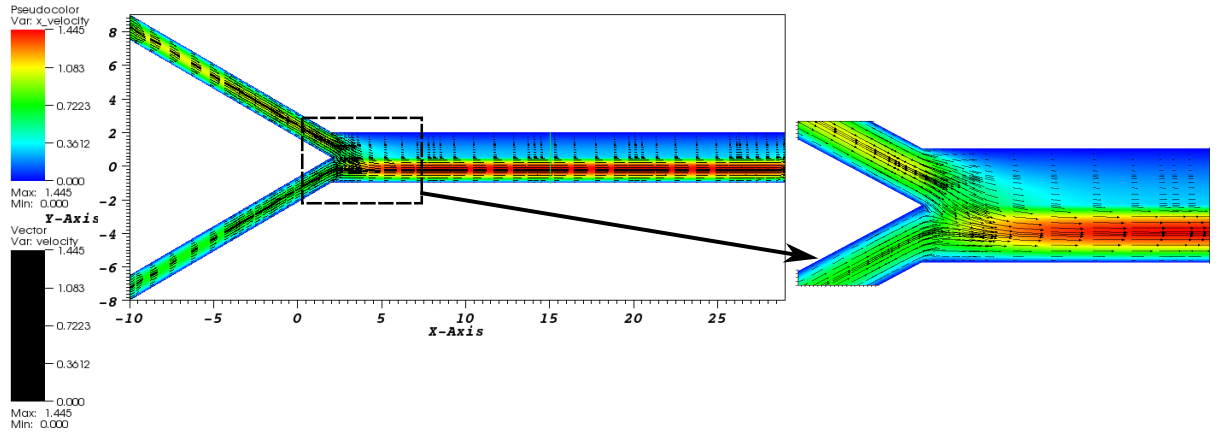


Figure A.8: Velocity distribution in the channel for the fourth test

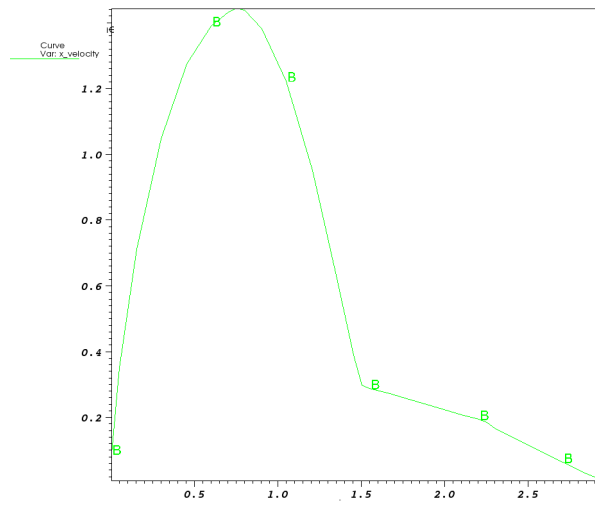


Figure A.9: Velocity profile extracted from the channel at $x = 23$ mm for the fourth test

this test the $P_N - P_{N-2}$ formulation is used to allow for variable viscosity. In order to visualize the viscosity distribution in the channel, the viscosity is treated as a passive scalar to be solved for and then used in the solution of the Navier-Stokes equations. This method allows us to determine the location of the interface between the two fluids layer since it is supposed to accommodate to the flow conditions. Fig. A.11 represents the velocity distribution within the channel. The velocity still reaches its maximum in the lower part of the channel as confirmed in Fig. A.12 (right part). However, the left part of the profile, which corresponds to the upper layer of the channel is not as linear as in the previous case detailed in section A.1.1.4. We can see in Fig. A.13, that the interface is still located in the middle of the channel, despite

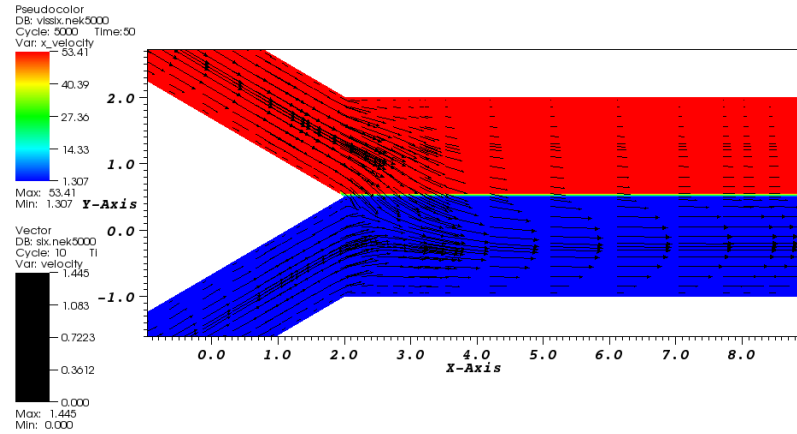


Figure A.10: Viscosity distribution in the channel for the fourth test

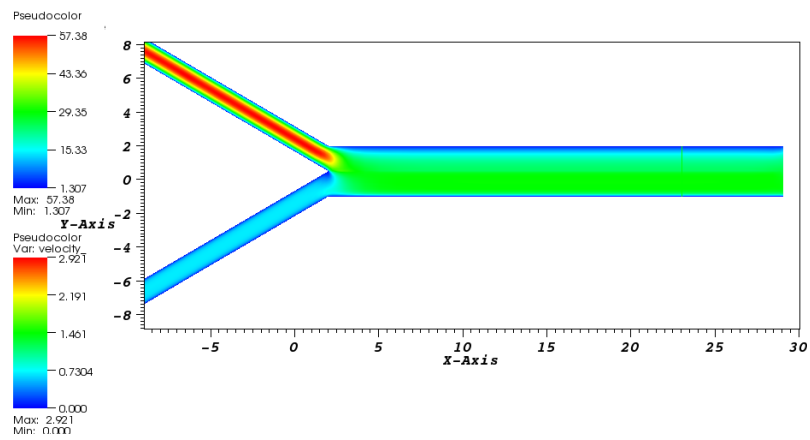


Figure A.11: Velocity distribution in the channel for the fifth test

the larger velocity ratio between the branches. From this case, we conclude that the oil is not ideal to entrain blood to create a controlled Couette blood flow. In addition, the interfacial tension between water and oil is very high which leads to instabilities within the flow, such as bubble formation. Although oil and water are two immiscible fluids as required for this application, at the micro level, two normally miscible fluids will tend to not mix due to the low Reynolds number. Therefore, another fluid with a smaller or null interfacial tension is preferred.

A.1.1.6 SIXTH TEST

From the previous tests, we noticed that the use of oil with a less viscous fluid is not appropriate for this application. Since the purpose of this application is to introduce

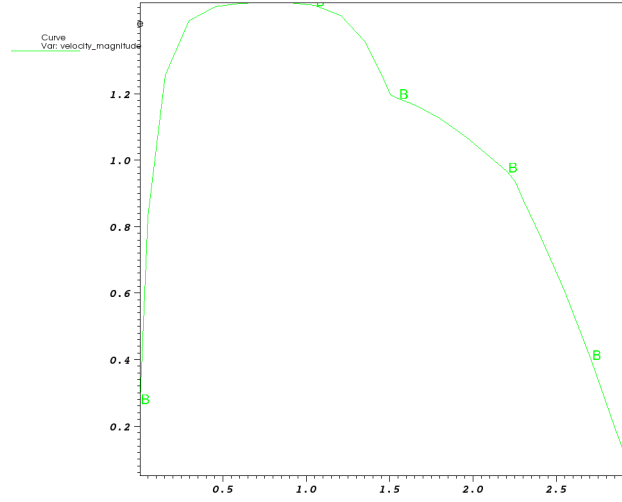


Figure A.12: Velocity profile extracted from the channel at $x = 23$ mm for the fifth test

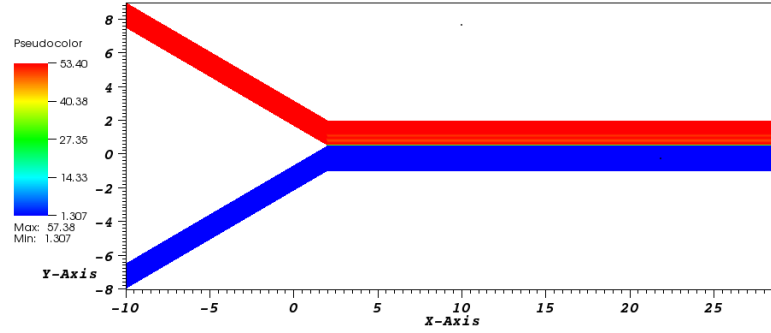


Figure A.13: Viscosity distribution in the channel for the fifth test

blood through one of the branches to analyze its flow, the apparent viscosity of blood at low shear rates, and the viscosity of water are used to simulate the two fluid flow for the next tests. The apparent viscosity of blood at low shear rates is about $\mu = 3cP$ [54] and its density is about $\rho = 1060 \frac{Kg}{m^3}$. Therefore, for these tests, blood is assumed to have a kinematic viscosity of $\nu = 3 \frac{mm^2}{s}$ and enters from the top branch of the channel at a velocity of $V_{top} = 0.7 \frac{mm}{s}$. The second fluid, assumed to be a water solution having a kinematic viscosity of $\nu = 1 \frac{mm^2}{s}$, is introduced from the bottom branch at the same uniform velocity of $V_{bottom} = 0.7 \frac{mm}{s}$. The stress formulation is used in this test to allow motion of the interface according to the flow conditions. However, the free-surface hydrodynamic formulation is used for this test. With this condition, the mesh is allowed to deform according to the flow. Therefore for this case, not only the interface between the two fluids accommodates to the flow conditions

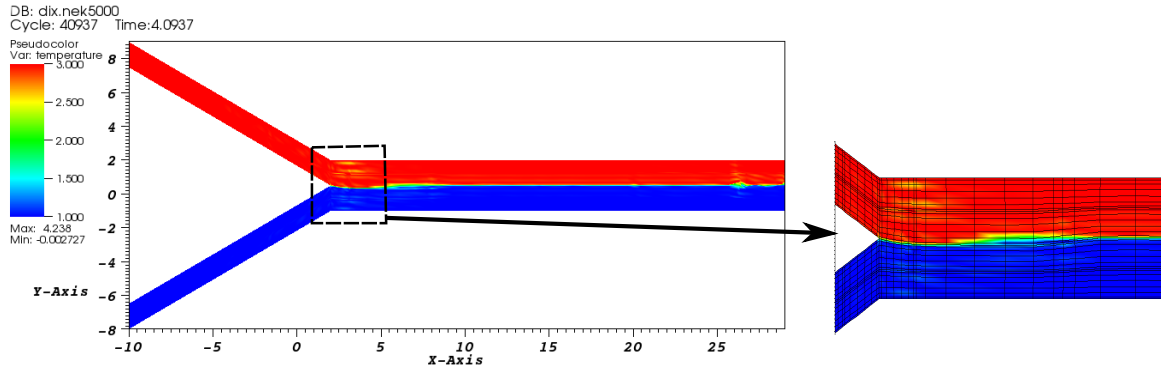


Figure A.14: Viscosity distribution in the channel for the sixth test and comparison with the mesh deformation. The interface can be located at $1.3mm$ from the bottom wall of the channel

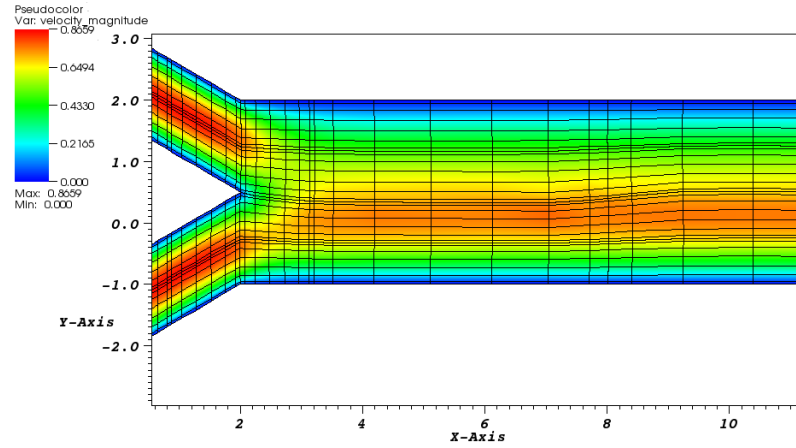


Figure A.15: Velocity distribution in the channel for the sixth test

but the mesh follows the same pattern. In order to use this condition, the surface tension, which in this case represents the interfacial tension, must be a fixed value or function. Since the two fluids used for this test, blood and a water solution, are assumed to be miscible, the interfacial tension was set to $\sigma = 10^{-4} \frac{Pa}{m}$ as an internal boundary condition in the middle of the channel. However in microcirculation, two miscible fluids do not mix because of the low Reynolds number, therefore, to minimize the diffusion layer, the diffusive term is set to be $\alpha = 10^{-3}$. In fact the diffusive term cannot exceed this value since it will cause problems of negative diffusivity when running the case.

Fig. A.16 shows the motion of the mesh according to the flow set for this case.

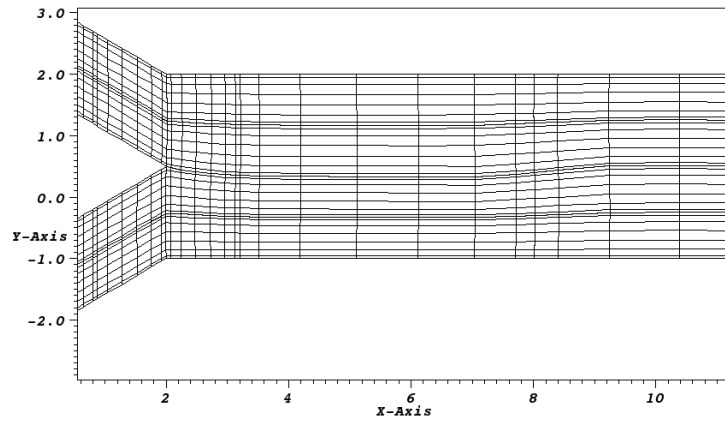


Figure A.16: Mesh deformation in the channel according to the flow conditions for the sixth test

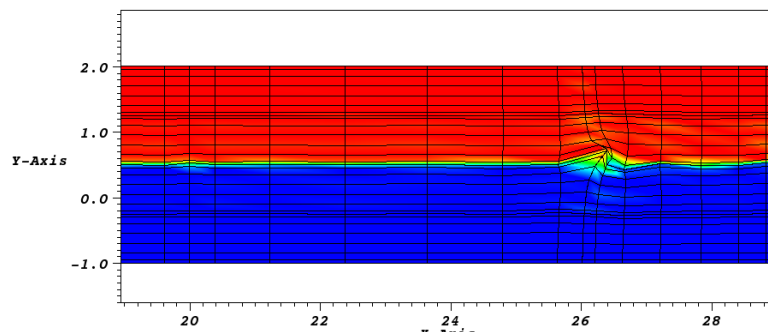


Figure A.17: Example of mesh distortion

We notice that the mesh boundary deforms in the channel from its original position (middle of the channel) so that the viscous fluid, entering from the top branch, is found to have a larger layer than the less viscous fluid. The interface between the two fluids is located at 1.3mm from the bottom wall of the channel. This results seems sensible since both fluids enter at the same velocity, and the viscous fluid tends to expand faster in the channel. Looking at the velocity profile, shown in Fig. A.18, the maximum value is located in the less viscous fluid layer of the channel (left part of the profile), while the velocity decreases when reaching the viscous fluid. A linear profile from the supposed "blood layer" is not expected since the two fluids are introduced in the channel with the same velocity. The advantage of using the moving boundary option is that not only the interface moves but the grid also accommodates to the boundary conditions, which helps to ensure the right direction for the interface motion. However, mesh distortion can occur as shown in A.17 and in some cases, mesh

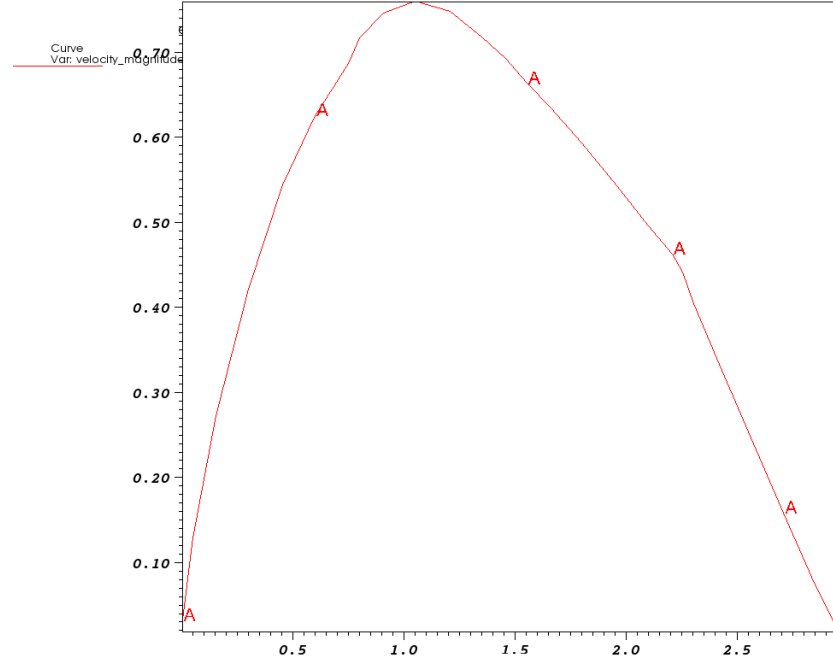


Figure A.18: Velocity profile extracted from the channel at $x = 23$ mm for the sixth test

overlap engender problems when running the case. We also notice, through Fig. A.13, the disturbance of the viscosity where the mesh deforms which can interfere with the results when calculating the shear rate within the "blood layer".

A.1.1.7 SEVENTH TEST

For this test, the same situation is repeated with the same two fluids used for the previous test detailed into section A.1.1.6. However, the the free-surface hydrodynamic formulation is not used here but only the stress formulation is activated. Also, the number of elements was increased to 344 elements for this case, while the order of polynomials remain $N = 8$ in order to obtain more accurate results. The purpose of this test is to see the difference between the two cases to choose the appropriate formulation to use. Viscosity of blood $\nu = 3 \frac{mm^2}{s}$ is set as boundary condition on the top branch as well as its velocity of $V_{top} = 0.7 \frac{mm}{s}$. A water solution having a kinematic viscosity of $\nu = 1 \frac{mm^2}{s}$ is introduced from the bottom branch at the same uniform velocity of $V_{bottom} = 0.7 \frac{mm}{s}$. The diffusive term is kept to be $\alpha = 10^{-3}$.

Through A.19, we can see that the results are unchanged from the previous case.

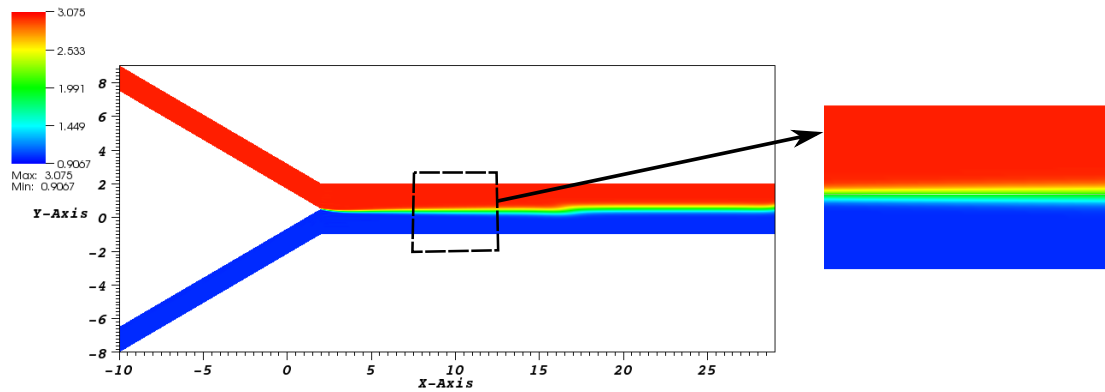


Figure A.19: Viscosity distribution in the channel for the seventh test

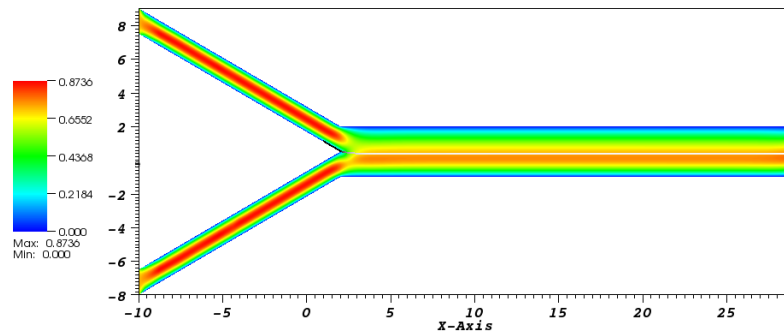


Figure A.20: Velocity distribution in the channel for the seventh test

In fact, the interface location is found to be at about 1.3mm from the bottom wall of the channel as in the previous case. This means that the "blood layer" represents 56% of the channel thickness. However comparing to the case in section A.1.1.6, the viscosity distribution is smoother. Also, the diffusion layer is shown to increase along the channel, as expected. Looking at the velocity profile presented in fig. A.21, the same conclusion can be drawn as previously where the maximum value of velocity is located in the less viscous fluid layer of the channel (left part of the profile), and then this velocity decreases when reaching the viscous fluid. Therefore, for this application, the moving boundary condition will not be used for the simulations since setting the viscosity as a passive scalar is sufficient to model the flow behaviour and is able to provide the same but smoother viscosity distribution. However using these specific parameters for velocity and viscosity in the channel did not provide a linear velocity

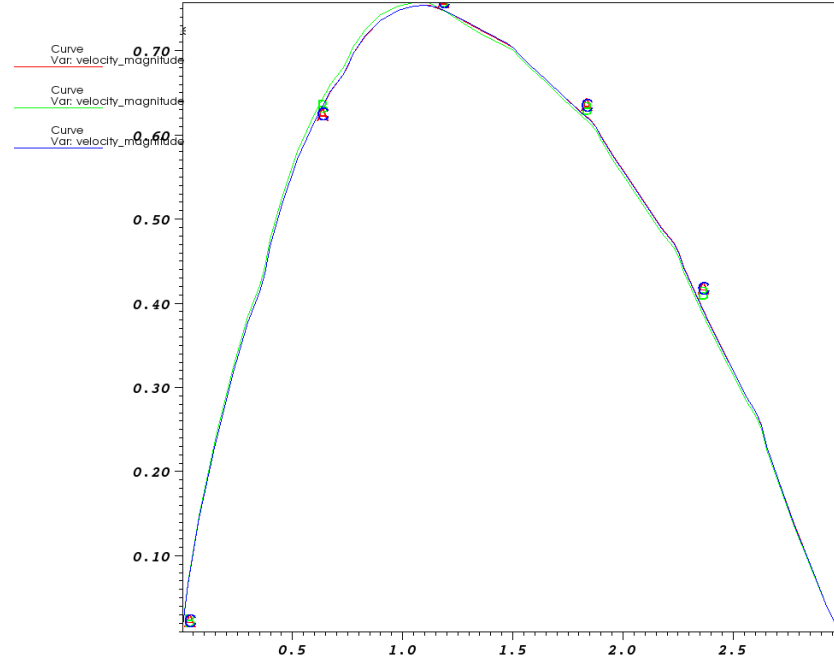


Figure A.21: Velocity profile extracted from the channel at $x = 23$ mm for the seventh test

profile in the blood compartment and therefore, more tests are required to determine the ideal case for such flow. This present case is tested experimentally and is detailed in section A.1.2 in order to be compared to the numerical results.

A.1.1.8 EIGHTH TEST

For this case the same geometry, mesh distribution and set up as in the previous case are used where 344 elements discretize the domain using a polynomial number of $N = 8$. However the velocity ratio is changed. Blood still enters from the top branch with a velocity of $V_{top} = 0.7 \frac{mm}{s}$, whereas the water solution coming from the bottom branch enters with a velocity three times higher than the blood velocity entrance, $V_{bottom} = 2.1 \frac{mm}{s}$. The diffusivity term remains $\alpha = 10^{-3}$.

The results obtained from this case are shown in the Figs A.23, A.24 and A.22. Since the ratio of velocity was modified so that the less viscous fluid enters with a velocity three times as high as the more viscous fluid (blood), the interface location changes and therefore adapts to the flow conditions. Through Fig. A.22, we can determine the interface location at around $1.85mm$, measured from the bottom of

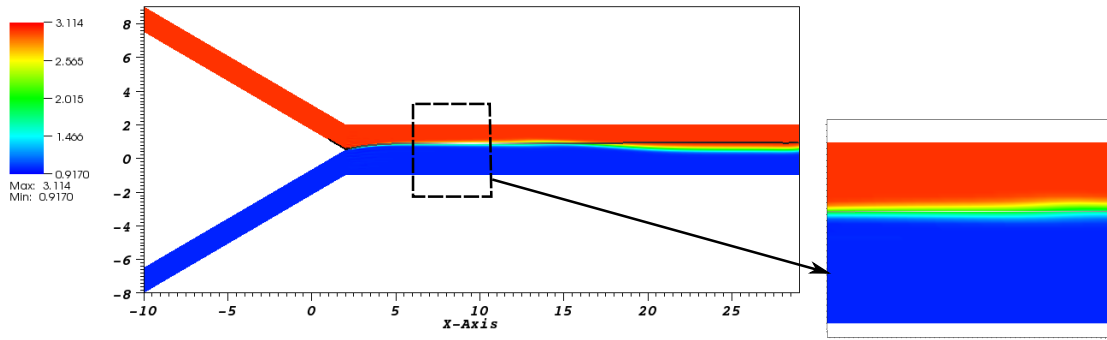


Figure A.22: Viscosity distribution in the channel for the eighth test

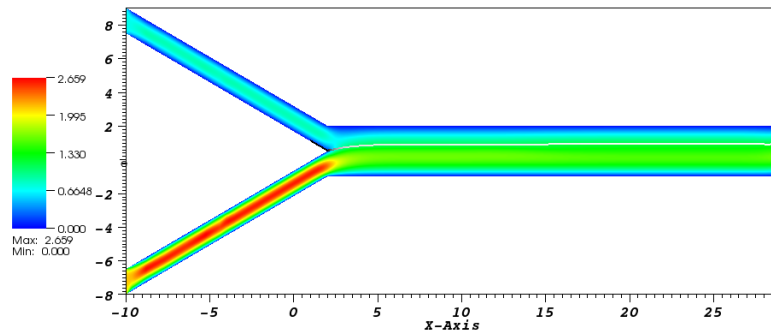


Figure A.23: Velocity distribution in the channel for the eighth test

the channel, which means that the "blood layer" now occupies 38% of the channel thickness. The velocity profile presented in Fig. A.24, shows that the supposed "blood layer" (the right part of the profile) velocity profile is almost linear, as desired. This experiment was tested as well in the laboratory to confirm the results.

A.1.2 LABORATORY EXPERIMENTS

The tests performed in the laboratory replicate both the seventh and the eighth tests described in sections A.1.1.7 and A.1.1.8 respectively. In order to simulate the fluid having the same viscosity as blood, a diluted solution of glycerol, with a mass fraction of $\Phi = 36\%$, is used for these experiments. The glycerol solution is inserted from the top branch at a flow rate of $Q_{top} = 0.042 \frac{ml}{min}$ to correspond to the numerical simulations of the seventh test. The second fluid, blue coloured distilled water enters from the bottom branch with the same flow rate as for the glycerol, $Q_{bottom} = 0.042 \frac{ml}{min}$, in order to obtain the same velocity from both branches. Tracer

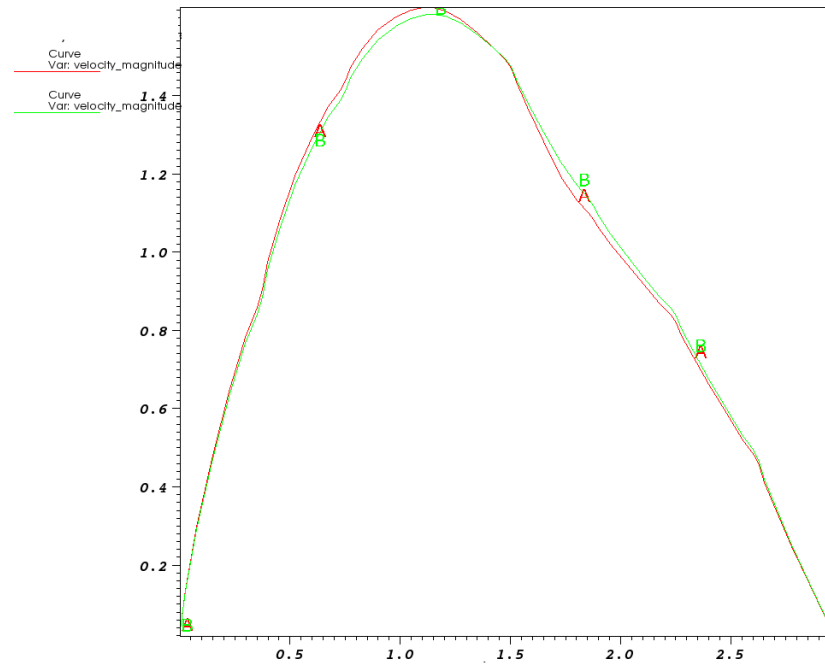


Figure A.24: Velocity profile extracted from the channel at $x = 15$ mm for the eighth test



Figure A.25: Experimental set up for the experiment with the same flow rate for both branches. The two fluid flow in the 3mm width channel can clearly be visualized.

particles are introduced into both fluids and diluted at 1% to visualize the velocity profile within the channel. The experimental set up for this test is shown in Fig. A.25.

Looking at the Ibidi channel under the microscope it was found that with the biggest objective available in the laboratory (10x objective lens) the whole width of the channel could not be completely visualized but only one tenth of the domain as seen in Fig. A.26.

The velocity profile corresponding to the visualized part of the channel, obtained experimentally for $Q_{top} = Q_{bottom}$, is shown in Fig. A.27. The velocity profile from

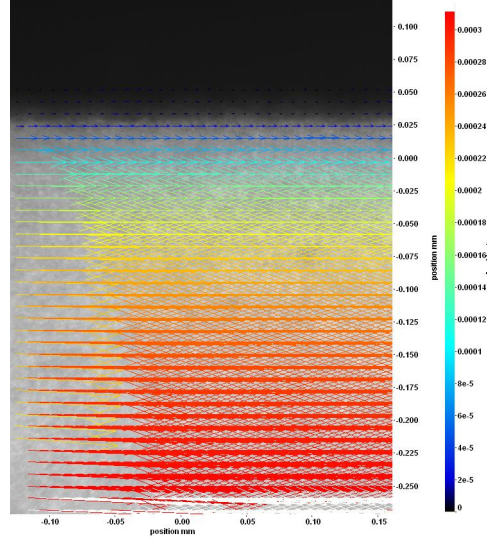


Figure A.26: The upper part of the channel width visualized under the microscope with the PIV system

the glycerol layer is not quite linear which is expected since the same velocity ratio between the two branches $V_{ratio} = 1$ and the glycerol (the viscous fluid) is expected to expand into the channel.

For the eighth test, water enters from the bottom branch with a velocity three times higher than the glycerol entering from the top branch. Therefore, for this second experiment, the same solution of glycerol, previously used, enters from the top branch at the flow rate of $Q_{top} = 0.042 \frac{ml}{min}$, while water enters from the bottom branch at a flow rate of $Q_{bottom} = 0.126 \frac{ml}{min}$. The experimental set up for this test is shown in Fig. A.28. However for this test, the results are not as successful as for the previous case since particles became lodged in the channel as seen in Fig. A.29. Because of the large channel dimensions, several other tests were performed on a smaller channel of $100 \times 15 \mu m$. Moreover the tests performed on the Ibidi channel were only performed in order to understand the behaviour of the two fluid flow when varying the viscosity and velocity ratios.

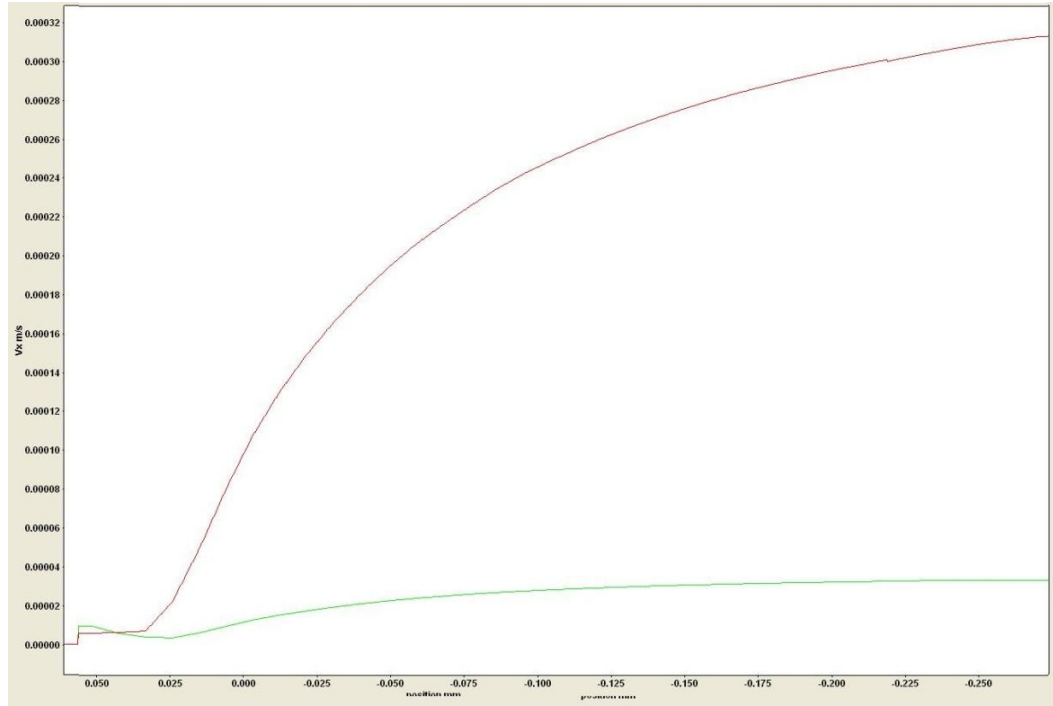


Figure A.27: Velocity profile corresponding to the part of the channel width visualized under the microscope with the PIV system

A.2 FIRST MICROCHANNEL PROTOTYPE

A.2.1 TWO DIMENSIONAL SIMULATIONS

Two different tests were performed with a microchannel fabricated in the laboratory using the photolithography method mentioned in Chapter (2) with dimensions based on the ibidi μ slide III³ⁱⁿ¹, where three branches serve as entry.

A.2.1.1 FIRST TEST

In order to perform the tests on the fabricated microchannel, the geometry of the channel in the simulation was adapted to match the dimensions and geometry of the channel. For this purpose the angle between the top and bottom branches was modelled to be $\beta = 62^\circ$ as for the Ibidi channel. However, for the simulations, the third branch was not considered since the third channel is blocked when performing the experiments in the laboratory. The same environment as for the Ibidi channel is recreated for this microchannel, where two fluids with different properties are introduced



Figure A.28: Experimental set up for the experiment with the different flow rate for both branches. The two fluid flow in the 3mm width channel can also clearly be visualized.

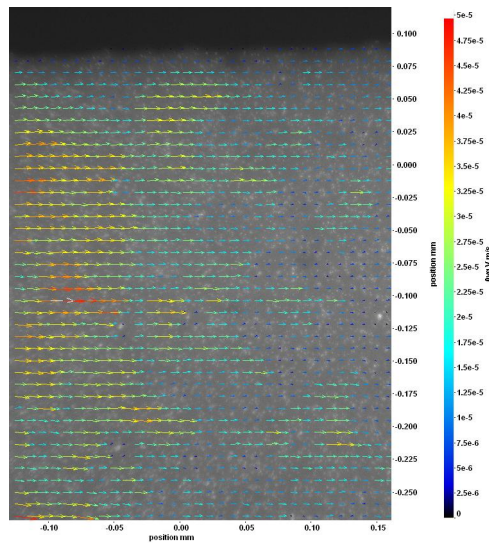


Figure A.29: The upper part of the channel width visualized under the microscope with the PIV system for the case with two different flow rates

to the channel at two different velocities to study the interface motion and ensure the linear velocity profile in the "blood layer". For the present test, the domain is discretized into 468 elements with $N = 8$. The velocity used for these test is based on the flow rate in microcirculation, more specifically in human capillaries, and was set to be $Q = 1 \frac{\mu l}{hr}$. Therefore, a fluid having a viscosity of $\nu_{top} = 3 \frac{mm^2}{s}$ enters from the top branch at a velocity of $V_{top} = 2.1 \frac{mm}{s}$, while another fluid having a viscosity of $\nu_{bottom} = 1 \frac{mm^2}{s}$ enters from the bottom branch at a velocity of $V_{bottom} = 0.7 \frac{mm}{s}$. The results of this test are shown in Figs. A.31 and A.30 that represent the viscosity and velocity distribution in the channel respectively. The layer of the viscous fluid (top layer) is clearly wider than the less viscous fluid layer. In fact we see that the layer

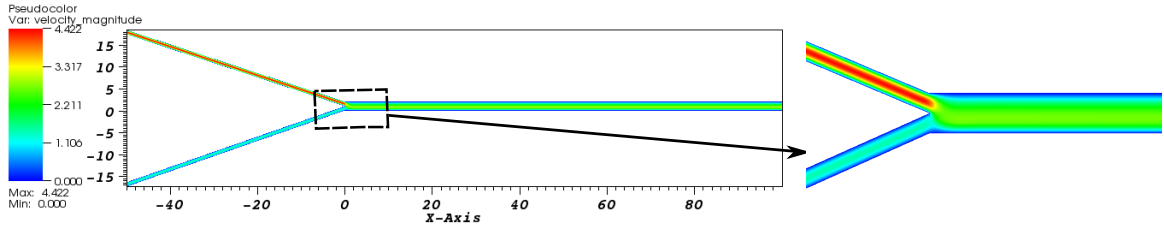


Figure A.30: Velocity distribution in the small elongated channel for the first test

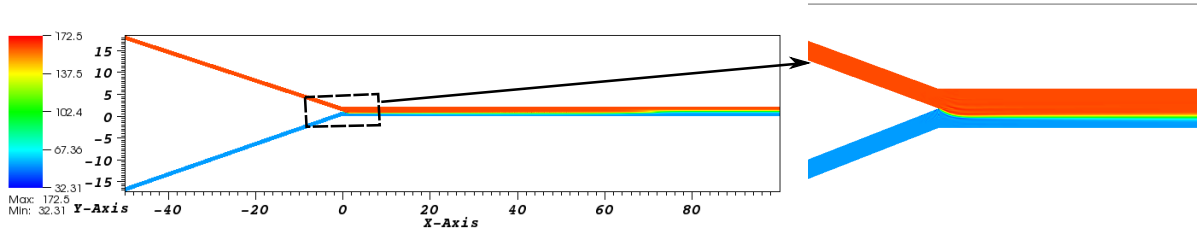


Figure A.31: Viscosity distribution in the small elongated channel for the first test

of the less viscous fluid is about 26% of the channel thickness. However when looking at the velocity profile obtained from this case, shown in Fig. A.32, the portion of the less viscous fluid (left part of the profile) is not linear. We therefore turn to a second test.

A.2.1.2 SECOND TEST

The second test consists of the same set up as in the previous first case, where the two fluids enter in the exact same geometry as previously described with two different velocities. The difference is that the viscous fluid now enters from the top branch with a viscosity 4 times lower than that of the fluid entering from the bottom branch. This means that the fluid having a viscosity of $\nu = 1 \frac{mm^2}{s}$ (bottom) enters with a velocity of $V_{bottom} = 0.7 \frac{mm}{s}$, while the fluid with a viscosity of $\nu = 3 \frac{mm^2}{s}$ (top) enters with a velocity of $V_{top} = 0.175 \frac{mm}{s}$. The results of this test are shown in Fig. A.33 and A.35 that represent the velocity and viscosity distribution in the channel respectively. The interface location shows the viscous fluid layer to be around 30% of the channel thickness. The velocity profile (Fig. A.34) in the viscous layer is almost linear now, as desired. This case is then tested experimentally to ensure the validity of the velocity profile and interface position found.

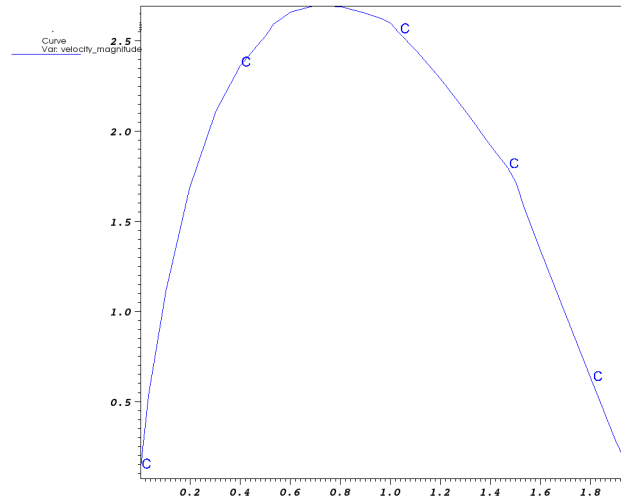


Figure A.32: Velocity profile extracted from the small elongated channel for the first test

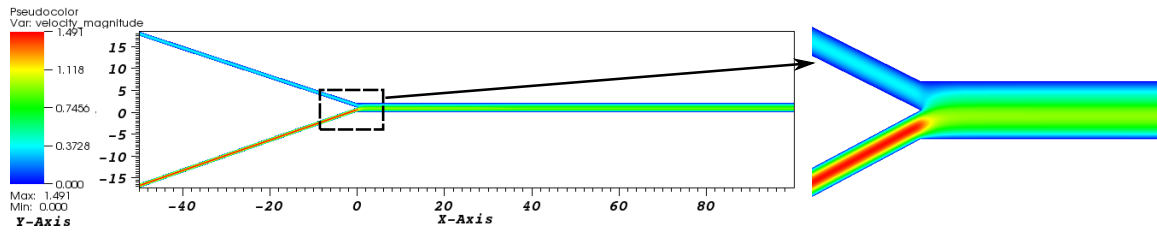


Figure A.33: Velocity distribution in the small elongated channel for the second test

A.2.2 LABORATORY EXPERIMENTS

The microchannel used experimentally consists of a small microchannel assumed to have a width of $100\mu\text{m}$, a depth of $15\mu\text{m}$ and a length of 20mm . It also comprises three entry branches based on the Ibidi channel. The branches are of $30\mu\text{m}$ in width, $15\mu\text{m}$ in depth and 17mm in length. When performing the experiments, the bottom and top branches are used to allow the two fluids to enter the microchannel and flow at a prescribed velocity. A whole for the tube connectors is not punched for the middle branch in order to plug the channel from this end. The same solution of glycerol prepared for the laboratory tests performed on the Ibidi channel detailed in section A.1.2, diluted with a mass fraction of $\Phi = 36\%$, is used for these experiments. The glycerol, mixed with 1% of fluorescent particles is inserted from the top branch of the microchannel at a flow rate of $Q_{top} = 4\frac{\mu\text{l}}{\text{hr}}$ with the Nexus 3000 pump, while distilled water mixed also with 1% of the tracer particles solution enters from the

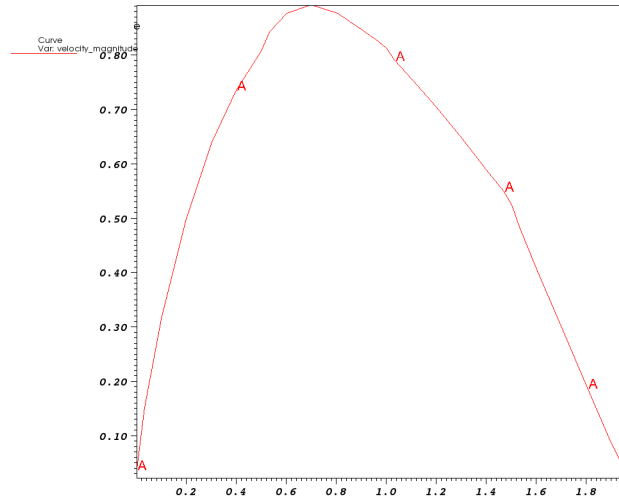


Figure A.34: Velocity profile extracted from the small elongated channel for the second test

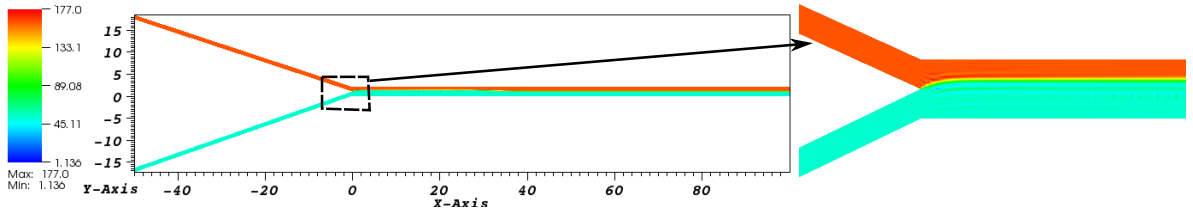


Figure A.35: Viscosity distribution in the small elongated channel for the second test

bottom branch at a flow rate of $Q_{bottom} = 4 \frac{\mu l}{hr}$ with the pico pump. A set of pictures are captured using the μ PIV system and processed with the cross-correlation method. A velocity field obtained for this case, shown in Fig. A.36, shows that the vectors are very small and does not correspond to the numerical solution performed for the same case. The time interval between the frames (dt) is reduced to allow the particles to move 5 to 6 pixels between two frames. However, the problem persists where the velocity field obtained is too small compared to the simulation. Looking at the intersection of the Y-channel, the glycerol flows from the upper branch backwards to the blocked middle branch. Therefore, a new design of a microchannel is needed to avoid complications with the third branch.

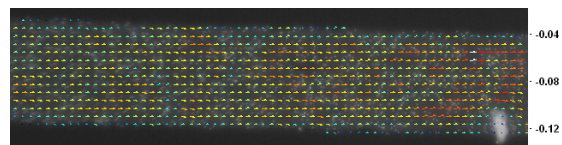


Figure A.36: Velocity distribution in the small fabricated channel for the first case tested experimentally using the μ PIV system