THE INFLUENCE OF SURFACTANTS ON CAPILLARY DRIVEN FLOW IN OPEN HYDROPHOBIC MICROCHANNELS TOWARDS CHIP-BASED FLUORESCENT DETECTION OF HSV


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Declaration

The substance of this thesis is the original work of the author, and due reference and acknowledgement has been made, where necessary, to the work of others. No part of this thesis has been submitted in candidture for any degree.

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Abstract

Herpes Simplex Virus (HSV) is widespread among the human population. HSV infection can have subtle symptoms and often go undiagnosed. There are two types of HSV infections. HSV type 1 (HSV-1) is associated with orofacial blisters transmitted through saliva whereas HSV type 2 (HSV-2) causes genital ulcers, genital herpes and in severe cases meningitis. HSV-2 is transmitted through sexual contact. Current methods of detecting HSV infection are labour intensive and time consuming. Patients that attend a doctor's surgery often have to wait weeks to obtain a result due to transportation of the patient's sample to a laboratory with suitable facilities and personnel with expertise to diagnose HSV infection. Currently, there is a need to develop a rapid point-of-care (POC) device that is capable of detecting HSV infections from a patient's bedside, doctor's surgery or a remote location. Currently, there are capillary flow driven devices for the detection of HSV infections but the microdevice developed in this study for the fluorescent detection of HSV infection has a novel method of detection via capillary flow through open microchannels made of a cyclo-olefin polymer. In this thesis, a capillary driven microdevice was developed with the goal of fluorescent detection of HSV-1 and HSV-2 as a first step toward developing a POC microdevice.

The first step in the development of this microdevice was to assess the influence of surfactants on the capillary flow through open hydrophobic microchannels and the hydrophilisation of the hydrophobic microchannels through the use of surfactant coatings which to the author’s knowledge has not been explored to date. An algorithm was developed which measured the meniscus position of the fluid in the microchannels by processing digitally captured high speed images of the fluid flow. It was found for the surfactant solutions flowing in hydrophobic open microchannels via capillary action did not follow conventional capillary flow theory possibly due the adsorption of surfactant molecules to the microchannel surfaces. To the author’s knowledge, the dynamic contact angles of fluids flowing in open microchannels have never been measured and in this thesis an algorithm was used to measure dynamic contact angles non-invasively by analysing digital images of the fluid flow. Surfactant coating of flat hydrophobic surfaces increased their wettability although surfactant coating of hydrophobic open microchannels promoted capillary flow of water but as the concentration of surfactant increased the flow through the microchannels decreased. The surfactant coating created a hydrophobic barrier that increased in strength with increasing surfactant concentration. Also, the meniscus shape started to deform from conventional capillary flow into a non-conventional shape which has not been documented before. This hydrophobic barrier and deformation of the meniscus shape may be due to the water adsorbing surfactant molecules from the surface of the microchannels.

The second step was to assess the performance of the fluorescent detection of HSV infection using the microdevice based on a miniaturised ELISA technique. The detection method was based on the immobilisation of 100-1000μg/ml of capture antibodies specific for either HSV-1 or HSV-2 antigens. The concentration of HSV specific antigens and fluorescent detection antibodies used were 75-200 and 20μg/ml respectively. The concentration of HSV antigens used was 4-5 orders of magnitude higher than a POC device would use. An algorithm was developed that eliminated background noise allowing accurate qualitative and quantitative measurement of fluorescent detection. The microdevice failed in fluororesently detecting both HSV-1 and HSV-2. This technique requires substantial modifications to improve its performance.
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Finally, I wish to thank my family for their loving support during the course of this thesis.
Dedication

I’d like to dedicate this to my loving family.
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<td>$A$</td>
<td>Surface area</td>
<td>$\text{m}^2$</td>
</tr>
<tr>
<td>$C$</td>
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</tr>
<tr>
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<td>Control surface</td>
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<tr>
<td>$E$</td>
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<tr>
<td>$F$</td>
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<td>Fab</td>
<td>Antibody binding element</td>
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<td>$b$, $c$, $d$, $e$, $f$</td>
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<td>Planck’s constant</td>
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<tr>
<td>$\bar{i}$</td>
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<tr>
<td>$f$</td>
<td>Frequency</td>
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<tr>
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<td>Boltzmann constant</td>
<td>$\text{m}^2\text{kg/K}^1\text{s}^2$</td>
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<td>$m$</td>
<td>Slope of a line</td>
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<td>$n$</td>
<td>Normal vector pointing to the outside of the control surface</td>
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<td>$p$</td>
<td>Pressure</td>
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<tr>
<td>$r$</td>
<td>Radius</td>
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</tr>
<tr>
<td>res</td>
<td>Reservoir</td>
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<td>$s$</td>
<td>Droplet arc length measured along the profile of the droplet</td>
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<tr>
<td>$sp$</td>
<td>Spreading coefficient</td>
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<tr>
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<tr>
<td>$v$</td>
<td>Velocity</td>
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<tr>
<td>β</td>
<td>Slope of the droplet profile</td>
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<tr>
<td>δ</td>
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</tr>
<tr>
<td>ε</td>
<td>Distance between two adsorption sites</td>
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<tr>
<td>θ</td>
<td>Contact angle</td>
<td>degree</td>
</tr>
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<td>σ&lt;sub&gt;rr&lt;/sub&gt;</td>
<td>Stress in the r-direction on a face normal to the r-direction</td>
<td>N/m&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>λ</td>
<td>Photon wavelength</td>
<td>nm</td>
</tr>
<tr>
<td>μ</td>
<td>Viscosity</td>
<td>Ns/m&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>ν</td>
<td>Kinematic viscosity</td>
<td>m&lt;sup&gt;2&lt;/sup&gt;/s</td>
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<tr>
<td>ρ</td>
<td>Density</td>
<td>kg/m&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
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<td>Critical Micelle Concentration</td>
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<td>Convective</td>
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<tr>
<td>IR</td>
<td>Reservoir inertia</td>
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<td>SV</td>
<td>Solid-Vapour</td>
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<td>b</td>
<td>Bottom wall</td>
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<td>c</td>
<td>Microchannel angle from horizontal</td>
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<tr>
<td>cap</td>
<td>Capillary flow</td>
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<td>ch</td>
<td>Wetted microchannel volume</td>
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<td>Equilibrium</td>
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</tr>
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</tr>
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<td>m</td>
<td>Meniscus</td>
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<td>Initial</td>
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<td>Inlet pressure</td>
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<tr>
<td>press</td>
<td>Pressure</td>
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<td>r</td>
<td>Spherical coordinate, right</td>
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<td>rel</td>
<td>Relative</td>
</tr>
<tr>
<td>s</td>
<td>Slip</td>
</tr>
<tr>
<td>surf</td>
<td>Surface</td>
</tr>
<tr>
<td>t</td>
<td>Top wall</td>
</tr>
<tr>
<td>v</td>
<td>Viscous (force)</td>
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<tr>
<td>vap</td>
<td>Vapour</td>
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<tr>
<td>l, 2</td>
<td>Phase</td>
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<tr>
<td>θ</td>
<td>Spherical coordinate</td>
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<tr>
<td>ψ</td>
<td>Spherical coordinate</td>
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<td>Definition</td>
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<td>----------------------------------------------------------</td>
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<tr>
<td>APTES</td>
<td>Aminopropyl-triethoxysilane</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical Micelle Concentration</td>
</tr>
<tr>
<td>COC</td>
<td>Cyclic olefin copolymer</td>
</tr>
<tr>
<td>COP</td>
<td>Cyclo olefin polymer</td>
</tr>
<tr>
<td>CPE</td>
<td>Cytopathic effects</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<td>FAM-X</td>
<td>Fluorescein-X</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>EIA</td>
<td>Enzyme immunoassays</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>HD</td>
<td>Hydrodynamic Theory</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>HSV</td>
<td>Herpes Simplex Virus</td>
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<tr>
<td>HSV-1</td>
<td>Herpes Simplex Virus Type-1</td>
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<td>HSV-2</td>
<td>Herpes Simplex Virus Type-2</td>
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<td>IFA</td>
<td>Immunofluorescence assays</td>
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<td>IgG</td>
<td>Immunoglobin G</td>
</tr>
<tr>
<td>MKT</td>
<td>Molecular Kinetic Theory</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
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<td>PC</td>
<td>Polycarbonate</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PDITC</td>
<td>Phenylene diisothiocyanate</td>
</tr>
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<td>PDMS</td>
<td>Polydimethylsiloxanes</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>POC</td>
<td>Point of Care</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SMP</td>
<td>Stealth Microarray Pin</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>TPC</td>
<td>Triple phase contact</td>
</tr>
<tr>
<td>gB</td>
<td>glycoprotein B</td>
</tr>
<tr>
<td>gC</td>
<td>glycoprotein C</td>
</tr>
<tr>
<td>gG</td>
<td>glycoprotein G</td>
</tr>
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</table>
Chapter 1

Microfluidic Devices

Modern microfluidics date back to the 1950’s with the development of the ink-jet printer at IBM and in the 1970’s with the development of a silicon based gas chromatography at Stanford University[41]. But it wasn’t until the 1990’s that integrated microfluidic devices began to emerge. Since then, there has been an exponential growth in microfluidic technology, at first only in academic research, but since the mid 1990’s also on a commercial basis[9]. Microfluidic devices are platforms consisting of channels ranging from a few microns to hundreds of microns in size that are connected to liquid reservoirs with volumes of microliters or lower[36]. Thus far, microfluidic devices have been proven to have the capability to perform deoxyribonucleic acid (DNA) sequencing, polymerase chain reaction (PCR) amplification, amino acid, peptide and protein analysis, immunoassays and cell sorting[43].

Microfluidic devices have several advantages when compared to conventional macro analytical methods such as DNA analysis, PCR and enzyme-linked immunosorbent assay (ELISA). These advantages are: reduced analysis time, high sensitivity, increased surface to volume ratio, personnel operating the microdevices require minimal technical training and eliminates the need for a single assay to be prepared and performed by numerous laboratory equipment. Furthermore, there is a reduction in the consumption of expensive reagents and analytes and the devices can be easily mass produced [85, 87, 64, 138]. The latter two allows the assays to become more affordable as the operating costs are reduced.

Microfluidic devices can have various fluid handling methods, detection techniques
and can be fabricated from a wide range of materials[64]. The selection of the fabrication method of the microfluidic device is affected by several factors, such as the available technology and equipment, cost, speed of fabrication, fabrication capabilities (desired feature size and roughness) and preferred material choice of the device. The technology for micromachining was first developed for the semiconductor industry and therefore the first generation of microfluidic devices were fabricated from silicon, quartz or glass. As the micromachining technology advanced, microdevices began to be fabricated from polymers permitting the fabrication of cheap microfluidic devices. There are several methods of fabricating microfluidic devices that are able to produce highly complex two and three-dimensional microstructures. Pumps, valves, mixers and other functional components in the macroscopic laboratory have been miniaturised onto microfluidic devices[35].

Due to the size of the channels in microfluidic devices, surface forces (surface tension, electrical effects, van der Waals interactions and surface roughness) play a significant role in the transport of fluids through the device[135]. There are several methods for fluid transport in microfluidic devices. These include: electroosmosis, mechanical pumps, vacuum pumps, centrifugal force, gravity and capillary action[13, 43]. The focus of this thesis was on the fluid flow through microchannels via capillary action. It is a passive method to induce flow in microchannels and is ideal for portable POC microfluidic devices because it does not require the use of cumbersome peripheral fluid pumping mechanisms, valves or actuators.

There are numerous detection techniques explored for microfluidic devices, light emission or absorption, electrochemistry, chemiluminescence, fluorescence, amperometric and radioactivity detection[64, 163]. Fluorescence is the most commonly used method of detection because of its high selectivity and sensitivity[118]. For fluorescent detection, fluorophores are attached to analytes which can be excited and detected selectively. The high sensitivity of fluorescence spectroscopy results in high signal-to-noise ratios[35].

The need for an accurate, rapid, low cost, easy to use, portable and disposable for the diagnosis and prognosis of infections and diseases has been the motivation for the development of POC microfluidic devices[138]. POC applications can be divided into 4 distinct categories:
1. Tests in which the results are immediately necessary to begin treatment of life threatening diseases, for example meningitis.

2. Results required to initiate treatment, for example the presence of a specific analyte in a sample.

3. Results required for a quick decision related to organism containment, for example methicillin-resistant Staphylococcus aureus (MRSA).

4. Tests performed on patients who do not return for follow up treatment, for example STIs.

POC devices have the capability to significantly reduce the costs involved in performing assays. Table 1.1 lists the steps taken in a conventional laboratory blood based assay compared to a POC device indicating a considerable reduction in sample handling and decision making steps, resulting in time and cost savings[140].

Table 1.1: Comparison of the steps taken in a conventional laboratory blood based assay compared to a POC device[140].

<table>
<thead>
<tr>
<th>Conventional laboratory assay</th>
<th>POC assay</th>
</tr>
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<tbody>
<tr>
<td>1. Test is ordered</td>
<td>1. Test is ordered</td>
</tr>
<tr>
<td>2. Test request is processed</td>
<td>2. Nurse draws blood sample</td>
</tr>
<tr>
<td>3. Nurse draws blood sample</td>
<td>3. Sample is analysed</td>
</tr>
<tr>
<td>4. Sample is transported to the lab</td>
<td>4. Results are reviewed by a nurse</td>
</tr>
<tr>
<td>5. Sample is labelled and stored</td>
<td>5. Clinician acts on results</td>
</tr>
<tr>
<td>6. Sample is centrifuged</td>
<td></td>
</tr>
<tr>
<td>7. Serum sample is sorted to analysers</td>
<td></td>
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<tr>
<td>8. Sample is analysed</td>
<td></td>
</tr>
<tr>
<td>9. Results are reviewed by the lab staff</td>
<td></td>
</tr>
<tr>
<td>10. Results are reported to the department</td>
<td></td>
</tr>
<tr>
<td>11. Clinician acts on results</td>
<td></td>
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</tbody>
</table>

1.1 Motivation for research

The motivation for this research was to investigate the influence of surfactants on the capillary flow of fluids through a polymer open hydrophobic microchannels and then evaluate
and assess the performance of a capillary driven microfluidic device capable of fluorescent detection of HSV.

The first step was to investigate the influence of surfactants on the capillary flow of fluids through a polymer open hydrophobic microchannels. Polymers are hydrophobic in nature and a method to increase its hydrophilicity was required to promote capillary flow through polymer microchannels. The addition of surfactant molecules to a fluid can reduce the fluid’s surface tension and thereby increase the fluid’s hydrophilicity and allow capillary flow through hydrophobic microchannels. The surfactant coating of hydrophobic microchannels have the potential to increase the hydrophilicity of the microchannels and allow capillary flow fluids through the microchannels. The influence of surfactant solutions and surfactant coatings of open hydrophobic microchannels on capillary flow were investigated.

The second step was to evaluate and assess the performance of a capillary driven microfluidic device capable of fluorescent detection of HSV. HSV infections are widespread among the human population and can have subtle symptoms that often go undiagnosed. These infections are persistent for life and once infected can recur at any time, up to 5 times a year[4]. The are 2 subtypes of HSV infections, HSV-1 and HSV-2. HSV-1 is associated with orofacial blisters and in some cases genital herpes and is usually acquired through saliva contact. It is estimated that 80-90% of adults show evidence of HSV-1 infection which is usually obtained during childhood[111]. HSV-2 is usually transmitted through sexual contact and can cause genital ulcers, genital herpes and in severe cases meningitis. HSV-2 has been proven to increase the risk of acquisition and transmission of HIV through the genital ulcers[4, 5, 111]. There is an increased risk of HSV-2 infection spreading to the central nervous system causing encephalitis, myelitis or meningitis in immunosuppressed patients. Immunosuppressed patients are patients undergoing chemotherapy, organ or bone marrow transplants or HIV infected people. In studies conducted on HSV-2, it was found that up to 90% of people that had HSV-2 infections were unaware that they were infected and it was estimated that 20% of the adult population were infected[111, 4].

There are three diagnosis categories of HSV infections; primary, non-primary first episode and non-primary. Primary case is when a patient acquired a HSV infection without
prior antibodies to HSV-1 and HSV-2 infections. Non-primary first episode of HSV-2 is when patient acquires a HSV-2 infection but has antibodies for HSV-1 from a previous infection. Non-primary case is where a patient has a recurring infection.

Most transmissions of HSV infection are through sexual contact or by maternal transfer due to people being asymptomatic. A rapid and accurate test is required to screen asymptomatic people, especially pregnant women in their third trimester to reduce the possibility of transmission of neonatal herpes to the infant during delivery. Pregnant women giving birth with a primary case of HSV-2 infection are significantly more likely to transfer the infection to the infant than women that have a non-primary, recurring, infection. Pregnant women with a primary case infection expose the infant to a larger viral inoculation, the cervix is more frequently involved and the infant lacks transplacentally acquired antibodies[61]. If the infection is undiagnosed there is up to 70% risk of the infant acquiring neonatal herpes, causing permanent neurological damage to the infant and a risk of infant morbidity[19, 61].

Genital herpes is the fourth most common sexually transmitted infection in the United Kingdom[45]. Genital herpes is a symptom of both HSV-1 and HSV-2 infections. HSV-1 infection is contributed to 30% of genital herpes cases with the remainder to HSV-2 infection[109]. HSV-2 infection is considered to be more severe than HSV-1 infections due to the higher rate of recurrence of the infection, HSV-2 recurs up to 4 times more frequently than HSV-1, has more severe health consequences and responds less to antiviral therapy[111].

There is a need for a definitive, rapid, point of care diagnostic device that can distinguish between HSV-1 and HSV-2[4]. Accurate tests can provide the basis for proper clinical management, timely treatment and appropriate counselling relating to the natural history and transmission risks associated with the infection[3].

1.2 Objectives

- Assess the factors that affect capillary flow of surfactant solutions in open hydrophobic microchannels.
• Assess the influence of surfactant coatings on the hydrophilisation of hydrophobic microchannels and its influence on the capillary flow of deionised water.

• Compare existing theory to experimental data on the prediction of capillary flow in open microchannels.

• Design a capillary flow driven microfluidic device incorporating immobilised capture antibodies for the detection of specific HSV viral antigens in samples via individually fluorescently labelled antibodies specific to HSV-1 and HSV-2.

• Evaluate and assess the performance of a capillary driven microfluidic device incorporating fluorescent detection HSV-1 and HSV-2.

1.3 Thesis Outline

The remainder of this research thesis is structured as follows: in Chapter 2 the basic principles of capillary flow of liquids in microchannels are outlined with an emphasis on capillary flow in open rectangular microchannels. Chapter 3 gives a brief literature review of current methods for the detection of HSV highlighting the need for a more rapid and low cost detection technique. Chapter 4 details the methodology of the design and development of a microfluidic platform for the rapid detection of HSV-1 and HSV-2. Chapter 5 describes the microfluidic experimental measuring techniques. This chapter is split into two sections; the fluidic measuring techniques for capillary flow in microchannels and HSV fluorescent detection. The first section describes the measuring techniques to characterise the fluid properties of the liquids used in the capillary flow experiments and the image capturing technique used to record the capillary flow of liquids through the microchannels. The second section outlines the methods for characterising the fluorescent marker for HSV detection and the HSV assay protocols which include the surface functionalisation of the microfluidic platform, capture antibody immobilisation and the HSV detection protocols. Chapter 6 outlines the data processing techniques for the capillary flow images and includes the edge detection procedure for the liquid meniscus in the microchannels, position, velocity and dynamic contact angle. The data processing techniques for fluorescent detection
of HSV-1 and HSV-2 are described which include the detection sensitivity, image noise reduction and quantifying fluorescent detection. Chapter 7 presents the results of the experiments performed for capillary flow in open microchannels described in Chapters 5 and 6 and are compared to theoretical equations provided in Chapter 2. Chapter 8 presents and discusses the results of the HSV assay protocols. Finally, chapter 9 states the conclusions of the experiments carried out followed by recommendations for future work.
Chapter 2

Capillary Flow In Microchannels

Capillary flow has many industrial and commercial applications that range from the microelectronic to the petrochemical, aerospace and textile industries[77, 143, 30]. With the new advancements in the fabrication techniques used for the manufacture of microfluidic devices, interest in capillary flow devices have increased dramatically because of the simplicity of design and operation. Capillary flow devices are particularly suitable to handheld, disposable, point of care clinical, chemical or biomedical diagnostic applications. It is of critical importance to understand capillary flow characteristics inside microchannels for accurate clinical diagnosis, chemical and biomedical analysis[112]. The capillary flow characteristics that must be studied in detail are the influences of microchannel geometry and the dynamic contact angle that is variable and dependent on the velocity of the fluid’s meniscus inside the microchannel[107].

In this chapter, the properties of fluids that effect the capillary flow of fluids in microchannels are described. Following this, the Young-Laplace equation which is used to determine the pressure on a curved surface is derived. Then, the Washburn equation is derived which was the first equation to describe capillary flow in cylindrical capillaries. The wetting characteristics of dynamic contact angles are discussed and two competing theoretical equations; Hydrodynamic theory (HD) and molecular kinetic theory (MKT) and an empirical equation are presented. Theoretical equations for the capillary flow in open rectangular microchannels are derived based on the conservation of mass and momentum. The fabrication process for the hot embossing of straight microchannels from a Topas substrate
was given and these microchannels were used to perform experimental investigations into
the validity of the theoretical equations of dynamic contact angles and capillary flow in
open microchannels.

2.1 Contact Angle

When a liquid droplet is brought into contact with a solid surface (e.g. polymer) its wetting
behaviour is generally characterised by the contact angle, \( \theta \), between the triple phase con-
tact (TPC) line as shown in Fig. 2.1. The TPC line is the interface between the liquid, solid
surface and the vapour phases. The liquid droplet has two competing forces; adhesive and
cohesive forces. The adhesive force between the liquid and solid surface causes the liquid
to spread along the surface while the cohesive forces within the liquid opposes the adhe-
sive force thereby trying to prevent the spreading of the liquid. The combination of these
forces results in the droplet having a spherical shape. The angle of the tangent between the
liquid and the solid surface is referred to as the contact angle. A wetting surface can be
classified into 4 distinct groups which are illustrated in Fig. 2.2; non-wetting, hydrophobic,
hydrophilic and perfectly wetting surfaces. A non-wetting, hydrophobic, hydrophilic and
perfectly wetting surface can be defined when the contact angle is equal to 180\(^\circ\), greater
then 90\(^\circ\), less than 90\(^\circ\) and 0\(^\circ\) respectively.

![Figure 2.1: Interfacial forces acting on a liquid droplet on a solid surface.](image)

![Figure 2.2: (a-d) A drop of liquid on a non-wetting, hydrophobic, hydrophilic and perfectly wetting surface.](image)
Young’s equation defines the surface force balance of droplet of liquid on a surface at the TPC line as:

\[ \gamma_{SL} + \gamma_{LV} \cos \theta_e = \gamma_{SV} \]  

(2.1)

where, \( \gamma_{SL}, \gamma_{LV} \) and \( \gamma_{SV} \) are the interfacial tensions for the solid-liquid, liquid-vapour and solid-vapour phase. The static/equilibrium contact angle is defined as \( \theta_e \) and is illustrated in Fig. 2.1. When the force balance is in equilibrium, the contact line does not move and its resulting contact angle is called the static contact angle. When the force balance is out of equilibrium, the contact line will move until it reaches its equilibrium state. In this case the contact angle is relaxing towards the static contact angle. This varying contact angle is called the dynamic contact angle. The dynamic contact angle is time dependent and can be initially up to a possible maximum of 180° and then relaxes to its static contact angle[34].

Dynamic wetting, the spreading of a liquid over a surface, is still the subject of some debate. The main reason for this uncertainty is a lack of agreement about the precise mechanism by which a contact line moves across a solid surface and how this mechanism interacts with the rest of the liquid flow[17]. Dynamic wetting operates on a scale that extends from macroscopic to the molecular. Typically, experimental measured quantities, such as, the wetting velocity, viscosity and surface tension are macroscopic while the contact angle formed between liquid and solid surface is measured at a resolution of a few microns[33].

There are still unresolved issues with dynamic wetting on the molecular scale because it is difficult to accurately measure phenomena on this scale. These issues are the nature and location of energy dissipation, the physical significance of the values of the parameters obtained from comparison of the theory and experiments and the effect of the geometry of the microchannels[114].

### 2.2 Surface Tension

On a solid surface a droplet takes a spherical shape and is subject to surface tension and normal pressures \( p_1 \) and \( p_2 \) at the interface between the phases as shown in Fig. 2.3 (a). Surface tension is the energy per unit area between the interface of two phases. It arises
from the unbalanced cohesive forces between molecules in a liquid which are represented by the blue and green dots in Fig. 2.3 (b). The molecules that are not on the liquid-vapour interface (blue dots) can distribute the cohesive forces in all directions amongst its neighbouring molecules but the molecules at the interface (green dots) do not have neighbouring molecules in all directions and therefore have stronger cohesive forces to distribute which will result in the interface contracting, as shown in Fig. 2.3 (b), to minimise its surface area and thereby reducing the free energy of the system. This cohesive force at the interface is called the surface tension, γ, of the liquid and is defined as:

\[ \gamma = \frac{\partial G}{\partial A} \]  

(2.2)

where, \( G \) is the free energy of the surface and \( A \) is the surface area. A drop of water in air has a spherical shape as this provides the lowest surface area per unit volume. The surface tension creates a film at the interface resulting in an object having to exert more force to penetrate this film into the liquid than when it would had to if it was completely submerged in the fluid. Surface tension is ordinarily measured in N/m but is commonly stated in dynes/cm. For example the surface tension of water is 72 dynes/cm at room temperature.

An alternative method for the calculation of the surface tension of a fluid was proposed by MacLeod (1923)[88] where the surface tension could be determined by the difference of the densities of the liquid and vapour phases multiplied by a constant, \( P \) (termed parachor), that is temperature independent but characteristic of the liquid:

\[ \gamma = P(\rho_l - \rho_v)^4 \]  

(2.3)
2.3 Surfactants

Microfluidic devices are usually fabricated from polymers such as polycarbonate (PC), poly(methyl methacrylate) (PMMA), polydimethylsiloxanes (PDMS) and cyclic olefin co-polymer (COC) which are hydrophobic to water. This hydrophobicity presents an obstacle to achieving flow within the device. To increase the polymer’s hydrophilicity, its surface can be treated with a surfactant which increases its wettability. A surfactant molecule consists of two parts; a hydrophobic tail and a hydrophilic head. Surfactant molecules tend to orientate themselves at the fluids interface with the hydrophilic head immersed in the liquid phase and is illustrated in Fig. 2.4. At a certain concentration of surfactant, the interface becomes saturated with surfactant molecules, the remaining molecules in the bulk increase and rearrange themselves into micelles. The concentration of surfactant that these micelles begin to form is referred to as the Critical Micelle Concentration (CMC).

The spreading coefficient, $sp$, of a liquid is given by\[72\]:

$$sp = \gamma_{SV} - \gamma_{LV} - \gamma_{SL}$$ (2.4)

If $sp \geq 0$, the liquid will spread across the surface creating a thin liquid film. If $sp < 0$, the liquid will partially wet the surface creating a drop of liquid on the surface. If the surface is treated with a surfactant, it will decrease the surface tension between the solid and liquid phases and therefore increase the spreading coefficient. Most of the previous studies on the influence of surfactants on wettability, in particular the contact angle, have been performed on flat smooth polymer substrates. This scenario is very different from studying the influence of surfactants on the wettability of microchannels. There is very little data on the interface shape and contact angle of a liquid in a microchannel after surfactant treatment[162].
CHAPTER 2

Capillary Flow In Microchannels

2.4 Young Laplace Equation

The interface between phases has a tendency to curve due to surface tension and the pressure difference across it. An expression for the pressure difference can be derived by considering the energy required to expand a curved surface as shown in Fig. 2.5. The curved surface moves a small distance $\delta r$ which results in a change in surface area[127]:

$$\delta A = \left(\frac{1}{R_1} + \frac{1}{R_2}\right)\delta r A_o$$  \hspace{1cm} (2.5)

where, $R_1$ and $R_2$ are radii of curvature and $A_o$ is the original curved surface area. There are two factors that change the free energy of the system. Firstly, the area of the curved surface is increased which results in an increase in the free energy at the surface, $\delta G_{surf}$, which is expressed as:

$$\delta G_{surf} = \gamma \delta A = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2}\right)\delta r A_o$$  \hspace{1cm} (2.6)

Secondly, there is a decrease in pressure energy due to the change in volume:

$$\delta G_{press} = -(p_1 - p_2)\delta V = -\triangle p \delta r A_o$$  \hspace{1cm} (2.7)

For the system to be in equilibrium, there must be no change in the net free energy which implies:

$$\delta G = \delta G_{surf} + \delta G_{press} = 0$$  \hspace{1cm} (2.8)
Substituting equations 2.6 and 2.7 into 2.8 gives an expression for the pressure difference which is also known as the Young-Laplace equation:

$$\Delta p = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right)$$  \hspace{1cm} (2.9)

![Diagram of a curved interface with labels for liquid, vapor, and contact angles.]

Figure 2.5: Mathematical representation of the change in the area of a curved interface.

The pressure difference at the interface of a liquid flowing through a rectangular microchannel, as illustrated in Fig. 2.6, can be determined based on the following equations assuming that the contact angle is the same for all the walls:

$$R_1 = \frac{W}{2\cos \theta}$$  \hspace{1cm} (2.10)

$$R_2 = \frac{H}{2\cos \theta}$$  \hspace{1cm} (2.11)

where, $W$ and $H$ are the width and height of the microchannel respectively. Substituting equations 2.10 and 2.11 into 2.9 gives an expression for the pressure difference in terms of the geometry of the microchannel:

$$\Delta p = 2\gamma \cos \theta \left( \frac{1}{W} + \frac{1}{H} \right)$$  \hspace{1cm} (2.12)
Figure 2.6: (a) A liquid flowing through a rectangular microchannel, (b) A liquid flowing through a rectangular microchannel as seen from the side and (c) A liquid flowing through a rectangular microchannel as seen from the top.

2.5 Washburn Equation

The first analytical studies of surface tension and capillarity were performed by Young and Laplace in the early 1800’s. Later, Hagen and Poiseuille investigated the flow of viscous liquids in capillary tubes and derived the Hagen-Poiseuille flow profile for laminar, fully developed, Newtonian flow\[24\]. The first analyses of dynamic capillary penetration of a liquid into a cylindrical tube were first carried out by Lucas, 1918, and Washburn, 1921 and is shown in Fig. 2.7. The Lucas-Washburn analysis assumed that the capillary flow of incompressible liquid from an infinite reservoir is laminar with negligible inertia effects, has a parabolic velocity profile and has a constant contact angle. The Lucas-Washburn analysis neglects the deviation from the Poiseuille flow velocity at the inlet and near the curved liquid meniscus surface\[106\][62]. The Lucas-Washburn analysis is based on the balance of interfacial forces; the capillary, gravitational and viscous forces. The driving force of capillary flow occurs when these forces are unbalanced and create a pressure difference. The Lucas-Washburn analysis begins by describing the capillary flow using the Hagen-Poiseuille equation for laminar flow, neglecting inertial effects:

\[
\frac{8\mu}{R^2} \frac{dL_f}{dt} = \frac{p_{in} - p_m}{L_f} \tag{2.13}
\]

where, \( R \) is the tube radius, \( L_f \) is the meniscus position, \( p_{in} \) is the pressure at the inlet, \( p_m \)
is the pressure at the meniscus and $\mu$ is the viscosity of the fluid. The pressure drop of the curved meniscus interface, the capillary pressure, is given by the Young-Laplace equation:

$$p_{\text{vap}} - p_m = \frac{2\gamma}{R} \cos \theta$$  \hspace{1cm} (2.14)

where, $p_{\text{vap}}$ is the pressure of the vapour and $\gamma$ is the surface tension between the liquid and the vapour. Substituting equation 2.14 into 2.13 an ordinary differential equation is obtained for $L_f$.

$$\frac{2\gamma}{R} \cos \theta = \frac{8\mu}{R^2} \frac{dL_f}{dt} L_f + p_{\text{vap}} - p_{\text{in}}$$  \hspace{1cm} (2.15)

The Lucas-Washburn analysis considers the contact angle constant and therefore equation 2.15 can be solved using the separation of variables to give an equation in terms of the liquid meniscus position:

$$L_f = \sqrt{\left(\frac{R\cos \theta}{2\mu}\right) + \frac{R^2}{4\mu}(p_{\text{in}} - p_{\text{vap}})t}$$  \hspace{1cm} (2.16)

The Lucas-Washburn equation indicates that the liquid meniscus position in a capillary tube is proportional to the square root of time and is a function of the wetting properties of the liquid and solid surface[24]. In comparing the Lucas-Washburn equation to experimental data it has been proven to give a good approximation of liquid penetration in long capillary tubes but it fails to describe the initial penetration of the liquid because
it neglects the inertial effects[30, 120]. Further modifications can be made to the Lucas-Washburn analysis to provide a more complete analysis. The hydrodynamics of the fluid flow in the reservoir near the capillary tube entrance and the departure from Poiseuille flow in the vicinity of the advancing meniscus were studied by Levine et al[81] who developed a comprehensive mathematical theory of these effects[159], which is discussed in detail in Appendix B.1.

2.6 Dynamic Contact Angle

The mechanisms behind the wetting characteristics of dynamic contact angles are widely debated. There have been numerous models developed based on the velocity dependence of the contact angle to measured properties such as liquid viscosity, surface tension and the static contact angle. There are two distinct theoretical models; hydrodynamic (HD) and Molecular Kinetic Theory (MKT). Both theories consider the out of equilibrium surface tension force as the driving force at the liquid meniscus which is balanced by some channels of dissipation. The HD theory is based on the liquid’s viscosity whereas the MKT relates to the solid-liquid molecular interactions at the vicinity of the TPC line and are shown in Fig. 2.8.

2.6.1 Hydrodynamic Theory

The HD theory describes the dynamic contact angle as a result of viscous bending at the vicinity of the contact line. The driving force at the contact line is therefore due to the unbalanced capillary force and the viscous resistance to the flow[34]. The discrepancy with the HD approach is that it breaks down with a moving contact line while using the “no-slip” boundary condition. To modify the HD theory to circumvent this it is assumed that the liquid near the contact line rolls over the surface and therefore a relaxation of the “no-slip” condition is required[16, 143]. The contact angle the liquid creates with the solid surface by allowing the liquid to roll over the surface is called the microscopic contact angle, $\theta_m$. It can be assumed that the liquid slips along the solid surface in a region referred to as the slip length, $L_s$, near the contact line and that microscopic contact angle does
not depend on the velocity of the contact line and can be set to equal the static contact angle[123].

Following the approaches of Blake et al[16], Lavi et al[77] and Popescu et al[107] and assuming that the viscosity in the vapour phase is negligible and the dynamic contact angle is dependant on the velocity of the flow, the dynamic contact angle is given by the following relation[123]:

$$\theta_d^3 = \theta_e^3 + 9\ln\left(\frac{L}{L_s}\right)Ca$$

(2.17)

2.6.2 Molecular Kinetic Theory

MKT is based on the kinetics of the molecular processes occurring at the TPC line. These molecular processes are either adsorption or desorption at the contact line. The MKT defines the dissipative effects of the solid-liquid molecular interactions through friction at the moving contact line in the process of a layer by layer advancement of liquid molecules on the adsorption site of the solid surface.

The wetting velocity, \(v\), of the contact line is defined as the adsorption frequency
multiplied by the average distance between two adjacent adsorption sites, $\varepsilon$[82]:

$$v = 2K\varepsilon\sinh\left(\frac{\gamma\varepsilon^2(\cos\theta_e - \cos\theta_d)}{2kT}\right)$$

(2.18)

where, $K$ is the equilibrium frequency of displacement of a liquid molecule between two adjacent adsorption sites occurring at the contact line, $\theta_d$ is the dynamic contact angle, $k$ is the Boltzmann constant and $T$ is the absolute temperature. $\varepsilon$ and $K$ are variable parameters that cannot be measured experimentally but are found by curve fitting experimental data.

The debate surrounding MKT is that there is no link to the wider hydrodynamics of the system, but MKT has been shown that it is a little more flexible and adaptable compared with the standard hydrodynamic theory. Although neither MKT and hydrodynamic theory can be universally applied to dynamic wetting since both molecular and hydrodynamic mechanisms are at work[16].

### 2.6.3 Empirical Dynamic Contact Angle

An empirical equation for the dynamic contact angle for capillary flow through microchannels by correlating the dynamic contact angle to the static contact angle have been used by Morales et al[95], Mawardi et al[91] and Han et al[55]

$$\cos\theta_d = \cos\theta_e(1 - e^{-\frac{\gamma}{M}})$$

(2.19)

where, $M$ is a characteristic length. This empirical equation can only be considered to be a good fit to the experimental data as it is based on experimental observations and has no theoretical basis.

### 2.7 Capillary Flow in Open Rectangular Microchannels

The objective of this analysis is to derive an equation which describes a fluid flowing through an open rectangular microchannel of width and height, $W$ and $H$ respectively, from a reservoir as shown in Fig. 2.9, via capillary action. Cartesian coordinates $x$, $y$ and $z$ are used which correspond to the direction of the fluid flow along the length, width and height
of the channel respectively. The fluid is considered as an incompressible, homogenous and Newtonian under isothermal conditions[95, 91]. The theoretical analysis presented in this section was developed by Morales et al. (2005)[95] and Mawardi et al (2008)[91] for nanoparticulate slurries filling micromoulds using capillary driven flow.

The theoretical analysis was based on the integral of the conservation of mass and momentum for an arbitrary deformable control volume, CV$_{ch}$, in a microchannel combined with a second conservation of momentum balance to obtain a hemispherical pressure field at the entrance to the microchannel. The control volume, CV$_{ch}$, for the first conservation of momentum balance is illustrated in Fig. 2.9 by the orange dashed lines. The integral of the conservation of momentum in the $x$-direction for this control volume can be expressed as:

$$
\sum F_x - \int_0^H \int_{-\frac{W}{2}}^{\frac{W}{2}} \int_{-\frac{L_f}{2}}^{\frac{L_f}{2}} \rho a_x dx dy dz = \frac{d}{dt} \left[ \int_0^H \int_{-\frac{W}{2}}^{\frac{W}{2}} \int_{-\frac{L_f}{2}}^{\frac{L_f}{2}} \rho v_x dx dy dz \right] + \int_0^H \int_{-\frac{W}{2}}^{\frac{W}{2}} v_x (-\rho v_x) dy dz
$$

Equation 2.20 compares the forces acting on CV$_{ch}$ minus the inertia forces due to the acceleration of CV$_{ch}$ (left hand side of equation 2.20) to the rate of the change of linear momentum inside CV$_{ch}$ minus the inlet momentum flux (righthand side of equation 2.20)[37]. There is no outlet momentum flux since the meniscus front moves with
a velocity of $v_x$. In equation 2.20, it is assumed that the fluid fills the entire height across the width of the microchannel and therefore neglects the fluid meniscus shape on the top of $CV_{ch}$\cite{95, 91} as illustrated in Fig.2.10. The objective of this theoretical analysis is to determine the fill length of the fluid in the microchannel, $L_f$ as a function of time, $t$.

![Figure 2.10](image)

**Figure 2.10:** (a) Fluid flowing in a microchannel with cross-section A-A. (b) Fluid in the microchannel assuming the fluid fills the whole microchannel and (c) diagram of the actual fluid flow meniscus shape in the microchannel.

In the literature\cite{81, 37, 38, 95, 91, 152, 32, 145, 146} the fluid undergoing capillary action in the microchannel is assumed to be laminar, fully developed and its velocity has a poiseuille shaped double parabolic profile due to the no slip boundary conditions and vanishing shear stress at the liquid-vapour interface. A finite volume simulation was carried out by Bouaidat et al (2005)\cite{18} on capillary flow in rectangular microchannels and it concluded that the deviations from its parabolic shape at the entrance of the microchannel and at the fluids flow front were negligible and that the assumption of the parabolic profile was justified\cite{18}. The double parabolic velocity profile is given by:

$$v_x(y, z, t) = D(t)\left(\frac{W}{2}\right)^2 - y^2\left(2Hz - z^2\right)$$

(2.21)

where, $D(t)$ is a time dependent function, which is obtained by determining the average velocity across the cross section of the microchannel:
\[ \vec{v}_x = \frac{1}{WH} \int_0^H \int_{-W/2}^{W/2} v_x dydz \]  

(2.22)

This results in \( D(t) \) being expressed as:

\[ D(t) = \frac{9}{(WH)^2} \frac{dL_f}{dt} \]  

(2.23)

Equation 2.23 satisfies the no-slip boundary conditions on the side, \( (y = \pm \frac{W}{2}) \), and bottom walls, \( (z = 0) \), of the microchannel and zero stress at the top surface, \( (z = H) \). The last two integrals in equation 2.20 can be solved using the velocity expression given in equation 2.21:

\[ \frac{d}{dt} \left[ \int_0^H \int_{-W/2}^{W/2} \rho v_x dxdydz \right] = \rho WH \left[ L_f \frac{d^2L_f}{dt^2} + \left( \frac{dL_f}{dt} \right)^2 \right] \]  

(2.24)

\[ \int_0^H \int_{-W/2}^{W/2} v_x (\rho v_x) dydz = -\frac{11}{25} \rho WH \left( \frac{dL_f}{dt} \right)^2 \]  

(2.25)

The forces acting on CV\(_{ch}\) are; pressure force at the flow front, \( F_{pf} \), the viscous forces, \( F_v \), along the sides and bottom of the microchannel in contact with the fluid and the pressure force at the inlet of the microchannel, \( F_{pi} \). The pressure force at the flow front, \( F_{pf} \), is derived using the Young-Laplace equation which is a force balance between the ambient pressure, fluid pressure and fluid surface tension. This force can be expressed as:

\[ F_{pf} = \left[ p_\infty - \gamma \cos \theta_d \left( \frac{2}{W} + \frac{1}{H} \right) \right] WH \]  

(2.26)

where, \( p_\infty \) is the ambient pressure, \( \gamma \) is the surface tension of the fluid at the liquid-vapour interface and \( \theta_d \) is the fluids dynamic contact angle.

The viscous forces on the bottom and side walls of the microchannel can be determined by integrating the wall shear stress over the side and bottom walls and using the differential of equation 2.21:

\[ F_v = \left[ 2 \int_0^{L_f} \int_0^H \left( -\mu \frac{\partial v_x}{\partial y} \right)_{y=\pm \frac{W}{2}} dxdz + \int_0^{L_f} \int_{-\frac{W}{2}}^{\frac{W}{2}} \left( \mu \frac{\partial v_x}{\partial z} \right)_{z=0} dxdy \right] \]  

(2.27)
\[ F_v = 3\mu L_f \frac{dL_f}{dt} \left( \frac{1}{W^2} + \frac{4}{H^2} \right) WH \]  

(2.28)

The pressure force at the inlet, \( F_{pi} \), is defined as the integral of the pressure at the entrance of the microchannel to the microchannel’s cross sectional area:

\[ F_{pi} = \int_0^H \int_{-\frac{W}{2}}^{\frac{W}{2}} p(0,y,z,t) dydz \]  

(2.29)

where, \( p(0,y,z,t) \) is the inlet pressure.

The pressure at the inlet to the microchannel, \( p(0,y,z) \), can be approximated to be the ambient pressure. However, Levine et al[81] and Dreyer et al[37, 38] performed similar analyses for a vertical cylindrical tube and parallel plates in contact with a reservoir that provide a more precise value for the inlet pressure. This was achieved by allowing for the effects of the inertia of the fluid in the reservoir and the losses as the fluid enters the channel by applying the integral momentum balance to a hemispherical control volume in the reservoir, \( CV_{res} \). The derivation of the pressure at the inlet is given in Appendix B.1 which results in the pressure at the inlet being expressed as:

\[ p(0,y,z) = p_\infty - \rho \left[ c_1 \left( \frac{d^2 L_f}{dt^2} \right) + c_2 \left( \frac{dL_f}{dt} \right)^2 + c_3 \left( \frac{dL_f}{dt} \right) \right] \]  

(2.30)

Substituting equations 2.29, 2.26, 2.28, 2.24 and 2.25 into 2.20 yields:

\[ \rho WH[2L_f + c_1]\left( \frac{d^2 L_f}{dt^2} \right) = WH[\gamma \cos \theta_d \left( \frac{1}{H} + \frac{2}{W} \right)] \]

\[ -\rho WH[c_2 - \frac{11}{25}] \left( \frac{dL_f}{dt} \right)^2 - WH[3\mu L_f \left( \frac{4}{W^2} + \frac{1}{H^2} \right) + \rho c_3] \left( \frac{dL_f}{dt} \right) \]  

(2.31)

To determine the fill length, \( L_f \), the non-linear second order differential equation, equation 2.31 can be solved numerically using a Runge-Kutta method with the initial conditions, \( L_f(0) = \frac{dL_f}{dt}(0) = 0 \). This results in a value of the fill length as a function of fluid properties and channel geometry. This equation can be reduced into its constituent forces which are as follows:

- Inertial force in the microchannel:
\[ F_{IM} = 2\rho WH L_f \left( \frac{d^2 L_f}{dt^2} \right) \] (2.32)

- Inertial force in the reservoir:

\[ F_{IR} = \rho c_1 WH \left( \frac{d^2 L_f}{dt^2} \right) \] (2.33)

- Capillary force in the microchannel:

\[ F_{CM} = WH\left( \gamma \cos \theta \left( \frac{1}{H} + \frac{2}{W} \right) \right) \] (2.34)

- Frictional force in the microchannel:

\[ F_{FM} = \mu WH L_f^3 \left( \frac{4}{W^2} + \frac{1}{H^2} \right) \left( \frac{dL_f}{dt} \right) \] (2.35)

- Frictional force in the reservoir:

\[ F_{FR} = \mu WH (\rho c_3) \left( \frac{dL_f}{dt} \right) \] (2.36)

- Reservoir convective force:

\[ F_{CF} = \rho WH [c_2 + \frac{11}{25} \left( \frac{dL_f}{dt} \right)^2] \] (2.37)

Equation 2.31 will be used in conjunction with the dynamic contact angle theories (Equations 2.17-2.19) to compare theoretical predictions with experimental values obtained for the capillary flow of surfactant solutions through various microchannel geometries in Chapter 7.

### 2.8 Microchannel Fabrication

To compare the theoretical capillary flow models to experimental data, microchannels of widths 100, 200, and 400\(\mu m\) and depths and lengths of 200\(\mu m\) and 20mm respectively were manufactured using a hot embossing technique. The microchannels were fabricated from a cyclic olefin copolymer named Topas (Grade 5013) by microLIQUID (Gipuzkoa, Spain) and its material properties are given in Appendix C.1. The hot embossing technique creates microchannels using an imprinting technique with controlled temperatures and pressures.
and is shown in Fig. 2.11. A master mould was manufactured with the inverse dimensions of the desired microchannels. This master mould and the Topas substrate were heated above the glass transition temperature (127°C) of the Topas substrate. The Topas substrate was then pressed against the master mould at a certain pressure. The master mould and Topas substrate were then cooled at a slow specific rate and then the Topas substrate was released from the master mould.

Figure 2.11: (a) Components for hot embossing (b) Master mould and Topas substrate heated above the Topas glass transition temperature and pressed against each other under a specific pressure and (c) Master mould and Topas substrate cooled below the Topas glass transition temperature and separated.

The widths and heights of the microchannels were measured by placing them under a microscope on a stage whose position was mechanically controlled in the x and y spatial directions using two M-227 DC-Mike Actuators and controlled in the z direction using a Tofrainc Focus Drive 101-26. The widths and heights of the microchannels were measured at five locations along the microchannels and the averaged measured widths of the 100, 200 and 400µm microchannels were 100.6±3.7, 190.5±2.1 and 392.6±2.1µm respectively. The heights of the 100, 200 and 400µm microchannels were 185±11.1, 195±11.8 and 196.6±11.4µm respectively.

2.9 Summary

In this chapter, the properties of a fluid that influence capillary flow were discussed and equations for the dynamic contact angle of a fluid under capillary flow in a microchannel were presented. A non-linear second order differential equation was derived to predict capillary flow in open rectangular microchannels. The process of fabricating open rectangular microchannels using a hot embossing technique was discussed. Experiments of capillary
flow through these microchannels will allow the validity of the derived non-linear second order differential equation for capillary flow coupled with the equations for the dynamic contact angle to be assessed.
Chapter 3

HSV Immunoassays

Immunoassays are currently the predominant analytical technology in which specific interaction of an antibody with its antigen is exploited to detect for analytes of interest in physiological fluids or other fluids[87, 22]. Antigens are substances which trigger the immune system to produce specific antibodies. Immunoassays are particularly effective at measuring trace levels of an analyte of interest. Immunoassays are frequently used for clinical diagnoses such as tumour markers for cancer types, environmental analyses such as water contaminants, biological threats and epidemic concerns[85]. There are numerous platforms that immunoassays can be based on which range from those that require skilled personnel to perform the assays to lateral flow assays used at home for pregnancy testing[165].

There is a need for a definitive, rapid, point of care diagnostic device that can distinguish between HSV-1 and HSV-2[4]. Accurate tests can provide the basis for proper clinical management, timely treatment and appropriate counselling relating to the natural history and transmission risks associated with the infection[3]. Many commercial assays are unable to discriminate between the subtypes of the HSV infection. This is due to the common antigens that the infection subtypes share. These common antigens result in cross-reactivity between the antibodies elicited by the immune system in response to the subtype infection. Assays that are unable to give a type-specific diagnosis and therefore type-specific treatment are of little use[63, 61]. These assays are unable to determine if HSV-2 is present in a patient’s serum that has HSV-1 present due to a prior oral infection or
if there is a dual infection. A type-specific, accurate, fast and reliable assays are required to allow the diagnosis of asymptomatic HSV-2 infection in patient’s serum with or without pre-existing antibodies to HSV-1 infection[61].

In this chapter, the HSV antigen structure and components are presented followed by the a discussion on the current type specific HSV assays currently in use and a brief review of microfluidic immunoassays.

### 3.1 HSV Antigen

The HSV antigen is a complex multilayered DNA virus. HSV-1 and HSV-2 antigen viral structures are quite similar. The antigens comprise of 4 components; the viral genome, capsid, tegument and envelope and are illustrated in Fig. 3.1. The viral genome is the core of the antigen. It is a double stranded DNA of HSV which is surrounded by a icosahedral protein shell called a capsid. The icosahedral capsid is a polyhedron with 20 identical equilateral triangular faces, 30 edges and 12 vertices. This is enclosed by a proteinaceous region named tegument. All this is contained by the envelope which is a bi-layer that contains 12 or more embedded types of glycoproteins which are located on the spikes on its outside[27, 160, 52].

![Figure 3.1: (a) Cryo-electron micrograph of HSV-1 antigen[60] (b) 3-D surface structure of HSV-1 antigen[161] (c) Cross-section of the HSV-1 antigen and (d) Icosahedral capsid shape.](image)

### 3.2 Type specific HSV Assays

Type-specific assays allows the identification of asymptomatic people with HSV-2 infection with or without the presence of HSV-1 antibodies from a previous HSV-1 oral infection. It
has been well documented that HSV-1 and HSV-2 infections share common epitopes\[3, 40, 61, 74, 109\]. Epitopes (glycoprotein spikes) are points on the antigen that antibodies can bind onto.

Recently HSV glycoprotein G (gG) was identified as being antigenically distinct in HSV-1 and HSV-2 infections. Most viruses trigger the immune system of an infected person to produce virus specific antibodies\[26\]. The immune system of a HSV infected person produces specific immunoglobin antibodies (IgG) in the presence of glycoprotein G (gG-1), a protein found only in HSV-1 infection. Similarly the immune system produces specific antibodies in the presence of glycoprotein G (gG-2), a protein found only in HSV-2 infection\[40, 109\]. This breakthrough allowed the development of type specific HSV assays.

Type-specific HSV assays have been developed in numerous formats that can detect the presence of HSV antigens; virus isolation in cell cultures (Western Blot), Enzyme linked immunosorbent assays (ELISA), enzyme immunoassays (EIA), immunofluorescence assays (IFA) and nucleic acid amplifications by PCR. The remainder of this section gives a review of these assay techniques for HSV detection.

### 3.2.1 Western Blot

Western Blot is an analytical cell culture technique for HSV isolation which is considered the diagnostic gold standard\[20, 115, 45\]. Western Blots are used to detect antibodies to specific proteins that are present in a patient serum. Western Blot technique starts by cell culturing HSV-1 and HSV-2 and breaking these down into protein lysates. The remaining steps of the Western Blot technique are shown in Fig. 3.2. The protein lysates are separated according to their molecular weight by gel electrophoresis creating protein arrays, green blots in Fig. 3.2, for HSV-1 and HSV-2 infected cell lysates\[4\]. These protein arrays cannot be seen visually. These protein arrays are transferred to nitrocellulose strips. A patient’s serum is reacted against the strip where specific antibodies in the serum will bind to target proteins on the strip if they are present in the serum. The strips are observed visually on a daily basis for the appearance of cytopathic effects (CPE). CPE are any detectable changes to the protein due to HSV infection, in this case the formation of visible blots (red blots
in Fig. 3.2), characteristic of HSV. Cultures are declared negative if there was no CPE observed over a 7-10 day period[110]. These tests are highly complex to perform and include a number of incubation steps and lengthy time for CPE to appear leading to long assay times.

![Figure 3.2: Steps involved in the Western Blot technique after HSV cell culture.](image)

There is a commercially available assay called HerpeSelect Immunoblot which is similar to the Western Blot technique and is FDA approved. Table 3.1 gives a selection of commercially available assays based on glycoprotein gG. The Western Blot technique is slow and labour intensive. It requires a minimum of 1 day to isolate and up to 10 days for possible CPE to appear and identify HSV-1 or HSV-2 presence in the patient’s serum[115, 4, 164]. Western Blot has a specificity of practically 100% but its sensitivity is influenced by the levels of virus shedding, quality of patient’s serum and serum refrigeration during collection and transport[45, 115]. Western Blot is highly accurate in discriminating between HSV-1 and HSV-2 infections but interpretation of results is subjective and profiles may not always be definitive[3].
### Table 3.1: Commerially available HSV assays.

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Format</th>
<th>Type</th>
<th>Sensitivity/specificity</th>
<th>Gold Standard</th>
<th>FDA approved</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tests based on purified glycoprotein gG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biokit HSV-2</td>
<td>Biokit USA</td>
<td>ELISA</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premier</td>
<td>Meridian</td>
<td>ELISA</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tests based on recombinant glycoprotein gG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HerpeSelect ELISA</td>
<td>Focus Diagnostic</td>
<td>ELISA</td>
<td>Yes</td>
<td>Yes</td>
<td>91.92[45]</td>
<td>96/97[45]</td>
</tr>
<tr>
<td>HerpeSelect Immunoblot</td>
<td>Focus Diagnostic</td>
<td>Immunoblot</td>
<td>Yes</td>
<td>Yes</td>
<td>99/95[45]</td>
<td>97/98[45]</td>
</tr>
<tr>
<td>CAPTIA HSV IgG Type Specific</td>
<td>Trinity Biotech</td>
<td>ELISA</td>
<td>Yes</td>
<td>Yes</td>
<td>88/98[50]</td>
<td>97/99[50]</td>
</tr>
<tr>
<td>Novagrost HSV-1 IgG</td>
<td>Dade Behring</td>
<td>ELISA</td>
<td>Yes</td>
<td>Yes</td>
<td>93-98/99[121]</td>
<td>100/95-98[121]</td>
</tr>
<tr>
<td>RIBA HSV Type 1/2 SIA</td>
<td>Chiron Corp.</td>
<td>Immunoblot</td>
<td>Yes</td>
<td>Yes</td>
<td>95/99[93]</td>
<td>98/99[141]</td>
</tr>
<tr>
<td>HSV-2 IgG</td>
<td>Kalon Biological</td>
<td>ELISA</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETI-HSVK-G2</td>
<td>Sorin Biomedica</td>
<td>ELISA</td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Western Blot: Yes
FDA approved: Yes
3.2.2 ELISA/EIA

Enzyme linked immunosorbent assays (ELISA) and enzyme immunoassays (EIA) are widely used to measure the presence and concentration of a particular antigen or antibody in a patient’s serum with an enzyme as the detection marker. These assays are usually performed on well plates or microscope slides. There are two types of ELISA assays, direct and indirect assays.

A direct, ‘sandwich’, assay determines the presence of a target antigen with antibodies that have an affinity for the target antigen. In this assay, see Fig. 3.3, the first step is to immobilise capture antibodies on the surface of the assay. It is then washed to remove any unbound antibodies from the surface. The second step, a patient’s serum containing target antigens is added. The target antigens bind to the immobilised capture antibodies creating antigen-antibody complexes. It is rinsed to remove any unbound antigens. The third step, antibodies conjugated with an enzyme, referred to as detection antibodies, that have an affinity for the target antigens attach to the target antigen-antibody complexes. The target antigen is ’sandwiched’ between a capture and detection antibody. It is washed again to remove any unbound antibodies. Finally, a substrate is added which causes a catalyst reaction with the enzyme causing a change in colour of the solution or surface which indicates the presence of the target antigens. The colour intensity gives a quantitative measure of the concentration of antigen present in the sample. The more detection antibodies that bind to the target antigen-antibody complexes the more intense the colour will be.

An indirect assay is slightly different. The first step, target antigens are immobilised to the surface of the assay. The second step, a patient’s serum containing antibodies is added. Only antibodies with an affinity for the target antigens will bind to the target antigens. Any unbound antibodies are washed from the surface. The third step, a second antibody conjugated with an enzyme is added which couples to the antibodies bound to the target antigens to form complexes. Any unbound antibodies are washed from the surface. Finally, a substrate is added which causes a catalyst reaction with the enzyme causing the solution to change colour.

There are a number of commercially available ELISA assays to detect HSV-1 and HSV-2. CAPTIA HSV Ig Type Specific, HSV-2 IgG ELISA, ETI-HSV-G2 and Novagnost.
HSV Immunoassays

3.2.3 IFA

Immunofluorescence assays (IFA) are quite similar to ELISA and EIA. The only difference between the assays is that the IFA uses a detection antibody conjugated with a fluorescent marker, a fluorophore, to indicate the presence of a particular antigen or antibody in a sample rather than an enzyme. A fluorophore is a fluorescent component of a molecule. When a fluorophore is excited by photons at a specific wavelength from an external source, such as a laser or mercury lamp, it will emit photons at a longer wavelength.

IFA assays can be direct or indirect assays and follow the same principle as ELISA and EIA assays that the detection antibodies bind to the target antigen-antibody complexes. To determine the presence of these complexes in IFA assays, the fluorophore needs to be
excited by photons at a specific wavelength which induces the fluorophore to emit photons at a longer wavelength. The emitted photons at the longer wavelength can be qualitatively and quantitatively measured using a fluorescent microscope or fluorescent reader equipped with the appropriate filter for the fluorophore used.

### 3.2.4 Lateral Flow Assay

Lateral flow assays have been developed for near patient, home testing and point of care testing which provide results in a matter of minutes. A typical lateral flow assay is performed by applying a patient’s serum to a substrate membrane with immobilised reagents and flows through it via capillary action. There are two types of assays, non-competitive and competitive assays. Competitive assays are usually used for smaller antigens[103]. Some assays include a control spot/line which indicates that the assay has performed correctly, the serum has flowed across the assay as intended. These assays comprise of 4 pads; sample, conjugate, reagent and wicking pads and are shown on the top of Fig. 3.4.

![Figure 3.4: Sandwich lateral flow assay operation.](image)

A typical non-competitive lateral flow assay has three basic steps of operation and are shown in Fig. 3.4. The first step, a patient’s serum, contains several types of antigens, is applied to the sample pad. The second step, serum is absorbed by the membrane and
the serum flows to the conjugate pad. The conjugate pad contains specific detection antibodies conjugated with an enzyme or fluorescent marker that bind to the target antigens in the serum. The third step, the target antigen-antibody complexes are carried forward by capillary action to the reagent pad. The reagent pad contains a test spot/line where capture antibodies with an affinity for the target antigens have been immobilised. The target antigen-antibody complexes bind to these antibodies. The reagent pad may also contain a control spot/line. This spot/line has control antibodies immobilised to catch any antibody which do not bind with the target antigens. The control spot/line indicates if the assay has performed correctly, the serum has transversed across the assay as intended. The remainder of the serum reaches the wick pad which pulls the serum through the reagent pad. A positive result for this assays occurs when there is a visual marker at the test and control line/spots. A negative result occurs when there is only a visual marker at the control line/spot. If there is no visual marker is present the serum needs to be re-tested. Shown in Fig. 3.5, are 3 lateral flow assays developed for HSV-2 detection with inconclusive, negative and positive result[74].

The competitive assay differs slightly to the non-competitive assay. The conjugate pad in the competitive assay contains target antigens bound to detection antibodies conjugated with a marker of known concentration before the serum is applied. When the serum flows through the conjugated pad the serum target antigens cannot bind to the detection antibodies. These unbound serum target antigens flow to the test spot/line where they compete with the antigens bound to the detection antibodies to attach onto the immobilised capture antibodies. Any serum target antigens that do not bind to the test spot/line travel to the control spot/line where they are free to bind to the antibodies immobilised there. The concentration of target antigen bound with detection antibodies attached to the immobilised capture antibodies at the test line/spot will decrease with increasing concentration of serum target antigen in the sample[103]. A positive result occurs if the concentration of target antigen bound with detection antibodies attached to the immobilised capture antibody at the test spot/line is lower than the known concentration, i.e. there is a visual marker at the test and control line/spot or just at the control line/spot. A negative result occurs when there is only a visual marker at the test line/spot. If there is no visual marker at either line/spot then
CHAPTER 3

HSV Immunoassays

Figure 3.5: (a) Lateral flow assay for the detection of HSV-2 giving an inconclusive result, buffer applied to the assay (b) Lateral flow assay for the detection of HSV-2 giving a negative result, negative HSV-2 sample applied to the assay and (c) Lateral flow assay for the detection of HSV-2 giving a positive result, positive HSV-2 sample applied to the assay[74].

the test did not perform properly.

These assays give a qualitative result but there can be some degree of quantification if a fluorescent marker was used in-conjunction with a fluorescent microscope or reader that could measure the intensity of the fluorescent signal of the bound fluorescent markers. Lateral flow assays can detect the infection without relatively time consuming and expensive cell isolation methods[51], easy to operate and require small amounts of reagents and sample.

There are two commercially available point of care membrane assays for HSV detection based on recombinant glycoproteins. BiokitHSV-2 is based on the gG-2 glycoprotein and is able to only detect HSV-2 in a sample. RIBA HSV Type 1/2 Immunoblot is based on
glycoproteins gG-1 and gB-1 for HSV-1 detection and gG-2 and gD-2 for HSV-2 detection. Biokit HSV-2 is the only FDA approved of the two assays.

3.2.5 PCR

Polymerase Chain Reaction (PCR) is a widely used technique used to amplify small quantities of target DNA with an enzyme reaction through thermal cycles. HSV detection by PCR is, to date, the most sensitive approach for detection of and differentiation of HSV subtypes[26]. PCR increases HSV detection rates by 11-71% compared with cell culture[45, 115, 153]. Detection of HSV DNA by PCR has primarily been used to test cerebrospinal fluid (CSF) samples in patients with suspected herpes encephalitis where low concentrations of HSV in CSF samples required the use of the most sensitive test possible[20]. Conventional PCR has some drawbacks, it requires experienced personnel, is prone to contamination and the operating costs are high when compared to simpler immunoassays[26, 115].

3.3 Microfluidic Immunoassays

The miniaturisation of immunoassays to microfluidic platforms has many advantages such as a reduction in: cost, sample volumes, reagent consumption and analysis time. To miniaturise immunoassays to microfluidic platforms, accurate fluid flow through the microfluidic immunoassays are critical to the performance of the device. Microfluidic devices commonly use the following forces for fluid transport: electrokinetic[155, 139, 76, 28], pressure[70], centrifugal [79, 39, 117] and passive forces[83, 47].

Flow produced by electrokinetic forces, also known as electroosmosis, is created by the chemical equilibrium at the solid-liquid interfaces between the surface charge of the solid and the counter ions in the fluid. This interface is referred to as the electric double or Debye layer as shown in Fig. 3.6. When an external electric current is applied across the solid and fluid, the counter ions in the fluid will move in the direction of the electric current. The bulk of the fluid moves with the counter ions due to the viscous forces of the moving counter ions to form a plug like flow profile. Microfluidic immunoassays use
capillary electrophoresis (the applied electric field) to separate analytes in a fluid according to their mass and ionic strength. This technique very effective in separating biological molecules, such as antibodies, for two reasons: the near flat plug flow profile and the small diffusion constant of the antibodies[7]. Microfluidic capillary electrophoretic immunoassays have been shown to be capable of detecting immunosuppressive drug cyclosporine A in blood samples[155], insulin[139], Staphylococcal enterotoxin A (causes food poisoning in humans)[76] and anabolic steroid metabolites[28]. The disadvantage of this type of microfluidic immunoassay for a point-of-care application is that is requires and external power source to create fluid flow.

![Figure 3.6: Electroosmosis flow[101].](image)

There are two commonly used methods for pressure driven flow through microfluidic devices: (i) by applying a vacuum pump to the outlet of the microdevice and the inlet open to the atmosphere or (ii) a mechanical syringe pump connected to the inlet of the microdevice. The pumps use a stepper motor to move the plunger in a syringe at a constant speed which controls the fluid flow rate through the microdevice[46]. Pressure-driven microfluidic immunoassays have been shown to detect blood analytes, such as, C-reactive protein, prostate-specific antigen, ferritin, and vascular endothelial growth factor[70]. Pressure-driven microdevices have a parabolic flow profile but electrokinetically driven microfluidic immunoassays have better reaction kinetics than pressure-driven ones, resulting from the flatter velocity profile[101, 66]. Pressure-driven microfluidic devices
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are not ideal for a point-of-care application because it requires an external power supply to produce fluid flow.

Centrifugal pumping microdevices are typically shaped like a compact disc and are rotated at speed. Pumping of fluids through these microdevices is achieved by centrifugally induced pressure (balance of rotational speed, microchannel geometry and fluid properties). Using this technique, fluids can be held in certain positions either through the use of capillary valves, sacrificial capillary valves or hydrophobic surfaces until the rotational speed is increased. The advantage of centrifugal over electrokinetic pumping is that it is insensitive to the fluid’s physiochemical properties (pH level and ionic strength)[89]. A centrifugal microdevice was developed for the detection of Hepatitis B virus from a whole blood sample[79] and is shown in Fig. 3.7.

Figure 3.7: Centrifugal pumping microfluidic immunoassay[79].

Passive-pumping uses capillary forces to propel fluids through microdevices. The fluid flow is dependent on geometry and fluid properties only. Passive pumping can be created either using porous capillary membranes or using microchannels and the latter provides more reproducible flow rates and volumes. A passive-driven microfluidic immunoassay using membranes has been developed for pregnancy test kits and the detection of nitrated ceruloplasmin, a significant biomarker for cardiovascular disease, lung cancer and stress response to smoking[83] which is shown in Fig. 3.8 (a). A passive-driven microfluidic immunoassay using microchannels has been developed for the detection of C-reactive protein (CRP), a general inflammation and cardiac marker, from a blood sample[47] and is illustrated in Fig. 3.8 (b). Passive-driven microfluidic devices are ideal for point-of-care applications because an external power source is not required to generate
flow although a power source may be required for detection.

![Microfluidic immunoassay using a membrane](image1)

![Microfluidic immunoassay using microchannels](image2)

**Figure 3.8:** (a) Microfluidic immunoassay using a membrane[83] and (b) Microfluidic immunoassay using microchannels[47].

### 3.4 Summary

Currently, there is a need for a cheap, disposable, rapid, point of care diagnostic device that can distinguish between HSV-1 and HSV-2. The current methods of detection require a large volume of costly reagents and detection of HSV can range from hours to weeks. This research thesis has the objective of designing, developing and assessing the performance in the detection of HSV type 1 and 2 using a microfluidic platform based on the combined operating principles of ELISA and lateral flow assays. This thesis will investigate the possible detection of HSV-1 and HSV-2 through fluorescence using the IFA the direct assay principle combined with the lateral flow of a patient’s sample through the microfluidic platform under capillary action (passive-pumping) which will be discussed in more detail in Chapters 4 and 5. Detection of HSV using this method could have the potential of reducing the volume of reagents consumption and reducing the detection time to a matter of minutes.
Chapter 4

HSV Capillary Chip Immunoassay Design & Development

Microfluidic devices have the potential to integrate the steps of a complex chemical process and detection into a single disposable point of care device[153, 116]. These devices are attracting a lot of attention in the academic and industrial world because they have the potential to improve the analytical performance by reducing cost, sample, reagent and power consumption. In addition, the surface-to-volume ratio of the microfluidic device can enhance mass transport, resulting in shorter assay times, increased detection sensitivity and integrating multiple processes in a single device compared to the conventional analytical methods[31].

Immunoassays are widespread and essential for clinical diagnoses but appear difficult to integrate into smaller, highly sensitive practical formats. ELISA is the most common immunoassay technique used in clinical diagnostics which is performed in microtiter plates. This technique is capable of detecting an antigen of interest in a sample through the use of capture antibodies immobilised on the surface of the microtiter plates but its sensitivity depends on the concentration of antibodies used. This method requires sample and reagents volumes of up to 100µl[13].

The ELISA technique was the standard laboratory technique for the last few decades. This technique requires the use of specialised equipment and is rarely performed outside of a centralised laboratory because of its cumbersome fluid handling techniques. It also
requires expensive and specialised instruments for optical detection[14]. The ELISA assay time can take several hours due to the numerous washing and preparation steps and the long diffusion time required for the antigen-antibody binding[85]. The long assay time can be mostly attributed to inefficient mass transport of the antigen/antibody from the solution to the surface of the microtiter plate whereas the immunoreaction itself is a rapid process[75]. The long assay time can be significantly reduced when the ELISA is used in conjunction with a shaker or stirrer. The ELISA technique has high sensitivity but requires a large sample volume[85].

Currently, Amic AB (Uppsala, Sweden) which has been acquired by Johnson & Johnson have the only commercially available open capillary flow microdevice (4castchip) shown in Fig. 4.1. Fluids flow through this chip by capillary action via highly ordered array of micropillars and has been used for the detection of C-reactive protein, myoglobin and troponin I. The flow rate through this chip can be controlled through its wetting properties or shape and spacing of the micropillars[69].

Figure 4.1: (a) Micropillar array and (b) Amic AB lateral flow device[69].

The open capillary driven microfluidic platform developed in this study was termed as the HSV capillary chip. It was designed to miniaturise the ELISA technique into a lateral capillary flow microdevice. This resulted in lower sample and reagent consumption by the virtue of the reduction in the size of the reaction chamber and thereby increasing the surface to volume ratio[14]. The sample or reagent consumption can be potentially reduced from 100µl to 1µl in microfluidic devices[65].

There are certain design criteria that microfluidic immunoassay devices need to satisfy, they must be capable of transporting a sample to specific locations on the device at specific time intervals, its material must allow optical detection and be bio-compatible with
the fluid sample and the assay reagents. For disposable point of care devices the fabrication costs must be kept to a minimum. Capillary flow devices are easy to fabricate, depending on the material, because of the low complexity of the design since there is no need for external fluid pumping connections.

In this chapter, the design considerations of the HSV capillary chip, such as, the material selection, fabrication and surface modification, will be outlined as well as the fluorescent marker for the HSV detection antibodies. The dimensions of the microchannels of the HSV capillary chip will then be discussed.

### 4.1 Design Considerations

The material choice for a microfluidic device is a significant factor to be considered when designing a passive driven microfluidic device that depends on the performance of chemical and physical tasks. The surface chemistry and optical transparency (depending on wavelength) of the material are two major considerations for microfluidic device fabrication. The requirement of surface modification and fluid handling within the capillary chip are primarily controlled by the surface properties of the material while the detection mode is governed by its optical properties[59].

#### 4.1.1 Material Selection

Depending on the application involved, microfluidic devices can be constructed from silicon, glass or polymers. Early biochemical microfluidic devices were fabricated from silicon as the micro-fabrication technology, patterning, etching and bonding of silicon wafers was already well established for the semiconductor industry[90, 10]. However, silicon is not optically transparent in the UV/visible region, which is normally the detection wavelength range of most bio-analysis[59]. Glass is an attractive material for microfluidic devices because of its moisture resistance, thermal stability, optical transparency and low background fluorescence, but the cost involved in the fabrication of these devices is turning manufacturers to seek other materials. There has been an increased usage of several types of polymers in the fabrication of microfluidic devices. Polymers are low cost, have a wide
range of surface properties and are easier to mass produce than glass or silicon microfluidic devices[10].

The fabrication techniques of glass and polymer microfluidic devices differ greatly. Glass microfluidic devices are fabricated through etching techniques which produces shallow rounded channels with low aspect ratios. Polymer fabrication techniques, such as, injection moulding, hot embossing and soft lithography, offer greater aspect ratio diversity of channel geometries and are not restricted to rounded channels[6].

For biomedical applications the use of reusable glass chips is economically unviable because of the lengthy cleaning steps involved between analyses and also because of the risk of cross contamination. The selection of disposable chip material must balance the inherent cost of the material with the processing costs[153]. For these reasons, point-of-care biomedical devices are better suited to be fabricated from low cost polymers which allow the device to be disposable and avoid the costly cleaning steps of glass devices.

There are a wide variety of polymers to choose from which have different material characteristics and several are listed in Table 4.1. When selecting a material for a disposable biomedical application there are many factors to consider; cost of bulk material, cost of fabrication, optical and physical properties, chemical resistance and surface chemistry. Polydimethylsiloxane (PDMS), Polycarbonate (PC) and Polymethyl methacrylate (PMMA) are the most commonly used polymers. More recently, cyclo olefin polymers (COP) have become commercially available and are now competing with PC and PMMA in the biomedical diagnostic market[124, 104, 56, 128].

COP has several distinct advantages over other polymers[1]. It has a lower viscosity at processing temperatures for injection moulding which allows for lower injection pressures and better filling of the mould. It has a lower water absorption, typically an order of magnitude lower than PC and PMMA. It has a superior UV transmittance and low auto-fluorescence when it was laser excited at 403, 488, 532 and 633nm[104]. It’s surface chemistry is also suitable for antibody deposition. For the above mentioned advantages the polymer selected for the chips for HSV detection in this study to be fabricated from was a COP named Zeonor (Grade 1060R) from Zeon Chemicals Europe, Dusseldorf, Germany.
Table 4.1: List of polymers and their properties (adapted from [157, 124, 150, 151]).

<table>
<thead>
<tr>
<th>Material</th>
<th>PMMA</th>
<th>PC</th>
<th>COP</th>
<th>PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabrication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot embossing</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Injection Moulding</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Soft Lithography</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Optical Properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission of visible light (%)</td>
<td>92</td>
<td>89</td>
<td>92</td>
<td>91</td>
</tr>
<tr>
<td>Low auto-fluorescence</td>
<td>Excellent</td>
<td>Fair</td>
<td>Good</td>
<td>Excellent</td>
</tr>
<tr>
<td>Chemical Resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Fair-good</td>
</tr>
<tr>
<td>Alkalis</td>
<td>Excellent</td>
<td>Poor</td>
<td>Good</td>
<td>Poor-fair</td>
</tr>
<tr>
<td>Solvents</td>
<td>Poor</td>
<td>Poor</td>
<td>Fair-poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Physical Properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition temperature (°C)</td>
<td>106</td>
<td>150</td>
<td>90-136</td>
<td>127</td>
</tr>
<tr>
<td>Water absorption (%)</td>
<td>0.1-0.5</td>
<td>0.35</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mould (linear) shrinkage</td>
<td>0.001</td>
<td>0.005-0.007</td>
<td>0.001</td>
<td>0.001-0.006</td>
</tr>
<tr>
<td>Surface charge</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

4.1.2 Chip Fabrication

Injection moulding was the fabrication technique used in the manufacture of the chips for several reasons. This technique is cost effective for mass production of disposable chips. It is also able to achieve very accurate shape replication and dimensional control. The injection moulding process begins by machining a negative mould of the microfluidic device. This negative mould was used as the template to create the capillary chips. Granules of the polymer, Zeonor, were dried in an oven to remove any moisture. These granules were then heated above the glass transition temperature of the polymer until the granules formed a molten resin. This was injected into the moulding cavity which was heated to just below the glass transition temperature of the polymer. The moulding cavity was heated to help uniform flow of the polymer through the moulding cavity. The molten polymer was injected into the mould under pressure and was subjected to a holding pressure to compensate for polymer shrinkage[6]. The mould was cooled to below the polymer glass transition temperature and the polymer solidified. Low viscosity polymers are better suited
to this technique because of their good flow properties which gives better filling of the mould cavity. The cycle time of this technique depends on the viscosity of the polymer, injection speed, melt and mould cavity temperature. Typically a cycle time can last seconds to minutes[1].

4.1.3 Surface Modification

Microfluidic immunoassays depending on immunoaffinity interactions have been used to detect specific antigens of interest from a patient’s sample based on the selective binding of specific antibodies to their relative antigens. These specific antibody-antigen interactions are dependent on the surface chemistry of the device.

Antibodies are types of glycoproteins that are produced in the presence of antigens. Immunoglobulin G, IgG, is the most commonly used glycoprotein in immunoaffinity devices and is shown in Fig. 4.2. IgG are 150 kD glycoproteins that consist of 4 polypeptides that are arranged in two identical 50-kD glycosylated proteins, heavy chains, and two identical 25-kD proteins, light chains. Disulphide bonds link the heavy and light chains together in a Y-shape. The antibody consists of a fragment crystallisable, Fc, and two fragment antigen binding, Fab, elements. The two Fab elements provide two locations for antigens to attach onto the antibody. Fab elements contain variable sequence of amino acids that are distinct from each type of antibody. This variation of the composition of the amino acids allows the immune system to produce different types of antibodies that have varying affinities and specificities for particular antigens. It is estimated that the immune system is capable of producing $10^6$-$10^8$ types of antibodies[54]. The Fc element contains the same sequence of amino acids in the same class of antibody, e.g. IgG antibody class[103, 54]. Fc element also contains carbohydrate residues that are covalently linked to the polypeptide chains. The carbohydrate residues and the amino acids are attachment sites for the antibodies to bind onto the surface of a solid support or attaching labels for detection[103].

Two types of antibodies used in immunoassays are polyclonal and monoclonal antibodies. Polyclonal antibodies are natural antibodies that are generated from immunising animals with a target antigen. Animals such as a mouse or rabbit are injected with the target
antigen and after a suitable time period a blood sample is taken from the animal. The antibodies produced in response to the target antigen are separated from the sample. These antibodies provide a heterogeneous mixture of antibodies that bind with varying strengths to the epitopes of the target antigen. Monoclonal antibodies are antibodies that are produced in a laboratory. Single antibody producing cells are isolated and are easy to culture and grow for long term antibody production. This process produces single types of antibodies that bind to a single specific epitope of a target antigen with the same strength[103, 53, 54].

In the assays developed in this thesis for HSV detection, polyclonal antibodies were used as the capture antibodies. Polyclonal antibodies were able to bind on to numerous epitopes of the antigen and therefore a greater quantity of antigens were able to bind to the capture antibodies than if monoclonal antibodies were used as the detection antibodies. The use of polyclonal antibodies as the detection antibodies gives the assay greater sensitivity. Ideally, monoclonal antibodies would have used as the detection antibodies for both HSV-1 and HSV-2 assays rather than polyclonal antibodies because monoclonal antibodies bind to a specific epitope of an antigen thereby giving an assay a greater specificity but only polyclonal detection antibodies for HSV-1 could be commercially sourced for the HSV-1 assay.

Polymer surfaces in their native form do not contain any functional groups that antibodies can attach to and therefore polymer surfaces need to undergo a surface modification.
This can be achieved through covalent bonding. For covalent bonding, the capture antibodies are generally bound to the support through the amino acids which are on the exterior of the capture antibodies[15, 119]. This can potentially create a layer of heterogeneous immobilised capture antibodies because the capture antibodies could attach to the surface simultaneously through several amino residues on the capture antibody. The capture antibodies could be bound to the surface in a more orderly manner by functionalising the surface so that the capture antibody can only attach to the surface through specific moieties of the capture antibody[119]. After the capture antibodies have been immobilised an incubation step at room temperature in humid conditions is sufficient in creating a covalent bond between the antibody and the surface. The incubation step must be in humid conditions so that the capture antibodies remain hydrated to ensure the integrity of their three dimensional structure[15, 137].

4.1.4 Fluorescent Detection Antibody

Detection antibodies in immunoassays are antibodies that are chemically labelled with reporter molecules such as enzyme or fluorophore molecules. Enzymes are regularly used because of their stability but require the use of carcinogenic or toxic substrates. To avoid this issue and also increase the assay sensitivity, antibodies can be labelled with fluorophores by chemical conjugation[108]. Fluorophores are fluorescent probes that are typically chemically bound to target molecules of interest, for example, DNA, proteins and Immunoglobulin[144].

A fluorophore can adsorb a range of wavelengths that result in varied excited states, this range is known as the adsorption spectrum of the fluorophore. These excitation states produce a range of emission photon wavelengths known as the emission spectrum. The excitation-emission spectrum of fluorescein isothiocyanate, FITC, is shown in Fig. 4.3. The peak on the left and right of the diagram represent the excitation and emission spectrum of FITC respectively. The excitation-emission spectra can overlap each-other but the adsorbed and emitted photons can be distinguished from each-other through the use of appropriate filters. The assays developed in this thesis for the detection of HSV-1 and HSV-2 used FITC as the chosen fluorophore to be conjugated to the detection antibodies because the
For fluorescence detection, there are several factors to consider; excitation photon intensity, excitation photon wavelength, fluorophore properties, filter selection and detection sensor sensitivity and dynamic range. The excitation photon intensity is a balance of required emission photon intensity and the rate of acceptable photobleaching. Excitation photon wavelength range should be as narrow as possible to avoid overlapping with the emission photon wavelength range. The fluorophore properties especially the rate of photobleaching need to be considered. Filter selection of appropriate filters that will allow the excitation and emission at the fluorophore’s peak wavelengths, or close as possible, to be distinguishable from each-other. The sensor of the detection system must be aligned properly to detect the vast majority of the emission photons. For the comparison of quantitative fluorescent data all these parameters must remain unchanged.

4.2 Design Development

The design of the capillary chip needed to incorporate passive fluid transport that allows sufficient time for antigen-antibody binding, contain two reaction chambers and waste fluid collection area. The design of the capillary chip is shown in Fig. 4.4. The capillary chip was originally designed for the detection of myoglobin and troponin I from a blood sample and the performance of the capillary chip was to be compared to a commercial
chip using its detection system. The capillary chip was designed to have the same overall dimensions of the commercial chip (which is also the same dimensions of a microscope slide) of 75, 25 and 1.2mm in length, width and thickness respectively so that it could be used in the commercial detection system. Due to funding constraints and the availability of the capillary chips, these were used for the detection of HSV-1 and HSV-2.

![Capillary chip with the microchannel filled with green dye and magnified images of the inlet, detection zones and outlet areas.](image)

**Figure 4.4:** Capillary chip with the microchannel filled with green dye and magnified images of the inlet, detection zones and outlet areas.

### 4.2.1 Sample Inlet

The inlet of the chip required a minimum volume of 20µl as this was estimated to be the maximum volume of fluid that would be applied to the inlet of the chip for each stage of the assay. The chip has a sample inlet area of 6.5x15mm and a depth of 0.5mm and a volume of 48.75µl. The volume of the inlet was almost two and half times the estimated maximum volume required which allowed flexibility in the assay volumes during the optimisation of the protocol for the assay.
4.2.2 Feed Channels

The original application of this chip required the filtration of red blood cells and platelets from a blood sample for the detection of myoglobin and troponin I. Therefore the chip needed to incorporate a blood filtration filter. This was positioned in-front of the 4 feed channels. The purpose of the 4 feed channels was to help pull the sample through the filter and into the micro-channels. The feed channels have a cross-section of 0.25x0.2mm and they channels merge into a single channel which transports the sample to the reaction chambers.

4.2.3 Detection Zone

The detection zone consists of two circular chambers, each with a diameter and depth of 0.4 and 0.2mm respectively and are placed in series. The first and second circular chambers were located 23 and 25mm respectively from the inlet. The detection zone was positioned at this location as this was the region that the commercial detection system scanned the chip for myoglobin and troponin I detection. The first chamber area was where the capture HSV antibodies were immobilised. The second chamber area can be used as a control area for the HSV assay.

4.2.4 Waste Collection

A waste area needed to be incorporated into the chip to collect the sample after the reaction chambers with a minimum volume of 20µl as this was estimated to be the maximum volume of fluid that would flow through the chip at each stage of the assay. This section had an area of 14.5x13mm and a depth of 0.2mm which gave a volume of 37.7µl. This volume was almost twice the estimated maximum volume required to allow flexibility in the assay volumes during the optimisation of the protocol for the assay. An adsorbent pad was placed in the waste zone for two purposes. Firstly to adsorb the sample fluid and secondly to help pull all the sample through the device.
4.2.5 Epoxy Prototype

Before out-sourcing the design to be mass produced, a prototype of the design was first fabricated in-house to verify that the geometry of the microchannels promoted capillary flow. An inverse mould of the chip design was manufactured from an aluminium block using a CNC machine. A prototype epoxy chip was created by curing an epoxy resin (Low Viscosity "Spurr" Kit, Tedpella, see Appendix C.1) in this mould. The aluminium mould and the epoxy prototype chip are shown in Fig. 4.5. The material properties of the epoxy resin were different to the Zeonor chips and the purpose the epoxy chips was to ensure that fluids would flow through the microchannels via capillary action before out-sourcing a final design to be manufactured using injection moulding.

![Figure 4.5](image)

Figure 4.5: (a) Inverse aluminium mould and (b) Epoxy chip with blue dye flowing through the chip via capillary action.

4.3 Summary

The design of the HSV capillary chip was based on the miniaturisation of the ELISA technique to a capillary driven microfluidic platform. The HSV capillary chip was fabricated from Zeonor because of its ease of manufacture using injection moulding, high optical transparency, low auto-fluorescence and suitable surface chemistry for antibody deposition after the surface has been chemically modified. The capillary chip fabrication and surface modification for capture antibody deposition were discussed. FITC was chosen as the
fluorophore for the HSV detection antibodies because the microscope used for fluorescent
detection had the appropriate excitation filters for FITC. Finally, the design features of the
capillary chip were outlined.
Chapter 5

Experimental Methods Part I: Fluidic Measuring Techniques

This chapter outlines the experimental measurement techniques used to assess the factors that influence capillary flow in open microchannels and the protocols used to perform HSV detection of the capillary chip immunoassays.

In the first section, the measurement techniques used to determine the physical properties of the fluids used for the capillary flow measurements are discussed. These properties are; the surface tension, static contact angle, density and viscosity of the fluid. This is followed by a presentation of the techniques used for capillary flow image visualisation and capture.

In the second section, the measurement techniques used for the HSV detection on the capillary chip immunoassays are described. The first procedure outlined is the FITC dye characterisation followed by the surface functionalisation of the capillary flow chips, verification of HSV capture antibody immobilisation and the HSV assay protocols.

5.1 Capillary Driven Flow In Straight Microchannels

The techniques used to measure the properties of a fluid (surface tension, static contact angle, density and viscosity) and capillary flow image visualisation and capture are detailed in this section.
5.1.1 Fluid Properties

The fluids used in the capillary flow experiments were deionised water and tween-20/deionised water solutions. The surface tension and static contact angle the fluids on a surface of a flat Topas substrate were measured using a KSV CAM 200 meter and CAM software. The densities were calculated using their molecular weights and the viscosity of the fluids were measured using a glass KPG Ubbelode U-bend viscometer.

5.1.1.1 Surface Tension

The surface tension the fluids were measured over a temperature range using KSV CAM 200 meter which is shown in Fig. 5.1. The technique used to measure the surface tension of the fluid was the pendant droplet technique which is independent of the contact angle. This technique is based on the profile of a droplet of liquid suspended in the vapour phase. The profile of the droplet is a result of the balance between gravitational and surface forces[96].

The surface tension of the fluid was calculated using geometrical features of the droplet arc length measured along the profile of the droplet, $s$, the radius of curvature at the apex, $r_{apex}$, droplet height, $z(s)$, and radius at the central plane, $x(s)$ which are illustrated in Fig. 5.2. Assuming that gravity and surface forces were the only forces acting on the droplet and the droplet was axisymmetric the relationships between the geometrical parameters and surface tension were obtained by solving the Young-Laplace non-linear ordinary
differential equations in three dimensions[156]:

\[
\frac{dx}{ds} = \cos \beta \tag{5.1}
\]

\[
\frac{dz}{ds} = \sin \beta \tag{5.2}
\]

\[
\frac{d\beta}{ds} = \frac{2}{r_{apex}} - \frac{\Delta \rho g z}{\gamma} - \frac{\sin \beta}{x} \tag{5.3}
\]

where, \(\Delta \rho\) was the density difference between the droplet and the vapour, \(\beta\) was the local slope of the droplet profile \((\frac{dz}{dr} = \tan \beta)\), \(\gamma\) was the surface tension of the liquid, \(g\) was the gravitational constant. The software solved equations 5.1-5.3 iteratively to determine the surface tension of the fluid.

![Figure 5.2: A pendant droplet on the tip of a needle.](image)

### 5.1.1.2 Static Contact Angle

To measure a contact angle of a fluid on a substrate, a droplet of a fluid was placed on the surface of a substrate from a syringe and a sequence of images were recorded using the KSV CAM 200 software. The contact angle was measured using the software by applying
the Young-Laplace equation to the profile of the droplet. The static contact angle was defined as the contact angle when the fluid stopped spreading across the surface of the substrate.

### 5.1.1.3 Density

The density of the tween-20/deionized water solutions were calculated using the molecular weight and density of tween-20 and water. A sample calculation is given in Appendix C.2.3.

### 5.1.1.4 Viscosity

The viscous forces are a measure of a liquid’s resistance to flow and are important forces that must be overcome to allow the transport of a liquid in a microchannel. The viscosities of fluids were measured using a glass KPG Ubbelode U-bend viscometer and a X-Stream XS-4 high speed camera which are shown in Fig. 5.3 (a). On one side of the viscometer there was a large expanded section with a marking just above it, labelled A in Fig. 5.3 (b). On the other side of the viscometer, there was a narrow capillary section and above this was a smaller expanded section than the other side. There was a marking above and below this smaller expanded section, labelled B and C in Fig. 5.3 (b). To measure the viscosity of the fluid the viscometer was placed in a vertical position and filled with a volume of the fluid so that it filled the viscometer to mark A. The fluid was then brought above mark B through the use of suction. The fluid was then released and let flow through the viscometer. The time taken for the fluid meniscus to move from mark B to C was measured and recorded using the high speed camera. This time was multiplied by a conversion factor particular for this viscometer and this gave the kinematic viscosity of the fluid. The viscosity of the fluid was calculated using the following formula:

\[ \mu = \nu \rho \]

(5.4)

where, \( \nu \) is the kinematic viscosity of the fluid.
5.1.2 Capillary Flow Image Capture

Images of capillary flow through the microchannels were recorded by a X-Stream XS-4 high speed camera mounted on an Zeiss Axioskop microscope with a 20x Olympus magnification lens. A Harvard Apparatus PHD 2000 syringe pump was used to place a fluid volume at the inlet of the microchannel and is shown in Fig. 5.4. Images of the capillary flow were recorded at five locations along the microchannel, 1, 3, 5, 7 and 9 mm, and this was done in triplicate. Data was extracted from these images which will be discussed in detail in Section 6.1.

The capillary flow testing can be split into two parts; the effect of a fluid’s surface tension and the effect of a surfactant coating on capillary flow in microchannels. To investigate the effect of surface tension of a fluid on capillary flow, three surfactant concentrations were added to deionised water (0.01%, 0.1% and 1% tween-20/deionised water (v/v) solutions) and were applied at the inlet of the microchannels. To examine the effect of surfactant coating on capillary flow, the microchannels were coated with 0.01%, 0.1% and 1% tween-20/deionised water solutions. Deionised water was then placed at the inlet of the channel. The volume applied at the inlet was varied from 10-20 µl to explore if this affected the capillary flow through microchannels with a surfactant coating. The testing procedure was as follows:

1. Microchannel cleaning:

The microchannels were cleaned in a 5% soap/deionised water (v/v) solution in an ultrasonic bath for 30 minutes followed by deionised water in an ultra sonic bath for
30 minutes.

2. Effect of surface tension of a fluid on capillary flow:

20 µl of a certain concentration of tween-20/deionised water solution was applied at the inlet of the microchannel by a syringe pump. An absorbent pad was placed at the outlet of the microchannel to draw all the fluid through the microchannel. The microchannel was then left to dry for 20 minutes at room temperature. When dry, the microchannels had a hydrophilic surfactant coating.

3. Effect of a surfactant coating on capillary flow:

10/20 µl of deionised water was applied at the inlet of the microchannel by a syringe pump. An absorbent pad was applied at the outlet of the microchannel to draw all the fluid through the microchannel.

*Figure 5.4: Capillary flow image capture set-up.*
5.2 HSV Capillary Chip Immunoassay

The methods used to develop the fluorescent detection of HSV in the capillary chip immunoassays are detailed in this section. The first procedure outlined is the FITC dye characterisation followed by the surface functionalisation of the capillary chip immunoassays and the HSV assay protocols.

5.2.1 FITC Dye Characterisation

The purpose of the FITC dye characterisation was to determine the molar concentration of the FITC labelled detection antibodies for HSV-1 and HSV-2. This was achieved by measuring the fluorescent intensities of serial dilutions of FITC. FITC was chosen as the detection fluorophore for the HSV assays because the IX-50 Olympus microscope used for the fluorescent measurement had the appropriate excitation and emission filters for FITC detection.

When FITC is excited at an appropriate wavelength it emits light at a longer wavelength. FITC has a peak excitation and emission wavelengths of 495nm and 525nm respectively and its excitation and emission spectra are shown in Fig. 5.5. The peak on the left and right of the diagram represent the excitation and emission spectra of the FITC respectively.

FITC isomer I (F7250) was acquired from Sigma Aldrich and 5.06mg of FITC was dissolved in 1ml of dimethyl sulfoxide (DMSO) acquired from Sigma Aldrich (D5879) to give a stock solution of 13mM FITC. This stock solution was serial diluted with PBS to
give FITC concentrations down to 0.1pM.

5.2.1.1 IX-50 Microscope Set-Up

The optical equipment used were an Olympus IX-50 microscope with a mercury lamp as a light source, an Olympus IB filter cube (U-MWB2) and an Olympus LCACHN 40xPH objective lens. A TSI power view plus 11 mega pixel camera (model no. 6301262) was attached to the microscope to capture images of the fluorescent signal and is shown in Fig. 5.6.

![Image of the IX-50 microscope set-up.](image)

In fluorescent microscopy, an excitation light is required to excite the fluorophore which causes the fluorophore to release an emission light of a longer wavelength. A filter cube was required to prevent the camera capturing the excitation light which can interfere with, causing background noise, the fluorescent signal trying to be detected. The filter cube comprised of three components; a bandpass filter, dichromatic mirror and a barrier filter and are shown in Fig. 5.7 (a). The band-pass filter transmits light from 460-490nm. The dichromatic mirror reflects light below 505nm and transmits light above 505nm and
the barrier filter transmits light above 515nm. Light from the mercury lamp enters the filter cube through the band-pass filter, the shaded blue region in Fig. 5.7 (a). This filter allows the excitation light of wavelength 460-490nm (blue light) through to the dichromatic mirror. This blue light is reflected by the dichromatic mirror through the objective lens where the light is focused on the microchannel of the device. This blue light excites the FITC present in the microchannel and releases the emission light of peak wavelength 520nm (green light). The green and blue light pass back through the objective to the dichromatic mirror. The mirror allows only the green light, light of wavelength above 505nm, through to the barrier filter. The barrier filter, illustrated by the shaded green region, allows light of wavelength above 515nm through to the camera connected to the microscope. The shaded blue region in Fig. 5.7 (b) represents the excitation light transmitted through band-pass filter. The shaded red region on Fig. 5.7 (b) represents the emission light transmitted through the barrier filter of the filter cube.

5.2.1.2 FITC Dye Characterisation Protocol

For the FITC dye characterisation, 10μl of each concentration of FITC diluted in PBS was placed at the inlet of the chip. Images were then captured using the TSI camera. These images were processed in a custom developed algorithm (which will be discussed in detail in Sections 6.2 and 6.3) to calculate the average fluorescent intensity for each concentration of FITC diluted in PBS.

5.2.2 Assay Protocol

This section provides the various optimised protocols for surface functionalisation, verification of capture antibody immobilisation and the HSV protocols. In the HSV protocols, polyclonal antibodies were used as the capture antibodies because they permit a greater sensitivity, capture more HSV antigens, but are not specific enough to discriminate between HSV-1 and HSV-2. Monoclonal antibodies were used as the detection antibodies to give a greater specificity for the HSV-2 assay but only polyclonal detection antibodies could be commercially sourced for the HSV-1 assay.
Figure 5.7: (a) Diagram of the excitation and emission filters of the Olympus IX-50 microscope and (b) The excitation and emission filters of the Olympus IX-50 microscope in relation to the excitation and emission spectra of FITC[148].
5.2.2.1 Reagents

A list of the reagents and buffers used is given in Tables E.1 and E.2 respectively. All of the reagents used are commercially available.

5.2.2.2 Assay Surface Functionalisation

The capillary chips were fabricated from Zeonor material which has excellent bulk properties (optical clarity, low cost, bio-compatible and easily mass produced) but its surface properties are not ideal for the immobilisation of capture antibodies. The surface functionalisation procedure modified the surface of the chip with chemical functional groups to increase its bio-compatibility with the immobilised capture antibodies, i.e. the capture antibodies would bind easier to the chip surface. The surface functionalisation protocol for these chips followed that of Raj et al.[113] which is illustrated in Fig. 5.8 and given below:

1. Surface cleaning:
   The initial step in the modification was to clean the surface with propanol.

2. Oxygen plasma treatment:
   The next step in the modification of the surface was to allow silane, that has a reactive amine group, to attach onto the surface. This was achieved by treating the surface with oxygen plasma and resulted in a surface that is more hydrophilic by the introduction of oxygen reactive groups, ester, hydroxide and carboxyl groups[113].

3. Silanisation:
   The surface was silanised using 3-Aminopropyl-triethoxysilane (APTES). The presence of water in APTES solution induced polymerisation of APTES, which was then chemisorbed onto the surface[113]. This coated the surface in amine reactive groups. APTES contained $NH_2$-terminated silanes which were used to bind onto a cross-linker.

4. Crosslinker:
A cross-linker was used to bind onto the amine surface groups on the chip while also providing a site for capture antibody attachment. The cross-linker used in these assay protocols was p-Phenylenedisothiocyanate (PDITC). PDITC is a crosslinker that contains two amine reactive isothiocyanate groups on a phenyl ring. It allowed the coupling of the capture antibodies to the surface of the chip in a two step process. The first step, one isothiocyanate group of the PDITC reacted with the amine groups at the surface through the formation of a thiourea linkage. The second step, the free isothiocyanate group reacted with the amine groups of the capture antibodies and formed a covalent bond.

The APTES and PDITC provided a thin amine reactive layer covalently attached on the surface which permitted the coupling of the antibodies to the surface[98, 12, 11, 113, 84]. A study was conducted on this immobilisation protocol and it was found that if either APTES attachment and/or oxygen plasma treatment steps were omitted it resulted in a lower concentration of immobilised capture antibodies to the surface of the chip[113].

5.2.2.3 HSV Capture Antibody Immobilisation

The initial step in the development of the assay protocol was to ensure the HSV capture antibodies were immobilised on the surface of the detection zone after the blocking and
wash steps of the protocol, i.e. the antibodies were not removed from the surface by the wash steps.

The HSV-1 and HSV-2 capture antibodies used in the assay protocols were purified polyclonal rabbit immunoglobulin antibodies that react with antigens common to HSV-1 and HSV-2. They react with all the major glycoproteins present on the envelope of the HSV antigen. The presence of the immobilised capture antibodies was assessed by flowing Quality Control (QC) antibodies labelled with Fluorescein-X (FAM-X) through the chip via capillary action. FAM-X has an excitation and emission of 495 and 520nm respectively. The QC antibodies were purified polyclonal goat anti-rabbit IgG antibodies which bind to rabbit IgG antibody’s heavy and light chains. Any fluorescence in the channel would indicate the presence of capture antibodies.

The general steps involved in confirming the presence of the immobilised capture antibodies after several wash steps are illustrated in Fig. 5.9 and given below. A more detailed and specific capture antibody immobilisation protocols for HSV-1 and HSV-2 are given in Appendix E.3.

1. HSV capture antibody immobilisation:

   The HSV capture antibodies of concentrations ranging from 100-1000µg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the capillary chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at room temperature in humid conditions for one-two hours to allow sufficient time to bind to the surface of the chip.

2. Wash:

   The chip was rinsed sequentially twice with a wash buffer and once with deionised water.

3. Blocking:

   The chip was immersed in a blocking buffer for an hour. This blocking buffer does not interfere with the assay reactions and its purpose was to prevent the non-specific binding of antibodies to the surface of the chip for the remainder of the protocol.
This improves the sensitivity of the assay by decreasing the background noise in the fluorescent signal and increasing the signal to noise ratio[113, 84, 99].

4. Wash:
The chip was washed and rinsed twice with wash buffer and once with deionised water to remove any unbound molecules.

5. QC antibody binding:
5µl of QC antibody of concentration 0.2µg/ml (diluted with PBS from stock solution) was applied to the inlet of the chip and flowed through the capillary chip via capillary action and was incubated at room temperature in humid conditions for 20 minutes. The QC antibodies bind to any antibodies present in the chip.

6. Wash:
The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of deionised water to remove any unbound antibodies.

7. Detection:
The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes after the last wash step.

5.2.2.4 HSV-1 & 2 Assay Protocols

The general HSV assay protocol is given in this section and more detailed and specific protocols for HSV-1 and HSV-2 assays are given in Appendix E.5. These protocols are similar to the HSV capture antibody immobilisation protocols. The general HSV assay protocol is shown in Fig. 5.10 and given below:

1. HSV capture antibody immobilisation:

The HSV capture antibodies of concentrations ranging from 100-1000µg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at
Figure 5.9: The steps in the HSV capture immobilisation protocol.
room temperature in humid conditions for one-two hours to allow sufficient time to
bind to the surface of the chip.

2. Wash:
The chip was rinsed sequentially twice with a wash buffer and once with deionised
water.

3. Blocking:
The chip was immersed in a blocking buffer for an hour.

4. Wash:
The chip was washed and rinsed twice with wash buffer and once with deionised
water to remove any unbound molecules.

5. Antigen-detection antibody binding:

5µl of HSV antigens of concentration 75-200µg/ml (diluted with PBS from stock
solution) were mixed with 5µl of HSV detection antibodies of concentration of 20µg/ml
(diluted with PBS from stock solution) labelled with FITC and left for 10 minutes
to allow the HSV antigens to bind to the HSV detection antibodies to create HSV
antigen-detection antibody complexes.

6. Capture-antigen-detection antibody binding:
The 10µl of HSV antigen-detection antibody mixture was applied to the inlet of the
chip and flowed through the chip by capillary action.

7. Wash:
The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of
deionised water to remove any unbound antibodies.

8. Detection:
The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes
after the last wash step.
In the HSV-1 protocol, goat polyclonal HSV-1 detection antibody labelled with FITC was used. Ideally, a monoclonal detection antibody labelled with FITC should have been used in the protocol to achieve maximum specificity but only a polyclonal HSV-1 detection
antibody labelled with FITC could be commercially sourced.

5.3 Summary

This chapter outlined the experimental plan which was designed in order to achieve the objectives presented for this thesis in Chapter 1. In the first section, the techniques of measuring fluid properties and capillary flow visualisation were described. In the second section, the protocols for fluorescent detection of HSV on the capillary chip immunoassays were outlined which included the characterisation of the FITC labelled HSV detection antibodies, surface functionalisation, verification of HSV capture antibody immobilisation to the surface of the HSV capillary chip immunoassay and the HSV protocols. Chapter 6 details the data processing techniques used for capillary flow measurements and fluorescent detection of HSV.
Chapter 6

Experimental Methods Part II: Data Processing Techniques

In this chapter, three image processing techniques developed during the course of thesis are outlined. The first technique involved creating an algorithm to analyse the flow characteristics of fluid flowing through an open microchannel under capillary action. This algorithm was able to establish the fluid’s meniscus shape, position, velocity and contact angles within the microchannel. The second algorithm determined the optimum exposure time that would provide the greatest sensitivity for HSV fluorescent detection. The third technique involved creating an algorithm that was capable of minimising noise in digitally captured images which permitted accurate qualitative and quantitative fluorescent detection.

6.1 Flow Profiles of A Fluid Flowing Through An Open Microchannel Under Capillary Action

An algorithm was devised which analysed the flow profile of the meniscus of a fluid flowing through a microchannel. This algorithm acted as a non-invasive technique allowing the measurement of the meniscus position, velocity and dynamic contact angle of a fluid flowing in a microchannel via the analysis of a sequence of digitally captured images using
a high speed camera, X-Stream XS-4, which has a resolution of 512x512 pixels. This algorithm provided fast and accurate analysis of fluid flow profiles through microchannels. This algorithm can be divided into four parts; edge detection, meniscus position, fluid velocity and dynamic contact angles.

6.1.1 Edge Detection

For edge detection, a sequence of digital images of the fluid flow through the microchannel was recorded and processed in the algorithm. The algorithm reduced the images to the region of interest (ROI), subtracted a background image to remove any anomalies, applied a median filter to reduce image noise, used a threshold value to determine if fluid was present in the microchannel and then determined the edge of the meniscus in the microchannel.

6.1.1.1 Region of Interest

In the sequence of images, the microchannel was horizontal and the fluid flowed from left to right. These images contained a large amount of data that was unnecessary and a typical image is shown in Fig. 6.1 (a). To reduce the computational memory required to run the algorithm, each image was reduced to the ROI which was the width and length of the microchannel. The ROI is shown in Fig. 6.1 (a) by the red dashed box and Fig. 6.1 (b) was the reduced image only containing the ROI created by the algorithm. This not only reduced the computational memory required to run the algorithm but also increased the speed of the analysis.

6.1.1.2 Background Image Subtraction

A background image, an image of the microchannel with no fluid, was subtracted from each of the sequence of images. This removed any anomalies, such as image noise, which will be discussed in further detail in Section 6.3.1, and dirt on the camera lens. If these anomalies were not accounted for they could interfere with the accuracy of the algorithm.
Figure 6.1: (a) Image captured by high speed camera (b) Image ROI (c) Image after background subtraction (d) Image after median filtering (e) Image after threshold applied and (f) Image after edge detection.
6.1.1.3 Median Filter

A median filter was applied to reduce noise while preserving the edges of the meniscus in the microchannel. The median filter operated by evaluating each pixel in the image and its neighbouring pixels. If the pixel was not representative of the neighbouring pixels it was replaced with the median value of the neighbouring pixels. The median filter was given by:

\[
\bar{i} = \text{Median}(i, a, b, s)
\]  

(6.1)

where, \(i\) was the initial image of \(M\times N\) pixels, \(\bar{i}\) was the filtered image, \(a = 0, \ldots, M-1\), \(b = 0, \ldots, N-1\) and \(s\) was the size of the filter centered at \((a, b)\). The influence of the application of the median filter to the images can be seen in Fig. 6.1 (d) with the reduction of noise in the area of the image where there was no fluid.

6.1.1.4 Threshold

A threshold was applied to the sequence of images so that the algorithm was able to identify the fluid flowing in the microchannel by creating a binary matrix whereby it assigned each pixel that the fluid occupied in the image to a value of 1 and the remaining pixels a value of 0. An image with a threshold applied is shown in Fig. 6.1 (e) with the fluid in the image represented by the white colour.

To determine the threshold value, the algorithm simultaneously displayed 3 figures; the median filtered image, the binary image of the median filtered image at a selected threshold value and its pixel intensity histogram and are displayed in Fig. 6.2 (a-c) respectively. The pixel intensity histogram displayed the number of pixels that have the same light intensity. The images recorded by the X-Stream XS-4 camera were 8-bit images. The pixel light intensity values for 8-bit images were rated from 0-255 (arbitrary units), the darkest and brightest pixel light intensity values were 0 and 255 respectively. A threshold value was selected using the pixel intensity histogram and this threshold value was applied to the median filtered image to create its binary image. The median filtered image and its binary image at the selected threshold value were displayed simultaneously to check the accuracy of the threshold value. An appropriate threshold value was found when the binary image
accurately mirrored the actual meniscus shape in the median filtered image. This threshold value was then applied to all the images in the sequence.

Figure 6.2: (a) Median filtered image (b) Binary image using the selected threshold value and (c) the histogram of the median filtered image.

### 6.1.1.5 Edge Detection

Edges in the image were detected where there was a sharp gradient in pixel intensity values between neighbouring pixels. In the sequence of images, the sharp gradient was found at the meniscus interface where the meniscus has a pixel value of 1 and the empty microchannel has a value of 0. An inbuilt function in the software was used to locate these gradients. The gradients found for a thresholded image using this function can be seen in Fig. 6.1 (f) indicated by the white colour. This function operated by first using a Gaussian filter to smooth the image. It then located edges by identifying pixel intensity gradients in the image. Finally, it determined strong and weak edges through the use of two threshold
limits. The higher and lower threshold limits corresponded to stronger and weaker edges respectively. An edge was determined when a pixel intensity value was above the higher threshold limit and stopped when its connecting pixel intensity was smaller than the lower threshold limit. Edges in the image may consist of connecting strong and weak edges and the use of two threshold limits helped preserve edges of the image. Unprocessed images and the meniscus edges detected using this algorithm are shown in Fig. 6.3 for the 101, 191, and 393\(\mu\)m width microchannels. The uncertainty associated with the edge detection technique was \(\pm 1\) pixel.

### 6.1.2 Meniscus Position and Velocity

The co-ordinates for the meniscus position were determined by obtaining the last pixel value that is equal to one in each row of the binary matrix. Shown in Fig. 6.4 (a-c) is a captured digital image, its binary matrix and the two overlapping each other respectively. The last pixel equal to 1 in each row is highlighted with the colour green. The column and row number gave the \(x\) and \(y\) co-ordinates of the meniscus position respectively. These co-ordinates were multiplied by a pixel scaling factor to give the meniscus position in a metric unit. The time for each meniscus position was determined by the recording frame rate. The meniscus velocity was determined by the difference in the meniscus position in a sequence of images.

### 6.1.3 Meniscus Dynamic Contact Angle

The dynamic contact angle was measured from the point of contact between the meniscus and the left and right side-walls of the microchannel. The dynamic contact angle was calculated by following an approach used by Heiskanen et al[58] but was modified in this algorithm to include a median filter step to reduce image noise in processing the digital images and also included measuring the angle of the microchannel to the horizontal to provide a more accurate meniscus dynamic contact angle.

In the ideal case, the meniscus shape of the fluid would be uniform across the width of the microchannel but in reality this does not occur because of the possibility of defects in
Figure 6.3: (a-c) Digital images of the meniscus shape in the 101, 191, and 393 μm width microchannels respectively. and (d-f) Images of the algorithm’s edge detection for the 101, 191, and 393 μm width microchannels respectively.
where, $x$ and $y$ are the meniscus co-ordinate points, $b_l$, $c_l$, $d_l$ are the constants of the left parabola and $b_r$, $c_r$, $d_r$ are the constants of the right parabola.

The slope of the tangent to the parabola at the left and right contact points, $m_l$ and $m_r$ respectively, were determined using the following formulae:

$$m_{pl} = 2b_l y_l + c_l$$  \hspace{1cm} (6.4)

$$m_{pr} = 2b_r y_r + c_r$$  \hspace{1cm} (6.5)
Figure 6.5: (a & b) The ideal and real mensicus shapes in microchannels respectively.

Figure 6.6: The points of contact, slope of the tangent and dynamic contact angles at the top and bottom walls.

The dynamic contact angles for the left and right contact points, $\theta_l$ and $\theta_r$ respectively, were calculated using the slope of half parabola curve fittings at the contact points using the following formulae:

$$\theta_l = \tan^{-1}(m_l) \quad (6.6)$$
$$\theta_r = \tan^{-1}(m_r) \quad (6.7)$$

The fluid in the image flowed horizontally from left to right. If the microchannel was not perfectly horizontal in the image, shown in Fig. 6.7, the calculation of the dynamic contact angle would be inaccurate. To correct for this, the angle the microchannel was to the horizontal, $\theta_c$, needed to be measured and added to the dynamic angle of the left and right contact points in equations 6.6 and 6.7 to give the following equations 6.8 and 6.9 for the true value of the dynamic contact angles.
\[ \theta_l = \tan^{-1}(m_l) \pm \theta_c \] (6.8)

\[ \theta_r = \tan^{-1}(m_r) \pm \theta_c \] (6.9)

Figure 6.7: A microchannel not perfectly horizontal.

6.1.3.1 Dynamic Contact Angle Calibration Curve

In order to validate the algorithm’s accuracy in determining the values of the contact angles, the algorithm was used on a sequence of 17 images of meniscus profiles of known contact angles which varied from 170-10°. These images were created and the contact angles measured using Adobe Illustrator CS3.

The digital images recorded for fluid flowing in the 101, 191, and 393 \( \mu \)m width microchannels had pixel widths of 34, 69 and 141 pixels respectively. For an accurate validation of the algorithm’s contact angle values, the sequence of images created in Adobe Illustrator CS3 were rescaled so that the pixel widths were identical to those of the digital images. Examples of the recorded digital images compared to the images created in Adobe Illustrator CS3 for each microchannel are shown in Fig. 6.8.

These rescaled images were processed in the algorithm and the resulting contact angle values were compared against those that were measured and are shown in Fig. 6.9. For contact angle values ranging from 50-120°, the algorithm’s values were in very good agreement with the measured values. To increase the accuracy of the values of the contact angles given by the algorithm for contact angle values less than 50° and greater than 120°
Figure 6.8: (a-c) Digital images captured for the 101, 191, and 393\(\mu\)m width microchannels respectively and (d-f) Images created in Adobe Illustrator CS3 for the 101, 191, and 393\(\mu\)m width microchannels respectively.

A calibration curve was required. A calibration curve was generated by plotting the algorithm’s contact angle values against the measured values. A fifth order polynomial was curve fitted to the data points and this was called the calibration curve. The calibration curves for the left and right wall contact angles are shown in Fig. 6.10 and the constants for the fifth order polynomial followed by their \(R^2\) value are given in Tables C.10 and C.11 for the left and right walls respectively.

The uncertainty associated with the edge detection technique was \(\pm 1\) pixel and to account for this uncertainty in the measurement of dynamic contact angle two extreme cases of 1 pixel infront and behind the algorithm’s edge detection were also measured. These two extreme cases were used to calculate the uncertainty in the algorithm’s dynamic
Figure 6.9: (a-c) Contact angles for the left and right walls calculated using the dynamic contact angle algorithm compared to the actual dynamic contact angles measured for 101, 191, and 393μm microchannels respectively.
Figure 6.10: (a-c) Contact angle calibration curves for the left wall for the 101, 191, and 393 µm microchannels respectively and (d-f) Contact angle calibration curves for the right wall for the 101, 191, and 393 µm microchannels respectively.
contact angle values.

6.2 Fluorescent Image Capture Pre-Processing

The pre-processing of fluorescent images can have a significant impact on the sensitivity of the detection of fluorescent signals and involves determining an appropriate image exposure time to allow the greatest sensitivity for detectable fluorescent signals and reduction in image noise.

6.2.1 Detection Sensitivity

In fluorescent imaging analysis, the pixel intensity histogram of an image is an important tool in determining the optimum exposure time (greatest sensitivity) for fluorescent image capture. It displays the number of pixels in the image that have the same pixel intensity. An algorithm was developed to analyse the histograms of the 12 bit images recorded by a TSI Power View Plus (11-mega pixel) camera (model no. 6301262) at the detection zone of the chip using 40x magnification. The algorithm transformed the captured image into a 2672x3938 pixel matrix. Each pixel had an individual light intensity value associated with it. The brightness of each individual pixel in the captured 12 bit images were rated on a pixel intensity scale of 0-4095 (arbitrary units). The darkest and brightest pixels in the image were 0 and 4095 respectively on the scale. Shown in Fig. 6.11 are the images of 10µl of 5x10^{-4} mM FITC diluted in PBS flowing through the detection zone of the chip for three different camera exposures of 0.5, 1 and 1.5 seconds, (a-c) respectively. On the right of these images are the resulting histograms of each image. Taking image Fig. 6.11 (a), its histogram has two distinct regions indicated by the red and green circles. The red area represents the number of pixels that are the darkest in the image. The green area represents the number of pixels that are the brightest in the image. To provide the greatest fluorescent detection sensitivity, the brightest pixels should be toward the upper end of the pixel intensity scale. This allows the pixel intensities to span the entire scale giving a greater sensitivity in the detection of fluorescent signals. It is evident from the histogram in Fig. 6.11 (a) that the green area is not near the upper end of the scale. To shift the
brighter pixels higher up on the scale a longer camera exposure time is required. Shown in Fig. 6.11 (c) is the image and its histogram for 10µl of 5x10^{-4}mM FITC diluted in PBS flowing through the detection zone with a camera exposure of 1.5 seconds. By analysing its histogram, the image can be concluded to be over saturated because the pixel intensities that were attempting to be recorded exceeded the maximum intensity, 4095, that could be captured by the camera. This is indicated by the single thin line on the histogram at the 4095 pixel intensity. Pixel intensities above this value cannot be assessed and therefore no quantitative data can be obtained. The difference between Fig. 6.11 (a) and (c) is that the images are under and over exposed respectively for optimum fluorescent image analysis. The ideal camera exposure time for fluorescent detection is within the camera exposure time for Fig. 6.11 (a) and (c). Shown in Fig. 6.11 (b) is the image and resulting histogram for 10µl of 5x10^{-4}mM FITC diluted in PBS flowing through the detection zone with a camera exposure time of 1 second. It can be seen from the image histogram that the brightest pixels are in the upper end of the pixel intensity scale but not too high that the pixels become over saturated. Therefore, this camera exposure time would be optimum time for fluorescent image analysis for this particular concentration of FITC dye diluted in PBS.

6.3 Fluorescent Image Post-Processing

Fluorescent image post-processing is an important step in enhancing fluorescent intensity data from captured digital images. The Post-Processing algorithm that was developed reduced image noise and calculated the mean pixel intensity of the image. The algorithm is discussed in the following section with the aid of the images captured of the 10µl of 5x10^{-4}mM FITC diluted in PBS flowing through the channel of the chip.

6.3.1 Image Noise Reduction and Mean Pixel Intensity Calculation

In digital images, noise is inevitably present in the recorded image due to the image capturing process. Noise results in pixel intensity values that do not represent the true value that the camera is attempting to capture. For accurate quantitative imaging analysis the
noise level should be eliminated or kept to a minimum. There are three main categories of 
image noise. The first is thermal noise, often referred to as dark current. It is caused by 
the generation of electrons in the imaging sensor due to thermal vibrations. The magnitude 
of thermal noise is effected by the temperature of the sensor. The cooler the sensor the 
less thermal noise present in the captured image[49]. The second is read noise which is 
generated by the electronic circuitry of the image capture process[105]. The third is shot 
noise which is caused by the random and unpredictable arrival of photons on the imaging
sensor of the camera[73].

The signal to noise ratio (SNR) was used to assess the performance of the image capturing process. It was the comparison of the fluorescent signal to the background noise of the image, i.e. the sensitivity of fluorescence detection. It was calculated by comparing the power ratio between the mean fluorescent signal and the background noise in the image[57]:

\[
SNR = \frac{\text{mean signal}^2}{\text{mean noise}^2}
\]  

\hspace{1cm} (6.10)

### 6.3.1.1 Image Noise Reduction and Mean Pixel Intensity Calculation Algorithm

The algorithm developed reduced the noise in the captured images which entailed background noise subtraction from the image, image thresholding and the application of a median filter to reduce shot noise in the image and calculated the mean pixel intensity of the image.

**Background noise subtraction** A captured image of 10µl of FITC of 5x10^{-4}mM concentration diluted in PBS flowing through the detection zone is referred to as the fluorescent image, shown in Fig. 6.12 (a). An image was captured with no fluid flowing through the channel. This image is referred to as the background image, shown in Fig. 6.12 (b). This image contains purely the noise of the image capturing process and was quantifiable by determining the mean pixel intensity of the image. The noise in the fluorescent and background images are clearly visible as random spikes in Fig. 6.12 (c-d) respectively.

To reduce the noise in the captured fluorescent image, the mean pixel intensity of the background image was subtracted from each individual pixel in the fluorescent image. Shown in Fig. 6.13 (a) and (d) are the fluorescent image with the mean background pixel intensity subtracted and the pixel intensities removed from the fluorescent image respectively.

The SNR was calculated before and after each step of the algorithm and the values are listed in Table 6.1. This step created a significant reduction in noise and resulted in the SNR of the fluorescent signal increased by a factor of 17.
Threshold A threshold was applied to further reduce the noise in the fluorescent image. The threshold was set to 3 times the maximum standard deviation of the mean intensity values of the background image. Any pixel below this threshold was removed from the fluorescent image. This removed most of the noise but was ineffective at reducing the effect of shot noise due to the extreme values of pixel intensities it created. These intensities were well in-excess of the threshold limit and are highlighted by the red dashed region in Fig. 6.13 (b). The intensities of the pixels below the threshold and were removed from the fluorescent image are shown in Fig. 6.13 (e). The combination of the image subtraction and threshold steps resulted in the SNR of the fluorescent signal being increased to $1.0136 \times 10^6$ times the SNR of the original image.

<p>| Table 6.1: SNR for the 3 steps of noise reduction. |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Image</th>
<th>Captured</th>
<th>Subtraction</th>
<th>Threshold</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNR</td>
<td>238.8</td>
<td>$4.0642 \times 10^3$</td>
<td>$2.4208 \times 10^3$</td>
<td>infinity</td>
</tr>
<tr>
<td>SNR Increase</td>
<td>1</td>
<td>17</td>
<td>$1.0136 \times 10^6$</td>
<td>infinity</td>
</tr>
</tbody>
</table>

Figure 6.12: (a & c) 2 & 3-D pixel intensities of the fluorescent image respectively. (b & d) 2 & 3-D pixel intensities of the background image respectively.
Figure 6.13: (a-c) Images of the fluorescent signal after background noise subtraction, thresholding and median filtering steps respectively and (d-f) Images of the pixel intensities removed by the background noise subtraction, thresholding and median filtering steps respectively.

**Median filter** To reduce the influence of shot noise in the fluorescent image a median filter was used which was a low pass filter. It operated by filtering individual pixel intensities by obtaining the median of its neighbouring pixels. The use of a median filter decreased the effect that shot noise has on a single pixel. The reduction of the shot noise is evident in Fig. 6.13 (c) with the removal of the pixel intensity spikes. The pixel intensities removed from the fluorescent image as a result of the filter are shown in Fig. 6.13 (f). After the
completion of this step, the SNR could not be calculated as the mean noise in equation 6.10 effectively went to infinity.

**Mean Pixel Intensity Calculation**  After the image noise reduction steps of the algorithm, the mean pixel intensity was then calculated by obtaining the mean of all the pixel light intensities in the image.

### 6.4 Summary

This chapter described the algorithms that were developed for the analysis of capillary flow images and fluorescence detection. The first section described an algorithm developed to analyse capillary flow images and determine the fluid’s position, velocity and dynamic contact angles. The second section outlined an algorithm that was used to determine the optimum exposure time that would provide the greatest sensitivity for HSV fluorescent detection. The third section described an algorithm that removed background noise and quantified the mean pixel intensity of the detected fluorescent signal. Chapter 7 and 8 will discuss the results obtained from these algorithms for capillary flow in open microchannels and the HSV capillary chip immunoassays respectively.
Chapter 7

Results & Discussion Part I: Capillary Driven Flow In Open Microchannels

To the author’s knowledge, there has been no experimental investigations into capillary flow of surfactant solutions (tween-20/deionised water) in open hydrophobic microchannels. The author also has not found any studies performed on the influence of surfactant coating of open hydrophobic microchannels on capillary flow of deionised water. The experimental results discussed in this chapter will help shed some light on these topics in the form of flow profiles and measurements of both dynamic contact angles and meniscus shape.

This chapter first describes the fluidic properties of deionised water and the surfactant solutions used in the capillary flow experiments. This is followed by presenting results for capillary flow of surfactant solutions through open hydrophobic microchannels with respect to the influence of microchannel geometry and surfactant concentration on the capillary flow. The experimental results are then compared to theoretical predictions for the dynamic contact angles and flow profiles. This section is then followed by an investigation into the capillary flow of deionised water through open hydrophobic microchannels coated with surfactants with regard to the meniscus shape, microchannel geometry, surfactant concentration and drop volume.
7.1 Fluid Properties

To assess the factors influencing capillary flow in open hydrophobic microchannels, the first step was to characterise the fluid properties used in the capillary flow experiments. The working fluids used in the capillary flow experiments were deionised water and 0.01%, 0.1% and 1% tween-20/deionised water solutions.

7.1.1 Static Contact Angle

The static contact angle of deionised water and the tween-20/deionised water solutions on the surface of a Topas substrate were measured using a KSV CAM 200 meter and the values obtained are given in Fig. 7.1 (a) for 19 and 24°C. Static contact angles were measured at these temperatures as these were the lower and upper temperatures of the fluid when measuring the capillary flow in the microchannels. As the concentration of the surfactant increased, the static contact angle decreased. Also, there was a slight decrease in static contact angle for each tween-20/deionised water solution with an increase in temperature. The contact angles decreased with increasing concentration because the addition of surfactant decreased the surface tension of the solution (see Section 7.1.2) and therefore according to Young’s equation (equation 2.1) the contact angle would also decrease.

The static contact angle of deionised water on the surface of a Topas substrate coated with 0.01-1% tween-20/deionised water solutions are given in Fig. 7.1 (b). As the concentration of the surfactant coating increased, the static contact angle of the deionised water decreased. The deionised water was able to wet more of the surface and when the substrate was coated with 1% tween-20/deionised water solution it could be said that the deionised water completely wetted the surface of the substrate.

The temperature used for the theoretical calculations was the average of the temperature measured for each of the experimental capillary flow measurements. To find the exact static contact angle at this temperature for the theoretical calculations a linear relationship was assumed between the contact angles measured at 19 and 24°C for each solution and a linear equation was found for each solution so that the contact angle could be calculated using the experimental capillary flow temperature. The constants of the linear equations
are given in Appendix C.2.1.

![Graph showing contact angle vs. surfactant concentration](image)

**Figure 7.1:** (a) Static contact angle of deionised water and 0.01-1% tween-20/water solutions on the surface of Topas and (b) Static contact angle of deionised water on the surface of Topas coated with 0.01-1% tween-20/water solutions.

### 7.1.2 Surface Tension

The surface tension for water and solutions of tween-20/deionised water using a KSV CAM 200 meter and the values obtained are given in Fig. 7.2 for a 19-28°C temperature range. There was a slight decrease in the surface tension of each fluid as the temperature increased because the Gibbs free energy of the fluid decreases with temperature.

The surface tension value used in the theoretical calculations corresponded to the average temperature measured for each of the experimental capillary flow measurements. This was found by applying a second order polynomial to the surface tension measurements and are shown by the lines in Fig. 7.2. The constants for these equations are given in Table C.6.

### 7.1.3 Viscosity

The viscosity of deionised water and the tween-20/deionised water solutions were measured using a glass KPG Ubbelode U-bend viscometer. The viscosity of a fluid was measured by recording the time taken for the fluid to flow between two marks using a high
speed camera and this time was multiplied by a conversion factor. There was little difference between the viscosities of the fluids. Using this method, it was not possible to measure the viscosity of the fluid over a temperature range. The calculated viscosities and uncertainties for these solutions are listed in Table 7.1. The uncertainty was calculated by first accounting for when the fluid flowed past the mark which would have been between 2 recorded image frames and therefore the viscosity of a fluid for the recording time between 2 frames was calculated. This was multiplied by two since there was two marks (B and C) and then this was added to twice the standard deviation (95% confidence) of the experimental measurements to give the overall uncertainty.

Table 7.1: The viscosities of deionised water and the tween-20/water solutions.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Viscosity (mPas)</th>
<th>Uncertainty (mPas)</th>
<th>Uncertainty (%)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.0</td>
<td>6.8333x10^{-7}</td>
<td>0.0654</td>
<td>21.67</td>
</tr>
<tr>
<td>0.01% Tween/water</td>
<td>1.1</td>
<td>7.0201x10^{-7}</td>
<td>0.0668</td>
<td>21.67</td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>1.1</td>
<td>7.3653x10^{-7}</td>
<td>0.0679</td>
<td>20</td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>1.1</td>
<td>6.9980x10^{-7}</td>
<td>0.0635</td>
<td>21</td>
</tr>
</tbody>
</table>

7.1.4 Density

The density of the tween-20/deionised water solutions were calculated using the molecular weights and densities of tween-20 and water. There was only a minor increase in the
density of the tween-20/deionised water solutions as the concentration of the tween-20 increased and the calculated densities for these solutions are listed in Table 7.2. A sample calculation is given in Appendix C.2.3.

Table 7.2: The densities of the tween-20/water solutions.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Density (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>998</td>
</tr>
<tr>
<td>0.01% Tween/water</td>
<td>998</td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>999</td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>1009</td>
</tr>
</tbody>
</table>

7.2 Capillary Flow In Open Microchannels

The capillary flow of 0.01%, 0.1% and 1% concentrations of tween-20/deionised water solutions were analysed for three microchannels of widths 101, 191 and 393 µm and a depth of 185, 195 and 197 µm respectively to determine the influence of microchannel geometry and surface tension of these solutions on capillary flow. Images of the capillary flow of these solutions were recorded at five locations along the microchannel, 1, 3, 5, 7 and 9 mm, and were grouped together to provide the fluid’s meniscus velocity and dynamic contact angle data along the length of the microchannels.

The data recorded for the tween-20/deionised water solutions were split into two distinct sections, the first compares the effect of the microchannel geometries on the flow profile of each of the tween-20/deionised water solutions, shown in Fig. 7.3, and the second compares the effect of surfactant concentration of the solution on the flow profile of the solution through each of the microchannels, shown in Fig. 7.5. The average value of the dynamic contact angle between the left and right side-walls was used in the theoretical calculations.

7.2.1 Effect of Microchannel Geometry On Capillary Flow

Examining the effect of the microchannel geometry on the flow profile of 20 µl of 0.01% tween-20/deionised water solution shown in Fig. 7.3 (a), it can be seen that as the microchannel dimensions increased, the meniscus velocity decreased. The flow profiles of
the 20µl 0.1% and 1% tween-20/deionised water solutions follow a similar trend as shown in Fig. 7.3 (c & e) respectively. For open microchannel capillary flow of surfactant solutions it can be concluded that as the width of the microchannels increased, the velocity of the solution decreased. This contradicts existing capillary flow theory whereby the velocity should decrease with decreasing microchannel dimensions due to higher frictional forces in the smaller microchannels and will be discussed in greater detail in Section 7.2.5.

To the author’s knowledge, there has been no direct measurement of the dynamic contact angle for any liquid flowing through an open microchannel under capillary action. Previous studies have either ignored the dynamic contact angle and used the static contact angle measured on a flat substrate[68, 8, 123, 122] or have derived a value for the dynamic contact angle from the meniscus position as a function of time and static contact angle measured on a flat substrate[162, 91, 95, 152, 37, 81, 2]. The dynamic contact angle is widely assumed to have an initial value of 90° and then relaxes towards its static contact angle[37, 91, 95, 152]. The dynamic contact angles for the 0.01% tween-20/deionised water solution are shown in Fig. 7.3 (b), there was little difference in the dynamic contact angles for the 101µm and 191µm microchannels but this could be due to the high uncertainty of the measured dynamic contact angle for the 101µm microchannel. For both microchannels, the dynamic contact angles were initially 90° and fall to approximately 85°. The dynamic contact angles for the 393µm microchannel were quite different, it had an initial contact angle of approximately 75° and reduced to 62°. The initial contact angle was 75° rather than 90° because the solution did not enter the 393µm microchannel perfectly in the centre from the inlet reservoir. Instead, the solution would make contact with one side-wall of the microchannel first, as shown in Fig. 7.4 (a), the solution made contact with the left wall first and the solution made a contact angle of approximately 75°. The meniscus of the solution would then make contact with the other side-wall and as indicated in Fig. 7.4 (b) when the meniscus made contact with the right wall it had an initial contact angle of roughly 90° and the contact angle quickly reduced to the same contact angle as the left wall as shown in Fig. 7.4 (c). The dynamic contact angles for the 0.1% and 1% tween-20/deionised water solutions follow a similar trend to each other and are shown in Fig. 7.3 (d & f). Both the 101µm and 191µm microchannels had initial contact angles of
90° and reduced to approximately 85° and 75° for the 101µm and 191µm microchannels respectively. For the 393µm microchannel, the initial contact angle was 67-75° and reduced to approximately 60° for the 0.1% and 1% tween-20/deionised water solutions.

Figure 7.3: (a-b) Recorded data for the meniscus position and averaged contact angle respectively for 20µl 0.01% tween-20/deionised water solution. (c-d) Recorded data for the meniscus position and averaged contact angle respectively for 20µl 0.1% tween-20/deionised water solution. (e-f) Recorded data for the meniscus position and averaged contact angle respectively for 20µl 1% tween-20/deionised water solution.

### 7.2.2 Effect of Surface Tension On Capillary Flow

To investigate the effect of surfactant concentration on the flow profiles of the 0.01-1% tween-20/deionised water solutions through the 101µm microchannel, the meniscus position of each of the tween-20/deionised water solutions were plotted against velocity in Fig. 7.5 (a). As the concentration of the surfactant was increased, the velocity increased. This was expected because as the concentration of surfactant increased, the surface tension and contact angle of the solution decreased which implied that the higher the concentration of surfactant, the faster the solution would flow through the microchannel. The flow profiles of the 0.01-1% tween-20/deionised water solutions through the 191µm and 393µm microchannels are shown in Fig. 7.5 (c & e) and they also followed the same trend as the
101µm microchannel, as the surfactant concentration increased, the velocity through the microchannels increased.

The measured dynamic contact angles for the 0.01-1% tween-20/deionised water solutions through the 101µm microchannel are shown in Fig. 7.5 (b) but due to the high uncertainty in the contact angle measurements, no real quantitative information was obtained in regards to the influence of surfactant concentration on the dynamic contact angle for this microchannel size. The dynamic contact angles for the 0.01-1% tween-20/deionised water solutions through the 191µm microchannel are shown in Fig. 7.5 (d), each of the tween-20/deionised water solutions initially had a contact angle of 90° and reduced to approximately 85° for the 101µm microchannel and 75° for the 191 and 393µm microchannels. The dynamic contact angles for the 0.01-1% tween-20/deionised water solutions through the 393µm microchannel are shown in Fig. 7.5 (f), each of the tween-20/deionised water solutions initially had a contact angle of 68-75° and reduced to approximately 57-64°. For the 0.1% and 1% concentrations of surfactant, the data indicates that the dynamic contact angle reached a plateau of a relatively constant value within the first 4mm of the microchannels. After this point, there were small oscillations in the dynamic contact angle which could be attributed to either surface contamination or surface roughness or a combination of both.

7.2.3 Theoretical Dynamic Contact Angles

The recorded data for the tween-20/deionised solutions were compared to curve fits for the theoretical and empirical equations for the dynamic contact angle from Section 2.6 and
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Figure 7.5: (a-b) Recorded data for the meniscus position and averaged contact angle respectively along the 101 μm microchannel for 20 μl 0.01-1% tween-20/deionised water solutions. (c-d) Recorded data for the meniscus position and averaged contact angle respectively along the 191 μm microchannel for 20 μl 0.01-1% tween-20/deionised water solutions. (e-f) Recorded data for the meniscus position and averaged contact angle respectively along the 393 μm microchannel for 20 μl 0.01-1% tween-20/deionised water solutions.

are shown in Fig. 7.6, where, Morales stands for the empirical equation (equation 2.19) used by Morales et al[95], HD stands for the Hydrodynamic Theory (equation 2.17) and MKT represents Molecular Kinetic Theory (equation 2.18). The fluidic properties of the tween-20/deionised water solutions and their static contact angles used for the theoretical calculations were obtained from the measurements made in Section 7.1 and are given in Table 7.3.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>θ₀ (°)</th>
<th>γ (mN/m)</th>
<th>µ (mPas)</th>
<th>ρ (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01% Tween/water</td>
<td>51.24</td>
<td>49.2</td>
<td>1.1</td>
<td>998</td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>43.18</td>
<td>40.8</td>
<td>1.1</td>
<td>999</td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>35.6</td>
<td>38.7</td>
<td>1.1</td>
<td>1009</td>
</tr>
</tbody>
</table>

The empirical equation for the dynamic contact angle depended on a single variable, $M$ the characteristic length in which the dynamic contact angle relaxes to the static contact angle, and was found through curve fitting. The empirical dynamic contact angles initially
began with a value of 90° and then reduced to the static contact angle. For the 101µm microchannel, the empirical equation agrees with the recorded data shown in Fig. 7.6 (a, d, g) for the 0.01%, 0.1% and 1% tween-20/deionised water solutions respectively but there is a high uncertainty associated with the contact angle measurements for this microchannel. For the 191µm microchannel, the empirical equation’s relaxation to the static contact angle occurred at a slower rate to the recorded data illustrated in Fig. 7.6 (b, e, h) for the 0.01%, 0.1% and 1% tween-20/deionised water solutions respectively. For the 393µm microchannel, the empirical equation gave an initial value of 90° and quickly reduced to the static angle measured on a flat substrate rather than trying to mirror the recorded data. This could be due to the fact that the empirical equation had to start with an initial value of 90° and was incapable of having an initial value of 68-75° as shown in Fig. 7.6 (c, f, i) for the 0.01%, 0.1% and 1% tween-20/deionised water solutions respectively.

The theoretical equation for the hydrodynamic theory depended on values for a variable, \( \ln\left(\frac{L}{L_s}\right) \), where \( L \) is the characteristic length of the capillary flow in the microchannel and \( L_s \) is the slip length of the microscopic contact angle, and was found by curve fitting. The hydrodynamic theory for the 0.01% tween-20/deionised water solutions in Fig. 7.6 (a, b, c) gave a much higher initial dynamic contact angle and relaxed to well below the recorded dynamic contact angle data. For the remainder of the microchannels and tween-20/deionised water solutions, the theory matched the data quite well except for the 0.1% and 1% tween-20/deionised water solutions in the 200µm microchannel where the initial contact angle was higher than the recorded data.

The theoretical equation for the molecular kinetic theory depended on determining the values of two variables, \( \varepsilon \) the average distance between two adsorption sites and \( K \) the equilibrium frequency of displacement of a liquid molecule between two adjacent adsorption sites, and these were found by curve fitting. This equation agreed reasonably well with the recorded data for the dynamic contact angles for all the microchannels and tween-20/deionised water solutions. Although, for the 393µm microchannel, this equation however had a lower initial dynamic contact angle than the recorded data.

Previous studies[162, 97, 125] carried out on the dynamic contact angles of fluids on flat substrates have demonstrated that the value of the dynamic contact angle was a
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Figure 7.6: (a-c) The experimental and theoretical dynamic contact angle values of 20µl of 0.01% tween-20/deionsied water solution for the 101, 191 and 393µm microchannels respectively. (d-f) The experimental and theoretical dynamic contact angle values of 20µl of 0.1% tween-20/deionsied water solution for the 101, 191 and 393µm microchannels respectively. (g-i) The experimental and theoretical dynamic contact angle values of 20µl of 1% tween-20/deionsied water solution for the 101, 191 and 393µm microchannels respectively.

function of the fluid’s spreading velocity and that the greater the fluid’s velocity, the greater the value of the dynamic contact angle. Other studies[42, 68, 112, 102, 162, 86] have demonstrated indirectly that the dynamic contact angle of capillary flow in microchannels was much larger than the static contact angle and varied with microchannel size. This was seen with the measured dynamic contact angle data in this study. The recorded dynamic contact angles reached a relatively constant value which was far above the value measured
for the static contact angle on a flat substrate. Also, these constant contact angles were different for each of the microchannels even though the same concentration of surfactant solution flowed through the microchannels. This occurred because the dynamic contact angle was a function of the solution’s velocity, as the width of the microchannel increased, the velocity decreased and therefore resulted in a lower dynamic contact angle value. This implies that the values for the static contact angle measured on the flat substrate in Section 7.1 should not be used for the theoretical equations for capillary flow of surfactant solutions in microchannels. To investigate this, the values that the dynamic contact angles became constant in the microchannels were used as the new static contact angles, which will be referred to as the capillary flow static contact angle, \( \theta_{cap} \), in the theoretical equations for predicting the dynamic contact angles. These were replotted against the recorded data in Fig. 7.7. The measured static contact angle on a flat substrate and the corresponding capillary flow static contact angle are given in Table 7.4.

<table>
<thead>
<tr>
<th>Microchannel (µm)</th>
<th>( \theta_s (^o) )</th>
<th>( \theta_{cap} (^o) )</th>
<th>( \theta_s (^o) )</th>
<th>( \theta_{cap} (^o) )</th>
<th>( \theta_s (^o) )</th>
<th>( \theta_{cap} (^o) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>51.24</td>
<td>85</td>
<td>43.18</td>
<td>82.5</td>
<td>35.6</td>
<td>82.5</td>
</tr>
<tr>
<td>191</td>
<td>51.24</td>
<td>85</td>
<td>43.18</td>
<td>75</td>
<td>35.6</td>
<td>75</td>
</tr>
<tr>
<td>393</td>
<td>51.24</td>
<td>62.5</td>
<td>43.18</td>
<td>57.5</td>
<td>35.6</td>
<td>57.5</td>
</tr>
</tbody>
</table>

Using the capillary flow static contact angle, there was an improvement in the initial values for the dynamic contact angles for the empirical equation for the dynamic contact angle as shown in Fig. 7.7. There was no significant change in the values of the dynamic contact angles given by the hydrodynamic and molecular kinetic theories. Only the values of the variables of the hydrodynamic and molecular theoretical equations changed which are given in Table C.12. The empirical equation was better suited to describe the measured dynamic contact angles than the hydrodynamic and molecular kinetic theories.

### 7.2.4 Theoretical Capillary Flow

To calculate the theoretical capillary flow in the microchannels, each dynamic contact angle equation using the capillary flow static contact angle were inserted into equation 2.31
Figure 7.7: (a-c) The experimental and theoretical dynamic contact angle values of 20µl of 0.01% tween-20/deionsied water solution using the capillary flow static contact angle for the 101, 191 and 393µm microchannels respectively. (d-f) The experimental and theoretical dynamic contact angle values of 20µl of 0.1% tween-20/deionsied water solution using the capillary flow static contact angle for the 101, 191 and 393µm microchannels respectively. (g-i) The experimental and theoretical dynamic contact angle values of 20µl of 1% tween-20/deionsied water solution using the capillary flow static contact angle for the 101, 191 and 393µm microchannels respectively.

individually and their resulting flow profiles are shown in Fig. 7.8. The experimental data recorded indicate that as the microchannel width increases, the velocity of the surfactant solution decreases and that is in direct contradiction to existing capillary flow theory for pure liquids (liquids with no surfactants). The shape of the theoretical velocity profile given when the Morales equation has been decribed by Morales et al (2005)[95].
Figure 7.8: (a) The experimental data for 20µl of 0.01% tween-20/deionised solution through 101, 191 and 393µm microchannels. (b-d) The experimental data for 20µl of 0.01% tween-20/deionised solution versus theoretical capillary flow profile for the 101, 191 and 393µm microchannels respectively. (e) The experimental data for 20µl of 0.1% tween-20/deionised solution through 101, 191 and 393µm microchannels. (f-h) The experimental data for 20µl of 0.1% tween-20/deionised solution versus theoretical capillary flow profile for the 101, 191 and 393µm microchannels respectively. (i) The experimental data for 20µl of 1% tween-20/deionised solution through 101, 191 and 393µm microchannels. (j-l) The experimental data for 20µl of 1% tween-20/deionised solution versus theoretical capillary flow profile for the 101, 191 and 393µm microchannels respectively.
According to the capillary flow theory, the smaller the dimension of the microchannel the higher the frictional forces within it and should result in a slower velocity than in a larger microchannel. The data recorded for surfactant flow in open microchannels is contradictory to this theory. This may be due to the fact that this capillary flow theory is based on the flow of a pure liquid and not a surfactant/deionised water solution. There is little literature on the spreading of surfactant solutions over hydrophobic surfaces. There were some investigations of surfactant solutions spreading over smooth flat surfaces[131, 130, 23, 8, 133, 134] and a handful of investigations of capillary flow into small cylindrical capillaries[80, 126, 132, 129, 29] and porous media[158] but none into capillary flow into rectangular or open rectangular microchannels.

### 7.2.5 Capillary Flow of Surfactant Solutions

Capillary flow of pure liquids in microchannels is dependent on the material and bulk fluid properties. Capillary flow of surfactant solutions in microchannels is more complex where extra factors such as surfactant concentration, surface tension gradients and the adsorption of surfactant in the region of the TPC line need to be considered. A complete understanding of the mechanisms involved in capillary flow of surfactant solutions is still lacking.

The mechanism behind the spreading of surfactant solutions over hydrophobic surfaces is complex and as of yet not fully understood. The combination of fluid flow, surfactant mass transfer and the fluid motion at the TPC line renders modelling of surfactant spreading a challenging fundamental problem[23]. The addition of surfactant to a pure liquid alters the surface forces, creates surface tension gradients and the adsorption of surfactant molecules on all the interfaces in the TPC region significantly affect the wetting characteristics of the solution[130].

The dominant process determining the spreading of a surfactant solution over a hydrophobic surface or the penetration into porous media seems to be the adsorption of surfactant molecules onto the hydrophobic substrate in front of the moving TPC line[80]. This results in a partial hydrophilisation of the hydrophobic surface in front of the TPC line.

The transfer of surfactant molecules from a water droplet by adsorption to the liquid-vapour, solid-liquid and solid-vapour interfaces on a hydrophobic substrate changes the
wetting characteristics in front of the droplet on the TPC line [131, 80]. A pure water droplet on a hydrophobic surface has a contact angle of greater than 90° and therefore is unable to penetrate into a microchannel. If the water droplet contains surfactants, i.e. a surfactant solution, the surfactant has three transfer processes which occur from the droplet to each of the interfaces on the TPC line: surfactant will adsorb at both (i) the liquid-solid interface and (ii) the liquid-vapour interface and (iii) the surfactant will transfer from the droplet to the solid-vapour interface in front of the droplet. Processes (i-ii) decrease the $\gamma_{LS}$ and $\gamma_{LV}$ respectively and process (iii) increases the surface free energy of the substrate. Process (iii) is a substantially slower process than the others due to the fact that the surfactants need to pass through a relatively high hydrophobic potential barrier from the liquid-vapour to solid-vapour interface[131].

The three processes increase the spreading power and decrease the contact angle according to Young’s equation[131]. At a critical concentration of surfactant, the contact angle of the solution with a hydrophobic surface will be less than 90° and the solution will penetrate into the microchannel. Depending on the concentration of surfactant in the solution there can be three possible flow regimes and are shown in Fig. 7.10: (i) when $C_o>C_m>C_{CMC}$, (ii) $C_m<C_{CMC}$ and (iii) $C_m=0$, where, $C_o$, is the surfactant concentration at the inlet of the microchannel and is assumed constant, $C_m$, is the surfactant concentration in the vicinity of the TPC line and $C_{CMC}$ is the surfactant concentration when surfactant micelles begin to form/destroy. For $C_o>C_m>C_{CMC}$, the surfactant solution contains individual surfactant monomers and surfactant micelles. The line plot represents the concentration of surfactant in the vicinity of the TPC line over the length of the microchannel. $C_m$ equals $C_o$ at the beginning of capillary flow in the microchannel but due to adsorption of surfactants to the microchannel walls $C_m$ will start to reduce. When $C_m<C_{CMC}$, the surfactant micelles begin to break-up and the balance of the interfacial forces at the TPC line will become dependent on $C_m$:

$$\gamma_{SV}(C_m)\cos\theta_d(C_m) = \gamma_{SV}(C_m) - \gamma_{SL}(C_m)$$  \hspace{1cm} (7.1)

When $C_m=0$, the surfactant concentration is zero (i.e. the same as the pure liquid) because the adsorption of the surfactant molecules to the microchannel walls occur faster than the
capillary flow into the microchannels and therefore no surfactant molecules reach the TPC resulting in the capillary flow ceasing.

Figure 7.9: (a) Flow regime when \( C_m > C_{CMC} \). (b) Flow regime when \( C_m < C_{CMC} \). (c) Flow regime when \( C_m = 0 \).

The CMC for tween-20 is 0.0804mM which corresponds to 9% tween-20/deionised water (v/v) solution[71]. The highest surfactant concentration solution used experimentally was 1% tween-20/deionised water solution and therefore the concentration of the tween-20/deionised water solutions were less than \( C_{CMC} \) (i.e. \( C_o < C_{CMC} \)) and contained no surfactant micelles. The solutions never stopped in the microchannels so the concentration of surfactant in the vicinity of the TPC line did not reach zero. The concentration of surfactants in the vicinity of the TPC line of the surfactant solutions over the length of the microchannels is shown in Fig. 7.10.

Theoretical equations for capillary flow of surfactant solutions in open microchannels must incorporate in the effect of surfactant adsorption near the TPC line because surface tension gradients begin to develop and due to these gradients the TPC line will have a tendency to move from regions of low tension (i.e. high surfactant concentration) toward regions of high tension (i.e. low surfactant concentration).[23].

In this study the value of the dynamic contact angles were measured, but in order to theoretically predict capillary flow of surfactant solutions in open hydrophobic microchannels, the concentration of surfactant in the region near the TPC line needs to be found. Future work could involve developing a diffusion/adsorption theoretical model to determine this surfactant concentration as a function of time.
7.3 Surfactant Coating of Hydrophobic Microchannels

To the author’s knowledge, there has been no studies performed on coating hydrophobic open rectangular microchannels with surfactants in order to increase the hydrophilicity of the microchannels and allow capillary flow of deionised water. There is also no experimental data on the interface shape of a liquid flowing through surfactant coated microchannels under capillary flow. Two volume sizes (10\(\mu\)l and 20\(\mu\)l) of deionised water were placed at the inlet of the 101, 191 and 393\(\mu\)m width microchannels with a surfactant coating of 0.01\%, 0.1\% and 1\% tween-20/deionised water. The flow rate and meniscus shape of the capillary flow was recorded at the 1, 3, 5, 7 and 9mm along the microchannels.

7.3.1 Surfactant Coated Open Microchannel Interface Shape

To the author’s knowledge there has been little or no studies carried out on the meniscus shape for capillary flow in surfactant coated microchannels. The meniscus shapes at the 3, 5, 7, and 9mm positions along the microchannels for 10\(\mu\)l of deionised water for each surfactant coated concentration are shown in Fig. 7.11-7.13. The meniscus shapes at the 3, 5, 7, and 9mm positions along the microchannels for 20\(\mu\)l of deionised water for each surfactant coated concentration are shown in Appendix C.3.5. The meniscus shape had the expected curvature for capillary flow in microchannels for each inlet volume and surfactant coating except for the 0.1\% tween-20/deionised water surfactant coating which is shown in Fig. 7.12. The meniscus shape for the 191 and 393\(\mu\)m microchannels at this surfactant
concentration started to transform into an unconventional shape for both inlet volumes, although, the change in the meniscus shape was more pronounced for the 10µl inlet volume but this was probably due to the fact that it had a slower flow rate than the 20µl inlet volume. The meniscus shape transformed from the curvature expected for capillary flow, where the centre of the meniscus started to flatten out and invert its curvature as the meniscus moved along the microchannel as shown in Fig. 7.14. Interestingly, this only occurred for the 191 and 393µm microchannels when coated with the intermediate surfactant concentration, although, the 393µm microchannel coated with 1% surfactant at the 9mm position did show signs of developing this alternative meniscus shape. As of yet, the reason for this is unknown but it would appear that the capillary forces were not the dominating forces of the fluid flow.

There have been several investigations into the capillary filling of fluids into corners of microchannels with particular attention to the meniscus shape[100, 25, 147, 67, 48]. Goldschmidtboeing et al (2006)[48] performed similar simulations of capillary filling in a 200µm square microchannels and found that in the corner of the microchannels, the fluid at the meniscus elongated along the corner into a sword-like shape ahead of the fluid meniscus and is shown in Fig. 7.15. Huang et al (2006)[67] performed capillary flow simulations on capillary filling of a 100µm square microchannels whereby in the simulations each surface of the microchannel where set to different contact angles ($\theta_1$, $\theta_2$, $\theta_3$, and $\theta_4$) shown in Fig. 7.16 (a) and also patterned each surface to have two different contact angles ($\theta^*_1$ and $\theta^*_2$) shown in Fig. 7.16 (b). The simulations provided some interesting meniscus shapes and are illustrated in Fig. 7.16 (c-f).

In comparing the meniscus shape deformation in the surfactant coated microchannels (Fig. 7.14) with the work carried out by Goldschmidtboeing et al (2006)[48] and Huang et al (2006)[67], the meniscus shape has the sword-like shape in the corners of the microchannel (but not of the same magnitude) and in the center of the microchannel the fluid behaved as if the surface was patterned which gave the fluid a higher contact angle than in the corners of the microchannel causing the meniscus shape to invert. To the author’s knowledge this meniscus shape has not been reported in any literature and as of yet the reason...
Figure 7.11: (a-d) Meniscus shape of 10 µl of 0.01% tween-20/deionised water solution flowing in the 100 µm at 3, 5, 7 and 9 mm respectively. (e-h) Meniscus shape of 10 µl 0.01% tween-20/deionised water solution flowing in the 200 µm at 3, 5, 7 and 9 mm respectively. (i-l) Meniscus shape of 10 µl 0.01% tween-20/deionised water solution flowing in the 400 µm at 3, 5, 7 and 9 mm respectively.
Figure 7.12: (a-d) Meniscus shape of 10µl of 0.1% tween-20/deionised water solution flowing in the 100µm at 3, 5, 7 and 9mm respectively. (e-h) Meniscus shape of 10µl 0.1% tween-20/deionised water solution flowing in the 200µm at 3, 5, 7 and 9mm respectively. (i-l) Meniscus shape of 10µl 0.1% tween-20/deionised water solution flowing in the 400µm at 3, 5, 7 and 9mm respectively.
Figure 7.13: (a-d) Meniscus shape of 10µl of 1% tween-20/deionised water solution flowing in the 100µm at 3, 5, 7 and 9mm respectively. (e-h) Meniscus shape of 10µl 1% tween-20/deionised water solution flowing in the 200µm at 3, 5, 7 and 9mm respectively. (i-l) Meniscus shape of 10µl 1% tween-20/deionised water solution flowing in the 400µm at 3, 5, 7 and 9mm respectively.
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Figure 7.14: (a-c) Interface shape of 20µl deionised water flowing in the 393µm microchannel coated with 1% tween-20 at 5, 7 and 9mm respectively.

Figure 7.15: Corner capillary flow ahead of the fluid meniscus in a square microchannel[48].

for this is remains unclear. Perhaps, some of the surfactants coated to the microchannels were adsorbed by the deionised water and this was altering the fluid flow characteristics.

7.3.2 Effect of Microchannel Geometry On Capillary Flow

The flow profile of 10µl of deionised water and the measured dynamic contact angles for each surfactant concentration coating for each microchannel are shown in Fig. 7.17. The flow profiles and the dynamic contact angles of the deionised water for the 0.01% tween-20 coated microchannels are shown in Fig 7.17 (a-b) respectively, there was little difference in the flow profiles between the 101 and 191µm microchannels. The flow profile for the 393µm was marginally slower than the other microchannels. Due to the high uncertainty in the measurement of the dynamic contact angle for the 101µm microchannel, no quantitative data could be obtained. The initial dynamic contact angle for the 191µm microchannel was approximately 80° and reduced to roughly 61° after 9mm. The dynamic contact angles
for the 393\(\mu\)m microchannel was considerably lower an initial and final values of approximately 55\(^\circ\) and 35\(^\circ\) respectively. The flow profiles and dynamic contact angles for the microchannels coated with 0.1% tween-20 are given in Fig. 7.17 (c-d) respectively. The flow profiles show that the 101\(\mu\)m had the fastest flow profile with the 191\(\mu\)m microchannel slightly slower and the 393\(\mu\)m microchannel considerably slower. The dynamic contact angles for the 191 and 393\(\mu\)m microchannels were omitted due to their irregular shape. The flow profiles and dynamic contact angles for the microchannels coated in 1% tween-20 are shown in Fig. 7.17 (e-f). The flow profiles follow a similar trend to the 0.1% tween-20 coated microchannels, as the width of the microchannel increased, the velocity decreased. The initial dynamic contact angles for the 101 and 191\(\mu\)m microchannels were approximately 90\(^{\circ}\) and 80\(^{\circ}\) for the 393\(\mu\)m microchannel and then relaxed to roughly 60\(^{\circ}\) and 50\(^{\circ}\) for the 191 and 393\(\mu\)m microchannels respectively.

The flow profile of 20\(\mu\)l of deionised water and the measured dynamic contact angles for each surfactant concentration coating for each microchannel are shown in Fig. 7.18.
Figure 7.17: (a-b) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the three microchannels coated with 0.01% tween-20 respectively. (c-d) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the three microchannels coated with 0.1% tween-20 respectively. (e-f) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the three microchannels coated with 1% tween-20 respectively.

The same trends were shown for the flow profiles of the 20µl deionised water.

### 7.3.3 Effect of Surfactant Coating Concentration On Capillary Flow

The flow profiles and the dynamic contact angles of 10µl of deionised water flowing through each of the 101, 191 and 393µm microchannels under capillary action based on the surfactant coating are shown in Fig. 7.19. The flow profiles and the dynamic contact angles for the 101µm microchannel are shown in Fig. 7.19 (a-b). The flow profiles indicate that as the surfactant concentration increased, the velocity decreased. This was not expected because the static contact angle measurements on a smooth flat substrate showed that as the concentration of the coated surfactant increased, the easier the deionised water would spread across the surface but this was contradiction with the capillary flow of deionised water through surfactant coated microchannels. The only explanation for this, is that the coated surfactants in the microchannels were creating some form of hydrophobic barrier.
and the mechanism behind it is as of yet unknown. There was little difference between the measured dynamic contact angles, there was only a slight increase the dynamic contact angle with an increase in surfactant coating concentration. The flow profiles for the 191 and 393µm microchannels follow a similar trend to the 101µm microchannel, as the concentration of the surfactant coating increased, the flow rate decreased. The dynamic contact angles measured also followed a similar trend that as the concentration of the coated surfactant increased, the dynamic contact angle increased.

The flow profiles and the dynamic contact angles of 20µl of deionised water flowing through for each of the 101, 191 and 393µm microchannels under capillary action based on the surfactant coating are shown in Fig. 7.20. The flow profiles and dynamic contact angles of the 20µl of deionised water followed similar trends to the 10µl of deionised water.
CHAPTER 7 Results & Discussion Part I: Capillary Driven Flow In Open Microchannels

Figure 7.19: (a-b) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the 101 µm microchannel coated with 0.01-1% tween-20 respectively. (c-d) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the 191 µm microchannel coated with 0.01-1% tween-20 respectively. (e-f) The flow profiles and dynamic contact angles for 10 µl deionised water under capillary flow through the 393 µm microchannel coated with 0.01-1% tween-20 respectively.

7.3.4 Effect of Inlet Volume of Capillary Flow

The flow profiles for the 10 and 20 µl deionised water are plotted for each microchannel and surfactant coating concentration in Fig. 7.21. The flow rate of the 20 µl deionised water was slightly faster than the 10 µl deionised water. Further study into the pressure at the inlet versus liquid volume is required to account for the difference in the flow rates.

The factors effecting capillary flow of deionised water through hydrophobic microchannels coated with surfactants warrants further study. The irregular shape of the meniscus and the fact that as the surfactant concentration of the coating increased, the flow rate through the microchannels decreased suggests that other as of yet unknown mechanism/s were influencing the fluid flow in a major way. A possible explanation maybe found through further investigations into the possible adsorption of surfactants into the fluid flow from the surfactant coating.
Figure 7.20: (a-b) The flow profiles and dynamic contact angles for 20µl deionised water under capillary flow through the 101µm microchannel coated with 0.01-1% tween-20 respectively. (c-d) The flow profiles and dynamic contact angles for 20µl deionised water under capillary flow through the 191µm microchannel coated with 0.01-1% tween-20 respectively. (e-f) The flow profiles and dynamic contact angles for 20µl deionised water under capillary flow through the 393µm microchannel coated with 0.01-1% tween-20 respectively.


Figure 7.21: (a-c) Flow profiles of the 10µl and 20µl deionised water through the 101, 191 and 393µm microchannels coated with 0.01% tween-20 respectively. (d-f) Flow profiles of the 10µl and 20µl deionised water through the 101, 191 and 393µm microchannels coated with 0.1% tween-20 respectively. (g-h) Flow profiles of the 10µl and 20µl deionised water through the 101, 191 and 393µm microchannels coated with 1% tween-20 respectively.

7.4 Summary

In this chapter, the fluidic properties of deionised water and surfactant solutions were measured. It was found that as the concentration of surfactant increased, the surface tension of the solution decreased. The static contact angle of surfactant solutions on flat hydrophobic surfaces decreased with increasing concentration. Flat hydrophobic surfaces with a surfactant coating reduced the static contact angle of deionised water on its surface.
The flow of surfactant solutions through hydrophobic microchannels were characterised in terms of flow profiles and dynamic contact angles. It was found that as the concentration of surfactant in the solution increased, the velocity through the microchannels increased due to the solution’s decrease in surface tension and static contact angle with increasing surfactant concentration. It was found that as the dimensions of the microchannels increased, the velocity decreased. This was in direct contradiction to conventional capillary flow theory for pure liquids which states that the velocity should decrease with decreasing microchannel dimensions due to higher frictional forces. The capillary flow of surfactant solutions differs to that of pure liquids and further study of the mechanics behind the adsorption of surfactants from the bulk surfactant solution to the hydrophobic microchannels might explain this difference in flow behaviours. To the author’s knowledge, the dynamic contact angle of surfactant solutions flowing in open microchannels via capillary action has not been directly measured until now. It was found that the dynamic contact angles reduced to a constant value that was significantly above the static contact angle measured on a flat substrate. It was also found that for the same concentration of surfactant solution, the dynamic contact angle values varied with microchannel dimensions. The smaller the microchannel dimensions, the higher the contact angle. This was due to the fact that the dynamic contact angle was a function of the velocity of the surfactant solution. The higher the velocity of the surfactant solution, the higher the dynamic contact angle. The various theories for the dynamic contact angles were investigated and it was found that the empirical equation best fitted the data obtained.

The surfactant coating of hydrophobic microchannels to promote capillary flow was investigated. To the author’s knowledge, there is little literature on the surfactant coating of hydrophobic microchannels and none on the meniscus shape of fluids flowing through these surfactant coated microchannels via capillary action. The meniscus shape of the deionised water significantly changed shape indicating that there are more than capillary flow mechanisms at work. As the microchannel dimensions increased, the velocity through the microchannels decreased. It was found that as the concentration of surfactant coating increased the velocity through the microchannels decreased which was in contradiction to the static contact angle measurements on a flat substrate with a surfactant coating. It
seemed that the surfactant coating was creating a hydrophobic barrier and the mechanisms behind this remains unclear but may be related to the adsorption of surfactants from the microchannel surface to the deionised water. The influence of inlet volume was investigated and it was found that when the volume was doubled from 10µl to 20µl there was a slight increase in velocity.
Chapter 8

Results & Discussion Part II: HSV Capillary Chip Assay

In this chapter, the results of the fluorescent detection of HSV-1 and HSV-2 using the capillary chip assay protocols are presented and discussed. This chapter is split into five sections. The first section outlines the process used to determine the optimum concentration of surfactant used to coat the HSV capillary chip assays in order to permit the fluidic steps of the assays to flow through the microchannels via capillary action. The second section describes how HSV fluorescent detection was optimised and quantified. The third section determines the FITC molar concentration of the HSV detection antibodies. The fourth section discusses the procedure used to verify that the HSV-1 and HSV-2 capture antibodies were immobilised on the surface of the detection zones of the capillary chips. The last section details the performance of the HSV capillary chip assay protocols.

8.1 Surfactant Coating For The HSV Capillary Chip Assays

In the previous chapter, the surfactant coating of Topas hydrophobic microchannels were investigated and in this section, the surfactant coating of the HSV capillary chip is discussed. The HSV capillary chips were fabricated from a different material, Zeonor, than
the Topas microchannels. Topas is a cyclic olefin copolymer whereas Zeonor is cyclo olefin polymer but they have very similar material properties. This section is split into two parts, the first investigates the flow profile across several surfactant coated capillary chips with no previous surface treatment and the second investigates the surfactant coating concentration required for reproducible flow rates for the HSV capillary chip assay protocols.

8.1.1 Surfactant Coating of Capillary Chip

The first step was to investigate if an unmodified capillary chip could be coated with a surfactant that promoted capillary flow. In this section, the influence of surfactant coating concentration on the flow of red dye through unmodified capillary chips was investigated. The three concentrations of surfactant, tween-20, used were 0.1%, 0.5% and 1% (v/v) tween-20 in a PBS solution. Five chips where cleaned with deionised water followed by ethanol for 30 minutes each in an ultra sonic bath and were then dried using nitrogen gas. A concentration of tween-20/PBS solution was used to coat the chips by placing 10µl of the solution at the inlet of the chip and through capillary action it flowed through the chip. An adsorbent pad was applied to the outlet of the chip to absorb the surfactant solution. After five minutes, 10µl of red dye was applied at the inlet of the chip. The meniscus position of the red dye was recorded using a high speed camera with an Optem zoom 70XL lens mounted on a tripod which allowed the recording of fluid flow along 38mm of the capillary chip. This was repeated three times for each chip and the same chips were used for each surfactant concentration. The recorded images were processed in the algorithm developed for analysing the flow profiles of the solutions flowing through the microchannels (see Section 6.1). The flow profiles produced by the red dye through the chips are shown in Fig. 8.1. As can be seen, the lower the concentration of the surfactant in the solution, the faster the red dye flowed through the chip. The red dye took approximately 8, 12 and 16 seconds to reach the end of the capillary chips that were coated with 0.1%, 0.5% and 1% surfactants respectively. This trend was the same for the surfactant coated Topas microchannels in the previous chapter.

The reproducibility of the flow profile of the red dye through several chips were also investigated. The mean meniscus position versus time of the five chips were plotted against
Figure 8.1: (a-e) The flow profiles of the red dye for five chips cleaned in ethanol and coated with 0.1-1% tween-20/PBS solutions.

each other for each concentration of surfactant coating in Fig. 8.2. It is clearly visible that for the three concentrations of surfactant the flow rates were consistent across five different chips as the flow time through the five chips are within a second of each other.

8.1.2 Surfactant Coating for HSV Capillary Chip Assays

It has been reported that decreasing the surfactant concentration or eliminating it can lead to higher assay detection sensitivities due to the fact that the surfactant coating can act as an additional blocking step[21]. Therefore, the surfactant coating for the HSV capillary chip
assays had to be the lowest concentration possible but still allow the fluid used for each step of the assays to flow through the chips under capillary action with a consistent and repeatable flow. From the previous section, five chips were cleaned with ethanol and coated with 0.1-1% tween-20/PBS solutions and this coating produced consistent and repeatable flow profiles for red dye and these concentrations were used as a starting point to determine the lowest concentration of surfactant that was needed to coat the capillary chips for the HSV assays.

To determine the lowest concentration of surfactant that could be used for the assay, five chips were blocked and washed according to the assay protocol and were then coated with 0.1%, 0.5% and 1% concentrations of tween-20/PBS solution. Next, the fluids and their volumes used in the assay steps were applied to the inlet of the chips (except for the HSV antigen/detection antibody step where PBS was used as a substitute). As the fluids used in the assay steps were transparent, 2% (v/v) of red dye was added to the fluids to help visualise when the entire fluid flowed through the chips and were absorbed by the pad

Figure 8.2: (a-c) The flow profiles of the red dye for five chips coated with 0.1-1% tween-20/PBS solutions respectively.
placed at the outlet. It was found that the 0.1% tween-20/PBS solution flow through the chip was not reproducible. During the PBS step, the 10µl of PBS applied to the inlet of the chips failed to reach the outlet of two out of the five capillary chips tested. For the higher concentrations of surfactant solutions, the fluid was able to flow through the chips for each step of the assay for all the five chips tested. The concentration of 0.5% tween-20/PBS solution was chosen as the concentration to be used for the HSV assay as this was the lowest concentration of tween-20 that consistently allowed the fluid for each step of the assay to flow through the chips. The time taken for the fluid to completely flow through the chips for this concentration are given in Table 8.1.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10µl 0.5% tween-20/PBS solution</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>10µl PBS</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>5µl Wash buffer</td>
<td>46.75 ± 3.5</td>
</tr>
<tr>
<td>5µl Wash buffer</td>
<td>36.25 ± 2</td>
</tr>
<tr>
<td>5µl Water</td>
<td>28.35 ± 2</td>
</tr>
</tbody>
</table>

### 8.2 HSV Fluorescent Detection Sensitivity

The detection sensitivity of fluorescent signals was dependent on finding the optimum camera exposure time to record the images. The optimum camera exposure times for the HSV-1 and HSV-2 detection antibodies were determined using the fluorescent pre-processing algorithm as explained in Section 6.2.1. An image was captured for 10µl of 0.02mg/ml of HSV-1 and HSV-2 detection antibodies flowing through the detection zone of the chips for various camera exposures. These images were converted to their relative histograms and the camera exposure time that allowed the greatest intensity sensitivity without the image becoming saturated was chosen as the optimum camera exposure time. The optimum exposure time for the HSV-1 and HSV-2 detection antibodies were found to be 3.85 and 3.7 seconds respectively. The images captured of the HSV-1 and HSV-2 detection antibodies and their resulting image histogram at a camera exposure of 3.85 and 3.7 seconds respectively are given in Fig. 8.3.
Figure 8.3: (a-b) Images of 10µl of HSV-1 and HSV-2 detection antibodies flowing through the detection zone of the chip at an exposure of 3.85 of 3.7 seconds respectively and (c-d) Image histograms of the 10µl of HSV-1 and HSV-2 detection antibodies respectively.

8.3 HSV-1 and HSV-2 Detection Antibody Molar Concentration

To determine the HSV-1 and HSV-2 detection antibodies FITC molar concentrations, images were captured of various concentrations of FITC diluted in PBS, at the optimum camera exposure time for the HSV-1 and HSV-2 detection antibodies. The FITC solutions were serial diluted down to a concentration of 1x10^{-8} mM of FITC. These images and those of the HSV-1 and HSV-2 detection antibodies at the same camera exposure were processed using the fluorescent image post-processing algorithms according to the procedures outlined in sections 6.3.1 and ?? and the average pixel intensity for each image was obtained. The average pixel intensities for the FITC concentrations were plotted on a log graph in Fig.
CHAPTER 8  
Results & Discussion Part II: HSV Capillary Chip Assay

Figure 8.4: (a-b) Curve fit for several concentrations of FITC dye for camera exposures of 3.85 and 3.7 seconds respectively.

8.4. The plotted points allowed the fitting an exponential curve which could be expressed by the following equation:

\[ \text{Intensity} = b(\text{molar concentration})^c \]  \hspace{1cm} (8.1)

where, \(b\) and \(c\) are the constants of the curve fit which given in Table E.6. The average pixel intensities for the fluorescent signal of the HSV-1 and HSV-2 detection antibodies calculated by the algorithm as \(3.4065 \times 10^3\) and \(3.5973 \times 10^3\) respectively. Using these pixel intensity values and equation 8.1, the FITC molar concentration of 0.02mg/ml of HSV-1 and HSV-2 detection antibodies were found to be \(2.9652 \times 10^{-4}\)mM and \(4.3394 \times 10^{-4}\)mM respectively.

8.4 HSV Capture Antibody Immobilisation

To investigate if the immobilised capture antibodies had sufficient binding strength to stay attached to the surface of the detection zone after several wash steps a HSV capture antibody immobilisation protocol was developed (see Section 5.2.2.3). Briefly, the capture antibodies were immobilised on the detection zone of the capillary chips and incubated in humid conditions. The chip was blocked and then rinsed with wash buffer to remove any unbound antibodies from the surface. 5\(\mu\)l of 0.2\(\mu\)g/ml QC labelled FAM-X antibodies
flowed through the chip via capillary action. The QC antibodies bound to any antibodies, i.e. capture antibodies, present in the microchannels of the chip. The chip was rinsed again to remove any unbound antibodies. The chip was placed under the Olympus IX-50 microscope to investigate if there was a fluorescent signal at the detection zone only. If there was no fluorescent signal, the binding strength was too weak to resist removal from the surface by either the numerous wash steps or the speed of fluid through the chip or both. The binding strength could be quantified by measuring the intensity of the fluorescent intensity of the signal. The stronger the fluorescent intensity, the greater the quantity of immobilised capture antibodies.

The HSV capture antibody protocol was performed for the HSV-1 and HSV-2 assays for concentrations of immobilised capture antibodies of 100, 250, 500 and 1000 µg/ml and a QC detection antibody concentration of 0.2 µg/ml. The images recorded and their fluorescent intensities of the HSV capture antibody protocols for each concentration of HSV-1 and HSV-2 capture antibodies are shown in Fig. 8.5 and 8.6 respectively. It can be seen that increasing the concentration of the spotted capture antibodies increased the quantity of immobilised capture antibodies. The quantity of immobilised antibodies present in the detection zone after the wash steps was relatively low when compared to HSV-2 assay. It is clearly visible from the fluorescent images in Fig. 8.6 that the HSV-2 capture antibodies were still present in the detection zone after several wash steps in reasonable quantities for HSV-2 antigen detection.

8.5 HSV Capillary Chip Assays

The protocols for the HSV-1 and HSV-2 capillary chip assays were given in Section 5.2.2.4. Briefly, the assays were performed by spotting and incubating capture antibodies to the surface of the chip in the detection zone. The chips were blocked and rinsed. 5 µl each of HSV antigens and detection antibodies labelled with FITC were mixed in solution. This solution was applied to the inlet and allowed to flow through the chip by capillary action. The chip was incubated in humid conditions. It was then rinsed and examined under a fluorescent microscope for a fluorescent signal. Typically, antigen concentrations in a patient’s
Figure 8.5: (a-d) Captured images of the HSV-1 capture antibody immobilisation protocol assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000µg/ml respectively and (e-i) Fluorescent intensities of the captured images of the HSV-1 capture antibody immobilisation protocol assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000µg/ml respectively.
Figure 8.6: (a-d) Captured images of the HSV-2 capture antibody immobilisation protocol assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000 µg/ml respectively and (e-i) Fluorescent intensities of the captured images of the HSV-2 capture antibody immobilisation protocol assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000 µg/ml respectively.
Results & Discussion Part II: HSV Capillary Chip Assay

A sample would be in the 1-500ng/ml range [154, 94, 136, 44, 92] and in the assay protocols developed in this thesis antigen concentrations 4-5 orders of magnitude higher were used to verify if the assay protocols were capable in principle of fluorescent detection of HSV.

8.5.1 HSV-1 Capillary Chip Assay

The HSV-1 capillary chip assay was performed for concentrations of immobilised capture antibodies of 100, 250, 500 and 1000µg/ml, 75-200µg/ml of HSV-1 antigen and 20µg/ml of HSV-1 detection antibodies. The images recorded and their fluorescent intensities of the HSV-1 capillary chip assays for each concentration of capture antibodies are shown in Fig. 8.7. These images were inserted into the fluorescent image post-processing algorithms to determine the average fluorescent pixel intensity of each image. The values of the average fluorescent pixel intensities for each concentration of immobilised HSV-1 capture antibody are shown in Fig. 8.9. It is evident that the assays performed poorly for immobilised capture antibodies concentrations of 100-1000µg/ml where the detected fluorescent signal for the presence of HSV-1 was marginally above the lower limit of detection. The fluorescent spot detected for the capture antibody concentration of 500µg/ml could be attributed to a dust particle. The lack of fluorescent detection was to be expected as the HSV-1 capture antibody immobilisation protocol for these concentrations indicated little or no immobilised capture antibodies after several wash steps. There was a trend that increasing the concentration of the capture antibodies resulted in a greater detectable fluorescent signal at the walls of the channel than the floor of the channel. This could have been due to a greater surface area being illuminated per pixel at the walls than the channel floor and therefore a greater quantity of bound capture-antigen-detection antibody complexes were being fluorescently excited.

8.5.2 HSV-2 Capillary Chip Assay

The HSV-2 capillary chip assay was performed for concentrations of immobilised capture antibodies of 100, 250, 500 and 1000µg/ml, 75-200µg/ml HSV-2 antigen and 20µg/ml of HSV-2 detection antibodies. The images recorded and their fluorescent intensities of the
Figure 8.7: (a-d) Recorded images of the HSV-1 capillary chip assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000µg/ml respectively and (e-i) Fluorescent intensities inside the red region of the captured images of the HSV-1 capillary chip assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000µg/ml respectively.
HSV-2 capillary chip assays for each concentration of capture antibodies are shown in Fig. 8.8. The values of the average fluorescent pixel intensities for each concentration of immobilised HSV-2 capture antibodies are shown in Fig. 8.9. It is evident that the HSV-2 capillary chip had a higher fluorescent detection than the HSV-1 capillary chip assays. There was a detectable fluorescent signal for the lowest concentration of immobilised HSV-2 capture antibodies and the detectable fluorescent signal increased for increasing concentration of immobilised HSV-2 capture antibodies although the detected fluorescent spots could be attributed to dust particles due to their non uniform distribution in the detection zone. Overall, this assay protocol performed poorly in the fluorescent detection of HSV-2. The poor performance in the fluorescent detection indicate that either the numerous wash steps could be removing the bound antigens from the surface or the HSV antigen-detection antibody binding was not strong enough to resist the force of the fluid flow rate through the microchannel. The possibility of reducing the number and volume of the wash steps was investigated but a further reduction from the assay protocols resulted in only partial removal of unbound molecules from the channels. The flow rate to a certain extent can be controlled through the use of surfactants but for a significant flow rate reduction a redesign of the chip is required.

The assays were unable to detect HSV-1 and HSV-2 antigens of concentrations of 75-200 µg/ml and a typical POC device measures antigen concentrations 4-5 orders of magnitude lower. This technique of fluorescent detection needs extensive modifications to improve its performance. There are several factors that can effect the assay’s performance. These include the binding strength of the capture antibodies to the surface, the binding of the HSV antigens to the capture antibodies and the binding of the detection antibodies to the HSV antigens. The combined binding strength of the capture-antigen-detection antibody complexes needs to be strong enough to stay bound to each other while withstanding the force of the flow of fluid through the channel and the numerous wash steps. The assays could be improved in several ways:

- Increasing the time for the incubation steps would allow more time for the formation of stronger bonds between the surface and the capture-antigen-detection antibody complexes.
Figure 8.8: (a-d) Recorded images of the HSV-2 capillary chip assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000 µg/ml respectively. (e-i) Fluorescent intensities inside the red circular region of the captured images of the HSV-2 capillary chip assay performed for immobilised capture antibodies of concentration 100, 250, 500 and 1000 µg/ml respectively.
Figure 8.9: The average pixel intensity of HSV-1 and HSV-2 fluorescent detection with varying capture antibody concentrations.

- Increasing the temperature of the incubation steps from room temperature to 37°C could improve the binding strengths. As the incubation temperature increases, the incubation time decreases.

- Eliminate the blocking step and check if there was an improvement in the strength of the fluorescent signal (improvement of the SNR).

- A redesign of the capillary chip could reduce the removal of bound capture-antigen-detection antibody complexes from the surface by reducing the flow rate of the fluid through the microchannel.

- The sensitivity of the assays could be improved by redesigning the detection zone of the capillary chips to give a higher surface-to-volume ratio. This would allow more sites for capture-antigen-detection antibody binding.

8.6 Summary

In this chapter, an optimum concentration of surfactant coating of the capillary chips was found which allowed all steps of the HSV capillary chips assays to flow through the chips via capillary action. The HSV fluorescent detection sensitivity was optimised through the imaging exposure time. The capture antibodies for HSV-1 and HSV-2 were successfully immobilised on the surface of the functionalised capillary chips but the quantity of the
HSV-1 detection antibodies was considerably lower than the HSV-2 detection antibodies. Overall, fluorescent detection of HSV-1 and HSV-2 did not occur to sufficiently high levels and the small levels of fluorescent that was detected could be attributed to dust particles settling in the detection zone of the chips. The assays were unable to detect HSV-1 and HSV-2 antigens of concentrations of 75-200µg/ml and a typical POC device measures antigen concentrations 4-5 orders of magnitude lower. This technique of fluorescent detection requires substantial modifications to improve the assays performance. Improvements could be made to the assay protocols such as increasing the time and temperature of the incubation steps, explore the possibility of removing the blocking step and redesign of the capillary chips to lower the flow rate through the capillary chips and also increasing the surface to volume ration in the detection zone of the capillary chips.
Chapter 9

Conclusions & Recommendations

The primary objectives of this work were to investigate the influence of surfactant capillary flow in open hydrophobic microchannels with an application toward fluorescent detection of HSV-1 and HSV-2. This chapter presents the conclusions of this work and recommendations for possible future work.

9.1 Conclusions

9.1.1 Capillary Flow In Open Microchannels

- The fluidic properties of deionised water and surfactant solutions were measured:
  
  - As the concentration of surfactant increased, the surface tension of the solution decreased.
  
  - The static contact angle of surfactant solutions on flat hydrophobic surfaces decreased with increasing concentration.
  
  - Flat hydrophobic surfaces with a surfactant coating reduced the static contact angle of deionised water on its surface.

- The flow of surfactant solutions through open hydrophobic microchannels was characterised in terms of flow profiles and dynamic contact angles:
– As the concentration of surfactant in the solution increased, the flow through the microchannels increased due to the solutions decrease in surface tension and decrease in static contact angle with increasing surfactant concentration.

– As the dimensions of the microchannels increased, the flow rate decreased. This was in direct contradiction to conventional capillary flow theory for pure liquids which states that the flow rate should decrease with decreasing microchannel dimensions due to higher frictional forces. The capillary flow of surfactant solutions differs to that of pure liquids and further study of the mechanics behind the adsorption of surfactants from the bulk surfactant solution to the hydrophobic microchannels might explain this difference in flow behaviours.

– To the author’s knowledge, the dynamic contact angle of surfactant solutions flowing in open microchannels via capillary action was directly measured for the first time. It was found that the dynamic contact angles reduced to a constant value that was significantly above the static contact angle measured on a flat substrate.

– For the same concentration of surfactant solution, the dynamic contact angle values varied with microchannel dimensions. The smaller the microchannel dimensions, the higher the contact angle. This was due to the fact that the dynamic contact angle was a function of the flow speed of the surfactant solution. The faster the surfactant solution flowed, the higher the dynamic contact angle.

– The various theories for the dynamic contact angles was investigated and it was found that the empirical equation best fitted the data obtained.

• The surfactant coating of hydrophobic microchannels to promote capillary flow was investigated.

– The meniscus shape of the deionised water significantly changed shape indicating that there are more than capillary flow mechanisms at work.

– As the microchannel dimensions increased, the flow rate through the microchannels decreased.
As the concentration of surfactant coating increased the flow rate through the microchannels decreased which was in contradiction to the static contact angle measurements on a flat substrate with a surfactant coating. It seemed that the surfactant coating was creating a hydrophobic barrier and the mechanisms behind this remains unclear but may be related to the adsorption of surfactants from the microchannel surface to the deionised water.

The influence of inlet volume was investigated and it was found that even though the volume was doubled from 10µl to 20µl these was little difference in the flow rate except for the 191 and 393µm width microchannels coated with 0.1% and 1% surfactant concentrations. The 10µl inlet volume was marginally slower than the 20µl flow rate.

9.1.2 HSV Capillary Chip Assays

- HSV-1 was not detected for concentrations of 100-1000µg/ml HSV-1 capture antibodies immobilised on the capillary chips with detection antibody and HSV-1 antigen concentrations of 20µg/ml and 75-200µg/ml respectively and any fluorescence that was detected could be attributed to dust.

- HSV-2 was not detected in sufficient quantities for concentrations of 100-1000µg/ml HSV-2 capture antibodies immobilised on the capillary chips with detection antibody and HSV-2 antigen concentrations of 20µg/ml and 75-200µg/ml respectively and any fluorescence that was detected could be attributed to dust.

9.2 Future Work

9.2.1 Capillary Flow In Open Microchannels

- The adsorption of surfactants from the bulk surfactant solution to the TPC line needs further study to help resolve capillary flow of surfactant solutions through hydrophobic open microchannels.
• Further study is required into the mechanisms behind the flow of deionised water through hydrophobic microchannels via capillary action in particular the adsorption of surfactants from the surfactant coating into the deionised water and the deformation of the meniscus shape from conventional capillary flow theory.

• As the fabrication techniques for microfluidic devices advances, more complex microchannel geometries can be produced and therefore the effect of varying the dimensions along a hydrophobic microchannel has on the capillary flow of surfactant solutions would warrant further investigation.

• The effect of varying the dimensions along a surfactant coated hydrophobic microchannel has on the capillary flow of deionised water would also warrant further investigation.

9.2.2 HSV Capillary Chip Assays

• The removal of the capture-antigen-detection antibody complexes from the surface of the capillary chips could be reduced by increasing the time for the incubation steps in the assays as this would allow more time for the formation of stronger bonds between the surface and the capture-antigen-detection antibody complexes.

• The binding strength of the capture-antigen-detection antibody complexes to the surface of the capillary chips could be further improved by increasing the temperature of the incubation steps from room temperature to 37°C. This increase in temperature could also reduce the incubation times.

• The quantity of HSV antigens binding to the capture antibodies may be improved with the removal of the blocking step in the assays. An increase in the quantity of capture-antigen-detection antibody complexes would lead to an increase in detectable fluorescence but non-specific binding would have to be addressed.

• A redesign of the capillary chip could reduce the removal of bound capture-antigen-detection antibody complexes from the surface by reducing the flow rate of the fluid through the microchannel.
• The sensitivity of the assays could be improved by redesigning the detection zone of the capillary chips to give a higher surface-to-volume ratio. This would allow more sites for capture-antigen-detection antibody binding.
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Appendix A

Published Work

A.1 Peer Reviewed Publications


A.2 Non-Peer Reviewed Publications

A.3 Publications in Progress


- O’Leary, F. & Griffin, P. “Capillary Flow of Water Through Surfactant Coated Open Hydrophobic Microchannels.”
Appendix B

Theoretical Capillary Flow In Microchannels

B.1 Pressure at the Inlet of the Microchannels

To calculate the pressure at the inlet to the microchannel, a second momentum balance was required in the reservoir control volume, $CV_{res}$ ($x<0$) where a three dimensional sink flow was assumed and is shown by the green dashed lines in Fig. B.1. The momentum equations where written using the same Cartesian coordinates as previous and spherical polar coordinates ($r, \theta, \varphi$) centred in the middle of the inlet of the microchannel.

![Diagram of the reservoir control volume.](image)

Figure B.1: Diagram of the reservoir control volume.
The velocity components of the momentum equations are $v_r, v_\theta, v_\phi$. Control Surface 1, $CS_1$, is defined as the curved surface of a hemisphere centred at the origin with a radius of $r_e$:

$$r_{eff} = 2 \sqrt{\frac{WH}{\pi}} \quad (B.1)$$

This radius results in the same area of projection as the inlet cross-sectional area of Control Surface 2, $CS_2$. $CS_2$ is defined as the surface at the inlet to the microchannel at $(0, y, z)$.

It is assumed that the volume flux across $CS_2$ is given by:

$$\dot{V}_{CS_2} = WH \frac{dL_f}{dt} \quad (B.2)$$

The volume flux across $CS_1$ is given by the continuity condition that the volume flux across $CS_1$ is equal to the volume flux across $CS_2$:

$$\int_0^{2\pi} \int_0^{\pi/2} r^2 v_r \sin(\theta) d\theta d\phi = \dot{V}_{CS_2} \quad (B.3)$$

which results in the radial velocity, $v_r$, outside the CV$_{res}$ given as:

$$v_r = -\frac{1}{4} \frac{WH}{\pi r^2} \left( \frac{dL_f}{dt} \right) \quad (B.4)$$

The Navier-Stokes equation for spherical coordinates for the velocity component, $v_r$, depending on $r$ and $t$ only reads:

$$\frac{\partial v_r}{\partial t} + v_r \frac{\partial v_r}{\partial r} = -\frac{1}{\rho} \frac{\partial p}{\partial r} + \nu \left[ \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial v_r}{\partial r} \right) - \frac{2v_r}{r^2} \right] \quad (B.5)$$

The velocity outside CV$_{res}$ has only one component, $v_r$, in the radial direction and therefore the other terms in equation B.5 are equal to zero. Therefore the pressure gradient can be expressed as:

$$\frac{dp}{dr} = -\rho \left( \frac{\partial v_r}{\partial t} + v_r \frac{\partial v_r}{\partial r} \right) \quad (B.6)$$

Integrating equation B.6 from $r=r_e$ to $r=\infty$ gives:
\[ p(r = r_{eff}) = p_\infty - \rho \left( \frac{1}{2} \frac{\sqrt{WH}}{\sqrt{\pi}} \frac{d^2L_f}{dt^2} + \frac{1}{8} \left( \frac{dL_f}{dt} \right)^2 \right) \]  

\[ \text{B.7} \]

The momentum balance for the \( CV_{res} \) is given as:

\[ \sum F_x = \frac{d}{dt} \left\{ \int_0^{2\pi} \int_0^\pi \int_0^{r_e} \rho \nu_r r^2 \sin \theta dr d\theta d\phi \right\} + \int_0^{2\pi} \int_0^\pi \int_0^{r_e} \rho (\nu_r \cdot n) r^2 \sin \theta dr d\theta d\phi \]

\[ + \int_{-W/2}^{W/2} \int_{-H}^{H} \rho \nu (\nu_r \cdot n) dy dz \]  

\[ \text{B.8} \]

where, the velocity, \( \nu_{rel} \), is the difference between the velocity of the control surface and the flow velocity. Because \( CV_{res} \) is constant, \( \nu_{rel} \) is equal to the flow velocity on the control surface. The velocity in the \( x \) direction in the reservoir can be described as \( v = -v_r \cos \theta \).

Equation B.9 now yields:

\[ \sum F_x = \frac{d}{dt} \left\{ \int_0^{2\pi} \int_0^\pi \int_0^{r_e} \rho \nu_r r^2 \sin \theta dr d\theta d\phi \right\} + \int_0^{2\pi} \int_0^\pi \int_0^{r_e} \rho (-v_r \cos \theta) v_r r^2 \sin (\theta) d\theta d\phi \]

\[ + \int_{-W/2}^{W/2} \int_{-H}^{H} \rho v^2 dy dz \]  

\[ \text{B.9} \]

There are two forces that act on the \( CV_{res} \) in the \( x \)-direction; the pressure force on the \( CS_2 \), \( F_{x2} \), and the pressure force on the \( CS_1 \), \( F_{x1} \). The pressure force on the \( CS_2 \), i.e. the entrance to the microchannel, is given by:

\[ F_{x2} = \int_{-W/2}^{W/2} \int_{-H}^{H} p(0,y,z,t) dy dz \]  

\[ \text{B.10} \]

\[ F_{x2} = -WHp(0,y,z,t) \]  

\[ \text{B.11} \]

The force acting on the hemisphere, \( F_{x1} \), in the \( x \)-direction is obtained from integrating the stress tensor at the hemisphere’s surface:

\[ F_{x1} = \int_0^{2\pi} \int_0^{\pi/2} (-\sigma_{rr} r^2 \cos \theta) \bigg|_{r=r_{eff}} \sin \theta d\theta d\phi \]  

\[ \text{B.12} \]
\[ F_{x1} = p_\infty WH - \rho \left[ \frac{1}{2} \frac{\sqrt{WH}}{\sqrt{\pi}} \left( \frac{d^2L_f}{dt^2} \right) + WH \left( \frac{dL_f}{dt} \right)^2 + \frac{2\mu}{\rho} \sqrt{WH} \sqrt{\pi} \left( \frac{dL_f}{dt} \right) \right] \] (B.13)

where, the stress tensor on the CS\(_1\) at \(r = r_{\text{eff}}\) can be defined as:

\[ \sigma_{rr} = -p(r) + 2\mu \frac{\partial v_r}{\partial r} \] (B.14)

The flux of momentum in the \(x\)-direction entering the hemisphere at CS\(_1\), \(\dot{M}_{CS1}\), at \(r = r_{\text{eff}}\) is:

\[ \dot{M}_{CS1} = \int_0^{2\pi} \int_0^{\frac{\pi}{2}} \rho (-v_r \cos \theta) v_r r^2 \sin(\theta) d\theta d\varphi \] (B.15)

\[ \dot{M}_{CS1} = \frac{1}{4} \rho WH (\frac{dL_f}{dt})^2 \] (B.16)

The flux of momentum in the \(x\)-direction leaving the CV\(_{res}\) at CS\(_2\), \(\dot{M}_{CS2}\), at \((x=0)\):

\[ \dot{M}_{CS2} = \int_{-\frac{w}{2}}^{\frac{w}{2}} \int_{-\frac{h}{2}}^{\frac{h}{2}} \rho v^2 dydz \] (B.17)

\[ \dot{M}_{CS2} = \rho WH (\frac{dL_f}{dt})^2 \] (B.18)

A uniform velocity distribution is assumed at CS\(_2\), since a certain flow length is required to develop a parabolic flow profile from the entrance of the microchannel:

\[ v_x = \frac{dL_f}{dt} \] (B.19)

The acceleration in the \(x\)-direction across CS\(_2\) is given by:

\[ \frac{dv_x}{dt} = \frac{d^2L_f}{dt^2} \] (B.20)

The flux of acceleration leaving CS\(_2\), \(a_{CS2}\), is:
\[ \dot{a}_{CS_2} = \int_{-\frac{W}{2}}^{\frac{W}{2}} \int_{-\frac{H}{2}}^{\frac{H}{2}} v_x \frac{dv_y}{dt} dydz \] (B.21)

To calculate the rate of change of momentum in CV\(_{res}\), a value for the acceleration in the CV\(_{res}\) needs to be obtained. Calculating the acceleration in CV\(_{res}\) is very difficult to achieve and therefore the acceleration in CV\(_{res}\) was taken as the mean acceleration across CS\(_1\) and CS\(_2\). The mean acceleration across CS\(_2\), \(\dot{v}_{CS_2}\), can be determined using equations B.2 and B.21:

\[ \dot{v}_{CS_2} = \frac{\dot{a}_{CS_2}}{V_{CS_2}} \] (B.22)

The acceleration at the hemisphere on CS\(_1\), \((r=r_{eff})\) radial outwards can be taken to be:

\[ \frac{Dv_r}{Dt} = \left( \frac{\partial v_r}{\partial t} + v_r \frac{\partial v_r}{\partial r} \right) \] (B.23)

The flux of acceleration inwards across CS\(_1\), \(\dot{a}_{CS_1}\), at \(r=r_{eff}\):

\[ \dot{a}_{CS_1} = 2\pi r_{eff}^2 \int_0^{\frac{\pi}{2}} \left( \frac{Dv_r}{Dt} \right) v_r \sin \theta \cos \theta d\theta \] (B.24)

The mean acceleration across CS\(_1\), \(\dot{v}_{CS_1}\), can be found by:

\[ \dot{v}_{CS_1} = \frac{\dot{a}_{CS_1}}{V_{CS_1}} \] (B.25)

\[ \dot{v}_{CS_1} = \frac{1}{4} \left( \frac{d^2L_f}{dt^2} + \frac{\sqrt{\pi}}{\sqrt{WH}} (\frac{dL_f}{dt})^2 \right) \] (B.26)

The mean acceleration inside the hemisphere, \(\dot{v}_{CV_{res}}\), was taken as the mean of the two mean accelerations at CS\(_1\) and CS\(_2\):

\[ \dot{v}_{CV_{res}} = \frac{1}{2} (\dot{v}_{CS_2} + \dot{v}_{CS_1}) \] (B.27)

\[ \dot{v}_{CV_{res}} = \frac{1}{8} \left[ 5 \left( \frac{d^2L_f}{dt^2} \right) + \frac{\sqrt{\pi}}{\sqrt{WH}} (\frac{dL_f}{dt})^2 \right] \] (B.28)
Theoretical Capillary Flow In Microchannels

The flux of momentum in $CV_{res}$ can be calculated using:

$$CV_{res\, momentum} = \rho \int_0^{2\pi} \int_{0}^{\frac{\pi}{2}} \int_{0}^{r_e} \dot{V}_{CV_{res}} r^2 \sin \theta \, dr \, d\theta \, d\phi$$ \hspace{1cm} (B.29)

$$CV_{res\, momentum} = \frac{1}{12} \rho WH \sqrt{\frac{WH}{\pi}} \left[ \frac{d^2 L_f}{dt^2} + \frac{(dL_f/dt)^2}{\sqrt{WH}} \right]$$ \hspace{1cm} (B.30)

Substituting equations B.12, B.10, B.30, B.16 and B.18 into equation B.9 allows the pressure at $CS_2$ to be found. The inlet pressure was obtained as:

$$p(0, y, z) = p_{\infty} - \rho \left[ 0.517 \sqrt{WH} \frac{d^2 L_f}{dt^2} + 0.958 \left( \frac{dL_f}{dt} \right)^2 + 3.545 \frac{\mu}{\sqrt{WH}} \frac{dL_f}{dt} \right]$$ \hspace{1cm} (B.31)

Rearranging equation B.31 with the following terms:

$$c_1 = 0.517 \sqrt{WH}$$ \hspace{1cm} (B.32)

$$c_2 = 0.958$$ \hspace{1cm} (B.33)

$$c_3 = \frac{0.545 \mu}{\sqrt{WH} \rho}$$ \hspace{1cm} (B.34)

This results in equation B.31 being expressed as:

$$p(0, y, z) = p_{\infty} - \rho \left[ c_1 \left( \frac{d^2 L_f}{dt^2} \right) + c_2 \left( \frac{dL_f}{dt} \right)^2 + c_3 \left( \frac{dL_f}{dt} \right) \right]$$ \hspace{1cm} (B.35)
Appendix C

Capillary Flow in Microchannel Calculations

C.1 Material Properties

Table C.1: Material properties of Topas (adapted from [150]).

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Unit</th>
<th>Test method</th>
<th>5013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume flow index MVR at 260°C, 2.16kg</td>
<td>ml/10 min</td>
<td>ISO 1133</td>
<td>48</td>
</tr>
<tr>
<td>Volume flow index MVR at HDT +115°C, 2.16kg</td>
<td>ml/10 min</td>
<td>ISO 1133</td>
<td>24</td>
</tr>
<tr>
<td>Density</td>
<td>g/cm³</td>
<td>ISO 1183</td>
<td>1.02</td>
</tr>
<tr>
<td>Water adsorption (24hr immersion at 23°C)</td>
<td>%</td>
<td>ISO 62</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Water vapour permeability (at 23°C and 85% relative humidity)</td>
<td>g.mm/m².d</td>
<td>DIN 53 122</td>
<td>0.030</td>
</tr>
<tr>
<td>Mould shrinkage (at 60°C and 2mm thickness)</td>
<td>%</td>
<td></td>
<td>0.4-0.7</td>
</tr>
</tbody>
</table>

Mechanical Properties

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>Test method</th>
<th>5013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strength (5mm/min)</td>
<td>MPa</td>
<td>ISO 527</td>
<td>46</td>
</tr>
<tr>
<td>Elongation at break (5mm/min)</td>
<td>%</td>
<td>ISO 527</td>
<td>1.7</td>
</tr>
<tr>
<td>Tensile modulus (1mm/min)</td>
<td>MPa</td>
<td>ISO 527</td>
<td>3200</td>
</tr>
<tr>
<td>Impact strength (Charpy)</td>
<td>kJ/m²</td>
<td>ISO 179/1eU</td>
<td>13</td>
</tr>
</tbody>
</table>

Thermal Properties

<table>
<thead>
<tr>
<th></th>
<th>°C</th>
<th>ISO 75</th>
<th>130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat deflection temperature HDT/B (0.45 MPa)</td>
<td>°C</td>
<td>ISO 11 359</td>
<td>0.6x10⁻⁴</td>
</tr>
</tbody>
</table>

Coefficient of linear thermal expansion | K⁻¹ | ISO 75 | 130 |

Electrical Properties

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>IEC 60250</th>
<th>2.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative permittivity</td>
<td></td>
<td>IEC 60112</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>

Optical Properties

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>ISO 13468-2</th>
<th>91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light transmission (2mm thickness)</td>
<td>%</td>
<td>ISO 13468-2</td>
<td>91</td>
</tr>
<tr>
<td>Refractive index</td>
<td></td>
<td></td>
<td>1.53</td>
</tr>
</tbody>
</table>
### Table C.2: Material Properties of Zeonor (adapted from[151]).

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>-</td>
<td>ASTM D792</td>
<td></td>
<td>1.01</td>
</tr>
<tr>
<td>Water absorption</td>
<td>%</td>
<td>ASTM D570</td>
<td></td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

#### Mechanical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear expansion coefficient</td>
<td>cm/cm°C</td>
<td>ASTM E831</td>
<td>7x10^-5</td>
</tr>
<tr>
<td>MFR (at 280°C, 21.18N)</td>
<td>g/10min</td>
<td>ISO 1133</td>
<td>60</td>
</tr>
<tr>
<td>MFR (at 230°C, 21.18N)</td>
<td>g/10min</td>
<td>ISO 1133</td>
<td>14</td>
</tr>
<tr>
<td>Flexural modulus</td>
<td>MPa</td>
<td>ISO 178</td>
<td>2100</td>
</tr>
<tr>
<td>Flexural strength</td>
<td>MPa</td>
<td>ISO 178</td>
<td>76</td>
</tr>
<tr>
<td>Tensile strength</td>
<td>MPa</td>
<td>ISO 527</td>
<td>53</td>
</tr>
<tr>
<td>Modulus of elasticity in tension</td>
<td>MPa</td>
<td>ISO 527</td>
<td>2100</td>
</tr>
<tr>
<td>Tensile elongation</td>
<td>%</td>
<td>ISO 527</td>
<td>60</td>
</tr>
<tr>
<td>Izod impact strength</td>
<td>J/m</td>
<td>ASTM 0256</td>
<td>18</td>
</tr>
<tr>
<td>Dupont impact strength</td>
<td>J</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>Rockwell hardness</td>
<td>-</td>
<td>ASTM 0785</td>
<td>20</td>
</tr>
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#### Thermal Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deflection temperature under 1.80MPa load</td>
<td>°C</td>
<td>ASTM D648</td>
<td>99</td>
</tr>
<tr>
<td>Glass transition temperature</td>
<td>°C</td>
<td>JIS K7121</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Electrical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dielectric breakdown strength</td>
<td>KV/mm</td>
<td>ASTM D149</td>
<td>70</td>
</tr>
<tr>
<td>Dielectric constant</td>
<td>-</td>
<td>IEC250</td>
<td>2.3</td>
</tr>
<tr>
<td>Dielectric tangent</td>
<td>-</td>
<td>IEC250</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

#### Optical Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Unit</th>
<th>Measurement method</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light transmittance</td>
<td>%</td>
<td>ASTM D1003</td>
<td>92</td>
</tr>
</tbody>
</table>
C.2 Fluid Properties

C.2.1 Static Contact Angles

The values and the uncertainty of the static contact angle for deionised water and the tween-20/deionised water solutions on the surface of a Topas substrate are given in Table C.4. The uncertainty was calculated using twice the quoted uncertainty value in the operational manual plus twice the standard deviation of the experimental measurements.

The constants for the equation of a line, \( y = mx + c \), for the static contact angle for
Table C.4: The values and uncertainties of the static contact angle for a tween-20/deionised water solution on the surface of Topas.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Static Contact Angle (º)</th>
<th>Uncertainty (º)</th>
<th>Uncertainty(%)</th>
<th>Static Contact Angle (º)</th>
<th>Uncertainty(º)</th>
<th>Uncertainty(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87.8350 ± 2.4188</td>
<td>±2.7314</td>
<td></td>
<td>87.4833 ± 3.9631</td>
<td>±4.5305</td>
<td></td>
</tr>
<tr>
<td>0.01% Tween/water</td>
<td>55.1767 ± 3.8131</td>
<td>±6.9114</td>
<td></td>
<td>48.6163 ± 2.3631</td>
<td>±4.8607</td>
<td></td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>47.7623 ± 1.5445</td>
<td>±3.2337</td>
<td></td>
<td>40.1223 ± 1.7690</td>
<td>±4.4907</td>
<td></td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>40.1907 ± 1.3858</td>
<td>±3.4479</td>
<td></td>
<td>32.5997 ± 5.4683</td>
<td>±16.7794</td>
<td></td>
</tr>
</tbody>
</table>

deionised and the tween-20/deionised water solutions on the surface of a Topas substrate over a 19-24ºC are given in Table C.5, where, \(x\) and \(y\) are the temperature and static contact angle of the fluid respectively.

Table C.5: The constants for the equation of a line for the static contact angle.

<table>
<thead>
<tr>
<th>Fluid/Constant</th>
<th>(m)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-0.2148</td>
<td>92.6385</td>
</tr>
<tr>
<td>0.01% Tween/water</td>
<td>-1.3121</td>
<td>80.1059</td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>-1.5280</td>
<td>76.7943</td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>-1.5202</td>
<td>69.0745</td>
</tr>
</tbody>
</table>

### C.2.2 Surface Tension

The constants of the second order polynomial, \(y = ax^2 + bx + c\), and values of the experimental uncertainty for surface tension for deionised water and the tween-20/deionised water solutions are given in Table C.6, where, \(x\) and \(y\) are the temperature and surface tension of the fluid respectively. The uncertainty was calculated using twice the quoted uncertainty value in the operational manual plus twice the standard deviation of the experimental measurements.

Table C.6: The surface tension polynomial fit constants and uncertainty values.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>Uncertainty (mN/m)</th>
<th>Uncertainty (%)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-0.0017</td>
<td>-0.0238</td>
<td>72.5003</td>
<td>±0.9568</td>
<td>±1.3582</td>
<td>0.99622</td>
</tr>
<tr>
<td>0.01% Tween/water</td>
<td>-0.0369</td>
<td>1.2219</td>
<td>40.2459</td>
<td>±4.2377</td>
<td>±9.3231</td>
<td>0.95026</td>
</tr>
<tr>
<td>0.1% Tween/water</td>
<td>-0.0155</td>
<td>0.4904</td>
<td>37.5579</td>
<td>±1.8203</td>
<td>±4.6606</td>
<td>0.96164</td>
</tr>
<tr>
<td>1% Tween/water</td>
<td>0.0019</td>
<td>-0.1521</td>
<td>41.1253</td>
<td>±0.5058</td>
<td>±1.3004</td>
<td>0.99421</td>
</tr>
</tbody>
</table>
C.2.3 Density

The density of the tween-20/deionised water solutions were calculated using the molecular weight and density of tween-20 and water. The molecular weight, $MW$, and density for Tween-20 is 1228g and 1.11kg/l respectively. The number of moles, $n_{tween}$, per litre is given by:

$$n_{tween} = \frac{\rho_{tween}}{MW_{tween}} = 0.9039 \quad (C.1)$$

The number of moles for 1% tween, $n_{1\%tween}$, per litre is given by:

$$n_{1\%tween} = 0.9039(0.01) = 0.9039 \times 10^{-2} \quad (C.2)$$

The density for 1% tween, $\rho_{1\%tween}$, is given by:

$$\rho_{1\%tween} = n_{1\%tween}(MW_{tween}) = 1.11 \times 10^{-2} \text{kg/l}$$

The density of water, $\rho_{water}$, is 0.998kg/l. The density of the tween dissolved in water is given by:

$$\rho_{1\%tween/water} = \rho_{1\%tween} + \rho_{water} = 1.0091\text{kg/l}$$

C.3 Capillary Flow Data Analysis

C.3.1 Capillary Flow Data Grouping

Images of the capillary flow were recorded at five locations along the microchannel, 1, 3, 5, 7 and 9mm, and this was done in triplicate. The average meniscus position versus time is shown in Fig. C.1 (a) for the five locations. The mean meniscus position versus time for the five locations are shown in Fig. C.1 (b). These were used to calculated the velocity of the meniscus over the 5 locations which were then able to be displayed on the same graph in Fig. C.1 (c). To obtain the mean time versus meniscus position over the 5 locations, the
velocity was divided by the meniscus position over the 5 locations and is shown in Fig. C.1 (d).

Figure C.1: (a) The 5 locations along the microchannel images were captured (b) The average meniscus position versus time for the 5 locations (c) The velocity of the meniscus versus position over the entire microchannel and (d) The mean position versus time over the entire microchannel.

C.3.2 Capillary Flow Data Uncertainty

The uncertainties in measuring the meniscus position and the dynamic contact angle of the fluid is given in Table C.7. There were two types of uncertainties in determining the fluid’s position; the pixel uncertainty and the experimental uncertainty. The pixel uncertainty refers to the algorithm determining the meniscus position using pixel intensity values. The
meniscus position could be found to within one pixel and therefore the absolute pixel uncertainty was taken as two pixels. The experimental uncertainty was taken as twice the standard deviation of the experimental measurements. To calculate the total position uncertainty the pixel and experimental measurement uncertainties were added together. For the dynamic contact angles, a calibration curve was applied during the algorithm and the uncertainty was only calculated using twice the standard deviation of the experimental measurements.

Table C.7: The average uncertainties for 20µl of tween-20/water solution capillary flow measurements through the microchannels.

<table>
<thead>
<tr>
<th>Microchannel (µm)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>±0.0522</td>
<td>±3.0598</td>
<td>±4.7359</td>
<td>±0.0456</td>
<td>±4.5036</td>
<td>±6.7268</td>
<td>±0.0229</td>
<td>±3.9671</td>
<td>±4.4137</td>
</tr>
<tr>
<td>191</td>
<td>±0.0453</td>
<td>±3.6588</td>
<td>±3.7791</td>
<td>±0.0530</td>
<td>±3.9574</td>
<td>±3.4155</td>
<td>±0.0489</td>
<td>±3.0414</td>
<td>±3.7888</td>
</tr>
<tr>
<td>393</td>
<td>±0.0279</td>
<td>±2.5613</td>
<td>±3.7187</td>
<td>±0.0364</td>
<td>±4.1573</td>
<td>±3.1838</td>
<td>±0.0402</td>
<td>±5.5198</td>
<td>±3.3031</td>
</tr>
</tbody>
</table>

Table C.8: The average uncertainties for 10µl of deionised water flowing through the microchannels coated with tween-20.

<table>
<thead>
<tr>
<th>Microchannel (µm)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>±0.0075</td>
<td>±4.9981</td>
<td>±4.8790</td>
<td>±0.0312</td>
<td>±3.8499</td>
<td>±3.261</td>
<td>±0.0240</td>
<td>±6.9559</td>
<td>±5.3643</td>
</tr>
<tr>
<td>191</td>
<td>±0.0020</td>
<td>±2.6104</td>
<td>±3.1527</td>
<td>±0.0162</td>
<td>±4.1138</td>
<td>±4.5268</td>
<td>±0.0216</td>
<td>±4.0617</td>
<td>±4.027</td>
</tr>
<tr>
<td>393</td>
<td>±0.0285</td>
<td>±3.2133</td>
<td>±3.6499</td>
<td>±0.0182</td>
<td>n/a</td>
<td>n/a</td>
<td>±0.0388</td>
<td>±3.0926</td>
<td>±2.7464</td>
</tr>
</tbody>
</table>

Table C.9: The average uncertainties for 20µl of deionised water flowing through the microchannels coated with tween-20.

<table>
<thead>
<tr>
<th>Microchannel (µm)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
<th>Position (mm)</th>
<th>θt (°)</th>
<th>θb (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>±0.0275</td>
<td>±6.0520</td>
<td>±4.7292</td>
<td>±0.0285</td>
<td>±4.1400</td>
<td>±4.2619</td>
<td>±0.0184</td>
<td>±7.3076</td>
<td>±7.4447</td>
</tr>
<tr>
<td>191</td>
<td>±0.0278</td>
<td>±3.2719</td>
<td>±2.9799</td>
<td>±0.0279</td>
<td>±4.5871</td>
<td>±4.6517</td>
<td>±0.0285</td>
<td>±3.5610</td>
<td>±4.5090</td>
</tr>
<tr>
<td>393</td>
<td>±0.199</td>
<td>±5.5993</td>
<td>±2.8499</td>
<td>±0.0214</td>
<td>n/a</td>
<td>n/a</td>
<td>±0.0173</td>
<td>±3.6080</td>
<td>±4.7470</td>
</tr>
</tbody>
</table>
C.3.3 Dynamic Contact Angle Calibration Curves

Table C.10: Constants of the fifth order polynomial equation for the calibration curve for the top wall contact angle and its $R^2$ value.

<table>
<thead>
<tr>
<th>Channel ($\mu$m)/Constant</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>2.79$x10^{-9}$</td>
<td>-1.01$x10^{-6}$</td>
<td>2.03$x10^{-4}$</td>
<td>-0.0271</td>
<td>3.01</td>
<td>-58.71</td>
<td>0.9939</td>
</tr>
<tr>
<td>191</td>
<td>3.57$x10^{-8}$</td>
<td>-1.59$x10^{-5}$</td>
<td>2.9$x10^{-3}$</td>
<td>-0.26</td>
<td>12.42</td>
<td>-201.99</td>
<td>0.9973</td>
</tr>
<tr>
<td>392</td>
<td>3.89$x10^{-10}$</td>
<td>-1.69$x10^{-5}$</td>
<td>3$x10^{-3}$</td>
<td>-0.26</td>
<td>12.04</td>
<td>-189.19</td>
<td>0.9957</td>
</tr>
</tbody>
</table>

Table C.11: Constants of the fifth order polynomial equation for the calibration curve for the bottom wall contact angle and its $R^2$ value.

<table>
<thead>
<tr>
<th>Channel ($\mu$m)/Constant</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>1.97$x10^{-8}$</td>
<td>-8.53$x10^{-6}$</td>
<td>1.4$x10^{-3}$</td>
<td>-0.12</td>
<td>6.17</td>
<td>-98.94</td>
<td>0.9981</td>
</tr>
<tr>
<td>191</td>
<td>3.95$x10^{-8}$</td>
<td>-1.76$x10^{-5}$</td>
<td>3.1$x10^{-3}$</td>
<td>-0.27</td>
<td>12.61</td>
<td>-205.63</td>
<td>0.9927</td>
</tr>
<tr>
<td>392</td>
<td>3.75$x10^{-8}$</td>
<td>1.62$x10^{-5}$</td>
<td>2.7$x10^{-3}$</td>
<td>-0.23</td>
<td>10.87</td>
<td>-172.11</td>
<td>0.9923</td>
</tr>
</tbody>
</table>

C.3.4 Capillary Flow Theoretical Dynamic Contact Angle Constants

Table C.12: Theoretical dynamic contact angle curve fit variables using the capillary flow static contact angle.

<table>
<thead>
<tr>
<th>Microchannel ($\mu$m)</th>
<th>0.01% Tween/water</th>
<th>0.1% Tween/water</th>
<th>1% Tween/water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M(m) ln $\gamma$</td>
<td>M(m) ln $\gamma$</td>
<td>M(m) ln $\gamma$</td>
</tr>
<tr>
<td></td>
<td>$\alpha$ (nm)</td>
<td>$\alpha$ (nm)</td>
<td>$\alpha$ (nm)</td>
</tr>
<tr>
<td></td>
<td>$K(10^6 s^{-1})$</td>
<td>$K(10^6 s^{-1})$</td>
<td>$K(10^6 s^{-1})$</td>
</tr>
<tr>
<td>101</td>
<td>22.89 792.8 2.27</td>
<td>4.25 259.7 0.134</td>
<td>8.65 207.5 0.134</td>
</tr>
<tr>
<td>191</td>
<td>10.99 3057.5 624.51</td>
<td>19.93 2488.5 0.134</td>
<td>11.09 1715.2 1.219</td>
</tr>
<tr>
<td>392</td>
<td>6.78 8966.2 0.127</td>
<td>3.36 10777 0.163</td>
<td>6.38 3331.2 0.098</td>
</tr>
</tbody>
</table>

C.3.5 Meniscus Profile Shape for Surfactant Coated Microchannels
Figure C.2: (a-d) Meniscus shape of 20µl of 0.01% tween-20/deionised water solution flowing in the 100µm at 3, 5, 7 and 9mm respectively. (e-h) Meniscus shape of 20µl 0.01% tween-20/deionised water solution flowing in the 200µm at 3, 5, 7 and 9mm respectively. (i-l) Meniscus shape of 20µl 0.01% tween-20/deionised water solution flowing in the 400µm at 3, 5, 7 and 9mm respectively.
Figure C.3: (a-d) Meniscus shape of 20µl of 0.1% tween-20/deionised water solution flowing in the 100µm at 3, 5, 7 and 9mm respectively. (e-h) Meniscus shape of 20µl 0.1% tween-20/deionised water solution flowing in the 200µm at 3, 5, 7 and 9mm respectively. (i-l) Meniscus shape of 20µl 0.1% tween-20/deionised water solution flowing in the 400µm at 3, 5, 7 and 9mm respectively.
Figure C.4: (a-d) Meniscus shape of 20µl of 1% tween-20/deionised water solution flowing in the 100µm at 3, 5, 7 and 9mm respectively. (e-h) Meniscus shape of 20µl 1% tween-20/deionised water solution flowing in the 200µm at 3, 5, 7 and 9mm respectively. (i-l) Meniscus shape of 20µl 1% tween-20/deionised water solution flowing in the 400µm at 3, 5, 7 and 9mm respectively.
Appendix D

Algorithms

D.1 Flow Profiles

clc
clear all

% set frame rate
frame_rate = input('frame rate: ');
frame_rate = 1/frame_rate;

% read images
files_chip = dir('Image_*.tif');
background_chip = imread('background_image.tif');
for a_chip = 1:length(files_chip)
data_chip = imread(files_chip(a_chip).name);
DATA_chip(:,a_chip) = data_chip(:,);
end

clear data_chip

x_th_limits = [155;196;323;350];

% ROI
figure, imagesc(DATA_chip(:,1));
colormap gray
title('Select ROI')
[x_chip,y_chip] = ginput(2);
close
x_chip=round(x_chip);
y_chip=round(y_chip);

%SCALE
pix_x_chip=[105.048387096774;142.53258064516;];
pix_y_chip=[162.365497076023;167.570175438596;];
pixel_mm = 0.1/(round(max(pix_x_chip))-round(min(pix_x_chip)));

%ROI
data_chip = (DATA_chip(round(min(y_chip)):round(max(y_chip)),round(min(x_chip)):round(max(x_chip))));

% LEVEL THE CHANNEL
figure, imagesc(DATA_chip(:,:,1));
colormap gray
title('Angle to the horizontal')
[w_chip,z_chip] = ginput(2);
close
w_chip=round(w_chip);
z_chip=round(z_chip);
dx_chip = gradient(w_chip);
dy_chip = gradient(z_chip);
C_angle_chip=(atand(dy_chip(1,1)/dx_chip(1,1)))*-1; %channel angle to the horizontal
Mid_image_chip=round(a_chip/1.5);

%BACKGROUND IMAGE SUBTRACTION
background_chip=double(background_chip);
background_chip=255-background_chip;
background_chip=background_chip(round(min(y_chip)):round(max(y_chip)),round(min(x_chip)):round(max(x_chip)));
for a_chip = 1:length(files_chip)
data_chip(:,:,a_chip) = double(data_chip(:,:,a_chip))+background_chip;
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end

x_th_limits=x_th_limits-x_chip(1,1);

x_th_limits_end=x_chip(2,1)-x_chip(1,1)+1;

% problems with uneven light illumination of the captured image -
% Split channel into 3 parts, 1/4, 1/2 & 1/4 because diff grad needed to be
% applied widthwise

quart_upper=round(size(data_chip,1)/8)-1;

quart_lower=size(data_chip,1)-quart_upper;

quart_end=size(data_chip,1);

% needed to be split into 3 sections lengthwise

section_1=round(x_th_limits(2,1)/2);

section_2=x_th_limits(2,1);

section_3=x_th_limits(3,1);

section_4=x_th_limits_end;

% THRESHOLD IMAGE

figure,

subplot(3,1,1) imagesc(data_chip(:,:,Mid_image_chip));

colormap gray

colorbar('location','east')

title('Original image')

subplot(3,1,2), imagesc(data_chip(:,:,Mid_image_chip)<th_chip);

colormap gray

title('Threshold image')

subplot(3,1,3) ksdensity(double(reshape(data_chip,[],1,1))); % Kernel hist

title('Kernel histogram')

th_chip = 200; % initial guess

while 1

[th1_chip th2_chip] = ginput(1);

if size(th1_chip) == [0 0], break; end

subplot(3,1,2) imagesc(data_chip(:,:,Mid_image_chip)<th_chip);

183
colormap gray

title('Threshold image')

th_chip = th1_chip;

end

close

Edge_chip(1:quart_upper,1:section_1,:) = data_chip(1:quart_upper,1:section_1,:)
< th_chip_section1_upper; % logical matrix

Edge_chip(quart_upper+1:quart_lower-1,1:section_1,:) = data_chip(quart_upper+1:quart_lower-1,1:section_1,:)
< th_chip_section1_middle;

Edge_chip(quart_lower:quart_end,1:section_1,:) = data_chip(quart_lower:quart_end,1:section_1,:)
< th_chip_section1_lower;

Edge_chip(1:quart_upper,section_1+1:section_2,:) = data_chip(1:quart_upper,section_1+1:section_2,:)
< th_chip_section2_upper;

Edge_chip(quart_upper+1:quart_lower-1,section_1+1:section_2,:) = data_chip(quart_upper+1:quart_lower-1,section_1+1:section_2,:)
< th_chip_section2_middle;

Edge_chip(quart_lower:quart_end,section_1+1:section_2,:) = data_chip(quart_lower:quart_end,section_1+1:section_2,:)
< th_chip_section2_lower;

Edge_chip(1:quart_upper,section_2+1:section_3,:) = data_chip(1:quart_upper,section_2+1:section_3,:)
< th_chip_section3_upper;

Edge_chip(quart_upper+1:quart_lower-1,section_2+1:section_3,:) = data_chip(quart_upper+1:quart_lower-1,section_2+1:section_3,:)
< th_chip_section3_middle;

Edge_chip(quart_lower:quart_end,section_2+1:section_3,:) = data_chip(quart_lower:quart_end,section_2+1:section_3,:)
< th_chip_section3_lower;

Edge_chip(1:quart_upper,section_3+1:section_4,:) = data_chip(1:quart_upper,section_3+1:section_4,:)
< th_chip_section4_upper;

Edge_chip(quart_upper+1:quart_lower-1,section_3+1:section_4,:) = data_chip(quart_upper+1:quart_lower-1,section_3+1:section_4,:)
< th_chip_section4_middle;

Edge_chip(quart_lower:quart_end,section_3+1:section_4,:) = data_chip(quart_lower:quart_end,section_3+1:section_4,:)
< th_chip_section4_lower;

% MEDIAN FILTER
for a_chip = 1:length(files_chip)
    Edge_chip(:,:,a_chip)=medfilt2(Edge_chip(:,:,a_chip));
end

Edge_chip(:,1,:)=[]; %median filter sets the corners of the matrix to NaNs
Edge_chip(:,end,:)=[];

%MENISCUS EDGE DETECTION
for a_chip = 1:length(files_chip)
    Edge_chip(:,:,a_chip) = edge(double(Edge_chip(:,:,a_chip)),'canny'); %canny function
end

for a_chip = 1:length(files_chip)
    for b_chip = 1:size(Edge_chip,1)
        e_chip = find(Edge_chip(b_chip,:,a_chip)); %find non zero elements
        if isempty(e_chip)
            Edge_pts_chip(b_chip,a_chip) = 0;
        else
            Edge_pts_chip(b_chip,a_chip) = e_chip(end);
        end
    end
end

DD=Edge_pts_chip(2:(end-1),:);
Edge_pts_chip=DD;
EE=data_chip(2:(end-1),:,:);
data_chip=EE;
b_chip = size(Edge_pts_chip,1);
c_chip=round(b_chip/2);
a_chip = length(files_chip);
b_chip = size(Edge_pts_chip,1);
Pos_chip_a=Edge_pts_chip*pixel_mm; %convert pixels to metric length (mm)
%find the point where the liquid enters & leaves which spans the ROI of each section
b=b_chip;
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Pos_chip_d = Edge_pts_chip * pixel_mm;

for a = 1:a_chip
    if Pos_chip_d(1:b,a) > 0 % spans the channel - changed to 1 for gradient threshold
        Pos_chip_d(1:b,a) = 1;
    end
    if Pos_chip_d(1:b,a) < 1
        Pos_chip_d(1:b,a) = 0;
    end
    if Pos_chip_d(c_chip,a) > 0 % find the start and end points
        Pos_chip_b(c_chip,a) = 1;
    else
        Pos_chip_b(c_chip,a) = 0;
    end
end

Pos_chip_start_pt1 = find(Pos_chip_b(c_chip,:));
Pos_chip_start_pt = Pos_chip_start_pt1(1,1);
Pos_chip_end_pt = Pos_chip_start_pt1(1,end);

% MENISCUS POSITION
Pos_chip = Pos_chip_a(c_chip,Pos_chip_start_pt:Pos_chip_end_pt);

Meniscus_span = Pos_chip_start_pt:Pos_chip_end_pt;

% TIME
a_chip = length(Pos_chip);
t_chip = [frame_rate:frame_rate:(a_chip*frame_rate)]; % time

figure,
plot(t_chip, Pos_chip, 'b');
grid on;
legend('Data', 'location', 'northwest');
xlabel('Time (sec)');
ylabel('Channel Position (mm)');

% CONTACT ANGLE
a_chip = length(files_chip);
size_width = size(Edge_pts_chip);
pixel_width = size_width(1,1);
b_chip = pixel_width;
c_chip = round(b_chip/2); %middle pixel

%top half of the channel
pixel_top = 1:(c_chip-1); %50 points
size_pixel_top = size(pixel_top);
pixel_top = reshape(pixel_top, size_pixel_top(1,2), 1);

%bottom half of the channel
pixel_bot = (c_chip+1):b_chip; %50 points
size_pixel_bot = size(pixel_bot);
pixel_bot = reshape(pixel_bot, size_pixel_bot(1,2), 1);

bb = size(Meniscus_span);
aa = round(linspace(1, a_chip, 6));

%MENISCUS POSITION

c_chip = round(b_chip/2); %middle pixel

a_chip = bb(1,2);

Edge_pts_chip_a = Edge_pts_chip;
clear Edge_pts_chip

Edge_pts_chip = Edge_pts_chip_a(:, Pos_chip_start_pt:Pos_chip_end_pt);
data_chip_a = data_chip;
clear data_chip

data_chip = data_chip_a(:, Pos_chip_start_pt:Pos_chip_end_pt);

Pixel_col_y_chip = 1:b_chip; %y-column

Pixel_col_y_chip = reshape(Pixel_col_y_chip, b_chip, 1);

Pixel_col_y_chip = repmat(Pixel_col_y_chip, Pixel_col_y_chip(1, a_chip));

cc_top = 1; %points taken away from centre point

cc_bot = 1;

Pixel_col_y_top_chip = 1:(c_chip-cc_top);
%top half of meniscus
Pixel_mat_y_top_chip = repmat(Pixel_col_y_top_chip, a_chip, 1);
Pixel_col_y_bot_chip = (c_chip + cc_top):b_chip; % bottom half of meniscus
Pixel_mat_y_bot_chip = repmat(Pixel_col_y_bot_chip, a_chip, 1);

% top CA
for a_chip = 1:bb(1, 2) % ployfit constants ax^2 + bx + c
p_t_m_chip_e2(a_chip,:) = [polyfit(Pixel_col_y_chip(Pixel_col_y_top_chip, a_chip),
                                 Edge_pts_chip(Pixel_col_y_top_chip, a_chip), 2)];
pv_t_m_chip_e2(Pixel_col_y_top_chip, a_chip) = polyval(p_t_m_chip_e2(a_chip, 1:3),
                                 Pixel_col_y_chip(Pixel_col_y_top_chip, a_chip)); % ployfit points
p_a_t_m_chip_e2(a_chip,1)=p_t_m CHIP_e2(a_chip,1);
k_t_m_chip_e2(a_chip,Pixel_col_y_top_chip)=2*p_t_m CHIP_e2(a_chip,1)*
Pixel_mat_y_top_chip(a_chip,:)+p_t_m CHIP_e2(a_chip,2); % top half parabola
end
slope_t_m_e2=k_t_m CHIP_e2;
tithe_t_m CHIP_e2=90+atand(slope_t_m_e2);

% correction for horizontal channel
if C_angle_chip>0
  tithe_t_m CHIP_e2=tithe_t_m CHIP_e2-C_angle CHIP;
else
  tithe_t_m CHIP_e2=tithe_t_m CHIP_e2+C_angle CHIP;
end

% bottom CA
for a_chip = 1:bb(1, 2) % ployfit constants ax^2 + bx + c
p_b_m CHIP_e2(a_chip,:) = [polyfit(Pixel_col_y_chip(Pixel_col_y_bot CHIP, a_chip),
                                 Edge_pts CHIP(Pixel_col_y_bot CHIP, a CHIP), 2)];
pv_b_m CHIP_e2(Pixel_col_y_bot CHIP, a CHIP) = polyval(p_b_m CHIP_e2
(a_chip,1:3), Pixel_col_y CHIP(Pixel_col_y_bot CHIP, a CHIP)); % ployfit points
pv_b_m reshape CHIP_e2(1:c CHIP,a CHIP)=[NaN];
pv_b_m reshape CHIP_e2(c CHIP+cc bot:b CHIP, a CHIP)=[pv_b_m CHIP_e2

...
(c_chip+cc_bot:b_chip,a_chip));

k_b_m_chip_e2(a_chip,:)=2*p_b_m_chip_e2(a_chip,1)*Pixel_mat_y_bot_chip
(a_chip,:)+p_b_m_chip_e2(a_chip,2); % bottom half parabola

slope_b_m_e2(a_chip,:)=k_b_m_chip_e2(a_chip,:);
tithe_b_m_chip_e2=90-atan(slope_b_m_e2);
tithe_b_m_reshape_chip_e2(1:c_chip,a_chip)=[NaN];
tithe_b_m_reshape_chip_e2(c_chip+cc_bot:b_chip,a_chip)=
[tithe_b_m_chip_e2(a_chip,:)];
end

if C_angle_chip>0

tithe_b_m_reshape_chip_e2=tithe_b_m_reshape_chip_e2-C_angle_chip;
else

tithe_b_m_reshape_chip_e2=tithe_b_m_reshape_chip_e2+C_angle_chip;
end

A(1:c_chip+cc_bot,1:a_chip)=NaN;
A(1:b_chip,:)=pv_b_m_reshape_chip_e2; pv_b_m_reshape_chip_e2=A;
clear A

tithe_e2_top=tithe_t_m_chip_e2(:,1);
tithe_e2_bot=tithe_b_m_chip_e2(:,end);
pixel_top_e2=Pixel_col_y_top_chip;
pixel_bot_e2=Pixel_col_y_bot_chip;
figure,
plot(tithe_e2_top,'-b.');hold all;
plot(tithe_e2_bot,'-r.);
title('Contact Angle At The Walls Of The Microchannel');
legend('Top pts e2','Bottom plot e2', 'location', 'southoutside');
grid on;
xlabel('Image');
ylabel('Contact Angle (deg)');
aa=round(linspace(1,a_chip,6));
skip=5;
figure,
imagesc(data_chip(:,:,aa(1,2)));hold all;
scatter(Edge_pts_chip(1:skip:end,aa(1,2)),1:skip:b_chip,'bo');
plot(pv_t_m_chip_e2(Pixel_col_y_top_chip,aa(1,2)),Pixel_col_y_chip(Pixel_col_y_top_chip,aa(1,2)),'.r-');
plot(pv_b_m_reshape_chip_e2(Pixel_col_y_bot_chip,aa(1,2)),Pixel_col_y_chip(Pixel_col_y_bot_chip,aa(1,2)),'.g-');
title('Edge Detection');
legend('Data points','Data e2 top', 'Data e2 bottom','Data e3 bottom', 'e2', 'e3', 'e4', 'location', 'southoutside', 'orientation', 'horizontal');
grid on;
xlabel('Pixel');
ylabel('Pixel');
colormap gray;
figure,
imagesc(data_chip(:,:,aa(1,3)));hold all;
scatter(Edge_pts_chip(1:skip:end,aa(1,3)),1:skip:b_chip,'bo');
plot(pv_t_m_chip_e2(Pixel_col_y_top_chip,aa(1,3)),Pixel_col_y_chip(Pixel_col_y_top_chip,aa(1,3)),'.r-');
plot(pv_b_m_reshape_chip_e2(Pixel_col_y_bot_chip,aa(1,3)),Pixel_col_y_chip(Pixel_col_y_bot_chip,aa(1,3)),'.g-');
title('Edge Detection');
legend('Data points','Data e2 top', 'Data e2 bottom','Data e3 bottom', 'e2', 'e3', 'e4', 'location', 'southoutside', 'orientation', 'horizontal');
grid on;
xlabel('Pixel');
ylabel('Pixel');
colormap gray;
imagesc(data_chip(:,:,aa(1,4)));hold all;
scatter(Edge_pts_chip(1:skip:end,aa(1,4)),1:skip:b_chip,’bo’);
plot(pv_t_m_chip_e2(Pixel_col_y_top_chip,aa(1,4)),Pixel_col_y_chip(Pixel_col_y_top_chip,aa(1,4)),’r-’);
plot(pv_b_m_reshape_chip_e2(Pixel_col_y_bot_chip,aa(1,4)),Pixel_col_y_chip(Pixel_col_y_bot_chip,aa(1,4)),’g-’);
title(’Edge Detection’);
legend(’Data points’,’Data e2 top’, ’Data e2 bottom’, ’Data e3 bottom’, ’e2’, ’e3’, ’e4’, ’location’, ’southoutside’, ’orientation’, ’horizontal’);
grid on;
xlabel(’Pixel’);
ylabel(’Pixel’);
colormap gray;
figure,
imagesc(data_chip(:,:,aa(1,5)));hold all;
scatter(Edge_pts_chip(1:skip:end,aa(1,5)),1:skip:b_chip,’bo’);
plot(pv_t_m_chip_e2(Pixel_col_y_top_chip,aa(1,5)),Pixel_col_y_chip(Pixel_col_y_top_chip,aa(1,5)),’r-’);
plot(pv_b_m_reshape_chip_e2(Pixel_col_y_bot_chip,aa(1,5)),Pixel_col_y_chip(Pixel_col_y_bot_chip,aa(1,5)),’g-’);
title(’Edge Detection’);
legend(’Data points’,’Data e2 top’, ’Data e2 bottom’, ’Data e3 bottom’, ’e2’, ’e3’, ’e4’, ’location’, ’southoutside’, ’orientation’, ’horizontal’);
grid on;
xlabel(’Pixel’);
ylabel(’Pixel’);
colormap gray;

R^2
%calulate R^2
d=1:Pixel_col_y_top_chip(1,end); e=Pixel_col_y_bot_chip(1,1):
Pixel_col_y_bot_chip(1,end);  
f=pixel_top;  
for a_chip = 1:bb(1,2)  
y_e2_top(:,a_chip)=Edge_pts_chip(Pixel_col_y_top_chip(1,1:end),a_chip);  
yhat_e2_top(:,a_chip)=pv_t_m_chip_e2(d,a_chip);  
R2_e2_top(:,a_chip) =1-(sum((y_e2_top(:,a_chip)-yhat_e2_top(:,a_chip)).^2)/sum((y_e2_top(:,a_chip)-mean(y_e2_top(:,a_chip))).^2)); % 1 - SSe/SSt  
y_e2_bot(:,a_chip)=Edge_pts_chip(Pixel_col_y_bot_chip(1,1:end),a_chip);  
yhat_e2_bot(:,a_chip)=pv_b_m_chip_e2(e,a_chip);  
R2_e2_bot(:,a_chip) =1-(sum((y_e2_bot(:,a_chip)-yhat_e2_bot(:,a_chip)).^2)/sum((y_e2_bot(:,a_chip)-mean(y_e2_bot(:,a_chip))).^2)); % 1 - SSe/SSt  
end  
figure,  
plot(R2_e2_top,’-b.’); hold all;  
plot(R2_e2_bot,’-r.’);  
grid on xlabel(’Image’);  
ylabel(’R^2’);  
legend(’top wall’,’bottom wall’,’location’,’eastoutside’);  
scrsz = get(0,’ScreenSize’);  
figure(’Position’,[scrsz(3)/4 25 scrsz(3)/2 (scrsz(4)-100)]),  
subplot(3,1,1), plot(t_chip,Pos_chip,’b.-’);  
xlabel(’Time (sec)’);  
ylabel(’Position (mm)’);  
grid on;  
subplot(2,1,2), plot(t_chip,tithe_e2_top,’b.-’);hold all;  
plot(t_chip,tithe_e2_bot,’r.-’);  
xlabel(’Time (sec)’);  
ylabel(’Contact Angle (^o)’);  
grid on;  
legend(’Top wall’,’Bottom wall’,’location’,’southoutside’,’orientation’, ’horizontal’)

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D.2 Detection Sensitivity of the Image Exposure

clc

clear all

Image_under = imread(‘image_under_exposure.TIF’);
Image_opt = imread(‘image_optimised_exposure.TIF’);
Image_over = imread(‘image_over_exposure.TIF’);

% IMAGE HISTOGRAM INFORMATION
[counts_Image_under x] = imhist(Image_under,65536);
[counts_Image_opt x] = imhist(Image_opt,65536);
[counts_Image_over x] = imhist(Image_over,65536);

screen_size = get(0, ’ScreenSize’);
figure(’Position’, [0 0 (screen_size(3)/2) screen_size(4)]),
subplot(3,1,1), imagesc(Image_under);
set(gcf, ’ColorMap’, map)
axis off;
subplot(3,1,2), imagesc(Image_opt);
axis off;
subplot(3,1,3), imagesc(Image_over);
axis off;

figure(’Position’, [(screen_size(3)/2) 0 (screen_size(3)/2) screen_size(4)]),
subplot(3,1,1), bar(counts_Image_under(1:4095,:));
xlabel(’Pixel Intensity’);
ylabel(’Number of Pixels’);
axis([0 4500 0 10000]);
subplot(3,1,2), bar(counts_Image_opt(1:4095,:));
xlabel(’Pixel Intensity’);
ylabel(’Number of Pixels’);
axis([0 4500 0 10000]);
subplot(3,1,3), bar(counts_Image_over(1:4096,:));
xlabel(’Pixel Intensity’);
ylabel('Number of Pixels');
axis([0 4500 0 10000]);

D.3 Image Noise Reduction

clc
clear all

%BACKGROUND IMAGE
background = imread('background_image.TIF');
matrix_size = size(background);
background_max = max(background);
background_max = max(background_max);
background_mean = mean(background);
background_mean = mean(background_mean);
background_mean = repmat(background_mean,2672,4008);
SD_background = 3.*std(double(background)); %3 times the standard deviation
SD_background_max = max(SD_background) + background_mean(1,1); %max standard deviation + mean value

%BACKGROUND NOISE SUBTRACTION
Image = imread('image.TIF');

Image(Image>=500)=500; %set the max intensity in images to 500 for clear images of the noise
Image_sub = imsubtract(double(Image),background_mean); %background subtraction
background_sub = imsubtract(double(background),background_mean);
Image_sub(Image_sub<=0)=0; %set negative numbers to zero
background_sub(background_sub<=0)=0;
Image_sub(Image_sub>=500)=500; %set the max intensity in images to 500 for clear images of the noise

%THRESHOLD
SD_Image_th_sub=Image_sub;
SD_Image_th_sub(Image_sub<=SD_background_max)=0; %3 times the SD
SD_background_th_sub=background_sub;
SD_background_th_sub(background_sub<=SD_background_max)=0;

%MEDIAN FILTER
SD_Image_th_sub_med=medfilt2(SD_Image_th_sub);
SD_background_th_sub_med=medfilt2(SD_background_th_sub);

%IMAGE CAPTURED
figure; imagesc(Image);
colormap gray; %image black & white
[f g] = ginput(1); %select center point of ROI
close

% REGION OF INTEREST
figure,
imagesc(Image); %image
colormap gray; %image black & white
t = 0:pi/20:2*pi; %circular section
R0 = 800; x0 = f; y0 = g; %radius and center point
xi = R0*cos(t)+x0;
yi = R0*sin(t)+y0;
LineHandler = line(xi,yi,'LineWidth',3,'Color',[.8 0 0]);
close
roimask = poly2mask(xi,yi, 2672,4008); %binary matrix of the ROI
size(Image,2);
pr_r = find(roimask); %pixel number of the 1’s in the ROI binary matrix
mean_ROI_Image_val=Image(pr_r); %mean of the ROI
mean_ROI_Image=mean(mean_ROI_Image_val(~isnan(mean_ROI_Image_val)));
%mean of the ROI not counting NaN
std_ROI_Image = std(double(mean_ROI_Image_val
(~isnan(mean_ROI_Image_val)))); %SD of the mean
ROI_image_mean_std=[mean_ROI_image std_ROI_image];

%BACKGROUND IMAGE
mean_ROI_background_val=background(pr_r);
mean_ROI_background=mean(mean_ROI_background_val
(~isnan(mean_ROI_background_val))); %mean of the ROI not counting NaN
std_ROI_background = std(double(mean_ROI_background_val
(~isnan(mean_ROI_background_val))));
ROI_background_mean_std=[mean_ROI_background std_ROI_background];

% IMAGE BACKGROUND SUBTRACTION
mean_ROI_image_val_sub=Image_sub(pr_r);
mean_ROI_image_sub=mean(mean_ROI_image_val_sub
(~isnan(mean_ROI_image_val_sub))); %mean of the ROI not counting NaN
std_ROI_image_sub = std(double(mean_ROI_image_val_sub
(~isnan(mean_ROI_image_val_sub))));
ROI_image_mean_std=[mean_ROI_image_sub std_ROI_image_sub];
mean_ROI_background_val_sub=background_sub(pr_r);
mean_ROI_background_sub=mean(mean_ROI_background_val_sub
(~isnan(mean_ROI_background_val_sub))); %mean of the ROI not counting NaN
std_ROI_background_sub = std(double(mean_ROI_background_val_sub
(~isnan(mean_ROI_background_val_sub))));
ROI_background_mean_std_sub=[mean_ROI_background_sub std_ROI_background_sub];

% IMAGE BACKGROUND SUBTRACTION & THRESHOLD
mean_ROI_image_val_th_sub=SD_image_th_sub(pr_r);
mean_ROI_image_th_sub=mean(mean_ROI_image_val_th_sub
(~isnan(mean_ROI_image_val_th_sub))); %mean of the ROI not counting NaN
std_ROI_image_th_sub = std(double(mean_ROI_image_val_th_sub
(~isnan(mean_ROI_image_val_th_sub))));
ROI_image_mean_std_th_sub=[mean_ROI_image_th_sub std_ROI_image_th_sub];
mean_ROI_background_val_th_sub=SD_background_th_sub(pr_r);
mean_ROI_background_th_sub = mean(mean_ROI_background_val_th_sub (~isnan(mean_ROI_background_val_th_sub))) % mean of the ROI not counting NaN
std_ROI_background_th_sub = std(double(mean_ROI_background_val_th_sub (~isnan(mean_ROI_background_val_th_sub))));
ROI_background_mean_std_th_sub = [mean_ROI_background_th_sub std_ROI_background_th_sub];

% IMAGE BACKGROUND SUBTRACTION, THRESHOLD & FILTER
mean_ROI_Image_val_th_sub_med = SD Image th_sub_med(pr_r);
mean_ROI_Image_th_sub_med = mean(mean_ROI_Image_val_th_sub_med (~isnan(mean_ROI_Image_val_th_sub_med))) % mean of the ROI not counting NaN
std_ROI_Image_th_sub_med = std(double(mean_ROI_Image_val_th_sub_med (~isnan(mean_ROI_Image_val_th_sub_med))));
ROI_Image_mean_std_th_sub_med = [mean_ROI_Image_th_sub_med std_ROI_Image_th_sub_med];

screen_size = get(0, 'ScreenSize'); % (left, bottom, width, height)
figure('Position', [(screen_size(3)/4) 0 (screen_size(3)/2) screen_size(4)]),
subplot(4,1,1), surf(double(Image(1:5:end,1:5:end)),'FaceColor','interp',
'edgecolor','none');
axis tight
xlabel('Every Fifth Pixel')
ylabel('Every Fifth Pixel')
zlabel('Pixel Intensity (a.u.)')
D.4 Image Processing Time Reduction

clc

clear all

load Image_Noise_Reduction %load data file

%IMAGE CAPTURED

figure,
imagesc(Image);
title('Select ROI Center Point')
axis off
set(gcf, 'ColorMap', map)
[f h] = ginput(1); %select center point of ROI
close

% REGION OF INTEREST
figure,imagesc(Image); %image
axis off
set(gcf, 'ColorMap', map)
t = 0:pi/20:2*pi; %circular section
R0 = 800; x0 = f; y0 = h; %radius and center point
xi = R0*cos(t)+x0;
yi = R0*sin(t)+y0;
LineHandler = line(xi,yi,'LineWidth',3,'Color',[.8 0 0]);
roimask = poly2mask(xi,yi, 2672,4008); %binary matrix of the ROI
size(Image,2);
pr_r = find(roimask); %pixel number of the 1’s in the ROI binary matrix
mean_ROI_Image_val=Image(pr_r); %mean of the ROI
mean_ROI_Image=mean(mean_ROI_Image_val(~isnan(mean_ROI_Image_val))); %mean of the ROI not counting NaN
Appendix E

HSV Capillary Chip Assay Protocols

E.1 Reagents

Table E.1: HSV protocol reagents.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Abbreviation</th>
<th>Supplier</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>BSA</td>
<td>Sigma</td>
<td>A7030</td>
</tr>
<tr>
<td>3-aminopropyliethoxysilane</td>
<td>APTES</td>
<td>ABCR</td>
<td>AB110815</td>
</tr>
<tr>
<td>p-phenylene disothiocyanate</td>
<td>PDITC</td>
<td>Sigma</td>
<td>78480</td>
</tr>
<tr>
<td>Methanol</td>
<td>MeOH</td>
<td>Sigma</td>
<td>34860</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>DMF</td>
<td>Riedel-de Haen</td>
<td>34903</td>
</tr>
<tr>
<td>Sodium Carbonate</td>
<td>Na&lt;sub&gt;2&lt;/sub&gt;CO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Fluka</td>
<td>71352</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>NaHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Riedel-de Haen</td>
<td>13433</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>Fluka</td>
<td>71380</td>
</tr>
<tr>
<td>Polyoxyethylene-sorbitan monolaurate</td>
<td>Tween-20</td>
<td>Sigma</td>
<td>P1379</td>
</tr>
<tr>
<td>Pyridine</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;N</td>
<td>Fluka</td>
<td>82701</td>
</tr>
<tr>
<td>1-propanol</td>
<td>C&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O</td>
<td>Riedel-de Haen</td>
<td>24135</td>
</tr>
<tr>
<td>Trizma-base</td>
<td>NH&lt;sub&gt;3&lt;/sub&gt;C(CH&lt;sub&gt;3&lt;/sub&gt;OH)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Sigma</td>
<td>T1503</td>
</tr>
</tbody>
</table>

Table E.2: HSV buffer ingredients.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash</td>
<td>20mM Trizma base, 150mM NaCl, 0.1% (v/v) Tween-20 at pH7.4</td>
</tr>
<tr>
<td>Block</td>
<td>3% (w/v) BSA in20mM Trizma base, 150mM NaCl at pH 9</td>
</tr>
</tbody>
</table>

Table E.3: Capture antibody immobilisation assay component.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Product Code</th>
<th>Product Name</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal Anti Rabbit IgG- FAM</td>
<td>AB47048</td>
<td>Anti Rab</td>
<td>1mg/ml</td>
</tr>
</tbody>
</table>
**Table E.4: HSV-1 Assay components.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Product Code</th>
<th>Product Name</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal capture antibody (Dako)</td>
<td>12H-B011402-2</td>
<td>Anti HSV-1 PAb</td>
<td>7.3mg/ml</td>
</tr>
<tr>
<td>Polyclonal HSV-1 detection antibody (Abcam)</td>
<td>AB20437</td>
<td>HSV1 antibody</td>
<td>1mg/ml</td>
</tr>
<tr>
<td>HSV-1 antigen (Meridian Life Sciences)</td>
<td>7305</td>
<td>-</td>
<td>0.15-0.4mg/ml</td>
</tr>
</tbody>
</table>

**Table E.5: HSV-2 Assay components.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Product Code</th>
<th>Product Name</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyclonal capture antibody (Dako)</td>
<td>13H-B011602-2</td>
<td>Anti HSV-2 PAb</td>
<td>9.9mg/ml</td>
</tr>
<tr>
<td>Monoclonal HSV-2 detection antibody (Abcam)</td>
<td>AB33040</td>
<td>HSV2 gB antibody</td>
<td>1mg/ml</td>
</tr>
<tr>
<td>HSV-2 antigen (Meridian Life Sciences)</td>
<td>7705</td>
<td>-</td>
<td>0.15-0.4mg/ml</td>
</tr>
</tbody>
</table>

**E.2 Surface Functionalisation Protocol:**

1. **Surface cleaning:**
   
   The chips were first cleaned by immersing them in 1-propanol for 10 minutes in an ultrasonic bath.

2. **Oxygen plasma treatment:**

   The chips were oxygen plasma treated using a March Plasmod apparatus using 100 Watts for 10 minutes at a pressure of 0.65 atmospheres.

3. **Silanisation:**

   The chips were silanised by submerging the chips in 3% APTES in a solution of methanol:deionised water (38ml:2ml) for 1 hour at room temperature in humid conditions. Following this, the chips were rinsed 3 times with methanol and dried with a stream of nitrogen. The chips were cured in an oven at 70°C for 30 minutes.

4. **Crosslinker:**

   The cross-linker, PDITC, was applied to the APTES modified surface by adding 0.196g of PDITC into a solution of DMF:Pyridine (36ml:4ml) and immersing the chips in this solution for 2 hours at room temperature while wrapped in aluminium foil. The chips were then washed sequentially once with DMF and twice with methanol and finally dried by a stream of nitrogen.
E.3 HSV Capture Antibody Immobilisation

E.3.1 HSV-1 Capture Antibody Immobilisation Protocol:

1. HSV-1 capture antibody immobilisation:

   The polyclonal HSV-1 capture antibodies of concentrations ranging from 0.1-1mg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at room temperature in humid conditions for one hour to allow sufficient time to bind to the surface of the chip.

2. Wash:

   The chip was rinsed sequentially twice with a wash buffer and once with deionised water.

3. Blocking:

   The chip was immersed in a blocking buffer (3%(w/v) BSA in 20mM trizma base, 150mM sodium chloride at pH 9) for an hour.

4. Wash:

   The chip was washed and rinsed twice with wash buffer and once with deionised water to remove any unbound molecules.

5. QC antibody binding:

   5µl of QC antibody of concentration 0.2µg/ml (diluted with PBS from stock solution) was applied to the inlet of the chip and flowed through the chip by capillary action and was incubated at room temperature in humid conditions for 20 minutes.

6. Wash:

   The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of deionised water to remove any unbound antibodies.

7. Detection:
The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes after the last wash step.

E.3.2 HSV-2 Capture Antibody Immobilisation Protocol:

1. HSV-2 capture antibody immobilisation:

   The polyclonal HSV-2 capture antibodies of concentrations ranging from 100-1000µg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at room temperature in humid conditions for one hour to allow sufficient time to bind to the surface of the chip.

2. Wash:

   The chip was rinsed sequentially twice with a wash buffer and once with deionised water.

3. Blocking:

   The chip was immersed in a blocking buffer (3%(w/v) BSA in 20mM trizma base, 150mM sodium chloride at pH 9) for an hour.

4. Wash:

   The chip was washed and rinsed twice with wash buffer and once with deionised water to remove any unbound molecules.

5. QC antibody binding:

   5µl of QC antibody of concentration 0.2µg/ml (diluted with PBS from stock solution) was applied to the inlet of the chip and flowed through the chip by capillary action and was incubated at room temperature in humid conditions for 20 minutes.

6. Wash:

   The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of deionised water to remove any unbound antibodies.
7. Detection:

The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes after the last wash step.

E.4 FITC Dye Characterisation

Table E.6: Constants for equation for determining the FITC molar concentration of HSV-1 and HSV-2 detection antibodies.

<table>
<thead>
<tr>
<th>Assay</th>
<th>a</th>
<th>b</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>3.769e4</td>
<td>0.2959</td>
<td>0.9817</td>
</tr>
<tr>
<td>HSV-2</td>
<td>3.592e4</td>
<td>0.2972</td>
<td>0.982</td>
</tr>
</tbody>
</table>

E.5 HSV-1 & 2 Assay Protocols

E.5.1 HSV-1 Assay Protocol:

1. HSV-1 capture antibody immobilisation:

The polyclonal HSV-1 capture antibodies of concentrations ranging from 100-1000µg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at room temperature in humid conditions for one hour to allow sufficient time to bind to the surface of the chip.

2. Wash:

The chip was rinsed sequentially twice with a wash buffer and once with deionised water.

3. Blocking:

The chip was immersed in a blocking buffer (3%(w/v) BSA in 20mM trizma base, 150mM sodium chloride at pH 9) for an hour.
4. Wash:

The chip was washed and rinsed twice with wash buffer and once with deionised water to remove any unbound molecules.

5. Antigen-detection antibody binding:

5µl of HSV-1 antigens of concentration 75-200µg/ml (diluted with PBS from stock solution) were mixed with goat polyclonal HSV-1 detection antibodies of concentration of 0.02mg/ml (diluted with PBS from stock solution) labelled with FITC.

6. HSV-2 antigen/Detection antibody:

The 10µl of HSV antigen-detection antibody mixture was applied to the inlet of the chip and flowed through the chip by capillary action.

7. Wash:

The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of deionised water to remove any unbound antibodies.

8. Detection:

The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes after the last wash step.

E.5.2 HSV-2 Assay Protocol:

1. HSV-2 capture antibody immobilisation:

The polyclonal HSV-2 capture antibodies of concentrations ranging from 100-1000µg/ml in a carbonate buffer at pH 9.4 were spotted on the detection zone of the chip using a SMP 9 Stealth Microarray Pin. The deposited capture antibodies were incubated at room temperature in humid conditions for two hours to allow sufficient time to bind to the surface of the chip.

2. Wash:
The chip was rinsed sequentially twice with a wash buffer and once with deionised water.

3. Blocking:
   The chip was immersed in a blocking buffer (3%(w/v) BSA in 20mM trizma base, 150mM sodium chloride at pH 9) for an hour.

4. Wash:
   The chip was washed and rinsed twice with wash buffer and once with deionised water to remove any unbound molecules.

5. Antigen-detection antibody binding:
   5µl of HSV-2 antigens of concentration 75-200µg/ml (diluted with PBS from stock solution) were mixed with purified mouse monoclonal HSV-2 detection antibodies of concentration of 20µg/ml (diluted with PBS from stock solution) labelled with FITC.

6. HSV-2 antigen/Detection antibody:
   The 10µl of HSV antigen-detection antibody mixture was applied to the inlet of the chip and flowed through the chip by capillary action.

7. Wash:
   The chip was rinsed twice by applying 5µl of wash buffer at the inlet followed by 5µl of deionised water to remove any unbound antibodies.

8. Detection:
   The fluorescent signal was measured using the Olympus IX-50 microscope 10 minutes after the last wash step.