Proton Transport in Aqueous Ionic Solutions

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Abstract

The anomalously fast transport of protons through water has been subject of research in chemistry, biology and physics for over 200 years. However, there is still little understanding on how solutes would influence this process. Reported here is an investigation on the mobility of protons in concentrated aqueous salt solutions in a microfluidic device. The influence of weakly and strongly hydrating cations and anions is investigated for solutions of 0.1 M HCl with varying concentrations of MgSO$_4$, Cs$_2$SO$_4$ and Mg(ClO$_4$)$_2$. The proton mobility is measured by determining the proton diffusion coefficient using a pH sensitive fluorophore, imaging the microfluidic device with a fluorescent microscope. Increasing the concentration of the salts was found to have an apparent suppressive effect on the proton mobility. The salts containing sulphate were found to influence the protons most and to a similar degree. However, this influence was largely traced back to the buffering effect of the sulphate ions, which decreases the free proton concentration, and therefore decreases the apparent proton diffusion coefficient. Initial re-analysis of these results surprisingly indicates an increase of the actual proton diffusion coefficient. Mg(ClO$_4$)$_2$, not possessing any buffering property, was found to have little suppressive effect for the lower concentrations (0.5 and 1.0 M), however for higher concentrations (2.0 and 3.0 M) proton diffusion was found to be up to 5 times slower compared to the reference measurements.
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Chapter 1

Introduction

Water is one of the most important substances on earth. Life depends on it: “For wheter you are a scorpion or a cucumber, a Salmonella bacterium or a bull elephant, water is literally your lifeblood, give or take a few additives.”[1] In other words, water is one of the most crucial parts of life moreover it consists of two of the most abundant building blocks in the universe, oxygen and hydrogen. From just the chemical formula - H$_2$O - it is hard to comprehend and impossible to predict the many anomalous properties - e.g. the wonders of the hydrogen bond network and the anomalously high proton conductivity - that water has.[2–5] And even though water is such an important substance, many of these anomalies are not completely understood, therefore water is still subject to extensive research and debate in chemistry, biology and physics.

The basic properties of proton transport in water were already discovered over 200 years ago by C. J. T. de Grotthuss.[6,7] This process has great importance in nature as well as in technology. Acid-base reactions, membrane proteins acting as transmembrane proton pumps[8], but also proton exchange membranes (e.g. Nafion in fuel cells)[9,10] rely on this process. Hence large research efforts have been devoted to this topic, leading to better understanding the mechanism behind proton transport at the molecular level.[3,4,7,11–16] The dynamics of the water molecules surrounding the moving proton are found to be crucial for the mobility of the proton.[12–17] Related research has shown that these dynamics can be influenced when adding species to the system, i.e. adding hydrophobic groups[18,19] or salts to water.[21,22] These additives can have a suppressive effect on the water dynamics, which in turn can affect proton mobility. In particular, it was shown that, by suppressing the reorientation dynamics of water with hydrophobic groups[19,20], the mobility of the proton can be suppressed as well.[20] Besides this hydrophobic hydration, also ions were found to influence the reorientation of water.[20,21] Therefore, the aim of this research project is to investigate if ions can influence the proton mobility in a similar way as hydrophobic groups do, and if possible investigate how different ions influence the proton mobility.

Water is not a mere solvent as was once thought, its function as a reaction medium is not trivial, for the role water plays is an active one.[2] e.g. when considering proteins in aqueous solution: "Many proteins make use of bound water molecules..., to mediate interactions with other proteins or with substrate molecules or to transport protons rapidly to locations buried inside the protein."[25] The active role of water can be traced back to its characteristic properties, many of which are anomalous, for water has 67 presently
known anomalous properties. Examples of these anomalies are a high melting point, a high boiling point, a high density and a high viscosity, whereas ice has a relatively low density. Most of these properties can be explained by the ability of water to form up to 4 hydrogen bonds per water molecule, leading to a (fleeting) hydrogen bond network in the liquid state. Other special properties, interconnected with the hydrogen bond network, are the polar nature and the high dielectric constant of water and together they govern the characteristics of the interactions of water. The origin of these properties can be understood from the structure of water molecules. This structure is often described starting from the oxygen atom in water, which has four, tetrahedrally arranged, sp3-hybridized electron pairs, two of which are associated to the hydrogen atoms, leaving two lone pairs on the other side. Considering a tetrahedron, this gives a structure with in the middle the oxygen atom, on two of the four corners covalently bonded hydrogen atoms and on the other two corners lone electron pairs. In reality this picture is less rigid, for the hydrogen atoms and especially the electrons are not so confined to the corners of the tetrahedron. The angle formed by the hydrogen atoms and the oxygen atom is $104.5^\circ$ to $109.5^\circ$. This angle is what determines the polar nature and with that the high dielectric constant of water. The oxygen atom has a net negative partial charge, and the hydrogen atoms both have a net positive partial charge. Hence, the water molecule overall is neutral, but the asymmetric charge distribution gives rise to a net dipole moment in the molecule. Due to its polar nature, water has a high dielectric constant, meaning that around charged species, i.e. ions, water molecules tend to turn their dipole moments such that they screen the electric charge. This decreases the attractive force between oppositely charged ions, increasing the solubility.

The distribution of charge over the water molecule, together with its tetrahedral configuration, determines the characteristics of hydrogen bonds. The negatively charged oxygen atoms interact with the positively charged hydrogen atoms of neighbouring water molecules, forming hydrogen bonds leading to the formation of a hydrogen bond network. A water molecule can form up to 4 hydrogen bonds, in which case two bonds are formed at the bare corners of each tetrahedron and the hydrogen atoms, at the other two corners, are bonded to neighbouring oxygen atoms. A hexagonal lattice structure can be built using many of these tetrahedrons, with each water molecule hydrogen bonded to 4 neighbours, which produces the main crystal structure of natural snow and ice on earth. However, in liquid water this picture is prone to perturbations, leading to a water molecule having on average 3.6 hydrogen bonds that are constantly prone to change. This change, or rather the rapid breaking and forming of hydrogen bonds is the core of water dynamics and water reorientation, and this is of great importance when considering the interactions of water with itself and its solutes.

The most common example of a solute in water is a salt, for about 97% of the water on earth sits in the oceans, i.e. water containing on average 3.5% of salt. Salts are ionic compounds consisting of cations (positive) and anions (negative) bound by an ionic bond, together forming a neutral substance. Dry salts are (powdery) crystals and most

\[\text{\footnotesize\*[Although many different types of ice exist: “There are sixteen or so crystalline phases (where the oxygen atoms are in fixed positions relative to each other but the hydrogen atoms may or may not be disordered ...) and three amorphous (non-crystalline) phases.”]}\]

\[\text{\footnotesize\[29\]Giving an overall average of 1.8 hydrogen bonds per water molecule, for two water molecules share one hydrogen bond together.}\]
salts dissolve, up to a defined concentration, in water. The potency of water to dissolved salts comes from the high dielectric constant, i.e. due to their dipole moment, the water molecules, after taking apart the salts, can form hydration shells around the individual ions as shown in figure 1.1. The oxygen atoms face the cation and the hydrogen atoms face the anion, water molecules hence shield the effective charge of the ions, separating them and lowering the probability of recombination.

This high solubility of salts explains why aqueous ionic solutions are so common, for besides seawater, ions can be found in many other aqueous systems. In biology ions play an important role in different systems and on different levels, e.g. in regulating the cell volume and in transmitting neuron signals. “Furthermore it is known that adding certain ions to solutions of biomolecules can significantly change the properties of these solutions. For example, the solubility of proteins depends strongly on the ions that are present in the solution.”[27] This effect depends highly on the specific ion and is the origin of the Hofmeister series[30][31], of which an incomplete ordering in strength of this effect is given as follows:

\[
\begin{align*}
\text{Anions:} & \quad \text{SO}_4^{2-} > \text{Cl}^- > \text{I}^- > \text{ClO}_4^- \\
\text{Cations:} & \quad \text{Mg}^{2+} < \text{Li}^+ < \text{Na}^+ < \text{Cs}^+
\end{align*}
\]

The ions on the left are typically strongly hydrating and the ions on the right weakly hydrating, where the degree of hydration indicates how strongly water molecules are attracted to and affected by the ions. It turns out that strongly hydrated anions and weakly hydrated cations have similar effects on solution properties, e.g. a decreased hydrocarbon solubility, aggregation effects (salting out or precipitating solutes), an increased protein stability and an increased surface tension. These ion-specific effects, even though they
(a) Hydronium cation at the bottom.  
(b) Proton is shared between two water molecules.  
(c) Proton has hopped over to the next water molecule.

**Figure 1.2: The Grotthuss mechanism:** The red balls are the oxygen atoms, the white ones are the hydrogen atoms and the connection rods indicate the covalent bonds.

are common to many different systems, are at present not very well understood, therefore extensive research efforts are invested in better understanding these effects. Particularly unclear is whether these effects are due to direct interactions between the ions and the dissolved (bio)molecules or that, through influencing the water solvent properties, the effect of the ions is expressed.

Ions exist in many different forms, composed of single or multiple atoms, single, double or even triple charge. The proton is a special ion, it is the most fundamental ion there is, for it consists of the smallest atom - hydrogen - and has a single positive charge. However, a proton in water behaves differently from other ions. Instead of a hydration shell forming around it, the proton will be covalently bonded to a water molecule, forming a hydronium cation - \((\text{H}_3\text{O}^+)\) - schematically depicted in figure 1.2a and 1.2c. The fact that the proton is an inherent part of the water network as soon as it is introduced in water, gives rise to the unique properties of proton mobility in water. For instead of the \((\text{H}_3\text{O}^+)\) physically moving through water, the excess proton can move by hopping over from the cation to a neighbouring water molecule as depicted in figure 1.2. For this hopping to take place, the excess proton of the \((\text{H}_3\text{O}^+)\) has to share a hydrogen bond with the neighbouring water molecule. What happens next is a charge transfer process where the covalent bond becomes a hydrogen bond (figure 1.2a) and the hydrogen bond becomes a covalent bond forming the new hydronium cation (figure 1.2c), just in between the proton briefly shares a hydrogen bond with both water molecules (figure 1.2b). During this process the proton barely has to move (\(\lesssim 1\,\text{Å}\)), therefore it is effectively electron motion in the reverse direction. The proton hopping process will be described in more detail in the next chapter (chapter 2).

Simulations done by Tuckerman et al. and experiments done by Tielrooij et al. have shown that, for an excess proton to move from one water molecule to the next, about 20 water molecules need to reorient. Intuitively, a proton can only hop from one molecule
to another when the sending and the receiving molecule have the proper orientation. These results indicate that the mobility of the proton is influenced by the dynamics of many of the surrounding water molecules as well, and not only those in the first but also those in the second hydration shell. These results imply that the movement of protons in water is influenced by the ability of the surrounding water molecules to reorient. Interestingly, another implication is that, when the reorientation of water molecules is hindered or suppressed, the movement of the proton will also be hindered. This effect was shown by Bonn et al., based on observations done by Rezus et al., among others, by investigating the suppression of proton mobility by hydrophobic hydration. When dissolving tetramethylurea (TMU) - a molecule with hydrophobic groups - in water, this reduces the reorientation rate of the water molecules surrounding the hydrophobic groups, resulting in a reduction of the proton diffusion coefficient by a factor of 10, for concentrations up to 5 M.

Similar to hydrophobic groups, also ions were found to limit the reorientation of water. Tielrooij et al. demonstrated that water molecules around strongly hydrating cations or anions are limited in their reorientation. The water molecules surrounding the ions are strongly attracted, limiting part of their degrees of freedom, which is represented in figure 1.1 for the cation on the left (figure 1.1a) and the anion on the right (figure 1.1b). However, the combination of strongly hydrating ion pairs - cation and anion - was found to have a more drastic effect on the reorientation of water molecules. The authors suggested that in this case, between the anion and cation, a group of water molecules is ‘locked’ in place, represented in figure 1.3 limiting all their degrees of freedom, leading to a stronger combined effect than could be concluded from the separate effects.

The decrease in freedom of reorientation of water molecules by the presence of ions has been demonstrated, in particular the influence of strongly hydrating ion pairs is much larger than a strongly hydrating cation or anion combined with a weak counterion. Since
the reorientation of water molecules and the mobility of protons are connected, it should be possible to see a similar effect on the mobility of protons due to the presence of ions, as was demonstrated for TMU. The aim of this thesis is therefore to investigate the effect of ions on the mobility of protons in salty water, and to give a quantitative analysis of this effect. Three salts were selected for this investigation, a strongly hydrating ion pair, MgSO$_4$, and a salt for each of these strongly hydrating ions with a weakly hydrating counterion, Mg(ClO$_4$)$_2$ and Cs$_2$SO$_4$.

The method used to investigate proton transport in salty water is based on the approach previously used by Bonn et al. for measurements performed on proton transport in presence of TMU. Proton mobility is measured in a microfluidic device (a device with micron sized channels) using a photoacid and a fluorescent microscope. The microfluidic device brings an acidic and a neutral solution together in a Y-shaped microchannel. Except for the difference in proton concentration, the conditions in both solutions are the same. At the point where the two solutions meet, the protons will diffuse from one side of the channel into the other side of the channel, driven by the concentration gradient. Protons are not so easily detected by direct measurements, therefore an indirect method is used. An acid sensitive fluorophore is used to detect the presence of the protons in the microchannel. This particular fluorophore is a fluorescent molecule with a sensitivity to acid or protons, for above a certain concentration threshold the fluorophore is protonated and quenched, which happens at a $pK_a \simeq 4.3$. When illuminating the microchannel under a fluorescence microscope, the parts of the channel with a proton concentration below the threshold will light up, and the parts above the threshold will be dark, as presented in figure 1.4. Even though the fluid in the channel is constantly flowing (at approximately $v \approx 0.2 \text{ m} \cdot \text{s}^{-1}$) this image does not change over time, due to the presence in the channels of laminar (non turbulent) flow even at high flow speeds. This property enables to visualize fast (diffusive) processes, e.g. proton transport in water.

This thesis discusses measurements on the mobility of protons in salty water. Chapter 2 gives some basic background information on proton dynamics in water, details on the microfluidics including flow and diffusion, and the measurement technique used, i.e. confocal fluorescence microscopy. The methods used for the measurements are given in chapter 3, the compounds, the setup - microscope and microfluidics - , a description of
the data acquisition and the model that was used to simulate the system. In chapter 4 follows a presentation of the results: the microfluidics data and the approach used for analysis, the calibration results of the fluorophore, the modelling and fitting of the data and finally the diffusion results. After that the diffusion results are discussed in chapter 5 followed by the conclusion in chapter 6 and future work suggestions in chapter 7. The appendices contain example Matlab files that were used for performing the analysis.
Chapter 2

Background

2.1 Proton transport in water

Protons in water are a special kind of ionic species, around regular ions a solvation shell is formed as explained in the introduction. However, protons in water are inherently part of the water network. It is generally accepted that a free proton in water does not exist, rather it will always be forming a hydronium ion ($\text{H}_3\text{O}^+$) (figure 2.1), as also mentioned before (figure 1.2). Therefore generally, in chemical physics or chemistry, it is accepted that, when talking about a proton in water, the hydronium cation is meant. However, the picture of a hydronium ion is more simplistic than the full story. Protons in water have been found to form several complexes or cations that give a more accurate description of their dynamics, these are the Eigen\(^{37} \text{ (H}_9\text{O}_4^+)\) and Zundel\(^{38} \text{ (H}_5\text{O}_2^+)\) cationic complexes as found in figure 2.1. The Eigen complex consists of a hydronium cation surrounded by three water molecules that are hydrogen bonded to the three hydrogen atoms of the hydronium. The Zundel complex consists of two water molecules both hydrogen bonded to one proton. Research done in recent years indicates that even these

![Eigen and Zundel cationic complexes](image)

Figure 2.1: The Eigen and Zundel cationic complexes: The red balls represent the oxygen atoms, the white ones represent the hydrogen atoms. The black solid lines are covalent bonds, the black dashed lines are hydrogen bonds.
Figure 2.2: Structural diffusion or Grotthuss mechanism: The red balls represent the oxygen atoms, the grey ones the hydrogen atoms and the blue dashed lines represent hydrogen bonds. The yellow balls represent oxygen atoms that are part of, on the left (a) a hydronium cation, in the middle (b) a Zundel complex and on the right (c) again a hydronium cation.\cite{15}

descriptions are insufficient for explaining fully the dynamics of a proton in water, however complete agreement on this subject has yet to be achieved\cite{14,15,39} and is beyond the scope of this thesis.

The movement of protons through water is known to take place through two different mechanisms, vehicle diffusion and structural diffusion. Regular diffusion of an ionic species in water is the process of vehicle diffusion. Here the species physically diffuses through the water in such a way that the water molecules have to move aside to let the species move past. Picture this for the proton such that the hydronium cation physically has to move through a tunnel of water molecules. This process is relatively slow compared to structural diffusion, which is also known as the Grotthuss mechanism\cite{6} or proton-hopping mechanism. A hydronium ion in water is surrounded by molecules that are almost the same, with the only difference, one excess proton. The proton-hopping mechanism is the process where this excess proton hops over to a neighbouring water molecule, as is pictured in figure 2.2. This process is in practice more tricky than it sounds, for it involves the formation of Eigen ($\text{H}_9\text{O}_4^+$) and Zundel ($\text{H}_5\text{O}_2^+$) complexes in order to pass on the excess proton from molecule to molecule. Structural diffusion of protons in water is however a much faster process than vehicle diffusion. That is because for structural diffusion the major entity that is transferred is the charge,\cite{12,13} which can easily be passed on from molecule to molecule, and there is only a very small amount of mass transferred each time a proton hops. Whereas, with vehicle diffusion, the whole hydronium ion has to move, which is at least 19 times heavier than a single proton, and this estimate of the effective mass excludes the water molecules that need to move aside. The major restriction for the speed of the proton hopping process is the reorientation of the water molecules. For as mentioned in the introduction, about 20 water molecules need to reorient for an excess proton to be able to hop over from one water molecule to the next.\cite{12,13} Let me explain, the excess proton can only hop over from one water molecule to the next if the water molecules are properly aligned, as shown in figure 2.2, which involves the formation of Eigen and Zundel complexes. The formation of these complexes requires that the surrounding water molecules move along as well. This is evident from figure 2.2, where the surrounding water molecules need to reorient for the formation of the Zundel complex - from (a) to (b) - and then the new Eigen complex - from (b) to (c). Besides the seven molecules pictured in this figure, also about 13 more surrounding
water molecules need to change their orientation to get from (a) to (c). Therefore, for a proton to hop over, all these surrounding water molecules have to reorient. Which is why structural diffusion is only about one order of magnitude faster than vehicle diffusion, as it is limited by the time needed for the water molecules to reorient, instead of by the time needed for the electron transfer. Even though structural diffusion is not quite like other diffusive processes on a molecular scale, for the purposes of this investigation we assume that structural diffusion of protons is a purely diffusive process on a mesoscopic scale. Therefore in order to understand how proton diffusion can be measured, some diffusion mechanics should be considered, but first follows a brief description of the system that is used for measuring proton diffusion, leading also to some fluid dynamics.

2.2 Diffusion in microfluidics

2.2.1 Microfluidics

Microfluidics refers to the research discipline dealing with transport phenomena and fluid-based devices at microscopic length scales. The microfluidic devices in this discipline are used to study reactions in fluids on small spatial but often also short time scales. These devices consist of microchannels for fluid flow, which are regularly tens to hundreds of micrometers wide and deep, but they can be even smaller. A measure for the turbulence of fluid flow in (such) a system is the Reynolds number. For low Reynolds numbers (below about 1,000) the flow is laminar, and if this number stays constant the flow conditions stay the same. The Reynolds number depends on several parameters (see subsection 2.2.2) but it includes the characteristic flow speed multiplied by the characteristic size of the channel. Therefore, when the size of the channel is decreased by a lot, the flow speed can be much higher while staying in the laminar regime. Because of the small sized channels used in these microfluidic devices, the flow speed can be very high without reaching turbulent flow. No turbulence means that the flow is in the laminar regime, and that means that it can be described as if the fluid were flowing in layers parallel to each other. Therefore, when bringing two fluids together, mixing is not caused by convection as a result of the flow conditions. The only mixing taking place will thus be due to a gradient in the concentration and possibly (for charged species) an electromagnetic potential.

The neat behaviour of the fluid makes that microfluidic systems are ideal for studying reaction dynamics at small length and short time scales, for example diffusion limited reactions or measuring a diffusion constant. A device that is used for microfluidics - a microfluidics chip - is therefore also called a microreactor or a lab on a chip (LOC). These chips often incorporate multiple functionalities at the same time. We, however, will only be looking at a kind of microfluidics chip used for flow experiments. To demonstrate what we are looking at in our microfluidic device, figure shows an image of one of our data sets. The figure shows the Y-shaped junction of our microreactor where two samples come together. One of these samples contains a fluorescent probe, the other sample contains both the fluorescent probe as well as a high concentration of protons (pH = 1.0). This particular probe is quenched in the presence of a high proton concentration (pH < 4.0). In figure it can be seen that the signal from the fluorescent molecules is being quenched - the signal width decreases along the channel in the direction of the flow.
which is caused by the protons that are diffusing into the fluorescent (upper) part of the channel.

Understanding this image and extracting numerical results from it is the main goal of this thesis, for measuring a diffusion constant of protons - under different circumstances - was the aim of this project, and for this we use images like the one in figure 1.4. Two important aspects in microfluidics are fluid flow and diffusion as was mentioned before, therefore the next part will discuss the fluid flow in our system.

2.2.2 Flow

In previous work\cite{20}, the flow profile in the channel was assumed to be constant for the middle part of the channel. This approximation holds reasonably well in the very middle of the channel (± 5 µm), however, some more towards the edges of the channel (± 10 µm) the error in this approximation is non negligible. Therefore, during this research, it became clear that the flow profile in the channel should be taken into account. Starting from the Navier-Stokes equation for an incompressible fluid (equation 2.1), which holds for fluids that are assumed to be incompressible, homogeneous and Newtonian, which is the case for water at room temperature.\cite{40,41}

\[
\rho \left( \frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \nabla \mathbf{v} \right) = -\nabla p + \eta \nabla^2 \mathbf{v} + \mathbf{f}
\]  

(2.1)

Here \( \rho \) is the density in \([kg \cdot m^{-3}]\), \( \mathbf{v} \) represents the flow velocity in \([m \cdot s^{-1}]\), \( t \) is the time in \([s]\), \( p \) is the pressure in \([Pa = kg \cdot m^{-1} \cdot s^{-2}]\), \( \eta \) is the dynamic viscosity in \([Pa \cdot s]\) or \([N \cdot s \cdot m^{-2}]\) and \( \mathbf{f} \) represents the body forces in \([N \cdot m^{-3}]\).

The Reynolds number is derived from the Navier-Stokes equations and is an indicator for the likelihood that there is turbulence in the system. For flow in a pipe this number is given by:\cite{40,41}

\[
Re = \frac{\rho D_H v_c}{\eta}
\]  

(2.2)

Here \( Re \) is the Reynolds number (a dimensionless quantity), \( v_c \) is the characteristic (average) speed in the channel\footnote{The maximum speed is used to give an upper limit to the Reynolds number.} and \( D_H \) is the hydraulic diameter of the pipe, which is given by:

\[
D_H = \frac{4A}{P_{wet}}
\]  

(2.3)

Where \( A \) is the cross-sectional area of the microchannel and \( P_{wet} \) is the wetted perimeter, which is the perimeter of the cross-sectional area that is wet. For our geometry and flow conditions \( Re \simeq 6 \). The flow in these kind of systems is generally found to be turbulent only for \( Re > 1,000 \) and the transition is expected to lie between 1,000 and 2,000, therefore we are dealing with laminar flow.\cite{40}

The Navier-Stokes equations have been solved for quite a few different geometries, however, an analytical solution for our geometry is not readily calculated. For more
symmetric systems the solution is more straightforward, so let us consider the solutions for a round tube, which is given by:

\[ u(r) = 2U \left( 1 - \left( \frac{r}{R} \right)^2 \right) \]  (2.4)

Here \( u(r) \) is the velocity at a radius \( r \) in the channel, \( U \) is the average velocity in the channel, and \( R \) is the radius of the channel. In our case this radius corresponds to half the hydraulic diameter from equation (2.3). The flow profile for a cross-section of the round tube, given by equation (2.4), is parabolic. However, our microchannel is twice as wide as it is deep, which makes it much more similar to a rectangular channel, that has consequences for the shape of the flow profile. For a cross-section in the middle of our microchannel, the flow profile looks somewhat parabolic, but it is a bit flattened in the middle, like in a shallow channel. An analytical solution for the flow profile in a rectangular shaped channel can be found in the book by Nguyen and Wereley. However, for the scope of this research it is enough to know what kind of flow profile is inherent to the system, an analytical solution is not mandatory. It is sufficient to have a numerical approximation of the flow, which can be determined using modelling software. The modelling software used can incorporate both diffusion and flow conditions, which gives a quantitative view of our system. For more details on the modelling procedure see subsection 4.1.2. Even though the software can model the diffusion as well, a brief description of diffusion is needed to know what behaviour can be expected.

### 2.2.3 Diffusion

Considering diffusive processes generally starts out from Fick’s laws of diffusion. Using these laws we will give a short introduction to diffusion in our system. From Fick’s laws a measure for determining the diffusive behaviour of protons can be obtained, in other words, a way of determining the diffusion coefficient, \( D_{H^+} \). Fick’s 1st law of diffusion (equation (2.5)) introduces the constant \( D \) which is a measure of the diffusion speed of a certain species. This law tells us that the diffusive flux is inversely proportional to the spatial change in concentration, which implies that a concentration gradient is the driving force of diffusion:

\[ J = -D \frac{\partial C}{\partial y} \]  (2.5)

Here \( J \) is the diffusion flux in \([\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}]\), \( D \) is the diffusion coefficient in \([\text{m}^2 \cdot \text{s}^{-1}]\), \( C \) is the concentration of the species in \([\text{mol} \cdot \text{m}^{-3}]\) and \( y \) is the position in \([\text{m}]\). The position is given by the \( y \)-coordinate since this is the direction along which diffusion is considered in our microchannel (figure 1.4). There is diffusion in the \( x \)-direction, but this is irrelevant, for this is the direction of flow. The system is constantly flowing, but without turbulence, therefore the image as seen in figure 1.4 remains static over time. Therefore, the \( x \)-direction can be considered as the time direction. Using the flow speed the \( x \)-coordinates can be converted into time coordinates: At the beginning of the channel we state that \( t = 0 \), then for a speed of \( v = 0.2 \text{ m} \cdot \text{s}^{-1} \), the position \( x = 200 \mu \text{m} \) corresponds to \( t = 1 \text{ ms} \). This way the microchannel can be considered as a one dimensional (1D) system evolving over time. But to avoid confusion, the direction of diffusion, which is
perpendicular to the direction of flow, is still considered being the \( y \)-direction (equation (2.5)).

Fick’s 2\textsuperscript{nd} law of diffusion can be derived from Fick’s 1\textsuperscript{st} law combined with conservation of mass. For our system we can assume that mass is indeed conserved, for no mass is converted into energy or vice versa, or such effects are negligible. Conservation of mass is described by the following continuity equation: \( \nabla \cdot J = -\frac{\partial C}{\partial t} \) where \( \nabla \cdot J = \frac{\partial J}{\partial y} \) for the assumption that the system is 1D. This equation says that the divergence of flux of a species \( (J) \) is proportional to the concentration change \( (C) \) over time \( (t) \) of that species (with a proportionality constant of -1). Plugging Fick’s 1\textsuperscript{st} law - equation (2.5) - into the continuity equation gives Fick’s 2\textsuperscript{nd} law:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial y^2}
\]  
(2.6)

Where \( t \) is time in [s]. A way to understand the diffusive behaviour in a system is by solving equation (2.6) for the boundary conditions of the system. However this equation is for one spatial dimension and our system is a 3D channel. As mentioned before, the diffusion in our system is approximately one dimensional, namely along the \( y \)-axis of the microchannel (figure 1.4), with the time dimension along the \( x \)-axis. The third spatial dimension of the microchannel is left out of this analysis, because diffusion along the \( z \)-direction is assumed to be irrelevant in our system (for the range of interest). Thus only the middle of the channel is considered for determining the diffusion.

In line with the above reasoning the following boundary conditions can be applied to our system. At a time \( t = 0 \), \( C_0 \) represents the initial concentration (of protons) on one side, i.e. for \( y < 0 \), \( C(y, 0) = C_0 \). On the other side the initial concentration starts at zero: so for \( y > 0 \), \( C(y, 0) = 0 \). These initial conditions correspond to the situation at the start of the channel, where both solutions are just brought together. Then the system evolves over time and the concentration gradient, the driving force of diffusion, does its job. The solution of Fick’s second law for these initial boundary conditions is acquired from the book "Random Walks in Biology"[44]:

\[
C(y, t) = \frac{C_0}{2} \left( 1 + \text{erf} \left( \frac{y}{2\sqrt{Dt}} \right) \right)
\]  
(2.7)

Here \( \text{erf}(x) \) represents the error function, which is a sigmoidal function, that, in its integral form, is defined as: \( \text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} du \), where \( x = \frac{y}{2\sqrt{Dt}} \). This function is cumbersome to work with analytically, but it can be evaluated numerically. Figure 2.3 shows two numerical solutions for equation (2.7) for a starting time \( t = 0 \) (figure 2.3a) and for a later time \( t = t^\ast \) (figure 2.3b). At the beginning of the channel, a proton distribution like in figure 2.3a can be expected, more towards the end of the channel (for \( x \simeq 200 \mu m \) or \( t \simeq 1 \text{ ms} \)) the distribution in the channel will look like figure 2.3b.

The probe used for detection will be explained further in section 2.3, suffice to say here that this probe indicates a specific concentration threshold (the pKa of the probe), giving information on where this threshold is reached, which indicates a specific concentration \( ([H^+]_\text{c}) \), say \( c_1 \). From the time evolution of the concentration profile as shown in figure 2.3 we will now track this specific concentration \( c_1 \) as it evolves over time, which can be defined as the evolution of a function over time. This function we call \( \Delta d \). For this we
Figure 2.3: Concentration profiles in 1D: These images show two numerical solutions of equation 2.7, the image on the left shows the concentration distribution at time $t_0 = 0$. The image on the right shows the situation for a time $t_1 = t^*$. The vertical axis, or $C(y,t)$-axis, represents the concentration of species $C$, the horizontal axis, or $y$-axis in this case, represents the direction along which $C$ is diffusing over time.

define $y_{c_1}$, which is the position where $C(y,t) = c_1$, and $y_0$ which is the middle of the channel. The difference between $y_{c_1}$ and $y_0$ is the distribution called $\Delta d$:

$$\Delta d(t) = y_{c_1}(t) - y_0$$

(2.8)

The progression of $\Delta d(t)$ over time is related to the square root of $D$ and $t$, $\Delta d(t) \propto \sqrt{Dt}$. This is a relation that holds for the 1D model with neat initial conditions as described above, however this system is too simplistic for a quantitative analysis of our results because it assumes a starting velocity that does not change for $t > 0$. However, as will be shown in subsection 4.1.2, there is a gradient in the flow velocity in the middle of the microchannel, just after $t = 0$, which complicates the picture considerably. Nevertheless we did introduce the concept of a concentration distribution in the microchannel (equation 2.7 and figure 2.3), and a concept measure that can be used to keep track of a specific concentration (equation 2.8), which are needed for the analysis of our data.

2.3 Fluorescence and Confocal Fluorescence Microscopy

Fluorescence, or rather confocal fluorescence microscopy, is the primary technique used for the purpose of this thesis, therefore fluorescence and the technique for imaging fluorescence is briefly described below.
2.3.1 Fluorescence and fluorophores

Fluorescence is a form of photoluminescence which is a special case of luminescence: the emission of light by a substance due to an (external) cause other than heating. Fluorescence is emission of (visible) light by a substance called a fluorophore, due to absorption of light. A fluorophore is a molecule that is sensitive to light - absorbs light - of a certain wavelength, and, as a reaction to this light, emits light of a slightly lower energy (longer wavelength). With light of a certain wavelength a fluorophore can be electronically excited, promoting one of its electrons from the electronic ground state ($S_0$) to the first excited state ($S_1$), or in some cases higher excited states ($S_2$, ...), as shown in figure 2.4. From the excited state the electron loses some of its energy through vibrational relaxation or internal conversion until it reaches the lowest excited state (figure 2.4). The excess energy is passed on as heat to the surroundings. From the lowest excited state the electron returns to the ground state by emitting a photon of lower energy than the one that was absorbed. A fluorescence cycle - absorption, relaxation and emission - typically takes place on a nanosecond ($10^{-9}$ s) time scale. Figure 2.4 also shows a second photoluminescence process, phosphorescence, which involves the conversion of electronic spin. This process takes much longer than fluorescence for completing one cycle, generally from $10^{-6}$ s to several seconds, but it can even be up to hours. The latter process has no use for us, for this cycle generally takes too long, therefore we use fluorescence.

2.3.2 Confocal Fluorescence Microscopy

Fluorescence microscopy is a common technique, widely used in biology where it is very popular e.g. for imaging cell structures. The fluorophores can be attached to specific structures in the cell, making it possible to selectively image specific parts. Imaging is generally done using confocal fluorescence microscopy, since this technique is very suited for 3D imaging. True 3D resolution is accomplished by actively suppressing any signal
coming from out-of-focus planes. This is achieved by using a pinhole in front of the detector in such a way that light originating from an in-focus plane, imaged by the microscope objective, can freely pass the pinhole, whereas light coming from out-of-focus planes is largely blocked by the pinhole. With this technique it is possible to image single layers in the middle of a fluorescent sample, with a focus of less than a micrometer thick. Noise due to signal coming from the surrounding sample is minimized through the filtering effect of the pinhole. A confocal fluorescent microscope is generally powered by a laser, or multiple if multiple fluorophores, with different excitation wavelengths, are being used. Other important parts include the objective of the microscope to focus the light on the sample and a device to collect the emitted fluorescent light, for example a charge-coupled device (CCD). The last important aspect besides the optics - mirrors, lenses and filters used for properly guiding and optimizing the light (from the laser to the sample and to the detector correctly) - is the way of acquiring an image.

Different techniques can be used for image acquisition, the most straightforward way is by moving the sample through the focus in small steps, this way a full image is acquired step by step. A more sophisticated way is using movable mirrors to move the focus and scan over an area of the sample, this way the sample can sit still while making the image. An even more advanced way is by using not one, but a whole array of pinholes, set in a disc which is spinning. Such a spinning disc is called a Nipkow disc after the German inventor Paul Nipkow, who developed it in 1884 as a means of dissecting an image into a linear analogue signal that could be electronically transmitted, and then reassembled at a remote site. Nipkow created a flat “imaging” disk by stamping a series of small squares arranged in an Archimedean spiral of constant pitch on each of two disks, one for the transmitter and another for the receiver. The first microscope based on this principle was developed by Petrán et al. in 1968. This microscope used one Nipkow disc for both excitation as well as detection. Later improvements to the principle added a second disc with a microlens array, focusing the light on the holes in the Nipkow disc, reducing excitation losses thereby reducing the needed laser power. Also the size, amount of pin holes and rotation speeds of the discs was improved, for the initial Nipkow disc microscopes were not very effective fluorescence microscopes due to a lack of signal. The spinning disc setup that we used is a commercial Yokogawa Spinning Disk Scanning Unit, more details on this setup can be found in subsection 3.2.1.

2.3.3 Proton sensitive fluorophore

Fluorescence microscopy is very effective for imaging specific compounds in the middle of a cell, but it can also be used for imaging fluorescence in a microfluidics device. Fluorescence microscopy was used in previous work to image the diffusion of protons, in the same system but under different conditions. This work was used as a basis for the research described in this thesis. The fluorophore used as a proton sensitive probe is fluorescein (disodium salt), see subsection 3.1.1 for more extensive details. Besides being a fluorophore this probe is sensitive to the pH of the solution, which is what in practice we want to measure.

Some states of fluorescein are fluorescent while other states are not. Fluorescein is a molecule that is sensitive to protons in such a way that a certain concentration of pro-
Figure 2.5: **Fluorescence quenching reaction**: The reaction depicted here is that of a fluorescent molecule that goes dark below a certain pH. Or in other words, it is quenched for a certain concentration of protons.

Protons will convert the fluorescent states into non-fluorescent states, therefore quenching the fluorescence. Figure 2.5 represents this quenching reaction: the fluorophore is quenched when its surroundings has a high enough proton concentration. The transition for fluorescein lies at a pH of about 4.5. The quenching image shows all we initially want to know about the fluorophore. Below a pH of about 4.5, the fluorophore will go dark, in practice this means that fluorescein can be used as a fluorescent probe for this specific proton concentration. Further details about fluorescein, its quenching reaction, the experimental setup and other methods can be found in the next chapter (chapter 3).
Chapter 3
Methods and materials

3.1 Samples

All samples described in this thesis have in common that they contain water, for which we used purified (Millipore) water. This water was treated with pure nitrogen (N\textsubscript{2}) to reduce the dissolved amount of oxygen (see subsection 3.1.4 samples for more details) and carbon dioxide. The compounds that we used to prepare the samples are disodium salt of fluorescein C\textsubscript{20}H\textsubscript{10}O\textsubscript{5}Na\textsubscript{2} (figure 3.1a), the acid HCl and the salts MgSO\textsubscript{4}, Mg(ClO\textsubscript{4})\textsubscript{2} and Cs\textsubscript{2}SO\textsubscript{4}. The substances were purchased at Sigma-Aldrich. All concentrations are given in M = mol/L (molar concentration = mole solute per litre solution).

3.1.1 Fluorescein sodium salt

The fluorophore that we used, for detecting proton diffusion in the microchannel, is fluorescein disodium salt, C\textsubscript{20}H\textsubscript{10}O\textsubscript{5}Na\textsubscript{2} also known as uranine. The structural formula of uranine is shown in figure 3.1a. In its dry form this salt forms a reddish powder, but when it is dissolved in water, it gives a highly fluorescent, yellow solution. When dissolving the uranine in water the following reaction takes place:

\[
\text{FlNa}_2(s) \overset{\text{H}_2\text{O}}{\rightleftharpoons} \text{Fl}^2^- (aq) + 2\text{Na}^+ (aq)
\] (3.1)

Where FlNa\textsubscript{2} represents uranine, Na\textsuperscript{+} represents the sodium cation and Fl\textsuperscript{2−} represents the dianion of fluorescein (where fluorescein is FlH\textsubscript{2} = C\textsubscript{20}H\textsubscript{12}O\textsubscript{5}, structural formula shown in figure 3.1b). The dianion of fluorescein has the highest fluorescent intensity with a quantum yield of 0.93.\textsuperscript{[35]} Lowering the pH (e.g. by adding HCl) causes first the phenol to protonate (pK\textsubscript{a} \simeq 6.4) forming the fluorescein anion, which shows still considerable fluorescence with a quantum yield of 0.37.\textsuperscript{[35,54]} Further decrease of the pH will protonate the carboxylic acid (pK\textsubscript{a} \simeq 4.3) to produce neutral fluorescein which is not fluorescent.\textsuperscript{[35,54]} Below pH \simeq 2 the neutral fluorescein can even get protonated further to form the cation, FlH\textsubscript{3}+, which is also not fluorescent. However, both the neutral and cationic species of fluorescein can, upon excitation, be converted to the anion and fluoresce with quantum yields of about 0.30 and 0.18, respectively.\textsuperscript{[35]} For the purpose of our

\textsuperscript{*}CO\textsubscript{2} can lower the pH through the following process: CO\textsubscript{2}(aq) + H\textsubscript{2}O ⇌ H\textsubscript{2}CO\textsubscript{3} which dissociates through H\textsubscript{2}CO\textsubscript{3}(aq) + H\textsubscript{2}O ⇌ HCO\textsubscript{3}− + H\textsubscript{3}O\textsuperscript{+}.  

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Figure 3.1: Uranine and Fluorescein: The image on the left (a) shows the structural formula of uranine (C\textsubscript{20}H\textsubscript{10}O\textsubscript{5}Na\textsubscript{2}) or fluorescein disodium salt. The image on the right (b) shows the structural formula of fluorescein (C\textsubscript{20}H\textsubscript{12}O\textsubscript{5}) in its neutral form.

experiments the fluorescein is considered to be quenched for a pH < 4, since the effective fluorescent intensity below this pH is about 10 times lower compared to that of the anion for pH > 4 (see also subsection 3.3.2 and section 4.2). The different species of fluorescein most important for this thesis, and the protonation to neutral fluorescein are shown in figure 3.2.

Initial experiments were done using a uranine concentration of 0.25 mM, similar to previous experiments\textsuperscript{20}. With this concentration fluorescein would start precipitating at the reaction interface, most likely due to the quenching reaction with the protons. The precipitated fluorescein would form a black cloud in the middle of the field of view, interfering with the measurements. It was found that using uranine in a 100 \times more diluted form was sufficient for our measurements. Lowering the concentration solved the precipitation issue completely. Therefore all further measurements were done using a uranine concentration of 2.5 \mu M. For the sake of this thesis, the fact that fluorescein is actually a salt, will be ignored. Because of the low concentrations compared to those of the other salts this is reasonable. It will be addressed as the fluorophore, the probe or just simply fluorescein in the remainder of this thesis.

3.1.2 Protons

Hydrochloric acid (HCl) was used to supply the protons in our samples. The HCl stock solutions were made up from concentrated (Fixanal) solutions, diluted in volumetric flasks of 1 L, to solutions of 1 M, 0.1 M and 0.01 M (pH = 0, 1 and 2). These pH values were tested using a Tamson pH-meter (CG 840). The 1 M HCl solution was used to prepare the microfluidics samples of 0.1 M (pH = 1) by dilution. The samples for the titration measurements, described in subsection 3.3.2\textsuperscript{3} were prepared using the less concentrated HCl solutions. These samples were all prepared by dilution, down to a proton concentration of 10\textsuperscript{-6} M (or up to pH = 6). The samples containing high (0.5

\textsuperscript{3}Note that the anion has two tautomers and the neutral species has three tautomers/isomers. The molecules that have the highest concentration in the equilibria are shown in figure 3.2.
Figure 3.2: Protonation of fluorescein: This image shows the protonation of fluorescein (C_{20}H_{12}O_{5}) from the dianion to neutral fluorescein. The dianion (on the left) of fluorescein is the most fluorescent (quantum yield 0.93), lowering the pH first causes the phenol to protonate (pKa ~ 6.4) forming the anion (in the middle), which is also very fluorescent (quantum yield 0.37). Further decrease of the pH will protonate the carboxylic acid (pKa ~ 4.3) to produce the neutral form of fluorescein (on the right) which is not fluorescent. Effectively this means that below pH ~ 4 fluorescein is quenched.

M or more) salt concentrations, were not checked for their pH, since it is not possible to measure the pH accurately in the presence of salts at high concentrations. Instead, the added HCl, or [H^{+}\text{total}] is the measure used for determining proton content. Here the effect of the salts on the exact proton concentration was neglected, for we assumed that the salts have no or little influence on this.

3.1.3 The salts

The salt samples were prepared with one of the following salts: magnesium sulphate (MgSO_{4}), magnesium perchlorate (Mg(ClO_{4})_{2}), caesium sulphate (Cs_{2}SO_{4}) and caesium iodide (CsI). Dissolving MgSO_{4} in water forms two ions: the cation Mg^{2+} and the anion SO_{4}^{2-}. Both are strongly hydrating ions therefore the effect on the proton mobility is expected to be the largest. The solubility of MgSO_{4} in water is high and a concentration of up to 2 M can be reached. The strong hydration of this salt is the reason for the concentration limit of about 2 M for this salt. Dissolving large amounts of MgSO_{4} in water at the same time causes water to heat up, since it is somewhat exothermic, so for dissolving larger quantities (for reaching 2 M solutions) the solutions are cooled down in ice water while dissolving the salt.

Dissolving one mole of Mg(ClO_{4})_{2} in water forms one mole of magnesium cations Mg^{2+} and two moles of perchlorate anions ClO_{4}^{1−}. The cation is strongly hydrating due to its double charge, the anion is weakly hydrating since it has a single charge. The structure of the perchlorate ion is similar to that of sulphate ion, so the major difference between the two is the difference in charge. The combination of weakly and strongly hydrating anion and cation is expected to show at least some effect on the proton mobility. The dissolution of magnesium perchlorate in water is more exothermic than that of magnesium sulphate, for this salt extra care was taken with preparing solutions. The solutions were cooled using icy water while dissolving the salt. The solubility of Mg(ClO_{4})_{2} is a little over 3 M.

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\[^{1}\] An erroneous assumption which will be extensively discussed in section 5.1.

\[^{2}\] Besides these two also MgOH^{+} and HSO_{4}^{−} are formed, this is an issue which is further discussed in section 5.1.
therefore the maximum concentration that we will use is 3 M.

Dissolving one mole of \( \text{Cs}_2\text{SO}_4 \) gives two moles of the cation \( \text{Cs}^+ \) and one mole of the sulphate anion \( \text{SO}_4^{2-} \). The caesium cation is a weakly hydrating ion since it has a single charge and is a heavy, relatively big ion, the sulphate anion is strongly hydrating as mentioned before. The combination of weakly and strongly hydrating cation and anion is expected to show at least some effect on the proton mobility. The dissolution of \( \text{Cs}_2\text{SO}_4 \) in water is not exothermic, making it the easiest salt to work with. The solubility of this salt is a little over 3 \( M \), therefore we will use this salt up to a maximum concentration of 3 M.

Dissolving one mole of CsI gives one mole of the cation \( \text{Cs}^+ \) and one mole of the anion \( \text{I}^- \). Both the cation and the anion are weakly hydrating ions, making this the reference salt where little or no effect should be detected. Initial measurements were done using this salt, however its influence on fluorescein was strong, suppressing the fluorescent intensity. Therefore the measurements done on CsI were useless, because the data was too noisy to provide a decent result. CsI was used last and there was no time left to overcome this problem.

### 3.1.4 Sample Ageing & Preparation

**Ageing of fluorescein:** During the experiments the fluorescent signal was getting lower over time, which is due to the fluorescein degrading over time. This degradation is due to photobleaching and or photooxydation, therefore the solutions were prepared in dark (brown) volumetric flasks, kept in the dark whenever possible and also partially deoxigenized water was used. A large stock of demineralized (Millipore) water was put to bubble for at least 12 hours with pure nitrogen. This was primarily done to reduce the oxygen content in the water, by replacing it with nitrogen.

**Sample preparation:** The samples are primarily made by mixing stock solutions of all the different compounds in volumetric flask. By mixing the different compounds they are diluted, which is taken into account. For making a 5 mL solution of 0.5 M MgSO\(_4\), 0.1 M HCl and 2.5 \( \mu \)M Fluorescein the following are the used amounts of stock solution: 1.25 mL of 2.0 M MgSO\(_4\) solution, 0.5 mL of 1.0 M HCl solution and 0.5 mL 25 \( \mu \)M Fluorescein. This combination gives 2.25 mL of solution, which is topped off with 2.75 mL of water giving a 5.0 mL solution, which was the goal. However, for the solutions with the highest concentrations of salt this method is not possible. In those cases the solutions are made by adding water and the other stock solutions together with the weighed off dry salt. Partially dissolving the salt in a few mL of water in a 5.0 mL flask, then adding the stock solutions and topping it all off with water, as mentioned above, to dissolve the salt completely.

### 3.2 Setup

#### 3.2.1 Spinning disc confocal microscope

The setup used for imaging the fluorescence in the microchannel is a spinning disc confocal microscope, also called a Nipkow disc confocal microscope\(^{[19]}\). It is a microscope generally
used for fluorescence microscopy of living cells and other biological systems. It is a confocal microscope which uses spinning discs with multiple pinholes in order to improve spatial resolution and reduce acquisition time. In a regular confocal microscope only one or two pinholes are used, and illumination of specific points in the sample is achieved by using movable mirrors. With these mirrors the sample is scanned point by point to acquire a full image. The pinhole size (diaphragm) can be adjusted to change the axial spatial resolution of the microscope, however the acquisition time is limited by the movement speed of the mirrors. The principle of a spinning disc confocal microscope is that it, instead of using movable mirrors, uses a disc with many pinholes. The disc is spinning and the holes are arranged in such a way that during one rotation the whole field of view is illuminated once or multiple times, depending on the exact disc design. Therefore the spinning of the disc makes sure the whole sample is scanned, or rather the entire field of view, which might look like the right image in figure 3.3b. However, if the exposure time is short compared to the rotation speed it can occur that not all the intended area of the sample was exposed long enough. This causes a pattern of dark(er) lines to appear in the image of your sample. The extreme example of this is when the discs are standing still, illustrating why they should be moving, since then only a limited view of the sample is visible, which would look like the left image in figure 3.3a.

The setup we used contains a Yokogawa Spinning Disk Scanning Unit, shown in figure 3.4. This is an advanced spinning disc unit using two spinning discs, one with a microlens array to focus the light onto the holes in the second disc in order to reduce losses and enable imaging with reduced laser power. The discs contain approximately 20,000 microlenses (in the first disc) and holes (in the second disc) of about 50 μm. The discs are designed such that a 30° rotation provides a single image, and thus a 360° provides 12 images. With a rotation speed of about 5000 rounds per minute this would yield a theoretical maximum frame rate of 1000 frames per second.

The setup we used allowed for different light sources to be used. The experiments were all performed using a laser with a wavelength of 488 nm and a maximum power of
10 mW. An Acousto-Optic Tunable Filter (AOTF) is used to control the input power of the laser. The AOTF can switch between lasers but we never used more than one laser. With the AOTF the laser power can be controlled precisely and rapidly. An optical fibre sends the light from the AOTF to the Yokogawa spinning disc unit. The laser light is shaped and collimated by several lenses and mirrors, before falling on the microlens array of the first spinning disc (figure 3.4). The microlenses focus a large part of this light, through a dichromatic beam splitter, onto the holes in the second disc. The spinning disc unit/module is connected to an inverted microscope (DMIRB Leica inverted microscope). After passing through the second spinning disc, the light goes into the microscope and is focused on the sample via a condenser and an objective. The laser light excites the fluorescein in the sample, which emits light at a wavelength of about 510 nm. Part of the light emitted by the fluorescent probe is then emitted back through the objective and the condenser and will pass through the holes in the spinning disc from the other side. This light is reflected by the dichromatic beam splitter and is focusing on a cooled EM-CCD camera (C9100, Hamamatsu Photonics).

The field of view is bounded by the objective and condenser that are used. The initial experiments were done using a 40× oil immersion objective and a 1.5× condenser, which yield a field of view of about 100 µm × 100 µm. The final experiments were all done using a 20× air objective and a 1.0× condenser. This combination yields a field of view of about 300 µm × 300 µm. The objective is mounted on a computer controlled piezo crystal, making it possible to set up measurements making a scan through the sample in the z-direction in well-controlled steps.

The laser power and exposure time used for the measurements depends on various parameters: the fluorescein concentration, the objective that was used and the specific measurement. For the microfluidics measurements with the 20× objective and a fluorescein concentration of 2.5 µM, the exposure time was 100 ms and the laser power 50% (of
The titration measurements (subsection 3.3.2 and section 4.2) were done using an exposure time of 100 ms and a laser power of 14% for the 40× objective. The exposure time of 100 ms is long enough to keep an interference pattern (an intermediate between figure 3.3a and 3.3b) from the spinning discs to appear in the images.

Sample holder The sample was placed in a custom made holder and then mounted on a microscope stage. The stage of the microscope could be controlled by a joystick to move it in the x and y directions. The movement was registered by the motor driving the stage, which displayed x and y coordinates in arbitrary units. The relation of these units to image pixels and micrometers was relevant for part of our research. While using the 40× objective and the 1.5× condenser the movable stage was used to image a sufficient part of the channel in the x-direction. Therefore the x displacement had to be determine as precisely as possible for stitching the channel images back together. Using the 20× objective and the 1.0× condenser, it was no longer needed to move the sample, since a large enough part of the channel was visible in just one full image.

Image recording Recording the images as well as controlling most of the setup was done using software called VoxCell. This software was used to control the spinning disc part of the setup as well as the AOTF and the camera settings. Also the piezo crystal was controlled and adjusted using this program. The control of the shutter and acquisition of the images was also controlled from VoxCell. The data images were saved in a stacked *.tif file format. These images could be opened and processed using ImageJ. This program was mostly used for a quick way to view the images. It was also useful for quickly determining, from the stacked images, which was the best layer to use for the analysis. This program was also used for determining the amount of pixels per image, or the pixel to micrometer ratio using images of a calibration slide with a predefined grid (Thor Labs).

3.2.2 Microfluidics setup

The experiments were performed using a glass microfluidic device tailor made by Micronit for our purpose. The microfluidic device is a glass microreactor with a Y-shaped microchannel etched into the glass, the design of the channel is shown in figure 3.5. The design is straightforward, two out of five possible inlets are used, these two inlets connect to the micro channels. The two arms of the Y-junction meet each other under an angle of 90°. From this Y-junction the channel goes on straight and ends at the middle most of 5 possible outlets. A cross-section of the channels looks like a rectangle with two rounded off corners (figure 3.5b). The straight part, at the coverglass, is about 48 µm wide and in the middle it is about 23 µm deep.

The microfluidic device is fed by two 500 µL Hamilton TLL (Teflon Luer Lock) syringes (Sigma-Aldrich). These syringes are driven by a dual syringe pump, which can drive max two syringes simultaneously at the same speed. The flow rate of the syringe pump for the 500 µL syringes ranges from 0.1 µL·min⁻¹ up to 17.65 µL·min⁻¹. The flow rate we used for all the microfluidic experiments is 5.0 µL·min⁻¹. Each syringe is pumped at this rate, meaning that the flow rate after the junction is twice this rate (10.0 µL·min⁻¹). The maximum speed after the junction is \( v_{\text{max}} \approx 0.2 \text{ m} \cdot \text{s}^{-1} \). This \( v_{\text{max}} \) was calculated for an approximation of the geometry of our channels by a rectangular
Figure 3.5: The design and cross-section of the microfluidic device: Figure (a) on the left shows the design of the microfluidic device. The circles represent the inlets and outlets (three used, others not used), the long black dashed line represents the middle of the chip. A cross-section of the channel along the $yz$-plane (along the middle line in figure (a)) is shown in green in figure (b) on the right.

The connections between the syringes, the silicon tubing and the microfluidics device use two different sets of connectors. The syringes and silicon tubing are connected together by Luer Lock screw connectors, these fix the tubing in the connector which is then screwed into the Teflon Luer Lock of the syringes. These connections should be screwed as tight and as dry as possible, to prevent leakage. Part of the experimental error in our measurements can come from differences in the flow due to (minor) leakage of the connectors. The other end of the tubing is attached to the microfluidics device using another set of connectors and a special holder for the glass device. The holder for the glass microchip was custom made by the workshop at AMOLF. The microchipholder consists of two parts, one part metal, one part a synthetic material (for protecting the glass chip), which are screwed together using 6 bolts. In the middle of the holder is a semi square hole, which is the window for the microscope objective. The microchip fits neatly in a space between the two parts, left out on either side of the objective window. There are screw holes positioned precisely above the inlets of the glass chip, which are the holes for the connectors to the microchip. These connectors consist of two separate parts, one part that sticks into the inlets, the other is a screw to pushes the first part into the inlet. The inlet part is a small black rubbery part with a hole where the silicon tube sticks through it. This part is placed at the end of the tube. The other part is a synthetic screw, with a hole for the tubing, and a grip part and a screw part. This screw is placed over the tube before the rubber part. This way the screw pushes the rubber part into the inlet of the microchip when it is screwed into the holder. Screwing the connectors tightly fixes the glass chip in place as well as ensures minimal leakage between the rubber connector and the glass chip. The speed in the microchannels is high and the channels are small, therefore the pressure is high, so the system is quite prone to leakage.
Tool tip  For keeping the system from leaking at the rubber connectors a hand rule is used. First, the screw connector is screwed finger tight. Then a special tool is used. With this tool, the screw is turned another one third of a turn. Here one should be careful: screwing "finger tight" does not mean, "as tight as you can" with just your fingers. Instead it means screwing it with loose fingers until it goes no further. Then the tool is used to screw the connector leakage free tight. I made the mistake of screwing too tightly twice. This caused the microchip from breaking off at the inlet, breaking it beyond repair.

Cleaning  Before and preferably also after use all parts should be cleaned to make sure that as little waste as possible is left behind in any part of the system. Cleaning the chip is done by flushing demineralized water through the channels at a high speed. At least an amount of 500 $\mu$L per syringe should be used for this. For the cleaning it is convenient to switch to larger syringes since this allows for a much higher flow rate. Using 1 mL syringes the flow rate is doubled, and the maximum flow rate per syringe is now 35.3 $\mu$L·min$^{-1}$. A good clean is done by flushing at least two times 1 mL per syringe. Then the tubing. This is done by keeping the side of the syringe connected and flushing through the tubes with demineralized water. Do this at least 5 times 1 mL per tube. This leaves the syringes and the connectors. The connectors can be put in a flask and soaked in demi water, then put in a sonicator to sonicate for about 10 minutes. This makes sure that remaining salt is solved and removed from the connectors. In order to get rid of the water on the connectors, the same thing can be done, however now using acetone instead of water.

3.3 Data acquisition

In this section the data acquisition for the microfluidics measurements and the calibration measurements are described.

3.3.1 Acquisition microfluidics

Thus far the spinning disc confocal microscope used for measuring the fluorescence in the microchannel and the microfluidics setup have been described. The technical part of acquiring the microfluidics data is described in short in this subsection. With the microfluidics set up and ready to go a measurement is done by letting the samples run trough the system for at least 10 minutes, in order to let the sample fill the entire system, flushing away all (if any) other fluids and or air bubbles. After this time the flow in the channel has settled to a static distribution over the channel. In that case, an image from the middle of the microfluidics channel at the Y-junction will look like the one in figure 4.1.

Measurements were done by making a z-scan through the microfluidic channel, using the piezo crystal in the confocal microscope. The scan is made by making 42 steps of about 1 $\mu$m starting below the channel (on the flat side) going trough the whole channel and finishing above the channel (the curved side). From this z-stack of images only one layer is selected for analysis per measurement. The reason for only analysing one layer is that this simplifies the analysis significantly. Also the modelling of our system becomes much
more straightforward if we only have one layer to analyse per measurement. However, since only one layer per measurement is used for analysis it is crucial to determine the position of this layer correctly. As was discussed in subsection 2.2.2, the flow speed through the channel is not one constant value, the flow depends a lot on the position in the microchannel. Therefore, to be able to treat each data set with the same analytical procedure, assuming one flow profile for each set, the layer used for analysis should be the same for each measurement. With the information from the z-scan it can be made sure that data is collected at the same z-coordinate for all the experiments. A more detailed description of this selection procedure is discussed in subsection 4.1.1. When the right layer is selected, the data to determine $\Delta d$ can be extracted from this layer (also described in more detail in subsection 4.1.1).

### 3.3.2 Acquisition for fluorescein calibration

The fluorescein calibration measurements, from this point on called the titration measurements or titrations, were performed to calibrate the response of fluorescein to the proton concentration. Fluorescein, a.k.a. the proton probe, is treated as an on-off indicator. The probe is ’on’ below a certain concentration threshold and it is turned ’off’ when the concentration of protons rises above this threshold. This quenching reaction is described in more detail in subsection 3.1.1. However, the presence of salts added to the mixture of water, fluorescein and hydrochloric acid, can change the exact threshold value. For different concentrations of salts, the probe might actually be indicating different concentrations of protons. This effect can change the values of $\Delta d$, which affects the interpretation and in the end the value we measure for the diffusion coefficient. Calibrating the probe for each individual salt concentration is therefore necessary for the correct interpretation of the microfluidics data. The titration procedure is described here, the results of these measurements can be found in section 4.2.

The titrations were done in a manner and with a purpose that is slightly different from regular titration measurements. Instead of adding a base to an acidic solution to increase the pH($= -\log[H^+]$), we prepared solutions of decreasing $[H^+]$ by diluting concentrated HCl solutions. The response of fluorescein was determined by preparing solutions with a range of pH = 2 to 6 to find the transition pH of fluorescein. From the literature we know that this transition lies at pH $\approx 4$.\[34,35\] However, the literature also suggests that the transition pH can change due to the presence of (other) salts.\[35\] Therefore the probe had to be calibrated for the presence of the different salts. Solutions with different concentrations of salt and HCl were prepared. Each sample was measured with the spinning disc confocal microscope to determine the intensity of the fluorescence as a function of $[H^+_{\text{total}}]$. The precision of the measurements was increased by first determining the $[H^+_{\text{total}}]$ of quenching roughly by using $-\log [H^+_{\text{total}}]$ steps of 0.5 to 1. Then the density of data points close to the quenching point was increased, decreasing the steps in the $-\log [H^+_{\text{total}}]$ to 0.1. A further decrease of the step size is much more time consuming, while not increasing the precision much. The transition generally takes place over about 4 steps of 0.1 $-\log [H^+_{\text{total}}]$ points.

The acquisition for the titration is done without using the microfluidics setup and is therefore much more straightforward. The samples were measured using small petri dishes with a thin glass window (of about 0.5 mm). A drop of about 70 µL was deposited
in the middle of this dish and then measured using the microscope. The measurement was done as fast as possible to keep the influence of sample to air exposure to a minimum, however some time was needed per measurement to determine the right focus. The correct focus was determined manually by checking for a sharp increase in intensity as the focus is moved from the glass petri dish (usually some signal from scattering) into the actual sample. Then a stack of 5 images was recorded, each going 2 µm deeper into the sample making sure that signal from the "bulk" sample is recorded. The average intensity of the middle area of the middle image of this stack was determined using Matlab (see appendix for code), unless there were indications that the measurements had gone wrong (specks of dust e.g.) then a more suitable area and or layer was selected manually.

3.4 Modelling (with Comsol Multiphysics)

The modelling software called Comsol Multiphysics was used to simulate the microfluidics system. This software can be used to simulate almost any kind of system, incorporating almost any physical and or chemical model. The software was used to combine flow and diffusion and make a 2D simulation of the microfluidics channel. In order to develop an accurate model, several boundary conditions had to specified. First of all, the structure of the microfluidic device, using some approximation, second, the flow conditions and last the initial conditions for diffusion.

The geometry of the microchannel was approximated assuming a rectangular cross-section of the channel with the following dimensions: 44 µm × 23.5 µm. Furthermore, only that part of the channel that was of interest was specified in the model, i.e. both inlets and about 230 µm of the main channel. Through this structure water was set to flow (at \( T = 20^\circ C \)), with input flow rates at both inlets set to \( 8.33 \cdot 10^{-11} \text{ m}^3/\text{s} = 5 \text{ µL/min} \), specifying laminar flow conditions. Given the details about the structure of the microchannel, the fluid properties and the initial flow conditions, the Comsol software allows to simulate the flow profile in the channel, of which the result is shown in figure 4.8 in subsection 4.1.2.

For the modelling of the flow in the microchannel certain approximations were made. To avoid the need for a server and extremely long calculation times the system was approximated by a 2D model, rather than a 3D one. For the flow in the channel this means that the boundaries that are most important for the flow profile generally get neglected, therefore a "shallow channel" approximation was applied. A volume force term is including in the Navier-Stokes equation (equation (2.1)), changing the fluid-flow equations, to account for the drag caused by these walls. This volume force term is of the form:

\[
F_\eta = -12 \eta \cdot \frac{\mathbf{V}}{(d_z)^2}
\]  

(3.2)

where \( \eta \) is the fluid’s dynamic viscosity \([\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}]\), \( \mathbf{V} \) is the velocity field \([\text{m} \cdot \text{s}^{-1}]\), and \( d_z \) is the channel thickness \([\text{m}]\). Equation (3.2) represents the resistance that the upper and lower boundaries place on the flow; however, it does not account for any changes in velocity due to variations in the cross-sectional area when the channel thickness differs. This is the most accurate approximation without having to model the system.
in 3D, without this approximation, the flow profile would be parabolic as in a cylindrical tube, rather than including a flattening at the top of the distribution (subsection 2.2.2).

The next step was specifying the initial conditions for the diffusion. This part was simplified as much as possible. The assumption made for simulating the proton diffusion in the channel, was that it could be described with a starting concentrations, of 0.1 M for one inlet and $10^{-7}$ M for the other inlet, of an arbitrary species with an adjustable diffusion coefficient $D$. By manually setting the diffusion coefficient each time, to a value range reasonable for proton diffusion (see section 4.3), the Comsol software could work out the diffusion of the protons from one part of the channel (initial proton concentration of 0.1 M or pH = 1), to the other part of the channel (initially filled with demineralized water and thus a proton concentration of about $10^{-7}$ M or pH = 7). The diffusion model produced concentration maps of the channel for the different $D$ (section 4.3). These concentration maps were translated to pH-maps (or $-\log [H^+_{\text{total}}]$, -maps), of which an example is depicted in figure 4.9 in the section 4.3. Using these concentration maps it was possible to simulate similar results as were obtained from the microfluidics measurements, which could then be compared for the range of diffusion coefficients used for modelling. The procedure used for the data analysis and the comparison with the model in order to determine a diffusion coefficient is described in detail in the next chapter (chapter 4).
Chapter 4

Results

4.1 Data analysis

In this section the data analysis of the microfluidics measurements is presented. Data from one of the measurements is used to demonstrate how each data set was treated. The data used for this demonstration comes from a measurement on 0.5 M of MgSO₄.

4.1.1 Data preparation

The data from the microfluidics measurements were acquired by making a z-scan through the microchannel (section 3.3) generating a stack of images of the channel. The analysis is performed on just one of the images or layers in this stack, simplifying the procedure considerably. However, it is important to analyse the same layer for each measurement because the flow profile is different for each layer, and unless this difference in flow is taken into account, the analysis gives a different result for each layer. Taking into account the flow for each layer would take a lot more time, therefore an approximation of the flow for one layer is made and only one layer is analysed. This approximation might be inaccurate, but as long as the same layer is selected for each measurement, the associated error is systematic, allowing for relative comparison of the measurements.

The z-scan is set to go from below the bottom to above the top of the microchannel, because this gives the most information about the location of each layer. The absolute z-position of the layers in the stack of images is extracted as follows. The average fluorescence intensity in the middle of the fluorescing inlet is determined for each layer in the stack. The intensity from each layer is plotted as a function of the layer or z-position, and from this plot the upper and lower edges of the channel are determined. With this information the layer situated 10 µm from the top layer of the channel is selected for analysis. The selected layer can now be used to determine the Δd-curve, which is our first goal. The code used for determining the Δd-curve and its analysis, including fitting to the model, was written in Matlab and can be found in Appendix A. The different steps in this code are described below using several images to illustrate the analysis procedure.

Figure 4.1 shows the image of the selected layer after loading from the stack of images. The figure shows the Y-junction of the microchannel as a fluorescence intensity map. The difference between the neutral (bright) and acidic (dark) inlet is clearly visible, although the dark part is completely dark, the fluorescein is completely quenched. The next step is
Figure 4.1: Microfluidics fluorescent image for 0.5 M of MgSO₄. This figure shows the Y-shaped part of the microchannel for a measurement on 0.5 M of MgSO₄. It is an image taken from the middle - in the z-direction - of the channel, selected from a stack of images like this one. The sides and the middle of the channel are indicated on the y-axis at 22, 0 and −22 µm. The grey-scale above the image indicates the fluorescent intensity in arbitrary units. The part of the channel shown is almost 350 µm in length.

to determine the edge of the microchannel, which is non trivial, for the channel is slightly tilted compared to the horizontal axis of the image. The angle of tilt is determined by fitting the edge of the fluorescent side of the channel. The final result of this fitting is shown in figure 4.2, which shows the edge of the channel as a bright white line. This white line - called the rounded cutting line - is shown, together with the cutting line, in figure 4.3. The cutting line is the initial result of fitting the edge of the channel, however, the image can only be cut out in whole pixels, therefore the rounded cutting line is made. Using this rounded cutting line we introduce a non-systematic error of 0.5 pixel in the data. A correction for this error, using the lines in figure 4.3, will be applied at a later stage.

Determining the edge of the channel is important for it is the anchor for determining the middle of the channel y₀, and the precision of Δd depends directly on the accuracy of y₀. Once the edges of the channels have been determined, the outside region can be discarded from the image. The lower edge of the channel is determined by taking into account a width of 44 µm. This cut results in figure 4.4, which depicts the main channel. Figure 4.4 is used to determine the Δd-curve. Along the main channel, for each x-pixel, a cross cut of the intensity profile is fitted with a sigmoid function $f_{\text{sigm}}(y)$, which is given by:

$$f_{\text{sigm}}(y) = I_0 + \frac{I_1}{1 + e^{-(y-y_1)/\delta}}$$  \hspace{1cm} (4.1)

In this equation $I_0$ is some base intensity, $I_1$ is the difference in intensity between the minimal and maximal intensity, $y$ is the y-coordinate corresponding to the $y$-position in the channel (figure 4.4), $y_1$ is the position corresponding to the intensity value at half maximum and $\delta$ is the width of the sigmoid. The sigmoid function (equation 4.1) is used
for fitting the intensity profiles in figure 4.4 rather than e.g. the error function, because we measure a certain \([H^+]\) thresh-hold (see also subsections 3.1.1, 3.3.2 and section 4.2), rather than the concentration profile of \(H^+\), for which the error function should be used.

The data used to determine \(\Delta d\) comes directly from the \(y_1\) values in the sigmoid function (equation (4.1)). Some of the fits to the intensity cross cuts are shown in figure 4.5. The cuts in this figure are taken at regular intervals along the channel (at \(x = 50, 100, 150, 200, 250\) and 300 \(\mu m\). In order to determine \(\Delta d\) data, the cross cut for every row of pixels along the channel in figure 4.4 is fitted. This leads to a data set \(y_1(x)\), which is used to determine \(\Delta d\) data \((x) = y_1(x) - y_0(x)\) (where \(y_0(x)\) is the middle of the channel). A correction is needed for the cutting performed in the previous step of the analysis, where we introduced a non-systematic error of 0.5 pixel, as can be seen from the jagged steps in figure 4.4. These steps are due to the tilt in the initial image as explained previously. A correction is made for these steps using the cutting line and the rounded cutting line, which are shown in figure 4.3, in order to obtain a smooth \(\Delta d\) data-curve in the final result. The correction function used to apply this correction is defined as:

\[
F_{\text{correction}} (x) = f_{\text{rounded cut}} (x) - f_{\text{cut}} (x)
\]  

(4.2)

The correction function is defined as the rounded cutting function \(f_{\text{rounded cut}}\) (figure 4.3) minus the cutting function \(f_{\text{cut}}\) (figure 4.3), and the correction function is shown in figure 4.6. The values of this function are subtracted from the extracted \(\Delta d\) data to let it line up properly.

The values \(y_1(x)\) are extracted from \(f_{\text{sigm}}(y)\), the difference with \(y_0(x)\) is determined and the correction function \(F_{\text{correction}} (x)\) is subtracted, giving finally \(\Delta d\) data \((x) = y_1(x) - y_0(x) - F_{\text{correction}} (x)\), where \(y_0\) is the middle of the channel, which is half the width of our image in figure 4.4. This procedure then leads to the results in figure 4.7. This figure
Figure 4.3: Cutting lines. The lines used for defining the cutting of the fluorescent edge of the microchannel. The blue line represents the edge of the channel as defined from fitting, the red line defines at what pixel in figure 4.2 the cut is made. The blue line defines the edge of the microchannel, the red line is the actual cutting line, for it is rounded to whole pixels.

Figure 4.4: Main channel image: This images shows the main channel as it is cut out form the microfluidics data. There are steps visible in the image, The steps are due to the limited resolution of the images and the fact that the channel is tilted with respect to the horizontal axes of the initial image. This non-systematic error of 0.5 pixel was introduced for creating this image and being able to analyse it. The correction for this error is applied in the next step.
Figure 4.5: Sigmoid fits of intensity profiles: Fitting of the intensity profiles for cross-sections at several points along the channel in figure 4.4. The blue circles represent the intensity data points, the red curves represent the sigmoid fits (equation (4.1)).

Figure 4.6: Correction function: In blue the correction function, used for correcting the cut done to obtain figure 4.4, is shown. This function, given by equation (4.2), is based on the lines shown in figure 4.3. The cutting procedure could only be done in steps of whole pixels, giving a stepped result (see also the red line in figure 4.3), this function is used to correct for these steps.
4.1.2 Modelling (in Comsol)

Comsol (for more details on Comsol see section 3.4) is modelling software that can simulate the flow and diffusion profiles in a simplified microchannel geometry. From the structure of the microchannel, the fluid properties and the initial conditions for the flow (section 3.4), comsol can model the flow in the channel. The result of this modelling is shown in figure 4.8 as a flow speed map through the microchannel. The colours in this figure indicate the different flow speeds in the channel. At the edges the flow speed approaches zero. In the middle of the channel the flow speed is the highest ($v_{\text{max}} \approx 0.2 \text{ m/s}$ for a flow rate of 10 $\mu\text{L/min}$).

Using the flow in the channel, the program determines the diffusion of the protons, from the side of the channel where protons flow in at a concentration of 0.1 M (pH = 1), to the upper part of the channel, where demineralized (neutral) water ($[H^+] = 10^{-7} \text{ M} \Rightarrow \text{pH} = 7$) is flowing in. Modelling of the diffusion combined with the flow produces a concentration distribution in the channel, which is converted to a concentration map of the protons in the channel on a logarithmic scale, pH. The resulting pH map is shown in figure 4.9. The upper part of the figure shows pH = 7 in bordeaux red, the lower part shows pH = 1 in blue, a rainbow of colours indicates the transition from high to low pH. The figure also shows the pH = 1 area is spreading along the channel, indicating the protons diffusing into the upper side of the channel. Using this map of the concentration of protons in the channel, the diffusion coefficient can be determined. For determining $D_H$, the diffusion coefficient in Comsol can be varied by manually inserting different
Figure 4.8: Comsol flow speed map of microchannel: The figure shows the flow in the microfluidic channel calculated by the model. The colors indicate the different flow speeds inside the channel. At the edges the speed is zero, or close to zero, this is depicted in blue. In the middle of the main channel, depicted in red, the speed is the highest ($v_{\text{max}} \simeq 0.2 \text{ m/s}$). The fluid in the middle of the channels, just after the Y-junction, experiences an acceleration. This is where the two flows meet and are ‘squeezed’ together into the main channel. In both inlets the flow speed is 5 $\mu$L/min, in the main channel the speed is 10 $\mu$L/min.

Figure 4.9: pH distribution map: This rainbow figure is a map of the concentration of protons in our model. It simulates the proton diffusion we see in our microfluidic channel. The colors in the channel indicate the concentration of protons in terms of pH. The blue at the bottom of the channel indicates a pH of 1 and the bordeaux red at the top indicates a pH of 7.
values, which results in different concentration maps. The next step is to determine which coefficient fits the data best. However, before fitting the results from the simulations to the microfluidics data, one more ingredient is needed. This ingredient has to do with the fluorophore used as a proton probe. Knowing the proton concentration that is probed is essential for the right interpretation of the data.

### 4.2 Titration measurements

The extraction of the $\Delta d_{\text{data}}$-curve, from the fluorescent images, along the $x$-direction of the microchannel (figure 4.7) was shown in subsection 4.1.1. To determine a proton diffusion coefficient ($D_{H^+}$) from this data, two more ingredients are needed. The first is the results from the titration measurements, to determine the concentration of protons that is being probed with the fluorescein (see also subsection 3.3.2). The second is the results from the modelling (section 3.4 and subsection 4.1.2) to quantify the results and extract a diffusion coefficient, which is only possible for each measurement by combining these two ingredients. Here follows the first ingredient.

**Figure 4.10: The titration curves for fluorescein**:
These curves represent the titration curves for fluorescein (2.5 µM) in H$_2$O. Intensity in arbitrary units plotted against, from left to right, decreasing HCl concentration. pH indicates [HCl] on an inverse log scale, since these are equivalent in this case. (b) The measurements were redone to check reproducibility.

The titration measurements give the response of the fluorescein to the concentration of hydrochloric acid (HCl). The measurement procedure is described in more detail in subsection 3.3.2. Figure 4.10 shows the titration curve for fluorescein in H$_2$O as a function of pH. Since HCl is a strong acid we can assume that it is completely dissociated, therefore [HCl] = [H$^+$], where [H$^+$] is the proton concentration in water. The concentration is represented on a logarithmic scale, plotting the fluorescein intensity as a function of pH = $-\log[H^+]$. So pH corresponds to the HCl concentration on an inverse logarithmic scale. For a solution with only fluorescein (2.5 µM) in H$_2$O we find a transition between the on-off state of fluorescence at pH = 4.0 – 4.5 (figure 4.10). These values are in
Figure 4.11: Titration curves for the sulphate salts: These curves show the results for the titration measurements for the sulphate salts, MgSO$_4$ in (a) on the left and Cs$_2$SO$_4$ in (b) on the right. The intensity (in arbitrary units) is plotted versus the concentration of added HCl on an inverse log scale: $-\log [H^+_{\text{total}}]$. The curve for no salt, in pink, is added as a reference.

Figure 4.12: Titration curves for Mg(ClO$_4$)$_2$: These curves show the results for the titrations done for Mg(ClO$_4$)$_2$ 0.5, 1.0 and 2.0 M, the intensity (in a.u.) is plotted versus the concentration of added HCl on an inverse log scale: $-\log [H^+_{\text{total}}]$. The curve for no salt, in pink, is added as a reference.
agreement with the values found in the literature\(^{34,35}\). Also the transition is sharp - the fluorescent signal goes up a factor of almost 10 between pH = 4.0 and pH = 4.4 - hence we can treat it as an on-off transition (see also chapter 3). The reproducibility of the results is exemplified by the comparison of figure 4.10a and 4.10b.

Sjöback et al. showed that NaCl has influence on the behaviour of fluorescein in aqueous solutions\(^{35}\). They measured significant changes in the absorption spectra of fluorescein for NaCl concentrations of up to 1.0 M\(^{35}\). These results indicate that the salts in our solutions are likely to have an influence on fluorescein as well. Therefore it was necessary to check if a change in behaviour was present, and if present to quantify the influence of the salts on the fluorescein. The titration measurements were repeated for each salt and concentration, primarily to determine the transition point for each case, but also to check the sharpness of the transition. Figure 4.11 and 4.12 contain the results obtained from the titration measurements: MgSO\(_4\) 0.5, 1.0 and 2.0 M in figure 4.11a, Cs\(_2\)SO\(_4\) 1.0, 2.0 and 3.0 M in figure 4.11b and Mg(ClO\(_4\))\(_2\) 0.5, 1.0 and 2.0 M in figure 4.12. The fluorescence intensity is plotted as a function of $-\log[H^{+}_{\text{total}}]$.

Figures 4.11a, 4.11b and 4.12 also contain the curve for the titration measurement in absence of salts. This allows to highlight the shift of the transition $[H^{+}_{\text{total}}]$. For example, the transition point for 0.5 of M MgSO\(_4\) (figure 4.11a) has shifted down to a value of $-\log[H^{+}_{\text{total}}] = 3.5 - 3.9$. For increasing salt concentration this shift is even larger. The other two salts (figure 4.11b and 4.12) show the same trend. The slopes of the transitions differ for each salt, but in general the transition is similarly sharp to the case with no salt.

### 4.3 Modelling and fitting

The results of the titration measurements (figure 4.11 and 4.12) indicate that a correction for the shift in the transition $[H^{+}_{\text{total}}]$ is needed. This correction is crucial for the correct interpretation of the microfluidics data. The modelling of flow and diffusion in the microchannel is described in subsection 4.1.2. The model gives a $[H^{+}_{\text{total}}]$ distribution through the channel, shown as a concentration ($[H^{+}_{\text{total}}]$) map in figure 4.9. From this concentration map, using the transition $[H^{+}_{\text{total}}]$ values from the titration measurements, an on-off image of the microchannel is obtained. Concentration values above the transition point correspond to the on-state (fluorescent-state), whereas values below the transition point correspond to the off-state (quenched-state). The result of this approach, as seen in figure 4.13, shows good agreement with the microfluidics data (figure 4.13 looks similar to figure 4.1). However, for exact comparison with $\Delta d_{\text{data}}$, the $\Delta d_{\text{model}}$-curve should be obtained. The on-off image displays how $\Delta d_{\text{model}}$ can be extracted. The $\Delta d_{\text{model}}$-curve is the difference between the middle of the channel along the x-direction and the boundary separating the on- and off-state in figure 4.13.

For determining the proton diffusion coefficient $D_{H^+}$, $\Delta d_{\text{data}}$ and $\Delta d_{\text{model}}$ should be compared. For this comparison the diffusion coefficient (D) of the model is set to 12 different values in a range that is likely for proton diffusion. The range used is $D = 5 \cdot 10^{-10}$ m\(^2\)/s to $D = 80 \cdot 10^{-10}$ m\(^2\)/s, with steps of 5 or $10 \cdot 10^{-10}$ m\(^2\)/s. The model gives a total of 12 concentration maps, each corresponding to a $\Delta d_{\text{model}}$-curve that can be compared to the $\Delta d_{\text{data}}$-curve.
Figure 4.13: On-off representation of the modelled microchannel: An on-off representation applied to the modelled $[H^+_\text{total}]$ map of the microfluidic channel (figure 4.9). The white part represents a $-\log [H^+_\text{total}] > 4.2$, the dark part represents a $-\log [H^+_\text{total}] < 4.2$ and quenched fluorescein. The boundary separating the on- and off-state corresponds to the $\Delta d_{\text{model}}$-line for the no salts measurements.

Direct comparison of the $\Delta d$-curves was found to produce unreliable results, as reproducing accurately the $\Delta d_{\text{data}}$-curve using the model is not straightforward. This can be explained by considering the shape of the on-off boundary; the first part of the channel - 0 to 40 $\mu$m after the Y-junction - is sensitive to minor (experimental) defects, because in this part of the channel there is a gradient in the flow speed (subsection 4.1.2). This acceleration gives rise to a shape in the $\Delta d$-curve which is non diffusive (subsection 4.1.1 and figure 4.7). Also, individual measurements, can present slight differences in the initial flow conditions due to a slight difference between the flow rate in the two inlets, which can lead to a lateral displacement of the $\Delta d$-curve. However, about 40 $\mu$m after the Y-junction, the flow has stabilized, as can be seen in figure 4.8. After this $x$-coordinate the diffusion of the protons dominates the shape of the $\Delta d$-curve. Therefore only the slope of the diffusive part of the curves (see also figure 4.7) should be used to compare the data and the model.

In order to illustrate the comparison procedure, figure 4.14 shows the slope of the $\Delta d_{\text{data}}$-curve for 0.5 M of MgSO$_4$, being the same curve as the slope shown in the lower panel of figure 4.7, plotted together with the corresponding $12 \frac{\partial \Delta d_{\text{model}}}{\partial x}$-curves for the range of D. The graph for $D = 20 \cdot 10^{-10}$ m$^2$/s shows the larger resemblance between data and model. Therefore $D_{H^+}$ for 0.5 M of MgSO$_4$ will lie close to this value. The difference between the data and model slopes is calculated using the sum of least squares ($\chi^2$):

$$\chi^2 = \sum_{i} \left( \frac{\partial (\Delta d_{\text{model}}(x_i))}{\partial x} - \frac{\partial (\Delta d_{\text{data}}(x_i))}{\partial x} \right)^2$$  \hspace{1cm} (4.3)

The result for 0.5 M of MgSO$_4$ is shown in figure 4.15. The $D_{H^+}$ is obtained by de-
Differential comparison of $\Delta d_{\text{data}}$ and $\Delta d_{\text{model}}$: $D_{H^+}$ is determined by comparing the data with the model. The slope ($\partial \Delta d_{\text{data}} / \partial x$) of $\Delta d_{\text{data}}$ for 0.5 M of MgSO$_4$, the same slope as shown in the lower panel of figure 4.7, is shown in blue. The slopes ($\partial \Delta d_{\text{model}} / \partial x$) of $\Delta d_{\text{model}}$ for a range of 12 diffusion coefficients ($D$) are shown in red. These curves are the slopes of third degree polynomial fits to the $\Delta d_{\text{model}}$ datasets. The graph for $D = 20 \cdot 10^{-10}$ m$^2$/s shows the best comparison.

Determining the minimum of $\chi^2$, in figure 4.15, which is done by fitting the curve with a parabola and finding its minimum. For 0.5 M of MgSO$_4$ this minimum is found for $D_{H^+} \simeq 23 \cdot 10^{-10}$ m$^2$/s. For every microfluidic measurement this procedure is repeated. The concentration maps are calculated using Comsol. Plotting and selecting the concentration ranges is done in Matlab. The code written and used for this procedure can be found in Appendix A.
Figure 4.15: Sum of least squares plot for the 0.5 M of MgSO₄ data: This graph gives a quantitative comparison for the graphs shown in figure 4.14. Determining the minimum of this curve gives the value of $D_{H^+}$ for this measurement. The minimum for this measurement lies at $D_{H^+} \simeq 23 \cdot 10^{-10}$ m²/s.
4.4 Diffusion coefficients

In figure 4.16 the results for all the microfluidics diffusion measurements are plotted together. These are the final results of this research project. Each point represents a measurement done on a separate microfluidics run, and for each point a freshly prepared sample was used. The figure shows the salt concentration in M along the x-axis and the proton diffusion coefficient $D_H^+$ in $10^{-10}$ m$^2$/s along the y-axis. On the left side of the figure, the points for measurements without salt are plotted in pink, these points are most numerous for these measurements were repeated most often. The data points for no salt show a very large spread, with a minimum value of $D_H^+ \simeq 38 \cdot 10^{-10}$ m$^2$/s, a maximum value of $D_H^+ \simeq 58 \cdot 10^{-10}$ m$^2$/s and an average of $D_H^+ \simeq 47 \cdot 10^{-10}$ m$^2$/s.

The error in these measurements is larger than for the other measurements, this has to do with the progression of $\Delta d$ (figure 4.7), which reaches values close (within 5 – 10 µm) to the edge of the microchannel, within the $x$-range of interest. At the edge of the
microchannel the flow speed is much lower (subsection 2.2.2), therefore the diffusion goes relatively faster, which causes the signal in this region to become more prone to noise than in the middle of the channel. For one the intensity of the signal is much lower than at the start of the channel, in part due to an inhomogeneous excitation intensity for the entire field of view but also due to photobleaching of the fluorophore. Secondly, because of the lower flow speed in this region, diffusion in the z-direction starts to play a more prominent role as well. The proton concentration in the surrounding layers is higher, which causes protons to diffuse in from above and below, reducing the signal intensity and increasing the noise in the data. The effect of diffusion in the z-direction was not taken into account in the model for the assumption was that the system could be described using a 1D approximation.

An additional effect that can explain part of the large spread in the no salt results, is due to the experimental error in the flow conditions. Slight differences in flow conditions, as mentioned in section 4.3 can cause errors, especially in measurements that are more sensitive to these conditions. The experimental error in the flow can be due to several conditions: the water tightness of the connectors, the conditions of the syringes or the overall cleanliness of the microchannel (some device were used many times, see also subsection 3.2.2). The first two conditions unavoidably vary slightly per measurement, but in an attempt to correct the latter effect, the measurements were performed in a sequence, starting with a no salt measurement, followed by a low concentration salt measurement, followed by measurements of increasing concentration (subsection 3.2.2). The first (no salt) measurements can then be used as a calibration measurement for the other measurements, assuming that the conditions for the subsequent measurements are identical to the initial conditions. For on average two out of three measurements this seems like a reasonable approach, however for the other one out of three this approach seems to cause an even larger error. The subsequent measurements are indicated in figure 4.16 by the dotted lines connecting the points.

The other results represented in figure 4.16 are as follows. The measurements done on Mg(ClO$_4$)$_2$ are represented in green, for concentrations of 0.5, 1.0, 2.0 and 3.0 M. The measurements for 0.5 M were done twice, the other measurements were done once. Between the two points for 0.5 M there is a difference of more than 10%, this could be due to the error in the flow conditions as discussed above. The results of these measurements fall in the same range as the no salt measurements ($D_{H^+} \approx 50 \cdot 10^{-10}$ m$^2$/s). When compared to their calibration measurements, on the same device with nominally the same flow conditions (the upper most point for no salt in figure 4.16 corresponds to the upper point for 0.5 M Mg(ClO$_4$)$_2$) however, a slight decrease of $D_{H^+}$ is visible. The measurement for 1.0 M is a bit lower $D_{H^+} \approx 43 \cdot 10^{-10}$ m$^2$/s, but the measurements for 2.0 and 3.0 M are a lot lower ($D_{H^+} \approx 20 \cdot 10^{-10}$ m$^2$/s and $D_{H^+} \approx 13 \cdot 10^{-10}$ m$^2$/s respectively).

In figure 4.16 the measurements done on Cs$_2$SO$_4$ are represented in blue, for 0.5, 1.0, 2.0 and 3.0 M. Here the measurements on 0.5, 1.0 and 2.0 M were done twice. The measurement on 3.0 M was done once. These measurements show the best consistency so far, the difference between two values is less than 10%. The 0.5 M measurements show already a significant drop from $D_{H^+} \approx 50 \cdot 10^{-10}$ m$^2$/s down to $D_{H^+} \approx 27 \cdot 10^{-10}$ m$^2$/s, a drop of almost 50%. The values for 1.0, 2.0 and 3.0 are $D_{H^+} \approx 17 \cdot 10^{-10}$ m$^2$/s, $D_{H^+} \approx 13 \cdot 10^{-10}$ m$^2$/s and $D_{H^+} \approx 5 \cdot 10^{-10}$ m$^2$/s respectively. The last value has a large error for it is on the lower edge of the range that we modelled for, it was beyond the
values we expected to reach.

The measurements done on MgSO$_4$ are represented in orange, for 0.5, 1.0 and 2.0 M and they were performed four, two and one times respectively. For the 0.5 and 1.0 M measurements the variance is extremely large, both showing a difference of more than 30%. The only plausible explanation for this large difference is again due to the experimental error in the flow conditions. However, when we consider the average of these data points the trend is extremely similar to the trend for Cs$_2$SO$_4$.

What I would like to emphasize are the three following aspects of figure 4.16: the overall trend, the specific effects of the different salts and especially the trend of the sulphate salts. The overall trend seen in the figure, is that for increasing salt concentration, D$_{H^+}$ is decreasing. For the highest concentrations (3.0 M) the decrease in D$_{H^+}$ is about a factor 5 compared to the average of the no salt measurements. The figure shows a drop from D$_{H^+} \simeq 50 \cdot 10^{-10}$ m$^2$/s for no salt down to an average of D$_{H^+} \simeq 10 \cdot 10^{-10}$ m$^2$/s for 3.0 M of salt.

For the effects of the different salts there are two different trends visible in the figure. Although both trends show a decrease in D$_{H^+}$ for increasing salt concentrations, Mg(ClO$_4$)$_2$ shows a different trend compared to Cs$_2$SO$_4$ and MgSO$_4$. A concentration of 0.5 M of Mg(ClO$_4$)$_2$ shows very little to no effect as described above, whereas 0.5 M of sulphate salt shows a very large effect. The diffusion coefficient is almost halved for the latter, going from D$_{H^+} \simeq 50 \cdot 10^{-10}$ m$^2$/s to about 25 – 30 \cdot 10^{-10}$ m$^2$/s. For higher concentrations the sulphate salts show the largest net effect, the diffusion coefficient is reduced to D$_{H^+} \simeq 5 \cdot 10^{-10}$ m$^2$/s. However for Mg(ClO$_4$)$_2$ the relative effect for the higher concentrations is much larger, since for a concentration of 3.0 M D$_{H^+} \simeq 13 \cdot 10^{-10}$ m$^2$/s, which is 4-5 times lower than for no salt.

The last thing I would like to emphasize is that for the sulphate salts there is no significant difference between the MgSO$_4$ and the Cs$_2$SO$_4$. Within the error margins these salts show the same effect on the proton diffusion. This is an unexpected result since we had expected a much larger effect for MgSO$_4$ than for Cs$_2$SO$_4$. Possible reasons for this unexpected outcome will be discussed in the next chapter.
Chapter 5
Discussion

There were two noteworthy findings presented in the previous chapter (section 4.4), determined from figure 4.16, which will be discussed here. First is the finding that the results of the sulphate salts are extremely similar. Second is the finding that the overall trend of the results indicates that the presence of salts causes proton transport to slow down.

5.1 The sulphate salts discussion

5.1.1 The sulphate salts show a similar trend

The diffusion results in section 4.4, figure 4.16 showed no significant difference between the sulphate salts, Cs$_2$SO$_4$ and MgSO$_4$. Our starting hypotheses was that MgSO$_4$ would have the largest suppressive effect on the proton mobility, because it was shown by Tielrooij et al. that this salt has the largest suppressive effect on the reorientation of water, for both cation and anion are strongly hydrating.\[22\] However no clear difference between MgSO$_4$ and Cs$_2$SO$_4$ could be determined from figure 4.16. In these results it appears that the anion is much more important in the suppressive effect of the salt on the proton mobility, and the cation seems to play only a minor role.

For an intuitive explanation we look once more at the process of proton hopping, which involves the hydronium ion and its surrounding water molecules. The water molecules are bound to the hydronium ion through hydrogen bonds, forming the Eigen complex. When the excess proton wants to hop over to the next water molecule, a Zundel complex needs to be formed (section 2.1). Now imagine a sulphate ion (SO$_4^{2−}$) that is present around the hydronium ion, hydrogen bonded in place of one of the water molecules as shown in figure 5.1. The SO$_4^{2−}$ has a double charge, and compared to the water molecules, which has no net charge, it will attract the positively charged proton much more. The SO$_4^{2−}$ therefore strongly obstructs the process of proton hopping.

However, this intuitive picture raises a question: How strong is the association between SO$_4^{2−}$ and H$^+$ really? This question was not addressed before until this point. The assumption was that this effect was negligible, since sulphuric acid (H$_2$SO$_4$) is a strong acid (with at most a $\text{p}K_a \simeq −2$)\[23\], and the association of H$^+$ and SO$_4^{2−}$ was overlooked. Nevertheless it turns out that this association is not something that can be ignored, for the $\text{p}K_a$ for hydrogensulphate ions (HSO$_4^−$) turns out to be $\text{p}K_a \simeq 2$.\[25\] So
although $\text{H}_2\text{SO}_4$ is a strong acid, $\text{HSO}_4^-\text{aq}$ is a rather weak acid, which means that protons will associate with the $\text{SO}_4^{2-}\text{aq}$ ion:

\[
\text{H}^+ (\text{aq}) + \text{SO}_4^{2-} (\text{aq}) \xrightleftharpoons[k_2]{k_1} \text{HSO}_4^- (\text{aq}) (5.1)
\]

Equation (5.1) indicates the chemical equilibrium reaction between $\text{H}^+, \text{SO}_4^{2-}$ and $\text{HSO}_4^-$, where $k_1$ and $k_2$ are the reaction constants of the forward and backward reaction respectively. The $pK_a \simeq 2$ is an indicator for where the equilibrium of this reaction lies, which is defined as:

\[
pK_a = -\log_{10} K_a \quad (5.2)
\]

Here $K_a$ is the acidity constant which is often written as the quotient of the equilibrium concentrations:

\[
K_a \simeq \frac{[\text{H}^+][\text{SO}_4^{2-}]}{[\text{HSO}_4^-]} \quad (5.3)
\]

The concentration of species $A$ is indicated by $[A]$ (in [M] = [mol/L]), furthermore, the approximation sign is used in this equation because the correct form of equation (5.3) involves the activity $a_A$ of species $A$. The approximation in equation (5.3) can still be used to give an indication of the worst case scenario. The first indication is that for a $pK_a \simeq 2$ - thus a $K_a \simeq 10^{-2}$ - the equilibrium in equation (5.1) shifts towards the $\text{HSO}_4^-$ ions in case of large initial $[\text{SO}_4^{2-}]$ and $[\text{H}^+\text{free}]$. This means that the sulphate is actually removing free protons from the mixture by forming $\text{HSO}_4^-$ ions, lowering the free proton concentration ($[\text{H}^+\text{free}]$), especially since $\text{SO}_4^{2-}$ is 10 times more abundant than $\text{H}^+$ for $[\text{MgSO}_4] = 1 \text{ M}$. The implications of this change are twofold, the initial conditions have changed, but also the diffusive process is altered because of the reduction of $[\text{H}^+\text{free}]$.

5.1.2 Revising the view on the sulphate salt results

The conditions in the sulphate experiments are different than initially assumed, instead of an initial proton concentration of $[\text{H}^+\text{free}] = 0.1 \text{ M}$, the actual concentration is lower. Evaluating equation (5.3) using the absolute concentrations used in the experiments gives inaccurate results, for the indication is that most of the protons should be associated with
the sulphate ions, reducing the proton concentration several orders of magnitude. However, the proton concentration is still quite high for we still see that the fluorescein is fully quenched in the acidic solutions. Therefore the activity coefficients of the salts need to be considered and possibly taken into account for more accurate results. These coefficients differ per salt and per concentration, generally decreasing with increasing concentration to a certain minimum, but rising slightly again for the higher concentrations. The activity coefficients for 0.5 M of Cs₂SO₄ and MgSO₄ are \( \gamma_{\pm, \text{Cs}_2\text{SO}_4} \approx 0.27 \) and \( \gamma_{\pm, \text{MgSO}_4} \approx 0.073 \) respectively. These activity coefficients are rather low, which gives an explanation for the amount of \( \text{H}^+_{\text{free}} \) still remaining in the (HCl) solutions, even for higher concentrations of sulphate salt. Effectively this means that, for a 0.1 M HCl solution with 0.5 M MgSO₄, only about 7.3% of the \( \text{SO}_4^{2-} \) can be considered to be active. Evaluating equations (5.3) for this example gives a free proton concentration of \( [\text{H}^+_{\text{free}}] \approx 0.069 \text{ M} \). Considering this first issue, the lowered initial \( [\text{H}^+_{\text{free}}] \) (or increased pH) changes the concentration gradient, which is the driving force of the diffusion (subsection 2.2.3), therefore the measured diffusion coefficient appears to be lower than it is in reality. However this effect is only part of the issue.

The second effect that changes the view on the sulphate results has to do with the diffusion of the \( \text{H}^+_{\text{free}} \) into the neutral part of the channel. Besides that their movement is hindered by the presence of the ions - as was the assumption - now a fraction of the protons is "caught" or absorbed by a fraction of the sulphate ions, reducing the effective local \( [\text{H}^+_{\text{free}}] \). Therefore reducing the apparent diffusion coefficient (even further), for there are less and less \( \text{H}^+_{\text{free}} \) to partake in the proton hopping. The implication of equations (5.1) and (5.3) together with the calibration measurements for the fluorophore (section 4.2) is therefore, that the measurements on Cs₂SO₄ and MgSO₄ do not determine the diffusion of \( \text{H}^+_{\text{free}} \), but rather that of the total amount of protons: \( \text{H}^+_{\text{total}} \). By \( \text{H}^+_{\text{total}} \) we mean both \( \text{H}_3\text{O}^+ \) and \( \text{HSO}_4^- \) ions. The calibrations were actually done for \( [\text{H}^+_{\text{total}}] \) (more discussion on this in section 5.3), but besides that the modelling was only done for one type of species. By neglecting the sulphate as a base we measured the diffusion coefficient of a combination of two species. Possibly this could be described as a product of the two separate coefficients, but the fact that this process involves a chemical reaction makes it increasingly complicated. In order to extract a diffusion coefficient for the \( \text{H}^+_{\text{free}} \) from these results, the chemical reaction should be explicitly included in the model, and then the diffusion coefficient should be determined that reproduces the results from these measurements.

### 5.1.3 Modelling the buffer reaction

To test how much the buffer reaction with the sulphate ions has affected the effective diffusion coefficient, the convection-diffusion model (subsection 4.1.2) was extended to include the buffer reaction and was evaluated for the measurements done on 0.5 M MgSO₄. The

\[ \text{Furthermore, a quick check with pH paper (although inaccurate due to the presence of the salts) gives an estimate that the proton concentration is reduced less than an order of magnitude for the 0.1 M HCl with sulphate salt solutions.} \]

\[ \text{†These activity coefficients were measured for a molality of 0.5 (mol/kg solvent), which is a slightly lower concentration than a molarity of 0.5 (mol/L solution), but in the case of MgSO₄ these concentration differ only a few percent.} \]
reaction was included in the diffusion model by adding two species (SO$_4^{2-}$ and HSO$_4^-$) and the reaction rates for all the species, defined as:

$$ R_{\text{H}^+} \equiv R_{\text{SO}_4^{2-}} \equiv -R_{\text{HSO}_4^-} \equiv -k_1 [\text{H}^+][\text{SO}_4^{2-}] + k_2 [\text{HSO}_4^-] $$

(5.4)

Here $R_A$ is the reaction rate of species $A$ (in [M/s]), $k_1$ is the forward reaction constant (in [1/(M·s)]) and $k_2$ the backward reaction constant (in [1/s]). Furthermore, the ratio of the reaction constants is equal to the equilibrium constant: $K = \frac{k_2}{k_1}$ (in [M]). The buffering reaction was assumed to be fast compared to the diffusion, making it a diffusion limited reaction, which allows for equilibrium to be establishing locally. The reaction constants were set to $k_1 = 1 \cdot 10^6$ 1/(M·s) $k_2 = 1.2 \cdot 10^4$ 1/s. The height of the reaction constant used in the model determines indirectly the amount of time necessary to run the simulation (as described in section 3.4 and subsection 4.1.2). For higher reaction constants a longer calculation time was required, the current values required several minutes for calculating the concentration profile for one value of $D$ (section 4.3) and about half an hour for the whole array. Going up one order of magnitude would require at least several hours to calculate the entire array of $D$-values. For an initial (quick) indication these values for the reaction constants were the highest achievable.

Equation (5.3) was used to determine the altered initial conditions, where, instead of $[\text{SO}_4^{2-}]$, the active concentration was used: $\{\text{SO}_4^{2-}\} = \gamma_\pm [\text{SO}_4^{2-}]$ (with $\gamma_\pm = 0.073$), the activity coefficients of $\text{H}^+$ and HSO$_4^-$ were considered to be 1. The initial concentrations for each inlet of the microfluidic device (based on the equilibrium of the buffer reaction) are given in table (5.1). The values in this table are given in [M] = [mol/L]. The diffusion coefficients for the sulphate and hydrogen-sulphate ions were assumed to be low compared to that of the protons and set an order of magnitude lower than the average value that we found for proton diffusion into pure water: $D_{\text{HSO}_4^-} = D_{\text{SO}_4^{2-}} = 5 \cdot 10^{-10}$ m$^2$/s.

The calibration measurements for the fluorophore (section 4.2) were done for $D_{\text{H}^+_{\text{free}}}$ meaning $\text{H}^+ + \text{HSO}_4^-$. The calibration measurements therefore indicate a transition point for a certain $[\text{H}^+_{\text{total}}]$, thus the modelled concentration profiles (subsection 4.1.2) corresponding to $[\text{H}^+_{\text{total}}] = [\text{H}^+_{\text{free}}] + [\text{HSO}_4^-]$ should be used to evaluate the data. Therefore the $\Delta d_{\text{data}}$-curve is compared to the concentration maps of $\text{H}^+_{\text{free}} + \text{HSO}_4^-$ using the transition $[\text{H}^+_{\text{total}}]$ determined in (section 4.2). From there on out the same procedure was applied as described in section 4.3.

Following the above procedure, the proton diffusion coefficients corresponding to the four points from figure 4.10 for 0.5 M MgSO$_4$ were re-evaluated. The results range from $D_{\text{H}^+_{\text{free}}} \simeq 56$ to $80 \cdot 10^{-10}$ m$^2$/s, the values that were found are not extraordinary for proton diffusion, the literature suggests proton diffusion coefficients of at least $87 \cdot 10^{-10}$ m$^2$/s

<table>
<thead>
<tr>
<th>Inlet 1</th>
<th>$[\text{H}^+_{\text{free}}]$ in M</th>
<th>$[\text{SO}_4^{2-}]$ in M</th>
<th>$[\text{HSO}_4^-]$ in M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet 1</td>
<td>0.0689</td>
<td>0.00542</td>
<td>0.03108</td>
</tr>
<tr>
<td>Inlet 2</td>
<td>$1 \cdot 10^{-7}$</td>
<td>0.0365</td>
<td>$1 \cdot 10^{-7}$</td>
</tr>
</tbody>
</table>

Table 5.1: Initial concentrations for the buffer reaction. The initial concentrations used for modelling the buffer reaction for 0.5 M MgSO$_4$, with an activity coefficient of $\gamma_\pm = 0.073$, are given above, per inlet, in [M] = [mol/L]. The equilibrium was assumed for initial conditions since the solutions arrive in equilibrium at the Y-junction in the experiments as well.
Table 5.2: Starting concentrations for the buffer reaction for 5.3% activity. The initial concentrations used for modelling the buffer reaction for 0.5 M MgSO₄, with an activity coefficient of $\gamma = 0.053$, are given above, per inlet, in [M] = [mol/L]. The equilibrium was assumed for initial conditions since the solutions arrive in equilibrium at the Y-junction in the experiments as well.

<table>
<thead>
<tr>
<th>Inlet 1</th>
<th>$[\text{H}^+_{\text{free}}]$ in M</th>
<th>$[\text{SO}_4^{2-}]$ in M</th>
<th>$[\text{HSO}_4^-]$ in M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.07707</td>
<td>0.00357</td>
<td>0.02293</td>
<td></td>
</tr>
<tr>
<td>1.0 x 10⁻⁷</td>
<td>0.0265</td>
<td>1.0 x 10⁻⁷</td>
<td></td>
</tr>
</tbody>
</table>

for low concentrations (pH=6.5). However, compared to our own reference measurements $D_{\text{H}^+_{\text{free}}} \approx 50 \cdot 10^{-10}$ m²/s these results are rather high.

Since these results were found to be strange, extra modelling was done to check for possible errors. First the reaction model was recalculated using an activity coefficient that was reduced to 0.1%, to check that the results for the $D_{\text{H}^+_{\text{eff}}}$ would be reproduced, and not largely altered because of the inclusion of a reaction mechanic. This approach successfully reproduced the results as shown in figure 4.16.

An extra round of modelling and analysis was done to check if a slightly lower activity coefficient would not yield results that were more in line with our expectations, that the results would turn out to be below the reference values. An activity coefficient of 5.3% was used for this check. A reasonable value for the literature suggest that this activity corresponds to a concentration of 1.0 M of MgSO₄ and the lowest activity is around $\gamma = 0.044$ for a concentration of 2.0 M. The initial concentrations used in this case are shown in table (5.2). The results corresponding to the lowered activity were found to be $D_{\text{H}^+_{\text{free}}} \approx 47$ to $68 \cdot 10^{-10}$ m²/s. These values are slightly equal to, but on average above, those found for the (corresponding) reference measurements (see also figure 4.16).

5.1.4 Extrapolating results

The reaction modelling was done for only one of the two sulphate salts and only one concentration. However, for this salt at this concentration the effects on the effective proton diffusion coefficient should be the least significant. Meaning that due to the low MgSO₄ concentration and the low activity coefficient, the reduction of the initial $[\text{H}^+_{\text{free}}]$ should be the lowest, and also the local $[\text{H}^+_{\text{free}}]$ should be least affected. For Cs₂SO₄, but also for the higher concentrations of MgSO₄, the (effective) $[\text{SO}_4^{2-}]$ is much higher and therefore the initial and local $[\text{H}^+_{\text{free}}]$ is reduced more. The damping of the proton diffusion coefficient as shown in figure 4.16 is therefore stronger in these cases, suggesting that the actual $D_{\text{H}^+_{\text{free}}}$ will be even higher than for the case that we have investigated so far. A complete re-analysis of all the other sulphate data was not possible at this point. However there remains a strong indication that for the reacting (buffering) proton diffusion process the $D_{\text{H}^+_{\text{free}}}$ is actually increased.

5.1.5 Artefact or real: A new hypothesis

The results from the diffusion-reaction model indicate an increase of the proton diffusion coefficient compared to that of the reference measurements. These results were recalculated using a lower activity of 5.3%, and still a slight increase of the diffusion coefficient
was seen. Although these results seem to contradict our initial hypothesis, there are indications that we are not looking at an artefact, but rather at a real phenomenon, for a similar increase in proton diffusion speed was seen by Shinohara et al.\cite{42}, who performed a similar experiment. A microfluidics experiment, almost identical to ours, was performed, however Shinohara et al. used a HCl solution in one inlet and a NaOH solution in the second inlet. The reference measurement was done with HCl in one inlet and pure water in the other. For the reaction diffusion experiment the authors found that the proton diffusion was about 10 times faster than for their reference measurement. Although the authors suggested that others had seen related increased diffusion rates for reaction diffusion experiments (Hibara et al.), no reasonable explanation for this phenomenon was presented.

A possible explanation that can explain both the increase in proton transport that we see as well as that seen by Shinohara et al. is the following. Rather than interpreting the results for the reacting systems as an increase in diffusion coefficient, we would like to suggest that in the case of the reference measurement the proton diffusion is slowed down. Let me explain: For the pure water (reference) measurements the protons diffuse into water with nearly no charge carriers, since the neutral part is practically pure H\textsubscript{2}O. Therefore in order to keep the local medium neutral, the chloride ions need to be dragged along as the protons diffuse into the neutral channel. Or rather, as the protons diffuse away from the chloride ions, they create an electromagnetic potential, which is effectively holding them back. Now, when both sides of the channel are filled with charged species, the electromagnetic potential caused by the diffusing protons is effectively negated by (many more) positively charged species from the proton poor part of the channel moving (slightly) in the opposite direction. Therefore the protons have more freedom of movement and can more easily diffuse, increasing the apparent diffusion coefficient. So rather than an increased diffusion coefficient caused by the buffer reaction taking place, in the pure water case the diffusion coefficient is effectively reduced because of the lack of other charged species to compensate for the induced electromagnetic potential of the diffusing protons. This hypothesis would also very well explain the rather low diffusion coefficient that was found for the reference measurements in our case, for these were done with a rather low pH, introducing a rather high potential upon proton diffusion. The extend and the implications of this hypothesis are more complex than can be fully described here, however in chapter 7 (future work) some experiments will be proposed to further test this hypothesis.

5.2 Non-reactive, concentrated salty water slows down proton transport

Even though further investigation is needed to determine which phenomena are precisely going on for the sulphate salts, the claim can be made that non-reactive concentrated salty water slows down proton transport. So far we had neglected the results for Mg(ClO\textsubscript{4})\textsubscript{2}, but for this salt we are actually looking at the D\textsubscript{H\textsuperscript{+}\text{free}}. The association strength of protons with the perchlorate ion (ClO\textsubscript{4}\textsuperscript{−}) is not an issue, as perchloric acid is a strong acid.\cite{58} Therefore the ClO\textsubscript{4}\textsuperscript{−} will not significantly lower the initial nor the local [H\textsuperscript{\text{+\text{free}}}]\textsubscript{free}. However, the results for this salt were not as spectacular as those of the other salts, especially not
considering the lower concentrations, but now that is set in a different perspective as the analysis that led to these results was incorrect and it now appears that the opposite effect was found for these salts. The results for Mg(ClO$_4$)$_2$ remains useful for testing our initial hypothesis, that salt slows down proton diffusion. The results for the lower concentrations of Mg(ClO$_4$)$_2$ were rather marginal, no significant reduction in $D_{H^{+}\text{free}}$ was determined, nevertheless, for higher concentrations $D_{H^{+}\text{free}}$ was found to be about 5 times lower compared to the reference measurements. This means that although the effect of the ions on the proton diffusion might not be as large as the initial results indicated, they do have a suppressive effect on proton diffusion. The measurements for Mg(ClO$_4$)$_2$ are a good indicator for that, since perchlorate is a strong acid, with a $pK_a < -2$. This means that perchlorate does not, like the sulphate, reduce the concentration of free protons. A point for discussion is however that there are only few measurements for this salt at higher concentrations. To verify that the effects for higher concentrations are real, these measurements should be repeated.

5.3 Calibration measurements of sulphate salts

From section 5.1 it is clear that the effective proton concentration was changed by the addition of the sulphate salts. This realization also puts the calibration measurements from section 4.2 in a different light. The measurements for Mg(ClO$_4$)$_2$ obviously do not change, this salt directly influences the fluorophore, making it less sensitive to the proton concentration. However for Cs$_2$SO$_4$ and MgSO$_4$ this issue has changed. From first indicative calculations, it seems very likely that the transition $[H^+_{\text{total}}]$ that we measured would actually correspond to an effective transition $[H^+_{\text{free}}]$ of about 4.0-4.5, which is the same transition as for the no salt case. Assuming that the activity coefficients are correct, an actual transition pH could be calculated from the titration results. Then using these the analysis for the sulphate salts could be done using the $[H^+_{\text{free}}]$ profiles instead of the $[H^+_{\text{total}}]$ ones. However, both should in principle yield the same results. This might be an extra check for the model that is used.

5.4 Discussion of experimental issues

The influence of several other parameters were investigated to see what the effect of neglecting these in the calculations were. The effect of the change in viscosity due to the presence of salts was investigated, this effect was found to be minor. This was also done for a change in the density due to addition of salts. None of these effects seem to significantly impact our results. So far the largest errors seems to be coming from differences in flow conditions between separate measurements, and the precision with which we are able to determine $\Delta d$. The errors coming from varying flow conditions can mainly be attributed to a limited amount of control over: 1. the state of the microchannels after several uses (removing salts from microchannels might not be as simple as it seems), 2. the state of (old) syringes that are being reused without cleaning them in between measurements. In principle, completely cleaning should not be necessary if the same salt is used and only the concentration is increased. This was done to increase the experimental efficiency for several measurements, always starting at low and going to high salt concentration, and
never mixing up the acidic and the neutral syringe. However, it was perceived that the plungers of the syringes started to run smoother every time they were being reused. When one of the two syringes runs more smoothly than the other, the continuity of the flow is affected, possibly completely disturbing the measurements, or less dramatic but more problematic for the error in the measurements, the flow conditions become asymmetric. The plungers primarily start running more smoothly when the sides of their heads become overly wet, especially for acidic solutions. Giving the syringes a final clean with acetone and afterwards blow them dry with pure nitrogen makes them run with some resistance, producing generally a steady flow. This indicates that the syringes should be cleaned thoroughly before each use. A way to do this when doing multiple measurements in a row, would be to use multiple syringes so that they can be switched for new measurements, instead of refilling used syringes without a proper cleaning.
Chapter 6

Conclusion

The aim of this investigation was to study the effect of salt on the proton mobility in water, specifically the effect due to strongly hydrating ions, i.e. Mg$^{2+}$ and SO$_4^{2-}$, either combined as an ion pair (MgSO$_4$) or in combination with a weakly hydrating counterion (Mg(ClO$_4$)$_2$ and Cs$_2$SO$_4$). It was found that the transport of protons is slowed down by the presence of (non-reactive, highly concentrated) salts, which is in agreement with the findings that salts suppress water reorientation$^{[22,23]}$, which in turn suppresses the proton mobility in water$^{[11,14,20]}$. The largest decrease in proton diffusion coefficient (about $5 \times$ lower) was observed for a salt concentrations of 3.0 M, from $D_{H^+} \simeq 50 \cdot 10^{-10}$ m$^2$/s for no salt down to $D_{H^+} \simeq 10 \cdot 10^{-10}$ m$^2$/s for 3.0 M.

It was found that each salt under investigation initially showed a suppressive effect on the proton mobility, however, the effect of the two sulphate salts was found to be very similar, furthermore, compared to the Mg(ClO$_4$)$_2$ the sulphate salts showed a much larger suppressive effect. The large suppressive effect for the sulphate salts was found to be caused by the buffering properties of SO$_4^{2-}$, causing an apparent suppressive effect on the proton mobility. A more proper treatment of the data gave a whole different view on these results, suggesting not a decrease but rather an increase of $D_{H^+}$. A possible explanation for this phenomenon was presented in subsection 5.1.5. One of the goals of this investigation was to conduct a quantitative comparison of the influence of salts, with different hydrating strength, on proton mobility in water. Although the investigation has produced some surprising and interesting results, possibly leading to new insights, the overall results were inconclusive and further investigation is needed. The continuation of this research is described in the next chapter (chapter 7 Future Work).
Chapter 7

Future Work

In order to be more conclusive in the investigation described in this thesis several issues should be investigated further. The most prominent of these issues is the buffering effect of the sulphate anion as described in section 5.1. The reaction modelling was performed for one salt and concentration leading to an increased $D_{H^+_{free}}$. Aim for the near future is to re-evaluate all the sulphate salt data sets using the reaction diffusion model. Since the initial conditions for each salt and concentration will be different, the data from the model will be different for each measurement, making the analysis a more time consuming process than it was before, when all the measurements were compared with the same modelled data sets. Besides re-evaluation using the current model - a simple reaction diffusion model - also alternate models should be considered to check the validity of these results, possibly more can be found in the literature in this regard.

The following step would be to set up several experiments to investigate the new hypothesis that we proposed in subsection 5.1.5. These experiments should include the following: - increasing the pH, to check if the $D_{H^+_{free}}$ is increases due to a reduction in the induced electromagnetic potential since less protons are moving. - measurements using a small amount of (non-reacting, weakly hydrating) salt, for different pH, to check if the $D_{H^+_{free}}$ is increased due to the overall presence of salt, which (partially) negates the induced electromagnetic potential by moving in the opposite direction. These salts could be NaCl or NaClO$_4$ (see also below).

Another issue, considering the original aim of this study, is the reproducibility of the measurements for Mg(ClO$_4$)$_2$. These measurements should be repeated for the higher concentrations - only one set per concentration was acquired so far - in order to improve the reliability of these measurements. Besides improving the reliability of the results, another addition can be made for better comparison of the current results. Measurements done on one other type of non-reactive salt would make it possible to compare the difference between a strongly and weakly hydrating cation, combined with a weakly hydrating anion. For this purpose it was already tried to do measurements on CsI solutions, however these experiments failed due to a bleaching effect of the iodide on fluorescein. Therefore another salt should be considered: NaClO$_4$ would be a good candidate to do a comparison with Mg(ClO$_4$)$_2$: it is a weakly hydrating ion pair and its solubility in water is very good.

The last issue I would like to discuss here concerns the reliability of the individual

*For all strongly hydrating anions do also strongly associate with protons.
measurements. It was seen in section 4.4 that the spread of some of the results was very large, and it was indicated that this effect could be due to issues with the flow conditions. Besides a way of improving flow conditions from the microfluidics pump, syringe and connector parts, flow conditions could be improved on the channel geometry part as well, by adjusting the geometry that was used in this investigation. The adjustment would be to use a channel with much larger aspect ratio (width versus height of the channels). Using a larger aspect ratio would mean an adjusted flow profile in the channel, for a very wide channel this means that the flow profile in the middle of the channel is flat along the y-axis, meaning a constant speed, instead of parabolic as in the channel used here. The flow profile would still have a parabolic shape at the edges, however the middle part of the channel would have a wide range where the flow speed is the same in every point along the y-axis, making it possible to approximate here with a constant speed, making it possible to use a 1D model approximation.
Appendix A

Matlab code for analysis of the microfluidics images

This appendix gives the Matlab code for analysing the microfluidics images acquired with the confocal fluorescent microscope. Besides calculations the code also includes comments to give an idea of the reasoning behind each particular step. The following code is the contents of the matlab *.m file used for the analysis of the microfluidics images. This file is specific for the measurements done on MgSO₄ 0.5 M measured on 18-08-2011:

```matlab
% ------------------------------------------------------------------------
% PROTON TRANSPORT ANALYSIS v4.0
% ------------------------------------------------------------------------
% Experiment: 110818 1A, data set: 003, layers used: all, (focus: layer 10)
% Sample used: MgSO4 - fluo - acid , 0.5 M - 2.5uM - pH1 (17-08-2011)
% Device used: A2, a custom made device, shiped on 18-03-2011
% ------------------------------------------------------------------------
% Version notes: implementation of cell use, makes it
% possible to read images using a loop instead of single lines for every
% data set. Also implemented single layer reading for stacked *.tif files.
% Notes:
% - changes made for accommodating the 20x objective images,
% mainly in the VARIABLES section below.
% - the correction line was set up properly:
% adding/subtracting instead of multiplying.

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% VARIABLES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% We need a conversion table to be able to compare the amount of pixels to
% the amount of micrometers or joystick variables. The simplest way to do
% this is to define a few different constants or ratio’s, namely, a
% joystick to pixel (jtp) ratio, a micrometer to pixel (utp) ratio, a ptj
% ratio, ptu ratio and finally also a jtu ratio, six in total:

% CONVERSION FACTORS/RATIOS (20x objective and 1.0x condenser):
```
utp = 1.2; % measured 1.2 pixels per micrometer with grid slide
ptu = 1/utp; % (about 0.83) micrometers per pixel
jtu = 992.5; % micrometers per joystick unit (jtp/utp)
utj = 1/jtu; % (about 1.007*e-3) joystick units per micrometer
jtp = 1191; % pixels per joystick unit (measured see below)
ptj = 1/jtp; % (about 8.4*e-4) joystick units per pixel

% The values for utp and jtp were determined using several grid images from
% "110818 grid" data. Images from a 10um and 50 um grid slide.
% Using the grid in the images, the amount of pixels per um could be
% determined. Also, by using the x positions from the text file, the
% joystick movement could be determined. Several values from different
% images were determined and the average was taken for these values.

% Setting the file path of the images
% %########### Adjust manually:
 Impath = 'D:\Maarten\Data\110818 1A';

N = 1; %number of tif files we want to evaluate
M = 20; %number of images per stacked tif file
layer = 10; % The layer we want to evaluate

data = cell(N,M); %define size of cell
Imfliplr = cell(N,M); %define size of cell

% The following loop loads all the images we are interested in into the
% predefined cells. In the second case, the images are flipped left to
% right for convenience:

for k = 1 : N;
 baseFileName = sprintf('110818 1A %3.3d.tif', k+2); % The 3.3 is important.
 fullFileName = fullfile(Impath,baseFileName);
 for l = 1 : M;
 Images{k,l} = imread(fullFileName,l);
 Imfliplr{k,l} = fliplr(Images{k,l});
 end
end
%% NEXT: get image out and show it.

%% We extract the image dimensions from the first image, they all have the
%% same dimensions, so we can use them for all images:
[d1I1,d2I1]=size(Images{1,1});

%% The following lines are obsolete unless you want to cut off a part of
%% the images before processing.

%% # Adjust manually:
marx = [1 1]; % margin we might want to ommit at the edges
mary = [1 1]; % margin we might want to ommit at the edges
xI = cell(1,N);
yI = cell(1,N);
xIm = cell(1,N);
yIm = cell(1,N);
STIM = cell(1);
for k = (1 : N);
xI{k} = marx(k):d2I1;
yI{k} = mary(k):d1I1;
xIm{k} = xI{k};
yIm{k} = yI{k};
STIM{1}(yIm{k},xIm{k}) = Imfliplr{k,layer}(yI{k},xI{k});
end

%%%%%%%%%%%%%%%%%%%%%%%%% SHOWING IMAGES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%% Determine the dimensions of the new STIM image
[d1STIM,d2STIM]=size(STIM{1});
%% Create figure
figure1 = figure('Colormap',...
[0:((1/255):1;0:((1/255):1']);
%% Create axes
axesSTIM = axes('Parent',figure1,'TickDir','out',...
'Position',[0.0752 0.0614 0.8084 0.8969],...
'Layer','top',...
'DataAspectRatio',[1 1 1],...
'Clim',[0 15535]);
%% Uncomment the following line to preserve the X-limits of the axes
xlim(axesSTIM,[-xZero*ptu ((d2STIM-1-xZero)*ptu)]);
%% Uncomment the following line to preserve the Y-limits of the axes
ylim(axesSTIM,[0 ((d1STIM-1)*ptu)]);
hold(axesSTIM,'all');
image([-xZero*ptu ((d2STIM-1-xZero)*ptu)],[((d1STIM-1)*ptu) 0],STIM{1},...
'Parent',axesSTIM,'DisplayName','Stitched Image of Channel',...
'CDataMapping','scaled');
We have our stitched image STIM, with this we now start with:
[dChI1, dChI2] = size(STIM{1});

Let us try to find initial points for the "cutting line" by using a loop, where we will use STIM. Point one should be taken around 100um to avoid the y-shaped part and point 2 at max-50um to avoid the dark "tail"

\[
xP1 = 70;
xP2 = dChI2-50*utp;
xP3 = round(dChI2/2);
\]

For calculating the average intensities of blocks of m by m squares:

\[
m = 5;
m1 = m-1;
m2 = m1/2;
n1 = 0;
n2 = 0;
\]

The following loops determine for point 1 and point 2, in two steps, the start of the channel. These points we will use to determine a line
representing the edge of the channel on the fluorescent side.

The 2 loops for point 1:
for yP1 = y
    LocI = STIM{1}(yP1:yP1+m1,xP1-m2:xP1+m2);
    AvI = sum(sum(LocI))/(m*m);
    if n1 < 1, Trh1a = 2*AvI; n1=2; end
    if Trh1a < AvI
        LocChI = STIM{1}(yP1+1:yP1+1+m1,xP1-m2:xP1+m2);
        AvChI = sum(sum(LocChI))/(m*m);
        Trh1b = AvChI;
        break
    end
end
for yP1 = y
    LocI = STIM{1}(yP1,xP1-m2:xP1+m2);
    AvI = sum(sum(LocI))/(m);
    if Trh1b < AvI, cpA = yP1; break, end
end

% We want to fit a sigmoid to the light side of the channel

SideW = 12; % the margin for which to fit
yChLSide = cpA-SideW:cpA+SideW; % the y range
xChLSide = xP1:xP2; %This variable "walks" from L to R

xc = yChLSide;
fitparamc=zeros(dChI2,4);
figure001=figure('Name','Fitting of the light side of the channel');
for k = xChLSide
    yc = STIM{1}(xc,k);
    % yc seems to be a somthing that is not a double, and our approach does
    % not work in that case, so we make yc a double....
    yd = double(yc);

    % Here we define the sigmoid function
    % It is a function depending on the variables pb(1) to pb(4).
    % We then subtract our data from this function.
    % The pb variables in the sigmoid tell us the following:
    % - pb(1) is the min or base value,
    % - pb(2) is the max or top value,
    % the values for 1 and 2 might be the other way around depending on
    % the way the sigmoid goes (from high to low or the other way)
    % - pb(3) is the x-mean value (position of the "middle" of the sigmoid)
    % - pb(4) gives the width of the sigmoid
    funb = @(pb) sum(( pb(1) + pb(2)./(1 + exp(-(xc-pb(3))./pb(4)) ) -yd').^2);
Then fminsearch is used to fit the sigmoid to our data. It minimize the difference between our data and the sigmoid function, by varying the different pb values. Starting values have to be inserted: pb(1)=0, pb(2)=11000, pb(3)=cpA , pb(4)=1
ppc = fminsearch(funb,[ 0 11000 cpA 1]);
The following line puts our fit in a function so we can plot it
fitc = ppc(1) + ppc(2)./(1 + exp(-(xc-ppc(3))./ppc(4)) )
Now we define a condition that for every nth value of k a figure will
pop up our fittings.
l=mod(k-min(xChLSide)+1,25);
if l < 1,
  L=mod(k-min(xChLSide)+1,24);
  if L < 7,
    subplot(2,3,L); plot(xc,yc,'bo',xc,fitc,'r-')

% Adjust manually:
title('Fit of intensity values along crosscut',...
  'FontSize',11,'FontName','Times New Roman');
xlabel('Channel crosssection (\mum)','FontSize',9,...
  'FontName','Times New Roman')
ylabel('Intensity (a.u.)','FontSize',9,'FontName','Times New Roman')
% Define axis range
  axis([min(xc) max(xc) 0 16383]);
end
end
% and then we put the values we found as a function of k in a matrix:
fitparamc(k,:)=ppc;
end
shg

% Now plot the pb(3) values for the part of the channel we evaluated.
% This gives a fit of where the side of the channel should be:
fitpb3uncor = fitparamc(xChLSide,3);
xChLSu = xChLSide * ptu;
fitpb3uncoru = fitpb3uncor*ptu;
figure; plot(xChLSu,fitpb3uncoru,'.')
% title('Centre values of the sigmoid fits uncorrected',...
  'FontSize',14,'FontName','Times New Roman')
% ylabel('Y (\mum)','FontSize',12,'FontName','Times New Roman');
% Define axis range
  axis([min(xChLSu) max(xChLSu)])
% NOW FIT THE CENTRE VALUES TO A LINE!
cpC = -0.010; % a good guess for the slope of our line

funline = @(pd) sum((xChLSide-pd(1))*pd(2) + pd(3) - fitpb3uncor'.^2);
pline = fminsearch(funline,[xP1 cpC cpA]);
fitline = (xChLSide-pline(1))*pline(2) + pline(3);
fitlu = fitline*ptu;

xCh = 1:dChI2;
fitLine = (xCh-pline(1))*pline(2) + pline(3);
xChu = xCh*ptu;
fitLu = fitLine*ptu;

figure; plot(xChLSide,fitpb3uncor,'.',xChLSide,fitline,'r-',...% xCh,fitLine,'b:')
% title('Centre values of the sigmoid fits uncorrected',...'
'_Fontsize',14,'FontName','Times New Roman')
xlabel('X (pixels)','Fontsize',12,'FontName','Times New Roman');
ylabel('Y (pixels)','Fontsize',12,'FontName','Times New Roman');

figure; plot(xChLSu,fitpb3uncoru,'.',xChLSu,fitlu,'r-',xChu,fitLu,'b:');
title('Centre values of the sigmoid fits uncorrected',...'
'_Fontsize',14,'FontName','Times New Roman')
xlabel('X (\mum)','Fontsize',12,'FontName','Times New Roman');
ylabel('Y (\mum)','Fontsize',12,'FontName','Times New Roman');

%% %%%%%%%%%%%%%%%%%%%% 2. Make the line+show %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

line(xCh) = fitLine;
% - rounding the values of our line to whole (pixel) numbers, creating a% sort of step function
liner(xCh) = round(line(xCh));
% - a controll plot of both lines we made
figure; plot(xCh,line(xCh),'b.',xCh,liner(xCh),'r-')
title('Cutting line and rounded to pixels step function',...'
'_Fontsize',13,'FontName','Times New Roman')
xlabel('X (pixels)','Fontsize',12,'FontName','Times New Roman');
ylabel('Y (pixels)','Fontsize',12,'FontName','Times New Roman');
% setting the axis range of the plot
axis([0 max(xCh) min(line(xCh))-10 max(line(xCh))+5])

%% %%%%%%% show line in STIM

STIMline=STIM{1};
for k = xCh
STIMline(liner(k),k) = 14535;
end
%% Create figure
figure1b = figure('Colormap',... 
[0:(1/255):1;0:(1/255):1;0:(1/255):1]);

% Create axes
axesSTIM = axes('Parent',figure1b,'TickDir','out',... 
'Position',[0.0752 0.0614 0.8084 0.8969],... 
'Layer','top','DataAspectRatio',[1 1 1],'CLim',[0 14535]);
% Uncomment the following line to preserve the X-limits of the axes
xlim(axesSTIM,[-xZero*ptu ((d2STIM-1-xZero)*ptu)]);
% Uncomment the following line to preserve the Y-limits of the axes
ylim(axesSTIM,[0 ((d1STIM-1)*ptu)]);
hold(axesSTIM,'all');
image([-xZero*ptu ((d2STIM-1-xZero)*ptu)],[((d1STIM-1)*ptu) 0],STIMline,... 
'Parent',axesSTIM,'DisplayName','Full Image of Channel',... 
'CDataMapping','scaled');
% Create title
title('fFull image with cutting line shown',... 
'FontSize',11,'FontName','Times New Roman');
% Create xlabel
xlabel('X (\it\mum)','FontSize',10,'FontName','Times New Roman');
% Create ylabel
ylabel('Y (\it\mum)','FontSize',10,'FontName','Times New Roman');
% Create colorbar
colorbar('peer',axesSTIM);
% Resize the axes in order to prevent it from shrinking.
set(axesSTIM,'Position',[0.0752 0.0614 0.8084 0.8969]);

%% %%%%%%%%%%%%%%%%%%%% 2b.Use the line to cut %%%%%%%%%%%%%%%%%%%%%%%%%%%%

% When we have the line, we can cut out the channel image:
% - define the channel width:
w=56;
% - define how big our image should become and fill it with zeros
ChIm00 = zeros(w,dChI2);
% - run the loop that will fill the matrix with the actual channel image
for xI = xCh,
    ChIm00(1:w,xI) = STIM{1}(liner(xI):liner(xI)+w-1,xI);
end
% - rename it for use in other parts of code
ChIv2=ChIm00;

%%%%%%%%%%%%%%%%%%%%%%%%%%%% 3.Show what we have made %%%%%%%%%%%%%%%%%%%%%%
% Create figure
figure2 = figure('Colormap',... 
  [0:(1/255):1;0:(1/255):1;0:(1/255):1]);

% Create axes
axesChI = axes('Parent',figure2,'TickDir','out','Position',... 
  [0.07525655644241 0.06140350877192 0.8084378563283 0.8969298245614],... 
  'Layer','top','DataAspectRatio',[1 1 1],'CLim',[0 15535]);

% Uncomment the following line to preserve the X-limits of the axes
xlim(axesChI,[-xZero*ptu ((d2ChI-1-xZero)*ptu)]);

% Uncomment the following line to preserve the Y-limits of the axes
ylim(axesChI,[0 ((d1ChI-1)*ptu)]);

hold(axesChI,'all');

image([-xZero*ptu ((d2ChI-1-xZero)*ptu)],[((d1ChI-1)*ptu) 0],... 
  ChIv2,'Parent',axesChI,'DisplayName',... 
  'Stitched Image of Channel','CDataMapping','scaled');

% ############# Adjust manually:
% Create title
title('fCut out channel image for 110818 1A S003L10',... 
  'FontSize',14,'FontName','Times New Roman');

% Create xlabel
xlabel('X (\it\mum)','FontSize',12,'FontName','Times New Roman');

% Create ylabel
ylabel('Y (\it\mum)','FontSize',12,'FontName','Times New Roman');

% Create colorbar
colorbar('peer',axesChI);

% Resize the axes in order to prevent it from shrinking.
set(axesChI,'Position',... 
  [0.07525655644241 0.06140350877192 0.8084378563283 0.8969298245614]);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% FUNCTION FOR FITTING
% of the resulting image ChIv2 (which is only the channel)
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%We have our ChIv2 and we want to fit a sigmoid to this image in the
%up-down direction, so that is the first index according to MatLab.
%We want to let our fitting run allong the left-right, so the second index
%of our image ChIv2.
%Let us get the dimensions of our image ChIv2
[d3,d4]=size(ChIv2);

xIM = 1:d4; %This variable "walks" from L to R
xIMu = -xZero*ptu:ptu:(max(xIM)-1-xZero)*ptu; %Conv to um's + shift(X0!)

yIM = 1:d3; %This variable "walks" from top to bottom
yImu = 0:ptu:(max(yIM)-1)*ptu; %Conversion to um's
%We want the light part of our channel image to be on the top side, in
%order to get the right variable out
Icfv2 = ChIv2; % again no need for flipping the image
%We try our fits over the whole image, we let k go through the whole range
%of xIM, however, we might also make the number of runs less by increasing
%the step size e.g. k= 1:50:max(xIM) with here a step size of 50
fitparamb=zeros(d4,4);
figure3=figure('Name',
'Fitting of the intensity data for crossections along the channel');

for k = 1:max(xIM)
    xa = yIM;
    xau = yIMu; %same as xa but in um's instead of pixels
    ya = Icfv2(xa,k);
    % ya seems to be a somthing that is not a double, and our approach does
    % not work in that case, so we make ya a double....
    yb = double(ya);
    %We define our sigmoid function here, as a function depending on the
    %variables pa(1) to pa(4) and subtract our data from it. (I think that
    %is more or less what is done here, but ask Johannes for details, it is
    %this way of using fits I believe, the sigmoid I got from the advanced
    %users forum of MatLab.
    %In the sigmoid the pa variables are as follows:
    % - pa(1) is the min or base value,
    % - pa(2) is the max or top value,
    % the values for 1 and 2 might be the other way around depending on
    % the way the sigmoid goes, (up or down?)
    % - pa(3) is the x-mean value (so distance to "middle" of sigmoid)
    % - pa(4) is the variable giving the width of the sigmoid
    funa = @funaeq( sum( ( pa(1) + pa(2)./(1 + exp(-(xa-pa(3))./pa(4)) ) -yb').^2 ));
    % then we do fminsearch which is I think the way to minimize the
    % difference between our data and the proposed function, by varying the
    % different pa values. We propose he starts looking near the values:
    % pa(1)=0, pa(2)=8000, pa(3)=60 (middle of the channel), pa(4)=1
    ppb = fminsearch(funa,[ 12000 -11000 30 1]);
    %I believe the following line is not necessary but for the plot below
    fitb = ppb(1) + ppb(2)./(1 + exp(-(xa-ppb(3))./ppb(4)) ) ;
    %Now we define a condition that for every nth value of k a figure will
    %pop up of the plots that we are making which will happen every time
    %this condition is met.
    l=mod(k,80);
    if l < 1,
        L=mod(k,79);
        if L < 7,
            subplot(2,3,L); plot(xau,ya,'bo',xau,fitb,'r-')
```
% #Adjust manually:
title('bbFit of intensity values along crosscut',...
'FontSize',11,'FontName','Times New Roman');
xlabel('Channel crossection (\mum)','FontSize',9,...
'FontName','Times New Roman')
ylabel('Intensity (a.u.)','FontSize',9,'FontName','Times New Roman')
% Define axis range
axis([min(xau) max(xau) 0 16383]);
end

%and then we put the values we found as a function of k in a matrix:
fitparamb(k,:)=ppb;
end

% Here a plot of pa(3) values over the whole channel, which we get from the
% matrix of pa values we made in the loop above, here still uncorrected for
% the crude channel cut we made before:
fitp3uncor = fitparamb(xIM,3);
fitp3uncoru = fitp3uncor*ptu;
figure; plot(xIMu,fitp3uncoru,'.')
title('Centre values of the sigmoid fits uncorrected','Fontsize',14,...
'FontName','Times New Roman')
xlabel('X (\mum)','FontSize',12,'FontName','Times New Roman');
ylabel('Y (\mum)','FontSize',12,'FontName','Times New Roman');
% Define axis range
axis([min(xIMu) max(xIMu) 10 33]);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 4.CORRECTION USING OUR LINE %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% - fill up the new function before loop
linec = zeros(1,numel(xCh));
for xJ = xCh,
    linec(xJ) = liner(xJ)-line(xJ);
end
% Show what our correction line looks like:
% Uncomment following line to show what the correction line is based on
% figure; plot(x,line(x),'b.',x,liner(x),'r-',x,linec(x),'b-')
figure; plot(xCh,linec(xCh),'b.'
title('Correction line','Fontsize',13,'FontName','Times New Roman')
xlabel('X (pixels)','FontSize',12,'FontName','Times New Roman');
ylabel('Y (ratio)','FontSize',12,'FontName','Times New Roman');
% setting the axis range of the plot
axis([0 max(xCh) min(linec(xCh))*1.1 max(linec(xCh))*1.1])
% And now to make the corrected data set:
% - fill up the new function before loop:
fitp3 = zeros(1,numel(xCh));
for xJ = xCh,
    fitp3(xJ) = fitp3uncor(xJ)+linec(xJ);
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 5. Show and fit the data we are interested in %%%%%%%%%%%%%%%%%%%%

fitp3u = fitp3*ptu;
figure; plot(xIMu,fitp3u,'r.
 title('Centre values of the sigmoid fits','Fontsize',14,...
 'FontName','Times New Roman')
xlabel('X (\mum)', 'Fontsize',12,'FontName','Times New Roman');
ylabel('Y (\mum)', 'Fontsize',12,'FontName','Times New Roman');
% Define axis range
axis([min(xIMu) max(xIMu) 10 33]);

% Representation of Delta_d:
% This is the same plot as above but now with the baseline being the middle
% of the channel instead of the side of the channel:
% Middle of the channel is:
Y0 = w/2;
Deld = (Y0 - fitp3)*ptu;
figure; plot(xIMu,Deld,'r.
 title('\Deltad along the channel in \mum','Fontsize',10,...
 'FontName','Times New Roman')
xlabel('X (\mum)', 'Fontsize',12,'FontName','Times New Roman');
ylabel('\Deltad (\mum)', 'Fontsize',12,'FontName','Times New Roman');
% Define axis range
axis([min(xIMu) max(xIMu) 0 12]);

% This part is for fitting the data, since we want to take the derivative,
% and it needs to be smooth to get some decent data out.
% Determine which part we want to fit and the order of the polynomial:
% "endmargin01" should be the number of pixels at the end of ChIv2 that you
% want to ignore in the fitting of the data. Depends on which data set you
% are using.

% ############# Adjust manually:
endmargin01 = 25*utp;
startfit01 = 50*utp + xZero; % Starting point for fitting
endfit01 = numel(xIMu)-endmargin01; % End point for fitting
fitrange01 = startfit01:endfit01;
polord = 3; % Use a low order of the polynomial to fit with
Deldp=polyfit(xIMu(fitrange01),Deld(fitrange01),polord);

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Deldfit = zeros(endfit01-startfit01+1,2);
Deldfit(:,2) = polyval(Deldp,xIMu(fitrange01));
Deldfit(:,1) = xIMu(fitrange01);
fig010=figure;
subplot(2,1,1);
plot(xIMu,Deld,'.',Deldfit(:,1),Deldfit(:,2),'.r-')
title('\Deltad along the channel in \mum','Fontsize',10,...
'FontName','Times New Roman')
xlabel('X (\mum)','Fontsize',12,'FontName','Times New Roman');
ylabel('\Deltad (\mum)','Fontsize',12,'FontName','Times New Roman');
% Define axis range
axis([min(xIMu) max(xIMu) 0 12]);

% The differential of the fit of our Delta-d, plus its range in the x-dir
Delddif = zeros(endfit01-startfit01,2);
Delddif(:,2) = diff(Deldfit(:,2))';
Delddif(:,1) = xIMu(startfit01:endfit01-1);
figure(fig010);
subplot(2,1,2);
plot(Delddif(:,1),Delddif(:,2),'.')
title('Slope of \Deltad along the channel in \mum','Fontsize',10,...
'FontName','Times New Roman')
xlabel('X (\mum)','Fontsize',12,'FontName','Times New Roman');
ylabel('Slope of \Deltad (\mum)','Fontsize',12,'FontName','Times New Roman');
axis([min(xIMu) max(xIMu) min(Delddif(:,2))*0.1 max(Delddif(:,2))*1.2]);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%% 6. Fit the data to the model %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Assuming we have imported the data from our model (COMSOL):
% The following data should be loaded in Matlab!!!
% - "Diffc02" & - "datx2"
% And also (for reference):
% - NE02 = numel(Diffc02);

indx = cell(NE02,1);
umx = zeros(NE02,1);
Delta_d = cell(NE02,1);
figure;
for g = 1:NE02
% For salt 1a (MgSO4 0.5 M) the transition pH lies around pH 3.75
 indx{g,1}=... 
    find(-log10(1e-3*datx2{g,1}(:,3))>3.6&-log10(1e-3*datx2{g,1}(:,3))<3.9);
umx(g)=size(indx{g,1},1);
 for i=1:numx(g)
    Delta_d{g,1}(i,1)= datx2{g,1}(indx{g,1}(i),1);
    Delta_d{g,1}(i,2)= -datx2{g,1}(indx{g,1}(i),2);
end
subplot(4,3,g);
plot(Delta_d{g,1}(:,1),Delta_d{g,1}(:,2),'or',xIMu*1e-6,Deld*1e-6,'.');
DifPlN = sprintf('D = \%d e-10 m^2/s', Diffc02(g));
title(DifPlN,'FontSize',10,'FontName','Times New Roman');
xlabel('x (m)','Fontsize',10,'FontName','Times New Roman');
ylabel('\Deltad (m)','Fontsize',10,'FontName','Times New Roman');
xlim([-10e-6 250e-6]);ylim([0 10e-6]);
end
shg;

% Once again stating the boundary conditions for our fit, now more
% explicitly for the model (02) and after also for the comparison (03)

% # Adjust manually:
startfit02 = 50*utp + xZero;
endmargin02 = 110*utp;
endfit02 = numel(xIMu)-endmargin02;
fitrange02 = startfit02:endfit02;
xum = xIMu(fitrange02);

% The X^2 aka comparison boundaries (03)

% # Adjust manually:
startfit03 = 2;
endmargin03 = 1;
endfit03 = numel(xum)-endmargin03;
fitrange03 = startfit03:endfit03;
Delta_d_pol = cell(NE02,1);
Delta_d_fit = cell(NE02,2);
pum = cell(NE02,1);
Fum = cell(NE02,1);
Fumdif = cell(NE02,1);
xumdif = xum(1:numel(xum)-1);
chi1 = cell(NE02,2);
chi2 = zeros(NE02,2);
fig001 = figure;
fig002 = figure;
fig003 = figure;
for g = 1:NE02
Delta_d_pol{g,1}=polyfit(Delta_d{g,1}(:,1)*1e6,Delta_d{g,1}(:,2)*1e6,20);
Delta_d_fit{g,1}=polyval(Delta_d_pol{g,1},xIMu);
figure(fig001); subplot(4,3,g);
plot(xIMu,Delta_d_fit{g,1},'or',xIMu(fitrange02),'
Delta_d_fit{g,1}(fitrange02),'
or',
Delta_d{g,1}(:,1)*1e6,Delta_d{g,1}(:,2)*1e6,'.b');
DifPlN = sprintf('D = %d e-10 m^2/s', Diffc02(g));
title(DifPlN,'FontSize',11,'FontName','Times New Roman');
xlabel('x (\mum)','Fontsize',10,'FontName','Times New Roman');
ylabel('\Deltad (\mum)','Fontsize',10,'FontName','Times New Roman');
xlim([-10 250]);ylim([0 12]); shg;
pum{g,1}=polyfit(xIMu(fitrange02),Delta_d_fit{g,1}(fitrange02),polord);
Fum{g,1}=polyval(pum{g,1},xum);

figure(fig002);subplot(4,3,g);
plot(xIMu(fitrange02),Delta_d_fit{g,1}(fitrange02),'.b',
    xum,Fum{g,1},'-r',Deldfit(:,1),Deldfit(:,2),'.k-');
DifPlN = sprintf('D = %d e-10 m^2/s', Diffc02(g));
title(DifPlN,'FontSize',11,'FontName','Times New Roman');
xlabel('x (\mum)','Fontsize',10,'FontName','Times New Roman');
ylabel('\Deltad (\mum)','Fontsize',10,'FontName','Times New Roman');
xlim([-10 250]);ylim([0 12]); shg;

% the differential of the model fit
Fumdif{g,1} = diff(Fum{g,1});
figure(fig003);subplot(4,3,g);
plot(Delddif(fitrange03,1),Delddif(fitrange03,2),'.',
    xumdif(fitrange03),Fumdif{g,1}(fitrange03),'r-')
DifPlN = sprintf('D = %d e-10 m^2/s', Diffc02(g));
title(DifPlN,'FontSize',11,'FontName','Times New Roman');
xlabel('x (\mum)','Fontsize',10,'FontName','Times New Roman');
ylabel('\delta\Deltad/\delta x (\mum)','Fontsize',10,'FontName','Times New Roman');
xlim([40 230]); ylim([-0.001 0.015]);

% sum of least squares for both differential and absolute versions
for q = fitrange03 
    chi1{g,1}(q,1) = (Delddif(q,2)-Fumdif{g,1}(q))^2; 
    chi1{g,2}(q,1) = (Deld(q)-Fum{g,1}(q))^2;
end
chi2(g,1) = sum(chi1{g,1});
chi2(g,2) = sum(chi1{g,2});
figure; subplot(1,1,1);plot(Diffc02,chi2(:,1),'xb:')
title('Differential comparison 110818 1A S003L10');
xlabel('Diffusion coefficient (\cdot 10^{-10} m^2/s)',...
    'Fontsize',10,'FontName','Times New Roman');
ylabel('\chi^2 (a.u.)','Fontsize',10,'FontName','Times New Roman');
Appendix B

Matlab code for analysis of the calibration images

This appendix gives the Matlab code for analysing the calibration images acquired with the confocal fluorescent microscope. Besides calculations the code also includes comments to give an idea of the reasoning behind each particular step. The following code is the contents of the matlab *.m file used for the analysis of the titration measurements. This file is specific for the measurements done on 110719 Fluorescein measurements, no salts:

```
% Titraions in confocal 110719 Fluo without salts sets 01 t/m 04:
% 110719 set 01
% Setting the file path of the images
% ##############################################
% ############# Adjust manually:
Impath = 'D:\Maarten\Data\110719 titration 0a';
pH=[40 41 42 44 45 46 47 48 49 50];
N = numel(pH); %number of tif files we want to evaluate
M = 5; %number of images per stacked tif file
TiIms = cell(N,M); %define size of cell
% The following loop loads all the images we are interested in into the % predefined cells. In the second case, the images are flipped left to % right for convenience:
for k = 1 : N;
    baseFileName = sprintf('110719 titration 0a%d 01.tif', pH(k));
    % Note the %d - it’s important.
    fullfile = fullfile(Impath,baseFileName);
    for l = 1 : M;
        TiIms{k,l} = imread(fullfile,l);
    end
end
[d1TiIms,d2TiIms]=size(TiIms{1,3});
xTiIms = round(2/5*d1TiIms):round(3/5*d1TiIms);
yTiIms = round(2/5*d2TiIms):round(3/5*d2TiIms); 
ImPar = cell(N);
ImSumA = cell(N);
```

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ImSumB = zeros(N);
NrOfElIm = zeros(1,N);
AvIm = zeros(1,N);
for k = 1:N;
    ImPar{k}=TiIms{k,3}(xTiIms,yTiIms);
    ImSumA{k} = sum(ImPar{k});
    ImSumB(k) = sum(ImSumA{k});
    NrOfElIm(k) = nnz(ImPar{k});
    AvIm(k) = ImSumB(k)/NrOfElIm(k);
end
AvIm0a01 = AvIm;
pHx0a01= pH/10;
% 110719 titration 0a set 02
% Setting the file path of the images
% ###################################################################
% ############# Adjust manually:
Impath = 'D:\Maarten\Data\110719 titration 0a';
pH=[40 41 42 43 44 45 46 47 48 49 50];
N = numel(pH); %number of tif files we want to evaluate
M = 5; %number of images per stacked tif file
TiIms = cell(N,M); %define size of cell
% The following loop loads all the images we are interested in into the
% predefined cells. In the second case, the images are flipped left to
% right for convenience:
for k = 1 : N;
    baseFileName = sprintf('110719 titration 0a%d 02.tif', pH(k));
    % Note the %d - it’s important.
    fullfile = fullfile(Impath,baseFileName);
    for l = 1 : M;
        TiIms{k,l} = imread(fullfile,l);
    end
end
[d1TiIms,d2TiIms]=size(TiIms{1,3});
xTiIms = round(2/5*d1TiIms):round(3/5*d1TiIms);
yTiIms = round(2/5*d2TiIms):round(3/5*d2TiIms);
ImPar = cell(N);
ImSumA = cell(N);
ImSumB = zeros(N);
NrOfElIm = zeros(1,N);
AvIm = zeros(1,N);
for k = 1:N;
    ImPar{k}=TiIms{k,3}(xTiIms,yTiIms);
    ImSumA{k} = sum(ImPar{k});
    ImSumB(k) = sum(ImSumA{k});
    NrOfElIm(k) = nnz(ImPar{k});
    AvIm(k) = ImSumB(k)/NrOfElIm(k);
end
AvIm0a02 = AvIm;
pHx0a02= pH/10;
% Plot the figure:
figure; plot(pHx0a01,AvIm0a01,'-bo',pHx0a02,AvIm0a02,':bo')
% Make a title
% ###############################################################################
% ############# Adjust manually:
title('Fluo Intensity as func of pH for Na_2Fl (2.5 \muM) without salts',...
'Fontsize',14,'FontName','Times New Roman')
% Make an x-label
xlabel('pH','Fontsize',12,'FontName','Times New Roman');
% Make a y-label
ylabel('Intensity (a.u.)','Fontsize',12,'FontName','Times New Roman');
% Define axis
axis([0 7 0 16383]);
Bibliography


Hoofdstuk 8

Nederlandse Samenvatting

Het abnormaal snelle transport van protonen - oftewel H⁺ - in water is al meer dan 200 jaar onderwerp voor onderzoek in scheikunde, biologie en natuurkunde. Er is echter nog veel aan dit fenomeen niet goed begrepen of bekend, onder andere hoe protonen zich in water gedragen zodra er andere (geladen) stoffen in het water zijn opgelost. Proton transport in water is een belangrijk proces in onder anderen biologie - voor de werking van cellen - en in technologie - voor de werking van waterstof brandstofcellen. Het is een proces dat overwegend plaats vindt in de nabijheid van andere opgeloste chemische stoffen, daarom is het van belang om te weten of en hoe deze stoffen het gedrag van protonen beïnvloeden.

Een H⁺ in water is altijd verbonden met zijn omgeving waarbij het een hydronium (H₃O⁺) kation vormt. Normale geladen deeltjes moeten, om te kunnen bewegen, tussen alle omringende watermoleculen door bewegen. Een H⁺ kan bij zijn beweging echter gebruik maken van de omringende watermoleculen, via het Grotthuss mechanisme of structurele diffusie, wat inhoudt dat het proton van het H₃O⁺ kation kan overspringen naar een naburig watermolecuul, wat over het algemeen bijna tien keer sneller gaat dan moleculaire diffusie. Voor dit proces moeten beide moleculen wel goed georiënteerd zijn, en het vinden van deze oriëntatie kost tijd. Het is gebleken dat de tijd die het kost limiterend is voor de snelheid waarmee een H⁺ kan bewegen door water. Ook is gebleken dat zouten invloed hebben op het gemak waarmee water moleculen zich kunnen heroriënteren. Hierbij is de mate van hydratatie van de ionen (mate waarin ionen water moleculen aan zich binden) bepalend voor de invloed van zout. Een sterk hydraterend kation of anion - relatief klein en of dubbel geladen ion - heeft een vertragend effect op de heroriëntatie van water. Worden een sterk hydraterend kation en anion gecombineerd dan is de invloed echter meer dan dubbel zo sterk. De verwachting is dat zouten, via hun onderdrukkende effect op de water heroriëntatie, tevens een onderdrukkend effect zullen hebben op de proton mobiliteit.

Wij hebben onderzocht hoe hoge concentraties zout de snelheid van proton transport in water beïnvloed, door te kijken naar proton diffusie in een microfluidics kanaal (hierna microkanaal). In de Y-splitsing van een microkanaal worden een zoutzuur oplossing met een pH van 1.0 (HCl, 0.1 M) en gedemineraliseerd water met een pH van 7.0 (H₂O, neutraal) bij elkaar gebracht, alwaar de protonen van de zure kant kunnen bewegen naar de neutrale kant van het kanaal, zie figuur [8.1]. Doordat de stroming in het kanaal laminair - niet turbulent - is, treed er alleen menging op door diffusie. Deze diffusie
**Figuur 8.1: Fluorescentie in een microkanaal:** De Y-splitsing van het microkanaal is hier weergegeven, de witte lijnen geven de wanden van het microkanaal aan, de stippellijn geeft het midden van het hoofdkanaal aan. Alle kanaaltjes zijn ongeveer 44 µm breed en 23 µm diep (in de z-richting). Bij de onderste tak van de Y stroomt zoutzuur met pH 1.0 naar binnen, bij de bovenste tak stroomt neutraal water met pH 7.0 naar binnen. Aan beide oplossingen is een fluorescerend, zuur gevoelig stofje toegevoegd, hierdoor licht het neutrale gedeelte van het kanaal op en is het zure gedeelte van het kanaal donker. De H^+ diffundeert van het donkere gedeelte naar het lichte gedeelte. De tijd t geeft de stroomrichting aan, want hoe verder in het kanaal, des te meer tijd is er voorbij gegaan, en des te meer H^+ is er naar het lichte gedeelte bewogen, waardoor de fluorescerende stof is uitgeschakeld. Er treed een meetbaar verschil op tussen het midden van het kanaal (de stippellijn) en het fluorescerende gedeelte van het kanaal, aangegeven met ∆d, en dit is een maat voor de diffusie snelheid. Het verloop van ∆d over de lengte van het kanaal hebben we gebruikt om de proton diffusie coëfficiënt (D_{H^+}) te bepalen.

wordt veroorzaakt door het verschil in de proton concentratie tussen de twee oplossingen. Aan alle oplossingen is een fluorescerende stof toegevoegd, welke een gevoeligheid heeft voor zure oplossingen. Bij een oplossing met een pH van minder dan 4.5 stopt het fluorescerende effect. Dit effect, zoals het optreed in het microkanaal, is weergegeven in **figuur 8.1**. Met behulp hiervan is de H^+ diffusie coëfficiënt (D_{H^+}) te bepalen.

Het doel van dit onderzoek was om de invloed van zouten op proton diffusie te onderzoeken. Deze invloed werd onderzocht door aan beide oplossingen in het hier-voor beschreven systeem, 0.5 M (mol/liter) tot maximaal 3.0 M zout toe te voegen (zie **figuur 8.7**). We gebruikten de zouten cesium sulfaat (Cs_2SO_4), magnesium perchloraat (Mg(ClO_4)_2) en magnesium sulfaat (MgSO_4) voor dit onderzoek. De D_{H^+} werd bepaald voor de drie verschillende zouten in toenemende concentraties. Het verwachte effect op de D_{H^+} is het grootst voor MgSO_4, omdat dit zout bestaat uit een combinatie van een sterk hydraterend kation en anion. Bij de andere twee zouten is ofwel het kation ofwel het anion zwak hydraterend.

De effectieve D_{H^+} (D_{H^+, eff}) die werd gevonden, geplot als functie van toenemende zout concentratie, was dalend tot sterk dalend voor alle zouten. Echter, de twee sulfaat zouten gaven identieke resultaten, beide een sterke daling van D_{H^+, eff} van bijna 80% voor de hoogste concentraties ten opzichte van de metingen zonder zout. Een onverwacht resultaat, wat bij nader onderzoek verband bleek te hebben met de bufferende werking van het sulfaat anion. Door de vorming van waterstof sulfaat ionen wordt de effectieve vrije proton concentratie sterk gereduceerd. Door expliciete modellering is onderzocht wat dit zou betekenen voor de eigenlijke D_{H^+} (of D_{H^+, free}). Hieruit blijkt dat D_{H^+, free} - waar deze aanvankelijk werd verondersteld af te nemen - lijkt toe te nemen voor de sulfaat zouten. In vergelijking met de referentie metingen - zonder zout - lijkt een reagerend of bufferend
zout dus niet een vertragend effect - zoals eerder gedacht - maar een versnellend effect op de proton diffusie te hebben. Verdere analyse van de resultaten betreffende de sulfaat zouten zullen volgen ter evaluatie van de impact van de buffer. Tevens zullen nieuwe experimenten worden gedaan om het achterliggende principe te kunnen doorgrijden.
Chapter 9

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