

Translational Diffusion of a Tracer Molecule in Nanoconfined Water

A thesis

Submitted in partial fulfillment of the requirements of the degree of

Doctor of Philosophy

by:

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To my **Pappa, Amma, Annan, and Chechi**

Certificate

Certified that the work incorporated in the thesis entitled “**Translational Diffusion of a Tracer Molecule in Nanoconfined Water**” submitted by **Ajith V. J.** was carried out by the candidate, under my supervision. The work presented here or any part of it has not been included in any other thesis submitted previously for the award of any degree or diploma from any other university or institution.

Date: February 3, 2022



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Declaration

I declare that this written submission entitled “**Translational Diffusion of a Tracer Molecule in Nanoconfined Water**” represents my ideas in my own words, and where others’ ideas have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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A handwritten signature in black ink, appearing to read 'Ajith V. J.', with a stylized flourish at the end.

(Ajith V. J.)

Acknowledgments

I was able to face the challenges in my Ph.D. and the COVID-19 pandemic with the help of a few people. I am using this opportunity to express my acknowledgments.

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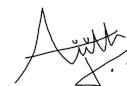
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Abstract

Water in nanoscale slits and pores has many unexpected features like fast permeation in carbon nanotubes, permeation in helium-tight membranes, suppression of freezing, etc. The knowledge of nanoconfined water finds applications in water filtration, nanofluidics, and studies of protein folding. There exist many studies which looked at the dynamics of confined water. Most of the spectroscopic measurements find the self-diffusion of water in nano-gaps similar to the bulk water. In contrast, the rheological measurements find the viscosity of surface-bound water to be many orders larger than its bulk values. It is not apparent how this large viscosity will manifest in the solute/tracer dynamics. Measuring tracer dynamics in the context of confined water have tremendous potential to bring clarity about the nature of nanoconfined water. However, such studies are lacking in the literature. This thesis tries to fill this gap.

A new instrument is made to probe tracer diffusion in nanoconfined water. This instrument integrates ideas of Atomic Force Microscope (AFM), Near-field Scanning Optical Microscope (NSOM), and Fluorescence Correlation Spectroscopy (FCS). We could create a nanoscale pore between the tip and glass coverslip with AFM-like actuation technology. With NSOM-like tips, we could locally illuminate a small volume in the nanoconfined water beneath the tip. We could measure the diffusion time scales of the fluorescent molecule crossing an observation volume beneath the tip by autocorrelation of fluorescent intensities from a tracer dye confined between the tip-substrate gap. The necessary instrument automation and data acquisition protocol are developed in-house. Furthermore, we developed a Monte-Carlo method to analyze the data from the instrument to obtain the diffusion coefficient of tracer dye under nanoconfinement.

We studied the diffusion of different fluorophores in nanoconfined water. Most of the studies are done for the fluorophore called Coumarin 343. Two-time scales are observed for the tracer molecule to cross the observational volume. From control experiments, we found the slow time scale is due to adsorption of fluorophore while the other time scale is due to diffusion. We found that even for confinements as low as 2nm, the translational diffusion of fluorophores is similar to the bulk diffusion. Measurements with another fluorescent molecule: Alexa Fluor 568, confirmed these conclusions. At the same time, we couldn't analyze the data from the molecule, Rhodamine 6G, as it is adsorbing strongly to the confining surfaces.

This thesis work establishes a new instrument with a new analysis procedure. We used the instrument to study the translational diffusion of fluorescent tracer molecules in single nanopore-confined water.

List of publications

- Translational diffusion of a fluorescent tracer molecule in nanoconfined water
V. J. Ajith, and Shivprasad Patil.
Langmuir, **38** (3), 1034-1044 (2022).
(Discussed in Chapter 5)
- Validity of Point-Mass Model in off-resonance Dynamic Atomic Force Microscopy;
S. S. Rajput, S. P. S. Deopa, **V. J. Ajith**, S. C. Kamerkar, and S. Patil
Nanotechnology, **32**, 405702 (2021).
- A new method for measurement and quantification of tracer diffusion in nanoconfined liquids;
V J Ajith and Shivprasad Patil,
Review of Scientific Instruments, **91**, 013702 (2020).
(Discussed in Chapter 3 and 4)
- The effect of boundary slippage and nonlinear rheological response on flow of nanoconfined water;
Amandeep Sekhon, **V J Ajith** and Shivprasad Patil,
Journal of Physics: Condensed Matter, **29**, 205101 (2017).

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Chapter 1

Introduction

This chapter introduces the thesis. Chapter starts by illustrating why it is important to study water near interfaces. Then the existing studies are reviewed. With this background, the scope and plans for the thesis-work are presented. The chapter ends with an outline of subsequent chapters.

1.1 Nano-confined Water

Water is the most commonplace liquid, composed of just three atoms: two hydrogens and an oxygen. Despite the small size and simple molecular structure, it is far from simple. It shows many anomalous properties like frozen form(ice) is less dense than liquid form, high specific heat capacity and latent heat, high boiling and freezing points, and many more. Life, as we know, shaped itself to utilize most of these properties. For instance, the high specific heat helps in regulating body temperature in animals and regulates Earth's climate in general. In another case, the high density of water compared to ice allows aquatic life possible in water bodies in cold-places. The unique properties of water are not limited to these bulk or large length-scales.

Water at an interface often surprises us with its unique behaviors, which its bulk counterpart does not have. Properties of interfacial water become apparent when multiple interfaces confine water in-between, mostly in nanometer length-scale. One such observation is the fast permeation of water in carbon nanotubes(CNTs). The flow rate of water in CNTs is few orders larger than expected [4]. Another interesting observation is that water can pass through a membrane that can even block light-weight Helium

gas [5]. Difficulty in freezing confined water is another intriguing observation. Water in nanoscopic pores does not freeze even at 173K [6], while bulk water freezes at 273K. One unique aspect of water near a solid interface is the periodic solvation force. This force is experienced on the confining walls while the walls are brought close to each other. Solvation force raises when the gap is roughly an integer multiple of molecular size of water [7]. These observations, with many others, make confined water special.

Other than having the thrill to unravel the rich physics of these observations, studying confined water has many applications. One of the applications is in water filtration and desalination. The depletion of freshwater sources has caused an ever-increasing demand for purification of water. Designing efficient membranes for filtering water needs a good understanding of how water behaves in small pores of membranes. Many studies are being done to use CNT embedded membranes for filtration and desalination [8]. The next application is in the making of efficient fuel cells. Fuel cells allow hydrogen gas to react with oxygen gas to generate electric potential and water. It consists of a membrane separating electrodes, which allows ion transport. The content of water in this membrane was found to change the performance of the fuel cell [9]. The most important application of the knowledge of interfacial water is in the study of protein folding. Proteins are long polymers of amino-acids. It does most of the functions in a cell. It folds by itself into a unique structure critical for its function without sampling all statistically possible configurations. Hydrophobic and hydrophilic interactions play a key role in this accurate and spontaneous folding [10]. Water transport across cell membranes is another process where its understanding is based on confined water dynamics. Such transport happens through a family of nanopores called aquaporins [11]. Concepts of confined water find applications in these areas. A better understanding of confined water will automatically impact these fields.

Considering all these unique properties and applications, many studies happened to refine and question our understanding of nano-confined water.

1.2 Studies on nano-confined water

1.2.1 Experimental realisation of nano-confined water

There are many systems available to study water in nanometer gaps or pores. Fine glass capillaries is one of the earliest system used to study confined water [12]. One could

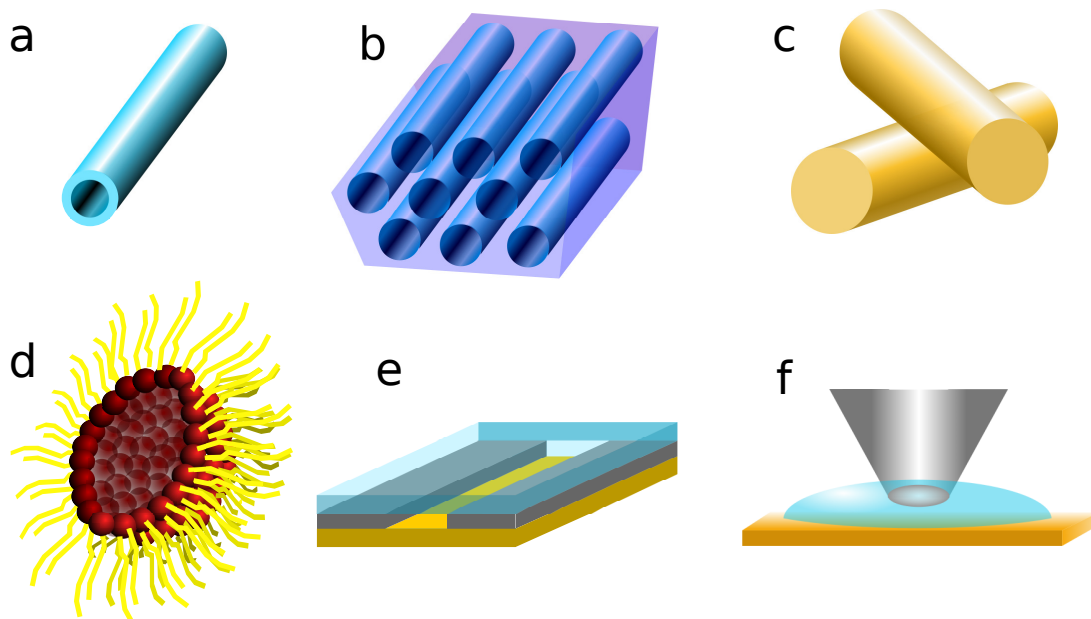


Figure 1.1: Experimental realizations of confined water (a) Capillaries and Nanotubes, (b) Pores in porous materials (c) Between the cross cylinders in Surface force apparatus(SFA), (d)Inside Reverse Micelles, (e)Fabricated nano-channels, (f)Between an Atomic Force Microscope tip and a substrate

look at the motion of water meniscus in a pressure-driven flow through the capillaries. Analogs to this system are used in current-day research. For instance carbon or boron-nitride nano-tubes [13] or fabricated nano-channels over silicon [14, 15]. Here confinement happens except along the flow axis of the channel. From early on there are studies tracking the diffusion of colloidal beads in micro-channels [16, 17]. Colloidal tracers are chosen as they are the smallest particle that could be tracked under an optical microscope due to diffraction limitation. Owing to the size of these particles, the least confinements possible were around $1\mu\text{m}$. At this scale, fluid-dynamic theories of the particle motion were found to be valid. With improved cameras and computational power, currently, people could track fluorescent beads as small as 50nm [18]. These measurements are not necessarily particle tracking, it can be intensity distribution tracking [15] or spectroscopic measurements [14]. These new measurements could work with small fluorescent molecules as well. Usage of fluorescent molecules as tracers and better fabrication techniques made sub-10nm channels feasible [15].

Porous materials are popular in the nano-confinement studies of water. One of the first porous material used to study confined water is “Vycor glass” from “Corning glass

works” [19, 20]. In this material, pores were not of uniform shape or having sharp size distribution. This issue is solved by using mesoporous silica materials called MCM41 and SBA15. These made from the sol-gel process has highly ordered cylindrical pores with mono-distributed diameter. Straight forward synthesis protocol allows fabricating materials with controlled pore diameters. Pores of 2.1nm to 5.4nm diameter [21] are used in various studies. Many spectroscopic studies like Infrared and Neutron diffraction are done on these systems. We will look into these studies in the next section.

Another important system is reverse micelles. These are nano-droplets of water surrounded by lipid molecules in oil like solvent. This system provides soft-wall confinement, which water might encounter in cells. Like in the case of mesoporous silica, many spectroscopic studies are possible in this system [22, 23].

In all these systems, if we need to measure properties as a function of separation, we need separate materials with different pore sizes. If all surface properties of pores in these separate materials are not the same, then it is difficult to compare the measurements. In such situations, a pore with controllable separation is desired. This requirement is facilitated in systems with parallel plate configuration. Here water is sandwiched between a pair of parallel walls separated by few nanometers. This configuration is possible in instruments like Surface Force Apparatus(SFA) [7] and Atomic Force Microscope(AFM) [24, 25]. One of the parallel walls can be moved lateral or perpendicular to the other surface in this configuration. This motion generates a reaction force in the water, and that can be measured using a force sensor attached to one of the surfaces. Analysis of this reaction force gives stiffness, viscosity, and relaxation time of the confined water. More about these measurements are presented in the next section.

The types of measurements possible with each of these systems are different. With mesoporous materials and reverse micelles, measurements are mostly spectroscopic. Furthermore, these measurements are done on an ensemble of nanopores or reverse micelles. Capillaries, nano-channels, and parallel-plate configuration allow measurements on a single pore. The next section is a review of these different types of measurements and their conclusions.

1.2.2 Different experiments and conclusions

Water is one of the popular topics of research from ancient times. Even today, we do not entirely understand all properties of water. The development of the field - “Fluid

dynamics” contributed a lot in understanding water’s behavior in bulk or large length scales. Molecular picture, along with theories of diffusion and hydrogen bonding, gave a more precise understanding of water. In the past 50 years, a new-found interest is there in studying water at interfaces. The newfound interest is due to the realization of interfacial water’s relevance in many fields like bio-molecules, water filtration, lubrication.

A plethora of experiments study the interfacial water and confined water. This review focus on experiments measuring the dynamical properties of confined water. All studies discussed here are done at room temperature. Thus, many interesting studies with temperature variation are not covered [26, 27]. Moreover, the studies on the non-polar liquids [28] are also not covered in this review.

In 1970, Churayev, Sobolev, and Zorin systematically studied viscosity dependence with the diameter of quartz capillaries [12]. They calculated effective-viscosity from the velocity of the pressure-driven flow by tracking the meniscus. Their measurements show that the effective water viscosity monotonically increases as the diameter of the capillary is reduced. For a capillary with diameter 80nm, the effective viscosity is found 40% larger than the bulk value. Such a behavior is not seen in non-polar liquids. This indicates that the hydrogen-bonding and other electrostatic interactions play an essential role in determining the effective viscosity. During this time, studies on the stability of soap films [29] and swelling of clays [30] indicated the presence of a dominating solvation force in nanometer scale.

Studies with Surface Force Apparatus

In 1976, Israelachvili and Adams introduced a very sensitive instrument for measuring forces between surfaces inside liquid [31]. This instrument and its variations are now called Surface Force Apparatus(SFA) (Also known as Surface Force Balance). In SFA, there is a pair of cross cylinders wrapped with mica, brought close together with a piezo-crystal. Either these cylinders are inside a liquid, or a small amount of liquid is added between them. The surface separation is measured with multiple beam interferometry. One of the surfaces is attached to a cantilever, whose deflection is used to measure forces. With SFA experiments in small gaps below 1nm, a highly repelling hydration force is revealed [32]. In 1983, careful experiments from Israelachvili and Pashley resolved the oscillatory nature of this hydration force [7]. They found that the force between two atomically flat mica sheets mediated by water is oscillatory with

respect to separation. Moreover, the period roughly equals to the size of the water molecules($0.25\pm0.03\text{nm}$). This solvation force is on the top of monotonic DLVO and van-der-waal forces, and it dominates below 1nm. This layering is not unique to water; it is also seen in other liquids [28].

SFA is further used to measure viscosity of confined water [33–38]. For finding viscosity the total force measured is decomposed as elastic and dissipative parts using appropriate force models. For this, different measurement strategies are used. One strategy is to measure the periodic stress developed due to a periodic strain given. The periodic strain is given by oscillating one of the surfaces normal [33] or parallel [35,37] to the other surface with a known amplitude and frequency. The stress thus developed is measured on the other surface via a force sensor along the direction of interest. This stress is modeled with the given strain to calculate viscosity. Another way to measure viscosity is from the drainage flow of water as the surfaces approach [34]. Most of these measurements indicates that viscosity of water only increase by 3 or 4 time from it's bulk value in nanometer gaps [33–35]. Raviv, Pierre, and Klein argued that even below 1nm, water viscosity could not be more than 3 or 4 times the bulk value [35]. On the contrary, one SFA measurement on the last bound water layer showed few orders(5 to 9) larger viscosity [36] compared to the bulk value of 0.9cP. For the different orientation of mica sheets(twist angles), this enhanced viscosity varied from 10^5 to 10^9 cP. Next year, Raviv and Klein published their work on aqueous NaCl instead of pure water used in the previous study. Here also they showed the water viscosity does not change drastically in confinement. Furthermore, they expressed that surface contamination might be why the large values of viscosity in other studies. To settle this issue, Sakuma, Otsuki, and Kurihara performed another SFA measurement on aqueous NaCl [38]. Here they recorded the frequency response of the measured shear amplitudes for different separations. As separation is decreased, below 1.8nm, the resonance peak in this frequency response shifted to larger frequencies, and the peak got broader. From the shape and peak position of this response, they estimated the elastic and dissipation constants, thus the viscosity. Their viscosity values were 2-4 orders larger than the bulk value when the gap is below 1nm.

Studies with Atomic Force Microscope

In parallel with SFA, scanning probe microscopy techniques like Atomic Force Microscopy (AFM) was evolving. Binnig, Quate, and Greber introduced AFM in 1986 [39]. Using AFM, we can measure forces experienced by a sharp tip over a surface. A force sensor, typically a cantilever, is attached to the tip. The deflection of this cantilever is monitored for measuring forces. By mapping the measured force from various points in space, we get a false image of the surface and thus, it is a microscope. AFM measurements are shown to work inside liquids as well [40–42]. In such cases, the interaction between the tip and substrate is mediated by the confined water.

There exist many variations in AFM. One type, called “transverse dynamic force microscope,” was used by Antognozzi, Humphris, and Miles in 2001. In this instrument, a tapered optical fiber is used as the tip as well as the cantilever. The fiber-tip is kept vertical to a mica surface and oscillated laterally. The oscillation amplitude of the tip is measured using a split-photodiode tracking reflected laser beam from the fiber. They observed periodic hydration force like in SFA. Besides, they estimated viscosity from the amplitude and phase of the tip oscillation. Moreover, the viscosity was around 3 orders larger than the bulk value for confinements below 1 nm. They also found that both elastic and dissipative force contributions are comparable in magnitude. Thus confined water is a viscoelastic material. A later SFA study also showed this viscoelastic character [38].

AFM measurements with small amplitudes and off-resonance oscillations gave direct and linear force measurements. Jeffrey et al. performed such a measurement on confined water in 2004 [43]. They also reported a large viscosity and water layering. An AFM with improved sensitivity to perform small-amplitude and off-resonance measurements was introduced by Patil et al. in 2005 [44]. With this instrument, a dynamic solidification of confined water was observed [45]. They observed that when the gap confining the water is reduced with a rate slower than 0.6 nm/s, the elastic and dissipative forces are in-phase. For a rate faster than 0.6 nm/s, this in-phase response shifted to an out of phase one, and beyond 1 nm/s, the elastic and dissipative forces are out of phase. This out of phase response gives periodic spikes in the calculated Maxwell’s relaxation time regarding gap-separation. The highest peak values are on the millisecond scale. Compared to the bulk relaxation time of water (a few fs), this is large. They called

this dynamic response as dynamic solidification.

Few AFM studies indicated confined water is a nonlinear viscoelastic material. In a study by Li and Riedo, confined water's relaxation time is found to be decaying with strain rate [24]. In a few other studies, confined water has a shear thinning character [46,47].

The nature of substrate surfaces confining water affects the properties of confined water. So far, all the studies we discussed are done over hydrophilic surfaces like mica, quartz, or glass. Studies are done over various surfaces with different hydrophilicity as per the contact angle. A less pronounced water layering or oscillatory force is observed over hydrophobic graphite than hydrophilic mica or glass [48]. Measurements on hydrophobic surfaces also revealed slippage of boundary water layer [47,49]. Instead of using different substrates, chemical modification of surfaces also changes the hydrophilicity [50,51].

It is also possible to build up a three-dimensional force map over a surface on the atomic scale. Water distribution over a mica surface has been measured using a special kind of frequency-modulated AFM [52]. A correlation with water molecules and underlying mica crystal structure is found in this study. They also observed site-specific variations in the hydration force. With another similar kind of AFM, water damping force over a calcite crystal has been measured [53]. Larger damping is observed over calcium sites.

Studies with Neutron Scattering

Neutron Scattering can be used to study the self-diffusion, density, and proton distribution of confined water. Water confined in porous materials or reverse micelles are used in these studies. A beam of neutrons is allowed to scatter over these samples, and the scattered beam is analyzed to extract the properties of confined water. For this purpose, inelastic and quasi-elastic neutron scattering is chosen.

Quasi-elastic Neutron Scattering (QENS) is used to measure the self-diffusion of water. We can extract translational and rotational diffusion coefficients. This technique allows measurement of time scales in the femtosecond to nanosecond range and length scale in angstrom. The translational diffusion coefficients of confined water is similar to the bulk value [19,23,54,55]. The rotational time scales are also comparable to the bulk value [23,54].

Deep Inelastic Neutron Scattering (DINS) is used to check the momentum distribution of protons. It is seen that the proton momentum distribution acquire a non-gaussian shape as the confinement pore size is reduced in the nanometer scale [56].

Neutron scattering is utilized to determine the structure of confined water [57]. In porous MCM41 samples, water seems to have a 20% lower density than bulk water. The water in these nanopores' core has slightly larger density, but still lower than the bulk value.

Studies with infrared pump-probe spectroscopy

Infrared(IR) pump-probe spectroscopy is used to check the hydrogen-bond dynamics of confined water. Besides, the anisotropy measurements with it reveal the reorientation or rotational time scales. The pump and probe frequencies are chosen around the vibration energy corresponds to a bond stretching of a dilute probe molecule, which is sensitive to hydrogen bonding in water. For this –OH stretch of dilute HOD in D₂O [22] or –OD stretch of dilute HOD in H₂O [58, 59] or –CN stretch of dilute SeCN[–] in H₂O [60] is used. A study by Dokter et al. on reverse micelles found that the change in transmittance of probe light decayed bi-exponentially as the delay time between pump and probe is increased [22]. Moreover, the amplitude of these two exponential contributions varied with the diameter of reversed micelles such that the amplitude of slower exponential is proportional to the surface area. With this, they concluded that the slower contribution is due to surface-bound water. This is consistent with the two-state model of confined water. In the two-state model, the water bound to surfaces has different properties than the confined water away from surfaces. They also measured the average reorientation time scale from anisotropy measurements. Furthermore, for diameters smaller than 1nm, the time scale was around 15-20ps, almost five times larger than the bulk value(2-4ps). In another study, it is seen that slow down in reorientation is more in reverse micelles with an anionic head group than with neutral head group [58]. Along with reversed micelles, certain surfactants can form lamellar structures. In lamellar structures, water is confined between two planar surfaces. Slow down in reorientation dynamics is seen for both of these configurations. This slow down is comparable when both lamellar and reversed micelles have the same number of water molecules per surfactant molecule. This criterion is matched for reversed micelles with a certain diameter larger than the gap in lamellar structure [59]. Similar measurements are also done on porous materials and

found a slowdown in reorientation dynamics in the same order [60, 61].

Studies using simulations

Molecular dynamics simulations are extensively used in studying interfacial water and nano-confined water [62–66]. Majority of the studies finds layering of water near interfaces. In one study the in-plane translational diffusion timescale for water in 2.44 and 1.65 nm gaps are found to be similar to bulk value [62]. Unlike translational diffusion the time scales of rotational diffusion is found to be larger than the bulk value [63].

Few other studies

There exist many other techniques that can be utilized to study the dynamics of confined water. Optical Kerr Effect spectroscopy can check the average reorientation dynamics of confined water [67]. Studies using this technique also found a slow down for confined water matching with the IR pump-probe results.

There are few diffusion measurements in single nano-channels. Unlike QENS, which measured self-diffusion of water, measurements are of the dynamics of a fluorescent molecule. We can gain insights into confined water from the diffusion of these tracer molecules. These measurements are done either with Fluorescent Correlation Spectroscopy(FCS) [14, 68] or by tracking the fluorescence distribution along nanochannels over time [15]. The study with FCS was done on channels having gaps 10nm, 20nm, and 30nm. They didn't find any significant slow down [14]. However, the study of fluorescence distribution tracking on a channel with an 8nm gap showed two orders reduction in diffusion coefficient [15]. The fluorophores used in these studies have a charge. So for singling out the effects of confined water in fluorophore diffusion, care should be taken to avoid the effects of these fluorophores' electrostatic interactions with surfaces.

1.3 Objectives of the thesis

We saw that even with so many experiments and independent repetitions of similar experiments, a clear picture of the confined water dynamics is yet to emerge. The drastic slowdown of more than four orders from bulk dynamics seen in AFM and few SFA studies are yet to be seen in diffusion studies. The majority of diffusion studies show a slow down within an order or a maximum slowdown by two orders. If there is an

instrument that can perform simultaneous AFM and diffusion measurements, we can understand these discrepancies better. Such instruments can also be used to check the influence of periodic strain on the diffusion. This thesis is the first step towards this future goal.

The majority of these diffusion experiments are done on an ensemble of reverse micelles or pores in porous materials. With reverse micelles, one has to worry about the motion of individual surfactant molecules, which composes the confining surface and the motion of the whole of the reverse micelle itself. These motions might interfere with water dynamics as we do not know if the time scales of all these independent motions are well separated. There are issues with porous materials, as well. These materials come in powdered form, and each powder particle has the pore structures. While filling water in the pores by adsorption or soaking, the presence of water outside the pores is unavoidable. Thus, such samples' measurements will have a mixture of water dynamics inside and outside the pores. These problems with ensemble measurements are not there in local measurements inside a single nano-channel. In all these cases, a gap dependence study is possible only by fabricating different samples with different confinement gaps. All these issues are avoided if we can measure diffusion in parallel plate configuration of AFM or SFA. Here gap can be controlled actively during the experiment, and local diffusion measurement can be made possible.

In this context, we list the objectives of this thesis as follows:

1. Build an experimental setup on a homebuilt AFM to perform translational diffusion measurements of a tracer molecule in controlled nanometer gaps having water.
2. Develop a way to quantify the apparent translational diffusion coefficient of the tracers from the observables in the setup.
3. Study confinement gap dependence on the translational diffusion of a tracer molecule in confined water

The experimental setup that will be built in this thesis has more scope than the listed objectives. This thesis establishes the instrument's capability to measure translational diffusion of a tracer molecule in confined water. More studies could be done with simultaneous AFM and diffusion measurements.

1.4 Organisation of the thesis

- Chapter 2 discusses the theoretical background relevant for the studies done in this thesis.
- Chapter 3 describes the experimental setup and instrument we build in detail.
- Chapter 4 describes the simulation-based procedure we developed to quantify tracer molecule's translational diffusion coefficient from the experimental data.
- Chapter 5 presents the gap dependent studies we conducted with the instrument
- Chapter 6 concludes the results and outlines the future outlook of this thesis work.

Chapter 2

Theoretical Background

This chapter briefly discusses various theoretical descriptions relevant to tracer diffusion in a liquid confined between two parallel boundaries. First, different models for diffusion and simulation strategies are presented. Then electrostatic interactions the tracer experience near a surface are outlined. Further, we describe a few expressions derived for Fluorescence Correlation Spectroscopy. These expressions are required for the analysis of our data.

2.1 Diffusion

This section lists the important attempts in describing and simulating diffusion. We start with Fick's law. Then Einstein's approach and more generalized Langevin's equations. For more details please refer to textbooks on diffusion [69].

2.1.1 Fick's Law

Diffusion is the motion of a tagged particle in a liquid. Fick's law is an empirical law that relates diffusion to concentration gradients. It is modeled from the observation that the solute particle moves from high to low concentration regions. We will use the number density and flux of solute particles in liquids to mathematically state the law. The number density $\rho(\vec{r}, t)$ is the average number of solute particles per unit volume in a small region around $\vec{r}$ at time t . The flux $\vec{J}(\vec{r}, t)$ is defined such that if $\hat{n}$ is a unit area at $\vec{r}$ and time t , then $\vec{J}(\vec{r}, t) \cdot \hat{n}$ will give the average number of particles crossing unit area in unit time along $\hat{n}$. According to Fick's law, the flux of solute particles is proportional

to the negative gradient of its concentration, and the proportionality constant is called the “Diffusion coefficient (D).”

$$\vec{J}(\vec{r}, t) = -D\nabla\rho(\vec{r}, t) \quad (2.1)$$

We can write another equation from this relation, which depends on number density. The flux and rate of change of number density are related via the continuity equation. According to the continuity equation, the change in number density in time equals the negative divergence of flux.

$$\frac{\partial\rho(\vec{r}, t)}{\partial t} = -\nabla\cdot\vec{J}(\vec{r}, t) \quad (2.2)$$

On substituting $\vec{J}(\vec{r}, t)$ from eq.2.1 we get,

$$\frac{\partial\rho(\vec{r}, t)}{\partial t} = D\nabla^2\rho(\vec{r}, t) \quad (2.3)$$

This equation is called the “diffusion equation.” For this differential equation, x,y, and z coordinates could be separated, and we can write identical independent differential equations for each coordinate. The solution then will be the product of all one-dimensional solutions. So for solutions and boundary conditions, let’s focus on the one-dimensional version of the diffusion equation.

$$\frac{\partial\rho(x, t)}{\partial t} = D\frac{\partial^2\rho(x, t)}{\partial x^2} \quad (2.4)$$

Now, let’s check the solutions for a few boundary conditions.

Case 1: Unbounded volume

In this case, the diffusion is in an unbounded volume.

Initial condition: $\rho(x, 0) = N\delta(x)$; at $t=0$ all N molecules are at $x=0$ position.

Boundary condition: $\rho(\pm\infty, t) = 0$

Solution:

$$\rho(x, t) = \frac{N}{\sqrt{4\pi Dt}} \exp\left(\frac{-x^2}{4Dt}\right) \quad (2.5)$$

Case 2: Semi-infinite volume(One reflecting boundary)

In this case, a reflecting boundary is kept at $x=0$.

Initial condition: $\rho(x, 0) = 2N\delta(x)$; at $t=0$ all N molecules are at $x=0$ position.

As $\int_0^\infty \delta(x)dx = 1/2$, normalisation constant for $\delta(x)$ is $2N$

Boundary condition: $\rho(\infty, t) = 0$ and $\frac{\partial \rho(x, t)}{\partial x} \Big|_{x=0} = 0$

The second boundary condition is due to the reflecting boundary. Due to the reflection, at $x = 0$, the flux will be zero. Then, Fick's law will give this boundary condition.

Solution:

$$\rho(\vec{r}, t) = \frac{2N}{\sqrt{4\pi Dt}} \exp\left(\frac{-x^2}{4Dt}\right) \quad (2.6)$$

Case 3: Bounded volume(Two reflecting boundaries)

In this case reflecting boundary is kept at $x = L/2$ and $x = 3L/2$. The diffusion happens only in $L/2 < x < 3L/2$. This choice of boundary positions helps in simplifying the solution.

$$\text{Initial condition: } \rho(x, 0) = \begin{cases} 0 & \text{if } 0 < x \leq L/2 \text{ or } 3L/2 < x \leq 2L \\ \rho_0 & \text{if } L/2 < x \leq 3L/2 \end{cases}$$

$$\text{Boundary condition: } \frac{\partial \rho(x, t)}{\partial x} \Big|_{x=L/2} = 0 \text{ and } \frac{\partial \rho(x, t)}{\partial x} \Big|_{x=3L/2} = 0$$

Solution:

$$\rho(\vec{r}, t) = \frac{\alpha_0}{2} + \sum_{n=1}^{\infty} \alpha_n \cos\left(\frac{n\pi x}{L}\right) \exp\left[-\left(\frac{n\pi}{L}\right)^2 Dt\right] \quad (2.7)$$

$$\alpha_n = \begin{cases} \rho_0 & \text{if } n=0 \\ (-1)^{\frac{n+1}{2}} \frac{2\rho_0}{n\pi} & \text{if } n=1,3,5,\dots \\ 0 & \text{if } n=2,4,6,\dots \end{cases}$$

Fick's law can't explain the motion of solute particles in equilibrium as number density is constant throughout the volume.

2.1.2 Einstein's Description

Einstein used concepts of probability to explain diffusion and argued that a sufficient number of collisions between solvent and solute particles drive diffusion.

Let the probability that a solute particle will move from $x = \xi$ to $x = \xi + d\xi$ in δt is $\Phi(\xi; \delta t)d\xi$ and initial condition be $\rho(x, 0) = N\delta(x)$. Then the time evolution of density of solutes $\rho(x, t + \delta t)$ could be expressed as,

$$\rho(x, t + \delta t) dx = \int_{-\infty}^{\infty} [\rho(x - \xi, t) dx] \cdot [\Phi(\xi; \delta t) d\xi] \quad (2.8)$$

Divide both sides with dx and replace $\rho(x - \xi, t)$ with its Taylor expansion. So we can write,

$$\begin{aligned} \rho(x, t + \delta t) &= \int_{-\infty}^{\infty} \Phi(\xi; \delta t) \rho(x - \xi, t) d\xi \\ &= \int_{-\infty}^{\infty} \Phi(\xi; \delta t) \left[\rho(x, t) + \sum_{k=1}^{\infty} \frac{(-\xi^k)}{k!} \frac{\partial^k \rho(x, t)}{\partial x^k} \right] d\xi \\ &= \rho(x, t) + \sum_{k=1}^{\infty} \frac{1}{k!} \frac{\partial^k \rho(x, t)}{\partial x^k} \int_{-\infty}^{\infty} (-\xi^k) \Phi(\xi; \delta t) d\xi \end{aligned} \quad (2.9)$$

Bring the first term to right and divide the equation with δt . Then in the limit $\delta t \rightarrow 0$ we get,

$$\frac{\partial \rho(x, t)}{\partial t} = \sum_{k=1}^{\infty} \left[\frac{1}{\delta t} \frac{1}{k!} \frac{\partial^k \rho(x, t)}{\partial x^k} \int_{-\infty}^{\infty} (-\xi)^k \Phi(\xi; \delta t) d\xi \right] \quad (2.10)$$

The probability, $\Phi(\xi; \delta t)$ is an even function. For k =odd number, the integrand is an odd function as $-\xi^k$ is an odd function and integral is zero. Einstein assumed the series converge fast and only $k = 2$ term contributes. Then the equation becomes,

$$\frac{\partial \rho(x, t)}{\partial t} = \left[\frac{1}{\delta t} \frac{1}{2} \int_{-\infty}^{\infty} \xi^2 \Phi(\xi; \delta t) d\xi \right] \frac{\partial^2 \rho(x, t)}{\partial x^2} \quad (2.11)$$

Comparing with diffusion equation from Fick's law, we can identify diffusion coefficient as,

$$D = \frac{1}{\delta t} \frac{1}{2} \int_{-\infty}^{\infty} \xi^2 \Phi(\xi; \delta t) d\xi \quad (2.12)$$

The integral is the second moment for ξ . As the mean=0 (the integral for, $k=1$),

the mean square displacement ($MSD = \langle (\xi - \langle \xi \rangle)^2 \rangle = \langle \xi^2 \rangle - \langle \xi \rangle^2 = \langle \xi^2 \rangle$) is equal to the second moment. Thus, Einstein's approach gives a relation connecting diffusion coefficient and mean square displacement of the solute particle.

$$D = \frac{\langle \xi^2 \rangle}{2\delta t} \quad (2.13)$$

On rearranging, we get,

$$\langle \xi^2 \rangle = 2D\delta t \quad (2.14)$$

This equation is for one dimension and $\rho(x, 0) = N\delta(x)$. We can generalize this equation as,

$$\langle \xi^2 - \langle \xi \rangle^2 \rangle = 2nD\delta t \quad (2.15)$$

Here $n = 1, 2, 3$ is the dimension number.

Now let's see the form of the probability $\Phi(\xi; \delta t)$. This probability is for the position of the particle after a time interval δt . This could be obtained from the instantaneous positional probability, $P_X(x, t|x_0, t_0)$. $P_X(x, t|x_0, t_0)dx$ gives the probability that the position of particle is between x and $x + dx$ at time t with an initial condition $X(t_0) = x_0$. This probability defines the random variable $X(t)$ which takes the position value x at time t . So $\Phi(\xi; \delta t) = P_X(x, t + \delta t|x, t)$. The number density and $P_X(x, t|x_0, t_0)$ are connected. It is easy to see the correlation, when $\rho(x, t) = 0$ we expect no particle at (x, t) so the $P_X(x, t|x_0, t_0)$ should be zero. Also, as $\rho(x, t)$ increases the probability of finding the particle at that point increases. The $P_X(x, t|x_0, t_0)$ and $\rho(x, t)$ are directly proportional. Thus, with normalization condition of probability we can write,

$$P_X(x, t|x_0, t_0) = \frac{\rho(x, t)}{N} \quad (2.16)$$

This proportionality implies, this probability also satisfies the diffusion equation,

$$\frac{\partial P_X(x, t|x_0, t_0)}{\partial t} = D \frac{\partial^2 P_X(x, t|x_0, t_0)}{\partial x^2} \quad (2.17)$$

The solutions we listed in the previous section also works for $P_X(x, t|x_0, t_0)$. So in free space with $P_X(\pm\infty, t|x_0, t_0) = 0$ and $P_X(x, t_0|x_0, t_0) = \delta(x - x_0)$ we have,

$$P_X(x, t|x_0, t_0) = \frac{N}{\sqrt{4\pi Dt}} \exp\left(\frac{-(x - x_0)^2}{4D(t - t_0)}\right) \quad (2.18)$$

Thus the random variable $X(t)$ is a normal random variable. The probability is the re-scaled or normalized histogram of all possible $X(t)$ values. So the distribution of all possible $X(t)$ is a normal distribution. $\mathcal{N}_X(\mu, \sigma^2)$.

$$X(t) = \mathcal{N}_X(x_0, 2D(t - t_0)) \quad (2.19)$$

The variance (σ^2) for this position random variable, $X(t)$ is nothing but the mean square displacement. $\langle (x - x_0)^2 \rangle = 2D(t - t_0)$.

This description of diffusion is an approximate one. The first issue is the approximation of the infinite sum with just two terms. This description doesn't give a convincing explanation for that. Another issue is about the lower limit of δt . Below some value of $(t - t_0)$, we expect solute moves ballistic without any collision. In this description, $X(t) = \mathcal{N}_X(x_0, 2D(t - t_0))$ is always normal distribution even when $(t - t_0) \rightarrow 0$. Thus, this description is not valid below a lower limit of time. We couldn't estimate this limit with this description.

2.1.3 Simulation strategy (Based on Einstein's results)

We found that the position random variable is normally distributed. Any normal distribution with mean μ and standard deviation σ can be written in terms of standard normal distribution ($\mu = 0, \sigma = 1$).

$$\mathcal{N}(\mu, \sigma^2) = \mu + \sigma \mathcal{N}(0, 1) \quad (2.20)$$

For $X(t)$ we can write,

$$\begin{aligned} \mathcal{N}(x_0, 2D(t - t_0)) &= x_0 + \sqrt{2D(t - t_0)} \mathcal{N}(0, 1) \\ x_t &= x_0 + \sqrt{2D(t - t_0)} n \end{aligned} \quad (2.21)$$

Here n is a random number from the standard normal distribution $\mathcal{N}(0, 1)$. The normalised histogram of 1000 random numbers drawn using this expression and the expected analytical form of the distribution given by eq. 2.18 are shown in the figure

2.1.

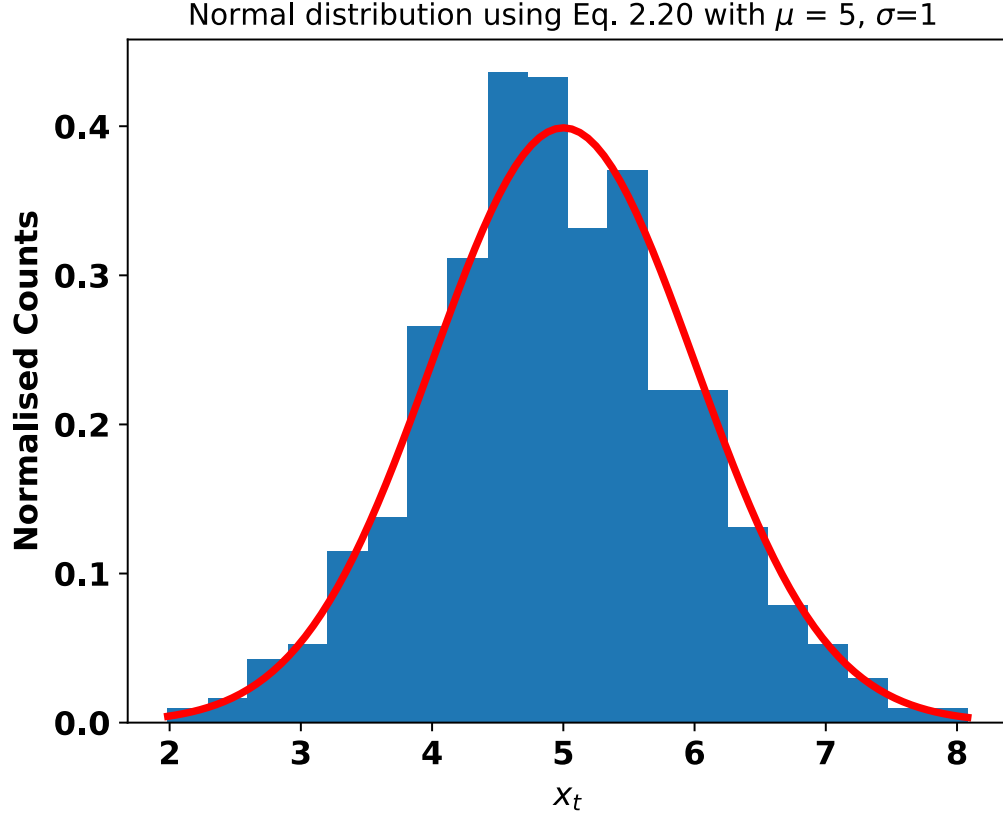


Figure 2.1: The normalised histogram of 1000 random numbers drawn using this expression eq. 2.20 and the expected analytical form of the distribution given by eq. 2.18

This updating formula can generate the trajectory of a diffusing particle. For getting a time series of positions(path for that particle) we consider t_0 as the previous time step(t_{i-1}) and “t” as the current time step(t_i). And $\Delta t = t - t_0 = t_i - t_{i-1} = \text{constant}$. And we used $1 \mu s$ as this time interval. Thus the position time series of a particle along x-axis is given by,

$$x_i = x_{i-1} + \sqrt{2D(t_i - t_{i-1})}n \quad (2.22)$$

Similarly we find y and z position for each time step; t_i

When we have reflecting boundaries, first, we find a tentative position with this formula. Then we will calculate the actual position in the bounded volume after considering all possible reflections.

For single reflecting wall at x_r :

Suppose we have a reflecting wall at x_r and accessible space is $x < x_r$. For this case, the algorithm for generating x_t is follows.

1. Generate tentative position in free space.

$$x_t^{tent} = x_0 + \sqrt{2D(t - t_0)}n \quad (2.23a)$$

2. Actual position

$$x_t = \begin{cases} x_t^{tent} & \text{if } x_t^{tent} < x_r \\ 2x_r - x_t^{tent} & \text{if } x_t^{tent} \geq x_r \end{cases} \quad (2.23b)$$

For two reflecting walls at $x = 0$ and $x = x_r$:

Suppose we have a reflecting wall at $x = 0$ and $x = x_r$ and accessible space is $0 < x < x_r$. For this case, the algorithm for generating x_t is follows.

1. Generate tentative position in free space.

$$x_t^{tent1} = x_0 + \sqrt{2D(t - t_0)}n \quad (2.24a)$$

2. Actual position

- (a) Due to the wall at $x = 0$, position is positive.

$$x_t^{tent2} = |x_t^{tent1}| \quad (2.24b)$$

- (b) Find the modulo of x_t^{tent2} with x_r

$$x_t^{mod} = x_t^{tent2} \bmod x_r \quad (2.24c)$$

- (c) Find number of reflections

$$n_r = \frac{x_t^{tent2} - x_t^{mod}}{x_r} \quad (2.24d)$$

(d) Actual position:

$$x_t = \begin{cases} x_t^{mod} & \text{if } n_r \text{ is even} \\ x_r - x_t^{mod} & \text{if } n_r \text{ is odd} \end{cases} \quad (2.24e)$$

2.2 Electrostatic interactions in confined solutions

In this thesis, we will be focusing on fluorescent dye molecules in confined aqueous solutions. The miscible dye molecules in water typically have partial charges or dissociate as ions in water. Moreover, the interfaces that confine the liquid also have charges. Here our interfaces are made out of glass or silica. These surfaces acquire negative charges in our experimental pH of 7 [70]. Thus, the dye molecules and confining interfaces have electrostatic interactions between them. This interaction cannot be ignored, particularly in nano-scale confinements, where the surface-to-volume ratio is significant. Our measurements will either be in pure water or NaCl solutions. The electrostatic interaction of charged tracer-dye in the confined volume will depend on the local electrostatic potential it experiences.

The local electrostatic potential in the confined electrolyte solution depends on the charges on the confining surfaces and the distribution of ions in the solution. The ions which have an opposite charge to the surface charge(counter ions) accumulate over the surfaces. This makes the solid-liquid interface electro-neutral. These charges are bound to the surfaces and form a ‘‘Stern layer.’’ Beyond the Stern layer, we have ions free to diffuse and form a diffuse layer away from the surface. This distribution of ions near a charged surface is called the ‘‘Electrical Double Layer’’(EDL).

We can use the formulae for electrochemical potential to derive an expression for potential near charged surface in electrolyte solution. The electrochemical potential of ion i ($\tilde{\mu}_i$) at constant temperature and pressure is given by:

$$\tilde{\mu}_i = \mu_i + z_i F \psi \quad (2.25)$$

Here, μ_i is the chemical potential, z_i is the valency of the ion i , F is the Faraday constant, the charge of Avogadro number of electronic charge(e), and ψ is the electric potential. At equilibrium, the electrochemical potential is constant throughout the

solution. That is $\nabla \tilde{\mu}_i = 0$. This gives:

$$\nabla \tilde{\mu}_i = -z_i F \nabla \psi \quad (2.26)$$

The chemical potential($\tilde{\mu}_i$) can be written in terms of standard chemical potential($\tilde{\mu}_i^0$) as:

$$\tilde{\mu}_i = \tilde{\mu}_i^0 + RT \ln(\gamma_a c_i / c^0) \quad (2.27)$$

where R is the gas constant, T is the temperature, γ_a is the activity coefficient, c_i is the molar concentration of ion i and c^0 is the standard molarity of 1 mol/l. The c_i can be converted to number density, n_i , as, $n_i = 1000 N_A c_i$. Now we can substitute $\tilde{\mu}_i$ in equation 2.26. This new equation is integrated from values in bulk of the liquid to the values over the surface. The ψ vary from 0 to ψ . And n_i from n_i^∞ to n_i . Then we get a Boltzmann equation for n_i .

$$n_i = n_i^\infty \exp(-z_i e \psi / K_B T) \quad (2.28)$$

Here we also used $e / K_B = F / R$. With this, we can calculate the charge density of all ions near to the surface;

$$\rho = e \sum_{i=1} n_i z_i \quad (2.29)$$

We can use this ρ in the Poisson equation to get the general relation for ϕ :

$$\nabla^2 \psi = \frac{d^2 \psi}{dz^2} = \frac{-\rho}{\epsilon_0 \epsilon_r} \quad (2.30)$$

$$\nabla^2 \psi = \frac{-e}{\epsilon_0 \epsilon_r} \sum_{i=1} n_i^\infty z_i \exp(-z_i e \psi(z) / K_B T) \quad (2.31)$$

This is the complete Poisson-Boltzmann equation for the potential near to the charged surface in an electrolyte.

2.2.1 Debye-Huckel approximation

If we consider the surface potential is small ($z_i\psi_i < 25$ mV at 25° C) and expand the exponential assuming $e^{-\alpha} = 1 - \alpha$, we get Debye-Huckel approximation [71]:

$$\frac{d^2\psi}{dz^2} = \kappa^2\psi(z) \quad (2.32)$$

where, κ is called the Debye-Huckel parameter. And it is given by:

$$\kappa = \frac{e^2 \sum_{i=1}^{\infty} n_i^{\infty} z_i^2}{\epsilon_0 \epsilon_r K_B T} \quad (2.33)$$

The solution for equation 2.32 is

$$\psi(z) = \psi_s \exp(-\kappa z) \quad (2.34)$$

where, ψ_s is the surface potential. This shows that the potential drops exponentially away from the surface. At a distance of $1/\kappa$ the potential fall to ψ_s/e , this defines the thickness of the EDL. The length scale $1/\kappa$ is called Debye length (λ_D). For a symmetrical $z_i : z_i$ electrolyte, λ_D is given by,

$$\lambda_D = \frac{3.04 \times 10^{-10}}{z_i \sqrt{c_i}} \quad (2.35)$$

So for 1:1 electrolyte like NaCl or KCl we have $\lambda_D=0.3$ nm for 1 M and $\lambda_D= 1$ nm for 100 mM.

2.3 Fluorescence Correlation Spectroscopy

Fluorescence Correlation Spectroscopy (FCS) is a technique that uses fluctuations in the observed fluorescence intensity to deduce different system properties. Measurements in our new instrument and FCS are similar. Also, we use a few expressions derived for FCS in our analysis. FCS is used for the measurement of diffusion coefficient, reaction kinetics and microrheology studies [72–76]. The technique is first proposed and realized by Elson and Mangde [72–74]. In this technique, an objective lens is used to focus a beam of excitation light in the bulk of a solution. The solution will have a fluorescent molecule in nM concentration. The fluorescence from these molecules is filtered and collected with a confocal setup. Single-photon detectors like Avalanche

Photodetectors(APD) or Photo Multiplier Tubes(PMT) are used to record the fluorescence intensity. The concentration of the fluorescent molecules is such that only around ten molecules will be present in the observational confocal volume in a given time. The diffusion cause this number to fluctuate. As the number is small, its fluctuations cause detectable fluctuations in the fluorescence intensity. Thus diffusion characteristics get encoded in these fluorescence fluctuations. A time trace of these intensity fluctuations is recorded, and its autocorrelation is calculated. We can estimate an average residential time for the fluorescent molecule from the autocorrelation. It is the average time the molecule takes to cross the observational volume. The residential time is extracted from the autocorrelation by fitting an analytical expression derived for FCS. The FCS expression considers the excitation profile, diffusion properties, and other experimental details.

If the diffusion is Fickian and the confocal observational volume approximated as a 3-D Gaussian, then the autocorrelation of the fluorescent intensity fluctuations is derived as

$$G(\tau) = \frac{1}{\langle N \rangle} \left(1 + \frac{\tau}{\tau_D}\right)^{-1} \left(1 + r^2 \frac{\tau}{\tau_D}\right)^{-1/2} \quad (2.36)$$

$$= \frac{1}{\langle N \rangle} \left(1 + \frac{4\tau D}{\omega_{xy}^2}\right)^{-1} \left(1 + \frac{4\tau D}{\omega_z^2}\right)^{-1/2} \quad (2.37)$$

where τ_D , the diffusion time, is the average residence time of the tracer molecule in the observation volume. $\tau_D = \frac{\omega_{xy}^2}{4D}$, where D is the diffusion coefficient and ω_{xy} is the radius in XY-plane, and ω_z is the height of the observation volume. ω_{xy} and ω_z are usually determined by recording autocorrelations for tracer dye whose D is known and fitting it to equation 2.37.

If the diffusion takes place only in a 2D plane then the equation 2.37 becomes.

$$G(\tau) = \frac{1}{\langle N \rangle} \left(1 + \frac{\tau}{\tau_D}\right)^{-1} \quad (2.38)$$

If we consider triplet transitions, one extra multiplicative factor is present in this expression.

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{\left(1 + \frac{\tau}{\tau_D}\right)} \left(1 + \frac{T}{1-T} e^{-\tau/t_t}\right) \quad (2.39)$$

Here, “T” is the fraction of fluorophores in triplet state and t_t is the triplet transition timescale. If there are two fluorescent species in the observation volume with different diffusion coefficients, the autocorrelation modifies as:

$$G(\tau) = \frac{1}{\langle N \rangle} \left[\frac{\phi_1}{\left(1 + \frac{\tau}{\tau_{D1}}\right)} + \frac{\phi_2}{\left(1 + \frac{\tau}{\tau_{D2}}\right)} \right] \left(1 + \frac{T}{1-T} e^{-\tau/t_t}\right) \quad (2.40)$$

Here, τ_{D1} and τ_{D2} are the residential time for species 1 and 2 respectively. And ϕ_1 and ϕ_2 are the corresponding weight factors for each species. The weight factor for a particular fluorescent species depends on its fluorescence yield and fraction of that species in the average number of total fluorescent species present in the observation volume ($\langle N \rangle$). If q_1 and q_2 are the fluorescence yield of molecules 1 and 2, “ f ” is the fraction of molecule 1. Then the weight factors; ϕ_1 and ϕ_2 are given by:

$$\begin{aligned} \phi_1 &= \frac{f q_1^2}{[f q_1 + (1-f) q_2]^2} \\ \phi_2 &= \frac{(1-f) q_2^2}{[f q_1 + (1-f) q_2]^2} \end{aligned} \quad (2.41)$$

In situations where the fluorescent molecule adsorbs or desorbs from the surface along with diffusion. The FCS expression for autocorrelation is given by [77]:

$$G(\tau) = \frac{1}{\langle N \rangle} \left[\frac{\phi_1}{\left(1 + \frac{\tau}{\tau_D}\right)} + \phi_2 e^{-k\tau} \right] \left(1 + \frac{T}{1-T} e^{-\tau/t_t}\right) \quad (2.42)$$

The weight factors ϕ_1 and ϕ_2 are defined exactly like in equation 2.41. Instead of subscripts 1 and 2 being different molecules, 1 indicates the molecules undergoing

diffusion, and 2 indicates the fraction of the same molecules undergoing adsorption-desorption events. And “ k ” is the rate constant of desorption.

2.4 Conclusion

This chapter outlines the theoretical description of diffusion. Moreover, the electrostatic interactions expected in the confined solutions are briefly discussed. The measurements from our new instrument are similar to those from a technique called Fluorescence Correlation Spectroscopy(FCS). So, a small introduction to FCS is given.

Chapter 3

Instrumentation

A part of this chapter has been published as:

“A new method for measurement and quantification of tracer diffusion in nanoconfined liquids”

V J Ajith and Shivprasad Patil

Review of Scientific Instruments, 91, 013702 (2020)

This chapter reports the development of a new instrument to measure tracer diffusion in water under nanoscale confinement. The chapter starts by explaining the idea of the instrument, its implementation, and its challenges. Then efforts for automating the instrument and measurements using a computer are briefly outlined.

3.1 Introduction

The physical properties of liquids under nanoscale confinement are debated in the literature. The behavior of nanoconfined liquids is relevant in areas ranging from tribology to biology [78–81]. The confinement by solid walls is reported to cause a significant change in liquid’s physical properties such as diffusion coefficient, viscosity, and dielectric constant [82–84]. It undergoes a phase transition leading to solidification at room temperature [83, 85]. However, both the phenomenon and its cause have been debated for decades [86–88]. In particular, the flow properties under confinement using different experimental approaches have led to contradictory results [89–93].

The Surface Force Apparatus(SFA) measurements have reported that water shows no change in viscosity for film thickness as small as 0.4 nm and below [92, 93]. However, many other measurements have reported orders of magnitude increase in viscosity under confinement [83, 84]. Nanoconfined thin films of non-associative fluids such as Octamethylcyclotetrasiloxane(OMCTS) exhibit significant non-linear effects in their viscosity measurements [83]. The small-amplitude AFM measurements of both water and OMCTS layers under confinement have revealed a speed dependant dynamic solidification [94, 95]. The lipidic cubic phases provide a versatile porous media to investigate the transport of molecules through the confined water [96–98]. More recently, it is argued that contradiction among different experimental reports stems from working with other experimental parameters such as shear frequency, shear rates, and wettability of confining walls [47, 88].

It is also essential to understand molecular mobility under confinement to gain insight into the mechanisms driving the change in flow properties under confinement. Many SFA or AFM-like techniques measure the viscous stress in confined liquids upon straining the liquid film. However, there are only a few tools for measuring translational or rotational diffusion under confinement.

There are scant reports of direct measurement of tracer diffusion under nano-confinement in literature. The nanochannels are used to confine liquid and optical methods have been employed to measure diffusion in ~ 10 nm confinement [99, 100]. Simulations are performed to understand the transport of solute molecules through a periodic array of nanopores formed by inverse bicontinuous lipidic cubic phases. [98] Experimentally, the diffusion of solute molecules through them is measured and was found to depend on solute molecular structure and confinement geometry [96, 97]. Molecular dynamics simulations are performed to understand the diffusion of water between two graphene sheets separated by 1 nm and in silica nanopores [101, 102]. In diffusion measurement using SFA the gap formed between two surfaces mounted on piezoelectric transducers is used for confinement [82]. In AFM-like confining geometry, the lateral confinement size is much smaller than the diffraction limit, and hence it isn't easy to access this volume optically for diffusion measurement. On the other hand, active feedback control can be used to hold the tip and substrate at sub 10 nm separations -a challenging task owing to the soft springs used to measure normal surface forces in the case of SFA. The

trade-off between sensitivity and stability makes employing servo control to hold the separation between surfaces at the desired value. We need an instrument that can maintain the separation between surfaces below 5 nm with active control and optical access to the probe-substrate gap.

3.2 The Idea

We want an instrument to measure the diffusion of tracer molecules in confined liquids. The confinement scheme we used for the instrument is illustrated in the figure 3.1. The confinement of liquid can be achieved using AFM like facility. Here an AFM tip is kept a few nm away from a substrate with feedback. The nanoconfined water will be between the tip and substrate. We can illuminate a local volume in the confined water with the same tip. Using fluorescent molecules as tracers will emit fluorescence photons as they diffuse through the illumination in confined water. We can collect these emitted photons using a confocal arrangement.

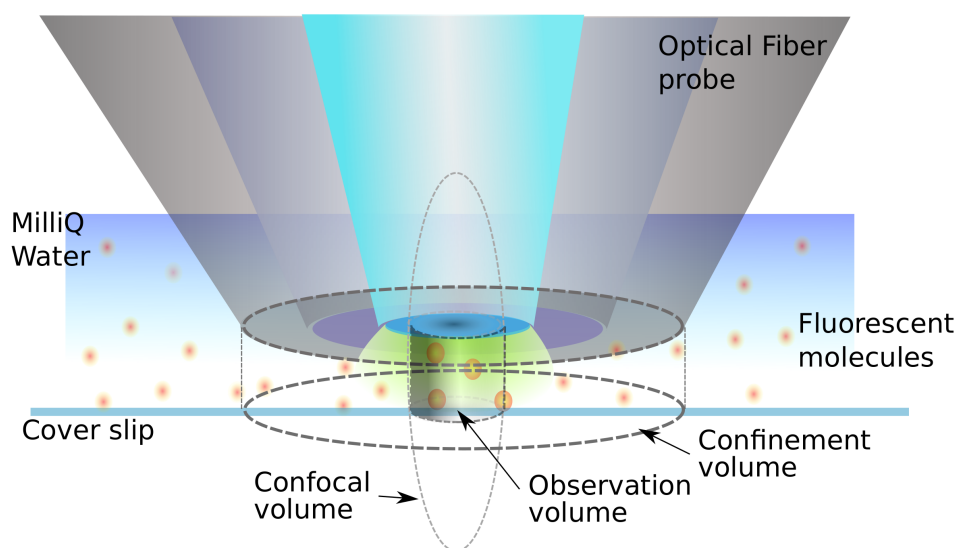


Figure 3.1: The schematic of the confinement gap formed by the tip-substrate junction and tracer molecules diffusing through it. The observation volume is the convolution of the confocal volume and the illumination profile. The height of this volume is determined by the tip-substrate gap (≤ 5 nm) and the lateral size by width of confocal volume.

The convolution of confinement, illumination, and confocal volume forms our observation volume. As the number of fluorescent molecules fluctuates due to its diffusion,

proportional fluctuations in the detected fluorescence intensity will be seen. We have to analyze this fluctuation to extract out-diffusion properties. This is the idea of the instrument and essential measurement we intend.

This idea is similar to Near-field Scanning Optical Microscope(NSOM) based Fluorescence Correlation Spectroscopy(FCS) [103–107]. Unlike NSOM based FCS, we are using tips with aperture larger than $\lambda/2$. And all these previous studies are done either to understand diffusion in the lipid bilayer or to demonstrate the tip illumination can extend the higher limit of dye concentration to a few 100 mM for an FCS measurement. None of these instruments are designed or used for studying dye-diffusion in nanoconfined water.

3.3 Implementation

To implement this idea, we need a special tip that can confine water and illuminate a local volume in that confined water. Also, we need provisions to actuate the tip, measure and control the separation between the tip and substrate and collect the fluorescent light from the confined water.

3.3.1 Special Tip

We need a special tip with an optical aperture at the end. For this, an optical fiber end is made into a sharp tip. Then the tip is coated with aluminum. And an optical aperture is opened by slicing the tip with Focus Ion Beam.

Fabrication of the tip

Step 1

The first step in fabricating the tip is to make sharp tips out of optical fiber. The optical fiber we use is Thorlabs 460HP. This optical fiber has 125 μm diameter without buffer and a 2.5 μm diameter core. We make tip out of this fiber using a laser-based pulling method. For this, we use the instrument Sutter 2000. In this method, a 20 W CO₂ laser is focused on a 1 mm spot over the fiber. And the fiber is kept under tension. As the laser heat up the fiber, fiber elongates. At a particular setpoint velocity of elongation, a hard pull is initiated, and the fiber breaks to form two sharp tips. Sutter 2000 is optimized to give tip of diameter ~ 40 nm for the following parameters:

A pull time of 0.13 or 0.14 s(shown on the instrument display after every pull) gives

Heat	Filament	Velocity	Delay	Pull
260	0	18	126	150

Table 3.1: Optimized parameters for Sutter 2000 fibre puller. The parameters values have units or meaning as defined by the user manual of Sutter 2000 instrument.

a consistently sharper tip with a short taper length and has a conical structure. If the parameter values didn't provide a pull time of 0.13 or 0.14 s, either the fiber is fixed in the instrument incorrectly, or there is dust accumulated on the focusing mirror of the device. The focusing mirror can be cleaned with Thorlabs lens cleaning tissue and Sticklers precision cleaning fluid(MCC-POC03M)

We use a 30 cm long fiber and focus the laser at 10 cm away from one end to get two tips as we pull and break this fiber. One piece is 10 cm long, and the other will be 20 cm. The longer one is used in further steps of the fabrication, while the shorter one is stored for SEM imaging to check the diameter of the tip.

Step 2

Next, the tip is coated with 150 nm of Aluminium(Al). For this, we used an RF-sputter coating setup. For coating all the surfaces, the tip is rotated along its axis while it is kept perpendicular to the Al-beam. The tip is rotated with a speed of ~ 5 rpm with a motor. The optimized parameters used for coating is given below:

RF Power	80 W
Pressure	$4.1 \text{ to } 4.4 \times 10^{-3} \text{ mbar}$
Time of coating	60 min

Table 3.2: Optimized parameters for RF-coating setup

Step 3

In order to get an optical aperture, we slice the fully coated tip using Focused Ion Beam(FIB). The FIB instrument we used is FEI Helios Nanolab 600i. The 20 cm long-coated fibers are loaded in this instrument after winding them over a stainless steel plate(5 x 5 x 0.2 cm) as shown in the figure 3.2. Using the SEM provision in the instrument, the tips are imaged. A rectangular area(600 x 600 nm) is selected over a tip such that the tip axis is perpendicular to one side of the rectangle. We used Gallium ions with

30 kV and 40pA to mill over this area for a depth of 1 μm . SEM images of the tip after this FIB milling are shown in figure 3.3.

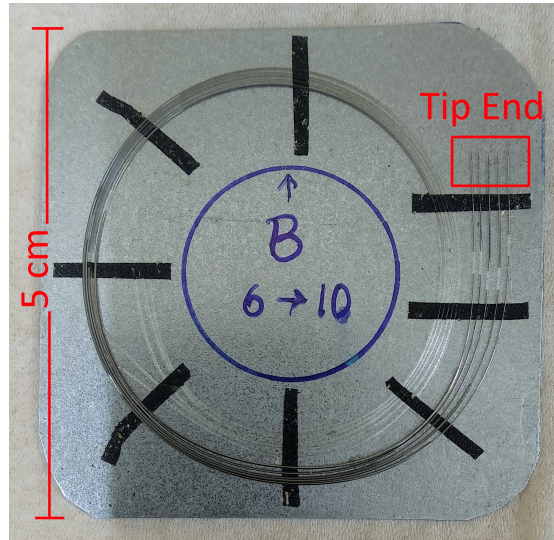


Figure 3.2: The 20 cm long Al-coated optical fibers are fixed on a stainless steel plate(5 x 5 x 0.2 cm) with strips of carbon tape. These steel plates are further mounted(with carbon tape) on FIB sample holder to load inside the FIB instrument. At at time 5 or 6 tips are mounted on such plates.

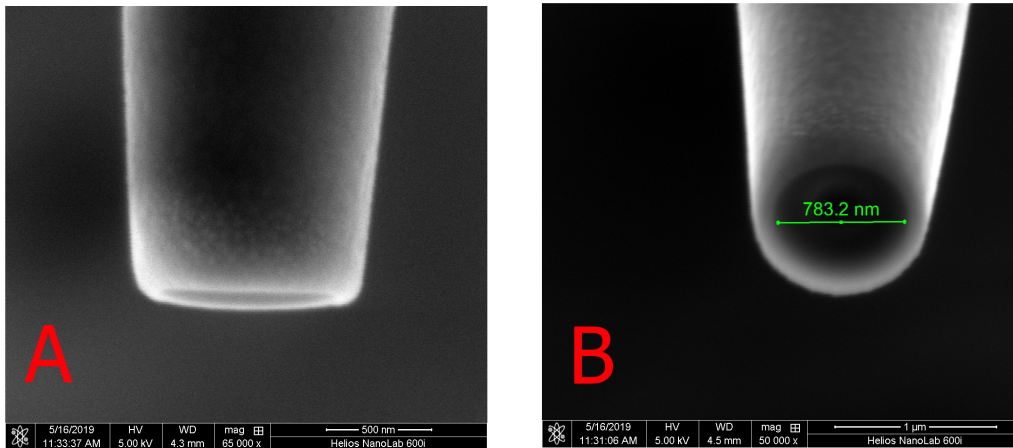


Figure 3.3: SEM images of a tip after slicing with FIB. A: Image when tip axis is approximately along focal plane, B: Image when tip is tilted 33° from focal plane

Specification of the tip

Ensuring 90° between tip axis and coverslip surface as shown in the schematic shown in figure 3.1 can be a challenge. There can always be a small tilt of $< 0.3^\circ$. With such a small tilt, there will be variation in the separation between tip and substrate at the different local points beneath the tip. This variation in confinement is reduced with sharper tips. At the same time, having a sharper tip means a small aperture. The efficiency of the tip to transmit light reduces drastically with a smaller aperture. Typical efficiency of tip with diameter of 60 nm is 10^{-7} [108]. With a $2\ \mu\text{m}$ blunt tip, we found that at least $3.2 \times 10^2\ \text{W/cm}^2$ of power density is required to generate correlated photons from observation volume for Alexa Fluor 568 fluorescent molecule at 532 nm excitation. For this power density at the 60 nm tip, we need $0.36\ \mu\text{W}$ at the tip. This means we have to couple 3.6 W light in the fiber. Coupling even much smaller 3.6 mW results in considerable heating of the tip and approximately 200°C of tip-temperature is reported [109]. For this sharp tip, a temperature of ~ 50 to 80°C is measured at the tip-end when a 0.5 to 1 mW of light is coupled [109]. Moreover, only the nearfield component of the light will be accessible from the tip, if the tip aperture diameter is less than $\lambda/2$. Modeling this nearfield illumination is not straightforward in the analysis later on. So we use tip with a diameter of 400-900 nm. Here we get 5 - $50\ \mu\text{W}$ at the tip when $\sim 1\ \text{mW}$ coupled. This means efficiency of these tips are 5×10^{-3} to 5×10^{-2} . In terms of power-density, this translates as 10×10^2 to $20 \times 10^2\ \text{W/cm}^2$. This power density is enough for our measurements. A tilt of 0.3° will give $\sim 2\ \text{nm}$ of systematic error in the gap measurements. We will discuss more about the systematic error later. Considering 80°C temperature for a tip with 10^{-7} efficiency, our fabricated tip with 10^{-3} efficiency and broad aperture will have a temperature comparable with room temperature.

3.3.2 Actuation of the tip

The tip actuation is done with a scanner and a hammer piezos. How the piezos are assembled, and the tip is fixed to them is shown in figure 3.4.

Fine-motion with Scanner Piezo

The fiber-tip is attached to the scanner piezo as shown in the inset of figure 3.4. The fiber is attached first to a quartz tuning fork which is fixed to the “drive piezo”. And drive piezo is mounted on “probe holder”. The need for quartz tuning fork and drive

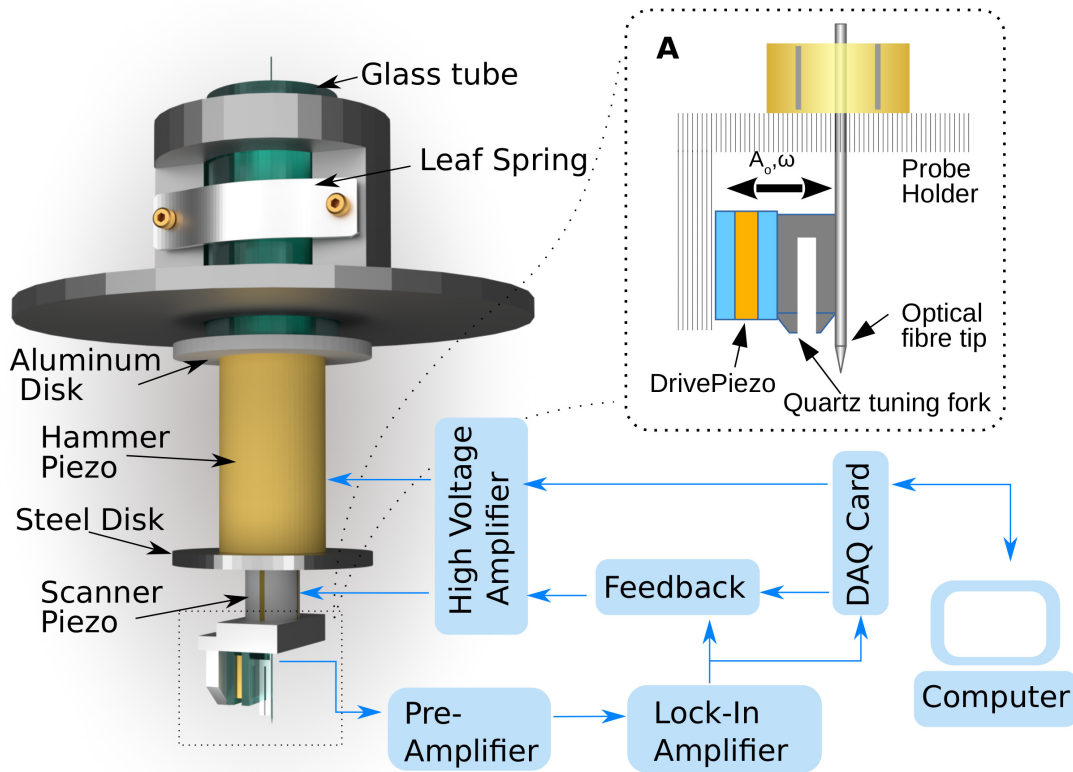


Figure 3.4: Piezo assembly for actuation.

piezo will be discussed in the next section. This “probe holder” is glued to one end of the scanner piezo. This ensures as the scanner piezo expand or contract, the same movement is obtained for the tip. This is how fine-movement of the tip is achieved.

The scanner piezo is a tube piezo with four outer and one inner electrodes (EBL #2, gold over copper electrodes). The dimension of the tube is 0.256” x 0.020” x 1.181”. Electrode arrangement is as shown in figure 3.5.

Fine motion along x, y, and z-directions are possible with this piezo. For z-motion, a voltage (V_z) is provided to all four outer electrodes while inner one is grounded. The pair of electrodes along the x-direction receives equal and opposite voltage (V_x) to move along the x-direction. Similarly, the pair of electrodes along y-direction receive equal and opposite voltage; V_y . Thus for positioning the tip at (x, y, z), the piezo receive ($V_z + V_x$, $V_z + V_y$, $V_z - V_x$, $V_z - V_y$) voltages on its electrodes starting with the electrode along positive x-axis and other electrodes in anti-clockwise direction.

Each of the voltages ($V_z + V_x$, $V_z + V_y$, $V_z - V_x$, $V_z - V_y$) given to scanner piezo is

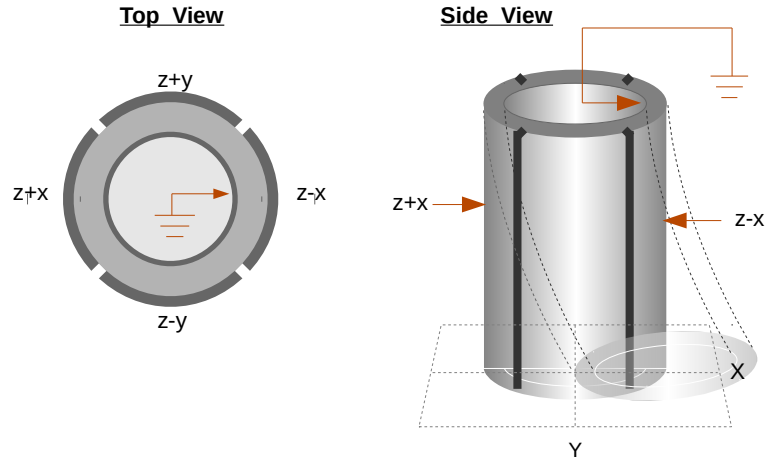


Figure 3.5: Scanner piezo electrode arrangement.

between -150 V to 150 V. First; a voltage is -10 to 10 V range is made with a DAQ card or other circuits then it is amplified with PA88 opamps. The amplification is done with a gain of 7 or 15. Independent PA88 opamps are used to amplify individual voltages. The circuit used for each PA88 opamps is shown in figure 3.6.

We use a transformer-based power supply for driving the PA88 opamps. The circuit used for this power supply is given in figure 3.7. The output of PA88 with this power supply and without piezo load gave a peak to peak noise of ~ 40 mV in $10 \mu\text{s}$. While with load, an additional ± 2 V periodic noise at 0.6 MHz is seen. This can be ignored as the scanner piezo is not sensitive to such a large frequency.

The unamplified voltage for z-motion, v_z , is generated either with a feedback circuit or from the analog output from a DAQ card. We will discuss more about the feedback circuit in the next section. The choice of v_z source is decided using a digitally controlled reed-relay. The instability resulting from the switching of this relay has to be avoided. For this purpose, a capacitor charged with the current v_z value is momentarily connected to the piezo with another relay before the switching of v_z source happens. The unamplified voltage for x and y-direction motion (v_x, v_y) is generated from analog outputs of a DAQ card.

From the three voltages; v_x , v_y , and v_z we calculate the actual voltage we require for amplification; $v_z + v_x$, $v_z + v_y$, $v_z - v_x$, and $v_z - v_y$. We utilize opamps for these

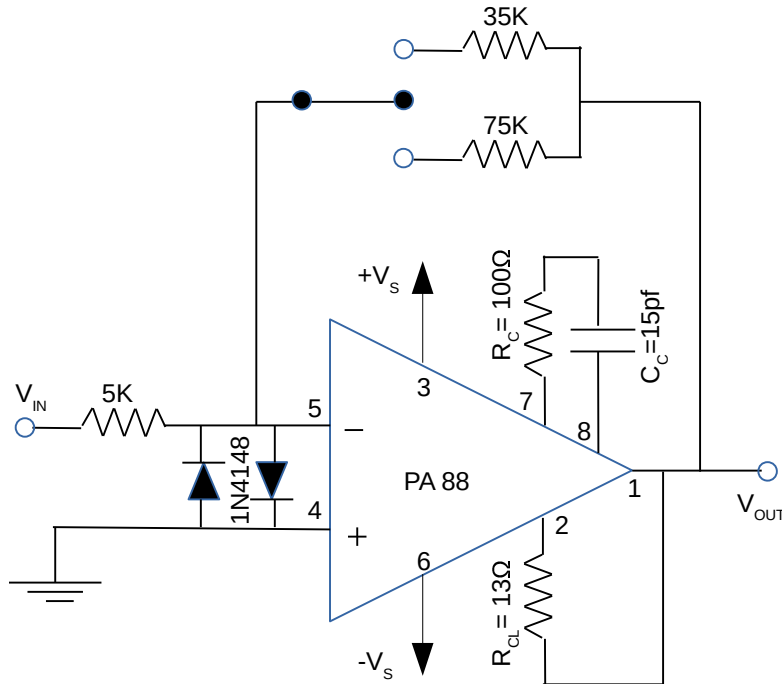


Figure 3.6: Circuit diagram for PA88 opamp high voltage amplifier for driving piezos.

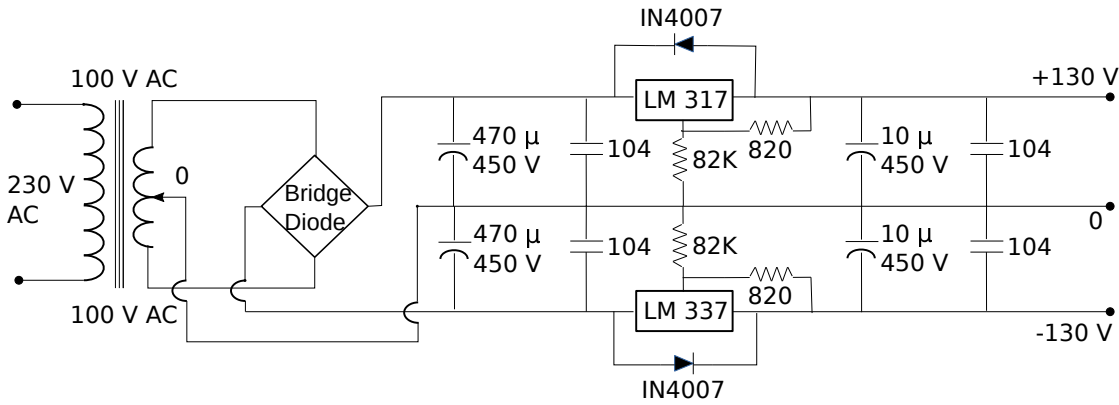


Figure 3.7: Circuit diagram for high voltage power supply.

arithmetic operations in real time. The circuit we used for addition and subtraction is shown in the figure 3.8. We use CA3140 opamps for this purpose.

This z-motion with the scanner piezo is calibrated with a fiber-based interferometer, and sensitivity is 16.44 nm/V. We get better than 1 nm resolution in this motion. With the scanner piezo, we get a range of $\sim 2 \mu\text{m}$ along the z-direction, while along x and y-direction, we get $\sim 20 \mu\text{m}$.

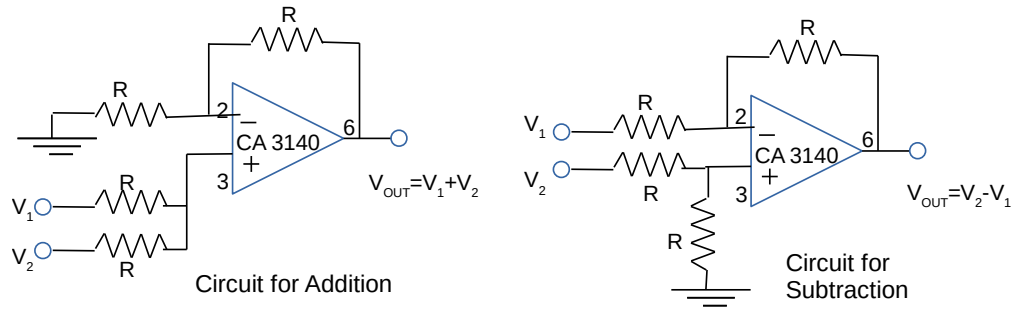


Figure 3.8: Circuit diagram for performing addition and subtraction of voltages with opamps.

Coarse-motion with Hammer Piezo

The tip is fixed to one end of the scanner piezo via probe holder, while the other end is fixed to an aluminum disk. And this aluminum disk is further connected to a glass tube which goes through a slot. The glass tube is held in the slot against gravity by pressing it with a leaf spring. The friction which holds the tube in place can be controlled by tightening/loosening the leaf-spring screws. The glass tube can be moved up or down if enough force is provided to overcome the friction. If the glass tube moves, then the tip also moves. We use an inertial sliding mechanism to move the glass tube. The inertial sliding is achieved by using the “Hammer piezo”. This will be used for coarse movement of the tip.

The top end of the hammer piezo tube is attached to the aluminum disk, while a slotted stainless steel disk is attached to the bottom end. The scanner piezo and hammer piezos are fixed on an aluminum disk such that they are concentric and there is enough gap between all electrodes. The stainless steel disk at the bottom end of the hammer piezo has a circular opening at its center, allowing the thinner and longer scanner piezo tube to project out. The probe holder is attached to the scanner piezo without hindrance from the hammer piezo or the steel disk.

The inertial sliding motion for the coarse movement of the tip is made in the following way. The hammer piezo is given a series of saw-tooth-like pulses. Figure 3.9 shows the motion of the hammer piezo for one of the pulse. As the voltage provided to hammer piezo exponentially increases from t_0 to t_2 , the piezo expands from length; L to L' . The bottom end of the piezo is free during this expansion. So, this end moves down with the steel disk. The steel disk is brought a step lower than its initial position. At t_2 , the voltage is suddenly dropped to 0. This makes the piezo contract back to its

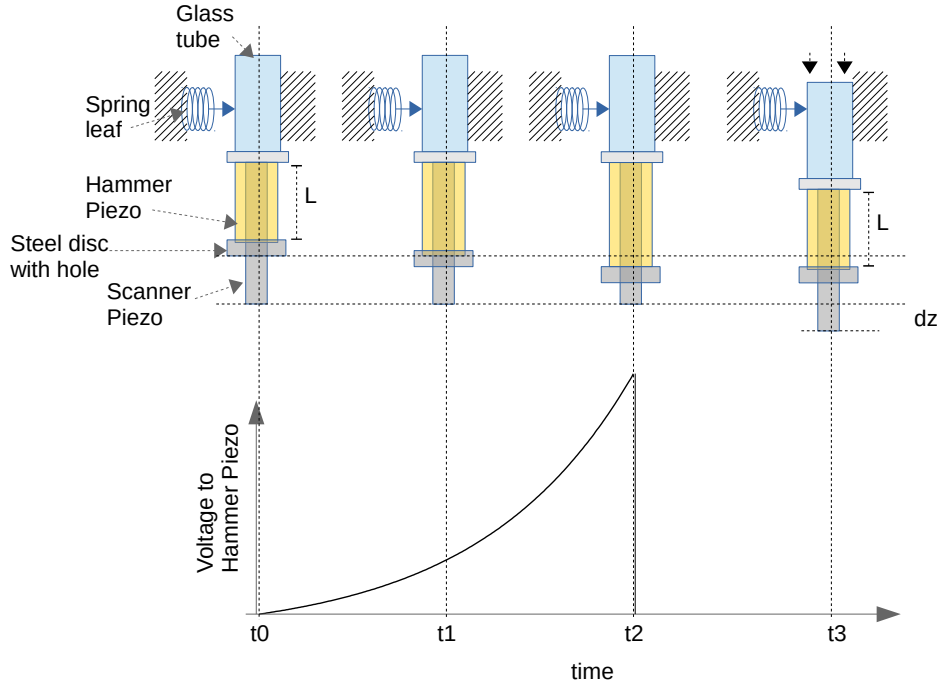


Figure 3.9: The inertial sliding mechanism used for the coarse movement of tip along z-axis

original length; L . The steel disk being slightly heavy than the aluminum disk with the glass tube makes the bottom end of the hammer piezo with more inertia. So during the contraction, the aluminum disk is brought down while the steel disk stays stationary due to its larger inertia. As the aluminum disk is brought down with this pulse, the attached tip via scanner piezo is also brought down a step. The motion can be reversed by giving a negative pulse. Depending on the number of these pulses, pulse height, frequency, and friction on the sliding the movement can be controlled. Typical pulse width is $1\mu s$ and height is 30 to 130 V. With a typical pulse, 50 nm to 100 μm steps are possible. A range of ~ 3 millimeters can be achieved with multiple pulses.

Coarse-motion with micro-positioning screws

The piezo assembly shown in figure 3.4 is further mounted on an optomechanical cage plate with a provision for x,y-motion with the help of spring-loaded micro-positioning screws. Moreover, this cage-plate is mounted on a cage assembly as shown in figure 3.10. The cage plate sits over four springs placed along the cage rods pressed down by

another micro-positioning screw. With this, coarse motion along the z-axis is achieved. We can manually move the tip with micro-meter precision along x,y,z-directions with these screws. Along x, y-direction, we have a range of 5 mm, and that along z-direction is 1 cm.

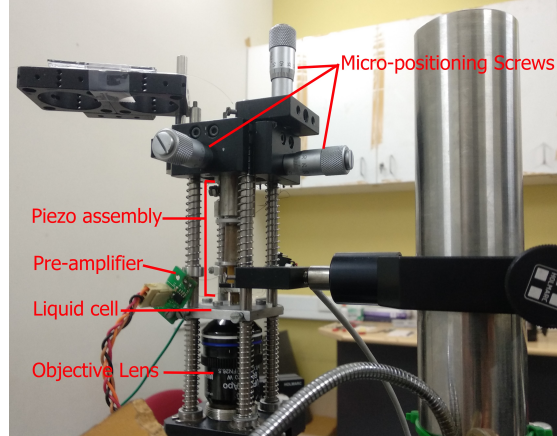


Figure 3.10: The piezo-assembly with micro-positioning screws for manual coarse movement of tip is visible in this part of cage assembly

3.3.3 Measurement and control of tip-sample separation

The fine and coarse movement of the tip allows us to change the separation between the tip and coverslip surface. The coverslip is mounted on a liquid cell, with water or a solution taken over it. As the tip-coverslip separation is controlled, we can adjust the thickness of the liquid between the tip and coverslip. We utilize a quartz tuning fork (QTF) for measuring and controlling the separation.

We already discussed how the fiber-tip is mounted over the QTF. As shown in figure 3.4A, the fiber is pasted over the one prong of the QTF, and other prong is attached to a drive-piezo via a piece of a glass slide (denoted by blue color in 3.4A). The glass slide isolates the electrodes of QTF and drive-piezo. The QTF is made out of piezoelectric quartz, and it is the same QTF used as the time-keeping element in quartz watches. These QTF has a resonance frequency of 2^{15} Hz (~ 32 kHz). And its dimensions are 6 mm x 1.5 mm x 0.3 mm. This QTF has mechanically oscillated with the drive piezo. The drive piezo is a compression mode (thickness polarized) piezo crystal (EBL #2, gold over copper electrodes, 0.197" x 0.197" x 0.029").

The QTF with fiber is driven at the resonance frequency of the combined system (33

to 34 kHz). A sinusoidal voltage is given to the drive piezo at the resonance frequency. Typically RMS drive voltage given to drive piezo is 50 to 100mV. This oscillates the tip along with the QTF laterally with respect to the coverslip. The oscillation of QTF prongs at its resonance causes a 90° out-of-phase bending between the prongs of QTF. This bending generates a piezoelectric current, and it can be accessed from the electrodes of the QTF. This current is in the nA range. This signal is preamplified with an opamp-based preamplifier and converted to voltage. The preamplifier we used is based on OPA656, and the circuit used is shown in figure 3.11. This preamplifier has a gain of 10^7 due to the $10\text{M}\Omega$ resistors used.

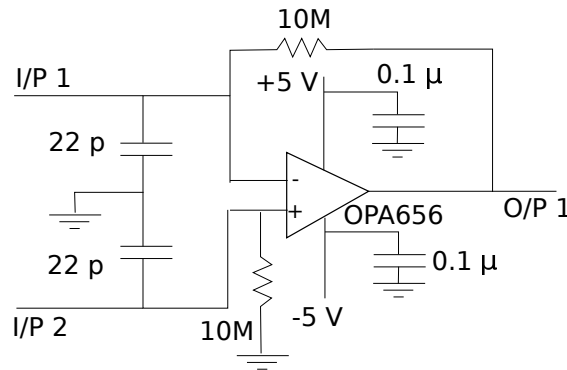


Figure 3.11: The preamplifier circuit used for preamplifying the piezo currents from quartz tuning fork

The preamplified voltage from the QTF is measured using a lock-in amplifier (Signal Recovery 7265). Using the lock-in amplifier, we can find the amplitude of the oscillating voltage from QTF and the phase difference of this voltage to the voltage given to the drive-piezo. As the tip is brought closer to the coverslip, the van der Waals forces become significant and manifest as a reduction in the QTF voltage amplitude. This reduction of voltage amplitude stops when the tip makes contact with the coverslip. This trend for a typical tip used in our experiments is shown in figure 3.12. This way, we can assign zero-separation to one scanner piezo voltage. If the drifts are negligible, we can provide a voltage to the scanner piezo and deduce tip separation from the coverslip.

Moreover, we could use the measured monotonic trend of QTF voltage amplitude as a feedback signal. The monotonic trend shown in figure 3.12 is unique for a given tip and the local x,y point of coverslip beneath the tip. Once such a curve is measured, it is enough to know the QTF voltage amplitude to see the separation. Thus, we can design

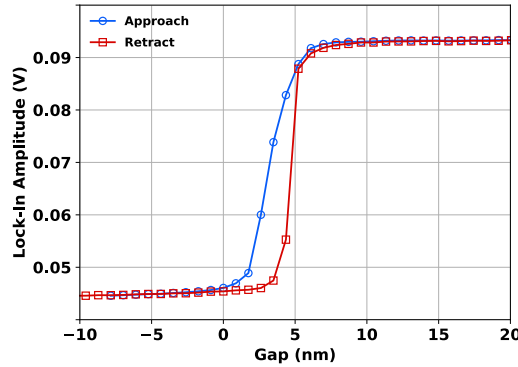


Figure 3.12: The amplitude of voltage generated in QTF as a function of tip-coverslip separation measured using Lock-in amplifier. In the raw measurement, x-axis is scanner-piezo voltage. It is converted to tip-coverslip separation by identifying the zero-separation voltage and converting the voltage to piezo extension in nm with the calibrated z-sensitivity value.

a feedback circuit with QTF voltage amplitude as the feedback signal to maintain a constant tip-coverslip separation. The feedback we used is made out of opamps. The circuit diagram is shown in figure 3.13. It is PI-controlled feedback, with p-gain fixed at 1/2 and a variable i-gain. The resistance(R) and capacitance(C) values for the integrator part of the circuit are chosen for the overdamped operation. The input signal(V_{IN}) for the PI-controller is from lock-in (QTF voltage amplitude) and we keep the lock-in time constant as 5ms. This choice of time constant provides a fast enough lock-in response for the signal changes while noise levels are kept low. The PI-output voltage is given as the z-voltage of the scanner piezo(v_z).

3.3.4 Optical Setup

The optical setup is illustrated in the diagram shown in the figure 3.15.

The components are mounted on an optical breadboard which is kept over an active vibration isolation(Accurion Nano_30). The components are arranged over three parallel horizontal planes; level 1,0,-1, and along a vertical line through a cage assembly. This arrangement is shown in the figure 3.15. Level -1 is the bottom plane, and its height matches the position of the beam-splitter in the vertical assembly. Level 1 is the top plane, and its height matches the dichroic filter in the vertical assembly. Level 0 has the height of the laser source, and it is in between levels 1 and -1.

We use a laser with $<40\text{mW}$ as the source of light. We use either Coherent Cube

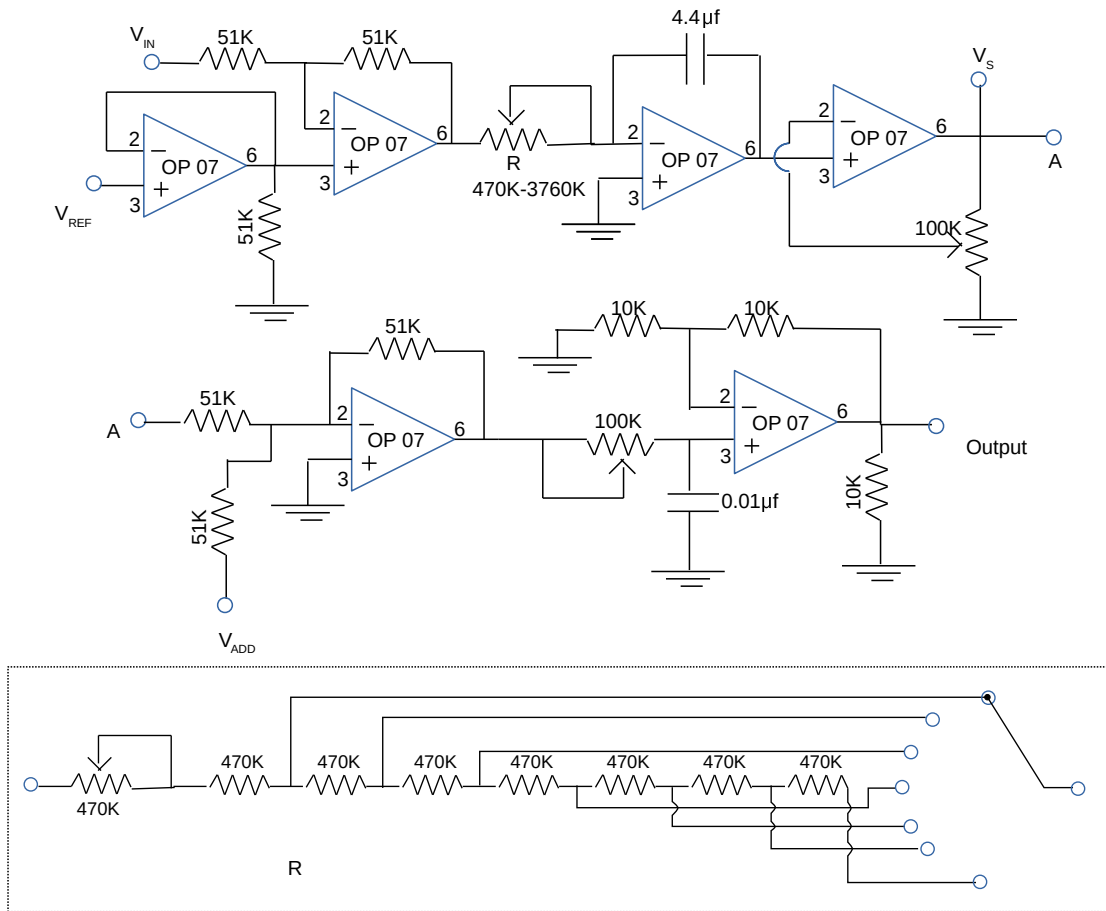


Figure 3.13: Circuit diagram for PI controller

446 nm / 40 mW or Coherent Compass 115M-15 532 nm / 15 mW. The selection of laser depends on the excitation wavelength required for the fluorescent molecule used in the experiment. The laser power is subsequently brought down to 5 mW to 10 mW with neutral density filters. The initial laser beam of 1-2mm width is expanded with a telescopic arrangement(between mirror 2 and 3 in figure 3.15). Using an iris, then a ~ 10 mm parallel gaussian beam is selected. Then this light is focused on an optical fiber end with a convex lens of $f = 25$ mm. We get around 1-2 mW of light in the fiber. The optical fiber used is the same single-mode fiber used in fabricating the fiber tip(Thorlabs 460HP). The other end of optical fiber is mechanically spliced with the non-tip end of the fiber-tip. We get more than 90% efficiency in splicing. We use AMP-Corelink mechanical splice for this purpose. The light emitted from the fiber tip then excites the fluorescent molecules. The fluorescent molecules are diffusing in the nanoconfined

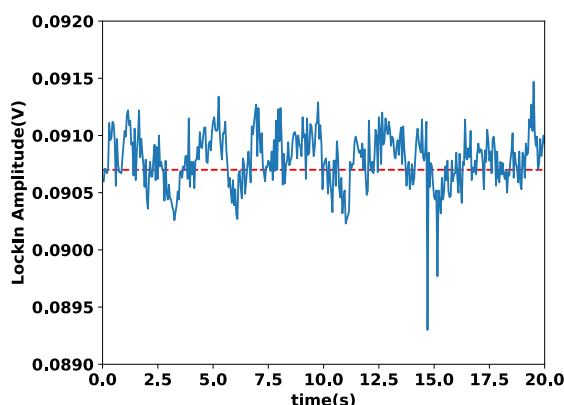


Figure 3.14: Stability of Feedback control. The fluctuation of lockin amplitude of QTF current indicates a fluctuation below ± 1 nm of gap. The setpoint voltage is indicated with red dashed line at 0.0907 V

water between the tip and a coverslip.

The fluorescent photons from the dye molecules are collected with the objective lens kept below the coverslip. We use 60x water immersion objective with a numerical aperture of 1.2 (Olympus 60X UPlanSApo). The objective lens is focused between the tip and coverslip. And it collects light from the tip as well as the light from the fluorescent molecules. We use a dichroic filter and an emission filter to extract-out fluorescent light. Then an achromatic convex lens(150 mm) is used to focus this light on one end of a multimode optical fiber(Dia. Core = 25 μm , Dia. with cladding = 125 μm , Thorlabs). The core of this fiber act as the pinhole for the confocal setup. The x,y-position of the pinhole is aligned to match the center of the tip. The other end of this optical fiber is connected to an Avalanche Photo Diode(APD; Perkin Elmer SPCM-AQRH-13-FC). The photon counts from APD are then fed into a correlator card (Flex990EM-12/D) to compute the autocorrelation of intensity fluctuations.

The filter combinations and corresponding excitation wavelength for different fluorescent molecules used are shown in the table 3.3.

Fluorescent Molecule	Excitation λ	Dichroic Filter	Emission Filter
Rhodamine 6G	532 nm	Omega 560DCLP	Thorlabs FD1R
Coumarin 343	446 nm	Omega 475DCLP	Omega 545AF75
Alexa Fluor 568 NHS Ester	532 nm	Omega 560DCLP	Thorlabs FD1R

Table 3.3: The filter combinations and corresponding excitation wavelength for different fluorescent molecules

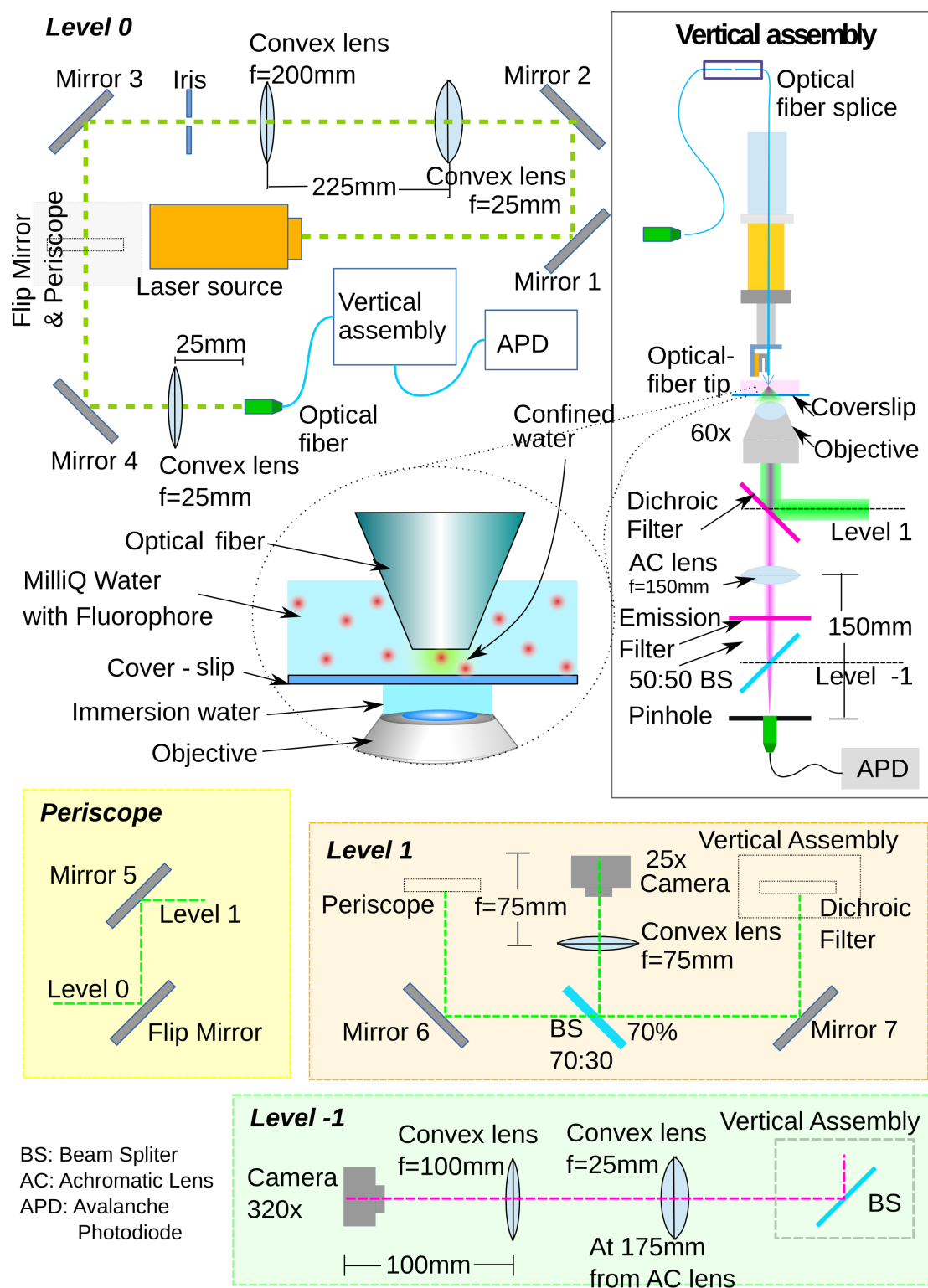


Figure 3.15: Optical setup of the instrument

The optical setup also has a facility to illuminate the fluorophores with the objective lens instead of the tip. For this, the parallel beam of light is rerouted using a flip mirror. While rerouting, the flip mirror act as the bottom mirror of a periscope, and the light beam is brought up to the height where the dichroic filter is mounted(level 1). Using a few mirrors, the light is brought to the dichroic filter, and it acts as a mirror for this excitation wavelength. The reflected light from the dichroic fills the back-aperture of the objective lens and gets focused on the solution with fluorescent molecules. The fluorescent photons are further collected with the same objective, and their intensity from the confocal volume is recorded with APD. The autocorrelation of the fluctuations in this intensity gives the bulk FCS data.

We also have two cameras for tip inspection and alignment. The camera-sensor used in level 1 is Thorlabs DCC1645C-HQ, a USB-based camera-sensor. Using this camera, we focus the objective over the coverslip surface. Moreover, the coarse tip-pinhole alignment, inspection of the tip, and inspection of tip illumination pattern are done with this camera. Details of these operations are discussed in the next section. There is one more camera sensor. This one is mounted at another horizontal plane(level -1), which matches the beam-splitter's vertical position in the vertical assembly. This camera-sensor is the "Raspberry Pi high-quality camera (Sony IMX477)". With the combination of lenses, we get an image magnification of $\sim 320\times$. This camera is used to inspect the tip for any structural changes and roughly estimate the tip's size. More discussion on the use of this camera is presented in the next section.

3.4 Challenges

We faced many technical hurdles in building this instrument. The major challenges will be discussed in this section. The challenges discussed here are regarding the tip-geometry modifications, drifts of the objective lens, and tip alignment.

3.4.1 Tip modifications and tip inspection

If the tip is pressed over the coverslip, the tip will buckle and break beyond a force. Such physical damage happens in many ways. It can happen while we manually bring the tip close to the coverslip and accidentally crash. It also occurs when pulses given to hammer piezo to get the tip closer to coverslip overshoots a safe range determined

by the searching done with scanner piezo. This can happen as the tip movement for a given pulse height or number of pulses will not be the same for different days. The sliding friction utilized in this inertial sliding can be different due to drifts in tightening screws of leaf spring, dirt or dust over sliding surfaces, temperature differences, etc. Another reason for the physical damage to the tip is the occasional misbehavior of the feedback, which maintains the constant separation between the tip and coverslip. In a few instances, the QTF amplitude changes measured using a lock-in amplifier are slower than either feedback time-constant or the mechanical noises the feedback is trying to correct. In such cases, the feedback can give an oscillating V_z , which can damage the tip. All these cases are rare and cause irreversible physical damage to the tip. Such damages cause irregular confining surface on the tip and ill-defined light illumination from the tip. We optimized the tip actuation algorithm to bring the tip safely near the coverslip. This algorithm is presented in the previous section. The feedback is used in overdamped conditions to avoid feedback oscillations.

We need to ensure that the tip we use for the experiments is not damaged. The quality of the tip is monitored in multiple ways. Using the camera on level 1, we can check the illumination pattern from the tip. The figure 3.16 shows the illumination pattern of light from the tip is pristine and mildly damaged tip after a few days of experiments. When the tip crashes, this pattern becomes asymmetrical.

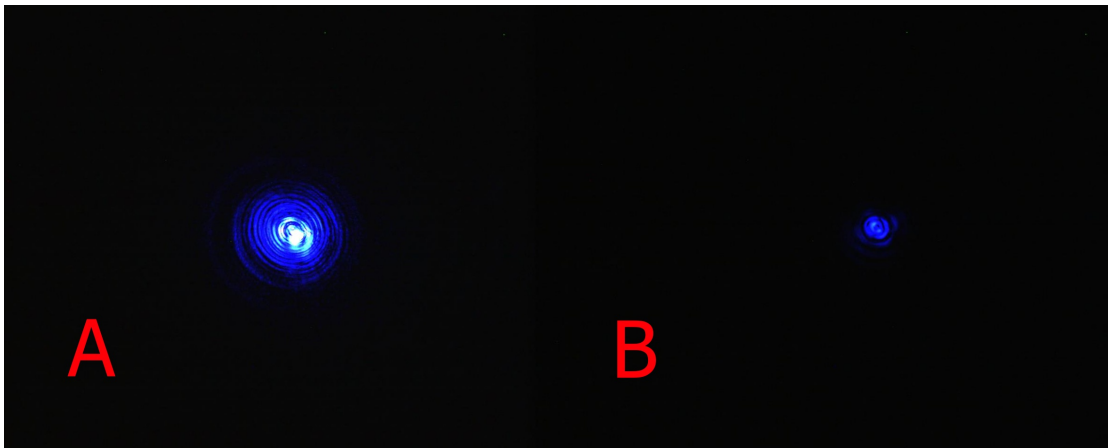


Figure 3.16: Illumination pattern observed using camera in level 1. A: Pristine tip illumination B: Tip illumination from a tip after multiple experiments

We use the camera on level -1 to know the rough shape of the tip. Ideally, we should

check the tip structure with the scanning electron microscope (SEM), but loading and unloading the tip in our instrument can bring impurities to the tip. Furthermore, the process can physically damage the tip. So we use a high magnification (320x) camera to monitor the physical structure of the tip in situ. In this camera, we image the shadow of the tip as shown in figure 3.17. Depending on the direction the light falls over the tip we can see the edge of the tip. An LED torchlight is used as the light source. We use the open-source program “Gwyddion” to measure the tip dimensions from the jpg images from the camera. The magnification factor and dimensions of the sensor pixels are used to calibrate the length scale of the image.

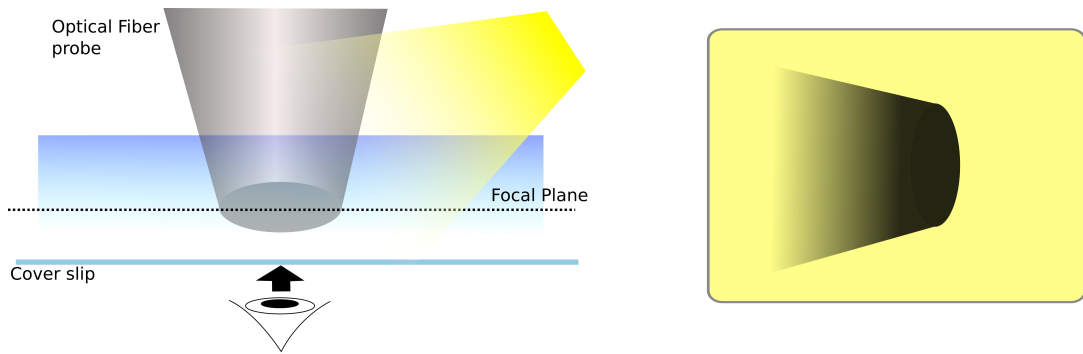


Figure 3.17: Imaging the shadow of the tip. The inset shows the expected image.

An image of a new tip and a slightly modified tip after a few experiments are shown in figure 3.18. These images are with light falling on the tip from the same direction. We take more images of the same tip by varying the light direction. Apart from tip structure, with this high magnification imaging (320x), we can see if there are any impurities accumulated on the tip.

With these facilities in the instrument, we can monitor the quality of the tip. If the tip is found to have asymmetric illumination and an irregular shape, the measurements from such tips are discarded.

3.4.2 Focusing with the objective lens and Focus-drifts

For our measurements, the objective is always focused on the top surface of the coverslip. The vertical position of the confocal pinhole is aligned for keeping the center of confocal volume on the top surface of the coverslip. The challenge is how to focus the objective exactly over coverslip and how to correct the objective drifts during ex-

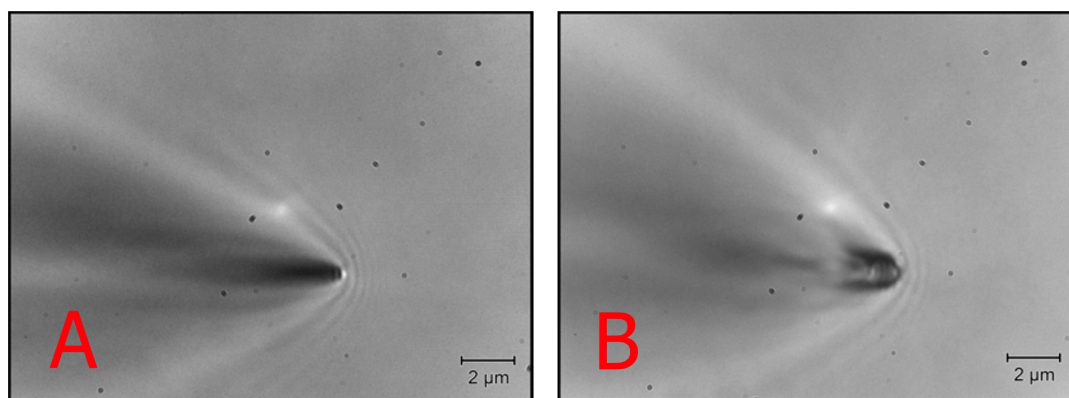


Figure 3.18: High magnification(320x) image of the shadow of the tip. A: Image of a pristine tip. B: Image of a tip slightly damaged after multiple experiments.

periments. With no impurities or visible structures on the coverslip, identifying the top surfaces by imaging the coverslip with objective and camera is difficult.

We utilize an interference pattern technique to track the objective movements and identify if it is focused over the coverslip. The flip mirror(Refer to the figure 3.15) is used to direct the parallel beam of laser light to the objective's back-aperture. As the beam is parallel, the light will converge at the focus of the objective and diverge beyond the focus. In the vicinity of the focus, the beam of light will have an hour-glass like shape. The surfaces of the coverslip intersect this beam, and the rays at the cross-section of the beam and coverslip surface will get partially reflected. Few of the reflected rays will be collected by the objective and can be imaged with the camera in level 1. The different rays from the cross-section interfere at the camera's image plane, and we observe an interference pattern. Depending on the vertical position of the cross-section, the pattern will be different. This is illustrated with actual images of patterns in the figure 3.19. When the focus of the objective is exactly on the surface of the coverslip, the pattern is smallest in size and intense. This way, we can identify the focus and align the focus exactly on the surface of the coverslip by moving the objective. Similar patterns are observed for both the bottom and top surfaces of the coverslip. Thus if we move the objective from a far way vertical position towards the coverslip, we can identify the bottom and top surfaces of the coverslip as the objective-focus cross them. The objective is moved with a micro-positioning screw with a piezo crystal at the end

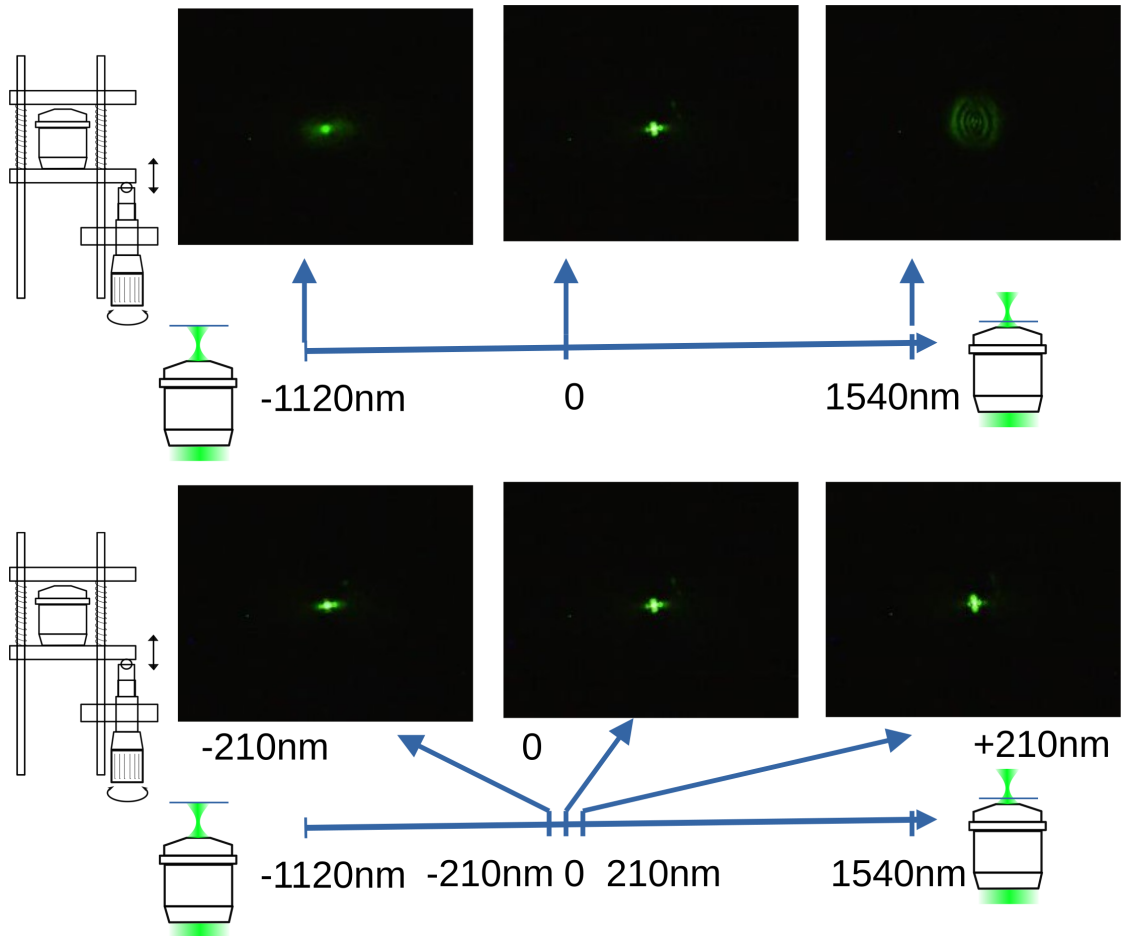


Figure 3.19: Variations in the interference pattern with the vertical position of the objective lens

of the screw(Thorlabs PE4). Thus we can move the objective with nanometer precision. And by visually observing the changes in the pattern, we can recognize the drifts in the relative distance between objective and coverslip. We are able to identify drifts as small as 200 nm(figure 3.19). We can correct such drifts by adjusting the voltage given to the piezo, which moves the objective.

The objective is focussed on the top surface of the coverslip using this procedure. Furthermore, we can monitor and correct any focus drifts in the system. Usage of piezo for the correction of focus-drifts doesn't introduce any mechanical disturbance, which can harm the tip when it is very close to the coverslip.

3.4.3 Tip alignment

The position of the tip should be inside the confocal volume for the measurements. And the center of the confocal volume is approximately on the top surface of the coverslip. Thus as the tip is brought a few nanometers above the coverslip, the vertical position of the tip will be well within the vertical range of confocal volume ($\sim 1\mu\text{m}$). The axis of the tip and x,y-position of the confocal pinhole has to be aligned on a straight line. As the confocal volume is on the top of the coverslip, the tip and pinhole's x,y alignment is possible only when the tip is very close to the coverslip. By moving either tip or pinhole, we can do the alignment. And for good alignment, fluorescence intensity collected by the pinhole will be maximum, and the autocorrelation of the intensity fluctuation will have the highest y-intercept. We cannot use micro-positioning screws to adjust the x,y-position of the pinhole or the tip. As the tip is very close to the coverslip, the disturbance caused by turning the micro-positioning screws can crash the tip. We can adjust the tip with piezos, but the range motion possible is small ($\sim \pm 10\mu\text{m}$). There can be instances for which this range may not be sufficient for the alignment. We need a range of a couple of millimeters for the pinhole x,y-motion. This range is owing to the magnification at the image plane where the pinhole is placed. And this has to be remotely controlled to reduce mechanical disturbance. For this purpose, a remote-controlled XY stage with stepper motors and 3D printed parts is made. The stage is controlled with Arduino. The figure 3.20 shows the photos of the XY stage. The core of an optical fiber is used as the pinhole. And the fiber is attached to the centerpiece of the XY stage.

3.5 Automation

The instrument control and data logging are performed with the few LabVIEW programs. We use NI PXI-6259 DAQ for generating and recording analog voltages. The lock-in amplifier (Signal Recovery 7265) is connected to the computer via RS232 interfacing. And a USB connection is used to interface the correlator card (Flex99OEM-12/D). The DAQ card, lock-in amplifier, and correlator card are accessible for writing programs in LabVIEW.

We wrote several LabVIEW programs for different purposes. We use these programs to auto approach the tip, check the frequency response of the QFT with the tip, align the tip inside the confocal volume, and perform experiments. The main "Data Acquisition"

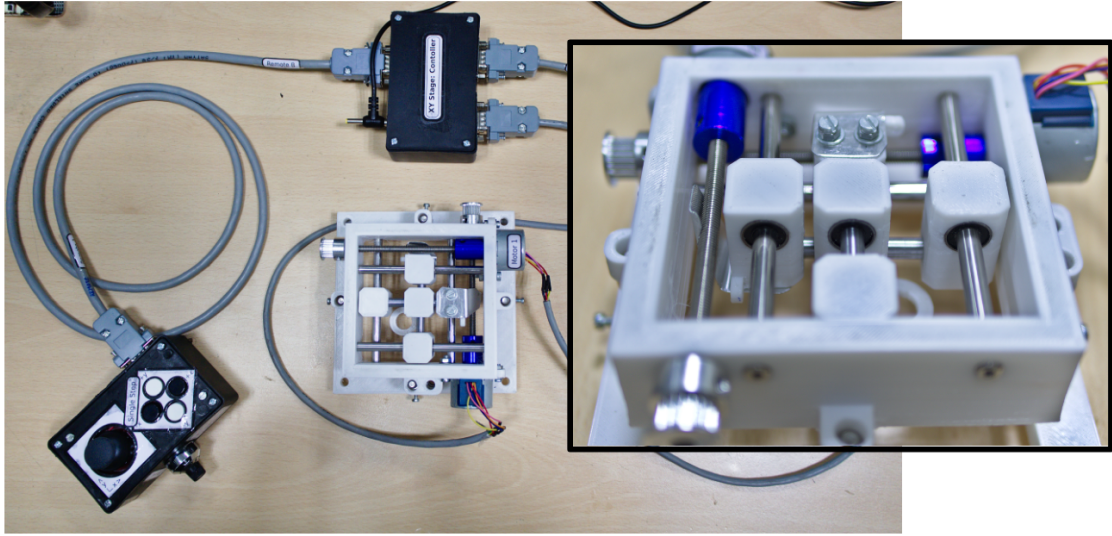


Figure 3.20: xy-stage for pinhole remote-alignment. The stage is made with stepper motors and 3D printed parts.

program has many facilities. The important ones are listed below:

1. QTF current/voltage amplitude and phase difference as a function of piezo voltage(v_z)
2. AFM and NSOM imaging
3. Feedback control
4. Confocal imaging of the tip illumination
5. Autocorrelation of fluorescent intensity fluctuations

The figure 3.21 shows a few snapshots of this main program.

Out of many algorithms we implemented with LabVIEW programs, bringing the tip safely near the coverslip is not so obvious. In the next section, this algorithm will be discussed.

3.5.1 Algorithm for the safe approach of the tip towards coverslip

The zero-separation should happen well within the range of the scanner-piezo actuation($\sim \pm 1\mu\text{m}$). Then only we can utilize the feedback to correct the drifts in the coverslip or tip-piezo assembly. Ideally, if the zero-separation happens at the natural length of scanner piezo($V_z = 0$), then piezo have more freedom to expand or compress to compensate

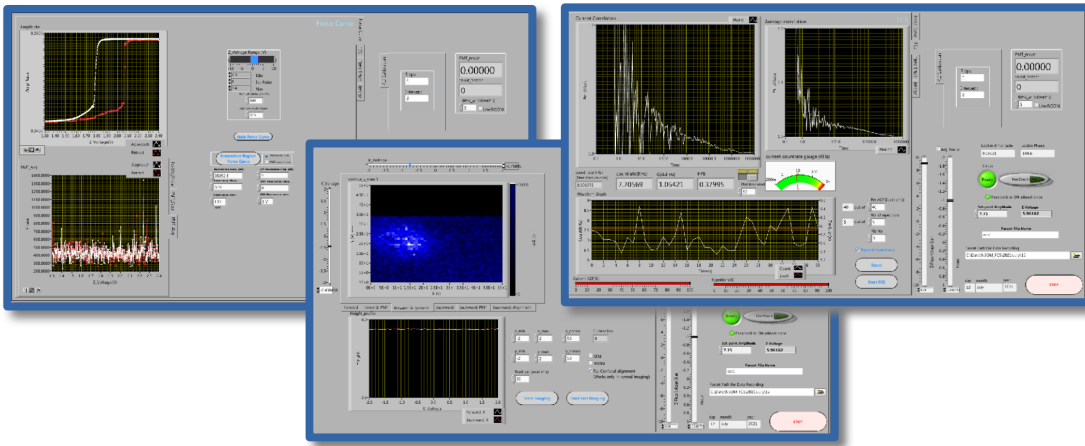


Figure 3.21: Snapshots of the main LabVIEW program

for the drifts in any direction. We have to use the actuation mechanisms to bring the tip just touching the coverslip when scanner piezo z-voltage is 0.

While we actuate the tip towards the coverslip, we need to consider that the tip is brittle. There is not much cushioning in the instrument once the tip press over the coverslip. If the tip comes down beyond the zero-separation, the tip can bend or buckle until a force. In such cases, if physical damage happens to the tip, that is irreversible. Such damaged tips will give irregular tip surface for confinement, and the light from such tips will be ill-defined. So we need an algorithm to bring the tip very close to the coverslip in a controlled way.

The following algorithm we use to bring the tip safely near the coverslip. First, the objective is focused few $100\ \mu\text{m}$ above the coverslip top surface. This is possible with a micro-positioning screw that pushes the objective lens upward in the cage assembly. The tip is brought down until a clear tip image is obtained from the camera at level 1. Using the camera in level -1, a high magnification image of the tip is taken. This image is used as a reference image to identify any tip modification during the approach or experiments. Then the feedback is turned on with a large setpoint for the QTF voltage amplitude, which is experimentally not achievable, say 10V. Then the feedback will compress the scanner piezo as much as possible (limited by gain and piezo voltage supply), and the tip will retract maximum from the coverslip. After this, a new setpoint is given. The new setpoint will be 90-95% of the QTF amplitude when the tip is far from the coverslip (free amplitude). This causes the feedback to expand the scanner piezo from the current

position until this QTF voltage is sensed. This QTF voltage amplitude is expected for a tip-coverslip separation of 5-10 nm. If the tip is far from the coverslip, the scanner piezo will attain its maximum expansion corresponding to the maximum possible V_z . Once feedback output for V_z saturates to its maximum value, the setpoint is reversed to the previous large value. And the tip will be retracted to the maximum limit. Now we essentially searched for the presence of coverslip in the full range of scanner piezo and found the coverslip is absent in the range. Once the tip is fully retracted, a specific series of pulses (for instance, 5 repeated pulses of height 35 V) is given to the hammer piezo. The inertial sliding due to the pulses will bring the entire piezo assembly with tip towards the coverslip. This step-motion of the tip towards the coverslip should be in the range of the scanner piezo. The choice of number of pulses and height of each pulse will be selected for an actuation step less than half of the range of the scanner piezo. Again a search for coverslip surface is performed with scanner piezo by changing the setpoints as mentioned before. The initial search with scanner piezo and subsequent step was taken with hammer piezo is the “search and step” sequence. Until the presence of coverslip is detected by finding a V_z for which QTF voltage amplitude reduces to 90-95% of QTF free amplitude, the “search and step” sequence is repeated.

The piezo assembly has to be further positioned such that the coverslip is felt near the natural length of the scanner piezo; near $V_z=0$. As a safety precaution for the tip, instead of bringing the tip such that the coverslip is felt exactly at $V_z=0$, the tip is positioned such that the scanner piezo has to expand around 100 nm further from its natural length to sense the coverslip. This ensures that if accidentally piezo gets $V_z = 0$ due to power failure or switching issues, it doesn't crash the tip on the coverslip. The fine adjustments in the tip-position are made with a couple of modifications in search and step sequence. The search will be performed only till 100 nm expansion, not till full expansion of scanner piezo ($\sim 1\mu\text{m}$). Then the retraction happens as before to the full compression limit of scanner piezo. Now the pulses of the hammer are selected for a step less than 100 nm. Repeating this modified search and step sequence ensures the coverslip is within 0-100 nm below the tip when $V_z = 0$. After completing these actuations of the tip, the scanner piezo is kept at a fully compressed state, which keeps the tip at a fully retracted state until experiments are performed.

A LabVIEW program is written to implement this algorithm

3.6 Conclusion

This chapter discussed the development of a new instrument. The instrument is designed to measure translational diffusion of a fluorescent molecule in nanoconfined water. The water is confined between a tapered fiber and a flat substrate (coverslip). Direct optical access to the confinement region is made possible by coating the fiber tip with metal and opening a small aperture ($0.1\ \mu\text{m}$ to $1\ \mu\text{m}$) at its end. A well-controlled cut using an ion beam ensures the desired lateral confinement area and adequate illumination of the confinement gap. The probe is mounted on a tuning-fork-based force sensor to control the separation between the probe and the substrate with nm precision. Fluctuations in fluorescence intensity due to diffusion of a dye molecule in water confined between probe and the sample are recorded using a confocal arrangement with a single photon precision. These fluctuations encode the diffusion characteristics. The autocorrelation of this fluctuation are calculated with a correlator card and are recorded in a computer for further analysis. Details of the instrument and challenges in building the instrument are described in this chapter. A few photos of the instrument are shown in the next page (figure 3.22).

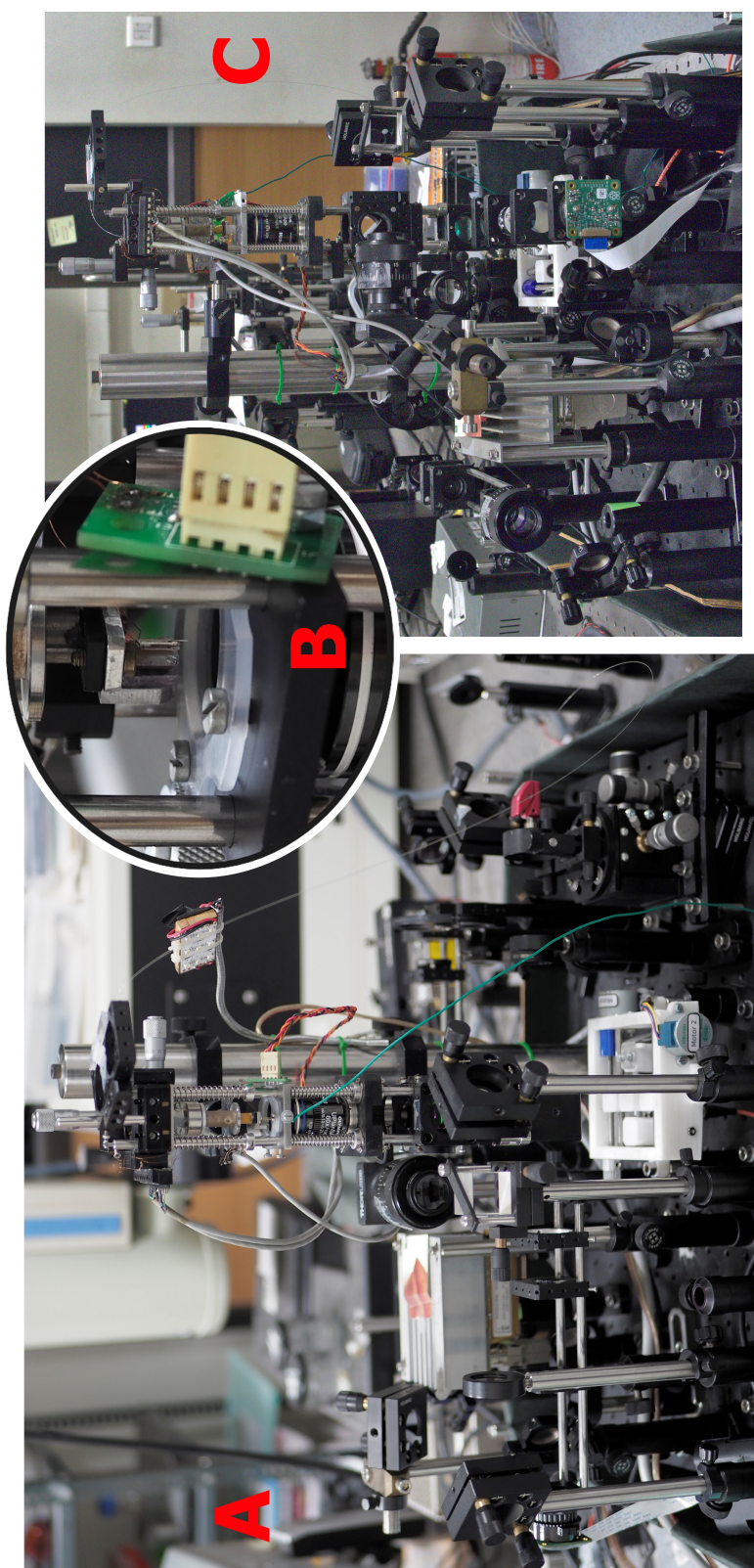


Figure 3.22: Photos of the instrument. A: Front view, B: Zoomed photo of the tip above the liquid cell, C: Side view

Chapter 4

Monte-Carlo Fitting Procedure

A part of this chapter has been published as:

“A new method for measurement and quantification of tracer diffusion in nanoconfined liquids”

V J Ajith and Shivprasad Patil

Review of Scientific Instruments, 91, 013702 (2020)

This chapter reports the development of a Monte-Carlo-based fitting procedure to be used for analyzing the data from the new instrument. With this new analysis technique, diffusion coefficient of fluorescent molecule in nanoconfined water is estimated.

4.1 Introduction

Our instrument’s autocorrelation of fluorescence intensity fluctuations needs to be analyzed to obtain the diffusion characteristics. We can use the FCS expressions mentioned in the “Theory” chapter to estimate time scales for diffusion in our measurements. The time scale values are obtained by fitting the expression on our data. While the time scale will capture the trend of diffusion coefficient as a function of the gap, the estimate of diffusion coefficient from it will have errors. This is because the FCS expressions are not derived for our setup. Unlike FCS, our observation volume is bounded by physical boundaries along the Z-direction. In our setup, the tracer diffusion in the Z-direction is restricted by the confining boundaries. The residence time τ_D is determined by both dif-

fusion and the restriction in z-motion. The other important factor is the excitation profile in our experiments. Equation 2.37 is derived for an objective illumination. In our setup, the illumination is done using the fiber-tip, and emission is collected using a confocal arrangement. The actual observation volume will be a convolution of tip-illumination and the confocal volume.

For the reasons mentioned above, it is necessary to develop a method to extract diffusion coefficient from experimental autocorrelation curves, which will accommodate our excitation profile and impose the physical boundaries on top and bottom within which the tracer molecules are restricted. We develop a Monte-Carlo method for analyzing our data which has the flexibility to change the illumination profile and accommodate the effects of confining boundaries. The autocorrelation curves obtained from multiple simulation runs are fitted to experimental data to extract accurate diffusion coefficients from the measurements.

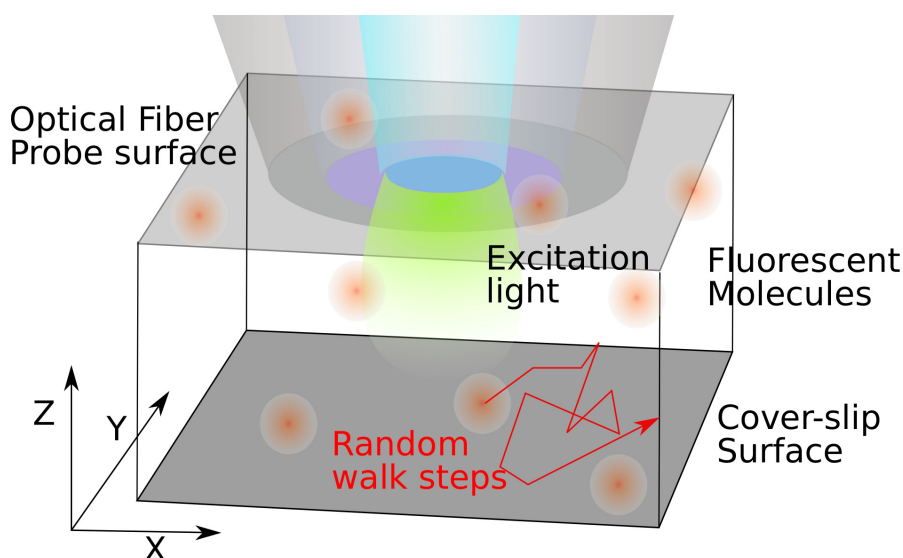


Figure 4.1: The simulation box. The fluorescing tracer molecules are moved to the next positions by a distribution dictated by the diffusion coefficient. A time series is generated by summing the fluorescence from all tracer molecules in the illumination volume in each time step. The autocorrelation of these fluorescence fluctuations is compared to experimental data. The best fit(least residues) provides the diffusion coefficient.

4.2 Monte Carlo Based Fitting

The simulations are performed by defining a rectangular volume with top and bottom surfaces as confining boundaries with reflecting boundary conditions. The lateral sides were considered to have periodic boundary conditions. Figure 4.1 explains the simulation box and parameters associated with it. Around ten fluorophores were placed randomly in the simulation volume and allowed to take a 3D-random walk. The length of each step Δr is picked from a Gaussian distribution with a diffusion coefficient(D).

$$\frac{1}{\sqrt{12\pi Dt}} \exp\left(\frac{-\Delta r^2}{12Dt}\right) \quad (4.1)$$

As x, y, z are independent, the distribution of displacements along any of these axis is also a similar Gaussian. For example for x -displacement the distribution is

$$\frac{1}{\sqrt{4\pi Dt}} \exp\left(\frac{-\Delta x^2}{4Dt}\right) \quad (4.2)$$

Using the equation 2.22, we can generate the positions of a diffusing molecule as a time series.

We can compute the positions of all fluorophores after every time step. Assuming a particular excitation intensity profile $I(x, y, z)$ and collection efficiency function $CEF(x, y, z)$, using the intensity profile and the absorption cross-section, we can calculate the number of photons absorbed by the fluorophore. Further, the number of emitted photons can be calculated by assuming a typical quantum efficiency. The product of the CEF with the number of emitted photons gives the fraction of photons detected from each fluorophore. And depending on the positions of each fluorophore, the value will vary. Summing the detected photons will give the fluorescent intensity of that instant. Thus, after every step, we can compute photon count from all fluorophores in the volume, and the time series of fluorescent intensity can be recorded. Simulations were repeated by varying diffusion coefficient until the resulting autocorrelation curve matched with experimental data.

In conventional FCS, an objective is used for both illumination and collection of photons. The illumination profile is given by [110].

$$I(r, d_{xy}) = \frac{2P}{\pi d_{xy}^2} \exp\left(\frac{-2r^2}{d_{xy}^2}\right) \quad (4.3)$$

where,

$$r = \sqrt{x^2 + y^2}$$

$$d_{xy} = \omega_o \sqrt{1 + \left(\frac{z\lambda}{\pi\omega_o^2}\right)^2}, \omega_o = \frac{0.61\lambda}{NA}$$

Here, P and λ are the power and wavelength of light, and NA is the objective's numerical aperture. This $I(r, d_{xy})$ is used to calculate the number of photons emitted from fluorophores in different locations of the simulation box. Governed by the confocal arrangement, a fraction of these emitted photons are detected. To calculate that fraction, we can define a collection efficiency function $CEF(x, y, z)$ as

$$CEF(x, y, z) = \exp\left(\frac{-2(x^2 + y^2)}{\Omega_{xy}^2} + \frac{-2z^2}{\Omega_z^2}\right) \quad (4.4)$$

where, Ω_{xy} and Ω_z are the Gaussian radius along XY-plane and z-direction where the collection efficiency falls to $1/e^2$. These parameters depend on the size of the pinhole and the wavelength of the light. For determining diffusion coefficient D by comparing simulations to experimental data, we need to determine Ω_{xy} and Ω_z for pinholes used in our experiments. The diffusion coefficient D of Coumarin 343 in bulk water is known to be $550 \text{ nm}^2/\mu\text{s}$ [111]. We fixed it in simulation runs and varied Ω_{xy} and Ω_z until it matches the experimental data obtained for bulk experiments. We obtained $\Omega_{xy} = 340 \text{ nm}$ and $\Omega_z = 7.8\mu\text{m}$ for 446 nm excitation. Similarly, for Rhodamine 6G with $D = 400 \text{ nm}^2/\mu\text{s}$ [112], we obtained $\Omega_{xy} = 486 \text{ nm}$ and $\Omega_z = 7.8\mu\text{m}$ for 532 nm excitation. See figure 4.2.

In the case where tip illumination is used, the illumination profile differs from conventional FCS. To describe it, the excitation intensity from the tip is approximated as a Gaussian along XY-plane with increasing width d_{xy} away from the tip, determined by a half-cone angle α . This intensity is normalized to have constant power(P) in XY-plane

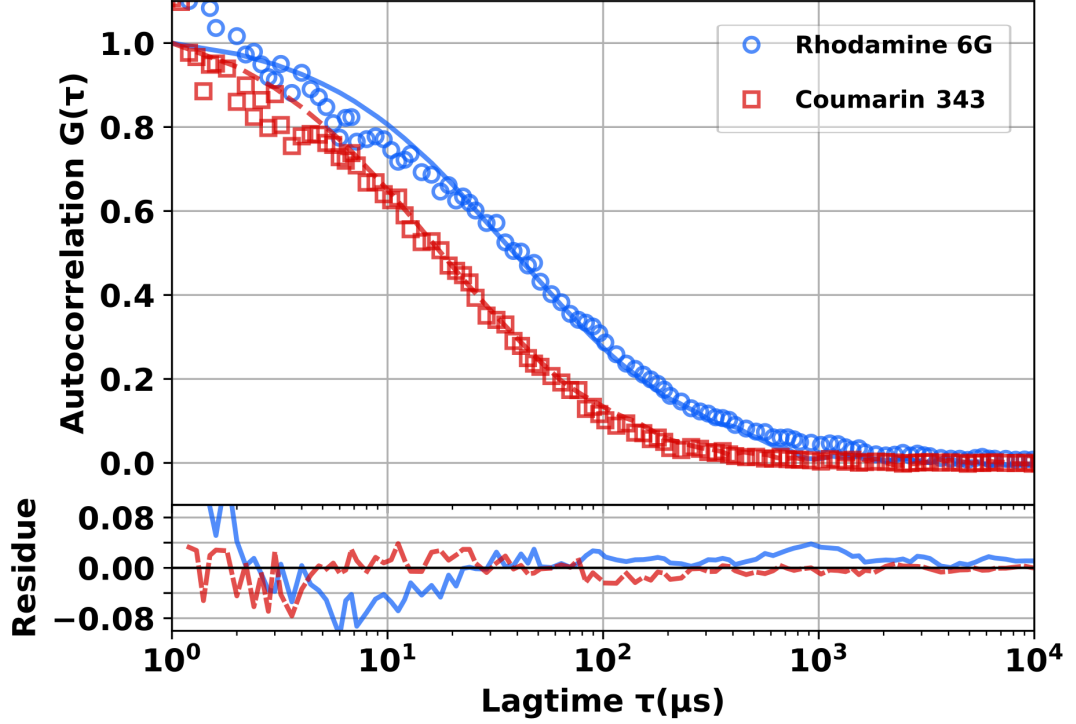


Figure 4.2: Conventional FCS measurements using Rhodamine 6G(open circles) and Coumarin 343 (open squares). The continuous lines are simulated autocorrelations curves by fixing the diffusion coefficient of these dyes to a value determined from previous bulk measurements. The calibration allows us to determine the parameters Ω_{xy} and Ω_z to be used in the simulations. $\Omega_{xy} = 486$ nm for 532 nm excitation used for Rhodamine 6G and $\Omega_{xy} = 340$ nm for 446 nm excitation used for Coumarin 343. Ω_z is $7.8 \mu\text{m}$

at any z-position. The half-cone angle(α) can vary from 5.6° (NA of the fiber used) to approximately 30° (for the tip). At a distance of a few nm away from the tip aperture, d_{xy} does not change appreciably from the radius of tip aperture r_{tip} for these values α . The illumination profile then becomes

$$I(r, d_{xy}) = \frac{2P}{\pi d_{xy}^2} \exp\left(\frac{-2r^2}{d_{xy}^2}\right) \quad (4.5)$$

where,

$$r = \sqrt{x^2 + y^2}$$

$$d_{xy} = r_{tip} + \tan(\alpha) |z|$$

The collection efficiency is given by equation 4.4

In this scenario, we need to use the tip radius r_{tip} in the simulation, which is determined from the size of optical aperture observed in the SEM pictures of the tip. We used Ω_{xy} and Ω_z determined from the conventional FCS experiments. To obtain diffusion coefficient D under confinement, we fixed Ω_{xy} , Ω_z , and r_{tip} , and performed simulation runs by varying D until the simulation data matches with experiments.

As Ω_{xy} , Ω_z depends on alignment, for a given tip and alignment, we have an alternate way to calibrate Ω_{xy} , Ω_z . In this way, autocorrelation from a large separation (> 100 nm) is used to calibrate Ω_{xy} , Ω_z . As separation is large, we use the bulk diffusion coefficient in the simulations. Then we use the calibrated values of Ω_{xy} , Ω_z for fitting the autocorrelations from the nanoconfinement. And this calibration will be valid only for the same tip and alignment.

For simulating our experiments, we used $3 \mu\text{m} \times 3 \mu\text{m}$ simulation box with a height of few nm. We found it important to take lateral length three times larger than the tip aperture diameter. It ensures that the simulated autocorrelation curve is independent of the lateral size of the simulation box and avoids artifacts owing to the periodic boundary conditions used. See figure 4.3 a. Similarly, the simulated autocorrelation should not depend on the vertical dimension of the simulation box as long as it is smaller than Ω_z . See figure 4.3 b. We used $1 \mu\text{M}$ as the fluorophore concentration, which corresponds to 11 fluorophores in the simulation box. Increasing the concentration decreases the intercept to the ordinate of the simulated autocorrelation curve. The normalized autocorrelations for different concentrations overlapped for the given diffusion coefficient (data not shown). The simulations shown here are generated fluorescent intensity time trace for 5 s with $5 \mu\text{s}$ time step. For data fitting we do simulations to generate 10 s long time trace with $1 \mu\text{s}$ time step. We performed few control runs to ensure the validity of our simulations. When we increased the separation between top and bottom boundary of the simulation box ($\geq 3 \mu\text{m}$) and used the collection efficiency function with $\Omega_{xy} = 340$ nm, the bulk measurements match the simulation data for the reported

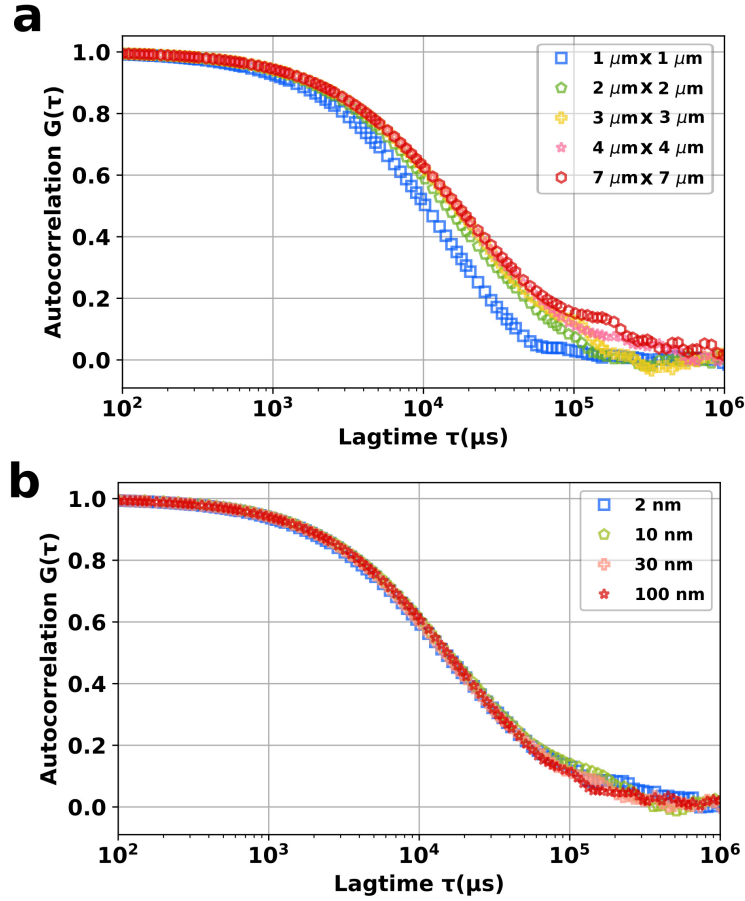


Figure 4.3: a) The simulated autocorrelation curves overlap each other if lateral area of the simulation box is larger than $3 \mu m \times 3 \mu m$. We choose the simulation box size to be larger than $3 \mu m \times 3 \mu m$, The height of the simulation box is kept fixed at 2 nm. b) the height to simulation box does not affect the autocorrelations if it is smaller than Ω_z . the lateral area is fixed at $3 \mu m \times 3 \mu m$. The diffusion coefficient used here is $1.3 \text{ nm}^2/\mu s$

value of bulk diffusion coefficient of Coumarin 343 ($550 \text{ nm}^2/\mu s$). A similar method is used by Wohland et al. [113] to quantify errors in estimating D in the context of conventional FCS.

As the values of concentration and fluorescence properties used in simulation and alignments in experiments can have slight variations from ideal expectations, the intercept of the simulated autocorrelation may not match with the experimental autocorrelation. So before fitting the simulated autocorrelation with the experimental one, the intercept is fitted. This is done by multiplying a factor to the simulated autocorrelation

to get a corrected one. The factor value is determined by finding the one that gives the least root mean square error between the simulated and experimental autocorrelation. This way, corrected autocorrelations from the simulation for different diffusion coefficients are obtained. Among these simulated autocorrelations, the one which gives the minimum root mean square error is the simulation fit. The diffusion coefficient corresponding to this fit is the diffusion coefficient of the fluorophore in the experiment.

4.3 Conclusion

In this chapter, we discussed a newly developed Monte Carlo-based fitting procedure. We developed it for our new instrument to estimate the diffusion coefficient from the autocorrelation data. This procedure obtains simulated autocorrelation for different diffusion coefficients by considering the experimental configuration. Then the simulated autocorrelations were compared with experimental ones, and RMS error between them were calculated. The diffusion coefficient corresponding to the simulated autocorrelation, which gives the least RMS error, is the fitted value.

Chapter 5

Fluorescent molecules in nanoconfined water

A part of this chapter has been published as:

“Translational Diffusion of a Fluorescent Tracer Molecule in Nanoconfined Water”

V J Ajith and Shivprasad Patil

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This chapter reports different experiments and measurements did with the new instrument. The diffusion and adsorption-desorption dynamics of the two different fluorescent molecules are explored. Moreover, viscosity of nanoconfined water is inferred

5.1 Introduction

Water is a an important solvent responsible for macromolecular organization, which is vital for life on earth [114]. For the most part, the water involved in these reactions is interfacial, or tightly confined to nanoscale cavities. Besides the scientific importance, investigation of nanoconfined water is useful in many applications such as filtration and desalination [8], fuel cells [9], properties of clays, minerals and food materials [115]. It also plays an important role in biological processes of protein folding [10] and transport in cellular pores [11].

In these situations, water is confined between either hydrophobic or hydrophilic

solid walls, which are roughly separated by a few nm. Such nanoconfined water, through many experiments, is known to exhibit curious response to external perturbations such as mechanical shear or electrical fields. Among these are fast permeation of water in carbon nanotubes [4], permeation of water through helium-leak-tight membrane [5], dynamic solidification of water layers next to the surface [45], suppression of freezing [6], and anomalously low out-of-plane dielectric constant [116]. Through decades of research, there is some understanding of physical origins of these curious properties of water at nanoscale. However, the subject is active and not free from controversies [24, 35, 36, 117]. Many spectroscopic measurements have been performed with the purpose of shedding light on behavior of water residing at interfaces, nanoconfined between walls or contained in nanoscale pores [26, 118].

In particular, viscosity or the damping coefficient of confined water is measured using Surface Force Apparatus [35], Atomic Force Microscope [43], and Capillary action [12]. Few dissipation measurements have claimed that confined water has a viscosity similar to bulk [35], while others concluded an 8-order increase in viscosity [24]. Nanoscale water also exhibits rheological response with characteristic timescales of the order of 1 to 10 μs and analogies are drawn with slow-down in supercooled water [24]. This means that, self diffusion of water molecules or tracer diffusion of dye molecule through it, is expected to show signatures of dynamic heterogeneity inherent to glass-forming systems. Hence, diffusion measurements become central to answer the question- How does water exhibit slow response to both electrical and mechanical perturbations? .

The slow-down in dynamics, however, is not an universal feature of all spectroscopic measurements of diffusion so far. For instance, measurements using Quasi Elastic Neutron Scattering (QENS) in room temperature [19, 55] conclude that the self translational diffusion of nanconfined water in porous materials is same as bulk water within an order. While at low temperatures below bulk-freezing point, nanoconfined water remains fluid and has almost 100 times reduction in translational diffusion [21, 23].

There are few measurements of tracer diffusion in water confined to single nanochannels. Using Fluorescence Correlation Spectroscopy (FCS) [14, 68], it was reported that there is no slow-down in diffusion of tracer dye. However, by tracking the fluorescence distribution along nanochannels over time, it was shown that diffusion is slowed down

by factor of 100 in organic solvents [15]. These measurements are relevant to the newly emerging field of nanofluidics [119–121]. It has applications in iontronics [122, 123], energy harvesting [124] and sequencing of biopolymers [125, 126]. There are few reports of subdiffusion of proteins in nanochannels [14]. In this context it is important to know about the factors affecting the dynamics of solute molecules in the solution confined in nanochannels. The interfacial charge, viscosity and confinement effects are expected to have an effect, however it is still unclear beyond what length scale we can ignore interfacial effects.

Over last few decades, the questions regarding dynamics of water confined between hydrophilic walls separated by less than 1 nm are addressed through experiments [24, 36, 45]. This question is also tractable using molecular dynamics simulation and large body of work exists in literature [62, 64]. However, there is a lack of clarity on properties of water where confining walls are separated by distance in the range of 1 -10 nm, particularly about the dynamics of solute molecules. This is of immense interest to the newly emerging field of nanofluidics which has tremendous technological impact.

Here, we report diffusion of tracer dyes, Coumarin 343, Rhodamine 6G and Alexa Fluor 568 in water confined to sub-10 nm separations using an instrument developed in our laboratory. An Aluminium-coated fiber tip, with an optical opening at its end is used to confine water between itself and the flat glass substrate. Using a servo control the gap between the tip and substrate is controlled with precision of less than a nm. The instrument allows us to probe diffusion of tracer dyes in the nanoscale gap which can be controlled in-situ. We clearly observed two timescales for diffusing dye indicating co-existence of slow and fast regions for diffusion of the dye.

The disappearance of slow component after addition of 1 M salt, indicates that it is likely due to fluorophores bound to the glass surface. Further, we show that the diffusion coefficient is bulk-like for sub-10 nm confinement gaps. The slow-down in tracer diffusion seen in the past can be attributed to the adsorption-desorption to the confining walls.

5.2 Experiments

All experiments are performed with a home-built instrument [127]. A schematic of the instrument is shown in figure 5.1. In order to create a small controlled gap in

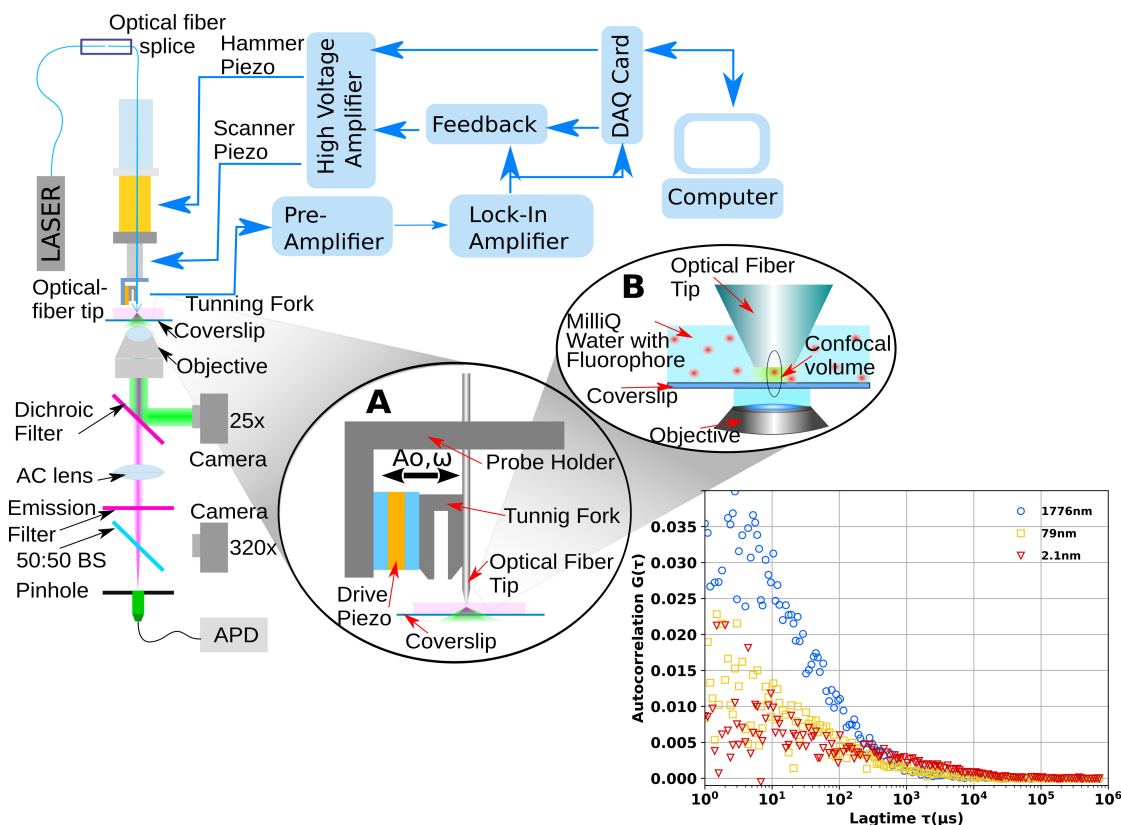


Figure 5.1: A schematic of the instrument used to perform measurements. A sharp tip made out of single mode optical fiber coated with Aluminium is loaded onto one prong of a quartz crystal tuning fork. A small optical aperture is opened at the end using focused ion beam. A servo control is used to keep the tip at a fixed separation from the substrate using amplitude of the tuning fork prong as input to the feedback controller. The excitation light from laser is coupled into the optical fiber and reaches the gap between the tip and substrate. The emitted light from the tracer dye is collected using an objective lens and is fed into an Avalanche Photodiode. Representative autocorrelation curves of fluctuations in fluorescence intensities from Coumarin 343 dye diffusing in the gap between the tip and substrate held at three fixed separations (2 nm, 79 nm and 1776 nm) shown. The lateral amplitude of the tip is used as an input to active feedback to control the separation. The data at large separations is collected without the feedback control. All separations are determined using voltages provided to the scanner piezo and have systematic errors of 2 nm as mentioned in the text.

which water is nano-confined, a sharp tip is pulled out of single mode optical fiber is used (Thorlabs 460HP, 125 μm diameter with cladding). The water is confined between the tip and the substrate. The tip is coated with 150 nm of aluminium and before mounting on one prong of Quartz Crystal Tuning Fork (QTF), an optical aperture (200-900 nm diameter) is opened at its end using Focused Ion Beam (FIB) milling. This constitutes one of the two flat confining surfaces. The other end of the fiber is used to couple the light from a 446 nm or 532 nm continuous wave lasers with 40 mW power. 446 nm is used for Coumarin 343 and 532 nm is used for Rhodamine 6G and Alexa Fluor 568. Before coupling, the power of the laser is reduced to 1 mW using neutral density filters. After coupling, around 5 to 50 μW of light comes out of the tip. The QTF is then mounted on a piezo drive fixed to the probe holder (Figure 5.1A). The liquid cell is fitted with a circular cover-slip which forms the other flat confining surface. This bottom confining surface is cleaned using UV-Ozone treatment. The cell is filled with pure water (MilliQ, 18 M Ω cm) having 250-500 nM concentration of tracer dye in it. The QTF is mechanically oscillated on its resonance (~ 32 kHz) using the piezo drive and amplitude of the prong with tip is < 1 nm. This oscillation generates a piezo electric current of same frequency and its amplitude monotonically decreases as the tip-cover slip gap decreases. Before every experiment the QTF current amplitude is recorded with lock-in amplifier as a function of piezo extension. In the experiments we monitor and record the QTF current amplitude and use it to calculate the gap. In a typical experiment, the tip is approached towards the substrate with servo-control, in which the QTF current amplitude serves as input parameter to control the separation between the tip and the substrate. This defines the thickness of the water confined between two walls and can be controlled using an active feedback. Since we use 16-bit resolution DAQ for our analog outputs for piezo extension, the thickness of the film can be controlled with precision of 0.1 nm. For gaps typically above 10-15 nm, the QTF current is not sensitive to the gap changes, so we cannot use the feedback. For such large separations, we give constant voltage to the piezo which corresponds to a specific gap. All separations are 'average' distances and are not directly measured in our experiments. They are calculated from the voltages provided to the scanner piezos.

The special optical fiber tip is capable of illuminating a local volume in the nanoconfined water beneath the tip. The diffusing fluorophores enter the nanoconfined water and

transit through the illuminated volume to give fluorescence. A confocal arrangement is used to collect these fluorescence photons and an Avalanche Photodiode (APD) is used to detect them. Typically, the tip diameter is larger than the cross section of confocal volume at the beam waist. It ensures that only the fluorescence from the nanoconfined region is detected. The observation volume is the convolution of confocal volume and the nanoconfined gap produced by the tip and substrate. It is a disc-like volume with height of tip-sample gap and diameter of the confocal volume. The number of fluorophores in the observation volume fluctuates due to diffusion. For low concentrations, these fluctuations will be seen as the fluctuations in the detected fluorescence intensity. These fluctuations are correlated for the average time the fluorophore spends in observation volume. This average residential timescale can be extracted by taking the autocorrelation of the fluctuations in the fluorescence intensity. If the diffusion under confinement is significantly different from the bulk, the slow-down should give increase in this residential timescale. If there is a fraction of fluorophores undergoing adsorption-desorption events at the interface along with diffusion then those fluorophores spend more time in observation volume. In this case the autocorrelation will have another slow residential time scale along with the residential time scale of the freely diffusing molecules.

Each autocorrelation of fluorescent intensity fluctuations for a specific gap is calculated for 100 seconds using a correlator card (Correlator Flex99-12). The correlator card uses multiple-tau algorithm to calculate the autocorrelation [113] and samples the time trace with a precision of 12.5 ns.

Using the instrument we performed experiments with different fluorescent molecules. Mainly we used Coumarin 343, Rhodamine 6G, and Alexa Fluor 568 (NHS Ester) for our experiments. The chemical structures of each of these molecules are shown in figure 5.2. Most of the analysis are done with data from Coumarin 343 molecules (11-oxo-2,3,5,6,7,11-Hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxylic acid; $C_{16}H_{15}NO_4$). In experiments, fluorescent molecules are mixed in water to get 250-500 nM concentration by series dilution. For a certain set of experiments fluorescent molecule is mixed in 1M or 100 mM NaCl water solution to avoid adsorption of tracer dye on the glass surface. The bulk diffusion coefficient of Coumarin 343 is $550 \text{ nm}^2/\mu\text{s}$ ($5.5 \times 10^{-10} \text{ m}^2/\text{s}$) [128]. As per the 3D visualising tool “Jmol” [129],

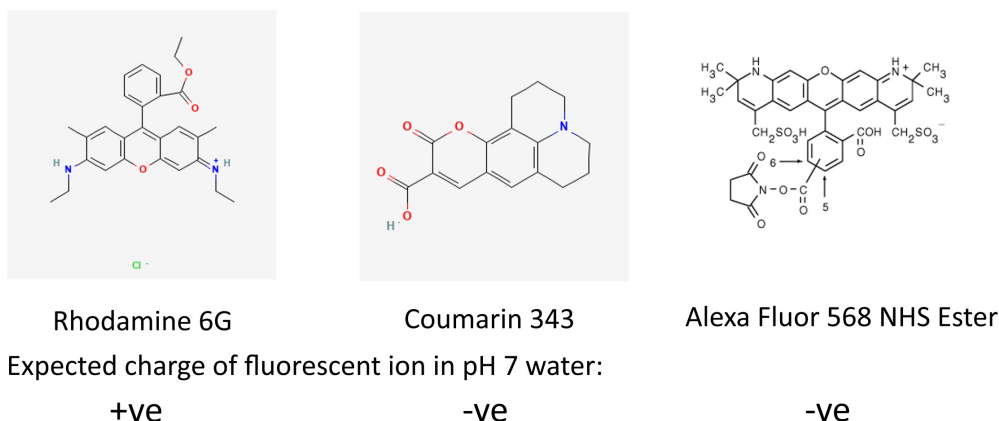


Figure 5.2: Chemical structure of the fluorophores used in the experiments. The expected charge-sign of the fluorescent ion in pH 7 water is also indicated. (Image Source: Structures of Rhodamine 6G and Coumarin 343- pubchem.ncbi.nlm.nih.gov [1, 2], Structure of Alexa Fluor-thermofisher.com [3])

maximum length along x,y,z dimensions of Coumarin 343 is (~ 1.15 nm, ~ 0.7 nm, ~ 0.3 nm).

The uncertainty in the gap between the tip and coverslip in our instrument is ~ 2 nm. This is mainly determined by roughness in the tip and roughness of coverslip for the area beneath the tip. Using a commercial AFM we measured the roughness of the coverslip. For an area of $1\mu\text{m} \times 1\mu\text{m}$, coverslip has a RMS roughness of 0.5 ± 0.2 nm. The optical fiber tips are sliced with Gallium ions (Ga^+) which are accelerated by 30 kV and current of 40 pA. From the Scanning Electron Microscopy images of the tips and roughness measure of coverslip, we estimate the uncertainty in tip-substrate separation to be ~ 2 nm.

Another important point, which affect the accuracy of the tip-substrate separation is a possible relative tilt between them. As mentioned before, we utilize the voltage given for the scanner piezo to find out the gap. In our measurements, piezo voltage for zero of the separations is determined from the sudden change in QTF current amplitude when tip is about to make a contact with the substrate. If tilt exists between tip and substrate, one edge of the tip is closer to the substrate and may cause this sudden change in amplitude. For such a scenario, even when we measure zero separation there may exist a

small gap between the substrate and center of the tip. This is likely to provide an underestimate in separation. To minimise the tilt, we take care to fix the tip perpendicular to the substrate and make FIB cut on the tip which is perpendicular to the axis of the fiber. Even with these cares, a slight tilt can be present. There is a way to check if surfaces are exactly aligned parallel to each other while performing measurements. If there is a tilt, as the tip oscillate laterally, the vertical force fields are also sampled. This contribution is largely due to van der Waals force between the tip and the substrate and it is expected to be attractive. As shown by Kim et al. [130], it introduces a phase lag in QTF current. This phase lag is present when the relative tilt between tip and substrate is more than 0.3° . In all our measurements such phase lag is not seen. This amounts to a possible tilt of 0.3° or less. Given the radius of our tip (~ 400 nm) and a tilt of 0.3° , the gap size is likely to be about 2 nm at the centre of the confinement region when one end of the tip is touching the substrate. Therefore, we consider a systematic error of 2 nm in all our measurements. All the measurements of separations shown in our graphs are calculated from the piezo voltages and may have this possible systematic error.

Figure 5.1 shows the representative autocorrelations obtained for Coumarin 343 diffusing through the gap between the tip and substrate held at three different separations. For separations of the order of $1\ \mu\text{m}$, the feedback control is turned off and the tip is pulled back from near-contact position by the distance mentioned in figure 5.1.

5.3 Theory

The average residential time spent by fluorophores in the observation volume determines the diffusion coefficient. It is estimated from the autocorrelations of fluorescence intensity fluctuations. A similar experimental technique called Fluorescence Correlation Spectroscopy (FCS) deals with autocorrelation of fluorescence intensity from the confocal volume in bulk. Elson and Magde have derived an expression for autocorrelation of intensities which accounts for geometrical details of the observation volume, quantum yield of the fluorophore and the detection efficiency of the instrument [131, 132]. Fitting this expression to experimental autocorrelation data yields the average residential time. However, this expression, which is derived for a typical Fluorescence Correlation Spectroscopy (FCS) experiments needs modification in order to apply to our experimental data. In a typical FCS, the confocal volume is placed well inside the bulk of the

solution and the movement of fluorophores are not restricted by any physical boundary. The illumination and collection is done using the same objective lens. We illuminate part of the confined region using the tip and emission is collected by an objective.

There are several attempts to derive an expression to fit autocorrelation data from FCS when there are two parallel boundaries present in the confocal volume [133]. For gaps much smaller than the height of confocal volume, this expression can be approximated by the one for two-dimensional(2-D) diffusion [133]. We use the expression describing 2-D diffusion in a typical FCS set up to fit our data and estimate the average residential time τ_D .

For a freely diffusing fluorescent species, the FCS expression for autocorrelation is:

$$G(\tau) = \frac{1}{\langle N \rangle} \frac{1}{\left(1 + \frac{\tau}{\tau_D}\right)} \left(1 + \frac{T}{1-T} e^{-\tau/t_t}\right) \quad (5.1)$$

Here, $\langle N \rangle$ is the average number of fluorescent molecules in the observation volume and τ_D is the residential time. “T” is the fraction of fluorophores in triplet state and t_t is the triplet transition timescale. If there are two fluorescent species in the observation volume with different diffusion coefficients, the autocorrelation modifies as:

$$G(\tau) = \frac{1}{\langle N \rangle} \left[\frac{\phi_1}{\left(1 + \frac{\tau}{\tau_{D1}}\right)} + \frac{\phi_2}{\left(1 + \frac{\tau}{\tau_{D2}}\right)} \right] \left(1 + \frac{T}{1-T} e^{-\tau/t_t}\right) \quad (5.2)$$

Here, τ_{D1} and τ_{D2} are the residential time for species 1 and 2 respectively. And ϕ_1 and ϕ_2 are the corresponding weight factors for each species. The weight factor for a particular fluorescent species depends on its fluorescence yield and fraction of that species in the average number of total fluorescent species present in the observation volume($\langle N \rangle$). If q_1 and q_2 are the fluorescence yield of molecule 1 and 2, and “f” is the fraction of molecule 1. Then the weight factors; ϕ_1 and ϕ_2 are given by:

$$\begin{aligned}\phi_1 &= \frac{fq_1^2}{[fq_1 + (1-f)q_2]^2} \\ \phi_2 &= \frac{(1-f)q_2^2}{[fq_1 + (1-f)q_2]^2}\end{aligned}\quad (5.3)$$

In situations where the fluorescent molecule adsorbs or desorbs from the surface along with diffusion. The FCS expression for autocorrelation is given by [77]:

$$G(\tau) = \frac{1}{\langle N \rangle} \left[\frac{\phi_1}{\left(1 + \frac{\tau}{\tau_D}\right)} + \phi_2 e^{-k\tau} \right] \left(1 + \frac{T}{1-T} e^{-\tau/t_t} \right) \quad (5.4)$$

The weight factors ϕ_1 and ϕ_2 are defined exactly like in equation 5.3. Instead of subscripts 1 and 2 being different molecules, here 1 indicates the molecules undergoing diffusion and 2 indicates the fraction of same molecules which are undergoing adsorption-desorption events. And “k” is the rate constant of desorption.

For typical FCS, the residential time is related to the radius of observation volume along lateral direction. This radius, ω_{xy} , is the distance from the peak of Gaussian which determine the objective illumination to the $1/e^2$ value of the peak. This typically matched with the radius of confocal volume for FCS. The relation between residential time (τ_D) and ω_{xy} is $\omega_{xy}^2 = 4D\tau_D$. Here, “D” is the diffusion coefficient of the fluorescent molecule. This follows from Einstein’s equation for mean square displacement in 2-D for the time period τ_D .

Similarly, in our setup we can define the width of the observation volume with the fit value of τ_D from the 2-D FCS autocorrelation expressions. In case where the tip illumination is broader than the width of confocal volume, the width of observation volume is mainly determined by the width of confocal volume and is fixed for a given tip and alignment. This implies that “D” is inversely proportional to τ_D for the measurements with same tip and same alignment. The estimate of τ_D at different separations with same tip and alignment, enables us to verify if diffusion is hindered due to nanoscale confinement.

For a given tip and alignment we can get an expression:

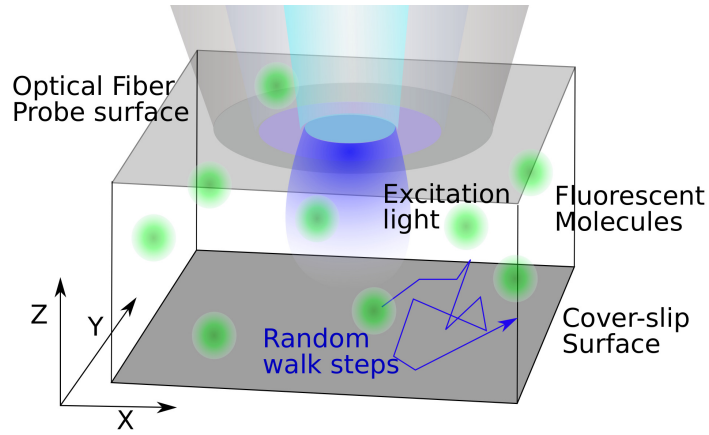


Figure 5.3: A schematic to describe the Monte Carlo simulation box.

$$\frac{D}{D_{bulk}} = \frac{\tau_D^{bulk}}{\tau_D} \quad (5.5)$$

τ_D^{bulk} is the value measured at large separations (>100 nm) with the same tip, where the diffusion is bulk-like.

5.4 Simulations

The models described in previous section have assumptions which deviates from the experimental situation. Models uses 2D diffusion instead of confined diffusion and illumination from objective rather than from the tip. The quantification of diffusion coefficient may have systematic errors and hence its determination may not have significant confidence. We can also estimate diffusion coefficients from the experimental data using a Monte Carlo based fitting procedure [127].

The schematic of the simulation box is given in figure 5.3. The top and bottom surfaces of the simulation box are the confining surfaces of tip and coverslip. Reflecting boundary conditions are used at these two surfaces. The separation between them is decided from the experiment. The other vertical sides of the simulation box have periodic boundary condition. The length and breadth of the box is at least $3 \mu\text{m}$ each to avoid the artifacts of periodic boundary conditions for a typical tip illumination of $1 \mu\text{m}$ diameter. Briefly, the simulation starts with a random distribution of fluorescent molecules with concentration used in the experiment. The fluorescent intensity obtained in confocal

volume from this distribution of molecules under the tip illumination is calculated by taking an excitation profile, detection efficiency and other experimental parameters. In the next time step, the positions of fluorescent molecules are changed according to a certain diffusion coefficient. The fluorescent intensity is calculated again for the new distribution of fluorescent molecules. By performing such calculations for each time step, a time trace of fluorescent intensity is obtained. The autocorrelation of the fluctuations in this time trace is calculated.

As the values of concentration and fluorescence properties used in simulation and alignments in experiments can have slight variations from ideal expectations, the intercept of the simulated autocorrelation may not match with the experimental autocorrelation. So before fitting the simulated autocorrelation with the experimental one, the intercept is fitted. This is done by multiplying a factor to the simulated autocorrelation to get a corrected one. The factor value is determined by finding the one which gives the least root mean square error between the simulated autocorrelation and experimental autocorrelation. This way, corrected autocorrelations from the simulation for different diffusion coefficients are obtained. Among these simulated autocorrelations the one which gives the minimum of root mean square error is the simulation fit. The diffusion coefficient corresponding to this fit is the diffusion coefficient of the fluorophore in the experiment. See ref. [127] for more details about the simulations.

5.5 Results

5.5.1 Diffusion in pure water

Figure 5.4a shows a representative autocorrelation of Coumarin 343 diffusing in 8 nm gap with best fits of three different equations; for single diffusing species (eq: 5.1), two diffusing species (eq: 5.2) and one single diffusing species performing adsorption-desorption events (eq: 5.4). Clearly, the single diffusing species poorly describes our data. The equation is not fitting well (blue dashed line).

The fit parameters are listed in the table 5.1. Both, equation 5.2 (green dotted line) and equation 5.4 (red continuous line) fit equally well. This means that both pictures are equally possible as far as quality of fit is concerned. However, since we are using

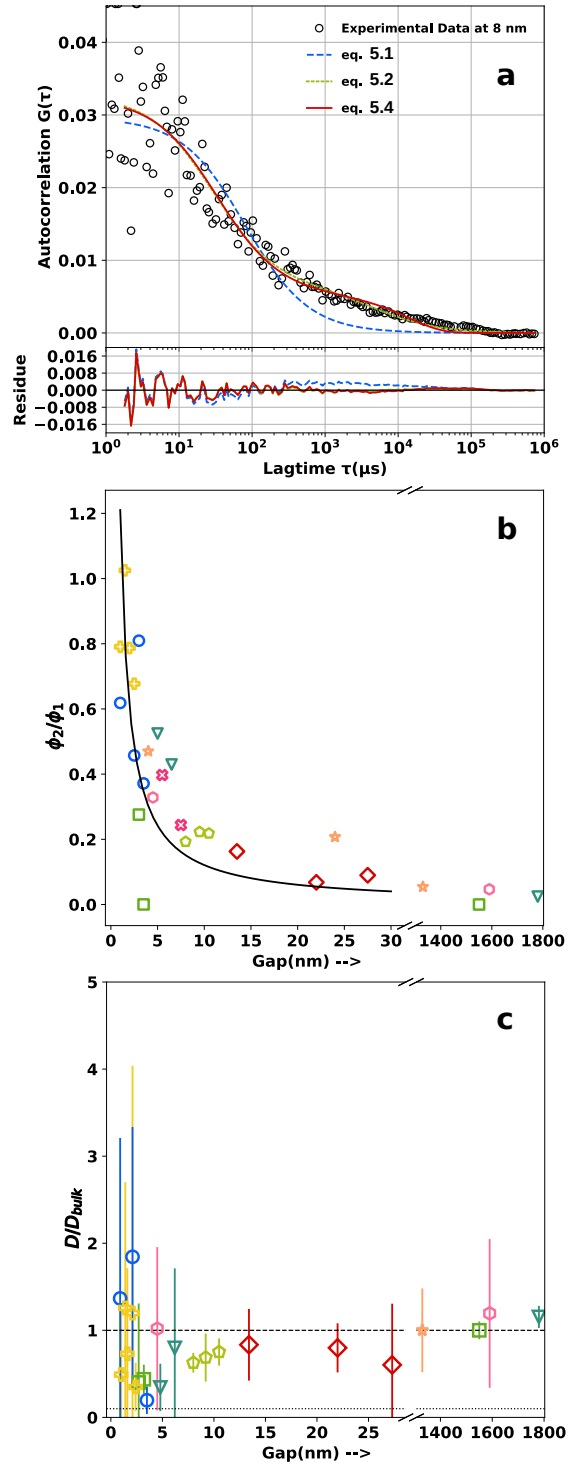


Figure 5.4: a) A representative curve for autocorrelation of fluorescence intensities from a dye diffusing in 8 nm gap with best fits using equation 1 (dashed blue line), equation 2 (dotted green line) and equation 4 (continuous red line). b) Ratio of weight factors of the adsorbing - desorbing fraction ϕ_2 to diffusing fraction ϕ_1 . Solid line is the fit to C_3/d , where C_3 is a positive constant and d is separation. c) D/D_{bulk} versus separation determined using equation 5.5, with the faster time scale τ_D

Parameters	Eq: 5.1	Eq: 5.2	Eq: 5.4
$1/ \langle N \rangle$	0.0296	0.0328	0.0207
τ_D or τ_{D1} (μs)	81	29	33
τ_{D2} (μs)	-	5037	-
$k(s^{-1})$	-	-	68.5
ϕ_1	-	0.81	1.312
ϕ_2	-	0.19	0.252
T^a	0.6	0.0001	0.0001
$t_t(\mu s)^a$	0.002	10	0.03
RMS residue	0.003702	0.003123	0.003150

Table 5.1: The parameters after fitting equation 5.1, 5.2 and 5.4 to a representative experimental auto-correlation curve in Figure 5.4a. The slow component time scale τ_{D2} varies from 500 μs to 5000 μs for different experiments.

^a The part of autocorrelation which gets affected by triplet state transitions are typically below $\sim 10\mu s$. Our data from confinement is very noisy below $\sim 10\mu s$. So the fit parameters values for T and t_t will not be reliable. This doesn't affect the fit values of other parameters.

a single fluorophore for these measurements, single diffusing species performing with adsorption-desorption events is more realistic model, provided the liquid under confinement is mostly homogeneous. In our experiments the confining walls are largely made of glass bearing negative charges. The large part of the tip is also made up of metal coating and may accumulate charges which are responsible for adsorption of negatively charged dye such as Coumarin. In this situation, the average number of molecules performing adsorption-desorption are same for different confinement gaps. This is because the area of interfaces in the observation volume from the tip and coverslip are the same for all the separation between coverslip and the tip. Thus, the number of adsorption sites accessed by the fluorescent molecules for a given concentration will be the same and number of molecules undergoing adsorption-desorption events; $\langle N_2 \rangle = C_1$, a positive constant. The number of molecules undergoing diffusion should increase linearly with the gap as the observation volume increases linearly with increase in separation. So, $\langle N_1 \rangle = \pi r^2 d c = C_2 d$, where “r” is the radius of observation volume, c is the concentration of the fluorescent molecule in the confinement region. This is likely to be different from the bulk concentration. $C_2 = \pi r^2 c$ is another positive constant. Now we can calculate the fraction of diffusing molecules $f = \langle N_1 \rangle / \langle N \rangle = \langle N_1 \rangle / (\langle N_1 \rangle + \langle N_2 \rangle)$. Substituting this “f”

in the expressions for ϕ_1 and ϕ_2 and taking the ratio ϕ_2/ϕ_1 , we can see that this ratio is inversely proportional to $\text{gap}(d)$ with a positive proportionality constant “ C_3 ”. And $C_3 = \frac{q_2^2 C_2}{q_1^2 C_1}$

$$\frac{\phi_2}{\phi_1} = \frac{C_3}{d} \quad (5.6)$$

Our data does follow this trend. Figure 5.4b shows plot of the ratio of the weight factors of adsorbed to the diffusing fraction ϕ_2/ϕ_1 from experiments at different tip-substrate separations. The values of ϕ_1 and ϕ_2 is obtained by fitting equation 5.4 to the autocorrelation data. Each colour or type of symbol represents data from experiments performed using different tips. The fit to C_3/d confirms that there are fluorophores homogeneously distributed over the confinement region except the ones adsorbed over the tip and substrate. The average residential time τ_D of the diffusing species can then be estimated by fitting equation 5.4 to all the data.

Using τ_D at different separations and τ_{bulk} (fit to data at separations ≥ 100 nm), we find D/D_{bulk} as mentioned in equation 5.5. Figure 5.4c shows D/D_{bulk} values with-respect to gap. Each colour or type of symbol represents data from experiments performed using different tips. The error values for this ratio are calculated from the standard deviation of the τ_D from the fitting procedure. We find that D/D_{bulk} fluctuates around the value 1 within 10 to 0.1. This means that the diffusion coefficient of Coumarin 343 in confined water, in gaps as low as 2 ± 2 nm, is of same order of magnitude to the it's bulk value.

In reference [127], we have reported development of the instrument with which we have later performed experiments to measure tracer diffusion under confinement. We had presented preliminary data, wherein the autocorrelations below $100 \mu s$ were noisy and excluded from fit procedures. This led to a single timescale over which intensities were correlated and we concluded that there is slow-down in the diffusion under confinement. We have improved the instrument and the experimental protocol to reduce the noise in the early delay times. As a result, the data is now fitted to equation 4. The present analysis also shows that the slow timescale is a result of adsorption-desorption of the dye molecules to the confining walls.

5.5.2 Diffusion in 1M salt solution

We further confirm our findings by mitigating the issue of fluorophore adsorption in a separate experiment. We performed experiments by adding 1M NaCl to the solution. Adding salt is known to reduce the charge mediated adsorptions by screening the local adsorption sites with counter-ions(ions of opposite charge compared to the adsorption site) [134]. We choose to add 1M NaCl as similar amount is found to enhance the probability of dynamic solidification of water [117]. Moreover, the Debye length at this concentration is 0.3 nm [71]. Representative autocorrelation curves for Coumarin 343 in confined 1M NaCl solution are shown in figure 5.5a and 5.6a for gaps 2.6 ± 2 , 4.8 ± 2 and 95 ± 2 nm. The equation for single diffusing species with no adsorption (eq. 5.1) fits well on all experimental autocorrelations. We deduce that adsorptions are negligible and the autocorrelation curve is mainly determined by diffusion. The τ_D values corresponding to these representative autocorrelations for 2.6 ± 2 , 4.8 ± 2 and 95 ± 2 nm were 46, 52, 39 μs respectively.

The τ_D values from the fit are used to calculate D/D_{bulk} . The D/D_{bulk} values are plotted against the confinement gap in figure 5.5b. The error value for each D/D_{bulk} is calculated from the standard deviation of τ_D from the fitting procedure. Similar to pure water, the D/D_{bulk} values of Coumarin 343 fluctuates around 1 between 10 and 0.1. So, the diffusion coefficient of Coumarin 343 in confined 1 M NaCl solution, for confinements as low as $\sim 2.6 \pm 2$ nm, is in the same order of magnitude of it's bulk value.

5.5.3 Monte Carlo to estimate Diffusion

The data from NaCl solution are further analysed with the Monte Carlo fitting procedure. A typical simulation fit to the autocorrelation from nanoconfined NaCl solution is given in 5.6a. This data is for Coumarin 343 diffusing in confinement gap of 2.6 ± 2 nm having 1 M NaCl solution. The diffusion coefficient corresponding to the auto-correlation data from the experiments is estimated from this fitting. For this particular data simulated autocorrelation for $D=550 \text{ nm}^2/\mu s$ gave the best fit. The figure 5.6b shows the fitted diffusion coefficient for different separations in two different experiments with different tips. The error bars for each diffusion coefficient is the minimum change in diffusion coefficient around the fitted value such that a significant

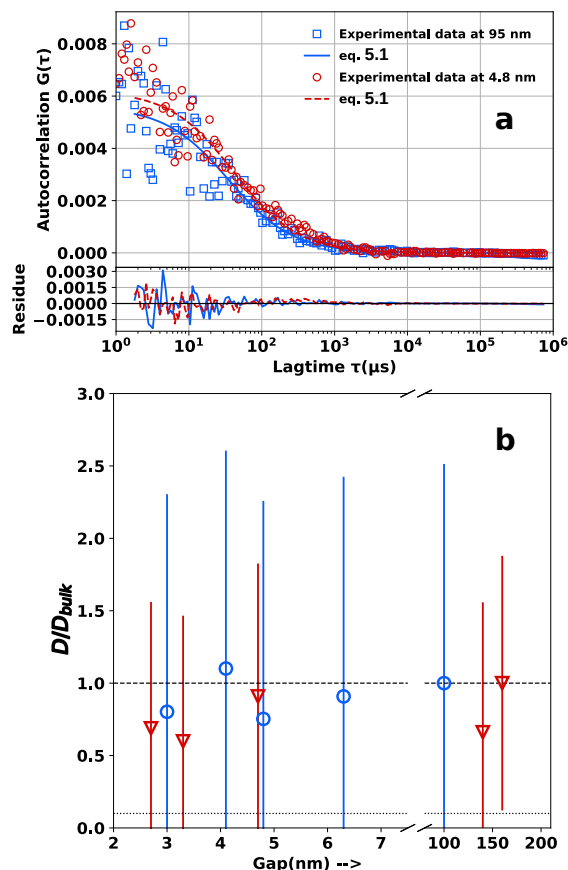


Figure 5.5: Representative autocorrelation curves of Coumarin 343 in 1 M NaCl at separations of 5 ± 2 nm and 95 ± 2 nm. The curves nearly overlap and do not have a component representing the adsorption-desorption kinetics of the fluorophore to the glass surfaces. The one component description by equation (2) fits the data well at both separations. The overlap of both autocorrelations indicate that there is only fast moving bulk-like component. b) D/D_{bulk} vs separation with 1 M NaCl. The horizontal lines are reference lines for $D/D_{\text{bulk}} = 1$ and 0.1 respectively.

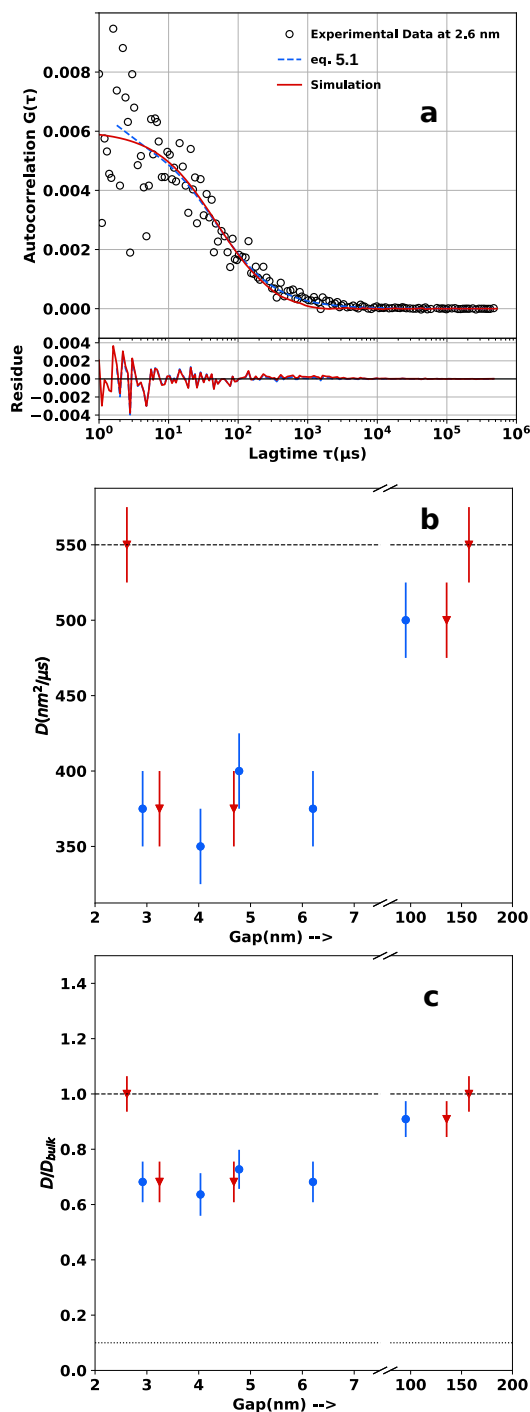


Figure 5.6: a) The fit of simulated data (red continuous line) to autocorrelation curve (black symbols) for confinement with tip-substrate separation of 2.6 ± 2 nm. The salt concentration is 1 M. Monte Carlo simulations are run with different diffusion coefficients till simulations and experiments fit well. The fit yields a diffusion coefficient of $550 \pm 25 \text{ nm}^2/\mu s$ b) Diffusion coefficient D versus separation. D is estimated by fitting simulations to the experiments, c) D/D_{bulk} versus separation. The horizontal lines are reference lines for $D/D_{bulk} = 1$ and 0.1 respectively.

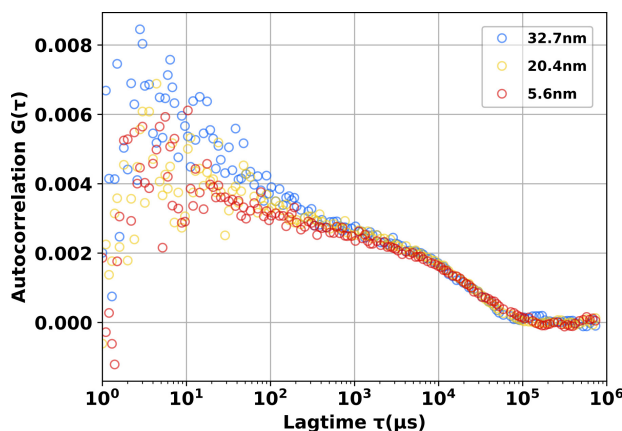


Figure 5.7: Representative autocorrelation curves for Rhodamine 6G diffusing in MilliQ water confined in different gaps.

change ($> 10^{-6}$) in root mean square error is obtained. We also find the D/D_{bulk} values. The figure 5.6c shows the D/D_{bulk} with respect to confinement gap. Compared to the D/D_{bulk} values from the equation fitting, the values from simulation fitting is more reliable. This is because the simulation procedure captures the specifics of the instrument. With this set of data we can find the order of magnitude of D/D_{bulk} . For finding the trend of D/D_{bulk} with separation more data is required.

5.5.4 Measurements with Rhodamine 6G

Rhodamine 6G is a positive fluorescent molecule in water ([9-(2-ethoxycarbonylphenyl)-6-(ethylamino)-2,7-dimethylxanthen-3-ylidene]-ethylazanium;chloride). The figure 5.7 shows a few representative autocorrelation curves for Rhodamine 6G. Strong adsorption contribution is present in autocorrelation curves. Even for large separation, this is the case. We found that even with 1M NaCl, adsorption is present.

5.5.5 Measurements with Alexa Fluor 568

The fluorescent molecule Alexa Fluor 568(NHS ester) is negatively charged in water ([13-[2-carboxy-4-(2,5-dioxopyrrolidin-1-yl)oxycarbonylphenyl]-7,7,19,19-tetramethyl-17-(sulfomethyl)-2-oxa-20-aza-6-azoniapentacyclo[12.8.0.0^{3,12}.0^{5,10}.0^{16,21}]docosa-1(14),3,5,8,10,12,15,17,21-nonaen-9-yl]methanesulfonate). The figure 5.8 shows the representative autocorrelations obtained with Alexa Fluor 568. The table 5.2 shows the fit parameters for these curves. The results from Alexa Fluor 568 are similar to

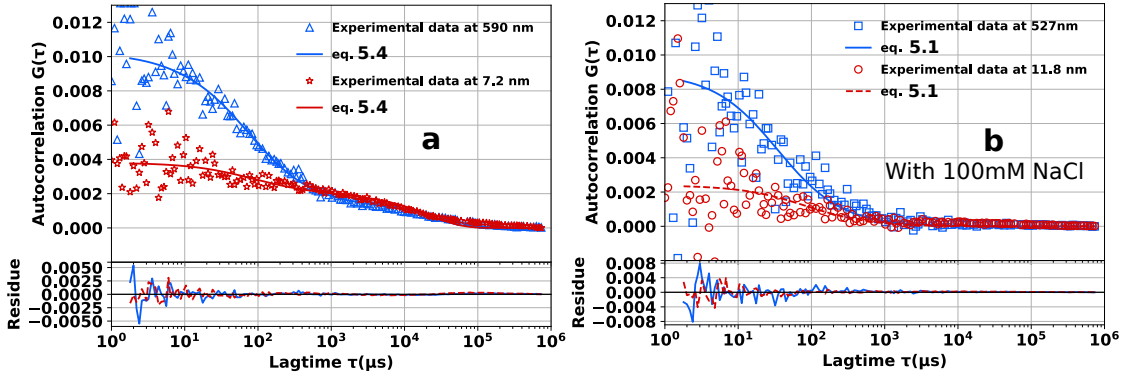


Figure 5.8: Representative autocorrelation curves for Alexa Fluor 568. a) data for MilliQ water. b) data for 100 mM NaCl. The fit parameters are listed in the table 5.2.

Coumarin 343. The adsorption of the dye molecules over the confining surfaces are avoided with the addition of NaCl. Moreover, the diffusion time scale in nanoconfined water (~ 10 nm) is in same order of its bulk value.

	In Water		In 100mM NaCl	
	7.2 nm	590 nm	11.8 nm	527 nm
τ_D or τ_{D1} (μs)	85	73	83.8	34
$k(s^{-1})$	44	24	-	-
D/D_{bulk}	0.86	1	0.41	1
$1/ \langle N \rangle$	0.001822	0.009192	0.002382	0.008904
ϕ_1	1.015	0.931	-	-
ϕ_1	1.074	0.142	-	-
T	0.00027	0.034	~ 0	~ 0
$t_t(\mu s)$	0.01	5	5	0.01
RMS residue	0.000579	0.000863	0.000936	0.00152

Table 5.2: The parameters after fitting equations 5.4 and 5.1 to the auto-correlation curves in figure 5.8a and b.

5.6 Discussion

The autocorrelation of fluorescence intensity fluctuations revealed that there are two timescales over which intensity is correlated for the Coumarin 343 molecules diffusing in nanoconfined water. It is found that fast time scale corresponds to the average

time required to cross the width of observation volume, provided the molecule has diffusion coefficient similar to its bulk value. The slower time scale disappeared in measurements with 1 M NaCl. Adding NaCl is known to avoid adsorption of solute molecules over substrates due to screening of electrostatic interactions. The Coumarin 343 molecule (11-oxo-2,3,5,6,7,11-Hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxylic acid; $C_{16}H_{15}NO_4$) has the carboxylic-acid group. It gains negative charge in pH ~ 7 . One of the confining surfaces, the cover-slip is cleaned with UV-Ozone before the experiments which enriches the surface with hydroxyl-terminations. This cover-slip in pH ~ 7 water gets negative charges [70]. The second confining surface, the tip-surface may accumulate unknown static charges owing to metal coating. By adding NaCl to pure water, we introduce co-ions and counter-ions. The counter-ions get attracted to the local charge centres on the surfaces and form an Electrical Double Layer (EDL). The Debye length associated for this EDL in 1 M NaCl is ~ 0.3 nm [71]. Thus ~ 0.3 nm EDL is present over both of the confining surfaces. The Coumarin 343 diffusing in this confined gap experiences highly shielded electrostatic potential due to EDL from the surfaces. This helps in avoiding the adsorption of Coumarin 343 over the confining surfaces. The disappearance of slower time scale in autocorrelation for 1 M NaCl confirmed that adsorption-desorption process is the reason for slower time scale in nanoconfined water without salt.

Furthermore, the ratio of weight factors of the fraction undergoing adsorption-desorption process and the fraction of molecules executing diffusion (ϕ_2/ϕ_1) show a trend with respect to confinement gap. This is consistent with our physical picture that number of molecules undergoing diffusion will increase linearly as the gap is increased while the number of molecules undergoing adsorption-desorption will be fixed. By fitting such a model, we could extract the residential time scale due to diffusion for various separations. Further, we could estimate the ratio of diffusion coefficient with its bulk value. We find that the diffusion coefficient of the tracer molecule is similar to the bulk value even for confinements as small as 2 ± 2 nm. Similar behaviour is also seen in 1 M NaCl solution confined till 2.6 ± 2 nm. This behaviour is independent of analysis method used to extract the diffusion coefficient. The estimate of diffusion coefficients at all separations using Monte Carlo simulation based fitting procedure, yields the same result.

In figure 5.1, we see that the y-intercept of autocorrelation from different gap are

different. From equation 5.4 we realise that the intercept is given by $(\phi_1 + \phi_2)/(\langle N \rangle (1 - T))$. And ϕ_1 and ϕ_2 are defined according to equation 3. This behaviour of intercept in figure 5.1 is not simple to explain because of the nonlinear dependence of ϕ_1 or ϕ_2 with the fluorescence yield and fraction of diffusing molecules. With a systematic study of this variation of intercept with gap, one could estimate the fluorescence properties of adsorbed and unadsorbed molecules. This is outside the scope of this thesis. Secondly, the estimate of uncertainty in the two time scales measured from autocorrelation curve is not possible by performing a statistical analysis presented in Wohland et al. [113] This requires the experiments to be performed multiple number of times at the same separation and experimental conditions. Such analysis is possible in a typical FCS experiment, wherein the observation volume is in the bulk of liquid and any number repetitions under the same conditions are possible. Although, the tip-sample separation can be held fixed over several seconds or minutes in our experiments, the same is not true about the objective and the cover-slip. Thus, it is difficult to estimate uncertainty in diffusion coefficient under confinement using a statistical analysis used in a typical FCS experiments. The errors in diffusion coefficient are determined through fitting procedures and Monte Carlo method.

We have performed measurement of diffusion coefficient for separation as small as ~ 2 nm. Below this separations the molecular effects, such as restriction in dye orientation and rotational diffusion are expected to affect the diffusion of the dye. The diffusion coefficient retaining a bulk-like value at 2 nm could be owing to the limitations in determining the gap size accurately. From our data and analysis we can conclude that diffusion is certainly bulk-like at separations of 5 nm and above.

The interfacial water has unique properties compared to bulk water. Yet, it is not clear under what length scales of confinements the interfacial properties become prominent. This may even depend on the physical property being measured. For instance, the out-of-plane dielectric coefficient of nanoconfined water over hydrophobic confining surfaces was measured to be ~ 2 , which is much smaller than the bulk value ~ 80 [116]. This so called electrically dead nanoconfined water is thought to be due to the reduction of rotational freedom of water molecules near interface [116, 135]. The capacitance measurements revealed that a gap size of more than 100 nm is required for water to have a bulk-like dielectric coefficient [116]. However, water's behavior when one mea-

sures the translational diffusion seems different. There are reports of many orders larger viscosity for interfacial water [24, 36]. So it is expected to exhibit a slow-down in self-diffusion as well as reduced tracer diffusion in water. The self-diffusion of water is probed in ensemble measurements using NMR or QENS [19, 54, 55, 136]. These measurements find that, at room temperature the translational diffusion of water is similar to the bulk value within an order for confinements as small as ~ 2 nm. In our study, the figure 5.4c, 5.5b, and figure 5.6c shows that the diffusion coefficient of Coumarin 343 in nanoconfined water for gaps as small as ~ 5 nm is within the same order of magnitude of the bulk value. This conclusion is consistent with NMR and QENS studies.

The majority of rheological measurements with AFM or SFA showed the large scale deviation of viscosity only below 1 nm of hydrophilic confinements [24, 36, 38]. Above 2 nm the average viscosity or dissipation measured were similar to the bulk values. Thus the AFM and SFA measurements also implies an average bulk-like diffusion for nanoconfined water in gaps more than 2 nm. Moreover, the orders of magnitude larger viscosity of water is seen only when the separation is multiple of the size of water molecule. The molecular dynamics simulations also agrees with our conclusion. In molecular dynamics simulation the in-plane translational diffusion timescale for water in 2.44 and 1.65 nm gaps are found to be similar to bulk value [62].

Like dielectric coefficient of interfacial water, the diffusion of tracer molecules can be anisotropic [137]. We measure the diffusion of fluorescent tracer molecules on an average moving parallel to the confining surfaces. The local out-of-plane diffusion might be different from what we measured.

There are few reports of measurement of translational diffusion of tracer dye in water confined at nanoscale. The measurement with varying gap size below 10 nm is not routine. For sub-micrometer confinements many particle tracking measurements are done using colloidal tracers [16, 17]. These measurements are mostly consistent with fluid dynamic description of motion of a particle near interface. The smallest possible confinement is limited by the size of the colloidal tracer particle. The choice of small fluorescent molecules as tracer allows the confinement to be as small as 1-10 nm. Zhong et al. measured diffusion of Rhodamine B in ethanol taken inside nanochannels of height 8 nm and found a reduction in diffusion coefficient by 2 orders [15]. Kievsky et al. measured diffusion of Rhodamine 6G in water in porous material with pore diameter

of 2.9 nm. They found an eight order reduction in diffusion coefficient [138]. Santo et al. measured diffusion of Rhodamine 6G in water in nanochannels of height 30, 20 and 10 nm. They found the diffusion coefficient to be similar to the bulk value in 30 and 20 nm channels and a 25% slow-down is seen in 10 nm channel [14]. Grattoni et al. measured diffusion of Rhodamine B in water in nanochannels having height 200 and 1 μm , as well as 13 and 5.7 nm [139]. They found a reduction of diffusion coefficient as the channel height is reduced. The lowest diffusion coefficient they measured (in 5.7 nm channel) is still within an order of the bulk value. Our measurements on diffusion of Coumarin 343 agrees well with Santo et al. and Grattoni et al.

The reasons for the drastic slow-down in the studies by Zhong et al. and Kievsky et al. is not clear. Usually this slow-down is attributed to the geometric confinement effects, surface interactions and possible viscosity difference of nanoconfined water. It is clear from our findings that the diffusion appears slowed down due to adsorption-desorption. Then the apparent diffusion coefficient calculated from the slower residential timescale due to adsorption will be few order less even for our data [127]. It is possible that the slow-down reported by Zhong et al. and Kievsky et al. is due to adsorption-desorption interactions.

The apparent diffusion coefficient which includes adsorption-desorption interactions can vary with separation even though the diffusion coefficient and timescale for adsorption-desorption interactions ($1/k$) are same for all separations. This is because the fraction of diffusing population changes with separations as we had seen in the trend of ϕ_2/ϕ_1 with gap. The time scale for this apparent diffusion can be estimated from the residential time scale for diffusing molecules and the residential time scale of the molecules undergoing adsorption-desorption. The timescales τ_{D1} and τ_{D2} from equation 5.2 can be interpreted as these timescales respectively. If we can extract out the values of f , the fraction of diffusing molecules, from ϕ_1 or ϕ_2 , the residential timescale of apparent diffusion coefficient (τ_{apparent}) can be estimated. It is the weighted average of τ_{D1} and τ_{D2} , given by $f\tau_{D1} + (1 - f)\tau_{D2}$. For estimating f from ϕ_1 or ϕ_2 one needs to know the fluorescence yields of the fluorescent molecule away (q_1) and near to the surfaces (q_2) independently. Qualitatively, we can see that for large separations we have f approaching 1 and τ_{apparent} will approach to bulk-like diffusion timescale, τ_{D1} . And for small separations, τ_{apparent} approaches the slow residential time scale due to adsorption-desorption.

For instance, if we consider τ_{D2} value shown in table 5.1 for a sub-10 nm confinement, it is two orders larger than the bulk diffusion timescale. Thus we expect two order slower apparent diffusion when adsorption-desorption interaction is included. Our goal in this paper is to understand the dynamics in the confinement which is not driven by the surface effects but is a result of confinement itself. We conclusively show that there is no alteration in diffusion coefficient due to confinement alone. The slow-down of 2 orders seen in our experiments in the absence of salt, which dominates the measurement of apparent diffusion, can actually be attributed to the adsorption effects.

In order to compare our measurements better with existing literature we also performed measurements with Rhodamine 6G. The cationic Rhodamine 6G is known to adsorb over glass surfaces [140]. This adsorption is due to electrostatic and hydrophobic interactions [141]. We found that, while we could avoid the mild adsorptions of Coumarin 343 by adding NaCl, Rhodamine 6G have strong adsorption even in 1 M NaCl. The autocorrelation we measured for Rhodamine 6G have large adsorption-desorption contribution even for 1 μm separations. As the contribution of diffusing species to the autocorrelation is less dominant, the average residential time estimated from fitting procedure is unreliable. This unreliability in fitting parameters from the adsorption-desorption FCS equation when adsorption is dominant has been pointed out before [77]. In the work of Kievsky et al., manipulating the pH or using organic solvent did not change the eight order reduction in diffusion coefficient. We also faced similar difficulties with Rhodamine 6G. However, the measurements performed using Alexa Fluor 568 were successful and supports the conclusions drawn using Coumarin 343. We observe that the choice of fluorescent molecules and interfaces matters in these type of experiments.

The length scale below 100 nm is particularly important in nanofluidics [119–121]. Nanofluidics is applied in fields of iontronics [122, 123], sequencing of biopolymers [125, 126], and energy harvesting [124]. In this field, the water at nanoscale is considered to have bulk-like viscosity in channels above 1 nm [120]. Our conclusions, which are arrived at from independent diffusion measurements, support this treatment of water at nanoscale. Usage of fluorescence correlation based measurements like Fluorescence Correlation Spectroscopy(FCS) is rare in nanofluidics. As FCS has been used to study reaction kinetics [131, 132], flow and diffusion measurements [131, 132], extending it to

nanofluidics will open-up countless new possibilities. Our study shows how to interpret the timescales in fluorescence autocorrelation. This is beneficial for fluorescence correlation based measurements in nanofluidics and close to an interfaces.

5.7 Conclusion

In conclusion, we measured diffusion of a tracer dye in water confined to nanoscale, where the separation between the confining walls is as small as ~ 5 nm. We found that the diffusion coefficient of the tracer dye remains unchanged for such small gaps. It indicates that viscosity of nanoconfined water is bulk-like for separations ~ 5 nm. These conclusions are consistent with molecular dynamics simulations of water confined to similar gaps and many reports of self-diffusion of nanoconfined water using quasi-elastic neutron scattering experiments. Moreover, we found that adsorption-desorption of fluorescent molecules can present a slow dynamics which might be the reason of previous reports of slow-down of diffusion in nanoconfined water.

Chapter 6

Conclusions and Future Outlook

This chapter summarizes the result of this thesis work. It further discusses the possible extensions of this work

6.1 Conclusions

It is essential to understand the properties and behavior of water in nanometer-scale gaps. In one nanometer gap, we can fit three layers of water molecules one after the other. The layer near the bounding surfaces of this gap have properties dictated by the interactions at the interface and geometrical constraints. Many intriguing properties of nanoconfined water have been observed in previous studies. This includes the fast flow of water in carbon nanotubes [4], water permeation in helium-tight membranes [5], and suppression of water freezing [6]. Along with studying these properties, one fundamental question that needs to be answered is how the dissipative nature of nanoconfined water is different from bulk water. The dissipative nature of water is studied with multiple measurements. The AFM and SFA-based studies estimated nanoconfined water's apparent viscosity [24, 36, 37] and relaxation times [24, 25]. The neutron diffraction studies probed the self-diffusion of water in nano-pores [19, 55]. Most of these studies found few orders larger viscosity for the surface-bound layers [24, 36]. In contrast, few studies didn't see any drastic slowdown [37]. More independent measurements are needed to clarify the nature of the dissipative properties. Moreover, it is not apparent how a solute molecule will diffuse in these bound layers or in its vicinity. Understanding the dynamics of solute particles in nanoconfined water is essential in the emerging

field of nanofluidics. In this context, we made an instrument to measure the translational diffusion of a fluorescent molecule in nanoconfined water.

The newly developed instrument uses a special optical fiber probe immersed in water taken over a coverslip. We can position the probe a few nanometers above the coverslip with the instrument. The water present in the separation between the probe and coverslip is the nanoconfined water. We can measure and control the separation using a millimeter-sized quartz tuning fork sensor and voltages given to the piezo, which moves the probe. The same fiber probe is used to illuminate an observational volume in the confined water. We add a trace amount (a few 100 nM) of fluorescent molecules in the water. Then a few fluorescent molecules diffuse through the illumination in the confined water. These molecules emit fluorescent photons. These photons are collected with a confocal setup. The number of fluorescent molecules in the observational volume fluctuates due to diffusion. Thus, a proportional fluctuation is observed in the fluorescent intensity. The autocorrelation of this intensity fluctuation decays with a timescale. This timescale corresponds to the average time the fluorescent molecule stays in the observational volume, the residential time. And it can be used to estimate the diffusion coefficient of the fluorescent molecule and changes in the diffusion coefficient as a function of confinement separation. We successfully developed an instrument that can do the described measurements.

The measurement is similar to the Fluorescent Correlation Spectroscopy(FCS) measurements. We can use the expressions derived for FCS to fit the autocorrelation from our instrument and get a value for the time scale. This time scale will vary according to the changes in the diffusion coefficient with confinement separations. However, we cannot use this time scale to estimate the diffusion coefficient. The expression for FCS doesn't incorporate the differences with our instrument. So we developed a Monte Carlo-based fitting procedure. The procedure extracts the apparent diffusion coefficient values from our measurements.

We used the instrument to measure the diffusion of different fluorescent molecules. For the fluorescent molecule Coumarin 343, we observe that the fluorescence intensities correlate over a fast($\sim 30 \mu\text{s}$) and slow ($\sim 1000 \mu\text{s}$) time components. The slow timescale is due to the adsorption of fluorophores to the confining walls, and it disappears in the presence of 1 M salt. The fast component is attributed to the diffusion of

dye molecules in the gap and is found to be bulk-like for sub-10 nm separations and indicates that viscosity of water under confinement remains unaltered up to confinement gap as small as ~ 5 nm. Our findings contradict some of the recent diffusion measurements under nanoconfinement; however, they are consistent with many estimates of self-diffusion using molecular dynamics simulations and measurements using neutron scattering experiments. The molecule Alexa Fluor 568 also showed the same results as Coumarin 343. At the same time, the addition of salt doesn't seem to remove the adsorption of the molecule Rhodamine 6G. The dominant adsorption contribution in autocorrelation made analysis difficult for Rhodamine 6G.

In summary, the main results from the thesis can be listed as the following:

- A new instrument is made which can measure translational diffusion of fluorescent tracer molecules in confined liquids
- A Monte-Carlo fitting procedure is developed to estimate the diffusion coefficient of fluorescent molecules in confined liquids from the fluorescent intensity autocorrelations obtained from the instrument
- Near the interface, motion of fluorescent molecule includes adsorption events and diffusion
- Adding NaCl avoided adsorption events in Coumarin 343 and Alexa Fluor 568 but not for Rhodamine 6G
- The confinement itself does not alter the diffusion coefficient of fluorescent molecules for separations as small as ~ 5 nm, indicating that water viscosity is bulk-like at this separation.

6.2 Future Outlook

The instrument we build can be used for many other studies. Moreover, we can consider a few improvements to the instrument.

- **More confined liquid studies.**

Currently, all experiments are done on pure deionized water. We can add viscogens like sucrose to manipulate the viscosity of the resulting solutions. We can check how

the diffusion coefficient changes with different viscogen concentrations for a given confinement separation. Moreover, we can see the variation in diffusion coefficient with a change in separation. Instead of water-based solutions, we can also check the fluorophore diffusion in other solvents in confinement.

- **Fluorescence properties near to an interface.**

The fluorescence properties like fluorescence yield depend on the fluorescent molecules' local environment. We can study how fluorescent properties change near the glass/silica interface using this instrument. We have seen that the intercept of autocorrelation depends on the fluorescence yield. We can measure the variations in the intercept with separation and deduce the change in the fluorescence yield near the confining surfaces.

- **Improvements in the instrument.**

Currently, the instrument doesn't have any means to adjust the angle between the tip and coverslip. As discussed in the previous chapter, we expect the angle between the normal to the coverslip and tip axis to be less than 0.3° . This angle restricts the least separation possible between the coverslip and the center of the tip. This limitation can be avoided by adjusting the angle between the tip and coverslip. One modification we can introduce to make the angle adjustments is as follows. The vertical assembly (figure 3.15) can be split into two halves: one part will be with tip and tip-actuation mechanisms and the other with coverslip and other optical components in the vertical assembly. The top half with the tip can be placed over the bottom half with three leveling screws as the legs. One possible implementation is illustrated in the figure 6.1

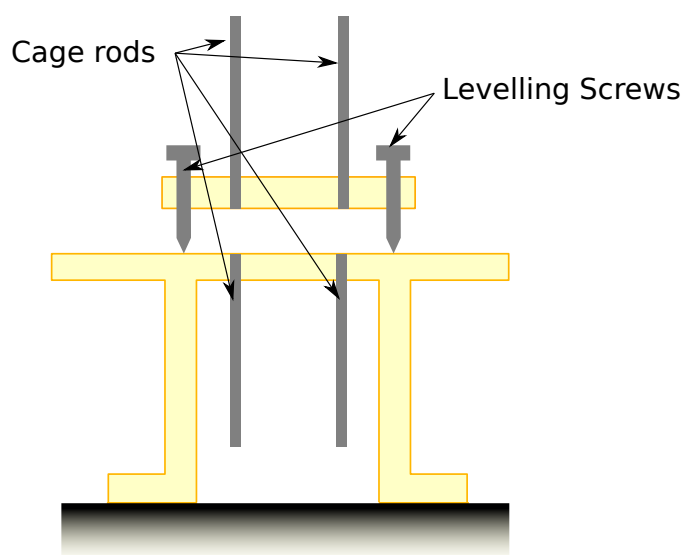


Figure 6.1: Modification in the instrument to adjust the angle between the tip and coverslip. The tip will be in the cage assembly along the cage rods of the top half. The coverslip with liquid-cell will be on the bottom platform. The cage rods extending downwards from the bottom platform will hold the objective lens and other optics of the vertical assembly. The top half has the adjusting screws to change the angle.

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