Robust Microfluidic Integration for shallow channel aperture Optical Tweezer

by

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B.E, Visvesvaraya Technological University, 2011

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Abstract

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The main objective of this thesis is to present a simple and robust hands-on technology for the fabrication of a microfluidic chip in a laboratory. The purpose of this new technology is to replace the existing PDMS based microfluidic chip used for optical trapping of diverse single nano particles. It also lists the different fabrication methods attempted and the successful integration of this chip to the optical trap system which is used to study binding at the single molecular level.

Microfluidics is a quickly growing field which deals with manipulating the fluids in channels whose dimensions are few tens of micrometers. Its potential has a major impact on fields like chemical analysis and synthesis techniques, biological analysis and separation techniques, and optics and information technology. One of the main application of these microfluidic chips is in optofluidics, which is the emerging field of integrated photonics with fluidics. This provides freedom to both fields and permits the realization of optical and fluidic property. It requires small volumes of fluids and connections and eventually performs better than conventional methods of robotic fluid handling.

Here, the microfluidic chip is targeted for optical trapping with double nano-hole aperture to trap a single protein. The double nanoholes integrated with this microfluidic chip show
that stable trapping can be achieved below flow rates of few µL/min. This has provided many possibilities of co-trapping of proteins and study their interactions.
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Dedication

To my family
Chapter 1

Introduction

Microfluidics is an enabling platform technology that allows automation and multiplexing of laboratory equipment, drug screening technologies, and in vitro diagnostic devices. Over the past two decades, several ventures have emerged to commercialize microfluidic technologies. Initially, these devices were envisioned to be used in biological analyses and chemical syntheses so that a range of substances could be prepared and analyzed at low volumes in order to replace manual processing and bulky benchtop equipment. Pioneering companies have argued that the efficient consumption of reagents, high-throughput analyses, miniaturization of components, and the ability of microfluidic devices to be produced from low-cost materials will reduce costs as compared to conventional benchtop equipment [1].

1.1 What is a microfluidic chip?

A microfluidic chip is a set of micro-channels etched or molded into a material (glass, silicon or polymer such as Polydimethylsiloxane). The micro-channels forming the microfluidic chip are connected together in order to achieve desired functions (mix, pump, sort, control bio-chemical environment). This network of micro-channels trapped in the microfluidic chip is connected to the outside by inputs and outputs pierced through the chip, as an interface between the macro- and micro-world. It is through these holes that the liquids (or gas) are injected and removed from the microfluidic chip (through tubing, syringe adapters or even simple holes in the chip) with external active systems.
(pressure controller, push-syringe or peristatic pump) or passive ways (e.g. hydrostatic pressure).

A microfluidic device can be identified by the fact that it has one or more channels with at least one dimension less than 1 mm. Common fluids used in microfluidic devices include whole blood samples, bacterial cell suspensions, protein or antibody solutions and various buffers. Microfluidic devices can be used to obtain a variety of interesting measurements including molecular diffusion coefficients [2], fluid viscosity [3], pH [4][5], chemical binding coefficients [6] and enzyme reaction kinetics [7][8][9]. Other applications for microfluidic devices include capillary electrophoresis [10], isoelectric focusing [5][11][12], immunoassays [13][14][15][16], flow cytometry [17], sample injection of proteins for analysis via mass spectrometry [18][19][20], PCR amplification [21][22][23], DNA analysis [24][25][26], cell manipulation [27], cell separation [28], cell patterning [29][30] and chemical gradient formation [31][32]. Many of these applications have utility for clinical diagnostics [33][34].

The use of microfluidic devices to conduct biomedical research and create clinically useful technologies has a number of significant advantages. First, because the volume of fluids within these channels is very small, usually several Nano-liters, the amount of reagents and analytes used is quite small. This is especially significant for expensive reagents. The fabrication techniques used to construct microfluidic devices, discussed more in depth in Chapter 3, are relatively inexpensive and are amenable to highly elaborate, multiplexed devices and also to mass production. In a manner similar to that for microelectronics, microfluidic technologies enable the fabrication of highly integrated devices for performing several different functions on the same substrate chip. One of the
long term goals in the field of microfluidics is to create integrated, portable clinical diagnostic devices for home and bedside use, thereby eliminating time consuming laboratory analysis procedures.

The current advancement in microfluidics technology allows us to fabricate microfluidic chips with the use of diverse materials such as polymers (e.g. PDMS), ceramics (e.g. glass), semi-conductors (e.g. silicon) and metal. This is currently possible because of the development of specific processes such as deposition and electrodeposition, etching, bonding, injection molding, embossing and soft lithography (especially with PDMS). Access to these materials makes it possible to design microfluidic chips with new features like specific optical characteristics, biological or chemical compatibility, faster prototyping or lower production costs, possibility of electro sensing, etc. The final choice depends on the aimed application.

Microfluidics has many applications. Some of the main ones are as listed below:

- In biomedical field, with the laboratories on a chip because they allow the integration of many medical tests on a single chip.
- In cell biology research because micro-channels have the same characteristic size as biological cells. Thus, microfluidic chips allow easy manipulations of single cells and rapid drugs change.
- In protein crystallization because microfluidic devices allow the generation on a single chip of a large number of crystallization conditions (temperature, pH, humidity)

And also many other areas: drug screening, glucose tests, chemical micro-reactor, electrochemistry, microprocessor cooling (fair return) or micro fuel cells.
1.2 History of Microfluidics

Over the 80’s, the use of silicon etching procedures, developed for microelectronics industry, allowed the manufacture of the first device containing mechanical micro-elements integrated on a silicon wafer. These new types of devices called MEMS (Micro Electro Mechanical Systems) gave rise to industrial applications, particularly in the field of pressure sensors and printer heads.

In the 90’s, many researchers investigated the applications of MEMS in biology, chemistry and biomedical fields. These applications needed to control the movement of liquids in micro-channels and have significantly contributed to the development of microfluidics. A major research effort was made to develop laboratories on a chip to enable the integration of almost all the processes required for complete biological, chemical and biomedical protocols on a single microfluidic chip. At that time, the majority of microfluidic devices were still made of silicon or glass, and thus, required the heavy infrastructure of microelectronics industry.

Starting the 2000’s, technologies based on molding micro-channels in polymers such as PDMS experienced strong growth. Cost reduction and production time decrease of these devices enabled a large number of laboratories to conduct researches in microfluidics [35].

1.3 Organization of This Thesis

This thesis focuses mainly on the fabrication of a microfluidic chip which successfully replaces the existing PDMS chip for aperture based optical trapping.
Chapter 2 is an overview of microfluidics basic principles, existing applications and common materials used for chip fabrication and highlights the disadvantages of these materials.

Chapter 3 gives a detailed summary of all the different materials experimented to replace a PDMS channel.

Chapter 4 presents different methods tried to integrate the new channel with a double nano-hole containing gold sample for optical trapping.

Chapter 5 concludes the thesis as well as making the suggestion for some possible future research directions.
2 Chapter 2

Microfluidics Overview

The manipulation of fluid at a micro scale often flowing through channels less than 1000µm is known as microfluidics. This field mainly focuses on the biochemical and medical applications. The microfluidic chip does have a smaller footprint compared to a giant machine in the lab, but the advantage is that it uses very less quantity of the fluids. To explain in lay man terms, why would one make a whole pot of curry just to dump it, on the other hand where one can make it as little as required and still devour it? Hence this is gainful that it uses less quantity of costly chemicals and reagents and also attractive due to the physics happening at such a small scale because matter does behave differently, most evidently in the way fluids flow.

2.1 Basics of Microfluidics

There are two types of microfluidic systems, continuous micro channel based flows and discrete droplet based manipulation. Here we mainly focus on the continuous flow based micro channels where the main transport mechanism or delivery of the fluid to the site of interest is either by pressure or electrokinetics. In this thesis, we concentrate on the pressure driven systems as it is the most robust technique, with minimum external infrastructure, having very little dependence on the fluid and surface properties. To actuate the fluid in a microchannel directly, off chip pneumatics or syringe pumps are used and to manipulate the flow locally, on chip valves are used. The main factors that affect the fluid flow in a small channel are discussed below.
2.1.1 Laminar versus turbulent flow

The flow of a fluid through a microfluidic channel can be characterized by the Reynolds number. The Reynolds number (Re) is a dimensionless quantity that describes the ratio of inertial to viscous forces in a fluid. Re is proportional to the characteristic velocity of the fluid and the length scale of the system; it is inversely proportional to the fluid viscosity. High Re (2,000) fluids have flow profiles that increasingly mix stochastically (turbulent flow). For microfluidic systems, Re is almost always in the laminar flow regime, allowing for highly predictable fluid dynamics [36].

Reynolds number is defined as

$$Re = \frac{L V_{avg} \rho}{\mu}$$

Where $L$ is the most relevant length scale, $\mu$ is the viscosity, $\rho$ is the fluid density, and $V_{avg}$ is the average velocity of the flow. For many microchannels, $L$ is equal to $4A/P$ where $A$ is the cross sectional area of the channel and $P$ is the wetted perimeter of the channel. Due to the small dimensions of microchannels, the Re is usually much less than 100, often less than 1.0. In this Reynolds number regime, flow is completely laminar and...
no turbulence occurs. The transition to turbulent flow generally occurs in the range of Reynolds number 2000. Laminar flow provides a means by which molecules can be transported in a relatively predictable manner through microchannels. Note, however, that even at Reynolds numbers below 100, it is possible to have momentum-based phenomena such as flow separation. Within the limit of the Reynold’s number, the Stokes’ equations are as follows:

\[ \nabla \cdot \mathbf{v} = 0 \]  
\[ 0 = -\nabla p + \eta \nabla^2 \mathbf{v} \]

Where \( \mathbf{v} \) is the velocity and \( p \) is the fluid pressure.

![Figure 2 Schematic diagram of two dimensional pressure driven flow through a microchannel](image)

The flow velocity in a pressure driven system can be calculated using the below equation with general assumptions. Figure 2 shows a channel with \( x \gg y \), with pressure applied along the \( x \) direction and the channel wide enough such that the sidewalls do not affect the flow profile. Considering the channel height as \( 2b \) and the fluid does not slip at the walls, the solutions to 1.1 is that for the Poiseuille flow, as given below:
\[ v_x = -\frac{1}{2\eta} \left( \frac{dp}{dx} \right) (b^2 - y^2) \]  \hspace{1cm} (1.3)

Where \( v_x \) is the fluid velocity in the \( x \) direction at position \( y \). Here \( dp/dx \) represents the applied pressure gradient along the direction of the flow, for a simple channel as shown in Figure 1, \( dp/dx = (p_2 - p_1)/L \). The velocity in a pressure driven flow exhibits a parabolic profile across the channel height such that the flow in the centre of the channel is faster than near the edges where it is zero as enforced by the no-slip condition.

From equation 1.3, the average velocity can be calculated as

\[ v_{avg} = \frac{1}{2b} \int_{-b}^{b} -\frac{1}{2\eta} \left( \frac{dp}{dx} \right) (b^2 - y^2) \, dy = -\frac{1}{2\eta} \left( \frac{dp}{dx} \right) \left( \frac{2b^3}{3} \right) \] \hspace{1cm} (1.4)

And the volumetric flow \( Q \) is given by,

\[ Q = \int_{-b}^{b} -\frac{w}{2\eta} \left( \frac{dp}{dx} \right) (b^2 - y^2) \, dy = -\frac{w}{2\eta} \left( \frac{dp}{dx} \right) \left( \frac{4b^4}{3} \right) \] \hspace{1cm} (1.5)

Where \( w \) is the width of the channel along the \( z \) direction. The equations are preceded by a negative sign since \( dp/dx \) is negative in the direction of the flow. From these above equations it is evident that the average velocity scales with the square of the height and the volume rate with its cube [35].

### 2.1.2 Surface and Interfacial tension.

Surface tension describes the tendency of a fluid in a surface to reduce its free energy by contracting at the surface–air interface. Interfacial tension is a similar phenomenon, but is generally applied to two immiscible fluids (for example, oil and water). These forces play more dominant roles on the microscale compared to gravity, which is much more dominant on the macroscale. Researchers have used these phenomena to conduct protein and cell sorting, perform nano reactions for protein crystallization, and passively drive fluids through microchannels.
2.1.3 Capillary forces

Capillary action describes the movement of a fluid through a narrow constriction, such as a narrow tube or porous material. At the microscale, capillary action is a more dominant force, allowing fluids to advance in opposition to gravity. Capillary forces have been used to manipulate fluids in many applications, the most famous examples perhaps being the at-home pregnancy test and portable glucometers to monitor blood glucose levels.
2.2 Existing microfluidic applications:

The growth of molecular biology has stimulated the development of systems like microfluidic systems for analysis of biomolecules, DNA and proteins [37]. Among the benefits resulting from the miniaturization of devices for use in these areas are: decreased cost in manufacture, use, and disposal; decreased time of analysis; reduced consumption of reagents and analytes; reduced production of potentially harmful by-products; and increased portability [37]. Single-molecule studies of protein interactions require precise temporal and spatial control over the chemical environment of the observed biological molecules [38].

Exploring the field of microfluidics because of its vast advantages listed previously and designing it for our requirement. The combination of both fluidic components and optical components in one setup contributes to optofluidics. Optofluidic Microsystems are key components towards lab-on-a-chip devices for manipulation and analysis of biological specimens. Optofluidics has roots from engineering and physics but applications can differ fundamentally from solid state devices to analytical chemistry and biology. In particular, the integration of optical tweezers in these devices allows stable sample trapping, while making available mechanical, chemical and spectroscopic analyses.

Our chip has some key advantages such as trapping smaller size nano particle with less power consumption (at least 2 times small particles, 10 times less power intensity). Besides that, there will be no need for florescent particles, which narrow down the applications for trapping. As a progress of our researched base result we demonstrate the potential conversion to a marketable product. The optical trapping chip can be used without any modification into existing optical trapping kits, which has already established
its user market like ThorLabs. Applications in which optical trapping play a promising role are vast, for example for biomarker detection in blood samples, for the study of protein-protein interactions for drug discovery, for the study of virus particles in virology/infection studies, and for the analysis of colloidal nanoparticles including quantum dots and metal nanoparticles.

We have demonstrated a microfluidic chip based on PDMS and its ability to trap against flow, which is essential for introducing additional nanoparticles. Trapping against flow is also essential to applications where we wish to move the trapped particle through a fluid.

We integrated the microfluidic channel into the optical trapping setup in order to examine the trapping stiffness against flow, and to introduce a second particle in the vicinity of the trapping area in order to observe nanoparticle interactions. It also demonstrates the dynamics of trapped particles in a double nano-hole optical trapping system and shows co-trapping of sub-50nm particles BSA and anti-BSA. This trapping setup helps to study the interaction dynamics between biomolecules and bio particles; for example, protein-protein interactions, interaction between sub units of ribosomes, DNA binding and protein denaturing events.

### 2.3 Materials used for chip fabrication

Polydimethylsiloxane (PDMS) has been the most widely used material in the research and development of microfluidics. PDMS is an optically transparent elastomer that allows rapid prototyping of devices. It is used for the fabrication of more complex microfluidic devices and helped ‘soft lithography’ become the most widely adopted method for fabricating microfluidic devices. Adoption of the material can be attributed to several key factors, including
1. The relatively cheap and easy set-up for fabricating small numbers of devices using PDMS in a university setting;

2. The ability to tune the hydrophobic surface properties to become more hydrophilic

3. The ability to reversibly and (in some cases) irreversibly bond PDMS to glass, plastic, PDMS itself, and other materials; and

4. The elasticity of PDMS, which allows for easy removal from delicate silicon moulds for feature replication.

PDMS microfluidic chambers have been used to trap nano particles in double nanohole apertures fabricated on gold samples [39] [40]. In those works, the gold sample was covered by a static fluidic chamber containing a diluted aqueous suspension of nanometre-sized polystyrene beads. Enabled by the integration of PDMS based flow channels with a double nanohole aperture trapping setup, the ability to trap nanoparticles against fluid flow for varying flow rates has been characterized [41]. It was experimentally demonstrated in that work that the flow rate at which the trapped particle is released has an empirical linear dependence of $1 \mu$L (min × mW)$^{-1}$. The volumetric flow rate is relevant here since it determines how much solution can be delivered to the trapping site. At the same time, this is limited by the local flow rate. In that typical case, the flow velocity at the centre of the trapped polystyrene particle was estimated to be 5.9 mm s$^{-1}$ which translated to 2 fN Stokes’ drag. It was shown that 10 mW of incident power in a double nanohole optical trap can hold on a particle for flow rates of up to 12 $\mu$L min$^{-1}$. This gave promising results for the delivery of additional nanoparticles to interact with a trapped nanoparticle; for example, co-trapping of BSA with anti-BSA by
sequential delivery of BSA and anti-BSA solutions to the trapping site using a microfluidic channel was achieved [42]. More recently, single protein binding was detected using the double nano-hole aperture integrated with PDMS based microfluidic channels [43].

Despite all the beneficial properties of PDMS that enabled its rapid adoption amongst university engineers, there are several limitations to implementing the material in biomedical research:

(i) At the molecular level, PDMS is a porous matrix of Si-O backbones covered with alkyl groups. This leads to poor chemical compatibility with several organic solvents as it tends to swell upon contact, making it mainly suitable for aqueous applications;

(ii) It also leads to adsorption of hydrophobic molecules that can release contaminants into the liquid;

(iii) This also changes the concentration of solution by water evaporation through the channel walls (this property has been exploited for protein crystallization)

(iv) Nanoparticles of silica in PDMS causes undesired scattering of light;

(v) Channel walls fabricated in deformable material like PDMS may oscillate under pressure, creating lensing effects and variable focal length in the detection system [44].

Various modification strategies have been introduced, but still cannot fully overcome these drawbacks [45] [46] [47] [48]. Therefore, applications of PDMS devices are restricted in aqueous solutions.
Researches have been going on to find suitable replacement material to PDMS. Materials like SU-8 (served as negative photoresist) were adapted for the fabrication of microchannel structures. The molecules cross link to form rigid network when heated that cannot soften before decomposition. These materials are stable even at high temperatures and are resistant to most solvents, and also optically transparent. Even though SU-8 is known for high strength, they are improper for diaphragm valve and also with their high cost, their application in microfluidics are limited [49].

One way to overcome these issues is to translate PDMS-based microfluidic channels to glass. In order to achieve microfluidic chip fabrication without bonding process, UV curable epoxy was reported to produce a sealed microfluidic channel without using a bonding process [44]. The UV epoxy based channel fabrication and advantages are discussed in detail in the next chapter.
3 Chapter 3
Microfluidic chip Fabrication Techniques

3.1 Sol-gel process:
The term sol-gel refers to a process in which solid nanoparticles dispersed in a liquid (a sol) agglomerate together to form a continuous three-dimensional network extending throughout the liquid (a gel). The term sol-gel is sometimes used as a noun to refer to gels made through the sol-gel process, but this is somewhat of an abuse of the term, since pretty much all gels are made through the sol-gel process.

Sol is a liquid. The continuous phase in a sol is a liquid and the dispersed phase is a solid. The difference between a sol and a non-colloidal liquid is that solid nanoparticles are dispersed throughout the liquid in a sol. If you put a sol in a centrifuge, you can force the nanoparticles dispersed in the liquid to precipitate out. This will not happen for a non-colloidal liquid solution, for example, salt dissolved in water. An example of a sol is black inkjet ink (carbon black dispersed in water).

A gel is a wet solid-like material in which a solid network of interconnected nanostructures spans the volume of a liquid medium. The continuous phase is a solid network and the dispersed phase is a liquid. Gels tend to be mostly liquid in composition and typically exhibit the density of a liquid as result but have cohesiveness like a solid. An example of a gel is Jell-O™ gelatin.

The Sol-Gel Transition
A sol can becomes a gel when the solid nanoparticles dispersed in it can join together to form a network of particles that spans the liquid. This requires that the solid nanoparticles
in the liquid, which are constantly bouncing around in random directions because of temperature (that is, they are undergoing Brownian motion), bump into each other and stick together when they do. For some nanoparticles this is easy, almost automatic, since they contain reactive surface groups that condense together to form bonds. For other nanoparticles, however, this can be tricky and requires the addition of an additive to “glue” the particles together or removal something from the particle surfaces so that they stick together when collide, either by bonding together or by electrostatic forces (static electricity).

As a sol becomes a gel, its viscosity approaches infinity and finally becomes immobile (that it is, it stops being able to flow and fill its container, although it might still wobble back and forth). This transition from sol to gel is called gelation. The point in time when the particle network extends across the entire volume of the liquid causing it to immobilize is called the gel point. The time required for a gel to form after mixing stuff together to make the gel is called the gel time.

The main objective is to demonstrate sol-gel bonding of an etched channel slide with inlet/outlet holes to a plain glass slide, and investigate microfluidic functionality. The conventional methods to follow are to create channels in glass slides using femtosecond laser sensitization and HF etching and to spin coat sol-gel precursor onto glass slide (attempt both flat slide and the one with the channels in it). Then bond it to the other slide, and test microfluidic capability. The test will be primarily for leakage (using dyes in water) and strength (using ad hoc methods, like attempting to pull apart manually).

To bond the two glass slides, the first precursor tried was sol-gel. The precursor sol can be either deposited on a substrate to form a film (e.g., by dip coating or spin coating), cast
into a suitable container with the desired shape (e.g., to obtain monolithic ceramics, glasses, fibers, membranes, aerogels), or used to synthesize powders (e.g., microspheres, nanospheres) [50]. The sol-gel approach is a cheap and low-temperature technique that allows for the fine control of the product’s chemical composition.

Three different methods are experimented to prepare sol-gel:

1. A weeklong process, the procedure needed a vacuum pump, and wasn’t available in the lab, and also the sol-gel was too sticky for spin coating and even drop coating. As it was time consuming, it was considered as an ineffective method.

2. A standard procedure followed by chemistry student under Brolo at UVic was referred to prepare the sol-gel. The procedure is day and a half, which is much better than the first one. We spun coat the sol-gel solution on the glass and heated, it didn’t bond the two glasses (because we think it formed a very thin layer as the solution is very viscous). However, when we drop coated the sol-gel on the glass it made a thin layer of glass and it bonded the two glasses. The main obstacle with this procedure is, it required the ethanol in the mixture to completely evaporate. As the two glasses bonded while heating, it did not leave any room for some parts of ethanol to evaporate and it hence did not solidify and the bonding was not liquid seal (tested with acetone and ethanol). Disadvantages: Ethanol did not evaporate, no proper liquid seal, time consuming process.

3. Through literature search, we found another fast, a day procedure, which resulted in an actual gel [the previous ones were either liquid or sticky glue]. But, when the gel was applied between the glass slides and heated in the vacuum oven it turned to a fine glass powder, which couldn’t bond the two glasses.
The sol-gel method has advantages such as growth of silicon oxide film at room temperature and controls of film thickness and capacitance through the adjustment of concentration. However, the poor quality of the film deposited by sol-gel method with porous structure has been a serious problem; not only integration in device process may be very difficult due to the higher wet etch rate but also making channels by this process are not working well due to no proper liquid seal and time consuming process.

### 3.2 TEOS

Tetraethyl orthosilicate abbreviated as TEOS, is the chemical compound with the formula $Si(OC_2H_5)_4$. It is a colorless liquid that degrades in water. TEOS is mainly used as a crosslinking agent in silicone polymers and as a precursor to silicon dioxide in the semiconductor industry [51].

One of the methods of preparing sol-gel is by polymerization of TEOS. It involves hydrolysis and condensation reactions. TEOS has the remarkable property of easily converting into silicon dioxide. This reaction occurs upon the addition of water:

$$Si(OC_2H_5)_4 + 2H_2O \rightarrow SiO_2 + 4C_2H_5OH$$

This hydrolysis reaction is an example of a sol-gel process. The side product is ethanol. The reaction proceeds via a series of condensation reactions that convert the TEOS molecule into a mineral-like solid via the formation of Si-O-Si linkages. Rates of this conversion are sensitive to the presence of acids and bases, both of which serve as catalysts. At low pH levels, that is, acidic conditions (slow hydrolysis), the silica tends to form linear molecules that are occasionally cross linked. These molecular chains entangle and form additional branches resulting in gelation. Under basic conditions (faster hydrolysis) more highly branched clusters form that inter-penetrable before drying and
thus behave as discrete species. Gelation occurs by linking of the clusters. With time colloidal particles and condensed silica species link to form a three dimensional network. The Stöber process allows the formation of monodisperse silica particles. One of the sol-gel process is the polymerization of TEOS in ethanol and water. Following addition of a catalyst to this homogenous solution undergoes a sol-gel transition to a rigid gel consisting of silica (Si O₂) and solvent filled pores. The chemical steps involved in sol-gel polymerization, hydrolysis and condensation, result in formation of a network of Si-O-Si chemical linkages from the alkoxy silane. In the subsequent step, the gel is processed by drying. During this time the volume of gel is reduced by approximately 80 percent resulting in silica gel.

The experimental procedure carried out is explained as follows. As TEOS is an irritant and highly water sensitive, the polymerization will require the use of concentrated acid and base solutions (HCl and NH₄OH).

1. Measure TEOS (30mL) using a graduated cylinder and place it in 250ml round bottom flask equipped with a reflux condenser and magnetic stir bar.
2. Measure ethanol (31mL) using a graduated cylinder and add to the TEOS in the flask. Stir the solution
3. In a graduated cylinder, add distilled water (38mL) and 3-4 drops of concentrated HCl together
4. Pour this acidic solution into the TEOS solution under constant stirring. The two solutions are initially immiscible but mix after a few mins.
5. Heat the solution to $60^\circ C$ and stir for 1.5 hours. After heating, cool the mixture to room temperature and place the solution in a polyethylene bottle. Place this bottle containing the sol-gel solutions in a drying oven ($60^\circ C$) for a day.

The polyethylene bottle when removed the drying oven after a day, contained sol-gel of the bottle shaped. This method was not preferred as the gel did not bond the two glass slides. Even when the solution was placed between the glass wafers instead of the polyethylene bottle, it did not bond the two glass wafers.

Second method with TEOS: At elevated temperatures ($>600 \, ^\circ C$), TEOS converts to silicon dioxide and the volatile coproduct is diethyl ether.

$$Si(OC_2H_5)_4 \rightarrow SiO_2 + 2(C_2H_5)_2O$$

Hence TEOS was spun coated on the glass slide, but it failed to stick the two glass slides. The reason might be because we were not equipped with a setup to heat the glasses up to 600$^\circ C$ degrees. The laser was used to heat it up more than 600 degree with assistance from one of the students of Prof. Martin Jun, Mechanical department at University of Victoria, but the two glass wafers did not glue to each other. We had to forego this method because the sample could not be heated more than 600$^\circ C$ and when heated with laser, there was no bondage.

Third method with TEOS: Silica precursor solutions were prepared by mixing tetraethoxysilane with absolute n-butanol, deionized water and HCl. The equivalent oxide concentration was 6% and [alkoxide]: [H2O]: [HCl] = 1: 7: 0.04. After stirring the solution was kept for 24 h at 60$^\circ C$. Before the deposition procedure the solution was filtered through a 0.2$\mu$m filter. Silicon wafers were used as substrates. Wafers was cleaned before use by:
i. \( H_2SO_4 : H_2O \) (3:2) solution at 140°C for 10mins;

ii. Rinse in deionized water;

iii. \( H_2O_2 : NH_4OH : H_2O \) (1:1:5) mixture at 65°C for 10mins;

iv. Rinse in deionized water again

The spin coating was performed at room temperature using a photoresist. The coating solution doses (1 ml) were dispensed onto the center of the stationary wafer (no special programmed path over the wafer was used) which was then spun at a speed of 500 rpm for 4s, and after this, the spin speed was increased up to 4000 rpm for 40 s. After film depositions the wafers were dried at\( T \sim = 200°C \) for 30mins in \( N_2 \) [52]. This method was discarded as it did not bond the two glass wafers as intended.

The TEOS was not an ideal material to use as an adhesive between the glass slides even after trying multiple methods and hence we had to forego this material as it did not serve the purpose we intended.

3.3 UV-epoxy

UV epoxy, is a high performance adhesive which is used in applications where high strength bonds are required. UV epoxy has many favorable properties, including high adhesion, a low electrical conductivity, high transparency, cationic polymerization reaction and so forth. Due to epoxy belongs to cationic polymerization, the oxygen has no effect on the polymerization process and hence vacuum conditions are not required. This epoxy which is cured from UV light is optically transparent and most commonly used in optics, fiber optics and microfluidics. They are heat and chemical resistant.

Microfluidic channels are often fabricated by silicon or glass bulk micromachining, which is expensive, relatively complex and shows limitations in the channel geometries.
In recent years polymers like polydimethylsiloxane (PDMS) or SU-8 photo-epoxy have become popular for microfluidic applications due to their high flexibility, ease of fabrication and high reproducibility by rapid prototyping methods [53]. Compared to PDMS, the most widely spread polymeric material for microfluidic channels, Norland optical adhesive (NOA81) has better chemical resistance to organic solvents, is impermeable to air and water vapor, is less prone to swelling upon contact with fluids, and surface treatments (for example oxygen plasma) are more stable [54] [55] [56].

We did not want to use the femtosecond laser to make channels, as using the laser made the glass surface rough and the channel lost its transparency. Hence other solutions were tried instead as follows along with UV epoxy:

### 3.3.1 Channel with Photoresist

A positive photoresist is spin coated on the glass slide. A mask (thin aluminum foil) is cut into rectangular shape of desired width and length and is placed on the photoresist glass slide. When the glass slide along with the mask is exposed to UV light, the exposed regions were removed when developed, leaving the unexposed rectangular shaped photoresist to make the channel. UV epoxy was drop coated around the photoresist channel uniformly. The top glass slide with two holes was aligned with the photoresist channel on the bottom slide. This setup is then cured under the UV light using the UV lamp for 5 minutes for the two glasses to bond. The aim is now to remove the photoresist so that it forms a channel and hence acetone was flown in the two holes from the top slide. The photoresist was removed but the UV epoxy around the channel was uneven as it was drop coated. This process needs more trial and error to get a better chip. Hence, it
did not result in a proper channel to flow the liquid. The drawback in using this method was that the photoresist did not form a neat straight channel for the liquid to flow.

3.3.2 Channel with Optical fiber

An optical fiber is used to make a channel through the glass wafers. Optical fiber is placed on the bottom glass substrate. The UV epoxy is coated uniformly on the other (top) glass substrate. It is then placed on the bottom substrate. Both the glass slides and the optical fiber sandwich is then placed under the UV lamp for curing for 5 minutes. The aim is to pull off the fiber to create a channel with UV epoxy after it is cured. It was difficult to pull off the fiber because the optical fiber glued to the epoxy. The optical fiber should be coated with a material which ensures that the fiber can be easily pulled off from the UV epoxy on curing. After experimenting with materials like photoresist, glycerin, PMMA, PDMS base and baked PDMS for the coating around the fiber, baked PDMS proved to be best coating material. The round channel created from the optical fiber has to be tested for the microfluidic flow capability. The fiber is aligned with microtubes and luers for inlet and outlet of the liquid. It is then sealed perfectly with fast set epoxy with generous amounts on both ends of the channel for inlet and outlet so that there is no leakage at the intersection. The fast set epoxy takes approximately 10 mins to harden and form a strong seal. The whole setup is then left to dry completely for half a day before pulling the fiber out. The fiber is then pulled off from either the inlet or outlet forming a perfect liquid seal round channel as shown in figure 5. The luer chosen for the inlet and outlet is such that it is easily screwed on to a syringe for easy liquid injection. The microfluidic chip is tested for leakages by flowing in water and ethanol and also to see if the channel holds its shape.
Figure 5 Optically transparent channel made from UV epoxy and optic fiber with inlet and outlet
4 Chapter 4
Integration with double nanoholes

After successful testing of the microfluidic channel with inlet, outlet and confirmation of no leakage, the next challenge is to integrate the existing gold sample with double nanoholes to this optical fiber channel as the main goal of this channel is to use it for optical trapping and to replace the existing PDMS microfluidic chip. Different methods experimented to achieve this goal are explained below.

4.1 Method 1

The procedure is listed in the steps below:

1. The top glass slide (3mm thickness) of the channel was cut into smaller glass square slides using diamond tip cutter. The bottom glass slide was replaced with 24 x 60 mm #0 size glass slide of thickness 0.08-0.13mm.
2. The optical fiber coated with baked PDMS was then placed on the #0 glass slide.
3. A thin layer of UV epoxy uniformly coated on one of the square cut glass slides and was placed on the left corner of the glass slide which has the fiber. This setup was baked under UV lamp for 5mins.
4. The gold sample containing double nanoholes was coated with UV epoxy on outer edges. This was then placed on top of the bottom glass substrate adjacent to the previously glued top square glass substrate, with double nanoholes facing the fiber and aligned along with it. This is an important step because this ensures that the DNH are present in the channel when the fiber is pulled out. This whole setup
is then baked under the UV lamp for 10 mins so that the gold sample sticks to the bottom plate.

5. Another square cut glass substrate was coated with UV epoxy of the same thickness as the previous top glass substrate. This was then placed on the right corner of the glass but adjacent to the already present gold sample. This setup had to be baked again under the UV lamp for 5 mins.

6. The luers are glued on both ends of the channel with fast epoxy.

7. After curing of the fast epoxy, the fiber was then pulled off to make the channel.

Even though this channel successfully tested for leakage proof liquid flow; the major disadvantage is that a layer of UV epoxy is present between the channel and the double nano holes. This is not the ideal condition for optical trapping of a particle. The trapping conditions require that a maximum distance between the lens and the DNH should not exceed 150µm. Since the layer of epoxy increases this limit, this setup cannot be used.
Figure 6 First method tried for integration of the gold sample along with the optic fiber channel (a) and (b) shows the right and left view of the channel with gold slide in between the two top glass slides.
4.2 Method 2

To overcome the disadvantage of the previous method, there should be no layer between the bottom substrate and the DNH. Hence, this method proved to be the best way to make a channel for optical trapping. The steps followed are as listed below:

1. In this method, a window was cut in the glass substrate with the size same as that of the gold substrate. A thin layer of UV epoxy is uniformly coated all over the substrate.

2. Similar to the previous methods, the top substrate was then placed on the bottom glass substrate (#0) that has the optical fiber.

3. This setup was cured under UV lamp for 5 mins. The space near the window on the glass substrate is cleaned thoroughly with acetone and ethanol if any epoxy is present.

4. A thin uniform layer of UV epoxy was coated in the window space and exposed to UV light for 1 min so that it is cured to a semi hard state.

5. The semi hardened state UV epoxy around the fiber in the window was cut with a blade and peeled off as shown in figure 7. This is an essential step as this ensures that there is no layer of UV epoxy between the bottom substrate and the DNH.

6. The gold sample coated with UV epoxy on the edges was placed in the window with DNH on the fiber and aligned with it so that the edges are sealed well and there’s no leakage. This setup was then cured under the UV lamp for 10 mins to make sure the gold sample with DNH is glued well sealing on all sides as it can lead to leakages.
7. The luers are again connected on both sides of the channel with fast epoxy. After curing of the fast epoxy, the fiber is pulled off.

8. The channel is as shown in Figure 7. It was tested successfully for a leakage proof by manual injection of acetone and ethanol with a lot of pressure.

![Figure 7 Second method for integration of gold sample with optic fiber channel.](image)

To make this more efficient, we made the channel vertical so that it uses less amount of liquid to flow through the channel. Following the same steps, but making the channel along the breadth of the rectangular glass slide, the final functional microfluidic channel for optical trapping looks as figure 8. This alternate way also makes it easier to mount this chip in our optical trapping setup.
Figure 8 The second method’s design was altered to make it vertical to make the channel efficient by reducing the usage of the liquid in the channel.

This method even though successful, was not completely satisfactory. The UV epoxy which is peeled off to expose the double nanoholes in the channel is very uneven and the ridges formed by the epoxy does not form a neat and clean channel as seen in figure 8. UV epoxy residue might be present in the channel when it is peeled off and cannot be cleaned efficiently because the UV epoxy is half baked. Also, fabrication was not easy since aligning the double nano holes along with the optical fiber is very difficult and cannot guarantee that the double nano holes would be present in the channel.
4.3 Method 3

The final method which proved to be the best is combining the advantages of the both methods 1 and 2 mentioned above. The channel was still made along the width of the glass slide. The steps to make the channel were as follows:

1. The #0 glass slide (24×60) was used as the bottom substrate. Another glass substrate (#0) was cut into square shaped glass slides using a diamond tip cutter.

2. One of the cut square shaped glasses was uniformly coated with UV epoxy and then placed on one end of the bottom substrate and baked under the UV light for 5mins. The second square cut glass slide coated with UV epoxy was placed adjacent at 0.2cm distance from the first one and baked again for 5mins to make a channel of width 0.2cm.

3. Using the regular gold glass slide containing DNH with UV epoxy coated on either side of the double nano holes and is placed as shown in Figure. This method did not close the channel completely as seen in Figure 9.

4. To overcome the problem, the simplest, clean, most efficient and hassle-free way was to use a bigger gold glass slide and hence the DNH were fibbed on them as shown in Figure 10.

5. The luers were attached to the ends of channel similar to that of methods 1 and 2 and tested for leakage and stability and cleaned with continuous flushing of acetone, ethanol and distilled water.

The resultant channel specifications are length – 24mm, width – 2mm, height – 1mm.
Figure 9 Third method for integration of gold sample with rectangular glass channel
Figure 10 Final design of microfluidic chip with integration of gold sample containing double nanohole and the rectangular glass channel (a) picture of the chip (b) side view of the schematic representation of the chip

4.4 Experiment results with the chip

The ultimate goal of the microfluidic chip is to replace the existing PDMS one which is integrated into our nano-aperture trapping setup and to characterize the ability to trap nanoparticles against fluid flow for varying flow rates. Hence the test is to check if this chip can be used for trapping under similar conditions as described from the previous paper of the PDMS microfluidic chip [41].

The chip is mounted on the optical setup integrated with the fluid delivery to the chip as shown in Figure 11. The setup is based on the inverted microscope configuration, modified from the Thorlabs optical tweezer kit (OTKB) that has been used in our past experiments [40] [39]. However, it includes a microfluidic assembly designed to fit in the trap. This setup is using a 830 nm continuous laser (Sacher Lasertechnik Group, Model TEC 120) that has better detection efficiency for the photodetector and helps with trapping smaller objects (due to the favorable wavelength dependent scaling). The
solution was flown into the channel using a Fusion Syringe Pump (Model Fusion200) which controls the flow rate.

Figure 11 Schematic diagram of the optical setup mounted nanoscale double-hole chip

Figure 12 shows the flowrate of the fluid to the trapping site as a function of the incident power for which the already optically trapped particle is released. To explain the linear relation between the power and flow rate at which the particle is released, we consider the interplay between the trapping force and Stokes’ drag. The trapping force scales linearly with power. The Stokes’ drag force scales linearly with flow rate. Therefore, as expected the flow rate scales linearly with power as seen in the PDMS chip from previous studies and with the UV epoxy chip.

From the figure, it can be observed that for incident powers of range 4mW-5.5mW and flow rates of range 1.2µL/min - 2µL/min stable trapping can be achieved. The straight
line is a linear fit of all the data which has a slope of 1.7 µL/ (min×mW). So far, we have not seen any departure from the linear behavior, as similar to that from the paper [41] but this is limited by the laser intensity achievable in our present setup.

![Figure 12](image)

**Figure 12** Flow rate as a function of the incident power studied using 20nm polystyrene spheres and a double nanoholes with 25nm separation

From the paper [41], we have seen that stable trapping of 20nm polystyrene particle is achieved with trapping powers of 3.5mW and larger for a flow rate of 1.7 µL/min. In comparison with the paper and Figure 4, we can see that we have achieved stable trapping for trapping powers greater than 4.2mW and flowrate from 1.3 to 1.9 µL/min.
The experiment was repeated three to four times and the results were similar. Hence we showed that the flow-rate in which the trapped particle is released depends linearly on the incident optical power. This analysis helps us in applications for studying the trapped particles and their interaction with other nano particles. One of the protein interactions, binding of biotin and streptavidin using the UV epoxy chip is discussed in detail in the next chapter.
Nanoaperture optical tweezers are emerging as an attractive method for protein–small molecule interaction (PSMI) studies. Some of the major advantages are working at the single molecule level without tethers or labels which in turn provides information about single molecule interactions, the good thermal conductivity of the metallic film of the nanoaperture dissipates any heat generated at the trapping site, hence successfully helping with studies of temperature sensitive protein-protein interactions.

Double nanohole optical tweezer has been used to observe the real-time dynamic variation in PSMI interactions with the primary focus on the effect of single and multiple binding events on the dynamics of the protein in the trap [57] [58]. As seen in the literature, it is shown that the double nanohole optical trap helps in extracting valuable and unique information about proteins. The results in this paper demonstrate that the double nano hole optical trap can differentiate between bound and unbound forms of proteins and also distinguish between multiple binding events. To prove that the same experiment produces similar results on a UV epoxy chip, the binding process of the biotin and streptavidin is repeated to check the consistency of the chip.

Biotin and Streptavidin has the strongest non-covalent bond in nature because biotin commonly known as vitamin B7 and vitamin H, has an extraordinary binding affinity to streptavidin, with a dissociation constant in the order of about $10^{-14}$ mol/L [59]. Streptavidin is a 53kDa protein purified from Streptomyces avidinii, which has widespread applications due to its very high affinity to the vitamin biotin. Streptavidin is
a tetrameric molecule, composed of 4 13kDa monomers, each of which can bind a molecule of biotin. The advantages of using streptavidin over avidin in binding biotin include no glycosylation and a lower isoelectric point [60]. These two factors mean that there is a lower risk of non-specific binding and this makes streptavidin often a more popular choice than avidin. Biotin is a small (244.3 Daltons), water-soluble B vitamin that is necessary for a variety of biological processes, such as the metabolism of amino acids and fats. Biotin binds to the tetrameric avidin proteins, including streptavidin and neutravidin, with exceptionally high affinity. The attachment of biotin to biomolecules is an important laboratory technique for protein purification or protein detection. Some advantages of the streptavidin biotin complex include: (a) It is the strongest known non-covalent interaction between a protein and ligand (b) Biotin and its co-factors bind very quickly (c) The streptavidin-biotin complex is very stable and is not affected by changes in temperature or pH.

![Figure 13 Schematic of the streptavidin-biotin interaction. Streptavidin protein consists of four identical subunits, each containing high affinity for biotin](image)
5.1 Experimental Setup

Streptavidin (Sigma-Aldrich, 85878, molecular weight 60 kDa) obtained in its powder form is then dissolved in phosphate buffered saline (PBS) to attain the desired concentration of 0.01% w/v solution. We then demonstrate protein binding by trapping biotin particles and then flowing streptavidin with a molecular weight of 60kDa.

A focused ion beam (Hitachi FB-2100 FIB) is used to mill a DNH in a 100 nm thick Au film on a glass substrate with a 5 nm titanium adhesion layer (EMF Corp.). Figure 14 shows a scanning electron microscope image (Hitachi S-4800 FESEM) of the DNH used in trapping experiments. The separation between the cusps is measured to be 30nm which is close to the optimal 25nm separation and is found to be the best for trapping 20nm PS particles according to our previous studies [40]. The gold sample is immersed in a 5mM aqueous solution of mPEG thiol, molecular weight 5kDa, overnight at room temperature and then rinsing it with distilled water. A monolayer of mPEG thiol is deposited on the gold surface which prevents streptavidin from adsorbing on the gold surface.
Figure 14 A scanning electron microscope image of the DNH used in the protein binding and control experiments

The optical trapping system is setup similar to that of the paper [43]. The main components of the system are: (i) An 830nm continuous laser (Sacher Lasertechnik Group, Model TEC 120) due to the favorable wavelength-dependent scaling, (ii) 100 × oil immersion microscope objective with 1.25 numerical aperture to focus the trapping beam onto the sample, (iii) To limit the power at the output of the objective lens, an optical density filter (ODF), (iv) A half wave plate (HWP) to rotate the polarization of the incident beam. This helps in aligning the electric field of the beam along the cusps of the double nanoholes which gives a large local field enhancement creating a strong trapping region, (v) A 10 × condenser microscope objective with 0.25 numerical aperture to collect the transmitted light through the DNH, (vi) A silicon based avalanche photodetector (APD) (Thorlabs APD110A) to measure the transmitted light. (vii) Data acquisition board to record the voltage values generated by the APD and sampling it at a frequency of 2 kHz. The setup is then modified to attach the inlet of the chip to the dual
Fusion Syringe Pump (Chemyx Inc., Model Fusion 200) for the microfluidic delivery of Streptavidin to the trapping site. With a channel dimensions of length – 2.4cm, width – 0.2cm, height – 0.013cm (~width of #0 glass slide), and a tube of length 55cm and radius 0.0125cm. Streptavidin was first injected into the channel with the help of a syringe and the chip is then mounted on the optical trapping setup. When the streptavidin particle gets trapped between the cusps of the double nano-hole, it increases the local optical field intensity and double nano-hole transmission as it dielectrically loads the region. Once the stable trapping of streptavidin is achieved, biotin is flowed in real time with the help of injection pump through a 55cm micro-tube of radius 0.0125cm at the rate of 0.003ml/min. It took the fluid about 9mins to reach the trapping site. The rate of the fluid 0.003ml/min is found ideal after a number of repeated experiments to flow in biotin without losing the trapping of streptavidin particle. Hence four biotin particle binds to the four different pockets of the trapped streptavidin causing further dielectric loading in the region thereby increasing the optical transmission. Similar results to the previous studies [43] were obtained.

5.2 Results

As seen earlier, streptavidin is a tetramer with four different monomer pockets and each of them binds to a single biotin molecule. The main objective of this experiment is to capture these four individual binding events of streptavidin and biotin. The binding of streptavidin and biotin are confirmed with the results obtained similar to that of the previous studies [43], we furthermore enlarged our data to see if we can capture the four individual bindings of these two proteins. The bond formation between biotin and avidin is very rapid, and once formed, is unaffected by extremes of pH, temperature, organic
solvents and other denaturing agents. These features of biotin and streptavidin are useful for purifying or detecting proteins conjugated to either component of the interaction. In this experiment, after the streptavidin is loaded on the chip, the data is recorded for 2 mins, then it is again recorded till the streptavidin in trapped in the double nanohole which is indicated by the sudden increase in the voltage level of the APD. Once the streptavidin was trapped, biotin was flowed in to the channel with an injection pump at a constant rate of 0.003ml/min so that the trapped streptavidin stayed intact. From the start of the flow, the data is recorded for 10mins. It takes about 8 to 9mins for the liquid to reach the trapping site (time is calculated based on the length of the pipe, area of the channel). The main objective of this data is to capture the multiple binding events and hence such low rate of the fluid was found ideal after multiple attempts to capture these events. Since the binding happens in less than a second, we needed sufficient data to process and to capture these bindings. The binding of streptavidin and biotin are confirmed by another significant increase in voltage as shown in the previous studies [43]. After the flow is stopped, the data is recorded again for the next 10 mins to observe the pattern of the biotinylated streptavidin.

Figure 15 (a) shows the recorded data from the DAQ after the streptavidin is trapped and the biotin is flown into the channel. Figure 15 (b) the sudden peak in the voltage after a period of time indicates the binding of the protein and the ligand. On further honing the data of the peak figure 15 (c), it shows a regular pattern.

Similar patterns were seen when the experiments were repeated over the next couple of days after cleaning the chip thoroughly with acetone, ethanol and distilled water and it
was also repeated with a new fabricated chip. Figure 16 demonstrates another set of results with peaks to show the binding of biotin and streptavidin.
Figure 15 Demonstration of the multiple binding events of biotin and streptavidin. (b) The encircled part from (a) is expanded to see the different bindings. The red lines marked indicate the four different binding of biotin to the four sites of streptavidin at almost the same voltage level (c) zooming the data on the peaks from (b), we can see the binding of the four biotins to the trapped streptavidin
Figure 16 Second data set recorded for the biotin flow after streptavidin is trapped in the channel. The peaks in the data represent the binding of four biotins to the four different pockets of a streptavidin
Autocorrelation is a mathematical representation of the degree of similarity between a given time series and a lagged version of itself over successive time intervals. Informally, it is the similarity between observations as a function of the time lag between them. It is a mathematical tool for finding repeating patterns, such as the presence of a periodic signal obscured by noise, or identifying the missing fundamental frequency in a signal implied by its harmonic frequencies. When the autocorrelation coefficient is close to 1, it means that there is no difference in the time series and that they are similar over a period of time.

Figure 17 Time traces of the trapping events - streptavidin is trapped (red colour), initial 10 mins of flow of biotin (multiple binding –green colour), after the flow of biotin (blue colour)
Figure 17 demonstrates the autocorrelation of time traces of the trapping events that are data of trapped streptavidin, data during the flow of biotin when the binding takes place and data recorded after the binding. This helps in better analysis of the binding of streptavidin and biotin. The autocorrelation function of the streptavidin (red data in figure 17) is very close to one indicating very high degree of autocorrelation and that the pattern is very similar to itself. Similarly the autocorrelation of data during the binding (green line in figure 17) and data after binding (blue line in figure 17) indicate the decrease in degree of autocorrelation as the similarity of the pattern changes with itself over time. Hence we can see the difference before, during and after the binding of streptavidin and biotin.
6 Chapter 6

Conclusion and Future Work

Optofluidic microsystems constitute as lab-on-chip devices for manipulation and analysis of biological specimens. In particular, the integration of nano-aperture optical tweezers – a double nanohole on a metal film in the microfluidic chips allows for stable sample trapping that helps in mechanical, chemical and spectroscopic analyses of single molecules and their interactions with other molecules when introduced in the same medium.

All chip based optofluidics are still in development phase, with most of them being experimented at the university laboratories. To make these chips commercially available, manufacturing cost constraints are one of the major factors. In the research world, most of the times the cost of the chip is disregarded as future block to overcome. Another major consideration for optofluidics is the packaging. For commercial purpose, we need to automate the fluid interactions and cannot rely on handheld syringes. Here, considering all the factors, we have successfully proposed a method which takes less manufacturing time and cost to produce a robust and automated fluid interface chip.

The main achievement of this work is the integration of microfluidics with existing optical setup replacing the PDMS chip for optical trapping which has been successfully used to trap nanoparticles such as polystyrene and co-trapping of protein and its ligand were explored because of the strong binding affinity between them. The three main factors to be considered while choosing the design or material for a microfluidic system are the function required, degree of integration and application. After a number of
different trials with other materials as mentioned in the thesis, UV epoxy which is inexpensive, is chosen as the best material as it gives us the advantage of physical characteristics such as flexibility, air permeability, nonspecific adsorption, cellular compatibility, solvent compatibility and optical transparency.

These low cost, hassle free microfluidic chip can be further extended to replace all the PDMS chip and help study DNA, single molecule with no label (without fluorescent markers) giving intrinsic information of the trapped particle (Raman modes) and operating in free solution with high speed.
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