Microfluidics for lab-on-a-chip applications

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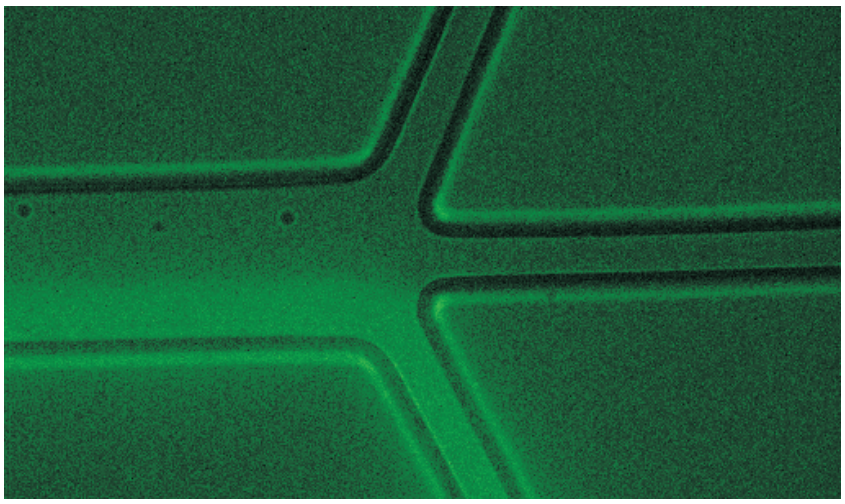

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Abstract

Integrated micro and nanofluidic lab-on-a-chip systems able to handle and analyse samples in the pico litre range are being realized and are certain to become an everyday item in biotechnology and biomedicine within a few years. Here we present an investigation of the development and implementation of a microfluidic device for assembly of sub micrometer objects, a factory-on-a-chip. Fabricated from poly(dimethylsiloxane), PDMS, and employing an optical tweezers system the device is demonstrated to trap and manipulate micron sized objects in microfluidic channels.

The thesis is a pilot study into the possibilities of integrating molecular motors as a transport system in a factory on a chip.

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Introduction

The field of microfluidics combines the fabrication methods of micro and nanotechnology with knowledge about the behavior of fluids on the fundamental microscopic level to give rise to very powerful techniques in controlling and measuring chemical reactions and physical and biological processes on the micro and nanoscale.
In the lab-on-a-chip, a miniaturized fluidic system, measurements or sample manipulations that otherwise requires considerable human involvement and sizeable laboratory equipment and space is performed on a chip that would fit in the palm of a hand. The development of the lab-on-a-chip also bring about possibilities of handling very small volumes, in the pico liter range, bringing about the opportunity to analyze samples that were previously beyond our reach. In addition it has proven to have the capacity to increase both speed and sensitivity. These qualities combined with the fact that this is a tool on the same scale as the single cell and many of the fundmental biological processes makes the lab-on-a-chip a well suited means for investigating and manipulation of these very processes.

When studying biological systems one of the great challenges is to approach and examine the vital parts of the organisms or molecules in their natural states without disturbing the function of the system. Two investigative tools that have the ability to approach these requirements are optical tweezers and microfluidic chips.

Optical tweezers utilize a very non-intrusive technique of manipulating objects on the micrometer level namely by means of tightly focused laser light. This provides the best way so far, in many instances, to manipulate biological objects in the cellular size range without corrupting their function to the degree where very little knowledge of biological importance can be deduced from subsequent measurements.
The characteristics of microfluidic systems can be designed to resemble those of the living cell while providing a controlled environment for the scientist to study their details. Most of the intricate parts of biological systems function in the very size range of these tools making them excellent candidates for studying biological systems.

Optical tweezers is a single object instrument which so far has found its uses in scientific investigation whereas microfluidics offers the benefits of higher throughput giving rise to the lab-on-a-chip where the well defined fluid handling characteristics and miniaturization of a microfluidic system allow for controlled handling of samples and opens up the possibility for serial manufacturing.
It has been shown that many different processes can be carried out in microfluid-

ic devices. Among these are separation processes such as capillary electrophoresis [1] and sorting of white blood cells[2], PCR[3], mass spectrometry [4] as well as detection devices and fluidic control components and transport systems for the microfluidic chip. Although new processes are added to the microfluidic toolbox at a rapid pace attention is now turning to integration of the different processes to build a useful lab on the chip.

Background to the problem

Taking the ideas of the lab-on-a-chip one step further would be to instead of analyzing micro and nanoscale objects trying to assemble molecules and macromolecular entities within the chip environment making the chips microscale production facilities or factory-on-a-chip devices.
Assembling molecules and other biological entities on a chip could enable us to construct complex molecular structures in a bottom-up high throughput process. This chip could utilize novel techniques such as molecular motors as a "conveyor belt" transporting the assembled parts through the factory. These possibilities are explored further in this thesis.

Presenting the problem

The objective of this diploma thesis project is: to design, develop and fabricate a microfluidic factory-on-a-chip device capable of assembling micro and nanoscale objects in a controlled sequential fashion. This entails selecting a chemical assembly process to perform on the chip, designing the structure of a chip which can accommodate this reaction and transporting the chemical ingredients to and from the assembly area on the chip and the assembled aggregates further so that they may be extracted or shuttled further to the next processing part of a factory-on-a-chip.

Outline

The following chapter of this thesis will describe the underlying theory of the methods and elements used in designing and implementing a microfluidic chip, with particular relevance to the device designed during this diploma project. This will start with a brief description of the microfluidics in general focusing on that which pertains especially to my chip design. The processes of fabrication by means of soft lithography will also be covered. Thereafter possible on-chip transport systems will be addressed and the theoretical aspects of potential chemical systems as well as optical tweezers will be described.

Following the theoretical chapter the specifics of the experiments and fabrication methods will be described. The last chapter contains the results of the study. Since this project involves development of a chip the discussion and the results

are described under the same heading. More generalized results are found under the heading conclusions followed by suggestions for further investigations and possible directions. Explained acronyms and references can be found on the last pages.

Theory

Fluid Flow

This section describes some of the most basic theoretical aspects of fluidic theory.

Laminar flow – low Reynolds number

The flow of a fluid is often described as belonging to one of two regimes, the laminar or the turbulent regime. The requirement for laminar flow states that the velocity at a particular position within the fluid can not be a random function of time as long as the boundary conditions are constant. [5] This implies that there is convective mass transfer only in the flow direction. A quantitative measure of whether or not a flow is laminar is given by the Reynolds number (Re). The Reynolds number is a measure of the ratio of inertial and viscous forces on the fluid.[5] When the viscous forces dominate the fluid flow the flow is said to be laminar. The Reynolds number is defined as:

$$\mathrm{Re} = \frac{vD_H}{\mu} \tag{1}$$

where μ is the kinematic viscosity of the fluid, v is a characteristic velocity of the fluid and D_H is the hydraulic diameter of the channel. The hydraulic diameter of the channel is a characteristic number of the volume to area ratio of the channel and is calculated as "four times the cross sectional area divided by the wetted perimeter"[5]. This yields the following expressions for some standard geometries.

$$D_H = \begin{cases} d & \textit{tube diameter} \\ \dfrac{2}{1/h + 1/w} & \textit{square channel height and width} \end{cases} \tag{2}$$

At Re above ~2000 the flow assumes turbulent behavior where convective mass transport take place in all directions[5]. However the exact value of this number is reported to be in the region 1500-2300 so caution needs to be taken when in the transition region[5] [6] [7].

Viscous drag

A measure of how the fluid flow affects a particle in the flow stream is supplied by the viscous drag force. In order to for the particle to maintain its position

while subjected to the flow this force must be counteracted. The viscous drag force F_{drag} for a sphere in a slowly moving fluid is described by [8]:

$$F_{drag} = 6\pi\eta a v \tag{2}$$

where η is the dynamic viscosity of the fluid, a is a characteristic size of the sphere and v is the average speed of the fluid flow. The viscous drag force on a rigid slender rod exposed to a flow orthogonal to the rod is [9]:

$$F_{drag} = \frac{4\pi\eta L}{\ln(L/2r) + 0.84} v \tag{3}$$

where L is the length of the rod and r is its radius.

Transport

One of the most important components in microfluidic devices is controlled mass transport. The following paragraphs deal with the two forms of transport, random and directed, from the perspective of microfluidics.

Diffusion

Diffusion is defined as the spreading of particles due to their Brownian motion. The phenomenon arises due to thermal energy. The simplest way of modeling diffusion is in one dimension. This is described by the following equation[10]:

$$x_{RMS} = \sqrt{2Dt} \tag{4}$$

where x_{RMS} is the root mean square displacement of a particle in a time t. D is the diffusion coefficient of the particle in the surrounding medium, typically 10^{-5} cm/s for a small molecule in water at room temperature. D is described by the Einstein-Stokes equation[11]:

(5)

in which k_B is the Boltzmann constant, T is the temperature, η is the viscosity of the medium and R is the radius of the particle which is assumed to be spherical.

As can be deduced from these relations both the characteristics of the particle as well as that of the surrounding medium has to be taken into account.

In the size regiment of microfluidics diffusion starts to play an important role as

a means of mass transfer.

Directed transport

The core process that needs to be controlled in any microfluidic device is the directed transport system. The requirements that a microfluidic device poses on the transport system can be quite diverse, both in the range of quantities transported and in how the transport system is allowed to influence the fluid that is transported. Hence, a number of different means of directed transport has been employed e.g. pressure driven flow [12], electroosmotic flow [13] and transport by utilizing motor proteins [14].

Pressure driven flow

Using pressure driven flow is probably the most straightforward way of moving fluids in a microfluidic device. Because of the simplicity in understanding the process a plethora of pressure driven microfluidic devices have been developed. There are two main approaches to introducing pressure into a microfluidic device. Either an external pump is used or the pump is incorporated into the microfluidic chip. The drawbacks of using pressure as a means of transport include the fact that the pressure driven flow will display a non-uniform velocity profile[5]. Also the smoothness of the flow can be hard to maintain when using pumps to drive flow in small microfluidic channels.

Electroosmotic flow

Another well known transport process in microfluidic devices is the use of electroosmotic flow. Electroosmotic flow arises when a polar liquid is brought into contact with the solid channel wall and subjected to an electric field[7]. Once the wall comes in contact with the polar liquid the wall surface acquires an electric charge. This will cause the relocation of charges inside the liquid yielding a thin layer of ions at the wall surface oppositely charged to the charge of the wall. This is known as the *Stern layer*. Outside the *Stern layer* another thicker layer of excess charges will form known as the *Diffuse layer*. This layer contains an excess of charges of the same polarity as those in the *Stern layer*. The ions within the *Stern layer* are fixed whereas those in the *Diffuse layer* are mobile.

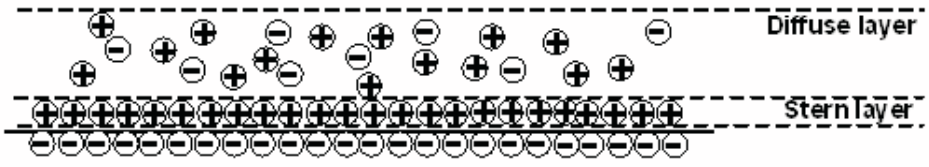


Fig 1. Schematic of the configuration of charges in a channel under electroosmoric conditions showing the mobile chrges n the diffusive and fixed charges in the Stern layer.

When an electric field is applied in the direction of the channel the diffuse layer will be mobile and controllable due to its net charge. For example a quartz surface is negatively charged due to the presence of hydroxyl groups found at its surface. If this surface comes in contact with a fluid positive counter ions in the fluid will assemble at the surface to counteract its negative charge. This will result in a diffuse layer which can be moved by an external electric field pulling the liquid along with it. The resulting flow will exhibit a straight velocity profile[15] the velocity of which can be calculated using;

$$v = \frac{\zeta \varepsilon_r E}{4\pi\mu} \tag{6}$$

from [5] where v is the bulk velocity of the fluid, ζ is the zeta potential at the channel wall, ε_r is the dielectric constant of the fluid, E is the electric field applied and μ is the viscosity of the fluid.

Transport using Motor Proteins

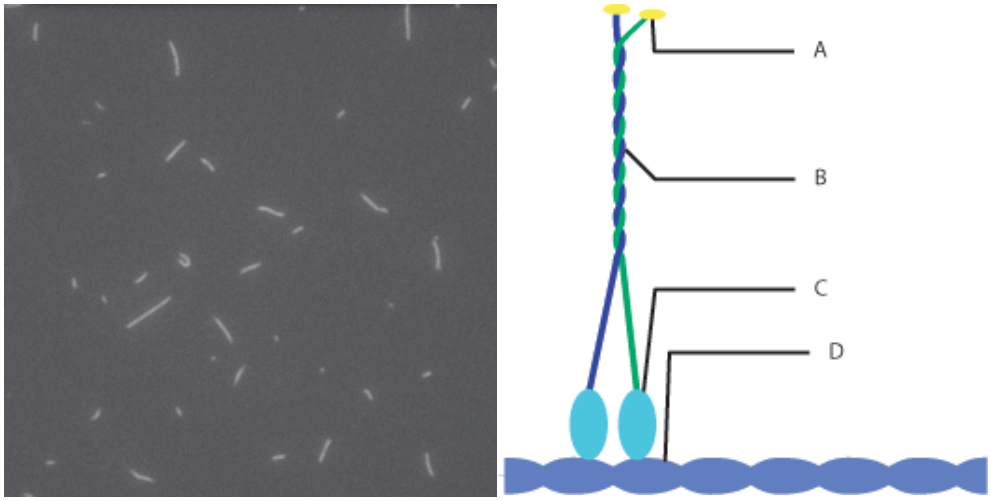
Another method of transport that has been used in experimental lab-on-a-chip setups utilizes members of either one of the processive molecular motor families' myosin or kinesin. Motor proteins are molecules which posses the ability of converting chemical energy, supplied by ATP, to directed mechanical work [9]. This allows the various species of molecular motors to move along filaments in a directed fashion.

Myosin is the name of a family of motor proteins which move along filamental actin fibers. Myosins II the most commonly found form of myosin are found in human muscle tissue. Kinesins instead move along tracks of microtubules, another filament. Both types of filaments have an inherent directionality and each type of motor protein move along its filament in a specific direction.

Both types of motor proteins are found in the cell where they e.g. take part in the mitosis machinery and transport cellular objects such as organelles and vesicles. Most processive motor proteins have a similar general structure. They consist of two head regions connected to one linker region each. The linkers are in turn intertwined and at the other end the tail region protrudes.

Motor proteins move in a stepwise fashion, one head region with an attached ATP binding to the filament track. The head region then undergoes motor action using the energy from hydrolyzation of the ATP moving the unattached head region forward along the filament strand. The other head region then acquires an ATP and is subsequently able to attach itself further along the filament. The head region no longer containing an ATP detaches and the cycle of movement can be

repeated. The tail region also has binding sections which enables the motor protein to attach itself to cargos. [9]
The capabilities of cargo attachment and directed motion make motor protein-filament system an excellent candidate for transporting cargos in a lab-on-a-chip environment.
A problem associated with the use of molecular motors *in vitro* is the particular conditions which they impose on their chemical environment. The aforementioned necessity of ATP presence to fuel movement is one but molecular motors also place other specific demands on several factors of a compatible chemical environment such as pH level and ionic strength. However the myosin family contains 18 different classes and the kinesin family 10. In turn each class includes several dozen members [16] many of which are only known by sequence.



Left:Fig.2 Images from an IVMA [32] showing sliding of fluorescently labeled actin filaments. Right: Fig 3: Schematic representation of the general structure of a processive motor in which A is the cargo biding tail region, B is the intertwined linker regions, C is the head region and D is the filament

Prototypes for guiding motor action have been realized using both kinesins [17] and myosins [18]. Both of these use the basic setup of the in vitro motility assay (IVMA) where movement is inferred on filaments by motor proteins immobilized at the tail region[19].
In [18] the velocities of actin filaments in such an assay are measured to ~5μm/s.

Chip Design & Implementation

When designing and fabricating a microfluidic device there are a number of approaches available. The specific approach used is determined by the desired characteristics of the device such as, geometrical and materials properties etc desired.

The set of techniques known as Soft lithography will be described here. Other techniques that can be used for micro and nanofluidics are standard Si- processing techniques such as Photolithography, Electron Beam Lithography, Reactive Ion Etching, Nano Imprinting etc. For more information on these see [20].

Soft Lithography

Soft lithography the general name for a number of different nanofabrication techniques such as; replica molding, micro-contact printing, micro molding in capillaries, micro transfer molding, solvent assisted micromolding and near field conformal photolithography using an elastomeric phaseshifting mask[20]. The common denominator for all of these methods is that they all in some way use an elastomer either as the end product or as an intermediate step as for example as a stamp. The most common elastomer used is PDMS which is described in much more detail in the following subsection. Soft Lithography was pioneered by George Whitesides at MIT and David Beebe at University of Wisconsin.

The basic outline of the process entails [5] that a master initially is produced on a silicon wafer by one of the Si-techniques mentioned above. The most commonly used is UV-photolithography which is capable of resolutions of >1μm. Here the device layout is printed on a transparency or on a chrome mask making some areas transparent and others oblique to UV-light. A Si wafer is then spin-coated with a photo curable resist which is exposed to UV-light through the mask. The wafer is then subjected to an etching solution that removes the uncured photoresist and the master is complete. The master is then used as a mould to cast a negative structure in an elastomer. This elastomer casting is either the end product or it in turn is used as a mould to make elastomer another generation of elastomer castings with structures similar to those of the Si-master. It is common that the surface of a master is treated to minimize elastomer adhesion by for example silanization[20].

The elastomeres produced can then in certain soft lithographical techniques be used as stamps or otherwise be further specialized[21].

One of the greatest advantages of soft lithography is that devices can be produced very quickly, inexpensively and the end product often has a high throughput quality. This makes the technique ideal for fabricating prototypes.

PDMS (Polydimethylsiloxane)

PDMS or Polydimethylsiloxane is a polymer which is often used for microfluidic applications. It has many advantages to other materials used in microfluidic assays such as glass or silicon. PDMS is flexible, optically transparent to wavelengths >230nm, impermeable to water, non-toxic to cells but is however permeable to gases.[22] It is also inexpensive and an excellent material for soft lithographical techniques.

PDMS is made up of repeating units of $-OSi(CH_3)_2-$. Its surface is hydrophobic in its unprocessed state due to the CH_3 groups. This is a conciderable drawback in microfluidic application since it renders the surface lacking in wettability with aqueous solvents and offers a channel surface prone to trapping air bubbles. In biological applications the hydrophobicity also makes the surfaces susceptible to unspecific binding of especially proteins which may cause serious problems in a biological assay. The problem of hydrophobic surfaces can however be overcome by treating the PDMS structure with an oxygen plasma to render the surface hydrophilic. The mechanism behind the change in susceptibility to water is due to an oxidation of the surface producing silanol groups (Si-OH) at the surface.
An oxidized PDMS surface will stay hydrophilic if kept in contact with water whereas in air the hydrophobic groups will once again dominate the surface behavior in ~30 minutes.[22]
The capacity to produce hydrophilic silanol surface groups also enables irreversible bonding to silicon based structures such as a glass slide or another PDMS structure.

A problem connected with the elastic properties of PDMS is the risk of elastic deformation. Because of the elastic nature of the material it will most likely deform slightly. Should however the aspect ratio of a structure in PDMS be too far from unity there is a definite risk of structural collapse [23].

Agarose

Agarose is a linear polymer which consist of alternate D-galactose and 3,6-anhydro-L-galactose monomers. It is refined from agar or marine algae carrying agar and is normally used as a gelling agent for electrophoretic as well as chromatographic separation and as a cell culturing medium [24]. Agarose has recently been used for replica moulding in order to produce stamps for stamping protein arrays and gradient.[25]

Optical Tweezers

An Optical Tweezers (also known as Optical Trap) is established by focusing a laser beam tightly using an objective lens with a high numerical aperture. The photons from the laser will induce a force on a dielectric particle near the focus point due to momentum transfer that occurs when the photons scatter upon hitting the particle. When implemented properly this force will cause the particle to become trapped at the focal point [26].

The trapping force is traditionally divided into two parts: the scattering force and the gradient force. The scattering force is caused by the incident photons colliding and subsequently being scattered or absorbed by the particle, thus transfer-

ring momentum. The resultant of all scattering events is a force pushing the particle towards the axis of the laser. In the dimension of the laser beam propagation the resultant force will normally be in the direction of the laser due to absorption of photons. For a highly focused laser beam however with a steep gradient however the gradient force will start to play a role counteracting the scattering force pulling the particle towards the focal plane.

A dipole in an inhomogeneous field experiences a force in the direction of the field gradient. This is the mechanism behind the gradient component of the trapping force. As the particle moves away from the focal plane the gradient force will increase pushing the particle back towards the focal plane.

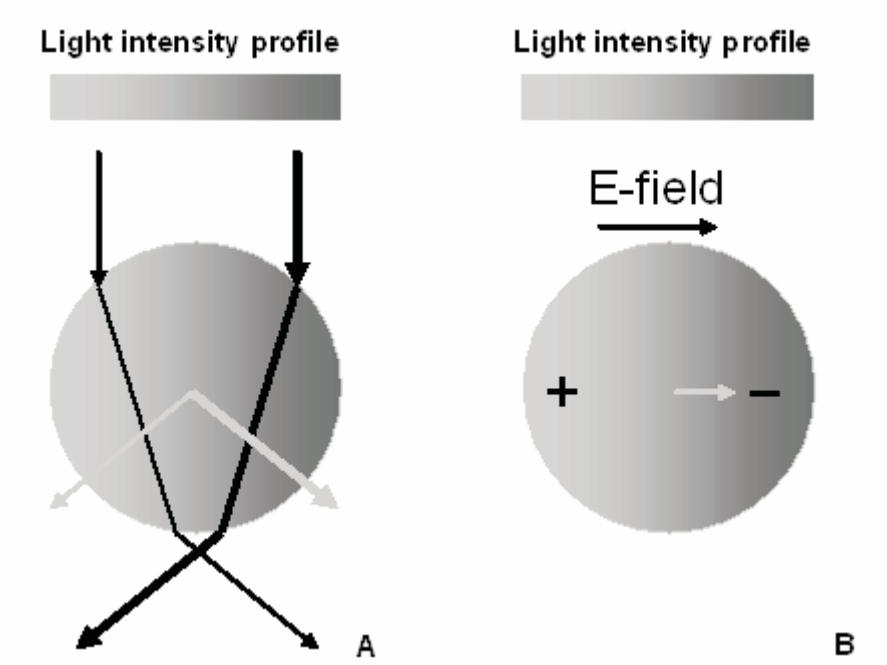


Fig 4. Description of the processes involved in optical trapping. (A) shows the ray optics description where illumination of a transparent bead with light of with increasing intensity from left to right resulting in a transfer of momentum from the light, represented as two black rays, to the bead pushing it in the direction of the increasing gradient and downwards in the direction of the focal point of the incoming light, represented by the lighter arrows. (B) shows the field representation where a force is excerted on the bead arising from the polarization of the particle by the field and following attraction to the stronger region of the electromagnetic field.

The forces in the optical tweezers can be calculated in a straightforward way for two limiting cases i.e. when the wavelength of the laser is much greater than or smaller than the diameter of a trapped spherical object. The first case, the so called Mie regime, can be described using ray optics (fig. 4.A) and the latter one one, the Rayleigh regime, can be described by viewing the particle as a point

dipole in a gradient field (fig 4.B) [26]. Mathematical description of the intermediate range is much more involved but the mathematical means of calculating the force on a sphere here has been developed [26].

Chemistry

The Biotin-Avidin system

The biotin-avidin system was first employed as a means of staining in the mid 1970s. The system is an example of protein-ligand binding. It consists of the glycoprotein avidin and the vitamin biotin. Because of the ability of these molecules to form an extremely stable compound with a binding affinity of $K_a=10^{15}$ M^{-1} it has found frequent use in the biochemical sciences as a means of attaching markers to target molecules or sites and commercially as a method for separating proteins [27]. It has also been used to attach cargo to microtubules in so called molecular shuttles[17]. The binding affinity of biotin to avidin is so strong that even biotin coupled to other proteins is available to form a bond with an avidin protein. The biotin-avidin bond can withstand forces of 5pN for 1 min and up to 170pN for 1ms [28]. This makes the system a good candidate for immobilizing particles in a microfluidic environment where viscous drag can exert forces in this range for prolonged periods of time.

An additional benefit with the system is the fact that one avidin has four binding sites for biotin which enables for several biotin-avidin systems to crosslink providing an opportunity for e.g. amplification when attaching fluorescent probes to a target.
Biotin, the smaller of the two, can be readily attached to many target molecules [29], in most cases with little or no disruption of the physical and biological properties of the target.

Lambda Phage DNA

The lambda phage or *Bacteriophage lambda* is a virus which, like all other bacteriophages, infects bacteria. The phage lambda is often used as a model organism in biology since it infects another well known model organism *Escherichia Coli*.
The virus consists of a head region which contains its 50 kbp DNA strand wrapped around a protein central part and a tail region which can attach itself to the host organism merging the tail with the hosts cell membrane injecting its DNA into it.[30]
Lambda phage DNA has the ability to assume both circular and rope like form since the DNA ends have 12 bases long complementary single strands called "sticky ends" with the sequence GGGCGGCGACCT [31].

Experimental

Fabrication

The fabrication process for the microfluidic chip is a five step process that utilizes a number of different techniques. The basic outline of the process is:

1. Device design
2. Mask fabrication
3. Master fabrication
4. PDMS micromoulding
5. Final assembly, bonding and activation

Device design

The first step in the process included designing the device taking into account all of the important characteristics of the system proposed to be contained within it. The masks to be used for UV-lithography were deigned using Adobe Illustrator CS (Adobe Inc).

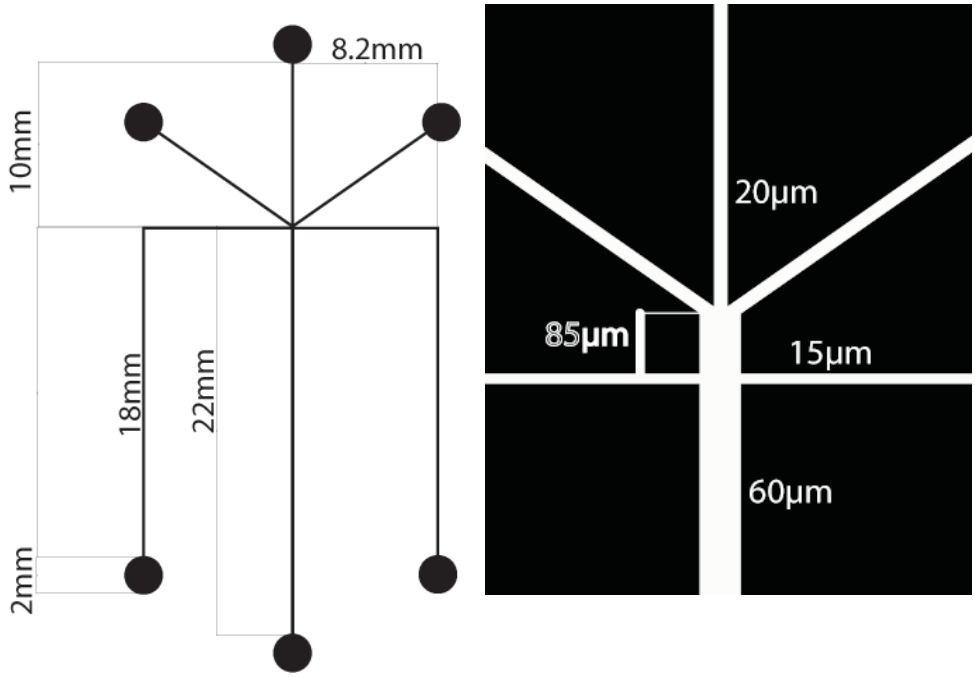


Fig. 5 The final design of the photomask (left) and channel intersecions in detail (right)

Mask fabrication

Three different masks designs were produced of which the final design is shown

in fig. 5. The first mask was designed to fit an existing chip holder that was connected to a pump and could distribute pressure over the different channel reservoirs. For the following two a new sample holder were produced (see fig 11)
Once designed the UV-masks were manufactured on chrome plated glass using a photomask laser writer (Heidelberg Instruments DWL 66). The masks were made positive leaving the channel areas transparent.

Master fabrication

The masters that were to be used as molds were fabricated on 2" standard silicon wafers. The relief structure on top of the wafer giving the channel structure were made from negative tone SU-8 photoresist formulations 2005, 2035 and 2050 (MicroChem, Newton, Ma). These formulations yield resist structures of heights ~5μm, ~35μm and ~50μm respectively. Since the process used to fabricate the masters closely resemble the method suggested by MicroChem only the specifics will be covered here.
The surface of the substrate silicon wafers were first dehydrated in a convective oven for 30 minutes at 200°C. Then the SU-8 resist was dispensed onto the surface covering it. The resist covered wafer was then placed in a spinner to achieve an even coating of well defined height.
After spin coating the substrate was soft baked on a hot plate and then exposed

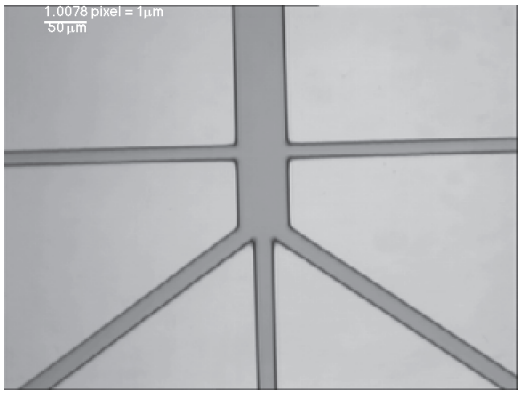


Fig. 6 Microscope image of details of the Silicon SU-8 masters showing structures for casting the channel intersections.

to UV-light from a mercury lamp through the chrome mask using a projection mask aligner (Karl Suess). Following exposure the resist covered wafer was post exposure baked on a hot plate and subsequently developed in SU-8 developer (MicroChem, Newton, Ma) to remove unexposed areas of SU-8 and then rinsed first in isopropyl alcohol and then in deionized water. Next the developed wafer with resist structure was dried using nitrogen gas. Finally the wafer was hard baked in a convection oven at 200°C for 45 minutes.

For a complete description of the specific times and temperatures used in the fabrication process see Appendix 1.

The finished master was then treated with an oxygen plasma for 10 seconds in order to clean the surface and make it susceptible for silanization. The master was then exposed to the vapors of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane in a mild vacuum environment for 2 hours to prevent adhesion of PDMS to the master during modeling.
The master to be used for agarose replica molding was not silanized [34].

Replica molding

Two different types PDMS prepolymers (RTV 615 kit, General Electric and Sylgard 184 kit, Dow Corning) were prepared by mixing PDMS base and curing agent in the weight ratio 9:1.

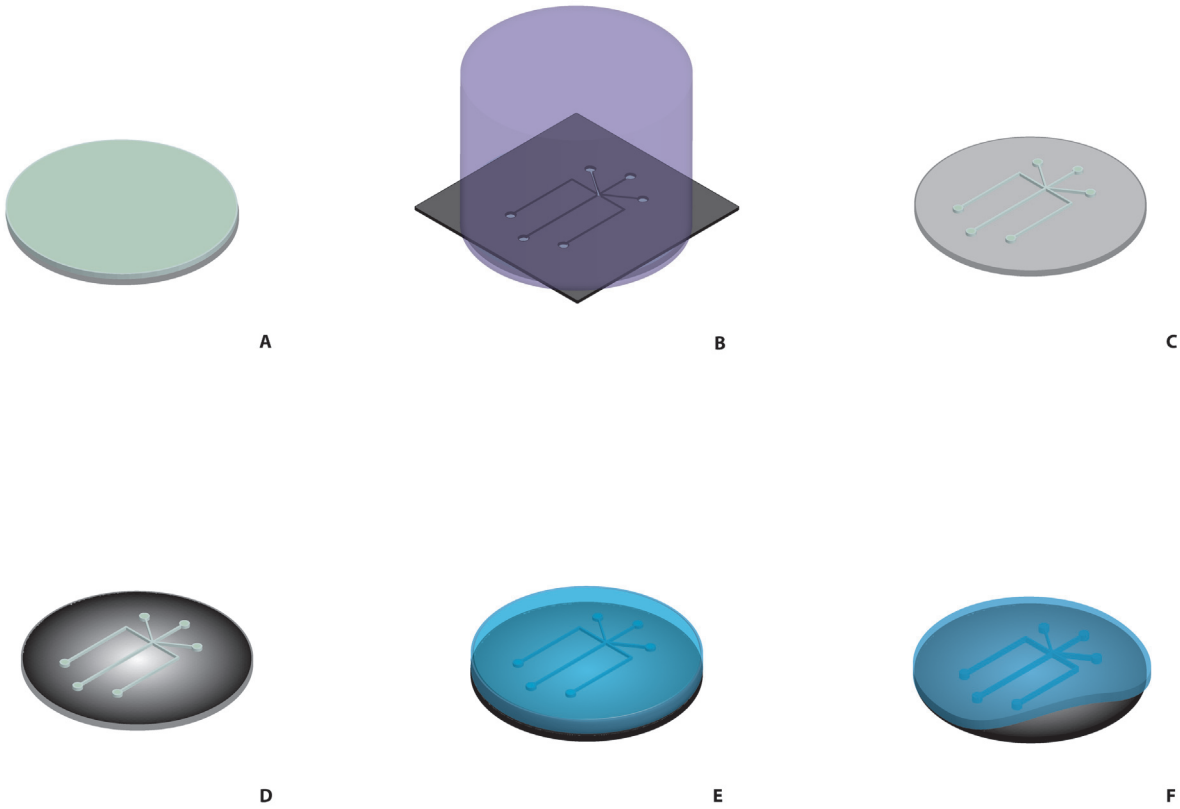


Fig. 7 Schematic of the different steps in the fabrication process of a replica molding master and susbequent molding of PDMS. (A) A silicon wafer is spin coated with SU-8 photoresist which after soft baking is subjected to UV-light (B) through a photomask. The unexposed areas of SU-8is then removed (C) by developing in a solvent. After further hard baking the master is silanized (D) and PDMS mixture is poured on top of it (E). After curing in a convection oven the elastmer can be peeled off the master(F)

The mixture was then thoroughly stirred to ensure optimal curing of the PDMS. Air bubbles which were formed during mixing were removed by degassing the PDMS mixture in a vacuum chamber, for 30 minutes or more, until no bubbles were visible.
The degassed PDMS mixture were then poured over the silanized master until completely covered and placed on a level holder in a convection oven at 80°C for 45 minutes. After baking the PDMS and mould were allowed to cool after which the PDMS was removed from the master by gently inserting a scalpel between PDMS and master and peeling the PDMS off. The master is then ready to be used again.

Initial tests of Agarose gel (USB, Cleveland, Oh)for replica molding were also conducted where a solution of 3% agarose were heated in a water bath until completely dissolved. The agarose gel was then allowed to cool down to 70°C and then poured on an SU-8 patterned silicon master. The agarose was then allowed to cure for one hour before removing the hardened gel from the master.

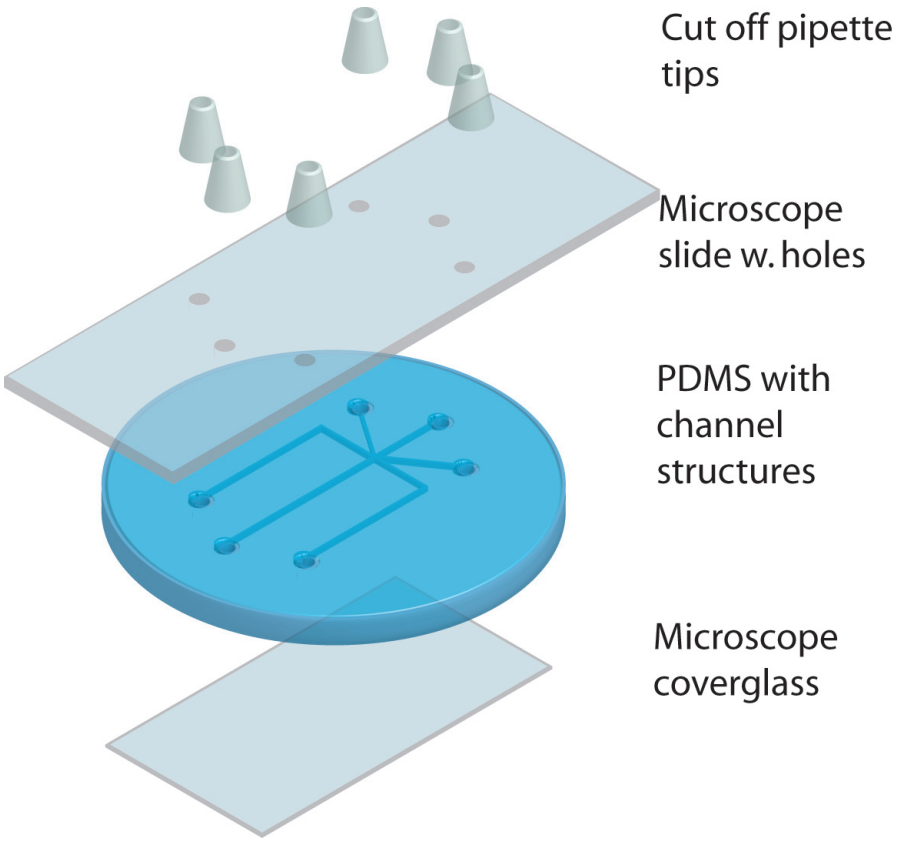


Fig. 8 Schmatic wiew of the final assembly of the microfluidic device. PDMS and glass slides were irreversibly bonded using an oxygen plasma and cut off pipette tips were glued to the device usig optical adhesive.

Final assembly, bonding and hydrophilization

To make a complete device the channels must be completed with a fourth side. Furthermore the mounting sections must be added so that the device can be mounted in the microscope and the hydrophobic channels must be made hydrophilic.

Holes matching the positions of the reservoirs in the PDMS structure were etched in a standard glass microscope slide using a sandblaster (MicroEtcher ERC, Danville Eng., San Ramon, Ca) and a copper mask to get well defined and positioned holes. Then cut off pipette tips were attached to the microscope slide concentric to the etched holes to make reservoirs capable of holding larger fluid quantities. In order to prevent unspecific binding to the glass microscope coverslips a 30-50 μm film of PDMS are deposited on one side of these by the following method. A droplet of PDMS were placed on one side of the coverslip which were carefully turned at a steep angle until completely covered. The coverslip was then placed vertically on one of its shorter edges and the elastomer was allowed to migrate to the lower part where it was removed manually using the side of a syringe needle. #0 coverslips were used in order to comply with the working distance requirement of 210 μm of the objective.

The PDMS casting, microscope slide and PDMS covered coverslip were exposed to an oxygen plasma for 60 seconds to activate the surfaces for bonding and make the channels hydrophilic and then immediately assembled (see fig 8). Finally the sample liqids were injected into the inlet reservoirs quickly after hydrophilization to prevent the channels from reverting to their hydrophobic state.

Macro Interface-Sample holder

A sample holder was fabricated from Plexiglas to connect a pump to any of the microfluidic reservoirs. Leak valves were installed on the tubing between the sample holder and pump in order to control the pressure inside the reservoirs.

Sample solutions

Fluorescent beads, emitting in the yellow-green and red wavelengths, of sizes 0.1, 0.49, 0.51 and 1μm (Duke Scientific, Palo Alto, Ca) in aqueous solution were used to characterize and test the device. The 0.1 (yellow-green), 0.49 (yellow-green), 0.51 (red) and 1 (yellow-green) μm beads were diluted to contain 0.001%, 0.01%, 0.01% and 0.01% beads respectively.

For the final device a buffer solution containing 5x PBS and 0.02% Triton X-100(Merck) were used.

Three different solutions of 10ppm 1 μm non-fluorescent polystyrene avidin labelled microspheres, .001ppm 0.04μm yellow-green fluorescent (wave-

length=505/515nm) biotin labeled polystyrene microspheres and 0.01ppm 0.04 μm red fluorescent (wavelength=580/605nm) avidin labeled polystyrene microspheres were suspended in buffer.
All labeled microspheres were acquired from Molecular Probes, Eugene, Or.

For experiments involving PLL-PEG a third set of buffer solutions containing 10 mM HEPES and 100mM NaCl med pH 7.4 were prepared.

Optical Tweezers and microscopy

A Nikon Eclipse TE 2000-U optical microscope was modified to house an opti-

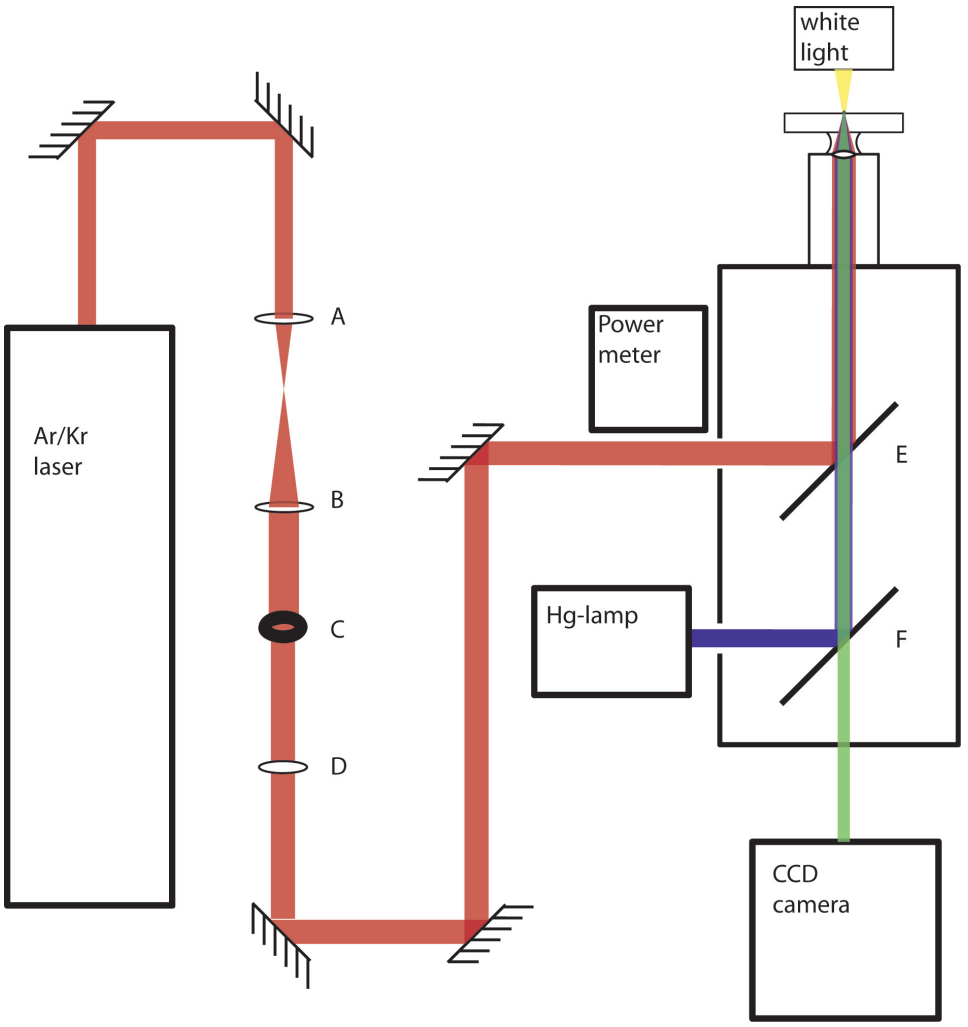


Fig. 9 The setupof the optical tweezers housed in the microscope. The laser is is guided through the beam expanding lenses (A) and (B) and subsequently passes an aperture (C) and a band pass filter (D) filtering out the undesired laser wavelengths.. A dichroic mirror (E) reflects the laser onto the back of the objective overfilling it. Another dichroic mirror (F) guide the UV-light into the objective.

cal tweezers system. An argon ion laser (Spectra Physics) that outputs in 647.1 nm and seven other wavelengths was used as the laser source. The laser beam is guided by mirrors into a beam expander consisting of two lenses of focal lengths 50 and 125mm expanding the beam to twice its original diameter. Then the laser hits an aperture, slightly overfilling it for a Gaussian beam profile. Following the aperture the expanded laser beam traverses a band pass filter (647/10 nm) and is guided by mirrors into the backside opening of the microscope.

At the backside opening of the microscope a fold away photo detector is positioned to enable measurement of the power of the laser light entering the microscope. Inside the microscope the beam is guided into the objective (NA 1.4, oil immersion, wd 0.21mm) via a dichroic mirror with almost perfect reflexivity for the 647.1 nm light. A short pass filter that excludes wavelengths above 600 nm is positioned between the objective and the ocular to filter out laser light potentially damaging to eyes. The setup also allows for the light to be collected in a CCD camera (iXON, Andor Technologies, Belfas, Northern Ireland)
The microscope also allows for the sample to be illuminated by from the top by white light and through the objective by UV-light from a side mounted mercury lamp. The latter to excite the fluorescent beads employed.
This setup uses a fixed tweezers and a sample chip that is moved on a motorized sample table. Hence the position of the optical trap can be moved relative to the chip channels.

Full Setup

For the complete setup of the experiments solutions were loaded into the reservoirs by pipette into the reservirs of the device. Fig 10 describes the proposed

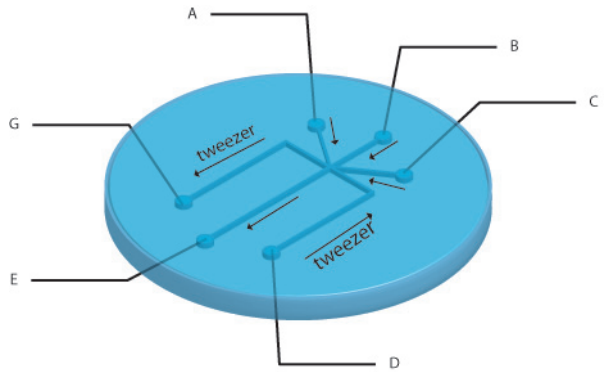


Fig. 10 Schematic describing the function of the device. The proposed transport processes of the device is described by the arrows where unlabeled arrows describe pressure driven flow and the tweezer labeled arrows describe transport by optical tweezer. The fully functional device would have 1 micron avidin covered beads transported from D to F using optical tweezers passing through flows of solution containing biotin covered beads from C, buffer from B and avidin labeled beads from A or solution containing biotinylated 12 bp lamda phage complementary DNA from C, buffer from B and lamda phage DNA from A.

function of the device. For most experiments attempted solution containing 40 nm flourescent biotin labeled beads were loaded into A, buffer solution into B and solution containing 1 micron avidin labeled beads into C. For these experiments D and F were not used.

The device was then placed on the microscope sample table and in some cases

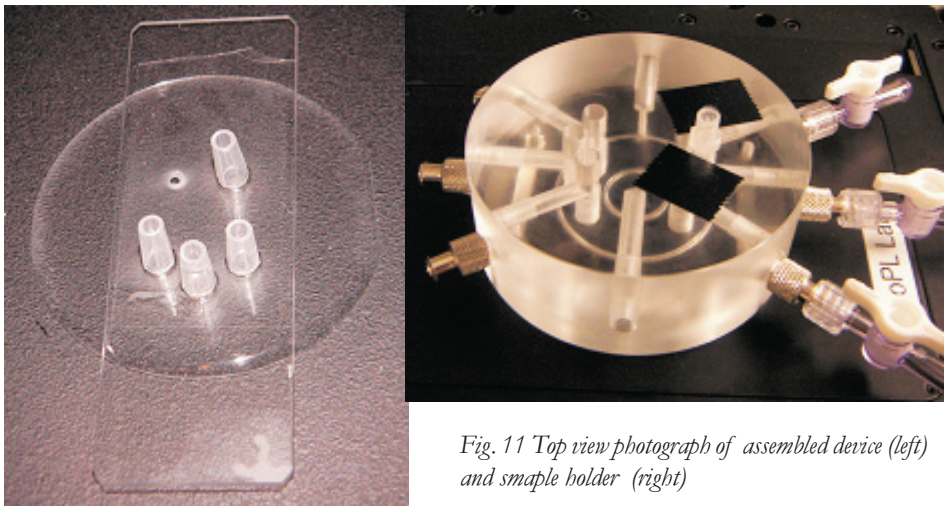


Fig. 11 Top view photograph of assembled device (left) and smaple holder (right)

connected to a pump via the sample holder. Particle speeds were measured from sequential images collected by the CCD camera.

Results and Discussion

In this thesis the development and implementation of a microfluidic factory-on-a-chip device is described. Different channel structures, transport systems, materials and chemistries are considered with the objective of constructing a microfluidic chip in which a sequential chemical process could occur in a controlled fashion.
A Si master is fabricated by means of UV-photolithography. After silanization the master is used as a mould to cast an elastomere containing channel structures. The elastomere is irreversibly bonded to a glass cover slip after having been functionalized in an oxygen plasma.
The device is then mounted in a sample holder creating a pressure difference between the different reservoirs in the structure. Different solutions containing microspheres is introduced into the channels and the device is studied in an inverted microscope in which an optical tweezers system housed capable of manipulating the microspheres. The microspheres which are not large enough to be resolved in the microscope are fluorescently labelled and studied using this method. Laminar flow conditions are established and attempts to controllably connect microspheres with functionalized surfaces are made trapping and subsequently moving an avidin covered microsphere through other flows containing biotin labelled fluorescent microspheres using the optical tweezers.

Design

The design of this chip is basically an integration project, where elements from different previous work are collected. The device design requires non-interfering transport along two orthogonal axes. This requirement rules out the combination of pressure driven flow and electroosmotic flow since these would interfere with one another as they are both bulk transport processes. The remaining options are to combine either pressure driven or electroosmotic flow with either one of the specific transport processes; transport using motor proteins or optical tweezers. Due to the added complications of including yet another set of biological molecules into the chip the decision was made to optical tweezers, however designing the rest of the chip to fit the conditions required by a motor protein, Myosin II so that the design could be further developed for these. Following these requirements pressure driven flow was chosen over electroosmotic flow since the electric field used in the latter would influence the function as both the actin strand and the myosin has a charge distribution.

The minimum width of the channels were limited by the laser ablation printer which could produce feature sizes >6μm using the current setup. In order to

prevent pressure losses and contamination the side channels meant to house the 1μm avidin covered beads were given this size in the final device design although when fabricated using 5μm SU-8 under etching in the development process proved a big problem for channels this narrow. The crossing between large channel containing the smaller beads were placed 100μm downstream from the fork in the larger channel to ensure that no diffusive mixing between avidin and biotin labelled beads could occur before this point.

Two candidates for assembly chemistries were considered for the device i.e. alternating avidin-biotin linking and avidin-biotin linking to a 12 bp DNA complementary to the lambda phage DNA followed by this DNA binding to the lambda phage DNA. Only the first were attempted due to time constraints.
Since the motor proteins are biomolecules their presence in a microfluidic assay imposes chemical requirements on the channel environment. This includes a neutral pH, specific ionic strength has to be maintained in all solutions that will come in contact with the motor proteins. Also temperature has to be kept in the vicinity of body temperature, 10-40°C [32].
Furthermore an adequate supply of ATP must also be supplied as a source of energy for the motor action to occur.
As to the geometrical structure of the chip in order to use molecular motor gliding assay the motor channels must be small enough so that the actin filaments are not allowed make u-turns within them. This requires that the width of channel in which actin is transported does not exceed 200 nm, which is the minimum radius at which an actin filament can bend without breaking [17].

Fabrication

Initial fabrication tests of agarose chips were made and well defined structures with feature sizes of 500μm were produced. Examination of the function of an agarose chip with channels loosely adhering to the bottom of a plastic Petri dish showed that steady laminar flows could be maintained within the channels. The possibility of ATP diffusing into the channels to maintain molecular motor function makes the usage of agarose as chip material appealing but the added complications of weak reversible binding to glass and accompanying problem of using the high NA oil immersion objective required for optical tweezers made us decide in favour of PDMS as chip material.
PDMS proved an easy to work with material with one exception. When cutting holes to access the reservoirs these would often collapse or torn off PDMS fragments would remain in the reservoirs sometimes clogging the channel entrances. Silanization of masters proved necessary to reduce adhesion between master and PDMS casting. No detrimental effects of the silanization were detected in the PDMS cast on these masters.

Device function and control

Upon introduction of solutions into the three reservoirs several parallel laminar flows could be established. The hydrophilic nature of the channels drew the liquid into the channel structure and once filled the flow remained constant for a long period of time >30 min. The proposed explanation for this is either that it is driven by the evaporation of liquid from the output reservoir or this in combination with the hydrophilicity of the output reservoir. The pump used proved to weak to influence the flow. Because of the difficulties to control the flow using the pump the flow influenced by controlling the amount of fluid in the outlet reservoir since the focus f the flow control was to limit the flow speed. Speeds of >40μm/s was attainable using this method of flow control. This is adequately slow so as not to disrupt the trapping of the beads in the optical tweezers.
The maximum power delivered into the microscope was measured to be 196mW. This corresponded to an output of ~200 mW from the laser in the 647.1nm line.

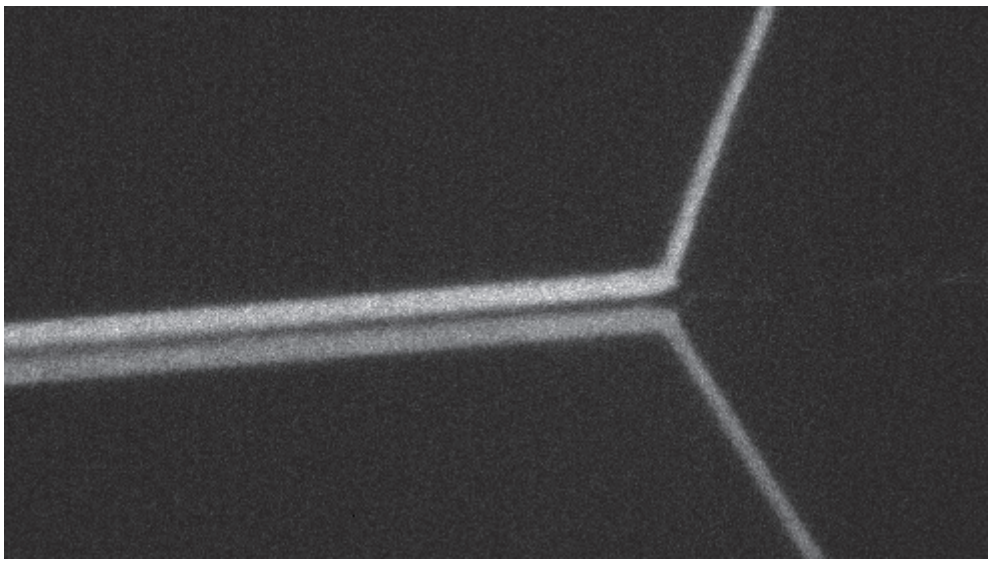


Fig. 12 Stabile laminar flows were obtained without pumping (each flow line is ca 20 microns wide). Buffer solution in the central flow keeps diffusing avidin and biotin labeled beads in the outer flows from aggregating before reaching the intersection where the optical tweezers transported beads can extract them.

Hence the optics outside of the microscope only causes very small losses.
Since the dichroic mirror inside the microscope is almost entirely reflects incident laser light of 647.1nm, at optimal conditions the optical tweezers setup can deliver close to 200mW in the specimen plane. As a general guideline an optical trap will sustain forces of 1pN per 10mW of laser power delivered to the speci-

men plane for micron sized beads. [26] This would imply that the optical tweezers system would be able to trap a micron sized bead as long as the forces acting on the bead were smaller than 20pN. This can be calculated to correspond to a flow velocity of 1 mm/s as an upper limit for trapping to occur, using (2).

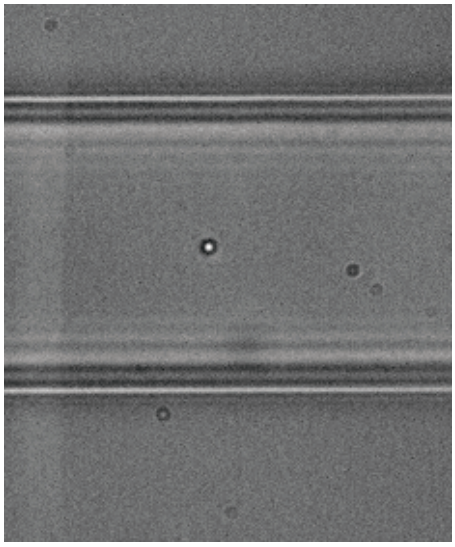


Fig. 13 Manipulation of optically trapped 1 micron sized beads were performed in the microfluidic channels.

Excellent trapping of 1μm beads were observed in the two directions of the species plane however the trapping in the direction of the laser beam proved harder to achieve. Instead the beads were trapped against the top of the channel.

Trapping the beads against the channel roof was uncomplicated for the beads which were not labelled with avidin. However for the avidin labelled beads the situation proved more problematic since the beads unspecifically adhered to the surface irreversibly.
Initial attempts to alleviate the problem by using a buffer solution containing PLL-PEG were unsuccessful.

The avidin-biotin linking of the beads were tested with satisfactory results but as optical trapping of the avidin labelled beads rendered them immobilized to the surface biotin-avidin linking in the channel were hard to achieve.

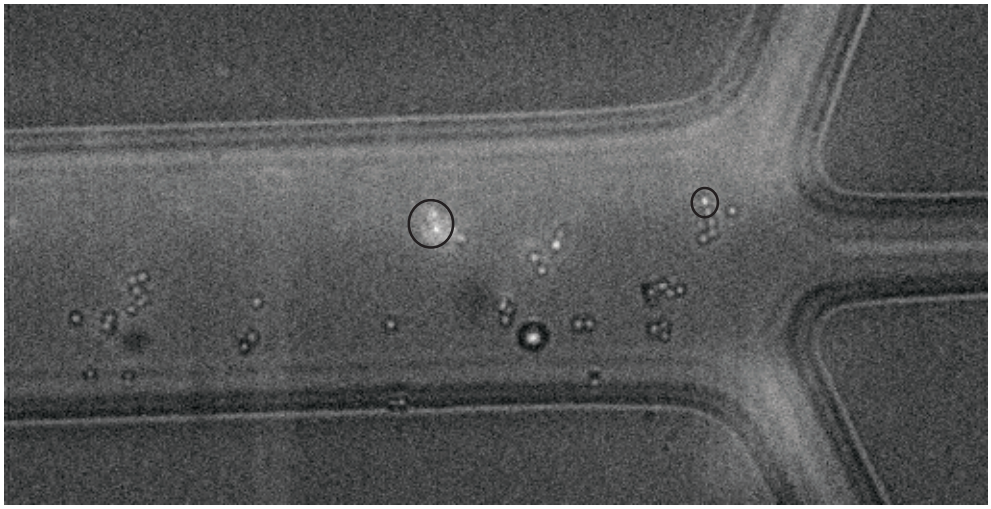


Fig. 14 Optical trapping of avidin covered micronsized beads were achieved but unspecific adhesion to the roof of the channels inhibited device function. Shown are a number of beads that were immobilized after trapping. Binding to fluoresecently labeled 40 nm biotinylated beads can be seen on the avidin beads immobilized closest to the upper third of the channel where the laminar flow of biotinylated beads is. (Encircled in image)

Conclusions

The device proposed is feasible since the involved processes were shown to work but some obstacles remain regarding integrating them into a chip. First of all either the unspecific binding of the avidin labeled beads or the insufficient optical trapping in the z-direction has to be alleviated, preferably both.

Future directions

Microfluidics for lab-on-a-chip and factory-on-a-chip applications is currently being widely researched. The promising aspects of low sample volume separation and testing as well as the high throughput of the devices will definitely find many useful applications both in the lab and in more industrial settings as micro and nanotechnology moves from the solid state arena to fluids and soft matter.
The device attempted was merely meant to serve as an experimental device mapping out problems and opportunities of the technology seeing as it has very little practical use. Including molecular motors in the device will surely offer even more challenges but much stands to be gained from this as this transport system is more selective and more specific than the bulk transport systems of pressure driven and electroosmotic flow. This would also be more in line with the high throughput system as this transport system can fit inside the chip.
The set of chemical reactions for factory-on-a-chip applications that would be the most appealing to be integrated into a microfluidic device are the native chemical ligation processes first realized by Dawson et al. [33]. This method allows large unprotected polypeptide segments to bind to one another, building pro-

teins. The process can be carried out at biological pH and in aqueous solution making it an excellent candidate for use in a motor protein driven factory on a chip able to produce complex proteins.
The possibility of casting microfluidic structures in agarose opens up the possibility for not only sustained motor protein action in conjunction with other transport techniques without specifically adding ATP to all solutions in the device but also the possibility of cell culturing in a very well specified environment. The ability to create chemical gradients and to grow cells in well defined flows at the same time could prove a helpful tool to biologists.

Acronyms

ATP	Adenosine Triphosphate
CCD	Charge Coupled Device
DNA	Deoxyribonucleic acid
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid
IVMA	In vitro motility assay
kbp	kilo base pairs
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PDMS	Poly dimethyl siloxane
PLL-PEG	Poly(L-lysine) - Polyethylene Glycol
pN	picoNewton
Re	Reynolds Number
Si	Silicon
SU-8	a negative photoresist

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Appendix 1

The process specifics for patterning of different thicknesses of SU-8 on 2" silicon wafers

SU-8 thickness	5μm	35μm	50μm
Spinner speed 1 (rpm) / time (s)	500 / 3	400 / 6	400 / 6
Spinner speed 2 (rpm) / time (s)	3000 / 60	500 / 10	500 / 10
Spinner speed 3 (rpm) / time (s)		1340 / 60	1340 / 60
Soft bake on hot plate	ramp from 65 to 95°C	3 min at 65°C	3 min at 65°C
		ramp from 65 to 95°C	ramp from 65 to 95°C
		4 min at 95°C	4 min at 95°C
Relaxation	10 min	10 min	10 min
UV exposure (11W/cm²)	7s	18s	22s
Post exposure bake on hot plate		ramp from 65 to 95°C and back to 65°C	
Relaxation	10 min	10 min	10 min
Development (ultrasonic bath 70V):			
in SU-8 developer	3 min	15 min	27 min
in IPA	30 s	5min	5 min
washing in deionized water	10s	10s	10s
drying in nitrogen gas	10s	10s	10s
Hard bake in convection oven 200°C	30 min	30 min	30 min

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