Towards the experimental realization of directed transport in equilibrium

A thesis submitted in partial fulfillment of the requirements for the

BS-MS dual degree programme

by

Arpit Yati

Under the guidance of

Dr. Shivprasad Patil

Assistant Professor

Department of Physics

Indian Institute of Science Education and Research, Pune

Dr. Umakant Rapol

Assistant Professor

Department of Physics

Indian Institute of Science Education and Research, Pune

Dedicated to the project assistants, the carriers of our project who receive a merge salary and have an uncertain future.

Contents

Certificate

This is to certify that this dissertation entitled "Experimental realization for directed transport in equilibrium" towards the partial fulfillment of the BS-MS duel degree program at the Indian Institute of Science Education and Research Pune, represents original research carried out by Arpit Yati at Indian Institute of Science Education and Research, Pune under the supervision of Dr. Shivprasad Patil during the academic year 2010-2011.

Name and signature of the student Arpit Yati
Date: 10 April 2011
Place: Pune
Supervisor Head Physics
Date:
Place:

Acknowledgment

We laughed when things did not work out, and they are not yet. Patil sir is patient. I did mistakes and I knew that I will be let off. Rapol sir's smile indicated that I was going the wrong way. Saying a thank you to them would undermine the effort taken by them to guide me.

I learned a lot and the credit goes to my guides, parents and friends. I am thankful to the people who left the hostel for their final year projects and went to greener pastures, giving us a lot of spatial luxury and peace.

List of Figures

Abstract

The document describes an attempt to experimentally verify noise driven directed transport. Symmetry broken systems will be tested for directed transport in a thermal bath. Two different sized coupled polystyrene beads of micrometer dimensions will be confined to move in a single dimension. A micro fluidic channel having inner diameter of the order of the larger polystyrene bead is constructed indigenously to hold the trapped system inside using an optical tweezers. The capillary plane is aligned to the horizontal to investigate the movement of the coupled beads. The beads will then be tracked to observe any movement in the absence of trapping laser.

Chapter 1

Introduction

The Smoluchowski-Feynman ratchet is a machine, proposed to perform perpetual motion, driven by Brownian motion of the molecules in a thermal bath [9, 11]. The machine consists of a ratchet in a thermal bath at temperature T1 with a pawl attached to it. The pawl restricts the rotation of the ratchet in one direction. The ratchet is connected to a paddled wheel by a mass less rod in a separate bath having temperature T2. It was stated by Feynman and later by Marcelo in 1993, that such system will not do work on its own if the temperature of the two baths are equal.

The Smoluchowski-Feynman ratchet proposes that extracting energy from the random motion in a heat-bath alone violates the second law of thermodynamics [11, 9, 5], since the entropy of the system will decrease. Brownian motion in a thermal bath cannot be biased by modifying structural features only. Moreover, it is postulated that there can be no net force on an object in a thermal bath by the Brownian motion of the particles.

This study, having a significant importance in Physics is also a crucial tool for understanding the movement of molecular structures in biological system such as protein movements in cell [7]. These molecules carry out motion by extracting energy from the surrounding medium [7]. The machines, as they may be called, doing Brownian motion behave differently from the macroscopic machines due the presence of energies in the order required for their motion and large enough to cause disturbances in the system. This energy is insignificant for the macroscopic machines.

It has been shown that energy can be extracted from a nonwhite thermal bath [5], but the study of perpetual motion of a system in a thermal bath alone has been studied recently[4]. The aim of this experiment is to verify directed transport of symmetry broken systems at equilibrium, proposed by [4].

The system consists of two coupled polystyrene beads of different diameter oscillating in a heat bath [Fig 1.0.1]. The system is held inside a glass capillary of inner diameter comparable to that of the larger bead to confine the motion of the system in one dimension. The inner diameter is kept low to prevent the toppling of the coupled beads inside the capillary, changing its course of motion

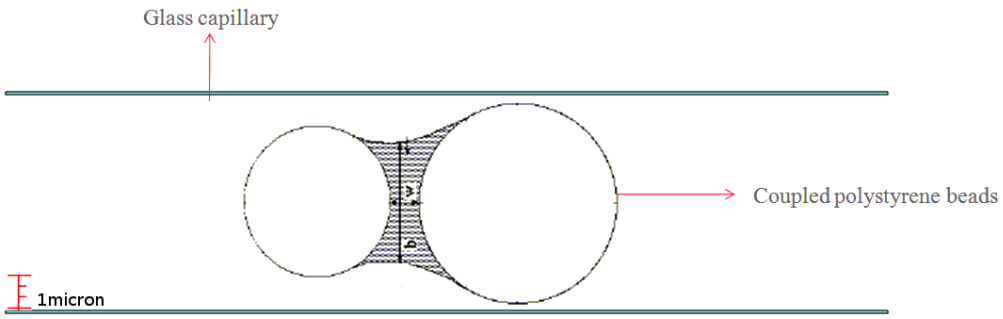

Figure 1.0.1: Schematic of the coupled oscillating beads inside a microfluidic channel.

and thus allowing Brownian motion in all dimensions. This will prevent us from observing any directed motion.

The system is dragged inside the capillary using a home built optical tweezer [10]. The motion of the system can be tracked using the tracking beam to verify directed transport in a thermal bath at equilibrium. Disadvantages of using tracking laser include heat generated due to the tracking laser which disturbs the equilibrium condition of the setup. Hence image processing of the trapped bead will be utilized to track the movements of the beads.

Position detection alone can be used to track the beads. Capillary action can pull the beads inside the micro fluidic channels. Optical tweezers are necessary because the system needs to be confined for a brief period before any further motion can be detected [4]. Furthermore tweezers give selective and directed maneuverability of the beads. Chemical treatment on the inner walls of the capillary to provide for the free flow of the beads, providing for the equilibrium conditions and the final tracking of the bead movements were impending till the submission of the thesis.

Chapter 2 of the thesis describes micro fluidic channels and their application in confining polystyrene beads in single dimension. It describes the procedure for making the channels in a laboratory and attaching them with an injector assembly to pump liquid through it. Chapter 3 deals with the theory, construction and calibration of optical tweezers. It describes the setup which in conjunction with the micro fluidic channels is used to study the confined polystyrene beads. Towards the end we have discussed the current status of the project, which includes calibration of the trap and trials made to move the polystyrene beads inside the micro fluidic channels. The codes used for processing the signals obtained from the quadrant photo diode have been provided in the Appendix.

Chapter 2

Micro fluidic channels

Microfluidic channels are thin capillaries having inner diameter of the order of a micron. Microfluidics deals with the flow, manipulation and analysis of fluids in microliter range. It assists life sciences studies related to microscale components in a biological sample. Other areas of application include chemical reactions involving drug delivery, low quantity reactions, DNA analysis, biosensors and chip based capillary electrophoresis. Advantages of using microfluidic channels include low sample usage, low Reynold numbers and high surface to volume ratio [1].

The pressure required to pump a liquid through a microfluidic channel is given by

$$P = Q.R_s\mu \tag{2.0.1}$$

where P is the pressure in bar, R_s is the flow resistance in mm^{-3}, Q is the flow rate ($\mu l/min$) and μ is the viscosity in mPa.s. Flow resistance of the fluid or channel is given by

$$R_s = \frac{6.79 * 10^{-9} * L}{d^4} \tag{2.0.2}$$

where L is the tube length (mm) and d is the pipe internal diameter.

Micro fluidic channels are commercially available in the market. The cost of these commercially available channels is quite high. There re-usability is subject to the fact that even stray objects like dust particles can block micro fluidic channels permanently.

2.1 Formation of microfluidic channels

Micro fluidic channels were built in our laboratory by heating and pulling cheaply available glass capillaries with inner diameter of .5 mm and outer diameter of 1 mm. The 10 cm long capillaries were clamped on one end with the other end attached to a weight. The thread attached on the other side of the capillary (with the weight) passes over a pulley. A Bunsen burner is used to heat

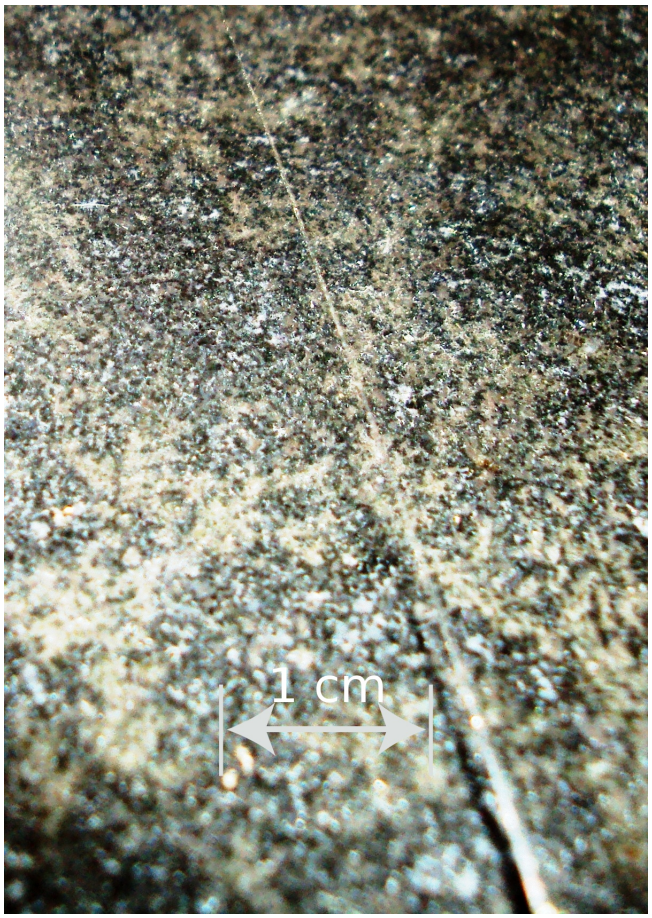

Figure 2.1.1: Glass micro fluidic channel (around 15cm long)

the capillaries in the middle. The glass gets pulled as it melts thus reducing the inner diameter. More weight increases the pulling force with which the glass is pulled which in turn reduces the inner diameter. The parameters are optimized to obtain capillaries of 2-5 micron inner diameter (Fig. 2.0.1). Polystyrene beads can be inserted inside the micro fluidic channel of inner diameter similar to that of the polystyrene beads, using an injector assembly, optical tweezer or capillary action. Optical tweezer is preferred as it gives control over the motion and selectivity of the trapped particles

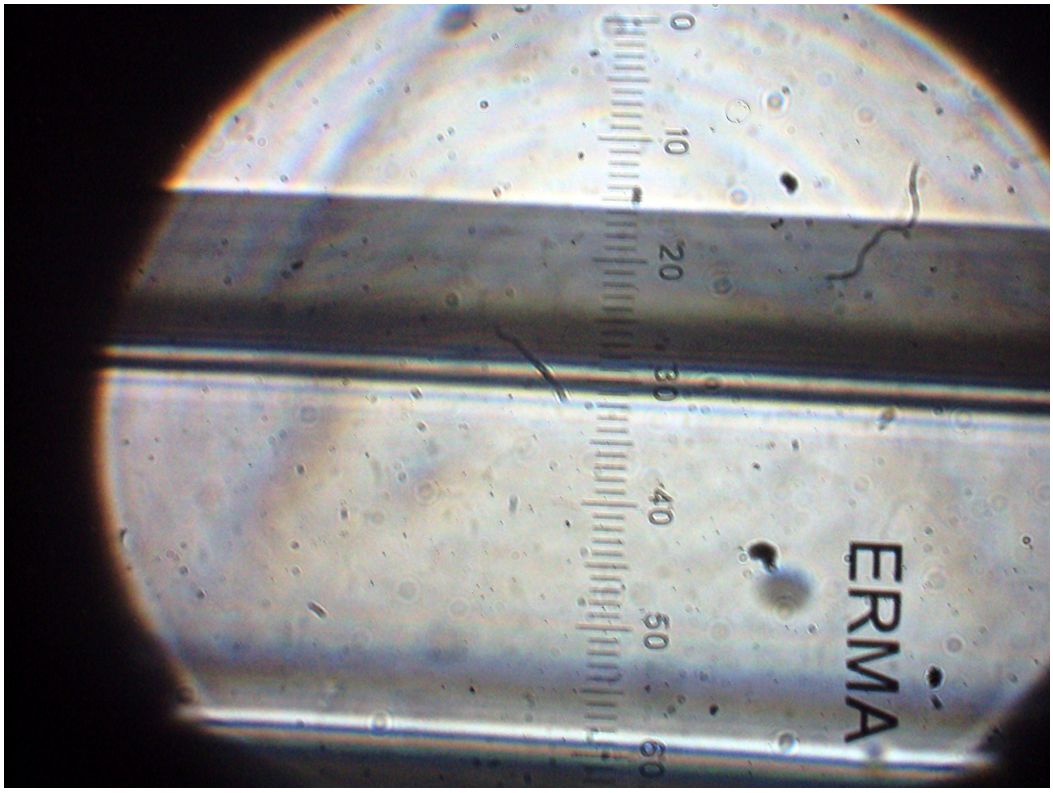

Figure 2.1.2: Micro fluidic channel Scale: 1div=1µm

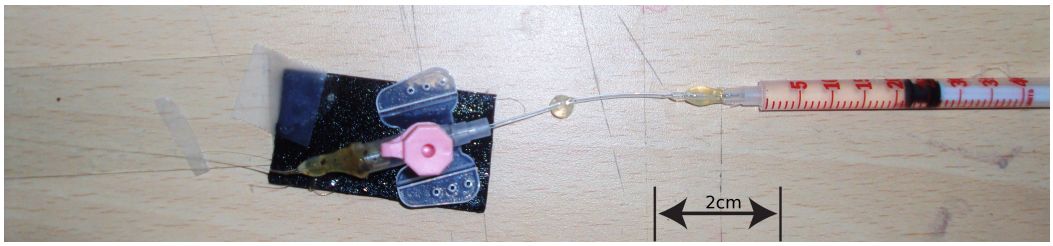

Figure 2.2.1: An injector assembly for pushing the beads inside the microfluidic channel. Various such methods were tried with satisfactory success in injection through direct capillary action.

2.2 Injector assembly

Fig. 2.0.1,2,3 shows another method for injecting fluids in micro fluidic channels is by an injector assembly. The indigenously build micro fluidic channel has a head (Original capillary size) and a tail part (stretched to micron size). An injector sealed with glue gun, is installed on the head of the capillary. Pressure needs to be applied to push the fluid through the micron size capillaries. This is contrary to the popular observation of capillary action which pumps the fluid on its own. Careful analysis reveals that the trapped air inside the head part through the tail requires application of pressure for a brief period of time (depending on the volume of air trapped), and not the drag force to push fluid through it. Injector assembly can be used for a regulated flow of fluid inside the capillaries, a possibility not available when the fluid is pumped through capillary action.

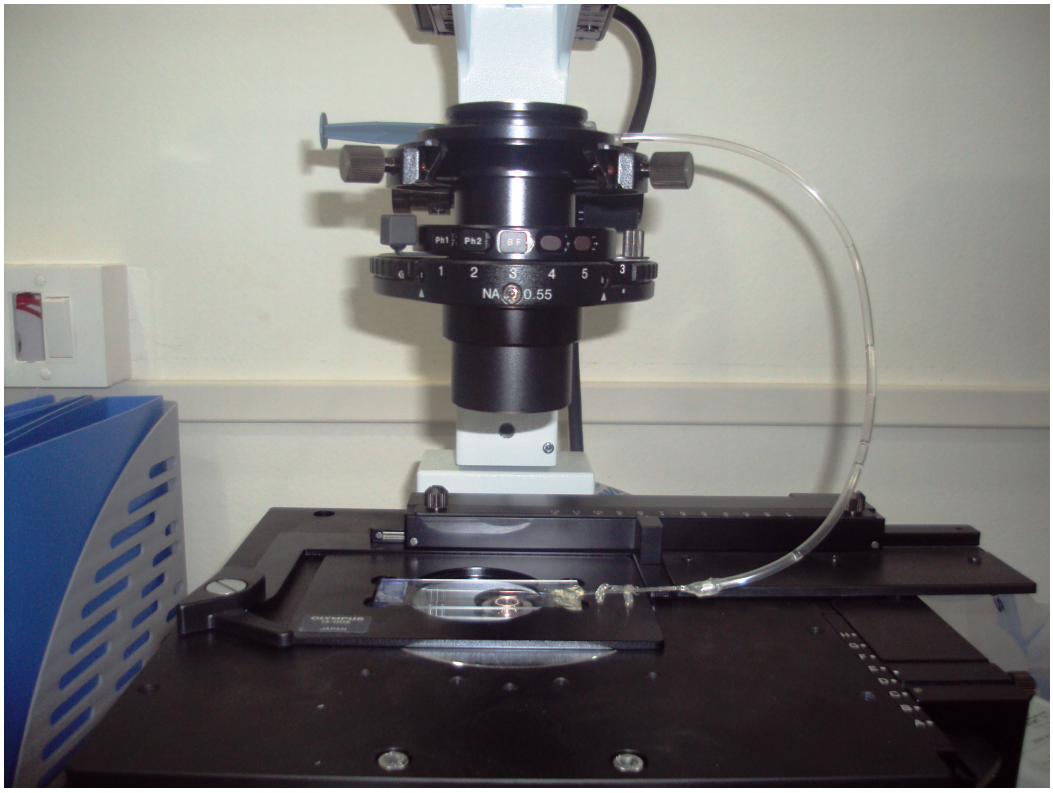

Figure 2.2.2: Injector assembly mounted on a IX71 Olympus microscope.

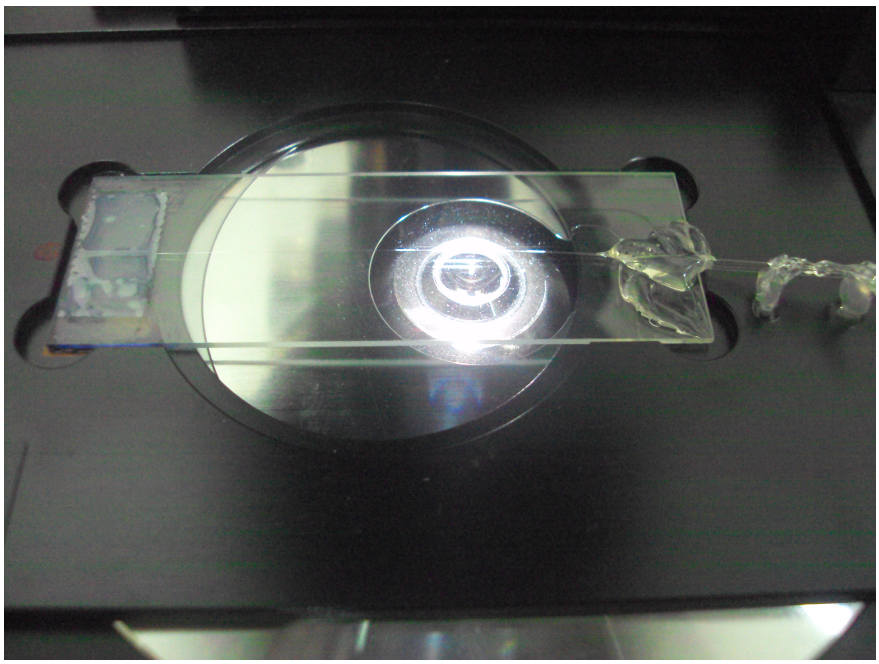

Figure 2.2.3: Microfluidic channel fixed on a slide holder.

Chapter 3

Optical Tweezer

Optical tweezers are devices which can hold micron size particles using an intense laser beam. As stated before, we needed to trap micron size polystyrene beads inside the capillaries and track their position as they move along in a thermal bath. Optical tweezers produce pico newton forces, sufficient to trap the beads in a liquid medium. Optical tweezers have position detection which can be exploited to track the confined coupled oscillating beads in a microfluidic channel.

Optical tweezers were first demonstrated by Arthur Ashkin [2, 3, 6]. The ability of a focused laser beam to form a three dimensional trap was exploited to trap micron sized particles from latex or polystyrene beads to studying the unfolding of protein molecules.

3.1 Formation of optical traps

A focused laser beam forms a trap with restoring force in all the three dimensions [2, 3]. A trap is formed by restoring force in all dimensions with a stable equilibrium at the center within the force limits. A focused laser beam exerts two kinds of forces on a trapped particle at its focus namely the scattering force and the gradient force. The scattering force is exerted by the photons colliding with the trapped particle, thus imparting an impulsive force on it. The net momentum change per unit time is transferred on the bead thus resulting in force which is directed towards the direction of beam propagation.

Gradient force is exerted by the spatial gradient formed from the Gaussian beam profile of the TEM_{00}mode laser. The force is directed towards the gradient of the Gaussian laser beam. Figure 3.1.1 shows two situations in which the particle experiences a restoring force. The laser beam in the first figure is does not falls on the center but is partially shifted rightwards. The right hand side intense region has more photons which get refracted than the left portion of the

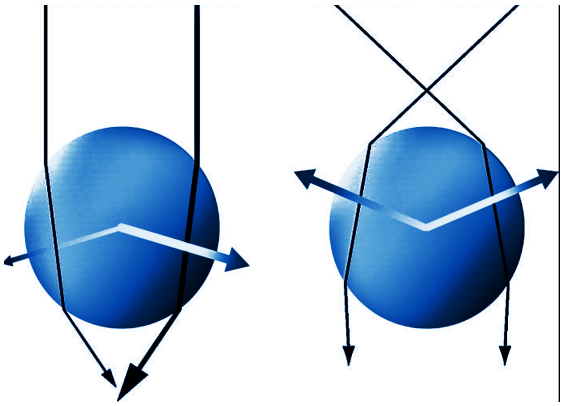

Figure 3.1.1: Formation of optical trap

beam. The particle will experience a net force in the right hand side direction towards the intense region of the beam. The particle is also shifted behind the focus and towards the laser source. A net force in the direction of propagation of beam will be experienced by the particle. In the second situation the particle is horizontally at the center of the laser beam hence there is no net force in the horizontal direction. But the particle is shifted away from the focus in the direction of propagation of laser beam. The free body diagram clearly shows net backward (towards the source) force acting on the particle.

The light ray refracted by the particle provides a net change in momentum, making the intensity variations the cause of a force directed towards the focus of the laser beam. The force is proportional to the gradient of the laser beam. The gradient force should be greater than the scattering force in order to form a stable trap. A high numerical aperture (NA) objective is used to make the gradient steeper, with its back aperture filled by the expanded laser beam[8].

The scattering force exerted by the beam:

$$F_{scatt} = \frac{\sigma n_m}{c} I_0 \tag{3.1.1}$$

where

$$\sigma = \frac{128\pi^5 a^6}{3\lambda^4} \frac{m^2 - 1}{m^2 + 2} \tag{3.1.2}$$

Here $I_0$is the intensity of the incident light, $\sigma$is the scattering cross section of the sphere, $n_m$is the index of refraction of the medium, c is the speed of light in vacuum, m is the ratio of index of refraction of the particle to the index of the medium (n_p/n_m),and λ is the wavelength of the trapping laser.

The gradient force is given by:

$$F_{grad} = \frac{2\pi\alpha\nabla I_0}{cn_m^2} \tag{3.1.3}$$

where

$$\alpha = n_m^2 a^3 \frac{(m^2-1)}{(m^2+2)} \tag{3.1.4}$$

is the polarizability of the sphere.

3.2 Requirements

3.2.1 Layout

The optical trap produces forces of the order of pico newtons. In order to avoid vibrations of this order, traps are built on vibration isolation tables. A trap consists of a trapping laser, tracking laser, beam expander, an inverted microscope with illumination, a CCD and a quadrant photo diode attached to it. A xy nano positioner can be installed for precise movements of the trapping chamber. An inverted is microscope preferably used to form a trap.

3.2.2 Trapping laser

The trapping laser should be powerful enough to generate a stable optical trap. Gaussian beams (TEM_{00}mode) are preferred over others since it forms a structurally adequate trap. Fluctuations in the power output of the laser can lead to instability in the trap. Infrared lasers are preferred for biological specimens to minimize the losses caused due to the intense laser beams [8]. Our system uses 1watt, 1064nm, diode pumped solid state laser for trapping and 15mW, 780nm Toptica laser for tracking the particle. The beams are mixed by a dichroic which allows the tracking laser to pass through it and reflects the trapping laser. DPSS lasers have their power supply detached from the laser, thus reducing vibrations and heat near the laser.

3.2.2.1 Beam width measurement

The Gaussian beam profile of the TEM_{00}mode laser is given by:

$$I(x,y,z) = \frac{2Pe^{-2(x^2+y^2)/w^2(z)}}{\pi * w^2(z)} \tag{3.2.1}$$

where P is the total power, w(z) is the z axis independent measure of the beam waist.

Knife edge method was used to measure the beam waist of the 1 watt, 1064 nm laser. The beam is blocked completely by a knife edge which is mounted on an x-y stage. Power is measured using a detector (Coherent, Lab max top) mounted on the beam path. The knife is moved out of the beam path with power measurements taken after .1mm.

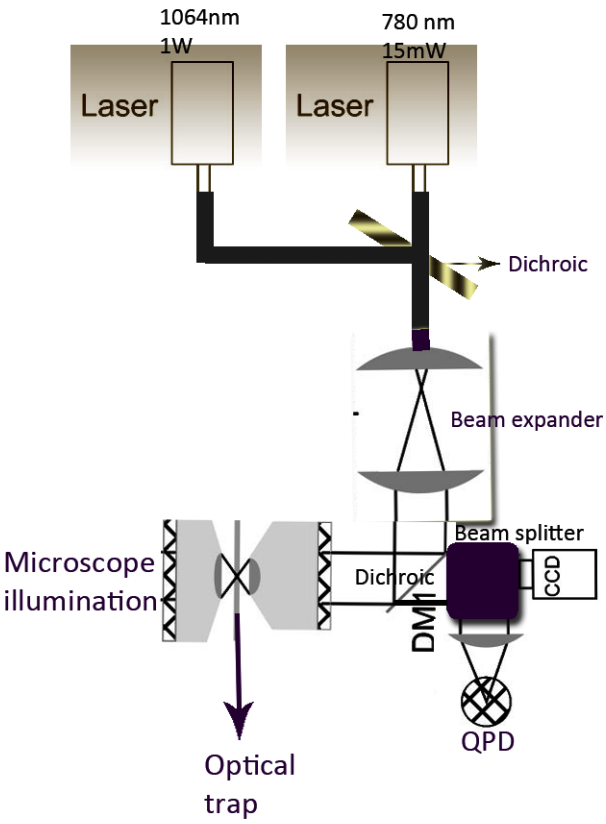

Figure 3.2.1: Schematic of our optical trap

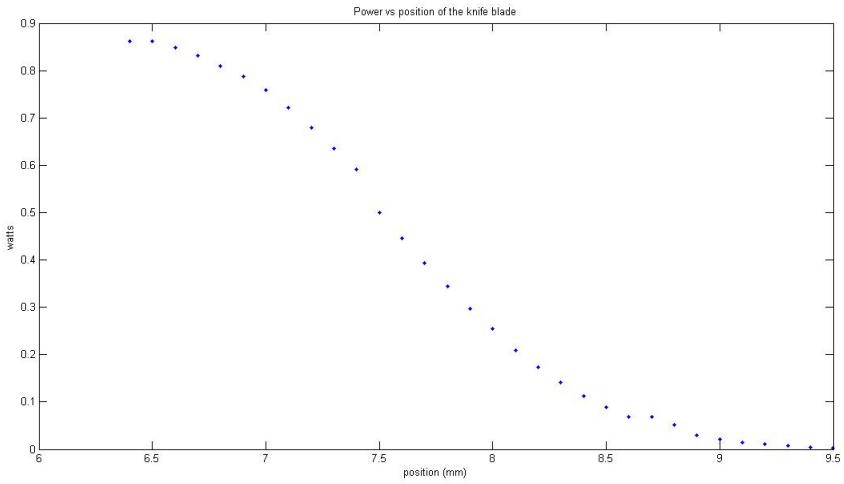

Figure 3.2.2: Beam power with respect to the position of knife edge

As the knife blade is moved the power in the detector rises. The fraction of power that is received at the position x_0 of the knife blade is given by

$$R = \frac{Pdetector}{P} = \int_{x=x0}^{inf} \int_{y=-inf}^{inf} \frac{2 * e^{-2(x^2 + y^2)} w^2}{\pi W^2} dydx \tag{3.2.2}$$

Beam waist is defined as the width at which the power drops to 10% of the maximum power. Putting x_0=$x_{10}$and x_0=x_{90}we get the beam width as

$$W = .7803(x_{10} - x_{90}) \tag{3.2.3}$$

The beam width comes out to be 1.248 mm. The numerical aperture of the 60 x objective is 1.2. The beam is expanded by a beam expander (100mm/50mm) in order to over fill the back aperture of the objective which forms a steep gradient at the focus.

3.2.3 Microscope

Microscopes reduce design complexities of optical traps, while reducing the freedom of manipulation. An inverted microscope is a preferred choice for the construction of a trap since it provides stage maneuverability instead of objective maneuverability, which provides for the fixation of the objective in the optical path. Our setup uses Olympus IX71 inverted microscope with 60x objective to with a Jenoptik CCD camera having a fire wire port. A filter which reflect 1064 nm laser completely is installed below the objective to receive signals at the QPD and camera from the tracking beam only. A xy nano positioner from

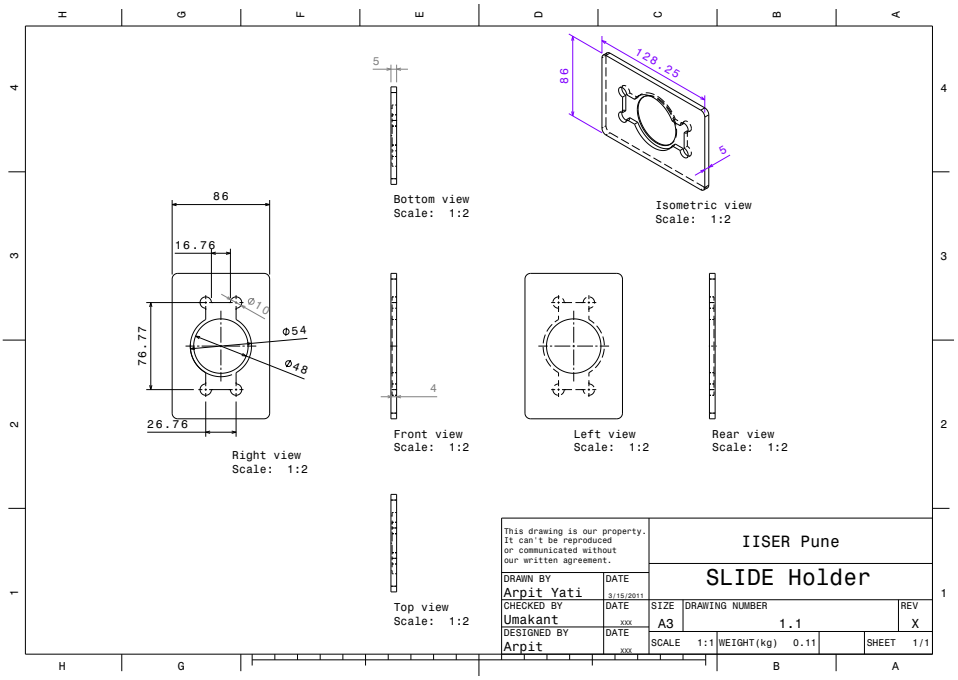

Figure 3.2.3: Slide holder with increased width for vibration damping

NEW FOCUS is mounted on the microscope for precise movements of the slide holder. A new slide holder with 5 mm thickness (more than the standard slide holder) is designed and build indigenously to reduce vibrations.

3.2.4 Position detection

The quadrant photo diode (QPD) is mounted on a xy manual stage in the optical beam path at the camera port of the inverted microscope. The QPD records the signal, amplifies it and sends it to the oscilloscope.

3.2.5 Sample preparation

The sample for the optical trap should not dry out immediately by the heat produced by the laser and the halogen lamp of the microscope. The beads in the sample were prevented from settling on the base of the sample holder by adding PbS buffer to the solution [12] . It is observed that once the polystyrene

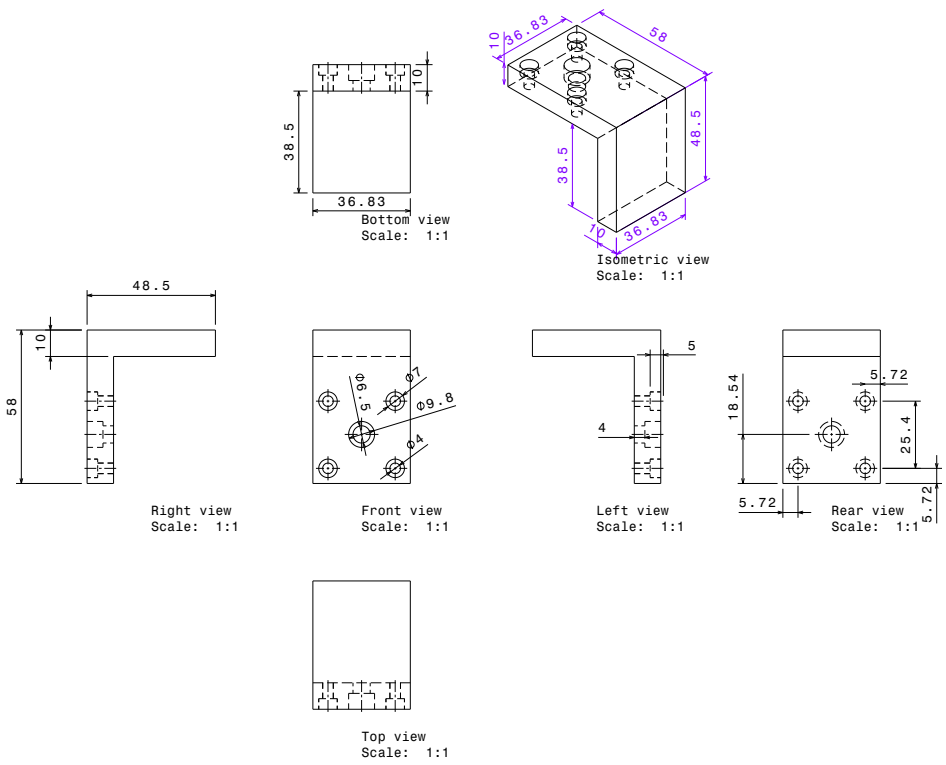

Figure 3.2.4: Right angle mount for NEW FOCUS XY positioner

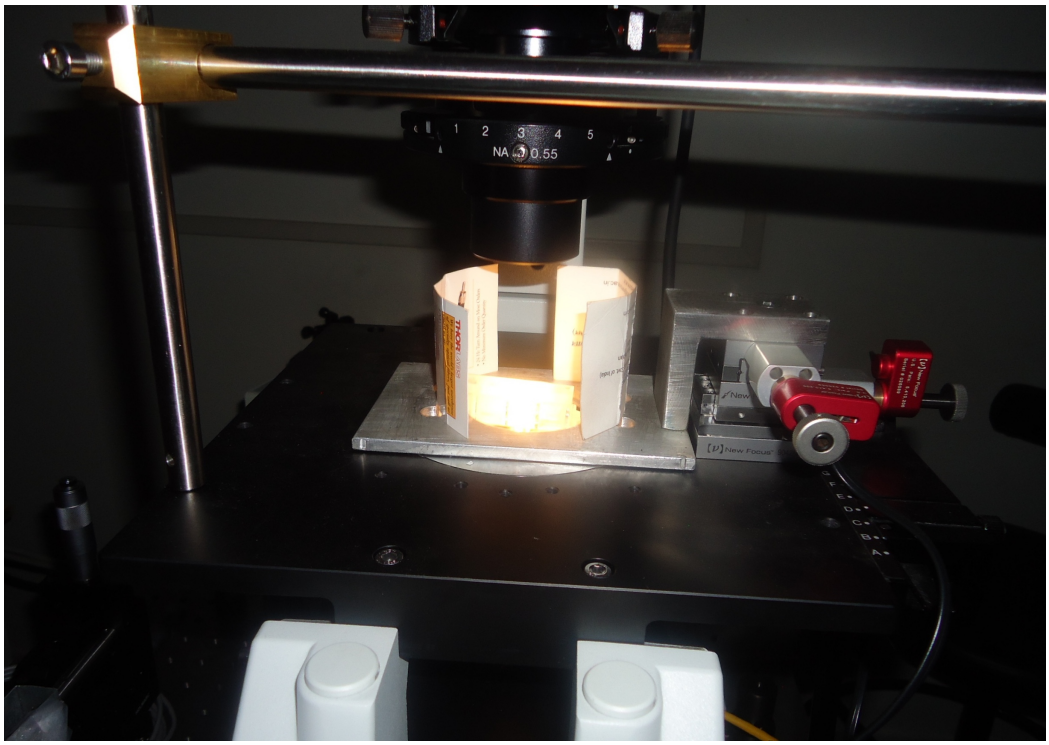

Figure 3.2.5: Side view: sample holder attached to the NEW FOCUS x-y stage

beads stick to the base of the holder, it makes the cell useless before it is washed again thoroughly. The stuck beads and other dust particles at the base scatter the incoming laser radiation before the trapped bead, thus increasing the noise in the final signal and reducing the signal to noise ratio. It is found that the cell can be made temporarily useful again by putting acetone or alcohol in the sample cell, which mobilizes the beads again.

3.3 Construction of Optical Tweezer

A 1 watt 1064 nm laser is mixed with a 780nm low power (15 mW) (tracking laser) using a dichroic. The laser beam is expanded using a telescope in order to fill the back aperture of the objective lens. The objective behind expanding the beam lies in the fact that the trapping force is proportional to the intensity gradient (eqn. 0.0.3). Expanding the beam overfills the back aperture of the focussing objective lens, thus increasing the intensity gradient. The objective focuses the laser beam with a 100x objective in a cell consisting of beads. The cell is mounted on an indigenously build Aluminium slide holder with increased thickness of 5mm to reduce vibrations. The slide holder is driven by a nano-positioner from NEW FOCUS. The positioner can move the slide by 30nm in each pulse. The light reflected back from the trapped beads is passed through a filter which selectively allows 780 nm laser beams to pass through it. A beam splitter allows the beam to be seen by a camera as well as recorded by a quadrant photo diode (QPD). The signal obtained from the QPD is amplified 20 times using an electronic amplifier and recorded in a digital oscilloscope. A QPD consists of four quarter moon shaped photo diode arranged in the four quadrants to make a circle with gaps in between them. When aligned, the laser spot falls in the center of the QPD. The three output signals from the QPD are the sum signal, i.e. the total intensity of the laser beam, delta x, the difference in the intensity of the left and the right quadrants and delta y, the difference in the intensity of top and the bottom quadrants. The oscillations of the beads can be recorded by fluctuations in the laser beam received by the QPD.

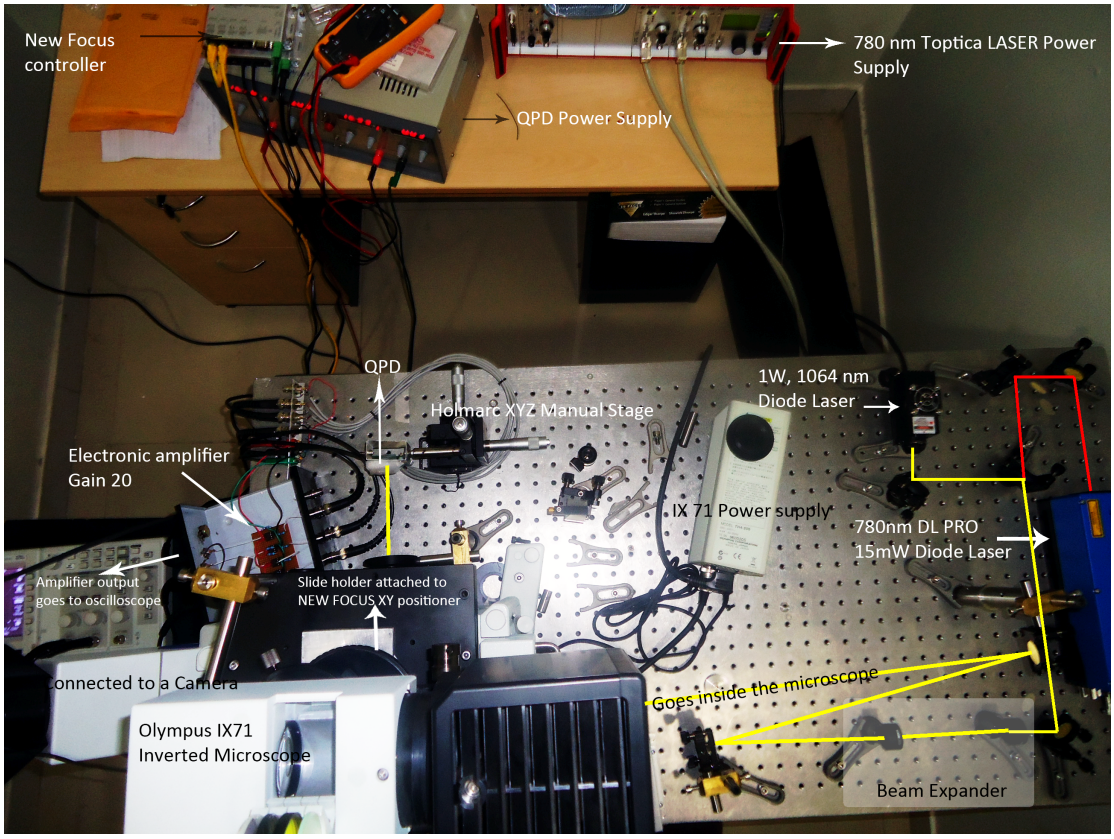

Figure 3.3.1: Optical Tweezer setup

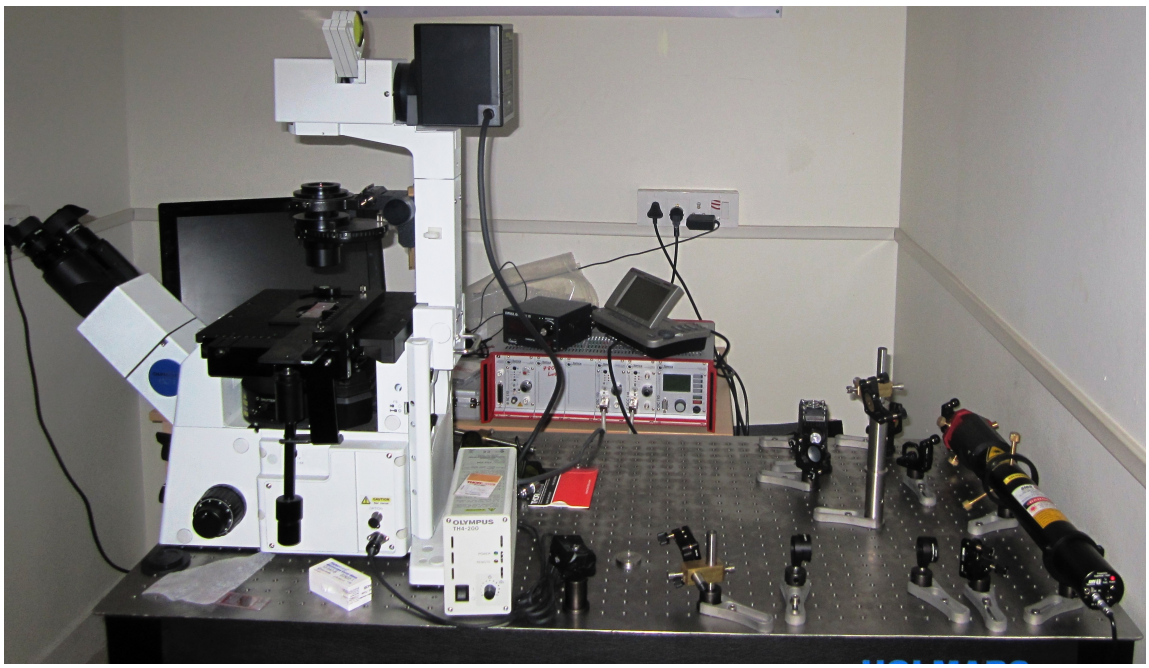

Figure 3.3.2: Optical tweezer setup: side view

Chapter 4

Results and discussion

Microfluidic channels and optical tweezers were constructed and calibrated to determine the trap stiffness. Pushing the coupled oscillating beads into the microfluidic channels, chemical or physical treatment of the inner walls of the capillary to reduce resistance during motion of the coupled system needs to be done.

4.1 Calibration of optical tweezer

For small regions the trap behaves according to the Hooke's law, F=-αx [8]. The trap constant α is determined by the roll off frequency in the power spectrum curve, $f_0 = \alpha(2\pi\beta)^{-1}$ (Fig. 4.1.1). The power spectrum is obtained by

$$S_{xx}(f) = \frac{k_B T}{\pi^2 6\pi\eta a(f_0^2 + f^2)} \tag{4.1.1}$$

where S_{xx}(f) is in units of displacement^2/Hz, η is the viscosity of the medium which in our case is water, a is the diameter of the bead and $f_0$is the roll off frequency. The detector measures the un calibrated power $S_{vv}(f)$=$\rho^2 S_{xx}(f)$ where ρ is the linear sensitivity of the detector. Sensitivity can be found by the product of power spectrum and frequency squared $S_{xx}(f)$.f^{2}which asymptotically approaches $k_B T/\pi^2 6\pi\eta a$. Hence $\rho$is given by:

$$\rho = [S_{vv}(f)\pi^2 6\pi\eta a/k_B T]^{1/2} \tag{4.1.2}$$

100K points at 100KSamples/second were recorded. The desired frequency range to be scanned is of the order of 1000Hz which is lower than our sampling frequency. According to Nyquist theorem the sampling frequency should be at least twice the frequency to be observed. The force constant for the setup is obtained by

$$\alpha = f_0 2\pi(6\pi n1) \tag{4.1.3}$$

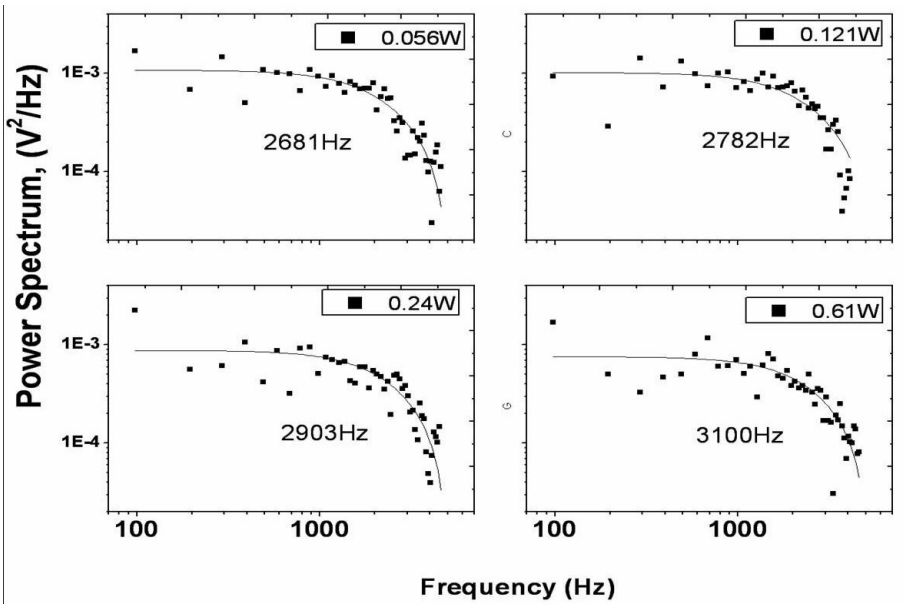

Figure 4.1.1: Power spectrum density

The force constant for a 6 micron bead, with a 1 watt laser comes out to be around .02pico Newton/nm.

Trapped beads can be directed inside the micro fluidic channel by holding the beads and moving the stage using the nano positioner. Presently single beads have been moved inside the channels. The beads stay trapped up to a few microns inside the channels.

4.2 Conclusion

Although we have some estimation of the parameters, with the existing techniques in our laboratory, it is difficult to construct micro fluidic channels with pre-determined inner diameter. We expect to make the inner diameter of the micro fluidic channels equivalent to the bead sizes. Coupling two beads of different diameter is a regular protocol work and can be done by our laboratory. Coupling is important because the expression for velocity of the coupled bead is is dependent on the stiffness of the coupling. Some modifications need to be done to improve the signal to noise ratio. We have planned to optically filter the laser beam received at the QPD to reduce the noise accompanying our signal. A lens to focus the beam has to be installed before the QPD to prevent the loss

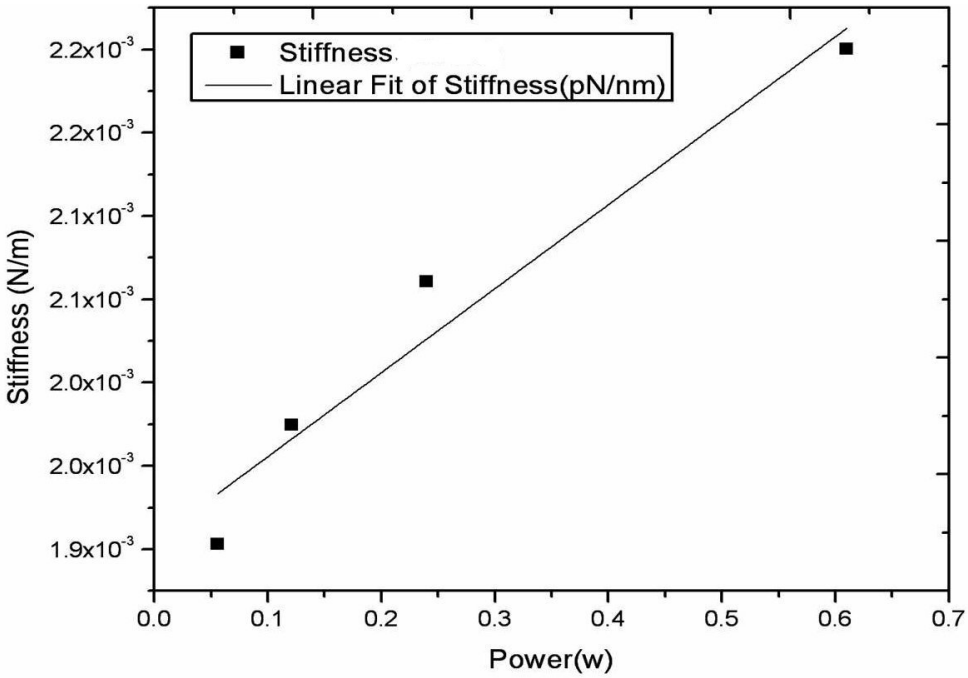

Figure 4.1.2: Force constant vs power

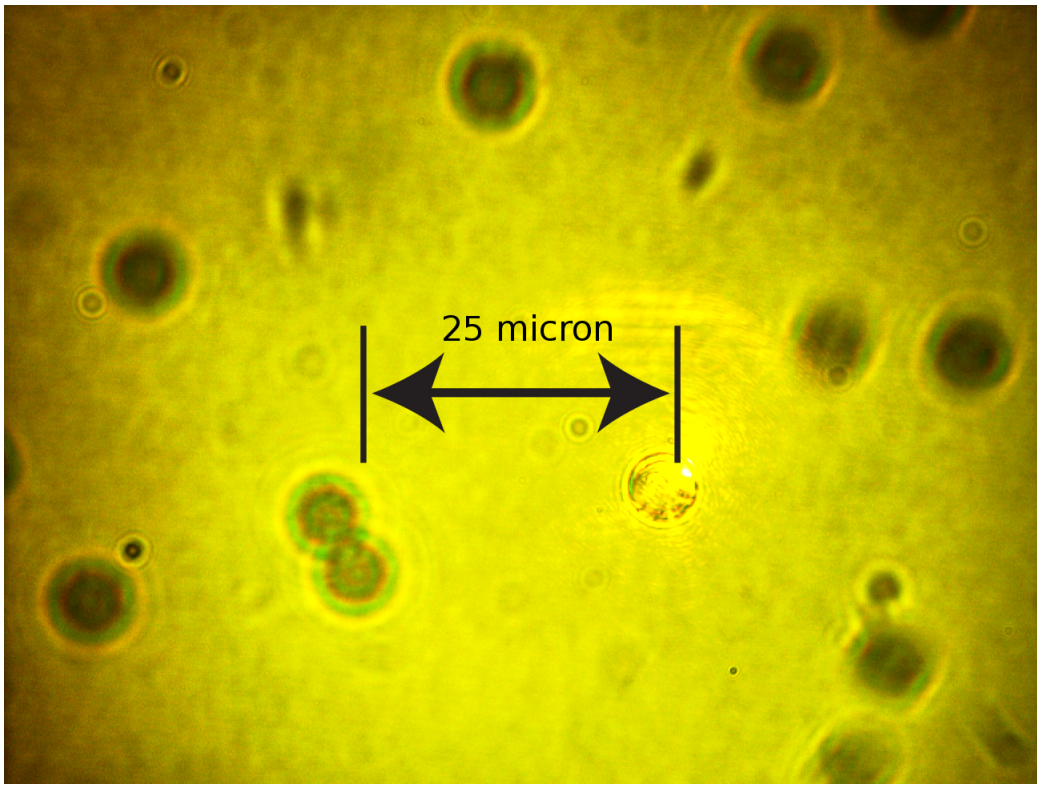

Figure 4.1.3: Trapped bead (Note the motion of other beads by their blurry image) diam=6μm

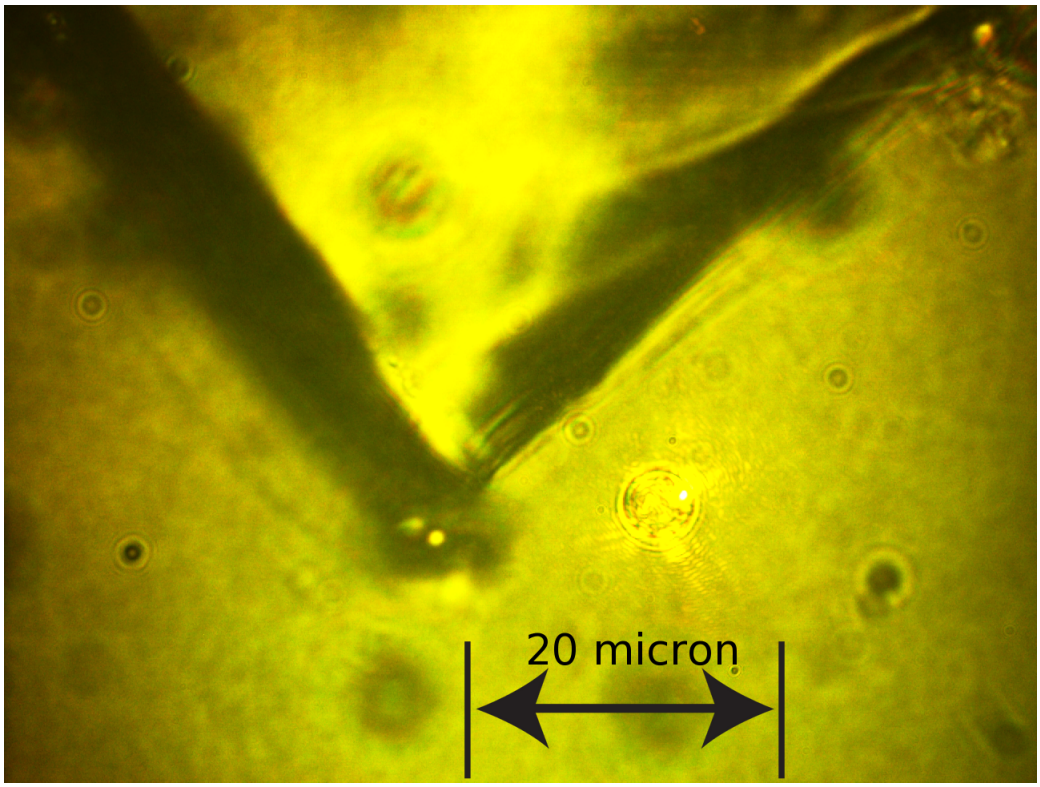

Figure 4.1.4: Trapped bead about to enter the microfluidic channel

of information by a scattered light. With the available setup, examining the coupled polystyrene beads and determining their motion will be possible within next few days.

In addition the calibrated optical tweezer can be used to pull proteins or other biological molecules. The protein folding forces can be found out using the stiffness of the trap.

Appendix

The code used for FFT and power spectrum density:

```
Fs = 5000; % Sampling frequency
T = 1/Fs; % Sample time
L = 100000; % Length of signal
t = (0:L-1)*T; % Time vector
size(data)
for j=1:L
t(j)=data(j,1);
%freq(j)=1/t(j);
ch2(j)=data(j,2);
ch3(j)=data(j,3);
ch4(j)=data(j,4);
ch2norm(j)=ch2(j)/ch4(j);
end
NFFT = 2^nextpow2(L); % Next power of 2 from length of y
ch2norm = fft(ch2norm,NFFT)/L;
f = Fs/2*linspace(0,1,NFFT/2+1);
spectrum=2*abs(ch2norm(1:NFFT/2+1))
% Plot single-sided amplitude spectrum.
plot(f,2*abs(ch2norm(1:NFFT/2+1)))
title('Single-Sided Amplitude Spectrum of y(t)')
xlabel('Frequency (Hz)')
ylabel('Power spectral density (V^2/Hz)')
```

Bibliography

[1] *Dolomite Microfluidics catalogue*, 2011.

[2] A. Ashkin. *Phys. Rev. Lett.*, 24:256, 1970.

[3] A. Ashkin and J. M. Dziedzic. *Appl. Phys. Lett.*, 19:283, 1971.

[4] Arijit Bhattacharya. Directed transport in equilibrium. *Archives*, 2011.

[5] 2 C. Van den Broeck, 1 R. Kawai and P. Meurs1. Microscopic analysis of a thermal brownian motor. *PHYSICA L R EVI EW L ET T ERS*, 93:9, 2004.

[6] A. Ashkin et al. *Opt. Lett.*, 11:288, 1986.

[7] Marcelo O. Magnasco. Forced thermal ratchets. *Physical Review Letters*, 1993.

[8] Keir C. Neuman and Steven M. Block. Optical trapping. *Review of Scientific Instruments*, 75:9, 2004.

[9] Feynman R.P. *The Feynman Lectures on Physics, Vol. 1*, 1, 1963.

[10] Optical tweezers for velocity mapping in microfluidic channels. Jing wu, daniel day, min gu. *Optical Society of America*, 2009.

[11] M. v. Smoluchowski. *Phys. Z.*, 13:1069, 1912.

[12] Robert F Marchington Nikolaus K Metzger & Kishan Dholakia Woei Ming Lee, Peter J Reece. Construction and calibration of an optical trap on a fluorescence optical microscope. *Nature Publishing Group*, 2:3026, 2007.