Development of 2D Optical Tweezing for Microscopy

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A dissertation submitted for the partial fulfillment of BS-MS dual degree in Science

Department of Physical Sciences
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Mohali

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Certificate of Examination

This is to certify that the dissertation titled "**Development of 2D Optical Tweezing for Microscopy**" submitted by Vishnu K P (Reg. No. MS15011) for the partial fulfillment of BS-MS dual degree programme of the institute, has been examined by the thesis committee duly appointed by the institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Ananth Venkatesan	Dr. Sanjib Dey	Dr. Samir Kumar Biswas (Supervisor)

Dated: June 15, 2020

Declaration

The work presented in this dissertation has been carried out by me under guidance of Dr. Samir Kumar Biswas at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have detailed in the bibliography.

Vishnu K P
(Candidate)

Dated: June 15, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Samir Kumar Biswas
(Supervisor)

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Dedicated to Vaidehi and Trilok.

Abstract

Optical tweezers find wide applications in many fields of science. The revolution created by it's invention by Arthur Ashkin sparked advance research in the field which led to the development of highly accurate devices for optical trapping. Although other methods are widely researched to develop more precise moving stages, electromagnetism based devices are often overlooked. These novel moving platform devices have the potential to bring in another revolution in the field of optical tweezing and nanotechnology.

The project aims to research on one such device which can potentially give the same accuracy and precision as the high end devices in existence. The approach is to automate the device to see how it function in the µm domain. The project also focuses on developing this idea into an optical tweezer for commercial purposes.

Optical Tweezers - An Overview

1.1 Introduction

Optical tweezers are powerful tools used to manipulate microscopic particles using highly focused laser beam. Since its invention in the early 1970s, optical tweezers have found numerous applications in Physics, biology and other allied fields. Early optical tweezers were mainly used for manipulating micron sized dielectric particles, trapping neutral atoms and studies on microorganisms. Recent advancements in the field paved way for more specific functions including study of mechanical properties of RBCs, trapping of gas microbubbles, study of chiral optical forces, applications in nanotechnology like assembly of nanodevices, optical force positioning, aggregation and spectroscopy, study on Casimir forces and research in stochastic thermodynamics[1].

The latest technological advancements led to the development of stages which can move in 3D with nanometer precision. These stages, when incorporated into the trapping device, resulted in higher spatial precision and improved calibration of both forces and displacements[2].

1.2 The working principle

Optical tweezers are used widely nowadays and are even capable of trapping particles as small as an atom.The laser beam is first allowed to pass through a microscopic objective with high angle of refraction. This focused laser beam exerts a radiation pressure which helps in trapping the particle. Two forces - the gradient force and scattering force - should be given emphasis at this point

1.2.1 Gradient force

The gradient force arises from the gaussian intensity profile of the laser beam used. Gradient force is a restoring force and acts to pull the particle to the centre of the beam.

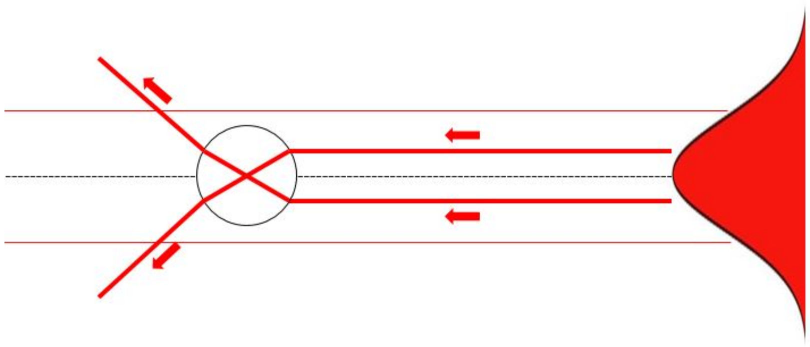


FIGURE 1.1: Laser beam passing through the particle which is present at the centre of the laser beam.

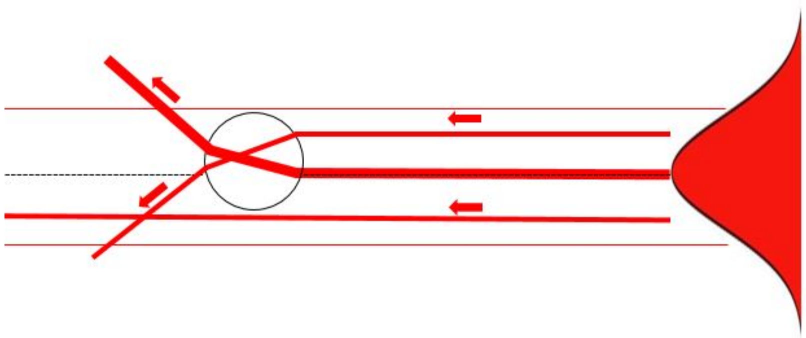


FIGURE 1.2: Laser beam passing through particle which is displaced slightly from the centre of the laser beam.

When the particle is at the centre of the laser beam, as given in figure 1.1, the momentum change in the upward direction and downward direction are the same. The resulting force due to conservation of momentum in the upward and downward direction balances each other. Thus the particle will continue to stay in equilibrium in the vertical axis of the figure.

However, a slight change in the position of the particle from the equilibrium will give rise to a force which is restoring in nature. In the example given in figure 1.2, the particle is displaced upward from the equilibrium. Thus the change in momentum will be more in the upward direction. In order to conserve the change in momentum, a force will act on the downward direction, which will try to pull back the particle to equilibrium. This force is the gradient force and is so called due to its origin from the gradient of laser beam.

1.2.2 Scattering force

Laser beam applies a force on the particle in the direction of propagation of the beam. This arises as a consequence of the conservation of momentum.

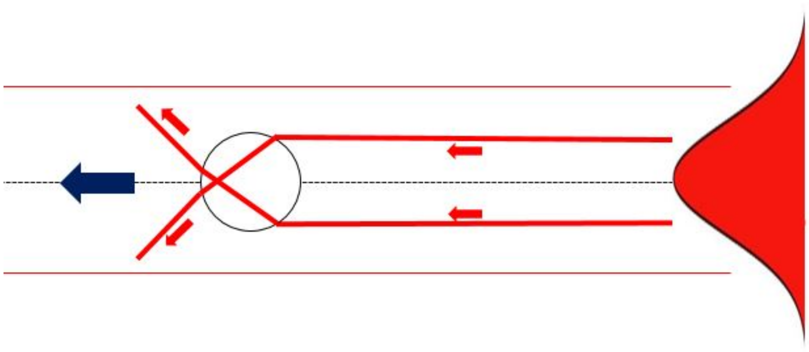


FIGURE 1.3: Laser beam passing through particle at centre of the laser beam. Direction of transfer of momentum is given.

In figure 1.3, some of the laser beam is absorbed by the particle while others get refracted. This absorbed beam transfers its momentum to the particle, thus enabling a movement in the direction of propagation of the laser (direction of blue arrow). Therefore the neutralization of gradient force alone will not give a stable trapping mechanism.

The problem caused by the instability of trap in the direction of the laser beam is solved by the introduction of a lens with high angle of convergence. When the lens is introduced, the light that comes out of the particle travels more in the direction of the laser beam than the light going into the particle. So the light coming out has more momentum in the direction of beam propagation than light going in. This implies that there is more momentum in the direction of propagation.

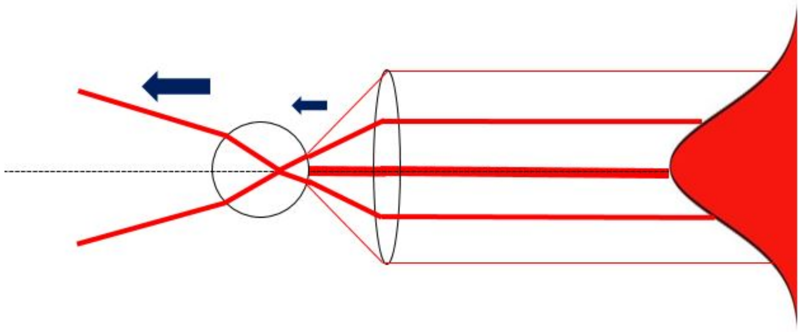


FIGURE 1.4: Principle of momentum change in direction of Laser beam.

In order to conserve momentum, a net force pulls back the particle in the direction opposite to the propagation of the beam. This holds the particle in equilibrium in the horizontal plane as well. The optical trap thus formed will be stable.

1.3 Conventional Optical Tweezer Setup

The beam emitted from the laser source travels through different components and enters the objective lens. It then converges to form a spot which is used to trap the particle. An insight into the different components of an optical tweezer proves helpful at this point.

- **Trapping laser** : A collimated trapping laser beam from a 1064 nm Nd:YAG laser is used mostly in the commercial systems. This laser is incredibly compatible with biological systems due to low absorption coefficient.
- **Half-wave plate** : Half wave plate is used to shift the polarization direction of linearly polarized light.
- **Acousto-optic deflector (AOD)** : AODs are used to deflect laser beams using sound waves created in TeO2 crystals by piezo-elements inside the device. The crystals are used in pairs.
- **Dichroic Mirrors** : Dichroic mirror allows passage of light of a certain wavelength and reflects light of other wavelengths.
- **Polarizer** : A polarizer is an optical filter which allows the passage of light of a certain polarization.

- **Wollaston prism** : A Wollaston prism seperates light into two different linearly polarized beams with orthogonal polarization.
- **Condenser** : Condenser lens are used to concentrate the light emitted from the lamp and focus it to illuminate the particle specimen.
- **Quadrant Photodiode (QPD)** : QPD is a detection device which can measure the centre of the interference pattern formed at the back focal plane of condenser by the laser beam and forward scattering by the particle[3]. This measurement allows it to track the position of the particle.

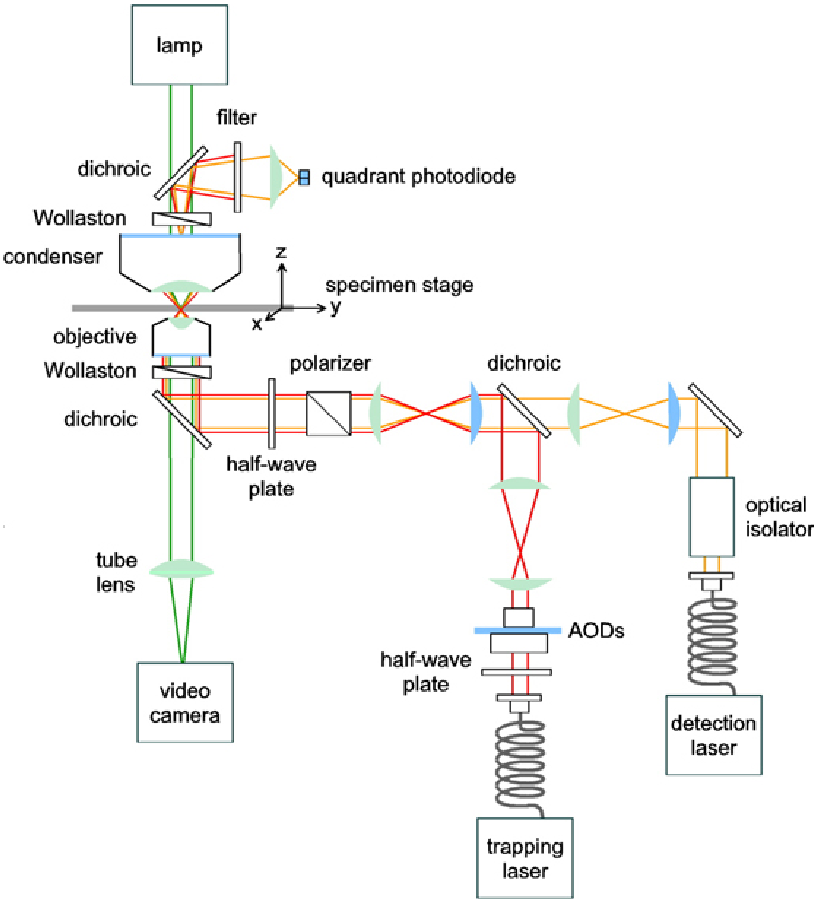


FIGURE 1.5: Modern optical tweezer apparatus. Image adapted from Block lab, Stanford University

1.4 Measurement of forces associated with trapping

Optical tweezers are capable of generating forces of the range 10^{-13} N to 10^{-10} N (piconewton range). Present day optical tweezers come with mechanism to estimate this force. Many methods exist to calibrate an OT to measure the force. A commonly found method is given below.

1.4.1 Virtual Spring Method

Virtual spring method treats the optical trap like a harmonic potential. In a harmonic potential, any object which is displaced a distance Δ x from the equilibrium will experience a restoring force given by:

$$F = \kappa \Delta x$$

In order to measure the force, the particle is displaced from the centre of the trap and the displacement is measured using the photodetector. In order to find κ, an external known force is applied to the particle and the equation above is used.
A fluid flow around the particle can create a drag force which displaces the particle from the centre. This drag force can be found using the Stokes equation given by:

$$F = 6\pi\eta a V$$

Once the stiffness of the trap is known, the associated force can be measured directly.

1.5 Aim

The advancement in the field of piezo electric stages and micromotors often tends to shadow the possibilities present in developing electromechanical devices for the same purpose. These novel devices, which make use of magnetic repulsion/attraction, is free from forces like friction. This opens up opportunity to develop highly accurate scanning devices, the stepsize of which can be lowered easily to the nanometer range. Such devices, which can be assembled using old electronic parts, give opportunities for labs to build optical tweezers at minimum cost.

The project aims to develop a scanning stage, which makes use of the principles of electromagnetism, with a step size almost touching the nanometer range. It further envisions to integrate this stage into an optical tweezer apparatus and acquire stable trapping.

Mechanical Design and Calibration of Automated 2D Scanning Stage

2.1 Introduction

The real revolution in the field of Optical manipulation came with the shift from manually controlled xy stages to fully automated motor-controlled stages and piezoelectric stages. The different vaieties used today include Piezo Nanopositioners, Piezo motor-driven Actuators and stages, motorized micropositioners and Hexapod 6-Axis stages. Three-dimensional (3D) piezoelectric stages with capacitive sensors have afforded unprecedented control of the position of a trapped object. Incorporation of such stages into optical trapping instruments has resulted in higher spatial precision and improved calibration of both forces and displacements[2].

(A) (B) (C)

FIGURE 2.1: (A) Long travel XY Piezo Linear-Motor Stage.(B) Piezo micromanipulator/ nanopositioning motors. (C) Miniature motorized linear stages & linear sliders. Images are adopted from Physik Instrumente(PI).

The XY stage setup designed and calibrated in this project makes use of an idea far removed from micro motors and piezoelectric stages. A collection of three solenoids placed inside two permanent magnets can provide high position resolution when appropriate current input is given to the solenoid. This assembly is then integrated into the optical tweezer to move the stage.

2.2 XY stage design using electromagnets

The original design of the system was conceived by Dr Samir Kumar Biswas. It works by the principle of mutual induction. An assembly of three solenoids is kept in between two permanent neodymium magnets. When current is passed through these solenoids, they generate a magnetic field which will cause a repulsion with the neodymium magnets. This repulsion is carefully calibrated to obtain the desired movement.

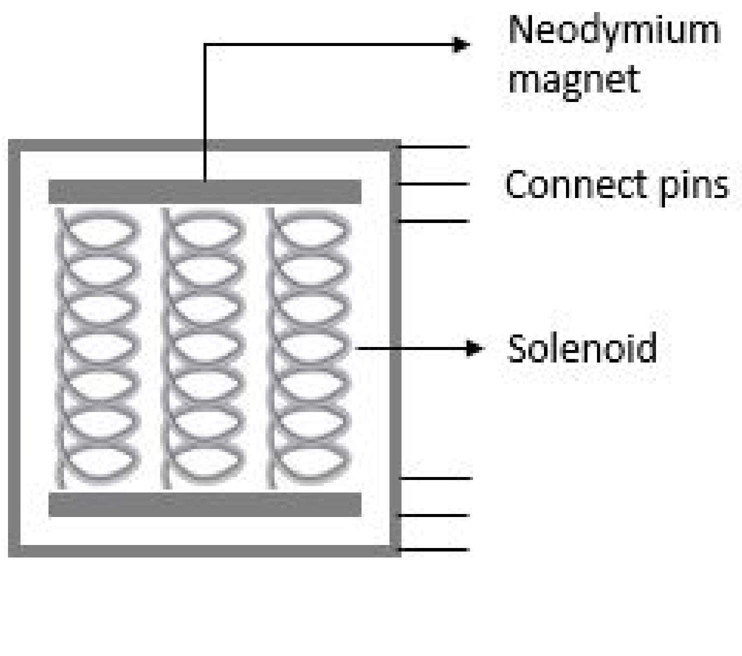
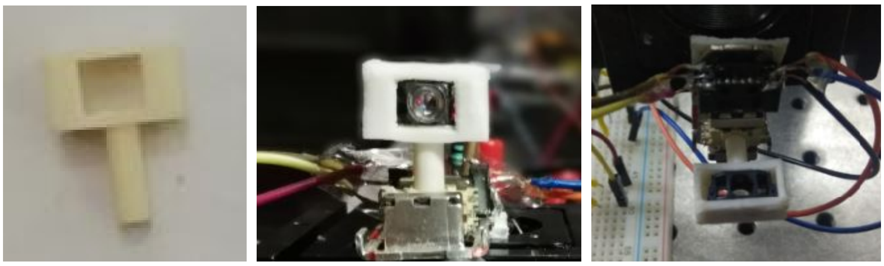


FIGURE 2.2: Schematic of solenoid-magnet assembly

The centre solenoid controls the movement of X direction and the solenoids at the ends work collectively to control movement in the Y direction. A small stage of dimension 20mm x 10mm x 4mm was designed using solidworks, 3D printed and attached to the apparatus earlier.



(A) (B) (C)

FIGURE 2.3: (A) 3D printed stage to hold the lens.(B) Stage integated into the setup. (C) Top view of stage integrated into the setup.

2.3 Setting Up The Device

The connecting pins of the solenoid was connected to Keithley 2230 power meter.

2.3.1 Keithley 2230 Power Meter

Keithley 2230 is a triple channel power supply which can provide power upto 195W. They have three programmable isolated channels, allowing the user to connect multiple circuits with different references. They support RS-232, USB and GPIB interfaces which make automated testing possible. This power source also provides 0.03% basic voltage accuracy and 0.1% basic current accuracy. The power meter can provide a minimum current of 1 mA.

(A) (B)

FIGURE 2.4: (A) Front view of Keithley 2230 .(B) Rear view of Keithley 2230 Power Meter.

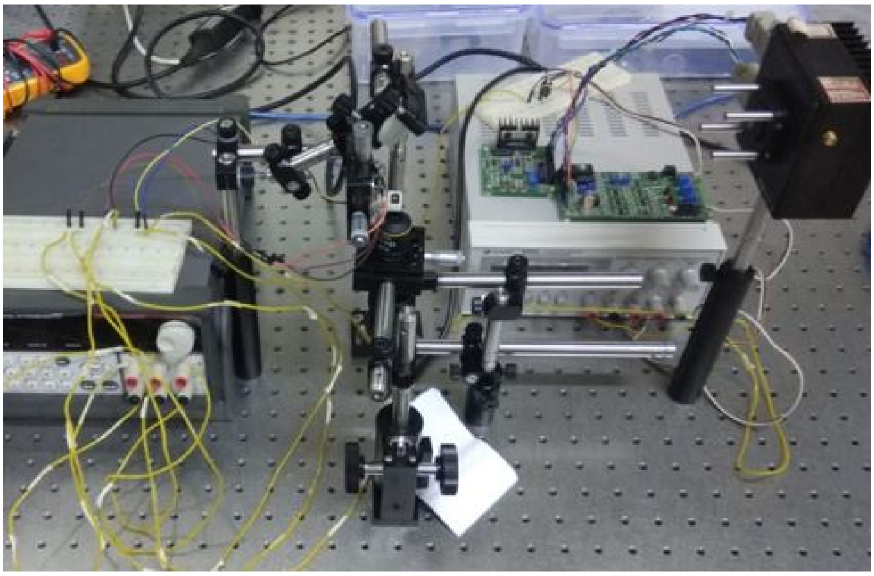
2.3.2 Programming the Power Meter and Interface using NI-VISA

A computer program was written using Python 3.0 for giving instructions to the programmable power source (Appendix A). The program was written to give a raster scan of dimensions 100 µm x 100 µm. The computer was connected to Keithley 2230 using USB connector and the program was implimented using NI-VISA driver.

NI-VISA(National Instruments VISA) is an NI driver used to interface with instrumentation systems comprising GPIB, VXI, PXI, serial (RS232/RS485), Ethernet/LXI, and/or USB interfaces.

2.4 Calibration of the Device

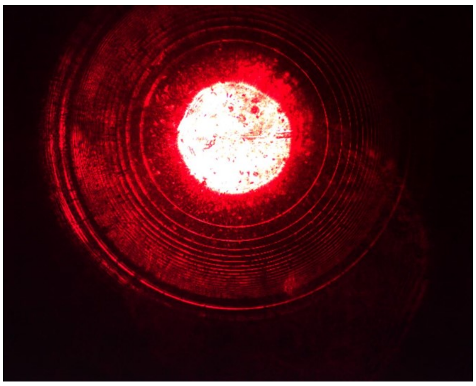
After integrating a 60x lens into the stage, a laser was allowed to pass through one side of the lens. The spot it created on the other side after converging was captured using a camera after zooming it with 10x objective. The movement of the spot when current was applied to different coils was recored and processed using the software 'Tracker'. Optical fibre of width 125 µm was used to calibrate the measuring tape in the software. This tool was then used to measure the distance the stage moved when different current input was given. A Current vs Displacement graph was plotted using the obtained data.



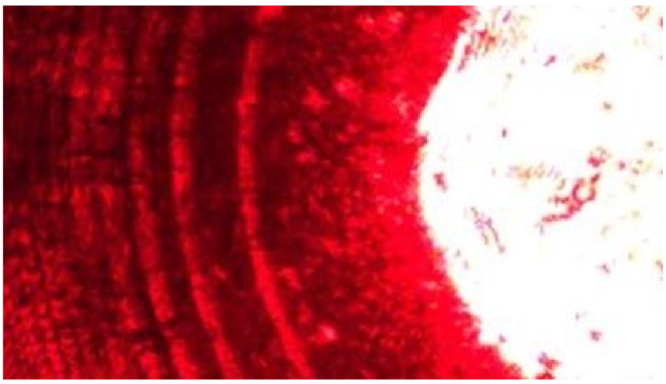
(A)

FIGURE 2.5: Top view of the apparatus.

The laser spot was aligned to be exactly at the centre of the lens. The video was zoomed to a reference point to measure the displacement. The video was recorded using ThorLabs ThorCam software.



(A)



(B)

FIGURE 2.6: (A) Centre aligned LASER spot obtained by the camera .(B) Zoomed in portion of the spot for measurement.

2.5 Results

2.5.1 Raster scan

A stable raster scan of dimensions 100 µm x 100 µm was successfully implimented using the device. The stage was stable and moved in equal step sizes in both X and Y directions.

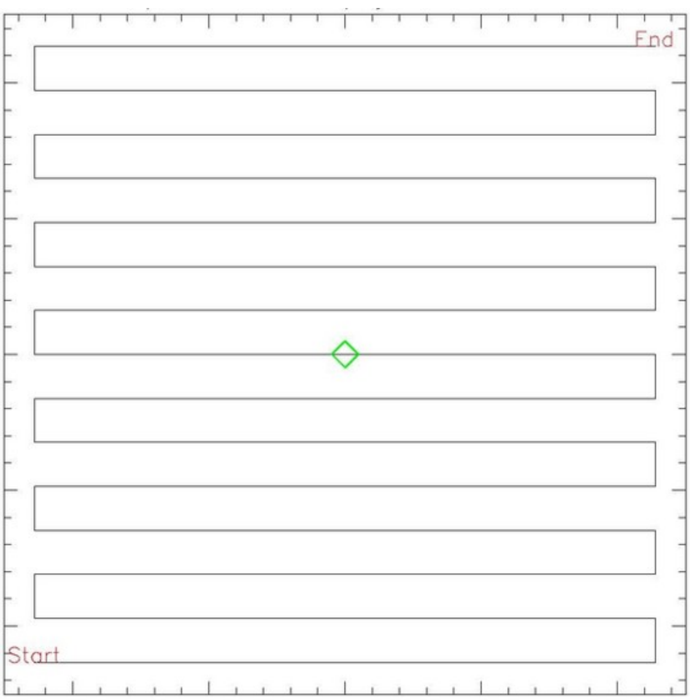
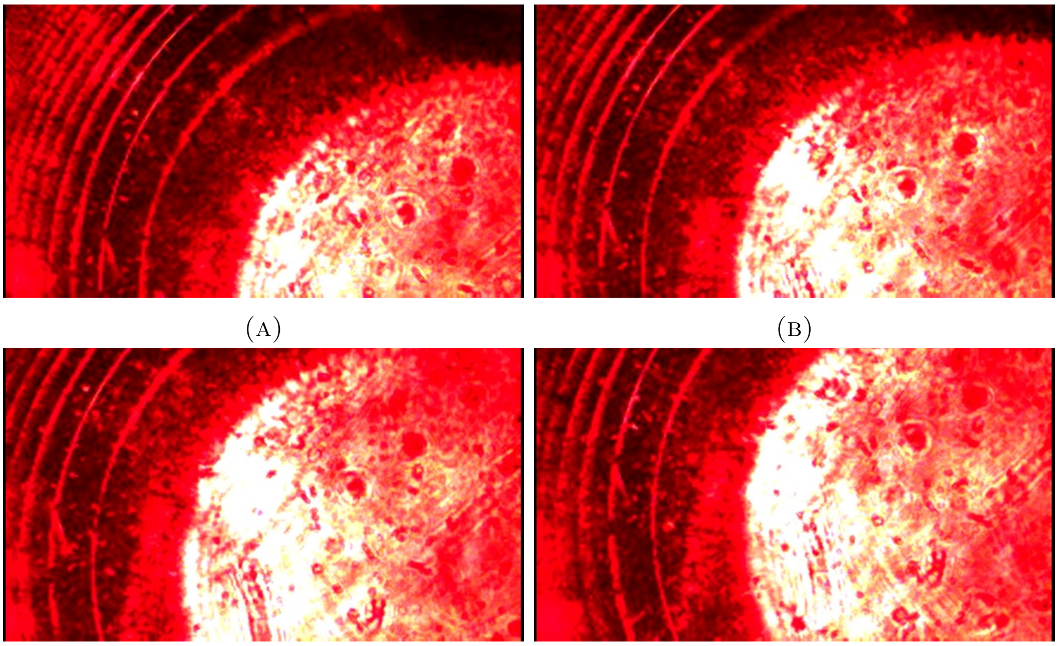


FIGURE 2.7: Raster scan path of dimensions 100 µm x 100 µm implimented using the device.

It is to be noted that beyond a length of 200 µm, the device was found to be unstable from time to time. It was especially found to be unstable above 400 µm, which can be attributed to the stress developed in the system.
Two programs were written each for 100 µm x 100 µm and 50 µm x 50 µm



(A) (B) (C) (D)

FIGURE 2.8: Movement of LASER spot during Raster scan at (A) t = 1 sec. (B) t = 4 sec. (B) t = 7 sec. (B) t = 10 sec.

2.5.2 Calibration of the device

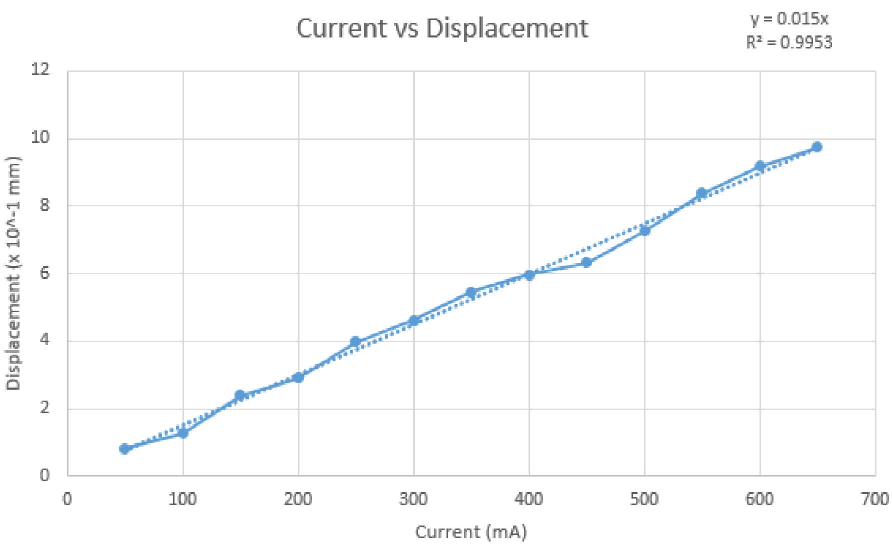


FIGURE 2.9: Calibration graph.

The graph drawn using the calibration data gave a straght line with equation y = 0.0015x and R value equal to 0.9953. Extrapolating this graph towards the origin gives a value of displacement 1.5 µm for a current of 1 mA.

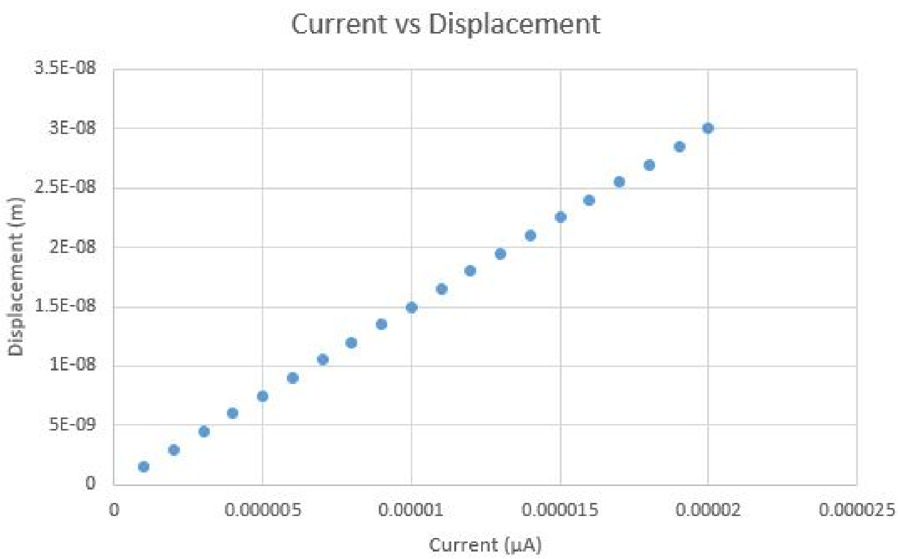


Figure 2.10: Theoretical prediction of the movement of the stage in nanometer domain.

The theoretical prediction assumes that the device will behave linear and stable in the lower domains. Accordingly, a current input of 1 µA can provide a step size of 1.5nm. This is quite possible using better power meters.

2.6 Discussion

The success in the execution of the electromechanical stage provides room for the development of more accurate devices using this novel idea. The domain of this device is not limited to the µm range. The stepsize could not be reduced below 1.5 µm due to the limitation of the power source. Keithley 2230 has a minimum current output of 1 mA. The use of later models of power source is recommended at this point. Keithley 2400 source meter is worth mentioning due to its features listed below.

V-Source	± 5 µV
V-Measure	± 1 µV
I-Source	± 50 pA
I-Measure	± 50 pA

TABLE 2.1: Keithley 2400 Sourcemeter Specifications

The theoretical prediction points to the need of 1 µA current for the device to achieve a step size of 1.5 nm. Thus theoretically, the use of Keithley 2400 can make the domain even smaller. But this should be verified experimentally as the exact response of the device to currents as small as 1 µA is unknown.

Optical Trapping and Measurement of Associated Forces

3.1 Optical Tweezer Apparatus

The main focus of the project after the implimentation of XY scan was to trap a particle. An apparatus was set up for this purpose keeping in mind the requirements of trapping.

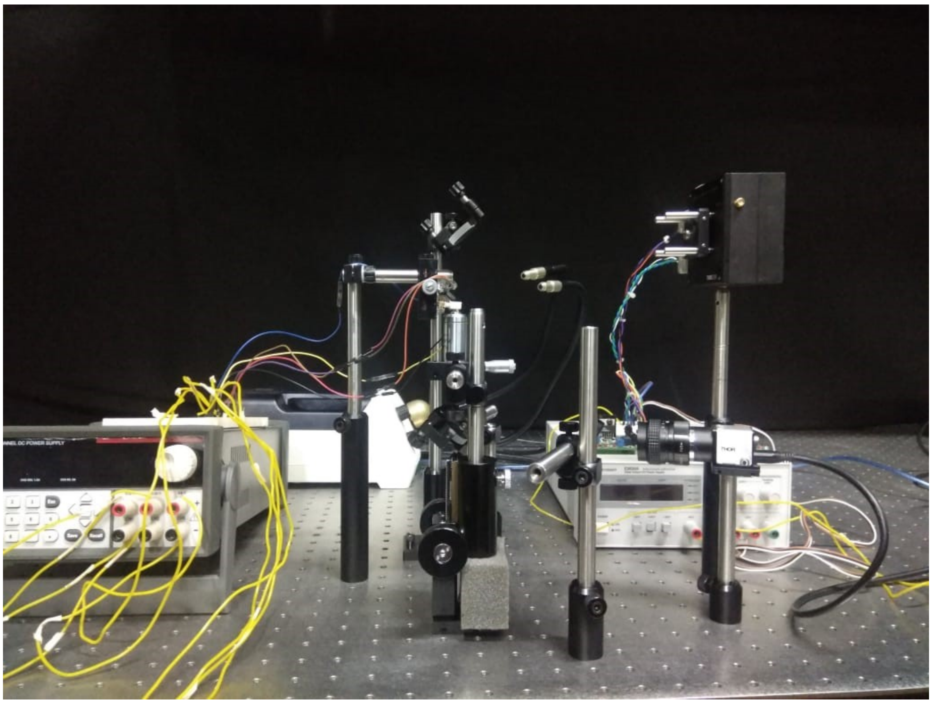


FIGURE 3.1: Detailed apparatus accoriding to the Schematic diagram given below.

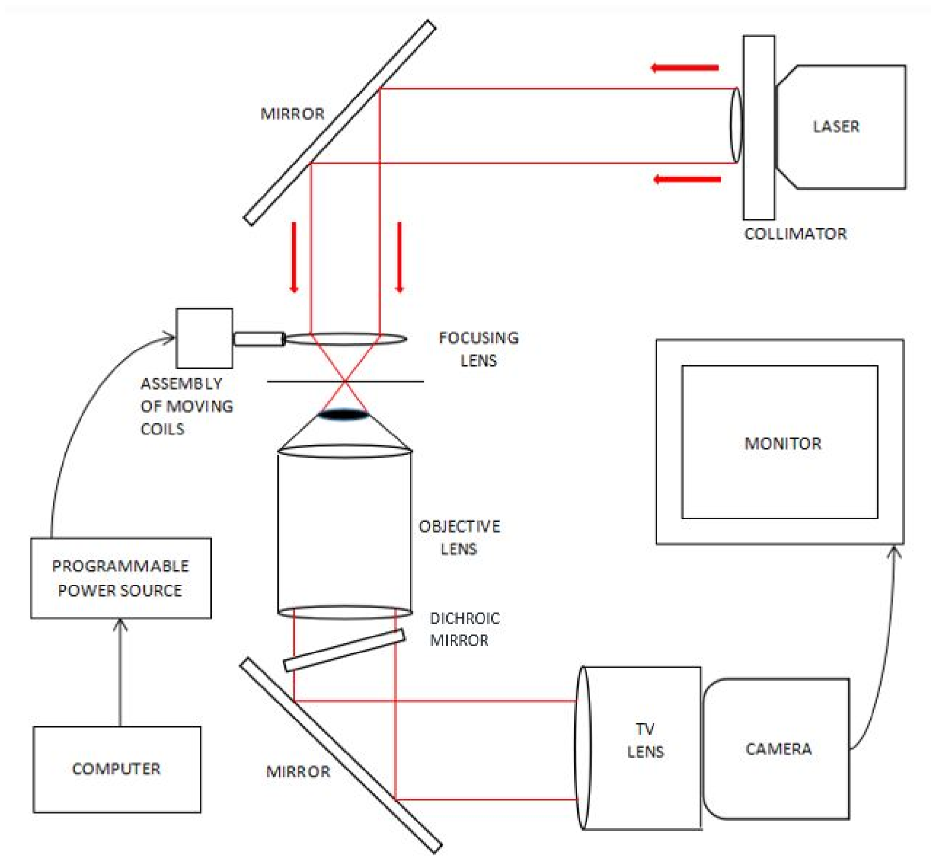


FIGURE 3.2: Schematic of the apparatus.

The apparatus consists of a LASER with output power set at 110 mW. This LASER beam is then passed through a collimator to obtain a collimated beam. The collimated beam is directed to a mirror which reflects it by a 90^0 angle into the scanning lens. The 60x scanning lens converges the beam into a tiny spot. A platform is placed exactly where the spot is formed. This platform is used for keeping the particle for trapping. This spot and the particle is then zoomed into a mirror by 10x objective lens. The image is further enlarged using a TV lens and captured by the camera.

The python program is loaded from the command prompt and passed to the power source using NI-VISA. The coil-magnet assembly moves, thereby moving the lens and thus the spot.

3.2 Measurement of Spot size

Minimum spot size plays a crucial role in the working of optical tweezer. The size of the spot determines which force will act dominant. At small convergence angles, less than approximately 30^0, the scattering force dominates over the gradient force and single beam trapping is either marginal or not possible. The 60x lens used in the apparatus has a high convergence angle and therefore produces a stable trap.

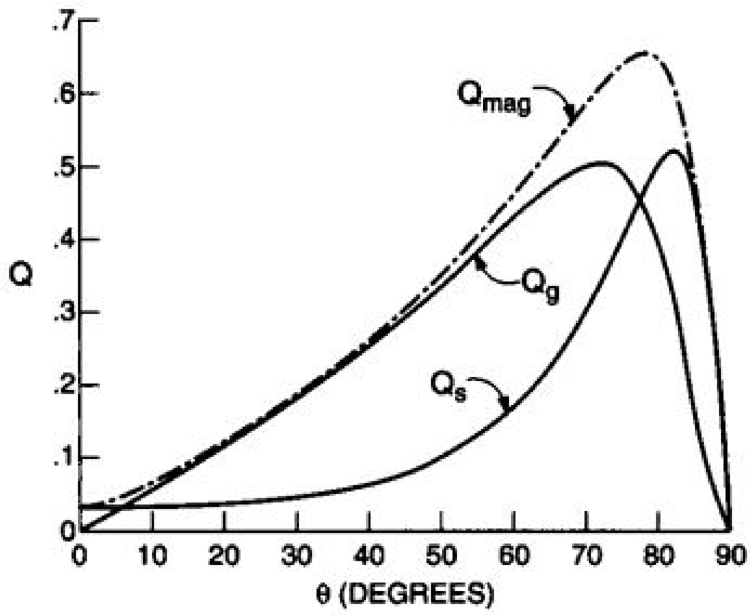


FIGURE 3.3: Values of the scattering force Qs gradient force Qg. and magnitude of the total force Qmag for a single ray hitting a dielectric sphere of index of refraction n = 1.2 at an angle 0[4]

In order to obtain the expected spot size, the laser beam, after reflecting from the mirror, should fall exactly perpendicular to the lens. To make sure this condition is satisfied, the mirror was kept at an angle exactly 45^0 from the horizontal plane. It was also made sure that the laser beam fell exactly at the centre of the lens.

The spot size was measured using two methods. In the first method, the spot was aligned along with a standard optical fibre and tracker software was used to measure the spot size. In the second method, the spot size was measured using a calibration slide.

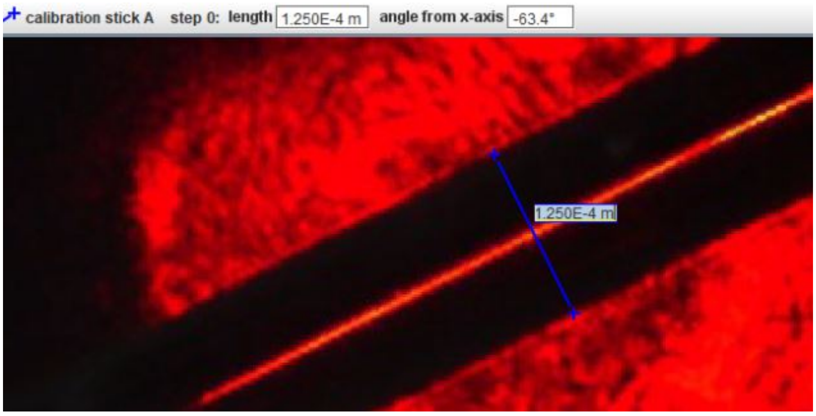


FIGURE 3.4: Tracker software screenshot of calibrating the calibration stick using optical fiber.

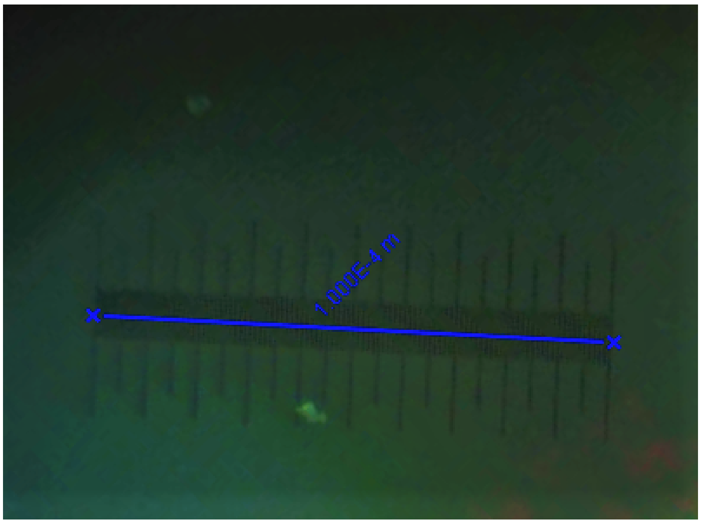


FIGURE 3.5: Calibration slide with measurement region of length 100 µm and 20 subdivisions.

Initial attempts resulted in obtaining a spot size of around 10 µm but proved useless because of the amount of scattering observed. Scattering indicates the need for better alignment of the apparatus.

(A)

(B)

FIGURE 3.6: (A) Spot sizes obtained initially. It is clearly visible that the spot sizes are comparable to the size of the optical fiber.

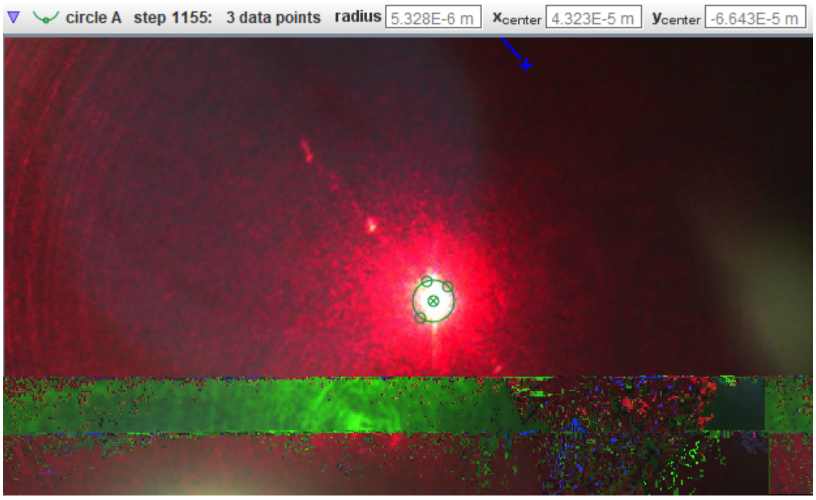


FIGURE 3.7: Spot size of 10 µm with scattering.

The intensity profile of the laser spot (Figure 3.9) proves that the adjusted spot has a Gaussian intensity profile. This is necessary as explained in the theory. A comparison of this intensity profile with the intensity profile of previous spots (appendix C) will help to understand the improvement.

FIGURE 3.8: Spot size as compared to 8 µm particle

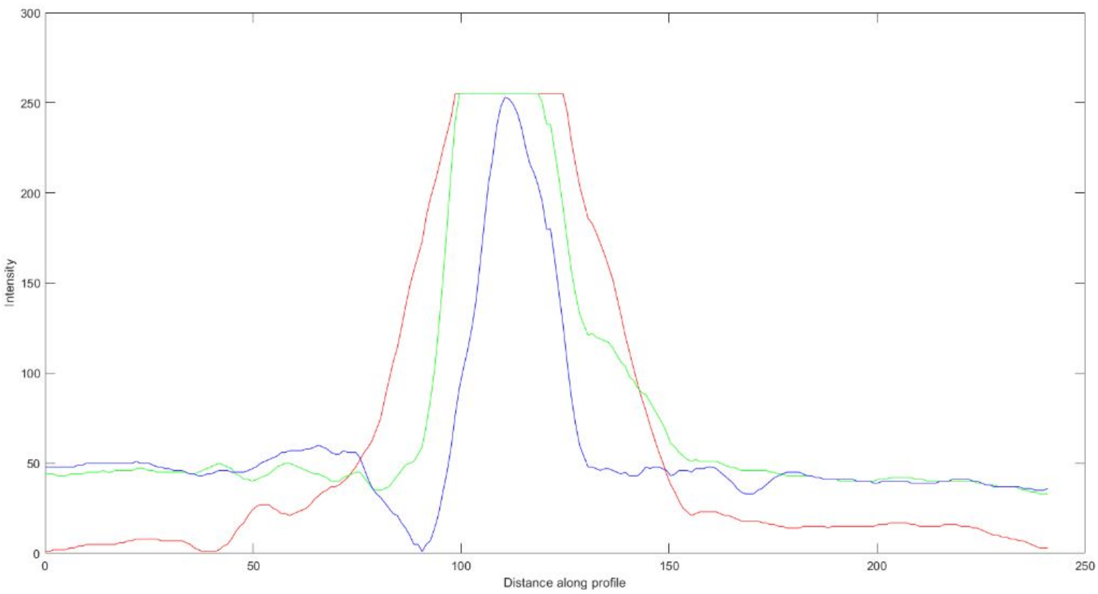
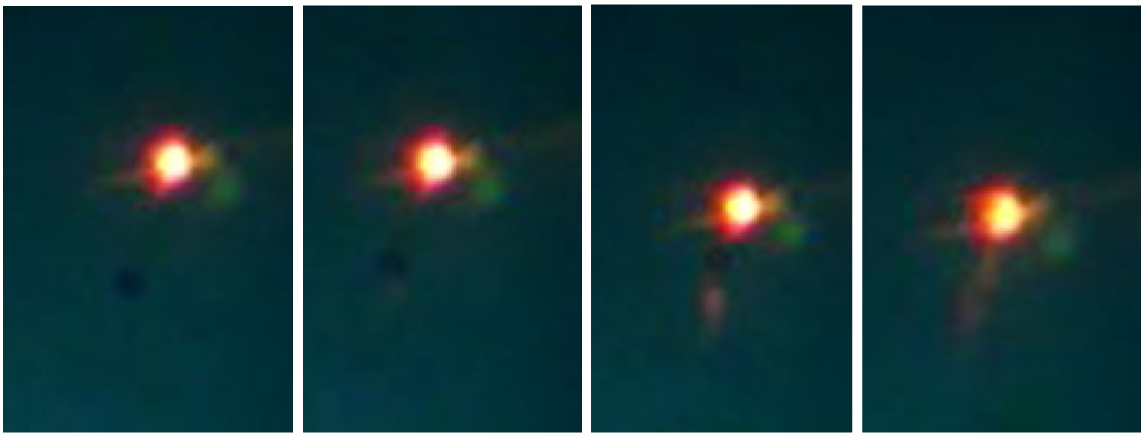


FIGURE 3.9: Intensity profile of laser spot.

3.3 Trapping The Particle

An aqueous solution of dielectric particles of size 8 µm in the ratio 1:2000 was prepared for the purpose of trapping. The power of the laser was adjusted to 125mW owing to the loss after refraction through the 60x lens. A small stage of thickness 50 µm was placed above the scanning lens to make the approach easy. A drop of the prepared aqueous solution was placed on this stage and the XY raster scan was implimented.



(A) (B) (C) (D)

FIGURE 3.10: LASER spot moving towards the particle.

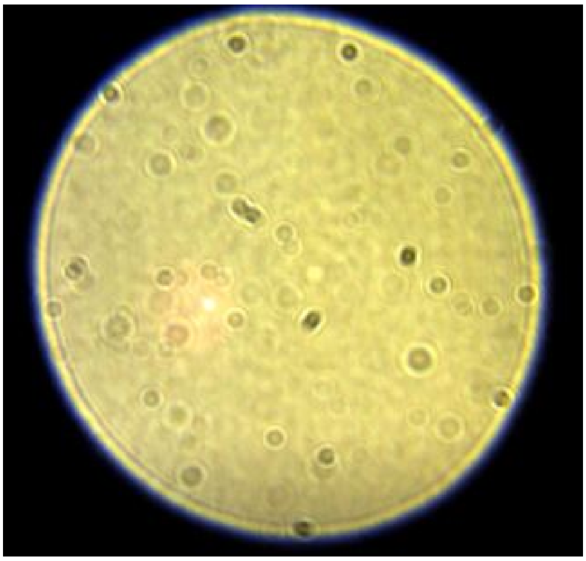
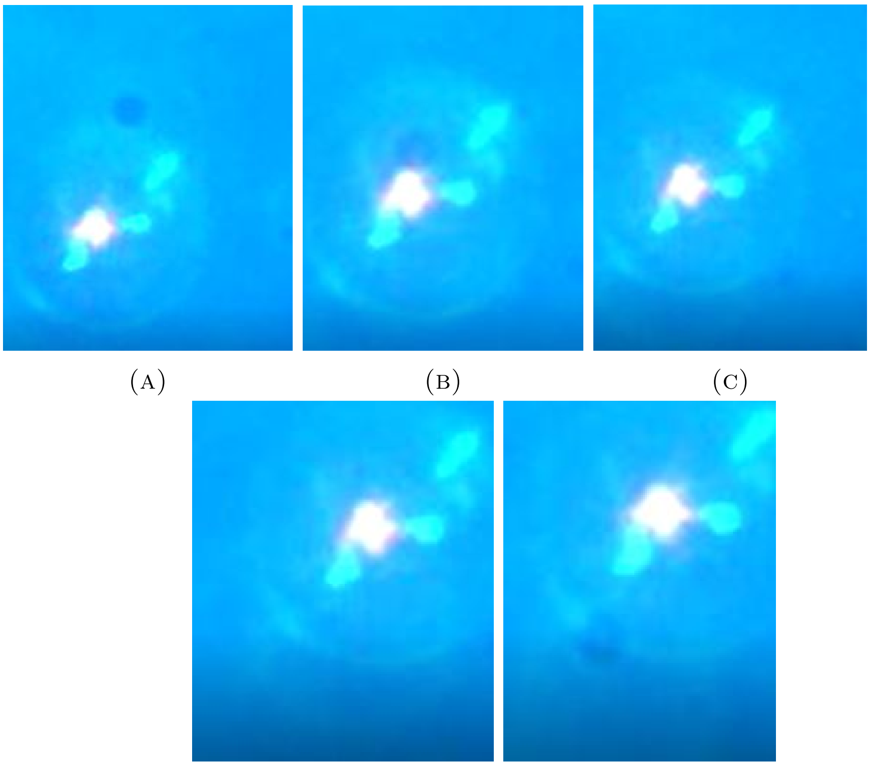


FIGURE 3.11: LASER spot in the field of dielectric particles.



(A) (B) (C) (D) (E)

FIGURE 3.12: Momentary trapping of particle.

3.4 Results

Figure 3.10 proves the production of a spot equal to or less in size than that of the 8 µm dielectric particle. Although the scattering could not be eliminated completely, an intense spot was made using the arrangement.

A momentary trapping of particle was achieved using the apparatus. The laser spot moved towards the particle, trapped it and carried it forward for a couple of seconds until an external shake made the particle move out of the trap. (The camera was not well focused to capture sharp images).

Measurement of associated forces could not be carried out due to the sudden unfortunate circumstances.

3.5 Discussion

It is doubted if the scattering happens due to the modification in the apparatus. The device is different from conventional apparatus in the sense that the particle is kept still and the lens is moved to bring the laser spot near the particle. While doing so, the laser beam can fall uneven on the lens and cause more intensity and scattering in a specific direction. The exact reason for the scattering has to be investigated.

The momentary trapping result given above is not a claim of achieving a stable trap but only a predeccessor for the same. Given a couple of days more work, a stable trap could be implimented.

The future improvements implimented on this apparatus can lead to the production of cheap and high quality optical tweezers.

Appendix A

Python program for XY raster scan

```
import math
import visa
from time import*
rm = visa.ResourceManager()
rm.list_resources()
kps = rm.open_resource(rm.list_resources()[0])


command1=open('left_solenoid.txt','r')
command2=open('right_solenoid.txt','r')
n=1
for j,k in zip (command1,command2):
        sleep(0.2)
        kps.write(j)
        kps.write('SOUR:CHAN:OUTP 1')
        sleep(0.2)
        kps.write(k)
        kps.write('SOUR:CHAN:OUTP 3')
        command3=open('midsol_left.txt','r')
        command4=open('midsol_right.txt','r')
        if(n%2==0):
                for l in command4:
```

```
                sleep(0.3)
                kps.write(l)
                kps.write('SOUR:CHAN:OUTP 2')
        else:
            for l in command3:
                sleep(0.3)
                kps.write(l)
                kps.write('SOUR:CHAN:OUTP 2')
        n=n+1
```

Appendix B

Sample text file used with raster scan program

sour : appl ch2 , 3 , 0.001
sour : appl ch2 , 3 , 0.002
sour : appl ch2 , 3 , 0.003
sour : appl ch2 , 3 , 0.004
sour : appl ch2 , 3 , 0.005
sour : appl ch2 , 3 , 0.006
sour : appl ch2 , 3 , 0.007
sour : appl ch2 , 3 , 0.008
sour : appl ch2 , 3 , 0.009
sour : appl ch2 , 3 , 0.010
sour : appl ch2 , 3 , 0.011
sour : appl ch2 , 3 , 0.012
sour : appl ch2 , 3 , 0.013
sour : appl ch2 , 3 , 0.014
sour : appl ch2 , 3 , 0.015
sour : appl ch2 , 3 , 0.016
sour : appl ch2 , 3 , 0.017
sour : appl ch2 , 3 , 0.018
sour : appl ch2 , 3 , 0.019
sour : appl ch2 , 3 , 0.020

Appendix C

Intensity profile of initial laser spot

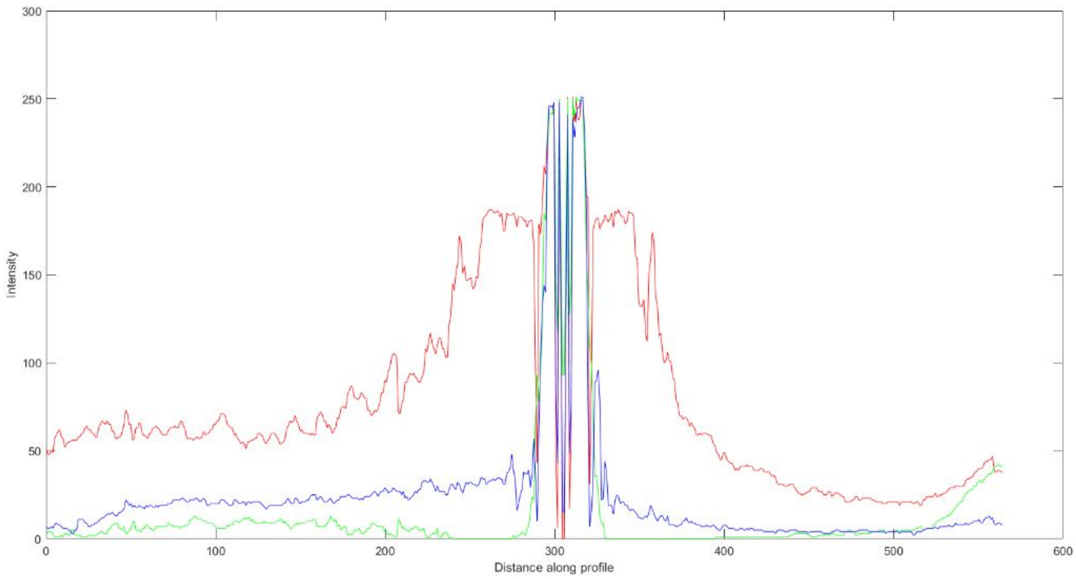


FIGURE C.1: Intensity profile of initial laser spot.

The graph proves that the initial spots had scattering associated with it.

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