Micro-manipulation using combined Optical Tweezers and Atomic Force Microscope

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ABSTRACT

In this paper, we report a novel approach to functionalize the tip of a micro-cantilever by selectively positioning a functionalized polystyrene bead using optical tweezers as a lever arm. We present a design that consists of an Atomic Force Microscope (AFM) combined with an optical tweezers setup to study specific interactions between complementary protein molecules. A BSA protein coated polystyrene bead, held stationary in an optical trap, is chemically grafted to AFM cantliever tip functionalized by the complementary protein- anti BSA. This arrangement also provides a flexible means of reversibly and irreversibly fusing polystyrene beads thermally at desired specified locations on the micro-cantilever, by heating the silicon tip with the focused laser beam of the optical tweezers. We use optical tweezers as a micro-manipulation tool for grafting pre-specified number of beads to cantilever in a controlled fashion as against the other widely used methods where an aggregate of molecules are chemically attached. Further, we study the changes in cantilever's resonant frequency and find it in good agreement with the expected change due to the additional bead mass. This study opens up opportunities in the area of biosensors by providing a method to standardize the calibration of chemically modified cantilevers.

Keywords: Atomic Force Microscope, Micro-cantilever, Optical tweezers, BSA, anti BSA

1. INTRODUCTION

The advent of micro and nano manipulation tools in the past two decades has ushered in an era of experimental studies of the building blocks of life. It is now possible to have single cell or single molecule studies and manipulation, which until recently was investigated by aggregate methods.

Some of these modern tools are Scanning Tunneling Microscope (STM)[1], Atomic Force Microscope (AFM)[2], Optical Tweezers[3], Single Molecule Fluorescence Microscope[4], and Scanning Electron Microscope (SEM).[5] These all have in common some of the advantages such as non invasive to the specimen, high resolution for imaging or manipulation, capabilitly of measuring forces in the nano-Newton or even pico-Newton range over long periods of time for in-vivo or in-vitro assays. Each of these tools is constrained by the kind of sample it can investigate, eg. the STM requires the sample to be electrically conducting, the AFM imaging requires the specimen to be immobilized, the trapping achieved via optical tweezers is good when the particle to be clamped is transparent. An integrated approach consisting of two or more of these modern tools in a single setup can help alleviate this constraint.

Atomic force Microscope (AFM) and Optical Tweezers, since their invention in mid 1980s, have had a significant impact in biological sciences and nano-technology. The AFM is most widely used to obtain the topographical images of samples with sub-nanometer resolution. Besides imaging, AFM is also used for manipulation and measuring of magnetic, biological and chemical properties at nanoscale. The AFM primarily uses a very sensitive mechanical member, of microscale dimensions, called a micro-cantilever with an ultra sharp tip, that has dimensions of a few nanometers, at its end that is used to probe/manipulate the specimen surface.

Optical Tweezers is an instrument that uses the radiation pressure of a focussed laser beam to trap and maneuver microscopic dielectric particles. The force exerted by tweezers is of the order of sub-piconewtons and can be used to investigate extremely sensitive dynamics between single molecules. The tweezer trap greatly checks the Brownian motion of the trapped particle and thus enable measurements with nanometer resolution.

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In this paper, we report an experimental setup that combines Optical Tweezers with an AFM[6]. This setup is capable of investigating an optically trapped specimen with the micro-cantilever of the AFM. The Infra red laser used for optical trapping provides a non invasive method for trapping and thus immobilizing biological specimens. In this integrated arrangement, this can be particularly useful in AFM studies for biological specimens that need to be immobilized. Further, with this setup, we present a novel technique to functionalize the micro-cantilever by selectively positioning a functionalized polystyrene bead using optical tweezers. The bead can be grafted both reversibly and irreversibly at desired locations along the length of the cantilever by either keeping the bead clamped in optical trap and moving the cantilever using the stage or rotating screws on AFM or by maneuvering the trapped bead using optical tweezers to the specific location along the cantilever beam. The effect of grafting beads to cantilever is studied by observing the shift in the resonant frequency of the cantilever beam.

The experimental setup and sample preparation is explained in Section 2. Section 3 describes the experimental procedure to graft the beads thermally and chemically. The power spectral density of thermal response of cantilever is also explained in Section 3, followed by analysis and results in Section 4.

2. EXPERIMENTAL SETUP

This section describes the sample preparation and the combined optical tweezers AFM setup.

2.1. Sample Preparation

A 10 mMolar solution of the protein Bovine Serum Albumin (BSA) and same molarity of its complementary protein anti-Bovina Serum Albumin (aBSA) is prepared in Phosphate Buffered Saline (PBS). 50-75 $/muL$ of BSA is added to a microtube containing a dilute colloidal solution of polystyrene beads (from Polysciences Inc.) sized 4.5 micron in diameter. Another sample is prepared by adding 50-75 $/muL$ of aBSA to another microtube containing dilute solution of the same polystyrene beads. The third sample contains a pure solution of polystyrene beads. The three microtubes after mild stirring are stored in the refrigerated environment for 12-16 hours. This allows proteins (BSA and aBSA) to form a coating on the polystyrene beads in microtubes 1 and 2.

The cantlievers used in the experiment are standard silicon cantlievers (from Asylum Research Inc., model AC 240TS) with a resonant frequency of approximately 70 kHz. Three sets of cantilevers are used for our experiment, viz. 1) uncoated cantilevers, 2) BSA coated cantilevers and 3) aBSA coated cantilevers. The cantilevers are coated with BSA and aBSA proteins by carefully placing them in seperate teflon wells containing BSA and aBSA respectively. The cantilevers are allowed to stay in the well for around 12 hours.

2.2. AFM Optical Tweezers setup

The two main components of the experimental setup are a single beam optical tweezers arrangement and an AFM (MFP-3D from Asylum Research Inc. Santa Barbara, CA). Figure 1 shows a schematic of the experimental setup where AFM sits atop a specially built stage for inverted microscope (Nikon TE2000U). The single beam optical tweezers is built using a linearly polarized infrared laser beam (shown in solid black line) from a continuous wave diode pumped Nd:YAG laser (CrystaLaser Inc., $/lambda$= 1064 nm, beam waist = 0.45 mm, maximum power output = 500 mW) is expanded using a 10 × beam expander (Thorlabs Inc.) and then passed through a half wave plate that modulates the angle of inclination of the linearly polarized light. A polarizing beam splitter cube (PBS, Newport Inc.) allows the p-polarized component of the laser beam to pass through and reflects the s-polarized component at right angle into the beam dump. The p-polarized beam passes through the telescopip lens assembly, lenses L1 and L2, and is introduced into the back-aperture of the 100 × objective (numerical aperture 1.4) of the inverted microscope through the epi-fluorescence port. The optical trap is formed at the focal plane of the objective lens inside the sample on the microscope stage. The sample as mentioned in Section 2.1, is colloidal solution of protein coated or plain polystyrene beads. For our purpose we use an open experimental cell for the sample, so that the cantilever can be introduced from the top. A simple coverslip can be used as experimental cell.

Atomic Force Microscope is placed on top of the microscope stage. Figure 2 is the picture of experiemntal setup showing AFM head on the microscope stage. Figure 1 shows the major functional components of the AFM,

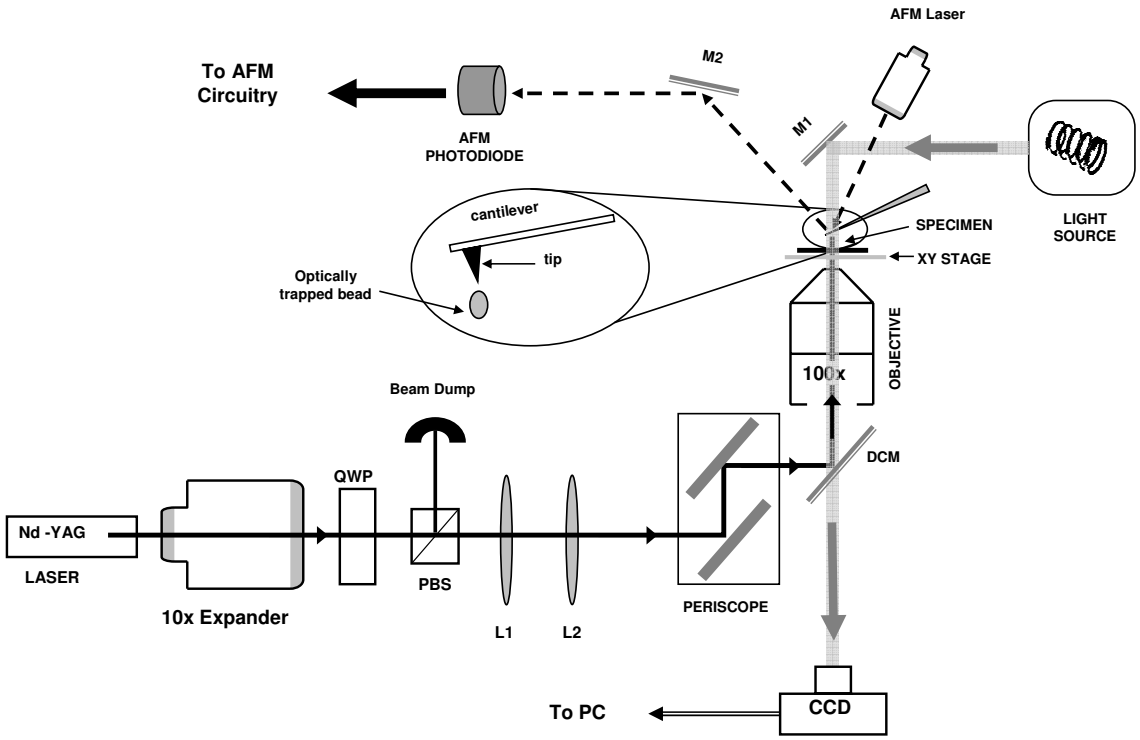

Figure 1. Schematic of setup showing Optical Tweezers with AFM. The solid black line represents the laser beam path of optical tweezers. The arrowheads show the direction of propogation. The optical trap is formed on X-Y stage inside the specimen. The AFM head consisting of the cantilever, the AFM laser and the photodiode is placed on top of the specifically built microscope stage. The laser beam of AFM laser is shown in black dashed lilne and the arrow shows its direction of propogation. A light source on the right is used to illuminate the sample and the cantilever. The illumination path is shown in grey along with arrows showing direction. A CCD camera is used to capture interactions in the sample plane. DCM is a dichroic mirror, PBS is Polarizing beam splitter, QWP is quarter wave plate, L1, L2 are convex lenses and M1, M2 are mirrors. The figure in inset shows interaction between tip of micro-cantilever and optically trapped bead.

viz. AFM laser, cantilever, photodiode (which primarily form the AFM head) and the circuitry. The operation of AFM involves shining the AFM laser beam (shown in dashed black line) on the back region of cantilever tip and capturing the reflected laser beam on the photodiode using mirror M2. Another mirror M1 was specifically placed inside the AFM head to illuminate the sample and cantilever by shining light from the side (shown in solid grey line), as depicted in the figure.

The illuminated sample and interaction between the trapped beads and cantilever tip is captured using a CCD camera.

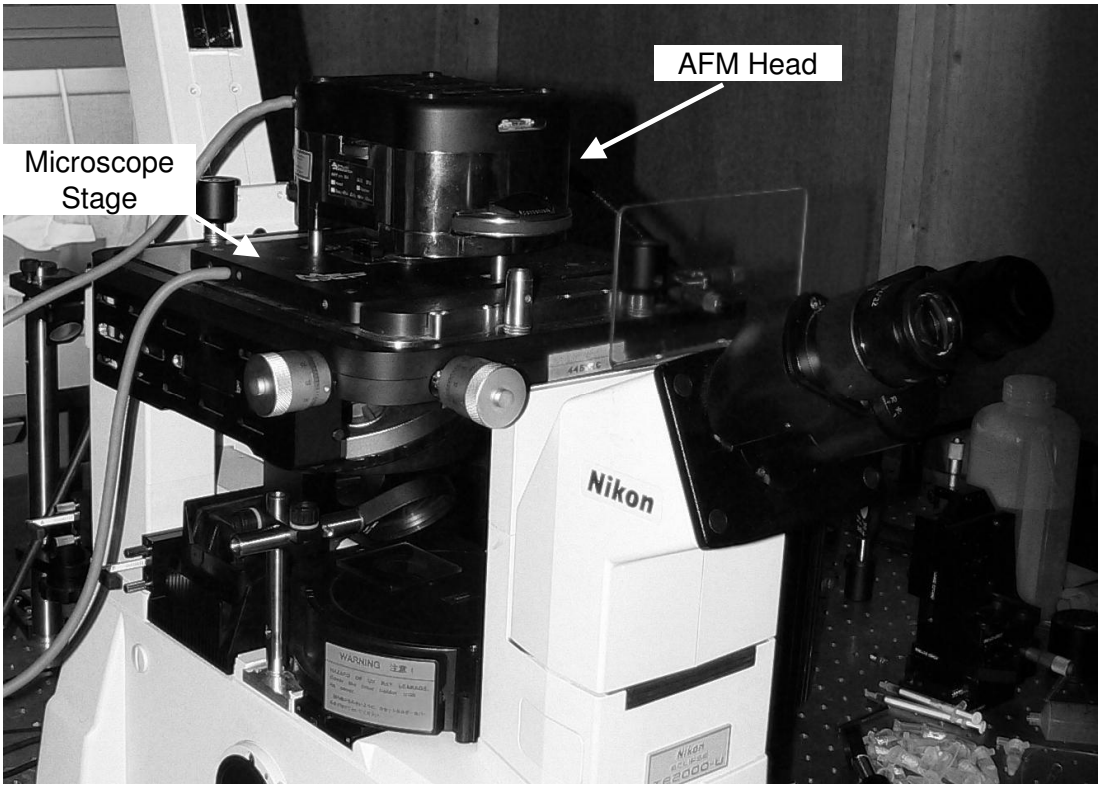

Figure 2. Picture showing AFM head placed on the Nikon microscope stage where optical trap is formed

3. EXPERIMENTAL PROCEDURE

A cantilever from the three sets, viz. BSA coated, aBSA coated and plain cantilevers, is selected and its thermal noise response recorded under water using MFP3d software. This gives the resonant frequency of the selected cantilever under water. The selected sample (colloidal solution of BSA coated or aBSA coated or plain polystyrene beads) is placed in the open experimental cell. The AFM head is placed over the stage such that the cantilever doesnt touch the sample meniscus. The cantilever is gradually lowered using the levelling screws of AFM head such that it gets submerged in the sample. This is done while keeping the closed loop "simple engage" option of AFM active. This is done to prevent cantilever tip from hitting the floor of experimental cell with great force, thereby breaking it. The CCD camera output is viewed intermittently by switching on the light source to check if the cantilever is visible in the field of view of microscope. The light source is not kept on continously as it interferes with the AFM laser reading on the AFM photodiode. The laser of Optical tweezers is switched on, once the cantilever is in the field of view and visible on CCD camera. A polystyrene bead is immobilized by optically trapping it. At this point both the trapped bead and cantilever are visible on the CCD camera. The cantilever tip is carefully brought over the trapped bead using the levelling screws on AFM head and moving the microscope stage. The cantilever tip is then carefully lowered and maintained in contact with the trapped bead

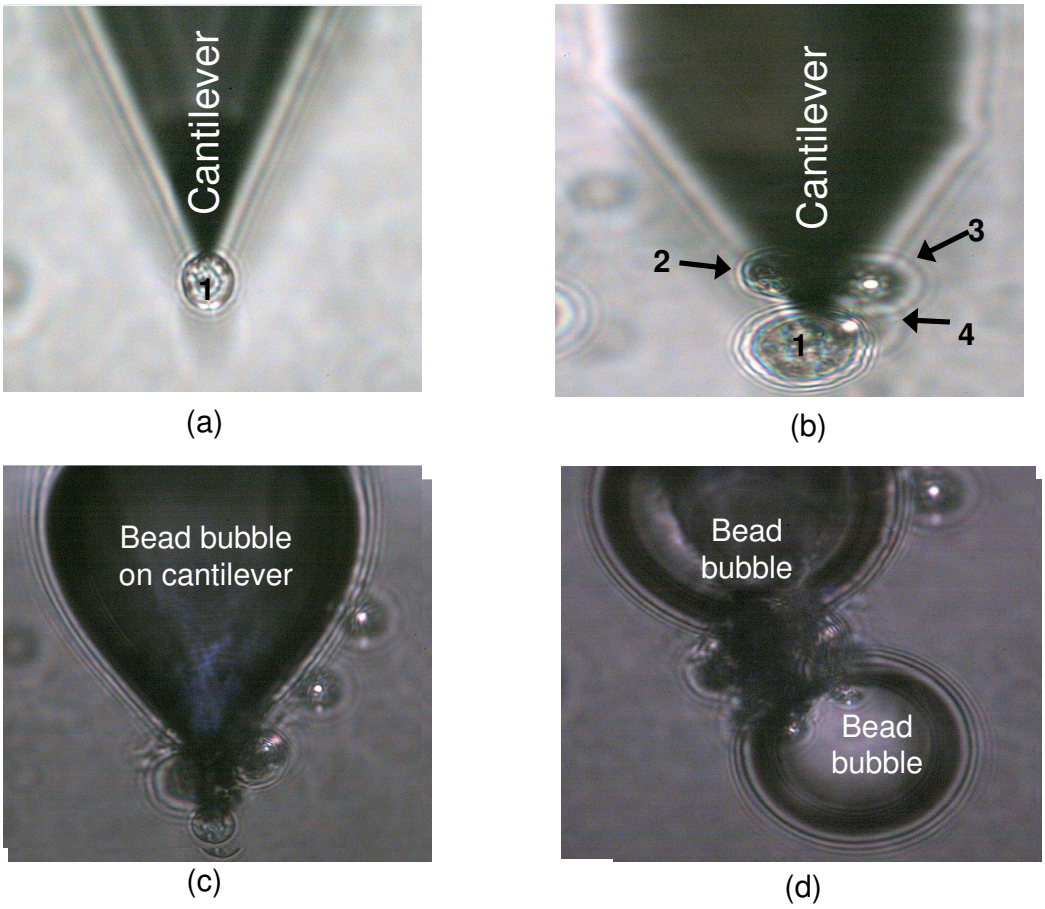

Figure 3. (a) This figure shows a BSA coated polystyrene bead attached to an aBSA coated cantilever tip. The picture shows the top view of the cantilever. (b) This figure shows 4 aBSA coated beads grafted to a BSA coated cantilever tip. The four grafted beads are numbered from 1 to 4. (c) and (d) These figures show the effect of heating an uncoated cantilever on the grafted bead. As the temperature of cantilever rises, the attached bead transforms into a bead bubble and clouds over the cantilever beam.

for sometime. The bead gets grafted on the cantilever tip either thermally or chemically as explained in Section 4. The modified cantilever is then raised using the levelling screws and thermal noise response of the cantilever measured. The above process is repeated for grafting more beads on the tip and then recording its time thermal noise response.

4. ANALYSIS AND RESULTS

The procedure to graft beads to the cantilever is described in Section 3. It is observed that the beads are grafted to the cantilever either thermally or via a chemical bond between BSA and its complementary protein aBSA. As the cantilever is brought in contact with the immobilized bead, it gets heated from the focussed laser of optical tweezers. As a result, the bead gets attached to the cantilever tip thermally. However, if a protein coated cantilever, say BSA coated cantilever, is brought in contact with a complementary protein coated bead, aBSA in this case, the bonding occurs instantly and we call it chemical grafting of the bead. It is observed that chemical

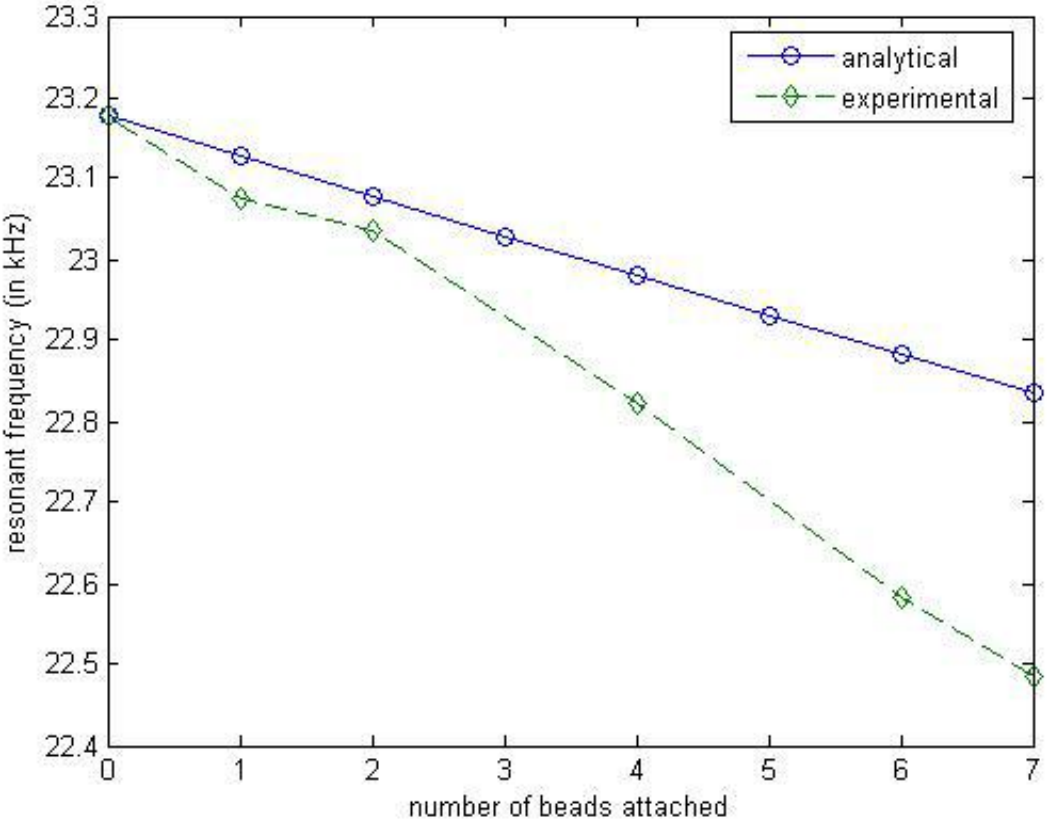

Figure 4. The figure shows plot between experimentally obtained resonant frequency of cantilever in water versus the number of beads attached and between analytically obtained resonant frequency versus the number of beads attached. The experimental data obtained, in kHz, was 23.177, 23.074, 23.036,22.806,22.854 and 22.485 for the number of beads attached to be 0, 1, 2, 4, 6 and 7 respectively.

grafting occurs much sooner than thermal grafting of the bead to the cantilever. Also, chemical grafting could be done at lower laser powers of optical tweezers, as the optical tweezers laser was only required to immobilize the bead, however thermal grafting required higher powers of laser. Figure 3 shows various cases where beads were attached to the cantilever. In Figure 3(a) a single functionalized BSA coated bead is grafted to an aBSA coated cantilever. Figure 3(b) shows 4 functionalized aBSA coated beads attached to a BSA coated cantilever. Figures 3(c) and (d) show a single bead attached to cantilever thermally. As this bond is formed by heat absorption of the silicon cantilever, the temperature of the tip can surge very fast resulting in formation of bead bubble that clouds a big part of cantilever beam. The Figures 3(c) and (d) were taken for the same cantilever as it got heated. It was also seen that thermal attaching of beads was reversible, that is the attached beads could be easily removed by raising the cantilever out of the sample meniscus and then lowering it to submerge it in the sample. For chemical grafting, the attached beads got removed when more than 3 or 4 beads were grafted to the cantilever tip. A possible reason for this could be smaller area of contact and hence a weaker bond between functionalized cantilever and complementary functionalized beads. The model of cantilever is well studied and is characterized as a second order spring mass damper system with a resonant frequency (free osciallation in air)

given by

$$f_{air} = \frac{1}{2\pi}\sqrt{\frac{k}{m_{eff}}}, \tag{1}$$

where f_{air} is the resonant frequency of cantilever in air, k is the spring constant and m_{eff} is the effective mass of cantilever. For a rectangular cantilever, like the one used in our experiment, effective mass is approximately 0.24 times the actual mass.

Under water, the damping is significant and the resonant frequency gets reduced by a constant, say γ, dependent on the damping factor. Therefore

$$f_{water} = \gamma f_{air}, \tag{2}$$

where f_{water} is the resonant frequency of cantilever in water.

Let m_b be the mass of the bead. As the bead is attached at the cantilever tip, the new effective mass becomes the summation of m_{eff} and m_b. Let $f_b(l)$ denote resonant frequency of cantilever under water with l beads attached to the cantilever tip. Therefore

$$f_b(l) = \gamma\frac{1}{2\pi}\sqrt{\frac{k}{m_{eff} + lm_b}}, \tag{3}$$

$$f_b(l) = f_{water}\frac{1}{\sqrt{1 + \frac{lm_b}{m_{eff}}}}. \tag{4}$$

The mass of bead, m_b, is much smaller than the effective mass of the cantilever beam. Therefore, first order approximation yields

$$f_b(l) = f_{water}\left(1 - \frac{1}{2}\frac{lm_b}{m_{eff}}\right). \tag{5}$$

Thus addition of beads linearly decreases the resonant frequency of cantilever.

The dimensions of cantilever beam (as provided by the manufacturer) are $240 \times 30 \times 2.7$ (μ^3). The density of Silicon is 2330 kg/m^3. This yields mass of cantilever (ignoring mass of tip) to be 4.53×10^{-11} kg. The effective mass, m_{eff} is 0.24 times the mass of cantilever. There $m_{eff} = 1.163 \times 10^{-11}$ kg. The mass of a single polystyrene bead (4.5 μm in diameter) is 5.01×10^{-14} kg. The resonant frequency of a BSA coated cantilever under water was found to be 23.177 kHz. aBSA coated beads were grafted to this cantilever and its resonant frequency recorded. Figure 4 shows the graph between resonant frequency of the cantilever obtained experimentally versus the number of beads attached (shown in green with diamonds and dashed line). The analytically obtained resonant frequency for corresponding number of beads attached is also plotted in Figure 4 (shown in blue with circles and solid line). AS expected, the experimental plot shows a linear decrease in the resonant frequency with addition of mass. However the rate/slope of decrease is found to be double that of analytical plot. this discrepancy can be attributed to uncertainty in the cantilever dimensions. The dimensions used for calculating the mass of the cantilever are generic dimensions given by the manufacturer and are usually inaccurate for thickness (quoted value of thickness is 2.7 microns). There could be other variables operating that needs to be investigated further.

5. CONCLUSIONS

A new method to functionalize the tip of micro-cantilever is presented by integrating AFM with optical tweezers. The functionalization thus obtained could be reversible or irreversible depending on the method employed, that is grafting done chemically or thermally. The combined optical tweezers AFM setup can thus be used as a powerful nano/micro manipulation tool. The change in the resonant frequency of cantilever is also studied as mass is deposited using optical tweezers. While the change in the resonant frequency determined experimentally is found to be linearly decreasing, it needs to be studied further to account for difference from the calculated values.

6. ACKNOWLEDGMENTS

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REFERENCES

1. G. Binnig, H. Rohrer, C. Gerber and E. Weibel, "Surface studies by scanning tunneling microscopy", Phys. Rev. Lett. **49, 1**, pp. 57, 1982.
2. G. Binnig, C. Gerber, E. Stoll, T.R. Albrecht and C.F. Quate, "Atomic Resolution with Atomic Force Microscope", Europhysics Letters, **3**, pp. 1281-1286, 1987.
3. A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm and S. Chu, "Observation Of a Single-Beam Gradient Force Optical Trap For Dielectric Particles." Opt. Lett. **11**, pp. 288-290, 1986
4. T. Funatsu, T. Harada, M. Tokunaga, K. Saito and T. Yanagida, "Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aquesous solution", Nature **374**, pp. 555-559, 1995.
5. Ardenne, Manfred, "Das Elektronen-Rastermikroskop. Theroretishce Grundlagen", Zeitschrift fur Physik,**108**, pp. 553-572.
6. G. V. Shivashankar and A. Libchaber, "Single DNA molecule grafting and manipulation using a combined atomic force microscope and an optical tweezer", Appl. Phys. Lett., **71 (22)**, pp 3727-3729.