2002

Utilization of Optical Tweezers for the Trapping and Manipulation of 1-3 um Polystyrene Latex Particles and Planktonic Staphylococcus epidermidis

Esther Kim

SOUTHERN SCHOLARS SENIOR PROJECT

Name: Esther Kim Date: 15 January 201 Major: Biophysics B.S.

SENIOR PROJECT

A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. The project is expected to be of sufficiently high quality to warrant a grade of A and to justify public presentation.

Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor three weeks prior to graduation. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is done as directed study or in a research class.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. You may attach a separate sheet if you wish:

My project will be building optical "tweezers" or an optical trap that will grab beads and eventually bacteria. Attached is a copy of my paper outline.

Linda Ann Foster

Signature of faculty advisor Chris Janse Expected date of completion 11 April

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Approval to be signed by faculty advisor when completed:

This project has been completed as planned: X CH

This in an "A" project: CH

This project is worth 2-3 hours of credit: CH

Advisor's Final Signature Linda Ann Foster

Chair, Honors Committee _____ Date Approved: _____

Dear Advisor, please write your final evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.

Esther has worked closely with Dr. Hanson and myself and has done an excellent job combining both areas in Microbiology (biofilms) and Physics (laser trapping). She was involved in laser design and testing the laser trap and developed the basis for bacterial studies in BIOL 397 Intro to Biological Research Based on the work she has done, I am delighted to give her an "A" for this project.

Linda Ann Foster

Esther Kim
Scientific Writing
Outline
Dr. Chris Hansen
15 January 2002

I) Introduction
   a. Background information (Previous experiments)
   b. Relevance
   c. Purpose
II) Theory
   a. Forces involved in the trapping
   b. Definition of "trapping"
   c. Applications that would result from successful experiment
III) Data (Same as the results?)
IV) Method
   a. Preparing bacteria
   b. Setting up the laser
   c. Trapping beads
   d. Trapping bacteria
V) Results
   a. Preparing bacteria
   b. Setting up the laser
   c. Trapping beads
   d. Trapping bacteria
VI) Conclusions
   a. Summary of results
   b. What the results mean
   c. Applications of the technique
   d. Further experiments
   e. Changes that could be made to the experiment

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ABSTRACT

An optical tweezers setup is being constructed to trap and manipulate *Staphylococcus epidermidis*. As a preliminary step, the capabilities of the optical trap were tested using small latex spheres (1-3 μm) in place of bacteria. Further studies will include manipulation of bacteria to compare adhesion forces between planktonic and biofilm bacteria.

INTRODUCTION

Optical tweezers or traps are formed by tightly focusing a single laser beam. The optical gradient force of the laser beam is then used to manipulate microscopic particles. This use of light force not only allows for the trapping and manipulation of single cells, but it also allows for work on living biological species[1]. The optical tweezers can be used to measure elasticity, force, torsion, position, surface structure, and the interaction between particles[2].

There are many other applications to this optical trap set-up. One of the foremost researchers in this field, Dr. Arthur Ashkin, has trapped a number of particles from polystyrene beads to bacteria to different proteins since 1986[3]. Since then several other researchers have used this tool for their own specific purposes. Current research is highly focused on genetic coding and DNA manipulation. A group at Rockefeller University, NY has been attaching DNA to polystyrene beads and then manipulating the DNA by trapping the attached beads[4]. Dr. Richard Dickinson, from the University of

Minnesota, is focusing on cell adhesion, as well as cell migration[5]. Their work is focusing on being able to predict cell adhesion forces as well as development of predictive models that will relate the extent and direction of cell migration. His work is of high interest because his work on predicting adhesion forces is applicable to this experiment.

The main application of the tweezers in this experiment will be to manipulate and eventually measure the adhesion forces between biofilm bacteria. Preliminary work has been done on the adherence preferences of *Staphylococcus epidermidis*. This species of bacteria was chosen due to its prevalence as a nosocomial pathogen of biomedical implants and devices and its ability to form biofilms[6]. Because each biofilm bacterium lives in a customized microniche in a complex microbial community, standard methods of killing bacteria are ineffective with biofilm bacteria. They are highly resistant to antimicrobial agents due to a slime layer they excrete that helps them adhere to surfaces[7] and because the communities are heterogeneous for different species of bacteria[8].

A timed-study was done on *Staphylococcus epidermidis* to discover how different surfaces promote or discourage the formation of biofilms. The two main surfaces were glass slides and gold-coated slides. A third surface of hydroxyapetite (an adhesive used in bio-implants) was attempted but the coating was non-uniform and was excluded from the study. It was noted that

Staphylococcus epidermidis had a tendency to form biofilms on glass surfaces (Figure 1a) while they remained fairly uniform on metal (gold-coated) surfaces (Figure 1b).

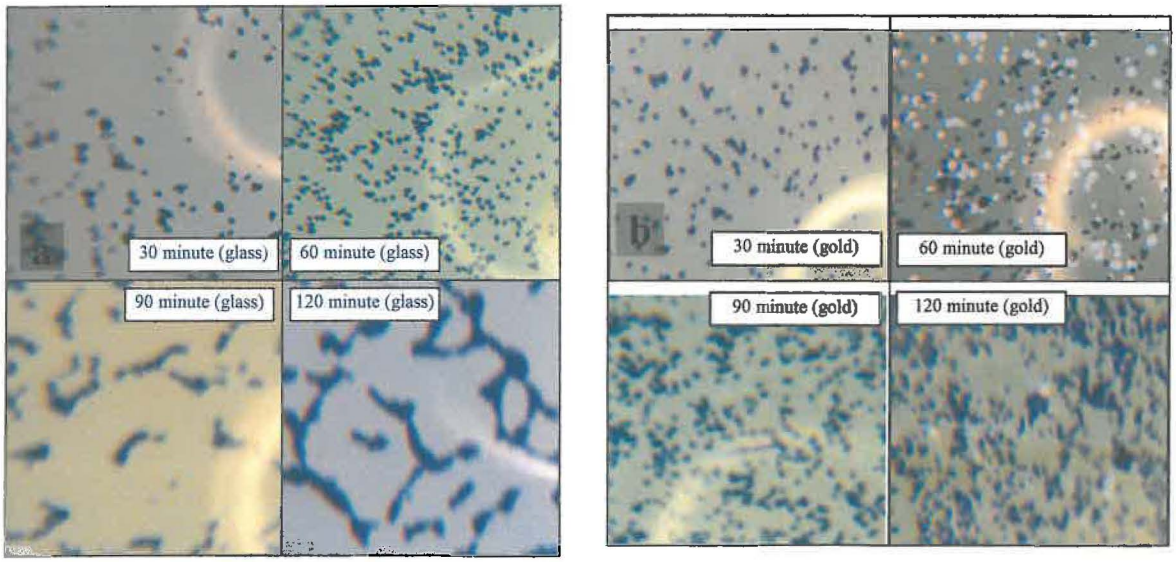

Figure 1 Aggregation tendencies of *Staphylococcus epidermidis* a) Timed-study on glass slides. b) Timed-study on gold-coated slides

The purpose of this experiment is to set up the basic model of the optical tweezers so that further experimentation can be done. By going through the necessary steps to construct the optical tweezers, many important experiments, including the manipulation of biofilm bacteria, can be performed without having to build the tweezers beforehand.

THEORY

The basic principle behind optical tweezers is that light carries momentum that is proportional to its energy. When light passes through a dielectric material, its path is bent by refraction which changes the momentum of the light (Figure 2)[9]. This change in optical path is due to the

fact that the particle (green sphere) has a higher index of refraction than its environment. This higher index of refraction causes the outgoing light to bend towards the optical axis (dotted line running down the center). If the particle had the same index of refraction as its environment, the light would follow the path marked by the dashed lines crossing through the sphere. Conservation of momentum requires that the momentum from the light must be transferred to the refracting particle which will then undergo an equal and opposite momentum change which is denoted by the green arrow in the bottom momentum diagram (p_{part}).

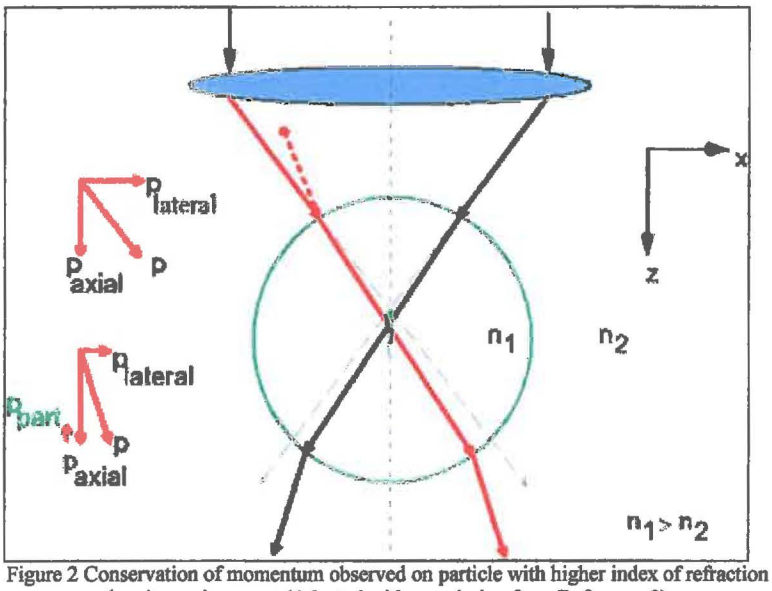

Figure 2 Conservation of momentum observed on particle with higher index of refraction than its environment (Adapted with permission from Reference 9).

Because optical tweezers must trap and hold particles in all three dimensions, they must trap the particles both laterally (x-y direction) and axially (z-direction)[10]. This can be accomplished by using a laser beam with a steep intensity gradient in all three directions. With its high intensity peak, a

Gaussian beam focused through a microscope objective provides the required intensity profile for this three dimensional trap (Figure 3).

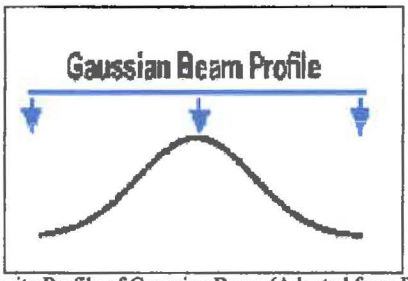

Figure 3 Intensity Profile of Gaussian Beam (Adapted from Reference 10)

Axial trapping occurs in the z-direction (Figure 4a). The scattering force acts to push the bead in the direction Pof the laser beam propagation which must be counteracted. When the objective lens has a high numerical aperture, it will produce a steep intensity gradient of the laser beam in the z-direction that produces a force towards the focal point of the lens. The gradient force (F_{grad}) will counteract the scattering force and trap the particle in the z-direction.

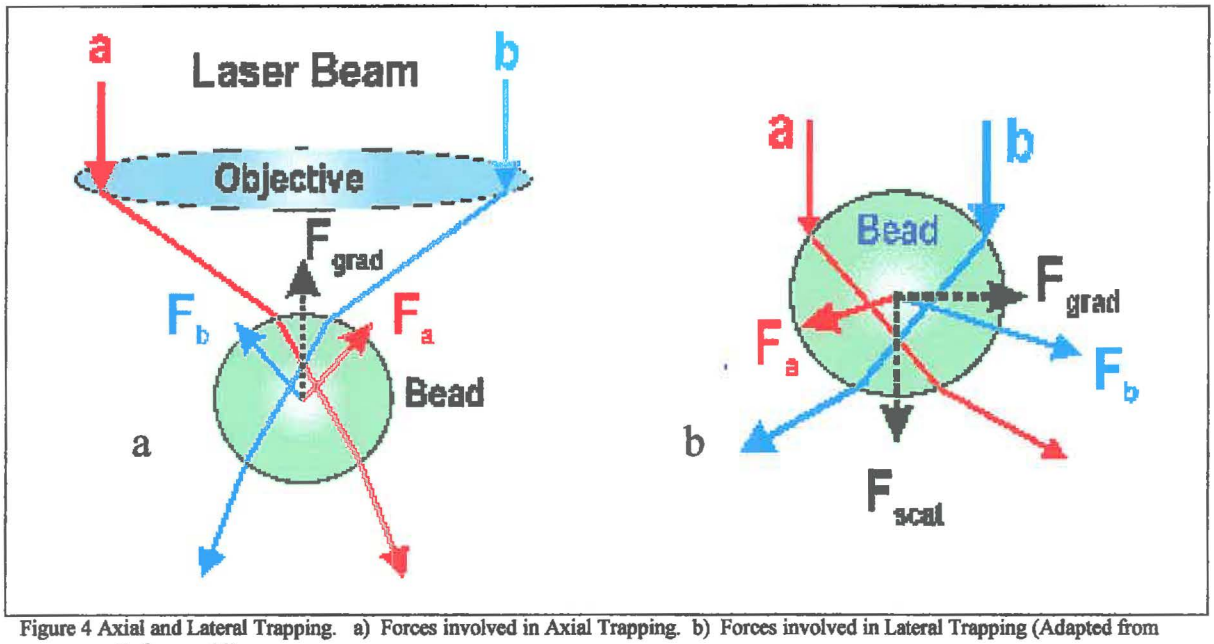

Figure 4 Axial and Lateral Trapping. a) Forces involved in Axial Trapping. b) Forces involved in Lateral Trapping (Adapted from Reference 10)

The particle will also experience a net lateral force pushing it toward the high intensity region of the beam (Figure 4b). The greater index of refraction of the particle causes the light to refract at a smaller angle than the incident angle. This again causes the particle to move towards the light focus, this time in the horizontal plane.

METHODS

An optical trap was built based on the setup suggested by a group at Harvard (Figure 5)[11]. The helium-neon laser (λ=632.8 nm) was expanded by the first two lenses (L1 and L2) in the setup which serve as a telescope. L1 (L45-296, Edmunds Industrial Optics) has a focal length of 4 cm and L2 (L32-904, Edmunds Industrial Optics) has a focal length of 40 cm. The expanded beam then passes through the third lens (L3) which focuses the beam at the pinhole. L3 (L32-877, Edmunds Industrial Optics) has a focal length of 17.5 cm. All the lenses in the setup are double convex lenses.

The laser beam then passes through a 50 μm pinhole (P50S, Thorlabs, Inc.) and focuses into the microscope (3001PL, AccuScope). The pinhole eliminates any low intensity, non-Gaussian light that will not focus properly, and it also serves as a point source that is imaged at the microscope's focal plane.

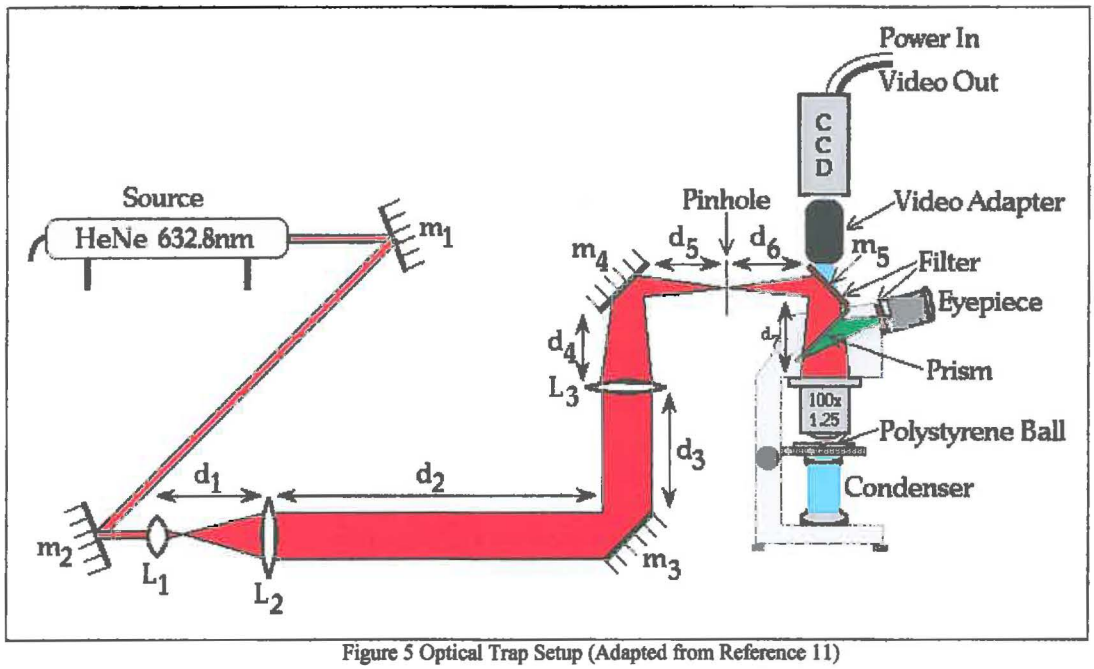

Figure 5 Optical Trap Setup (Adapted from Reference 11)

The laser beam was scanned at 4 different distances from the source to determine 1) its divergence, 2) the spot size at each point, and 3) whether it exhibited a Gaussian beam profile. A power meter (S20MM, Thorlabs, Inc.), masked with either a pinhole, was mounted on a translation stage and scanned through the center of the laser beam profile at each location. The intensity of light was recorded as the pinhole was scanned through the laser beam.

Using this information, the location of the focus and the beam size at the focus were determined. The beam size is characterized by the "waist", the distance from the beam center to where the intensity falls to 1/e of its peak value. The waist at any point can be calculated using the following equation[12]:

$$w^2 = w_o^2 \left[1+(\lambda/\pi w_o^2)(z-z_o)^2\right] \qquad \text{(Equation 1)}$$

where w is the waist a distance z from the source, w_0 and z_0 are the waist and the location of the focus respectively.

After determining the profile of the beam, the lenses were placed at the appropriate distances from the source and the laser beam was focused into the microscope. In order to monitor the laser beam safely, a 45° dichroic beamsplitter was used to absorb the red light reflecting from the microscope while allowing the blue light to pass through so that observations could be made. These beamsplitters were placed on the microscope eyepiece and in front of the lens of the CCD camera (ST7, Santa Barbara Instrument Group Astronomical Instruments) as shown in Figure 5.

Once the optical trap was built, polystyrene latex beads were prepared in phosphate buffer saline (pH 7). These beads were used to optimize the function of the trap before experimentation with bacteria. Two beads sizes were prepared. The 1μm beads (Lot 100K1366, Sigma Chemical Company) were chosen for the first test-run because they were closer to bacterial size. However, it was later determined that the 3 μm beads (Lot 40K0572, Sigma Chemical Company) would be easier to trap even though they were larger in diameter1[2].

Serial dilutions of the beads were prepared with phosphate buffer saline until the bead concentration of the final dilution was 0.01% by volume. The

diluted beads were then placed in concavity slides and cover-slipped. The coverslips were sealed with Mounting Medium X (Lot 0272, ACCRA Lab) to increase the lifetime of the slides.

The prepared slide was placed on the microscope stage and viewed. Once the beads were brought into focus along with the laser beam, trapping was attempted. This was done by placing the bead in the focus of the laser and translating the microscope stage.

RESULTS

In order to characterize the laser beam, the beam profile was measured at four distances from the source. The results of these scans are shown in Figure 6. Plotting the light intensity as a function of distance traveled across the beam by power meter showed that the laser beam was a Gaussian beam. The solid red line is a theoretical (Gaussian) fit to the data (black squares). It is important to note how nicely the data points follow the curve, especially near the peak. The waist at each distance is indicated on each curve by the black horizontal line. Note the non-gaussian tails that stray from the theoretical curve. These points are eliminated in the optical trap by the use of a pinhole as a spatial filter. The data has been plotted on a logarithmic scale so that the details of the non-gaussian tails are enhanced. Figure A shows a poor fit to the curve but this data point was thrown out as detailed below.

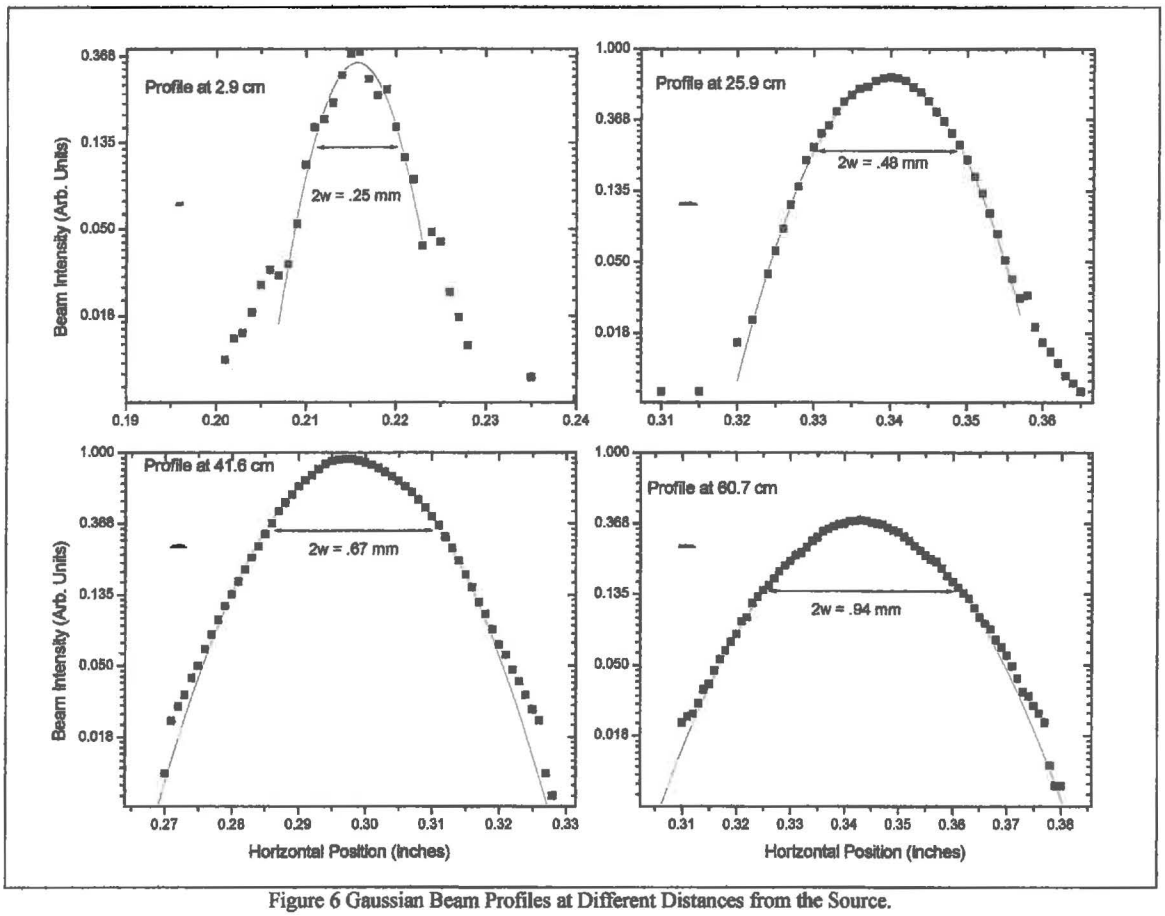

Figure 6 Gaussian Beam Profiles at Different Distances from the Source.

The results of the beam characterization are listed in Table 1. These data were fit to the theoretical curve (Eq. 1) using Least Squares and two free parameters, w_0 and z_0. The fit resulted in values for w_o and z_o of 0.22 mm and 14.5 cm, respectively. Values in the third column were calculated using equation 1 and these parameter values. The predicted beam waist as a function of position is shown below in Figure 7.

Table 1. Beam waist at various distances from the source.

Distance from Source (mm)	Measured Beam Waist (mm)	Calculated Beam Waist (mm)
29	0.13	0.25
259	0.24	0.24
416	0.34	0.33
607	0.47	0.47

The poor agreement at z = 29mm is believed to be a result of scanning through an off-center axis of the laser beam. When fit to the data a solution could not be obtained when this point was used, although a very close fit could be obtained using only the other three points. Thus, the first point was not used in the fit. It is also of interest that that the laser beam appears to focus 14.5 cm after the source rather then at the exit of the source.

By measuring the spot sizes at the different points, the divergence of the beam, calculated by taking the difference between the spot sizes and dividing this by the distance between the measurements, was determined to be 0.6 mrad. This is equal to the slope of the graph at large distances (Figure 7).

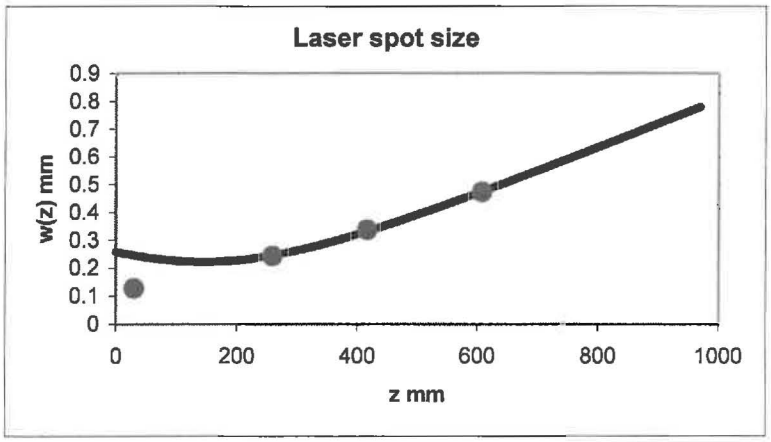

Figure 7 Laser Spot Sizes as a Function of Distance from the Source

Efforts to trap the beads were unsuccessful. However, focus of the laser into the microscope and onto the bead was accomplished (Figure 8). In Figure 8a, the 1 μm beads are brought into focus. The bead of interest is noted by the red arrow. In the next frame, the laser beam enters the

microscope but is not yet focused. In Figure 8c, the laser beam is focused onto the bead. In Figure 8d, the background diffraction pattern of the laser has been eliminated to clearly show the particle scattering the focused beam.

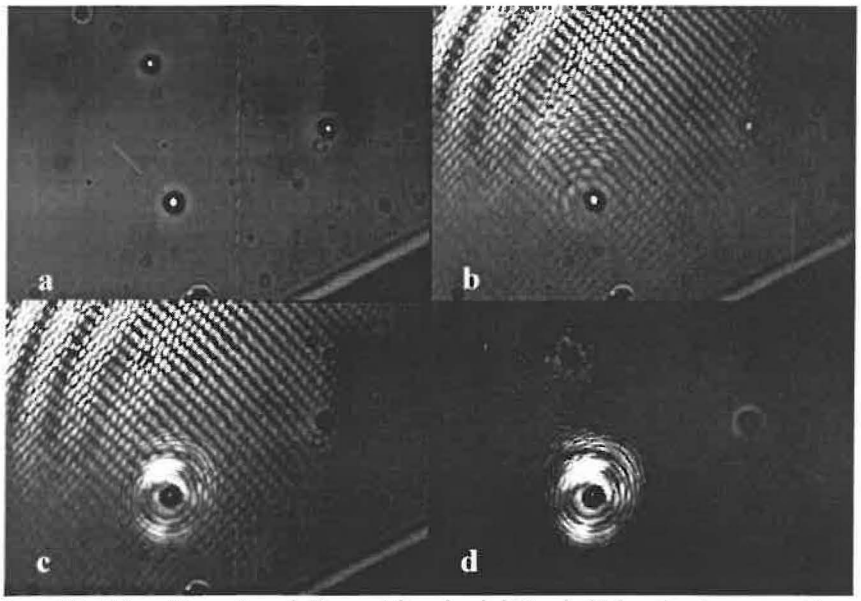

Figure 8 Particle and Laser Beam in Focus a) 1 μm bead. b) Bead with laser in microscope. c) Laser focused on bead. d) Laser focused on bead minus background diffraction.

CONCLUSIONS

The laser beam was successfully characterized as a Gaussian beam and carefully expanded to focus into the microscope. Though trapping was unsuccessful, the basic trap has been constructed and further work will only entail fine-tuning the working system.

The limitations of the helium-neon laser prevented actual trapping from occurring. It was determined that only a quarter of the intensity from the 10 mW laser was focusing into the microscope. At least 10 mW is required to trap a particle. In order to get more light intensity into the microscope, further work will be done to expand the laser beam even more to get a higher

percentage of light through the pinhole. More intensity will result in a greater trapping force. Dr. Dickinson has successfully begun to chart a force-distance profile to predict cell adhesion forces[5]. He has been using a 100 mW laser which is 10 times stronger than the helium neon laser of this setup. He also utilizes a computerized stage/focus control which allows for a more precise focus and movement of the stage. Improvements need to be made to the setup of this experiment to reflect these ideas.

Also, the 50 μm pinhole will be replaced with the 25 μm pinhole (P25S, Thorlabs Inc.). This will allow for better focus of the laser beam by providing a better point source of light. Also, more of the low intensity beam will be filtered out.

Once the beads have been trapped, experimentation will continue on *Staphylococcus epidermidis*. To do this, the helium-neon laser will be replaced with an infrared laser. The purpose of this switch is to increase the power of the light. The helium-neon laser has a power of approximately 10 mW. The infrared laser has double the power at approximately 20 mW. More importantly, the bacteria will absorb the red light from the helium-neon laser. This will cause them to be destroyed. By using the infrared laser, the bacteria can safely be trapped without any adverse effects[13].

Eventually, the project will entail manipulating the bacteria in order to measure different adhesion forces. The main comparison of interest is the

difference in adhesion forces between planktonic and biofilm bacteria. Determining the difference in adhesion strength will allow for further research on ways to remove these highly resistant bacteria.

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