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Optimization of Optical Tweezers

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Abstract

Recently our group demonstrated the ability to trap bacteria using optical tweezers, but low laser power limited trapping ability. I will report on improvements made to both laser power and beam quality, which lead to improved trapping ability. Initial results were seen to increase laser intensity at the sample, but the beam quality was severely degraded. The solution to this problem was to better shape the laser beam using an anamorphic prism pair. This process was repeated for a higher power laser diode. We were able to maintain a high quality beam while more than doubling laser power at the sample. This was evidenced by a larger fraction of light passing through the spatial filter and by higher translational speeds than previously observed.

Introduction

In the 1960's, Arthur Ashkin showed the initial viability of an optical trap, an instrument used to study and manipulate μm sized particles down to atoms[1]. A wide range of applications were then developed for this new instrument including the study of kinesin motors[2], RNA polymerase[3], nucleic acid folding[4], cooling of atoms to the μK range[1], and a host of other uses[1].

The physics department at Southern Adventist University began building one of these so-called optical tweezers setups in 2000. Initial set-up work as well as beam profiling was completed by Kim in the winter of 2001[5]. While much of the power was retained through the entire system, the laser power was too weak to trap. Allen then replaced the laser, worked on the current pinhole system, and achieved tenuous trapping[6]. Most recently, Liu trapped beads as well as two different types of bacteria, *Staphylococcus epidermidis* and *Micrococcus luteus*, in both tryptic soy broth (TSB) and a phosphate buffer saline (PBS)[7]. With this trapping, initial proof of concept work was complete. Liu also developed methods for beam alignment and determined baseline trapping strength using frame by frame analysis of recorded video footage. While trapping of various samples in different media was achieved, the optical tweezers was operating at the limit of its capability at the highest available laser intensity.

This project focuses on improving the trapping strength primarily by increasing beam intensity at the sample. To accomplish this, more laser power must be brought to the sample, while maintaining a Gaussian beam profile to provide for uniform trapping. In this paper I will report on the adjustments made to the beam path and improvements made to initial beam shaping optics and how they affected both beam intensity and beam quality.

The purpose for this work is to increase the dynamic range of the instrument, with which we will be able to trap a wider range of samples, including larger and more oddly shaped bacteria and larger beads. However, there is a concern that biological samples may become overheated and lyse due to the increased power output. This is why it is necessary to increase the dynamic range of the instrument so that it can be set to a to an appropriate power level for the specimen being trapped.

Optical Tweezers Theory

The basic concept behind the optical tweezers is that a change of momentum in light will hold some small particle confined in the focus of the trapping laser beam. This change in the momentum of light is due to the difference between the refractive indices of the particle being trapped and the surrounding medium. As light crosses this interface, it provides a very small force (~ 0 pN) which pushes the particle toward the center of the trap. This force varies with distance and can be modeled using the common spring formula: $F = -kx$ where F is the restoring force, k is the spring constant, and x is the distance from the center of the object to the center of the trap.

One way to estimate the lower bound of the trapping strength, f, is to analyze the motion of a trapped particle moving through a fluid environment and determine the viscous drag force needed to remove the object from the trap. This force on the particle is then given by $v_{drift} = f / \zeta$ where f is the force on the particle, v_{drift} is the speed at which it travels and ζ is the viscous friction coefficient. Stokes formula then relates ζ, the viscous friction coefficient, to η, the viscosity of the fluid and R, the radius of the trapped particle in Stokes equation[5]: $\zeta = 6\pi\eta R$. In general terms this means that for a given ζ, the faster one translates a particle, the greater the force that is needed to hold the particle in the trap. This is what would be expected if two similar particles were used in the same aqueous solution. In practice, one records video footage of a trapped particle, of known radius, being translated through a fluid of known viscosity. Then the video is analyzed to determine the maxim speed of translation before the particle is forced out of the trap. This speed can be used, along with the equations listed above, to estimate a lower bound on the trapping force.

To provide the best trapping force, a focused beam with a Gaussian profile at the target site[6] is desired because it allows for the most tightly focused beam. It also allows for a symmetric trapping force with the peak trapping strength in the middle of the beam. Any aberrancies in the beam lead to variable trapping strengths in different directions and to an overall loss of trapping strength. To prevent these aberrancies, a method called spatial filtering[7] is employed, which uses a pinhole to remove non-

Gaussian elements in the laser beam. Our means of spatial filtering is done by using a manually adjusted 25-micron pinhole, which when entirely filled by the laser beam ensures a Gaussian beam profile. The goal then is to get the most light, correctly focused, through the pinhole, which results in higher power throughput.

Adjustments to the beam can be made through any of the directional mirrors and the anamorphic prisms at the beginning of the beam path, shown in Fig. 1. The directional mirrors are used to align the beam so the maximum amount of power can pass through the pinhole. The anamorphic prisms at the beginning of the beam path expand the beam in only one dimension – horizontal in our setup. This expansion converts the ellipsoidal beam into a round and approximately Gaussian beam.

Experimental

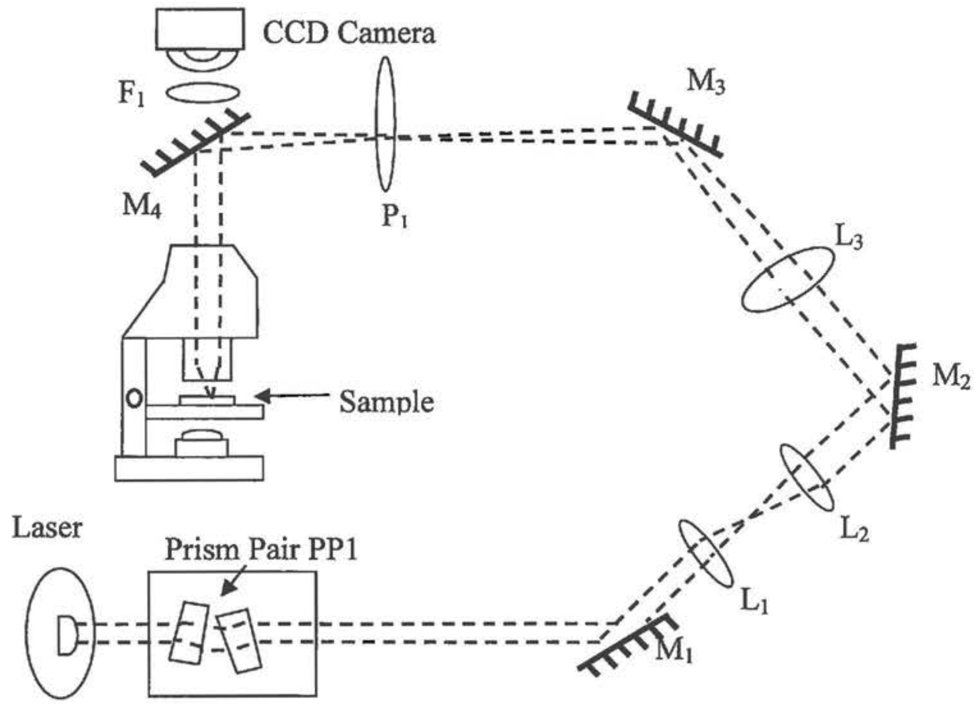

Figure 1. Shown above is the design of the optical tweezers. The mirrors M_{1-4} are directional mirrors, lenses L_1 and L_2 are used as a reverse telescope expanding the beam, and L_3 is a 17 cm lens which allows the light to be focused through the pinhole, P_1. The light is then passed through the dichroic filter, F_1.

Starting with a setup as described in Liu's report[8] and shown in Fig. 1, improvements were made to primarily increase trapping strength while maintaining beam quality. At first the optical path was adjusted to increase power through the pinhole and hence to the sample. By adjusting the mirrors (M1, M2, M3) and pinhole (P1) the power was increased by a factor of- 2-3, but an elliptically shaped aberration was introduced to the beam. This aberration introduced an asymmetry to the beam at the sample and thus greatly reduced the ability to trap. With further adjustments, including the temporary removal of the reverse telescope (L1 and L2) and optimization of the mirrors, we were unable to remove this aberration while providing an acceptable increase in power.

This problem was solved by adjusting the anamorphic prism pair (PP1). An initial beam scan without the prism pair in place was completed to determine the needed expansion factor. A Thorlabs model S20MM light power meter with a 50 micron pinhole (Thorlabs, Newton, New Jersey) connected to a Radio Shack True RMS multimeter (Radio Shack, Fort Worth, Texas) was used to manually scan across the beam in both the vertical and horizontal directions, to map out the laser intensity profile. The resulting readings were plotted in Microsoft Excel (Microsoft, Redmond, Washington) to make the graph of the beam which gives intensity as a function of position as shown in Fig. 2. The radius of the beam, at 1/e from the peak, is determined by fitting the curve $y = Ae^{\frac{(x-x_0)^2}{w^2}}$ to the data, where w is the radius of the beam. The width was determined to be .72 mm and the height to be 2.09 mm. Therefore the horizontal dimension needed to be expanded by a factor of 2.09/0.72 x or 2.9 x to make the beam nearly circular.

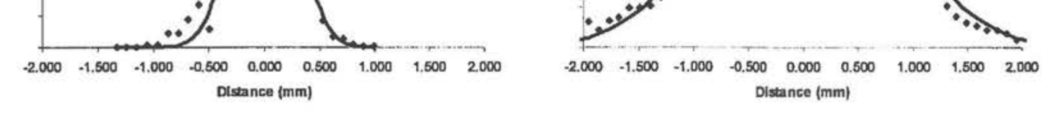

FIGURE 2. Beam scans prior to placing prisms. Beam width was determined to be 1.44 mm vertically and 0.82 mm horizontally.

In order to achieve the correct expansion, the prisms were then placed at the angles indicated by the design sheet[9] for the Thorlabs PS871-B anamorphic prisms (Thorlabs, Newton, New Jersey). Subsequent beam scans demonstrated that the resulting beam was nearly circular with horizontal and vertical diameters of 2.30 mm and 2.25 mm respectively. This process removed the elliptical aberration in the beam as it passed through the pinhole, while allowing most of the power to pass to the sample.

Just after this beam scan, the laser diode, a Hitachi HL 6535MG, failed. It was being driven over its specifications, burned out, and then was replaced with a similar Hitachi HL 6535MG laser diode, which required an additional adjustment to the beam profile. An initial beam scan showed a width, 0.82 mm, and a height, 1.43 mm as shown in Fig. 2. This led to an expansion factor of 1.74x. The Thorlabs specification sheet did not include the values for this magnification. Using a fourth-degree polynomial, the lines were extended as shown in Fig. 3 to include the desired magnification of 1.74x.

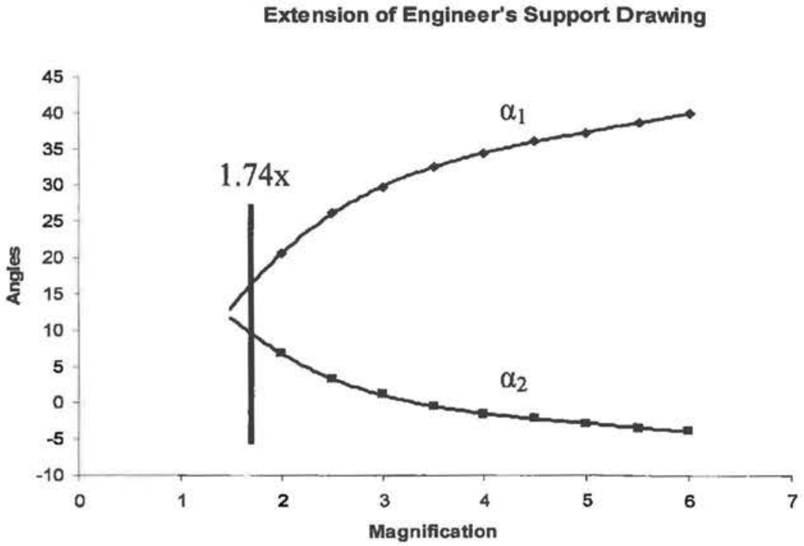

Figure 3. Extension of the design parameters (data points) for the prism pair, as given by Thorlabs' engineering support drawing for these prisms[9]. Desired magnification was 1.74 times.

Upon placing the prisms, a new beam scan was completed to show the improved Gaussian profile of the beam. The horizontal and vertical beam widths were determined to be 1.79 mm and 1.43 mm, respectively, as shown in Fig. 4. This yielded a sufficiently circular beam to provide the desired beam quality.

Figure 4. Beam scans done after placing prisms, beam width was determined to be at 1/*e* from the peak power and for the vertical section that was 1.57 mm and for the horizontal section 1.75 mm.

The work described in this paper in conjunction with work by another student

[10] resulted in a five-fold increase in useful laser power at the sample. Paige upgraded the steering mirrors and lenses with antireflection coated optics to minimize absorption of the laser light and redesigned the reverse telescope (L1 and L2) to optimize throughput at the pinhole. Table 1 shows the improvements made by our combined efforts. With better optical components, we were able to get 20% more laser power to the pinhole. The improved beam quality then allowed us to get 60% of this light through the pinhole, which is approximately five times better than previous work. This is primarily because the light coming to the pinhole is more circular in shape, and therefore more power goes through rather than being blocked by the pinhole.

Position of Power Reading	Liu 2006	% of Total Power	Knecht, Paige 2007	% of Total Power
Beginning of the beam	73 mW		85 mW	
Before the pinhole	38 mW	52%	~ 60 mW	~ 70%
After the pinhole	8 mW	11%	40 mW	47%
Microscope Stage	<6 mW	<8%	27mW	32%

Table 1. Lui 2006 power values as reported in Ref 8. The laser was replaced during this time, and so % of total power values provides a better comparison.

As well as showing improvement by increased power through the pinhole, the increase in trapping strength can be determined by analyzing video as discussed in the theory section. The video has not been analyzed in detail yet, but it does show a bead being translated much faster than in Liu's 2005 video. This faster speed of translation gives a preliminary indication that the trapping force has been increased, demonstrating that the improvements made to the instrument have increased overall trapping strength.

Conclusion

From these results, it was learned that it is critical to position the prisms accurately to maintain laser beam quality. Previously without this quality, the power loss through the pinhole was caused by incorrectly aligned prisms, which delivered a poorly shaped beam to the pinhole. The continuation of the Thorlabs Engineer's Support drawing was feasible and to worked well. Also a process was developed for placing the prisms, which allows for all these adjustments to be completed quickly with good results.

The ultimate goal of the project is to study biological samples. An aspect of this goal is to study *Staphylococcus epidermidis* and its ability to adhere to different substrates. With the ability to trap individual specimens, we could very accurately place bacteria on specific sites on a surface. Once placed, different qualities, such as adhesiveness or neighbor selectivity can be studied individually, rather than statistically as in previous studies. Also, further down the line, a second laser could be added to study interactions between two unique biological samples.

Further work is being done to provide a flowcell which will provide a constant flow of a fluid by a trapped sample. This will allow for long-term experiments that a mechanical stage would not allow. Also, the flowcell has the ability to introduce different samples at different times which will expand the range of experiments for which this instrument can be used.

Acknowledgements:

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Appendix 1: Prism Alignment Procedure

To correctly align the beam through the first element in the path, the prisms, a procedure was developed which includes: 1) initial laser beam scans to determine necessary expansion factor, 2) modification of support drawings, if necessary, to accommodate the needed exapansion, and 3) drawing a positioning template and final placement of the prisms.

First, vertical and horizontal intensity profiles were determined by scanning a pinhole (50 μm diameter) through the collimated laser beam in both directions . Measurements of optical power were taken every 0.093 mm, and the data collected was then plotted in Microsoft Excel. The beam width in each dimension was approximated by the 1/e full width of a Gaussian fit to the data. Using this width, an expansion factor was determined, and the Thorlabs sheet consulted to determine the necessary angles, α_1 and α_2 as shown on the Thorlabs specification sheet[9], to place the prism pair.

Upon determining the angles, α_1 and α_2, the prism placing procedure goes as follows:

1. On each of two separate pieces of graph paper, draw a reference line coincident with one of the grid lines. Then draw a second line subtending the angles α_1 and α_2, one on each sheet, as shown in Fig. 5.
2. Place the prisms, in the correct orientation as determined from the spec sheet, along those angles, using double stick tape to hold the prisms down.
3. Place the first prism in the path of the beam.
4. Place the second prism in the path of the beam, making sure that the grid lines on the two pieces of graph paper align, which will ensure that the two parallel lines that α_1 and α_2 are based on correctly align, as in Fig. 6.
5. Tape the pieces of graph paper together and remove the excess paper and ensure that the system is secured to the stand so the prisms will not be bumped out of their position.

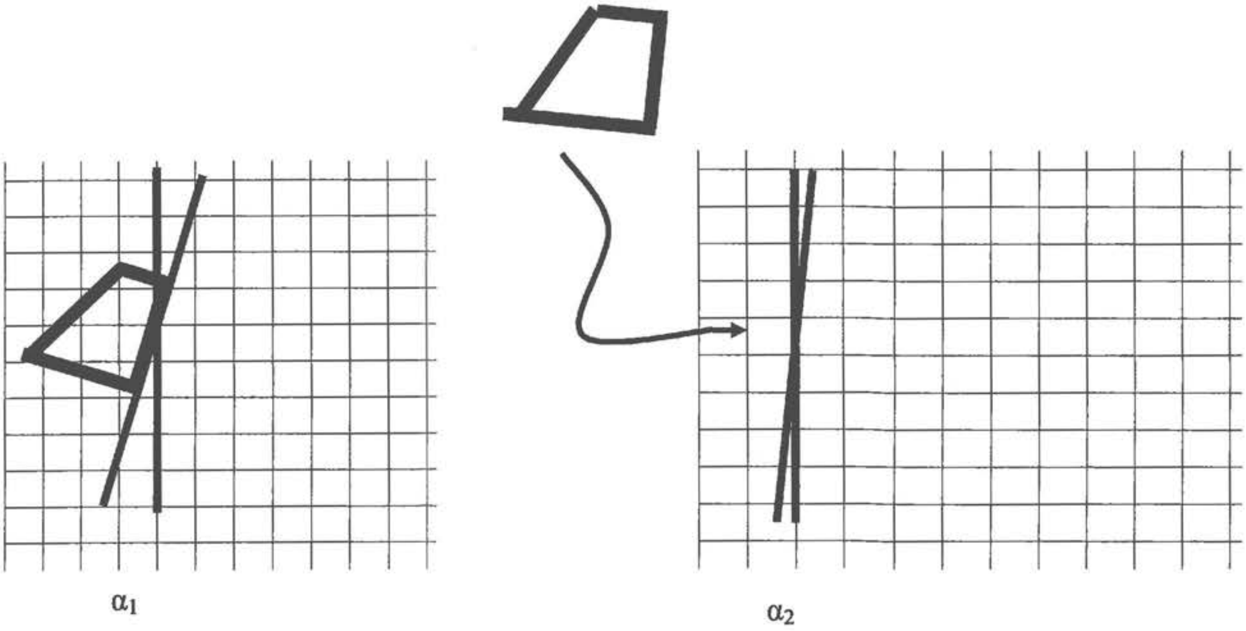

Figure 5 Graph paper with angles α_1 and α_2 drawn and the prisms being placed.

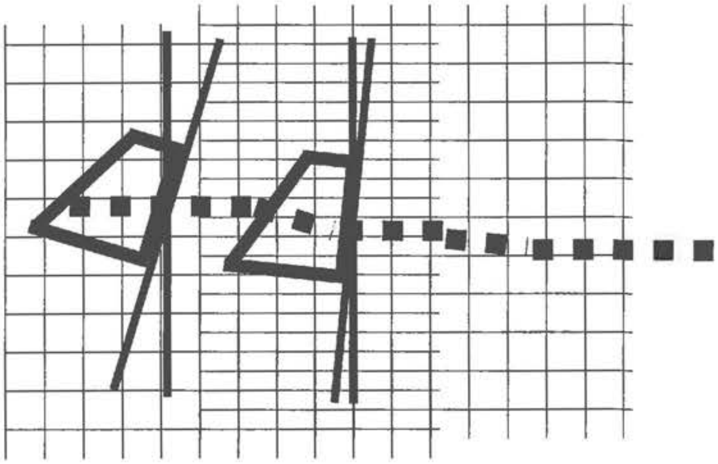

Figure 6 The first prism has been placed in the path of the beam and while the second prism is placed the vertical lines, parallel to the reference lines, must align to ensure that the magnification is correct.

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Southern Scholars Honors Program
Senior Project Proposal Information Sheet

SOUTHERN
ADVENTIST UNIVERSITY

Name Matthew Knecht Date______

Major Physics: Bio physics

Southern Scholars
southernscholars.southern.edu
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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 four weeks prior to the last day of class for the semester the project is turned in. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is usually done as directed study or in a research class.

NOTE-Senior Project Proposal Due Date: The senior project proposal is due in the Honors Program Director's office two weeks after the beginning of the semester the project will be completed. The proposal should be a detailed description of the Honors Project's purpose and proposed methodology.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. Attach a separate sheet of paper.

Signature of faculty advisor ______

Expected date of completion April, 2007

NOTE: An advisor's final project approval does not guarantee that the Honors Faculty Committee will automatically approve the project. The Honors Faculty Committee has the final vote.

Approval to be signed by faculty advisor when the project is completed:

This project has been completed as planned (date) 16 April, 2007

This is an "A" project yes.

This project is worth 2-3 hours of credit 2 hrs

Advisor's Final Signature ______ Date: 17 April, 2007

Chair, Honors Committee Mark Peach Date Approved: 1 May '08

Dear Advisor,

(1) 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.

(2) Please include a paragraph explaining your specific academic credentials for advising this Senior Project.