



Laser Tweezers Manipulation

Cellular and sub-cellular microbiological force application methods may be divided into two categories; those that can apply relatively high forces through the use of a physical connection to a probe (ex: AFM, microneedles), and those that apply smaller forces with a detached probe (ex: laser tweezers, magnet-based manipulators). Detached probe techniques typically rely on the use of a microbead that is either free to move throughout the specimen volume or has been functionalized to attach to a particular molecular group or proteins. In this laboratory a specific detached probe technique, laser tweezers, will be introduced. The goal of this station is to become familiar with the design considerations for developing such a system as well as applications of such a system.

Laser tweezers have been applied to a wide variety of biological problems, for example, measurements of the forces generated during DNA transcription, the properties of neuronal membranes, and the forces generated by the molecular motors dynein, kinesin and myosin. Its limitations are in the achievable force (generally less than 200 pN), specimen heating at higher forces (approximately 5.6°C/Watt of laser power at 975nm and 15°C/Watt of laser power at 1064nm laser wavelength in water) which can cause convection forces in the pN range, and the non-specificity of forces which act on all refracting particles and macromolecules within the range of the optical trap. Conversely, the ability to apply force selectively to a single particle within a set of other particles in a specimen is possible with optical tweezers.

Procedure

The first part of the lab, and one of the most interesting, is to calibrate the forces exerted by the trap. Our system uses a quadrant photodiode to track the position of the bead in 3D. The QPD registers the amount of light falling on each quadrant, and updates this information 10,000 times/sec. In order for the QPD to report the position of a bead, the forward scattering of bead must be aligned within the approximate center of the QPD.

Figure 1 (next page) shows the diagram of light path through the microscope. The QPD receives light from the tracking laser only. The wavelength of the trapping laser is of 1000 nm, while the tracking laser is ~820 nm. A 900 nm longpass filter and several neutral density filters assure this. After columnating each, both lasers travel the same path into the epifluorescence path of the inverted microscope. Only one mirror in the trapping laser is independent of the tracking laser, and thus that is the one used to align them.

Step 1. Align the trapping laser and tracking lasers within the field of view. To keep things moving, the lasers will be aligned before the lab starts, but we will check them first thing. For optimum alignment a power meter is placed on the objective port, and both lasers are positioned to give maximum power output. Alternatively, and more visual, we can see the spot of reflection





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of each laser on the specimen glass. By changing filters and blocking one at a time, we can align the spots using video. This works acceptably well in practice. Check that this is so.

Step 2. Position a bead fore-scattered image within the QPD. Using the coarse stage controls and then the XYZ piezo stage as fine control, position a bead in a central location within the QPD. Both the scattered image and voltage levels of each quadrant are used for this process. To start, we will use 1 μm polystyrene beads in 2M sucrose as a standard reference slide. The trapping laser is blocked by closing its shutter.

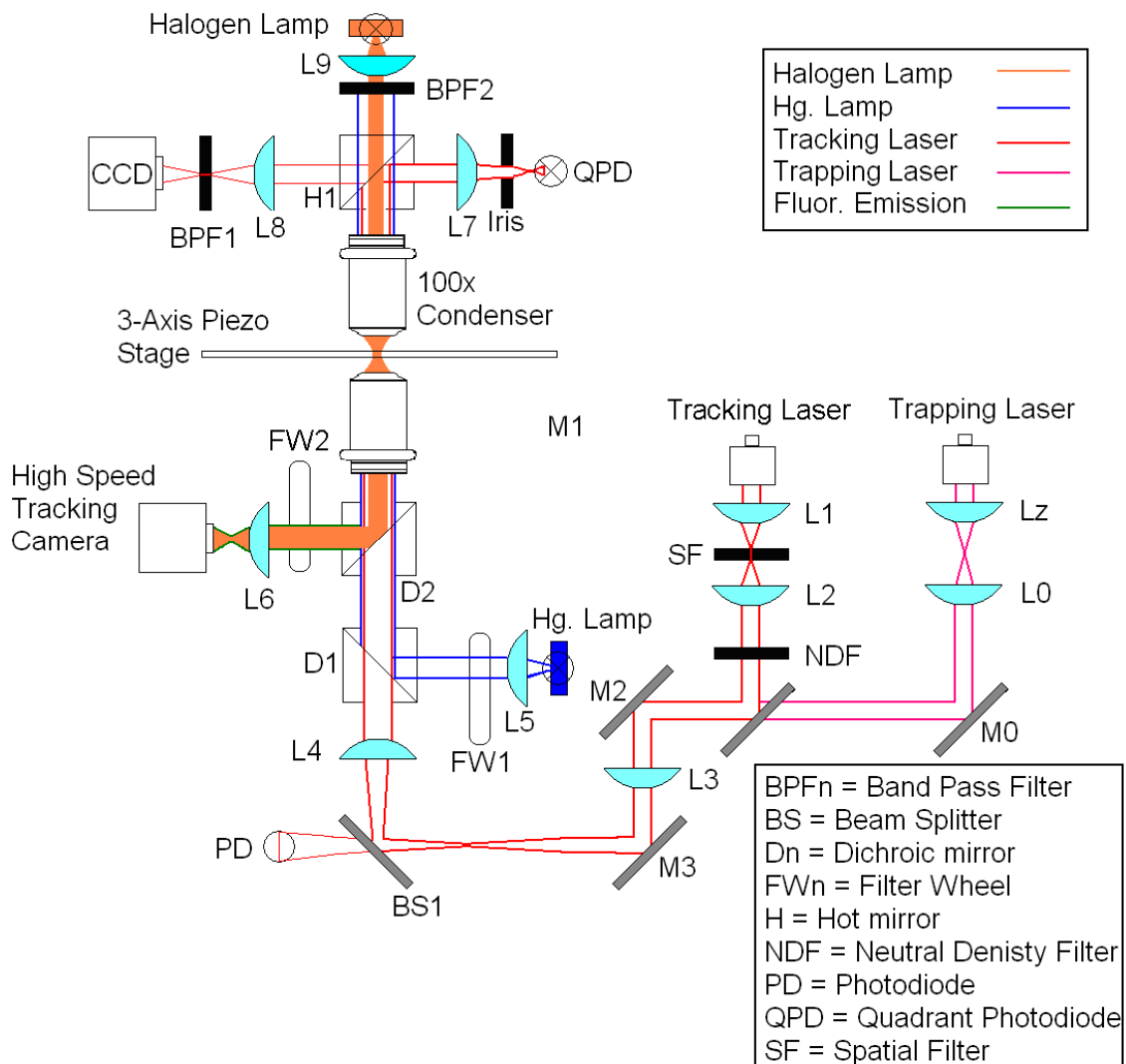


Figure 1. Optical Path Diagram, based on inverted microscope stand.





Figure 2 (Right) shows the view of the QPD from the CCD camera positioned across from it. A bead in the center of the QPD will appear as a diffuse light spot. This diffraction changes in intensity as the bead moves in Z (up-down) relative to the laser focus, and from side to side as the bead or stage moves in X and Y. Thus the position of the bead can be mapped as voltages by the A/D boards within one of the three computers used for this experiment.



Step 2. Begin tracking the bead. Using the Laser Tracking software, we will tell the computer to begin tracking the bead. The interface for this program is shown below. The Align Tether and Trap Origins button is circled, but we will use the Track XYZ button for now. The Align button will be discussed below. Tracking can be done with the trapping laser off or on, but it works more consistently with the trapping laser off. [Note: theoretically tracking need not be established before using the trap, but in practice the tracking algorithm uses the

XYZ piezo stage to keep the bead centered on the QPD. The feedback process, called “agnostic tracking,” moves the stage randomly in all three dimensions and assesses voltage changes associated with each movement. It then uses this map to quantify bead motion and to keep the bead centered. For this reason, when we actually use the trap, we will have to turn off the tracking program.

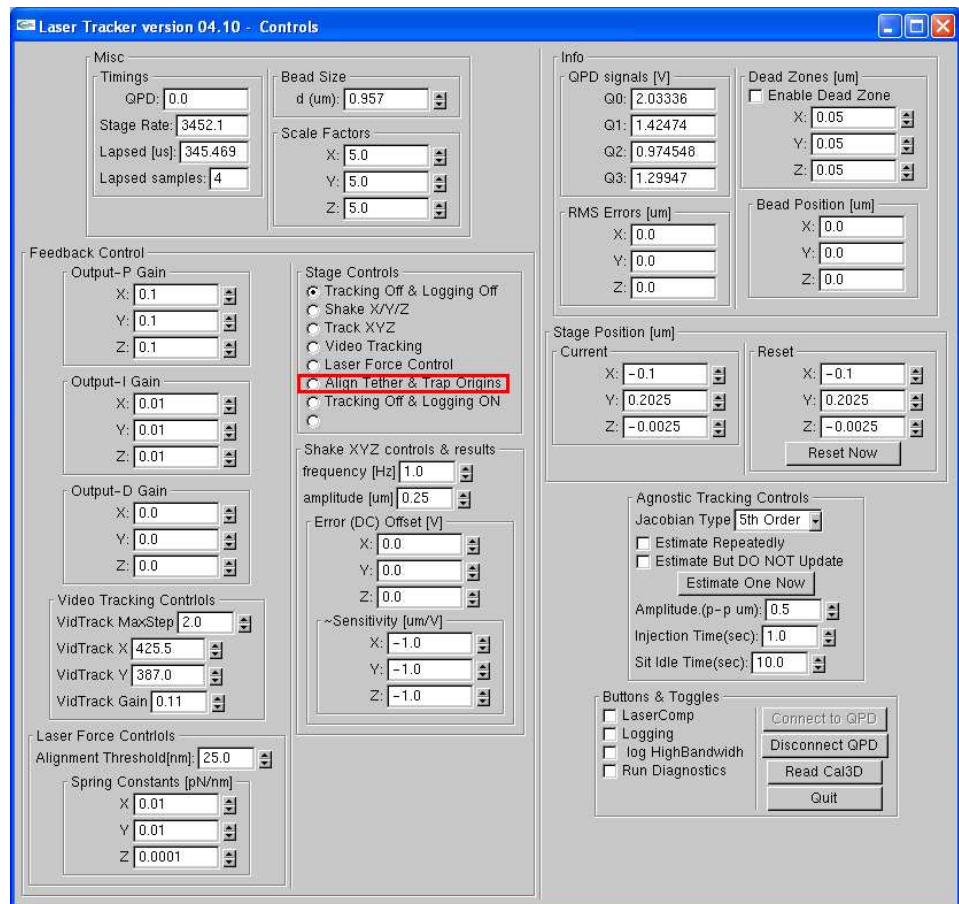


Figure 3 shows the User Interface for the Tracking program. Readouts of stage position, voltage on the QPD, data logging bandwidth, and several other functions are displayed.

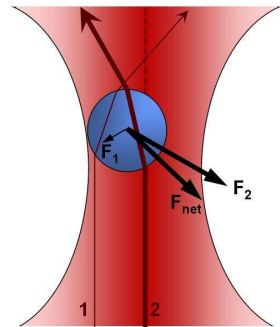




To start tracking, click **Track XYZ**. If the tracking is established, go to Step 3, if not, readjust the bead position to maximize energy on all quadrants and try again.

Step 4. Once tracking is established, turn on the trapping laser. This is done by opening the shutter on that laser. At present we use the actual shutter controller box for this. If alignment was good, and the bead was not fluctuating too far from center when you turn on the trap, then tracking will continue. If not then try again by repeating Steps 2-4. To actually use the trap, tracking cannot be on, so as long as the QPD readouts are close, and the bead is centered, calibration can continue even with tracking off. This is a judgment call.

Ideally with tracking and trapping both working, one would click the “Tracking off, logging on” button. This turns tracking off, and the program no longer sends signals to the stage to keep the “image” of the bead in the center of the QPD. Any stage movement would now move the bead relative to the beam waist of the trapping laser, and act to “pull” the bead in the direction of the stage motion. This will be used in the next section.

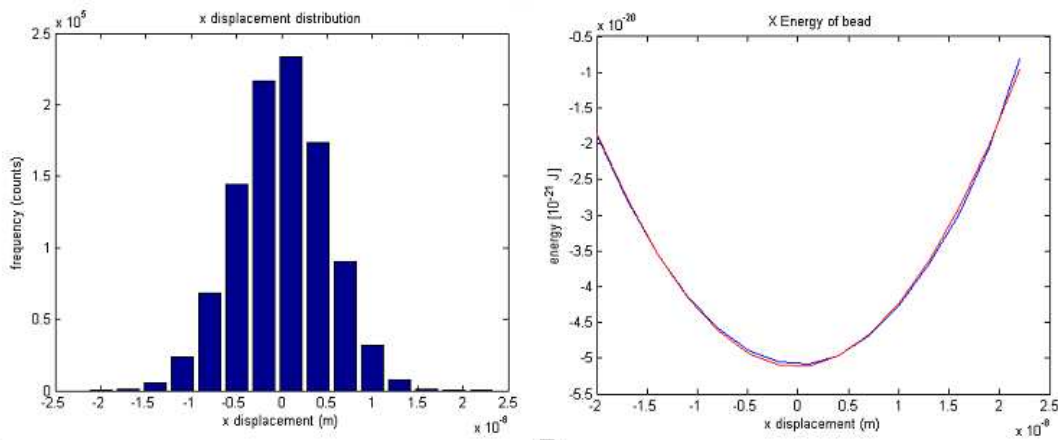


Step 5. Click “logging” and “high band width logging” to start a file.

Step 6. Cut the file, obtain spring constants. After about 60 sec of trapping without tracking, cut the data to make a file for analysis to calculate the stiffness of the trap. Data is cut by clicking the logging button.

Analysis of the calibration data does not directly require knowing the viscosity of the solution the bead is in, but in practice 2M sucrose works well. The analysis looks at the frequency of movements of a specific distance from the center of the trap. Trap stiffness is calculated from the steepness of this distribution. This is described in more detail in the Appendix, but for the present, we convert the data from the QPD into matlab format, and analyze the displacements using a program called ApolloK. This generates data like that shown below. In the time domain, the position histogram for a thermally excited bead in the trap shows a Boltzmann distribution. This histogram may then be converted to an energy profile by multiplying the log of the counts in each bin by the temperature and Boltzmann’s constant, and fit to a quadratic ($F = \frac{1}{2}kx^2$) to determine the spring constant. In Figure 4 (A), the position histogram for a 1 μm polystyrene bead in a laser trap is shown. In Figure 4 (B), this histogram has been converted to energy (blue line) and fit to a quadratic to determine the spring constant (red line). The resulting spring constant is in units of pN/nm.





A Once the data is obtained, we use the time and distance frequency information to calculate k . These are entered into our analysis programs.

Experiment with Cells or other specimen

Now that we have the spring constants, we can use the trap to move a bead that is tethered in some way to a cell. Tethers can be non-specific, or be specific links via antibodies against cell surface markers, and so on. Thus questions of specific interest can be addressed. We may start with non-specific tethers, or try something more interesting, or assess what altering the cell cytoskeleton does to the tether response, depending on time.

Step 7. Place a new specimen onto the microscope, with 1 μ m beads and cells present, and obtain focus. Next a bead is located that is attached to a cell of interest.

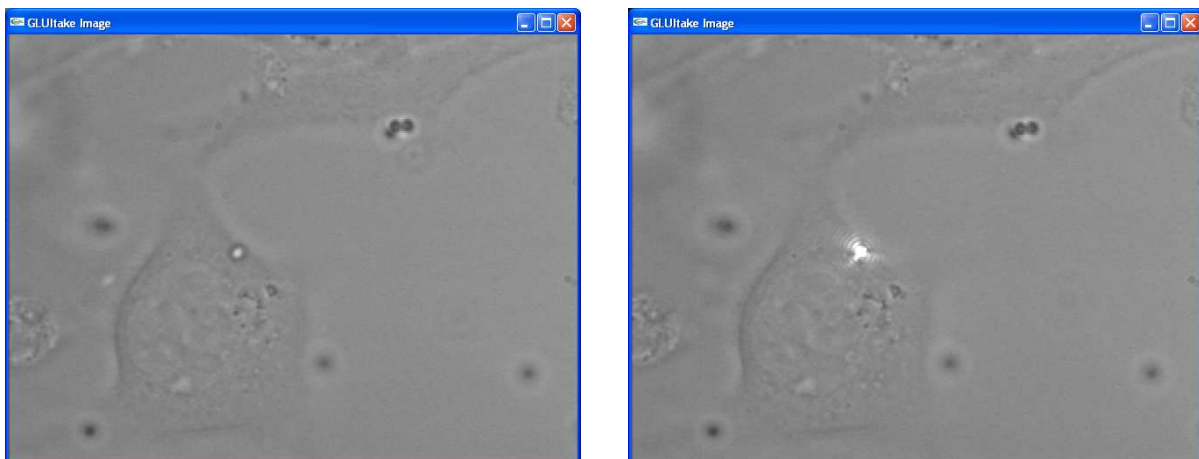


Figure 5 above shows a MEF cell with a 1 μ m bead attached, with the trap off (left) and the trap on (right). The light to the camera is filtered in this case through out Texas Red fluorescence cube. Otherwise it would dominate the entire scene. This will be shown during the lab.





Step 8. Once a bead has been selected, block the trapping laser using the UniBlitz shutter and move the bead to the location of the trapping laser/trapping beam waist. This provides to provide a gross alignment of the DNA tether origin and the center of the trapping laser, and ensures that the experiment will begin with 0 force applied to the tether.

Step 9. After completing the gross alignment of the tether and trap origins, click on the ‘Align Tether & Trap Origins’ radio button in the Laser Tracker program (as shown in Figure 3 above). This program will execute a fine alignment procedure to ensure that the origins of the tether and trap are within a specified distance. This program executes 3 main tasks:

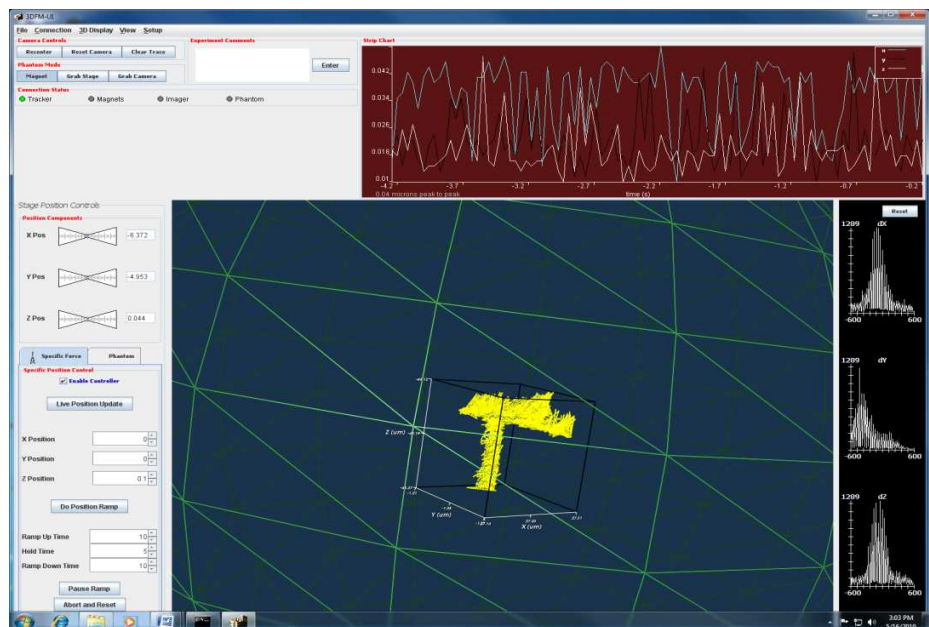
- Calibration of the detection system-This converts the voltages measured by the quadrant photodiode into distances
- Alignment of tether and trap origins
- Data logging-for this experiment we will check the ‘log HighBandwidth’ checkbox to collect data at approximately 10 kHz.

Step 10. With the user interface program (program can be found on the desktop of Argon-cs), select the desired distance to move the stage as well as the duration of the extension. Stage controls can be found in the ‘Specific Force’ section of the 3DFM-UI (shown below). This program will be running in the proper mode when you reach the station. Make sure to click on the ‘Enable Controller’ checkbox prior to beginning the experiment (this tells the program that you will be controlling the stage through the interface). A screenshot of this section of the user interface is below.

Figure 6: 3DFM-UI used to program the DNA extension.

11) On the Laser Tracker program interface, click the ‘Logging’ checkbox to start a new data file.

12) To begin stretching the tether (move the bead), click on the ‘Start Position Ramp’ button on the 3DFM-UI. This will cause the piezo stage to move in the manner specified by the user.





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13) Once the stage has stopped moving, click on the 'Logging' checkbox in the Laser Tracker program to cut your data set. This data set will be located in the D:\Data\Tracking\Ver05_13 folder with the file name corresponding to the time when the data set was cut. This file is in the .vrpn format and will need to be converted to the .mat format for data analysis

14) Convert the .vrpn file to a .mat file by dropping it on the vrpnLogToMatlab03_04 program on the desktop of Aurum-cs. This program will put the converted data file in the same folder as the original .vrpn.

15) Finally the data can be viewed in Excel, or manipulated in Matlab. Jay Fisher wrote a Matlab script to graph the force extension data from the vrpn files. It plots the stage motion in x, Y, and Z in terms of the movement or displacement of the bead from the center of the trap. This distance

Analysis Details

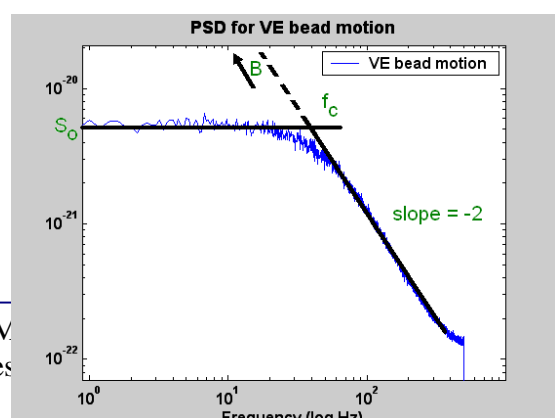
Force calibration

The force calibration of the laser tweezers system can be accomplished using three techniques. In each case, the goal of the calibration routine is to determine a spring constant, k . This quantity describes the restorative force that is applied to the probe as it moves away from the center of the trap. The first technique relies on time domain data of the amplitude of the bead's position fluctuations inside the trapping beam. This is similar to the second method, which uses the same data, but instead of analyzing the data in the time domain the analysis takes place in the frequency domain. For the third method, the specimen containing the trapped particle is moved at a known velocity in order to determine the drag force that is required to overcome the trapping force. This technique is best for determining the maximum force that may be applied by the trap, but does a poor job of trap calibration. As a result, further discussion will focus on the first and second techniques.

In the time domain, the position histogram for a thermally excited bead in the trap shows a Boltzmann distribution. This histogram may then be converted to an energy profile by multiplying the log of the counts in each bin by the temperature and Boltzmann's constant, and fit to a quadratic ($F = 1/2kx^2$) to determine the spring constant. This was already shown in Figure 4 above. The resulting spring constant is in units of pN/nm.

Figure 7: Example PSD showing components used to determine the viscosity and spring constant.

In the frequency domain, the cross-over frequency (f_c) of the power spectrum along with the 1 Hz intercept





(S_0) may be used to determine the spring constant using the following relation:

$$k = \frac{2K_b T}{\pi^2 S_0 f_c^2}$$

where K_b is Boltzmann's constant, and T the temperature in Kelvin. In addition to the spring constant (but unrelated to this discussion) the viscosity (η) of the media may be determined from the following expression:

$$\eta = \frac{K_b T}{6\pi 10^B r_{bead}}$$

where B is the 1 Hz intercept of the portion of the data with a slope of -2 and r_{bead} is the radius of the bead. An example data set (simulated data) with these parameters labeled is shown in Figure 46. In practice this approach is more difficult to apply in instances where the spring constant is weak due to the fact that the cross over will be at a low frequency where system drift has a tendency to corrupt the data.

Data analysis

Three main types of data will be collected during this experiment. They are position data for the bead inside the laser trap (x,y,z), piezo stage position data (x,y,z), and time stamps corresponding to the bead and stage data. The position data for the bead inside the laser will be converted in to a force using a spring constant obtained prior to this experiment. Both the stage position data and the bead position data will be used to determine the extension of the DNA. Force vs. extension data will be plotted and fit using the worm-like chain model to determine the persistence length and contour length of the DNA. The model equation is below:

$$f(r) = \frac{KT}{P} [25(1-r)/L] \exp(-2) - .25 + r/L$$

K = Boltzmann's constant

T = Temperature

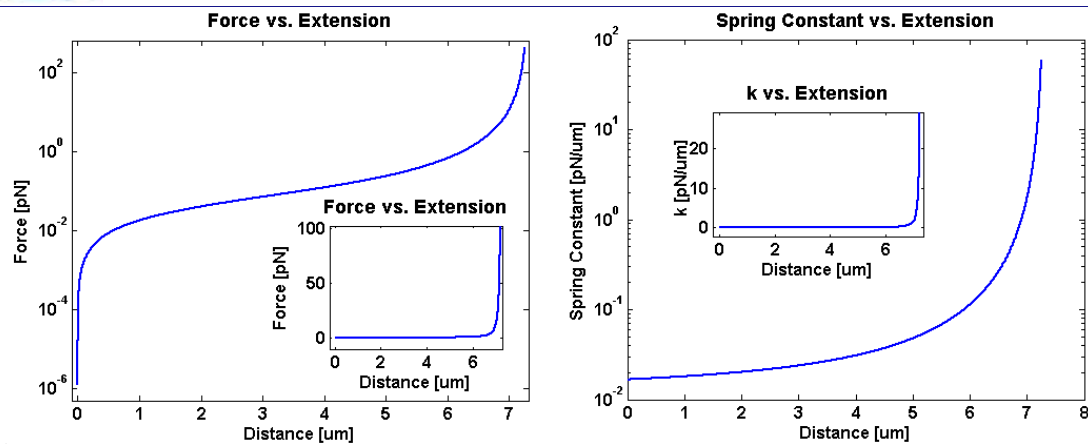
r = length of extension

P = persistence length

L = contour length

This model describes the elastic behavior of DNA by treating it as a flexible rod of length L that curves as a result of thermal fluctuations. Example Force vs. Extension plots for the worm like chain model are shown below.





A B
Figure 7: Force vs. Extension plot using the worm like chain model for a molecule with a persistence length of 50 nm and a contour length of 7.3 microns.

References

1. Block S. M. 1992. "Making light work with optical tweezers." *Nature* 360(6403):493-5.
2. Svoboda K., Block S. M. 1994. "Biological applications of optical forces." *Annu Rev Biophys Biomol Struct* 23:247-85.
3. Simmons R. M., Finer J. T., Chu S., Spudich J. A. 1996. "Quantitative measurements of force and displacement using an optical trap." *Biophys J* 70(4):1813-22.
4. Visscher K., Gross S. P., Block S. M. 1996. "Construction of multiple-beam optical traps with nanometer-resolution position sensing." *IEEE Journal of Selected Topics in Quantum Electronics* 2(4):1066-1076.
5. Gittes F., Schmidt C. F. 1998. "Interference model for back-focal-plane displacement detection in optical tweezers." *Optics Letters* 23(1):7-9.
6. Peters I. M., de Grooth B. G., Schins J. M., Figdor C. G., Greve J. 1998. "Three dimensional single-particle tracking with nanometer resolution." *Review of Scientific Instruments* 69(7):2762-6.
7. Smith S. P., Bhalotra S. R., Brody A. L., Brown B. L., Boyda E. K., Prentiss M. 1998. "Inexpensive optical tweezers for undergraduate laboratories." *Am. J. Phys.* 67(1):26-35.
8. Visscher K., Block S. M. 1998. "Versatile optical traps with feedback control." *Methods Enzymol* 298:460-89.
9. Neuman K. C., Chadd E. H., Liou G. F., Bergman K., Block S. M. 1999. "Characterization of photodamage to escherichia coli in optical traps." *Biophys J* 77(5):2856-63.
10. Pralle A., Prummer M., Florin E. L., Stelzer E. H., Horber J. K. 1999. "Three-dimensional high-resolution particle tracking for optical tweezers by forward scattered light." *Microsc Res Tech* 44(5):378-86.





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11. Ashkin, A. 2000. "History of Optical Trapping and Manipulation of Small-Neutral Particle, Atoms, and Molecules." *IEEE Journal of Selected Topics in Quantum Electronics* 6(6):841-856.
12. Ishii Y., Ishijima A., Yanagida T. 2001." Single molecule nanomanipulation of biomolecules." *Trends Biotechnol* 19(6):211-6.
13. Kuo S. C. 2001. "Using optics to measure biological forces and mechanics." *Traffic* 2(11):757-63.
14. Lang M. J., Asbury C. L., Shaevitz J. W., Block S. M. 2002. "An automated two-dimensional optical force clamp for single molecule studies." *Biophys J* 83(1):491-501.
15. Peterman, E., Gittes, F. & Schmidt, C.F. Laser-induced heating in optical traps. *Biophysical Journal* **84**, 1308-1316 (2003).

