

## Nanoscale Manipulation and Characterization Using Optical Tweezers

“Optical tweezers” may be used to manipulate and characterize objects at both the micrometer and nanometer scales. The basic idea is that a tightly focused beam of laser light may trap and manipulate objects, such as nanospheres or living biological cells, *in vitro*. The laser light acts on the difference in the index of refraction between the tweezed object and its surrounding medium. By carefully controlling and characterizing the trapping laser beam(s), microscopic objects can be positioned with nanometer precision and forces from  $\sim 0.01$  pN to 100 pN can be measured. The optical tweezer apparatus itself is based on an inverted microscope. The microscope objective produces the tightly focused laser used to tweeze objects. In addition, we use the microscope to watch the experiments.

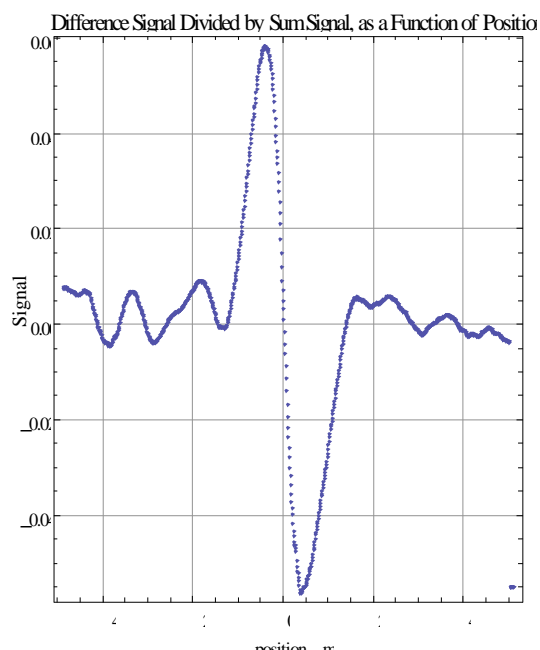
This past year we have been developing apparatus and procedures to allow for four specific projects:

- 1) measuring the binding force between potentially “therapeutic” nanoparticles and a targeted cell’s membrane;
- 2) measuring statics and dynamics of intermolecular forces  $f_{iml}$  for a single DNA molecule entangled in a polymer solution;
- 3) optical tweezer positioning cells precisely on a microfluidic platform to allow accurate studies of electroporation in a realistic environment;
- 4) characterizing forces applied by a novel magnetic micro-tweezer.

The experimental requirements of the individual projects overlap one another. And so we are developing a toolkit of the needed capabilities. We will next describe the highlights of this work and then return to a more detailed look at the individual projects.

Frequently in optical tweezer studies, one needs to know the position of an object to high precision—much better than the  $\sim 200$  nm resolution we get from the microscope itself. We use the forward scattered light that passes through the microscope’s condenser for this purpose. Imaging the back focal plane of the condenser onto a quadrant photodiode allows a very high resolution determination of an object’s position relative to the laser focus. Figure 1 shows data acquired from a 750 nm nanosphere as the microscope stage moves it through the focus of the tweezer beam. Near-single-nanometer resolution is possible for a suitably stable subject.

A second common requirement for optical tweezer work is to accurately measure *forces* in the pN range. The way this is done is to measure the displacement of an object from the center of a calibrated tweezer “trap.” For small displacements, the trap will be harmonic and the *force* is given by Hooke’s law:  $f = -kx$ . To measure the force requires measuring the displacement from the trap center/laser focus and finding the force constant,  $k$ . The displacement measurement goes as discussed above.



**Figure 1.** Signal from quadrant photodiode. Points are taken at 10 nm increments as a 750 nm nanosphere is passed through the focus of the trapping laser. For small displacements, this calibration allows using the detector signal to determine position **relative to the laser focus** to  $\sim 1$  nm for a suitably stable object.

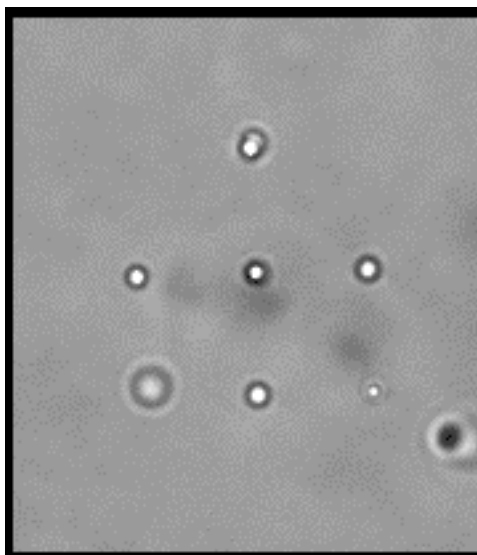
We have measured the force constant for several traps using the Brownian motion of a trapped particle. The average displacement,  $\bar{x}$ , for this motion satisfies

$$\frac{1}{2} k \bar{x}^2 = \frac{1}{2} k_B T$$

where  $k_B$  is Boltzmann’s constant and  $T$ , the absolute temperature. By measuring the mean displacement, again as described above, the trap’s spring constant is obtained.

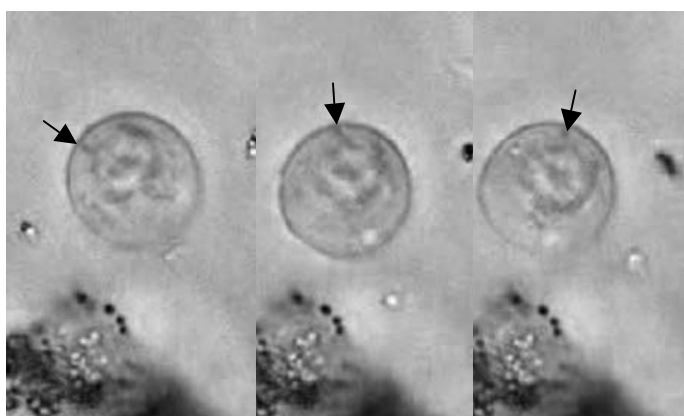
Another common requirement for optical tweezer experiments is to simultaneously manipulate two or more objects. We do this in two ways. First, we can create multiple traps by rapidly switching the trapping beam between trapping positions in the specimen plane. An acousto-optic modulator allows the laser beam to be scanned between traps at kilohertz frequencies. In Figure 2, we show five nanospheres being simultaneously manipulated. One consideration is that the light from all traps image to the same location on the condenser back-focal-plane quadrant photodiode used to measure the position of an object and, in this first method of creating multiple traps, distinguishing the positions of the individual traps is difficult. We use a “polarization” method of creating multiple traps where this is necessary. This method creates two different traps by splitting the beam into two orthogonal polarizations. Downstream, a polarizer just before the quadrant photodiode position-detector allows for distinguishing between the two traps.

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**Figure 2.** Five 750 nm nanospheres simultaneously trapped by rapidly scanned the tweezing laser.

We also have been working on techniques to manipulate single biological cells. We find that we are able to non-destructively trap and move most cells. Though, as a rule-of-thumb, the easier it is to see a cell under the microscope, the easier it is for us to manipulate the cell. This is likely because the trapping force depends on a difference in the index of refraction between the cell and the surrounding medium. A large index difference allows both easier viewing and stronger trapping. In Figure 3, we show an example in which we used two tweezer beams to orient a single leukemia cell.



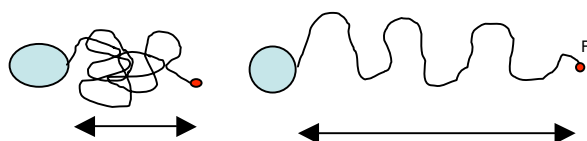
**Figure 3.** Rotating a human leukemia cell. Two optical tweezer traps are used to orient a cell by turning it through an angle of  $\sim 50^\circ$ . The rotation occurred over about 12 s in time. The arrow points to a cell feature that allows rotation tracking.

We next discuss the individual projects listed above in the context of the tools we have developed.

The first project is to measure the binding force of a nanoparticle to the membrane of a target cell. The nanoparticles, developed via NSEC collaboration, will

ultimately be used to deliver therapeutic agents to the cell. Characterizing the binding is useful for understanding and optimizing that process. The nanoparticles are coated with an antigen to bind to the targeted cells. Our initial work will target leukemia cells similar to that shown in Figure 3. One set of tweezers will control the cell and a separate trap will be used to manipulate, track, and measure the forces on a nanoparticle. Our first experiments will measure binding to the cell membrane using (easy-to-tweeze) polystyrene nanospheres coated with the appropriate antigen as stand-ins for the nanoparticles. Later, manipulating and measuring membrane binding forces for actual, potentially therapeutic nanoparticles will require embedding a high index “handle” within the nanoparticle. We have studied the tweezing characteristics of 100 nm gold particles and will use them as “handles.”

The second project is a collaboration with Prof. S. Q. Wang to study fundamental problems of entangled polymer solutions. The basic idea is to measure the forces on a DNA molecule entangled in a polymer solution both as it is stretched from equilibrium and also as it is released from an initially stretched condition. The experimental arrangement is shown schematically in Figure 4. A large polystyrene anchor bead will be attached to one end of the DNA and a 100 nm gold sphere to the other. Each end would be held in a tweezer beam, and displacements and forces on the molecule will be measured *via* the beads as described above.



**Figure 4.** Measuring forces on a DNA molecule entangled in a polymer solution will be measured by attaching a polystyrene anchor and a small gold probe to ends of the DNA.

The third project simply uses the tweezer apparatus to controllably position biological cells in an electroporation test stand to reliably characterize the electric fields in that device.

The fourth project is a collaboration with Prof. Sooryakumar to develop a novel magnetic tweezer apparatus they are developing. Their idea is to use domain walls in magnetic films to manipulate magnetic nanoparticles. We are using our position and force measurement capabilities to help calibrate the apparatus they are developing. We find we are able to optically trap the magnetic beads *they* manipulate. Hence we can measure the displacements and forces they affect and, in turn, calibrate their apparatus.