Stretching DNA with Optical Tweezers

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Abstract:

Optical tweezers are a modern day tractor beam capable of manipulating and studying the forces acting on microscopic objects. The laser is safe with biological samples; providing an non-invasive method of studying the forces acting on whole bacteria, protein coated glass beads, and more. Protein coated beads will be used to link DNA from one bead to another to study its elastic properties. One bead is firmly secured using micropipettes, while the other is manipulated using the optical tweezers. By moving the laser, this forces the DNA stretch like a spring. Our goal is to discover whether the stiffness changes with the number of base pairs, or if it remains constant regardless of the length of the DNA strand

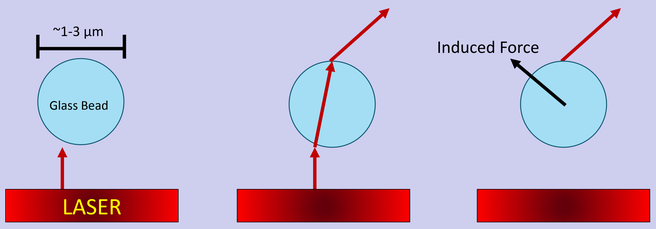
Optical Tweezers:

Optical tweezers are able to generate forces on microscopic, refractive objects by using the momentum of a laser light. Photons, the individual particles of light, are massless but they still have a momentum proportional to their wavelength given by the equation:

$$p = \frac{h}{\lambda}$$

Where h is Plank's constant and λ is the photon's wavelength

Lasers beams are a collection of photons which travel in the same direction, therefore laser beams carry momentum as well. The optical tweezers uses this property to trap objects by altering the path of the laser beam. When the laser refracts through the object, its undergoes a change in momentum. Due to the conservation of momentum a reaction force is generated, pushing the object in the opposite direction.



The incoming light has an initial momentum, Light near the center of the laser is more intense

As the light refracts through the bead, its direction of motion is changed.

When the light changes direction, it imparts some momentum to the glass bead

A gradient laser intensity generates unsymmetrical forces on the object, making the total force directed towards the center of the laser beam. This inward force can be modeled as a simple harmonic oscillator, providing a way to measure external forces using the position of the bead relative to the laser center. This approximation is governed by the equation:

$$F = -kx$$

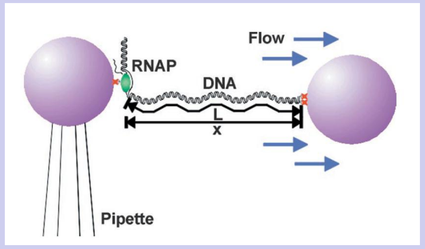
Where F is the induced force, x is the distance from the laser center, and k is the stiffness coefficient.

Using this approximation, we plan to study the stretching properties of DNA by attaching it between two refracting plastic beads.

Stretching DNA:

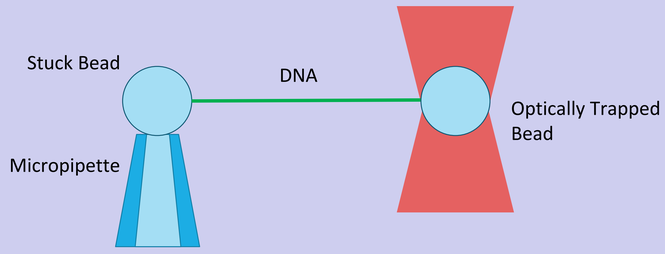
DNA is a long, elastic strand of paired nucleotides bounded by an iconic double helix backbone. These strands arte stable despite their incredible length. In a typical human cell, the DNA inside the nucleus can be over a meter in length. Using DNA's can create interesting samples for the optical tweezers, allowing us to connect silica beads over a distance. Furthermore, proteins can be attached to the ends of DNA, letting the optical tweezers measure their mechanical properties.

For example, the transcription rate of RNA Polymerase has been studied using an optical tweezers setup [(1)] by attaching DNA to a glass bead and attaching the DNA to another bead coated in RNA polymerase proteins.



Davenport et al. [(1)]
This diagram shows the setup for an experiment using RNA Polymerase (RNAP). As the RNAP processed the DNA, the tweezers recorded how the right bead moved over time.

The goal for the optical tweezers here at UNC is to attempt similar experiments using protein coated beads and DNA strands. In order attempt these experiments we will first study the elastic properties of DNA to gain experience and understand how the DNA behaves under tension.



DNA can be stretched by fixating one protein coated bead with a micropipette, and pulling on another bead with the optical tweezers. DNA will be attached to both beads using two proteins: biotin and streptavidin.

Method:

- DNA will be attached to glass beads using a method called biotinylation. Biotin is a small protein which has an extremely high affinity to streptavidin, a larger protein complex. Biotin will be attached to both the 5' and 3' ends of DNA, and streptavidin coated beads have been purchased online.
- The DNA we will use comes from the M13mp18, a widely available DNA plasmid from the M13 bacteriophage. This strand of DNA is around 7,249 base pairs long, or around 2.5 um fully stretched. The DNA will be cut using restriction enzymes.
- One bead will be fixated using a micropipette and another will be trapped using the optical tweezers. The tweezers will then stretch the DNA between the two beads and record the resulting elastic force. This process will be repeated many times to ensure accuracy.

Future Possibilities:

- This method could be used to study the binding forces between proteins. The tweezers could pull on the linked protein until the protein bond breaks, or until the DNA breaks.
- DNA Origami are folded DNA structures created using small DNA strands called staples. A variety of shapes can be created, including possible shapes which change under tension generated by the optical tweezers.
- Bacteria, organelles, and other small biological samples can be safely trapped using the optical tweezers. This allows studies on how they propel themselves and navigate their environment.

References:

- [1] Davenport, John, Gijs Wuite, Robert Landick, and Carlos Bustamante. "Single-Molecule Study of Transcriptional Pausing and Arrest by E. Coli RNA Polymerase." *Science* 287 (2000): 2479-500. Print.

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