

Slide Preparation for DNA Attachment for use in Optical Tweezers

D. Kirkeby¹, K. Carlson², E. R. Jones⁴, and E. Grace¹

Northwestern College

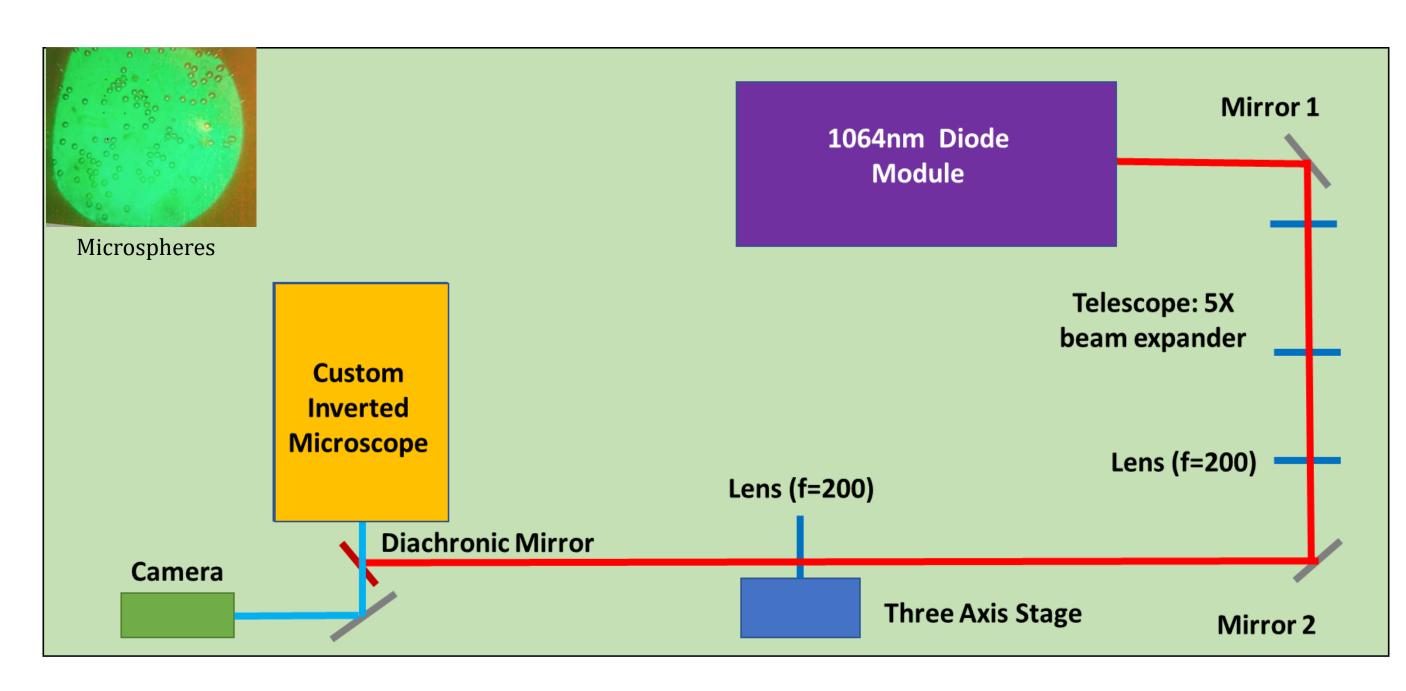
¹Department of Physics, ²Department of Biochemistry and Chemistry, ³Department of Biology. ⁴Department of Physics & Astronomy, Stony Brook University

Abstract

Our research team, ISLAND CURE, is a multidisciplinary team of professors and undergraduate students with the goal to design and build instruments to make biological measurements on a limited budget. One of the apparatuses we are designing, is optical tweezers, which are a Nobel Prize-winning technology capable of trapping microscopic and sub-microscopic particles using a laser beam. Using a 1064 nm beam, we will trap a single strand of DNA using beads and this will enable us to exert minute forces upon the DNA. This experiment will give us a better understanding of the forces on damaged DNA; specifically, the damages that lead to mutations and cancer. With this knowledge our goal is to be able to provide insight into mutagenesis and cancer development, and ideally how to treat and prevent them. Our job was to find a way to prepare a slide in which a single piece of DNA can attach to be used in the inverted microscope setup.

Optical Tweezers

- Highly sensitive device for manipulation of microscopic particles
- A series of lenses and mirrors clean the beam before entering the inverted microscope
- The LED provides us with the image of the sample via the CCD since 1064 nm light is not in the visible spectrum
- Force precisely monitored, ideal for Biomolecular DNA
- Comparing forces on damaged and undamaged DNA

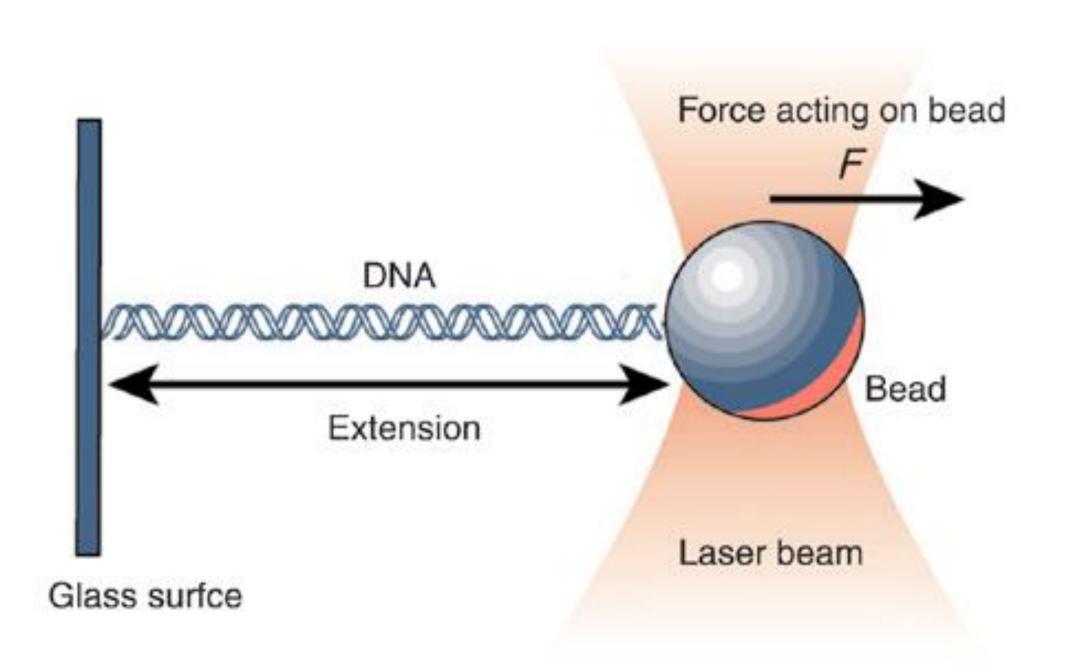


Our Optical Tweezer Setup

Our Current Inverted Microscope

Tweezing DNA

- A single piece of DNA is prepared with one end attached to the slide and the other to a microsphere
- The microsphere is trapped in the 1064 nm beam
- Slight adjustments to the beam move the microsphere, manipulating the DNA



Slide Preparation It is crucial that the Start with a precleaned slide times in the Alternate rinsing witl Place Slide in 10M KOH and ozonator and ozonator for 30 Ambion water 3x minutes each desiccator are followed exactly PEG coating Ouickly rinse mold Peg/Peg-Biotin acts 7x with Ambion water Apply adhesive mold as "DNA Glue" to slide to remove peg/pegbiotin For best results, the protocol must be precisely followed Preform acetone Place slide in rinse by pipetting up desiccator for 15 and down in the mol minutes at 90°C Protein /

• This protocol gives us a slide that is prepared to have a specialized piece of DNA attached to it

Place slide in

desiccator for 5

minutes at 90° C

Air dry in fume hood

with air nozzie

• The DNA will be attached to the slide on one end and to the microsphere on the other

Apply 110 microliters of peg/peg-biotin to the mold

Acknowledgements

- Thank you to David Arnett in the NWC Chemistry Department for providing support with the slide prep
- Thank you to Phoebe Laser Research Group at NWC including Martha Stein, Daniel Nordquist, Daiki Jonouchi, Tim Kaltenberger, Jacob McDonald, Tyler Nhan, Gabriel Andres, Alexa Olguin, and Alaena Trevino for their input.
- Thank you, Brett Freudenthal and Tyler Weaver, and the rest of the Freudenthal Lab at Kansas University Medical Research center.
- Thank you to the Laser Teaching Center and the Hal Metcalf research group at Stony Brook University for your input and support.