Optical Trap Alignment and Calibration for Force Measurements Using Viscous Drag

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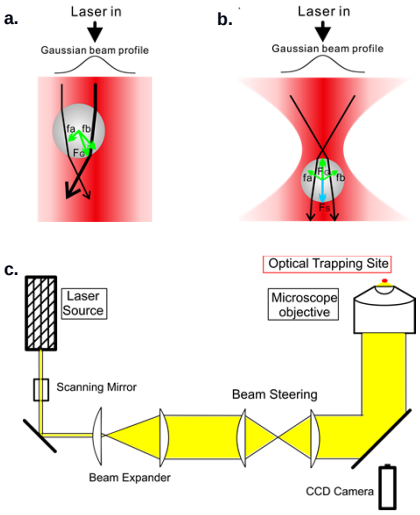
ABSTRACT

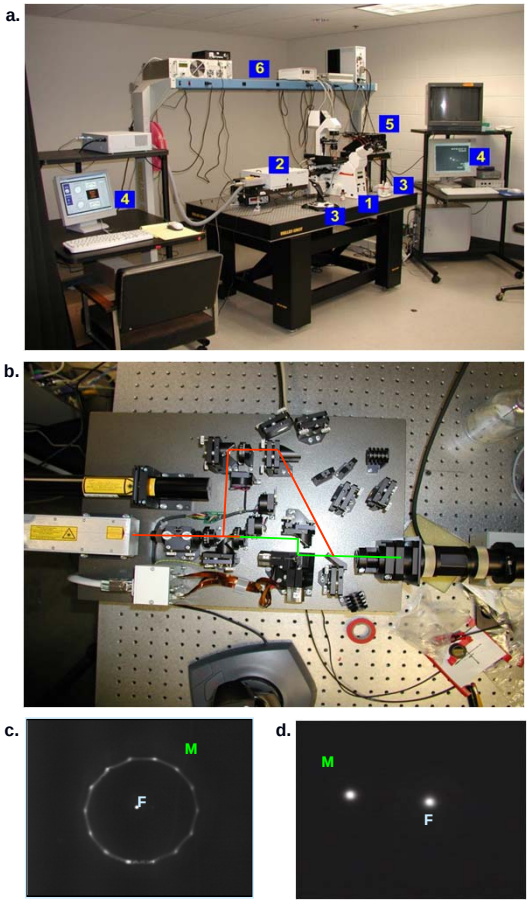
Optical tweezers, or optical traps, have become a versatile tool in the biological sciences. It can trap or "tweeze" objects in the nanometer (to micrometer size range, which lets experimenter study the behavior of single molecule. This became one of the most successful single-molecule techniques used in biological science, due to its preciseness, ability to trap various small objects and ability to measure forces acting on small objects. In this poster, the various methods used to align optical tweezers are demonstrated. The, the method used to calibrate force measurement using viscous drag experienced by a small object is shown. The careful alignment of the optical traps combined with the system calibration, will be used to measure stall forces for the translocating RecBCD enzyme.

Optical Trap: Theory



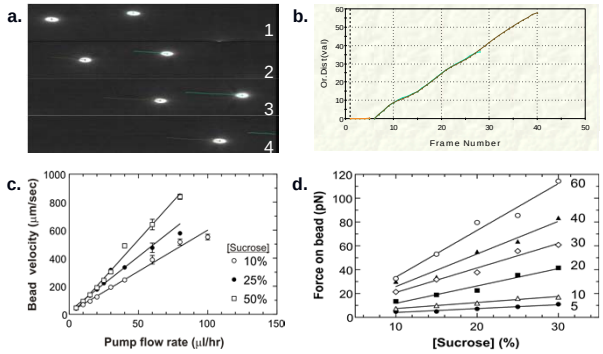
(a). Optical force experienced by a small object inside the laser beam. Force exerted by laser with lesser intensity (fine black arrow): f_a. Force exerted by laser with stronger intensity (bold black arrow): f_b. Net optical force exerted by the laser on the object: F_G
(b). Optical force around the focus. F_G is the net optical force in counter-propagating direction of the laser, and F_S is the force in propagating direction.
(c). Schematic of a simple optical trap.

Optical Trap Alignment



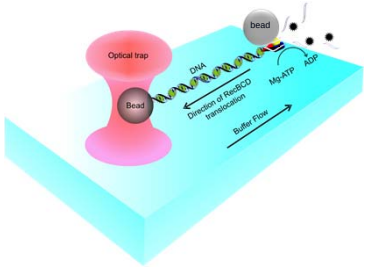
(a). Laboratory set up of the optical trap: (1) Optical table with air-floating stage (2) Laser manipulating optics (3) Joysticks (4) Computers equipped with appropriate software to control the kit (5) Syringe pumps connected to a flow cell (6) Overhead cabinet with laser rack mount on top
(b). Laser path of Nd:YAG laser. Red: s-polarized fixed beam. Green: p-polarized scanning beam
(c). (d). Laser reflections on the glass slide. F is fixed laser beam, and M is mobile or scanning beam. Mobile beam can be scanned through a circle with set radius.

Force Calibration: Drag Force Measurements



(a) Sequential movie frames of fluorescent beads being tracked. Fluid flow is toward the right; green and brown lines show the tracking of the bead centroids.
(b) Bead displacements versus frame number. Green and brown lines overlap as displacement of the both beads are same.
(c) Relationship between pump flow rate and bead velocity. A minimum of five 1um beads were tracked per pump flow rate per [sucrose]. Data were analyzed by linear regression.
(d) F_{drag} on 1um beads as a function of [sucrose]. The values to the right of each curve indicate the pump flow rate.

Future Direction: RecBCD Enzyme



Bead attached to a DNA and a RecBCD enzyme is captured by an optical trap. The buffer solution contains Mg-ATP. As a DNA is unwound, bound fluorophores dissociate and are washed away in the flow. RecBCD must pull the bead against the fluid flow (opposing force).

SUMMARY

- The optical trap theory was studied to understand the alignment procedure.
- The optical trap is aligned with the accuracy of micrometers.
- Force measurements using optical trap are calibrated using viscous drag force method.
- The translocating RecBCD enzyme will be studied using this optical trap.