Abstract

Optical tweezers are important tools for studying cellular and molecular biomechanics. We present a robust optical tweezers device with advanced features including multiple optical traps, acousto-optic trap steering, and back focal plane interferometry position detection. We integrate these features into an ultra-high contrast microscope, with no compromise to its capabilities (differential interference contrast microscopy, fluorescence microscopy, etc.). An Nd:YAG laser is split into orthogonally polarized beams that form separate traps. Acousto-optic deflectors (AODs) independently direct each beam, custom software controls trap position over a range of (20 μm)^2 at the focal plane, and the beam can be rapidly switched between locations to create additional traps. The beams terminate onto a quadrant photodiode detector (QPD). The present optical tweezers design uses advanced technologies (AODs, QPDs) to create a flexible, sensitive instrument. Further, the design features a novel incorporation of optical tweezers into an existing interferometer system. This design integrates a new flexibility of experimental techniques available with unimpaired optical trapping.

Introduction

Optical tweezers use focused light to trap and maneuver microscopic objects, and can detect mechanical events that reveal the mechanisms of:
- Cell locomotion
- Chromosome movements
- RNA and DNA binding
- RNA transcription
- Protein action
- Gene expression
- Chromosome position
- Chromosome orientation

We present the construction and calibration of a robust optical tweezers device with advanced capabilities:
- Multiple independent optical traps
- Acousto-optic trap steering
- Back focal plane interferometry (BFP) position detection
- Full range of microscopy techniques available with unimpaired optical trapping

Optical tweezers are ideal for transducing optical forces because they behave as a linear spring in the image plane: the trap force is a restoring force, F = −kx.

BFP detection using quadrant photodiode detectors to determine an object’s displacement. The critical system parameters for BFP detection are QPD sensitivity, β in [V/μm], and trap stiffness, r in [N/m]. We present calibrations for a single trap in a four-quadrant detector arrangement as well as for a wide range of trap stiffnesses between 1 and 100 N/m.

Sensitivity

- Determination of QPD sensitivity, β
  - Immobile bead to cover glass
  - Use nano-positioning stage to step bead through low power trap
  - Measure positions, an QPD signal at each position
  - Fit central linear region with a line

Eq. Partition and Power Spectral Analysis

Using thermally generated motion to measure trap stiffness

- Measuring the variance of the position of a trapped particle provides a second estimate of trap stiffness:
  \[ \frac{1}{4} \int_0^L \left( \frac{1}{t} \right)^2 dt \]
- Measuring wave disturbance to the expected form at the growth force

Acceptance Between Methods-Stiffness vs. Laser Power

The value of k from a single trap and equipartition methods agree within 5%.

Power spectral analysis of displacement

- Viscous Drag Method
- Three calibrations for a system detect trap level forces

Viscous Drag Data Processing
- Full uniform velocity stage movements digitized at 1kHz (gray trace)
- Low pass filter cut off set to 25 Hz (black trace)
- Select regions of interest (boxes)
- Subtract average unforced bead position from average displaced bead position yielding displacement in Vx

Viscous Drag Calibration

- Important parameters:
  - k: trap stiffness
  - Δx: displacement
  - γ: drag coefficient
  - V: fluid velocity

- By applying several velocities, the displacement for several forces is measured
- Plot force as a function of displacement
- Slope of fit line is stiffness

Trap Steering

- Stable over (25 μm)^2 range:
multiple traps from time-sharing

Discussion

- Flexible, sensitive instrument

- Acousto-optic trap steering controls trap position easily. A steered trap maintains comparable detection capabilities to a non-steered trap.

- Back focal plane interferometer has two primary advantages over image-based detection techniques:
  - Position can be measured without knowing absolute trap position
  - Measurement is not affected by changes in microscope image (due to stage drift, illumination changes, etc.)

- Agreement between the three methods of force calibration demonstrates a high level of accuracy and precision in the absolute measurement ability of the system
- Sources of error differ for each calibration method
  - Viscous drag and equipartition methods rely on accurate QPD calibration
  - Power spectral analysis relies on calculated drag coefficient, the equipartition method does not

- Integrating the QPD signal with AOD control allows force-clamping or position-clamping of trapped objects

- Power spectral analysis verifies the absence of low-frequency noise in the detector signal. The most significant source of error appears to be drift in the position of the condenser lens.

Conclusion

The present optical tweezers design uses advanced technologies (AODs, QPDs) to create a flexible, sensitive instrument. Further, the design features a novel incorporation of optical tweezers into an existing interferometer system to meet the demands of precise micro biomechanical experiments. Our laboratory will employ the optical tweezers to study the biomechanics of chromosome movements in mitosis.

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Advanced Optical Tweezers for the Study of Cellular and Molecular Biomechanics

Gary J. Brouhard, Henry T. Schek III, Alan J. Hunt

University of Michigan, Department of Biomedical Engineering, Ann Arbor, MI

References


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