Another example demonstrated the ability of dual-beam FCCS to measure the effective charge of DNA molecules interacting with various counterions in continuous flow capillary electrophoresis (Fogarty et al. 2009). The effective charge, Z_{eff}, of a molecule is related to the electrophoretic mobility, μ_e, and diffusion constant, D, according to the equation:

$$Z_{eff} = \frac{\mu_e k_B T}{De} \quad (3)$$

Hence, both the electrophoretic mobility and diffusion constant must be known to accurately measure the effective charge. Dual-beam FCCS was used to accurately measure these parameters simultaneously based on its ability to resolve the flow and diffusion properties of the molecules. Thus, the interactions of DNA with various counterions can be studied.

In summary, dual-beam FCCS serves an important role within the family of FCS-related techniques due to its ability to analyze molecular processes taking place in liquid solutions undergoing unidirectional flow. Such techniques will continue to play a role in characterizing such biophysical processes as DNA, RNA, and protein binding, biomolecule folding, and the electrostatic processes of biomolecules and their associated ions.

Cross-References

- ▶ Fluorescence Correlation Spectroscopy
- ▶ Fluorescence Cross-Correlation Spectroscopy
- ▶ Fluorescence Fluctuation Spectroscopy
- ▶ Microfluidics for Single Molecule Detection
- ▶ Single Fluorophore Blinking
- ▶ Single Fluorophores Photobleaching

References

Brinkmeier M. Cross-correlated flow analysis in microstructures. In: Rigler R, Elson ES, editors. Fluorescence correlation spectroscopy: theory and applications. Berlin: Springer; 2001. p. 379–95.

Fogarty K, Van Orden A. Two-beam fluorescence cross-correlation spectroscopy for simultaneous analysis of positive and negative ions in continuous-flow capillary electrophoresis. Anal Chem. 2003;75:6634–41.

Fogarty K, Van Orden A. Fluorescence correlation spectroscopy for ultra-sensitive DNA analysis in continuous flow capillary electrophoresis. Methods. 2009;47:151–8.

Fogarty K, McPhee JT, Scott E, Van Orden A. Probing the ionic atmosphere of single-stranded DNA using continuous flow capillary electrophoresis and fluorescence correlation spectroscopy. Anal Chem. 2009;81:465–72.

Jung J, Van Orden A. A three-state mechanism for DNA hairpin folding characterized by multiparameter fluorescence fluctuation spectroscopy. J Am Chem Soc. 2006;128:1240–9.

Jung J, Ihly R, Scott E, Yu M, Van Orden A. Probing the complete folding trajectory of a DNA hairpin using dual beam fluorescence fluctuation spectroscopy. J Phys Chem B. 2008;112:127–33.

LeCaptain DJ, Van Orden A. Two-beam fluorescence cross-correlation spectroscopy spectroscopy in an electrophoretic shift assay. Anal Chem. 2002;74:1171–6.

Schiro PG, Kuyper CL, Chiu DT. Continuous-flow single-molecule CE with high efficiency efficiency. Electrophoresis. 2007;28:2430–8.

Van Orden A, Jung J. Fluorescence correlation spectroscopy for probing the kinetics and mechanisms of DNA hairpin formation. Biopolymers. 2008;89:17–6.

Widengren J, Mets U. Conceptual basis of fluorescence correlation spectroscopy and related techniques as tools in bioscience. In: Zander C, Enderlein J, Keller RA, editors. Single molecule detection in solution: methods and applications. Berlin: Wiley-VCH; 2002. p. 69–120.

Dual-Beam Optical Tweezers

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Synonyms

Molecular motors; Optical trap; Optical tweezers; Single-molecule methods

Definition

An optical trap is a device that uses a focused laser beam to create a potential well, in which a small object (100 nm to a few micrometers) whose refractive index is greater than that of the surrounding fluid can be

trapped. The object experiences a restoring force that is directed toward the center of the potential well, which is directly proportional to the distance of the object from the center of the potential well. An optical trap can be used to apply piconewton forces to biological marcomolecules such as proteins and DNA. A dual-beam optical tweezer consists of two focused laser beams that are used to trap two different objects and move them independently in solution. A common biological application of the dual-beam optical tweezer is the measurement of the biophysical properties of molecular motors.

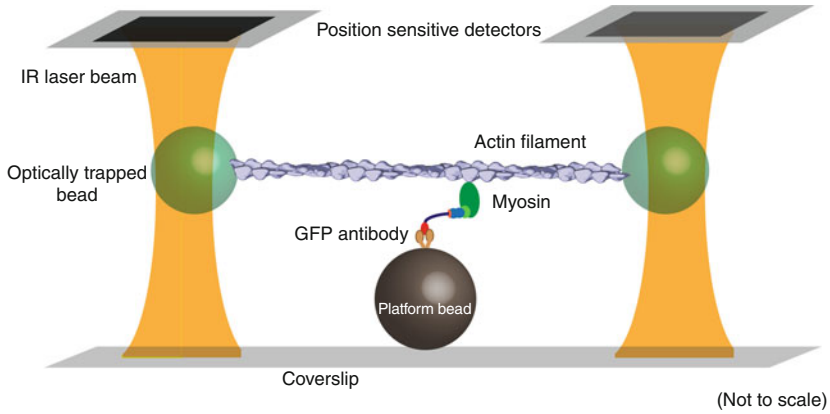
Basic Concept of an Optical Trap

Light has momentum and a force is generated when the momentum is changed. When light passes through an interface between two different media, reflection and refraction occur at the interface. Consider the example of a laser beam that is focused at the center of a spherical object that has a higher refractive index (n) than that of the surrounding aqueous medium. Reflection of light at the surface of the object generates a force (radiation force) on the object, in the direction of light propagation, with a magnitude proportional to the beam power. Refraction of light, on the other hand, produces a force (gradient force) that attracts the object to the focal point of the laser beam, with a magnitude proportional to the gradient of beam power. The object is "trapped" at a point where the vector sum of the gradient and radiation forces is zero (Ashkin 1992). This point is referred to as the center of the trap and is located near the focal point of a high numerical aperture (NA) microscope objective lens (NA $>$ 1.2, and most often 1.4).

For biological applications, where the optical trap is used to apply a force on protein/DNA molecules, the trapped object is usually a polystyrene (n = 1.57) sphere ranging in size from several hundred nanometers to a few micrometers. The force experienced by the trapped object increases linearly with distance from the trap center. The strength of the trap is expressed in terms of the "trap stiffness" and varies for most applications from 0.005 to 1 pN/nm. The force experienced by the trapped object is therefore the product of the trap stiffness and distance from the trap center. A more detailed theoretical description of how the laser traps such particles can be found in Svoboda and Block (Svoboda and Block 1994). The specific layout of the optical trap elements is dependent on the biological question. In this entry, the details of a dual-beam optical trap are discussed, based on the three-bead assay to study myosin molecular motors and originally developed by Finer et al. (1994); for advancements see Sung et al. (2010).

Myosin Function Studied Using a Dual-Beam Optical Trap

The broad goal of this instrument is to investigate the coupling between protein structure and function. In this entry, the setup of a dual-beam optical tweezer as it relates to investigating the mechanical stroke and the chemical ATPase cycle of the myosin motor is discussed. A detailed description of the setup, alignment, and calibration of different optical components can be found in Sung et al. (2010). With minor modifications, the dual-beam optical trap setup can be applied to a variety of biological applications. The dual-beam optical trap has been used to study the workings of myosin at the single molecule level, with high spatial (∼nm) and temporal (∼ms) resolution (Finer et al. 1994). Figure 1 shows the geometric configuration of different elements to investigate the interaction between a single actin filament and a single myosin molecule. A single biotinylated actin filament is attached at each end to a 1 μm polystyrene bead, through a neutravidin-biotin linkage. The two polystyrene beads are held in two independent optical traps. The optical traps are positioned such that the actin filament is stretched taut. Myosin molecules are attached to 1.5-μm platform beads using either an antibody linkage or nonspecific adsorption. The platform bead is moved using the microscope stage such that the actin filament is positioned close to the myosin molecule. Myosin binding to actin results in a stroke of its lever arm. Since the myosin is anchored to the surface, the myosin stroke displaces the actin filament along its length. This in turn causes a displacement of the optically trapped beads at the two ends of the actin filament. Bead displacement is monitored by a quadrant photo diode or a position-sensitive detector and is a measure of the stroke size of the myosin motor. Following each stroke, the myosin exchanges ADP for ATP and detaches from the actin filament. For processive myosins, each of the two heads alternately

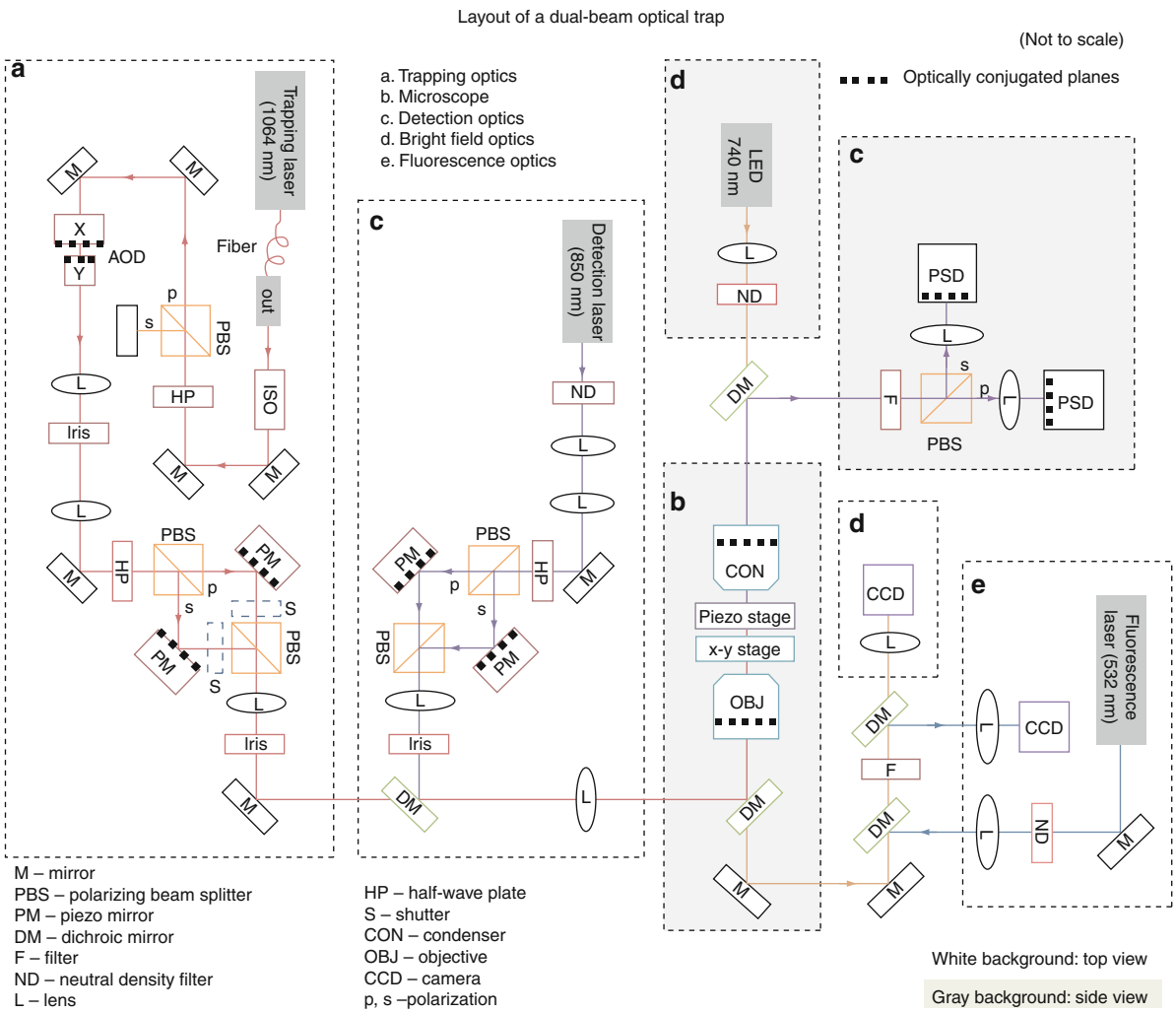


Dual-Beam Optical Tweezers, Fig. 1 Configuration of actomyosin in a dual-beam optical trap. An actin filament is attached at each end to a 1-μm polystyrene bead. The polystyrene beads are held in two separate optical traps. The actin filament is stretched taut and positioned close to a platform bead (1.5 μm). A myosin molecule, attached to the platform bead through an antibody linkage, interacts with the actin filament. A myosin stroke results in the displacement of the actin filament along its length. Position-sensitive detectors are calibrated to read out the displacement of the trapped beads

binds and releases from the actin filament, resulting in a processive movement of the actin filament relative to the myosin, which translates into a stepwise movement of the optically trapped beads.

Layout of a Dual-Beam Optical Trap

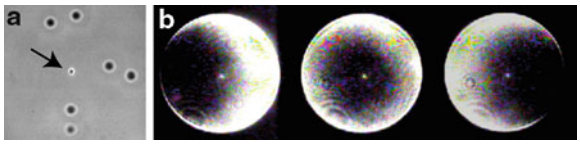
A dual-beam optical trap can be divided into five modules: (a) Trapping laser with beam steering; (b) microscope with objective and condenser; (c) detection laser with bead position detector; (d) bright field illumination and detection; and (e) fluorescence illumination and detection (Fig. 2). A 1,064-nm laser beam with high power (100 mW–5 W) is used for optical trapping. The laser beam is guided by a set of mirrors (M) through an isolator (ISO), followed by telescopic lenses (L) that expand the beam. The position of the optical trap in the sample plane can be varied by a combination of piezo mirrors (PM) and acousto-optic deflectors (AOD). PMs provide a large working distance in the sample plane (>10 μm) with lower response frequency (~100 Hz). AODs provide a smaller working distance (~1 μm) with a higher response frequency (>10 kHz). A polarizing beam splitter (PBS) is used to split the trapping beam into two components ("p" and "s"). The position of the two beams in the sample plane is controlled separately by two different PMs. Another PBS is used to combine the two trapping beams to generate a dual-beam optical trap. An 850-nm laser is used for detection of the optically trapped beads. Similar to the trapping laser, the detection laser is steered using PMs and is split and recombined using PBSs. The trapping and detection lasers are steered into the back focal plane of the objective (OBJ). Each of the two trapping beams is focused by the OBJ to form independent optical traps in the sample plane. An interference pattern is formed in the back focal plane of the condenser (CON) as a result of the interaction of the detection laser with the trapped object. The shape of the interference pattern changes in response to a change in the position of the trapped object relative to the trap center (Fig. 3). A position-sensitive detector (PSD) responds to this change in interference pattern and its output can be calibrated to directly read out the movement of the trapped object relative to the trap center. Brightfield illumination (740-nm LED) and detection (CCD camera) are used to visualize the sample plane while steering either the coverslip or trapping laser to facilitate trapping of objects in solution. For a dual-beam optical trap, a separate object is trapped in each of the two trapping beams. A series of dichroic mirrors (DM) is used to sort the different light paths to their respective detectors. Fluorescent macromolecules including DNA or proteins can be visualized using a separate fluorescence illumination and detection path. Figure 2 shows the layout of a 532-nm laser that is used for epifluorescent illumination of the sample plane. Using appropriate DMs, the corresponding emission is captured by a CCD camera.



Dual-Beam Optical Tweezers, Fig. 2 Layout of components in a dual-beam optical trap. The optical trap can be partitioned into five modules as follows: (**a**) Trapping optics – This includes the trapping laser, isolator (*ISO*) and steering components (piezo-mirrors (*PM*) and acousto-optic deflector (*AOD*)). The two beams in the dual-beam trap are derived from a single laser beam by using polarizing beam splitters (*PBS*). (**b**) Microscope – The microscope objective (*OBJ*) focuses the laser beam to form an optical trap in the sample plane. The condenser (*CON*) is used for detection of the trapped beads and also brightfield illumination of the sample plane. (**c**) Detection optics – This includes the detection laser and piezo mirrors (*PM*) for steering the detection beams. Polarizing beam splitters (*PBS*) are used to derive two separate beams for detecting the two optical traps. (**d**) Brightfield optics – This includes the brightfield illumination through the condenser and the CCD camera to display the sample plane. (**e**) Fluorescence optics – This includes a laser of appropriate wavelength for epifluorescent illumination of the sample plane and a CCD camera to capture the emission of fluorophores in the sample plane

Insights into Macromolecular Function Using a Dual-Beam Optical Trap

The dual-beam optical trap configuration has been used to dissect the workings of the molecular motor myosin. Finer et al. (1994) first developed the dual-beam optical trap and used it to measure the displacement of the actin filament relative to the tail of myosin, also known as the stroke size, during a single ATPase cycle of this molecular motor. For processive myosins, including myosin V and myosin VI, several research groups have measured myosin step sizes using a dual-beam optical trap (Trybus 2008; Spudich and Sivaramakrishnan 2010). The optical trap has been used to apply piconewton forces on myosin heads following a mechanical stroke or a processive step.



Dual-Beam Optical Tweezers, Fig. 3 Images of the sample plane and back focal plane. (**a**) Snapshot of the sample plane captured on a CCD camera. *Arrow* points to a trapped bead. Platform beads appear as unfocused dots with dark centers. (**b**) Snapshot of the interference pattern in the back focal plane of the condenser acquired using a CCD camera. *Center* – bead aligned with the center of the trap, *left* – bead displaced to the left of the trap center, *right* – bead displaced to the right of the trap center

The force can either be in the direction of myosin movement (forward) or opposed to myosin movement (backward). Changing the power of the trapping beam in the sample plane alters the magnitude of the trapping force. The force applied can be increased until the myosin can no longer stroke or step and is a direct measure of the maximum force that can be generated by the myosin (stall force). In addition to the size of the stroke or step, the optical trap also measures the length of time that the myosin remains strongly bound to the actin filament. The mean value of the strongly bound time is a measure of a rate-limiting step in the ATPase cycle of the myosin. The dual-beam optical trap can therefore directly measure the rates of events in the ATPase cycle, including the ADP release rate and the ATP re-binding rate. The dual-beam optical trap is the only known method to measure these rate constants in the presence of either a forward or backward load on the myosin, which in turn provides insight into the physiological function of myosin. For instance, in myosin VI a backward load on the processive motor results in a significant increase in the ADP release rate and transforms the motor from a transporter to an anchor (Chuan et al. 2011). In the case of myosin V, forward and backward loads on a myosin head have different effects on the kinetic cycles and induce asymmetry in a myosin V dimer, which facilitates long-range processive movement (Trybus 2008). Last but not least, the dual-beam optical trap configuration with acto-myosin allows application of piconewton forces on protein elements that are fused to the myosin tail. For example, the mechanical properties of a single, unusual, ER/K α-helix can be directly measured by fusing different lengths of this protein structural element to the tail of myosin VI and subjecting it to varying bending forces in a dual-beam optical trap (Sivaramakrishnan et al. 2009). Beyond acto-myosins, the dual-beam optical tweezer configuration has been used to directly detect base-pair stepping by RNA polymerase (Abbondanzieri et al. 2005) and nonequilibrium thermodynamics of the unfolding of RNA hairpins (Liphardt et al. 2002).

Cross-References

- ATPase: Overview
- Molecular Motors
- Myosin: Fundamental Properties and Structure
- Optical Tweezers

References

Abbondanzieri EA, Greenleaf WJ, et al. Direct observation of base-pair stepping by RNA polymerase. Nature. 2005;438(7067):460–5.

Ashkin A. Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. Biophys J. 1992;61(2):569–82.

Chuan P, Spudich JA, et al. Robust mechanosensing and tension generation by myosin VI. J Mol Biol. 2011;405(1):105–12.

Finer JT, Simmons RM, et al. Single myosin molecule mechanics: piconewton forces and nanometre steps. Nature. 1994;368(6467):113–9.

Liphardt J, Dumont S, et al. Equilibrium information from nonequilibrium measurements in an experimental test of Jarzynski's equality. Science. 2002;296(5574):1832–5.

Sivaramakrishnan S, Sung J, et al. Combining single-molecule optical trapping and small-angle x-ray scattering measurements to compute the persistence length of a protein ER/K alpha-helix. Biophys J. 2009;97(11):2993–9.

Spudich JA, Sivaramakrishnan S. Myosin VI: an innovative motor that challenged the swinging lever arm hypothesis. Nat Rev Mol Cell Biol. 2010;11(2):128–37.

Sung J, Sivaramakrishnan S, et al. Single-molecule dual-beam optical trap analysis of protein structure and function. Methods Enzymol. 2010;475:321–75.

Svoboda K, Block SM. Biological applications of optical forces. Annu Rev Biophys Biomol Struct. 1994;23:247–85.

Trybus KM. Myosin V from head to tail. Cell Mol Life Sci. 2008;65(9):1378–89.

Dual-Color Fluorescence Correlation Spectroscopy

- Fluorescence Cross-Correlation Spectroscopy