Optical Trapping

Introduction

Optical Trapping, also known as Optical Tweezers, is a technique that uses light scattering to hold an object in place. When a laser beam is directed at a particle, cell, or other microscopic object, the target's shape can cause a scattering of the beam. This scattering represents a change in momentum of the light, which in turn exerts a force on the target. This force traps the target in the focal point of the beam, allowing the microscopist to control the x, y and z position of the target with remarkable precision. And, as optical trapping typically uses near-infra-red lasers with wavelengths beyond typical fluorescence wavelengths, this versatile technique can be used alongside a wide range of microscopy techniques, such as epi-fluorescence, confocal imaging, TIRF, FRET, single-molecule and super-resolution techniques.

Uses of Optical Trapping

When targeting objects of well-defined shape and optical properties, the force exerted upon the object can be very precisely calculated, meaning not only can this technique be used to move and trap targets, but also to measure the mechanical properties of surfaces such as cell membranes.

Alternative techniques to measure mechanical properties of membranes, such as Atomic Force Microscopy (AFM), are common. However, AFM relies upon physical contact between a probe on a stiff cantilever and the substance of interest, which can easily damage the soft tissues found in biological samples. Measuring force through a probe in an optical trap, however, can be exceedingly gentle in comparison, with the force adjustable through changing laser power.

Another powerful companion technique for optical trapping is single-molecule fluorescence microscopy, whereby the fluorescent response from single molecules is detected. Fluorescent yields for single molecules are typically very low, so the trapping of cells in a known position without the need to bind the cell to a surface can allow for clearer imaging. Combining the two techniques can provide information about the presence, identity, spatial dynamics, and conformational dynamics of single biomolecules, alongside observation and control with ~nm spatial resolution, ~ms temporal resolution and ~pN-scale applied forces (Hashemi Shabestari *et al.* 2017).

Further, optical trapping can be used to hold biological samples in place for super-resolution imaging techniques such as PALM/STORM, which require long image sequences to build a composite image of sub-diffraction-limited structures. Optical trapping can take the place of biologically invasive adherence of cells to glass, or cell fixing (Diekmann *et al.* 2016).

Mechanism

The trapping laser beam is tightly focused onto a single point in 3-D space, through a microscope objective. The beam typically has a Gaussian profile, and the gradients in intensity are what cause the trapping effect as the particle scatters the beam. Figure 1 illustrates a particle experiencing a force due to the applied beam.

If a particle would move to be offset perpendicularly from the center of the beam, the most intense light would be scattered in the direction of that offset, resulting in a net restoring force. A similar effect provides a restoring force parallel to the beam. However, the momentum of photons that are absorbed or randomly scattered by the particle exert a small force, moving the equilibrium position of the particle slightly downstream of the beam waist.

For a spherical particle of a radius larger than the wavelength of the light, the theoretical model is known as Mie scattering. For small displacements, this force is linear, analogous to a spring. Particles smaller than the wavelength of the light undergo Raleigh scattering, which has a more complex mathematical form but is also well understood. These models allow researchers to calculate the forces experienced by particles very precisely, with the limitation being how closely the particle matches the 'ideal' sphere (Mie) or dielectric particle (Raleigh).

This precision can be exploited by attaching biological molecules or tissues to spherical probes, which are then controlled with the optical trap to measure forces or pin objects in place. Many factors affect the applied force such as the power, spread, and polarisation of the beam, and the size, structure and refractive index of the target.

When trapping biological objects such as cells directly, particles are seldom spherical and contain variations in refractive index that remove the possibility of exact analytical models of the response of the particle to the beam. However, such particles can still be trapped and approximate models can be formulated. (Favre-Bulle *et al.* 2017)
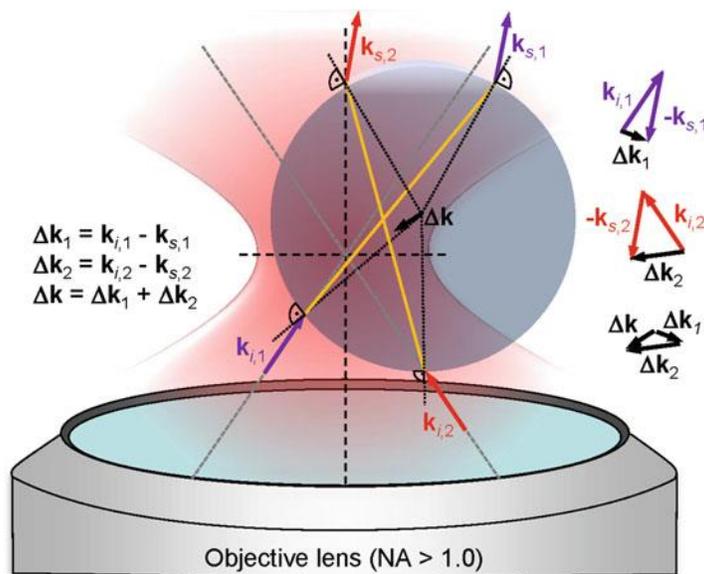

Figure 1: Force vectors for example lightpaths of a particle in an optical trap. The particle is sufficiently large compared to the wavelength of light that this 'Ray Tracing' approach is valid. From (Hendricks & Goldman, 2017)

Systems

An optical trapping system is typically built on top of a commercial brightfield or fluorescence microscope. A near-infrared (NIR) laser of typically 1068 nm wavelength is most commonly used (Hendricks & Goldman 2017, p78), to avoid absorption of light by water, and damage to biological tissues.
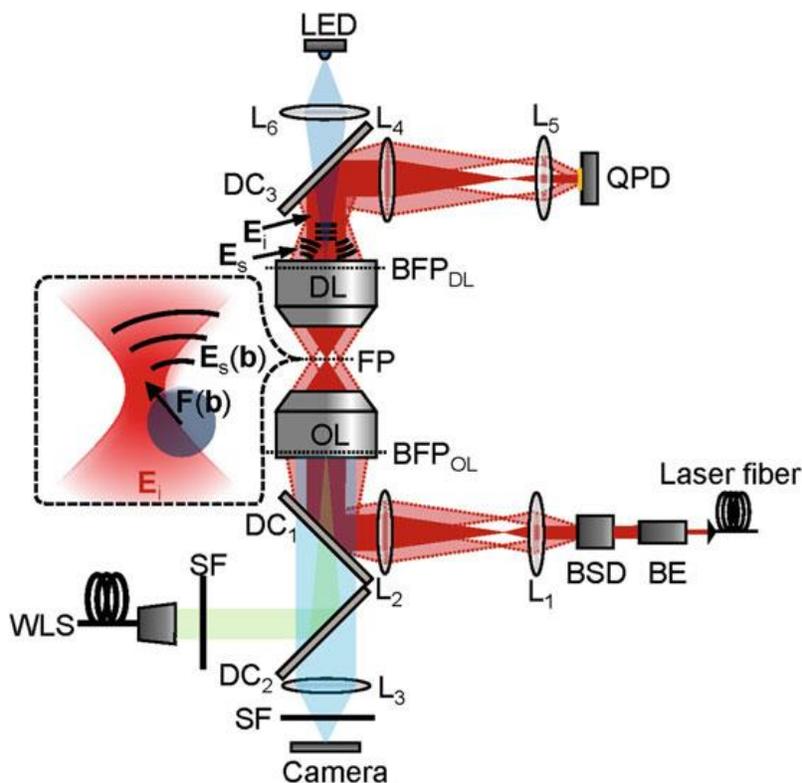
Figure 2: Schematic of an Optical Trap system, including a brightfield illumination and observation lightpath, complete with microscope camera. The trapping laser, shown in red, exerts a force on the particle in the centre. The scattered light from the particle is detected by the Quadrant Photodetector (QPD), for providing feedback to the trapping laser. From (Hendricks & Goldman 2017)

Multiple trap systems can be produced from a single beam using beam splitters, via spatial light modulators (SLM), acousto- and electro-optic deflectors, scan mirrors or combinations of these devices, allowing simultaneous control of multiple trapped objects. SLMs even allow the transference of angular momentum to trapped objects, applying torque.

The light from the trapping laser is detected by either a quadrant photo-detector (QPD) or specialist NIR camera, for alignment and analysis.

Applications

The technique was invented by Arthur Ashkin and colleagues in Bell Labs in 1986, and its potential to be used with biological specimens was immediately seen (Ashkin *et al*. 1986). The ability to hold single cells in a fixed position and/or apply a well-defined force opened new doors for biological microscopy. It was first utilized to study *E. coli* flagella to measure the torque they produced (Block, Blair, and Berg 1989), and single kinesin motors, measuring the speed and binding forces of the kinesin attached to microtubules.

For modern in-vivo studies of single cells, the ability to trap the cell in a known position without adherence to a surface is hugely beneficial for studying the natural behaviors and structures of the cell, and for allowing continuous observation over long timescales.

In recent years, optical traps are used increasingly in vivo, inside living organisms, to move biological objects up to a few microns in size, such as red blood cells (Zhong *et al.* 2013), erythrocytes, macrophages (Johansen *et al.* 2016) and injected nanoparticles.

A recent study also demonstrated the ability of optical trapping to move large biological objects in vivo. Favre-Bulle *et al.*, in their publication in Nature Communications 2017 (Favre-Bulle *et al.* 2017), were seeking to characterize neural circuits related to the zebrafish vestibular system (for detection of movement). In larval zebrafish, the developing vestibular system consists solely of the force-sensing otolith or 'ear stone', the movement of which indicates to the zebrafish that it is experiencing a force. Conventionally, to stimulate the vestibular system, an organism would have to be physically moved. As most imaging methods rely on immobile specimens, this would rule out simultaneous stimulation and imaging.

Using an optical trap, the researchers were able to move the 55 µm-diameter, roughly spherical otolith non-invasively and with precision, through the body of the zebrafish. The researchers were then able to simultaneously image the zebrafish brain and behavior.

Super-Resolution microscopy

Optical trapping can also be used to directly control cells for use with advanced imaging techniques, including super-resolution techniques such as dSTORM, in which a super-resolution image is composited from a large number of individual frames. Such techniques require long acquisition timescales for capturing multiple frames, and so typically require invasive fixing of cells or adherence to glass. In 2016, Diekmann *et al.* demonstrated that holographic optical trapping can be used to immobilize rod-shaped bacterial cells, for the first time allowing dSTORM imaging of a bacterial cell free in space (Diekmann *et al.* 2016). Further, the position and orientation of individual cells can be directly controlled, opening the door for deeper insights into the nanoscale structures of biological samples.

Camera Requirements

The use of optical trapping does not itself influence the camera requirements of a microscopy technique, as trapping systems can be used alongside a huge variety of microscopy techniques, including brightfield, epi-fluorescence, spinning disk and laser scanning confocal, STED, single-molecule fluorescence, and PALM/STORM super-resolution techniques.

For brightfield applications, a camera with a large field of view and smaller pixels to improve resolution may give the best results. For more demanding applications such as super-resolution imaging (PALM/STORM and companion techniques), a more sensitive camera with high QE and low noise, capable of high speeds, would be recommended.

References

Ashkin, A., J. M. Dziedzic, J. E. Bjorkholm & Steven Chu. (1986) **Observation of a Single-Beam Gradient Force Optical Trap for Dielectric Particles**. *Optics Letters* 11(5): 288. https://www.osapublishing.org/abstract.cfm?URI=ol-11-5-288.

Block, Steven M, David F Blair & Howard C Berg. (1989) **Compliance of Bacterial Flagella Measured with Optical Tweezers.** *Nature* 338(6215): 514–18.

Diekmann, Robin *et al.* (2016) **Nanoscopy of Bacterial Cells Immobilized by Holographic Optical Tweezers.** *Nature Communications* 7.

Favre-Bulle, Itia A., Alexander B. Stilgoe, Halina Rubinsztein-Dunlop & Ethan K. Scott. (2017) **Optical Trapping of Otoliths Drives Vestibular Behaviours in Larval Zebrafish.** *Nature Communications* 8(1). http://dx.doi.org/10.1038/s41467-017-00713-2.

Hashemi Shabestari, M. *et al.* (2017) 582 Methods in Enzymology *Recent Advances in Biological Single-Molecule Applications of Optical Tweezers and Fluorescence Microscopy*. 1st ed. Elsevier Inc. http://dx.doi.org/10.1016/bs.mie.2016.09.047.

Hendricks, Adam G. & Yale E. Goldman. (2017) 1486 Methods in Molecular Biology *Optical Tweezers, Methods and Protocols*.

Johansen, Patrick Lie *et al.* (2016) **Optical Micromanipulation of Nanoparticles and Cells inside Living Zebrafish**. *Nature communications* 7: 10974.

Zhong, Min-Cheng *et al.* (2013) **Trapping Red Blood Cells in Living Animals Using Optical Tweezers**. *Nature communications* 4: 1768.