Dual-beam Laser Traps in Biology and Medicine: When One Beam is Not Enough

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ABSTRACT

Optical traps are nowadays quite ubiquitous in biophysical and biological studies. The term is often used synonymously with optical tweezers, one particular incarnation of optical traps. However, there is another kind of optical trap consisting of two non-focused, counter-propagating laser beams. This dual-beam trap predates optical tweezers by almost two decades and currently experiences a renaissance. The advantages of dual-beam traps include lower intensities on the trapped object, decoupling from imaging optics, and the possibility to trap cells and cell clusters up to 100 microns in diameter. When used for deforming cells this trap is referred to as an optical stretcher. I will review several applications of such traps in biology and medicine for the detection of cancer cells, sorting stem cells, testing light guiding properties of retinal cells and the controlled rotation of cells for single cell tomography.

Keywords: optical trap, optical tweezers, optical stretcher, optical cell rotator, cell mechanics, retina optics, tomography

1. INTRODUCTION

1.1 History

Van de Hulst wrote in 1957: "Radiation pressure will never be relevant in the lab (only extraterrestrial affairs) because there is no light source available that could create the light intensities required". But the invention of laser by Maiman exactly 50 years ago changed everything and opened the door to contactless, optical manipulation. The early pioneer of optical manipulation was Arthur Ashkin at Bell Labs in the early 70s. He was the first to demonstrate that microscopic objects can be manipulated with light (and not heating). He went on to show first levitation of objects with one vertical laser beam and to create a stable trap using two-counterpropagating laser beams – the first dual beam laser trap (DBLT)[1].

Further developments came in 1986 in the form of optical tweezers[2] (an optical trap created from a single, tightly focused laser beam), 16 years after dual-beam trap also by Ashkin.

Building on Ashkin's dual-beam work, Mara Prentiss created the first dual-beam fiber trap in 1993[3], using optical fibers to deliver the two beams, simplifying the experimental setup significantly.

1.2 Optical Forces

Ashkin discovered that dielectric particles in proximity of a laser beam featuring a Gaussian intensity profile are pushed away from a divergent laser source and pulled towards the beam center[4]. The forces responsible for this behaviour are often divided into two. The force that pushes the particles away is called *scattering force* because it can be explained by the scattering of the incident light. The force that pulls the particle towards the beam center arises due to the Gaussian intensity profile of the laser beam: The particle is pulled along the gradient into the region of the highest field intensity. This force is therefore called *gradient force*. These forces arise from interactions at the surface of the object as the light moves from one refractive index to another. By considering the change in momentum of the light as it passes through an object, a map of the forces exerted on the object can be created (Figure 1). It can be seen that surface stresses are created at the front and back surfaces and if the object is compliant, it will be deformed. This effect is the source of the optical stretcher[5-7], a fibre based dual beam optical trap which is capable of deforming compliant objects and cells.

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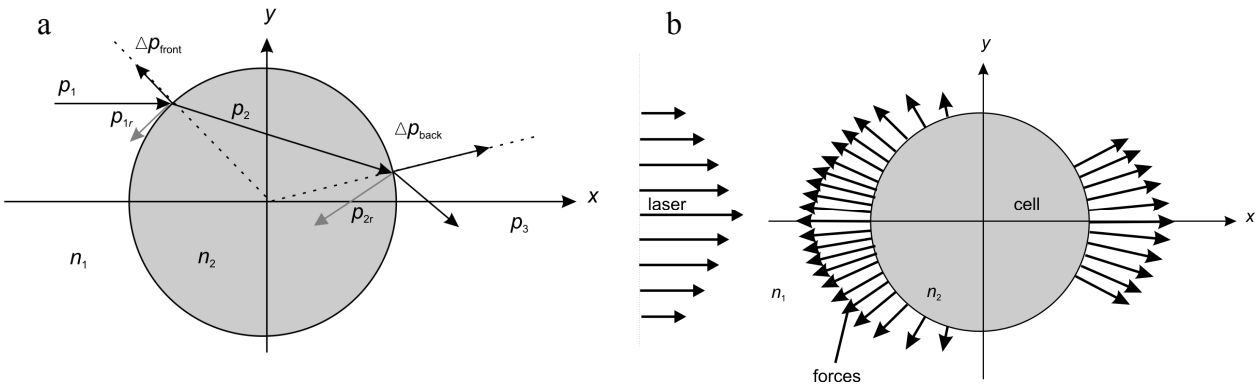

Figure 1 Schematic of the change in the momentum of light as it passes through a spherical object with a higher refractive index (n2>n1). The conservation of momentum means that as the light's net momentum changes at each surface, momentum is transferred to the object Δp. b) net forces on a spherical object due to a laser beam with a Gaussian intensity profile. Adapted from Ref 7.

In order to understand the forces exerted on an object, several models have been proposed[7-10]. With objects much larger than the wavelength of light, a geometrical or ray optical (RO) technique can be used as a first approximation. Mie theory can be used to further refine the forces (and stresses) and for spheroids, an analytical solution can be obtained[10].

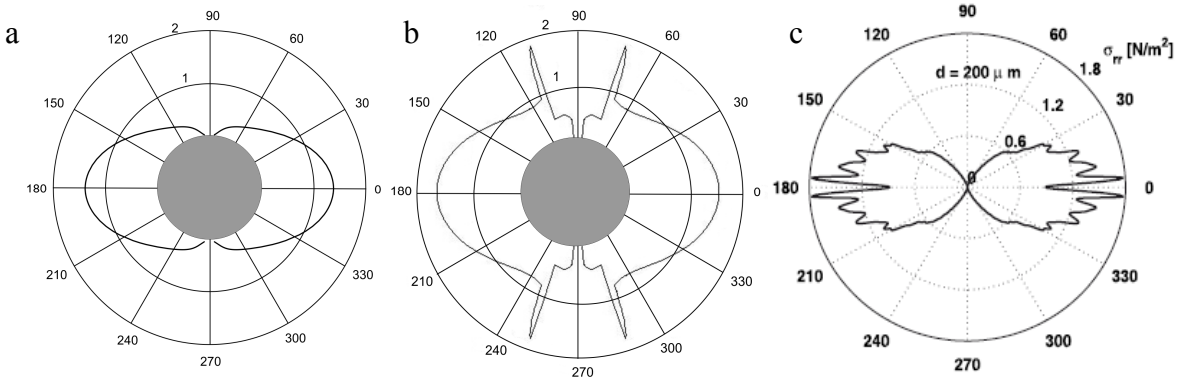

Figure 2 Representations of the resultant forces upon spherical objects in a dual beam trap. a) Ray optics technique with parallel rays b) Ray optic model with divergent beams c) Mie optics model. (a) and (b) adapted from Ref. 8, c) adapted from Ref 9

1.3 Optical Traps

In optical tweezers, the tightly focused laser beam generates a gradient force high enough to stably trap an object in 3D against gravity and Brownian motion with only a single beam[2]. However the high gradient also results in a region of very high power density near the trap which both limits the size of object which can be stably trapped and can induce photo-damage to sensitive samples. In contrast, dual-beam traps use a second beam to balance the scattering forces and create a stable trapping region. By using the balance of the two beams to create a trap along the optical axis, the requirement for tightly focused light is removed and traps can be formed using divergent light. One method often used is to trap an object between the end-faces of two optical fibres carrying counter-propagating beams[3]. Such systems do not require any further optics, decoupling the optical trap from the imaging microscope and allowing integration of the trap with other optical manipulation and measurement techniques.

Dual beam traps, with divergent beams, have much lower intensities (at the same trapping power) as optical tweezers, reducing the risk of damaging the sample as well as increasing the size of the trapping region, allowing for much larger objects (up to 100 microns) to be trapped. These advantages make dual beam traps very suited to manipulating sensitive biological samples.

In the remainder of this paper we will present and discuss several applications of this DBLT in biology and medicine.

2. DUAL-BEAM TRAPS IN BIOLOGY

2.1 High-content analysis

Recently there has been much emphasis on extracting as much information from each sample under study as possible and this is even more important with biological samples, which often have a high variability from sample to sample. Most cells grow and have been characterized while attached to a suitable surface and it is possible to probe many aspects while the cells are attached. However it becomes difficult to test a high number of cells. But even when many are analyzed then it is not possible to sort them to be able to remove unwanted cells or to keep and study particular cells. In contrast to this is flow cytometry, such fluorescence-activated cell sorting (FACS) where cells are analyzed and sorted based on fluorescence signal as the cells flow through the system. As this is a fly-through technique the throughput is very high, but the number of different tests performed simultaneously is rather limited. DBLTs especially in combination with microfluidics[6,11,12] offer the possibility of trapping many individual cells successively in suspension, holding each cell until the analysis is sufficiently unambiguous and the subsequently sorting the cells. The analysis can be also fluorescence (essentially everything that can be done with FACS) but in addition other spectroscopic techniques or even cell mechanical analysis can be used.

2.2 Living cells as optical fibers

A special type of the cell analysis mentioned above was used to help answer a biological question related to vision that has been puzzling researchers for a long time: inverted retina. Some evidence that light gets through the retina only along certain type of cells – Müller glial cells[13,14]. Refractive index and scattering measurements (Figure 3a,b) suggested the potential that these cells act as optical fibers. Other techniques to probe these cells have suffered problems being able to hold and align the fragile cells while testing their light guiding ability. A DBLT however is well suited to trap, align and stretch out cells with two counterpropagating NIR laser beams[15]. By including an additional VIS laser beam and detector, the light guiding ability of the cell can be studied while the cell is held in the trap (Figure 3 c,d)

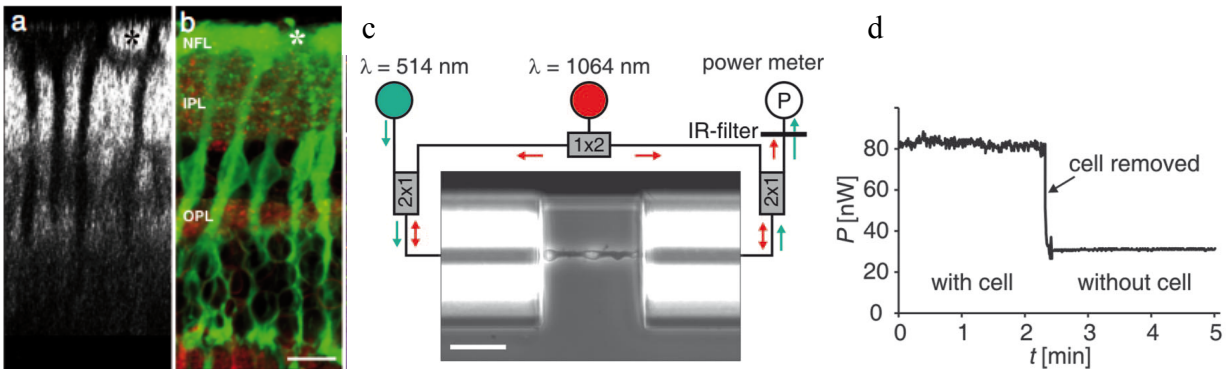

Figure 3. Reflection (a) and fluorescence (b) image of the retina showing the large distance and complex path light (from above) has to follow to reach the photoreceptors (bottom of image) through the nerve fiber layer (NFL) and inner and outer plexiform layers (IPL and OPL respectively). The low reflecting structures spanning the retina are Müller cells. (c) Representation of the dual-beam trap used to probe the light guiding capabilities of Müller cells using a 1064nm NIR laser to trap and a 514nm laser and detector to probe the transmission. (d) Measured transmission as a Müller cell is removed from the trap, showing the light guiding effect. [scale bars *a-b*: 10 µm, *c*: 50 µm] Adapted from Ref 15.

2.3 Single-cell tomography

Another recent development in DBLT, is of the optical cell rotator[16]. This uses a modified BDLT which has one non-rotationally symmetric beam to trap a cell a specific orientation. By rotation of one of the optical fibers, the trapping orientation can be controllably changed and the cell is rotated around the optical axis which is perpendicular to the imaging axis, allowing for contactless, single-cell tomography.

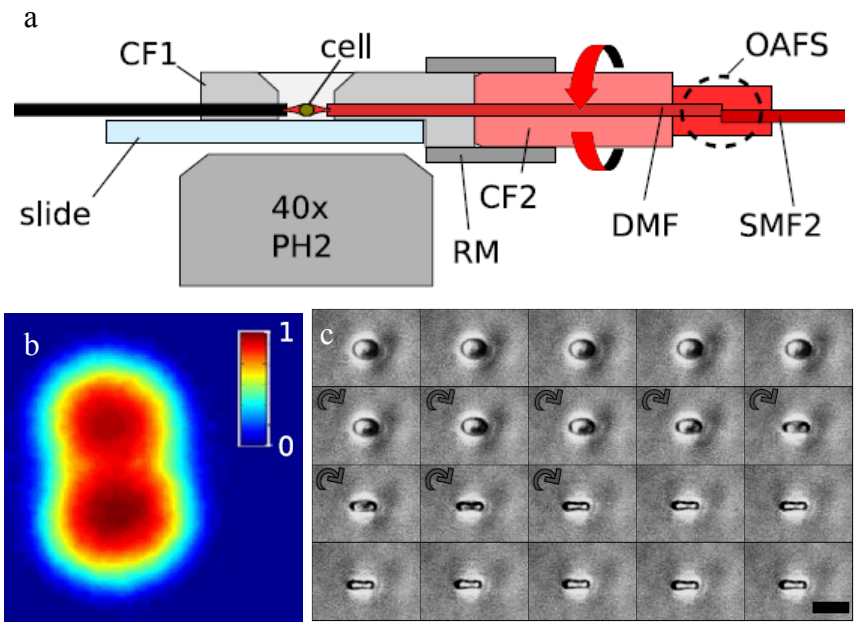

Figure 4 The optical cell rotator. (a) Schematic of the optical cell rotator. A cell is held in a dual beam trap in which one beam has a non rotationally symmetric profile (b) created by an offset arc fusion splice (OAFS) into a dual mode optical fiber (DMF). The trapped object can be rotated by rotating the ceramic ferrule (CF) which holds the dual mode fiber with a rotation mount (RM). (c) Phase contrast images of a red blood cell as the dual-mode fiber is rotated by 90 degrees. Scale bar: 10 μm. Adapted from Ref. 16.

2.4 Cell mechanical measurements with dual-beam laser traps

Because DBLTs use lower intensities at the same power, compared to optical tweezers, (1-2 orders of magnitude) correspondingly higher powers can be used in these traps. At this power, the forces applied to the cell surface are sufficient to cause an appreciable deformation of the entire cell without endangering cell integrity and viability due to high photon fluxes. If used for the purpose of this controlled deformation of cells dual-beam laser traps are also referred to as optical stretcher[5-8].

Cell function is intricately linked to cytoskeleton, which in turn determines the mechanical properties of cells. Thus, when cells change their function this is reflected in an altered mechanical phenotype, which can be measured with relatively high-throughput using an optical stretcher.

One example of such measurements have shown that differentiation of acute promyelocytic leukemia cells following the neutrophil lineage is associated with an increase of cell deformability, which appears to be regulated by the actin cytoskeleton[17]. This softening of cells facilitates cell mobility, which is crucial for physiological neutrophil function.

While there are potentially many applications of DBLTs in medicine (high-content analysis), the one that is furthest advanced is the use of cell mechanical properties measured with an optical stretcher as an inherent cell marker. As discussed in 2.4, cell mechanics and function are intricately linked so that functional changes can be monitored by mechanical measurements. This does not only hold true for physiological changes, such as migration, division, or differentiation, but also for pathological changes.

2.5 Cancer diagnosis

Changes in the cytoskeleton are often linked to disease states with cancer being one of the most striking (changes in the morphology and migration being diagnostic of cancer). It is possible that cytoskeletal changes could result in the deformability of cells changing which could be measured using the optical stretcher. In fact, cancer cells are more compliant than healthy cells and as they become more aggressive they become softer[18]. This is likely due to their increased ability to migrate as the cancer develops. This label-free inherent measure has been seen with leukemia cells, breast epithelial cancer cells and oral cancer cells.

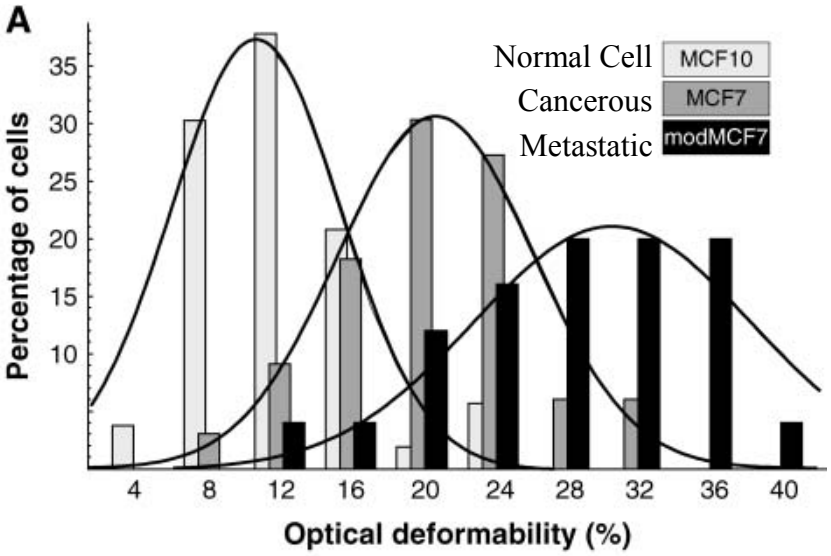

Figure 5 Optical deformability measured with the optical stretcher for different stages of breast cancer showing the cancerous MCF7 cells are more deformable than normal, healthy cells and the metastatic modMCF7 cells are more deformable still. The three populations can be distinguished with 99.9% confidence (Student's t-test) and require very few cells (~30 cells per population). Adapted from Ref 18.

2.6 Therapy and drug screening

If cells need to be compliant to be able to migrate then consequently it should be possible to prevent them from migrating, or at least to slow them down, by making them stiffer. This was demonstrated in the context of retinoic acid syndrome (RAS). Recently ATRA (all-trans retinoic acid) differentiated acute promyeocytic leukemia (APL) cells can infiltrate tissue and cause problems including death.

By changing the relaxation ability of these cells with the addition of taxol, drug to stabilize microtubules, the cells also slowed down[17,19]. Pore entry (mimicking trans-endothelial migration) is the rate limiting step. Since these cells are neutrophil-like and only have up to 8 hours before they get cleared from the blood, any delay in their entry into tissue will help to reduce the severity of RAS. If this can be confirmed in clinical trials this would be an important proof of concept that could then be applied to other infiltrative disorders, including cancer metastasis. DBLTs in the form of optical stretchers could be used in this context to identify drugs that alter the rheological properties of cells and, thus, could be investigated further. The optical stretcher could also be employed to monitor the effect of such drugs on individual patients to adjust timing and dosage of such drugs for optimized efficacy.

3. CONCLUSION

Since the first demonstrations of optical traps in 1970, the ability to trap and manipulate objects without contact has held many great potentials and the two methods developed by Ashkin have diverged into fields in their own right which complement each other well. Dual beam laser traps with their lower intensities, larger trapping volume, ease of

implementation and integration, offer a unique platform for biological and biomedical research across a wealth of areas from measuring inherent mechanical and optical properties of cells through high resolution, 3D tomographic imaging to disease diagnostics and drug development.

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