



Optical Tweezers and their Application to Biological Systems

Nobel Lecture, December 8, 2018 by
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IT IS A PLEASURE to present this summary of my 2018 Physics Nobel Lecture [1] that was delivered in Stockholm, Sweden, on December 8, 2018 by my fellow Bell Labs scientist and friend René-Jean Essiambre. This summary is presented chronologically as in the Lecture. It starts with my fascination with light as a youngster and goes on to the invention of the optical tweezer and its applications to biological systems, the work that was recognized with a Nobel Prize. Along the way, I provide simple and intuitive explanations of how the optical tweezer can be understood to work. This document is a personal recollection of events that led me to invent the optical tweezer. It should not be interpreted as being complete in the historical attribution of the scientific discoveries discussed here.

CROOKES RADIOMETER: THERMAL EFFECTS

I have always been fascinated by the forces that light can exert on objects. I started to play with a Crookes radiometer [2] in my early teenage years and tried all kinds of experiments with it. I learned that thermal effects can explain the motion of the Crookes radiometer.

* Arthur Ashkin's Nobel Lecture was delivered by René-Jean Essiambre.

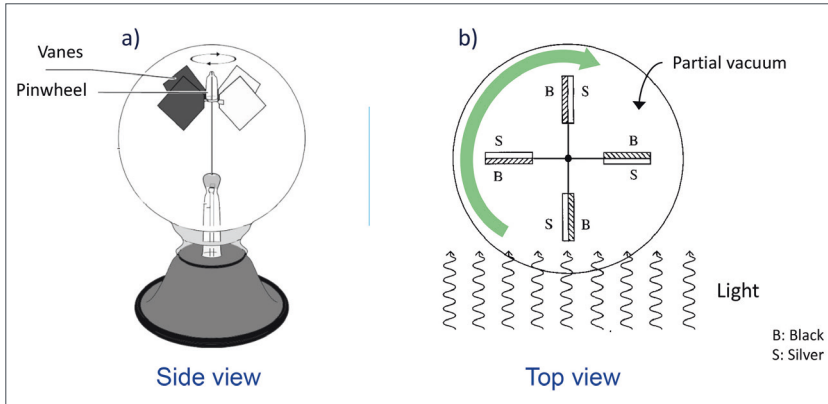


Figure 1. Crookes radiometer: a) side view b) top view showing the direction of rotation. Thermal effects are responsible for the rotation of the vanes.

Black surfaces absorb light which heats up the surface. In contrast, metallic surfaces reflect light with negligible heat generation. A Crookes radiometer, represented schematically in Fig. 1a, is a set of four vanes on a spindle with each vane having a black and a metallic side, everything being placed in a low-pressure glass bulb. They are disposed at 90-degree angles so as to always show the black side on the left and the metallic side on the right when looking at the radiometer from any side. Upon shining light on the radiometer, with light from the sun for instance, the vanes will turn in the direction where the black side moves away from the light. This direction of rotation originates from the heating of the black surface (thermal effect) by the absorption of the photons that in turn heat up the air around the black side of the vanes. The heated air moves around in the bulb resulting in a net force on the black surface that makes it move away from the light as represented in Fig. 1b.

LIGHT PRESSURE

The motion of the Crookes radiometer can be explained by thermal effects alone. During my teenage years I was acquainted with the experiment of Nichols and Hull [3], which demonstrated the effect of light pressure. This was accomplished with the development of high vacuum pumps that achieved lower pressure, therefore reducing the thermal effects until radiation pressure became the dominant effect. With a device like the Crookes radiometer, better vacuum results in the vanes rotating in the opposite direction as the one indicated on Fig. 1b.

HIGH-POWER MAGNETRONS

Shortly after the entry of the United States in World War II, I was drafted and asked to help with building a high-power magnetron in the Columbia Radiation Laboratory for radar application. I learned to make and solder high-power magnetrons for use with radar. I built a magnetron named the “rising-sun” magnetron because its geometry resembled the rays of the sun at sunrise. It operated at about 10 GHz, a frequency about 50,000 times smaller than visible light. The magnetron emitted high-intensity pulses at a frequency of 1000 cycles per seconds. If one shined it on a metallic vane of a phone earpiece and detected a thousand cycle frequency on an oscilloscope matching this frequency. I interpreted this as possibly the effect of light pressure on the receiver plate.

ARRIVING AT BELL LABS

I joined Bell Labs in New Jersey, in 1952, tasked to work on vacuum tube amplifiers. It was both interesting and time-consuming work. I joined Calvin Quate’s sub-department, as it was called in those days, and was assigned to do an experiment on cancelling noise. This was a flawed idea, but it was not known at the time I started. Of course, it was doomed to failure, and I ended up being blamed for lack of positive results. After one year working fruitlessly on this topic, I was almost fired. But, fortunately, Quate saved me. This endeavor came to an end a few months later, with the arrival of Neville Robinson, a student of Rudolf Kompfner, who showed that the cancellation of noise was impossible. He showed that rather than being cancelled, the noise was transferred to other frequencies. I was barely saved, and thereafter started work of my own.

THE ADVENT OF THE LASER

Shortly after the first laser was demonstrated in 1960, I began to work with light pressure again, now with much more powerful and well-collimated light sources than previously available. The experiments performed in the early to mid-1960s were primarily exploring various linear and nonlinear effects of laser light on different materials and waveguides. This new tool that was the laser enabled a fast pace of discovery.

THE FIRST LIGHT PRESSURE EXPERIMENTS WITH A LASER

In 1966, I attended the International Quantum Electronics Conference (IQEC) in Phoenix, AZ. At the conference, Eric Rawson and his mentor Professor A.D. May from the University of Toronto presented a video showing particles behaving like “runners and bouncers” in the internal

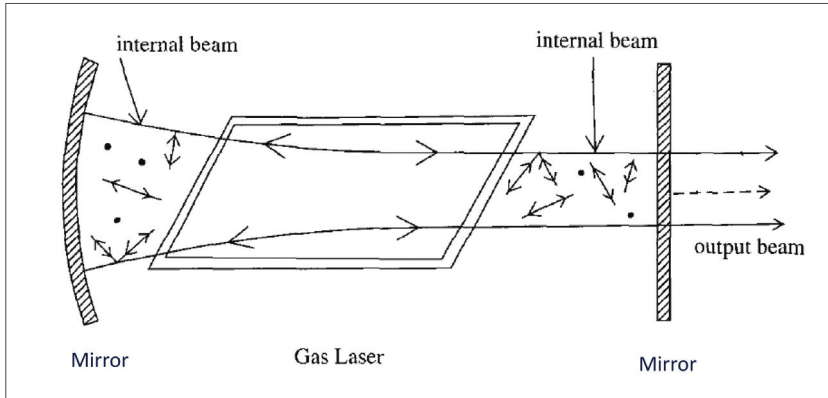


Figure 2. "Runners and Bouncers" in the internal beam of a laser cavity as observed by Rawson and May.

beam of a laser cavity [4], as depicted in Fig. 2. This curious behavior piqued my curiosity. One among various other possible explanations given for this phenomenon was light pressure. I figured out from a back-of-the-envelope calculation that light pressure could not be responsible for this behavior. Not long after the talk, all persons involved with the question agreed that the heating of the particles through absorption of light was responsible for the observed motion. The main impact of this experiment on me, however, was to reignite my desire to explore how light pressure from lasers could be observed and used to move small particles.

LIGHT PRESSURE ON A MIRROR AND ON A TRANSPARENT SPHERE

Let's consider the effect of light pressure on two different types of objects that do not absorb light: a highly reflective mirror and a highly transparent sphere. The mirror and the transparent sphere are assumed to be tiny (micrometer sized) and the effects of any other forces other than light pressure are considered negligible. First, what happens when a particle of light, a photon, hits a perfectly reflecting mirror at normal incidence as represented schematically in Fig. 3a? Of course, the photon is reflected, and its momentum is reversed. The mirror experiences a slight recoil, opposite to the change of momentum of the photon, so that the overall momentum is conserved. Now, let's consider a transparent object such as a small sphere made of glass or polystyrene as depicted in Fig. 3b. What follows is the core principle on which the operation of optical traps and optical tweezers is based. When a photon is incident on the sphere on its outer side, it generally goes through the sphere and is deflected towards the center of the sphere. This change of direction of the photon corre-

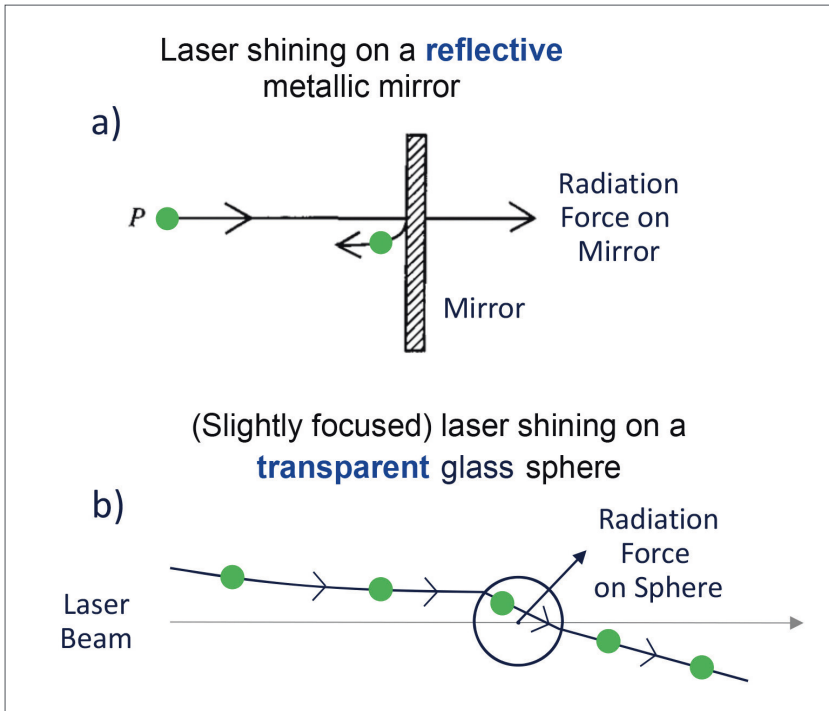


Figure 3. Two scenarios that show the impact of light pressure: a) a photon incident on an ideal reflective mirror; b) photons incident on a transparent sphere. When the mirror or the sphere are small (micrometers in size) and many photons are incident, the radiation pressure forces exerted on these objects can exceed gravity by several orders of magnitude.

sponds to a change of momentum. From conservation of momentum, the transparent sphere reacts and acquires a motion. Thus, a photon hitting the upper part of a sphere exits the sphere in the downward direction as shown in Fig. 3b. This change of direction of the photon induces a change of momentum that results in a force that makes the sphere move both forward and upward. On the other hand, when a photon hits the lower part of the sphere, the photon is deflected in the upward direction. The sphere then experiences a force that has a forward and a downward component. When two photons symmetrically located on each side of the sphere hit it simultaneously, the sphere moves only in the forward direction as the downward and upward components cancel. I gave the label “scattering force” to the component of the light pressure force on a transparent sphere as it arises from the scattering of light. As a consequence, the small sphere moves in the direction of the beam. An estimate of the scattering force exerted by a one-watt laser on a glass sphere of one micrometer in diameter gives a force that is six orders of magnitude larger than gravity. This is a tremendous force.

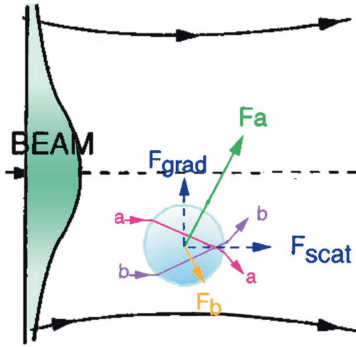


Figure 4. Forces exerted on a transparent sphere located off-axis in a laser beam. The force F_a resulting from photons hitting the upper part of the sphere is larger than the force F_b coming from the photons arriving on the lower part of the sphere due to a larger number of photons incident on the upper part of the sphere (higher laser intensity).

MOTION OF A SPHERE WITHIN A LASER BEAM

A laser beam is not uniform in the transverse direction as it follows the distribution of the spatial modes of the cavity. Let's consider what the net effect of light pressure is on a transparent sphere located off-center within a laser beam as depicted in Fig. 4. A typical laser beam has its highest intensity near its center, with the intensity decreasing gradually with the distance from the center. We refer to this difference in light intensity as light gradient, with a positive gradient being defined as the direction from low to high light intensity. In the case of the transparent sphere located off-axis as in Fig. 4, there is more light intensity hitting the upper part of the sphere (path a) closer to the beam center than the lower part (path b) further away from the beam center because it is closest to the region of the beam that has the highest intensity. Because there are more photons near the center of the beam, the net effect of the gradient radiation pressure is to push the sphere towards the center of the beam. I refer to this light pressure force as "gradient force" as the sphere is pulled towards the region having the highest gradient of intensity. As a result of light pressure, the sphere in Fig. 4 will move forward due to the scattering force and, at the same time, will move upward toward the center of the beam due to the gradient force. It will then stop when it hits the vertical microscope slide in Fig. 4 and be trapped at the center of the beam. It is trapped from the left by the laser beam and from the right by the microscope slide and can be referred to as an opto-mechanical trap.

TWO-BEAM OPTICAL TRAP IN LABORATORY NOTEBOOK

In 1969, I thought that placing a particle between two laser beams would create the first all-optical trap due to light pressure. Figure 5 shows two parts of an entry to my notebook. Figure 5a shows the schematic of the two-beam all-optical trap. In this figure, it uses a donut-shaped mode to trap a metallized sphere. Transparent spheres are discussed later in this

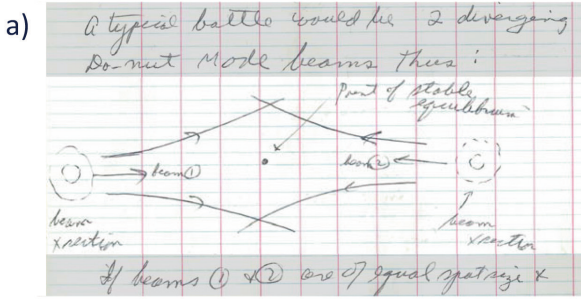
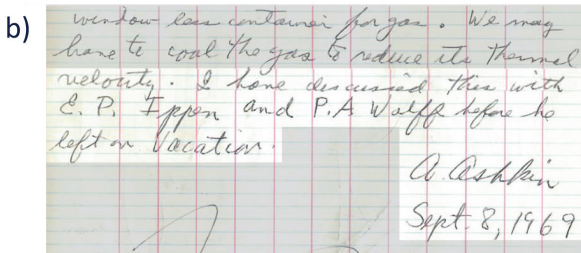


Figure 5. Two excerpts of an entry in my laboratory notebook at Bell Labs on September 8, 1969. It presents the idea of a two-beam all-optical trap. From Bell Labs archives.



entry. Figure 5b also shows the two witnesses of this entry, Erich P. Ippen, who became Professor at MIT and Peter A. Wolfe, who was my boss at the time. This led to the first paper on all-optical trapping in 1970 [5].

OPTICAL LEVITATION

In the two-beam trapping experiment, each laser provides the opposing force to trap the sphere. By using a single laser oriented upwards, one can compensate for gravity. As a result, the sphere is levitated in the air. Because light pressure can produce forces much larger than gravity, only low powers are necessary to achieve optical levitation. Figure 6 shows pictorially how the scattering force of a slightly diverging laser compensates for the gravitational force on the sphere. By changing the power of the laser one can change the height of the sphere.

The light pressure from the laser beam oriented upward can be used to levitate particles. Consider a glass ball resting on a microscope slide. If a low power laser beam goes through the ball from below, the ball experiences an upward

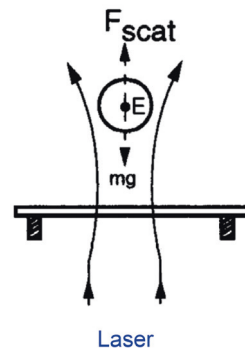


Figure 6. Transparent sphere levitating above a microscope slide as a result of light pressure from a laser pointing upwards.

force but does not move. As the power is increased above a certain threshold the scattering force exceeds gravity (neglecting van der Waals forces), and the ball will start to levitate. The final height reached depends on the laser power and its divergence.

OPTICAL TWEEZERS

Finally, in 1983, I discovered that a transparent sphere could be trapped by a single highly focused laser beam. I named this single-beam trap “optical tweezers”. It is generated by using a large numerical aperture microscope objective. Such an objective produces a strong gradient of intensity along the direction of the laser beam near the laser focus as depicted in Fig. 7. This axial gradient generates a light pressure force in the backward direction relative to the beam and of a magnitude that can exceed the forward scattering force. At a certain distance downstream but close to the beam focus center, both the gradient and scattering light pressure forces are equal. This is the equilibrium point where a sphere is trapped. This invention of the optical tweezers is cited in the 2018 Nobel Prize for Physics.

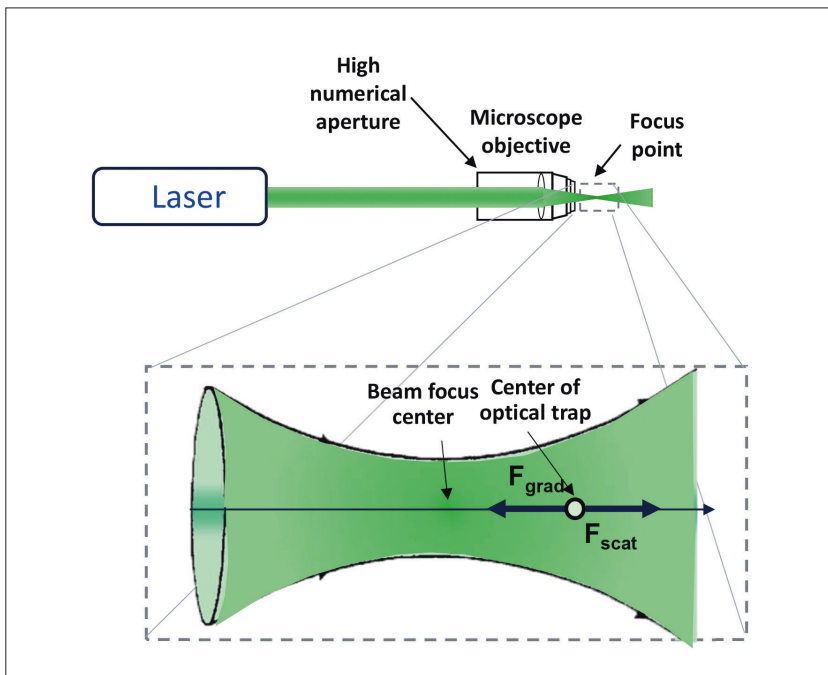


Figure 7. The layout of an optical tweezer (above) along with a magnification near the beam focus. A transparent sphere settles on the beam axis just beyond the beam focus where the gradient and scattering forces are equal.

OPTICAL MANIPULATION OF MICROORGANISMS

The understanding and development of optical tweezers were based on transparent spheres but were not limited to this ideal shape. They can also apply to other objects that can greatly depart from sphericity. It simply requires that the sum of the rays traversing a portion of an object is such that a net gradient force becomes comparable in magnitude to the scattering force. When this happens, the object can be “grabbed” by the optical tweezers and freely manipulated. The only requirement is that the object lets some light through. Note that most “small living things” are highly transparent at certain wavelengths where high-power lasers exist.

One of the first microorganisms manipulated by optical tweezers was the protozoan, *Paramecium*: a large, single-celled organism measuring between 50 to 330 μm in length. The internal components are called organelles and they range in size from a few μm to a few tens of μm . A picture of *Paramecium* with its organelles is shown in Fig. 8. The dimensions of these organelles are on the same order as the beam waist of a 1-micrometer laser, and therefore a natural subject to trap optically. Soon after we put together the set-up, we were able to manipulate its organelles. The circle, indicated by the arrow in Fig. 8, shows the organelle that could be moved around inside *Paramecium* or trapped in space. In the latter case, the trapped organelle eventually escapes the optical tweezer trap when it hits the walls of the moving cell.

In a period of over a year, we went on to use optical tweezers to manip-

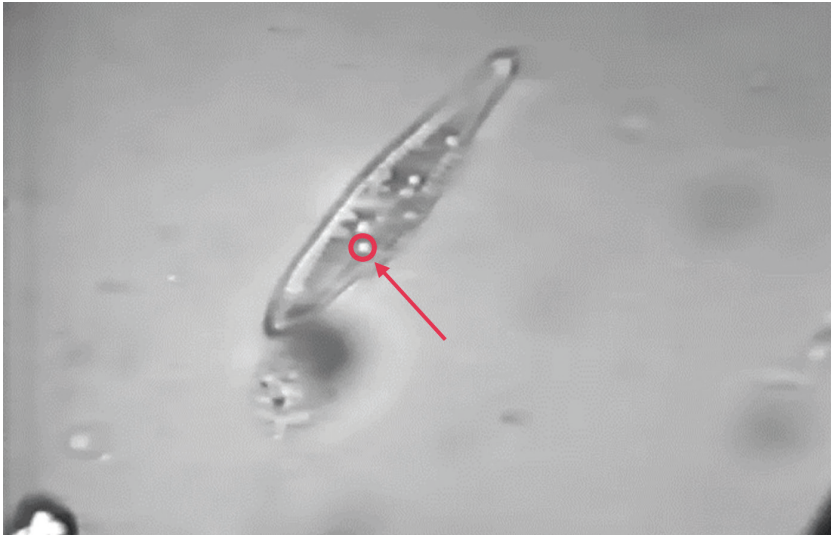


Figure 8. Picture of a paramecium where one can see the internal organelles. The arrow points to the specific organelle that we manipulated using an optical tweezer (see Nobel Lecture [1] for the video).

ulate “all sorts of living things”. This included tobacco mosaic viruses (TMVs) that can be trapped easily by its ends. We also trapped sperm cells, manipulated chlorophyll in onion cells, and trapped various other microorganisms including bacteria.

OPTICAL TWEEZERS TO MEASURE THE MOTION OF KINESIN

Motor proteins are enzymes that move objects inside living cells. Optical tweezers are particularly well suited to measure the displacement of individual motor proteins, revealing their dynamics and properties.

Figure 9a shows a single motor protein, called kinesin, attached to a tiny plastic bead, about half a micrometer in diameter [6]. The bead is first captured by an optical trap and then positioned directly over a microtubule. The kinesin molecule binds to the microtubule and begins to walk out of the trap, carrying the bead with it. Its motion is fueled by molecules of adenosine triphosphate (ATP) in the solution. The displacement of the bead is measured using a position-sensitive detector that monitors tiny deflections of the laser beam produced by the bead moving inside the trap. Figure 9b shows three different records of displacement, all showing that kinesin takes discrete steps as it moves along the microtubule. Each step measures just 8 nm.

Steven Block is one of the pioneers who studied the properties of individual biomolecules using optical tweezers, and his lab was the first to measure directly the steps taken by kinesin motors. He started his research with optical traps within days of the publication of our study in *Nature*, which reported that living bacteria, such as *E. coli*, could be

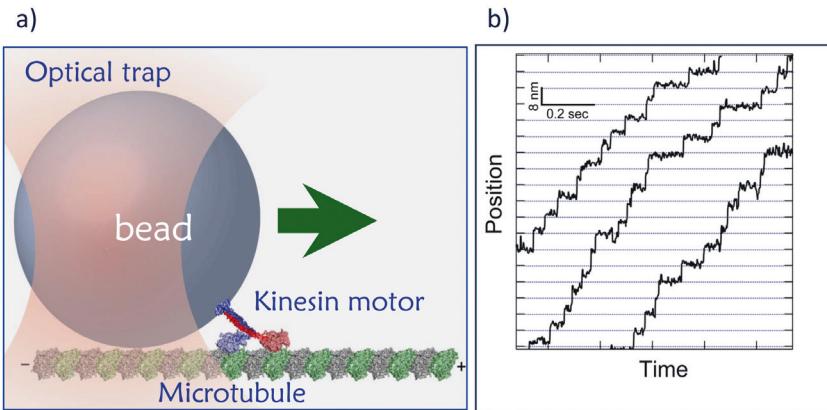


Figure 9. a) A schematic representation of the transport of a transparent bead by a kinesin molecule walking on a microtubule; b) three measurements of the position of the bead with time showing that the motion occurs by discrete steps of 8 nm. With permission from Steven Block.

trapped noninvasively by optical tweezers. Block first worked with his mentor, Howard Berg (at Harvard), to measure the nanomechanical properties of individual bacterial flagella, which propel swimming bacteria. He later built a series of sensitive optical trapping instruments that allowed him to study individual motor proteins, such as kinesin and myosin, nucleic acid enzymes, such as RNA polymerase, exonuclease, and helicase, and folded RNA molecules, like hairpins and riboswitches.

MEASUREMENTS OF GENE TRANSCRIPTION

– DUAL OPTICAL TWEEZERS

Gene transcription by a single molecule of ribonucleic acid (RNA) polymerase (an enzyme) can be followed as it moves along a template of deoxyribonucleic acid (DNA), synthesizing a corresponding RNA [7]. In a technique represented in Fig. 10, one end of the DNA template is attached to one bead, while a single RNA polymerase enzyme is attached to the second bead. This forms a bead – DNA – bead chain, also called a single-molecule “dumbbell” assay. Each bead is trapped by a separate optical trap, with one trap being stronger than the other. In this configuration, the assay can be used to measure both the force and the displacement that the polymerase enzyme experiences. As the enzyme proceeds along the DNA template, transcribing the corresponding RNA, the distance between the two beads changes. The video shown in the Nobel Lecture [1] displays how the separation between the beads, and therefore the location of the RNA polymerase, changes over time. High-resolution records of the relative motion of the beads reveal interesting RNA polymerase behavior, including transcriptional pausing, stepping, backtracking, and termination.

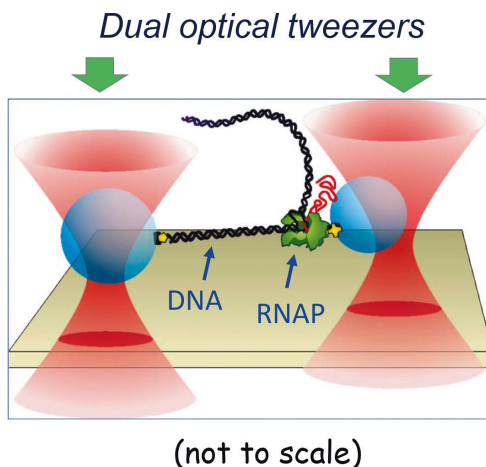


Figure 10. Two optical tweezers hold transparent spheres with one attached to a DNA template and the second attached to an RNA polymerase (RNAP) enzyme, actively transcribing RNA. With permission from Steven Block.

MEASUREMENT OF BASE-PAIR STEPPING BY RNA POLYMERASE

Figure 11 shows a measurement of the extension between the two beads of the single-molecule dumbbell assay as a function of time [7]. Note the transcriptional pauses, which occur reproducibly, at specific locations in the DNA template. This is an extremely sensitive apparatus that allows very precise measurements of displacement. At the large magnification shown in Fig. 11, we notice that the distance between the beads always changes in discrete increments of 3.4 Ångströms, which corresponds exactly to the distance between successive base pairs of the DNA double-helix. This was the highest-resolution measurement ever made directly on a single enzyme at that time. Optical tweezers, in combination with atomic force microscopy, is now being used to study these and other DNA-dependent processes, such as the work of Thomas Perkins at JILA/University of Colorado.

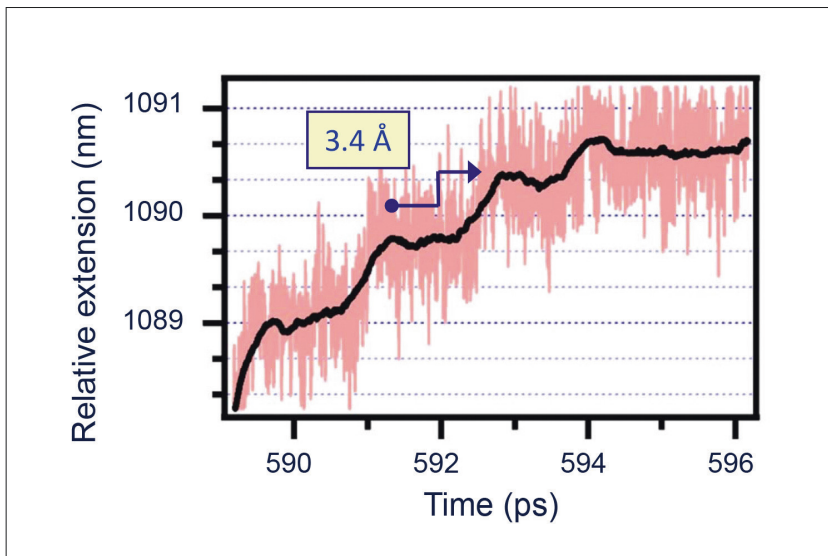


Figure 11. Measurements of the extension between the spheres as a result of the transcription of DNA by the RNA polymerase. A step size of 3.4 Ångströms was measured, which corresponds to the distance between the adjacent base pairs in the DNA double helix. With permission from Steven Block.

OTHER BIOLOGICAL APPLICATIONS OF OPTICAL TWEEZERS

There are many other applications of optical tweezers in biology such as

- 1) Measuring the properties of biopolymers produced in living organisms such as DNA and RNA
- 2) Measuring motion and forces of molecular motors, linear and rotary, that are agents of movement in living organisms
- 3) Studying the folding of proteins and structured nucleic acids
- 4) Studying the binding and assembly of biomolecular components
- 5) Micro-manipulation of small objects in general, including cells and organelles
- 6) ... and probably many other applications yet to come.

There are now several companies commercializing optical tweezers for diverse applications.

CONCLUSIONS

Since the invention of the optical tweezer in 1983, the technique has proven to be applicable to a wide range of particle sizes, from small numbers of atoms up to entire living cells. A key advantage has been the formidable power with which optical tweezers are able to manipulate and measure the motion of tiny objects. This has proven to be of particular value in studying the behavior of biomolecules. However, much work remains to be done in this area. Many of the nanomechanical properties of even the most essential proteins and nucleic acids remain unknown, and further investigation is needed to elucidate important processes involving these biomolecules. Advanced physical techniques, including optical tweezers, scanned-force microscopy, and the like, offer greatly improved capabilities to understand life's fundamental mechanisms.

ACKNOWLEDGEMENTS

I would like to acknowledge the contributions of my dear wife, Aline, my long-time research partner Joseph Dziejczak with whom many of the optical trapping experiments were performed, a close colleague, John Bjorkholm, and many other collaborators. I owe special thanks to René-Jean Essiambre for helping prepare and delivering the Nobel Lecture and for contributions to this summary. I am also indebted to Steven Block for graciously sharing materials, providing comments, and for a careful reading of the manuscript.

BOOK

I wrote a comprehensive book on optical trapping [8] with the help of my wife, Aline. It contains a description of the discovery and evolution of optical trapping from its inception until 2005. It also includes reprints of key articles on optical trapping. Of course, work continues as new discoveries are made. One of the motivating factors for writing the book were assertions made by certain researchers to the effect that magneto-optical traps (MOTs) were “much more important” than optical tweezers for trapping. I did not accede to this school of thought, and therefore decided to write a book demonstrating the power of using radiation pressure to trap tiny objects.

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