NS190-Paper

Optical Tweezers

in Biophysics Studies

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CONTENTS

ABSTRACT

Optical tweezers, or optical trapping, was invented in the 1970s by Ashkin et al [1]. It has now become a powerful tool for single molecular studies. During its years of development, people have achieved very high spatial and temporal resolution and have developed various novel approaches in its applications in biology or biophysics. This paper gives a review of the principles of optical tweezers and some examples of how it can be used in biology, or biophysics field. A discussion of its perspective in future studies is also included to show the potential of this promising technique.

INTRODUCTION

Arthur Ashkin first demonstrated the capability of optical forces to stably hold and slightly move micron-sized dielectric particles in water and air [1], and later he developed the single-beam gradient force optical trap, or known as optical tweezers [2]. Since its invention, Ashkin and his colleagues did various experiments with this technique. Today optical tweezers still plays an important role in both biology and physics studies. It is widely adopted in single molecular studies for its capability to apply pico-Newton-level forces to micron-sized dielectric particles and to measure angstrom-level displacement with millisecond-level temporal resolution [3]. Both its theoretical background and experimental applications are popular research topics and many researches are involved in for a better understanding and use of it. However, some factors such as the resolution was and still remains restrictions which need to be broken.

The purpose of this paper is to give a review of the theoretical background and newly developed techniques based on optical tweezers, and illustrate some interesting examples of applications in biophysics.

In the first part the basic principles of optical tweezers will be introduced, followed by a discussion of how the resolution is restricted and how can it be improved. The third part deals with how a real setup is assembled and how people do measurements with it. After that is a general introduction of how novel approaches derive from combining optical tweezers with other popular methods for specific purposes. Then a big part of the paper will give detailed examples of how optical tweezers were used in real studies and how it helped. In the end some further discussions and scientific perspectives are included.

PRINCIPLES

Theoretical background

The physical principles behind the optical tweezers are actually quite simple. It utilizes an objective lens with high numerical aperture (NA, defined as $NA = n \sin\theta$ where n is the refractive index of the medium and θ is the half angle of the maximum cone of light that can enter or exit the objective.) to focus a laser beam into a very small diameter (half wavelength). The scattering of the photons (i.e. reflecting and refracting of light) will transfer the momentum to any dielectric particle at the focus (Figure 1a). Therefore the particle will experience optical forces. This optical forces can be categorized into two components: scattering forces, which push particles in the light propagation direction, and gradient forces, which pull particles in the spatial light gradient direction. When gradient optical forces is larger than the scattering forces, the particle will be pulled over to the focus point and can stay stably at that position (Figure 1b) [2]. In practice a near-infrared laser beam is used (will be explained later), the high NA microscope objective lens helps to create the large spatial gradient in light intensity so that a stable trap can be formed. As a result, the trapped particle will be located slightly down beam of the focus. The optical trap behaves like a Hookean spring at the central area, i.e. the restoring force is linearly proportional to the offset from the equilibrium position for small displacements (~150 nm). And the stiffness of such a spring is determined by the light intensity [3].

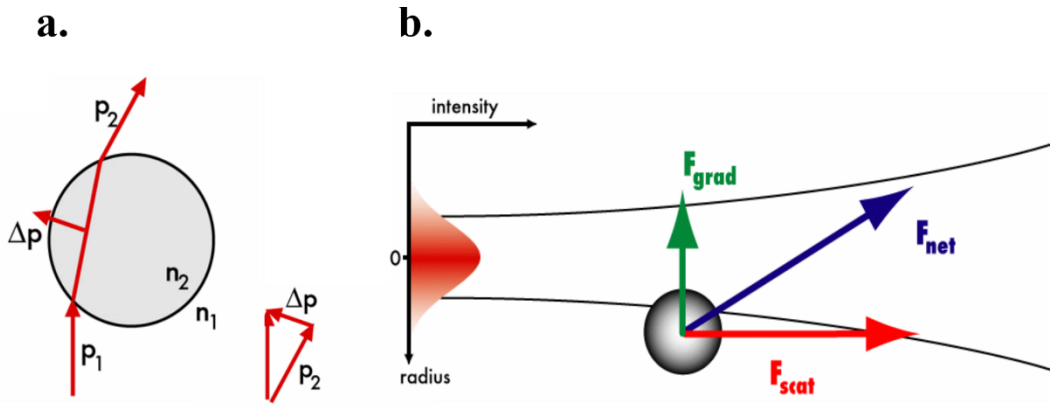

Figure 1. Schematic of optical momentum and force in an optical trapping system. (a) Momentum change when light is refracted, or in other words, photons are inelastically scattered, by the trapped particle. The incident photons have momentum $\boldsymbol{P_1}$*, and the scattered photons have momentum* $\boldsymbol{P_2}$*,* $\boldsymbol{\Delta P}$ *represents the change of momentum caused by the scattering. According to Newton's law, a same momentum change with an opposite direction will be given to the particle. (b) Optical forces in an optical trap. Scattering force* $\boldsymbol{F_{scat}}$ *is in the laser propagation direction, gradient force* $\boldsymbol{F_{grad}}$ *is in the intensity gradient direction, giving a total force* $\boldsymbol{F_{net}}$ *pulling the trapped particle to the beam center. Pictures are from internet.* not specific enough!

To estimate the force on a sphere two limiting cases need to be considered. If the size of the trapped particle is much larger than the wavelength of the trapping laser, the optical trapping system is in a regime of Mie scattering. It is not difficult to obtain the optical forces in this regime from simple ray optics [4]: the momentum of the incident light will be transferred to the trapped particle, and the value of this momentum can be estimated from the refraction of the laser. The direction of the optical force depends on the relative value of the indexes of refraction of the particle and the surrounding medium, if $n_{particle} > n_{medium}$, the force will be in the same direction as the intensity gradient, and vice versa. The scattering of light by the trapped particle, both elastic and inelastic (specular reflection and absorption of light) gives rise to the scattering forces. One thing to note is that the trapping efficiency can be improved if the cross

section of the incident laser beam is large enough to overfill the objective entrance pupil [4], because the central rays are those responsible for the scattering.

If the size of the trapped particle is much smaller than the wavelength of the trapping laser, the trapping system is then in the regime of Raleigh scattering, and the particle can be modelled as a point dipole inside a strong electric field. The scattering force is thus determined by the absorption and reradiation of the dipole and can be given as [3]:

$$F_{scatt} = \frac{I_0 \sigma n_m}{c}$$

$$\sigma = \frac{128\pi^5 a^6}{3\lambda^4}\left(\frac{m^2-1}{m^2+2}\right)^2$$

where a is the radius of the trapped particle, I_0 is the intensity of the incident light, σ is the scattering cross section of the particle, n_m is the index of refraction of the medium, m is the ratio of the index of refraction of the particle to that of the medium, c is the speed of light in vacuum and λ is the wavelength of the trapping laser. The scattering force is along the laser propagation direction and is proportional to the laser intensity. The gradient force is due to the interaction of the dipole with the field and can be written as [3]:

$$F_{grad} = \frac{2\pi p}{c n_m^2} \nabla I_0$$

where

$$p = n_m^2 a^3 \left(\frac{m^2-1}{m^2+2}\right)$$

is the polarizability of the sphere. The gradient force is proportional to the gradient of light intensity and in the direction along the gradient when $m > 1$ [3].

In practice, it is always the non-extreme case that the size of the trapped particle is comparable to the wavelength of the trapping laser. Unfortunately estimating the optical forces in this situation is not easy and requires a lot more electromagnetic theories.

Experimental preparation

The particles inside the optical trap are not the molecules which we want to study. Usually dielectric beads (such as silica or polystyrene) are used as handles. They can be connected to the molecules via biochemical methods. They typically have the size of ~0.2-5 μm [3]. The other end of the molecule that is being studied should also be connected, typically to the sample chamber surface (Figure 2a), a second bead which is held and controlled by a micropipette (Figure 2b), or a bead which is held in another optical trap (Figure 2c) [5].

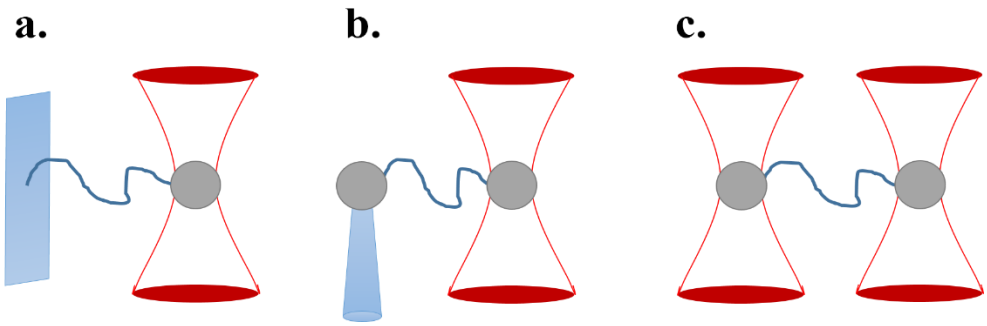

Figure 2. Three typical geometries of optical tweezers. (a) The molecule, or other biological systems, is attached to the surface of the sample chamber and the optically trapped bead on its both ends. (b) One end of the biological system is attached to another bead that is mechanically attached to the end of a micropipette. (c) The second bead is held in a second optical trap.

The motion of the system is monitored from the motion of the beads in the traps. Thus the beads also play roles of probes. To detect the bead position and thus the displacement and force is crucial and many methods were carried out. One of them, back-focal-plane interferometry technique has become the standard in the field for its sensitivity, linearity and speed in all dimensions [6]. The idea is simply to detect the change of the interference pattern formed between the trapping laser and the scattered light in order to get information of the movements of the trapped bead. However the result is tremendous, the resolution is only limited by the background electronic noise of the detector, and thus can reach angstrom and millisecond in spatial and temporal resolutions respectively [7] .

To do quantitative measurements with optical tweezers, the raw data, which usually contains various voltages from photo-detectors and other components of the instrument, need to be translated into physical parameters such as forces and displacements. This calibration can be done by measuring the corresponding offset of the trapped bead from its equilibrium position under an accurately-known force. There are also ways to measure the optical forces directly [8]. However, these force-displacement data are still insufficient to understand the actual motion of the biological system. Both the extension (distance between the two ends) and tension (linked to the optical forces on the bead) of the tethered molecule need to be measured to analyse the elastic property of the molecule. For the situation where one of these data is not able to be measured, a technique called force-feedback is powerful. The idea is to adjust the position of the bead, and thus the distance between the two ends of the molecule, to maintain a constant optical force on the bead, and measure the movement of the bead [9].

The ability of detecting the movements and shape change of the tethered biological system, i.e. the ability to detect the movements of the beads, determines the spatial resolution of the instrument. And the timescale of fastest detectable motions defines its temporal resolution. [5] These two concepts are of crucial importance and are inextricably linked, they are the key factor of judging a good optical tweezers system, and determines whether an optical tweezers system can be used in a certain experiment. Many scientists devote themselves into advancing the spatial and temporal resolution of an optical tweezers system.

RESOLUTION

Many biological processes contain different discrete physical movements and thus have corresponding displacements. The value of such displacements varies as the different inherent periodicity of the substrate on which these processes take place. For example the microtubules

have a periodicity of ~8 nm and the distance between adjacent base pairs in double-stranded DNA (dsDNA) is ~3.4 Å. To directly observe and measure these discrete displacements and deviations from this discreteness can reveal important details of these processes. The discrete steps makes it possible to measure the dwell times, the time spacing between these steps, and give their probability distribution. The dwell time distribution provides a statistical measure of the kinetics behind the processes and can give more information than the average rate of such steps [10]. The obstacle in front of the direct observation of such steps and dwell time distributions is the resolution. The advancement of high spatial and temporal resolution have allowed researches to study such steps and thus understand the physical change behind various biological processes.

Spatial resolution

In an experiment the biological system is positioned at a balance of three forces: the trapping force applied by the laser, the tension inside the system itself, and random thermal fluctuations. An accurate measurement depends on reliable data of all these three forces, and noise which can cause an uncertainty in these measurements is responsible for dragging the spatial resolution to a lower level. Generally two types of noises are discussed: experimental or Brownian. The experimental noise arises from components of the instrument, while the Brownian one comes from the fundamental thermal instability [5]. Experimental noise is usually caused by drift and fluctuations of the sample stage, the micropipette, loose mechanical connections, or the fluctuation of the laser power. It can generate movements of the trapped beads and for the detector it is impossible to distinguish such movements from that generated by the biological system, which is the one we are interested in. Such problems can, and can only, be solved by developing better optical tweezers instruments [7]. On contrary, Brownian noises cannot be avoided by making better instruments. Thermal forces thus provides the fundamental limit to the spatial resolution.

Fortunately it is possible to estimate the influence of the Brownian forces and minimize it by tuning the experimental parameters. In practice removing the experimental noises as much as possible is the first task before the limiting of the Brownian noise starts to show its influence.

Experimental noise can be largely reduced by using a novel and stable optical tweezers system, which typically contains a component to isolate the system from the environment (will be discussed later). For a mechanically stable setup which does not have an isolation system, the experimental noise mainly comes from environmental factors. Temperature drift, mechanical and acoustic vibrations in the room, as well as background electronic noise, all can couple into the instrument and affect resolution [7]. The trick to remove these disturbance is usually just to monitor such disturbance and subtract it from the measurement data. A second laser which works as a probe is often involved for this purpose [11]. For the three cases in Figure 2, a cross on the surface of the chamber (Figure 2a), a mark on the micropipette (Figure 2b), or a second bead (Figure 2c) can all play the role of the track point of this probing laser. The movements of these track points will be recorded and be cancelled out from the movement of the bead by an active feedback.

The effect of thermal forces on resolution can be minimized because it depends on several experimental variables such as bead size and tether stiffness. It is thus helpful to understand this dependence. The signal-to-noise ratio (SNR) is a dimensionless ratio of the strength of signal to that of the noise, in our case the strength corresponds to the displacement. SNR is widely used to give a quantitative estimate of the resolution.

For a single trap system in slow measurements the SNR can be given as [5]:

$$SNR \leq \frac{\kappa_{tether}\Delta l}{\sqrt{4k_B TB\gamma}}$$

where κ_{tether} is the stiffness of the biological tether, which is the slope of the force-extension curve of the tether at a given tension, Δl is the size of the displacement of the bead, T is the temperature, k_B is Boltzmann constant, B is the bandwidth of the measurement, and $\gamma = 6\pi\eta r$ is the drag coefficient of the trapped bead. η is the viscosity of the medium, and r is the radius of the trapped bead [5]. The two sides of the equation are equal in perfect instruments without any experimental noise.

With this expression it is now possible to illustrate how different parameters influence the resolution. It is clear that the SNA is positively related to the tether stiffness and displacement, while negatively related to the temperature, the drag coefficient of the bead, or the measurement bandwidth. Among these factors the displacement is obtained through the measurement and cannot be adjusted forehand, and the temperature should be controlled in a small range due to the allowed temperature range of biological systems. Decreasing the bead size can reduce the drag coefficient and can in principle help. However, it is mechanically difficult to use very small beads and the bead size can affect the reliability through other parameters. For example by optimizing, instead of minimizing, the bead size the errors in force measurements can reach a minimum. An ideal size is approximately equal to the beam waist of the trapping laser [12]. A notable fact is there is no dependence in the equation on trap stiffness, despite the fact that beads in stiffer traps fluctuate less. It is because that the bead in stiffer traps are also less sensitive to the movements of the biological system, and the two effects cancel out [5].

Recent research gives 1 Å resolution in three dimensions over 100 s ($\Delta f = 0.01 - 10$ Hz) using forward-scattered light in a geometry similar to Figure 2a. Using back-scattered light the same resolution can be achieved but in a higher bandwidth ($\Delta f = 0.1 - 50$ Hz). By improving the rate of feedback even sub-Angstrom stabilities over 100 s ($\sigma_x = 0.3$ Å; $\sigma_y = 0.2$ Å; $\sigma_z = 0.6$ Å; $\Delta f = 0.01 - 10$ Hz) can be observed [13].

For the dual trap geometry as shown in Figure 2c a similar expression can be given. When the two beads are treated independently from each other the total noise will be increased by the existence of the second bead. However, in reality, the two beads are always tethered together and their behaviours influence each other and such correlation helps to reduce the effect of Brownian noise [14]. The idea is to divide the fluctuations of the beads into symmetric and anti-symmetric regimes. Symmetric fluctuation influence the beads in the same direction and is thus irrelevant to the tension on the tether. Anti-symmetric fluctuations will stretch or compress the molecule and plays a role of limiting the spatial resolution. Thus by monitoring the motion of both beads simultaneously, it is possible to discard the noise caused by symmetric motion with a differential measurement, improving the resolution of the system [14]. The SNR equation for this differential fashion remains the same as before, only with γ replaced by $\gamma_{eff} = \gamma_1\gamma_2/\gamma_1+\gamma_2$, where γ_1, γ_2 are the drag coefficients of each of the beads [14]. Because γ_{eff} is smaller than either γ_1 or γ_2, the second trap actually helps improve the spatial resolution.

Temporal resolution

The temporal resolution of optical tweezers is determined by various limitations, such as the relaxation time, thermal noise and the dead time [15]. It can even be limited by the electronics when the sample rate exceeds the capability of the electronic system. Though this rarely happens.

The relaxation time of the system is defined as the time constant $\tau = \gamma/\kappa$ of the exponential process of the system changing from one equilibrium to a new one after an external perturbation. Where γ is the viscous drag coefficient and κ is he stiffness of the system [16]. In an optical tweezers system the relaxation time is determined by the trap stiffness. A bead inside a trap with smaller stiffness moves faster and has shorter relaxation time. The relaxation time provides an intrinsic limitation to the temporal resolution because faster processes cannot be expressed by the movements of the beads and are thus invisible to the observers.

As for spatial resolution, thermal noise also plays a role for temporal resolution. Actually it provides an external limitation to the temporal resolution. As shown by the SNR expression, a large bandwidth, which means worse temporal resolution, helps improve the spatial resolution. A compromise between spatial and temporal resolution is required according to the goal of individual experiments, but usually the spatial resolution is the one that draws more attention.

Additional temporal limitations arise from the dead time, i.e. the lifetimes of some fast process such as weak molecular bonds or non-processive motors with short interaction lifetime. These processes usually have the duration of millisecond or sub-millisecond [15]. This limitation is also intrinsic.

INSTRUMENTS

The typical components for an optical tweezers system are trapping laser, beam expansion and steering lenses, a high NA objective, a sample holder, and some means for observing the trapped sample (Figure 3). Optical traps are often built from an inverted microscope, allowing the microscope providing imaging, trapping chamber manipulation, and objective focus functions. Additional elements such as position and force clamp systems would help to achieve more control than simply trapping and manually manipulating objects. Commercial optical tweezers systems with some limited capabilities are accessible. But they have fewer means of measurement. A home-built optical tweezers system is cheaper, more flexible and provides more control, but it costs much more time and consideration.

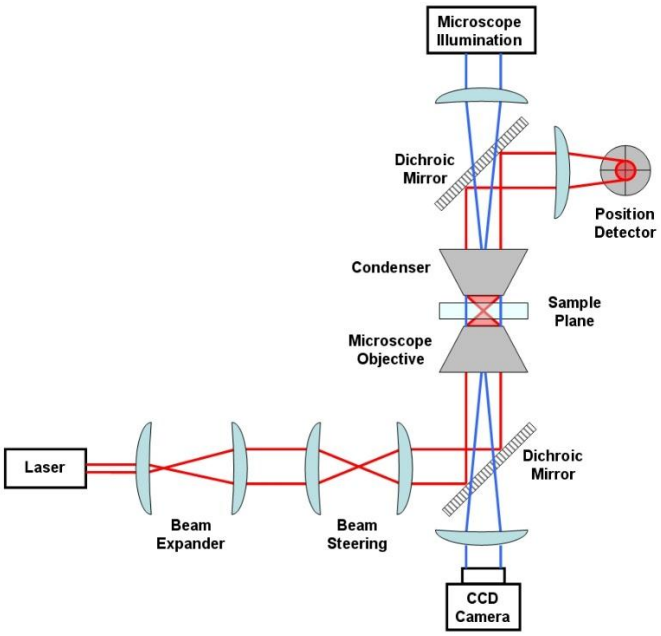

Figure 3. Experimental setup of an optical tweezers system. The beam expander expends the laser beam to overfill the aperture of the objective. The beam steering lenses steer the position of the trap in the sample plane. The dichroic mirror reflects the laser wavelength but transmits the illumination wavelength. The laser beam is focused by the objective to form the optical trap. The position detector is for back focal plane detection and the camera for imaging.

Ref to origin lacking?

Trapping laser

The key factors of a trapping laser are single mode output (typically Gaussian TEM_{00} mode), good pointing stability and low power fluctuations. Gaussian mode provides the smallest diameter of focused beam waist and will lead to the most efficient trap. Good pointing stability eliminates unexpected displacements of the trap position in the sample plane. And low power fluctuation gives few temporal variations in the optical trap. Solutions for achieving a lower pointing instability usually increase the chance of amplitude fluctuations. Both of them bring noises to the system. Many other factors such as the output power and the laser wavelength thus become the criteria of choosing a suitable trapping laser.

The trapping stiffness and the optical forces are determined by the output power of the trapping laser at the sample plane. Generally speaking, maximum trapping forces on the order of 1 pN per 10 mW of power delivered to the specimen plane can be achieved with micron-scale beads [3]. For biological studies there are usually many restrictions of the available wavelength. The light need to be able to go through the cell or other environmental medium, and not be absorbed by the proteins or water. This restricts the wavelength ~750-1200 nm. The optical damage to the biological system and the heating effect to the medium and trapped particle bring more restrictions. Observations show a minimum damage for bacterial cells of *Escherichia coli* at 970 and 830 nm [17]. The optical properties of the objective, mirrors and detectors at a certain wavelength are also important factors to be considered. The type of laser and its availability of providing a certain power at a certain wavelength are restrictions from the laser system, and actually often determines the wavelengths of lasers which can be used.

In practice the mostly often used lasers are neodymium: yttrium-aluminium-garnet (Nd:YAG) laser and its close cousins, neodymium: yttrium-lithium-fluoride (Nd:YLF) and neodymium: yttrium-orthovanadate ($Nd:YVO_4$). These lasers operate in the near infrared region at 1047, 1053, or 1064 nm, which behave good regarding the restrictions. Diode pumped versions of these lasers are mostly used because they are able to provide high power (up to 10 W or even more) and very nice amplitude and pointing stability. And due to its structure the heat generating part can be isolated from the trapping system. The main drawback is their high cost.

Microscope

As mentioned before and shown in Figure 3, inverted microscopes with some modifications are used for optical tweezers. The modifications involve inserting two dichroic mirrors on both sides of the microscope, separating laser beam from illuminating light, guiding the beam to the objective and position detector respectively. Inverted microscopes are often preferred because their flexible objective provides stable coupling of the trapping light. It is also easier to extend the microscope to other imaging tools because for these cameras the microscope is up-straight and helps get larger images of the trapped molecule. The mechanical structure of the microscope may bring experimental noise as was discussed before, therefore further extensive modifications such as using a single objective holder instead of the rotating multi-objective holder or using a stable piezoelectric stage are optional.

Objective

The efficiency of an optical system is defined as the ratio of stiffness over input power [3]. The stiffness depends on the NA of the objective lens, while the input power depends on the trapping laser and the transmittance of the objective. Therefore a nice chosen objective lens will largely improve the efficiency of the whole system. A commonly used NA range is 1.2-1.4 NA, such values are enough to produce a large intensity gradient for trapping. In addition to the NA of the lens, immersion medium in which the lenses and the trapped molecule are immersed into, is used to enlarge the NA. Water, oil or glycerol are all typical choices, but different mediums together with a reasonable working distance give different limits on the depth the bead can be trapped. However, the refractive index mismatch between the immersion medium and the trapping medium gives rise to spherical aberrations, which draws back the trap performance. The larger the mismatch is, the worse the trap is. Therefore for aqueous systems the water immersion medium is always used. A practical problem when considering transmittance is that the commercial microscope objectives are mostly made only for visible light. Which is not the wavelength used for trapping. As the trapping laser is in the infrared region, those lenses for infrared microscopy give better performance.

Position detection

With the above components the optical tweezers system can already trap and operate particles and observe the change via cameras. However, without a position detecting element we can never obtain experimental data of the displacements and forces, thus are not able to study the biological process behind the conformational change. Unfortunately precise position detection and force calibration are currently only available for spherical objects [3]. This provides another reason to use dielectric microspheres as beads. The position detection methods discussed here are all designed for such beads.

1. Video based position detection. As shown in Figure 3, a camera is always included in an optical tweezers system, and it is often sufficient for simple purposes. Biological processes can be directly observed via this detection, the size of the molecule and its movements can also be measured by calibrating the size of a pixel to the real spatial distance. The resolution depends on the number of pixels. The temporal resolution is limited by the video acquisition rates. For conventional cameras this rate is 25 to 120 Hz, and for specialized high speed camera the rate can reach or even exceed 25 MHz. But the availability of such high speed cameras is limited by the computer speed or memory capacity, and the required exposure time under certain illumination conditions which are suitable for biological systems.

2. Imaging position detector. A quadrant photodiode (QPD) is used in imaging position detectors. The trapped particle will be imaged on to the QPD, and the movements of the particle will be reflected in the movements of its image in x and y components. The four coupled diode detectors can then detect the x or y displacement by using differential calculations. To improve spatial resolution a large magnification is needed, however the intensity of the image will be reduced. This provides a limitation of temporal resolution.

3. Laser based position detection. Laser based position detection uses a single laser for both trapping and detecting, and it is now widely used in optical tweezers systems. The light scattered by the trapped particle is collected by a stable condenser and a dichroic mirror on the condenser side. There are in general two types of laser based position detections. One is the back-focal-plane interferometry, the other is based on polarization interferometry. Both of them require a very nice co-alignment of all components in the optical path.

As introduced before, the back-focal-plane interferometry depends on the interference between the scattered light and the un-scattered light. A QPD is used for monitoring the change of the interference pattern during the movements of the trapped bead. Movements on both x and y directions can be precisely detected.

The polarization interferometry involves the using of two Wollaston prisms, one for splitting the incoming plane wave laser beam into two spatially separated orthogonal polarized beams before the trap, another for recombining the two beams after the trap. When the trapped particle is deviated from its equilibrium position, it will bring different phase shift to the two beams, giving rise to a change of polarization states in the recombined beam. This change can be calibrated by using known displacements and can thus return precise displacement measure results. A shortcoming of this technique is that it can only reflect movements in one dimension.

4. Axial position detection. A common drawback of the approaches discussed above is that all of them are only able to measure lateral displacement of objects within the sample plane, but not the axial motion. It is sufficient for traditional optical tweezers applications. However now optical tweezers have seen much more application in various fields of studies and detecting axial motion becomes a must. A good method for this purpose is to detect the total laser intensity in the back focal plane of the condenser. This technique utilizes the property that a laser beam will gain a phase shift of π when passing through the focus, known as the Gouy phase. While the light which is scattered and did not pass through the focus won't have this phase change, but a phase change gained during the propagation until it meets the scattering particle. Thus the far field interference pattern contains information of the axial position of the bead and makes it possible for axial position detection. The drawback of this technique is that this axial information may couple to lateral information, and bring uncertainty of both measurements. And unlike for lateral position detections, the axial position detection is inversely proportional to the NA of the detector [3]. A compromise must be made if the same detector is used for both lateral and axial detections.

too brief, too superficial to be useful

Dynamic control

?

In real measurements a dynamic control over the position and stiffness of the trap is needed. Then we can achieve real time control of the forces applied on the trapped particle, and provide mechanical conditions suitable for the biological systems to be studied. Moreover, if the relaxation time of the processes are slower than the reaction time of the dynamic control, one trapped laser can be shared with multiple traps in the same experiment.

how? Why not without dyn control?

Scanning mirrors, acousto-optic deflectors (AOD) and electro-optic deflectors (EOD) are the three mostly used dynamic control techniques. Scanning mirror uses a mirror on a fast mechanical component to scan the trapping laser to the wanted direction. AOD uses an acousto-optic crystal which diffracts light into different orders at different angles, the diffraction angle can be adjusted by an acoustic signal. EOD is similar to an AOD but the controlling signal is electrical. These techniques work under different principles and are controlled by different signals, but they can all deflect the input light to a certain angle with a short switching time.

Piezoelectric stage

Piezoelectric (PZ) stage technique provides an easy way to achieve stable, linear, reproducible, precise positioning in three dimensions. The stage can move precisely in a certain direction under a controlling electrical signal. The integration of a feedback loop will adjust the position precisely in real time and reduce unwanted drifts largely. Since the position is the key parameter

of an optical tweezers experiment and PZ stages can adjust positions fast and precisely, it has brought numerous difference to various aspects of optical tweezers systems. For example they allow precise, three-dimensional control and detection of the trap position. They allow fast, reproducible and precise calibration of position, force and interferometry pattern. They also allow to apply a constant force or constant displacement beyond the limitation of other components. The drawbacks of PZ stages are the high cost and the relatively (compare to AODs or EODs) slow communication with the stage controller.

Environmental isolation

As discussed in the resolution part, many environmental factors, such as temperature, acoustic noise, mechanical vibrations and air fluctuation will all influence the measurements of the optical tweezers. Various things are done to isolate the system from these unwanted external noises. For example air tables are used to offer mechanical isolation, enclosing all free space optics will reduce air currents, housing the system in an environment with lower index of refraction can lower the effect of possible fluctuation, reducing the length difference of optical paths in an multi-trap system can result in a same deviation in all beams and producing no difference for the trapped beads, taking away power supplies and heat sources helps to reduce thermal and acoustic noise, and adopting acoustic isolated and temperature controlled experimental rooms can provide a stable macro environment.

NOVEL TECHNIQUES AND APPLICATIONS

To study biological processes with optical tweezers the conventional single-trap or dual-trap optical tweezers systems begin to show their limitations. First of all, the motion of the molecules or molecular motors is complicated and in three dimensions. While as mentioned before, for all the position detection methods the lateral and axial motion cannot be measured with high resolutions simultaneously. Moreover, the motion may be accompanied by some conformational changes within the protein. Which cannot be understood simply by detecting force and displacement. Furthermore, inside the cell, molecular motors do not work separately but are part of larger and more complex structures which has tight coordination between its components. A single- or dual-trap optical tweezers may not be sufficient to observe all the motions at the same time. Therefore traditional optical tweezers will be poorly suited to study a phenomenon involving any if these complicity, new manipulations and measurement capabilities need to be integrated to the system, forming various hybrid or novel optical tweezers approaches.

Such novel technique integration includes new mechanisms of attaching molecules to surfaces or beads, applying torque to gain information on more degrees of freedom, multi-trap optical tweezers for simultaneous study of more than one molecules, microfluidics and fluorescence, STED microscopy, etc. This section will give examples of how a traditional optical tweezers and these novel techniques work in real biological studies. Most of these studies relate to the mechanical properties of DNA and the protein-DNA interaction mechanisms.

Stretching and unzipping DNA with traditional optical tweezers

In its relaxed mode DNA molecules are in worm-like shapes and occupy little space. However they can be stretched into tens of microns length. The elastic property stands central of its mechanical properties, and thus plays important roles in its cellular functions, including folding,

packaging, regulation, recombination, replication and transcription. The elastic stiffness of DNA can be characterized by its contour length under zero tension L_O, the persistence length L_P, and the elastic modulus K_O.

Wang et al. have published a method of obtaining these parameters of DNA molecules using traditional single-trap optical tweezers [18]. The experiment is done in a regime similar to Figure 2a, the DNA molecule is tethered between a polystyrene bead (~520 nm) and the surface of a microscope cover-glass. The trapping laser is the first order diffracted Nd:YLF light (1047 nm) from an AOD. The AOD is used for modulating the laser intensity and to isolate the power source from the trapping system. The same laser is also used for position detection. A feedback circuit is added to the system to control the AOD to provide clamps.

In the experiment the bead is held in the trap while the PZ stage moves. When the DNA tether is stretched, the bead starts to be pulled away from the equilibrium point and the restoring force starts to increase. When the bead moves to a clamp position the feedback circuit is activated and controls the AOD to give higher laser intensity to hold the bead in a constant position. When the DNA continues to be stretched, the bead stay still and the force measurement is done by the laser intensity. This experiment allows rapid determination of the force-displacement relation [18]. Experimental data are fitted with entropic elasticity theories based on worm-like chain models. Results of Wang's experiment gives a persistence length L_P of ~47 nm in a buffer containing 10 mM Na^+. The buffer solution has certain influence on the elastic properties of the molecules, L_P will be reduced to ~40 nm at the present of multivalent cations such as Mg^{2+} or spermidine $^{3+}$. The elastic modulus is shown of ~1100 pN by an elasticity theory which fits the experimental results perfectly [18].

This experiment provides an example of the application of a conventional single trap optical tweezers system in the study of DNA mechanical properties, proving the ability of measuring nanosclae forces and displacements of such a simple optical trap. Another example of single trap optical tweezers used in DNA studies is unzipping DNA [19], which was done with a similar geometry of optical tweezers.

The difficulty sits in the unzipping of DNAs is that the separation of the two strands (~ 2 nm) is too small for a bead which is suitable for trapping. Therefore a special DNA construction was designed for the purpose of this experiment. Additional linker arms are attached to both strands of the DNA to form a λ shape. The length of the DNA is ~16 μm, and the lengths of the linker arms are ~2.5 μm length each. Also in a regime similar to Figure 2a, one of the two linker arms is connected to a silica bead which is trapped with Nd:YAG laser (1064 nm), and the other is attached to a cover-glass on a PZ stage. As the PZ stage moves, the arm attached to it moves further from another arm, the DNA is then unzipped. Forces and displacements were recorded [19].

Results show the forces in different stages of the unzipping process. The force remains zero before the two arms are totally stretched, and then it starts to rise rapidly. The force stops to rapidly rise at 10-15 pN, this is when the mechanical unzipping starts. The force then exhibits a very rapid variation with an amplitude of ~10%, this is due to the differences in the pairing and stacking energies among the different basepairs, and thus reflects the sequence of the DNA molecule [19].

High-force optical tweezers using gold-thiol chemistry

The above examples and some other studies, such as characterizing molecular motors, unfolding protein, rupturing protein-ligand bonds, make use of the trapping force to stretch and

pull DNA molecules. The range of such force is typically 0.01-100's pN [20]. These experiments use a similar experimental apparatus (Figure 2a) which requires a surface (coverglass) that biomolecules can couple to. The properties of the surface can thus influence the experimental result. A good surface which can maintain the biological activity of the attached molecule and can minimize unwanted sticking will provide an insurance of the experimental result. Gold-thiol chemistry is used to provide a better surface and a stronger attachment (~1.4 nN) [20]. It was not widely used in previous researches because the gold can be ablated by the trapping laser. Paik et al. used an array of gold nanoposts with radius of about 50-250 nm, height of about 20 nm to minimize the contact of gold surface with laser [20]. In their experiment the posts separate from each other at 8 μm. In order to stably anchor the DNA to the nanoposts, three repetitions of dithiol phosphoramidite (DTPA) are used as the "glue". Another end of the DNA molecule is attached to the bead held in an optical trap [20]. DNA molecule with the DTPA linkers are incubated immediately after the nanoposts are deposited, and then the surface is passivated with methoxy-(polyethylene glycol)-thiol (mPEG-SH) to make it excellently non-stick [20].

In the experiment two lasers were used, a high power one (400 mW at focus) for trapping, a low power one (250 μW) for measuring bead position using back-focal-plane detection. A simple way to verify that only one DNA molecule is being studied is to check if the fitted persistence length is around 50 nm, which is a characteristic figure, also proved in the previous example. In their experiment a persistence length L_P of 44.6 nm is obtained at moderate forces (< 10 pN).

The high forces (> 100 pN), and thus high laser power (400 mW) are used in studies such as overstretching DNA. DNA undergoes a special structural transition at ~65 pN in which its extension increases to 1.7 times its contour length under a small force increase (~ 5 pN) [21]. This is another characteristic property and can be used to prove whether a single DNA molecule is under stretching. This transition is also observed in Paik's experiment. The experiment was designed to overstretch the DNA molecule till a high force (~100 pN), however the binding between the bead and the DNA molecule failed and unfortunately stopped the experiment.

Though failed, the experiment is sufficient to show the strong binding effect brought by the gold-thiol chemistry, at least stronger than the binding between the bead and the DNA molecule. It might be possible to use gold nano-particles that have been passivated from laser ablation as beads to achieve a stronger bead-DNA connection.

Torque applied with optical tweezers and DNA under torsion

We have discussed examples of stretching DNA molecules in one direction and it is already good enough to show many intrinsic properties of DNA molecules. It is also possible to apply such translation operation in all three dimensions. However, a three dimensional translation movement is not all that happens in nature. People have devoted many efforts to use optical tweezers to measure additional degrees of freedom, for example rotations.

A sketch can say more than a 1000 words ...

Rotating biological structures and measuring the associated torque with an optical tweezers apparatus will bring extra information of the tethered molecules. Strick et al. provided a smart way to do this. Again using the apparatus similar to Figure 2a, they attached one end of the dsDNA molecule to a flat surface, and another end to a bead made from paramagnetic material. This magnetic bead can rotate while a rotating external field is applied [22]. Since the other end of the molecule is not able to rotate along, the DNA molecule will be under torsion and became supercoiled. The authors then determined how the elasticity depended on the twist. Results show that for slightly twists (< 1% change in linking number), the contractility of the DNA

molecule will increase, regardless of the direction of the twist. Such an effect is predictable from a rod model that the rod will minimize the torsional energy by forming spring-like structures [23]. Stretching the rod will lead to extra tension in the molecule. The tension reaches a critical value of ~0.5 pN and then stops to grow. The DNA strands now separates to dissipate this energy. This is consistent with theoretical reports [24] that negatively supercoiled DNA cannot change its twist by more than approximately 1%. Molecules with large positive linking numbers display a different force plateau at a higher critical force of around 3 pN [25].

There are of course other techniques to demonstrate for rotating microscopic particles. In a geometry of Figure 2b, the rotation of the micropipette (left bead, with the rotation axis on the direction of connecting the two beads) allows the addition of torque to a single molecule of DNA [26]. Particles with irregular shape can be rotated by azimuthally asymmetric beams or combinations of beams [27]. Polarized light can apply torque to birefringent crystals such as calcite or quartz particles [28]. Rotation provides an extra degree of freedom and therefore a clearer means to illustrate many biological behaviours. The integration of torque control broadened the application of optical tweezers systems.

Quad-trap optical tweezers and condensation of bacterial chromosome

All the experiments that have been discussed so far focused on the mechanical properties of DNA molecules. The approaches that were used all involved manipulations of a single DNA molecule and the experimental setups were all as simple as Figure 2a. However, this is not sufficient for more complex studies. Some processes, for example the condensation of the bacterial chromosome, involves interactions between many DNA and protein molecules. One of these proteins is the nucleoid structuring protein H-NS. It is a dimeric protein that can bind to two DNA segments simultaneously but independently. Dame et al. developed a quad-trap optical tweezers technique to manipulate two DNA molecules at the same time and allows the observation of how these H-NS proteins bind to DNA molecules, in order to understand their role in the condensation process [29]. As illustrated in Figure 4, the four traps are formed by splitting a single laser into two polarization states, using one of them to form one of the optical traps, and then time sharing the other one with an AOD to form the other three traps [29]. A requirement of successful time sharing is a high scan rate. The laser has to return to a trap before the bead in this diffuse away, typically on the order of tens of milliseconds.

For simplicity, the force was only detected in the single trap that is not time shared (Figure 4b). Results show that for DNA strands which are already overlapped, H-NS can form a bridge between. But the bridge will not form if a single strand is covered with H-NS and then another strand is brought together. The results also implies that the H-NS proteins bridges DNA segments which are closely together and thus condenses the chromosome. With the same setup the authors also measured the unzipping force of such a DNA-protein-DNA bridge. Due to the high resolution of their apparatus, they have seen steps of this unzipping process. These researches can never be done with a single- or dual- trap system.

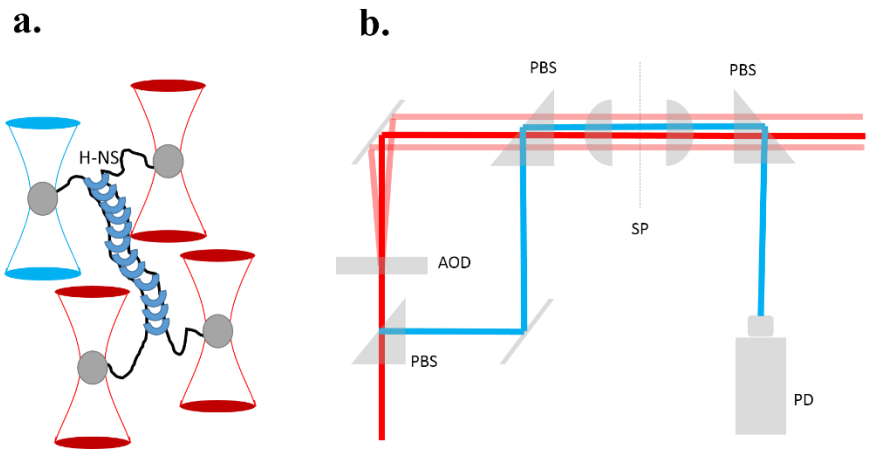

Figure 4. Quad-trap optical tweezers. (a) Sketch of the quad trap system manipulating two DNA molecules, with a H-NS bridge between the molecules. The blue and red traps represent different polarizations, and the blue one is used for position detecting. (b) The setup of the quad-trap system. The incident laser is split into two polarized beams through a polarizing beam splitter (PBS). One of them (red) is rapidly shared between three traps by an AOD. Another one (blue) is not shared and projected onto a position detector (PD) after the trapping system. The four traps are formed on the sample plane (SP).

Optical tweezers in protein-DNA interaction studies

Protein-DNA interaction is a concept that contains much more aspects than a DNA-H-NS-DNA bridge. A huge number of studies were done in various regimes of protein-DNA interactions and with various novel techniques. Microfluidics and fluorescence microscopy are popular companions of optical tweezers when studying protein-DNA mechanics.

Microfluidic flow cells can provide precise temporal and spatial control of the chemical environment of the molecule. It uses channels to separate different environments for different manipulations. It can be categorized by the number of channels, the material for the structure, and the fluid delivery system [30]. Single-molecule fluorescence microscopy provides a tool to observe the location and dynamics of labelled bio-molecules directly. It can even provide information of chemical reactions and conformational changes of the observed molecule [31]. To generate fluorescence the molecules need to be illuminated with excitation light. There are three ways of illumination: epi-illumination where the entire sample plane is illuminated, confocal illumination where only a selected small area is illuminated by a focused beam, or total internal reflection where an evanescent wave is used to excite a small region near the surface of the sample slide [32].

Various researches have been done by combining optical trapping, fluorescence microscopy and microfluidics. The first example here is the sequential isolation and visualization of a single DNA molecule using YOYO-1 dye. A multi-channel microfluidic flow cell is the crucial equipment in this experiment, different steps are done in different channels. Before the experiment the cell should be prepared with components suitable for each step into corresponding channels. The laminar flow ensures that the components in different channels won't mix with each other. The first step, done in the first channel, is trapping the beads. Two beads which were already in the first channel are trapped automatically soon after the laser is turned on. Then the two beads will be moved to the second channel to be connected with DNA molecules which are prepared in this channel. Whether the movement of one bead can influence another bead can be a criteria to judge if a DNA molecule is caught. If so, the molecule can be brought into the third channel, the buffer channel. The force-extension measurement is done in this channel, whether the stretching behaviour show any of the two characteristic elastic

properties mentioned in the gold-thiol example can tell if only one DNA molecule is caught. Then in the fourth channel the DNA is incubated with the YOYO-1 dye. In the experiment the DNA is bathed in a solution containing 20 nM YOYO-1, 10 mM Tris-HCl and 50 mM NaCl for 5-10 s. Then under the illumination of 473 nm excitation light in the epi-illumination mode the bright fluorescence signal of the dye can be observed [33].

Another example that makes the DNA-protein interaction visible is the binding of fluorescently labelled Rad51 to dsDNA. Rad51 protein helps repair DNA double strand breaks and is thus a very important protein to maintain the DNA integrity. It can form nucleoprotein filaments on both ssDNA and dsDNA. In the experiment the Alexa Fluor 555 is used to label the Rad51 protein. Before the experiment all the label work were done and the DNAs already carries the labelled Rad51 filaments. At the beginning of the experiment, similar as the previous example, two beads are trapped and a DNA molecule is caught in the first three channels. Then in the buffer channel the illumination light of 532 nm is turned on and the fluorescence images can be observed. The force can also be measured at this step. Next the beads start to separate from each other and a tension will be applied on the DNA molecule. The fluorescence of this experiment exhibits an alternation of dark and bright regions, this shows the conformation of the protein filament on the DNA. Together with the force-extension relation of this complex it is possible to find how the protein filaments form and bind to DNAs [34].

The next example is an extension of such combinations, it dedicates in improving resolution. Heller et al. provide a method of observing and measuring the mechanism of individual protein binding to a densely covered DNA. The approach combines optical tweezers with multicolour confocal and stimulated emission depletion fluorescence microscopy (STED, a process that provides super-high resolution by selectively deactivating fluorophores to enhance the imaging in that area [35]) and provides high spatial and temporal resolutions (50 nm, 50 ms) [36]. As shown in Figure 5, the experiment is done in a dual-trap regime and both beads are included for force measurement. The DNA tethered between the beads is stretched so that the confocal excitation and STED beam which has to scan along DNA is only needed to move in one direction. The scan rate is limited by a tip-tilt PZ stage at 200 Hz.

A STED nanoscopy experiment is done using this setup. Unlike normal confocal illumination methods, STED can provide with focal-intensity distribution that features a 1D central line of nearly zero intensity. Theoretically, since the DNA molecule, the target object being detected, and the line-shape STED focus, the detecting tool, are both one dimensional, it is possible to achieve a zero dimensional detecting point which can return information with infinite spatial resolution. However due to the finite size of actual molecule and focus shape, this can never happen. But the resolution can be enhanced by minimizing the width of the overlapped area of these two, it can be done by orienting the central line perpendicular to the DNA molecule. At the STED power of 26 mW, the resolution of ~50 nm was obtained, which is comparable to the persistence length of a single DNA molecule, much better than normal confocal imaging (six-fold enhancement).

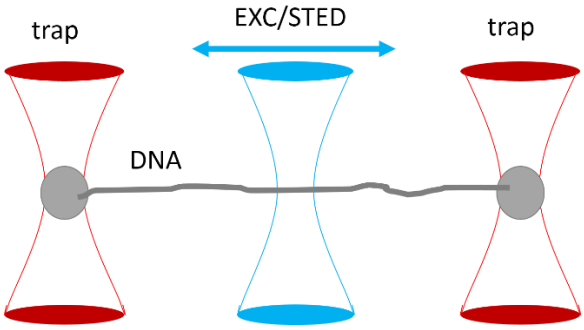

Figure 5. Experimental layout of a STED nanoscopy in protein-DNA study. A dual-trap regime is adopted. A DNA molecule is tethered between the beads trapped in the two optical traps (red). An excitation (EXC) beam (and superimposed STED beam) can scan over the DNA and is shown in blue [36].

An example experiment showing the advantage of this technique is the study of DNA-binding dynamics of the human mitochondrial transcription factor TFAM labelled with ATTO 647N. A real-time observation of how individual TFAM-ATTO 647N molecule bind to DNA in a low concentration (up to ~100 nM TFAM-ATTO 647N) solution is achievable with normal confocal fluorescence microscopy. After binding the TFAM-ATTO 647N will diffuse on the stretched DNA. However, when the protein density is high, for resolving individual protein or its trajectories, normal confocal fluorescence microscopy shows its limitation. Fortunately STED makes it possible. The super-high resolution of STED allows individual protein to be tracked, even at high protein density. It can also provide statistics for large amount of particles and allows studies on a protein-crowded DNA. The result in this experiment show that the TFAM molecules diffuse rapidly and will oligomerize after collision with other TFAM molecules. The result of such oligomerization is that a crowded DNA exhibits a lower diffusion constant D than a sparsely covered DNA [36].

These examples demonstrate how optical tweezers is used in associate with other methods to study the protein-DNA interactions. For this purpose the optical tweezers system is indeed used as “tweezers” which can catch and hold the DNA molecule, making it easier for other techniques, rather than a tool for precise displacement and force detection as in previous examples.

CONCLUSIONS AND PERSPECTIVES

It has been four decades since Ashkin and co-workers first invented the single beam, gradient force optical trap technique. Researches have witnessed tremendous improvement and new applications of this useful technique. People have been searching for a comprehensive understanding of conformational changes of proteins and DNAs in smallest spatial and fastest temporal scales and have been pushing the resolution of optical tweezers to a new height. Now it is possible to directly observe many of these movements in a single molecule level. By observing DNAs and proteins in angstrom level people have been able to understand various processes that happens inside a cell, such as enzyme catalysis, cell signalling, and ion channel gating. However, there is never an end in improving the resolutions. Remaining low experimental noise for a long time, improving temporal resolution to several orders of magnitude higher, or achieving high spatial and temporal resolution at the same time are all potential directions of study which will all help for a better understanding of biological processes and are all not yet well studied.

Another trend in the advancement of optical tweezers is to combine it with various novel techniques to provide additional readouts, such as force and displacement along complementary axes, torque, and angle, or fluorescence position and orientation, in order to analyse and understand more complex systems. Efforts in combining more advanced physical or chemical methodologies with optical tweezers and find their application in real biological, or even physical or chemical studies need to be done.

Due to the simplicity of the optical tweezers apparatus, many popular topics, much more than what we have discussed, can be used along with an optical tweezers experiment, forming new combination of probing methods and bring better comprehension of biological processes. For example femtosecond laser pulses is a popular tool for spectroscopy for its high peak intensity and nonlinear behaviour. It has been verified that for large beads (comparable or even larger than trapping wavelength) it is the average power rather than the peak power determines the quality of optical trapping [37], Therefore it is possible to use low power femtosecond laser pulses to trap and use its time delay replica for interferometry probing, by adjusting the light path the time delay can be controlled in nanoseconds or even higher resolution. If applicable, this would bring information of the trapped particle at the very first moment after the change of trapping force and improve the temporal resolution to a new level. A recent research shows that carbon nanotubes can bind to some DNAs, depending on the sequence and chirality [38]. An experiment similar to the one unzipping DNA with optical tweezers may help understands which exact basepairs are responsible for this binding. Carbon nanotubes can also be introduced into optical tweezers studies as a DNA selection tool, or other potential applications.

Improvements of the example experiments may also lead to new discoveries. For example applying torque on the beads. The methods now include one of using polarized light to rotate birefringent particles. The electronic component of the light was used. However the magnetic component, or the interaction of electronic and magnetic fields may also be capable for this purpose. With the quad-trap setup, it is possible to study the non-covalent interactions between two DNA molecules, or protein and DNAs, to understand the mechanism of their folding and identifying each other. In another example, gold-thiol chemistry was used to improve high force performance, however the beads failed. This might be improved by adopting the gold-thiol chemistry also in the bead side. Gold can also be replaced with silver, which also bounds with thiols, to study the influence brought by the metal. The thiol molecules are also replaceable.

Other properties of DNA molecules, or other molecules, are new directions to devote into. For example, electronic properties. Conductive beads can be transferred into electrodes so that voltage can be applied on a DNA molecule. The conductivity change of the DNA molecule during stretching can thus be measured. If gold-thiol chemistry is again involved, by choosing π conjugated thiol molecules whose electronic status can be changed with external light stimulation, for example change between different resonant structures which have different electronic behaviour, an optical control over the DNA current can be achieved, and the influence on electronic properties from the thiol molecules to the DNA molecules can also be studied.

Today optical tweezers is a mature field, but important technical advances will continue to be made, and more applications will be seen. By combining more advanced hybrid techniques with the highest spatial and temporal resolution a brand new world of biology will be exposed to us.

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1-1 Sep 28, 2014, 11:47, Erik Van der Giessen

- essay delivers what it promised

- nice overview of huge field

- author has struggled with enormous amount of data; however, instead of restricting attention, it seems that all information needed to be included, this giving a collage of fragments rather than a story.

- referencing figures inadequate

If perspective own ideas: 7
If not: 6.5

2-1 Sep 28, 2014, 11:47, Erik Van der Giessen

If you can fit so many subsections on a single page, then they are probably too small.