Laser manipulation of bio-/biomimetic materials


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We demonstrate a few examples of laser trapping and manipulation technique being applied for the measurements of small forces in the range of fN-pN and for laser microfabrication. We discuss prospective principles applicable for future nano/micro-mechanical tools. Elastic properties of microtubules of 24 nm in diameter were determined, a gold particle of 0.25 μm in diameter was laser trapped and manipulated. Paramagnetic microspheres were DNA-anchored to a glass substrate for measurement of elastic properties of DNA by evanescent light scattering. Also, a gold particle was attached to DNA and laser manipulated. The motion of the DNA molecule was controlled. The spring constant of the DNA was determined by using thermodynamical analysis of evanescent light scattering.

Keywords: laser trapping, laser manipulation, ponderomotive action of light, light pressure, elastic properties of DNA molecules, evanescent light field, microtubule

1. Introduction

The impulse carried per photon is \( p = \hbar k \), here \( \hbar \) is the Plank constant and \( k \) is the wavevector of light. Whenever the light is absorbed and refracted (this also includes reflected and scattered) or, in the most general description, when any change occurs in direction of the light propagation, the corresponding change in the impulse will cause a force to be exerted by light on the object, where those changes took place. This is the modus operandi of laser trapping and manipulation of sub-micrometer-sized objects by laser tweezers. Ponderomotive (mechanical) force of light, \( F \), exerted upon a single particle can be calculated from Maxwell-Bertoli law, which defines light pressure as \( F = I C \), where \( I \) is the light intensity, \( c \) - light speed, and \( C \) is the scattering cross section of the particle.

2. Experimental

Here the description of the common features of laser trapping setup is given. A detailed information on samples is provided in separate sections of Part 3.

Laser trapping and manipulation setup1 was extended to allow handling of two particles. Also, a complementary pulsed laser irradiation was introduced to have a possibility of microfabrication over a trapped particle as shown in Fig. 1. Laser trapping force, \( F \), was calculated from the release velocity, \( v \), of a particle dragged in solution using Stokes law: \( F = 6 \pi r v \), where \( r \) is the radius of microsphere. This method allow to measure forces down to several pN. For smaller forces, the measurements of evanescent light scattering by a microparticle were carried out with photomultiplier (sensing of axial movement) or by segmented detector2,3 (sensing of lateral and axial movement). This technique allows to measure lateral movement of a laser trapped microparticle as small as 10 nm (an axial precision is typically twice lower due to a larger axial extent of the waist of the trapping beam). This corresponds to the force of tens-of-fN or, in terms of energy, the potential changes as small as few kT can be easily sensed at room temperature (\( k \) denotes the Boltzman’s constant and \( T \) is the absolute temperature).

Fig. 1 Setup of laser manipulation with additional possibility of laser assembling. PBS is the polarized beam splitter, DM - dichroic mirror, OL - objective lens, GM - galvanic mirrors, and \( \lambda/2 \) is the \( \lambda/2 \)-plate.
3. Results and Discussions

Laser trapping typically exerts gradient forces of pN-nN on a sub-micrometer-sized dielectric particle at typical laser trapping power of 50-200 mW.

3.1 Assembling by photo-polymerization

Assembling micrometer-sized objects is an attractive possibility provided by the microfabrication. Two polystyrene beads were trapped by two separate laser traps using laser power of 100-150 mW per beam, as shown in Fig. 2(a). The beads were drawn close to each other (b), and welded together at the point of contact by a single laser pulse with 355 nm wavelength and 20 ps duration. The energy density was 2 J/cm². Afterwards, the newly fabricated two-bead structure was re-trapped several times to make three- and four-bead structures (c-e). This type of micro-welding by a single pulse ablation is technically similar to a multibeam laser manipulation and fixation technique where the assembling of several laser trapped latex particles was achieved via polymerization.

Fig. 2 Optical images of laser manipulated polystyrene beads in water. The welding of beads was made by a single 20 ps duration and 355 nm wavelength laser pulse pointed to a contact spot between beads.

We have also developed a modification of this technique, which uses laser-manipulation to make extended, flexible gel structures. In water solution of acrylic acids, photo-polymerization results in a gelified material. Very low friction forces existing between gels as well as between gels and solid surfaces, make them interesting candidates for the applications in artificial joint implants, and other bio-mechanical applications. In the experiments silica beads were laser trapped and manipulated in aqueous solution of the acrylic acid (AA, 2 mol/dm³) monomers, photoinitiator, and conjugator/crosslinker. For the initiation of polymerization by two-photon absorption, the 2,2-diethoxy-1-phenylethanone (DEPE, 0.1 mol/dm³, standard illumination wavelength λ < 270 nm) was used as photoinitiator. We have also found that single-photon photoinitiator, 4,4'-dihydroxybenzophenone (DHBP, 0.8 mol/dm³), can be used as well at the same wavelength of 532 nm. The conjugator in both cases was N,N'-methylene-bis-acrylamide (MBA) at the concentration of 0.03 mol/dm³ for two-photon, and 0.1 mol/dm³ for single-photon initiation reaction.

The trapped beads were synchronously moved along the axis aligned through their centers, and illuminated by laser pulses at 532 nm (Fig. 3). As a result, an AA-gel formed between the silica beads. The surface of the silica beads remained chemically unmodified, and the gel-silica contact was maintained solely by physical forces. However, the contact was strong enough to withstand laser manipulation forces up to 200 pN (the strength of the joint was not known to us precisely). There was no obvious difference in the appearance and flexibility of the structures polymerized using single-photon or two-photon initiation.

Further experiments were carried in an effort to produce even more complex silica-AA-gel structures. First, we trapped two silica beads, approached them by manipulation to make a contact, and then the contact region between them was gelified by third beam. A flexible link, such as shown in Fig. 3, was created between the two separately held bead structures (Fig. 4). These preliminary results...
show the applicability of this technique for the fabrication of complex and flexible bio-compatible structures. Modification of the glass bead surface could help to fabricate even more mechanically robust constructions.

3.2 Elastic properties of microtubules

Microtubules, which are flexible biopolymers, can be used for nanotechnology applications (e.g., nano-actuator) as they have a rigidity similar to that of plexiglass and other plastic materials. The flexural rigidity, or bending stiffness, of microtubules can be measured using a laser trapping technique and dark-field microscopy. One end of a microtubule rod was chemically bound to a glass microsphere, while the other end was bound to a silica glass substrate. Then, the microsphere was laser-trapped and manipulated to exert three different deformation modes on the microtubule. The flexural rigidity is defined as a product of the Young's modulus E and the geometrical moment of inertia of the cross-section. Flexural rigidity was calculated by measuring the deformation of a microtubule rod subjected to an external force or by measuring the critical force of a certain deformation.

The values of flexural rigidity for different deformations were between $10^{-25}$ and $10^{-23}$ Nm² as measured for the 5-25 μm length microtubules. We found that a $E I \propto l^2$ dependence exists as shown in Fig. 5(c), which might be explained by segmental structure of microtubule. Scattering of $E I$ values (Fig. 5(c)) can be explained by the following facts: unknown geometry, area, and mechanical properties of the both contact points - at the microsphere and at the substrate.

During the procedure of microsphere binding to a microtubule immobilized at one end, we used stage movement to squeeze the free end of microtubule between substrate and microsphere. The exact area of contact was out of control. However, those substrate-microtubule-microsphere structures, which were once formed, showed sustainable elastic properties and the same values of flexural rigidity were measured repeatedly. This allowed us to conclude, that the contact regions of microtubules are not mechanically degrading during the measurement and the scattered data are explained primarily by the geometry (area) of the contacts at both ends of microtubules. Other source of uncertainty could be caused by the size difference of the microspheres, what in turn determine the laser trapping force. The 7% deviation of diameter of silica microspheres causes corresponding uncertainty in the radiation force assessment.

Any inhomogeneity in morphology of microtubules (>100 nm), if exists, may bring about the differences in $E I$ values, too. It was demonstrated recently that the microtubules have a specialized, randomly distributed points along their length, which are involved in an anchoring microtubules to the substrate. Indeed, it is expected that the microtubule, which has its own well organized and composite structure on the scale of nanometers, would possess a distributed rigidity on a similar scale. Additional existence of the “anchoring points” separated by 10-100 nm can feasibly alter the flexural strength of a microtubule on the relevant scale. At present, the structure and nature of those “anchoring points” is still under debate. The experimentally observed dependence $E I \propto l^2$ can be explained by the presence of such segments along the length of microtubule.

To have an idea, how differs the long and short microtubules in terms if rigidity, we summarized the data available from our experiments in a histogram shown in Fig. 5(d). Gaussian shape of a distribution, despite a small number of measurements, implies that the measure $E I(l)$ for the 3-20 μm microtubules obeyed normal distribution. In other words, the structural morphology of long and short microtubules was similar from the point of view of elastic properties.

Once the elastic properties are established, the movement of the microtubule can be determined and predicted under the known force, F. Let us consider the force acting on the free end of the microtubule in the direction y (vertical in Fig. 5(c)). The shape then is given by:

$$y(x) = \frac{F l^3}{6 E I} \left( -\frac{x^3}{l} + 3 \left( \frac{x}{l} \right)^2 \right),$$  (1)

where $x$ is the coordinate along the microtubule. The shape of microtubule is known only for small deflection angles. Eqn. 1 for $x=l$ and correspondingly $y(l)=d$, was used to calculate flexural rigidity $E I$.

The relaxation of microtubule after its deflection ($d = F l^3/(6 E I)$), when the deflecting force is released (the laser trapping force in our case) can be described by a first-order differential equation, which is solved by a simple exponential decay of the form:

$$y(t) = y(0) e^{-t/\tau},$$

where $\tau$ is the relaxation time.
where $t$ is the time, $y(0)$ is the maximum initial deflection, $\beta = 60EI/(l(2d))$ is the elastic constant with $d$ being the diameter of microtubule, and $\gamma = 11\pi \eta l^2$ is the hydrodynamic friction between the microtubule and environment (buffer solution in our experiments), in which it moves ($\eta$ denotes the dynamic viscosity). Eqs. 1 and 2 define a shape (static) and relaxation (kinetic) properties of microtubule, respectively. These properties can be applied for a development of future micro-/nano-mechanical systems driven by small forces of 0.1-10 pN.

### 3.3 Brownian motion of particle anchored by DNA

Currently, measurements of mechanical properties of DNA molecules are critical and challenging task of science and technology. In this section we describe an experiment of the measurement of spring constant of ds-DNA.

First we describe the procedure of anchoring of microsphere to a glass substrate by a double stranded (ds) DNA of 1000 bp (338 nm in length). Anti-DIG (DIG is for digoxigenin) was immobilized onto slide glass substrate by procedures described in refs. [8,9]. The glass substrate was immersed in concentrated nitric acid at 100°C for 3 h. After rinsing with water, the substrate was dried for 4 h at 500°C in oven to burn organic adsorbates. The cleaned surface of the substrate was etched in a 10 w/w% hydrofluoric acid solution for 20 s, and then, immersed in concentrated nitric acid at 100°C for 3 h. Then silanization of slide glass was carried out in 5 w/w% mercaptopropyltrimethoxysilane ethanol solution by immersion for 3 h. After rinsing with ethanol several times, the substrate was dried by moderate nitrogen flow. The silanized substrate was treated with crosslinker, N-succinimidyl3-male-imide-propionate, solution (2 mM in ethanol) for 2 h, and then, washed in phosphate buffered saline (PBS, from Wako) buffer. Immobilization of protein G was done by immersion of substrate into protein G solution (0.1 mg/ml in PBS buffer) for 3 h, and then, washed in PBS buffer. This accomplished a substrate preparation.

Then, 5′ biotylated DNA (1000 bp) was prepared by polymerase chain reaction (PCR). λ-DNA was used as template in PCR. After PCR, DIG was attached to 3′ end of the by using DIG nucleotide 3′ end-labeling Kit (from Roche Diagnostics). Avidin coated microspheres (diameter 0.5-1 μm) were obtained from Promega (Streptavidin Magnetic Particles). 10 ml of DNA solution (5 μg/ml) was mixed with 30 ml of the microsphere solution (10 mg/ml), and the mixture was stored for 3 h at 4°C. The mixture was dropped onto the anti-DIG coated glass substrate and rinsed with PBS buffer solution thoroughly. Schematic structure of a sample is shown in Fig. 6(a). DNA molecule prepared had the only one binding site to a streptavidin coated microsphere. This preparation procedure insured for a single microsphere to be bound to a substrate by a single DNA molecule.

Measurement of evanescent light scattering was performed in PBS buffer solution. The sample was mounted onto prism and a He-Ne laser emission was directed to the glass/water interface under total internal reflection geometry. Evanescent light scattered by a microsphere was observed in an optical microscope. Fluctuations of the light intensity in time were monitored using photomultiplier tube for the free, immobilized, and DNA-anchored microspheres. Such detection scheme is sensitive to the vertical movement of a DNA-
anchored microsphere. Time sequence of a scattered light intensity should be recorded with sampling rate faster than the mechanical response time of a particle, which is \( \tau = 4 \pi r^2 \rho / (9 \eta) \), where \( r \) and \( \rho \) are the radius and mass density of a particle, respectively, and \( \eta \) is the dynamic viscosity of solution. By taking \( \rho = 1.19 \, \text{g/cm}^3 \) (PMMA), \( \eta = 1.002 \, \text{Pa-s} \) (water), and \( r = 0.5 \, \mu \text{m} \), one can find that sampling rate should be faster than 2.5 MHz, since \( \tau \) is 0.4 \( \mu \)s. In our experiments the microspheres were heavier, made of an iron derivative, and 2 MHz sampling rate would be sufficient. However, the present measurement setup allowed us to measure only the time averaged height fluctuations of a microsphere, since the sampling was carried out at 30 Hz. The actual penetration depth of evanescent field at our experimental conditions was 450 nm as measured in an independent experiment with quadrant photodiode and laser manipulation technique\(^2\). This well corresponds to an incident angle \( \alpha = 62.1^\circ \) used in our experiments. Penetration depth, \( z_p \), of the evanescent mode can be calculated by \[ z_p = \frac{n_1}{4 \pi r n_2^2 \sin(\alpha) - n_2^2} \]. where \( n_1 = 1.51 \) and \( n_2 = 1.33 \) are the indices of glass and water, respectively.

Histogram of the scattered light intensity, which is related to the vertical Brownian movement of a microsphere anchored by DNA, can be obtained from time sequence of evanescent light scattering intensity. An envelope of the histogram is, in fact, the probability density function, \( p(z) \), for the axial position of a microsphere. For a fixed particle, this distribution depicts the instrumental function of a setup. Since the DNA-anchored microsphere is randomly moved with thermal energy, the position distribution \( p(z) \) can be related to the potential-energy profile \( V(z) \) exerted on the microsphere via Boltzmann distribution. Then, an axial potential \( V(z) \) is given by

\[ V(z) = -kT \ln(p(z)). \]  

(5)

The axial displacement of a particle was calibrated according to the known penetration depth of evanescent field. For a free microsphere, an axial displacement by \( z_p \) corresponds to a change in potential by \( kT \) (the intensity of scattered light drops by factor of exp(-1)).

Restoring force of DNA spring can be calculated from the axial potential profile of DNA as \( F = -\text{grad}V(z) \). This is plotted in Fig. 8. The slope of the linear fit to experimental data yields in a spring constant \( k = 1.4 \times 10^3 \, \text{N/m} \) according to Hook's law \( F = -kA \). This value can serve as an approximate one, since the depth of penetration of evanescent mode was measured in the different experiment, also, the sampling rate in recording of light scattering was slower than the position change of a microsphere. The value of spring constant is more than by one order of magnitude larger than that determined for much longer DNA molecules\(^{10} \). The other assumption inherently present in the determination of a DNA spring constant discussed above is that the distance substrate-microsphere is only changing as a consequence of longitudinal movement of DNA molecule. However, this distance is also fluctuating in a Brownian field due to tilting/bending of DNA molecule. If combined, the 3D position

\[ \text{Fig. 9 Micro-optical image of 3D laser trapping of Au-particle in a single laser trap. The brightest particle was laser manipulated and maximally introduced into evanescent field, the scattering of which is the source of the contrast in this image. Scale bar, 10 \mu \text{m}.} \]

\[ \text{Fig. 10 Intensity of scattered evanescent light detected by quadrant detector vs. lateral (a) and axial (b) displacement of a laser trapped Au particle. Squares — experimental data, lines — theoretical dependences. Diameter of particle was 0.25 \mu \text{m.} } \]

3.4 Manipulation of Au-particle

Laser trapping and manipulation of metallic particles is usually impossible since the light pressure, the so-called scattering force, is larger than the gradient force due to high reflectivity of metal. Consequently a metallic particle is repelled out of focus. However, it was demonstrated that Au-colloidal particles (diameter about 40 nm) can be successfully laser trapped\(^2\) and even used as a near-field probe for microscopy\(^1\). Recently, even larger Au-colloidal particle of 250 nm in diameter was successfully laser trapped in a gradient trap and manipulated\(^\text{14} \).
Figure 9 shows 3D laser trapping of Au particle and its insertion into an evanescent field by laser manipulation. Quadrant detector was used to establish spatial coordinates of the laser trapped Au particle according to the procedures reported elsewhere\textsuperscript{2-14}. Figure 10 shows an intensity of scattered evanescent light for lateral and axial displacement of the laser manipulated Au particle. Experimental data follow closely the theoretical curves. This proves that sub-micrometer sized metallic objects can be hold in a single beam laser tweezers and manipulated.

Obviously, the occurrence of a gradient-force in a single beam laser trap needs to be examined in more details to explain the mechanisms causing laser trapping of metallic particles. The key parameter is a skin depth, $\delta$, of light penetration into a metallic surface, which is given by\textsuperscript{15}:

$$\delta = \frac{\lambda}{\sqrt{c \pi \sigma \mu_0}},$$

where $\lambda$ is the wavelength of light, $c$ is the speed of light, $\sigma$ is the conductivity of metal, and $\mu_0$ is the vacuum permeability. Let us evaluate eqn. 6 for our experimental conditions with $\lambda = 1.064 \mu m$ and gold resistivity $\rho = 2.44 \text{ m\Omega-cm}$ ($\sigma = 1/\rho$). One can find $\delta = 148 \text{ nm}$. This concludes that as long as Au colloidal particle is smaller in diameter as 0.3 $\mu m$ it behaves as a dielectric one. This explains the phenomenon of laser trapping and manipulation of Au-colloidal particles.

Next, Au-particle was tethered by DNA molecule to a substrate by a similar chemical procedure described in a previous section. First experimental data have shown that such the Au-particle can be laser manipulated in the same ways as a free Au particle (Fig. 10). We hope to measure elastic properties of DNA molecules for different deformation modes by this setup in the nearest future.

4. Conclusions

We have demonstrated several applications of laser trapping technique: (i) assembling of polymer beads into complex 3D structures by single shot welding (ablation), (ii) formation of flexible 3D structures where silica beads were interconnected by AA-gel photo-polymerized via one- and two-photon absorption, (iii) determination of flexural rigidity on 24-nm-diameter microtubules, and (iv) laser trapping and manipulation of Au-colloidal particle of 250 nm in diameter by single beam laser tweezers. The mechanism of laser trapping of metallic particles is explained by the skin depth of light penetration. The measurement of elastic properties of ds-DNA by evanescent light scattering is demonstrated. The spring constant of a ds-DNA of 1000 bp was evaluated to be $k = 1.4 \cdot 10^{-3}$ N/m.

References