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OPTICAL MANIPULATORS

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Optical manipulators of microparticles using femtosecond laser radiation

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***Abstract*. Two setups for manipulating (translocation, stretching, rotation) microscopic objects (with the dimensions from several nanometers to tens of micrometers) are developed based on optical trapping by femtosecond laser radiation. The possibility of single-cell translocation is shown. The possibility of destructing malignant cells as well as of cutting off a fragment from a malignant cell cluster due to the rapture of bonds is demonstrated in multiphoton absorption of femtosecond light pulses. The possibility of the holographic control to move several particles simultaneously is shown.**

Keywords: *femtosecond holography, optical trap, optical manipulator*.

1. Introduction

At the end of the twentieth century a significant progress in the investigation methods lead to the possibility of studying processes proceeding during very short times in microscopic volumes. This is caused by the appearance of such an instrument as femtosecond laser pulses, which allowed one to study the processes at femtosecond time resolution, and of different microscopic techniques, primarily optical microscopy, making it possible to perform investigations at nanometer spatial resolution. As a result, this aroused great scientific interest in biological research (especially, in cytology and molecular biology), which resulted in great demand for optical manipulation of microscopic objects. The original paper of Ashkin [1] armed the researchers with 'an optical arm' for trapping separate micrometer particles or a set of nanometer particles. New measuring techniques made this 'arm' sensitive to different properties of the sample. Optical manipulation of microscopic objects in combination with the action of femtosecond pulses on them is a promising investigation method enjoying a wide scope of applications in physics, chemistry and biology.

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The main idea of the method consists in the fact that optical trapping of objects whose dimensions lie in the range from several nanometers to several micrometers can be ensured by focused laser beams. If the laser beam focus is displaced, then along with the laser beam, the trapped object or its fragment is also displaced, which makes it possible to manipulate the trapped objects. In this case, the main prospects arise when this method is applied to nanoparticles and biological objects. Today this method finds many applications. It is used to move gold nanoparticles with the dimensions larger than 18 nm [2]; to sort out cells, bacteria, etc. by different criteria [3]; to measure adhesion forces between two cells [4]; to create complex three-dimensional biological structures [5]; to develop addressed delivery and target action on biological targets; to manipulate separate organelles inside a living cell [6], etc. At present, the problem on application of the optical manipulator and scalpel in oncology and microsurgery is being actively studied.

Description of physical processes during trapping depends on the ratio of the particle size to the wavelength of radiation involved in trapping. There exist two limiting cases: the particle size is much smaller and much larger than the wavelength.

It is assumed in the first case that under the action of laser radiation the particle in the objective focus becomes uniformly polarised and then is treated as a point dipole. The particle of this type experiences forces caused by light absorption and scattering ($\boldsymbol{F}_{\rm abs}$ and $\boldsymbol{F}_{\rm sc}$) as well as by the radiation intensity gradient ($\boldsymbol{F}_{\rm grad}$). In this case,

$$\boldsymbol{F}_{\rm grad} = \frac{|\alpha|}{2}\,\overline{\nabla |E|^2},$$

where α is the polarisability; E is the electromagnetic field strength. The force $\boldsymbol{F}_{\rm grad}$ is directed along the intensity gradient towards the region of its maximum (if the particle polarisability is higher than that of the surrounding environment). This force can exceed other forces ($\boldsymbol{F}_{\rm abs}$ and $\boldsymbol{F}_{\rm sc}$), which leads to the 'trapping' effect: the particle is trapped in the potential well formed due to its gradient force.

The behaviour of the transparent particles, whose dimensions are much larger than the incident radiation wavelength, can be described in terms of the classical theory of refraction and reflection. The result of this consideration coincides with the above described: the particle is captured by the potential well and becomes 'trapped'.

Initially, an optical manipulator represented a device making it possible to focus laser radiation on a microscopic

object. The positions of the focus and microscopic object were superimposed mechanically. The particles were trapped and relocated either by moving the sample on the stage or the elements of the optical system. The stage in this case was moved 'manually' or with the help of motorised translation stages (in the latter case, the movement accuracy of the order of ten nanometers was achieved).

A holographic optical manipulator is a refinement of the optical manipulator. Mainly, its operation consists in using a spatial optical modulator, which is a mirror liquid-crystal matrix. The principle of its operation is based on the fact that each element of this matrix can be programmed for a specific phase delay of laser radiation. After the laser pulse is reflected from this modulator, the initially plane laser radiation front is converted. Then, this converted beam propagates through the objective lens and is focused in a three-dimensional 'pattern' programmed in advance. Thus, we can obtain not one trap but a set of traps controlled independently and three-dimensionally, by programming the modulator for beam focusing to the required number of points. The number of traps is limited by the modulator parameters (the ultimate power of incident radiation, the dimensions of the liquid-crystal mirror).

By using this technique, one can manipulate gold nanoparticles (more than 10 – 20 nm in size), carbon nanotubes, transparent microscopic particles (0.5 – 10 μm), cells, cell clusters, bacteria, etc.

This paper is devoted to the development of the optical manipulator and scalpel employing femtosecond pulses (a similar setup is being also developed at the Joint Institute for High Temperatures, RAS [7]). One of the specific features of femtosecond pulses consists in the fact that due to their small duration, it is possible to obtain a high peak power at a low energy. This gives a number of advantages when using femtosecond lasers in optical manipulators and scalpels. First, at a small pulse energy, heating of the object under study decreases. Second, multiphoton absorption making it possible to visualise the movement of the trapped object by the fluorescence is easily realised. Third, an optical trap can be successfully used as a nanoscalpel, which reveals new possibilities for nanosurgery. In this case, such a scalpel can operate not due to the sample heating but due to the rapture of bonds in the multiphoton absorption of the femtosecond light pulse. The authors of paper [8] demonstrated the destruction of one mitochondrion inside the Hela cell by using focused radiation from a femtosecond laser.

2. Behaviour of a particle in a potential well

In considering the translocation of a Brownian particle by pulsed femtosecond radiation, it was obtained that when the condition $D\tau < l_w^2$ is fulfilled (where τ is the interpulse time; l_w is the width of the potential well caused by the interaction of a particle with an electromagnetic field; and D is the diffusion coefficient of the particle), optical trapping by pulsed laser radiation is possible because the particle has no time to move substantially from the trapping point during the interpulse time. Using the Einstein relation $D = k_B T\mu$ between the diffusion coefficient and mobility μ as well as $\mu = 1/(6\pi\eta a)$ calculated by the Stokes formula (where η is the solvent viscosity and a is the particle radiu), we can find the diffusion coefficient for a spherical particle: $D = k_B T/(6\pi\eta a)$. For particles with radii $a \approx 10^{-7}$ cm in a nonviscous solvent (for example, water), this expression yields $D \sim 10^{-6}$ cm^2 s^{-1} at room temperature. The condition $D\tau < l_w^2$ is fulfilled for a laser with a pulse repetition rate ~ 1 MHz. The majority of femtosecond lasers operate at a frequency of several tens of megahertz. The following conclusion was drawn: when the output power of a cw laser is equal to the output power of a pulsed laser, the behaviour of the Brownian particle in the optical trap for these two lasers is the same. The ultimate velocity V of the particle relocation with the help of an optical trap is estimated under the assumption that the friction force acting on the particle and caused by the trap movement should not exceed the maximal gradient force attracting the particle to the trap centre:

$$V = \mu U/l_w = [U/(al_w)]/(6\pi\eta),$$

where U is the potential well depth. The ultimate velocity is proportional to the laser radiation intensity and inversely proportional to the particle radius and the trap width. This estimate is valid if $l_w \gg a$. A more detailed mathematics is presented in our previous paper [9].

3. Experimental setups

Within the framework of this paper we developed an experimental setup (its scheme is shown in Fig. 1) for optical trapping. The basis of the setup is an inverted optical microscope (Olympus IX71) into which laser radiation was coupled. The microscope focuses radiation with the help of the objective with a high numerical aperture (in the experiments we used the objectives Olympus Apo 100$^\times$/1.4, Olympus LCAch N 40$^\times$/0.55). A Mai Tai femtosecond laser (690 – 1000 nm, 3 W,

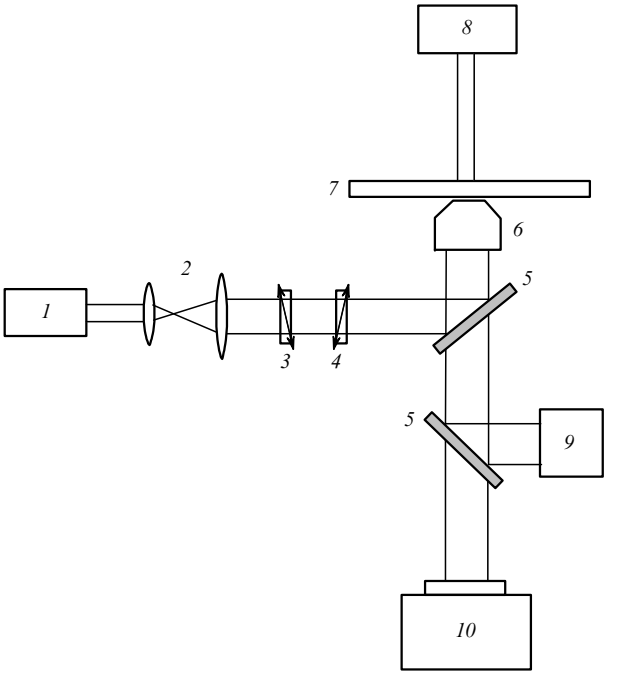

Figure 1. Scheme of the setup: (*1*) laser; (*2*) beam expanding lenses; (*3*) and (*4*) polariser and analyser; (*5*) dichroic mirrors; (*6*) objective; (*7*) object stage; (*8*) illuminator; (*9*) spectrometer/photon counter; (*10*) camera.

80 MHz) is used in the setup. Focused radiation falls onto the object plane where the object under study is located.

A CCD camera (Sony ExwaveHAD) was used for visual control. The sample on the stage was moved manually or with the help of motorised translation stages (in the latter case, the movement accuracy of the order of ten nanometers was provided).

The setup also included a spectrometer, which allows one to manipulate the objective and measure the fluorescence spectrum simultaneously. To record the nanoparticle fluorescence, use can be made of the photon count system, which makes it possible to detect the optical trapping of the particles that cannot be seen in the optical microscope.

The holographic manipulator was fabricated on the basis of the microscope (Fig. 2). A semitransparent mirror reflecting laser radiation and transmitting visible light was mounted between the objective and the eye-piece. The planes of the liquid-crystal spatial light modulator (SLM) and the entrance pupil of the objective conjugate. This is achieved with the help of lenses (*1*) and (*2*) between which the given radiation field in the sample is formed. For this purpose, a computer-controlled SLM is used.

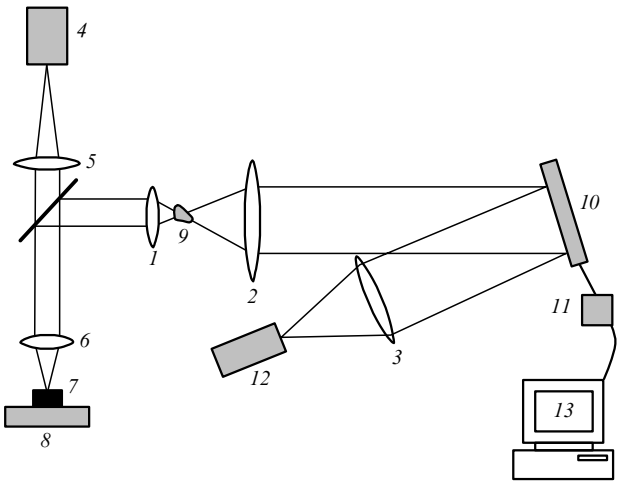

Figure 2. General scheme of the holographic setup: (*1, 2*) and (*3*) biconvex (collecting) lenses; (*4*) videocamera; (*5*) eye-piece; (*6*) objective; (*7*) sample; (*8*) object stage; (*9*) image; (*10*) liquid-crystal transparency; (*11*) interface module; (*12*) laser radiation source; (*13*) computer.

The spatial light modulator (HOLOEYE Photonics) used in the paper represented a 15.36×8.64-mm liquid-crystal reflecting screen with a resolution of 1952×1088 pixels. Voltage to separate cells of the liquid-crystal matrix is applied through an interface module providing 256 discrete voltages. The phase shift in the SLM cell in the range from 0 to 2π corresponds to each voltage. The upper boundary of the voltage range providing the phase delay 2π depends on the radiation wavelength and can be adjusted with instrumental methods. The hologram image with 256 grey levels is supplied to the interface module input from the computer display. Lens (*3*) provides the plane radiation front and complete irradiation of the entire transparent area.

Lens (*2*) forms the first real image near the focus of lens (*1*). A reduced image is formed near the focus of the microscope objective. The object is observed using the scheme of a traditional microscope with the help of a digital videocamera.

A computer program was developed for constructing the hologram of the specific volume object. The particle manipulation was controlled by using the special control window on the monitor screen. The possibility of trapping real particles and their translocation to the required place as well as implementation of other actions (orientation and change in the shape of macromolecules, cell deformation, photochemical reactions, etc.) in real time was provided. The particle translocation was provided either with the help of a pointer or by specifying the motion trajectories for the selected particles. Three-dimensional trajectories are given with the help of the mouse pointer or analytically.

4. Results of the experiments

We performed experiments on manipulating separate cells and cell agglomerates. In the first case, we used a blood cell (echinocyte) as an object under study, while in the second experiments we used malignant cells of the epithelial tissue.

In the first experiment we performed translocation of a single cell. The laser radiation wavelength was 800 nm and the output power was 25 mW. Figure 3 presents consecutive video frames obtained in the experiment.

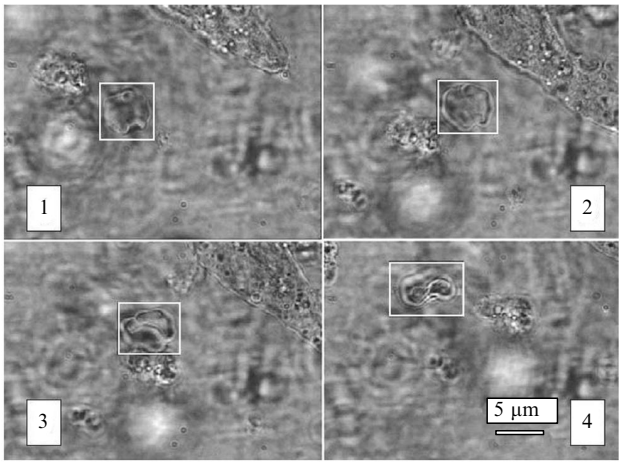

Figure 3. Experiment on translocation of a single cell (echinocyte): frame 1 – initial position of the echinocyte; frame 2 – upward translocation of the echinocyte; frame 3 – the echinocyte is moved to the left and its conformation starts changing in this case; frame 4 – the echinocyte is moved to the left and its conformation significantly differs from the initial.

The change in the position of the echinocyte in the trap during the experiment was obviously caused by the non-coincidence of the centre of gravity of the cell and the trap centre. A significant three-dimensionality of the laser radiation intensity gradient along the laser beam axis also adds to this. The centre of gravity of the cell tends to fall onto the intensity maximum; however, the gradient force also affects the peripheral parts of the cell. As a result, there appears a torque and finally, the cell acquires the position of a stable equilibrium (frame 4). After the laser action terminates, the cell acquired the initial plane conformation (as in frame 1). Thus, using optical trapping, we can manipulate a separate cell.

The second experiment demonstrates that when the laser radiation power is increased, the optical tweezers can be used as a scalpel. Figure 4 presents the consecutive video frames showing the action on the agglomerate of epithelial malignant cells. In this experiment the laser radiation wavelength was 800 nm and the laser power was 50 mW.

At present, the methods of surgical treatment of cancer patients are being constantly improved. Modern surgery is distinguished by a more precise approach and reconstructive and plastic component, which obligatory requires the use of hi-tech technologies. The obtained results indicate the principle possibility of the influence of the 'femtosecond optical manipulator' method on the cell structure of a malignant tumour as well as on the prospects for using this method in studying the intracellular processes of a malignant cell.

Use of femtosecond pulses interacting with the biological objects has the advantage consisting in the fact that the high intensity (peak power) of femtosecond light pulses (at their small energy) makes it possible to realise effectively multiphoton processes of light absorption. In these cases the absorbed energy is spent not on heating but on the rupture of chemical bonds and ionisation [10]. This opens up new possibilities for developing micro- and nanosurgery. Note also that the character of the action being exerted significantly depends on the energy, phase parameters, and the pulse repetition rate.

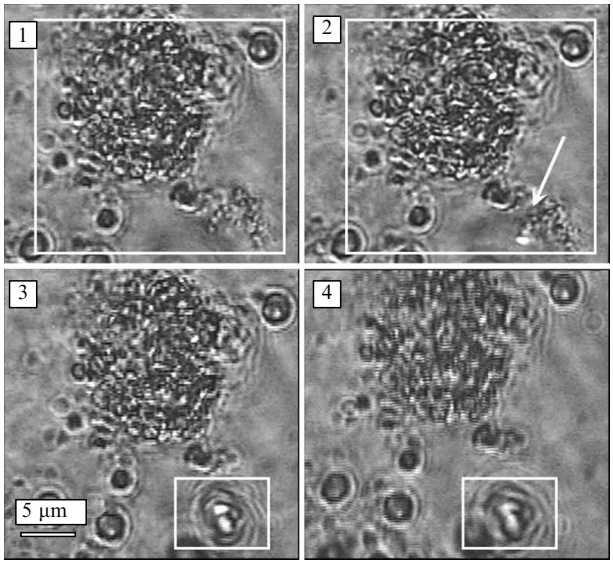

Figure 4. Microsurgical treatment (due to multiphoton processes) of a malignant cell cluster in the epithelial tissue upon focusing femtosecond laser radiation: frame 1 – initial state of the cell cluster; frame 2 – femtosecond laser radiation is focused to the spot shown by the arrow; frame 3 – a small fragment of the cell cluster is separated from the main part and captured by the optical trap, the fragment being slightly deformed (deformation is caused by the high intensity of laser radiation); frame 4 – the fragment of cell cluster is separated and relocated aside from the main part.

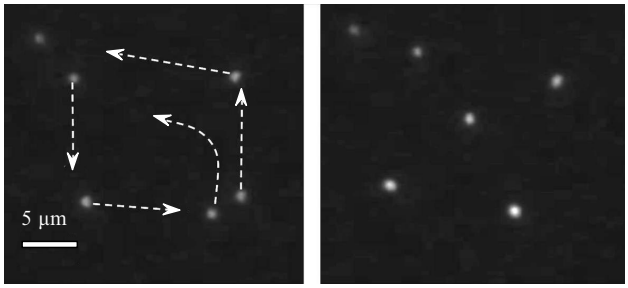

Figure 5. Focusing femtosecond radiation into several optical foci, visualised by two-photon fluorescence microscopy. Two video frames of the experiment (first and last) are shown; dashed curves are the initially set trajectories of the focus point movement.

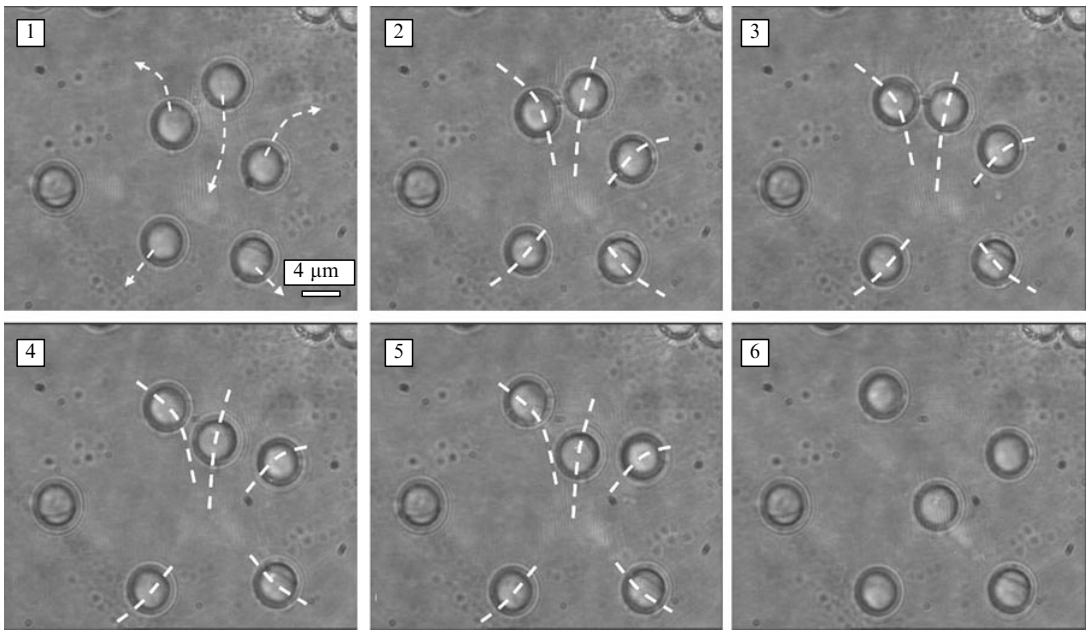

Figure 6. Simultaneous manipulation of polymer balls (4.4 μm in diameter) with the help of the holographic setup: dashed curves are the initially set motion trajectories of the particles.

Figure 5 and 6 demonstrate the possibility of using femtosecond light pulses for simultaneous manipulation of several objects. In our experiments, we used the femtosecond holographic setup. In the first experiment, several optical traps were formed in a plate with a fluorescent paint applied to it (the absorption maximum at the wavelength close to 400 nm). Two-photon fluorescence was excited by 100-fs, 40-mW femtosecond laser pulses at 800 nm.

Using the computer we set the motion trajectories of several traps. They moved simultaneously and independently of each other. The directions of relocations are shown by the arrows in Fig. 5, which presents two video frames of the experiment – the first and the last. A beamsplitter transmitting visible radiation and reflecting IR radiation into the videocamera was used in the optical scheme (exposure of the camera by reflected IR radiation was virtually absent).

In the second experiment (Fig. 6), we used 4.4-μm polymer balls. We managed to manipulate five balls simultaneously, each ball being relocated independently of others. For this purpose, we used 100-fs, 80-mW pulsed radiation at 725 nm. The position of the balls was controlled automatically by the computer. Figure 6 presents six video frames of the experiment. In frame 1, the arrows show the directions of the ball movements.

5. Conclusions

We have developed, under laboratory conditions, two setups for manipulating (translocation, stretching, rotation) microscopic objects (with dimensions from several nanometers to tens of micrometers), in which use is made of optical trapping by femtosecond laser radiation. We have shown the possibility of moving single cells, destructing malignant cells, and cutting off a fragment from a cluster of malignant cells due to the rupture of bonds in multiphoton absorption of femtosecond light pulses with the help of the developed setups. We have demonstrated the possibility of a holographic control to move several particles simultaneous by using femtosecond laser radiation.

Dynamic control of femtosecond laser radiation allows one to manipulate microscopic particles, thereby actively influencing them physically and chemically. The efficiency of multiphoton processes is responsible for the application of femtosecond pulses for treating biological materials, for studying microscopic particles or their aggregations in an optical trap by the method of multiphoton fluorescence microscopy, for visualising the trapped object translocation by two-photon fluorescence microscopy.

The holographic control of radiation significantly expands the scope of its possible applications because it allows one to manipulate many particles simultaneously and independently. In particular, we assume that of interest is the use of many traps as optical scalpels, i.e. treating the malignant tissue (destruction, cutting off of fragments) simultaneously in its different parts. Promising is also application of a laser holographic manipulator in oncology both as a micromanipulator and a highly precise microscalpel.

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