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Optical Coherence Microscopy Combined with Optical Tweezers for Studying Cellular Mechanics

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Abstract—A method combining phase-sensitive optical coherence microscopy (OCM) and optical tweezers for studying mechanical properties of microobjects including biological cells is implemented. The process of microbead trapping was studied using the developed method. The response of the red blood cell (dyscocyte and spherocyte) membrane to the optical tweezer excitation is measured.

Keywords: optical coherence microscopy, optical tweezers, red blood cells, cellular mechanics

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Currently, a topical problem is the study of micromechanical properties of single erythrocytes. Deformation and aggregation properties of erythrocytes control the blood rheology at the microcirculatory vessel level, i.e., strongly affect blood microcirculation and are associated with such pathologies as anemia [1] and poikilocytosis [2]. For this reason, of particular interest is the development of techniques allowing the determination of micromechanical properties of single living cells.

Main methods for studying mechanical properties of single cells are: atomic-force microscopy, micropipette aspiration [3], cytometry under ac magnetic field [4], backscattered light measurements [5], optical tweezers [6], and optical coherent microscopy [7]. The objective of this study is to develop a method which would make it possible to induce mechanical perturbations on the cell surface and to simultaneously detect its response. To this end, a setup combining two methods, optical tweezers and optical coherent microscopy (OCM), for studying microobjects was designed and fabricated. The optical tweezers makes it possible to control objects and to create a mechanical perturbation with submicrometer precision on their surface [6]. OCM allows detection of displacements to within 10 nm and construction of three-dimensional object images [7]. The main advantage of the combination of two methods is the feasibility of mechanical excitations on the cell surface by a tweezers, simultaneously detecting a membrane displacement using phase-sensitive OCM and studying the three-dimensional cell structure.

2. METHOD

2.1. Setup

To induce a mechanical perturbation using an optical tweezers, an infrared laser (the radiation power in the optical trap is 20–40 mW, the wavelength is 1050 nm), a chopper for periodic signals (frequency is 2–10 Hz), and movable lenses for displacing the trap position was used (Fig. 1). Radiation was focused by a water-immersion objective with aperture NA = 1.2.

To detect cellular motion using OCM, a wide-spectrum source was used, fabricated using a photonic crystal fiber (the supercontinuum spectrum width is 320 nm, the total power is 10 mW), reference and signal beams for interference, and galvanomirrors for scanning (the scan field width and height are 50 μm) was used.

The obtained interference spectrum can be processed in two ways; by extracting the Fourier amplitude which provides information about the sample reflectance as a function of depth (amplitude OCM) with resolutions on all axes of 1 μm. Or by extracting the Fourier phase which characterizes the cell membrane relief (phase or phase-sensitive OCM). The axial resolution of the latter phase-sensitive method is 100 times better, it is 10 nm [7].

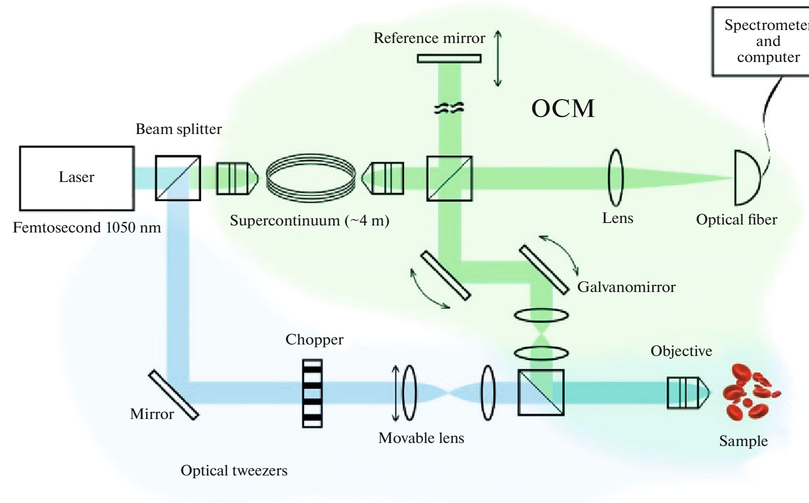

Fig. 1. Schematic diagram of the setup.

The combination of OCM and optical tweezers methods was already used to trap cells and their imaging in a trap [8]; however, the combination of two techniques is first used to determine mechanical properties of biological cells. In the presented method, the optical tweezers induces a mechanical perturbation on the cell surface, simultaneously with which phase-sensitive OCM records its response.

2.2. Preparation of Biological Cells

To perform experiments with discocytes (normal erythrocytes), fresh venous blood (no more 1–2 storage days) was used. Blood was separated into plasma and erythrocytic mass by centrifuging; in what follows, only plasma was used as a natural buffer solution. Immediately before experiments, 5 μl of fresh capillary blood (of one of the authors) was added to 1 ml of plasma; 40 μl of the obtained solution were used as the sample.

To prepare a sample with spherocytes (spherical erythrocytes), a solution with a lowered osmolarity, consisting of 1 part of a sodium-phosphate buffer, 12 parts of distilled water, and 32 parts of physiological solution was used.

3. RESULTS

3.1. Microbead Motion

As a preliminary, the developed method was used to study motion of nonliving objects. We used an optical tweezers for trapping a polystyrene microbead 10 μm in diameter and phase-sensitive OCM for detecting its trajectory in the trap potential.

Figure 2 shows the microbead trajectories measured using phase-sensitive OCM during its trapping by an optical trap at various different radiation powers in the optical trap.

As a result of calibration experiments with microbeads, the stiffness k of the harmonic trap potential was estimated as 0.1–0.5 pN/μm (the stiffness in experiments with living cells was not estimated).

3.2. Study of Living Cell Motion

In the following experiment, the combination of phase-sensitive OCM and optical tweezers was used to study mechanical properties of cells.

We used optical tweezers to induce identical mechanical excitations on the discocyte and spherocyte surface, i.e., periodic displacements of the cell edge due to ponderomotive forces (Fig. 3). It was found that the response amplitudes of discocyte and spherocyte differ significantly.

In other studies, (e.g., [9]) it was already shown that the spherocyte membrane rigidity is several times higher than the discocyte rigidity; however, the spherocyte response amplitude we measured appeared about threefold larger than the discocyte response amplitude. This is probably caused by that the cell

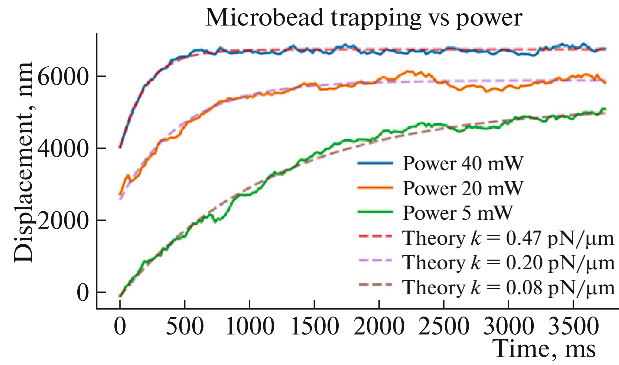

Fig. 2. Microbead trapping trajectories at various radiation powers in an optical trap (experiment and theory). k is the trap potential stiffness.

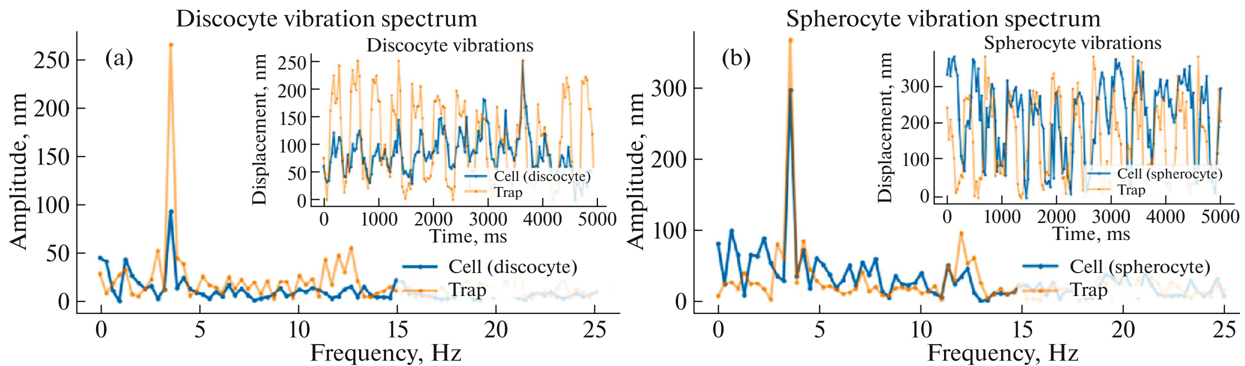

Fig. 3. (a) Discocyte and (b) spherocyte responses to cell edge elevation.

shape and the external force application point have a larger effect on the response in our experimental configuration, rather than the membrane rigidity.

Furthermore, the difference between compositions of media in which discocyte and spherocyte were studied can also have an effect.

3. CONCLUSIONS

The developed method makes it possible to noninvasively induce a mechanical perturbation of a cell and to detect its response by recording a three-dimensional phase signal, which allows obtaining information about mechanical properties of cells.

The method made it possible to detect that the amplitude of the discocyte response to an external influence is three times lower than the spherocyte response amplitude, which can be explained by different shapes of cells. The developed method can also be used in the future to study the motion of not only the cellular membrane, but also internal cell structures such as the core and organelles.

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