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Visualisation of the oscillation dynamics of cytoplasm in a living cell of Physarum mixomycete plasmodium by the method of optical coherence Doppler tomography

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***Abstract*. The method of optical coherence Doppler tomography is used for the first time to visualise the oscillatory amoeboid mobility in strands of Physarum polycephalum mixomycete plasmodium and to record periodic radial contractions of the strands and spatiotemporal variations in the velocity of the cytoplasmic flow inside them.**

Keywords: *optical coherence Doppler tomography, cytoplasm, living cell, plasmodium.*

Optical coherence tomography (OCT) and its Doppler modification (OCDT) are being rapidly developed at present, involving the expansion of functions and improvement of the parameters of experimental setups and the expansion of their applications [1]. The visualisation of the structure of scattering media and directional microflows in them with high spatial and temporal resolution makes it possible to study in detail the structure and functions of the microvascular part of human and animal bloodstream systems, the development of embryos, and the mobility dynamics of large cells strongly scattering the light.

In this paper, we report the first application of OCDT for studying the functions of the contractile system of the Physarum plasmodium, which is a single-cell organism. Paper [2], which is closest in its topic to our paper, is devoted to the visualisation of cytoplasmic flows in amoebas by the method of phase microscopy.

Physarum in the plasmoidal stage of the living cycle represents a time-varying system of almost cylindrical tubes (strands) connecting the frontal zones of organism with its main body [3]. Autowave variations of intracellular pressure gradients produce shuttle flows inside the sol-like part of the cytoplasm (endoplasm) along these strands [4]. The typical oscillation period for strands a few hundreds micrometers in diameter at room temperature is about a minute.

The dynamics of wall contractions in the directional endoplasmic flows in the plasmodium was studied from the point of view of the mechanisms of self-organisation of the amoeboid mobility by many authors by various methods, including laser Doppler microscopy (LDM) [5] and mathematical modelling [4]. The amoeboid mobility is universal and forms the basis of the vital activity of many types of living organisms, beginning from amoebas and leucocytes to fibroblasts and cancerous cells.

Plasmodium was cultivated by the Camp method [6] and was placed on a thin transparent agar – agar layer in a Petri dish for measurements. Measurements were performed by using the OCDT setup built in our laboratory [7] (Fig. 1), in which a radiation source was a 840-nm, 6-mW superluminescent diode emitting light with the bandwidth of 50 nm. The setup is based on a Michelson interferometer. Unlike the conventional OCDT scheme, the reference mirror is immobile. The depth scan is performed by moving a piezoelectric stage on which the interferometer is mounted. The scanning stage movement velocity is 1.6 mm s^{-1}, which corresponds to the Doppler modulation frequency of 3810 Hz.

When a probe beam is directed at an angle to the endoplasmic-flow velocity vector (in our case, at 40°), the system allows one to obtain the distribution of Doppler frequency shifts in radiation backscattered along the probe

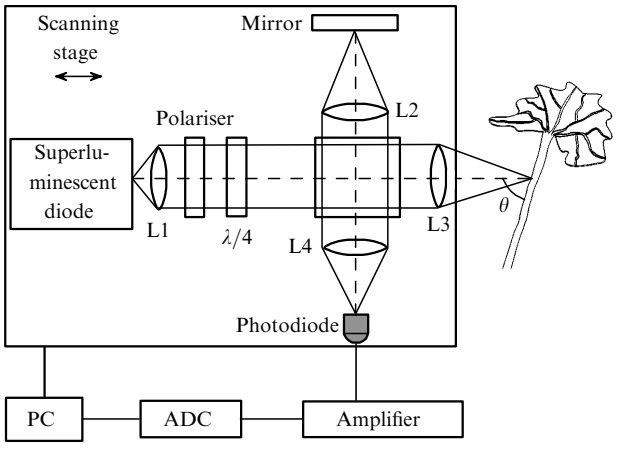

Figure 1. Scheme of the OCDT setup.

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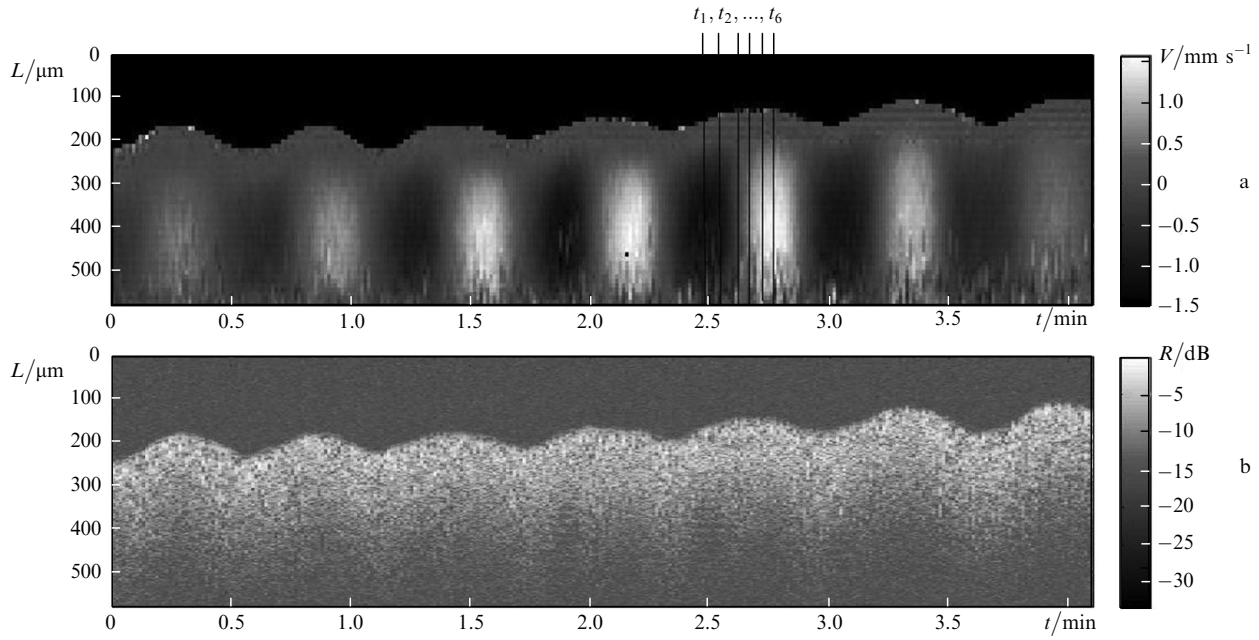

Figure 2. Spatiotemporal velocity distribution of the endoplasmic flow in the Physarum plasmodium strand recorded with the OCDT setup during the time interval of 4.1 min (pulsations of the external boundary of the strand wall are well observed at the shade of grey corresponding to the absence of the flow, which is also characteristic for the strand walls) (a) and time pulsations of the external strand wall obtained during the amplitude detection of the interference signal (b).

beam by optical inhomogeneities (nuclei, mitochondria, etc.) moving in the flow with respect to the carrier Doppler frequency (in our case, 3810 Hz), which is determined by the scan velocity. The spatial resolution of the velocity measurement along the probe beam is determined by the coherence length of the probe radiation (in our case, 6 μm).

Figure 2a shows the velocity distribution of the endoplasmic flow in a plasmodium strand of thickness ~ 220 μm. The optical thickness L along the probe line is plotted on the ordinate, and the time t – on the abscissa. The velocity values at different points of the scan line at different instants correspond to different levels of the shades of grey. Because the detection of the interference signal at the carrier modulation frequency during each scan begins from the external wall of the strand, radial pulsations of this wall at the point being probed are well manifested in the figure. However, the use of the amplitude recording of the interference OCDT signal along with the frequency recording improves the sharpness of pulsation recording (Fig. 2b).

Note that the visualisation of the flow velocity distribution across the strand and in time has a high sharpness. The obtained pattern allows the reconstruction of the temporal dynamics on the axis and at different distances from the wall. Figure 3 shows an example of the time

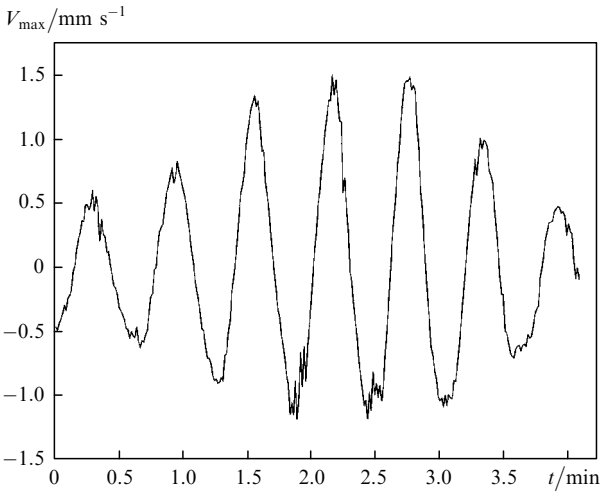

Figure 3. Time dependence of the axial velocity V_{max} with the pronounced low-frequency modulation of velocity oscillations characterising the distributed and self-oscillation nature of the contractile activity of plasmodium.

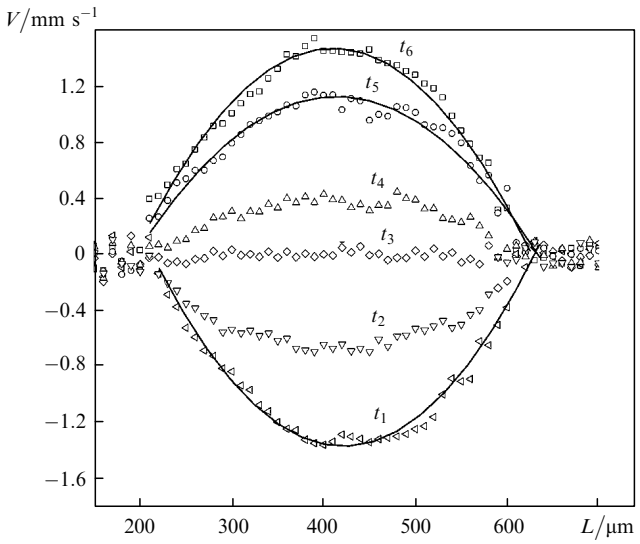

Figure 4. Instant radial distributions (profiles) of the endoplasmic flow velocity in the Physarum plasmodium strand obtained in $t_1 - t_6$ cross sections (see Fig. 2).

dependence of the axial velocity V_{max}. The low-frequency modulation of velocity oscillations, which was earlier observed by the LDM method [4], is distinctly manifested. This modulation demonstrates the distributed and self-oscillation nature of the contractile activity of the plasmodium [3].

Figure 4 presents several instant radial distributions (profiles) of the endoplasmic flow velocity obtained by the reconstruction of the initial image in $t_1 - t_6$ cross sections (Fig. 2a). One can see that these profiles can be well approximated by quadratic parabolas. This means that the non-Newtonian properties of the endoplasm in strands of this size are manifested negligibly.

By comparing the amount of information obtained by the OCDT and LDM methods, we can conclude that the former method offers considerable advantages and is promising for further studies of the amoeboid mobility.

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