OPTICAL DETECTION OF ALIVE MICROORGANISMS IN WATER

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The technique and equipment for measurements of the alive microorganisms mobility in water are described in the paper. The technique is based on the recording of self–beating of scattered radiation spectral intensity. The results of measurements of the average speed of microorganisms motion in the surface samples of the river Volga water are presented.

INTRODUCTION

Both theoretical and experimental optical methods of investigation of the condensed media properties and the processes taking place there are rather fully developed at present. By the value of the characteristic shift (broadening) of the frequency spectrum these methods can be divided into two groups[1].

1) The first group includes the cases with the relative frequency shifts being of 10^{-7}–10^{-5} and more of the incident radiation frequency, which is of the order of 10^{15} Hz for the visible radiation. They are characteristic for the processes connected with the intra–atomic and intramolecular interactions. Such shifts are measurable by classic interferometeric instruments.

2) The second group includes the cases with the relative frequency shifts being of 10^{-8}–10^{-15} of the incident radiation frequency. They are characteristic of the motion of a medium as a whole, the vortex motion, the Brownian motion etc. Such shifts are inaccessible in resolution for interferometeric instruments (even for Fabry and Perot interferometer), but, on the other hand, the characteristic temporal scales of those are already measurable by the radiophysical methods of correlative spectroscopy. In particular, in the spectroscopy based on the intensity self–beats, the amplitude frequency spectrum of scattered radiation is measured either indirectly from the correlation function or directly by a spectrum analyzer. The measurements presented in this paper are concerned just with the latter case.

MEASUREMENT TECHNIQUE

Figure 1 presents the geometry of recording the spectrum of light scattered by medium inhomogeneities. The field E_S scattered by jth element to point R_0 at the distance r from the scattering volume with the characteristic size a ($r \gg a$) is as follows:

$$E_s = \sum_j A_j \exp\left(i\left(\varphi_j - \omega_0 t\right)\right), \qquad (1)$$

where A_j and φ_j are the amplitude and phase of polarized jth component of scattered radiation, respectively, and ω_0 is the circular frequency of the incident radiation. The phase φ_j depends on the position of jth element relative to the origin of the coordinate system[1]

$$\varphi_j = \mathbf{q} \cdot \mathbf{r}_j, \qquad (2)$$

where $\mathbf{q} = \mathbf{k}_0 - \mathbf{k}_s$ is the wave vector of scattered light (we consider here only the single scattering). Since in our case $|\mathbf{k}_0| = |\mathbf{k}_s|$, the wave vector $\mathbf{q}$ takes the form

$$|\mathbf{q}| = |\mathbf{k}_0 - \mathbf{k}_s| \approx 2|\mathbf{k}_0| \sin(\Theta/2) = (4\pi n/\lambda_0)\sin(\Theta/2), \qquad (3)$$

where λ_0 is the wavelength of incident radiation in vacuum and n is the refractive index of the medium.

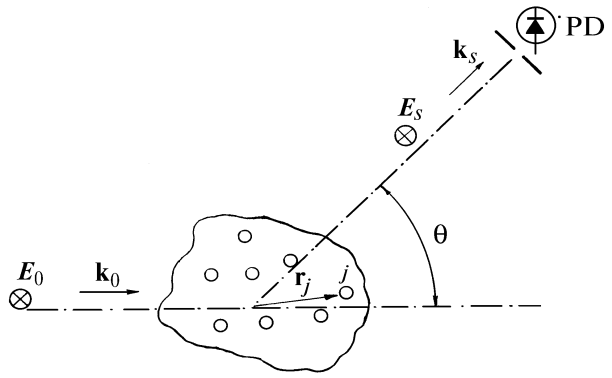

FIG. 1. Geometry of the light scattering experiment. The distance from the detecting photodiode PD *is far longer than the characteristic dimension of a volume sounded.*

The summation over all refractive elements yields the average intensity of the radiation incident onto the photodiode.

$$I_s = \langle |E_s|^2 \rangle = \langle \sum_j \sum_k A_j A_k \exp\left(i\,\mathbf{q}\left(\mathbf{r}_j - \mathbf{r}_k\right)\right) \rangle . \qquad (4)$$

If the centers of mass of scattering elements move, the $(\mathbf{r}_j - \mathbf{r}_k)$ factor varies in time and, accordingly, the intensity I_s fluctuates.

It is just these fluctuations we are interested in. Using their frequency spectrum it is possible to define the spectrum of speed of the scattering particles motion.

As follows from Ref. 1, if there are N equal noninteracting spherical scattering particles, each contributing by I into the scattered intensity at an angle Θ, the spectrum of optical signal is of the Lorentzian shape

$$I(\omega) = N \cdot I\,(\Omega/\pi) \,/\, ((\omega - \omega_0)^2 - \Omega), \qquad (5)$$

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with the maximum at the frequency ω_0. The half–width determined at the half–maximum is connected with the particles translational diffusion coefficient D_t by the following equation:

$$\Omega = D_t \cdot \mathbf{q}^2 . \quad (6)$$

The diffusion coefficient is related to the medium parameters by the Stokes–Einstein equation

$$D_t = k\,T \,/\, 6\pi\,\eta\,r_h , \quad (7)$$

where η is the medium viscosity, r_h is the hydrodynamic radius of the particles, k is the Boltzmann constant, and T is the absolute temperature.

The optical spectrum (5), if recorded by a photodiode, yields the spectrum of photocurrent power, which is experimentally measured. It is also of the Lorentzian shape, but with the maximum at zeroth frequency and half–width being two times greater than that from Eq. 5

$$P(\omega) = N^2\,I^2\,(2\Omega/\pi) \,/\, (\omega^2 - 2\Omega^2) . \quad (8)$$

The spectrum (8) half–width measured in Hz for the spherical particles in water at room temperature (as follows from Eqs. 3, 6, and 7) is

$$f = 2\pi\,\Omega \approx 9.8\,\sin(\Theta/2) \,/\, \lambda_0\,r_h . \quad (9)$$

We used in our experiments a diode laser emitting at $\lambda_0 = 0.86$ μm and scattering angle of 20°. Under these conditions the spectrum half–width for Brownian particles with 4 μm diameter is only 0.2 Hz. However, if there are microorganisms moving at a speed dozens times greater than that of Brownian particles in the medium, the spectrum (8) half–width is about 10–100 Hz. Figure 2 presents the spectra calculated according to Eq. (8) at various Ω. It is seen from Fig. 2, that the greater the spectrum half–width (i.e., the average speed of microorganisms in water) the wider the initial part of spectrum, which is practically horizontal in logarithmic coordinates. The curve bend point (more accurate, the decrease of signal amplitude by 6 dB) corresponds to the half–width of the function (8).

In our experiment just the frequency spectrum (8) of photocurrent power was recorded, and the average absolute speed of microorganisms motion (the migration speed) was obtained from this spectrum half–width. Because the rms displacement of particle is linear in time (Einstein formula)[2]

$$\langle X^2 \rangle = 2\,D_t\,t . \quad (10)$$

The average migration speed (see Eq. (6)) is, in our experiment

$$V = \sqrt{\langle X^2 \rangle} \,/\, t \approx 0.85\,\sqrt{f} , \quad (11)$$

where the half–width f is measured in Hz and the speed V is measured in μm/s. To verify the measurements the cell with the water sample under investigation (with the volume of about 0.1 cm^3) after measuring V was heated in boiling water for 1–2 minute, then cooled, and the spectrum was recorded again. In doing so the spectrum of pure Brownian motion of the mechanical pollutants and dead microorganisms motion in water was recorded.

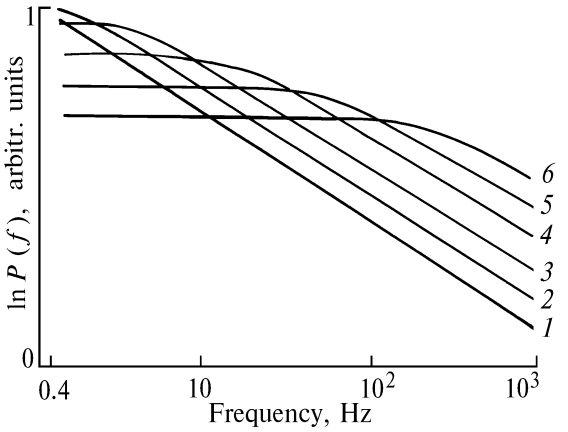

FIG. 2. Calculated spectrum of photocurrent power (8) in logarithmic scale, the curves 1–6 correspond to the half–widths of 0.25, 1.00, 4.00, 16.00, 64.00, and 256.00 Hz, *respectively.*

EXPERIMENTAL RESULTS

The typical picture of experimental spectra is presented in Fig. 3. It is seen from the figure that in spite of the poor precision of measurements (the spectrum was recorded point–by–point using selective voltmeter of V6–9 type with averaging of 10 s at each point) the spectrum half–width proved to be quite a measurable value. The signal–to–noise ratio for real samples of river water was 20–30 dB and the spread of measurements was mainly connected with the statistical nature of the measured value. The distribution half–width in our experiments was too narrow, so the average size of Brownian particles (after boiling the sample, see above) could not be measured.

In our experiments the lowest frequency was of 3 Hz. This is good explanation of the fact that the maximum of Brownian motion spectrum is lower at the experimental curves than that of corresponding to alive microorganisms spectrum.

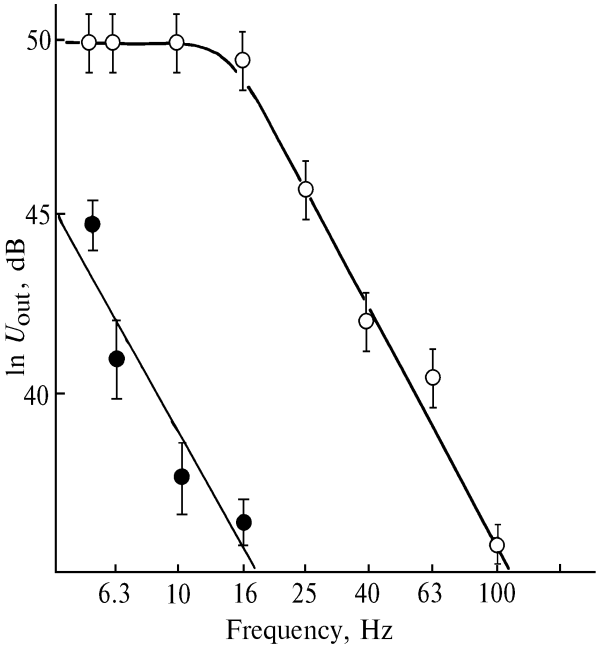

FIG. 3. Typical shape of the experimental curves: sample No. 10 (1) and the same after boiling (2).

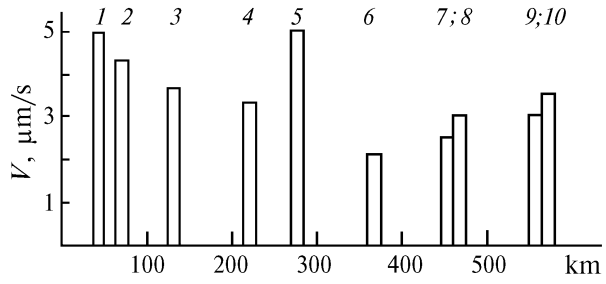

FIG. 4. Measured mobility of microorganisms as a function of the distance from Moscow downstream of the river Volga: 1 – in an hour after departure from the North River Station; 2 – in 2.5 *hours after departure; 3 – Dubna town region; 4 – Kalyasin town region; 5 – Uglich town region; 6 – exit from the Rybinsk lock; 7 and 8 – Yaroslavl town raid, the surface and bottom layers of water; 9 – Kostroma town region; and, 10 – Kostroma river near the flowing into the river Volga.*

Figure 4 presents the measurement results on the average speed of microorganisms motion in the surface samples of the river Volga water obtained in the frame of ecological expedition Moscow–Nizhny Novgorod–Moscow taking place on June 27–July 7, 1993. It is seen that the moving microorganisms were detected in all samples, i.e., the instrument sensitivity is quite sufficient to work with natural water without any preliminary sample preparation.

For a comparison it should be noted that practically no moving particles were observed with testing inspection using the 900X microscope, i.e., there was less than one particle within the field of observation.

At the same time, our measurements are not very informative because they do not contain the information about the type of microorganisms detected (are they phytoplankton, bacterium, or something else). That is why it is not possible to answer even the question: is the high mobility good for any given body of water, or not?

Nevertheless, the method presented could be efficient enough for monitoring, for example, of bio– and phytoplankton in open sea water, where it is known they are the main part of biomass and their vital functions are directly connected with an ecological state of water.

REFERENCES

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