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Computer-aided Assessment of Protozoan Motility Parameters from Video Data

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Abstract—A method of assessing the motility of *Paramecium caudatum* is proposed as a tool for studying magnetobiological effects as well as the impact of other physical and chemical factors. A video record of protozoan movement under the microscope is processed with a special computer program; motility is determined as a function of the mean velocity of movement over a certain interval of time. The main advantages of this technique are its versatility (different parameters of movement can be readily determined) and the possibility of repeated use of video data.

Key words: biotesting, infusoria, *Paramecium caudatum*, cell motility, magnetobiology

Our interest in the motility of *Paramecium caudatum* (slipper animalcule) is largely due to its possible use in revealing magnetobiological phenomena, which are quite topical but insufficiently studied (see, e.g., [1]). Paramecium is a convenient object for biotesting of electric, magnetic, and electromagnetic fields [2–5]. Further, it is quite sensitive to certain chemical agents [6], and is therefore often used as a biosensor of chemical pollution.

In biological toxicity tests, the criterion of protozoan death—registered visually as cessation of movement—is time-consuming, subjective and unreliable. Another criterion is the decrease in cell motility [6]. This index can be determined 'manually,' either by videotaping the movement of paramecia and measuring their displacements frame by frame [7] or by photographing them in a dark-field microscope with stroboscopic lighting [5]. Automated techniques also exist, but they require sophisticated special equipment and software [3, 4].

In the literature, the motion of protozoans is described as a set of standard locomotor reactions. Thus the escape reaction (an abrupt change in the speed and trajectory of movement) has been presented [8] as a sequence of still simpler movements associated with particular biochemical stages. From the trajectory it can be inferred how the intracellular events proceed and which of these processes are involved in the particular locomotor abnormalities caused by unfavorable or stress factors.

To assess various parameters of protozoan locomotor activity, it is convenient to use video recording, which saves time on staging repeated experiments. If image processing does not allow calculation of several characteristics simultaneously, one and the same video record can be run as many times as necessary to determine these values separately.

To date, only a few characteristics of locomotion and their correlation with the factors affecting paramecia have been considered. For instance, copper ions and menadione decrease their motility [6]; an alternating magnetic field makes them gather at the upper bound of the volume [3]; hyperpolarization of the cell membrane lowers the frequency of spontaneous escape reactions so that paramecia move at a constant speed along the vertical chamber walls [4].

Video recording may help establish the correlations of locomotor parameters with each other and with the environmental factors. Though only the mean motility has been measured in the present work,

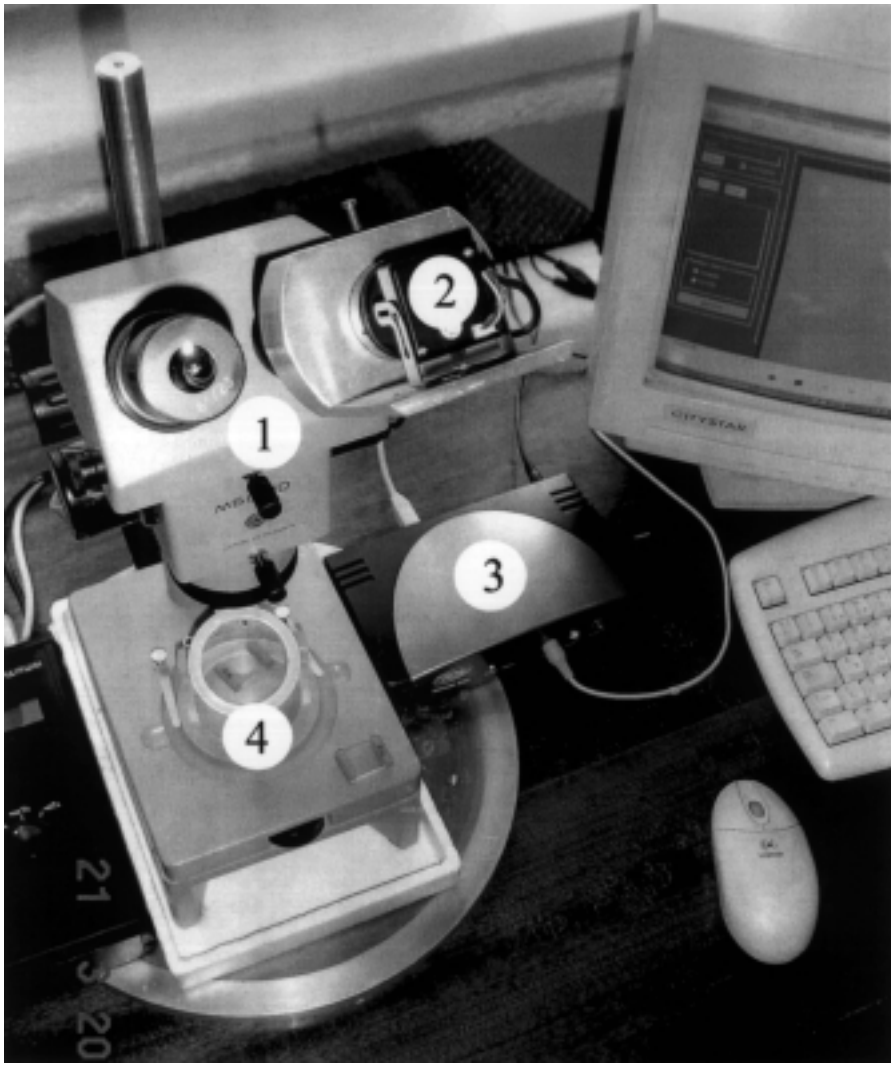

Fig. 1. Computerized system for estimating the microbial locomotion parameters: (1) microscope, (2) modular video camera, (3) digitizing unit, (4) set of Helmholtz coils.

modification of the program allows determination of other parameters as well.

The monoculture of *Paramecium caudatum* was maintained at room temperature in shaded glass vessels with 500 ml nutrient medium (0.2 g dried banana skin and 10 mg yeast per 100 ml distilled water) changed every fortnight; the population density thereby remain practically constant.

Paramecia were observed with a light transmission long-focus microscope MBS-10 where the magnetic exposure system (*vide infra*) could be conveniently accommodated under the objective lens. The culture medium was freed of large particles (over 0.1 mm). Paramecia in a drop of this solution were placed into a thin-walled inert polymer chamber 1×1 cm in the plane; the depth was varied from 0.04 to 0.16 cm. To minimize evaporation and prevent contamination, the chamber was covered with a glass slip fastened at two sides.

The background magnetic field was measured with a TMI-01 magnetometer (Physical Instrument Design Center, IGP RAS) accurate to 0.1 μT for permanent and 1 nT for alternating fields. The permanent magnetic field (mainly geomagnetic) at the experimental location was 43.9 ± 0.5 μT, mean inclination 69.6°; the deviations were caused by the magnetic noise of the urban electric transport. Field nonuniformity was less than 0.1 μT/cm. The alternating magnetic field did not exceed 40 nT over the 10–200 Hz range.

Paramecia were exposed to uniform magnetic field using a pair of Helmholtz coils: each of 25 turns

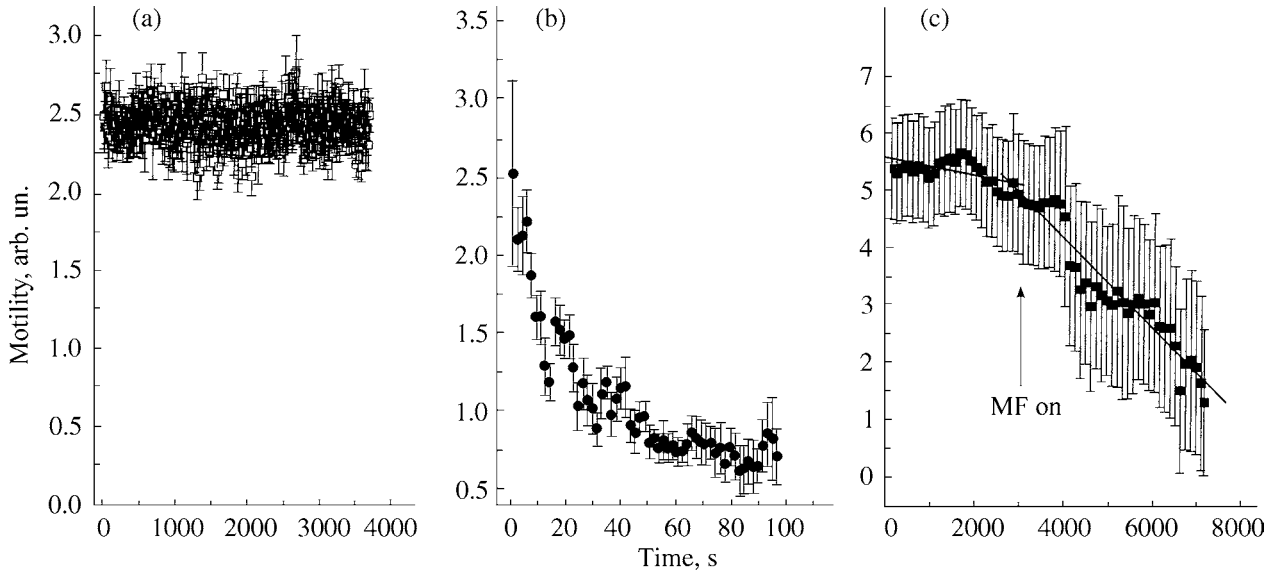

Fig. 2. Time dependences of protozoan motility: (a) stable reference conditions, (b) during drying of the medium, (c) in alternating magnetic field (sine 9 Hz, 79.1 μT); straight lines in panel (c) are linear regressions before and during magnetic exposure.

of 0.5-mm lacquered copper wire wound 2.5 cm apart on a 5-cm hollow Plexiglas bobbin; coil resistance about 1 Ω, inductance about 10^{-5} Hn; coil pair constant 0.895 μT/mA. The coil cylinder could be turned ±45° about the horizontal axis, its support could rotate about the vertical axis, and had in its middle a hollow cylindrical stand for the specimen admitting the microscope light beam from below. The chamber with paramecia was placed onto the stand and into the coil set.

One of the microscope eyepieces carried a modular video camera (K-2005C, LG, Korea) connected via a digitizing unit (LL5-USBAV-700, ADS Technologies, UK) to a computer (Fig. 1). The image was recorded on the hard disk in MPEG format at a frame rate of 29 s^{-1}.

Further, the video file was processed with a specially designed program, calculating the motility simultaneously over all trajectories. Motility was determined as averaged displacement of paramecia between two consecutive frames in the processed image resulting from bitwise subtraction of the bit matrix of the preceding frame from that of the next frame. This displacement is characterized by the sum of points of the videocam optical field that are distinct from the background in color and brightness. As the program parameters, one could set: (i) the starting frame for calculating the motility; (ii) the averaging interval (expressed as the number of values to be averaged, calculated for adjacent frame pairs); (iii) the 'porosity' expressed as the relative size of the skip interval following the averaging interval (frames ignored in calculation). In this way, motility values were calculated for equal time intervals. As a result, we obtained the time dependence of a locomotor parameter (motility in this case) and its dispersion,

A typical reference curve in displayed in Fig. 2a: one can see that over one hour under standard conditions there was practically no change in locomotor activity barring fluctuations. The mean motility was 2.5 arbitrary units, corresponding to a mean velocity of 0.98 ± 0.05 mm/s, which fits nicely into the range reported in the literature (0.3 to 2.5 mm/s) [4, 7].

Figure 2b shows the data on another experiment where paramecia died because of evaporation of the medium: their motility is seen to drop quite quickly.

To test the technique in magnetobiological studied, as a part of a more comprehensive experiment we assessed the influence of a low-frequency sine (9 Hz) magnetic field on protozoan motility. The amplitude of magnetic induction in the center of the Helmholtz coil pair was 79.1 μT. First, a 50-min reference recording of cell motion was made in the absence of applied field, and then the field was switched on (arrow in Fig. 2c) for another 70 min. One can see only a slight decline in motility prior to but a pronounced decrease throughout the exposure.

Thus, the proposed method of measuring protozoan motility yields reproducible values consistent

with the literature, is highly automated, can produce temporal series with very short averaging (down to 1/29 s), and is readily adapted by modifying the program to determine other locomotor parameters. The latter can be done without repeating the experiment since the video data are stored as a file.

The software permits processing recorded sequences of up to 5 h duration, which may be necessary in order to obtain statistically significant results for parameters more subtle than the motility of paramecia. The proposed procedure makes maximal use of the possibilities of a contemporary office computer by efficiently combining the operation of its interface during video recording with the operation of its processor and co-processor in data treatment. Thus mathematical processing of a 2-h sequence (up to 700 Mb) would take several minutes to several hours depending on the desired calculation accuracy. Another advantage is the possibility of joint experiments with other laboratories via Internet. An abridged version of the program for calculating microbial motility is available (www.biomag.info/paramecia).

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