Study of effects of electromagnetic factors on the early development of biological organisms using comprehensive multispectral optical and scanning acoustic microscopy approach

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Abstract. The effects of electromagnetic field on development of biological organisms were studied in this work by multispectral optical microscopy and scanning acoustic microscopy. The living embryos of loach Misgurnus fossilis at early stages of development were used as biological objects. The electric field applied to the living organism was generated by a specially designed unit that can operate independently or in combination with the acoustic microscope. It was shown that the sequentially recorded multispectral optical and acoustical images reveal dynamics of the development. The long lasting experiments confirm minor effect of the optical and acoustic radiation on the embryos. The scanning acoustic microscope was used for in vivo visualization of three dimensional structure of the egg, estimation of the acoustical properties of the yolk tissue and visualization of slow substance flows inside the object. The speed of motion depends on the location of the investigated area, status of the organism and its development stage. The estimated valued of the speed is in a range of 0.1 – 1 µm/s. It was shown that the application of the electric field causes deceleration of the embryos development.

1. Introduction
The study of the influence of various physical factors on the early stages of the development of biological organisms is of considerable interest [1, 2]. Particular attention is drawn to the influence of the weak endogenous and exogenous electric field (EF). Special EF generators with real-time adjustment of the amplitude and frequency parameters are required for the detailed study of the dynamics of spatial changes in biological structures under the external field. On the other hand the detection, visualization and monitoring of such changes in living biological objects require appropriate scientific instrumentation. Ordinary optical microscopy is an excellent method, but sometimes it suffers insufficient contrast of observed structural organs. Ultrasound is non invasive and posses deep penetration. However commonly used medical scanners demonstrate insufficient spatial resolution. In
this work we use approach based on combined multispectral optical microscopy and scanning acoustic microscopy.

2. Materials and methods

There are two different methods used in this study for the in vivo monitoring of the developing small biological objects: multispectral optical microscopy and scanning acoustic microscopy. The electromagnetic field was generated with a specially designed electronic unit.

2.1. Preparation of biological objects

In this work, embryos of loach *Misgurnus fossilis* were selected as standard biological objects commonly used in developmental biology. Females were caught from nature and were kept in a refrigerator before the experiments. Chorionic gonadotropin (CG) hormonal stimulation was conducted to accelerate the maturation of females at room temperature. Production of sexual products and artificial insemination were carried out in accordance with standard methods described in detail elsewhere [3]. All procedures involving animals and their care conform to the institutional guidelines and with approval from the Government of Russian Federation.

2.2. Multispectral optical microscopy

The microscopic acousto-optic spectral imager based on a BW Optics MF-606 microscope with the acousto-optic module designed at the Scientific and Technological Center of Unique Instrumentation of RAS [4] was used in the experiments. The images shown in figure 1 as an example were obtained at wavelength of 660 nm. Images (a) and (b) were measured at different stages of the embryo development. Processing of the multispectral data reveals the relationship between the development stages and spectral data. More details about this technique can be found in another paper presented at the conference.

![Figure 1. Acousto-optic spectral images of the embryo.](image)

2.3. Scanning acoustic microscopy

The ultrasonic visualization of the biological objects in the course of the present study has been carried out by scanning acoustic microscopy (SAM). Ultrasound has a minor impact on the living organisms [5, 8], so the acoustic microscope can be considered as a suitable and effective tool for a long lasting observations of the structural changes in the evaluative bio object. In the acoustic microscope, high frequency, sharply focused ultrasonic waves are employed for the visualization. The microscope was designed in accordance with a traditional confocal scheme presented in figure 2.

The ultrasonic transducer 1 generates in immersion liquid (water) the wideband pulse wave focused inside the object 2. The waves scattered by the structural discontinuities propagate back and are converted by the same ultrasonic transducer to the electrical signal. The received signal is recorded as a function of the time of flight \( t \) which is proportional to the distance between the transducer and the
reflectors. To produce the three-dimensional data set the transducer is mechanically scanned in a raster manner over lateral coordinates \(x, y\).

The central frequency and the bandwidth of the ultrasonic transducer used in the experiment were 50 MHz and 50 %, respectively. The aperture angle of the focused ultrasonic beam was \(\theta_0 = 40^\circ\). In accordance with the classical criteria \([6]\), the lateral resolution in the focal plan of this confocal imaging system is about 30 \(\mu\)m. The spatial resolution along the vertical axis \(z\) is determined by the duration of the acoustic pulse, and it can be estimated at 20 \(\mu\)m.

\[\text{Figure 2. Scanning acoustic microscope and generator of electromagnetic field.}\]

\[\text{2.4. Generator of electric field}\]

One of our tasks was the creation of a specialized generator of constant or alternative electric field with a flexible control of the field time parameters, its polarity, precise setting of the output pulse voltage and a programmable timer to implement a specified research protocol. Since the impedance of the "electrodes – biological object" system varies in a wide range of \(10 \text{ to } 10^5\) Ohms, the output amplifier of the designed device operates in the voltage source mode \([9]\). The controller of the device is built based on a microprocessor. It equipped with a display and autonomous power supply. The main specifications of the generator are as follows: output voltage – 0 ... 2500 mV with step of 50 mV, pulse duration – 0.1...10.0 ms with a step of 0.05 ms, pulse repetition rate – 0... 2000 Hz with a step of 1 Hz. Accuracy of all parameters setting is equal to 1%. The overall view of the generator is shown in figure 2 \([10]\).

Electromagnetic stimulation of the organism was conducted \textit{in vivo} by applying of the appropriate voltage to the electrodes 3 immersed in the water tank of the acoustic microscope (figure 2). Alternatively the biological object can be placed in a special separate electrode cell.

\[\text{3. Experiment}\]

In this work, loach eggs have been used as live convenient biological objects. The eggs were placed in the immersion liquid for the ultrasonic visualization. As an example, figure 3 shows ultrasonic images of the one live egg taken at different times. The images represents the cross sections of the object in the lateral dimensions using grayscale coding of the amplitude of the recorded echo. The plane of visualization was set approximately in the middle of the yolk. The size of the field of view is \(2 \times 1.6\) mm.
Figure 3. SAM images of the loach egg taken in the middle of the yolk at 2-hour intervals.

Continuous changes in the internal structure of the egg can be detected in the images. The external ring of the bright dots indicates the position of the skin. The dark circle in the middle of each picture (a) – (c) is produced by the yolk; and the bright noisy area around it is generated by the blastoderm. The observed contrast can be explained by the fact that there are many strong reflectors located inside the blastoderm.

Figure 4. Ultrasonic images of the loach egg taken at various vertical positions of the focal plane with a step of 180 µm.

SAM is capable of visualization at various depths giving information about 3D structure of the object. Figure 4 (a) – (c) shows the cross sections of the well developed embryo as an example. Having a set of the scans of this kind, a volumetric reconstruction of the organs and calculation of their volumes and mutual positions are possible.

4. Results and discussion
To evaluate the influence of the electric field on the development of the embryo the ultrasonic images have been sequentially recorded. Figure 5 shows images of the blastoderm top of the loach egg at early stage of development taken at 10 min intervals. There was no electromagnetic field application between images (a) and (b) recorded with a delay of 30 min. Rapid changes in the blastoderm profile can be observed in the images. After image (b) was recorded, the alternative voltage 10 V with a frequency of 10 Hz was applied to the electrodes. After another 30 min interval since the voltage application image (c) was measured. Comparison of pictures (a) – (c) shows that the changes between (b) and (c) are much less pronounced then the changes between (a) and (b). Therefore it is possible to conclude that the electric field causes a slowdown in the development of the embryo.
Scanning acoustic microscope is an attractive tool for detection and monitoring slow changes in elastic discontinuities inside living objects. To visualize these tiny variations the output signal of SAM is recorded as a function of the lateral coordinate $x$ and the “slow” real time $T$. Often such data set $s(x,t)$ is called M–scan [6, 7]. Figure 6 presents the M–scans measured before (a) and after (b) electromagnetic field treatment. In this case a frequency of 100 Hz is used for the embryo stimulation.

In these scans the horizontal and vertical axes represent the lateral coordinate $x$ and time $T$, respectively. The length of $x$ axis is equal to 1.5 mm, and the duration of $T$ axis is 8 min at time step of 4 s. The changes in the image pattern in the vertical direction indicate the movement of the discontinuities inside the object. Generally it is possible to estimate the speed of the motion. Measurements conducted in a separated experiment shows that the reported speed is in a range of $0.1–1 \mu m/s$. Comparison of data (a) and (b) presented in figure 6 leads to observation that the activity of the object is much lower after the electromagnetic stimulation.

5. Conclusion
The technique developed for study of development process in living biological objects is based on combination of the multispectral optical microscopy and scanning acoustic microscopy. In this work the influence of some electric fields on the development was successfully studied. It was shown that the complementary information provided by both instruments is valuable. Therefore it can be suggested that the proposed approach could be useful for much wider application area.

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