Processing of acousto-optic images at early diagnosis of the functional state of the developing biosystem

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Abstract. The task of studying the embryo development at an early stage is considered. For this, hyperspectral imaging using an acousto-optical tunable filter is proposed. Acousto-optic visualization of the early developmental stages of the loach Misgurnus fossilis embryo showed a regular change in the spectral characteristics of different image areas corresponding to the tissues and body fluids, depending on the functional state of the embryo. This is manifested in the regular arrangement of a discrete set of maxima in the optical absorption spectrum with a distance of 20 and 30 nm between them. Value 20 nm corresponds to the normal development of biological tissue. The appearance of the value 30 nm between the maxima of neighboring optical absorption bands indicates the development of pathological processes in the biosystem. Such deviations from the norm are characteristic of germinal tissues, and they are absent in the perivitelline fluid. Therefore, for the early diagnosis of the physiological state of a developing biosystem, special attention should be paid to optical absorption spectra, in which 30 nm intervals between the maxima of the neighboring absorption bands prevail.

1. Introduction

At present, numerous studies are devoted to the fundamental mechanisms of developmental biology, since changes occurring during lifetime from the moment of fertilization of an ovum to an adult multicellular organism cause many questions related to the formation of a complex multi-level system that is constantly becoming more complex [1].

The use of the embryonic model for the research is very promising, since it provides a possibility to analyze the functional features of the various stages (especially the initial ones) of embryogenesis, when many processes are concentrated on a short period of time that determine the subsequent vital activity of organisms (active cell division, differentiation, morphogenesis). Even the smallest impact in the early development stages may entail the appearance of various delayed effects. In addition, embryonic models have unique advantages for studying the preservation of the completeness of genetic information, which can only be found in early ontogenesis, since many of the most important genes for population conservation are expressed during this period, and a change in the regulatory mechanisms of gene expression occurs in the initial period of embryogenesis [2].

To study mentioned processes non-destructive methods that allow not to interfere the vital activity of a living biological system are needed. Acousto-optic spectral imaging is an example of such a method

[3]. The purpose of this work was to measure the optical absorption spectra from different image areas corresponding to fluids and tissues of the loach embryo using acousto-optic spectral imaging and to analyze them in detail.

2. Methods and materials

Acousto-optic spectral imaging is the registration of spectral image series using sequential tuning of the acousto-optic filter based on the phenomenon of anisotropic Bragg diffraction of wideband optical radiation on the ultrasound wave. The central wavelength of the filter transmission band is defined by the period of ultrasound wave which is driven by a radio-frequency signal and can be controlled via PC. The microscope-based spectral imager used for our research is shown in figure 1 [4]. The device is designed to allow simultaneous visual observation of the specimen, registration of wideband (color) image and spectral image series necessary for the measurement of the absorption spectra distribution.

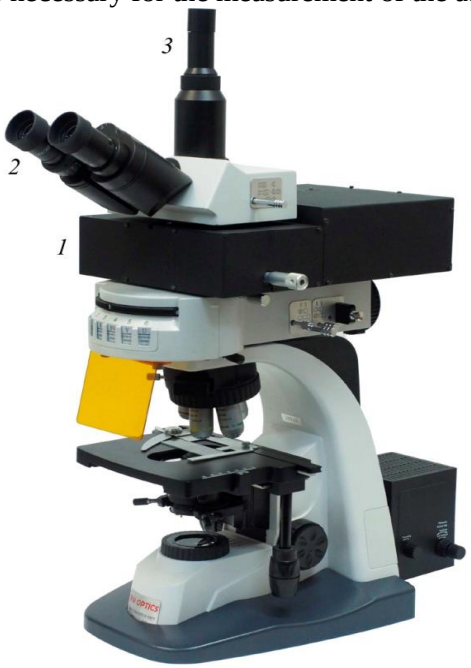

Figure 1. Microscopic acousto-optic spectral imager. 1 – acousto-optical spectral imaging unit, 2 – eyepieces for visual observation, 3 – color camera for wideband image registration.

The experiments were carried with the embryo of the loach Misgurnus fossilis which is considered to be a classical object of developmental biology. Females caught from nature inhabitant were kept in a refrigerator at 4-5°C. Accelerated maturation of females was performed by hormonal stimulation of chorionic gonadotropin at room temperature, the artificial insemination was performed according to the standard method [5]. The fertilized eggs were thoroughly washed with two portions of fresh water. After that some of the embryos (50 pcs) were placed in an isolated storage with a stabilized temperature of 17°C. Development stages were determined according to the tables of normal development of the loach [6,7]. The embryo of a certain developmental stage was thoroughly washed with two portions of fresh water and placed in the object plane of a microscopic spectral imager. Spectral image series of the developing embryo were registered twice a minute for approximately an hour.

3. Results and discussion

Figure 2 shows the example of a single spectral image of the loach embryo at 33rd development stage. The areas used for spectral analysis are highlighted in red. These regions represent different parts of embryo (myotomes, different parts of yolk and perivitelline space). The spectral distributions of optical density in different regions are presented in figure 3.

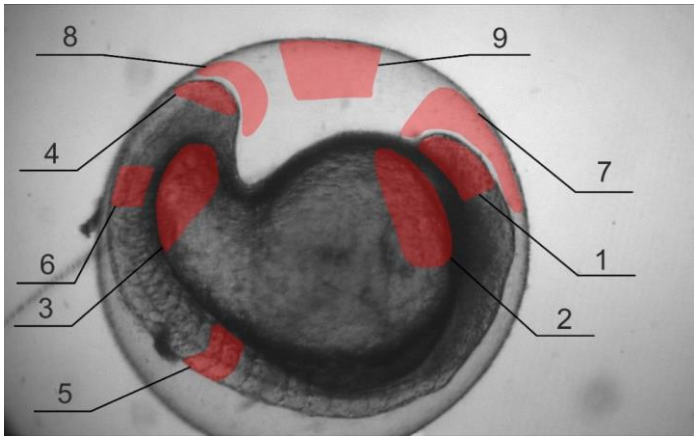

Figure 2. Spectral image of the loach embryo at 33rd stage of development. 1 – head section (eye area); 2 – yolk near the head; 3 – yolk near the tail; 4 – tail section; 5 – differentiated myotomes; 6 – differentiating myotomes; 7 – perivitelline space near the head; 8 – perivitelline space near the tail; 9 – perivitelline space.

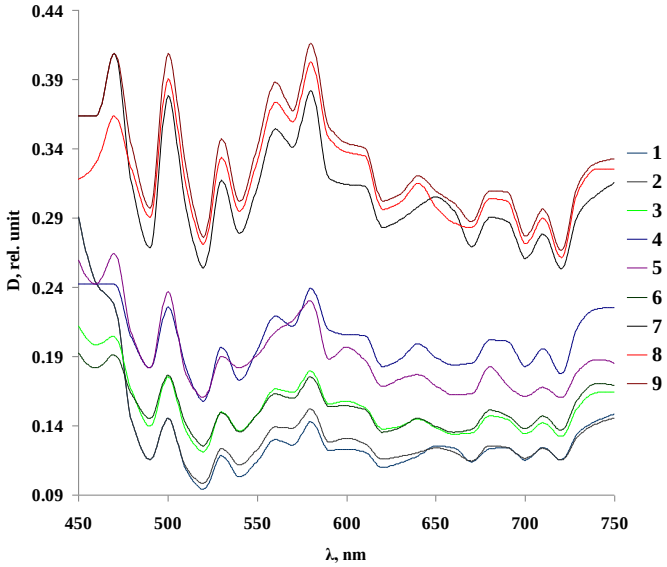

Figure 3. Optical density spectral distributions for different regions (number of the curve corresponds to the number of region in the figure 2).

The first feature in the optical absorption spectra for different regions of the loach embryo (maxima or their absence) falls in the wavelength region nearby 477 nm. Light at this wavelength plays an important role in many physiological processes occurring in all living organisms [4]. In the case of the head section (eye area) and yolk in the area of the head section, there is a sharp drop in optical density up to 485 nm. In this case, in different areas of the perivitelline space, a significant increase in the optical density is observed with a maximum at 477 nm, followed by a sharp drop in optical absorption to the minimum, at 485 nm. We note that in the region of differentiating myotomes, the optical density in this region of wavelengths does not change. A characteristic feature for all spectra is the greatest change in optical density in the wavelength range from 450 to 590 nm. In the wavelength range of 590–720 nm, insignificant changes in the optical density are observed for all selected regions of the loach embryo.

More detailed analysis of the features of the spectra for different regions of the loach embryo emphasizes the simultaneous growth of optical density at a wavelength of 500 nm for all regions of the embryo. In the wavelength region of 580–620 nm, the spectra can be divided into two groups: with a slight change in optical density (perivitelline space and tail region in the tail part of the embryo) and spectra with well-defined maxima (other tissues shown in figures 2 and 3). In the spectral range from

640 to 670 nm it is possible to conclude about peculiar resonance rearrangements in the tissues of the embryo by the appearance of the spectra. In the wavelength range from 670 to 720 nm the spectra are naturally divided into two groups according to the criterion for the presence of a local maximum in the spectrum.

For each spectrum, a pattern was found in the location of the maximum values of the optical absorption intensity in different regions of the embryo, determined by a discrete set of uniform intervals (the distance between adjacent maxima), among which the values of 20 and 30 nm dominate. The intervals of 20 nm correspond to regular sequences of uniform strokes known for the scale of characteristic dominant sizes in natural environments [5,6], while the presence of an interval of 30 nm seems to be associated with the process of the pathological development of a biosystem. It is noted that during the evolution of a biological system, states are fixed in which the intervals of 30 nm clearly dominate, occupying up to 3/4 of the analyzed wavelength range (450 ÷ 750 nm). Such states appear rarely and are represented by single measurements in the analyzed region of the embryo.

4. Conclusion

The analysis shows that the biggest fluctuations in optical density are observed in various areas of the perivitelline space (glycoprotein solution). For other areas of the image, corresponding to the tissues of the loach embryo (tail section, differentiating myotomes and yolk in the area of the tail section), a stable change in optical density is observed. It is known that the yolk in the tail section is actively consumed for the energy needs of the embryo and the yolk in the region of the head section is preserved until the late stages of development. The difference in spectral characteristics for these areas of the yolk qualitatively confirms its utilization. Thus, the use of an acousto-optic spectral imager demonstrates optical spectral properties of a living system at a certain moment as well as the unique opportunity analyse the dynamics of the biochemical processes of a developing embryo by means of analysing the time changes of absorption spectra.

5. References

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