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Application of hyperspectral imaging for analysis of embryonic development of lower vertebrates

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Abstract. The task of analyzing the characteristics of the embryonic development of lower vertebrates using non-invasive optical methods is considered. We propose to apply hyperspectral imaging for segmentation of embryo zones with different physical and chemical properties. This approach allows one to obtain information on the spatial distribution of optical spectral properties of the observed object. An experimental setup based on a microscope coupled with a spectral imaging add-on module utilizing a tunable acousto-optic filter has been created to implement this approach. Hyperspectral data arrays are formed as a series of spectral narrowband images obtained by successive tuning of the acousto-optic filter within the working spectral range. A series of experiments was conducted in which the development of embryos of the Misgurnus fossilis loach during their transition from the 32$^{nd}$ to the 33$^{rd}$ developmental stage was observed using the described experimental setup. Characteristic spatial regions corresponding to certain parts of the loach embryo were identified. The time dependences characterizing the change in the spectral properties of the selected regions were obtained. It is shown that joint processing of a series of spectral images obtained in different spectral bands within the working spectral range of an acousto-optical spectral imaging module allows one to effectively identify differences in the kinetics of the optical transmission spectral density for various regions of the axial mesoderm.

1. Introduction

The use of the embryonic development model in experimental research is promising since it gives an ability to trace the influence of various factors on living systems in a period of rapid complication, in which even minor disturbing impact can lead to disruptions in the development program and the appearance of visible anomalies. According to this approach lower vertebrate embryos are objects of
special interest as their development occurs in the surrounding water and is available for direct observation. Bony fish eggs are polylecithal. Their yolk is formed during the gametogenesis to support the embryo development at the early stages. At further stages of development, yolk transforms into the yolk sac, which is retained until the end of embryogenesis. The main function of the yolk is to provide the metabolic processes of the embryo. It contains energetic substrates, vitamins and different proteins such as vitellogenin, lipovitellin, phosphitin etc. Recent studies show that the mentioned yolk components take part in congenital immune defences as proteins act like polyvalent recognition receptors [1-4]. Vitellogenin and phosphitin provide antioxidant properties and protect the embryo from the oxidative stress [2, 4]. During embryogenesis, certain specific fish egg yolk regions perform functions of organs being developed further, such as blood formation, osmoregulatory and excretory functions [5-7] etc. All these processes are connected with the embryo development stages.

The conditions and the dynamics of yolk components local changes must be traced accurately with respect to the embryo morphogenesis features and the changes of physical and chemical properties of the yolk itself as the extraembryonic systems are being formed. This task requires the use of invital non-invasive research methods. Hyperspectral imaging is a kind of non-invasive methods successfully tested for studying the structure of animal organs and tissues and their vital cycles [8-12]. This method allows quantitative characterizing the spatial distribution of spectral properties. In this paper, we analyzed the functional conditions of different regions of Missgurnus fossilis loach embryo yolk before the beginning of spontaneous motor activity using acousto-optic (AO) spectral imaging.

2. Experimental setup

Different scientific instruments and methods can be used to obtain spectral images, but the method based on tunable optical filters (liquid crystal tunable filters, acousto-optic tunable filters, etc.) is considered to be one of the most versatile. AO tunable filters (AOTFs) provide high optical throughput, the ability to vary the transmission function, rather high spectral and spatial resolution and small tuning time, while being completely PC-controlled devices with no need of mechanical adjustment. AOTF operation principle is based on anisotropic Bragg diffraction of wideband optical radiation on the ultrasound wave [13]. AO cells installed in the AOTF used in the described setup are made of a uniaxial birefringent crystal. The ultrasonic wave excited by the piezotransducer in the AO cell induces the Bragg grating due to elasto-optic effect. The transmission wavelength of the AOTF is defined by the period of the grating which can be controlled by tuning the frequency of the ultrasonic wave. Imaging AOTFs conserve the spatial structure of the image for each narrow spectral band. In this case, one can electronically adjust the frequency of the ultrasonic wave and scan the working wavelength range of the AOTF to implement narrow-band imaging (NBI) and hyperspectral imaging (HSI) techniques.

Hyperspectral imaging data arrays are formed from the series of spectral images obtained by the acousto-optic spectral imaging add-on module [14]. The schematic drawing of the used module is presented in figure 1. The experimental setup is created on the basis of a conventional bright-field microscope with a wide-band Koehler illumination system which consists of wide-band light source (1) (halogen bulb), collector lens (2), field diaphragm (3), mirror (4), condenser aperture diaphragm (5) and condenser (6). Koehler illumination system is commonly used in modern scientific light microscopy to provide even illumination of the observed object. The specimen (8) (loach embryo) is installed on the 2-axis linear translation stage (7) to allow position alignment and located in the front focal plane of the infinity-corrected microscopic objective (9). Light emerging from this objective forms a parallel beam. Beamsplitter cube (10) splits the input light power into two optical trains. Beamsplitting ratio is 90:10 for optical trains (11-18) and (19-20), respectively. Elements (19) and (20) form a wide-band channel used for specimen orientation and positioning. Projection lens (19) focuses light from an objective (9) and forms a color image captured by the RGB camera (20). Spectral channel of the microscopic acousto-optic imager consists from elements (11-18). The key element of the spectral channel is the AOTF (12-16). To provide high image quality, AOTF operates in parallel beams scheme [15]. To reduce spectral drift two identical AO cells (13) and (15) are
installed and rotated at 180° in the diffraction plane. As the AO cells implement anisotropic diffraction, the appropriate polarization is selected by the polarizer (12). Polarizers (14) and (16) cut off the undiffracted light. The coupling system 11 conserves the structure of the parallel beam and allows correct optical matching of the microscopic objective (9) and the AOTF. Projection lens (17) focuses the narrow-band light from the AOTF and forms an image captured by the monochrome camera (18). AOTF controller unit (21), monochrome camera (18) and RGB camera (20) are connected with a personal computer (PC) (22).

Figure 1. Microscope-based setup with AO spectral imaging module. 1 – wide band light source; 2 – collector lens; 3 – collector lens field diaphragm; 4 – mirror; 5 – field diaphragm; 6 – condenser lens; 7 – 2-axis linear translation stage; 8 – specimen; 9 – microscopic objective; 10 – beamsplitter cube; 11 – coupling system; 12, 14, 16 – crossed polarizers; 13, 15 – identical AO cells; 17, 19 – projection lenses; 18 – monochrome camera; 20 – RGB camera; 21 – AOTF controller unit, 22 – PC.

The embryo development between 32nd and 33rd stages of the loach Misgurnus fossilis which is considered to be a typical object of developmental biology was observed in the experiment. Females brought from natural inhabitant were kept in a refrigerator at the temperature of 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, described by Kostomarova in [16]. The fertilized eggs were washed with two portions of fresh water. After that, some of the embryos (50 pcs) were placed in isolated storage with a stabilized temperature of 17 °C. Developmental stages were determined according to the data tables of normal development of the loach [17, 18]. The embryo of a desired developmental stage was washed with two portions of fresh water and placed in the object plane of a spectral imager. Spectral image series of the developing embryo were registered every 2 minutes for approximately an hour. We analyzed the yolk optical transmission spectra in four regions of interest shown in figure 2. The regions of interest (ROIs) were located near the head of the embryo (1), near the tail (2), near the differentiated myotomes (3) and in the ventral part of the egg (4).
Figure 2. Spectral images of the loach embryo ($\lambda = 635$ nm) at the beginning (32$^{nd}$ stage – a) and in the end (33$^{rd}$ stage – b) of the experiment with highlighted ROIs analyzed in the yolk: 1 – near the head; 2 – near the tail; 3 – near the differentiated myotomes; 4 – in the ventral part of the egg.

3. Results and discussion

Having analyzed the obtained optical transmission spectra for the described ROIs, we noticed the different character in spectral distributions during the transition of the embryo from the 32$^{nd}$ to the 33$^{rd}$ stage of development. The differences first appeared at the certain moment corresponding to the growth of the number of body somites. Several transformations took place through the experiment, are described below. The embryo length increased and the tail started differentiating. As the yolk continued transforming and its shape became more “pear-like”, the intensive movement of the yolk granules occurred. The eye formation began from the creation of the lens placodes. The motor activity of the embryo began at the end of the experiment (at the 33$^{rd}$ stage of development).

Figure 3. Time series of optical transmission spectra obtained for the ROIs analyzed in the yolk: (a) – near the head (1); (b) – near the tail (2); (c) – near the differentiated myotomes (3); (d) – in the ventral part of the egg (4).
The entire time series of optical transmission spectra obtained for the regions of interest are demonstrated in figure 3. A change in the character of the transmission spectra that occurs at the 30th - 32nd minute of the experiment is common for all of the ROIs. This change is conditioned by the regulative features of the central nervous system. Time series demonstrate partially matching local extrema of optical transmission for different ROIs. Figure 4 illustrates the time series cross-sections corresponding to the chosen wavelengths. The local minima common for regions near the head and in the ventral part of the egg indicate the synchronization of the transformation processes occurring in the yolk. Though, the additional local extrema for the ROIs near the tail and near the differentiated myotomes and the difference between the extrema positions indicate a specific heterogenic time delay of biochemical reactions in the yolk. Such heterogeneity may occur due to forming a hierarchy of connections in the central nervous system of a developing embryo.

![Figure 4](https://via.placeholder.com/150)

**Figure 4.** Cross-sections of the optical transmission spectra time series (see figure 3) corresponding to the wavelengths of 490 nm, 530 nm, 580 nm, 630 nm and 720 nm for the ROIs analyzed in the yolk: (a) – near the head (1); (b) – near the tail (2); (c) – near the differentiated myotomes (3); (d) – in the ventral part of the egg (4).

4. **Conclusion**

Analysis of the experimental results shows that spectral imaging of a developing biosystem is a promising technique able to provide data characterizing the features of specific morphological structures. The observation of the developing embryo indicated synchronization and the local differences of the biochemical processes in different areas the yolk. The implementation of the described technique can lead to a better understanding of the processes inside the biological system during its development.
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