Live imaging of mammalian embryonic development

Kirill Larin, Irina V. Larina, and Mary E. Dickinson

Optical coherence tomography shows promise in assessing cardiovascular development in mammalian model systems.

Understanding the genetic basis of congenital heart defects in humans relies on the analysis of mammalian model systems, such as mouse and rat embryos. Hundreds of mouse mutant analogs of human birth defects and diseases have been reported, helping to advance our understanding. However, the primary structural characterization of these mutant phenotypes has been based on static analysis of histological sections. Since the major function of the heart is to pump blood, static analysis does not provide information about blood flow patterns. As a result, very little is known about the cardiodynamic processes of early embryonic mammalian development. Thus, there remains a pressing need to develop tools and methods for visualizing and analyzing blood flow dynamics, which can only be performed by live embryonic imaging. To address this problem, we are developing methodology for live imaging of mouse and rat embryos—combining live embryo culture techniques, optical coherence tomography (OCT), and advanced computational modeling. 1–4

OCT is a noninvasive 3D imaging technique with micrometer resolution and imaging depths of a few millimeters. 5 Thus, it is an ideal tool to visualize the structures of whole, early embryos at the resolution of single cells. This technique is based on the detection of backscattered photons from a tissue within a coherence length of the source, and it does not require the use of contrast agents. Additionally, Doppler OCT is a functional extension of this technology that allows quantification of blood flow. Combined, these features potentially allow for high-throughput, live visualization of wild-type and mutant embryos.

We focused our analysis on mouse embryos at 7.5–10.5d. This time frame allowed us to follow the development of the cardiovascular system while the heart tube formed, began to beat, looped, and formed chambers and valves. During these stages the embryos can be cultured on the imaging stage for up to 24h while maintaining a strong, stable heartbeat and blood circulation. This embryonic stability allowed us to analyze the cardiodynamics and blood flow pattern of the embryos. 1,4 Figure 1 shows an example of live mouse embryo imaging with OCT at 9.5d. Structural OCT is very effective in producing 3D reconstructions of live cultured embryos—we easily resolved the internal structures and the whole embryo was within the imaging view.

Continued on next page
Our next step was to optimize 4D (3D plus time) imaging of the beating embryonic heart.\textsuperscript{3,7} We used a non-gated 4D reconstruction algorithm because sequential acquisition of the volumes did not allow for a reasonable frame rate to analyze heart wall dynamics. We successfully produced a 4D reconstruction of the beating embryonic rat heart at 10.5d (see video\textsuperscript{6}). Figure 2 shows representative frames from the reconstruction for different phases of the heartbeat cycle. While the reconstruction provided sufficient detail, efforts are now focused on tracking heart wall movements during the cardiac cycle.

In summary, we demonstrated that Doppler OCT can be successfully used to reconstruct spatially and temporally resolved blood flow profiles in cultured mouse and rat embryos.\textsuperscript{1,2,4} At early stages of circulation—when the majority of blood cells are still within blood islands—hemodynamic measurements can be performed on individual circulating blood cells.\textsuperscript{2} Additionally, when flow is established, the analysis can be performed on the bulk movement of blood cells.\textsuperscript{4} We are now using these tools to characterize cardiodynamics and blood flow patterns in wild-type and mutant embryos with cardiac abnormalities. Also, we are developing tools for improved classification and quantification of these abnormalities. This work can potentially contribute to the understanding, prevention, and treatment of cardiovascular diseases and disorders.

The project is supported by the National Institutes of Health (R01HL095586) and the American Heart Association (10SDG3830006).

Author Information

Kirill Larin
Department of Biomedical Engineering
University of Houston
Houston, TX

Kirill Larin is an associate professor with joint appointments at the Department of Molecular Physiology and Biophysics at Baylor College of Medicine, Houston, and the Department of Optics and Biophysics at Saratov State University, Russia. His research is focused on developing optical methods for imaging and sensing of biological and non-biological materials and processes. He has authored more than 50 papers and books and has several patents in the area of optical imaging.

Irina V. Larina and Mary E. Dickinson
Department of Molecular Physiology and Biophysics
Baylor College of Medicine
Houston, TX

Irina Larina is an assistant professor whose research is focused on developing methods for live dynamic characterization of mammalian embryonic development.

Mary Dickinson is an associate professor who is studying the role of mechanical forces in vascular remodeling and heart morphogenesis in early vertebrate embryos.

References
6. Video shows 4D reconstruction of the beating embryonic rat heart at 10.5d. http://spie.org/documents/newsroom/videos/5581/Larin.wmv