Refining imaging strategies to enhance understanding of congenital anomalies

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Optical coherence tomography in live animal models offers dynamic imaging capabilities for analyzing how aberrant morphologies and other birth defects occur during embryogenesis.

Studying the mechanisms that regulate normal and abnormal development in murine (mouse) embryos can yield deeper insights into how and why anomalies sometimes appear. A great deal of knowledge can be gained from imaging studies of murine cells, tissues, and organs during early development that could ultimately lead to improved strategies for the prevention and treatment of birth defects in humans. In the past, such imaging has involved tissue staining to enable researchers to visualize cellular development. Today’s noninvasive optical coherence tomography (OCT) relies on natural tissue contrast and is capable of resolution of a few micrometers and imaging depth of a few millimeters. These qualities make it a uniquely dynamic approach to the study of embryogenesis in living animals. For example, OCT enables us to observe murine embryos just as their hearts have begun to beat. At that stage, the animals are only about one millimeter in size.

In collaboration with researchers from the University of Houston and the Texas Medical Center, we are refining live murine embryonic OCT imaging strategies and dynamic analysis methods to assess various aspects of normal and abnormal embryonic development. One of our strategies is to visualize whole cultured embryos during embryonic days 7.5–10 with a primary focus on cardiodynamic analysis, and another is to image in utero superficial embryonic structures at later stages. In the murine embryo’s early development, we can dissect it out of the uterus, with the yolk sac intact, and culture it on an OCT imaging stage (see Figure 1). As the embryonic vascular system is unaffected, this approach gives us the ability both to see the beating embryonic heart and the developing circulatory system as well as to characterize hemodynamics while the embryo—for up to 24 hours—continues to develop in the static culture wherein it remains in the same position during the imaging session.

Figure 2 shows an example of a 4D (3D plus time) image of the murine early embryonic heartbeat. Together with Michael Liebling at the University of California, Santa Barbara, we have developed a number of computational approaches: these include nongated acquisition and synchronization, de-noising, and mozaicing of cardiodynamic OCT data sets. We are using these methods now to understand early embryonic cardiodynamics and perform functional characterization of mutant embryonic phenotypes with cardiac defects.

To further our understanding of embryogenic mechanisms at later stages of gestation, we developed OCT techniques to visualize live murine embryonic organs beginning at embryonic day 12 through the remainder of embryogenesis in utero. For longitudinal imaging, we anesthetize the female and lift the uterine horn through an abdominal incision that allows access. After imaging, we close the incision with surgical sutures. Thus

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we can repeatedly image the same living embryo to characterize temporal changes in organ development at unprecedented spatial resolution. We have also demonstrated high-resolution OCT imaging of other embryonic organs, such as the brain, eye, and limbs.\(^3,4\) Moreover, we can adapt this approach to analyze embryos from genetic screens both to avoid histological sectioning of multiple litters and to study the effects of pharmacological and toxicological agents on embryo development.

We are currently working with the National Institutes of Health (NIH)-funded BaSH (Baylor College of Medicine, the Wellcome Trust Sanger Institute, and MRC Harwell) consortium to incorporate newly developed dynamic embryonic OCT approaches into phenotyping applications for embryonic lethal lines. These high-throughput methods, in addition to reducing the number of mice required for analysis, will help us deduce structural features and contribute valuable cardiodynamic and blood-flow information not accessible with any other currently available approach. Our ongoing work will focus on gaining further knowledge about how these imaging studies have the potential to contribute to the prevention, diagnosis, and treatment of congenital defects in humans.

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References