Label-free in utero imaging of mouse embryos

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An optical coherence tomography technique enables, high-resolution 3D imaging of mammalian embryos through the uterine wall without harm.

The mouse is a classic mammalian model used to study the anatomical and physiological development of different organ systems. Hundreds of mouse mutants associated with human diseases have been reported, helping to advance our understanding of the genetic basis of development and disease. Moreover, the proven value of genetic approaches to study gene function in the mouse, and the efficiency with which individual mutations can be mapped and cloned, has led to several large-scale, international, genome-wide screens for new and advanced models of human disease.

Traditionally, primary analysis of embryonic mutant phenotypes has been based on static examination of histological sections, requiring many litters at different time points to deduce phenotypic changes. However, methods that allow for the direct assessment of embryonic phenotypes in utero would obviate the need for sectioning multiple litters, provide dynamic information about development, and enable higher throughput analysis for use in large-scale screens. Currently available non-invasive methods suffer either from poor resolution or long acquisition times, which limits their use for live imaging of embryos in utero.

Recently, we reported the superior capability of optical coherence tomography (OCT) for imaging live cultured embryos at E7.5–10.5 days post coitum (see Figure 1). However, embryos grown in culture can only be maintained for 24–48 hours, and embryos beyond E10.5 will not survive because they need maternal support. This method therefore excludes the possibility of following long-term processes in the same embryo. Moreover, currently no high-resolution technique allows repetitive live embryonic imaging in the mouse at later stages of gestation.

To fill this gap, we demonstrated the possibility of live-imaging mouse embryos in utero beginning at E12.5 through the remainder of embryogenesis using OCT. Prior to E12.5, each embryo in the uterus is surrounded by a thick, highly scattering layer of decidua (maternal tissue), which makes it inaccessible to OCT imaging. As the embryos grow and the placenta forms, the decidua thins and degenerates by E12.5, enabling visualization of embryos through the uterine wall.

For repetitive imaging, the pregnant females were anesthetized with isoflurane by inhalation and placed on a heated platform to maintain the body temperature at 37°C during the whole procedure. Abdominal fur was removed with an electric clipper. The gravid uterine horn was externalized through a 1–2cm incision made in the lower abdomen and was covered with transparent plastic wrap. After imaging, the

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incision was closed using surgical sutures. Up to three repetitive imaging sessions were performed with the same embryo.

A short video shows a 3D OCT image of E15.5 mouse embryo. Figure 2 shows examples of 3D images of mouse embryos in the uterus at three different stages: E13.5, E15.5, and E17.5. Each data set for the images was acquired over a 10×10×2.2mm volume. For visualization purposes, the top layers—corresponding to the uterine wall and yolk sac—were removed from the images with a clipping plane. In this figure, the head and forelimb are distinguishable in all the images, and there is a clear morphological and size difference between the stages shown. At E13.5—see Figure 2(a)—nearly the entire embryo is within the imaged area, while at E17.5—see Figure 2(c)—only parts of the head and the forelimb are visible. Craniofacial details are distinguishable at E15.5 and E17.5.

We used this technique to image development of different embryonic organs, such as the eyes, brain, and limbs. For example, Figure 3 shows 3D reconstructions of the embryonic forelimb from E12.5 to E18.5 acquired through the uterine wall using OCT. The insets in the figures show scanning electron micrographs of the forelimb at the corresponding embryonic stages from the Atlas of Mouse Development. The morphological differences among the various stages are clearly evident. The limb has a polygonal shape at E12.5: see Figure 3(a). At E13.5—Figure 3(b)—the digits become evident as the interdigital zones undergo indentation. At E14.5—see Figure 3(c)—the digits are splayed out. By E15.5 and E16.5—see Figure 3(d) and (e)—the digits become longer and more parallel to each other, while the webbing between the outgrowing ones undergoes programmed cell death. The development of the nails (claws) is evident starting at E15.5: see Figure 3(d–g). These images correlate very well with histological and electron microscopy analysis of mouse embryos at the corresponding embryonic stages.

In summary, we have described a method for vital, 3D imaging of mouse embryos in utero from E12.5 to E18.5. Although semi-invasive, this method allows us to repeatedly image the same living embryos without harm from E12.5 to E18.5 days post coitum, enabling characterization of temporal changes in organ development at previously unprecedented spatial resolution. Our next step will be to validate this imaging technology in mouse mutant models with congenital defects.

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

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