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MICROSCOPY

Mapping mouse development

Adaptive light-sheet microscopy enables imaging of mouse development through early organogenesis with single-cell resolution.

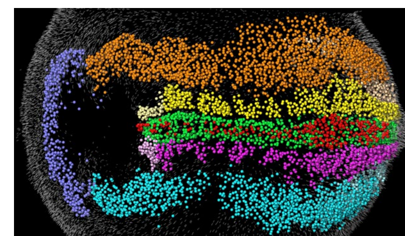
The ability to monitor and track all cells and their fates throughout mouse embryogenesis would provide crucial insight into mammalian development. Although embryogenesis has been studied extensively in organisms such as zebrafish and Drosophila using light microscopy, the study of mouse embryogenesis has largely been limited to the very early stages because these samples are difficult to image.

Philipp Keller, along with Kate McDole, Léo Guignard, and colleagues at Janelia Research Campus, addressed this challenge by using a combination of technical innovations. Keller notes that unlike other commonly imaged model organism embryos, mouse embryos are much more light sensitive, require precise culturing conditions, have challenging optical properties, are large, and undergo large changes in size and shape during embryogenesis. "All of these problems make mouse embryos a very difficult target for high-resolution, long-term live imaging," he says.

Overcoming these myriad challenges required methods development at nearly every stage of the imaging workflow. To grow the embryos appropriately, the team had to build a custom sample chamber for longitudinal embryo culture during imaging, which allowed them to control temperature and atmosphere. To maximize image quality, they made a derivative of their AutoPilot light-sheet microscope, which offers gentle imaging with a high degree of automated control over image acquisition to maximize image quality over time.

The AutoPilot microscope was redesigned with custom objectives that were relatively small and had short working distances. This allowed high-numerical-aperture objectives to be used in the right geometry and minimized the optical path length through what Keller calls the "optically problematic" culturing medium. In addition, the already flexible microscope was improved by a redesign of the control framework that allowed the scope to adapt itself to a sample that was constantly changing in size, shape, and position, increasing in volume by a factor of 200 during the acquisition period. Keller notes that this was perhaps the most important of all of the innovations.

The researchers also exploited the ability of longer wavelengths of light to better penetrate tissues, which scatter and distort light, by using red and near-infrared fluorescent proteins. "We observed major improvements in depth penetration when switching from GFP to mKate2, as well as when switching from mKate2 to miRFP703," says Keller. "We are thus quite excited at the prospect of using even further improved markers and further red-shifted reporters in the future."



Reconstruction of an embryo at the early somite stage. Reproduced from McDole et al. (2018), with permission from Elsevier.

Once these changes were implemented, the researchers were able to generate unprecedented images of the post-implantation mouse embryo, particularly in the period from gastrulation to organogenesis, a developmental period that is of high interest for biological research because of its implications in stem cell research and tissue engineering. Furthermore, they were able to do so with very high cellular resolution. Once the images were acquired, however, the team's remaining challenge was in the biological interpretation of the data. For this, they had to develop software tools for automated identification and tracking of cells, reconstruction of fate maps, and registering of embryos in time and space. With these in hand, they produced an invaluable atlas of post-implantation mouse development that is now available as a resource.

Although Keller says that there are still many biological questions and technological advances to pursue, this work represents a landmark achievement for microscopy in living organisms and developmental biology.

Rita Strack

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Research papers

McDole, K. et al. In toto imaging and reconstruction of post-implantation mouse development at the single-cell level. *Cell* **175**, 859–876 (2018).