MICROSCOPY

Be still, my beating heart

An optical gating strategy allows day-long light-sheet imaging of the developing heart in zebrafish embryos.

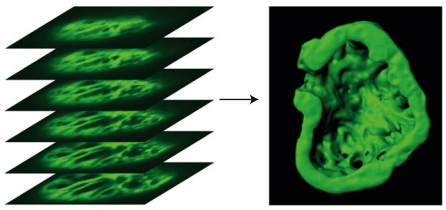
Imaging is a powerful approach for studying embryogenesis. Decades of advances in microscopy have yielded methods that enable fast and high-resolution longitudinal imaging of development, especially in small animals such as embryonic zebrafish. Nevertheless, some structures are more challenging to image over time than others. The developing heart is a particularly difficult target because it is constantly in motion and also grows larger over time.

Imaging the beating heart has been a long-standing interest for Jonathan Taylor at the University of Glasgow and his colleagues. "The real power of live imaging lies in time-lapse imaging: watching the processes of life unfolding in an unperturbed organism," says Taylor. "Having seen the developmental insights coming from direct time-lapse imaging in other organs, we wanted to bring this same capability to the dynamic environment of the heart."

But imaging an organ that beats over 120 times per minute requires special considerations. "The fact that the heart beats constantly makes it impossible to image in [three dimensions] without some sort of image synchronization," notes Taylor. However, existing strategies that use image synchronization were not sufficient for extended time-lapse three-dimensional (3D) imaging of the heart at speeds required to monitor interesting biological details, such as tracking cells moving and dividing over time.

To address this issue, Taylor's team started by triggering image acquisition in time with the motion of the beating heart to capture 3D images specific to certain points in the cardiac cycle, which effectively removed the beating motion. However, this alone was not enough. According to Taylor, "the big challenge for continuous day-long imaging was to ensure that, in eliminating the rapid beating motion, we did not blind ourselves to the much slower changes taking place over hours and days, which are the very thing we wanted to see and study."

For this, the team had to develop a sophisticated algorithm to keep the images in phase while the heart changed substantially in size and shape over the full day. This was a challenge, and existing algorithms were effective at synchronizing imaging for less than an hour. "We had to develop computer algorithms that were fast enough to analyze video images in real time, but robust enough to deal with whatever surprises might come up while running unsupervised for 24 hours," recalls Taylor. Their approach, which they call adaptive prospective optical gating, regularly updates the brightfield reference images that are used to maintain this fixed phase lock.



Light-sheet imaging is used to generate 3D images of the embryonic zebrafish heart. Reprinted with permission from *Nature Communications*, Springer Nature.

Using their method, the team monitored the development of a beating zebrafish embryo heart over a full day with little photodamage, which is another important concern for longitudinal imaging. They obtained highly detailed insights into how cells arrange and divide during a developmental process known as trabeculation, providing strong evidence that two proposed models are occurring together and suggesting a 'mixed' model for the process. Taylor recalls, "as soon as we were able to sit there and just watch it happening in 3D video, we realized that what we were seeing was not what people had thought was happening."

Taylor and his team are dedicated to making the tools available for researchers to do their own experiments and note that their strategy should be easy to implement on any customizable microscope. They hope that future work allows for studying arrhythmic hearts and monitoring other organs.

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