Heart stopping moments with zebrafish: imaging inside the living, beating heart

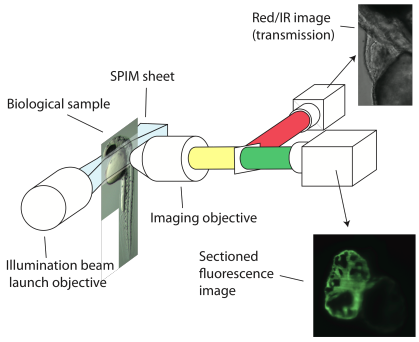
Jonathan M. Taylor, Glasgow University jonathan.taylor@glasgow.ac.uk

"Realtime Optical Gating for 3D Heart Imaging", Journal of Biomedical Optics **16** 116021 (2011).
"3D adaptive optics in a light sheet microscope", Optics Express **20** 13252 (2012).
"High-resolution 3D optical microscopy inside the beating zebrafish heart...", Biomedical Optics Express **3** 3043-3053 (2012).

The ability to image inside the naturally-beating zebrafish heart is becoming increasingly attractive for developmental and functional biological investigations, but the beating motion of the heart presents significant challenges.

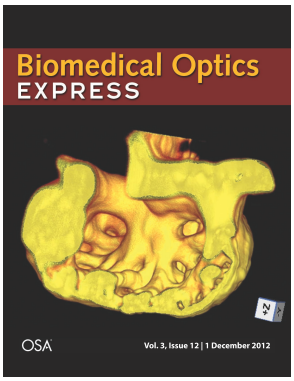
Conventional approaches require pharmacologically stopping the heart, or synchronizing acquisition to an ECG signal. ECG measurement is an enormous challenge in zebrafish embryos, and pharmacological stopping of the heart not only perturbs normal development but also means the heart muscle loses tone and relaxes into a shape not observed at *any* point in its normal cycle.

By continuously acquiring brightfield images and performing image processing in real time we are able to achieve **real time optical gating** to the motion of the heart. This allows us to trigger acquisition of fluorescence images at a fixed point in the heart cycle.

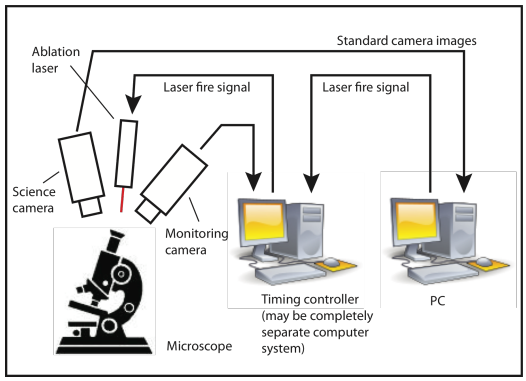


Using a custom-built SPIM imaging system (able to acquire optically sectioned widefield fluorescence images), we trigger image acquisition at a fixed point in the heart cycle as the microscope scans down through the volume of the heart. This means that as the images are acquired it appears as if the heart is **"frozen in time"**, but all the while the heart is beating normally and the embryo remains completely healthy. Specifically designed optics allow us to maintain a consistent brightfield focus for reference, while the fluorescence focus is scanned down through the heart.

This work has been supported in part by British Heart Foundation funding via the QMRI, University of Edinburgh. By developing new technologies such as this technique for **non-invasive 3D imaging** of living animals, we unlock new possibilities for studying cardiac development in unprecedented detail, watching the structure of the heart continuously as it grows.



This work, featured on the cover of Biomedical Optics Expess, has allowed us to obtain high resolution images of the beating heart of a zebrafish embryo, revealing the detailed structure of the trabeculae (folds of muscle within the ventricle of the heart) and their appearance during contraction. We are currently extending this to acquire a "4D" movie showing both the motion throughout the heartbeat and also the developmental changes over slower timescales



Seamless integration of our synchronization platform with a commercial photo-ablation workstation. We have demonstrated this application, enabling researchers to carry out precision "healing" studies on the moving heart by targeting moving cells for laser-induced damaging. It also has potential to be used for photoactivation, offering exciting potential for fluorescent tagging and tracking of individual cells over the first few days of development

We have demonstrated that we can acquire **high resolution 3D reconstructions** of the beating zebrafish heart, unaffected by the rapid motion of the heartbeat: our innovative real-time gating system ensures all images are taken at exactly the same point in the cycle.

We have also shown that the system can be used for **laser intervention** in the normally-beating heart, making possible experiments that would previously have been completely impossible.

The next steps are to enhance the reliability and ease of use of the system so it can serve as an accessible tool for biological research. We plan to apply the technique to such areas as fluid flow in the heart, heart performance and development, as well as investigating its potential for photoactivation and optogenetics in the beating heart.

University of Glasgow
EPSRC Pioneering research and skills
British Heart Foundation
The University of Edinburgh
Durham University