Contribution ID : **30** Type : **Short Talk**

Hybrid Optically-Gated Light Sheet Microscopy Allows Long-term Timelapse Imaging in the Developing Zebrafish Heart

Tuesday, 14 August 2018 12:30 (15)

Although the low-impact nature of light sheet microscopy has opened up new avenues for developmental timelapse imaging, the heart remains a particularly challenging organ to image in 3D timelapse. To image processes on timescales of minutes to hours (such as heart development, cell migration, repair and regeneration) demands some form of synchronized image acquisition in order to separate the high-frequency heartbeat motion from the lower-speed morphological changes of interest. Although current postacquisition synchronization methods are attractive for imaging the beat process, or for acquiring small numbers of timepoints, the accumulated light dose precludes longer-term timelapse imaging. Indeed, we will show that this rapidly induces catastrophic photobleaching, phototoxicity and heart arrhythmia.

We have previously developed prospective optically-gated light sheet microscopy, to allow synchronised 3D imaging of the in vivo beating zebrafish heart with a laser dose no higher than required for imaging static tissue. However, sustained timelapse imaging over 24h or more presents significant additional challenges, since the dramatic morphological changes undergone by the heart frustrate existing synchronization approaches. We will describe how we have been able to overcome this barrier by using hybrid prospective-retrospective optical gating technologies, and present 24h 3D-timelapse video imaging of cardiac development and immune response to cardiac injury.

Just as light sheet microscopy minimizes the distribution of the light dose to the specimen in the spatial domain, our approach offers the same gain in the time domain. Our work opens up the unperturbed, beating heart to direct timelapse imaging studies that have until now been restricted to stationary organs. Our new approach also points the way towards integrated light-sheet microscopy studies of the developmental coupling between heart structure, fluid flow and electrical activity.

Affiliation

Glasgow University, UK

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Primary author(s) : TAYLOR, Jonathan; Dr NELSON, Chas (Glasgow University, UK)

Presenter(s) : TAYLOR, Jonathan

Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy