Development of a flexible light sheet fluorescence microscope for high speed 2D and 3D imaging of calcium dynamics in isolated cardiac myocytes and cardiac tissue slices

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We report a new custom-developed flexible light sheet fluorescence microscope (LSFM) for studying dynamic events in cardiac tissue at high speed in 2D and 3D and the correlation of these events to cell microstructure. The system has been designed to be flexible and to allow the illumination and detection objectives to be oriented with their optical axes lying in either the vertical or horizontal planes. This has been achieved by mounting the whole optical assembly on a breadboard that can be rotated and allows it to be applied samples mounted horizontally in a dish or in a vertically held tube.

The system builds on previous work and provides multiple types of illumination for different applications. For slower, higher resolution imaging, we have implemented a scanned line illumination that can be combined with 1-D confocal detection via the rolling shutter of the sCMOS detection camera [1] and that can also be scanned along the illumination beam propagation direction [2] to allow images to be acquired at multiple positions of the beam waist [3]. This provides a reasonably thin effective light sheet across the whole field of view without the need for deconvolution in post processing. For lower phototoxicty and photodamage when imaging the specimen at higher speeds, motorized flip mirrors allow the system to be switched to a 2D light sheet illumination mode coupled with angular dithering of the beam to reduce shadow artefacts [4].

High speed 3D imaging is achieved by scanning the position of the detection focal plane using the folded remote refocusing approach [5] in synchrony with an axial sweep of the illumination beam. The folded remote refocussing configuration is used so that the moving mass is minimised, allowing higher refocussing speeds and a larger axial scan range to be achieved compared to moving an objective lens.

We present a characterisation of the system's performance in terms of spatial resolution and imaging speed together with calculations of light dose and collection efficiency for each imaging mode. We also present data obtained with this system demonstrating time-lapse imaging of calcium dynamics in isolated cardiac myocytes. The system is used to obtain a higher resolution structural map of the t-tubule network followed by high speed 2D and 3D imaging of calcium dynamics that can then be correlated to the t-tubule structure.

References
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