MODIFIED GAUSSIAN LIGHT-SHEET FOR TILED SPIM

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Compared to other popular microscopes used for 3D imaging, such as confocal microscopes, light-sheet microscopes offer both higher imaging speed and lower photo-damaging effects on the specimen. A wide range of modifications to the classical light-sheet design have been proposed, many imposing more complex structure on the light-sheet. The aims here include: improving image quality and resolution, further increasing imaging speed and decreasing photobleaching.

We will present our recent SLM-SPIM concept [1], a Selective Plane Illumination Microscope (SPIM) in which a Spatial Light Modulator (SLM) is introduced into the optical path of the illumination beam, providing great versatility for implementing these advanced light-sheet designs on a single software-reconfigurable instrument. This offers a platform for trialling recently-published techniques before investing time and expense in a dedicated custom-built system, and also for developing novel techniques.

To illustrate the utility of our platform for developing new techniques, we will present our work on a modification to the *tiling* technique in light-sheet microscopy. Tiling [2,3] was developed to work around the trade-off between light-sheet thickness and length in light-sheet microscopes, which limits their optical sectioning abilities. While an ideal light-sheet would stay thin across the full imaging Field of View (FoV), giving good optical sectioning throughout, real Gaussian light-sheets only remain thin over the beam's Rayleigh length. Tiling offers a way to use a thin, short sheet while assuring good optical sectioning beyond its Rayleigh length. However, this comes at the cost of extra excitation light (and hence photobleaching) since any point in the sample is exposed to laser light at times when it is not actually being imaged. We have developed a modified light-sheet profile which can be applied using a simple binary amplitude mask (implemented in our case using our SLM-SPIM system), to minimize this undesirable laser exposure. The result is that, when performing tiled imaging over a restricted volume, easily-bleached samples can be imaged with less photobleaching.

We will present details of our new tiling technique, including simulations and preliminary experimental results, and discuss the pros and cons of using the SLM-SPIM platform to develop and evaluate a new technique such as this one.

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[3] Dean, K. M., Roudot, P., Welf, E. S., Danuser, G., and Fiolka, R., "Deconvolution-free subcellular imaging with axially swept light sheet microscopy," *Biophysical journal* **108**(12), 2807-2815 (2015).