CORRECTION OF ATTENUATION ARTEFACTS IN LIGHT SHEET FLUORESCENCE MICROSCOPY

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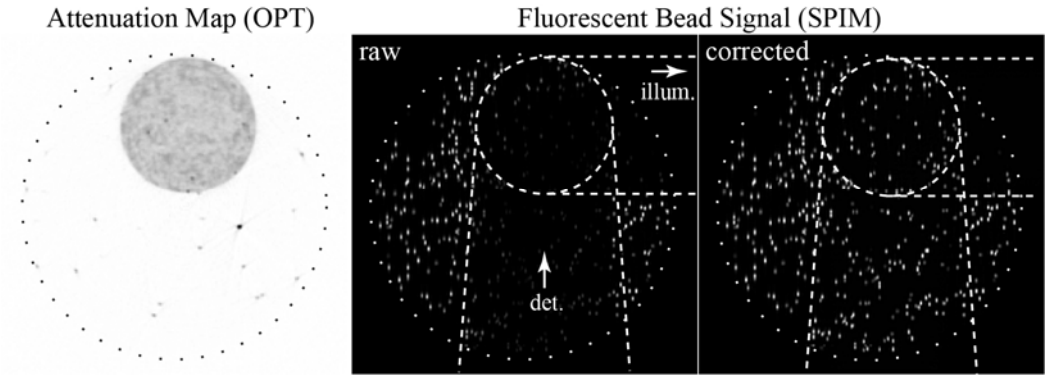
Light-Sheet Fluorescence Microscopy (LSFM) has recently become the accepted imaging technique for many applications in fields such as developmental biology, neurology, and immunology. Although LSFM techniques can provide high resolution images in 3D mesoscopic samples while minimizing photo-damage, they often suffer from "stripe" or "shadow" artefacts caused by the attenuation of light by features of the sample. Various methods to reduce these effects have been proposed (e.g. multi-view imaging [1] & mSPIM [2]), but in general the problem remains.

We recently introduced the OPTiSPIM [3], combining Optical Projection Tomography (OPT, the implementation of the concept of x-ray computed tomography at visible wavelengths), and Selective Plane Illumination Microscopy (SPIM, a version of LSFM). While SPIM generates high-resolution 3D fluorescence data sets, OPT can provide a 3D map of the distribution of a sample's attenuation. As well as being useful in its own right, this attenuation map can be used to computationally correct the shadow artefacts in the SPIM images. I will discuss how this is done, and present examples illustrating the usefulness of the method.



Fluorescent bead phantom with attenuating inclusion.

[1] J. Swoger, P. Verveer, K. Greger, J. Huisken & E.H.K. Stelzer. "Multi-view image fusion improves resolution in three-dimensional microscopy", *Opt. Express*, 15, 8029-8042 (2007).
[2] J. Huisken & D.Y.R. Stainier. "Even fluorescence excitation by multidirectional selective plane illumination microscopy (mSPIM)", *Opt. Lett.*, 32, 2608-2610 (2007).
[3] J. Mayer, A. Robert-Moreno, R. Danuser, J.V. Stein, J. Sharpe & J. Swoger. "OPTiSPIM: integrating optical projection tomography in light sheet microscopy extends specimen characterization to nonfluorescent contrasts", *Optics Lett.*, 39, 1053-1056 (2014).