IN-DEPTH EXPLORATION OF THE ROLE OF THE BESSEL BEAM IN SINGLE AND MULTI-PHOTON LIGHT SHEET MICROSCOPY

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1. INTRODUCTION
Selective plane illumination microscopy (SPIM), an optical sectioning technique, enables the imaging of dynamic processes as they occur in a three-dimensional biological sample. By exciting fluorescence in a single plane, orthogonal to the detection axis, rapid wide-field image acquisition is possible with minimal background fluorescence and photo-damage.

Isotropic high resolution imaging is paramount when imaging sub-cellular processes, yet a wide field-of-view (FOV) is also crucial to the understanding of intra-cellular processes in biological organisms. Recently it was shown that the FOV can be extended significantly by employing quasi non-diffracting Bessel beams (see Figure 1) to create the light sheet [1].

2. RESOLUTION VERSUS IMAGING VOLUME
We investigated the resolution of Bessel beam light sheet microscopy, both for single and two-photon fluorescence excitation. An inverse relationship is demonstrated between the FOV extension and the axial resolution achievable with single photon experiments. In stark contrast, for two-photon Bessel beam excitation we show that the contrast is enhanced at high spatial frequencies compared to that achievable with conventional SPIM. Two-photon Bessel beam light sheet excitation therefore not only extends the FOV it also enhances the experimentally achievable axial resolution, also when image deconvolution is taken into consideration [2].

3. CONCLUSION
Our experiments provide new insight into why, and under what conditions, beam shaping should be used in light sheet microscopy. We demonstrate a clear relationship between the FOV extension and axial resolution of a Bessel beam light sheet microscope. We also report a fundamental difference in this relationship when two-photon instead of single photon excitation is used. We show how these findings enable the optimization of existing techniques, as well as significant enhancements afforded by incorporating a spatial light modulator in the illumination path.

REFERENCES