Physical Properties of Square and Hexagonal Lattice Light-Sheets

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Light-Sheet Fluorescence Microscopy (LSFM) is an indispensable tool for imaging diverse specimens such as single cells, spheroids, organoids, tissue sections, developing embryos, and more. For most types of LSFM, the sample is illuminated with a light-sheet from the side, and photons are collected in a widefield detection format. However, the performance of a LSFM is heavily influenced by the properties of its illumination beam: thinner light-sheets provide better axial resolution and optical sectioning but are typically only useful over a small field of view since such light-sheets also become shorter.

In an effort to overcome this tradeoff between resolution and field of view, lattice light-sheet microscopy (LLSM) uses light-sheets that are derived from two dimensional optical lattices. However, only a few articles quantitatively report the physical properties of the lattice light-sheets and how they compare to standard high numerical aperture Gaussian optics[1,2]. In our previous work we have explored the physical properties of the dithered square lattice light-sheets and concluded that they are indeed very similar to Gaussian based light-sheets in terms of thickness, confocal parameter, propagation length and overall imaging performance[2]. Here, we perform analogous experiments on the hexagonal lattice light-sheets, including light-sheet measurements in transmission, point spread functions and optical transfer functions (see Figure 1). In doing so, we found gaps in the OTF for hexagonal LLSM, which we explore in detail. Thus, we provide a systematic evaluation of propagation length, resolution, and optical sectioning, for hexagonal lattice light-sheets. And lastly, we also present biological images acquired with hexagonal lattice and Gaussian light-sheets.

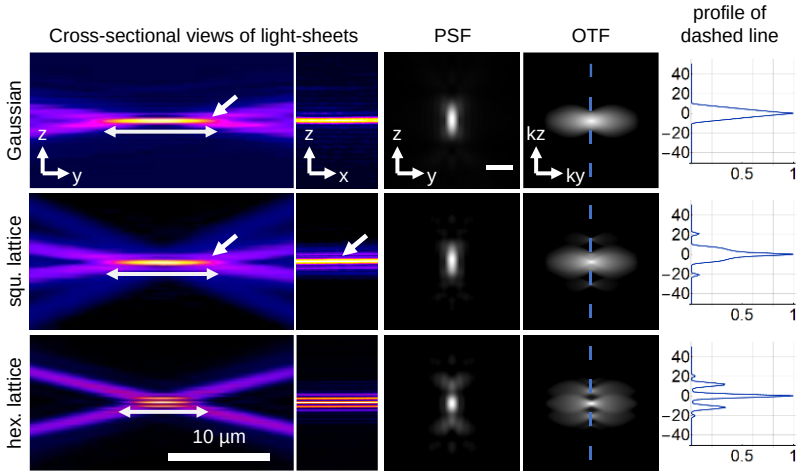

Figure 1: Brief comparison of Gaussian (NA0.24), square lattice (NA0.55,0.44), and hexagonal lattice (NA0.55,0.44) light-sheets. The PSF is generated by multiplying a simulated widefield PSF with the experimentally measured light-sheets. A gamma correction of 0.5 was applied to PSF and OTF images.

1. *Biomed. Opt. Express* **11**, 8 (2020).
2. *Opt. Express* **28**, 27052 (2020).