Optical Properties of Lattice Light-Sheet Illumination

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Lattice light-sheet microscopy (LLSM) coherently superimposes multiple Bessel beams to generate two-dimensional optical lattices that are non-diffracting in their propagation direction. In most applications, these lattices are dithered laterally to generate a time-averaged light-sheet. Compared to digitally scanned Bessel beams, LLSM offers a greater illumination duty-cycle and decreased side lobe intensity, which minimizes photodamage and improves optical sectioning, respectively. As such, LLSM has been successfully used to gain biological insight into clathrin mediated endocytosis, immune cell migration, and heterotypic organelle interactions, to name just a few examples. However, little is known about key parameters in LLSM, including the tradeoff between illumination beam width, propagation length, and side lobe strength. For example, Bessel beam light-sheets suffer from increased power density in their side lobes when scaled up to longer propagation lengths for a given light-sheet thickness, which decreases optical sectioning due to heightened out-of-focus illumination. However, it remains unclear how lattice light-sheets behave under similar circumstances.

Here, we systematically explore the theoretical and empirical properties of commonly used illumination beams in LLSM (e.g., square and hexagonal lattices). For optical experiments, the illumination train of LLSM was replicated, and light-sheet properties were measured in transmission by stepping a high-numerical aperture objective through the illumination lattice (Figure 1). This allowed us to unambiguously evaluate the relationship between annulus radius, annulus width, lattice thickness, lattice propagation length and illumination confinement (ratio of energy in the main lobe to total energy). Like other propagation invariant beams, we found that excitation confinement is gradually lost for hexagonal lattices of high aspect ratio, as prominent side lobe structures grow in intensity. In contrast, we found that square lattices have a more Gaussian character, *i.e.* the beam width increases for larger propagation lengths, but excitation confinement deteriorates less than for hexagonal lattices. As such, the intra-Bessel beam spacing defines if the lattice is truly non-diffracting (e.g., like a Bessel beam) or not (e.g., like a Gaussian beam). Ultimately, we hope that through this study we can clarify some of the properties and capabilities of lattice light-sheets.

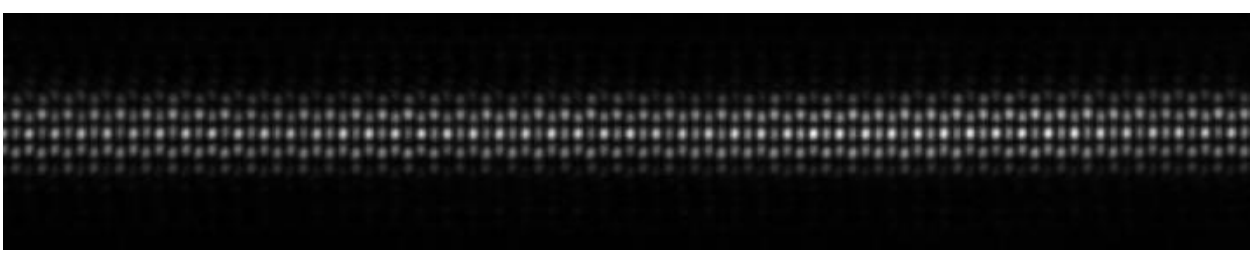

Figure 1: Example of a hexagonal lattice light-sheet pattern as observed in transmission.