Positive and negative light-sheets – an attractive combination

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Ideally, Light-Sheet Fluorescence Microscopy (LSFM) would employ an excitation intensity distribution that approximates a thin and long sheet of light. However, the length of a Gaussian light-sheet decreases dramatically and nonlinearly with the thickness of the light-sheet. In contrast, scanning a propagation-invariant Bessel beam laterally can create a longer and thinner light-sheet. However, Bessel beams suffer from strong side lobes structures that degrade axial resolution and the confinement of excitation, with the latter increasing photobleaching in volumetric imaging.

Here, we leverage the recently discovered Field Synthesis theorem to address the sidelobe problem. In short, Field Synthesis states that scanning a focused line over an illumination pupil recreates the same time-averaged light-sheet as it is obtained by conventional beam scanning in real space [1]. Field synthesis is a flexible mechanism to generate any scanned light-sheet, which allows us to create both a positive and a novel negative light-sheet with the same optical train. This negative light-sheet is generated by introducing a pi-phase shift to one half of the pupil, which then results in a sharp minimum along the focal plane (Figure 1).

In this presentation, we will discuss our method to create complementary pairs of positive and negative light-sheets by solely changing the polarization state of the laser light. We will also discuss applications of the two light-sheets, such as 1) synthesizing a high-aspect ratio light-sheet with less side lobes by a linear combination of positive and negative light-sheets, and 2) producing super-resolution in the axial direction by deactivating photo-switchable fluorescent proteins with the negative light-sheet.

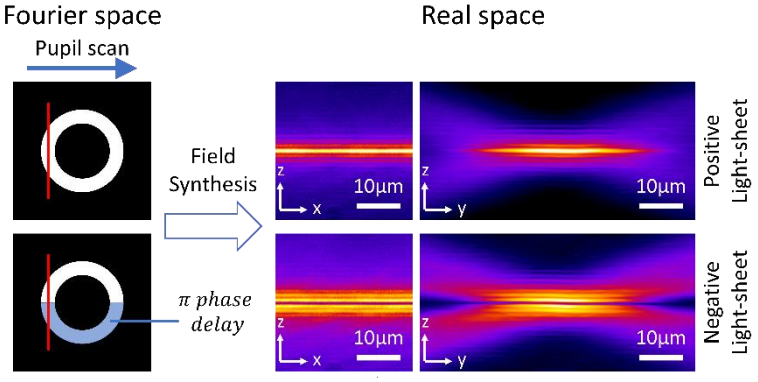

Figure 1: generation of positive and negative light-sheets with Field Synthesis

1. B.-J.Chang, M.Kittisopikul, K. M.Dean, P.Roudot, E.Welf, and R. P.Fiolka, "Universal Light-Sheet Generation with Field Synthesis," bioRxiv 427468 (2018).