The Awkward Reality of Focal Shifts in Imaging

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1. FOCAL SHIFTS PROBLEMS IN OPTICAL IMAGING

There is a global race in biological imaging to reach ultimate limits for many performance criteria simultaneously (resolution, depth, speed, volume and more) but progress is impeded by fundamental tradeoffs. One option to improve resolution, penetration depth and imaging speed meanwhile is to combine spatial focusing and temporal focusing in nonlinear microscopy. However, there will be focal shift problems in both kinds of focusing: the beam shapes determine not only the axial optical sectioning thickness but also the focal plane positions.

2. Strategy to catch near-diffraction limited axial resolution

In the spatiotemporal focusing, focusing occur in perpendicular directions: temporal focusing along the spectral components overlapping direction and spatial focusing across it. In both directions, theoretically one expects both focal planes sit at the focal plane of the objective lens. However, if the aperture sizes differ for the temporal (spectral) and the spatial focusing directions, the focal shifts are different and the focal planes do not overlap [1, 2]. This focal shift gap degrades the axial resolution and contrast. The solution is to restore a fully-filled circular aperture illumination when possible, or use to tune the temporal focal shift by chirp. Fluorescence imaging 1 mm deep into mouse lung demonstrates a doughty nonlinear microscopy with wide field of view, high contrast, fast acquisition-rate and near diffraction-limited axial resolution based on an ordinary ultrafast oscillator and spatiotemporal focusing nonlinear microscopy design. The key idea is that focal shift matching promotes near-diffraction-limited axial fluorescence optical sectioning for both low and high NA objectives by laser intra-cavity spectrum modulation.

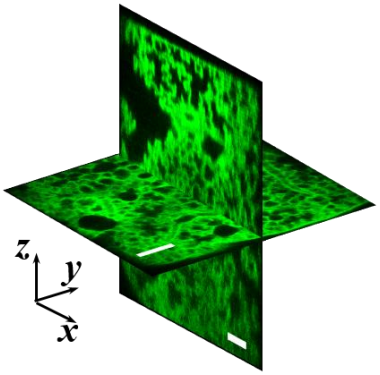

Figure 1: Volumetric imaging in mouse lung. Scale bars, 100um.

[1] W. H. Carter, "Focal shift and concept of effective Fresnel number for a Gaussian laser beam," *Appl. Opt.* **21**, 1989-1994 (1982).
[2] J. J. Stamnes, "Waves in focal regions : propagation, diffraction, and focusing of light, sound, and water waves," The Adam Hilger series on optics and optoelectronics (A. Hilger, Bristol ; Boston, 1986), pp. xviii, 600 p., 604 p. of plates.