Fast three-dimensional confocal microscopy with Lissajous scanning

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KEY WORDS: fast volumetric imaging, acoustic lens, confocal, resonant mirror, Lissajous.

Confocal laser scanning microscopy (CLSM) is an essential tool in biology for non-invasively studying three-dimensional (3D) phenomena at sub-cellular resolution. However, image acquisition speed in CLSM is compromised by the need of pixel-by-pixel scanning. Efforts to address this issue include parallelizing the illumination/detection or enhancing scanning speed along the lateral and optical axis[1,2]. Alternatively, it is possible to reduce the spatial sampling rate to even below the Nyquist criterion. The latter helps capturing an entire 3D image at shorter times, but because all points are not properly sampled, key details for image reconstruction may be lost.

Here, we present a novel CLSM architecture that combines unsurpassed scanning speed with sparse sampling to significantly enhance volumetric imaging rate. Our approach consists of using a resonant mirror for x-scanning at 8 kHz and an ultrafast varifocal lens for z-scanning at 456 kHz. Because the excitation beam follows an independent sinusoidal curve for each axis, the overall scanned trajectory corresponds to a spatio-temporally varying Lissajous curve across the XZ plane, as shown in Fig. 1. To address the low sampling rate caused by the Lissajous-based scanning, we use two complimentary strategies: (i) an advanced computational interpolation[3], which requires *a priori* information and; (ii) an accumulation of multiple time frames, each containing different sampled points. As a result, the number of added frames, and hence the corresponding effective time required to capture a volume, becomes an additional degree of freedom that determines the quality of the final image. Thus, we can adjust the desired temporal resolution and quality of the image in a post-processing step.

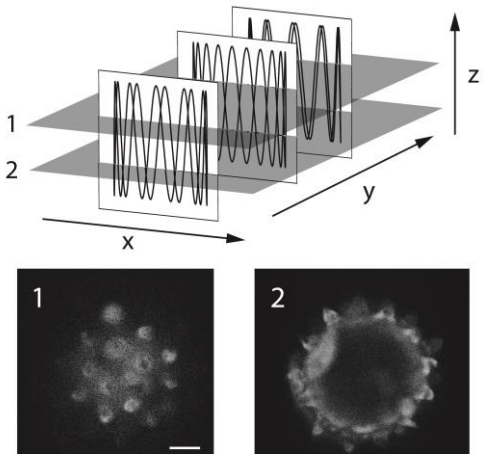

Figure 1. Above: Schematic of our confocal microscope, showing spatio-temporally varying Lissajous curves in the XZ plane. Note that the slow axis is located along the y direction. Below: XY images of a flower pollen at the two different z-positions indicated above. Scale bar 5 um.

We demonstrate ultrafast cross-sectional imaging and multi-color volumetric imaging of artificial and biological samples (Fig. 1). We also prove the suitability of our approach for live imaging by recording the calcium activity of neural networks from a 3D cultured *in vitro* murine brain model over a volume of 60 × 30 × 50 μm^3, at sub-cellular resolution, and at a rate above 30 volumes per second.

References:

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