Fast and Light-Efficient Volumetrically Parallelized Fluorescence Microscopy

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In fluorescence microscopy, 3D volumes are typically constructed by the serial acquisition of multiple 2D image planes, which becomes rate-limiting at fast image acquisition rates. To circumvent this problem, optical techniques based upon refraction and diffraction were developed that acquire multiple 2D focal planes in parallel [1, 2]. However, these approaches are plagued by poor light efficiency and increased image blur. For example, if 3 focal planes are imaged simultaneously, each image plane receives 1/3rd of the light captured by the objective (in addition to diffractive losses). Further, the remaining 2/3 of the light is detected as out-of-focus fluorescence. Thus, threefold longer integration times are needed to reach an equivalent signal to noise ratio as a single plane imaging system, albeit, with increased blur.

Here we address these shortcomings and introduce the first, to our knowledge, volumetrically parallelized imaging scheme with negligible cross-talk between image planes and a collection efficiency comparable to widefield microscopy. We refer to our method as Parallelized Light-Sheet Fluorescence Microscopy (pLSFM), which combines the uncompromised sensitivity of widefield detection, the gentle illumination of light-sheet microscopy, and speed (up to 14 Hz) afforded by 3D parallelization, all the while maintaining ~300 and ~600 nm lateral and axial resolution, respectively. Unlike other parallelized imaging approaches, the photon flux is not reduced by the degree of parallelization. Therefore, pLSFM overcomes both technological and photophysical limitations of other volumetric imaging methods.

In its current implementation, pLSFM simultaneously images three focal planes, allowing us to perform imaging of very sensitive processes routinely for 1000's of z-stacks as well as very rapid imaging of intracellular dynamics at volumetric image acquisition rates up to 14 Hz (see also Figure 1). Importantly, we show that owing to the lossless parallelization, the ~threefold increased volumetric acquisition rate does not increase photo-toxicity.

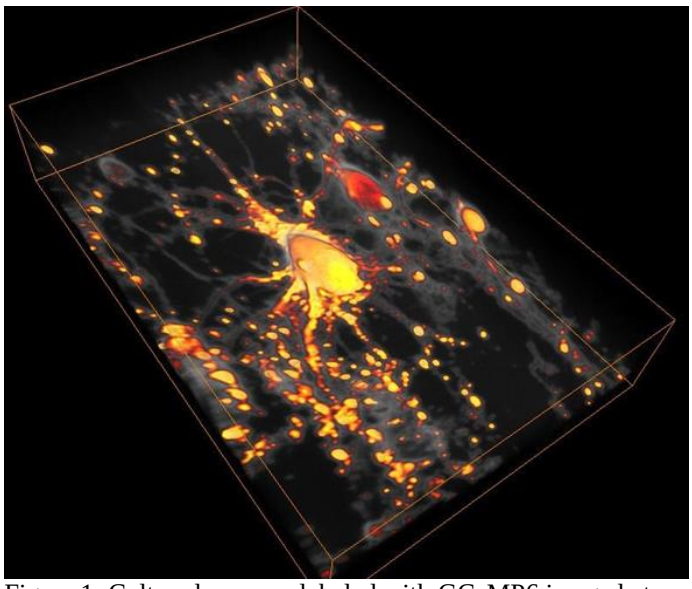

Figure 1: Cultured neurons labeled with GCaMP6 imaged at a volumetric acquisiton rate of 14Hz for 400 timepoints.

1. **S. Abrahamsson, et al "Fast multicolor 3D imaging using aberration-corrected multifocus microscopy," Nature Methods 10, 60-63 (2012).**
2. **S. Geissbuehler, et al"Live-cell multiplane three-dimensional super-resolution optical fluctuation imaging," Nature Communications 5, 5830 (2014).**