FAST VOLUMETRIC IMAGING WITH TAILORED ORTHOGONAL ILLUMINATION

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KEY WORDS: light-sheet microscopy, 3D imaging, beam shaping, axial scanning

A crucial challenge in optical microscopy techniques is increasing the volumetric imaging speed while maintaining submicrometric lateral resolution. To this end, several successful methods have been developed, including spinning disk confocal microscopy or temporal focusing. However, Light Sheet Microscopy (LSM) is the only approach capable of producing optical sectioning with reduced photobleaching at speeds high enough to track the evolution of rapidly evolving phenomena [1]. Unfortunately, orthogonality between excitation and detection arms and the requirement of confocality between the respective focal planes seriously constrain volumetric imaging rates in LSM. In particular, in order to obtain a 3D image, one must acquire multiple optical sections at different positions within the sample by either translation of the entire specimen or by moving both illumination and detection planes. In any case, the need to mechanically move optics or sample is inherently limited by inertia, and even if high actuation speeds could be achieved, such displacements could induce vibrations that interfere with volumetric recording. Here, we present a novel imaging modality that solves the current speed limitations of LSM while preserving its core advantages in terms of optical sectioning, resolution and reduced photobleaching. Our approach uses orthogonal illumination and extended depth of field detection [2,3] in a configuration that obviates the need of mechanical moving parts. In addition, tailored excitation patterns can be generated for improved signal-to-noise ratio and further reduction in photobleaching. We demonstrate the feasibility of our approach by 3D-tracking fluorescent beads at rates up to 200 volumes per second and by imaging the fast dynamics of unicellular organisms. These results open the door to diffraction-limited volumetric imaging at an unprecedented temporal resolution, offering researches a new tool to explore new fundamental processes in life sciences.

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