HIGH-SPEED VOLUMETRIC IMAGING USING VOXEL RE-MAPPING MICROSCOPY

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3D fluorescence microscopy is a powerful tool in biological and medical research as it allows investigating the whole structure of biological tissues and cells, but its low data acquisition speed critically limits its applications, especially in real-time observation of dynamic samples. Here we propose and demonstrate voxel re-mapping microscopy that enables volumetric imaging at unprecedentedly high volume rates of >1,000 volumes/sec. In our method, multiple 2D images at different depth positions are simultaneously captured by a single-frame readout of a CMOS camera (Fig. 1). To maximize the readout speed, an optical image on an image sensor is scanned by an image scanner (e.g., a galvanoscanner) in the direction of the camera's readout line during z-scan such that the multiple 2D images are aligned in this direction. Then, a volumetric image is obtained by a single-frame 2D image, enabling a substantial increase in the volume rate. For example, assuming a standard scientific CMOS camera (e.g., HAMAMATSU ORCA-Flash 4.0), a volume rate of 1,000 volumes/sec can be achieved at ~200 × 200 × 10 voxels. In addition, we can obtain a volumetric image using multiple frames, each of which includes multiple 2D images at slightly shifted positions in the depth direction. This image acquisition sequence enables flexible adjustment of the number of voxels in the three dimensions while retaining the maximum volume rate determined by the readout speed of a camera. As a proof-of-concept demonstration, we obtained volumetric images of neurons of *Caenorhabditis elegans* that express Yellow Cameleon 2.60 at a volume rate of 60 volumes/sec, demonstrating the increase of the volume rate by a factor of ~10 (Fig. 2).

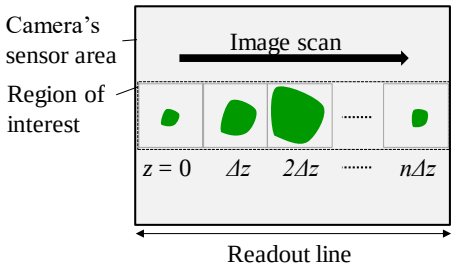

Fig. 1: Principles of voxel re-mapping microscopy.

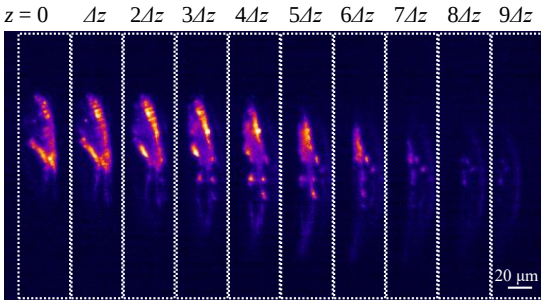

Fig. 2: Volumetric image of neurons of *Caenorhabditis elegans*.