PIXEL BASED Z-FOCUS CONTROL IN SCANNING MICROSCOPY

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Optical microscopes, arguably the most commonly used tools to characterize the building blocks of nature, are optimized for acquiring two-dimensional information. In particular, they are designed to capture images from the plane perpendicular to the optical axis, namely the XY plane. This represents a strong limitation, because the inherent 3D properties of samples require truly volumetric characterization tools [1]. Indeed, the particular phenomena of interest may occur along the axial direction, but remain hidden by the difficulty in retrieving signal from outside the imaged XY plane. Here, we propose an alternative approach for laser scanning microscopy (LSM) that provides an additional degree of freedom to these microscopes, enabling fast acquisition of information along the Z axis. Our method relies on coupling an acoustic varifocal liquid lens into a LSM setup in order to axially scan the focus at kHz rates [2]. Because the time to complete an axial scan can be in the microsecond time scale, multiple z-focus scans can be performed on a pixel by pixel basis. Thus, our systems operates in an analogous way to traditional resonant scanners, but instead of achieving high-speed X line scanning, we obtain fast Z-line scanning. Importantly, appropriate spatiotemporal demultiplexing of the collected photons, which can be performed by using a fast acquisition card [3], leads to the reconstruction of each pixel along the z-scanned line, as shown in Fig. 1. This approach leads to a suitable architecture in order to perform A scans, B scans or C scans [4], which can be of interest for fluorescence correlation spectroscopy, imaging flow cytometry or fast volumetric imaging, respectively. We demonstrate our technique by imaging a calibration sample made of fluorescent beads and by showing the performance of our technique when imaging a biological sample.

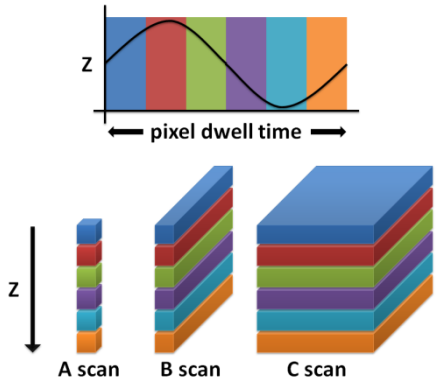

Fig 1: Simultaneous capture of multiple focal planes in A, B and C scans.

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