SCANNINGLESS DEPTH RESOLVED MICROSCOPY

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ABSTRACT: The ability to perform optical sectioning is one of the great advantages of laser-scanning microscopy, be it confocal or multiphoton microscopy. This comes however, at a cost of long image acquisition times, of an order of tens of milliseconds, due to the serial acquisition of data points. We show that by introducing spatiotemporal pulse shaping techniques it is possible to obtain full-frame depth resolved imaging without scanning, in a very simple setup. In multiphoton laser scanning microscopes, the depth resolution is achieved by spatially focusing an ultrashort pulse to achieve a high intensity at the focal plane [1]. Due to the nonlinear dependence of the signal on the intensity this results in superb rejection of the out-of-focus signal. In contrast, our method relies on temporal focusing of the illumination pulse. The pulsed excitation field is compressed as it propagates through the sample, reaching its shortest duration (and highest peak intensity) at the focal plane, before stretching again beyond it. This method is applied to obtain depth-resolved two-photon excitation fluorescence (TPEF) images of drosophila egg-chambers with nearly 100,000 effective pixels in a scanningless setup using a standard Ti:Sapphire laser oscillator. A single cross-section obtained by this method is compared to a full-frame multiphoton image obtained by plane-wave illumination in Fig. 1. This method can be particularly useful for multiphoton time-resolved studies such as full-frame depth resolved fluorescence lifetime imaging.

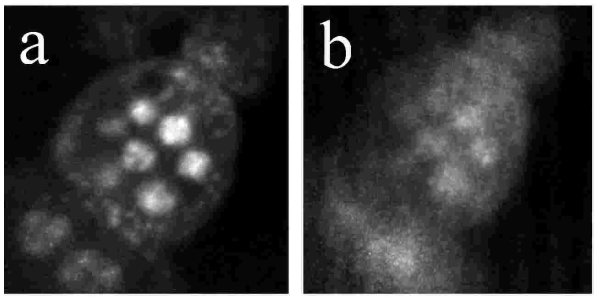

Figure 1: Scanningless images of a drosophila egg-chamber stained with DAPI. Image (a) was taken with a temporal lens in the microscope path. Image (b) shows, for comparison, a non-depth resolved image, illuminating with a plane wave. The area of each image is 140x140μm. The excellent rejection of the out-of-focus fluorescence in (a) is apparent.

1. W. Denk, J. H. Strickler, W. W. Webb, "Two-photon laser scanning fluorescence microscopy", *Science* **248**, 73-76 (1990).