Extended Depth of Field in Confocal Microscopy

Amaury Badon, Jerome Mertz
Department of Biomedical Engineering, Boston University,
44 Cummington Mall, Boston, MA 02215, USA
abadon@bu.edu

Abstract: We present a new type of confocal microscope that simultaneously image a specimen at four different depths, thus providing volumetric imaging at video rate. Our technique is an attractive tool for *in vivo* high-speed volumetric calcium imaging.

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1. Introduction

Confocal microscopy offers the ability to image a three dimensional sample thanks to its optical sectioning, or in others words its ability to discriminate between the desired in-focus and the out-of-focus signal. This feature relies on a point scanning scheme where the incident light is focused at a given location inside the sample, the light from this location is then propagated to an image plane where a pinhole is placed in front of a detector. A section of the sample is obtained by scanning the input beam in the transverse plane (X-Y) which can be done fast enough to study millisecond signals in brain imaging [1]. However, volumetric imaging in confocal microscopy requires an axial displacement (Z) of the sample or a remote focusing approach which can be order of magnitude slower or difficult to implement [2, 3]. Here, we propose a simple and new type of confocal microscope that simultaneously image a specimen at four different depths without additional mechanical movement, thus providing volumetric imaging at a rate fast enough for calcium imaging in *in vivo* mice.

2. Principle and proof of concept experiments

Inspired by differential confocal microscopy where the use of two detectors at different positions in the image plane improves the axial resolution in confocal microscopy [4], we propose here to use multiple detectors in the image plane to perform volumetric imaging at video rate (30 Hz). In practice, we have developed a confocal microscope with four detectors associated with different depths in the specimen plane. With a depth difference Δz=25 μm between two consecutive detectors, we achieve a total depth of field of the order of 100 μm.

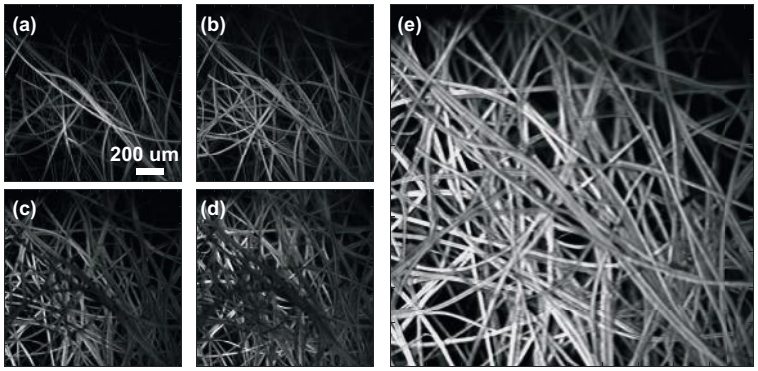

Fig. 1. Multi-Z confocal images of stained lens paper. (a-d) Simultaneously acquired confocal images corresponding to depth z=+37.5, +12.5,-12.5 and -37.5 μm. (e) EDOF image obtained by combining the four previous images.

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As a first proof of concept, we stained lens paper with fluoresceine. From figure 1 (a-d), the four images obtained simultaneously are clearly associated with different depths inside the specimen. Additionally, an extended depth of

field (EDOF) confocal image can be obtained by a simple sum of the different images [5]. The resulting image contains features over the whole volume while keeping both the diffraction limited lateral resolution and the axial sectioning of a confocal microscope (see 1 (e)).

3. Preliminary *in vivo* results

To go further and demonstrate the assets of our multi-Z confocal microscope we imaged jRGECO-labeled neurons in an *in vivo* mouse. From the figure 2 (a,b), we see the benefit to image simultaneously a whole volume. While some neurons are present on both images, the two images mostly display different neurons. As a result, the EDOF image allows to see a larger number of neurons compare to a regular confocal microsocope. Additionally, calcium transient on indivial neurons over a large volume can be recorded at video rate without being sensitive to axial motion as the depth of field of the EDOF is of the order of 100 μm (see Fig.2 (d)).

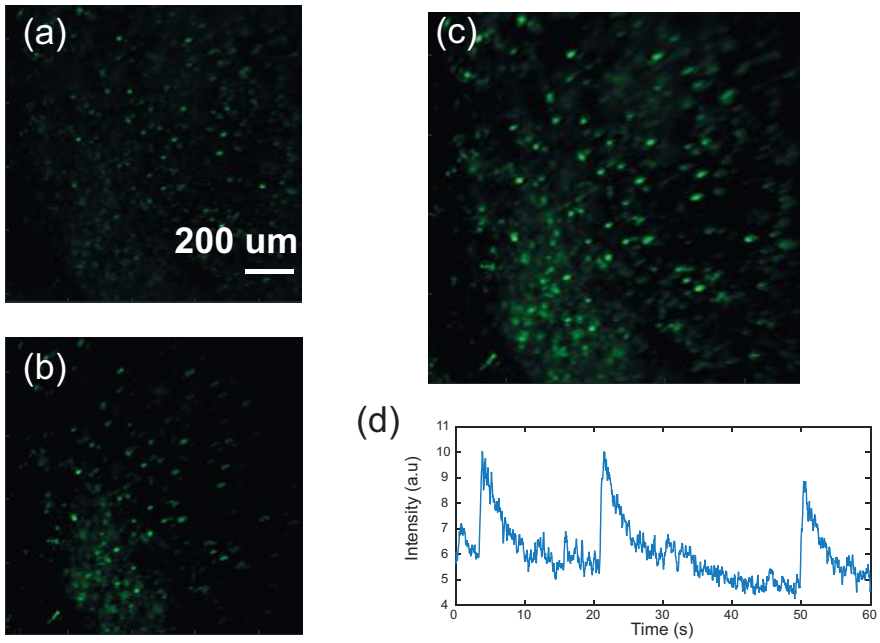

Fig. 2. *In vivo* volumetric functional imaging of mouse brain. (a,b) Image associated with z=+37.5 and z=-37.5 um. (c) EDOF image obtained by combining the images at different depths. (d) Typical calcium transient of an individual neuron measured over one minute.

In conclusion, these preliminary results demonstrate the ability of the proposed approach to perform high speed volumetric imaging *in vivo* by simultaneously recording four confocal images at different depths.

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