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« Deep-3D-multiphoton imaging of whole biological structures with a computational assistance and the pipeline FAMOUS »

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We have developed a new instrumental and computational pipeline based on multiphoton microscopy [1] imaging thick biological samples on whole organs without slicing or labeling procedure. This strategy delivers a new viewpoint on the tested biological structures [2]. For the post-processing strategy, we are developing a specific regularized image restoration algorithm [3, 4]. The first step is to led an accurate 3D estimation of the point-spread function (PSF) of the instrument all over the entire depth of the structures [5-6]. This latter approach is named FIGARO. An advanced model fitting algorithm measure the depth-variant distortions of the image resulting from the combination between the heterogeneities of the structure resulting into scattering and absorption phenomena and distortions associated to the instrument.

The resulting approach is a pipeline called FAMOUS whose performance is evaluated in case of a heterogeneous medium constituted by a whole mouse muscle. The whole EDL (Extansum Digitorum Longus) is resected from tendon to tendon, is unsliced and is imaged with a multiphoton microscope thanks to the emission of a second harmonic generation (SHG) signal, emitted from the myosin assembly of muscle. The images of the PSF are recorded simultaneously in the second recording channel of the multiphoton microscope. Thanks to the pipeline FAMOUS, optical artefacts resulting from the acquisition chain are evaluated, including medium heterogeneities in the 3 dimensions. We are keeping a specific attention to the axial structuration of myosin whose structuration is lost in blur and noise due to the optical resolution lost especially in case of a deep 3D imaging. The restored images reveale for the first time an axial level of structuration unexpected until now.

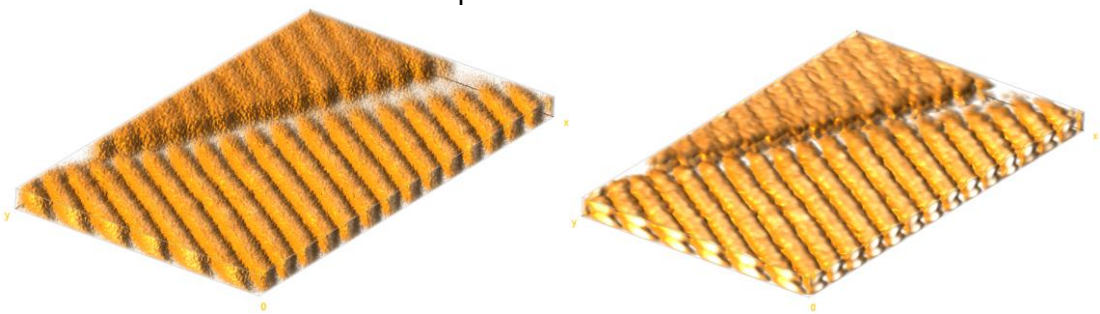

Figure 1: 3D image of a mouse muscle structure. Left. Raw acquisition of MPM. Right. Restored image with FAMOUS computational and instrumental pipeline.

Striated skeletal muscles are organs with a highly ordered structure resulting in an optically heterogeneous medium. The deep imaging of SHG from myosin in a whole mouse muscle, unsliced, lose the axial information of the striated structuration. Figure 1 presents the raw image (A) and the result of the pipeline FAMOUS (B). Figure 1 reveals the high impact of FAMOUS pipeline in the image quality, here revealing the fibrillar organization of the myosin of a wild mouse muscle.

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