

« FAMOUS pipeline: multiphoton microscopy and computational strategy for 3D imaging of biomedical sample »

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We present the new instrumental strategy based on multiphoton microscopy [1] approach computationally aided for improving visually the image quality of thick biological samples delivering a new viewpoint on biological structures [2]. The post-processing approach relies on a regularized image restoration algorithm [3, 4], fed with an accurate 3D estimation of the point-spread function (PSF) of the instrument all over the entire depth of the structures [5-7]. This latter step amounts to measure, thanks to an advanced model fitting algorithm, the depth-variant distortions of the image resulting from the combination between the instrumental contribution and the medium heterogeneities.

The performance of the proposed pipeline, called FAMOUS, is evaluated in two situations: in case of a homogeneous medium and in case of a heterogeneous medium constituted by a whole mouse muscle. The second harmonic generation (SHG) signal, emitted from the myosin assembly of muscle is recorded. Optical artefacts resulting from the acquisition chain including medium heterogeneities in the 3 dimensions are estimated with the specificities related to each sample. In case of a raw acquisition of SHG signal from myosin of muscle, the axial structuration of myosin is lost in blur and noise due to the deep 3D imaging. Thanks to our image restoration pipeline, the image resolution theoretically expected is now revealed for the first time. Figure 1A presents the raw acquisition of SHG, recorded at 400 nm with an excitation at 800 nm with a Titanium-doped sapphire laser system. The axial visualization of the muscle organization presently disclosed was never shown until now due to the lack of adapted instrumental solution.

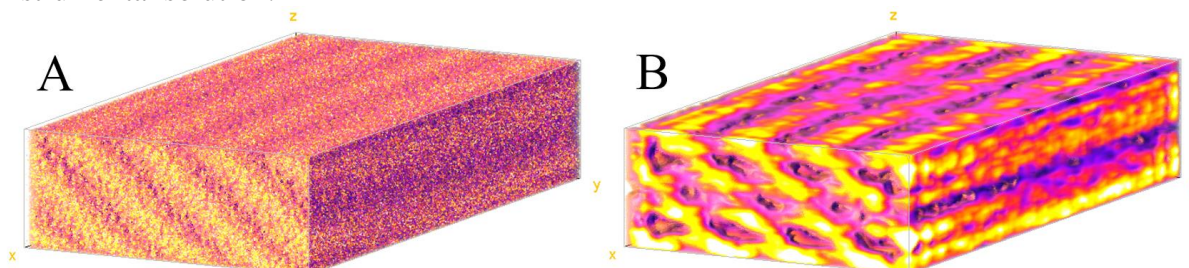


Figure 1: 3D image of a mouse muscle structure. A. Raw acquisition of MPM. B. Restored image with FAMOUS computational and instrumental pipeline.

Striated skeletal muscles are optically heterogeneous media presenting a highly ordered structure. SHG from myosin in a whole mouse muscle is visualized in the sample depth, on the whole muscle unsliced. Figure 1 illustrates the raw acquisition (A) and the image generated with the pipeline FAMOUS (B). The high impact of FAMOUS pipeline in the image quality reveals the fibrillar organization of myosin of muscle.

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