



**HAL**  
open science

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

Claire Lefort, Mathieu Chalvidal, Alexis Parente, Véronique Blanquet, Henri Massias, Laetitia Magnol, Emilie Chouzenoux

► **To cite this version:**

Claire Lefort, Mathieu Chalvidal, Alexis Parente, Véronique Blanquet, Henri Massias, et al.. Deep-3D-multiphoton imaging of whole biological structures with a computational assistance and the pipeline FAMOUS. Physics and Biological Systems 2021, Jun 2021, Grenoble, France. hal-03274262

**HAL Id: hal-03274262**

**<https://hal.science/hal-03274262v1>**

Submitted on 8 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Claire Lefort<sup>1</sup>, Mathieu Chalvidal<sup>2</sup>, Alexis Parenté<sup>3</sup>, Véronique Blanquet<sup>3</sup>,  
Henri Massias<sup>1</sup>, Laetitia Magnol<sup>3</sup>, Emilie Chouzenoux<sup>4</sup>

Affiliation: <sup>1</sup> XLIM Research Institute, CNRS UMR 7252, Université de Limoges, France

<sup>2</sup> CerCo, UMR CNRS 5549, Université Paul Sabatier, Toulouse, France

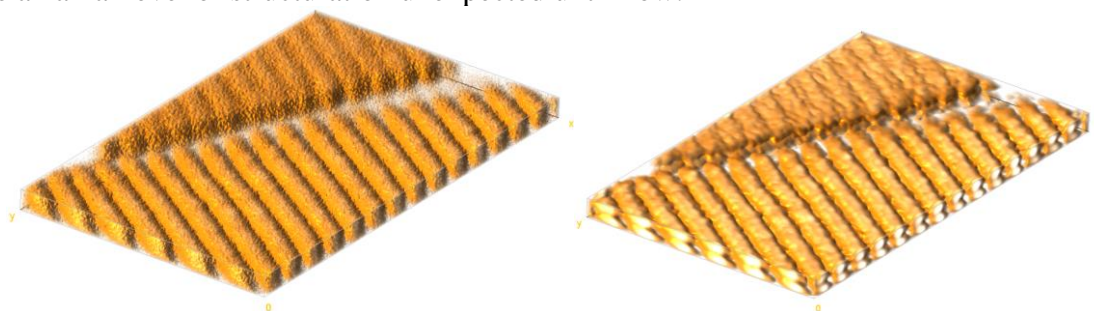
<sup>3</sup> PEIRENE, EA7500, USC 1061 INRA, Université de Limoges, France

<sup>4</sup> Center for Visual Computing, CentraleSupélec, Inria Saclay, Université Paris-Saclay, France

[claire.lefort@cnrs.fr](mailto:claire.lefort@cnrs.fr)

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 lead 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 reveal 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.

[1] A. M. Larson, Nature Photonics 5 1, doi.org/10.1038/nphoton.an.2010.2 (2011)

[2] C. Lefort et al, J. Phys. D: Appl. Phys. **54** 274005, doi.org/10.1088/1361-6463/abf8f2 (2021)

[3] Hortholary et al., Microscopy Res. And Tech., doi.org/10.1002/jemt.23712 (2021)

[4] B. de Monvel et al, Biophysical journal. 85. 3991-4001, 10.1016/S0006-3495(03)74813-9 (2004)

[5] Chouzenoux et al., J. of Math. Imag. and Vis. 61 (7), 1037-1050, doi.org/10.1007/s10851-019-00884-1 (2019)

[6] W. Liu et al., J. Biomed. Opt. 18 26005, 10.1117/1.JBO.18.2.026005 (2013)