Intravital imaging of biomaterials integration in tissues using a multicolor multiphoton microscope: Applications in Mechanobiology

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Different mechanisms are triggered when tissue is exposed to a biomaterial. The success of the biomaterial targeted process, like the release of chemicals, promoted angiogenesis, tissue regeneration, etc. depends on its integration in the tissue [1]. Studying this interaction in vivo requires the ability to image simultaneously deep immersed proteins and biomaterials with high resolution and low damage. Several methods offer solutions but only multiphoton microscopy (MM) has the ability to image with high resolution deep inside the sample. Why is not MM more extensively applied as a platform for investigating biomaterial integration in vivo? The high cost of the typical source for multiphoton microscopy is a clear limitation. Furthermore, imaging several channels simultaneously becomes out of reach for most of the labs.

Here, I present a novel and low cost multiphoton imaging system that allows simultaneous excitation of several fluorophores and non labeled sources like second harmonic (SHG), produced by collagen fibers, and third harmonic generation (THG), produced at the interfaces. In the last years similar systems [2] based on highly nonlinear photonic crystal fibres for generating more broadband pulses have been proposed but we present, for the first time to our knowledge, results for deep imaging (more than 200 um) of tissues with such source. Fluorescently labeled electrospun fibers of polylactic acid (PLA) forming a network were introduced in the chorioallantoic membrane (CAM) of the chicken embryo. After 4 to 7 days the chicken was injected with fluorescent labels and time-lapse images were taken for several hours (Fig. 1).

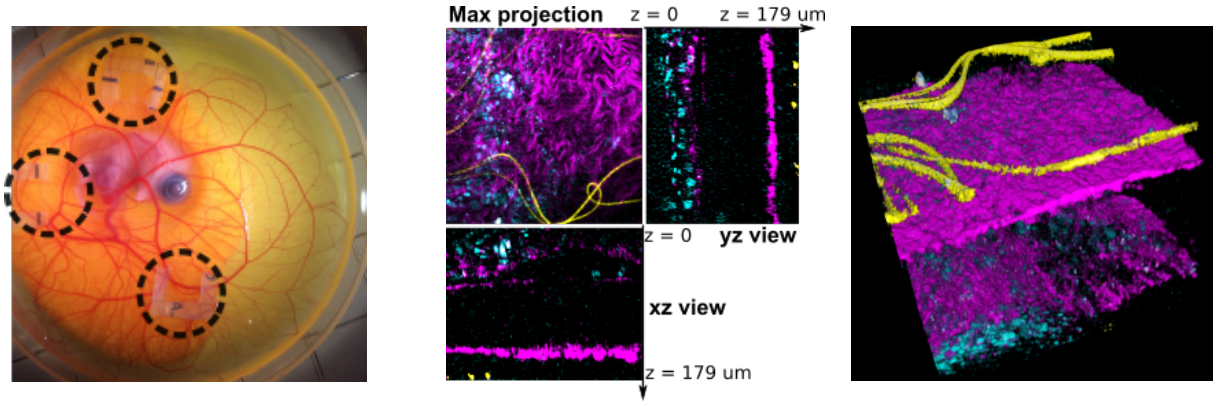

Fig. 1 Left: PLA fibers immersed in the CAM of the chicken embryo. Central: Maximal projection and lateral views of the MM images of the PLA fibers immersed in the CAM of the chicken embryo after 5 days. 3D image of the same fibers showed on the left. Nuclei labeled with Hoechst 33342, SHG from collagen structure, PLA fibers labeled with Rhodamine.

Using this novel platform we visualized PLA fibers inside the CAM simultaneously with nuclei, capillaries and the extracellular matrix. We also followed the dynamics of the interplay of fibers/cells/extracellular matrix for several hours with no observable damage.

References

[1] K. G. Defrates "Protein-based fiber materials in medicine: A review" Nanomaterials 2018, **8**, 457
[2] S. You et al. "Intravital imaging by simultaneous label-free autofluorescence-multiharmonic microscopy" Nat Comm. 2018, 9, 2125..