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Application of non linear optical imaging as a tool for biomedical studies

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Introduction

Nonlinear optical (NLO) imaging is a recent powerful tool in the field of microscopy because of its sensitivity to specific molecules or biological structures. For instance, multiphoton fluorescence (2 or 3 photons), second and third harmonic generation, and coherent anti-Stokes Raman scattering (CARS) has allowed the development of fast, safe and costless biomedical imaging to improve the diagnostic in a broad range of clinical disciplines: gastroenterologists, dermatologists, neurologists...

Indeed, the use of light for imaging has been these last decades largely improved by the emergence and development of ultrafast lasers, which allow deeper imaging in biological tissues with higher spatial resolution.

In this work, we expose different medical or biological samples that have imaged by multiphoton microscopy in comparison with standard classical histology.

Methods

Multiphoton fluorescence Anesthetized mice and samples were positioned on a stage of a customized Olympus multiphoton microscope BX61WI/FV1200MPE with a 25X immersion objective (1.05NA, 2.0mm working distance) coupled with a tunable femtosecond Ti:Sapphire pulsed laser (Chameleon Ultra II, Coherent) for the excitation. Image stacks were taken at 2µm steps, acquired under 810nm excitation for second harmonic generation (SHG, collagen) and auto-fluorescence (elastin) wavelength with FluoView FV1200 software (v4.1.1.5, Olympus). Rhodamin Dextran 70K was also injected into the bloodstream and samples were imaged under 870nm excitation. The different components of the emitted light from the sample were separated using a dichroic mirror (570nm) and detected by a pair of photomultiplier tubes preceded by fluorophore specific emission filters (BA 575-630 for elastin, 405/10 for SHG). The images obtained were analyzed with Imaris software (Bitplane AG) or Fiji/ImageJ (NIH).

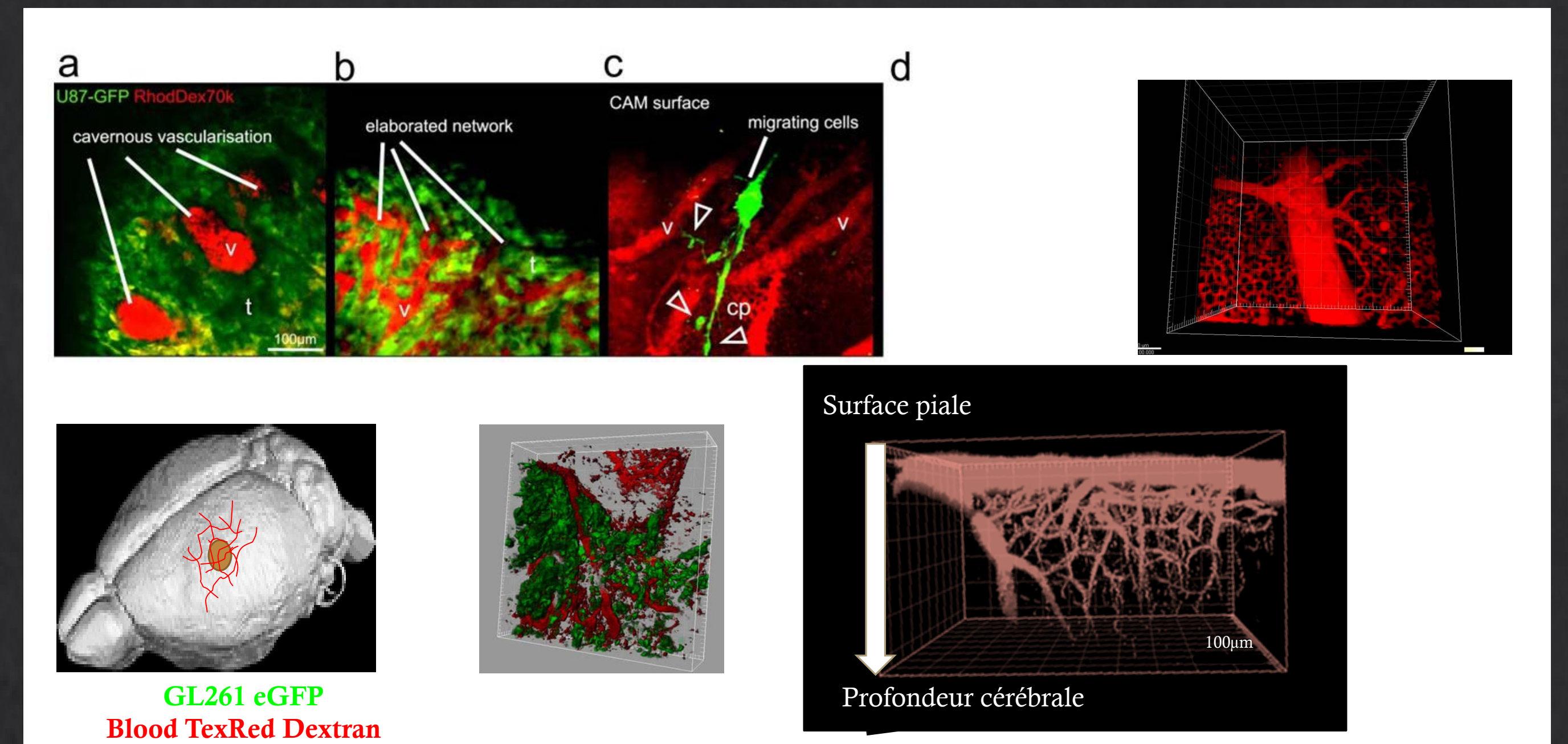


Fig.4 Graft of U87 cells in quail or GL261 in mice shows complex irrigation patterns that can be depressed by nsPEF. Intravascular rhodamine dextran and GFP-U87 grafted on CAM were observed with multiphoton microscope and average Z-stacks are (a-c, e-i). v = vessels, t = tumor mass, cp = capillary plexus. Scale bar in (a) = 100µm applied to all.

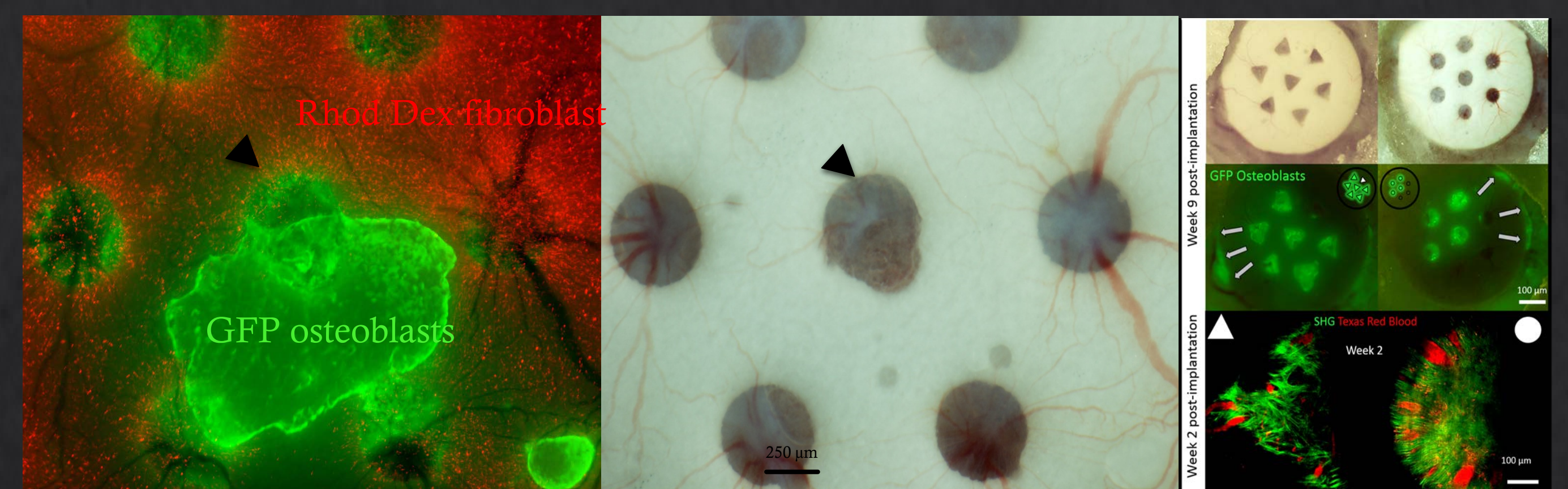


Fig.5 Development of a new generation of calcium phosphate bioceramics for bone tissue engineering in mice. 3D multiphoton microscopy reveals by Second Harmonic Generation (SHG) the extracellular matrix (collagen, green) in the round and triangular pores in a vasculature network (red)

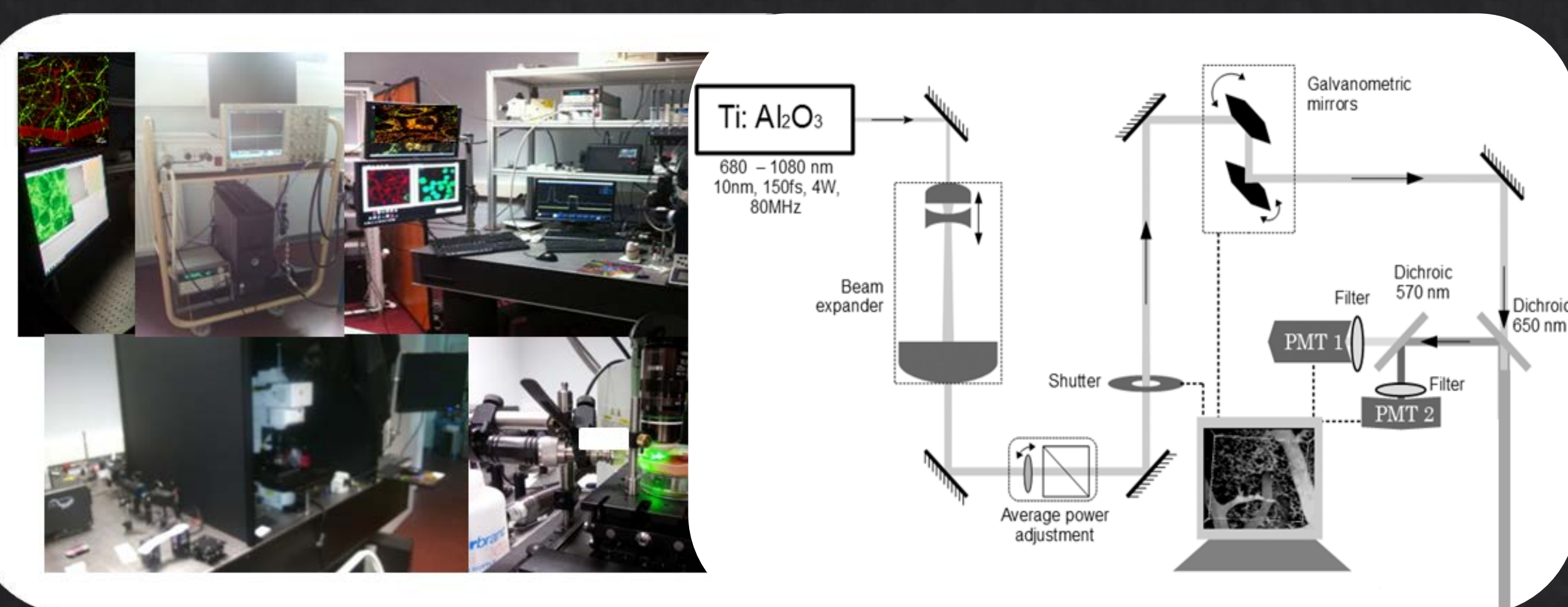


Fig.1 Complete *in vitro* or *in vivo* exposure system coupled to a multiphoton imaging station for real time observation

Histology and Immunohistochemistry

Tissues were collected and embedded in paraffin. Sections (12 µm) were cut, stained with hematoxylin-eosin-safran (HES) and for immunohistochemistry (CD3, CD4, CD8) on an automate BenchMarck XT (Roche®, Meylan, France), to be analyzed for the presence of tumor nodules.

Results

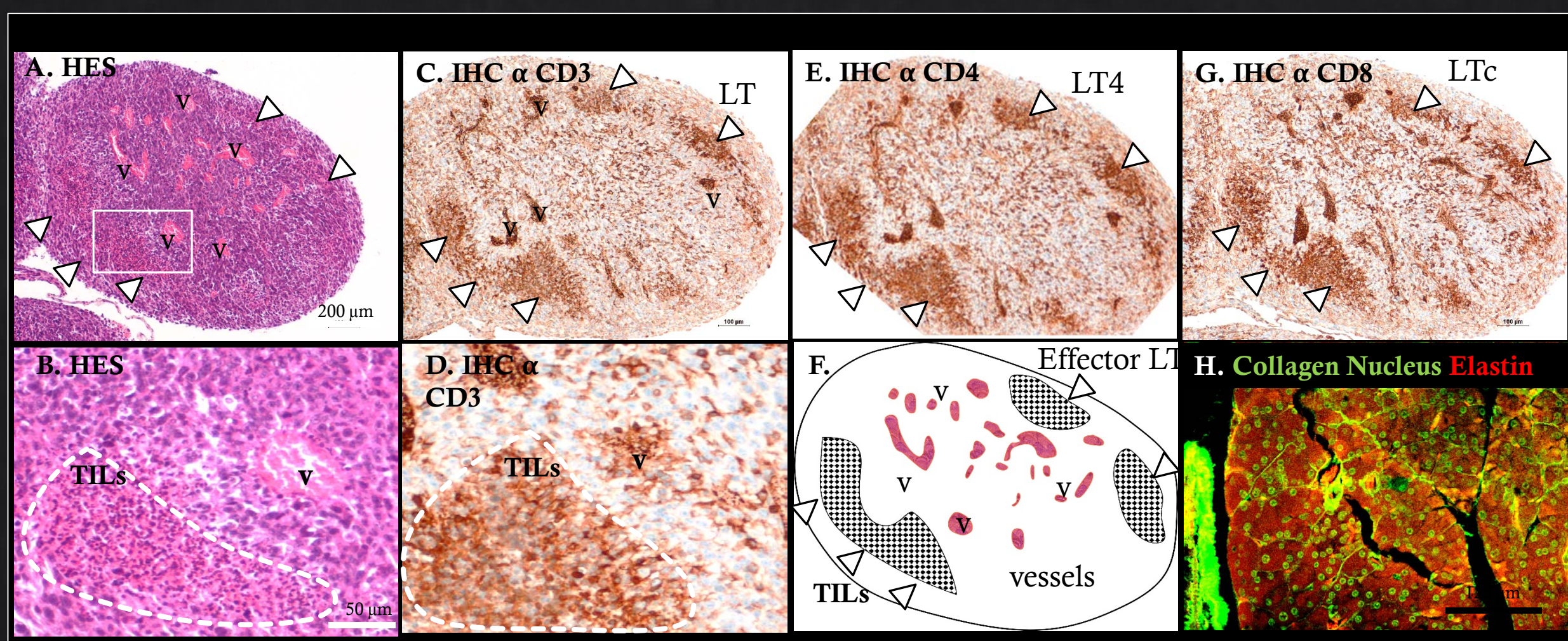


Fig 2. Grafted CPC nodules in mice reveal high number of cells including Tumor infiltrating Lymphocytes (TILs) among a complex tumoral micro-environment (high vascularization, poor fibrosis, dense tissue). Scale bar in A applied to C,E,F,G and in B applied to D

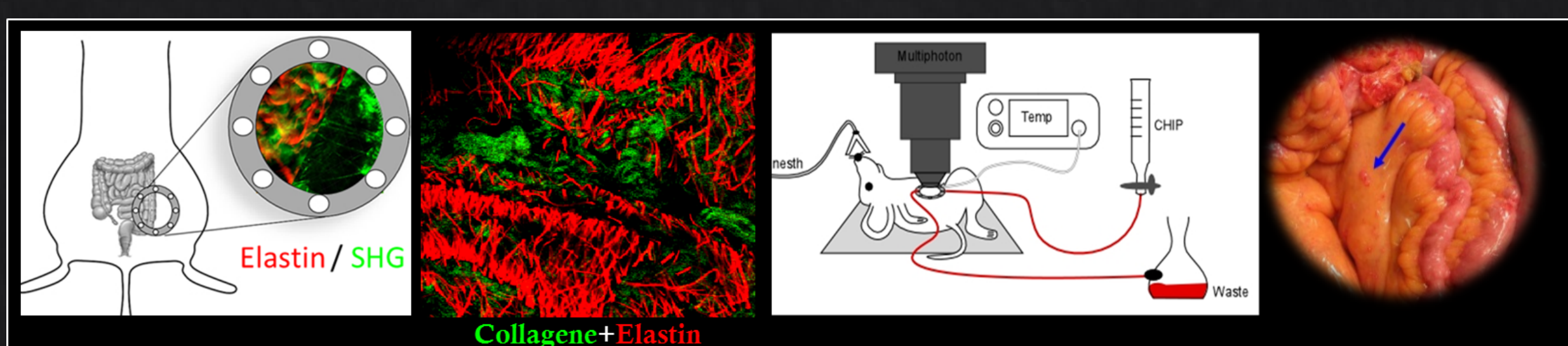


Fig 3. *In vivo* multiphoton microscopy allows the follow-up of digestive tumoral development on peritoneum. Different treatments (electric therapy, chemotherapy or HIPEC therapy) can be evaluated in real-time by imaging in a mouse model.

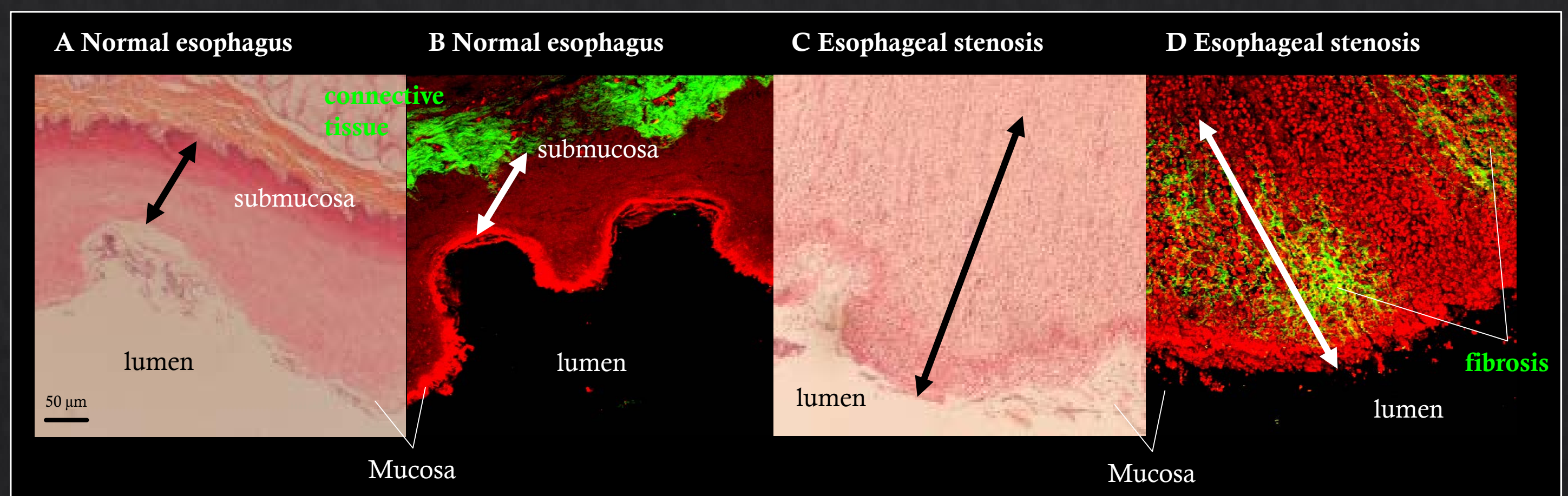


Fig.6 Treatment of esophageal mucosal lesions is associated with a risk of esophageal stenosis, leading in this pig model to acute inflammation, deep ulcers, local submucosal fibrous connective tissue proliferation, collagen deposition, esophageal wall fibrosis, and even esophageal stricture formation.

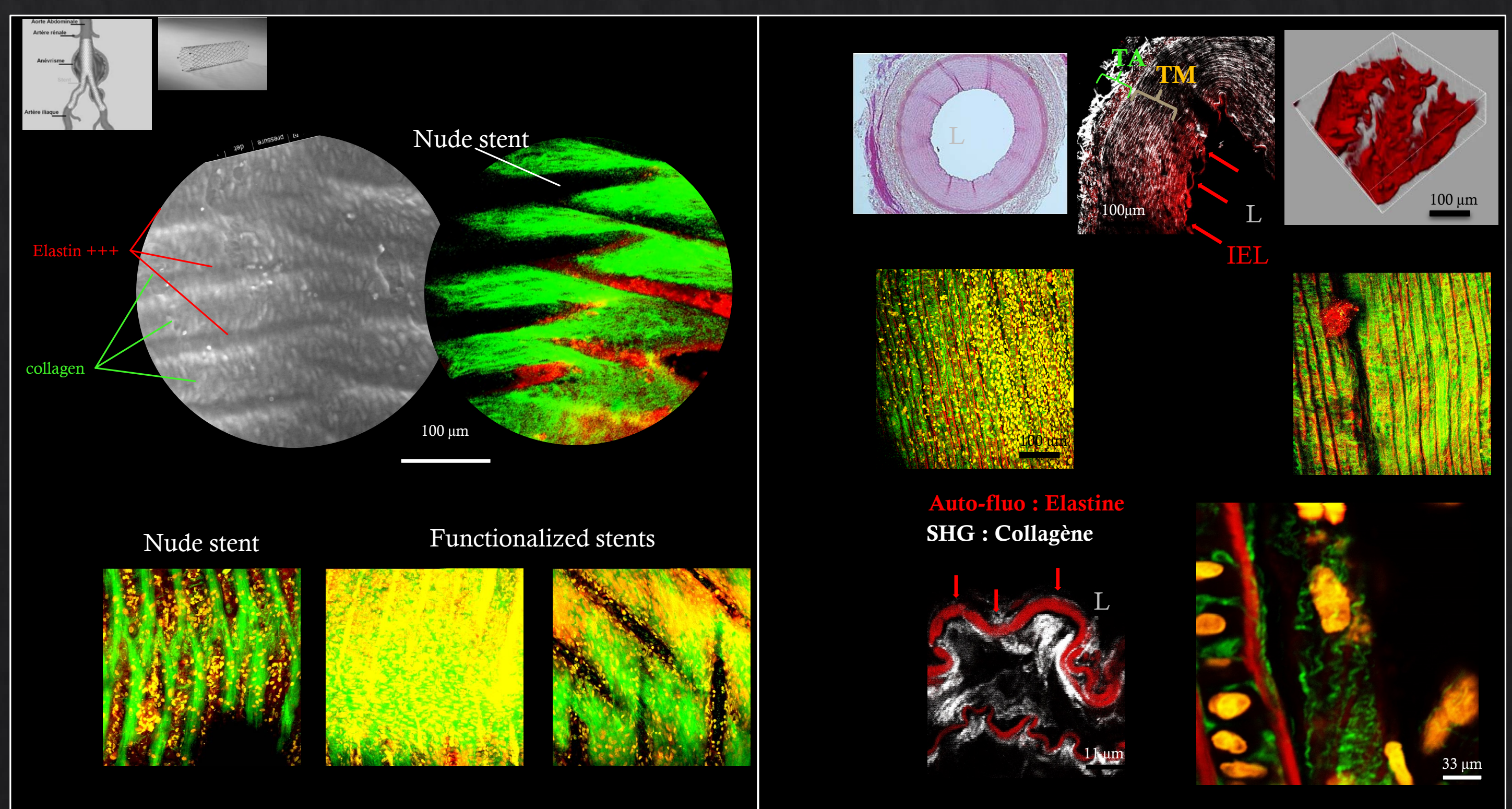


Fig.7 Optimisation of vascular stent biocompatibility «flow-diverters» in a rabbit model. Abdominal aorta is observed for collagen (SHG in green), elastin (auto-fluorescence in red) and cells (DAPI positive nuclei in yellow).

Fig.8 Endovascular anatomy inspection after the passage of diagnostic or therapeutic micro-instruments to the site of interest by intravascular in a pig model. Artery are observed post mortem for collagen (SHG in green or grey), elastin (auto-fluorescence in red) and cells (DAPI positive nuclei in yellow).

Conclusions - Future studies

In this work, we expose the advantages provided by multimodal NLO imaging of *in vivo* tissues and medical biopsies from our medical center over classical Anatomical pathology analysis (e.g. HES). The ability to employ both label and label-free imaging on peritoneum tissue, carcinomas nodules, vascular stent in aorta or fibrosis, that are complex medical samples, will drive to better understand pathology processes and the efficiency of their therapies.