Organ clearing and fast non-linear microscopy to investigate biodistribution of gene or cell therapeutic agents for neuromuscular diseases with exploration of GFP and Harmonic signals

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3D imaging of the whole intact organ at the microscopic level is essential to investigate disease-associated morphological changes or to assess a therapeutic efficacy. However, this tissue 3D exploration is highly limited because of the opacity of the investigated tissues. Developments of numerous methods in tissue clearing represent innovative solutions for investigation of the organs at the cellular scale [1]. Furthermore, technological advances in non-linear microscopy with the input of High Defined Fast Resonant Scanner 1Kx1K allow rapid acquisition in depth with high resolution to detect Harmonic Nanoparticles (HNPs) in whole organ and low photobleaching of GFP fluorescence.

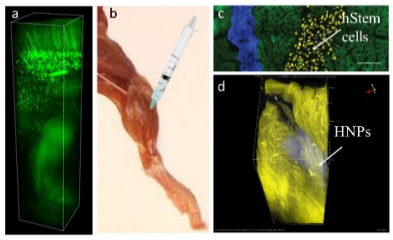

Figure 1 : a) Cleared GFP mouse brain using passive Clarity method and mounted in RapiClear. b) Injection of HNPS labelled human Stem Cells in gastrocnemius of mouse. c) Yellow immunolabeling of labelled HNPs hStem Cells on muscle cryosection. d) Detection of HNPS in whole cleared muscle of mouse.

In this work, we demonstrate for the first time how it is possible to preserve GFP used as reporter of gene expression and harmonic signals generated from collagen and HNPs [2], imaged on few mm in cleared samples by using aqueous solution with high refractive index. To do that, 2,2"-Thiodiethanol (TDE) and RapiClear reagent were used with a defined refractive index according to the clarified organ. Fluorescence from GFP and Second Harmonic Generation (SHG) from collagen were imaged in cleared brain, spinal cord, skeletal muscle (Figure 1) and liver on 5 to 1 mm of thickness. Labeled cells with HNPs were tracked and imaged in whole cleared *Gastrocnemius* muscle of mice with fast A1R HD MP+ multiphoton microscope. These methods are very promising tools to assess new therapeutic strategies on neuromuscular diseases in animal models, using AAV vector encoding fluorescent proteins and HNPs-labeled stem cells.

References;
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