Multiphoton/Resonant scanner HD/RapiClear® for 3D investigation of Central Nervous System

Romain Fleurisson, Johan Deniaud, Elina Varaillon, Julien Pichon, Laurence Dubreil, Marie-Anne Colle
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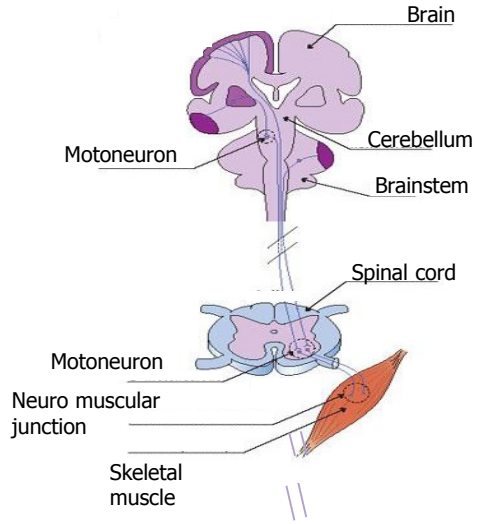
INTRODUCTION

3D imaging of the central nervous system is essential to investigate **morphological changes** of **diseases** or to assess the **efficacy of a treatment** (Denk et Horstman, 2004). However, microscopic 3D exploration is limited due to the opacity of tissues and light scattering. Technological advances in microscopy and **tissue clearing** represent innovative solutions for **investigating organs** (*Hama H. et al., 2011 ; Chung K. et al., 2013*). These methods are promising tools to **assess new therapeutic strategies** on neurodegenerative diseases developed by our UMR703 Research Unit using animal models and AAV vector encoding fluorescent proteins.

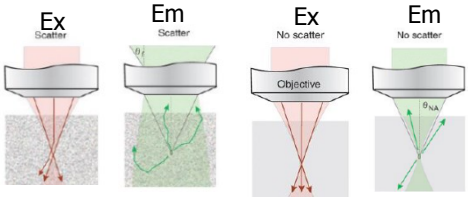


Organisation of the nervous system (www.institut-myologie.org)
GFP expression in adult mice rAAV9-eGFP (Bey et al., 2016)
Multiphotonic and Harmonic generation microscopy ...(Dubreil et al., 2017)

In this work, we demonstrate that combination of MP imaging, Resonant scanner and RapiClear solutions is efficient for GFP and harmonic signals investigations in brain, spinal cord and muscle in depth.

• Clearing method principle

Tissue Opacity | *Tissue clearing*



Light scattering due to variant RI in tissue
Optics in instruments applications in Biology and Medicine, JP Goure, John Wiley & Sons. 2013 - 352

Medium	Refractive Index RI
Proteins	1.6
Melanin	1.7
Cytoplasm	1.37
Membrane	1.46

• Four main clearing methodologies

1.Organic solvents

which dehydrate sample (BABB, 3DISCO, iDSCO) (Ertürk et al., 2012, Renier et al., 2014)

2.Aqueous solutions

with high refractive index (SeeDB, FRUIT, TDE) (Ke MT et al., 2013) (Costantini I. et al., 2015)

3.Hyper-hydrating solutions

that eliminate lipids (CUBIC, Scale)

4.Tissue-transforming processes

such as the production of a polyacrylamide gel and electrophoresis (CLARITY) (Feng Y. et al., 2017)

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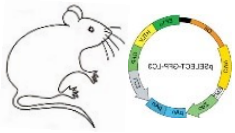
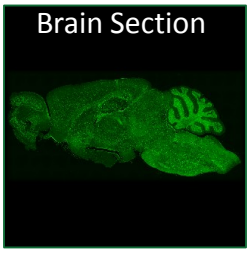
MATERIAL AND METHOD

Animal model for GFP fluorescence and Harmonic signals investigation

6neo/6neo Pompe mice expressing GFP-coupled LC3 protein, markers of autophagy.



PBS/PFA Perfusion



Clearing method investigated

Samples :
Brain, spinal cord, muscle
Thickness : 500µm to 5 mm

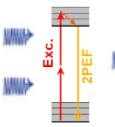
PBS washings
2% PBS Triton X100 at RT 3 days
RapiClear 1.52 clearing at RT 2 hours – O/N-few days

SunJin Lab
Optical Clearing Innovation
RapiClear® 1.52

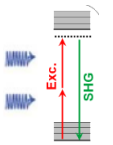
Brain | Spinal cord | Muscle

Multiphotonic excitation

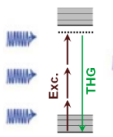
Fluorescence and Harmonic generation



Fluorescence
(GFP, Alexa immunolabeling)



Second Harmonic Generation
(collagen, myosin)



Third Harmonic Generation
(lipidic vesicules, myelin)

3D Image acquisition

CFI Plan Apo 10xC Glyc

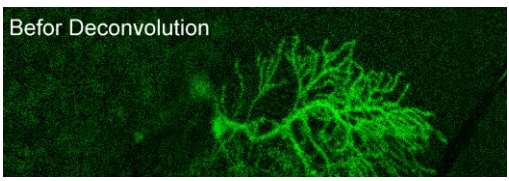
Mag	NA	WD
10x	0.5	5.5mm

Research, Confocal, Multiphoton

- A1 RMP NiKon
- Insight Deepsee laser 680-1300 nm
- Resonant scanner 1K
- 3 GaAsp detectors
- Objective X10; NA 1,1; WD 5.0 mm

NIS-Elements

- **Deconvolution**
- Increase signal noise ratio



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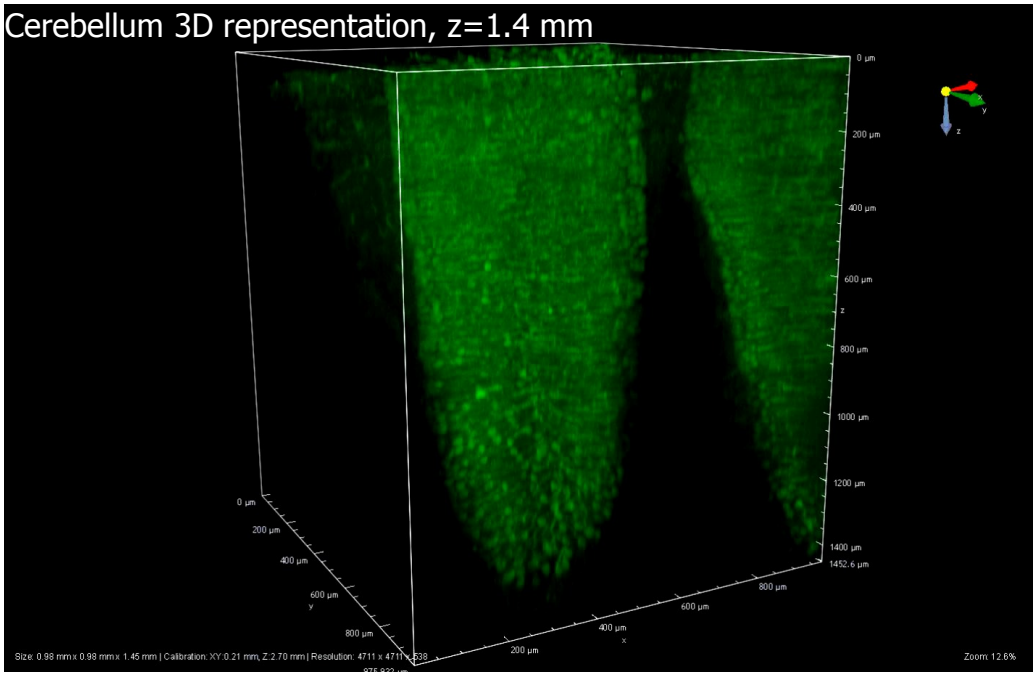
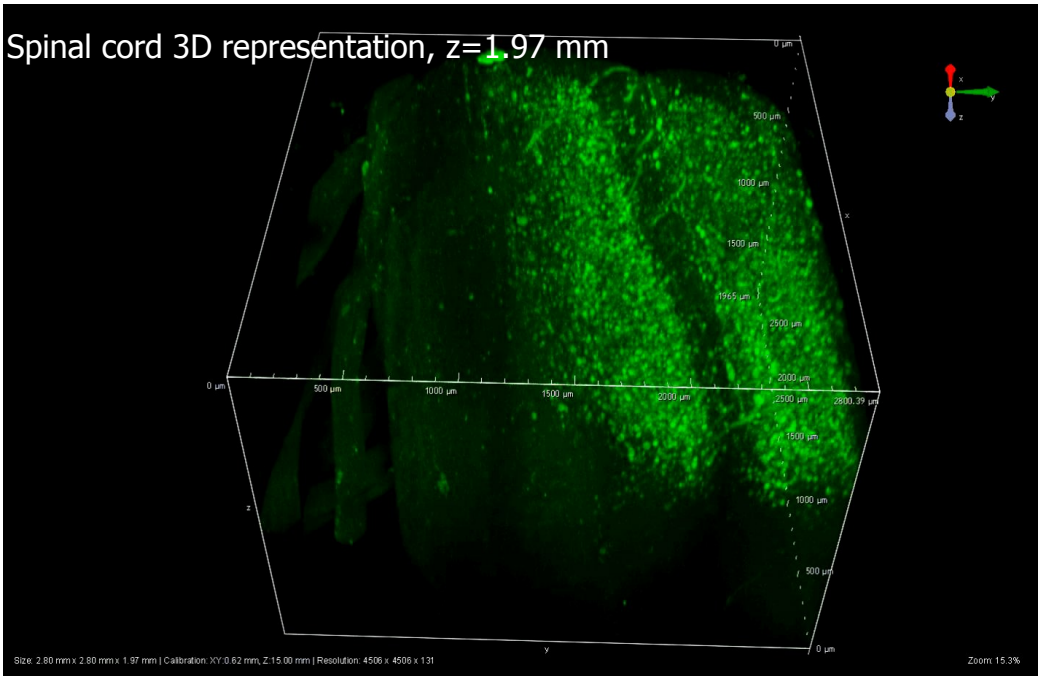
RESULTS

Optical clearing with RC 1.52, λ exc 950 nm

Cerebellum 3D representation, z=1.4 mm



Spinal cord 3D representation, z=1.97 mm



GFP-LC3 transgenic Mouse

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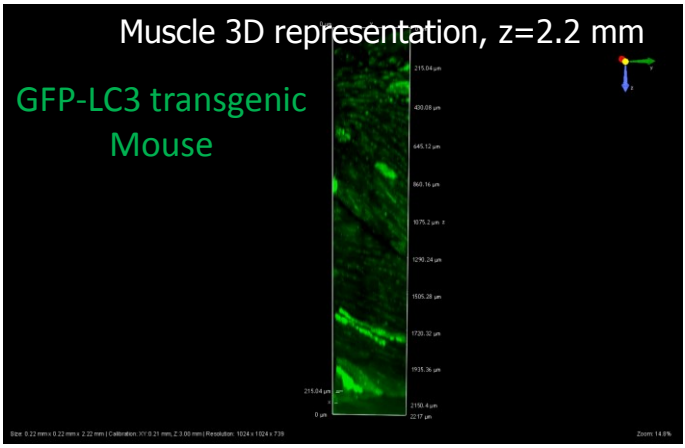
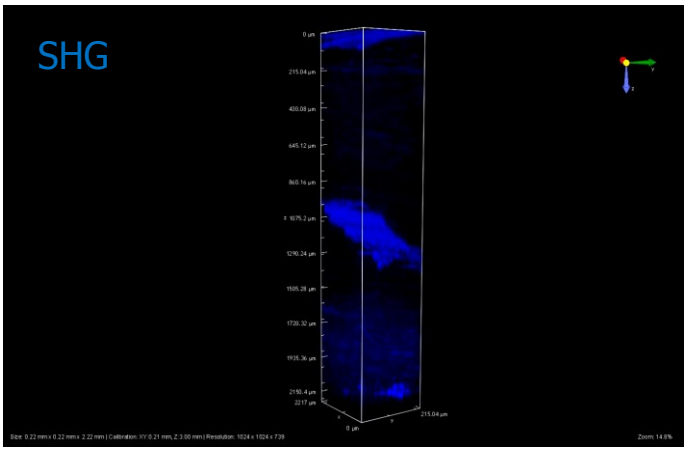
Muscle Optical clearing with RC 1.52, λ exc 950 nm





CONCLUSION

In the past decades, a large number of methodologies were introduced for tissue clearing with specific advantages and disadvantages (Azaripour et al., 2016). Here, we demonstrate that RapiClear is a good compromise between clearing efficacy, time consuming and preservation of endogenous fluorescence and harmonic signals in brain, spinal cord and muscle with 2 mm deep investigation. Combination between biphotonic microscopy, resonant scanner 1K and organ clearing solution is promising tool for fluorescent protein or cells tracking in whole organ to assess therapeutic strategies.

ACKNOWLEDGMENTS

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