Organ clearing and biphotonic microscopy to investigate the central nervous system and peripheric organs with preservation of GFP and Harmonic signals

Fleurisson, R.[1], Pichon, J.[1], Deniaud, J.[1], Dubreil, L.[1] and Colle, M.[1]

[1] UMR703 PAnTher INRA Oniris Nantes Atlantic College of Veterinary Medicine Food Science and Engineering, France

3D imaging of the central nervous system (brain, spinal cord) and peripheric organs as skeletal muscle and cardiac muscle at the microscopic level is essential to investigate morphological changes of various diseases or to assess the efficacy of a treatment (Denk et Horstman, 2004). However, this 3D exploration of the tissues is very limited because of the small volumes and the opacity of the tissues, which does not allow the passage of the light. Technological advances in fluorescence bio-imaging with the development of numerous methods in tissue clearing represent innovative solutions for exploring the organs at the cellular level (*Hama H. et al., 2011; Chung K. et al., 2013; Chenchen P. et al., 2016*). The clearing methods can be classified according to the following categories: organic solvents which dehydrate the sample (BABB, 3DISCO) (Ertürk et al., 2012), aqueous solutions with high refractive index (SeeDB, FRUIT, TDE) (Ke MT et al., 2013) (Costantini I. et al., 2015), hyper-hydrating solutions that eliminate lipids (CUBIC, Scale) or tissue-transforming processes such as the production of a polyacrylamide gel and electrophoresis (CLARITY) (Feng Y. et al., 2017).

We demonstrate for the first time how it is possible to preserve both native GFP and harmonic signals generated from collagen and myelin, image 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 prepared with a defined refractive index according to the clarified organ. Furthermore, we demonstrated the input of the resonant scanner coupled to sensitive detectors to image thick cleared samples with visible light and / or infra-red light allowing rapid acquisition and therefore low photobleaching. Harmonic and fluorescence signals were imaged in cleared brain, spinal cord and muscle on 5 to 1 mm of thickness (Figure1).

These methods are very promising tools to assess new therapeutic strategies on neurodegenerative diseases using animal models and AAV vector encoding fluorescent proteins.

Acknowledgements: This work was supported by a grant from Investissement d'Avenir (ANR-11-INBS-0011-NeurATRIS: A Translational Research Infrastructure for Biotherapies in Neurosciences). The support by Pays de la Loire is gratefully acknowledged. Images have been performed with an A1R-MP biphotonic microscope from the APEX platform UMR703 INRA Oniris, Center of Excellence Nikon Nantes, France. Thanks to SFmu for his financial help to participate to this congress.

References

Denk W. and Horstmann H. (2004) PLoS Biol 2(11) e329.

Hama H, Kurokawa H, Kawano H, Ando R, Shimogori T, Noda H, Fukami K, Sakaue-Sawano A, Miyawaki A. (2011) Nature Neuroscience14:1481-88.

Chung K, Chung K1, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AK, Pak S, Bernstein H, Ramakrishnan C, Grosenick L, Gradinaru V, Deisseroth K. (2013) Nature 216;497(7449):332-7.

Pan C, Cai R, Quacquarelli FP, Ghasemigharagoz A, Lourbopoulos A, Matryba P, Plesnila N, Dichgans M, Hellal F, Ertürk A. (2016) Nature Methods 13:859-867

Ertürk A, Bradke F. (2012) J .Exp. Neurol 242:57-64.

Ke MT, Fujimoto S, Imai T. (2013) Nat Neurosci.16(8):1154-61.

Costantini I, Ghobril JP, Di Giovanna AP, Allegra Mascaro AL, Silvestri L, Müllenbroich MC, Onofri L, Conti V, Vanzi F, Sacconi L, Guerrini R, Markram H, Iannello G, Pavone FS.(2015) Sci Rep.May 7;5:9808.

Hu W, Tamadon A, Hsueh AJW, Feng Y.(2017) J Vis Exp. 2017 10;(130).

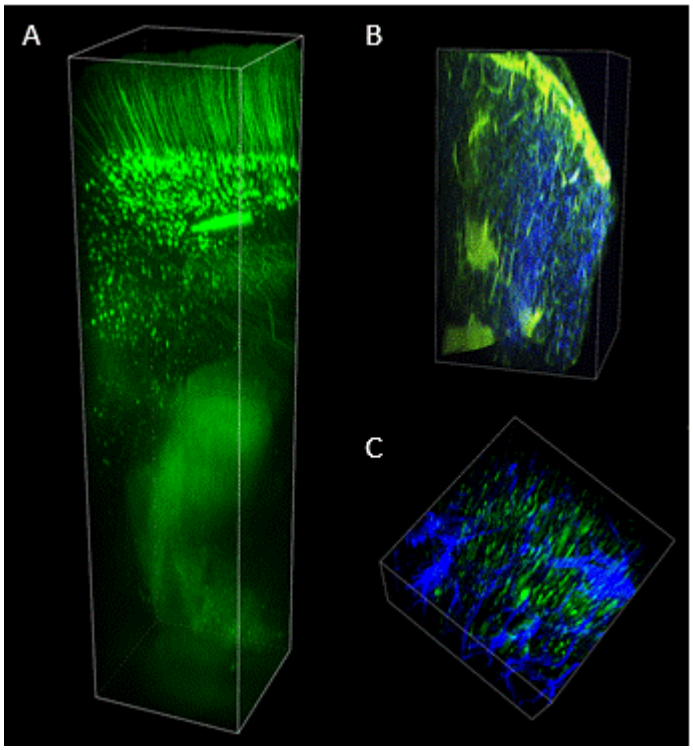

Figure 1: A) 1.21mm X 1.21mm X 5.05mm volume imaged from an intact cleared GFP mouse brain using passive Clarity method and mounted in RapiClear (Ryu Nakamura, Nikon Japan).B) 512.33m X 512.33m X 0.971mm cleared mouse spinal cord using RapiClear 1.52 (blue : THG, yellow autofluorescence). C) 1.27mm X 1.27mm X 0.824 mm cleared mouse muscle using RapiClear 1.49 (blue : SHG, green : GFP)