Organ clearing and biphotonic microscopy - an innovative complementary technological approach to investigate the central nervous system

Laurence Dubreil [1,2], Romain Fleurisson[1,2], Johan Deniaud[1,2], Francesca Daniela Franzoso[1,2], Marie-Anne Colle[1,2]

1 INRA UMR703 PAnTher, F-44307 Nantes, France

2 LUNAM Université, Oniris, École nationale vétérinaire, agro-alimentaire et de l'alimentation Nantes-Atlantique, Nantes, F-44307, France

3D imaging of the central nervous system 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 biphotonic and light-sheet microscopy together 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*). These methods are very promising tools to assess new therapeutic strategies on neurodegenerative diseases developed by our research unit UMR703 using animal models and AAV vector encoding fluorescent proteins.

For our study we used Scale, Clarity and uDISCO clearing methods and biphotonic imaging on thick brain sections. Harmonic and fluorescence signals conservation results will be discussed.

,

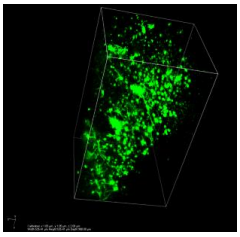

***Figure1**:Primate Brain cleared with Scale Method. GFP signal (green) in neurons. Z stack,1 mm depth.*

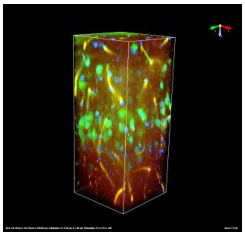

***Figure2:**Mouse brain cleared with Clarity Green Fluorescence. Neurons immunolabelling on 430 µm depth with NeuN antibody* (green, Alexa Fluor 488)

References

Denk W. and Horstmann H. (2004) PLoS Biol 2(11) e329.
Hama H. et al. (2011) Nature Neuroscience14:1481-88.
Chung K. et al. (2013) Nature 216;497(7449):332-7.
Chenchen P. et al. (2016) Nature Methods 13:859-867

Acknowledgements The support by Pays de la Loire and NeurATRIS is gratefully acknowledged. Images have been performed with an A1RMP biphotonic microscope from the APEX platform UMR703 INRA Oniris, Center of Excellence Nikon Nantes, France. Thanks to the APEX Plateform Staff.