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Organ Clearing and Biphotonic Microscopy: an Innovative Complementary Technological Approach to Investigate the Central Nervous System

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CLEARING AND 3D IMAGING TO INVESTIGATE SNC

3D imaging of the central nervous system at the microscopic level is essential to investigate **morphological changes** of **diseases** or to assess the **efficacy of a treatment** (Denk et Horstman, 2004). However, 3D exploration of tissues is limited due to the opacity of tissues, which doesn't allow the light transmission on large volume. Technological advances in biphotonic and light-sheet microscopy together with the development of methods in **tissue clearing** represent innovative solutions for **investigating organs** at the cellular level (*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 research unit UMR703 using animal models and AAV vector encoding fluorescent proteins.

In the present work, we used **SCALE**, **CLARITY**, **uDISCO**, **TDE** clearing methods and **biphotonic imaging** on brain and spinal cord. Harmonic and fluorescence signals conservation results are discussed.

CLEARING METHODOLOGIES INVESTIGATED

- ScaleA2, 4 M urea, 10% glycerol and 0.1% Triton X-100, (Hama et al., 2011)
- Clarity, acrylamide and bisacrylamide, clearing tissue, (Chung et al., 2013)
- uDISCO, BENZYLE ALCOOL (BA), BENZYLE BENZOATE (BB) ET DBE, (P. CHENCHEN ET AL., 2016)
- TDE, 2,2'Thiodiethanol (Y. Aoyagi et al., 2015)

BIOLOGICAL SAMPLES

- Brain and spinal cord fixed in PFA 4% from mouse
- Brain from fixed in PFA 4% from Non Human Primate (NHP)

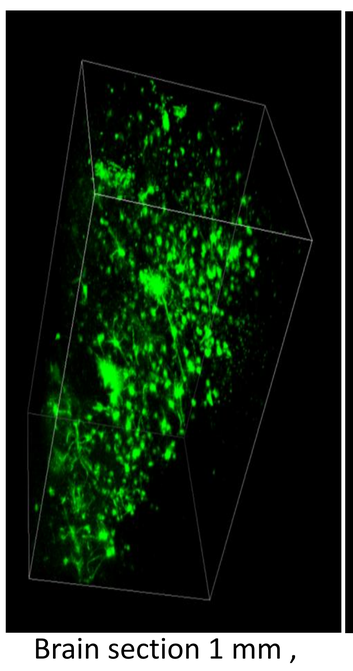
PARAMETERS CONSIDERED TO ASSESS PERFORMANCE OF CLEARING METHODS

- Sample retraction due to clearing methods
- Depth investigation of the sample
- Preservation of fluorescent probes used for immunolabeling
- Preservation of the signal from endogenous fluorescent protein and harmonic signals

RESULTS

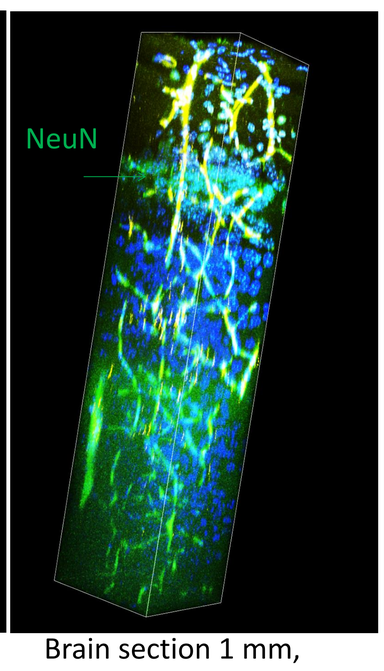
BRAIN

SCALE



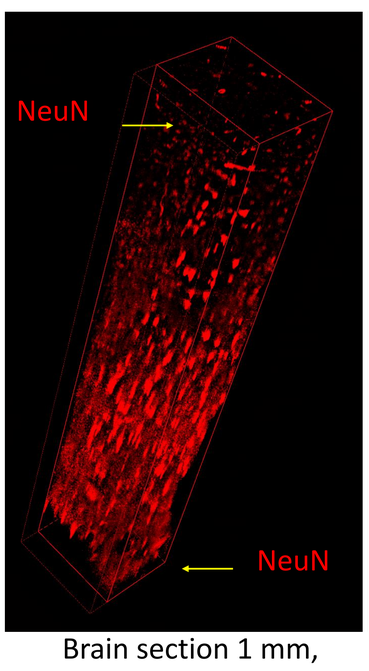
Brain section 1 mm , **Neurons GFP**

CLARITY



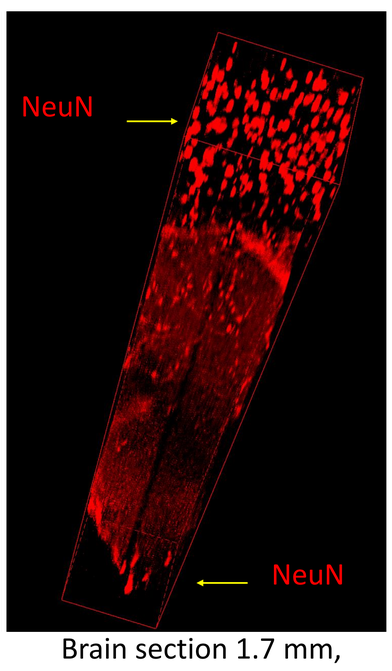
Brain section 1 mm, **Neun-AL488**

uDISCO



Brain section 1 mm, **Neun-AL555**

TDE

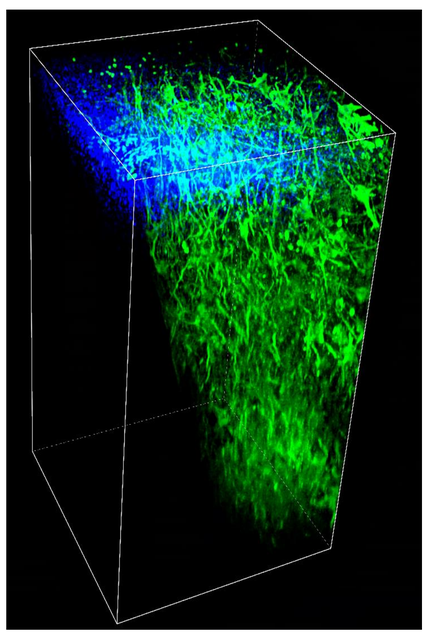


Brain section 1.7 mm, **Neun-AL555**

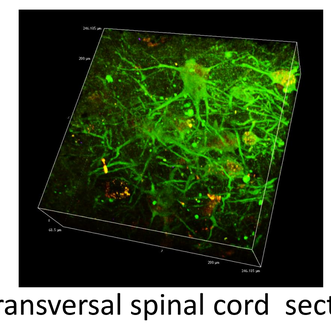
Penetration of antibodies in thick samples is a limit

SPINAL CORD

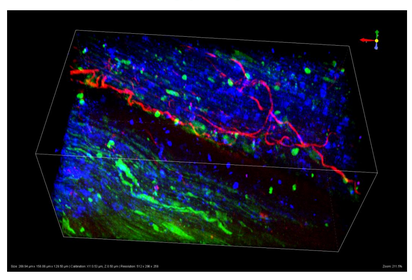
TDE



Transversal spinal cord section 0.9 mm **Neun GFP, THG, myelin**



Transversal spinal cord section **Neun GFP**



Longitudinal spinal cord section 0.2 mm **GFP, THG myelin, SHG collagen**

Clearing Method	Time (Day)	Size reduction	Deep (µm)	Fluorescence immunolabeling	GFP	SHG	THG	Equipment required for clearing
SCALE	30	No	1000	++ (FI before clearing)	+++	+	+	No
CLARITY	1	No	950	++ (FI after clearing)	ND	+	-	Yes
uDISCO	3-4	Yes, 50%	1000	++ (FI before clearing)	+/-	+/-	-	No
TDE	3 -6	No	1700	++ (FI before clearing)	++	+	+	No

TDE	Time (Day)	Size reduction	Deep (µm)	GFP	SHG	THG
Spinal Cord	3-6	No	900	++	+	+

BIPHOTONIC MICROSCOPY

- A1R-MP, Nikon
- Insight Deepsee 680-1300 nm
- X25 MP1300 objective lens (NA 1.10 WD 2,0 mm)

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, in addition to the most studied parameters (endogenous fluorescence, size sample change ect…), we report the preservation of harmonic signals in brain and spinal cord cleared from Scale, Clarity, uDISCO and TDE .

- Intensity of SHG from collagen and THG from myelin was not increased by using clearing methods and limited to the first 100 µm in depth.
- THG was not retained with uDISCO and Clarity methods.
- SHG was retained by using Scale, Clarity, uDISCO and TDE.
- **TDE seems to be most interesting clearing methods for spinal cord (0.9 mm) allowing preservation of GFP, SHG from collagen, and THG from myelin.**

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