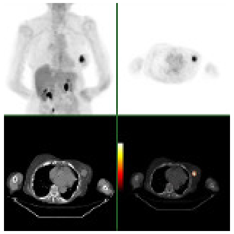

France Life Imaging

Workshop
« Avancées récentes en analyse d'images médicales multi-modales »

22-23 mars 2018 Orsay (France)

Workshop WP4

« *Intérêt de l'analyse des imageries* in vivo *et post mortem du cerveau en recherche préclinique* »

Thierry DELZESCAUX

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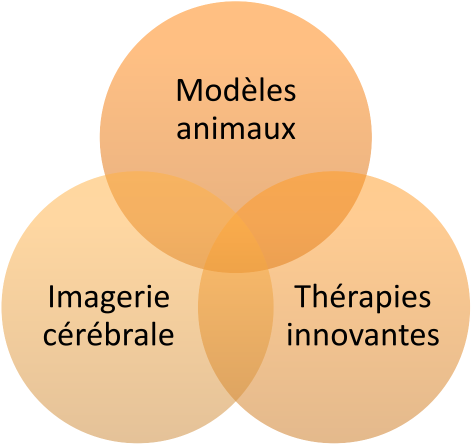
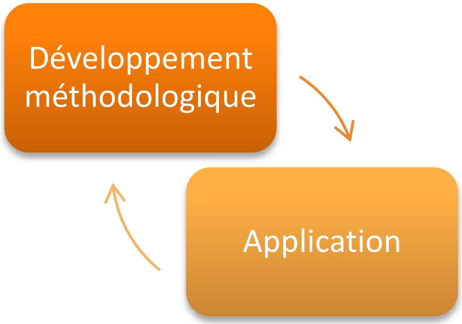
Molecular imaging research center

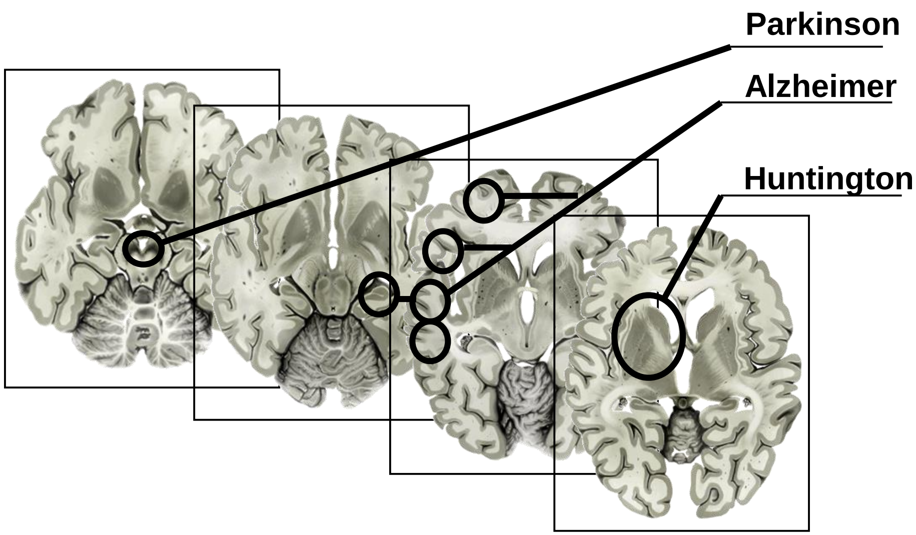
Une plateforme dédiée à la recherche translationnelle

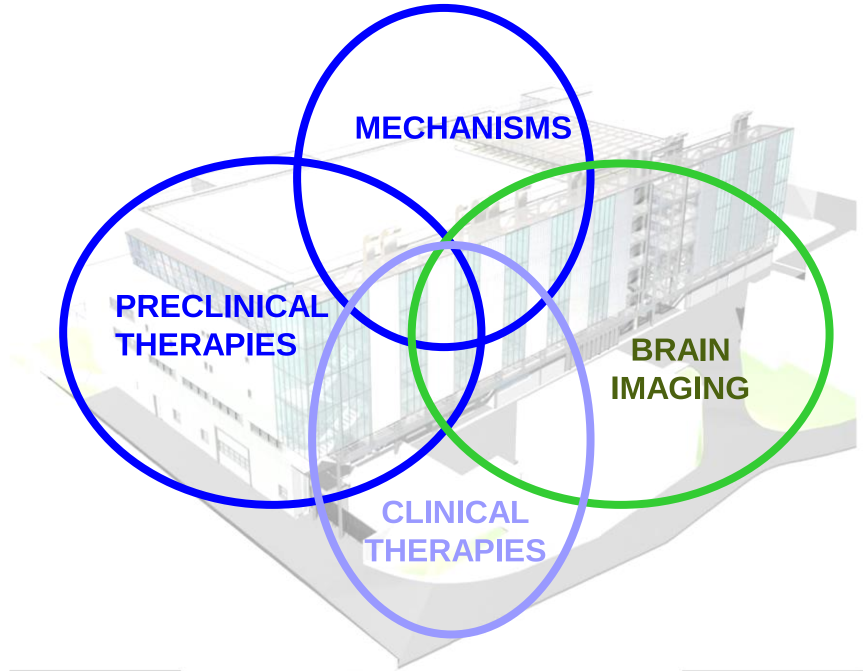
- Hébergement et Comportement
- Analyses Biologiques
- Laboratoires L2/L3
- Histologie
- Microscopie
- Imagerie TEP
- Imagerie et Spectroscopie RMN

Laboratoire des Maladies Neurodégénératives





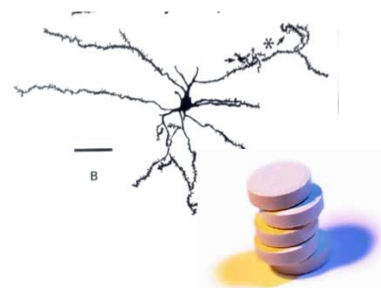


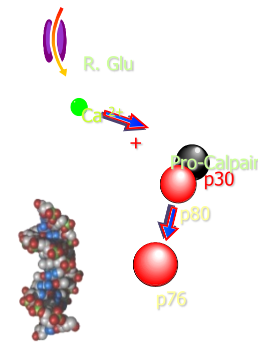


→ **Medical and socio-economic impact**

→ **Challenges**:

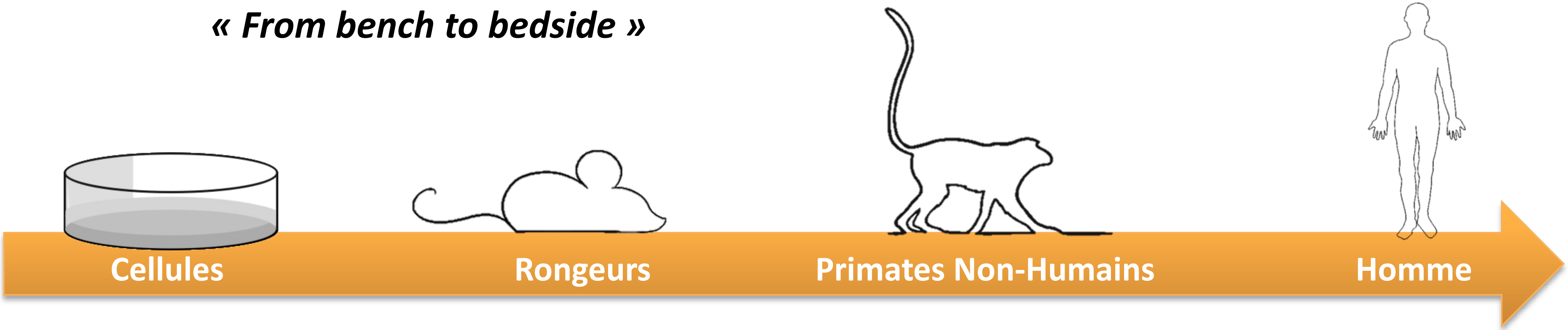
- **Mechanisms**
- **Therapies**
- **Brain Imaging**





Translational research

« From bench to bedside »



	Cellules	Rongeurs	Primates Non-Humains	Homme
Similarité avec l'Homme	--	-	+	
Durée d'étude	Jours	Mois	Mois/Années	Années
Nombres de sujets	++++	> 10	< 10	> 100
Coût	----	--	++	++++

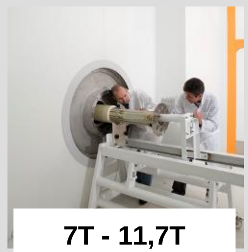
Cerebral imaging in rodents. MIRCen facilities

In vivo imaging: *Function* – *Anatomy*

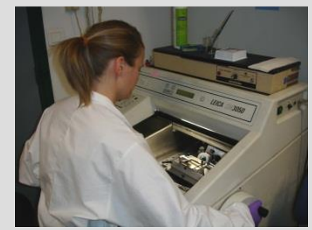
Post mortem imaging: *Anatomy* – *Function*

PET	MRI	Blockface	Histology	Autoradio-
PET FDG, etc…	**MRI** 7T	**Blockface photographs**	**Histology** Cresyl, Nissl	**Autoradio-graphy**

µ-PET (rodent)



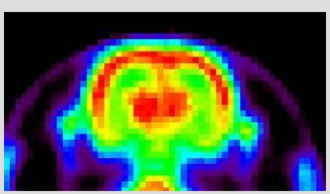
7T - 11,7T

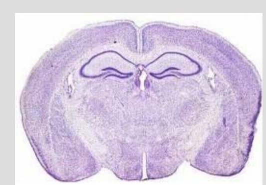


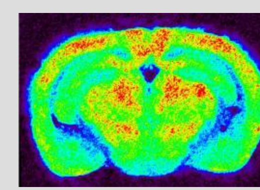
Cryostat

Dimension	3D	3D	3D/2D	2D	2D

Images







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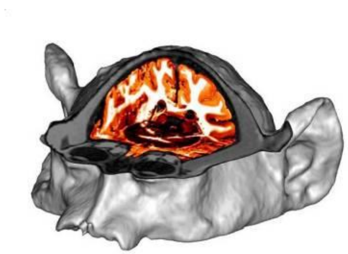
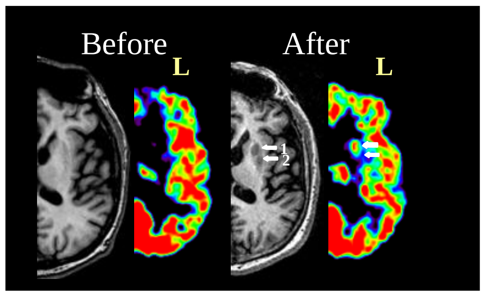
In vivo / *post mortem* imaging techniques

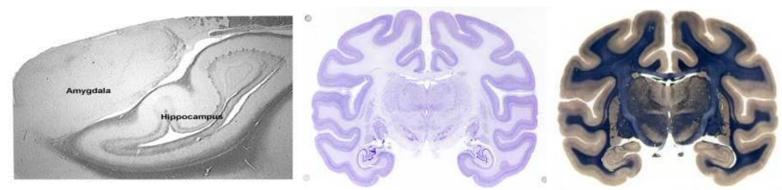
Advantages / Disadvantages

In vivo imaging

Post mortem imaging







+

- ✓ Longitudinal studies
- ✓ 3D imaging
- ✓ Fast acquisition time

–

- ✓ Technical limitations
- ✓ Difficult to use
- ✓ Recent
- ✓ High cost

Complementary

+

- ✓ High spatial resolution
- ✓ Easy to use
- ✓ Low cost
- ✓ Variety of stainings

–

- ✓ Single observation
- ✓ 2D imaging
- ✓ Tedious

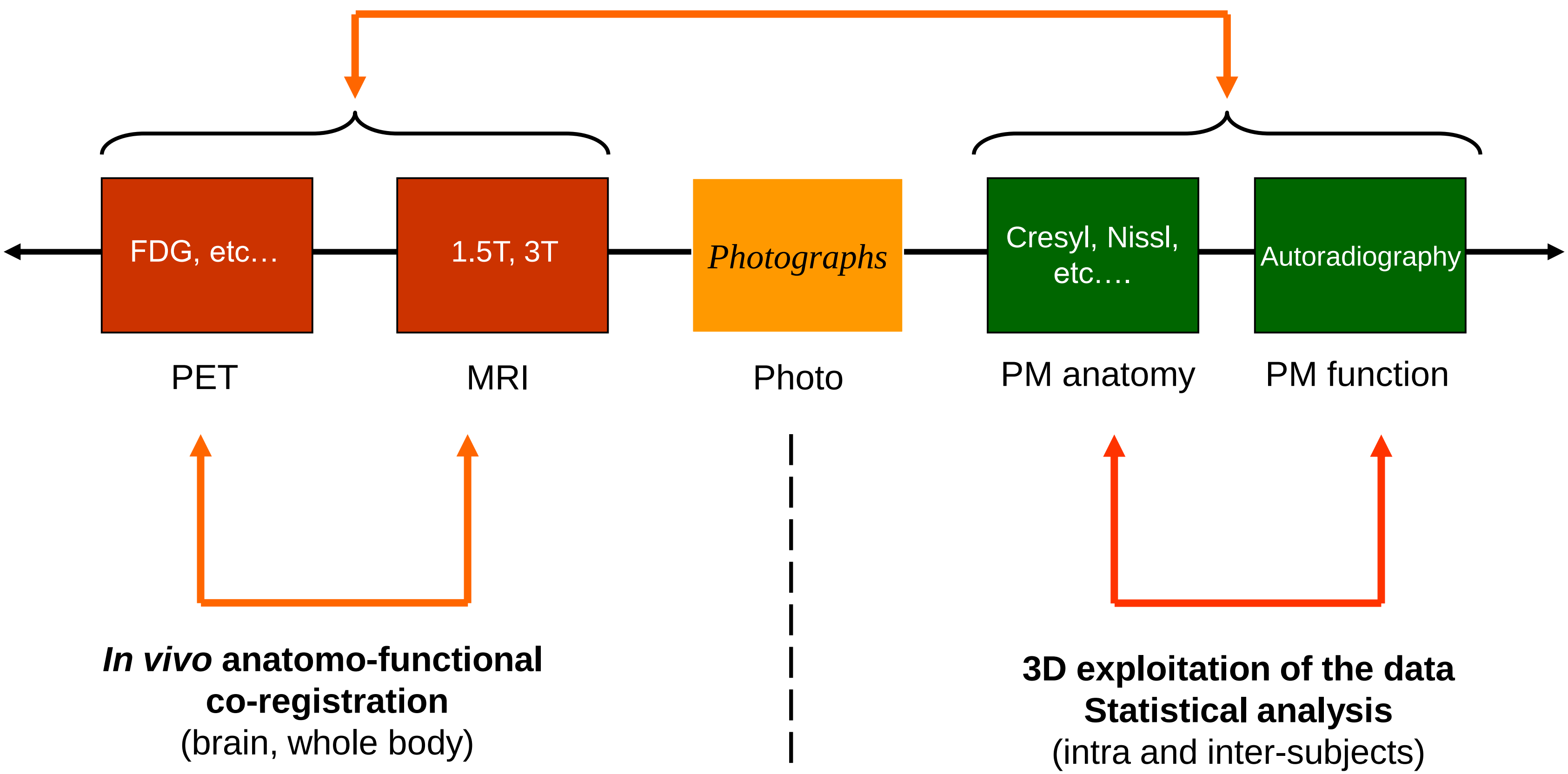
Background: research / applications

Merging of information acquired *in vivo* and *post mortem:*

- *at microscopic and macroscopic level,*
- *anatomy and function.*

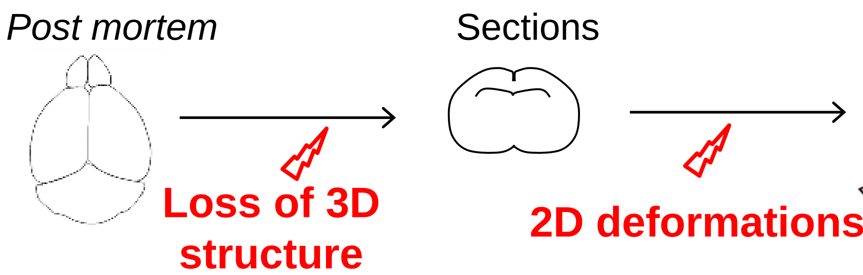
Quantitative validation of PET (instrumentation)

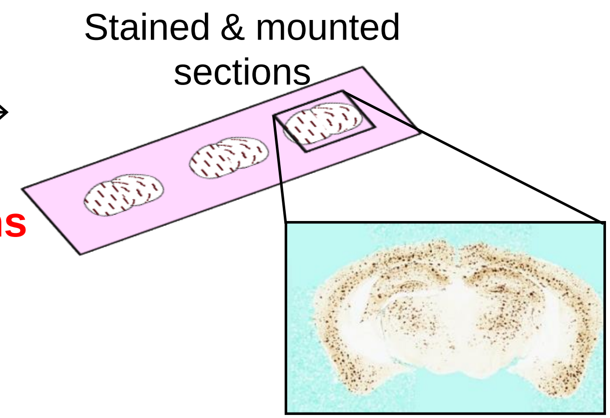
Evaluation of PET abilities (follow-up)



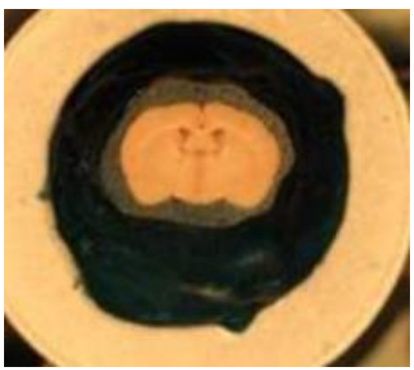
3-D *post mortem* reconstruction

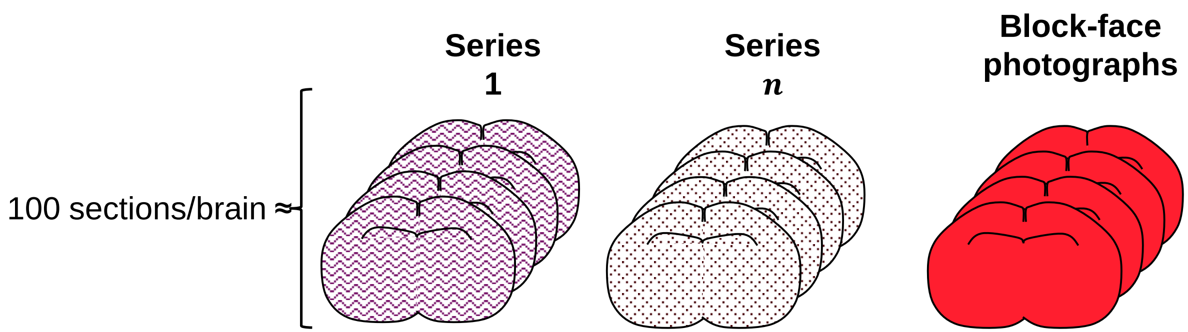
Serial histology processing





1. **Tissue sample collection**
2. **Tissue sectioning and block-face photography**
3. **Serial section staining**
4. **Tissue section imaging**



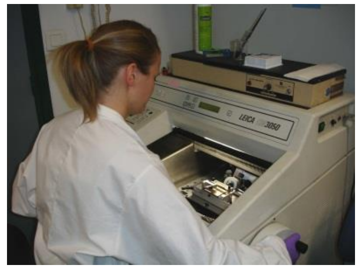


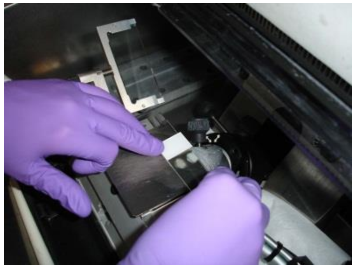
3D photographic modality

Cryostat

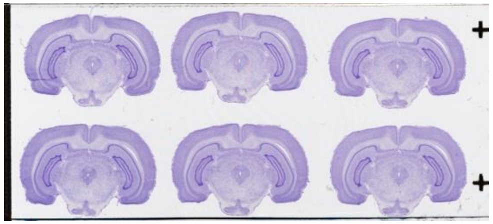
Cutting process

B
R
A
I
N





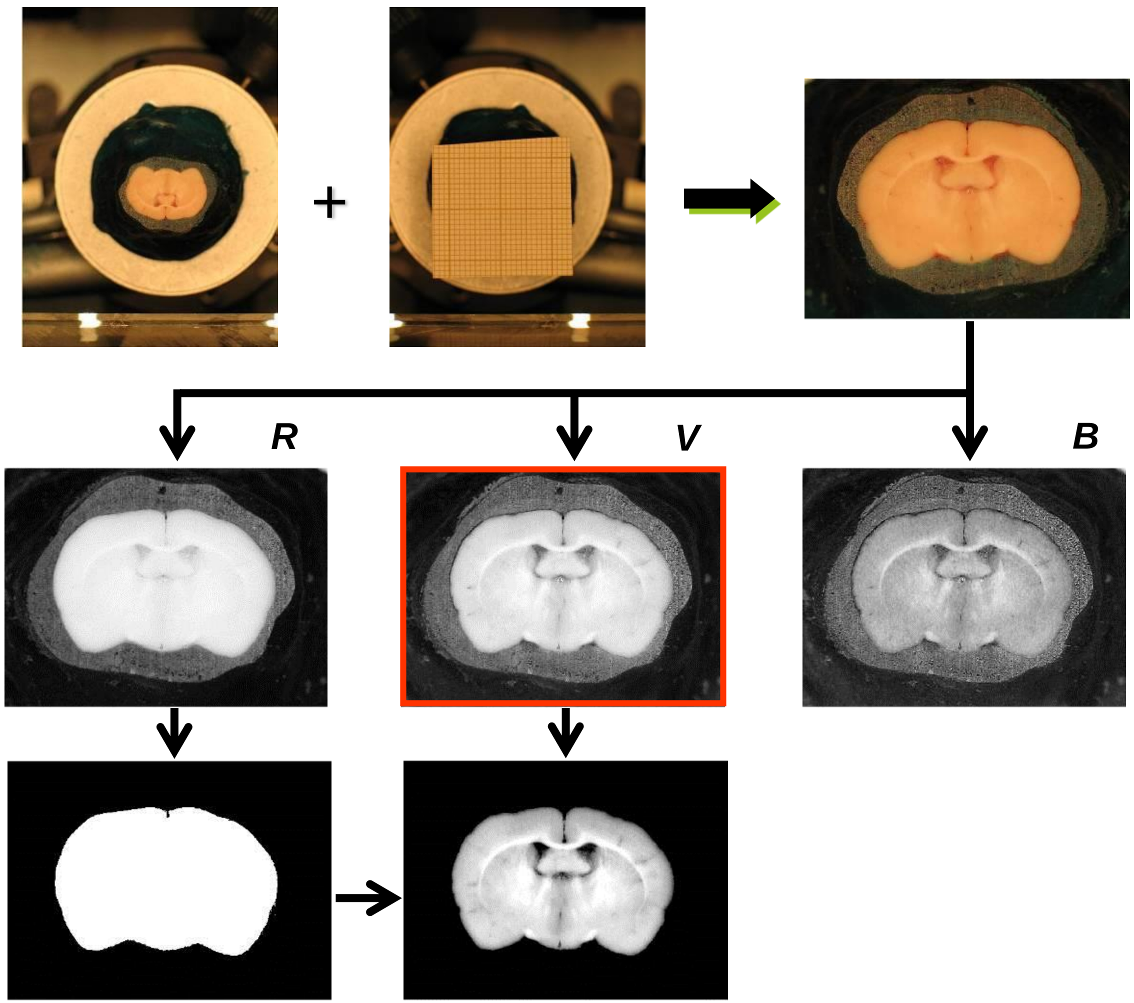
Histological staining



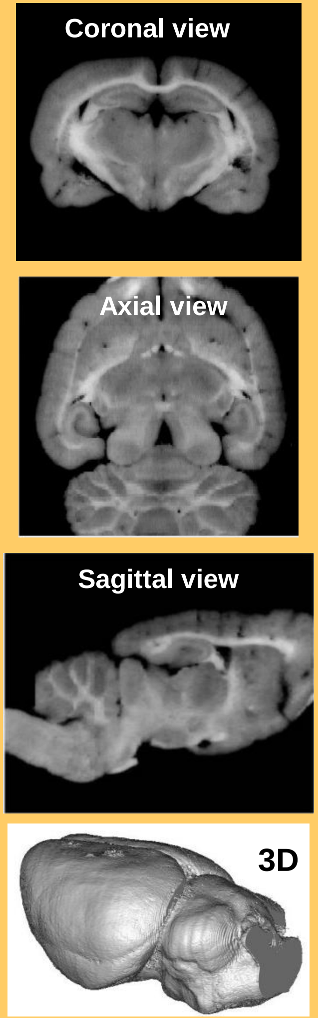
"Banana effect"

First prototype developed

3D photographic modality



3-D photographic volume

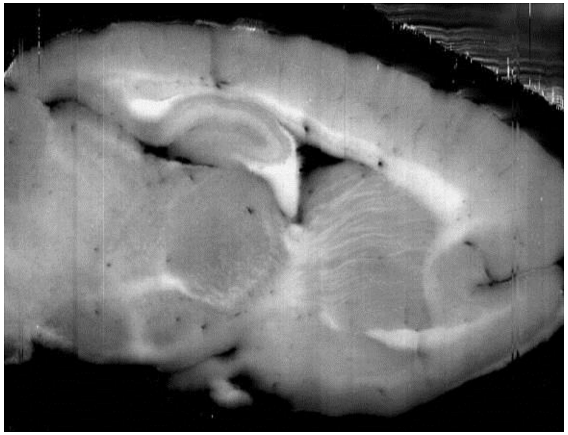


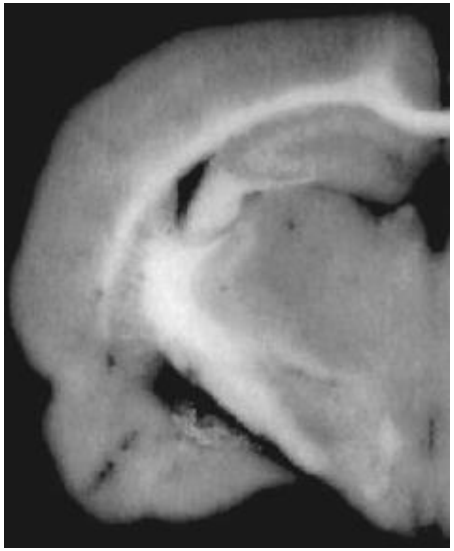
3D photographic modality

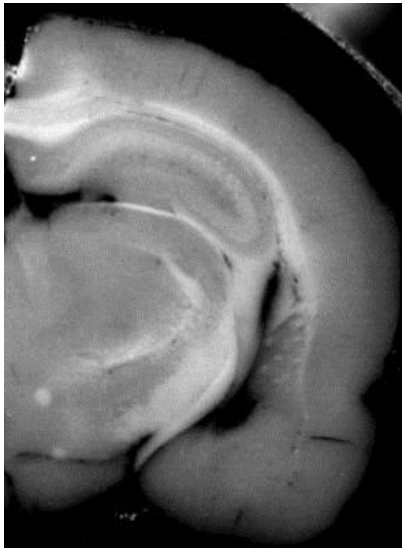
Photographs

New prototype:
Improved performances

Setup

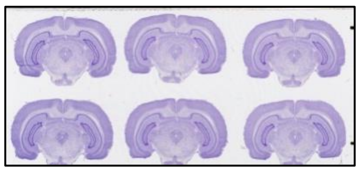






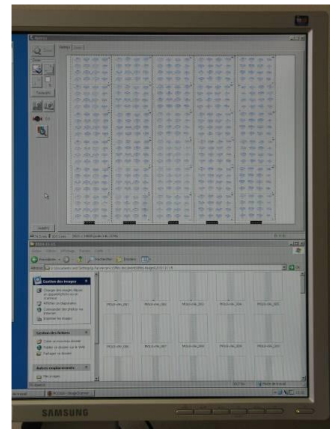
Tissue section digitization

❑ Optimized acquisition of the data

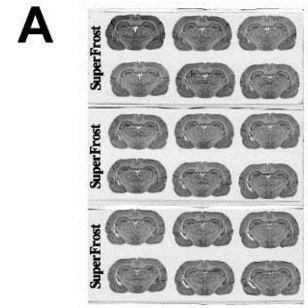


Histological staining

Flatbed scanner



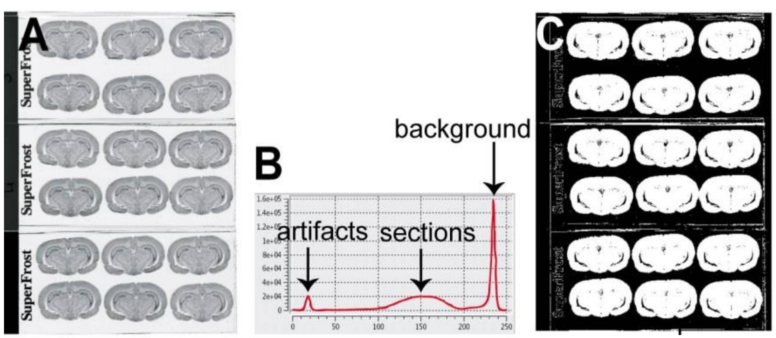
Anatomical information

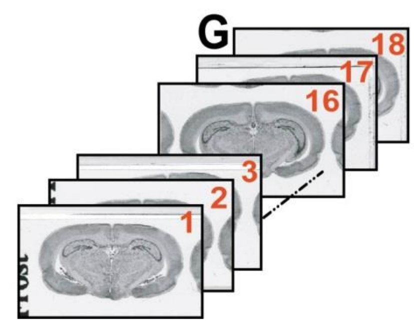


Histological stained sections (cresyl violet)

"Column" acquisition
Several dozens of sections can be digitized at once

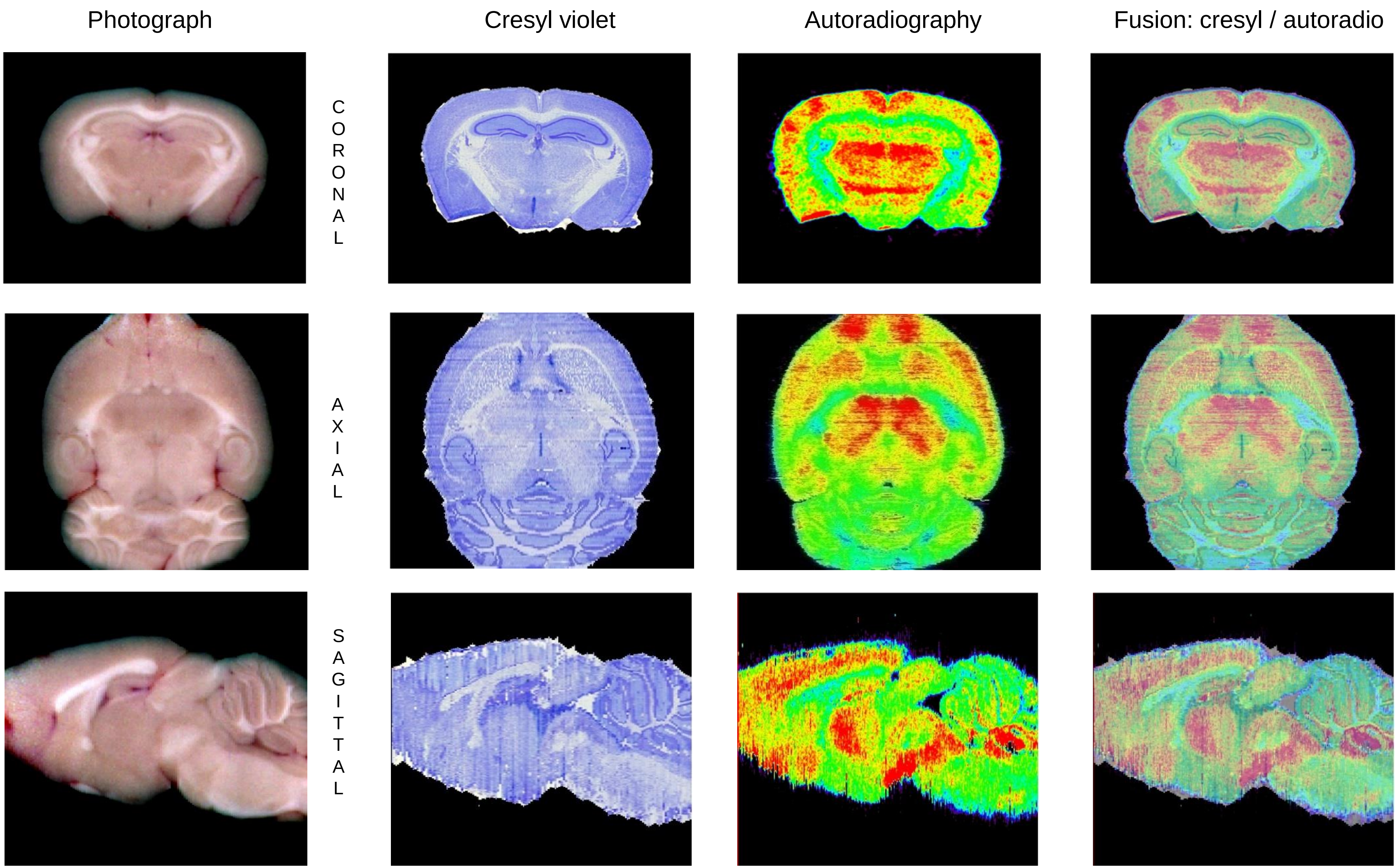
❑ Automated extraction and stacking of the sections





3D *post mortem* reconstruction – HD mouse model

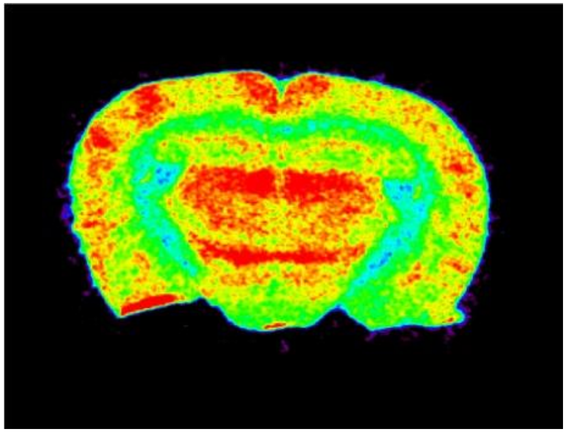
Boussicault *et al.*, JCBFM, 2014



Applications in Alzheimer’s disease

1) Metabolism changes
2) Amyloid load assessment
3) In vivo / post mortem co-registration
4) Future research

1) Metabolism changes



1) Metabolism changes in Alzheimer’s disease

• **Material:**

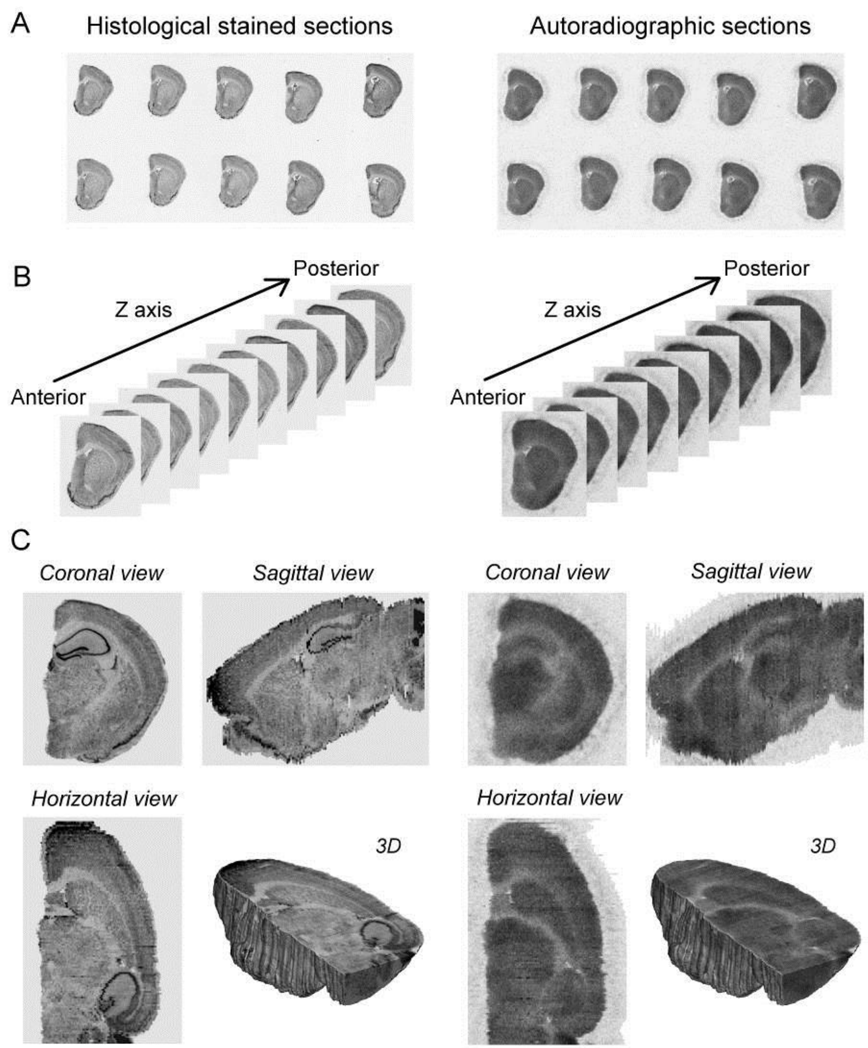
- APP-PS1 (n=3),
- PS1 control (n=4).

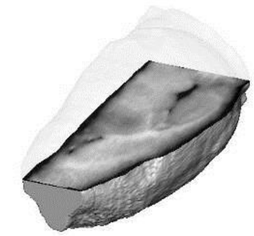
Hemibrain studied

• **Reconstruction strategy:**
- Use of reference volume,

- Multimodality 3-D consistency,

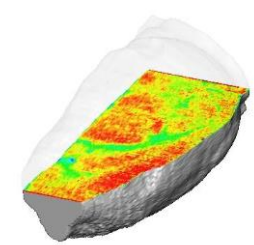
- Photography : reconstruction + spatial normalisation.





Photographic volume

Histological volume

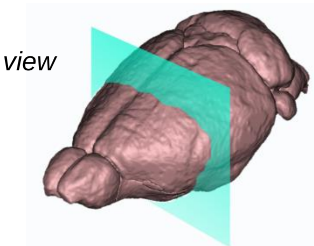


Autoradiographical volume

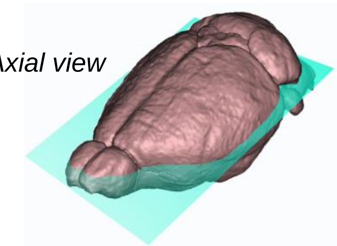
Construction of a digital atlas of mouse brain

- **Pre-processing applied to the downloaded files**

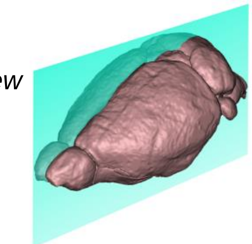
Coronal view



Axial view

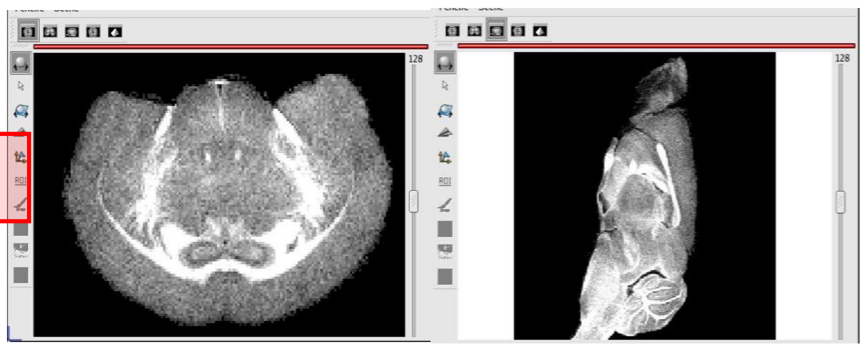


Sagittal view

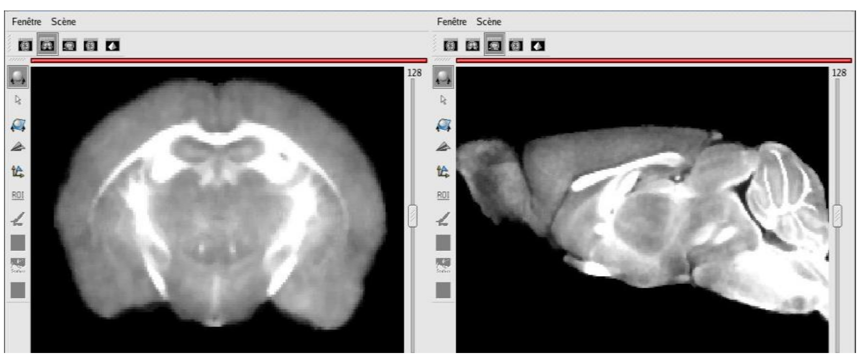


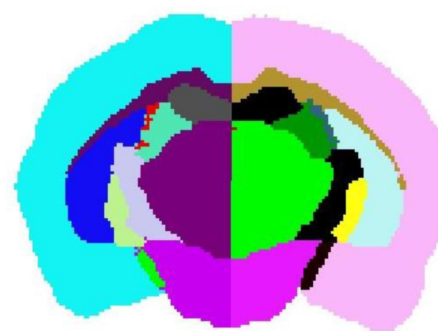
- MRI volume
- Labeled volume

Downloaded volumes

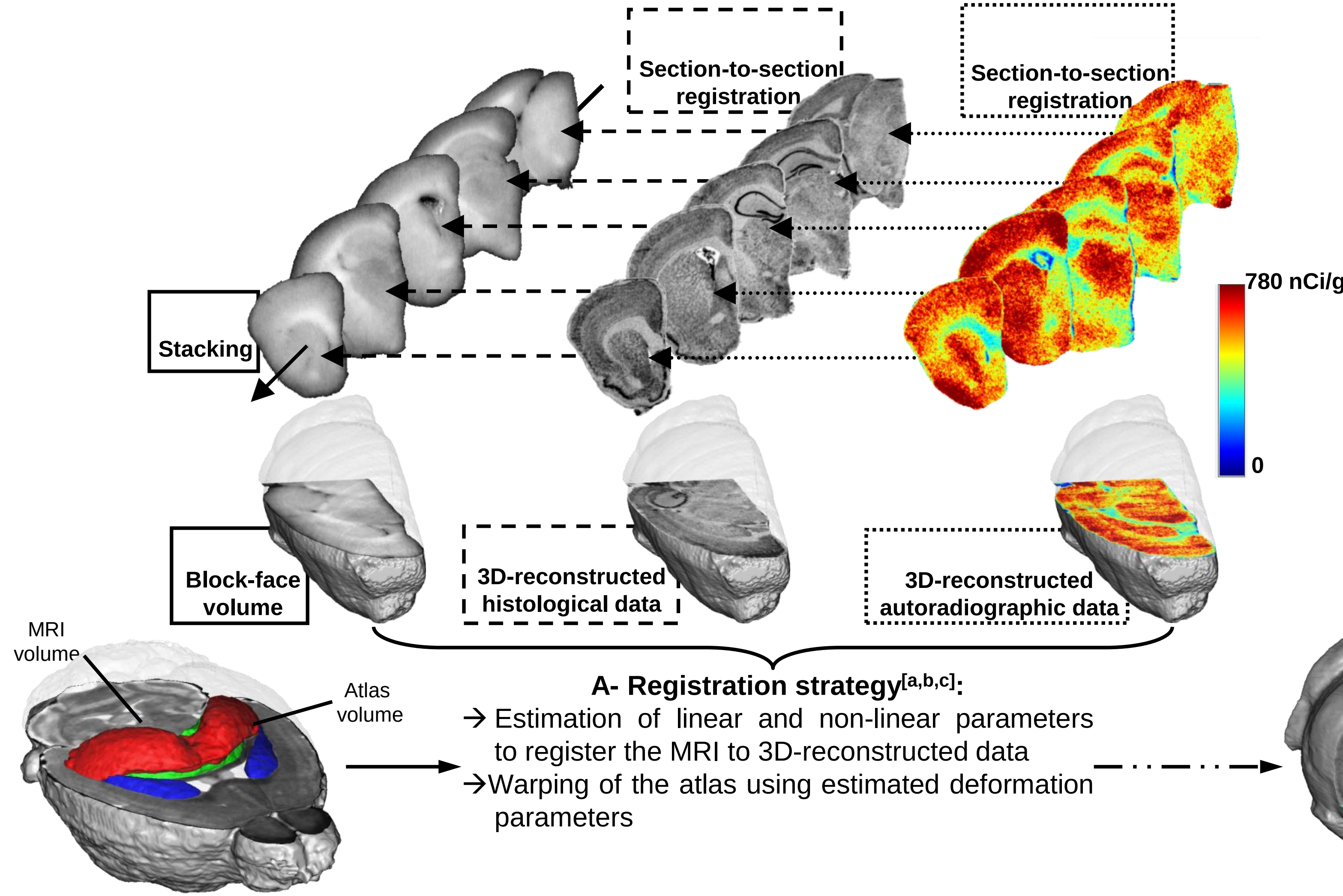


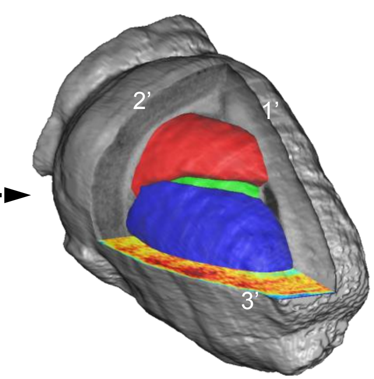
Processed volumes





Analysis of PM dataset using a 3D digital atlas



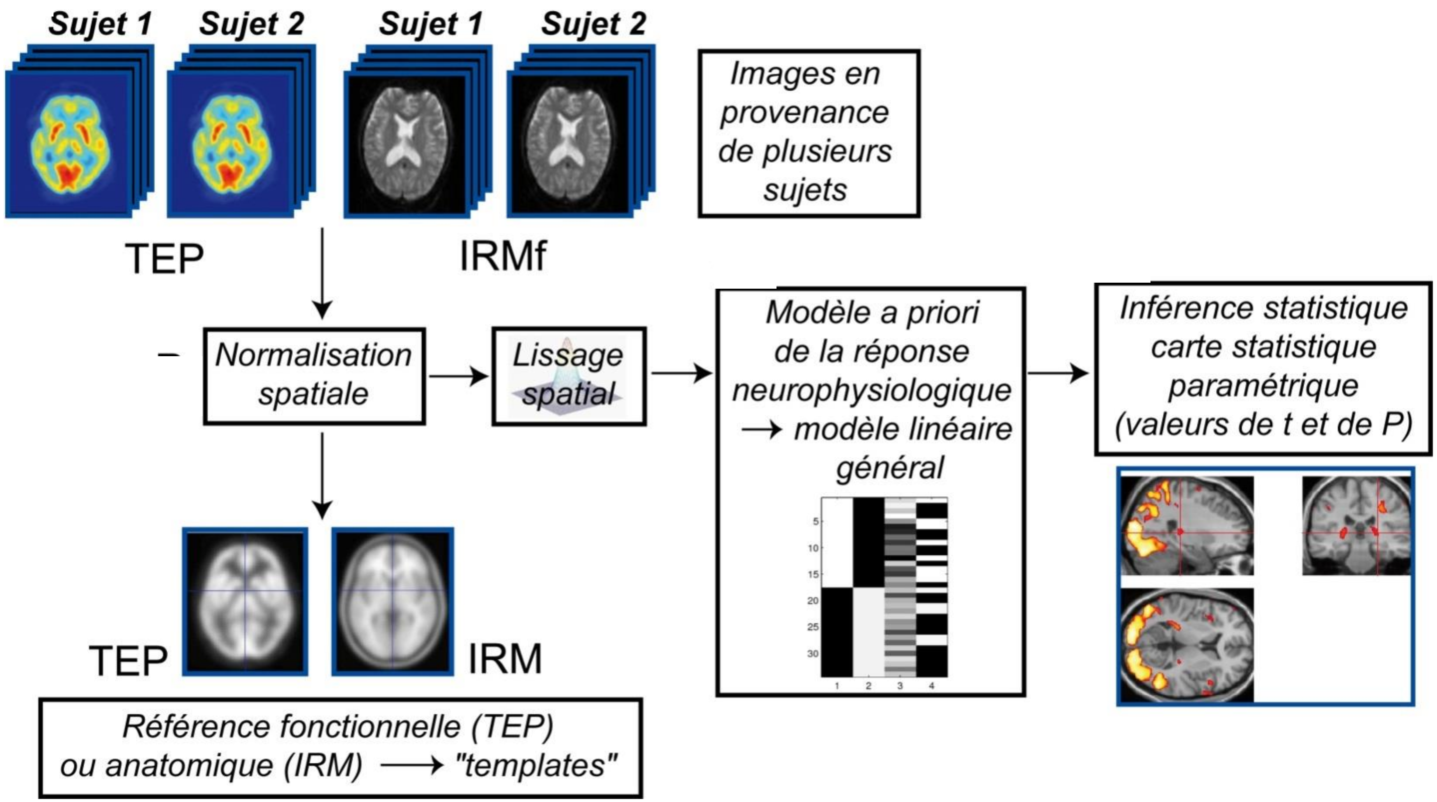


Lebenberg *et al.*, ISMRM and ISBI conferences 2009

Lebenberg *et al.*, NeuroImage 2010

Analysis of PM dataset using voxel-wise approach

Détection automatique et sans *a priori*, à l'échelle des voxels,
de différences significatives d'intensité entre deux groupes d'images



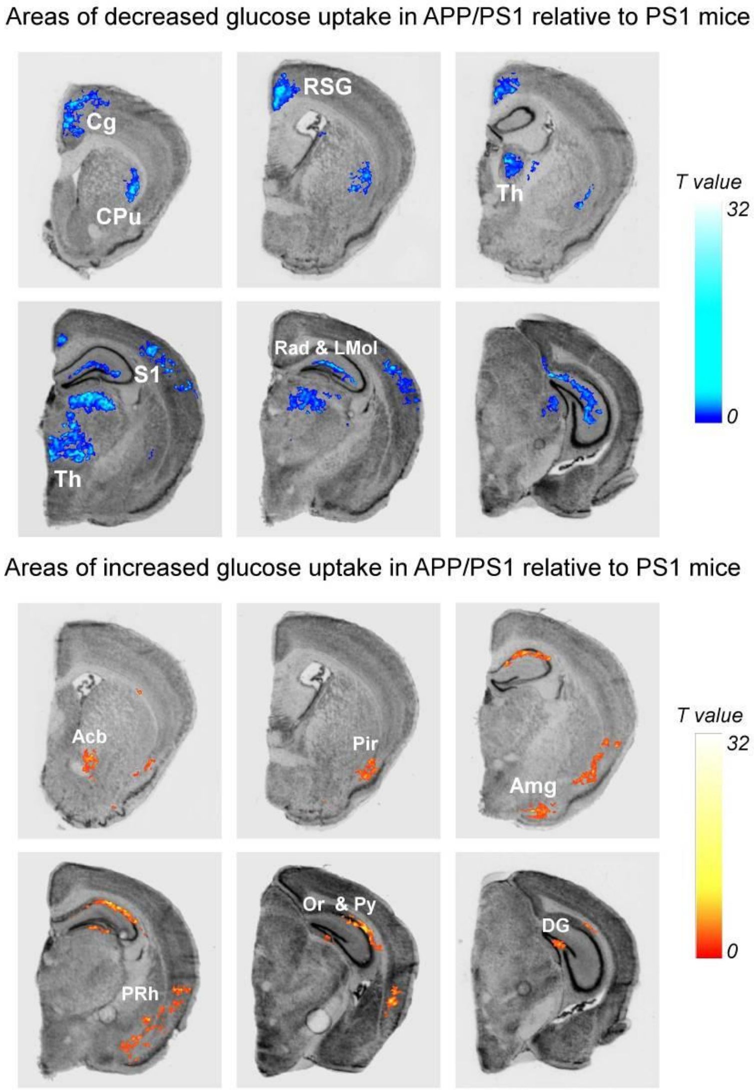
Technique principalement développée et utilisée chez l'**Homme** en imagerie *in vivo*

Voxel-wise metabolism analysis [1/2]

- **Synthesis of glucose changes detected:**
  - ↘ : cortex Cg, hippocampus Rad and Mol, thalamus Th,
  - ↗ : hippocampus CA1, CA3, Pir cortex,

- **Limitations:**
  - Huge amount of data,
  - Reconstruction step,
  - A single measurement.

- **Interests:**
  - Without anatomical *a priori*,
  - Possibility to perform exploratory studies *(identification of structures / sub-structures involved)*,
  - Anatomo-functional data, high resolution *(~20-40 µm)*.



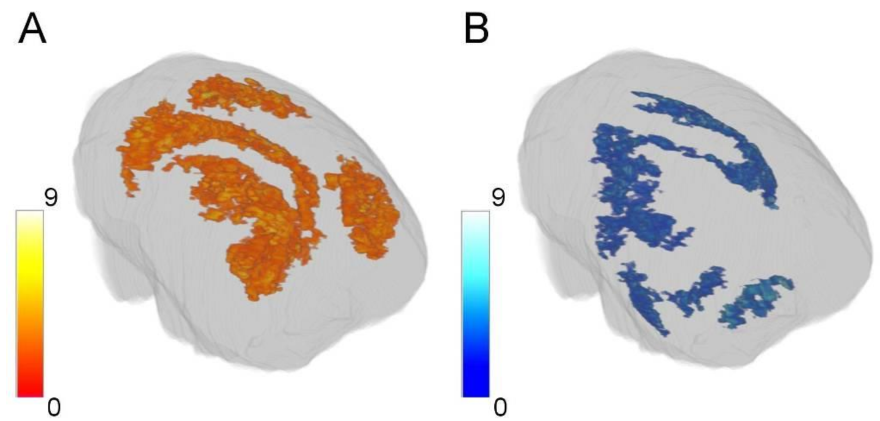
Voxel-wise metabolism analysis [2/2]

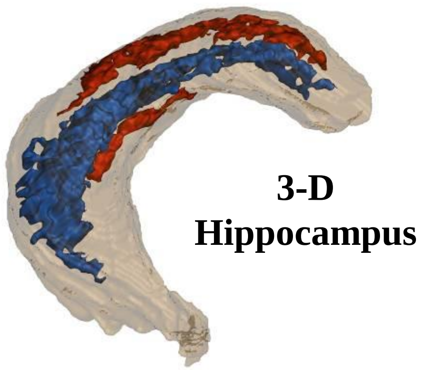
- **Surface rendering**

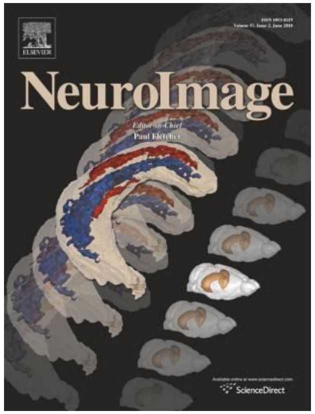
⇨ Improve our understanding of **pathophysiological processes**

⇨Possibility to quantitatively evaluate **drug efficacy** / **new therapeutic strategies**

⇨ Possibility to apply this methodology to **other species** (mouse, rat, microcebe, etc.)





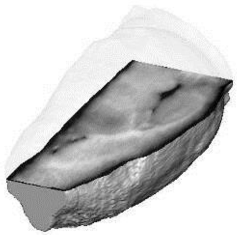


Dubois *et al*, NeuroImage, 2010

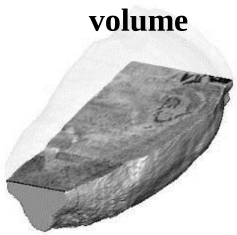
Detection of metabolic changes in AD mouse model

Lebenberg *et al*, NeuroImage, 2010

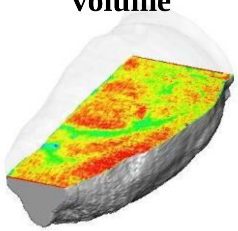
Dubois *et al*, NeuroImage, 2010



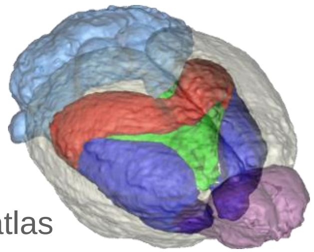
Photographical volume



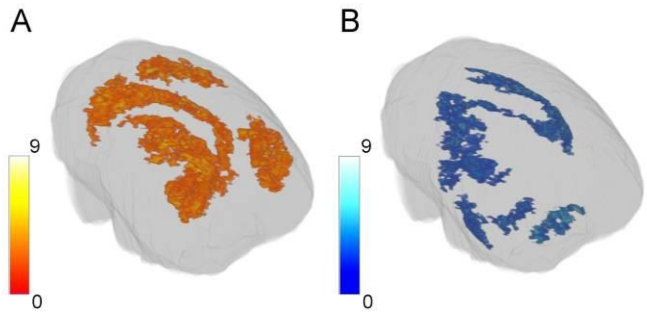
Histological volume



Autoradiographical volume

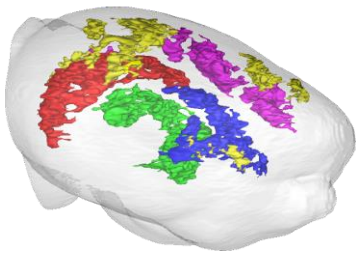


3D digital atlas



Statistical Parametric Mapping analysis (SPM) without anatomical a priori

Combined approaches

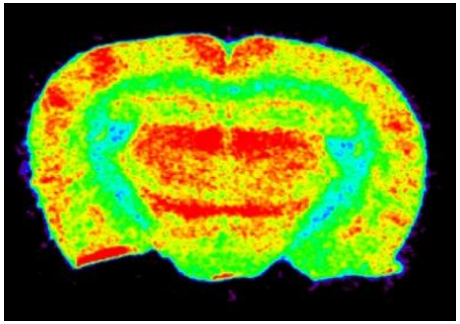


Lebenberg *et al*, NeuroImage, 2011

- ⇨ 3D *post mortem* reconstruction
- ⇨ Automated detection of regions presenting metabolic changes
- ⇨ Automated labelling of these regions based on the use of digital atlas

Extend 3D analysis from quantitative mesoscopic to qualitative microscopic data

- First group studies using 3D histology were performed:
  1) *in rodents (rats, mouse),*
  2) *on autoradiographic data.*



Autoradiographic section

- Continuous / quantitative information / mesoscopic scale / 3D reconstruction

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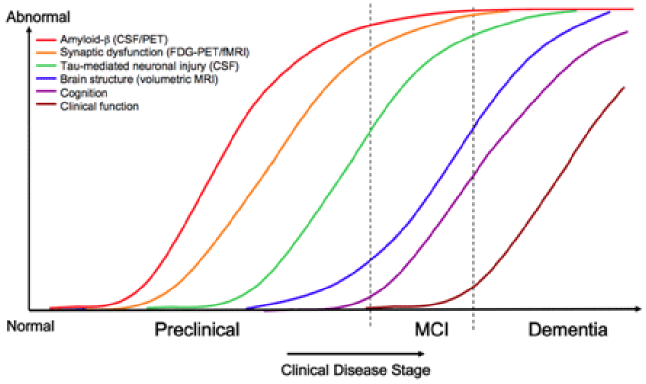
- Sparse / qualitative information / meso-micro scale / 3D reconstruction

AD biomarkers



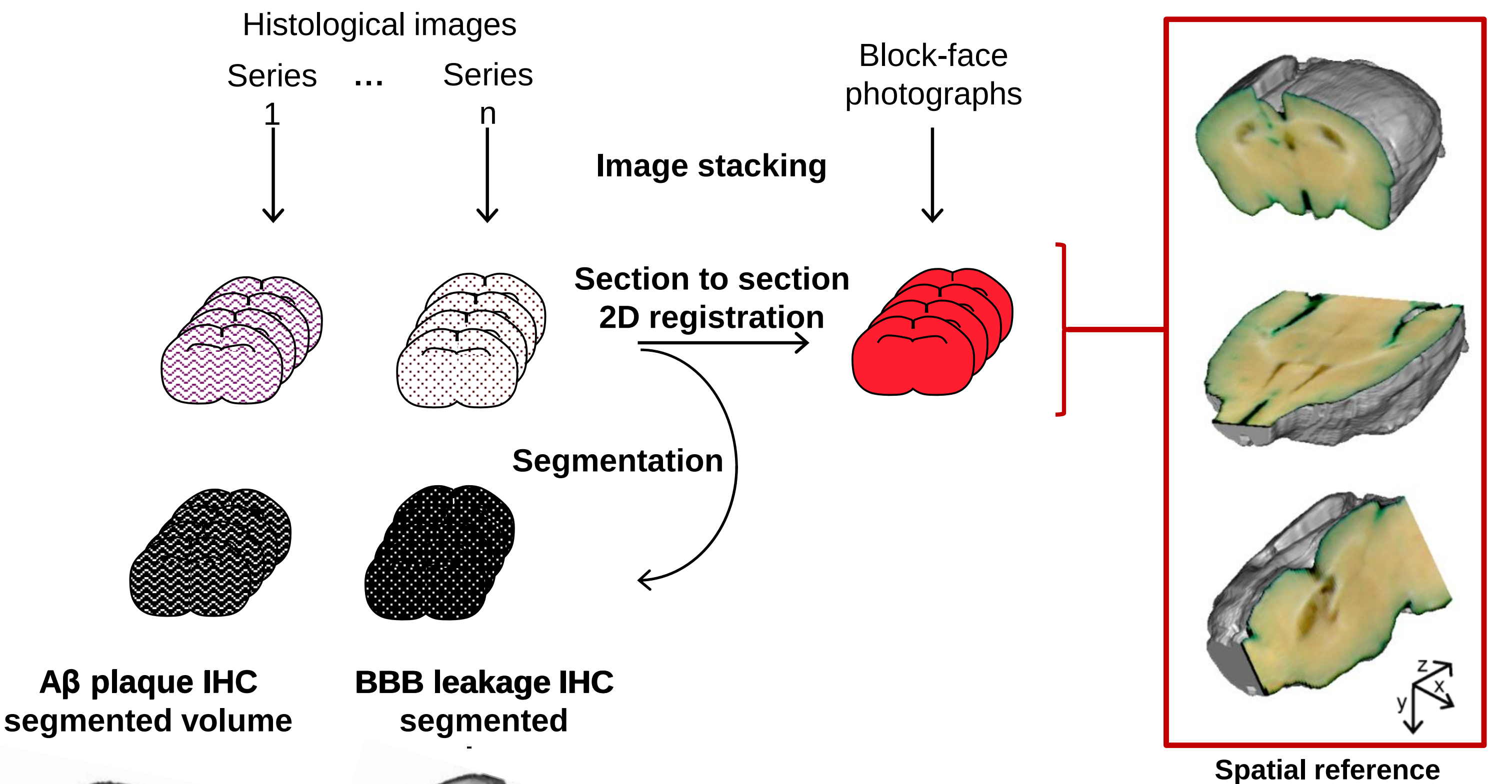
Amyloid plaques staining

2) Amyloid load assessment

High throughput *post mortem* studies

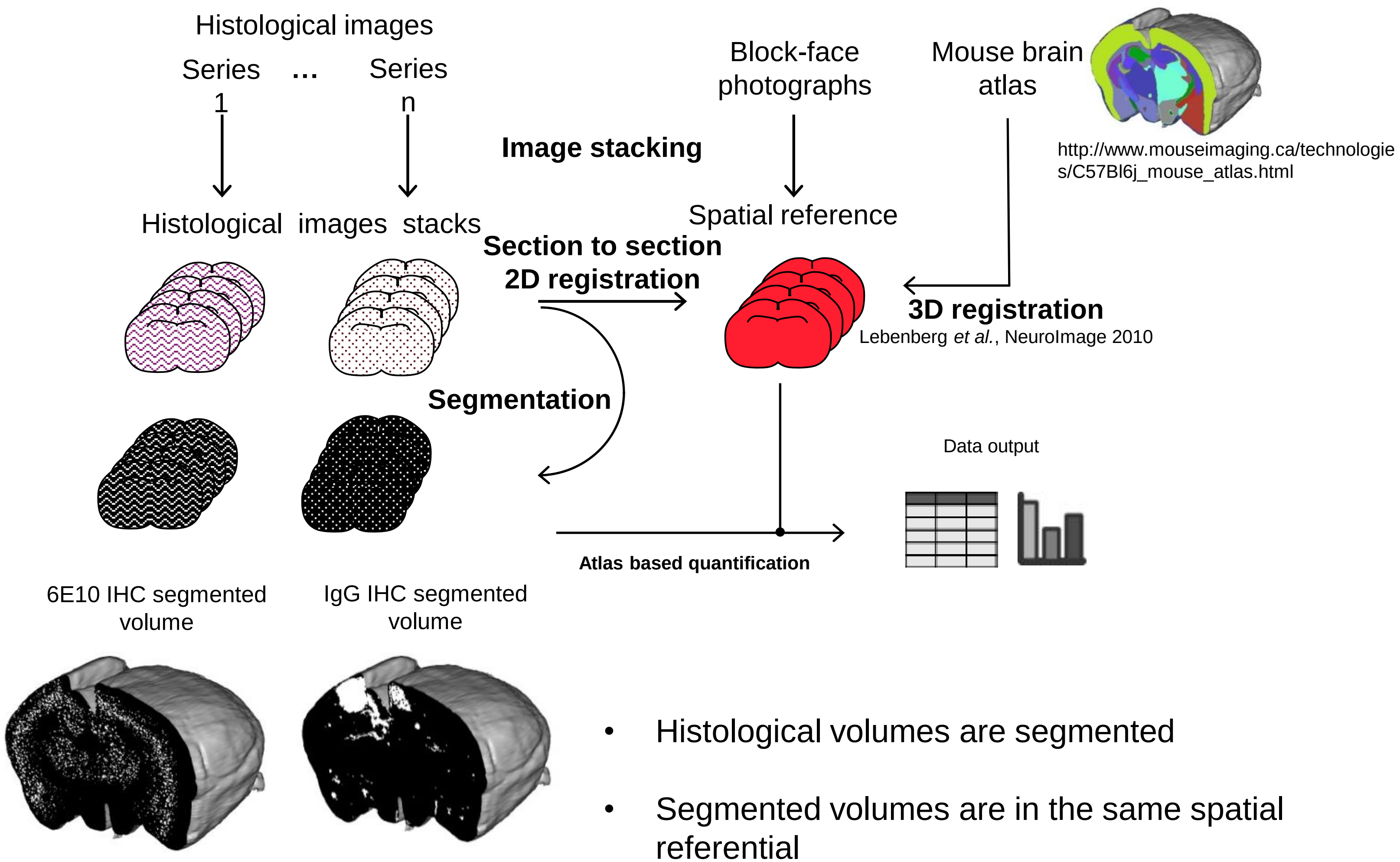
1) Histology production
2) Digitization process
3) Hardware facilities

Neuropathology image segmentation



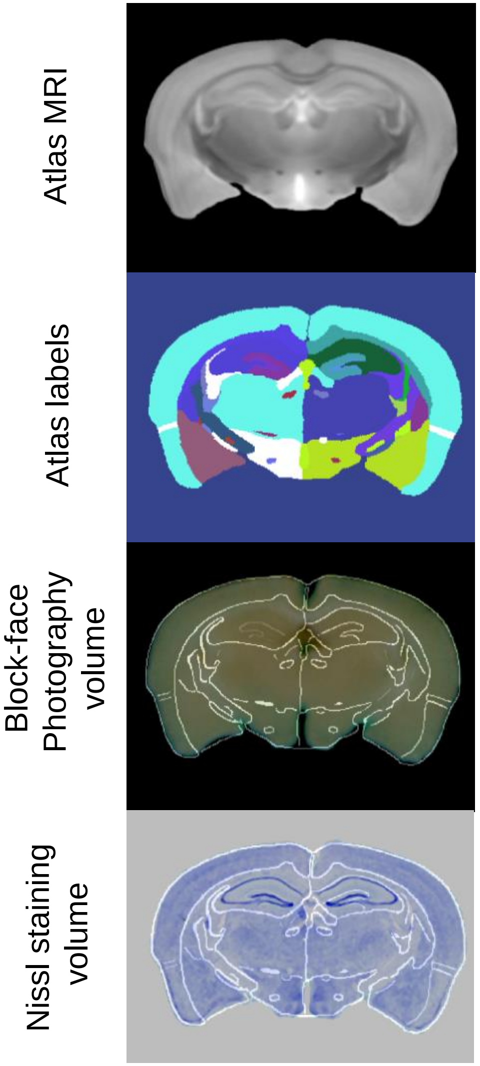
- Histology volumes are in the same referential
- **Histology volumes are segmented**

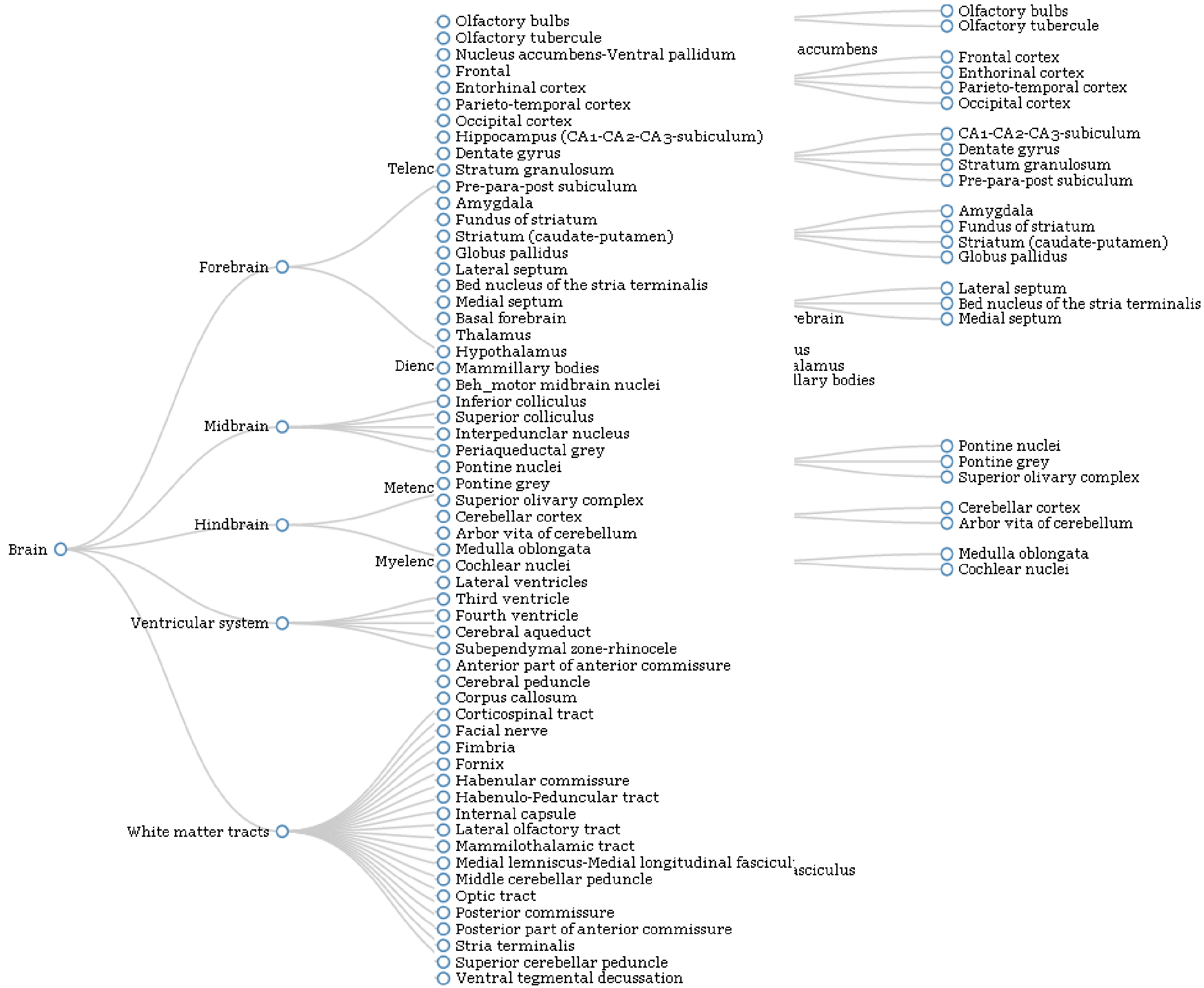
Ontology-based analysis (1/3)



- Histological volumes are segmented
- Segmented volumes are in the same spatial referential
- **Regional measures output**

Ontology-based analysis (2/3)





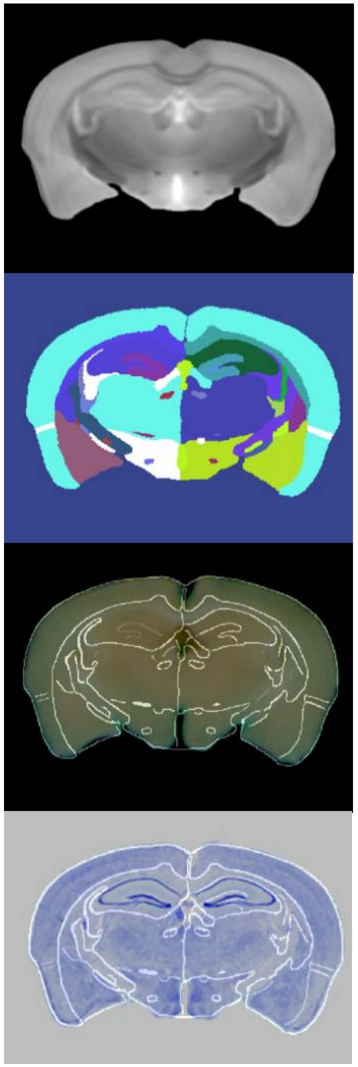
Ontology-based analysis (3/3)

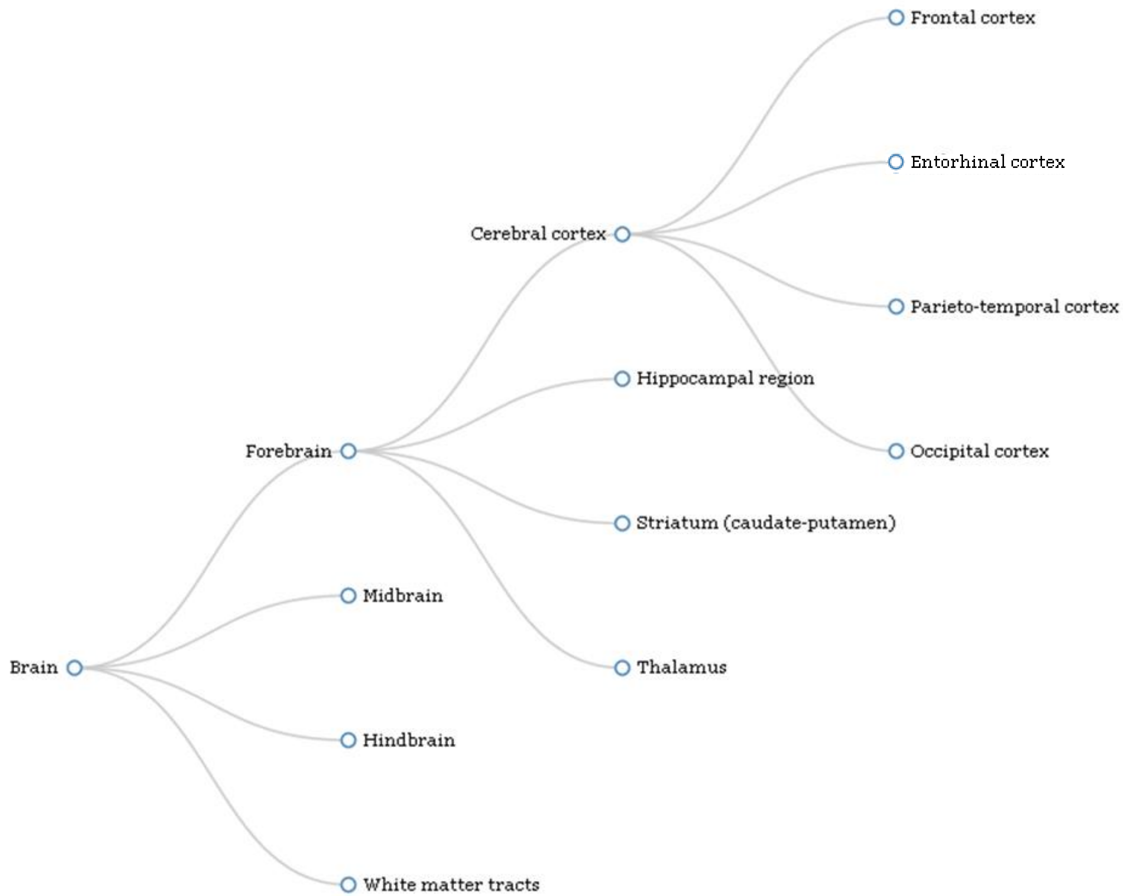
Atlas MRI

Atlas labels

Block-face Photography volume

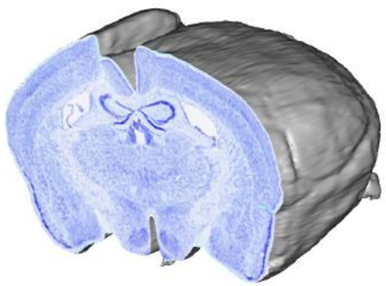
Nissl staining volume



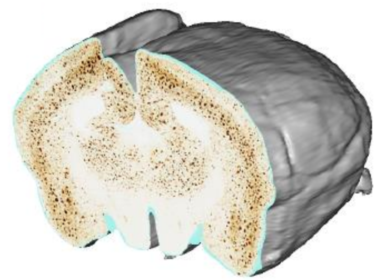


3D analysis – Amyloid load assessement

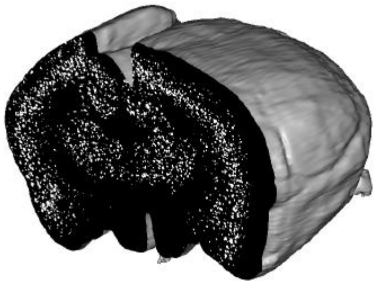
Violet de crésyl



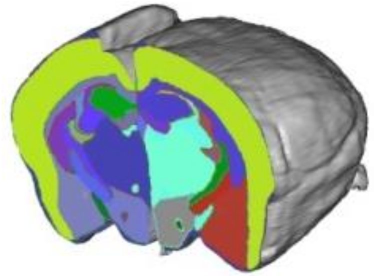
6E10 IHC



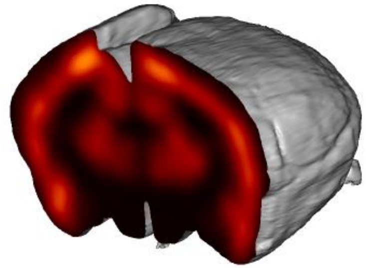
Plaques amyloïdes



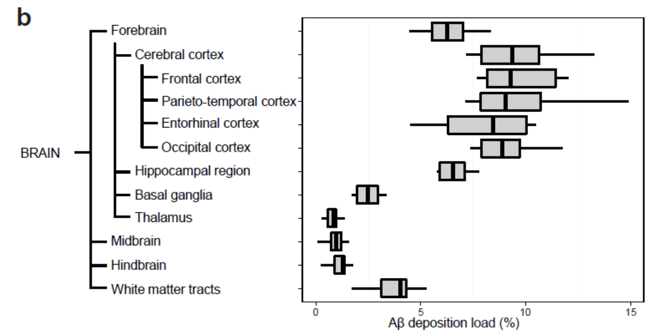
Atlas cerveau souris

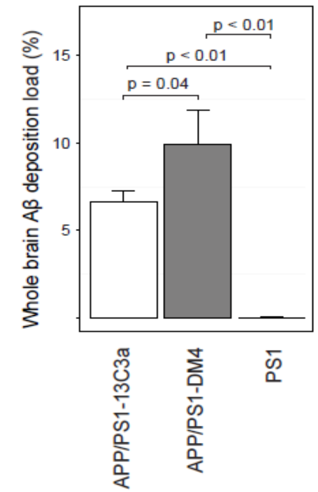


Carte quantitative



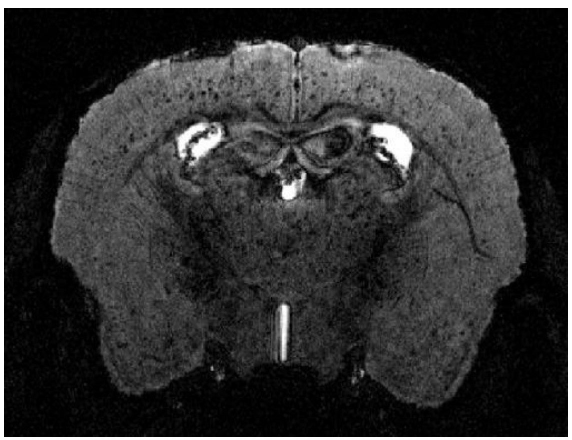
Vandenberghe *et al.*, Sci Rep, 2016

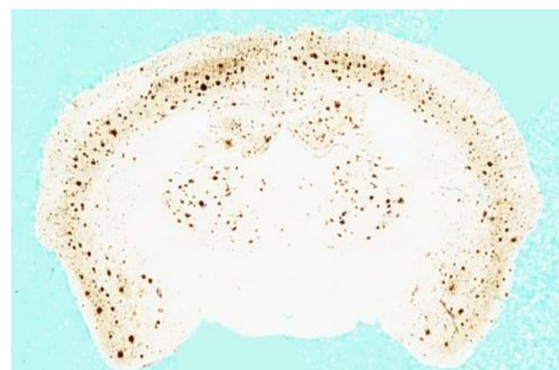




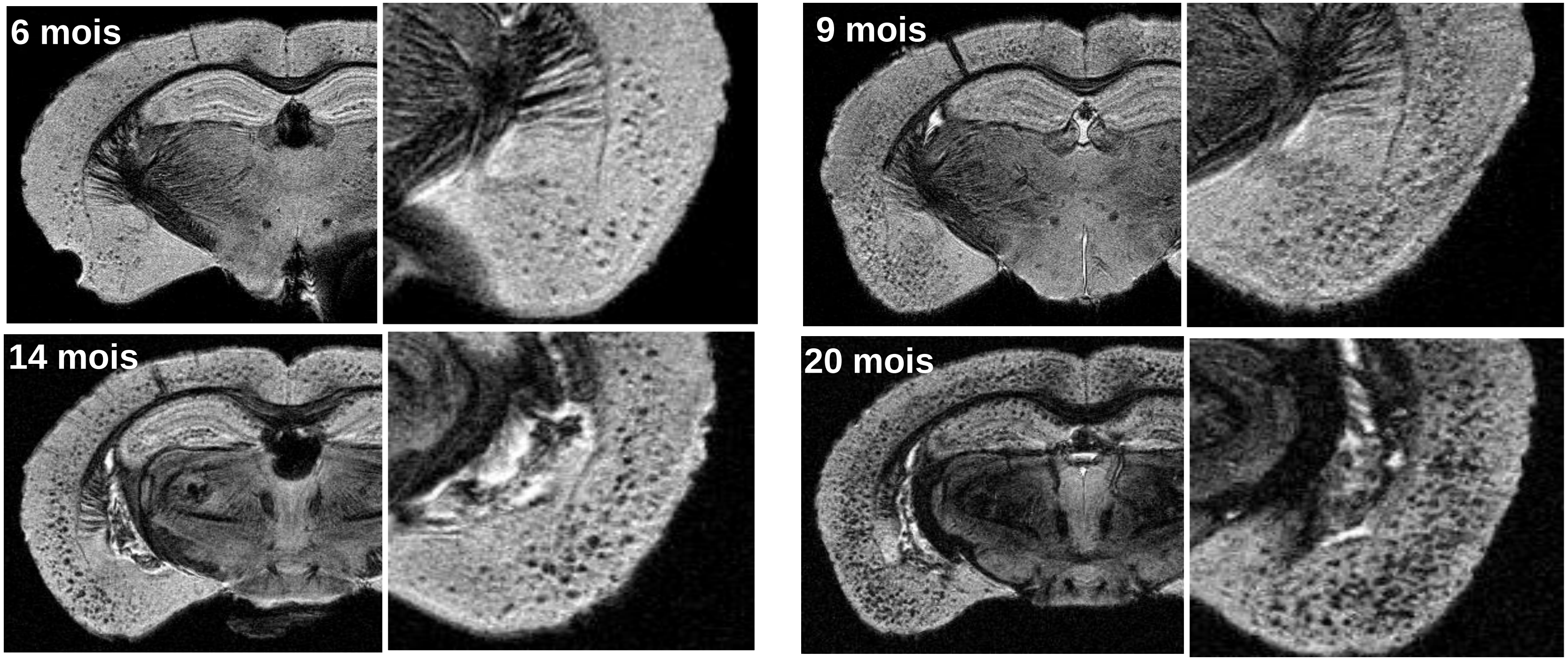
3) *In vivo – post mortem*

Co-registration

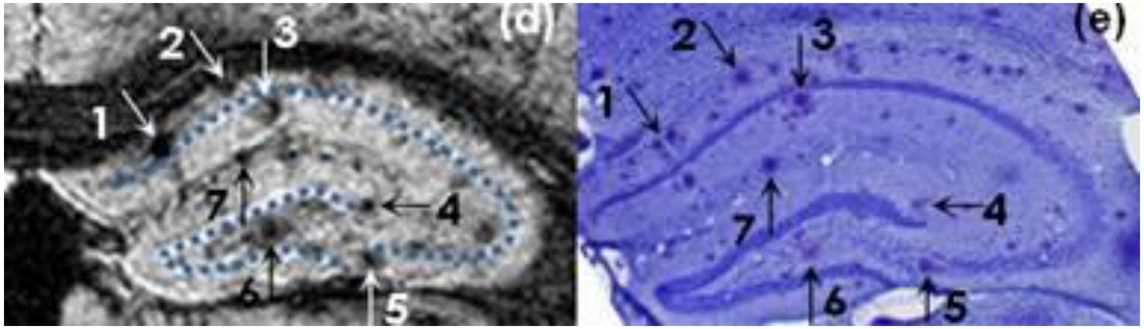




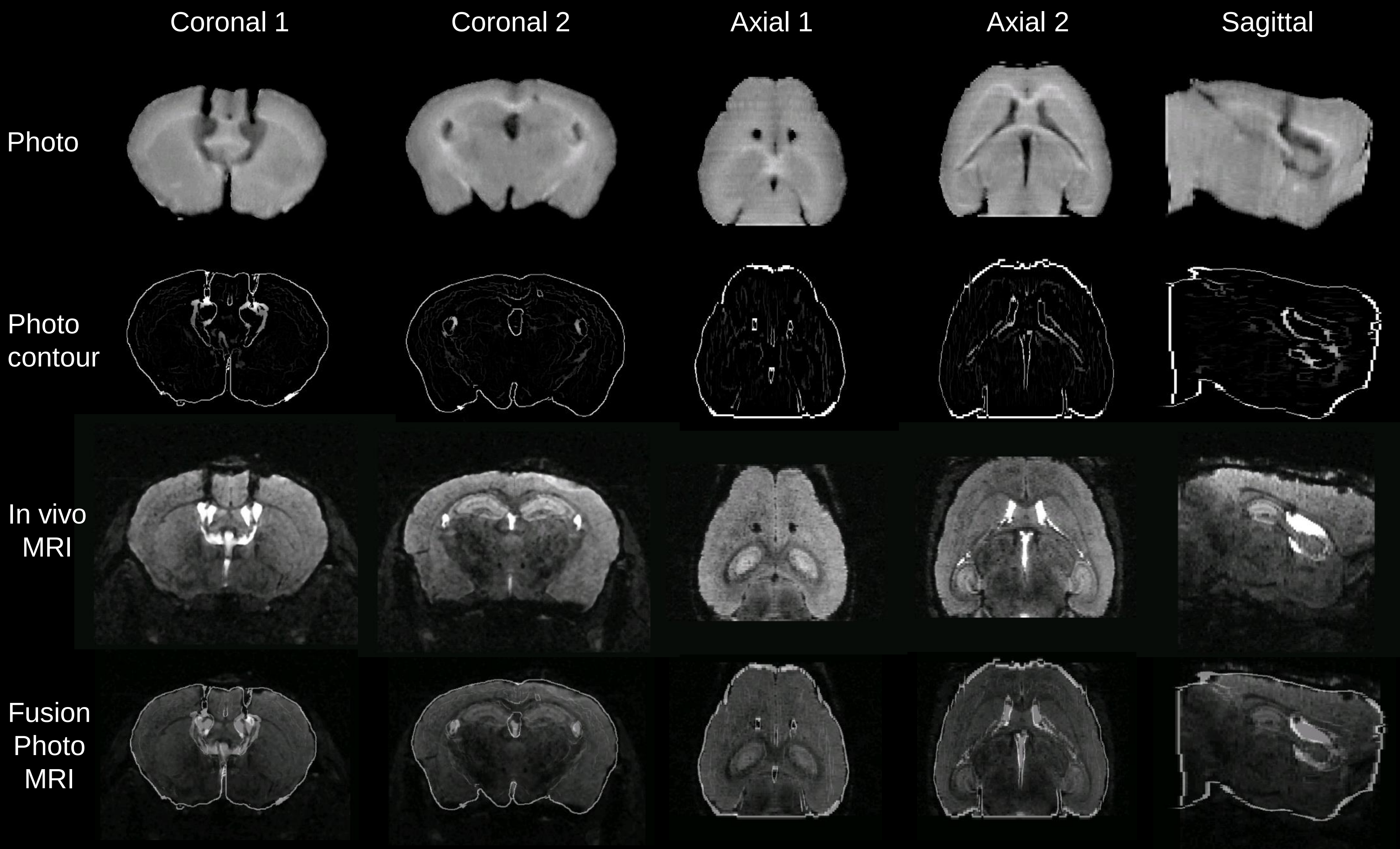
In vivo / post mortem co-registration



M. Dhenain team
(*post mortem* MRI)



In vivo / post mortem 3D *co*-registration



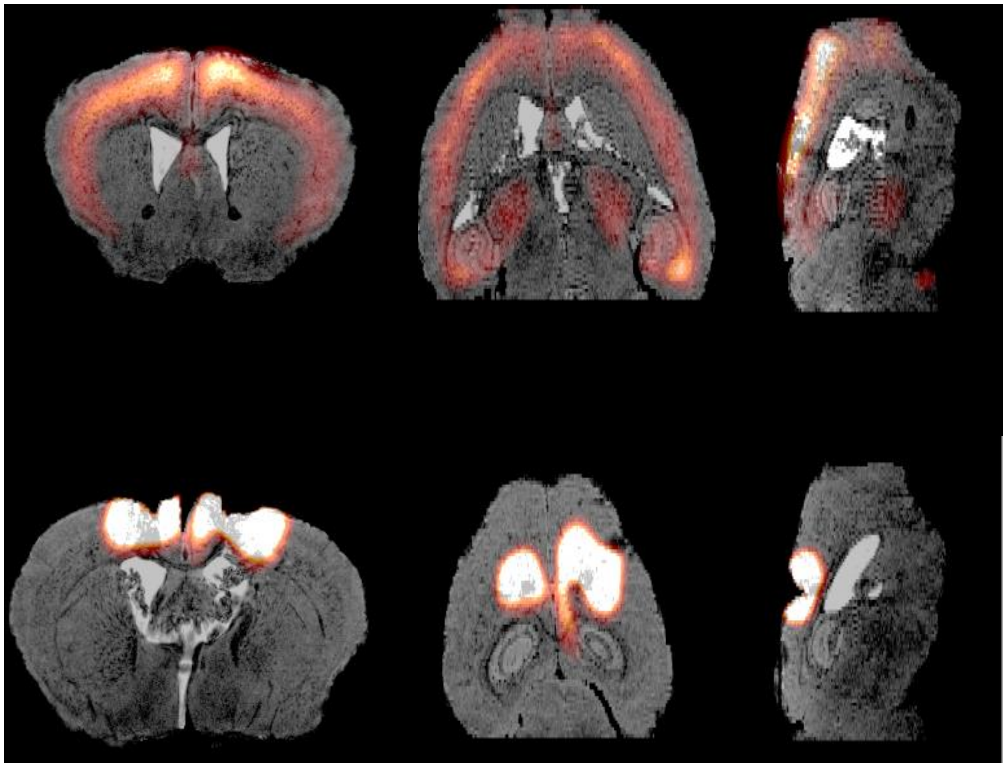
In vivo / *post mortem* 3D *co*-registration

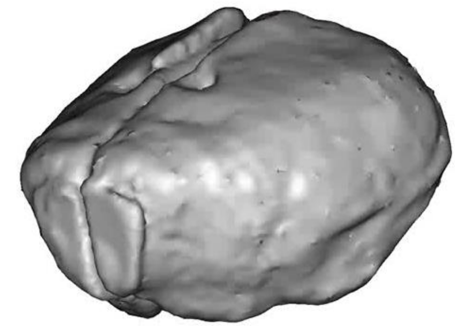
3D view

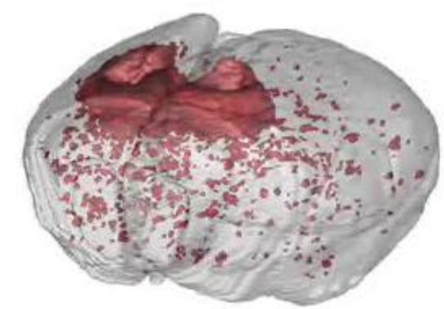
Coronal | Axial | Sagittal

Amyloid density + MRI

BHE leakage + MRI







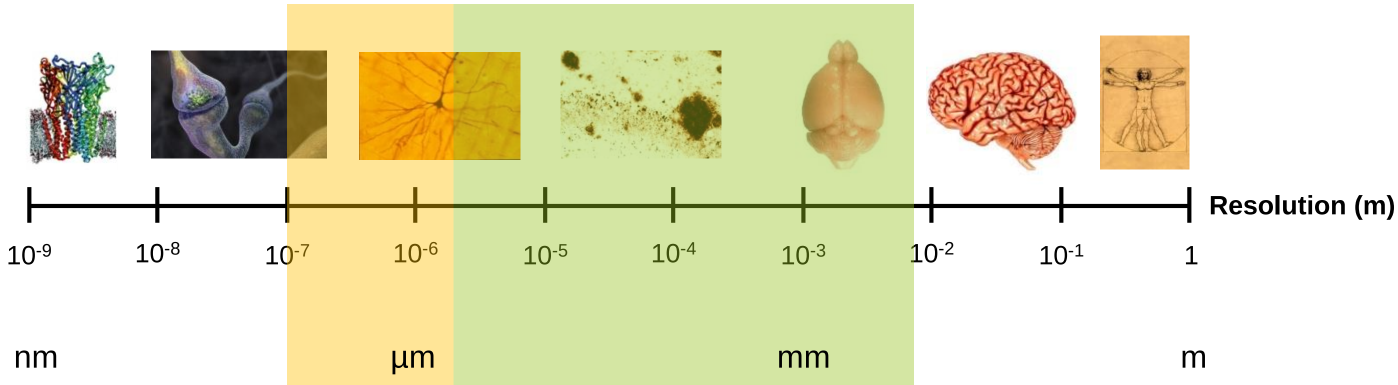
Vandenberghe *et al.*, Sci Rep, 2016

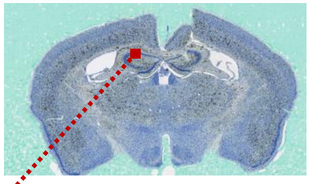
Santin et *al.*, Front Aging Neuro, 2016

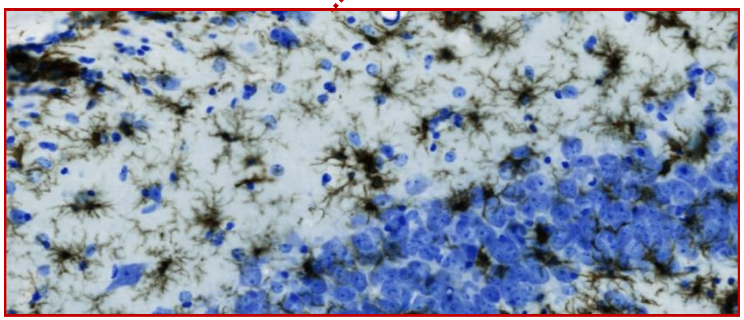
4) *Future research*

Microscopic level

3D whole-brain histopathology



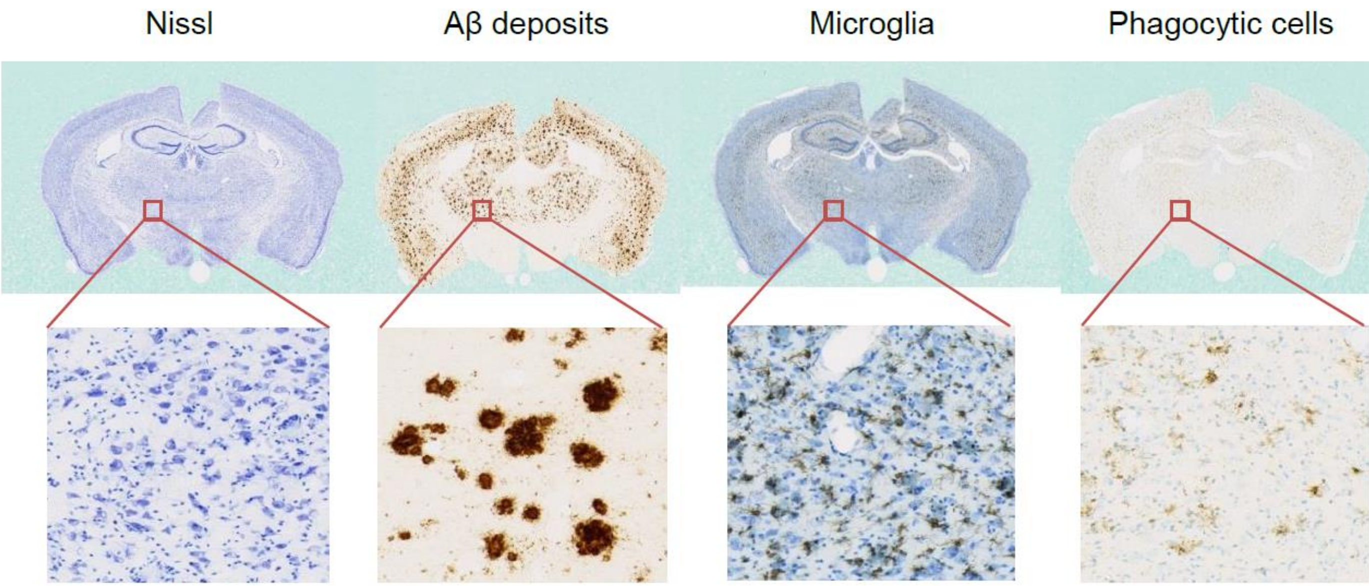




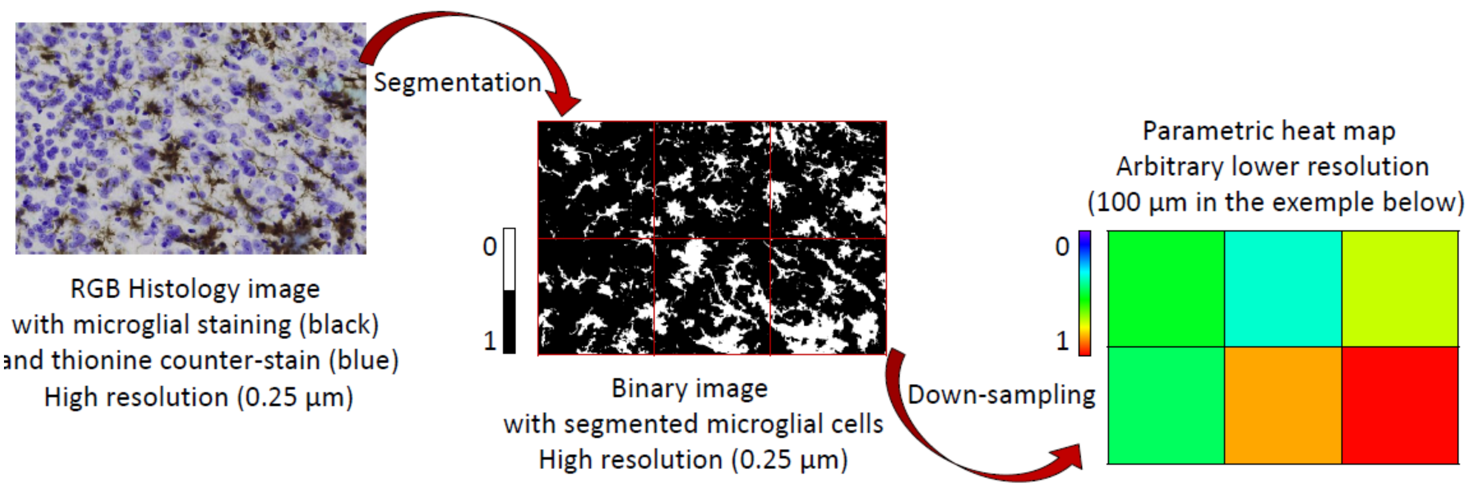
Images	Resolution (xyz, µm)	Approximate number of voxels	Approximate file size (gygabytes)
7T mouse brain MRI scan	30 30 30	10^7	0.02
Block-face photography volume (100 sections)	30 30 120	10^7	0.02
Mesoscopic IHC volume (100 sections)	5 5 120	10^8	1
Microscopic IHC volume (100 sections)	0.20 0.20 120	10^{11}	1000

Multiple markers 3D histopathology [1/3]

Information acquired at cellular level

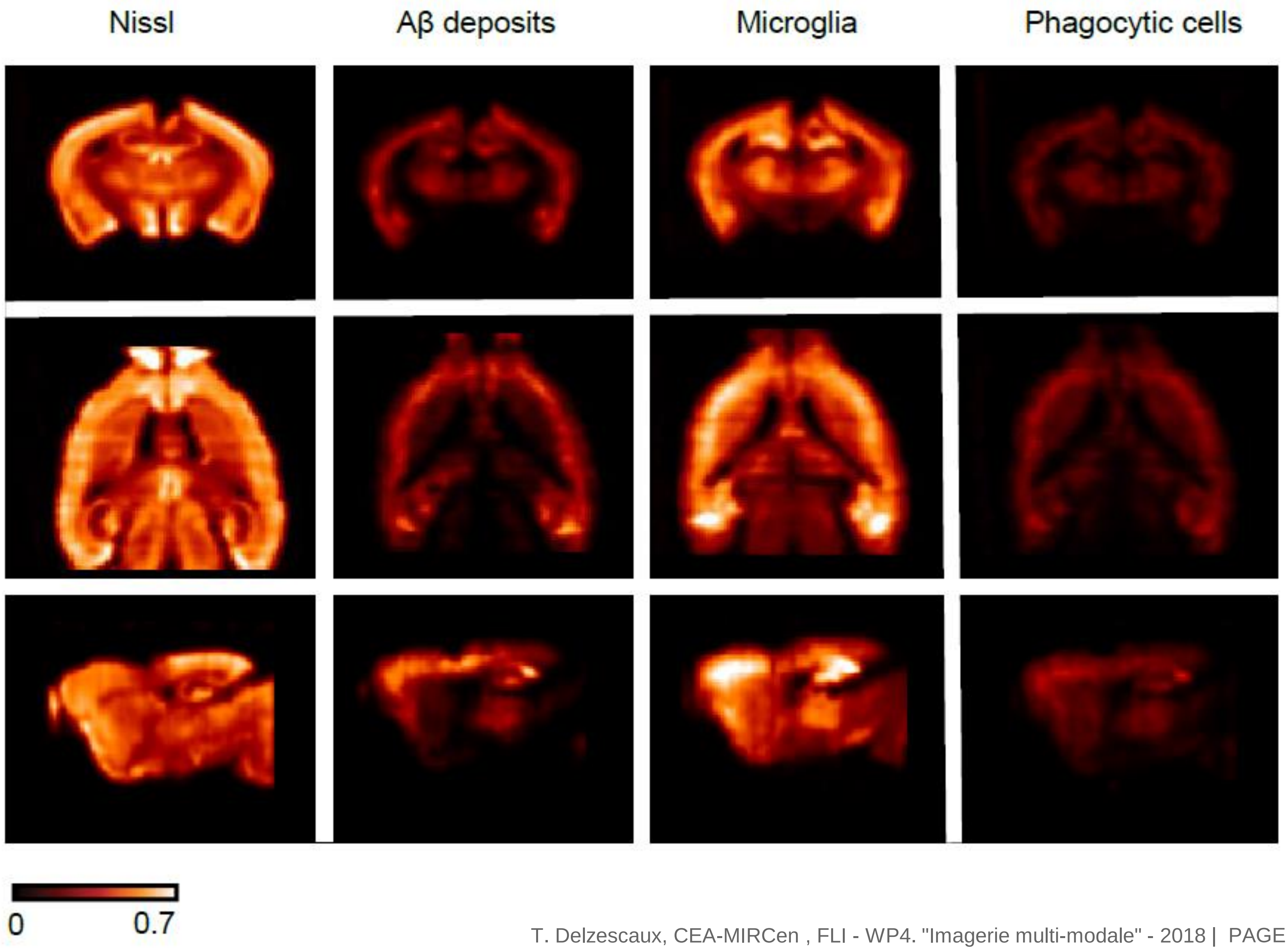


Analysis

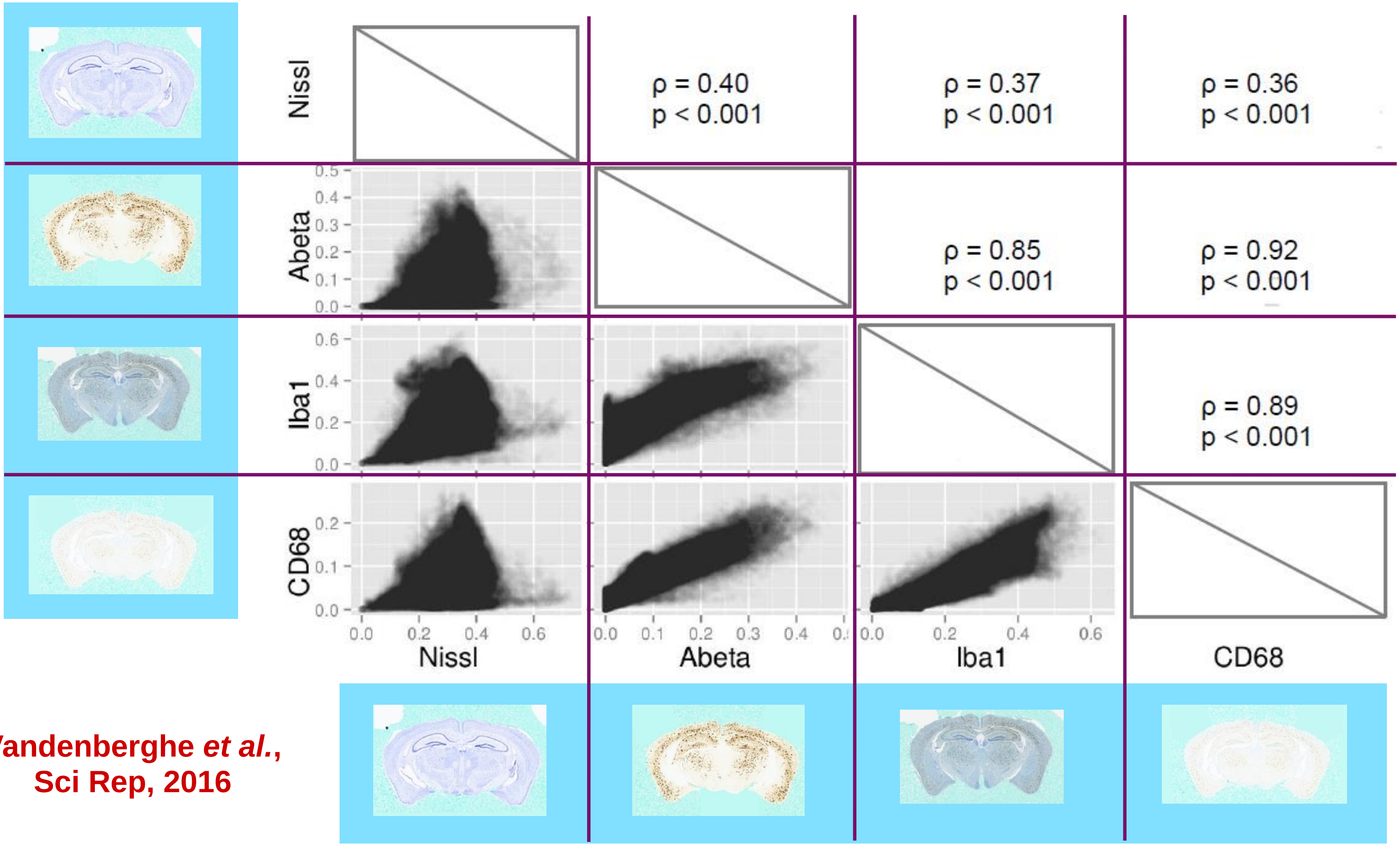


Deriving mesoscopic quantitative information from high-resolution histology images.

Multiple markers 3D histopathology [2/3]



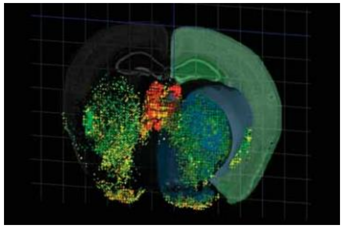
Multiple markers 3D histopathology [3/3]



Vandenberghe *et al.*, Sci Rep, 2016

Bridging the gap between microscopic and macroscopic scale

3D histology

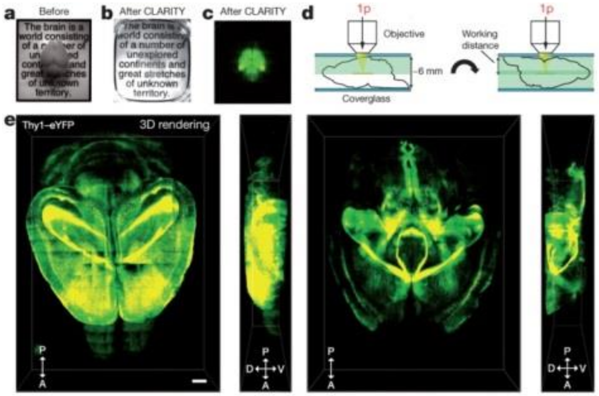


Lein *et al*, Nature 2007

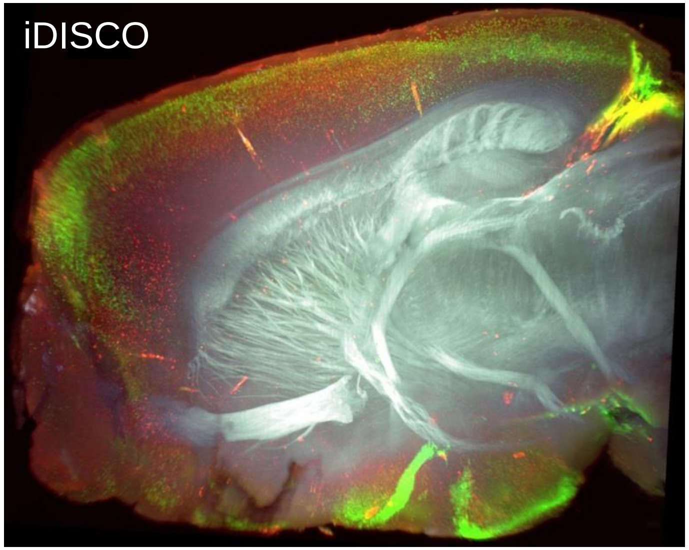
Yang *et al*, Frontiers in Neuroanatomy, 2013

Dauguet *et al.*, J Neurosci Methods 2007
Dubois *et al.*, Neuroimage 2010
Lebenberg *et al*, NeuroImage 2010

Clarification



Chung *et al*, Nature 2013



High performance computing

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- Carole Escartin
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Programme transversal
Technologies pour la Santé (CEA)