DE LA RECHERCHE À L'INDUSTRIE

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HIGH-THROUGHPUT 3D WHOLE-BRAIN QUANTITATIVE HISTOPATOLOGY IN RODENTS

GdR – ISIS : Analyse de tissu biologique et histopathologie numérique

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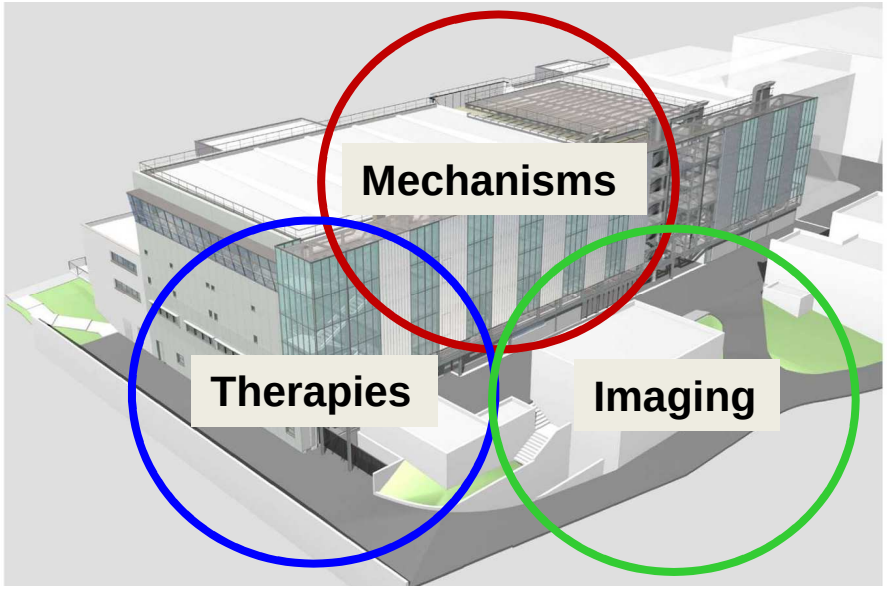
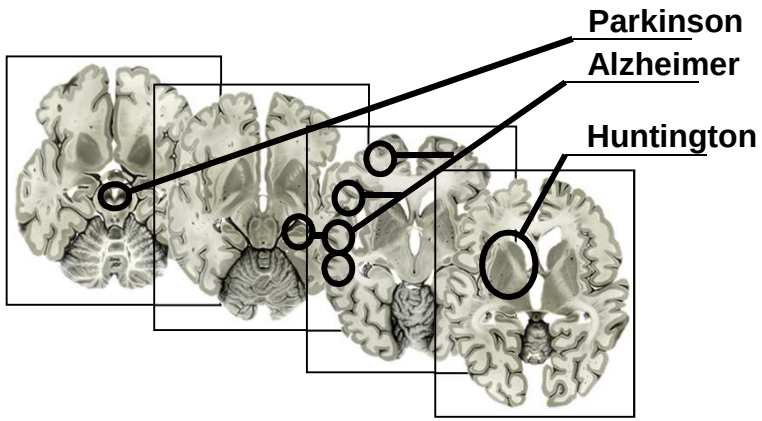
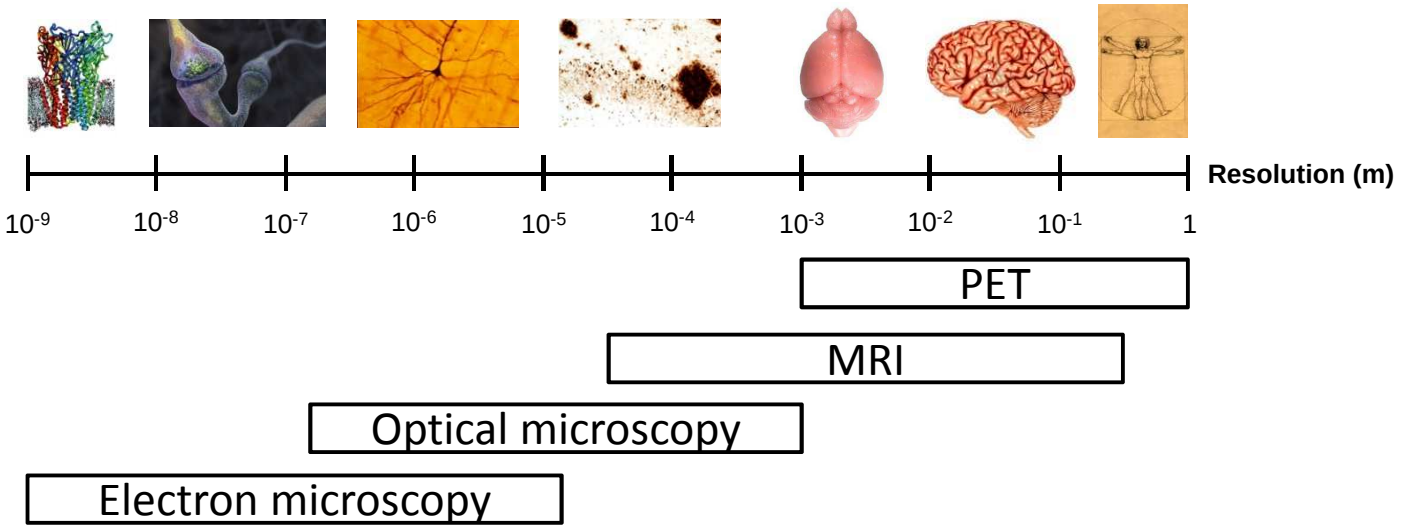




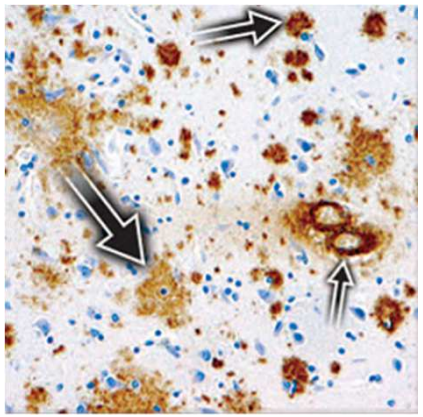
An integrated platform:

- Housing and experimentation A1/A2/A3
- MRI – MRS
- PET
- Histology
- Microscopy
- Image analysis

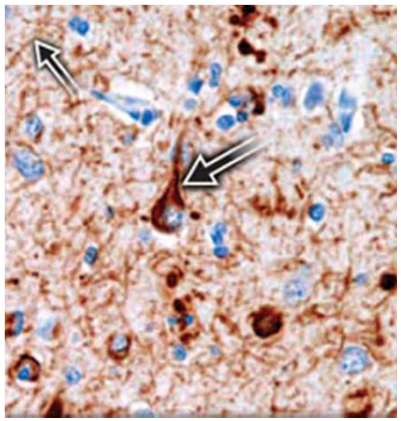
Histopathology in Alzheimer's Disease (AD)



Amyloid IHC



Tau IHC

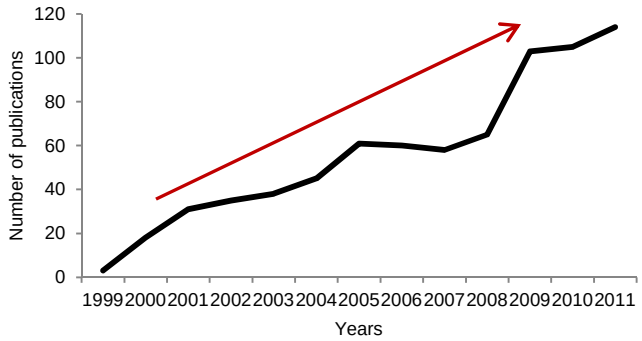


- **High-specificity** +
- **High-resolution**
- ***Post mortem*** -
- **Two-dimensional**

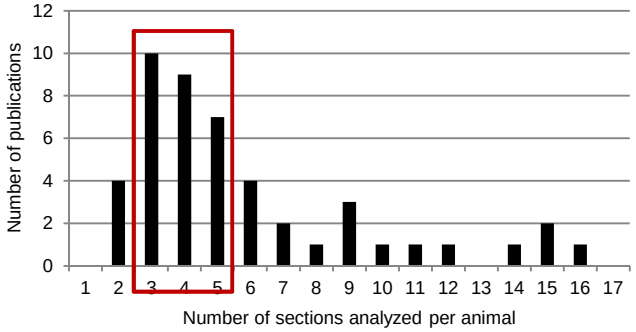
Holtzman *et al*, 2011

Literature survey: amyloid load quantification

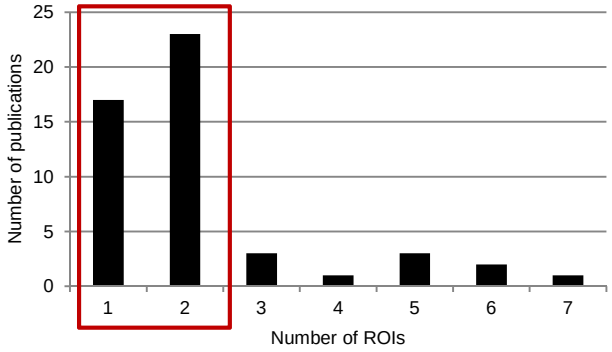
Publications with amyloid load quantification



How many sections ?

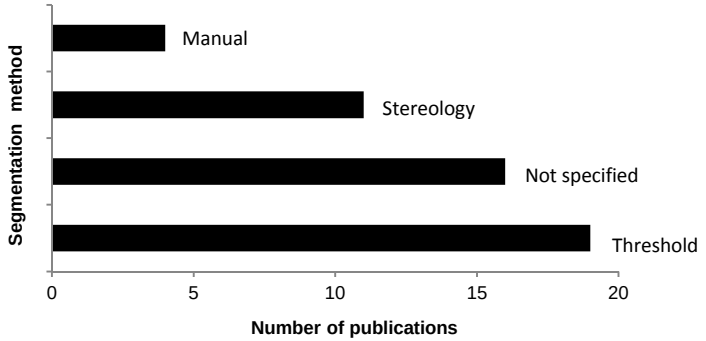


How many ROIs ?



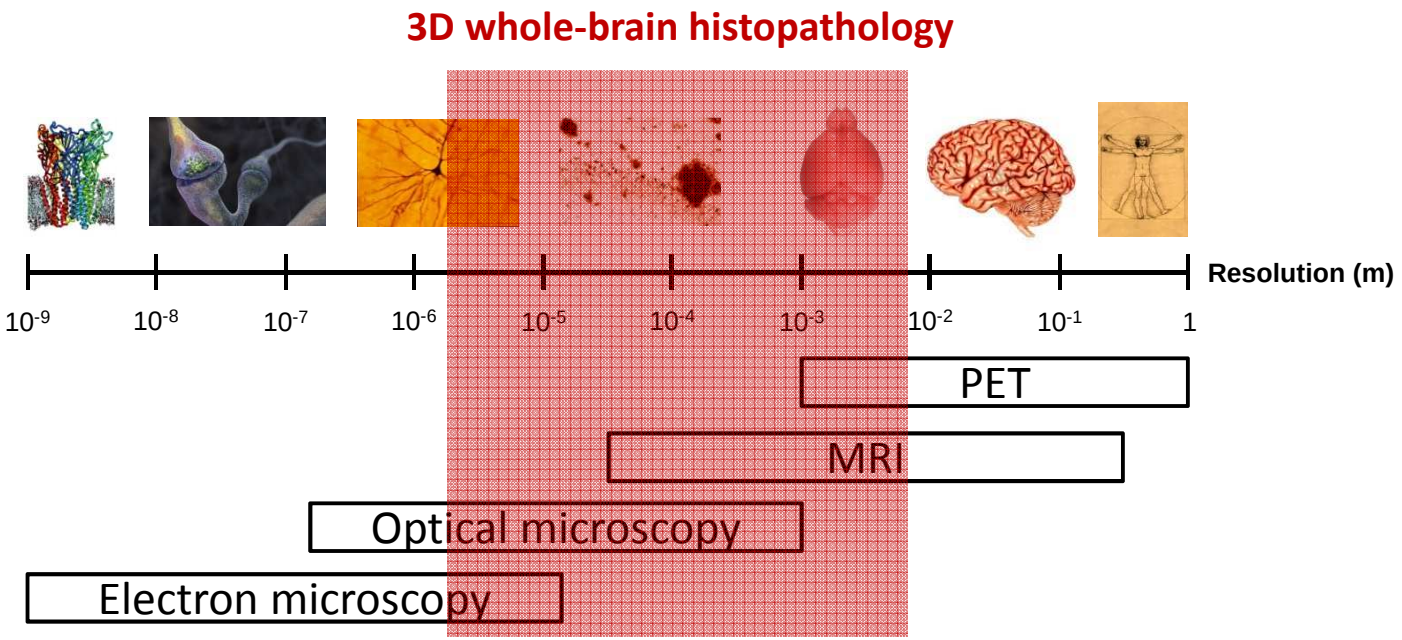
→ Cerebral cortex & hippocampus

How are amyloid plaques segmented ?



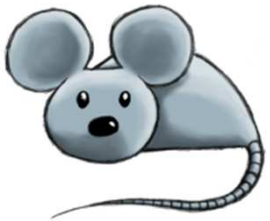
Interest to extend the analysis to 3D ?

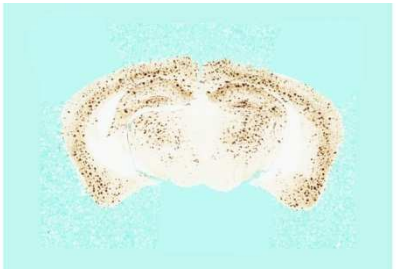
3D whole-brain histopathology: amyloid load



Applications of brain-wide quantitative histopathology at the mesoscopic scale:

- Mouse model characterization
- Preclinical evaluation of drug candidates
- Gene effect on amyloidosis
- *In vivo* imaging validation





Material and methods

Experimental designs

- **First study:**
  - 13.5 months old APP/PS1dE9 mice (n=6)

  → **Model characterization**

- **Second study:**
  - 12 APP/PS1 mice:
    - 8 were treated with 13C3a
    - 4 were treated with DM4 (treatment control group)
  - 4 PS1 mice (amyloid plaque free mice).
  - Mice were imaged by MRI for amyloid plaques before euthanasia (Marc Dhenain, Mathieu Santin).

  → **Method validation and comparison with classical quantification approach**

  → **13C3a immunotherapy evaluation**

  → **Spatial correlation of pathological and cellular markers**

  → ***in vivo* - *post mortem* confrontation**

- **Third study:**

  APPswe mice (n=5), APPswe-ADAM30 mice (n=5), APPswe-ADAM30-Cre (n=10), other groups…

  → **What is the effect of ADAM30 expression on amyloid load?**

Histology processing

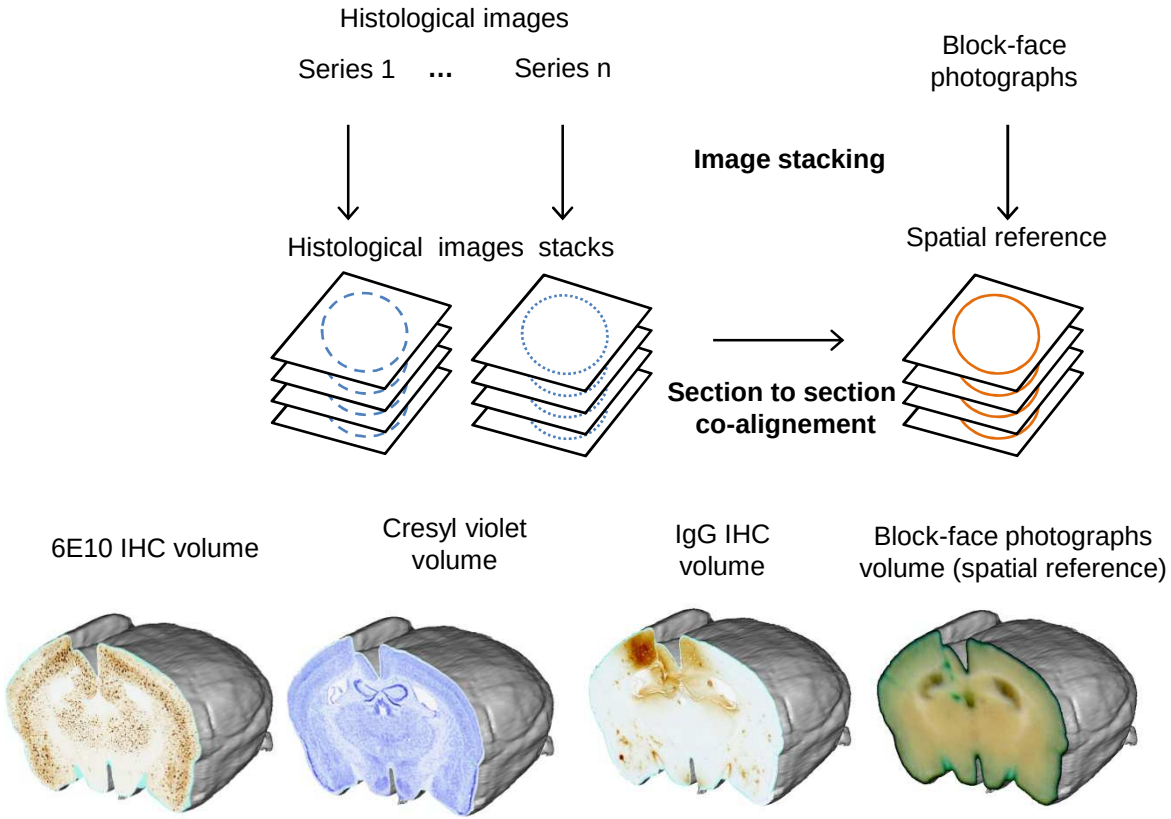
- Block-face photographs were taken during brain cutting (lateral resolution: 33 µm)

- Sections were stained for :
  - Anatomy (Nissl staining)
  - Aβ plaques (6E10 IHC)
  - Mouse IgG1 (GAM IHC)
  - Microglia (Iba-1 IHC)
  - Activated microglia (CD-68 IHC)

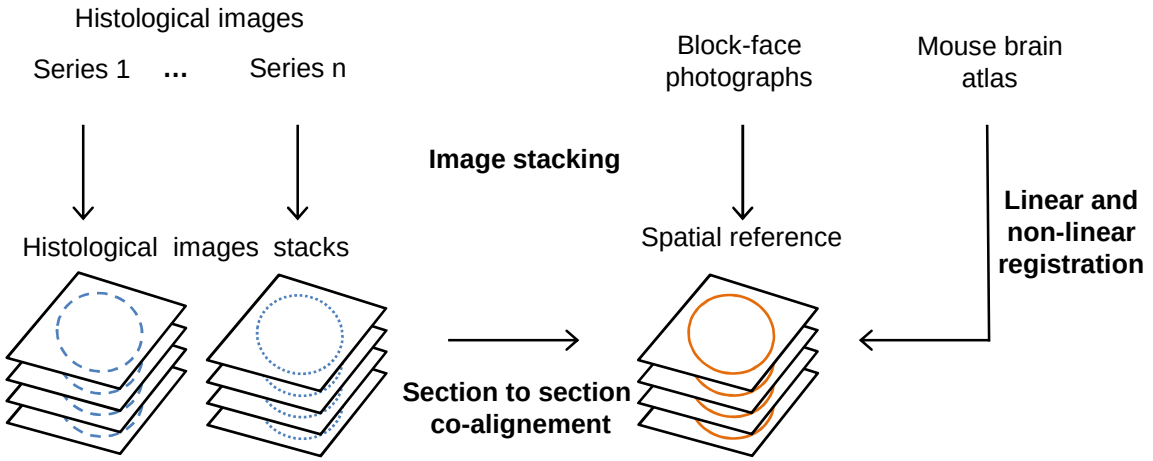
- Images are acquired at 21 µm/pixel for Nissl series and at 5 µm/pixel for the other series.

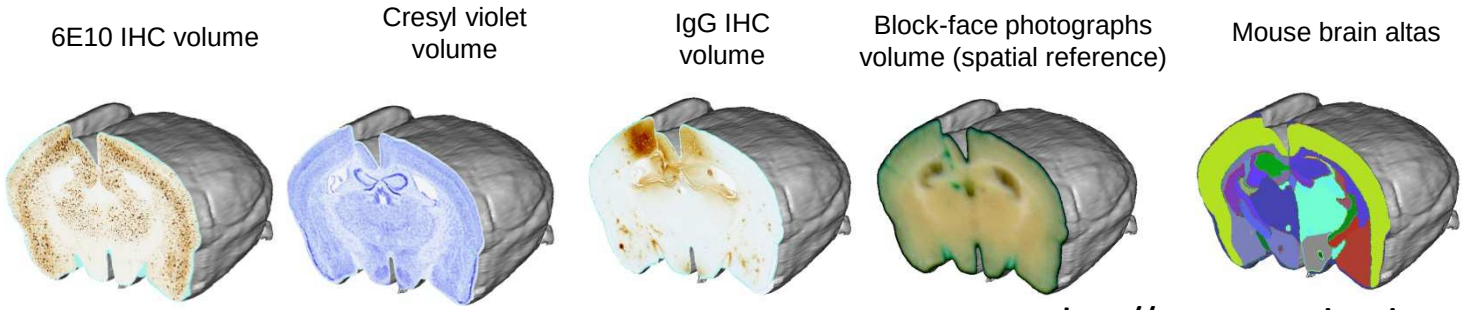
Nissl 6E10 IgG1 Iba-1 CD-68

3D reconstruction



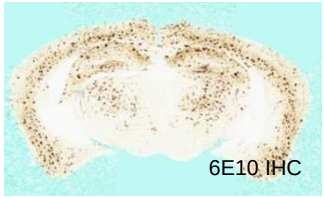
Atlas based analysis (1/3)

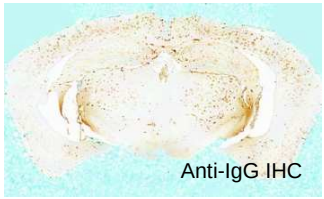


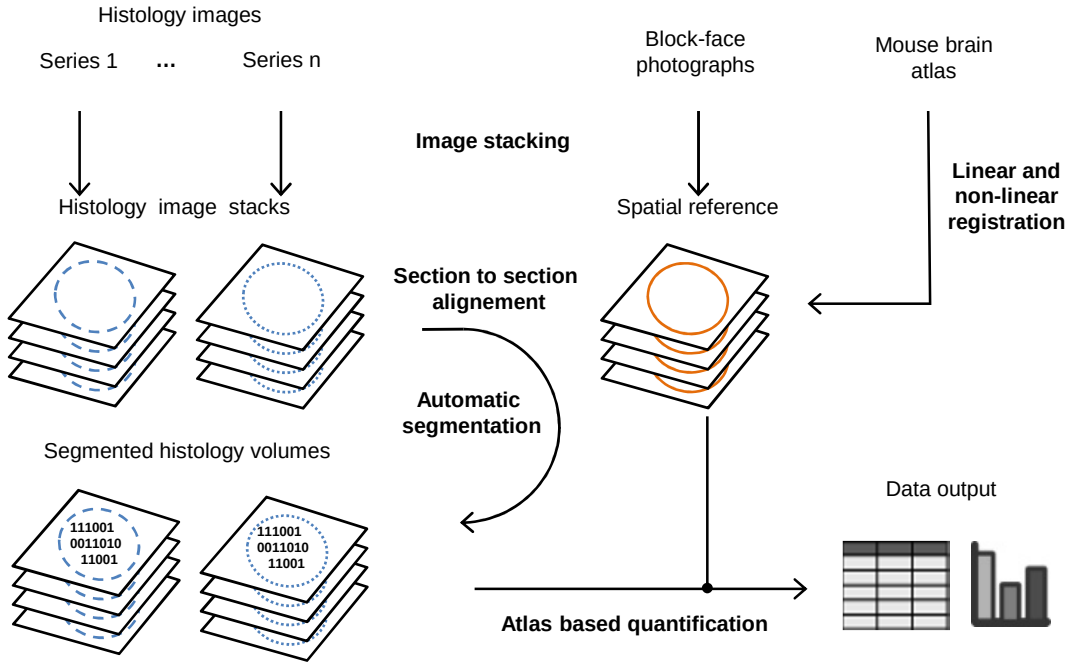


http://www.mouseimaging.ca/technologies/C57Bl6j_mouse_atlas.html

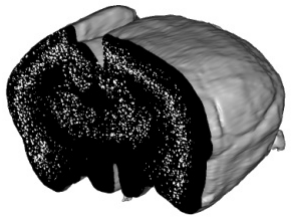
Quantification of histological markers



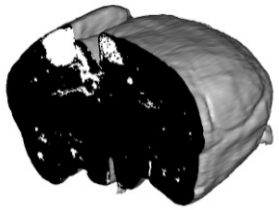




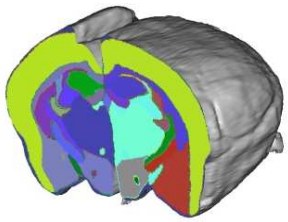
6E10 IHC segmented volume



IgG IHC segmented volume



Mouse brain altas



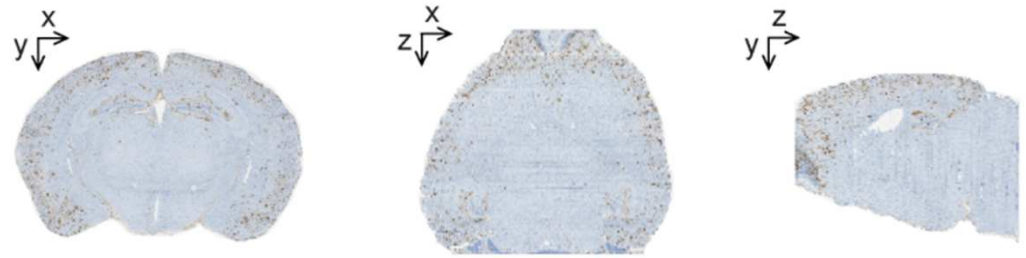
→ Histological volumes are segmented

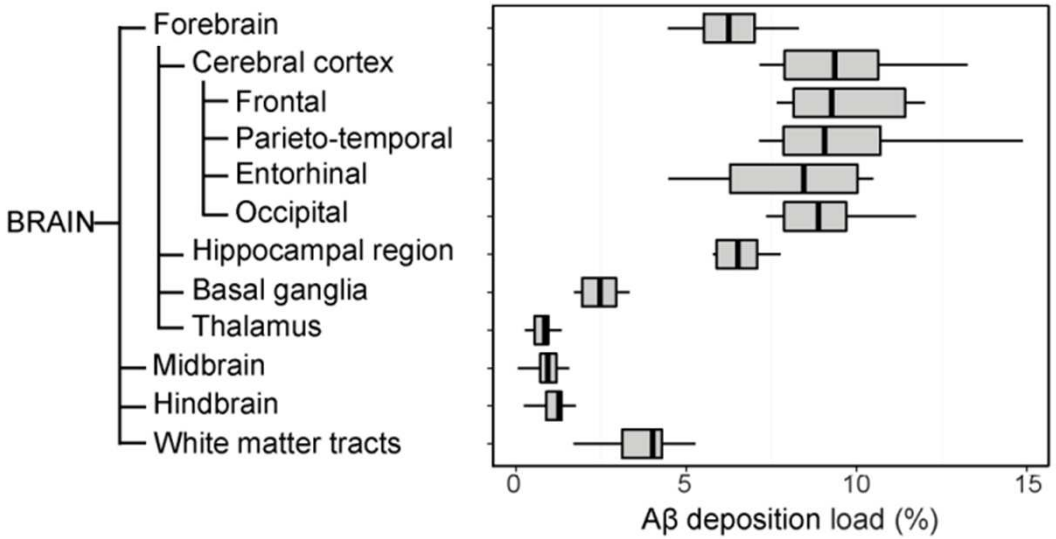
→ Segmented volumes are in the spatial reference

→ Data output compatible with statistical software (Statistica, R…)

Applications

Mouse model characterization

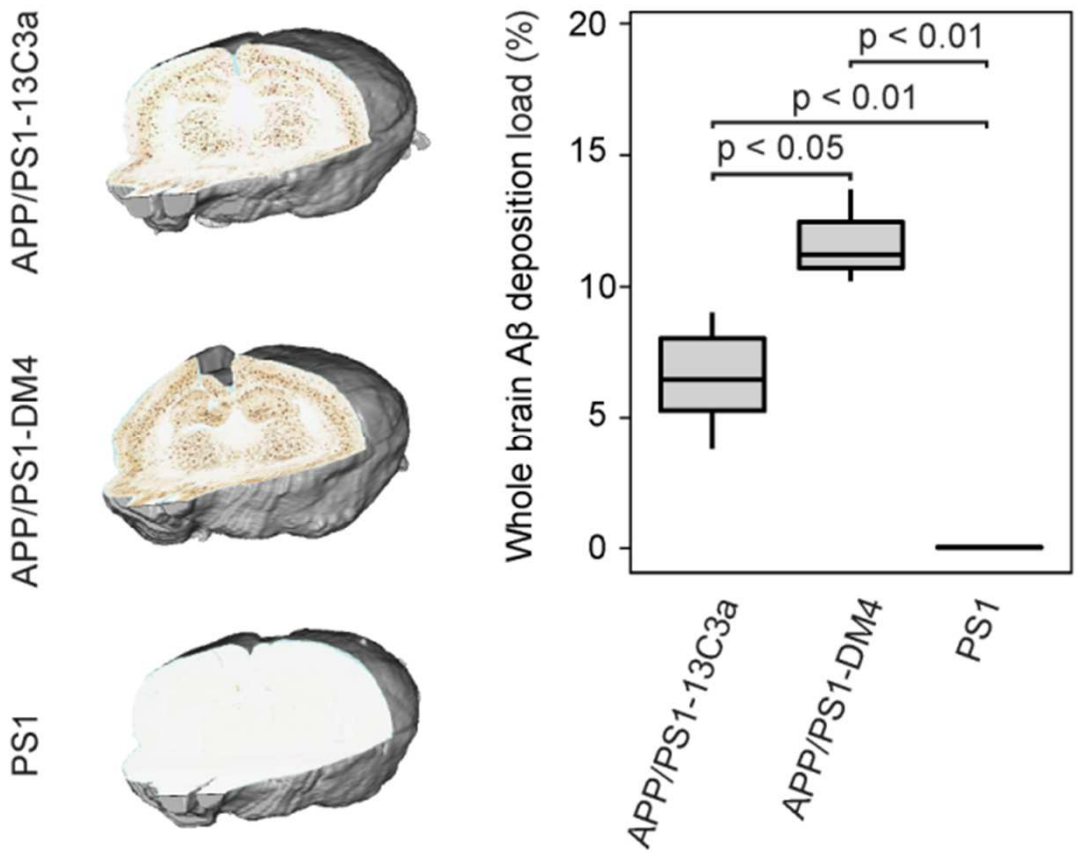




→ Amyloid load deposition +++ in cerebral cortex and hippocampal region

→ Amyloid deposition detected in basal ganglia and white matter tracts

Immunotherapy evaluation

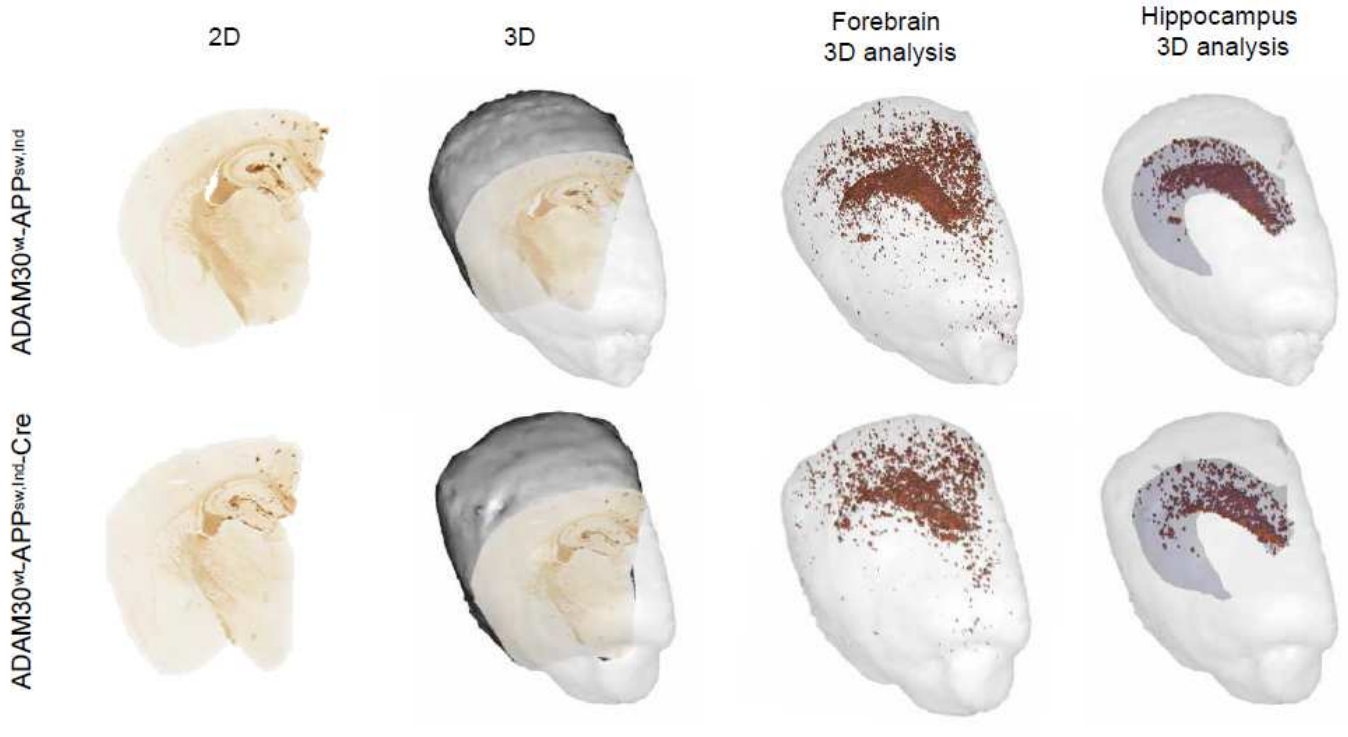


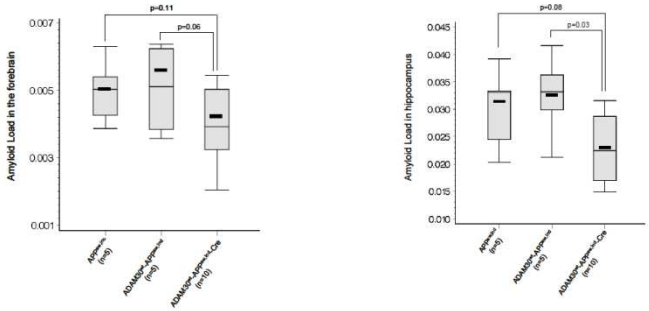
Vandenberghe *et al.*, in revision.

→ **Amyloid load is close to none in PS1 mice**

→ **13C3a lowered amyloid load in APP/PS1 mice.**

ADAM30 overexpression effect on amyloid load





Institut Pasteur de Lille

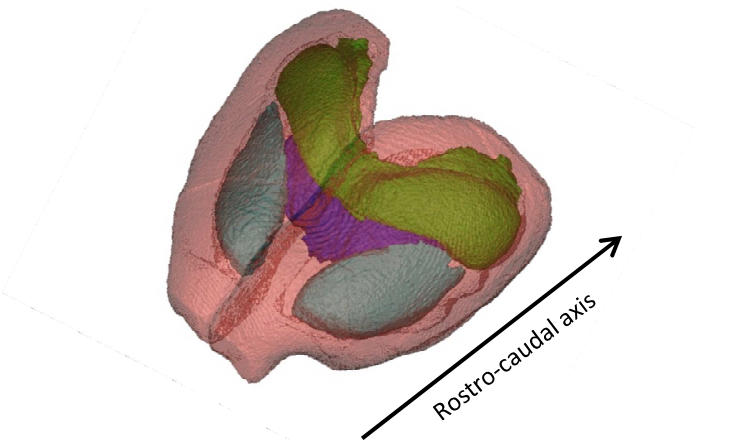
Letronne *et al.*, in preparation
Vandenberghe *et al.*, in preparation.

Atlas-based analysis

Validation and comparison with current standards

Validation and comparison with current standards

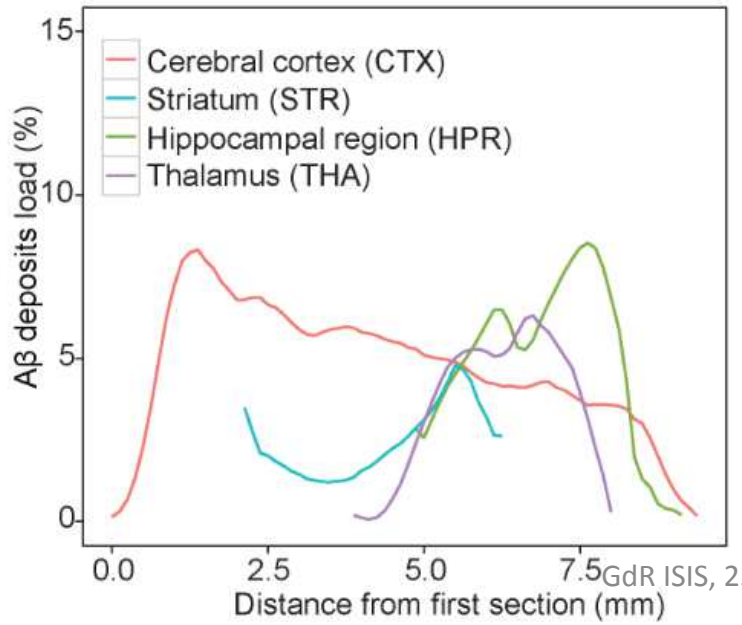
Mouse brain ROIs in 3D

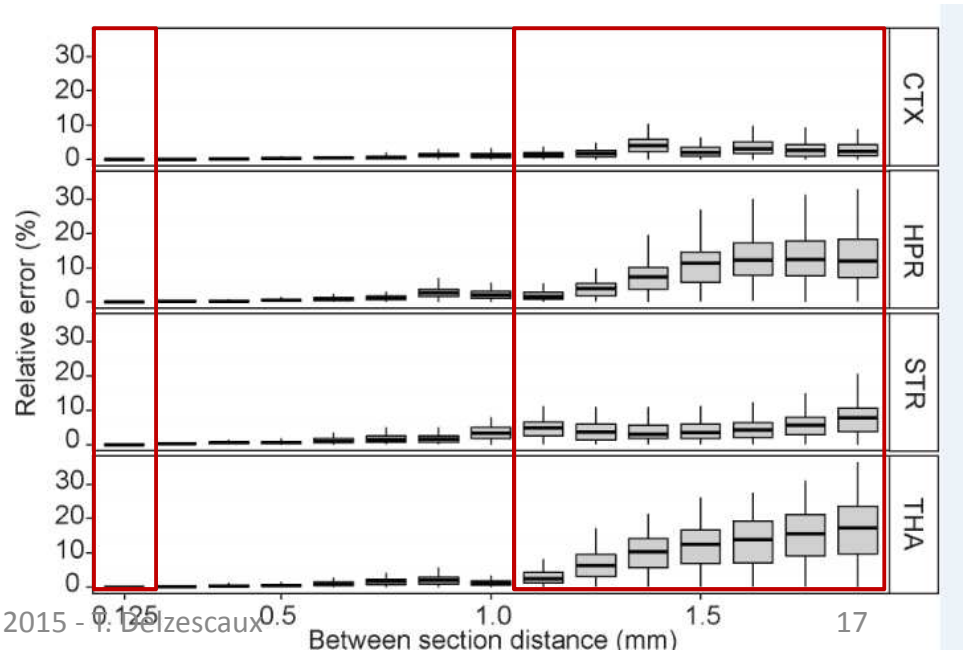


Amyloid load variation along the rostro-caudal axis (n=12 APP/PS1 mice)

ROI	Mean coefficient of variation (n=11)
Cerebral cortex	0.39
Hippocampal region	0.44
Striatum	0.38
Thalamus	0.55

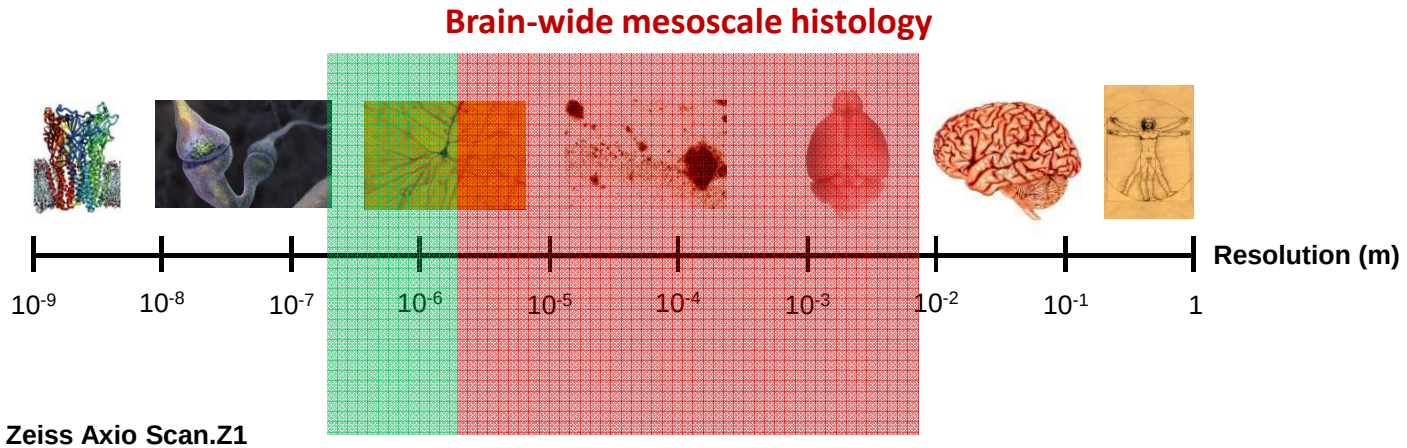
Profiling amyloid load distribution along the rostro-caudal axis

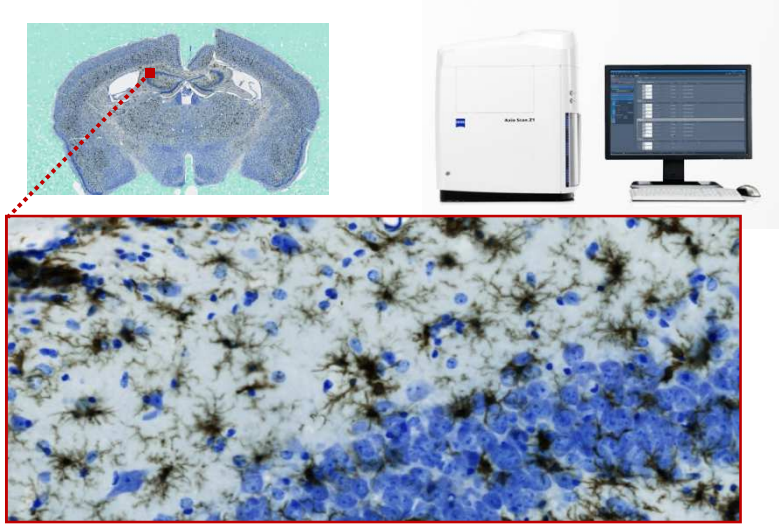




Perspectives and preliminary results

Perspective: microscopic image analysis

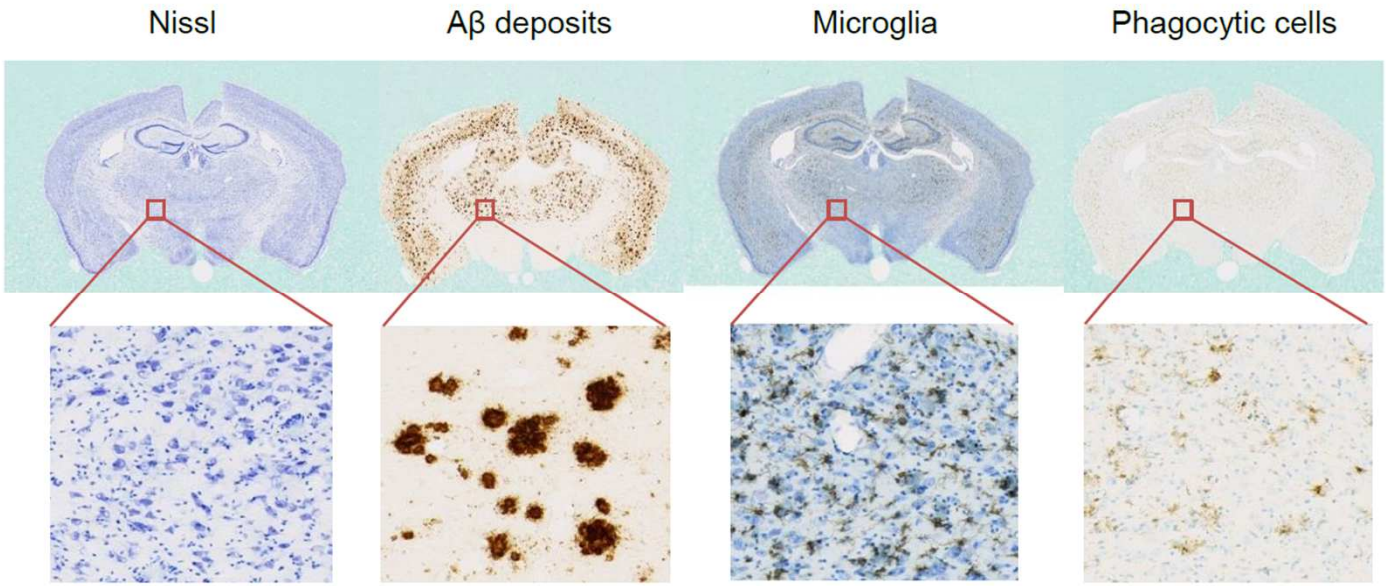




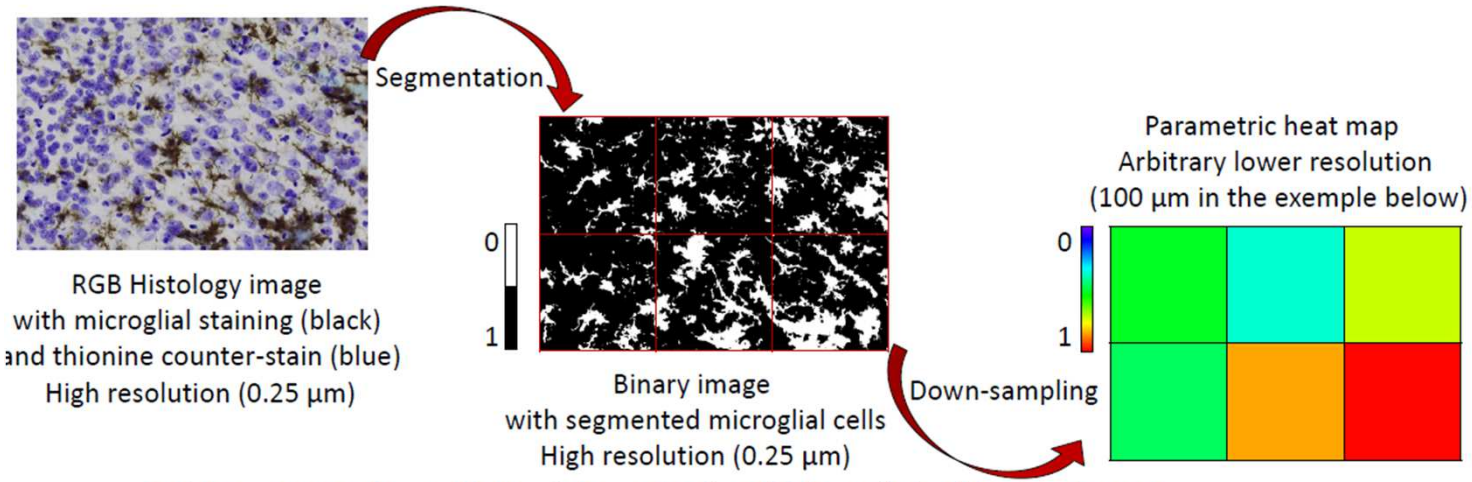
Images	Resolution (xyz, µm)	Approximate number of voxels	Approximate file size (gygabytes)
7T mouse brain MRI scan	30 30 30	10^7	0.02
Block-face photography volume (100 sections)	30 30 120	10^7	0.02
Mesoscopic IHC volume (100 sections)	5 5 120	10^8	1
Microscopic IHC volume (100 sections)	0.20 0.20 120	10^{11}	500

From mesoscopic to microscopic scale (1/4)

Information acquired at cellular level

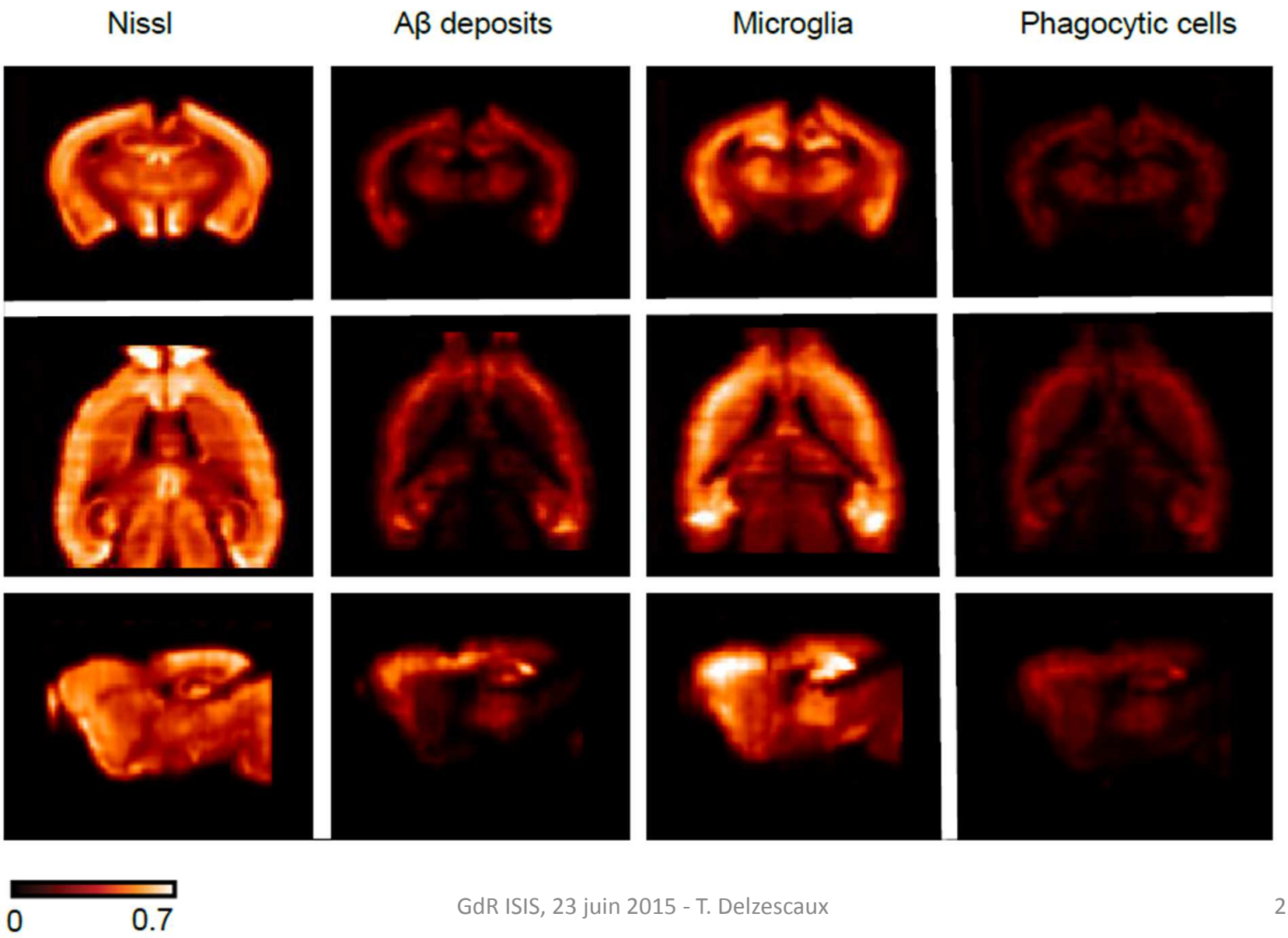


Analysis

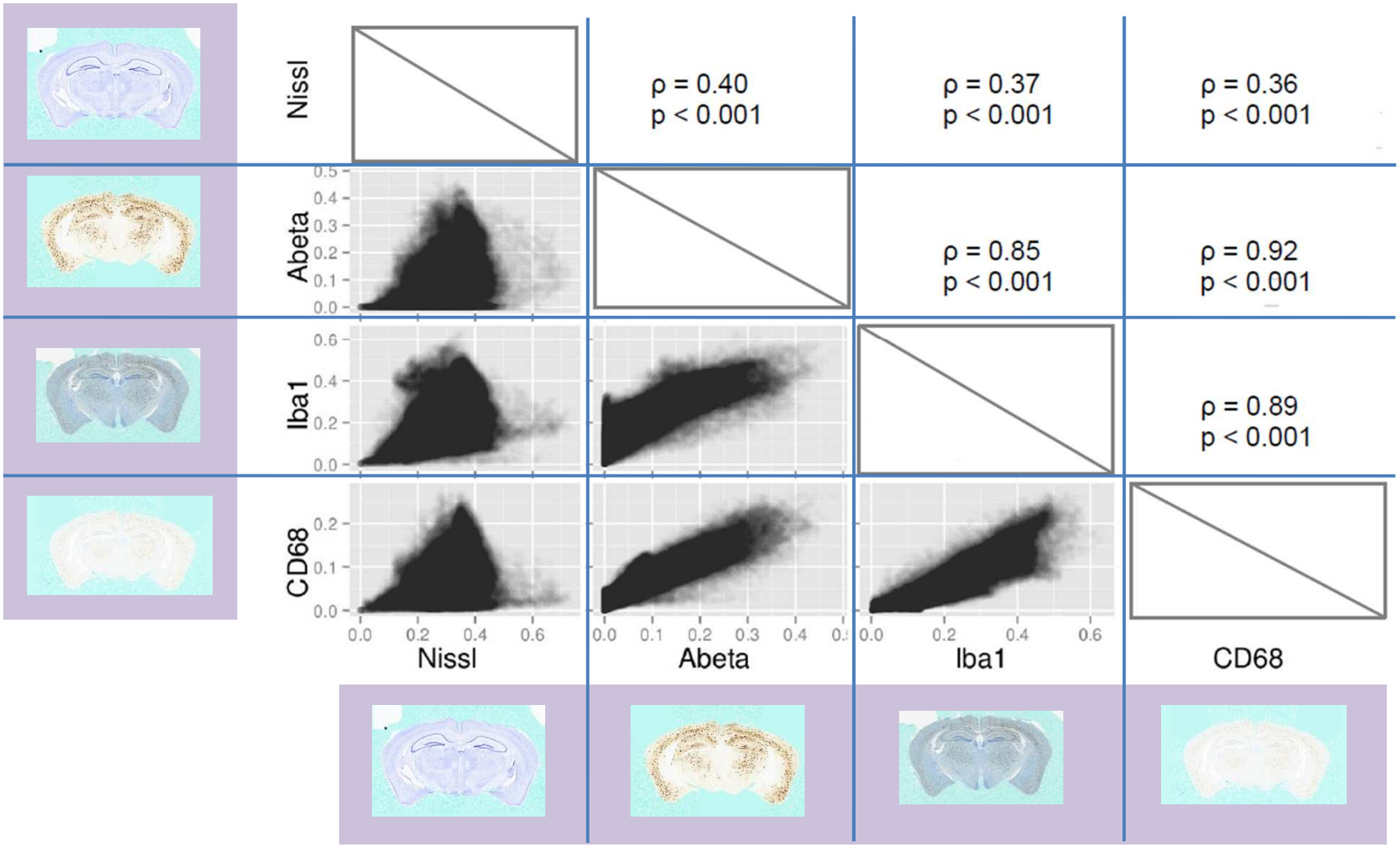


Deriving mesoscopic quantitative information from high-resolution histology images.

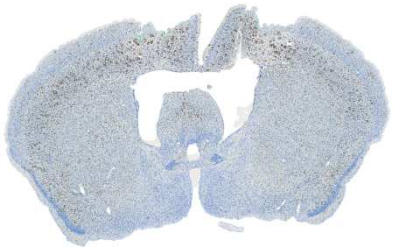
From mesoscopic to microscopic scale (2/4)



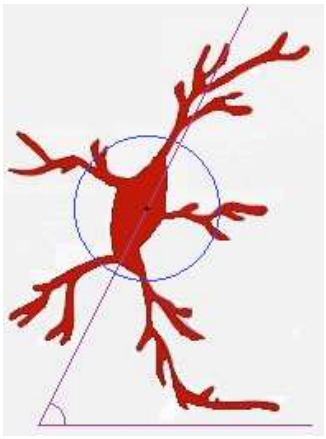
From mesoscopic to microscopic scale (3/4)



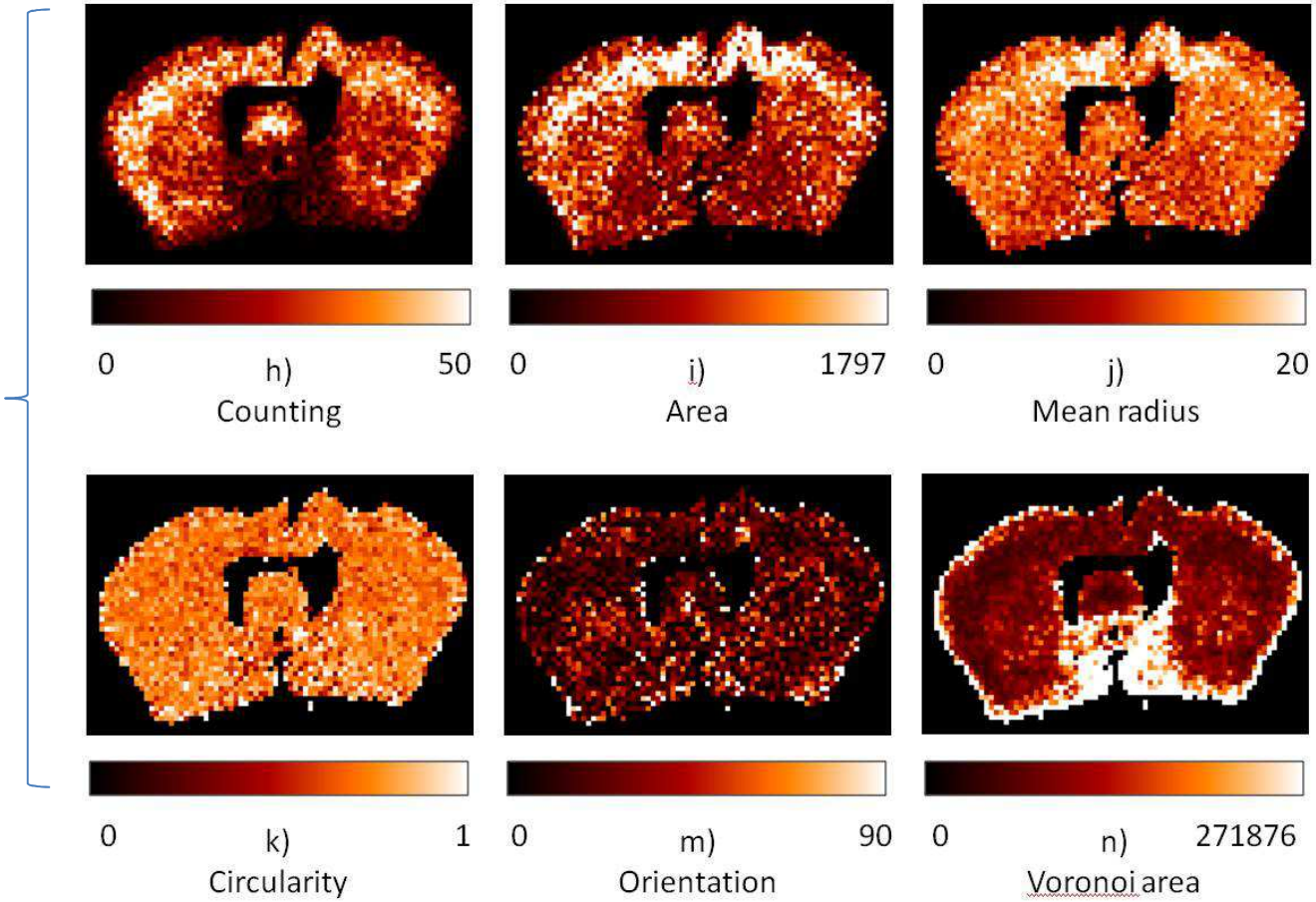
From mesoscopic to microscopic scale (4/4) (Zhenzhen YOU)



Mouse brain section (Iba1)



Microglia cell



New challenges to adress: cell indidualization, big data processing, etc...

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Thomas Debeir
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