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Le 23ème Colloque Médecine et Recherche en Neurosciences de la Fondation IPSEN

Cellular morphometric analysis: from microscopic scale to whole mouse brains

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Context

Neurodegenerative diseases occur when neurons in the brain and spinal cord begin to deteriorate.

In certain cases such as Alzheimer's disease, cell morphology and function are disturbed *(Fig. 1)* and **pathological aggregates** form in the brain. Characterizing the relationship between these anomalies is important to understand the mechanisms involved in this pathology.

Quantifying the morphological changes is crucial and **Whole-slide imaging (WSI)** offers the unique opportunity **to analyze whole brain sections at the cellular level** using various histological markers. However, this technique generates terabytes of data which can be difficult to analyze.

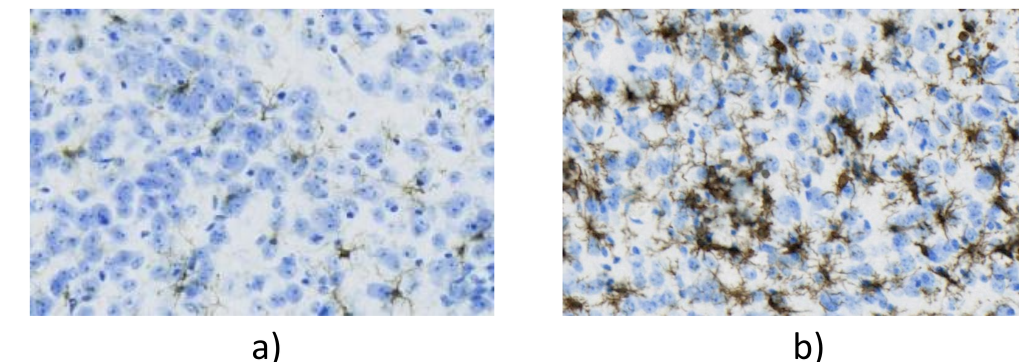

Figure 1*: a) microglial cells (brown) in a normal mouse*
b) activated microglial cells in a mouse model of Alzheimer's disease

Materials

- We worked on an **APP/PS1 mouse model** of Alzheimer's disease.
- A mouse brain was cut into 20-µm-thick sections, yielding a total of about 400 sections.
- Sections were stained with **Iba-1 antibody to mark microglial cells** and scanned using an AxioScan.Z1 (Zeiss) at a resolution of **0.44 µm (x10)** *(Fig. 3a)*.

Methods

- Each section *(Fig. 3a)* was **segmented to detect microglial cells** by a machine learning classifier[1] *(Fig. 3b)*.
- **The microglial cells were labelled** *(Fig. 3c)* and corresponding **Voronoi partitions were calculated** to estimate their spatial influence i.e. each point was attributed to the closest cell *(Fig. 3d)*.
- Based on the original RGB image, labelled image and Voronoi image, **parameters of interest** *(Fig. 2)* were calculated for each microglial cell and **stored in the form of a table** *(Fig. 3e)*.
- According to this parametric information, we spatially summarized information *(Fig. 3f-g)* by generating **quantitative heat maps** *(Fig. 3h-n)* at a lower resolution (**112 µm**) for each parameter of interest.

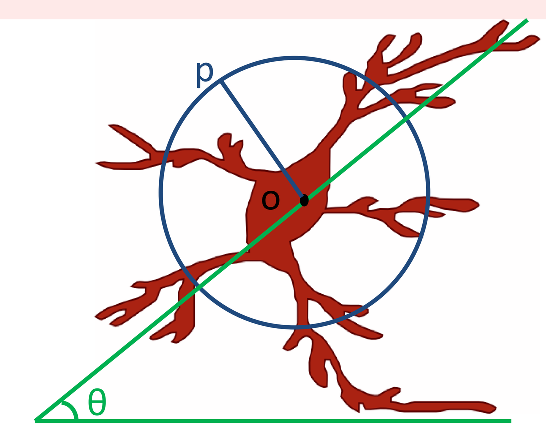

Figure 2: *Parameters of interest. o: mass center, $\overline{op}$: mean radius, Orientation (θ): angle between the direction of maximal Feret diameter and horizontal axis*

Results

Figure 3: *General flow chart, method proposed to integrate mesoscopic quantitative information from high-resolution histology images*

Microscopic image

In plane resolution: 0.44µm

Segmentation of microglial cells

Labelling of microglial cells

Calculation of Voronoi partitions corresponding to each microglial cell to estimate their spatial influence

a) RGB histology image of microglial staining

b) Binary image presenting segmented microglial cells

c) Labelled image of microglial cells

d) Voronoi image corresponding to labelled microglial cells

Parameters	Cell 1	Cell 2	···	Cell n	Unit
Mass center x	17258	10010	···	14747	px
Mass center y	5378	4982	···	4877	px
Area	14904	5146	···	447	px²
Mean radius	85.8	49.4	···	12	px
Circularity	0.03	0.06	···	0.95	/
Orientation	-2.8	-83.7	···	84.3	°
Mean red color	105.8	111.1	···	98.1	/
···	···	···	···	···	···
Area of Voronoi	35619	18616	···	2373	px²

e) Parametric table (px: pixel; °: degree)

f) → g) : Extract information from several pixels in a high resolution image and summarize it in a low resolution image in both horizontal and vertical direction

Max

Min

f)

g)

Summarize information *(f-g)* based on c) and e)

Mesoscopic heat map image

In plane resolution: 112µm

0 h) Counting 50

0 i) Area 1797

0 j) Mean radius 20

0 k) Circularity 1

0 m) Orientation 90

0 n) Voronoi area 271876

...

Discussion and perspectives

- This original approach enables:
  — to extract and summarize pertinent information from high-resolution qualitative images,
  — to dramatically reduce (ratio = 65536) the amount of information to be processed.
- Analysis has been extended to other staining of interest (*Fig. 4* cells: Nissl staining, amyloid plaques: 6E10 staining) and from brain sections to the entire reconstructed brains in 3D using our in-house software BrainVISA (http://brainvisa.info).
- 3D Voxel-wise statistical studies will be realized to investigate cellular structural alterations without *a priori* between groups as already performed on autoradiography data[2].
- The possibility to correlate 3D whole-brain parametric maps with *in vivo* imaging modalities (MRI, fMRI, PET, *in vivo* microscopy, etc.) will improve the understanding of the relationship between brain structure and function in disease.

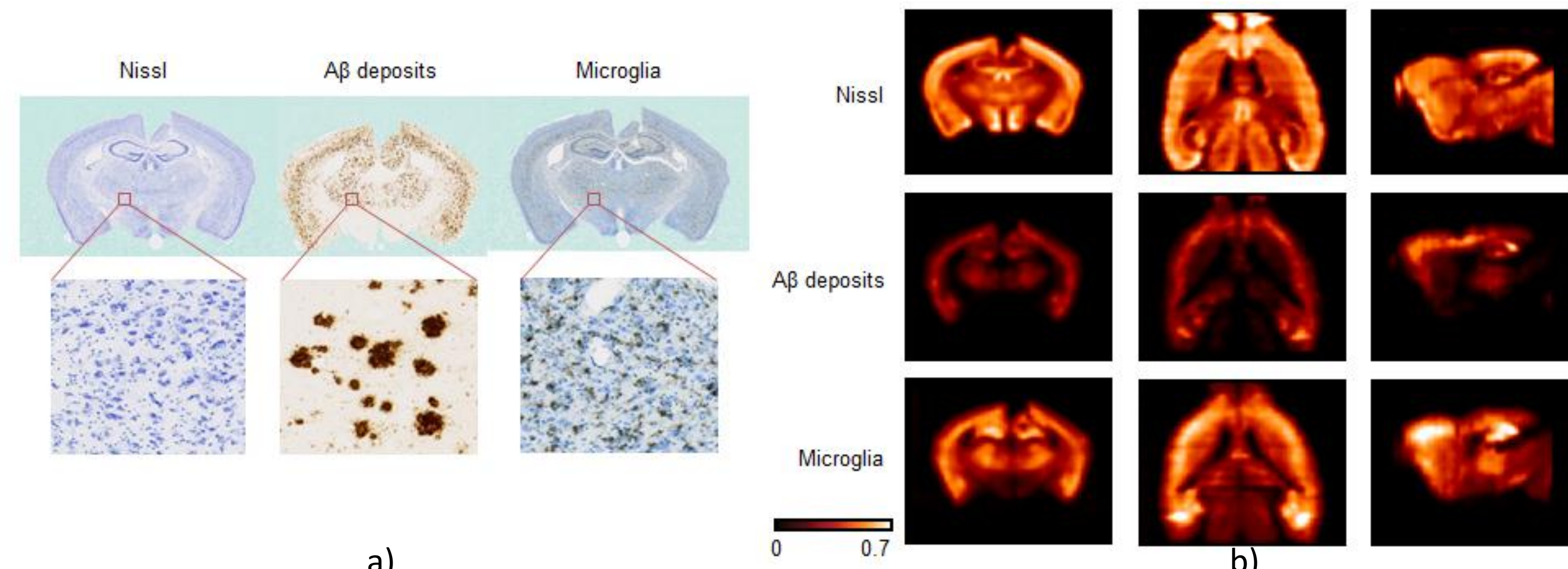

Figure 4: *a) Supplementary staining tested, b) 3D density maps obtained in whole mouse brains*

References :
[1] Vandenberghe et al., (2015) EMBC (submitted)
[2] Dubois et al. (2010) NeuroImage

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