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Neuroscience methodology

Research Topic

Study of glial heterogeneity impact on neuronal circuits in the context of development, aging, pathological conditions (neurodegenerative and neurodevelopmental diseases)

Applicable research context

- Investigation of the implication of central nervous system (CNS) non-neuronal cells in the building, regulation and maintenance of the neuronal network of the brain.
- Dysfunction of the cellular properties of glial cells leading to pathologies impacting cognitive functions.

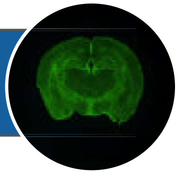
Scope of this methodological protocol

The following methodological workflow will help to speed-up whole brain sections immunofluorescence studies such as:

- Underscoring of glial heterogeneity in physiological states, as well as in pathological conditions including neurodegenerative and neurodevelopmental diseases;
- Investigation of regional differences and functional diversity in glia-neuron interactions;
- Structural characterization of glial heterogeneity across sex, age, and brain regions.

Speed-up whole slide immunofluorescence brain section imaging and analysis using **InnoQuant fluorescent slide scanner**

SAMPLES TYPE



- Free-floating mouse brain sections 40µm-thick
- mIF markers : Iba1 (microglia), P2YR12 (microglia), NeuN (neurons), GFAP (astrocyte)

ISSUES

- Speed-up acquisition currently slowed down due to focus issues on a widefield motorized microscope
- Count the number of double positive cells on whole brain sections
- Identify the number of microglia on brain slices

OUR SOLUTION

- Accelerate by 2 the whole tissue section imaging using automated content-based focus and a 0.5 µm pixel size
- Cells of interest can be identified and numbered reliably using a trained image analysis object classifier

USER BENEFITS

- Time-saving scanning speed
- User-friendly acquisition workflow
- Seamless analysis with a suitable image quality

Key words

- Aging and age-associated disorders
- Microglia, astrocytes, neurons
- mIF free-floating whole brain sections
- Biomarkers : NeuN, Iba1, P2RY12, GFAP

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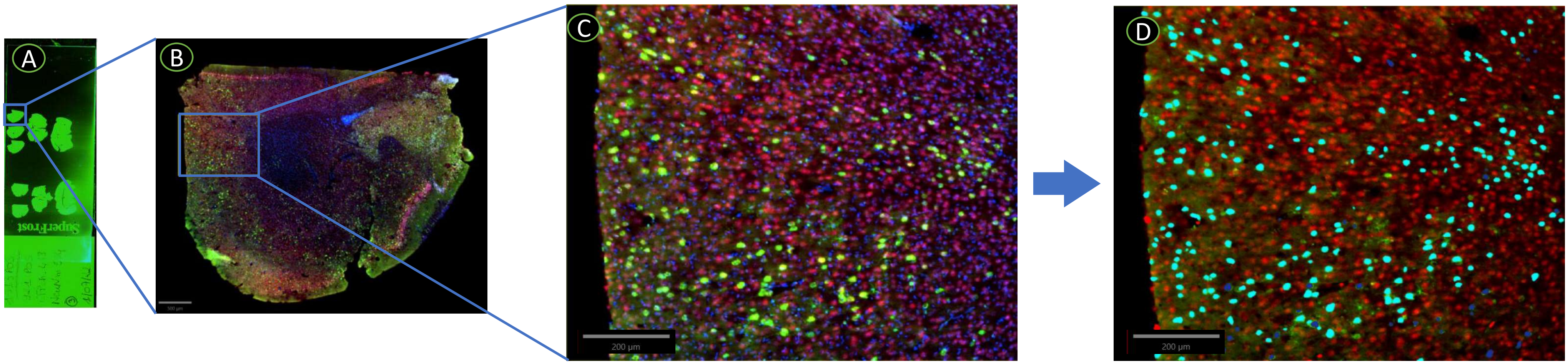
Goal: identify the number of double positive cells

1st case study



Mouse brain sample. Imaged with InnoQuant at 0.5 µm/pixel. (A) Sample detection. (B) Whole tissue acquisition. (C) ROI chosen for analysis. ROI area: 959887 µm² *(D) QuPath analysis results (DAPI channel has been hidden). Double positive cells are displayed in cyan.*

QuPath analysis workflow: identification of the double-positive cells

Markers:

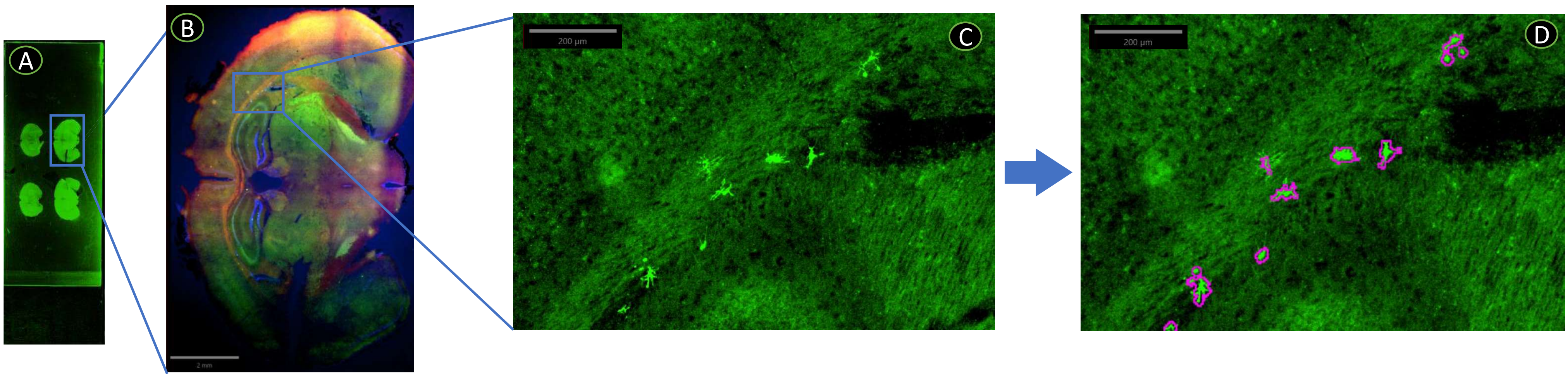
- GFP on the CD9 gene
- mIF markers: Iba1-AF594, NeuN-AF594, GFAP-AF594
- DAPI

Cell detection (Stardist) → Pixel thresholder to classify the CD9-GFP positive cells / Pixel thresholder to classify the AF594 positive cells → Merge of both classifiers → **Results: 222 double positive cells on the ROI**

Scan duration (focus included)
~8 min @0,5µm/pix

Goal: count the number of microglia on the full slice after exposition to PLX

2nd case study



Mouse brain sample. Imaged with InnoQuant at 0.5 µm/pixel. (A) Sample detection. (B) Whole tissue acquisition. (C) ROI for better visualization. DAPI and Texas Red channel have been hidden. (D) QuPath analysis results. Microglia are identified in magenta.

Markers:

- mIF marker: Iba1-AF488, P2RY12-AF488
- Texas Red
- DAPI

Scan duration (focus included)
~18 min @0,5µm/pix

QuPath analysis workflow: number of microglia on the slice after exposition to PLX

Creation of a training image → Training of a model (Machine Learning). Use of filters and thresholders. → Use the model on the full slice → **Results: 49 microglia on the full slice**