AI-driven whole-brain cellular profiling using 3D microscopy

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Background & Aim

Neurodegenerative diseases are characterized by complex, large-scale structural and cellular alterations across the brain. Understanding these changes requires comprehensive, whole-brain analysis at high spatial resolution.

Three-dimensional light sheet fluorescence microscopy (3D LSFM) enables rapid imaging of intact brains at micrometer resolution. Through robotic sample handling and automated acquisition, more than 500 brains can be scanned per week, generating 20–1000 GB of data per sample. However, the sheer volume and complexity of these datasets make conventional image analysis approaches non-scalable and impractical.

AI-driven image analysis provides a high-throughput, automated solution for extracting quantitative information from large-scale 3D brain datasets, enabling systematic and unbiased characterization of neurodegenerative pathology.

Methods

Whole brains are labelled using multi-modal staining strategies and imaged using automated high-throughput light sheet fluorescence microscopes (LSFM). Volumes are registered to a standardized anatomical atlas and subdivided into 3D patches for scalable processing. AI-based segmentation is performed using a 3D U-Net architecture with residual blocks implemented in Python. Custom-built Python pipelines are developed for preprocessing, atlas mapping, region-wise aggregation, endpoint-specific voxel modelling (counts, coverage and accumulated intensity), and automated reporting. Quantification includes hemisphere-resolved region-wise signal aggregation, volume normalization, and generation of tables, statistical summaries, and publication-ready figures.

Conclusions

We present a scalable, atlas-driven AI framework for automated whole-brain quantification across diverse biological endpoints. By integrating volumetric imaging, deep learning-based segmentation, and standardized region-wise aggregation, this pipeline enables high-resolution, reproducible, and decision-ready analysis of large 3D datasets.

The modular architecture supports multiple staining modalities and disease models, facilitating unbiased and high-throughput characterization of CNS pathology in preclinical research.

1 Overview of automated sample preparation and light sheet fluorescence microscopy pipeline

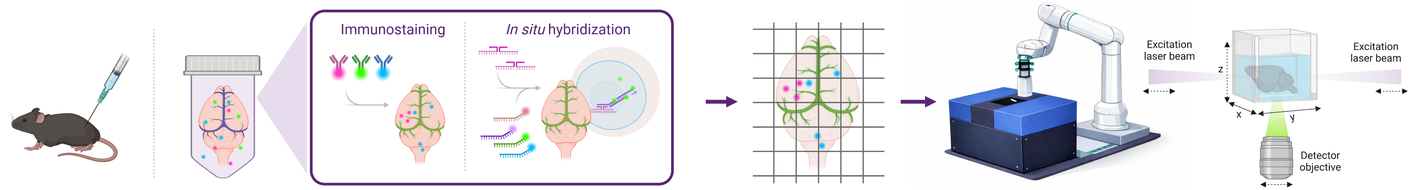

Figure 1. Whole brain labelling is carried out by immunohistochemistry (IHC) or mRNA *in situ* hybridization (ISH) using hybridization chain reaction (HCR), followed by tissue clearing in ethyl cinnamate (ECi). Custom built robotic systems and light-sheet fluorescence microscopes (LSFM) enable imaging of hundreds of samples at single-cell resolution, generating data for quantitative image analysis.

2 Visualization of cell types and pathological changes by LSFM

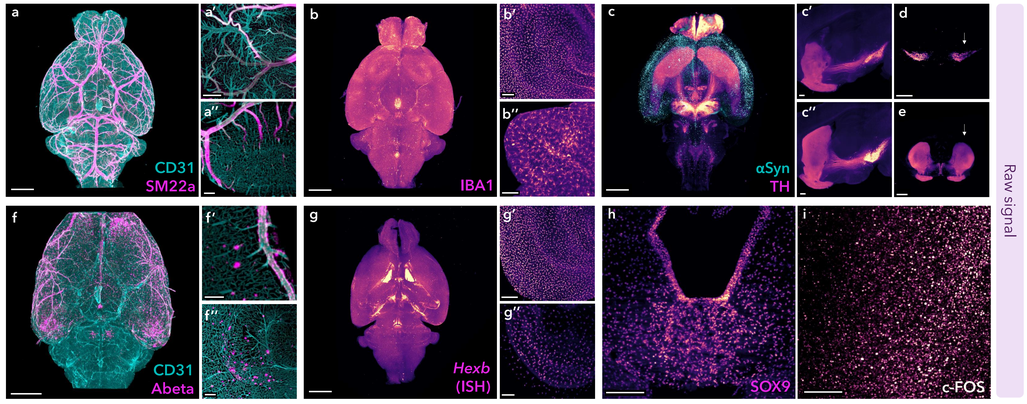

Figure 2. Example LSFM data. a. Visualization of large blood vessels (SM22a) and capillaries (CD31) by whole brain IHC. **a'-a''**, magnified optical planes (cortex, cerebellum). **b.** Microglia (IBA1) detected by IHC. **b'-b''**, magnified optical planes (hippocampus, cerebellum). **c.** Visualization of α-synuclein (αSyn) and tyrosine hydroxylase (TH) by IHC in a mouse model of Parkinson's disease, 26 weeks following unilateral injection of preformed fibrils in the striatum. **c'.** Loss of TH signal is evident in injected side in comparison to the control hemisphere, in **c''**. LSFM enables holistic visualizing Substantia nigra (SNc) connectivity with the striatum. **d.** Optical coronal plane at the level of SNc and in **e**, striatum, demonstrating weaker TH signal in the ipsilateral side (arrow). **f.** Abeta and vasculature imaging in female 30 weeks old ARTE10 model of Alzheimer's disease. **f'-f''**, magnified optical planes (cortex, hippocampus). **g.** Microglia (*Hexb*) detected by ISH. **g'-g''**, magnified optical planes (hippocampus, cerebellum). **h.** Astrocytes, whole brain IHC for SOX9; view of area postrema region**. i.** Neuronal activation imaging by c-FOS IHC. Scale bars: a, b, c, f, g, 2 mm; a'-a'', b'-b'', c'-c'', f'-f'', g'-g'', h-I, 300 µm; d-e, 1 mm.

3 3D image segmentation

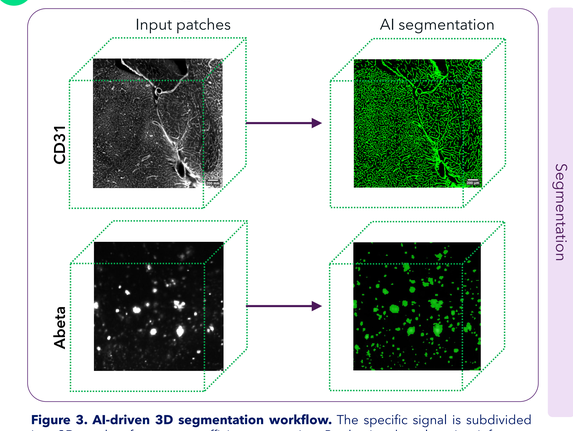

Figure 3. AI-driven 3D segmentation workflow. The specific signal is subdivided into 3D patches for memory-efficient processing. Patch-wise deep learning inference is then applied to generate high-resolution segmentation maps across the whole brain.

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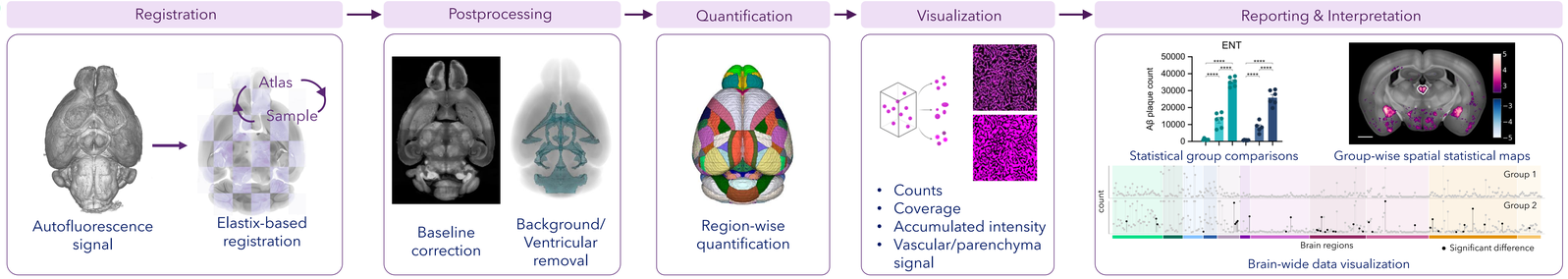

Figure 4. Scalable, atlas-driven whole-brain quantification. Whole-brain autofluorescence volumes are registered to a reference atlas. Optional postprocessing enables correction for background signal and removal of ventricular fluorescence. 3D signal is mapped voxel-wise to a standardized atlas for automated region-wise endpoints, which can be normalized to region volume. Endpoint-specific voxel modelling supports spatial interpretation without altering quantitative outputs. Data is delivered in publication ready figures and via interactive online data browser.

Endpoints for CNS research

Parkinson's Disease

- Count of TH positive neurons
- TH signal intensity
- αSyn volume fraction

Alzheimer's Disease

- Plaque count
- Plaque volume
- Plaques associated with vasculature
- Plaques in parenchyma
- Compound accumulation in plaques

General CNS

- Volume fraction of microglia (IBA1, *Hexb*)
- Microglia activation (*Trem2* upregulation)
- Compound biodistribution, accumulated signal per brain region
- Blood brain barrier (BBB) penetration: relative accumulation of compound in parenchyma and vasculature
- Vascular density
- Neuronal activation (c-Fos, pSTAT3)
- Cell-type-specific changes (glial or neuronal populations)
- Changes in brain region volume
- Gene expression characterization at mRNA and protein level