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A novel whole-brain imaging pipeline for high resolution spatial and temporal measurements of amyloid-beta plaque dynamics in pre-clinical Alzheimer's Disease animal models

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PURPOSE

- Alzheimer's Disease (AD) has a strong spatial-temporal component to its progression in which the deposition of amyloid-beta (Aβ) plaques varies according to brain region and time point.
- This variation cannot be studied using standard imaging and analysis platforms, as they often cannot provide both region-specific quantification and high-yield whole-brain imaging.
- Furthermore, many Aβ analyses require destructive homogenization of tissue, preventing secondary analysis.

OBJECTIVE

- To address common limitations in standard workflows, we have developed a novel high-throughput whole-brain imaging pipeline for pre-clinical AD models.
- With this pipeline, plaque progression can be measured quantitatively for individual brain regions across time.
- Our processing platform also produces indexed tissue sections which are ideal for secondary staining and analysis.

METHODS

Tissue Preparation

Aβ plaques in the well characterized 5XFAD mouse model of AD at four time points (t= 2, 3, 4, 6 months; n= 8 male, 8 female) were labelled with an intra-peritoneal injection of 0.5 mg/kg of methoxy-X04. Twenty-four (24) hours after injection, mice were euthanized with 0.1 mL/10 g ketamine-xylazine and perfused with 1× PBS and 4% PFA. Intact brains were then dissected and post-fixed in 4% PFA for 12–24 h at 4°C. Samples were embedded in an agarose block and polymerized in an embedding matrix to provide stability for sectioning.

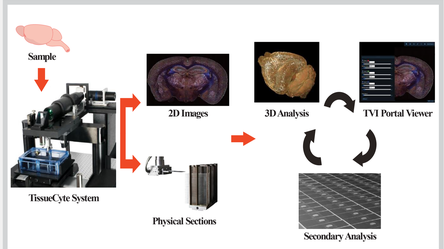

Figure 1. TissueVision's Serial Two-Photon Plus (STP[2]) pipeline.

STP[2] Imaging

Brain samples were sectioned serially with 100 um thickness and imaged with a TissueCyte Serial Two-Photon (STP2) system **(Figure 1)**. With an excitation wavelength of 780 nm, autofluorescence of anatomical features could be visualized within channel 1 (emission spectrum > 560 nm) and channel 2 (500-550 nm). Methoxy-X04 labelling was visualized primarily within channel 3 (< 500 nm). Secondary analysis of Iba1 (channel 1) and LAMP-1 (channel 2) was performed on select physical sections **(Figure 2)**.

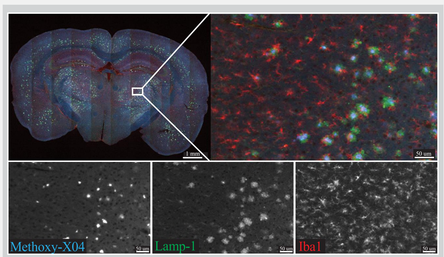

Figure 2. Full section image and zoom (Methoxy-X04:blue, LAMP-1:green, Iba1:red).

Plaque Analysis

Each brain was registered to the Allen CCF average template brain using a multi-resolution series of transforms for coarse-to-fine alignment[1,2]. To evaluate plaque localization, custom algorithms were developed to compute average plaque density across samples for all regions annotated in the Allen Reference Atlas. 3D heat map visualizations of the regional density data were also generated.

Validation

Our novel plaque labelling and quantification platform were validated using standard ELISA protein detection. Six sections with varying levels of plaque density were probed (t=4 months, n=8 females). Aβ concentration for the targeted sections was then quantified using commercially available ELISA kits. (Anaspec-AS-55554). Special attention was given to probing the longer form of Aβ containing peptides 1-42 (Aβ42), which has shown a greater tendency to aggregate and therefore is believed to contribute most heavily to Aß plaque formation.

RESULTS

Plaque Analysis

The analysis of whole-brain plaque distribution in the 5XFAD mouse model revealed distinct spatial-temporal changes across the brains. STP[2] imaging, combined with CCFv3 mapping and the secondary analysis of vasculature, Lamp-1 and Iba1, allowed for targeted evaluation of Aβ plaques and a comparison of regional molecular changes with age or treatment **(Figure 3, Figure 4)**.

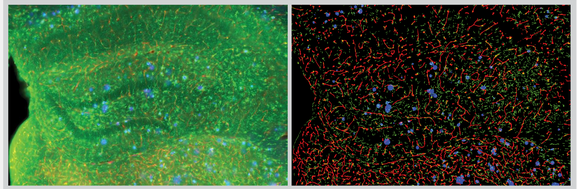

Figure 3. Left: Secondary analysis image of vasculature (red), Iba1 (green) and plaques (blue). Right: Triple colocalization segmentation of the three signals.

Using data from the 4 time points, we are able to characterize the rate of plaque accumulation across brain regions during disease onset and observe a progressive increase in plaque accumulation across various brain regions **(Figure 5)**. The highest growth rates were consistently observed across disease progression in regions associated with the hippocampus, thalamus, pons, and cortical subplate, whereas lower growth rates occurred across many disperse brain regions. Together, these results demonstrate the capacity to track AD regional densities and determine regions for targeted additional analysis.

Validation

A positive correlation was observed between our plaque regional density measures and those obtained through ELISA (R^2= 0.739), demonstrating strong applicability of our method to measuring brain levels of Aβ42. Results indicate that the novel analysis also provides lower variability than ELISA according to the computed average coefficient of variation (0.345 versus 0.525, respectively). In addition, visually distinguishable Methoxy-X04 labelled plaques could be identified in STP[2] section images even when ELISA was unable to detect Aβ42 within the same tissue.

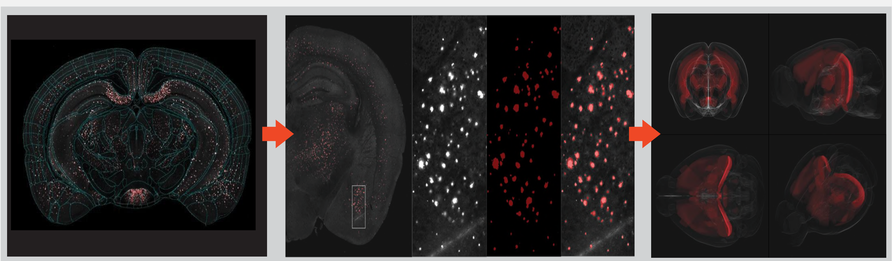

Figure 4. Analysis pipeline for regional quantification of plaques. Left: Regional Allen CCF overlay. Middle: Plaques, mask segmentation, and overlay. Right: 3D heatmap average plaque density

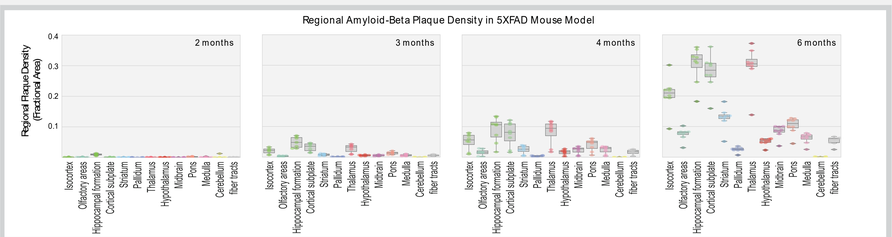

Figure 5. Plots of plaque density in major brain regions (as colorized in Figure 3) across time points of female 5XFAD mice, demonstrating a significant density accumulation (* p < 0.05).

CONCLUSION

- We demonstrated the capacity to map the progression of AD pathology in the 5XFAD mouse model by quantifying the density distributions of Aβ plaques at defined time points.
- Validation shows that this novel method is more precise and sensitive than standard ELISA, especially at the level of single tissue sections.
- This technique reveals the colocalization of vasculature and Aβ, providing a way to assess cerebral amyloid angiopathy (CAA).
- This novel technology has great promise for quantifying the spatial-temporal Aβ plaque efficacy of AD animal models, and for producing translatable pre-clinical AD data for drug discovery. The high sensitivity and precision of the STP[2] platform can benefit region-specific disease progression compared to standard laboratory approaches.
- Future spatial biology assays will involve secondary molecular profiling using multiplexed IHC based on MALDI mass spectrometry (MALDI-IHC) from Ambergen[3] **(Figure 6).**

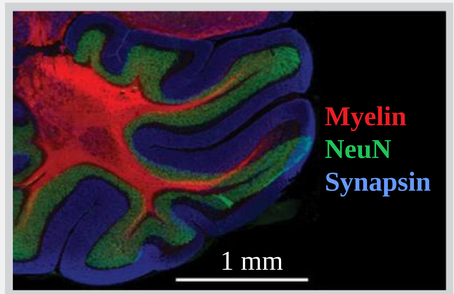

Figure 6. MALDI-IHC brain image adapted from Yagnik et al[3].

CITATIONS

[1]Allen Institute. (2017). *Allen Reference Atlases: Atlas Viewer*. Allen Reference Atlases: Atlas Viewer. Retrieved July 7, 2022, from https://atlas.brain-map.org/

[2]Wang, Q., Ding, S.-L., Li, Y., Royall, J., Feng, D., Lesnar, P., Graddis, N., Naeemi, M., Facer, B., Ho, A., Dolbeare, T., Blanchard, B., Dee, N., Wakeman, W., Hirokawa, K. E., Szafer, A., Sunkin, S. M., Oh, S. W., Bernard, A., … Ng, L. (2020). The Allen Mouse Brain Common Coordinate Framework: A 3D reference atlas. *Cell*, *181*(4). https://doi.org/10.1016/j.cell.2020.04.007

[3]Yagnik, G., Liu, Z., Rothschild, K. J., & Lim, M. J. (2021). Highly multiplexed immunohistochemical MALDI-MS imaging of biomarkers in tissues. *Journal of the American Society for Mass Spectrometry*, *32*(4), 977–988. https://doi.org/10.1021/jasms.0c00473

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