Optimized fixed-formalin paraffin-embedded spatial transcriptomics of post-mortem Alzheimer's disease human brain tissue

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Introduction

Understanding the molecular mechanisms driving neurodegeneration requires tools that can interrogate gene expression within the spatial context of intact tissue. Bulk and single-cell RNA sequencing have identified transcriptome-wide changes in Alzheimer's disease (AD), but these approaches require tissue homogenisation, sacrificing the spatial relationships between gene expression and disease-defining pathology. Spatial transcriptomics enables genome-wide transcriptional profiling while preserving tissue architecture, offering an unprecedented opportunity to map gene expression changes surrounding AD pathology. However, applying spatial transcriptomics to post-mortem human brain tissue is hindered by RNA degradation during routine formalin fixation and paraffin embedding (FFPE). Brain banks typically store dissected tissue blocks in formalin for days to months prior to paraffin processing — a post-fixation interval that progressively fragments RNA through cross-linking, rendering tissue incompatible with sensitive transcriptomic workflows. Here, we evaluated UMFIX (Universal Molecular Fixative) as an alternative storage medium during the post-fixation interval for tissue already perfused with 15% formalin. We systematically compared RNA and DNA quality and tested the suitability of UMFIX-stored tissue for Visium HD spatial transcriptomics.

Methods

- Compared molecular characteristics of tissue stored in UMFIX (60% ethanol, 20% methanol, 13% polytethylene glycol, 7% water) to samples stored in 15% formalin before paraffin-embedding (Fig 1).
- Conducted Visium HD spatial transcriptomics (10x Genomics) to investigate gene expression changes around amyloid plaques in AD.

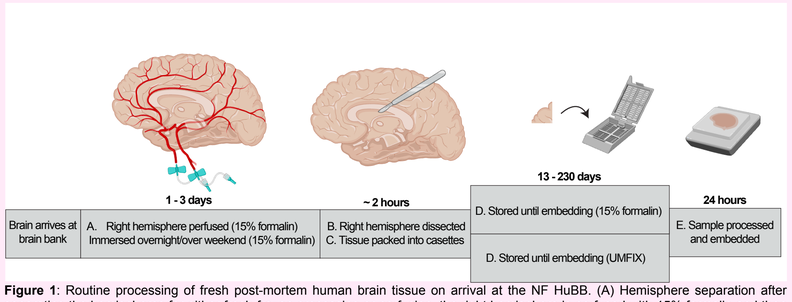


Figure 1: Routine processing of fresh post-mortem human brain tissue on arrival at the NF HuBB. (A) Hemisphere separation after separating the hemispheres for either fresh-frozen processing or perfusion, the right hemisphere is perfused with 15% formalin and then immersed in 15% formalin overnight at RT. (B) Dissection: 1-3 days post-perfusion, the perfused hemisphere is dissected into regional blocks. (C) Casette packaging: during dissection, blocks are placed into paraffin processing casettes. (D) Post-fixation storage interval: casettes are held until paraffin processing and embedding can be scheduled. In an ideal workflow, this interval would be ~ 24 h, but in practice it ranges from days to months (13 - 230 days for the samples in this study) due to pathology batch processing, external laboratory scheduling and technician availability. In our standard workflow, this interval is spent in 15% formalin. In this study, we compared the molecular characteristics of case-matched adjacent blocks kept in 15% formalin (standard) versus blocks kept in UMFIX for the same interval, before identical paraffin processing and (E) embedding. Created using Biorender.

Results

- UMFIX stored tissue had significantly higher DV200 scores in all regions investigated
- Lower Ct thresholds for the housekeeping gene ACTB in UMFIX stored samples versus formalin stored samples - at low amplicon lengths, no difference between UMFIX and fresh
- Enhanced in situ hybridisation signal for the astrocytic marker GFAP in UMFIX samples
- Overall, suggests RNA integrity is improved in UMFIX stored samples, suggesting a greater likelihood of success with spatial transcriptomics

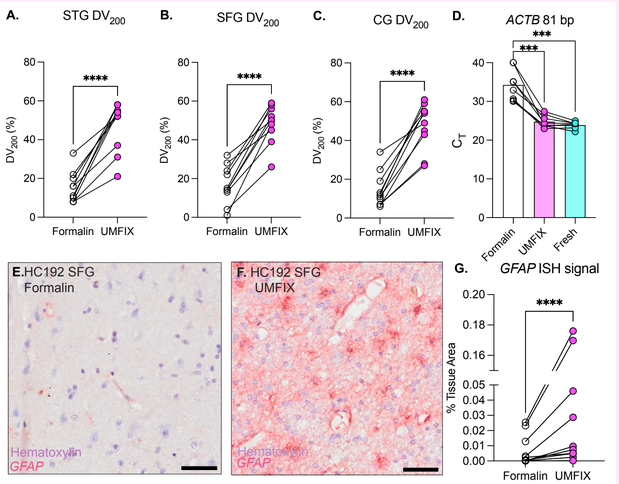


Figure 2: RNA quality is improved in UMFIX stored samples compared to formalin stored samples. Improved DV200 scores were observed in the STG (A), SFG (B) and CG (C). (D) UMFIX stored samples and fresh-frozen samples (opposite hemisphere) had significantly lower cycle threshold values for qPCR for the housekeeping gene ACTB. (E-F) In situ hybridization with RNAscope for GFAP shows a statistically significant increase in UMFIX samples compared to formalin samples in both white and grey matter, quantified in (L). Scale bars = 100 um.

Results Continued

- Given improved RNA integrity in UMFIX samples, proceeded to Visium HD spatial transcriptomics in an AD case with amyloid plaques. Achieved meaningful spatial transcriptomics results, able to cluster cells. Then proceeded to map gene expression changes around amyloid plaques (adjacent section), giving 166 DEGs around plaques. Now validating these hits at the protein level with IHC.

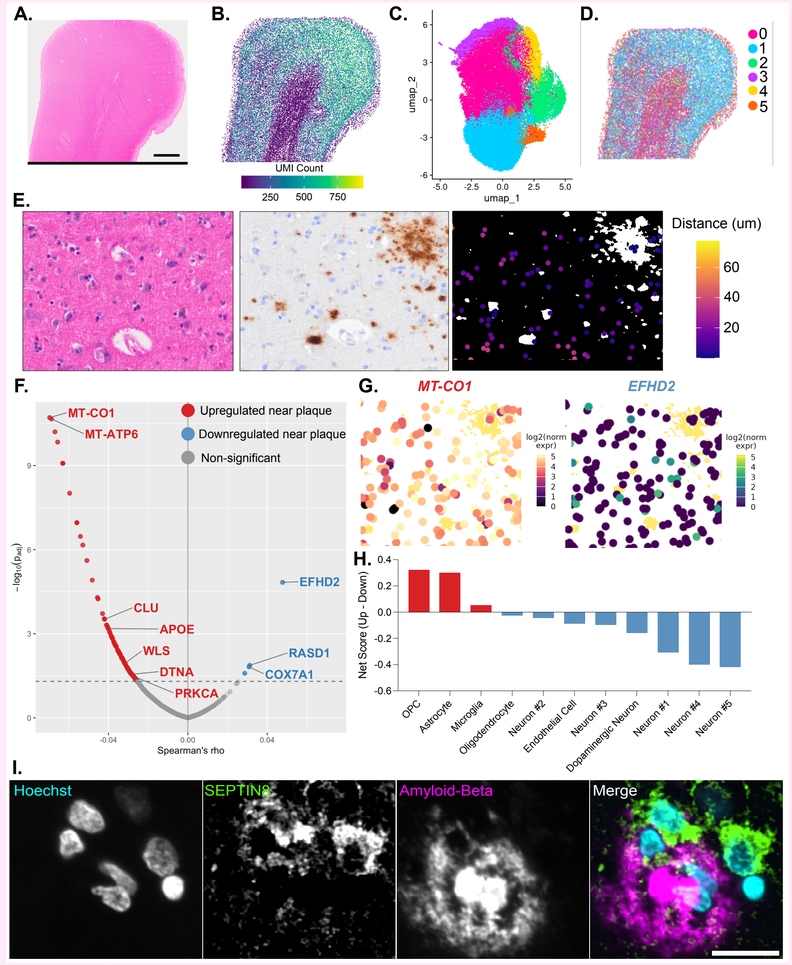


Figure 3: Spatial transcriptomics of Alzheimer's disease human brain tissue reveals gene expression changes around amyloid plaques. (A) Hematoxylin and eoisin image (B) filtered unique molecular identifier counts show a high number of unique transcripts per cell. (C-D) Uniform manifold approximation projection and spatial distribution of clusters. (E) Alignment of hematoxylin and eosin spatial transcriptomics image (left panel) with adjacent slide stained for AB (middle panel) allows for binary segmentation of amyloid plaques (white on black background), and distance each cell is from an AB plaque is calculated (right panel). (F) Volcano plot displaying Spearman's rho analysis of differential gene expression around AB plaques. 162 genes were found to be upregulated near AB plaques (red), while 4 genes were found to be down-regulated around AB plaques (blue). (G) Spatially mapping the most upregulated and downregulated genes around AB plaques (yellow) clearly reflects patterns seen in Spearman's analysis. (H) Anchoring differentially expressed genes to a reference single-cell RNA-seq dataset displays up-regulation of genes expressed by oligodendrocyte precursor cells (OPC), astrocytes and microglia, and downregulation of genes expressed by all other major brain cell types. (I) Immunohistochemistry of one of the differentially expressed genes, SEPTIN8 (green), shows clear proximity labelling near AB plaques (magenta).

Disussion/Conclusion

- Across all ten donors and three brain regions, UMFIX storage consistently preserved RNA integrity to a level comparable to fresh-frozen tissue, as evidenced by significantly higher DV_{200} scores, lower qPCR cycle thresholds for ACTB (amplicons up to 440 bp from cDNA; 876 bp from gDNA), and substantially enhanced RNAscope GFAP signal — none of which were achievable from matched formalin-stored blocks. These findings establish that the post-fixation storage interval is a critical determinant of molecular quality in banked FFPE brain tissue.
- Critically, UMFIX-stored tissue supported successful Visium HD spatial transcriptomics, with >50,000 cells captured per case at high read depth, clear identification of major brain cell types, and appropriate spatial localisation of cortical layer markers. Integration of Visium HD data with adjacent Aβ immunohistochemistry enabled distance-dependent gene expression analysis, revealing 162 genes significantly upregulated and 4 downregulated in proximity to amyloid plaques. We are now validating selected targets at the protein level.
- Upregulated genes included established AD-associated targets MT-CO1, MT-ATP6, APOE and CLU — validating our spatial approach — alongside novel candidates WLS, DTNA and PRKCA, with no prior reported association with AD plaque biology. Cell-type deconvolution revealed upregulation of genes expressed by oligodendrocyte precursor cells (OPCs), astrocytes and microglia near plaques, consistent with established glial responses to Aβ, while neuronal gene expression was broadly downregulated — reflecting local synaptic and axonal pathology
- Tissue shrinkage was observed in UMFIX samples (~52% increased cell density in grey matter; ~32% in white matter), which should be considered in morphometric analyses. Routine immunohistochemistry remained compatible with UMFIX storage, though antigen retrieval was required for some targets.
- **Taken together, these data demonstrate that substituting UMFIX for formalin during the post-fixation storage interval is a practical, brain-bank-compatible strategy that unlocks Visium HD spatial transcriptomics in archived FFPE tissue. This opens the way for a scaled spatial transcriptomics study across AD disease severity (Braak stages III–VI), w. The identification of spatially resolved molecular signatures surrounding Aβ pathology offers a new route to disease-relevant therapeutic targets in AD.**

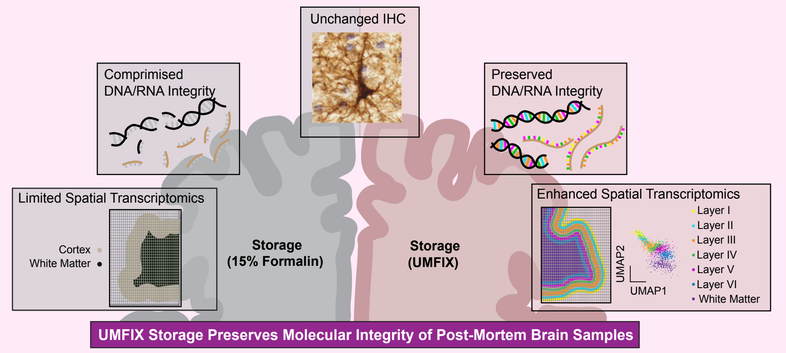


Figure 4: Storing tissue blocks perfused with 15% formalin in UMFIX before paraffin-embedding preserves the molecular integrity of post-mortem human brain tissue samples, and allows identification of gene expression changes around amyloid plaques in AD.

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