Single-Section Multiomics Mapped Across FFPE Tissue

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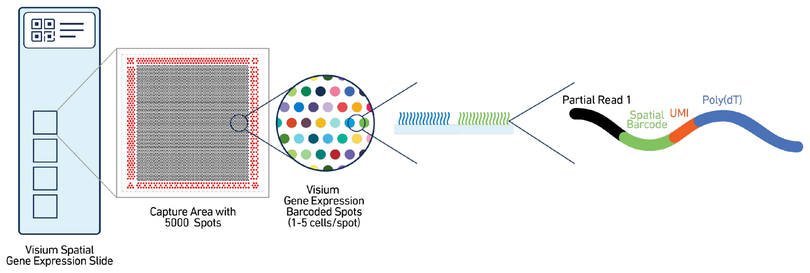
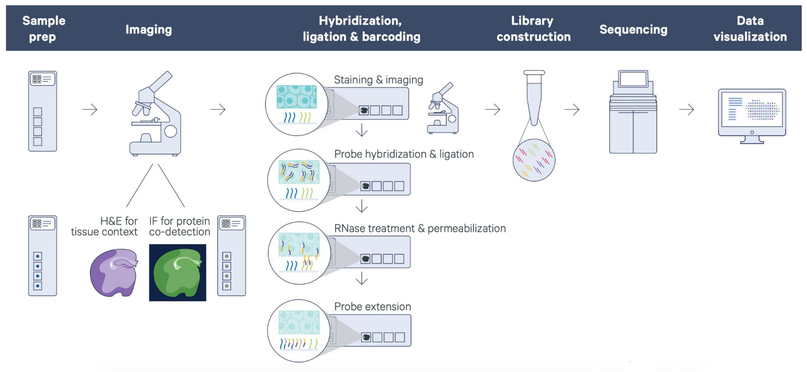
1. Introduction

Recent research indicates that spatially defined protein mapped together with mRNA information can predict clinical outcomes more accurately than either of these analytes alone (Vathiotis et al., 2021). We successfully applied and optimized the immunostaining protocol for the Visium Spatial Gene Expression for FFPE assay, which is designed to measure mRNA in tissue sections derived from formalin fixed & paraffin embedded (FFPE) tissue samples and requires a Visium Spatial slide with intact tissue sections as input. Immunostaining tissue sections with fluorescently labeled antibodies enables simultaneous protein detection. This protocol outlines deparaffinization, decrosslinking, immunofluorescence (IF) staining, and imaging of tissue for use with 10x Genomics Visium Spatial Gene Expression for FFPE assay. Deparaffinized, decrosslinked, and stained tissue sections are inputs for the downstream Visium Spatial Gene Expression for FFPE workflow.

2. Immunofluorescence staining and unbiased spatial gene expression

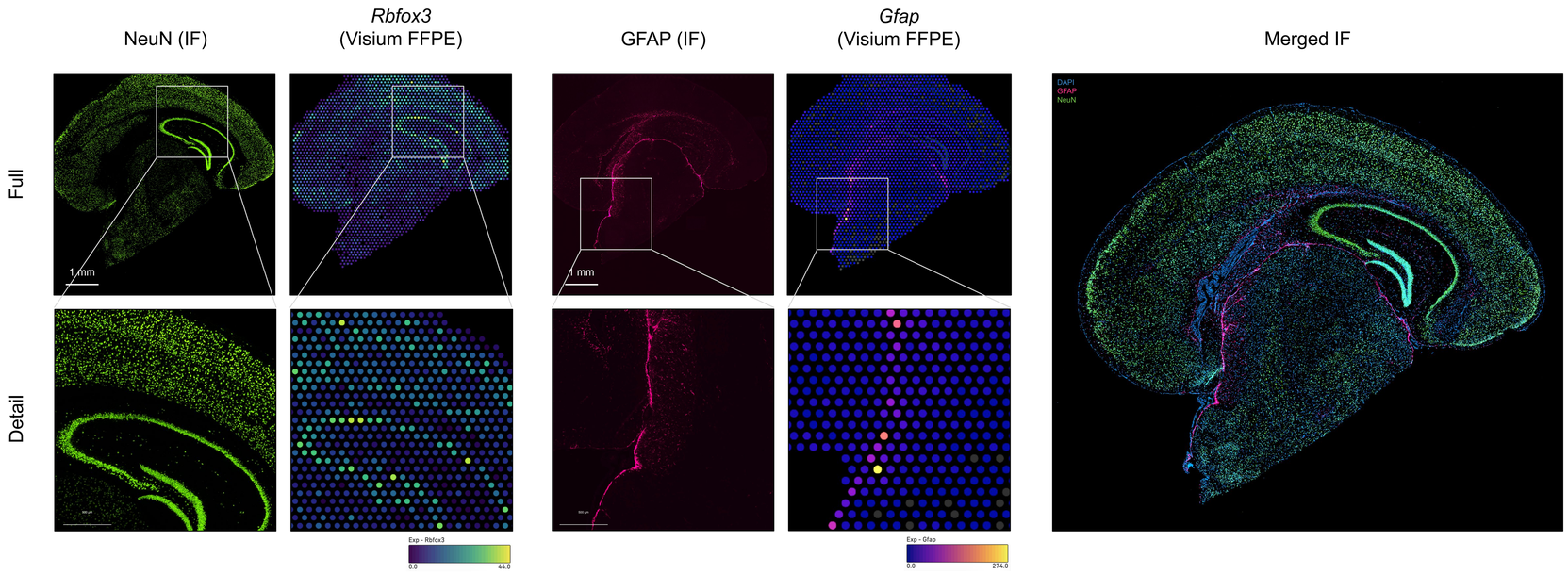
The technology enables FFPE tissue analysis by performing decrosslinking of the analytes and in contrast to Visium for Fresh Frozen tissues, where total mRNA is captured on the surface, here the human or mouse whole transcriptome probe panels covering nearly 20,000 specific genes are used to target the analyte inside the tissue. These probe pairs hybridize to their gene target and are then ligated to one another. The ligation products are released from the tissue upon RNase treatment and permeabilization. The ligated probe pairs bind with spatially barcoded oligonucleotides present on the Capture Area. All the probes captured by primers on a specific spot share a common Spatial Barcode. Libraries are generated from the probes and sequenced and the Spatial Barcodes are used to associate the reads back to the tissue section images for spatial mapping of gene expression. For tissue visualization a conventional H&E or immunofluorescence staining technique can be used.





3. Experimental conditions

A Visium Gene Expression slide with 5 µm FFPE mouse brain sections was obtained and deparaffinized according to demonstrated protocol “Visium Spatial Gene Expression for FFPE – Deparaffinization, Decrosslinking, Immunofluorescence Staining & Imaging” (CG000410). Tissues were first de-crosslinked for 1 hour at 70 ºC in TE pH 9.0 buffer, blocked in blocking buffer and stained in the same buffer supplemented with fluorophore-conjugated antibodies and DAPI counterstain for 1 hour at room temperature. Staining solution contained 200 ng/mL (1:5000) DAPI (Thermo Scientific, Cat., No. 62248), 1:200 dilution of anti-GFAP (Cy3™-labelled; Sigma, Cat., No. C9205-.2ML) and 1:100 dilution of anti-NeuN (Alexa Fluor® 647-labelled; Abcam, Cat., No. ab190565).



After staining tissue sections were washed, slide removed from slide holder, coverslipped using SlowFade™ Diamond Antifade Mountant (Thermo Scientific, Cat. No. S36967) and imaged using Metafer imaging system from MetaSystems (incl. Carl Zeiss AxioImager microscope). Imaging was carried out adhering to the technical note “Visium Spatial Gene Expression for FFPE Imaging Guidelines” (CG000436). Images exported as a multi-page TIFF and later used for manual fiducial frame and tissue alignment.

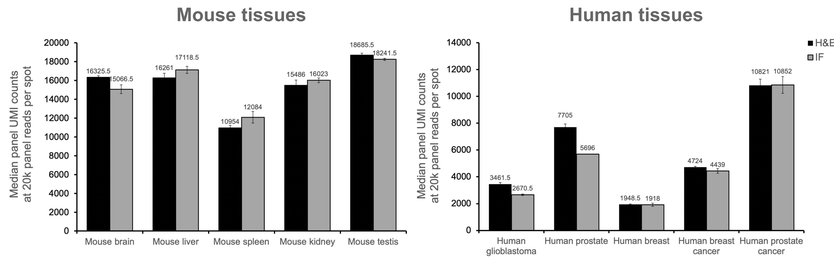
After imaging, the coverslip was removed and the slide returned in its holder. Gene expression libraries were then prepared using Visium Spatial Gene Expression Reagent Kit for FFPE and adhering to the guidelines (CG000407). A mouse whole transcriptome probe panel covering ~20,000 genes was used to capture the unbiased transcription profile of a mouse brain. Libraries were then quantified using qPCR and sequenced using NovaSeq instrument. For data analysis, IF images and corresponding manual alignment files, containing coordinates of fiducial frame and the tissue were used to map the barcoded molecules back onto the 2D coordinate plane.

4. Simultaneous transcriptome and proteome co-detection on a FFPE mouse brain section

The data integrates into Space Ranger where single section whole transcriptome readout + IF protein data is correlated. This can be visualized using the Loupe Browser. Individual genes, in this case, Gfap gene for GFAP (Glial fibrillary acidic protein, an astrocyte marker) or Rbfox3 gene for neuronal nuclei (NeuN) can be selected to be displayed individually and correlations with the IF stain can be observed. Loupe Browser is also equipped with a function allowing to navigate between different layers in the image and disable or enable them at any time. In conclusion, a more accurate representation of the interactions between protein and RNA can be derived from a single tissue section, than methods that utilize adjacent serial sections.

5. Data comparison with H&E staining protocol

The IF staining protocol is compatible with broad range of tissues - both of human and mouse. Sometimes it may result in a slight decrease in the number of unique transcripts detected with no impact on the fraction of reads mapped confidently to the transcriptome, as compared to the H&E staining protocol. This, however, should not affect interpretation of experimental results.



6. Conclusion

Here, we demonstrated the ability to use 10x Genomics Visium Spatial Gene Expression Solution for FFPE for measuring two analytes (RNA and protein) from a single tissue section. In contrast to current spatial multiomic techniques that require two sections to detect RNA and protein, this new technique provides a more accurate representation of interactions between the two analytes in a single section. The protein co-detection is enabled by immunofluorescence staining prior the library preparation workflow.