



Sequencing-Based High-Throughput Imaging of Proteins and RNA in FFPE Tissue Sections

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Background

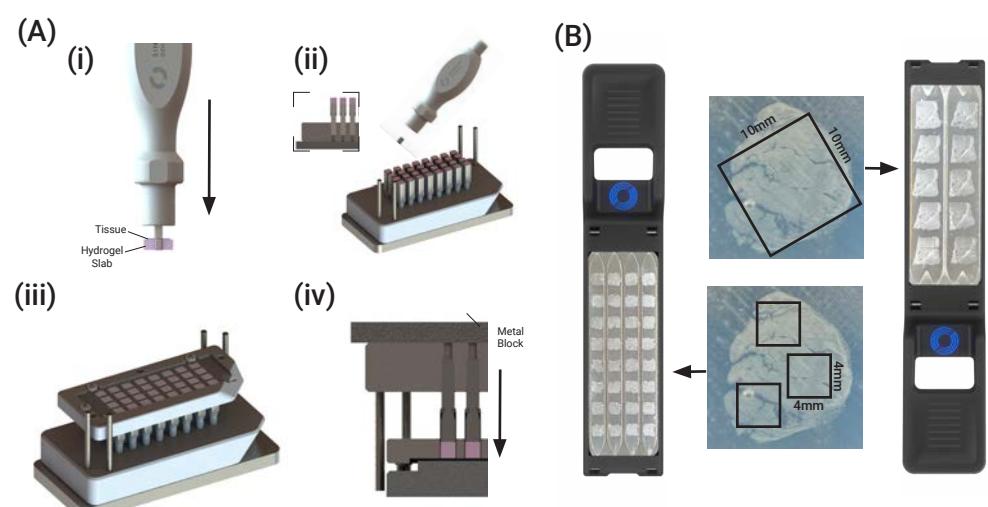
In situ multiomics is revolutionizing research in oncology, immunology, and neurobiology. However, speed, throughput, and cost are major bottlenecks for these critical studies. Here, we present data from the G4X™ Spatial Sequencer. With sub-micron resolution and ultra-high throughput capacity, G4X employs rapid 4-color sequencing-by-synthesis (SBS) chemistry to profile RNA transcripts and proteins in FFPE tissue and generates fluorescent H&E (fH&E) images, producing multi-modal spatial images of 40 cm² of tissue across 4 flow cells in less than 24 hours.



Figure 1 G4X Spatial Sequencer, flow cells, and performance capabilities

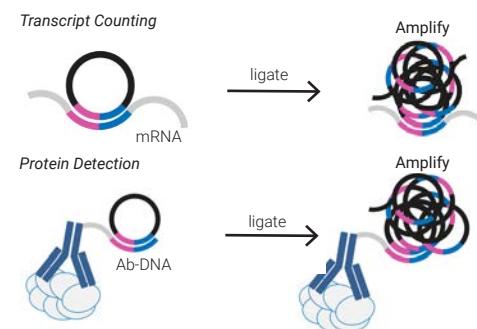
Fast and Simple High-Throughput Workflow

(A) Tissue transfer workflow. (i) A tissue sample on a hydrogel slab is punched with a sharp cutter. (ii) The cutter is removed and loaded to a ‘pusher’ pillar array. (ii-insert) cross-section view of a cutter with hydrogel/tissue on a ‘pusher’ pillar. (iii) A glass slide on a holder is aligned over the tissue array. (iv) The whole assembly is flipped 180°, and weight and heat are applied to selectively attach tissue samples on the glass surface. (B) Example images of flow cells with high-density tissue placement. We transferred either small (4 mm) or large (10 mm) tissue samples onto a flow cell depending on the application and tissue size.



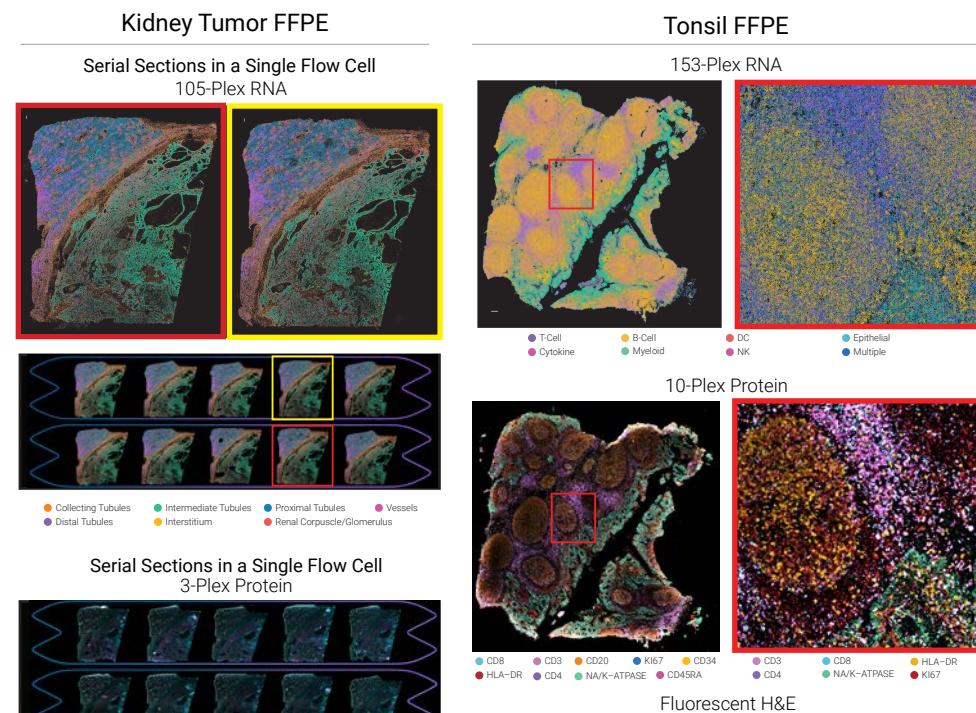
Sample Prep for Combined RNA and Protein Detection

Transcripts are detected by annealing a padlock probe sequence to a target RNA and additional specificity is conferred by requiring the 3' and 5' end to be adjacent for ligation. Proteins are detected by staining with oligo conjugated antibodies, which are then targeted with padlock probes that use the ab-oligo as a splint. All ligated padlock probes are then amplified by rolling circle amplification.



Unified Readout for Multiomic Profiling in the Same Section

G4X allows for concurrent proteomics and transcriptomics profiling *in situ* plus fH&E in the same section.

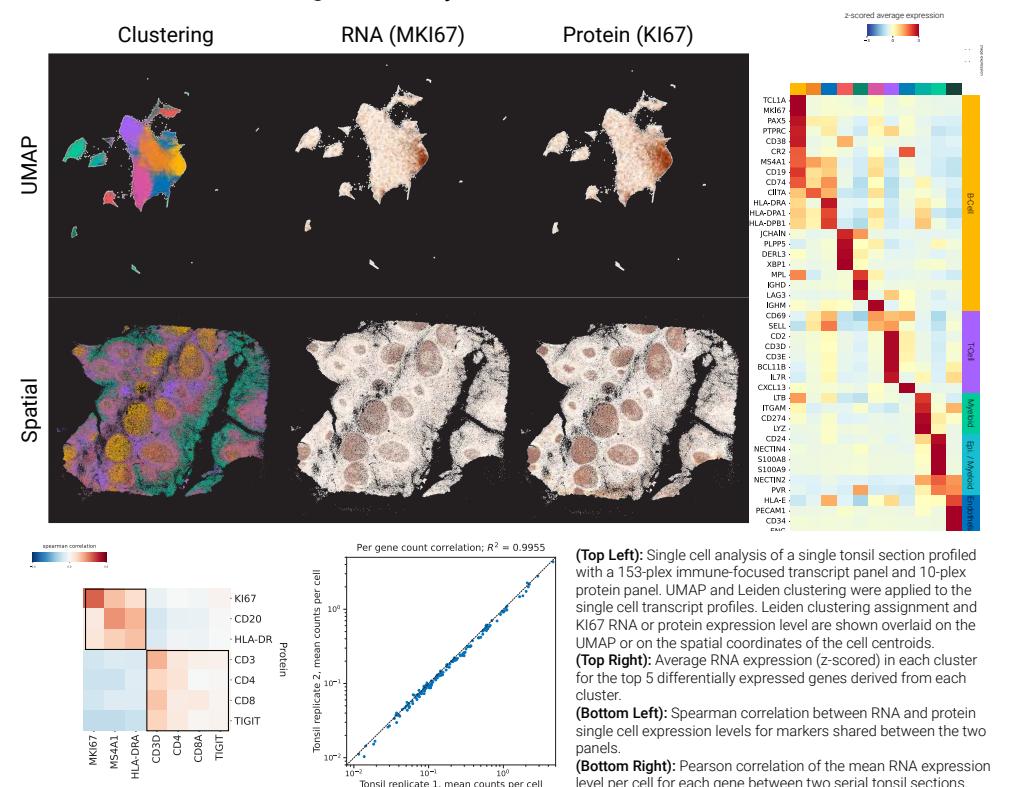


Quantification and Analysis (cont.)

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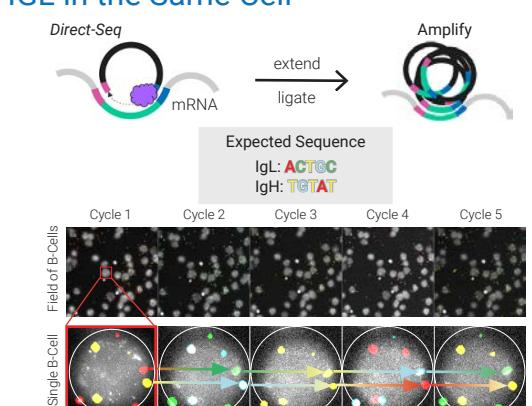
Dimensional reduction and unsupervised clustering of transcriptional profiles from ~250k cells from a single tonsil section demonstrated clear delineation of immune cell subpopulations (T-Cells, B-Cells, and Myeloid Cells) with spatial localization matching the expected pattern of dense germinal centers surrounded by T-Cells and epithelial cells lining along the exterior of the tissue. Additionally, we observed concordant expression profiles at both the RNA and protein level.

Single Cell Analysis



Case Study: Acute Myeloid Leukemia (AML) in Bone Marrow

Bone marrow is exceptionally important for studying hematologic malignancies such as AML, but decalcification during sample prep leads to RNA fragmentation. Here, we demonstrate strong performance in this difficult sample type by profiling 150 transcriptional targets across 35 bone marrow samples from human AML patients provided by Dr. Catherine Wu at the Dana Farber Cancer Institute. Data from all samples was captured in one run.

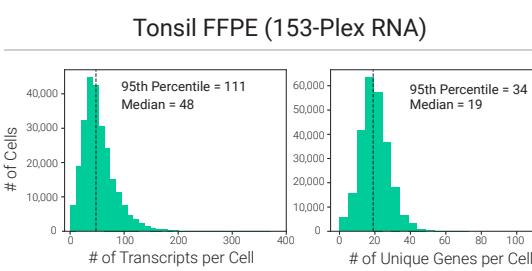


Direct-Seq™: Profiling IGH and IGL in the Same Cell

We leveraged the sequencing-based readout of G4X to enable sequencing of unknown regions of RNA. We used padlock probes with a gap rather than a ligation junction after hybridizing to a target RNA (**top panel**). The unknown sequence was incorporated into the padlock probe before ligation and amplification by RCA. As an example, we show Direct-Seq applied to profiling the CDR3 segment of IGH and IGL in a B-cell line (**bottom panel**; only 5 of 30 cycles shown).

Quantification and Analysis

In this study, we used 3 high-level metrics to evaluate the transcriptomic results: transcripts per cell, unique genes per cell and false discovery rate. The first two are shown on the right. The false discovery rate was ~2% but is dependent on sample quality and other factors. ~10 million cells/flow cell were detected, varying with tissue type and format.



Conclusion

We present an SBS-based approach for *in situ* detection of targeted gene transcripts and proteins at an unprecedented throughput, with applicability to the analysis of human kidney, tonsil and bone marrow FFPE tissue samples. The capability to deeply profile tissues at high throughput will enable larger translational studies, and comprehensive assembly of 3D maps of gene and protein expression at subcellular resolution.