Whole-transcriptome profiling of developing human ovaries with 10X Atera WT *in situ* spatial transcriptomics

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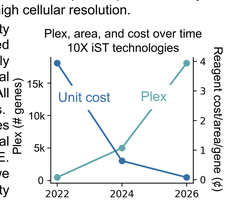
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Introduction and study design

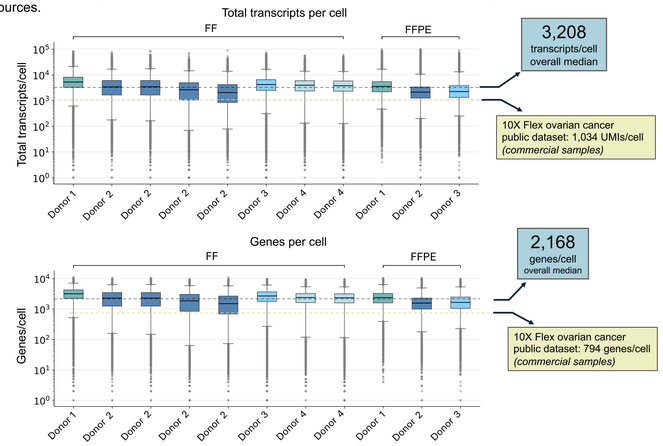
Targeted methods for imaging-based *in situ* spatial transcriptomics (iST) have been limited to profiling up to a few thousands of genes across modest areas (~2 cm^2). This has historically created a trade-off between plex and spatial specificity, namely between **capture-based whole-transcriptome** (WT) measurements (subject to transcript diffusion) and **low-plex imaging measurements** (true cellular-level resolution). However, the recent release of imaging-based 10X Atera enables whole-transcriptome profiling across whole slide areas (>6 cm^2), substantially re-defining the trade-off ceiling between plex and area in *in situ* approaches with high cellular resolution.

Study: We leverage the new 10X Atera WT platform to compare data quality between matched fresh frozen (FF) and formalin-fixed, paraffin-embedded (FFPE) of ovaries and fallopian tubes from human donors. We additionally profiled a Hodgkin's lymphoma FFPE clinical specimen for technical comparisons to previously acquired Xenium 5K data on the same cohort. All samples were sectioned at the Broad Institute and profiled at 10X headquarters.
Evaluation: We show the high detection sensitivity and low false positive rates across the Atera WT panel as well as high reproducibility between technical replicates. We show a higher rate of sensitivity of FF in comparison to FFPE. When comparing to previously acquired data on the control sample cohort, we find that Atera achieves more than a **5-fold improvement** in detection sensitivity on a per-gene basis than Xenium Prime (FFPE).



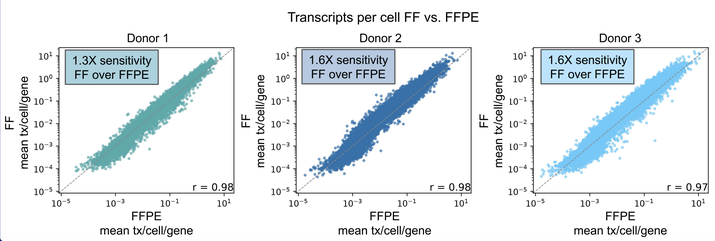
Overall assay sensitivity

The Atera whole-transcriptome panel achieves high overall sensitivity and breadth across the WT panel, achieving a median of **3,208 transcripts/cell** and **2,168 genes/cell** across all of our datasets. For reference, 10X public datasets for single-cell RNA sequencing on commercial ovarian cancer samples showcase a median of 1,034 UMIs per cell and 794 genes per cell with Flex, which is generally considered the gold-standard for high-sensitivity single-cell profiling. In future work, we will compare Atera to Flex single-cell profiling on identical sample type and sources.



Fresh Frozen vs. FFPE sensitivity

While both FFPE and FF sample types achieve high detection sensitivity with the Atera assay, we find that FFPE samples achieve 65-75% of the detection sensitivity as FF (matched; same tissue bisected before preservation) .



Acknowledgments

We would like to thank the Ardlie lab at the Broad Institute and the Shipp lab at the Dana Farber Cancer Institute for collaborating with us on this study. We would also like to thank 10X Genomics for facilitating Early Access to Atera.

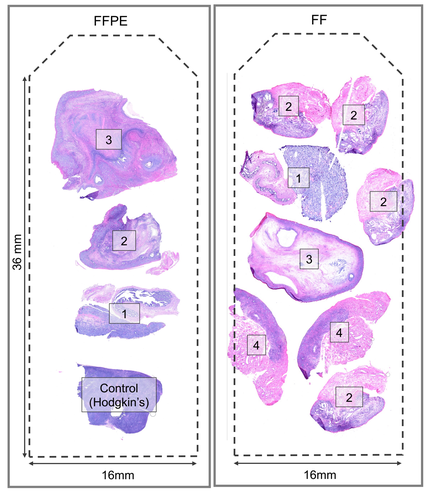
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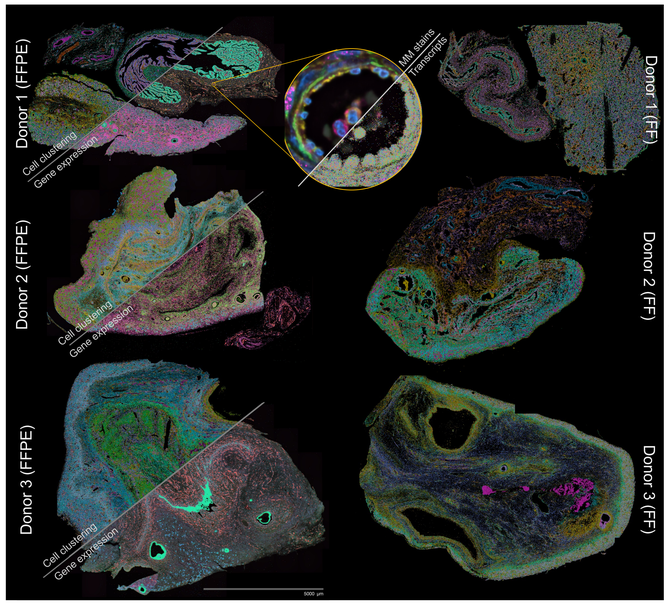
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Study layout and visual overview of results

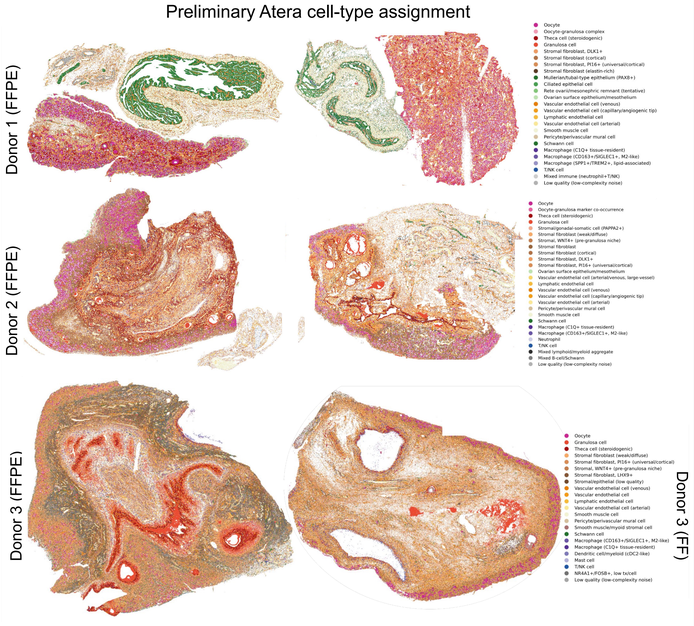
Atera slide layouts (1 FFPE, 1 FF)
Human ovaries and fallopian tubes (donor #)
and Hodgkin's lymphoma control



Gene expression and cell type clustering

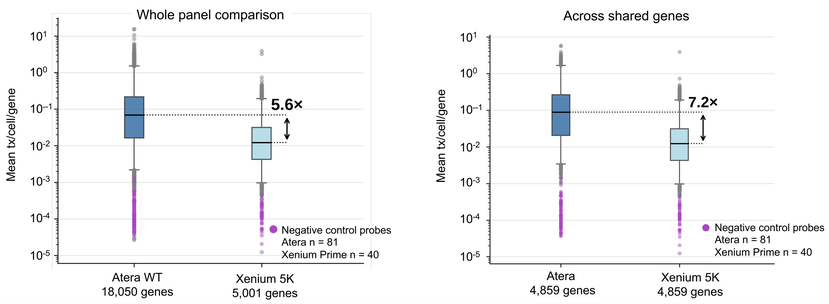


Preliminary Atera cell-type assignment



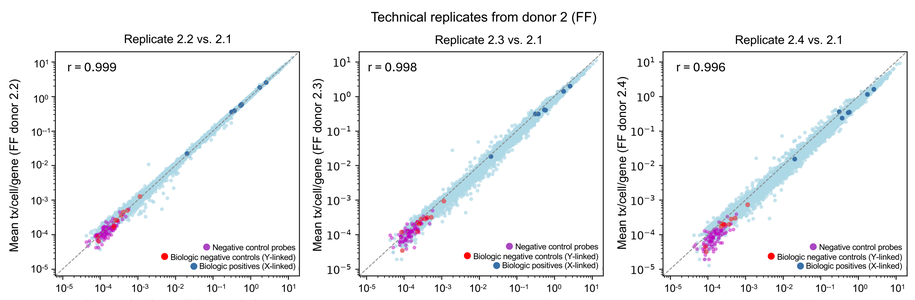
Atera WT vs. Xenium Prime

Our Atera WT pilot study included an FFPE block of Hodgkin's lymphoma from a clinical cohort that we previously profiled with the 10X Xenium Prime (5K) assay (n = 4) in collaboration with the Shipp Lab at DFCI. All samples in this comparison are of the same disease status, histological subtype, and collection period. We show that the **Atera WT platform achieves over 5X the sensitivity as the Xenium Prime assay**, both at the whole-panel level and across the subset of overlapping genes. Negative control probe rates are overlaid to show similarity between assays.

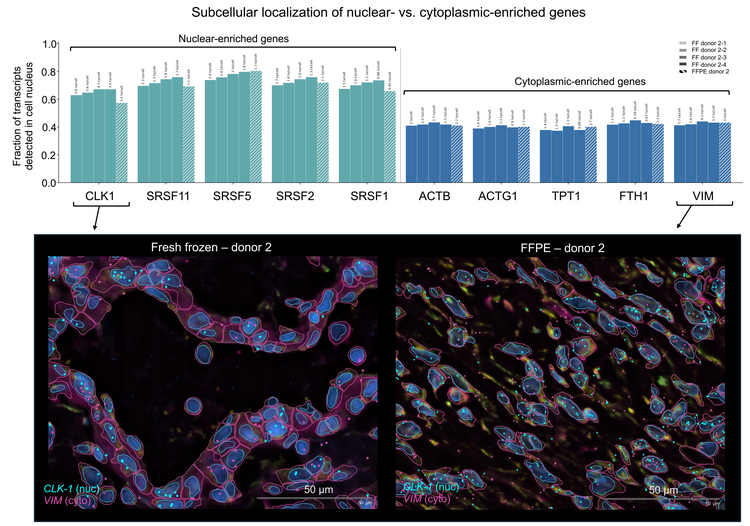


Same-slide technical replicates

Here we show very high reproducibility across technical replicates (sections from the same donor and tissue) profiled on the same slide with the Atera platform. Spearman correlation coefficients across all paired comparisons, including those not shown here, are r = 0.99.



Subcellular localization of nuclear- vs. cytoplasmic-enriched genes



Future work

Here we demonstrate that the Atera platform achieves high sensitivity across the WT panel with low false positive rates. We demonstrate the concordance between matched FFPE and FF samples and the superior sensitivity of Atera over the Prime assay. In future work, we will explore the expression profiles that emerge and persist in primary follicles throughout ovarian development. We will additionally compare the 10X Atera pipeline to the low-plex Xenium assay on matched tissues.