Visium HD enables spatial discovery in FFPE human breast cancer at single-cell scale

Monica Nagendran, Jerald Sapida, Joey Arthur, Govinda Kamath, David Patterson, Augusto Tentori
10x Genomics, Pleasanton, CA

1. Introduction

Advances in spatial transcriptomic technologies have led to a greater understanding of the complexity of cellular interactions in development and disease. Technologies with increased spatial resolution are required to more precisely distinguish and characterize cellular diversity and disease states within tissues. The Visium HD Spatial Gene Expression assay is a novel, high-resolution spatial technology that utilizes a whole transcriptome probe panel and resolves data at single-cell scale within intact tissue sections.

2. Methods

We applied 10x Genomics Visium HD Spatial Gene Expression assay to FFPE human breast ductal carcinoma in situ (DCIS), an early stage breast cancer tissue with high clinical relevance as well as complex spatial organization. Tissue sections mounted on standard glass slides were stained with Hematoxylin & Eosin (H&E) and imaged prior to the Visium HD workflow, allowing for morphological assessment in conjunction with single cell scale whole transcriptomic profiling on the same tissue section.

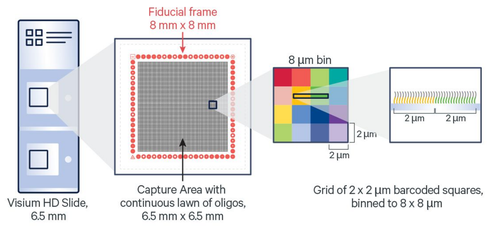

Figure 1. Visium HD Spatial Gene Expression slide. The HD array consists of ~12 million 2 µm x 2 µm spatially-barcoded areas without gaps and the data is binned to 8 µm x 8 µm for visualization and analysis.

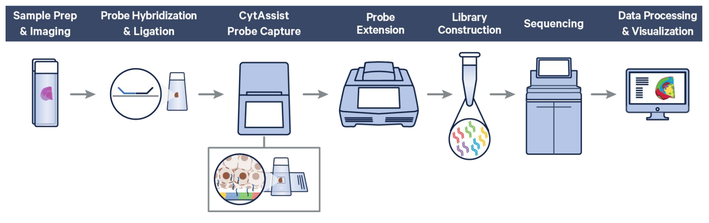

Figure 2. Visium HD Spatial Gene expression assay workflow. Visium HD is compatible with H&E staining of FFPE tissues on standard glass slides using routine histological workflows. This provides the flexibility to image and select target regions prior to whole transcriptome analysis using probe-based fixed RNA profiling. Visium HD assay relies on the Visium CytAssist instrument for probe capture. This is followed by probe extension, library construction, sequencing, and data visualization.

Acknowledgements

Thanks to the Visium HD development team at 10x Genomics, Pleasanton, CA. Special thanks to Syrus Mohabbat and Miriam Valencia for sample identification and obtaining pathologist annotations.

3. Spatial organization of major cell types

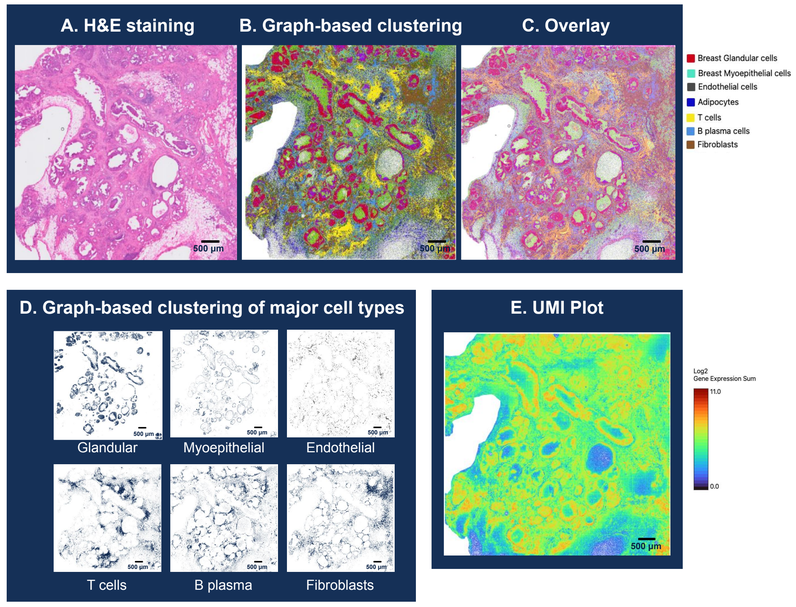

Figure 3. A) High resolution H&E staining of FFPE human breast DCIS tissue section. B) Louvain graph-based clustering analysis and C) Overlay of H&E and clustering identifies several major cell types at expected locations. D) The breast glandular and myoepithelial cell clusters mimic the distinct spatial localization of these cells in the ductal regions identified by H&E. These breast epithelial cells are surrounded by immune and fibroblast cell clusters. E) UMI plot mimics several tissue features seen in the H&E image and shows high transcript density in the ductal cells as expected.

4. HD resolves fine anatomical structures and their cell type composition

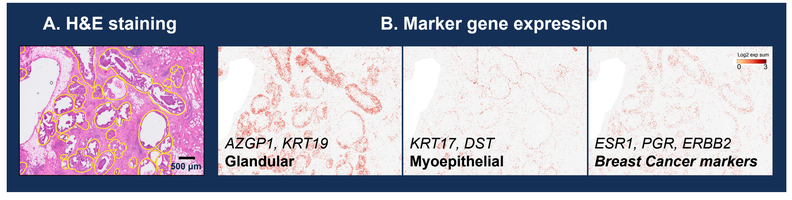

Figure 4. A) Overlay of H&E with pathology annotations (yellow outlines) indicating DCIS regions. B) The small bin size (8 µm x 8 µm) and full tissue coverage of Visium HD, allows the classification of key cell types in fine anatomical structures like DCIS based on marker gene expression. The spatial localization of these markers match the DCIS regions annotated on the H&E image as expected.

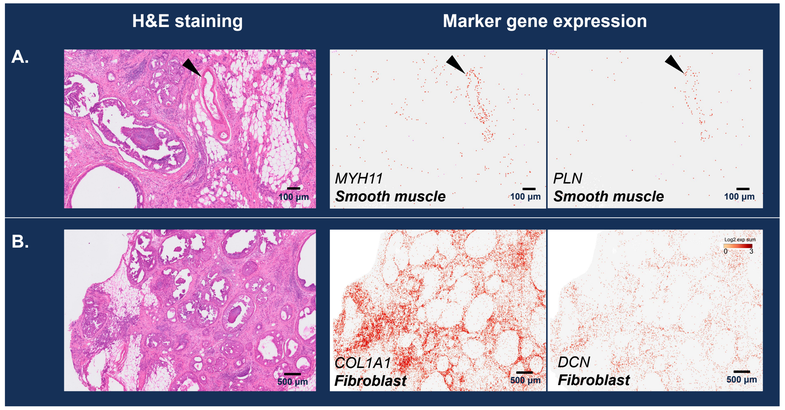

Figure 5. The HD array, workflow, and consumables ensure accurate transcript localization, enabling utilization of the improved feature size to achieve single cell scale resolution. A) The black arrowhead indicates a blood vessel in the H&E image and the correct localization of MYH11 and PLN, markers of smooth muscle cells that line the blood vessel. B) H&E images of human breast DCIS and Visium HD UMI heat maps of COL1A1 and DCN show fibroblast localization in the regions surrounding the ductal cells as expected.

5. Spatial profiling of immune cells

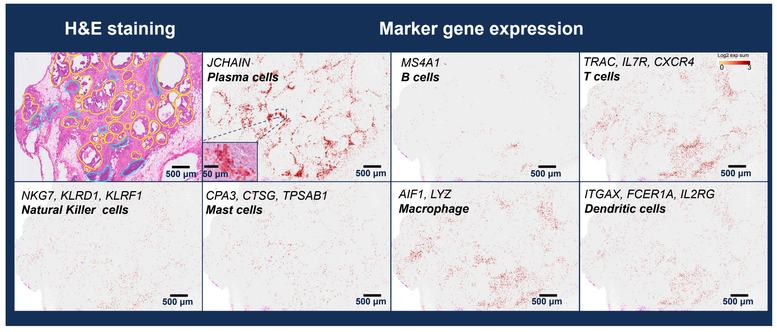

Figure 6. Overlay of H&E with pathology annotations indicating DCIS (yellow outlines) and immune rich (cyan outlines) regions. Spatial distribution of several immune cell types (Plasma, T, B, Natural killer, Mast, Macrophage, and Dendritic cells) is shown above. Plasma, T cells and Macrophage marker genes are abundant in immune rich regions around the DCIS as inferred from the H&E annotation. Inset shows the overlay of H&E showing an immune rich region and JCHAIN, a marker for Plasma cells. Note the tightly packed 8 µm x 8 µm square bins in the inset image.

6. Conclusion

The combination of single cell-scale resolution, full tissue coverage, and precision of transcript localization in Visium HD enables spatial mapping of major cell types in human breast DCIS. The Visium technology enables researchers to gain novel insights on normal development, disease pathology, and clinical translational research. Visium HD will further these capabilities and enable unprecedented insights to expand biological applications.