Novel spatial tagging of single nuclei spatializes single-cell ATAC-seq and V(D)J sequencing with gene expression for tumor microenvironmental analysis

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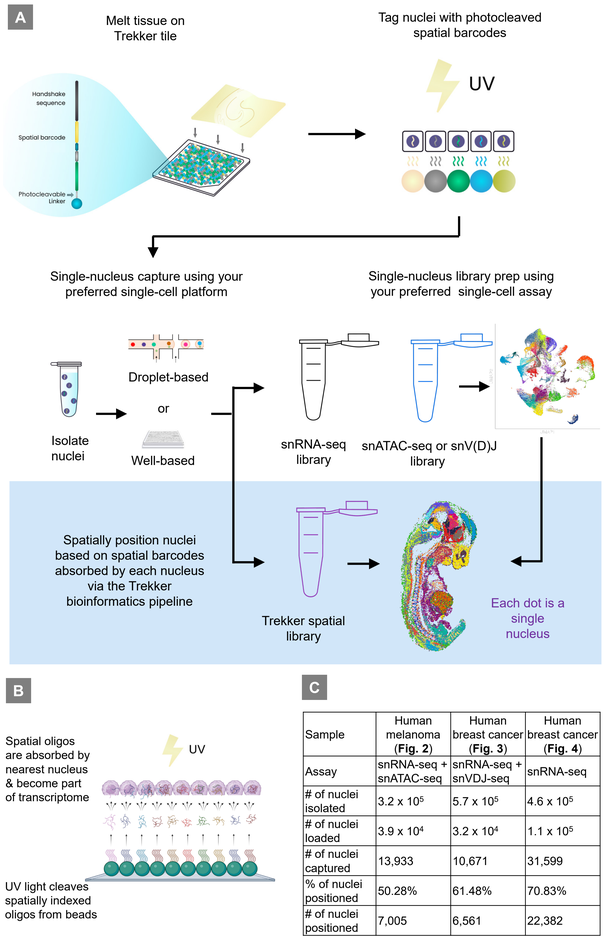
ABSTRACT

Advances in single-cell multiomic technologies such as RNA-seq, ATAC-seq, or V(D)J sequencing have enriched our understanding of tumor gene expression, chromatin accessibility, and immune receptor diversity, but they fall short in capturing the critical spatial context of cells within the tumor microenvironment (TME). Existing spatial methods, whether microscopy- or sequencing-based, primarily focus on gene or protein expression only. Additionally, they often lack true single-cell resolution, requiring computationally involved cell segmentation or deconvolution, and offer limited molecular complexity. To address this gap, the Trekker™ single-cell spatial mapping kit, based on Slide-tags technology, provides a paradigm-shifting solution by transforming single-cell sequencing assays into spatially resolved single-cell data. Trekker spatially labels individual cells within a tissue section by releasing the DNA barcodes from a spatially indexed bead array surface into nearby nuclei. The spatially tagged nuclei are then dissociated from the array and processed on conventional single-cell sequencing assays and platforms. The technique expands spatial measurement beyond gene expression alone, enabling spatial multiomic analyses from chromatin and V(D)J profiles. Moreover, given that the spatial measurements are at true single-cell resolution, the resulting datasets are easier to analyze and interpret.

In this study, we applied Trekker to existing single-nuclei (sn) ATAC-seq and snV(D)J-seq assays combined with gene expression to spatially analyze human melanoma and human breast cancer fresh frozen tissues. These previously unattainable combinations enable simultaneous spatial exploration of gene expression and epigenetic regulation within the spatial context of tumors and mapping of immune receptor clones within the TME. Joint unsupervised clustering on snRNA-seq and snATAC-seq data identified spatially defined tumor, stromal, immune, and vasculature cell types driven by both transcriptomic and epigenetic signatures. These cell types were further classified spatially into different tumor zones. We linked tumor-zone-specific genes with accessible chromatin domains and binding motifs. Tumor, stromal, and immune interactions were inferred within their native spatial context using spatially aware ligand-receptor analysis. V(D)J and immune composition analysis revealed spatial distribution of different T-cell clones. Our findings demonstrate the potential of Trekker to advance oncology and immunotherapy by providing a comprehensive spatial view of tumor ecosystems.

1 The Trekker workflow



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Sample	Human melanoma (**Fig. 2**)	Human breast cancer (**Fig. 3**)	Human breast cancer (**Fig. 4**)
Assay	snRNA-seq + snATAC-seq	snRNA-seq + snVDJ-seq	snRNA-seq
# of nuclei isolated	3.2 x 10^5	5.7 x 10^5	4.6 x 10^5
# of nuclei loaded	3.9 x 10^4	3.2 x 10^4	1.1 x 10^5
# of nuclei captured	13,933	10,671	31,599
% of nuclei positioned	50.28%	61.48%	70.83%
# of nuclei positioned	7,005	6,561	22,382

Figure 1. Trekker workflow. Panel A. Schematic representation of experimental and computational steps in the Trekker workflow. **Panel B.** Illustration of how Trekker oligos spatially tag individual nuclei. **Panel C.** Summary of datasets analyzed in Figures 2–4. In this study, only a subset of isolated nuclei were loaded onto single-cell assay platforms. The Trekker bioinformatics pipeline was used to extract spatial coordinates and integrate spatial information with snRNA-seq, snATAC-seq, and snV(D)J-seq data.

2 Trekker brings spatial dimensions to multiomic snRNA-seq and snATAC-seq analysis of primary human melanoma tissue

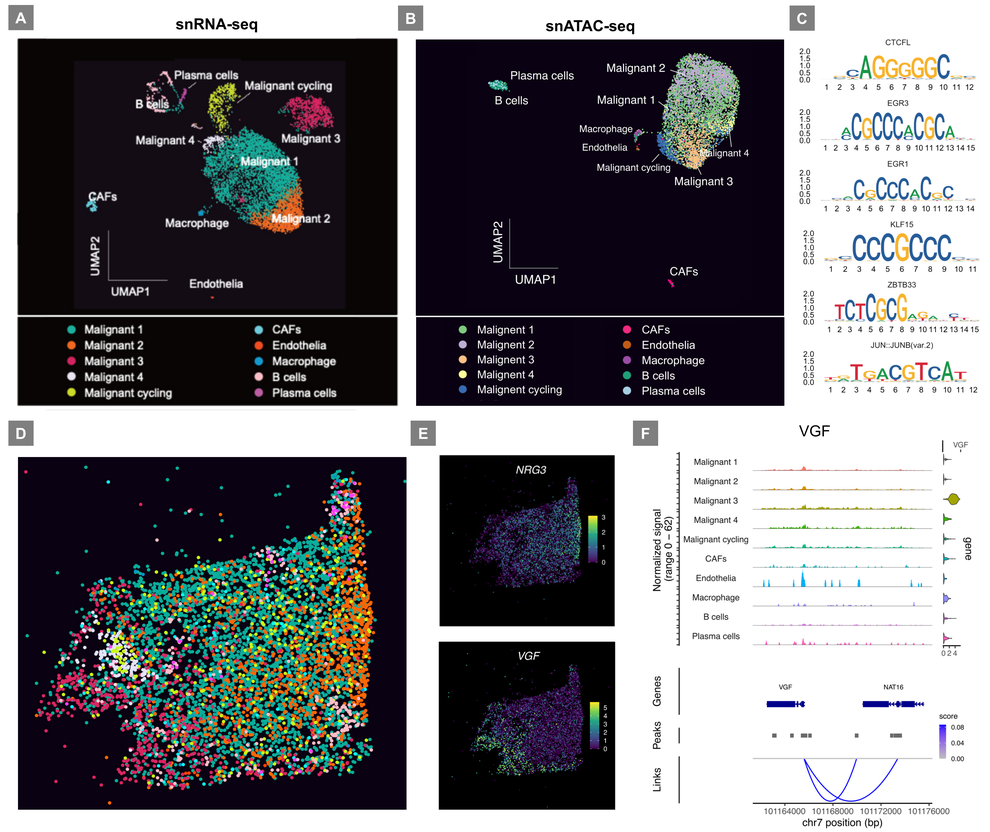
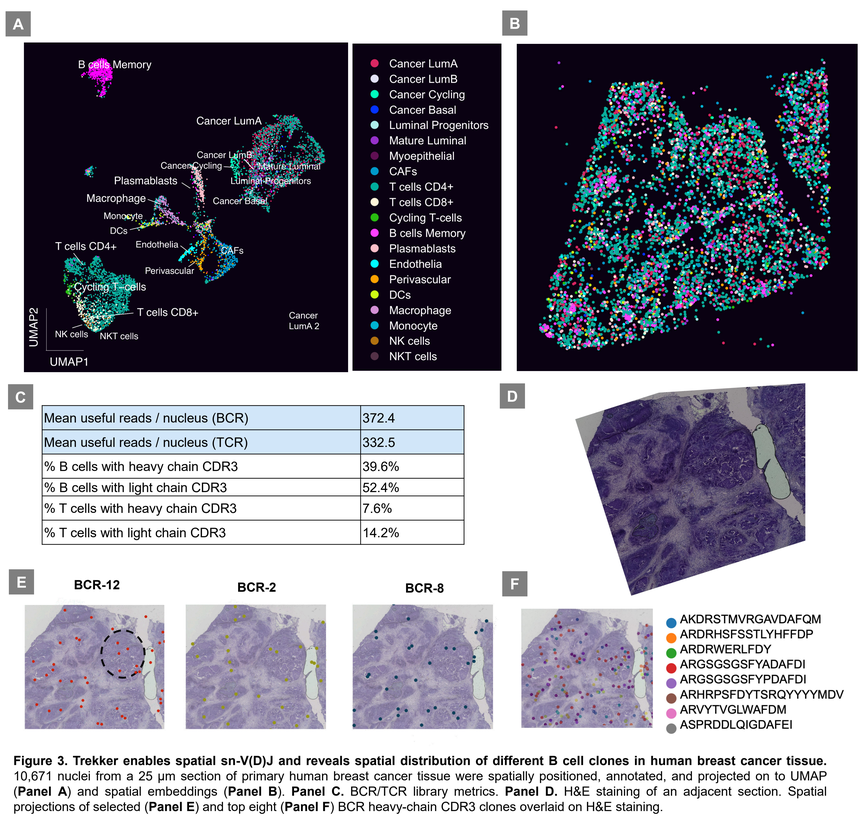


Figure 2. Trekker brings spatial dimensions to multiomic snRNA-seq and snATAC-seq analysis of primary human melanoma tissue. 13,933 nuclei from a 25 µm section of primary human melanoma tissue were spatially positioned using a 10 mm × 10 mm Trekker tile. Nuclei were annotated via label transfer from a published single-cell dataset, with all cell types annotated based on snRNA-seq data. Cell-type-annotated nuclei were projected onto dimensionally reduced space using snRNA-seq gene expression (**Panel A**) and snATAC-seq accessibility peaks (**Panel B**). **Panel C.** Sequence logos of the top six overrepresented DNA motifs found in differentially accessible ATAC peaks within the 'Malignant 3' tumor subtype. **Panel D.** Spatial projections of cell-type-annotated nuclei, colored as in Panel A. **Panel E.** Spatial expression patterns of malignant cell subtype-defining genes *NRG3* and *VGF*. Counts were normalized using SCTransform. **Panel F.** Genomic accessibility and gene expression of VGF in all annotated cell types, highlighting its association with the 'Malignant 3' tumor subtype. Accessibility data spans 500 bp upstream and 10,000 bp downstream of the *VGF* gene and is annotated with gene loci (**middle top**), reference peaks (**middle center**) and statistically correlated peaks (**bottom**).

3 Trekker enables spatial snV(D)J profiling and reveals the spatial distribution of different B-cell clones in human breast cancer tissue



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Mean useful reads / nucleus (BCR)	372.4
Mean useful reads / nucleus (TCR)	332.5
% B cells with heavy chain CDR3	39.6%
% B cells with light chain CDR3	52.4%
% T cells with heavy chain CDR3	7.6%
% T cells with light chain CDR3	14.2%

Figure 3. Trekker enables spatial sn-V(D)J and reveals spatial distribution of different B cell clones in human breast cancer tissue. 10,671 nuclei from a 25 µm section of primary human breast cancer tissue were spatially positioned, annotated, and projected on to UMAP (**Panel A**) and spatial embeddings (**Panel B**). **Panel C.** BCR/TCR library metrics. **Panel D.** H&E staining of an adjacent section. Spatial projections of selected (**Panel E**) and top eight (**Panel F**) BCR heavy-chain CDR3 clones overlaid on H&E staining.

CONCLUSIONS

We demonstrated Trekker can be easily integrated upstream of existing single-nucleus assays, adding spatial dimensions to previously hard-to-achieve multiomic data analysis. Even with nuclei subsampling, we were able to simultaneously delineate spatial locations, gene expression, and chromatin accessibility states of diverse tumor, immune, and vascular cell subtypes. Spatial context revealed locations of V(D)J clonotypes. Spatially-aware ligand-receptor analysis facilitated investigation of cell-to-cell communication between spatially proximate cells. Trekker provides a more comprehensive spatial approach to characterize the TME.

4 Trekker identifies differential spatial patterns and interactions of immune and tumor cell populations in human breast cancer tissue

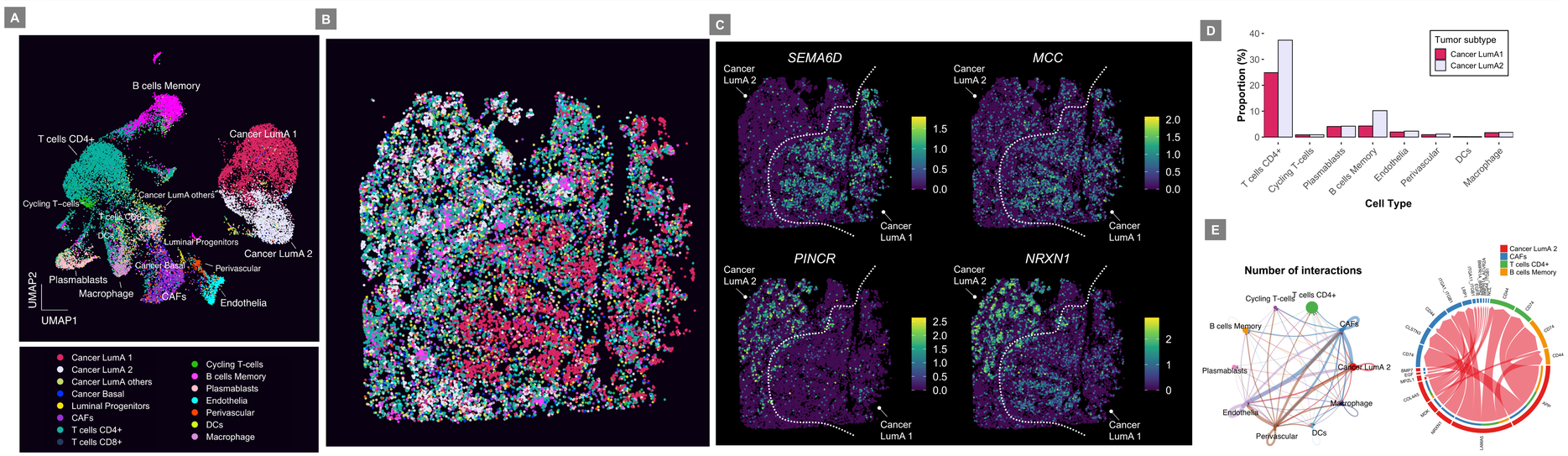

Figure 4. Trekker identifies differential spatial patterns and interactions of immune and tumor cell populations in human breast cancer tissue. 31,599 nuclei from a 25 µm section of primary human breast cancer tissue were spatially positioned using a 10 mm x 10 mm Trekker tile. Nuclei were annotated via label transfer from a published single-cell dataset. Annotated cell types were projected onto UMAP (**Panel A**) and spatial (**Panel B**) embeddings, revealing two spatially segregated tumor subtypes (Cancer LumA 1 vs LumA 2). The boundary between these subtypes can be marked by spatially variable genes. Counts are normalized by SCTransform (**Panel C**). **Panel D.** CD4+ T cells and memory B cells were relatively enriched in the LumA 2 subtype compared to LumA 1. **Panel E.** Number (left) and identify (right) of statistically significant ligand–receptor interactions involving immune cells, vascular cells, epithelial cells, and cancer-associated fibroblasts (CAFs) within the LumA 2 tumor region (as defined in Panel C).

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