

# Spatial Organization of the Mouse Kidney in 3D

## Introduction

Single-cell genomics, particularly single-cell RNA sequencing (scRNA-seq), has transformed nephrology by providing a detailed view into the cellular heterogeneity of the kidney, a complex organ with numerous specialized cell types. By profiling the transcriptomes of thousands of individual cells, researchers have generated comprehensive cellular atlases of healthy and diseased human and mouse kidneys<sup>1</sup>.

By comparing the single-cell transcriptomes of healthy kidneys to those with conditions like diabetic nephropathy, acute kidney injury, and renal cell carcinoma, researchers have begun to pinpoint specific cell subsets that are most affected by disease. This has led to the identification of disease-associated gene signatures and signaling pathways that are dysregulated during pathology<sup>2</sup>. However, cells do not exist in isolation and scRNA-seq data misses crucial cell-cell interactions that occur in the three-dimensional space of the kidney in its biological state.

Now, single-cell spatial analysis in the kidney moves into 3D. In this study, we report on a 64-gene panel that subsets key cell populations in 50  $\mu\text{m}$  thick adult mouse kidney tissue using Pyxa<sup>®</sup>. Three-dimensional insights will be crucial for identifying new therapeutic targets and developing more precise diagnostic and prognostic biomarkers for kidney diseases based on the structure of the kidney and cell-cell interactions in situ.

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## Methods

### *Samples and Tissue Preparation*

Adult C57BL/6 mouse kidney tissue was collected, fresh-frozen, and embedded in OCT compound. Tissue was cryosectioned at 50  $\mu\text{m}$ , mounted in well plates, and stored at  $-20\text{ }^{\circ}\text{C}$  until processing.

### *STARmap Sample Prep Protocol*

Spatial transcriptomic profiling was carried out using the STARmap<sup>™</sup> protocol. Sections were pretreated and permeabilized before adding SNAIL<sup>™</sup> probes in Hybridization Buffer using a 64-gene panel, as described below (Table 1). Unbound probes were removed with Post-Hybridization Wash Buffer. Hybridized probes

were subsequently ligated and amplified in situ, then embedded in hydrogel to preserve spatial architecture. The remaining tissue was cleared in Clearing Buffer with Clearing Enzyme and evaluated with Sample Verification Solution. Multiple sequencing rounds were imaged with DAPI to label cell nuclei and fluorescent probes to detect amplicons representing individual transcripts.

### *Imaging*

Sequencing rounds were imaged on Pyxa<sup>®</sup>, a high-resolution confocal microscope with z-stack acquisition to capture three-dimensional transcript distributions.

## Methods (cont'd)

### Data Processing

Image registration, transcript decoding, and cell segmentation were conducted using the computational pipeline. Cell boundaries were defined via DAPI nuclear staining followed by transcript density dilation.

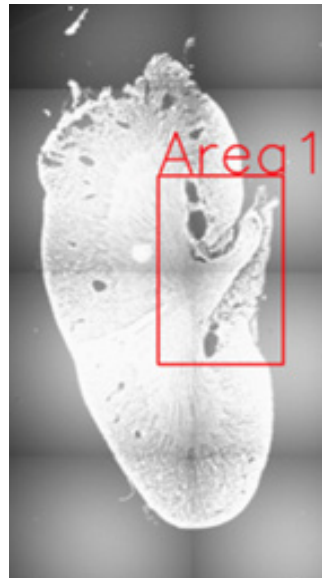
To ensure high-quality data for downstream analysis, the following filtering was applied:

- Transcript Count: Only cells containing between 5 and 350 transcripts were retained.
- Cell Volume: Only cells with a total volume between 200 and 10,000  $\mu\text{m}^3$  were included.

Post-segmentation analysis was performed using the Scanpy library. Transcript counts were normalized by cell volume and log transformed. Clustering was performed in Scanpy using the Leiden algorithm, and cell types were annotated manually based on canonical marker genes.

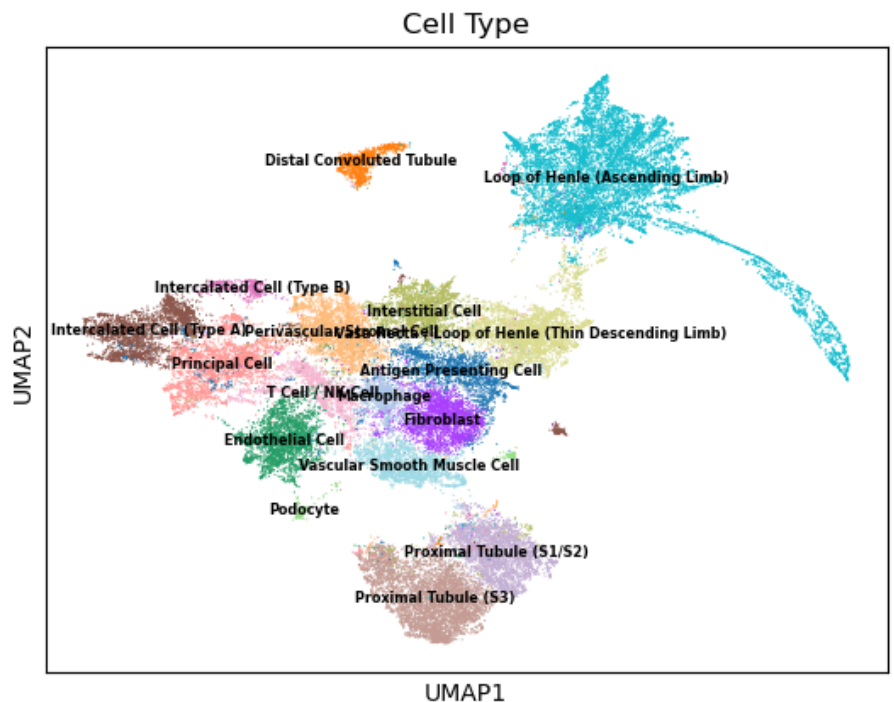
## Results

Gene Name	Gene Name
<i>Aldob</i>	<i>Cxcl12</i>
<i>Slc5a2</i>	<i>Pecam1</i>
<i>Slc22a6</i>	<i>Cdh5</i>
<i>Aqp1</i>	<i>Emcn</i>
<i>Havcr1</i>	<i>Kdr</i>
<i>Acsn1</i>	<i>Acta2</i>
<i>Umod</i>	<i>Tagln</i>
<i>Slc12a1</i>	<i>Rgs5</i>
<i>Clcnka</i>	<i>Myh11</i>
<i>Kcnj1</i>	<i>Pdgfra</i>
<i>Slc12a3</i>	<i>Col1a1</i>
<i>Pvalb</i>	<i>Dcn</i>
<i>Trpm6</i>	<i>Lum</i>
<i>Aqp2</i>	<i>Cd68</i>
<i>Hsd11b2</i>	<i>Adgre1</i>
<i>Avpr2</i>	<i>C1qa</i>
<i>Scnn1a</i>	<i>Aif1</i>
<i>Scnn1b</i>	<i>Cd3e</i>
<i>Scnn1g</i>	<i>Cd8a</i>
<i>Fxyd4</i>	<i>Nkg7</i>
<i>Atp6v0d2</i>	<i>Cd19</i>
<i>Atp6v1b1</i>	<i>Cd79a</i>
<i>Slc4a1</i>	<i>Ms4a1</i>
<i>Slc26a4</i>	<i>Itgax</i>
<i>Foxi1</i>	<i>Xcr1</i>
<i>Nphs1</i>	<i>Flt3</i>
<i>Nphs2</i>	<i>Foxd1</i>
<i>Podxl</i>	<i>Col4a3</i>
<i>Wt1</i>	<i>Il7r</i>
<i>Mafb</i>	<i>Ccl21a</i>
<i>Pdgfrb</i>	<i>Cd79b</i>
<i>Itga8</i>	<i>Mzb1</i>

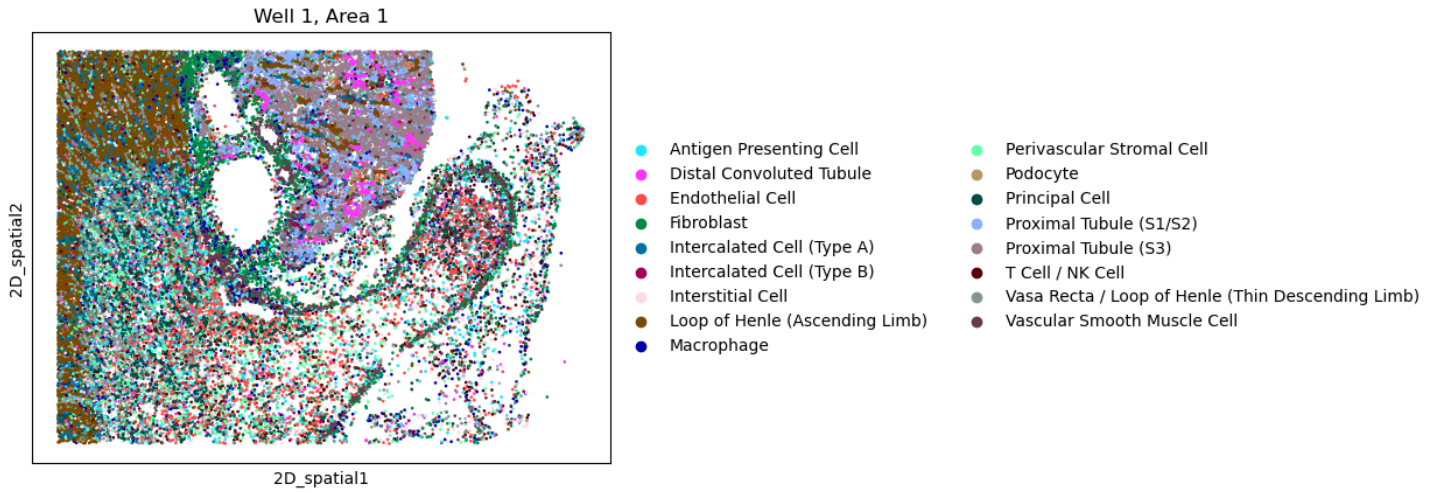


**Figure 1 (left).** Imaging was performed on the area of kidney outlined as Area 1.

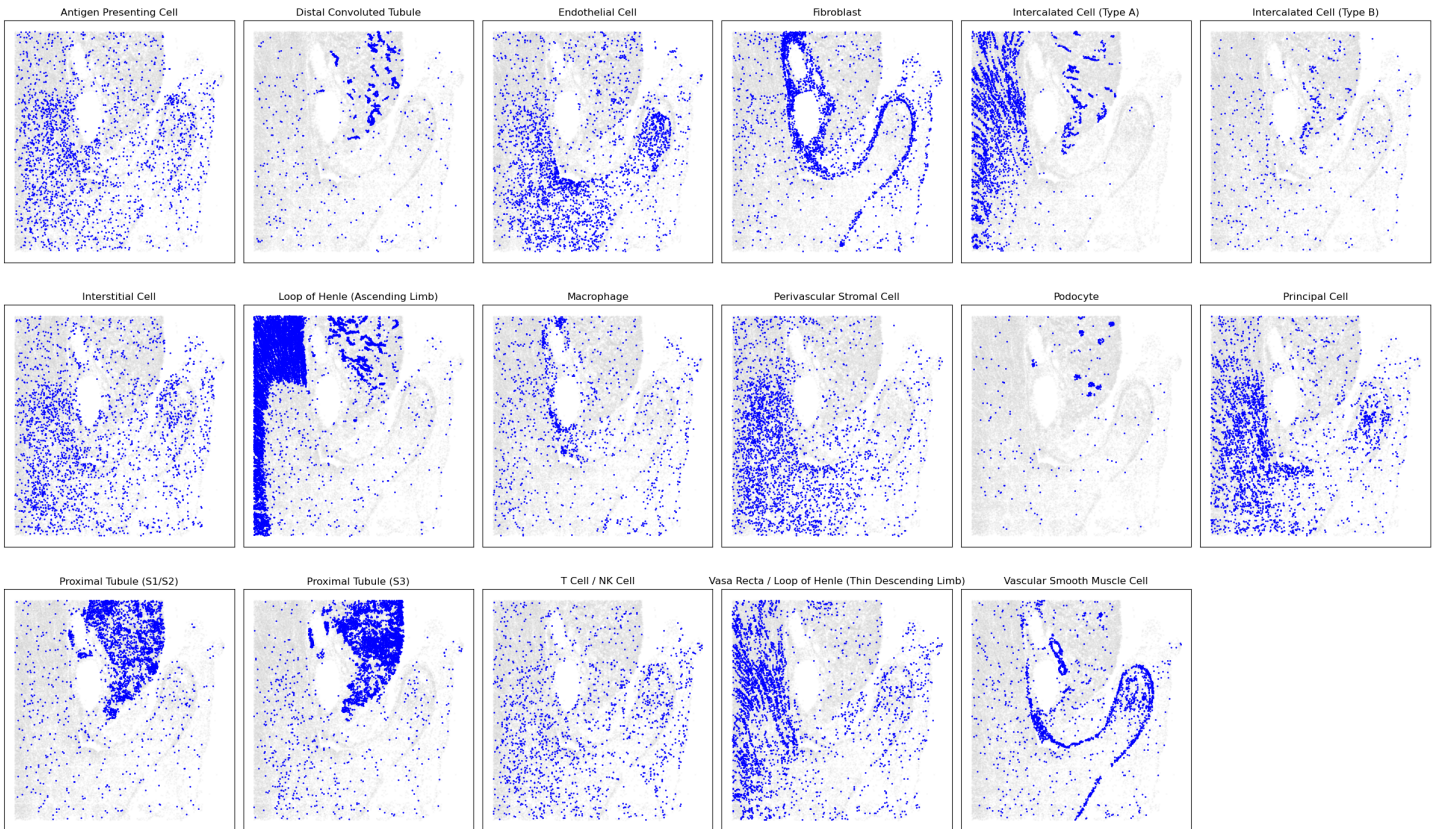
**Figure 2 (bottom).** Key cell populations in the adult mouse kidney can be subset using a 64-gene gene kidney cell typing panel. Each dot in the UMAP represents a single cell, where dots are color-coded and assigned cell typing based on gene expression patterns.



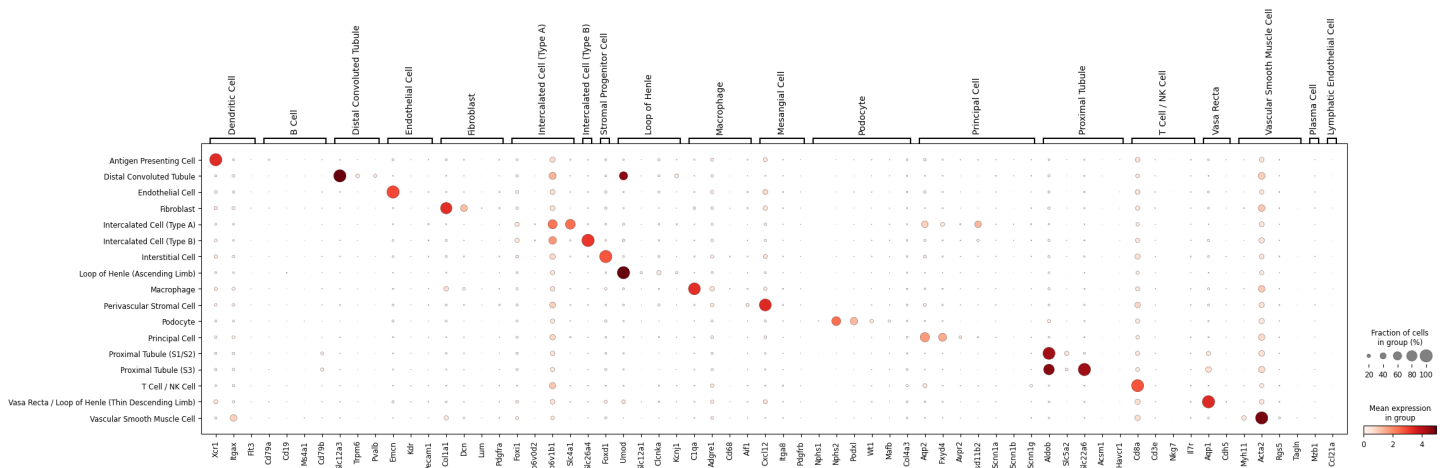
**Table 1.** Gene panel used in this study.



**Figure 3.** Spatial map of cells in the adult mouse kidney, pseudocolored by cell type. Each dot represents a single cell. Three-dimensional data was collapsed down to two dimensions for ease of visualization.



**Figure 4.** Spatial mapping of individual cell types in the adult mouse kidney shows cell type localization within the kidney structure.



**Figure 5.** Gene expression patterns define key cell populations and structural elements in the kidney.

## Conclusion

Here, we describe a 64-gene kidney panel that was used with Stellaromics® commercial STARmap™ sample preparation reagents to subset common cell types in the adult mouse kidney. Cell typing and spatial mapping revealed the expected distribution of each cell type throughout the structure of the kidney. This demonstrates the compatibility of mouse kidney tissue to STARmap sample preparation and sequencing on Pyxa®.

## References

1. <https://doi.org/10.1016/j.isci.2023.106877>
2. <https://doi.org/10.1038/s41591-018-0194-4>

## Acknowledgements

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