

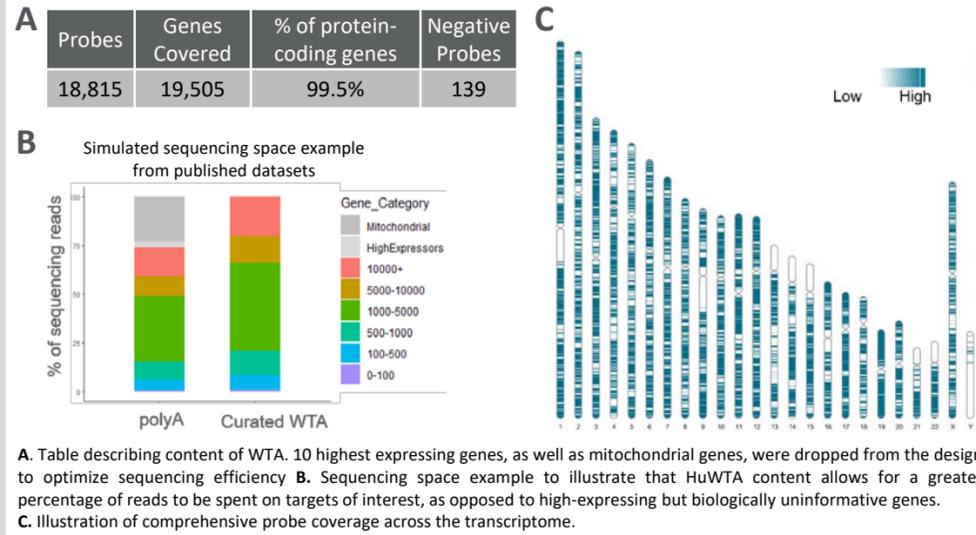
# Illuminating the Transcriptome: Design and Technical Performance of Whole Transcriptome Atlas for GeoMx Digital Spatial Profiler

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## Abstract

- High-plex spatial technologies are needed to profile gene expression while maintaining information on tissue architecture
- The Human Whole Transcriptome Atlas (HuWTA) for GeoMx<sup>®</sup> DSP uses a curated approach to target 99.5% of protein-coding genes while optimizing readout efficiency
- Here, we demonstrate the capabilities of HuWTA by benchmarking against other bulk and spatial technologies
- We also examined unique morphological structures in multiple tissues to outline guidelines for use of HuWTA.
- Finally, we used these guidelines in experiments profiling directed AOIs across healthy and diseased kidney samples
- Our results demonstrate that HuWTA successfully integrates transcriptome-scale spatial biology with sophisticated AOI design to enable flexible discovery with spatial context.

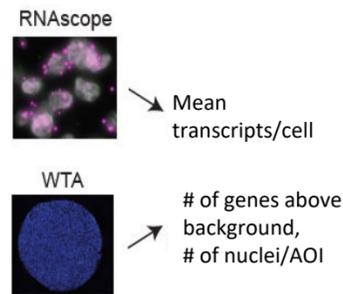
## HuWTA covers 99.5% of protein coding genes



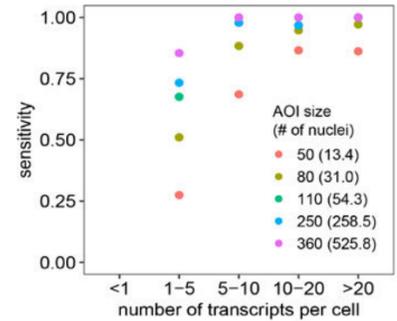
## HuWTA demonstrates high sensitivity, and concordance with RNAseq

### Absolute transcript number sensitivity for HuWTA

### RNAscope and HuWTA data from the same cell lines used to determine per cell sensitivity

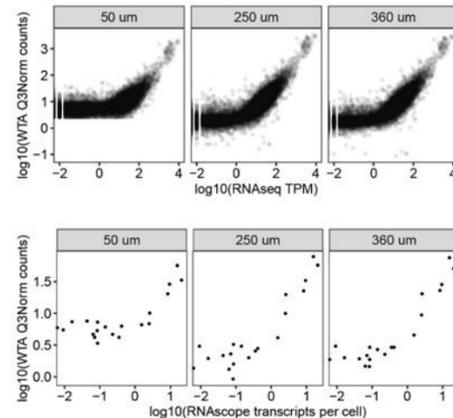


### Defining absolute transcript detection thresholds for HuWTA

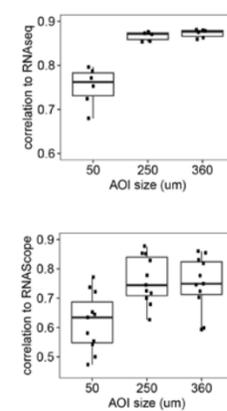


### Concordance with RNAseq and RNAscope

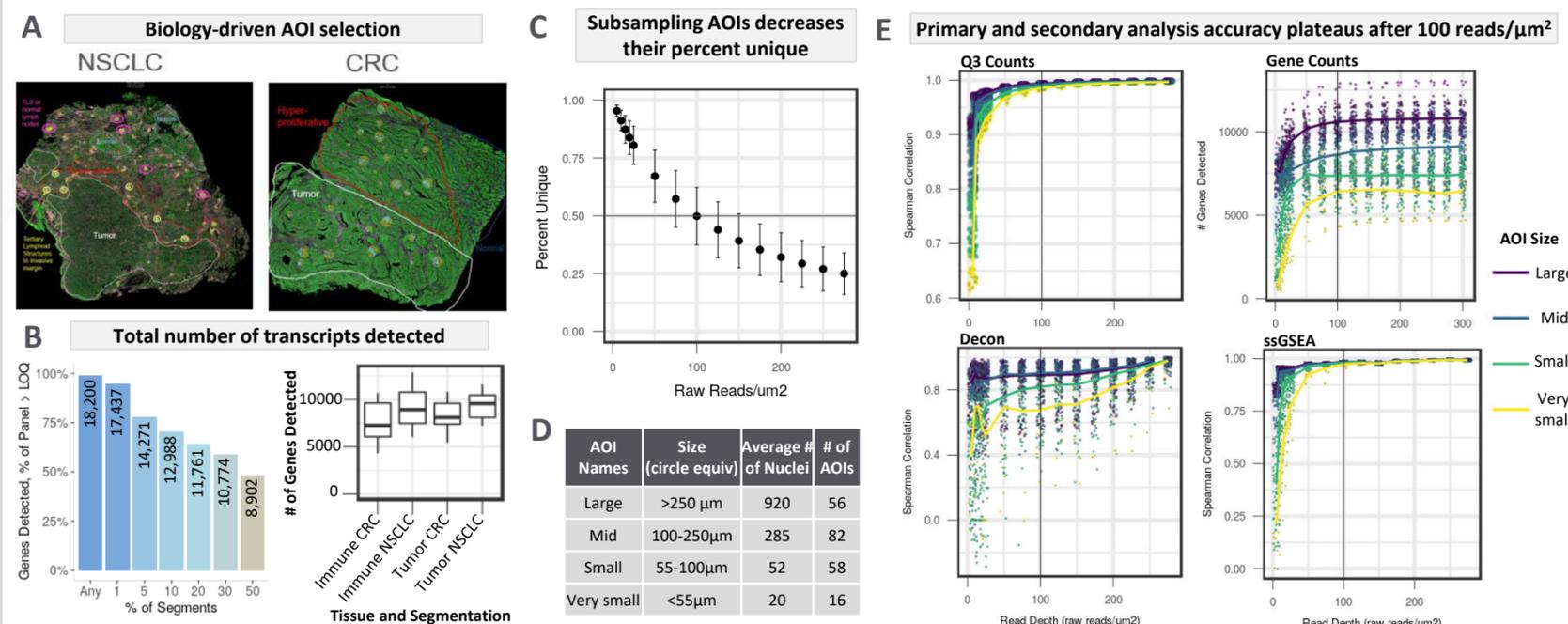
### Scatterplots for a representative cell line (faceted by AOI size)



### Spearman's correlations across cell lines

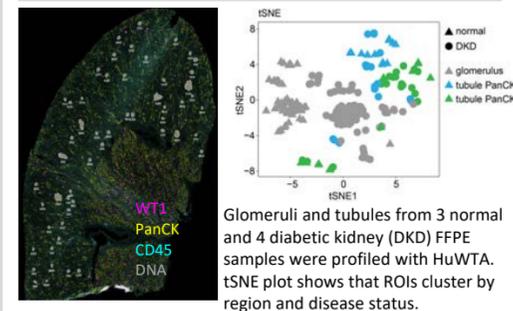


## Technical benchmarking in tissue: subsampling to determine ideal read depth

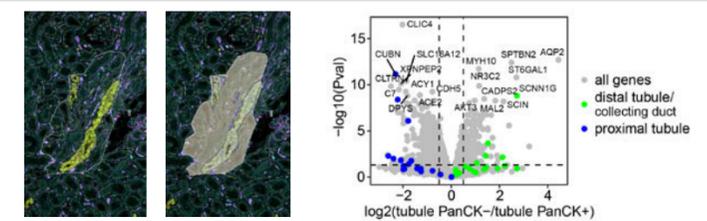


## Discovering spatial biology in the kidney: HuWTA uncovers heterogeneity in health and disease

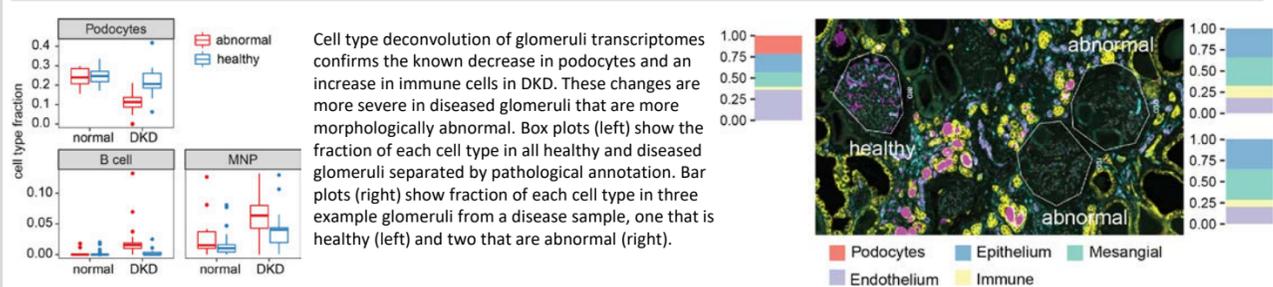
### Targeted AOIs capture relevant biology



### HuWTA identifies gene expression changes in regions of the normal kidney



### HuWTA reveals changes in cell populations in disease and spatial heterogeneity across diseased glomeruli



## Conclusions

- Curated HuWTA content covers 99.5% of protein-coding genes
- HuWTA is concordant with other platforms
- HuWTA can detect transcripts present at 1 copy per cell
- HuWTA measures the whole transcriptome in a single AOI
- HuWTA results are optimized with 100 sequencing reads/ $\mu$ m<sup>2</sup>
- Flexible AOI selection captures gene expression differences in biological structures in healthy and diseased kidney
- Cell type deconvolution reveals spatial heterogeneity across diseased samples

## GeoMx DSP chemistry and workflow

