Updating immune cell deconvolution for the spatial genomics era



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High MKI67 in Tumor

ligh MKI67 in <mark>Microen</mark>

Tumor regions expressing

PD-L1 are not evident

from whole region data.

Separate profiling of Tumor and Microenvironment segments from regions of a NSCLC tumor Summary Confirming accuracy: Segmented profiling: measure gene Importance of segmented profiling: A spatially-resolved gene expression dataset cannot be fully understood without a 191 400 x 400um region expression only within cell types of Tumor/ Immune markers Canonical driver and immuno-oncology genes are expressed rom a NSCLC tumor. census of the cell types present in each profiled region. Here we introduce the interest (guided by fluorescent markers) by both Tumor and Microenvironment. Only with segmented are highly specific to Separate profiling of profiling can we attribute their expression either cell type. appropriate segments NanoString Quantitative Single Cell Deconvolution (qSCD) algorithm for using gene expression data to quantify mixed cell types. We validate the algorithm in tumor tissues, and we demonstrate its use in a NSCLC tumor profiled with the GeoMx[®] Expression **whole** regio W/2 == Cancer Transcriptome Atlas RNA panel. Naive approach: Single Region of profile whole region GeoMx DSP reagents and workflow Interest B Protein detection probe **RNA detection probe** Α indexing olig egion-specific indexing oligo egion-specifi JV-dependen UV linker Segmented profiling: only tumor PanCK **CD45** Segmented profiling: 191 regions of tumo only microenvironment

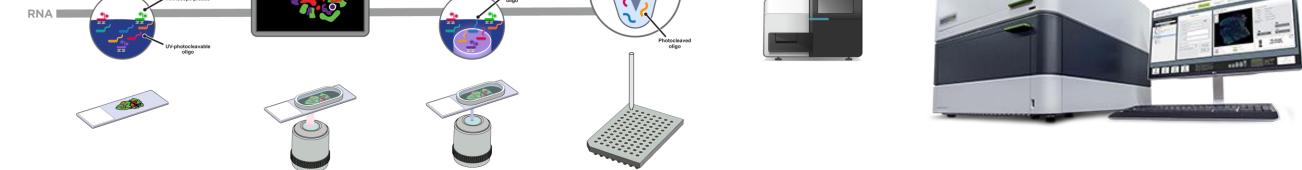
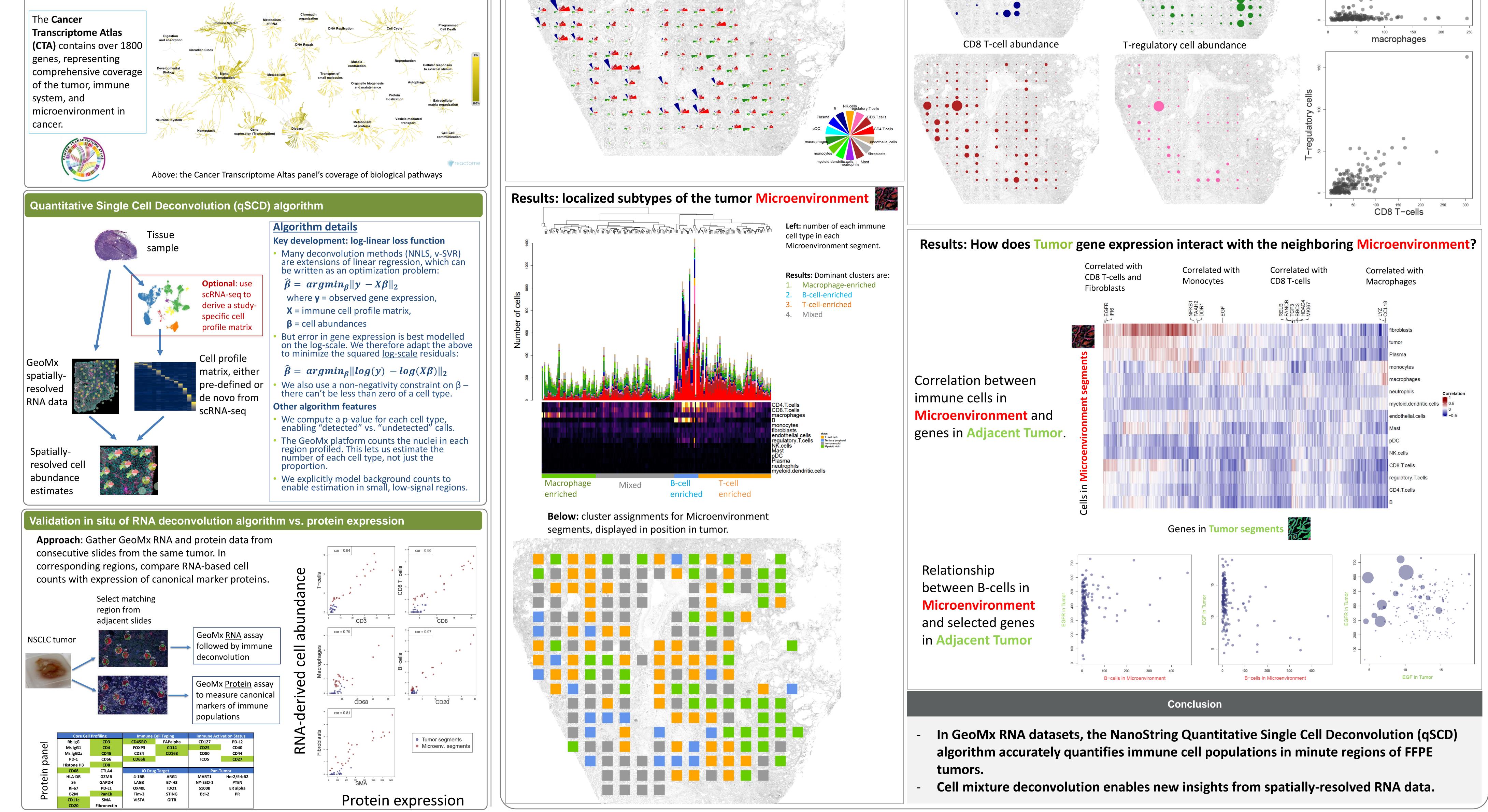


Fig 1. The GeoMx Digital Spatial Profiler (GeoMx DSP) is based on barcoding technology that enables spatially resolved, digital characterization of proteins or RNA in a highly multiplexed (over 2,000-plex) assay. The oligonucleotide tags cleaved from discrete regions are quantitated by NGS or nCounter, and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance. Using a UV-cleavable linker, epitope-specific antibodies (A) or in-situhybridization probes (B) are conjugated with unique DNA-oligo tags. GeoMx DSP shapes and illuminates UV lights over user-defined tissue subregions of interest only to cleave & collect DNA-oligo tags and records xy coordinates of the subregions (C). Cleaved tags from each ROI are collected and counted using nCounter or a NGS sequencer.

Spatial RNA profiling with the Cancer Transcriptome Atlas

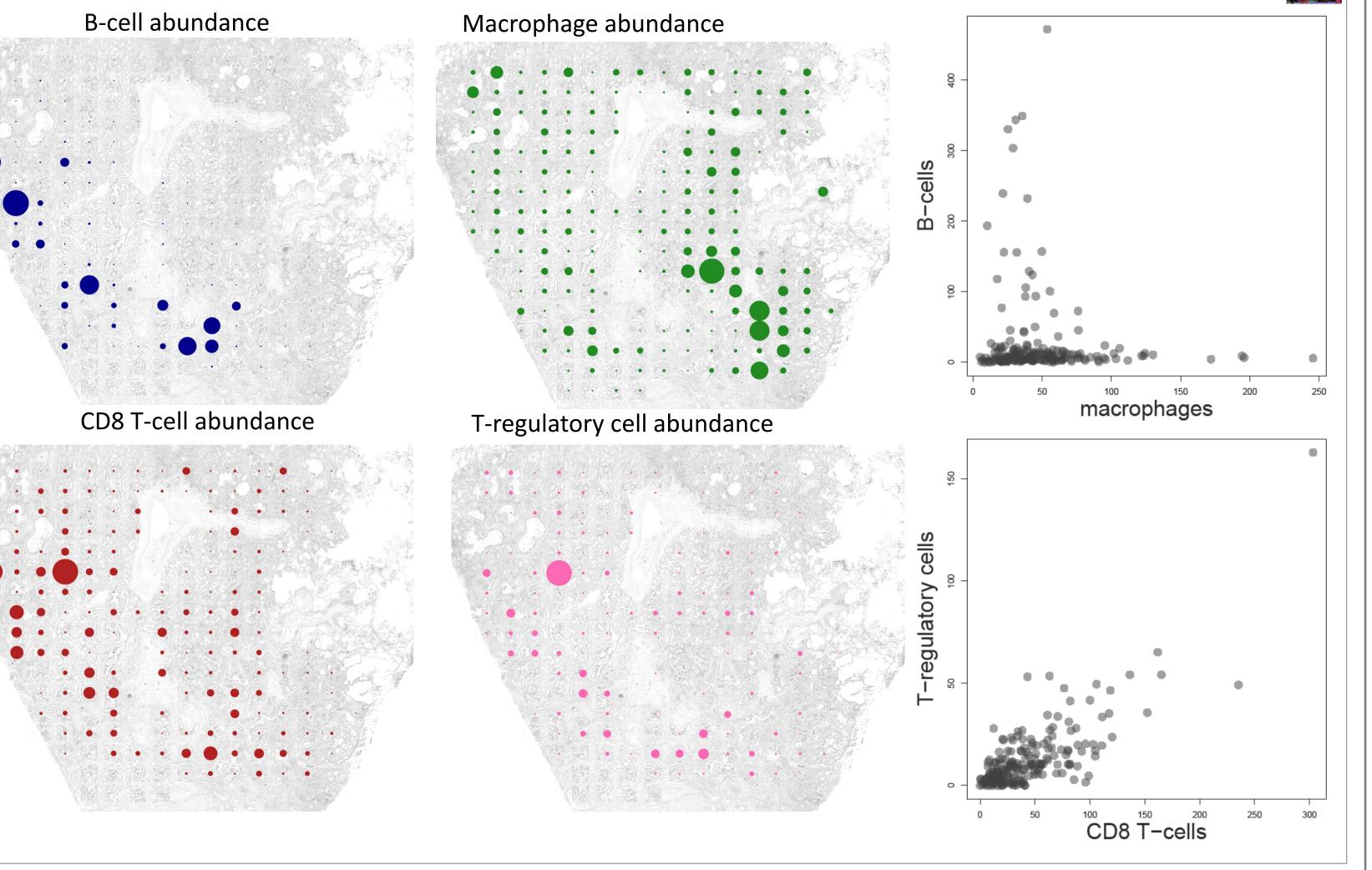


Spatially-resolved immune cell deconvolution in microenvironment segments

Results: Abundance of immune cells in Microenvironment nol_ **Below:** Estimated counts of 14 cell types in microenvironment segments from 191 regions.

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Results: How do immune cells co-localize across Microenvironment segments?



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Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact us at <u>TAP@nanostring.com</u>. Patrick Danaher, Sarah Church, Chris Merritt, Dan Zollinger, Jason Reeves, Youngmi Kim, Joseph Beechem*

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