Spatial Transcriptomics

Digital Pathology to Investigate Intratumor Heterogeneity in Breast Cancer

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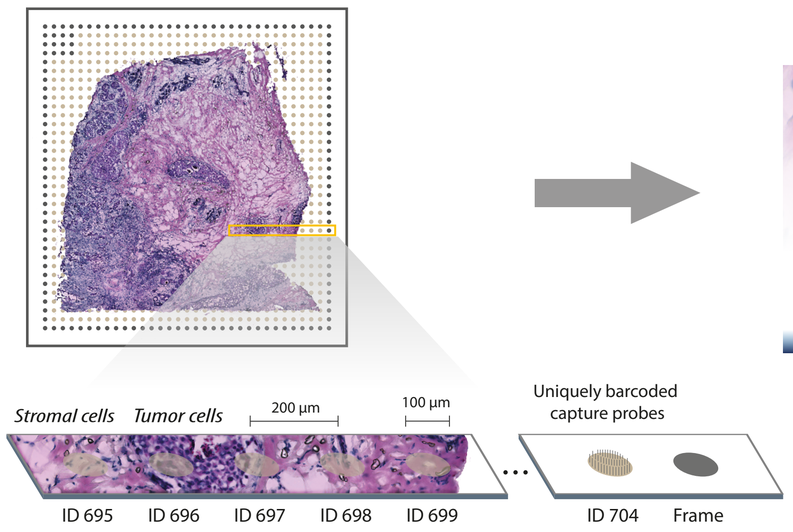
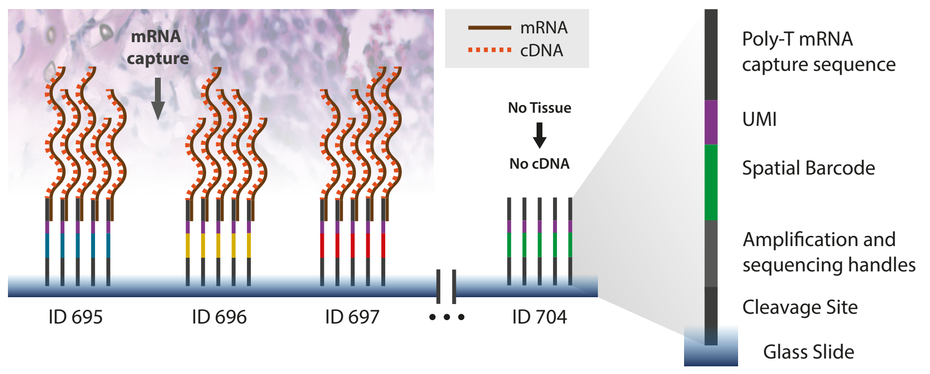
An Overview of the Spatial Transcriptomics Method

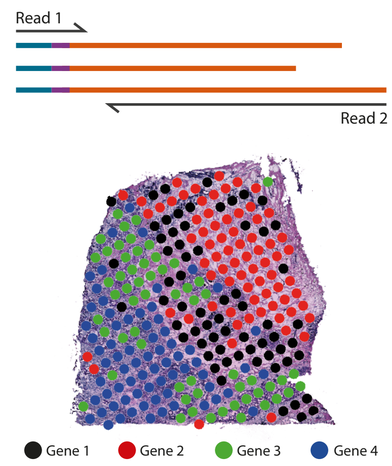
1. Sectioning and Imaging on Spatially Barcoded Array



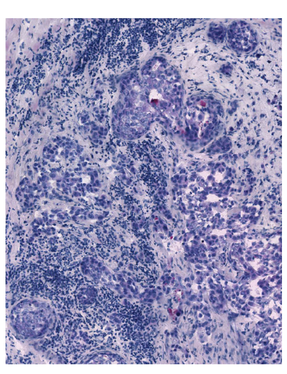
2. Permeabilization and cDNA Synthesis



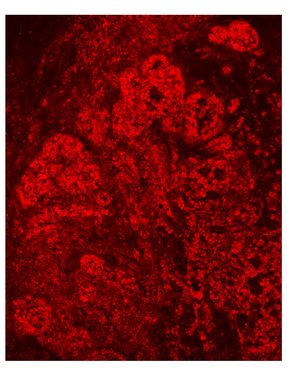
3. Sequencing, Analysis and Visualization



Fluorescently Labeled cDNA Proves Localized RNA Capture



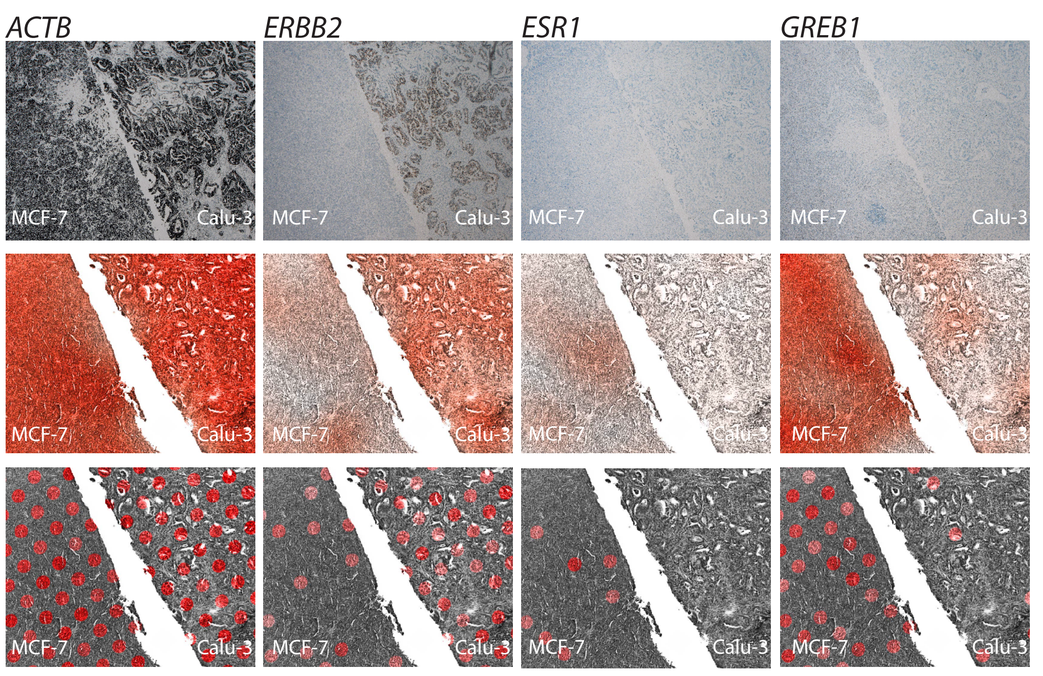
Tissue was initially stained with hematoxylin and eosin (HE). The staining shows spatial localization of cells and reveals tissue morphology.



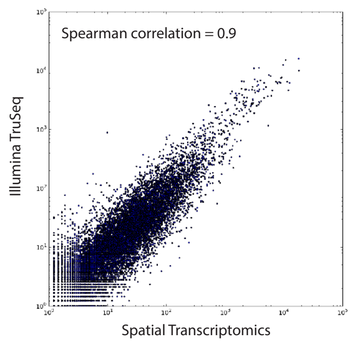
After cDNA synthesis, the tissue was degraded and removed before imaging. Fluorescently labeled cDNA "footprint" on chip surface indicates successful cDNA synthesis and low horizontal diffusion of RNA molecules (low signal in between cells).

Detection of Tissue Specific Genes in FFPE Xenograft Samples

Spatial Transcriptomics can be applied to tissue samples with low amount of RNA and poor quality such as FFPE samples. However in such cases the yield and number of genes that can be detected could be reduced due to inaccessible poly-A tails and degraded RNA compared to fresh frozen samples. Right hand images show examples of four marker genes with specific expression in two different xenograft models (MCF-7 and Calu-3) placed on the same array. Top images show the detected expression from in situ hybridization (ISH) experiments (Ventana Medical Systems, Inc), where black or brown color indicates detected expression of that gene. Center and bottom images show the expression patterns detected by Spatial Transcriptomics, where strong red color indicate a high expression in that spot or region. In the center images we used the coordinates of each spot (as seen in the bottom images) and the number of normalized reads to generate a levelplot directly in the tissue.



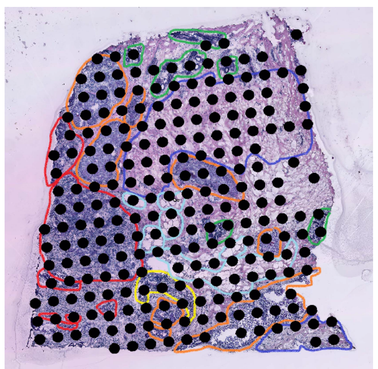
Compared to Regular RNA-seq



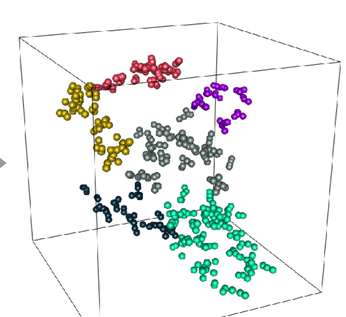
Total RNA was extracted from fresh frozen MCF-7 Xenografts samples and libraries were prepared according to Illumina TruSeq protocol. The data was compared to Spatial Transcriptomics using one tissue section on a spatially barcoded array. Approximately 15K genes were detected in each dataset, of which about 14K genes were shared between the datasets. The gene expression levels were highly correlated when counting 3'-tags (Spearman correlation = 0.9).

Analysis of Clinical Breast Cancer Sampes

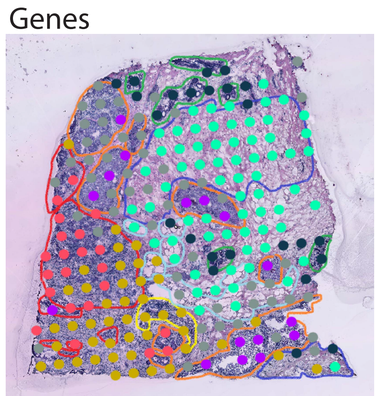
Selecting Spots in Areas of Interest



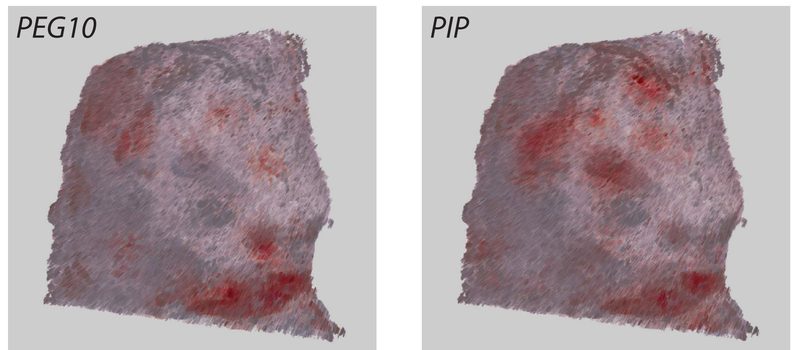
Dimesionality Reduction and Hierarchical Clustering



Visualize the Location of Clusters and Find Marker Genes



Combine Several Sections and Visualize Spatial Expression Patterns in 3D



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