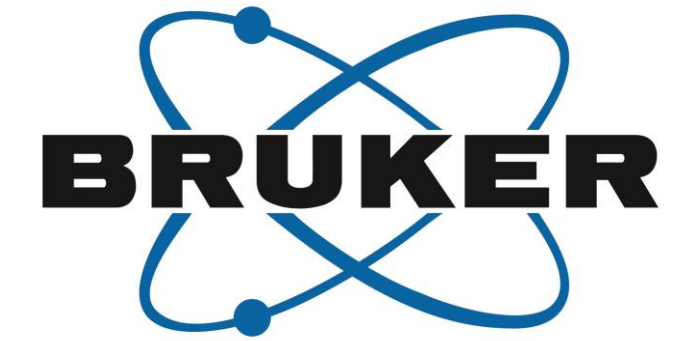


High plex spatial single-cell microRNA profiling of human colon cancer tissue using Spatial Molecular Imaging (SMI)



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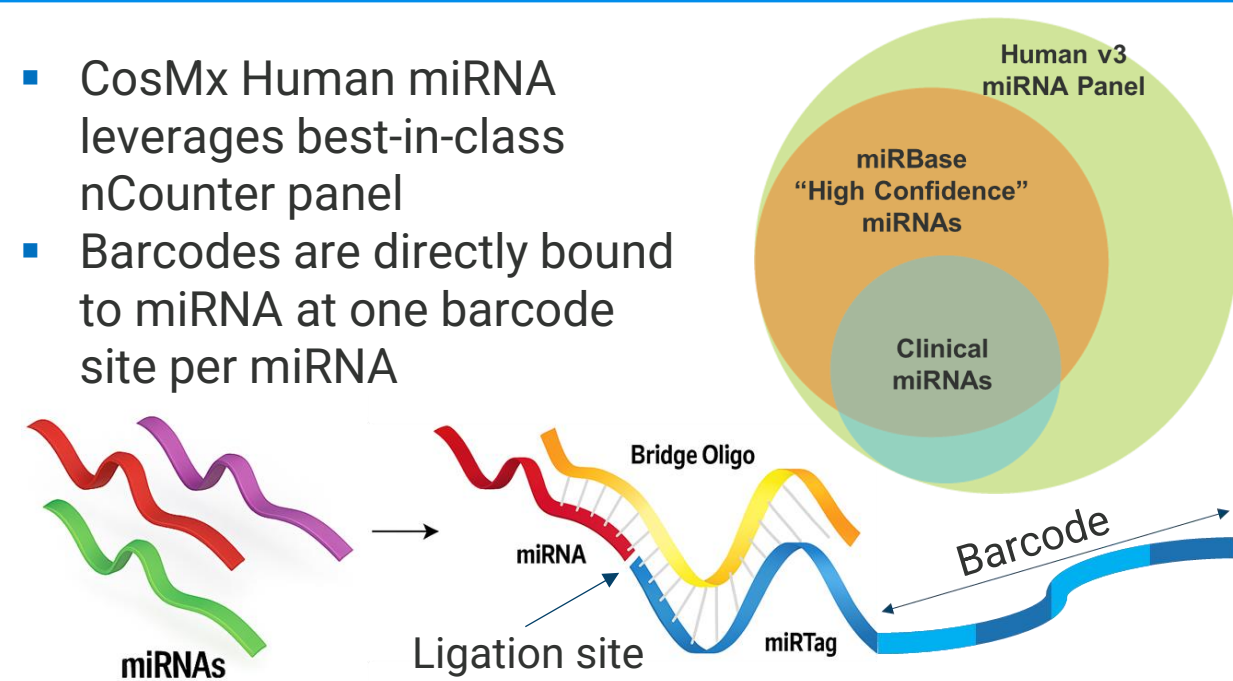
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Introduction

High-plex spatial miRNA detection has (historically) not been possible due to short sequence length, which poses a challenge for conventional in situ hybridization probe design. CosMx[®] SMI addresses this limitation by using a strategy to directly attach readout barcodes to miRNA targets in tissue. Detection is achieved through multiple rounds of reporter binding and fluorescence imaging. Here, we describe miRNA detection at the highest plex and highest spatial resolution ever achieved in sections of formalin-fixed paraffin embedded (FFPE) colon adenocarcinoma.

CosMx 800 plex miRNA Assay

- CosMx Human miRNA leverages best-in-class nCounter panel
- Barcodes are directly bound to miRNA at one barcode site per miRNA



CosMx miRNA Assay Workflow

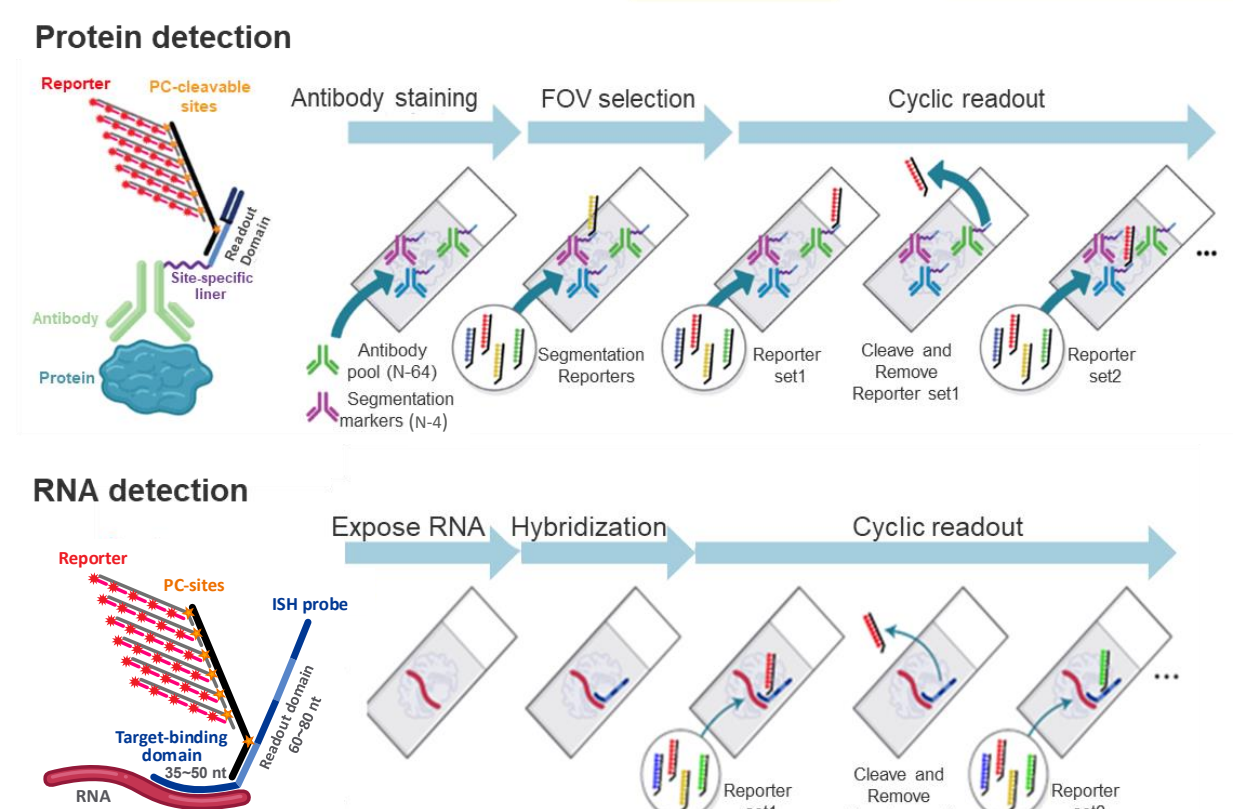
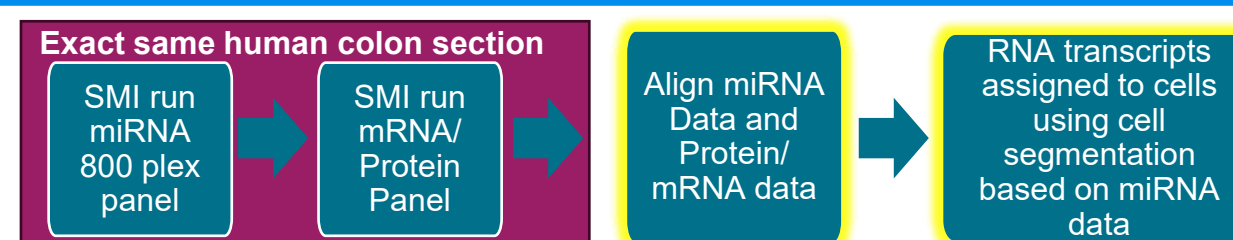


Fig 1. The SMI assays sequentially detect protein and RNA targets with oligonucleotide barcode-conjugated antibodies and barcoded RNA probes via several rounds of reporter binding and fluorescence imaging. Cells are segmented based on morphology stains and decoded RNA targets are assigned to individual cells

Highly specific miRNA panel proven with synthetic targets in FFPE tissue

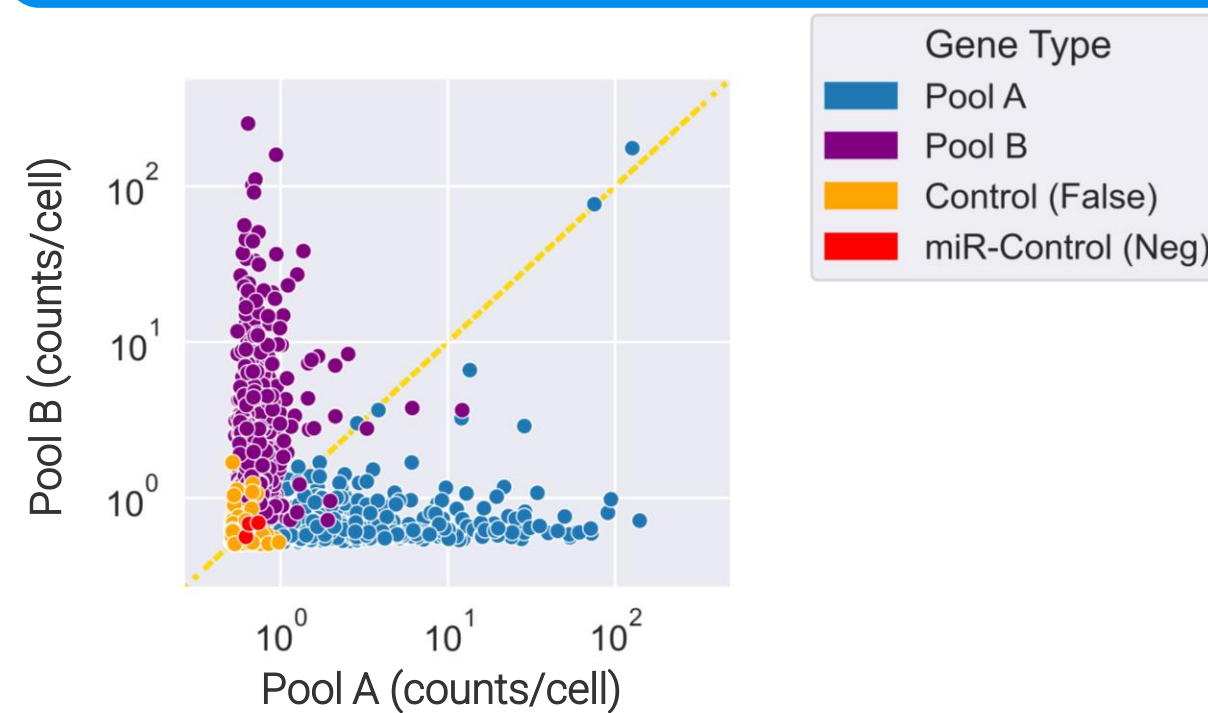


Fig. 2. Each miRNA design was evaluated using synthetic targets in FFPE tissue. The 800 miRNA synthetic targets were divided equally into Pool A and Pool B, and both were tested with the 800-plex miRNA Panel. Correlation between counts/cell for Pool A vs. counts/cell for Pool B suggest that probe designs for the vast majority of the 800 plex panel are highly specific for their targets.

Unmatched 38 nm high plex miRNA spatial subcellular resolution

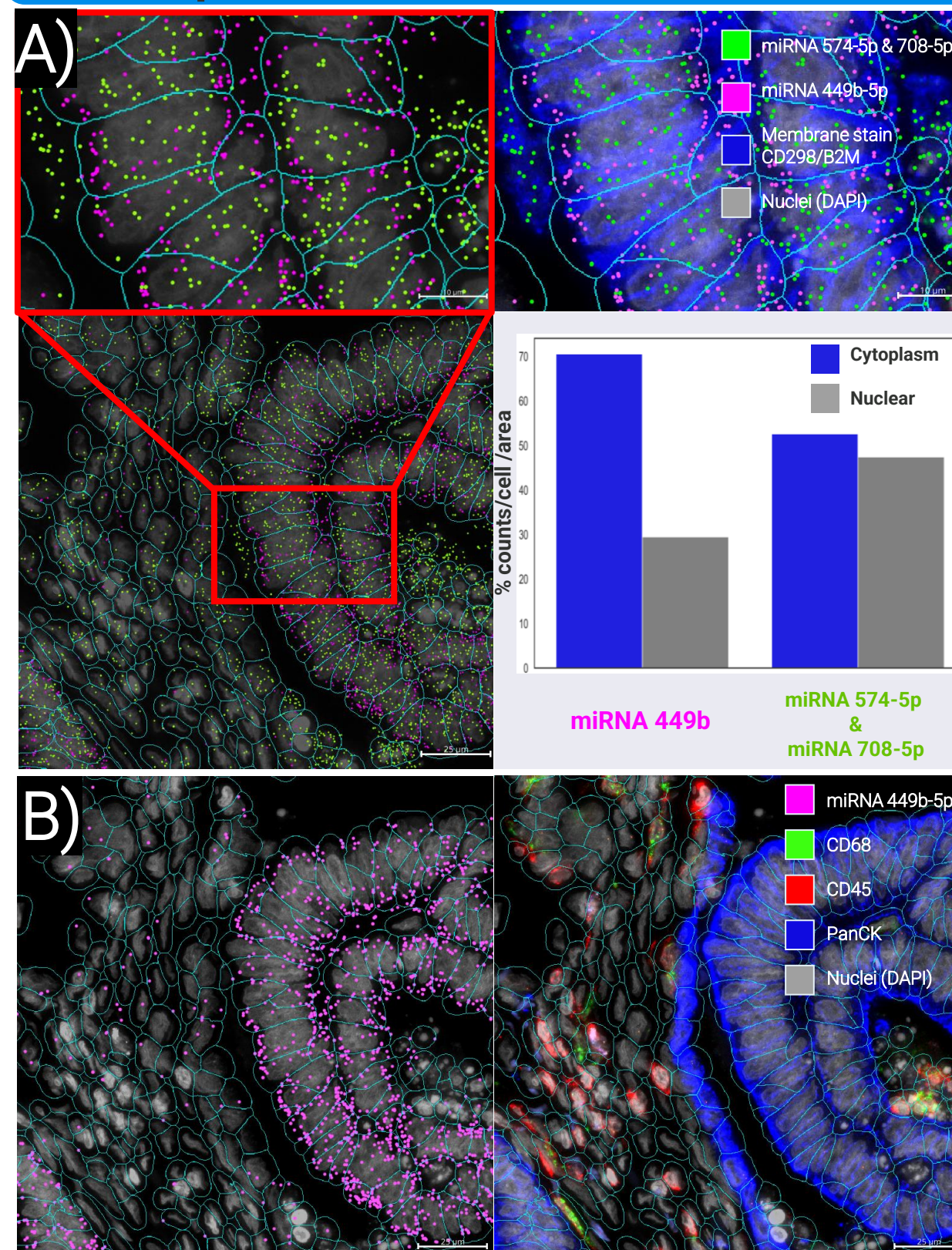


Fig. 3 miRNA can be detected at 38 nm subcellular resolution in nuclear and/or cytoplasmic compartments. (A) miRNA 449b (magenta) shows cytoplasmic enrichment in contrast to other miRNAs (574-5p & 708-5p; green) that show even distribution in nucleus and cytoplasm. Bar plot shows percentage of counts per cell per compartment area. (B) miRNA 449b - 5p specifically shows up in epithelial cells (PanCK+) as expected per its known tumor suppressor role in colon cancer (1)

Distinct miRNAs exhibit cell type specific expression.

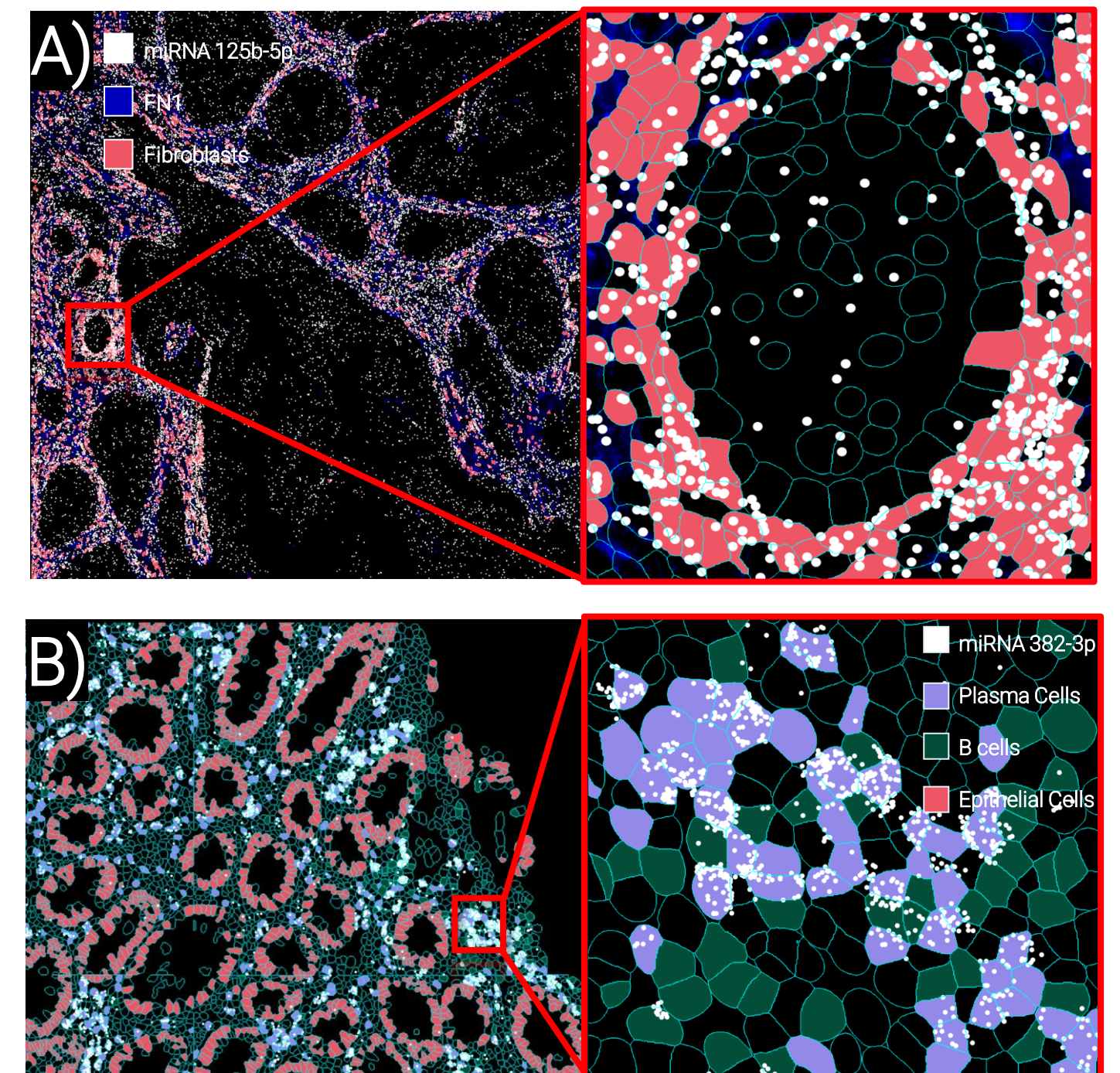


Fig 5. (A) miRNA 125b-5p specifically shows up in fibroblasts identified via protein data cell typing. This is consistent with regulatory relationships identified between Fibronectin and miRNA 125b-5p (2), as well as with evidence that miRNA 125b-5p is necessary for the transition of fibroblasts to myofibroblasts (3). (B) miRNA 382-3p is highly localized to plasma cells identified via RNA based cell typing (1K plex), but not in B type immune cells. Although not much is known about this enrichment in immune cells, it supports the observation that miRNAs are often localized to specific cell types and is an exciting area for further investigation.

Conclusions

- CosMx SMI showcases spatial miRNA detection at the highest plex ever achieved at subcellular resolution in FFPE tissue
- The CosMx 800-plex miRNA panel:
 - Leverages content and designs from the best-in-class nCounter miRNA panel
 - Is highly specific for synthetic targets in FFPE tissue
 - Shows cell type specific expression for specific miRNAs as expected

References

- (1) Fang Y et al. miR-449b inhibits the proliferation of SW1116 colon cancer stem cells through downregulation of CCND1 and E2F3 expression. PMID: 23674142.
- (2) Martinucci B et al. Fibronectin modulates the expression of miRNAs in prostate cancer cell lines. PMID: 35898539.
- (3) Nagpal V et al. miR-125b Is Critical for Fibroblast-to-Myofibroblast Transition and Cardiac Fibrosis. Circulation. PMID: 26585673.

