MOLECULAR PIXELATION: 3D SPATIAL PROTEOMICS OF SINGLE-CELLS BY SEQUENCING

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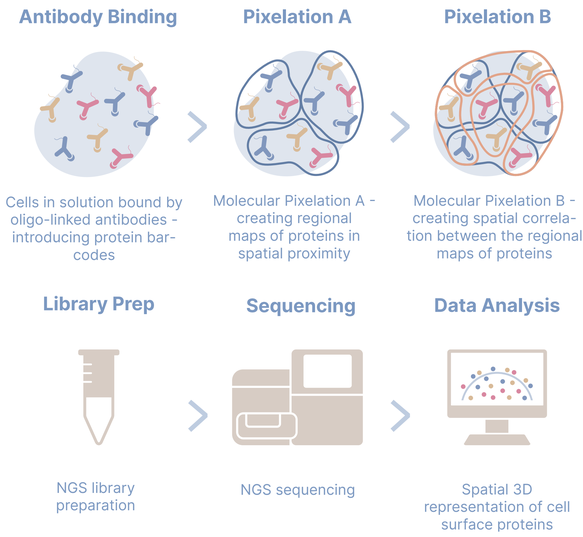
GO BEYOND WHAT YOU CAN DO TODAY - UNLOCK THE SPATIAL SURFACE PROTEOME

The cell surface proteome is spatially dynamic and changes with the state of the cell, which in turn determines its activity in health and disease. Understanding differential gene regulation, post-transcriptional changes and variations in protein translation alone is insufficient to fully comprehend what causes the onset of disease, progression and response to treatment.

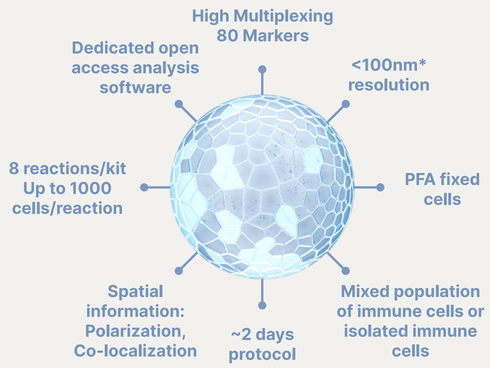
MOLECULAR PIXELATION™ (MPX™) ENABLES YOU TO VISUALIZE CHANGES IN THE SPATIAL ARCHITECTURE OF MEMBRANE PROTEINS ON SINGLE CELLS

- Opening up for detailed analysis of vital processes of the immune system, such as cell-cell communication and mobility
- Detecting marker abundance with polarization and co-localization patterns in 3D space

Molecular Pixelation (MPX) Workflow



FEATURES



*nanometer size as measured by scanning electron microscopy.

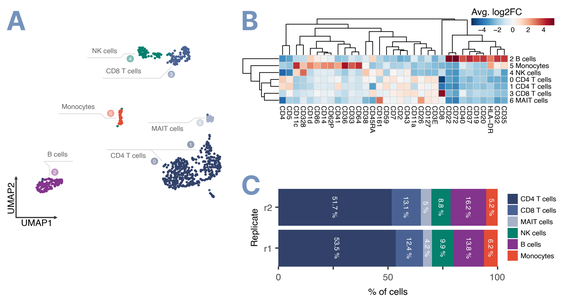
APPLICATIONS

Molecular Pixelation is a novel addition to the single cell analysis community to reveal new insights into cellular life.

Our pioneering Molecular Pixelation technology and its dedicated open source software enables simultaneous detection of 80 human immune cell surface protein markers. This allows for visualization of receptor abundance, polarization and co-localization of thousands of cells in 3D, at nanoscale resolution.

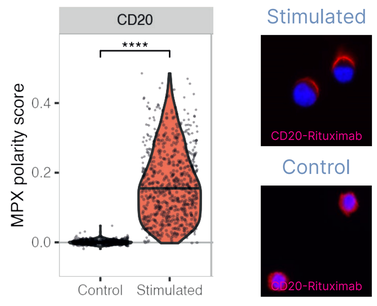
HALLMARKS OF MOLECULAR PIXELATION

I Analysis of abundance levels



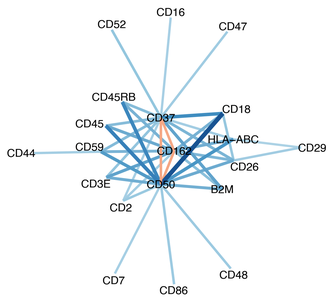
MPX can analyze protein abundance using MPX single cell protein count data, similarly to other single cell technologies, exemplified above by an MPX experiment with healthy PBMCs; **A)** UMAP of PBMC following Molecular Pixelation. **B)** Heatmap of relative expression of differentially abundant proteins. **C)** Frequencies of annotated cell types per replicate.

II Analysis of spatial clustering



MPX Polarity Scores measures the level of spatial clustering of each protein one each cell. Above is shown a violin plot of CD20 Polarity Scores from Raji cells upon Rituximab stimulation. The increased MPX polarity scores compared to control cells suggest clustering of CD20 upon stimulation, which was confirmed by fluorescene microscopy (right).

III Analysis of spatial co-lolcalization

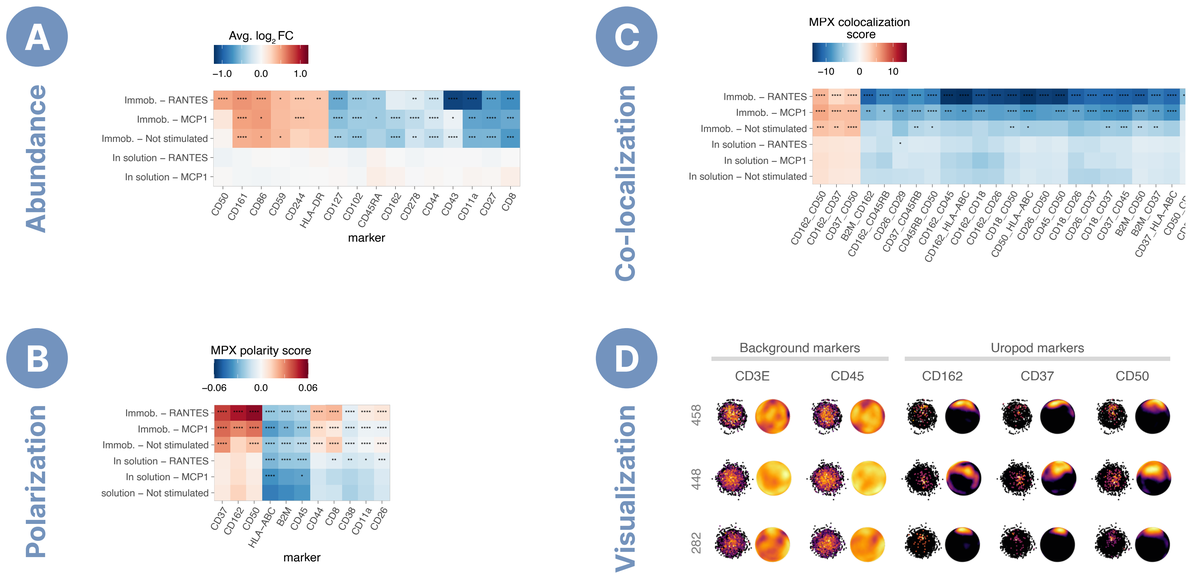


MPX co-localization scores measures the level of co-localization between protein pairs. Figure III shows a graph representation of differentially co-localized proteins between CD54 immobilized T-cells treated with RANTES in comparison to control. Protein pair links are colored by the average difference in co-localization.

IV 3D Visualization of spatial localization on single cells

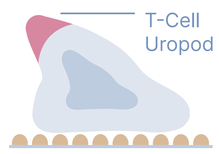
Left: T-cell with high CD162/CD37 co-localization from RANTES stimulated cells. **Right:** T-cell with low CD162/CD37 co-localization from untreated population. Each node is colored by a score summarizing the abundance of three Uropod markers: CD162, CD37 and CD50.

CHEMOKINE-STIMULATED T-CELLS ANALYZED WITH MPX SHOW KNOWN AND NOVEL PATTERNS OF PROTEIN SPATIAL ORGANIZATION

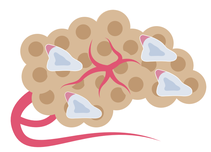


- **Uropods** are critical for cytotoxic T-cells to infiltrate tumors and their formation is associated with immune checkpoint inhibition efficacy and overall cancer survival.
- **MPX** was performed on cells either in suspension or immobilized to a plate coated with CD54 (ICAM1) or in combination with chemokine stimulation by either CCL2 (MCP1) or CCL5 (RANTES) to promote Uropod formation.
- **The effects of the stimulations** were analyzed from three distinct perspectives: protein abundance, polarization, and co-localization:
  - **16 proteins** with significantly different protein abundance in any of the conditions (A).
  - **11 markers** exhibiting significant differentiation in protein clustering (B)
  - **40 marker** pairs (20 shown) displayed significant differences from the differential co-localization analysis (C).
- **CD50, and CD162,** which are known to be located within the T-cell uropod exhibited differential spatial arrangement in CD54 immobilized and stimulated samples, with increased polarization and increased co-localization.
- **CD37** has previously been shown to participate in uropod formation in B-cells, neutrophils, and dendritic cells, and is here observed to increase in polarization, and co-localization together with CD50 and CD162 upon CD54 immobilization and chemokine stimulation in T-cells.

These findings exemplifies how MPX can be employed to identify patterns of protein spatial organization and their potential roles in cellular processes as well as MPX potential to be used to study and define cell states to study and define cell states from the spatial arrangement of proteins.



Uropods are a signature of migratory T-cells that are essential for them to infiltrate tumors.



MPX was used to detect uropod formation, providing important insights into T-cell motility, which could help the development of new immune therapies.