PIXELGEN TECHNOLOGIES

PRODUCT SHEET

Pixelgen Single Cell Spatial Proteomics Kit
Immunology Panel I, Human

THE DYNAMIC CELL - SPATIAL SURFACE PROTEOME UNLOCKED

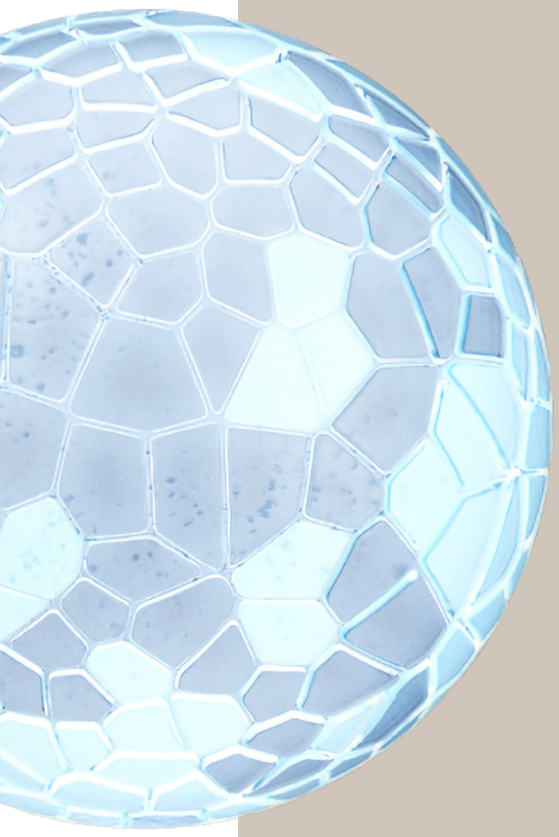
The cell surface proteome is spatially dynamic and changes with the state of the cell, which in turn determines its activity in health and disease.

Protein spatial architecture enables cell-cell communication, mobility, structure, and immunological activities.

Molecular Pixelation enables the study of these fundamental aspects of cell biology at an unprecedented scale enabling data driven research into immunology, drug development and future diagnostics.

TO ADDRESS THIS NEED

Pixelgen Technologies has developed the Molecular Pixelation (MPX) workflow for single cell analysis of immune cells which generates location data on spatial cell surface proteins.



3D Spatial Single Cell Proteomics

Enables deep phenotyping of immune cells to provide a more profound understanding of cell biology, disease causing mechanisms and drug mode-of-action.

Unprecedented Spatial Protein Multiplexing

High multiplex analysis of cell surface proteins with validated target specificity.

In Focus without a Microscope

Single tube, partitioning-free sample preparation, providing spatially resolved abundance data similar to Confocal and Flow Cytometry, always in focus from every angle.

Leverage your existing NGS Workflows

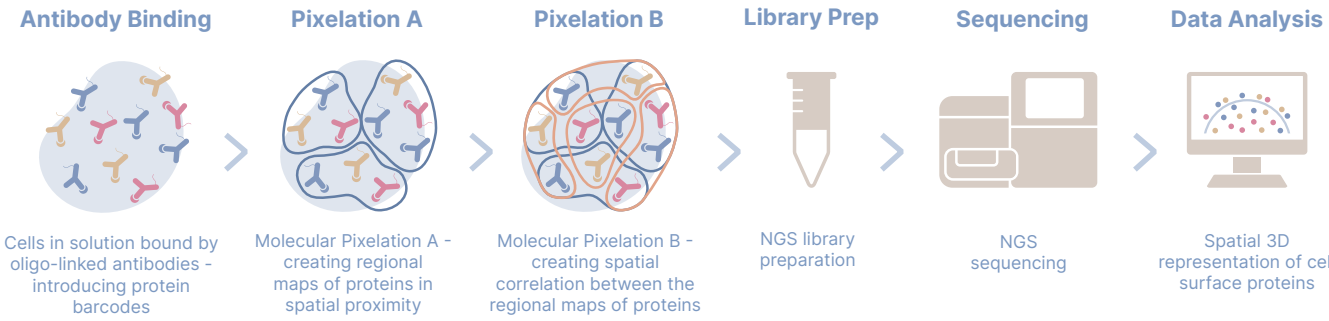
Obtain powerful single cell protein abundance, polarization and colocalization data with a simple, scalable solution available to any size cell and molecular biology laboratory.

GO BEYOND WHAT YOU CAN DO TODAY

Understanding of up and down regulation of genes, post-transcriptional changes as well as variations in protein translation is insufficient to fully comprehend what causes the onset of disease, progression and response to treatment.

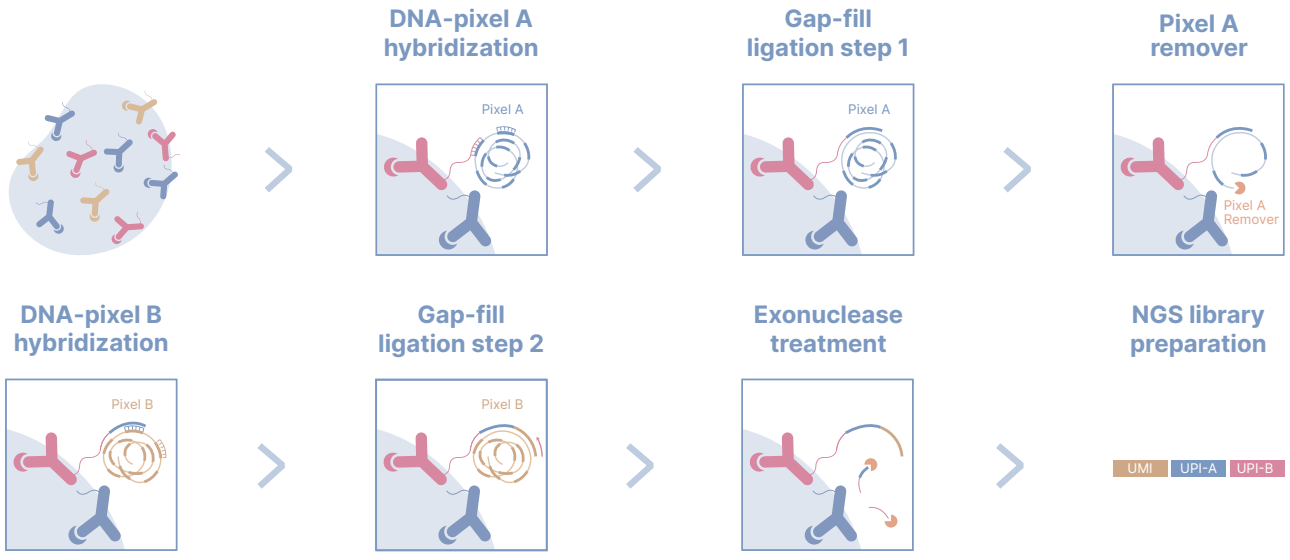
MOLECULAR PIXELATION ENABLES YOU TO VISUALIZE THE SPATIAL ARCHITECTURE OF MEMBRANE PROTEINS ON A SINGLE CELL

- Opening up for detailed analysis of the vital processes of the immune system, such as cell-cell communication and mobility
- Delivering 76 immune-cell-specific proteins with spatial resolution in a subcellular multiplex assay panel



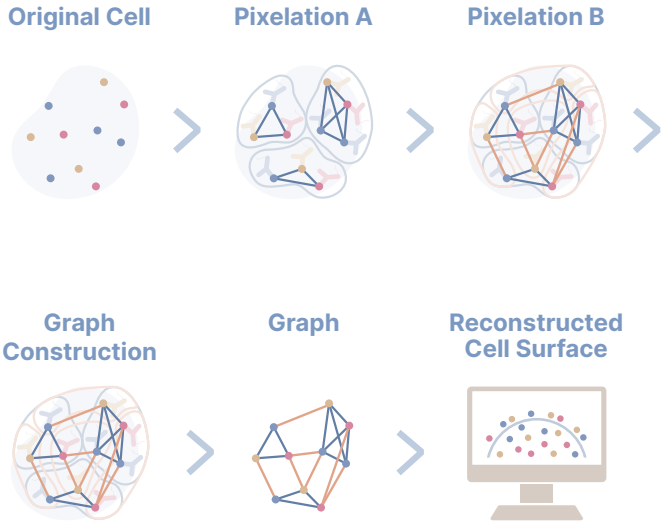
WORKFLOW

- The protocol is initiated with Antibody-Oligo Conjugates (AOCs) binding to proteins on the surface of PFA-fixed cells.
- Pixelation A involves the addition of DNA-pixels A, where each A pixel binds to many AOCs in proximity, generating small connected protein neighborhoods.
- Pixelation B uses DNA-pixels B to link the local neighborhoods, constructing one global protein map of the cell surface.
- A standard PCR-based, Illumina compatible library is generated.
- After a Quality Control (QC) step, Next Generation Sequencing (NGS) is carried out.



NGS DATA ANALYSIS

- The NGS FASTQ files are imported to Pixelgen's software Pixelator, undergoing several steps of QC and analysis.
- The read sequences from the Unique Molecular Identifier (UMI), the antibody barcode on the AOC, and the Unique Pixel Identifier A (UPI-A) and Unique Pixel Identifier B (UPI-B) of the amplicon, are build to produce a graph, ultimately generating a network of protein connections.
- Each graph is a reconstruction of the surface of a cell. Pixelator returns two main outputs: a web report with summaries and plots, and a .pxl file for downstream data analysis.

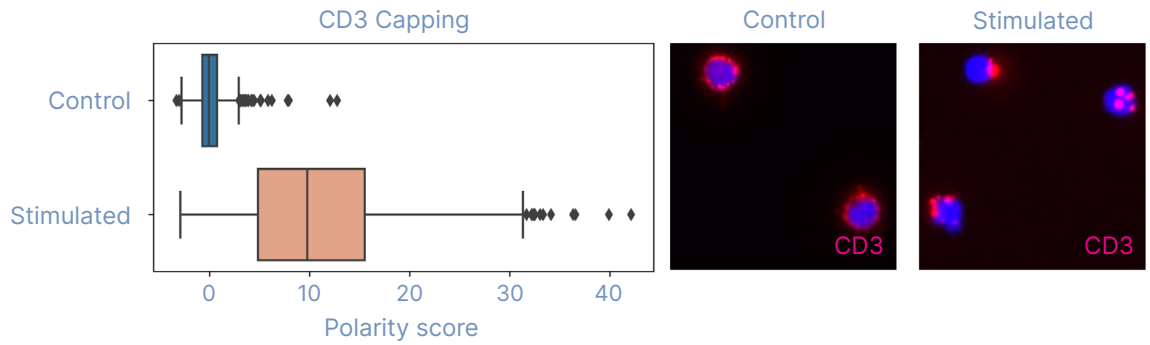


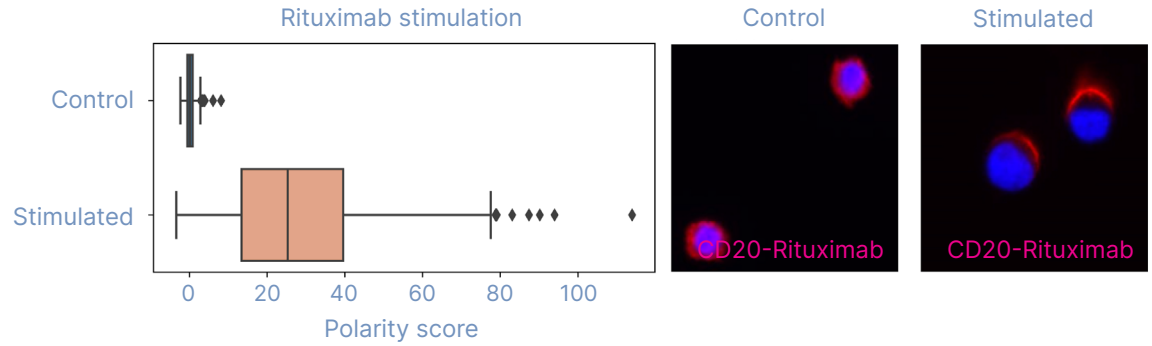
INTRODUCING: THE POLARITY SCORE

Polarity Score quantifies each target proteins non-randomness of spatial distribution for each single cell.

CD3 capping spatially clusters CD3 on T cells and the therapeutic antibody Rituximab clusters CD20 on B cell lymphoma cells.

Orthogonal fluorescent microscopy shows the spatial distribution of the target proteins upon stimulation.

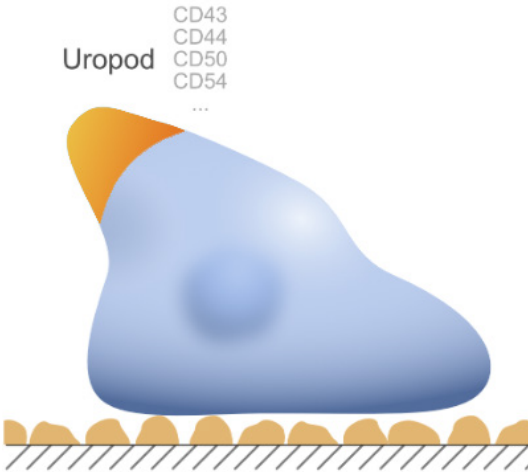


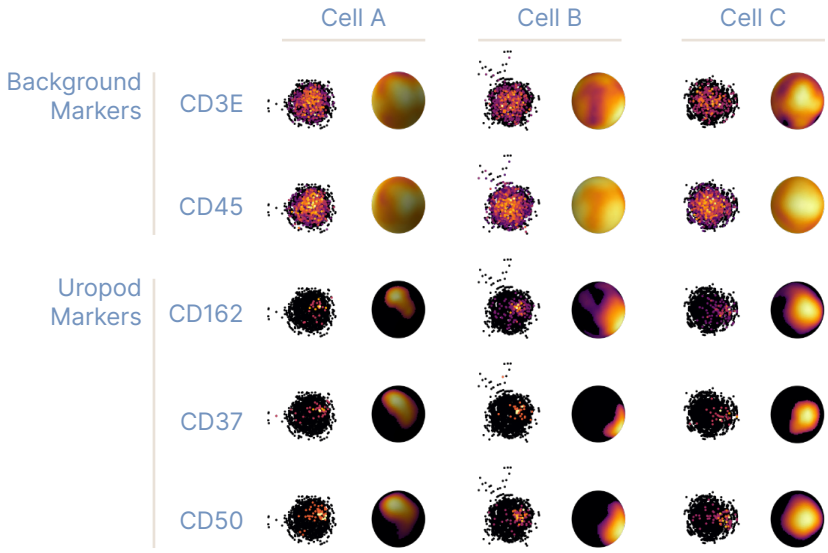


COLOCALIZATION OF PROTEIN PAIRS IN UROPODS OF CHEMOTACTIC T CELLS

Uropod formation is an essential mechanism for cytotoxic T cells to infiltrate tumors, which correlates to immune checkpoint inhibition efficacy and overall cancer survival.

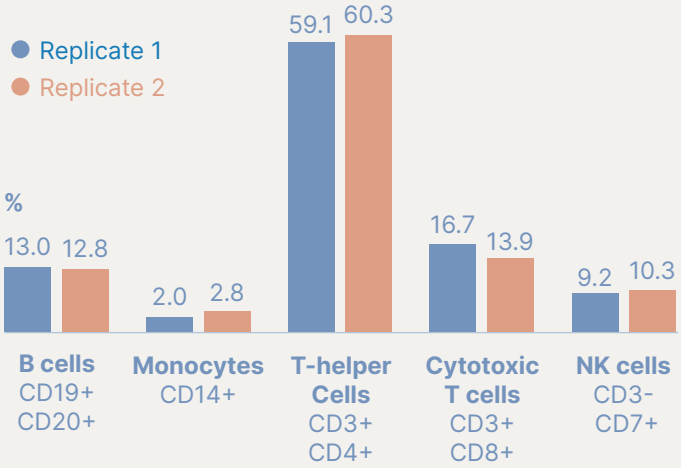
The applicability of MPX is illustrated in the detection of uropod formation in migrating cells. The high multiplexing ability of the method and the graph data generated enable objective discovery of new colocalization patterns, or the opposite, segregation of proteins.





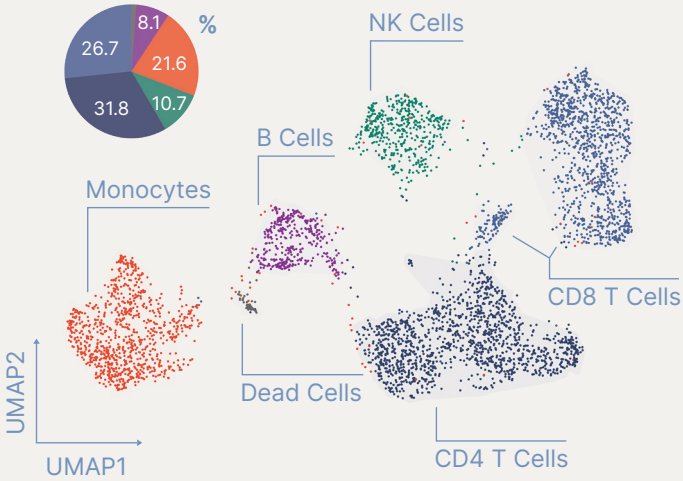
SHOWCASING REPRODUCIBILITY

Data from a PFA fixed PBMC sample processed in two separate MPX runs, using the same reagent kit lot. Replicate 1 and 2 demonstrates workflow reproducibility across samples.



DEMONSTRATED ANNOTATION OF 4000 PBMC CELLS

Using Molecular Pixelation we find the major Peripheral Blood Mononuclear Cells (PBMCs) cell types and annotate them from well-known markers. This UMAP shows expected fractions of immune cells annotated at single cell resolution.



UNRAVELING IMMUNE CELL SURFACE PROTEIN CONSTELLATIONS LEADS TO NEW BIOLOGICAL DISCOVERIES

The **Pixelgen Single Cell Spatial Proteomics Kit, Immunology Panel I, Human** unlocks the ability to map immune cell surface proteins with an unprecedented multiplex, can offer a better understanding of the different stages of human health and disease as well as the mode-of-action of drugs.

PRODUCT FEATURES

Research area:
Immunology

Protein panel:
80 proteins in multiplex (76 protein assays + 4 controls)

DNA-Pixel size:
~200 - 300 nm

Data type:
Digital spatial protein correlation from NGS output

Sample compatibility:
Mixed population of immune cells or isolated immune cells

Sample type:
PFA fixed (cell surface not permeabilized)

Cell input:
500 000 cells for fixation, up to 1000 cells to library preparation

Data output:
Up to 1000 cells per reaction generating one cell surface map per cell

Protocol time:
2 days

PRODUCT	PRODUCT NUMBER
Pixelgen Single Cell Spatial Proteomics Kit, Immunology Panel I, Human Reagents Kit, 8 reactions	PXGIMM001

MOLECULAR PIXELATION REVEALS NEW INSIGHTS INTO CELLULAR LIFE

For research use only

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