

## THE DYNAMIC CELL - SPATIAL SURFACE PROTEOME UNLOCKED

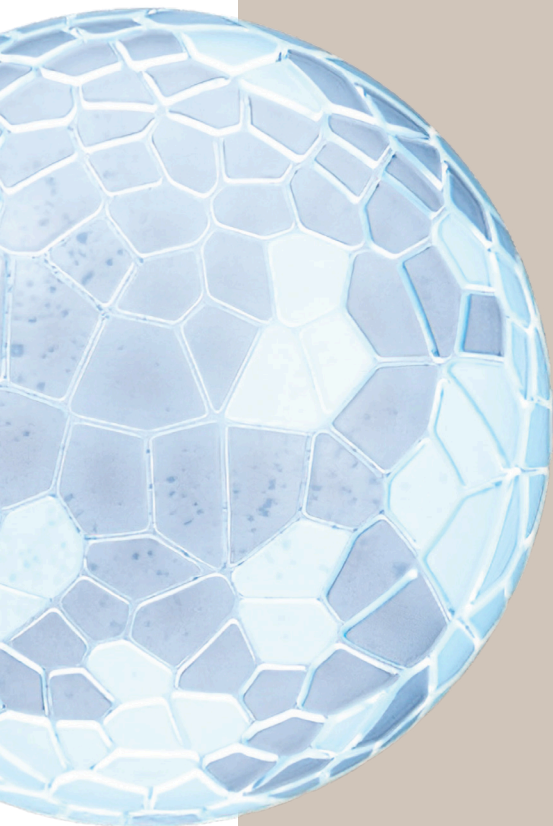
The cell surface proteome is spatially dynamic and changes with the state of the cell, which in turn determines its activity in health and disease.

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 Proteomics of Single Cells

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

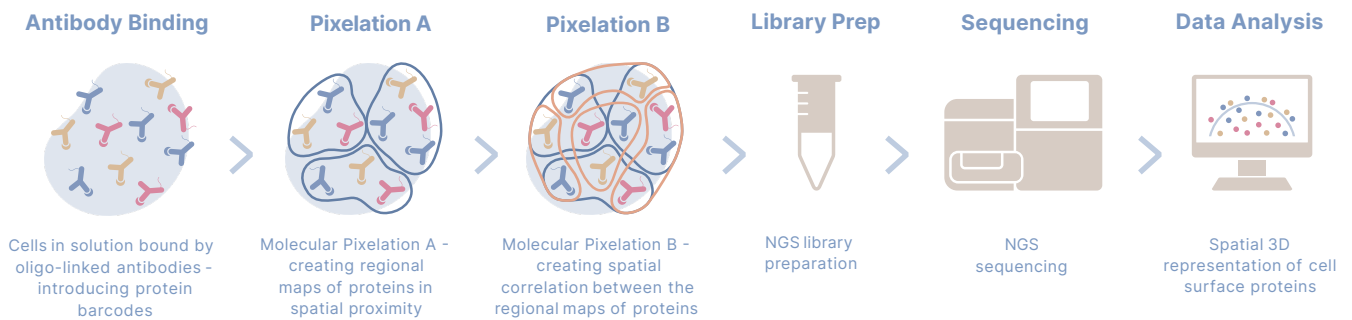
Obtain powerful single cell protein abundance, polarization and colocalization data with a simple, scalable solution available to any size cell and molecular biology laboratory.

# 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 progression and response to treatment.

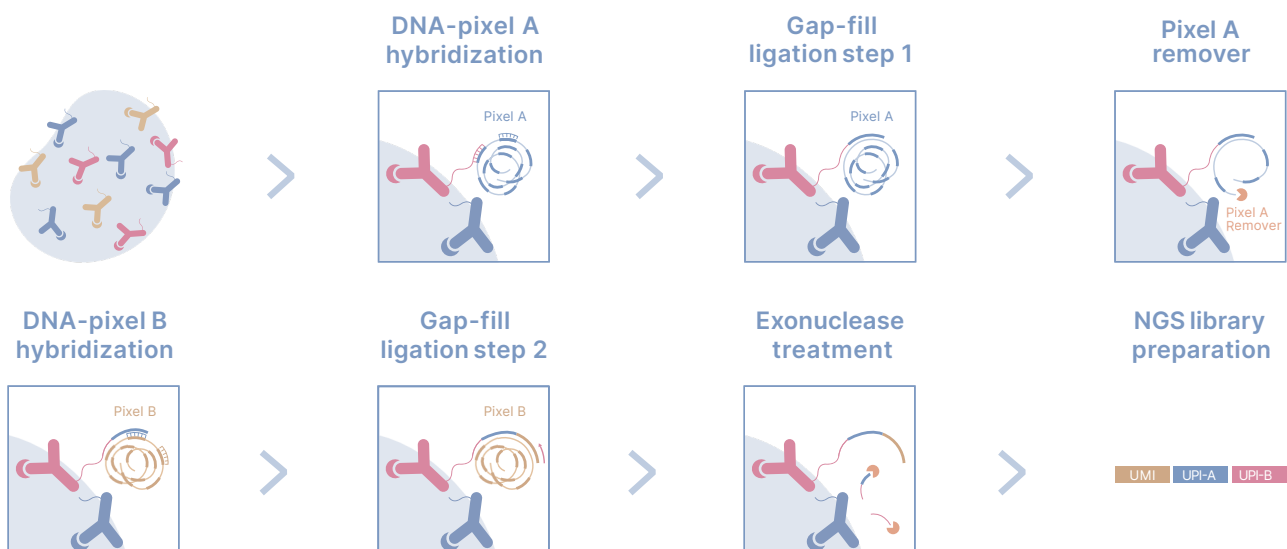
## MOLECULAR PIXELATION ENABLES YOU TO VISUALIZE THE SPATIAL ARCHITECTURE OF MEMBRANE PROTEINS ON A SINGLE CELL

- Opening up for detailed analysis of the vital processes of the immune system, such as cell-cell communication and mobility
- Delivering 80 immune cell specific protein assays using DNA-based nanometer sized molecular pixels in a subcellular multiplex assay panel



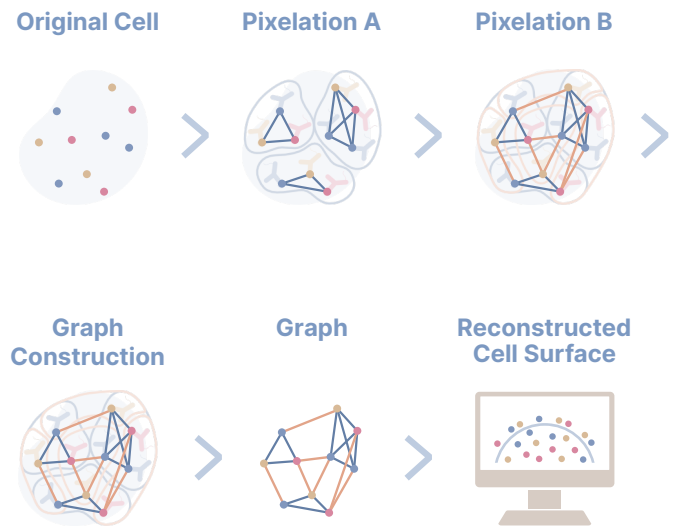
## WORKFLOW

- 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

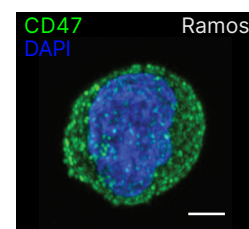
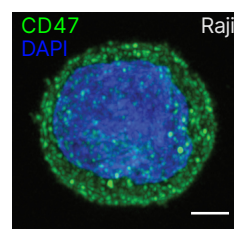
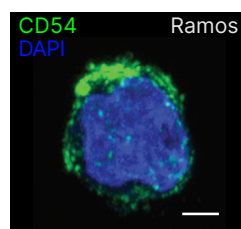
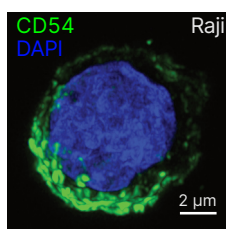
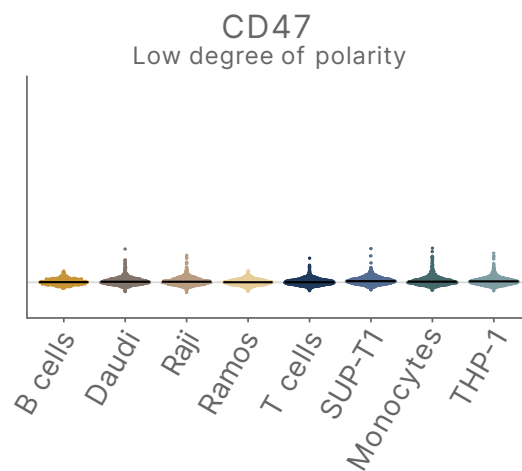
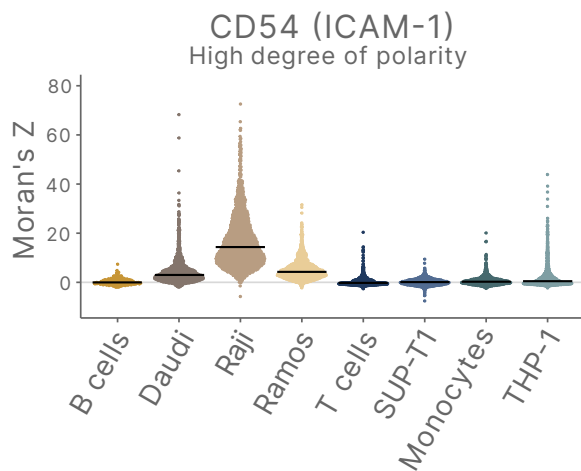
- The NGS FASTQ files are imported to Pixelgen's software Pixelator, undergoing several steps of QC and analysis.
- The read sequences from the Unique Molecular Identifier (UMI), the antibody barcode on the AOC, and the Unique Pixel Identifier A (UPI-A) and Unique Pixel Identifier B (UPI-B) of the amplicon, 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.



# USING THE POLARITY SCORE TO UNDERSTAND CANCER

The polarity score, which measures a protein's degree of non-random distribution, was used to discover polarization of the adhesion molecule CD54 (ICAM-1) in Burkitt's lymphoma cell lines.

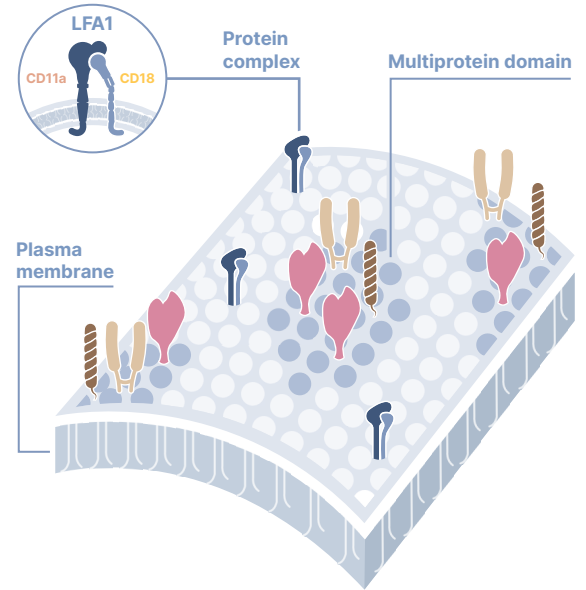
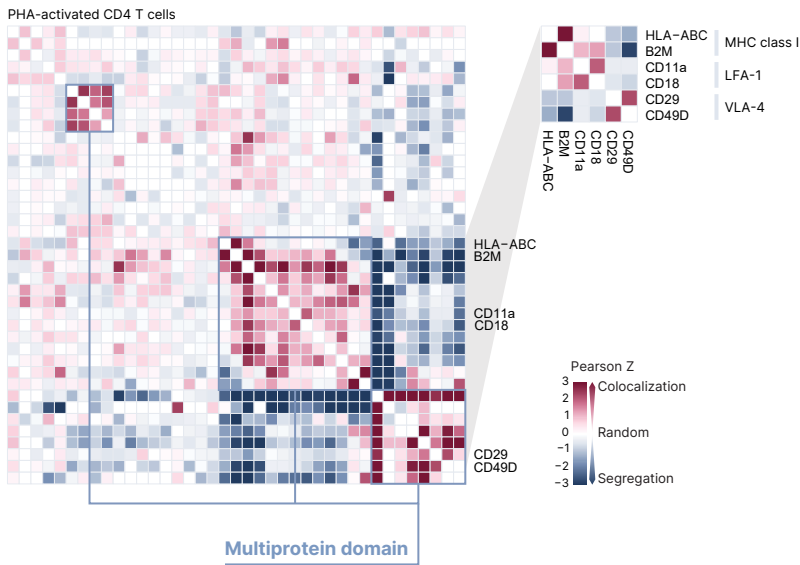
Orthogonal fluorescence microscopy comparison shows the spatial distribution of the polarized CD54 and the non-polarized CD47 in two of the cell lines.



# MOLECULAR PIXELATION DISCOVERS PROTEIN COMPLEXES AND MULTIPROTEIN DOMAINS

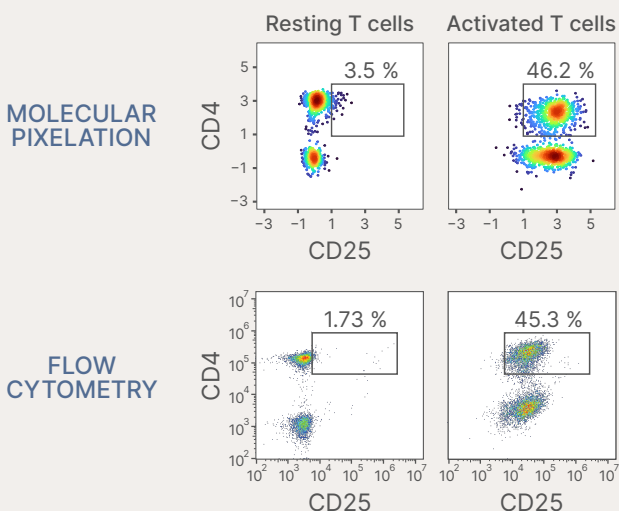
The MPX colocalization score evaluates protein colocalization at different scales, from simple protein-protein complexes, such as LFA-1, to intricate multiprotein domains.

Apart from colocalization, MPX additionally reports on protein segregation, providing an even deeper understanding of the membrane architecture.



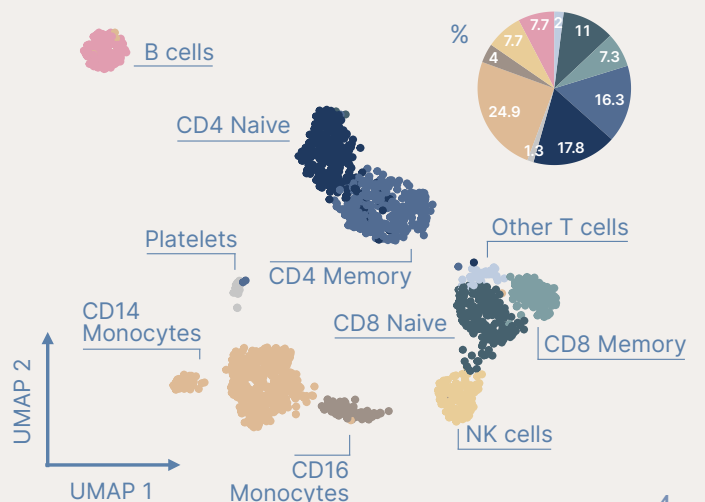
## SHOWCASING THE CORRELATION BETWEEN MOLECULAR PIXELATION AND FLOW CYTOMETRY

The IL2 receptor subunit  $\alpha$ , CD25, is upregulated in T cells during activation. Molecular Pixelation and Flow Cytometry display high correlation for the detection of CD25-positive CD4 T cells.



## DEMONSTRATED ANNOTATION OF 2000 CELLS

Using Molecular Pixelation, major PBMC subsets can be efficiently detected and annotated using well-known markers. This UMAP shows the 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 2, Human v2** 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:**  
84 proteins in multiplex  
(80 protein assays  
+ 4 controls)

**DNA-Pixel size:**  
<100 nm\*

**Data type:**  
Digital spatial protein  
counts from NGS  
output

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

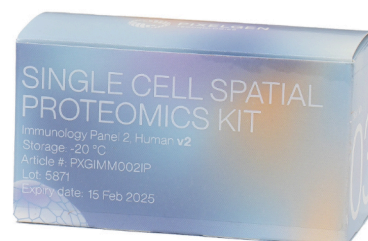
**Sample type:**  
PFA fixed (cell surface  
not permeabilized)

**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

\*nanometer size as measured by scanning electron microscopy.



### PRODUCT

**Pixelgen Single Cell Spatial Proteomics Kit,  
Immunology Panel 2, Human v2**

Reagents Kit, 8 reactions

### PRODUCT NUMBER

PXGIMM002

MOLECULAR PIXELATION OFFERS A NEW DIMENSION  
IN THE EXPLORATION OF CELLULAR ACTIVITY



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