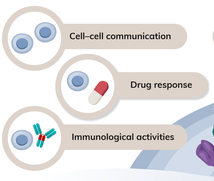
BioTechniques In Focus

PIXELGEN TECHNOLOGIES

Studying the cell-surface proteome of single cells

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.

The location and spatial arrangement of membrane proteins in relation to one another influences cell function:



The challenge

Current technologies for analyzing the spatial organization of cell-surface proteins have limitations:

Imaging-based techniques e.g fluorescence microscopy and imaging flow cytometry – typically only provide data in one focal plane and have limited multiplexing capacity and throughput.

Super-resolution microscopy – limited in multiplexing and throughput. Considered expensive.

Single-cell targeted proteomics using DNA-tagged antibodies e.g CITE-seq – requires single-cell compartmentalization and only provides relative expression levels without any subcellular spatial information.

The solution: Molecular Pixelation

Molecular Pixelation (MPX™) is a new technology that overcomes these limitations by capturing 3D spatial maps of surface proteins on thousands of single immune cells simultaneously in a highly multiplexed manner.

How it works:

1. DNA-tagged antibodies are bound to **proteins** on the surface of thousands of PFA-fixed immune cells.

2. DNA pixels, which are nanometer-sized single-stranded DNA molecules with a unique pixel identifier (UPI), hybridize to several DNA-tagged antibodies simultaneously, creating **protein neighborhoods** where the antibodies share the same UPI sequence.

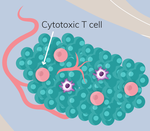
3. A second set of DNA pixels is incorporated, linking the protein neighborhoods to create a **protein map**.

4. The generated amplicons are analyzed using next-generation sequencing technologies.

5. A dedicated software package called Pixelator™ uses sequencing reads to create **graphs** of protein maps. Each graph is a reconstruction of the surface of a cell, quantifying proteins and describing their spatial distribution and protein colocalization with unprecedented resolution.

Detecting T-cell uropod formation with MPX

Uropods are the hind part of polarized cells that stabilize and move the cell during migration.



They are essential for cytotoxic T cells to **infiltrate tumors**, and their formation correlates to immune checkpoint inhibition efficacy and patient survival.

MPX was used to detect uropod formation in migrating immune cells, providing important insights into T-cell motility, which could help the development of **immune therapies**[1].

1. Karlsson F, Kallas T, Thiagarajan D et al. Molecular Pixelation: Single cell spatial proteomics by sequencing. *bioRxiv* doi:10.1101/2023.06.05.543770 (2023).

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