PIXELGEN TECHNOLOGIES

Increased sensitivity with MPX v2

Technical Note

TN00001

ABSTRACT

Molecular Pixelation (MPX) employs DNA-barcoded antibodies and molecular pixels to reconstruct the membrane protein landscape of single immune cells using bulk sequencing. The method simultaneously provides information about protein abundance, polarity and colocalization in individual cells, for 80 membrane proteins. This breadth of information gives MPX a unique angle to, for instance, identify new cellular states and sub-populations, or to characterize mechanistic pathways behind immune cell signaling.

Here we present the latest upgrade of Molecular Pixelation, version 2 (MPX v2). Building upon optimized chemistry, MPX v2 provides an increased protein detection sensitivity, leading to higher signal-to-noise ratios for protein abundance measurements, reduced background levels, improved cell gating capabilities as well as an enhanced potential to detect protein colocalization events. MPX v2 can be efficiently applied to study a wide variety of immune cell types, enabling deep, multiplexed phenotyping with excellent sensitivity.

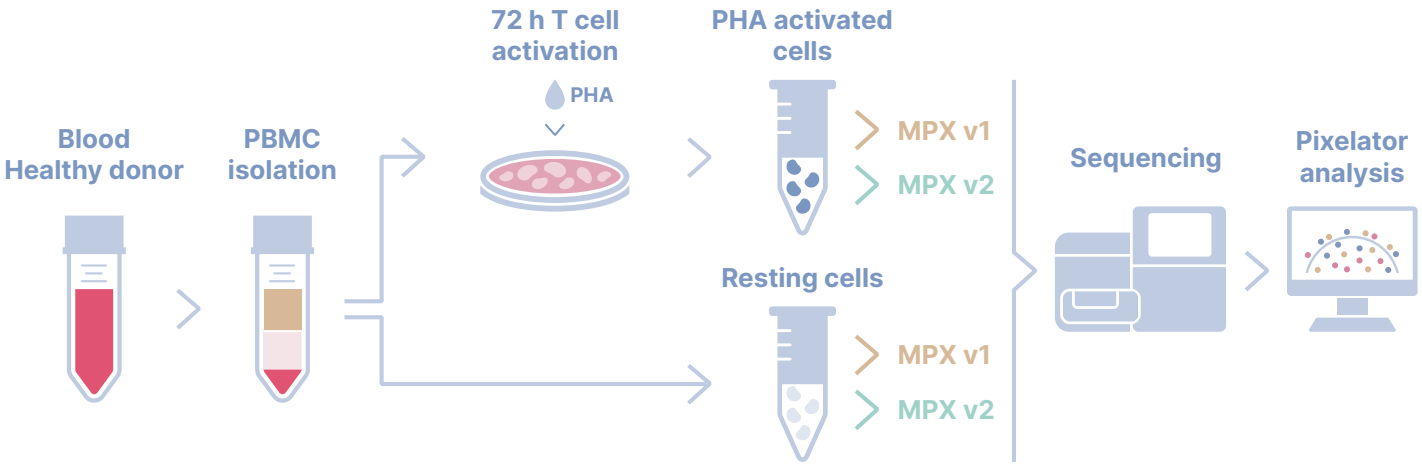

Figure 1. Experimental workflow for the comparison of MPX v1 and v2. For the evaluation of the performance of MPX v2, PBMCs isolated from a buffy coat of a healthy donor were simultaneously used for Molecular Pixelation using v1 and v2. In order to detect as many proteins as possible in the panels, both resting and 72h PHA activated PBMCs were included in the analysis. Samples were sequenced on a NextSeq2000 and analysis was performed using Pixelator 0.18 with the nf-core/pixelator pipeline 1.3.0.

BACKGROUND

For the evaluation of immune cell function and phenotype, analysis of single cell protein expression and localization plays a fundamental role. Many protein-based analysis methods make use of antibodies to identify proteins of interest. The antibodies are in turn detected using DNA oligos and sequencing (e.g. Molecular Pixelation [1] and CITE-seq [2]), heavy metals and mass spectrometry (e.g. mass cytometry [3]) or fluorescence (e.g. flow cytometry and microscopy). The potential of these methods to identify biologically relevant differences in protein abundance and/or localization is highly dependent on the detection sensitivity of the method, the possibility for signal amplification as well as the specificity and sensitivity of the antibodies themselves. Improving the separation between true signals and noise levels is thus key to increasing the potential for novel biological discoveries using these methods.

INCREASED SENSITIVITY

In this tech note, we illustrate how the upgraded chemistry deployed in the MPX v2 panels provides enhanced detection specificity and sensitivity, offering a unique platform to explore the spatial organization of surface proteins on immune cells. We compared the performance of MPX v1 and MPX v2 following the experimental setup described in Figure 1.

In MPX, individual cells and their surface maps are reconstructed through the detection of unique molecules grouped into spatial zones using molecular pixels [1]. The optimized chemistry drastically boosted the number of unique molecules detected at the surface of single cells (Fig 2A), with a more than two-fold median increase. Additionally, the density of detected molecules per A pixel, i.e. per spatial zone, was enhanced (Fig 2B), producing a wealth of information for reconstructing spatial relationships between proteins.

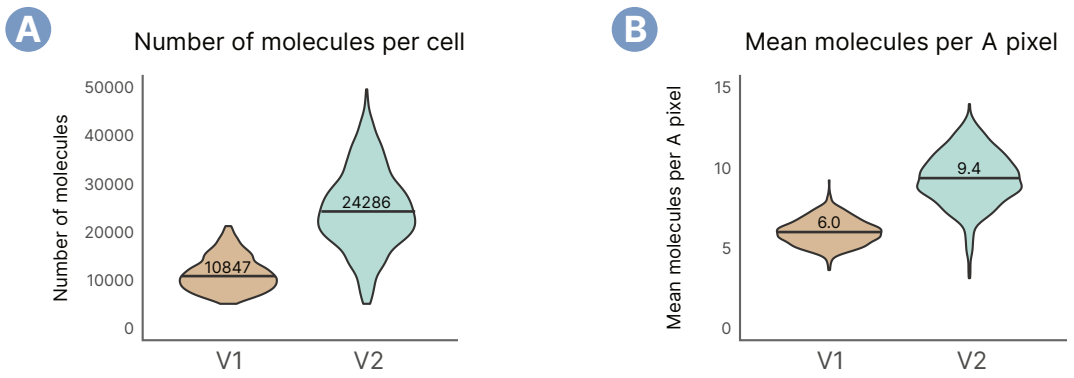

Figure 2. MPX v2 exhibits enhanced surface molecule detection sensitivity. (A) Number of unique molecules detected per cell for resting PBMCs analyzed using either the MPX v1 (left) or MPX v2 (right). (B) Average number of unique molecules detected per A pixel using either MPX panel version. The horizontal lines and values indicate the median of the metric distributions taken from ~1000 cells per condition.

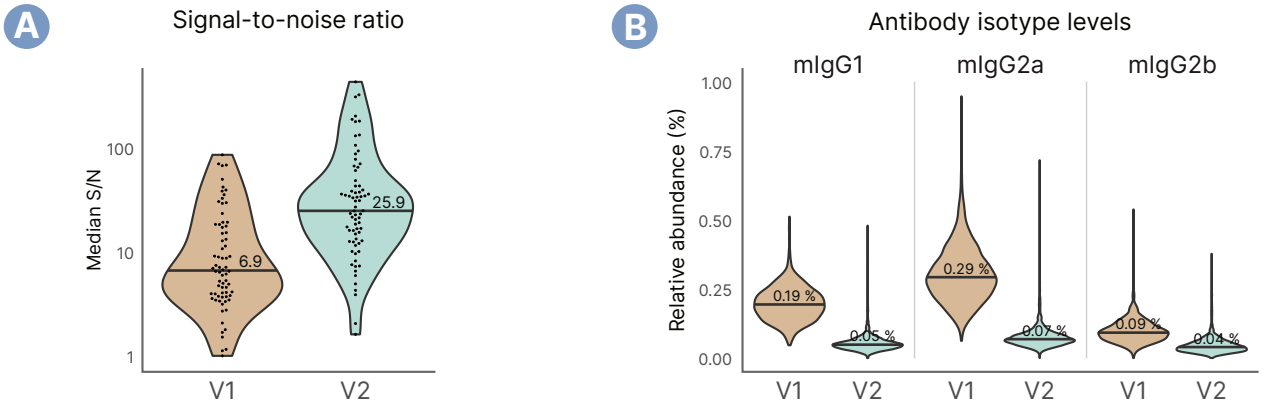

Figure 3. MPX v2 provides increased signal-to-noise levels. (A) Signal-to-noise (S/N) ratio for individual target proteins. For a given protein, positive cells were gated and the S/N was calculated as the median ratio between protein abundance and the corresponding antibody isotype level in each cell. Since the markers are differentially expressed between resting and PHA-activated PBMCs, only the maximum S/N obtained for these two cell populations is plotted here. (B) Antibody isotype levels detected by MPX v1 and v2 in single cells, relative to the total molecule count obtained for the same cell. The horizontal lines and values indicate the median for each condition.

ENHANCED SIGNAL-TO-NOISE LEVELS

We investigated whether the optimized chemistry would also provide upgraded specificity to MPX measurements. By gating the cells expressing a particular marker, we measured each markers's signal-to-noise ratio, defined as the average marker abundance divided by the detected level of the corresponding antibody isotype (Fig 3A). We found a 3.7-fold increase in signal-to-noise ratio between MPX v1 and v2. Importantly, this was not solely due to a higher number of observed molecules per marker, as we also observed reduced background binding as detected by the isotype control antibodies (Fig 3B).

Gating immune cells based on marker expression makes it possible to further study subpopulations of interest. A prerequisite for efficient gating is an effective separation between populations expressing various levels of the protein. For this reason, we confirmed that the improved signal-to-noise ratio in MPX v2 also resulted in an enhanced separation between positive and negative cell populations, as exemplified using three markers, PD-L1, CD14 and 2B4 (Fig 4A-C). Thus MPX v2 further improves the specificity of MPX in measuring the abundance of surface markers on immune cells.

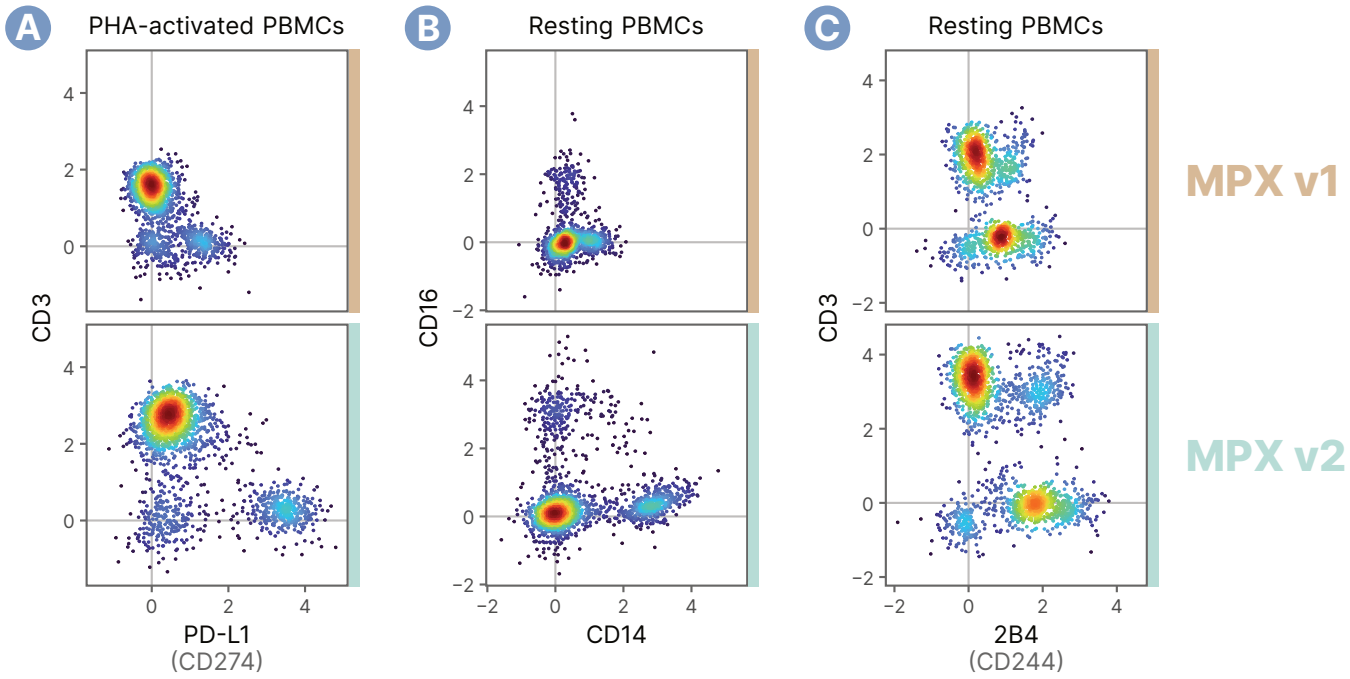

Figure 4. MPX v2 achieves better separation of positive and negative populations. Example plots of dsb-adjusted [4], single-cell protein abundance levels, as measured using either MPX v1 (top row) or v2 (bottom row). (A) Abundance levels of PD-L1 and CD3 in PHA-activated PBMCs. (B) Abundance levels of CD14 and CD16 in resting PBMCs. (C) Abundance levels of 2B4 and CD3 in resting PBMCs.

IMMUNOLOGY PANEL II, HUMAN v2

- 84-plex panel containing antibodies recognizing 80 surface proteins, 1 intracellular control protein and 3 isotype controls
- High versatility, targets major cell types in PBMCs including CD8 T cells, CD4 T cells, B cells, Monocytes and NK cells
- 3.7-fold increase of signal-to-noise levels and reduced background detection thanks to upgraded chemistry in MPX v2
- Improved sensitivity for the detection of protein complexes and multiprotein domains

Single Cell Spatial Proteomics Kit, Immunology Panel II, Human v2

Product number: PXGIMM002

IMPROVED DETECTION OF PROTEIN COLOCALIZATION

One unique feature offered by MPX is the capacity to simultaneously measure abundance and localization for all panel proteins in thousands of single cells. The generated spatial localization maps of the cells can be displayed and viewed in three dimensions, or summary measures can be readily generated, including protein polarity and protein pairwise colocalization scores. It has been previously shown that protein colocalization heavily affects the functional capacity of membrane proteins [5-7]. Hence, the potential to uncover interaction partners or multiprotein domains can enable deeper understanding of signaling cascades, or aid in the discovery of novel cellular states.

In order to evaluate if the optimized MPX v2 chemistry provides a superior sensitivity also for spatial measurements, we compared the average pairwise colocalization scores for 32 proteins expressed in PHA-activated CD4+ T cells, generated using either MPX v1 or v2 (Fig 5). Overall, the two versions presented similar colocalization patterns, including the detection of the same multiprotein domains. However, the v2 version showed a greater range of colocalization scores, both for positive scores (indicating colocalization of two proteins) and negative scores (indicating segregation of two proteins). MPX v2 thus offers a higher sensitivity in the analysis of protein organization.

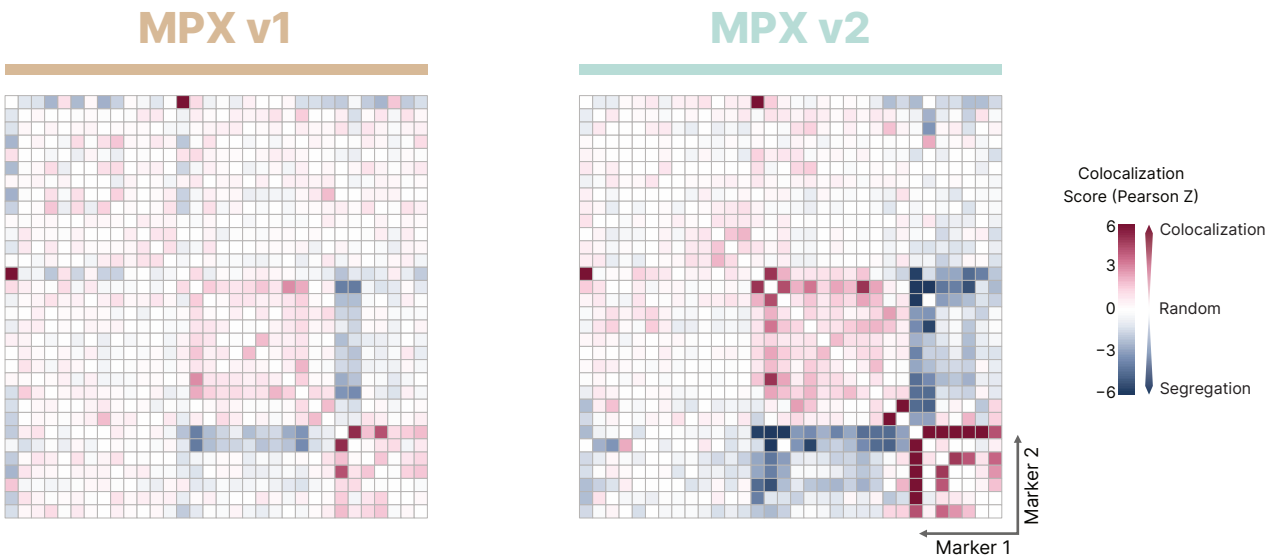

Figure 5. MPX v2 reveals new details in the spatial organization of T cell surface markers. Pairwise MPX colocalization scores for 32 markers expressed at the surface of PHA-activated CD4+ T cells, measured using either MPX v1 (left) or v2 (right). Positive scores indicate colocalization of protein pairs, whereas negative scores indicate segregation. Scores close to zero denote random relative distribution of the two proteins.

SUMMARY

Molecular Pixelation (MPX) v2, built on an optimized chemistry, provides superior signal-to-noise ratios, reduced background levels and enables a more accurate gating of cell populations. Not only does this improved performance affect the efficiency of protein abundance measurements, but it additionally yields a higher sensitivity for the determination of protein organization. Ultimately, MPX v2 can be used to shed light on multiple aspects of immune cell biology, revealing new mechanistic pathways or distinct cellular states.

REFERENCES

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