

Highly multiplexed, single-cell imaging of cell suspensions using a novel platform

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Introduction

Analyzing populations at the single cell level has become increasingly important in the study of cancer and autoimmune disorders due to high levels of population heterogeneity and rare cell phenotypes that can drive disease pathogenesis and progression. Until recently, characterizing protein markers on single cells was limited to a handful of markers due to the technical and logistical challenges of flow cytometry platforms. New advancements in single cell analysis technologies have enabled researchers to study more than 30 parameters per cell. But these platforms are expensive and require significant panel design, thereby limiting access and usability. Here we demonstrate the use of the recently launched CODEX system to generate an in-depth immune profile of human PBMC samples. This CODEX method offers a less expensive method, simpler experimental design and faster alternative to some of the established techniques.

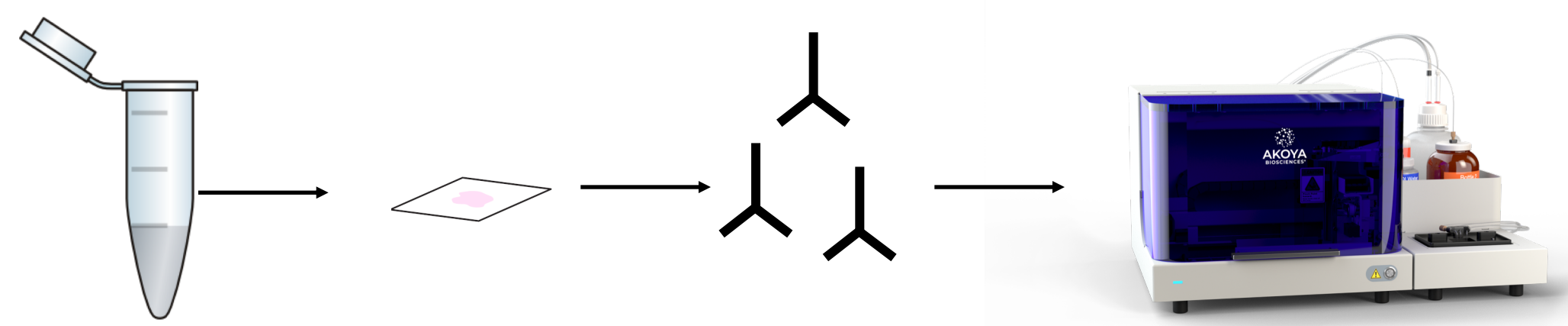


Figure 1: PBMC's were fixed and spread on a poly-lysine coated coverslip. Staining was performed using 26 Akoya inventoried markers for fresh frozen tissue. After staining a multicycle imaging CODEX run was performed.

Highly multiplexed Imaging Platform

CODEX Technology

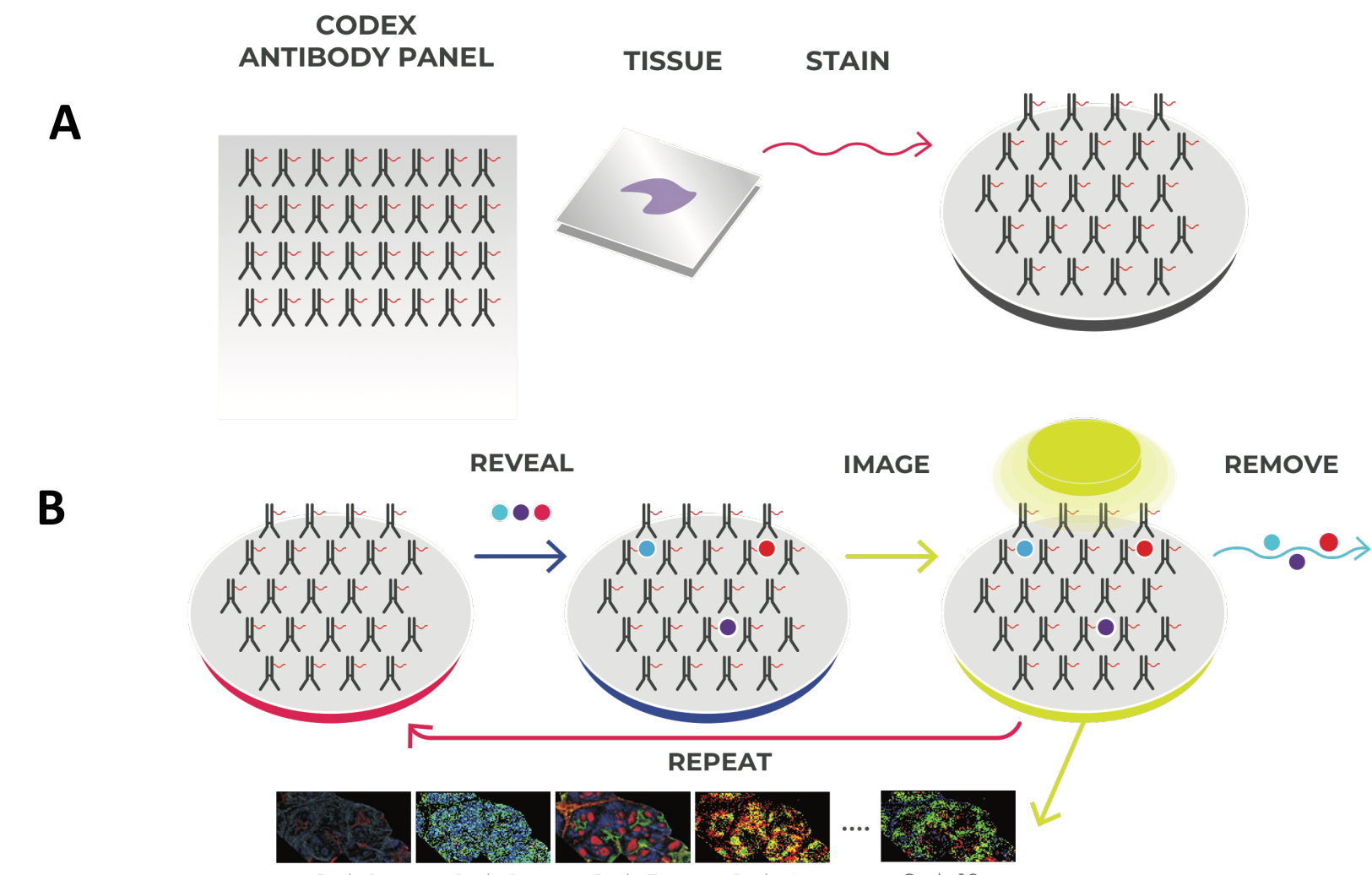


Figure 2: CODEX uses antibodies conjugated to unique and proprietary oligonucleotide barcodes. Staining is performed in a single step with up to 50 customizable antibodies (A). The sample is then imaged iteratively using the CODEX microfluidics platform. During each cycle three CODEX reporters with spectrally-distinct dyes are applied to the sample and imaged.

Fluidics



Figure 3: The iterative process is automated through Akoya's microfluidics platform (left) that can be integrated with a widefield epifluorescence microscope as shown above (right).

Immune marker staining for PBMC's

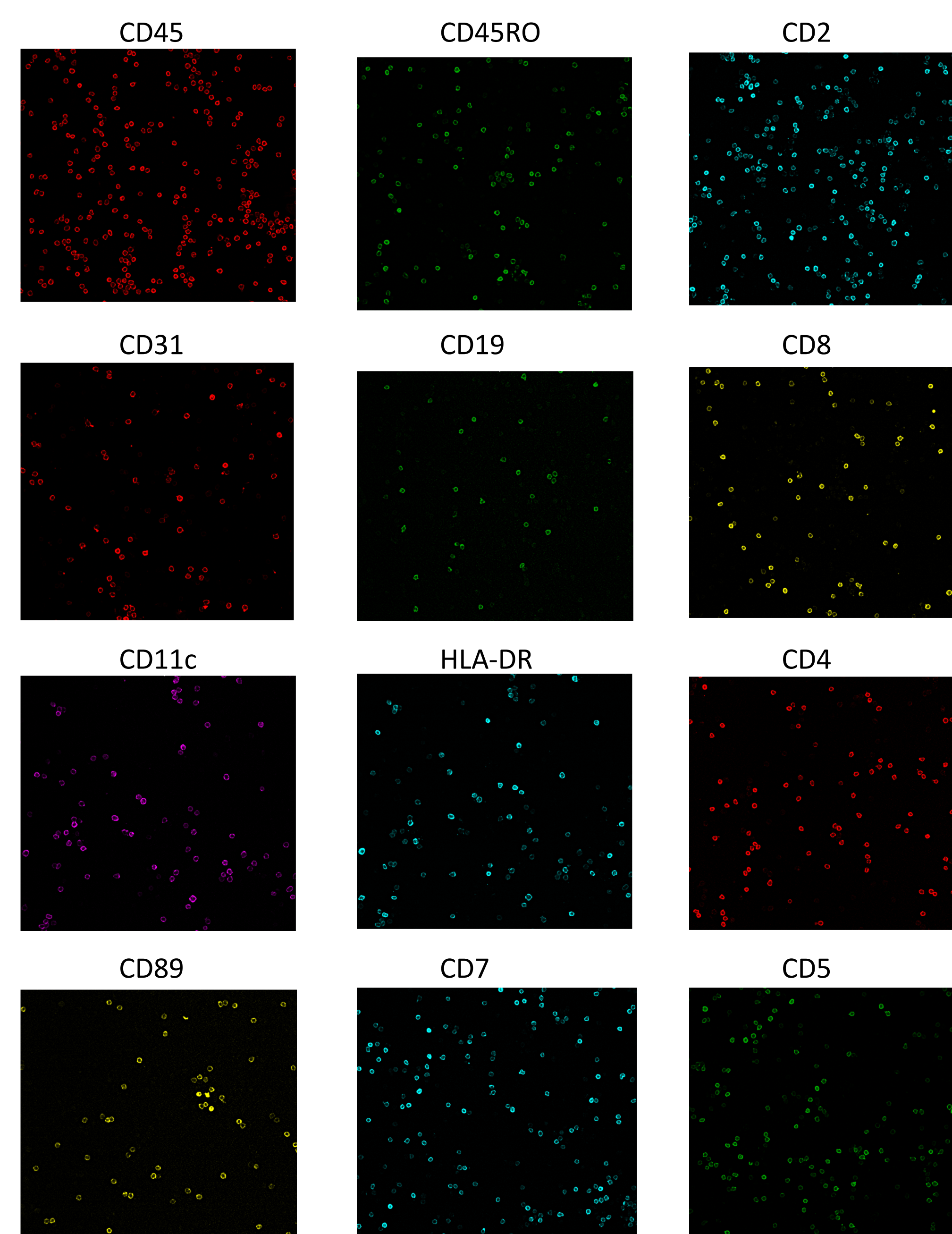


Figure 4: CODEX multiplex experiment was performed on human PBMC's staining for 26 different markers. All antibodies were used at a 1:200 dilution. Staining pattern for some of the markers is shown above.

Assessing CODEX performance on cell spreads

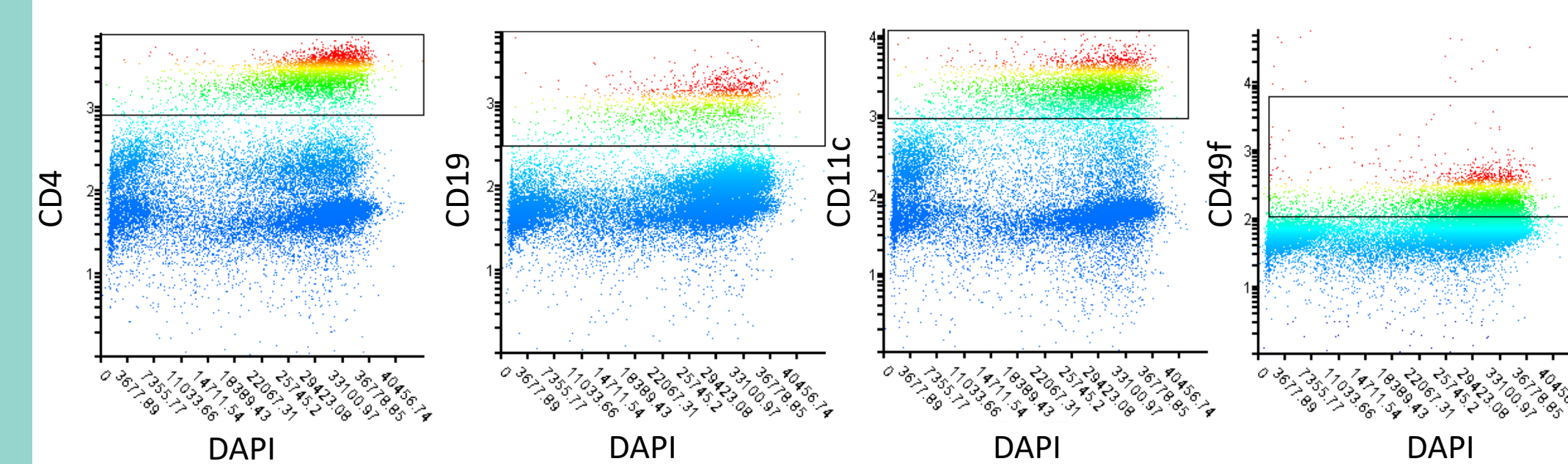


Figure 5: Most markers stained well and displayed good separation between signal and noise on a scatter plot. However, some markers need to be optimized for staining since the separation wasn't well defined, e.g. CD49f.

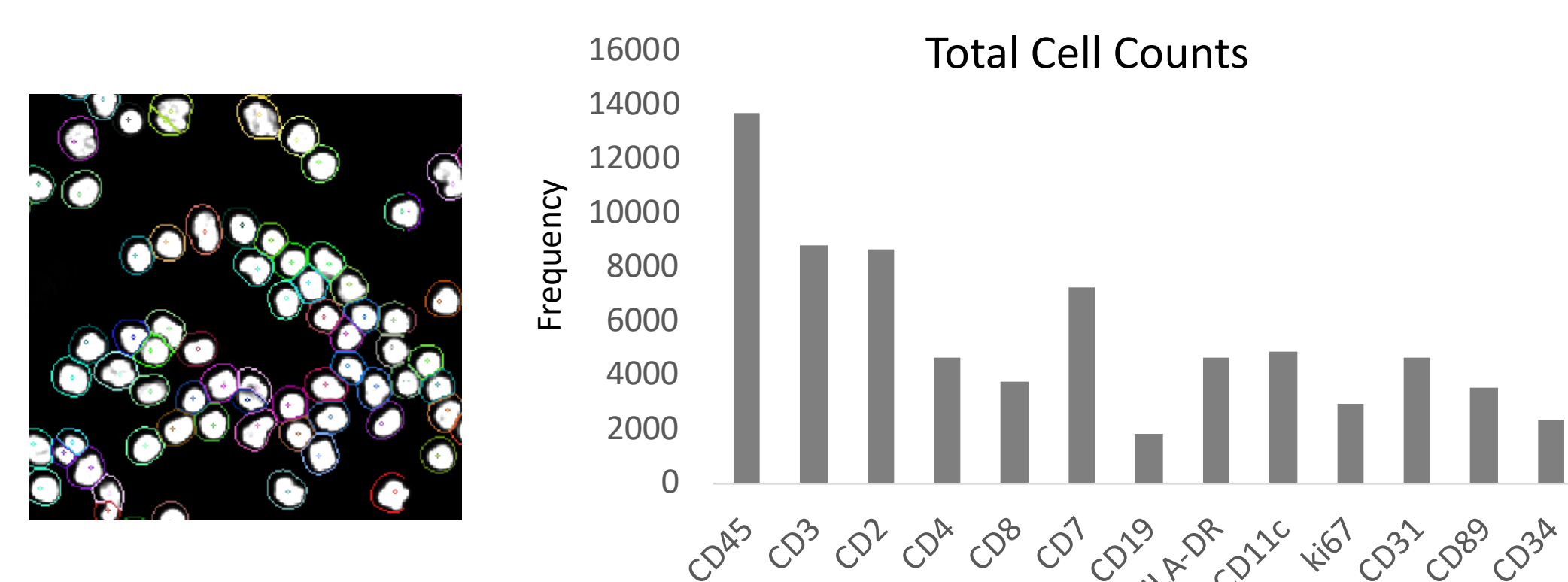


Figure 6: Segmentation was performed using a watershed based algorithm.

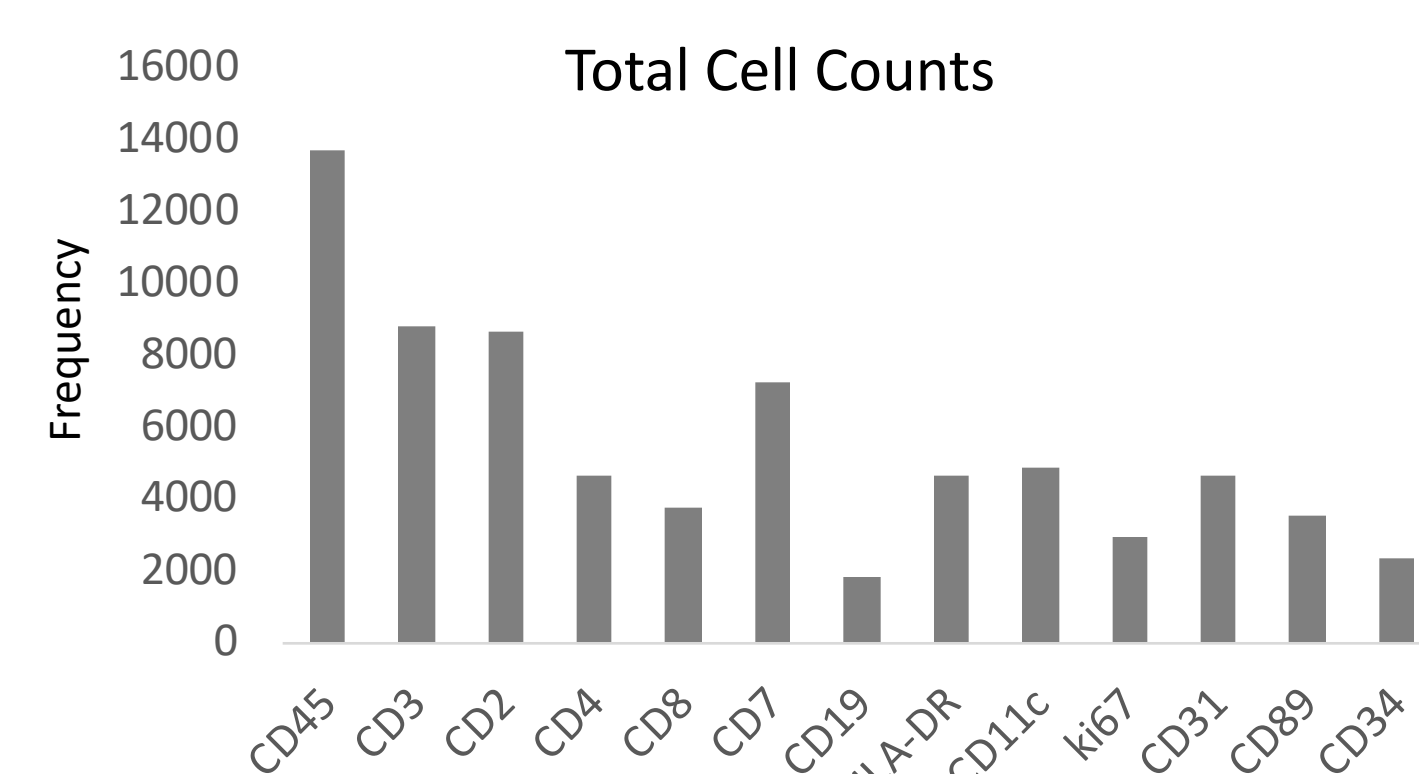


Figure 7: Segmentation results were used with intensity based thresholding to get total cell numbers for various marker expression on a 7X7 tile image.

Qualitative Assessment of marker staining

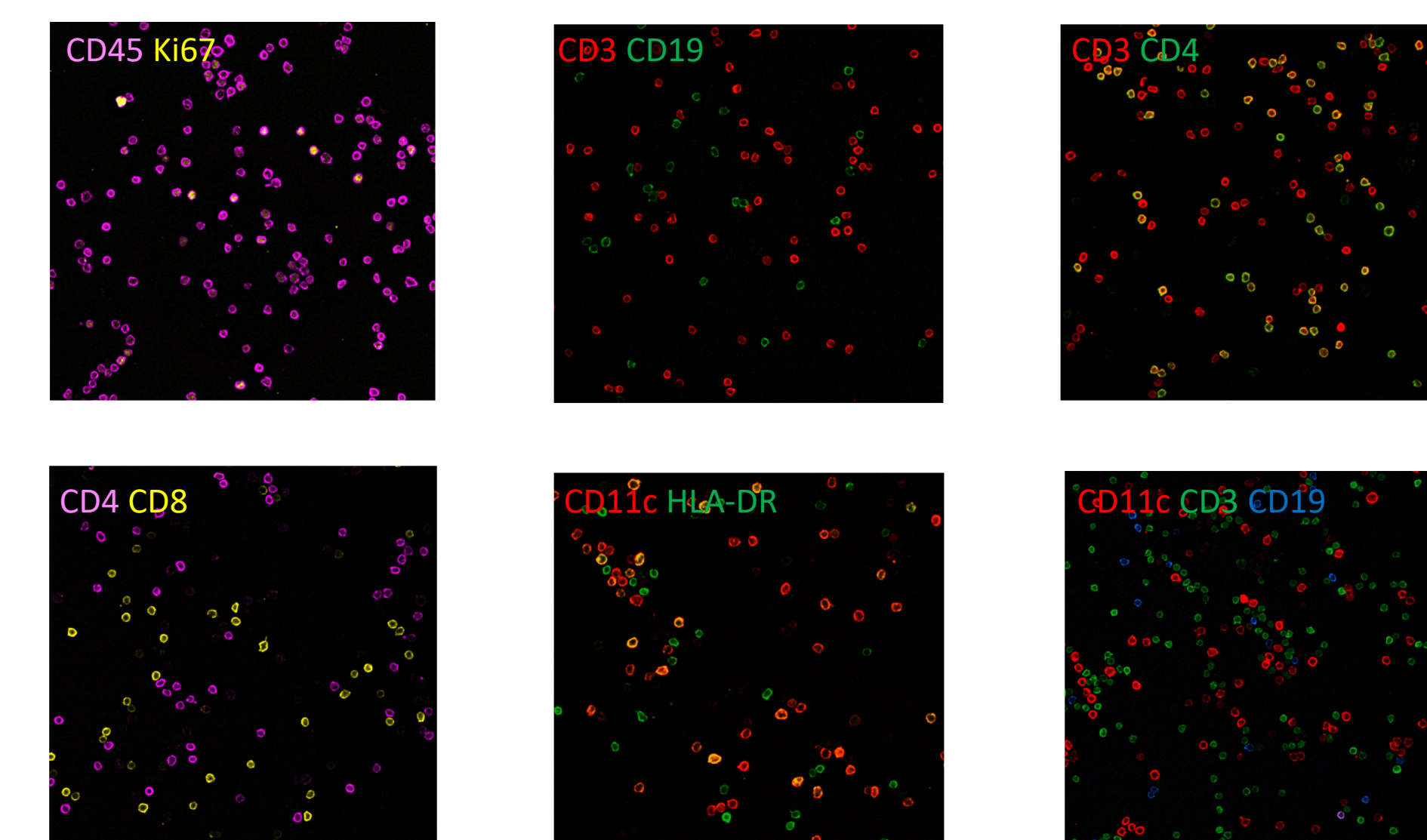


Figure 8: Staining pattern for some of the epitopes was checked visually. Mutually exclusive markers such as CD19 (B cells) and CD3 (T cells) did not show overlap in staining. Co-expression of epitopes for similar cell types could also be observed. For e.g. HLA-DR and CD11c displayed an overlapping population of dendritic cells.

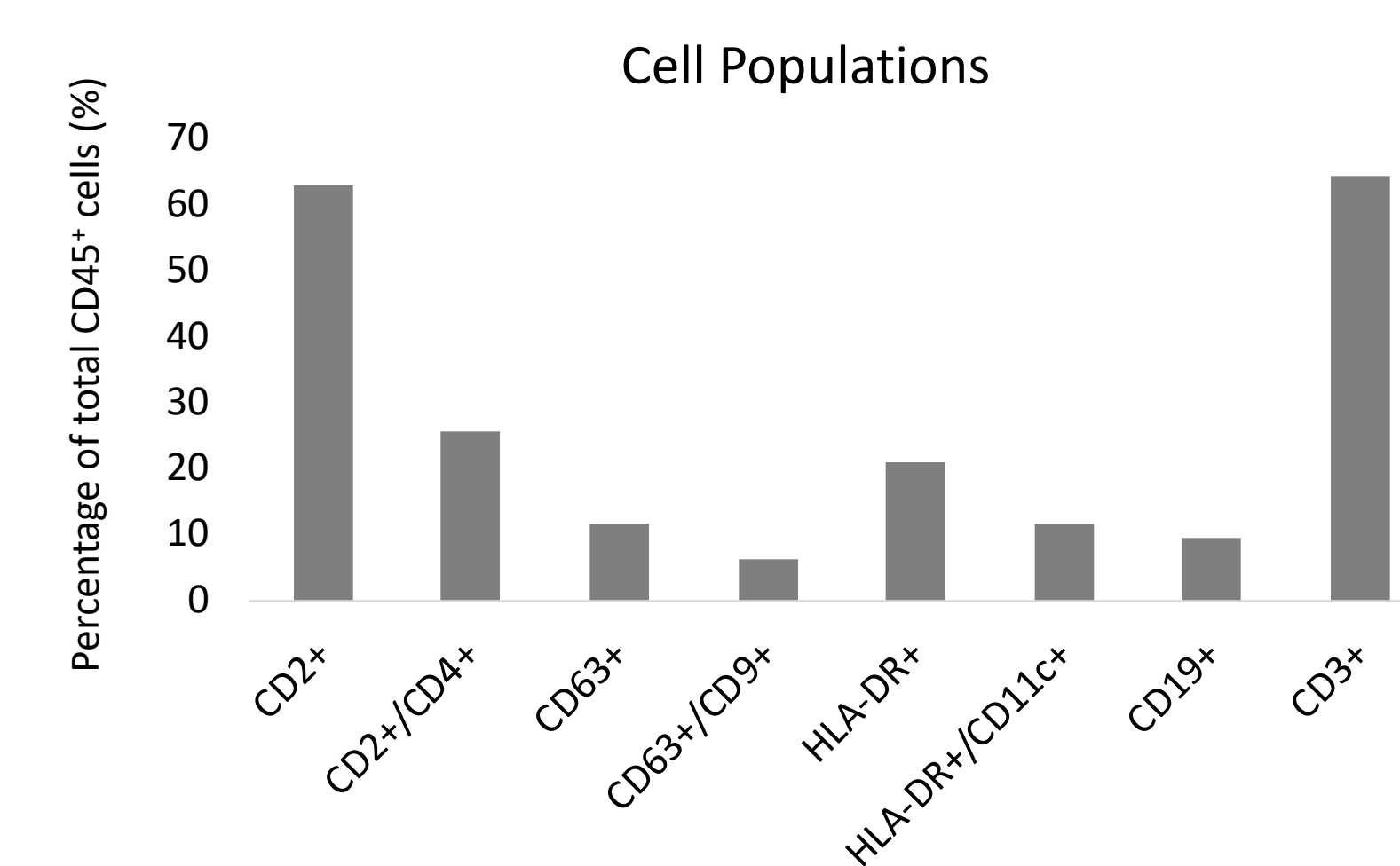


Figure 9: Cell populations positive for different marker expressions are displayed as a percentage of the total immune cell (CD45+ cells).

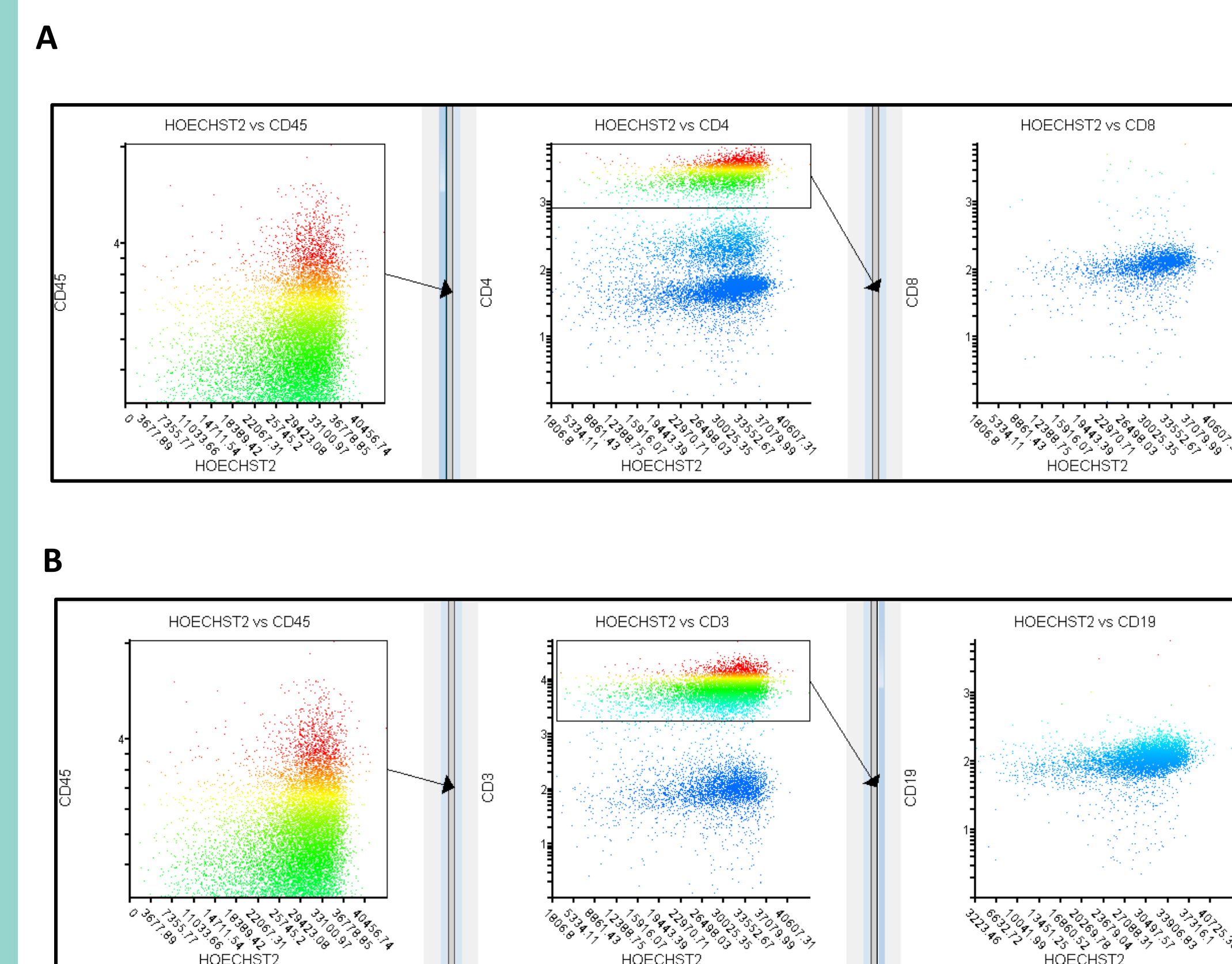


Figure 10: Different cell types such as T cells (CD3) and B cells (CD19) did not show an overlapping population when evaluated through sequential gating (A). Similarly different T cell subtypes could be identified and did not display an overlapping population for CD4 and CD8 (B).

No loss in signal over multiple cycles

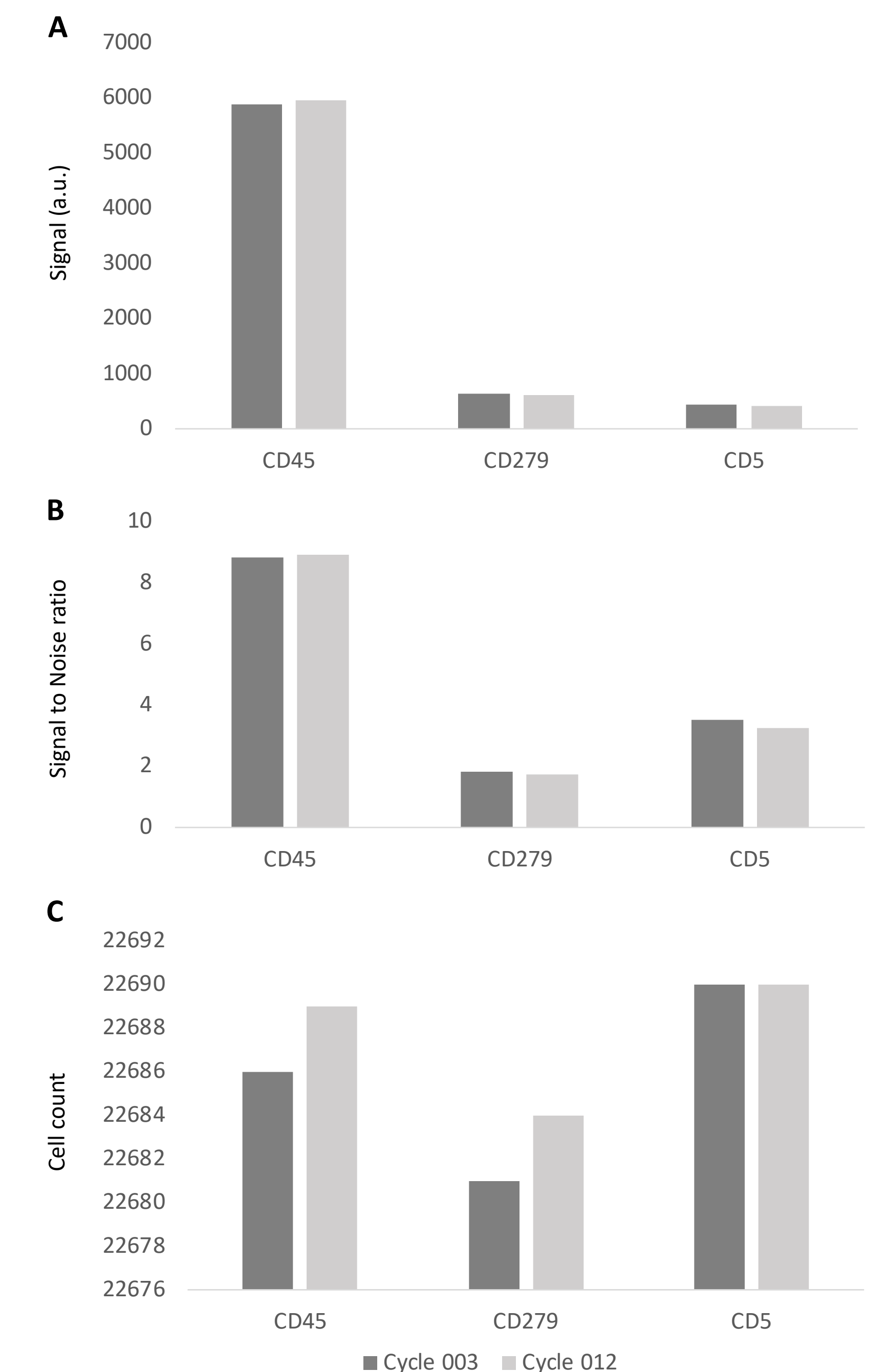


Figure 11: Signal intensity was tested for 3 different markers over a multicycle experiment. The signal and SNR do not show a difference for the 3 markers tested with 9 intervening CODEX cycles in between (A, B). The number of cells being detected did not change over 9 cycles (C).

Conclusions

- Simultaneous analysis of tens of markers in blood or plasma samples can have several applications in the discovery of cellular biomarkers, immune monitoring and drug discovery and development. This preliminary study shows the compatibility of the CODEX system with cell suspensions for highly multiplexed, single-cell analysis and offers a more cost-effective method for immune profiling of blood samples.
- CODEX also offers the advantage of visualizing cell morphology associated with different markers which maybe beneficial for diagnostic purposes in some disease types.

Future Directions

- The CODEX Inventoried Antibodies used in this panel were validated for tissue samples and not cell suspensions. Additional validation and optimization of some markers is needed to improve staining performance with PBMC samples.
- Compare CODEX data with a well established technique like flow cytometry for validation
- Correlative studies using 10X genomics platform should be performed to compare transcriptomics data with protein expression

*DW and JC provided PBMC's for this study