Expanding Mass Cytometry Discoveries and Applications with Cadmium-Labeled Antibodies

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Materials and Methods

Sample Preparation, Staining, and Analysis

- All seven cadmium isotopes were conjugated to purified anti-CD45 antibodies using the Maxpar MIPCP Antibody Labeling Kit (Figure 3A).
- Live-cell barcoding using cadmium-labeled anti-CD45 (Cd-CD45) antibodies was tested on frozen human PBMC from healthy donors (rPBMC) from Cellular Dynamics International (CA) and Cell-1 PBMC from Biotrin (Ireland) with the following antibodies: 110Cd, 111Cd, 116Cd, 170Er, 187Re, 209Bi, and 162Dy.
- Live-cell barcoding with six different Cd-CD45 antibodies was tested on frozen human PBMC from healthy donors (rPBMC) from Cellular Dynamics International (CA) and Cell-1 PBMC from Biotrin (Ireland) with the following antibodies: 110Cd, 111Cd, 116Cd, 170Er, 187Re, 209Bi.
- Pre-barcoding and post-barcoding controls were observed as expected, indicating that the Cd-CD45 antibodies were effective for staining PBMC.
- Post-barcoding controls showed no overlap between the positive and negative barcode channels as depicted in the histogram on the separation plot.
- Samples were acquired on a Helios instrument running CyTOF Software V2.0.
- The normalized FCS files were debarcoded using open source software packages Premessa (github.com/PerkinEl/C/premessag)
- Debarcoded FCS files were analyzed using Cytobank (cytobank.org).
- For additional information, contact your local Fluidigm field application specialist.

Results

Debarcoding the 35-Plex FCS File

- We observed the expected signal from Cd-loaded PBMC. We observed the expected signal from Cd-loaded PBMC and products.
- A particularly important integration and application of Cd-labeling into previously defined panels is seen with cell barcoding (BC). Cell barcoding enables sample multiplexing for single-cell analysis in mass cytometry (Figure 2), leading to improved data consistency and workflow efficiency. The Cd-CD45-20plex PIT Barcoding Kit (Cat. No. 201060) utilizes six stable palladium (Pd) isotopes for barcoding up to 25 samples into a single tube.
- Pre-barcoding and post-barcoding controls were observed as expected, indicating that the Cd-CD45 antibodies were effective for staining PBMC.
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Conclusions

- The identification of seven cadmium (Cd) isotopes has enabled mass cytometry to expand the multiparametric characterization of single cells to 56 parameters.
- The possibilities afforded by Cd-labeling antibodies can be seen in their ability to expand existing panels and support new applications, such as live-cell barcoding described in this work. Notably, this allows for pre-stain barcoding for fixation-sensitive targets.
- PBMC samples barcoded with all permutations of 7-choose-3 Cd-CD45 antibodies (35-plex) can be successfully mixed together and debarcoded using available open source software such as Premessa.
- Combined barcoded samples can be effectively stained in a single tube with equal functional gating outcomes across all debarcoded samples.
- Overall, we have demonstrated that using Cd-CD45 antibodies is an appealing choice for live-cell barcoding applications on PBMC and provides an alternative workflow to traditional barcoding for multiplexing mass cytometry samples.

References

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