Antigen Density Quantification by Mass Cytometry Using Quantum™ Simply Cellular Bead-based Calibration

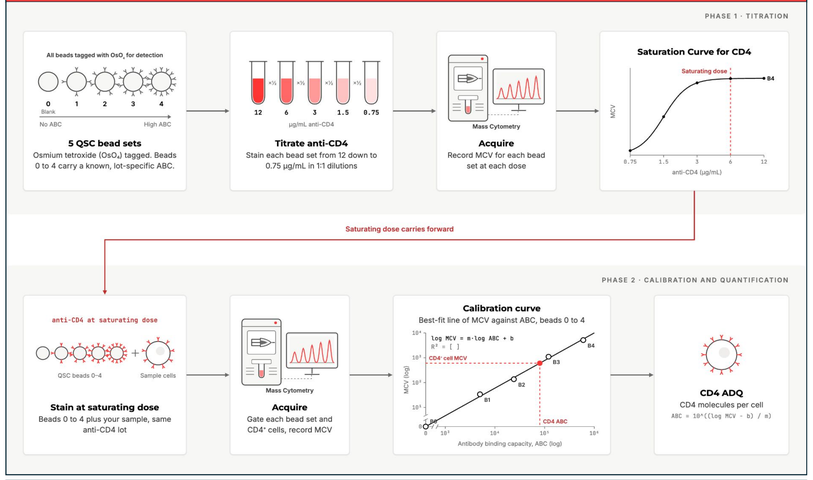
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Teiko

Background

Owing to a lack of standardized and interlab-validated methods, antigen-density quantification (ADQ) has less than 0.5% penetration in clinical trials. Each run needs its own calibration, and controls are not reliably available for every time point. Without comparable and quantifiable values, targeted therapeutics including antibody-drug conjugates (ADCs), bispecific T-cell engagers, and CAR-T developers cannot set antigen density-engagement thresholds for successful immune responses. Cytometry-based approaches, including conventional mass cytometry, primarily provide relative measurements of antigen expression, limiting their utility for ADQ. To address this limitation, we developed a bead-based calibration workflow using Quantum Simply Cellular (QSC) beads with defined antibody binding capacities (ABC) to convert mass cytometry signal into standardized estimates of antigen density (molecules per cell). This approach expands the utility of mass cytometry for quantitative target characterization, supporting therapeutic target selection, drug development, and translational research.

Methods



Optimization of Antibody Saturation on Quantum™ Simply Cellular (QSC) Beads

A

QSC Bead Tube	Antibody Binding Capacity
(Blank)	0
1	1510
2	13810
3	30630
4	69845

B

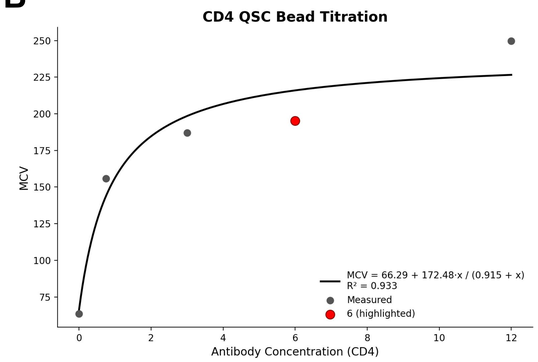


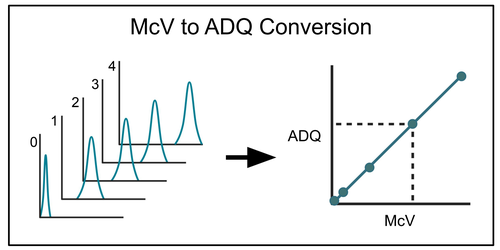
Figure 1. CD4 Antibody Titration Curve Using QSC Beads.
A) QSC beads and their respective antibody binding capacity (ABC). **B)** CD4 antibody was titrated from 12 to 0 µg/mL using QSC beads with the highest antibody binding capacity. The saturating antibody concentration was identified prior to generating linear regression curves using QSC beads with increasing ABCs. Metal-conjugated antibodies were serially diluted and incubated with QSC beads of the highest antibody binding capacity (ABC = 69,845) for 30 minutes at room temperature. Following washing, beads were acquired by a Helios® mass cytometer (Standard BioTools), and antibody signal intensities were evaluated to determine the optimal saturation concentration.

Generation of Linear Curves Using Quantum™ Simply Cellular Beads

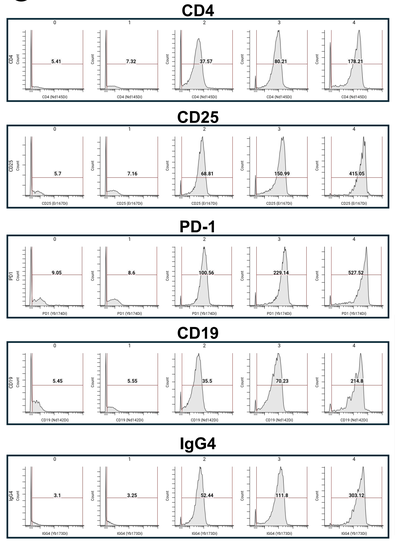
A

Marker	Clone
CD4	RPA-T4
CD25	M-A251
CD19	HIB19
PD-1	EH12.2H7
IgG4	HP6025

B



C



D

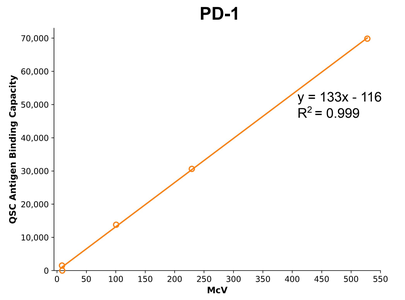


Figure 2. Generation of Marker-specific Linear Curves
A) Selected metal-conjugated antibodies and their respective clones tested. **B)** Diagram depicting the conversion of McV values of tested antibodies on QSC beads acquired by mass cytometry. Marker-specific calibration curves were generated by linear regression of signal intensity against known antibody binding capacity values. **C)** Gated McV of CD4, CD25, PD-1, CD19, and IgG4 Beads 0-4. **D)** Representative linear regression of signal intensity of PD-1 used downstream to convert McV to ADQ.

Gating Scheme

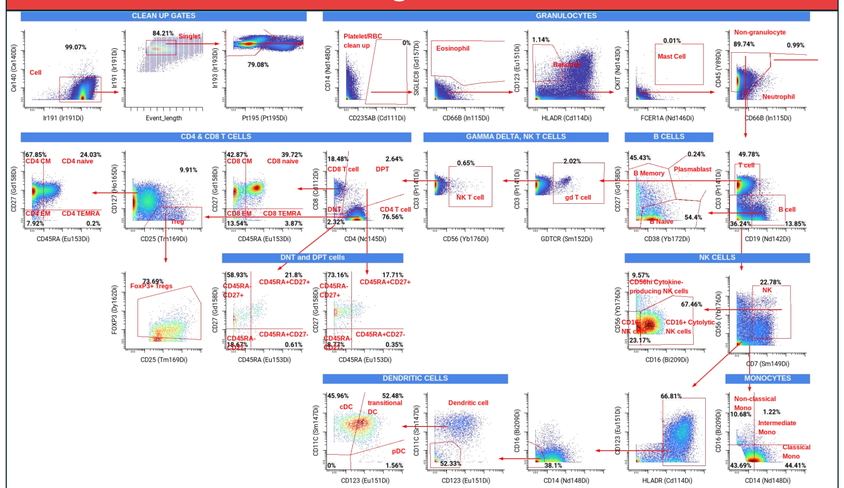
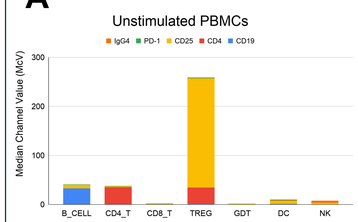


Figure 3. PBMC Gating Strategy: Gating strategy representing a PFA-fixed PBMC sample thawed and labeled with a 20-plex palladium barcode, pooled, and stained with the 42-marker antibody cocktail. Cells were labeled with iridium intercalator and acquired on a Helios® mass cytometer (Standard BioTools). FCS files were normalized using EQ bead-based batch normalization and debarcoded prior to analysis in CellEngine (CellCarta). Cell populations were identified by manual gating.

Antigen Density Quantification on Unstimulated PBMC Samples

A



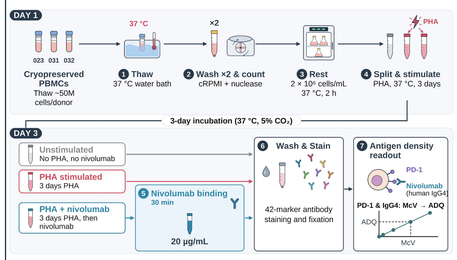
B

	Antigen Density Quantification (ADQ)				
	CD19	CD4*	CD25	PD-1	IgG4
B_CELL	**11267 to 13172**	-1641 to -1641	1386 to 3119	-92 to 4	1372 to 1473
CD4_T	1472 to 1662	**8688 to 15031**	1295 to 1760	-97 to -16	1320 to 1455
CD8_T	1410 to 1566	-1641 to -1641	1295 to 1573	-116 to 86	1320 to 1361
TREG	1556 to 1832	**7987 to 13954**	**30635 to 51620**	-47 to 137	1373 to 1468
GDT	1482 to 1632	-1641 to -1641	1295 to 1599	-116 to 15	1320 to 1360
DC	1767 to 1875	-1641 to -1559	1407 to 2846	-12 to 99	1500 to 1797
NK	1410 to 1410	-1641 to -1641	1581 to 2395	-112 to -42	2042 to 2320

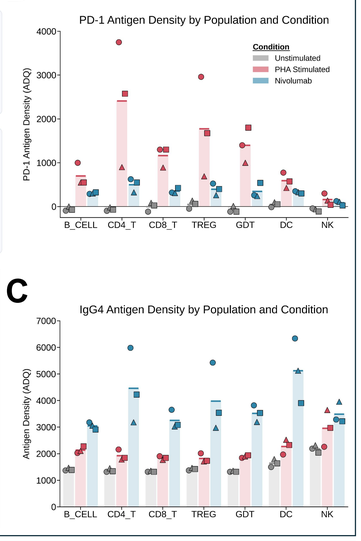
Figure 4. Antigen Density Quantification in 3 PBMC Donors A) Median channel value (McV) of CD19, CD4, CD25, PD-1, and IgG4 using antibody clones from **Figure 2A**. Bar chart values represent the average of three healthy PBMC donors prepped and stained as described in **Figure 3**. **B)** Table displaying the min and max ranges of ADQ for 5 tested markers across B-cells, CD4+ T-cells, CD8+ T-cells, FoxP3+ T-regulatory cells, γδ T-cells (GDT), Dendritic cells, and NK-cells. Bold values represent expected marker–population pairs. Previously reported ADQ ranges in designated cell populations for bolded markers: [1]CD19: ~9-13k, [2]CD4: ~45-60k, [3]CD25: ~30-55k. *Previous reported ADQ for CD4 utilized Leu-3a clone.

Proof of Concept: PD-1 Antigen Density Quantification in Response to Nivolumab Treatment

A



B



C

Figure 5. Antigen density upon nivolumab treatment in stimulated PBMCs. A) Schematic demonstrating workflow performed on three PBMC donors each split across three treatment conditions. Cryopreserved PBMCs were thawed, washed, and assessed for cell count and viability. Cells were resuspended at 2 × 10^6 cells/mL, rested for 2 hours at 37°C, and left unstimulated, stimulated with phytohemagglutinin (PHA) for 3 days, or stimulated with PHA for 3 days followed by treatment with 20 ug/mL Nivolumab. **B-C)** Antigen Density Quantification by immune population across unstimulated, PHA-stimulated or PHA+Nivolumab treatment of PD-1 (**B**) or IgG4 (**C**). Bar represents average ADQ of the three donors in each respective cell population.

Conclusions

- **QSC bead calibration converts mass cytometry signal into antigen density.** Five marker-specific linear curves (CD19, CD4, CD25, PD-1, IgG4) convert median channel values (McV) into molecules per cell (ADQ).
- **ADQ in resting PBMCs reflects expected lineage expression.** CD19 ADQ on B-cells fell within previously reported ranges, and CD4 and CD25 ADQ were highest on their expected populations and close to their expected ranges. PD-1 was at background levels in unstimulated cells.
- **ADQ captures nivolumab target engagement.** After nivolumab treatment, PD-1 ADQ dropped below PHA-only levels in every donor and cell population, while IgG4 ADQ increased, consistent with detection of PD-1–bound nivolumab.

References

1. Wang L, Bhardwaj R, Mostowski H, et al. Establishing CD19 B-cell reference control materials for comparable and quantitative cytometric expression analysis. *PLoS One*. 2021.
2. Wang M., et al., Quantifying CD4 receptor protein in two human CD4+ lymphocyte preparations for quantitative flow cytometry. *Clin Proteomics*, 2014.
3. Ohmes J, Monne LR, et al. Interleukin-2 deprived state of regulatory T cells and their recovery by low-dose interleukin-2 in patients with inflammatory myopathies. *Arthritis Rheumatol*. 2026.