Evaluación de Memoria Celular Utilizando Citometria de Flujo Espectral de 45 Colores

CYTEK®
TRANSCEND THE CONVENTIONAL

Memory T Cell Profiling Using Spectral Cytometry

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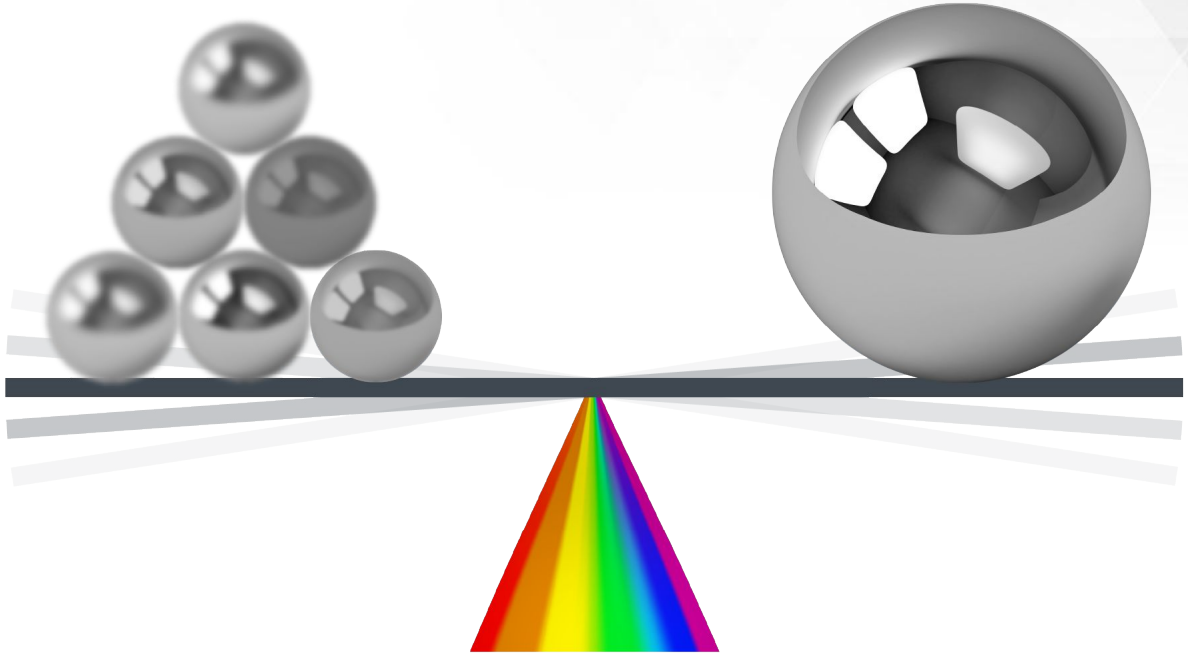
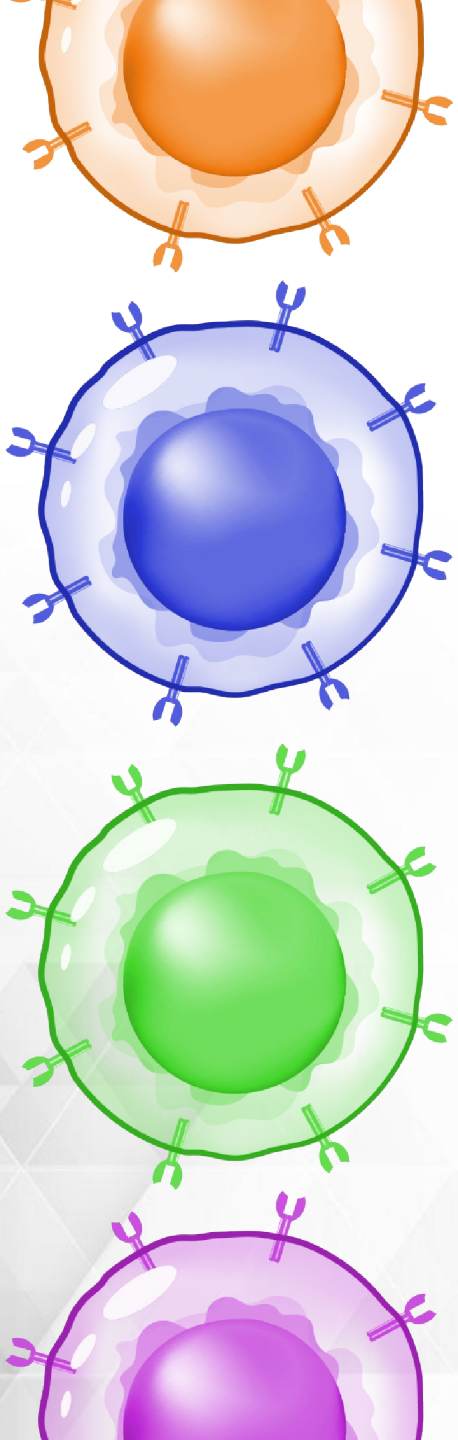
Scientific Director
Cytek Biosciences
Oct 5th 2024





Flow Cytometry

A technology used to detect structural and functional characteristics of cells in suspension, examining thousands of events per second and using fluorescence as a readout.

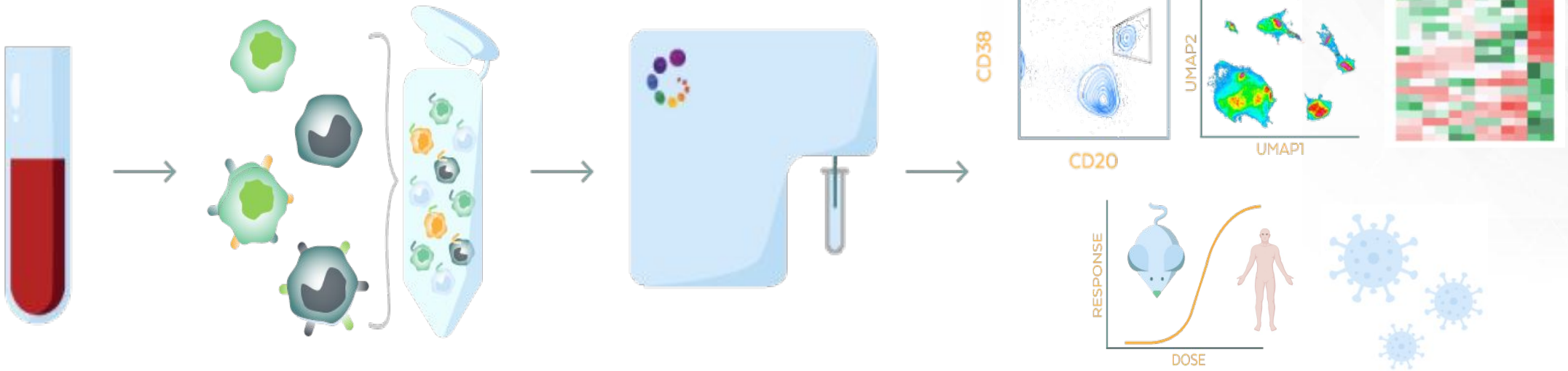
Flow Cytometry

Collect and prepare samples

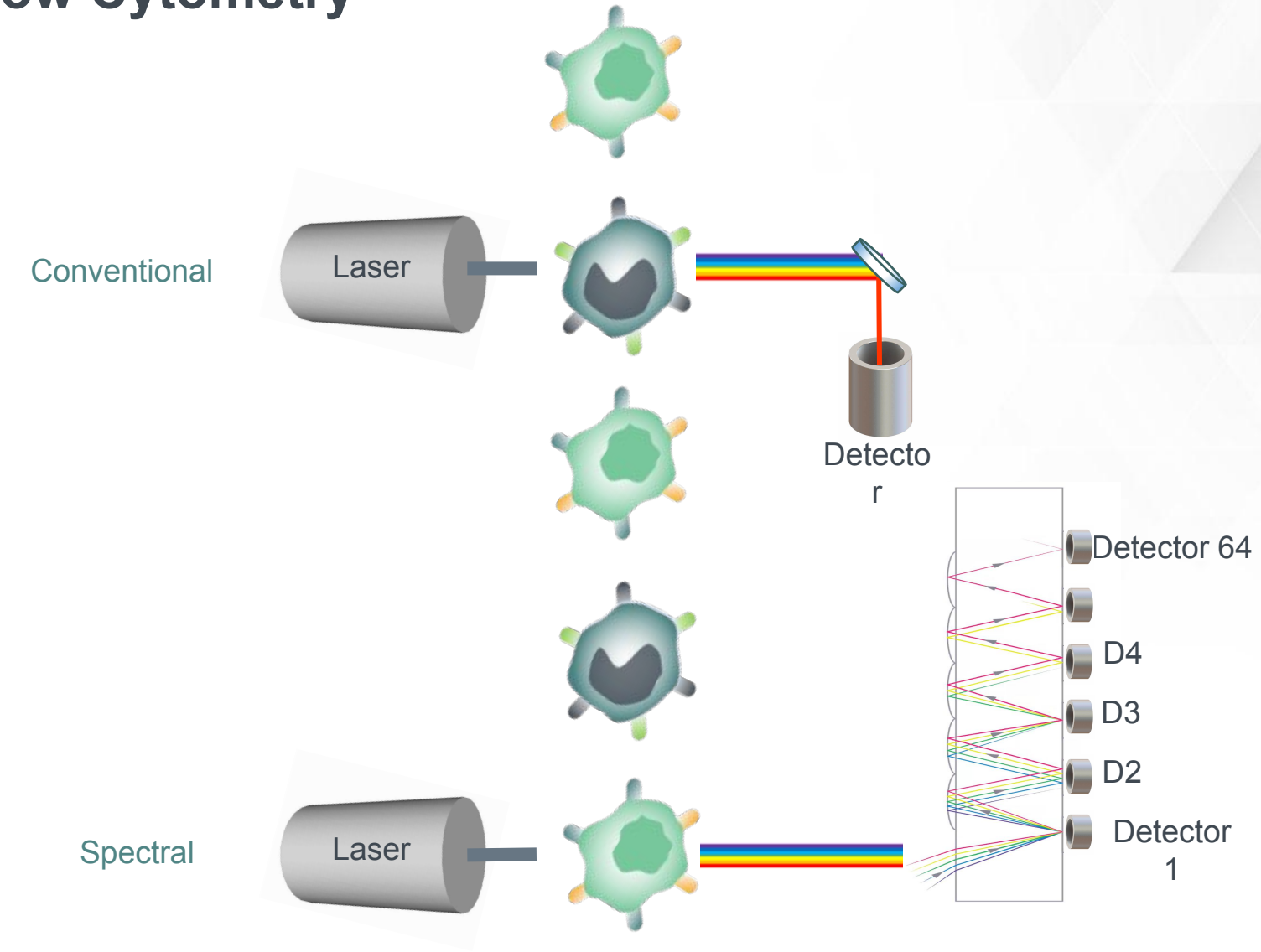
Stain cells with labeled antibodies

Acquire samples on a cytometer

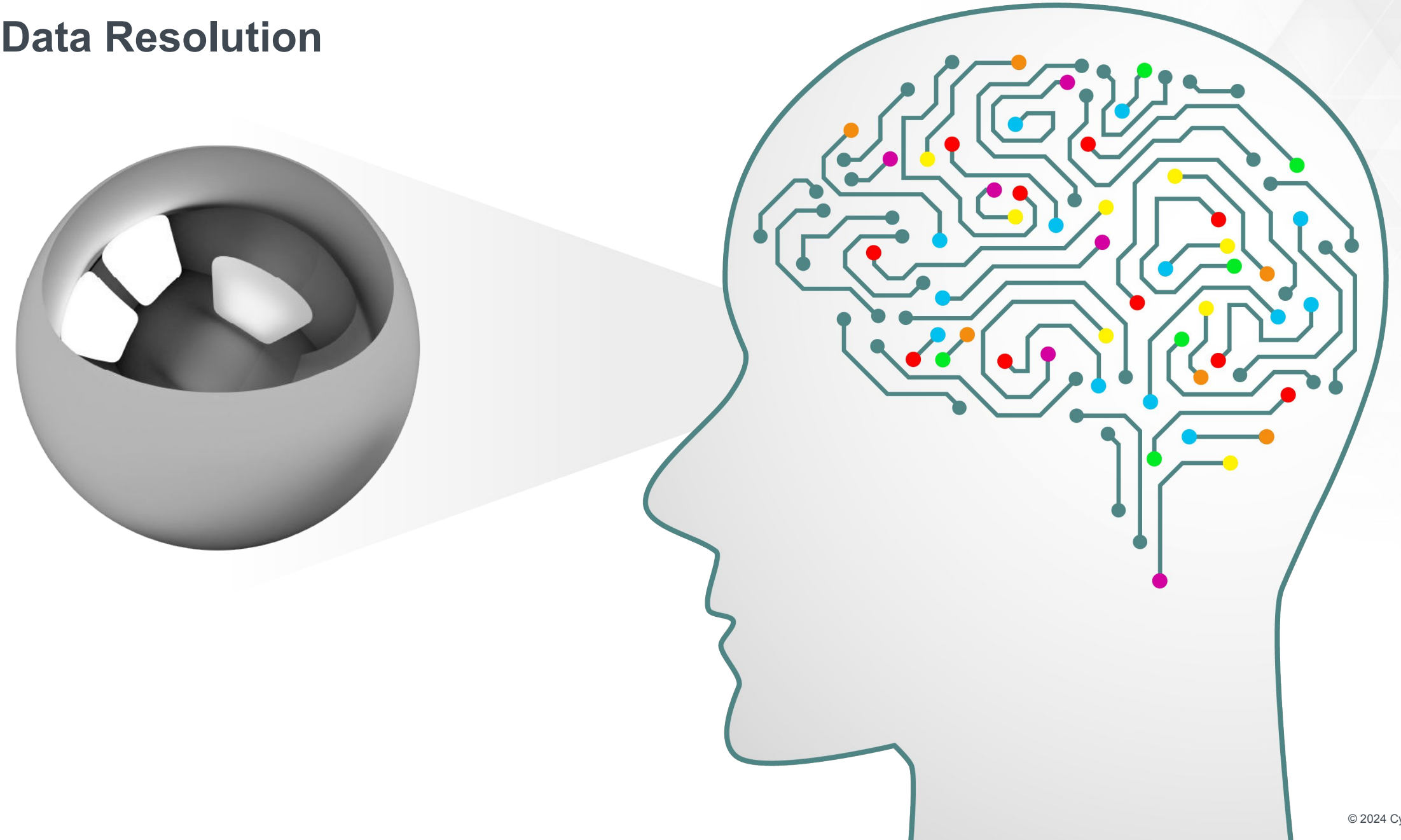
Analyze and interpret results



Spectral Flow Cytometry



Data Resolution



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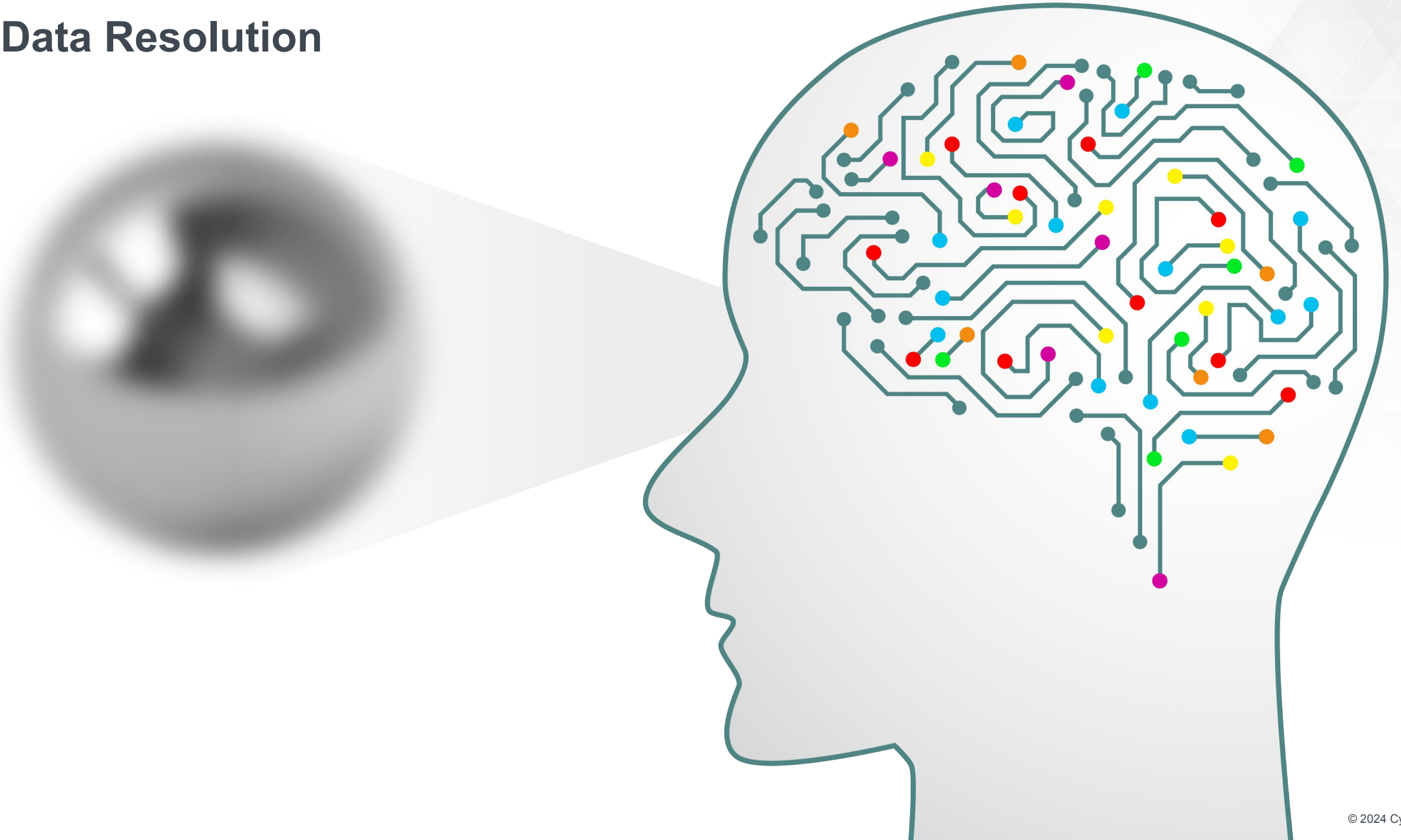
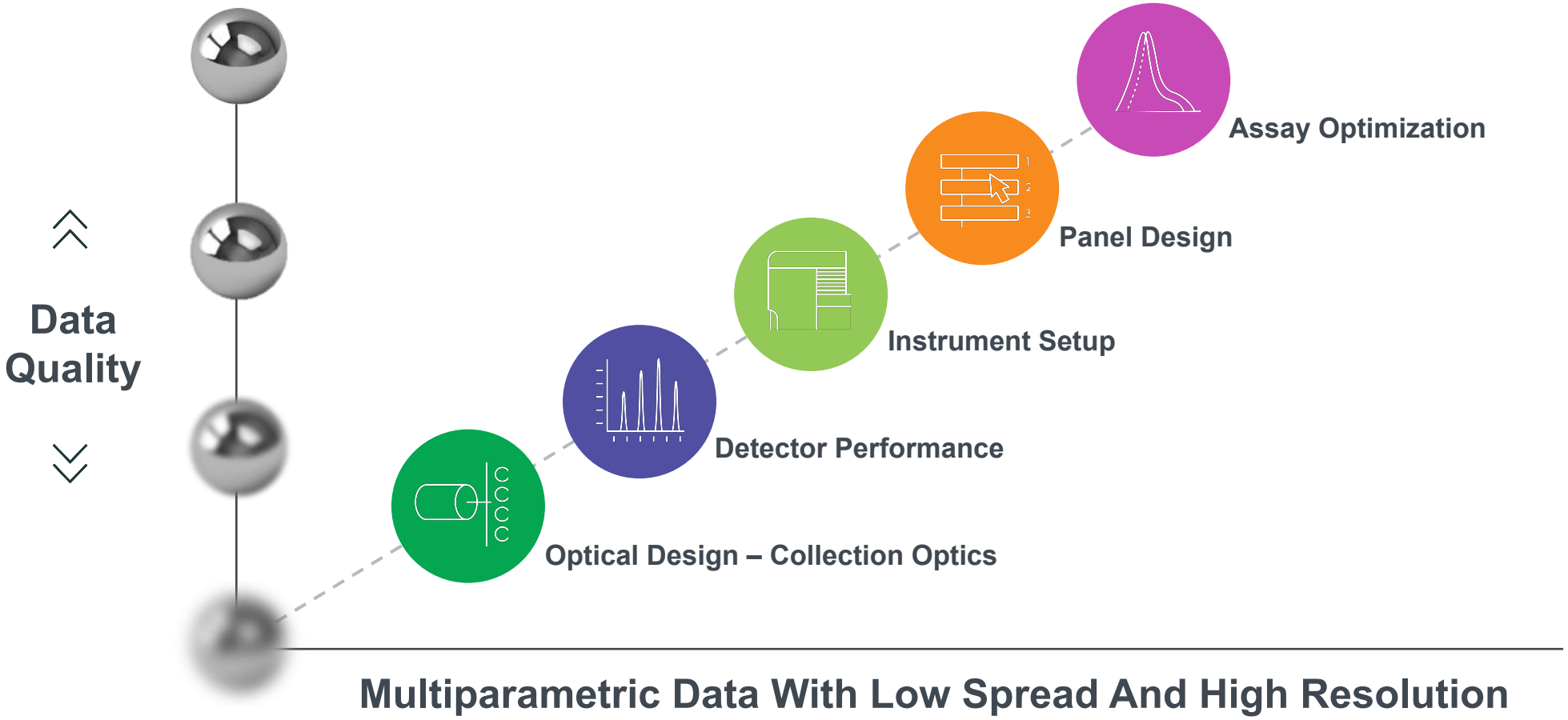
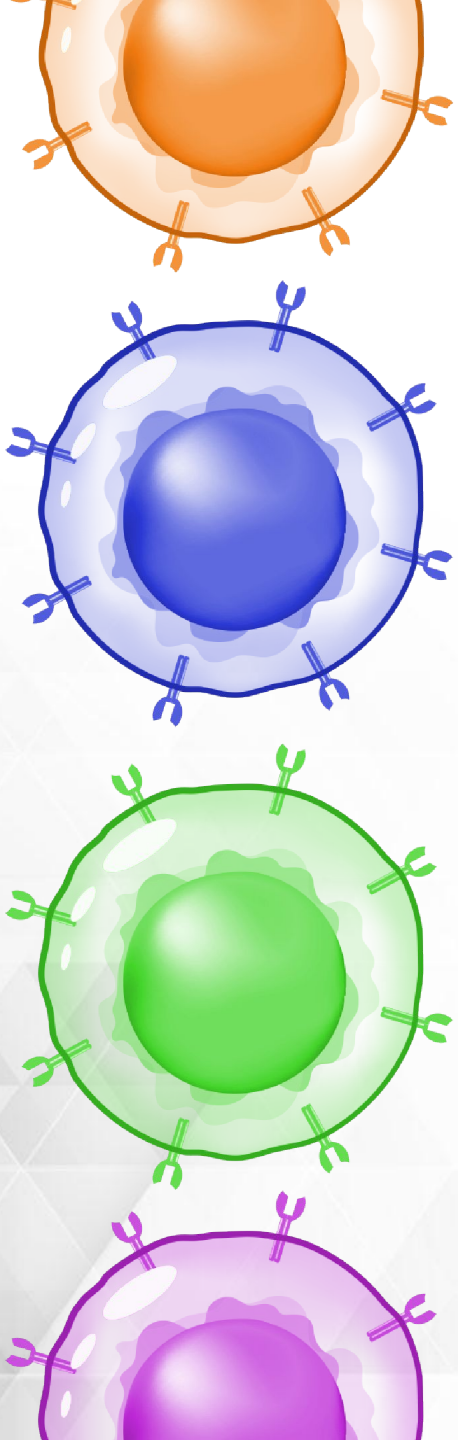


The Journey To Outstanding Resolution in Spectral Flow Cytometry

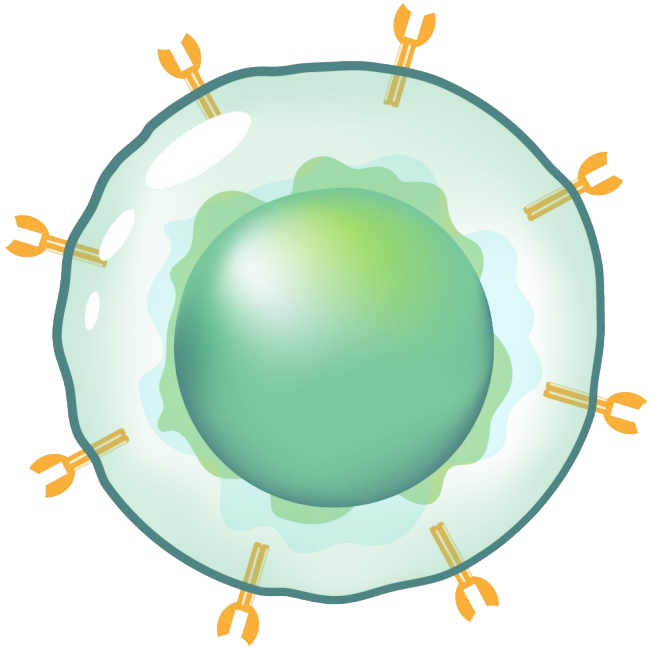


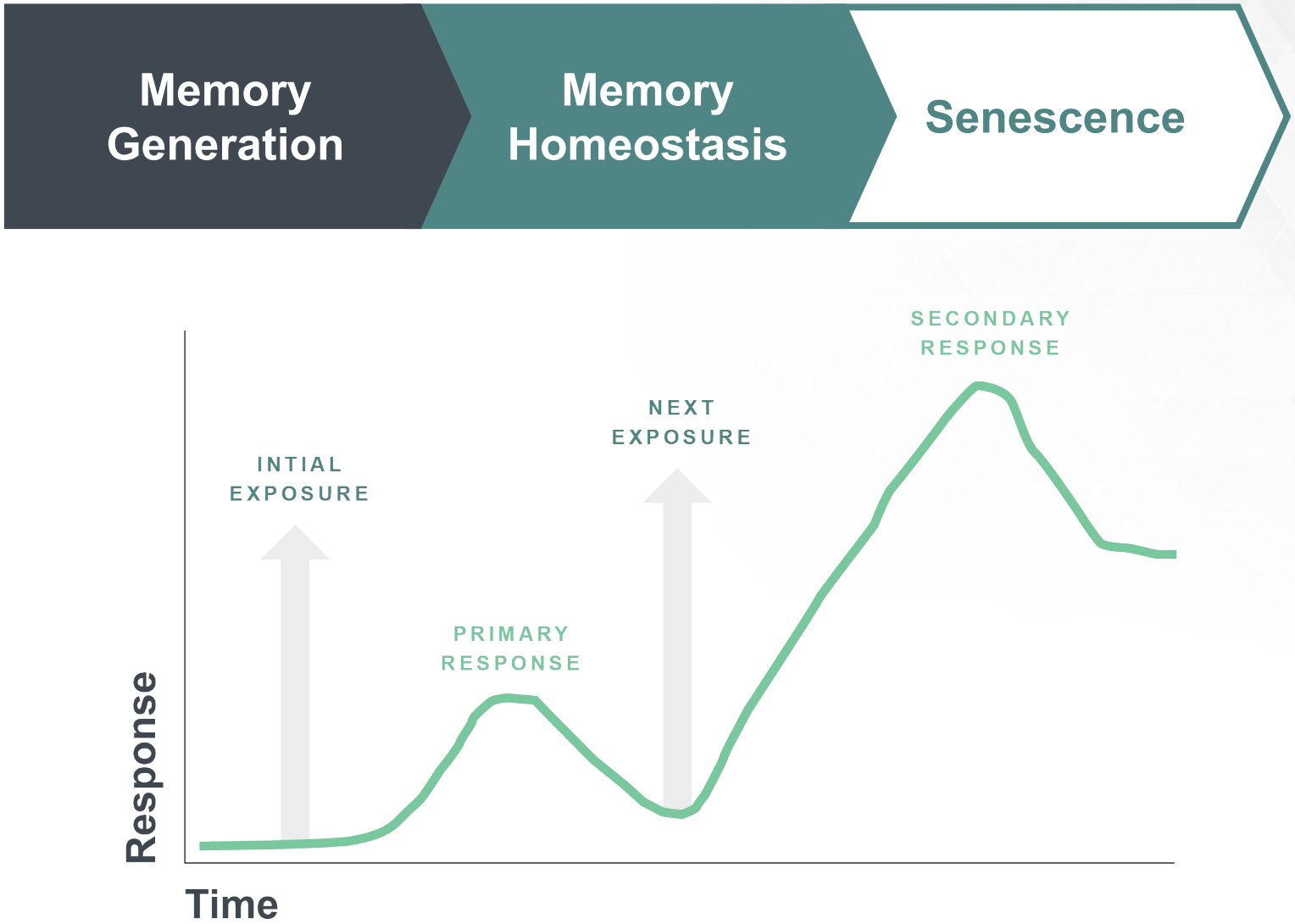


Assay For 45-color Memory T Cell Profiling

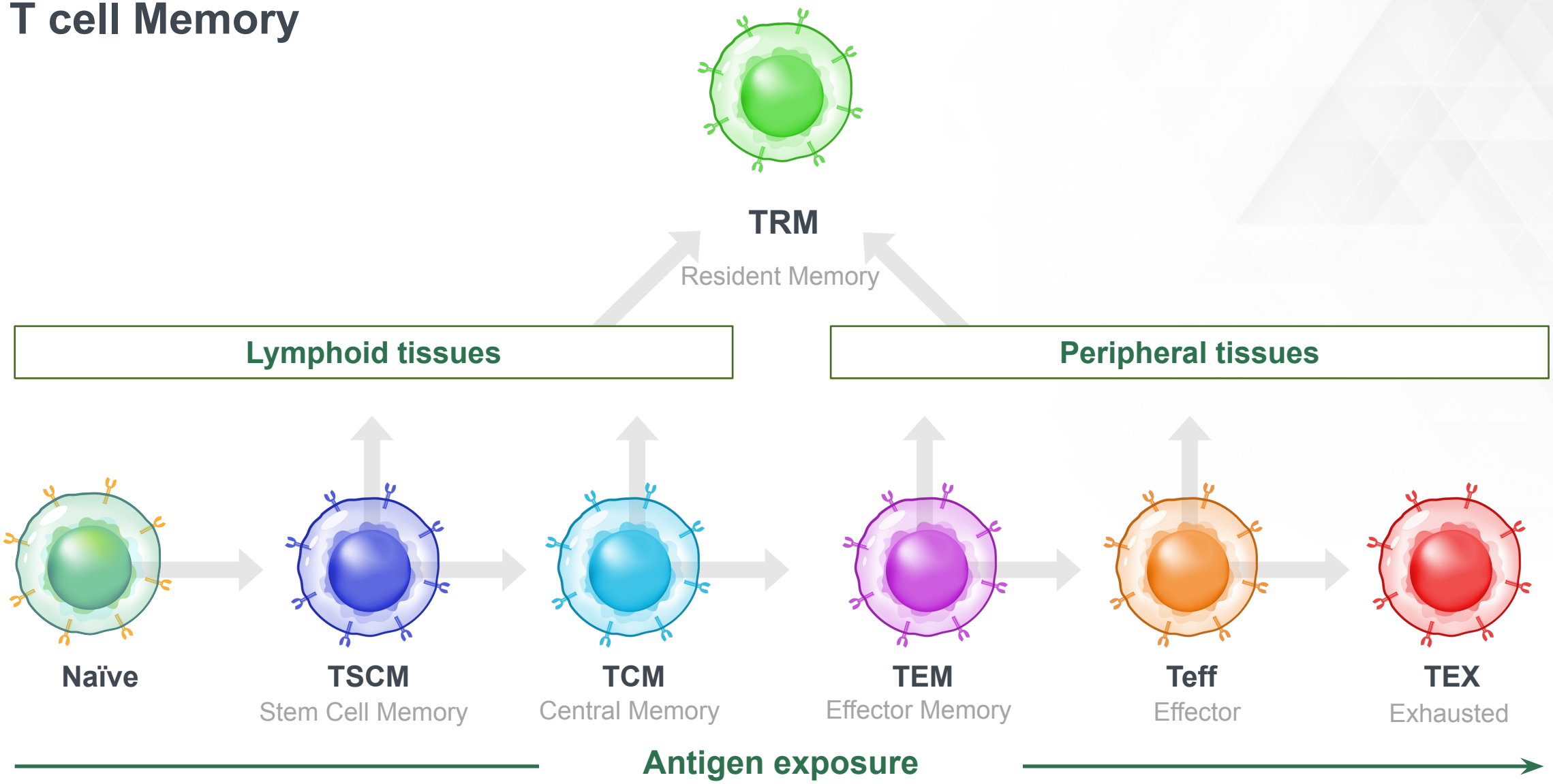
For Deep Immunophenotyping Of The Major Lineages Present In Human Peripheral Blood Mononuclear Cells With Emphasis On The T Cell Memory Compartment

T cell Memory





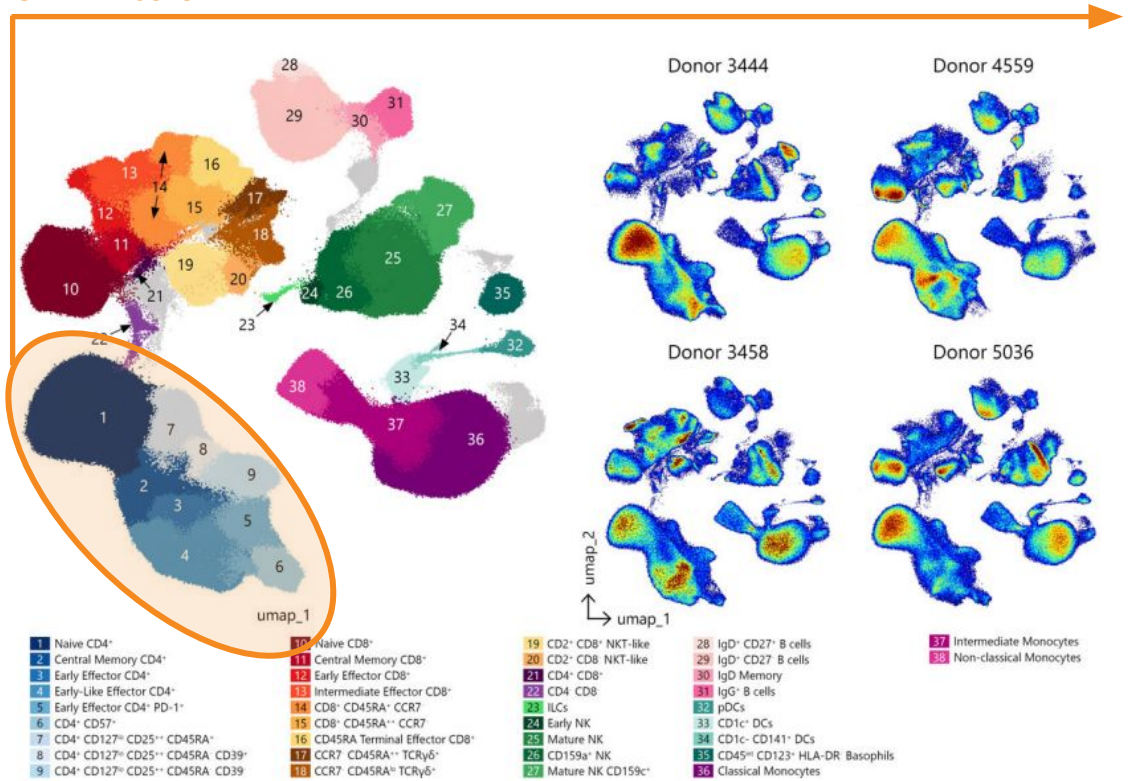
T cell Memory

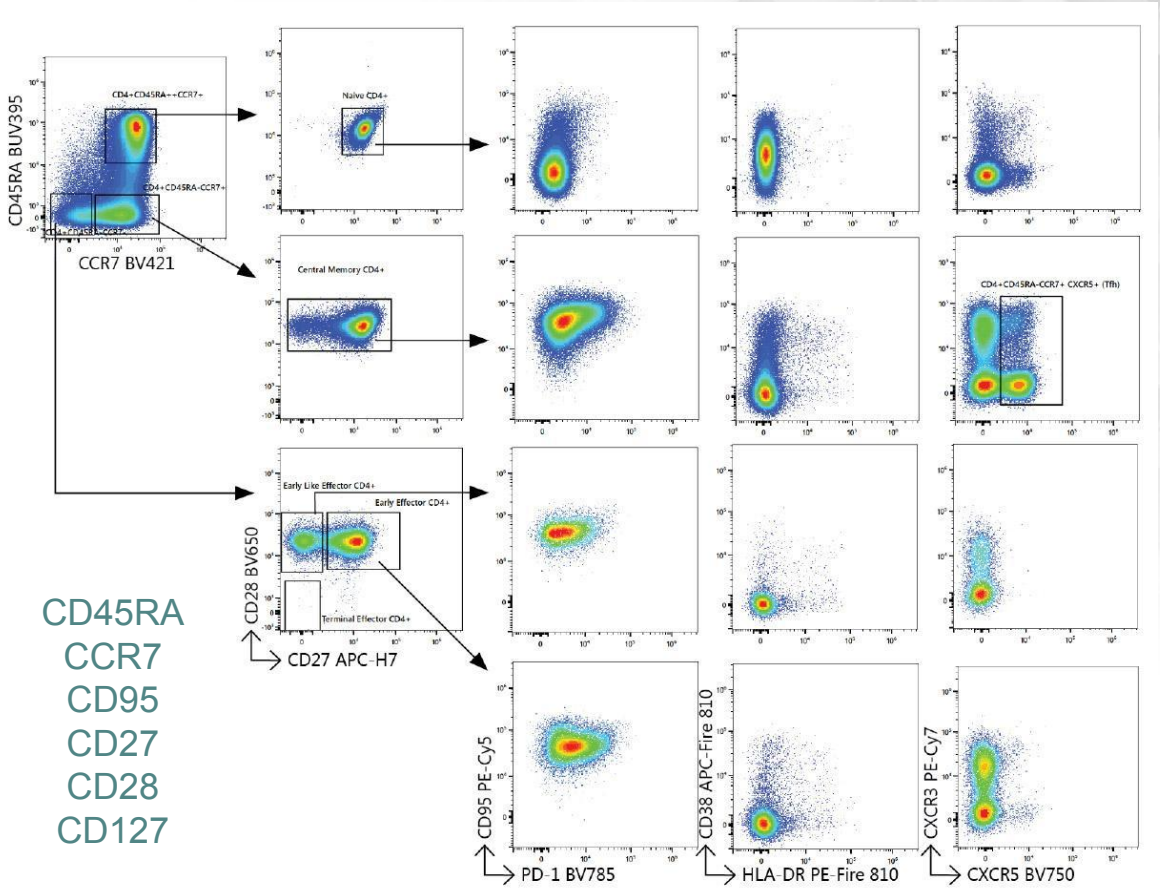


Preliminary T cell Memory Evaluation

OMIP-069 publication set a high-quality 40-color panel

CD4+ T cells

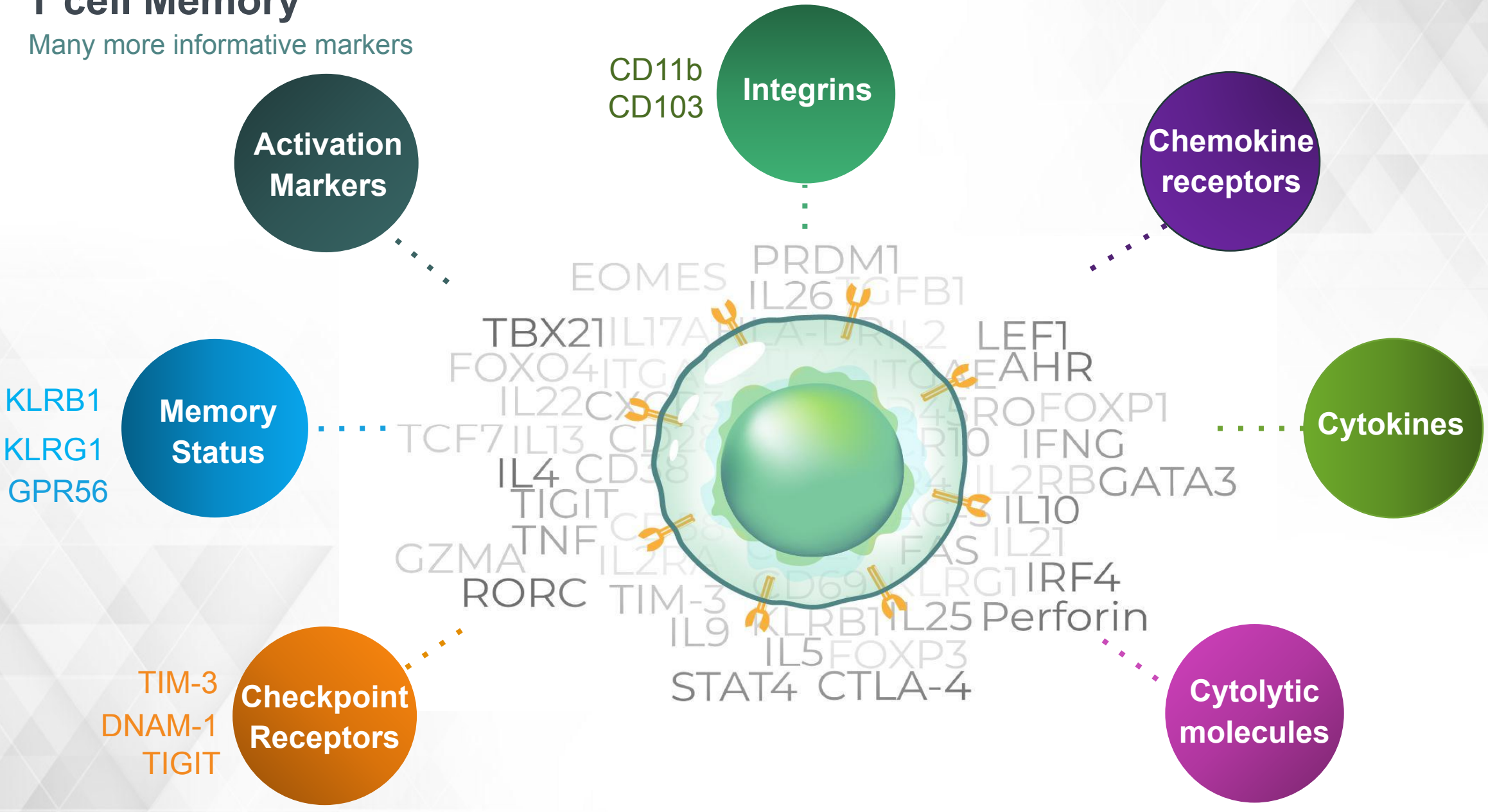




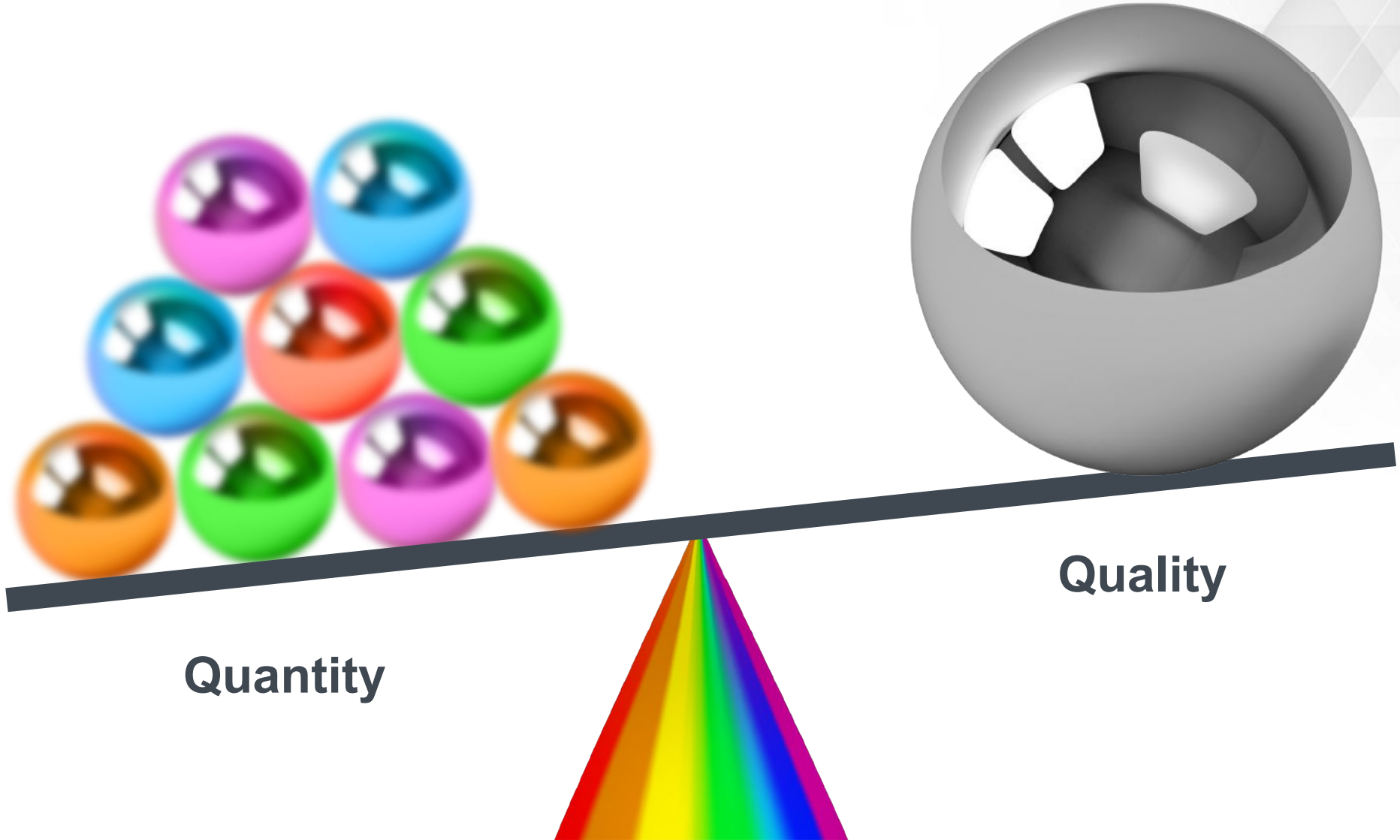
CD45RA
CCR7
CD95
CD27
CD28
CD127

T cell Memory

Many more informative markers

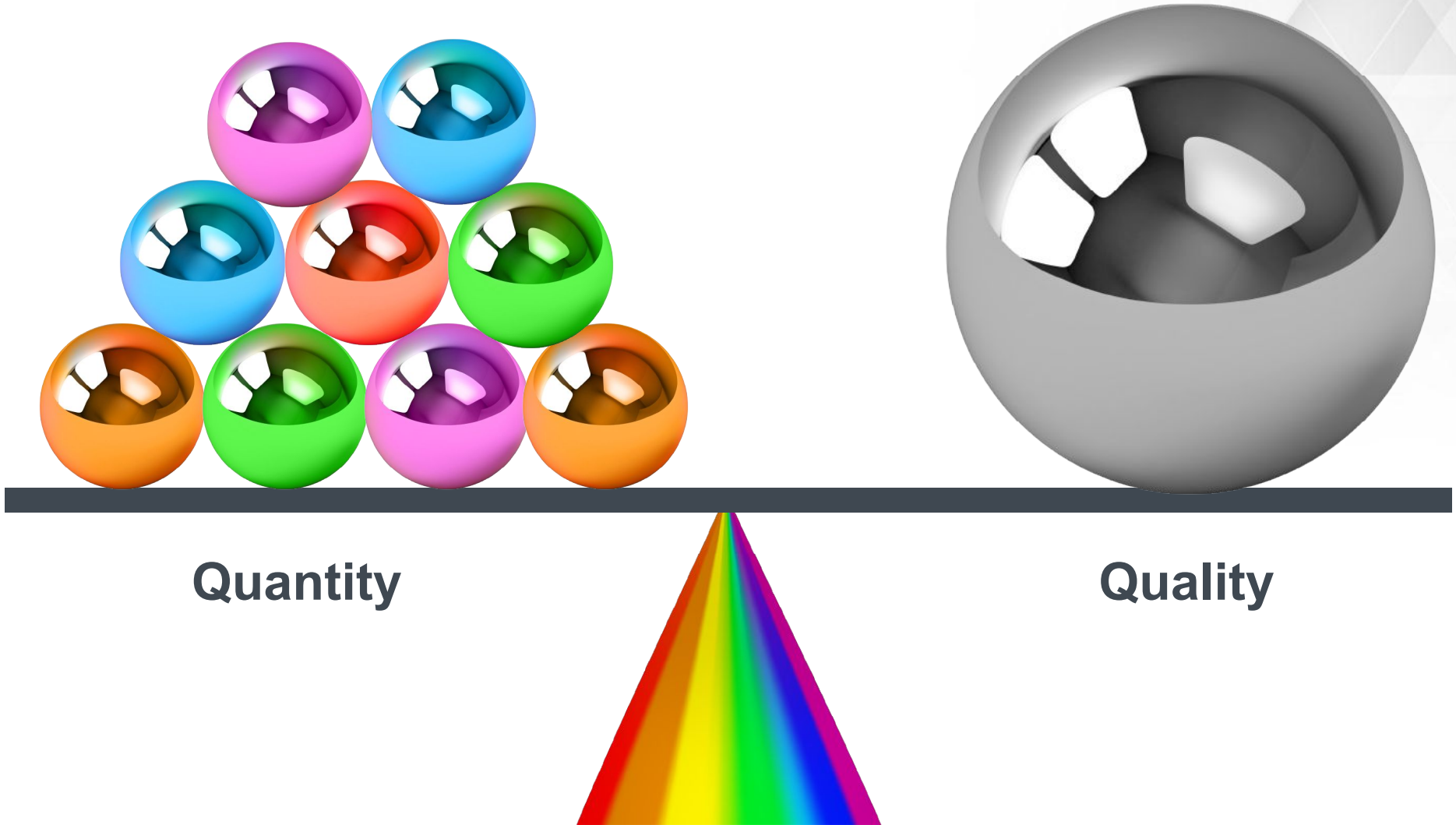


What If You Want To Go Beyond 40-colors In Spectral Cytometry?

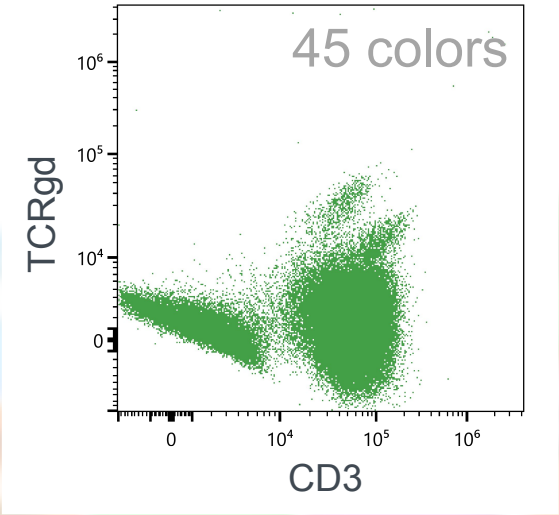


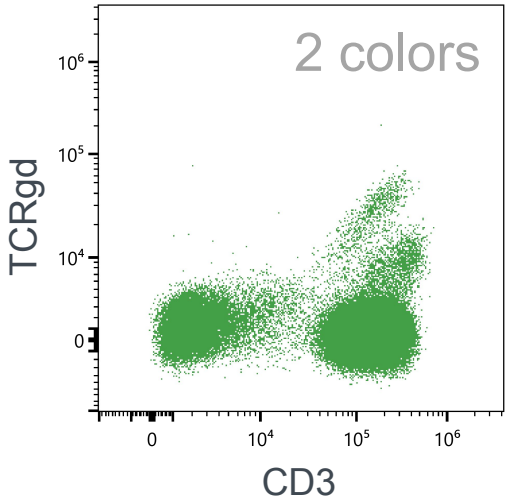
What If You Want To Go Beyond 40-colors In Spectral Cytometry?

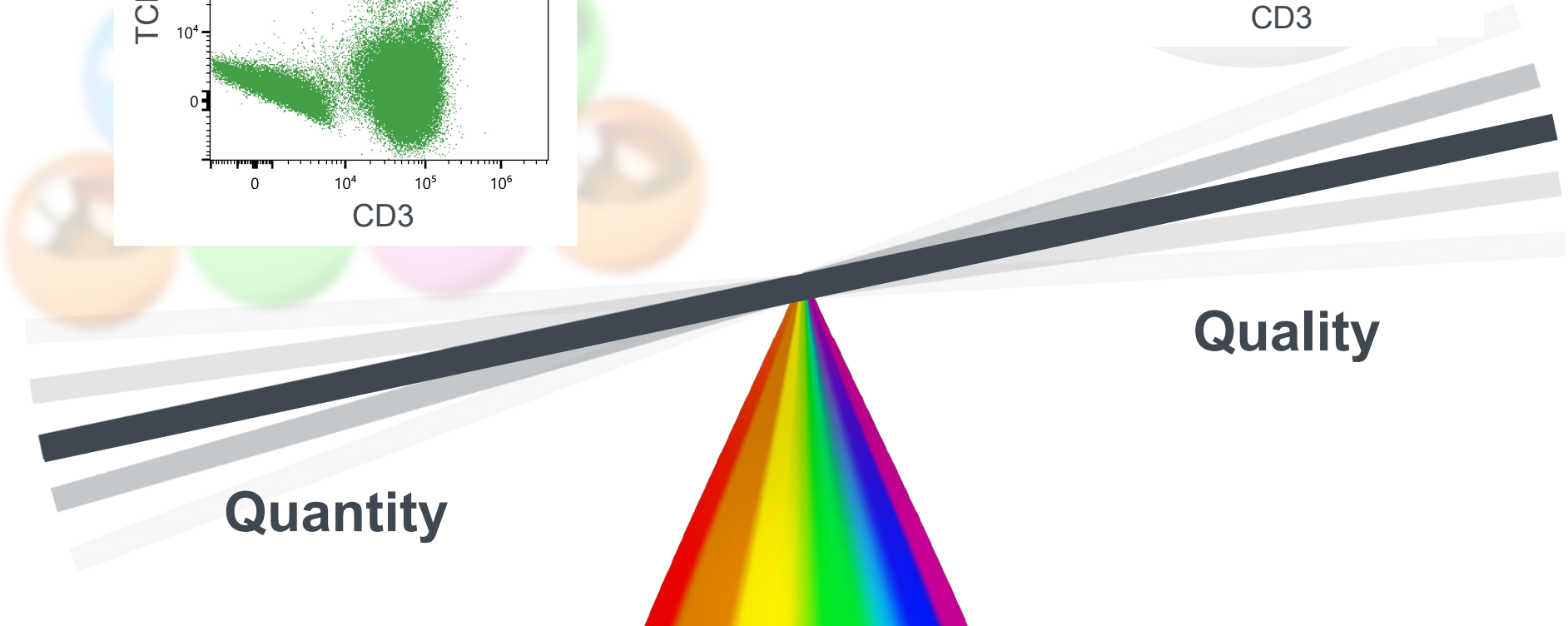
Need to balance quality and quantity



Why Quality Matters?



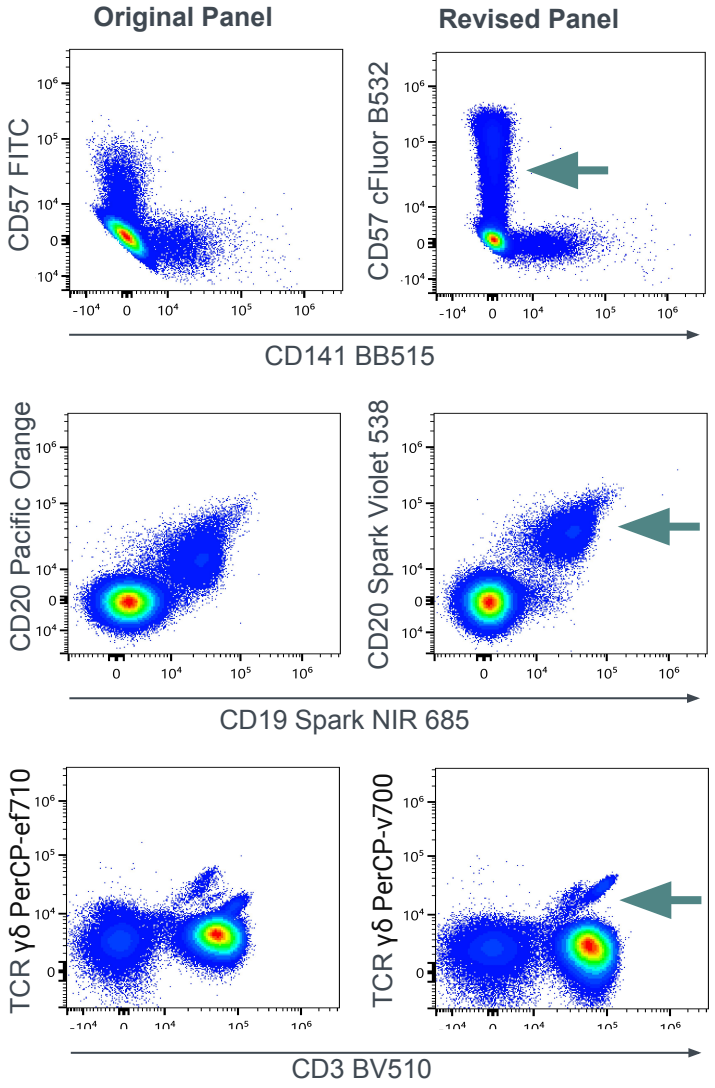




Reagent Selection

Resolution was preserved or improved with fluorochrome replacements in the full panel

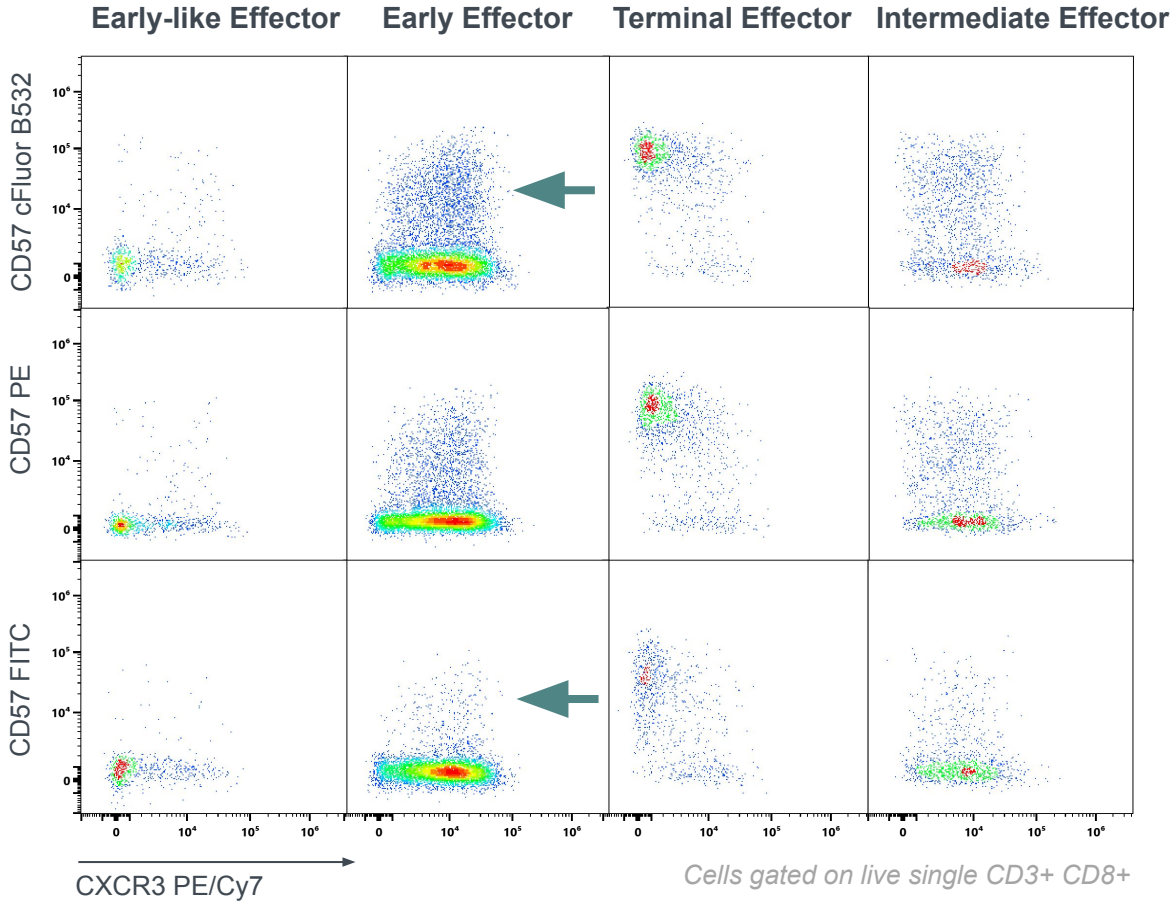
Original Fluor	Replacement
Pacific Orange	Spark Violet 538
PE-Alexa Fluor 610	cFluor® YG610
PE/Fire 700	cFluor® BYG710
PerCP-eFluor710	PerCP-Vio 700
APC-R700	cFluor® R720
FITC	cFluor® B532
PE/Fire 810	BV480
BV480	cFluor® BYG750



Reagent Selection

Comparable staining pattern, resolution and spectrum profile between original and replacing reagents

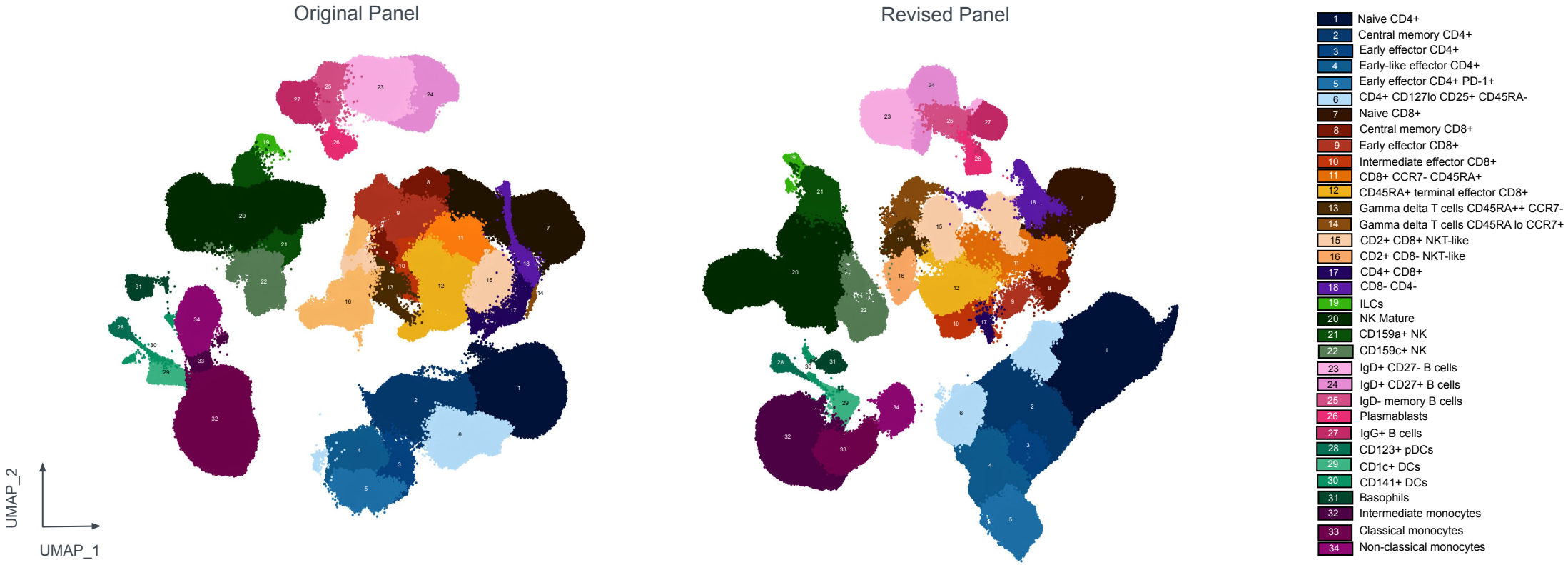
Original Fluor	Replacement
Pacific Orange	Spark Violet 538
PE-Alexa Fluor 610	cFluor® YG610
PE/Fire 700	cFluor® BYG710
PerCP-eFluor710	PerCP-Vio 700
APC-R700	cFluor® R720
FITC	cFluor® B532
PE/Fire 810	BV480
BV480	cFluor® BYG750



Cells gated on live single CD3+ CD8+

Revised 40-color Panel Performance

Similar populations are identified by clustering when comparing original and revised panels



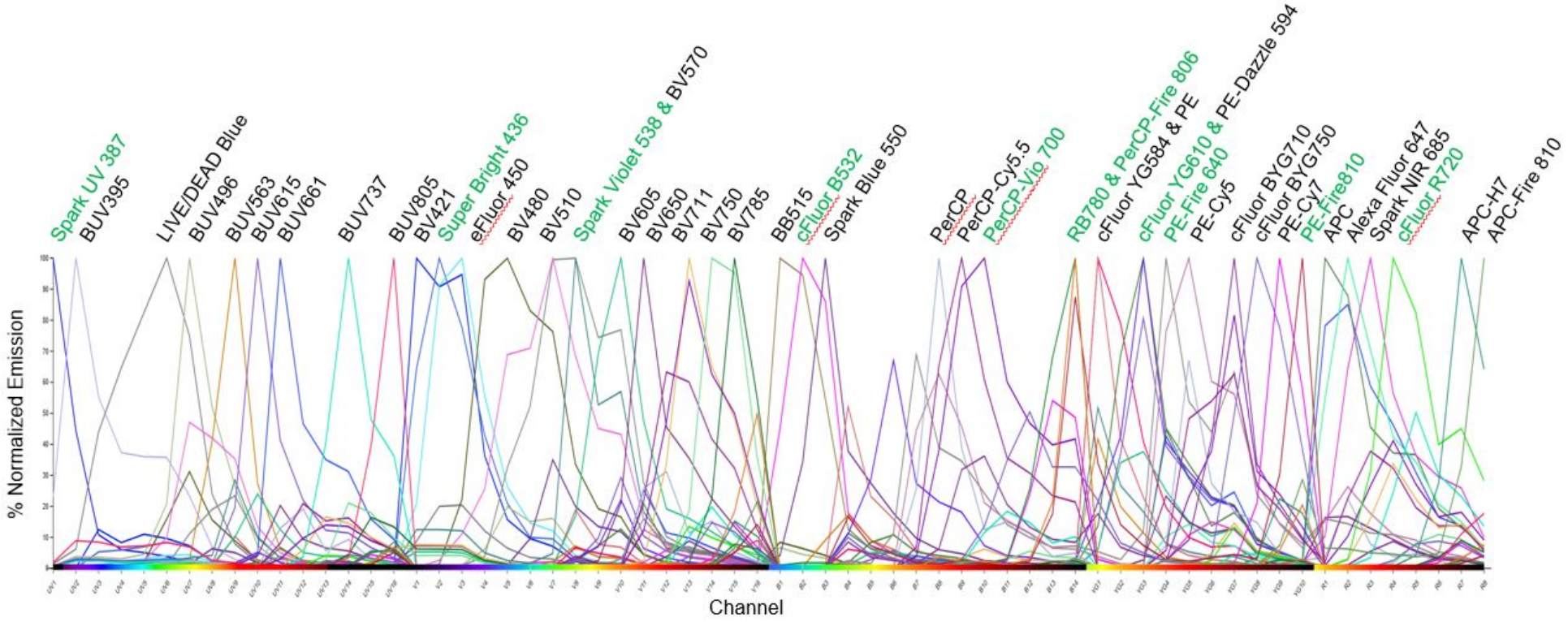
Design Of A 45-color Panel

How to decide what new fluorochromes can be added to the panel?

Cytek Cloud

Design Of A 45-color Panel

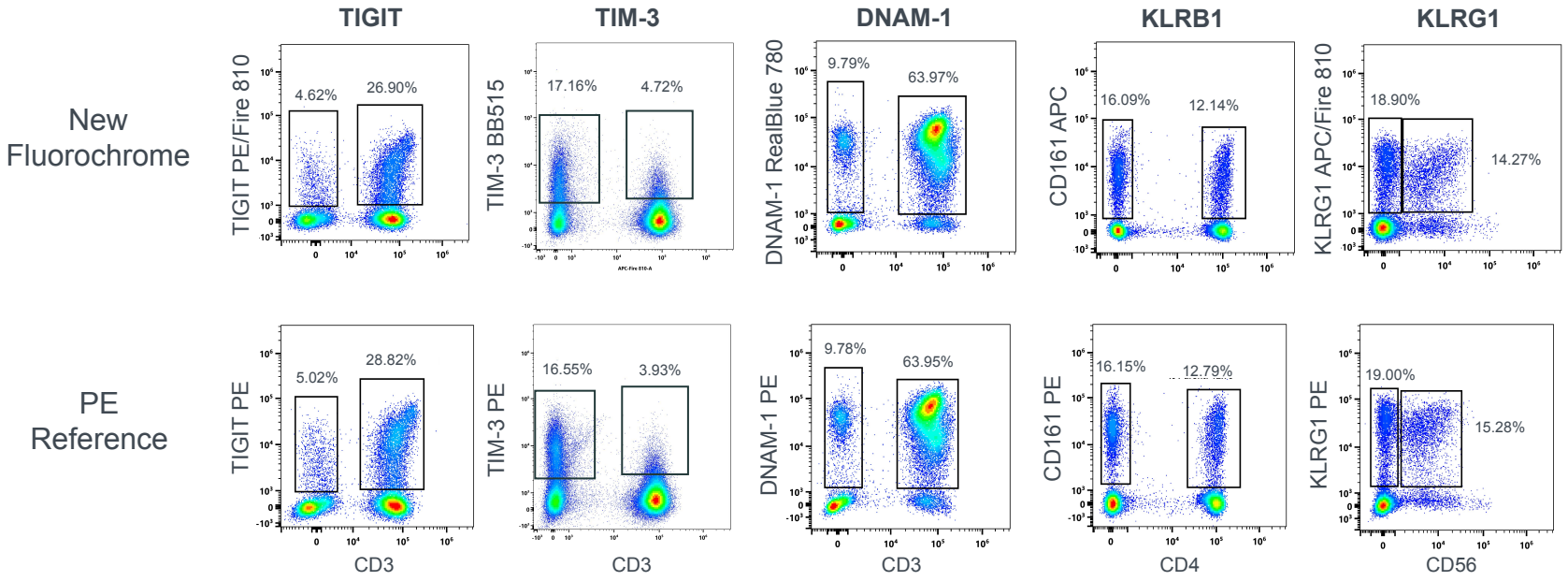
Spectral signatures overlaid using the Cytek Cloud



Evaluation Of New Reagent Performance

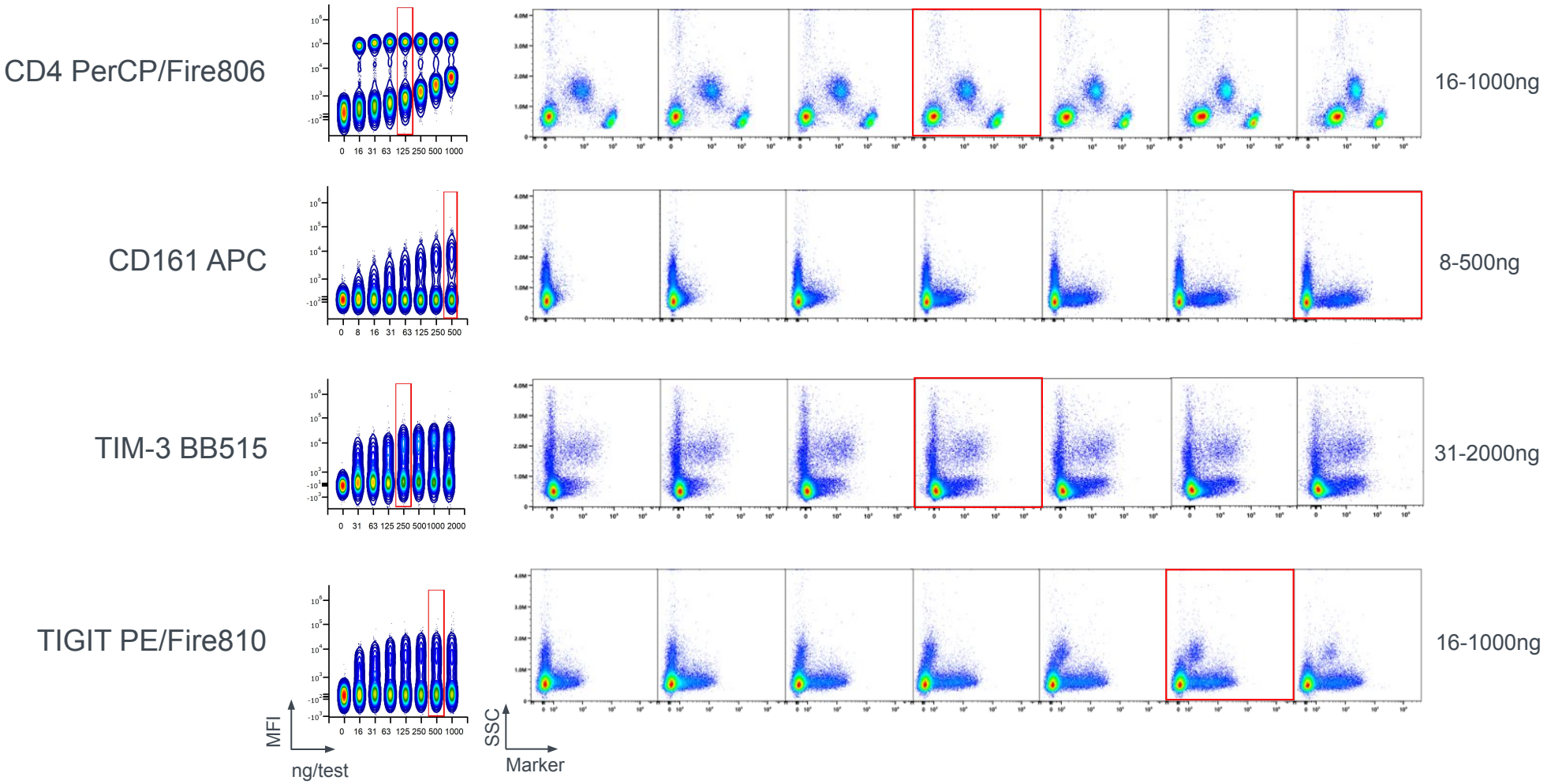
Newly introduced reagents showed equivalent performance when compared to the same clone conjugated to PE

New marker	Fluorochrome
CD11b	Spark UV 387
TIM-3	BB515
DNAM-1	RB780
KLRB1	APC
KLRG1	APC/Fire810
CD103	PE/Fire 640
TIGIT	PE/Fire 810
GPR56	PE



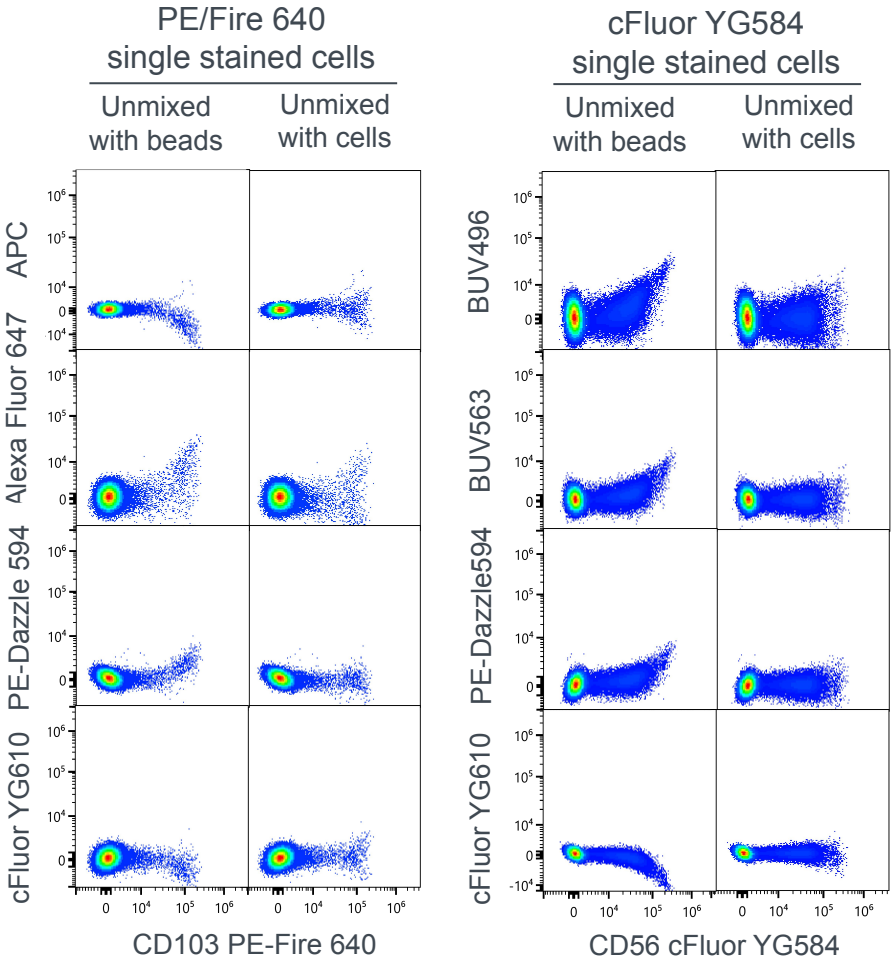
Antibody Titrations

Every reagent showed the expected staining pattern, with reduced non-specific binding. Optimal titers were determined



Reference Control Optimization

15 cell reference controls are required for accurate unmixing



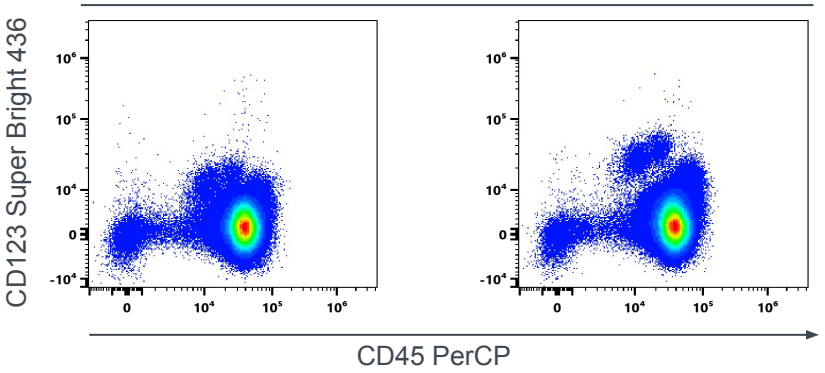
Staining Protocol Adjustments

Addition of sequential staining steps improved data resolution

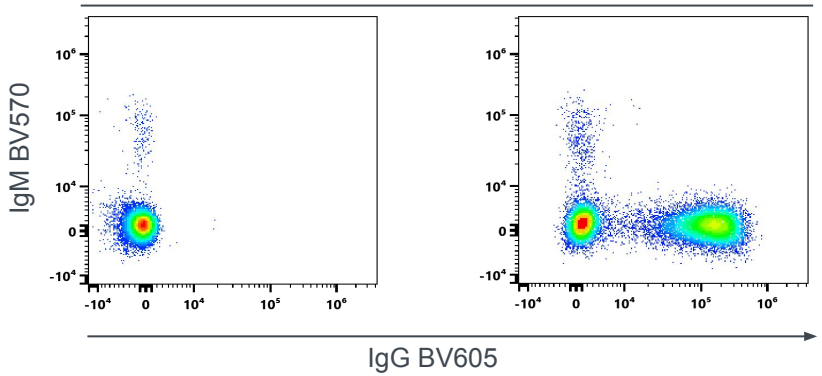
Antibody added with full cocktail

Antibody added separately

Live Gate



IgD Memory Gate



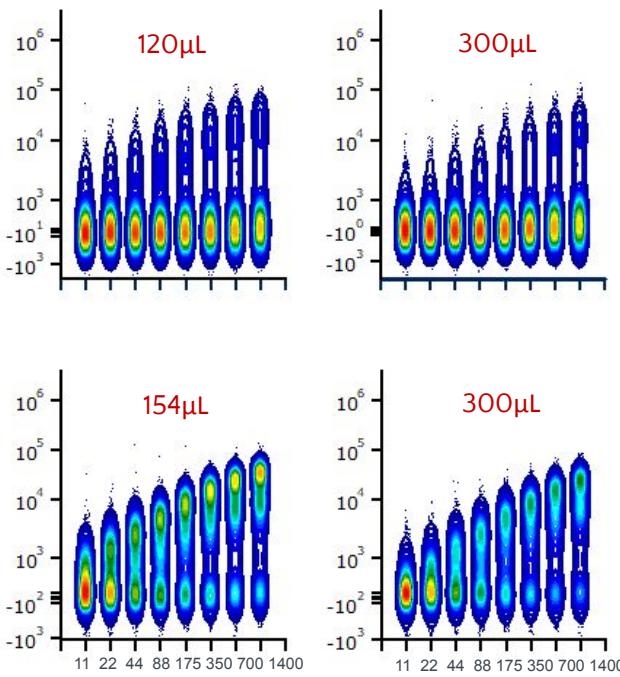
CCR5 BUV563

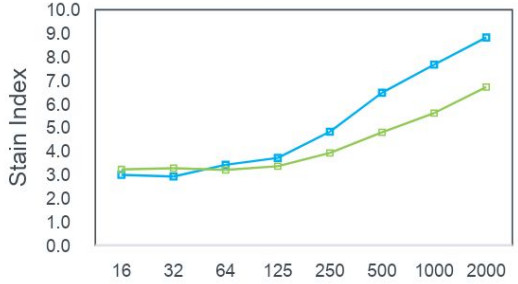
Gated on single low SSC events

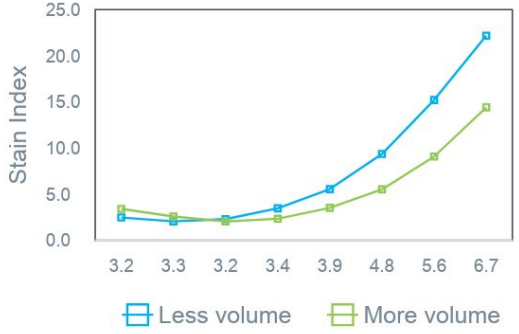
CCR7 BV421

Gated on single low SSC events

Volume at addition **Final volume**

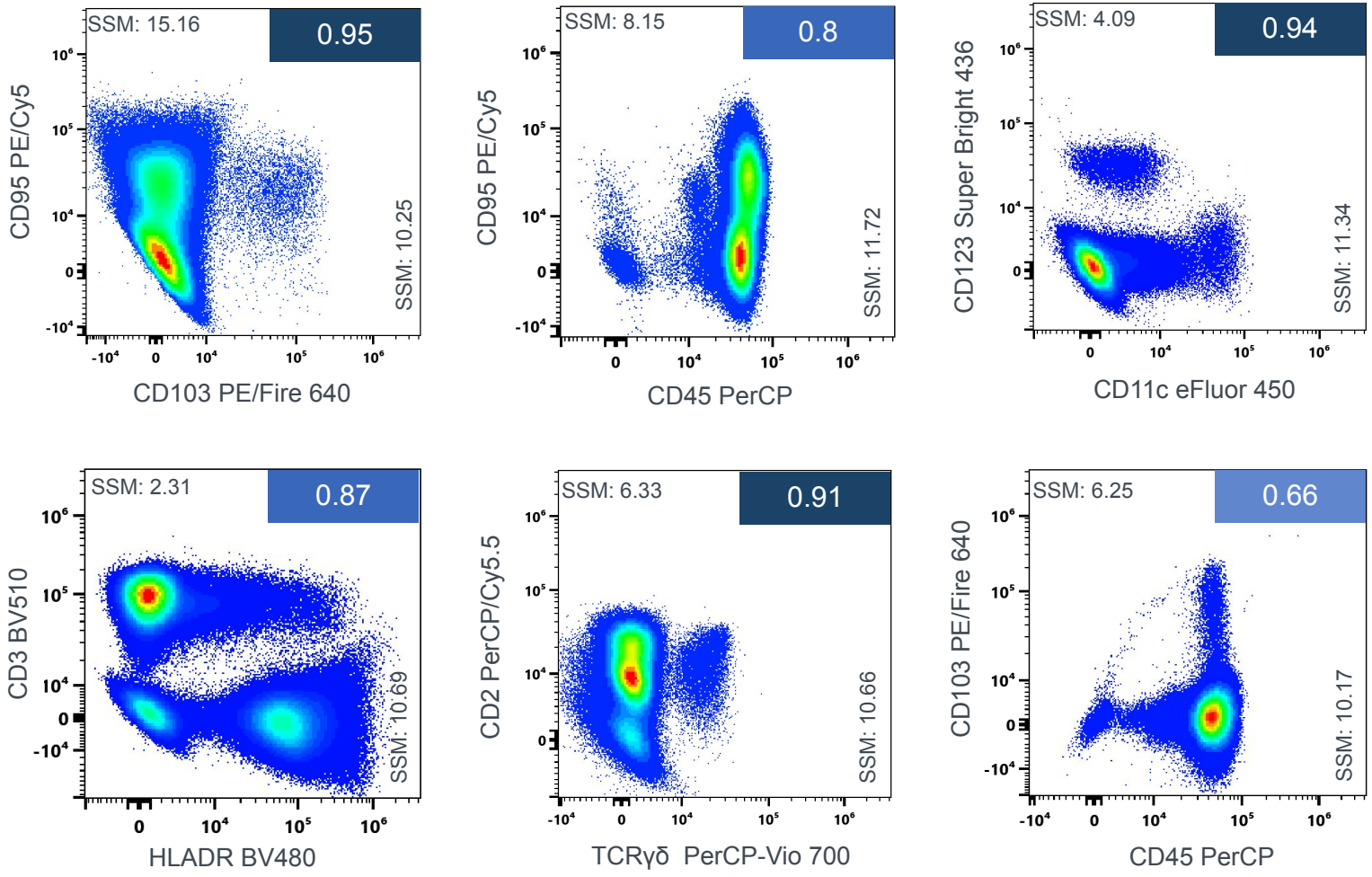






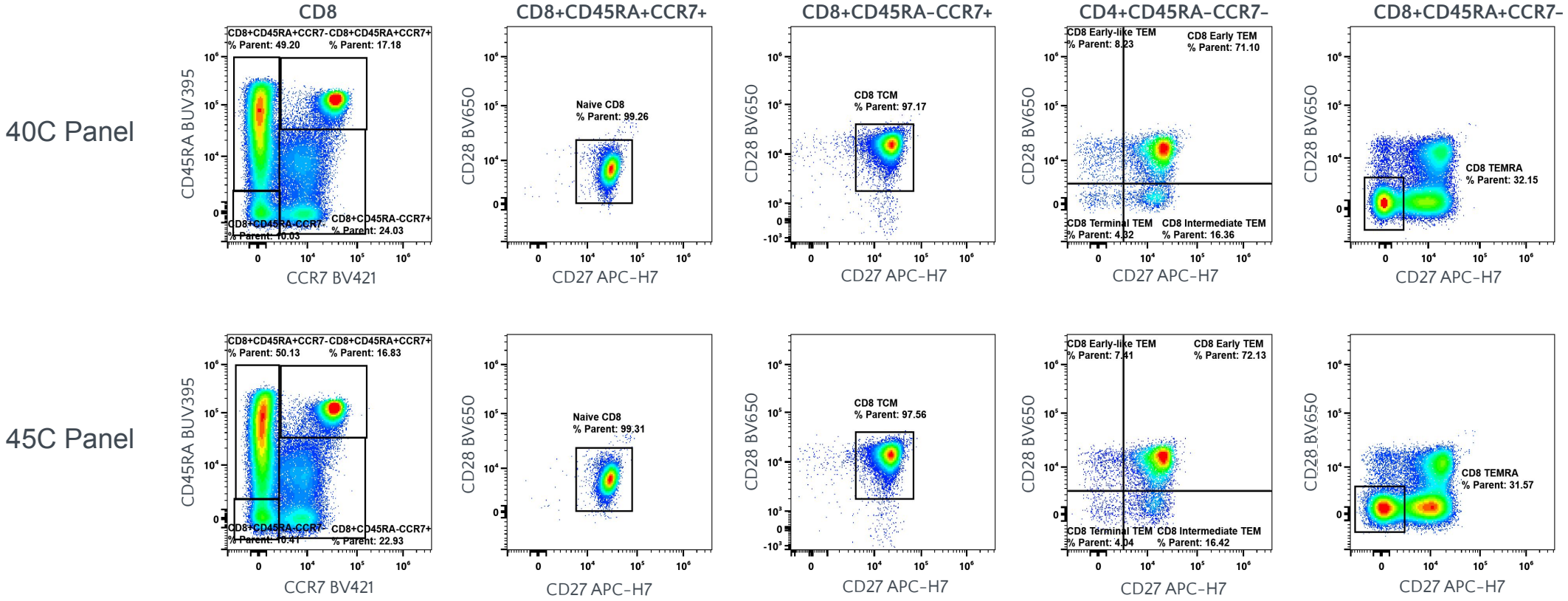
Resolution of Highly Overlapping Fluorochromes

The use of highly overlapping dyes did not impact data quality



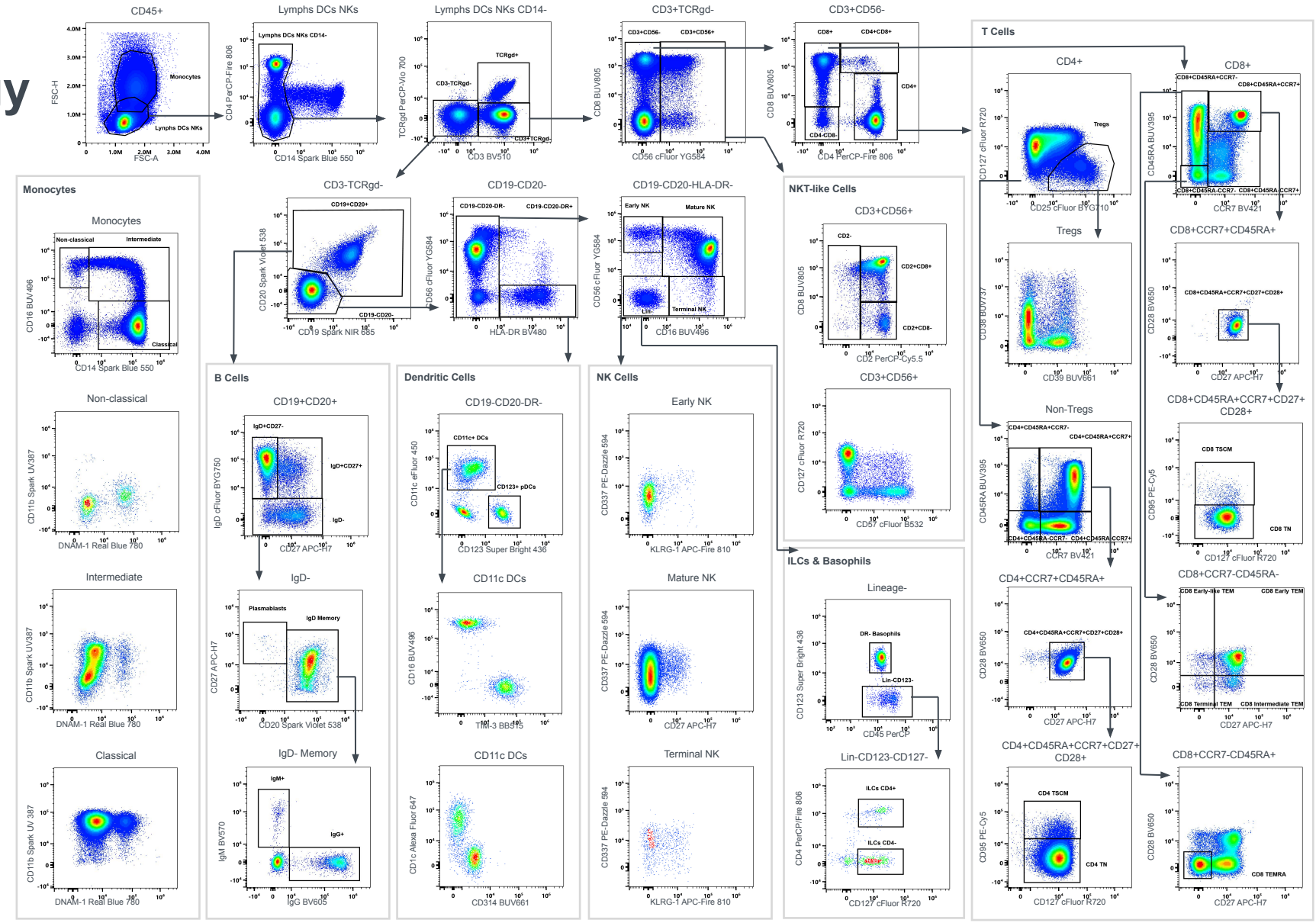
Resolution of OMIP-069 Existing Populations

Marker resolution and population frequencies were preserved for all the original reagents



Overall Gating Strategy

Each population of interest was identified and gated successfully



STEP 1 Challenges with reagents

STEP 2 Fluor substitutions

STEP 3 Panel Design

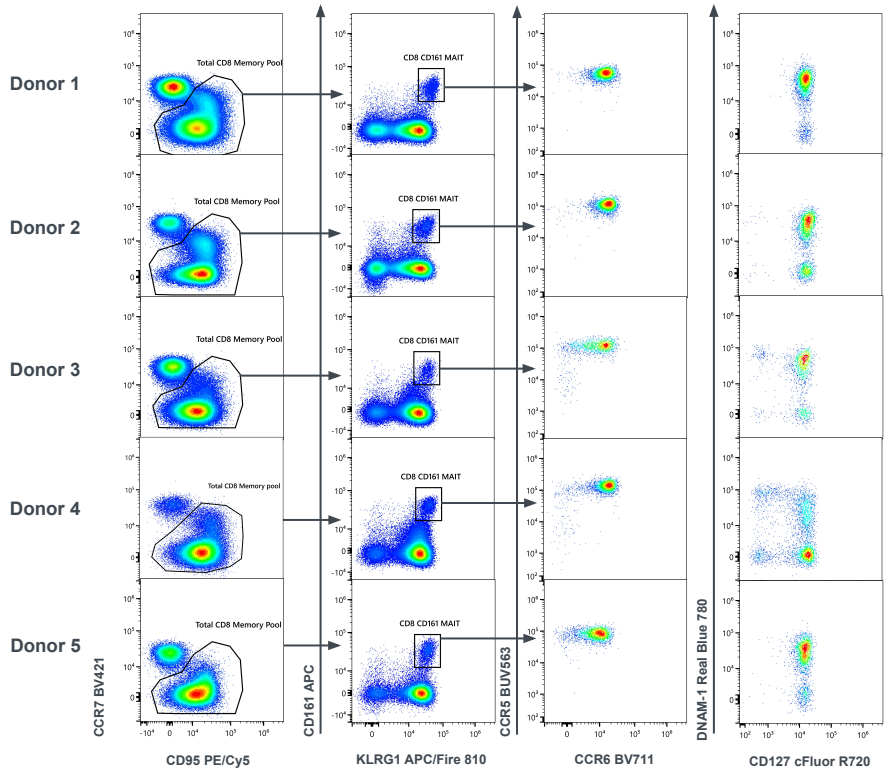
STEP 4 Panel Optimization

STEP 5 Data Analysis

STEP 6 Functional Evaluation

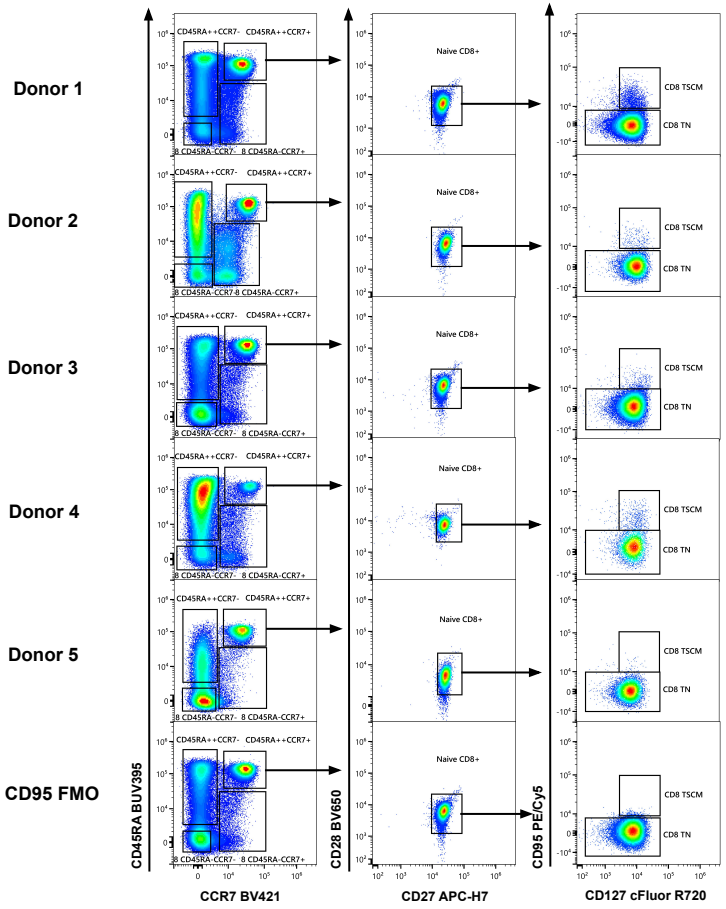
CD8+ MAIT Cell Identification

Mucosa-associated invariant T cells characterized by expression of CD161 and KLRG1



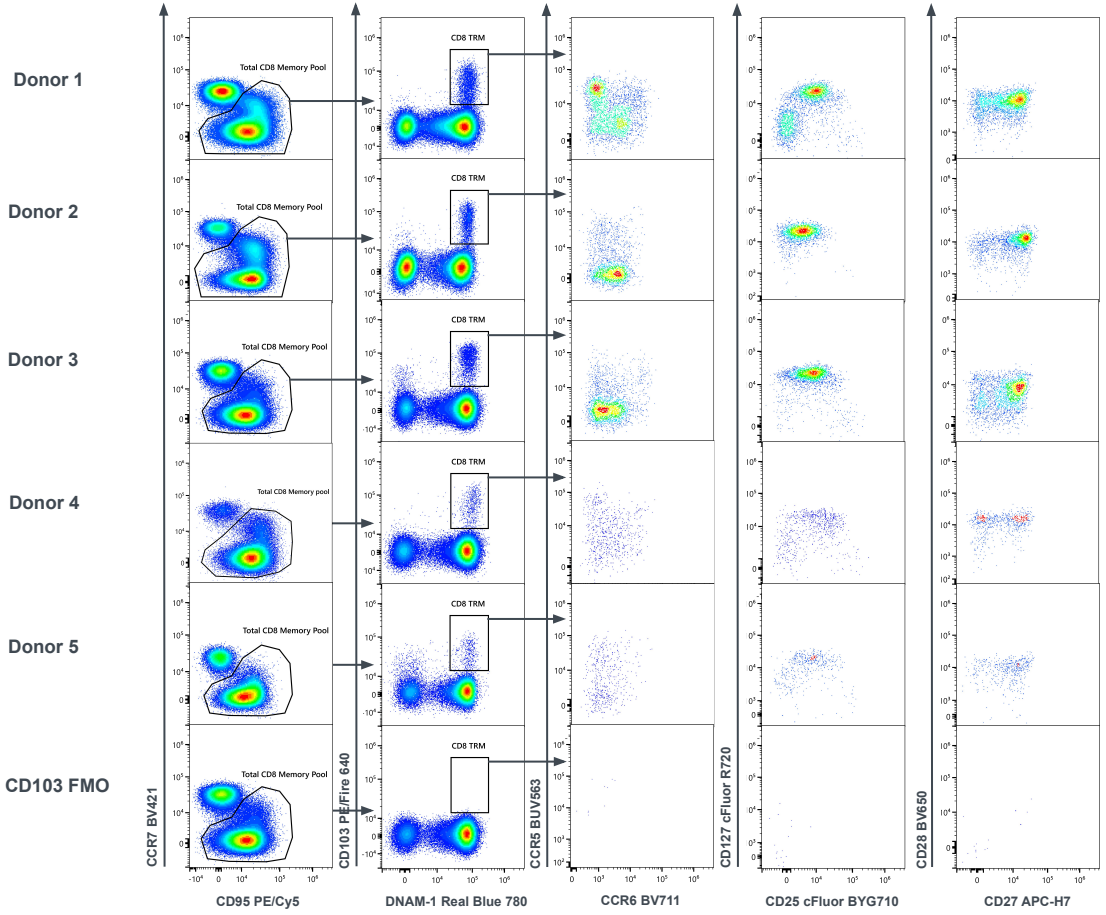
TSCM Cell Identification

T stem cell memory cells identified by expression of CD95, CD45RA, and CCR7



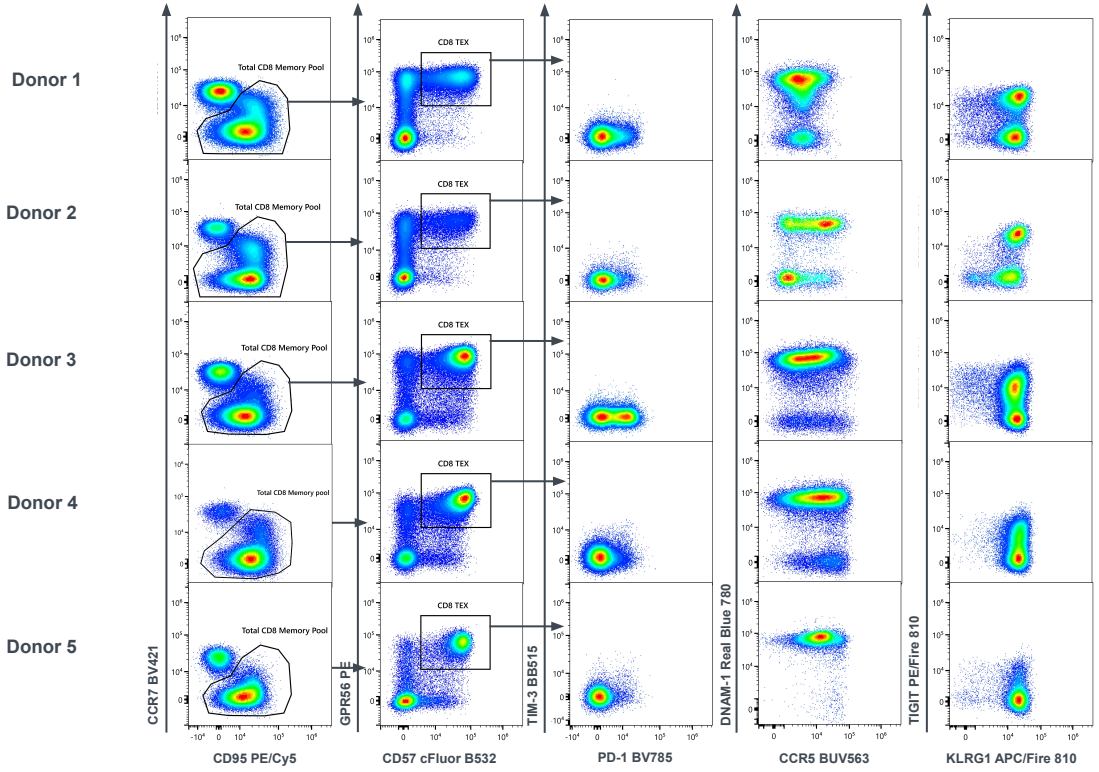
TRM Cell Identification

Tissue-resident memory T cells characterized by expression of CD103 and DNAM-1



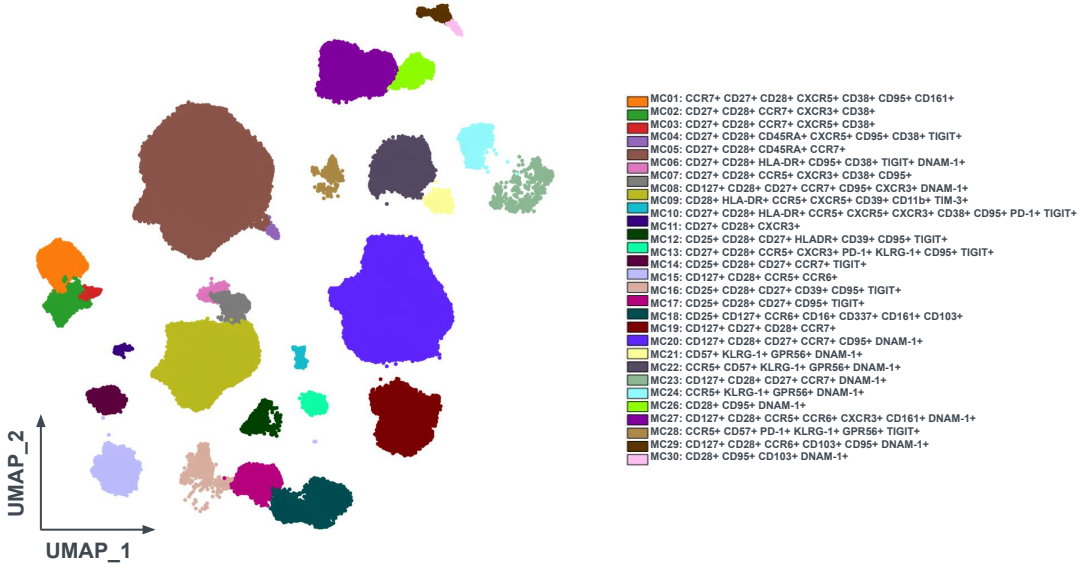
TEX Cell Identification

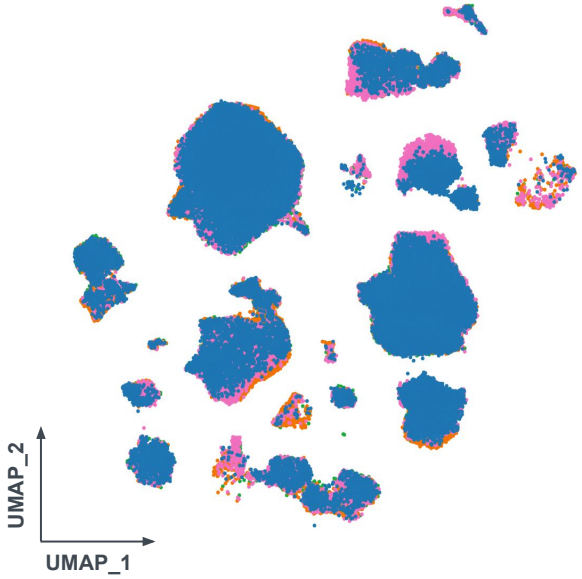
Terminal exhausted T cells identified by expression of CD57 and GPR56



Clustering Analysis

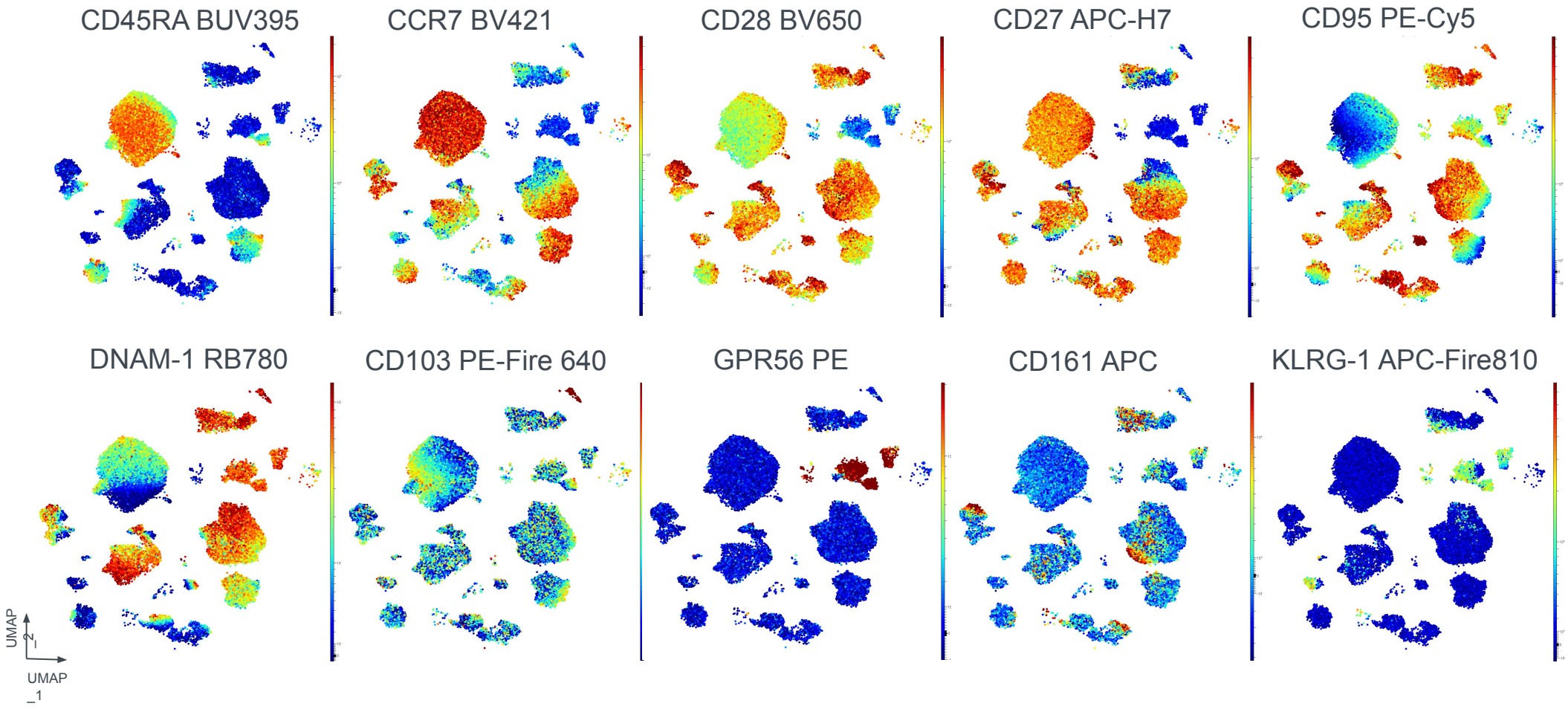
30 unique CD4 T cell metaclusters, identified using FlowSOM and UMAP, evidence the heterogeneity of the memory compartment



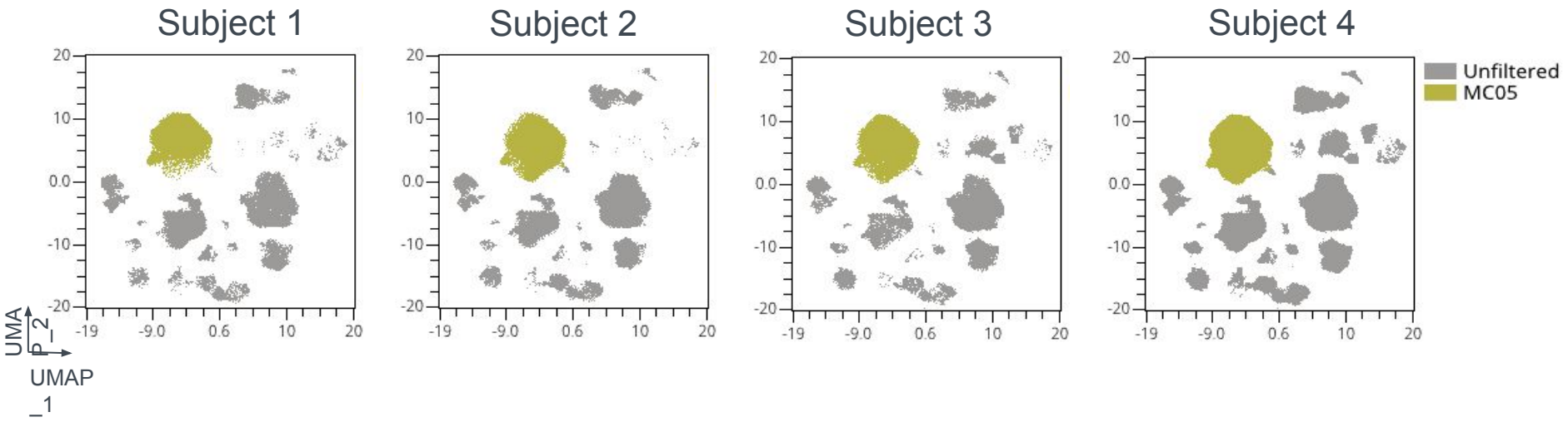


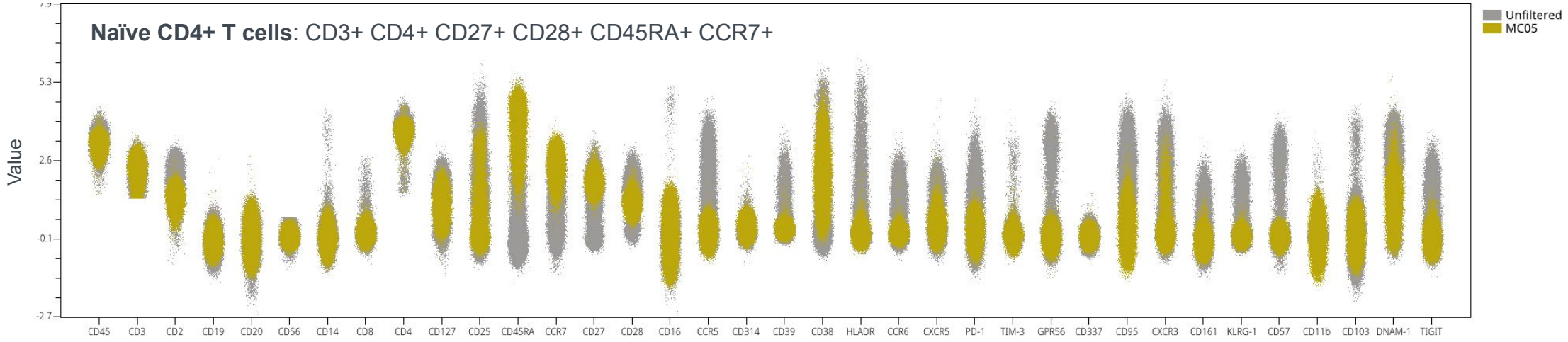
Clustering Analysis

Expected marker expression was confirmed for each metacluster

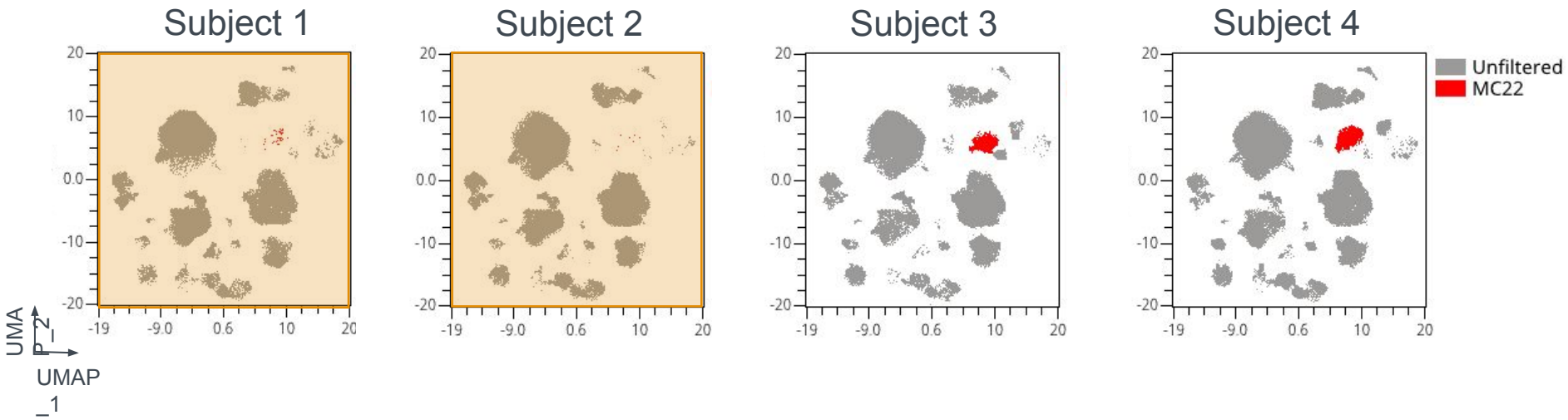


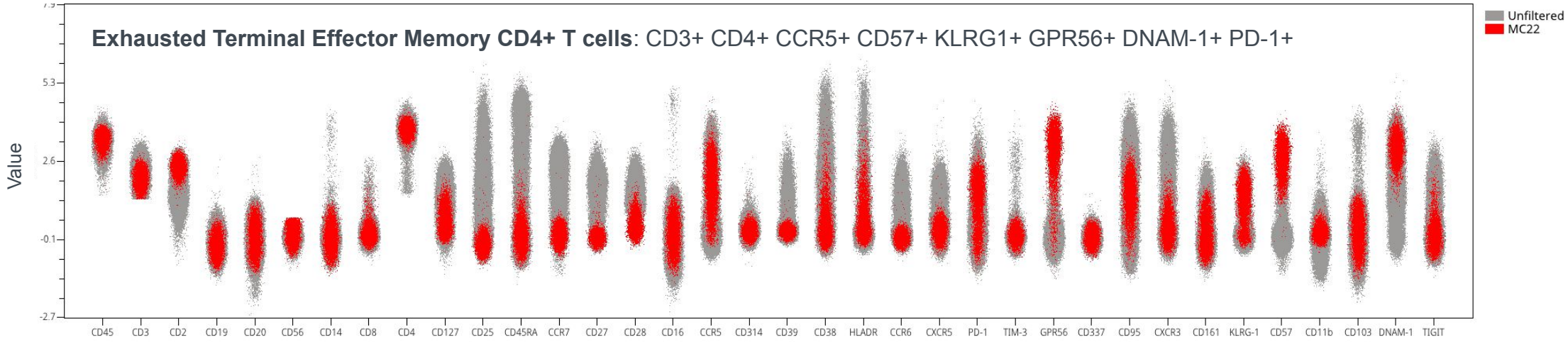
Unsupervised Multidimensional Analysis Facilitates Memory Classification





Unsupervised Multidimensional Analysis Facilitates Memory Classification



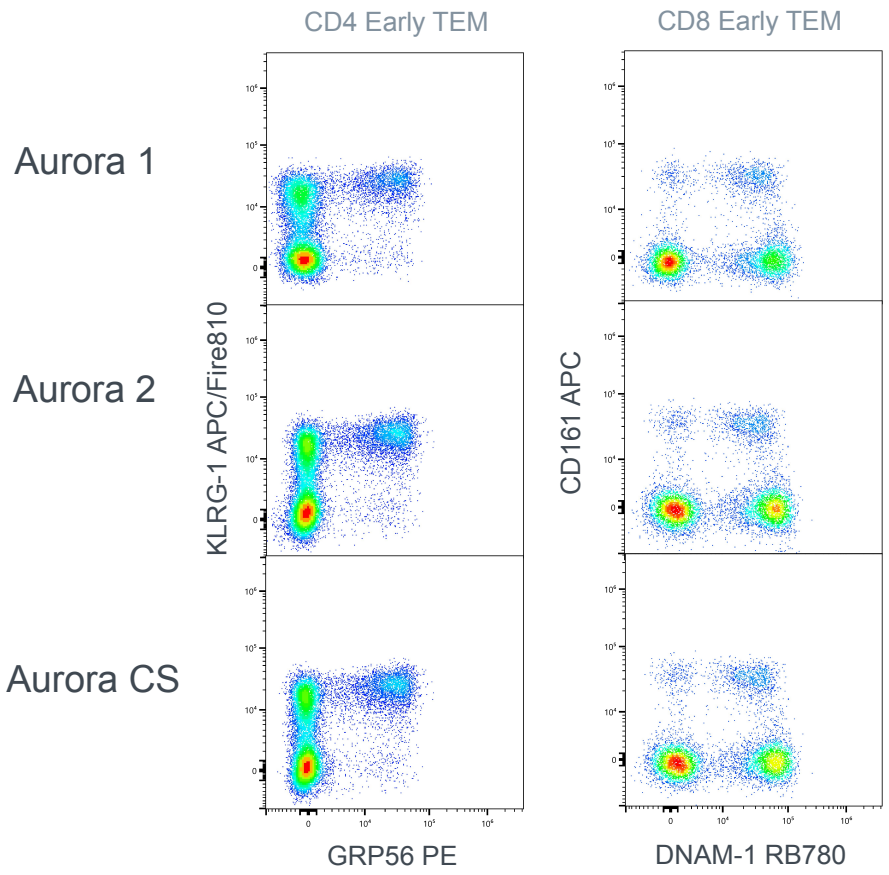


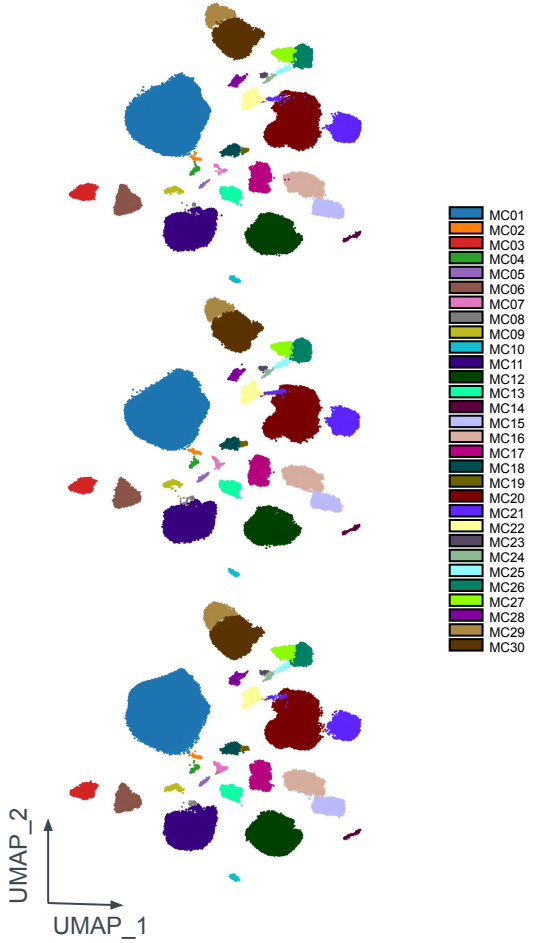
Downstream Evaluation of Memory Populations

Consistency in resolution across instruments with similar optical configuration

CYTEK AURORA™ SYSTEM

CYTEK AURORA™ CS SYSTEM

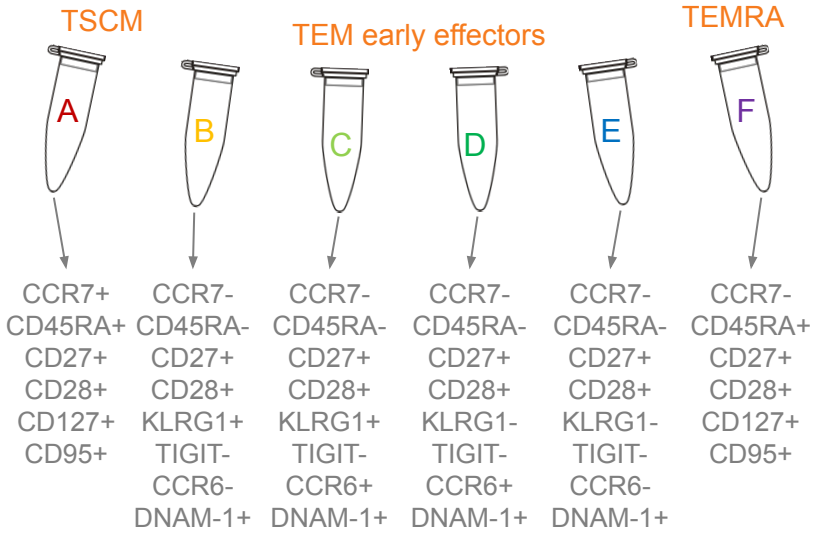


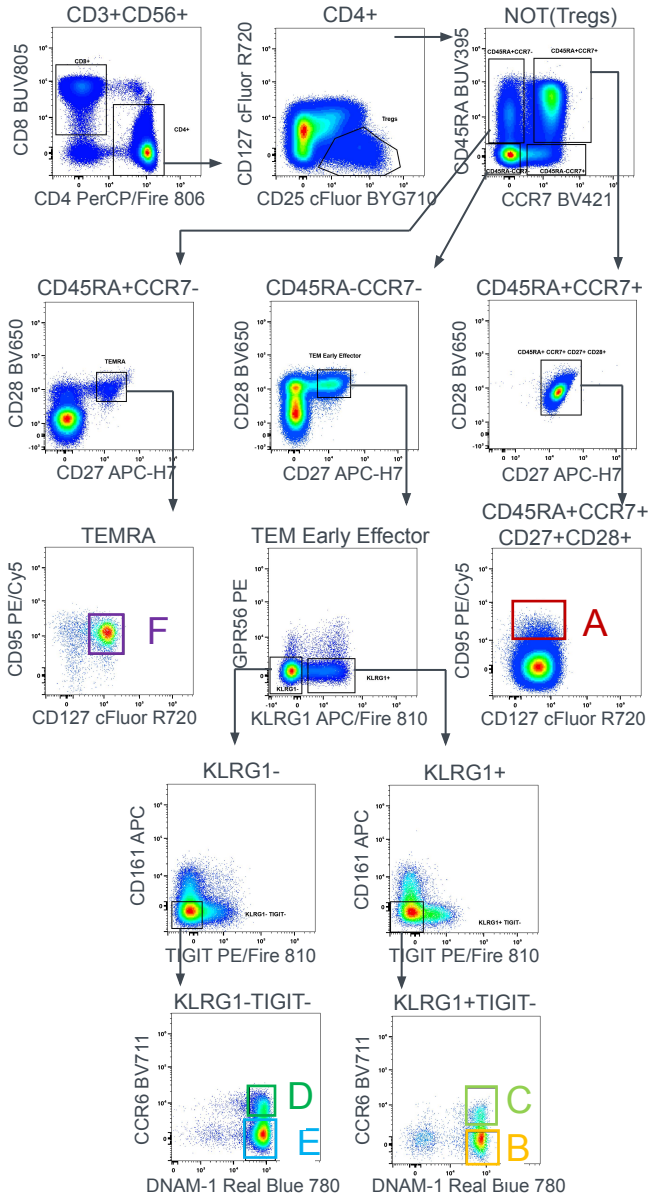


Functional Evaluation of Memory Subsets

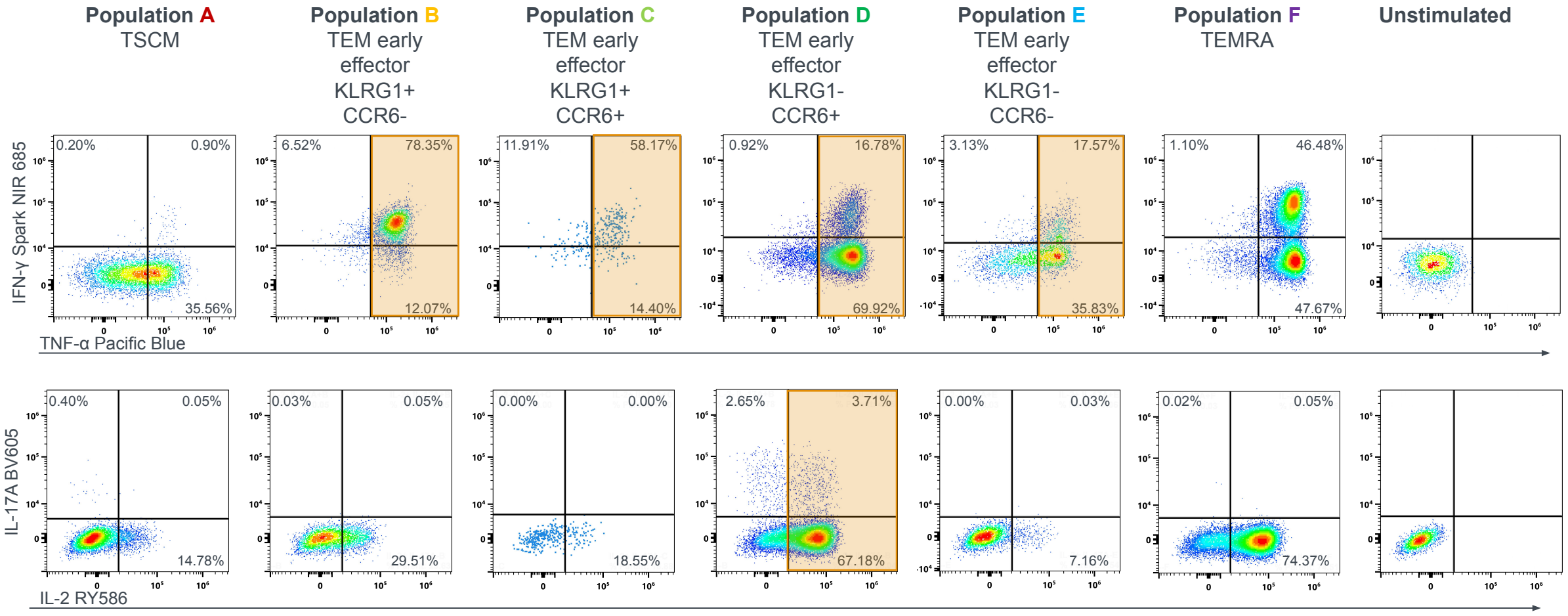
6 populations were identified for functional characterization

Sorted from CD45$^+$ CD3$^+$ CD19$^-$ CD56$^-$ CD14$^-$ CD4$^+$ CD8$^-$





Functional Evaluation of Memory T-Cell Subsets



Conclusions

1. Data quality should guide multiparametric assay optimization.

2. We developed a 45-color panel with outstanding performance across all markers.

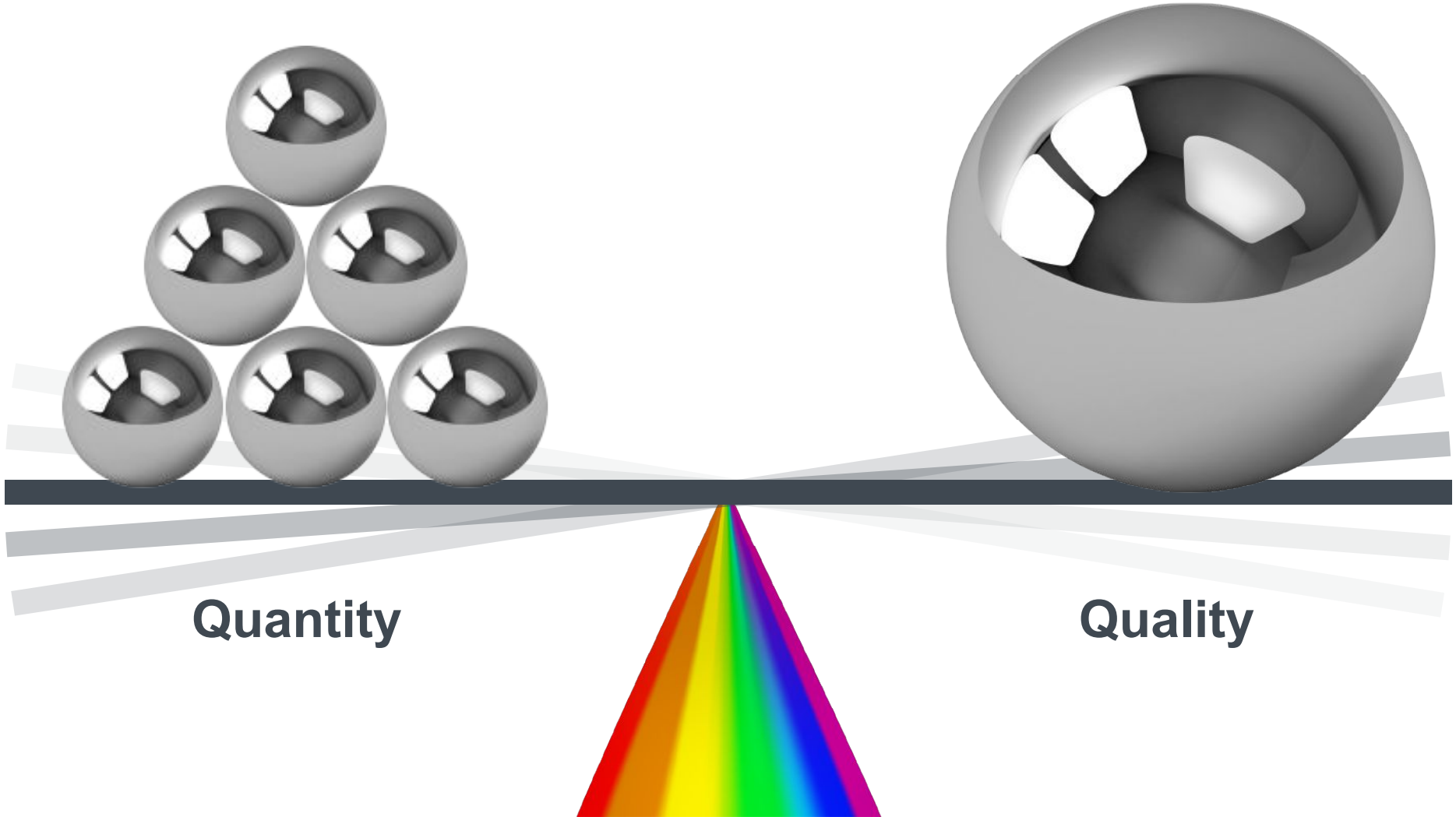
3. This panel enables deep understanding of the T cell memory compartment.

4. The combination of spectral technology and careful assay design and optimization results in a high-resolution panel with deeper and robust insight into immune monitoring for multiple diseases.

Quantity

Quality

Data quality should guide flow cytometry assay optimization



Gracias

PAF
Purificación y Análisis de Fluidos S.A.S.

VI CONGRESO LATINOAMERICANO DE BIOQUIMICA CLÍNICA

II CONGRESO INTERNACIONAL DEL COLEGIO NACIONAL DE BACTERIOLOGÍA

¡El riesgo es que te quieras quedar!

Cartagena, Colombia 3 al 6 OCTUBRE 2024

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