CHI FLOW CYTOMETRY UNIT CAPABILITIES

Up to 40-color panels for precision immunophenotyping of PBMC, T-cell subsets, and specific tissues.
Flow sorting to enrich viable, rare, and antigen specific populations for sequencing applications.

HIGH PARAMETER PHENOTYPING

A 36-color panel using a Cytek Aurora is established for characterization of cell populations spanning the broad lineages present in PBMC, from which 98 populations are routinely quantified by manual gating (Fig. 1A). This granularity of cell population quantification has been used to screen differences in PBMC phenotypes when comparing between biological conditions, or to validate changes observed by orthogonal assays (Fig. 1B-D). Additionally available in an exploratory context where optimization and validation would be required:

- 38-color T-cell focused panel with functional subsets detected by staining for transcription factors and cytokine production after PMA stimulation.

- Customization of panels to include markers for additional cell types such as neutrophils, or antigens of interest for specific tissues.

- Analysis approaches incorporating all markers assayed in unbiased or semi-supervised strategies rather than manual gating.
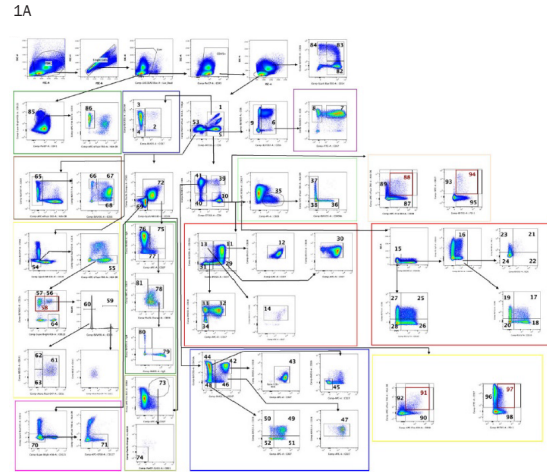
1A

Fig. 1A: Manual gating of populations
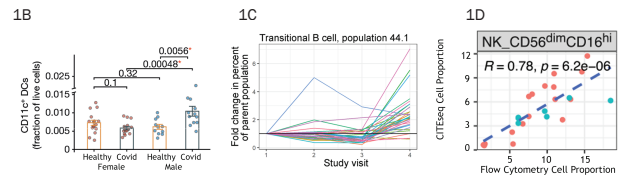
1B   1C   1D

Fig. 1B-C: Detection of differences in populations
Fig. 1D: Correlation with CITE-seq3

FLOW SORTING FOR SINGLE CELL SEQUENCING

Up to 4-way sorting based on 12 fluorescence parameters is available using a Sony MA900. This is typically used for enrichment of cell populations prior to single cell sequencing, where examples have included:

- Selection of viable cells from preparations of tissue biopsies.

- Enrichment of low frequency populations of interest such as γδ T cells.

- Antigen-specific cells with optimization to obtain sequencing data from <10,000 sorted cells.

Markers in 36c phenotyping panel	
CD1c	CD57
CD3	CD95(Fas)
CD4	CD123
CD8	CD127
CD11c	CD141
CD14	CD161
CD16	CD183 (CXCR3)
CD19	CD185 (CXCR5)
CD20	CD196 (CCR6)
CD21	CD197 (CCR7)
CD24	CD278 (ICOS)
CD25	CD1279 (PD-1)
CD27	HLA-DR
CD28	IgD
CD38	IgG
CD45	IgM
CD45RA	TCRgd
CD56	Viability

References:
1. Sparks et al., Nature, 2023;
2. Apps et al., JCI Insight, 2020;
3. Liu et al., Cell, 2021

NIH Center for Human Immunology, Inflammation and Autoimmunity

National Institute of Allergy and Infectious Diseases

James M. Cherry, Ph.D., Chief of Operations | Iyadh Douagi, Ph.D., Scientific Director

TO ACCESS, CONTACT:

Dr. Richard Apps | Richard.Apps@nih.gov

NIH Center for Human Immunology, Inflammation and Autoimmunity