Automated and high-throughput T-cell immunophenotyping assays using Celigo and Cellaca® PLX image cytometers

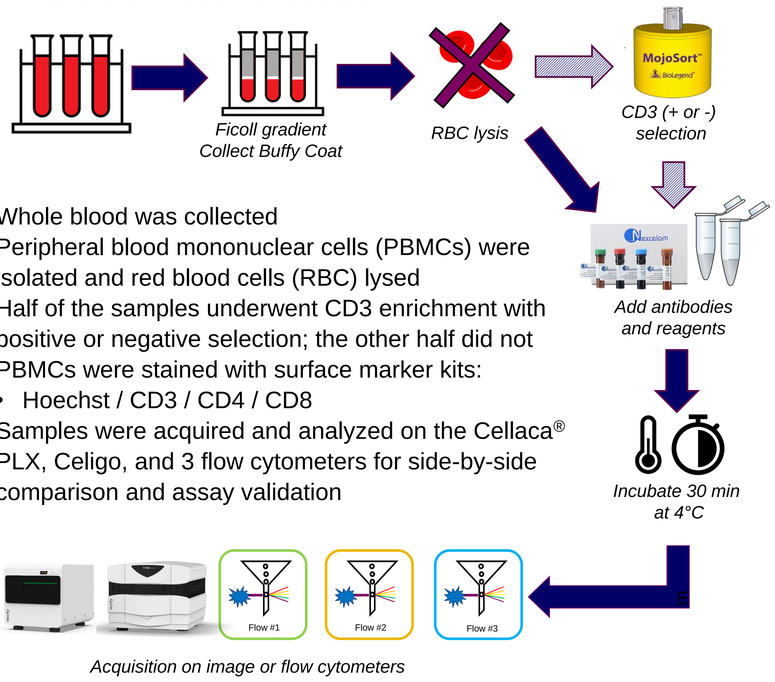
Daniel Browe[1], Carolina Franco Nitta[1], Mackenzie Pierce[1], Jeanne Elia[2], Jen Ruiz[2], Art-Danniel Hipol[2], Henry Qazi[1], Sarah Kessel[1], Dmitry Kuksin[1], Eunice Mejia[2], Bo Lin[1], Timothy Smith[1], Natalie Schwartz[1], Josh Croteau[2], Nicolas Schrantz[2], Xifeng Yang[2], and Leo Li-Ying Chan[1]

[1]Department of Advanced Technology R&D, Revvity, Lawrence, MA 01843, USA
[2]Department of Cell Analysis, BioLegend, Inc., San Diego, CA 92121, USA

1 Introduction

The importance and impact of cell therapies is growing in the field of medicine, underlining the need for speed and accuracy in cell counting and phenotyping. The safety and efficacy of cell therapy products depends on accurately quantifying cell identity, purity, viability, and concentration. Automated cell counters are essential for satisfying these needs within the aggressive timelines of the clinic. We previously validated the Cellaca PLX image cytometer against three different flow cytometers, where freshly isolated PBMCs were assayed for T cell markers (CD3, CD4, and CD8) on all instruments. The corresponding population percentages between the four instruments were within 5.0%, indicating that the image cytometer results can match the accuracy of flow cytometers. The Celigo image cytometer is capable of whole-well brightfield and multi-channel fluorescent imaging (up to 4 channels) for both adherent and suspension cells within numerous vessel types. Coupled with a low auto-fluorescent slide, we demonstrate here that it can also be used for immunophenotyping, where results were validated using paired stained samples.

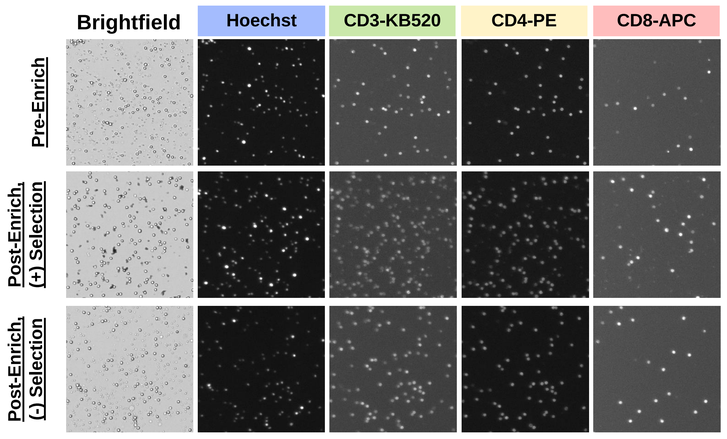
2 Materials and Methods



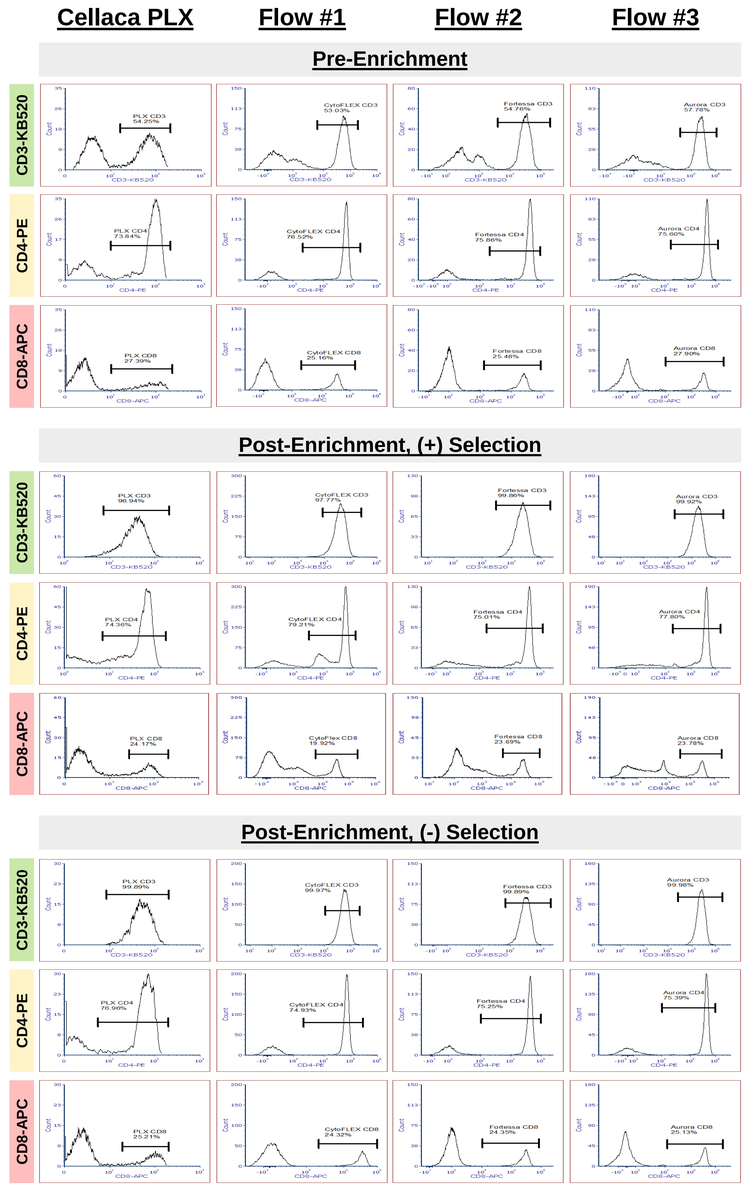
1. Whole blood was collected
2. Peripheral blood mononuclear cells (PBMCs) were isolated and red blood cells (RBC) lysed
3. Half of the samples underwent CD3 enrichment with positive or negative selection; the other half did not
4. PBMCs were stained with surface marker kits:
   - Hoechst / CD3 / CD4 / CD8
5. Samples were acquired and analyzed on the Cellaca® PLX, Celigo, and 3 flow cytometers for side-by-side comparison and assay validation

3 CD3 / CD4 / CD8 / Hoechst on Pre- and Post-enriched PBMCs in Image and Flow Cytometry Platforms

Cellaca PLX Image Cytometer



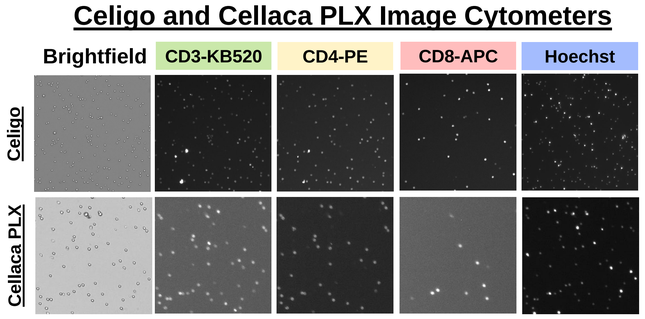
CD3 Enrichment		Cellaca PLX	Flow #1	Flow #2	Flow #3
Pre	CD3-KB520	54.25%	53.03%	54.76%	57.78%
	CD4-PE	73.84%	76.52%	75.86%	75.60%
	CD8-APC	27.39%	25.16%	25.48%	27.90%
Post, (+) selection	CD3-KB520	96.94%	97.77%	99.86%	99.92%
	CD4-PE	74.36%	79.21%	75.01%	77.80%
	CD8-APC	24.17%	19.92%	23.69%	23.78%
Post, (-) selection	CD3-KB520	99.89%	99.97%	99.89%	99.98%
	CD4-PE	76.96%	74.93%	75.25%	75.39%
	CD8-APC	25.21%	24.32%	24.35%	25.13%

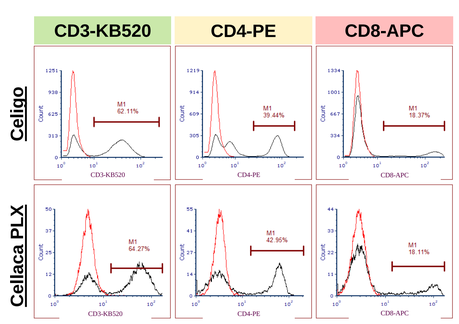


- Freshly isolated PBMCs post-RBC lysis were enriched for CD3 using the MojoSort system for either negative (-) or positive (+) selection
- All samples were stained with CD3-KIRAVIA Blue 520™ (KB520), CD4-PE, and CD8-APC (or their respective isotype controls), and Hoechst (total) following manufacturer's protocol
- Paired stained samples were imaged on the Cellaca PLX and three different flow cytometers
- CD3 / CD4 / CD8 populations are highly comparable for each sample type and between each system (image or flow cytometer) independent of negative or positive enrichment for CD3
- Prior to enrichment, ~55% of PBMCs were CD3+, while post-enrichment that population increased to ~100%, indicating that both positive and negative enrichment processes are successful at separating cell populations

4 CD3 / CD4 / CD8 / Hoechst Comparison of Image Cytometer Systems

Celigo and Cellaca PLX Image Cytometers





Antibody	Celigo	Cellaca PLX
CD3-KB520	62.11%	64.27%
CD4-PE	39.44%	42.95%
CD8-APC	18.37%	18.11%

- Freshly thawed PBMCs were stained with CD3, CD4, and CD8 antibodies (or their respective isotype controls), and Hoechst following the manufacturer's protocol
- Paired stained samples were imaged on the Cellaca PLX and Celigo

- T-cells are marked by CD3-KIRAVIA Blue 520™ (KB520) in the green channel, which are then sub-divided into helper T-cells (CD4-PE; orange channel) and cytotoxic T-cells (CD8-APC; far-red channel)
- CD3 / CD4 / CD8 population percentages were highly consistent in the single stained controls (data not shown) and multiplexed samples in the Cellaca PLX or Celigo
- The corresponding T-cell population percentages between the two image cytometers were within 5.0%, indicating that results are comparable
- The signal (stained cells) and background differs between the two instruments, as the settings are specific to each system
- All antibodies tested showed high signal-to-background ratios (above 10) in both the Cellaca PLX and Celigo

5 Conclusions / Future Directions

- Cell populations were highly comparable (within 5%) among the systems tested
  - Cellaca PLX and three flow cytometers
  - Cellaca PLX and Celigo
- Results suggest that image cytometers may provide a rapid alternative and orthogonal method for immunophenotyping, cell counts, and viability measurements
- The proposed method can streamline T-cell therapy workflows from sample staining to data analysis quickly moving precious patient samples downstream within development processes
- Image cytometers, capable of automated simple surface marker detection, can be used in the fields of cell & gene therapy and immuno-oncology (IO) without the need for highly complex flow cytometers
- Additional immunophenotyping panels for NK cells, B cells, monocytes, macrophages, and other genetically modified cell types, such as CAR T, CAR NK, or T cell receptor (TCR) are critical for future image cytometric application development in cellular therapy fields

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Revvity, 360 Merrimack Street, Builiding 9, Lawrence, MA 01843, USA (978) 327-530 www.revvity.com