Development of a high-throughput image cytometric screening method as a research tool for immunophenotypic characterization of patient samples from clinical studies

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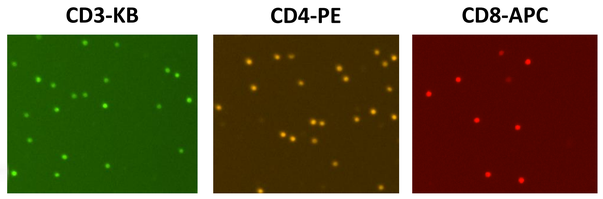
1. ABSTRACT

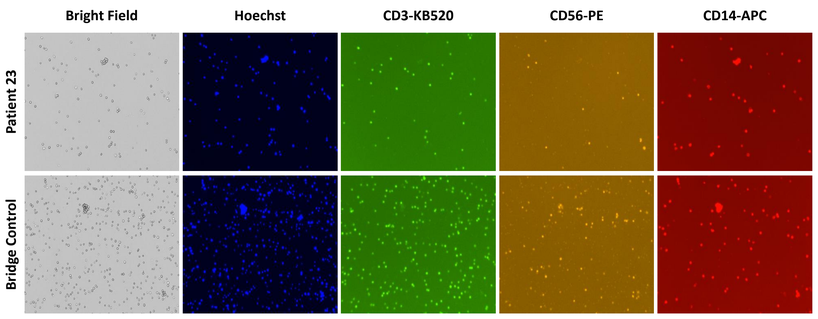
Immunophenotyping has been the primary assay for characterization of immune cells from patients undergoing therapeutic treatments in clinical research, which is critical for understanding disease progression and treatment efficacy. Currently, flow cytometry is the main methodology for characterizing surface marker expression for immunological research and has been proven to be an effective and efficient method for immunophenotyping; however, it requires highly trained users and a large time commitment. As such, there is a need for a higher throughput method for routine surface marker expression analyses that have been identified to determine early indicators of disease development, disease prognosis, or treatment effectiveness. Recently, a novel image cytometry system (Cellaca® PLX Image Cytometer, Revvity Health Sciences, Inc., Lawrence, MA) was developed as a complementary method to flow cytometry for performing high-throughput immunophenotyping. Effectively combining these two immunophenotyping methodologies will allow for a comprehensive analysis and continuous monitoring of critical surface markers of interest in a high-throughput manner. In this work, we demonstrate a high-throughput image cytometric screening method to characterize immune cell populations, streamlining the analysis of routine surface marker panels. The T cell, B cell, NK cell, and monocyte populations of 46 primary PBMC samples from subjects enrolled in autoimmunity and oncology disease study cohorts were analyzed with two optimized immunophenotyping staining kits: Panel 1 (CD3-Kiravia Blue™, CD56-PE, CD14-APC) and Panel 2 (CD3-Kiravia Blue™, CD56-PE, CD19-APC). We validated the proposed image cytometry method by comparing the Cellaca® PLX and the Aurora (Cytek®) cytometer, which generated bright field and fluorescent images, as well as scatter plots for the 46 primary PBMC samples and 2 controls. In addition, the image cytometry method can directly determine cell concentrations for downstream assays. The results demonstrated comparable CD3, CD14, CD19, and CD56 cell populations from the primary PBMC samples, which showed an average of 5 – 10% differences using the Bland-Altman statistical analysis. The proposed image cytometry method provides a novel research tool to streamline immunophenotyping workflow for characterizing patient samples in clinical studies.

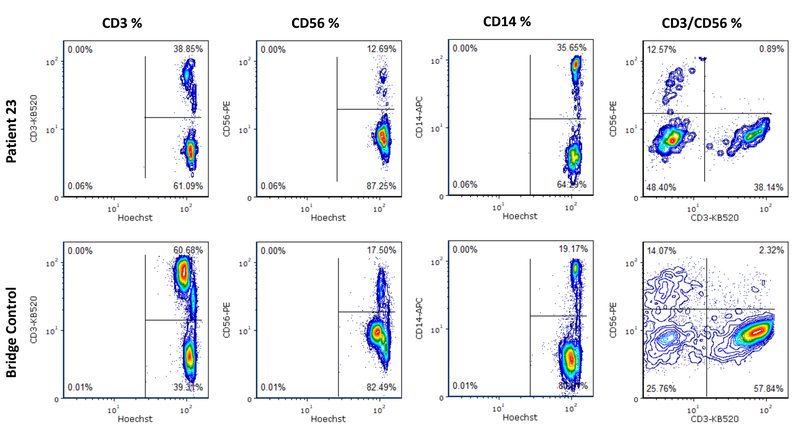
2. CELLACA® PLX IMAGE CYTOMETRY



1. Cellaca® PLX is a high-throughput image cytometer that uses an epifluorescence optical path for brightfield and fluorescence-based image cytometric analysis
2. The system uses four excitation LEDs (365, 470, 531, and 620nm) and five emission filters (452, 534, 605, 655, 692 nm) to acquire images using the Cellaca® PLX software (Matrix 5.0)
3. Two immunophenotyping panels were used to stain T cells, NK cells, B cells, and Monocytes in primary PBMC samples
    1. CD3-KB/CD56-PE/CD14-APC
    2. CD3-KB/CD56-PE/CD19-APC
4. Applications: Immunophenotyping, cell and gene therapy, apoptosis, viability
5. Requires only 15uL of sample for immunophenotyping analysis .

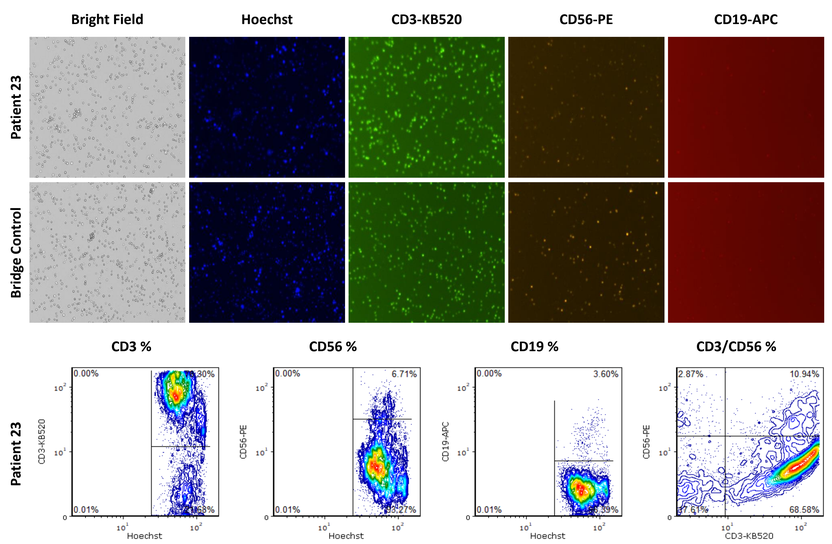
3. T CELL, NK CELL AND MONOCYTE PANEL

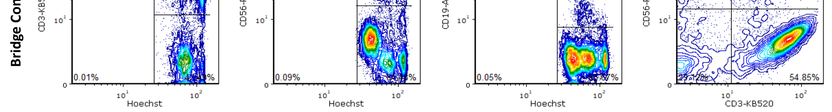




- Two antibody panels were used to stain 23 patient samples for both cohorts and one bridging control. A total of 46 patient samples and two bridging controls .
- The Brightfield, Hoechst+, CD3+, CD56+, and CD14+ images were captured using the Cellaca® PLX. Antibodies were chosen based on select variety of Revvity available kits that were compatible with the flow cytometry protocol chosen.
- The dot plots were also acquired using the Cellaca PLX results made using FCS Express
- Patient samples acquired from two disease cohorts for analysis (Multiple Myeloma and Rheumatoid Arthritis)
- Hoechst stains all nucleated cells, CD3-KB stains all CD3+ cells, CD56-PE stains all CD56+ cells, and CD14-APC stains all CD14+ cells.

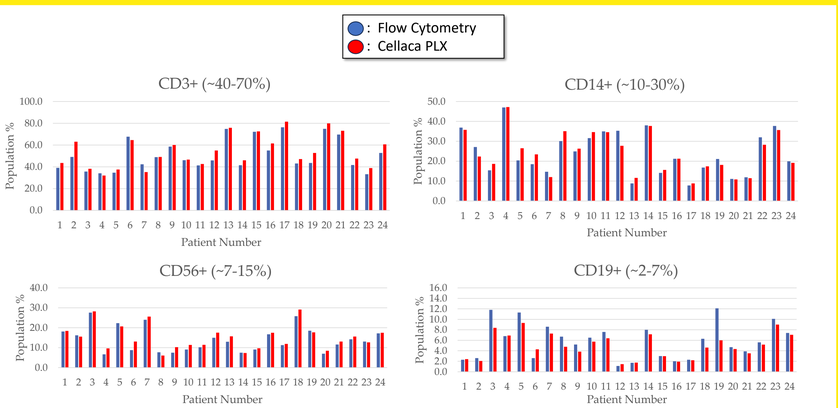
4. T CELL, NK CELL, AND B CELL PANEL





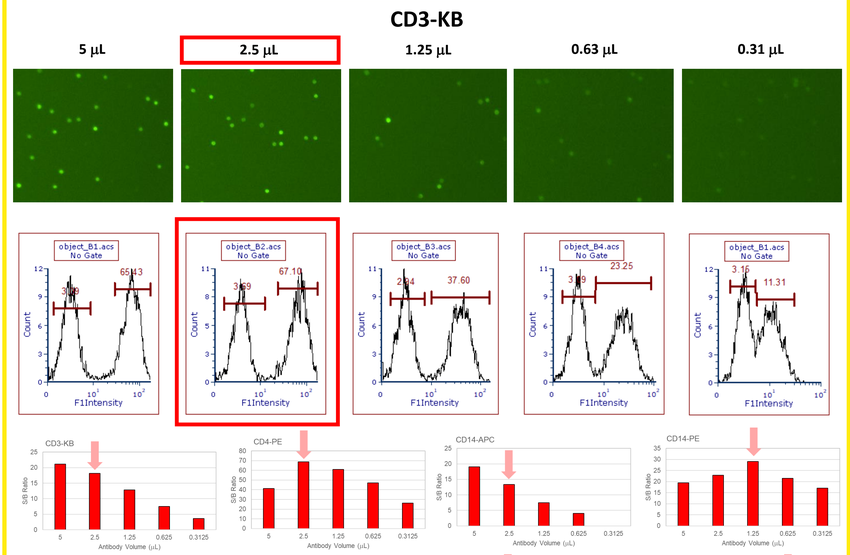
- Images above shown were captured using the Cellaca® PLX in each channel
    - Brightfield, Hoechst+, CD3+, CD56+, and CD19+
- Two different samples are shown where Patient 23 is shown in the 1st row . The bridging control is shown in the second row.
- Patient samples were acquired from two disease cohorts for analysis (Multiple Myeloma and Rheumatoid Arthritis)
- Hoechst stains all nucleated cells, CD3-KB stains all CD3+ cells, CD56-PE stains all CD56+ cells, and CD19-APC stains all CD19+ cells.
- Cellaca PLX analysis can show percentage of negative and positive population variations using antibodies for both kits.

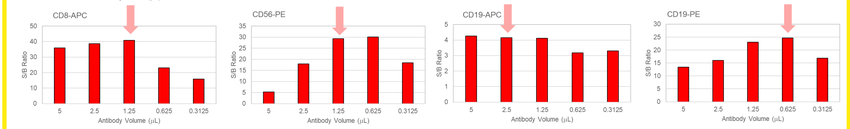
5. ORTHOGONAL COMPARISION WITH FLOW



- Populations of CD3+, CD14+, CD56+, CD19+ cells were characterized using both the Cellaca® PLX and flow cytometry
- The results demonstrate comparable populations measured of CD3+, CD14+, CD56+, and CD19+ cells between the two analytical methods (~5-10% difference)

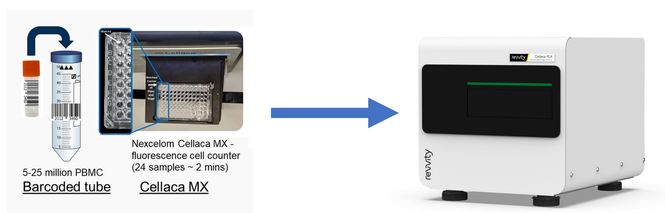
6. ANTIBODY TITRATION OPTIMIZATION





- Images captured and analyzed using the Cellaca® PLX for CD3-KB
- The images and histograms demonstrate antibody titration experiments can be performed using the Cellaca® PLX to determine the minimum amount of antibody required to immunophenotype cells isolated from patient samples
- Additional antibodies tested include CD4-PE, CD8-APC, CD56-PE, CD14-APC, CD19-APC, CD14-PE, and CD19-PE
- The calculated Signal/Background ratios are plotted and compared in the bar graphs and each titration chosen indicated by the arrow.

7. PLX WORKFLOW



1. Immune cell isolation from patient primary PBMCs
2. Cell counting and viability analysis can be performed using the Cellaca® MX
    - 15uL of each sample were analyzed using slides
3. Following acquisition of concentration and viability, the Cellaca® PLX can be used for immunophenotyping

8. CONCLUSION

- The Cellaca® PLX is a novel, image-based approach to immunophenotyping
- Easy to operate, increases accessibility and decreases time from assay to data
- Smaller panels allow for a targeted approach
- Imaging allows for visual confirmation
- The flow cytometer and Cellaca® PLX showed highly comparable cell frequencies for CD3+, CD14+, CD56+, and CD19+ immune cells from 46 patient PBMC samples and two bridging controls.
- Confirmed suitability for new surface marker/fluorophore combinations commonly used in immunophenotyping
- Rapid panel optimization with antibody titration

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