APPLICATION NOTE

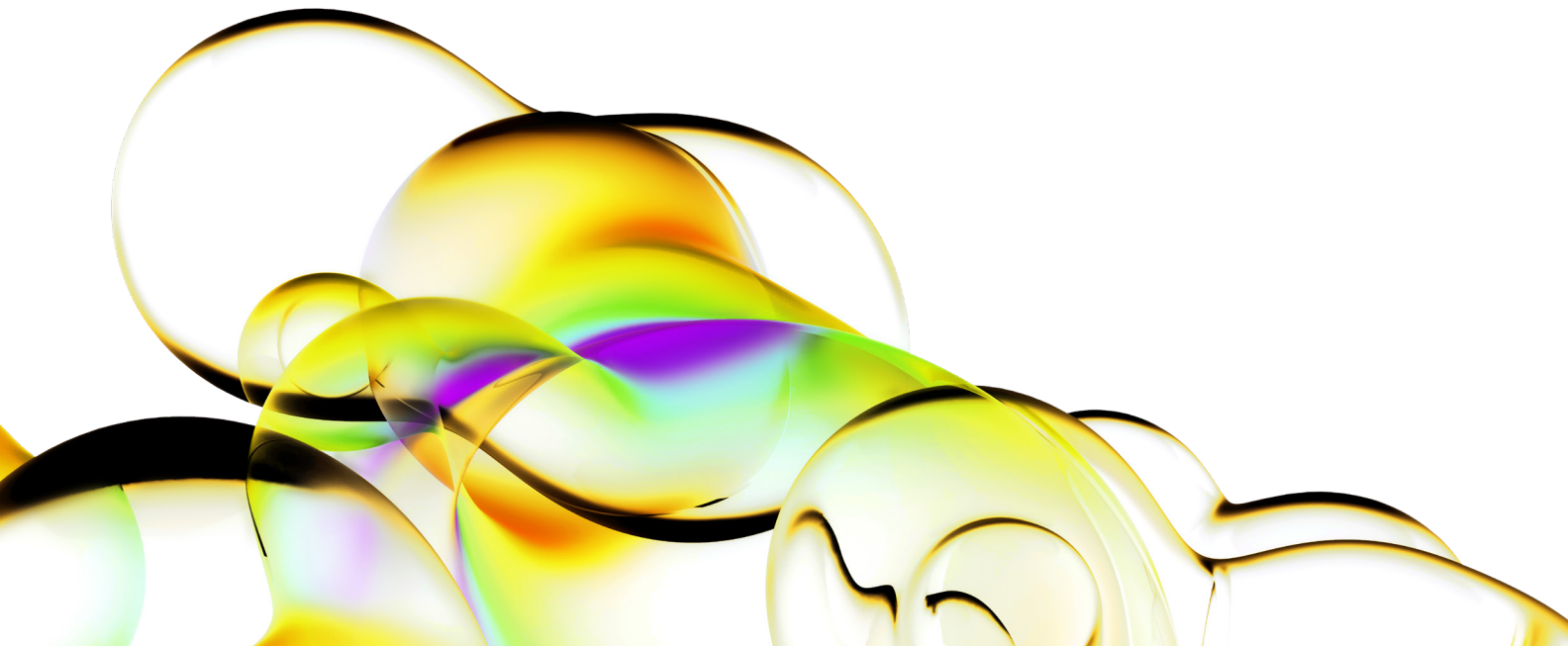
Novel method for T cell immunophenotyping using image cytometry.

Introduction

To quantitatively determine the antigen receptors in a sample, immunophenotyping is the most widely used technique by laboratories particularly focused in immuno-oncology, CAR T-cell manufacturing, and cell viability. Typically, immunophenotyping has been carried out using flow cytometry.

Flow cytometry challenges

Flow cytometry has a few distinct disadvantages that can impede research progress (Couvillion et al., 2019; Alvarez-Barrientos et al., 2000; Davey & Kell, 1996), including the fact it requires a very expensive instrument that, for many laboratories, can only be accessed via a core facility. Therefore, despite providing a wealth of information, data acquisition via flow cytometry can be extremely slow, not only due to the time required to prepare and run samples, but also because it requires an expert user and access to a flow cytometer. Additionally, a relatively large amount of sample is required, and sample viability and quality might be compromised due to unavailability of the flow instrument. Even with the disadvantages, the advantages of flow cytometry, notably the ability to measure many parameters on each and every cell in a large heterogenous population, means it is still often the best option for immunophenotyping (Robinson, 2018).



Alternative method for 2-color immunophenotyping assays

The Cellometer® Spectrum image cytometer is an ideal complementary tool to address the disadvantages of flow cytometry. The Cellometer Spectrum image cytometer is a customizable cell counting and cytometry instrument that provides flow-like data on a simple 2-color assay directly from the laboratory bench – no core facility required. It allows for measuring both cell viability and concentration without having to complete a time-consuming flow cytometer run. Each assay requires only a small amount of sample (20 µL) and provides accurate and consistent results without the need for additional sample manipulations, such as utilizing FACS buffer, resulting in a faster assay set up and run time. Importantly, the Cellometer Spectrum image cytometer produces fast results: users are able to obtain cell images, cell counts, size measurements, and viability calculations in less than 30 seconds and can plot cell population data as a histogram, scatter plot, dot plot, or contour plot. With this output speed, researchers are able to quickly perform a cell purity check at the bench and continue with downstream assays without waiting for a flow cytometer. Other advantages include user-changeable filters, dual-fluorescence to eliminate interference from red blood cells, low sample volume, and algorithms to determine the concentration and viability of various sophisticated cell types such as apheresis, bone marrow, splenocytes, and many other primary cell types. Whether using it as a complementary method for additional data or as the first stop before starting downstream assays, image cytometry can save laboratories time and samples. Furthermore, by performing simple 2-color assays at the bench, laboratories can reduce the reliance on a busy flow core facility, saving flow cytometry for more complicated or detailed assays.

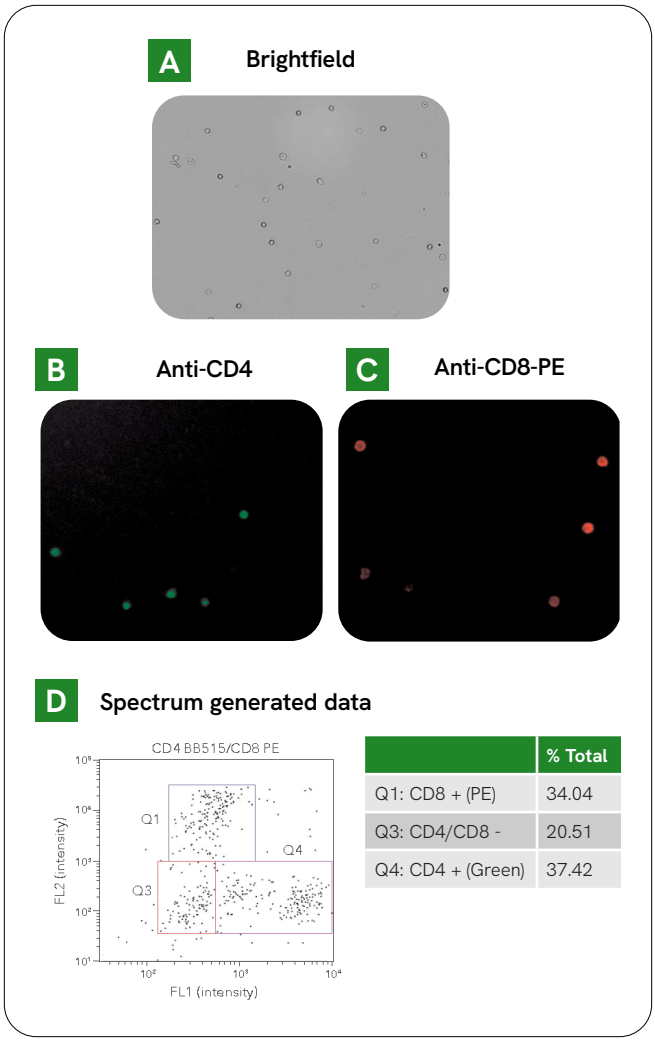
Approach and results

A comparative study was performed using a flow cytometer and a Cellometer Spectrum image cytometer to examine the T cell populations from primary human PBMC samples.

First, peripheral blood mononuclear cells (PBMCs) or an apheresis sample are obtained at a cell concentration of 2 x10^6 cells/mL in 20 µL. Surface marker-specific antibodies (10 µL) and buffer (20 µL) are added to bring the total volume to 50 µL. The volumes of antibody and buffer will differ based on the antibody quantity and quality, but the amount needed of the sample is consistently 20 µL, a major advantage of the Cellometer Spectrum image cytometer in terms of conserving precious samples.

This mixture is then incubated at 4°C for one hour and an additional 250 µL of buffer is added. The sample is centrifuged for 5 minutes at 1500 rpm, decanted from the tube, and re-suspended in 50 µL of PBS. Only 20 µL of this prepared sample is required to load into the Cellometer Spectrum image cytometer for analysis. This protocol takes less than 2 hours from start to finish, and the results are available in a matter of minutes.

To demonstrate the utility of the Cellometer Spectrum image cytometer, thawed PBMCs were analyzed using both the Cellometer Spectrum image cytometer (Fig. 1) and a flow cytometer (Fig. 2). Following the protocol described above, human anti-CD4-BB515 and anti-CD8-PE antibodies were used (Fig. 1A-C) to label and analyze cell population for downstream CAR-T manufacturing (Fig. 1D). The ability to perform a quick and efficient cell purity check before and/or after cell sorting greatly helps maintain sample quality and viability by decreasing the time required to obtain this data.



	% Total
Q1: CD8 + (PE)	34.04
Q3: CD4/CD8 -	20.51
Q4: CD4 + (Green)	37.42

Figure 1: A brightfield image (A) of the cells as well as those stained with human anti-CD4-BB515 (B) and anti-CD8-PE antibodies (C) were imaged on the Cellometer Spectrum image cytometer. Population analysis was then performed using FCS Express™ Flow Cytometry software to determine the percentage of CD4 and CD8 positive cells as well as the percentage of cells that were double negative (D).

To compare the results obtained using the Cellometer Spectrum image cytometer, the same human PBMC sample was then analyzed using a flow cytometer (Fig. 2). The results obtained on the two instruments showed similar quantification of the CD4 and CD8 populations. It is important to note that, to yield the same results, the flow cytometer required a sample volume 50 times greater than the Cellometer Spectrum image cytometer (1 mL versus 20 µL per sample). Furthermore, bright field and fluorescent images that are automatically generated and saved by the Cellometer Spectrum image cytometer provide additional verification that the stained objects are indeed the cells of interest and not cellular debris.

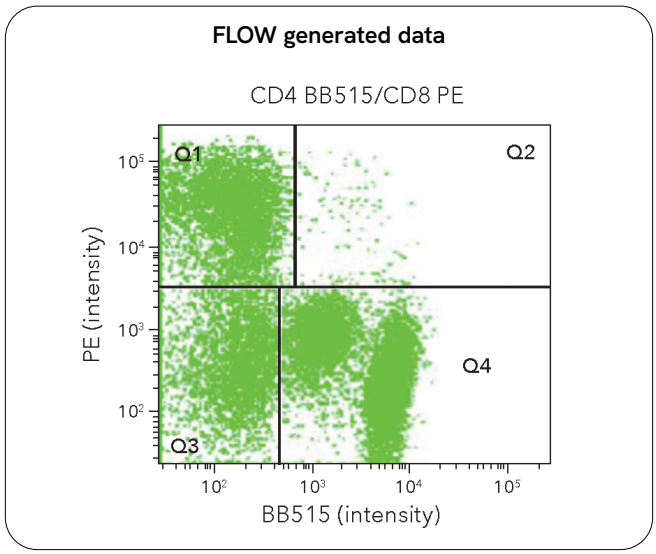


Figure 2: Human PBMCs analyzed using a flow cytometer. The same sample used in Figure 1 on the Cellometer Spectrum image cytometer was analyzed by flow cytometry. The percentage of CD4 and CD8 cells in the sample was similar when comparing data obtained by the Cellometer Spectrum image cytometer and data generated on the flow cytometer.

Conclusions

The Cellometer Spectrum image cytometer is an all-in-one system that is capable of performing basic cell counting, primary cell viability, and cell-based assays with accurate and consistent results. Researchers are able to quickly assess the viability and bulk percentage of a population of interest at the bench in order to quickly continue with downstream assays such as magnetic bead separation. This type of cell purity check has been accessed for human anti-CD3, CD4, and CD8 surface markers. The powerful imaging cytometry capabilities of the Cellometer Spectrum enables laboratories to quickly perform a 2-color assay in order to identify various populations of T cells from bulk PBMCs and apheresis samples without the need for complicated flow cytometry.

References

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