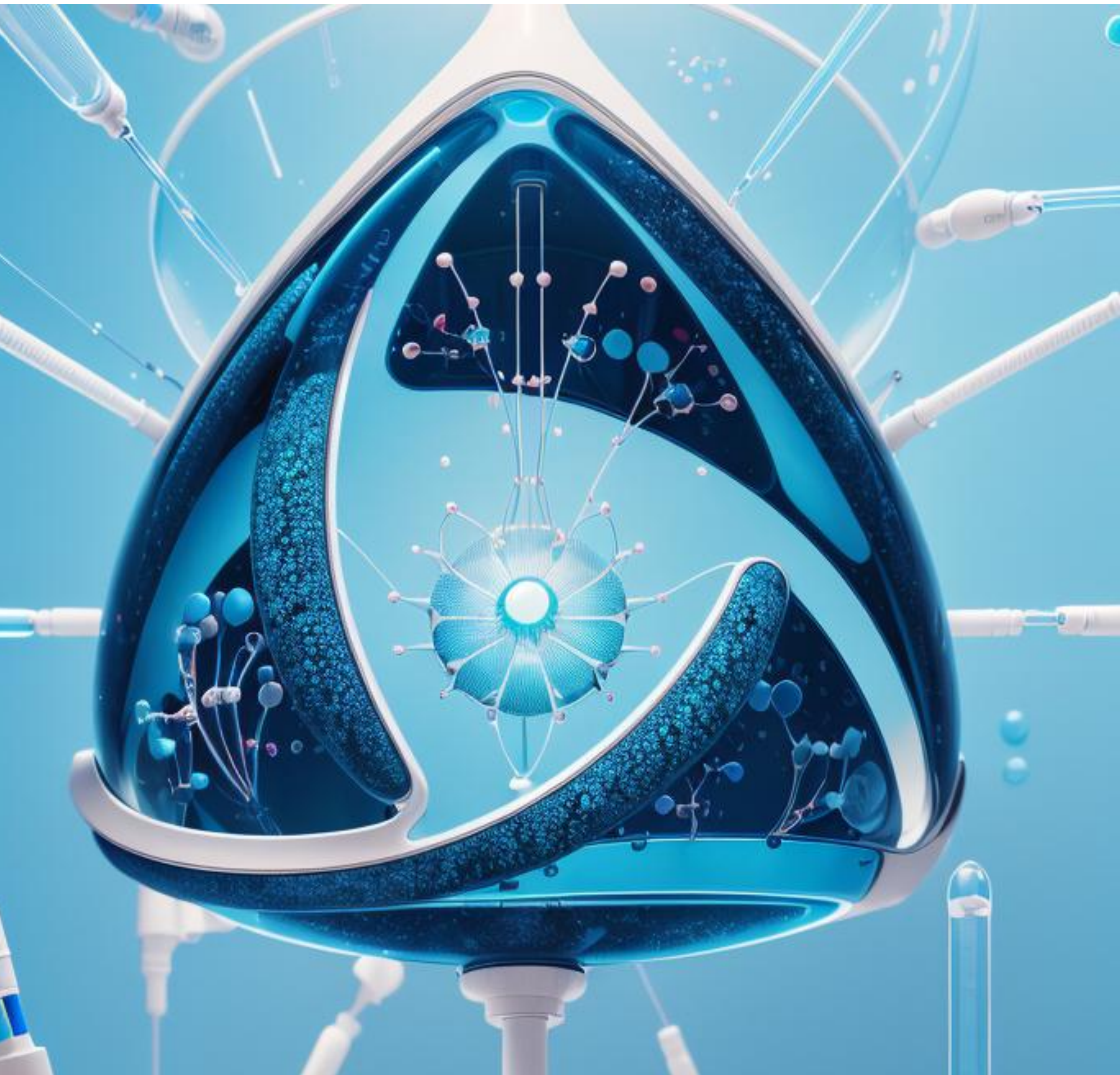
ORFLO Personal Cell Analysis



Unlocking the Next Stage in CAR-T Cellular Analysis With The Moxi Go II

ORFLO | Personal Cell Analysis

Introduction

The field of immune-based therapies has exploded in the last few years. With an ever growing arsenal of tools for leveraging a patient's own immune system to tackle diseases specific to them, we have begun to see a true burst of progress in personalized medicine. One particularly popular approach has been leveraging a Chimeric antigen receptors (CARs) to genetically modify a person's immune cells to enhance their ability to detect the patient's cancer cells[1]. CARs are synthetic receptors that have been engineered to redirect lymphocytes, most commonly T cells (CAR-T), to recognize and eliminate cells expressing a specific target antigen. CAR-T therapies against large B-Cell lymphoma were approved by the US Food and Drug Administration (FDA) in 2017[2], and we have seen a rapid increase in other similar therapies since. These techniques typically involve extracting immune cells from the patient, treating them *in-vitro*, and reintroducing them into the body post-modification.

There are a variety of ways to monitor this process in cell culture, the two most prominent being cell counting via imaging-based and coulter-based cell counting methods and higher-level cellular analysis with fluorophore-tagged antibodies and fluorescent reporters via flow cytometry. While each instrument used to achieve these two things has its advantages and disadvantages, few and far between can do both at once. Out of all your options, no other instrument has the power to do both with the speed, precision, and ease that ORFLO's Moxi Go II can. In this document, we will take you through how you can unlock the next stage in your cellular analysis process for CAR-T with our instruments.

1. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*. 2017;377(26):2531-2544. doi:10.1056/NEJMoa1707447
2. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*. 2021;11(4):69. doi:10.1038/s41408-021-00459-7

Moxi Cell Analyzers

ORFLO's proprietary cell counting technology can count tens of thousands of cells in seconds, providing first-in-class data to you fast. Normally, accuracy is negatively impacted by acquisition time. However, by leveraging the current gold standard Coulter Principle, we bring you both with no compromises to either. All of this in a self-contained, easy-to-use, and benchtop-sized machine.

Microfluidics Meets Cell Counting

Don't like wasting half of your sample on figuring out whether your experiment worked correctly? Don't like spending time and money on a large-scale flow cytometry run for a simple transfection efficiency or cell viability check? Our innovative microfluidics cassettes have created a simple, effective, and self-contained way to get everything you need, all for the low, low cost of 60µL! Just insert the cassette, pipette in a small amount of your precious sample, and let the microfluidics and our proprietary cell counting technology do the rest.

Gold Standard Count Accuracy

Moxi GO II was compared to the gold standard Beckman-Coulter Z2 system, an image-based cell counter, and a hemocytometer, with respect to counts of serial dilutions of a range of particle types (beads, yeast, mammalian cells) and sizes (~4µm to ~16µm in mean diameter). The results showed that the count-to-count variability, as measured by the coefficient of variation (CV), was on the order of 20-30% for the imaging system, compared to 4% for the Moxi system.

Moxi GO II - Next Generation Dual-Channel PMT Fluorescence Cell Analyzer

With our Moxi Go II system, we have coupled our cell counting technology with the ability to detect two channels of fluorescence to revolutionize the way that cell counting is done. Our 488nm laser and Photo Multiplier Tubes (PMTs) gives the accuracy and detection capabilities of a normal flow cytometer for both a 525/45nm (Green) channel and 561nm/LP (Orange/Red) channel. This combination gives you unparalleled benchtop power when working with any cell types marked with any of your favorite fluorescent cell markers!

Initial PBMC Quality Checks

The first step in any CAR-T workflow is assessing the sample that was extracted from the patient. The cells of interest will lie in the PMBC portion of the blood taken, and the purity, quality, and viability of this subset of cells is the critical first stage for ensuring a successful downstream workflow.

Identifying Specific PBMC Cell Populations With Size Alone:

The core of all of our instruments is our microfluidics-based Coulter counting technology. With the speed and accuracy of the gold-standard method for cell counting for decades at the heart of the Moxi Go II, you can be sure that you will always get the most repeatable and accurate cell counts of any cell counter. With the ability to get sub-µM sizes, we can readily distinguish between cell populations in the PBMC fraction that other cell counters cannot.

PBMC Cell Sizes		
Cell Type	Average Size (literature)	Average Size (Moxi)
Red Blood Cell	6-8µm	5.5µm
Lymphocyte	6-9µm	7.5µm
Monocyte	9.5µm	9.5µm

Table 1: Average cell size for PBMC cell types. The literature ranges were taken from reported papers and online references. The Moxi ranges were calculated from a repository of PBMC data recorded on all of our Moxi instruments

With the Moxi Go II, you can not only distinguish cells based on size, but easy Acridine Orange (AO) staining seamlessly separates real cells from debris, guaranteeing the highest quality cell counts for each cell type.

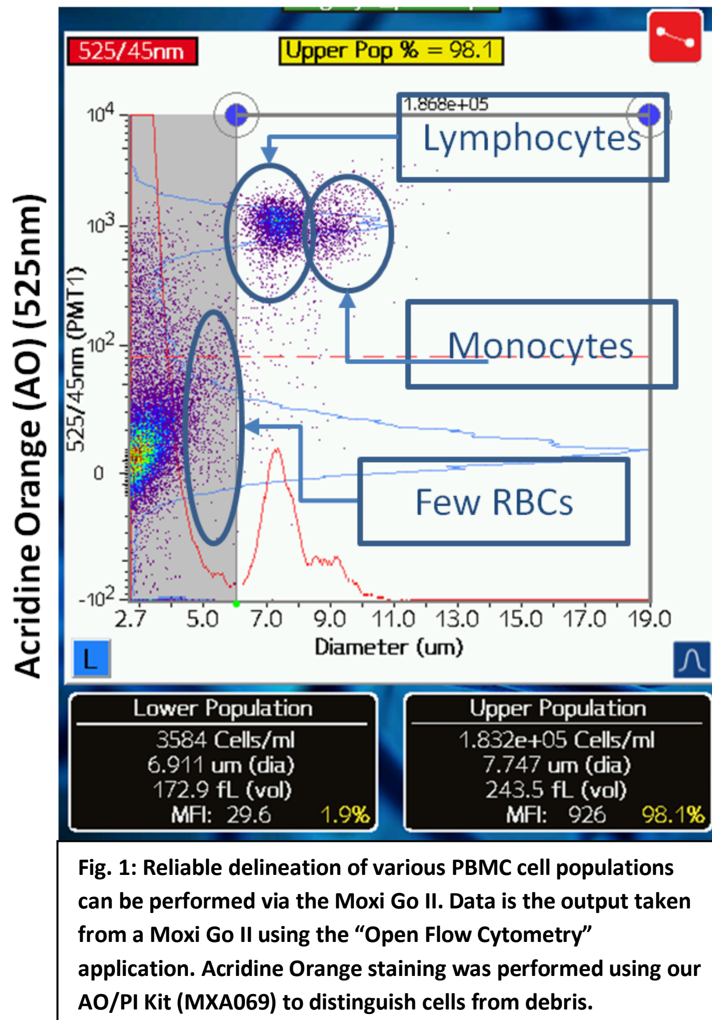


Fig. 1: Reliable delineation of various PBMC cell populations can be performed via the Moxi Go II. Data is the output taken from a Moxi Go II using the "Open Flow Cytometry" application. Acridine Orange staining was performed using our AO/PI Kit (MXA069) to distinguish cells from debris.

Viability Measurements Using PI:

The Moxi Go II is uniquely primed to address both the size-based identification and quantification of specific cell populations in your PBMC and determine percentages of live cells all in one run. With the ability to resolve cells in two dimensions by size on the x-axis and by fluorescence intensity on the y-axis, you can get viability analytics via fluorescent dyes such as PI and total cell counts at the same time.

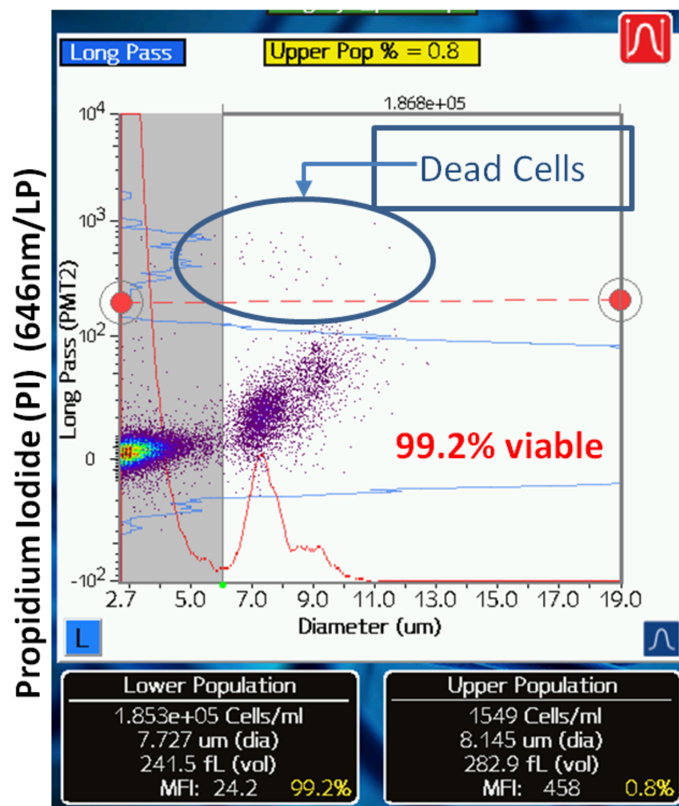


Fig. 2: Two-dimensional analysis of viability and cell size on a single sample run. Output image was generated on a Moxi Go II using the "Cell QC" application. PBMC cells were stained with Propidium Iodide (PI) to determine live vs dead cells.

Gold Standard T-Cell Activation Monitoring

CD3/CD28 Dynabeads are one of the most commonly used ways to activate T cells ex-vivo once they have been plated in cell culture[3]. They involve small beads coated in anti-CD3 and anti-CD28 antibodies that stimulate the plated T cells and cause them to activate and expand.

Our Moxi Go II is primed to give you everything you could need to monitor this expensive and time-sensitive process:

Assessing Dynabead Potency:

The first check you need to perform before starting anything is how potent your Dynabeads still are. This can be done quickly and easily with the Moxi Go II by simply labeling a small diluted subset of them with Goat Anti-Mouse-FITC (IgG FC) and checking how many are fluorescently positive on the 525/45nm channel.

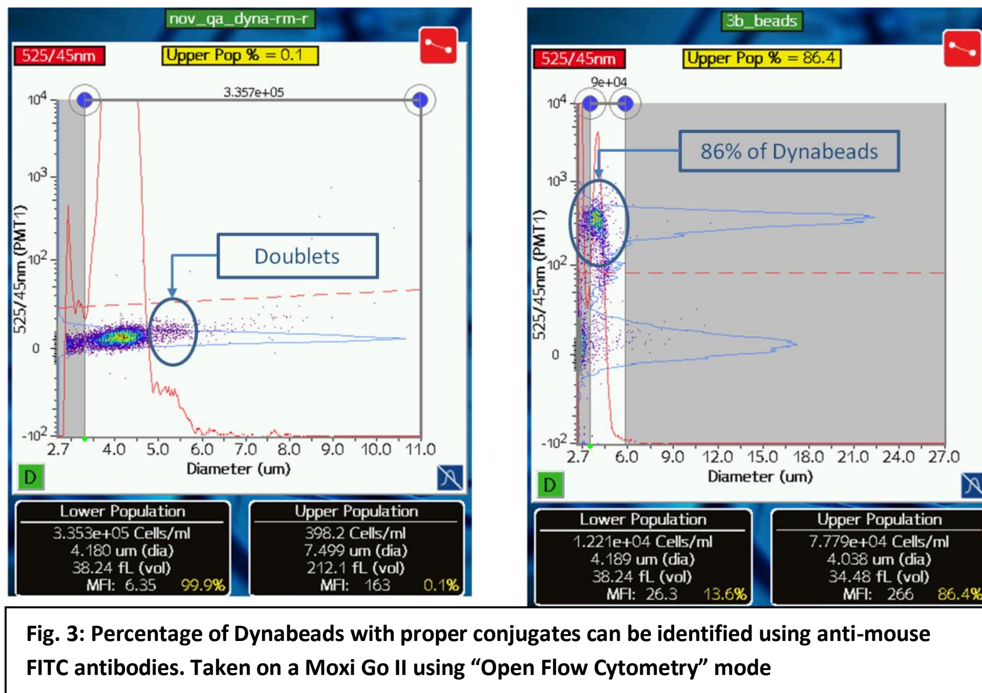


Fig. 3: Percentage of Dynabeads with proper conjugates can be identified using anti-mouse FITC antibodies. Taken on a Moxi Go II using "Open Flow Cytometry" mode

Monitoring of Dynabead-T cell interaction:

Once you are sure your Dynabeads are good, the next step is to incubate them with your T Cells to start the activation process. With the FITC-labeled antibodies you had previously, the Moxi Go II can identify your T cell population based on size and your Dynabeads based on fluorescence. Because of this two-dimensional analysis, you can clearly and easily see which T cells are currently interacting with Dynabeads. A run on the Moxi Go II takes 30 seconds and takes only 60µL of sample to complete for counting tens of thousands of cells, so you can get continual updates on this process!

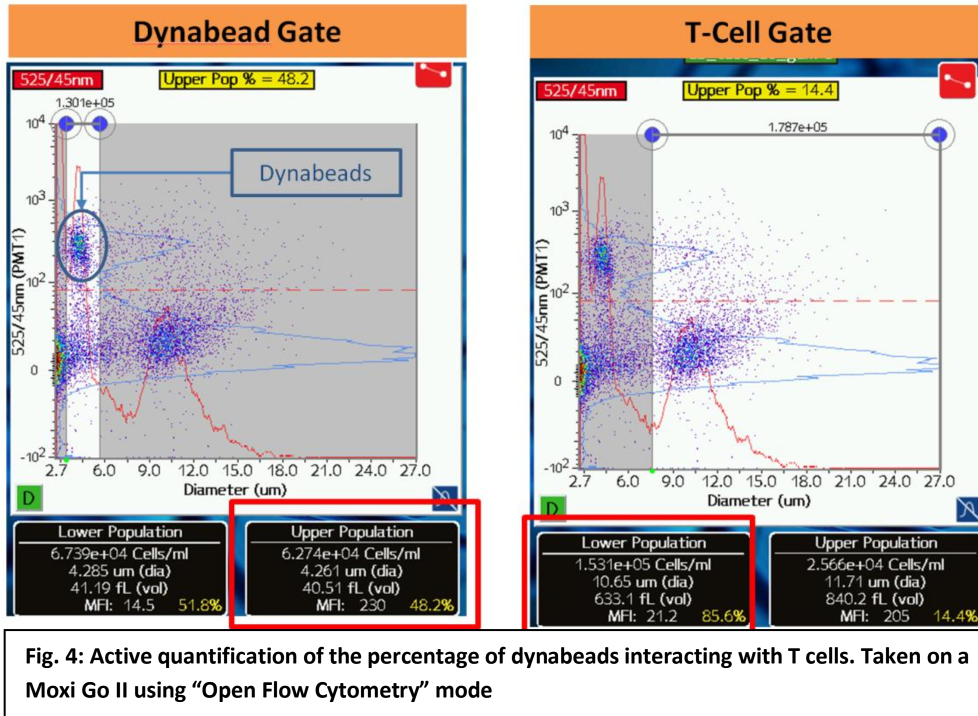


Fig. 4: Active quantification of the percentage of dynabeads interacting with T cells. Taken on a Moxi Go II using "Open Flow Cytometry" mode

Longitudinal Measurement of Viability:

With minimal sample used per test and a run that counts tens of thousands of cells in less than 30 seconds, it makes repeated longitudinal monitoring of cell culture viability a simple addition to your normal culture protocols. With comparable accuracy to a flow cytometer, there is no need to leave the bench and commit to an expensive flow cytometry run when checking the status of your CAR-T cells in culture.

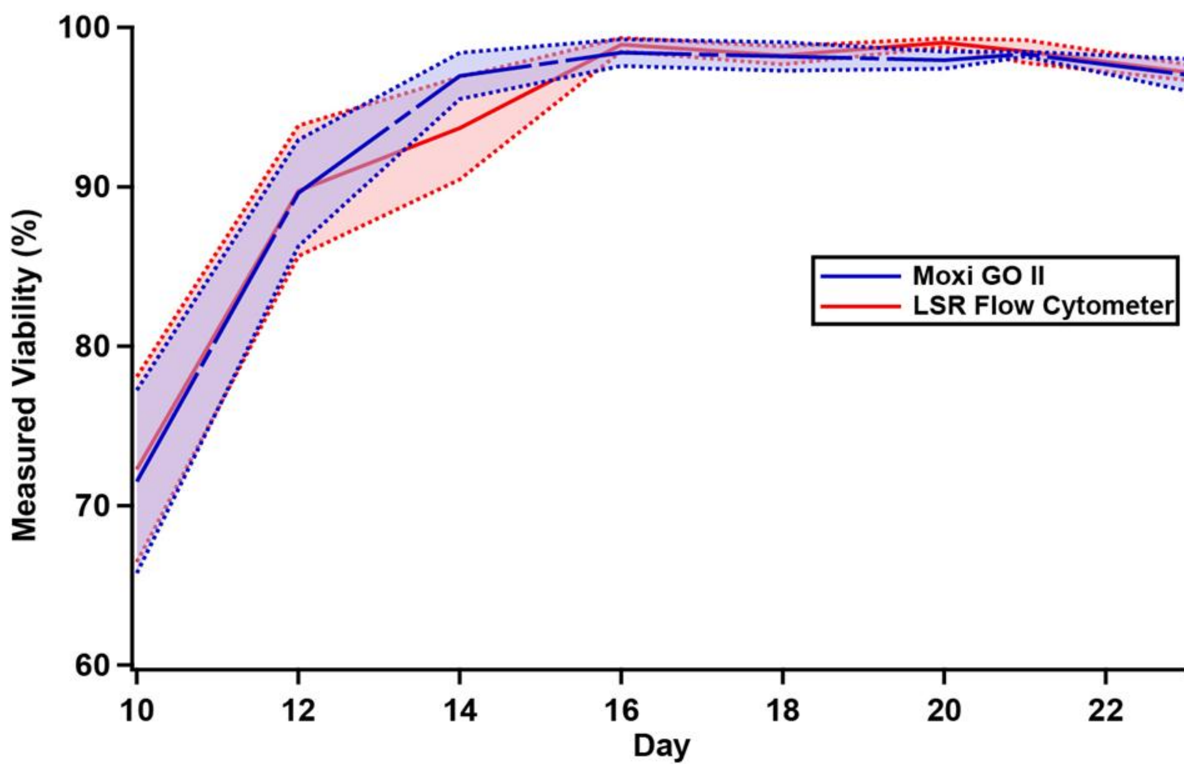


Fig. 5: Time course data during T-cell activation shows the qualitatively analogous curves of the Moxi Go II and the LSR Flow Cytometer.

Two-Color Monitoring of T Cell Activation:

Once your Dynabeads have been cleared and your T cells have begun the activation process, it will be extremely important to monitor them via changes both in their physiology and in their gene expression profile. With the Moxi Go II, you can use a dual fluorophore profiling of your cell population with both the 525/45nm and 561nm/LP channels to identify T cells in general and quantify the subset of those T cells that are expressing an activation marker like CD25.

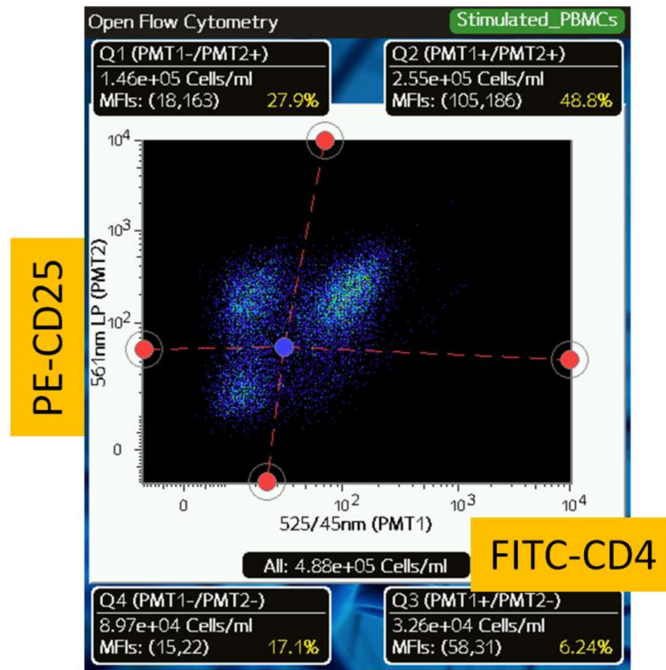


Fig. 6: Identification of active T cells using dual-color fluorescent profiling. Taken on a Moxi Go II using "Go FLOW" mode

CAR-T Cell Expansion

One of the most important indicators of when it's time to harvest your CAR-T cells is its expansion. With our best-in-class cell sizing technology, we are the only instrument that can give you this reliable metric quickly and with minimal sample loss. We have the proven accuracy of the gold standard Coulter multisizer in a square foot of space so you know that you will be able to get the sub-µM measurements you need the first time. Plus, with the Moxi Go II, you can couple it with fluorescent labeling to give you unparalleled quantification abilities. Scientists and industry professionals agree that there is no easier, faster, and more reliable platform for knowing when the optimal time for harvesting your CAR-T cells!

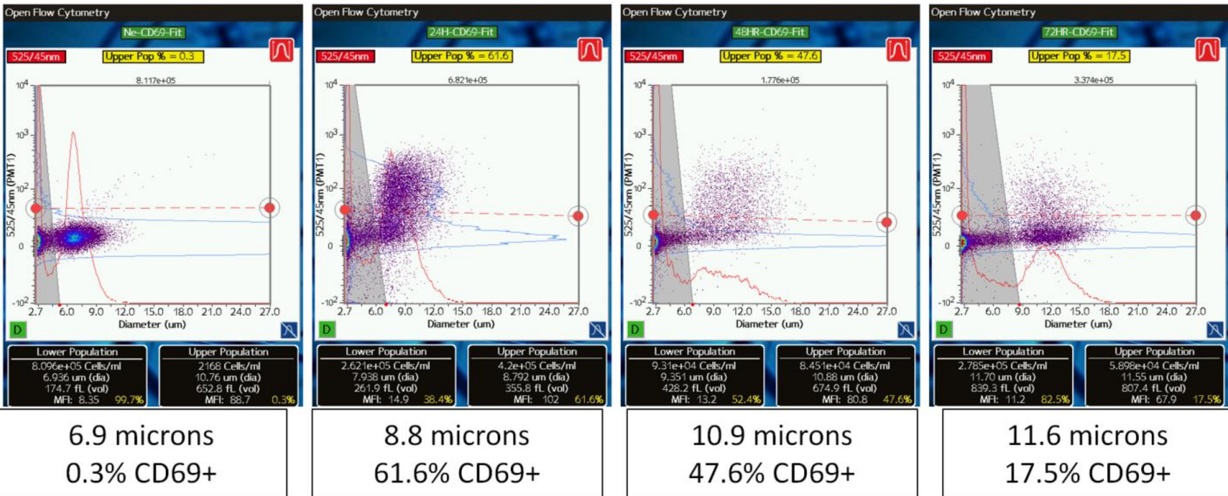
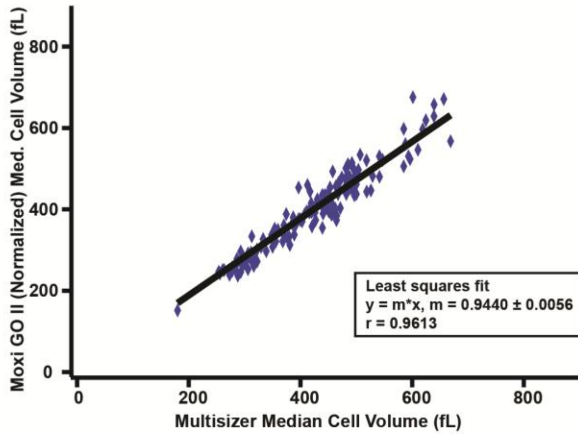
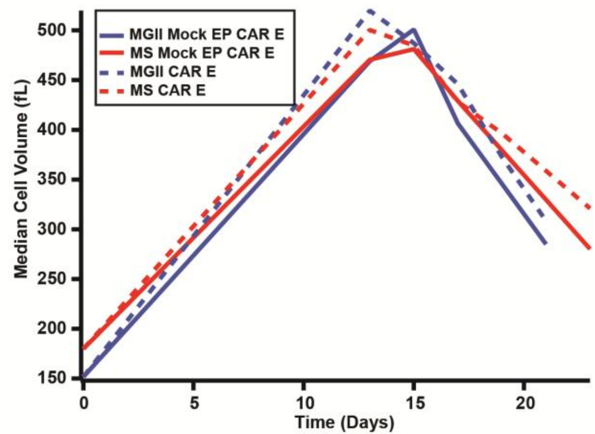
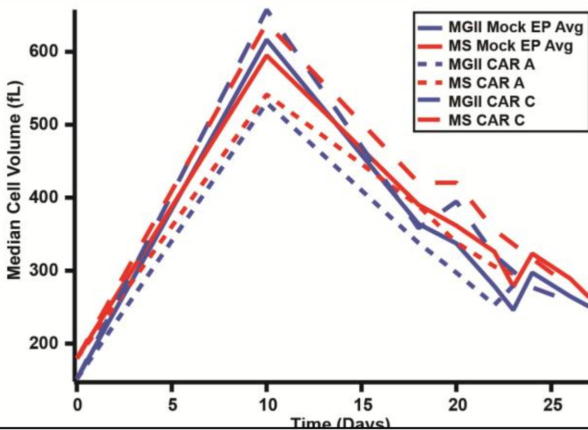


Fig. 7: Identification of active T cells using a combination of fluorescence labeling and cell sizing. Fluorescent marker = FITC-labeled CD69. Taken on a Moxi Go II using "Open Flow Cytometry" mode







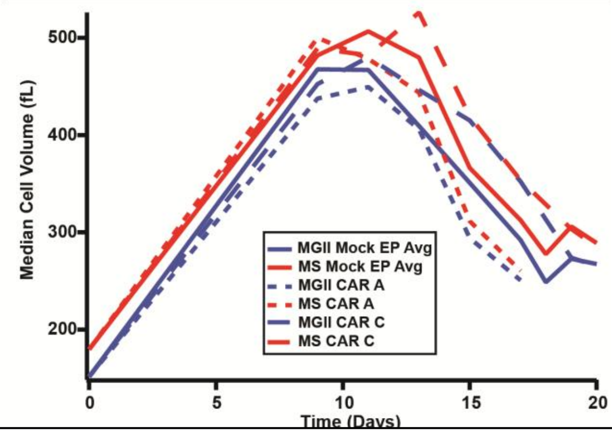


Fig. 8 a.) Direct, scatter plot comparison of Moxi GO II reported (normalized) median cell volume to Multisizer reported cell volume. b.) Scatter plot graph of the Moxi GO II reported cell concentrations vs. the Multisizer reported cell concentrations for identical samples. A linear (least squares) fit of the data reflects the high degree of correlation (Pearson's r = 0.9576) between the output of the two systems. b .), c.) & d.) Time course curves graphing the expected size shifts reflected by T-Cell activation. Figures compare various CAR transfections to mock data for separate donors (b.) ND429, c.) ND520, d.) ND548

Conclusion

For any type of work involving cells, Moxi cell analyzers are being used. For CAR-T research and product development, we are the trusted machine to bring you everything you could need for achieving the specific pivotal tasks in your workflow required for success. From initial PBMC counting to viability checks to monitoring dynabead assays to easily quantifying to cells as they activate and expand, we bring you the power of a cell counter and flow cytometer in a square foot of space.

The Moxi Go II is perfectly and uniquely suited to fulfil all of these requirements. At the price of a single years' service contract for a flow cytometer, unlock the next stage of cellular analysis with first-in-class technology at a fraction of the cost for traditional cell analysis methods.

Scan to contact us

email us at info@orflo.in

ORFLO Technologies, 260 Northwood Way, Ketchum, ID 83340, USA

ORFLO_App_C_2023_01

@Copyright 2023 ORFLOTechnologies,. All Rights Reserved.