Elucidating Complex Flow Cytometry Studies with the Speed and Sensitivity of the ZE5 Cell Analyzer

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Abstract

Recent advancements and innovations in flow cytometry instrumentation have enabled scientists to perform increasingly complex experiments and to obtain biologically relevant data in a high-throughput manner. Utilizing speed and flexibility, the ZE5 Cell Analyzer from Bio-Rad Laboratories takes flow cytometry from a technique to a powerful tool that goes beyond instrumentation. With the capability to sample up to 100,000 events per second and with five lasers with 30 detectors to choose from, the ZE5 has previously been shown to aid in the quantification of T-cell subsets. To demonstrate the superior ability of the ZE5 Cell Analyzer to examine a diverse range of biological applications, exosome and immunophenotyping studies were performed. The exosomes were isolated and used to show the subcellular detection capabilities of the ZE5. Published studies show measurements of cell health using more than 20 parameters at one time with the ZE5; here we used peripheral blood to demonstrate simultaneous detection of 19 parameters. The ZE5 Cell Analyzer, designed with an understanding of sample integrity and with a combination of speed, automation, and innovative design, will show that a cytometer can be used for complex data analysis to easily resolve rare or dim populations with high-quality data, as well as routine laboratory work.

How Does the ZE5 Cell Analyzer Help You Do More?

Unmatched Speed and Stability

The ZE5 Cell Analyzer combines improved speed, enhanced electronics, and a cell laser transit time that is five times faster than other systems to create a unique and indispensable cell analysis system for any laboratory (Figure 1A). The ZE5 Cell Analyzer can run at high speeds without compromising data quality due to enhanced fluidics that deliver a stable flow rate up to 3.5 µl/sec. To match this rapid flow rate, the ZE5 Cell Analyzer's faster laser transit time creates a shorter pulse width, which is then sampled two times more frequently than comparable systems. The increased sampling rate, coupled with fast, low-noise electronics, results in a tighter coefficient of variation (CV) and higher-resolution data (Figure 1B). The speed of the ZE5 Cell Analyzer enables more in-depth investigation and more specific analysis in applications, such as identification of specific cell subsets undergoing apoptosis, real-time activation and proliferation of cells due to immune or drug response, or detection of cells in different stages of the cell cycle.

Obtain Better Reproducibility with No Carryover

Conventional flow cytometry requires manual washing of the sample probe between samples to reduce sample-to-sample carryover. The ZE5 Cell Analyzer automates this activity with its flying collar wash feature that automatically washes the sample probe between samples. Automation of crucial steps reduces carryover and potential contamination, thus removing a primary source of data variation (Figure 2) and providing results you can trust.

Study Small Samples with Ease

The ZE5 Cell Analyzer has small particle detection capability (forward scatter [FSC] from the 405 nm laser), permitting analysis of difficult-to-study samples such as exosomes, which are generally smaller than 250 nm (Figure 3). Exosomes are extracellular vesicles (EVs) that have been implicated in intracellular communication and are a key area of interest in disease research. Conventional exosome analysis using flow cytometry can require manual hardware adjustments and advanced instrument calibration. The ZE5 Cell Analyzer now provides a user-friendly platform with built-in capabilities that allows any lab to study these and other small particles. Using both surface and internal markers, centrifugation, beads, or other staining methods, the ZE5 displays optimal sensitivity for small particle identification.

Publish, Don't Rerun Experiments

The low-noise electronics in the ZE5 Cell Analyzer also contribute to great resolution and consistent, reproducible data even at the high speeds only the ZE5 can achieve (Figure 4). The ZE5 Cell Analyzer instills confidence in results and enables quicker experiment-to-publication time. With reduced carryover, faster speed of acquisition, and multiple parameters, the ZE5 Cell Analyzer supports the robust exploration of novel assays while improving the quality and reproducibility of ongoing experiments.

Analyze Cell Populations You Couldn't Study Before

With up to five lasers and 30 detectors, the ZE5 Cell Analyzer easily improves upon standard assays, supports new panels, and is an excellent aid in difficult-to-study rare cell analysis. The option for multiple spatially separated lasers facilitates the study of highly complex samples. Numerous detectors expand options and allow for larger panels, enabling critical data acquisition of whole samples (Figure 5). No longer do samples need to be split, which increases the sensitivity of measurement.

Results

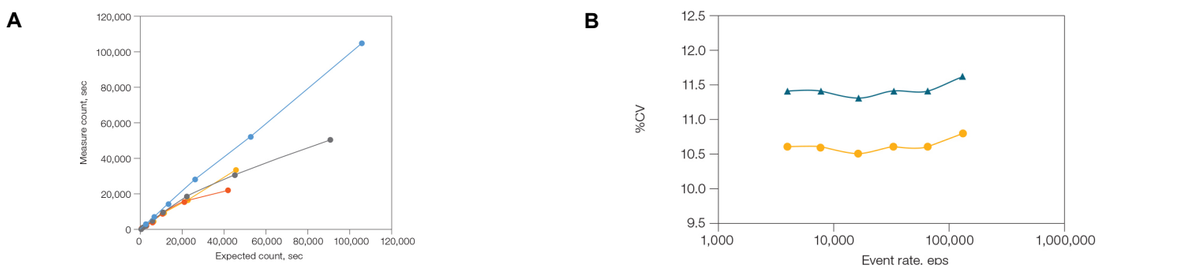

Fig. 1. Speed and signal stability. A, the ZE5 Cell Analyzer outperforms other systems at higher acquisition speeds because it continues to acquire data in the 100,000 events per second range, whereas other systems drop off around 20,000 events per second. Dragon Green Beads (Bangs Laboratories, Inc.) were used in a serial dilution to determine when the observed acquisition rate falls off the theoretical limit. ZE5 Cell Analyzer (—); cytometer 1 (—); cytometer 2 (—); cytometer 3 (—). **B**, beads were read at increasing events-per-second rates, and singlet beads were gated using pulse geometry gating. The percentage CV of side scatter (SSC) and fluorescein isothyiocyanate (FITC) was plotted over approximately 4,000–129,000 eps. The mean and standard deviation (SD) of CVs over this range were FITC-A (●): mean = 10.6, SD = 0.063; SSC-A (▲), mean = 11.4, SD = 0.063.

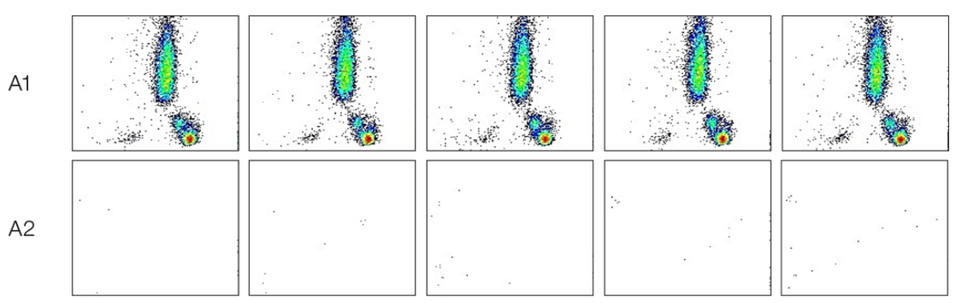

Fig. 2. Carryover reduction. Lysed whole blood was run on the ZE5 Cell Analyzer in high-throughput mode. After each sample, the system performed an automatic wash cycle of 0.25 sec outside and 1.75 sec inside the sample injection port. A clean tube of water was run immediately after the wash to evaluate carryover. Row A1 shows the populations acquired with the corresponding well A2 carryover demonstrated underneath. Representative plots are shown for five separate runs.

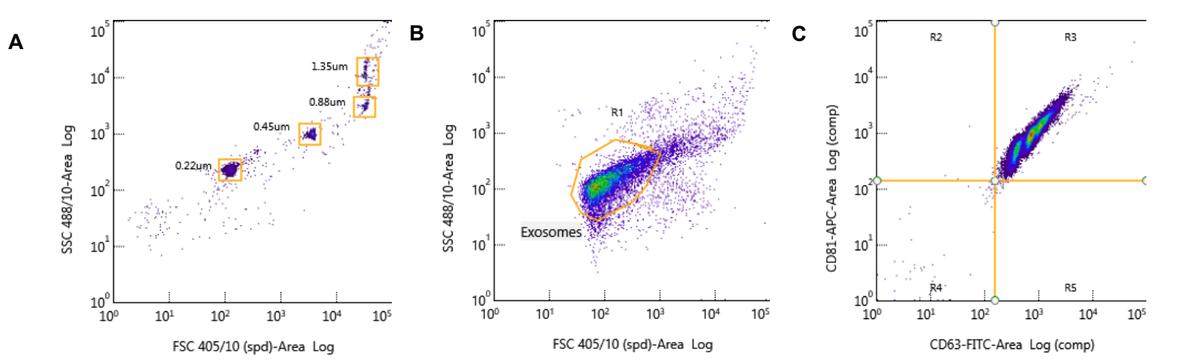

Fig. 3. Exosome analysis. A, yellow fluorescent beads ranging from 0.22 to 1.35 µm were used to set up the voltage of a ZE5 Cell Analyzer; **B**, the size of exosomes purified from MCF-7 cells was determined by Zetasizer ZSP (Malvern Panalytical Ltd) to be in the range of 185 to 300 nm. Exosomes were gated on FSC from the small particle detector off the 405 nm laser vs. SSC. **C**, dual staining with CD63-FITC and CD81-APC was performed using exosome-coated SureBeads Protein G Magnetic Beads (Bio-Rad Laboratories, Inc.), which had been incubated with unconjugated Anti-Human CD63 (Bio-Rad) prior to the fluorescent conjugation for 30 min at room temperature and washed twice with PEB–0.1% Tween 20.

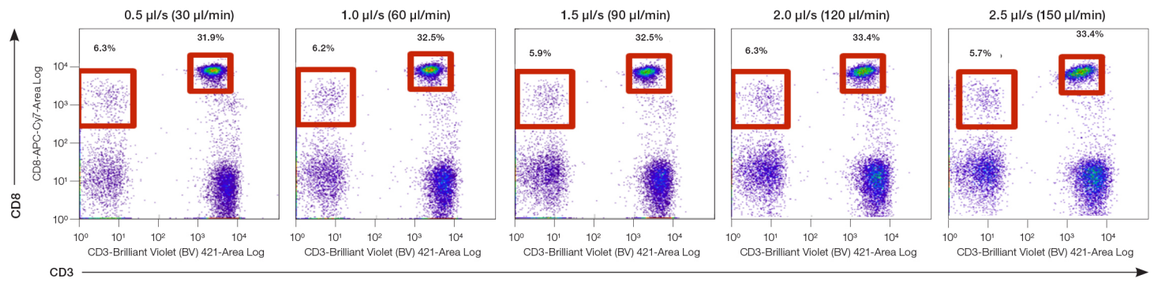

Fig. 4. Robust reproducibility. Whole blood was lysed and stained with CD45–Alexa Fluor 488/CD3-BV421/CD8-APC-Cy7. Twenty thousand events were acquired per run to evaluate various population percentages over multiple event rates.

A

Laser/Filter		Marker	Fluorophore
355	700LP	CD25	BUV737
	387/11	CD45RO	BUV395
	447/60	Live/Dead	Zombie UV
	525/50	CD19	BUV496
405	420/10	CD3	Brilliant Violet 421
	460/22	CD4	V450
	625/24	CD20	Brilliant Violet 605
	525/50	CD27	V500
	750LP	CD16	Brilliant Violet 785
	720/60	CD123	Brilliant Violet 711
	670/30	CD14	Brilliant Violet 650
488	525/35	IgD	Alexa Fluor 488
561	670/30	CD127	PE-Cy5
	615/24	CCR7	PE–Dazzle 594
	577/15	CD11c	PE
	750LP	CD33	PE-Cy7
640	720/60	CD8	Alexa Fluor 700
	670/30	CD56	APC
	775/50	HLA DR	APC-H7

B

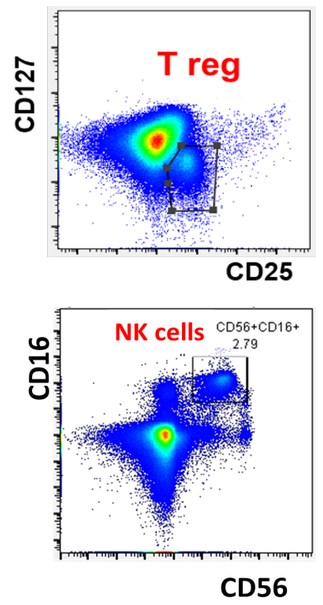

Fig. 5. Large panel analysis. Whole blood was lysed and stained and 20,000 events were acquired to evaluate various populations. **A**, chart showing a 19-color staining panel; **B**, representative plots showing data analyzed with Everest Software for T regulatory (T reg) and natural killer (NK) cell populations. Initial gates were set using Zombie UV Live/Dead Dye to exclude dead cells.

Conclusions

Using the ZE5 Cell Analyzer we can detect particles as small as exosomes to cancer cells to produce biologically relevant data in a high-throughput manner. Our results with the ZE5 Cell Analyzer demonstrated:

- Superior speed
- Excellent reproducibility
- Optimized rare cell analysis

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