

Next-generation cell mimics double as apoptosis controls and efficient flow cytometry training tools

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ABSTRACT

The increased application of flow cytometry in research, drug development and clinical diagnostics has paralleled a challenge for flow cytometry scientists and technicians. Biological samples and calibration beads are extremely useful for teaching skills such as compensation, gating and instrument setup. However, the use of biological samples is not always feasible for reasons associated with the potential risk of biohazard, while beads do not represent a perfect cell model. To address this challenge, Slingshot Biosciences focused on the question of whether non-biohazardous synthetic cells could replace biological samples or beads for flow cytometry training and other applications. By applying the principles of biochemistry, high-precision manufacturing and polymer chemistry, Slingshot Biosciences designed cellular mimics to resemble biological samples that researchers may encounter in real experiments. These cell-like controls are readily available to use immediately with little preparation, thereby facilitating fast and efficient training for users new to flow cytometry, without any risk of biohazard. For specific detection of apoptosis, Slingshot has developed cells that mimic the staging of apoptosis without the need to induce cell death. Here we provide evidence that the apoptosis cell mimic can be used to distinguish live, apoptotic and necrotic cells at consistent percentage composition across all populations. Furthermore, the cell mimic can be used with DNA dyes such as 7-AAD or PI and can be run on both spectral and conventional flow cytometers. Accordingly, this innovative cytometry reagent can be applied to setting up compensation or for spectral unmixing. All together, these synthetic cells are designed to reliably reproduce intra and inter-laboratory results with the added benefit of improving the tools available to train scientists new to flow cytometry.

INTRODUCTION

Unlike beads, Slingshot's synthetic cell mimics can match the output of biological cells. This breakthrough technology creates synthetic cells that match the optical, fluorescence and biochemical features of any cell type.

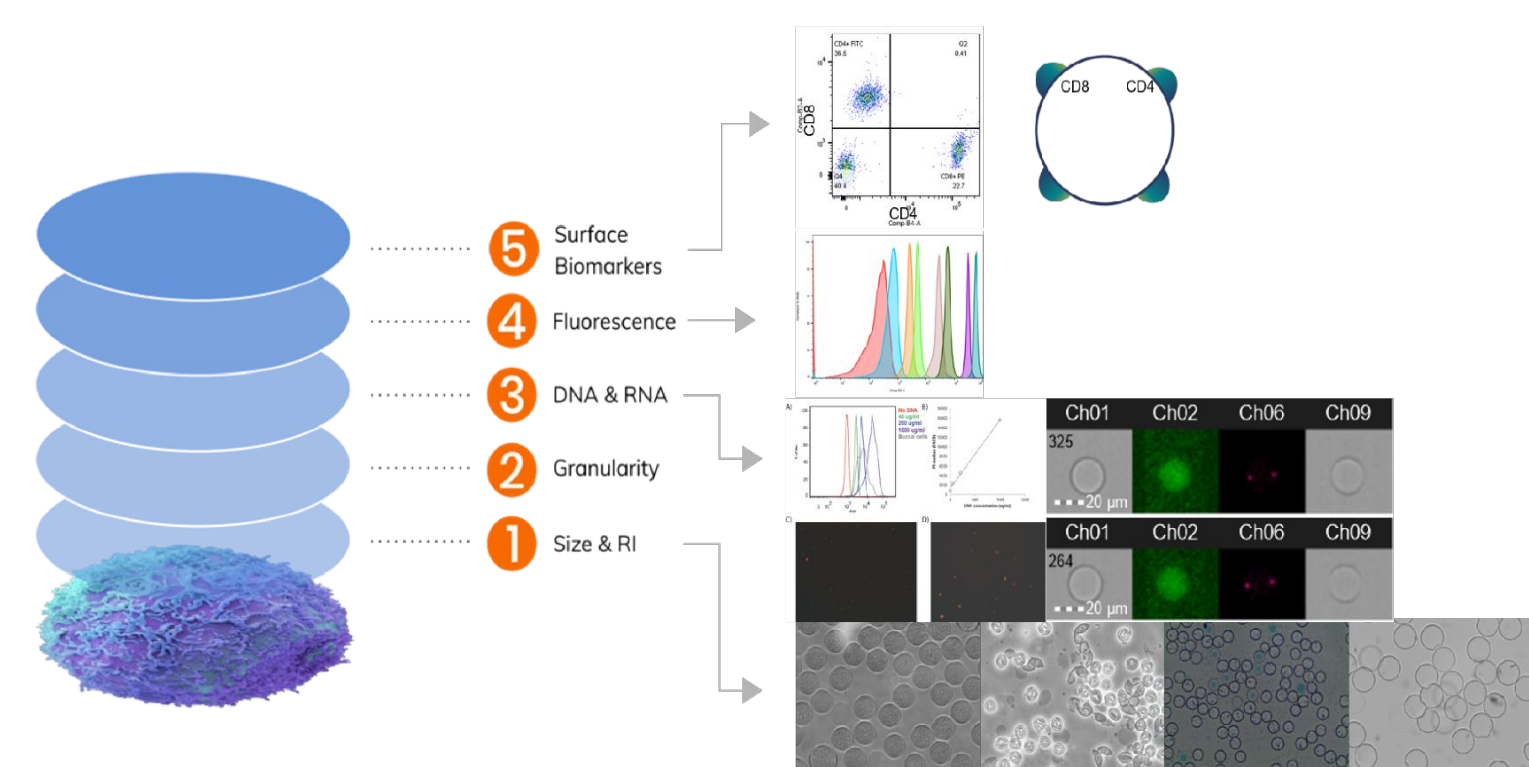


Fig 1. Schematic of Slingshot technology capability to match biological cells

RELIABLE SAMPLES FOR TRAINING NEW FLOW CYTOMETRY USERS

Combining synthetic cell mimics for compensation, viability staining and cellular whole blood mimics, Slingshot Biosciences created a training kit designed to make it easier for flow core lab directors and managers to reliably train scientists new to flow cytometry.

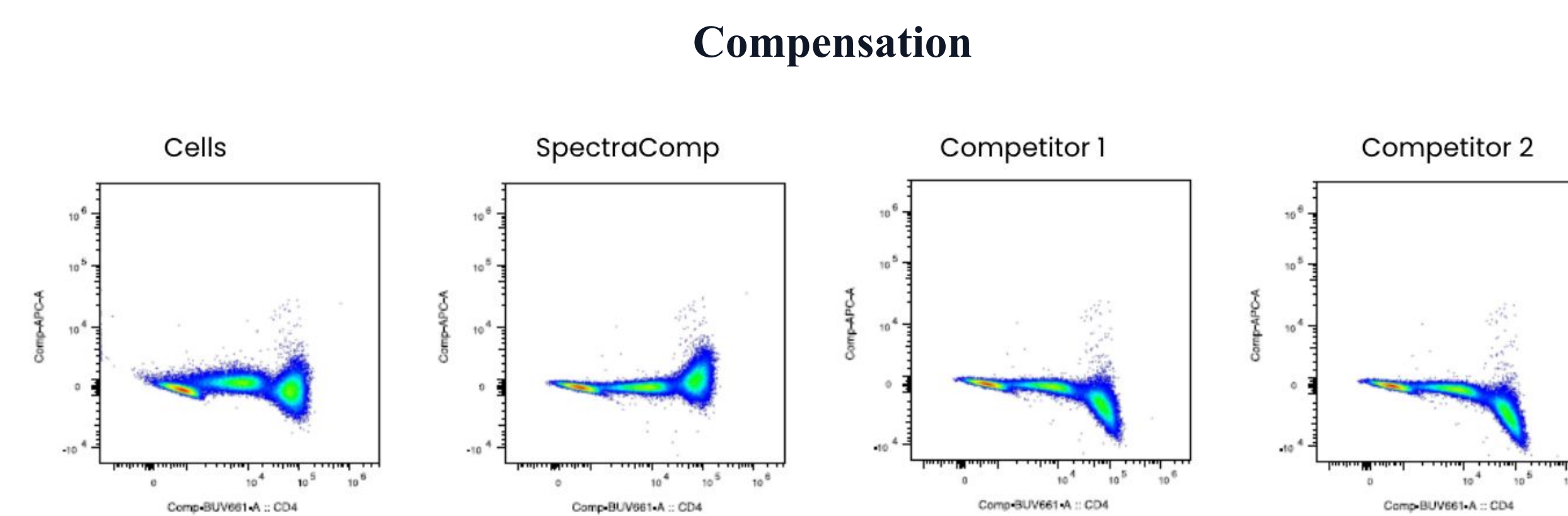


Fig 2. The compensation performance of Slingshot's SpectraComp™ matches stained cells (current gold standard)

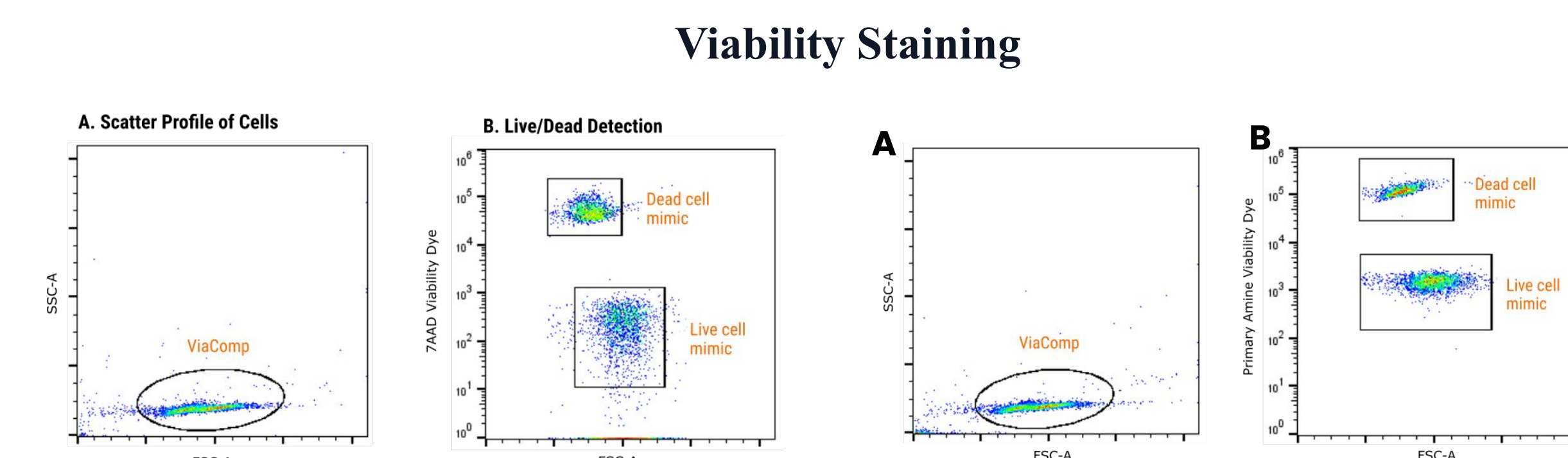


Fig 3. Slingshot ViaComp™ shows binding to DNA (7-AAD) intercalating dye. **A.** ViaComp mimics the scatter profile of lymphocytes. **B.** ViaComp is stained with 7-AAD to distinguish live and dead cell populations.

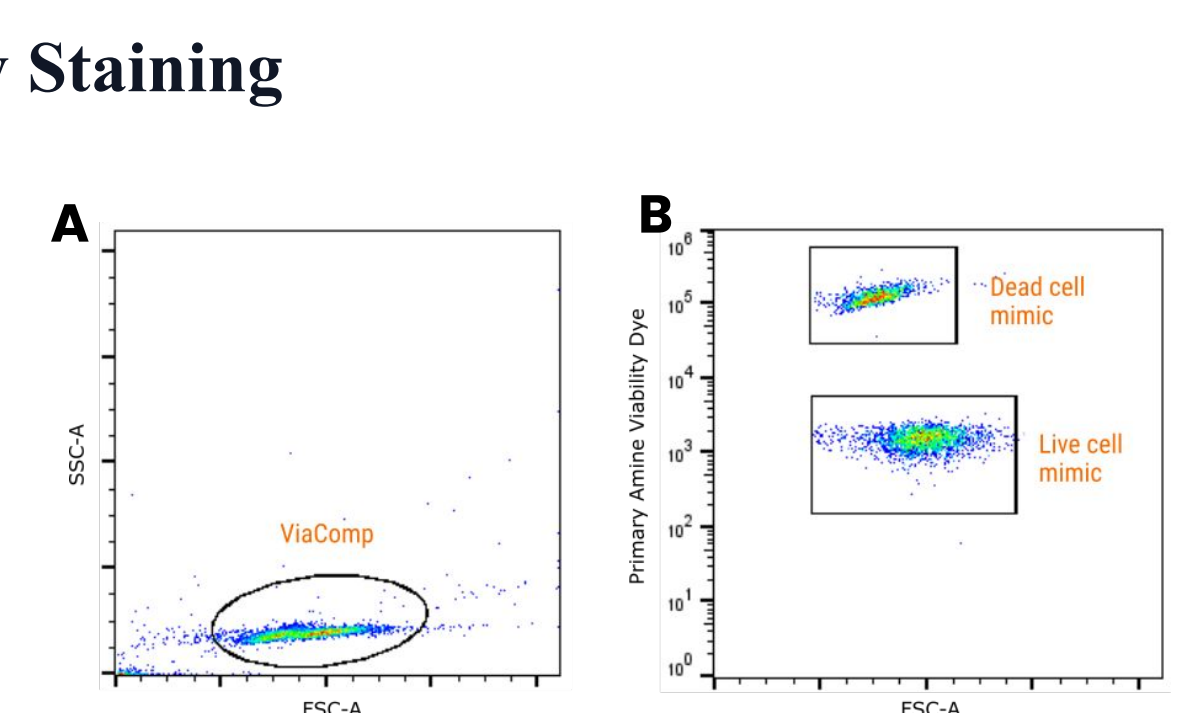


Fig 4. Slingshot ViaComp™ shows binding to primary amine dye. **A.** ViaComp mimics the scatter profile of lymphocytes. **B.** ViaComp is stained with a primary amine dye (488 nm) to distinguish live and dead cell populations.

Gating and Analysis

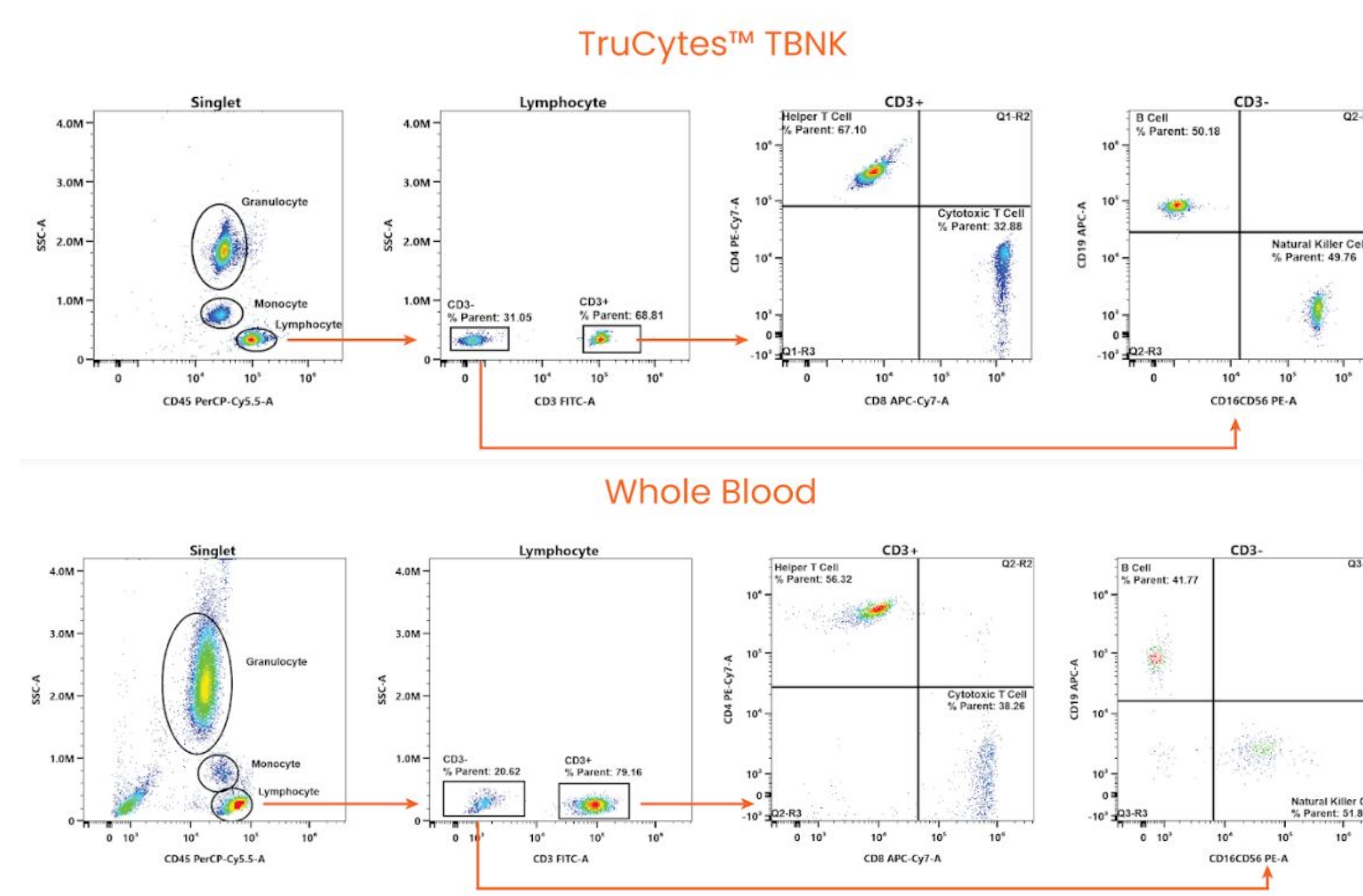


Fig 5. Slingshot's TruCytes™ match the scatter and biomarker profile of TBNK subsets of whole blood.

ALTERNATIVE TO APOPTOSIS INDUCTION

Slingshot Biosciences has created a synthetic apoptotic cell mimic which binds annexin V. With double stranded DNA added to the hydrogel, it can also be used to distinguish between live, apoptotic and dead populations.

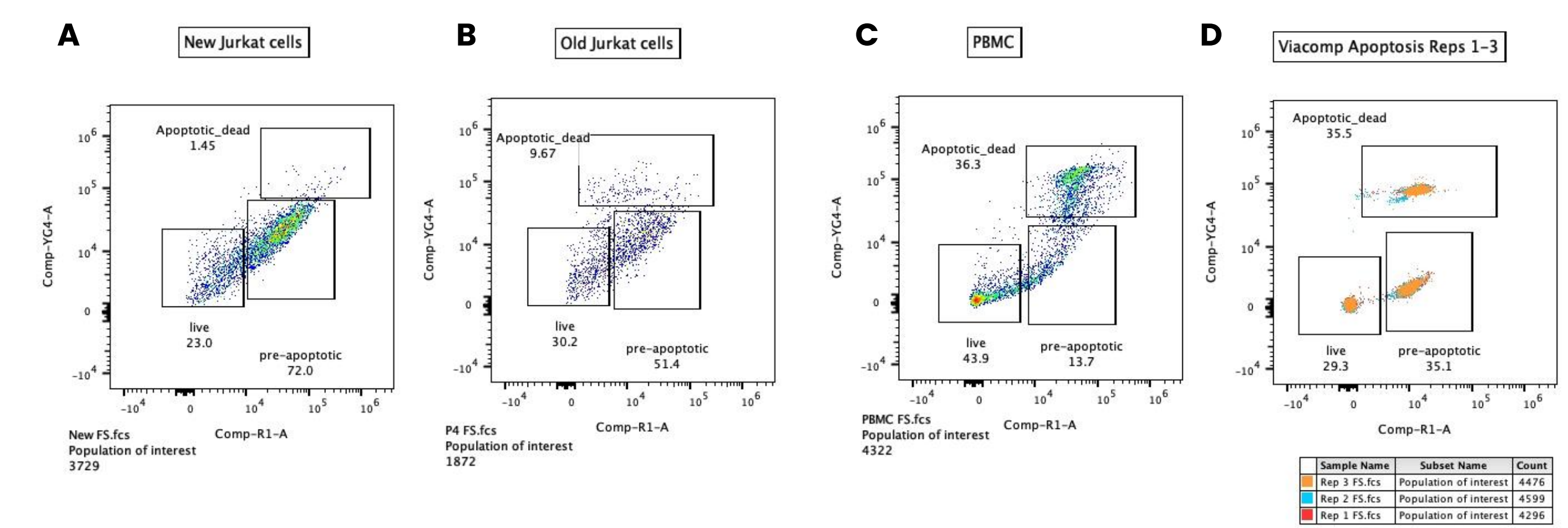


Fig 6. Slingshot's ViaComp Apoptosis cell mimics consistently demonstrate live, pre-apoptotic, and dead populations at consistent percentages. **A.** 5 day old apoptotic Jurkat cells. **B.** Apoptotic 1 day old Jurkat cells. **C.** Apoptotic PBMCs. **D.** Three replicates of Slingshot's ViaComp apoptosis cell mimics. Apoptosis was induced in biological samples with CD95 at 0.0625ug/ml for an hr at 37°C

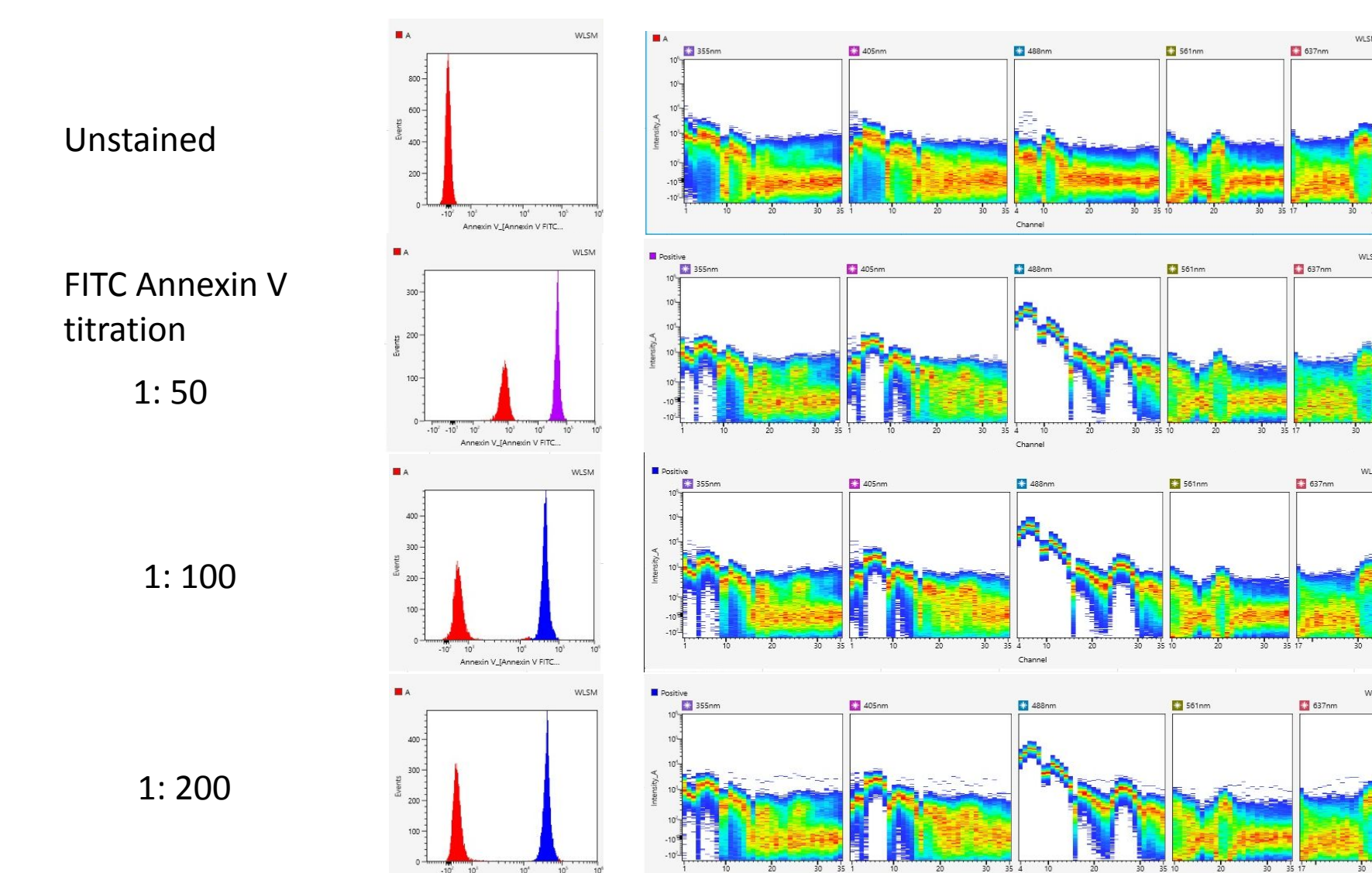


Fig 7. Spectral analysis with Slingshot apoptosis cell mimics showing titration of annexin V. Performed on 5-L Sony ID7000 cytometer.

CONCLUSIONS

Slingshot Biosciences is addressing the training needs of flow cytometry core labs with synthetic cell mimics that can be used for viability, compensation and instrument setup or gating. These cell mimics are non-biohazardous and simple to prepare, so flow cytometry leaders can focus on teaching techniques rather than troubleshooting.

Apoptosis induction is time consuming and inconsistent with biological samples. Slingshot apoptosis cell mimics allows detection of apoptosis for training, as well as spectral unmixing, compensation or use as a process control.



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