

Polymer-based synthetic cells for next-generation, multi-modal viability staining and analysis

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SUMMARY

We have developed a synthetic cell that binds both DNA intercalating and amine-reactive dyes to simulate the viability staining of real cells. Biomarkers can be added to the surface of the particles to create custom controls, such as an apoptotic cell mimic.

BACKGROUND

Viability staining is the process by which live and dead cell populations within a sample are differentiated. Viability controls are generally a mixture of live and dead cells stained with a differentiating dye. These dyes generally fall into one of two broad categories: DNA-intercalating and primary amine (protein) stains. Despite the fact that several DNA binding dyes are commonly used to identify live cell populations, compensation beads for DNA dyes are rarely available. In addition, existing polystyrene beads used for amine-reactive dyes have fundamentally different properties when compared to cellular material causing difficulties when trying to work with certain major classes of dyes in key fluorescence channels. Here we present data highlighting particles that are independently tuned along optical, fluorescent, genomic and biochemical parameters, generating a highly-flexible synthetic cell for viability characterization.

TUNABLE SCATTER PROFILE

Optical properties of hydrogel-based synthetic cells can be precisely tuned to match the forward and side scatter profiles of various cell types.

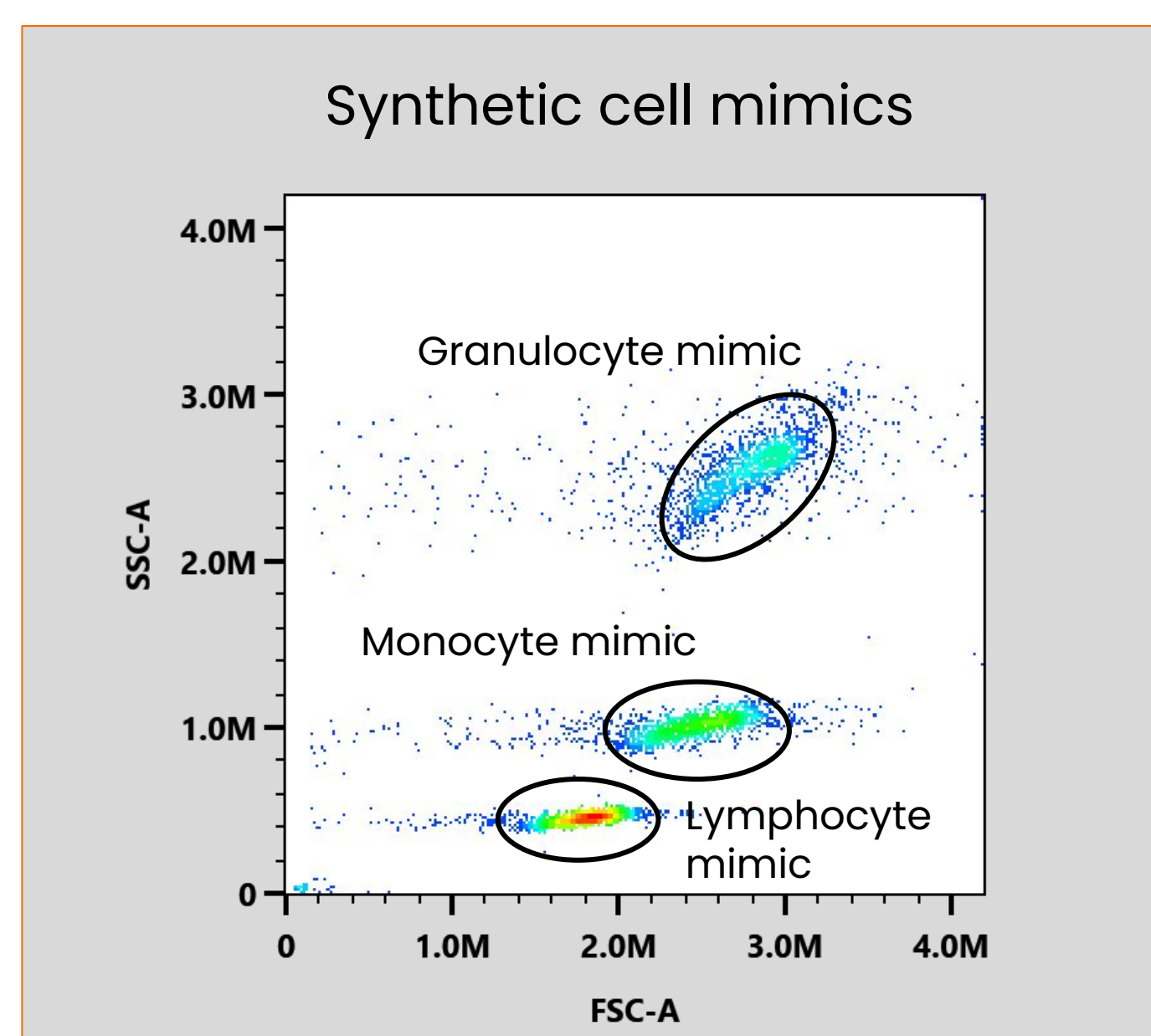


Fig. 1. FlowCytos® white blood cell mimics match the scatter profiles of unfixed lymphocytes, monocytes, and granulocytes.

VIABILITY COMPENSATION CONTROLS

Both double stranded DNA and primary amines are embedded in ViaComp® synthetic cells, making them compatible with a variety of viability stains including DNA-intercalating and amine-reactive dyes. Combining with a negative bead yields clearly separable positive and negative fluorescence populations which can be used for compensation and assay controls. ViaComp® optical properties tuned to match the scatter profile of fixed lymphocytes.

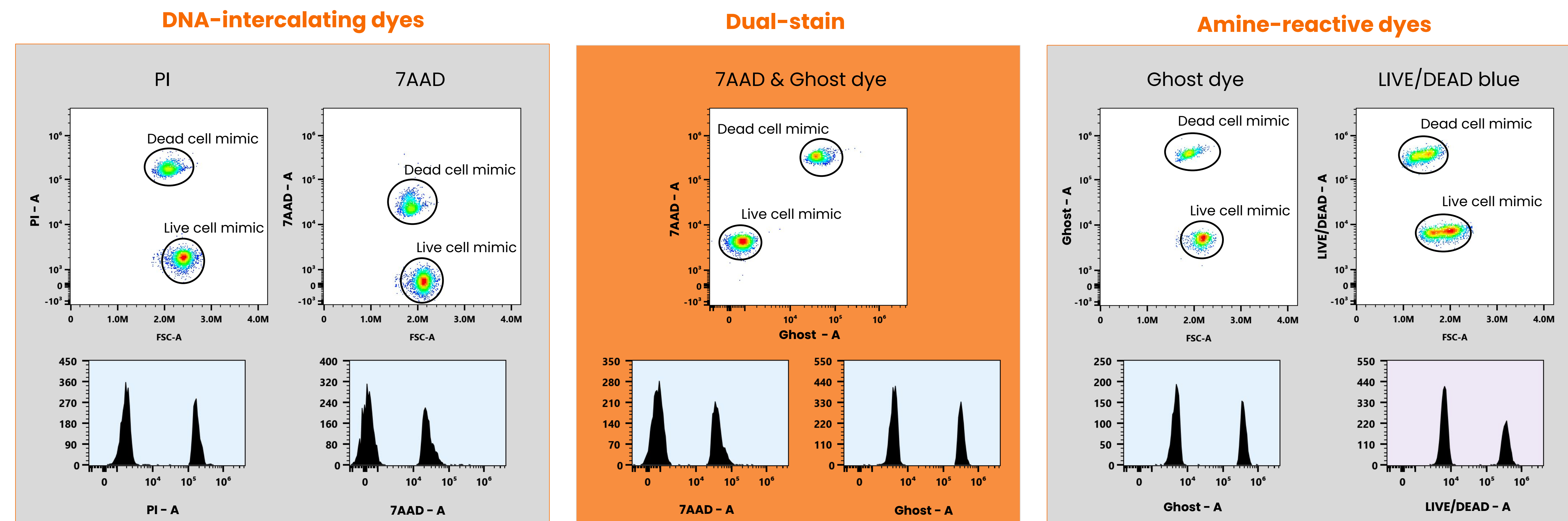


Fig. 2. (Left) ViaComp® synthetic cells stained with DNA-intercalating dyes propidium iodide (PI) and 7AAD. (Middle) ViaComp® simultaneously stained with 7AAD and amine-reactive ghost dye. (Right) ViaComp® stained with amine-reactive ghost dye and LIVE/DEAD fixable blue dead cell stain.

ALTERNATIVE TO HEAT-KILLED CELLS

Viability controls generally rely on killing a portion of the sample with heat or chemical methods. This can be time-consuming, potentially wasteful, and often relies on harsh treatments to facilitate cell death. Dead cells also emit higher autofluorescence compared to live cells and tend to experience much higher levels of non-specific binding. Using synthetic cells for viability controls mitigates these issues and improves consistency between experiments.

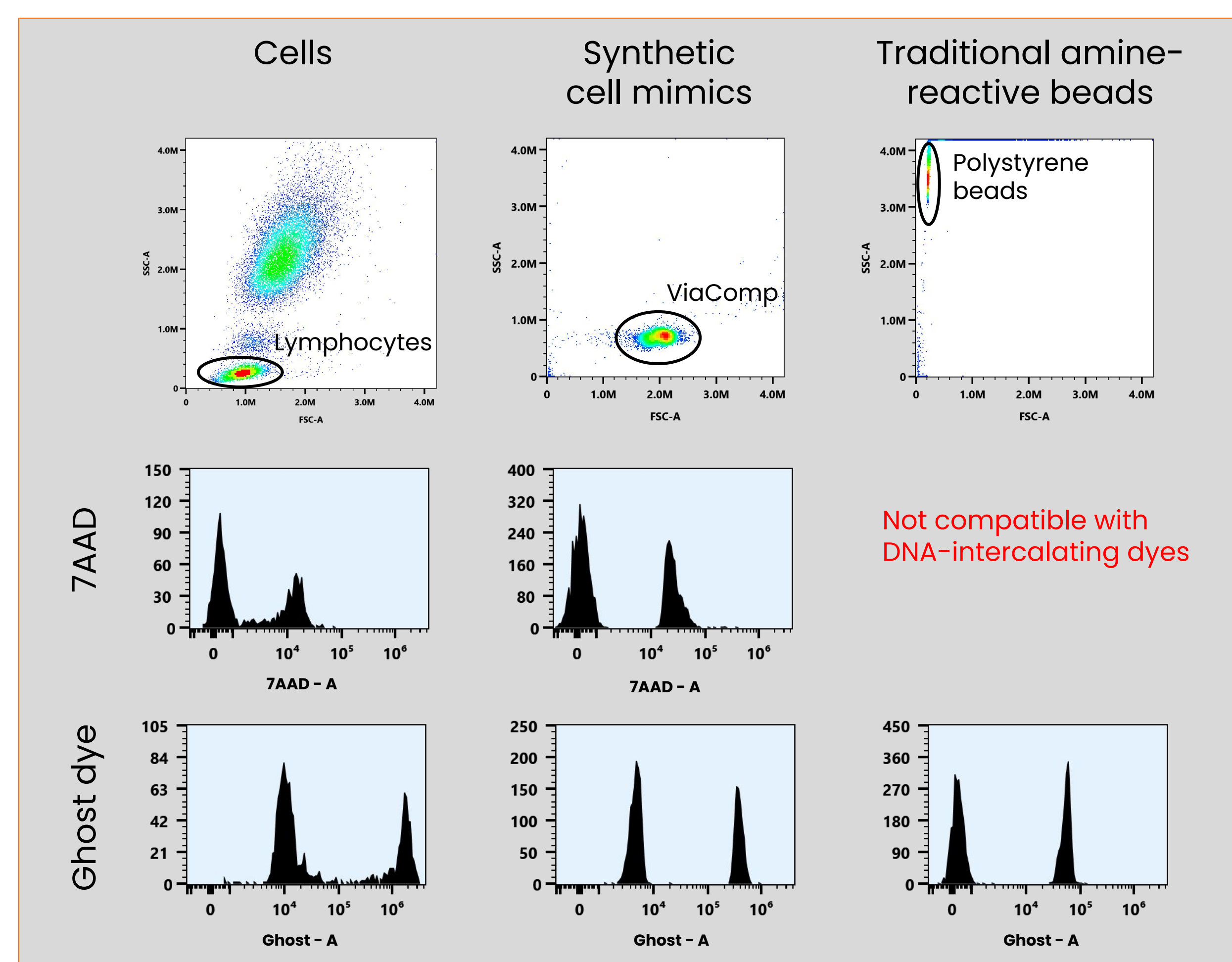


Fig. 3. Staining profiles for 7AAD and amine-reactive ghost dye for (left) a mixture of live and dead lymphocytes, (middle) ViaComp® synthetic cells, and (right) a traditional amine-reactive bead. Dead cells were heat-killed at 65°C for 5 minutes.

SYNTHETIC APOPTOSIS MIMIC

Annexin V conjugates are commonly used to detect phosphatidylserine (PS) present on the extracellular side of an apoptotic cell's plasma membrane. We have created a synthetic apoptotic cell mimic which binds annexin V. When double stranded DNA is added to the bead, it can be also used to distinguish between apoptotic cells and dead cells with compromised membranes.

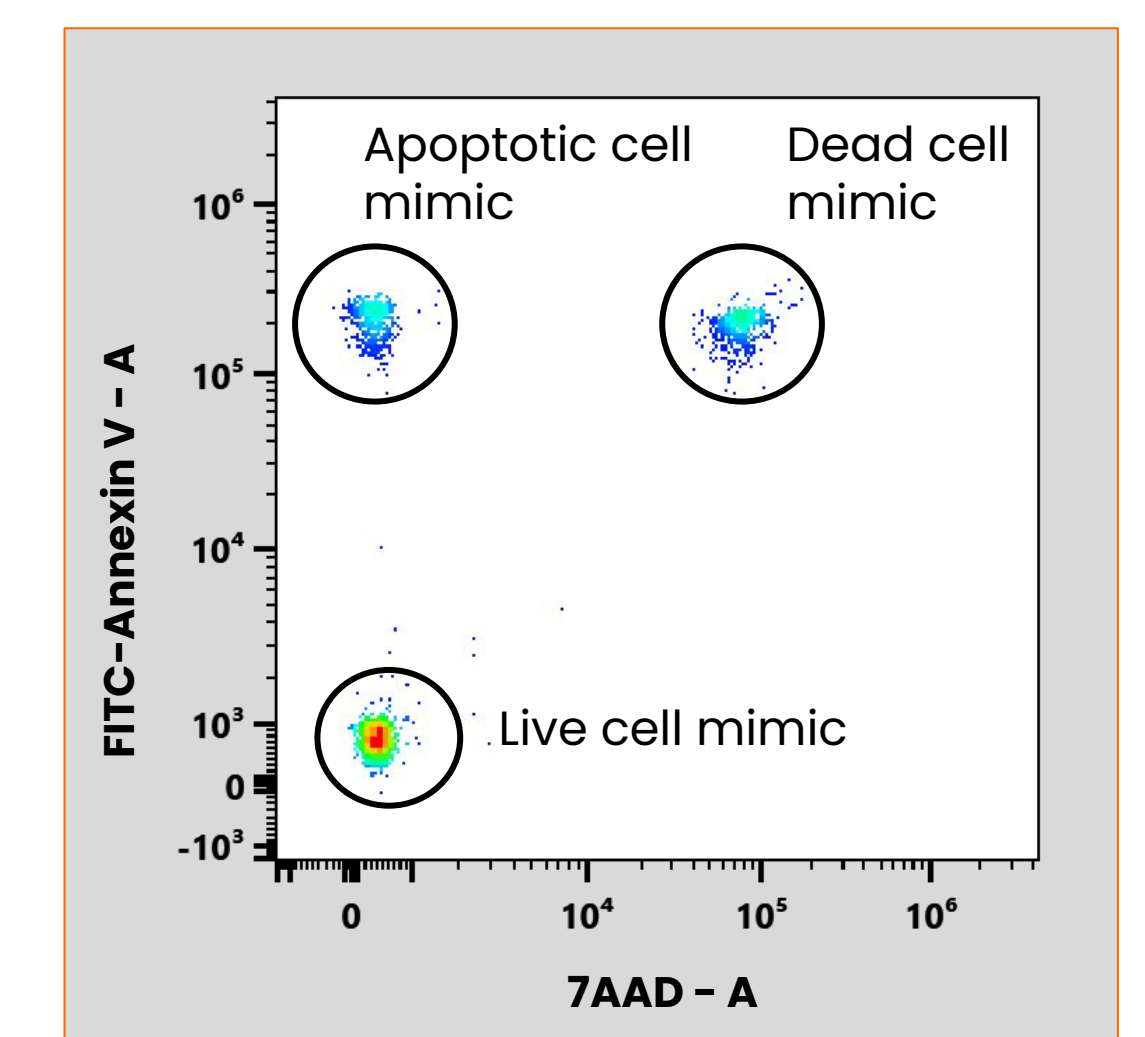


Fig. 4. Apoptosis cell mimics stained with FITC-Annexin V and 7AAD show three distinct populations, (1) annexin V+/DNA- apoptotic cell mimics, (2) annexin V+/DNA+ dead cell mimic, and (3) annexin V-/DNA- live cell mimics.

CONCLUSIONS

Viability staining is important both in experimental research and therapeutic applications of flow cytometry. Currently, common viability staining methods require researchers to kill a portion of their cells in an added step that is wasteful, time-consuming, and often still does not achieve the desired standard of control. We have created a viability control that reacts with both DNA and amine-reactive dyes to provide clear separation between positive and negative signals for compensation or spectral unmixing. When decorated with other biomarkers, the beads mimic properties of biological cells for flow cytometry applications but in a consistent manner. This first-in-class technology enables a new paradigm for quantitative flow cytometry.