

Introduction

Amine-reactive dyes, such as Viability™ Fixable Dyes, have established themselves as a class of reagents for discrimination of dead cells from living cells. By entering cells with compromised membranes and reacting with the amino groups of cytoskeletal proteins, dead cells are covalently labeled throughout the cell volume. The resulting fluorescent signal from dead cells is therefore much brighter than the signal from living cells with intact membranes, which are labeled only on the surface. The label is stable and does not suffer in downstream applications such as fixation and permeabilization unlike some of the traditional DNA intercalating dyes, such as propidium iodide or 7-aminoactinomycin D (7-AAD). The reactivity against amino groups, however, requires cells to be labeled in a medium without amino groups, i.e., in a protein-free medium to avoid

premature reaction and deactivation of the amine-reactive dye. Typically, cells are transferred into phosphate-buffered saline (PBS) prior to labeling. To assess the viability of whole blood samples, it is common to lyse red blood cells (RBCs) and wash away debris and serum components with PBS before using an amine-reactive dye as fixable dead cell stain. However, RBC lysis is stressful for all cells and reduces cell viability. Here we present a method and protocols using Viability Fixable Dyes, which allow probing of whole blood cell viability in the presence of serum proteins without prior lysis of RBCs. By this method, it is possible to assess the viability of the whole blood sample before any downstream application and without detrimental effects on cell viability by RBC lysis.

Method

For use with whole blood, Viability Dyes were reconstituted in 20 μ L anhydrous DMSO (5 \times stock) and for use with lysed blood in 100 μ L (1 \times stock). For staining of dead cells in whole blood 1 μ L of the 5 \times Viability Dye solution is added to a 100 μ L whole blood sample and incubated for 10 min at RT.

Directly after incubation with Viability Dye antibody fluorochrome conjugates can be added for surface staining. Further downstream processing such as RBC lysis, fixation, and permeabilization can follow the initial staining.

Results

1 Viability™ 405/520 Fixable Dye detects dead cells in whole blood spiked with heat-treated cells

Dead white blood cells (WBCs) were prepared from whole blood by RBC lysis and heat treatment at 95 °C for 10 min and spiked into whole blood from the same donor. 100 μ L whole blood samples were titrated with different volumes of Viability™ 405/520 Fixable Dye (5 \times stock solution) and compared with a 7-AAD staining. Prior to analysis the samples

were stained with CD45-APC, subjected to RBC lysis, and analyzed by a MACSQuant® Analyzer 10. Viability 405/520 and 7-AAD detected dead cells in whole blood at comparable frequencies (fig. 1). Use of more than 1 μ L of dye solution led to additional cell death due to DMSO toxicity and increased background staining of living cells.

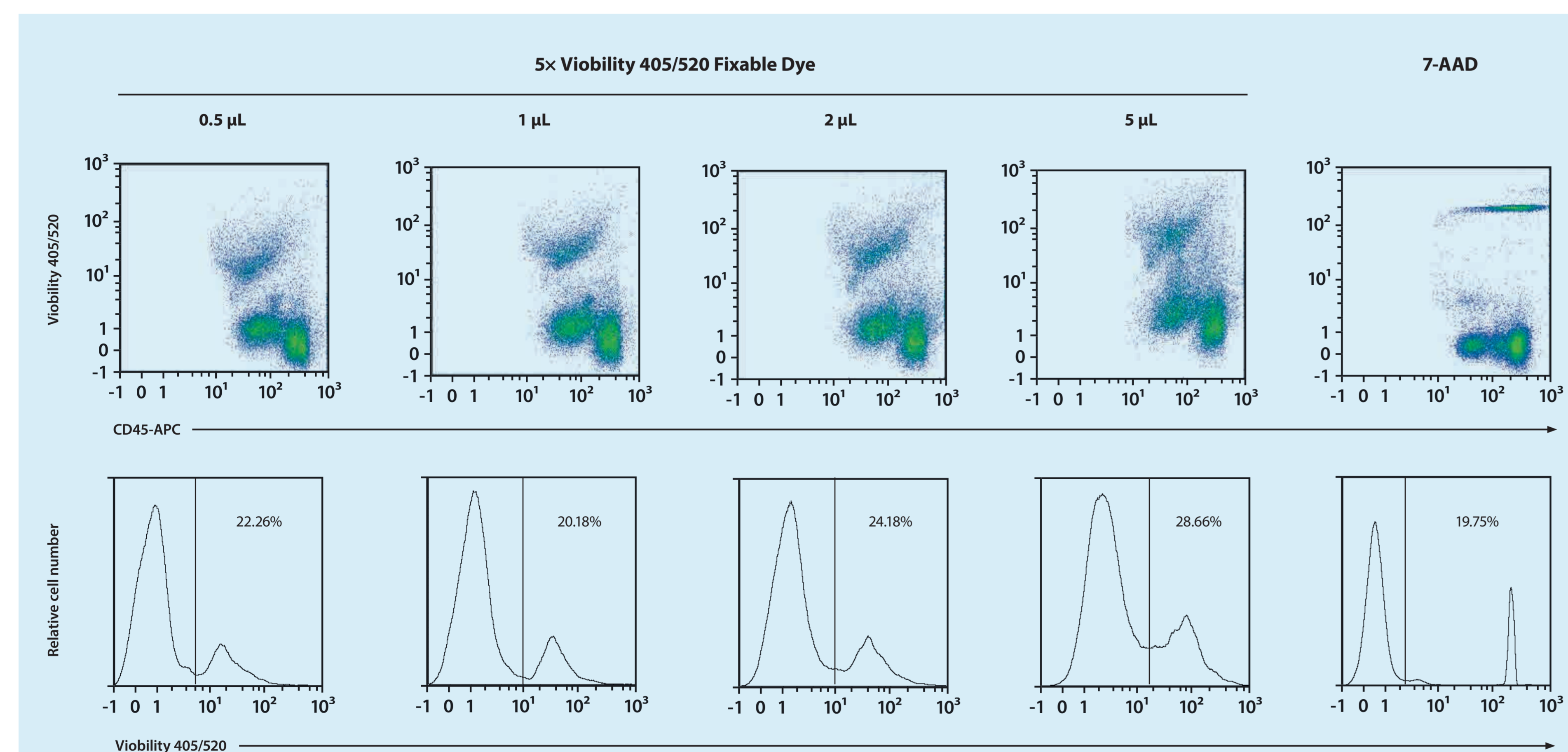


Figure 1

2 Comparison of Viability™ Fixable Dye staining of whole blood vs. lysed blood

100 μ L whole blood samples were subjected to RBC lysis, washed with PBS, and stained with 1 \times stock solutions of Viability™ 405/452, Viability 405/520, or Viability 488/520. The cells were counterstained with CD45-APC and analyzed by a MACSQuant Analyzer 10. In comparison 100 μ L whole blood samples were stained with 1 μ L of 5 \times stock solutions of Viability 405/452, Viability 405/520, or Viability 488/520.

The whole blood samples were counterstained with CD45-APC, subjected to RBC lysis, and analyzed by a MACSQuant Analyzer 10. Membranes of cells subjected to lysis prior to labeling with Viability Dyes appeared to be more compromised than those of cells stained in whole blood prior to lysis.

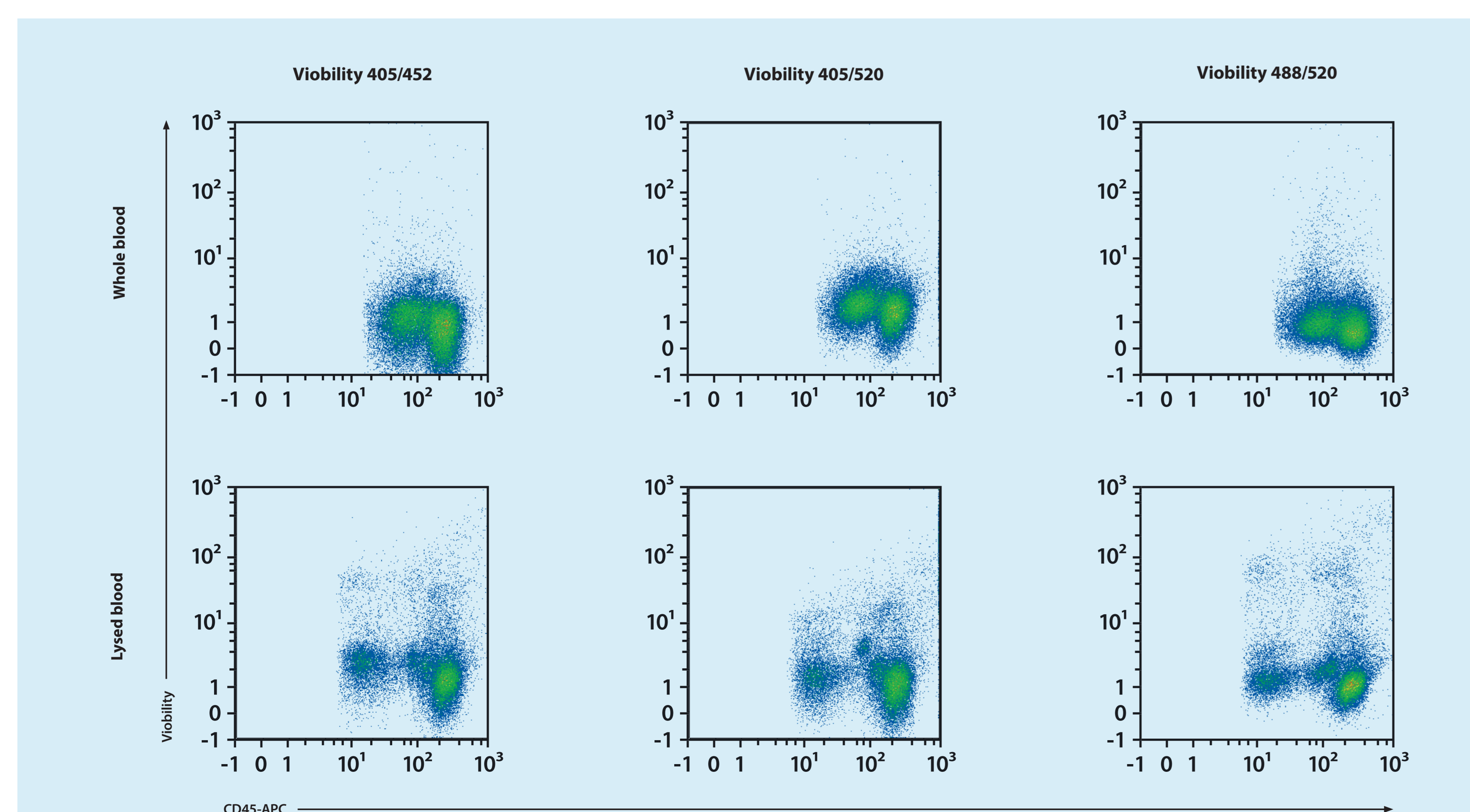


Figure 2

3 Detection of dead cells in aged whole blood

Whole blood stabilized with EDTA was stored at RT for several days to simulate sample aging between collection and analysis. At different time points 100 μ L samples were drawn and stained with 1 μ L Viability™ 405/520 (5 \times stock) in comparison to 7-AAD. Prior to analysis the samples were stained with CD45-APC, lysed and analyzed by a MACSQuant® Analyzer 10. Viability 405/520 detected cells with compromised membranes corresponding to dead and late apoptotic cells in aging whole

blood (fig. 3A). Frequencies were higher than with 7-AAD staining which does not label apoptotic cells (fig. 3B). On day 2, monocytes were already stained by Viability 405/520, but were still 7-AAD-negative. On day 3, similar frequencies of dead cells (mostly granulocytes and monocytes) were recognized by both dyes. Therefore, Viability 405/520 detects dying cells earlier than 7-AAD.

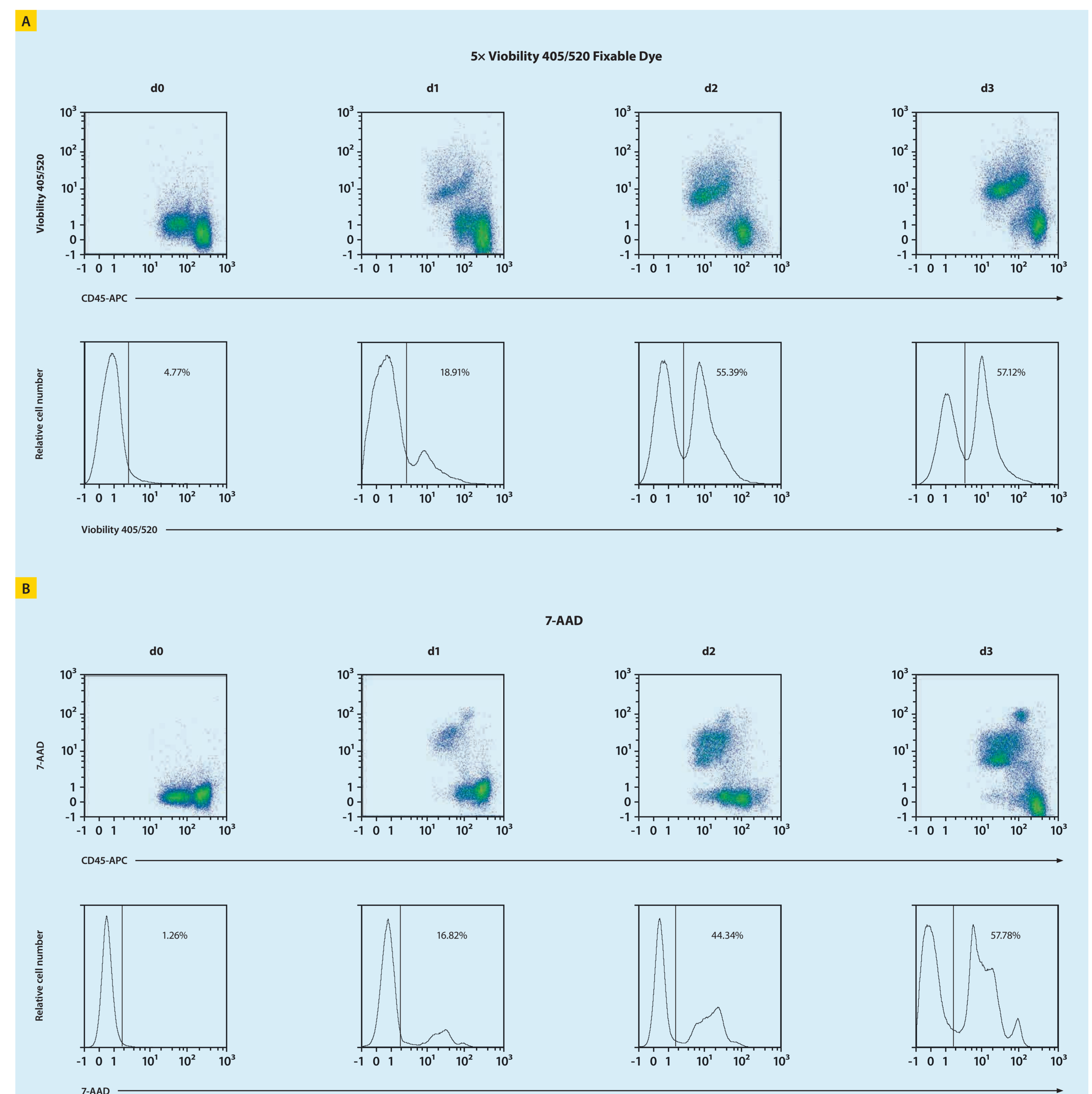


Figure 3

4 Staining of whole blood with a T cell antibody conjugate panel

A volume of 70 μ L of whole blood was stained with 1 μ L of Viability 405/520 (5 \times stock). After 10 min incubation at RT 40 μ L of a cocktail of fluorochrome-conjugated antibodies, including CD4-FITC, CD8-PE, CD3-PE-Vio®770, and CD45-APC, was added and incubated for another 10 min. For evaluation cells were subjected to RBC lysis and analyzed by a

MACSQuant® Analyzer 10. Lymphocytes were gated by scatter properties. Viability™ 405/520-positive cells were excluded as dead cells.

Viability 405/520 allows for the exclusion of dead cells within whole blood and does not interfere with subsequent staining of T cell surface antigens.

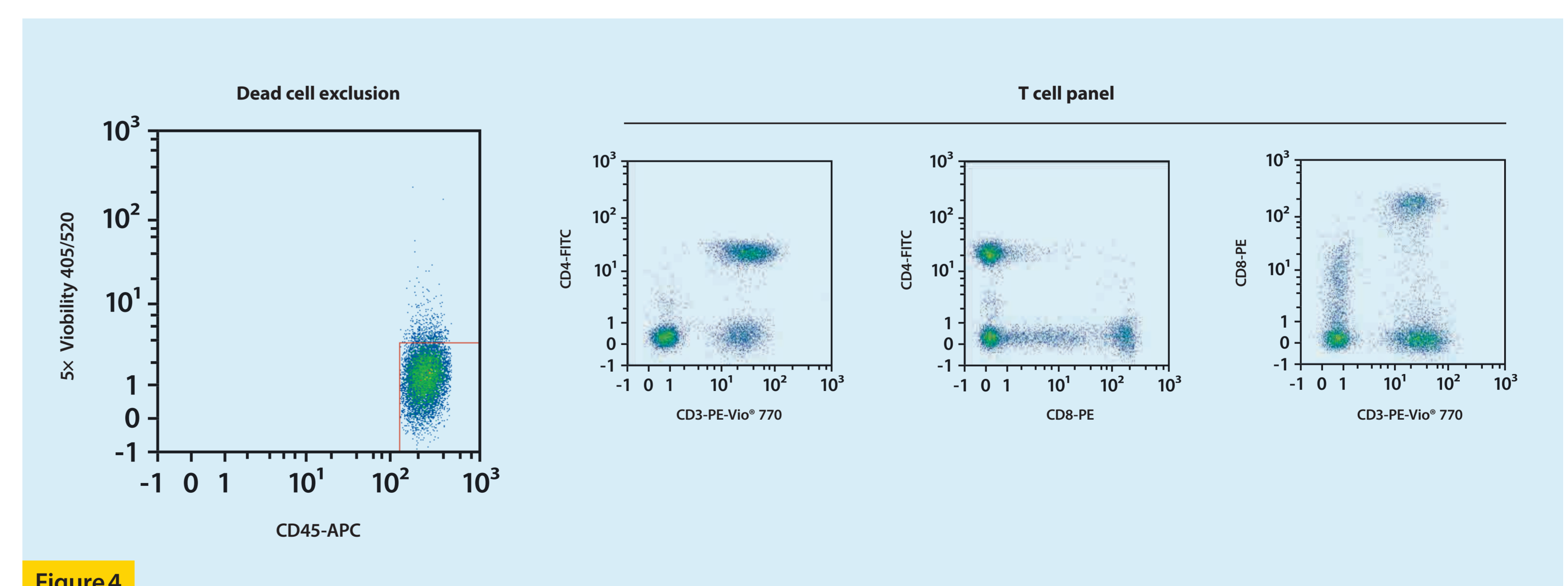


Figure 4

Conclusion

- Reliable detection and exclusion of dead and dying cells in whole blood by using Viability™ Fixable Dyes at a five-fold higher concentration to compensate for the presence of solutes containing amino groups.
- Compromised cells in aged whole blood samples are detected earlier with Viability Fixable Dyes than with 7-AAD.
- Viability Dyes do not interfere with subsequent staining of surface markers, giving the possibility of labeling and excluding dead cells that are prone to non-specific binding of antibody-fluorochrome conjugates and present in the whole blood sample at the time of surface staining.
- Viability Dyes are suitable for analysis of cells sensitive to whole blood processing procedures such as RBC lysis or density gradient centrifugation.