invitrogen

New fixable viability dyes and applications for flow cytometry

Brandon Trent, Kelly Lundsten, Thiago Alves da Costa, and Chris Langsdorf
Thermo Fisher Scientific, Eugene, OR, USA

Background

Flow cytometry provides many advantages including single-cell quantitative analysis, high sample throughput, and multiplex cell characterization. Accurate discrimination of live and dead cells is a fundamental component of most flow cytometry experiments, but many viability dyes were optimized using legacy instruments and outdated model systems. Here we provide an updated overview of methods and reagents to assess cell viability with flow cytometry. Results and discussion are based on our recent efforts to expand the color palette of fixable viability dyes. We introduce six new dyes, each with narrow and unique emission spectra ideal for expanding high-parameter conventional and spectral cytometry experiments. Additionally, this data demonstrates updated workflow recommendations for modern cytometers, improving stability, efficiency, and sample throughput. Novel insights and methods presented here will streamline complex experiments and provide deeper insight into cell behavior.

Figure 1. Fixable Live/Dead Dyes

A.

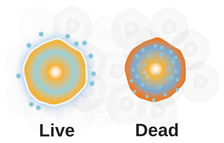

Fig 1A. Amine fixable dyes work by allowing reactions to occur between proteins and amine-reactive dyes. Live cells with intact cell membranes have proteins on their membrane/extracellular matrix that will bind the amine dye and fluoresce dimly. Fixable amine-dyes penetrate the compromised membranes of dead cells, labeling the proteins inside the cell, generating a much brighter fluorescent staining pattern. **B.** Fixing cells with some conventional DNA-staining dyes (i.e. Propidium Iodine) can lead to problems resolving the live/dead populations. Live/Dead amine fixable stains solve this issue and allow for fixed cells to be analyzed, even months after fixing.

B.

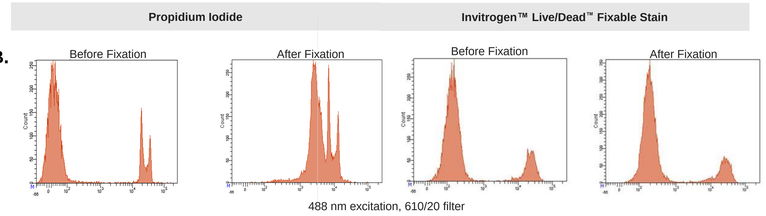

Figure 2. Non-specific staining by dead cell populations

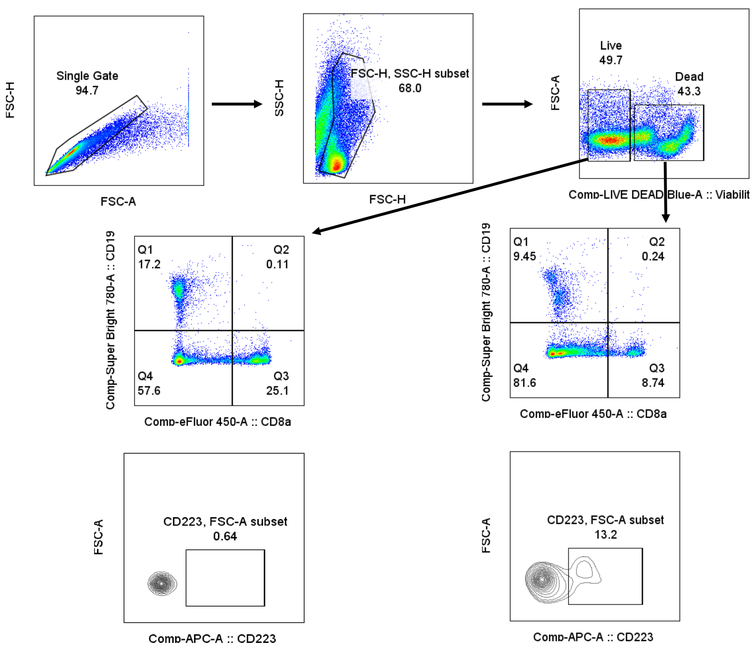

Fig. 2. Panel for T cell analysis in stimulated human PBMCs demonstrating the importance of live dead staining. Dead cells are able to bind non-specifically to fluorescently labeled antibodies, leading to false positives in downstream analyses. This is a particular concern when staining for rare markers or markers with a low separation index. The bottom panels display non-specific CD223 staining in a dead CD8 T cell population.

A.

Figure 3. Expanding the detection of live dead cellular populations

Invitrogen™ LIVE/DEAD™ Fixable (emission) Viability kits (laser line)	Laser	Emission
LIVE/DEAD Fixable Lime (506) Viability kit for 405 nm excitation	Violet	506 nm
LIVE/DEAD Fixable Olive (557) Viability kit for 488 nm excitation	Blue	557 nm
LIVE/DEAD Fixable Orange (602) Viability kit for 561 nm excitation	Yellow	602 nm
LIVE/DEAD Fixable Scarlet (723) Viability kit for 633 nm excitation	Red	723 nm
LIVE/DEAD Fixable Near-IR (780) Viability kit for 633 nm excitation	Far Red	780 nm
LIVE/DEAD Fixable Near-IR (876) Viability kit for 808 nm excitation	808	876 nm

B.

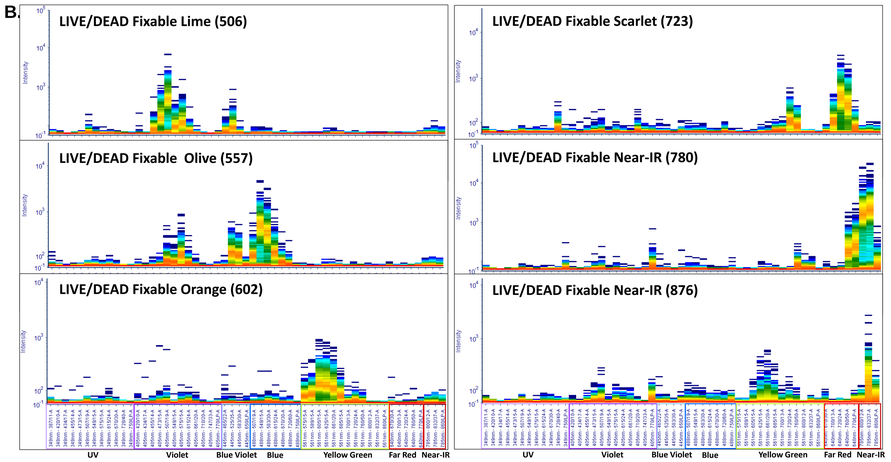

Figure 3A. New options in the fixable LIVE/DEAD catalog allow for more greater options when designing flow panels. New options in the violet blue, yellow, red, far red, and IR lasers provide numerous options to incorporate new viability dyes. **B**. Full spectral analyses of new fixable viability dyes collected on the Invitrogen™ Bigfoot™ Spectral Cell Sorter.

Figure 4. Distinction of viable cell populations corroborated with use of high-definition flow imaging

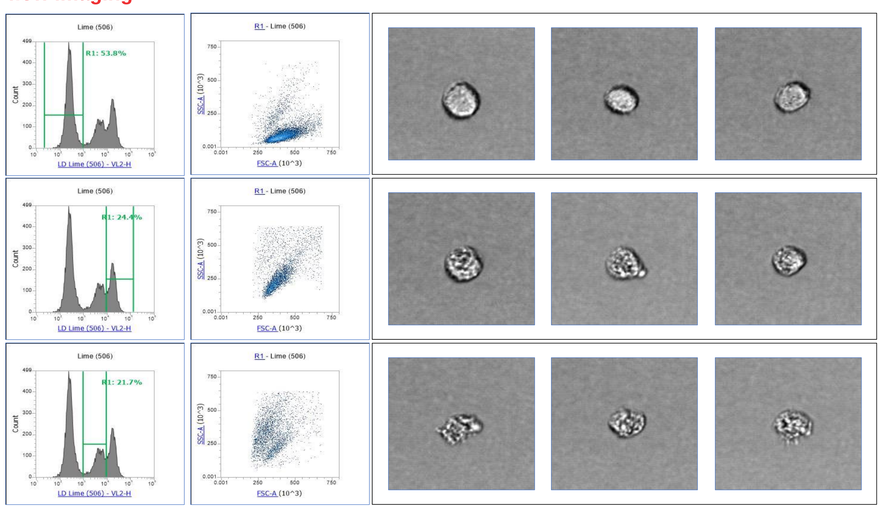

Figure 4. Live/Dead populations often display varying degrees of positivity depending on the integrity of the cellular membrane, leading to different positive populations. Here we used LIVE/DEAD Lime (violet laser excited) to determine cellular viability in CAR-T cells. Using the Attune CytPix flow imagine capability, we observe that differences in the positive live dead populations dependent on how intact the cell membrane appears, thus allowing us to appropriately set our live/dead populations.

Figure 5. New fixable live/dead dyes sorted using spectral cytometry

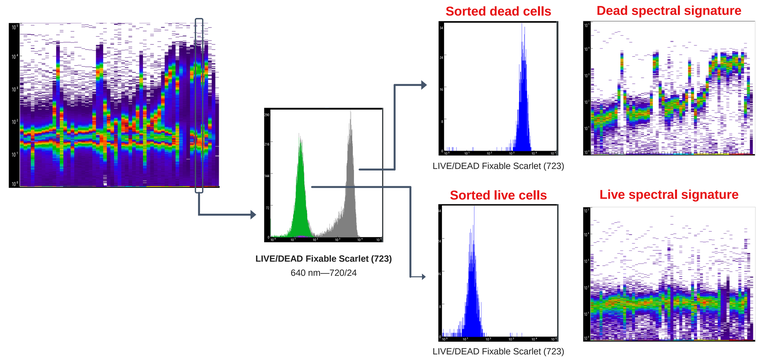

Figure 5. CAR-T cells stained with LIVE/DEAD Scarlet were sorted into dead and viable populations using the Bigfoot Spectral Cell Sorter. Left – full spectra showing the both the stained live and dead populations (box placed around peak in the red channel emitted by LIVE/DEAD scarlet). Right – after compensation, cells are sorted by cellular viability and the spectral signature of either the live or dead cells is analyzed. As expected, live cells display little to no spectral signature of any viability dye, while dead cells have a distinct LIVE/DEAD Scarlet signature.

Figure 6. New fixable Live/Dead dyes can be visualized using fluorescence microscopy

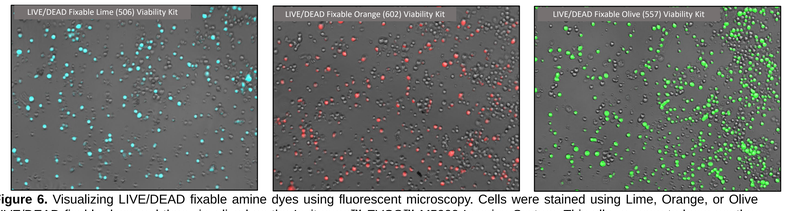

Figure 6. Visualizing LIVE/DEAD fixable amine dyes using fluorescent microscopy. Cells were stained using Lime, Orange, or Olive LIVE/DEAD fixable dyes and then visualized on the Invitrogen™ EVOS™ M5000 Imaging System. This allows users to have another visual confirmation of their live/dead staining and approximate % of live and dead cells they should expect in downstream flow analysis or sorting.

Figure 7. New fixable viability dyes to be utilized in any workflow

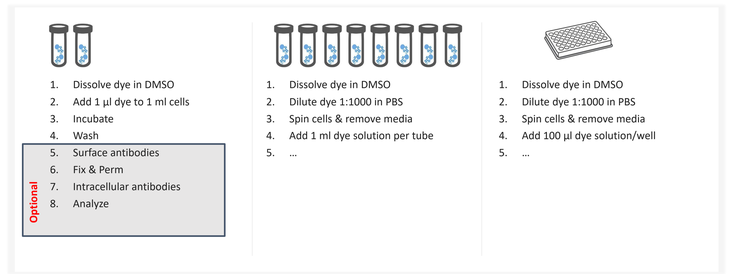

Figure 7. Basic workflow protocol that can be incorporated into any workflow. Recommended concentrations of LIVE/DEAD dyes shown here but should be titrated with your particular cell type.

Conclusions

- Panels in flow cytometry are becoming increasingly complex with the advent of spectral phenotyping and sorting.
- New fixable viability dyes can provide viability staining with unique spectral signatures in the violet, blue, yellow, red, and IR lasers.
- Analysis of new fixable dyes using spectral sorting and high-definition flow imaging corroborates the importance of viability staining to avoid analysis of dead cells and non-specific staining.

ThermoFisher SCIENTIFIC