New fixable viability dyes and applications for flow cytometry

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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component of most flow cytometry experiments, but many viability dyes were optimized using legacy instruments and outdated model systems.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 palettes of fixable viability dyes. We introduce six new fixable 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. Live/Dead dyes with distinct spectral properties to readily differentiate live and dead in an experiment.

B. Non-specific staining by dead cell populations

Figure 2. Non-specific staining by dead cell populations

Figure 3. Expanding the detection of live dead cellular populations

A. Table 1: Spectral properties of new fixable dyes.

B. Flowchart depicting the staining process and sample preparation for flow cytometry.

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

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

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

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

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.