There are several methods for analysing live, dead, and apoptotic cells by flow cytometry. These methods can be divided into three classes: membrane impermeant dyes, fixable dead-cell dyes and vital dyes.

Membrane impermeant dyes

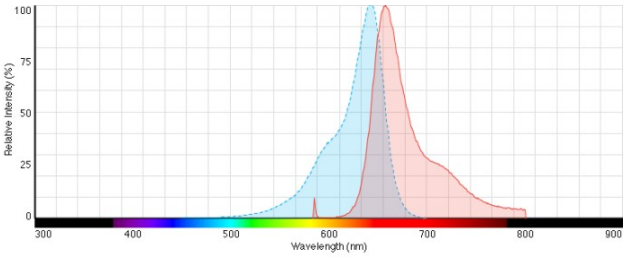
Loss of membrane integrity is the ultimate indicator of cell death, and is easily detected with impermeant DNA dyes. These dyes are excluded from cells with intact membranes, but enter cells with compromised membranes and undergo significant fluorescence enhancement when they bind DNA.

Classic DNA dyes are the first type of live dead cell dyes that most scientists and flow cytometrists consider for their experiments.

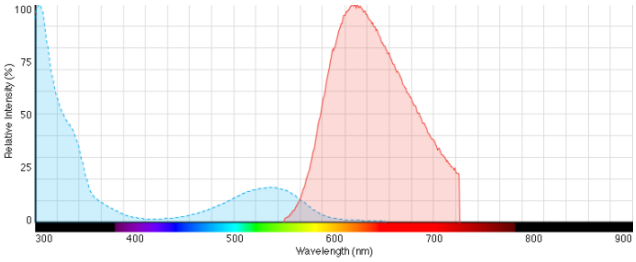
Examples of these dyes include:

Sytox dyes

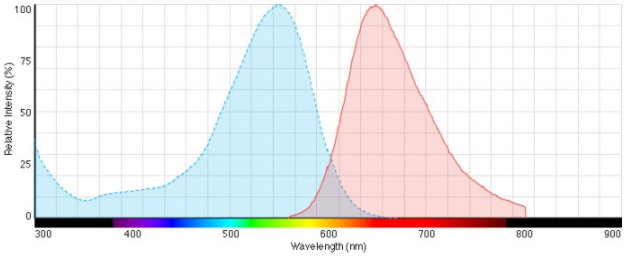
TO-PRO-3 Excitation⁄Emission: 642⁄661 nm.



Propidium Iodide (PI) Excitation⁄Emission: 493/636 nm (in aqueous solution).



7-aminoactinomycin D (7-AAD) Excitation⁄Emission: can be excited by the 488 nm laser and has an emission maxima of 647 nm.



PI and 7-AAD in particular have a long history in flow cytometry applications. This is because the method of action of PI and 7-AAD are very similar. They are both DNA binding dyes that are membrane impermeant, meaning living cells with intact membranes will exclude these dyes and exhibit little to no fluorescence. Classic DNA dyes are easy to use and typically added at the end of staining, which means they require minimal incubation. Because these dyes bind in equilibrium with DNA, external dye concentration must be maintained during analysis, and the dye should not be washed out.

Cell impermeant dyes are not appropriate for fix perm staining applications

Usefull links:
https://www.thermofisher.com/it/en/home/life-science/cell-analysis/fluorophores/to-pro-3.html

FIXABLE DEAD-CELL DYES

Amine dyes are fixable, so whether you're traditionally staining your cells or fixing and permeabilizing your cells, the fluorescence is maintained. Like impermeant dyes, the fixable dyes are excluded from the cytosol of healthy cells.
The dyes react with surface proteins of healthy cells, but also label proteins throughout the cytoplasm of cells with damaged membranes, causing dead cells to have at least 50-fold greater fluorescence than live cells. Because they covalently interact with available amino groups of cellular proteins, labeled cells can be aldehyde fixed and permeabilized without losing viability.

Useful links:

https://www.thermofisher.com/it/en/home/life-science/cell-analysis/flow-cytometry/cell-health-and-viability-assays-for-flow-cytometry/cell-viability-assays-for-flow-cytometry/flow-fixable-viability-dyes.html

https://www.biolegend.com/NewsLegend/020614/index.htm

VITAL DYES

This class of dyes measures viability by fluorescing when acted upon in metabolically active cells. Calcein acetomethoxyis membrane permeable, yet, due to its attached acetomethoxy group , does not fluoresce. However, once inside a metabolically active cell, cellular esterases cleave the acetomethoxy group yielding calcein. Once free, calcein readily binds intracellular calcium and fluoresces brightly green. As a result, viable cells appear bright green, while dead cells do not.