#3 | October 2024

NEWSLETTER

Core Facility Cytometry

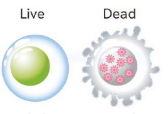
Contents of this issue:

Why viability dyes?

Viability dyes for dead cell exclusion offer many advantages in flow cytometry:

- **Reduction of non-specific staining and autofluorescence**
- **Better results in functional assays**
- **More efficient sorting**
- **Easier identification of cells after fixation**

Classes of viability dyes



DNA-intercalating dyes: can only pass through permeable membranes of dead cells. *E.g.: DAPI, propidium iodide, 7-AAD*

+ simple application, inexpensive
- broad emission spectrum, cannot be combined with fixation and intracellular staining

Enzyme-activated dyes: only living cells have functional esterases and are stained. *E.g.: Calcein-AM*

+ specific, non-cytotoxic, sharper emission spectra
- more expensive than DNA dyes, cannot be combined with fixation and intracellular staining

Amine-reactive dyes: bind to amino termini of proteins. Dead cells have permeable cell membranes, therefore more proteins are stained. *E.g.: Horizon FV Dyes, Zombie Dyes, Ghost Dyes, LIVE/DEAD*

+ specific, fixable, non-cytotoxic, sharper emission spectra, ideal for multicolor
- bind free serum protein (do not use BSA when staining!), more expensive than DNA dyes

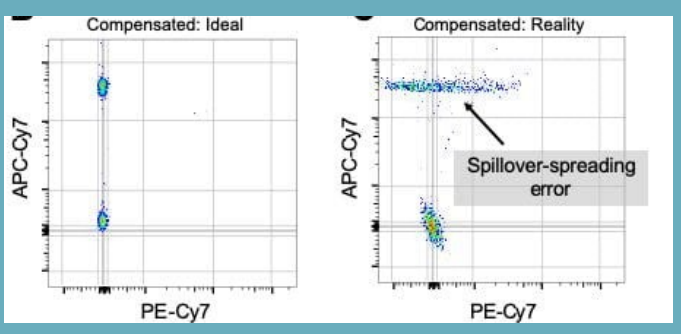
<u>ATTENTION</u>: DNA-intercalating dyes are very “sticky” and remain in the sample line of the flow cytometer for a long time! Please note the following when using these dyes:

1. Always measure unstained samples at the beginning!
2. Thoroughly rinse the flow cytometer after the measurement!
3. Make a note on the user log to avoid nasty surprises for subsequent users!

The Core Facility also provides unstained dead cells to test for possible residues!

Spillover Spreading Matrix (SSM)

The more signals from fluorescent dyes spill into a detector, the more the nominally negative distributions scatter (= spillover spreading). The degree of spreading increases with the fluorescence intensity as well as the extent of spectral overlap. This ultimately leads to a reduced sensitivity of the detector.



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A spillover spreading matrix (SSM) is used to quantify the extent of spillover spreading between detector pairs and is a useful tool for comparing device performance and for designing multicolor panels (TIP: see also our guide Panel Design).

In order for the Core Facility to create an SSM, we need as many different fluorophore-tagged antibodies as possible!

<u>Can you spare a few µl of your antibody?</u>

<u>Then please get in touch with us!</u>

TIP: Until October 31, 2024 there is an antibody sample promotion from Szabo Scandic:

<u>Order up to 3 Samples here :)</u>

If you have any further questions, simply contact us at:
ZMF-CF-Zytometrie@jku.at

Thank you very much and good luck with your experiments!