Channel-Free Dead Cell Exclusion with DRAQ7™

In flow cytometry dead cells are often removed from analysis to exclude cells that absorb antibody unspecifically, or are counted to assess sample quality. For fresh samples there is a choice of fluorescent viability dyes, mostly DNA binding dyes that label nuclei when cell membranes are compromised. However, this expends at least one valuable fluorescence channel, limiting number and choice of chromophores for multi-colour phenotypic analysis.

Described here is a method that allows exclusion of dead cells and/or sample quality evaluation without using a channel, releasing all available channels for phenotypic analysis on dual-/multi-laser cytometers.

This procedure can even be applied to a pre-existing phenotypic panel that subsequently needs dead cell exclusion. It relies upon **DRAQ7™** - a water-soluble, far-red fluorescing DNA probe that has no overlap with common visible-range fluors such as FITC or R-PE. DRAQ7 is entirely membrane impermeant, yet rapidly enters "leaky" cells to label nuclear DNA. Therefore, DRAQ7 is an ideal replacement for DAPI, propidium iodide and 7-AAD.

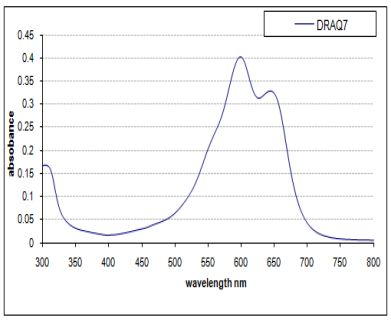

Fig. 1a. Absorbance spectrum for DRAQ7. Exλmax: 599nm, 646 nm

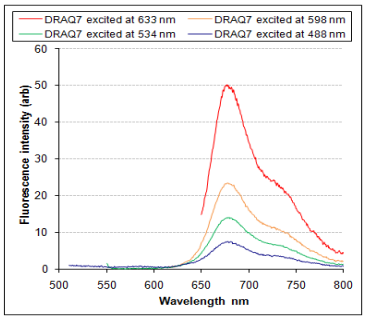

Fig. 1b. Emission profiles for DRAQ7 from common excitation wavelengths

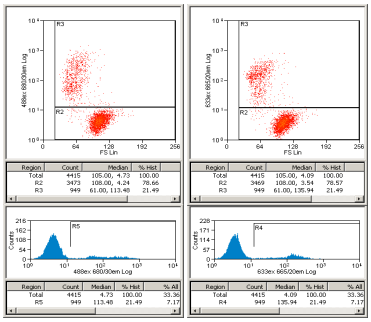

Fig. 2. DRAQ7 detection with blue (L) & red (R) lasers on the Beckman Coulter CyAn™ ADP flow cytometer.

Uniquely, spectral properties of DRAQ7 permit its detection using excitation between blue and red wavelengths (figures 1a,b) and exemplified in figure 2: dead cells clearly separated from intact (viable) cells with DRAQ7 using either blue or red excitation on a CyAn™ flow cytometer.

In principle, this means DRAQ7 events occupy more than one fluorescence channel, which might appear undesirable. However, to the contrary, on a dual-/multi-laser flow cytometer it is possible to create bivariate plots for pairs of fluorescence channels where DRAQ7 is uniquely detected; crucially, DRAQ7 is the only red/far-red dual-excited chromophore detected in this manner. Accordingly, one can detect and exclude DRAQ7+ dead cell "events" in a bivariate plot that generates an additional or "virtual" channel.

To demonstrate this, freshly isolated human PBMCs (in PBS/0.5% BSA/5mM EDTA) were incubated with anti-CD3 (PerCP) antibody for T cells and anti-CD19 (AlexaFluor 647, AF647) antibody for B cells, pre-compensated against each other, and DRAQ7 (3 µM final conc.) for 10 minute at 4 °C, washed and resuspended at 10^7 cells/ml. The sample was analysed on a BD Biosciences Accuri C6 flow cytometer (integrated with the HyperCyt® System, Intellicyt Corp.) configured with three 488 nm laser line detection filters: FL1 530/30, FL2 585/40, FL3 >670 and one 640 nm laser line detection filter: FL4 675/25. In figure 3, the excitation-separated PerCP (FL3) and AF647 (FL4) signals are plotted against other. A unique and distinct population of double-positive events (i.e. the

DRAQ7+ leaky/dead cells) in the region labelled "Dead cells" is shown with the mutually-exclusive T & B cell populations. The morphology of this region identifies a DNA distribution - ranging typically from G2/M down to sub-G1 events with degraded DNA - and is oriented such that it bisects the axes reflecting the dual positivity.

Accordingly, it is easy to exclude dead cell events using a gate drawn in a bivariate plot, for any specific antibody panel. Compensation for the DRAQ7 signal is not required. One should be aware that it is not possible to use a bivariate plot for this dead cell gating that has coincidence of antigens for two red / far-red fluorophore-tagged antibodies on cells (e.g. CD4 & CD3) since these events would co-locate with DRAQ7 double-positive events. However, on most research-grade flow cytometers a number of bivariate plot options may be visualised for the best region for dead cell exclusion e.g. on a cytometer with 4 channels relevant to DRAQ7 there are 6 bivariate plots to display the double-positive DRAQ7 events. Usefully, this also make it possible to add DRAQ7 to a pre-existing antibody panel, enabling dead cell exclusion without disturbing the pre-existing antigen / antibody / chromophore matrix.

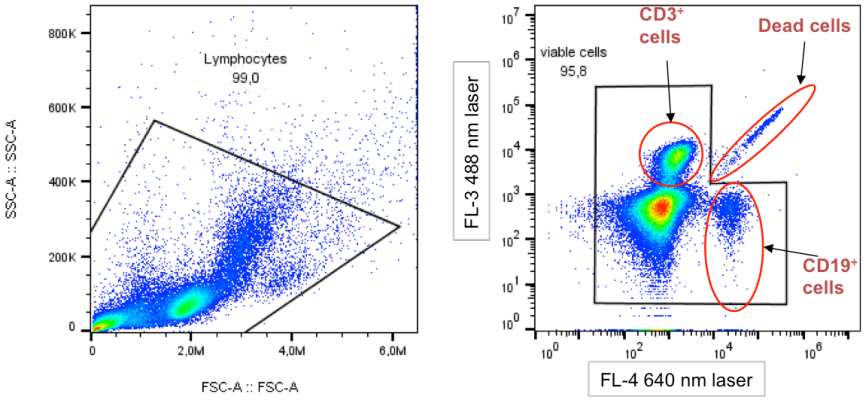

Figure 3. Flow cytometric analysis of PBMCs stained with PerCP-anti-CD3, AF647-anti-CD19 & DRAQ7 Left plot: forward scatter versus side scatter. Right plot: bivariate plot of FL3 (blue-excited, 670nm longpass detection channel) & FL4 (red-excited, 675/25 nm bandpass.

This simple method enables a flow cytometry user to add an additional channel to a flow cytometer for the exclusion of dead cells during panel design or retrospectively, and without need for compensation.

Acknowledgements

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References

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If you would like to know more about DRAQ7™ or any other BioStatus product get in touch ..

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