An evaluation of spectrally distinct succinimidyl ester dyes for tracking cell proliferation by Flow and Image Cytometry

David McDonald[1], Audrey Clarissa[1,2], Jolene Bradford[3], Annette Meeson[2], Andrew Filby[1]

1. Flow Cytometry Core Facility, Faculty of Medical Sciences, Newcastle University, Newcastle-upon-Tyne, UK.
2. Institute of Genetic Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle-upon-Tyne, UK.
3. Flow Cytometry Systems, Thermo Fisher Scientific, Eugene Oregon USA

Introduction

While cellular proliferation is important for positive biological outcomes such as embryogenesis and protective immune responses it also drives cancer and autoimmunity. As such understanding how proliferation is controlled at the cellular and molecular level is essential to numerous fields of biological research. Succinimidyl ester (SE) dyes can be used in conjugation with image and non-image based cytometry to track the proliferative history of any single cell both *in vivo* and i*n vitro*. SE dyes freely enter live cells, bind to cellular proteins via amine reactive chemistry and are rendered fluorescent by cleavage by intracellular esterases. As the cell divides, the fluorescently labelled proteins should be equally shared between daughter progeny giving rise to distinct peaks with approximately half the fluorescent intensity of the parental population. In order to exploit the incredible multi-parameter nature of cytometry, a number of SE-family dyes have been developed with unique spectral properties (see table 1 below).

CellTrace™ Dye	Excitation Laser and Wavelength	Detection filter set
CellTrace CFSE (CSFE)	Blue 488 nm	530/30 nm
CellTrace Violet (CTV)	Violet 405 nm	450/50 nm
CellTrace Blue (CTB)	UV 355 nm	379/28 nm
CellTrace Yellow (CTY)	Yellow 561 nm	584/14 nm

Table 1: A list of the SE dyes used in this study with excitation wavelength and emission filters used

Methods

Using the established experimental framework for testing the efficacy of SE-family dyes for tracking cell proliferation by flow (Begum et al, Cytometry Part a, 2013 & Filby et al, Methods 2015) we evaluate members of the CellTrace Proliferation dyes from Thermo Fisher Scientific.

Human PBMC or Jurkat T cells were stained with titrated amounts of Cell Trace dyes in serum free conditions for 20 minutes at a density of 2-4 million per mL before quenching with FCS (10% v/v) for 10 minutes (all done at 37ºc). For Jurkats, the post-stained Jurkat distribution was reduced by cell sorting using an AriaIIu cell sorter to 5 channel widths (binned to a 256 scale). PBMC were activated with 2.5 ug/mL PHA in the presence of IL-2. Samples were then analysed for division at 24 hr time points (up top 96 hrs) using a BD FACSsymphony A5 or a LSRFortessa using the detection parameters shown in table 1 for each dye. Depending on the dye, either DAPI or PI was used to exclude dead cells. Data analysed using FCS Express 6.

THE Flow Cytometry Core Facility

Results

Determining optimal labelling conditions:

In all cases (dyes and cell types) a titration was performed and viability, intensity and uniformity of labelling was determined as described in Begum et al and Filby et al. The concentration that gave the best viability, the highest MedFI and lowest CV was chosen (see figure 1).

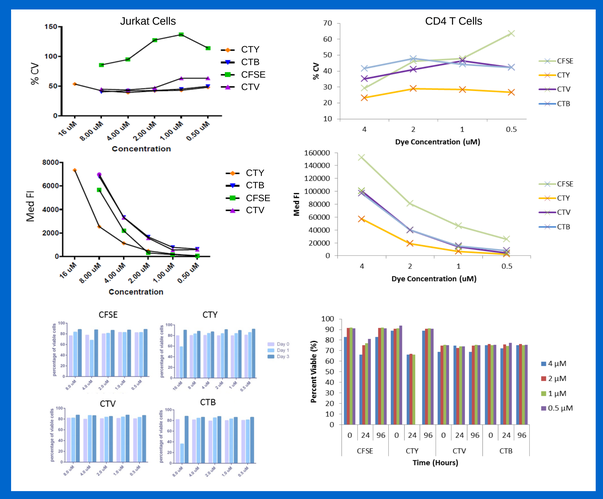

Figure 1: Data showing the %CV (top graphs), MedFI (middle garphs) and viability by DNA dye exclusion (bottom graphs) for Jurkats (left column) and CD4 T Cells (right column). Optimal concentrations of 8 uM were selected for CTB and CTY, 4 uM for CTV and CTFR.

Next we took the optimised dye concentrations and labelled Jurkat cells for cell sorting to narrow the input width using a 5 channel sort gate (256 channel binning). The CTY post sort distribution was wider than the others due to slightly more intrinsic measurement error in that channel as determined by 6 peak beads (data not shown). We also labelled PBMCs with the optimised dye concentrations and determined the input width at 24 hrs before any division had occurred but allowing enough time for the dyes to stabilise in the cells.

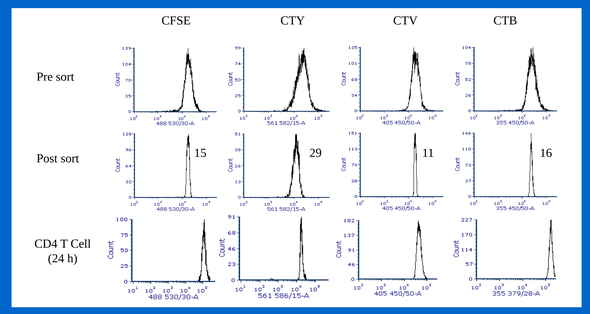

Figure 2: Data showing the profile of Jurkat cells post labelling with the optimised dye concentrations and then after narrowing the distribution by cell sorting (post sort channel widths shown on plots). The pre-division labelling distributions are also shown for PBMCs 24 hours after labelling before cell division.

Performance in culture:

Sorted Jurkat cells were analysed every 24 hrs for 96 hrs to follow the evolution of the population using the % divided, proliferation index and mean division metrics. Interestingly we noted that CFSE labelled cells lagged behind in the proliferation programme compared to other dyes with CTY labelled cells proliferating the fastest. We also noted that peak quality was again poor with CFSE-labelled cells and did not match input. PHA-stimulated primary Human CD4 T cells followed over the same time course we're in keeping with above. CTY labelled cells proliferated the fastest and committed to division equally with CTV. Slow proliferation was observed with CFSE and CTB with the former again giving poor peak resolution in subsequent generations.

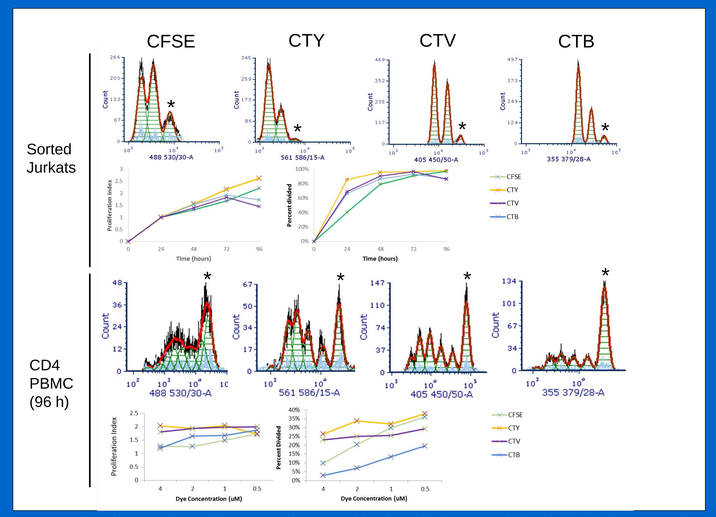

Figure 3: Data showing the proliferative performance of Jurkat cells (upper panels) and PBMCs (lower panel). Undivided peak highlight (*).

Discussion

The CellTrace SE dyes generally provide an excellent tool for tracking cell division by Cytometry technologies. Our study however highlights that not all SE dyes are equal in terms of the proliferative performance of the cells. Specifically we found that:

1.Cells labelled with CellTrace Yellow (Jurkat or PBMCs) proliferate better than the same cells labelled with other SE dyes.

2.CellTrace CFSE is particularly poor as the division peak "quality" does not match the initial input width, particularly evident with sorted Jurkats cells. We have seen this previously (see Begum et al).

3.Overall we found that the two new spectrally distinct SE dyes, CellTrace Blue and CellTrace Yellow are very useful additions to the cytometry proliferation toolkit.

References:

1. Begum et al, Cytometry part a, 2013. 2. Filby et al, Methods, 2015