Improving Flow Cytometry Data with StarBright Dyes; Fluorophores with High Stability and Assay Compatibility

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Fluorophore instability is a common issue that can affect data quality. Tandem dyes are well known to have poor photostability, causing them to break down into their constituent donor and acceptor molecules, altering their spectral profile. Fixation can also alter profiles or even degrade fluorophores completely to leave no signal. An example of this can be seen in PE and PE-tandems when using alcohol-based fixatives.

Multiplexing with these unstable fluorophores can impact data quality in both conventional and spectral cytometry. The performance of a well-designed panel giving excellent data and cell resolution may decline if any of the fluorophore's spectral profiles are subsequently altered. The panel may no longer be compensated or unmixed correctly if new controls using the degraded fluorophores were not acquired and the original algorithm was applied. Increased spreading can also occur, which results in poorer resolution of cell populations.

Not all fluorophores are compatible with every assay, for example, intranuclear and phosphoflow protocols contain an alcohol fixation step. Therefore, any fluorophores used in these assays must be able to withstand this method of fixation, reducing the fluorophore choice available.

Data presented here show StarBright™ Dyes (Figure 1) are highly stable, maintaining consistent spectral profiles and brightness. They show lot-to-lot and within lot stability (Figure 2), with minimal variation in all standard staining buffers tested (Figure 3), and after fixation in both PFA and alcohol-based fixatives (Figure 4). They have the same or improved photostability than alternative fluorophores (Table 1 and Figure 5). This improved spectral stability means consistent data is generated, with no increased spillover and spread introduced from dye breakdown.

StarBright UltraViolet, Violet, Blue, Yellow, and Red Dye Range

StarBright Dyes are a range of 32 superior dyes excited by the most common laser lines; 355 nm, 405 nm, 488 nm, 561 nm, or 640 nm.

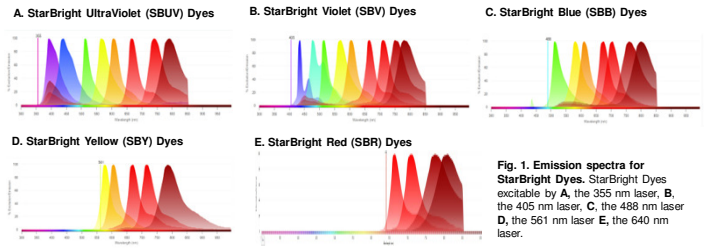

Fig. 1. Emission spectra for StarBright Dyes. StarBright Dyes excitable by **A,** the 355 nm laser, **B**, the 405 nm laser, **C**, the 488 nm laser **D,** the 561 nm laser **E,** the 640 nm laser.

Lot Stability

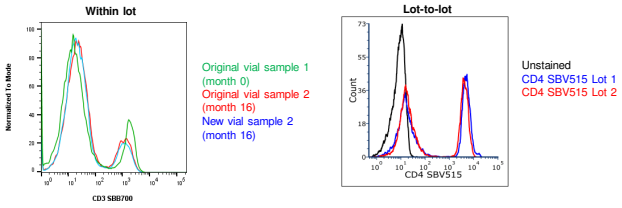

Fig. 2. Consistent staining using StarBright Dye conjugated antibodies within and between lots. Red blood cell lysed human peripheral blood was stained with Mouse Anti-Human CD3 (MCA463SBB700) or Mouse Anti-Human CD4 (MCA1267SBV515) conjugated to StarBright Dyes and acquired on a 5 laser ZE5 Cell Analyzer (Bio-Rad).

Suitable for all Buffers

StarBright Dyes are compatible with common staining buffers, as shown by a consistent brightness and stable spectral profile. An example of a StarBright Dye excited by each laser line is shown.

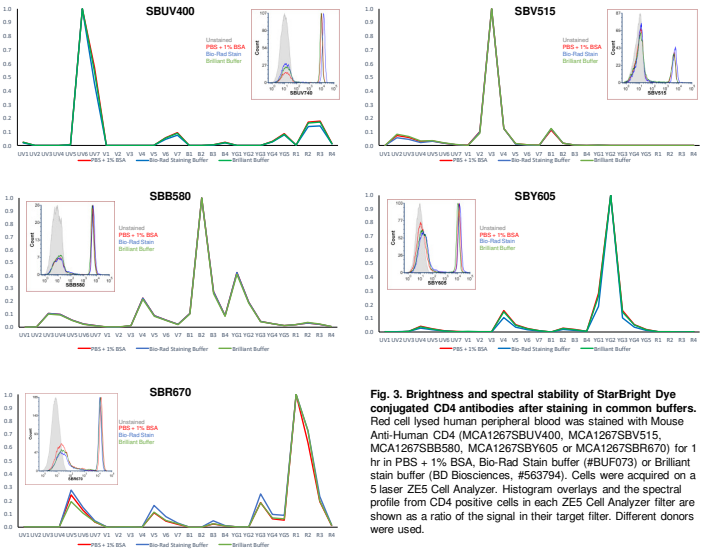

Fig. 3. Brightness and spectral stability of StarBright Dye conjugated CD4 antibodies after staining in common buffers. Red cell lysed human peripheral blood was stained with Mouse Anti-Human CD4 (MCA1267SBUV400, MCA1267SBV515, MCA1267SBB580, MCA1267SBY605 or MCA1267SBR670) for 1 hr in PBS + 1% BSA, Bio-Rad Stain buffer (#BUF073) or Brilliant stain buffer (BD Biosciences, #563794). Cells were acquired on a 5 laser ZE5 Cell Analyzer. Histogram overlays and the spectral profile from CD4 positive cells in each ZE5 Cell Analyzer filter are shown as a ratio of the signal in their target filter. Different donors were used.

Fixable in PFA or Alcohol

StarBright Dyes remain stable after fixation. The brightness after staining with a StarBright Dye conjugated CD4 antibody is shown before and after fixation in common fixatives. An example of a StarBright Dye excited by each laser line is shown.

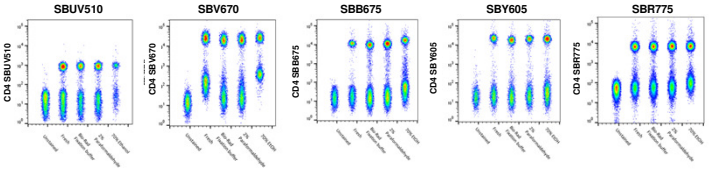

Fig. 4. Stability of StarBright Dye conjugated antibodies after fixation. Red cell lysed human peripheral blood was stained with Mouse Anti-Human CD4 (MCA1267SBUV510, MCA1267SBV670, MCA1267SBB675, MCA1267SBY605 or MCA1267SBR775) and acquired on a 5 laser ZE5 Cell Analyzer before and after fixation in Bio-Rad Fixation buffer (BUF071), 2% PFA or 70% EtOH.

Photostability – Stain Index

StarBright Dyes and other fluorophores conjugated to mouse anti-human CD4 were left out in the light for up to 14 days. Many fluorophores lost brightness by day 14, as would be expected. StarBright Dyes show the same or an improvement in stain index stability compared to comparison fluorophores.

A. 355 nm excitable fluorophores

Fluorophore	SBUV400	BUV395	SBUV445	SBUV510	BUV496	SBUV575	BUV563	SBUV605	BUV615	SBUV665	BUV661	SBUV740	BUV737	SBUV795	BUV805
ZE5 Filter	387/11	387/11	477/60	508/25	508/25	577/15	577/15	615/24	615/24	615/24	670/30	747/33	747/33	780LP	780LP
1 day	100%	100%	100%	100%	100%	100%	100%	100%	72%	89%	97%	98%	100%	95%	90%
4 days	100%	100%	95%	100%	100%	95%	100%	100%	54%	100%	100%	100%	100%	95%	0%
14 days	84%	93%	84%	85%	95%	72%	100%	100%	47%	75%	100%	100%	100%	70%	0%

B. 405 nm excitable fluorophores

Fluorophore	BV421	SBV440	PB	BV480	SBV475	SBV515	BV510	SBV570	BV570	SBV610	BV605	SBV670	BV650	SBV710	BV711	SBV760	BV750	SBV790	BV785
ZE5 Filter	420/10	460/22	460/22	460/22	525/50	525/50	525/50	615/24	615/24	615/24	615/24	670/30	670/30	720/60	720/60	750LP	750LP	750LP	750LP	750LP
1 day	87%	100%	100%	83%	100%	92%	99%	58%	100%	93%	100%	96%	100%	57%	100%	55%	100%	77%	68%
4 days	100%	69%	94%	87%	52%	7%	61%	19%	5%	36%	7%	26%	0%	0%	1%	1%	2%	5%	0%
14 days	100%	18%	89%	83%	4%	1%	11%	3%	0%	13%	0%	6%	0%	0%	0%	0%	0%	0%	0%

C. 488 nm excitable fluorophores

Fluorophore	SBB580	SBB615	SBB675	SBB700	BB700	PERCP Cy5.5	SBB765	RB780	SBB810
ZE5 Filter	593/52	593/52	692/80	692/80	692/80	692/80	750LP	750LP	750LP
1 day	90%	100%	47%	76%	84%	94%	86%	86%	93%
4 days	16%	30%	2%	8%	72%	85%	20%	67%	84%
14 days	1%	5%	0%	3%	56%	44%	2%	8%	18%

Key

Relative stain index compared to control
100-75
75-50
50-25
25-5
5-0

D. 561 nm excitable fluorophores

Fluorophore	SBY575	PE	RY586	SBY605	SBY665	PE-Cy5	PE-A647	SBY720	PE-A700	PE-Cy5.5	SBY800	PE-Cy7
ZE5 Filter	577/15	577/15	583/30	615/24	670/30	670/30	670/30	726/60	720/60	726/60	750LP	750LP
1 day	97%	100%	98%	100%	47%	99%	95%	80%	21%	NT	69%	NT
4 days	100%	100%	97%	88%	2%	84%	74%	9%	11%	12%	31%	89%
14 days	100%	100%	95%	86%	1%	28%	25%	0%	5%	6%	1%	11%

E. 640 nm excitable fluorophores

Fluorophore	SBR670	A647	A700	SBR715	SBR775	APC-Cy7	SBR815
ZE5 Filter	670/30	670/30	670/30	720/60	775/50	775/50	800LP
1 day	100%	99%	100%	100%	100%	99%	99%
4 days	100%	99%	92%	93%	100%	86%	82%
14 days	100%	85%	39%	73%	100%	40%	67%

Table 1 A-E. Photostability of the stain index of StarBright Dyes and other fluorophores conjugated to mouse anti-human CD4. Red blood cell lysed human peripheral blood was stained with antibodies left out 1, 4 or 14 days in light at room temperature and a control antibody stored at 4°C in the dark. The stain index was calculated for each antibody after staining and expressed as a percentage of the control antibody. NT = not tested.

Photostability – Spectral Profile

StarBright Dyes maintain a stable spectral profile when exposed to light, even with a reduction in stain index. Spectral profiles at 4 and 14 days for StarBright Dyes and alternative dyes with similar max excitation and emission max are shown compared to control. The profile for antibodies that lost all positive signal could not be determined. An example from each laser line is shown.

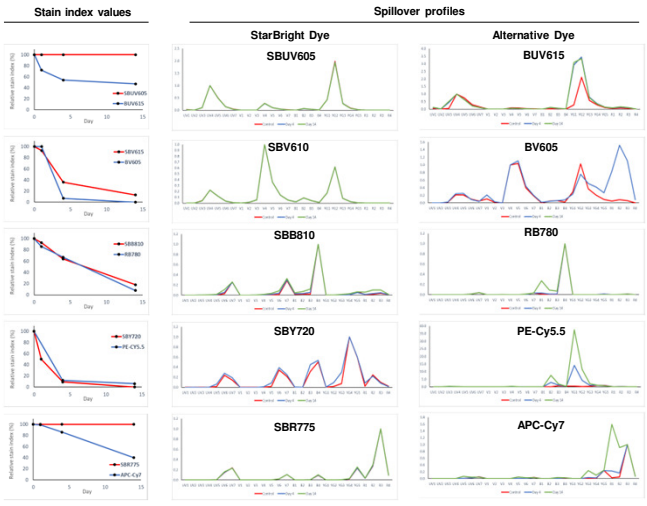

Fig. 5. Spectral photostability of StarBright Dyes and other fluorophores conjugated to mouse anti-human CD4. Red blood cell lysed human peripheral blood was stained with antibodies left out 1, 4 or 14 days in light at room temperature or a control antibody stored at 4°C in the dark. Cells were acquired on a 5 laser ZE5 Cell Analyzer. The stain index was calculated and expressed as a percentage of the control antibody. A spectral profile was generated by plotting the signal in each filter as a ratio of the signal in their target filter.

Conclusions

- StarBright Dyes are compatible with common buffers and fixatives giving stable reproducible results
- They have improved photostability over many common fluorophores and maintain a stable spectral profile even when the signal is reduced by photobleaching. This allows consistent data to be generated, with minimal experimental changes observed/introduced from dye breakdown
- StarBright Dyes are suitable for all protocols and ideal for multiplexing assays in both conventional and spectral flow cytometry

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