#178

Rare Cell Population Detection With StarBright Dye Antibody Conjugates

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Introduction

Rare cell populations, like hematopoietic stem cells (HSCs) and circulating endothelial cells in peripheral blood, are cells with extremely low frequency (usually less than 0.01% of the total population). Although rare, they are important cell subsets for self-repair and potential biomarkers of disease diagnosis. It is challenging to detect and characterize these cells by flow cytometry, due to their rareness and complex phenotype, as well as the limitation of current fluorescent dyes on the market. Many conventional dyes are dim, and tandem dyes have lot-to-lot consistency issues and may easily degrade under certain conditions, which are challenges for building consistent multiplex panels, especially for rare cell population detection on flow cytometry.

Here, we demonstrate how new StarBright™ Dye antibody conjugates, with unique features, facilitate the detection and characterization of rare populations. StarBright Dyes are novel fluorescent nanoparticles with superior brightness over conventional dyes. StarBright Dyes also have **narrow excitation and emission spectra**, leading to reduced spillover. The **StarBright Dye series (UltraViolet, Violet, Blue, Yellow, and Red)** are suitable for all instrumentation with the appropriate lasers and filters in conventional flow cytometry and have been shown to work in full spectrum flow cytometry. Based on the stain index data of StarBright Dyes, their **broad range of brightness** provides greater **flexibility** for building multiplex panels. Also, the **overall greater brightness** of StarBright Dyes allows more options for detecting low-abundance antigens in multicolor panels. **High stability** is another advantage of StarBright Dyes, as methanol treatment (an important cell permeabilization step for downstream intracellular staining), can seriously reduce tandem dye performance, but did not affect the surface staining with StarBright Dyes. Moreover, we were able to detect rare cell subsets — HSCs in human peripheral blood mononuclear cells (PBMCs), with an eight-color multiplex panel combining StarBright Dye antibody conjugates and conventional fluorophore-conjugated antibodies.

In summary, StarBright Dyes are superior choices for detecting and characterizing rare cell populations, due to their brightness, stability, and consistency.

Materials and Methods

Excitation and Emission Spectra: The StarBright Yellow (SBY) spectra were generated using Bio-Rad's Spectraviewer available at: bio-rad-antibodies.com/spectraviewer

Staining Conditions for Stain Index Data: Human peripheral blood mononuclear cells (PBMCs) were stained in a 96-well plate for 1 hr at 4°C in the dark with a mouse anti-human CD4 StarBright Dye antibody conjugate, washed three times, and resuspended in FACS Buffer. All antibodies were titrated to determine the optimal staining concentration prior to use.

Data Collection and Analysis: Data for these studies were collected on a 5-laser 30-parameter Bio-Rad ZE5 Cell Analyzer and analysis was performed using FlowJo 10.8 (Becton, Dickinson & Company) and FCS Express (De Novo).

Stain Index (SI) Calculation: Single cell lymphocytes were gated to identify positive and negative populations on the target channel. The Median Fluorescence Intensity (MFI) of both populations was quantified and the negative population's robust Standard Deviation (rSD) calculated. Stain Index (SI) was calculated using the following formula:

$$SI = \frac{MFI_{pos} - MFI_{neg}}{2XrSD_{neg}}$$

Methanol (MeOH) Treatment on Staining Stability: Human peripheral blood mononuclear cells (PBMCs) were stained with anti-CD4 StarBright Yellow SBY605 and anti-CD4 PE-Dazzle 594 (as control) for 1hr at 4°C, followed with fixation (2% PFA RT for 15 mins) and methanol treatment (ice-cold pure MeOH at 4°C for 30 mins). Cells were washed two times and resuspended in the FACS buffer prior to data acquisition.

Multiplex panel: PBMCs were stained with antibodies dye conjugates, as shown in Table 1. All antibodies were titrated to determine the optimal staining concentration prior to use. Single staining and fluorescence minus one (FMO) controls were set up for compensation and gating. Hematopoietic stem cells (HSCs) were identified as Lin-(CD3-/CD19-/CD33-)/CD38-/CD45-/CD34+/CD90+.

Table 1. Bio-Rad reagents used in the multiplex panel.

Target	Fluorophore	Bio-Rad Catalog#
CD38	SBV760	MCA1019SBV760
CD45	SBV670	MCA87SBV670
CD3	SBV515	MCA463SBV515
CD19	SBUV665	MCA1940SBUV665
CD33	SBV610	MCA1271SBV610
CD34	SBB765	N/A
CD90	SBY575	N/A
Viability	DAPI	1351303

4',6-diamidino-2-phenylindole, DAPI; SBB, StarBright Blue; SBUV, StarBright UltraViolet; SBV, StarBright Violet; SBY, StarBright Yellow.

StarBright Dyes Have Narrow Excitation/Emission Spectra and Minimal Spillover

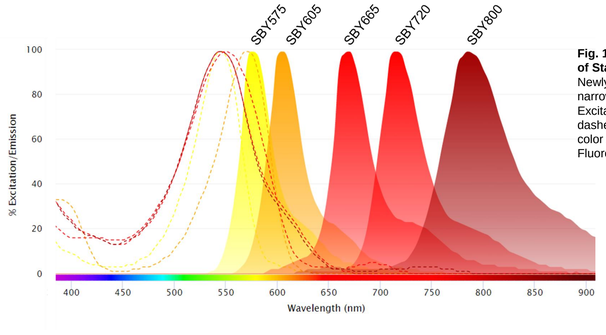

Fig. 1. Excitation and Emission Spectra of StarBright Yellow (SBY) Dyes.
Newly launched StarBright Yellow Dyes have narrow excitation and emission spectra. Excitation spectra are represented as dashed lines and emission spectra as solid color on the graph. Data are from Bio-Rad's Fluorescent Spectraviewer.

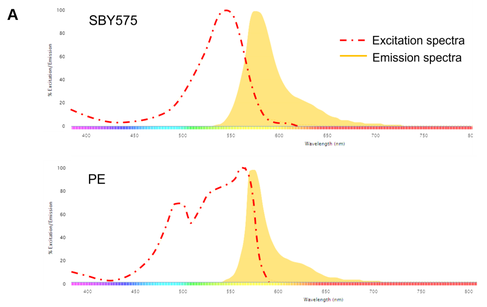

Fig. 2. Spectra and spillover value (SOV) comparison between SBY575 and phycoerythrin (PE).
A, SBY575 has narrower excitation spectra and comparable emission spectra (data from Bio-Rad Spectraviewer), compared to the conventional dye PE. Also, unlike PE, SBY575 is not significantly excited by **488 nm** laser.

B, The narrow excitation spectra of SBY575 contribute to less spillover into other channels when compared to PE, especially on the 488/593_52nm channel. Spillover data was collected by Bio-Rad ZE5 cell analyzer and analyzed by FlowJo (Becton, Dickinson & Company).

High Stability of StarBright Dye Antibody Conjugates

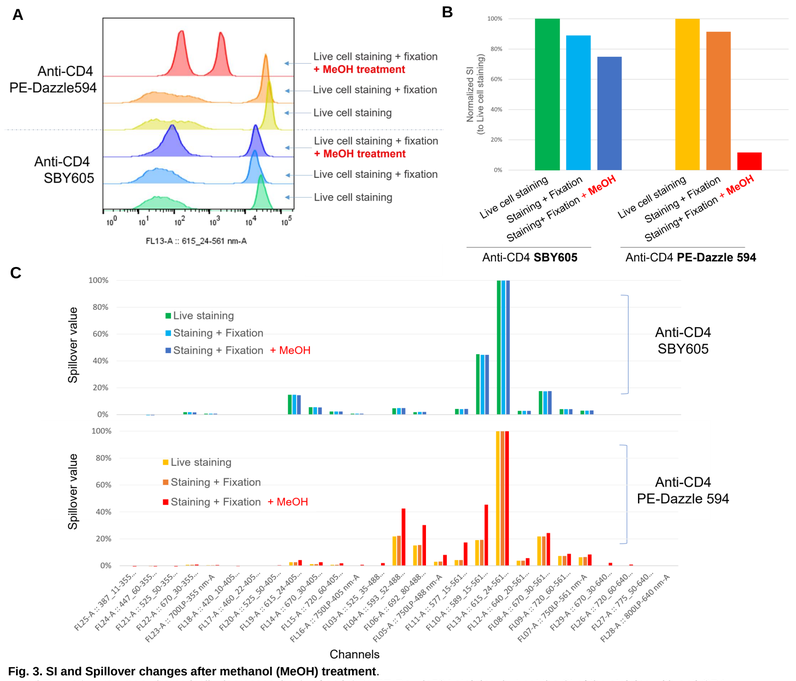

Fig. 3. SI and Spillover changes after methanol (MeOH) treatment.
A and **B,** MeOH treatment dramatically decreases the SI of anti-CD4 PE-Dazzle594 staining, but not the SI of the staining with anti-CD4 SBY605. **C**, MeOH treatment changes spillover in Anti-CD4 PE-Dazzle 594 staining, not in Anti-CD4 SBY605 staining, which indicates the high stability of StarBright Dyes, but not PE-Dazzle594, with MeOH treatment.

StarBright Dye Brightness (Stain Index) Ranking

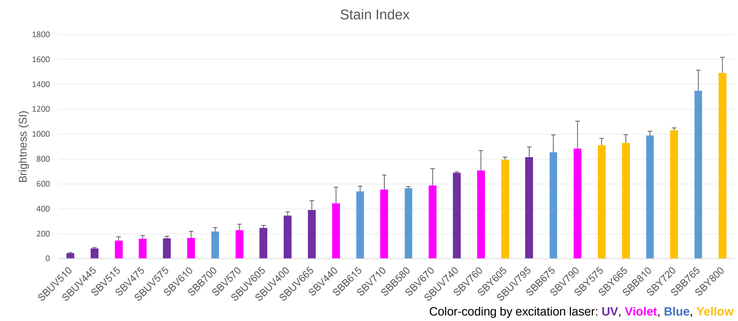

Fig. 4. Stain Index values for StarBright Dyes.
Stain Index (SI) values for most StarBright Dyes, covering StarBright UltraViolet, Violet, Blue, and Yellow series. The broad range of brightness provides greater flexibility for building multiplex panels. SI is calculated based on mouse anti-human CD4 StarBright Dye staining of human peripheral blood cells, gated on live, single cell lymphocytes. The stain index represents the relative brightness of the fluorochromes. Data are acquired on a ZE5 Cell Analyzer.

Multiplex Panel for Hematopoietic Stem Cell (HSC) Identification

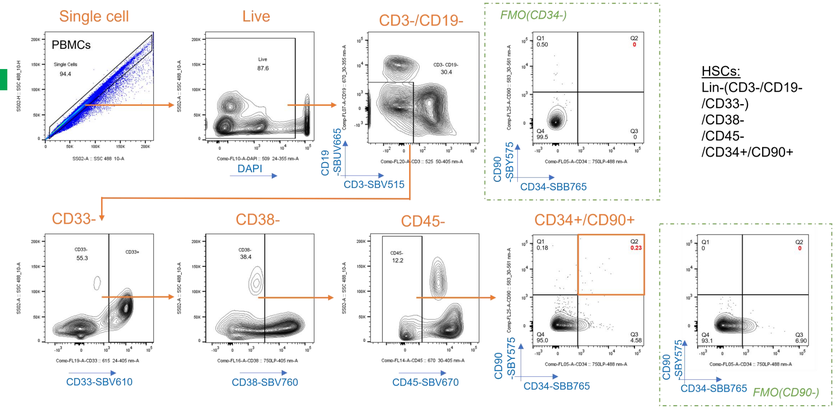

Fig. 5. 8-color multiplex panel.
Human peripheral blood mononuclear cells (PBMCs) were stained with a 7-color antibody panel, and a live/dead dye (DAPI) allowing for identification of the HSC population. HSCs were identified as Lin-(CD3-/CD19-/CD33-)/CD38-/CD45-/CD34+/CD90+. Gating was based on single staining and fluorescence minus one (FMO) controls. The percentage of HSCs in the total cell population is approximately 0.002% for this donor.

Conclusions

Features of StarBright Dyes:

- Narrow excitation and emission spectra
- Bright
- Highly stable
- Flexible to build multiplex panels for rare cell identification

StarBright Dye Antibody conjugates are superior choices for detecting and characterizing rare cell populations.