StarBright Dyes and the ZE5 Cell Analyzer: Generating Fast, Reproducible, High Resolution Flow Cytometry Data

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Flow Cytometry is an important and versatile tool for drug discovery. It can be used to build our fundamental understanding of disease processes, identify target molecules and lead candidates, as well as to provide crucial data as part of pre-clinical and clinical trials. Data quality, sensitivity, reproducibility, and speed are important considerations in any experimental program, but are especially important in drug discovery applications, where erroneous or delayed data can have significant consequences.

StarBright™ Dyes from Bio-Rad are unique fluorophores specifically developed for flow cytometry applications. They offer superior brightness and narrow excitation and emission spectra allowing for more comprehensive multiplexing whilst minimizing loss of data resolution. Their unique properties allow antibody panels to be prepared months prior to use, such that antibody panels may be prepared once and used multiple times, removing an important source of experimental error.

The ZE5 Cell Analyzer from Bio-Rad is a flexible, high-speed, high-parameter flow cytometer with up to five lasers and 30 detectors. Its fast sample acquisition and rapid plate handling enable complex immunophenotyping data to be acquired at the speed of a dedicated screening instrument. In this study we aimed to develop a 27-color immunophenotyping panel using StarBright Dyes and analyzed at high-speed using the ZE5 Cell Analyzer. We also examine the effect of extended pre-mixing of antibody panels on data reproducibility.

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

After the launch of the StarBright Red Dye series later this year, we will have a range of 32 superior dyes excited by the most common laser lines; 355 nm, 405 nm, 488 nm, 561 nm, or 640 nm. These are available conjugated to antibodies against common immunophenotyping targets.

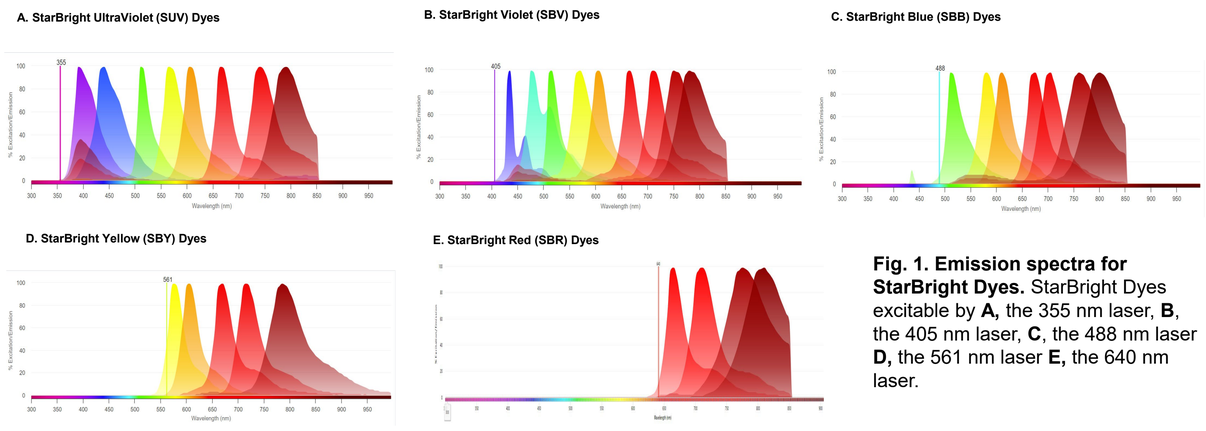

Fig. 1. Emission spectra for StarBright Dyes. StarBright Dyes excitable by **A,** the 355 nm laser, **B**, the 405 nm laser, **C**, the 488 nm laser **D,** the 561 nm laser **E,** the 640 nm laser.

Materials and Methods

Staining conditions. Red blood cell lysed human peripheral blood was blocked with 10% human serum. Cells were incubated with an antibody panel or a single antibody for compensation control tubes. Cells were stained in a 96-well plate for 1 hr at room temperature, washed 3X in PBS + 1% BSA and resuspended in PBS + 1% BSA. 4′,6-diamidino-2-phenylindole (DAPI) (#1351303, Bio-Rad) or propidium iodide (PI) (#1351101, Bio-Rad) was added 5 min prior to acquisition. All antibodies were titrated to determine the optimal staining concentration prior to use.

Premixing panel. Antibodies used in the panel (Table 1) were combined in a mastermix six months previously and stored at 4°C in the dark. The premixed panel was compared to a freshly prepared panel.

27-color multiplex panel. Antibodies used in the large panel are shown in Table 2.

Data collection and analysis. Cells were acquired on a 5-L, 30-parameter UV option A, ZE5 Cell Analyzer (Bio-Rad) in normal or high throughput mode. Analysis was performed using FCS Express 7 Software (De Novo Software).

StarBright Dye Premixes Are Stable for Six Months

For experiments that require repeated measurements over several days, preparing and storing a mastermix for an extended period has clear advantages in terms of reproducibility. In this example we show that data from a panel containing nine StarBright Dye conjugated antibodies, that were stored for six months, were comparable to a freshly prepared panel (Figure 2). Premixing allows you to save time, money, and reduce errors when using the same panel over an extended period.

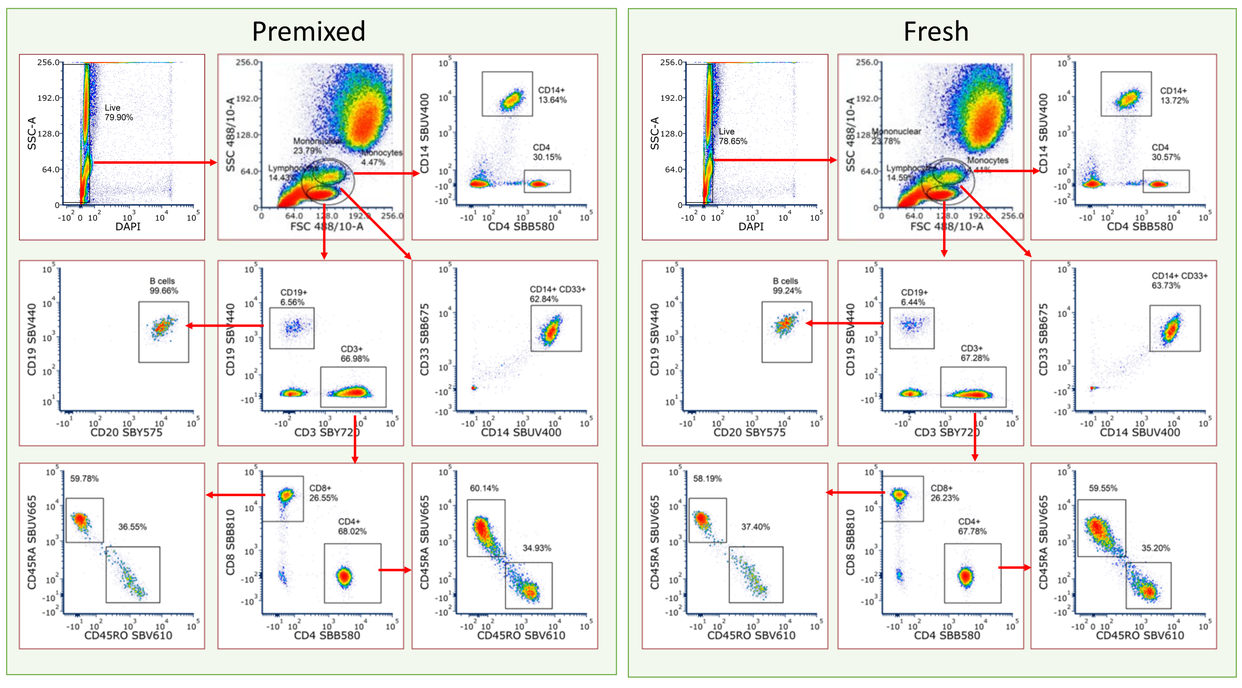

Table 1. Bio-Rad antibodies and live/dead dye used in then premixed panel.

Fluorescent Dye	Target	Catalog Number
SBUV400	CD14	MCA1568SBUV440
SBUV665	CD45RA	MCA88SBUV665
SBV440	CD19	MCA1940SBV440
SBV610	CD45RO	MCA461SBV610
SBB580	CD4	MCA1267SBB580
SBB675	CD33	MCA1271SBB675
SBB810	CD8	MCA1226SBB810
SBY575	CD20	MCA1710SBY575
SBY720	CD3	MCA463SBY720
DAPI	Live/dead	1351303

Fig. 2. Flow cytometry data using a premixed antibody panel stored at 4°C for six months, compared to a freshly made antibody panel. Red blood cell lysed human peripheral blood was stained with the antibody panels and acquired on a ZE5 Cell Analyzer. Data were analyzed using FCS Express 7.

27-color Immunophenotyping Panel

An immunophenotyping panel containing 22 StarBright Dyes, and other common fluorophores, was successfully acquired without the use of a special buffer. Major T cell, B cell, monocyte, and granulocyte lineages were identifiable (Figure 3). Various subsets within these lineages can also be clearly distinguished. The data were acquired on a ZE5 Cell Analyzer in both standard mode and high throughput mode. There was minimal variation in cell populations between the two acquisition speeds (Figure 4).

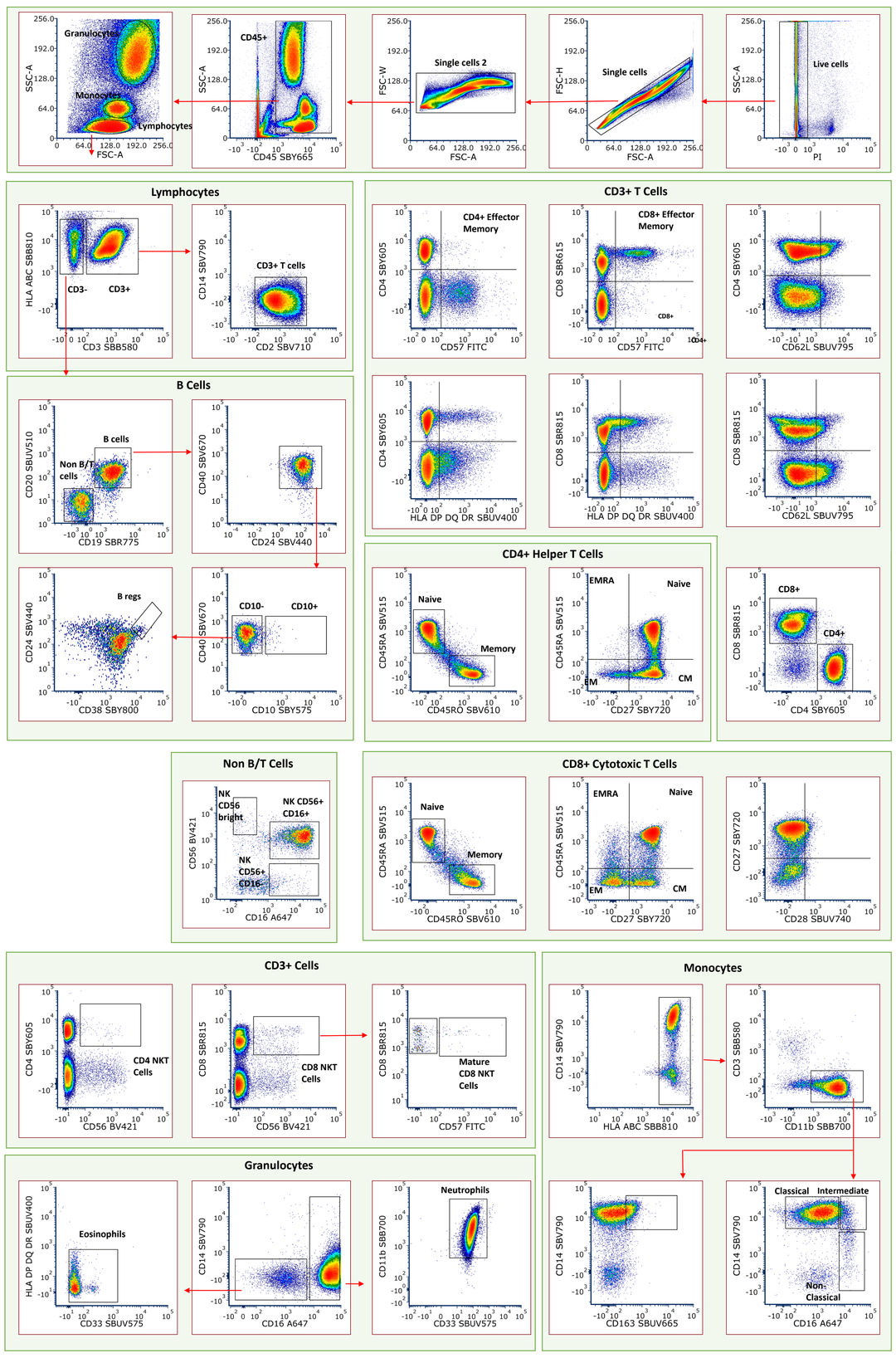

Fig. 3. 27-color multiplex panel. Red blood cell lysed human peripheral blood was stained with a live/dead dye and a 26-color antibody panel (Table 2) in PBS + 1% BSA allowing identification of multiple cell lineages and subsets.

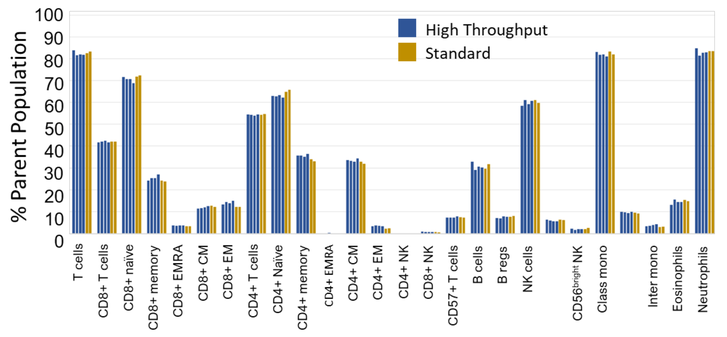

Fig. 4. Comparison of data acquired in standard and high throughput mode on the ZE5 Cell Analyzer. Cell lineages are expressed as a percentage of their parent cell population. Each bar represents an individual replicate collected in high throughput mode (blue bars) and standard mode (yellow bars). B regs, regulatory B cells; CM, central memory; EM, effector memory; EMRA, terminally differentiated effector memory cell re-expressing CD45RA; NK, natural killer cells; NKT, natural killer T cells.

Table 2. Bio-Rad antibodies and live/dead dye used in the 27-color panel.

Fluorescent Dye	Target	Catalog Number
SBUV400	HLA DP DQ DR	MCA477SBUV400
SBUV510	CD20	MCA1710SBUV510
SBUV575	CD33	MCA1271SBUV575
SBUV665	CD163	Coming soon
SBUV740	CD28	MCA709SBUV740
SBUV795	CD62L	MCA1076SBUV795
BV421	CD56	N/A
SBV440	CD24	Coming soon
SBV515	CD45RA	MCA88SBV515
SBV610	CD45RO	MCA461SBV610
SBV670	CD40	Coming soon
SBV710	CD2	MCA1194SBV710
SBV790	CD14	MCA1568SBV790
FITC	CD57	MCA1305F
SBB580	CD3	MCA463SBB580
SBB700	CD11b	MCA551SBB700
SBB810	HLA ABC	MCA81SBB810
SBY575	CD10	MCA1556SBY575
SBY605	CD4	MCA1267SBY605
SBY665	CD45	MCA87SBY665
SBY720	CD27	MCA755SBY720
SBY800	CD38	MCA1019SBY800
A647	CD16	MCA5665A647
A700	CD31	MCA1738A700
SBR775	CD19	Coming soon
SBR815	CD8	Coming soon
PI	Live/dead	1351101

Conclusions

- The excellent spectral properties of StarBright Dyes allow for complex immunophenotyping while minimizing spectral overlap and preserving data quality
- StarBright Dyes can be premixed up to six months prior to use without affecting data reproducibility, and reducing experimental error associated with multiple mastermix preparations
- The ZE5 Cell Analyzer is capable of collecting complex 27-color immunophenotyping data in as little as 8 seconds/well with no loss of data reproducibility
- StarBright Dyes and the ZE5 Cell Analyzer are an ideal combination enabling superior data quality, sensitivity, speed, and reproducibility in drug discovery

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