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New fluorochromes expand the possibilities for spectral flow, leveraging detection beyond 800nm

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Poster# P185

Abstract

The adoption of spectral flow cytometry and advancements in near-infrared (NIR) detection have increased the demand for additional fluorochromes. Leveraging the BD FACSDiscover™ A8 and S8 platforms' ability to detect signals up to 861 nm, BD recently introduced BD Horizon RealBlue™ 824 and RealViolet™ 828—two fluorochromes with emission maxima above 820 nm—and continues to expand possibilities on this wavelength range with BD Horizon RealYellow™ 825-P, BD Horizon RealRed™ 820-P, and BD Horizon RealUV 840-P (prototype names; final names pending), presented here for the first time.

Brightness and stability are critical for large-Stokes-shift fluorochromes emitting at long wavelengths. We show that the BD dyes >800 nm are bright and stable, demonstrating robust photo- and thermal stability. Each dye can be paired with its nearest neighbor (e.g., dyes in the 780 nm region), creating a new spectral position that can be added to existing multicolor panels to support higher plexity or used as an alternative to 780 nm dyes in smaller panels for improved peak separation. This approach reduces panel complexity scores and minimizes resolution loss caused by spillover spread from adjacent emissions.

Introduction

Dye development in the NIR space has historically been challenging due to a variety of factors, including:

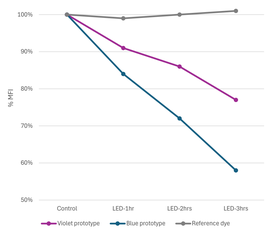
- Limitations of PMTs
- Instrument configurations
- Stability of NIR emitting dyes
- Broad emission curves for NIR emitting dyes

BD Horizon RealBlue™ 824 and RealViolet™ 828, as well prototypes BD Horizon RealYellow™ 825-P, BD Horizon RealRed™ 820-P, and BD Horizon ™ RealUV 840-P, in conjunction with new instrument technology, overcome these challenges.

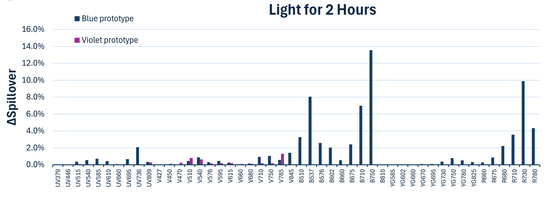
Dye development methods

During our development process, dye prototypes are screened for sufficient brightness, clean spillover, as well as hardiness. Hardiness tests include photostability, thermal stability, and buffer compatibility. Dye prototypes that do not meet our standards are rejected. For dyes with emission maxima above 800 nm, due to the specific challenges of these positions, particular focus is placed on robust photo- (both pre- and post-staining) and thermal stability.

A



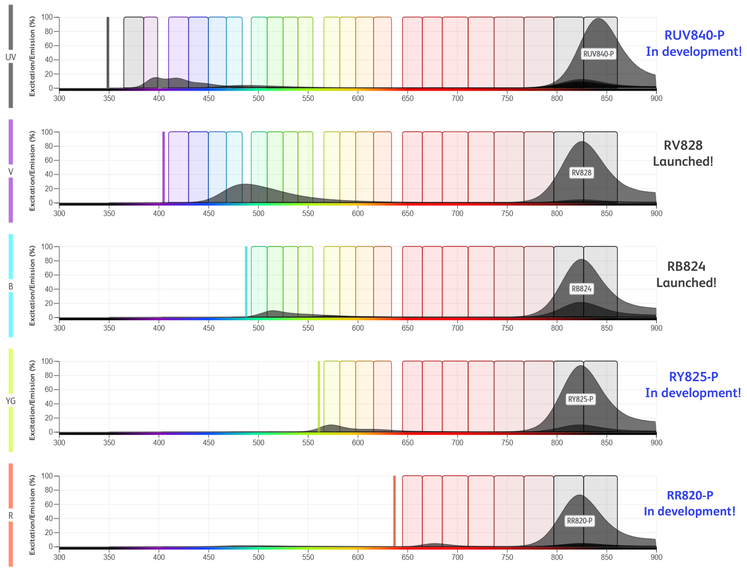
B



Reagents are exposed to 600 lux LED light, average lab lighting, for 0, 1, 2, or 3 hours and cells are stained and analyzed on an A5SE. A) shows MFI change over the three-hour exposure and B) shows spillover change at the 2-hour time point. Dye candidates are rejected if they do not meet our standards for MFI or spillover changes. The violet prototype shown was rejected based on MFI change and the blue prototype shown was rejected based on MFI change and spillover change.

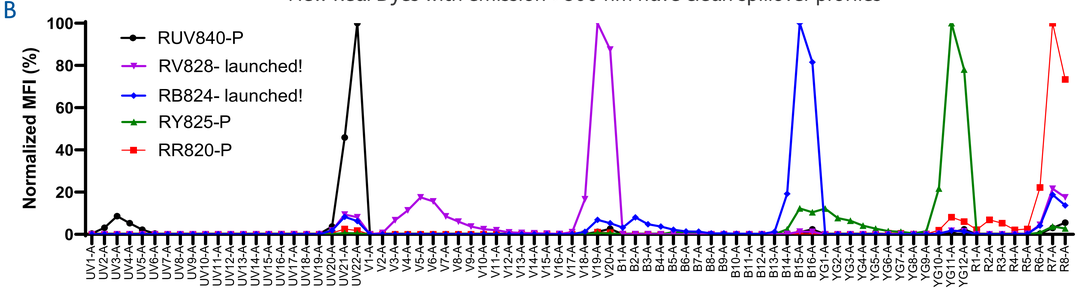
Results (1)

1A The BD FACSDiscover ™ platform enables development of dyes in the NIR space



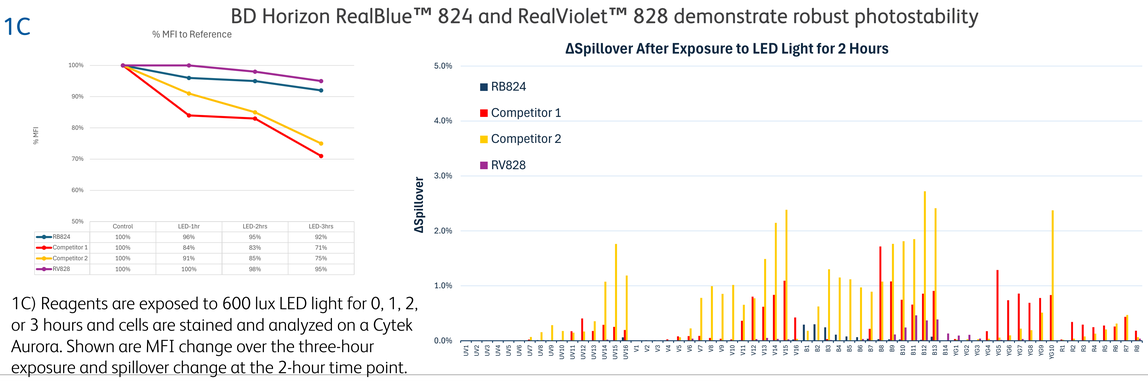
1A shows the configuration of a 5L BD FACSDiscover™ A8 or S8 with the emission spectra of RB824, RV828, RUV840-P, RY825-P, and RR820-P. Image generated using BD®Spectrum Viewer.

1B



1B shows normalized emission profile of hCD4 SK3 RB824, RV828, RUV840-P, RY825-P, and RR820-P on a BD FACSDiscover ™ S8 run on lysed whole blood.

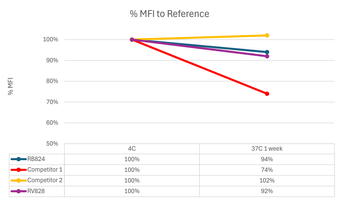
1C



1C) Reagents are exposed to 600 lux LED light for 0, 1, 2, or 3 hours and cells are stained and analyzed on a Cytek Aurora. Shown are MFI change over the three-hour exposure and spillover change at the 2-hour time point.

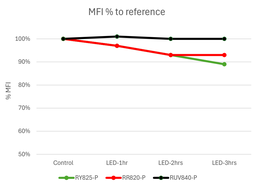
Results (2)

2A BD Horizon RealBlue™ 824 and RealViolet™ 828 demonstrate robust thermal stability



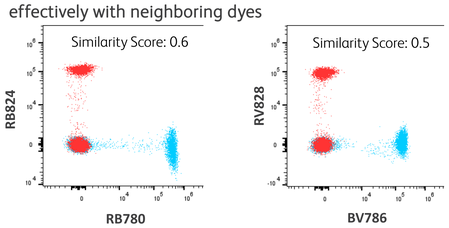
2A) hCD4 conjugates are stored at 4C or 37C for 1 week. Lysed whole blood is stained and run on a Cytek Aurora.

2B BD Horizon RealBlue™ 824 and RealViolet™ 828 demonstrate robust photostability



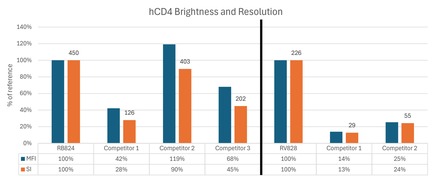
2B) Reagents are exposed to 600 lux LED light for 0, 1, 2, or 3 hours and cells are stained and analyzed on a Cytek Aurora. MFI change over the three-hour exposure is shown.

2C BD Horizon RealBlue™ 824 and RealViolet™ 828 can be used effectively with neighboring dyes



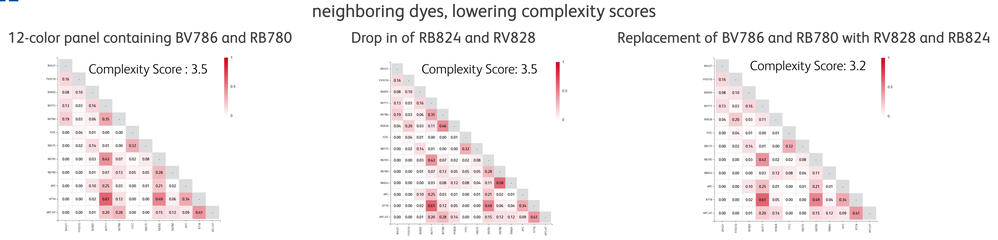
2C) hCD4 stained lysed whole blood run on BDFACSDiscover™ S8 and unmixed in FlowJo. Single color overlays are shown.

2D BD Horizon RealBlue™ 824 and RealViolet™ 828 are comparable to or brighter than other same position dyes on the market



2D) hCD4 brightness and resolution at optimal concentration. Lysed whole blood staining run on A5SE.

2E BD Horizon RealBlue™ 824 and RealViolet™ 828 can be dropped into existing panels or used as replacements for neighboring dyes, lowering complexity scores



2E) Similarity index tables and complexity scores on the BD FACSDiscover™ A8 for a 12-color panel, with RV828 and RB824 dropped in or used as replacements for BV786 and RB780. Data generated using BD® Spectrum Viewer.

Conclusions

- New instrument platforms, such as the BD FACSDiscover A8 ™ and S8, enable development of NIR dyes.
- BD recently introduced BD Horizon RealBlue™ 824 and RealViolet™ 828 and has BD Horizon RealYellow™ 825-P, BD Horizon RealRed™ 820-P, and BD Horizon RealUV 840-P (prototype names; final names pending) in development.
- BD Horizon RealBlue™ 824 and RealViolet™ 828 meet our stringent standards for photo-and thermal stability, as well as brightness and spillover.
- Prototype dyes emitting >800 nm demonstrate robust photostability.
- The stability of our new dyes will enable customers to use these dyes in panels and automation protocols, mitigating concerns about breakdown of NIR dyes.
- BD Horizon RealBlue™ 824 and RealViolet™ 828 are compatible with neighboring dyes and can be dropped in to existing panels with minimal impact on complexity score or used as replacement dyes for BV786 and RB780, reducing the impact of adjacent dye spillover.