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## Evaluation of ‘Super Bright’ Polymer Dyes in 13-16-color Human Immunophenotyping Panels

**Anna Belkina, Riley Pihl, Jennifer Snyder-Cappione**

Flow Cytometry Core Facility, Boston University School of Medicine, Boston, MA, United States

Sirigen Group Limited developed unique polymer ‘Brilliant’ dyes that have become a staple of modern multicolor panel design. Polymer-based conjugates are often 4-10 times brighter than conventional fluorochromes with similar excitation/emission parameters. A new group of polymer fluorochromes, the ‘Super Bright’ dyes, was recently launched by eBioscience. The performance of these new dyes in large polychromatic panels is unclear to date. Therefore, we tested several preparations of the Super Bright dyes (such as Super Bright 436 and Super Bright 600) in two polychromatic fluorescent panels (one 13- and one 16-color). Specifically, we evaluated the spillover spread matrices of both panels to evaluate the compatibility of Super Bright dyes with other fluorochromes in a setup with tight placement of fluorochrome emissions over the spectrum. We have also matched Super Bright conjugates with comparable Brilliant Violet-labeled antibodies of same specificity in an existing 13-color panel where those conjugates are staining relatively dim targets, such as CCR6 and CD25, on resting human PBMC cells. Our results show that Super Bright dyes inflict a modest spillover spread in neighboring channels. In a 16x16 spillover spread matrix (3-UV, 5-VIOLET, 5-BLUE, 3-RED) Super Bright dyes demonstrate low to moderate spillover that is very close quantitatively to the Brilliant Violet dyes. In a 13-color human immunophenotyping panel that we previously developed to quantify T cell subsets, the “brightness” (i.e. the staining index of the Super Bright-conjugated antibodies) appears to be lower than comparable Brilliant Violet dyes when titrated, although stained populations in a full panel are still well separated. As the use of up to nine Brilliant polymer dyes simultaneously in large panels is not uncommon, we also tested the performance of Super Bright dyes in staining protocols that include Brilliant Buffer (BD Biosciences) to prevent polymer dye interactions and found them compatible. Overall, we found Super Bright dyes to perform well in large polychromatic panels. This expansion of commercially available conjugated antibody repertoire with the addition of Super Brights is timely and will greatly facilitate the success of larger (13+ color) fluorescent panel design.