Introduction

The InFusion™ NovaFluor™ Platform. Our DNA-based nanostructure acts as a scaffold to arrange fluorophores into a PEBL system with engineered spectra. This allows us to create labels with significantly lower overlap across excitation and detection channels, opening detectors for scientists to increase panel complexity and reducing spillover spreading error.

Antibody Conjugation Simplified. Our conjugation workflow is a simple and highly adaptable process that involves attaching a single stranded DNA oligo handle to an antibody, then purifying to remove excess oligo and unlabeled antibody. The DNA nanostructure can then be annealed to the antibody and labeling saturates at 1:1 labeling.

High Lot-to-Lot Consistency. The NovaFluor nanostructure incorporates dyes at defined locations and thus highly reproducible from lot to lot, enabling <5% variance in maximum emission intensity. In combination with the 1:1 labeling to the antibody we observe, this translates to consistent performance in flow of the antibody conjugates.

DNA-Based Dye Nanostructures Enable New Directions in Spectral Flow Cytometry

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Abstract

To increase the complexity of flow cytometry panels and allow scientists to dig deeper into the biology made accessible by these experiments, more antibody conjugates are needed with spectrally unique dyes that can be easily differentiated on spectral cytometers. Using DNA-based macromolecules, dyes can be attached at defined positions, enabling the design of highly efficient FRET networks with tunable fluorescence properties that minimize cross-excitation and spectral overlap. Unlike PE or APC tandem dyes, our DNA nanostructure displays a highly significant lot-to-lot variability due to differences in the degree of labeling or FRET efficiency. Our approach allows for high signal-to-noise consistency (>95% spectral difference between lots) due to the highly specific attachment of the dye at defined positions on the DNA nanostructure. Combined with the controlled 1:1 labeling of antibodies that our chemistry affords, we were able to demonstrate a wide antibody library for each of these novel dyes and their respective conjugate. In addition, our testing shows that individual antibody-dye conjugates are stable for at least three years when stored at 4 ºC and that antibody-dye conjugates are compatible when stored mixed together in solution for up to 14 days. Furthermore, the DNA nanosstructures are compatible with methanol as well as 2% formaldehyde fixation and the antibody-conjugates exhibit high stability on cells after fixation, displaying no appreciable change in signal in up to 14 days when stored in fixant buffer.

Results

Conclusions

The NovaFluor platform enables highly tunable spectra to fully maximize the capabilities of spectral flow cytometry instruments for building increasingly complex panels. Here we demonstrate additional advantages of this platform:

- >5-year stability of the NovaFluor structure and its antibody conjugates and >2 week stability when stored in fixative with no observable change in spectrum.
- Compatibility with mechanical fixation and identical spectra on beads and cells (PBMCs).
- Stable when stored in master mixes for >1 month at 4 ºC.
- General conjugation protocol enables rapid testing of new antibody clones.

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