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

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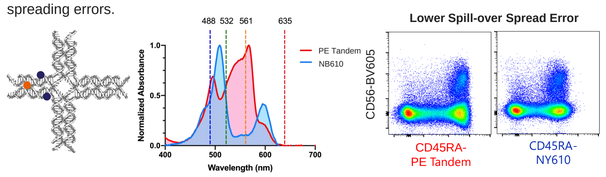
Abstract

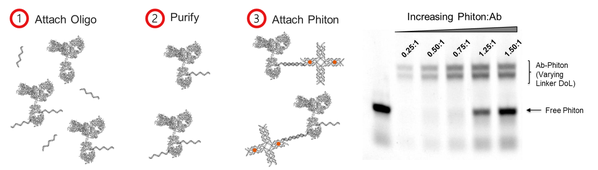
To increase the complexity of flow cytometry panels and allow scientists to dig deeper into the biology made accessible by these experiments, more antibody conjugates are needed with spectrally unique dyes that can be easily differentiated on spectral cytometers. Using DNA-based macromolecules, dyes can be attached at defined positions, enabling the design of highly efficient FRET networks with tunable fluorescence properties that minimize cross-excitation and spectral spillover. Unlike PE or APC tandem dyes, which can display significant lot-to-lot variability due to differences in the degree of labeling or FRET efficiency, our approach allows for high lot-to-lot consistency (<5% spectral difference between lots) due to the highly specific attachment of the dyes at defined positions on the DNA nanostructure. Combined with the controlled 1:1 labelling of antibodies that our chemistry affords, we were able to demonstrate a relative similarity ≥0.99 for each of these novel dyes when comparing the spectral signatures on cells to those on compensation beads. In addition, our testing shows that individual antibody-dye conjugates are stable for at least three years when stored at 4 ºC and that different antibody-dye conjugates are compatible when stored mixed together in solution for up to 14 days. Furthermore, the DNA nanostructures 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 for up to 14 days when stored in fixation buffer.

Introduction

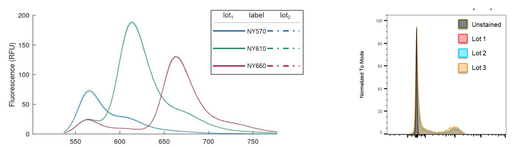
The Invitrogen™ NovaFluor™ Platform. Our DNA-based nanostructure acts as a scaffold to arrange fluorophores into FRET networks with engineered spectra. This allows us to create labels with significantly lower spillover across excitation and detection channels, opening detectors for scientists to increase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



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-dye nanostructure can then be annealed onto the antibody and labeling saturates at 1:1 labeling.

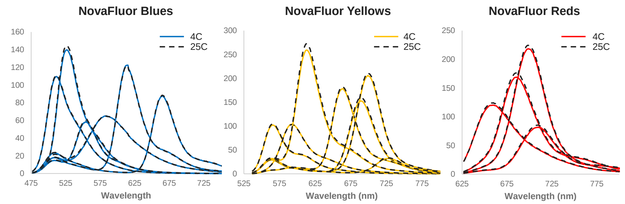


High Lot-to-Lot Consistency. The NovaFluor structure incorporates dyes at defined locations and thus is highly reproducible from lot to lot, exhibiting <5% variance in maximum emission intensity. In combination with the 1:1 labeling to the antibody we observe, this translates to consistent performance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

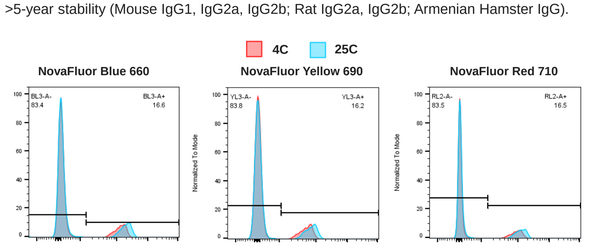


Results

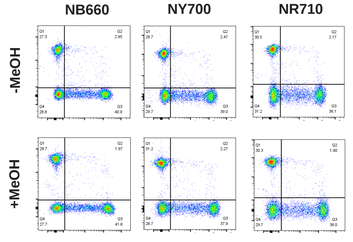
NovaFluor Dyes exhibits >5-year stability. The NovaFluor structure shows exceptional stability stored in PBS at 4 ºC. Accelerated aging at elevated temperature (25 ºC) results in negligible change in the spectrum of the dyes over an accelerated 5 years of storage, with <5% variance in the maximum emission intensity for 19 dyes across 3 excitation lasers.



NovaFluor conjugates exhibit >5-year stability. Accelerated aging at elevated temperature (25 ºC) was carried out on the Antibody-NovaFluor conjugates to determine the shelf life of the conjugates at 4 ºC. Our results demonstrate that our conjugates perform within a defined passing criteria of +/- 5% log(MFI) of the positive population for the aged sample relative to the normally stored sample out to at least 5 years. Shown are conjugates of anti-Human CD4 (SK3) to representative dyes excited by the blue, yellow, and red laser lines. Six different common antibody isotypes were tested as part of this study and all exhibit >5-year stability (Mouse IgG1, IgG2a, IgG2b; Rat IgG2a, IgG2b; Armenian Hamster IgG).



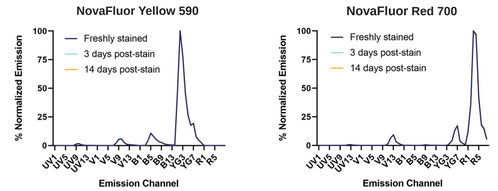
Antibody- NovaFluor conjugates can be used in conjunction with methanol fixation for simultaneous observation of extracellular and intracellular targets. Cells were stained with Anti-Human CD4 (SK3) conjugated to a representative dye from each laser line (y-axis) and Anti-Human CD8a (OKT8) eFluor450 (x-axis), then were fixed with 2% PFA and compared with and without subsequent treatment with methanol. No loss of signal is observed after methanol treatment.



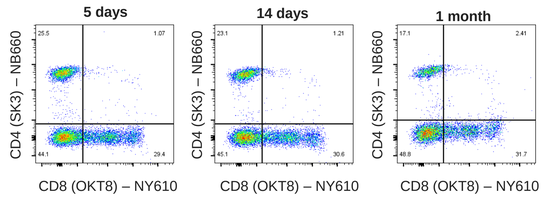
Color	MeOH	MFI+	SI
NB660	-	23905	47.9
	+	33325	72.5
NY700	-	94294	45.8
	+	129672	65.3
NR710	-	47043	59.2
	+	54359	69.5

Results

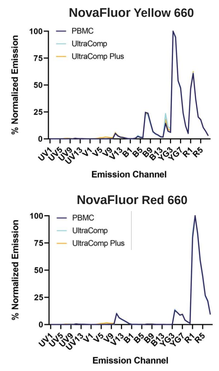
NovaFluors are stable stored in fixative for 14 days. Human PBMCs were stained with NovaFluor conjugates to Anti-Human CD4 (SK3), then were fixed with Invitrogen™ eBioscience™ IC Fixation Buffer. Data was collected on day 1, day 3, and day 14. Shown are the spectral signatures for a representative yellow and red dye at the three time points. The similarity index was calculated at day 3 and day 14 relative to day 1 and is >0.99 for all dyes tested, meaning the spectra are indistinguishable.



Antibody- NovaFluor conjugates are stable in master mixes for >1 month. Antibody-NovaFluor conjugates can be mixed and stored as master mixes at 4 ºC with little change in the performance over 1 month when staining cells. This is despite the noncovalent linkage of the antibody to the DNA nanostructure and the use of the same linkage sequence, which speaks to the high thermal stability of the double-stranded DNA linkage.



NovaFluor spectral signatures are nearly identical on cells and beads. Human PBMCs and Invitrogen™ Ultracomp or Ultracomp Plus eBeads™ were stained with CD4 (SK3) NovaFluor conjugate, then analyzed on a 5-laser Cytek® Aurora. The spectral signatures between cells and beads have similarity indices >0.99 for all colors tested, indicating that they have essentially identical spectra, enabling compensation beads to be used for unmixing for all colors instead of cells.

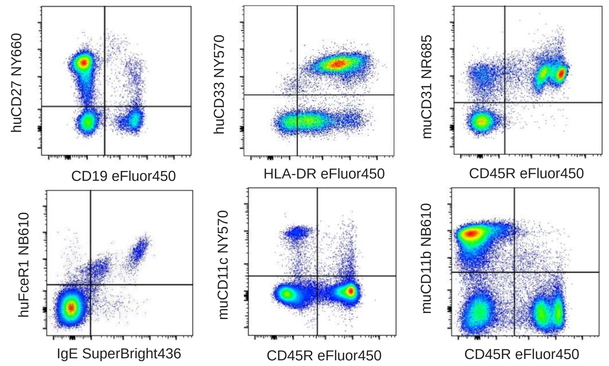


Similarity Index vs. Cells

	Ultracomp	Ultracomp Plus
NB510	1.00	1.00
NB530	1.00	0.99
NB555	0.99	1.00
NB585	0.99	1.00
NB610-30S	0.99	0.99
NB610-70S	1.00	1.00
NB660-40S	1.00	1.00
NB660-120S	1.00	1.00
NY570	1.00	1.00
NY590	1.00	1.00
NY610	1.00	1.00
NY660	1.00	1.00
NY690	1.00	1.00
NY700	1.00	1.00
NY730	0.99	0.99
NR660	1.00	1.00
NR685	1.00	1.00
NR700	1.00	1.00
NR710	1.00	1.00

Results

An adaptable workflow for rapidly generating new antibody conjugates. Our simple conjugation workflow allows us to rapidly generate new Antibody- NovaFluor conjugates and test them in different model systems. Shown are six representative clones staining a variety of cell types on mouse and human tissue. We are excited to continue to expand the number of clones that can be studied with our dye conjugates.



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 methanol 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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