# Designing spectrally clean fluorescent dyes for high dimensional biological analysis

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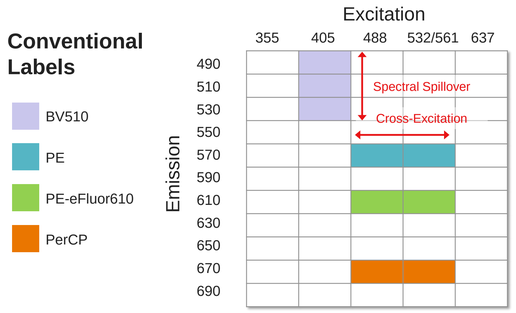
## Abstract

In flow cytometry, there exists a growing demand for spectrally clean dyes to increase the number of biological parameters that can be analyzed simultaneously. Cross-laser excitation and spectral spillover of conventional dyes remain a challenge in meeting this demand. For decades, protein based fluorochromes such as PE and APC, along with their tandems, have enabled countless discoveries in biology. Although bright, these fluorochromes have considerable cross-laser excitation that blocks complete use of other detector channels. Another challenge is the inability to control the tandem dye placement translating to variable FRET efficiencies, leading to unwanted spectral spillover.

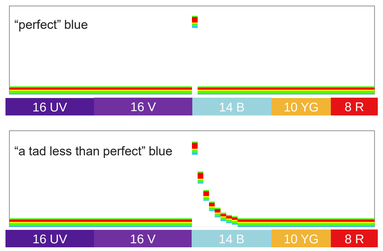
To address challenges with a dye's spectral properties, we have designed a novel DNA-based platform that enables precise control over dye composition and placement. Control over these properties reduces both the spectral spillover and cross-laser excitation. As part of the design process, we have demonstrated the ability to fine tune the resultant spectral signature through iterative dye design. Dyes with cleaner spectra translate to less compensation or spread because there is less unwanted fluorescence in secondary channels. As a result, additional detector channels are freed up for more labels to be added to the analysis. Furthermore, through our design process, we targeted empty channels that until now have not been filled by any commercially available dyes. The unique attributes of the DNA-based platform for designing spectrally cleaner dyes affords the ability to obtain higher content data and will enable novel discoveries in biology.

## Introduction

### Conventional labels exhibit cross-laser excitation and spectral spillover that use up multiple detection channels:



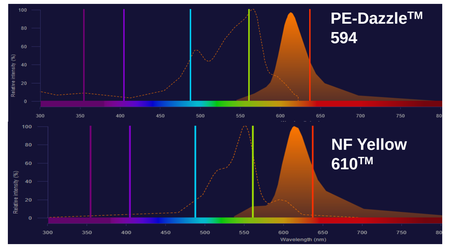

### A "perfect" label will be excited by one laser and emit in one detector channel:



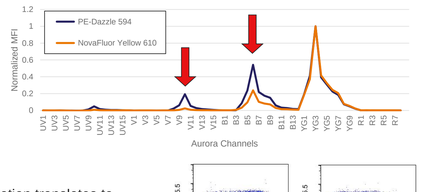

## Results

### Cross-laser excitation and spectral spillover create spread:

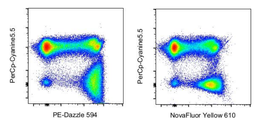
- PE-Dazzle™ 594 strongly excited by the blue and yellow lasers
- NFY610 exhibits significantly less blue cross-laser excitation




- Another way to visualize cross-laser excitation (red arrows) is with the Aurora's MFI Vector

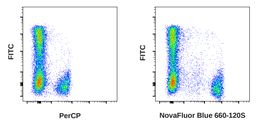



- Less cross-laser excitation translates to significantly less spreading from NovaFluor Yellow 610 into the PerCP-Cy5.5 channel than PE-Dazzle 594.

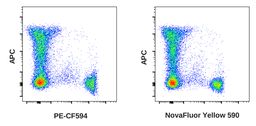



### Examples of lower spread from other NovaFluor labels:

- Brighter signal with NovaFluor Blue 660 - 120S than PerCP, without significant increase of spreading into FITC channel




- Less spreading of NovaFluor Yellow 590 into APC channel than PE-CF594

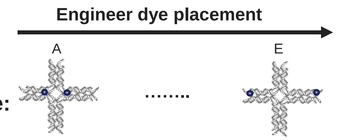



### Numerically Quantify Spread for comparing labels:

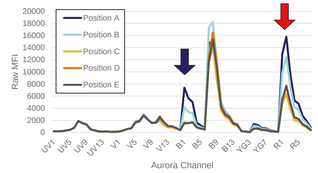
| Fluorescent label | Emission max (nm) | Primary detector | Laser line | Spread | Separation Index (SI) |
|---|---|---|---|---|---|
| **NovaFluor Blue 660-120S dye** | 665 | B7 (652-669) | 488 | 5971 | 119 |
| PerCP | 678 | B8 (669-687) | 488, 561 | 1585 | 13 |
| **NovaFluor Yellow 590 dye** | 592 | YG2 (588-608) | 561 | 1878 | 154 |
| PE-CF594 | 614 | YG3 (605-625) | 488; 561 | 14204 | 260 |
| **NovaFluor Yellow 610 dye** | 612 | YG3 (605-625) | 561 | 4257 | 117 |
| PE-Dazzle™ 594 dye | 610 | YG3 (605-625) | 488; 561 | 13497 | 280 |

## Results

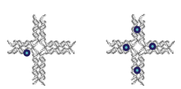
### Precise control over the dye placement, number, and composition cleans up the spectral signature:

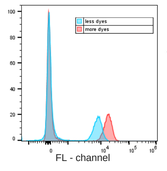



- By engineering the placement of dyes, both the spectral spillover (blue arrow) and the cross-laser excitation (red arrow) are lower. As shown previously this leads to less spread.




### Engineer number of dyes



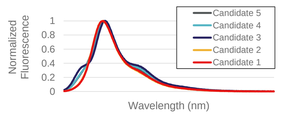



- The brightness of the label is adjustable by adding more dyes to the structure.

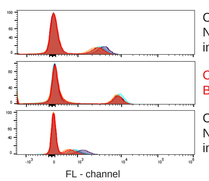
### Engineer dye composition




Example 1: Spectral Sculpting for a primary channel




- Engineering the dye network enables sculpting of the emission peak shape to remove the shoulders on either side of the main peak

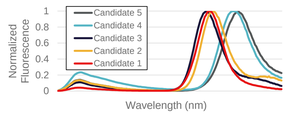



Candidate 1: Nearly dimmest in secondary

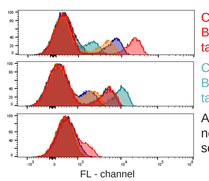
Candidate 1: Brightest in primary

Candidate 1: Nearly dimmest in secondary

Example 2: Tuning to different target channels




- Engineering the dye network enables tuning of the emission peak to a target detection channel




Candidate 1: Brightest in its target channel

Candidate 4: Brightest in its target channel

All dim in nearest secondary

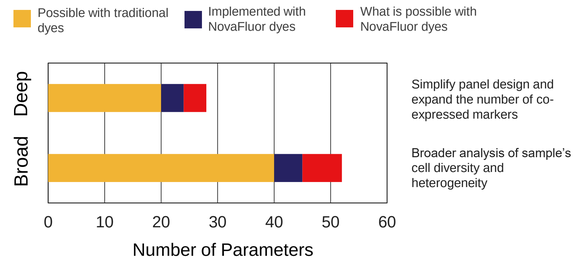
## Results

Our DNA-based dye nanostructure platform enables control over the dye composition and placement to rationally engineer labels with the following key attributes:

1. The label emits in the intended primary channel
2. Lower cross-laser excitation and spectral spillover to ensure the primary channel is brighter than any secondary channel

## Conclusions

1. Thermo Fisher offers 19 different NovaFluor labels across the blue, yellow, and red laser lines on a variety of antibodies.
2. The possibilities for high dimensional biology with spectrally clean fluorescent labels engineered for low spread include:




## Acknowledgements

We would like to acknowledge the whole team at Thermo Fisher Scientific dedicated to researching this DNA-based dye nanostructure platform and expanding its applicability.

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