Exposure of cells to laser light in flow cytometers causes molecules such as **NADPH & flavins** to emit fluorescence. This intrinsic emission of light is what we call **autofluorescence** (AF). Particles or cells can have varying levels of autofluorescence, and each cell or condition type should have a fully **unstained control** to account for this.



Conventional

Spectrally Unmixed

Spectrally Unmixed + AF as a separate parameter
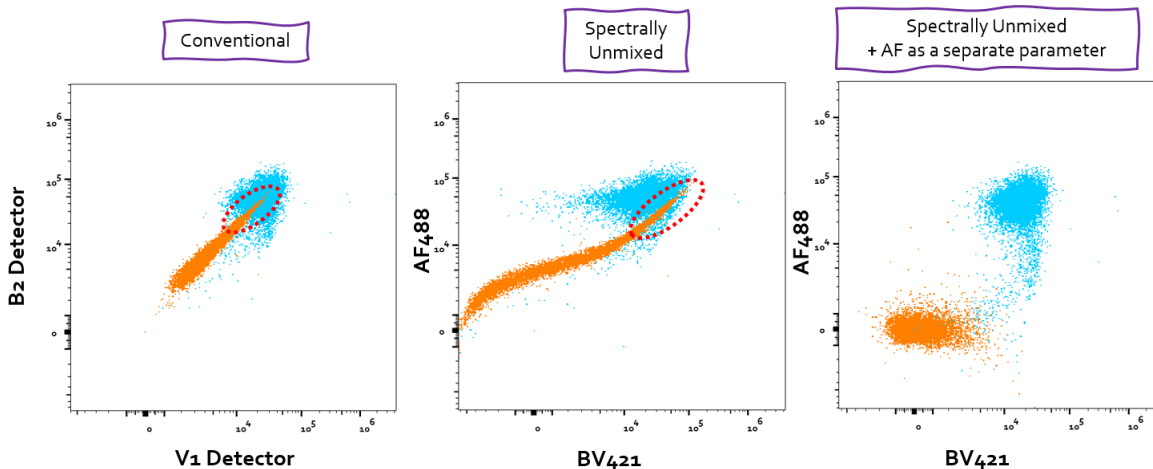
Overlay of **unstained** and **fully stained** samples recorded using the Cytek Aurora full spectrum flow cytometer.

- *Conventional* Plot shows peak channel for the BV421 (V1) vs. peak channel for AF488 (B2). Unable to determine positivity due to high AF.
- *Spectrally Unmixed* Plot shows data unmixed without AF as a separate parameter. Resulting data shows a skewed unstained population and inability to determine positivity.
- *Spectrally Unmixed + AF as separate parameter* Plot shows data which incorporates the AF signature into the unmixing algorithm. Clear separation of the populations is seen.

💡 **Helpful Hints**

- AF tends to be higher when excited by the 335, 405 and 488 nm laser and in the emission wavelength range of 400-600 nm.
- Use of fluorochromes that emit in longer wavelengths (>600 nm) can result in improved population resolution.
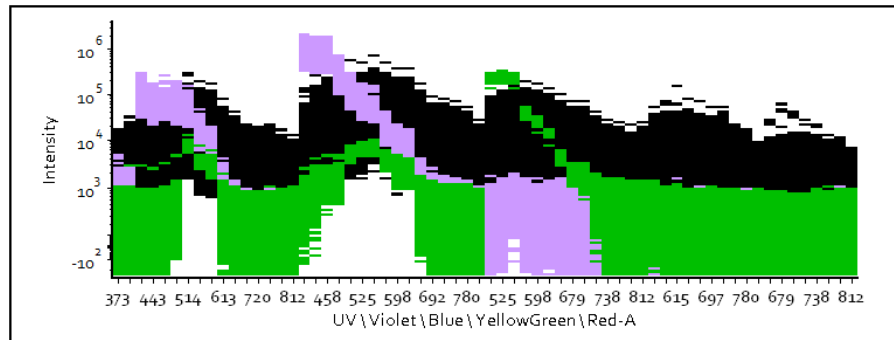


### Full Spectrum Overlay Plot

Overlay showing the fluorescent signatures of **BV421**, **AF488** and **autofluorescence of the unstained cells** represented above. It is clear that the AF of the cells is very high, especially in the peak channels for the fluorochromes in this experiment, leading to difficulties in resolving populations.

When working with certain cell types, **high autofluorescence can impact data** interpretation. If using a conventional flow cytometer, the use of fluorochromes that emit in wavelengths less impacted by AF is suggested. When working with a full spectrum flow cytometer, unmixing algorithms have the ability to **reduce the impact of autofluorescence** through the unique AF signature of cells.

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