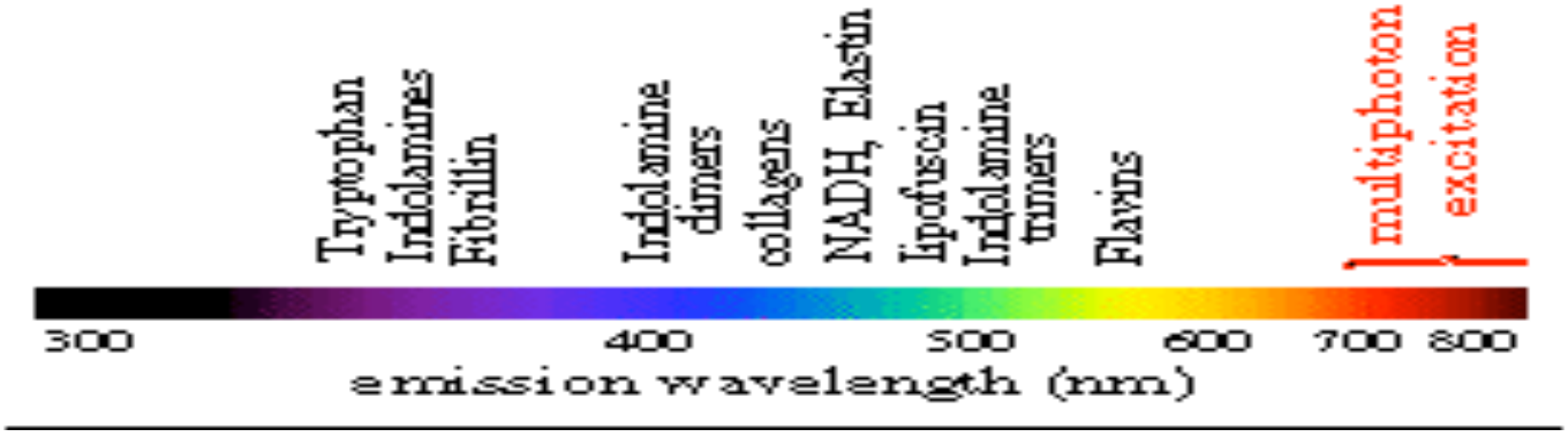
AUTOFLUORESCENCE
MIT Flow Cytometry Core Facility

Autofluorescence

Cells contain molecules, which become fluorescent when excited by UV/Visual radiation of suitable wavelength. This fluorescence emission, arising from endogenous fluorophores, is an intrinsic property of cells and is called auto-fluorescence. Autofluorescence is different from fluorescent signals obtained by adding exogenous markers like FITC, GFP, or PE. It is usually strongest with short wavelength excitation (UV or Blue) and short wavelength emission (Blue and Yellow).

Major Causes of Autofluorescence

Intracellular autofluorescence is often dominated by the reduced pyridine nucleotides (NAD(P)H) and the oxidized flavins (FMN, FAD), both of which are potentially useful as cellular metabolic indicators.



Mitochondrial NADH autofluorescence can be directly used as an indicator of cellular respiration (Piston et al.,1995). Since only the reduced form has an appreciable fluorescence yield, hypoxia, which causes an increase in the NADH/NAD+ ratio, can be detected as an increase in mitochondrial autofluorescence.

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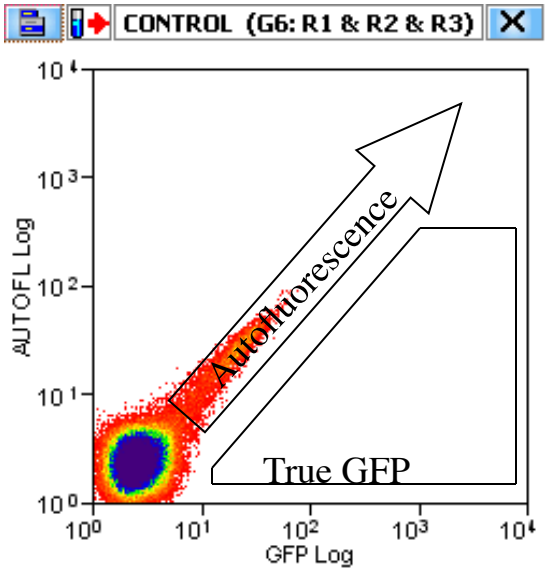
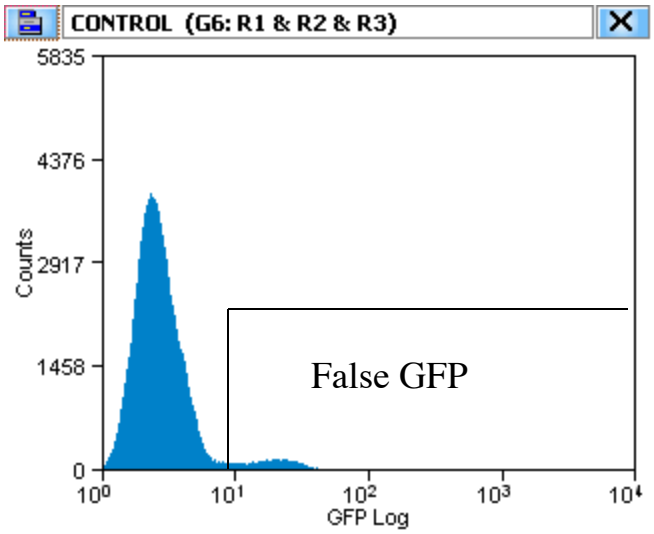
A false positive population that is due to autofluorescence can be detected.

Autofluorescence typically has similar excitation and emission characteristics to fluorescein & PE and will, therefore, interfere with the detection of FITC and GFP fluorescence. This is why it is best to measure GFP or FITC on a FL1 vs FL2 plot instead of a histogram of FL1.





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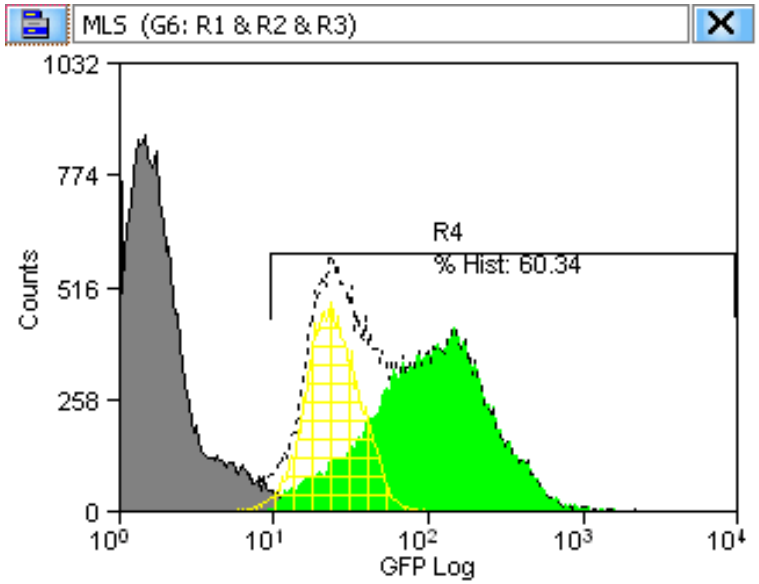
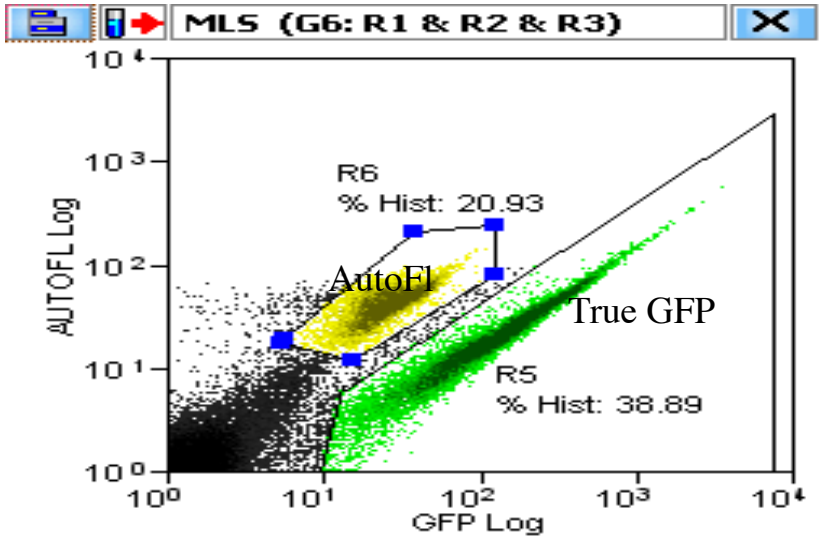
Autofluorescence increases the percentage of positive cells.

Depending on the cell type, signal intensity, and percentage positive using a histogram (left) instead of a FL-1 vs FL-2 plot (right) can lead to an large inaccurate increase of the positive population.





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How to address the autofluorescence problem?

The best ways to address the issue of autofluorescence:

1. Gating it out- Not always possible but sometimes can be done if gates are very tight.
2. Chemically remove it or quench it- This can also reduce "real" signal. Trypan Blue is a possibility.
3. Using a ratio of green to yellow-If green or yellow is not available try using red in your ratio.

Ratio	Auto fluorescence	FITC/GFP	PE
Green/ Yellow	= 1:1	> 1:1	< 1:1

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