AUTOFLUORESCENCE

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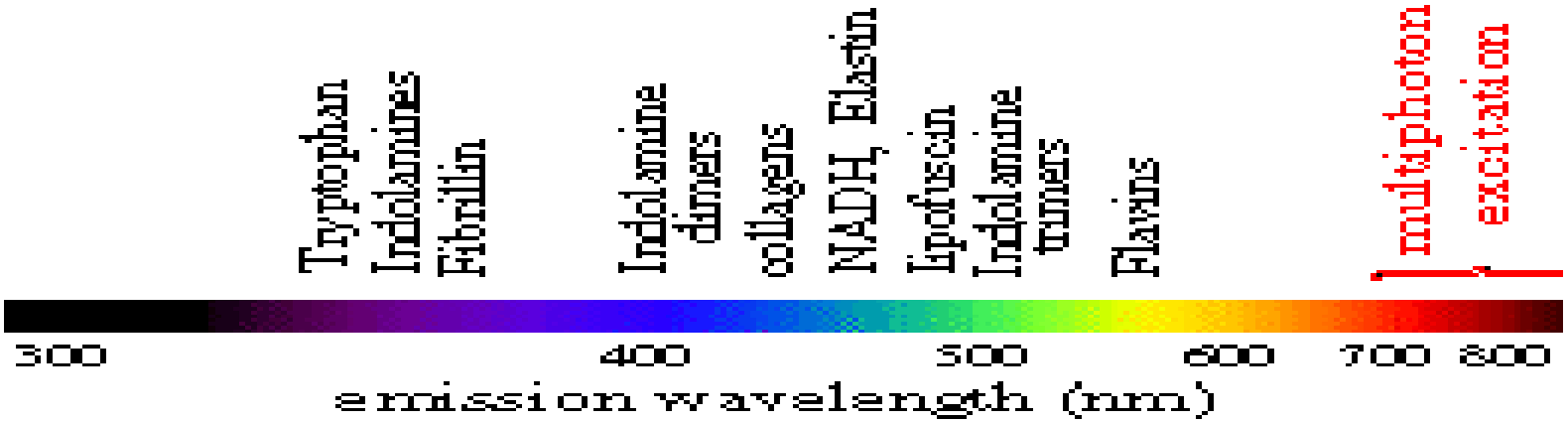
Autofluorescence

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 which is different from fluorescent signals obtained by adding exogenous markers like FITC, GFP, or PE.

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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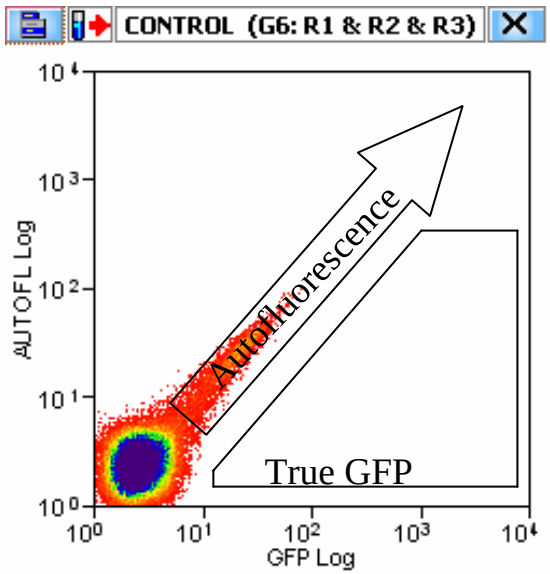
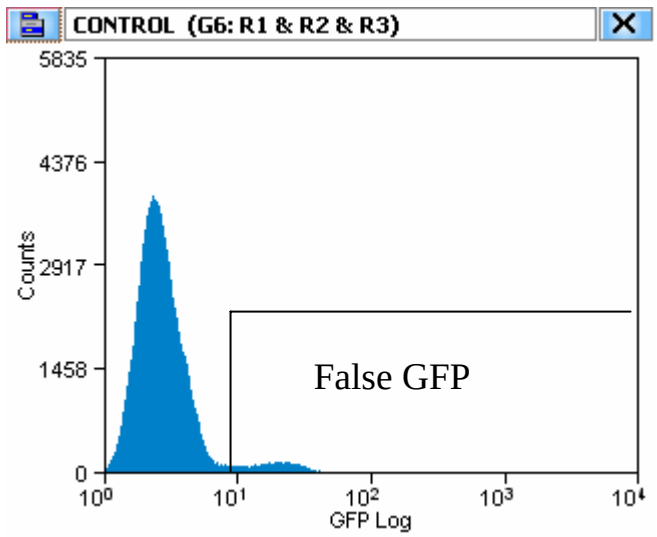
What is the problem with autofluorescence?

autofluorescence typically has similar excitation and emission characteristics to fluorescein & PE and will, therefore, interfere with the detection of FITC and GFP fluorescence that 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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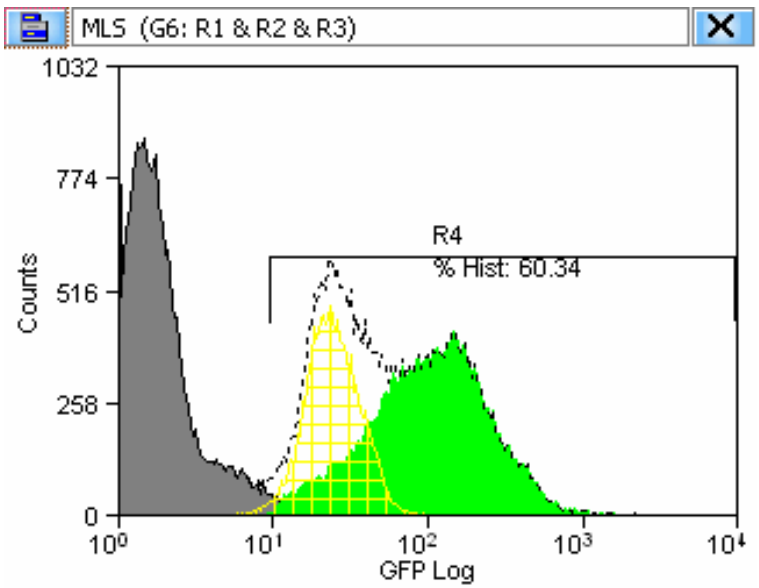
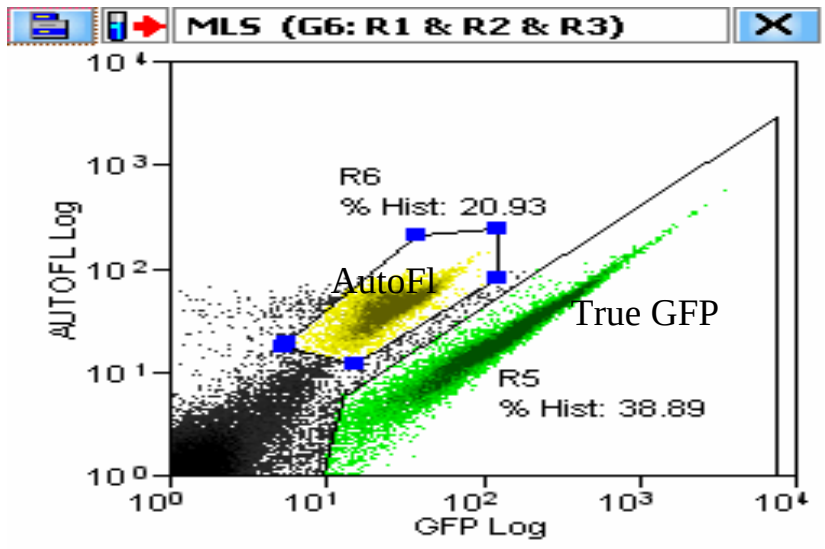
Autofluorescence increase % of positive

Depending on the cell type, using a histogram (left) instead of a fl-vs-fl2 plot (right) can lead to an increase of 50% more positive.





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How to fix autofluorescence problem?

The best ways to address the issue of autofluorescence:

1. Gating it out

- not always possible but can be done .

2. Using broad-pass filter:

difficult due to the broad emission spectrum.

3. Chemically remove it

-can also reduce “real” signal.

4. Using a ratio of green to yellow

ratio	auto	Fitc/gfp	PE
G/Y	= 1	> 1	< 1

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