



Using a Spectral Viewer

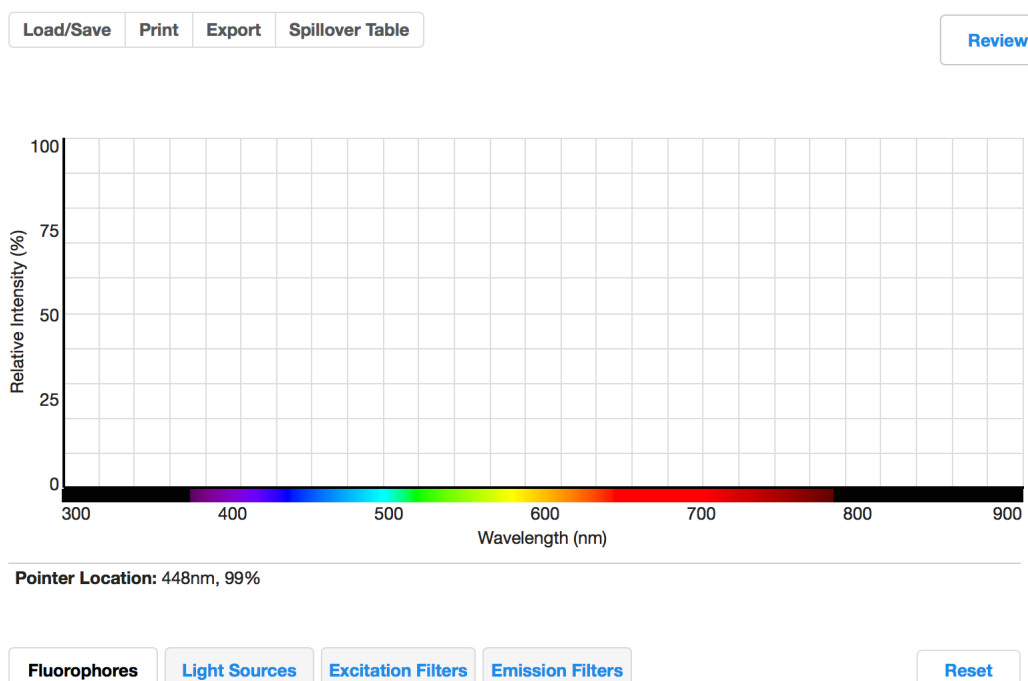
A spectral viewer is a tool to analyse the excitation and emission spectra of fluorochromes, fluorescent proteins and dyes commonly used for flow cytometry. Is very useful to identify the best excitation laser and bandpass filter to collect the maximum emission signal of a given fluorochrome. Additionally, a spectral viewer could be used to predicts the potential spillover between fluorochromes present in a sample in order to anticipate compensation needs and improve the experimental design.

They are several spectral viewers available on line, some of the most relevant are:

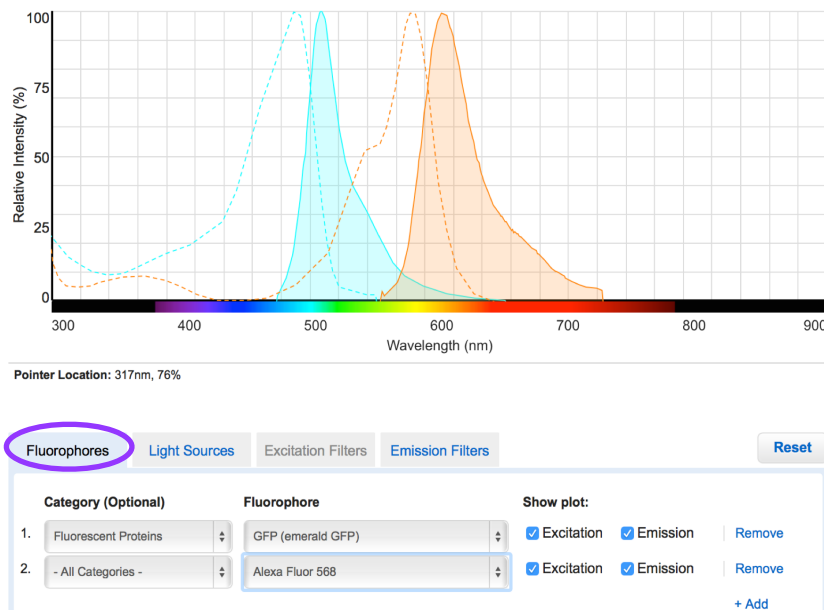
- BD Bioscience Spectrum Viewer
- Biolegend Spectra Analyser
- ThermoFisher Fluorescence Spectra Viewer

All of them are free and very easy to use; you just need to specify the fluorochrome name, excitation lasers and the filter configuration of your instrument. Below the steps to use the ThermoFisher Fluorescence Spectra Viewer:

1. Follow the link: <https://www.lifetechnologies.com/de/de/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html>



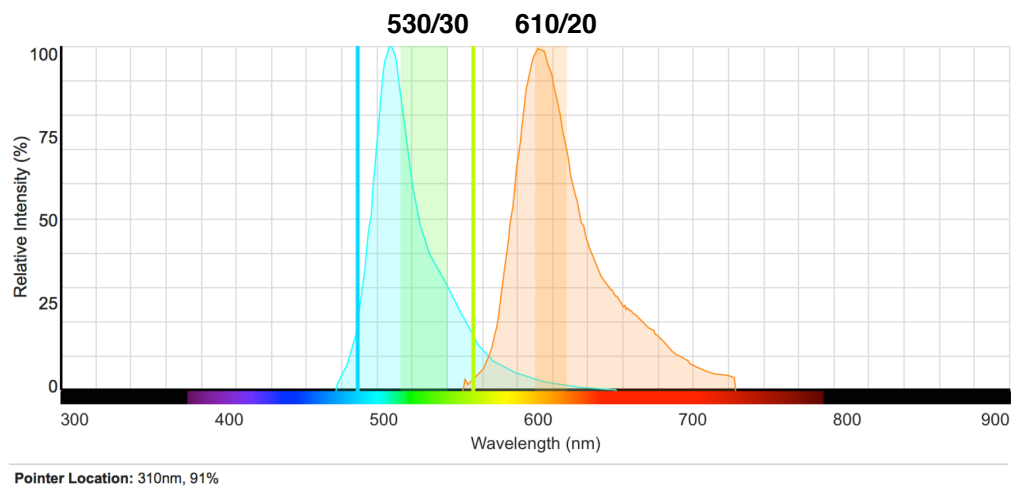
2. In the Fluorophores tab, choose the fluorochrome or fluorescent protein in order to visualise the excitation and emission profile.



3. Specify the excitation lasers:



4. Select the bandpass filters:



Fluorophores Light Sources Excitation Filters **Emission Filters** Reset

Filter	Wavelength	Bandwidth	Display on plot	Remove
1. Custom Filter	530	30	<input checked="" type="checkbox"/>	Remove
2. Custom Filter	610	20	<input checked="" type="checkbox"/>	Remove

+ Add

if you hover over the filter, it will report the percentage of the emission curve that is contained within that filter. In this case, the 530/30 bandpass filter captures about 35% of the GFP emission curve.

5. Click on Spillover Table to visualise the spillover of mGFP into the Alexa 568 detector (BP 610/20) and the one of Alexa 568 into mGFP.

Load/Save Print Export **Spillover Table**

Fluorophore / Filter	530/30	610/20
GFP (emerald GFP)	35.0%	1.0%
Alexa Fluor 568	0.0%	31.3%

The table indicated that 1% of the mGFP fluorescence is captured by the 610/20 filter.