CTAC / ICBR SOP : Setting the Excitation Filter for a Fluorescent Scan using the IVIS Software Control Panel

Title: Setting the Excitation Filter for a Fluorescent Scan using the IVIS Software (Living Image™) Control Panel
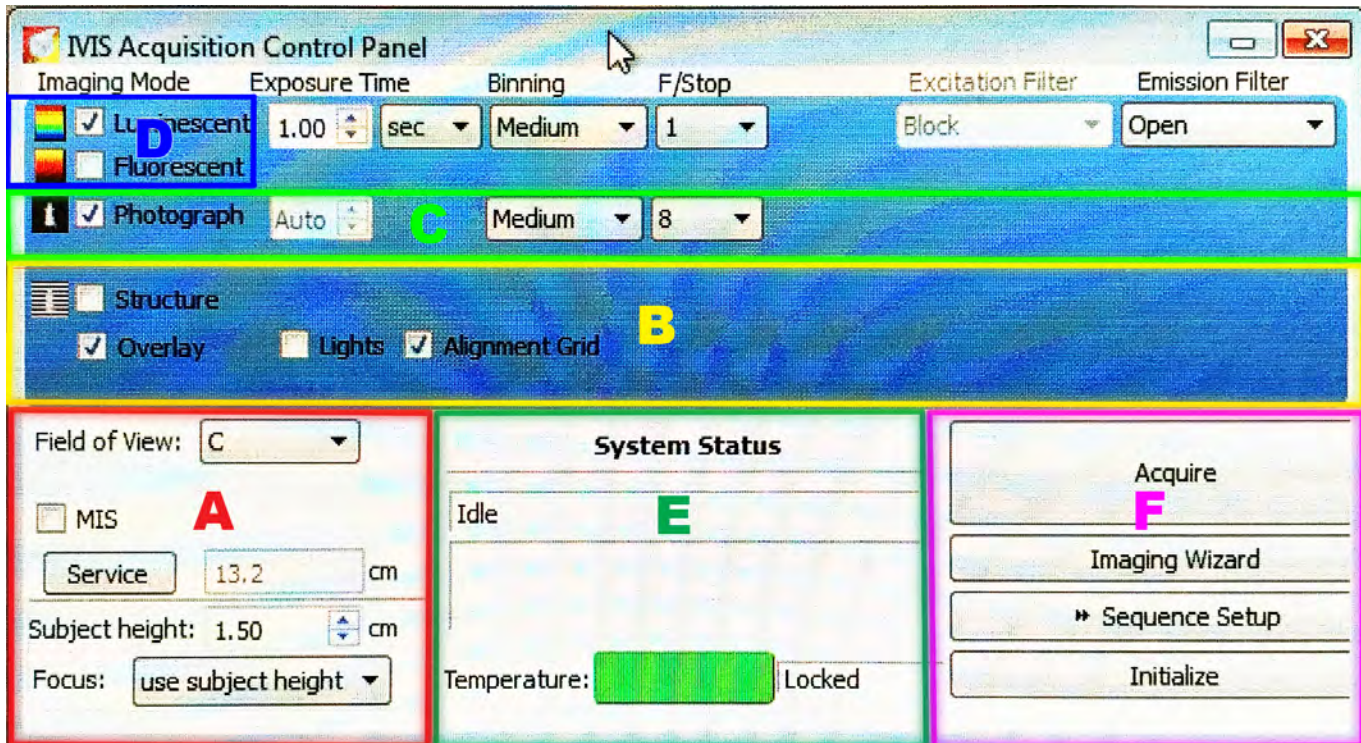
Materials Required:

Computer

Living Image™ software

Purpose:

To detail the use and selection of an excitation filter on the software Control Panel to conduct a scan for fluorescence.
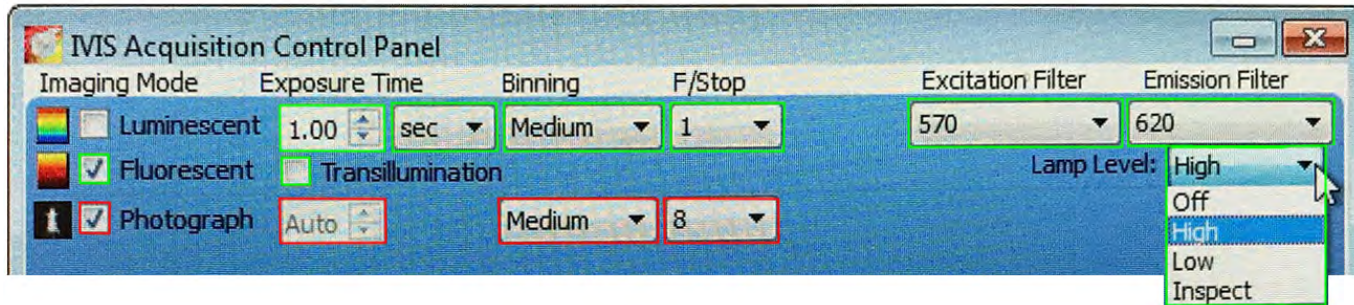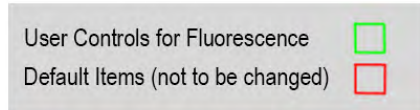
Software component identification:

The fluorescent setting toggle is found in section D.

To conduct a fluorescent scan, the "Fluorescent" toggle in section D must be checked.

The fluorescence scanning controls comprise the areas noted in GREEN
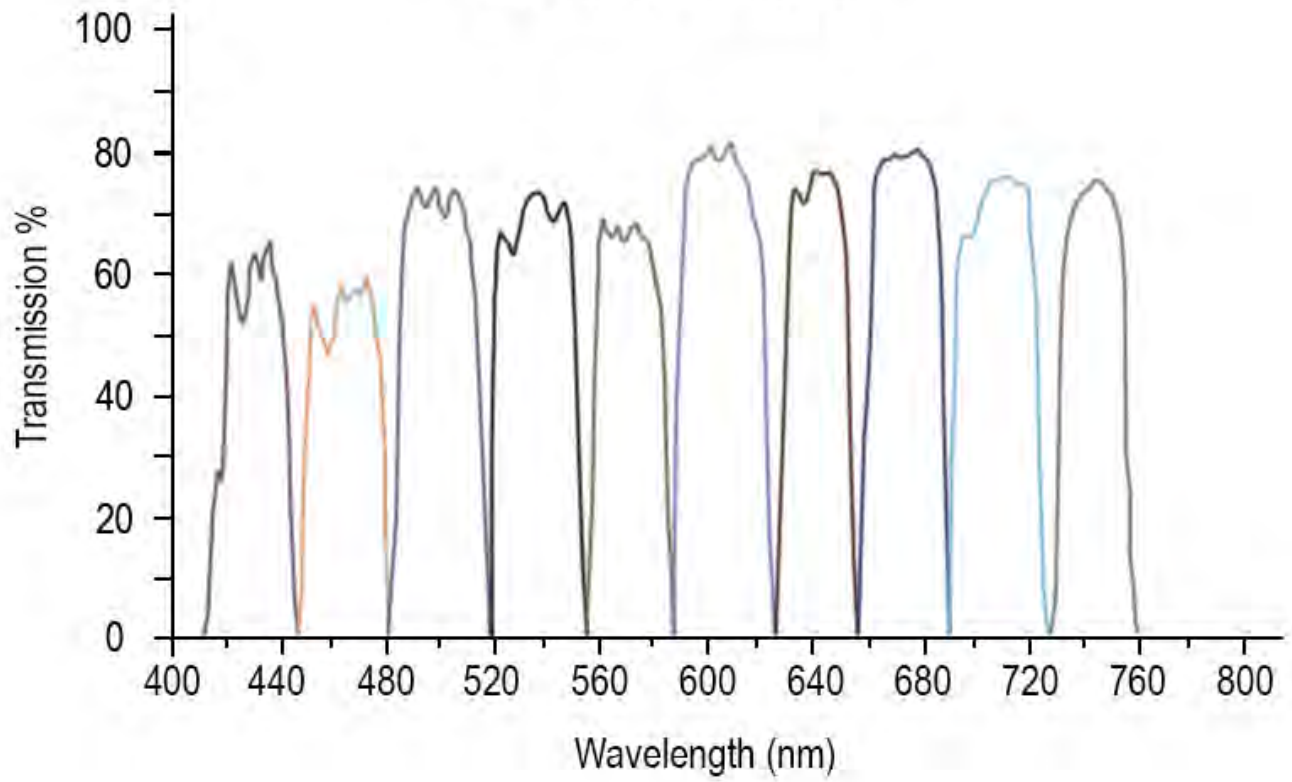

Excitation Filter

Fluorescence detection requires that energy is delivered into the subject in the form of selected wavelengths of light. The fluorescent molecules within the subject return this light at a different wavelength, which is detected by the instrument when scanning. The correct input wavelengths must be used to be able to excite the fluorescence under detection, in order for light to be emitted from the subject.

The instrument's Excitation Filter setting is a drop down menu, which allows a number of settings. These include "Open" (all wavelengths), "Block" (no light, used to assess any naturally emitting light if required), and 10 filters with bandwidths of 35 nanometers.

The following images depict common fluorophores and strategy for filter selection based on their excitation and emission spectra.

The main goals where possible are to select the best filter that does not overlap the emission spectrum of the fluorophore used, and cover the highest amount of energy from the excitation spectrum.
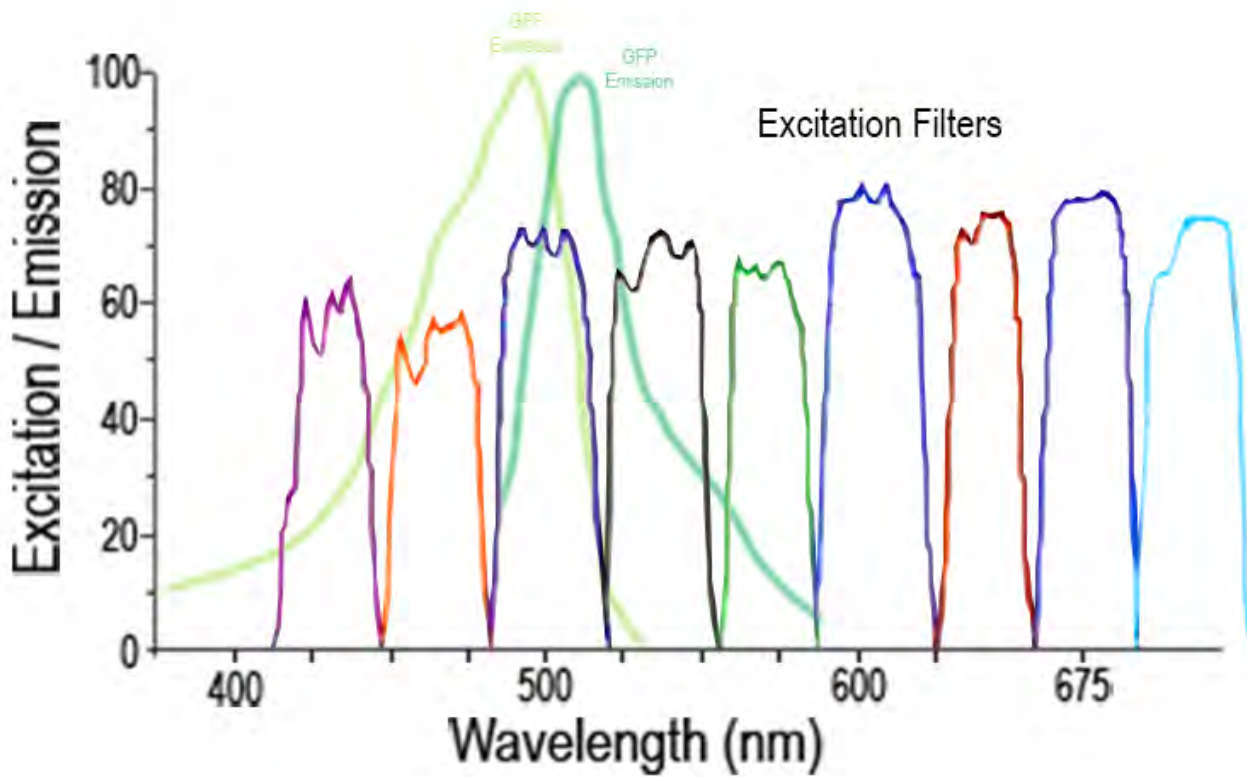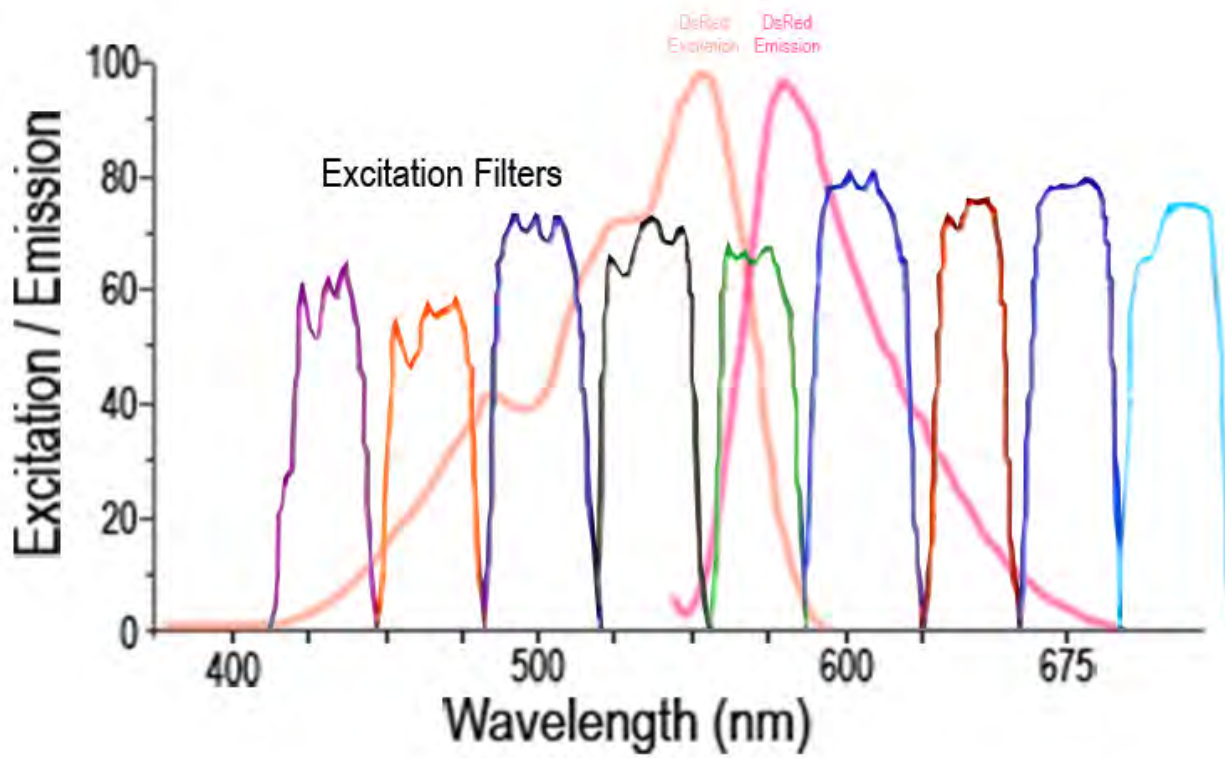
Excitation Filters

This image shows the different filter ranges for excitation on the IVIS Spectrum instrument. The covered wavelengths are from 410nm-760nm in 35nm increments
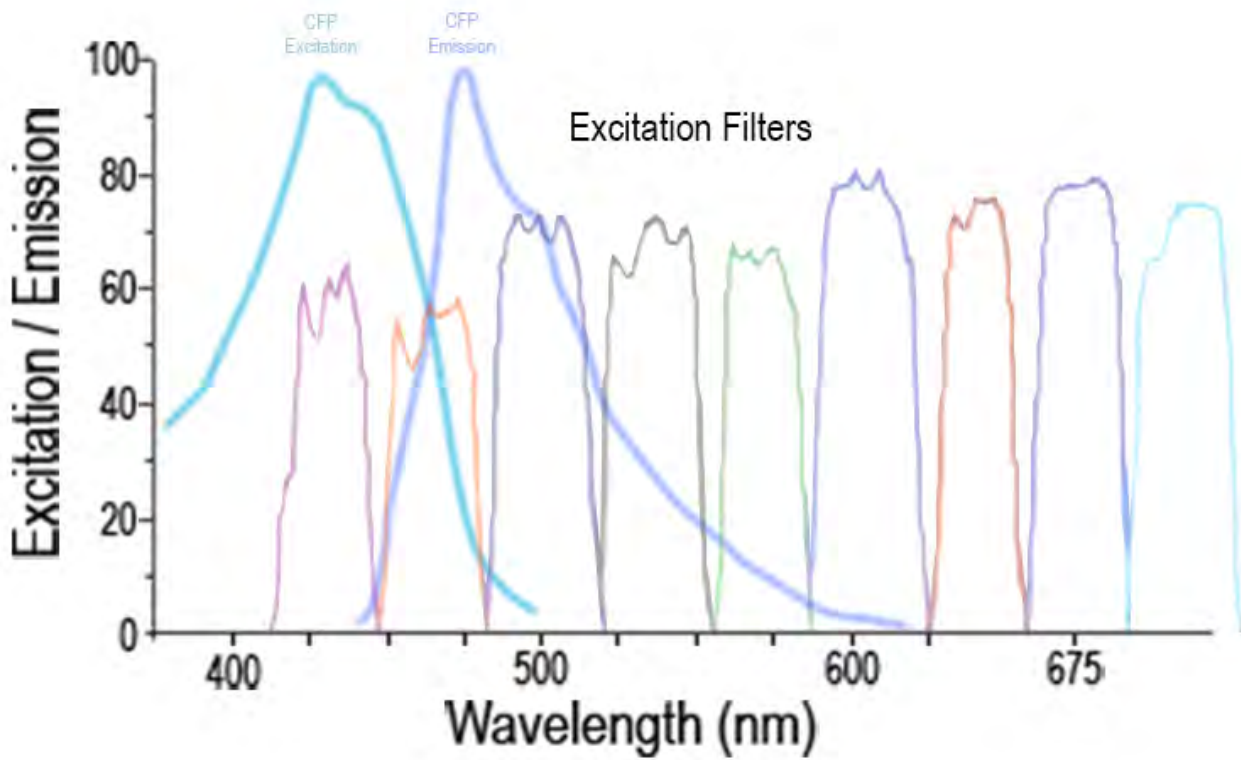
The two curves for CFP (Cyan Fluorescent Protein) are shown, the excitation curve is to the left of the emission curve. While there are 3 filters that cover the excitation curve, the second and third are within the same range as the emission curve. The first filter on the left should be selected.
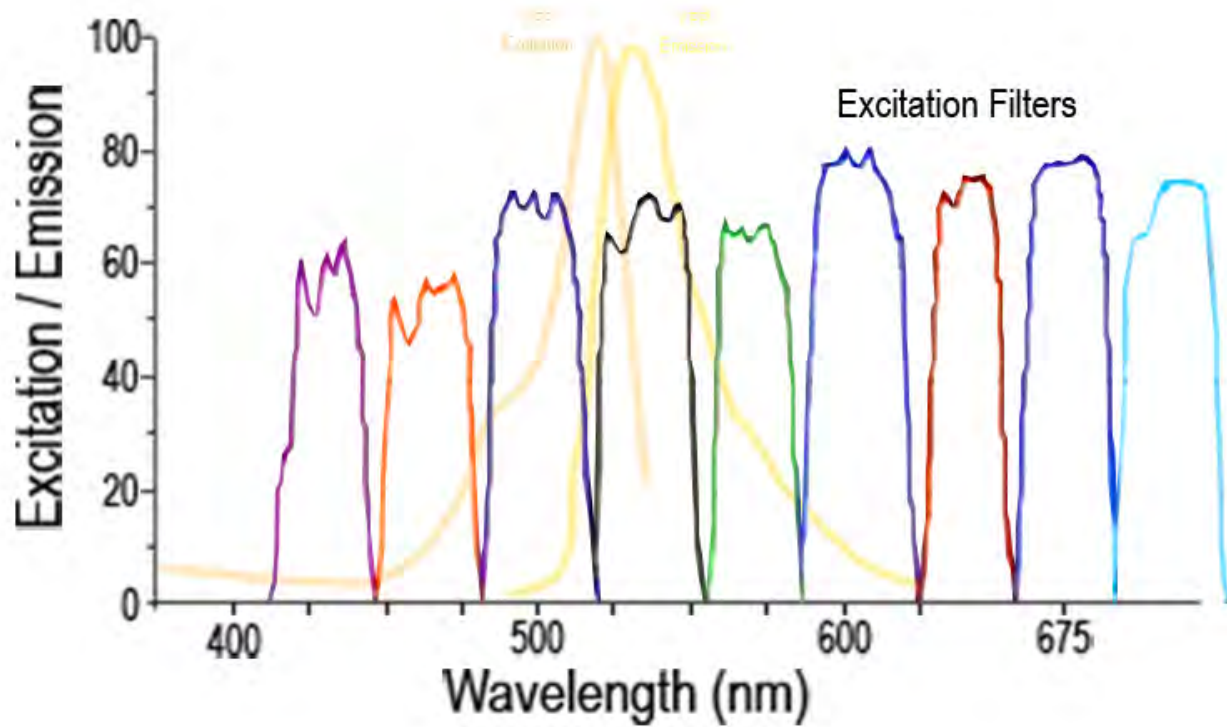
The two curves for GFP (Green Fluorescent Protein) are shown, the excitation curve is to the left of the emission curve. While there are 3 filters that cover the excitation curve, the first is at the lower slope of the curve, the third is within the same range as the emission curve. The second filter on the left should be selected.

The two curves for DsRed (Discosoma Red Protein) are shown, the excitation curve is to the left of the emission curve. While there are 5 filters that cover the excitation curve, the first three are at the lower slope of the curve, the fifth is within the same range as the emission curve. The fourth filter on the left should be selected.

The two curves for YFP (Yellow Fluorescent Protein) are shown, the excitation curve is to the left of the emission curve. YFP is a challenging fluorophore to use, as its excitation and emission curves have a lot of overlap in their peak values. While there are 4 filters that cover the excitation curve, the first two are at the lower slope of the curve, the fourth is within the same range as the emission curve. The third filter on the left should be selected.