

# UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

(<https://biotech.ufl.edu>)

## CTAC / ICBR SOP : Camera Control During a Luminescence Scan Using the IVIS Software Control Panel

**Title:** Controlling the Camera During a Luminescent Scan using the IVIS Software (Living Image™) Control Panel

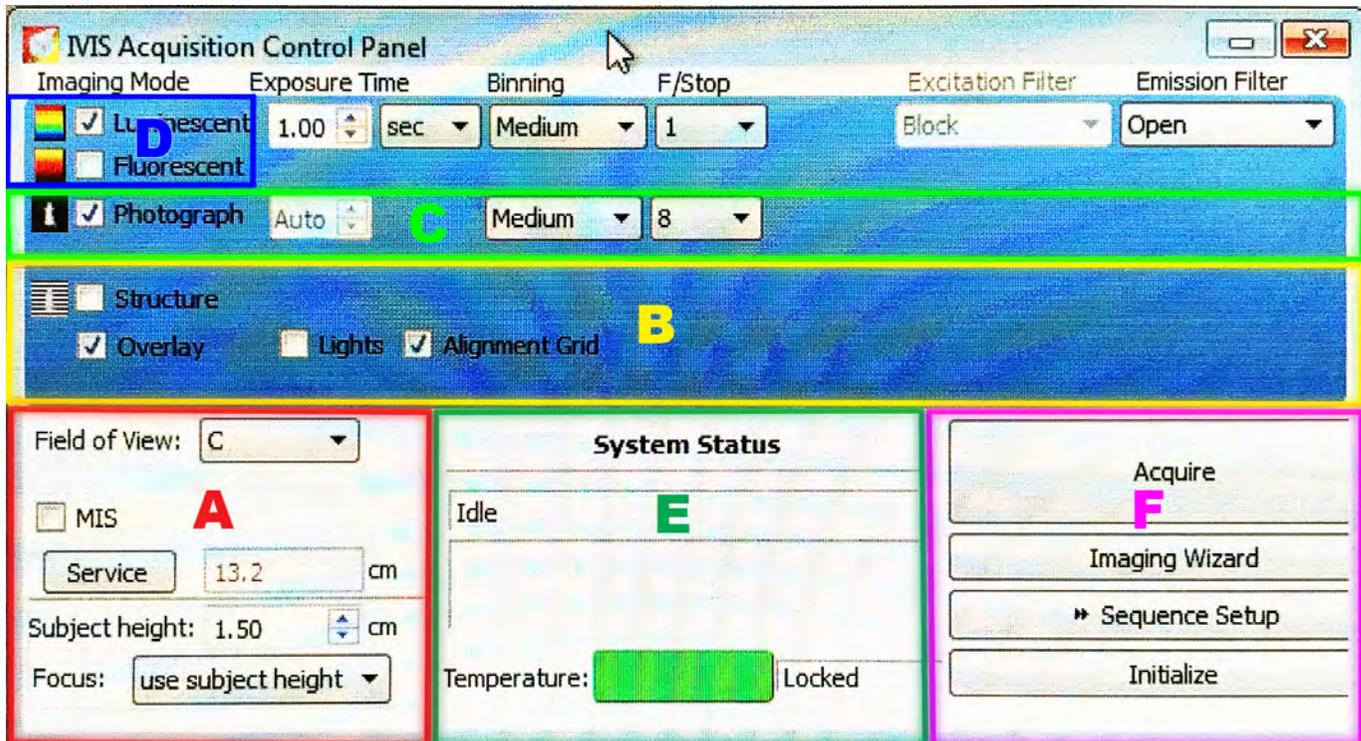
### Materials Required:

- Computer
- Living Image™ software

### Purpose:

To detail the camera controls and actions on the software Control Panel necessary for conducting a scan for fluorescence.

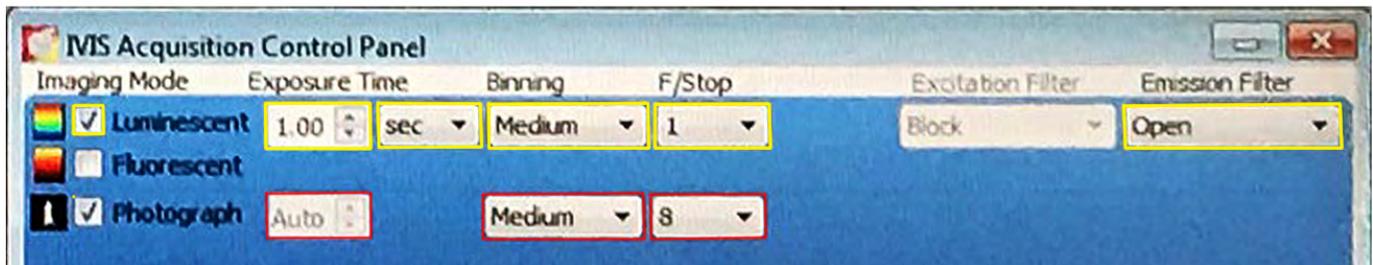
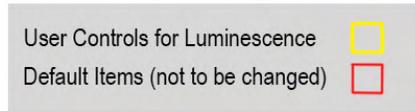
### Software component identification:



The fluorescent setting toggle is found in section D.



To conduct a luminescent scan, the “Luminescent” toggle in section D must be checked.



The luminescence scanning controls comprise the areas noted in yellow.

From left to right:

### **Exposure Time:**

This is the length of time set for the scan by the user. Using the UP and DOWN arrows, the value may be changed by increments of 1.00. More discrete values can be entered manually, by highlighting the numerals intended for change and overwriting them.

The unit of measure may also be changed by toggling the drop down control to toggle between seconds and minutes, dependent on the expected signal strength.

### **Binning:**

This refers to the degree to which an averaging algorithm is applied to the signal data captured. The more that it is applied, the lower the resolution of the data. However, the time of data capture will be shorter with high binning.

The binning algorithm looks at a pixel, and the 8 pixels surrounding it. With low binning (high resolution), this will be 9 bytes of information, detailing color value and position for each pixel.

However, if all 8 pixels surrounding the center pixel are the same color, binning allows a compression where, for all 9 pixels only the center position values are noted, and that the surrounding pixels have the same value. This can be represented as 2 bytes of information. Additionally, it allows for increasing the pixel size.

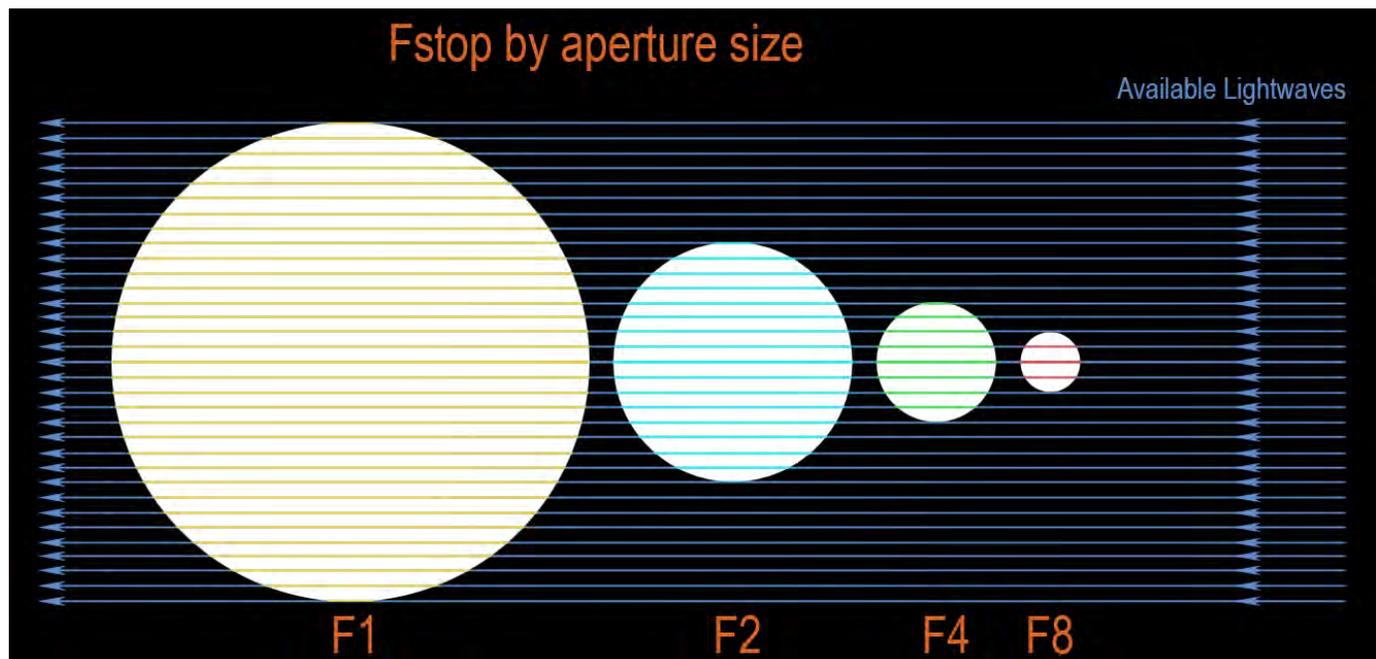
Reduction of data size and increase of pixel size are consequences of high binning, with the advantage of faster scanning and throughput but the disadvantage of resolution and sensitivity.

## F Stop:

This value is a standard photography term to refer to the size of the aperture for the camera, which is like the iris in the human eye. Its function is to admit or reduce the amount of light entering the camera.

A low F Stop value is a wider, more open aperture, which will capture more of the available light. This is useful when the signal strength is too low.

A high F Stop value is a narrower, more restrictive aperture which will capture less of the available light. This is useful when the signal strength is too high.



As can be seen, the capture area is different for each setting. The most open setting of the aperture is F1. All other aperture settings are relative to this standard. Therefore, each is a **Factor (F)** of division relative to F1. F2 is 50% of the capture area of F1. F4 is 25% of the capture area of F1. F8 is 12.5% of the capture area of F1.

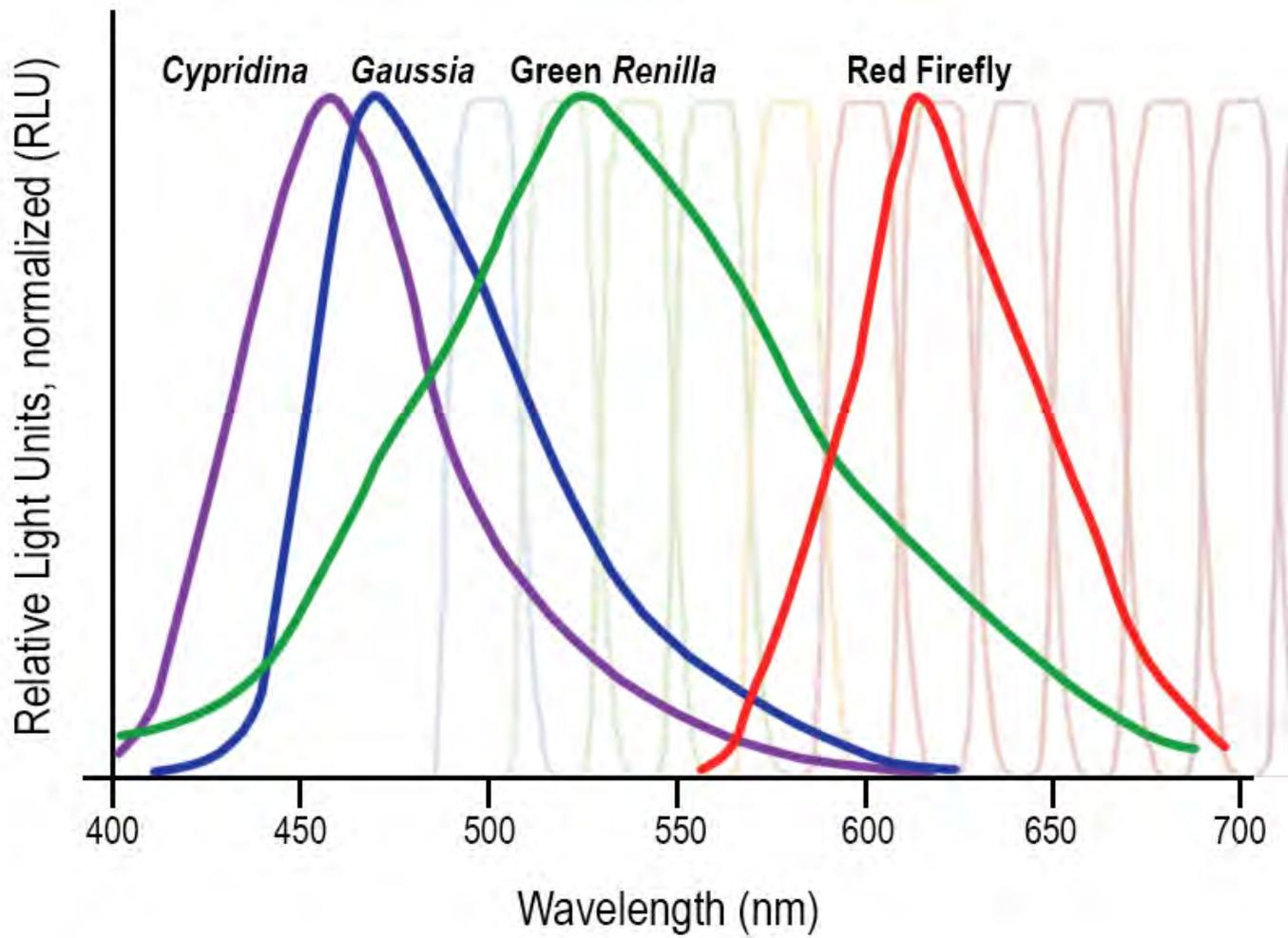
## Excitation Filter

This is not required for a luminescent scan, and will be greyed out and unavailable.

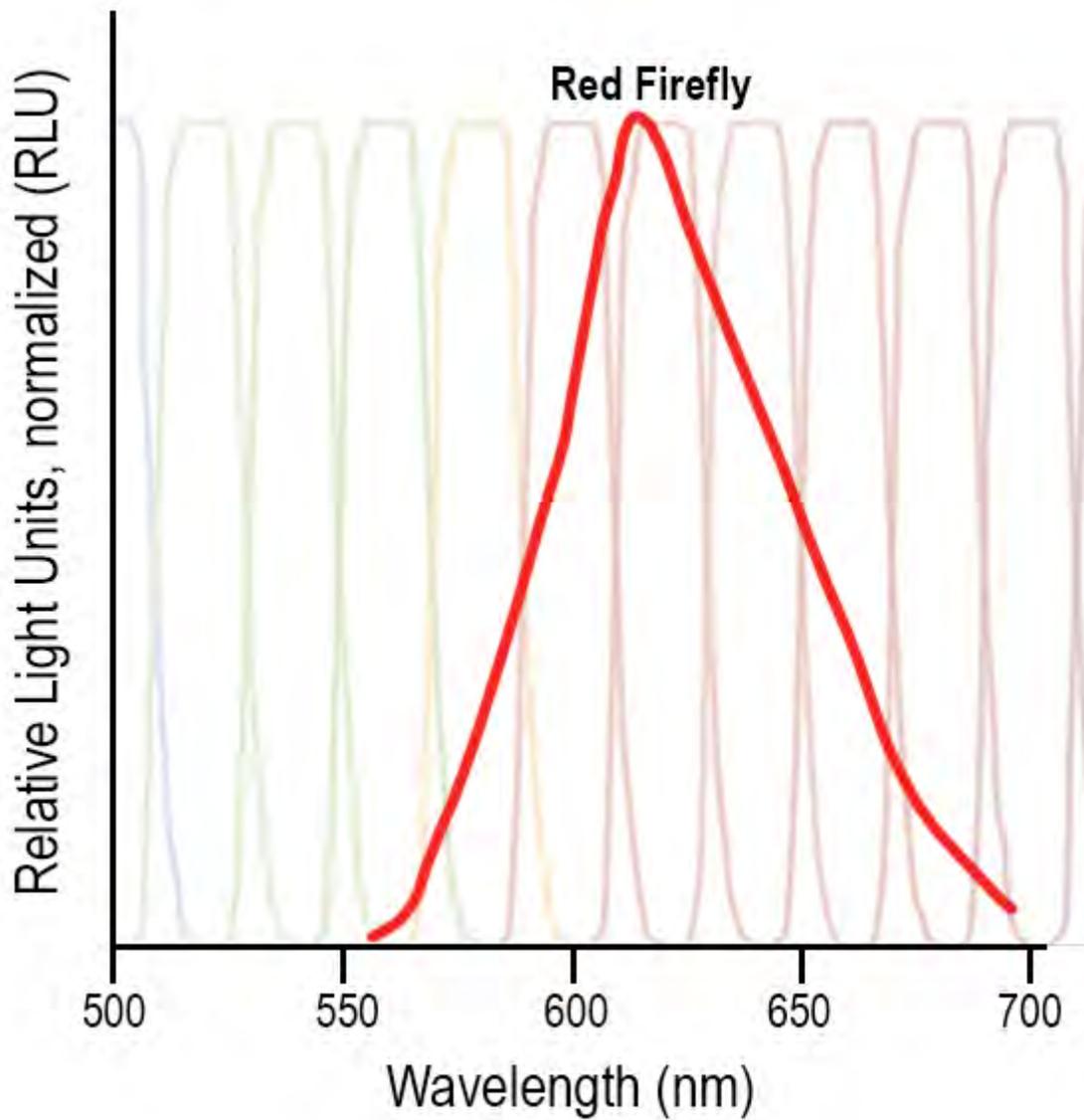
## Emission Filter

The instrument's Emission 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 18 filters with bandwidths of 20 nanometers.

The user will select the filter band most appropriate for capturing the most energy from the luminescent tracer of interest, dependent on its emission curve.

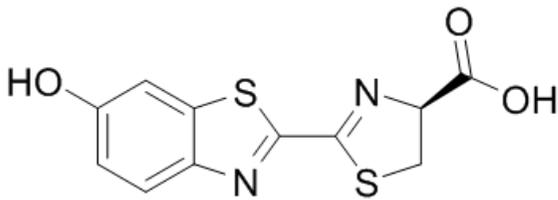


Examples



This figure shows the emission spectrum of red firefly. The filter seventh from the left, at 625nm, is the filter that will capture the most discrete and energetic emissions.

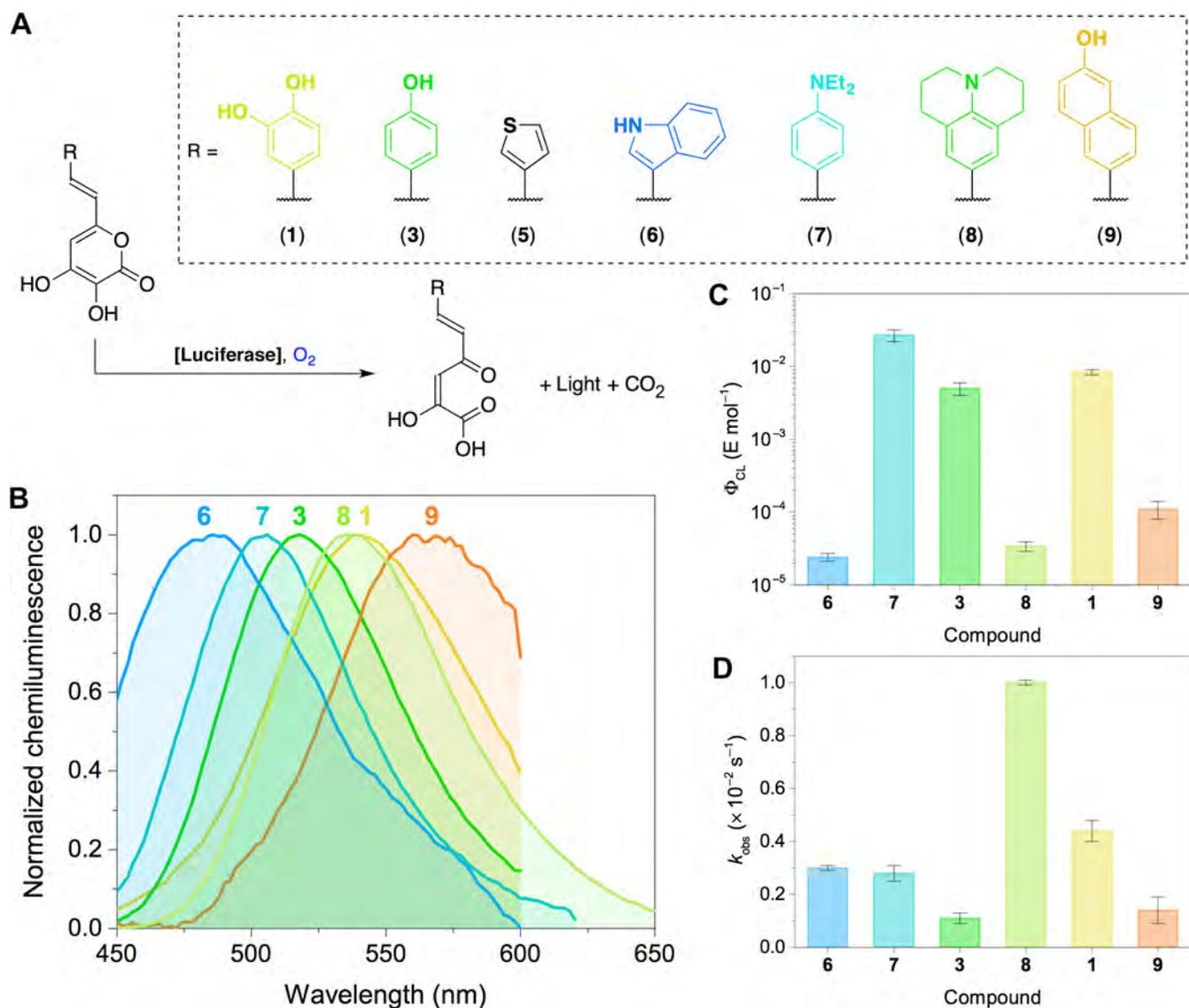
Most luminescence scans are the result of the detection of D-luciferin and its localization to where it is being metabolized.



There are a number of different luciferins available commercially, with modification groups attached that shift their emission curves. Prior to a scan, the product emission optimum setting should be determined from the documentation associated with the product used.



Bioluminescence can also be naturally occurring, and is common in fungi. Different modifications of luciferin may present a number of different spectral values. These are dependent on the modification, and in turn the modification has a varying effect on signal output and intensity.



What to do if you are not provided with the luminescent substrate's emission value?

There are certain conditions where the emission value of the luminescent substrate is not known. These include lost documentation, detection of an unknown luminescent signature, using a research product that has not been commercially characterized, etc. In such an instance, the IVIS Spectrum can be used to assess a subject's full spectrum output and, using this as a benchmark, run a Sequence of differing filter values to determine to most effective for the experiment.

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