

# UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

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

## CTAC / ICBR SOP : Controlling the Camera During a Fluorescent Scan using the IVIS Software Control Panel

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

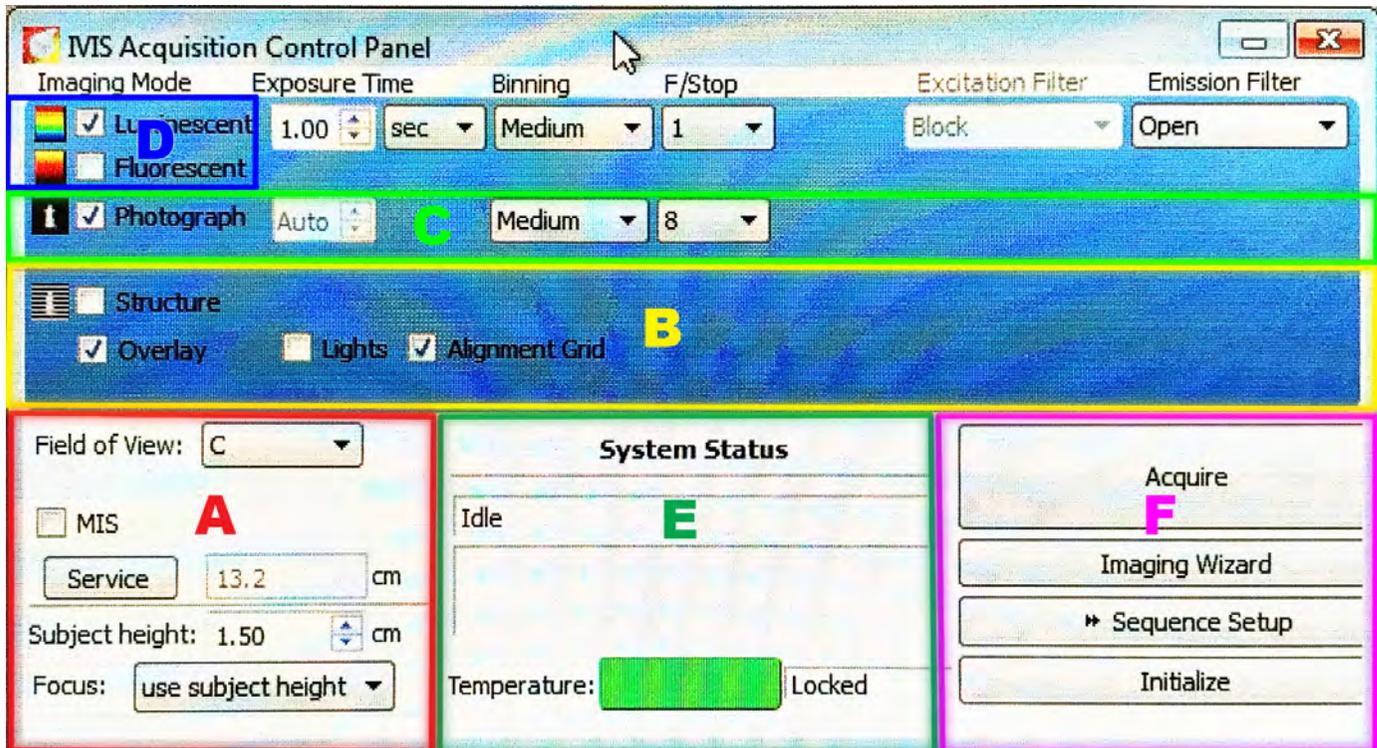
### Materials Required:

- 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:

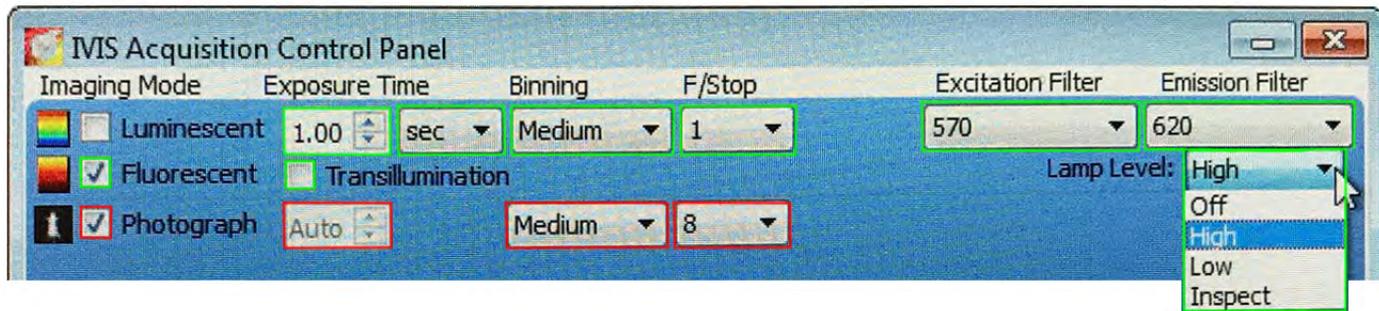
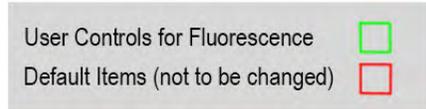


The fluorescent setting toggle is found in section D.



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

The fluorescence scanning controls comprise the areas noted in green.



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.

### Transillumination:

This is the toggle for transillumination, which is where the excitation light source is located below the stage.

### 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.

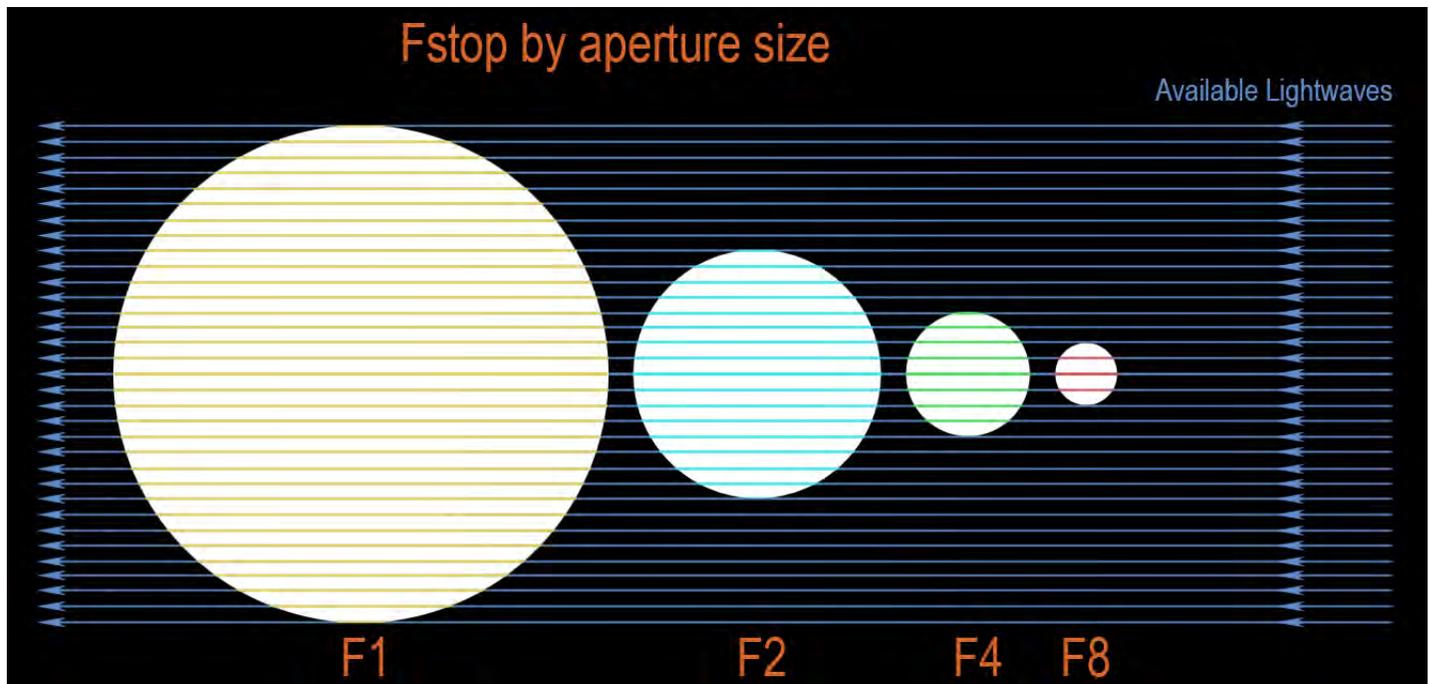
Reduction of data size and increase of pixel size are consequences of high binning, with the advantage of faster scanning and throughput but the disadvantage of resolution and sensitivity.

### 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.

### Lamp Control

The lamp providing the light spectrum can be set to 4 positions, of which 3 are user selectable.

Off – ensures lamp is off

High – ensures lamp setting is on high intensity

Low – ensures lamp setting is on low intensity

Inspect – for instrument service – **CTAC staff only**.

These elements of light control are additionally covered in [CTAC / ICBR SOP Light Control During a Fluorescent Scan \(https://ctac.mbi.ufl.edu/ctac-icbr-sop-controlling-the-light-captured-during-a-fluorescent-scan-using-the-ivis-software-control-panel/\)](https://ctac.mbi.ufl.edu/ctac-icbr-sop-controlling-the-light-captured-during-a-fluorescent-scan-using-the-ivis-software-control-panel/)

## **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.

More detailed information and examples can be found in [CTAC / ICBR SOP Setting the Excitation Filter for a Fluorescent Scan \(https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-setting-the-excitation-filter-for-a-fluorescent-scan-using-the-ivis-software-control-panel/\)](https://ctac.mbi.ufl.edu/ivis-spectrum-how-to-documents-and-sops/ctac-icbr-sop-setting-the-excitation-filter-for-a-fluorescent-scan-using-the-ivis-software-control-panel/)

## **Emission Filter**

The emission filter allows the peak energy being emitted from the subject to be isolated from wavelengths outwith the 20nm span of the filter. This cuts down on any unwanted signaling "noise" or autofluorescence at other wavelengths in the spectrum (signal-to-noise), and increases the fidelity of the data acquired.

The emission filter is used in both fluorescent scans and luminescence scans, as the subject in both cases is emitting photons requiring detection.

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.

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