

UF | ICBR Cytometry

University of Florida, Interdisciplinary Center for Biotechnology Research

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

CTAC / ICBR SOP : Running an Exposure Sequence

Title: Running an Exposure Sequence using the IVIS Software (Living Image™) Control Panel

Materials Required:

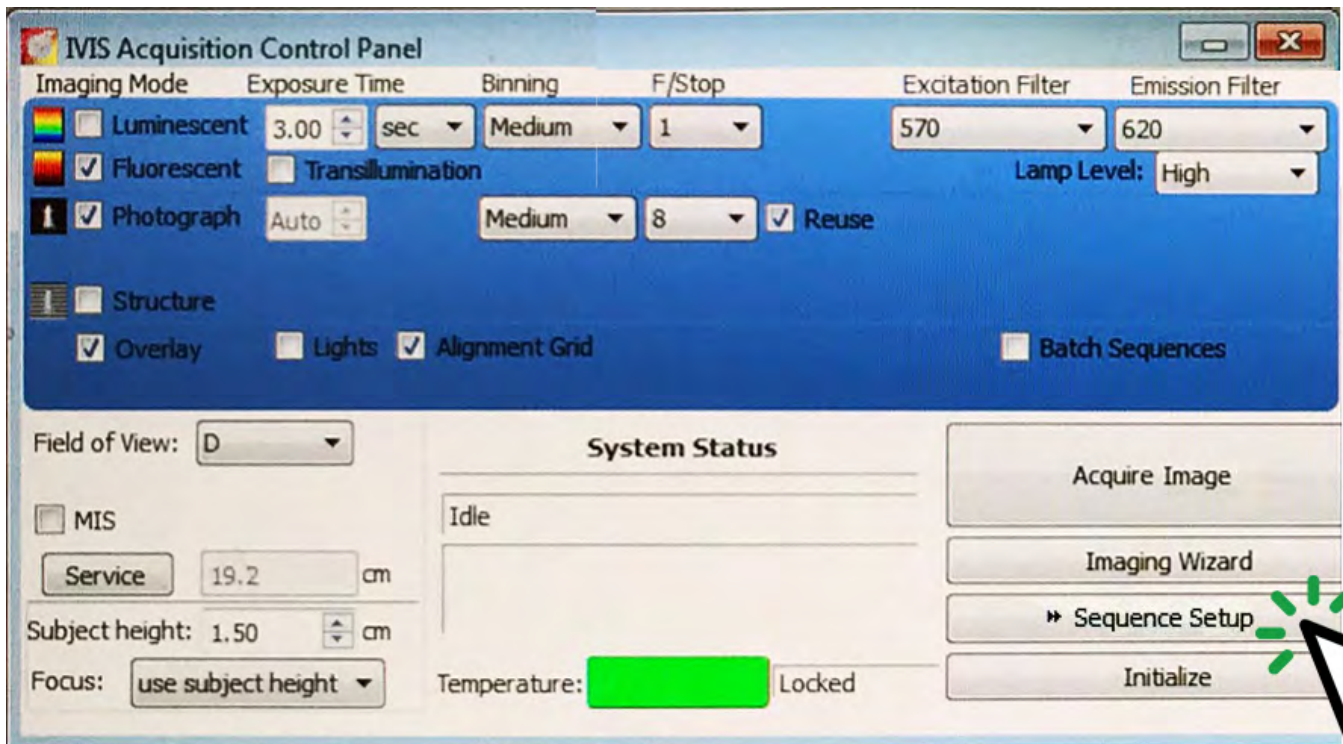
- Experimental subject
- IVIS instrument & Living Image™ software
- Bottle of Isoflurane (anesthetic) as required
- Vaporizer filler tube and screw neck mount as required
- Oxygen source as required

Purpose:

To understand the function and utilize the Living Image™ software in Sequence Mode while controlling the IVIS instrument to Optimize Exposure Time.

Sequence Scanning:

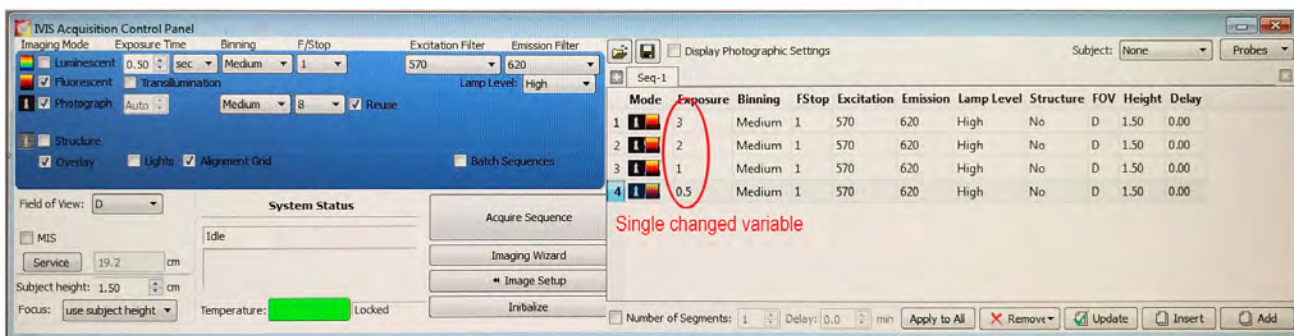
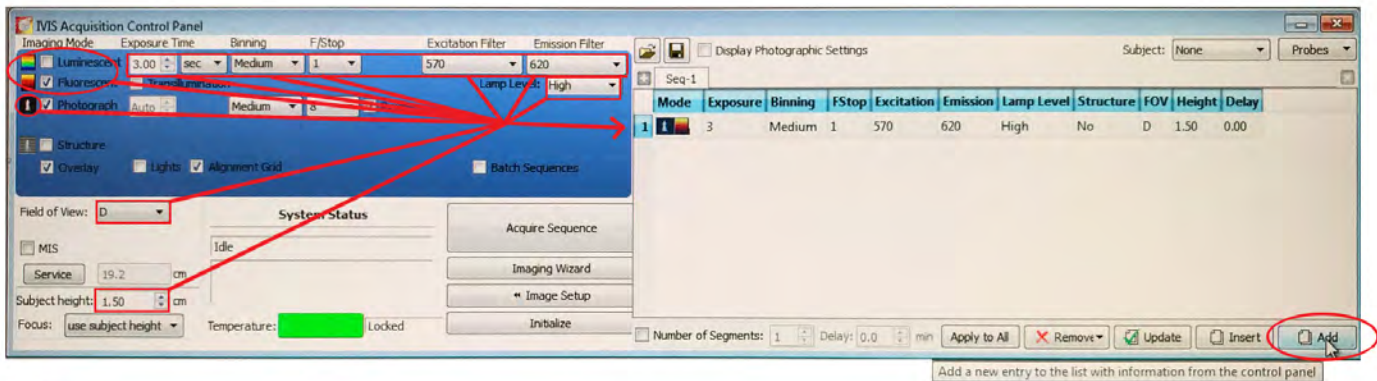
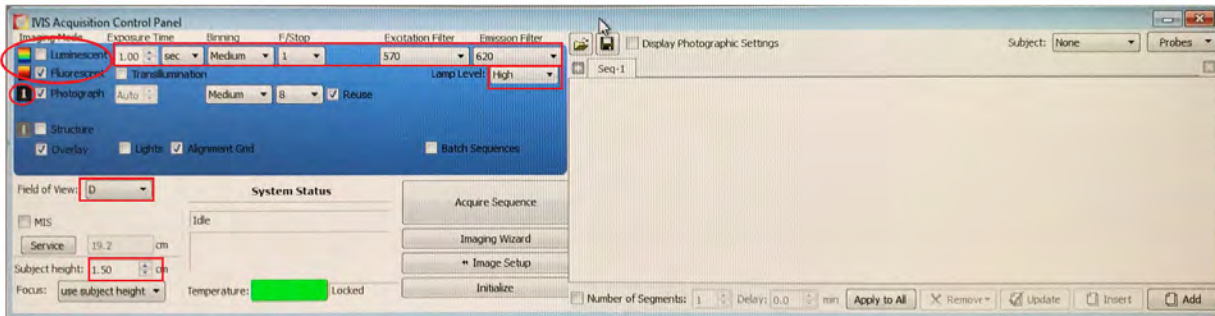
To set the machine into sequential scan mode, the Sequence Setup button should be clicked.



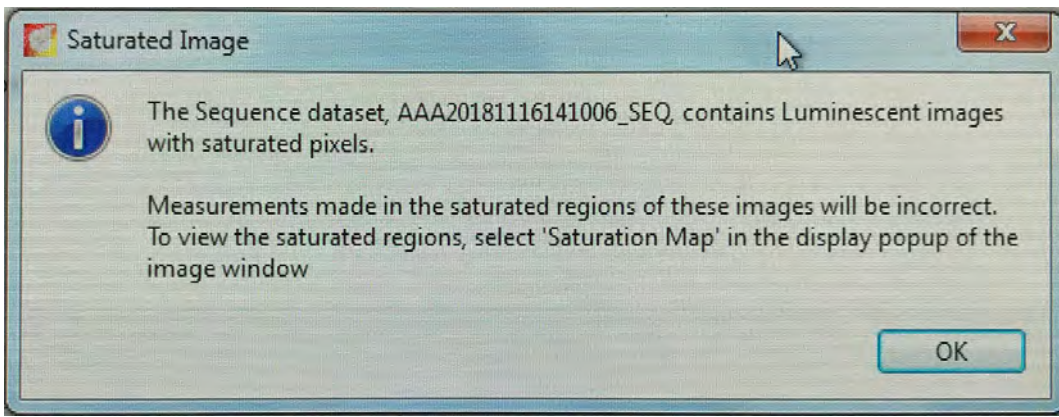
Setting up a sequence can be a valuable diagnostic or optimization tool. A number of identical scans excepting a single variable can be run back-to-back, allowing direct and visual comparison of the effects of parameter change.

A sequence can be used to determine the optimum exposure necessary for the subject to be scanned.

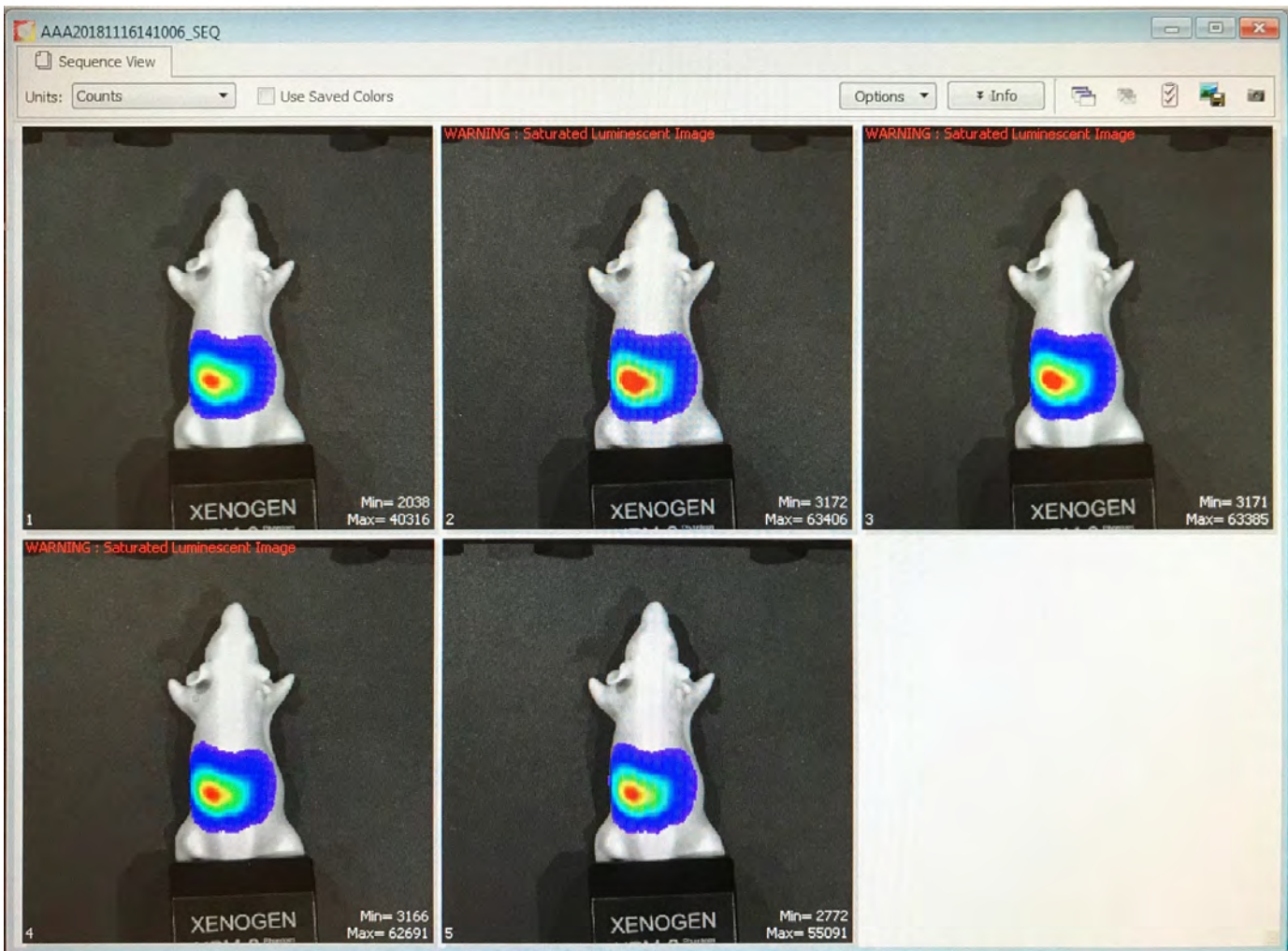
First, the subject should be scanned with the exposure setting to Auto. This is located by hitting the down arrow beside Exposure Time when the display shows 0.00. This will provide a reference image with which to compare additional scans. A range of times may be selected, and using the Add button on the right bottom of the sequence window, each line may be added in turn.



The use of the auto exposure setting as a reference gives a scanned picture, where the instrument has automatically closed the camera once the **maximum quantity of photons that will not saturate the pixels generated** has been reached. The machine does not provide this numerical value, only a generated picture of the scan. Using the sequence function, a number of potential exposure times may be tested in comparison to the auto exposure. Where the exposure is too great, these scans will exhibit saturation of the pixels. There will be a Saturated Pixel warning window that pops up to alert the user.



This sequence of scans is shown below.



This means that the detectors inside the instrument are overloaded from the signal and cannot show the intensity correctly. You will see this message on top of each of the images that are saturated. A lower exposure should be selected. However, the most ideal setting will that exposure that is the highest that may be achieved without pixel saturation.

We can, using the information from the first sequence scan, further refine the next sequence.

When a number of images are acquired during a sequence scan, the full size picture can be seen by double clicking on the image itself.

Optimum exposure is between 0.78s and 0.76s. One additional short sequence with only querying 0.76s (right, as known standard), and 0.77 seconds (left) resolves more accurately to a value of 0.77 seconds as neither displays a saturation warning.

This derived exposure time, if gained using known and confirmed negative and positive controls, can be used consistently through the experimental series or project.