CTAC / ICBR SOP : Running an Emission Filter Sequence

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

Materials Required:

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

Purpose:

To detail the steps necessary to run a sequence of scans using the Control Panel to Optimize the Best Emission filter.

Sequence Scanning:

To set the machine into sequential scan mode, the Sequence Setup button should be clicked.
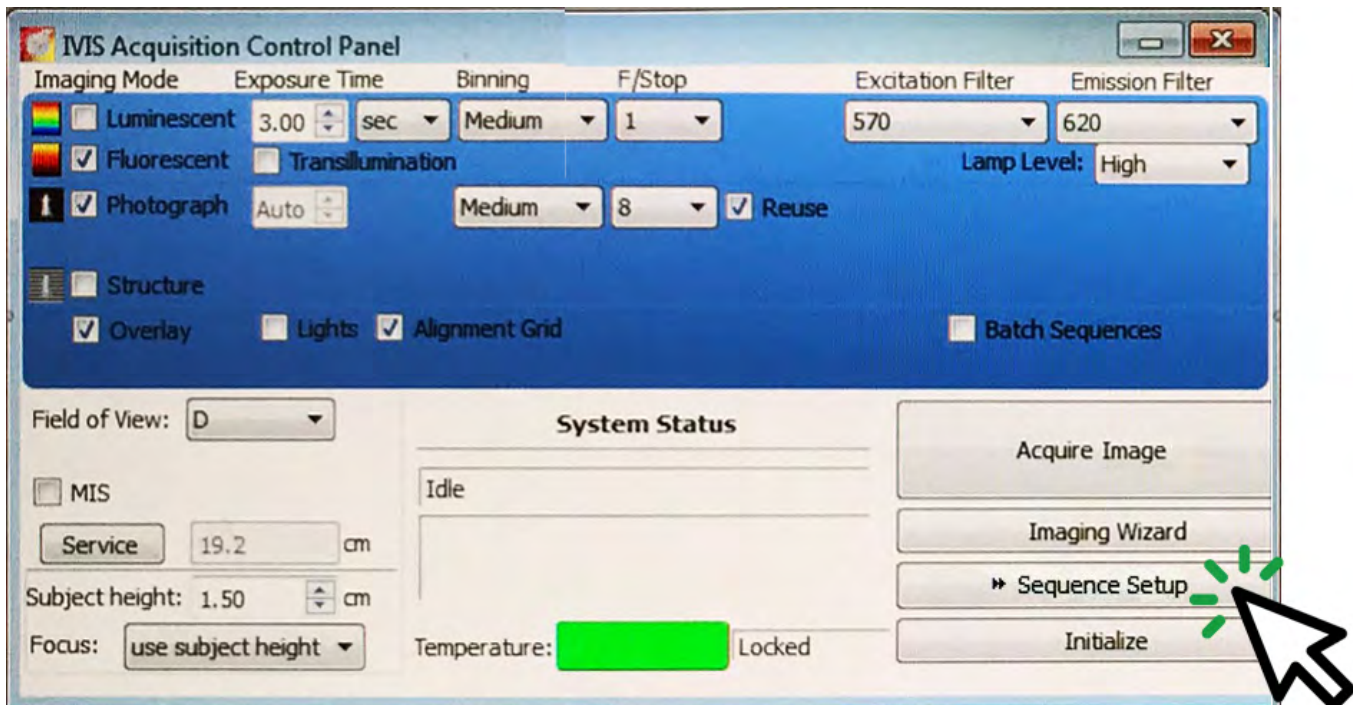
Setting up a sequence can be a valuable diagnostic or optimization tool. A number of identical scans excepting a single variable can be run back-to-back, allowing direct and visual comparison of the effects of parameter change.

Setting up a sequence using the different filters available allows optimization of the emission filter to be used for a given excitation input, whether a biochemiluminescence or fluorescence (requiring excitation).
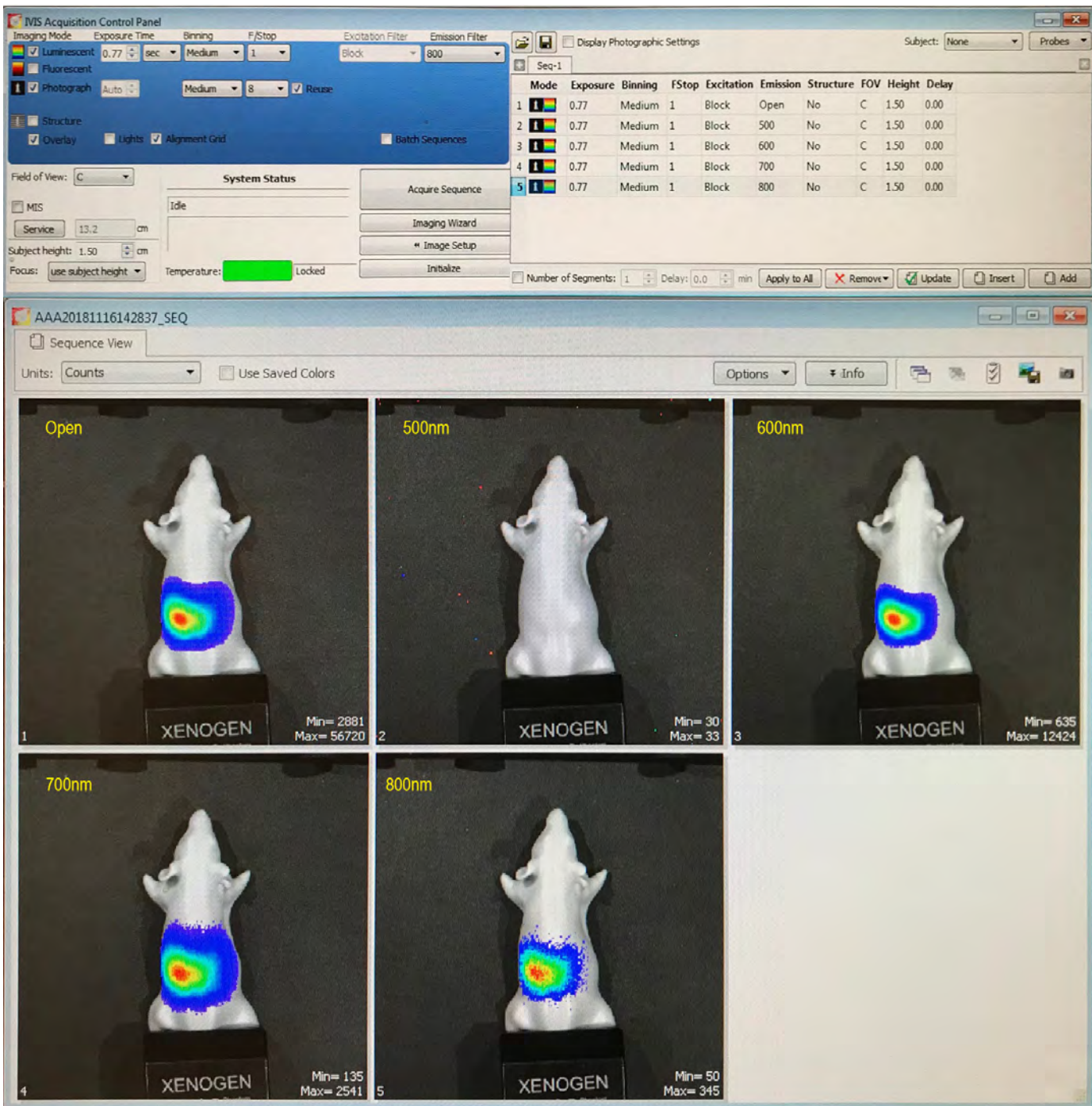
If a fluorophore or a bioluminescent agent is intended for use whose emission values are unknown, the IVIS Spectrum can allow the user to determine the optimal settings.

If the signal to be detected is the result of biochemiluminescence, such as luciferase or firefly, this reaction will sustain its own energy signature as long as the reaction is underway. The enzyme kinetics and time course should be understood for any imaging session, as this type of signal rarely lasts for greater than 25 minutes from inception, and varies over this time course. Consequently, this must be kept into consideration. If the signal is fluorescent, an appropriate wavelength of excitation energy must be delivered to cause the fluorophores to emit photons for detection.

A sequence may be set up to find where the peak values of emitted light are found on the spectrum. All parameters are maintained without change, excepting that of the selected emission filters. These are contrasted against an excitation value of OPEN for a luminescent inquiry, which means that the instrument will detect and record light from across all of the visible spectrum, or the known fluorophore excitation band. If the fluorophore's optimum excitation is unknown, this should be determined before proceeding. An optimization for excitation sequence should be conducted, detailed in SOP – Running an Excitation Sequence

After the base parameters have been copied into the Sequence pane, the emission filter valuesy may be changed (only one at a time!). Recommended is to select a number of well spaced (every $3^{rd} – 4^{th}$ filter) filters to compare against the Open reference line. You will build up several lines, each which will correspond to a scan in the sequence. If the user makes an error on the parameter settings these can be all clicked and directly edited within the sequence pane. There is no need to redo the parameter line.

Once the sequence has been inputted, the Sequence scan can be started by clicking Acquire Sequence on the Control Panel. The resultant scan will have a picture for each sequence line. These can be directly compared to each other to refine further scans. The goal will be to see which of the filters allows the majority of the excitation energy to be emitted, without degradation of the core signal. The user should select the parameters that best approximate the reference parameter setting, and conduct further scanning to refine the excitation filter that targets the fluorophore most efficiently.

IVIS Acquisition Control Panel

	Mode	Exposure	Binning	FStop	Excitation	Emission	Structure	FOV	Height	Delay
1		0.77	Medium	1	Block	Open	No	C	1.50	0.00
2		0.77	Medium	1	Block	500	No	C	1.50	0.00
3		0.77	Medium	1	Block	600	No	C	1.50	0.00
4		0.77	Medium	1	Block	700	No	C	1.50	0.00
5		0.77	Medium	1	Block	800	No	C	1.50	0.00

AAA20181116142837_SEQ

Sequence View

Units: Counts

Open — XENOGEN — Min= 2881 Max= 56720
500nm — XENOGEN — Min= 30 Max= 33
600nm — XENOGEN — Min= 635 Max= 12424
700nm — XENOGEN — Min= 135 Max= 2541
800nm — XENOGEN — Min= 50 Max= 345

Here the first image is the energy returned with no emission filters in place. This encompasses photons from 480 nm all the way to 840 nm. This is our reference. We wish to see a narrow band (20 nm) filter image that preserves as much of this signal as possible. No signal is detected at 500 nm, whereas 600 and 700 nm respectively have considerable signal. 800 nm has signal, but it is more fragmented. By visual inspection, the next sequence to be run should more closely interrogate the 560 – 700 nm with more filters. The 700 nm image shows some degradation of the maximum signal area, and additionally has low non-specific photon acquisition at the periphery, suggesting that at this wavelength and above there will be less available signal.
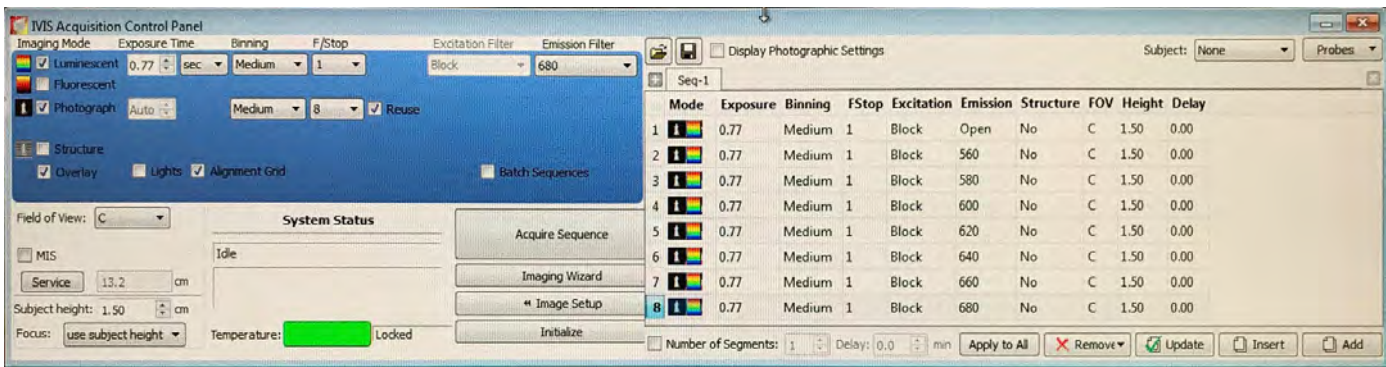
The above window shows the next level of inquiry to further refine the optimum filter band. The resultant scan images should be compared to the OPEN filter value and selected based on a blend of:

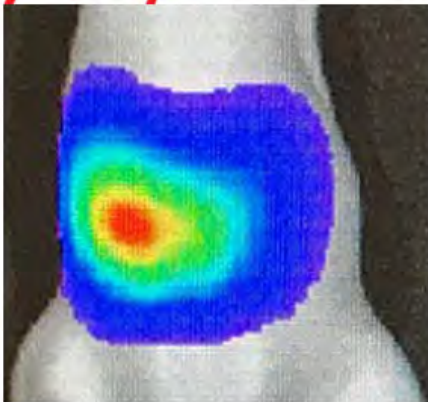Smooth signal gradients versus fragmented / pixelated boundaries
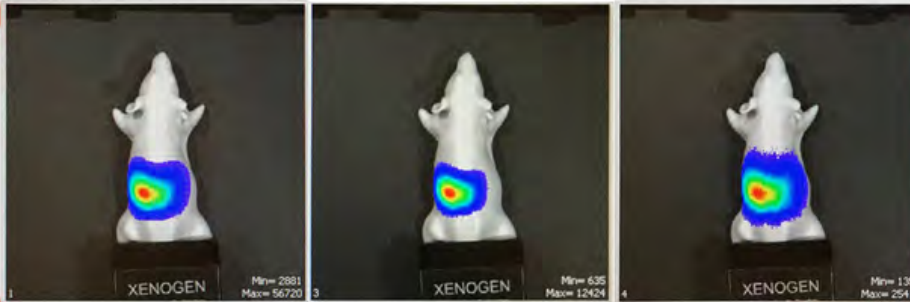
Adherence to versus departure from core intensity

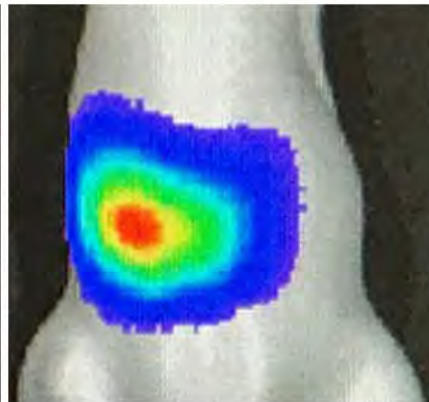Less versus more low threshold signal.
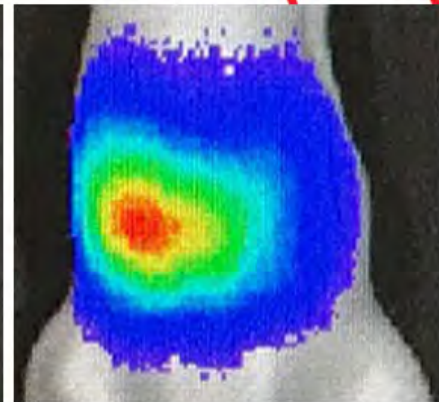

Reference: OPEN filter   600nm   700nm

Reference

Comparison to ref:
Good smoothness in gradient
Core signal well preserved
Minimal minimum threshhold signal

Comparison to ref:
Poor smoothness in gradient
Core signal diffuse / fragmented
Plentiful minimum threshhold signal

The instrument defaults different color scales for fluorescence and luminescence. The color scale for fluorescence is defaulted to "Red/Yellow", which is a gradient between just two colors. The default color scale for luminescence is "Rainbow", which is a five color gradient (shown above). As a generality, using multiple colors in the color scale gradient allows the assessment detailed above to be more visually accurate. The IVIS Spectrum system has a number of different color scales available for use, and all may additionally be reversed. Commonly, a multiple color scale will be used to optimize the scanning conditions, after which the color scale used will be more dictated by the needs of the user and the experiment.

When a useful sequence is generated, it may be saved as a .seq file and can be imported to use on multiple occasions. Several laboratories maintain sequence files for quick optimization of their studies.