



## 2D Bioluminescence Imaging (IVIS Spectrum)



*IVIS Spectrum control panel provides the image acquisition functions.*

### Turn on Sequence

1. Log into computer using netID and password
2. Start Living Image Software.
3. Input user ID: **./zeiss** and password *Spectrum1*
4. Initialize Software (1).
5. Wait until camera temperature box turns green (~ 7 min). The default temperature is 37°C
6. Open chamber and make sure black plastic mat is in place or use a sheet of black Artagrain paper instead. If imaging plants, use the black plastic mat intended for plants.

### Quick Image Acquisition

1. Put specimen in chamber.
2. Put a check mark next to Luminescent (3) and select Auto exposure (4).
3. Checking Luminescent automatically sets Excitation filter to *Block* and Emission Filter to *Open*.
4. Select a Field of View, FOV (5). A-D represent progressively larger FOVs that you can visualize by checking the alignment grid inside the chamber. Make sure Alignment Grid is ticked in the control panel (6).
5. Set Subject height (7).
6. Click Acquire (8).

- For the first image of the session, you are prompted to enable the autosave function. When this is enabled, all images acquired during the session are automatically saved to a user-selected location. You can also choose to manually save images.
- Enter whatever information you want in the dialog box that appears.
- An image window will appear when acquisition is finished. Units (9) are displayed as Counts or Radiance.



Image window displays an overlay of photograph and luminescence with features relevant to an acquired image. The Units and Display option tools are zoomed in.

## Shut down

- If data was saved in the D drive, copy/transfer to the fileshare.
- Exit Living Image program.
- Clean black mat (or dispose artagain sheet) and induction chamber with disinfectant wipes and follow shut down procedure for anesthesia (if applicable). **Do not spray alcohol inside chamber!**
- Log out of computer.

## Field of View (FOV) Settings

FOV Setting	Imaging area, cm <sup>2</sup>
A	4
B	6.5
C	13
D	22.5

## Temperature Box Colors

Color	Size of stage area, cm
	System is not initialized.
	System is initialized, but CCD camera temperature is out of range and not ready for imaging.
	System is initialized and CCD camera is at or within acceptable range of the demand temperature and locked. The system is ready for imaging.

## Control Panel Settings

Parameter	Description
Exposure time	The length of time that the shutter is open during image acquisition
Binning	Controls pixel size on the CCD camera. Large binning increases pixel size & sensitivity, but reduces spatial resolution. Small binning = less sensitivity, higher resolution Recommended binning: 1-4 (Small) for imaging of cells or tissue sections, 4-8 (Medium) for in vivo imaging of subjects, and 8-16 (Large) for in vivo imaging of subjects with very dim sources.
F/Stop	Sets the size of the camera lens aperture, which controls the amount of light detected and the depth of field. <ul style="list-style-type: none"> <li>larger f/stop = smaller aperture = lower sensitivity</li> </ul>
Excitation Filter	For luminescent imaging, <i>Block</i> is selected by default.
Emission Filter	For luminescent imaging, the <i>Open</i> position (no filter) is automatically selected by default.
Lights	Turns on the lights located at the top of the imaging chamber
Alignment Grid	Shows the sizes and positions of the possible fields of view
Field of View	Width of the square area (cm) to be imaged. A smaller FOV gives a higher sensitivity measurement
Subject height (cm)	Distance above the stage that you are interested in imaging. E.g. for a mouse leg joint, subject height = a few mm; for uppermost dorsal side of a mouse, set subject height to 1.5 - 2.0 cm. The default subject height is 1.5 cm.