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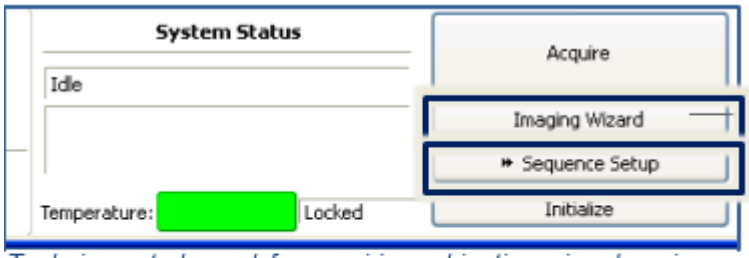
Bioluminescence Kinetic Series (IVIS Spectrum)

2D Image Sequence Acquisition

For quantitative imaging, the photon flux will be dependent on the time after luciferin injection. Thus, it is recommended that you acquire a **kinetic image series** and use the image with maximal radiance values for analysis (usually occurs ~ 10 min post-injection).

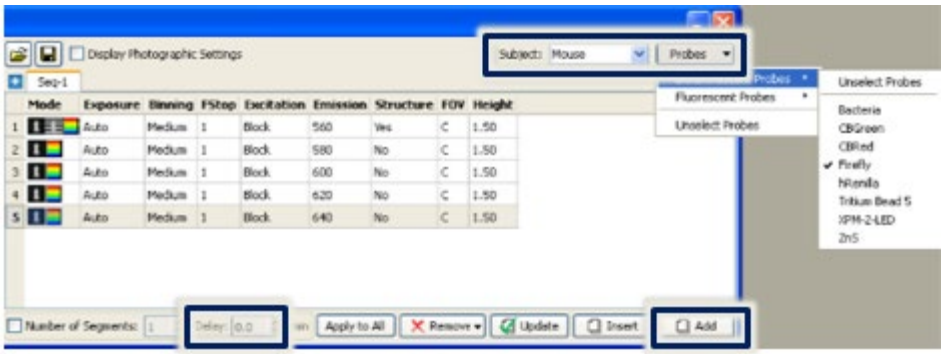
Manual Setup

1. Confirm that the IVIS Spectrum is initialized and the CCD temperature is locked.
2. Have available D-luciferin stock solution at 15 mg/ml in sterile PBS.
3. Setup kinetic series by selecting Sequence Setup.



Tools in control panel for acquiring a kinetic series: Imaging Wizard provides a step-by-step to create optimal sequences for bioluminescent imaging. The Sequence Setup option allows the user to manually set up image sequence parameters.

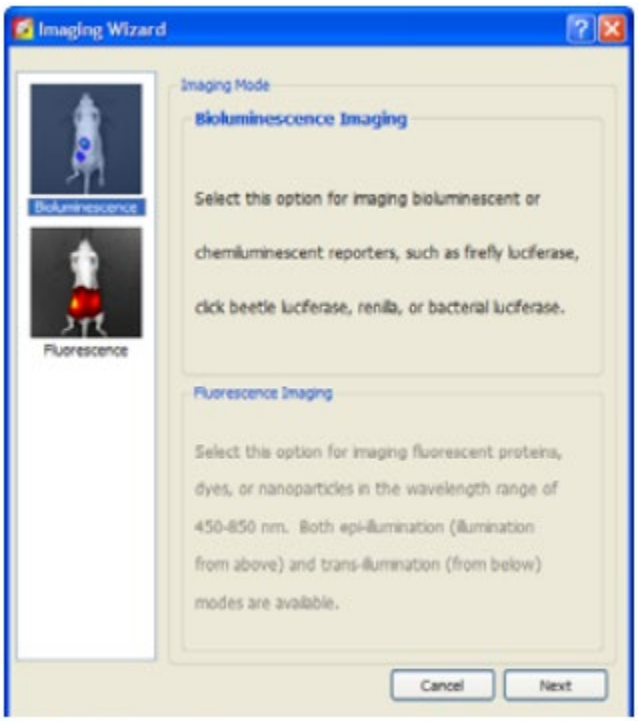
4. A sequence editor appears.
5. Choose a subject and probe from the drop-down list.
6. Specify image settings for the first image in the sequence.
7. Click *Add* button.
8. Repeat steps 5 and 6 until you have around 10 images, while adjusting parameters as needed.
9. Set a time delay between each acquisition: enter a time (minutes) in the Delay box in the sequence table. 2-minute intervals is a good start.
10. After setting up your series, induce mouse (or mice). Weigh each mouse and inject IP with 150 mg/kg D-luciferin (10 μl stock solution/g mouse)
11. Turn on imaging flow and transfer mouse to imaging chamber. If imaging multiple mice, put baffles in between.
12. Acquire Sequence.
13. Save images if autosave was not previously enabled.



The Sequence Editor is used to manually setup/add/edit parameters settings for an image sequence, including choosing a subject and probe from drop down lists. The Add and Delay buttons are highlighted.

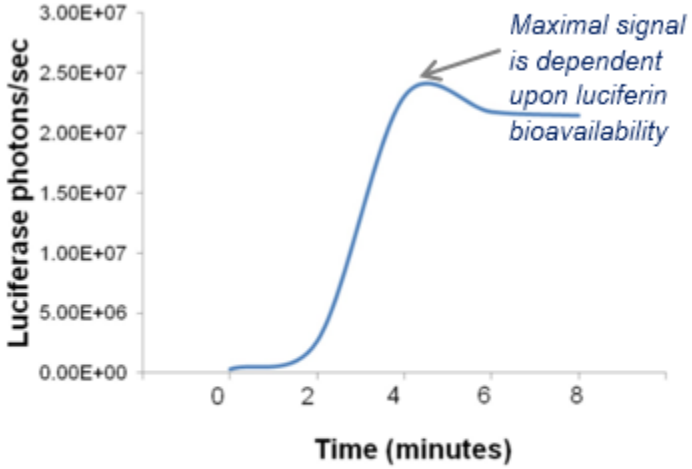
Setup via Imaging Wizard

1. Have available D-luciferin stock solution at 15 mg/ml in sterile PBS.
2. Setup kinetic series by selecting Imaging Wizard in the control panel.
3. Click Bioluminescence in the Imaging Wizard tool, then, click *Next*.
4. Choose the type of image sequence to acquire. Open Filter is the default option.
    - Open Filter: Acquires a luminescent image at maximum sensitivity
    - Spectral Unmixing: Acquires an image sequence for analysis using the Spectral Unmixing tools to analyze images when more than one reporter is used in the same animal (or plant) model.
    - DLIT: for 3D image reconstruction
5. Step through the rest of the wizard. Each page of the wizard guides you with step-by-step instructions and descriptions.
6. When you finish the wizard, it sets up the sequence to acquire in the Sequence editor.
7. After setting up your series, induce mouse (or mice). Weigh each mouse and inject IP with 150 mg/kg D-luciferin (10 µl stock solution/g mouse)



To set up an image sequence, step through the Imaging Wizard

8. Turn on imaging flow and transfer mouse to imaging chamber. If imaging multiple mice, put baffles in between.
9. Acquire Sequence.
10. Save images if autosave was not previously enabled.
11. Take note of time post-injection where luminescence is at maximum. For subsequent imaging of similar specimens, wait this amount of time before imaging.



Kinetic plot shows a maximum radiance value (photons/sec) obtained at a certain time post-injection that can be used for subsequent image acquisition and analysis