Perkin Elmer *In Vivo* Imaging System - Spectrum

Standard Operation Protocol

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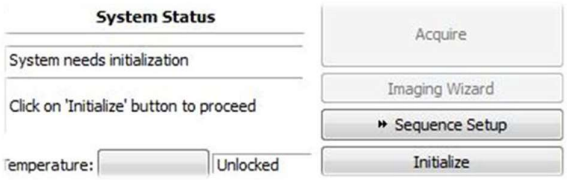
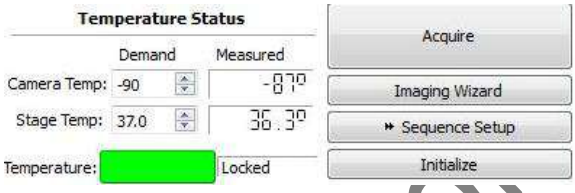
General Start-up Procedure

1. Sign on log sheet once you enter the Small Animal *In Vivo* Imaging Room.
2. Clean the Lab Benches, Sample Mat and Imaging Chamber with 70% Ethanol. Switch on the Air Purifier.

3. Switch on the monitor of the computer connected with IVIS Spectrum. (The computer and the software (Living Image 4.4) should be always on, shutdown/restart of the system or software is not recommended)
   If the software is incidentally closed, launch **Living Image 4.4** and Log in as CLS (Caliper Life Science) and then Initialize the system. Wait until system "Idle"

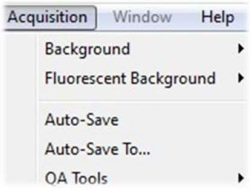


4. Check the camera's temperature by clicking the green/red status box. The system is ready to use when status box is green, i.e the camera temperature ≤-87°C. Otherwise, wait unit system is ready.

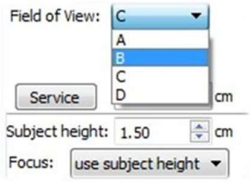


5. Prepare your sample during waiting. ** For animal Specimens, please make sure all the animal handling procedures are CULATR-approved and comply with L.A.U regulation.

6. Select Acquisition>Auto Save To
   Select Destination for saving image data as your folder in D: Drive> User>



7. In Field of View in the control panel, choose a calibrated position/area of the sample stage
   - A: 4 x 4 cm; (Closest to the Camera; Highest sensitivities)
   - B: 6.5 x 6.5 cm;
   - C: 1 3 x 13 cm;
   - D: 22.5 x 22.5 cm; (Farthest from the camera; Lowest sensitivities)



8. When the specimen is ready, open the Imaging Chamber Door.
9. Place the Specimens or anesthetized animal on the sample mat (use Perforated Plate whenever 3D Trans-Illumination Fluorescent acquisition is needed; otherwise use the 30cm x 30cm Sample Mat) and within the green laser grid on the stage. The cross indicates the center of the imaging area.
10. Close the Imaging Chamber Door.

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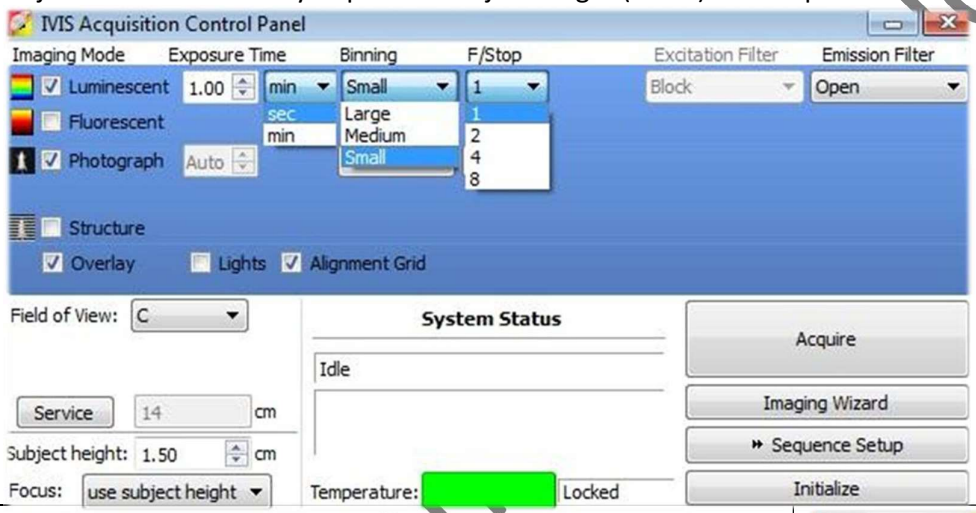
Basic Operation for 2D Bioluminescence Imaging

1. In control panel, check the box of Luminescent image
   Set up the acquisition parameters automatically or manually
   a. For automatic set-up, select Auto in Exposure box, the software will determines exposure time, binning and F/stop setting,
   b. To manually set up,
      Adjust Exposure Time (0.5 sec to 5 min) according to the signal intensity
      Adjust Binning if necessary.
      Small (4) for High resolution; Low sensitivity;
      Medium (8);
      Large (16) for Low resolution; High sensitivity;
      Adjust F/stop if necessary. 1 for large aperture; 8 for small aperture
      Adjust Focus if necessary. Input the subject height (in cm) of the specimen.



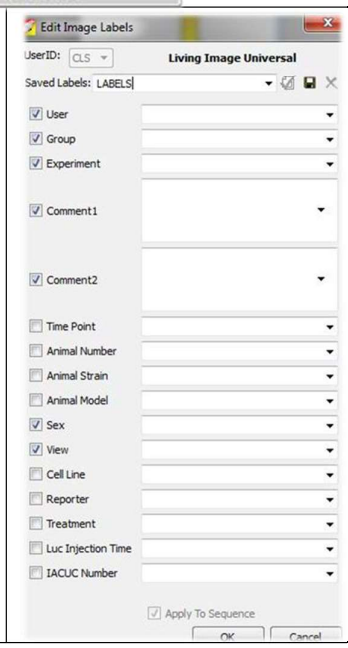
2. Check the box of Photograph for a Bright field image

3. **Acquire** to capture the Photography and Luminescent Image

4. During the Acquisition, fill in the Sample information and click OK.

5. Save the data in D:Drive>User>Your Folder if Auto Save is not activated.

6. Perform Image Display Adjustment, ROI Measurement, etc. analysis in the offline software (Please reserve Spectrum Offline session in FCF Online Booking System; Free of Charge) according to the Living Images analysis Protocol.



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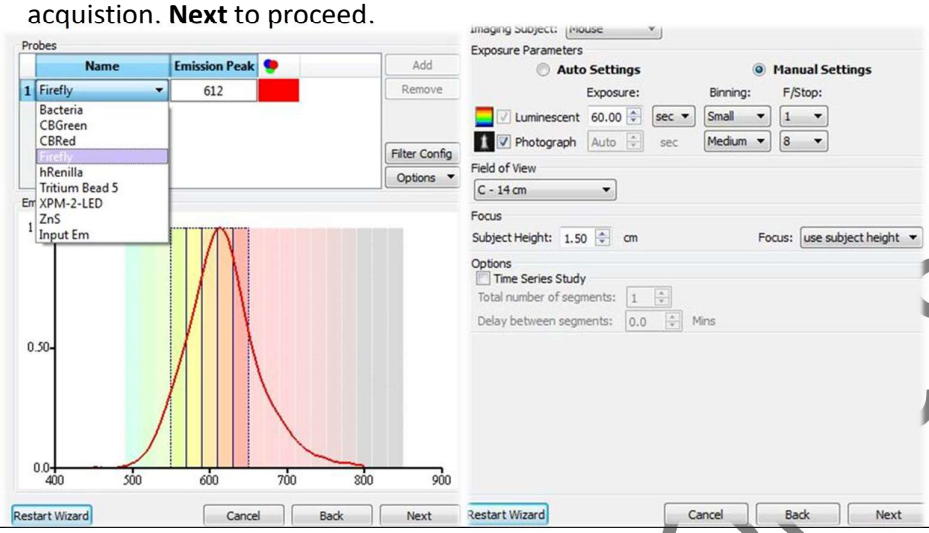
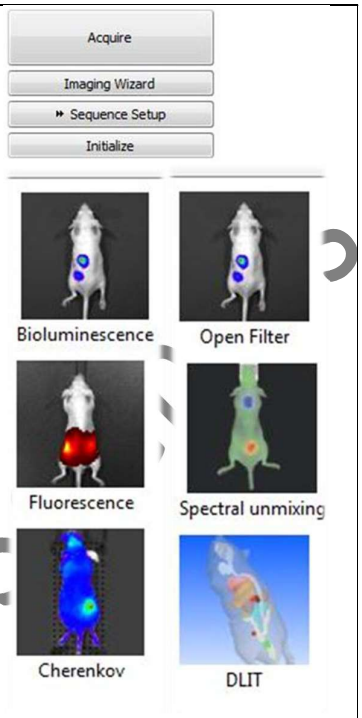
Basic Operation for 3D Bioluminescence Imaging

1. Launch Imaging Wizard. **Restart Wizard** if necessary. Select Bioluminescence and then Diffuse Light Tomography (DLIT).

2. Choose an appropriate probe (e.g. Firefly Luciferase) and Emission filters. 5 filters is recommanded for accurate estimation of the signal depth. Use filters with longer wavelength (660nm & 680nm) for weak signal acquistion. **Next** to proceed.



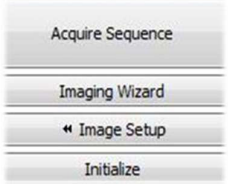


3. Select Image Subject (Mouse/ Plate/Other).
4. Set up the acquisition parameters automatically or manually
   a. For Auto Settings, select Auto in Exposure box, the software will determines exposure time, binning and F/stop setting,
   b. For Manual Settings,
      Adjust Exposure Time according to the signal intensity
      Adjust Binning if necessary.
      Adjust F/stop if necessary.
5. Select appropriate Field of View.
   Adjust Focus if necessary. Input the subject height (in cm) of the specimens.
6. **Next** to generate the Image Sequence. **Acquire Sequence** to capture all Images in the sequence window.

7. During acquisition, fill in the Sample information and click OK.

8. Save the data in D:Drive>User>Your Folder if Auto Save is not activated.

9. Perform Image Display Adjustment, ROI Measurement, 3D Image Reconstruction, etc. analysis in the offline software (Please reserve Spectrum Offline session in FCF Online Booking System: Free of Charge) according to the Living Images analysis Protocol.



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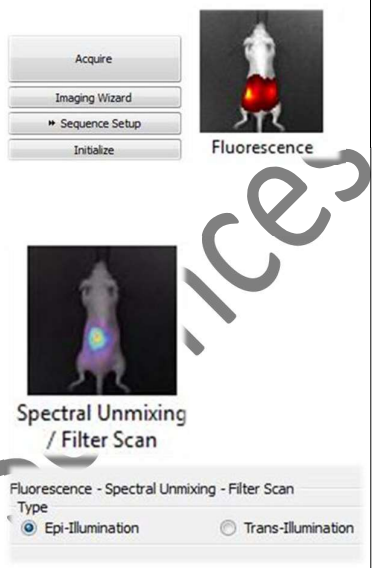
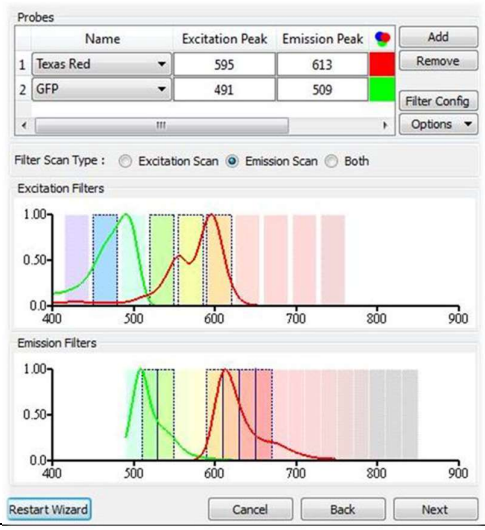
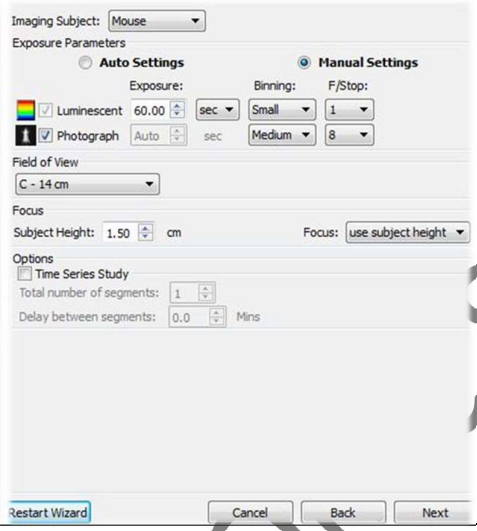
Basic Operation for 2D Fluorescence Imaging (Epi-illumination)

1. Launch Imaging Wizard. **Restart Wizard** if necessary. Select Fluorescence and then Spectral Unmixing/ Filter Scan. Choose Epi-illumination and **Next**.
2. Choose an appropriate probe/fluorophore(s), **Add** if using more than one probe . Select suitable Excitation and Emission filter(s) for optimized detection or spectral unmixing. **Next** to proceed.







3. Select Image Subject (Mouse/ Plate/Other).
4. Set up the acquisition parameters automatically or manually
   a. For Auto Settings, select Auto in Exposure box, the software will determines exposure time, binning and F/stop setting,
   b. For Manual Settings,

      Adjust Exposure Time according to the signal intensity
      Adjust Binning if necessary.
      Adjust F/stop if necessary.

5. Select appropriate Field of View.

   Adjust Focus if necessary. Input the subject height (in cm) of the specimens.

6. **Next** to generate the Image Sequence. **Acquire Sequence** to capture all Images in the sequence window.
7. During acquisition, fill in the Sample information and click OK.

8. Save the data in D:Drive>User>Your Folder if Auto Save is not activated.

9. Perform Image Display Adjustment, ROI Measurement, Spectral Unmixing etc. analysis in the offline software (Please reserve Spectrum Offline session in FCF Online Booking System; Free of Charge) according to the Living Images analysis Protocol.

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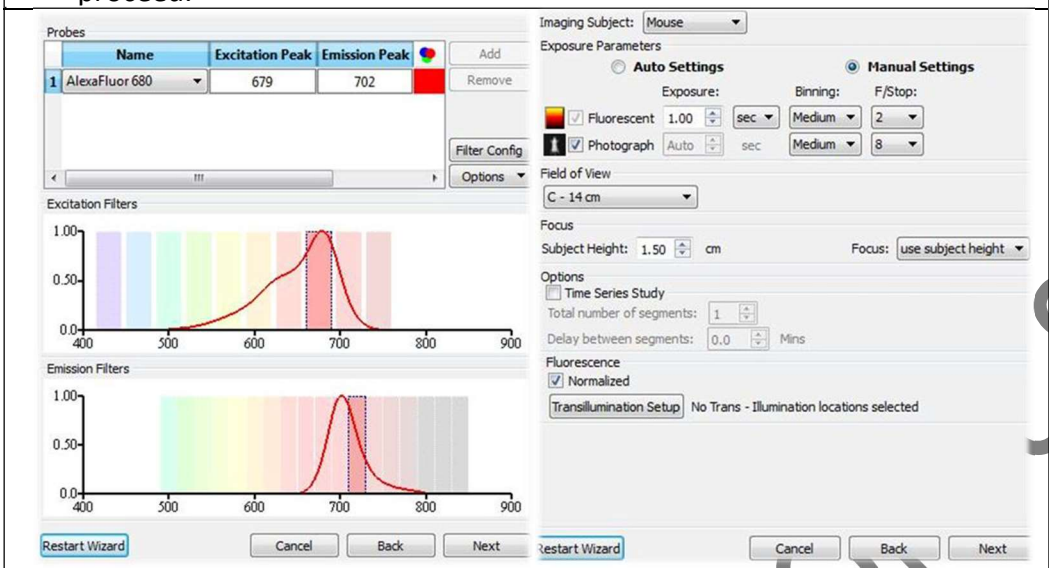
Basic Operation for 3D Fluorescence Imaging (Trans-illumination)

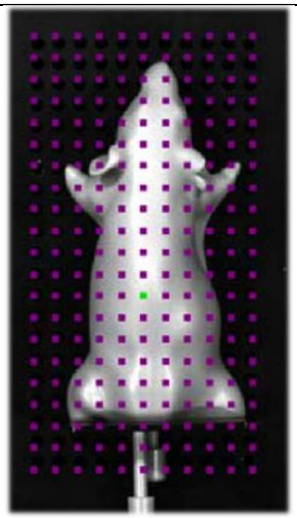
1. Launch Imaging Wizard. **Restart Wizard** if necessary. Select Fluorescence and then Fluorescent Tomography Algorithm (FLIT).
2. Choose an appropriate probe and excitation/emission filter set, add excitation\emission filters if spectral unmixing is needed. **Next** to proceed.



3. Select Image Subject (Mouse/ Plate/Other).
4. Set up the acquisition parameters automatically or manually
   a. For Auto Settings, select Auto in Exposure box, the software will determines exposure time, binning and F/stop setting,
   b. For Manual Settings,

   Adjust Exposure Time according to the signal intensity
   Adjust Binning if necessary.
   Adjust F/stop if necessary.

5. Select appropriate Field of View.
   Adjust Focus if necessary. Input the subject height (in cm).
6. In Transillumination Setup, select the grid under the expected signal location.
7. Holding Control Key to select discontinuous area
8. Use Epi-illumincation or Raster scan as a preview first if signal location is not determined.
9. Click Next to generate Image Sequence. Acquire Sequence to capture all Images in the sequence window.
10. During acquisition, fill in the Sample information and click OK.
11. Save the data in D: Drive> User if Auto Save is not activated.
12. Perform Image Display Adjustment, ROI Measurement, 3D Reconstruction, Spectral Unmixing etc. analysis in the offline software (Please reserve Spectrum Offline session in FCF Online Booking System; Free of Charge) according to the Living Images analysis Protocol.



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General Cleaning and Finishing Procedures

1. Close all the Images but **Do Not Exit Living Image** software
2. Transfer image/data files using FCF network server according to the Data Transfer Instruction. Close all the browsers but not Living Image after the transfer.
3. Remove all your samples from the Imaging chamber. Clean the stage and Sample Mat with 70% Ethanol and Kimwipes.
4. Clean the bench with 70% Ethanol and paper towel.
5. Important! : The system runs calibration at mid-night for accurate intensity measurement for the next day use. Please do
   a. **NOT** Exit Living Image software but Close **all** other software and images
   b. Make sure the **Chamber Door is Closed** properly.
   c. Turn off the monitor only (***NEVER turn off*** the computer or any parts of the system!!!)
6. Sign out in log book according to the Actual leaving time.
7. Turn off light and close/lock the door when leave.

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