Caliper IVIS Spectrum

Quick Operation Guide
The instrument and computer should be left on all the times. Background readings are acquired each night.

Each user will log on to IVIS instruments using the Kerberos username and password. You will be billed for the greater of your reserved time or your logged time. Please have the same person make reservations and log on/off of the instruments.

Launch Living Image.
- Double-click icon on desktop

Log in using your initials, (bypass the request to make a password, you don’t need to add an additional password as you are logged on with Kerberos).

Initialize (bottom right corner of the control panel).
- On IVIS1 and 2 the temperature bar should be green (camera temperature is always set at -90). If this light is red, contact AIPT staff immediately.
Pre-Image Preparation

- **Bioluminescence:** inject luciferin (i.p. or subcutaneous) 10 minutes before imaging (see Luciferin Protocol for more information).
  - The 10 minute wait allows luciferin to achieve peak distribution in the mice.
  - Luciferin levels remain stable for ~20 minutes and return to near baseline levels by 60 minutes post injection.

- Anesthetize mice in the anesthesia chamber

- Place **black paper** on the stage for bioluminescent imaging or **Lexan plastic sheet** (matte side up) for fluorescent imaging.

- Glass nose cones and dividers are available in the drawer of the IVIS workstation.

- Place anesthetized mice in imaging chamber.
  - Place mice close to the center of the field of view.
  - Place the midline of the mice or area of interest along crosshairs of alignment grid. Position mice uniformly.
  - Use dividers between mice to block adjacent bioluminescent/fluorescent signals.
  - Note: Black mice will have a ~10X reduction in the bioluminescent signal and ~20X reduction in fluorescence as compared to white, shaved or nude mice.
Image Acquisition-Control Panel Settings

- **Bioluminescence:**
  - select exposure time, f-stop and binning.
  - for new imaging protocols use auto exposure, medium binning and f-stop 1.
  - Auto exposure will adjust the exposure time to acquire at least 3000 counts.

- **Fluorescence:**
  - select fluorescence type: *Epi-Illumination* (most common, shallow source) or *Transillumination* (for deep sources; contact Scott Malstrom for transillumination training).
  - Select exposure time, binning, f-stop, excitation and emission filters.
  - for new imaging protocols start with auto exposure, medium binning, f-stop 2, and appropriate excitation/emission wavelength filters.
  - If you do not know which filter set would be optimal for your fluorophore, click on the **Sequence setup** button on the control panel, then click on **Imaging Wizard** to guide you through the process.
Choose the **Field of View (FOV)**, A-D; D being the largest field of view that can image up to 5 mice (the default FOV is C).

Once all parameters are set, and the mice are in position, click the **Acquire** button.

The **Image label box** will appear for you to input information about the image.

You should acquire between **1,000-60,000 counts** for each image. If the maximum counts are outside this range, adjust the exposure time.

If you see a warning that you have a **saturated image**, acquire an image using less exposure time. If you try to quantify an ROI from a saturated area, your photon values will not be accurate.
Saving and Accessing Image Data

- The software will ask you if you want to **autosave** your images, this feature will save all images to the specified location. You can also **manually save** images. If an image is not saved, when you try to close the image or software a warning will indicate that you should save the image.

- Image data consists of **folders**, not individual files. All of the text and visual elements in the folder are required to reconstruct the data set in the Living Image software on this or any other computer.

- Each image has a unique identification code. For example: SM20110612091222 [initials][year][month][day][hour][min][sec]

- To open images use **Browse**. By using this function, you can open whole data sets at once

- To place images into a presentation, you can directly cut and paste Living Image images into PowerPoint or excel documents. Alternatively, you can export images by choosing **File>Export**.
The Tool Palette contains information about the active image and organizes the image analysis tools.

Image Adjust: You can change the appearance of the images by adjusting the Min and Max color bars. These adjustments do not change the quantitative photon data associated with the image.
You can select regions of interest (ROI) to determine how many photons are radiating from the source.

- ROI types are circles, squares, grids or free form.
- Select the units for measurement with the Unit tab.
  - Photons (bioluminescence)
  - Radiant Efficiency (fluorescence)
- Create an ROI, then hit the Measurements tab (pencil icon).

- A window will appear with a table of ROI Measurements.
  - This data can be directly cut and pasted into an excel spreadsheet.
  - Use the Total Flux (p/s) column for calculating your results.
Data storage and retrieval

Data Transfer to the server

- To transfer your data to the ccrbi2 file server you will need to map the network drive.

**IVIS1**
- Open “my computer”, look for a tab “map network drive”
- Folder: `\ccrbi2\ccrbi2_Data1\atwai`
- Click “finish”
- Type in user name: CCRBI2\malstrom and for
- Type in password: Ray4eiph
- Create a folder for yourself in the IVIS folder on the server

**IVIS 2**
- Open “my computer (ATWAI IVIS2)” look for a tab “Tools” then “map network drive”
- Folder: `\ccrbi2\ccrbi2_Data1\atwai`
- Click “finish”
- Type in user name: ccrbi2\malstrom
- Type in password: Ray4eiph

- Create a folder for yourself in the IVIS folder on the server.
- Make sure to compress data so that image files are intact when you transfer them to your own computer for analysis.
- You may obtain a copy of the Living Image software and information about downloading you data from the ccrbi2 server from Scott.

Please talk with Scott Malstrom about which image analysis tools would be best to use for your experiment; as well as an other imaging questions. (malstrom@mit.edu or 617-715-4312; Building 76-188)