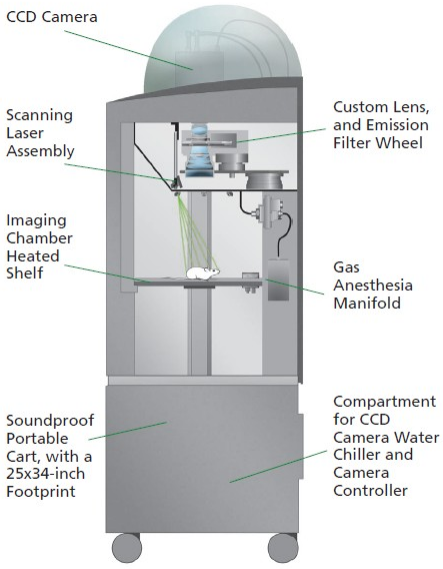
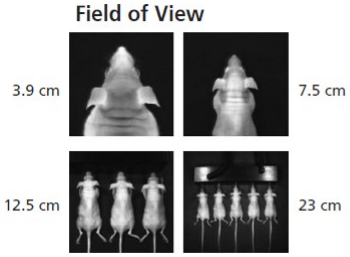
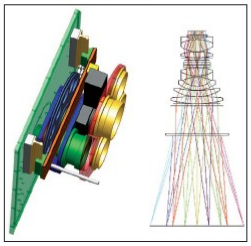
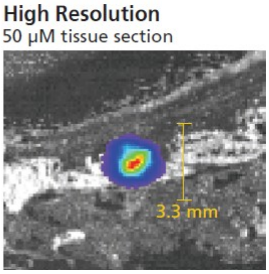
XENOGEN IVIS 200 USER GUIDE

The Xenogen IVIS system combines the ability to image in vitro or in vivo, fluorescence or bioluminescence, and 2D or 3D. All imaging components are contained within a portable cabinet occupying a 25x34 inch footprint. The system includes a highly sensitive, cooled, back-thinned CCD, a light tight imaging chamber with complete computer automation, and the Living Image software package for image acquisition and analysis. The lens provides high light collection at f/1, an adjustable field of view from 4-23 cm, uniform light collection, and superior resolution with single cell sensitivity for in vitro or ex vivo use.
Imaging Chamber: *Light-tight, Heavy-duty castors, Integrated gas anesthesia, Integrated fluorescence, LED lamps for photographic images, Heated stage to maintain optimum body temperature, Electromagnetic door latch, Motor controlled stage, filter wheel, lens position, and f-stop, Scanning laser for mouse alignment and surface topography*
CCD Camera: *Back-thinned, back-illuminated grade 1 CCD provides high quantum efficiency over the entire visible to near-infrared spectrum, 13.5 micron pixels, 2048 x 2048, 16 bit digitizer delivers broad dynamic range, CCD is thermoelectrically (Peltier) cooled to -90 °C ensuring low dark current and low noise*
Custom Designed Lens: *5 inch diameter optics, f/1– f/8, High-resolution - down to 20 microns, Emission filter wheel, 12 or 24 slots*









I. Animal preparation and anesthesia

1. Turn ON the 'Evacuation Pump' using the switch on the front panel.
2. Turn on the oxygen supply.
3. Turn ON the gas flow handle on the anesthesia system.
4. Set the vaporizer value to 0%.
5. Turn ON the toggle named 'IVIS Flow on/off' and the toggle named 'CHAMBER on/off', then set the flow gauge to 2.5 L/min.
6. Once the flow rate to both channels has been set, turn off both toggles (IVIS Flow on/off and CHAMBER on/off).
7. Set the vaporizer value to 2.5%.
8. Weigh mouse/mice
9. Place the mouse in the induction chamber, then close and lock the chamber.
10. Turn ON the toggle named 'CHAMBER on/off' and allow approximately 5 minutes for the animal to become fully anesthetized.

11. Inject mouse with Luciferin.

    Luciferin is a chemical substance found in the cells of various bioluminescent organisms. When Luciferin is oxidized under the catalytic effects of luciferase and ATP, a bluish-green light is produced. Because the reaction is dependent on ATP, it allows researchers to determine the presence of energy or life. Firefly luciferin is a particularly good reporter for in vivo biophotonic imaging due to properties of its emission spectra.

12. Put mouse back in cage. Allow Luciferin to distribute in mouse for **10 minutes**; *After luciferase injection, the bioluminescence signal gradually increases and reaches a plateau at 10 min, which lasts for 15–20 min, so the best time window of imaging is 10–25 min after luciferin administration (unless you are doing kinetic experiments)*
13. After 10 minutes Luciferin distribution, place each mouse back in anesthesia box (2.5% isoflurane).

II. Imaging

Log on to the computer and start the Living Image® 2.6 software program from the Windows Start Menu/Desktop shortcut. Log in by selecting your initials from the drop down menu, or if you are a new user, enter your initials and click DONE. A system control panel will appear in the lower right corner of the monitor.

Click the Initialize IVIS system button in the camera control panel. After initialization, the Temperature Status box in the center of the panel should be green, indicating that the CCD camera is adequately cooled. Door of imaging chamber is locked (light is red) during initialization.

1. When mice are in a moderately deep plane of anesthesia (lying on its side and breathing rhythmically), remove them from induction chamber and close the lid. Stick nose of each mouse in nose cone of anesthesia manifold in the imaging chamber.
2. Turn ON the toggle named 'IVIS Flow on/off'. (Turning on the second toggle will effectively flush the dead volume that exists between the anesthesia Machine and the imaging chamber.)
3. Adjust vaporizer value to 1.5-2.0 %.
4. Select the desired Field of View from the pull down menu on the left side of the control panel. Enter the approximate Subject Height in the lower left entry box. Check the Overlay box in the control panel and set the Exposure Time, binning, and f/stop for the luminescent image. If unsure of what exposure time to use, it is best to start with low sensitivity settings (10 sec, Medium, f/1) and increase/decrease as necessary.
5. Click the Acquire button on the control panel.

    Note: During the luminescent image acquisition, the Acquire button becomes a Stop button, which can be used to terminate the exposure, if desired.

6. After the exposure is complete, the overlaid image is displayed. Edit the information in the Change Information window and click Done. Always click Done in the Image Information window before proceeding.
7. Confirm that the signal of interest is above the noise level (recommend >600 counts) and below CCD saturation (~60,000 counts). If the signal level is unacceptable, adjust Exposure Time, Binning or f/stop and repeat the image acquisition.
8. Measure number of photons/counts for both the signal and background.
9. Upon acquisition, an image is not automatically saved, but it is automatically named using the operator's initials, and a date and time stamp. To save the image, choose Save Living Image Data (under the Living Image menu item), select the Format "Save all Living Image Data Files", check the box "Save Lab Book", then click Save.

   This completes the data acquisition. If desirable, adjust the display with the Max Bar or Min Bar slider. Subsequent analysis can be conducted immediately or deferred until later, after additional images of the sample have been acquired.

III. Post-imaging

1. Exit the program. Log off.
2. Take mice out of imaging chamber. Return to cage. Dispose black paper from imaging chamber and clean/wipe chamber with disinfectant wipes. Put in a clean black paper to replace the old one.
3. Turn off anesthesia manifold controls as well as O_2 supply valve.

Imaging Parameters:

1. ***FOV:*** *width (in cm) of the square area on the stage that is to be imaged. Standard calibrated FOV positions are indicated by A, B, C, D, E, and correspond approximately to the ff. widths: 4, 6.5, 13, 20, 25 cm*
2. ***Exposure Time:*** *controls the length of time that the shutter is open for photographic and luminescent images. In the photographic settings, check "Auto" so exposure time will automatically be adjusted to produce a good photograph. Luminescent images typically have longer exposure times that need to be adjusted, depending on the brightness of the subject.*
3. ***Binning:*** *increases the pixel size on the CCD, which delivers higher sensitivity at the expense of spatial resolution; binning a luminescent image results in a significant improvement in the signal-to-noise ratio. Binning pulldown menu choices for luminescent images are small, medium, and large, with the default set to medium. Each change in binning changes the sensitivity by a factor of four.*
4. ***f/stop:*** *controls the lens aperture on the camera. A larger number indicates a smaller aperture setting and lower sensitivity. The smallest f/stop (f/1) corresponds to the widest*

lens opening and the most sensitive setting. The detected signal scales inversely with the square of the f-number. A smaller aperture results in reduced sensitivity but improved depth of field, which produces a sharper image of an object with varying height. Typically, the photographic image is taken with a small aperture (f/8 or f/16) to give the sharpest image. The luminescent image is taken with a large aperture (f/1) to maximize sensitivity.

Analysis Parameters:

Counts *allows the user to select the basic uncalibrated, raw data mode of image display. In this mode, image pixel contents are displayed as the numerical output of the charge digitizer on the CCD. This numerical output is often referred to as counts, analog digitizer units (ADU), or relative luminescence units (RLU). It is a number that is proportional to the number of photons detected in the pixel.*

Photons *displays image data in terms of absolutely calibrated photon emission from the source. It is important to understand that while raw data (Counts) is a measurement of photons reaching the CCD detector, the data displayed in Photons mode is calibrated for all instrument settings and parameters, and thus represents the actual physical emission from the sample being imaged. Therefore, it is possible to compare measurements made with different instrument settings (e.g., differing exposure times, binning, FOV, etc.) as well as to compare measurements made on different instruments. In Photons mode, the quantity displayed is termed "radiance" and the units are photons/s/cm^2/sr.*