

# How to use Xenogen IVS 200 Imaging Unit in Carlson Animal Facility

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## NOTE:

- 1). You can read more detailed information about the background and instrumentation of Xenogen from the UChicago's Core Facility site: <http://oicf.bsd.uchicago.edu/index.html>
- 2). The IVIS 200 unit is located in Carlson ARC basement. All imaging during regular business hours (**8:30 AM - 5:00 PM**) has to be scheduled using the online scheduler: <http://oicf.uchicago.edu/phpscheduleit/index.php>. Imaging can only be conducted by the Animal Imaging Specialist or the certified/trained users of our lab. After-hours unsupervised use of the OICF will be permitted only to certified users and individuals who have undergone OICF training and testing. We have a copy of the key (**kept in J-611**, labeled as **Xenogen**) to get access to the imaging unit.
- 3). Please bring a **blank CD-ROM** or **USB flash drive** (>250MB, depending on how many animals you want to image) with you to copy the acquired imaging data.
- 3). If you have any technical questions, please contact OICF staff at **(773) 834-8465**, or Dr. Souris at **(773) 834-7316**, or e-mail: [sourisj@uchicago.edu](mailto:sourisj@uchicago.edu)

## STEP ONE: ACQUIRE XENOGEN IMAGING DATA

1. Turn on the computer, and log in: (TCHE/TCHE)
2. Switch on the O2 cylinder
3. Open the pump in the right side of the gas anesthesia machine.
4. Open the pump of chamber.
5. Place the mice in. After the mice has been anaesthetized, 100ul (each side 50ul) of substrate is intraperitoneally injected, and then count time. [you have 10-15min after injection]
6. Open the software Image 2.5.
7. Initialize Xenogen IVIS system in the inferior right corner dialog box, wait at least 5min until the "idle" sign appears, then check the green box and ensure the camera has been keeping cool lower than -105F.
8. Click the pull down menu of "living image", --- dark change --- view available dark change. Wait 10s.
9. Inspect the default imaging settings; only change the two settings according to the request.
  - f/stop 1/ 8
  - Bin - medium
  - exposure time - 1min
  - Excitation - block
  - Emission filter - open
  - Photographic 0.2
10. Check the small [high resolution] *Sometimes we need the larger scale to take wide view picture, we need choose "D" for more than 3 animals (generally, we choose C for 2 mice) in the inferior left dialog box.*
11. After verifying the parameter, place the animal in the right place, and then acquire the picture at 10min post injection.

## SAVING THE DATA

1. Firstly, go to the desktop and create the new folder in the TC folder.

2. Then go back to activate the window containing the data! , click the “living image” pull down menu--- saving living image data---check the upper option in the pop-up menu---browse the folder and save in the specific location.
3. If you want to get the Bitmap picture directly, activate the window again, save again, but remember check “save copy” in the pop-up menu.
4. After saving, we just close the current window (not main window), then image the next animal.

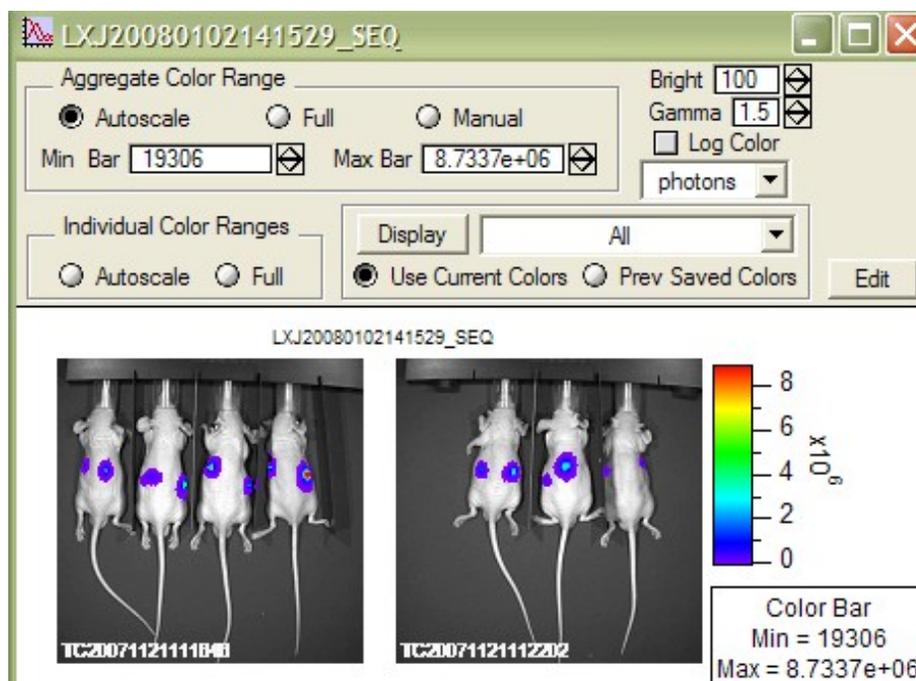
### **CLEANING AFTER IMAGING**

1. Clean the desk, cleaning the machine wherever touched; clean the chamber outside and inside.
2. Clean the Xenogen machine, clean the column mask and slide.
3. Wipe the desktop and keyboard, and change the film on the mouse and the machine handle.
4. Animals should be returned to the **Return Room JS-45A**.

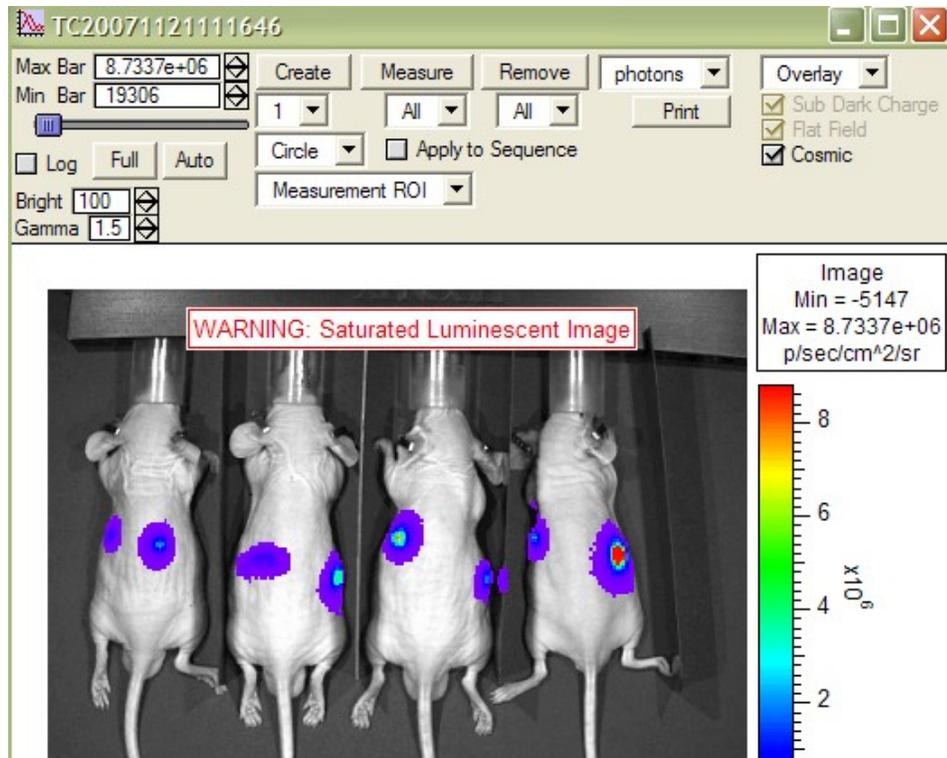
### **STEP TWO: ANALYSIS OF XENOGEN IMAGING DATA USING LIVING IMAGE**

**NOTE:** Our lab has a copy of the **Living Image** software.

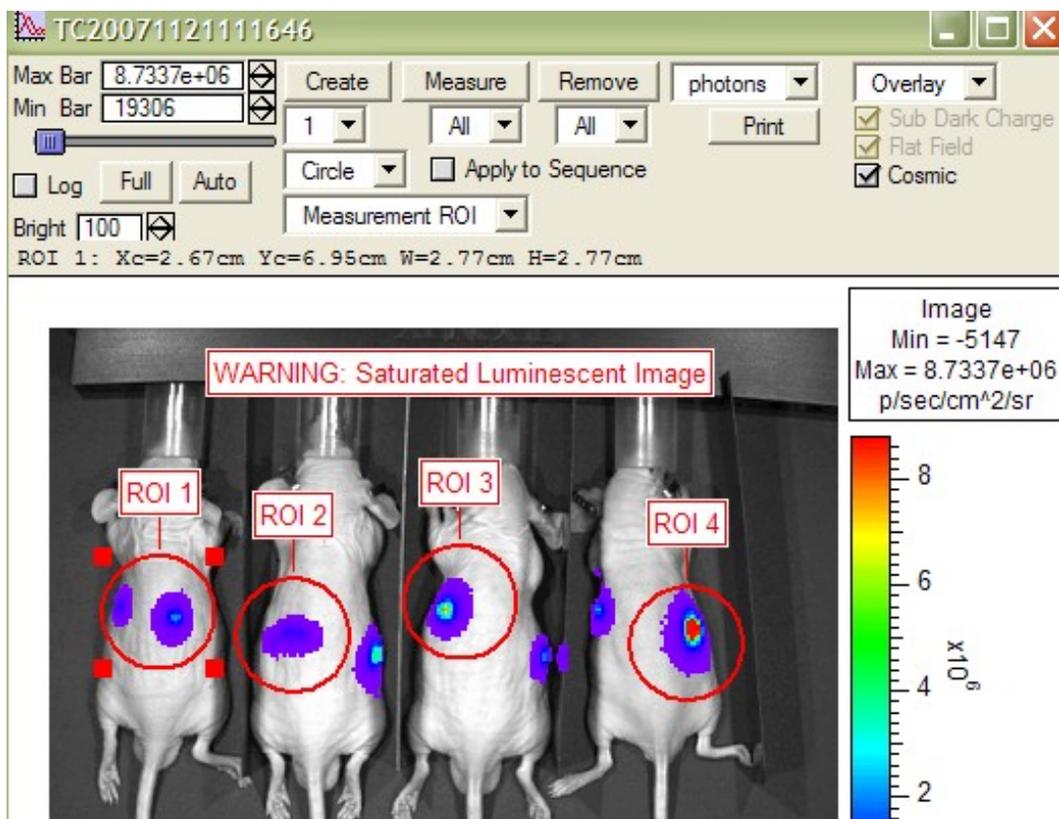
1. double click “Living Image 2.50”
2. choose “living image” in the menu bar,--- click “Browse for LI Data”--- then choose uses you want( it’s your computer name generally)
3. In the new pop-up window, click “add” to choose the image files from computer, you can just choose the folder that includes all the image files.
4. Select all the files, then Click “Load as Group”.
5. Wait several seconds until the all the files has been loaded, the scale bar has been automatically set by the computer. If you want to have the different scale, the **Min bar** and **Max bar** in the upper-left corner of the windows can be adjusted, or you can choose checking “Log Color”.



6. Single click for each picture to activate each file.

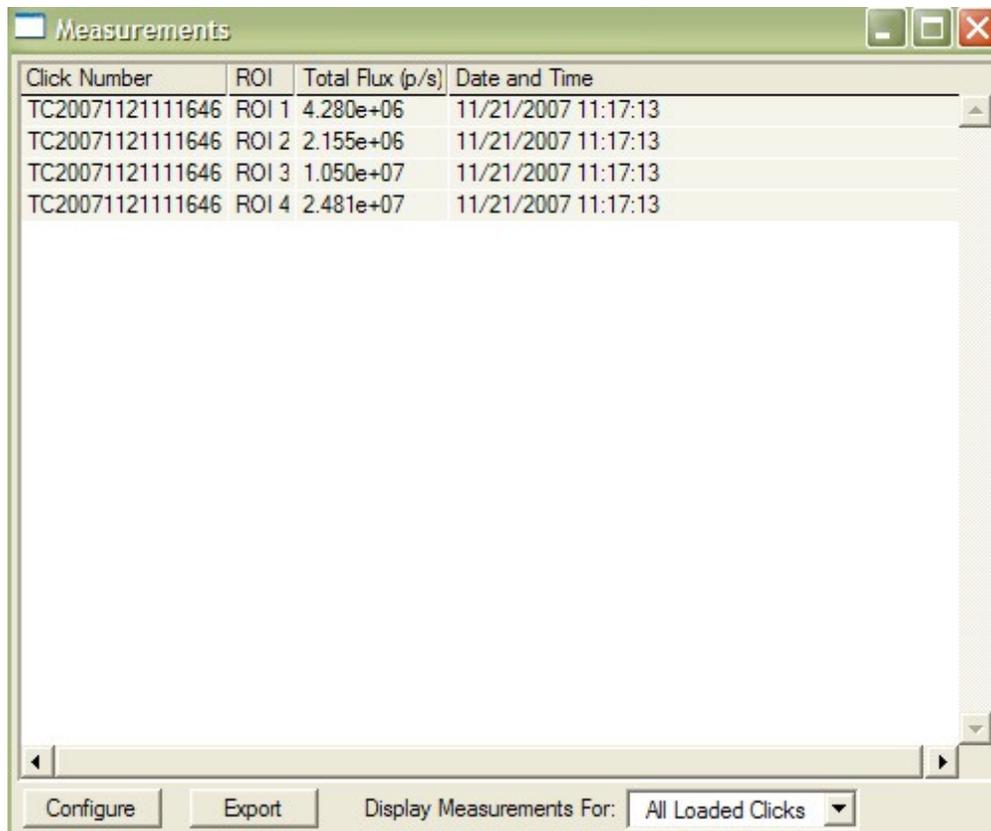


7. Click "Create" to create the measure circle, and move each circle to cover the signal zone of the animals.



8. Click "measure" to calculate the intensity of the signal.

- Click "Export" in the measurements window to output all the data in excel files.



Click Number	ROI	Total Flux (p/s)	Date and Time
TC20071121111646	ROI 1	4.280e+06	11/21/2007 11:17:13
TC20071121111646	ROI 2	2.155e+06	11/21/2007 11:17:13
TC20071121111646	ROI 3	1.050e+07	11/21/2007 11:17:13
TC20071121111646	ROI 4	2.481e+07	11/21/2007 11:17:13

Configure    Export    Display Measurements For: All Loaded Clicks

**NOTE**

- If the signals are saturated, that means you better decrease the cells you injected.
- Analyzing all imaging files of one batch experiment in the same scale will be recommended.