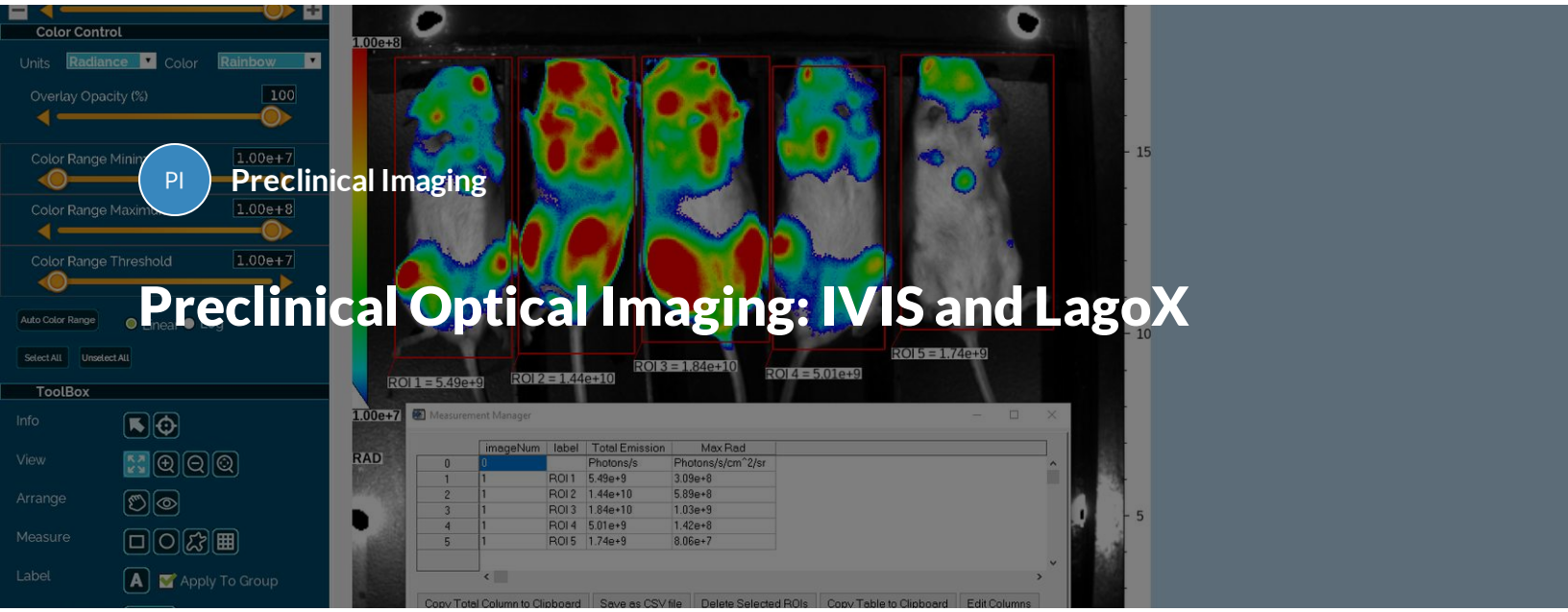


PI Preclinical Imaging

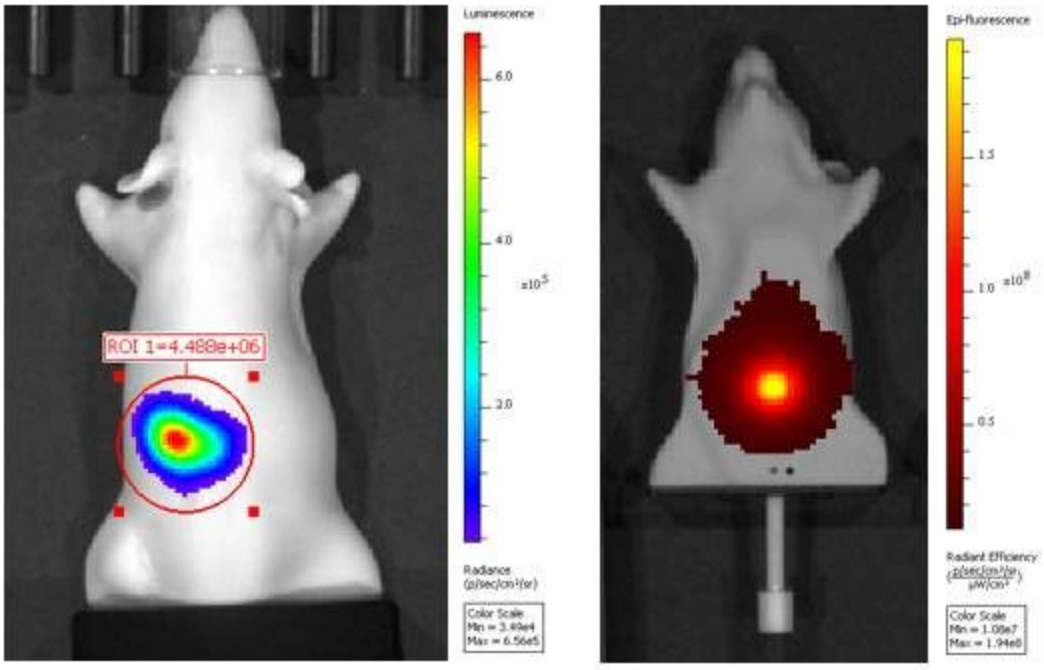
Preclinical Optical Imaging: IVIS and LagoX

This online training is required for any researchers who wish to use the IVIS Spectrum and/or LagoX for mouse imaging studies using bioluminescence or fluorescence. This online training should be completed before in-person training, as a refresher-course, and as a reference for future use of the equipment.

- **Overview**
- **Scientific Background**
- **Getting Started**
- **Imaging Procedure**
- **Wrapping up**
- **Further Resources**
- **BSL-2 Procedure**

Overview

PI Preclinical Imaging

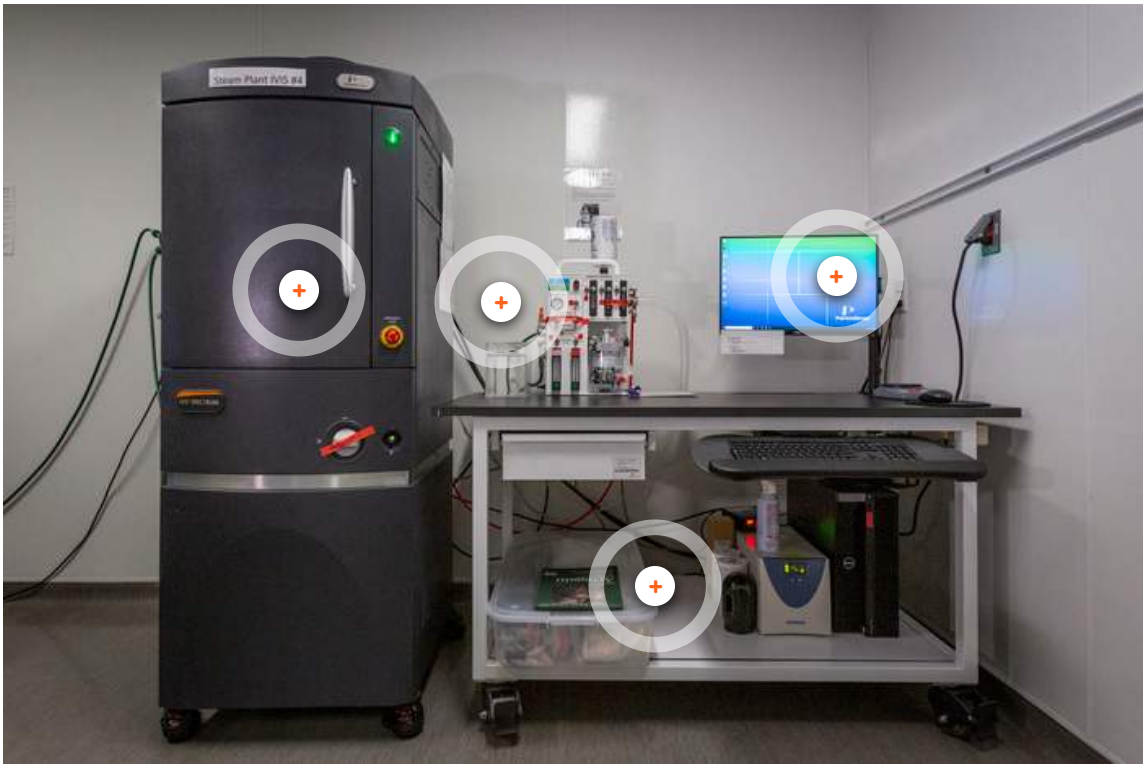


Bioluminescence (left) and fluorescence (right) image from a mouse phantom acquired using the IVIS Spectrum machine in the Comparative Medicine Vivarium

Our optical imaging instruments can collect either bioluminescence or fluorescence images from small animals or tissue sections. It is great for applications that require high-throughput imaging, as it can acquire images in less than a minute, and can image up to 5 mice at a time (10 mice on the LagoX). This modality offers a spatial resolution between 1 and 5 mm, depending on the application.

Reservations on these systems can be made up to 30 days in advance on iLab. The machine can be reserved on weekends and holidays, with 24/7 access. Full access is granted after completing a Comparative Medicine facility tour, this online training, and a brief in-person training.

Click on each of the components in the images below to see the different features of the systems.



Optical Imager

The IVIS instruments are located in BE-039 (shares room with biosafety cabinet and a downdraft table), BSL2 in BE-243 procedure room, and S4-225 (at the Steam Plant - shares room with micro-CT).
The LagoX instrument is located in BE-357 (shares room with MRI and Echo).

Supplies provided

Matte black art paper is provided. This paper can be used to reduce glare off of reflective surfaces in the images (including the stage platform) and to cover injectate and syringes as luciferin is light sensitive.

Anesthesia setup

Each system has an oxygen tank and anesthesia system, each of which differ slightly from each other. Each room has an anesthesia system guide that can be used for reference.

Turning on the monitor

To use the system you will need to reserve time on it in iLab. You cannot turn on the monitor unless you have a reservation in iLab and have logged in on to your iLab reservation.

On the LagoX, the green button on the left will raise the door. In the center is the X-ray indicator lights. The red button on the right is the emergency stop button.

Minor differences between the systems

CONTINUE

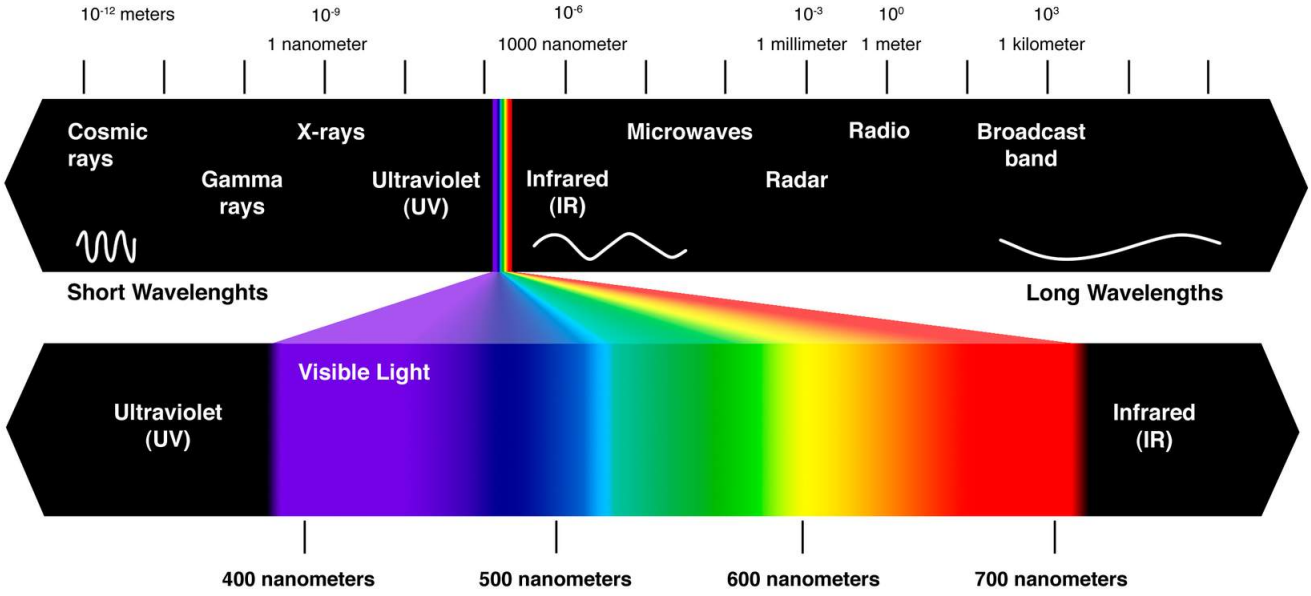
Scientific Background

PI Preclinical Imaging

What is Optical Imaging?

Optical imaging methods rely on the detection of light, or photons, to form an image. The energy of a photon determines what its wavelength is. Most optical imaging methods use photons within and around the visible spectrum of light, from 350 nm wavelength (ultra-violet) to 1300 nm (near-infrared).

The Electromagnetic Spectrum of Light



Full Electromagnetic Spectrum. Optical imaging methods like bioluminescence and fluorescence typically only use light at wavelengths between 350 nm (ultraviolet) to 1200 nm (near infrared).

The IVIS and LagoX can perform two different optical imaging methods

This modality can perform both bioluminescence and fluorescence. Both these methods fall into the category of "optical imaging". However, bioluminescence and fluorescence are different from each other in several key ways.

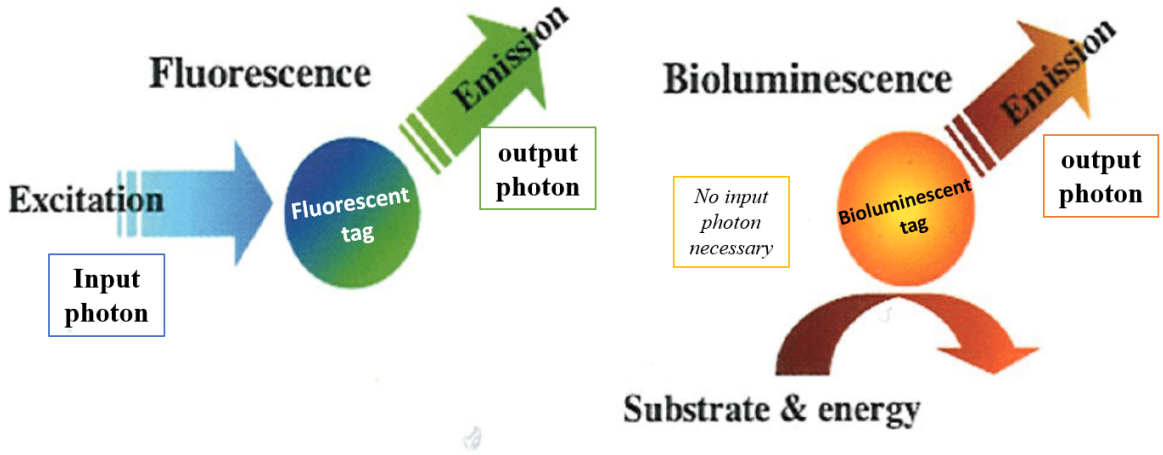


Figure adapted from *Biomedical Optical Imaging*, Oxford Press 2009

Basic differences between fluorescence and bioluminescence mechanisms of light production

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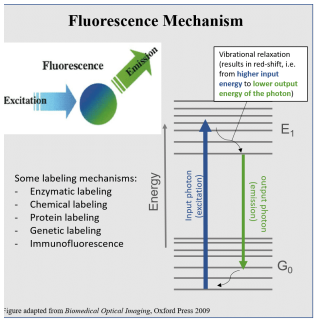
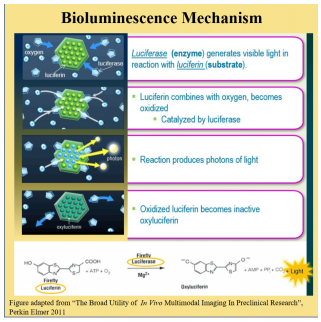


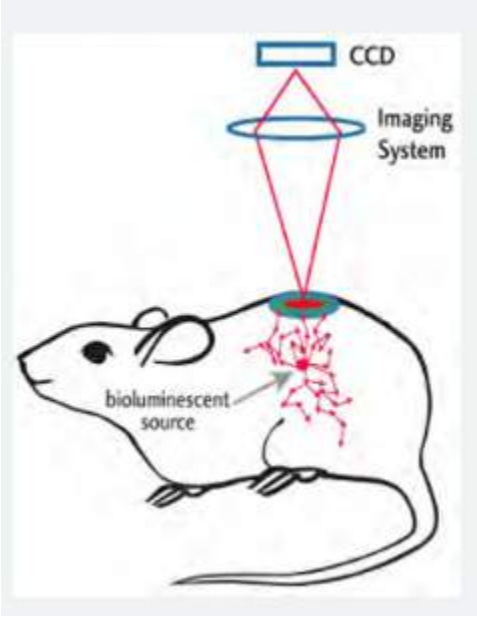


Fluorescence process diagrams. Click to expand photo.

Bioluminescence process diagrams. Click to expand photo.

Fluorescence is an optical process where input photons from an excitation source interact with a fluorophore. Some of those photons undergo an excitation process where they enter a higher energy electronic state, and during the process of relaxing back to a ground state energy they emit a photon of a lower energy (called the emission photon). This is our fluorescent signal that is detected by our CCD camera.

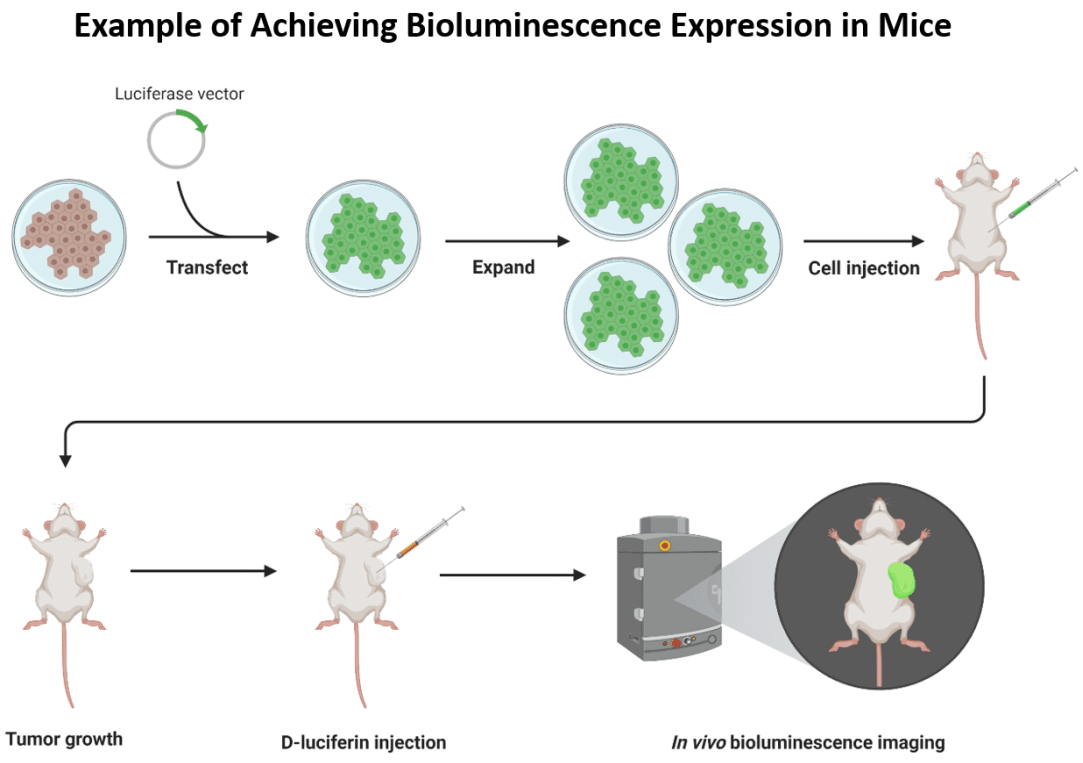
Bioluminescence, unlike fluorescence, does not require an input photon nor any light source to generate signal. The enzyme luciferase is expressed in the animal model (this requires preparation ahead of time! Luciferase does not naturally express in any standard mouse model). Only when luciferin, the substrate, is injected into an animal expressing luciferase and localizes to those specific areas where luciferase has been expressed, a chemical reaction occurs, and light is produced. This produced light is detected by the CCD camera.



Schematic of light collection with the CCD camera

To visualize the areas we are interested in seeing in our mice, we will need to tag our cells of interest with optical tracers that can track the position and quantity of these cells. These optical tracers can be either fluorescent tags or luciferin that attaches to luciferase enzyme.

These optical tracers emit photons, which diffuse through the tissue and the camera captures the signal.



Reprinted from "Bioluminescence Imaging of Tumor Mass" by BioRender.com (2021).

Where does the luminescent signal come from to make the images?

- Refractive index differences in the tissue that result in sound waves reverberating differently
- The ratio of hydrogen's different spin states which are measured with the response to the magnetic field
- Photons emitted from either a bioluminescent or fluorescent tag in the animal, some of which are detected by the camera
- Density differences between tissue sources which the X-ray tube detects

SUBMIT

How many *input* photons are necessary for fluorescence?

- No input photons are necessary for a fluorescence excitation process

- ○ 1 or more excitation photons
- ○ 1 or less emission photons

SUBMIT

Which of the below statements are **true**? *Select all that apply:*

- ☐ No input (aka excitation) photons are needed for a bioluminescent process
- ☐ There is no laser or light source used to generate bioluminescence
- ☐ A green laser (533 nm) is used to generate luciferin signal
- ☐ Luciferin is the most common injectate used for bioluminescence

- [ ] A light source is needed to generate photons for a fluorescence excitation process

- [ ] If you inject luciferin into a standard C57BL mouse without any modification or preparation you will see bioluminescence

SUBMIT

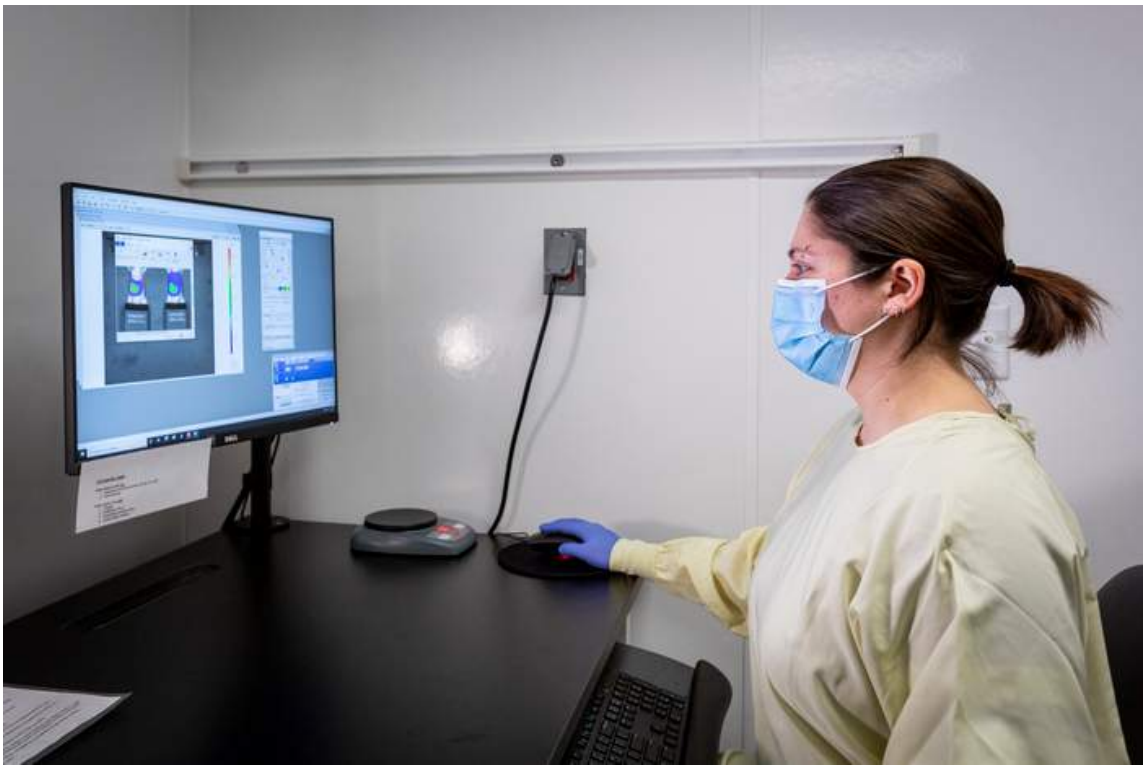
Complete the content above before moving on.

Lesson 3 of 7

Getting Started

PI Preclinical Imaging

Lets Begin



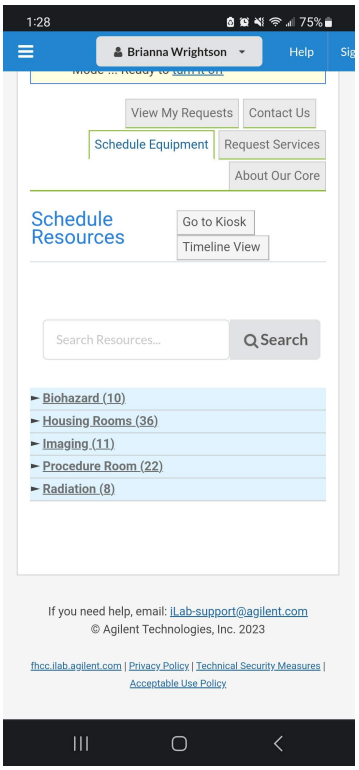
Step 1

Reserve time on iLab

For instructions on how to register for iLab if you have not yet done so, click here.

Step 2

Log in on iLab



Log in at the start of your session to turn the monitor on. Go to Kiosk from the Schedule Equipment tab. This is where you will see your reservation to start your session within 30 mins of the scheduled time. After 30 mins your reservation will become a no-show.

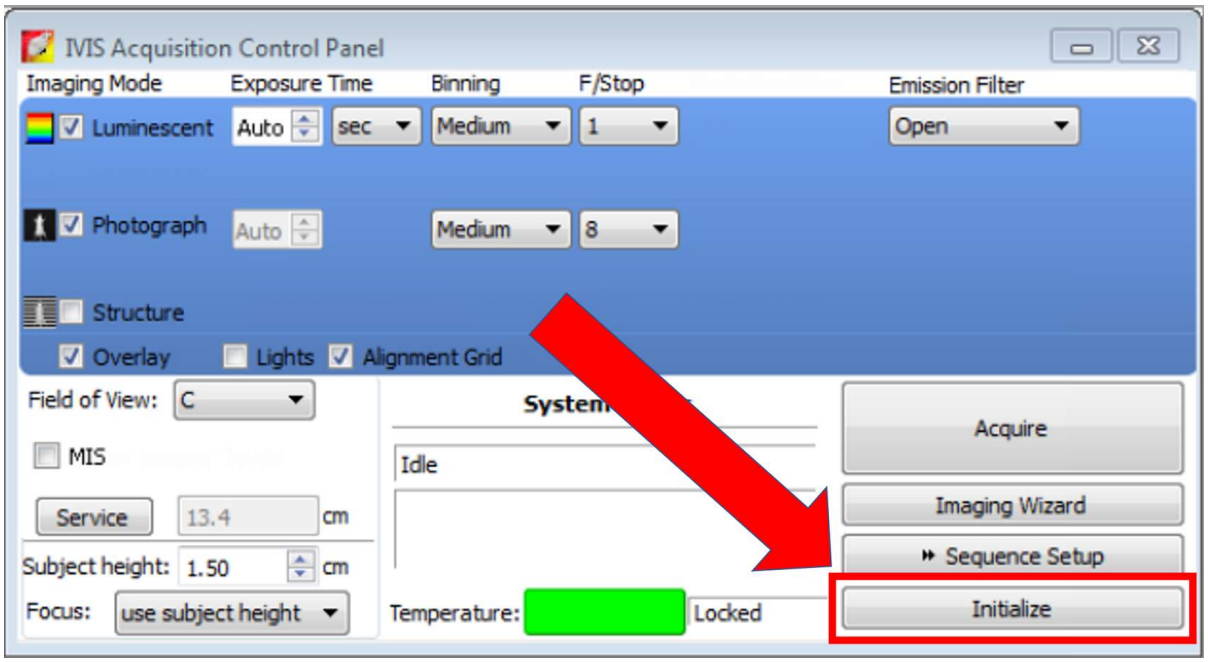
Step 3

Open Living Image Software on the IVIS or Aura software on the LagoX

- Open Software
  - if this is your first time using the software, set up a new user account
- For the IVIS, current users can find their user name in the drop down.
- For the Lago, just type in user name. This will be the name of your data folder.

Step 4

Initialize Machine

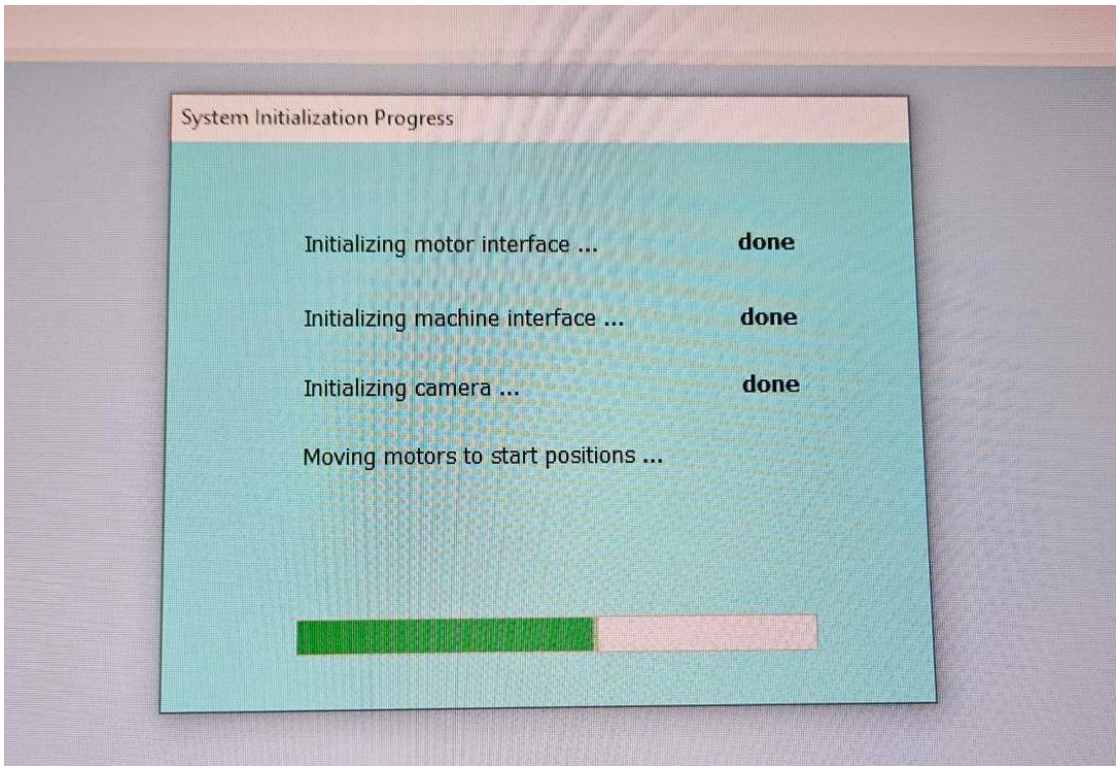


- The IVIS Acquisition Control Panel should appear in the bottom right-hand corner.
- Press Initialize to begin the warm-up and cool-down process for the IVIS machine. This process will take about 10-15 minutes, so you should do this step first.
- You cannot image any mice until the bar to the left of the 'Initialize' button switches to green.
- To check on the status of the machine warm-up and cool-down process, click on the red (or green) bar to see the temperature of the bed and the temperature of the camera.
    - Bed temperature needs to warm up to 38 °C

- Camera needs to cool to -90 °C (will be ready around -86)

Step 5

LagoX



- The Lago will automatically start to initialize once logged in. The status will become "idle" when system is ready to image.

Move on to animal prep!

While waiting for the machine to initialize, now is usually a good time to do all the animal preparation, including weighing the animals, (if doing bioluminescence) prepare luciferin injections, and beginning to anesthetize some animals and injecting the luciferin.

Bioluminescence animal preparation:

Here we will discuss factors to consider when performing bioluminescence imaging. Then we will give an example of a general procedure for bioluminescence imaging after I.P. injection of luciferin.

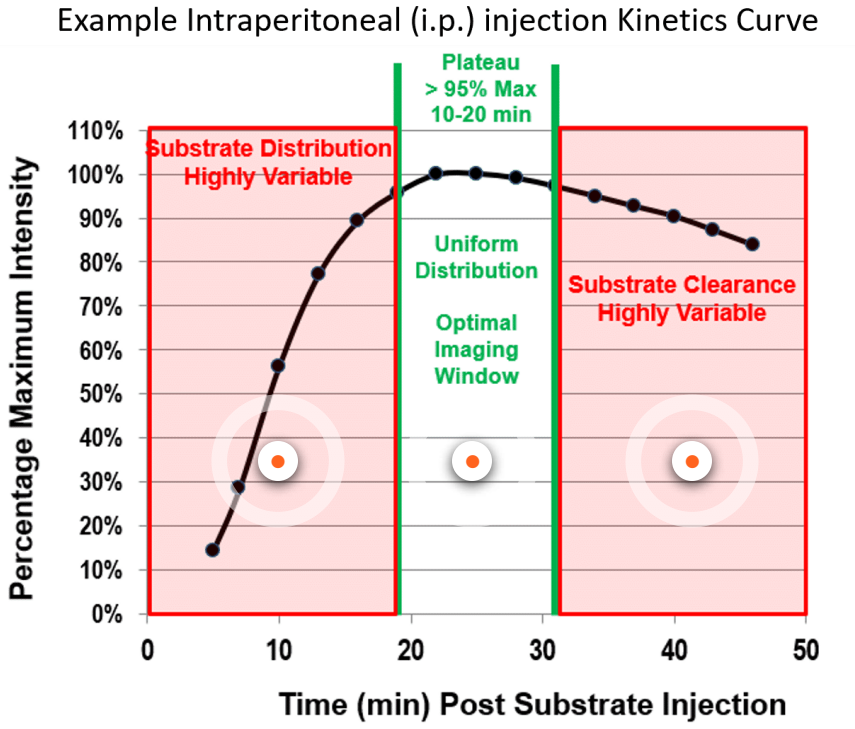
Note: Here we use the terms luciferin and substrate interchangeably, as the majority of the time those terms are interchangeable. Please also note that other study variables like using another injection method (like retro-orbital instead of intra-peritoneal) will require a different procedure. We recommend developing lab-specific SOPs for bioluminescence imaging.

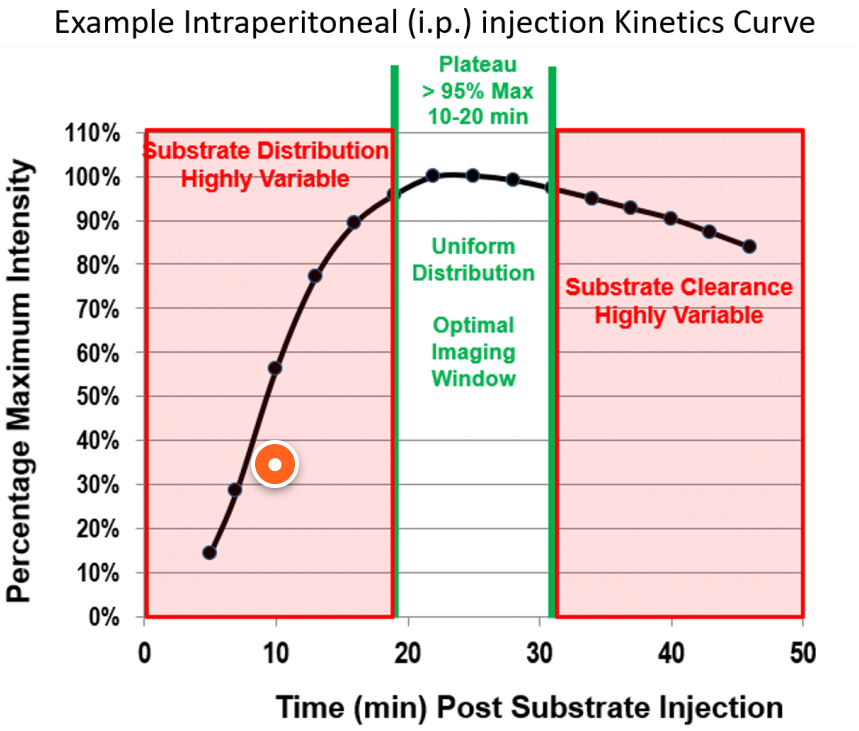
Establishing a Kinetics Curve

You will need to use a kinetics curve to determine when the best imaging time is, post- luciferin injection. See the below example bioluminescence kinetics curve for an I.P. injection of luciferin.

It is important that at the beginning of every study that you generate your own kinetics curve for your own mice, as the method of luciferin injection (I.P., subQ, or R.O.) will affect the optimal imaging window time dramatically. Additionally, every mouse model is different and even with the same mouse model, mice may have different renal clearance times depending on their health status.

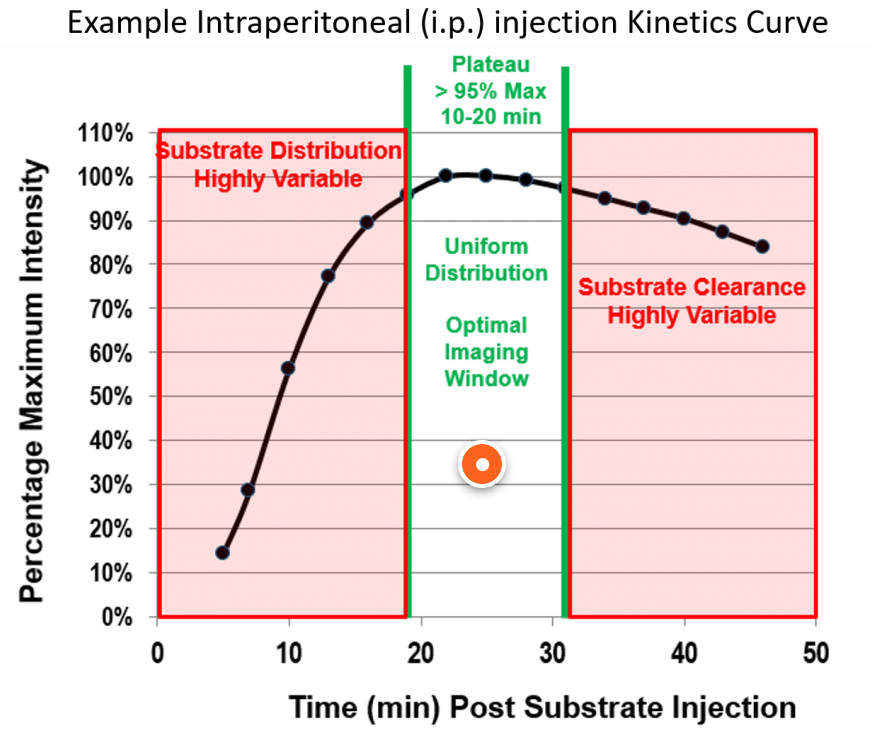
To generate a kinetics curve you inject a mouse with the substrate in the manner of your choosing, then image it at many different time points. Then plot the total signal from each image time point against the time, to generate a curve similar to that shown below. Use this data to determine your optical imaging window and to guide your future experiments.





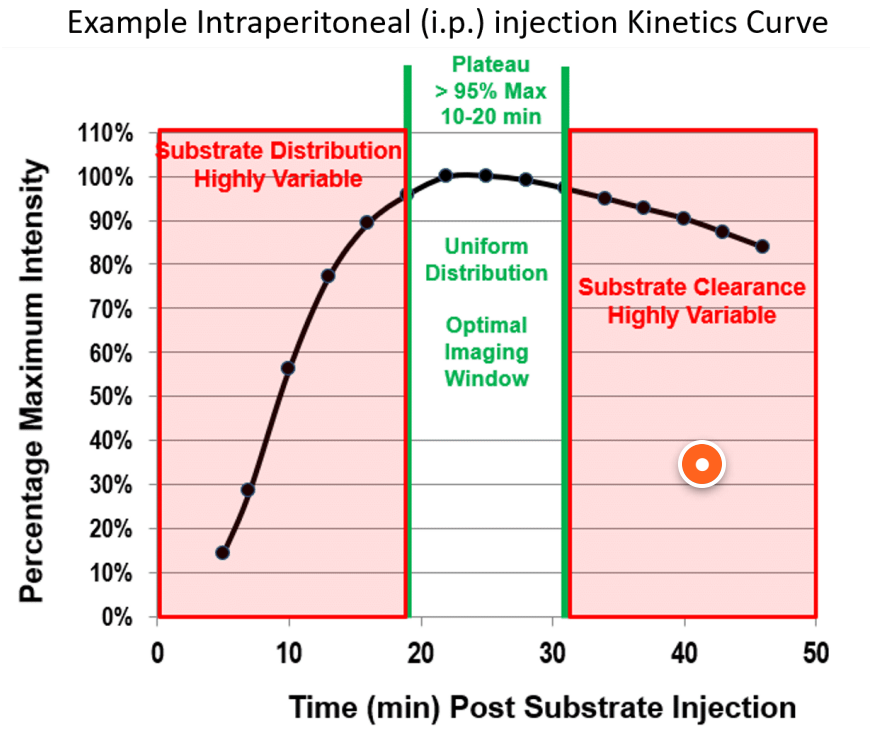
Substrate distribution initially is highly variable

Immediately after injection there is much variability in how evenly distributed the substrate is throughout the mouse's body. Avoid imaging the animals during this period.



Optimal Imaging Window during distribution plateau

You want to image during the "Optimal Imaging Window" here, when the signal & distribution is at a plateau. You want to image the mice during the period (green window) when the substrate's distribution is as steady as possible.



Substrate clearance highly variable

If you wait too long to image, the mouse's renal clearance induces more variability and may result in low signal or un-reproducible data.

Example procedure for bioluminescence imaging with luciferin

Step 1

Weigh your mice then prepare luciferin for injections

Prepare luciferin at 10 µl/g of body weight. Generally, each mouse should receive 150 mg Luciferin/kg.

Step 2

Inject substrate and note the time of injection

- For IP: Inject 10 to 20 mins before imaging (determined by kinetic curve, an example of an I.P. kinetics curve is shown above)
- For RO: inject 3-5 min before imaging
- For Subcutaneous: inject ~15-25 before imaging

Step 3

Anesthetize mice

- For IP, put mice in anesthesia induction box and wait ~5 minutes to let luciferin circulate through their body, then put the mice to sleep.
- Once mice are asleep, transfer each mouse to the imaging chamber and wait for the ~15 minute post-injection time point. Then acquire the image. (see next sections on how to acquire images)

You don’t need to make a kinetics curve, all mice have the same renal clearance time regardless of their health status.

- ○ True
- ○ False

SUBMIT

Which factors affect the optimal imaging window? *Select all that apply.*

- ☐ Substrate injection method
- ☐ Mouse model
- ☐ Mouse disease burden

SUBMIT

Fluorescence Imaging animal preparation

Expressing fluorophores in animals that are meant to be imaged with the IVIS or Lago is often done further in advance, although exceptions apply. Contact preclinicalimaging@fredhutch.org to discuss

Complete the content above before moving on.

Lesson 4 of 7

Imaging Procedure

PI Preclinical Imaging

IVIS Preparation

Open oxygen tank

Oxygen is the carrier gas for the anesthetic agents.
Please make sure to turn this off when you are finished imaging!

Turn anesthesia system on

Turn on exhaust pump

Turn oxygen lever on anesthesia system to “on”

Send isoflurane to anesthesia chamber

Set gas flow rate to 1 L/min

Anesthetize animals

Prepare your animals. When they are ready, anesthetize at 2-2.5% isoflurane.

Turn on IVIS manifold flow

when animals are ready to be transferred to IVIS chamber, open the IVIS manifold gas flow which distributes isoflurane to the nosecones in the IVIS chamber.
Set the gas flow rate to 1 L/min.

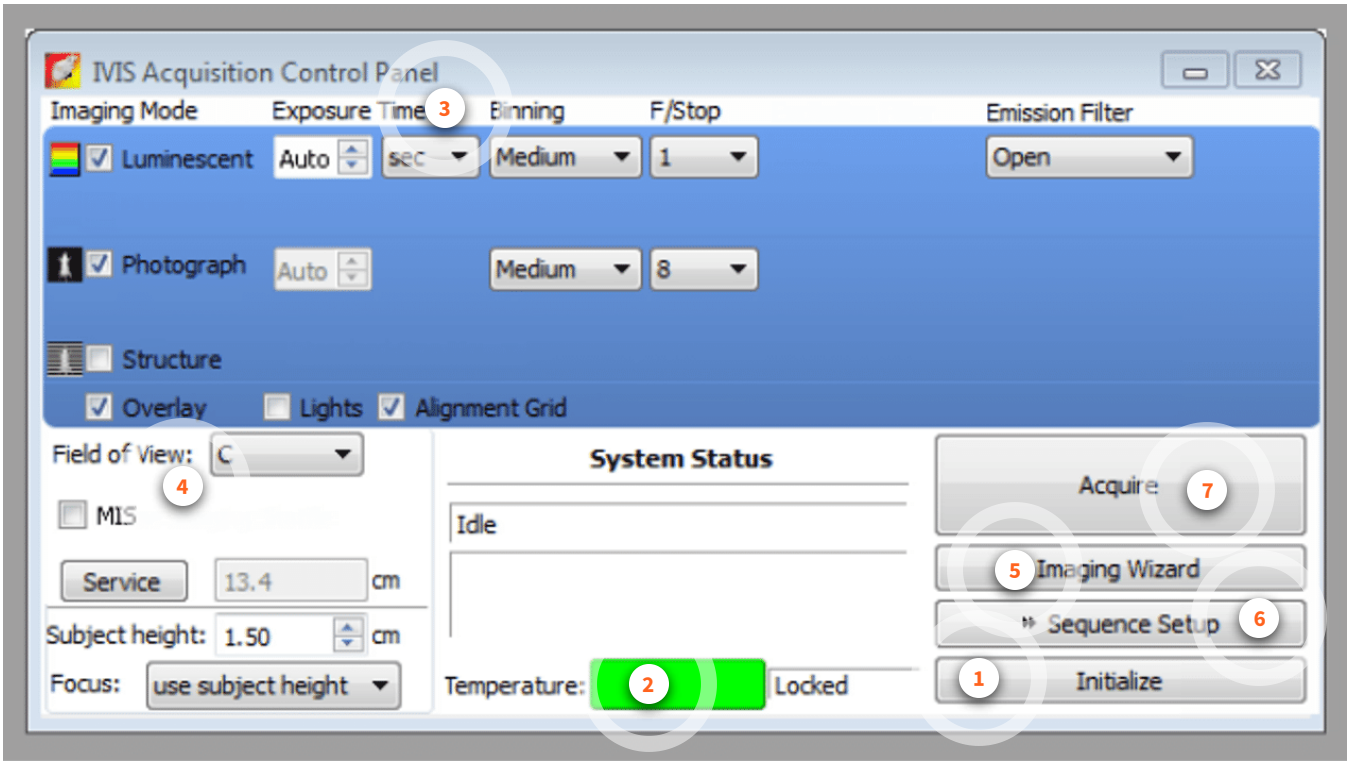
Transfer animal to IVIS imaging chamber

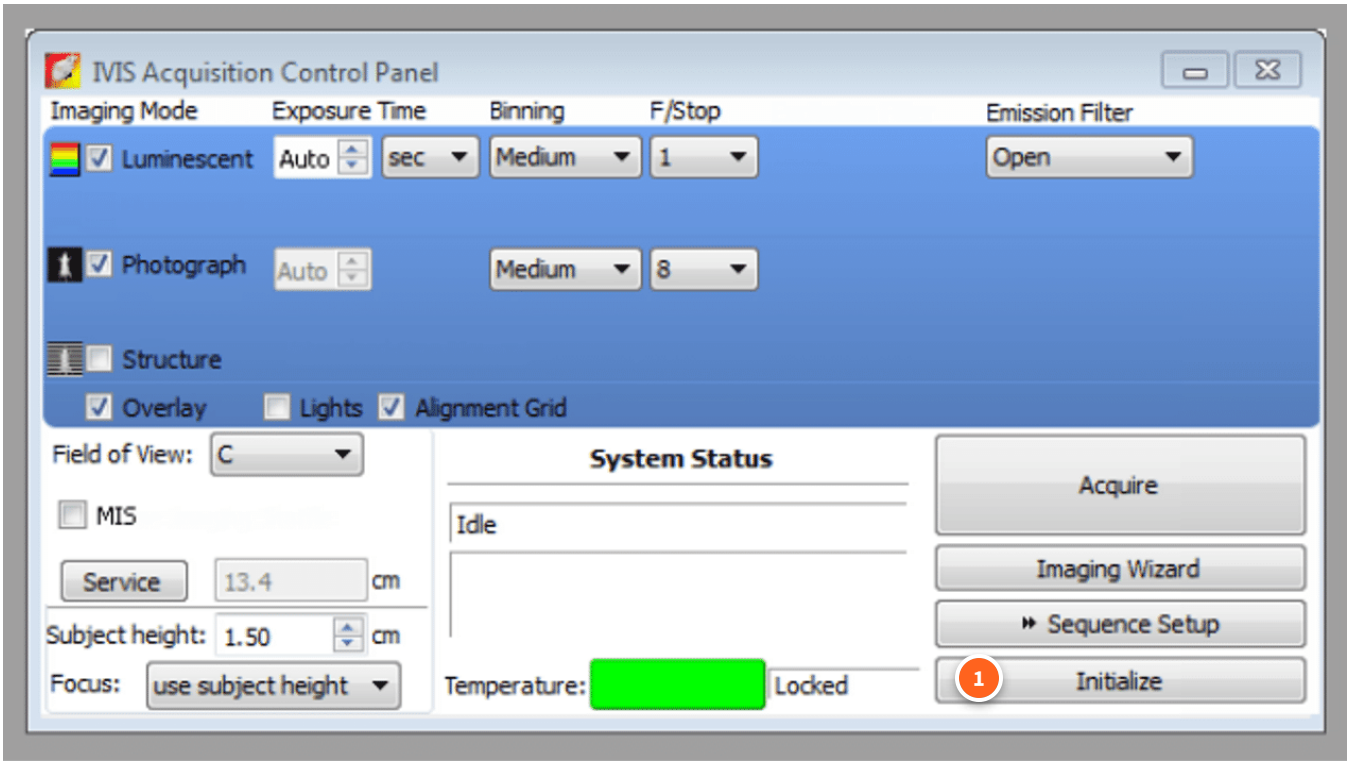
IVIS Acquisition

After the system is initialized and animals are in the chamber, you can now collect your images.

The first image you Acquire will prompt the software to ask if you want to "Auto Save" your data. Say **YES**. Set the file location where you want the images to be saved.

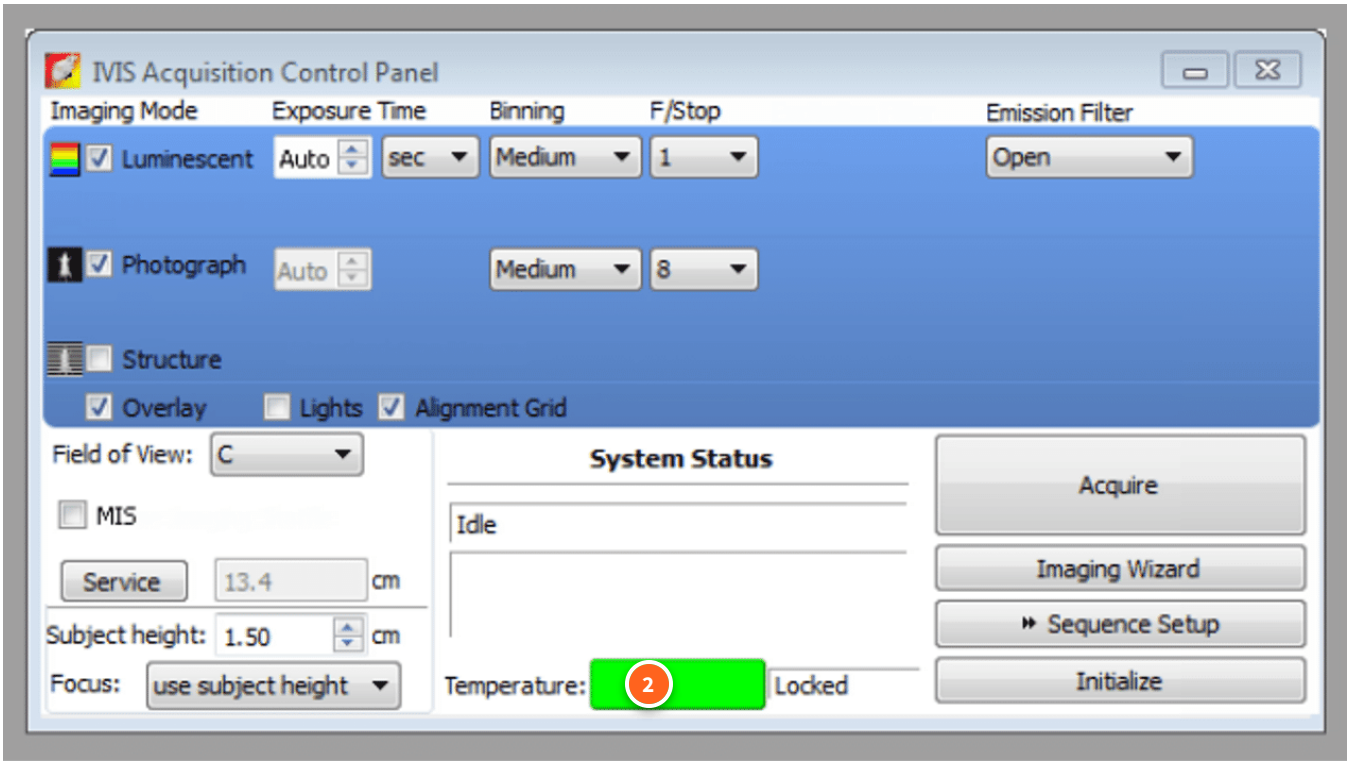
Click on the steps below to explore the different imaging parameters and the way that images are acquired in the Living Image software.


IVIS Acquisition Control Panel
Imaging Mode
Exposure Time
Binning
F/Stop
Emission Filter
Luminescent
Auto
sec
Medium
1
Open
Photograph
Auto
Medium
8
Structure
Overlay
Lights
Alignment Grid
Field of View:
MIS
Service
13.4
cm
Subject height:
1.50
cm
Focus:
use subject height
System Status
Idle
Temperature:
Locked
Acquire
Imaging Wizard
Sequence Setup
Initialize
1
2
3
4
5
6
7



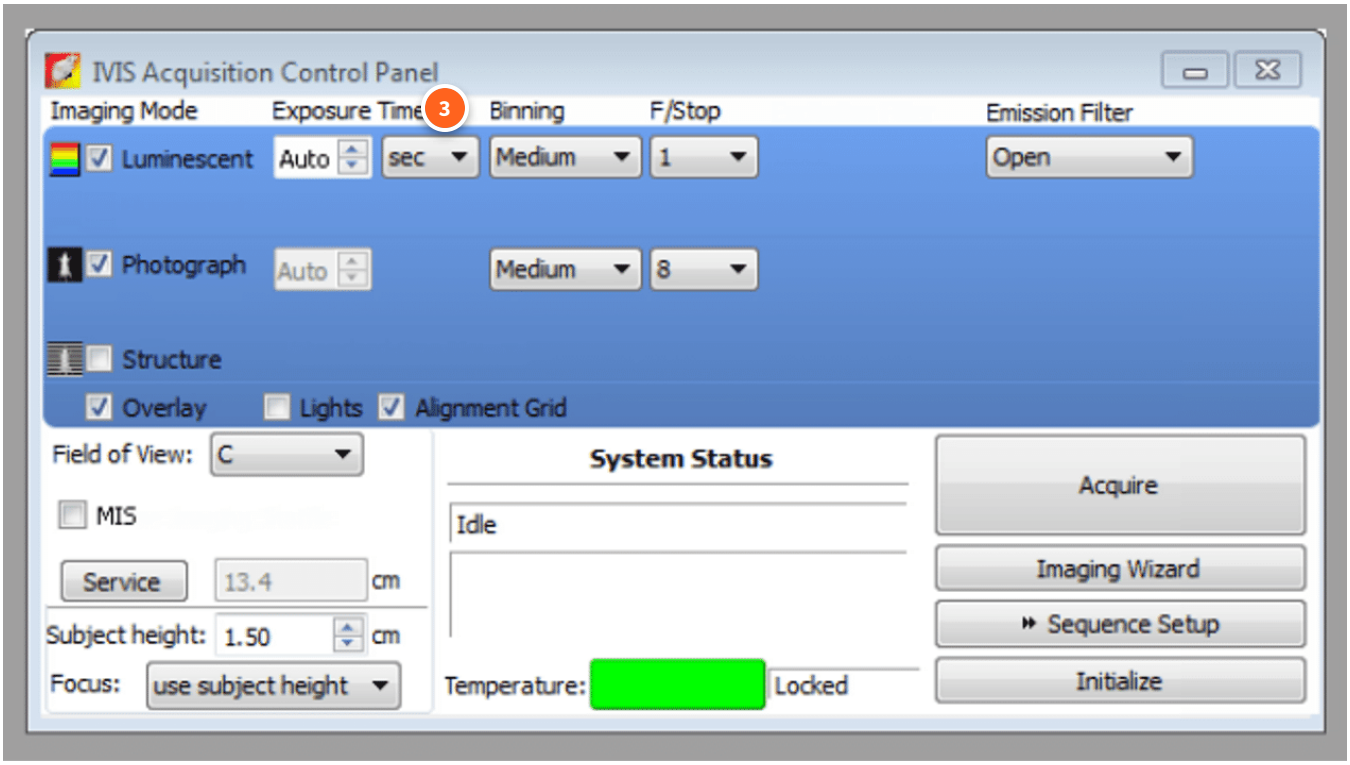
Initialize

As discussed in section 3, start by pressing initialize. Do this at least 10 minutes before your animals need to be imaged.



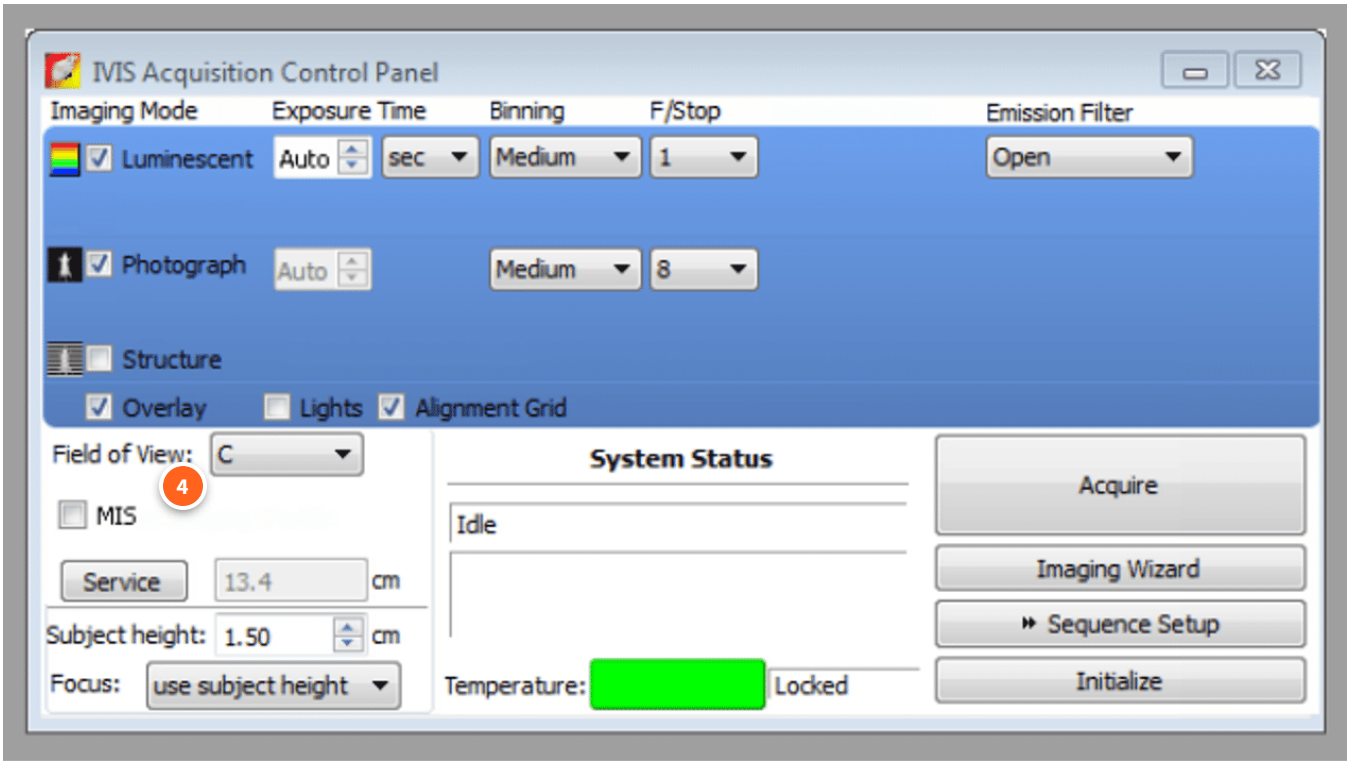
Ready status

Green bar indicates imaging can proceed as the temperature of the camera is at (or close to) -90 °C. Click on this bar to see the temp status, once at -86 you can proceed with imaging.



Exposure Time

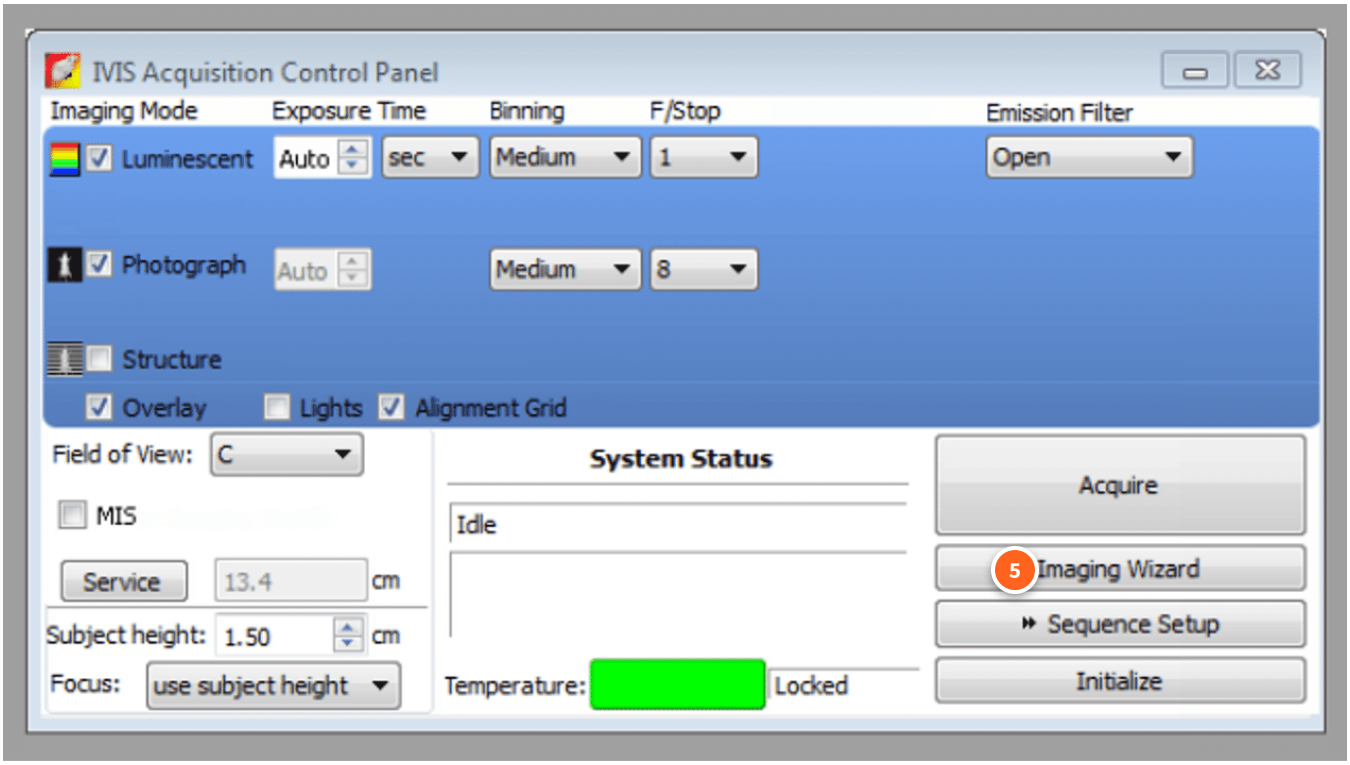
For most applications we recommend setting the exposure setting to "Auto". This acquires an image until the signal acquired is optimized by the dynamic range of the camera, which is at ~600-60,000 counts. This will ensure an optimal signal-to– noise ratio for images.
However the time of exposure may vary between subjects you can image at several timepoints. However you *must* make sure you are acquiring enough signal so that the dynamic range of the sensor is optimized, that way the data can be quantitative. Contact Preclinical Imaging if you would like further advice or discussion on this topic.



Field of View (FOV)

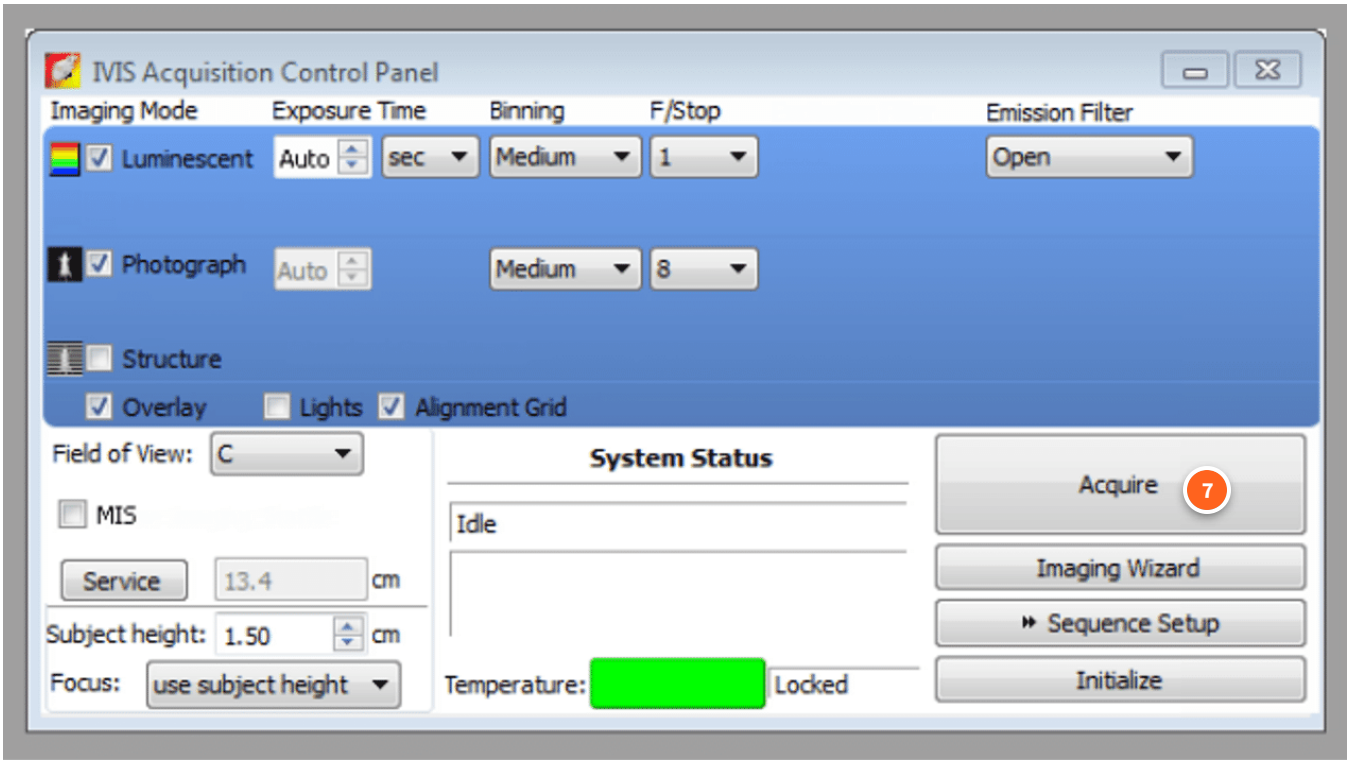
Green laser projects the imaging area onto the stage. This setting affects the subject height. Recommended field of view settings are given below:

Number of mice being imaged at a time	FOV setting
5 mice per image	D
3 mice per image	C
1 mouse per image, zoomed in on particular location	B
Small tissue sample only	A



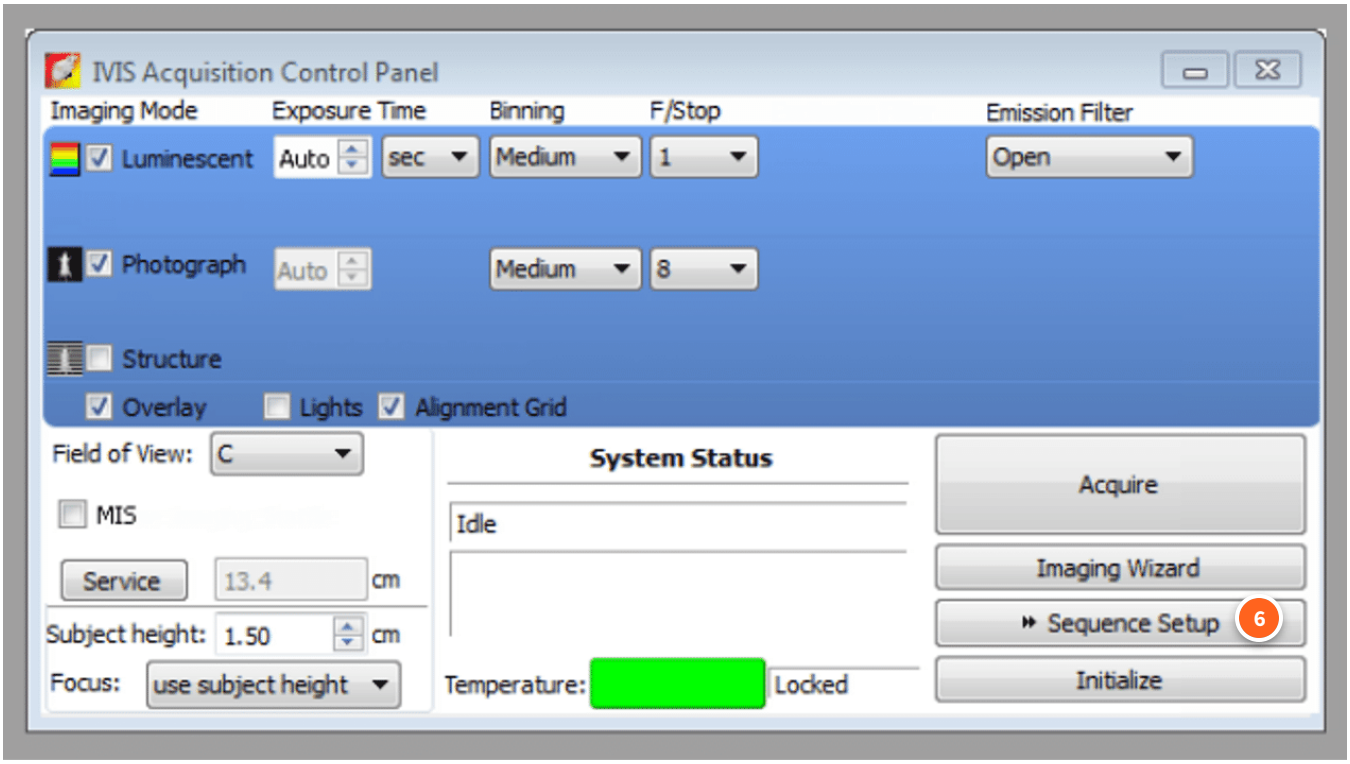
Imaging Wizard

The Imaging Wizard provides step-by-step help with setting imaging parameters. We strongly recommend using this when performing fluorescence imaging.



Acquire

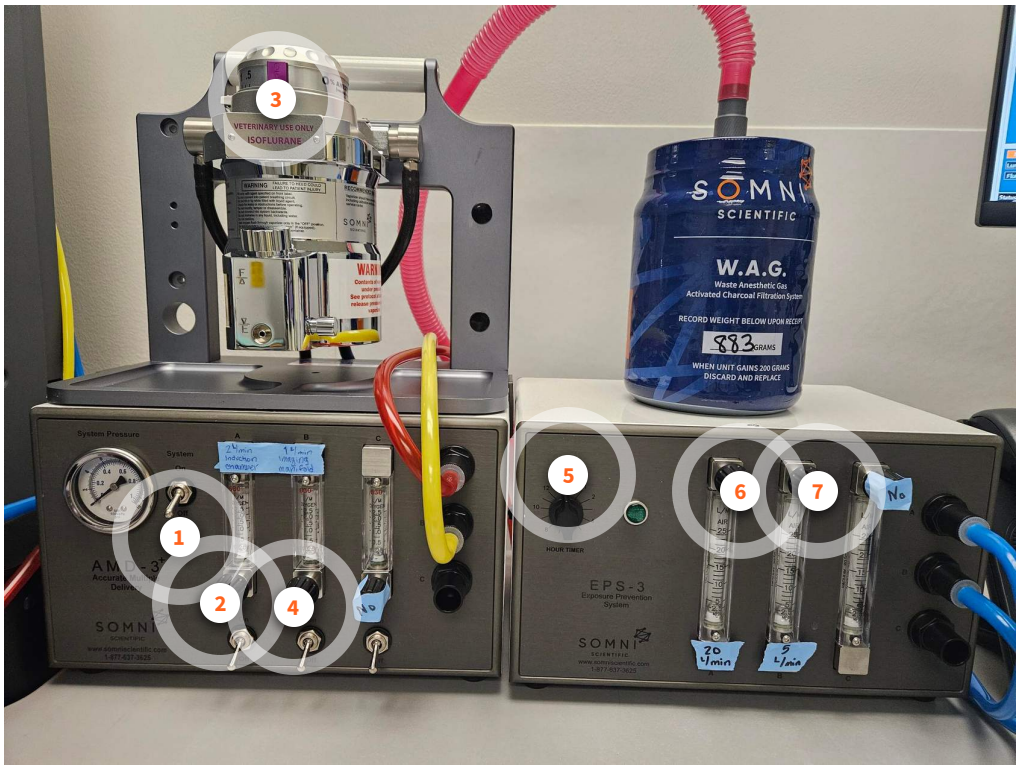
Once all other parameters are set, Acquire will take the image. At first a pop up box will appear asking if you want to autosave images. Say yes and set the file path. Then input the sample information

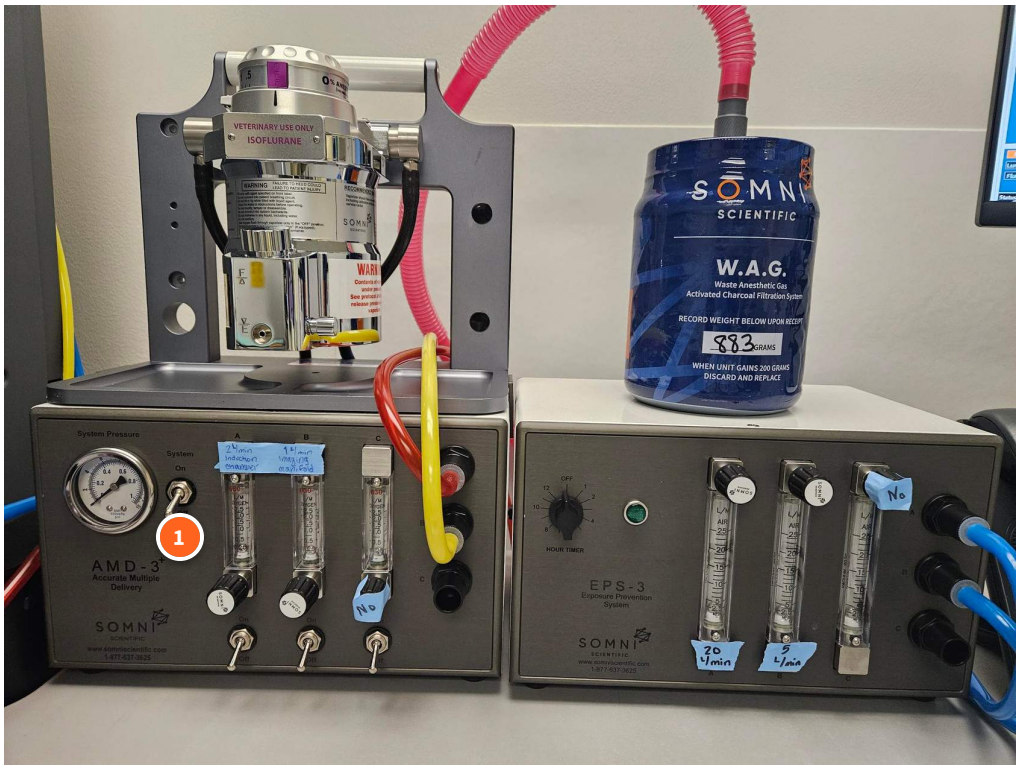


Sequence Imaging

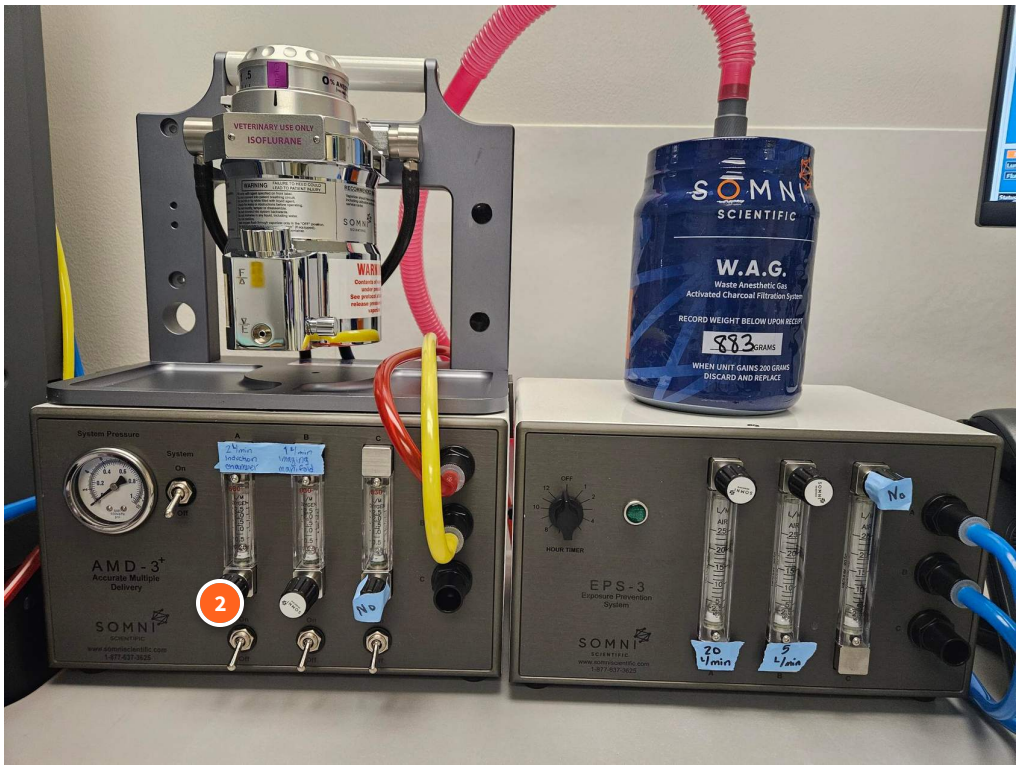
You have the capability to set up multiple images to acquire in succession varying in parameters.

LagoX Preparation


3
VETERINARY USE ONLY
ISOFLURANE
SOMNI
SCIENTIFIC
W.A.G.
Waste Anesthetic Gas
Activated Charcoal Filtration System
RECORD WEIGHT BELOW UPON RECEIPT
883 GRAMS
WHEN UNIT GAINS 200 GRAMS
DISCARD AND REPLACE
System Pressure
1
2
4
5
6
7
EPS-3
Exposure Prevention System
SOMNI
20 L/min
5 L/min
No

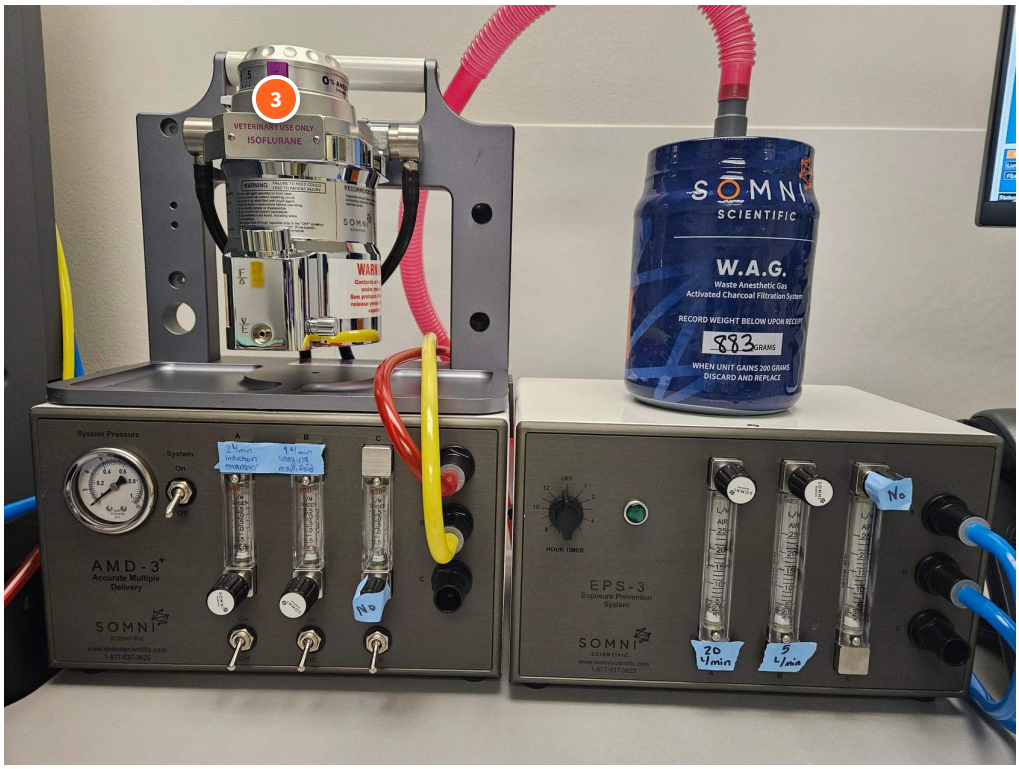


Open O2 tank and anesthesia system on



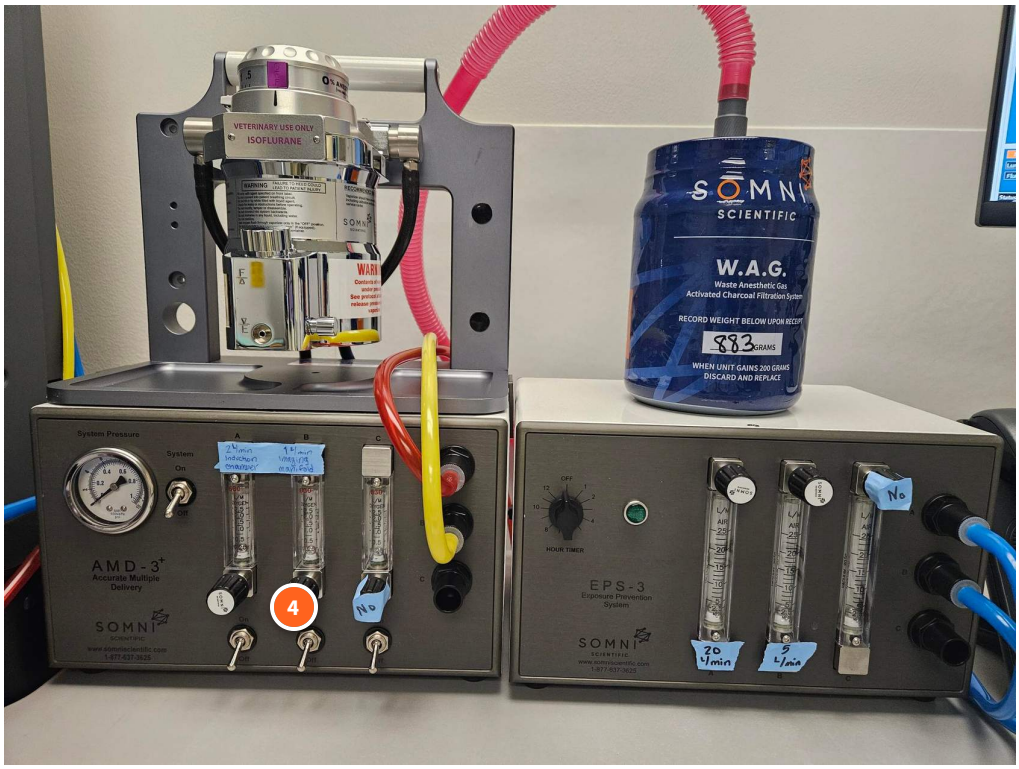
Switch on Induction Chamber

set to 2 L/min



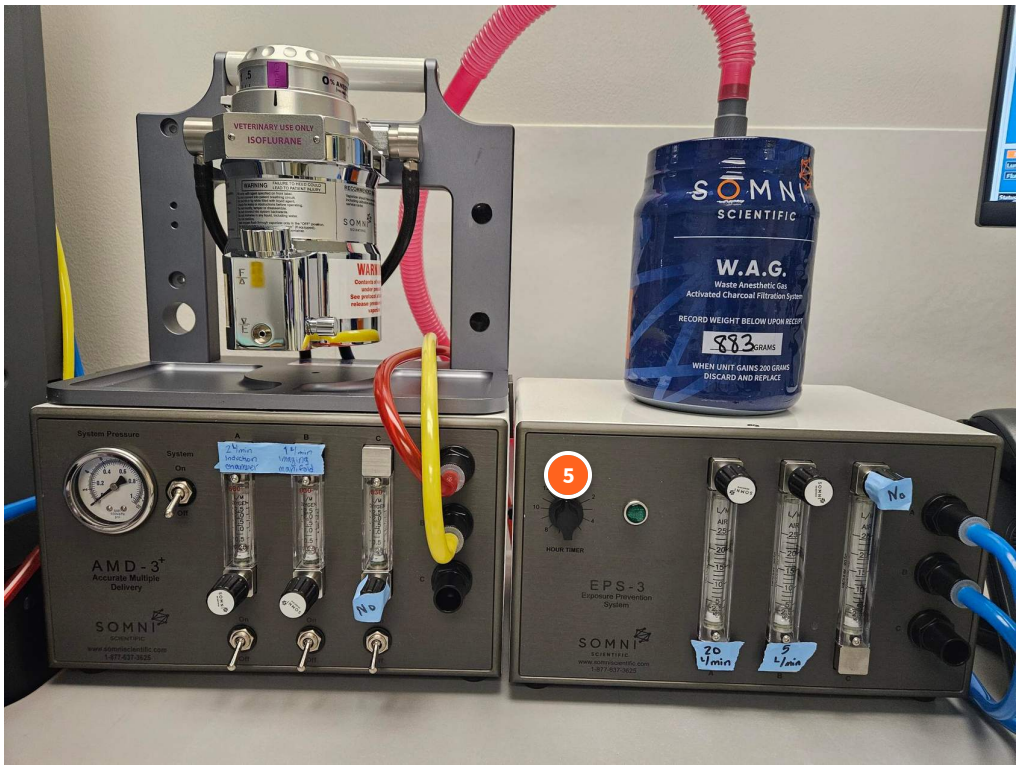
Turn on vaporizor

2.5% iso for induction



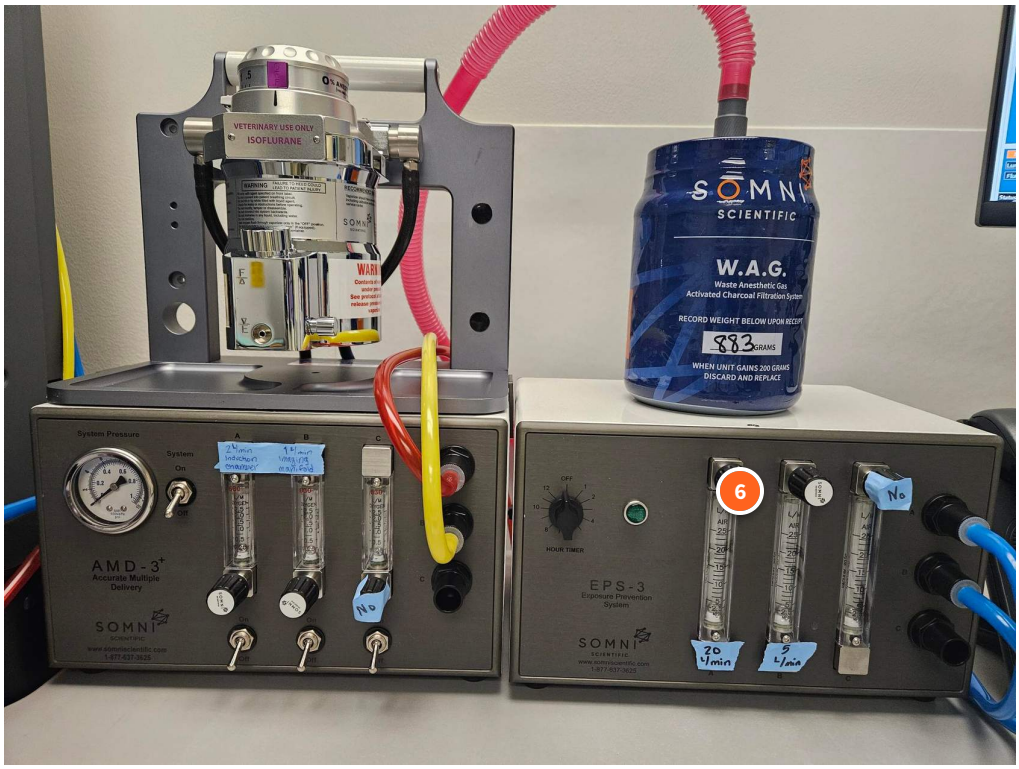
Switch on Imaging Manifold

Once mice are in the Lago set this to 1 L/min



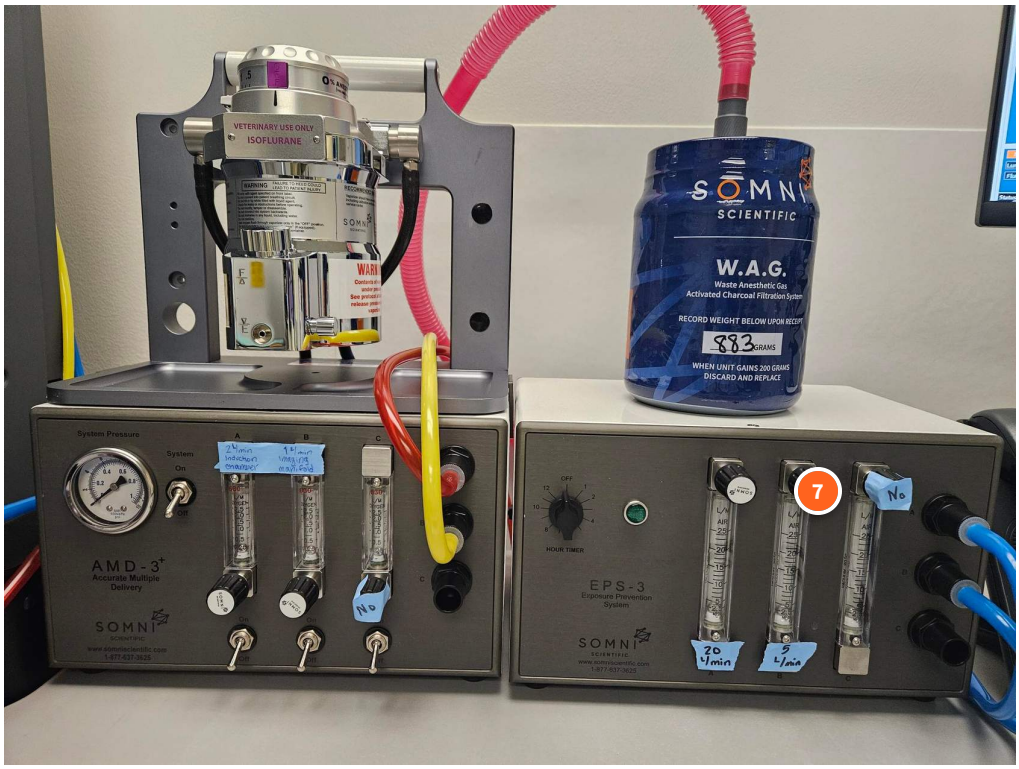
Turn on exhaust

exhaust can be switched on to 1+ hours



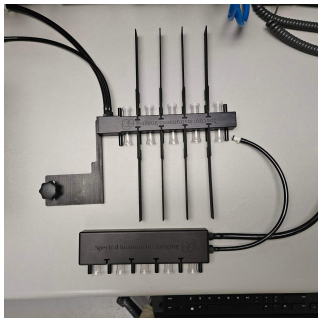
Induction Chamber gas exit port

set to 20 L/min

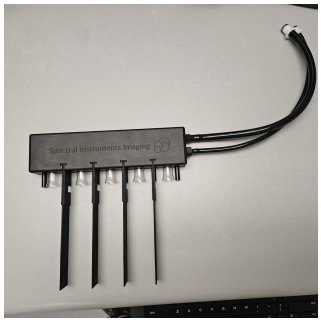


Imaging Manifold gas exit port

set to 5 L/min

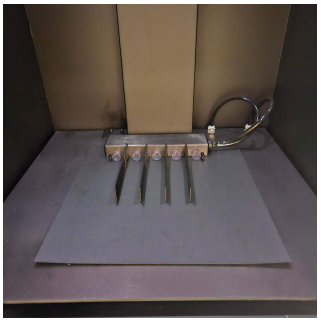


10 mouse manifold



5 mouse manifold

Calibration device and plate holder. Used to center device and well plates, Plate holder connects on the pins to the left



Manifold within Lago: gas and exhaust cables connect on the right

LagoX Acquisition

Your user name is your data folder name, which is automatically saved in the location PC > OS (C:) > Users > SII > user.

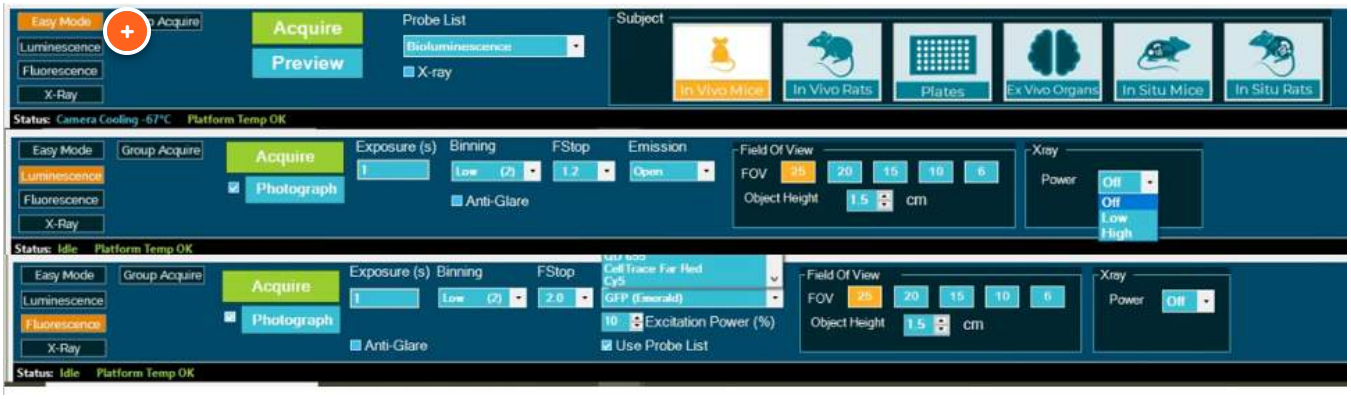
TIP: Make a shortcut on the desktop to your data folders

Below are the 3 options for image acquisition. Note : X-ray can be included in all 3 or as a stand alone option.

Easy Mode

Luminescence

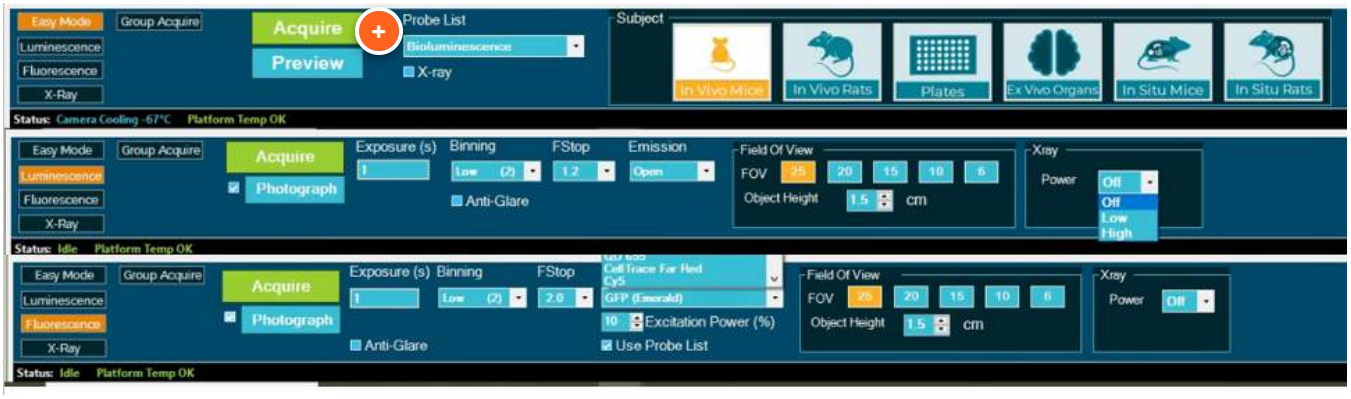
Fluorescence



Easy Mode

Set to Easy Mode, this acquires an image so the brightest pixel in each mouse is approximated to the specific target max

YouTube video demonstrating this: https://www.youtube.com/watch?v=v07OPtxcPKY

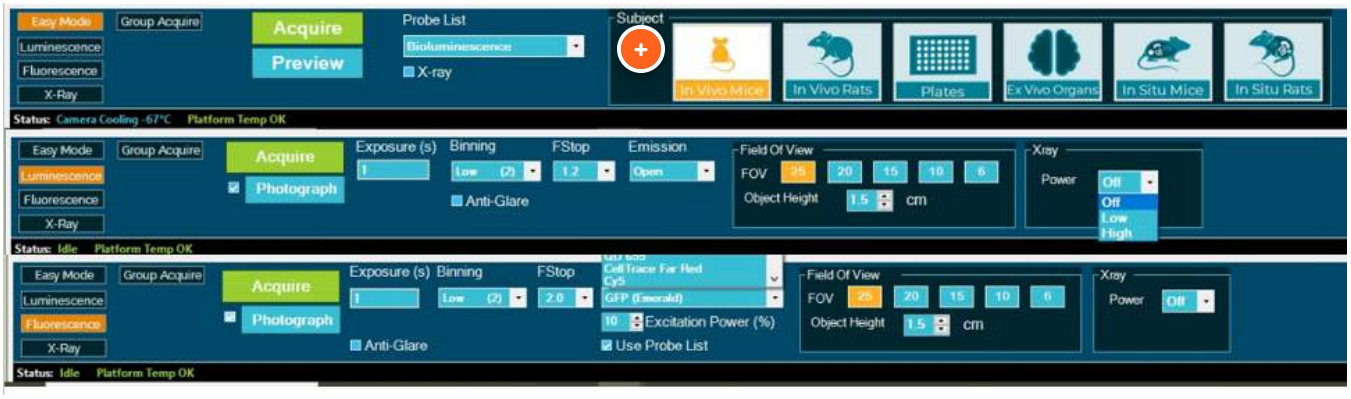


Aquire

Imaging system is ready when status is "idle" on bottom left of the screen.

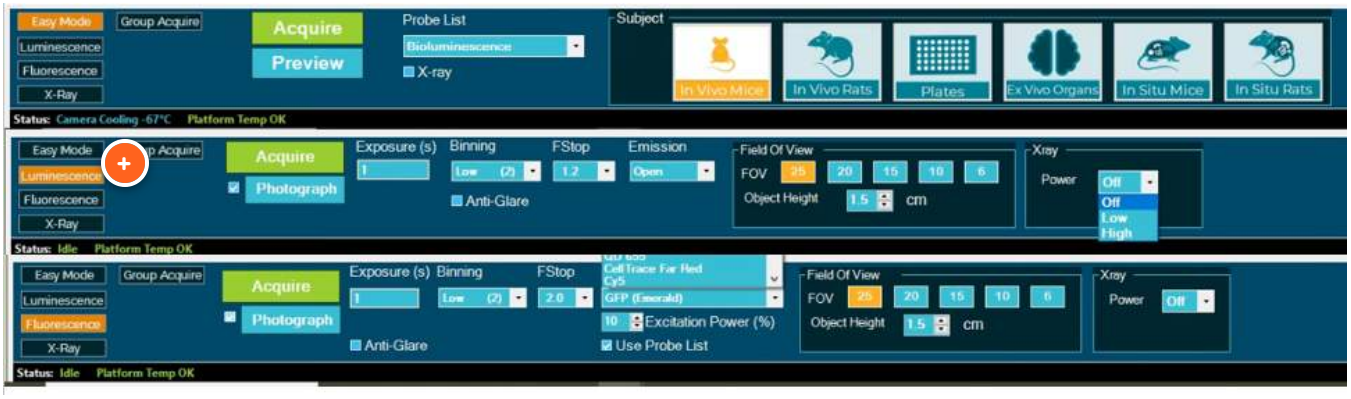
After selecting imaging mode(s) you can acquire the images.

To acquire multiple images choose group acquire. You will first need to define a group protocol.
https://www.youtube.com/watch?v=5jnD93ZONns



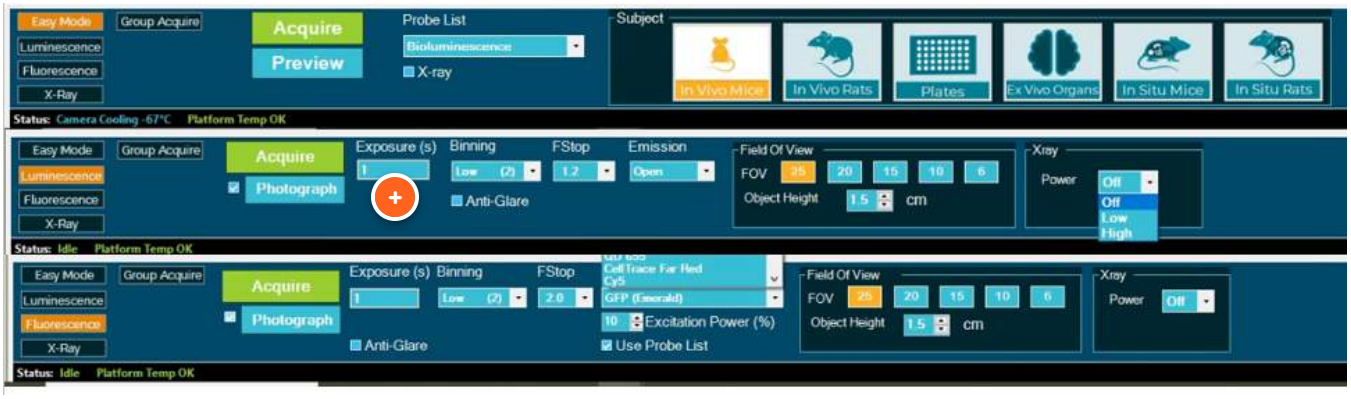
Choose Imaging Subject

For Easy Mode you will have the option to choose your subject. This will ensure that accurate subject height is registered.



Luminescence

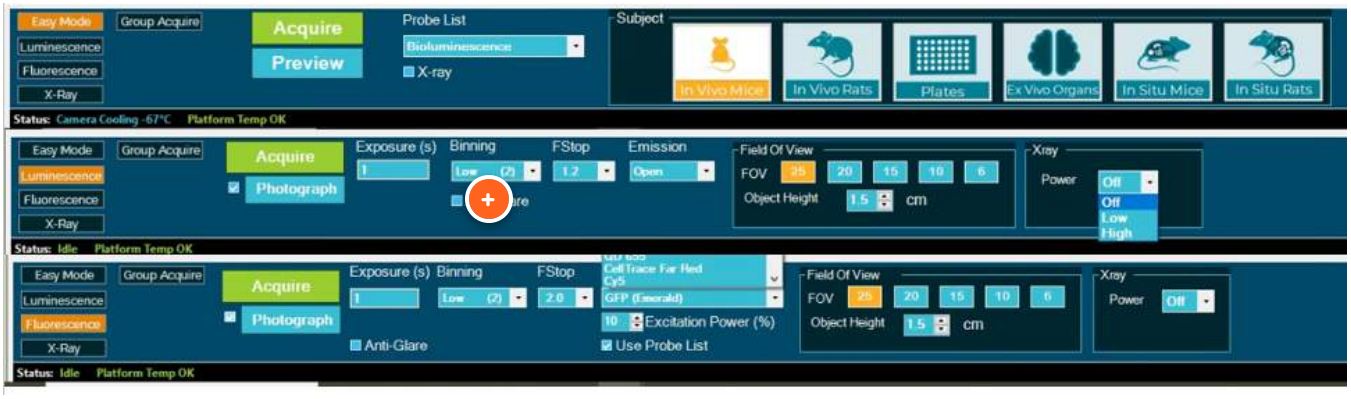
Luminescent mode acquires an image of light emitted from an Unilluminated sample, for example, bioluminescent emission from plants, animals, or in vitro sources such a cells in microplate wells. There are no lights on inside the imaging chamber during acquisition.
https://www.youtube.com/watch?v=HILyzfeTE7I



Exposure Time

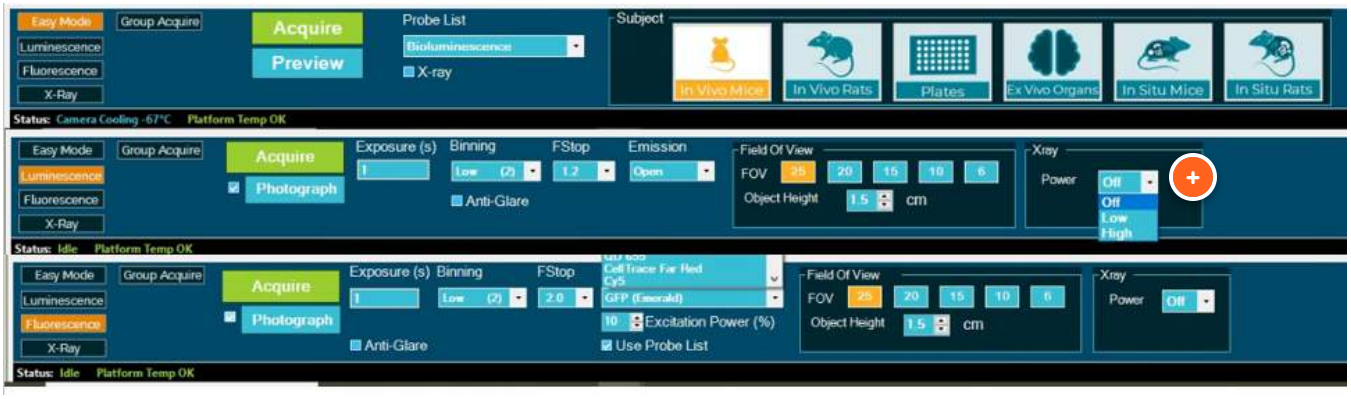
The amount of time (seconds) the shutter is open during image acquisition. Longer exposure times result in higher sensitivity but might result in a saturated image.

Reach out to the PCI team to discuss the best exposure time setting for your study.



Binning

Sets the pixel size of the CCD camera. A higher level of binning increases sensitivity but reduces spatial resolution. Higher sensitivity is due to a higher signal-to-noise ratio resulting from binning. Also, binning increases signal. An 8 x 8 binning level produces a super pixel that is 64 times as large with 64 times the signal compared to a non-binned pixel.



X-Ray

you need IACUC approval before using this setting - reach out to PCI team for assistance getting IACUC approval

Low Power: 44kV tube voltage and 175uA tube current.
High Power: 50kV tube voltage and 200uA tube current

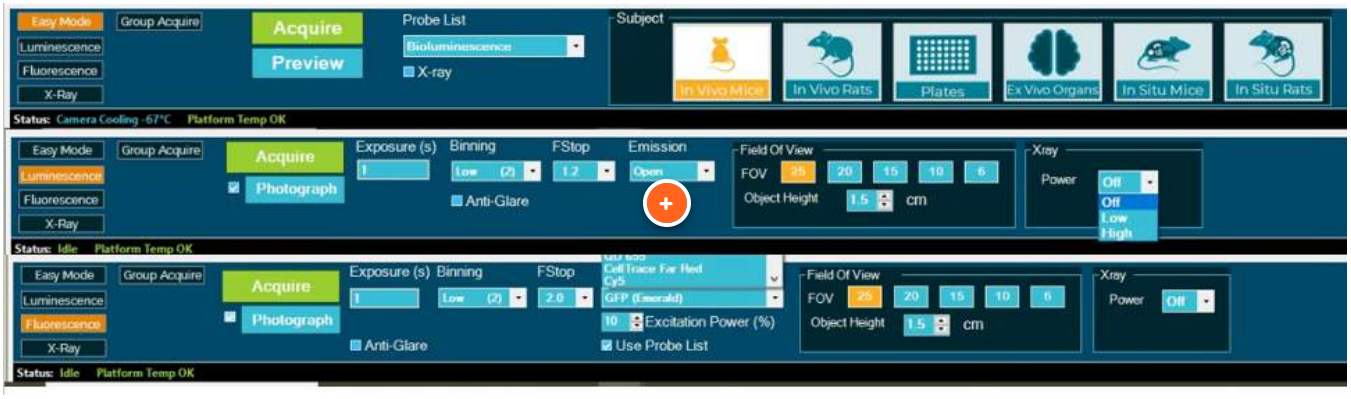
Resolution - Binning level for the X-ray image.

High resolution = no binning
low resolution = 2 x 2 binning.

FStop

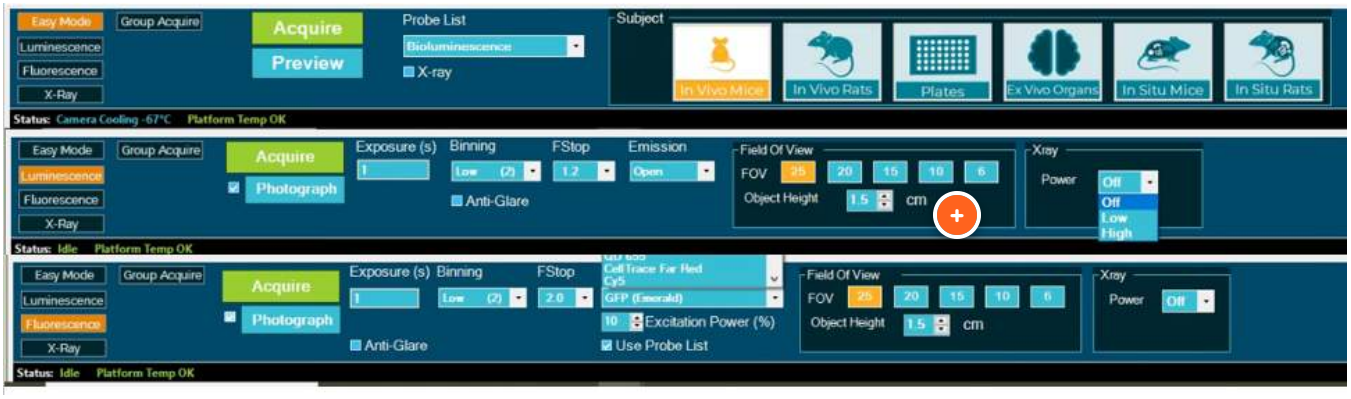
the FStop determines the size of the lens aperture. F/1.2 is the largest aperture setting, and provides maximum sensitivity, but also the smallest depth of field (the range over which the image is in focus). As the F Stop number increases, the aperture size decreases and the depth of field increases.

Almost always the FStop 1.2 setting is suitable for mouse imaging experiments



Emission

Leave this set to open



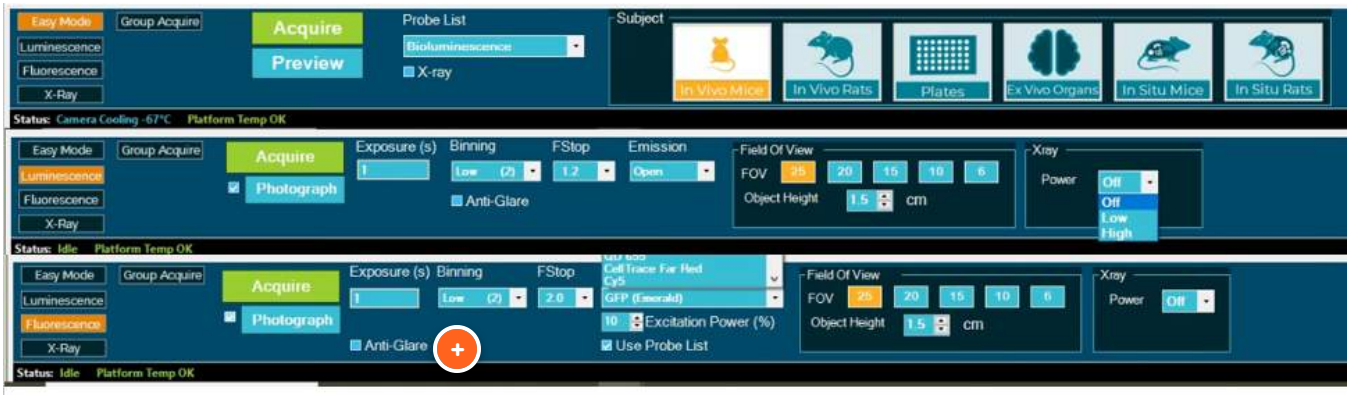
Field Of View

Field of view is the width of the area to be imaged. It is good practice to set the FOV no larger than is necessary to include the subject(s) because sensitivity decreases as the FOV increases. This is because the imaging platform moves farther from the camera as the FOV increases.

25cm= 5-10 mice

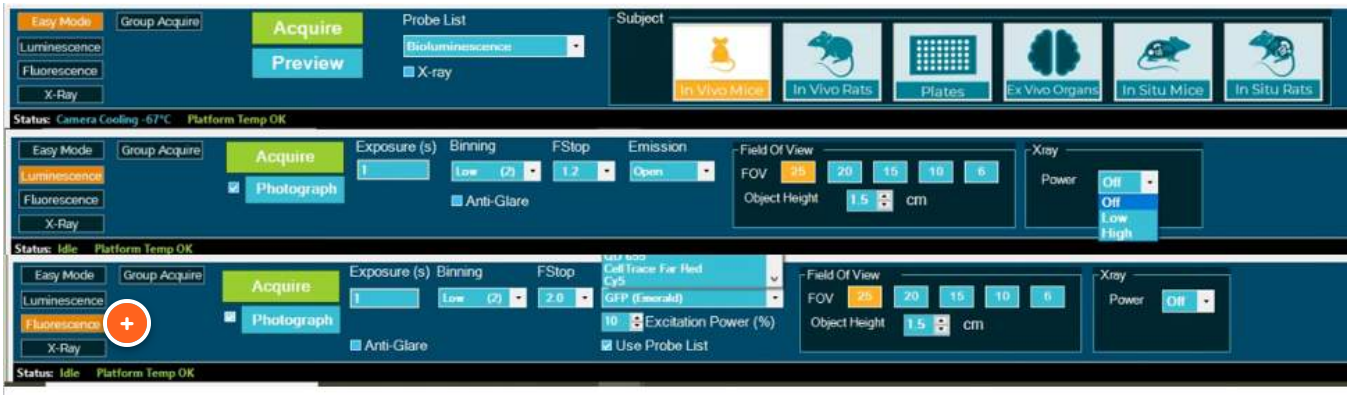
20cm= 5 mice

15cm = 3 mice



Anti-Glare

NOTE: If photographs are saturating with the normal photograph settings (due to shiny surfaces, for example), click to check the box next to anti-glare. The anti-glare photograph mode will take about four times as long, but will result very little or no saturated pixels or blooming in the photograph. When using this special anti-glare photograph mode, the exposure time of the photograph usually needs to be increased to prevent grainy photographs.



Fluorescence

The LagoX offers significantly improved fluorsence capability than the IVIS!

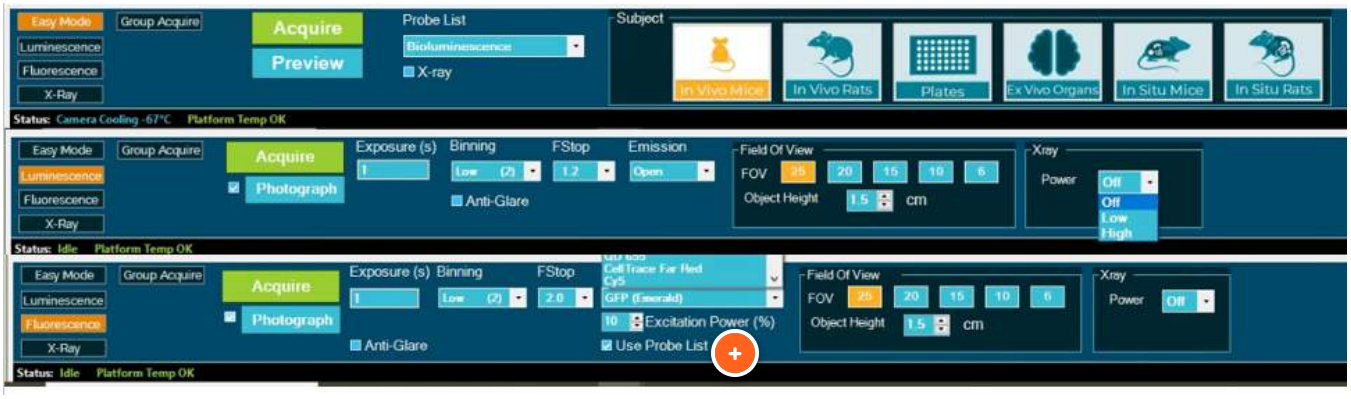
Youtube video demonstrating how to acquire in fluoresence mode: https://www.youtube.com/watch?v=BDfH3vV2MRY

You can also see this video for background on fluoresence imaging principles with the Lago and choosing the right filter set and excitation wavelength for your animals: https://www.youtube.com/watch?v=crIMOJHxSFA&list=PLXFBpuxnMStcO7n-xggFsjogsMgJ5vHI8&index=2

The LagoX has **emission** filters for: 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, and 870 nm.

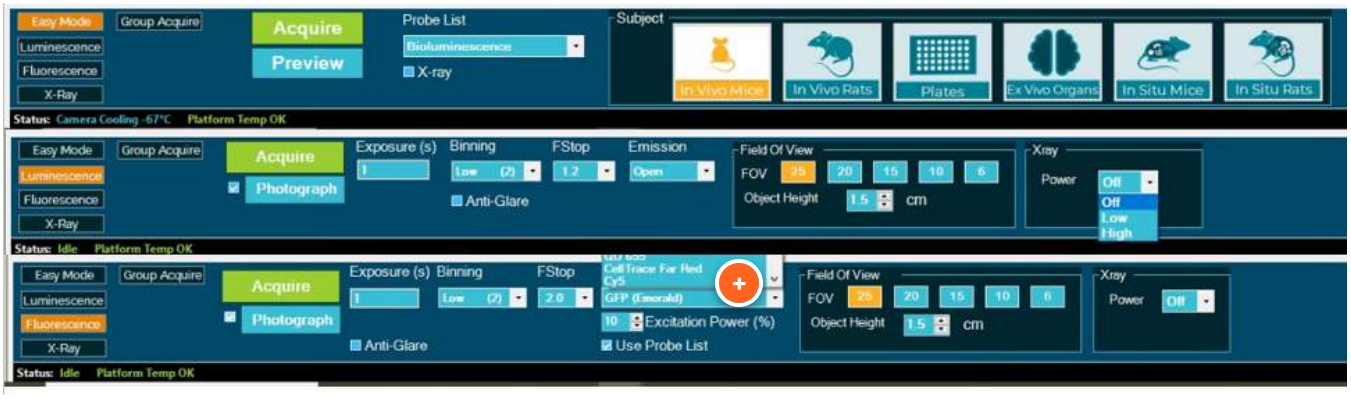
Each emission filter has a 20 nm transmission window, thus a 670 nm emission filter transmits all light from 660 to 680 nm.

Pairs of excitation LEDs positioned around the lens provide the excitation energy necessary for fluorescence imaging. The LED light is diffused and filtered to give uniform illumination at 360, 405, 430, 465, 500, 535, 570, 605, 640, 675, 710, 745, 770 and 805nm. The full spectral width of an excitation LED source is 20 nm, so for example, when 570 nm excitation light is selected, the emitted wavelengths will include 560 -580 nm.



Excitation Power

Investigators can select a power level, 0-100%, for the LED that they are using in their FLI. Choosing % Power is simply a means of adjusting the photon flux rate (total photon/sec) emitted by the LED of choice. If you double a LED's % Power, its flux rate is doubled. Our general recommendation if to start with % Power settings between 10-50 % (...you can use 10% as a default for all FLI). If fluorophore molecules remain undetected, then higher LED % Power settings may be successful in getting signal from weaker FLI models. Bottomline: It is good to use only the number of excite photons/sec needed in a given FLI model. Excess excite photons can challenge the camera system emission filter, as low incident angle excite photons can occasionally get through the emission filter to the camera sensor...and minimizing noise of this nature is always a good idea.



Probe List

Editing FLI Probe List: To add to the FLI Probe List pull down menu, proceed as follows:
·Go the gear icon in the bottom right corner of the Aura main page (see 1st image below),
·Select "User Acquisition Preferences (see 2nd image below),
·Select "Probes" tab (see 3rd image below),
·Select probes of interest from the "Master Probe List" by highlighting the probe name (see 4th image below),
·Select "Add Item," and
Select "User Probe List" to be sure that the selected probe(s) have been added to your newly expanded probe list.

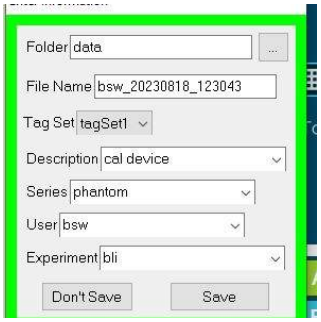
Image Label

When you start to acquire a red highlighted info box will appear (on the right of the screen) and does not allow info to be saved till after imaging.

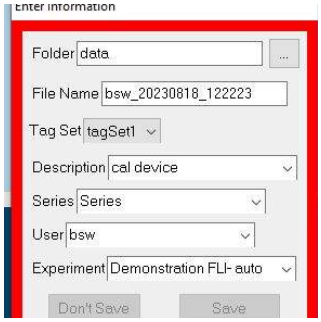
Enter image info once the box is green (and on the left of the screen). This is when you will be able to save info.

It is recommended to not change file name, TagSets can be created for reoccurring study cohorts.

Examples of info: Experiment (area of study), Series (animal IDs), Description (specific image conditions).

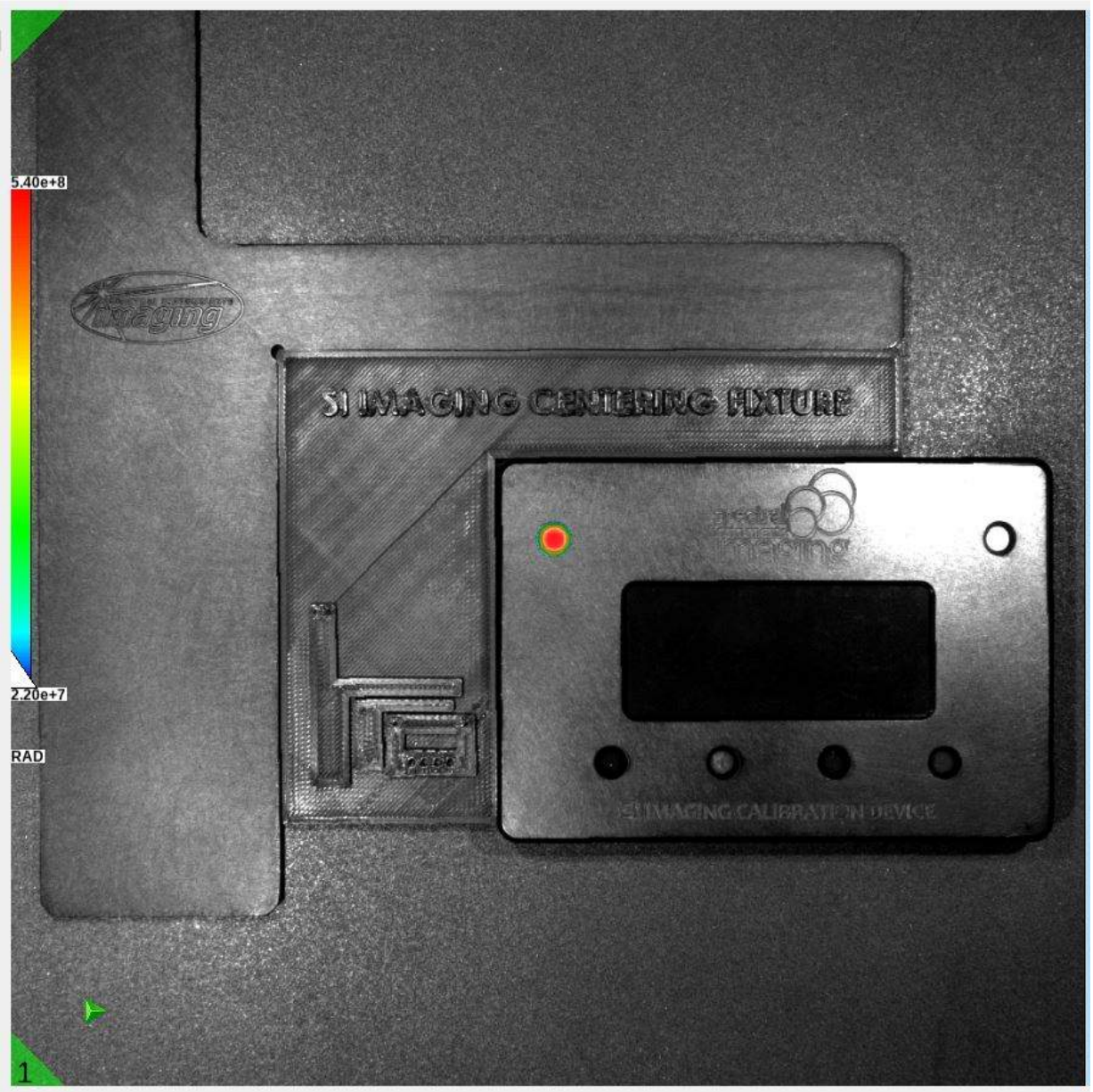


Info box appears on the left after imaging, able to save



Info box appears on the right during imaging

Recommend to start and end every study with an image of the calibration device for future reference.



Take an image of the calibration device using using Easy Mode

---

Why is "auto or easy mode" the recommended exposure time for most basic imaging settings?

- It is faster
- It gives more quantitative comparative images
- Because the machine will acquire the image until the camera has detected enough bioluminescence or fluorescence light to give a good signal-to-noise ratio

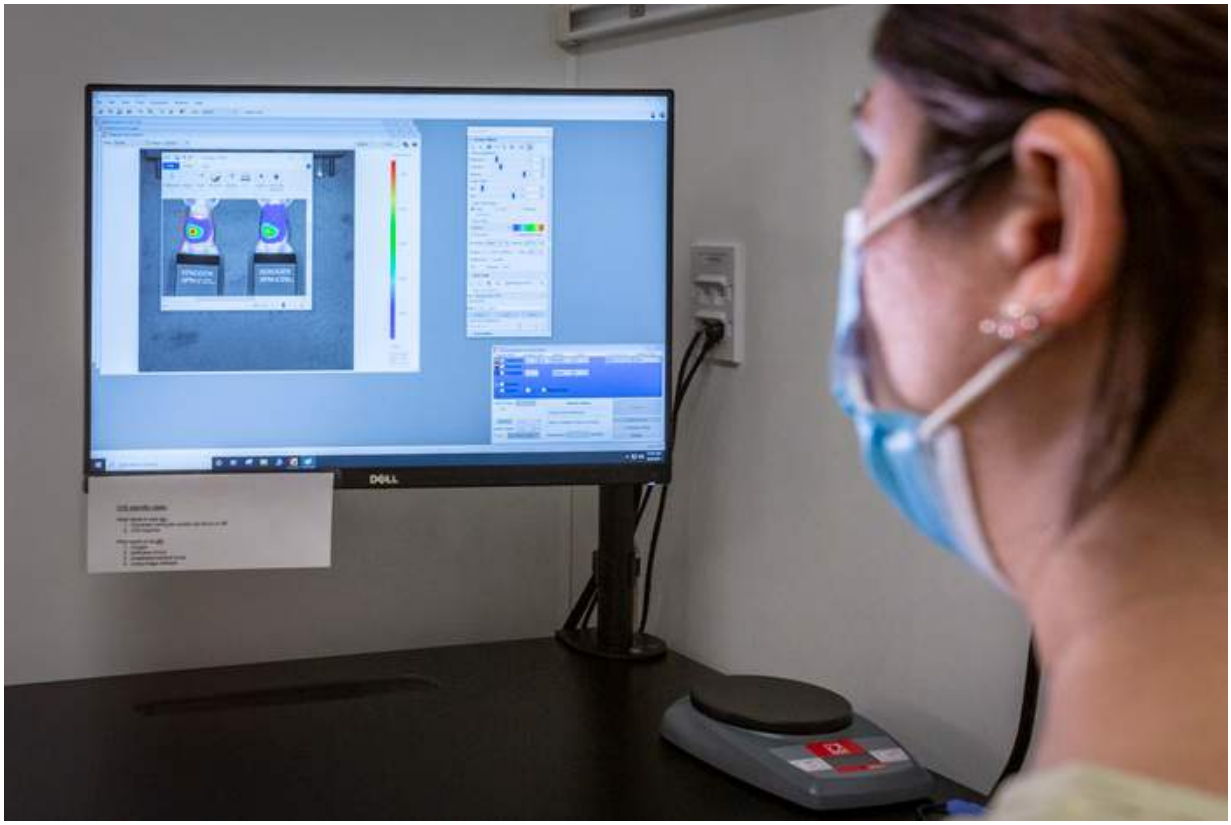
SUBMIT

Complete the content above before moving on.

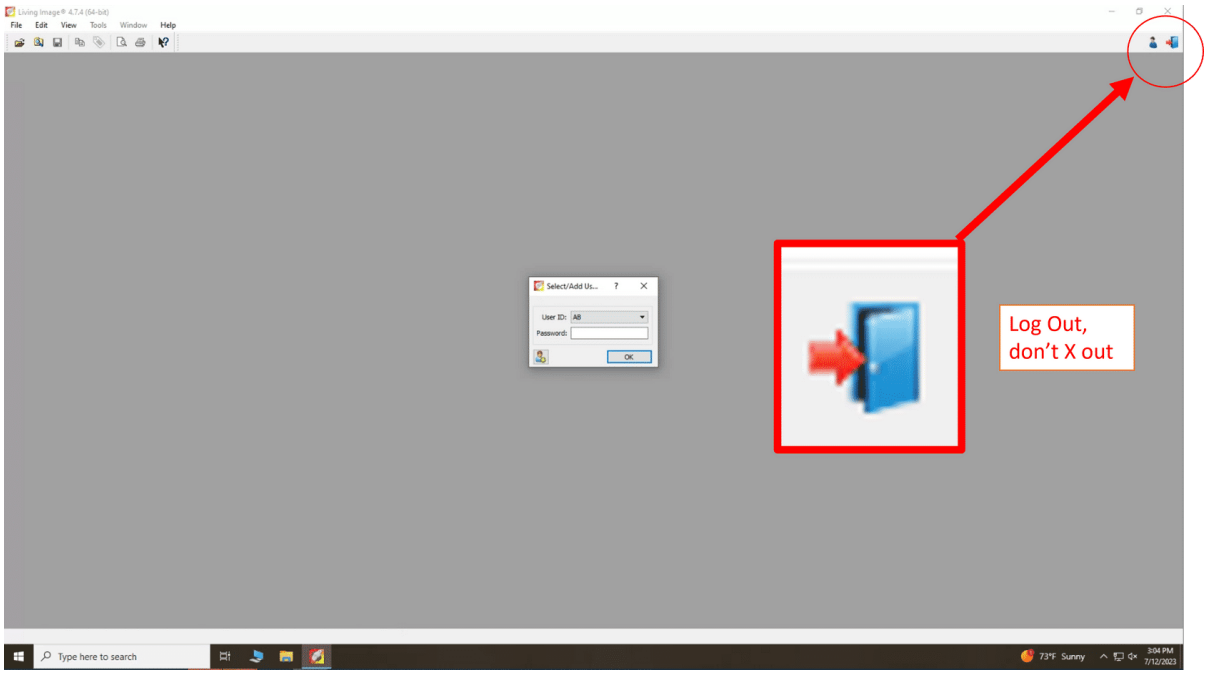
Lesson 5 of 7

Wrapping up

PI Preclinical Imaging



Shut down procedure



First you must recover your animals, then turn off the anesthesia equipment used. Leaving imaging system on. For IVIS please note that we recommend leaving software on and open, logging out of your account.

Step 1

Recover animals

Remove animals from anesthesia, return to a warm cage and monitor as they are waking up.

Step 2

Turn off components

- Close O2 tank and isoflurane vaporizer off and allow O2 to flush through the system
- Close switch to "Manifold" and "Induction Chamber"
- Turn oxygen lever off
- Turn exhaust pump to "off"

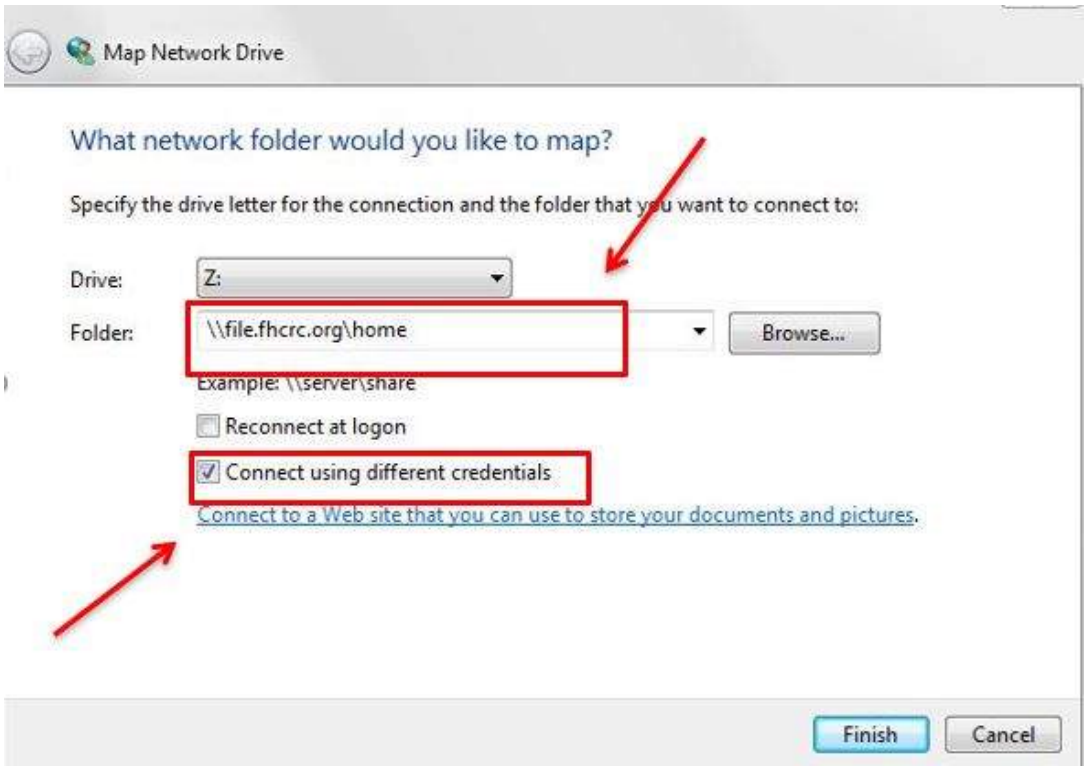
Step 3

Clean up!

- Clean chamber with ethanol or C-Dox do not spray directly into chamber
- Clean anesthesia box, please use C-Dox (not ethanol)
- Refill vaporizer with isoflurane

Step 4

Save your data!



Preclinical Imaging is not responsible for saved data. It is the responsibility of the user to copy and store their data in a different location.

1. Open File Explorer
2. Go to This PC
3. Click on "Map Network Drive".
4. Fill in "network location" with: \\file.fhcrc.org\home "Z:" drive should be selected. Box labeled "connect using different credentials" should be checked.
5. Log on with your hutch user ID (followed by @fhcrc.org) (your ID should look like username@fhcrc.org.) and password
6. Once connected to the network, go to the location in your personal Z: drive where you would like to save your images.

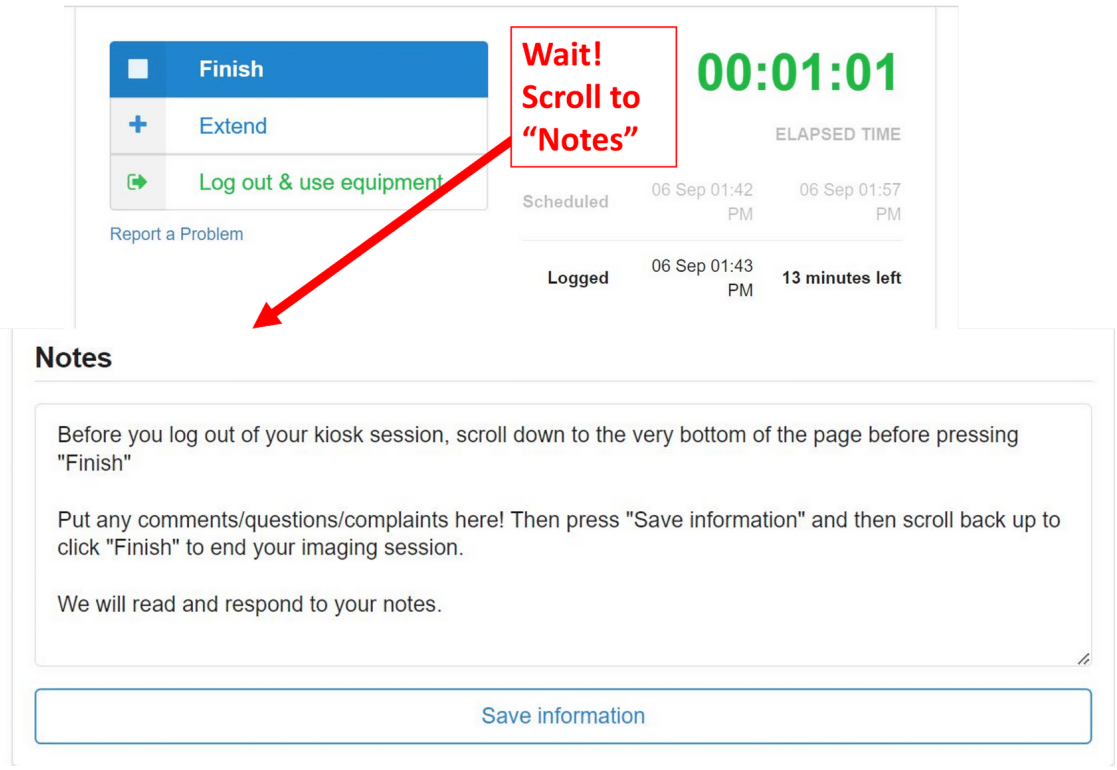
7. In a new File Explorer tab, open the folder on the desktop where you saved your IVIS images or the data folder your Lago images automatically saved from this day's sessions.
8. Transfer the files from the Desktop folder to the desired folder in the Z: drive.
9. Once data transfer is complete, go to "This PC" and right click on your Z: drive. Press "delete network drive" to disconnect.

These instructions are also printed out in the room.

When you return to your computer you should see the data you transferred in your Z: drive (under "This PC").

If you do not see a Z: drive at your home computer follow the above steps on your own computer to map to your network drive.

log out of iLab equipment kiosk



Go back to the equipment kiosk and click "Finish"

Put any questions or complaints within the notes section when you log out of the iLab kiosk!

- We read these and will follow up with you

CONTINUE

Further Resources

PI Preclinical Imaging

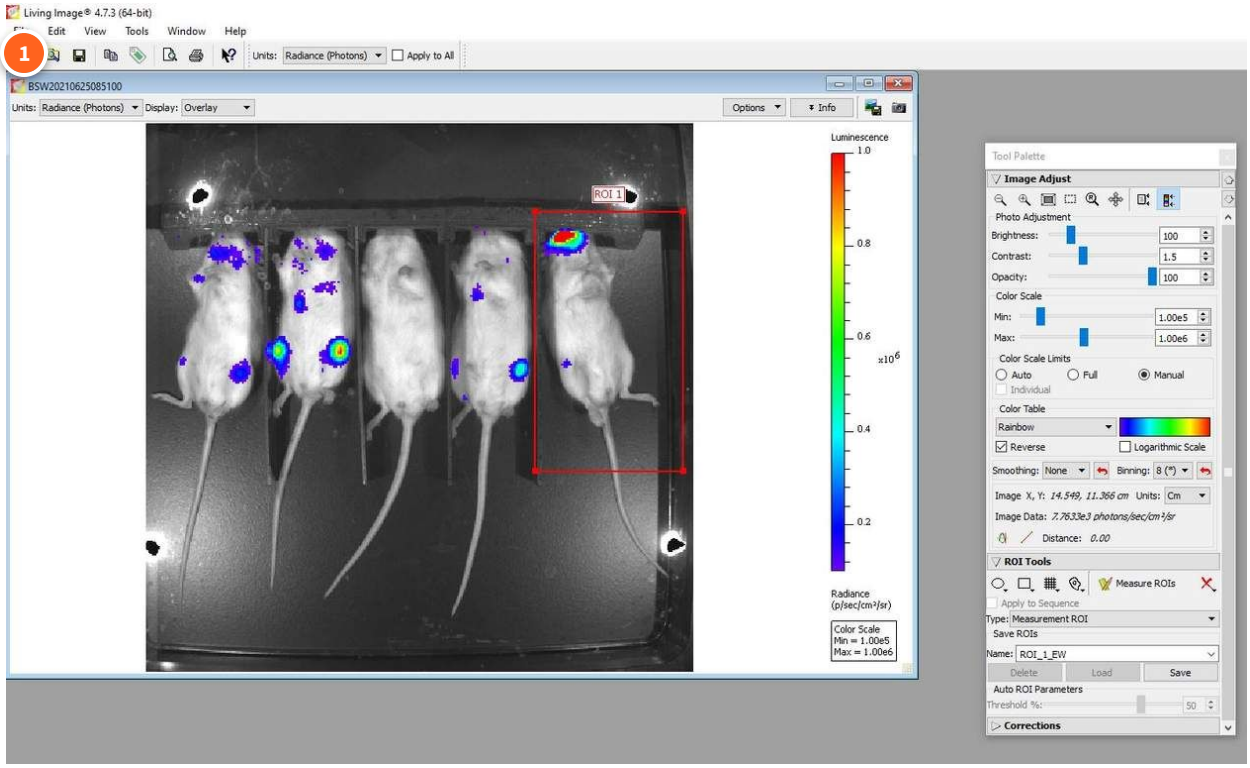
How do I analyze the data I have taken?

After you transfer your data to your personal network drive, the images can be viewed in the Living Image or Aura software depending on system used. You will need to copy the data on the Z: drive to the computer desktop in order to load the images into the software.

Copies for Living Image and Aura are on all Arnold Library Computers and the Steam Plant PC #8. Aura is free to download on your office desktop.

For IVIS


Living Image® 4.7.3 (64-bit)
File Edit View Tools Window Help
Units: Radiance (Photons)
Apply to All
Units: Radiance (Photons)
Display: Overlay
Options
ROI 1
Luminescence
1.0
0.8
0.6
x10^6
0.4
0.2
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 1.00e5
Max = 1.00e6
Tool Palette
Image Adjust
Photo Adjustment
Brightness: 100
Contrast: 1.5
Opacity: 100
Color Scale
Min: 1.00e5
Max: 1.00e6
Color Scale Limits
Auto
Full
Manual
Individual
Color Table
Rainbow
Reverse
Logarithmic Scale
Smoothing: None
Binning: 8 (*)
Image X, Y: 14.549, 11.366 cm Units: Cm
Image Data: 7.7633e3 photons/sec/cm²/sr
Distance: 0.00
ROI Tools
Measure ROIs
Apply to Sequence
Type: Measurement ROI
Save ROIs
Name: ROI_1_EW
Delete
Load
Save
Auto ROI Parameters
Threshold %: 50
Corrections
1
2
3
4
5
6
7
8
9

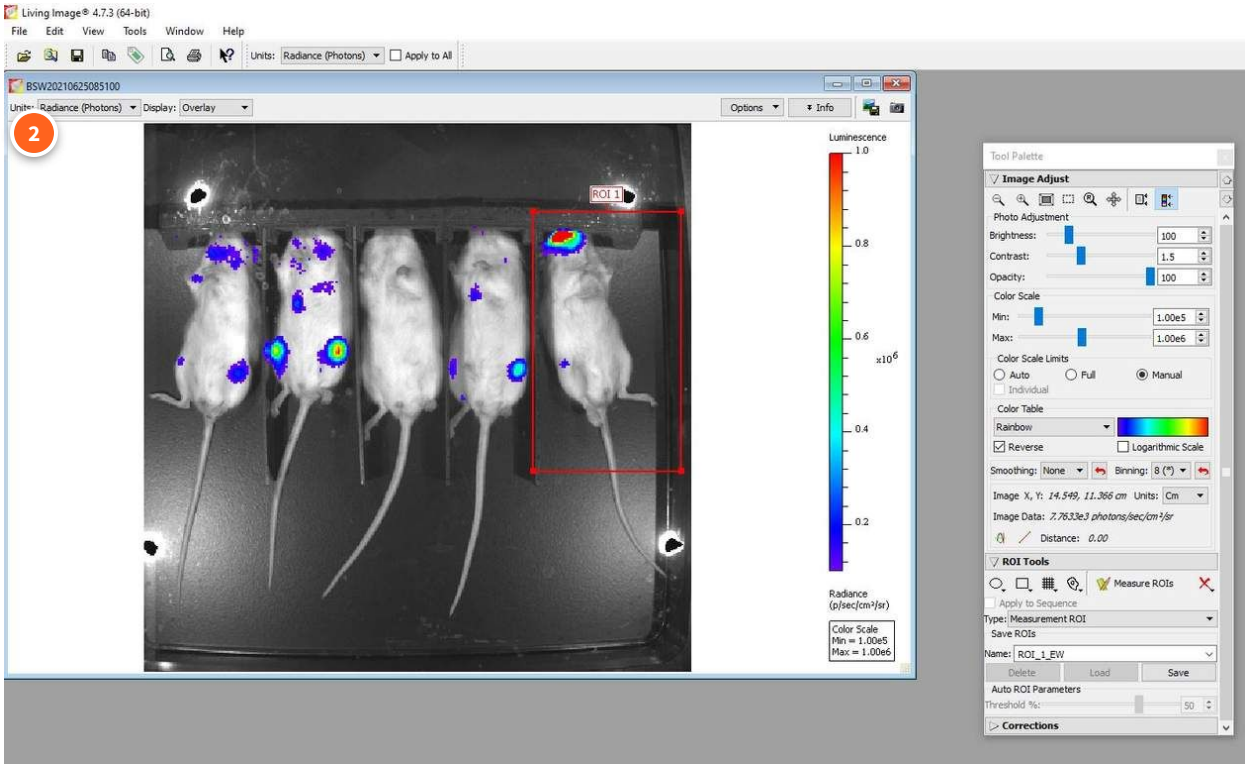


Load in data

File> Open (Folder icon) then Navigate to the folder containing your IVIS data on your desktop.

The folder naming format saves your user initials, followed by the date, followed by the timestamp on the image.

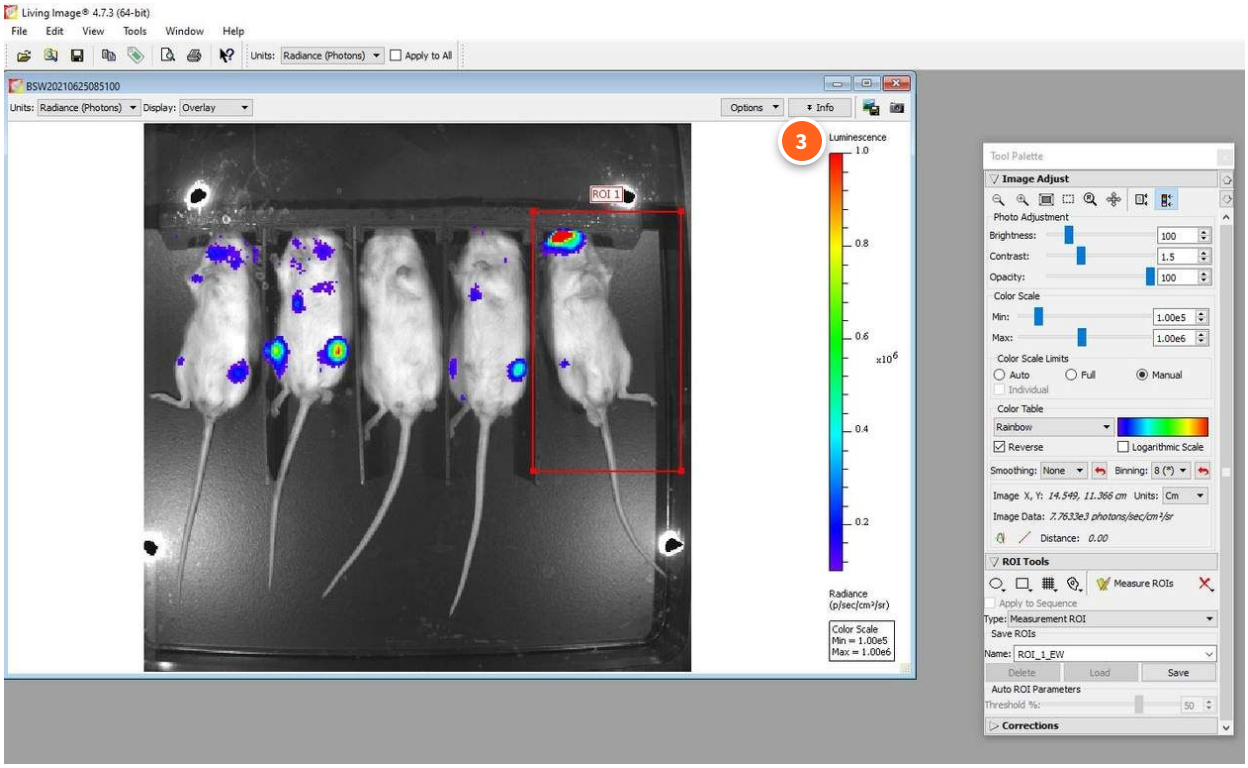
Select the "ClickInfo.txt" file. This will open the photograph and luminescence photos for you.



Change Units

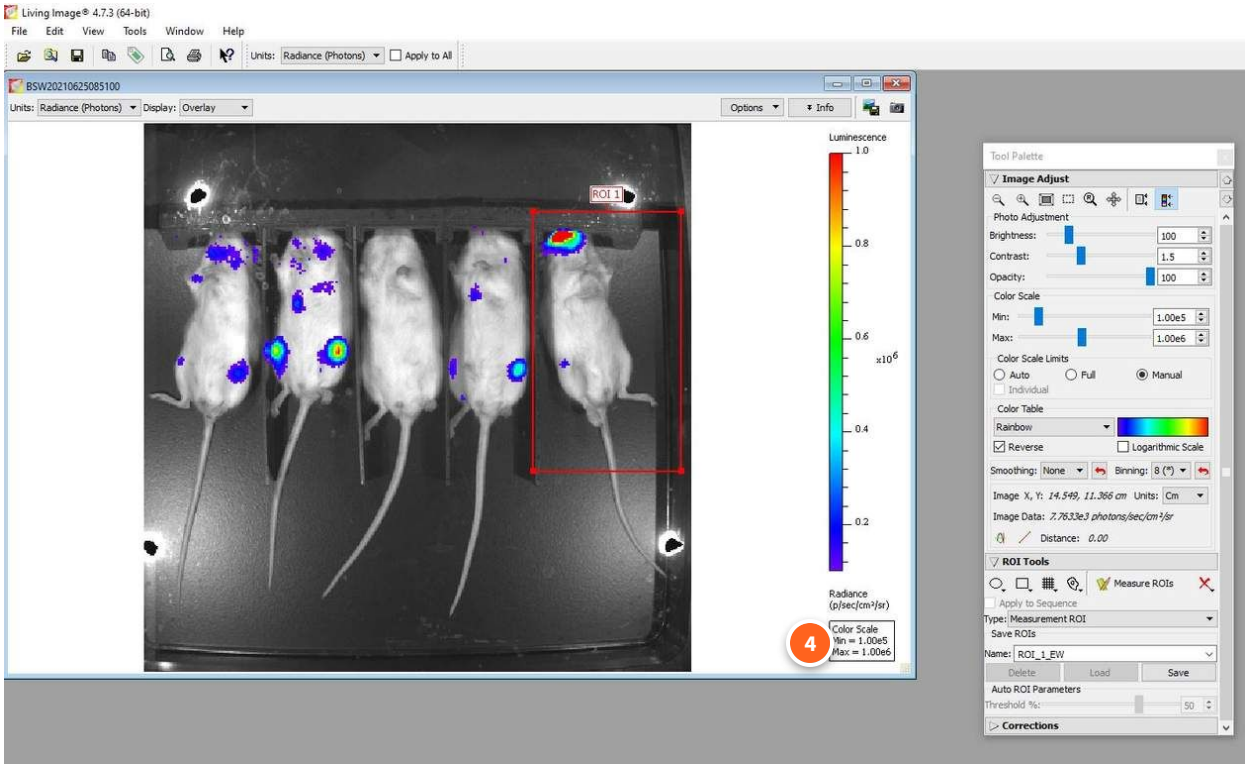
By default the software will give you units in counts. This is only to give you an idea how well your signal is and if it will be quantifiable later. Min counts should be over 600 while Max counts should not be over 60,000.

Change units to Radiance (Photon) when performing analysis to ensure quantitative measurements.



Info Tab

Here you will be able to see the info you applied to the image during acquisition.
Time stamp, image parameters, animal IDs...



Color Scale Bar

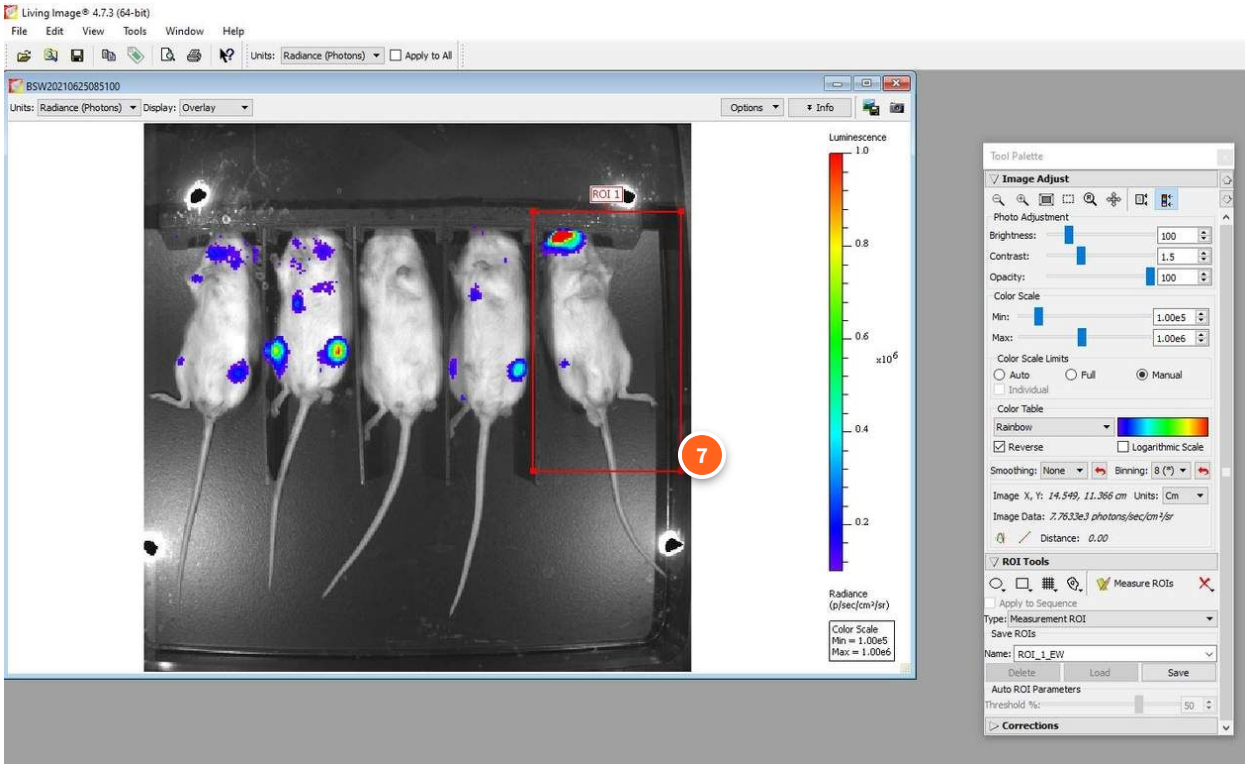
This provides your min max values of either counts or radiance.

Adjust Color Scale

To normalize all of your images, first change the units to Radiance, then you can adjust color scale in the tool palette to a color scale range that would work for all the images that you're analyzing together. This is great for longitudinal studies using images but not necessary for quantifying signal.

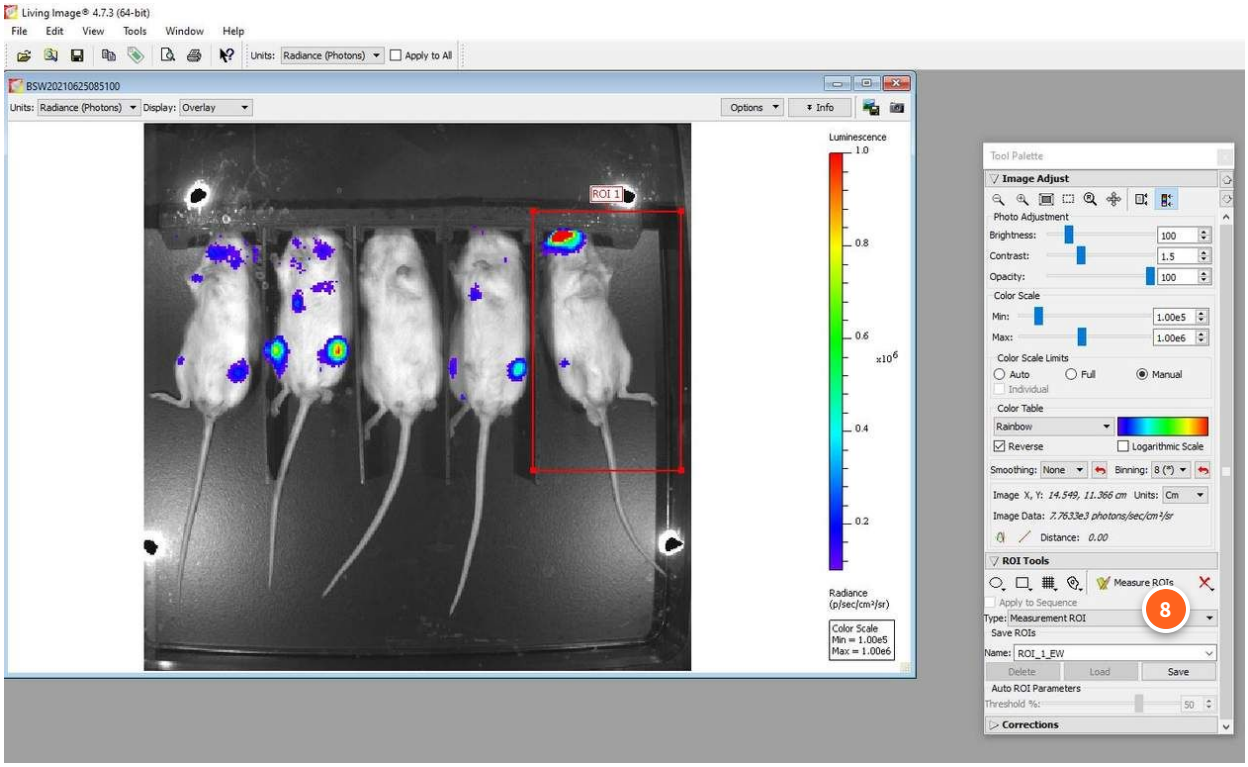
ROI tools

You have a few options on ROI tools, chose whichever tool you like to use.



Drawing ROI

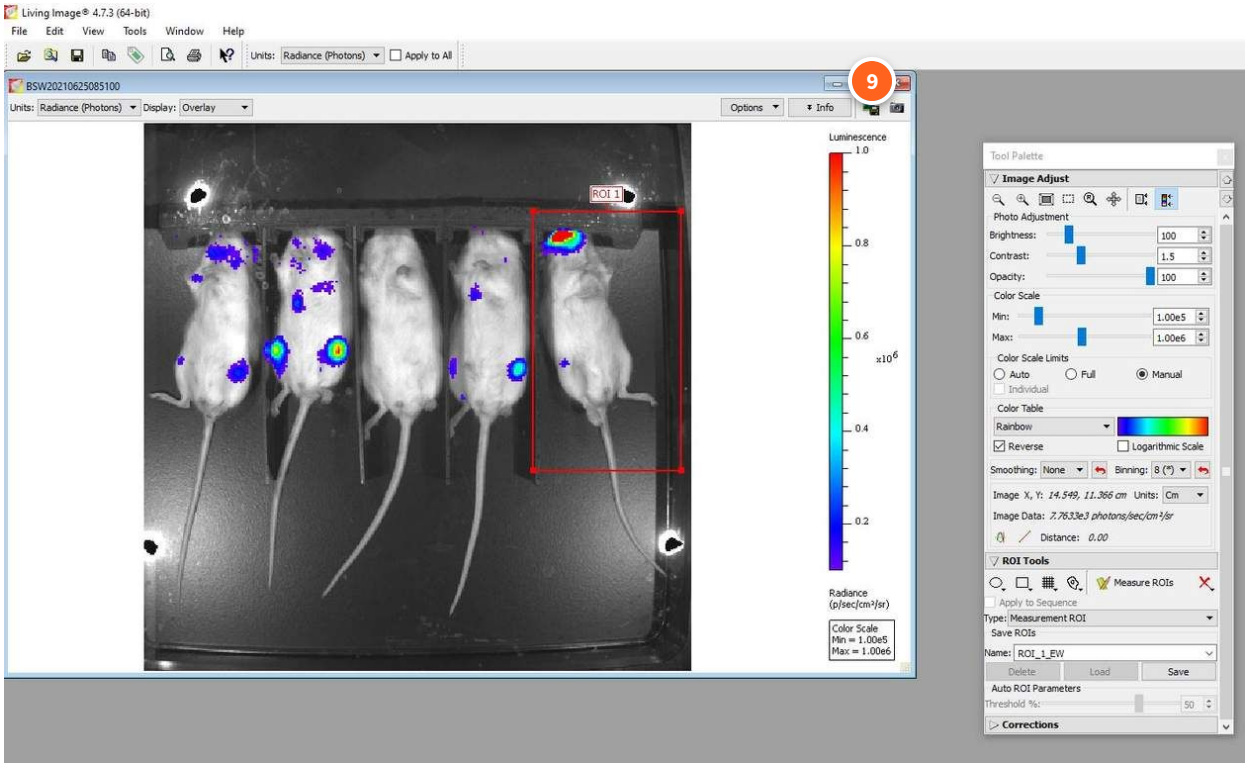
You can either draw around the signal (visual bioluminescence) or the whole mouse, depending on what your lab is looking at.



Measure and save ROIs

While still in this window with all your ROIs in place, if you click the Measure ROI's button, you'll see this mini-spreadsheet show up that will show you the values in spreadsheet form for ROI 1, ROI 2, and so on. While you still have the first window with ROI's open, go ahead and open the next file to analyze the same way. When you click the Measure ROIs button, now you'll see the cumulative measurements in the mini-spreadsheet.

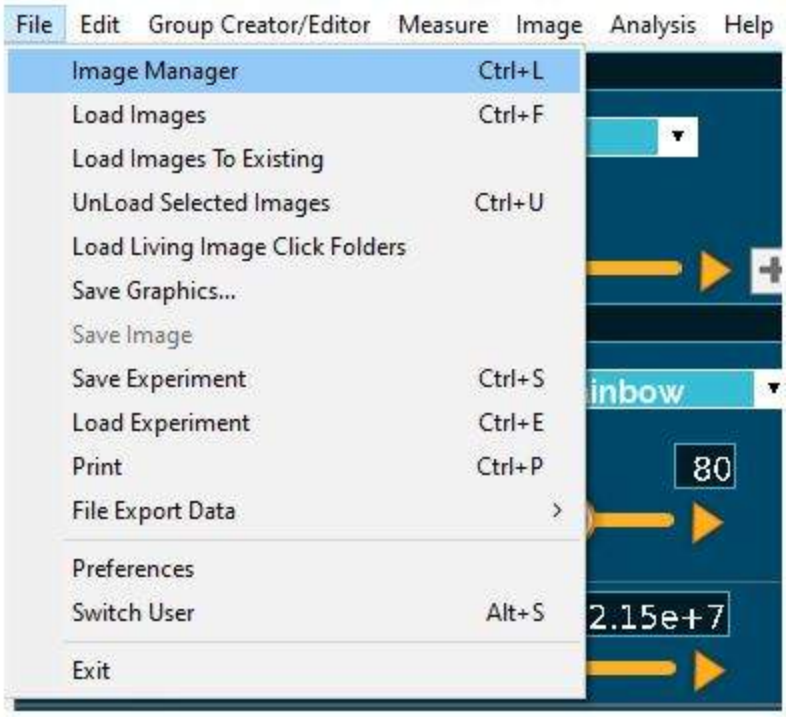
When you have finished all your measurements and all your measured images are stacked up, click Measure ROIs, and then click Export and save as Csv before you save to your desktop folder.



Save Photo

Click the Save Photo icon at the top of the image if you would like to later open image in paint and use in a PowerPoint.

For LagoX



Lago images can be loaded from the load images options or from the Image Manager

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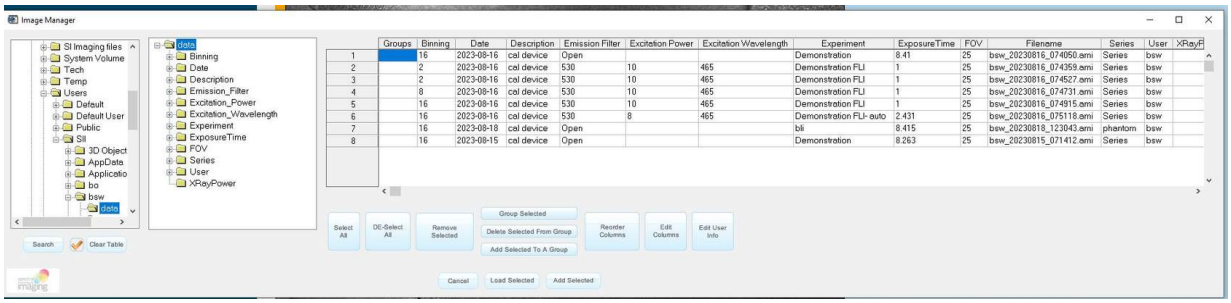


The Image Manager uses tags to organize and search image data from the info you provided during imaging. Multiple images can be organized and loaded together.


File Edit Measure Image Analysis Help
Display
Image Type
Photo Overlay
Size of Images to Display
Color Control
Units
Radiance
Rainbow
Overlay Opacity (%)
80
Color Range Minimum
9.88e+6
Color Range Maximum
2.47e+8
Color Range Threshold
9.88e+6
Auto Color Range
Linear
Log
Select All
Unselect All
ToolBox
Info
View
Arrange
Measure
Label
Apply To Group
Measurements
2.50e+8
9.90e+6
RAD
ROI 1 = 2.66e+9
Exposure time = 16.718
Binning = 16
Spectral Instruments Imaging
spectral instruments imaging
0
5
10
15
20
25

Image Type

You have the option to visualize your images overlay, photo, Fluor/Lumin, X-ray, and info separately from one another.
This can also be visualized by scrolling the mouse wheel.

Units

Radiance will give you your total flux (tumor burden) in photons/sec.

Counts will give you the raw data of the images which you will help you determine the significance of the signal. Low counts (<1000) means low signal.

Color Range

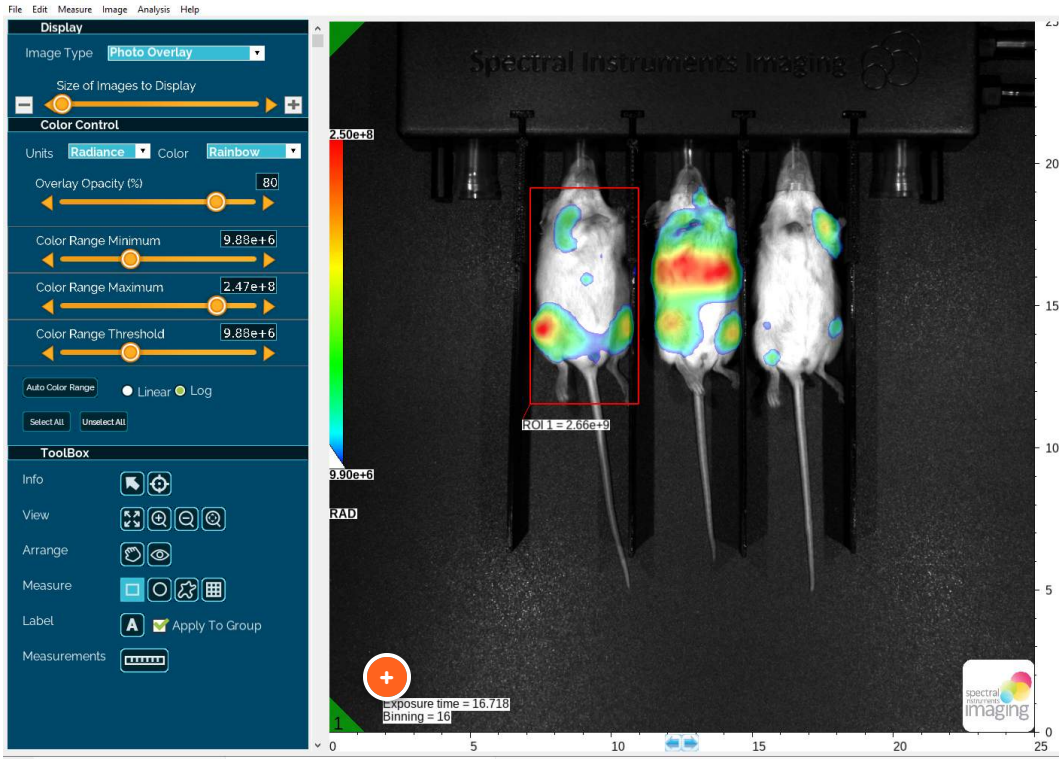
To normalize all of your images, adjust the color scale range that would work for all the images that you're analyzing together.
This is great for longitudinal studies using images but not necessary for quantifying signal.

ROI

Region of Interest measure tool

Measurements

Download a cvs (excel file) of all the ROI measurements.



Add info to image

If you want to add info to be seen on the imaged, find the small green triangle on the bottom left. This will list image parameters, info, and animal IDs you can choose to view.

Application Guides

Explore some further application guides by selecting any areas below that are relevant for your research.

IVIS: Fluorescence

Adaptive Fluorescence Background Subtraction

Spectral Unmixing

Transillumination Fluorescence 1 - Setup

Transillumination Fluorescence 2 - Raster Scan

Transillumination Fluorescence 3 - Normalize

Image Analysis with Living Image

Living Image Software guide

AutoExposure

Overlaying Images 2D

Working with Image Math

Drawing ROIs

Subject ROIs

Determining Saturation

How to Open Living Image in Aura

IVIS: Advanced Image Acquisition

High resolution / binning

Advances in 3D Optical Imaging Quantifications and Sensitivity

Image Overlay - 3D

3D Multimodality Images

IVIS: Tomographic Measurements

Setup and Sequence Acquisition

Topography

Source Reconstruction and Analysis

Fluorescence Tomography and Topography

Fluorescence Tomography – Source Reconstruction and Analysis

Animal Handling and Protocols

IP injections

Using the Gas Anesthesia System

Luciferin Prep and Dosing of Animals

Kinetic Analysis of Bioluminescent Sources

Misc for IVIS

Loading groups of images

Well Plate Quantification

Stem Cell Research

Cerenkov Imaging of Radioisotopes in IVIS Systems

Lago Tech Notes

7 reasons to choose Sii
10 Tips and tricks
Comparison of Preclinical Imaging Modalities
How to quantitate
Reporter Expression
Rodent Depilation
Science of Optical Imaging

Spectral Instruments Imaging YouTube Channel

https://www.youtube.com/@spectralinstrumentsimaging2594/featured

Click below if you would like to save a PDF of this training for future reference or to print out for your records

FILE

File Attachment Block

No file added

Can I reach out the PCI team if I have ANY questions about analysis, equipment settings, experimental design?

Type your answer here

SUBMIT

Thank you for completing this part of the IVIS training!

You will need to complete a brief in-person component of this training before being able to access this modality. Please contact preclinicalimaging@fredhutch.org with any questions or feedback regarding this training.

IF you plan to use IVIS imaging in the BSL-2 facility, please continue to the "BSL-2 Procedure" section.

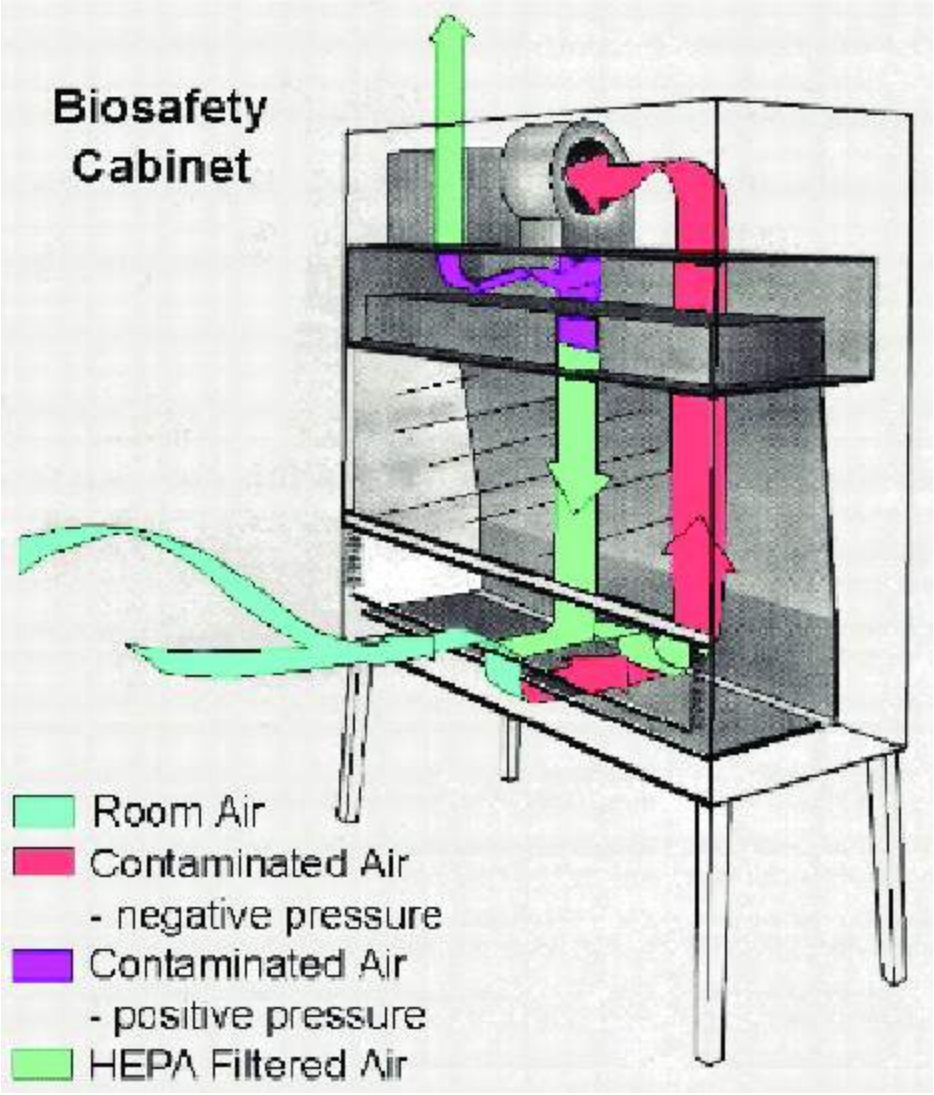
CONTINUE

BSL-2 Procedure

PI Preclinical Imaging

The procedure to use the BSL 2 IVIS is largely the same as standard BSL 1 level IVIS imaging, but there are a couple of key differences that will be described here.

IVIS Imaging & BSL-2 Safety



Airflow diagram in a Biosafety Cabinet. *Sreenivas Reddy, Bathula & Rakhimol, A.. (2017). Global Trends in Biorisk Management. BioRisk. 12. 1-23. 10.3897/biorisk.12.12156.*

Due to the airborne hazards associated with some mice housed in BSL-2, the mice are not permitted to be out in the open of the room or in the IVIS chamber without an additional form of protection for workers in the room.

This means all mice must be kept within a biosafety cabinet or an imaging box or chamber with appropriate HEPA filters attached.

Mice will need to be put to sleep in an anesthesia box in the biosafety cabinet, then transferred to an imaging box equipped with HEPA filters.

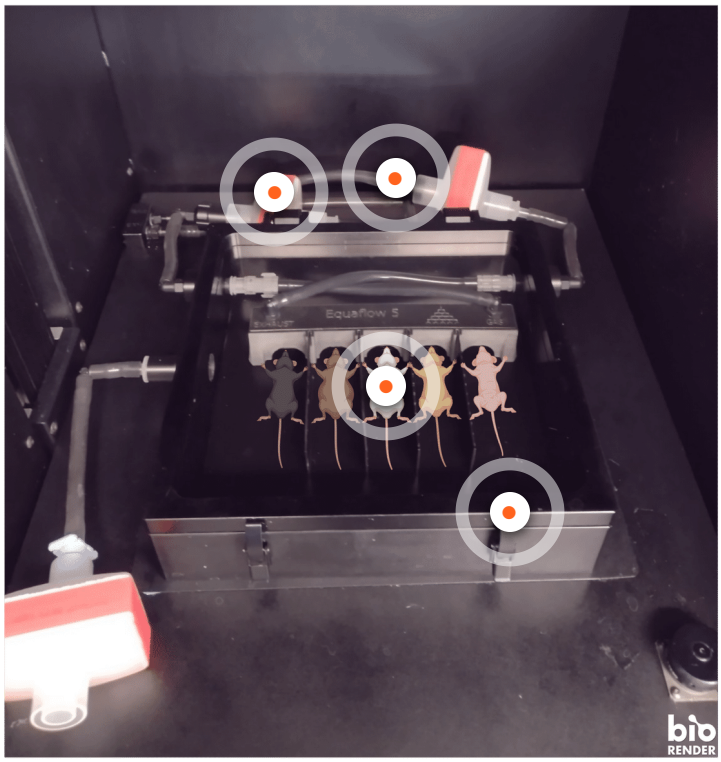
BSL-2 IVIS imaging box open inside a biosafety cabinet

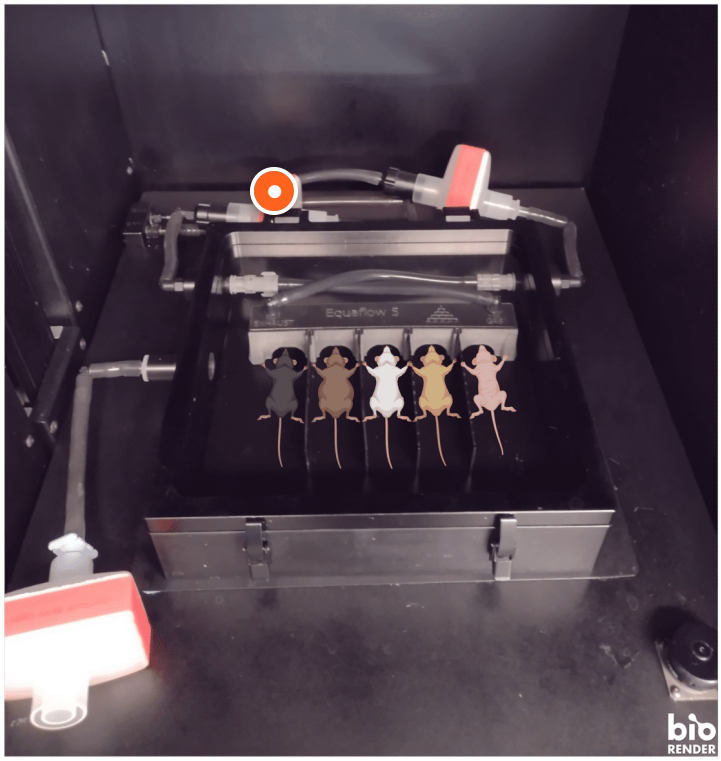
When the mice are deeply asleep, you can transfer them to this box and position them appropriately. Then close the lid and move the box into the IVIS imaging chamber. All of this is done while the mice are asleep. The imaging chamber lid must be closed before moving it out of the biosafety cabinet.

With HEPA filters attached, the chamber's gas lines can be connected to anesthesia lines in the IVIS imaging chamber.

The positions of the mice can ONLY be adjusted within the box if they are in the biosafety cabinet. In other words, you CANNOT open the lid in the IVIS imaging chamber if the mice need to be repositioned. If the mice start to wake up in the box, you need to move the box back to the hood, place the mice back in the anesthesia induction chamber, and then wait for them to fall back asleep to position them again correctly.

Click on the icons below to learn more about how the BSL-2 Imaging Chamber operates.

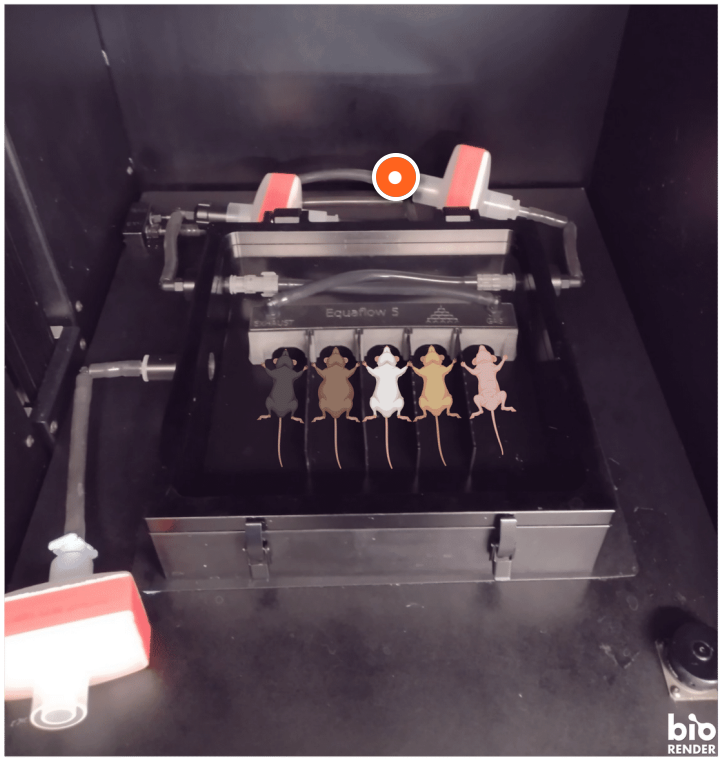




HEPA filters

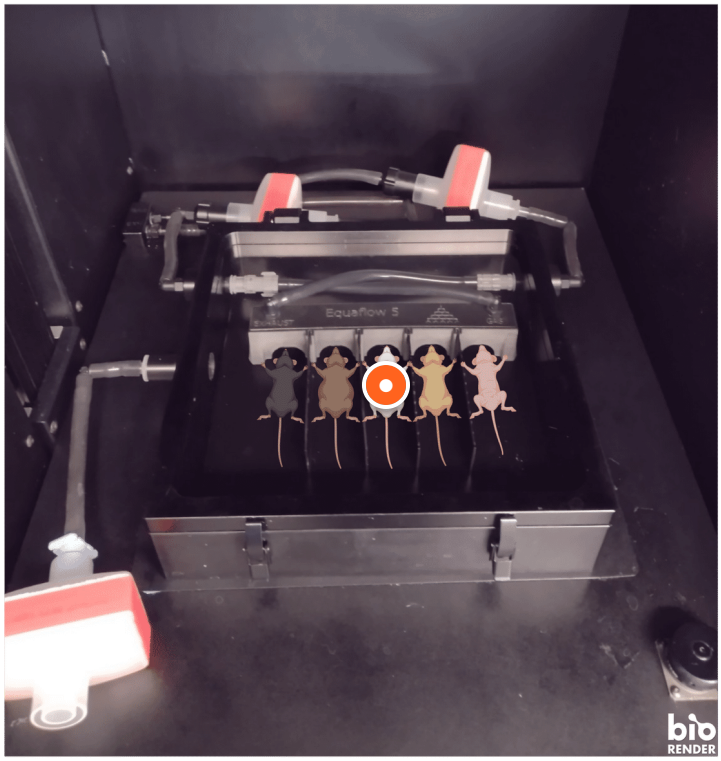
Chamber *must* be equipped with HEPA filters at all times! This is to prevent airborne pathogens from being released into the room where people are working.

Prior to beginning the study, consult with EH&S and Preclinical Imaging to determine what type of HEPA filter is appropriate and safe for your study.



Gas & Exhaust lines

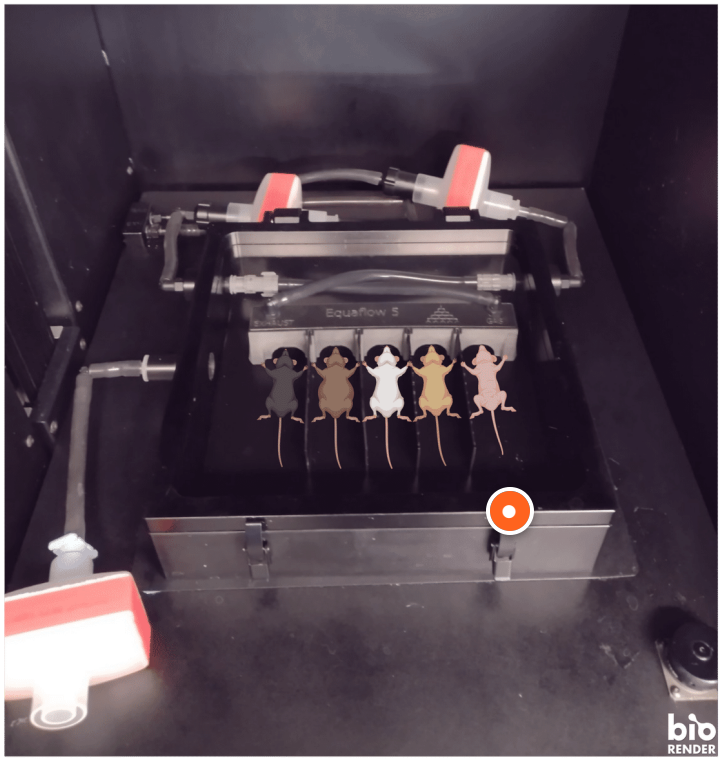
Connect the HEPA filters, which are connected to the box, to the input and output gas lines. This delivers isoflurane to the box and provides an exhaust line to outside of the chamber.



Mice lay in chamber

Mice must be well asleep before being put in the chamber so that they do not wake up while moving from the BSL2 hood to the IVIS imaging chamber.

Mice can be laid in any orientation so long as their noses are within the nose cone. Here they are shown supine but can be laid prone or on their sides.



Box Latch

Close box latch over mice to keep isofluorane and any airborne pathogens *in*.