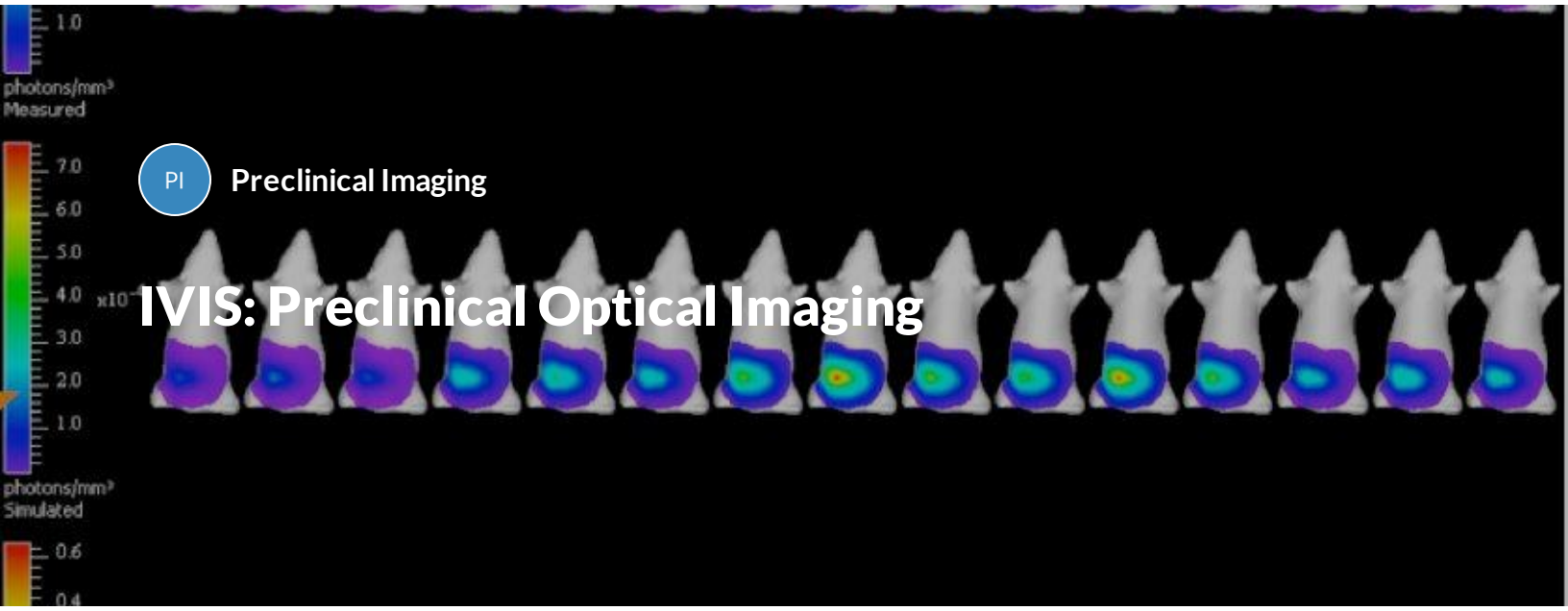


This online training is required for any researchers who wish to use the IVIS Spectrum for mouse imaging studies using bioluminescence or fluorescence. This online training should be completed before in-person training, as a refresher-course, and as a reference for future use of the equipment.

Overview

Scientific Background

Imaging Procedure

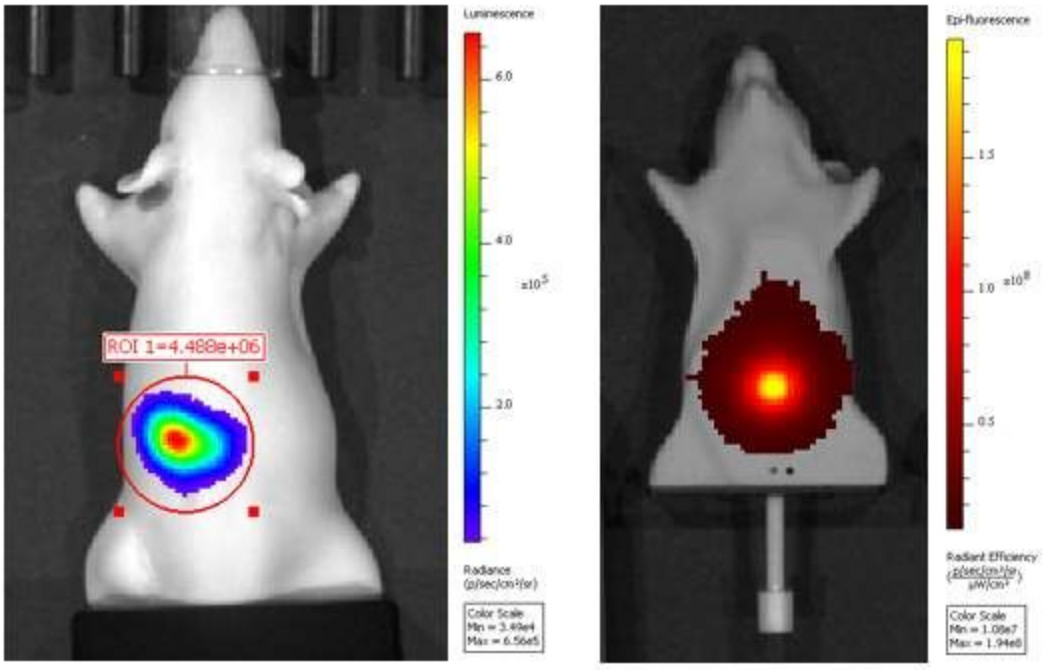
Wrapping up

Further Resources

BSL-2 Procedure

Overview

PI Preclinical Imaging

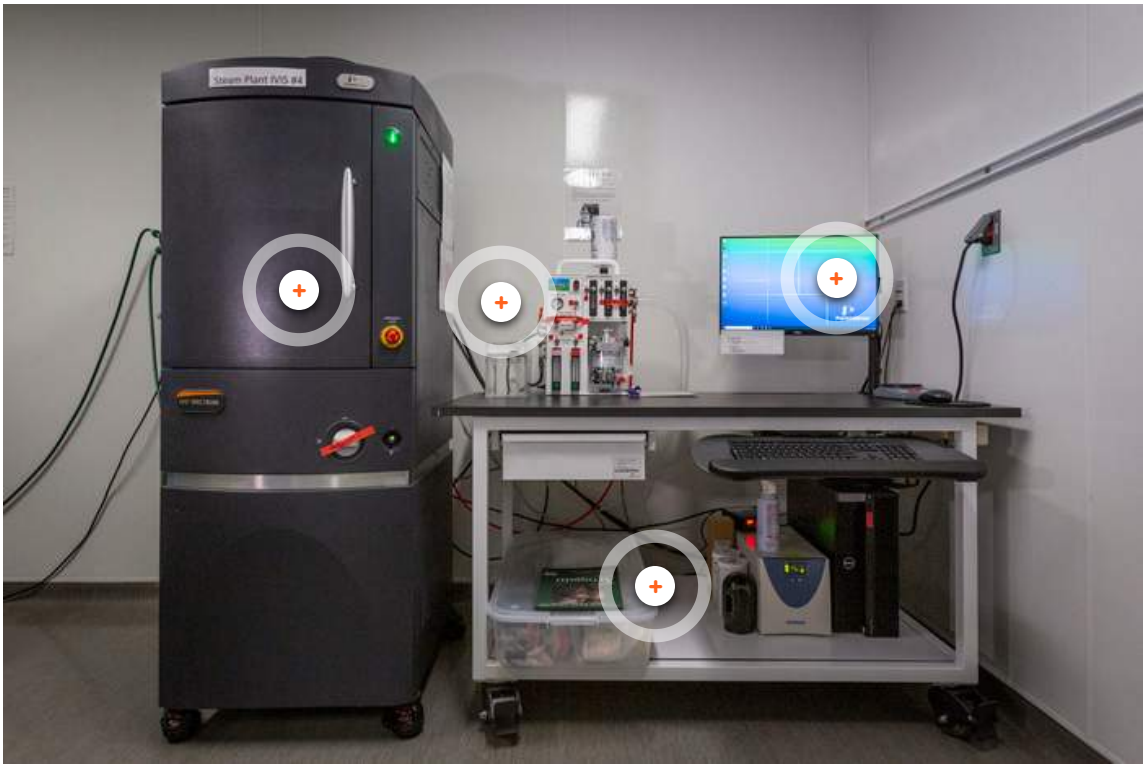


Bioluminescence (left) and fluorescence (right) image from a mouse phantom acquired using the IVIS Spectrum machine in the Comparative Medicine Vivarium

The IVIS Spectrum instrument 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. The IVIS offers a spatial resolution between 1 and 5 mm, depending on the application.

Reservations on the IVIS 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 IVIS training.

Click on each of the components in the image below to see the different features of the IVIS system.



IVIS

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

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 IVIS room 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 IVIS 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.

CONTINUE

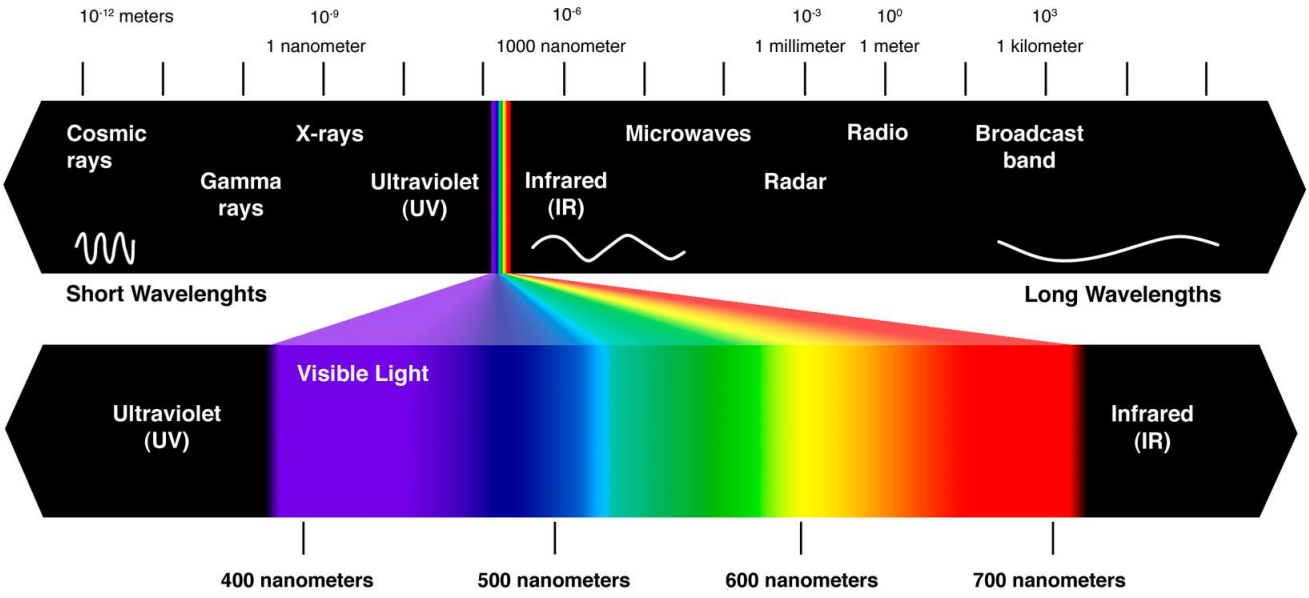
Scientific Background

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 can perform two different optical imaging methods

The IVIS spectrum 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.

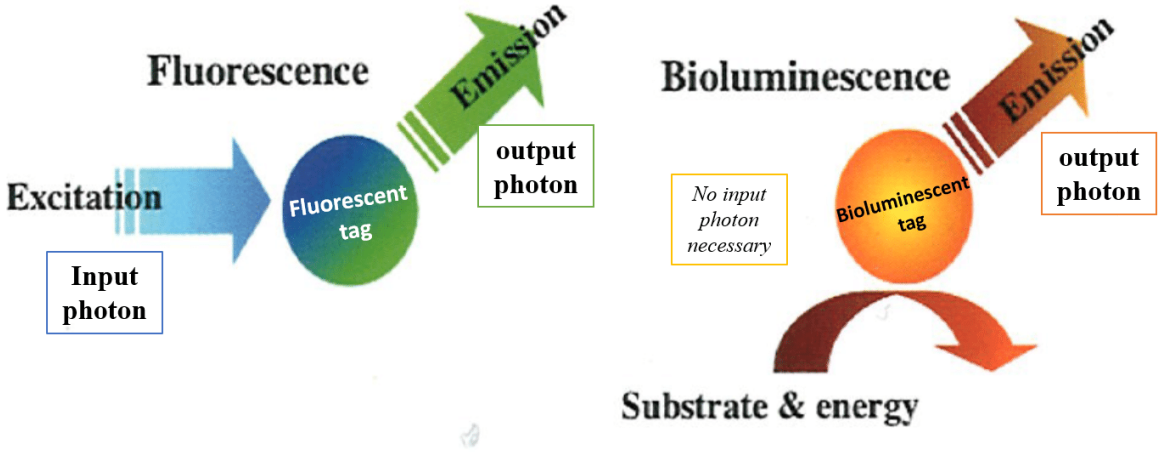
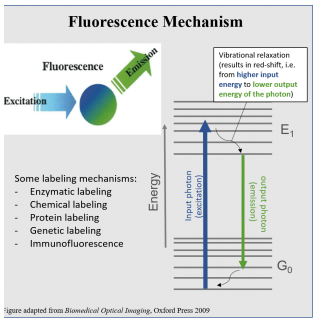
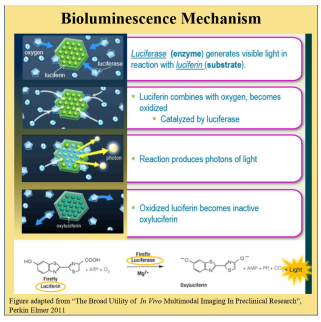


Figure adapted from *Biomedical Optical Imaging*, Oxford Press 2009

Basic differences between fluorescence and bioluminescence mechanisms of light production



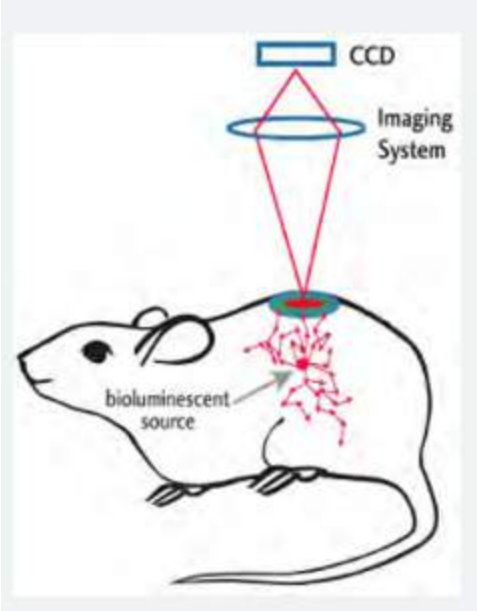
Fluorescence process diagrams. Click to expand photo.



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.

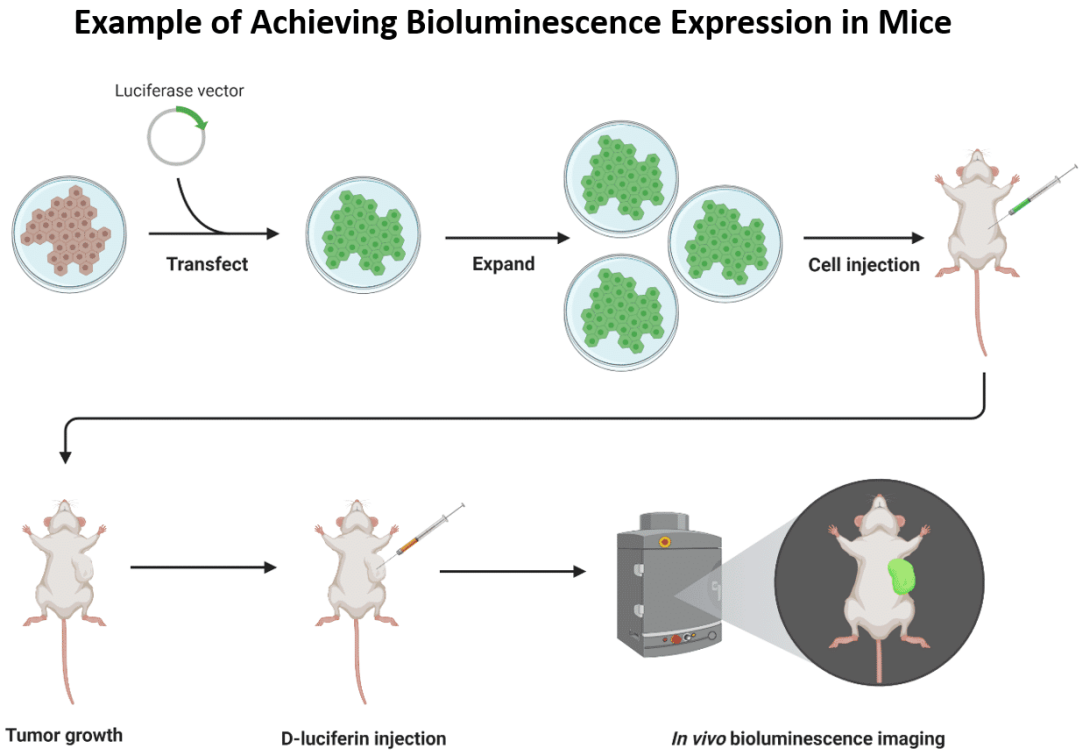
Bioluminescence, unlike fluorescence, does not require an input photon nor any light source to generate signal. The enzyme luciferase is expressed in the animal model (this requires preparation ahead of time! Luciferase does not naturally express in any standard mouse model). Only when luciferin, the 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 IVIS camera.



Schematic of light collection with the IVIS 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 IVIS 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 IVIS 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

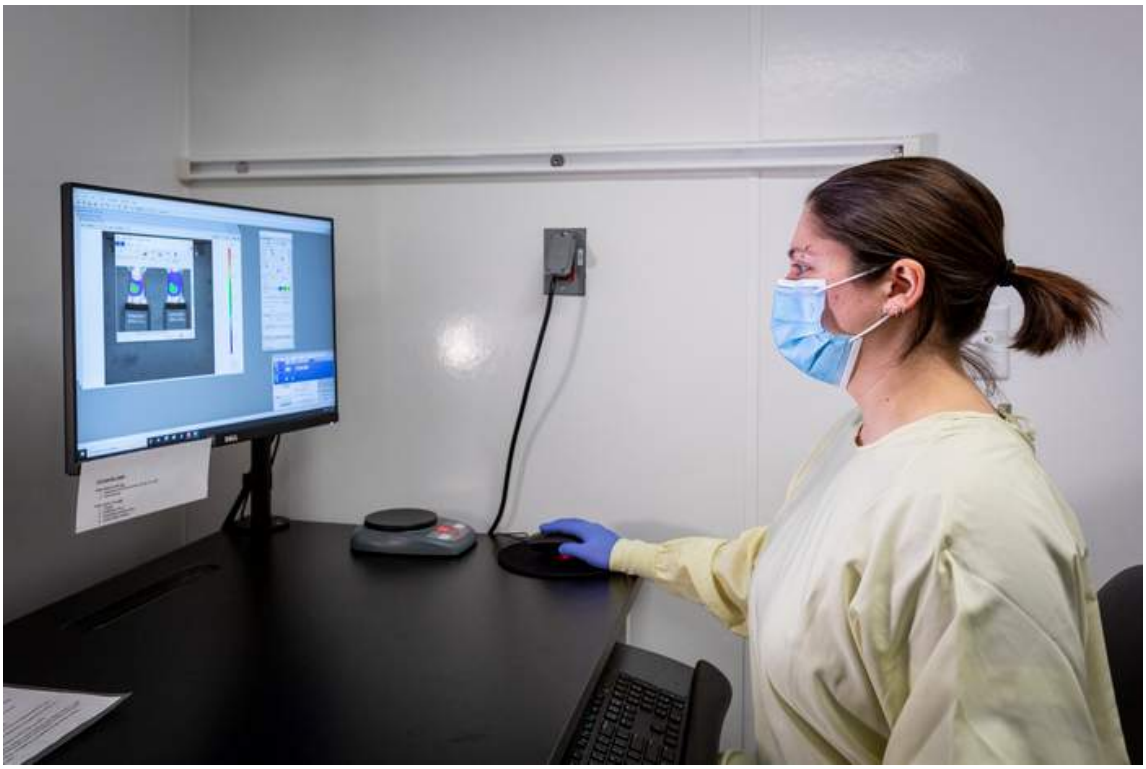
SUBMIT

Complete the content above before moving on.

Imaging Procedure

PI Preclinical Imaging

Getting Started



Step 1

Log in on iLab

Reserve time on iLab and log in at the start of your session to turn the monitor on.

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

Step 2

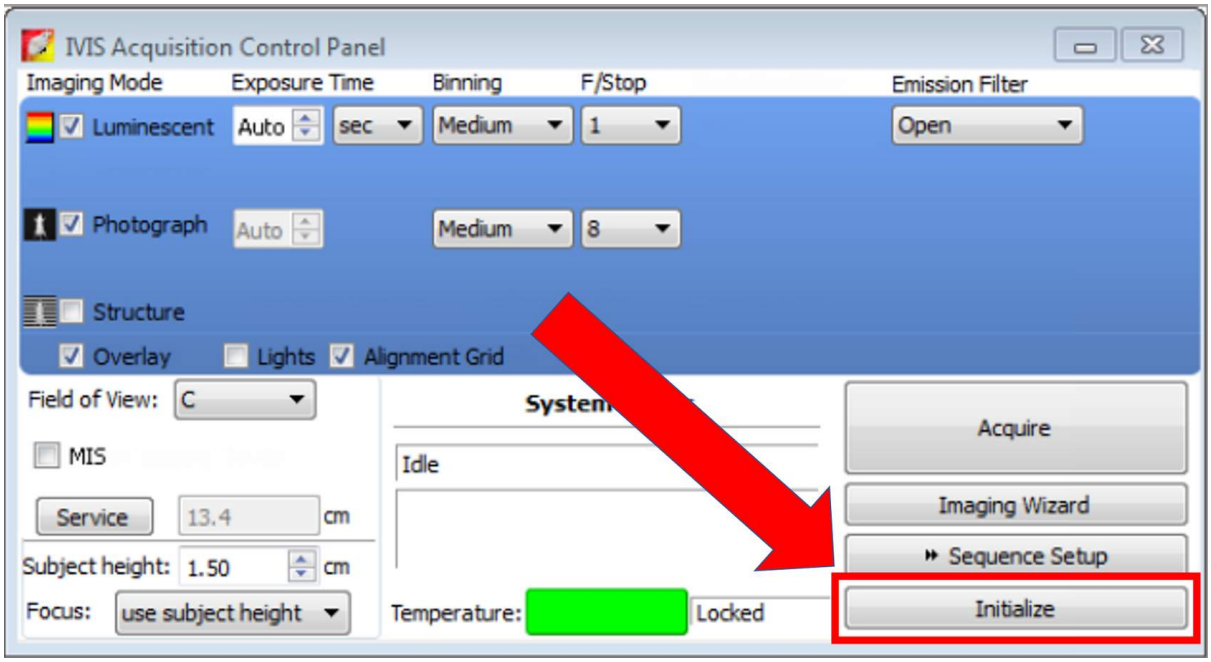
Open Living Image Software

- Turn on computer
- We recommend restart computer to avoid software crashing
- Open Living Image Software
  - if this is your first time using the software, set up a new user account and create

  - if re-logging back in, scroll to your user initials, select, and press 'OK"

Step 3

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

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.

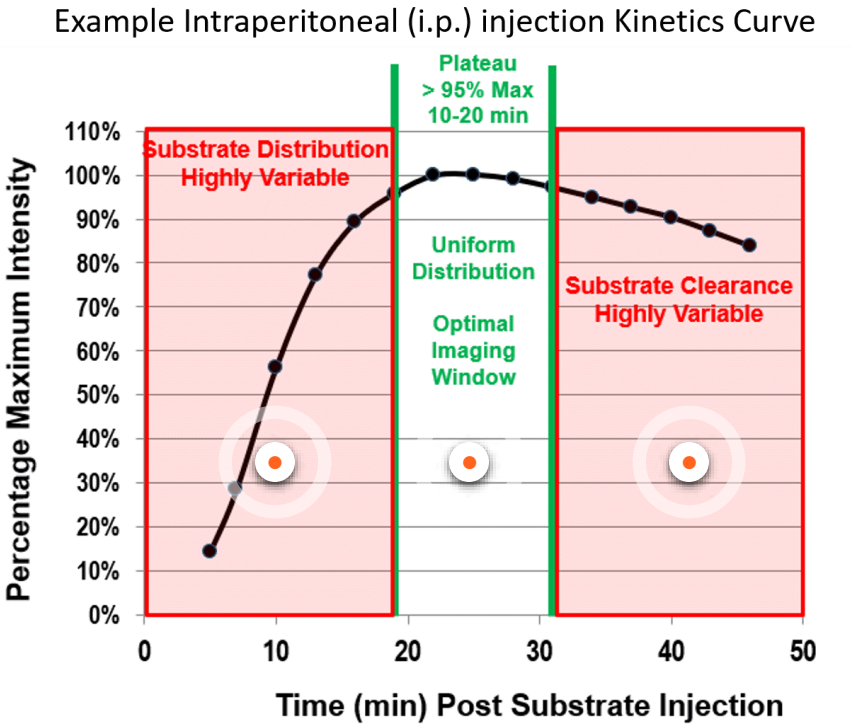
Note: Here we use the terms luciferin and substrate interchangeably, as the majority of the time those terms are interchangeable. Please also note that other study variables like using another injection method (like retro-orbital instead of intra-peritoneal) will require a different procedure. We recommend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

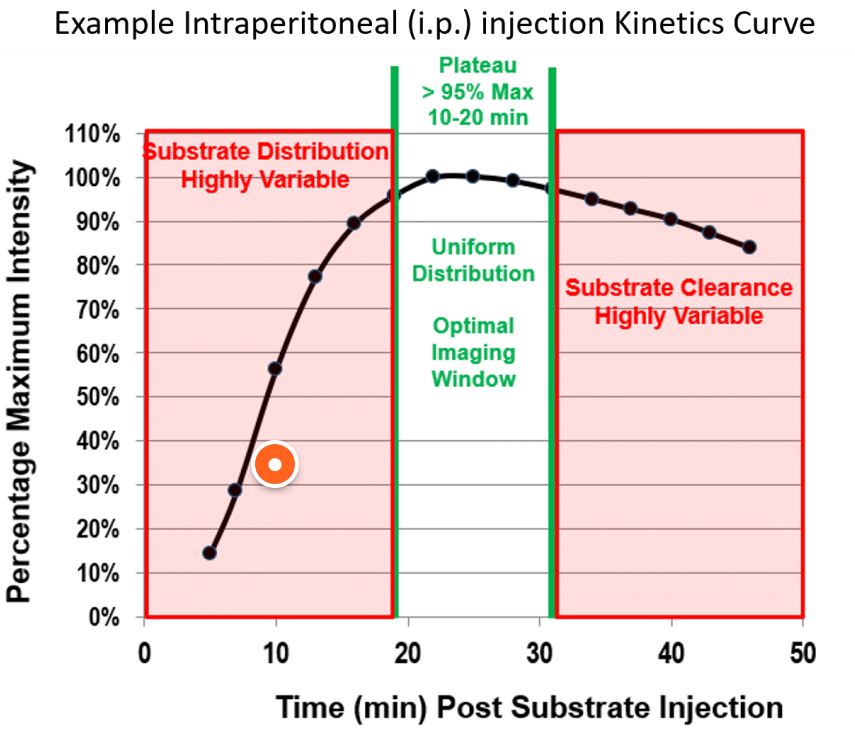
Establishing a Kinetics Curve

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, I.M., 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.

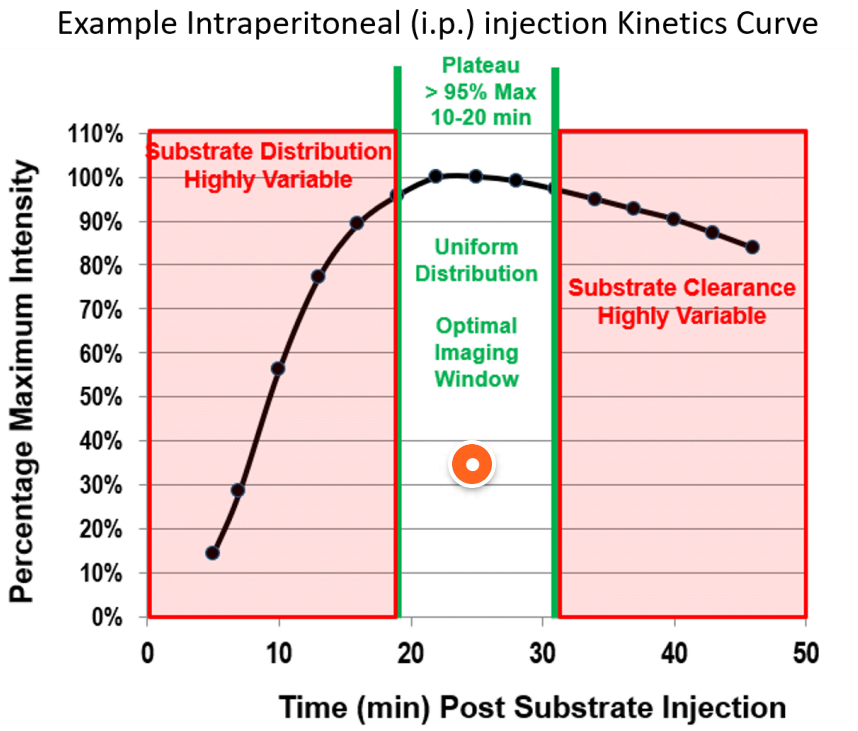
To generate a kinetics curve you inject a mouse with the substrate in the manner of your choosing, then image it at many different time points. Then plot the total signal from each image time point against the time, to generate a curve similar to that shown below. Use this data to determine your optical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





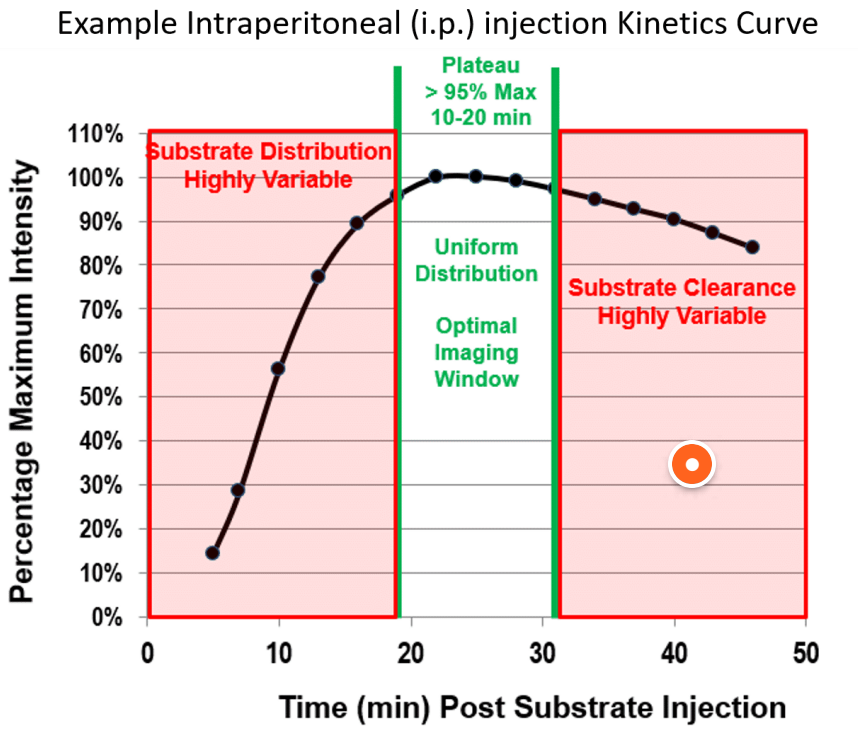
Substrate distribution initially is highly variable

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 IVIS imaging chamber and wait for the ~15 minute post-injection time point. Then acquire the IVIS 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 is often done further in advance, although exceptions apply. Contact preclinicalimaging@fredhutch.org to discuss

Complete the content above before moving on.

Image Acquisition

Preparing the equipment for animal imaging

Click on each item in the photo below to follow the steps to prepare animals for imaging.

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

*When using the Primary IVIS, only do this when you are about to open the induction box or the imaging chamber.

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.

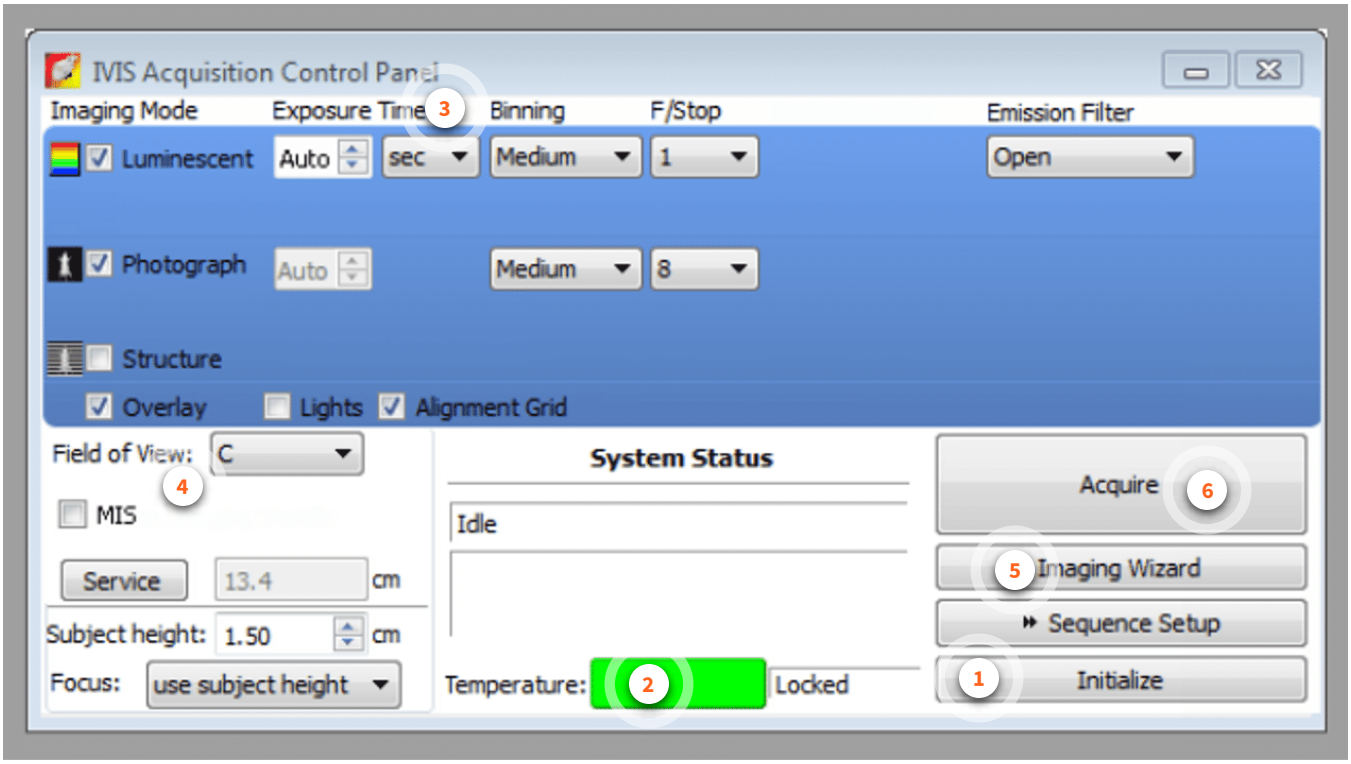
Transfer animal to IVIS imaging chamber

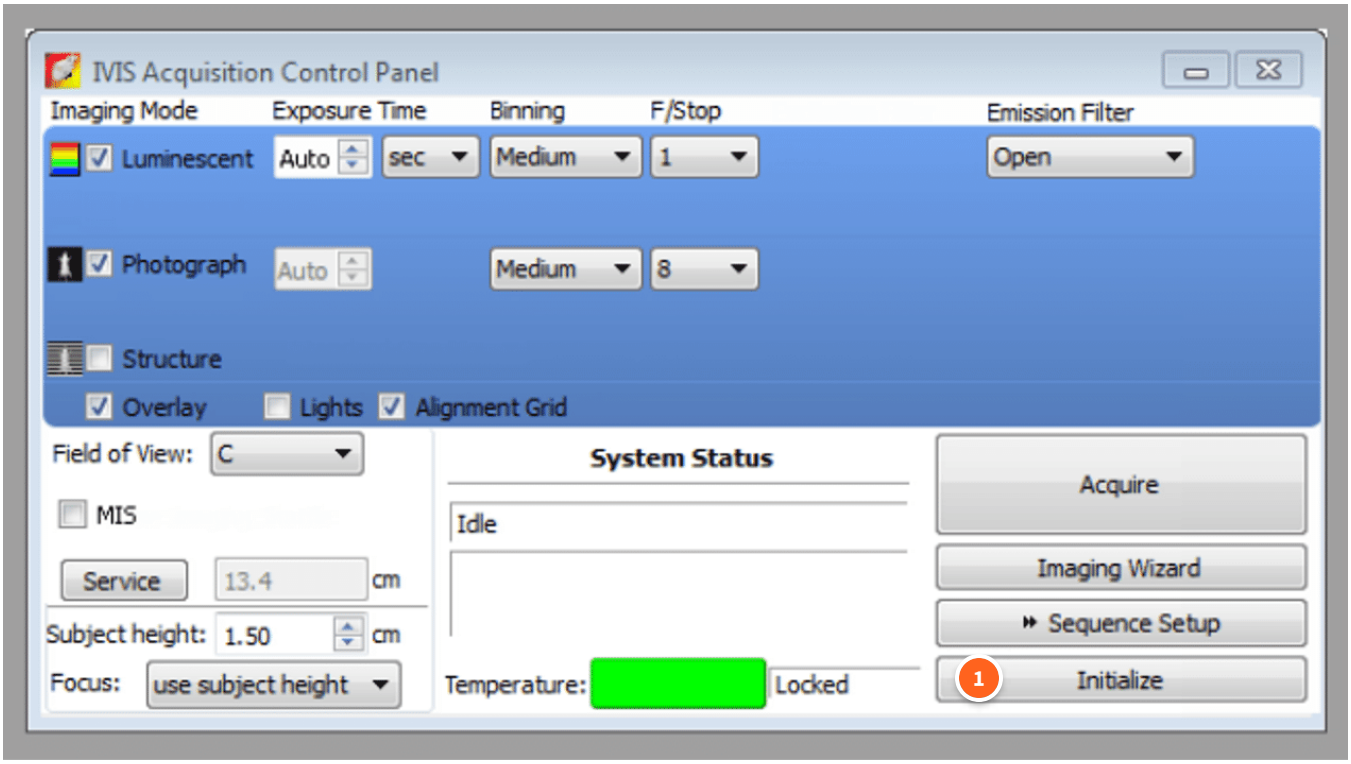
Acquiring animal images

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.

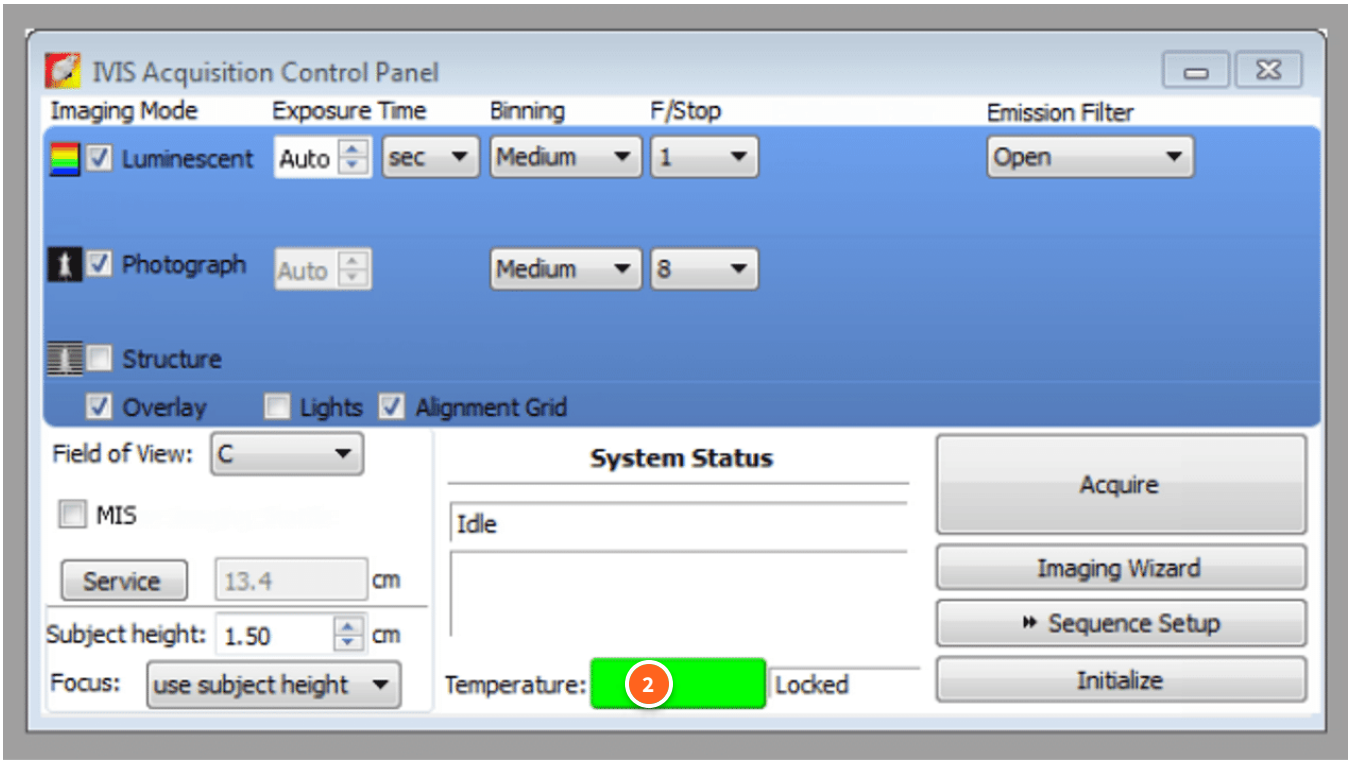
Click on the steps below to explore the different imaging parameters and the way that images are acquired in the Living Image software.


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:
C
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



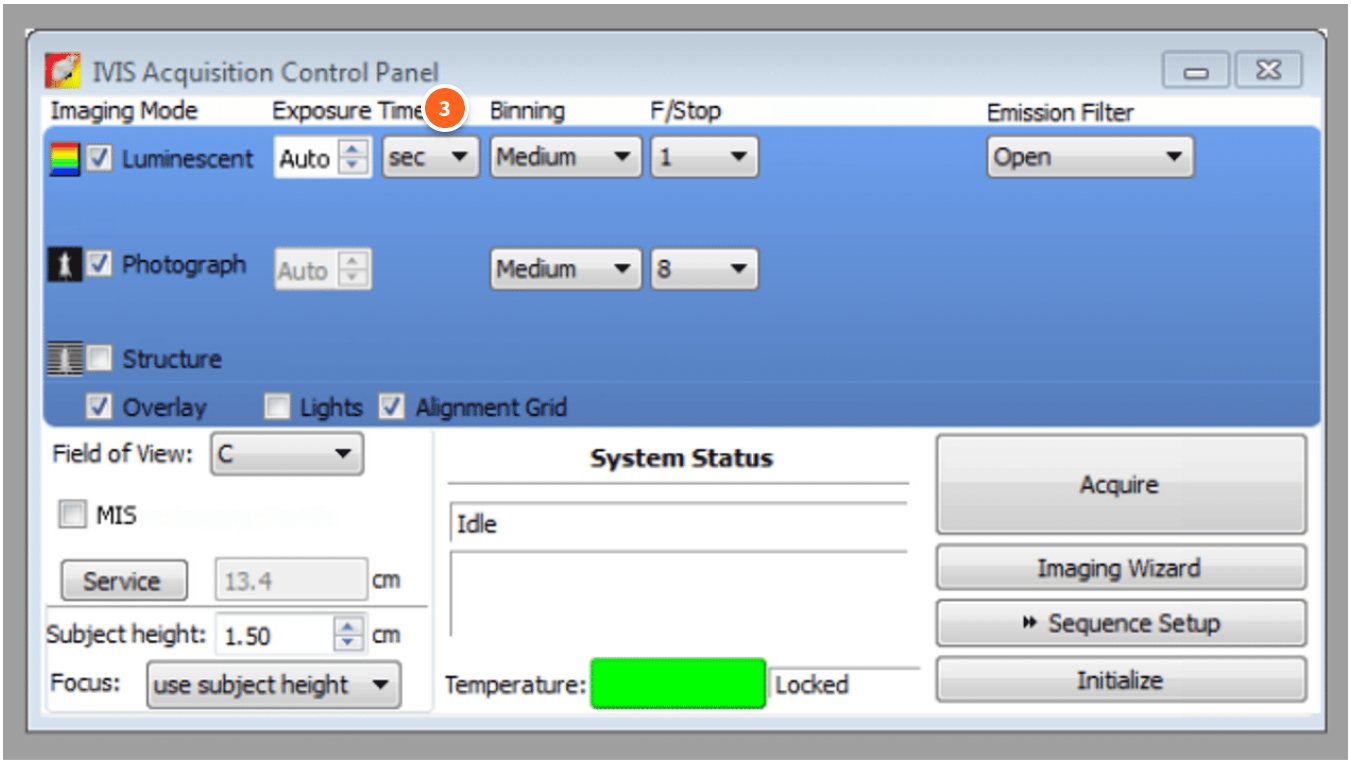
Initialize

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



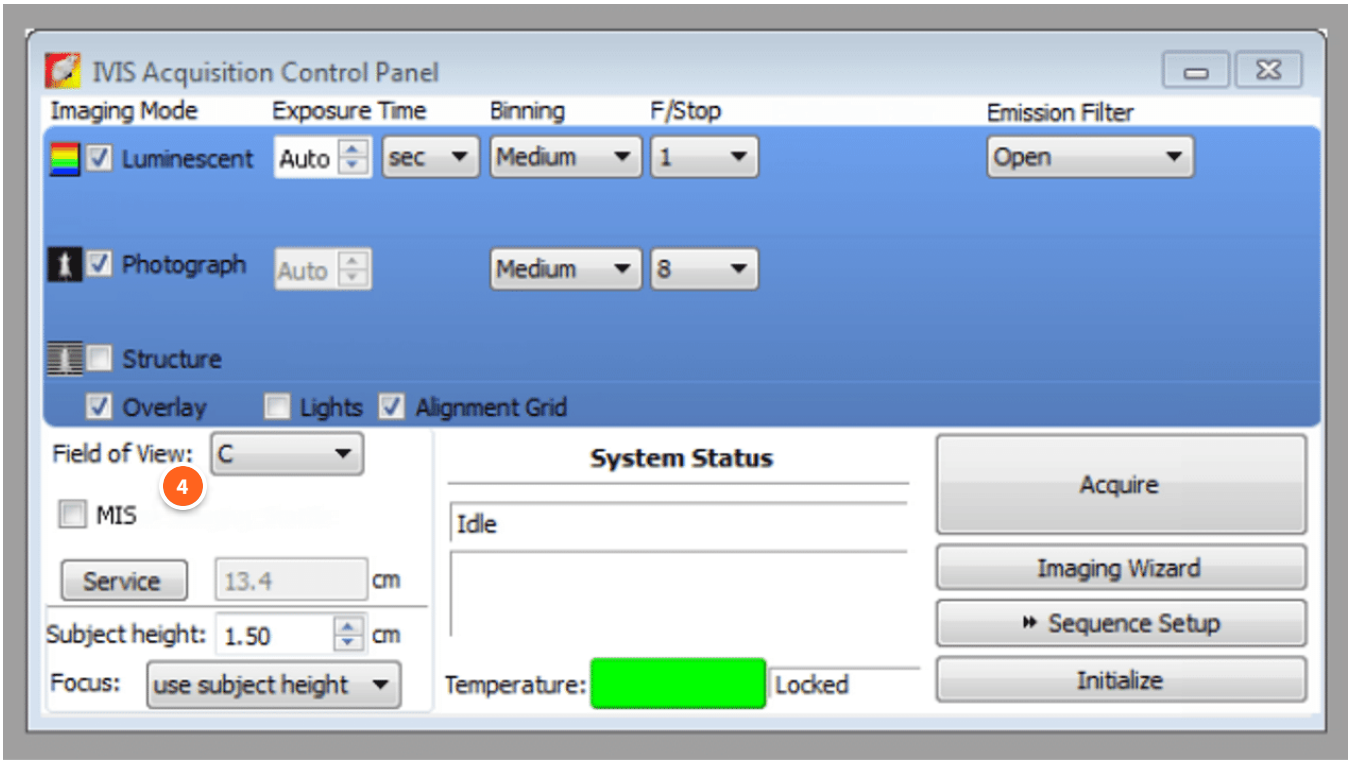
Ready status

Green bar indicates imaging can proceed as the temperature of the camera is at -90 °C.



Exposure Time

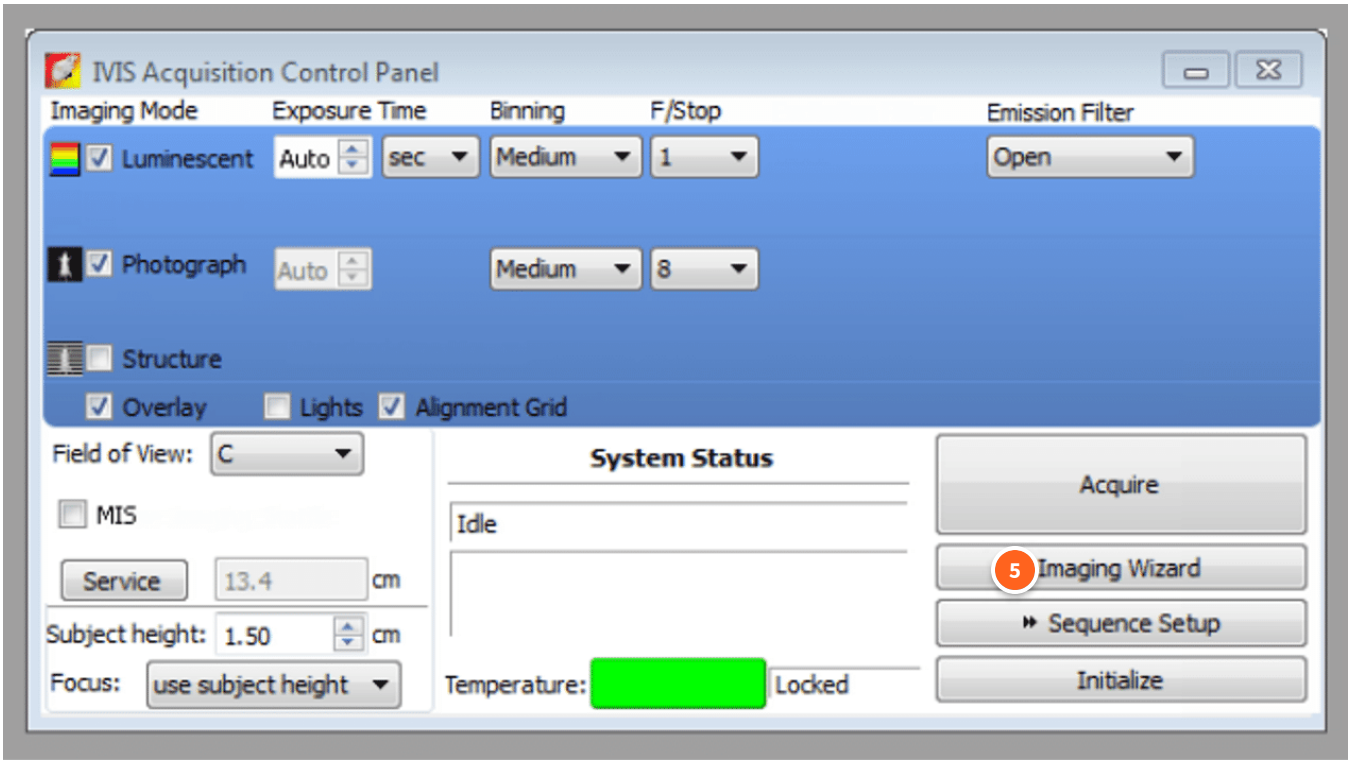
For most applications we recommend setting the exposure setting to "Auto". This acquires an image until the signal acquired is optimized by the dynamic range of the camera, which is at ~600-60,000 counts. This will ensure an optimal signal-to– noise ratio for images.
However the time of exposure may vary between subjects so if you want a reproducible, consistent acquisition time you can input a time here. 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)

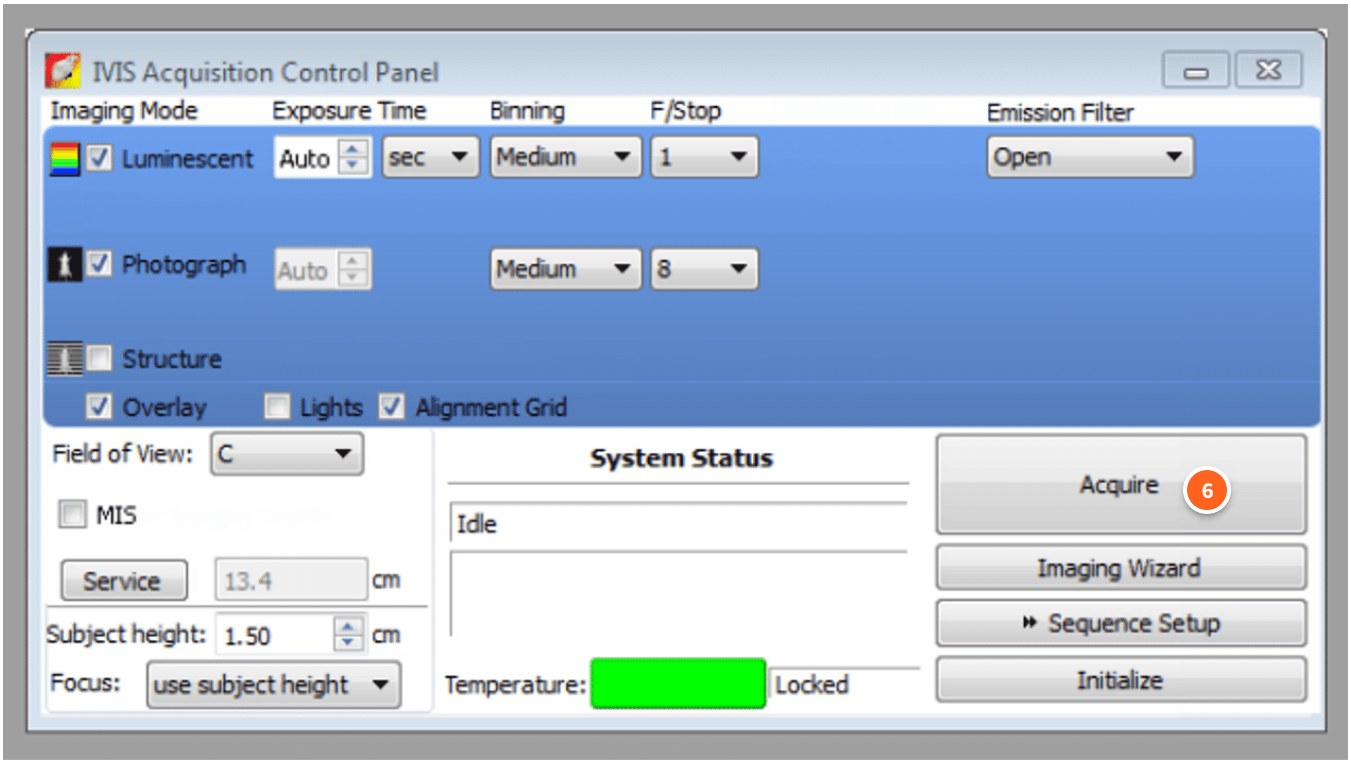
Green laser projects the imaging area onto the stage. This setting affects the subject height. Recommended field of view settings are given below:

Number of mice being imaged at a time	FOV setting
5 mice per image	D
3 mice per image	C
1 mouse per image	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

Why is "auto" 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

What Field of View (FOV)setting should you use if you want to image 5 mice in the chamber at the same time?

- A
- B
- C
- D

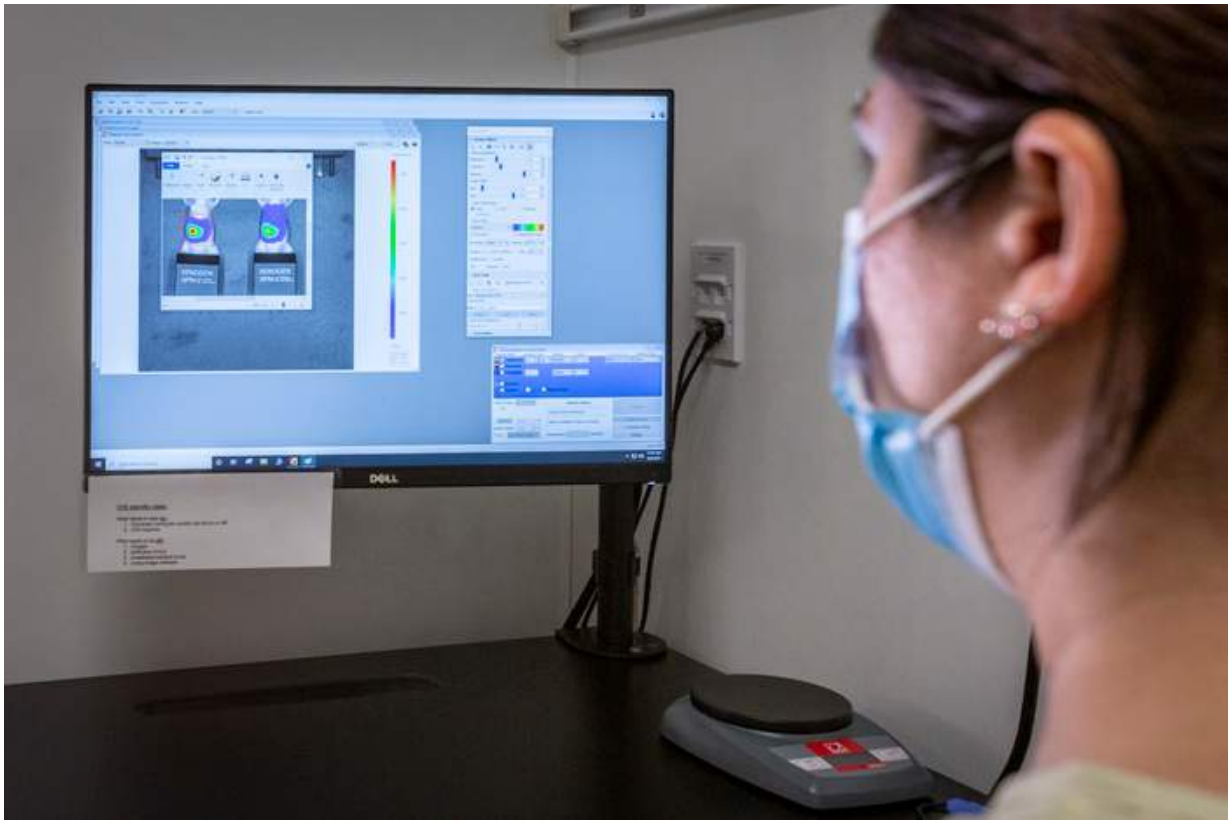
SUBMIT

Complete the content above before moving on.

Lesson 4 of 6

Wrapping up

PI Preclinical Imaging



Shut down procedure

First you must recover your animals, then turn off the instruments used.

Step 1

Recover animals

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

Step 2

Turn off machine & components

- Close O2 tank and isoflurane vaporizer off and allow O2 to flush through the system
- Close switch to “IVIS manifold” and “Induction Chamber”
- Turn oxygen lever off
- Turn exhaust pump to “off”

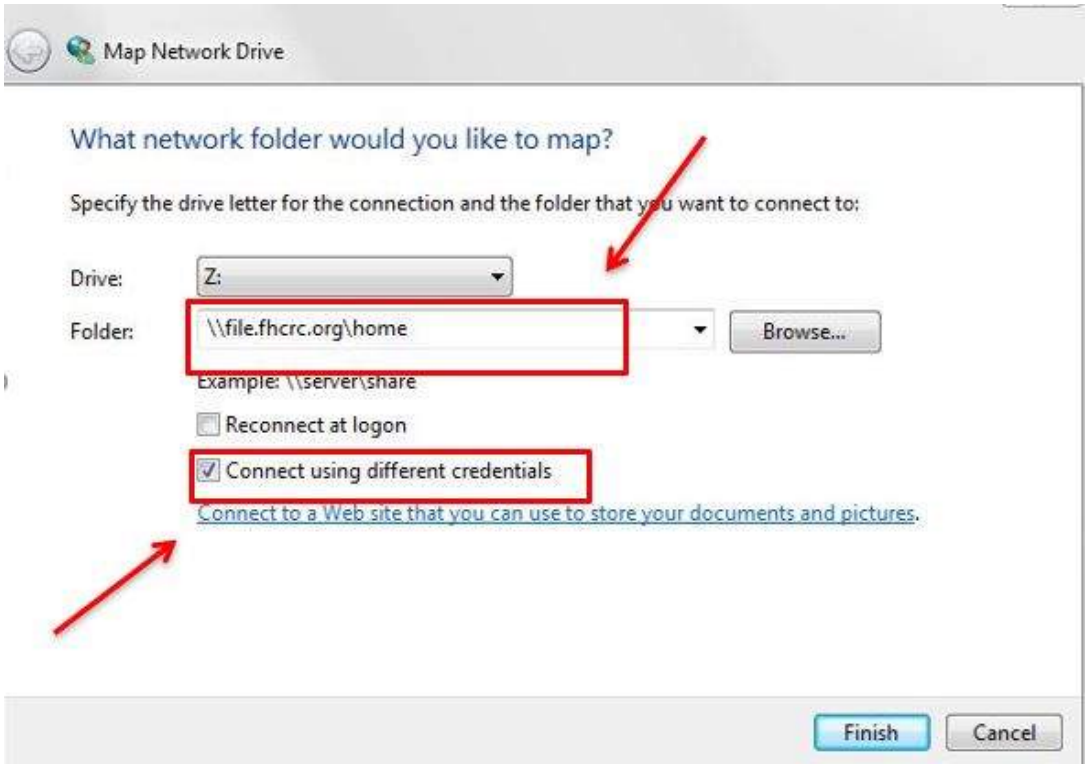
Step 3

Clean up!

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

Step 4

Save your data!



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

1. Click "Prefs"
2. Select the “Network” tab.
3. Click on “Map Network Drive”.
4. Fill in “network location” with: \\file.fhcrc.org\home “Z:” drive should be selected. Box labeled “log on with 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 IVIS images.

7. In a new File Explorer tab, open the folder on the desktop where you saved your IVIS images 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 off

iLab

Log out of iLab to avoid being charged for further time.

CONTINUE

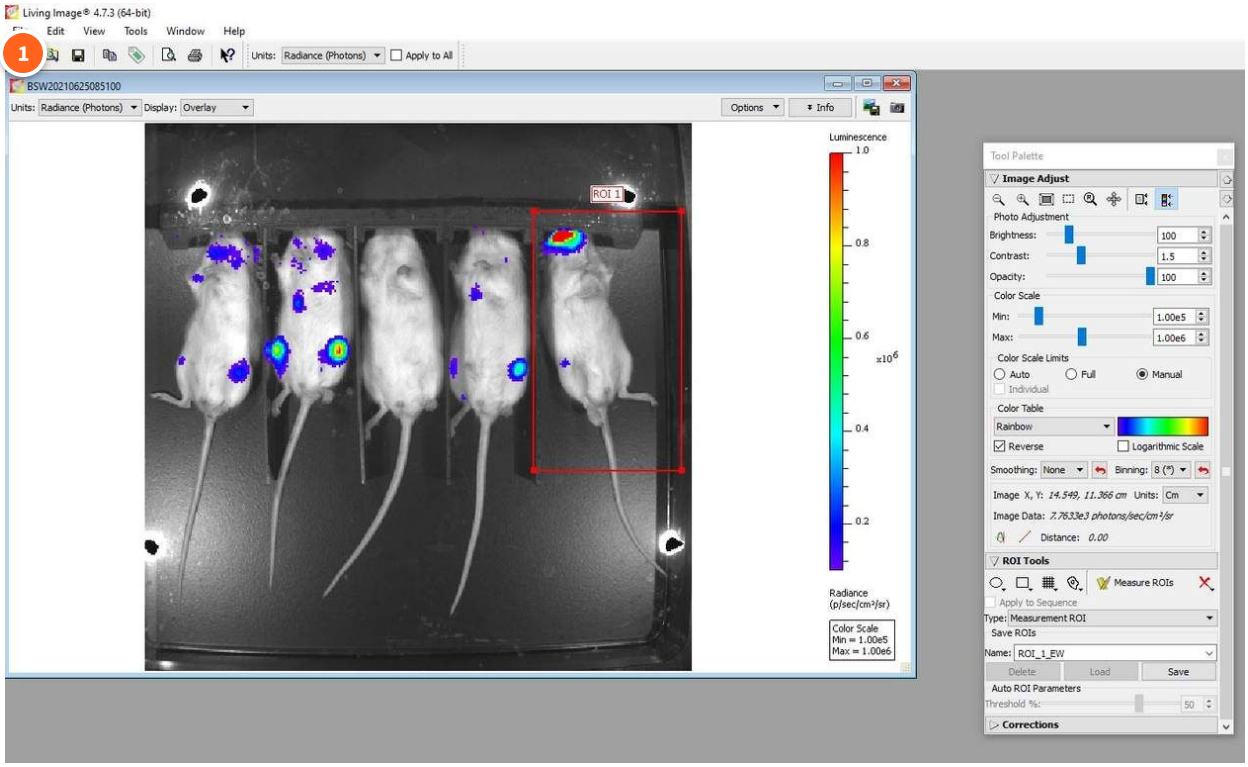
Further Resources

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 software. You will need to transfer the data off the Z: drive to the computer desktop in order to load the images into the Living Image Software.

Copies for Living Image are on Arnold Library Computers #2, #6, #7, and the Steam Plant PC #8.

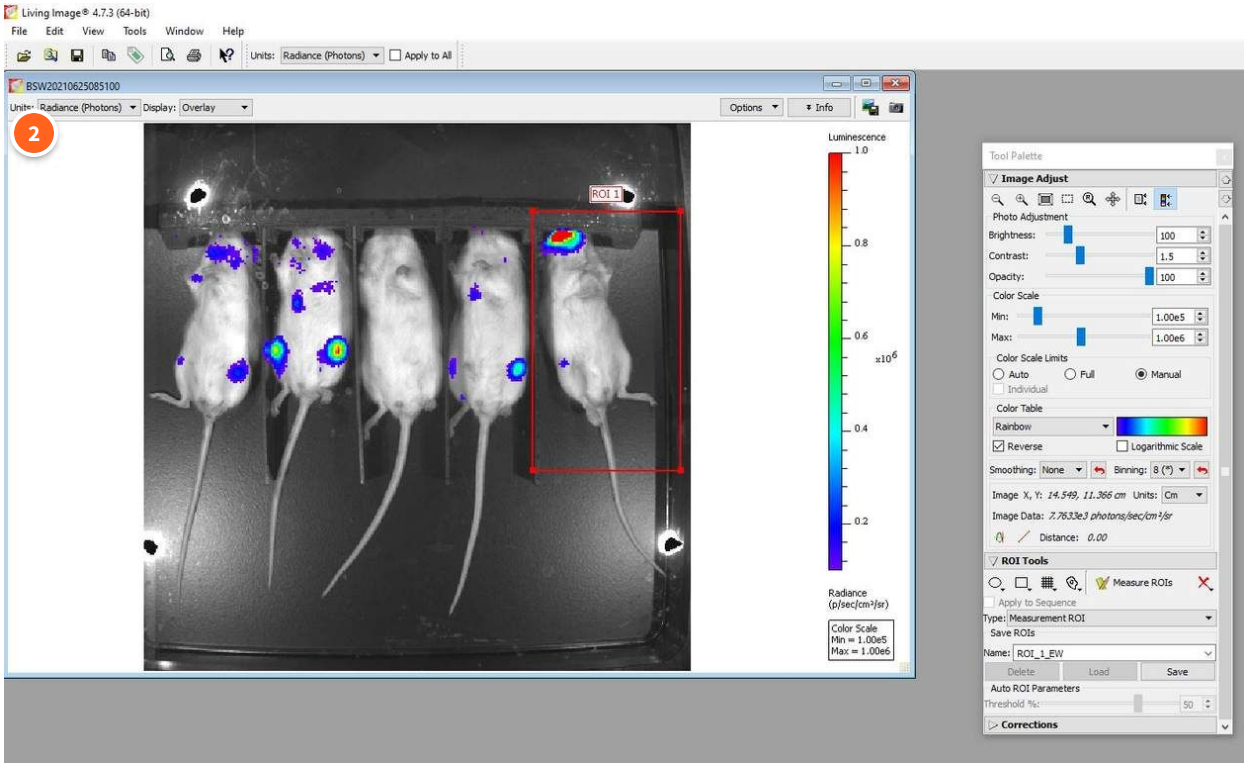


Load in data

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

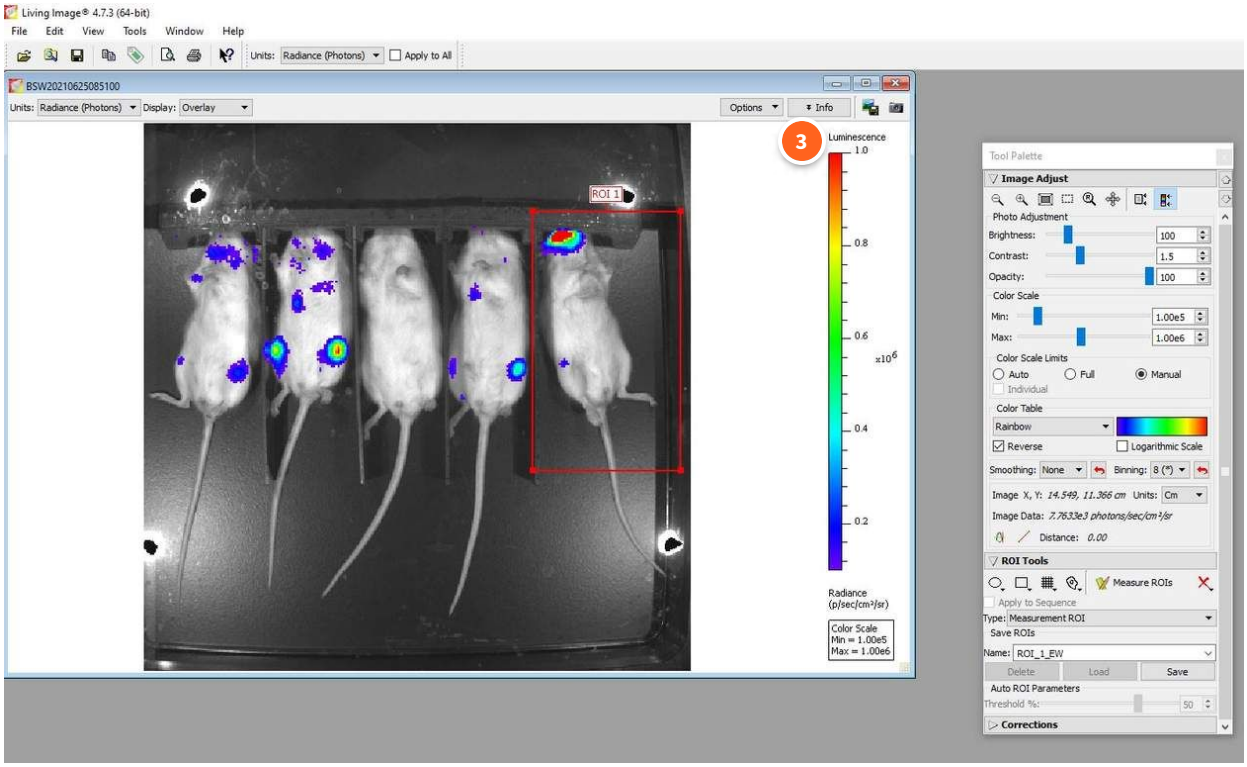
The folder naming format saves your user initials, followed by the date, followed by the timestamp on the image.

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



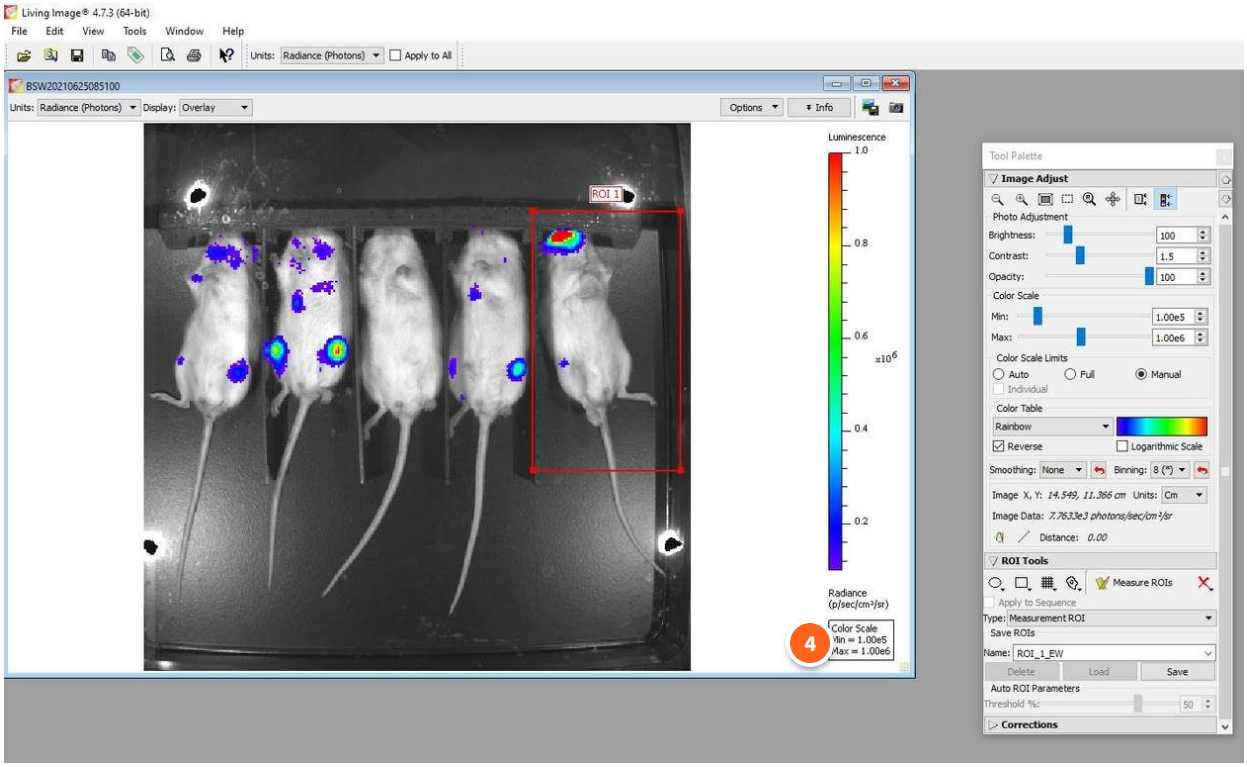
Change Units

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, animal IDs...



Color Scale Bar

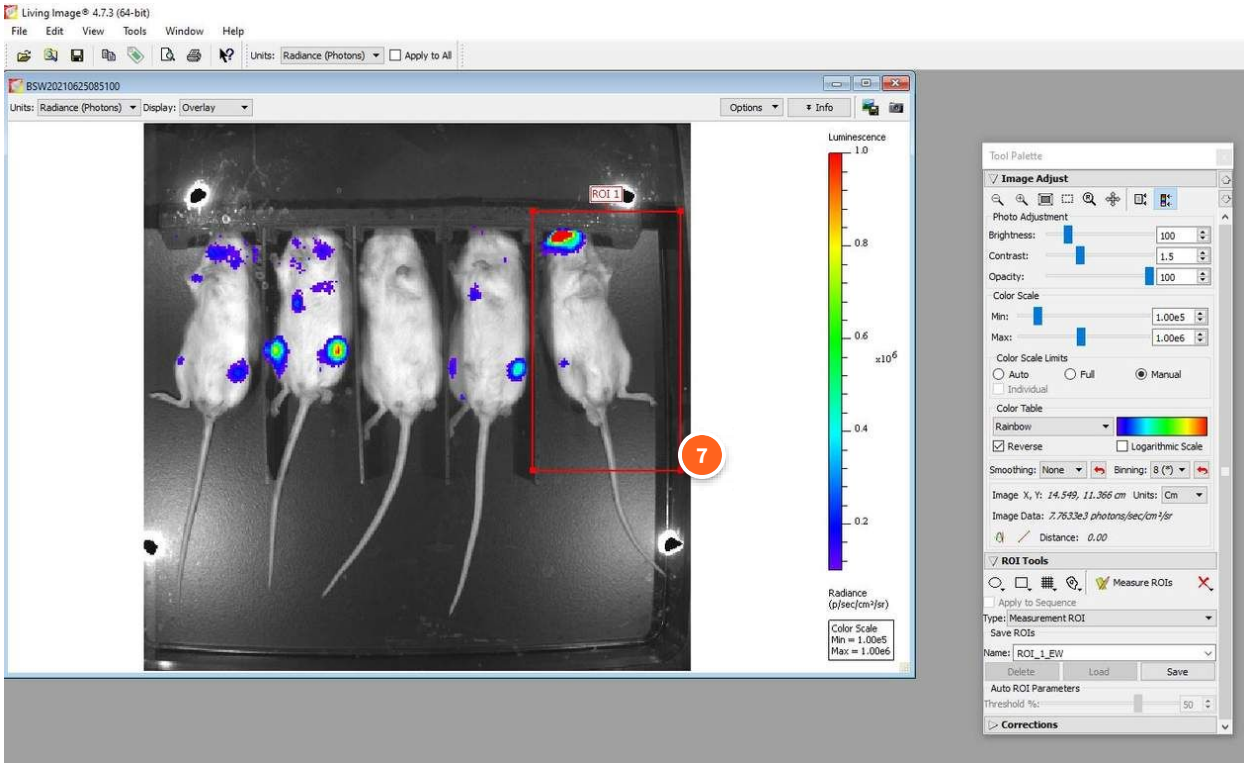
This provides your min max values of either counts or radiance.

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.

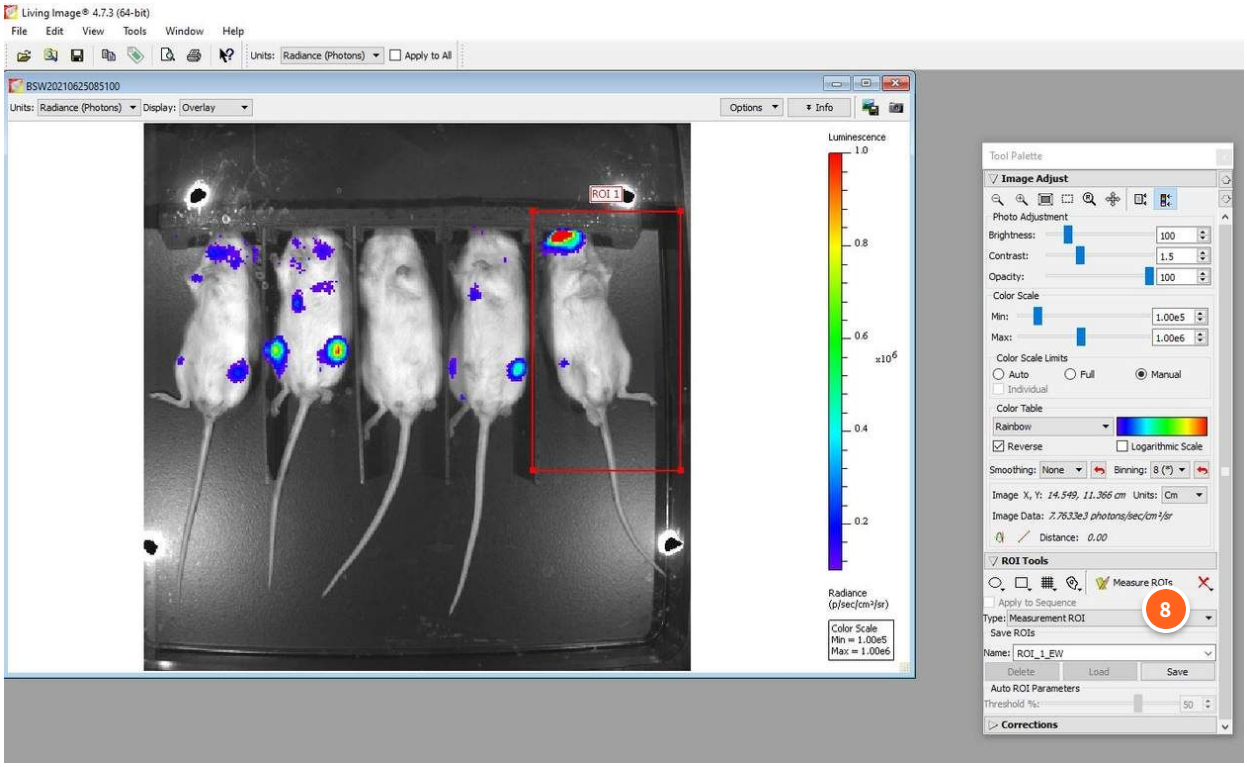
ROI tools

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



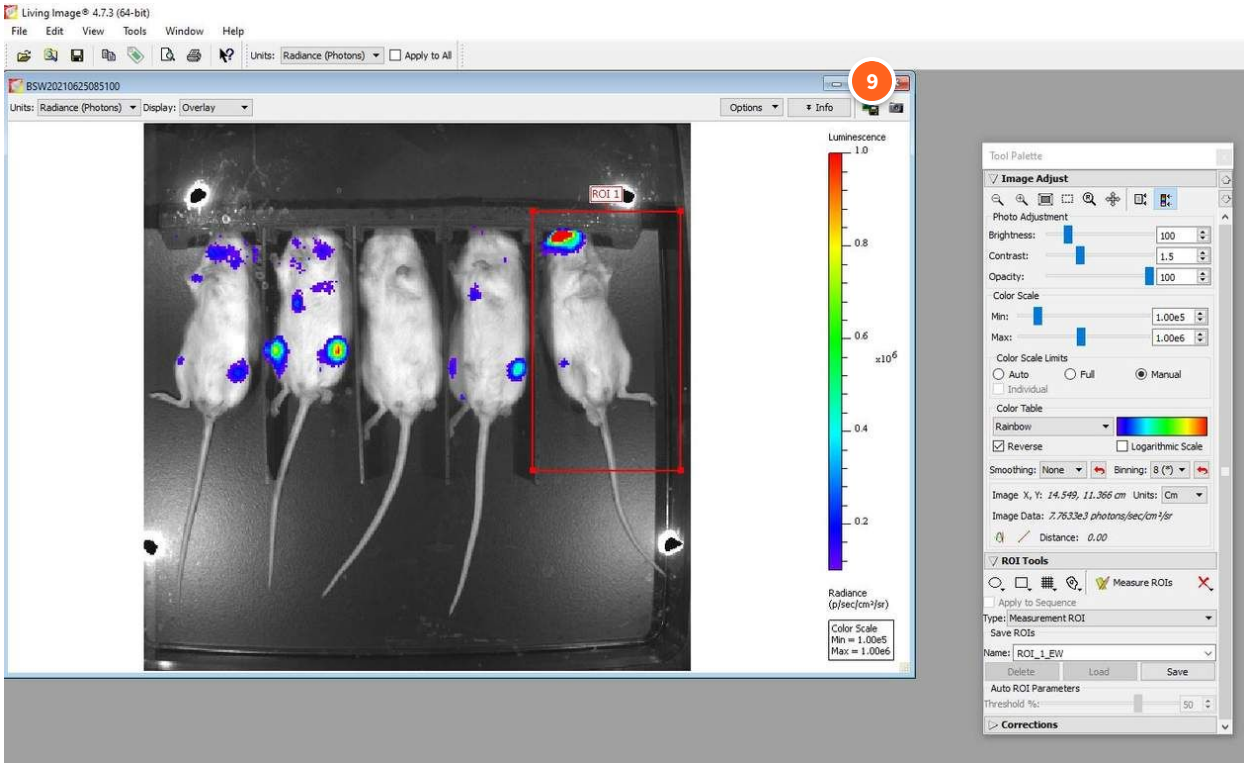
Drawing ROI

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. Set your color scale to the same as the first image, get your ROIs. 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.

Fill in the blank for the following question:

To quantitatively analyze my IVIS images I need to change my analysis units to _____ mode.

Type your answer here

SUBMIT

Application Guides

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

Fluorescence

Adaptive Fluorescence Background Subtraction
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/1db545ca-fe5a-4380-bda9-4f863f393fff_Adaptive%20Fluorescence%20Background%20Subtraction.pdf

Spectral Unmixing https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/a2e71d03-f8fb-47e1-8177-c02230f07349_Spectral%20Unmixing.pdf

Transillumination Fluorescence 1 - Setup
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/e4b1ab64-c997-4b80-96b2-c122921be89b_Transillumination%20Fluorescence%20Setup.pdf

Transillumination Fluorescence 2 - Raster Scan
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/c24c1095-5e65-4cb3-be6a-ab41d0b526ff_Transillumination%202-%20Raster%20Scan.pdf

Transillumination Fluorescence 3 - Normalize
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/6aad9bc8-5802-4e67-a03c-9ba561d61786_Transillumination%203-%20Normalized.pdf

Image Analysis with Living Image

Living Image Software guide
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/c5ca27b6-6d21-4655-8a83-bcea7c55b405__Living%20Image%20Software.pdf

AutoExposure https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/ce2659dd-e4e6-4318-8831-e3e5c0276d82__AutoExposure%20Pre-clinical%20imaging%20systems%20technical%20note%20for%20IVIS%20systems.pdf
Overlaying Images 2D
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/e0a4a246-640d-4e09-8373-559f4e9c7dca__Image%20Overlay-2D.pdf

Working with Image Math
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/261b1850-1e99-4a16-929b-e1a5da4991d0__Working%20with%20Image%20Math.pdf

Drawing ROIs
https://fredhutch.csod.com/ui/lms-learning-details/app/material/2551f77d-5e7c-40f9-9b95-2aee828bcca9

Subject ROIs https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/35187616-4b68-4c2c-b116-e71a319f1caa__Subject%20ROIs.pdf

Determining Saturation
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/934eacc9-c76f-41bb-b7e2-1d47519288ca__Determine%20Saturation.pdf

Advanced Image Acquisition

High resolution / binning
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/d26affea-08e6-42e6-bf83-8b6802e743fa__Acquisition%20of%20High%20Resolution%20Images.pdf

Advances in 3D Optical Imaging Quantifications and Sensitivity
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/b16c12b9-778a-429e-ab89-3965973bd066__Advances%20in%203D%20Optical%20Imaging%20Quantification%20and%20Sensitivity.pdf

Image Overlay - 3D
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/137412e0-5054-4eaf-ab9f-571fcdc13b10__Image%20Overlay-3D.pdf

3D Multimodality Images
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/83a7c6f8-2253-40a5-ab86-41a7588ec954_3D%20Multimodality%20Imaging.pdf

Tomographic Measurements

Setup and Sequence Acquisition
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/1b8ebf8a-ef73-4aa7-b2ef-aac1edc5096f_DLIT%201%20Setup.pdf

Topography
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/5eb2f047-6c0f-4a76-bbb1-e12424b4e722_DLIT%202%20Topography.pdf

Source Reconstruction and Analysis
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/e8dc99d3-2abd-4259-b918-33155e4de43f_DLIT%203%20Reconstruction.pdf

Fluorescence Tomography and Topography
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/e9ef947a-8615-4ebb-ad92-6b6caceadaff_FLIT%202%20Tomography.pdf

Fluorescence Tomography – Source Reconstruction and Analysis
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/67ff6ce5-56e2-4348-aaa6-15c5dfc156f3_FLIT%203%20Reconstruction.pdf

Animal Handling and Protocols

IP injections
https://fredhutch.csod.com/ui/lms-learning-details/app/material/5bc65203-8bd2-4b04-a792-b66b0a28acab

Using the Gas Anesthesia System
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/d30f1098-b29f-4e24-9fe7-2f557b3ede51_Gas%20Anesthesia.pdf

Luciferin Prep and Dosing of Animals
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/ca1a5d56-4d1b-4af4-911d-b71d1d332b6a__Luficerin%20prep%20and%20dosing%20animals.pdf

Kinetic Analysis of Bioluminescent Sources
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/0276c758-9769-4975-8c0a-43cfad97680a__Kinetic%20Analysis%20of%20Bioluminescent%20Sources.pdf

Misc

Loading groups of images
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/425de39f-b48b-4805-8c3b-98a3269a4121__Loading%20groups%20of%20images.pdf

Well Plate Quantification
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/62c38301-ca98-4bd3-b3ca-bcb9e11a676b__Well%20Plate%20Quanitification.pdf

Stem Cell Research
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/42f486f0-e7ce-4682-9caa-c7468fa54968__Stem%20Cell%20Research%20and%20Regenerative%20Medicine.pdf

Cerenkov Imaging of Radioisotopes in IVIS Systems
https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/e83e24e9-2063-4dae-9382-062db86de096__Cerenkov%20Imaging%20of%20Radioisotopes%20in%20IVIS%20Systems.pdf

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

PDF

ivis-preclinical-optical-imaging-_N94fDrL (1).pdf

9.2 MB

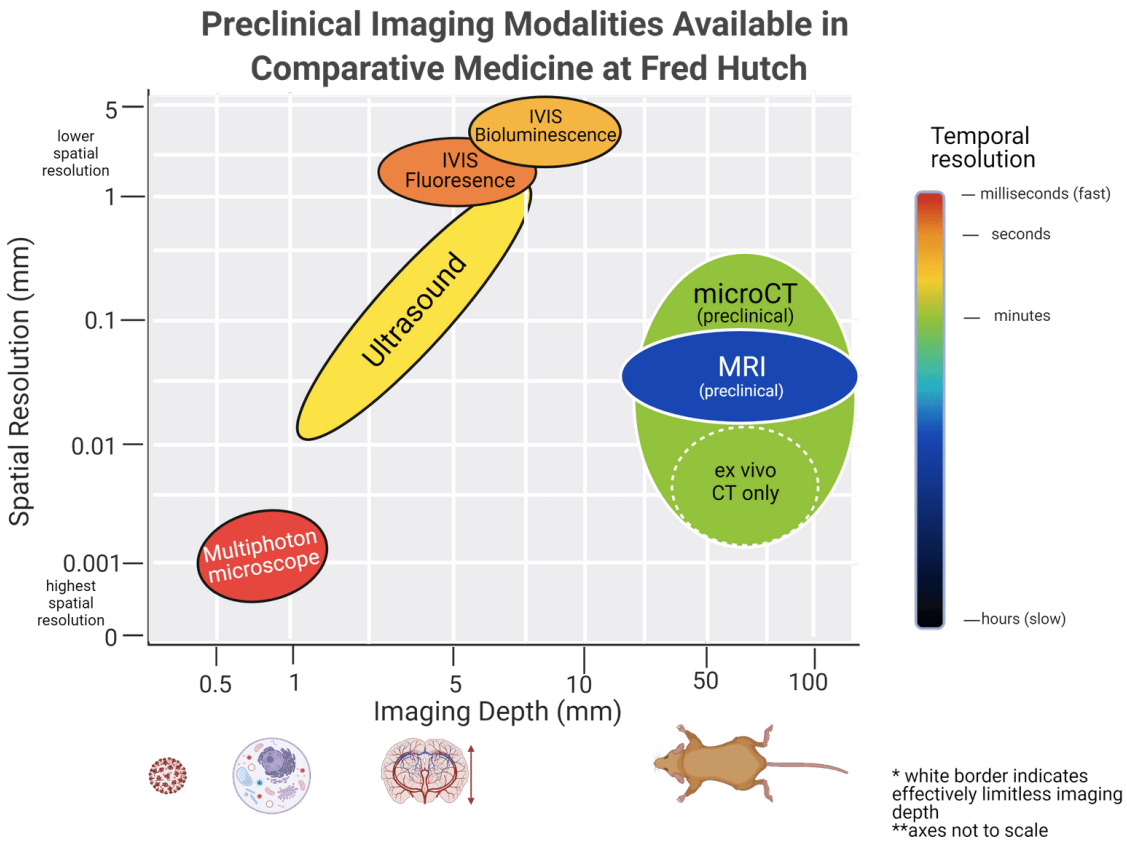
Thank you for completing this part of the IVIS training!

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

Have questions or want further information?

Preclinical Imaging offers an array of imaging services, including one-on-one consultations and analysis advice!

See below charts for further information regarding the services provided by Preclinical Imaging and feel free to send an inquiry to us at preclinicalimaging@fredhutch.org.



Spatial, temporal, and depth imaging capabilities of the imaging modalities available in Preclinical Imaging

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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.

Check coolant level before every imaging session

The BSL-2 IVIS uses 25% ethanol as coolant. This evaporates more quickly and thus it is ***imperative*** that the coolant level is ***always*** checked before every imaging session.

Not doing so may permanently damage the camera and thus render the machine useless. Then there will be no more BSL-2 IVIS.

This image shows the chiller at the back of the BSL-2 IVIS.

As shown in the photo, unscrew the cap from the top of the chiller to check the coolant level.

If the fluid level is *below* the top of the cross-hairs, re-fill with ethanol provided in the room.

Then, importantly, **return the cap** back on!

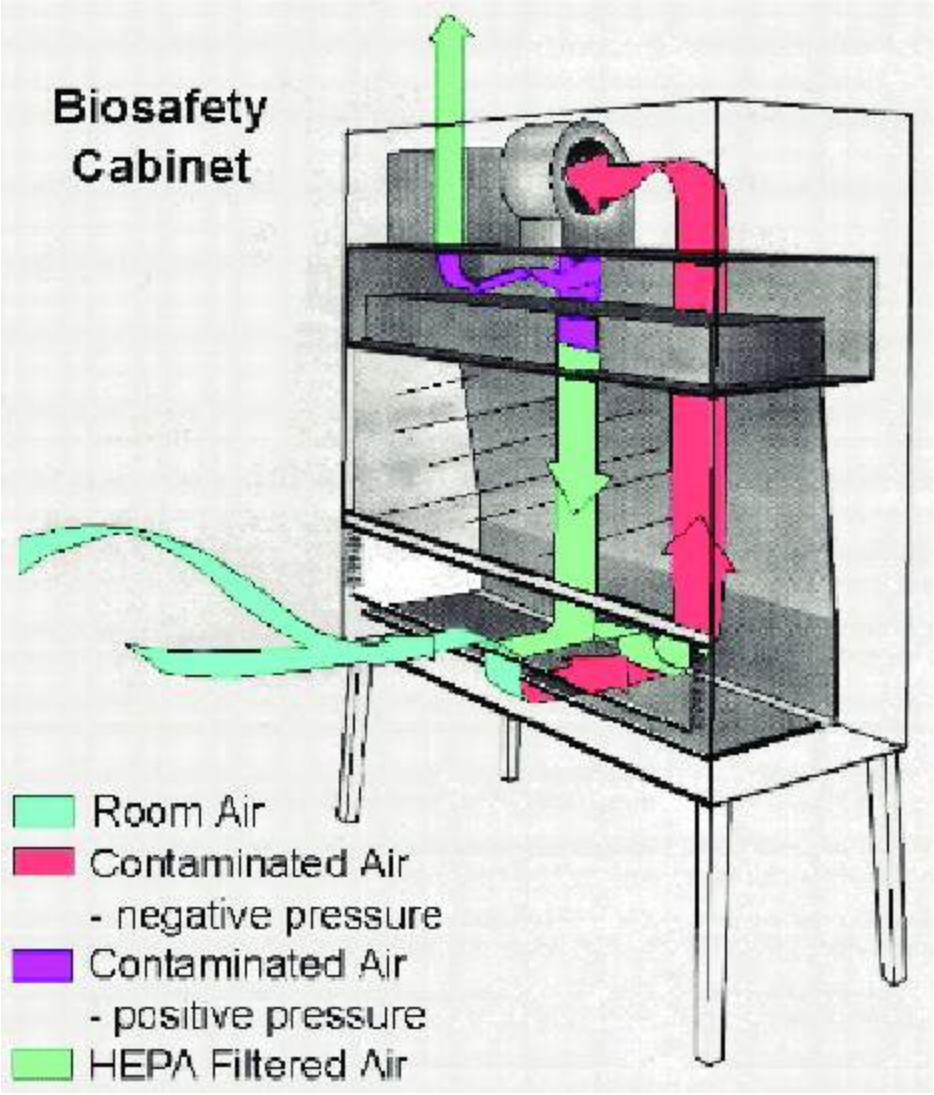
Before initializing the machine, make sure that nothing, including the BSL-2 specific imaging chamber (described below) is in the BSL-2 IVIS chamber.

Match the statements below correctly:

I will always check the coolant level	BEFORE turning the IVIS machine on in BSL 2
If the ethanol level is low I will...	Unscrew the cap at the back and add more ethanol
Under no circumstances will I...	Image in BSL2 UNLESS I check the ethanol coolant level FIRST

SUBMIT

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.

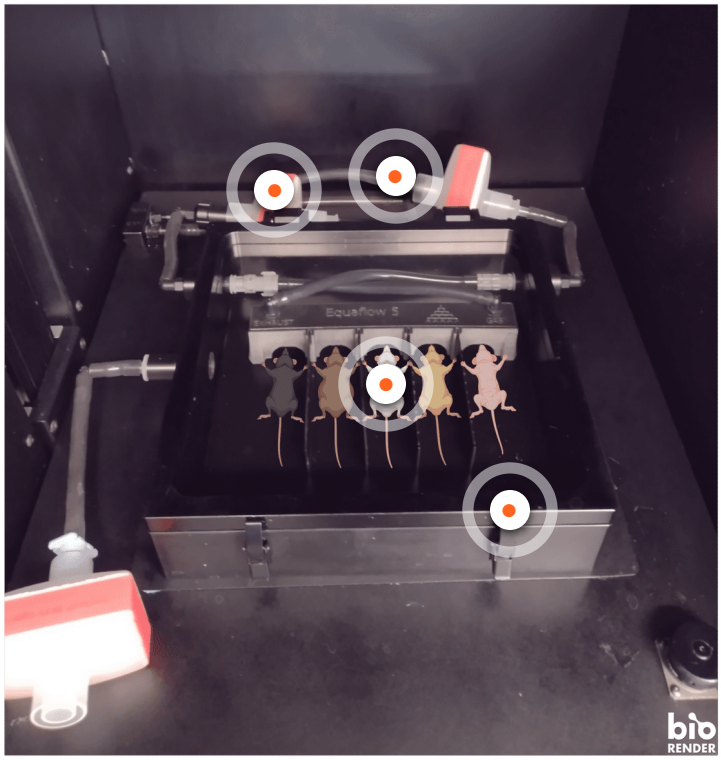
BSL-2 IVIS imaging box open inside a biosafety cabinet

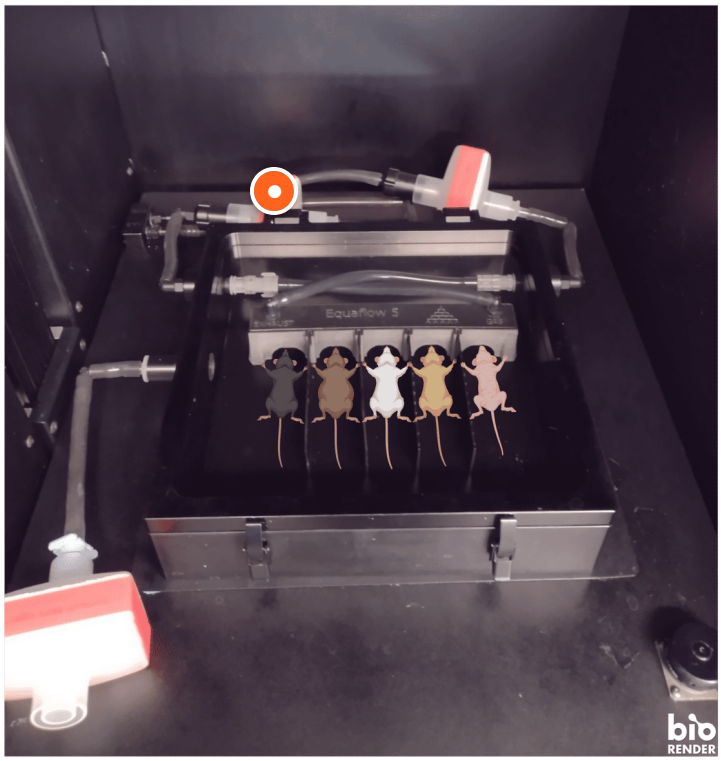
When the mice are deeply asleep, you can transfer them to this box and position them appropriately. Then close the lid and move the box into the IVIS imaging chamber. All of this is done while the mice are asleep. The imaging chamber lid must be closed before moving it out of the biosafety cabinet.

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.

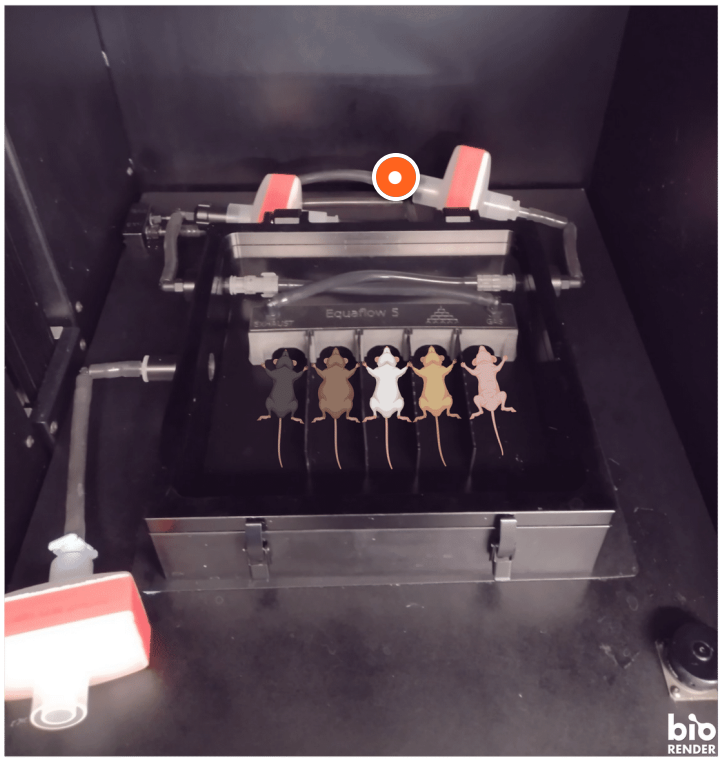




HEPA filters

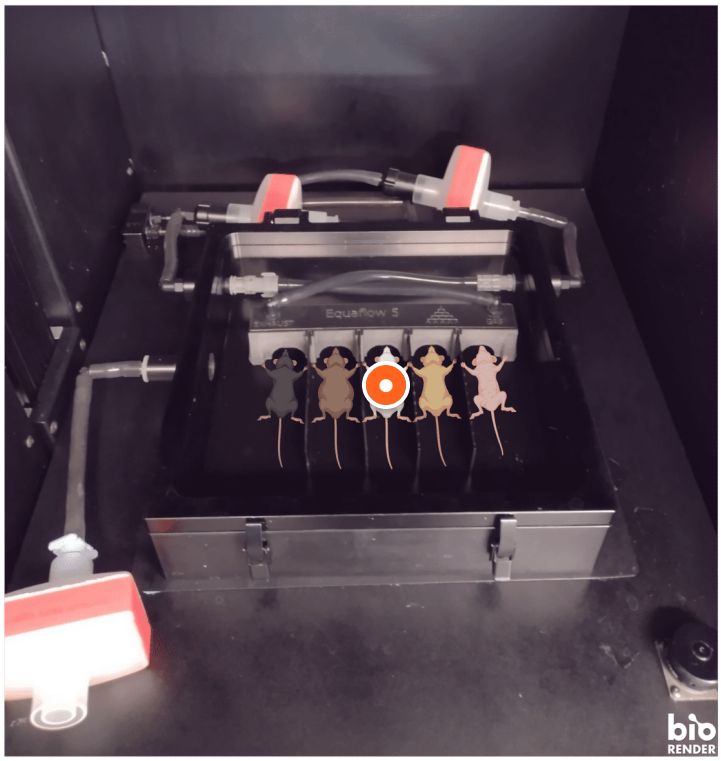
Chamber *must* be equipped with HEPA filters at all times! This is to prevent airborne pathogens from being released into the room where people are working.

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

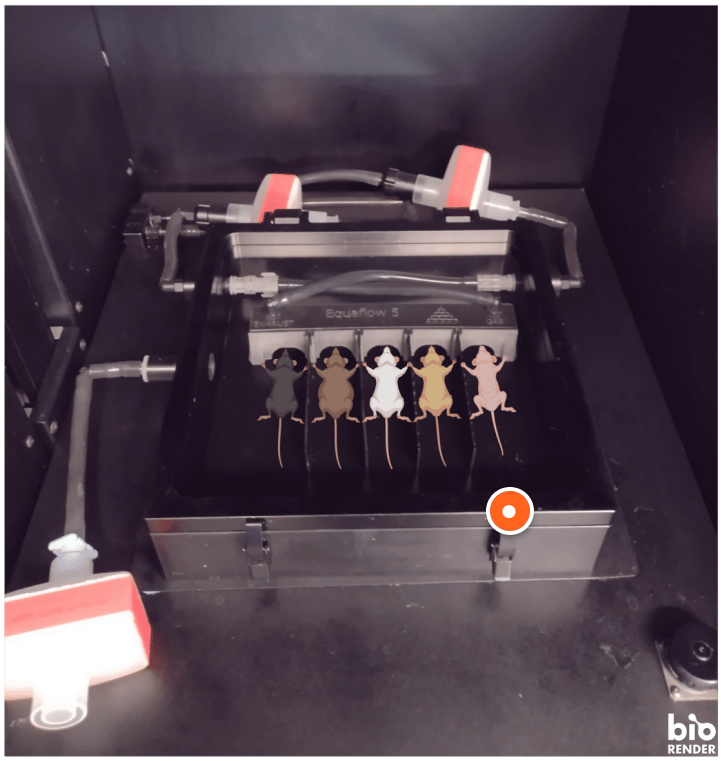
Connect the HEPA filters, which are connected to the box, to the input and output gas lines. This delivers isoflurane to the box and provides an exhaust line to outside of the chamber.



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*.