

# Spectral Instruments Imaging FAQ Knowledge Base

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## Biological Considerations for In Vivo Imaging

### Which applications are best suited for BLI versus FLI?

BLI is best for tracking cell populations, monitoring tumor growth, or measuring gene expression due to its sensitivity and low background. FLI is well suited for biodistribution studies, nanoparticle tracking, and multiplexed imaging using different fluorophores.

### What is the difference between bioluminescent and fluorescent imaging?

Bioluminescence imaging relies on luciferase enzymes that emit light when exposed to a substrate (for example, D-luciferin). It produces very low background noise and is ideal for sensitive detection of cells or molecular activity. Fluorescence imaging uses fluorescent probes or proteins excited by an external light source, offering multiplexing capabilities and more flexibility in probe selection.

Bioluminescence is an enzymatic biological process involving a luciferase that catalyzes a luciferin substrate in the presence of oxygen to produce light. This process results in a high signal-to-noise ratio because the animal produces very little background light (some chemiluminescence and phosphorescence) and no external excitation light is needed. Fluorescence requires an external light source to excite a fluorescent target molecule/reporter (e.g., proteins or dyes). In vivo fluorescent imaging has a lower signal-to-noise ratio compared to bioluminescent imaging. In addition, to better optimize fluorescent imaging it is necessary to excite and capture light at defined wavelengths corresponding to the peak excitation and emission wavelengths of the fluorochrome being used (e.g., using defined wavelength LED and bandpass filters).

### How sensitive is bioluminescence compared to fluorescence?

BLI is generally more sensitive because it does not rely on external excitation light, resulting in extremely low background noise. This allows detection of very small numbers of viable (live) cells in vivo, even in deep tissues. Fluorescence imaging (FLI), by contrast, has a higher background due to excitation light, but it enables multiplexed detection and can be used with samples that are no longer alive, making it valuable for endpoint studies, tissue sections, and assays where cell viability is not a requirement.

### How do I choose the right reporter gene or fluorophore for my application?

If your goal is to monitor live cell dynamics or longitudinal processes such as tumor growth, infection, or gene expression, choose a luciferase-based reporter (BLI). For fixed tissues, ex vivo samples, or studies involving protein, peptide, or small molecule tagging, fluorescence imaging (FLI) is more suitable. When using FLI, consider fluorophores with minimal spectral overlap and, for in vivo work, those in the near-infrared (NIR) range to minimize signal attenuation.

## What is the best wavelength of light to use for in vivo imaging?

In general, long wavelength red light (especially > 650 nm) will penetrate tissues better than short wavelength blue/green light (400 – 500 nm), especially at depth and in tissues with a high level of hemoglobin. However, there are several additional factors to consider, including:

1. short wavelength blue/green light is usually brighter (higher quantum yield) than long wavelength red light;
2. both luciferases and fluorescent proteins can possess broad wavelength distributions (up to several hundred nm, from blue to red). Thus, although a predominantly red light source will work most efficiently at depths, in some instances a brighter predominantly blue light source will penetrate better at shallow (e.g., subcutaneous) tissue depths.

## Should I use a bioluminescent or fluorescent reporter/probe for my experiment?

This choice depends principally on the biological process and disease model being imaged. Bioluminescence is significantly more sensitive than fluorescence, especially at depth in an animal. However, most luciferase-based reporters need to be genetically encoded and expressed inside a cell, making labelling slower and more laborious than fluorescent imaging with chemical fluorochromes (fluorescent proteins require similar effort to luciferases). In addition, fluorescent reporters, which are available in a wider array of wavelengths, are better for tracking multiple targets simultaneously, as well as for translational models (e.g., image guided surgery). For more info on optical imaging probes and reporter expression, please read: <https://spectralinvivo.com/wp-content/uploads/2021/06/Reporter-Expression.pdf>

## What is the best luciferase to use in my animal model?

There are several luciferases, of both terrestrial and marine origin, that have been used for preclinical imaging of disease in small animals. Factors such as availability, cost, substrate distribution (PK/PD) in vivo, wavelength, brightness, toxicity (substrate), immunogenicity, oxygen tension dependency, energy dependence (e.g., ATP), and size, are all important considerations.

**Firefly luciferase:** By far the most widely used luciferase is North American firefly luciferase (FFluc), whose mammalian optimized DNA, found in plasmids, viral vectors, engineered cell lines and transgenic animals, is relatively easy to obtain and whose luciferin substrate is inexpensive, with very good in vivo biodistribution. Moreover, at 37°C, this luciferase has a peak wavelength of 612 nm, with a significant proportion of this light emitted beyond 650 nm. Brighter variants of FFluc, such as AkaLuc, have been engineered, which, in combination with its novel substrate, Akalumine, has a peak bioluminescent wavelength of 650 nm. However, this substrate is more expensive.

**Red and green click beetle luciferase:** Both red and green click beetle luciferase are strong contenders with firefly luciferase, working equally well in vivo using firefly luciferin as a substrate. At shallow depths, the two click beetle luciferases can be used in combination and discriminated due to their different wavelengths (green – 537 nm, red – 613 nm), but this ability disappears at depth. Both click beetle luciferases are more stable than firefly luciferase at low pH.

**NanoLuc luciferase:** This very small, very bright luciferase is a great complementary reporter to firefly luciferase (for dual imaging methodologies, such as a NanoLuc labelled CAR T-cell targeting a FFluc labelled tumor) and superior BRET (e.g., NanoLuc fused to a long Stokes shift fluorescent protein) capabilities. Although its peak wavelength of 460 nm is far from ideal for imaging at depth, its superior brightness (100x – 1000x brighter than firefly luciferase) makes it a viable in vivo reporter, particularly at shallow depths. Moreover, because this luciferase is energy independent (not requiring ATP), it can be used to tag targeting antibodies and can be expressed on the surface of cells, unlike firefly luciferase which requires ATP. The in vivo substrate for NanoLuc, fluorofurimazine, although significantly more expensive than firefly luciferase, also has good in vivo biodistribution.

**Renilla luciferase:** Conventional renilla luciferase (peak bioluminescence at 480 nm) is not as bright as NanoLuc and its substrate, coelenterazine, is somewhat toxic and has poor biodistribution. Derivatives of renilla, such as Super Rluc8 (peak bioluminescence at 540 nm), have been developed that are considerably brighter, but more difficult to acquire.

**Gaussia luciferase:** Like NanoLuc this is a small, bright luciferase with a peak bioluminescent wavelength of 460 nm. However, the substrate for this luciferase is coelenterazine, which makes it less ideal for in vivo imaging, as discussed above.

**Bacterial luciferase:** The bacterial lux operon (luxCDABE) from *Photobacterium luminescens* has been engineered into a vast array of both Gram-positive and Gram-negative bacteria. This luciferase operon functions to give bioluminescence (peak wavelength of 480 nm) without the need for exogenous substrate. Versions of this operon have been generated that work in eukaryotic/mammalian cells, but this reporter construct hasn't been extensively studied or adopted for animal imaging studies.

## What type of animal model should I use for my study?

Optical imaging performance can be influenced by the animal's pigmentation, fur, and skin thickness, which affect light scattering and absorption. Hairless, albino, or white-furred animals (such as BALB/c or nude mice) are often preferred for optimal signal sensitivity and transmission. If these strains are not feasible, hair can be removed by shaving and/or using a depilatory cream, and red-shifted/NIR reporters (for fluorescence imaging) can help compensate for signal loss in pigmented animals.

Beyond optical properties, it is also essential to choose an animal model that is biologically relevant to your research question. This means selecting a strain, genetic background, or disease model that accurately reflects the physiology or pathology you aim to study; whether that's immune competence, tumor growth behavior, or organ-specific responses.

Consulting existing literature and previous imaging studies in similar models can help guide this choice.

### **Do I need hairless mice for in vivo optical imaging?**

Not always. Hair and pigmentation absorb and scatter light, especially for fluorescence. Nude or albino mice improve signal detection, but black-coated mice (e.g., C57BL/6) can still be used successfully with optimized settings, particularly in BLI. For details on the impacts of animal fur and removal when imaging continue reading: [https://spectralinvivo.com/wp-content/uploads/2022/10/rodent\\_depilation\\_v3.pdf](https://spectralinvivo.com/wp-content/uploads/2022/10/rodent_depilation_v3.pdf)

### **How does pigmentation or tissue depth affect fluorescence imaging?**

Pigmentation and tissue depth attenuate excitation and emission light, reducing sensitivity. Near-infrared (NIR) fluorophores penetrate deeper and are less affected by pigmentation.

### **Can I image luciferase in black coated mice (e.g., C57BL/6)?**

Yes, but signals may appear attenuated due to melanin absorption.

### **Should I use anesthesia during imaging sessions, and which types are compatible?**

Yes, anesthesia is required to immobilize the animals during quantitative optical imaging acquisition. Isoflurane is most commonly used because it is quick, safe, and compatible with repeated imaging. Sevoflurane, or several injectable methods can also be used but it is best to check with your Vet before proceeding.

### **How do I prepare my animal for imaging?**

In addition to potentially removing hair from the area of interest, it is advisable when performing fluorescent imaging to use a diet that doesn't contain plant material to prevent non-specific fluorescence from chlorophyll, which can interfere with a wide range of imaging probes (especially those in the 700 – 800 nm range). For additional suggestions, continue reading: [https://spectralinvivo.com/wp-content/uploads/2020/06/10Tips\\_Tricks\\_5\\_5\\_2020.pdf](https://spectralinvivo.com/wp-content/uploads/2020/06/10Tips_Tricks_5_5_2020.pdf)

### **What types of controls should I include in my optical imaging experiments?**

Recommended controls include:

- Non-transfected or unlabeled cells or animals (background control)
- Vehicle-only injection controls
- Reference animals with known levels of reporter expression

- Spectral Instruments Imaging Calibration Device (SIICD) as a positive control to confirm the system is functioning properly before imaging

These controls help with normalization, interpretation of results, and reassurance that unexpected signals are biologically related rather than due to instrument variability.

### **Can I perform both BLI and FLI in the same imaging session?**

Yes. Many researchers collect fluorescence first, followed by bioluminescence imaging. Aura software makes it easy to organize and analyze both data types in a single project. You can use Aura's Composite Image Manager feature to merge both images into one.

### **Can optical imaging be used for longitudinal studies in the same animal?**

Yes. Both BLI and FLI are noninvasive and allow repeated imaging of the same animal over weeks or months, reducing variability and animal use.

### **Can I image GFP in vivo?**

Yes, GFP can be imaged in vivo, but there are important considerations. GFP emits in the green spectrum, which overlaps with tissue autofluorescence and is strongly absorbed by hemoglobin, reducing sensitivity in deep tissues. For superficial imaging (such as subcutaneous), GFP may give good signal, but for deeper or whole-body imaging, near-infrared (NIR) fluorophores are generally recommended for better signal-to-background ratio and tissue penetration.

For more details on how tissue absorption and probe selection affect in vivo imaging, see our tech note: <https://spectralinvivo.com/wp-content/uploads/2021/06/Science-of-Optical-Imaging.pdf>

### **Why aren't quantum dots used more often as fluorophores?**

Quantum dots are highly effective fluorophores widely used in various applications. However, a significant drawback in preclinical imaging is that their absorption peaks often fall in the violet or ultraviolet range of the electromagnetic spectrum, where tissue penetration depths are shallow.

### **How and when should I administer the imaging agent (probe or substrate)?**

Delivery methods for fluorescent probes or bioluminescent substrates (luciferins) can include intraperitoneal, subcutaneous, intraorbital, or intravenous injection. The timing depends on the specific agent used:

**Substrate (luciferin) delivery:** Some protocols recommend imaging a few minutes after injection of the substrate (e.g., 5 – 10 min after subcutaneous injection or 1 – 2 min after IV injection) to capture a peak signal. However, it is good practice to run a substrate/luciferin kinetic study when first developing an animal model to more accurately identify when a peak signal occurs after substrate administration. For example, when using FFluc

engineered cells in an animal model, it is recommended that 150 mg/kg of firefly luciferin is administered subcutaneously, and the animals are imaged sequentially every 1 – 2 minutes for a period of 20 minutes. If a signal is particularly weak, 300 mg/kg luciferin can be administered subcutaneously. This same protocol can be used for other substrates (e.g., fluorofurimazine for NanoLuc, or coelenterazine for renilla or gaussia luciferase). However, the route of administration may vary due to the formulation of the substrate.

Establishing the kinetic curve for your specific model is essential, it ensures imaging is performed at the true signal peak for your enzyme/substrate system and accounts for biological factors such as tissue perfusion, substrate delivery efficiency, and reporter expression. This process is now straightforward using the Kinetics Mode in Aura software, which automatically acquires and plots signal changes over time, making it easy to visualize and determine the optimal imaging window.

**Luciferin can be administered subcutaneously.** This same protocol can be used for other substrates (e.g., fluorofurimazine for NanoLuc, or coelenterazine for renilla or gaussia luciferase). However, the route of administration may vary due to the formulation of the substrate.

**Fluorescent probe delivery:** There is a vast array of fluorescent probes readily available. These come in a multitude of different wavelengths and chemical formulations. Those that have high quantum yield with excitation and emission wavelengths between 650 nm and 850 nm are most desirable for in vivo imaging. In most instances, these chemical fluorochromes are injected IV and remain in the animal's tissues for 24 – 72 hrs. If targeted (e.g., attached to an antibody) or activated (e.g., containing a proteolytic cleavage site), imaging is usually possible 6 – 48 hrs after administration.

Establishing the kinetic curve for your specific model is essential, it ensures imaging is performed at the true signal peak for your enzyme/substrate system and accounts for biological factors such as tissue perfusion, substrate delivery efficiency, and reporter expression. This process is now straightforward using the Kinetics Mode in Aura software, which automatically acquires and plots signal changes over time, making it easy to visualize and determine the optimal imaging window.

For more details on our kinetics feature, check out this poster: [https://spectralinvivo.com/wp-content/uploads/2024/03/kinetics\\_poster\\_emim\\_2024\\_print.pdf](https://spectralinvivo.com/wp-content/uploads/2024/03/kinetics_poster_emim_2024_print.pdf) and webinar: <https://www.youtube.com/watch?v=0TbgVHyF8lg>

## **How does in vivo optical imaging compare to other imaging modalities like PET, SPECT, CT, or MRI?**

Optical imaging is fast, cost-efficient, and highly sensitive, making it ideal for high-throughput screening and real-time data acquisition. Unlike modalities such as PET or SPECT, which rely on radioactive tracers, optical imaging uses non-ionizing light and

bioluminescent or fluorescent reporters, offering a safer and more convenient workflow for longitudinal studies.

While PET and SPECT provide high sensitivity and excellent depth penetration for quantitative functional imaging, they require radiochemistry facilities and have higher operational costs. CT and MRI, on the other hand, offer detailed anatomical information with superior spatial resolution and 3D reconstruction capabilities, but they are slower, more expensive, and less suited for dynamic molecular imaging.

Optical imaging complements these techniques by providing rapid functional and molecular readouts that can guide or validate findings from other imaging modalities. As a result, multimodal imaging; combining optical imaging with CT, MRI, PET, or SPECT, has become increasingly common, offering researchers a more comprehensive understanding of both molecular and anatomical changes within the same animal.

For a more thorough comparison of preclinical imaging modalities, continue reading this tech note: <https://spectralinvo.com/wp-content/uploads/2021/07/Comparison-of-Preclinical-Imaging-Modalities.pdf>

## **What are the main limitations of in vitro imaging compared to in vivo imaging?**

In vitro imaging provides high control and sensitivity but lacks the complexity of living systems. In vivo imaging captures whole-animal physiology but must account for tissue absorption, scattering, and biodistribution.

## **How do I transition from in vitro to in vivo optical imaging experiments?**

Start by verifying your reporter system works in vitro, then move to small pilot in vivo studies. This ensures proper substrate activity, fluorophore stability, and imaging sensitivity. Read this tech note more on this topic: [https://spectralinvo.com/wp-content/uploads/2024/09/why\\_move\\_from\\_in\\_vitro\\_to\\_in\\_vivo2.pdf](https://spectralinvo.com/wp-content/uploads/2024/09/why_move_from_in_vitro_to_in_vivo2.pdf)

## **Why is my bioluminescent signal intensity low?**

Potential causes of low signal intensity when imaging cells or animals (transgenic) genetically engineered with a luciferase may include:

1. inefficient or ineffective reporter delivery (transfection, viral transformation);
2. weak promoter or response element;
3. DNA methylation (especially with viral promoters);
4. insufficient dose of the substrate;
5. incorrect delivery of the substrate (missed tail vein injection with IV delivery or injecting into a fat pad with IP delivery);

6. Inadequate PK/PD delivery of the substrate (reduced ability to cross BBB or physical structure/tissue matrices in the animal).

### **Why is there high background fluorescent signal?**

High background fluorescence can be due to a multitude of reasons including:

1. autofluorescence from the animal (e.g., diet containing plant material);
2. phosphorescent materials (e.g., plastics or dye markers) in the imaging chamber;
3. dirty equipment.

### **What if I get an inconsistent signal in my animals?**

Variability can arise from uneven administration of the imaging agent. For the most consistent results, standardize handling protocols and ensure the substrate is distributed evenly. For bioluminescent studies, use Aura software's Kinetics feature to identify and capture images during the reporter enzyme's peak plateau time.

### **Which luciferase substrates should I use, and how do I administer them?**

D-luciferin is standard for firefly luciferase, while coelenterazine is used for Renilla luciferase. Substrates are typically administered intraperitoneally (ip.), subcutaneously (subQ), or intravenously (iv). Read this tech note for more details on optimizing substrate dosing: [https://spectralinvivo.com/wp-content/uploads/2025/01/Optimizing\\_Substrate\\_Dosing\\_for\\_Reliable.pdf](https://spectralinvivo.com/wp-content/uploads/2025/01/Optimizing_Substrate_Dosing_for_Reliable.pdf)

### **How do I determine the optimal time point to image after substrate injection?**

Perform a kinetics experiment! Aura's Kinetics Feature automates peak/plateau detection, helping you identify the best time to image for consistent results. For more details, watch this webinar on the Kinetics feature in

Aura: [youtube.com/watch?v=0TbgVHyF8lg&feature=youtu.be](https://www.youtube.com/watch?v=0TbgVHyF8lg&feature=youtu.be)

### **How do I minimize autofluorescence during fluorescence imaging?**

Use near-infrared probes and place animals on an alfalfa-free diet at least 5 days prior to imaging. For additional suggestions, continue reading: [https://spectralinvivo.com/wp-content/uploads/2020/06/10Tips\\_Tricks\\_5\\_5\\_2020.pdf](https://spectralinvivo.com/wp-content/uploads/2020/06/10Tips_Tricks_5_5_2020.pdf)

### **How can I minimize variability when comparing longitudinal BLI signals?**

Keep substrate dose, route, and timing consistent across sessions. Use Aura's Kinetics feature during pilot experiments to determine the signal's plateau phase, then fix this imaging window for all time points. Maintaining consistent anesthesia protocols and imaging temperature also reduces physiological variability.

## **Biological Considerations for In Vivo Imaging**

### **What imaging modalities are supported by Spectral Instruments Imaging systems?**

Spectral Instruments Imaging systems are all designed for Bioluminescence and Fluorescence imaging; with select models including planar X-ray. As part of Bruker, some Spectral data can be coregistered with other Bruker and Molecubes preclinical imaging modalities, such as mCT, PET, SPECT and MRI.

### **How many animals can I image at once in my system?**

This depends on your system model. The Lago/X accommodates up to 10 mice simultaneously. The Ami HT/X accommodates up to 5 mice simultaneously. The Kino accommodates up to 3 mice simultaneously. Rats, hamsters, guinea pigs, NHP, Zebra Fish and many other animal models can also be imaged.

### **Do I need calibration before each experiment?**

No calibration is required, as the systems are factory calibrated. Instead, use the Spectral Instruments Imaging Calibration Device (SIICD) as a reference check.

### **What is the recommended procedure for imaging multiple animals at the same time?**

To achieve consistent and reliable imaging results when imaging multiple animals simultaneously:

1. Anesthetize and position uniformly: Ensure all animals are under similar levels of anesthesia and placed in consistent orientations on the imaging platform. This reduces variability in signal due to movement or positioning.
2. Injection timing and consistency: Administer substrates (for BLI) or fluorescent probes at the same time and via the same route for all animals. This ensures each animal is imaged at comparable stages of substrate uptake or reporter expression.
3. Animal handling systems: For studies with larger cohorts, consider using an animal management systems to improve workflow and positioning accuracy:
  - DecaFlow: Enables imaging of up to 20 mice efficiently, with precise positioning and the ability to quickly swap trays for continuous imaging. [https://spectralinvivo.com/wp-content/uploads/2023/10/decaflow\\_flyer.pdf](https://spectralinvivo.com/wp-content/uploads/2023/10/decaflow_flyer.pdf)
  - PentaFlow: Supports up to 10 mice with the same efficiency and positioning benefits. [https://spectralinvivo.com/wp-content/uploads/2023/10/pentaflow\\_flyer\\_v5.pdf](https://spectralinvivo.com/wp-content/uploads/2023/10/pentaflow_flyer_v5.pdf)

## How long can I safely keep animals under anesthesia for imaging?

Most sessions last 5–20 minutes. Longer sessions are possible with proper monitoring and enabling the Heated Platform feature on your Spectral Instruments Imaging system to prevent hypothermia. Refer to your animal handling protocol for your project and consult your veterinarian.

## How do the Kino, Ami HT, and Lago systems differ?

While all Spectral Instruments Imaging systems share the intuitive Aura software, they are designed to meet different workflow needs and experimental scales. Key differences include field of view, sensitivity, LED excitation wavelengths, emission filter options, and upgrade paths:

- **Kino:** A benchtop system for imaging three mice simultaneously. It offers 10 LED excitation wavelengths and 5 emission filters, making it suitable for standard BLI and basic FLI applications. Ideal for small-scale studies where space and throughput are limited.
- **Ami HT:** Also a benchtop system, the Ami HT supports up to five mice at once. It features 10 LED excitation wavelengths and 10 emission filters, providing enhanced flexibility for multiplexed imaging and studies requiring higher sensitivity than the Kino. Can be equipped/field upgraded with X-ray. The system has an upgrade path to expand capabilities as experimental needs grow.
- **Lago:** A floor standing system designed for high throughput, demanding preclinical workflows, capable of imaging up to ten mice simultaneously. The Lago provides 14 LED excitation wavelengths and 20 emission filters, along with the highest sensitivity in the lineup. Can be equipped/field upgraded with X-ray. It supports advanced workflows such as 3D BLI/tomography and can be co-registered with Bruker/ Molecubes MRI,  $\mu$ CT, PET, and SPECT for multimodal imaging.

For a full comparison of specifications, features, and upgrade options across all systems, visit: <https://spectralinvivo.com/imaging-systems/>

## What emission filter options are included for fluorescence imaging?

You can select from this standard set of available emission filters: 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, & 870nm

## What kind of cooling system is used for the camera?

All Spectral in vivo imaging systems feature a deeply cooled, custom-built camera that quickly cools its CCD sensor to -90°C in less than 5 minutes using an air-cooled Peltier system. This design improves efficiency, enhances image quality by reducing noise, & improves reliability by eliminating the risk of leaks found in gas or liquid cooling systems. More on this via this link: <https://spectralinvivo.com/data/#no-leaks>

## **What determines how sensitive an imaging system is? How do you measure this?**

The sensitivity is largely dictated by the amount of background noise in a system... as sensitivity is essentially the minimum signal that can be resolved above that noise.

## **How do you measure the sensitivity of these imaging systems?**

The gold standard to measure the sensitivity of an imaging system is Minimum Detectable Radiance (MDR).

## **How do I clean the imaging system?**

Proper cleaning helps maintain both performance and longevity of your system. Follow these guidelines:

Exterior surfaces:

- Use a lint-free wipe with soap and water or one of the following approved cleaning solutions:
  - 70% alcohol (ethyl or methyl) / 30% deionized water
  - Clidox®-S
  - Sporidicin®
  - Trifectant® Disinfectant
  - MB-10

Interior surfaces (imaging chamber and platform):

- Do not spray or pour liquids directly into the chamber.
- Saturate a lint-free wipe with the cleaning solution and gently wipe the platform or chamber walls.
- To minimize potential fluorescence artifacts from some cleaning agents, wipe surfaces afterward with deionized water. Avoid pooling water on the platform.

Important:

- Do not clean the ceiling of the imaging chamber without contacting Spectral Instruments Imaging first. Extreme care is required to avoid damaging sensitive optical components.

## **What kind of maintenance schedule is required?**

Spectral Instruments Imaging systems are designed to require minimal user maintenance. There are no routine calibrations needed; the system is calibrated from the factory.

Preventive maintenance visits are typically performed annually by certified service engineers.

### **Do I need to turn off the imaging system every night?**

No, it's not necessary to power down the system each night. Most users simply close Aura and leave the system in Green Mode, which places it in a low-power standby state. This helps maintain camera temperature stability and allows for faster startup the next day.

### **How long does it take for the imaging system to be ready for imaging?**

From startup, the system is ready within a few minutes. The most time sensitive component is the camera cooling: the camera cools to its operating temperature ( $-90^{\circ}\text{C}$  absolute) in about 5 minutes. You can begin setting up your acquisition session while the camera cools, so by the time you're ready to image, the system will be as well.

### **How quickly can an imaging system be delivered?**

Systems are typically available to ship from inventory within 48 hours of order processing.

### **Can I demo one of these systems?**

Yes. You can request a remote demonstration of our imaging systems by submitting a request here: <https://spectralinvivo.com/live-demo>. Remote demos allow you to see the system in action, review software workflows, and ask questions to an applications scientist in real time.

### **I have an IVIS™, what should I expect when transitioning to a Spectral Instruments Imaging system?**

Transitioning from an IVIS™ system to a Spectral Instruments Imaging system is straightforward and comes with several advantages. All Spectral systems use Aura software, offering an intuitive interface for BLI and FLI while maintaining familiar quantitative workflows. You can also load your old LivingImage™ files and analyze them directly in Aura. Like IVIS™, Spectral systems are absolutely calibrated to a NIST-traceable photon standard, enabling direct comparison of datasets across instruments and platforms. Expanded fluorescence capabilities with LED excitation wavelengths improve multiplexing and tissue imaging. Kinetics mode allows automated peak/plateau detection for bioluminescent substrates, ensuring reproducible longitudinal studies. Comprehensive support, documentation, tech notes and tutorials guide users in probe selection, animal handling, and imaging best practices.

## Software: Acquisition & Analysis

### **I have never used Aura software. What resources are available to help me get started?**

Yes. Aura Analysis Software is free to download and available for both Mac & PC users.

### **I have never used Aura software. What resources are available to help me get started?**

In addition to the manual (which can be found under Help in the main menu), our [learning center](#) has several video tutorials and tech notes to help you get started. You can also request a free, remote training here: [spectralinvivo.com/contact-us/](https://spectralinvivo.com/contact-us/)

### **Can I analyze my old Living Image data in Aura?**

Yes. Aura can import Living Image files directly. To do this, click File → Image Manager, select the desired file, and click Load. Your images will appear in Aura for processing and quantification. Follow this link for a video tutorial:

<https://www.youtube.com/watch?v=PvDJO-ps1AQ&feature=youtu.be>

### **Do the Kino, Ami HT, and Lago use the same software?**

Yes. All Spectral Instruments Imaging systems use Aura software, ensuring a consistent user experience across platforms. Data acquired on any system can be analyzed, processed, and compared within the same workspace or via our license free analysis software.

### **Can I expect comparable results between different Spectral models or sites?**

Yes. Every Spectral Instruments Imaging system is absolutely calibrated to the same NIST-traceable photon standard, so datasets can be directly compared between instruments, even across facilities or collaborators. This consistency ensures reproducible, quantitative measurements and is a key advantage for collaborative or multi-site studies. In addition, because of this absolute calibration, data from Spectral systems can also be compared to other absolutely calibrated platforms, such as IVIS™, enabling broader cross-platform analysis.

### **How do I draw regions of interest (ROIs)?**

Select the ROI tool from the Toolbox and draw shapes around areas of interest. ROIs can be adjusted, copied, or applied across multiple images for consistent measurements. For BLI, use Total Emission (Photons/Second) to quantify signal within the ROI. For fluorescence, use Total Efficiency (cm<sup>2</sup>). For a step-by-step guide, watch this short tutorial on ROI analysis: <https://www.youtube.com/watch?v=GaASXN8aRTQ>

## **Can I compare data from different imaging sessions?**

Yes. Aura enables side-by-side comparison of images and quantitative data. To do this, launch Aura, go to File → Image Manager, select multiple .amix files, and click Load.

## **How do I save Images for presentations or publications?**

In Aura, you can save images by clicking on File → Save Graphics, selecting the file type you prefer (PNG, TIFF, JPEG, BMP, or PPM), and then clicking Save. This allows you to export high quality images suitable for presentations, manuscripts, or reports.

## **What camera settings should I use?**

In most cases, you can start with Easy Mode, which automatically determines the optimal acquisition settings for your image. This mode balances sensitivity and dynamic range, helping you capture high quality data.

If you wish to use manual mode for bioluminescence imaging (BLI), you can fine tune the exposure time, binning, and f-stop as needed, taking care to avoid saturation. If you receive a message about overexposed pixels, reduce the exposure time, binning level or close the f-stop slightly.

If you wish to use manual mode for fluorescence imaging (FLI), you should first adjust the excitation and emission filter selection as well as the LED% power. Optimize these settings along with exposure time, binning and f-stop to maximize signal-to-background ratio.

## **Do I need to normalize signal intensities to exposure time or binning?**

No. Because Spectral Instruments Imaging systems are absolutely calibrated, all total emission and total efficiency measurements are independent of camera settings, making normalization unnecessary.

## **How does absolute calibration improve reproducibility in publications?**

Absolute calibration allows quantitative photon-based measurements (photons/sec) that are independent of acquisition parameters. This ensures reproducibility of published data, making figures and datasets traceable and comparable between studies; something often missing in systems relying on arbitrary “intensity units.”

## **What exposure times should I start with for bioluminescence imaging?**

Start with short exposure times 10-15 seconds and adjust as needed. If signals are strong, reduce exposure to prevent saturation; if weak, increase exposure and/or use binning to enhance sensitivity. You can also use Smart AutoExposure, enabled in Aura’s Easy Mode.

## **How do I avoid signal saturation in fluorescence imaging?**

Check for overexposed pixels in Aura’s Overexposed Pixel Map (under Image Type) and reduce: exposure time, LED excitation power percentage, or binning levels. Adjust excitation and emission filter sets to improve signal-to-noise.

**Between the total emission (photons/s) vs. Mean Rad (Photons/s/cm<sup>2</sup>/sr), which one should I use?**

Use Radiance as the measurement unit and report your BLI data in Total Emission (photons/second).

Even if the exposure time or other camera parameters (e.g., binning, f/stop) vary between acquisitions, you'll still be able to directly compare your Total Emission values across different time points. This is because all Spectral Instruments Imaging systems are absolutely calibrated.