APPLICATION NOTE

Optical imaging on the IVIS SpectrumCT system: General and technical considerations for 2D and 3D imaging.

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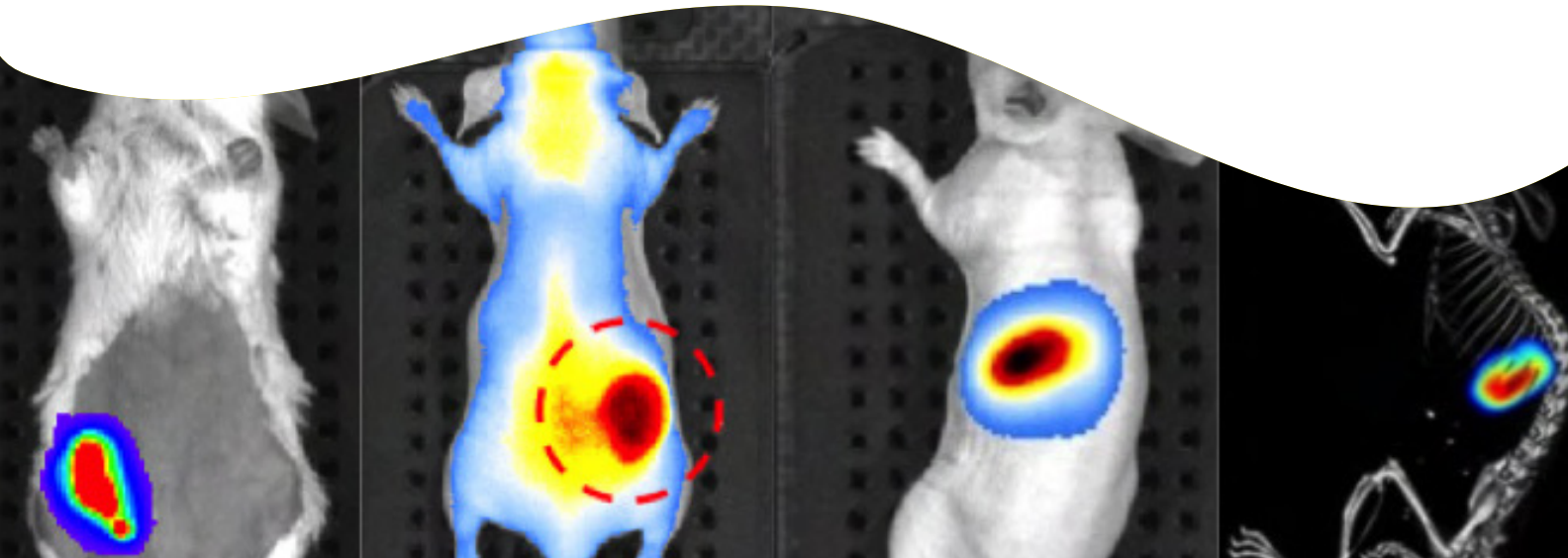
Abstract

The IVIS® SpectrumCT is a powerful and versatile optical imaging platform for small animal imaging in pre-clinical settings. With a relatively small floor footprint, it provides two key optical imaging functionalities: Bioluminescence (BLI) and Fluorescence (FLI). Its advanced optical components, including an ultra-sensitive CCD camera and two full range excitation/emission filter sets, enable the system to operate in a broad spectral range, extending from the visible to near infrared (NIR) light. Simple 2D BLI/FLI scanning for surface signal offers a powerful tool for many applications in preclinical research, and the 3D BLI/FLI scanning modes expand this system's capabilities beyond standard optical imaging. Bioluminescence acquired in 3D scanning mode provides sufficiently bright spectral signatures, differing with source depth, to enable 3D localization and accurate reconstruction. The 3D fluorescence scanning mode, which uses a transillumination strategy for delivering excitation light, more effectively excites deep tissue signal, which allows fluorescence detection and quantification in challenging models, like orthotopic cancer and pulmonary inflammation, that cannot be imaged as effectively in 2D. Pairing these 3D optical datasets with the system's X-ray computed tomography (CT) component provides biologically relevant, anatomically accurate context to best interpret your imaging results. Together with the Living Image® software for image analysis and a great variety of optical imaging agents for biological investigations, Revvity's IVIS SpectrumCT provides an all-around optical imaging solution that fits many different research needs. The goals of this technical note are: (1) to provide the users a general, comprehensive understanding of the operational principles and the underlying differences between BLI and FLI; and (2) to explain and demonstrate technical considerations and animal preparations that can assure optimal 2D/3D optical imaging using the IVIS SpectrumCT system.



Materials and methods

Fluorescence imaging agents

Seven Revvity fluorescent agents (IVISense™ Folate Receptor 680, IVISense Integrin Receptor 750, IVISense Bombesin Receptor 680, IVISense Transferrin Receptor 750, IVISense MMP 680, IVISense Annexin-V 750, IVISense Gastrointestinal 750) were used to image tumors and tissues under indicated experimental conditions. The imaging dose for these agents was as recommended in the product insert (2 nmol/25 g mouse for most agents, except for IVISense Gastrointestinal 750 probe whose typical dosage is 0.25 nmol/25 g mouse).

Animal diet

Mice were fed ad libitum with common alfalfa-containing food pellets obtained from KAYTEE (Chilton, WI), or with special alfalfa -free pellets obtained from Harlan Laboratories (Teklad Global Diet 2019X) in order to reduce food fluorescence in the stomach and intestines.

Tumor xenograft models

Six- to eight-week-old nu/nu and BALB/c mice were obtained from Harlan Laboratories (Madison, WI). Experimental procedures on laboratory animals were performed in accordance with Revvity IACUC guidelines. 2 x 10^6 of human HCT116/luc or Colo205/luc cells were subcutaneously inoculated onto nu/nu mice to initiate xenograft tumor growth. 1 x 10^6 murine 4T1/luc cells were injected into the lower right mammary fat pad on syngeneic BALB/c mice to initiate tumor growth. 1 X 10^5 of human HCT116/luc cells were surgically implanted into the spleen of nu/nu mice to induce intrasplenic tumor growth.

Depilation

Mice were anesthetized under 2% isoflurane-mixed oxygen prior to trimming with a hair clipper in the region of interest. To achieve optimal depilation, we then applied Nair depilatory cream and gently rubbed it into residual fur. We waited for 1-2 min and rinsed the skin with warm water to remove the depilatory cream. The skin was wiped dry with gauze and the animal was placed back into a cage on a heating pad for recovery from anesthesia.

Bioluminescence imaging (2D BLI and 3D DLIT)

Images were acquired using the IVIS SpectrumCT and were analyzed using Living Image® (v4.4) software. To generate bioluminescence signals, IVISbrite™ D-Luciferin Potassium Salt bioluminescent substrate (Revvity) was injected IP into mice at 150 mg/kg prior to bioluminescence imaging. Animals were then anesthetized under oxygen containing 2% isoflurane and placed into the imaging chamber. For 2D BLI imaging, the system was set to acquire images without any emission filter (open) to maximize sensitivity and to improve detection limit. For 3D DLIT imaging, the system first performed CT imaging (to establish the animal's surface boundary for tomography reconstruction) and then acquired a series of 2D bioluminescence surface radiance images at different emission wavelengths. The emission filters used for a typical 3D DLIT imaging scanning for firefly luciferase are: 560, 580, 600, 620 and 640 nm.

Fluorescence imaging (2D epi-FLI and 3D FLIT)

Prior to imaging, fluorescent agents were injected into animals in order to generate fluorescent contrast. The route of delivery depends on the biological application and the particular imaging agent used. For a typical 2D epi-fluorescence (epi-FL) imaging session, a single pair of excitation and emission filters were used in alignment with the fluorescent agent's optimal ex/em wavelengths. For 3D FLIT imaging, a CT dataset was acquired to establish the animal's surface boundary for tomography reconstruction. Then a series of fluorescent surface radiance images were acquired at multiple transillumination locations encompassing the region of interest.

Spectral unmixing imaging (SPUM)

The Living Image software is capable of spectral unmixing (SPUM) analysis on a series of 2D fluorescent surface radiance images (see technote "Spectral Unmixing"). SPUM analysis require the series to be acquired with a range of excitation/emission filters to distinguish two or more fluorescent sources provided that the image series covers the fluorescent spectra of all responsible sources. A common application of SPUM is to separate the interfering tissue autofluorescence and food fluorescence from the true fluorescence signals contributed by the agent of interest. Typical SPUM analyses involves the use of multiple excitation lights for the background autofluorescence and the specific fluorescent agents.

CT contrast agents

ExiTron U, V and ExiTron nano 12000 were obtained from Miltenyi Biotech. To establish liver and spleen CT contrast, 100 µL of ExiTron nano 12000 was IV injected 16 hours prior to imaging. For establishing kidney CT contrast, ExiTron U or V (100 µL) was IV injected one hour prior to imaging.

BLI or FLI? Which modality will work better for your study?

Table 1 provides general comparisons of the two commonly used optical imaging methods. As these two methods utilize different energy sources for signal generation, they have different biological applications and different technical considerations. Firefly luciferase-based bioluminescence imaging is very specific, due to the fact that its light production is mediated by ectopic expression of a foreign enzyme, and that the chemical energy internally stored within the substrate is solely responsible for light production. During its light-producing reaction, firefly luciferase specifically oxidizes its unique substrate D-luciferin in the presence of ATP. Without the enzyme, normal tissues virtually have no light emission and thus present a very low background. In general, BLI has a superbly high signal-to-noise ratio, a high sensitivity and a rather broad detection range.

Interestingly, the requirement for cellular ATP means that BLI only visualizes viable luciferase-expressing cells, making it suitable for pre-clinical evaluation of anti-cancer interventions in animal cancer models.

On the other hand, FLI takes a different approach to generate light signal. FLI uses external light as the energy source for fluorophore activation. Of course, one simple approach for *in vivo* FLI is to introduce expression of red and far-red recombinant fluorescent proteins into animals or cells in a manner similar to luciferase-based BLI. However, ectopic expression may not always be technically feasible and the ultimate signal to noise ratio may be lower and less attractive in comparison to luciferase-based BLI. One very practical approach to FLI is to use exogenous NIR imaging agents specific for particular cellular/biological markers.

Table 1: General comparison between bioluminescence (BLI) and fluorescence (FLI) imaging.

	Bioluminescence	Fluorescence
Imaging Mechanism	Specific oxidation of the D-luciferin substrate by the **firefly luciferase** enzyme.	Selective retention or activation of **fluorescent agents.**
Energy Source	Internal chemical energy stored within the D-luciferin and ATP.	External light energy for fluorescent excitation.
Interpretation	**Highly specific and sensitive, but more limited** Only cells or genes that express firefly luciferase can be visualized.	**Highly versatile, but more complex** Fluorescence imaging visualizes the contrast of agent distribution in living animals, which is determined both by specific targeting and the drug-like properties of these agents.
Biological Readouts and Applications	**Cell location, growth and viability** Luciferase utilizes its specific substrate D-luciferin for light production. As normal tissues and cells do not express luciferase, BLI is highly sensitive and has a very low background levels. In addition, BLI measures cell viability as the light-producing reaction requires intracellular ATP. Specific promoters can be used for luciferase expression to investigate gene regulation in living animals, and split luciferins can be used as biological sensors.	Three major strategies have been used to established fluorescent contrast *in vivo*. ***Vascular agents:*** Suitable for perfusion and vasculature imaging. ***Targeted agents:*** Fluorescent dyes can be conjugated to a variety of targeting moieties (small molecule, proteins, or peptides) for specific binding to surface bio-markers. ***Activatable agents:*** Fluorescently-quenched agents that contain specific enzyme substrate linkers can be activated by specific enzymatic cleavage.
Experimental Considerations	BLI requires genetic modification to introduce expression of luciferases.	FLI commonly requires imaging of control animals receiving the same fluorescent agent in order to establish the agent's background fluorescent levels at sites associated with its normal metabolism and clearance, such as the liver, kidneys and bladder. In some cases, autofluorescence background mice are required.

This approach has an advantage in situations in which 1) expression of luciferase or fluorescence in your particular model is either challenging or not possible (e.g. spontaneous tumor models, patient derived tumor models, applying imaging readouts to an established non-imaging preclinical model), 2) you are avoiding genetic modification of your cell line/animals, or 3) you are interested in detecting biological changes and mechanisms underlying disease (e.g. inflammation underlying cancer or infection).

Revvity offers three types of IVISense fluorescent probes for a variety of *in vivo* imaging applications: **1. Vascular, 2. Targeted,** and **3. Activatable** fluorescent agents. Vascular imaging agents are passively distributed through blood vessels to enable imaging of vascularity, blood pooling, and vascular leakage in tumors and inflammation. Targeted agents actively target and bind to key biomarkers. Activatable agents change from non-fluorescent (optically silent via intramolecular quenching) to highly fluorescent through enzyme-mediated release of their fluorophores.

The highly versatile and flexible nature of FLI makes it easier to simultaneously obtain multiple biological readouts by simply using agents differing in both specificity and fluorescence spectra. Nevertheless, as FLI utilizes an external light source, other experimental considerations, which will be covered in the following sections, must be taken into account prior to imaging.

The IVIS SpectrumCT

The IVIS SpectrumCT is a powerful multimodal imaging platform for small animal imaging in pre-clinical settings. With a relatively small floor foot print, it provides bioluminescence (BLI), fluorescence imaging (FLI) and X-ray computed tomography (CT). Its advanced optical components include an ultrasensitive CCD camera for light detection, a broad excitation light source capable of either surface epi-illumination or transillumination, and a full range of emission filters to capture light ranging from the visible to near infrared (NIR) spectra. In addition, the CT component, paired with advanced optical imaging modes, enables the system to transform traditionally straightforward 2D optical photography (BLI and FLI) into 3D tomography (DLIT and FLIT) with anatomical context. Combined with the advanced 3D analysis Living Image software and a great variety of optical imaging agents we currently offer, Revvity's IVIS SpectrumCT provides an all-around optical imaging solution that fits many different research needs. This technical note will first provide a general, overall description of its imaging capabilities and then provides detailed technical guidance and considerations for users when performing small animal imaging research using the system.

Bioluminescence imaging (BLI)

BLI visualizes light produced by luciferase within a living subject. Luciferases are unique light-producing enzymes capable of using luciferin as a substrate. The most commonly used luciferase for imaging applications is firefly luciferase, which catalyzes luciferin oxidization in the presence of oxygen and ATP. The DNA coding sequence of firefly luciferase has been identified and further optimized for mammalian expression. With the aid of molecular biology techniques, a luciferase-expressing cassette can be genetically introduced into viruses, cancer cells, or even the whole animal (transgenic) for BLI imaging research. One great advantage of luciferase-based bioluminescence imaging is the very low background signal. Since the substrate has uniquely evolved together with its partner enzyme, D-luciferin is not a substrate for any mammalian enzymes. Thus BLI imaging is extremely specific and highly sensitive; only luciferase-expressing cells are able to generate light signal.

The IVIS SpectrumCT is equipped with an ultrasensitive, thermoelectrically-cooled CCD camera (-90 °C) to capture any light emitted from a living subject. Figure 1 depicts the 2D approach for BLI imaging, in which a camera is positioned over the animal to detect all light coming from the animal surface. In order to maximize light capture and imaging sensitivity, 2D BLI imaging can be acquired without an emission filter (open) due to the absence of background noise signal from normal tissues. This relatively simple, yet powerful, method has high throughput, providing a convenient tool for assessing bioluminescent tumor and infectious burden in cohort studies of large sample sizes. Nevertheless, it should be noted that 3D depth information should never be assumed from 2D images due to light absorption and scattering.

Fluorescence imaging (FLI)

Unlike bioluminescence imaging, fluorescence imaging requires an external light source for fluorophore excitation. As each fluorophore has its unique excitation and emission profile, the IVIS SpectrumCT is equipped with a full range of excitation and emission filters to capture light from the visible to near infrared (NIR) spectrum, a feature that allows the use of a great variety of commercially-available and lab-made near infrared fluorescent agents.

Revvity offers a broad range of fluorescent molecular imaging agents for many *in vivo* applications, including arthritis, cancer, pulmonary inflammation, liver injury, CNS inflammation, and bacterial infection. In order to establish sufficient signal contrast for visualization, Revvity NIR agents were designed for systemic delivery and specific retention and/or activation in target tissues, in other words incorporating drug-like properties. This is important, because once in the body, NIR IVISense fluorescent probes, like drugs, are subject to xenobiotic metabolism and excretion. Agent clearance can result in accumulation in some organs normally involved in drug excretion, such as liver, GI tract, kidneys and bladder. Thus, when designing FLI studies, it is paramount to include agent-injected, undiseased control animals. In addition, some studies may require uninjected control animals for defining, and correcting for, autofluorescence background.

Figure 2A depicts the general imaging approach for 2D epifluorescence imaging. In this mode, both the camera and the stationary excitation light source are positioned above the animal. In order to generate optimal fluorescents signals, FLI typically uses a single pair of excitation/emission filters according to the agent's spectral Properties. 2D images are acquired by illuminating the surface of the mouse (epi-illumination) and collecting the animals' surface fluorescent signal. The imaging system is equipped with excitation and emission filter wheels that cover a wide range of light wavelengths, enabling multiplex imaging.

Figure 2B and C illustrate the complexity of fluorescent signal typically seen with imaging agents known to show some metabolic background either in the liver (B) or kidneys (C). An HCT116/luc tumor-bearing nu/nu mouse (from Figure 1) was injected with IVISense MMP 680 and IVISense Annexin-V 750 for targeting tumor matrix metalloprotease activity and cell death, respectively. Importantly, we included a tumor-free animal that also received the same agents as the tumor-bearing mouse.

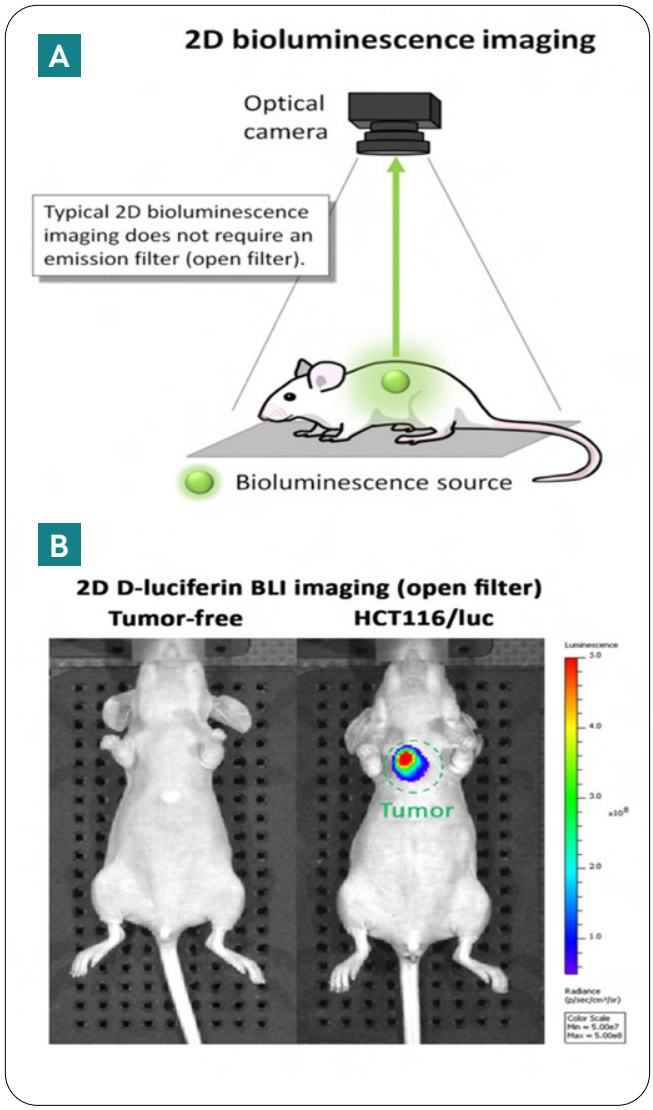


Figure 1: **BLI imaging specifically visualizes firefly luciferase expression** *in vivo*. (A) Diagram of the 2D BLI imaging approach. (B) BLI imaging of a tumor-free nu/nu mouse and a mouse bearing a human HCT116/luc xenograft tumor on the chest. Note the absence of signal in the tumor-free control mouse.

Both agents showed excellent tumor targeting 16 h post-injection. However, unlike the BLI results (which only visualized the tumor), IVISense MMP 680 produces both tumor fluorescence and background signals in the liver and bladder. As different agents are metabolized differently, IVISense Annexin-V 750, exhibited no liver signals but showed excretion exclusively through the kidneys and bladder. As in other types of molecular imaging (like ^{18}F-FDG PET), these signal patterns can be easily interpreted by careful comparison to the negative control which merely shows metabolic signals and no tumor signal.

Interpreting fluorescence imaging complexity

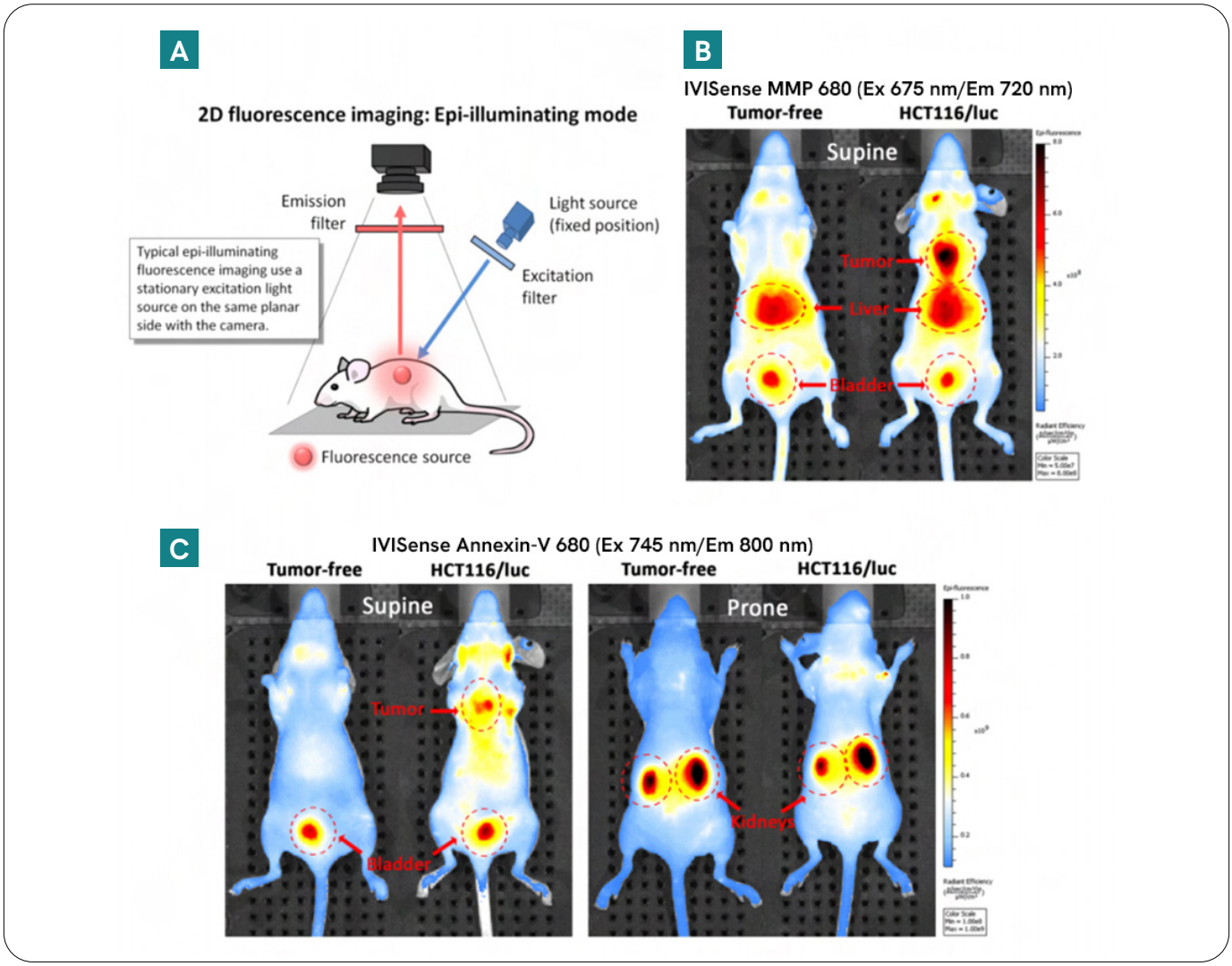


Figure 2: **Fluorescent imaging agents generate fluorescent contrast in target tissues and also in organs involved in agent metabolism and excretion.** (A) Diagram of the 2D FLI imaging approach. IVISense MMP 680 (B) and IVISense Annexin-V 750 (C) imaging was performed in a tumor-free nu/nu control mouse and a mouse bearing a subcutaneous HCT116/luc tumor on the chest. Note that both tumor-free and tumor-bearing mice exhibited agent-related signals in organs normally involved in drug metabolism: the liver and bladder for IVISense MMP 680 (supine), and the kidneys (prone) and bladder (supine) for IVISense Annexin-V 750. Only the tumor-bearing mouse showed upper chest fluorescence associated with MMP activity or cell death within the tumor mass.

Food fluorescence interference

Plant-based rodent food pellets commonly contain alfalfa, and the chlorophyll component in alfalfa shows fluorescence in the red and far-red light wavelengths. When performing FLI, endogenous food fluorescence can strongly interfere with NIR agent imaging, particularly when using 645 nm or 680 nm agents. Using a 605 nm excitation filter, Figure 3A compares the fluorescent emission spectra of common and alfalfa-free food pellets together with the spectra of two representative fluorescent agents, far-red IVISense Bombesin Receptor680 and NIR IVISense Transferrin Receptor 750. 3B shows epi-FLI imaging of the food pellets and vials containing the fluorescent agents using two separate channels (Ex640/Em680 and Ex745/Em800) to mimic the standard *in vivo* approach for FLI with far-red 680 nm and NIR 750 nm agents. In the Ex640/Em680 channel, we observed a significant reduction in food fluorescence in the alfalfa-free pellet. By switching further into the NIR wavelengths at Ex745/Em800 channel, food fluorescence interference is much reduced.

What is the impact of alfalfa-containing chow on *in vivo* FLI? To examine this, we subcutaneously inoculated nu/nu mice with a mixture of IVISense Bombesin Receptor 680 and IVISense Transferrin Receptor 750 on both the chest and abdomen. Figure 3C (left panel) shows that the standard chow interferes with the emission spectrum of IVISense Bombesin Receptor 680, preventing clear detection of fluorescent signal in the abdominal region. However, if alfalfa-free chow is used for at least 2-3 days prior to imaging, there is little interference. Figure 3C (right panel) shows that imaging of a 750 nm agent (IVISense Transferrin Receptor 750) was not affected by food fluorescence at all, as no chow signal was evident when using Ex745/Em800.

Thus, users should be cautious when performing fluorescence imaging involving fluorescent agents in the spectral range of 650-750 nm. It is of great importance to maintain the animals on alfalfa-free diet during the course of imaging study in order to reduce any potential signal interference from the food. To further prevent such interference, users can also use agents that absorb/fluorescence further into the near-infrared range (>750 nm).

Alternatively, it is possible to take advantage of the spectral unmixing (SPUM) functionality of the IVIS SpectrumCT and Living Image software to separate food and imaging agent fluorescence (see technote "Spectral Unmixing"). To examine this feature, we used the mice from Figure 3 and acquired sequential fluorescent emission from 660 to 840 nm wavelengths, covering both the far-red and NIR spectra. We performed SPUM analyses using multiple excitation lights for the background autofluorescence and the specific fluorescent agents. As depicted in Figure 4, SPUM was able to reconstruct and distinguish the IVISense Bombesin Receptor 680 activity in the abdominal region with virtually no food interference. When using the redder IVISense Transferrin Receptor 750 agent, SPUM was not required for visualizing the agent. Nevertheless this approach still worked well to separate the fluorescent signals.

Use of proper mouse chow can eliminate interference with FLI

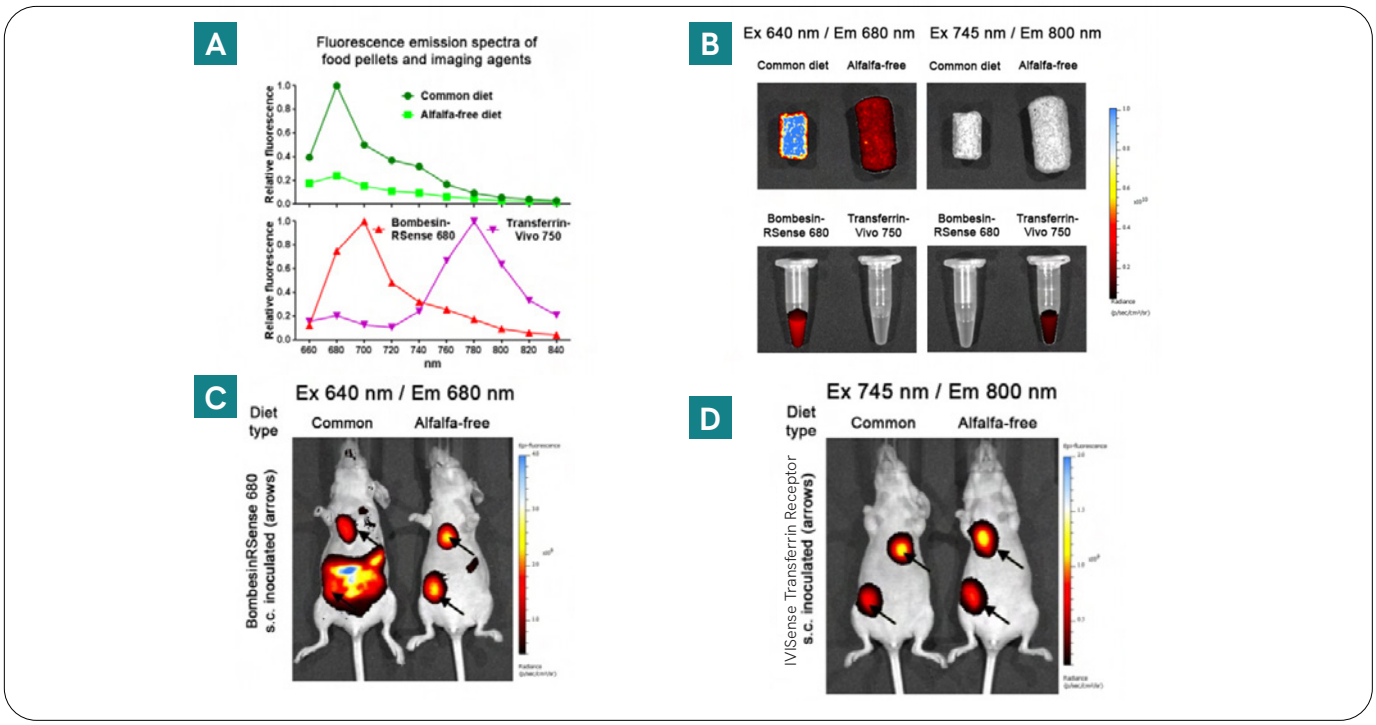


Figure 3: **The alfalfa in common rodent food pellets produces considerable fluorescence interference in the red 660-720 nm region**.
(A) Fluorescent emission spectra of common and alfalfa-free food pellets (top), and two representative fluorescent agents of different emission properties (bottom): IVISense Bombesin Receptor 680 and IVISense Transferrin Receptor 750, when excited with a 605 nm filter.
(B) Epi-fluorescence images of the food pellets and the fluorescent agents with a far-red Ex640/Em680 nm or a near-IR Ex745/Em800 nm filter set.
(C) Imaging of nu/nu mice subcutaneously inoculated with a mixture of IVISense Bombesin Receptor 680 and IVISense Transferrin Receptor 750 both on the chest and on the abdomen (arrows). The left animal was maintained with common pellets while the right was on an alfalfa-free diet. Note that common food caused strong fluorescent interference in the guts when using a far-red Ex640/Em680 nm filter set for IVISense Bombesin Receptor 680. In contrast, no interference was observed when using a redder Ex745/Em800 nm filter set for IVISense Transferrin Receptor 750.

Spectral unmixing to distinguish fluorescence sources

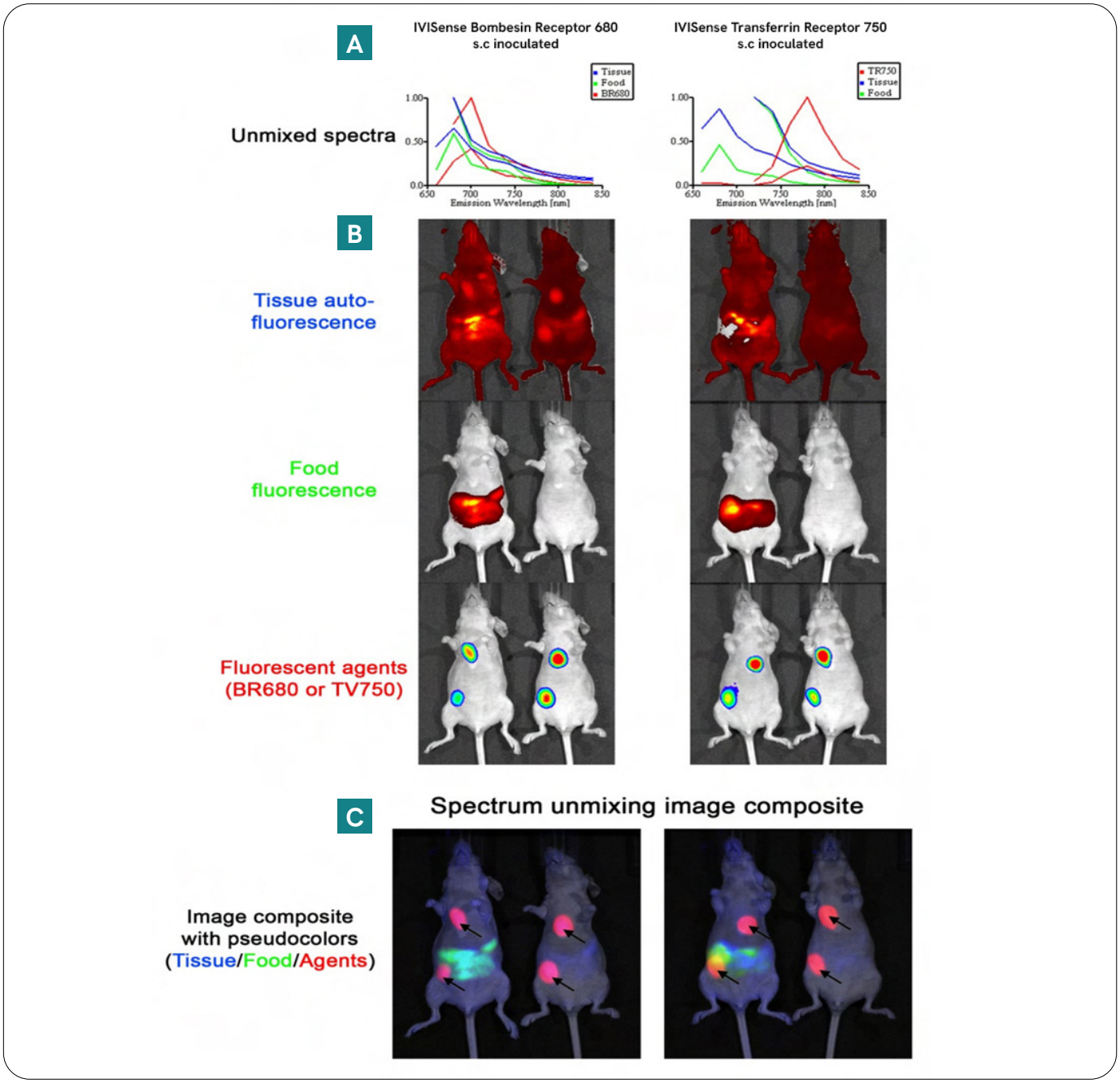


Figure 4: **Spectral unmixing (SPUM) to distinguish agent fluorescent signals from food fluorescence and tissue auto-fluorescence**. To compare the effectiveness of SPUM in the far-red (IVISense Bombesin Receptor 680) and NIR (IVISense Transferrin Receptor 750) regions, the same animals shown in Figure 3 were excited with 605, 640 and 675 nm filters, and fluorescent emissions were sequentially acquired from 660 to 840 nm (see technote "Spectral Unmixing"). To separate IVISense Bombesin Receptor 680 fluorescence from tissue and food fluorescence, the 605 and 640 nm excitation filters were used to establish emission profiles for SPUM analysis (left). The redder IVISense Transferrin Receptor 750 fluorescence can be readily identified by SPUM using the 605 and 675 nm excitation filters (right). The image data were then SPUM-analyzed with the Living Image software for visualization of tissue, food and fluorescent agents. (A) Unmixed spectra of each fluorescent component, where the blue lines indicate the unmixed tissue auto-fluorescence; the green lines indicate the food fluorescence and the red lines show the fluorescence of the indicated imaging agents. (B) Images of mice in which SPUM successfully separated tissue autofluorescence, food fluorescence and agent fluorescence. (C) Pseudo-colored composite images of all fluorescence components. The arrows indicate the sites of 680 and 750 nm agent inoculation.

Depilation (Hair removal)

Mouse hair, whether black, brown, grey, or white, absorbs and scatters light in both the visible and the NIR spectrum. Thus it is recommended to shave and/or chemically depilate mice in order to minimize interference with the imaging signals. For a practical reason, where possible, it is recommended to use hairless mouse strains such as nu/nu and immunocompetent SKH-1E mice.

BLI: To highlight the impact of hair on BLI, we imaged BALB/c mice bearing syngeneic 4T1/luc tumors in the lower right mammary fat pad. As shown in Figure 5, the same animal was imaged prior to and after depilation. The presence of white fur resulted in a >50% loss of signal output compared with the image acquired with hair removed in the abdominal region. It is important to note that many researchers acquire BLI images without depilation, however, the major limitation of such practice could be loss of imaging sensitivity and accuracy. For optimal imaging quality, it is strongly recommend to fully depilate the region of interest, especially when imaging small and/or dim imaging targets.

FLI: Similar to what is observed with BLI, there is a significant effect of depilation on 2D FLI. To illustrate this, BALB/c mice were subcutaneously (SC) injected with IVISense Transferrin Receptor 750 (20 pmol) in a mixture solution containing Matrigel (100 mL, 50% by volume). This approach, shown in Figure 6, provides a close parallel to the BLI model shown in Figure 5, and the results clearly show that the presence of hair decreases fluorescence at the injection site by ~50%.

There are a couple of other issues to note regarding depilation. First, darker hair, as compared to white hair, is likely to have an even more significant light scattering/blocking effect on both BLI and FLI. Secondly, for FLI special care needs to be taken when removing the hair using clippers and depilatory cream, as minor skin damage may cause additional unwanted signal, especially when using inflammatory or vascular imaging agents.

Mouse hair can interfere with both BLI and FLI

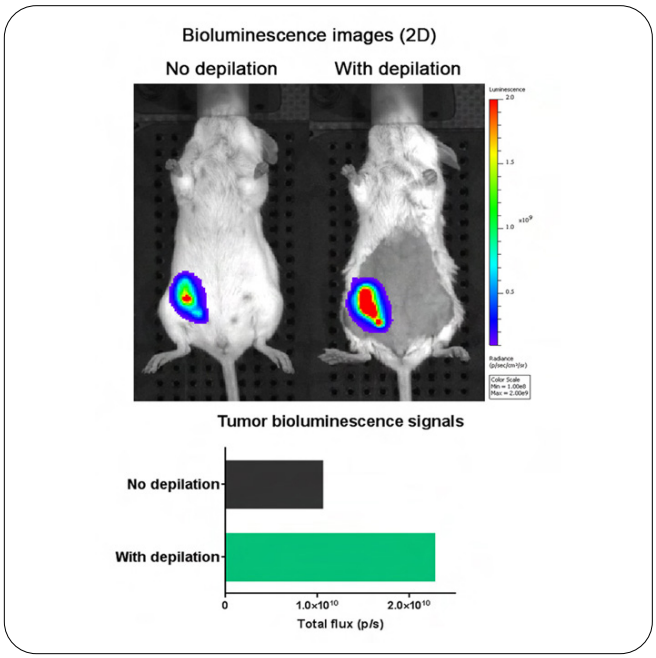


Figure 5: **Hair reduces bioluminescence output.** BLI imaging of a 4T1/luc tumor in the mammary fat pad of a BALB/c mouse. The same tumor-bearing animal was imaged before and after hair removal without any emission filter. Hair removal indeed enhances light capture by the ultrasensitive CCD camera.

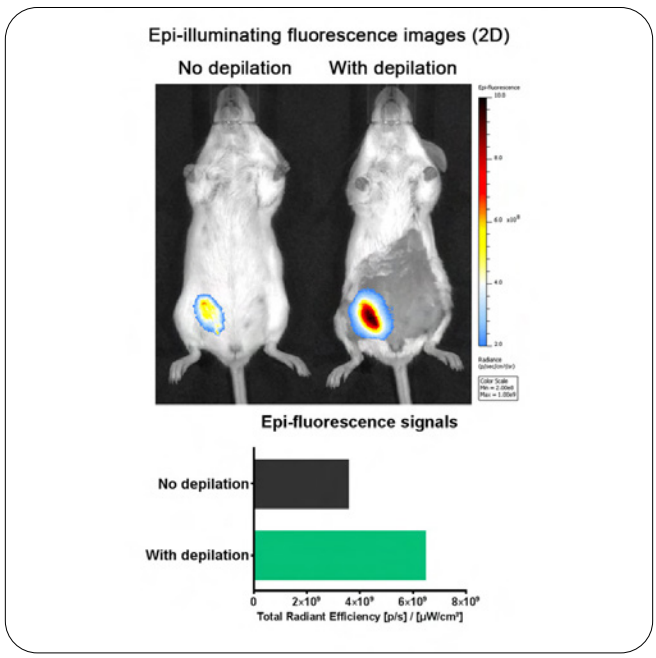


Figure 6: **Hair reduces epi-fluorescence signal output.** A BALB/c mouse was injected SC with 20 pmol of IVISense Transferrin Receptor 750 in 100 µL of Matrigel (50% by volume), and was then imaged prior and after abdominal depilation. The presence of hair reduces FLI imaging signals of the injection site by blocking both the excitation and emission light. With the hair removed, we observed considerable enhancement in fluorescent signals.

Animal positional effects on 2D BLI and FLI

In this section, we evaluated the impact of animal positioning on 2D BLI and FLI imaging using two tumor xenograft models. In both cases, we examined its effects in terms of both imaging quality and signal quantification.

For BLI imaging, we established an intrasplenic cancer model, by injecting 1 x 10^5 HCT116/luc cells into the spleen of nu/nu mice. Two days after cell injection, 2D BLI imaging of a representative tumor-bearing mouse was performed in various positions (Figure 7). The animal showed the highest bioluminescence output in the side position (~90 ° relative to the supine position), a position in which the whole spleen region was exposed to the camera's field of view. Signal decreased somewhat as the spleen region was moved laterally away from the center, with variable attenuation seen relative to the optimal position.

Imaging position also affected the results of epi-fluorescence imaging, given its additional requirement of an external energy light source. To demonstrate this, we used a nu/nu mouse implanted with human Colo205/luc tumor cells on the right flank.

The tumor-targeting NIR fluorescent probe, IVISense Integrin Receptor 750, was used to detect surface $\alpha_v\beta_3$ integrin expression in these tumor cells. As shown in Figure 8, 24 hr after agent injection the optimal imaging position placed the tumor centrally within the boundaries of the animal and facing the camera (90 °). This generated both the ideal image and maximal quantification due to optimal exposure for excitation and light capture. When the target tumor was not directly/centrally exposed to the light source and camera (45 °), or was blocked (0 °) from the excitation light sources, very little or no fluorescent signal was observed. Surprisingly, positioning of the animal in the prone position (180 °, which achieves apparently reasonable tumor alignment to light source and camera) showed loss of image quality and a 25% loss of signal.

Thus, for longitudinal studies involving repetitive imaging of the same subjects for a long period of time, it is of great importance to be very consistent with regard to imaging position in order to reduce variations and to achieve accurate measurements.

Consistent mouse positioning is very important for 2D BLI and FLI imaging

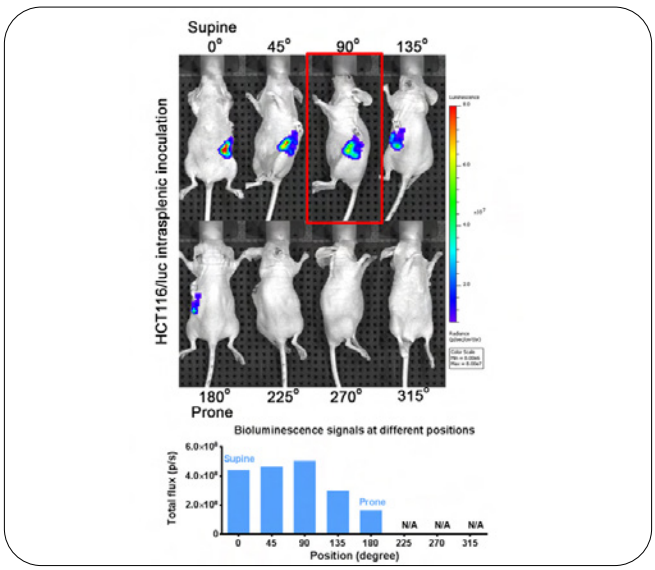


Figure 7: **The effects of animal positioning on 2D bioluminescence imaging.** Human HCT116/luc cells (1 x 10^5) were injected into the spleen of a nu/nu mouse, and BLI imaging was performed two days after cell injection. The mouse was imaged at various angles (0 ° - 315 °) with regard to the supine position (0 °). Maximal BLI signal was observed when the animal was imaged on its side with the whole spleen in the view of the camera. Tumor BLI signals were unable to be quantified in positions where the spleen was completely obscured by the body (225 ° - 315 °).

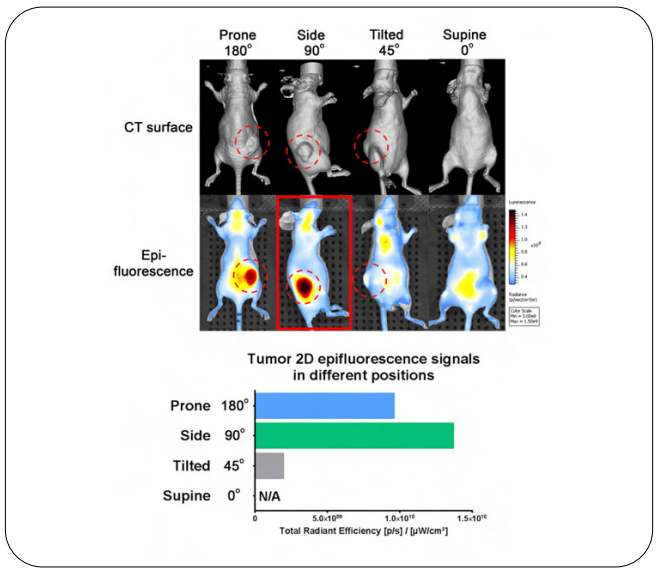


Figure 8: **The effects of animal positioning on epi-fluorescence imaging.** A nu/nu mouse bearing a human Colo205/luc tumor was injected IV with a fluorescent tumor imaging agent, IVISense Integrin Receptor 750. 24 h later, the animal was imaged in various body positions. Optimal tumor fluorescent signal was observed when the tumor was completely exposed in the field of view.

3D optical tomography by bioluminescence and fluorescence

The IVIS SpectrumCT features two advanced modes for acquiring optical 3D tomography in bioluminescence and fluorescence. In both modes, the system uses the built-in CT to establish both the animal surface and tissue/bone anatomical context for image reconstruction. Figure 9 and 10 depicts the imaging principles of these two modes.

Diffuse Light Tomography (DLIT, Figure 9) is a 3D bioluminescence imaging module built into the Living Image software (see DLIT 1,2, and 3 technotes). In tissue, firefly luciferase has a broad emission range from 540 to 660 nm, and the maximal emission is about 620 nm. Lights with different wavelengths scatter and attenuate differently in tissue, with red-shifted wavelengths showing better tissue penetration, so source bioluminescence from deeper positions will show more loss of the bluer 540 signals than the redder 660 signals. DLIT generates 3D tomographic images by acquiring a series of 2D bioluminescence surface radiance images with different emission filters. Typical firefly luciferase DLIT imaging involves acquiring a series of 560-640 nm images in 20 nm increments. Using the CT data to define animal boundaries, the Living Image software then reconstructs a 3D tomographic image using a light diffusion algorithm that models depth localization based on the variations of light signals at different emission wavelengths.

Fluorescence Imaging Tomography (FLIT, Figure 10) uses a different approach for determining 3D localization of light signal (see FLIT 1/2/3 technotes). Unlike conventional 2D epifluorescence (which uses epi-illumination), FLIT imaging uses a transillumination excitation light source placed on the opposite side of the camera and close to the animal's body. As a result, the system can deliver more energy to compensate for possible light attenuation and scattering, ideal for deep tissue tomography. For optimal excitation and emission, FLIT uses distinct pairs of excitation/emission filters aligned to each fluorescent agent used. In order to establish the distribution of fluorescent agent in the body, a series of 2D fluorescent surface radiance images are acquired with the light source at various transillumination positions throughout the region of interest. The positional information from the variety of distal and proximal light source positions, combined with the surface data generated by the CT imaging, are analyzed by the Living Imaging software to reconstruct a 3D distribution map of the agent within the body.

In the following section, we will discuss technical considerations when acquiring 3D DLIT or FLIT images.

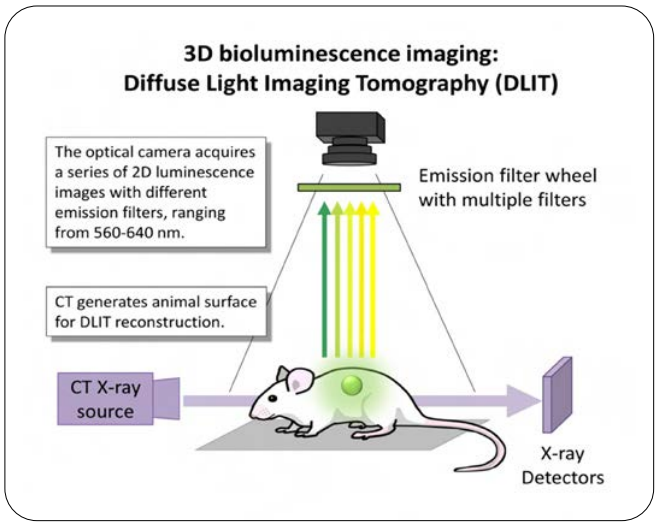


Figure 9: **DLIT imaging principle.** Diffuse Light Imaging Tomography (DLIT) is a bioluminescence-based imaging technique. The light produced by firefly luciferase is not monochromic. Instead, firefly luciferase has a broad emission profile ranging from 540 to 660 nm. As light diffuses differently at different wavelengths in tissue, DLIT can determine the 3D distribution of luciferase activities in living animals by analyzing a series of 2D surface radiance images acquired at different wavelengths. For the IVIS SpectrumCT, such analysis is performed in conjunction with the surface information provided by CT for a more accurate result.

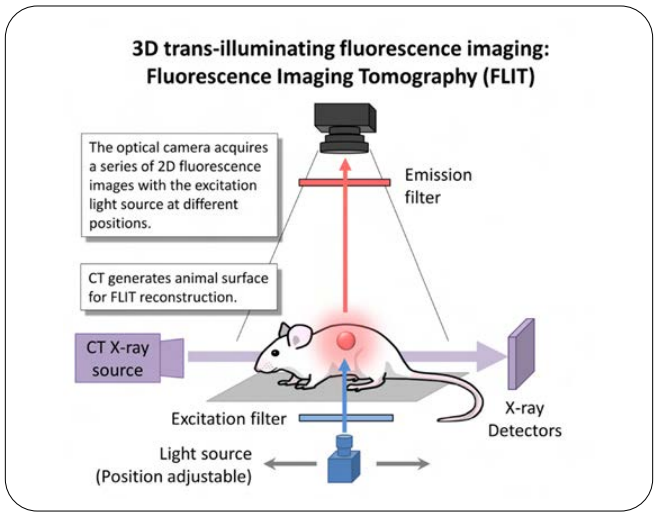


Figure 10: **FLIT imaging principle.** Fluorescent Imaging Tomography (FLIT) is a transillumination fluorescent imaging method, where the excitation light source is placed on the opposite side of the camera. The key advantage of this arrangement is that the light source is placed closer to the animal for better tissue penetration. As a result, FLIT is more suitable for deep tissue imaging in comparison to the conventional epi-illumination method. The tissue distribution of the fluorescent agent can be determined by 2D scanning the area of interest at various XY positions, and then using the CT animal surface to reconstruct a 3D tomography image.

Depilation (Hair removal)

Depilation shows clear improvement of 2D bioluminescence and fluorescence imaging, so it is not surprising that hair can also adversely affect the imaging quality of 3D tomographic BLI (DLIT) and FLI (FLIT).

For bioluminescent DLIT imaging, we established syngeneic orthotopic breast cancer, using implantation of 4T1-luc tumor cells in the lower right mammary fat pads of BALB/c mice. Mice bearing established tumors (seven days) were imaged before and after depilation of the abdomen (Figure 16). Although the presence of hair did not completely prohibit 3D reconstruction of the DLIT images, abdominal hair indeed caused lower signal output (decreased ~50%) in comparison to the image acquired after hair removal. Nevertheless, the presence of hair has effects on 3D reconstruction detail and tumor co-registration.

We then evaluated the impact of hair on FLIT imaging using a fluorescent kidney model. BALB/c mice were injected IV with IVISense Folate Receptor 680 to establish fluorescent contrast in the kidneys as the agent is actively metabolized and excreted via the renal pathway. 24 hr after agent injection, little fluorescent signal is apparent within normal tissues, but high fluorescent signal remains in the kidneys.

Animals were then imaged with different degrees of depilation (abdominal, dorsal, or both sides). As shown in Figure 12, the presence of hair adversely affected visualization of kidney fluorescence, and complete torso depilation (i.e. both abdominal and dorsal) significantly improved the overall 3D tomography imaging results. Hair caused light scattering and thus resulted in poor image quality with a multiple reconstruction artifacts. Partial depilation (either abdominal or dorsal) modestly improved the appearance of the 3D reconstruction, however only full depilation provided optimal 3D reconstruction quality. Quantitatively, the presence of hair has a dual impact on FLIT, both attenuating the penetration of excitation light and the emission of fluorescence from the target tissue. Although signal could be detected and quantified to some degree in all conditions, only full depilation of the imaging region provided interpretable images, minimal artifacts, and accurate quantification.

As an additional note, complete depilation needs to be achieved without any skin damage. Researchers should use caution when removing hair using clippers and/or depilatory cream. Both approaches could cause skin damage and rupture if not performed properly. The normal wound healing processes can cause unwanted fluorescent signal contrast in the region of interest especially when using inflammation or vascular imaging agents.

Depilation is also important for 3D BLI and FLI imaging

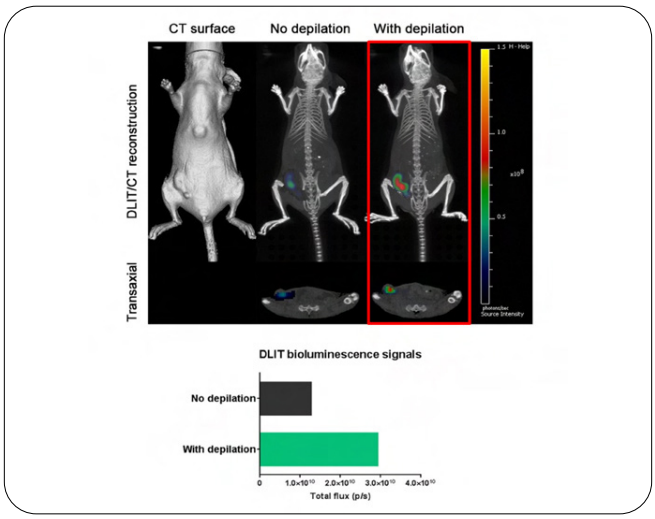


Figure 11: **Hair reduces DLIT signal output and reconstruction accuracy.** 3D DLIT imaging of a 4T1/luc tumor in the mammary fat pads of a BALB/c mouse before and after abdominal depilation. The presence of hair not only reduces the total bioluminescent signal output, but also negatively affects the image quality when comparing the transaxial DLIT/CT fusion images.

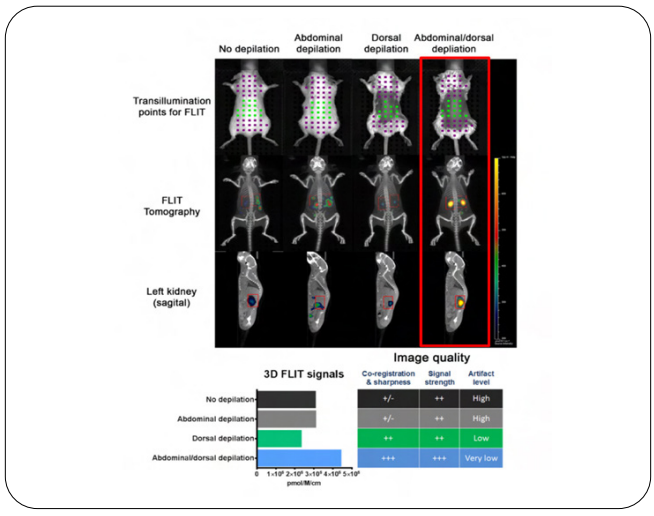


Figure 12: **Hair affects FLIT imaging quality and quantitative result.** BALB/c mice were IV injected with IVISense Folate Receptor 680 to generate fluorescent contrast in the kidneys. Animals receive abdominal, dorsal or depilation of both sides in regions with regard to the kidneys. The green markers indicate the FLIT scan areas. Mice with both abdominal and dorsal hair removed showed the strongest signal output and the best image quality.

Area selection for FLIT imaging

As FLIT is based on a series of surface fluorescent images acquired at different transillumination positions, correctly selecting the dimensions and positioning of the scan field are critical for the 3D imaging quality and the final quantitative results. We explored the impact of scan fields on FLIT using nu/nu mice with IVISense Folate Receptor 680 fluorescence in the kidneys (Figure 13). After systemic delivery of the fluorescent agent, conventional 2D epi-illumination imaging confirmed the presence of fluorescent signal in the kidneys and bladder. The mice were then imaged with a minimal scanning area of 1 X 4 right across the kidney region, or in a larger 3 X 4 scanning areas that was encompassed the entire kidney region. Alternatively, we placed the 3 X 4 scan field either above or below the kidneys in a way that completely excluded the organs.

With FLIT imaging using the minimal (1 X 4) scan field across the kidneys, we were able to achieve reasonably accurate and satisfactory kidney reconstructions. The FLIT reconstructions were improved when we used the 3 X 4 scan field capturing the entire kidney region. This scan field generated a more concentrated and detailed kidney reconstruction. FLIT imaging was also performed with the alternative placement of the 3 X 4 scan field, either above or below the target organs. Despite the fact that no masking was used to limit the reconstruction areas, FLIT imaging with these scan fields yielded little kidney signal and no kidney reconstructions.

As a practical note for FLIT imaging, we recommend selection of scan fields slightly larger than the target being imaged and designed with some margin around the target. Proper setup and consistency in selecting FLIT scan fields ensures the most accurate and repeatable results.

Considerations for proper scan field setting in FLIT

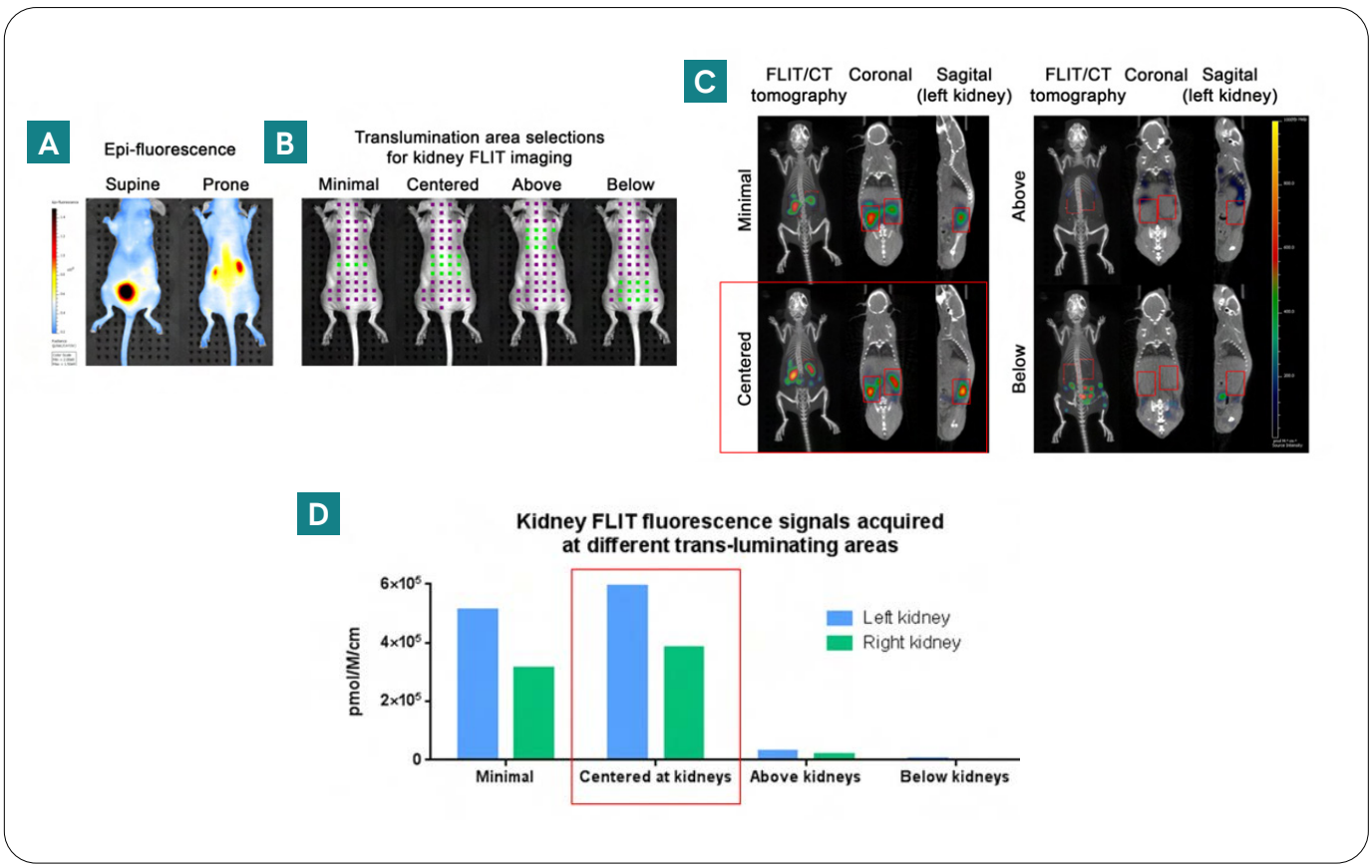


Figure 13: **FLIT area selection**. (A) Epi-fluorescence imaging of nu/nu mice injected IV with IVISense Folate Receptor 680 to generate fluorescent contrast in the kidneys and bladder. (B) The green points indicate different scan fields selected for FLIT imaging. (C) Kidney FLIT reconstructions using the four different scan fields. Images are represented as 3D tomography as well as appropriate coronal and sagittal cross-sections. (D) Quantification of FLIT signal output within left and right kidney ROIs with the four different scan fields.

3D optical tomography: Tips for effective FLIT imaging

The basic procedures for FLIT acquisition on the IVIS Spectrum and IVIS SpectrumCT are covered in prior technotes (series 6 technotes). However, successful FLIT acquisition and reconstruction can at times be challenging, requiring a marriage of biology, fluorescent agent, animal, and imaging system. All four of these parameters contribute significant complexity and can be contributors to both success and failure. Although the default settings within Living Image will work well for many imaging scenarios, optimal scan field set-up, fine adjustment of pre-reconstruction thresholds, and correct setting of masks (to refine reconstruction regions) can assure maximal signal detection and image quality.

A representative nu/nu mouse bearing two 4T1 mammary fat pad tumors was injected IV with IVISense MMP 680 to detect tumor matrix metalloprotease activity. Typical FLIT imaging involves the acquisition of multiple surface radiance images from different light source positions throughout the region of interest, and these images are used in the 3D reconstruction algorithm. Figure 14 depicts a composite "overview" to illustrate the effectiveness of the transillumination scanning pattern in revealing tumor fluorescence at the surface of the animal.

Here the data is shown as an image depicting the average of multiple surface radiance images but the same data can be analyzed by a mathematical algorithm in Living Image to generate 3D tomographic images. The very first step in FLIT imaging is to define the scan field that is composed of multiple transillumination points throughout the region of interest (Figure 14A). After image acquisition, the software generates a visual summary of all the data from every image of the sequence, each representing the fluorescence radiance derived from one light source position (Figure 14B). Examination of each individual sequence image reveals that 4-9% of the lower grade fluorescence is automatically removed from the image (Figure 14B, expanded panel). This default thresholding is often excessive for effective FLIT reconstruction, however each sequence image can be manually adjusted to lower this threshold % (different for each panel) in order to retain more signal for reconstruction. Application of the mask drawing tool (Figure 14C), defines the area of reconstruction as a region slightly larger than the scan field. This appropriately limits the size of the reconstruction to the properly transilluminated field and provides good apparent tumor size/definition when voxels are properly adjusted to 0.62 mm (Figure 14D).

The impact of different degrees of thresholding on optimal reconstruction is examined in the next section.

Pre-reconstruction image adjustment

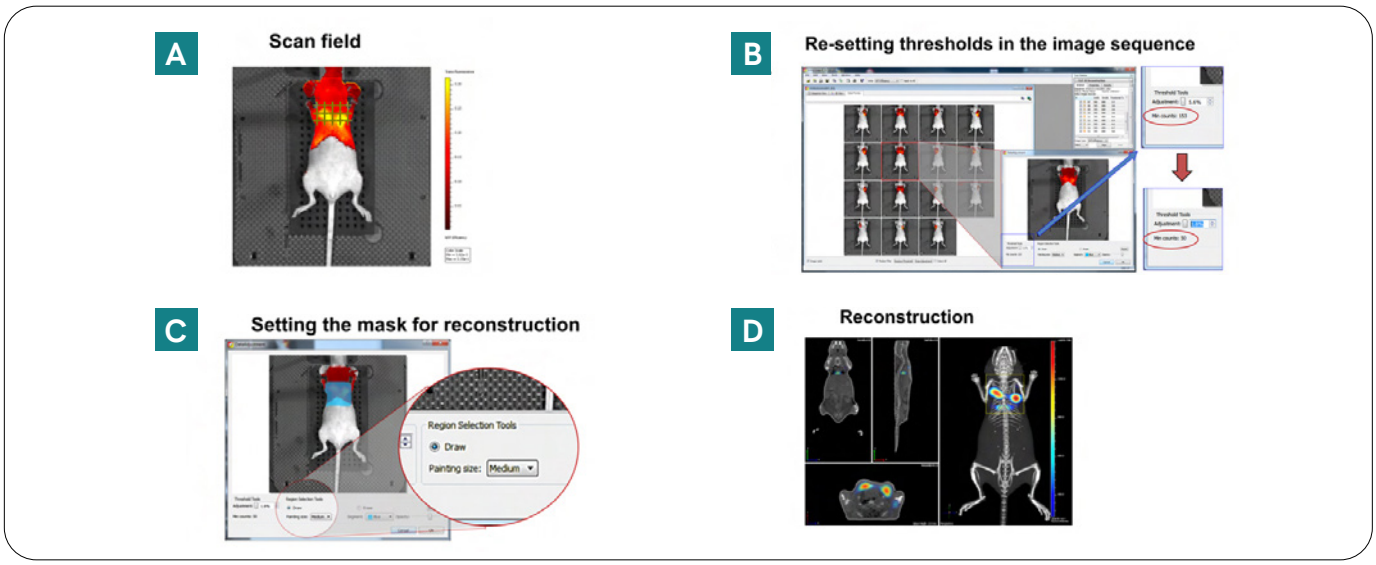


Figure 14: **Pre-reconstruction thresholding and masking.** (A) A 15-point scan field was placed to encompass the upper mammary fat pad region of a representative nu/nu mouse where two tumors were implanted. (B) FLIT imaging acquires multiple image panels, each representing the result from single point transillumination FLI. (C) Double-clicking one of the panels enables adjustment of thresholds (red circle) and reconstruction field masking (blue shade). (D) Use of the newly thresholded/masked panel of transillumination images in reconstruction provides an appropriately constrained and thresholded 3D tomography image. The yellow box highlights the reconstructed region.

3D optical tomography: Tips for effective FLIT imaging (continued)

Use of the default settings within Living Image (average of ~2000 counts/panel, ranging from 2 - 6% threshold) effectively identified and reconstructed IVISense MMP 680 fluorescence in the two 4T1 tumors implanted in the upper mammary fat pads (Figure 15A) of nu/nu mice. However, the well characterized liver background for this imaging agent was not detected. Therefore, we applied threshold ranging (Figure 15B), by decreasing average pre-reconstruction thresholds to 800, 400, 100, and 50 counts/panel (achieving this by custom thresholding each panel from 0.1-2%). With the lowest thresholding (i.e. 50 counts/panel), tumor localization agreed well with the mCT cross-sectional images (not shown), and liver signal was appropriately positioned centrally within the ribcage (Figure 15B).

Regions of interest (ROIs) were placed to capture left and right tumors as well as the tip of the liver that falls within the lower region of the reconstruction field. Living Image 4.4 software quantified the total radiant efficiency within each ROI as a means for identifying the optimal conditions for reconstructing both tumor and liver signal (i.e. all of the signal that is actually present within the scan field). There was an obvious improvement in quantification of liver background as thresholding decreased (Figure 15C), with appropriate liver signal seen at 50-100 count/panel thresholding.

Optimal pre-reconstruction was identified as 50 counts per acquisition panel (generally a range of 30-70 in individual panels), as this allowed the reconstruction to yield ROI quantification levels that agreed well with an independent measurement of fluorescent concentration (in nM) in tumors and liver (Figure 15D).

Reconstruction images with different pre-recon thresholds

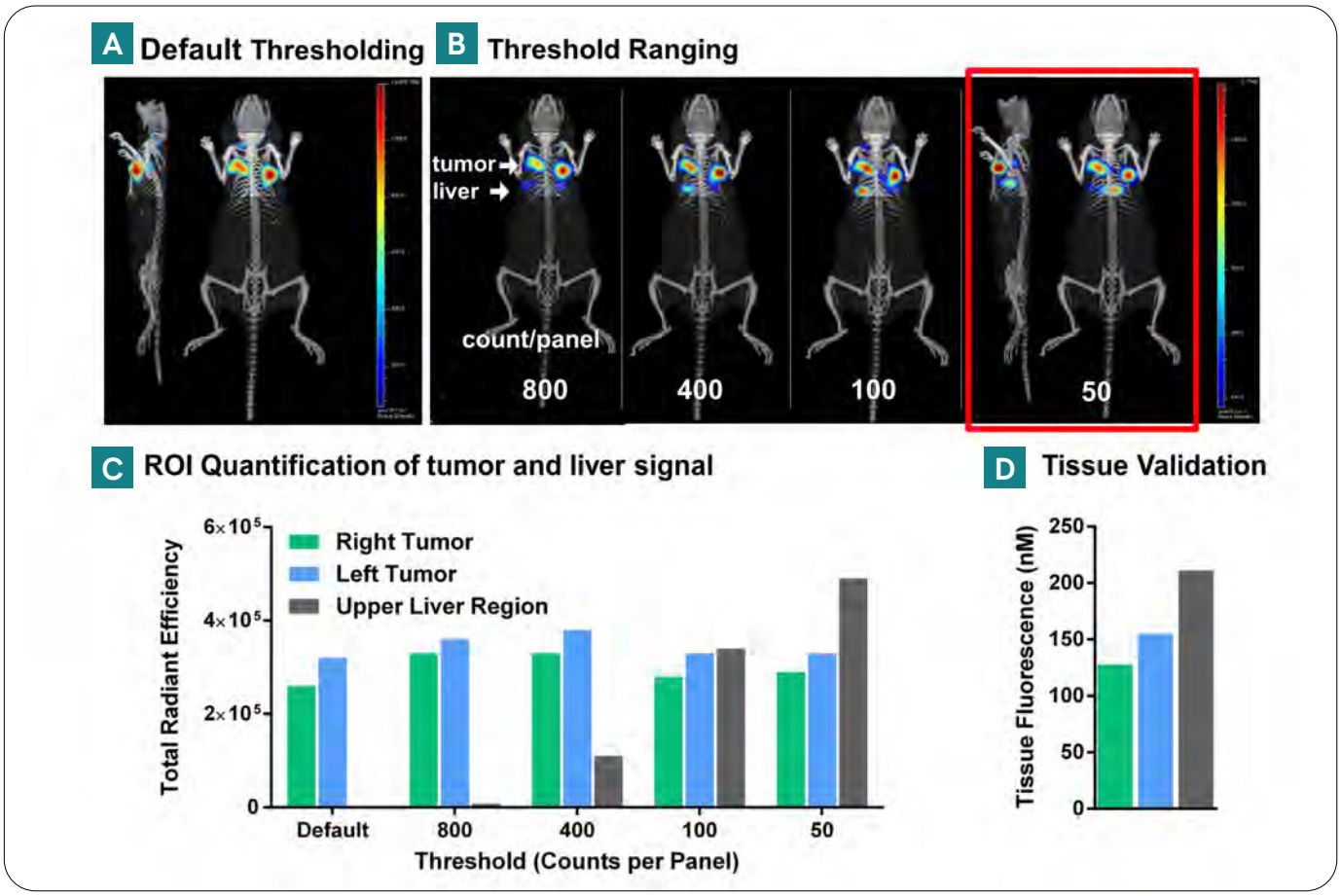


Figure 15: **The impact of thresholding on proper tumor and liver fluorescence reconstruction**. (A) FLIT reconstruction of the same mouse from Figure 14 with default thresholding conditions. (B) FLIT reconstructions with different manually-applied reconstruction thresholds, ranging from 800 down to 50 counts/panel). (C) Quantification of FLIT fluorescent signals in tumor and liver ROIs in terms of total radiant efficiency under different thresholds. (D) Independent measure of tumor and liver fluorescence in nM.

Advantage of FLIT imaging: 3D fluorescence distribution with accurate anatomical context

One major advantage of FLIT tomography imaging is that it provides 3D tissue localization of the fluorescent agent signal with anatomical context. In order to demonstrate its anatomical accuracy, we performed FLIT imaging in animals co-administered with validated CT contrast agents that specifically highlight the target organs. IVISense Gastrointestinal 750 probe was mixed with a CT contrast agent ExiTron U prior to oral gavage into a nu/nu mouse (Figure 16). Fluorescence in the stomach can be readily visualized by conventional 2D epi-fluorescence imaging. We later performed FLIT imaging to validate that the fluorescent signals originated from the stomach. With the help of the ExiTron U contrast agent, CT imaging clearly revealed the location of stomach. In addition, FLIT imaging precisely placed the IVISense Gastrointestinal 750 fluorescence within the anatomical structure of this organ.

In another example, we used FLIT imaging to co-visualize IVISense Folate Receptor 680 signals and CT contrast agent in the kidneys. As mentioned previously, IV delivery of IVISense Folate Receptor 680 results in specific kidney signals as the agent is actively cleared via the renal excretion. This fluorescent signal in the kidneys could be visualized by conventional 2D epi-fluorescent imaging, however signal scattering into the spinal column region reduced the accuracy of the imaging. To better map the internal organs for precise localization of the FLIT fluorescent signals, we took advantage of two CT contrast agents, ExiTron nano 12000 and ExiTron V (both obtained from Miltenyi Biotech). IV injection of ExiTron nano 12000 16 h prior to FLIT imaging enables CT visualization of the liver and spleen (Figure 17). On the other hand, systemic delivery of ExiTron V establishes specific kidney contrast within one hour of IV injection. By spacing the injection time of each contrast agent, we used CT to simultaneously visualize the liver, spleen, and the kidney in the same living subject. Using this method, we were able to co-visualize the CT contrast (ExiTron V) and fluorescent 3D signals IVISense Folate Receptor 680) in the Kidneys with great accuracy.

FLIT imaging with CT contrast agents to demonstrate anatomical accuracy

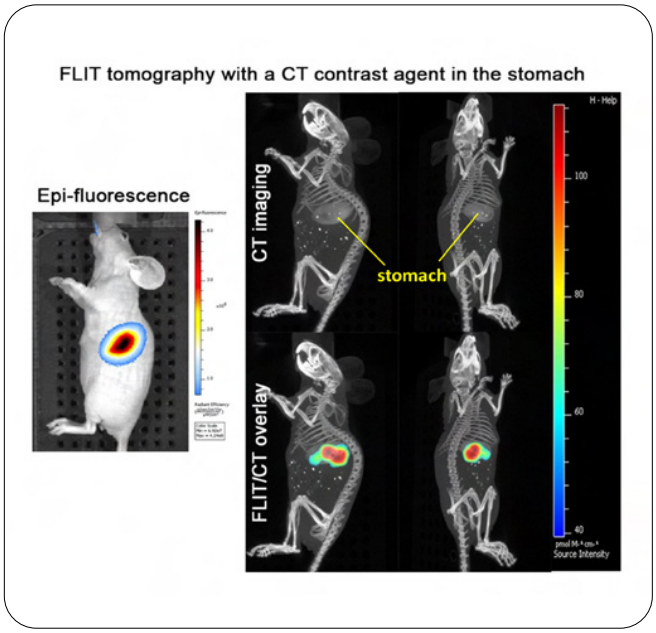


Figure 16: **IVISense Gastrointestinal 750 FLIT imaging with a CT contrast agent in the stomach.** A nu/nu mouse received an oral gavage of a mixture of IVISense Gastrointestinal 750 and CT contrast agent ExiTron U (Miltenyi Biotech). Conventional 2D epi-fluorescence imaging revealed the presence of fluorescence signals in the stomach (Left). The animal was then subject to FLIT imaging. The CT contrast helped reveal the true location of the stomach. FLIT/CT overlay clearly placed the fluorescent signal in the contents of stomach.

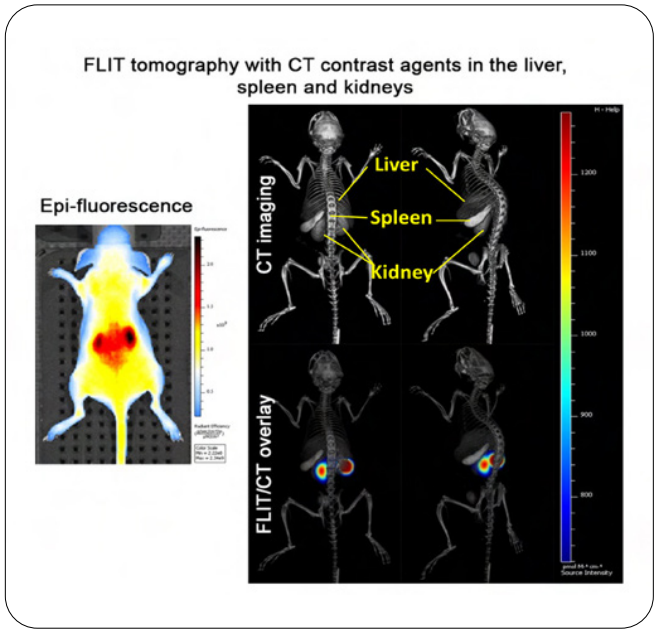


Figure 17: **Renal IVISense Folate Receptor 680 FLIT imaging with CT contrast agents.** Two CT contrast agents were used to visualize the liver, spleen and kidneys. A nu/nu mouse was first IV injected with ExiTron nano 12000 16 h prior to FLIT imaging to establish CT contrast in the liver and spleen. Four hours before FLIT/CT imaging, the animal received IV injection of IVISense Folate Receptor 680 to introduce kidney fluorescence. ExiTron V was then injected IV into the animal one hour prior to imaging to establish kidney contrast. FLIT/CT overlay clearly placed the fluorescent signal in the kidneys.

Advantage of FLIT imaging: Visualizing deep tissue fluorescence with better consistency

Another major advantage of FLIT tomography imaging is that it provides better quantitative consistency as compared to 2D epi-fluorescence when imaging deep tissues. FLIT is based on a transillumination approach to excite fluorescence (Figure 10). As the excitation light source is placed close to the animal, FLIT provides better tissue penetration and minimizes surface background signal capture by the camera (on the other side of the animal). To compare 2D and 3D imaging performance and quantification, we imaged mice receiving oral gavage of IVISense Gastrointestinal at different concentrations. IVISense Gastrointestinal 750 probe was undiluted (1X, 0.25 nmol in 100 ul) or serially diluted (x 1/4 to x 1/32) prior to oral gavage into nu/nu mice (Figure 18A).

Fluorescence images were optimized for each dose (set to maximum pixel/voxel) to compare accuracy of localization and signal pattern, showing significant dose-related qualitative changes by 2D imaging but not by 3D imaging. Fluorescence signals obtained from both modes were also analyzed and compared to the expected values based on the known dilution factors.

Using NIR excitation light, epi-fluorescence mode provides sufficient excitation light penetration for visualizing deep IVISense Gastrointestinal 750 fluorescence in the stomach. However, when the agent was extensively diluted to (32-fold) from its original strength, the image quality was significantly reduced (Figure 18B). In addition, quantitative analysis based on surface epi-fluorescence signals may not truly reflect the actual fluorescence levels in deep tissue. For example, in animals receiving serially diluted IVISense Gastrointestinal 750 with known dilution factors, we generally observed 2~3 fold lower epi-fluorescence than expected. In contrast, FLIT is more consistent and we observed good 3D fluorescence tomography reconstruction in the stomach. More importantly, FLIT provides consistent quantitative analysis throughout the range of dilutions tested.

FLIT provides consistent quantification when imaging deep tissue

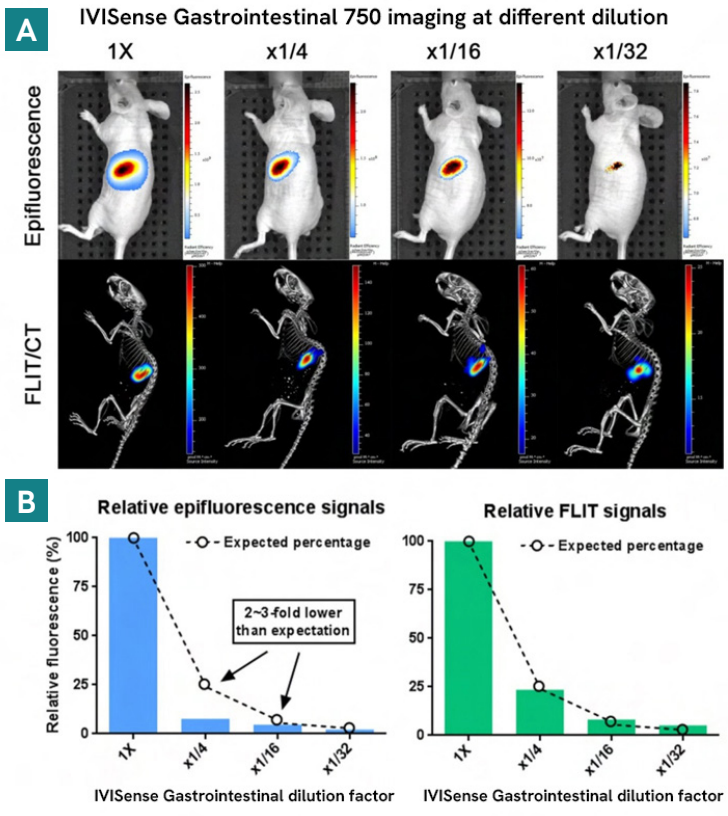


Figure 18: **Comparison of epi-fluorescence and FLIT imaging of deep fluorescence in the stomach**. (A) IVISense Gastrointestinal 750 was serially diluted and then orally gavaged into nu/nu mice. The animals were then subjected to conventional 2D epi-fluorescence and 3D FLIT imaging. (B) Fluorescence signals were quantified and compared to expected values.

Revvity *in vivo* imaging agents

Fluorescent imaging using Revvity's comprehensive suite of NIR fluorescent imaging agents allows researchers to detect and quantify the complexity of disease biology when imaging on the IVIS Spectrum or SpectrumCT.

Protease-activatable agents are optically silent upon injection but are activated to fluoresce *in vivo* following cleavage by specific protease biomarkers of disease. Benefits include biologically specific readouts and high signal-to-noise at the target biology. Targeted agents can use a variety of small molecule or biomolecule targeting moieties to achieve highly specific localization to sites of biological importance. Physiologic agents include Revvity's non-targeted NIR fluorescent agents that localize or clear from tissue with performance characterized by their physical/chemical design.

These agents (Table 2) are developed for *in vivo* use, incorporating drug-like properties for optimal selectivity and performance, but these agents also have rich supporting *in vitro* and microscopy datasets for validation of mechanism and performance. Some common applications are listed for each of the agents.

Table 2: Panel of IVISense NIR Fluorescent *In Vivo* Imaging Agents and Applications.

Type	IVISense probe	Mechanism	Some applications
Protease-activatable	Cat B 680 FAST Cat B 750 FAST	Lysosomal Cathepsin B activity	Cancer, arthritis, pulmonary neutrophilia/eosinophilia, atherosclerosis, general acute/chronic inflammation
	Cat K 680 FAST	Cathepsin K activity	Osteoclastic bone resorption (osteoporosis), soft tissue calcification, tumor associated macrophages
	MMP 645 FAST MMP 680 MMP 750 FAST	Secreted and membrane pan-matrix metalloprotease activity	Cancer, arthritis, pulmonary neutrophilia/eosinophilia, atherosclerosis, general acute/chronic inflammation
	Neutrophil Elastase 680 FAST	Secreted neutrophil elastase activity	Pulmonary inflammation, acute neutrophilia
	Pan Cathepsin 680 Pan Cathepsin 750	Lysosomal pan-cathepsin activity	Cancer, arthritis, pulmonary neutrophilia/eosinophilia, atherosclerosis, general acute/chronic inflammation
	Renin 680 FAST	Mouse renin activity	Elevated renin-angiotensin system (RAS) activity associated with hypertension or hyper-reninemia
Targeted	Annexin V 750	Phosphatidyl serine "flip: to outer cell membrane	Cell death (apoptosis/early necrosis): Cancer& treatment, drug-induced liver injury
	Folate Receptor 680	Folate receptor	Tumor/inflammation metabolism marker, cancer, arthritis, general acute/chronic inflammation
	Hypoxia CA IX 680	Carbonic Anhydrase IX	Tumor hypoxia in CAIX-expressing cancer
	Integrin Receptor 645 Integrin Receptor 680 Integrin Receptor 750	Alpha V beta 3 integrin	Cancer (tumors and neovasculature), atherosclerosis (foam cells)
	Osteo 680 Osteo 750 Osteo 800	Hydroxyapatite	Bone turnover (growth and resorption): Osteoporosis, fracture healing, arthritis, soft tissue calcification
	Tomato Lectin 680	Endothelial glycoproteins	Cancer vascularity
	Bombesin Receptor 680	Bombesin receptor	Tumor proliferative marker
	Transferrin Receptor 750	Transferrin receptor	Tumor metabolism marker

Type	IVISense probe	Mechanism	Some applications
Physiologic	Vascular 680 Vascular 750	IV agent	Cancer vascular leak/perfusion agent, arthritis, general acute/chronic inflammation/edema
	Vascular NP 680 Vascular NP 750	IV agent	Vascularity imaging by intravital microscopy
	Gastrointestinal	PO agent	Gastric tox imaging (gastric emptying rate, GI transit)
	Acute Vascular 750	IV agent	Vascular leak/local perfusion by intravital microscopy
	GFR 680	NIR-labeled inulin	Glomerular filtration rate by kinetic blood assessment
	Edema 680	IV agent	Acute vascular leak/edema in cancer and inflammation

Conclusions

The Revvity IVIS SpectrumCT imaging system is a versatile molecular imaging platform capable of bioluminescence, fluorescence and CT imaging. In this technote, we explain and demonstrate the significance of proper animal preparation and the impact on overall imaging quality. In particular, we discuss the effects of diet, depilation, animal position and imaging area selection on bioluminescence and fluorescence imaging. Most procedures are relatively easy, with the potential to significantly improve imaging quality, but they are frequently overlooked. For example, when operating in the 650-700 nm range, simply placing animals on a low-fluorescent alfalfa-free diet can dramatically improve fluorescent imaging quality by reducing interfering chlorophyll fluorescence. In addition, we provided comprehensive, in-depth assessments of the depilation effects on various imaging modes – ranging from direct 2D BLI and epi-fluorescence, to 3D tomographic DLIT and FLIT imaging. Hair removal in the imaging areas can significantly improve signal strength, localization and visual quality across all optical modes. For longitudinal imaging studies that involve repetitive imaging of the same subjects, we showed the importance of consistent animal positioning and correct scan field selection in order to reduce variation and thus enhance accuracy.

This technical note provides IVIS SpectrumCT users with a quick overview of practical considerations when imaging in different acquisition modes and will hopefully encourage users to fully explore its functionalities in their scientific pursuits.

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