Multiplex 2D *in vivo* imaging of NIR fluorescent agents

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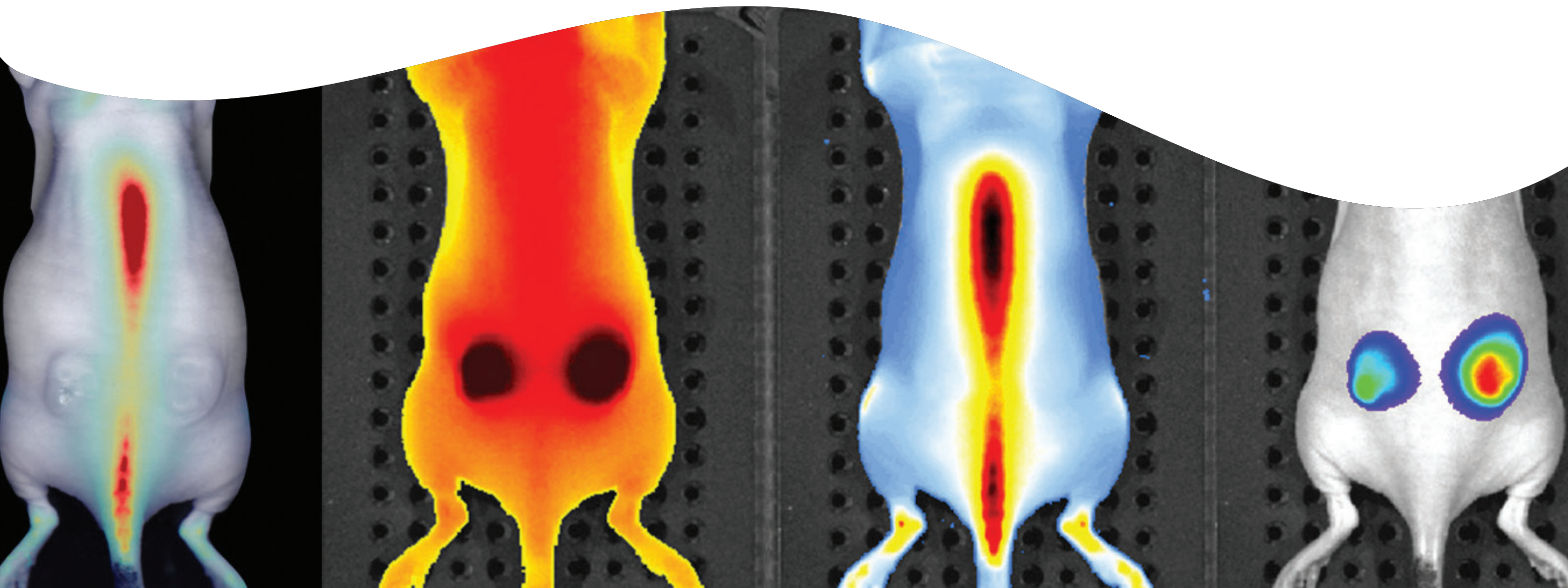
Abstract

Epifluorescence (2D) imaging of superficially implanted mouse tumor xenograft models offers a fast and simple method for assessing tumor progression or response to therapy. This approach for tumor assessment requires the use of near infrared (NIR) imaging agents specific for different aspects of tumor biology. This application note highlights the ease and utility of multiplexing several NIR fluorescent imaging probes to characterize the complex biology within tumors growing in a living mouse. IVISense™ Integrin Receptor 750 detects $\alpha v\beta 3$ integrin expression, IVISense Bombesin Receptor 680 is used to detect the upregulation of bombesin receptor (associated with tumor proliferation), IVISense Transferrin Receptor 750 detects increases in transferrin receptor (due to increased iron metabolism), IVISense MMP 680 is activated by disease-related matrix-metalloproteases (secreted by tumor cells and tumor associated macrophages), and IVISense Pan Cathepsin 750 detects increases in cathepsin activity (elevated in lysosomes of tumors and inflammatory cells). IVISense Osteo 680, a bone turnover imaging agent, was used as a non-tumor imaging control. These, and multiple other Revvity imaging agents, can be used to characterize tumor biology, and in this set of studies the data shows that two different tumors, HeLa (cervical tumor cell line) and 4T1 (breast tumor cell line), can differ in their pattern of labeling intensities for six distinct biological imaging agents. Such an approach is likely to prove valuable for the full biological characterization of tumors during progression, metastasis, or response to treatment.



Materials and methods:

Fluorescent agents

Multiple NIR fluorescent agents (see Table 2) from Revvity (Waltham, MA) were used to image tumors. These agents were developed for systemic intravenous injection and were rigorously designed for both biological targeting as well as for favorable drug-like properties of pharmacokinetics and biodistribution. All agents are extensively validated *in vitro*, *in vivo*, and ex *vivo* to assure proper mechanistic performance *in vivo*. As a control for the multiplex NIR imaging studies, the bioluminescent signal from the tumors (which express luciferase) was assessed to corroborate tumor size and location. The imaging dose for these agents was as recommended in the technical data sheet.

Luciferase-expressing tumor lines

HeLa-luc cells (human cervical adenocarcinoma) and 4T1-luc2 cells (murine breast adenocarcinoma) were acquired from Revvity (Waltham, MA). These tumor cell lines were generated by stable transfection of parent cells with luciferase genes expressed under the SV40 (HeLa) and human ubiquitin C (4T1) promoters. These cell lines can be implanted in nude mice for the purpose of longitudinal detection and imaging following bolus luciferin (the substrate for luciferase) injection. The resulting bioluminescent signal provides intensity measures that correlate with tumor burden and progression.

Tumor models

Six to eight week-old female athymic nude mice were purchased from Charles River Laboratories (Wilmington, MA) and maintained in a pathogen-free animal facility with water and low-fluorescence mouse chow (Harlan Teklad, Madison, WI). Handling of mice and experimental procedures were in accordance with Revvity IACUC guidelines and approved veterinarian requirements for animal care and use. To induce tumor growth with 4T1-luc2 mouse breast adenocarcinoma cells, mice were injected in both upper mammary fat pads with $1x10^6$ cells/site (Revvity, Waltham, MA), yielding 130-250 mm^3 tumor masses within 8-9 days. To induce tumor growth with HeLa-luc cervical cancer cells, mice were injected in two flank sites on the lower back with $2x10^6$ cells/site (Revvity, Waltham, MA), yielding 500-1000 mm^3 tumor masses within 13-19 days. See Figure 3.

These models provided a tool to assess the ability of multiplex imaging to define biological changes associated with tumor growth.

***In Vivo* imaging systems**

The IVIS® SpectrumCT and FMT® 4000 imaging systems (Revvity, Waltham, MA) were both used in parallel for all tumor imaging studies. The IVIS SpectrumCT is an optical imaging system designed for fast, high-throughput (multi-animal) 2D imaging of both bioluminescence and fluorescence signal with tomographic capabilities. The FMT 4000 is a dedicated, single-animal rapid fluorescence tomography system with epifluorescence capabilities. See Table 3.

***In Vivo* 2D imaging and analysis**

Mice were imaged on the IVIS SpectrumCT for bioluminescence signal, 15 minutes after intraperitoneal luciferin injection, as a means to corroborate the size and location of the 4T1 and HeLa tumors. Two-wavelength (680 and 750 nM) NIR epifluorescence imaging was performed on both the IVIS SpectrumCT and FMT 4000 to detect the 6 different NIR imaging agents injected intravenously as 680/750 pairs in individual mice. Fluorescence was imaged 24 h following agent injection. IVIS SpectrumCT data was analyzed using Living Image® 4.3.1 software. ROIs were placed around the two tumors in each mouse, and a background region was selected in a nearby region. Background was automatically subtracted from tumor signal. FMT 4000 epifluorescence data was analyzed by TrueQuant software, also subtracting background fluorescence from tumor signal. All data was represented as mean fluorescence per tumor region as the best means to normalize for differences in tumor size.

Table 1: *In Vivo* imaging filters

System	680 nm	750 nm	BLI
IVIS SpectrumCT	Ex 675 nm Em: 720 nm	Ex: 745 nm Em: 800 nm	Ex: N/A Em: Open
FMT 4000	Ex: 670 nm Em: 690 nm	Ex: 745 nm Em: 770 nm	———

Table 2: Basic Properties of Six Different Revvity Fluorescent Tumor Imaging Agents

Imaging agent	MW (g/mol)	Characterization
IVISense Osteo 680	1,471	NIRF-labeled small molecule bisphosphonate drugs detect exposed hydroxyapatite in bone, indicating regions of bone turnover (growth or loss). Used to detect normal regions of bone turnover as well as regions of bone pathology.
IVISense Bombesin Receptor 680	24,000	NIRF-labeled bombesin peptide binds to upregulated bombesin receptors associated with increased tumor cell proliferation
IVISense Transferrin Receptor 750	106,000	NIR-labeled transferrin detects transferrin receptor upregulation associated with the increased cell metabolic need for iron in cancer and inflammatory cells. Also detects normal iron metabolism in the liver.
IVISense Integrin Receptor 750	1,278	NIRF-labeled small molecule integrin antagonist detects $\alpha v\beta 3$ integrin upregulation associated with cancer and inflammation
IVISense MMP 680	~450,000	Pan-MMP activatable agent that detects abnormal upregulation of MMP secretion associated with cancer and inflammation.
IVISense Pan Cathepsin 750	~450,000	Pan-cathepsin activatable agent that detects abnormal upregulation of cathepsin secretion associated with cancer and inflammation.
IVISense Folate Receptor 680	1,606	NIRF-labeled folate binds to folate receptors that are upregulated in cancer and inflammation

Agent Summary. Characteristics of the agents (wavelength, MW) are shown, as well as a general overview of the biology and utility based on both imaging studies and known biology of the respective targets.

Introduction and results

Fluorescent agents

Preclinical imaging of NIR imaging agents in mouse models offer a unique opportunity to capture important biological processes such as disease relevant biomarker upregulation, changes in protease activity, vascularity and vascular leak, inflammation, cell death, and metabolism, in situ and in a living animal. Revvity offers a variety of NIR imaging agents, and a suite of unparalled imaging instrumentation platforms, to detect biological changes associated with disease as measured by quantification of fluorescent signal. The IVIS SpectrumCT and FMT 4000, provide useful visualization capabilities (a variety of color palettes) and quantification tools for epifluorescence screening of mice. The IVIS SpectrumCT offers a range of 2D and 3D imaging capabilities, including bioluminescence imaging, a broad range of wavelengths for fluorescence excitation (10 filters ranging from 415-760 nM), and the ability to perform advanced spectral unmixing for superior separation of fluorescence signals.

For these studies, we focused on simple and rapid epifluorescence imaging, comparing human cervical adenocarcinoma tumor xenografts (HeLa-luc) to orthotopic mouse mammary adenocarcinoma tumors (4T1-luc2) in nude mice. Both tumor lines express $\alpha v\beta 3$ integrin, MMP and cathepsin protease activity, and bombesin and transferrin receptors. Use of appropriate imaging agents (see Table I) allows the detection of these biomarkers non-invasively *in vivo* in living mice. All mice were maintained on low fluorescent chow, and all agents selected for these studies are excited at either 670 nM or 745 nM, allowing optimal imaging conditions with an expectation of minimal background and no cross-talk between the two agent wavelengths on either system.

Table 3: IVIS SpectrumCT and FMT 4000 Imaging Systems

Imaging agent	IVIS SpectrumCT	FMT 4000
System description	A broad utility imaging system with high throughput, high quality 2D bioluminescence and epifluorescence capabilities. Integrated CT for co-registration with 3D bioluminescence (DLIT) data and 3D fluorescence (FLIT) capabilities. 2D spectral unmixing can separate agent signal from autofluorescence or chow fluorescence.	A dedicated, rapid NIR fluorescence tomography (3D) system with high-quality epifluorescence capability, as well. This system is designed for single animal imaging at four discrete Red/NIR wavelengths optimal for *in vivo* imaging. Not designed for bioluminescence imaging.
Light source	Epifluorescence & Tomography: 150 Watt Quartz tungston halogen	Epifluorescence: Four pairs of LED (635, 660, 750, and 780 nm) Tomography: Four 0.5-80 mW laser diodes (635, 670, 746, and 785 nm)
Excitation filters	430, 465, 500, 535, 570, 605, 640, 675, 710, and 745 (30 nm bandwidth)	632, 680, 760, and 780 nm (6-22 nm bandwidths)
Emission filters	500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840 (20 nm bandwidth)	647-673, 690-740, 770-800, 805+
# mice for epifluorescence Imaging	1-5 mice at a time	1 mouse at a time
2D imaging time for 5 mice	~ 1 minute	~ 5-6 minutes
Camera	TE-cooled. Back-thinned, back-illuminated Grade 1 CCD. Low noise.	TE-cooled. Back-illuminated 16-bit dynamic range CCD
Bioluminescence	Yes	No
Epifluorescence	Yes	Yes
# mice for fluorescence tomographic imaging	1 mouse at a time	1 mouse at a time
Fluorescence Tomography	Small region scans only, 15-20 minutes	Small region scans, 2-3 minutes Whole body, 12 minutes
CT co-registration	Integrated CT. Automatic co-registration.	Requires separate CT system
Dimensions	Floor: 203 x 163 x 214 cm (w x d x h)	Bench top: 46 x 48 x 89 cm (w x d x h)

No crosstalk between 680 nM and 750 nM NIR imaging agents

When performing multiplex fluorescence imaging in mice, spectral crosstalk is an important issue to consider, depending on the spectral wavelength(s) to be imaged, whether the mice have been fed low fluorescent chow, and whether the fluorophores overlap each other in their absorption and emission profiles. In particular, autofluorescence or chow related fluorescent signal can be an issue when imaging 635 or 680 nM fluorescent agents. However, if you keep your mice on low fluorescent chow, background signal in mice is low enough to be negligible when imaging at 680 or 750 nM wavelengths, the two most common NIR wavelengths for *in vivo* imaging agents.

To test the two imaging systems for their potential for interfering crosstalk between 680 and 750 nM agents, we assessed normal, or tumor-bearing mice, maintained on low fluorescent chow and simultaneously injected with both 680 and 750 nM agents. The agents were selected based on their clear differences in localization, providing the cleanest opportunity to detect abnormal distribution (indicative of channel crosstalk). For example, one study (shown in Figure 1) used hairless SKH-1E mice that were injected with IVISense Folate Receptor 680, IVISense Osteo 750, or both. Mice were imaged on the IVIS SpectrumCT, with an uninjected mouse used as a control. The folate agent, designed to detect

inflammation or cancer,
will merely clear through the kidneys of normal mice, with no predisposition for accumulation in bone regions. In contrast, IVISense Osteo probe labels regions of high bone turnover, generally in the vertebral column, skull, and knees, with no accumulation in the kidneys.

Figure 1 shows that imaging the IVISense Folate Receptor, IVISense Osteo, and Folate Receptor/Osteo mice at 675 nM yields only the signal derived from IVISense Folate Receptor. There is no indication of spine signal in the third mouse, which received only IVISense Osteo. Similarly, the same mice imaged at 745 nM show no indication of channel crosstalk, with the only evident signal coming from IVISense Osteo injected mice. There is no indication of kidney signal in mouse two, which received only IVISense Folate Receptor. In addition, the negative control mouse (mouse 1) shows no evidence of 675 or 745 nM excited fluorescence. Figure 1B shows quantitatively the absence of cross-talk and, further, that there is minimal crosstalk even using suboptimal emission filters.

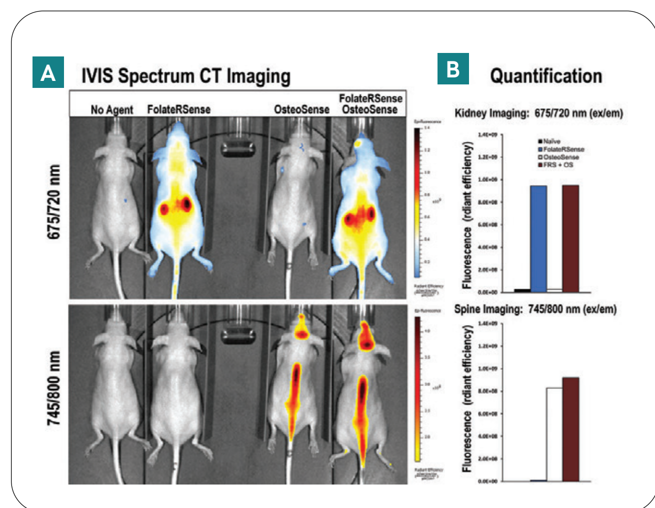


Figure 1: Assessment of crosstalk between 680 and 750 nm agents. SKH-1E mice, maintained on low fluorescence chow, were injected intravenously with either IVISense Folate Receptor 680 or IVISense Osteo 750 or both agents. An uninjected mouse was used as a control. Mice were imaged 3h later to assess IVISense Folate Receptor clearance through the kidneys and IVISense Osteo incorporation into the bone of the vertebral column. (A) IVIS SpectrumCT images showing 680 nm excitation (top) and 750 nm excitation (bottom). (B) Quantitative results of kidney and spine fluorescence, showing different emission filters used for the two agents.

In a similar study for the FMT 4000 (shown in Figure 2) HeLa tumor xenograft-bearing mice were injected with IVISense Osteo 680 and IVISense Integrin Receptor 750 to detect bone and tumor mass, respectively. Mice were imaged for epifluorescence on the FMT 4000 at 680 and 750 nM, and the images clearly show no obvious tumor region signal at 680 nM and the near absence of spine signal at 750 nM.

The quantification shown in Figure 2B further supports this finding, showing negligible crosstalk between IVISense Osteo 680 and IVISense Integrin Receptor 750; no tumor signal is seen at 680 nM imaging, and no spine signal is seen at 750 nM imaging.

It is worth noting that the IVIS SpectrumCT (as well as the IVIS Spectrum and IVIS Lumina III) offer 2D spectral unmixing capabilities that may be important when attempting to separate imaging agents at 645 nM either from chow signal or from agents excited at 680 nM. In this unmixing process, a large range of filter sets must be used for fluorescence acquisition, and libraries to define the spectral behavior of each fluorophore are also required. However, we have found in our current studies that a simpler and more streamlined process can be used for imaging mice maintained on low fluorescence chow and using combinations of 680 and 750 nM agents. Just the appropriate filter pairs for IVIS SpectrumCT and FMT 4000 are required, and the analysis requires only appropriate placement of tumor and background ROIs.

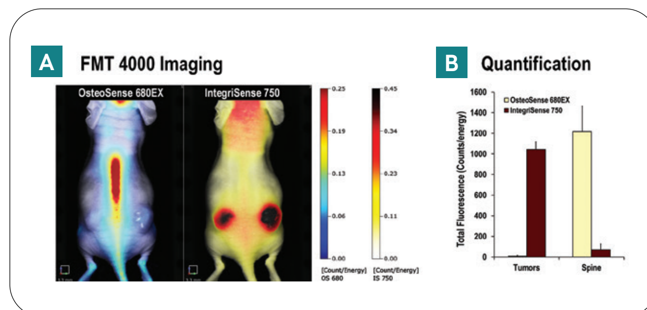


Figure 2. Assessment of crosstalk between 680 and 750 nm agents. HeLa tumor-bearing nude mice, maintained on low fluorescence chow, were injected intravenously with both IVISense Osteo 680 and IVISense Integrin Receptor 750 agents. Mice were imaged 24h later to assess IVISense Integrin Receptor tumor labeling and IVISense Osteo incorporation into the bone of the vertebral column. (A) FMT 4000 images showing 680 nm and 750 nm excitation. (B) Quantitative results of tumor and spine fluorescence, showing negligible crosstalk between the two fluorescent channels.

Tumor model: image representation

To best illustrate the utility of Revvity's NIR fluorescent imaging agents for profiling tumor biology, mice were injected superficially with either 4T1-luc2 tumors (both upper mammary fat pads) or HeLa-luc tumor cells (two lower dorsal flank sites) (see Figure 3). These two tumors grew to optimal size within 2 weeks and offered visual size and location corroboration. The tumors were profiled by imaging with 6 different imaging agents, detecting different biomarkers such as protease upregulation, metabolic activity, and integrin expression.

Image representation is a critical aspect of data analysis and interpretation, and both the IVIS SpectrumCT and FMT 4000 offer a variety of color scales to highlight differential bodily distribution of bioluminescence and fluorescence signal. For bioluminescence imaging, the signal is limited exquisitely to the regions in which luciferase expressing cells are localized. Figure 4A shows 4T1-luc2 tumors implanted on the upper mammary fatpads, providing corroboration of tumor localization and size. The same mouse received the NIR fluorescent imaging agent IVISense Integrin Receptor 750 to detect $\alpha v\beta 3$ integrin expression in these tumors, and these images are represented from IVIS SpectrumCT imaging (Figure 4B) and FMT 4000 imaging (Figure 4C) using a variety of color scales available on Living Image® and TrueQuant® software, respectively. Upper panels represent images thresholded for optimal viewing of tumors (only), and the lower panels show images with low-level thresholding that shows the whole body signal present following the systemic injection of this NIR imaging agent.

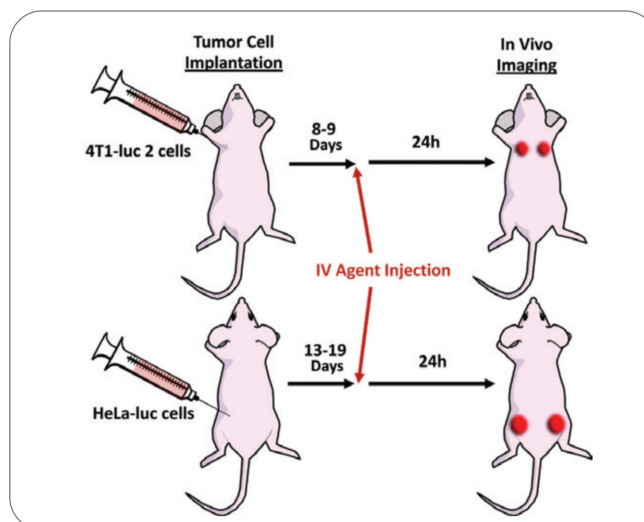


Figure 3: Schematic diagram of 4T1-luc2 and HeLa-luc tumor model imaging studies. Models were performed as mammary fat pad and flank tumor implantation models, respectively.

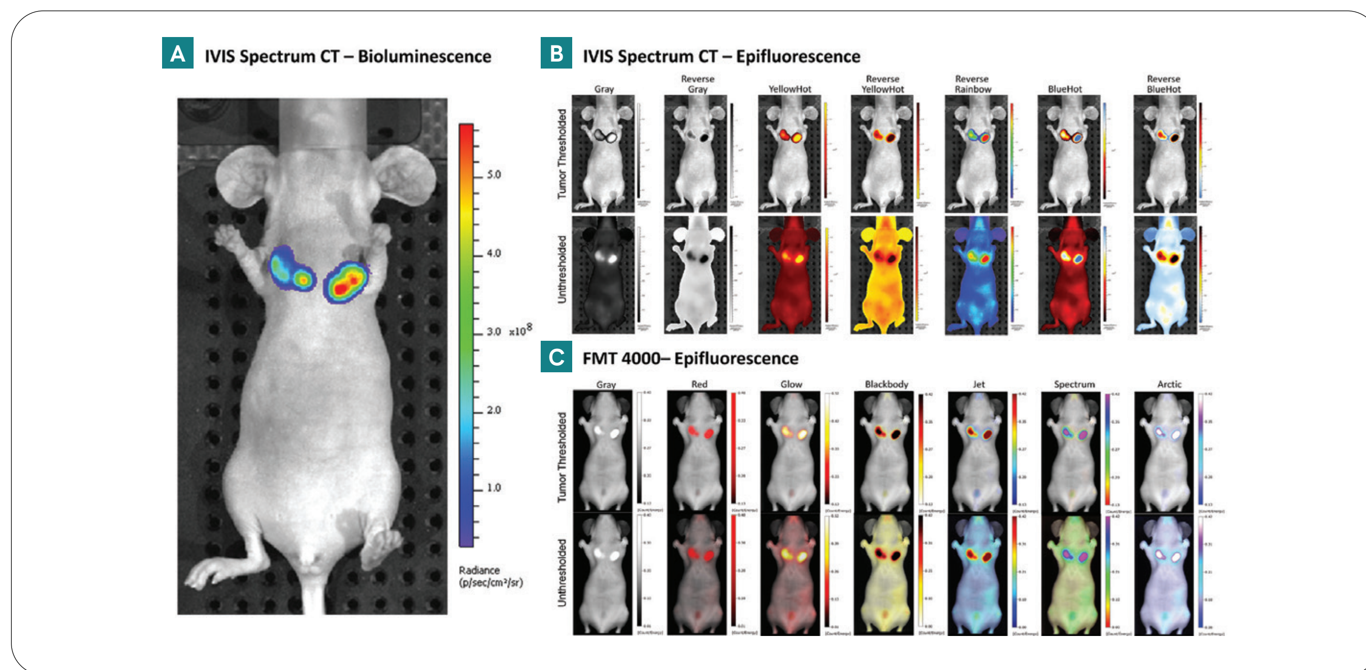


Figure 4: Color scales for image representation. A representative mouse bearing two established 4T1-luc2 tumors implanted superficially in the upper mammary fat pad was imaged by bioluminescence to show tumor localization (A). IVISense Integrin Receptor 750 was used to image the same mouse on the IVIS SpectrumCT (B) and FMT 4000 (C) to highlight some of the color palettes available for image representation on both systems. Thresholding can be applied to the images to show just the tumors, or the bodily signal can be retained to best represent the systemic signal you would expect with injection of an exogenous fluorescence agent.

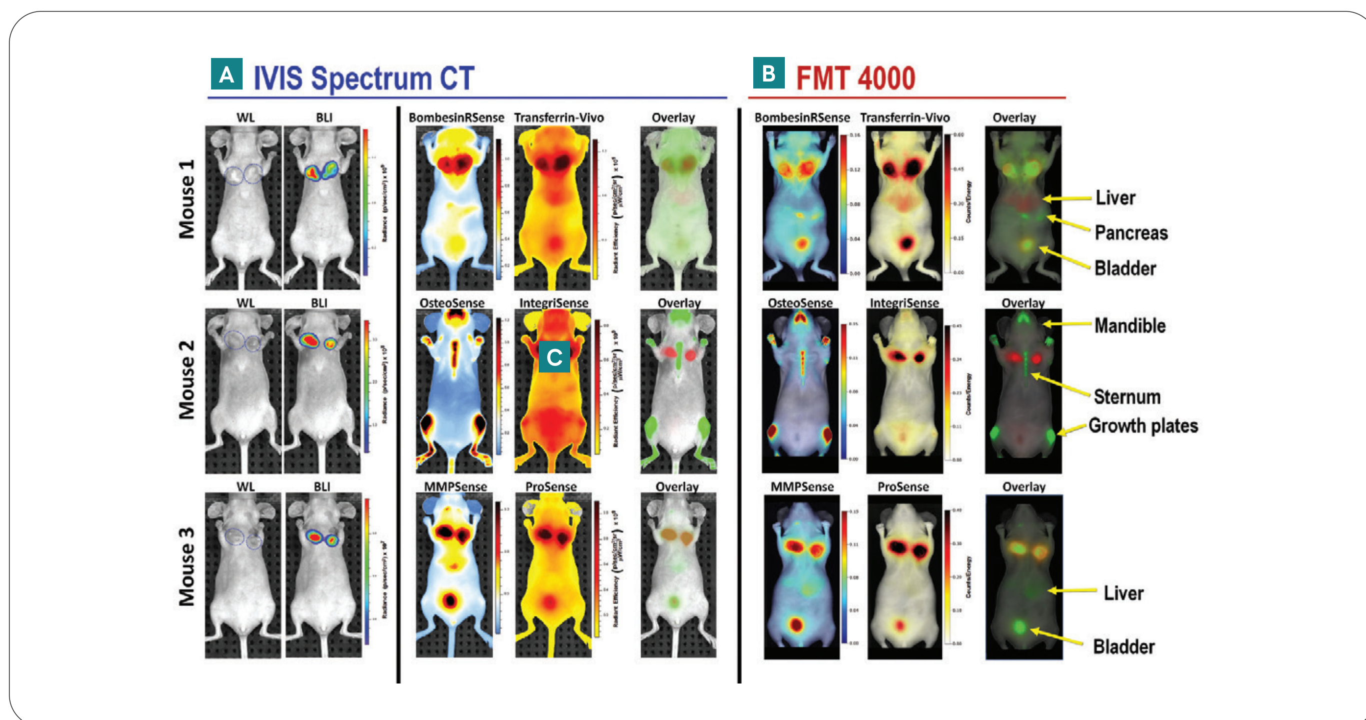


Figure 5: 4T1 Tumor Imaging. Three representative mice bearing two established 4T1-luc2 tumors implanted superficially in the upper mammary fat pads were injected with the indicated pairs of imaging agents (680 and 750 nm agents). (A) Mice were imaged by IVIS SpectrumCT for bioluminescence to show tumor localization and for fluorescence to show specific biomarker detection. (B) The same animals were imaged on the FMT 4000 for fluorescence detection. Composite images in A and B show co-localization of each pair of imaging agents.

Tumor model: biomarker profiling

Revvity's NIR *in vivo* imaging agents provide important tools for non-invasively detecting and quantifying a variety of tumor biomarkers, including protease activity (MMPs and cathepsins), changes in vascularity and vascular leak, expression of metabolism-related biomarkers, tumor necrosis/apoptosis, as well as specific or general markers of tumors. As these agents are available with different fluorophores of differing NIR wavelengths, it is possible to image two different agents simultaneously within the same mouse. This opens the door to more extensive tumor profiling by using 6-10 different imaging agents across a cohort of tumor bearing mice.

To illustrate the potential of broad tumor profiling, we used groups of HeLa and 4T1 tumor-bearing mice and injected them with three pairs of imaging agents; IVISense Osteo 680 & IVISense Integrin Receptor 750, IVISense Bombesin Receptor 680 & IVISense Transferrin Receptor 750, and IVISense MMP 680 & IVISense Pan Cathepsin 750. IVISense Osteo was used as a control agent not expected to localize appreciably to tumor tissue. The other agents were used to detect relative differences, comparing HeLa and 4T1 tumors, in integrin expression (general tumor burden marker), bombesin receptor and transferrin receptor upregulation (increased proliferation and metabolism), and protease activity (associated with tumor aggression).

Figure 5 shows a composite figure with bioluminescence and epifluorescence imaged on the IVIS SpectrumCT and epifluorescence imaging (only) on the FMT 4000. Each image was optimized to show the best tumor definition for each agent. Both systems showed excellent NIR agent imaging and very good agreement with the tumor regions identified by bioluminescence imaging. The IVIS SpectrumCT allowed the simultaneous imaging of five mice at a time. The only agent unable to define tumor regions was IVISense Osteo, an expected finding for this bone imaging agent. This agent also confirmed the absence of channel cross-talk between 680 nM and 750 nM image acquisitions.

Imaging by both systems also showed evidence of agent clearance through the bladder for most of the agents, some liver localization of IVISense MMP and IVISense Transferrin Receptor and some pancreas localization of IVISense Bombesin Receptor. Both the IVIS SpectrumCT software (Living Image) and the FMT 4000 software (TrueQuant) offered overlay capabilities for co-registering the two different agents injected in each mouse.

Figure 6 represents the same experiment performed in HeLa-luc tumor xenografts. Images for each agent are represented on the same color scales as for the 4T1-luc2 tumor mice to facilitate easy comparison of agent performance in these two tumor types. HeLa and 4T1 tumors showed comparable IVISense Integrin Receptor, IVISense Bombesin Receptor and IVISense Transferrin Receptor signal, however the HeLa tumors were dramatically reduced in IVISense MMP and IVISense Pan Cathepsin activity relative to the 4T1 tumors. It is important to note, however that even these low levels were readily imaged and could provide good tumor definition and localization. This is shown in the IVIS and FMT overlay images.

Quantification of these imaging datasets was performed using each system's software and involved the careful placement of ROI's encompassing the tumor regions. Control ROI's were placed in nearby region away from tumor, liver, or bladder signal. Background was subtracted from each tumor ROI, and quantification was represented as average radiant efficiency for IVIS datasets. For FMT analysis, the total counts per energy for each tumor were divided by the ROI area in mm^2, generating counts/energy/mm^2 (FMT). These measures represented tumor "brightness", a measure relatively independent of tumor size.

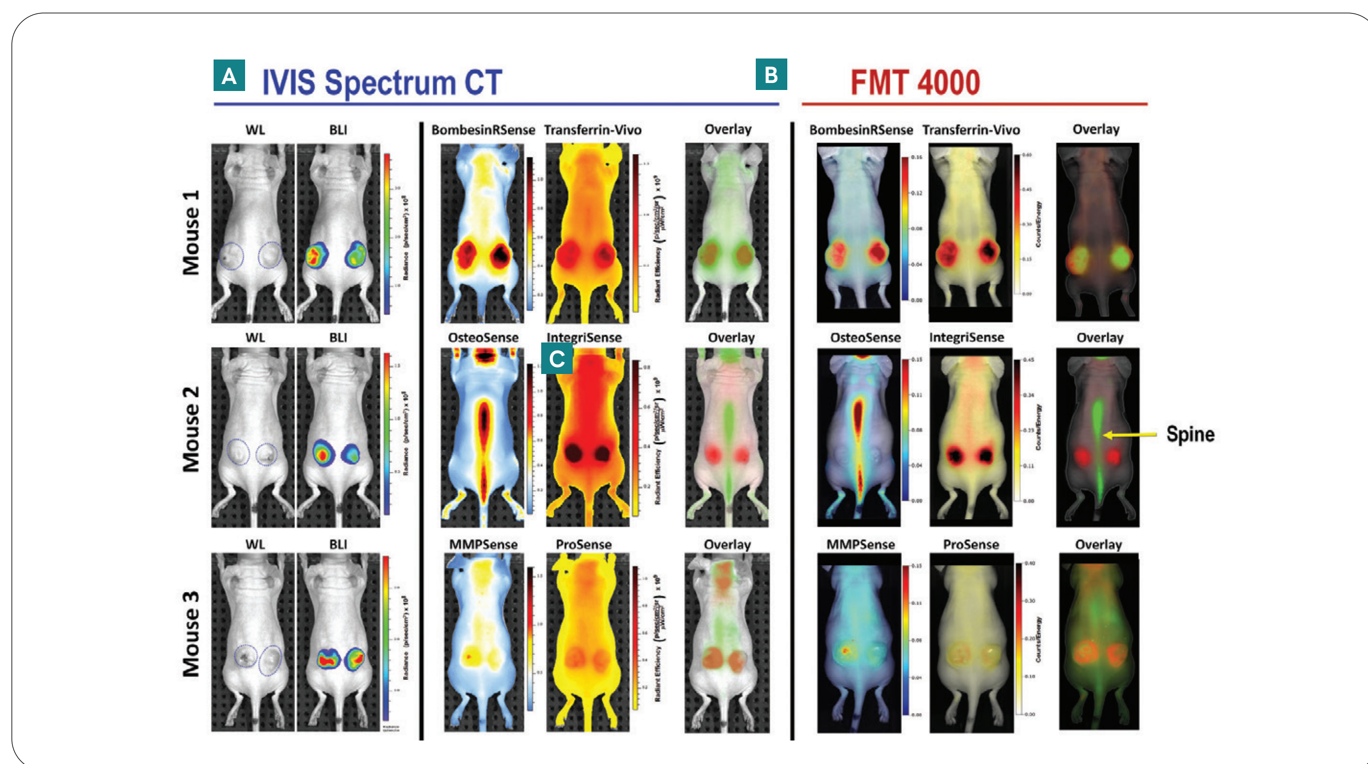


Figure 6: HeLa Tumor Imaging. Three representative mice bearing two established HeLa-luc tumors implanted superficially on the lower dorsal flank were injected with the indicated pairs of imaging agents (680 and 750 nm wavelengths). (A) Mice were imaged by IVIS SpectrumCT for bioluminescence to show tumor localization and for fluorescence to show specific biomarker detection. (B) The same animals were imaged on the FMT 4000 for fluorescence detection. Composite images in A and B show co-localization of each pair of biomarkers.

Figure 7 shows that the two different tumor types were quite similar in IVISense Integrin Receptor, IVISense Bombesin Receptor, and IVISense Transferrin Receptor fluorescent signal, indicating similar levels of integrin, bombesin, and transferrin receptors. In contrast, HeLa cells were 50-70% lower in protease activity as assessed by IVISense MMP and IVISense Pan Cathepsin imaging. This lower protease activity in HeLa cells may account for the slower initial growth rate of HeLa tumors (Figure 8), suggesting the importance of proteases early following implantation of cells. At later time points, the HeLa tumors rapidly achieve the size of the 4T1 tumors. Each agent is represented on it's own Y axis scale, because different agents/ fluorophores may vary in their performance, particularly when comparing agents of different wavelengths..

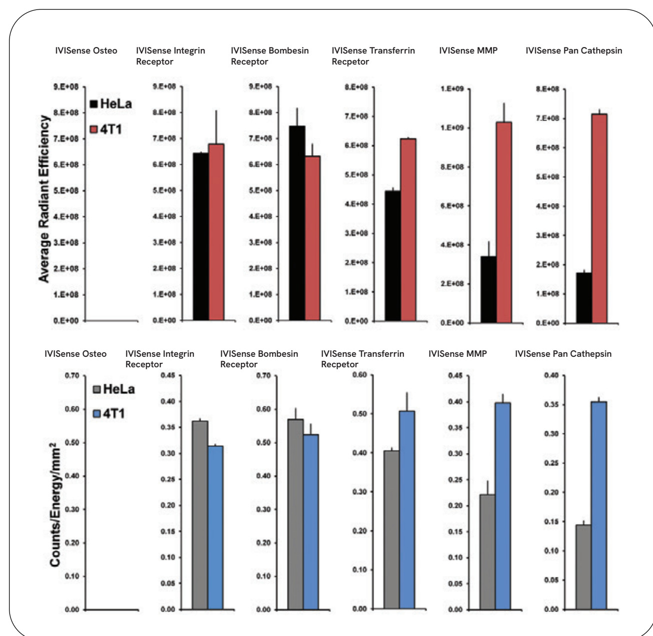


Figure 7: Fluorescence Quantification. ROI's were placed to capture tumor and control region fluorescence, and following background subtraction the data was represented as the average signal within the 2D ROI. (A) IVIS SpectrumCT quantification. (B) FMT 4000 quantification.

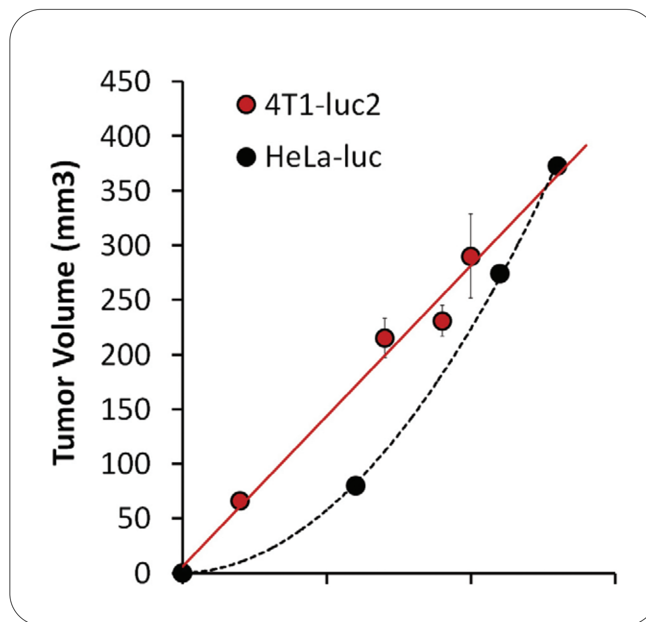


Figure 8: Tumor Growth Rates. Caliper measurements of 4T1-luc2 and HeLa-luc tumors were measured over time to assess growth kinetics. Length and width measurements were used to calculate tumor volumes in mm^3.

Conclusions

Fluorescent imaging using Revvity's NIR fluorescent imaging agents allows researchers to capture the complexity of tumor biology. These studies showed profiling of two different tumors by imaging agents that detect different types of protease activity as well as surface receptor expression of markers for cell burden, proliferation, and metabolism. Fluorescence acquisition was simple and sensitive on both the IVIS SpectrumCT and the FMT 4000, and simultaneous imaging of two NIR agents (680 and 750 nM) was easily achieved with no appreciable channel crosstalk. This detailed tumor profiling approach is likely to prove valuable for the full biological characterization of tumors, and there are a number of other Revvity agents (Table 4) that can provide additional assessments of changes during progression, metastasis, or response to treatment.

Table 4: Revvity NIR Imaging Agents for Oncology

Type of Target	IVISense Agent	4T1-luc2 tumors	HeLa-luc tumor	Target Activity
Proteases	Cat B 680 FAST Cat B 750 FAST	++	+	Lysosomal cathepsin B activity (tumor aggression, inflammation)
	Pan Cathepsin 680 Pan Cathepsin 750	++	+	Lysosomal pan-cathepsin activity (tumor aggression, inflammation)
	MMP 645 FAST MMP 680 MMP 750 FAST	++	+	Extracellular pan-matrix metalloproteinase activity (tumor aggression, inflammation)
	PSA 750 FAST*	-	-	Extracellular prostate specific antigen proteolytic activity
Cell surface receptors	Integrin Receptor 645 Integrin Receptor 680 Integrin Receptor 750	++	++	Integrin $\alpha v \beta 3$ expression (tumor burden)
	Annexin Vivo 750	+/- to + a	+/- to +a	Phosphotidylserine expression (cell death)
	Folate Receptor 680	+/-	'+/-	Folate receptor expression (folate metabolism)
	Hypoxia CA IX 680	+/- to +b	+/- to ++b	Carbonic anhydrase 9 (CA IX) expression (tumor hypoxia)
	Tomato Lectin 680	+/-	+/-	Vascular Endothelium - N-actylglucosamine expression (vascular burden)
	Bombesin Receptor 680	++	++	Bombesin receptor expression (tumor proliferation marker)
	Transferrin Receptor 750	++	++	Transferrin receptor expression (iron metabolism)
Vascular	Vascular 680 Vascular 750	++	++	Vascularity, perfusion, and vascular permeability
	Vascular NP 680 Vascular NP 750	++	++	Vascularity, perfusion, and vascular permeability - long pharmacokinetic profile
	Edema 680	+/-	+/-	Acute perfusion, and vascular permeability

* FAST (Fluorescent Activatable Sensor Technology) agents have faster kinetics and a broader imaging window [a] requires cytotoxic treatment

[b] depends on hypoxia status of tumor

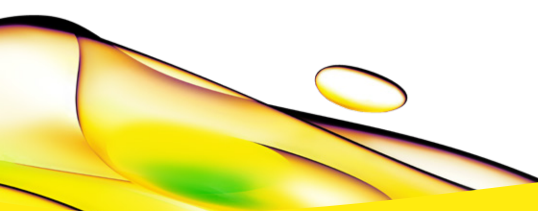
+ + very good tumor definition, very high signal

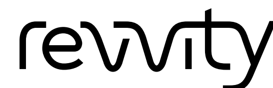
+ good tumor definition

+/- some tumor definition

- no clear tumor definition; very low signal

Tumor Imaging Agents: Multiple agents offered by Revvity are listed with relative scoring for 4T1-luc2 and HeLa-luc imaging. A "-" indicates no clear tumor definition and very low signal, a "+/-" indicates some tumor definition, a "+" indicates good tumor definition, and "++" indicates very good tumor definition accompanied by high tumor fluorescence.





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