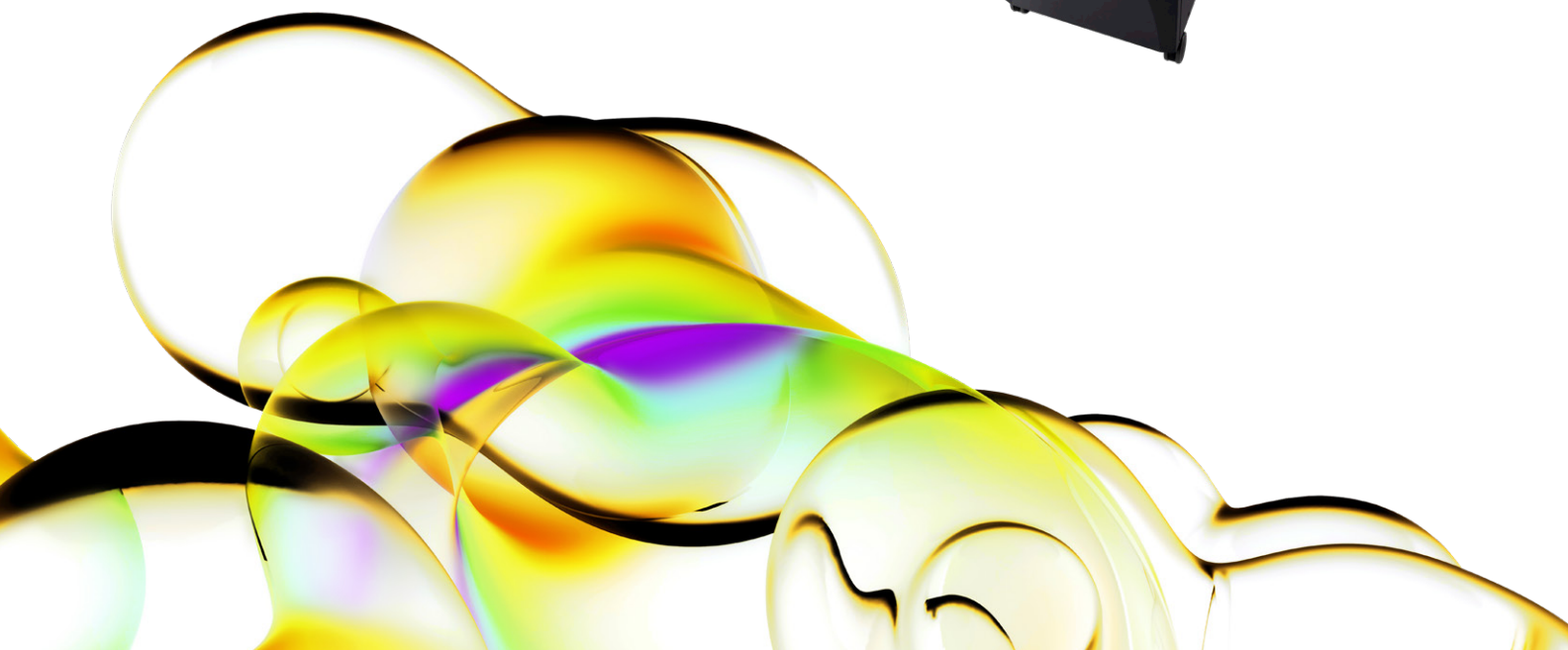
# Advanced pre-clinical optical imaging.

## Key features

- High Sensitivity *in vivo* fluorescence and bioluminescence imaging
- 3D tomographic reconstruction
- Absolute calibration
- High throughput
- High resolution (to 20 microns) with 3.9 cm field of view
- Twenty eight high efficiency filters spanning 430-850 nm
  - Multispectral imaging with superior spectral unmixing properties
  - Ideal for distinguishing multiple bioluminescent and fluorescent reporters
- Ability import and automatically co-register CT or MRI images yielding a functional and anatomical context for your scientific data

The IVIS® Spectrum advanced pre-clinical optical imaging system combines high throughput and full tomographic optical imaging in one platform. The system uses leading optical imaging technology to facilitate non-invasive longitudinal monitoring of disease progression, cell trafficking and gene expression patterns in living animals. Take full advantage of bioluminescent and fluorescent reporters across the blue to near infrared wavelength region using optimized set of high efficiency filters and spectral unmixing. It also offers true 3D tomography for both fluorescent and bioluminescent reporters that can be analyzed in anatomical context against a Digital Mouse Atlas or registered with other tomographic technologies such as MR, CT or PET through the multimodality module.

**IVIS Spectrum**



## World-leading optical imaging system: uncompromised sensitivity and flexibility

### Transmission and epi-illumination imaging

Emitted light from the excitation filter wheel feeds through a fiber optic bundle to illuminate the specimen from either the top, in epi-illumination (reflectance) mode, or from underneath the stage, by means of an automated bundle switch. Transilluminating the subject from below at precise x,y-locations allows for transmission imaging, enabling more sensitive detection and accurate quantification of deep sources. Transmission fluorescence imaging also reduces the effects of autofluorescence.

### Narrow band excitation and emission filters

The IVIS Spectrum excitation and emission filters enable spectral scanning over the blue to NIR wavelength region.

- 10 narrow band excitation filters: 415 nm – 760 nm (30 nm bandwidth)
- 18 narrow band emission filters: 490 nm – 850 nm (20 nm bandwidth)

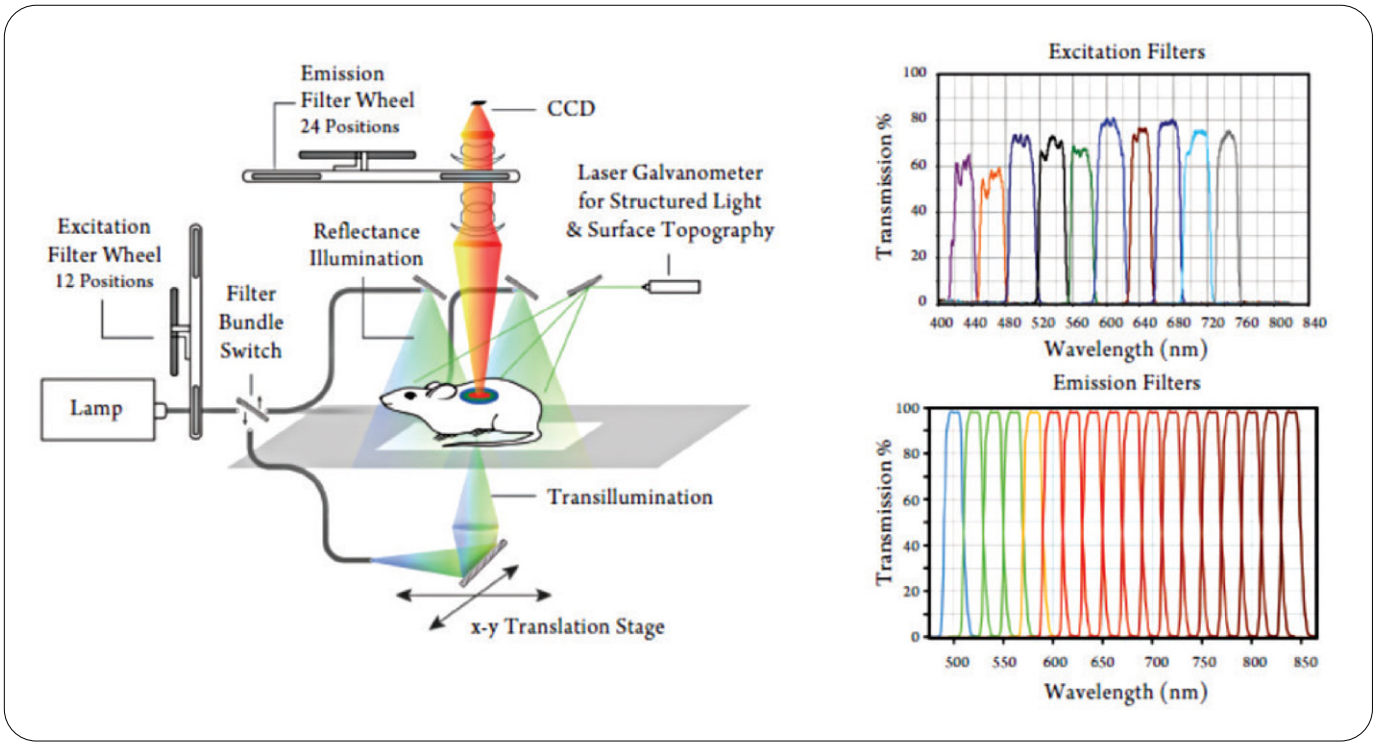



Figure 1: Narrow band excitation and emission filters

## Inside the IVIS Spectrum

### Imaging chamber

- Light tight imaging chamber
- Heavy duty castors
- Integrated gas anesthesia
- Integrated fluorescence
- LED lamps for photographic images
- Heated stage to maintain optimum body temperature
- Electromagnetic door latch
- Motor controlled stage, filter wheel, lens position, and f-stop
- Scanning laser for mouse alignment and surface topography

**CCD camera**

- Back thinned, back illuminated grade 1 CCD provides high quantum efficiency over the entire visible to near-infrared spectrum
- 13.5 micron pixels, 2048 x 2048
- 16-bit digitizer delivers broad dynamic range
- CCD is thermoelectrically (Peltier) cooled to -90 °C, ensuring low dark current and low noise

**Custom-designed lens**

- 6-inch diameter optics, f/1– f/8
- High resolution – down to 20 microns
- Emission filter wheel with 24 slots

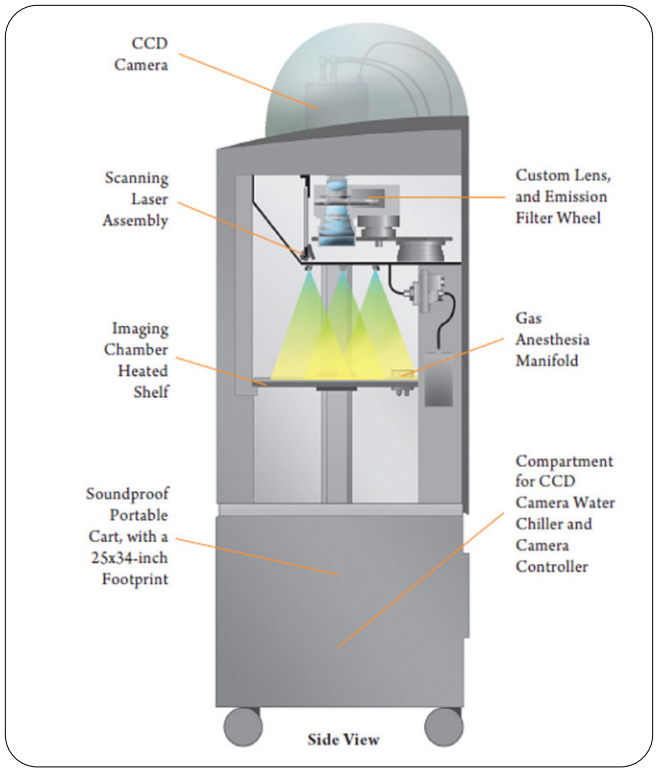



Figure 2: Imaging chamber and CCD camera

## Versatile field of view – single cell to five mice

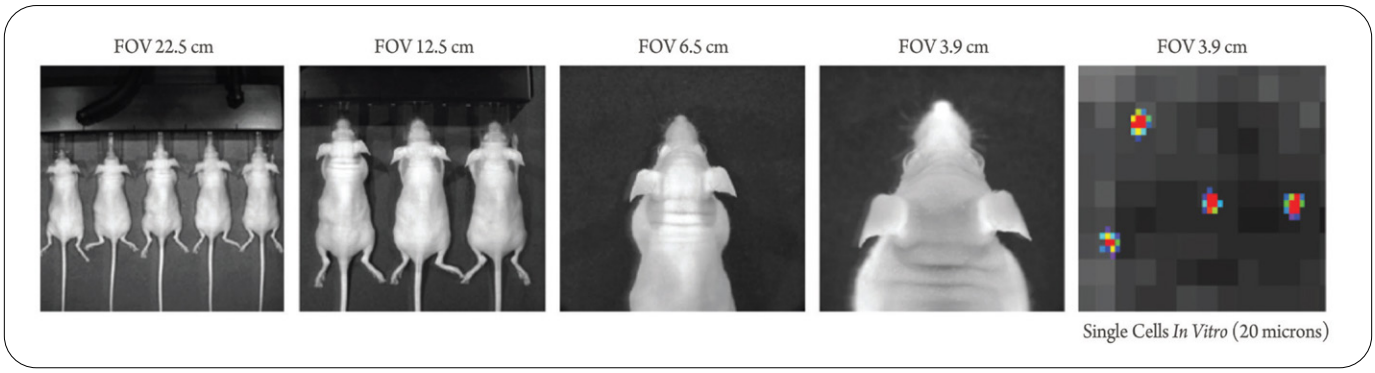



Figure 3: From 20 microns to localize single cells to five whole mice, the IVIS Spectrum gives you the automated flexibility, throughput and resolution required to quantitate functional developments in whole animals down to a single cell.

## Bioluminescence imaging- best in class *in vivo* sensitivity

IVIS Spectrum has a cooled (-90 °C) camera with large CCD chip area and low F-stop for high sensitivity bioluminescence light detection. Image multiple bioluminescent reporters like firefly luciferase, Renilla luciferase and bacterial luciferase *in vivo* at depth rapidly and quantitatively. The ultra sensitive camera optics allows the detection of as few as five cells (Figure 4), earlier monitoring of micormetastais *in vivo* and track tumor development longitudinally *in vivo* (Figure 5).

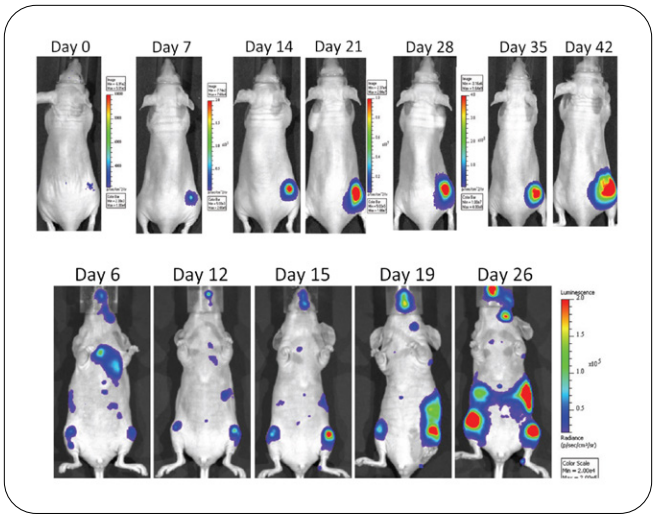



Figure 4: Detection of five 4T1-luc2 cells injected subcutaneously in nude mice (top) and monitoring metastasis post intracardiac injection of MDA MA-231-luc2 cells (bottom) longitudinally.

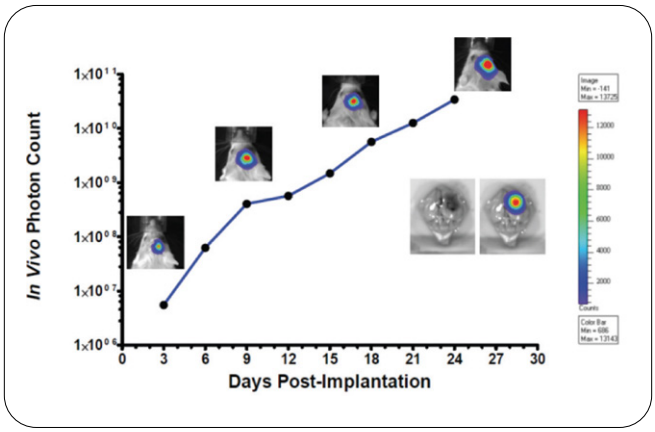



Figure 5: Monitoring tumor growth after intracranial implantation of GL261-luc2 cell line in immuno-competent C57BL/6 mice.

## Fluorescence imaging – versatility in fluorescence

The IVIS Spectrum can image and quantify all commonly used fluorophores, including fluorescent proteins, dyes and conjugates. IVIS Spectrum achieves superior spectral unmixing through a wide range of high resolution, short cut-off filters and advanced spectral unmixing algorithms. Spectral unmixing not only allows detection and separation of multiple reporters, but greatly reduces the effects of tissue auto-fluorescence.

Revvity offers the broadest portfolio of fluorescent agents and dyes for *in vivo* applications. The IVIS Spectrum is the most sensitive system to visualize these fluorescent agents in various *in vivo* applications (Figure 6, 7).

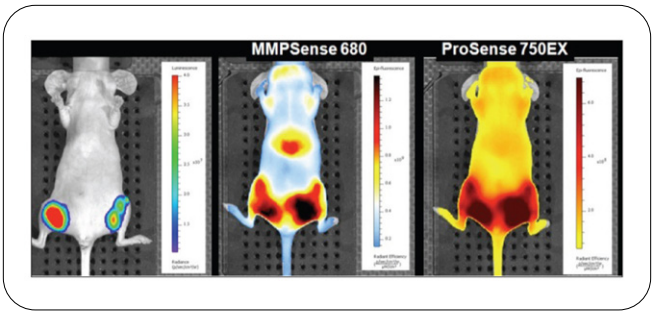



Figure 6: Multispectral imaging of two biological events in the same tumor model. Monitoring MMP and cathepsin activity in mouse with 4T1-luc2 induced bone metastasis.

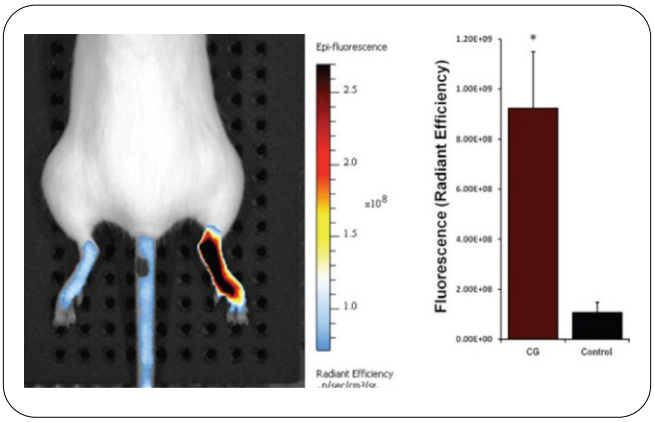



Figure 7: Measurement of VivoTrack 680 labeled macrophage recruitment to carrageenan induced inflammation in right paw. Chart shows the difference in fluorescence intensity between control and inflamed paws.

## Multispectral imaging with advanced spectral unmixing algorithms

Living Image® Software is designed to simplify advanced and complex biological models by intuitively guiding the user through experiential setup and analysis. The imaging wizard with the newly added probe library will help design imaging settings and select the right filter pair for fluorescence studies. The software also offers a step by step guide for spectrally unmixing multiple fluorescent signals from the same animal.

Advanced spectral-unmixing algorithms and a broad range of high spectral resolution filter sets minimize autofluorescence and provides the opportunity to image a wide variety of targeted and activatable fluorescent probes and reporters. Figure 8 shows unmixing of four different fluorescent reporters simultaneously.

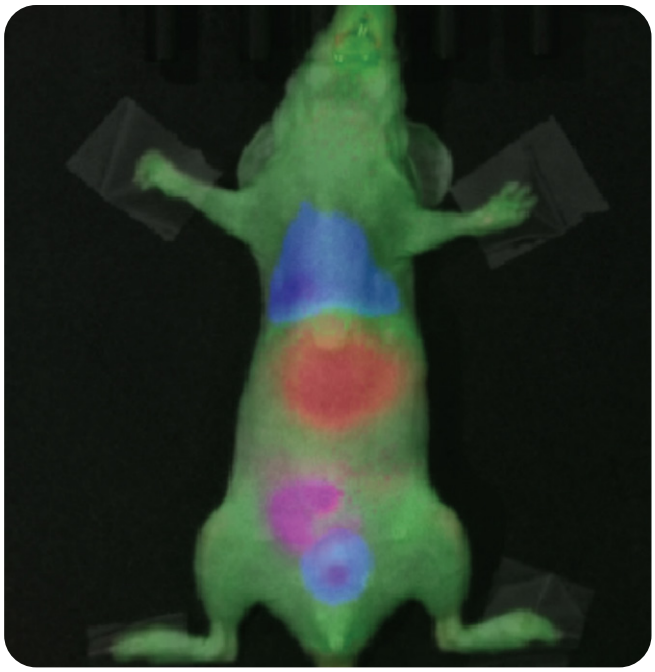


Figure 8: Spectral unmixing of four different fluorescent roproters. Tissue autofluorescence, VivoTag 680 in liver, VivoTag 750 in the lungs and ICG in the gut.

## Absolute localization in optical imaging – 3D analysis

Look deeper, see further, and take science to a new level of sophistication with the 3D technology from Revvity. 3D diffuse tomography utilizes structured light data with bioluminescence or fluorescence images to reconstruct three dimensional representations of light emitting reporters and compute signal strength (Figure 9). Take the next step and analyze 3D sources in an anatomical context with the Digital Mouse Atlas. The tomography tool allows the quantification of number of cells and dye concentration in a tumor and 3D coregistation of both bioluminescent and fluorescent reporter (Figure 10).

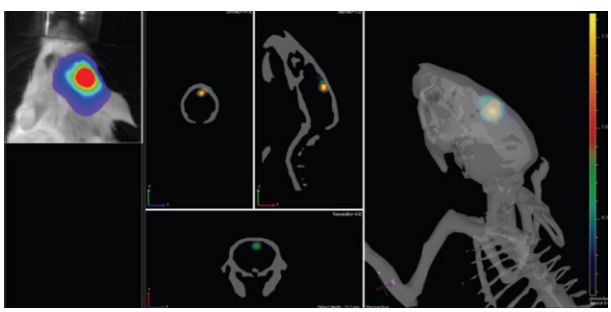


Figure 9: DLIT 3D reconstruction shows precise localization of GL261-luc2 brain tumor using digital mouse atlas.

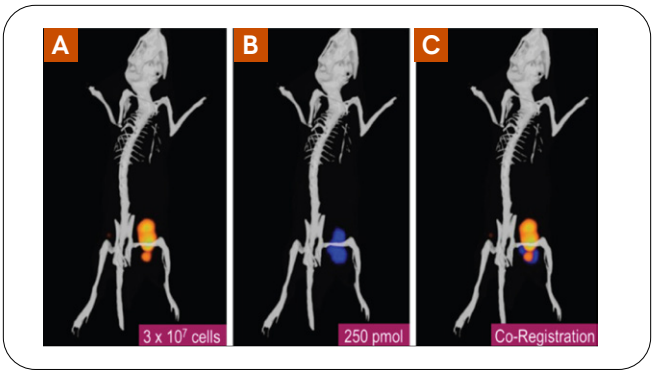



Figure 10: FLIT reconstruction of implanted P3CM cells expressing tdTomato (a) targeted with an therapeutic antibody-750 probe (b). Coregistered image with both reporters (c).

## Advanced tomographic 3D analysis on the IVIS Spectrum

- Determine geometry and quantify the depth and intensity and of fluorescent sources in 3D space using FLIT (Fluorescent Imaging Tomography) or bioluminescent sources using DLIT (Diffused Luminescent Imaging Tomography) (Figure 11)
- View optical sources in anatomical context. Automatically co-register organs of interest from the Digital Mouse Atlas on a 3D image
- Import and automatically co-register a CT or MRI radiograph (Open Inventor format) on a 3D image
- Export 3D images to DICOM compliant formats
- View sagittal, coronal and transaxial sections through a 3D image
- View optical sources from multiple perspectives by converting a static 3D image into a movie of a rotating subject

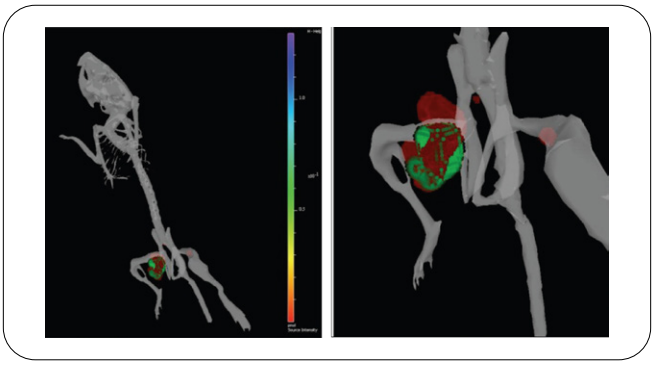


Figure 11: DLIT and FLIT reconstructions overlaid of implanted P3CM cells expressing luciferase (GREEN) and targeted with a 750 nm fluorescent agent (RED).

## Multimodal co-registration

The IVIS Spectrum is the most advanced *in vivo* imaging system available on the market today – not only can it quantitate and localize 3D fluorescent and bioluminescent sources *in vivo*, but it can import and automatically co-register CT (Figure 12, 13 and 14) or MRI images yielding anatomical context. No need to confine or morph your subject, structured light measurements provides clear anatomical reference for co-registration. Maximize the content extracted from preclinical models by integrating diverse imaging modalities in a single longitudinal study.

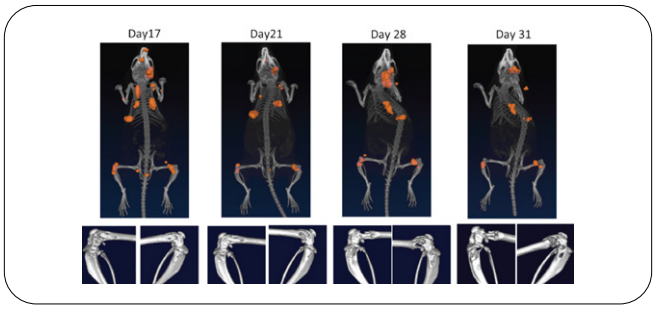



Figure 12: MDA-MB-231-luc2 bioluminescent tumors imaged on the IVIS Spectrum (orange) were co-registered with the subject's skeletal anatomy imaged with the Quantum FX microCT.

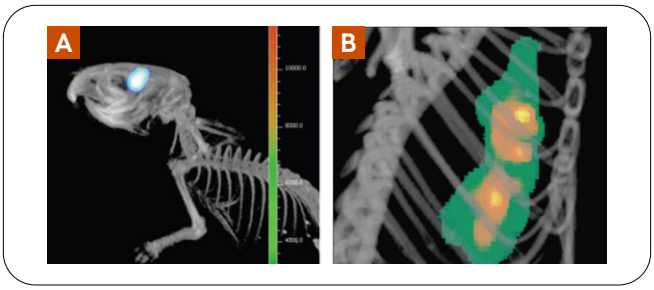



Figure 13: A) U-87MG-luc2 BLI signal from the brain co-registered with microCT. B) Streptococcus pnuemoniae Xen10 BLI signal in lungs co-registered with microCT.

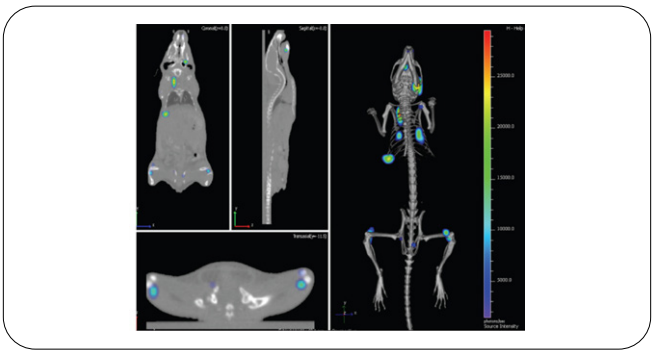


Figure 14: Optical co-registration of MDA-MB-231-luc-D3H2ln metastases with microCT.

**The Spectrum Series platform is tailored to your workflow and is available in three models: IVIS SpectrumBL, IVIS Spectrum and IVIS SpectrumCT**

| Features | IVIS SpectrumBL | IVIS Spectrum | IVIS SpectrumCT |
|---|---|---|---|
| Animal Capacity | 10 mice | 5 mice | 5 mice |
| Bioluminescence | ✓ | ✓ | ✓ |
| Fluorescence | | ✓ | ✓ |
| Full Spectral Tunability | | ✓ | ✓ |
| Epi-Illumination | | ✓ | ✓ |
| Trans-Illumination | | ✓ | ✓ |
| 3D Fluorescence Tomography | | ✓ | ✓ |
| 3D Bioluminescence Tomography | ✓ | ✓ | ✓ |
| Quantification | ✓ | ✓ | ✓ |
| Absolute Calibration | ✓ | ✓ | ✓ |
| 3D Multimodal Co-Registration (PET, CT, MRI) | ✓* | ✓ | ✓ |
| Integrated X-Ray and microCT | | | ✓ |
| Compute Pure Spectrum - Spectral Unmixing | | ✓ | ✓ |
| Optimized NIR Excitation Lightsource | N/A | Extended NIR Range 150W Tungsten EKE | |
| Detector type | | 1" Back-thinned, back-illuminated Grade 1 CCD | |
| Camera Temp | | -90 °C | |
| Imaging Pixels | | 2048 x 2048 | |
| Accessory Line | | Isolation chamber, Anesthesia, calibration tools, phantom mice, Multimodality Software and Mouse Imaging shuttle, DyCE Imaging, Multi View Imaging | |

* For bioluminescent reporters, chemiluminescent, and Cerenkov sources only

| Specifications | |
|---|---|
| **Imaging system components** | |
| Camera Sensor | Back-thinned, back-illuminated Grade 1 CCD |
| CCD Size | 2.7 x 2.7 cm |
| Imaging Pixels | 2048 x 2048 |
| Quantum Efficiency | > 85% 500 - 700 nm; > 30% 400 - 900 nm |
| Pixel Size | 13.5 microns |
| Min. Field of View (FOV) | 3.9 x 3.9 cm |
| Max. Field of View (FOV) | 23 cm x 23 cm |
| Min. Image Pixel Resolution | 20 microns |
| Lens | f/1 - f/8; 1.5 x, 2.5 x, 5 x, 8.7 x magnifications |
| Read Noise | < 3 electrons for bin = 1, 2, 4; < 5 electrons for bin = 8, 16 |
| Dark Current (Typical) | < 100 electrons/s/cm$^2$ |
| Fluor. Excitation Filter Slots | 12 |
| Fluor. Emissions Filter Slots | 24 |
| Excitation Fluorescence Filters | 10 |
| Emission Fluorescence Filters | 18 |
| Fluor. Background Subtraction Filters | Yes |
| Heated Stage | Yes |
| Diffuse Tomography Software | Yes |
| Gas Anesthesia | Yes |
| Workbench | Yes |
| CCD Operating Temperature | -90 °C |
| Imaging Chamber Interior Size | 43 x 50 x 60 cm (W x D x H) |
| Imaging System Space Requirement | 203 x 163 x 214 cm (W x D x H) |
| Power Requirements | 20 Amps for 120 VAC or 10 Amps for 230 VAC |
| Stage Temperature | 20 - 40 °C |
| Computer (Min. Specifications) | Windows 7, 4 GB RAM, nVidia Quadro 600, 250 GB and 1 TB HD, 24" widescreen LED Monitor |

For more information, please visit our website at www.revvity.com

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