DOI:
https://doi.org/10.61900/SPJVS.2025.04.18

ADVANCED IN VIVO OPTICAL IMAGING: IVIS SPECTRUM TECHNOLOGY AND ITS APPLICATIONS IN PRECLINICAL AND VETERINARY RESEARCH

Aniță Dragoș Constantin[1], Aniță Adriana Elena[1]

e-mail:adriana.anita@iuls.ro

Abstract

The IVIS Spectrum (PerkinElmer) represents a benchmark platform for non-invasive, quantitative optical imaging in small-animal models and has become an essential tool in translational biomedical and veterinary research. By integrating ultrasensitive bioluminescence and fluorescence detection with advanced spectral unmixing and three-dimensional tomographic reconstruction, the system enables longitudinal, *in vivo* monitoring of molecular and cellular processes with high spatial and temporal resolution. This review provides a comprehensive synthesis of the physical principles underlying IVIS Spectrum technology, critically evaluates its major applications in oncology, infectious diseases, inflammation, and regenerative medicine, and discusses its contribution to the implementation of the 3Rs (Replacement, Reduction, and Refinement) in animal experimentation. Particular emphasis is placed on the operational use of the IVIS Spectrum system at the ROVETEMERG Research facility, highlighting its role in complex oncology and infectious disease models relevant to veterinary medicine and the One Health paradigm.

Key words: IVIS Spectrum, *in vivo* optical imaging, bioluminescence imaging, fluorescence imaging, veterinary research, preclinical models

1. FUNDAMENTAL PRINCIPLES OF IVIS SPECTRUM TECHNOLOGY

The IVIS Spectrum system is designed for the detection of extremely low-intensity visible and near-infrared photon emission originating from within living tissues. Its architecture combines high-performance optoelectronic hardware with advanced computational algorithms, enabling sensitive, quantitative, and reproducible *in vivo* imaging.

1.1 DETECTION HARDWARE AND SENSITIVITY

At the core of the IVIS Spectrum platform is a back-thinned, back-illuminated charge-coupled device (CCD) camera, thermoelectrically cooled to approximately −90 °C. This deep cooling substantially reduces dark current and thermal noise, thereby maximizing the signal-to-noise ratio and enabling near single-photon detection. The system supports multiple fields of view (FOV), typically ranging from approximately 3.9 cm to 23 cm, allowing flexible experimental configurations from high-resolution single-animal imaging to high-throughput imaging of multiple animals simultaneously. These hardware features form the basis for absolute quantification of emitted light, reported as radiance (photons/s/cm²/sr), independent of acquisition parameters such as exposure time or binning (Rice et al., 2001; Xenogen IVIS Spectrum Documentation).

1.2 BIOLUMINESCENCE IMAGING (BLI)

Bioluminescence imaging relies on enzymatic light production resulting from the oxidation of a substrate by a luciferase enzyme. Commonly employed systems include firefly luciferase (luc/luc2), *Renilla luciferase*, and bacterial luciferases encoded by the *lux* operon. Firefly luciferase remains the most widely used reporter for mammalian models due to its red-shifted emission spectrum (approximately 560–610 nm), which provides improved tissue penetration compared with blue-emitting reporters. Because mammalian tissues do not generate endogenous bioluminescence, background signal is negligible, conferring exceptional sensitivity and making BLI particularly suitable for longitudinal monitoring of viable cells, tumor burden, and pathogen dissemination (Close et al., 2011; O'Neill et al., 2009).

[1] Regional Center of Advanced Research for Emerging Diseases, Zoonoses and Food Safety, Faculty of Veterinary Medicine, „Ion Ionescu de la Brad" Iași University of Life Sciences, Romania

1.3 FLUORESCENCE IMAGING AND ILLUMINATION MODES

Fluorescence imaging requires external excitation of fluorophores followed by detection of emitted photons. The IVIS Spectrum uniquely supports both epi-illumination and trans-illumination modes (Fig. 1). Epi-illumination, in which excitation light is delivered from above the animal, is optimal for superficial or subcutaneous targets. In contrast, trans-illumination delivers excitation light from below the imaging stage, allowing photons to traverse deeper tissues and enabling fluorescence molecular tomography (FMT). This dual-illumination capability significantly enhances depth localization and quantitative accuracy in deep-seated targets (Koba et al., 2011).

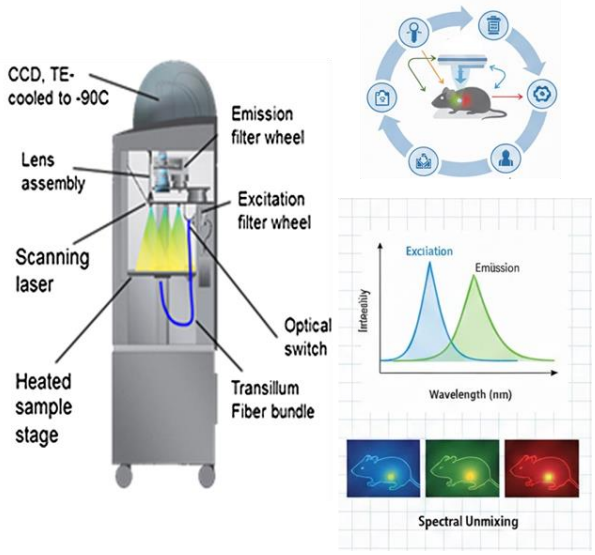

Figure 1. Schematic representation of the IVIS Spectrum Imaging System technology

1.4 SPECTRAL UNMIXING AND MULTIPLEXING

A major challenge in fluorescence imaging is tissue autofluorescence and spectral overlap between reporters. The IVIS Spectrum addresses these limitations through multispectral acquisition using a comprehensive filter set spanning approximately 430–850 nm. Spectral unmixing algorithms implemented in Living Image® software mathematically separate specific reporter emissions from autofluorescence and allow simultaneous resolution of multiple fluorophores or reporters within the same animal (Living Image® Software Manual, 2017). This capability is critical for complex experimental designs involving multiplexed reporters or activatable probes.

1.5 ABSOLUTE QUANTIFICATION AND DATA REPRODUCIBILITY

Unlike arbitrary photon counts, IVIS Spectrum data are expressed as absolute radiance values, enabling direct comparison across time points, experiments, and research centers. This feature is particularly important for longitudinal studies and multi-site collaborations, where standardization and reproducibility are essential (Weissleder, 2001).

2. PRECLINICAL AND VETERINARY APPLICATIONS

Optical imaging using the IVIS Spectrum has become a cornerstone technology in veterinary and translational research, enabling dynamic, non-invasive interrogation of disease processes *in vivo*.

2.1 ONCOLOGY AND TUMOR BIOLOGY

In oncology research, IVIS-based imaging is extensively used to monitor tumor initiation, growth, metastasis, and therapeutic response. Bioluminescent tumor models, including mammary carcinoma (e.g., 4T1) and orthotopic glioblastoma xenografts, allow sensitive quantification of viable tumor burden over time. Changes in bioluminescent signal often precede anatomical alterations detectable by conventional imaging, providing an early indicator of treatment efficacy (Frenster and Placantonakis, 2018; Lim et al., 2009).

2.2 INFECTIOUS DISEASES AND VIROLOGY

Because luciferase signals can be monitored non-invasively, the same animals can be imaged repeatedly, enabling longitudinal studies in individual mice over time. Furthermore, three-dimensional image reconstruction can be performed, providing detailed information on the anatomical localization of the molecular reporters. Reporter-labeled bacteria and viruses have transformed the study of infectious diseases by enabling real-time visualization of pathogen dissemination within the same living host. IVIS imaging has been successfully applied to bacterial infections using *lux*-expressing strains, as well as to viral pathogenesis studies involving neuroinvasive and hemorrhagic viruses. Notably, IVIS-based detection can reveal viral spread and tissue tropism days before the onset of clinical signs, offering unique insights into host–pathogen interactions (Doyle et al., 2004; Hutchens & Luker, 2007; Poussard et al., 2012; Davies et al., 2023).

2.3 INFLAMMATION AND TOXICOLOGY

Inflammatory responses and drug-induced toxicities can be non-invasively monitored *in vivo* using IVIS imaging, which combines bioluminescence, such as luciferase reporters, with near-infrared fluorescence and activatable probes

like luminol. Luminol, for example, reports myeloperoxidase (MPO) activity associated with neutrophil infiltration and has been validated in models of uveitis, acute liver injury, and systemic inflammation, providing a sensitive, quantitative measure of inflammatory burden over time (Gutowski et al., 2017; Sirbu et al., 2019). This platform enables longitudinal tracking of immune cell dynamics, oxidative stress, and cell death, while also supporting toxicological studies by detecting early signs of drug-induced toxicity, assessing pharmacokinetics, and evaluating mechanisms such as protease activity, tissue calcification, or impaired physiological functions like glomerular filtration (Peterson, 2021). High-throughput imaging further allows simultaneous analysis of multiple animals, facilitating rapid comparison of compounds, doses, or experimental conditions and providing an integrated framework to study both disease progression and therapeutic effects *in vivo*.

2.4 STEM CELL AND REGENERATIVE MEDICINE

In regenerative medicine, IVIS imaging enables longitudinal tracking of transplanted stem or progenitor cells, providing critical information on their survival, proliferation, migration, and engraftment. Seminal studies using luciferase-expressing embryonic and hematopoietic stem cells have demonstrated the capacity to identify functional niches and assess therapeutic outcomes *in vivo* (Cao et al., 2006; Xie et al., 2009). Longitudinal assessment of engraftment and survival is essential for determining therapeutic persistence and efficacy, while correlating imaging signals with functional readouts allows evaluation of intended effects, such as tissue regeneration or inflammation reduction. This approach also supports safety monitoring by detecting abnormal proliferation or potential tumorigenicity, particularly in genetically modified cells. Moreover, high-throughput imaging enables simultaneous analysis of multiple subjects, thereby accelerating comparative studies across cell types, doses, or experimental conditions (Hong et al., 2023).

3. TECHNICAL CONSIDERATIONS AND THE 3RS PRINCIPLE

3.1 ADVANTAGES

The IVIS imaging platform combines high sensitivity and versatility, detecting both bioluminescent reporters (e.g., luciferase) and fluorescent signals (e.g., GFP, near-infrared) with red-shifted probes enabling deep-tissue imaging. High-throughput capabilities allow simultaneous imaging of multiple animals, while spectral unmixing and a broad filter range (430–850 nm) facilitate multiplexed detection and reduce autofluorescence. Its non-invasive nature permits repeated measurements in the same animal, minimizing stress, reducing animal numbers, and enhancing statistical power, thereby aligning with the principles of Reduction and Refinement in preclinical research. Furthermore, the absence of surgical procedures minimizes animal stress and improves welfare.

3.2 LIMITATIONS

Despite its versatility and widespread use, optical imaging is subject to several tissue- and substrate-related limitations that can affect data quality and interpretation. Light scattering and absorption, particularly by hemoglobin and water, restrict tissue penetration, making deep organs or structures challenging to visualize, especially at visible wavelengths. Endogenous tissue autofluorescence can further interfere with signal detection, although spectral unmixing capabilities, such as those offered by the IVIS Spectrum, can partially mitigate this issue. Animal pigmentation also poses a significant challenge: dark fur, as in C57BL/6 mice, absorbs light and reduces signal-to-noise ratios, often necessitating shaving or the use of albino strains, which can in turn alter skin pigmentation and complicate imaging. In addition, substrate pharmacokinetics—such as luciferin biodistribution and blood–brain barrier penetration—must be carefully controlled to ensure reproducible quantification (Rice et al., 2001).

4. IMPLEMENTATION OF IVIS SPECTRUM AT THE ROVETEMERG RESEARCH PLATFORM

The IVIS Spectrum (Fig.2) system installed at the ROVETEMERG Research Facility from Iași University of Life Sciences (IULS) constitutes a central component of its advanced preclinical imaging infrastructure. Operating under controlled experimental conditions, the system supports high-impact translational research in oncology and infectious diseases. Standardized imaging protocols based on luc2-expressing tumor cells enable precise longitudinal monitoring of tumor progression.

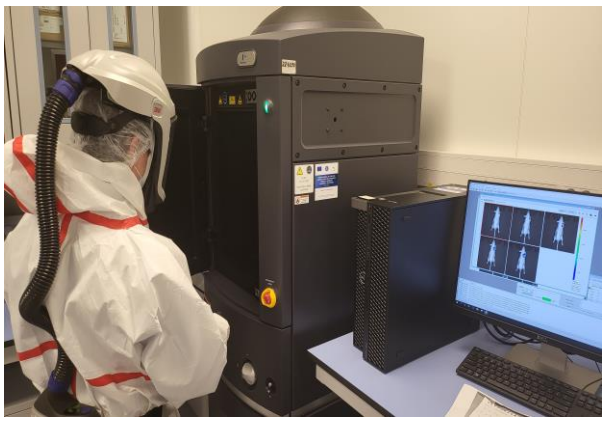

Figure 2. IVIS Spectrum optical imaging system installed at the ROVETEMERG research facility. The system enables non-invasive bioluminescence and fluorescence imaging of small-animal models under controlled experimental conditions, supporting longitudinal studies in veterinary and translational research.

Quantitative analyses are performed using Living Image® software (Fig.3), with regions of interest (ROI) placed over anatomically defined areas and acquisitions standardized at peak signal intensity, typically 15–20 minutes after luciferin administration. This approach ensures sensitive, reproducible assessment of therapeutic efficacy and disease dynamics *in vivo.*

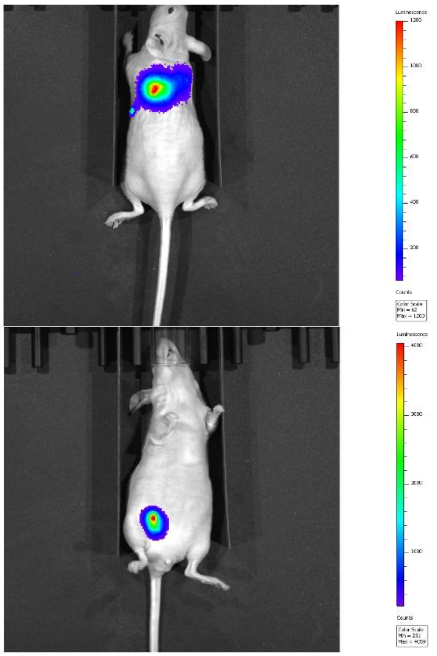

Figure 3. Representative *in vivo* bioluminescence imaging of luciferase-expressing cells in a murine model. Color-coded photon emission maps illustrate spatial distribution and intensity of the bioluminescent signal, quantified as radiance (photons/s/cm²/sr).

5. FUTURE PERSPECTIVES AND TRANSLATIONAL RELEVANCE

Within veterinary research and the broader One Health framework, IVIS Spectrum technology offers a powerful platform for studying zoonotic pathogens, comparative oncology, and host–pathogen interactions across species. Future developments are expected to focus on multimodal imaging integration, combining optical imaging with CT or MRI to provide complementary molecular and anatomical information. Advances in near-infrared fluorophores and activatable probes promise improved tissue penetration and specificity, further enhancing the translational relevance of IVIS-based imaging from preclinical models to clinical and veterinary applications.

REFERENCES

Baban, C.K., Cronin, M., Akin, A.R., et al. (2012). Bioluminescent bacterial imaging in vivo. *Journal of Visualized Experiments.* (69):e4318

Close, D.M., Xu, T., Sayler, G.S., Ripp S. (2011). In vivo bioluminescent imaging (BLI): noninvasive visualization and interrogation of biological processes in living animals. *Sensors (Basel)*.11(1):180-206.

Davies, K.A., Welch, S.R., Jain, S., et al. (2023) Fluorescent and Bioluminescent Reporter Mouse-Adapted Ebola Viruses Maintain Pathogenicity and Can Be Visualized in Vivo. *The Journal of infectious diseases*. 228(Suppl 7):S536-S547

Doyle, T.C., Burns, S.M., Contag, C.H. (2004). In vivo bioluminescence imaging for integrated studies of infection. *Cellular Microbiology*, 6, 303–317

Frenster, J.D., Placantonakis, D.G. (2018). Bioluminescent in vivo imaging of orthotopic glioblastoma xenografts in mice. *Methods in Molecular Biology*, 1741, 217–228.

Gao, C., Zhou, Y., Chen, Z., Li, H., Xiao, Y., Hao, W., Zhu, Y., Vong, C.T., Farag, M.A., Wang, Y., Wang, S. (2022). Turmeric-derived nanovesicles as novel nanobiologics for targeted therapy of ulcerative colitis. *Theranostics,* 12(12), 5596-5614. https://doi.org/10.7150/thno.73650.

Gupta, A., Sircombe, K. J., Stevenson, J. D., Wardell, S. J. T., & Pletzer, D. (2025). Protocol for a murine skin abscess model to study bacterial infection dynamics, microbial interactions, and treatment efficacy. *STAR protocols*, 7(1), 104291.

Gutowski, M. B., et al. (2017). In vivo bioluminescence imaging for longitudinal monitoring of inflammation in animal models of uveitis. *Investigative Ophthalmology & Visual Science*, 58, 594–602.

Hong, S., Lee, D.S., Bae, G.W., Jeon, J., Kim, H.K., Rhee, S., Jung, K.O. (2023). *In Vivo* Stem Cell Imaging Principles and Applications. *International journal of stem cells*. 16(4):363-375.

Hutchens, M., & Luker, G. D. (2007). Applications of bioluminescence imaging to the study of infectious diseases. *Cellular Microbiology*, 9, 2315–2322.

Koba, W., Kim, K., Lipton, M.L., et al. (2011). Imaging devices for use in small animals. *Seminars in Nuclear Medicine*, 41(3), 151–165.

Weissleder, R. (2001). A clearer vision for in vivo imaging. *Nature Biotechnology*, 19, 316–317.

Rice, B.W., Cable, M.D., Nelson, M.B. (2001). In vivo imaging of light-emitting probes. Journal of Biomedical Optics, 6, 432–440.

Lim, E., Modi, K.D., Kim, J. (2009). In vivo bioluminescent imaging of ma mary tumors using IVIS Spectrum. *Journal of Visualized Experiments*. (26):1210.

Living Image® 4.5.4 Software Manual. (2017). *PerkinElmer Health Sciences*.

Munteanu, R. A., Tigu, A. B., Feder, R., Tatar, A. S., Gulei, D., Tomuleasa, C., & Boca, S. (2024). *In vivo* imaging system (IVIS) therapeutic assessment of tyrosine kinase inhibitor-loaded gold nanocarriers for acute myeloid leukemia: a pilot study. *Frontiers in pharmacology*, *15*, 1382399.

O'Neill, K., et al. (**2009**). Bioluminescent imaging: a critical tool in pre-clinical oncology research. *The Journal of Pathology*, 220, 317–327.

Peterson, J.F. (2021). Chapter 25 - Noninvasive in vivo optical imaging models for safety and toxicity testing, Editor(s): Ramesh C. Gupta, Rajiv Lall, Ajay Srivastava, Nutraceuticals (Second Edition),Academic Press, 363-377, ISBN 9780128210383,

Poussard, A., Patterson M., Taylor K., et al (2012). In vivo imaging systems (IVIS) detection of a neuro-invasive encephalitic virus. *Journal of Visualized Experiments.*

Sirbu, D., et al. (2019). Enhanced in-vivo optical imaging of the inflammatory response to acute liver injury using near-infrared dyes.

Xenogen IVIS Spectrum Noninvasive Quantitative Molecular Imaging System. *Technical Documentation.*

Xie, Y., et al. (2009). Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature*, 457, 97–101.

.

.