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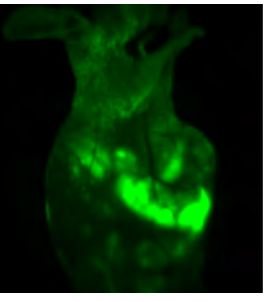
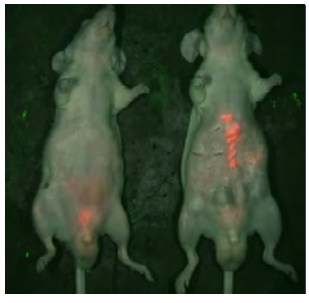
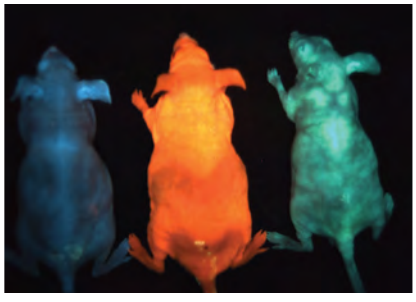
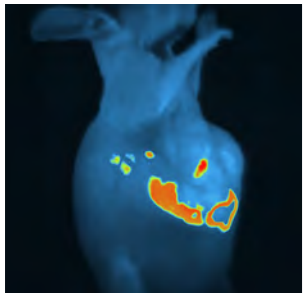
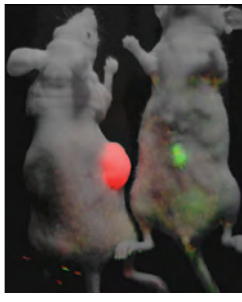
GETTING THE INSIDE STORY: IN VIVO IMAGING

UVP iBox® Imagers

In Vivo Imaging

Bioluminescence and fluorescence in vivo applications

Whole Animal In Vivo Imaging Systems

- Cancer research
- Tumor studies
- Heart disease
- Near IR
- Metastasis
- Immunology
- Small animal imaging
- Bacterial/viral infections
- Gene expression
- Nanomedicine
- Inflammation
- Agriculture

Website: http://us.analytik-jena.com
info@us.analytik-jena.com

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Reach and Flexibility: In Vivo Imaging with the iBox®

Biological organisms are highly complex, with even normal functions governed by underlying mechanisms involving interplay between multiple elaborate systems. Historically, researchers have been forced to investigate organismal function and malfunction by developing in vitro experimental models designed to mimic one mechanism or system. While in vitro work, bit by bit, has greatly improved our understanding of biology, it has been generally unable to capture the intricate interplay so vital to regulating function and homeostasis.

Animal models have been used for decades in modern biological research, but for the vast majority of that time, organ-, cellular-, and molecular-level data could only be obtained postmortem. This kept researchers in the dark as to whether they were observing the beginning, middle, or end of a process, as well as what transpired before and what would have transpired after. In vivo imaging represents a solution to that uncertainty. Made vastly more accessible by significant technological advances in recent years, in vivo imaging gives scientists the ability to bridge the gap between the petri dish and the animal model.[1,2]

Reach: Seeing the Unseen

One of the major obstacles facing in vivo imaging has been signal detection and resolution. In contrast to in vitro imaging, where emitted light signal can be focused towards the detector and reach it relatively unimpeded, in vivo signal scatters due to the longer distances between emitter and detector, as well as the three-dimensional nature of imaging an organism. Moreover, this already diminished signal can be further blocked, distorted, or absorbed by impediments such as other organs, fluids, skin, and hair.[3] The decreased signal magnitude makes it difficult to obtain detailed spatial signal distribution information beyond tissue level.

Modern imagers—with more sensitive detectors and larger repertoires of compatible probes—have largely overcome detection issues. Combined with in vivo surgical techniques to create skin flaps to peer deep into animals at high resolutions, instruments such as the UVP iBox Explorer$^{2}_{TM}$ are capable of cellular-level spatial resolution without sacrificing whole-body imaging, meaning that researchers can use a single instrument to reveal cellular behaviors in their natural environments and correlate them to systemic or whole-body responses.

Flexibility: Keeping Up with Creativity

Historically, in vitro assays have outpaced in vivo imaging complexity and data output—immunofluorochemistry and flow cytometry can readily multiplex over a dozen different markers. However, several improvements in experimentation and technology have narrowed the gap.

Probe wavelength range as well as imager detection capabilities frequently hampered researchers in the past. Now, technology has expanded the researcher's toolbox. Modern imagers can detect light wavelengths ranging from violet to infrared, giving scientists more room to maneuver when it comes to minimizing spectral overlap.

The iBox Scientia^{TM} takes it one step further and can multiplex, making it possible to observe discrete cell-cell interactions and protein-expression dynamics—in real time and in the physiological environment. In addition to possessing extensive fluorescent and near-infrared (NIR) imaging capabilities, the iBox Scientia can perform bioluminescent imaging. Thus, providing unparalleled flexibility for the researcher.

Simplifying and Streamlining: Improving Throughput and Accessibility

Standard protocols dictate that in vivo imaging be performed on a small number of animals at a time, which, when combined with the time spent preparing each animal one at a time prior to imaging, increases hands-on time beyond what is reasonable for the amount of data collected. Instruments such as the UVP iBox Studio and Scientia possess fields of view large enough to accommodate the simultaneous imaging of multiple animals (up to three at a time for the Studio and five at a time for the Scientia). Combining multi-animal with multi-probe imaging has exponentially increased the throughput potential of in vivo imaging.

Historically, in vivo imaging has typically required a considerable financial commitment, especially in light of in vitro alternatives. In response, manufacturers have developed compact imager models such as the UVP iBox Studio. While the Studio lacks the cell-level resolution of the Explorer^{2} or the bioluminescent capabilities of the Scientia, it still offers high sensitivity fluorescent imaging capabilities at a cost roughly half that of its iBox counterparts, making it ideal for researchers just starting to explore in vivo imaging or those using in vivo imaging as a complement to their other established research techniques.

For references, please see page 7.

Transgenic mice expressing fluorescent proteins.[2,4] Orthotopically implanted tumors are designed to express a different color than the transgenic mouse, enabling the differentiation between tumor and normal tissue. Pseudo-color composite, AnalytikJena iBox Scientia (AntiCancer, Inc.)

Illuminating Answers: Fluorescence vs. Bioluminescence Imaging

Fluorescent and bioluminescent light sources are popular choices in the life sciences to identify targeted molecules. While it can be convenient to think of them as interchangeable–"a light is a light"–there are clear differences in the two modalities that researchers must account for when alternating between them or using both concurrently.

Harnessing Nature: Bioluminescence for Imaging Biological Mechanisms

Broadly speaking, bioluminescence refers to light produced and emitted by a living organism. This light is typically produced by a lucigenic chemical reaction and should be considered separate from biofluorescence, which is the presence of an endogenously lucigenic molecule or protein (e.g., GFP). These lucigenic reactions are usually catalyzed by specific enzymes (e.g., luciferase converts luciferin to oxyluciferin). To harness this technology, researchers generate transgenic cells expressing these catalytic enzymes and introduce them into animals. Alternatively, transgenes can be introduced directly into the animal, under which expression of the transgene is controlled by host machinery. In either case, light production follows the addition of an enzyme-specific substrate.[1]

Because bioluminescent reactions require multiple components to interact, they produce a highly specific and quantifiable signal with minimal background interference.[2] They also allow researchers to design a lock-and-key style experiment where the luminescent substrate can be attached to one element and the enzyme attached to another. Here, similar to FRET, light will only be produced when the two elements interact and bring the substrate into proximity with the enzyme, allowing scientists to monitor not only the presence and abundance of a target molecule, but also its activity.

Bioluminescent signal is advantageous for deep-tissue imaging, with penetration depths of several centimeters.[1] Since the signal is endogenously generated, researchers need not worry about lot-to-lot variability in signal or probe accessibility differences when performing repeated imaging. However, developing a model suitable for bioluminescence imaging requires more effort and time than simply exogenously applying a fluorescent probe. Researchers must also keep in mind that the lucigenic reactions are subject to chemical and enzyme kinetics, and the presence of co-factors, inhibitors, and other modulatory factors.

Tracking Solutions: Fluorescence for Locating Specific Markers

Imaging in vivo with fluorescent tags enables fast capture high-resolution acquisition. Fluorescent images can be acquired using millisecond exposure times, in contrast to bioluminescence which requires up to 30 minutes or longer. Another important difference is that because of the long exposure and low noise requirements, bioluminescent cameras are more sophisticated, more highly cooled, and thus more expensive.

Fluorescence is emitted by substances (fluorophores) that have already become excited by absorbing light. Unique excitation and emission wavelength spectra allow different fluorophores to be easily distinguished from one another, thus allowing researchers to probe dozens of different signals in a single experiment. This has greatly contributed to the ever-growing popularity of fluorescent imaging for both in vitro and in vivo applications.

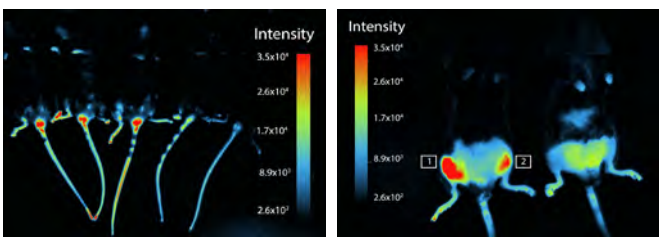
For in vivo imaging, fluorophores can be produced endogenously or exogenously introduced. The former entails using genetic engineering to facilitate inducible production of fluorescent proteins in target cell types, while the latter involves introducing fluorophore-conjugated antibodies or nanoparticles to target specific molecules (and cells) of interest. In both cases, fluorophore signal is generally stable over prolonged periods of time, allowing for more logistical flexibility for the researcher.

However, fluorescent signal is typically noisier than bioluminescent signal, since light scatters as it exits the body. Confounding autofluorescence can also be a significant problem, as many key proteins abundantly expressed within in vivo models (e.g., NADPH, collagen, riboflavin, folic acid) are naturally fluorescent. The recent shift in favor of near-infrared (NIR) imaging has been made with the aforementioned factors in consideration. Very few endogenous molecules within in vivo models emit fluorescence in the NIR spectrum,[3] and NIR light scatters less as it exits the body, permitting greater imaging penetrability.[4]

Why Not Both? Combining Bioluminescence and Fluorescence

Bioluminescence and fluorescence clearly both have their strengths and weaknesses. Rather than accept this trade-off and select one or the other, researchers are now seeking to combine the two modalities in order to use the strengths of one to offset the weaknesses of the other.[2,5] Performing in vivo imaging using both modalities helps to confirm the veracity of the data individually generated from each and expands the potential scope, breadth, and depth of a given study.

For references, please see page 7.



(Left) Wild-type black mice expressing luciferase after luciferin was injected via tail vein to generate bioluminescent signal. *(Right)* Ai9 mice expressing TdTomato, a red fluorescent protein variant, after an intramuscular injection. Region 1 is the positive control with region 2 showing the formulation under investigation. Both bioluminescent and florescence images were taken using the iBox Scientia 900 by Ali Jazayeri, VIR Biotechnology (2018).

Let's Get Physiological: In Vivo Imaging Applications

The ability to visualize phenomena within their natural physiological and/or pathological environments and the ability to facilitate repeated sampling within the same experimental animal at different time points are the two main advantages of in vivo imaging. These make the modality well suited for research applications concerning disease pathogenesis and progression, natural response mechanisms, and the development of novel treatment agents and/or methodologies.

Shedding a Light on Cancer: Imaging Tumor Progression and Treatment

Arguably, in vivo imaging is most popularly used in cancer research. Cancer is a very individual-specific disease and, while there are certainly commonalities among tumors in a specific organ or cell, key mechanisms such as metastasis and apoptosis are heavily dependent on how the tumor interacts with the surrounding tumor environment. In vivo imaging not only allows researchers to monitor tumor progression over time in its natural environment without inter-individual variability, it also allows them to evaluate the effectiveness of anticancer mechanisms. Technology is progressing in a direction where researchers, through long-term imaging studies, will be able to track an individual cell and phenotypic shifts within a tumor over time.[1]

Developing clinically relevant strategies for personalized cancer treatments is critical, and patient-derived orthotopic xenografts (PDOX)—where a patient tumor is implanted in the same location in the test mouse as it originally came from in the patient—are key to this. This ensures that cancer progression in the mouse mimics that seen in the patient, providing a test bed for specialized antimetastatic treatments customized for that particular patient.[2-4] Fluorescently tagged tumor cells provide an effective strategy for tracking the effectiveness of antimetastatic therapies.

First Responders: Imaging the Immune System

Given that immune responses and the cells that govern them can change with remarkable rapidity, the discovery of numerous immune cell subtypes and sub-subtypes using in vitro methods has generated more questions. In vivo imagers are now able to identify and track individual cells, allowing researchers to observe the macro (e.g., immune response activation) and the micro (e.g., the presence/absence of specialized cell subsets at a given site of immune activation) using a single instrument.

The Heart of the Matter: Imaging for Cardiovascular Research

Cardiovascular research has relied on anatomical parameters (e.g., ventricular wall thickness) rather than the underlying mechanisms underpinning abnormal pathophysiologies. While ultrasound remains the gold standard for visualizing cardiac anatomy, fluorescent and bioluminescent in vivo imaging can be used to investigate key phenomena. These include postcardiac injury inflammation and scar formation,[5] atherosclerotic plaque development,[6] cardiomyocyte electrical signal propagation,[7] and leukocyte mobilization/adhesion/extravasation.[8] The capacity of in vivo imagers to perform repeated imaging in the same animal is critical here, as cardiovascular physiology differs significantly between individuals, even in inbred mouse strains.[9]

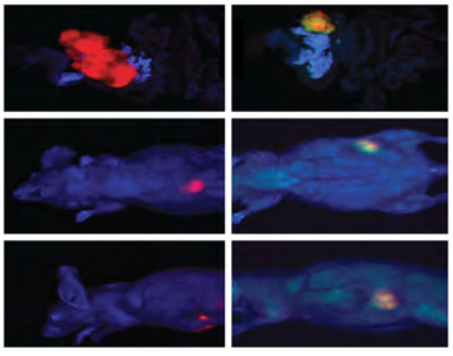
Payload Delivery: Imaging for Therapeutic Discovery

The success of any treatment method hinges upon the ability of the agent to reach its intended site, and in vivo imaging can be used to determine this, whether directly through linking a tracer with the agent in question (e.g., nanoparticle delivery systems) or indirectly by evaluating morphological changes post-treatment (e.g., measuring tumor extent). In addition, in vivo imaging is used to examine agent distribution patterns in the body, evaluating bioavailability, identifying potential unintended accumulation sites, and monitoring clearance kinetics. There are numerous examples of monitoring nanoparticle delivery, including tagged lipid nanoparticles targeting tumors.[10]

Getting the Whole Picture: Inter-System Interactions

Ultimately, pathology does not exist in a vacuum, but rather arises as a consequence of interactions between multiple systems. For example, the immune system plays a key role in both regulating and accelerating tumor development, as well as mediating cardiovascular injury extent. By observing pathophysiological phenomena in their natural environments, in vivo imaging allows researchers to see the big picture—to not only see singular pathogenic mechanisms, but also to see how the body responds to these homeostatic alterations and identify potential therapeutic methods based on weaknesses in endogenous responses.

For references, please see page 7.

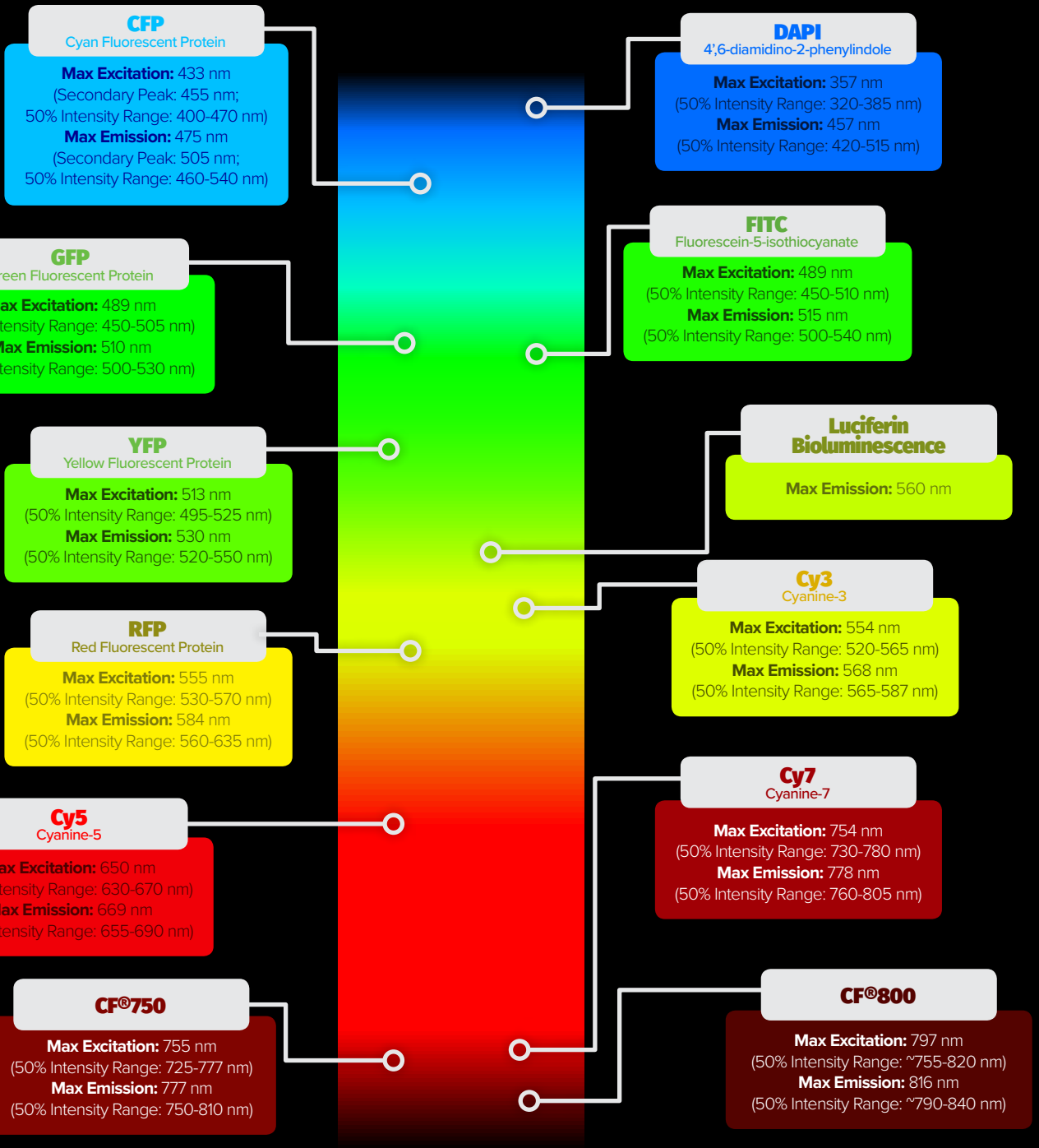


Orthotopically implanted pancreatic cancer tumors in a CFP nude mouse. The red fluorescent tumor is readily evident in contrast to the blue background of the whole animal. Dual-color tumor cells with green nuclei and red cytoplasm appear yellow and are also readily detected against the blue "normal" background of the CFP mouse.[11] AnalytikJena iBox Scientia.

SPECTRALLY CLEAR

OPTIMIZING MULTIPLEX IN VIVO IMAGING

When planning an imaging experiment, it can be tempting to think that a probe only emits light at a single specific wavelength. However, the reality is that both fluorescent and bioluminescent probes produce light across a range of wavelengths, making spectral overlap a significant issue. For in vivo imaging, which is already subject to more confounding factors than in vitro modalities, minimalizing spectral overlap during multiplex experiments is key to optimizing signal intensity.



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Article 2 - Illuminating Answers: Fluorescence vs. Bioluminescence Imaging

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Article 3 - Getting Physiological: In Vivo Imaging Applications

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UVP iBox® Studio

In Vivo Fluorescence Imaging

- Sophistication of in vivo imaging, now available at prices within your budget
- Optimize your application by choosing between two high performance cooled camera options
- Included warming plate for up to 3 mice on an ergonomic slide out tray
- Multiple illumination sources – RGBW LEDs and NIR lasers, making the system extremely versatile
- Automated control of the five-position emission filter array to allow addition of emissions for visible, IR and UV to meet all spectral requirements
- Unlimited selection of filters enables users to image in the fluorescent, visible and NIR ranges for multiple applications (RFP/GFP included, others optional)
- Small footprint and compact form maximize the use of laboratory bench space
- Optional UVP anesthesia kit for immobilizing small animals

UVP iBox® Explorer^2

In Vivo Imaging Microscope

- Magnification ranges of 0.17x - 16.5x enables easy transition from the macroscopic to the microscope scale
- Ability to image organs and cells subcutaneously and within the body cavity of living mice
- Optical configurations are parcentered and parfocal, allowing seamless imaging through the magnification ranges
- Leading-edge high frame rate cooled color camera enables quick detection, image capture and high throughput
- Bright illumination of samples with the UVP eLITE produces an intense fluorescent signal and fast exposure times
- Unlimited selection of filters enables users to image in the fluorescent, visible and NIR ranges for multiple applications (RFP/GFP included, others optional)
- Optional UVP anesthesia kit for immobilizing small animals

UVP iBox® Scientia

In Vivo Bioluminescence Imaging

- Selection of high resolution, high sensitivity CCD cameras for fluorescence and bioluminescence in vivo imaging
- Motorized optics for setting the aperture, zoom and focus
- Power lift is adjustable to any setting in a ten inch travel range; Automated calibrating home position synchronizes the power lift at start-up
- Bright illumination of samples with the UVP eLITE produces an intense fluorescent signal and fast exposure times
- Automated control of the five-position emission filter array to allow addition of emissions for visible, IR and UV to meet all spectral requirements
- Unlimited selection of filters enables users to image in the fluorescent, visible and NIR ranges for multiple applications (RFP/GFP included, others optional)
- Optional UVP anesthesia kit for immobilizing small animals

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