TECHNICAL NOTE

Smart Tray accessories for higher throughput optical imaging on the IVIS Spectrum 2 and IVIS SpectrumCT 2.

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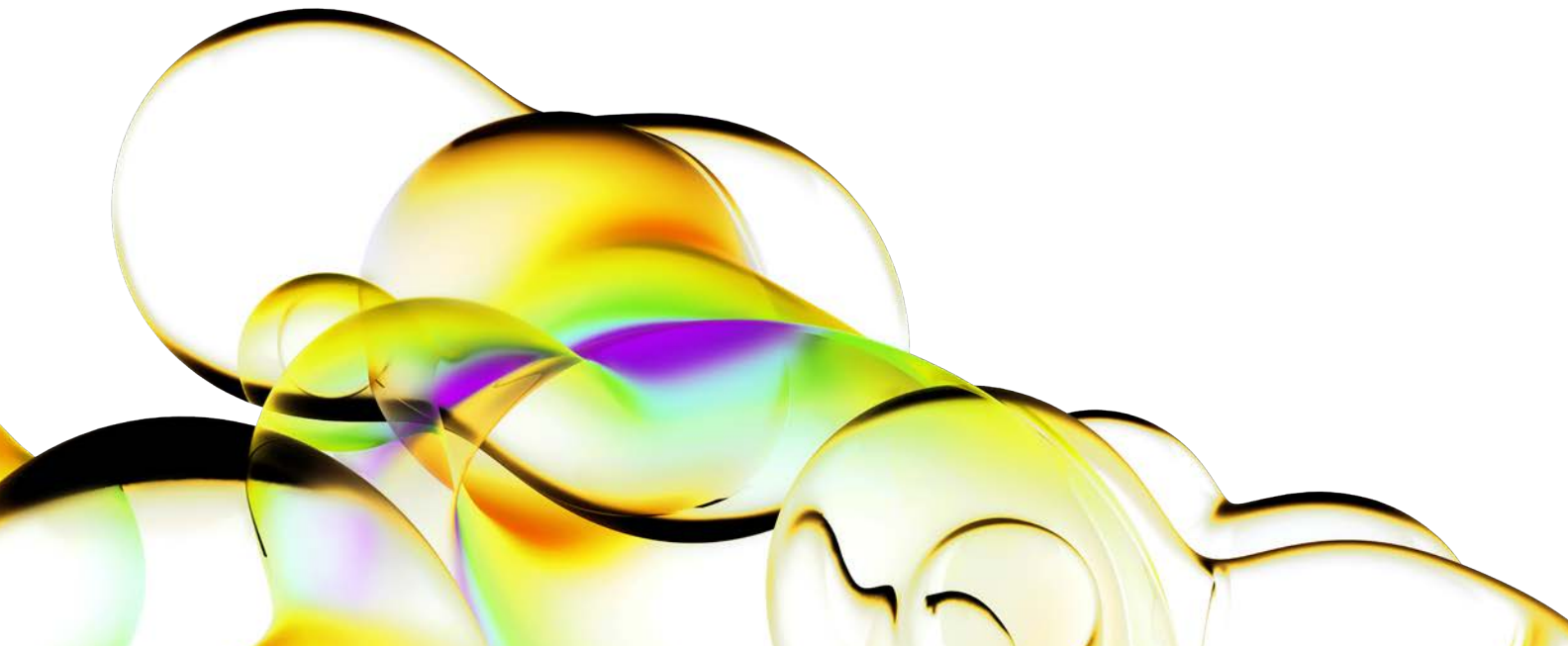
Abstract

The IVIS™ Spectrum 2 and SpectrumCT 2 in vivo optical imaging systems incorporate a CCD camera with eXcelon® coating that provides improved detection of optical signal across a broader spectrum of wavelengths. These systems integrate both 2D and 3D bioluminescence and fluorescence imaging, as well as high performance spectral unmixing and intuitive visualization and analysis software.

The large field of view of these imaging systems is now paired with Smart Tray accessories, including two 5-mouse Smart Tray manifold systems with a benchtop docking/anesthesia posing station. This is designed to increase through-put and quality of in vivo imaging of disease progression and therapeutic intervention across a wide range of disease models.

This technical note focuses on the benefits of the Smart Tray system and benchtop posing station, by offering easier positioning of the animals and more rapid imaging as compared to the use of standard 5-mouse and 10-mouse manifold strategies.



The Smart Tray system uses two 5-mouse trays (SMT-5) that magnetically snap into a benchtop docking station for delivery of anesthesia and warming as well as full view and easy access to each animal for optimal positioning. The SMT-5 trays are designed with ease of use and user safety in mind with carefully-designed nose "bays", and calibrated scavenging, to minimize isoflurane leakage.

Once animals are positioned properly, tray 1 is disconnected from the posing station and magnetically locked into the dock inside the IVIS Spectrum 2 or IVIS SpectrumCT 2. While the animals on tray 1 are being imaged, preparation of the next set of mice can be performed in parallel by docking tray 2 onto the benchtop posing station.

Comparison of a standard 5-mouse manifold, an expanded 10-mouse manifold, and the SMT-5 was performed to test the speed and workflow in imaging a 30-mouse study with 1 or 2 users. Testing showed significant speed enhancement and ease of use for fluorescence and bioluminescence imaging in comparison to the two conventional manifolds.

Materials and methods

Fluorescence imaging agents

The fluorescent probe IVISense™ Integrin Receptor 750 (Revvity), was used to image tumors in mice under indicated experimental conditions. The imaging dose for this probe was as recommended in the product insert (2 nmol/25 g mouse).

Animal diet

Mice were fed ad libitum with alfalfa-free chow from Harlan Laboratories (Teklad Global Diet 2019X) to reduce food fluorescence in the stomach and intestines.

Tumor model

Six- to eight- week-old nu/nu mice were obtained from Charles River Laboratory (Wilmington, MA). Experimental procedures on laboratory animals were performed in accordance with Revvity IACUC guidelines. IVISbrite™ 4T1 Red Fluc bioluminescent mouse breast adenocarcinoma cells (Revvity) were injected (1 x 10^6 cells) subcutaneously (s.c.) into the mouse flanks to initiate tumor growth.

2D bioluminescence imaging (2D BLI)

Images were acquired using the IVIS Spectrum 2 and data was analyzed using Living Image® software (v4.8). For 2D BLI imaging, the system acquired images without any emission filter (open) to maximize sensitivity and to improve detection limit. To induce bioluminescence signal, IVISbrite D-Luciferin potassium salt (Revvity) was injected intraperitoneally (i.p.) 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. BLI images were acquired 10 minutes post D-luciferin injection to achieve optimal luminescence output.

2D fluorescence imaging (2D FLI)

One day prior to imaging, the fluorescent probe, as described, was injected intravenously (i.v.) to generate targeted fluorescent contrast. For 2D epifluorescence imaging, a single pair of excitation and emission filters were used in alignment with the fluorescent agent's optimal ex/em wavelengths. The filter pairs for IVISense Integrin Receptor 750 are 745/800 (ex/em).

Figure 1: IVIS Spectrum 2 and SpectrumCT

Mouse preclinical imaging: Options for mouse anesthesia manifolds

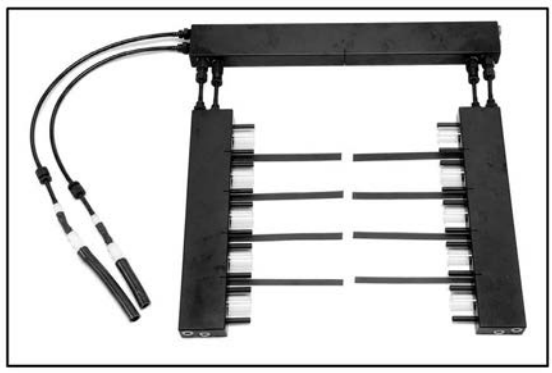
Preclinical small animal optical imaging is used as a non-invasive strategy for acquiring data in living mice. As such, it is important that the imaging systems are designed for animal welfare, optimal animal positioning, safe anesthesia delivery, and data quality. Minimizing the time for image acquisition is also an essential component to minimize animal welfare concerns, and high throughput imaging approaches serve to decrease use of anesthesia and maximize the amount of data that can be acquired.

Researchers use different strategies for mouse positioning in the imaging system, often depending on the system they use. Most commonly, the anesthesia delivery manifolds are attached to anesthesia lines within the imaging chamber of the system, providing the means to image 3-5 mice at a time. Many companies will also provide alternative manifolds that can increase the number of mice placed in the imaging chamber. These replacement manifolds can offer the placement of up to 10 mice at a time in the imaging system.

Below, you can see the three types of manifolds that were assessed for speed and efficiency of mouse imaging. The standard 5-mouse manifold which offers basic imaging capabilities, the 10-mouse manifold which allows the acquisition of twice as many mice at the same time, and Revvity's Smart Tray system which not only enables imaging of five mice at a time but uses a system of benchtop and imaging chamber docking stations amenable to using multiple Smart Trays for streamlined imaging of large mouse studies.

5-Mouse manifold

10-Mouse manifold



5-Mouse smart tray (SMT-5) and Benchtop posing station

Figure 2: Types of Imaging Manifolds or Trays

Imaging throughput and quality: Images

When considering throughput and imaging outputs, it is important to note that there are different ways to maximize data acquisition. However, each approach can also lead to compromises in 1) ease of use, 2) quality of data, or 3) the potential for errors.

A standard 5-mouse manifold offers optimal mouse placement, but of only 5 mice. Placement of individual mice at the manifold requires the researcher to reach fully into the imaging chamber, moving and positioning each mouse. The researcher, in the process of leaning in to see within the chamber, risks some incidental exposure to small amounts of residual isoflurane that may have accumulated in the sealed imaging chamber.

A standard 10-mouse manifold maximizes the number of animals placed within the imaging system, however it again requires direct positioning of each animal within the imaging chamber. There are added challenges with the cramped positioning of the mice and the need to carefully tuck each mouse tail out of the way, especially for larger mice. The time for animal positioning can be more than twice as long as for the 5-mouse manifold.

This approach also requires the researcher to very carefully keep track of animals, as two cages of animals will be open simultaneously. This means that a reliable animal labeling strategy should be used for using the 10-mouse manifold system, and a larger anesthesia induction chamber may be needed.

Revvity's uniquely-designed Smart Tray magnetically snaps into the benchtop docking/anesthesia station, which allows the positioning of animals on the tray on the benchtop, outside the imaging chamber. This design and setup means the researcher now has full access to the mice and freedom to use both hands for proper positioning. When animals are ready to be imaged, the tray disconnects from the benchtop dock and is then magnetically snapped into the docking port in the imaging chamber without disturbing the positioning of the mice. While the mice are in the imaging chamber, a second Smart Tray is used to prepare the next set of five mice. This creates an efficient workflow easily managed by a single scientist.

Below are images of mice using the various manifold systems inside the imaging chamber of the IVIS Spectrum 2.

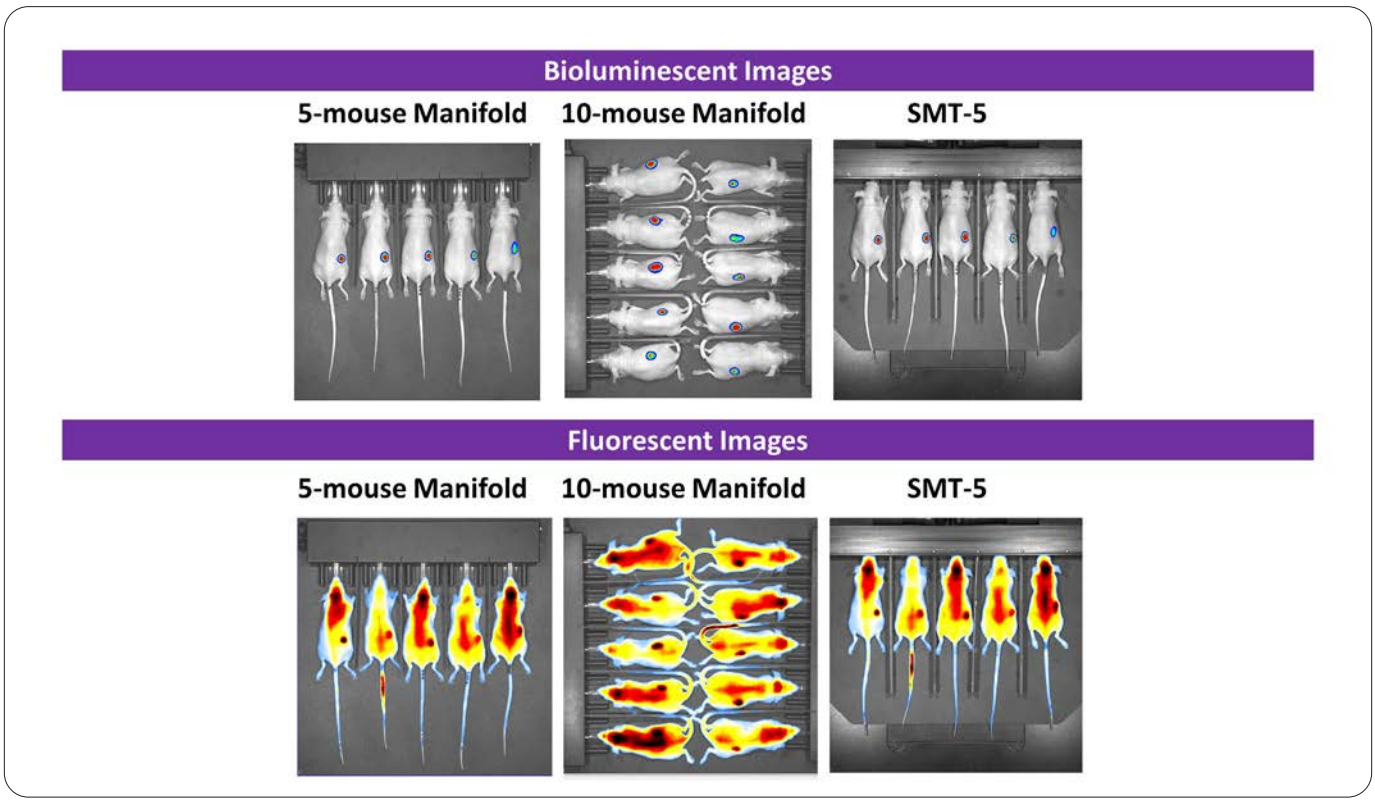


Figure 3: Mice inside the imaging chamber of the IVIS Spectrum 2, comparing the 5- and 10- manifolds with the Smart Tray (SMT-5) using bioluminescent imaging (Upper row) and fluorescent imaging (Bottom row). Bioluminescent imaging uses IVISBrite Red F-luc tumors 4T1 tumors, and fluorescence imaging incorporates an IVISense Integrin Receptor 750 detection of αVβ3 integrin expression in these tumors.

Timing throughput of various manifold systems for FLI

To compare the different manifold systems for their imaging throughput for bioluminescence and fluorescence, 4T1 RedFLuc tumor-bearing mice were imaged for bioluminescence and fluorescence, making careful note of the timing required for each step of the process (summarized in Table 1). To ensure accuracy of timing measurements, personnel rehearsed imaging using the different manifolds so that they had significant experience with each manifold. In addition, a typical 30-mouse study was performed to best mimic real world imaging scenarios in the research setting. Benefits and limitations of the different manifolds are summarized in Table 2.

SMT-5 System: This approach offered the fastest imaging process due to the parallel access to positioning on two separate manifolds, quicker positioning due to benchtop access, and less overall mouse exposure to anesthesia. See Figure 4.

Standard 5-mouse Manifold: This imaging approach was slower than the SMT-5 tray system due to waiting time for access to the manifold, only available for the next batch of mice after completion of imaging of the prior set. Positioning mice directly in the imaging system also took slightly longer.

Standard 10-mouse Manifold: This approach created the largest efficiency challenges despite the ability to image twice as many mice at one time. For two cages worth of mice (10 mice) good positioning of mice on the large manifold was very slow and cumbersome. Overlapping activities were only possible during the brief image acquisitions.

Timings shown are for a single scientist and two 5-mouse anesthesia induction chambers in order to make a fair comparison to the 10-mouse manifold (which would be handicapped in performance with a single anesthesia chamber). Detailed timing of critical steps were made, breaking down procedural steps, including injection, anesthesia, animal positioning, and imaging. These are color coded for easy visualization of the timing (Figure 4). Each arrow represents a single acquisition process from start to finish for one set of mice. Arrow overlaps indicate parallel activities as possible for a single scientist.

Image Timing Metrics per 5 mice (minutes)

Table 1: Comparison of SMT-5 system with standard 5- and 10-mouse manifolds for timing of each imaging step in the preparation of mice and image acquisition. Green boxes indicate improved performance, whereas red shading indicates compromised performance.

	SMT-5	5 mouse manifold	10 mouse manifold
BLI: Collect mice and inject substrate	2.0	2.0	2.0
FLI: Collect mice for anesthesia	0.5	0.5	0.5
BLI: Luciferin time*	10.0	10.0	10.0 – 12.5
FLI: Anesthesia time	2.0	2.0	2.0
Mouse positioning at manifold	1.0	1.5	2.5
Imaging Time	1.0	1.0	1.0
Swap mice in the imaging system	0.5	2.5	3.5
BLI: Time saving with 2 trays	10-20%	na	na
FLI: Time saving with 2 trays	12-40%	na	na
Ease of use	+++	++	+

* Timing depends on standardized imaging time post-luciferin injection and must be tracked to assure acquisition is initiated at the right time.

Table 2: Comparison of SMT-5 system with standard 5- and 10-mouse manifolds on limitations and benefits for optical imaging. Green boxes indicate optimal performance.

	SMT-5 System	5-mouse manifold	10-mouse manifold
Manifold Assembly	No assembly required	No assembly required	Complicated
Alignment to FOV	Yes	No	No
Animal Loading Speed	Fast (mice can be pre-loaded on the second tray on bench)	Moderate. Rate limiting step is waiting for available manifold	Slow. Rate limiting step is waiting for manifold and longer time for animal positioning.
Animal Positioning	Easy. Working in open space allowing free use of both hands for mouse positioning	Moderate. Working in limited space in the imaging chamber. Use one hand for mouse positioning	More difficult to place and position each mouse in the imaging chamber, avoiding limb and tail overlap.
Isoflurane Leakage	Low	Moderate	Higher
Average anesthesia time (per mouse)	BLI: 13-15 min FLI: 4-5 min	BLI: 15-18 min FLI: 5-7 min	BLI: 17-21 min FLI: 8-11 min
Likelihood of Error	Low	Low	Increased
Image Quality	Good	Good	Can be compromised by animal crowding and longer range of luciferin time between mice with BLI
Image Analysis	Good	Good	Slightly faster with fewer images to analyze.

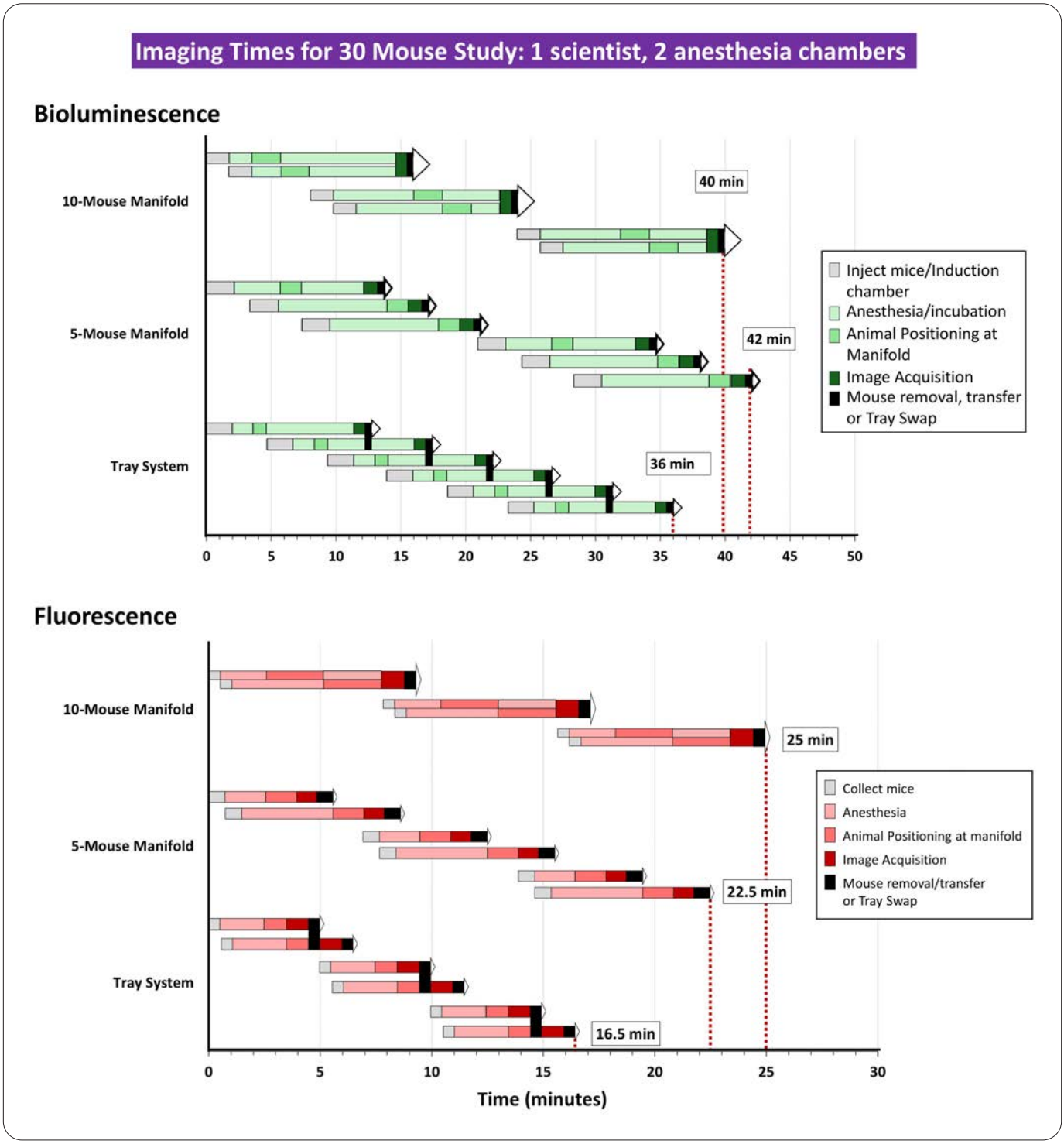


Figure 4: Comparison of SMT-5 system with standard 5- and 10-mouse manifolds on throughput for bioluminescence and fluorescence imaging.

Manifold performance with 1 or 2 scientists

Imaging throughput can also be improved by maximizing efficiency of mouse handling, placement, and image acquisition by increasing to two scientists. Tables 3 and 4 list the time required to image a 30-mouse study with 1 scientist and 1 anesthesia chamber, 1 scientist with 2 anesthesia chambers, and 2 scientists with 2 anesthesia chambers.

An additional scientist offers the most significant benefit for all the manifold systems, as it improves general animal handling and allows simultaneous activities. Small time benefits in BLI for the 10-mouse manifold over the 5-mouse manifold are due to less stringent control over image timing post-luciferin injection, ranging 10-15 minutes within each group rather than precisely 10 minutes.

The SMT-5 system performs up to ~15% faster in BLI and up to ~35% faster by FLI under optimal conditions, than the 5- or 10-mouse manifolds. It further offers more accurate and easy positioning on top of the through-put benefits.

Tables 3 and 4: Comparison of SMT-5 system with standard 5- and 10-mouse manifolds with 1 or 2 scientists and 1 or 2 anesthesia chambers.

Table 3	1 scientist 1 anesthesia chamber	1 scientist 2 anesthesia chambers	2 scientists 2 anesthesia chambers
Tray System	22.5 minutes	16.5 minutes	13.0 minutes
5-mouse manifold	25.5	20.5	20.5
10-mouse manifold	31.5	25.0	21.0

Table 4	1 scientist 1 anesthesia chamber	1 scientist 2 anesthesia chambers	2 scientists 2 anesthesia chambers
Tray System	39.0 minutes	36.0 minutes	30.0 minutes
5 mouse manifold	48.0	42.0	34.0
10 mouse manifold	45.0	40.0	38.0

Conclusions

Revvity's IVIS Spectrum 2 and IVIS SpectrumCT 2 imaging systems are versatile molecular imaging systems capable of bioluminescence, fluorescence and integrated microCT (IVIS SpectrumCT 2 only) imaging. In this technical note, we highlight the Smart Tray accessories which greatly enhance the user experience with regard to animal preparation and handling.

While the traditional manifold systems are singly affixed within the imaging chamber, the Smart Tray system provides two detachable and interchangeable trays for animal placement. One tray can be magnetically snapped onto the docking port in the chamber while the other can be attached to the docking/positioning station on the bench. This unique feature makes it possible to position the second batch of mice on the benchtop while simultaneously acquiring images for the first batch of animals in the IVIS imaging chamber.

Of note, unlike the traditional mouse imaging workflow where animal positioning is typically done with a single hand due to limited space in the IVIS imaging chamber, the secondary tray on the bench allows free access to use both hands for animal placement and positioning, with a much better angle of view (top-down) to fine-tune animal posture.

All of these improvements reflect significant time saving, ease-of-use, minimizing of animal exposure to isoflurane, and an overall better user experience.

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