TECHNICAL NOTE

High-throughput imaging systems and smart tray accessory for faster acquisition of optical imaging data.

Authors

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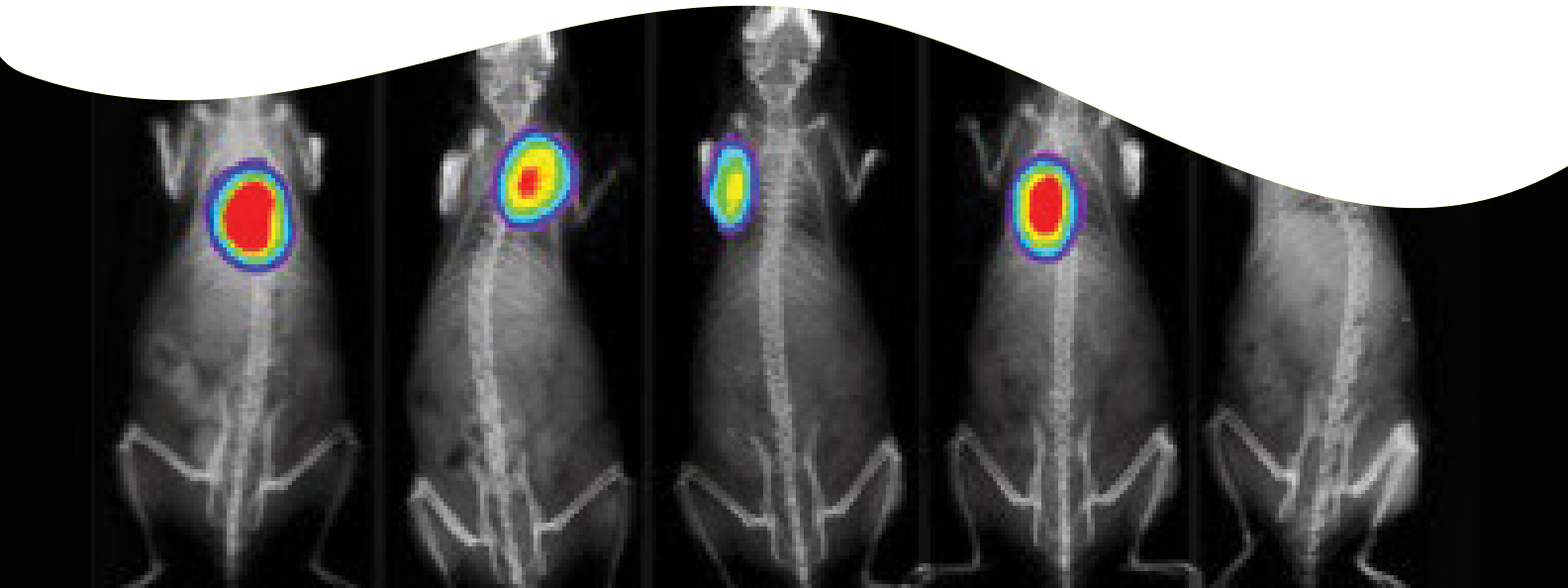
Abstract

The IVIS® Lumina™ S5 and X5 2D high throughput *in vivo* optical imaging systems offer a larger CCD camera to support an expanded field of view enabling the acquisition of 2D high-sensitivity bioluminescence and fluorescence images of 5 mice simultaneously. This expanded field of view, paired with a unique line of "Smart" accessories designed to accelerate setup and labeling, offers a streamlined approach to get robust data – and biological answers – regarding disease progression and therapeutic intervention across a wide range of disease models. These accessories include, 1) a next generation anesthesia unit (RAS-4) with improved multi-connection capabilities and gas scavenging, 2) PharmaSeq p-Chip® scanner to facilitate animal labeling, which includes software support for automatic animal ID import into Living Image® software, 3) MVI-2™ rotational imaging device that permits 360° surface capture of optical imaging datasets from two mice simultaneously, and 4) the 5-mouse Smart Tray manifold system with benchtop docking/anesthesia posing station.

This technical note focuses on the particular benefits of the Smart Tray system and benchtop docking station and how they increase through-put for *in vivo* imaging studies.



The Smart Tray system allows users to position animals on a 5-mouse tray that magnetically snaps into a benchtop docking station for delivery of anesthesia and warming as well as full view and easy access to each animal. Animal trays are designed with ease of use and user safety in mind; the newly designed nose "bays", with calibrated scavenging, make mouse positioning simple while minimizing isoflurane leakage. The bay design further makes cleanup easier. When animals are positioned properly, Tray 1 is disconnected from the posing station and magnetically locked into the dock inside the IVIS Lumina S5 or X5. While the animals in Tray 1 are being imaged, preparation of the next set of mice can begin by docking Tray 2 into the benchtop posing station. Comparison of a standard 5-mouse manifold, an expanded 10-mouse manifold, and the Smart Mouse Tray-5 (SMT-5) was performed to test the speed and workflow in imaging a 30 mouse study.

Testing showed significant speed enhancement and ease of use for fluorescence in comparison to the two conventional manifolds. For bioluminescence imaging, 30 mice could be imaged with the SMT-5 approximately 30% faster than the 5-mouse manifold and in the same time frame as the 10-mouse manifold but with better ease of use and improved ability to time imaging with luciferin injections.

Materials and methods

Fluorescence imaging agents

Fluorescent agents, IVISense™ Folate Receptor 680 and IVISense Integrin Receptor 750 (Revvity), were used to image mice under indicated experimental conditions. The imaging dose for these agents 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) in order to reduce food fluorescence in the stomach and intestines.

Tumor models

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 F-luc bioluminescent mouse breast adenocarcinoma cells Revvity were injected at 1 x 10^6 subcutaneously (s.c.) into the mice to initiate tumor growth.

2D bioluminescence imaging (2D BLI)

Images were acquired using the IVIS Lumina S5 or IVIS Lumina X5 and data was analyzed using Living Image® software (v4.5). For 2D BLI imaging, the system typically 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 intraperitenealy (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)

Prior to imaging, fluorescent agents as described in the following sections were injected intravenously (i.v.) to generate targeted fluorescent contrast. For 2D epifluorescence imaging session, 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 Folate Receptor probes are 660/710 and IVISense Integrin Receptor 750 are 740/790 respectively.

Figure 1: IVIS Lumina S5 and IVIS Lumina X5 High-throughput 2D imaging systems.

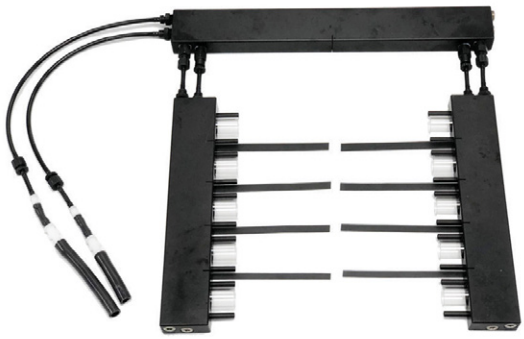
Mouse preclinical imaging: Options for mouse anesthesia manifolds

Preclinical small animal optical imaging, offers non-invasive imaging strategies for acquiring data in living mice. As such imaging systems need to be designed with special attention to animal welfare, animal positioning, anesthesia, and data quality. Minimizing the time for image acquisition is also an essential component to achieving the concerns listed above, and high throughput imaging approaches serve to minimize stress to animals (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, most often providing the capability of imaging 3-5 mice at a time. Many companies will also provide alternative manifolds that can replace standard manifolds to 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 used to explore the speed and efficiency of mouse study 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 and 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 involve compromises in either 1) ease of use, 2) quality of data, and 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. 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 opened 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 Smart Tray design magnetically snaps into the benchtop docking/anesthesia station, which allows the positioning of animals on the tray on the benchtop, outside of 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. 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 5 mice using the various manifold systems inside the imaging chamber of the IVIS Lumina X5 imaging system.

Inside the imaging chamber

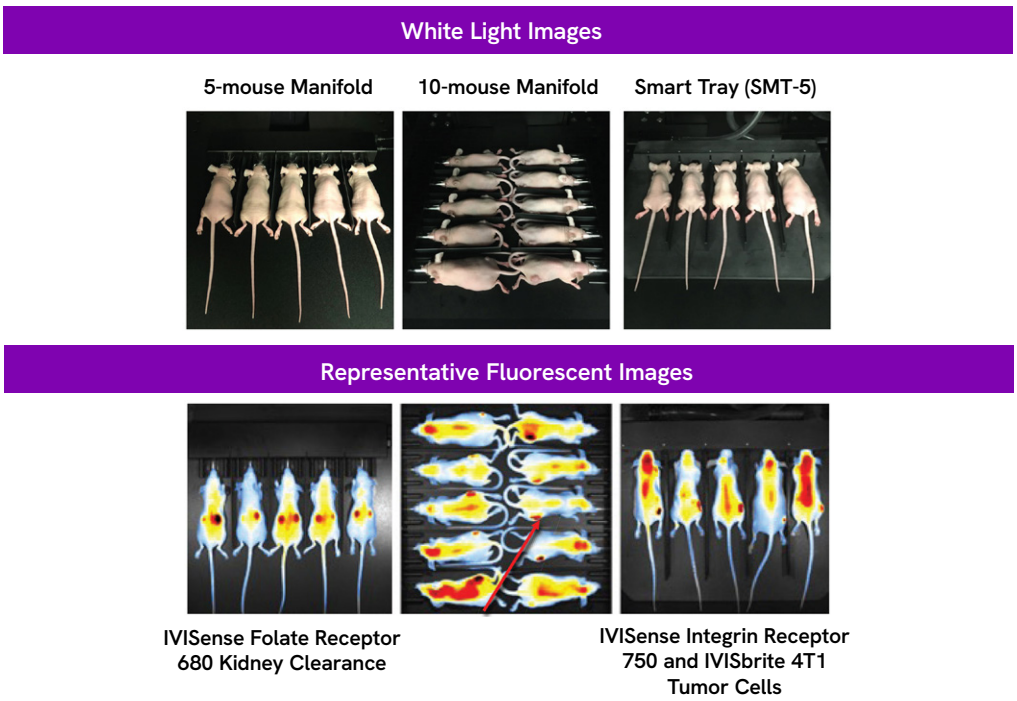


Figure 3: Mice inside the imaging chamber of the IVIS Lumina X5. (Top row) Comparison of the 5- and 10- manifolds with the Smart Tray (SMT-5) manifold under white light. (Bott om row) Fluorescence imaging output using IVISense Integrin Receptor 750 and IVISbrite 4T1 Red F-luc tumors 4T1 tumors using 5- and 10-mice manifolds in comparison to the Smart Tray system.

Timing throughput of various manifold systems for FLI

To compare the different manifold systems for their imaging throughput for fluorescence imaging mice received either IVISense Folate Receptor 680 (kidney imaging) or IVISense Integrin Receptor 750 (tumor imaging) fluorescent agents. To assure 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.

Detailed timing of critical steps were made, breaking down procedural steps to 1) anesthesia induction, 2) animal positioning, and 3) image acquisition. 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.

Smart Tray System: This approach allowed imaging of 30 mice in 16 minutes, with each subsequent tray of mice initiated while imaging the group before.

The critical time-saving was due to the the quickness and ease of animal positioning on the benchtop. **Imaging time 16 minutes.**

Standard 5-mouse Manifold: This approach offered the same overlap of activities as the Smart Tray, however the efficiency and speed of animal positioning was significantly lower, adding a total of 4.5 additional minutes for 30 mice. **Imaging time 20.5 minutes.**

Standard 10-mouse Manifold: This approach created the largest efficiency challenges despite the ability to image twice as many mice at one time. Two cages worth of mice (10 mice) still required anesthesia of only five at a time, and once the first five were positioned, the next five were anesthetized. Good positioning of mice on the large manifold was very slow and cumbersome. Overlapping activities were only during the brief image acquisitions. **Imaging time 28 minutes.** *[Note that overall time would be improved to about 22-24 minutes with a larger anesthesia induction chamber.]*

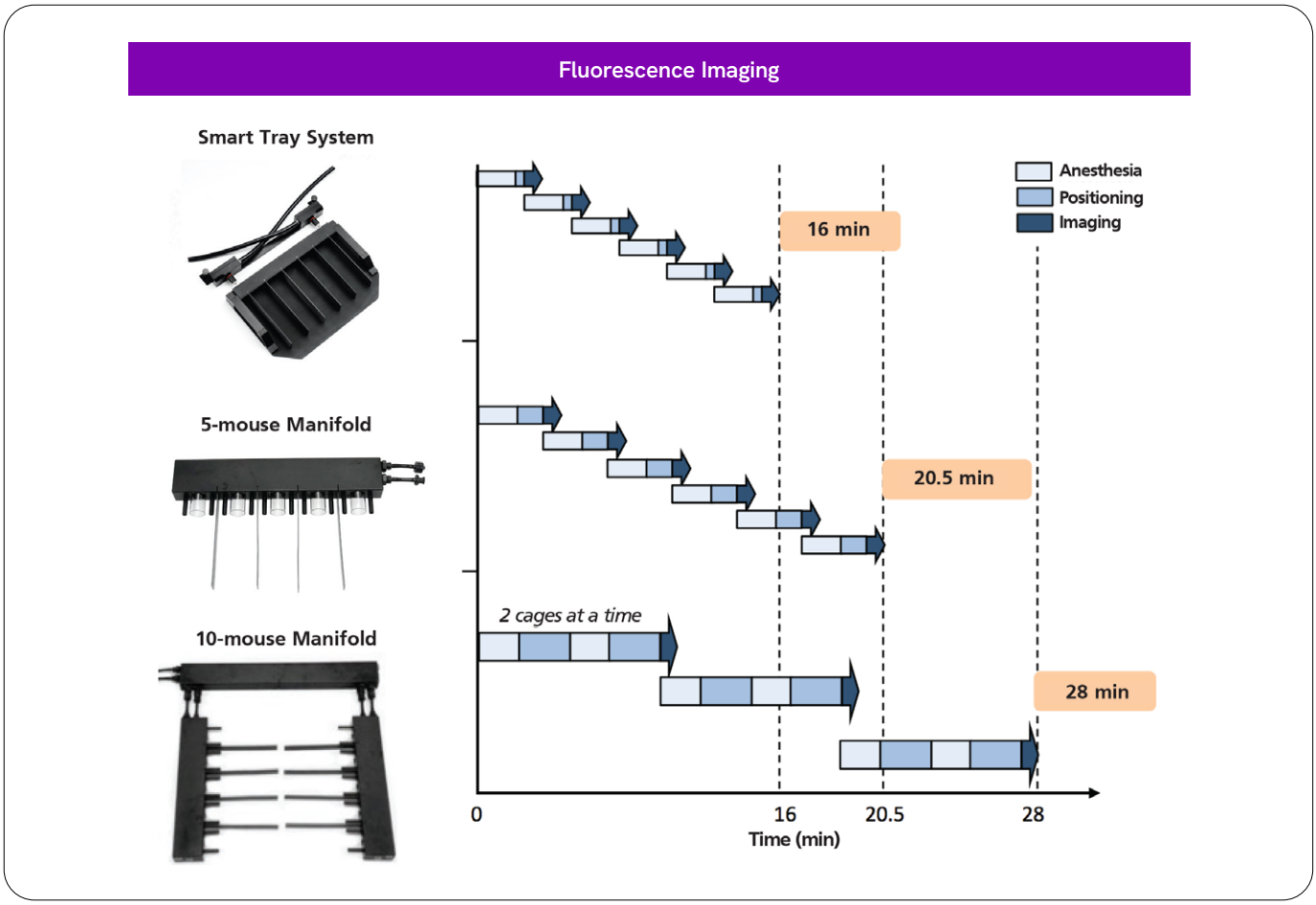


Figure 4: Comparison of Smart Tray system with standard 5- and 10- manifolds on throughput for fluorescence imaging.

Timing through-put of different manifold systems for BLI

When comparing the different manifold systems for BLI imaging throughput, mice were implanted with luciferase-expressing tumors. Detailed timing of critical steps were made, breaking down procedural steps to 1) luciferin injection and anesthesia induction, 2) animal positioning, 3) incubation time to allow proper luciferin/ luciferase kinetics, and 4) image acquisition. These are color coded for easy visualization of the timing (Figure 5). Each arrow represents a single acquisition process from start to finish for one set of mice. Arrow overlaps indicate parallel activities.

Smart Tray System: This approach allowed imaging of 30 mice in 31 minutes, with each subsequent tray of mice initiated during the incubation time of the group before. Similar to FLI imaging in the previous section, the critical time-saving was due to the quickness and ease of animal positioning on the benchtop. This strategy allowed uniform imaging timing relative to luciferin injection. **Imaging time 31 minutes.**

Standard 5-mouse Manifold: This approach offered the same type of overlap of activities as the Smart Tray, however temporal overlap was shorter due to waiting for removal of mice from the manifold. Positioning took twice as long, but the majority of the additional 15 min was due to awaiting the free manifold in the imaging chamber. **Imaging time 46 minutes.**

Standard 10-mouse Manifold: This approach was very fast overall, however it required handling only five at a time, and once the first five were positioned, the next five were anesthetized. Good positioning of mice on the large manifold was very slow and cumbersome. Overlapping activities were only during the brief image acquisitions. Unfortunately, this strategy made it difficult to control the imaging time post-luciferin injection, and the two sets of five mice differed by about five minutes. **Imaging time 32 minutes**. *[Overall time would NOT be improved much with a larger anesthesia induction chamber (accommodating 10 mice at a time) as a short incubation time would likely need to be added.]*

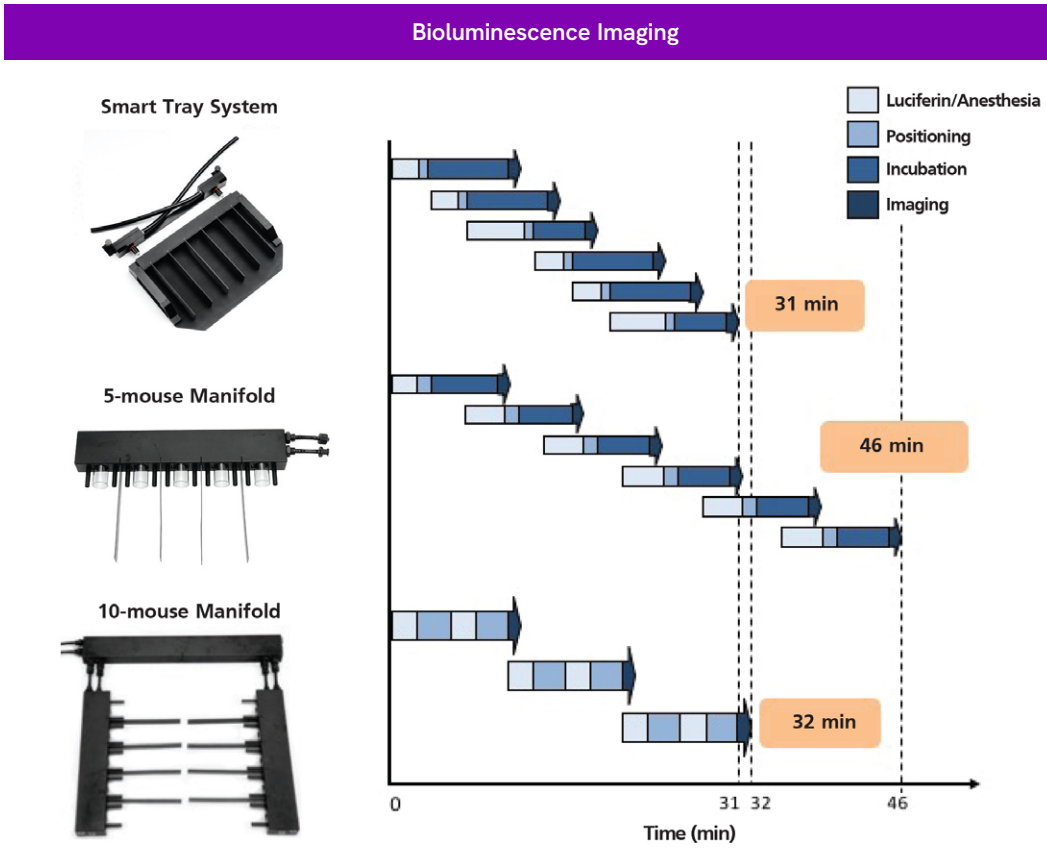


Figure 5: Comparison of Smart Tray system with 5- and 10- manifolds on throughput for bioluminescence imaging.

Conclusions

Revvity's IVIS Lumina S5 and X5 imaging systems are versatile molecular imaging systems capable of bioluminescence, fluorescence and integrated X-ray (IVIS Lumina X5 only) imaging. In this technical note, we highlight improvements that have been made to the IVIS Lumina imaging platform and accessories to streamline and accelerate the imaging process. In particular, the upgraded optics enhance image sensitivity and enable simultaneous imaging of 5 mice, compared to the typical 3 mouse capacity of the previous IVIS Lumina systems. Further, the newly designed Smart Tray system greatly enhances 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, and an overall better user experience. The following table summarizes the comparison between the Smart Tray system and the traditional 5-mouse or 10-mouse manifolds.

Table 1: Comparison summary of the Smart Tray system with standard 5- and 10- mouse manifold systems.

	Tray system	5-mouse manifold	10-mouse manifold
Manifold Assembly	No assembly required	No assembly required	Complicated
Pre-Alignment for Precise FOV	Yes	No	No
Animal Loading Speed	Fast (Can be pre-loaded on the second tray on bench)	Moderate	Slow
Animal Placement	Easy. Working in open space allowing use of both hands for placement	Moderate. Working in limited space in the chamber. Use only one hand for animal placement.	More difficult to place and position each mouse in the chamber. Also need to curl the tails.
Isoflurane Leakage	Low	Moderate	Higher
Likelihood of Error	Low	Low	Increased
Data Tracking	Fiducials incorporated for mouse ID by software when paired with PharmaSeq pChip implantation	N/A	N/A

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