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Guide to the Use of the Multispecies Imaging Module (MSIM) for Imaging Rats on the FMT Imaging System

Abstract

Non-invasive preclinical imaging techniques have become important tools in biomedical research, advancing the researcher's ability to monitor disease progression and the impact of therapeutic intervention in a variety of disease areas. With the recent advances in NIR tomographic imaging technology it has been possible to demonstrate the capabilities of fluorescence molecular tomographic (FMT®) imaging to detect and quantify fluorescent biomarkers of disease in deep tissue sites of cancer, inflammation, and infectious disease.

The development of the Multispecies Imaging Module (MSIM) now allows the power of deep tissue FMT imaging to be applied to preclinical rat research models. The MSIM allows the researcher to rapidly switch between mouse and rat imaging through the addition or removal of the mouse cassette adapter. With the adapter in place, the standard mouse imaging cassette can be inserted for mouse imaging. Removal of the adapter allows the insertion of a larger imaging cassette suitable for rats or other animals of similar size (i.e. 200-450 g body weight), with gas anesthesia delivered effectively to the animal during imaging. The MSIM allows 6 different positions of rat cassette placement, facilitating imaging of discrete regions of the animal, from nose to tail. In addition, the entire animal can be imaged in 2D and 3D mode by capturing a series of scans at each cassette position, and the TrueQuant™ system software will combine these images in one composite view for easy analysis.

| A. Front View | B. Side View | C. Multispecies Cassette Insertion |
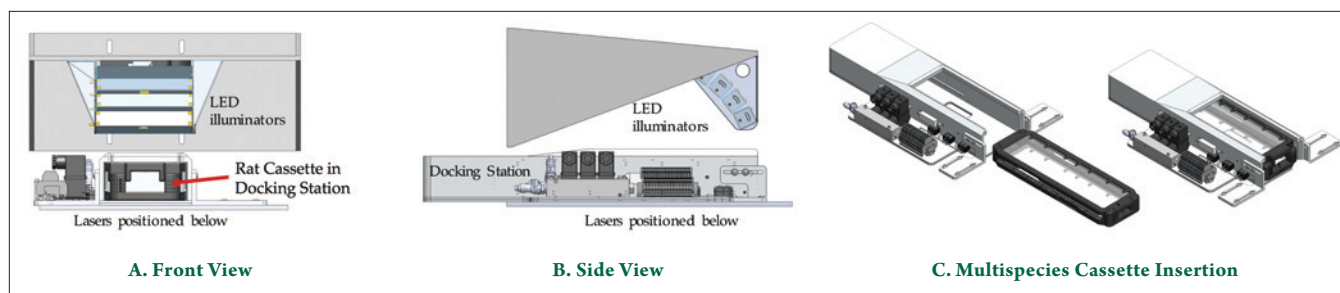
Figure 1. **MSIM Docking Station.** The multispecies docking station is positioned centrally within the FMT system, above the lasers and below the LED illuminators. This position allows both 2D and 3D imaging utilizing LED and laser light sources, respectively.

A. The front view.

B. The side view of the system shows where the docking station is positioned within the FMT system.

C. The multispecies imaging cassette, can be easily inserted and removed, and for mouse imaging this can be substituted easily with the mouse adapter and mouse casette.


| A. Top View | B. Narrow Side View |
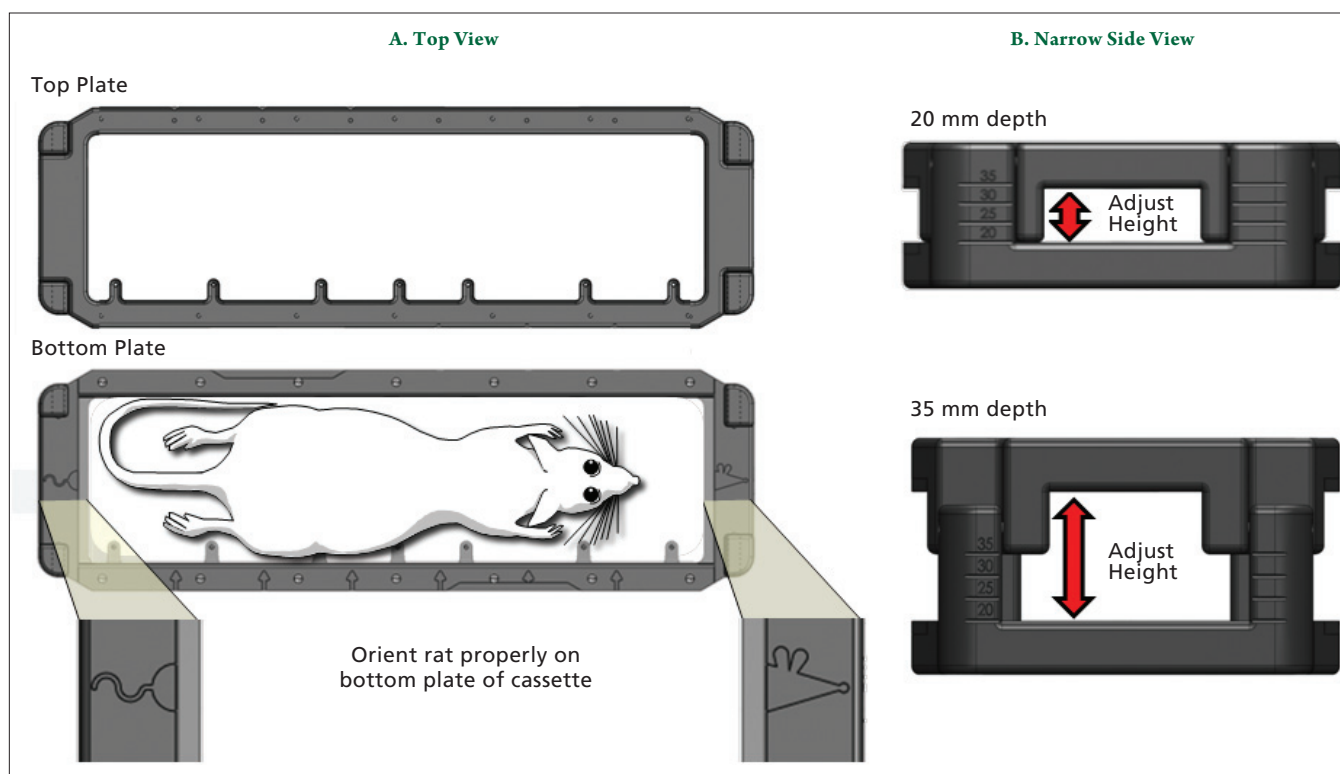
Figure 2. **Rat positioning in the cassette.** The anesthetized rat is positioned on the bottom plate of the imaging casette, with the nose placed toward the side with the "head" icon and the tail positioned toward the "tail" icon. The top plate can then be placed over the bottom plate and adjusted for proper height to achieve gentle restraint of the rat.

Rat Imaging Procedure:

1. Anesthetize the animal for approximately 10 minutes using the RC2+ Anesthesia System or similar gas anesthesia induction chamber. If using the RC2+ Anesthesia System, it is recommended to set the vaporizer to position 4 for isoflurane delivery.

2. Make sure that the mouse cassette adapter has been removed from the FMT system (Figure 2). This will allow insertion of the multispecies imaging cassette.

3. Place the anesthetized animal on the bottom plate of the MSIM cassette (shown in Figure 2A) with the head of the animal oriented towards the head positioning marker on the MSIM cassette.

4. Replace the top plate by holding onto the sides of the cassette then lowering it onto the bottom plate with the fiducial markers lined up. *[Note: the top and bottom cassettes will only fit together in the correct orientation].*

5. Graduated scales located on both sides of the cassette (Figure 2B) indicate the depth or distance between the internal acrylic plates of the MSIM. The graduations are 20, 25, 30, and 35 mm. Select the proper depth for the animal on the cassette. Both sides of the cassette should indicate the same depth, making the top and bottom plates parallel to each other.

   For a 300 g rat, 25 mm depth is recommended. The animal should be able to breathe comfortably within the cassette.
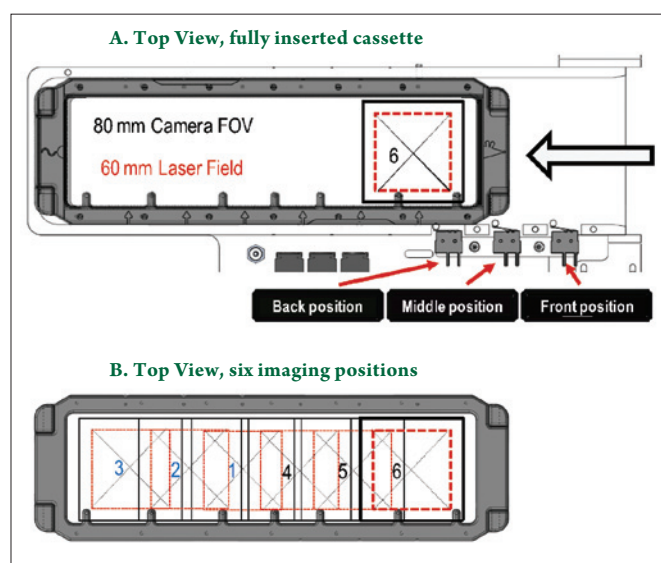
Figure 3. **Casette positioning in the Docking Station.** The imaging cassette can be inserted at 3 different positions into the docking station, front, middle and back. The camera field of view (FOV) and localization of the laser field regions are indicated. Head-first insertion provides positions 1, 2, and 3, and tail-first insertion provides positions 4, 5, and 6. These six positions allow the FMT system to capture fluorescence signal in nearly the entire cassette volume. The laser scan-field is smaller that the camera FOV, but the reconstruction volume is roughly the same size as the FOV.

6. Insert the cassette containing the anesthetized rat in the internal docking station with the animal in either the supine or prone position. It is recommended to focus on only one animal position (prone or supine) until you generate the complete desired dataset. Then change to the other animal position if desired.

7. To achieve a full body image using the MSIM, the animal has to be imaged in 5-6 segments, depending on the size of the animal. For simplicity, image the animal with the head first orientation (3 positions) and feet first orientation (3 positions). The orientation is defined by which part of the animal (head or feet) enters the internal docking station first.

Orientations:

HFP = Head First Prone   **HFS =** Head First Supine

FFP = Feet First Prone   **FFS =** Feet First Supine

8. Push the MSIM cassette head-first to the front position to image and close the chamber door.
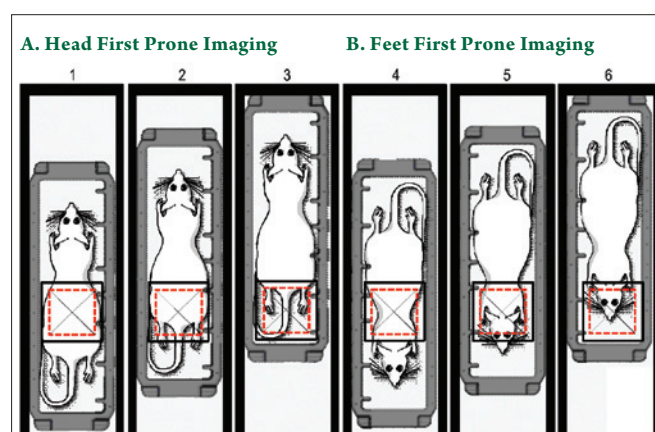
Cassette Positions (Figure 3):

Front: The cassette is at the entry end of the internal docking station. Resistance to moving further into the chamber will be felt when the position is *locked* in place.

Middle: From the *Front* position, push the cassette further in to the next position of resistance. Again, resistance to moving further into the chamber will be felt when the position is *locked* in place.

Back: From the *Middle* position, push the cassette all the way to the back of the chamber.

[Note: proper anesthesia delivery ports will be activated depending on the positioning of the imaging cassette (not shown).]

9. If only a single position is to be imaged, then position the cassette in the internal docking station at the proper location. Position can be confirmed by capturing a reflectance image only ("Reflectance Images Only") on the scan tab.

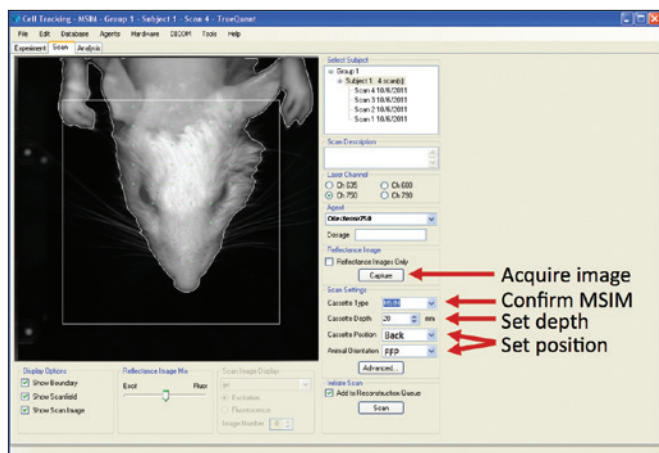*Figure 4.* **Casette positioning in the Docking Station.**

Figure 5. **Acquiring Scan.**

10. To acquire images, verify that the correct Cassette Type, Cassette Depth, and Cassette Position are selected in the Scan Tab of the TrueQuant software (see Figure 5). Capture a reflectance image of the animal to confirm positioning for the proper anatomical scan region, if doing a single scan. (This scan can be repeated if the animal is not in the right imaging position). Otherwise proceed through imaging all 6 rat cassette positions.

11. Imaging head first at the first 3 positions will generate images for positions 1, 2, and 3, as illustrated in Figure 4, capturing lower/mid torso through the feet/tail. Repeat the 3 imaging positions with the cassette inserted feet first to generate images for positions 4, 5, and 6 (thoracic region, upper back, and head).

A. Planar reflectance imaging
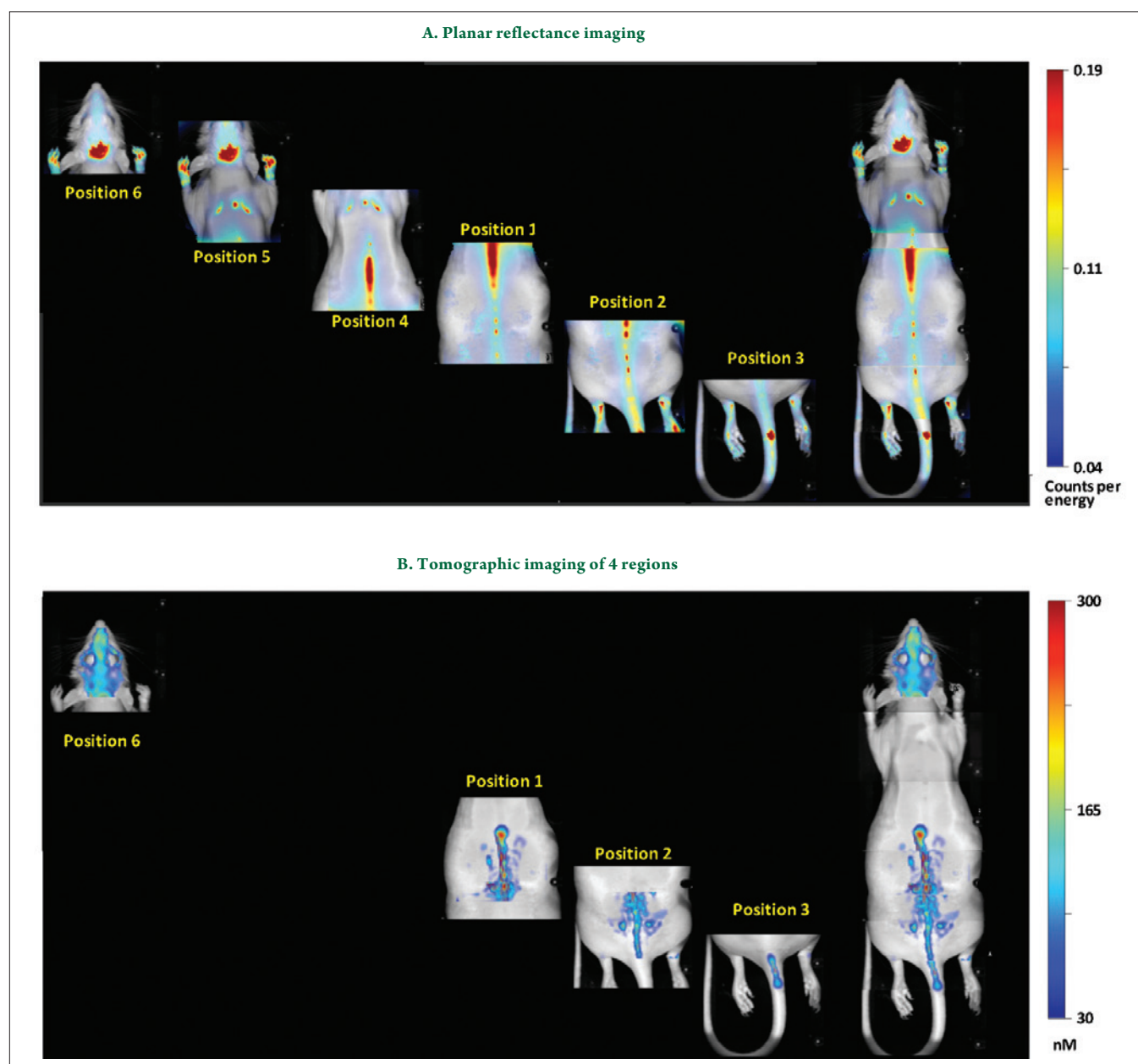
B. Tomographic imaging of 4 regions

Figure 6. **Host PC** – Given the amount of image data that is collected for whole body rat imaging and the amount of memory needed to adequately reconstruct a 3D image, we strongly recommend that you make sure that your version of TrueQuant is running on a 64-bit Windows(R) 7 computer.

12. Planar image (2D) acquisitions for each position are very fast. For tomographic imaging, MSIM scans require approximately 10-20 minutes per position, depending on the number of scan points across the region of tissue. If imaging all 6 positions, expect about 60-90 minutes of scan time.

13. When imaging is completed, remove the animal from the cassette and replace the MSIM mouse adapter in the FMT.

14. Figure 6 shows a representative rat imaged using OsteoSense® 750 Fluorescent Pre-clinical Imaging Agent to reveal regions of normal bone turnover in the spine and calvarium. TrueQuant software can show each imaged region separately or combined in a single window. The software automatically corrects for differences in cassette orientation if all settings during imaging are entered correctly.

15. If only a single region will be scanned tomographically, 2D reflectance images can be acquired rapidly for all other positions to provide a full rat image in the background for reference.

16. The same docking station used for rat imaging can be used to image the mouse by inserting the mouse cassette adapter into the docking station (Figure 7). This creates a smaller sized docking port suitable for inserting the mouse imaging cassette. The insert also serves two additional functions; 1) it is heated by the system to provide an appropriate imaging environment, and 2) it re-routes the gas anesthesia to allow for efficient delivery to the mouse cassette.
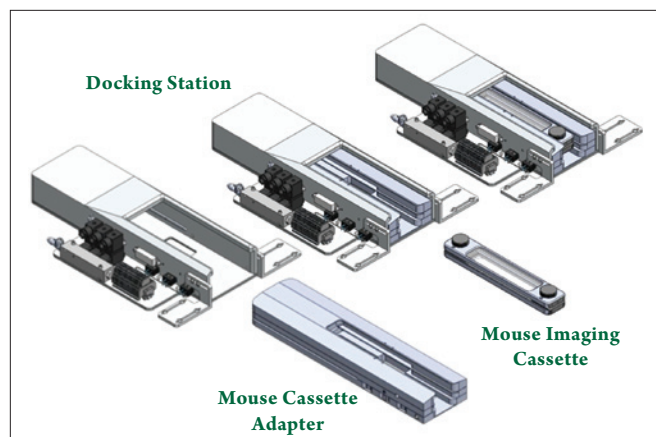

Figure 7. **Diagrams representing MSIM and associated components.**

MSIM Features

Internal Docking Station

– 130 mm width, 463 mm length, 73.35 mm height (5.1" x 18.2" x 2.88")

– Internal anesthesia and exhaust channels

– Internal heaters to maintain subject body temperature

– Spring plungers provide positive MSIM cassette positioning

– Mouse imaging possible with the use of a mouse cassette adapter

Cassette

– Interior height: 20-35 mm, adjustable

  • Height Indicator marks, 20, 25, 30 and 35 mm

– Asymmetrical:

  • Different size posts, for system determination of orientation

  • Markings for head and tail

Host PC

The quantity of image data that is collected for whole body rat imaging and the amount of memory needed to adequately reconstruct a 3D image requires that your TrueQuant software is running on a 64-bit Windows® 7 computer.

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