Supplemental Materials and Methods

CT Module. The CT module consisted of an X-ray source (XRB011, Spellman High Voltage), collimator, X-ray detector (X-Scan0.4C4, Detection Technology, Finland), rotating gantry (DZWN04RA300M, Winner Optics, Beijing, China), and power supply modules. The CT scanner type was the 2-D helical scan mode of a 68 mm transverse FOV. The X-ray source had a tungsten-made anode and a focus spot of 33 µm with a 40-degree cone beam geometry. Nominally, X-ray tube voltage was adjustable between of 35 kV – 75 kV; and the maximum tube power was 15 watts. A 1-mm copper collimator was used to filter the beam cone into a fan beam. The X-ray detector was a line-type detector, which had an energy detection range of 20 keV – 160 keV. The detector has an active length of 102 mm, consisting of 512 pixels at the size of 0.4 mm, and a USB interface support. The X-ray source and detector were oppositely mounted on the rotating gantry and generated slice data while rotating (normally 7 rpm) around the object. A low-cost rotary transmission module was designed, allowing transmission of power and electrical signals from the devices assembled on a rotating gantry to the stationary power supply modules and a host PC. This module consists of a slip ring (SRDZ60190, Prosper Mechanical & Electrical Technology, Hangzhou, China) and a fan-less embedded box PC system (ARK-3360, Advantech, California, USA). The control power supply and data transmission of the CT and SPECT modules relied on the rotary transmission module. The CT module has a spatial FWHM resolution of 150µm, a density resolution of 1%.

SPECT Module. The SPECT module was also mounted on the same rotating gantry with CT, sharing the same transverse FOV as the CT module. With an axial displacement to increase the axial FOV, the two opposing anti-parallel gamma cameras offer a 40 mm transverse FOV and an 80 mm axial FOV. Each gamma camera consisted of a LYSO scintillator array (Yibo Industrials, Shanghai, China), a PS-PMT (H8500, Hamamatsu Photonics, Hamamatsu, Japan), a parallel-hole collimator

(Nuclear Fields, St. Marys, Australia) and electronics modules. The lead low-energy-high-resolution (LEHR) parallel-hole collimator was 34.8 mm thick with 1.5-mm-diameter cylindrical holes that could filter out scattered gamma rays. The LYSO scintillator arrays were coupled to the PS-PMT through optical silicone oil. Each LYSO scintillator array had a 22 × 22 crystal matrix [1.90 (± 0.05) × 1.90 (± 0.05) × 3.00 (± 0.1) mm^3]. The crystal surfaces were polished and the crystals are optically isolated from each other by reflective materials. The Hamamatsu H8500 PS-PMT is an 8 × 8 matrix (64 channels) flat panel photomultiplier tube with 12-stage dynode made of bialkali photocathode material, and allows for a big active area of 49 × 49 mm^2 with small dead space and fast time response. Before processing by preamplifier and shaping amplifier circuits, the position and energy signals from the 64 anodes were de-coupled and combined into 16 stripe readout channels. Digitized output was acquired by the host computer. The power supply module can supply the H8500 with an operating voltage of -1000 V to achieve a pixel gain of ~1.5×10^6. The SPECT module has a spatial FWHM resolution of 2.5 mm and an energy resolution of 14%.

PET Module. The PET module consisted of a stationary gantry, detector ring, electronics modules, and power supply modules. The detector ring consisted of 27 detector banks arranged in a circle 114 mm in diameter, resulting in a 60 mm transverse and 26 mm axial FOV. Each PET detector bank has two blocks in the axial direction. In each block, there is a LYSO (Yibo Industrials, Shanghai, China) scintillator block coupled to a 4 × 4 SiPM array (SPMArray4, SensL Inc., Cork, Ireland) through the optical silicone oil. The SPMArray4 is a 16-element SiPM array that has a 3.16 mm × 3.16 mm pixel chip area and each bank has two blocks in the axial direction. Each of the scintillator blocks had a 4 × 6 matrix of crystals [2.10 (± 0.05) mm × 3.16 (± 0.05) mm ×15 (± 0.1) mm], whose surfaces are polished and optically isolated to each other by reflective materials. With a total of 54 detector blocks, the PET imaging system has eight crystal rings with 162 crystals per ring. The electronics module contained circuitry for pulse shaping, timing, timing measurement, coincidence-detection, communication. The power supply consisted of several DC power supply modules and a supply circuit module with a ± 5V and ± 30V output. The PET module has a spatial FWHM resolution of 2.55–3.225 mm in the transaxial FOV and 3.3–4.15 mm in the axial FOV, an on average energy resolution of 21%, and a maximum sensitivity of 0.76% at a coincidence window of 12 ns and an energy window of 300–650 keV.

FMI Module. The FMI module consisted of three main parts: excitation source, fluorescence detection, and rotating gantry. The excitation source consisted of a Halogen lamp (7ILT250, 7-star, Beijing, China) with a continuous spectrum of 300-2500 nm, a six-position motorized excitation filter wheel (FW102C, Thorlabs, New Jersey, USA), and a custom-designed excitation fiber. By using different band-pass filters mounted in the excitation filter wheel, the excitation source could excite various fluorescent agents. The fluorescence detection consisted of a charge coupled device (CCD) camera (PIXIS 512B, Princeton Instrument), a machine vision lens (C3516-M, Pentax Ricoh Imaging, Tokyo, Japan) and a six-position motorized emission filter wheel (FW102C, Thorlabs). The fluorescent detection and excitation sources were mounted on opposite sides of the rotating gantry (WN03RA200H, Winner Optics). Cables and fibers that connect the stationary and rotational parts were aligned using a cable drag chain. The cable drag chain wrapped around a custom-designed hub disk that was mounted on the gantry. The FMI module incorporated light shielding in order to minimize extraneous light interference and was placed on other side of the animal bed opposited to the PET-SPECT-CT modules. The FMI module, which offers both fluorescence molecular tomography and reflection scanning mode, could perform fluorescence excitation and detection around the entire animal, with a 10 cm × 10 cm FOV.

Animal Bed Module. The motor-controlled animal bed module is composed of a motorized precision linear stage (WN250TA300L, Winner Optics, Beijing, China) with servo motors (SGMAV-02ADA61+SGDV-1R6AO1A, Yaskawa Electric), a motorized precision rotational stage (WN02RA100M, Winner Optics, Beijing, China), and a transparent poly methyl methacrylate (PMMA) animal bed. During imaging, the animal bed can not only move along the axial direction to pass through different modalities, but also reverse the head-tail orientation by a rotational stage to shift from FMI to other imaging modalities (precision of 5 μm and 0.01°). In addition, the vertical height can be adjusted manually; limit and zero switches were also installed. The animal bed is made from a transparent PMMA pipe for transporting both exciting and fluorescence light in FMI, and it's further modified to facilitate gas anesthesia.