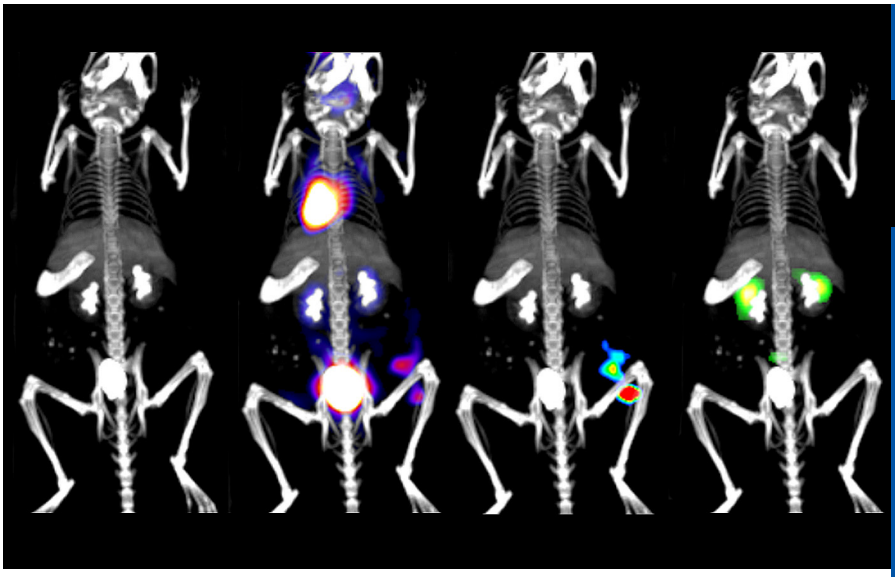

TECHNICAL NOTE

Preclinical *In Vivo* Imaging

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Multimodal Co-registration Using the Quantum GX, G8 PET/CT and IVIS Spectrum Imaging Systems

Abstract

PerkinElmer offers a suite of molecular imaging solutions (Optical, microCT and PET) for visualizing bio-physiological events in small laboratory animals. Each of these advanced imaging modalities uses unique principles for generating 3-dimensional tomographic images, and are capable of non-invasive imaging of specific biological functions. Therefore, an imaging strategy combining more than one imaging modality could provide not only multiple biological readouts, but also their correlation in the 3-dimentional space of an animal model. The goal of this technical note is to provide and perform a general workflow for exporting 3D images acquired from various PerkinElmer imagers and performing spatial co-registration of these images using a single software platform, VivoQuant™ (Version 2.5, InviCRO Inc.). VivoQuant is the standard image visualization and analysis software suite currently included with the G8 imaging system. The software is capable of handling multiple DICOM image inputs from different modalities and allows users to adjust the relative spatial position for each image input. This technical note illustrates the steps for registering 3D tomographic images generated by the Quantum GX for microCT, the G8 for PET and IVIS® Spectrum for optical (bioluminescence and fluorescence) imaging.

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Multimodal Imaging: A Comprehensive View of Disease

The goal of molecular imaging is to non-invasively visualize biological events or physiological changes in living subjects. There are currently several molecular imaging methods available to achieve this goal. For example, the imager may apply external energy such as radiation or magnetic field (X-ray CT, MRI) in order to generate high-resolution anatomical images of the body. Alternatively, images can be acquired that assess special biological functions by functional imaging, generally involving the use of fluorescent probes or radioactive tracers. After administration, spatial distribution of such molecules can be determined using radiation detectors (PET, SPECT) or with optical cameras with fluorescence capability (FLIT). Furthermore, optical imaging of genetically-encoded intrinsic light production (bioluminescence, DLIT) can be used to assess specific tumor growth or unique gene expression events. As each imaging method uses different imaging principles for visualizing anatomical structures or specific biological functions, combining one or more imaging modalities, or multimodal imaging, can provide a more comprehensive view of the particular animal model.

Of the modalities utilized in this technical note, microCT provides the highest resolution for structural and anatomical images. In principle, microCT uses X-rays to penetrate and generate images based on differences in tissue electron density. Therefore, microCT is best suited for visualizing dense tissues such as bones with great resolution. Nevertheless, Quantum GX can be used for soft tissue imaging with the help of contrast agents (iodine-based or nanoparticles) which enhance target tissue density for detection. Alternatively, PET imaging depends on the use of radioactive tracers, whose tissue targeting capability and distribution patterns are largely determined by their chemical structure. Thus, by designing targeted molecules, PET imaging can be used to visualize specific biological target expression or metabolic functions. Optical imaging is a versatile modality for functional imaging since many of its signal-generating mechanisms directly couple with specific biological events. By specific incorporation of the firefly luciferase gene into a target tissue, the resulting substrate-driven bioluminescent light signal provides a means to measure the abundance of that tissue with the advantage of low background signals. All of these imaging modalities are capable of generating 3D tomographic images, allowing visualization of physiological changes and biological events in living animals. This technical note will provide general workflows for (1) how to prepare and export necessary 3D images from the Quantum GX, the G8 PET/CT and IVIS Spectrum; and (2) how to perform spatial co-registration of images using the VivoQuant software.

Preclinical *In Vivo* Disease Model

All multimodal imaging datasets were generated using nude mice implanted with subcutaneous 4T1-Redluc tumor cells on the right flank. Four types of images were acquired for multimodality image co-registration as indicated in Figure 1.

1. Quantum GX microCT provides anatomical reference with dense bone tissues, and as well as soft tissues such as the liver, spleen, and kidneys using ExiTron™ nano6000 and Iodixanol contrast agents,
2. ^{18}F-FDG, a glucose PET tracer, was used to image tissues with increased metabolic uptake of glucose. These organs include the brain, heart, kidney, and tumor,
3. 3D FLIT imaging was performed on the IVIS Spectrum using FolateRSense™ 680 (PerkinElmer, NEV10040) fluorescent agent to highlight the kidneys,
4. Bioware® Brite Cell Line 4T1-Red-FLuc (PerkinElmer, BW124087) flank tumors produced bioluminescence signals for 3D DLIT imaging using the IVIS Spectrum system after injection of D-luciferin (PerkinElmer, 122799).

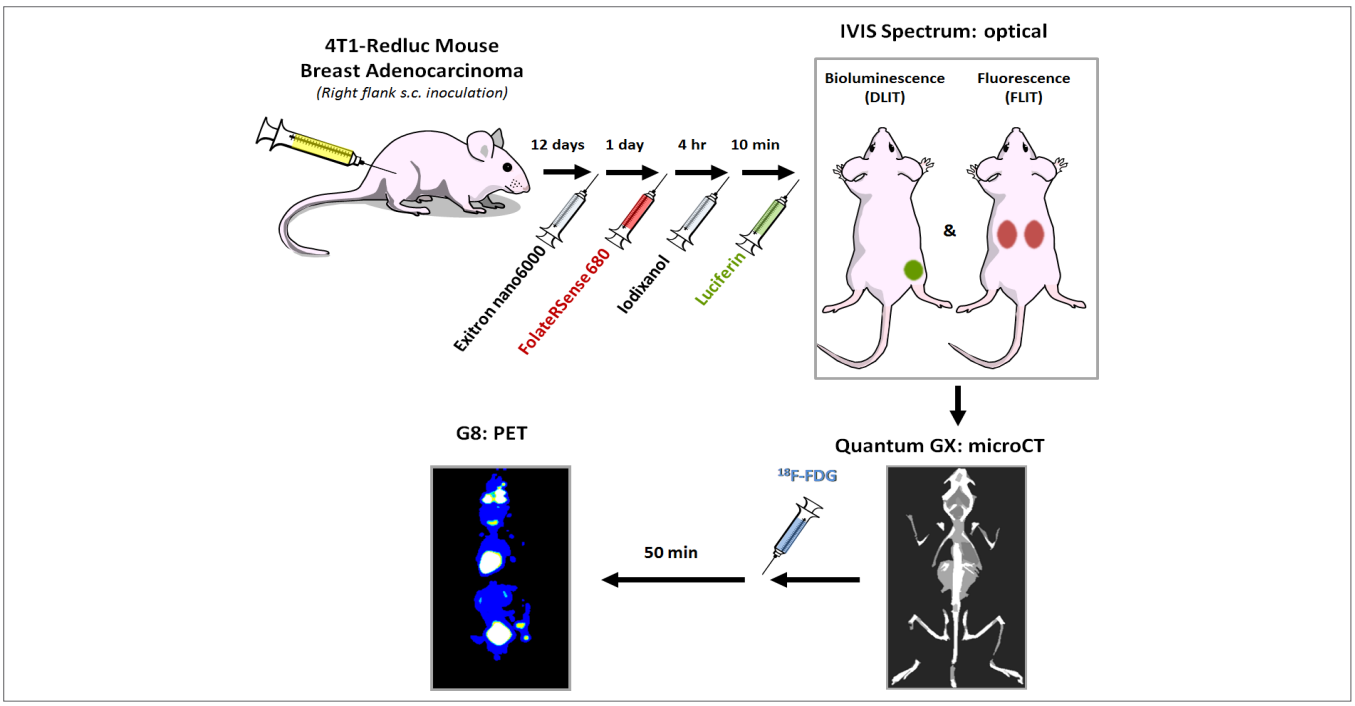
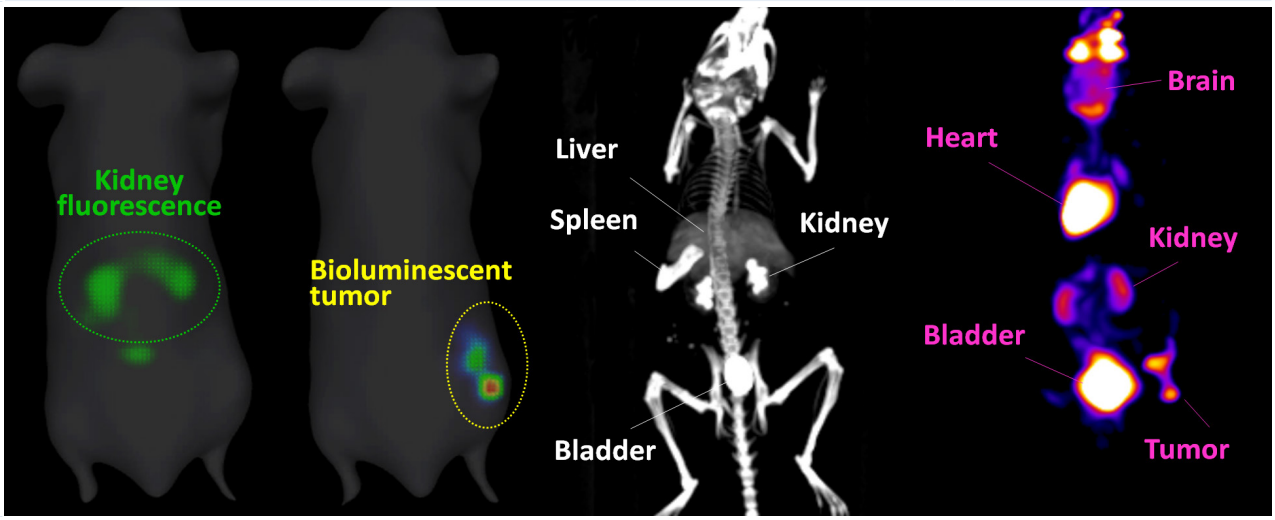


Figure 1. Animal model preparation and multimodal imaging procedures.

Table 1. Modality comparison and the animal model used for multimodal imaging co-registration.

	IVIS Spectrum		Quantum GX	G8
Modality and Function	**Fluorescence (FLIT)**	**Luminescence (DLIT)**	**X-ray microCT**	**MicroPET/CT**
	3D tomographic imaging of fluorescent agent distribution (Functional: targeting, activation)	3D tomographic imaging of luciferase activity (Functional: growth, gene expression)	Low-dose, high-resolution anatomical imaging of bones and contrast agents (Anatomical: bone and soft tissues)	Visualizing radioactive tracer distribution in living animals. The on-board CT scanner provides general anatomical imaging (Functional: tracer uptake)
Example *In Vivo* Images	The kidney fluorescence was induced by systemic administration of FolateRSense 680, which is normally excreted via the renal pathway.	The 4T1-RedFluc tumor cells exogenously express luciferase activity for direct assessment of tumor viability and growth upon injection of its d-luciferin substrate.	In addition to the bone image, two contrast agents were used: (1) ExiTron nano6000 is injected 24 hr before to produce the liver and spleen contrast; (2) Iodixanol was injected 30 min prior to induce the kidney and bladder contrast.	^{18}F-FDG, a glucose analog, was delivered i.v. to visualize tissues with elevated glycolytic activity, such as the brain, heart, kidney, and tumor. As the tracer is metabolized and excreted, PET signals can be detected in the bladder.



Overview of General Co-registration Workflow Using VivoQuant Software

Recommended image import sequence for multimodal co-registration in VivoQuant, illustrated in Figure 2.

1. It is recommended to import the microCT image as the first reference image frame due to the high resolution quality of the scans that provide general bone structure for positioning and orientation.
2. The second import (termed Input 1) should be the PET image. Normal background uptake can be observed in organs such as the heart, kidneys and bladder in addition to the tumor, sources of signal that can be used as reference points for co-registration.
3. Optical images should be the last to be incorporated into the image set (Input 2) as they offer fewer reference points of alignment.
4. As VivoQuant offers only three modality co-registration, swapping the Input identity of different datasets will allow different combinations of multimodality representation. [Most commonly, the two different optical datasets can be swapped as Input 2].

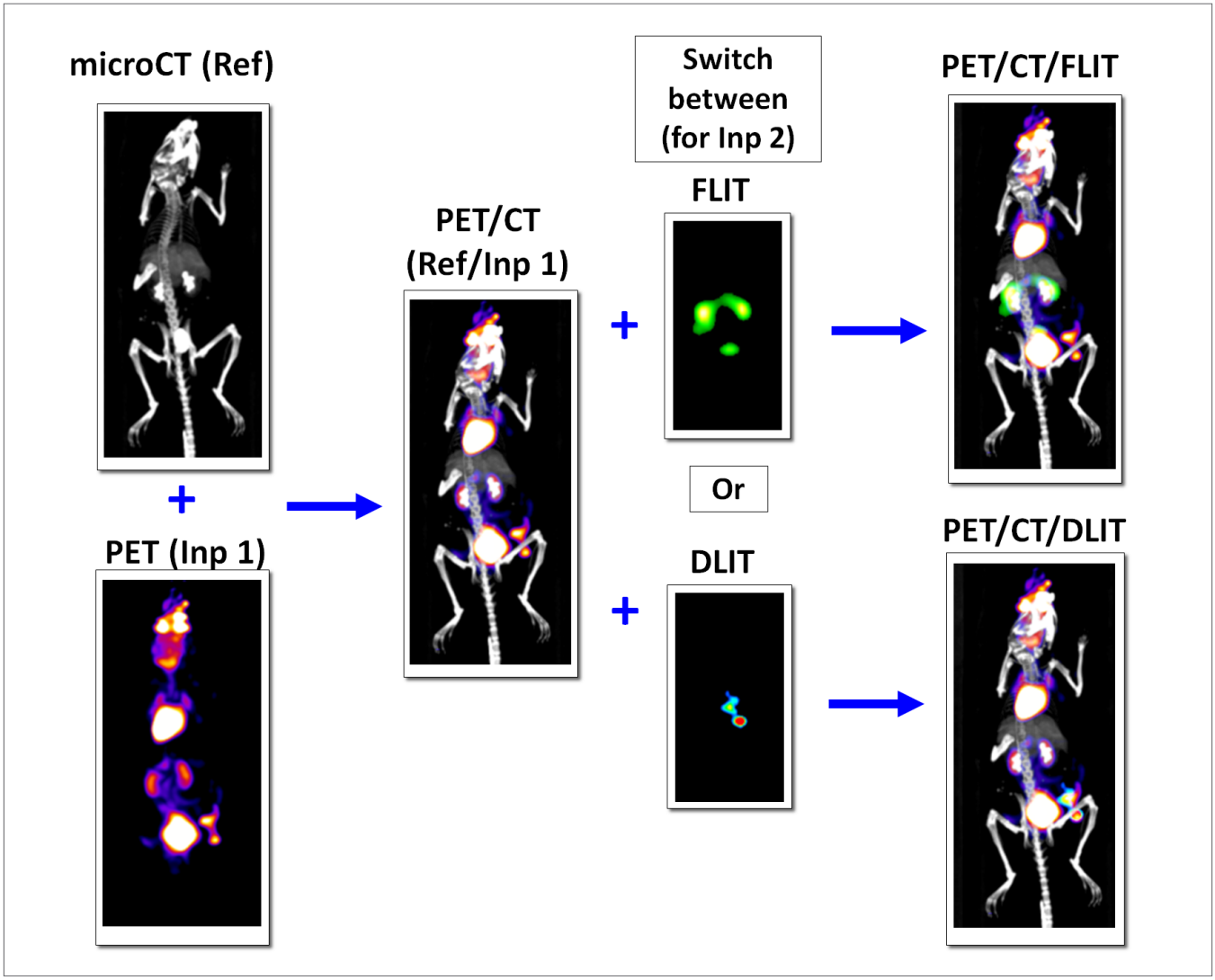
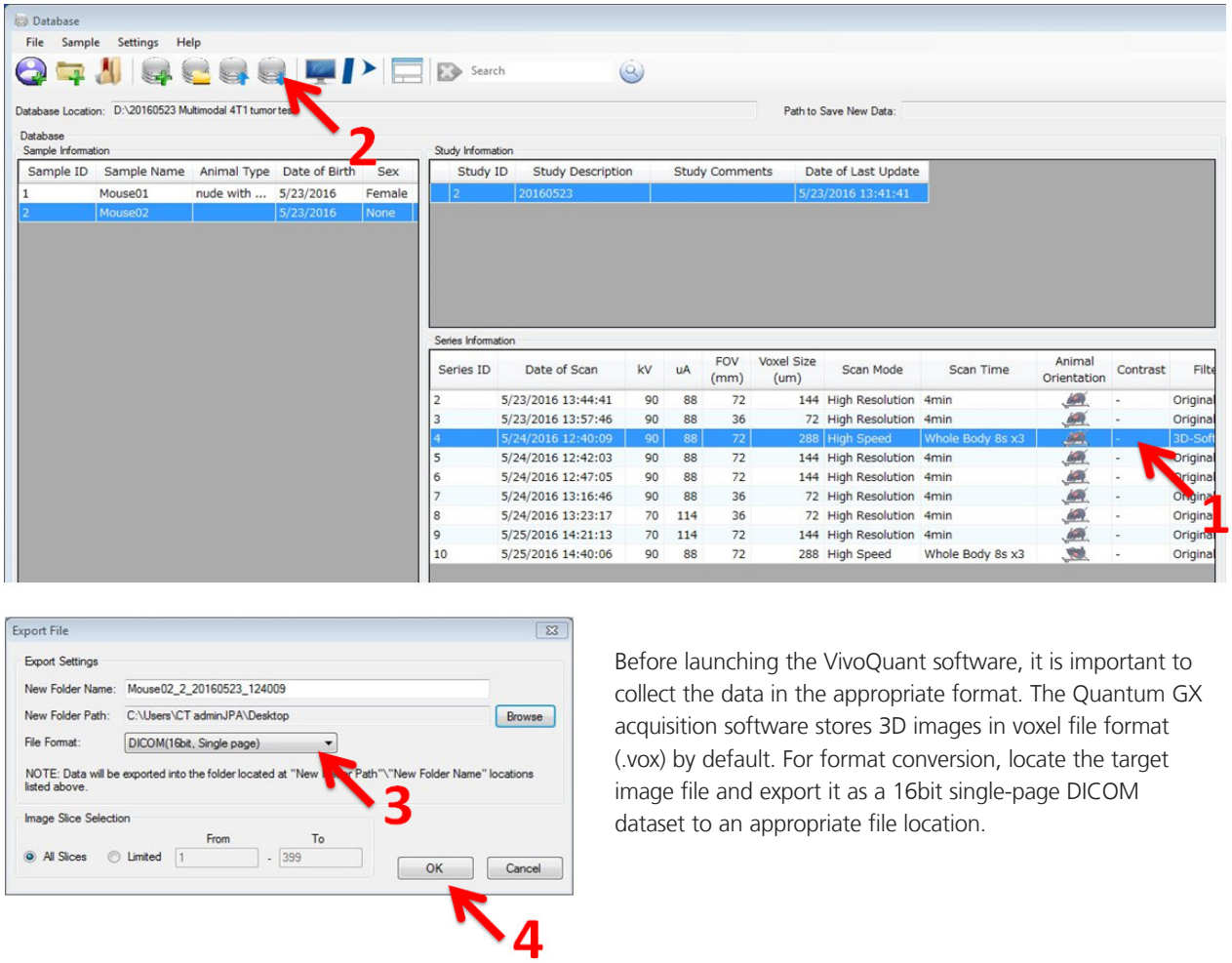


Figure 2. Suggested workflow for multimodal co-registration.

Methods for Exporting DICOM Files from Different PerkinElmer Imaging Modalities

Quantum GX MicroCT DICOM Export

1. Using the Quantum GX system software, left click in the database window to identify the image you would like to export.
2. Left click the "Export File" button and a window will show up requesting the file name and path for this export.
3. On the File Format dropdown menu, select "DICOM (16 bit, Single page)" .
4. Click OK and the DICOM files will be exported to the designated location.



Before launching the VivoQuant software, it is important to collect the data in the appropriate format. The Quantum GX acquisition software stores 3D images in voxel file format (.vox) by default. For format conversion, locate the target image file and export it as a 16bit single-page DICOM dataset to an appropriate file location.

Figure 3. How to export a DICOM image from the Quantum GX microCT imager.

G8 PET/CT DICOM Files

The G8 PET/CT system acquires both PET and microCT images. The onboard microCT provides good image resolution for general co-registration needs, however, in this technical note a high-resolution microCT generated by Quantum GX will be used for co-registration instead.

After reconstruction, the G8 system software stores all images in default DICOM format, located in the StudyData folder in the D drive (D:\\DATAPART1\StudyData\Users). Thus, the user simply needs to identify and copy the target DICOM image folder to a new destination for later use as shown in Figure 4.

The G8 stores all 3D images in DICOM format by default. In this example the ^{18}F-FDG uptake image was collected using the PET only mode. The image files are typically stored at a default location D:\\DATAPART1\StudyData\Users\. In the instance in which a CT image was acquired using the onboard CT system together with PET, both image datasets will be stored in the same DICOM folder that can be viewed separately by the VivoQuant software. The user can elect to copy only the PET DICOMs in this case.

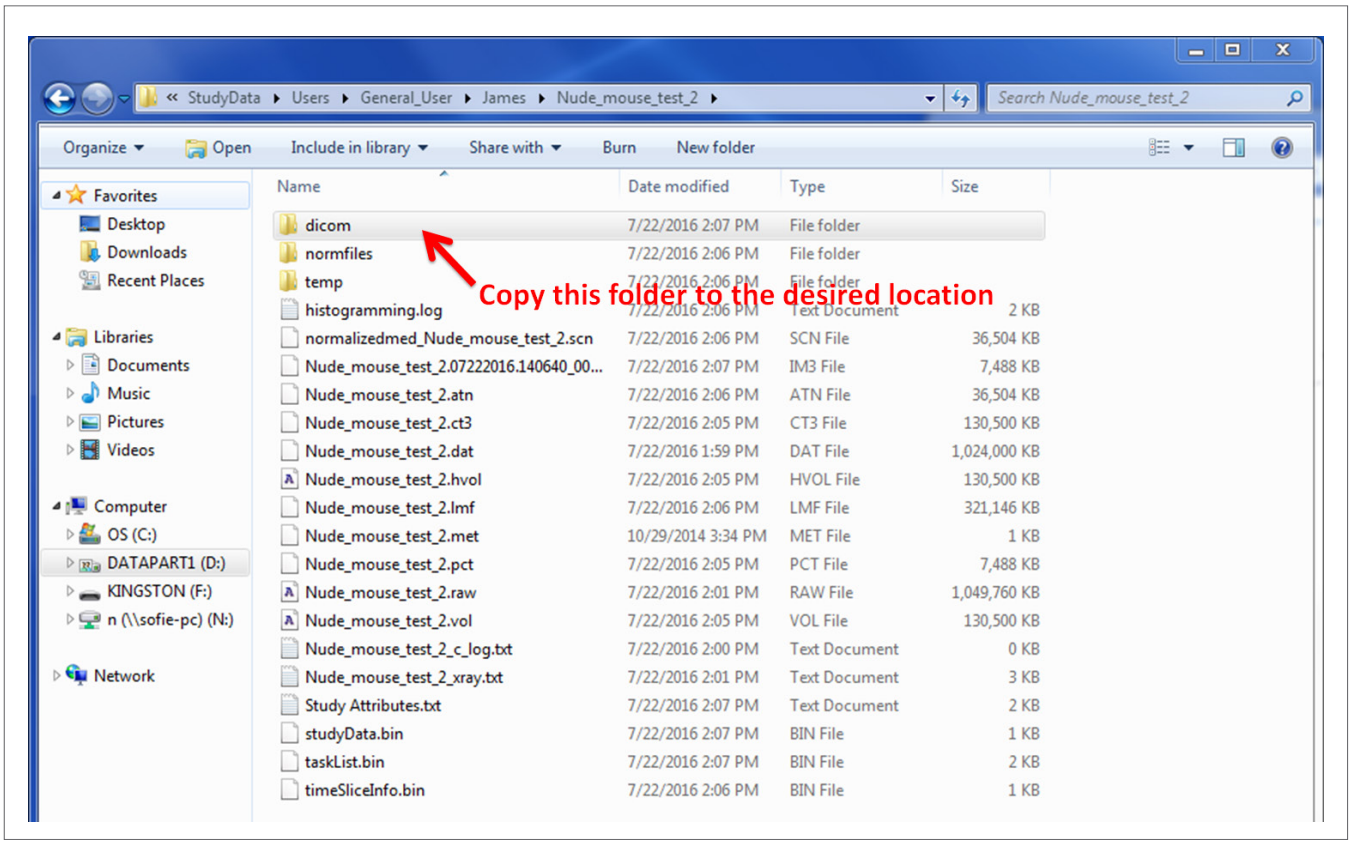


Figure 4. G8 DICOM image file location.

IVIS Spectrum: Exporting Optical DICOM Files

The IVIS Spectrum system acquires two types of 3D optical images: (1) DLIT for bioluminescence imaging, and (2) FLIT for fluorescence imaging.

1. Open the each imaging dataset (either DLIT or FLIT images) in the IVIS Spectrum Living Image® software.
2. Using the Tool Palette window, open "3D Optical Tools". After optimizing the image, uncheck "Display Subject Surface" (as shown in Figure 5) since the animal surface is not needed.
3. Go to "File>Export" and select "3D Scene as DICOM". A "3D Scene Exporter" window will appear.
4. On the "Save DICOM as" dropdown menu, select "Multi-Frame DICOM".
5. On the "Slice Resolution" dropdown menu, select "High".
6. Click the "Export" button to save the DICOM folder at a desired location.

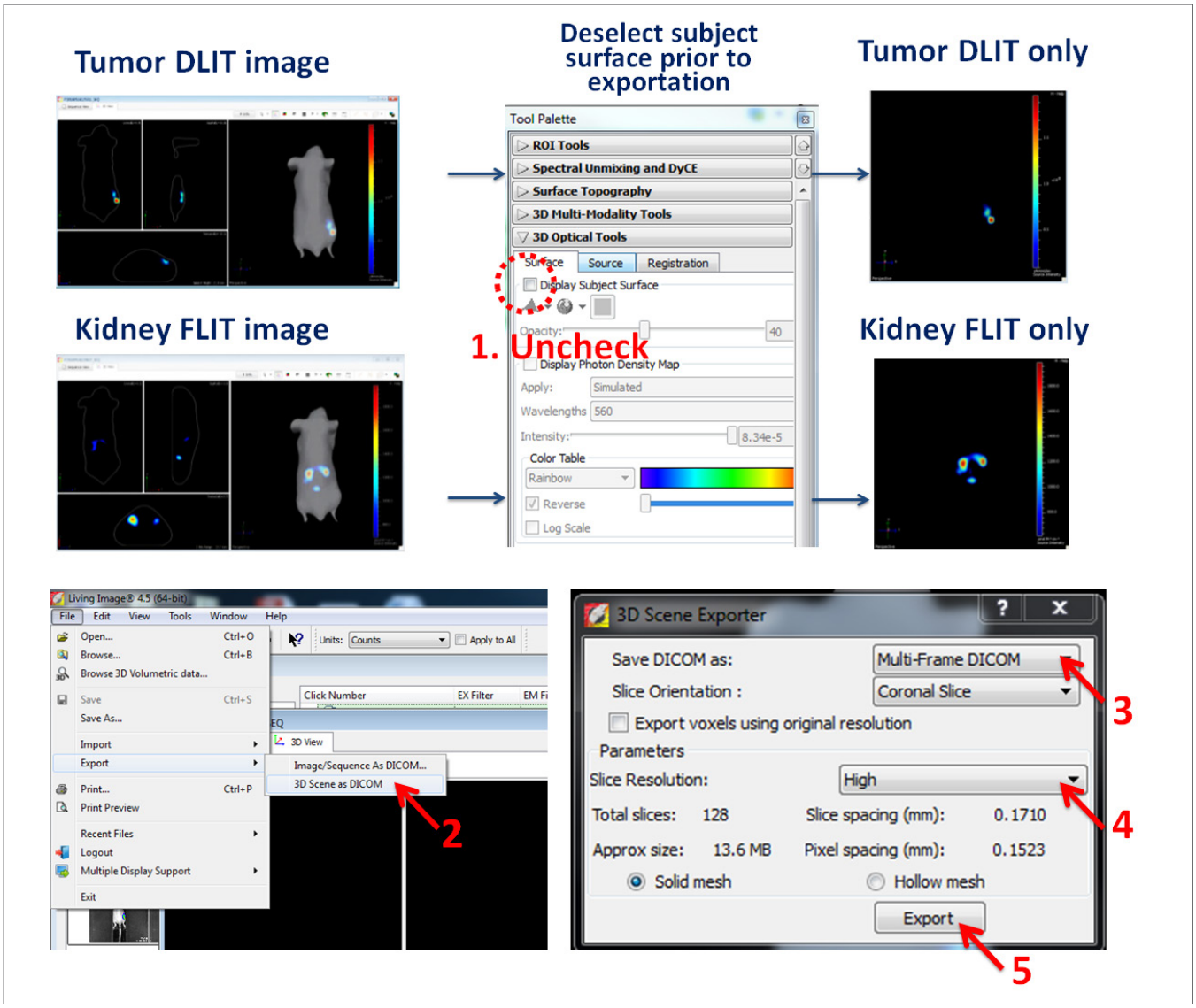


Figure 5. Exporting optical DICOM files using the Living Image software.

The IVIS Spectrum system acquires two types of 3D optical images, bioluminescence (DLIT) and fluorescence (FLIT). In both cases, the data export processes are similar. It is important, however, to remove the attached "Subject Surface" as it is not needed. The user should also adjust the visual representation (by going to the "Source" tab in the "3D Optical Tools" window) prior to export.

Co-registration of Multiple DICOM Images Using VivoQuant Software

1. Click the "Data Browser" button on the main tool bar of VivoQuant as shown in Figure 6. A Data Browser window will appear.
2. Click the "Open local folder" button and select the target folder containing the DICOM image.
3. A single left click to highlight the target image for image import.
4. Click the "Open" button. The image will be loaded.

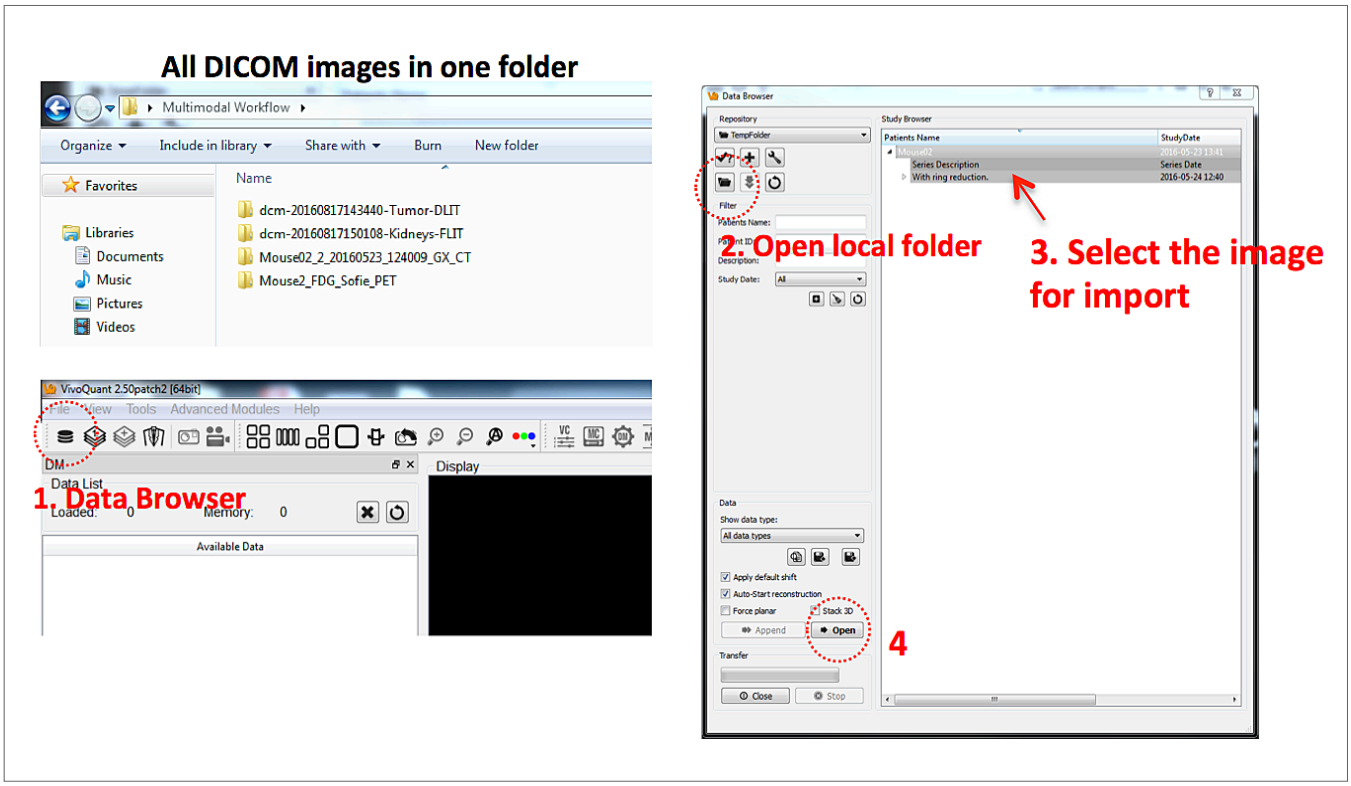


Figure 6. Import the first CT DICOM dataset into VivoQuant.

After gathering all the DICOM images needed from different modalities, it is recommended to organize and properly name all necessary DICOM folders to include information such as subject name, modality and other details to avoid confusion. It is also suggested to put all associated DICOM images in the same folder.

Adjusting microCT Data Using the VivoQuant Viewing Interface

Once the microCT image is loaded, four image panels will be present on the right-hand side in the Navigation window of VivoQuant. Within these four image panels, the one on the left is the 3D image in the maximum intensity projection (MIP) mode. The other three panels are sagittal, coronal and transverse slice views.

For threshold adjustment, use the color scale bar on the left side of the window to adjust the minimum and maximum threshold for visualization. In this case, raise the minimal threshold to hide most of soft tissue signal before importing next modality. Use this high resolution microCT image as the spatial reference image (Ref) when importing PET and optical data.

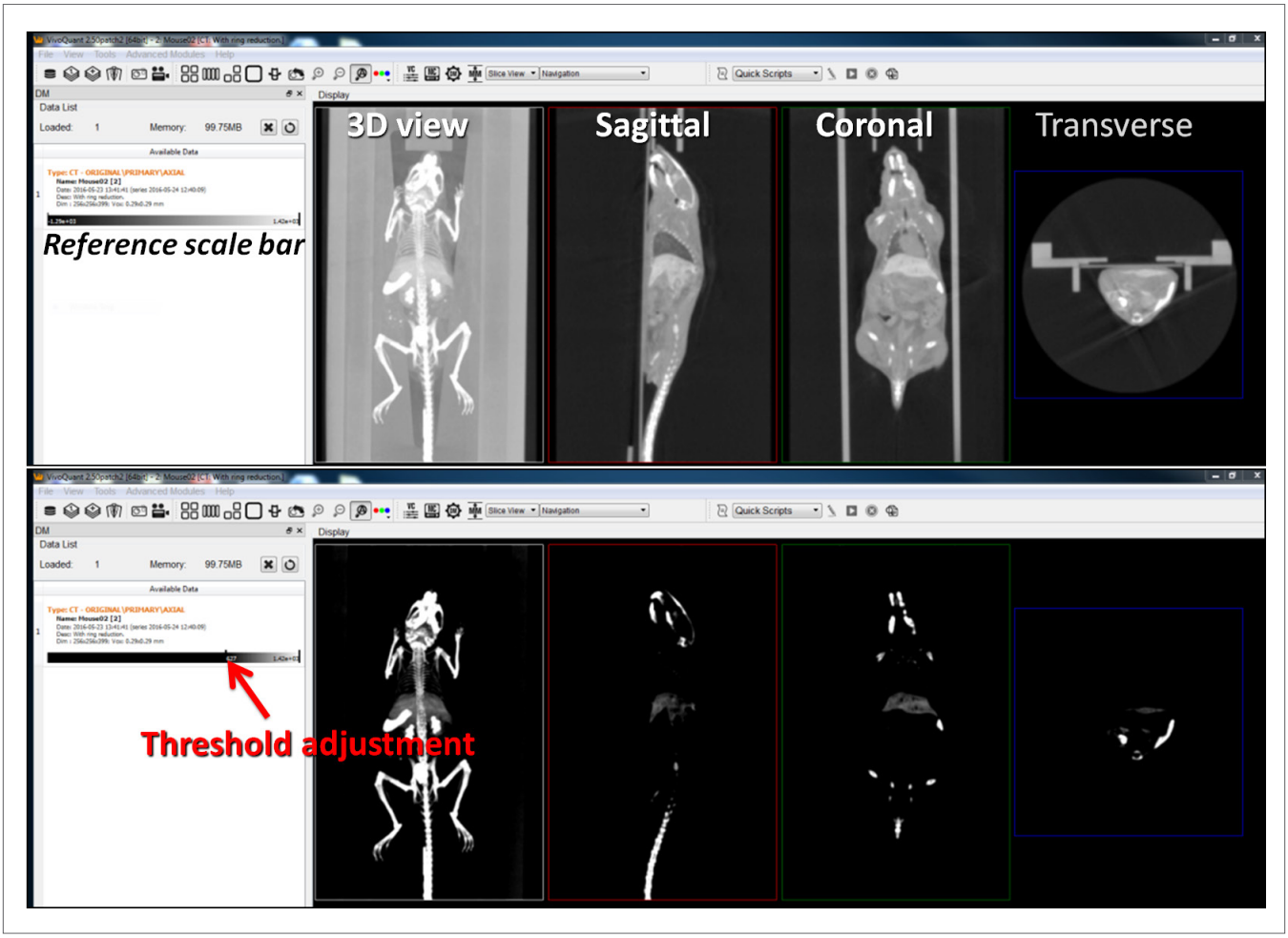


Figure 7. microCT images opened in VivoQuant.

Importing a PET DICOM Dataset for Image Co-registration with MicroCT

1. In the VivoQuant Data Browser window, click "Open local folder" and select the PET data folder as shown in Figure 8.
2. Make sure to click once to highlight the target image.
3. Click "Append" to import the PET image. The image is now listed under the CT reference image.
4. Adjust the color bar for the PET image and reduce the threshold to better define the body outline.

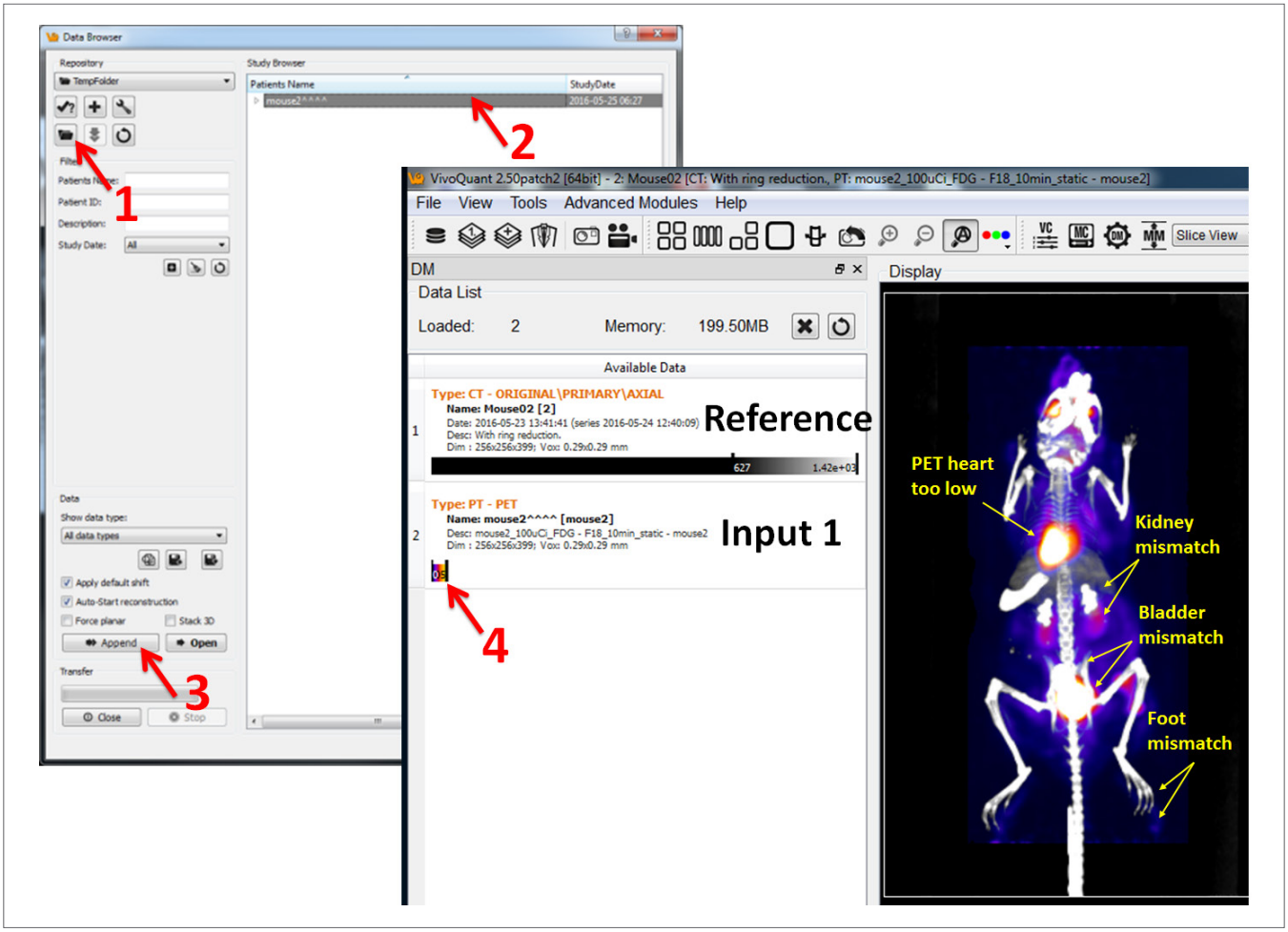


Figure 8. Add a second PET DICOM image to the reference CT image before co-registration.

The previously imported microCT dataset serves as the "reference" image. Importing the PET dataset differs from that of the reference image in that the user will need to click the "Append" button instead of "Open" to superimpose the PET image onto the reference CT image. This PET image will serve as the Input 1 (Inp 1) of this particular image datasets. Since these two datasets are not registered to each other yet, the PET image is slightly misaligned with the reference microCT image.

Using the Reorientation/Registration Tool to Manually Align Two Image Datasets

1. Select "Reorientation/Registration" from the dropdown menu on the main tool bar of VivoQuant.
2. Make sure the "Inp 1" check box is selected, and uncheck the "Ref" box. In this case, Ref is the CT image and Inp 1 is the PET image.
3. Both images were acquired using the multimodal cassette that keeps the animal in the same posture, so there is no need to rotate or scale the images. Adjust only the Translation settings (X-, Y-, Z-axis) to align both images using appropriate visual landmarks, i.e. body boundary and kidney signal in this case.
4. Click the "Accept" button to save the settings.

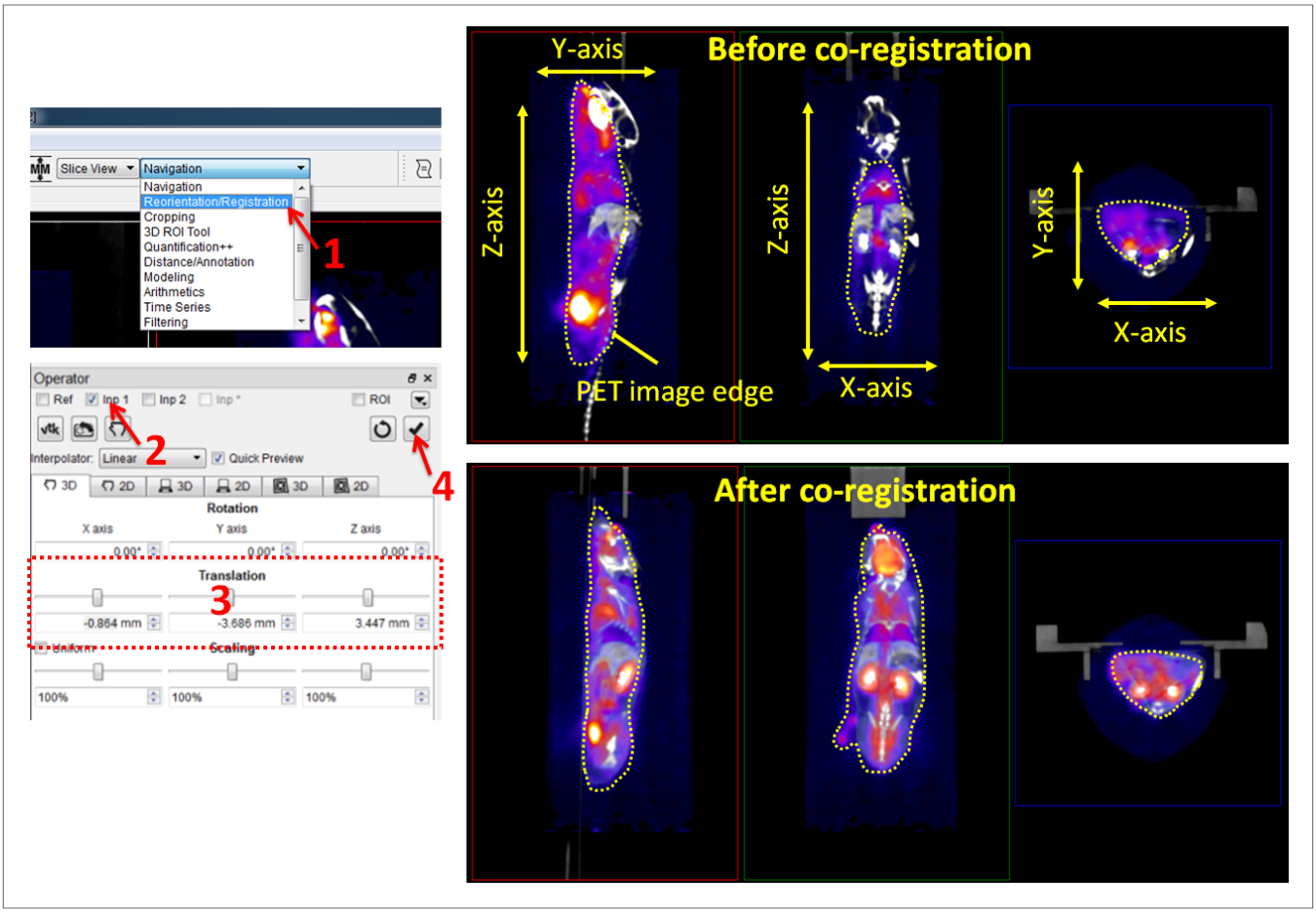


Figure 9. Registration of the G8 PET image to the Quantum GX microCT image.

With both PET and microCT images loaded in the software, it is simple to manually register the functional PET image to the reference CT image. The PET image body shape (obvious with reduction of maximum threshold) and kidney signal provide useful references for alignment to the CT reference image.

Adding and Adjusting the FLIT DICOM Dataset to the Co-registrated PET/CT Image

1. Click the "Open local folder" button to search for the FLIT DICOM folder.
2. Click to high-light the image you want to import in the Study Browser window.
3. Click the "Append" button and the FLIT image will be loaded as Input 2 (Inp 2) in the viewing window.
4. Adjust the color bar threshold if necessary.
5. For co-registration, select "Reorientation/Registration" in the dropdown menu.
6. Adjust the relative position using the Translation tool.
7. Click "Accept" to save the settings.

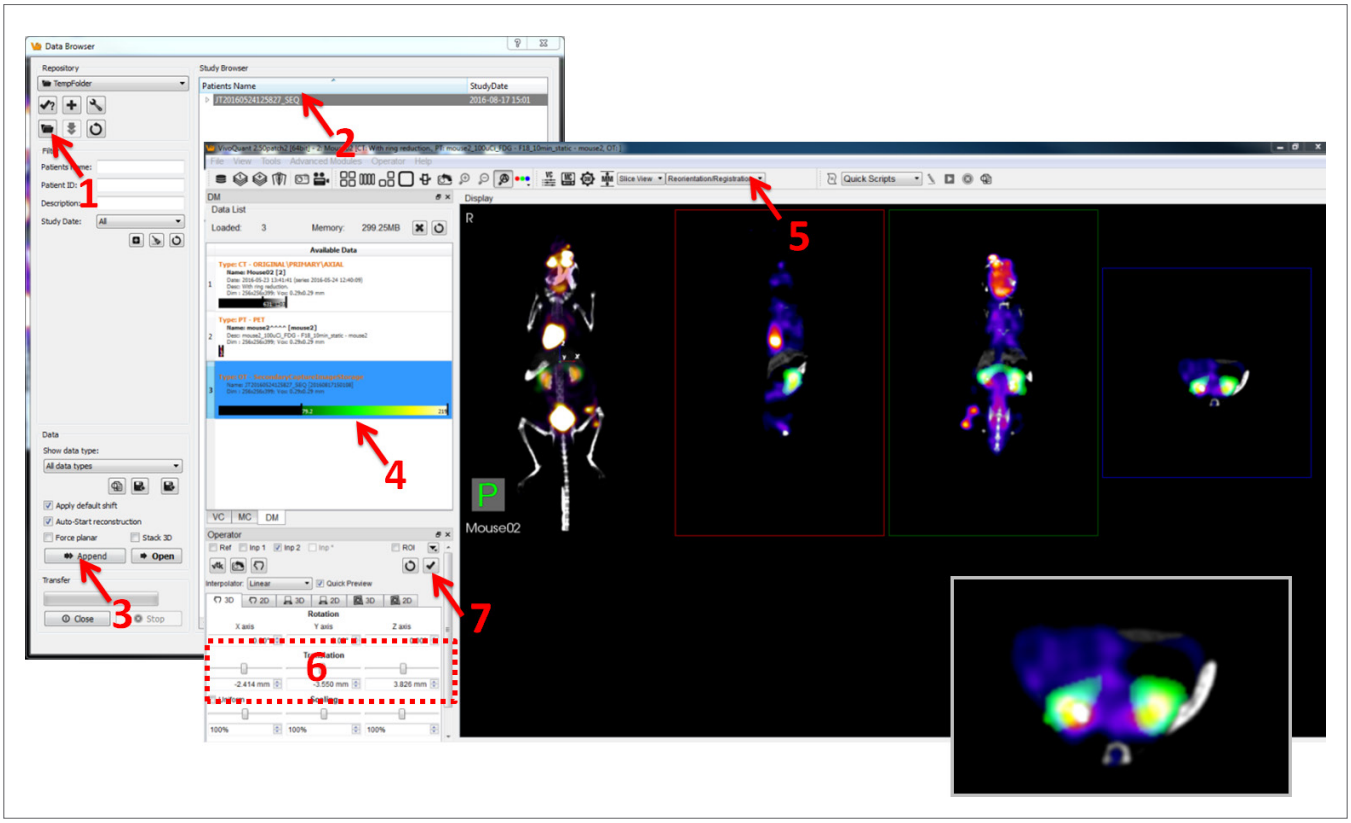


Figure 10. Appending the FLIT DICOM image to the PET/CT images.

With both the microCT and PET images aligned at this point, an optical DICOM image can be imported as a third component (Input 2). FLIT kidney signal for this particular dataset aligns well with both PET signal and microCT contrast agent, and the FLIT alignment procedure to PET/CT is the same as PET to microCT alignment.

Switching Visualization Priority and Changing Color Scheme When Importing More than Three Modalities

At this point, three DICOM image datasets have been imported into the VivoQuant software: 1. microCT (Ref); 2. PET (Input 1); and 3. FLIT (Input 2). Before importing the fourth and final optical DLIT DICOM, it is important to note that, although VivoQuant allows multiple DICOM dataset imports, the software can only visually present three image sets in the viewing window. To replace the FLIT dataset with the DLIT dataset:

1. Use "Open local folder" to look for the target DLIT DICOM folder as shown in Figure 11.
2. Select to highlight the image name.
3. Click the "Append" button to import the image. You will see the color bar of the import will be in gray scale and at the fourth position from the top.
4. Since VivoQuant can only display three datasets for viewing. Right-click on the image title to switch viewing priority.
5. Select "Use as Input 2" to switch with the FLIT image for viewing priority. The DLIT image will show in gray color scale by default.
6. To change the color scheme, right-click the color bar and a list of available color schemes will appear.
7. Select the desire color scheme. In this case, we use Prism 2 for the DLIT image.
8. The process for alignment of the DLIT image (not shown) is identical to the other cases shown above. In this case alignment is performed using PET and DLIT tumor signal.

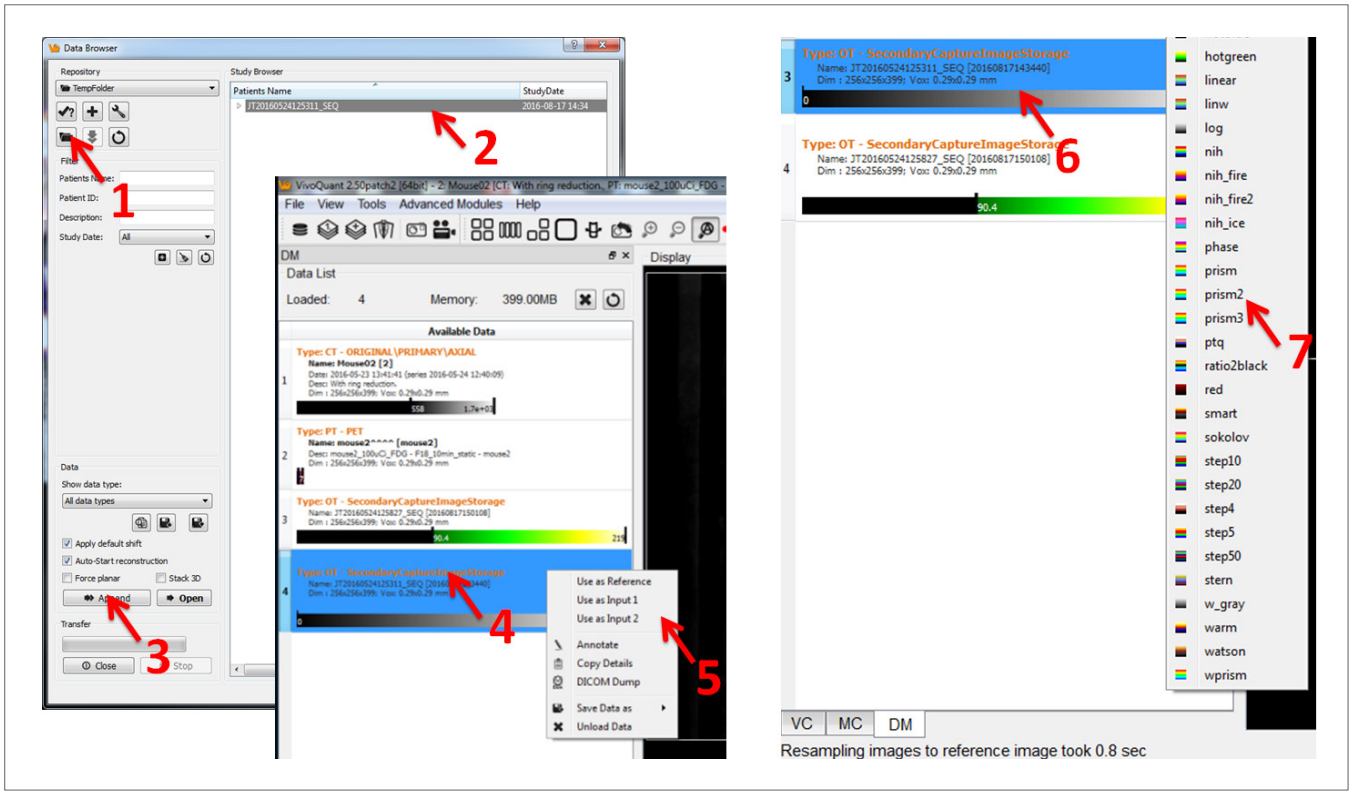


Figure 11. Switching viewing priority between image sets.

There is no visual representation for imports beyond Input 2. Different three modality visualizations can be achieved by switching visualization priority between datasets when more than three datasets are present. The import process is the same as described earlier, however attention must be paid to assigning appropriate Input identities. Color themes can be changed to improve visualization of the different combined modalities.

Saving the Completed Multimodal Image Session for Future Review

At this point, all DICOM images have been compiled and aligned in a single viewing session. Since the entire process involves multiple steps, it is recommended to save the entire imaging session in case the user wishes to review it at a later date. VivoQuant provides a convenient way to save the entire imaging session. Most importantly, the saved session will contain all positioning and thresholding settings in addition to the image datasets. This useful tool can potentially save considerable time and effort if the user decide to come back and wishes to work with the datasets again on the same animal subject again. The session save function is under the File>Session>Save on the main menu bar.

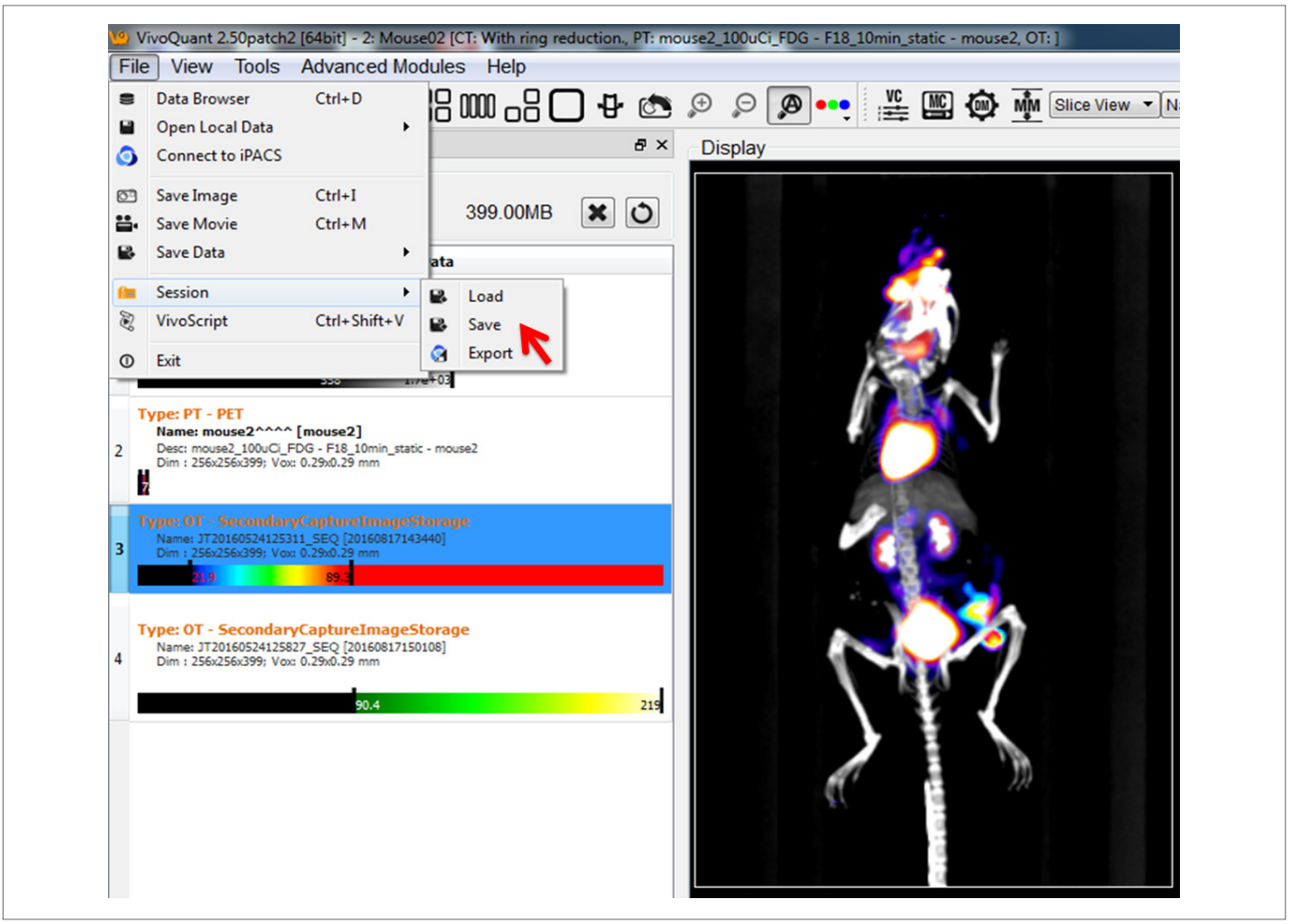


Figure 12. Saving the whole imaging session for future review.

Conclusions

This technical note details the procedures to export DICOM images and combine them into a multi-modality imaging data set using VivoQuant. Since each modality uses different methodology to generate images, the combination of more than one imaging modality could provide a more comprehensive view of the animal model. This technical note demonstrates how to export suitable 3D image files (DICOM) from a variety of PerkinElmer imagers such as the Quantum GX for microCT, the G8 PET/CT for microPET, and the IVIS Spectrum for optical imaging. Detailed, step-by-step procedures of how to align all these images using the VivoQuant software are provided. The note also demonstrates the advantage of multimodal imaging for comprehensive investigation of living subjects.

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