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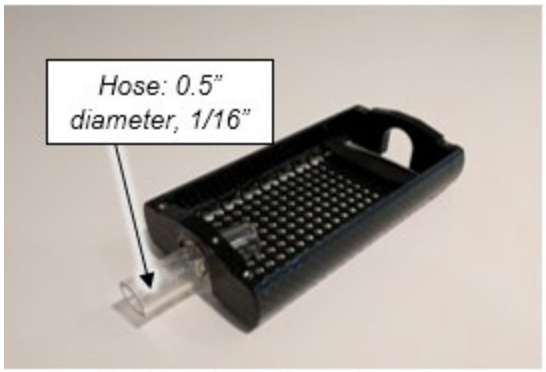
Acquiring Combined 3D Bioluminescence and CT Data (IVIS Spectrum & Bruker Skyscan)

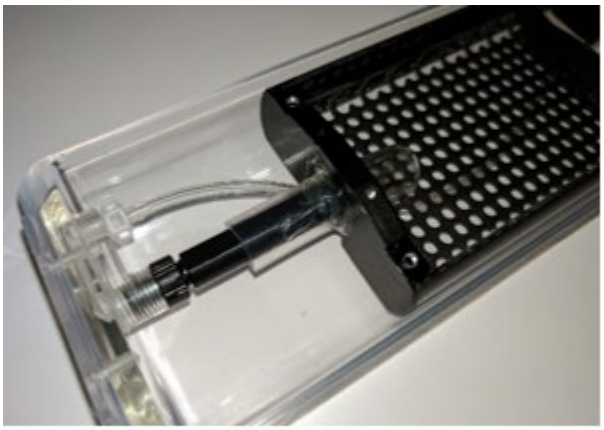
Setup

1. Make sure that the IVIS-Spectrum and Skyscan 1276 are both on and initialized.
2. Block off all but one port in the IVIS anesthesia manifold.
3. Set up DLIT (diffuse luminescence imaging tomography) sequence using the imaging wizard.
   a. Bioluminescence
   b. DLIT
   c. RatChoose type of bioluminescence
   d. Imaging subject > Mouse (Do Not check MIS)
   e. Set everything else as default.
4. Setup Skyscan including calibrating dosage,
   a. Ensure that X-ray source is warmed up.
   b. Slide the tube from the dual imaging cassette (DIC) over the black anesthesia tube in the Rat Imaging Cassette (RIC). See DIC in RIC figure.
   c. Insert the RIC into the instrument.
   d. Set low dose filter.
   e. Set 504 x 336 image mode.
   f. Actions > Scout and batch scanning
   g. Double-Click *ff* to disable flat field (*ff*) correction.
   h. Double-Click empty area to work in.
   i. Ctrl-alt-shift-s to disable safety mode.
   j. Options > Scanning modes > Change exposure time to yield pixel average of approximately 60%
   k. Options > Update *ff* for current mode (Uncheck update only central)
   l. Dosage calibration
   m. Move stage to where mouse will be and go to Options > Dose meter.
   n. Turn off the X-rays and remove the stage.



Dual Imaging Cassette (DIC)

Rat Imaging Cassette (RIC)



DIC in RIC

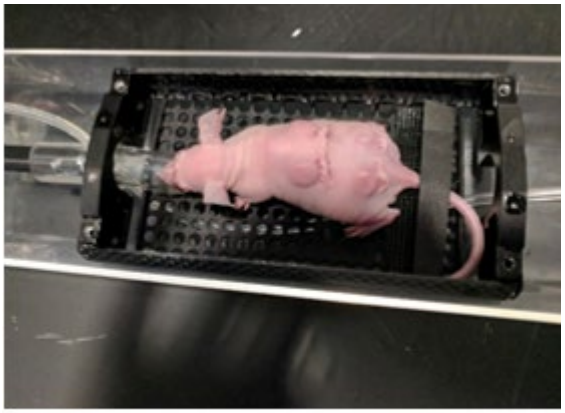
3D Bioluminescence

1. Induce mouse in anesthesia box.
2. Inject mouse with luciferin. Start timer if you are comparing mice.
3. Direct anesthesia flow to imaging chamber and organize mouse in DIC. Slide DIC tube in IVIS anesthesia tube and wait until ~5 min. (Note that if you are comparing mice, this timing must be the same between animals.)
4. Acquire sequence.
5. Tool Palette (usually at upper right) > DLIT 3D Reconstruction > Analyze > Reconstruct
6. Does it look OK? Mouse shape with luminescent blob?

DIC connection to IVIS anesthesia

CT

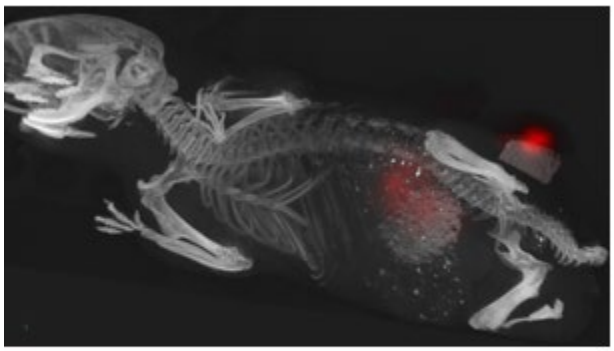
1. Gently slide DIC out of IVIS imaging chamber.
2. Slide DIC hose over anesthesia delivery tube in RIC. See Mouse in DIC figure.
3. Rt-click in scout view > Rescan scout view
4. Ctrl-left-click-drag to determine view (release mouse button first).
5. Fill out scan form that shows up and start scan.
6. Turn on light bulb to get live view.



Mouse in DIC

Reconstruction & Data Alignment

1. Find the recon icon (looks like a candelabra).
2. Open projection images.
3. Set red lines to determine boundaries.
4. Fine tuning > post alignment
5. Save data (need Bitmaps, bmp, for Coreg).
6. Export 3D bioluminescence data: File > Export > 3D Scene as DICOM > Single frame DICOMs
7. Alignment is done using the Coreg module.
8. Output from this module is bioluminescence (red) over CT (greyscale).
9. Visualize with available 3D analysis program.



Bioluminescent cancer cell proliferation within varying tissue implants (Fischbach Lab, Cornell)