MicroCT Reconstruction Protocol

Last updated: 22Apr21 by SN

1. Open GPURecon Server
   a. This software must be open for NRecon to work
2. Open NRecon from desktop
3. Check X/Y alignment : Start Tab → Preview fastest slice → Settings Tab → Show Profile Window (Blue box with curving lines)
   a. If black/green lines match up, no action needed.
   b. If the graph is a complete mess, rescan the fish.
   c. If scan needs XY correction → click action → X/Y alignment w/ reference scan → adjust box to become the volume of tube (fish are horizontally in the fish)), click through all the pictures and make sure is in the box and click the match button. Then hit preview again once completed
4. Switch to the Output tab, then right click and drag to draw a line bisecting the fish sagittally (splits the fish into left+right sides), then hit "update rotation" and confirm to align the fish horizontally
5. Check Beam Hardening: Right click and drag to draw a line through the wall of the tube - tangent lines are longest. This creates a graph of an area that has uniform density.
   a. "Cupping" - graph is bent in a concave manner. Beam Hardening needs to be increased
   b. "Dome" - graph is bent in a convex manner. Beam Hardening needs to be decreased
   c. 20%-40% is standard - our machine usually is set to 41%
6. Ring Artifacts Reduction: Set to 10 in the settings menu → Start → Fine Tuning → Ring Artifacts Reduction → 5 trials with a parameter of 5. Minimize the number of circular distortions in the sample.
   a. If you are trying to minimize ring artifacts in a section of the scan with little tissue, check to see if artifacts manifest in the walls of the tube. Different sections will generally be consistent with one another.
7. Misalignment Compensation: Fine Tuning → Post-alignment → 5 trials with a parameter of 0.5. Minimize the appearance of stretched/distorted tissues or bone and out of focus sections of the sample.

     a. As with ring artifact reduction, Misalignment compensation can be difficult in sections with low area (e.g. tail fin). When in doubt, keep consistent with the base value predicted by the computer and with previous sections.

8. Smoothing: Fine Tuning → Smoothing → 5 trials with a parameter of 1. Adjust dynamic range until you can see noise around the outside of the scanning tube (usually between the origin and the first peak). Minimize the noise visible around the outside of the tube wall.

9. For scans with multiple sections, only the ring artifact reduction and misalignment compensation need to be repeated - smoothing and beam hardening are consistent throughout the scan. Be aware that the software will predict a new value for misalignment compensation for each section - do NOT assume the estimated compensation is identical for each section.

10. File format is set in the Output tab - should depend on what the reconstructed files will be used for. When in doubt, reconstruct as a .BMP file.
     a. Quantitative Analysis = .BMP
     b. Presentation = .JPEG
     c. Email = .PNG
     d. Hi-Res = .TIF

11. Confirm that the Region Of Interest (ROI) encompasses the entire profile of the fish. Preview the widest points in the full X/Y plane, then adjust the edges of the ROI to fit. Do NOT shift the center point of the ROI after initially placing it around the fish.

12. Adjust the Dynamic Range by previewing the densest sections of the fish (should be the two sets of small paired ear bones). Adjust the dynamic range so that the right bar is aligned with the far end of activity displayed on the graph. Be sure to check both sets of bones - while one pair may look denser than the other, the actual density can vary between the two.

13. Adjust the Volume Of Interest by dragging the red lines at the top and bottom of the scan until they are close to the ends of the fish. It's a good idea to rotate the sample to confirm that the fish is within these boundaries at all points.

14. To begin the reconstruction, go to the Start tab and click "Start." Confirm that "Find Z-Connection" and "Apply Fusion" are the only two options selected, then hit OK.

15. When the reconstruction is finished, open the folder of reconstructed images in CTVox (should be in the same folder as the scanned images). Click on one of the black images

- NOT the preview image of the fish in NRecon - and wait for the model to construct. Refer to the CTVox manual for information on how to manipulate the model.

16. OPTIONAL STEP - if you wish to obtain 2D images of the coronal, transverse, and sagittal planes, or if you wish to adjust the X/Y/Z axes of the image, open DataViewer and load the reconstructed dataset.
    a. Adjust the dynamic range or color scheme using the slider and dropdown menu in the box at the top left of the window. Make sure the sample is clearly visible.
    b. Click "Load for 3D Viewing," and open all 3 viewing planes for the sample.
    c. Center the axes on the sample in each plane by double-clicking. Ctrl+LMB and move the mouse to change the *angle* of the axes.
    d. Ctrl+Shift+V to crop the region of interest.
    e. Actions → Save → Select a VOI to save.
        i. Resize should be 1; anything else loses resolution.
        ii. Make sure "Retain Original Intensity" is checked
        iii. Select view of choice.
        iv. Save: filename_rotated