

# MicroCT Scanning Setup Protocol

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## Micro CT Preparation

1. Before preparing sample, turn on microCT computer and run program SkyScan1275.
  - a. Additional window will pop up, select “yes” and proceed
2. Turn on X-Ray to warm up. If the x-ray has been turned off for 8 hours, it will need 15 minutes to warm up. If it has been a few days, scanner will require 45 minutes. If it has been 40+ days, the x-ray will require 2 hours to warm up. Plan scans accordingly.

## Sample Preparation

1. Samples are typically fixed at least 24 hrs in 4% PFA in PBS in the 4C fridge
  - a. Samples can also be fixed in ethanol or stained with iodine or PTA, see staining protocol for more details
2. Select a container that best fits the size of sample, such as a centrifuge tube, 15 mL or 50 mL conical tube. You can prepare a conical tube for this purpose by using a razor blade to *carefully* remove the pointed end.
  - a. Note - these conical tubes should be saved and kept on the microCT bay shelf for future imaging
3. Dry sample with kimwipes and place sample in low density foam (purple color) to prevent movement in imaging container. There should be enough foam so that it is wider than the sample and snug against the walls of the tube. Add a bit of gauze at the bottom of the tube or container and wet with a small amount of PBS (2-3 drops).
  - a. This will prevent the sample from drying out too much during the scan
4. Cover the open top of tube with parafilm if exposed

5. Mount sample onto imaging stage by covering the stage in clay and placing the imaging container in the exact center of stage.
  - a. Extra clay is found in the white microCT tray on the bay shelf where samples are prepped
6. Check for perfect centering of sample by holding stage at eye height arm's length from face. Rotate and look for wobbling. Recenter if necessary.
  - a. Parafilm bottom of tube to stage once it is perfectly centered

### **Imaging Preparation**

1. Turn off x-ray when finished warming up.
2. Place stage into center of rotary nut and turn to the right until it is fully tightened. Close chamber door.
  - a. Take caution that the sample is not touching anything on the inside of the microCT, especially the x-ray source on the left wall of the instrument
3. Turn on x-ray and turn on continuous snapshot. You can lower sample using green arrow buttons at bottom of screen, and make larger by bringing sample closer to the x-ray (therefore adjusting the scanning resolution).
  - a. Scanning resolution settings:
    - i. 1.5 mL centrifuge tube: 8.0um
    - ii. 15 mL conical tube: 10.5um
    - iii. 50 mL conical tube: 35.5um
    - iv. Gelato jar: 48.5um
  - b. Note: Do not update sample position and magnification at same time, update elevation first, then magnification
4. Rotate sample and check that there is white space (air) on both sides of the tube at all times in field of view (FOV). This is to ensure it is centered.
  - a. Small margin of air is fine, but there MUST be air on either side
5. Optimize contrast. Type shift+ctrl+alt+s (this unlocks scanning setting; locked every time you open SkyScan) and open up scanning settings. Adjust voltage.

Use 20-30 kv for soft tissues, and 40-45 kv for hard tissues. Use the maximum amount of current for the voltage (typically set to 500mA).

6. Add a resolution - standard or high, located at the bottom right of the scanning screen. High resolution scans will take much longer than standard resolution scans.
  - a. Generally we only scan at high resolution
7. Add a filter if desired. Filter toggle is located inside the scanning chamber in the back right corner
  - a. Generally not needed for our zebrafish scans
8. Perform a flat field correction-
  - a. If using a small container, lower stage until out of FOV. If using a larger or taller tube, turn off x-ray and remove from scanning chamber.
    - i. Place sample in wooden holder when it is not in machine so that it does not move
  - b. Turn off flat field correction by double clicking "ff" in top left corner
  - c. Right click in the middle of the field of view and change avg exposure so it is as close as possible to 61-62%.
    - i. options -> scanning settings -> increase exposure
  - d. Update flat field references
    - i. Click options- > update flat field references -> and select first and last boxes -> enter
  - e. Turn flat field correction back on, should be an avg. of 90-92%.
  - f. Place sample back in scanning chamber and adjust to correct scanning resolution

### **Scanning Sample**

1. Set Upper and lower bounds of scan. (Double right-click a little above and below the fish)
  - a. Necessary for multi-part scans

2. Hit Start Scan and name file by clicking blue rotating arrow button at the top of the screen
  - a. NOTE: Place an underscore after the file name!
  - b. Create a new folder with the same file name
3. Adjust degree of rotation. Should be between 0.1-0.3 degrees. (This is the degree that that stage turns between taking images.)
4. Adjust Averaging Frame #. Should be 4-6 for hard tissue, and 2-3 for soft tissues.
5. If performing an overnight scan or a scan of something asymmetrical, a 360 degree scan is preferable.
6. Click random movement. (This will minimize ring artifacts.)
7. Hit Scan to start. If doing an overnight scan, select shut down scanner and/or computer after scan
  - a. NOTE: If SkyScan window will not open/maximize, open Task Manager, click on SkyScan program and right click to expand. Select sub-program (SkyScan), right click again and select maximize.