

Recommended sample preparation procedure for samples to be scanned by MicroCT

Collect bones of interest using the standard protocol for bone collection (Ideally, the whole femur or tibia is to be collected to study cortical and trabecular bone but other regions of interest are up to the investigator and the project.

A) Sample Preparation

1. Collect bones of interest using the standard protocol for bone collection.
 - a. Collected bones can be fixed in formalin for 48 hours, followed by 70% Ethanol, then store at 4 degrees until you scan). Any other solution that is appropriate for your specimens (e.g. PBS) is good for the scanner as long as it is immersed in a liquid. Plastic embedded specimens can also be scanned but with some limitations. Note that some of these decisions **will be driven by later histology requirements**.
 - b. Alternatively, remove your bones and wrap it in Phosphate Buffered Saline (PBS) soaked gauze. Freeze/Thaw as necessary. – ***Recommended for samples used for mechanical testing later*** –

B) Scanning Preparation

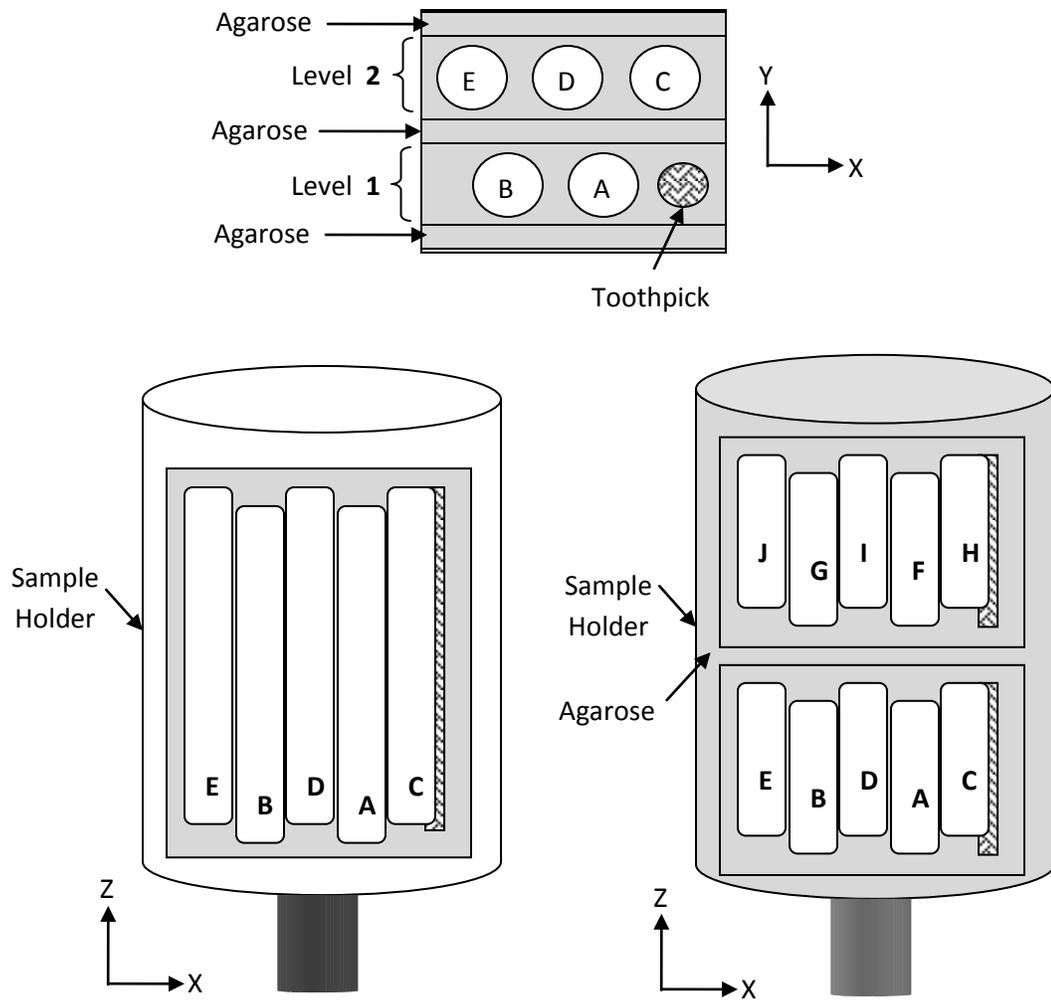
It is of great importance to properly fix the sample in the sample holder, such that there is no movement during the measurement. If a sample changes position during the measurement, the final image will most likely display motion artifacts. We usually use agarose to hold the samples in the tube/vial but it's also possible to use materials like Styrofoam that has low absorption for x-rays. When soft-tissue information is required, agarose may overwrite the soft-tissue boundary information and Styrofoam is recommended. **Within an experiment, use the same scanning medium for all specimens.** The scanning medium significantly affects the x-ray attenuation, with measurements in air being significantly different from the ones scanned in water, saline or Styrofoam.

1. Boil agarose until transparent in a microwave or on a hot plate and allow it to cool before using. Microwave a jar of agarose to liquefy it. It is important for later histology that you **DO NOT COOK YOUR SAMPLES**. Wait until the jar is warm (not hot!) to the touch before continuing.
2. Bone placement:
 - a. Place a short toothpick/pipette tip (about the length of one of your bones) into a dish to serve as an orientation marker or landmark and note down which bone is close to the marker (this will help defining which bone is where).
 - b. Place the bones in level 1 (try to make all the bones as vertically straight (relative to the toothpick) as possible). Make sure all the bones fit into the selected tube/sample holder.
 - c. If it's possible to accommodate more than one layer, place next set of bones on level 2 after level 1 cools down, and pour some more agarose to hold those in place.

Note: Scanning tibia – Try to align the distal TFJ in line for all samples

Scanning femurs – Try to align either mid-point of bones or distal end of bones

Scanning radii – Try to align mid-point of the bones.



3. After agarose cools down (becomes little harder), use a spatula or scalpel to cut out the sample and place it in the selected tube. **Make sure your sample is not sticking out of the tube.**
4. If the tube is long enough, you can also place another layer of samples with layer of agarose in between the two layers.
5. If the sample is loose in the tube or if you're not scanning immediately, fill the tube with agarose and let it harden. If you are doing very long scans or your samples will sit in the machine overnight consider putting a thin cap over the top of the tubes to lessen dehydration effects.