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Histology and Histomorphometry Core

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PROCESSING AND EMBEDDING OF DECALCIFIED FROZEN SECTIONS

1. Always dissect tissue, including bone, in ice cold buffer (saline or PBS) to prevent drying and preserve tissue morphology

2. Place freshly dissected tissue in cold 4% paraformaldehyde (PFA) or 10% formalin in an ice bucket ASAP after harvesting. Use fixative volume at least 20x of the volume of the tissue
   (To make PFA, wamp up 440 mL of dH2O to 60°C on hot plate, do not let the temp get up to 65°C, If it does , cool to 60°C. Weigh 20 g of PFA in hood and stir into prewarded water on hot stir place. Never let the temp get up to 65°C. If it does discard and start over. Add 50 uL of 10N NaOH to help PFA dissolve. After PFA dissolves, add 50mL of 10x PBS, pH 7.4. Check final pH – should be 7.4 and total volume at 500 mL. Store in 4°C overnight or freeze in -20 for up to 2 weeks

3. Decalcify skeletal tissue- Follow decalcification protocol

4. After decalcification (if needed), rinse in PBS 3x
   a. Transfer bone tissue to 5% sucrose/1x PBS for 4 hours at room temp on shaker
   b. Transfer bone tissue to 30% sucrose/1x PBS overnight in 4°C, older bone can be stored 2-3 days on shaker
   c. Transfer bone tissue to a solution that is 50% of the 30% sucrose/PBS and 50% OCT and put into 4°C on shaker – older bones need 2-3 days
   d. Embed in OCT
   e. Submit samoples to Histocore on dry ice with electronic requistion form