



**Title:** Long Bone MicroCT Scanning Followed by Histology

**Revision  
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**Notes:**

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<https://musculoskeletal.wustl.edu/cores/core-b/>

<https://musculoskeletal.wustl.edu/cores/core-c/>

1. Harvest samples fresh and place into 10% NBF. We recommend 15mls volume per bone for fixation of long bones. The bones will remain in the NBF for 24 hours. Samples can be left at room temperature and do not need to be placed on a rocker. For samples other than long bones we recommend placing fresh tissue in 15-30x tissue volume neutral buffered formalin for 24-48 hours.  
([https://musculoskeletal.wustl.edu/files/2021/01/Fixation\\_protocols\\_2021.doc](https://musculoskeletal.wustl.edu/files/2021/01/Fixation_protocols_2021.doc))
2. After 24 hours, rinse the bones 6 times for 15 minutes each with PBS and then put into 70% ETOH. Bones can be stored in 70% ETOH until scanning.
3. Immediately before scanning, embed bones in 2% agar (\*). It is very important that the agar be cool so that it does not cook the samples. We recommend using agar that is about 38 degrees Celsius in temperature.
4. Scan the samples in the MicroCT machine.  
*Note: for our validation study mentioned below, we left the bones in agar for 15 hours.*
5. After removing the bones from agar (and removing all the agar from the bones) place them in 15mls PBS for 3 minutes to hydrate them.
6. If you know you plan to decalcify the bones, place them into the appropriate decalcification solution at this time.  
([https://musculoskeletal.wustl.edu/files/2021/01/Decalcification\\_protocol\\_2021.doc](https://musculoskeletal.wustl.edu/files/2021/01/Decalcification_protocol_2021.doc))
7. If you plan to store the bones for histology at a later date, place them into 15mls each 30% ETOH (30 min) then 50% ETOH (30 min) then finally into 70% ETOH.

\*= See Michael Brodt for further instruction about specimen preparation/scanning if needed.

<https://musculoskeletal.wustl.edu/cores/core-b/notes/>

***Continue reading below for additional background information (experiment and images).***



## Introduction:

The efficient and thorough phenotyping of bone tissues often require multiple evaluations to be performed on the same tissue. For example, it is common to first conduct microCT analyses and then histological evaluation on the same tissue. In such a pipeline, the samples undergo embedding in agar and exposure to room temperatures during the microCT scan process. We thus sought to determine whether agar-embedding and microCT scanning can affect two routine bone histological stains: Hematoxylin and Eosin (H&E), and Tartrate Resistant Acid Phosphatase staining (TRAP).

## Methods and Results:

We used 2 male C57Bl/6 mice at 16 weeks of age. We harvested intact femur/tibia bilaterally and lumbar vertebral motion segments (L1-2 and L3-4) and fixed them all immediately in 10% neutral buffered formalin, rinsed them in PBS, then stored them in 70% ethanol.

One sample of each pair per mouse went straight down the histology preparation pipeline for routine decalcification in 14% EDTA, paraffin embedding and staining in the Morphology and Morphometry Core (Core C). The second sample was routed to the Structure and Strength Core (Core B), where it was embedded in 2% agar (38°C) and scanned with our MicroCT scanner (70kVp, 57uA, 7.4um resolution, ~3 hours scan time). After scanning, the sample was left in the scanning holder at room temperature (20°C) which meant the sample was in agar for 15 hours. After removal from agar the sample was given to the histology core to follow the identical pipeline as the first samples. Both batches of samples were decalcified, sectioned, stained and slides were imaged. We observed there was no detrimental effect from the intermediate step of embedding in agar and MicroCT scanning. The scanned samples looked comparable to the samples sent to histology alone (see Images below).

There were no appreciable, qualitative differences in the appearance of either H&E or TRAP stains, with similar discernment of architecture and cellular detail. Separation artifacts between marrow and bone tissues were also equivalent between the 2 methods. This evaluation was not set up for a quantitative comparison, but the intensity of TRAP staining and distribution of identified osteoclasts was very similar. Since all samples in a given experiment should be treated the same way (ie. with or without prior microCT), the prior scanning of bones should not affect experimental outcomes.

## Conclusion:

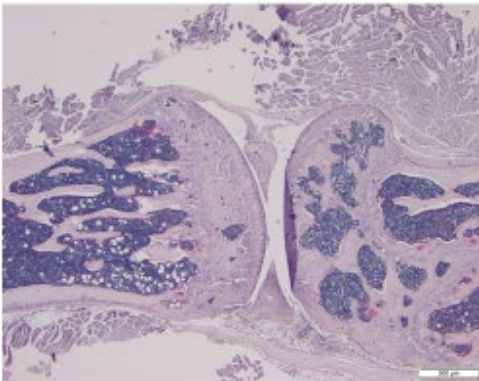
There are no appreciable differences between scanned and unscanned samples that would affect the outcome of an experiment.



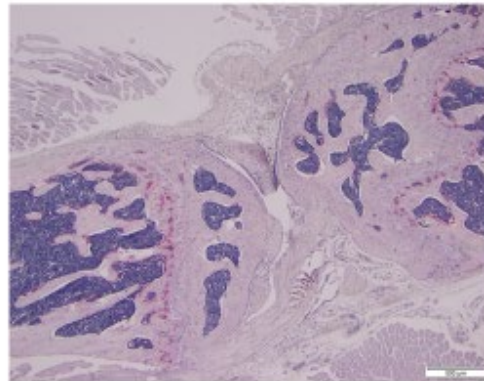
Images from our in-house experiment

## TRAP Staining

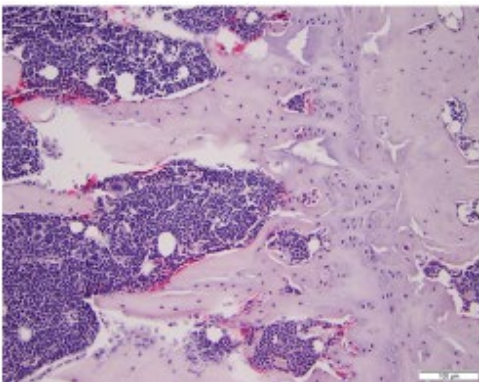
**Knee No MicroCT 4x**



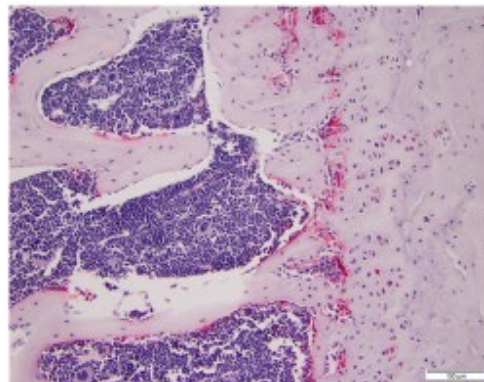
**Knee With MicroCT 4x**



**Knee No MicroCT 20x**



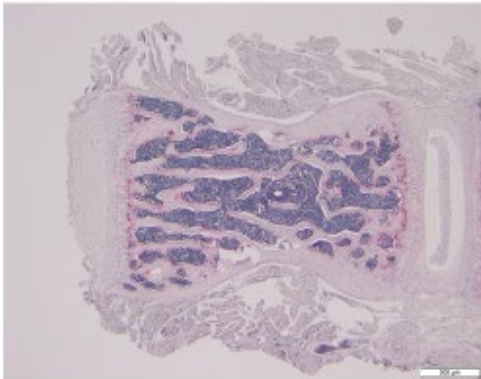
**Knee With MicroCT 20x**



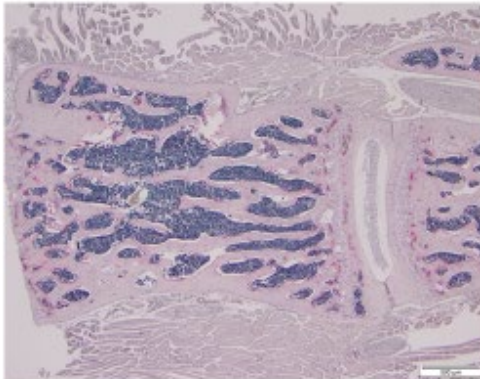


## TRAP Staining

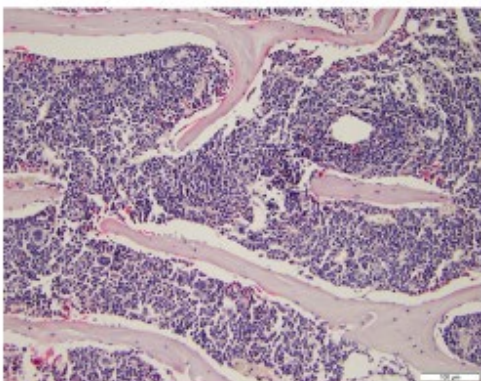
Vertebra No MicroCT 4x



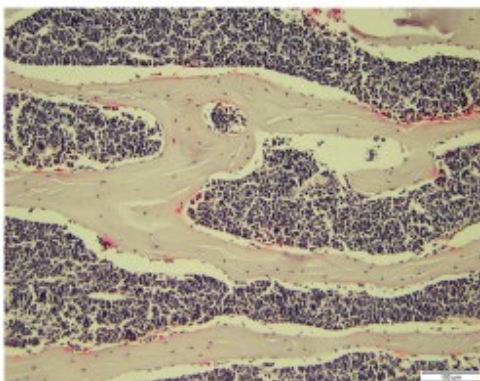
Vertebra With MicroCT 4x



Vertebra No MicroCT 20x



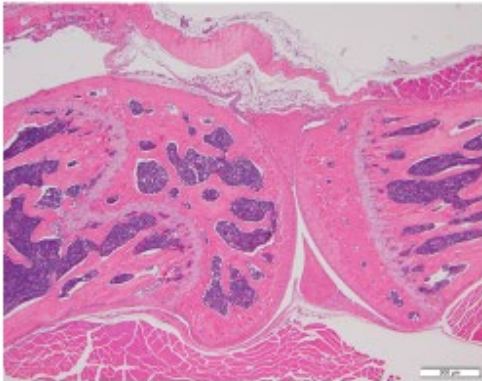
Vertebra With MicroCT 20x



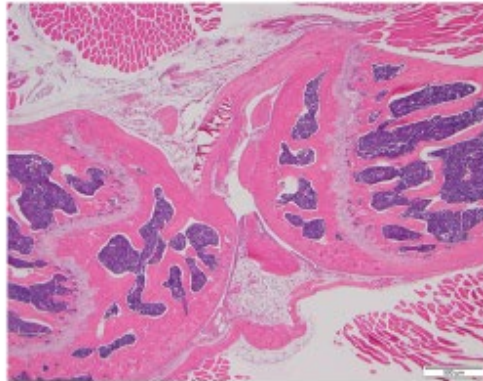


## H&E Staining

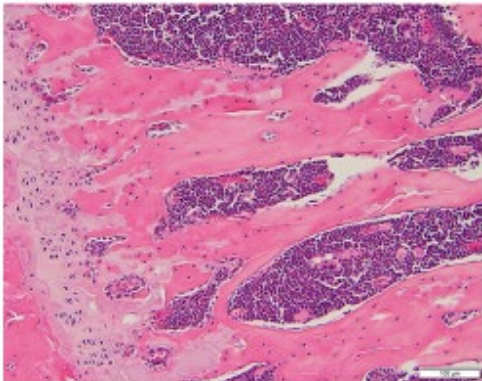
Knee No MicroCT 4x



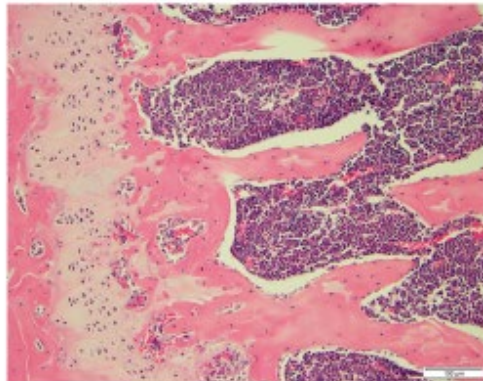
Knee With MicroCT 4x



Knee No MicroCT 20x



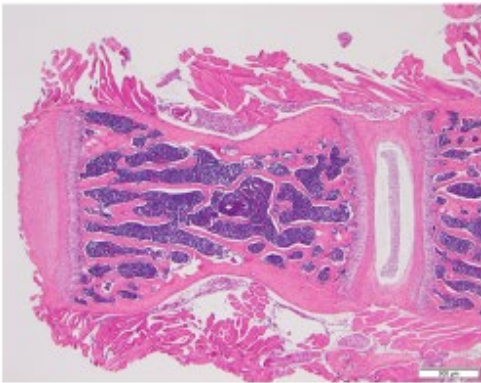
Knee With MicroCT 20x



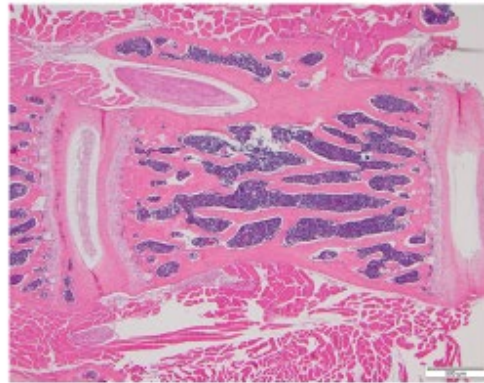


## H&E Staining

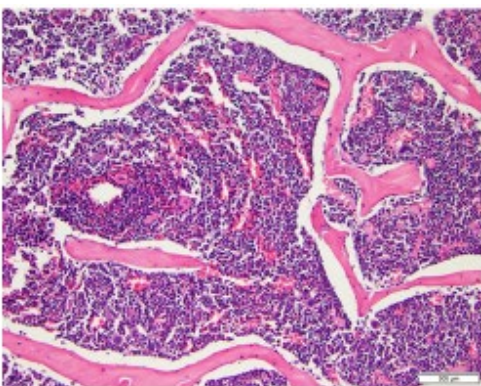
Vertebra No MicroCT 4x



Vertebra With MicroCT 4x



Vertebra No MicroCT 20x



Vertebra With MicroCT 20x

