PCMD Histology Core Learning Lunch Series

Bone Histomorphometry

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Bone Histomorphometry

- Histological methods to assess bone phenotype
- Methylmethacrylate (MMA) embedding (Erben 1997)
  - Mineralized (undecalcified) bone
  - Good penetration into tissue
  - Easy to be removed
- Static histomorphometry (5-μm section)
  - Goldner's Trichrome staining
  - Toluidine blue staining
  - Von Kossa staining
- Dynamic histomorphometry (8-μm section)
  - Fluorochrome labeling

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Standardized Nomenclature

Recommended Reading:

Standardized Nomenclature, Symbols, and Units for Bone Histomorphometry: A 2012 Update of the Report of the ASBMR Histomorphometry Nomenclature Committee

Dempster DW, Compston JE, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR, Parfitt AM.

J Bone Miner Res. 2013 Jan;28(1):2-17. doi: 10.1002/jbmr.1805.

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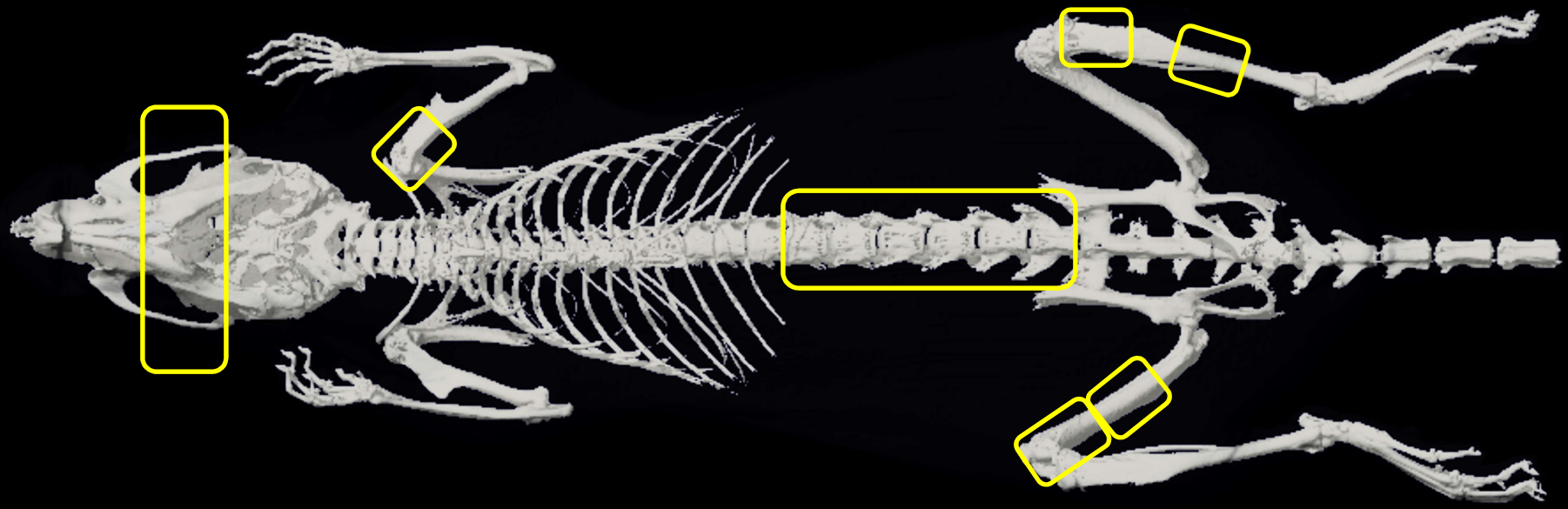
Choice of Skeletal Sites



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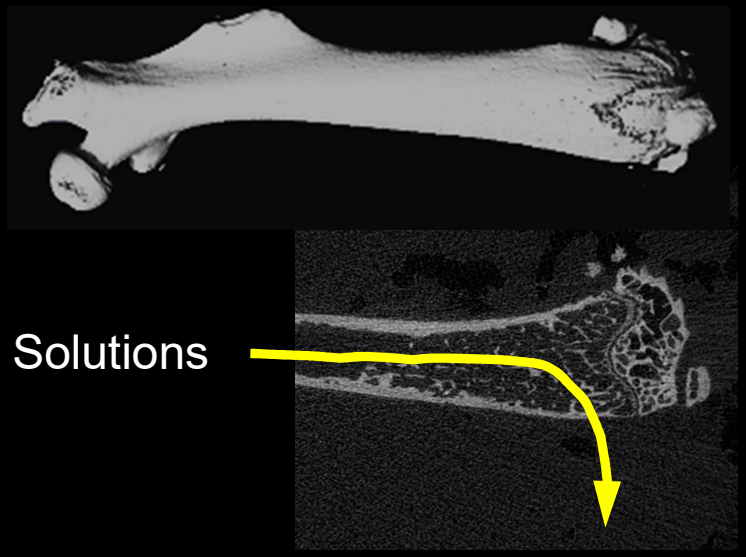
Sample Preparation and Sectioning

- Specimens
  - Harvest (Open the samples)
  - Fixation
  - Dehydration
  - MMA embedding
- Preparation Time
  - 10-14 days



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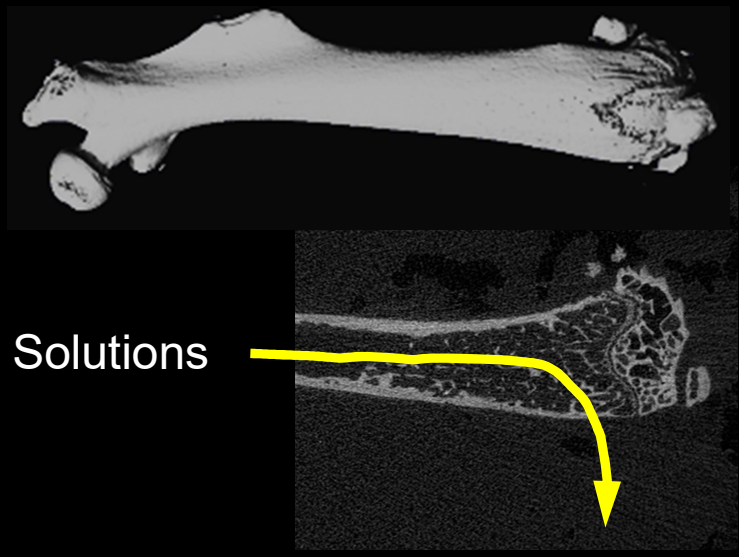
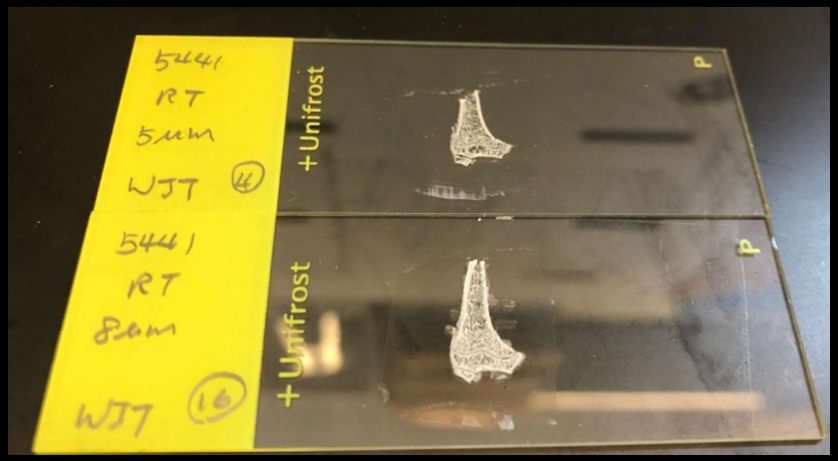
Sample Preparation and Sectioning

- Specimens
  - Harvest (Open the samples)
  - Fixation
  - Dehydration
  - MMA embedding
- Preparation Time
  - 10-14 days
- Sectioning
  - Polycut-S motorized microtome





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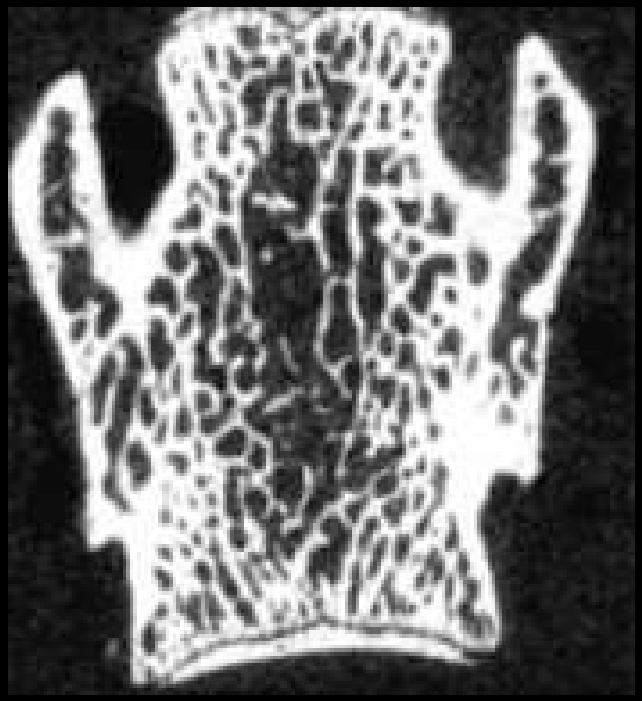
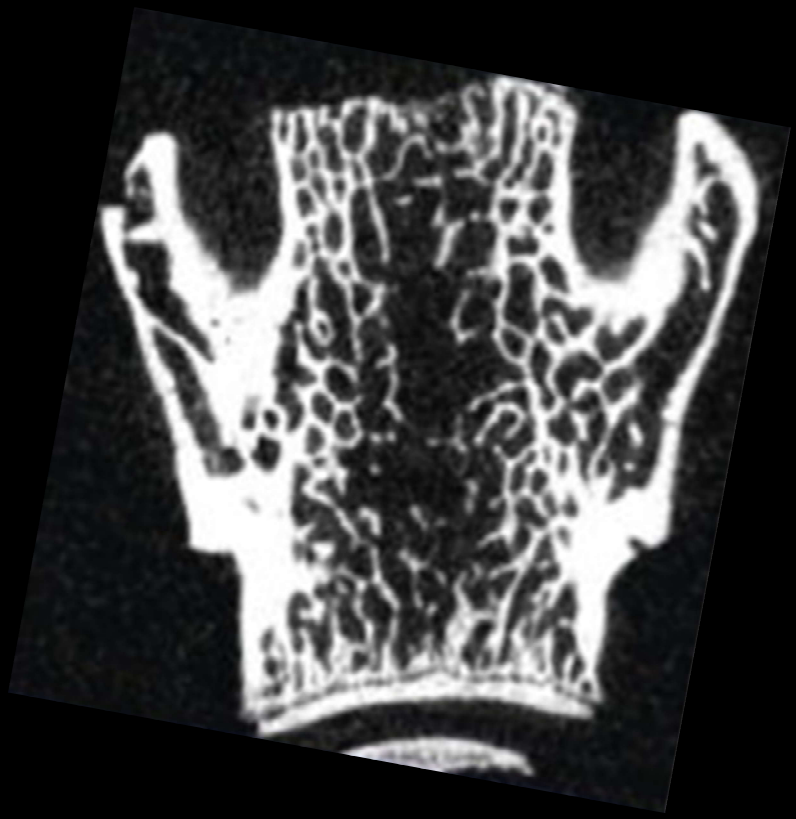
Region of Interest (ROI)





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Lin *et al. Bone Res. 2015; 3: 15028.*

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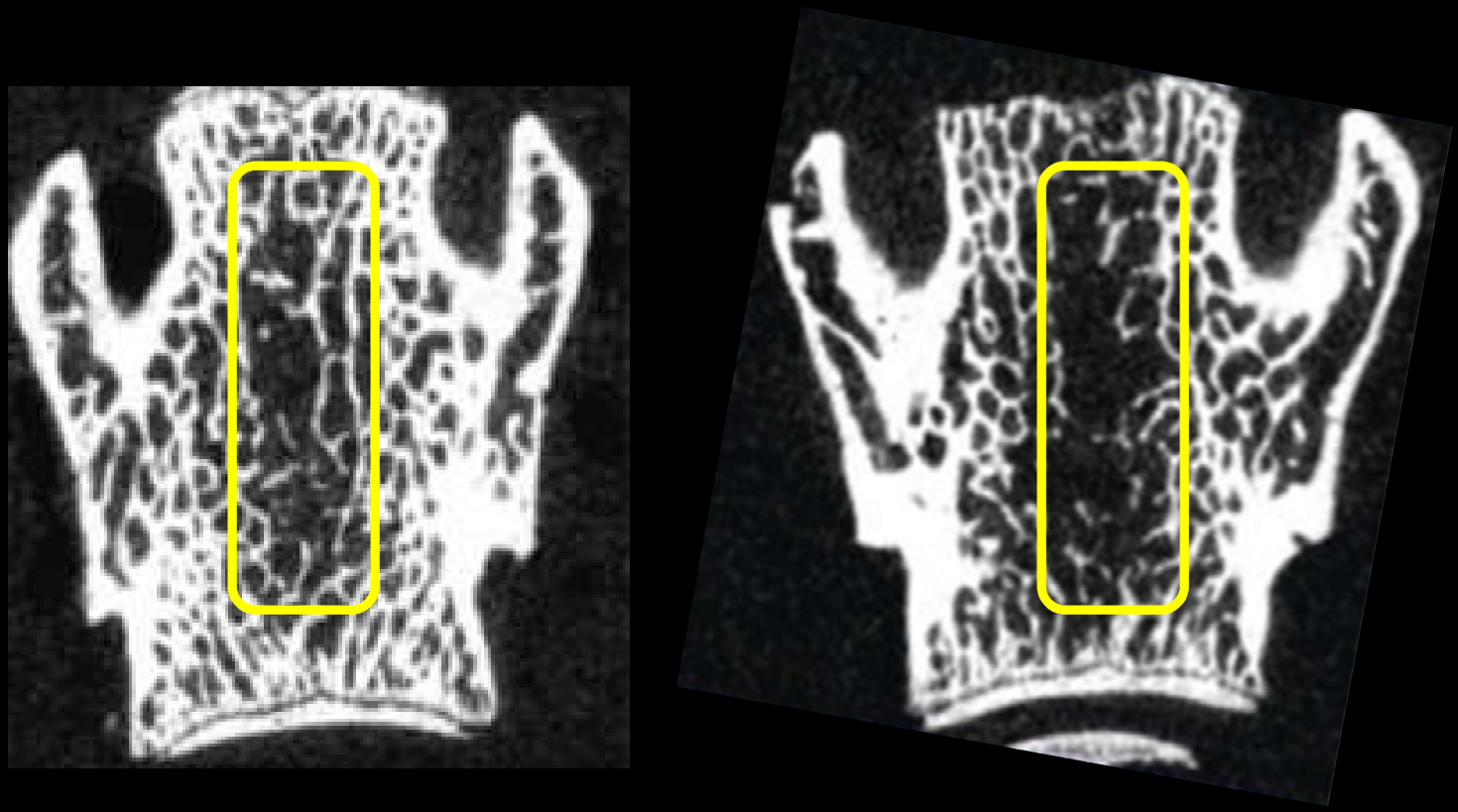

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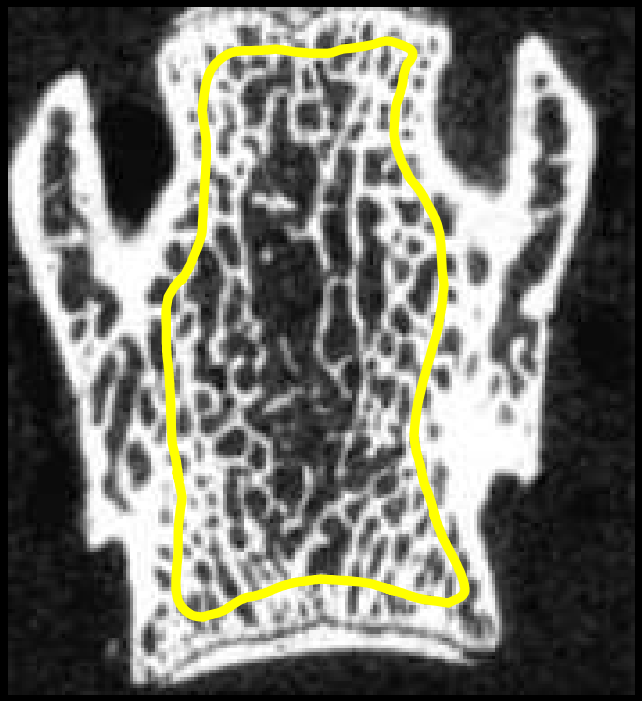

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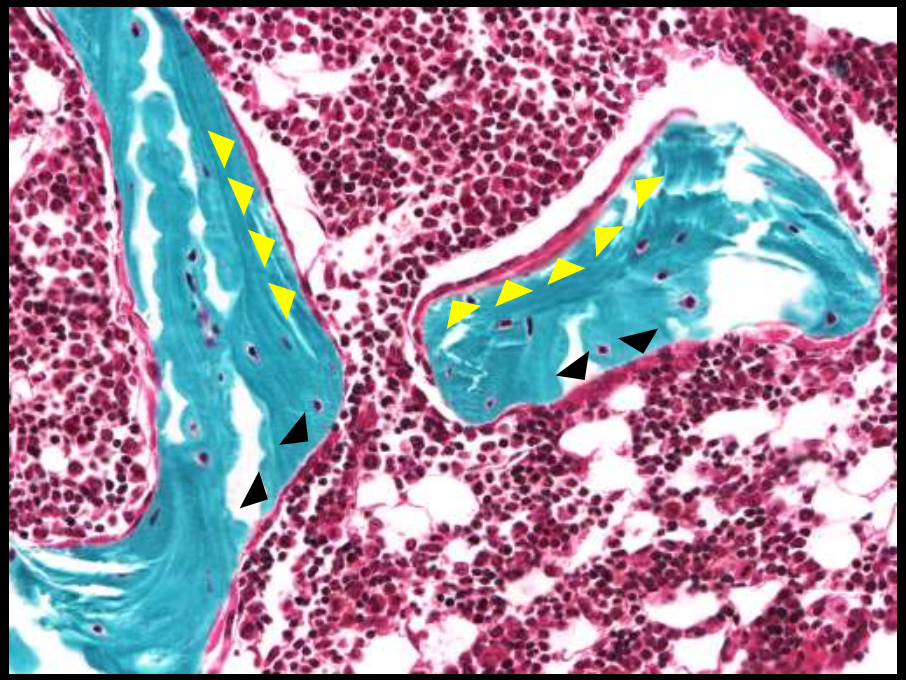
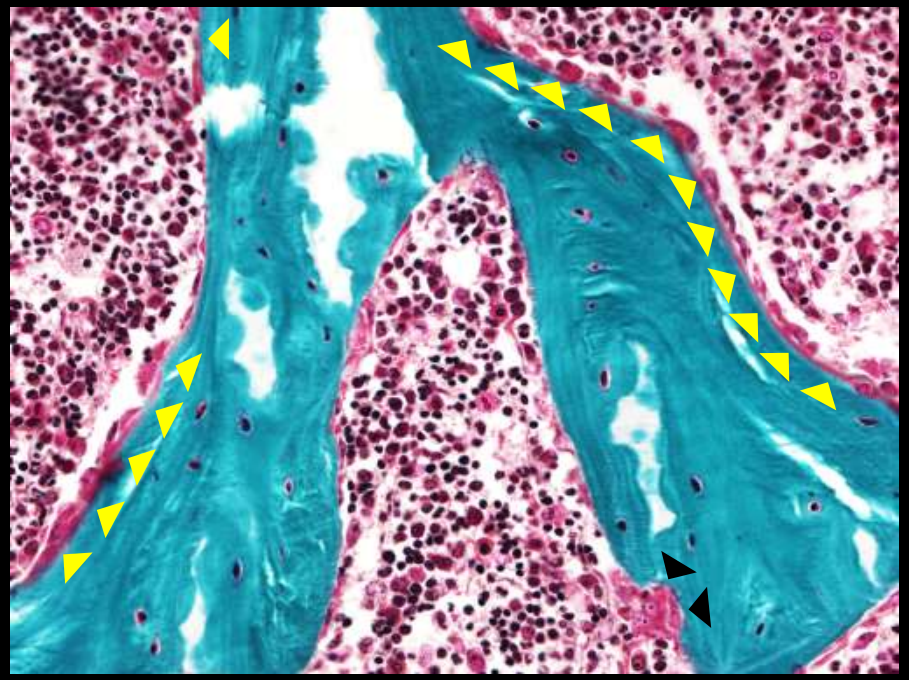
Lin *et al. Bone Res. 2015; 3: 15028.*

Static Histomorphometry

- Goldner's Trichrome staining
  - Osteoblasts, Osteoclasts, Osteoid
  - Parameters: N.Ob/BS, Ob.S/BS, N.Oc/BS, Oc.S/BS, OS/BS





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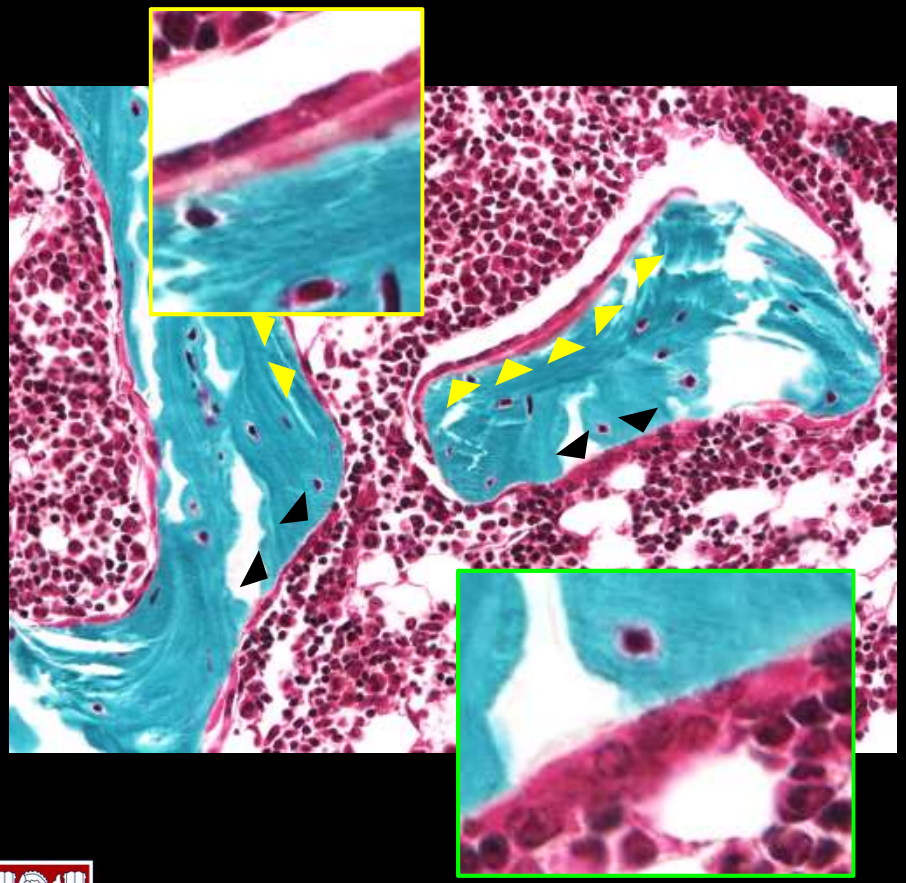

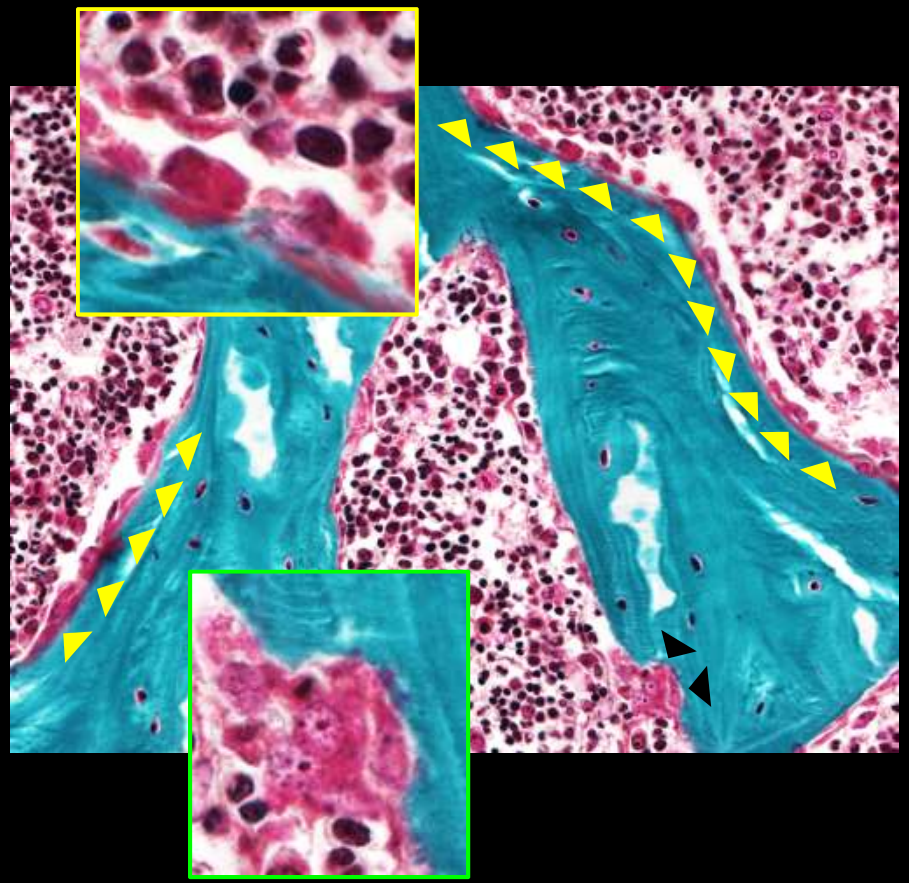

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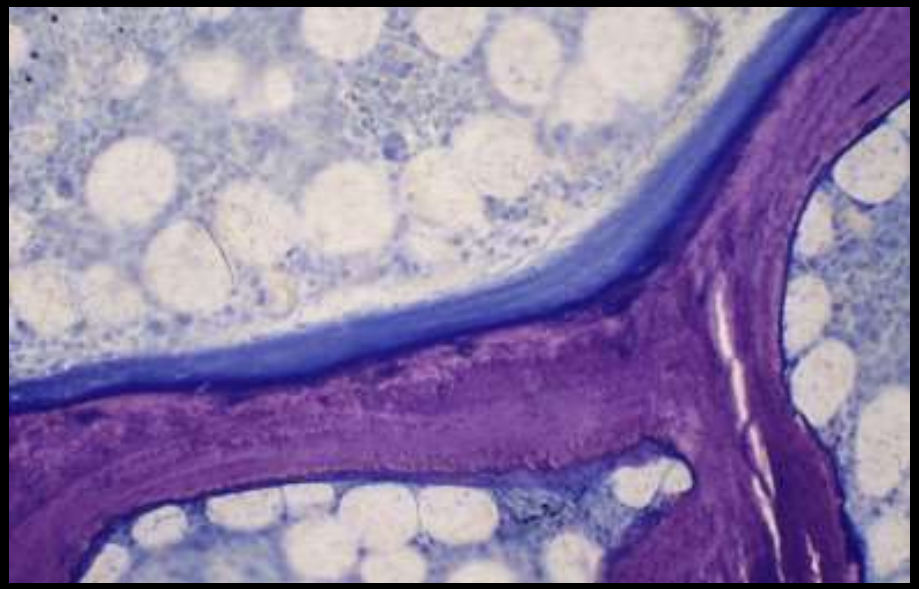
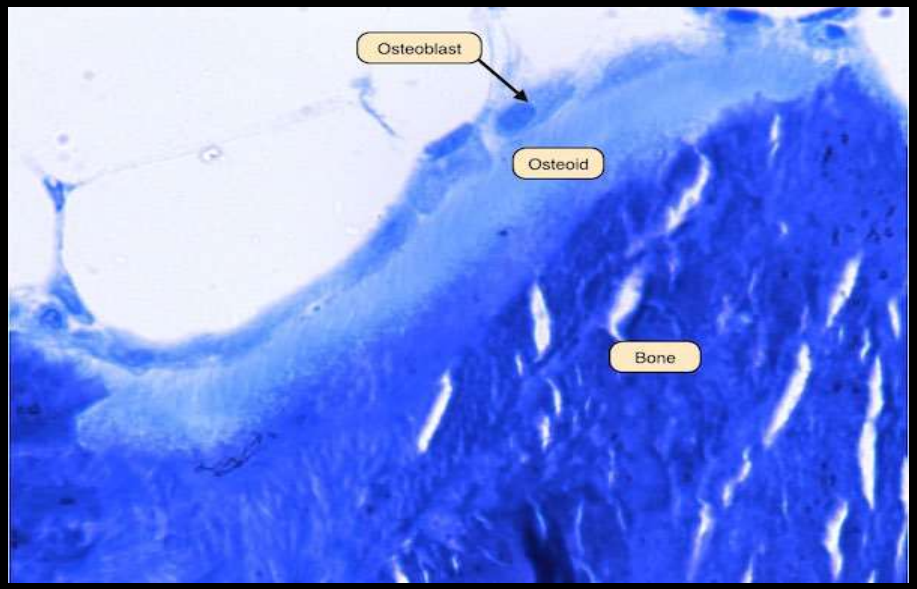
Static Histomorphometry

- Toluidine staining
  - Osteoblasts, Osteoclasts, Osteoid
  - Parameters: N.Ob/BS, Ob.S/BS, N.Oc/BS, Oc.S/BS, OS/BS



https://www.sciencedirect.com/topics/medicine-and-dentistry/osteoid



http://medcell.med.yale.edu/systems_cell_biology/bone_lab.php

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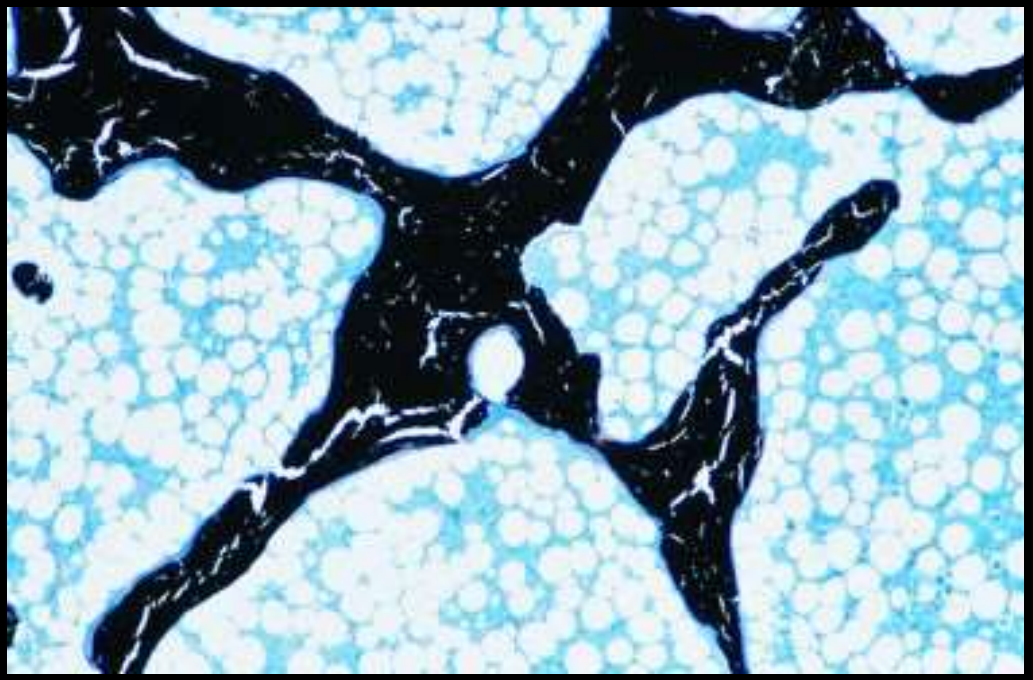
Static Histomorphometry

- Von Kossa staining
  - Osteoid
  - Parameters: OS/BS



https://www.leicabiosystems.com/pathologyleaders/an-introduction-to-decalcification/

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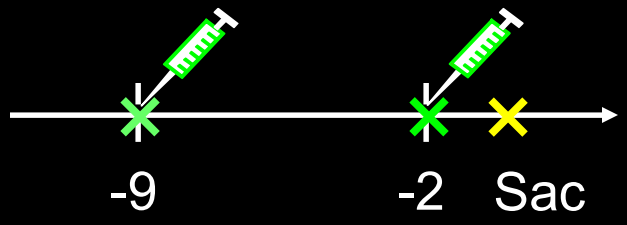
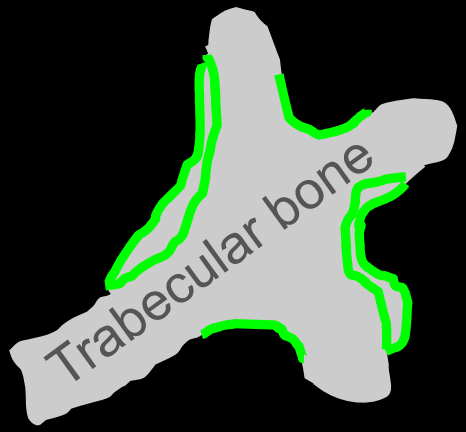
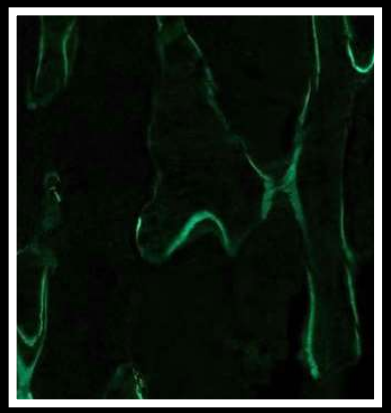
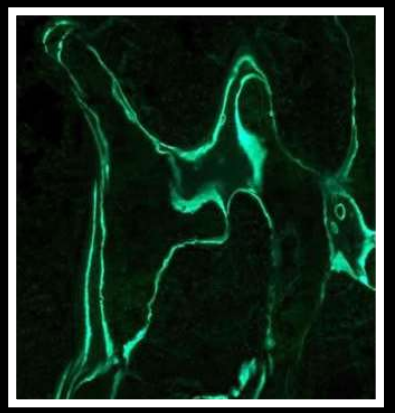
Dynamic Histomorphometry

- Measurement of bone formation
- Fluorochromes
  - Calcium-seeking molecules that bind to the mineralization fronts in bone formation sites
- Fluorochrome labeling injection
  - Calcien (Subq or IP) (5-25 mg/kg)









Altman *et al*. Bone. 2014 Apr; 61: 149–157.

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- Measurement of bone formation
- Fluorochromes
  - Calcium-seeking molecules that bind to the mineralization fronts in bone formation sites
- Fluorochrome labeling injection
  - Calcien (Subq or IP) (5-25 mg/kg)
  - Alizarin Red (IP) (25 mg/kg)
  - Xylenol Orange (IP) (70-90 mg/kg)
  - Tetracycline (IP) (30 mg/kg)

Fluorochromes	Excitation (nm)	Emission (nm)
Calcein Green	505	532
Alizarin Red	530-560	580
Xylenol Orange	546	580
Tetracycline	390	560

http://microspheres.us/microsphere-basics/fluorochromes-excitation-emission-wavelengths/248.html

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Dynamic Histomorphometry

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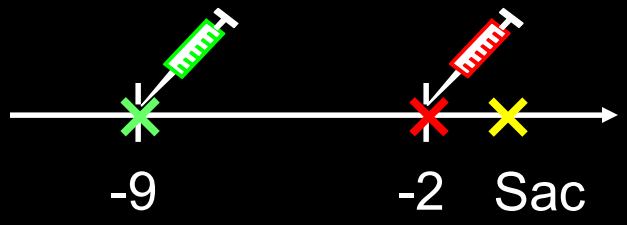

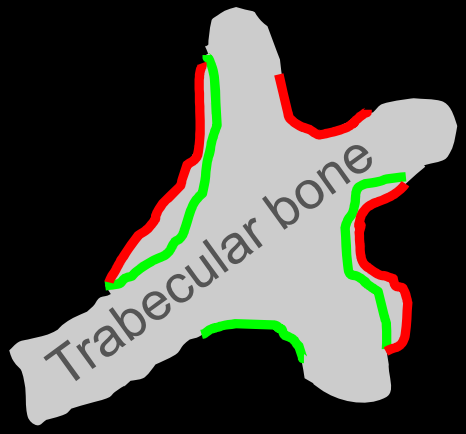

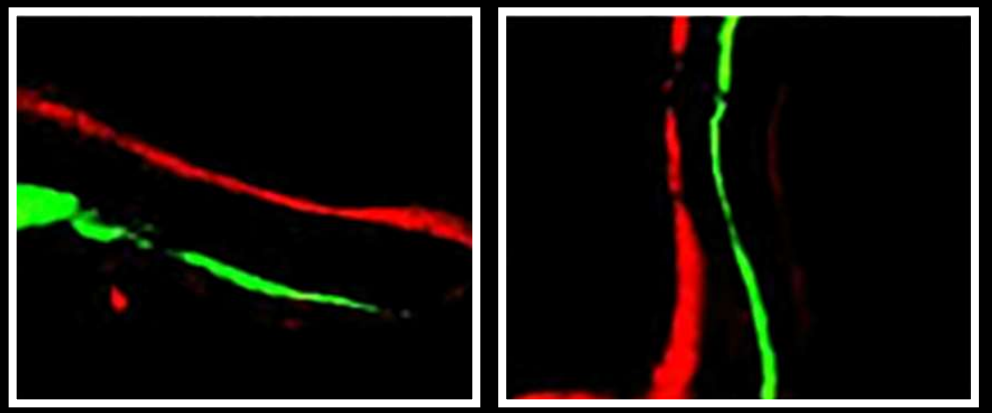

Chandra *et al*. FASEB J. 2018 Jan;32(1):52-62.

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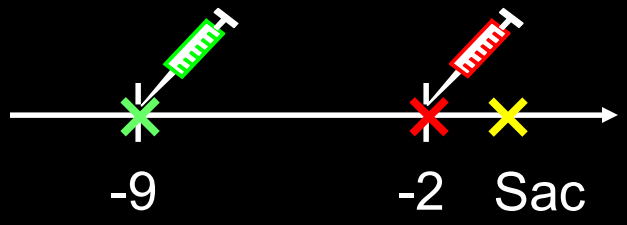

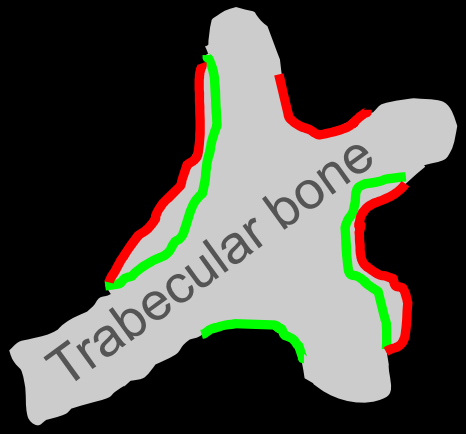

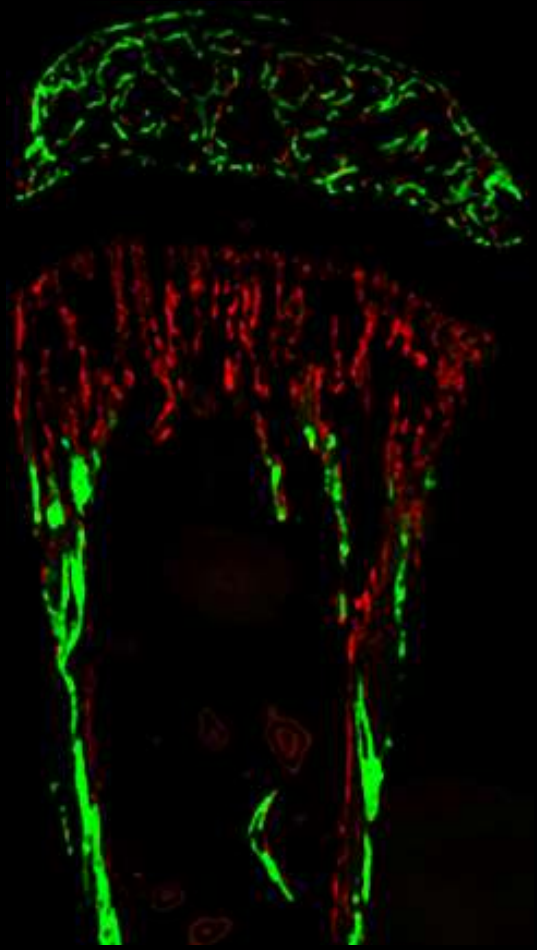

Courtesy by Yu Shi

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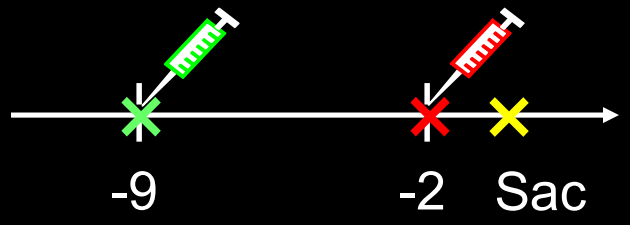
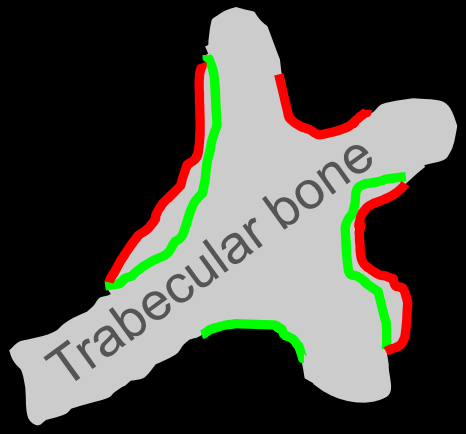
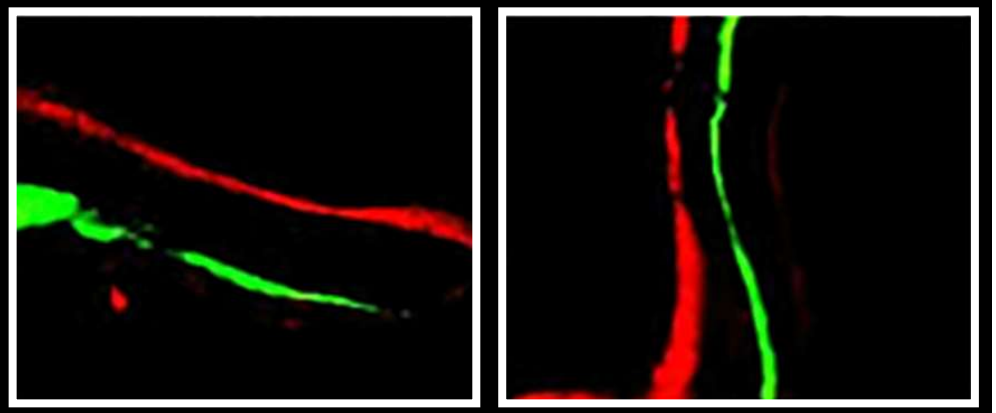
Dynamic Histomorphometry

- Measurement of bone formation
- Fluorochrome labeling injection
  - Calcien (Subq or IP) (5-25 mg/kg)
  - Alizarin Red (IP) (25 mg/kg)
  - Xylenol Orange (IP) (70-90 mg/kg)
  - Tetracycline (IP) (30 mg/kg)
- Injection interval based on the age the animals
  - Young animals: 2-4 days
  - Adult animals: 5-7 days
  - Old animals: 7-10 days







Chandra *et al*. FASEB J. 2018 Jan;32(1):52-62.

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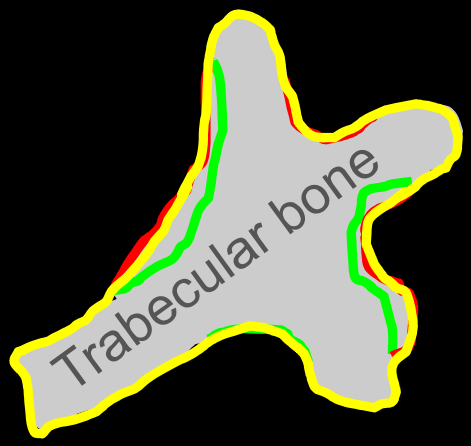
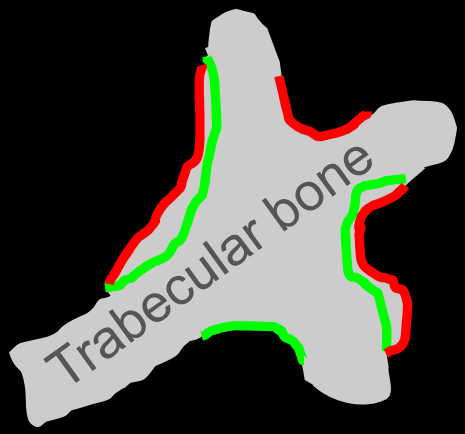
Dynamic Histomorphometry

- Dynamic histomorphometry analyses
  - Parameters: Bone Surface (BS)



Dynamic Histomorphometry

- Dynamic histomorphometry analyses
  - Parameters: Single Label Surface/Bone Surface (sLS/BS)



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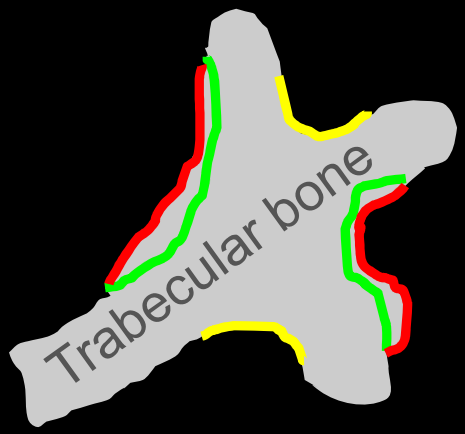
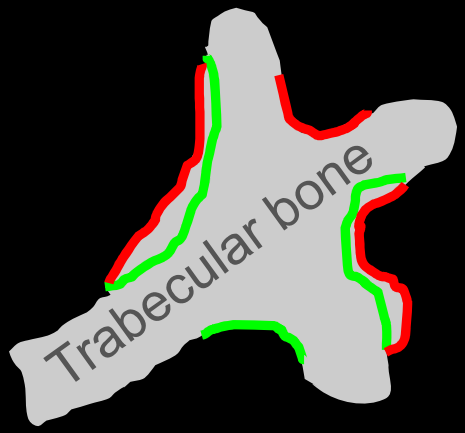


Dynamic Histomorphometry

- Dynamic histomorphometry analyses
  - Parameters: Mineralizing Surface/Bone Surface (MS/BS)
    - The extent of bone surface actively mineralizing



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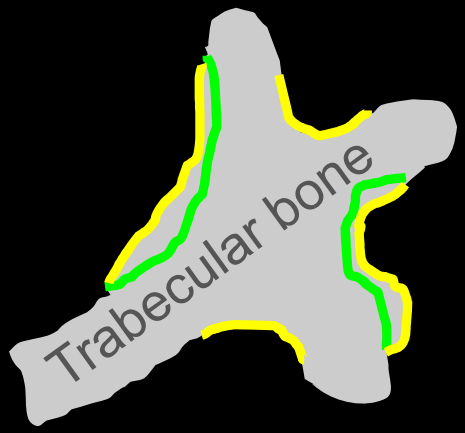

    - MS/BS
      - report actual value (zero is proper)

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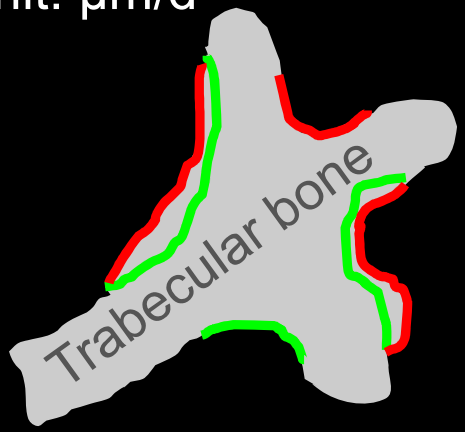
Dynamic Histomorphometry

- Dynamic histomorphometry analyses
  - Parameters: Mineral apposition rate (MAR) = Ir.L.Th/Ir.L.T
    - The distance between the midpoints or between the corresponding edges of two consecutive labels (Interlabel thickness, Ir.L.Th), divided by the time between the midpoints of the labeling periods
  - Unit: μm/d



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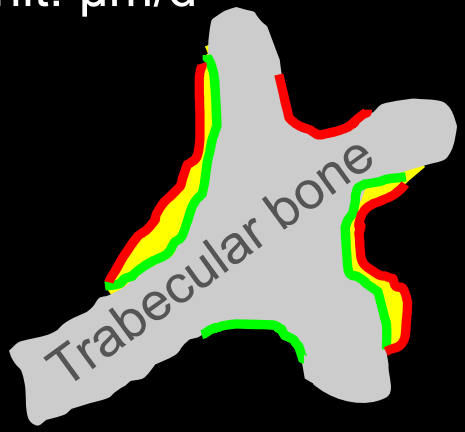

    - MAR:
      - report “missing value” when no double labels
      - the option of assigning (imputing) a minimum value to MAR
        - » 0.1 μm/d or 0.3 μm/d (lowest measurable average value)
      - report actual data but with notes with insufficient double labels

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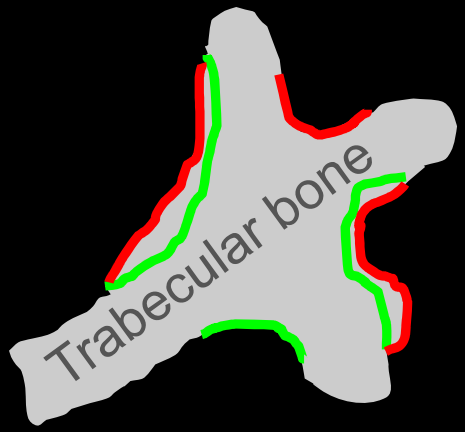
Dynamic Histomorphometry

- Dynamic histomorphometry analyses
  - Parameters: Bone formation rate (BFR/BS) = MAR*(MS/BS)
  - Unit: $\mu m^3/\mu m^2/d$



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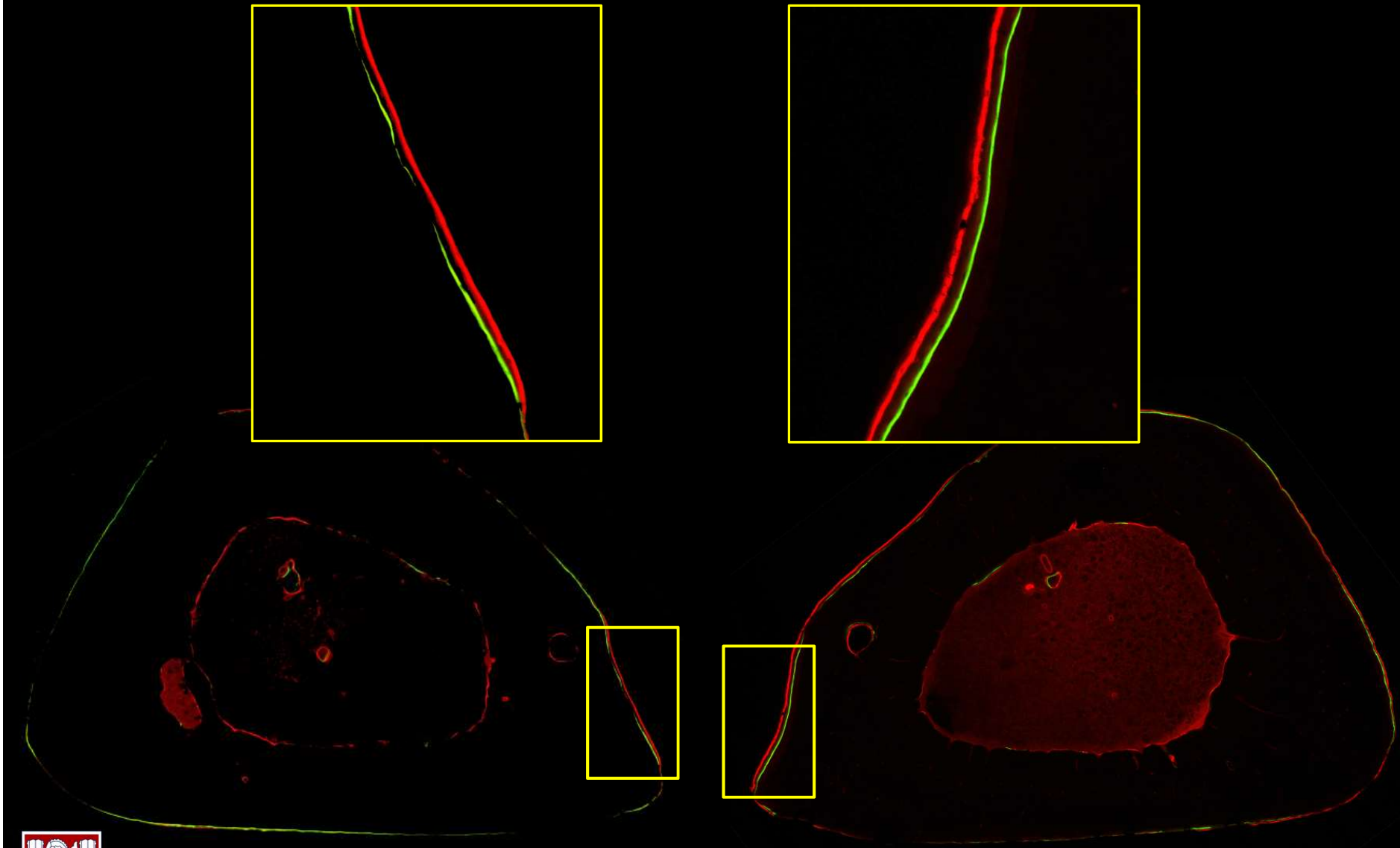
Dynamic Histomorphometry



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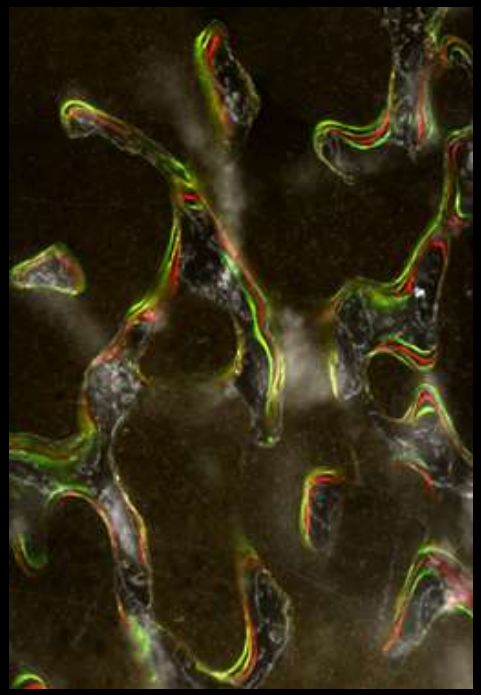
Courtesy by Yihan Li

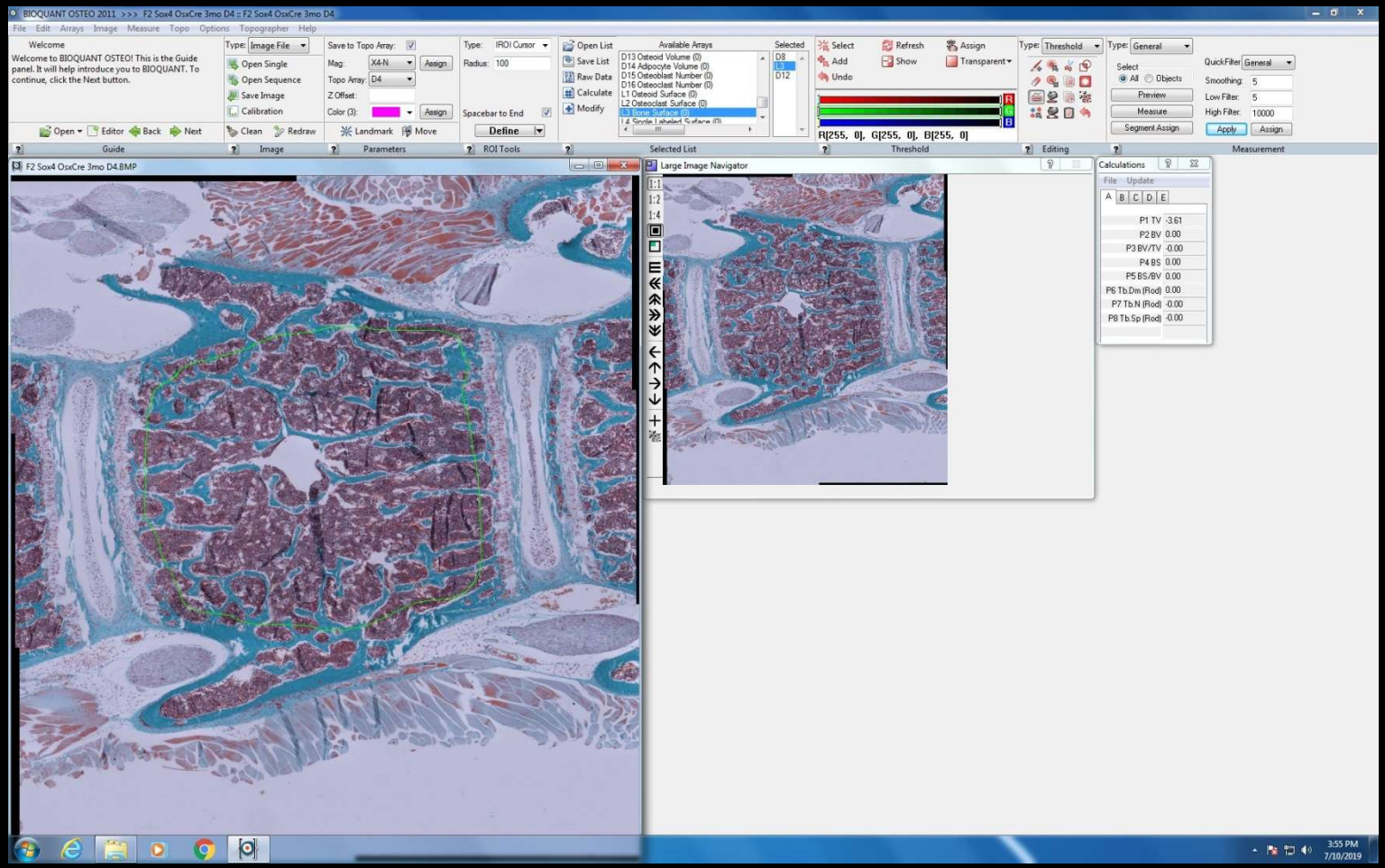
Alternative Methods

- Plastic (methylmethacrylate) embedding/sectioning
  - mineralized (undecalcified) bone
  - 8μm section (dynamic histomorphometry)
  - 5μm section (static histomorphometry)
- Cons
  - 10-14 days to process the samples (ready to section)
  - difficult to section (wrinkling)
- Frozen sectioning with tape technique
  - 2-3 days to process the samples
  - relatively easy to section
  - only for dynamic histomorphometry



Analysis Software

- ImageJ software
- Bioquant Osteo software
  - PCMD Histology Core



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Questions?

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