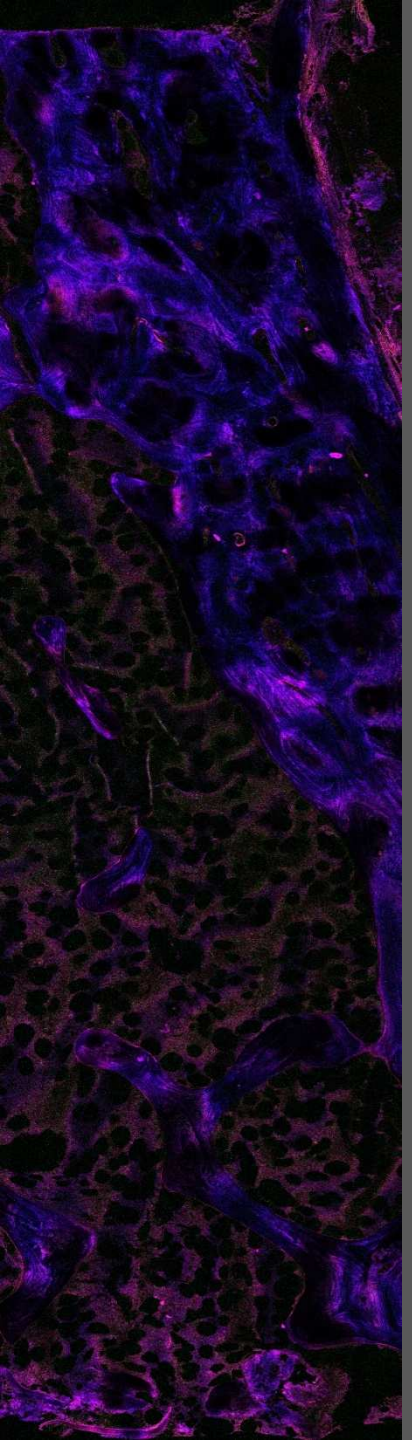

Use of Laser Scanning Confocal Microscopy to Detect Diagenetic Changes in Bone

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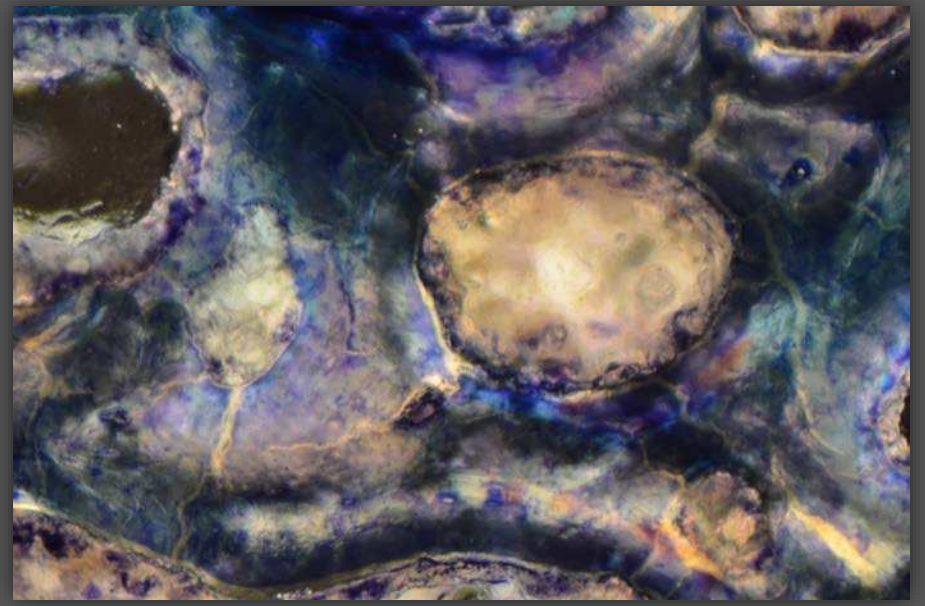
Austin, TX

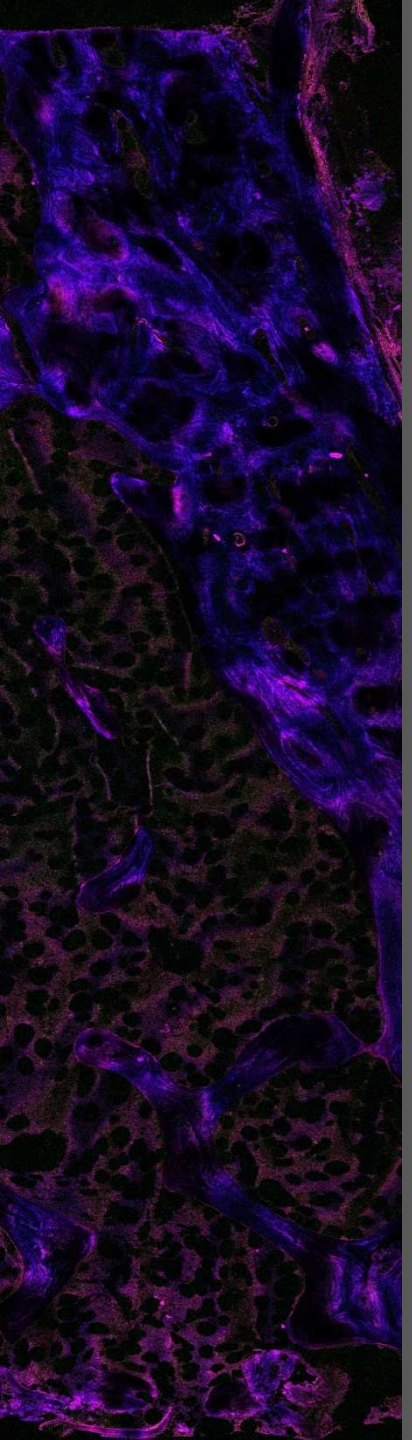
UNIVERSITY OF TORONTO MISSISSAUGA

Diagenesis

- Histological postmortem alteration of bone
- Mineral and Biological origin
  - Often seen as destruction or mineral changes
- Need to isolate diagenetic alterations from antemortem bone formations
- Allows for recovery of usable sections of diagenetically altered bone

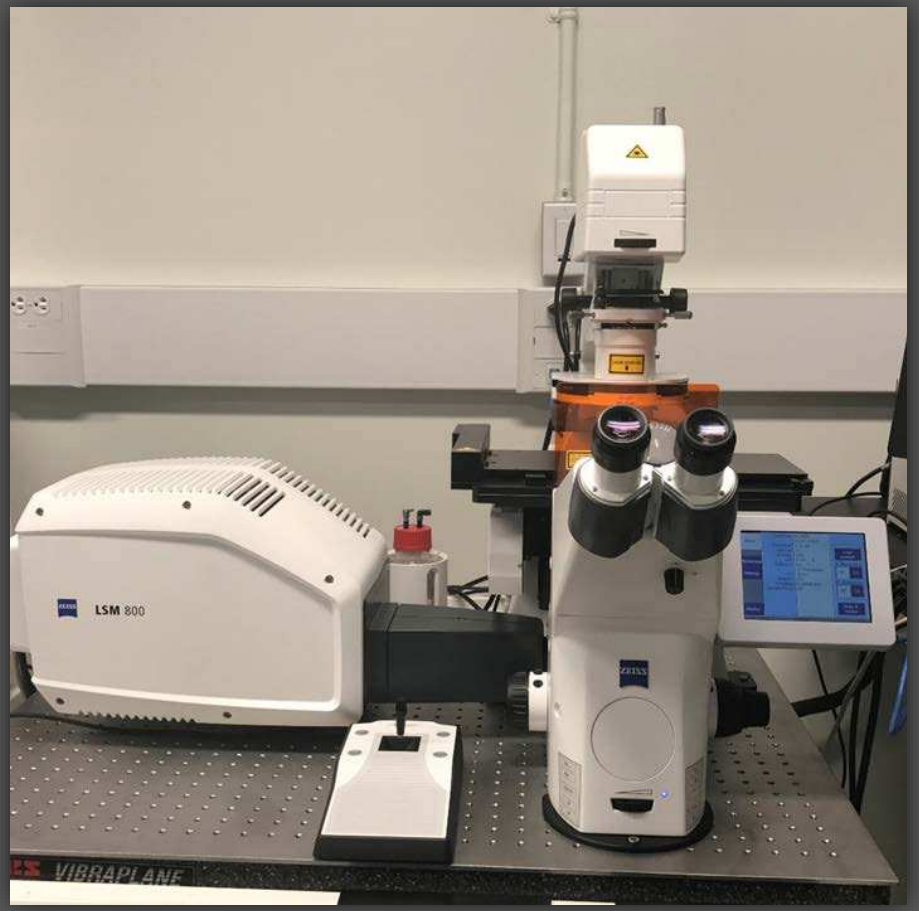


Apollonian rib sample 5th – 2nd Century BCE

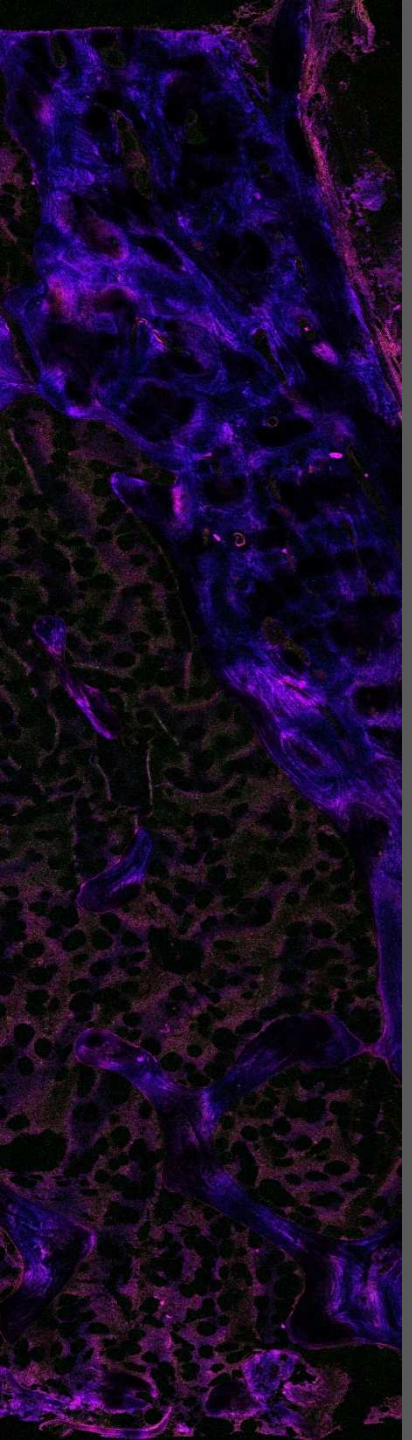


Laser Scanning Confocal Microscopy

- Principles of Light & SEM
- Laser light
  - Specific excitation & emission wavelengths (λ)
    - 405nm, 488nm, 543nm, 633nm λ
- Targeting of specific materials
  - Quantification
- High-resolution, high-magnification images
- Images can be taken in multiple slices along the Z-Axis
  - Allows for 3D microscopy

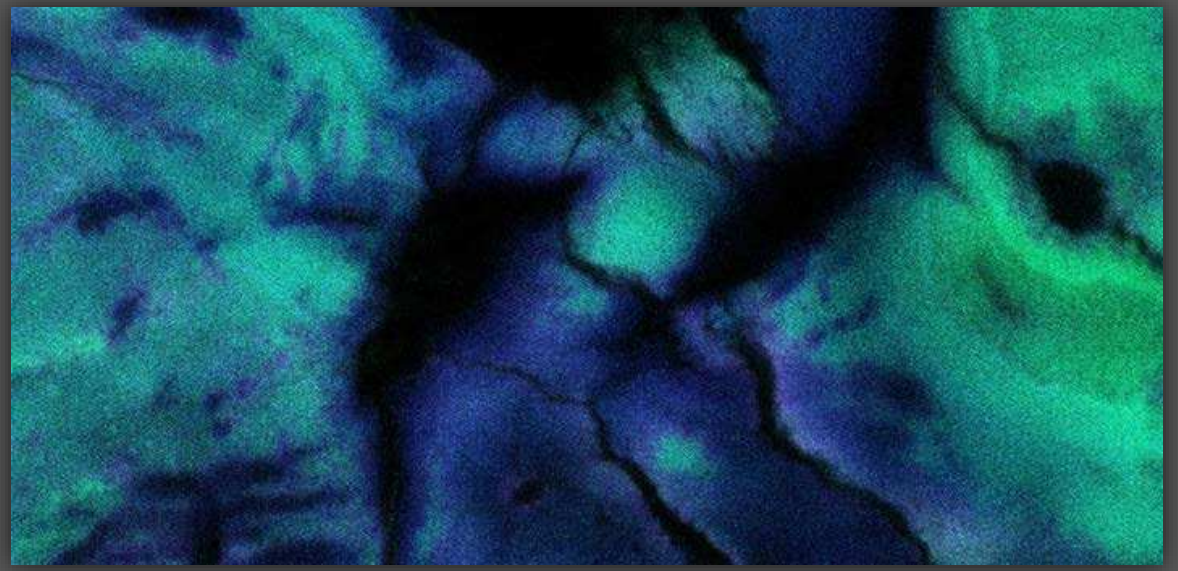


LSM 800 (University of Toronto)

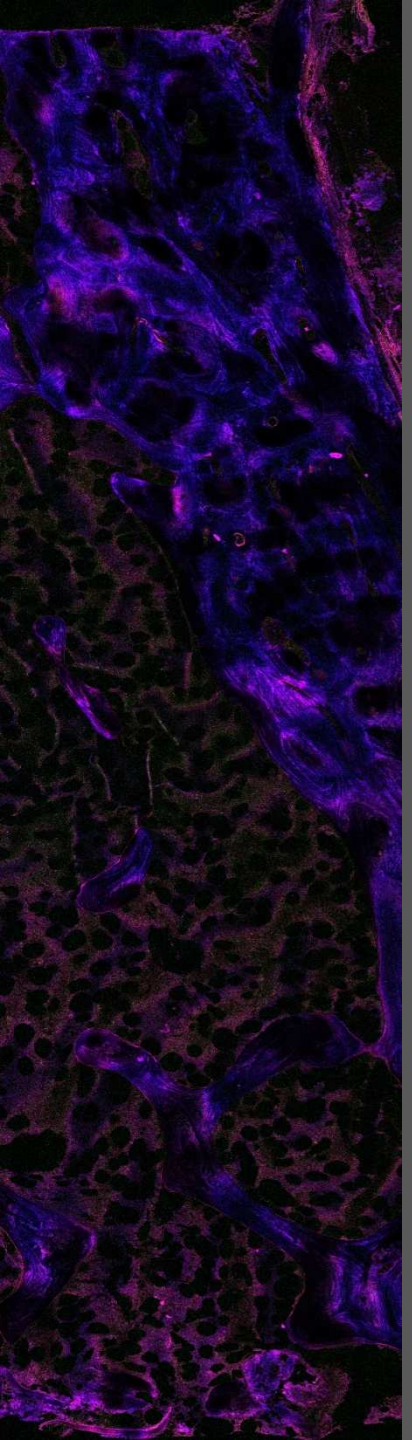


Impetus for the Project

- Accident
- Demonstrating power of LSCM
- History of using clinical samples
- Diagenetically altered sample
- Discovered anomaly

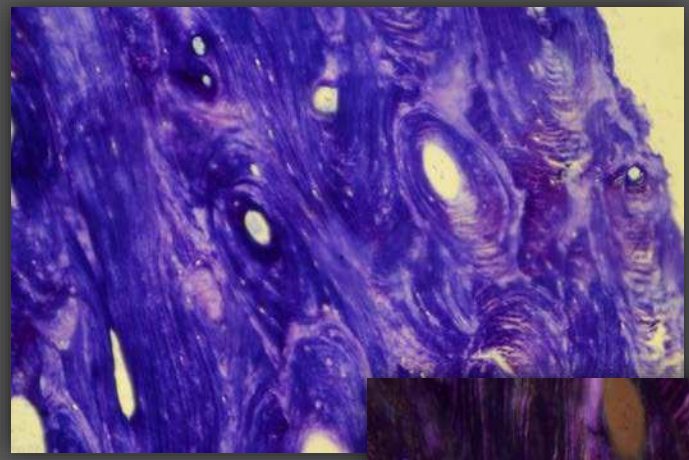


Diagenetically altered rib (5th – 3rd C BCE Apollonia), LSCM & Toluidine Blue, 64x Mag

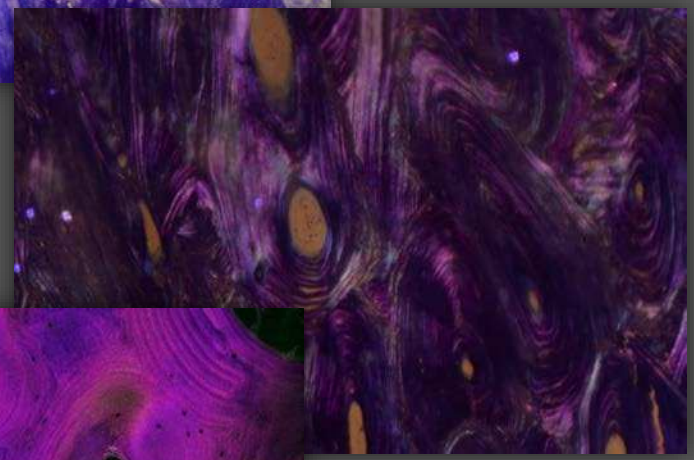


Methodology

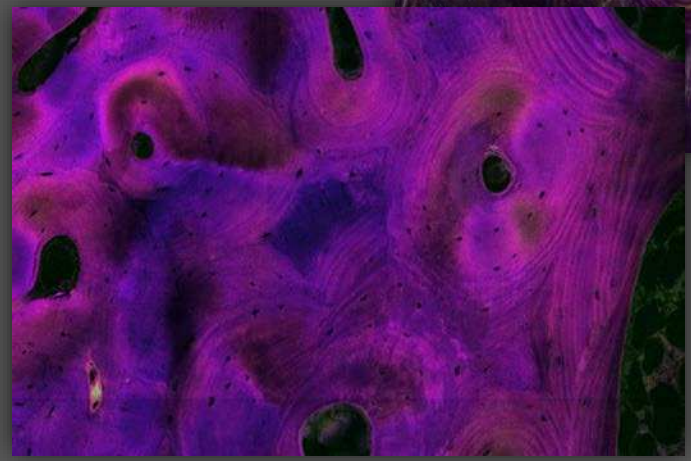
- *N*=27
  - Clinical samples *n*=16 (Iliac)
  - Apollonian samples *n*=11
    - 5th – 2nd Century BCE (Rib)
- Stained with toluidine blue
  - Acid etched with formic acid for stain penetrance
- Clinical samples pre-stained by CHUM



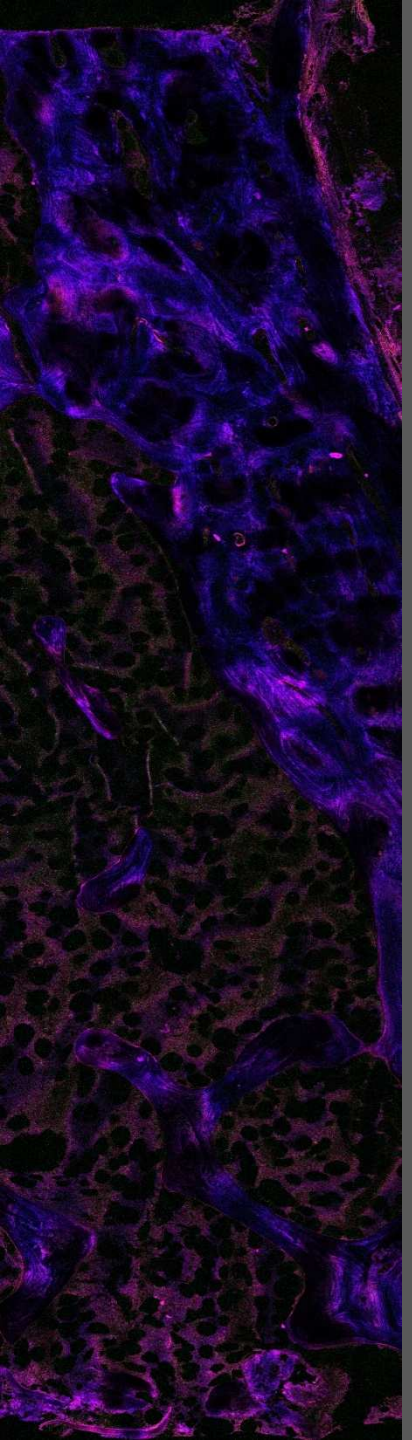
Non-polarized Clinical



Polarized Clinical

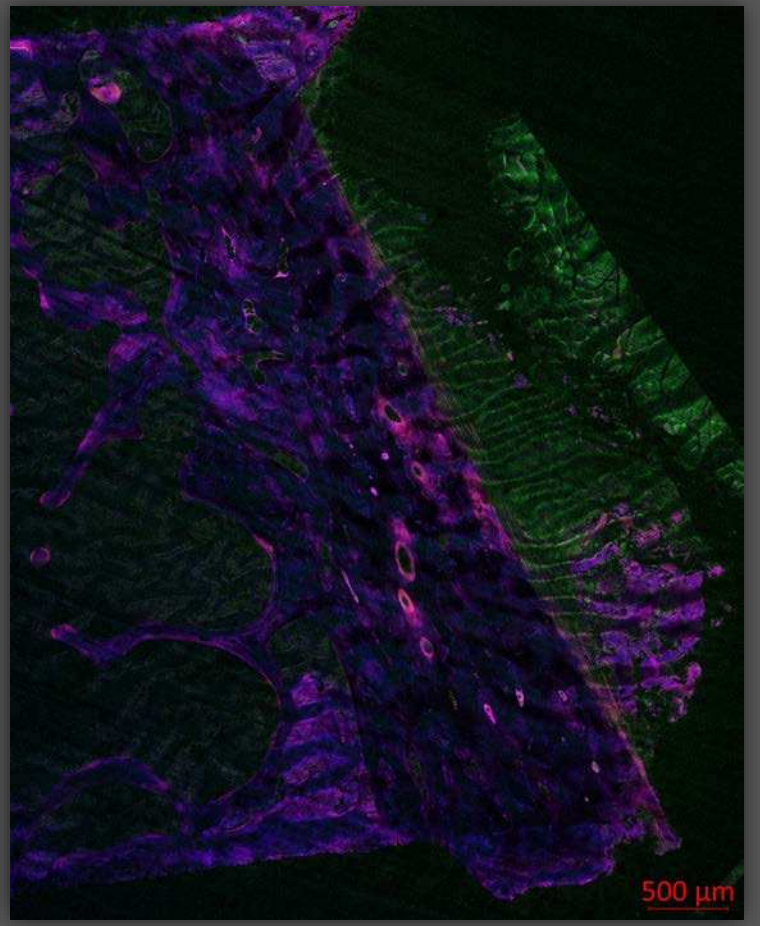


LSCM Clinical

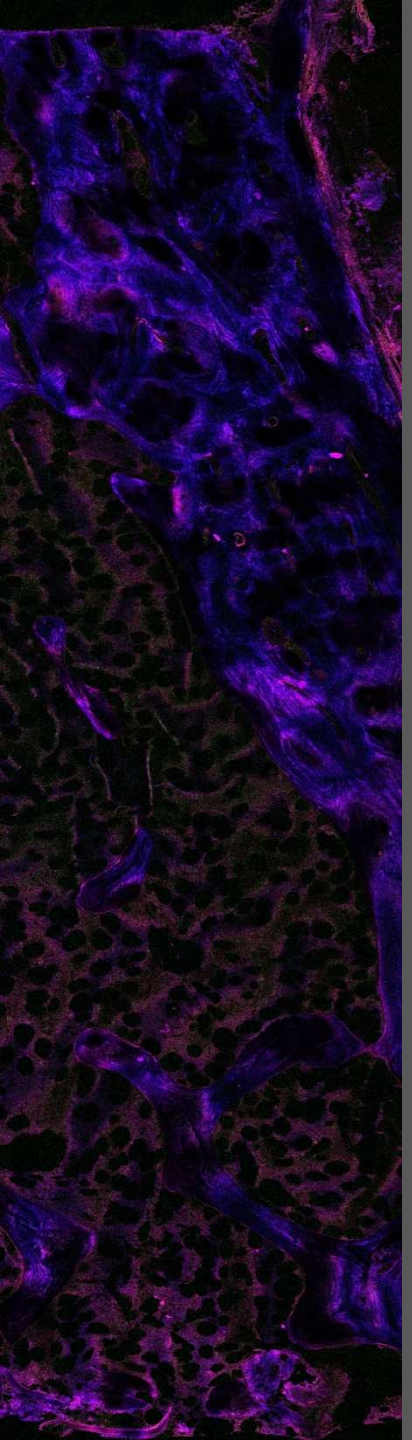


Methodology

- Imaged using Carl Zeiss™ LSM 800 with an Observer.Z1 inverted scope
- Pinhole set to 1AU
  - Pixelization set at 2661px x 2661px
  - Scanning speed of 7
- Tile set
  - 40x – 63x Magnification
  - Section of cortical bone
  - Random
- Comparisons taken on Nikon Eclipse E200 & Keyence VHX2000 Digital Microscope



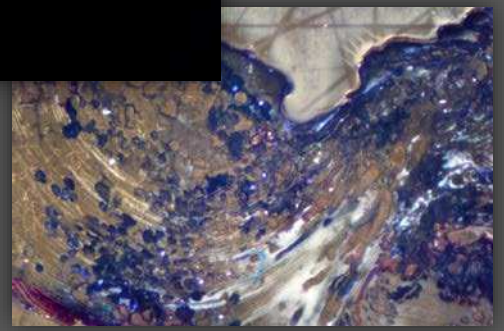
Tile set of clinical sample at 40x Mag

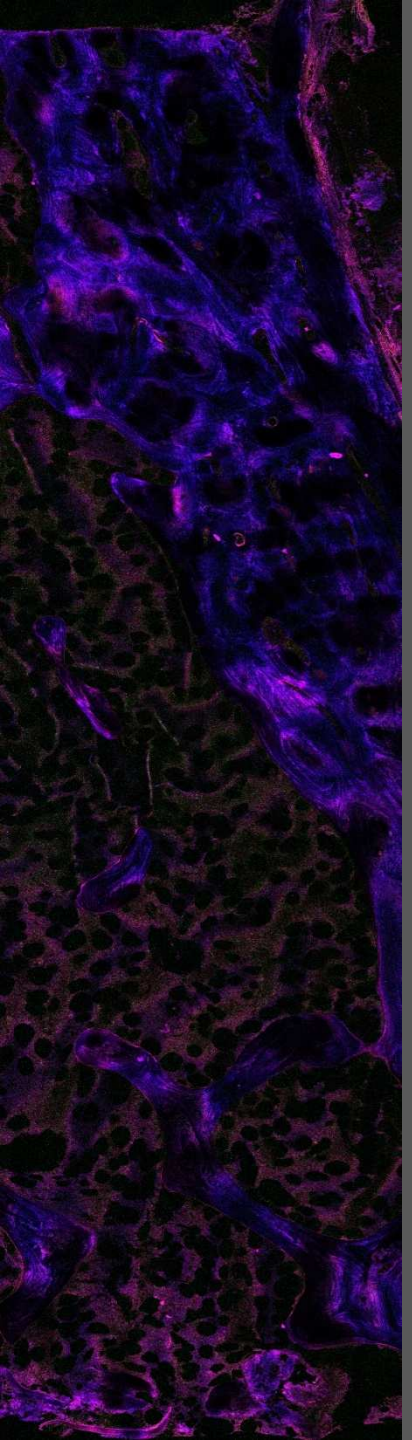


Results

- All samples registered on the 543nm & 633nm λ
- Clinical samples
  - 543nm > 633nm
- Apollonian samples
  - 543nm < 633nm
  - **Localized registration** 405nm & 488nm λ at cortical margins & Haversian canals
  - "Thicker" mineralization surrounding Haversian canals

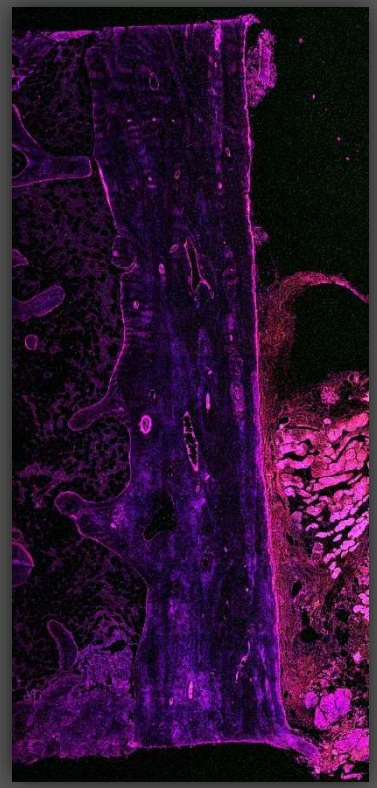
Diagenetically altered sample under LSCM and polarized light

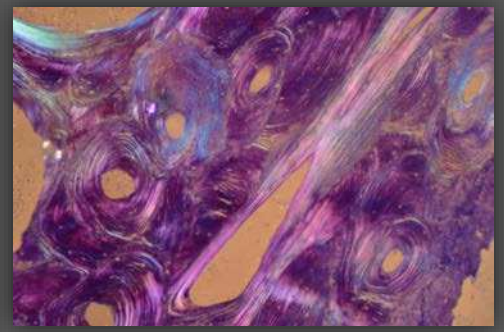


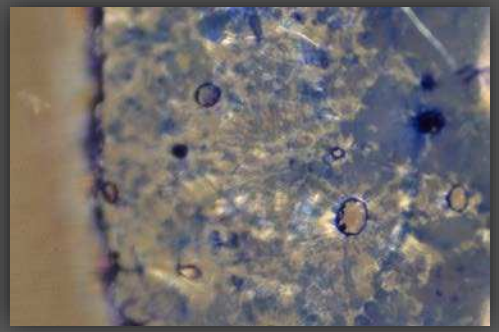


Results

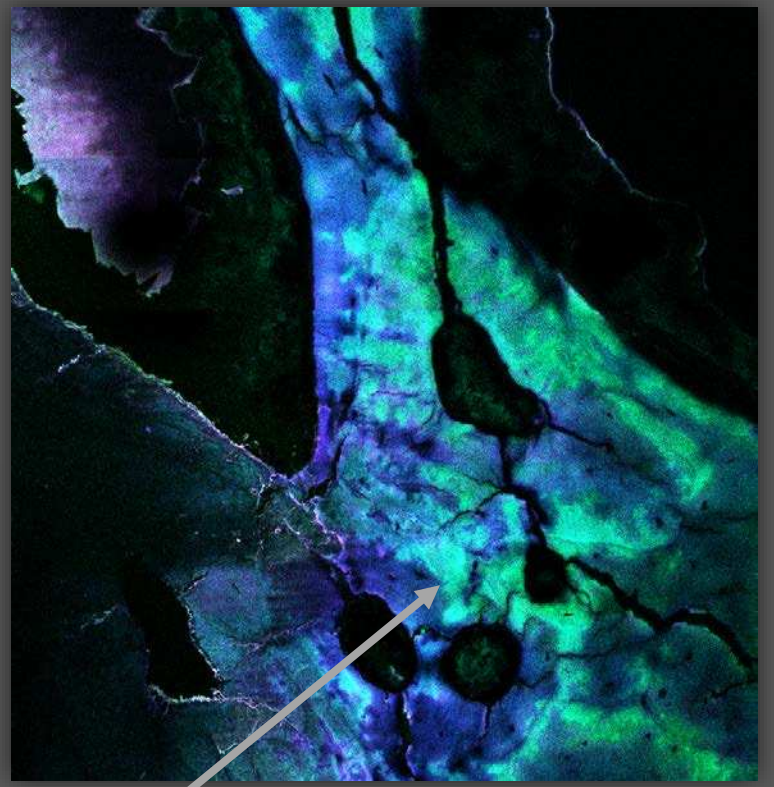
Clinical Iliac Sample

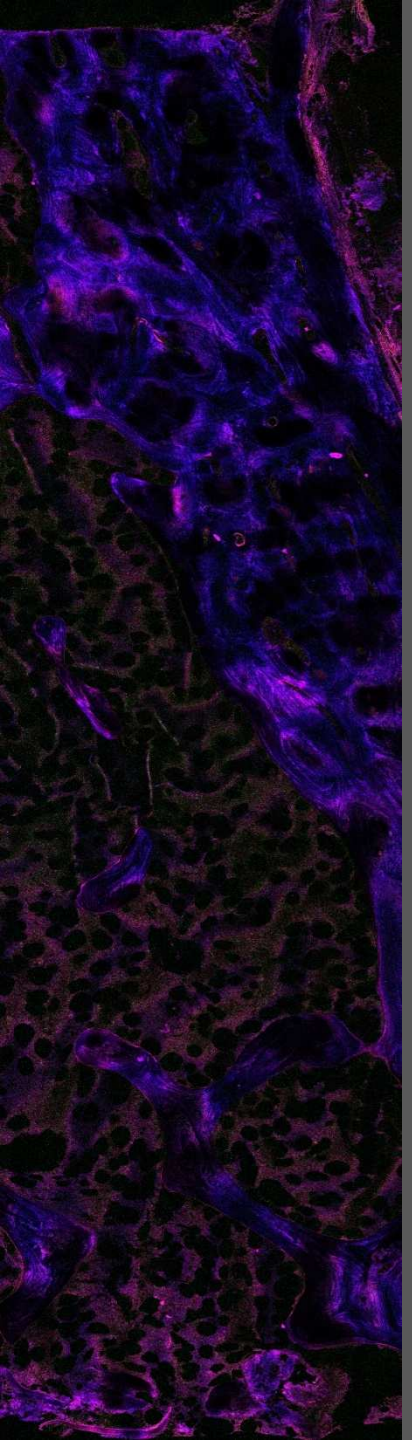






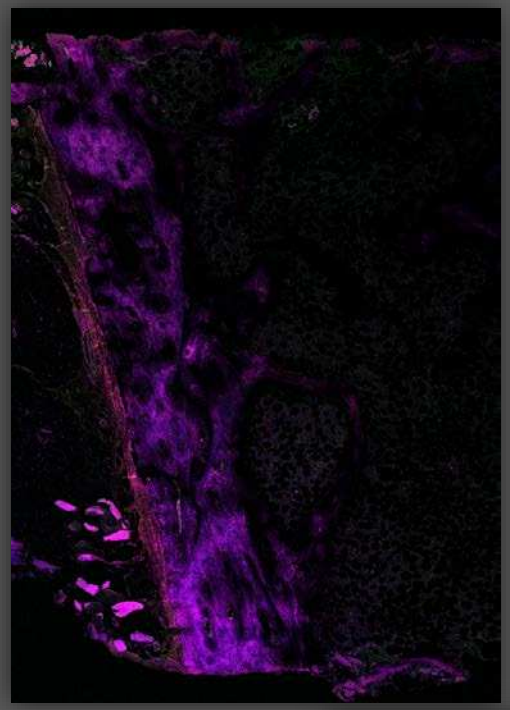
Diagenetically Altered Rib

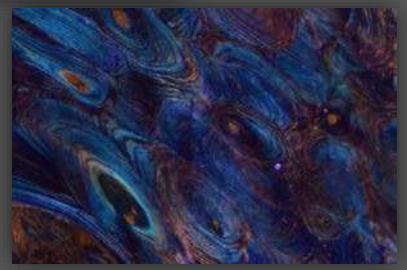




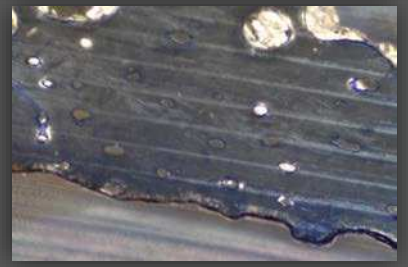
Results

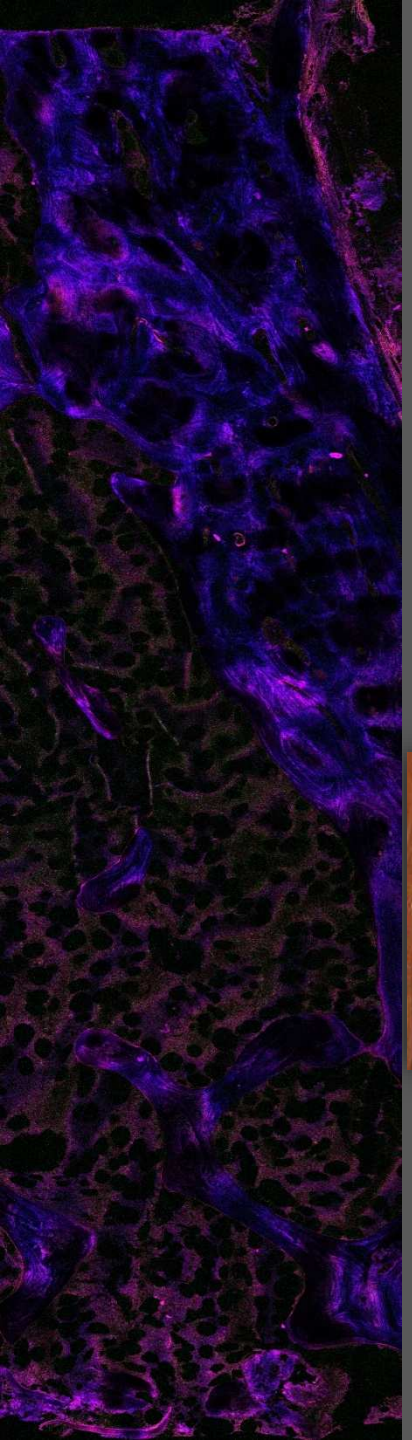
Clinical Sample





Diagenetically Altered

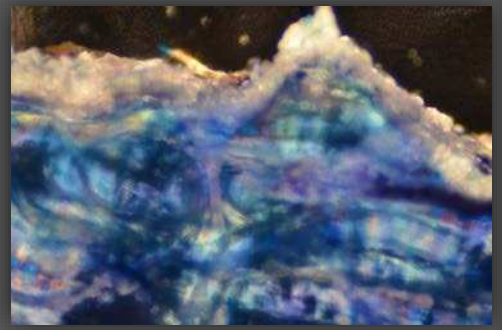


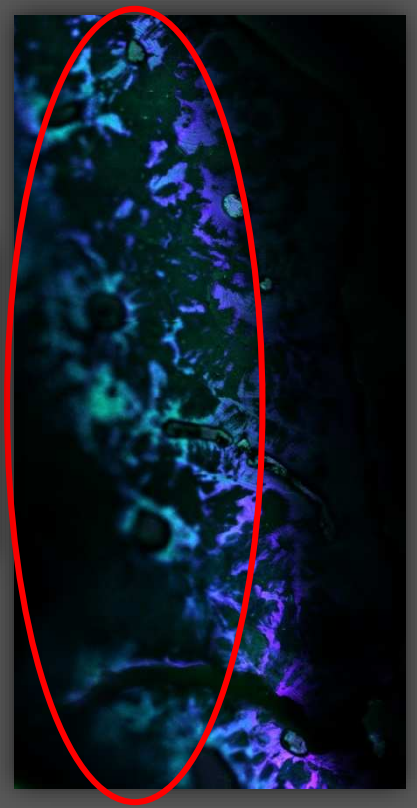


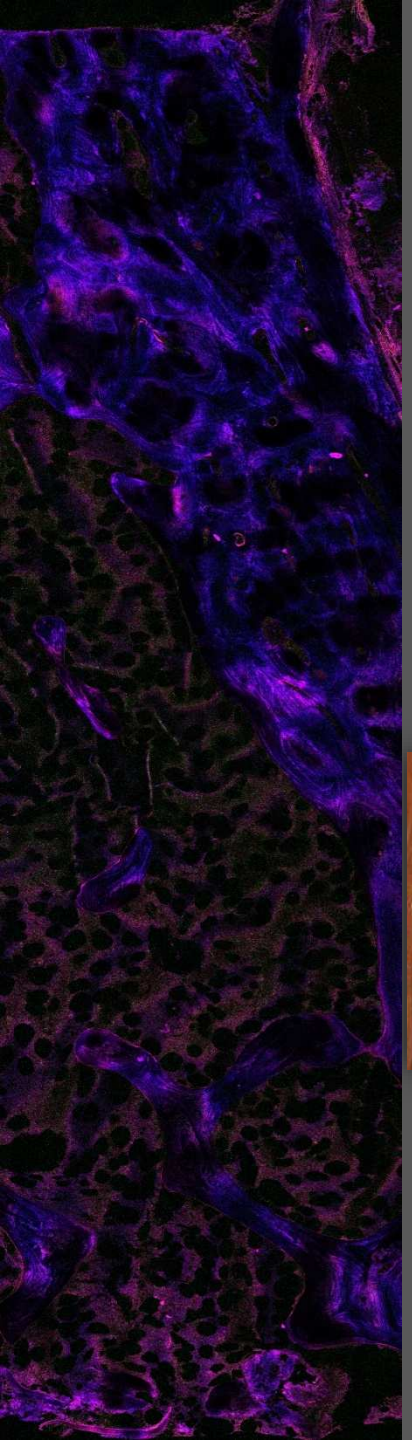
Results

Clinical Sample

Diagenetically Altered



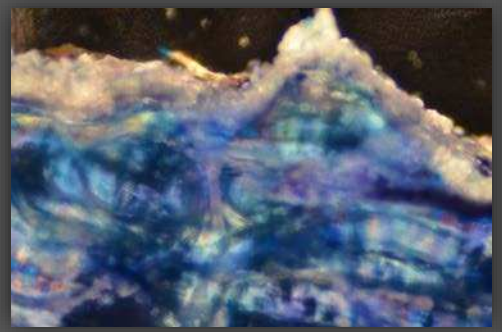


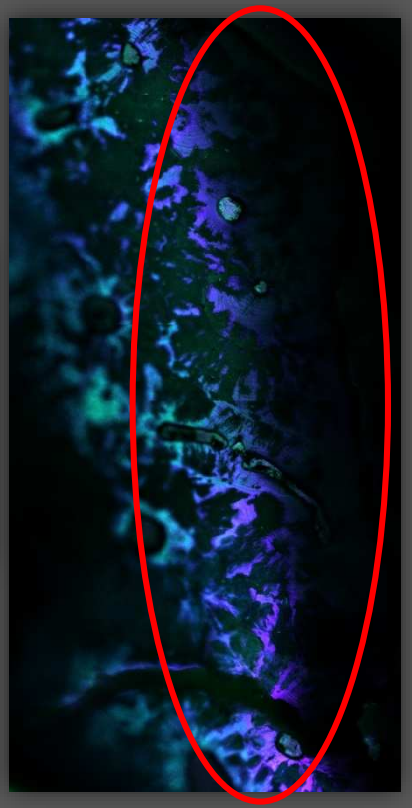


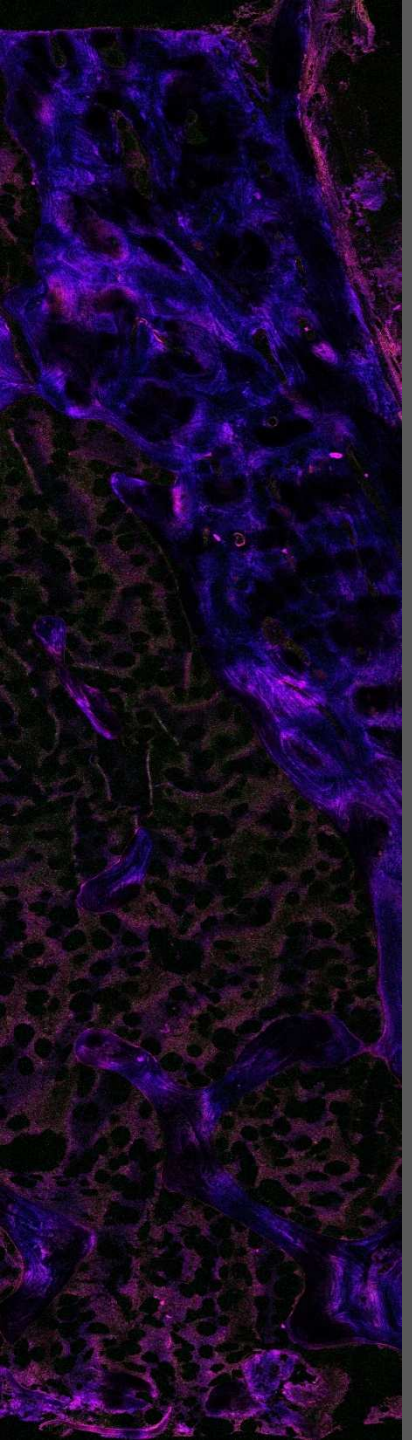
Results

Clinical Sample

Diagenetically Altered

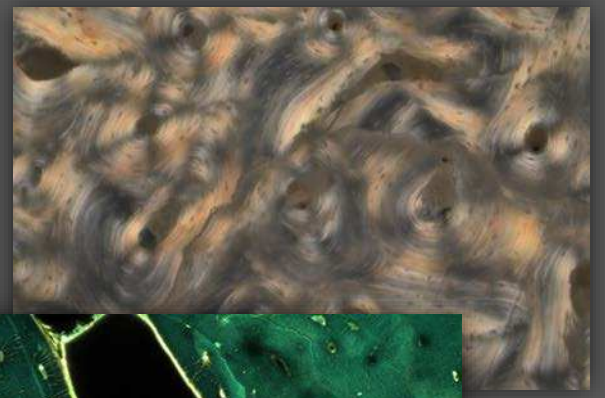


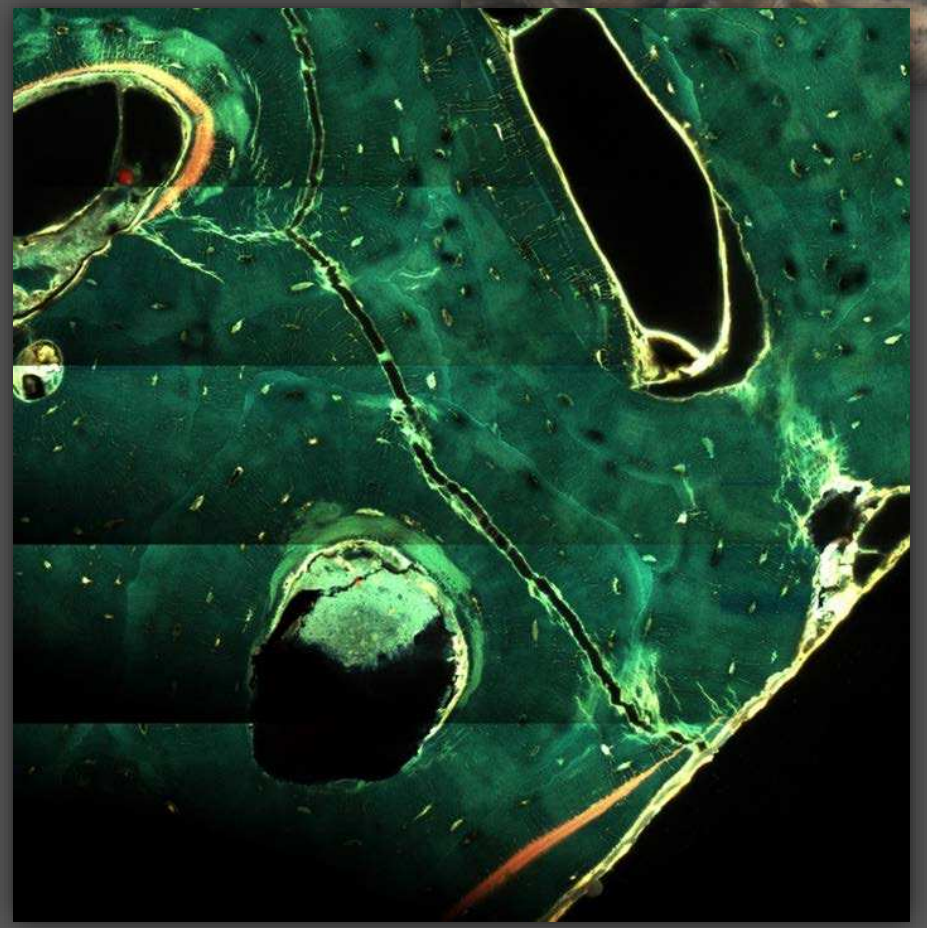




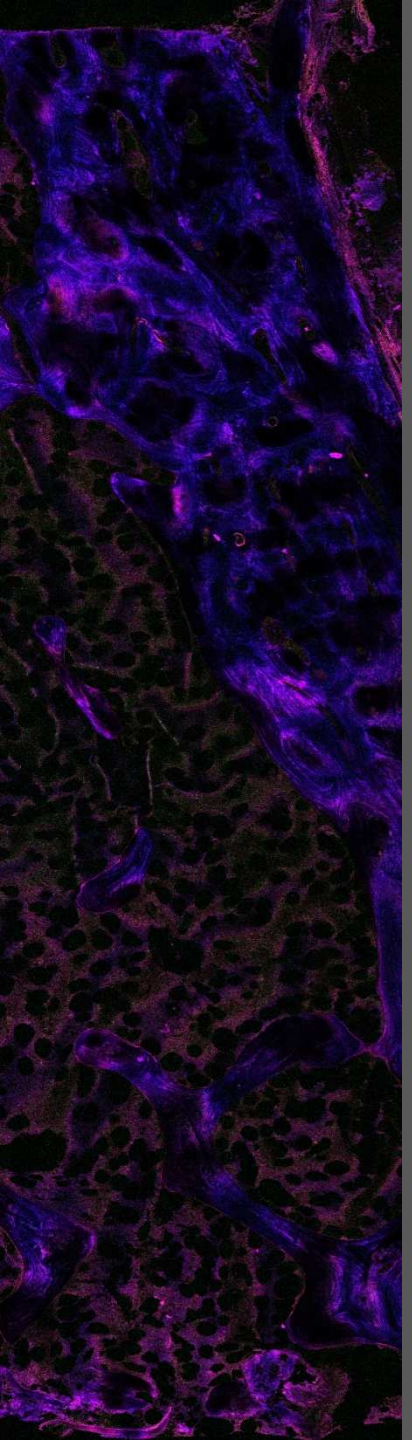
Unstained Bone

- Eliminate areas of less staining
- Unstained bone registers on 405nm and 488nm λ
- Toluidine Blue registers on 543nm and 633nm λ
- Areas of diagenetic alteration registering mainly on 405nm and 633nm λ





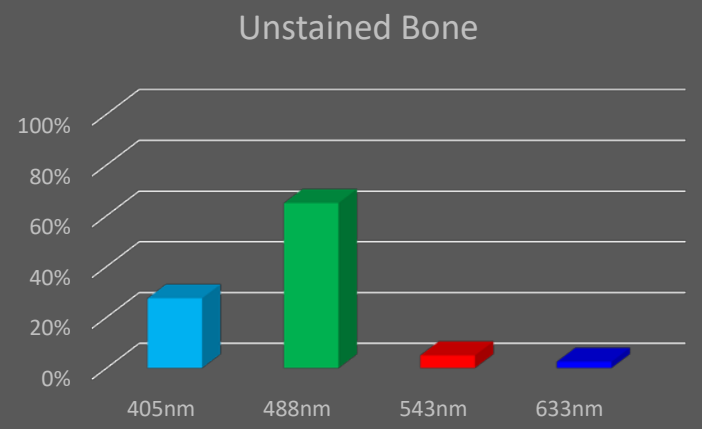
Unstained femur, 64x Mag. (PLM at 40xMag)

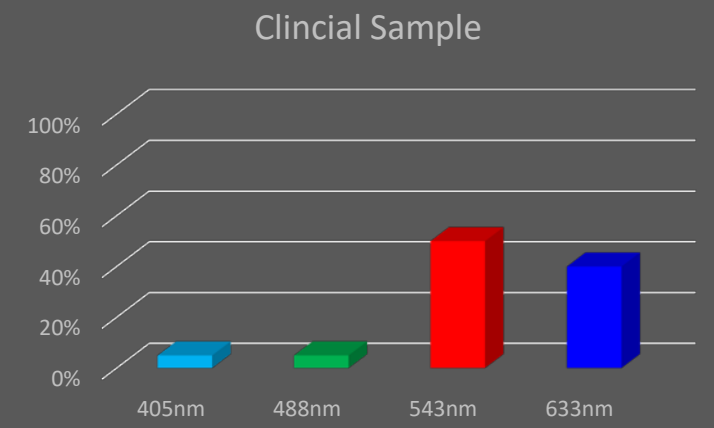


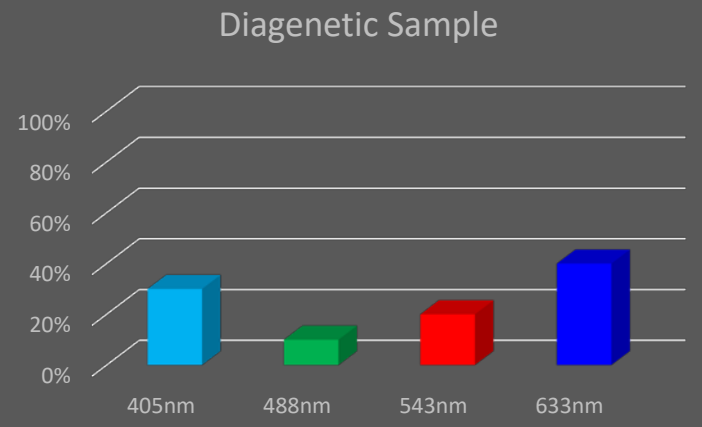
Unstained bone

- Not just the registration
  - Ratio of registration
- Unstained higher on 488nm
- Toluidine Blue clinical 543nm
- Diagenetic bone 405nm and 633nm

- Can eliminate poor staining

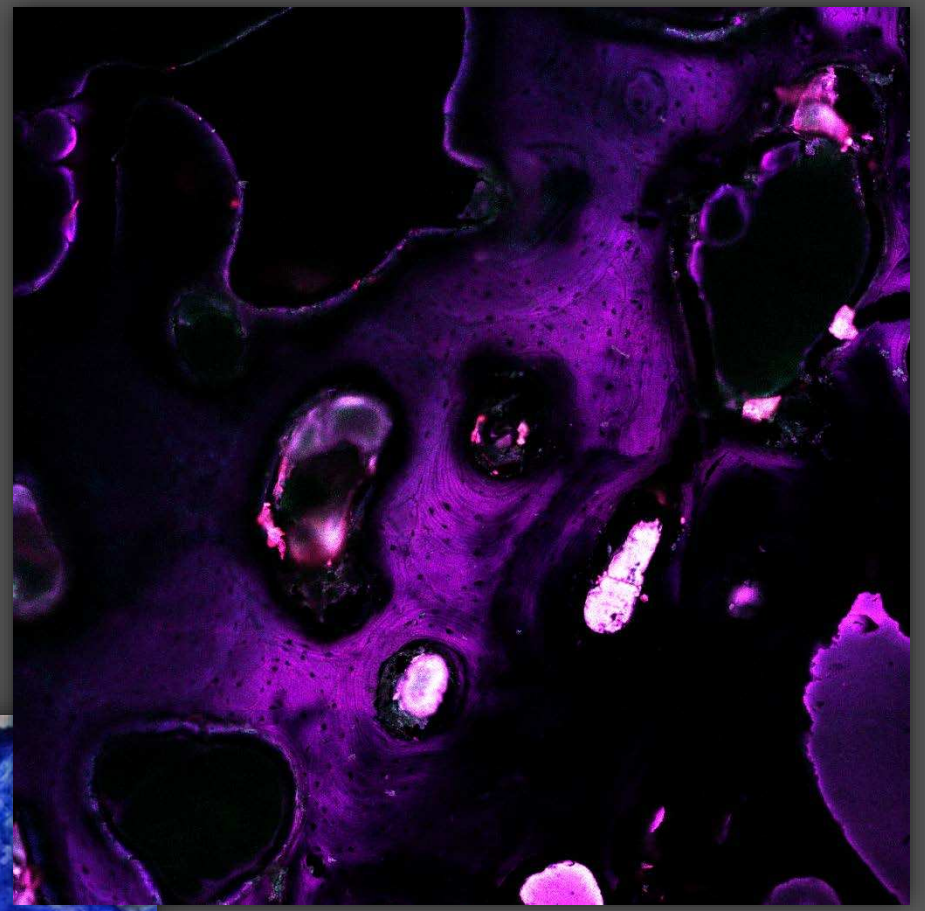




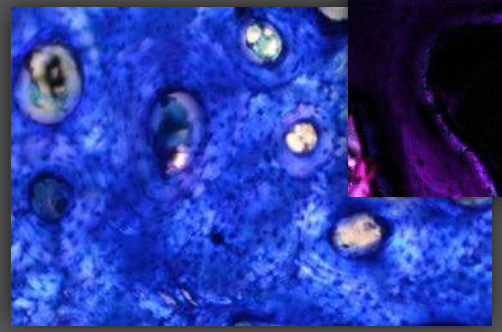


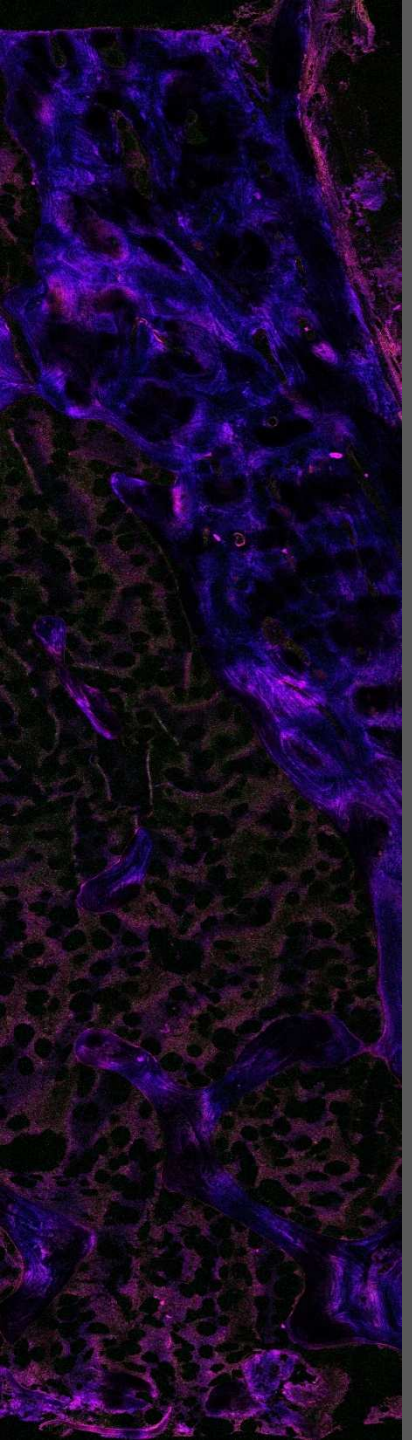
Bone Differences

- Different bones may stain differently
- Femoral control
  - Anatomical Sample
- Imaged similar to clinical iliac samples
- No differential staining



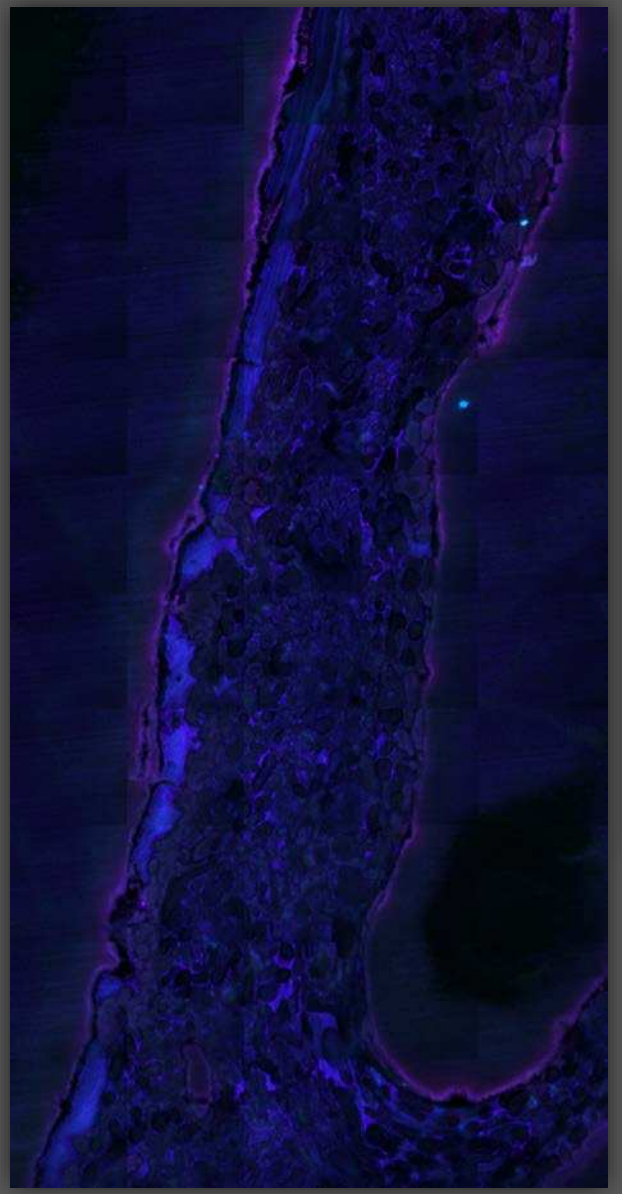
Toluidine blue stained femur

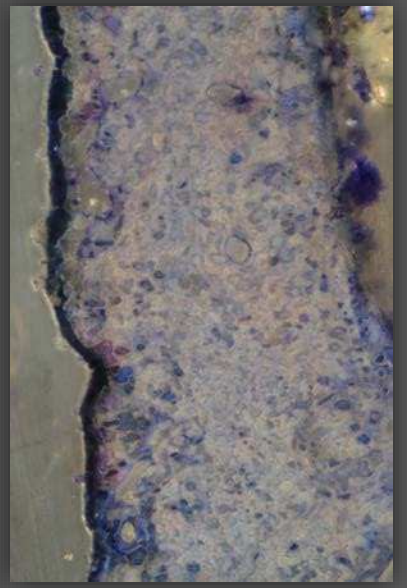


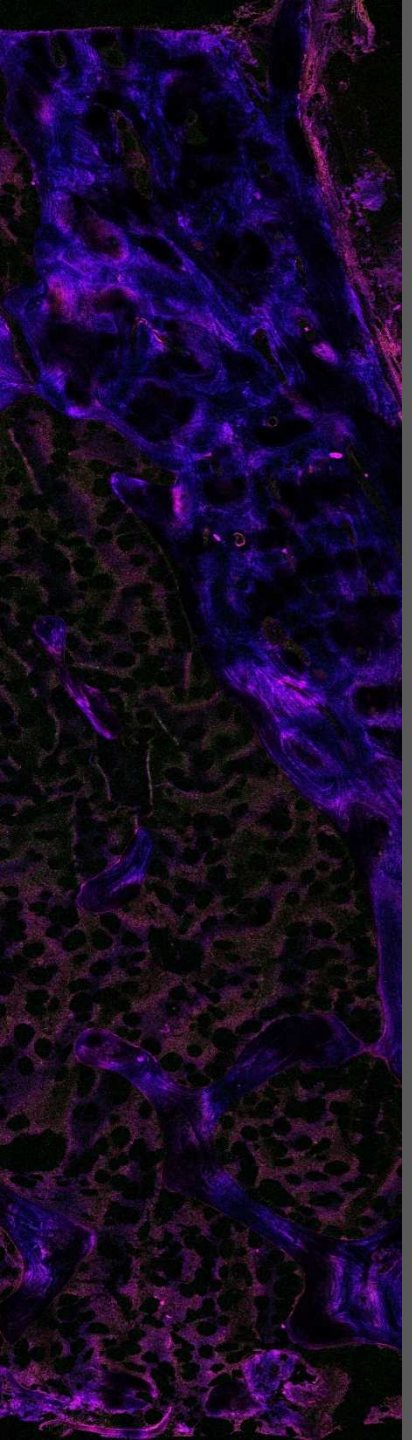


Conclusions

- Diagenetic alterations can be detected using LSCM
- We see diagenetic incursions into cortices and Haversian canals
- Cannot determine if mineral or biological
  - Differential staining should aid
- LSCM good tool in histo-osteological studies







Thank You

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- LCSM Group, Department of Biology, University of Toronto at Mississauga
- Dr. Marc LaFlamme, Dr. Jochen Halfar, Department of Earth Sciences, University of Toronto at Mississauga
- Vanessa Rossi & Alex Saly, University of Toronto at Mississauga

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