Use of Laser Scanning Confocal Microscopy in Osteological Examinations

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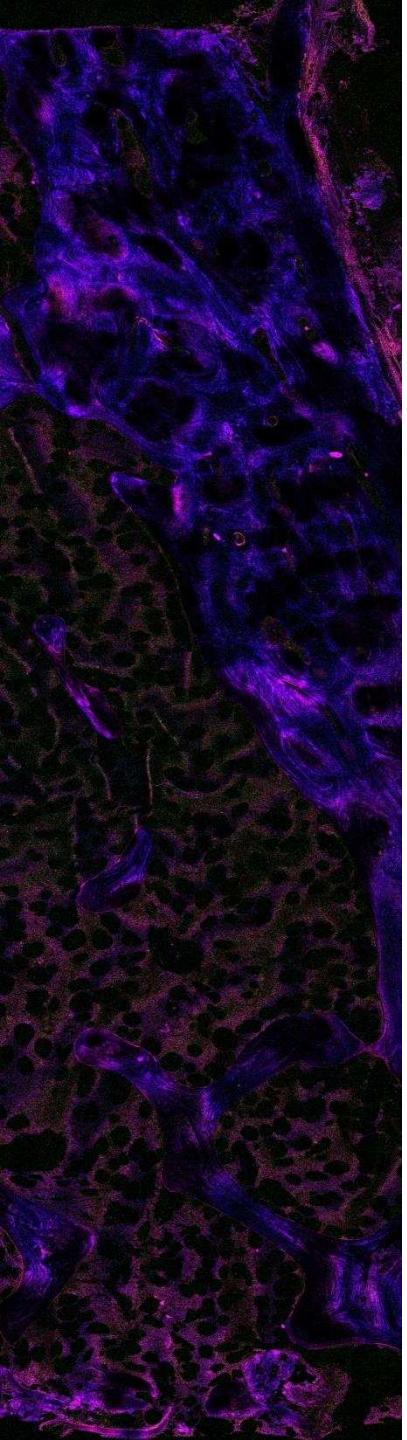
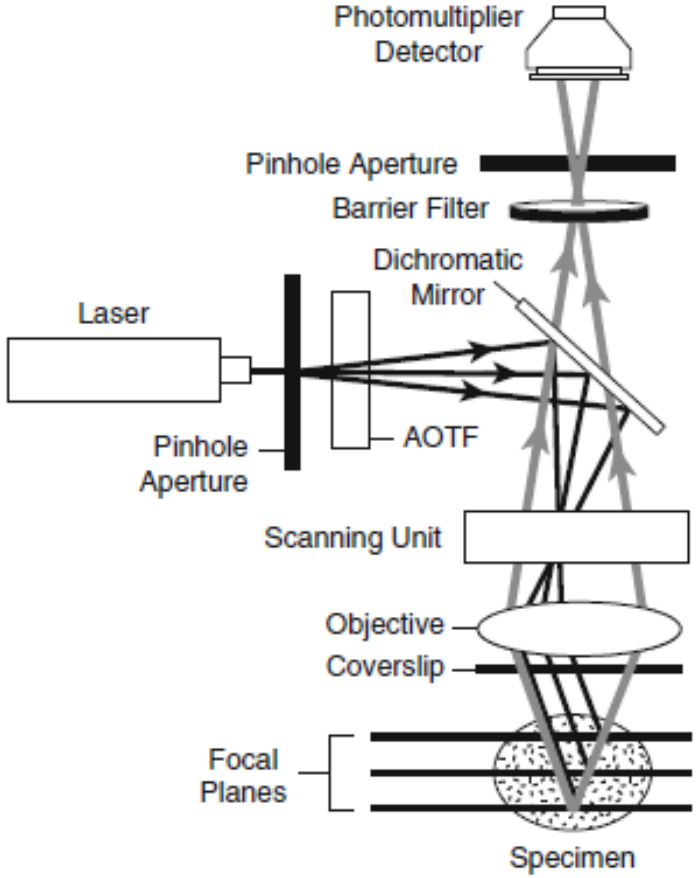
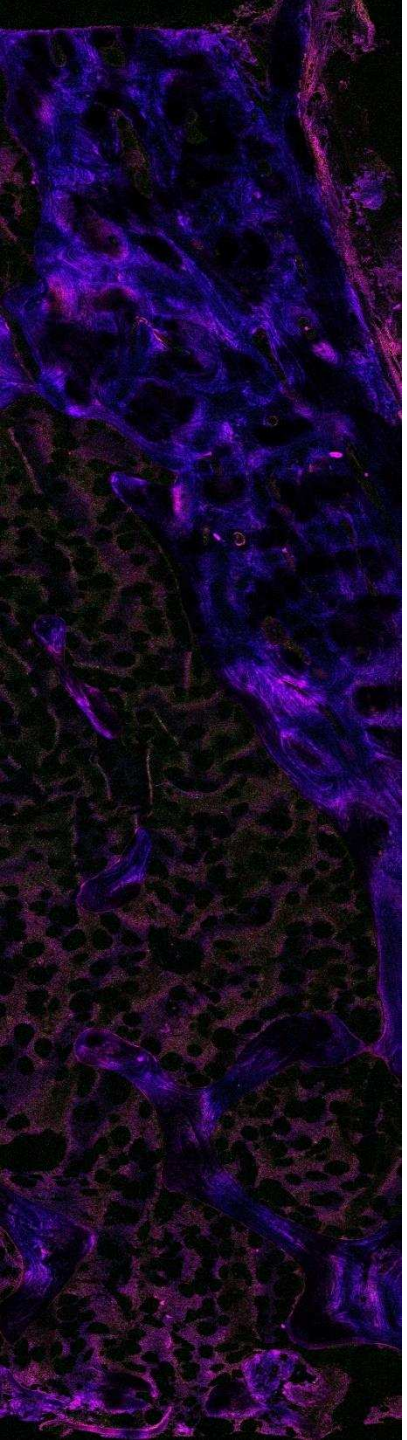


Confocal Microscopy

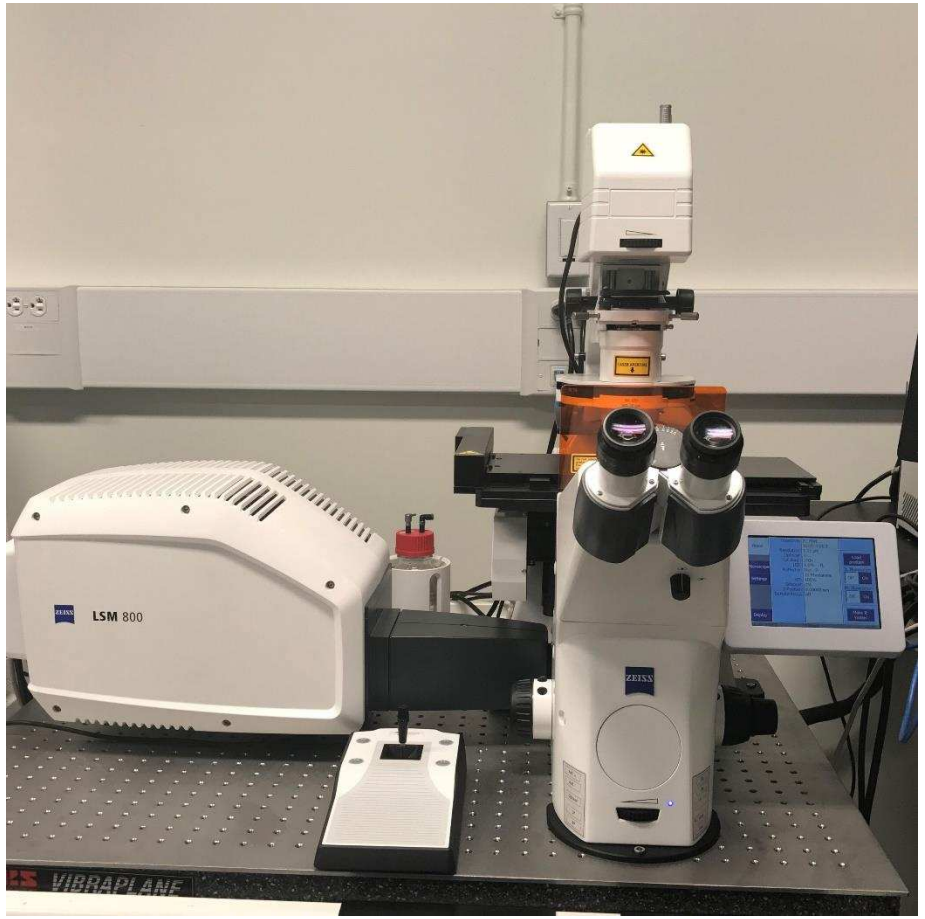
- *In vivo* imaging
- 2 focal objectives
  - Same objective twice (Modern)
- Specific focal planes
  - 3D Imaging
- Pinhole technology
  - Removes superfluous light



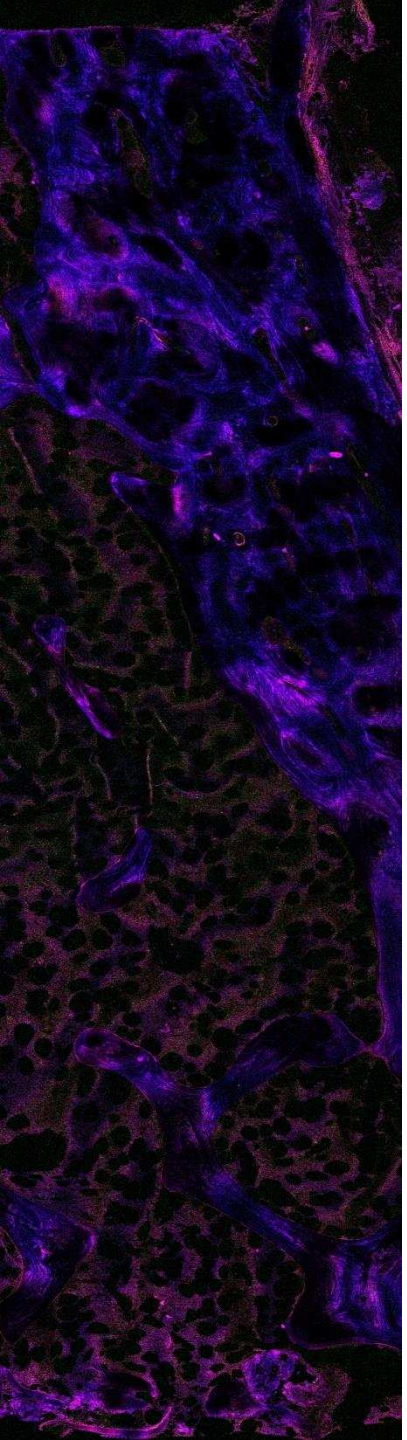


Laser Scanning Confocal Microscopy

- Principles of Light & SEM
- Laser light
  - Specific excitation & emission wavelengths (λ)
- Photon-multiplier registers emission λ at specific pixels
  - Pixel intensity generates image
- Targeting of specific materials
  - Quantification
- High-resolution, high-magnification images



LSM-800, University of Toronto - Mississauga



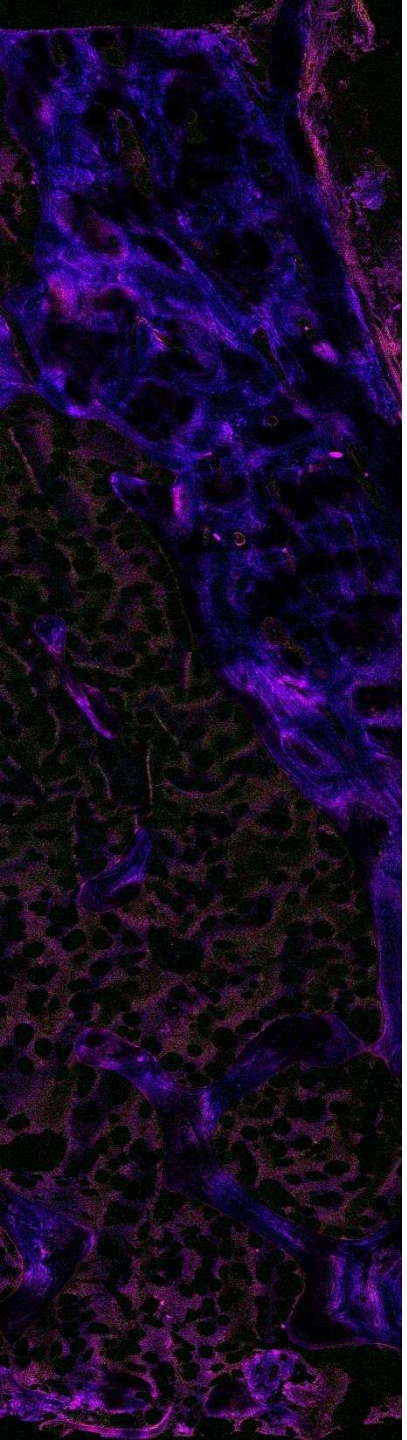
Carl Zeiss™ LSCM Models

LSM 510-META

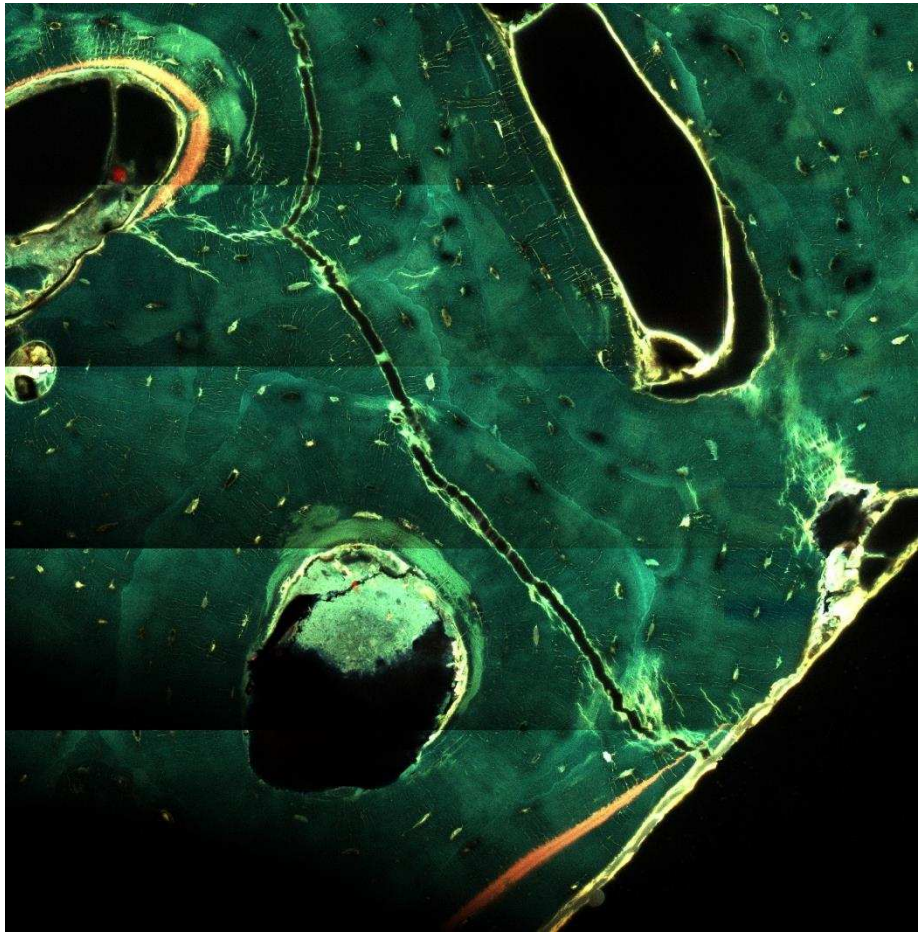
- Cheaper (older)
- 4-Laser system
  - 7 λs
    - 405nm – 633nm
- Gas-powered laser system
  - Argon, Helium-Neon
- Multiple light-filter system
- 3-D imaging/modeling

LSM 800 with Airy-Scan

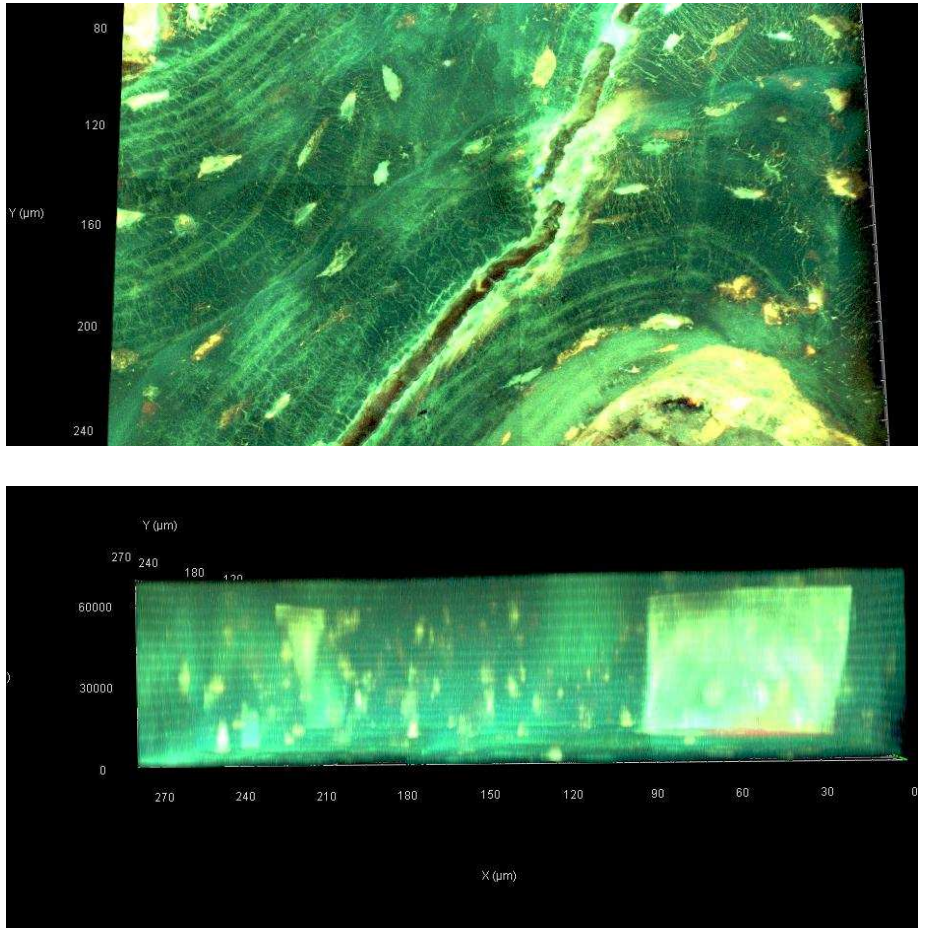
- Motorized stage
  - Allows for tile images
- 4-laser system
  - No-gasses required
  - Gradient
- More powerful lasers
  - Less energy
- Limited light-filters
- Better 3D imaging/modeling
  - Over wider area
- Higher resolution (>5k x 5k)



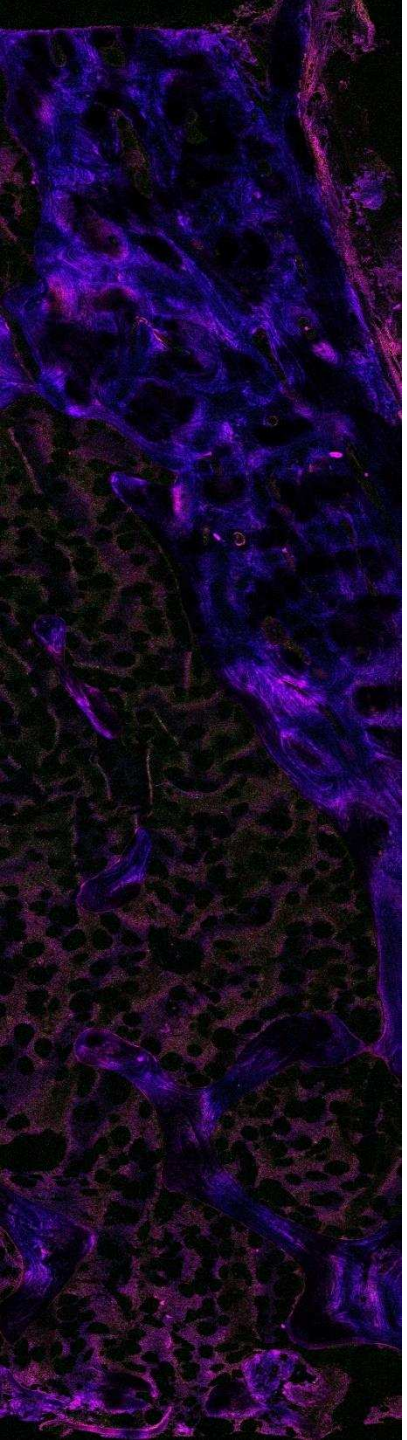
LSCM and Bone (Unstained)



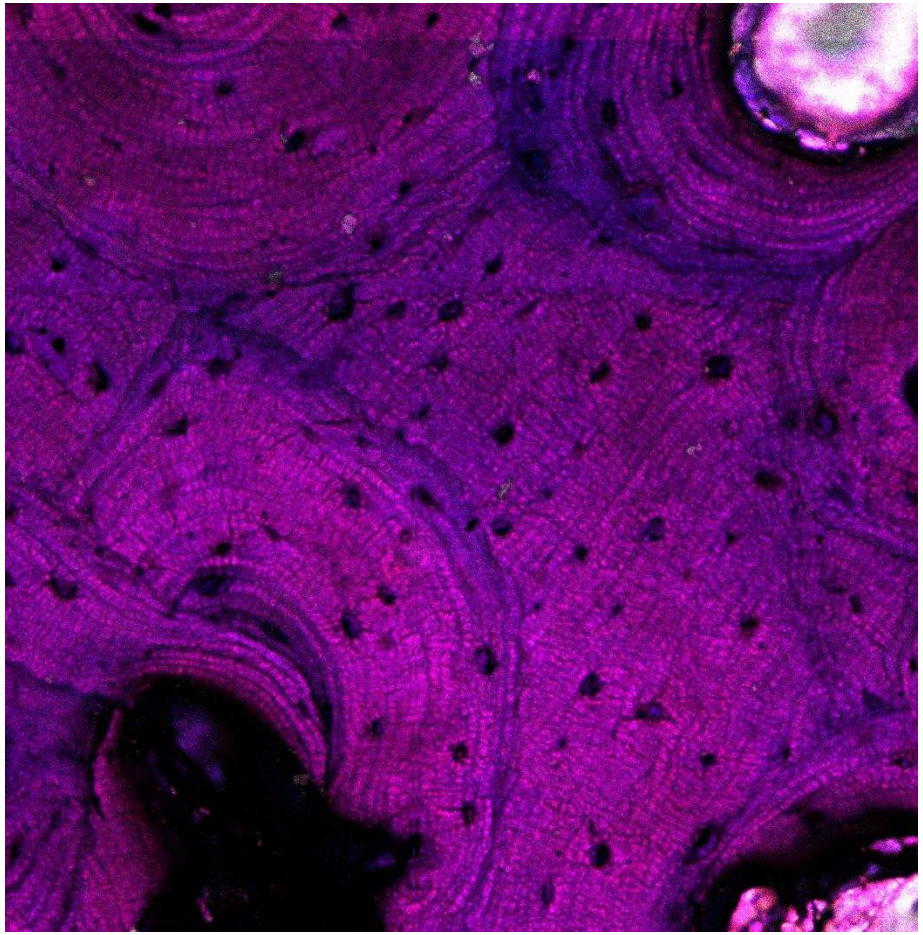
Femoral Cortical Section (Endosteal) 40x Mag Tile Image



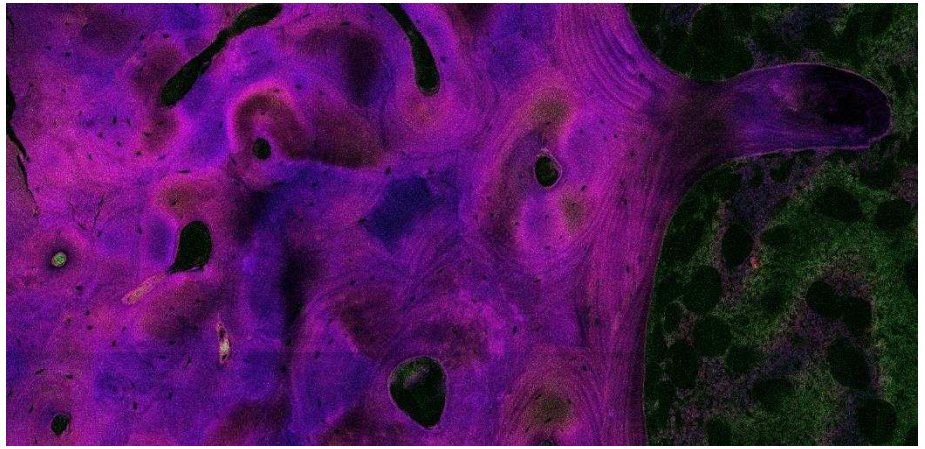
Femoral Cortical Section 40x Mag Z-Stack Tile Image (Top); Z-axis view (Bottom)

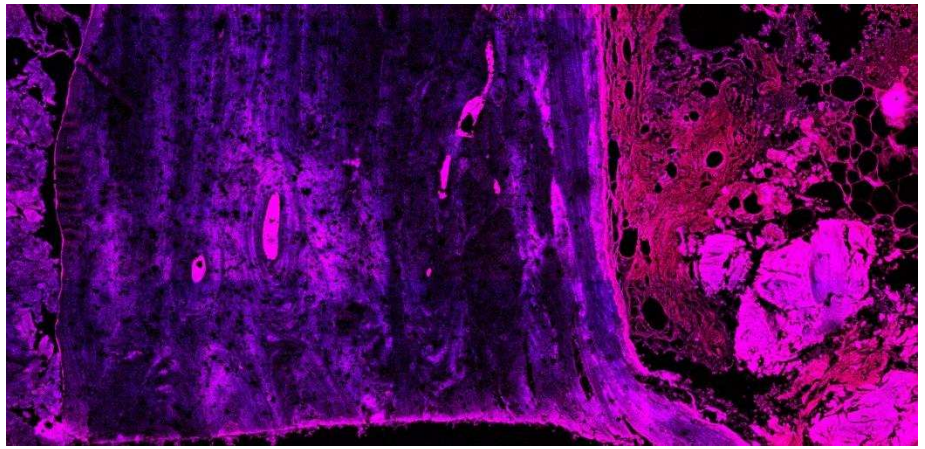


Gross Structure Visualization

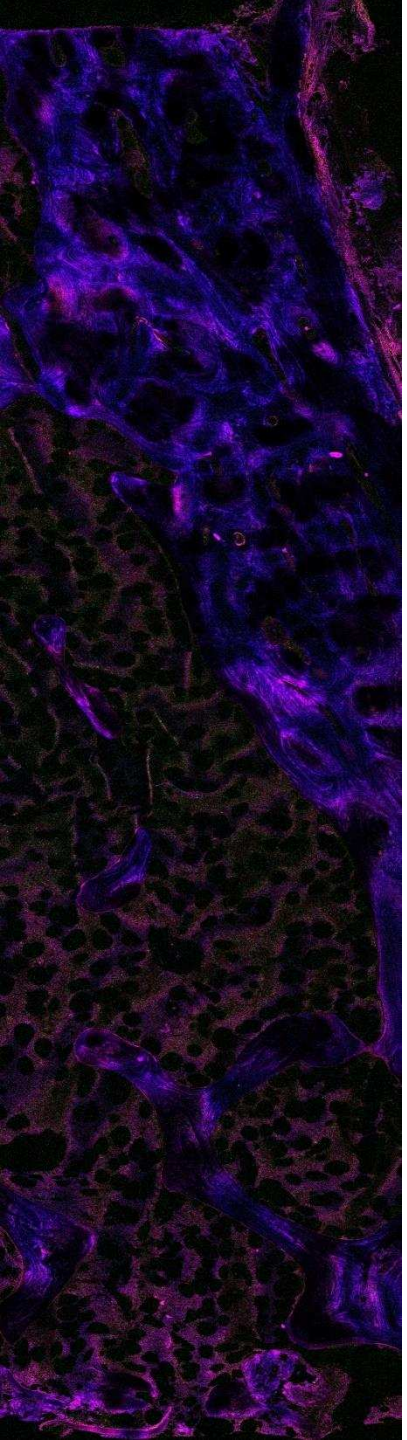


Femoral Section Stained in Toluidine Blue

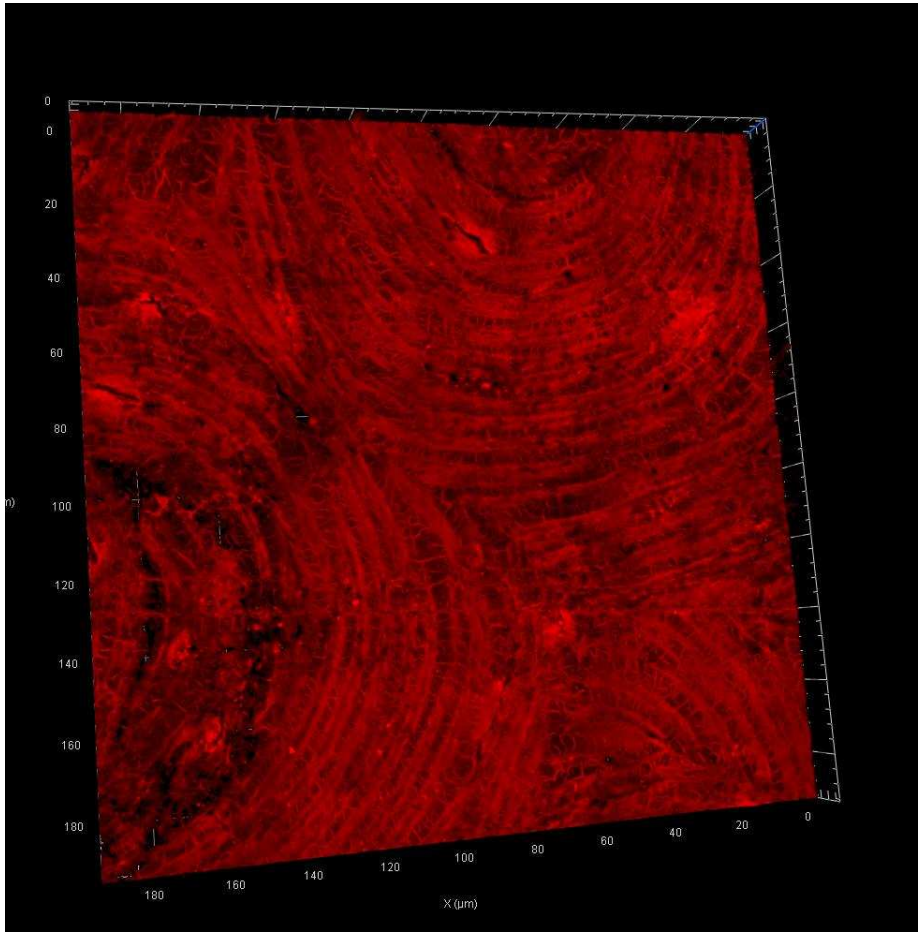




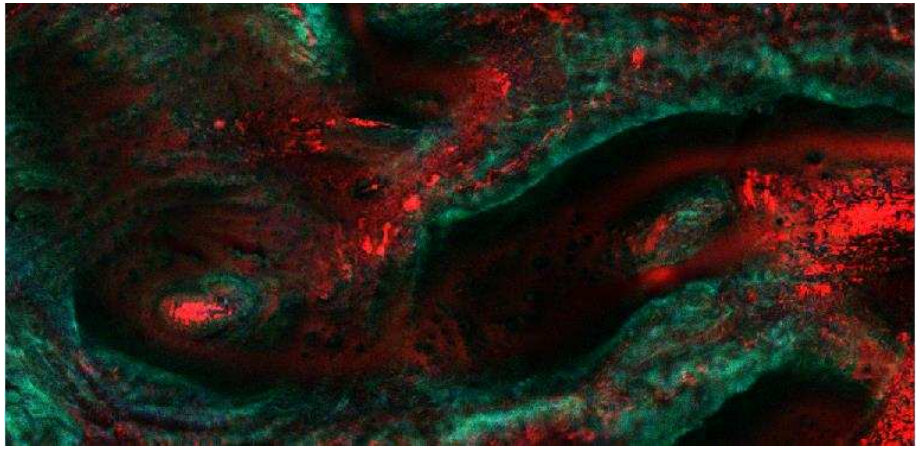
Iliac Cortical Sections Stained in Toluidine Blue

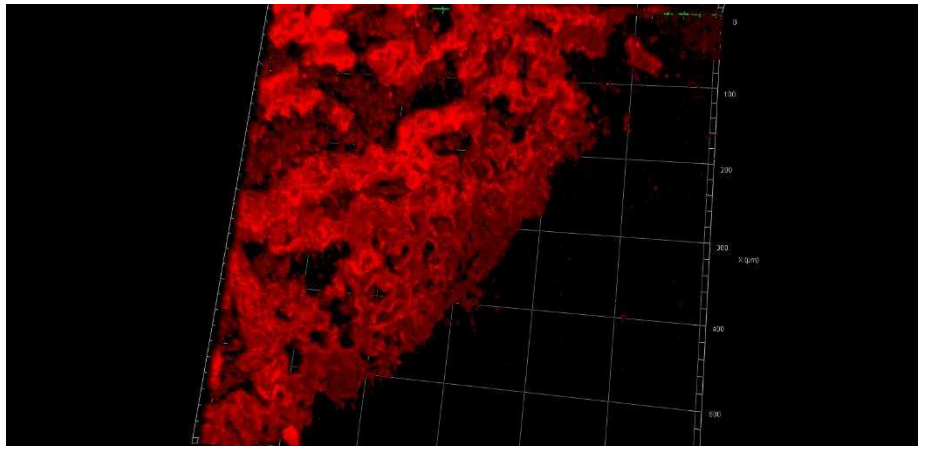


Visualization of Microstructure

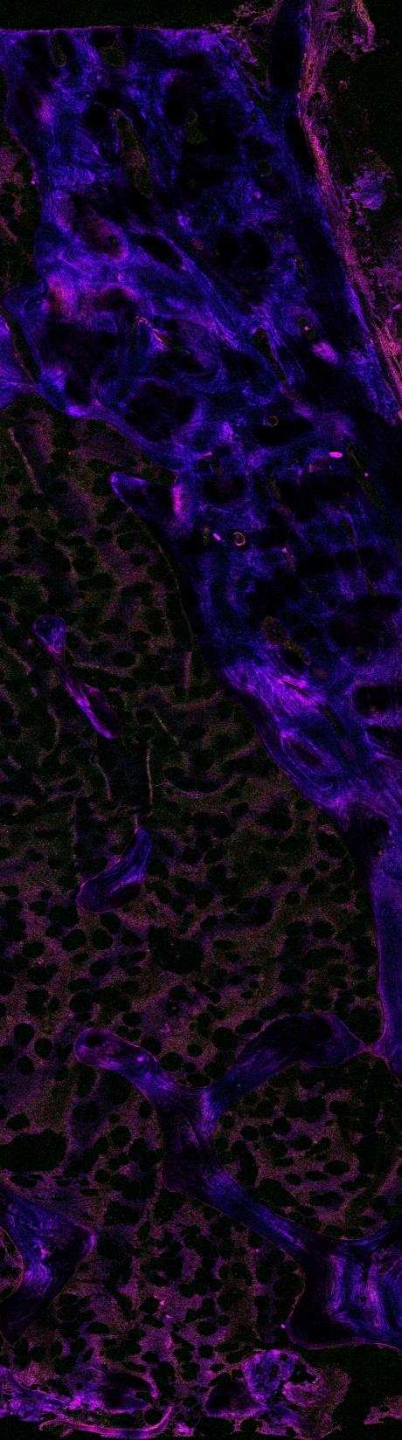


3-D Model of Mineral Microstructure, 63x, Femoral Cortex

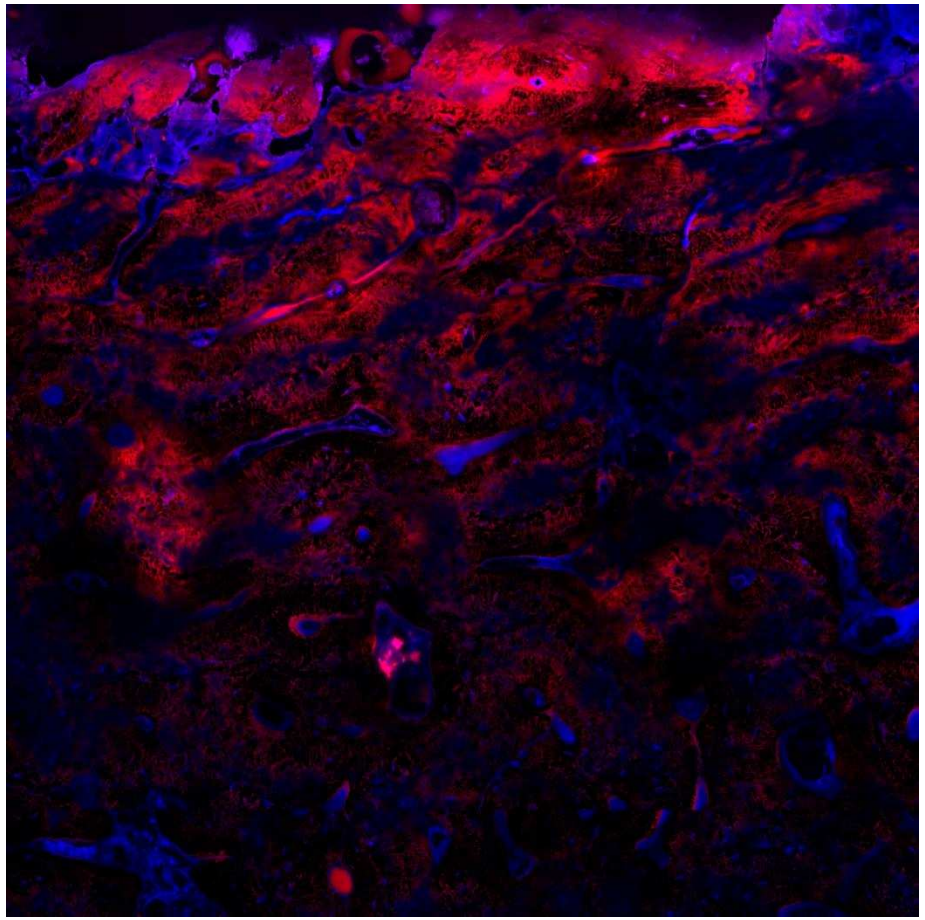




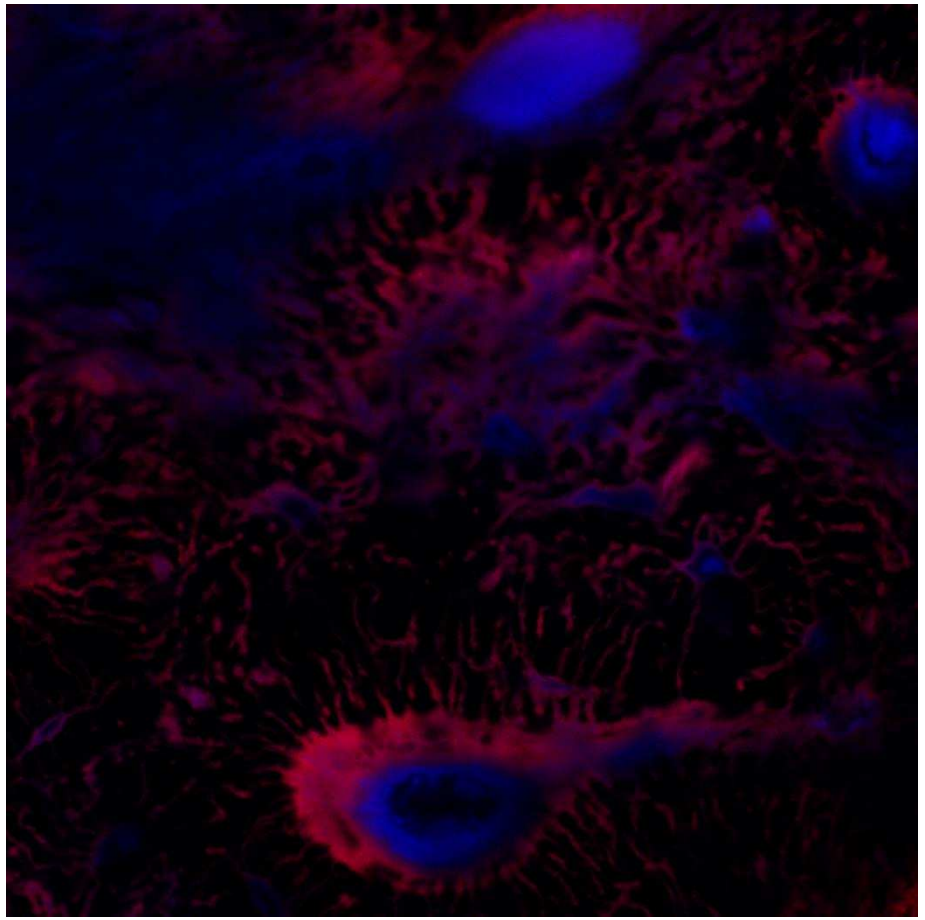
Iliac trabeculae 63x (Top); 3-D fractured edge of pig cortex 63x, 4 tile image



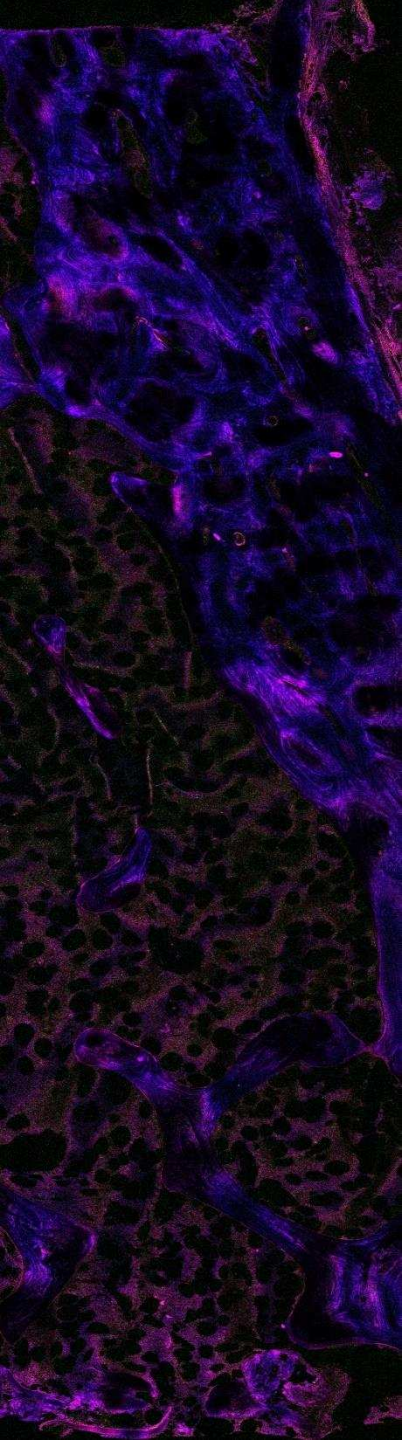
Visualization of Microstructure



Tile Image Set of Pig Cortical Bone

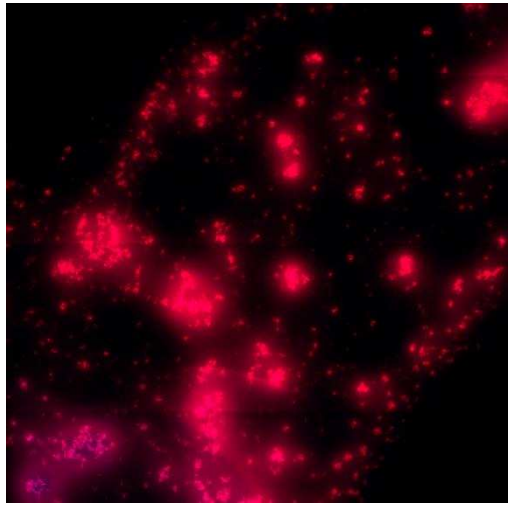


Close-up of Haversian canal from Left



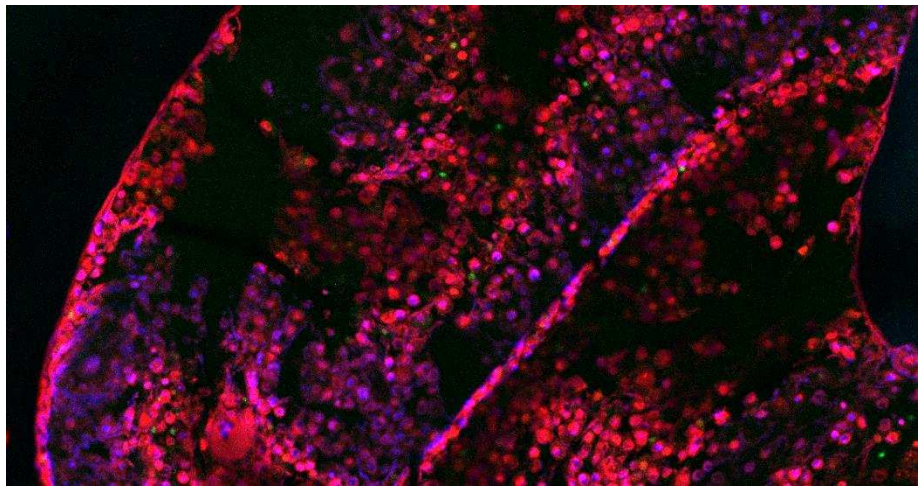
Protein & Cell Isolation/Count

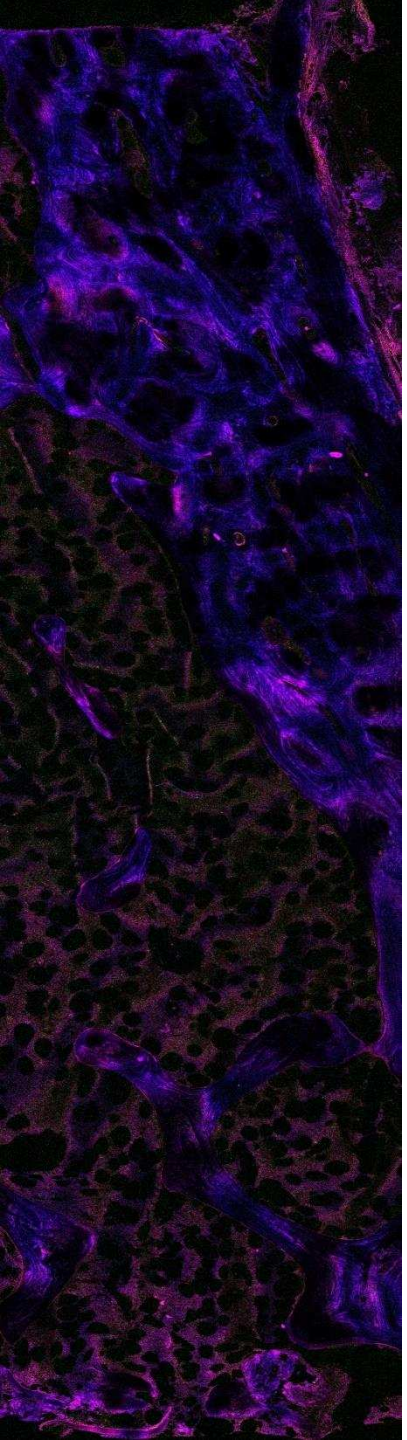
- Different dyes
  - Differential staining
  - Can dye specific proteins/cells
    - Osteocalcin
    - Osteopontin
    - Osteoclast/Osteoblasts
- Isolation of λ
  - Image just target material
- Quantification
  - Number present
  - Intensity
    - Good for degradation studies



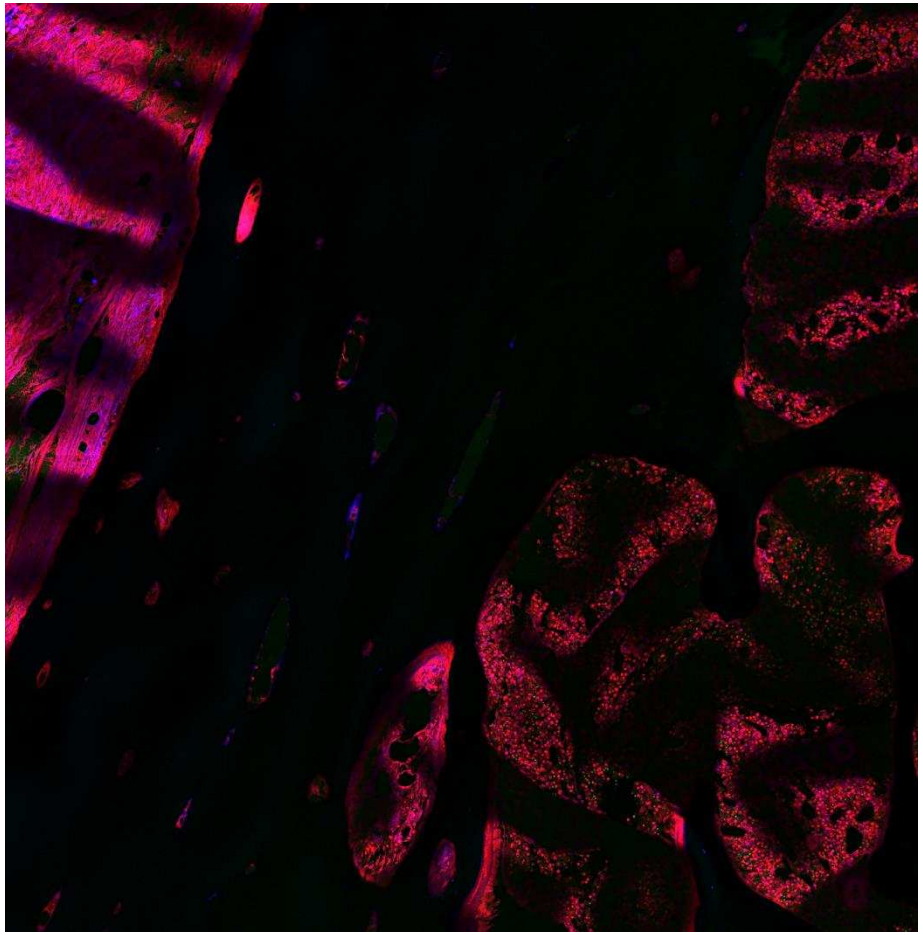
Intra-cortical Protein (pig) stained in Slow-Fade Gold (Left)

Intra-marrow Protein (Human) stained with Goldner's Tri-Chrome (Bottom)

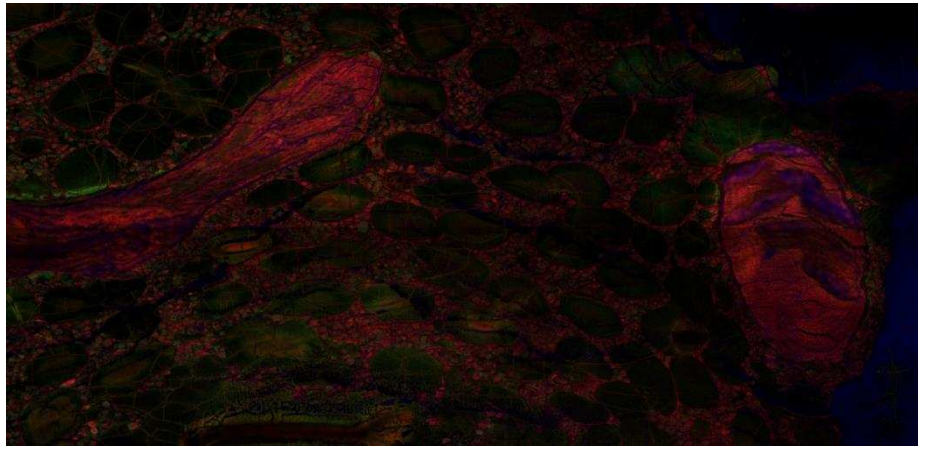


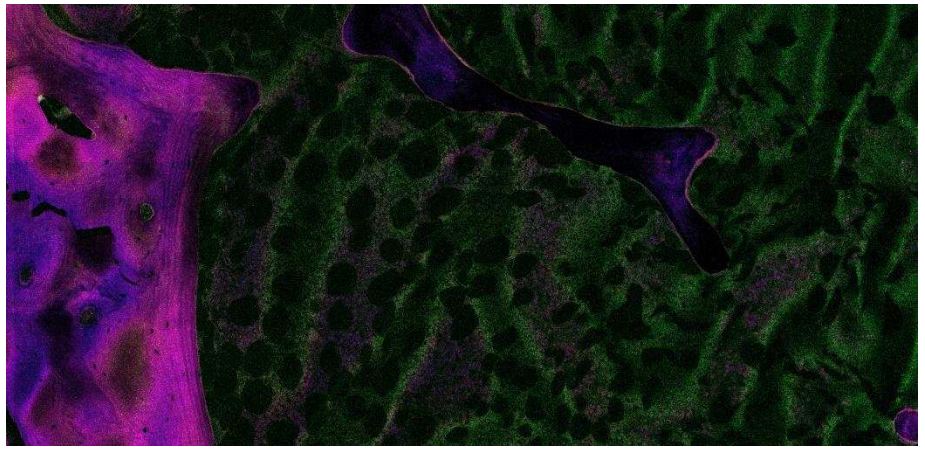


Soft-Tissue/Marrow Visualization

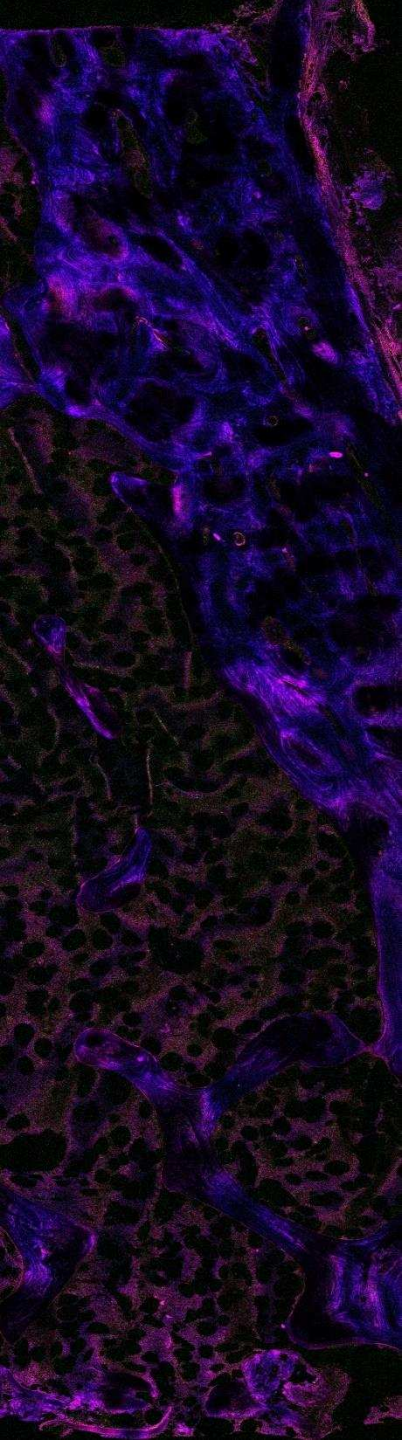


Goldner's Tri-Chrome

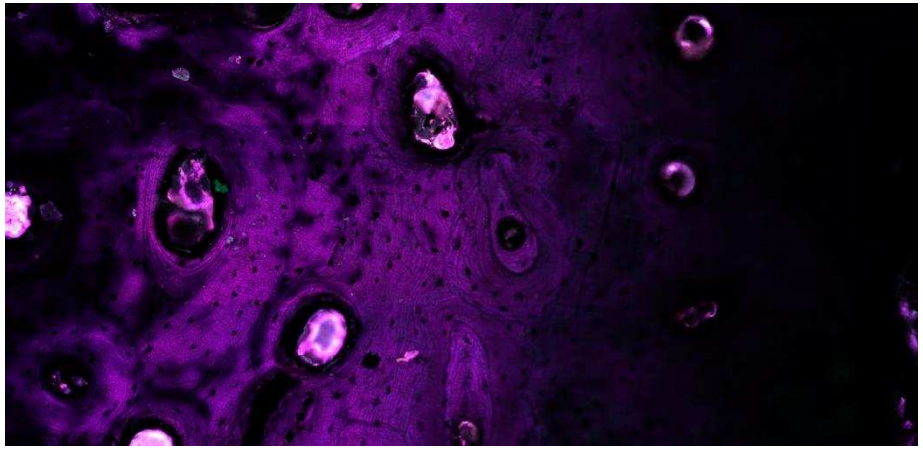


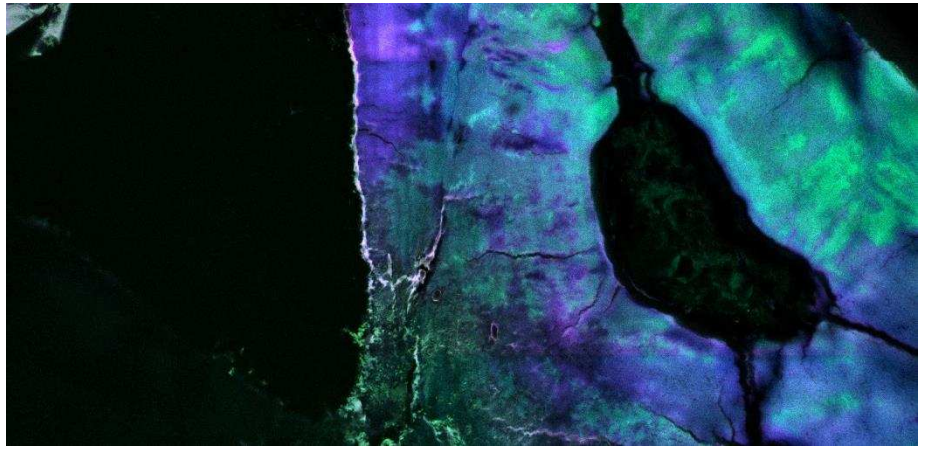


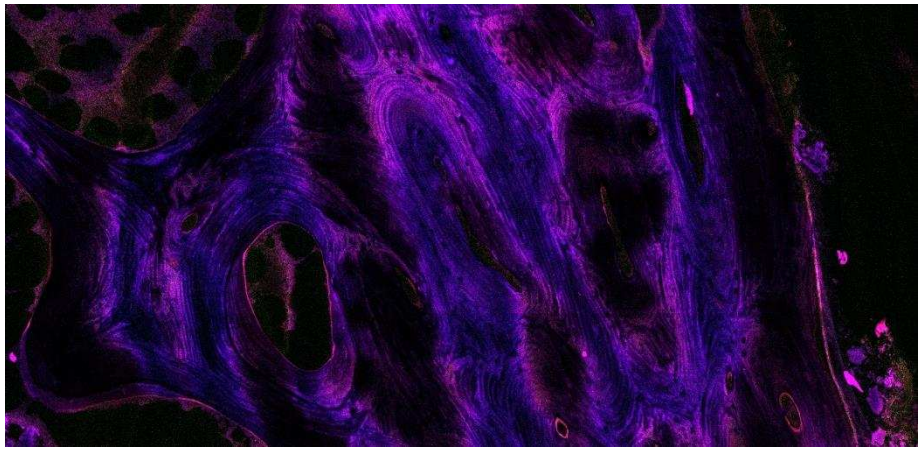
Basic Fuschin (Top); Toluidine Blue/Natural (Bottom)



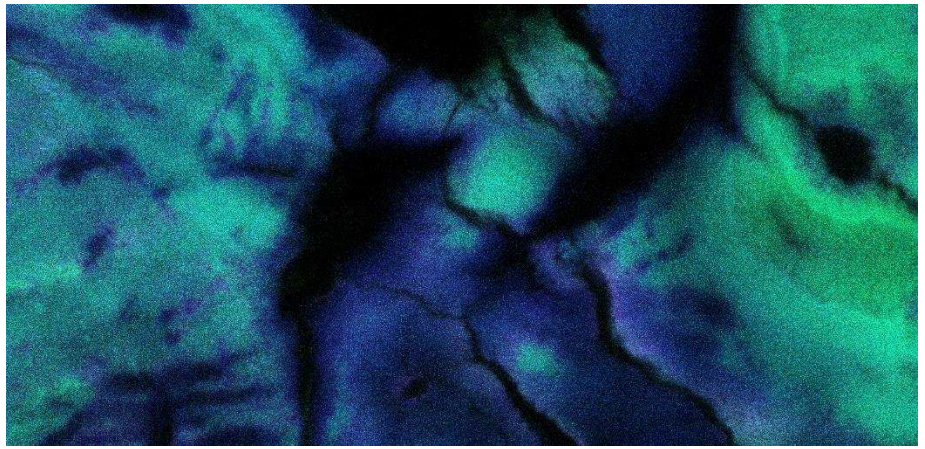
Diagenetic Alterations



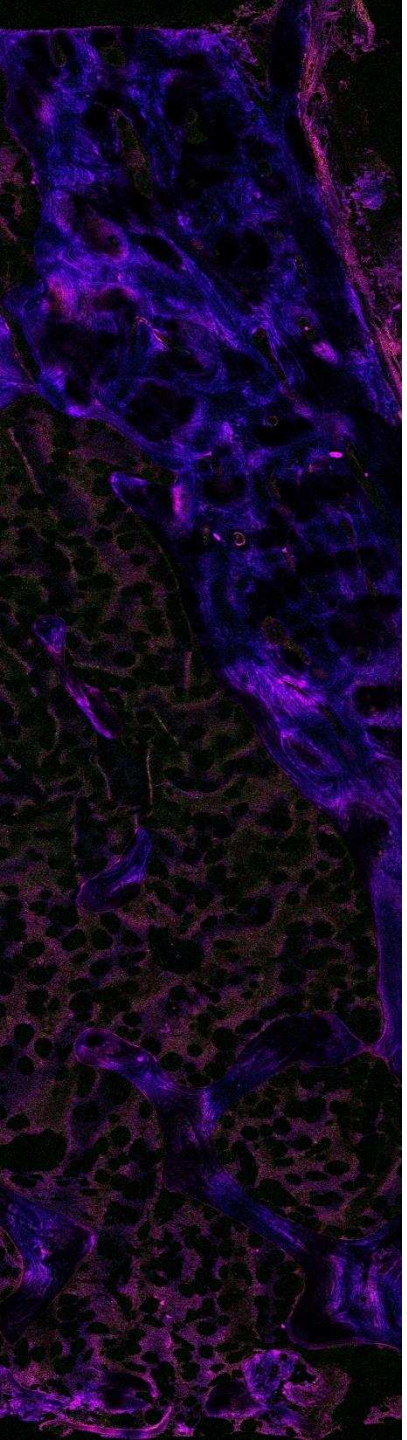




Non-Diagenetically Altered Bone (20x)



Diagenetically Altered Bone (40x)



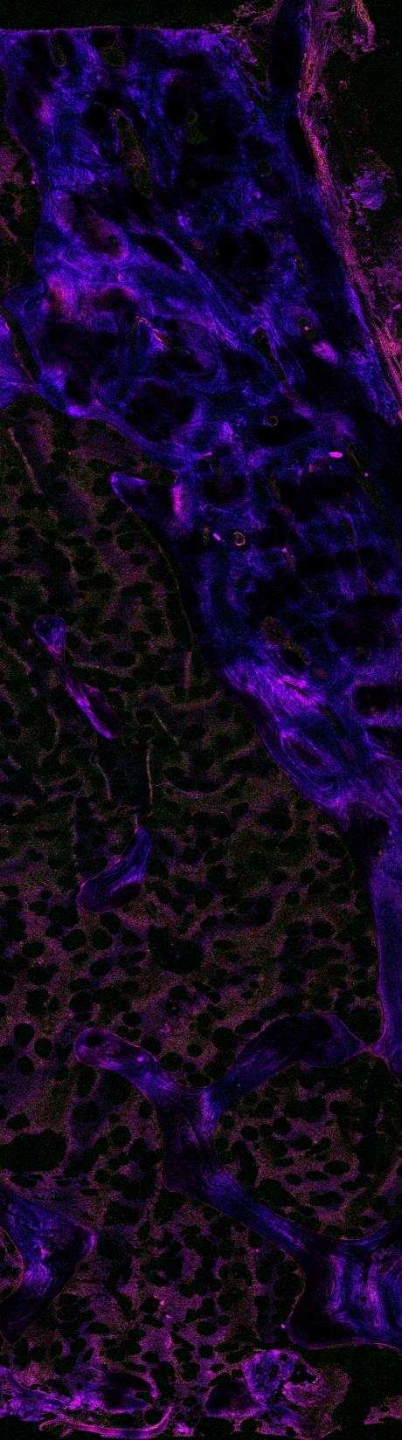
Conclusion

Benefits

- Higher resolution
- Microstructural examination
- Three-dimensional modeling
- Isolate λ = isolate target material
- Greater opportunities in examinations & mapping

Drawbacks

- Costly
  - Interdepartmental collaboration
- Time consuming
  - Can take hours depending on task/quality
  - Set-up does not correlate across samples



Thank You

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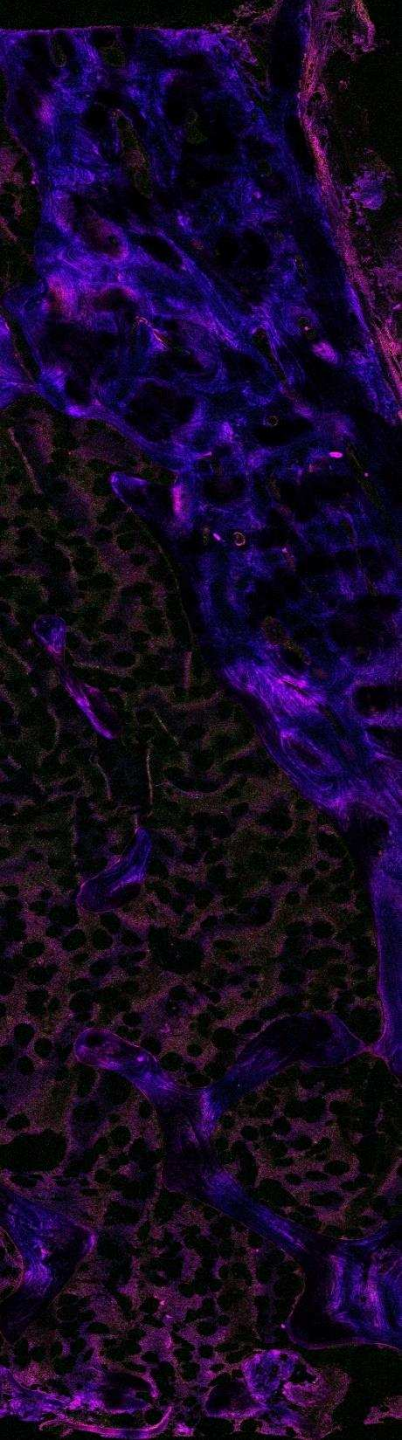
McMaster University

UNIVERSITY OF TORONTO MISSISSAUGA

CHUM Centre hospitalier de l'Université de Montréal



References & Further Reading

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