

Reconsidering Special Stains In Forensic Histo-Anthropology

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DISCLOSURES

- Lelia Watamaniuk, McMaster University: NO DISCLOSURES
- Ashley Smith, University of Toronto: Funding partially provided by SGS Fellowship

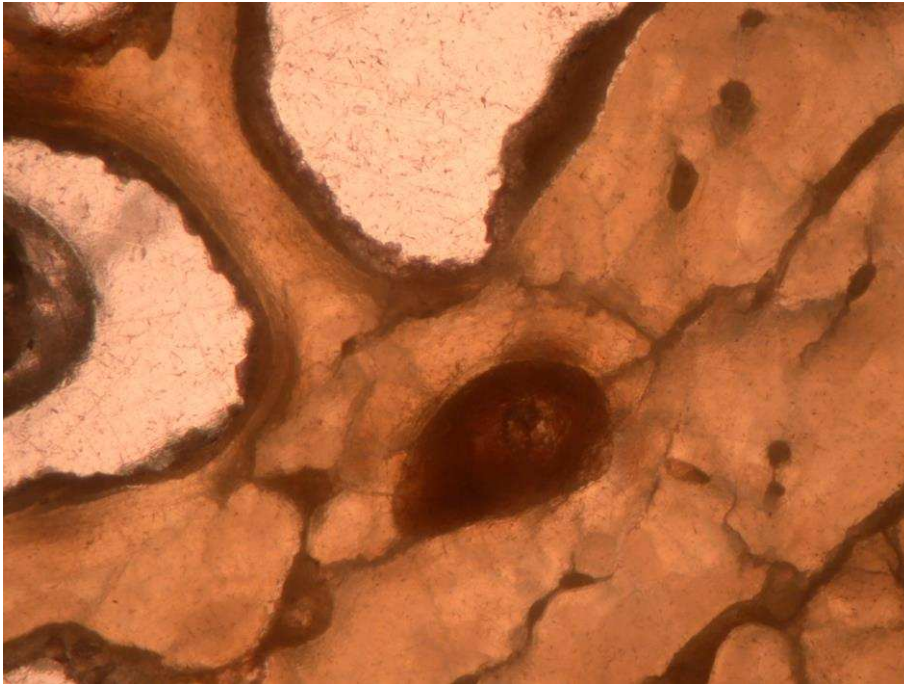
HISTOLOGY & (FORENSIC) ANTHROPOLOGY

- Light Microscopy (LM)
 - Plane & polarized light
- Scanning Electron Microscopy (SEM)
- Age estimation
- Specimen identification (human/non-human)

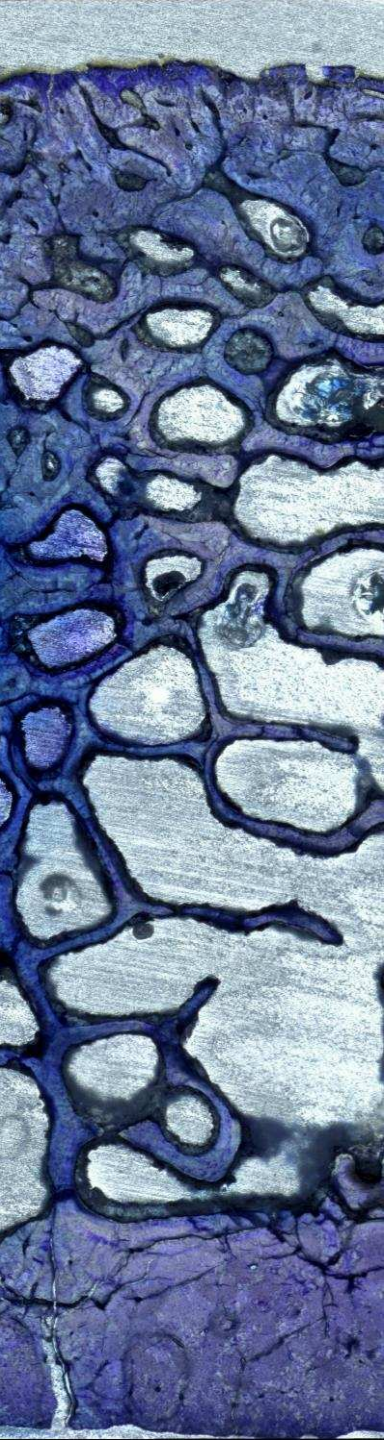


Human Rib (3rd-4th C, Apollonia)

PLAIN & POLARIZED LIGHT MICROSCOPY



Human Rib (3rd-4th C, Apollonia) (l: plain light, r: polarized light)



DIGITAL MICROSCOPY

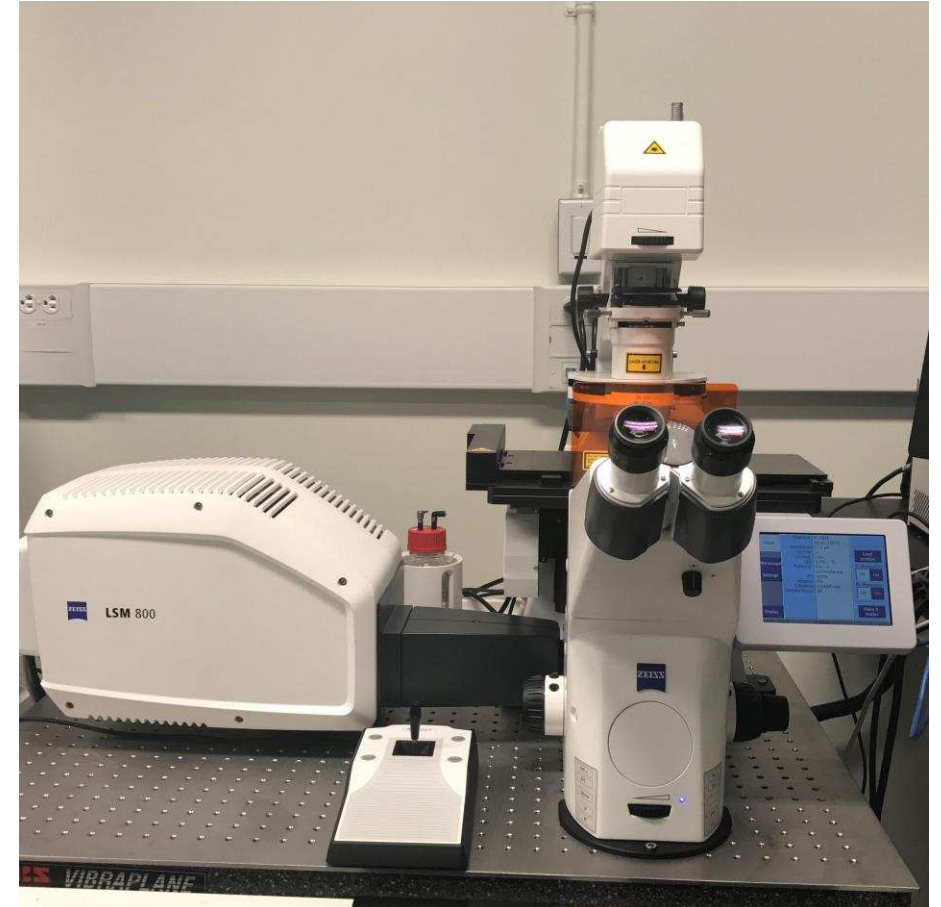
- Light microscopy
- Overhead or transmitted
- 2D & 3D measurements
- Lenses: 10x – 1000x
- Polarized light filters



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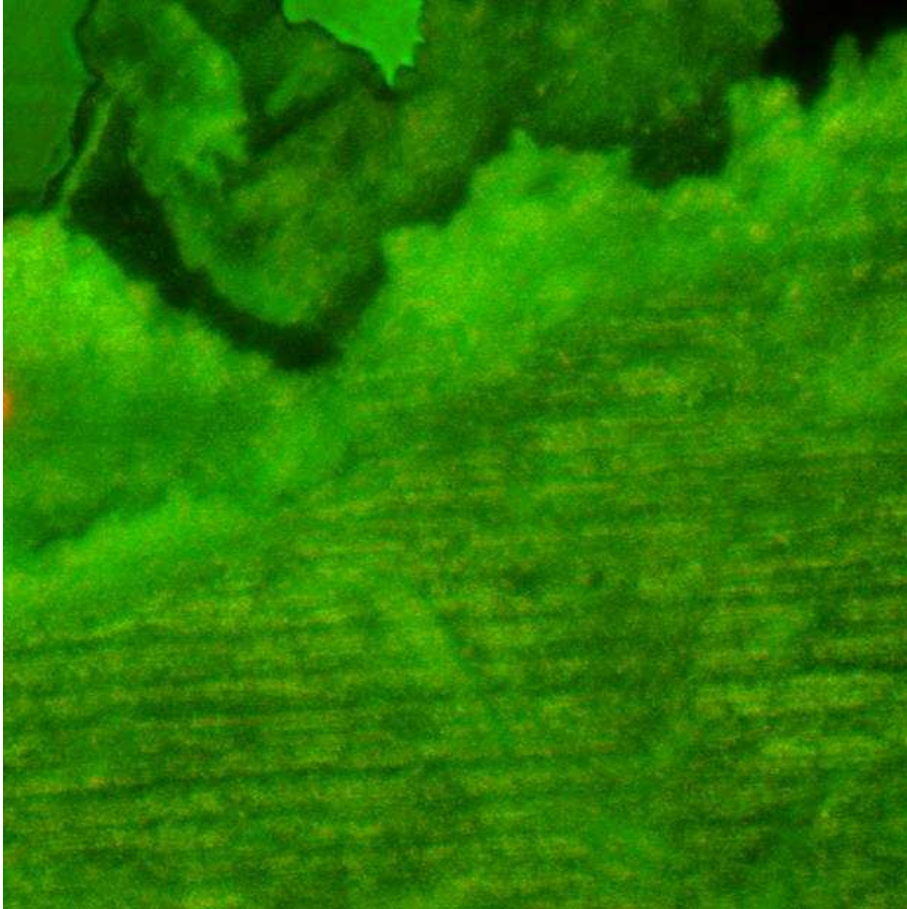
LASER SCANNING CONFOCAL MICROSCOPY (LSCM)

- Uses laser light to excite photons in a sample
- Each material excites at selected wave lengths
- Targeting selected wavelengths allows for quantifying specific materials
- Images can be taken in multiple slices along the Z-Axis
 - Allows for 3D microscopy

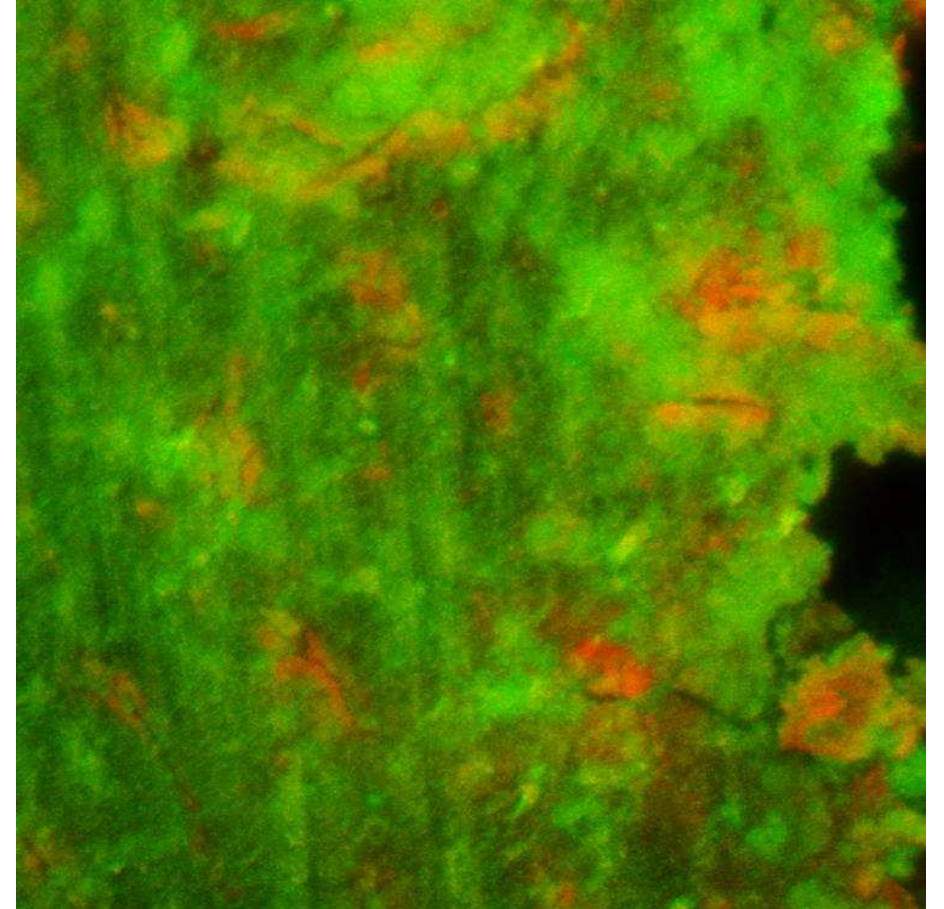


Carl Zeiss LSM 800. University of Toronto - Mississauga

LSCM & UNSTAINED BONE



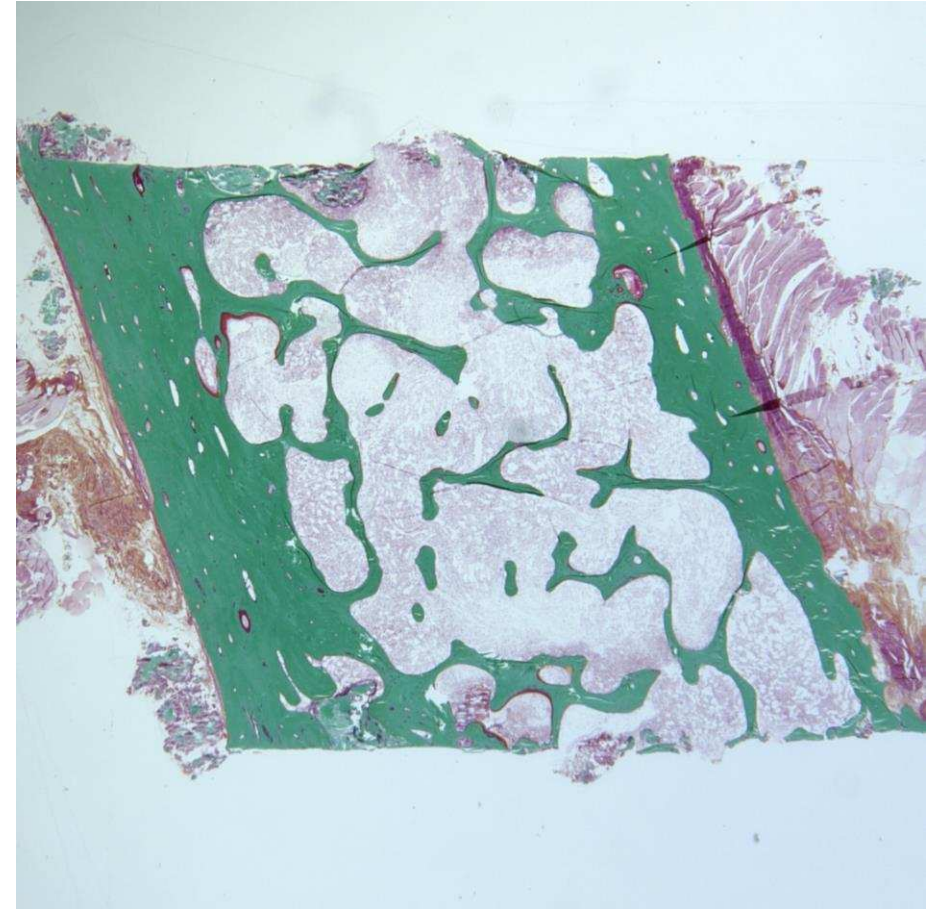
Fresh Pig Bone unstained/undyed with natural autofluorescence



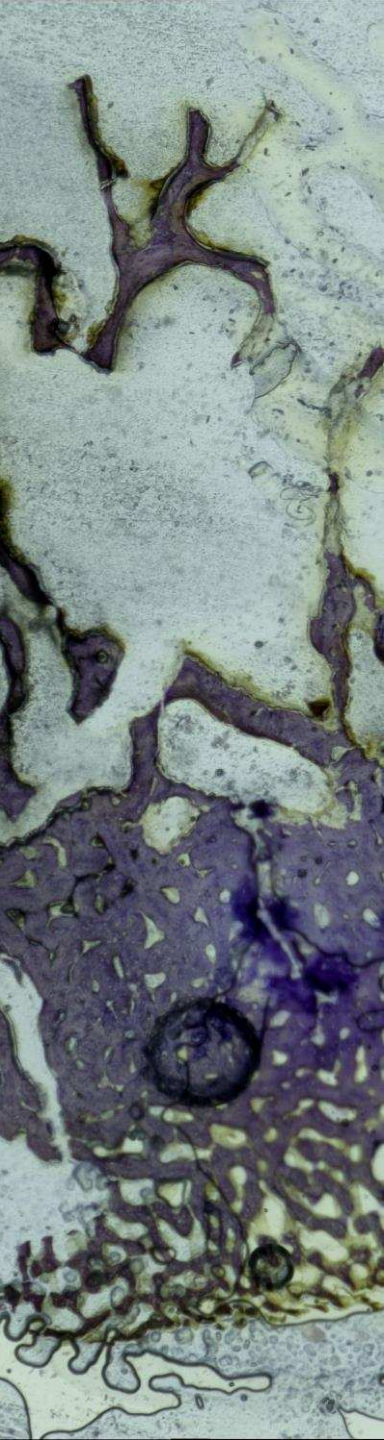
5y Pig Bone unstained/undyed with natural autofluorescence

STAINING in PATHOLOGY

- General staining
 - Hematoxylin & Eosin
 - Toluidine Blue
 - Component stains – cells, nuclei, connective tissues
- Special staining
 - Goldner's Trichrome
 - TRAP
 - Prussian Blue



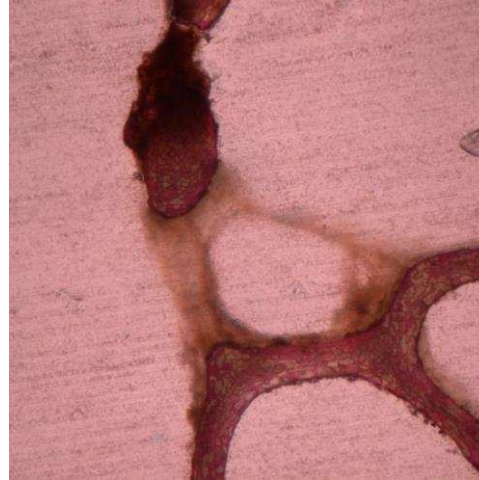
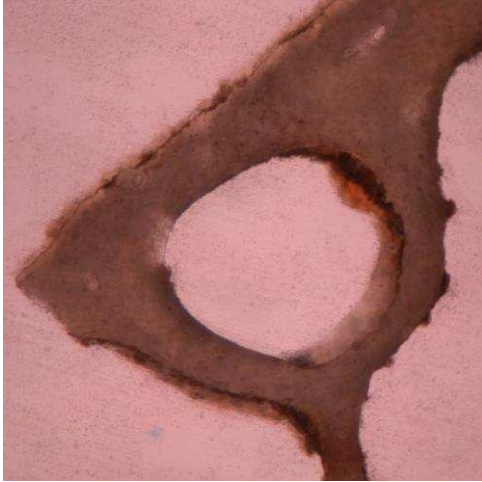
Biopsy Human x10 – Goldner's Trichrome



SPECIAL STAINS for BONE

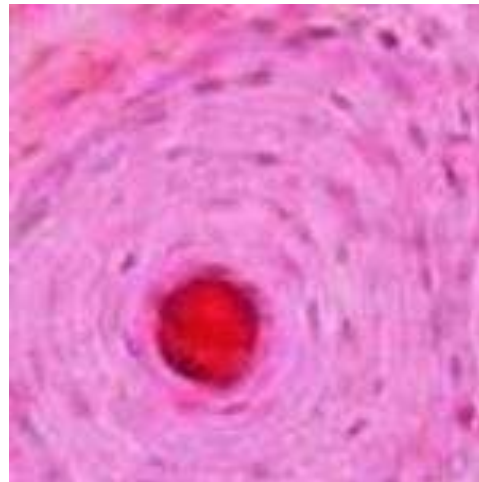
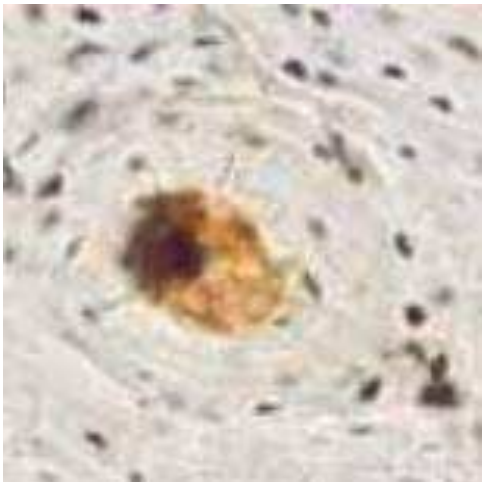
- **H&E:** Routine stain for cellular detail, best on paraffin sections
- **Toluidine Blue O:** Stain utilized for optimal demonstration of mineralized bone and osteoid seams, osteoblasts, osteoclasts.
- **Alkaline Phosphatase:** Enzyme histochemical stain for osteoblasts. This stain is diminished in formalin fixed tissues.
- **Tartrate Resistant Acid Phosphatase (TRAP):** Enzyme histochemical stain specific for osteoclasts.
- **Von Kossa:** Stain best for demonstration of mineralization in bone and other tissues. Also used to demonstrate areas of mineralization in cell cultures.
- **Gomori's trichrome:** Stain used to visualize collagen in tissues
- **EVG (Elastin/Van Gieson):** Demonstrates presence of elastic fibers in tissues
- **Prussian Blue:** Demonstrates presence of iron in tissues
- **Goldners Trichrome:** Stain utilized for differentiation of mineralized and non-mineralized areas in bone.
- **Alizarin red/Alcian blue staining of whole tissues:** Stain for differentiation of mineralized vs. unmineralized bone in whole tissues

HEMATOXYLIN & EOSIN



Human Rib (3rd-4th C, Apollonia)

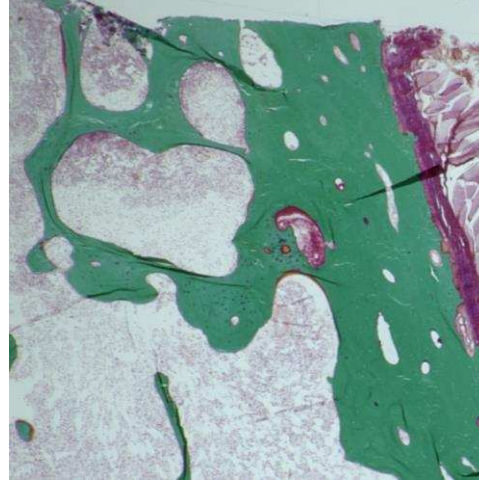
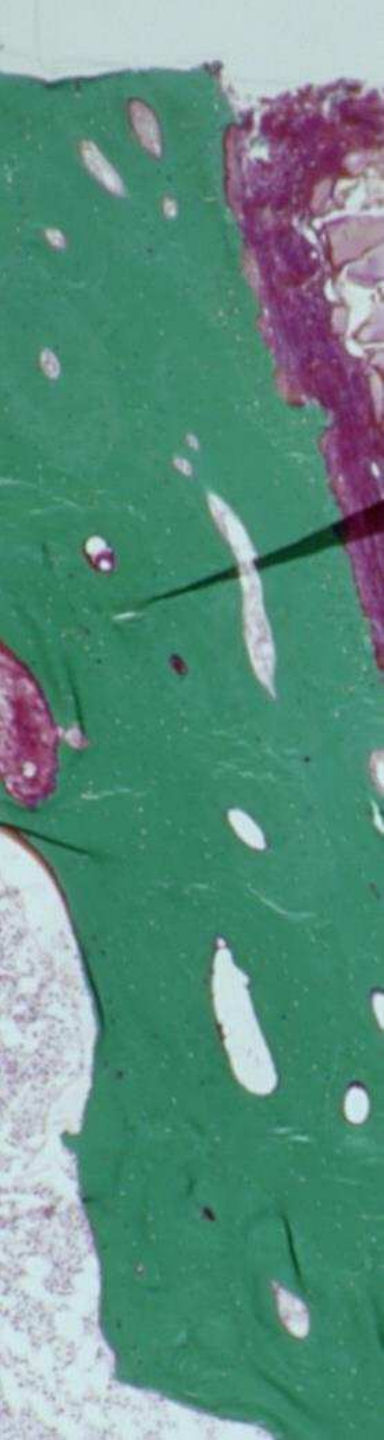
- Highlights areas of diagenetic change



Human Archaeological Bone
(Fig. 3, c, d respectively)
de Boer et al. (2010)

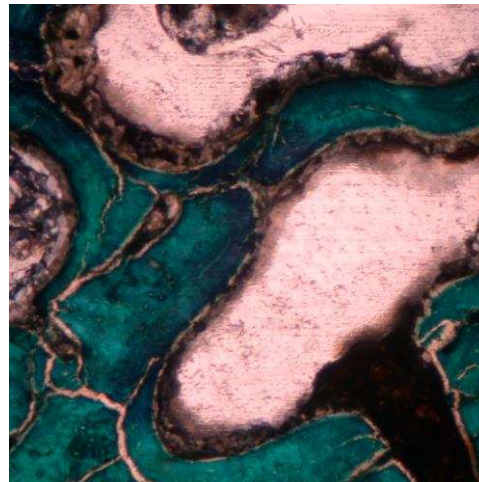
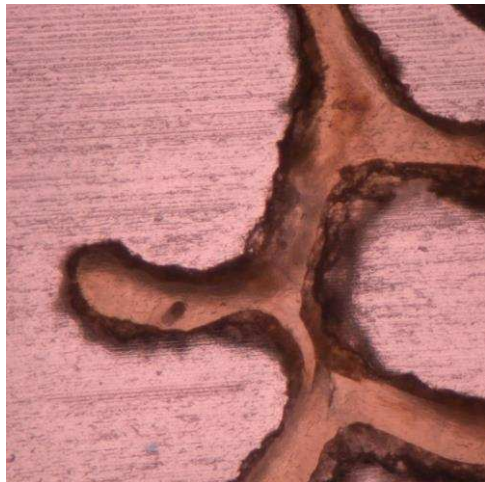
- Structures more clearly visible eg lamellae

GOLDNER'S TRICHROME



Biopsy Human x10 – Goldner's Trichrome

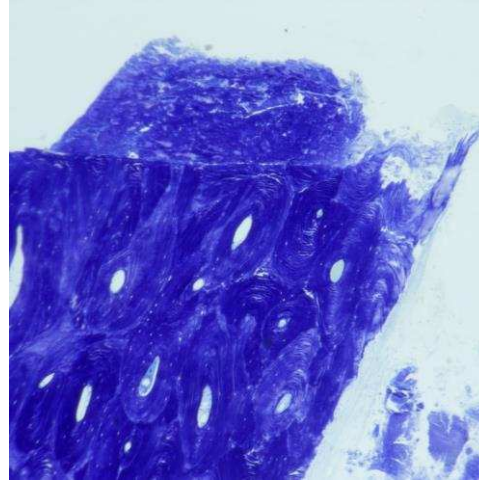
- Mineralized v unmineralized (osteoid)
- Stained marrow



Human Rib x10 (3rd-4th C, Apollonia)

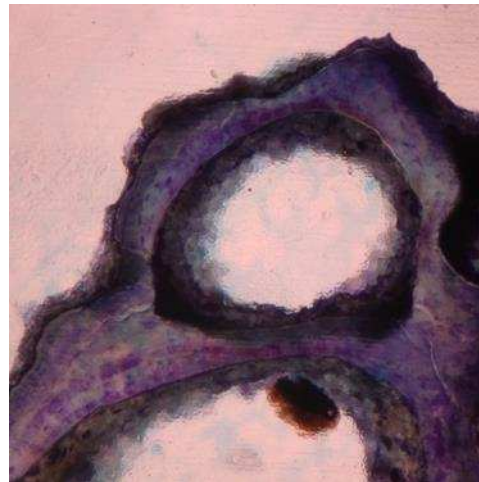
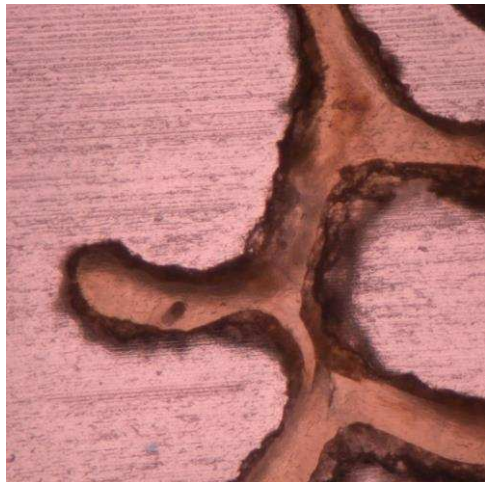
- Preparation artefacts versus diagenesis/damage clear

TOLUIDINE BLUE - PLAIN LIGHT



Biopsy Human x10 – Toluidine Blue

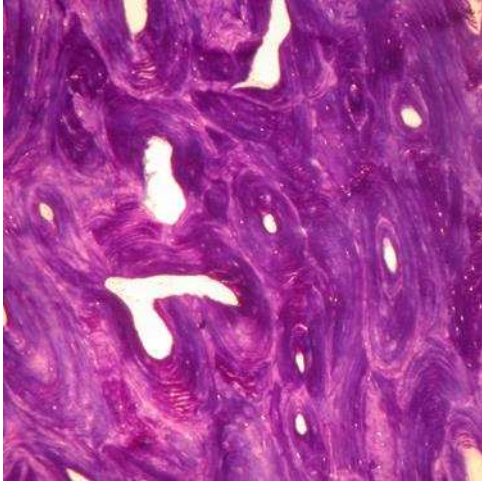
- Osteons visible
- Stained marrow (faint blue)



Human Rib x10 (3rd-4th C, Apollonia)

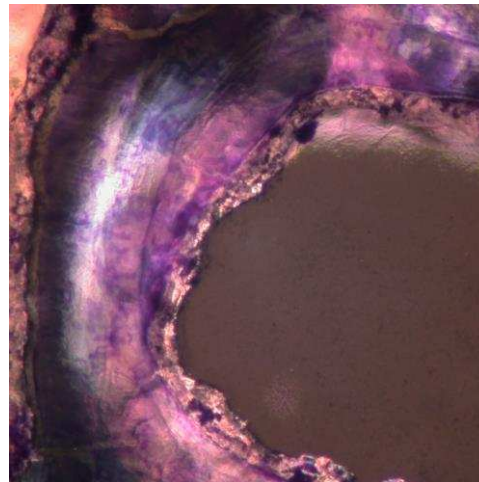
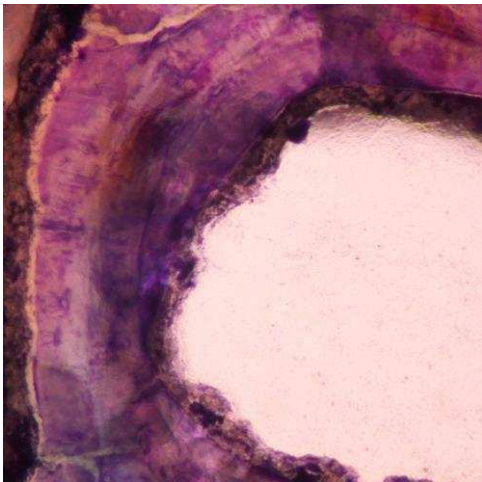
- Structural features visible
- Differential staining depending on diagenesis

TOLUIDINE BLUE – POLARIZED LIGHT



Biopsy Human x10 – Toluidine Blue (r: polarized light)

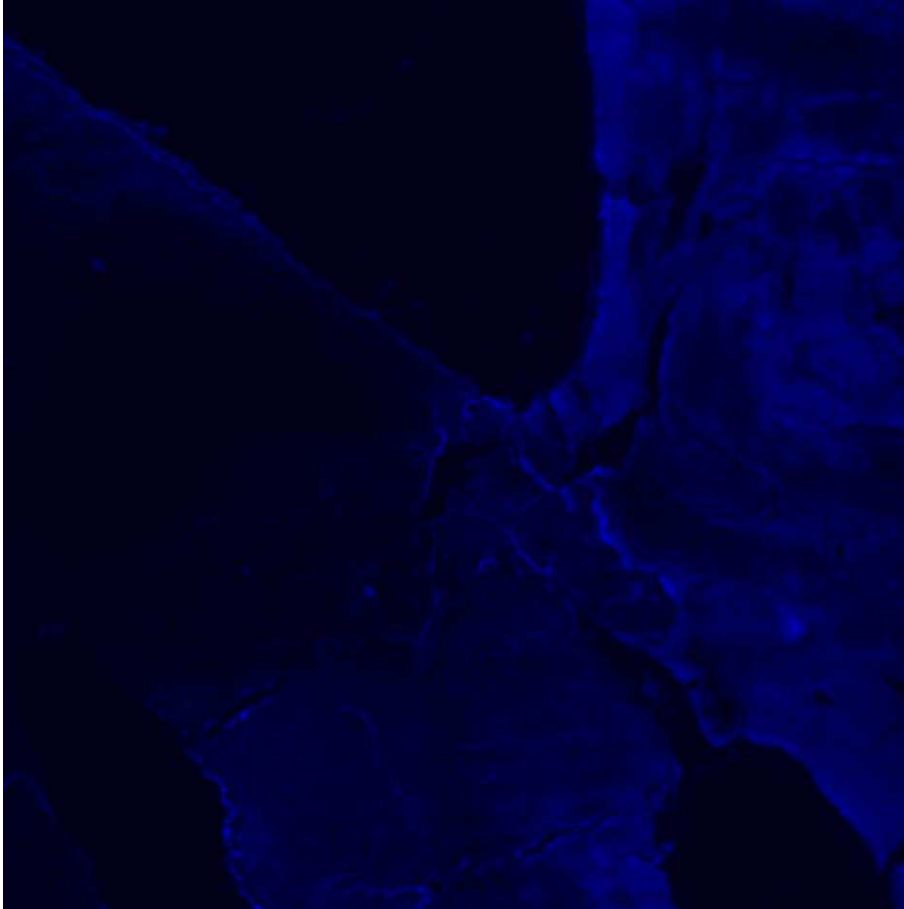
- Osteons visible
- Clear lamellar structure



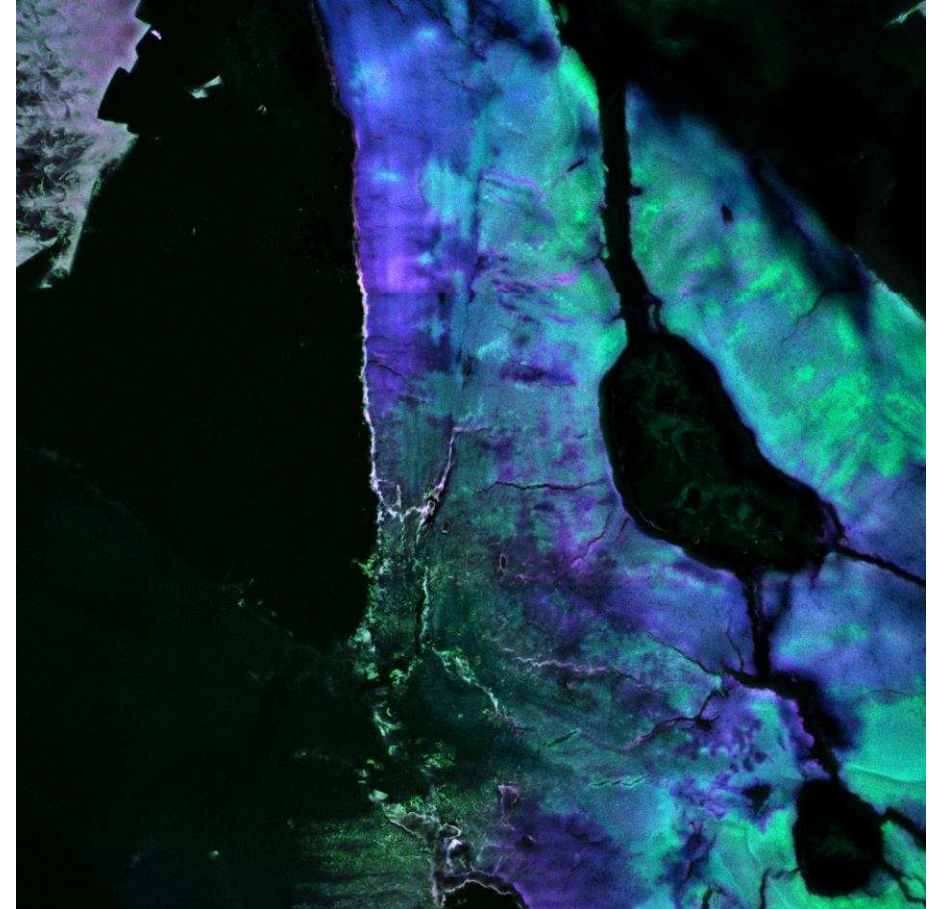
Human Rib x10 (3rd-4th C, Apollonia) Toluidine Blue (r: polarized light)

- Structure versus damage clear
- Retained cement lines visible

LSCM & TOLUIDINE BLUE

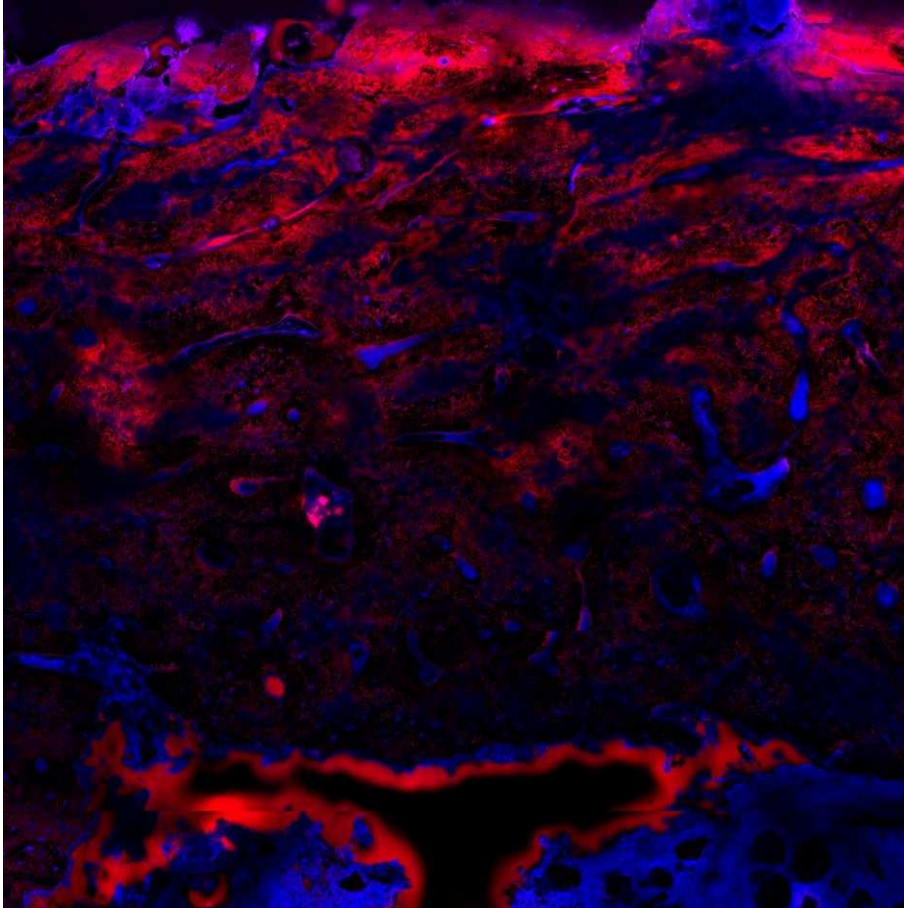


GIF Human Z-Stack 4-Tile Image; 40xMag;
633nm Channel

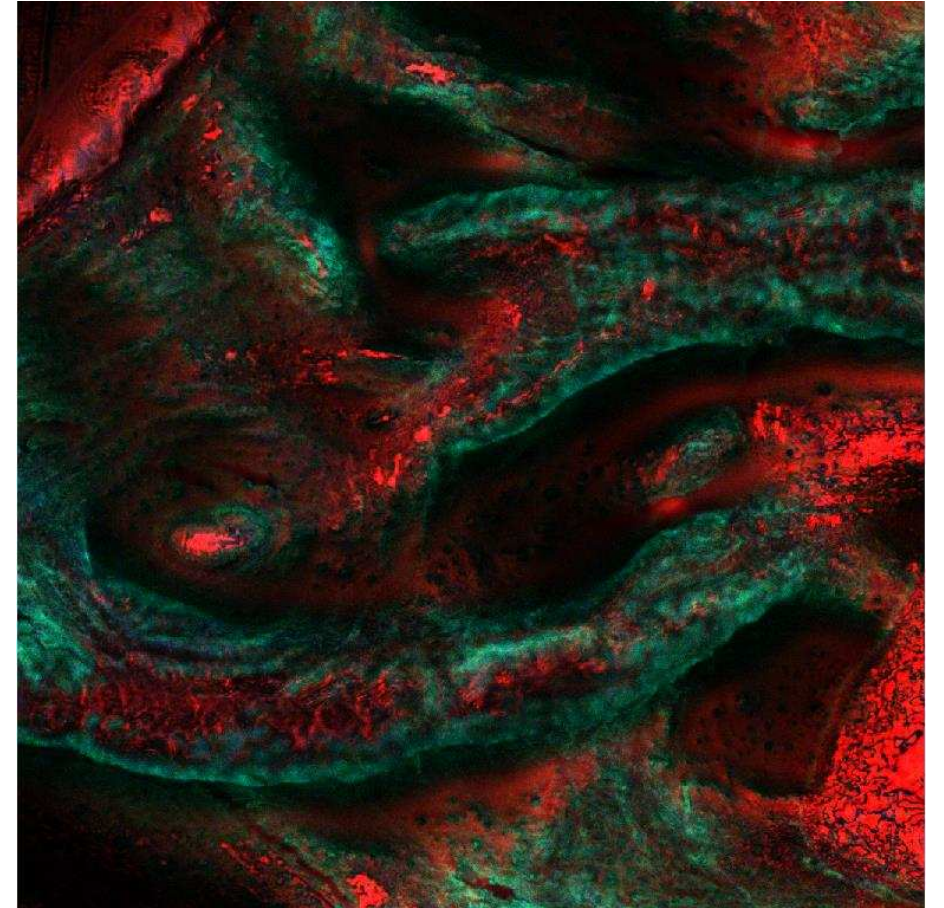


Human 9-Tile Image; 40xMag; Multichannel

BASIC FUSCHIN

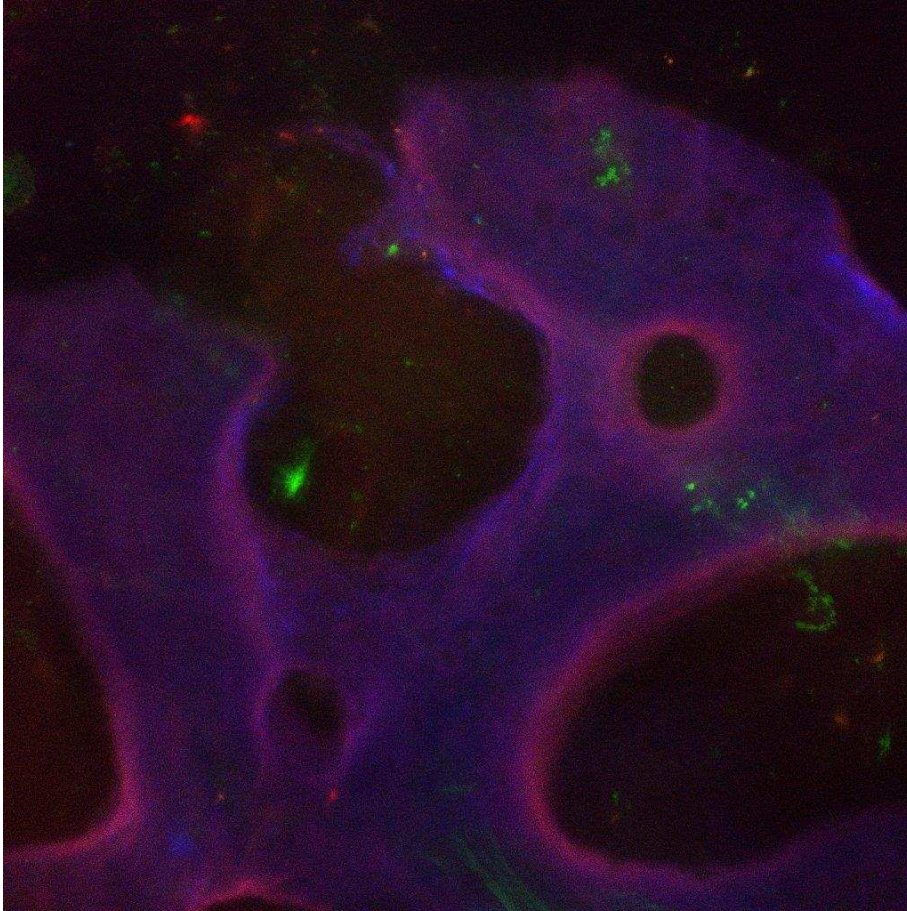


Fresh Pig Bone Tile Set at 40x Mag

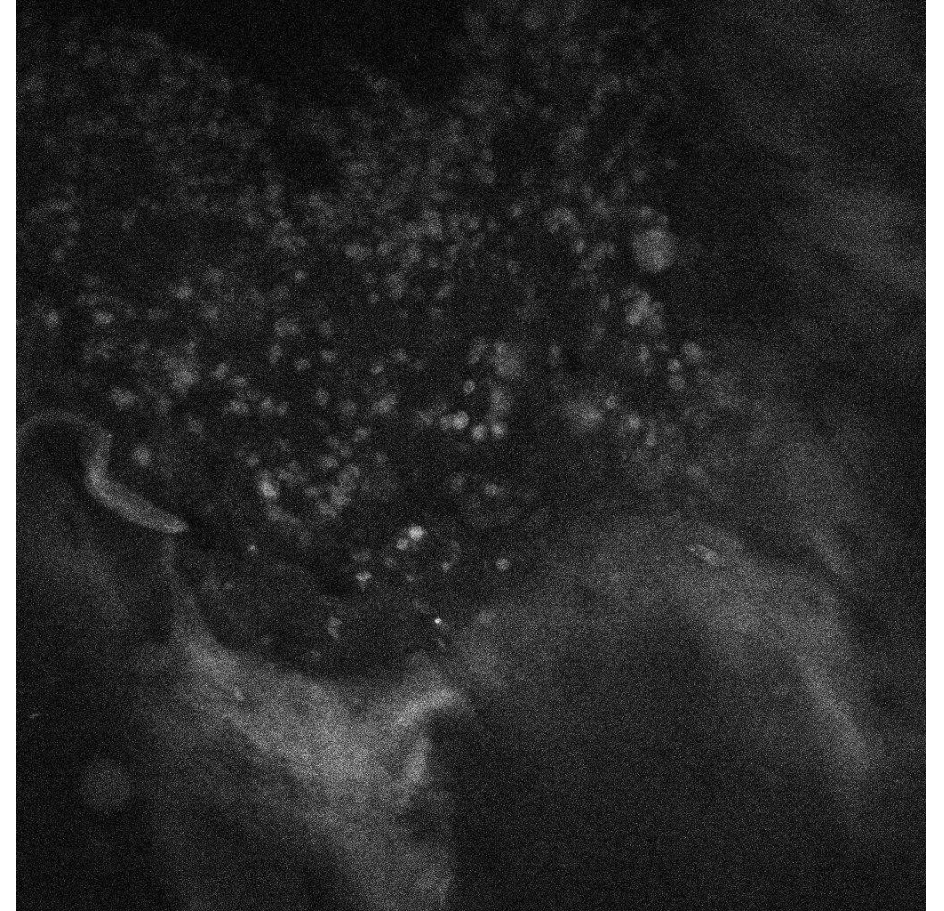


Biopsy Human Bone at 63x Mag

TRAP 87

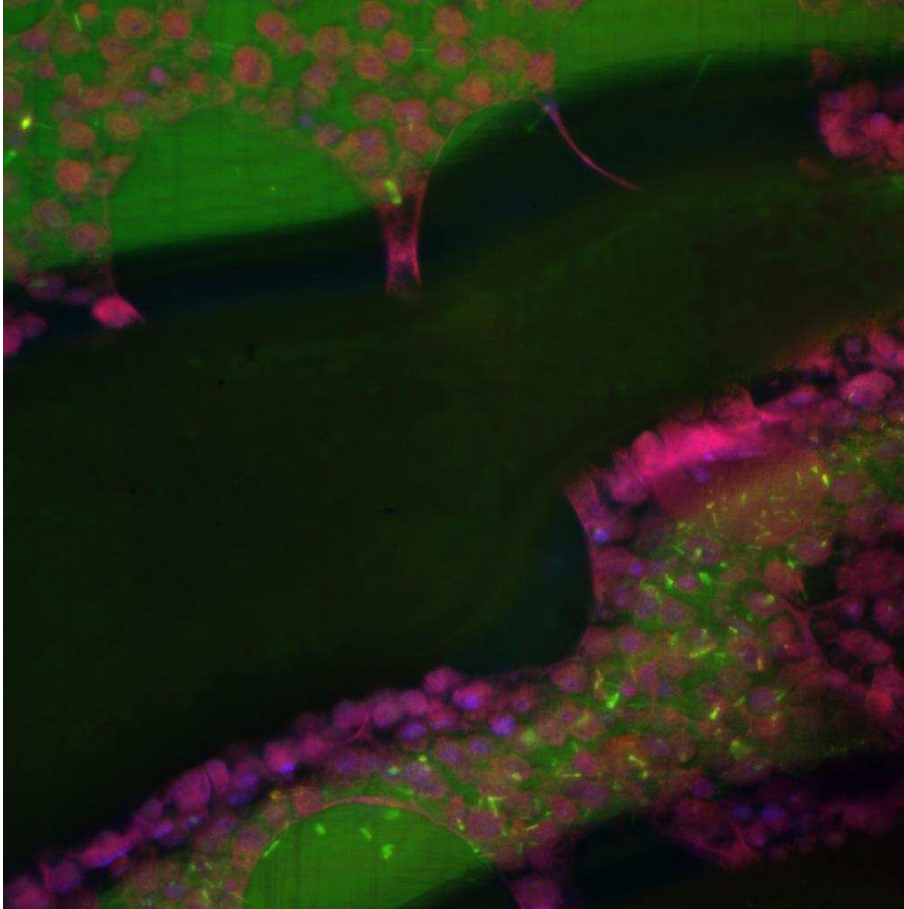


TRAP 87 with Dry Pig Bone, 20x Mag

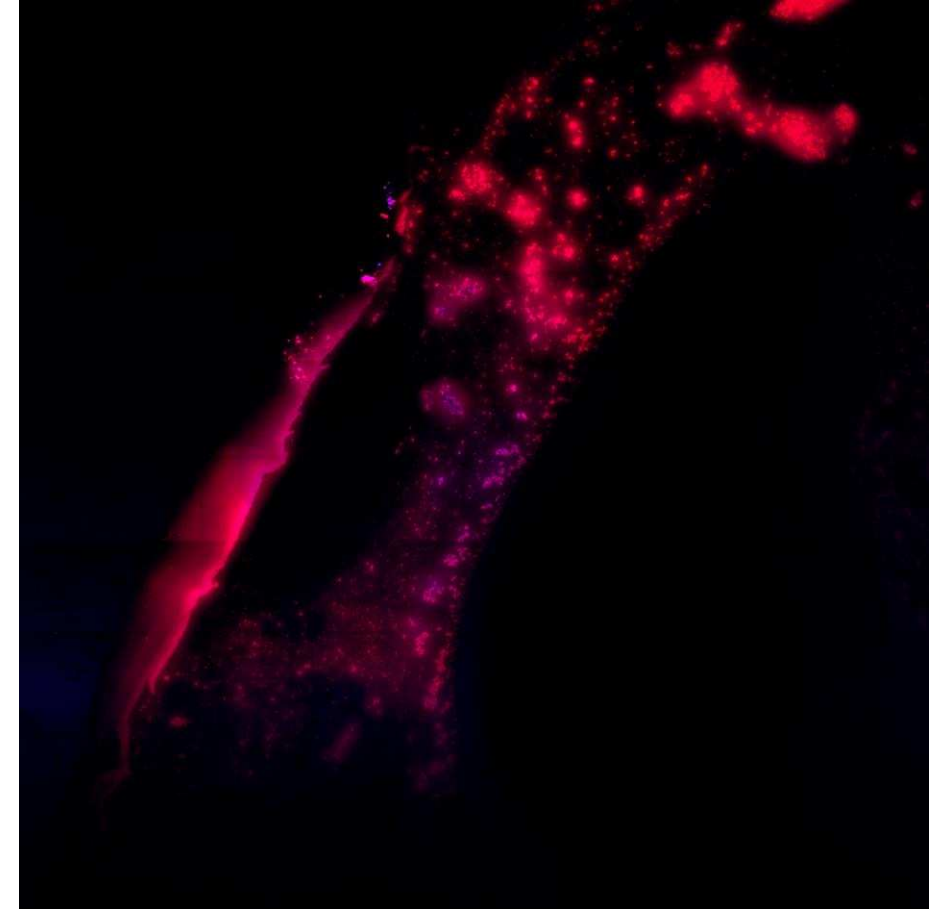


Fresh Pig Bone at 40x Mag; Alexafluor 488
with TRAP 87 (Greyscale)

FLUORESCENCE DYES



5-y Pig Bone at 63x Mag; Alexafluor 488 with
SlowFade Gold (Basic Fuchsin)



Fresh Cortical Pig Bone Tile Set at 40x Mag;
Alexafluor 488 with SlowFade Gold

REFERENCES

- de Boer HH, Aarents MJ, Maat GJR. 2012. Staining Ground Sections of Natural Dry Bone Tissue for Microscopy. *Int J Osteoarchaeol.* 22(4):379-386.



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