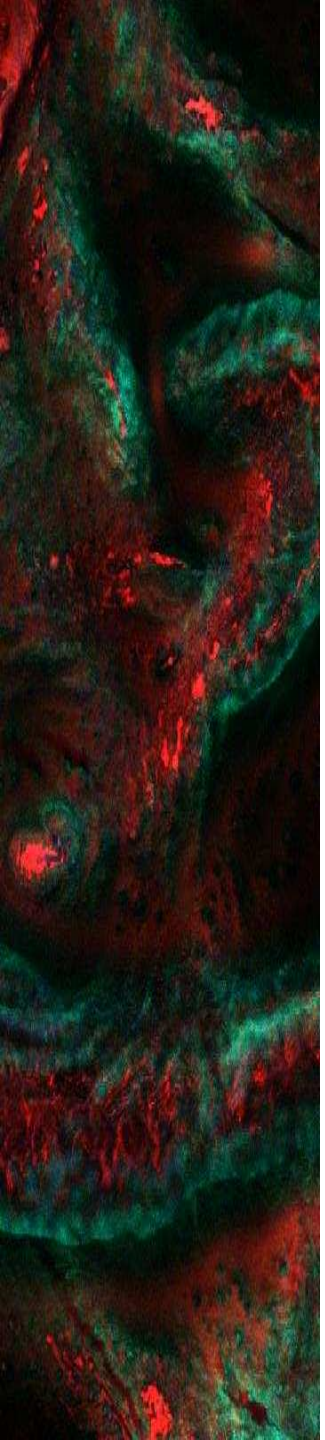

Laser-Scanning Confocal Microscopy Distinguishing Peri-Mortem & Post-Mortem Damage Using Histotaphonomic & Histochemical Techniques

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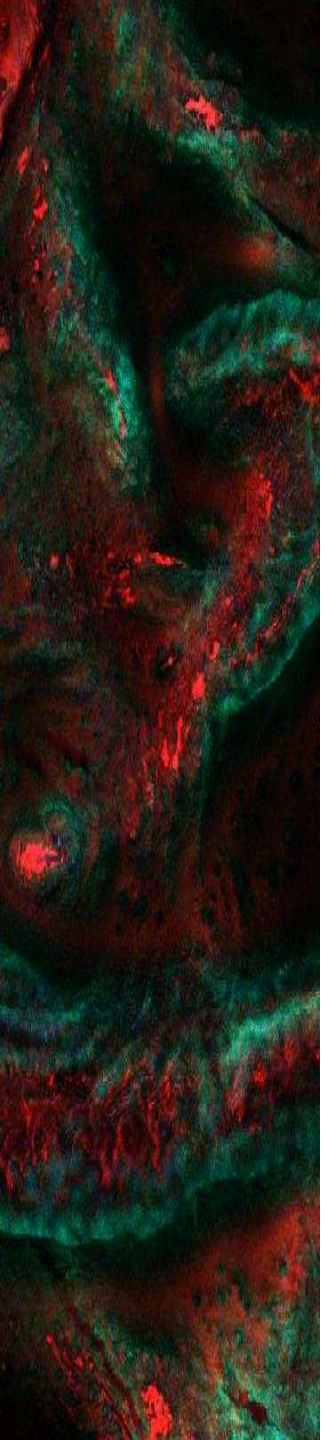
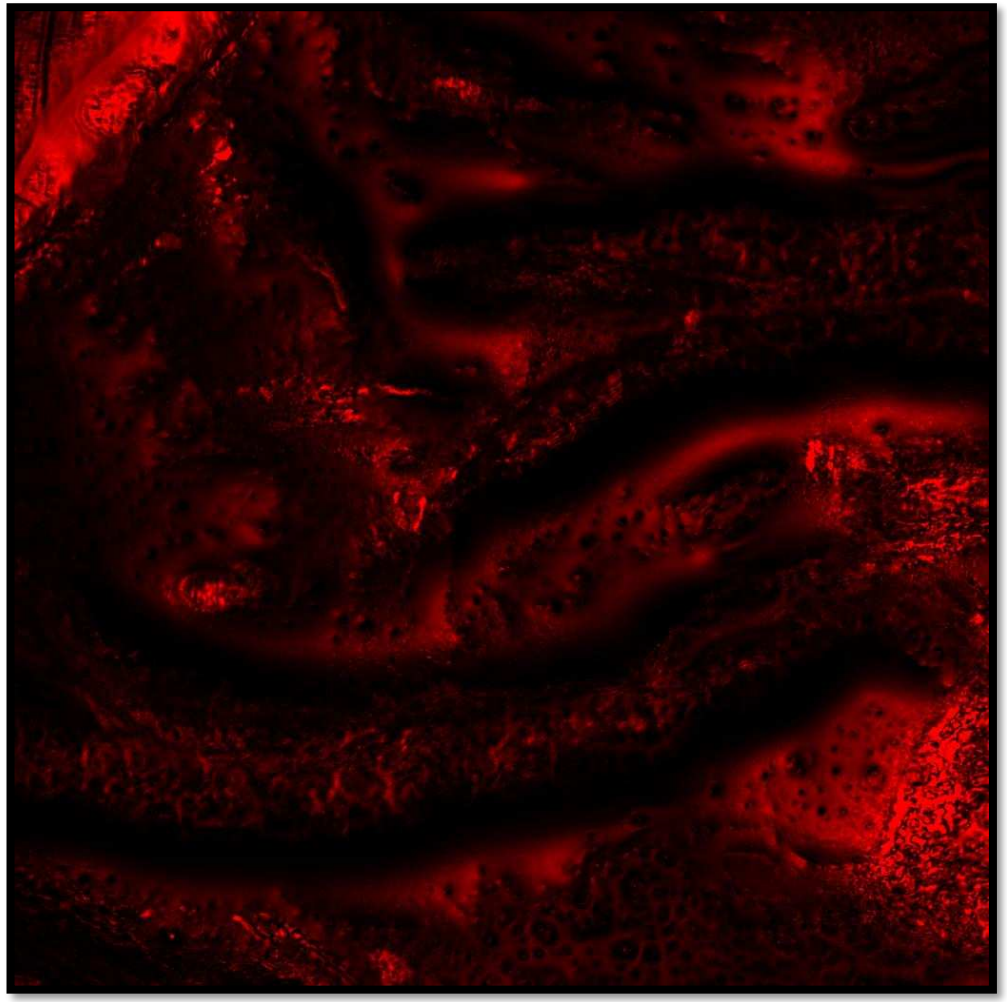
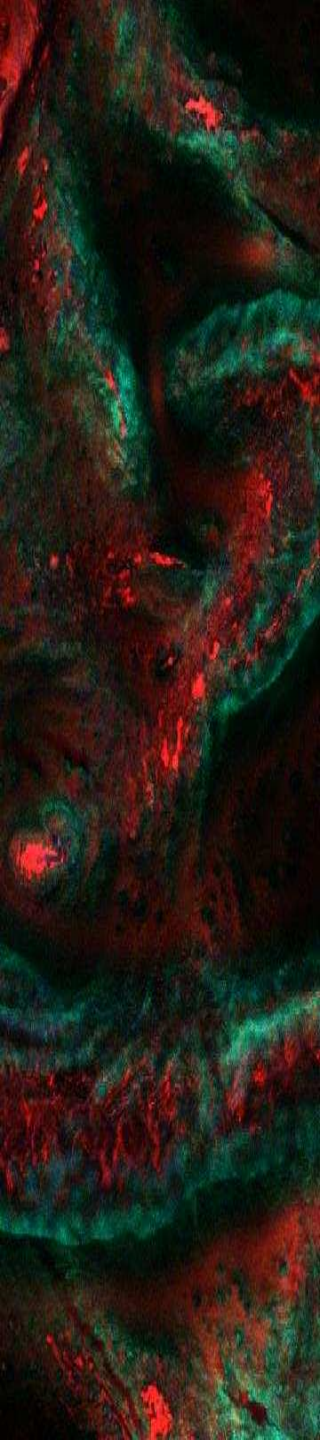


Purpose

- Usefulness of Laser Scanning Confocal Microscopy (LSCM)
- Differentiate perimortem from postmortem damage
- Histotaphonomic & histochemical
  - Microstructural
  - Protein degradation
  - Osteoclast degradation



Basic fuchsin stained ilia cortex 40x mag., 488nm Argon laser



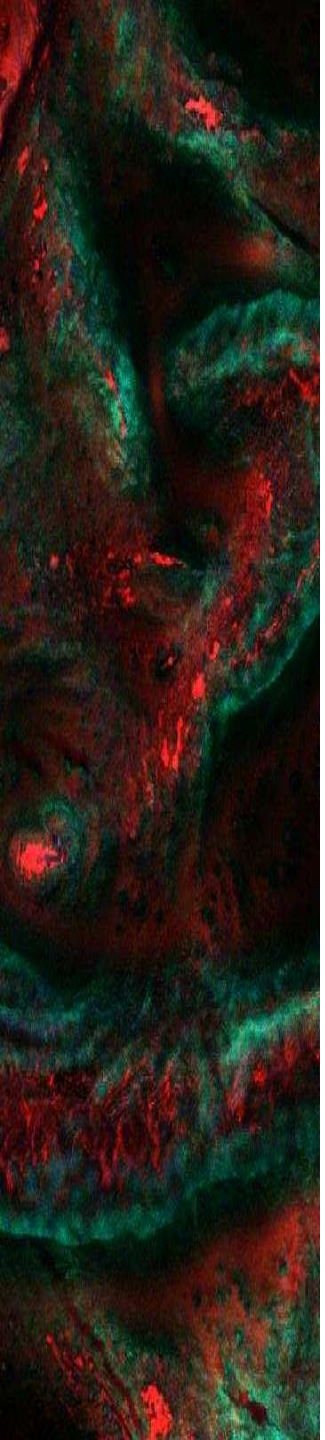
SIGNIFICANCE

- Better Reconstruction of death event
  - injuries associated with death
  - damage occurring PM
    - concealing body
    - dismemberment
- Legal Implications
- Determine PMI

PERI- VS POST-MORTEM BREAKAGE

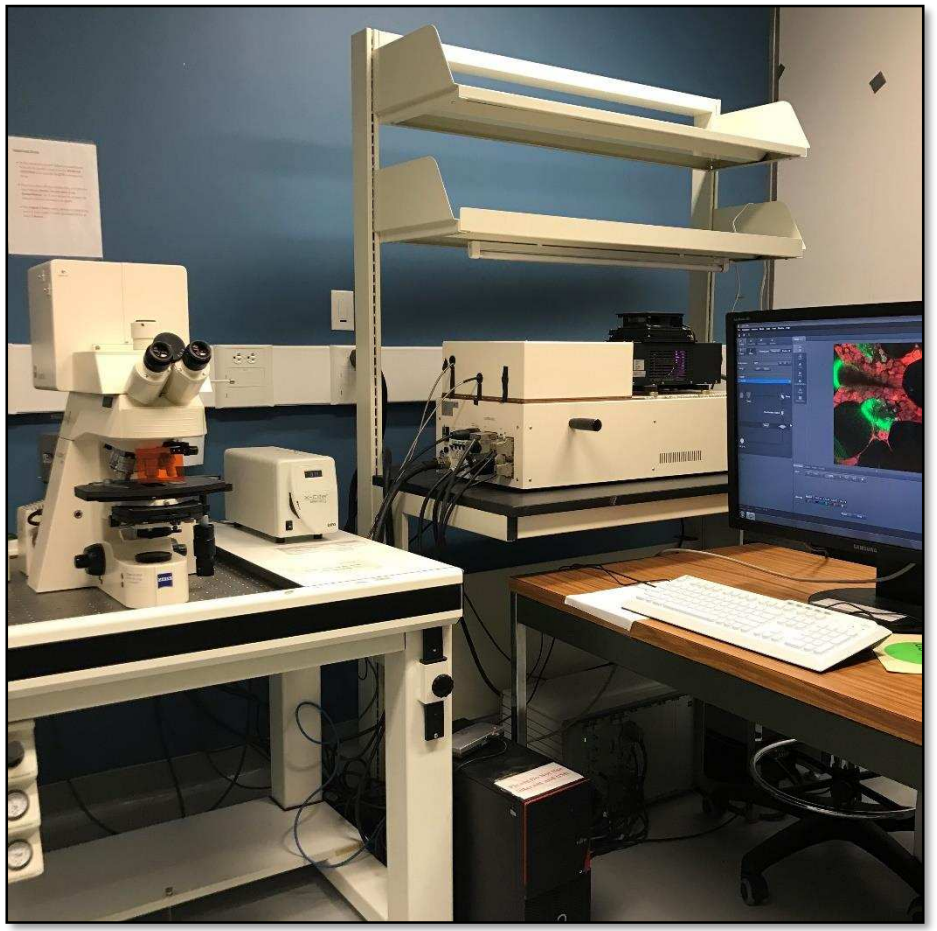
- Gross Taphonomic Differences
  - Organic Component
  - Moisture Content
- Break Morphology
- Fresh
  - Gross Appearance
    - Jagged, Uneven, Spiralled
  - Microscopic
    - Smooth
- Dry
  - Gross Appearance
    - Straight, Perpendicular to shaft
  - Microscopic
    - splintered

Perimortem trauma of human femur vs postmortem damage of human tibia



Laser Scanning Confocal Microscopy

- Combines principles of scanning electron, white light, and epifluorescence microscopy
- Different laser wavelengths
  - Fluoresce dyes and stain
- Photonmultiplier Detector
  - Converts light intensity into image
- Generates 3D Image
- Protein/Cell Isolation
- Quantified Microscopy

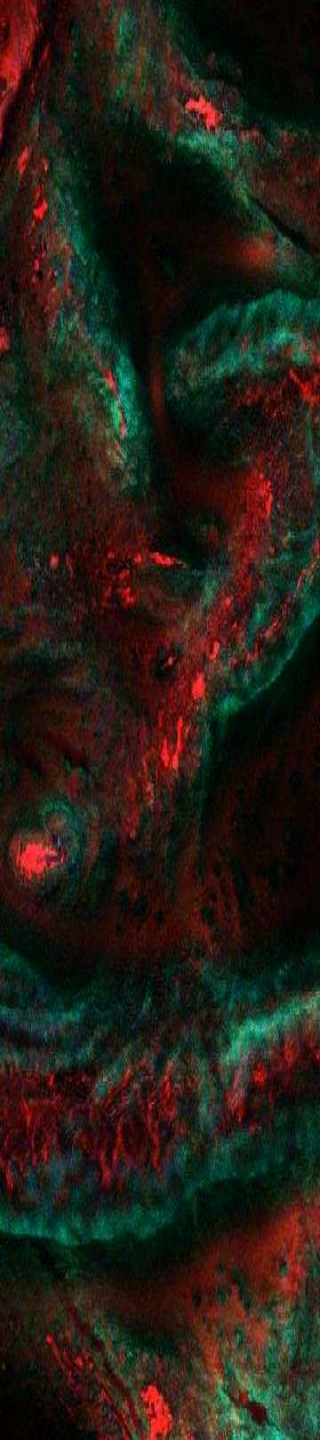


Laser Scanning Confocal Microscope at the University of Toronto Mississauga

METHODOLOGY

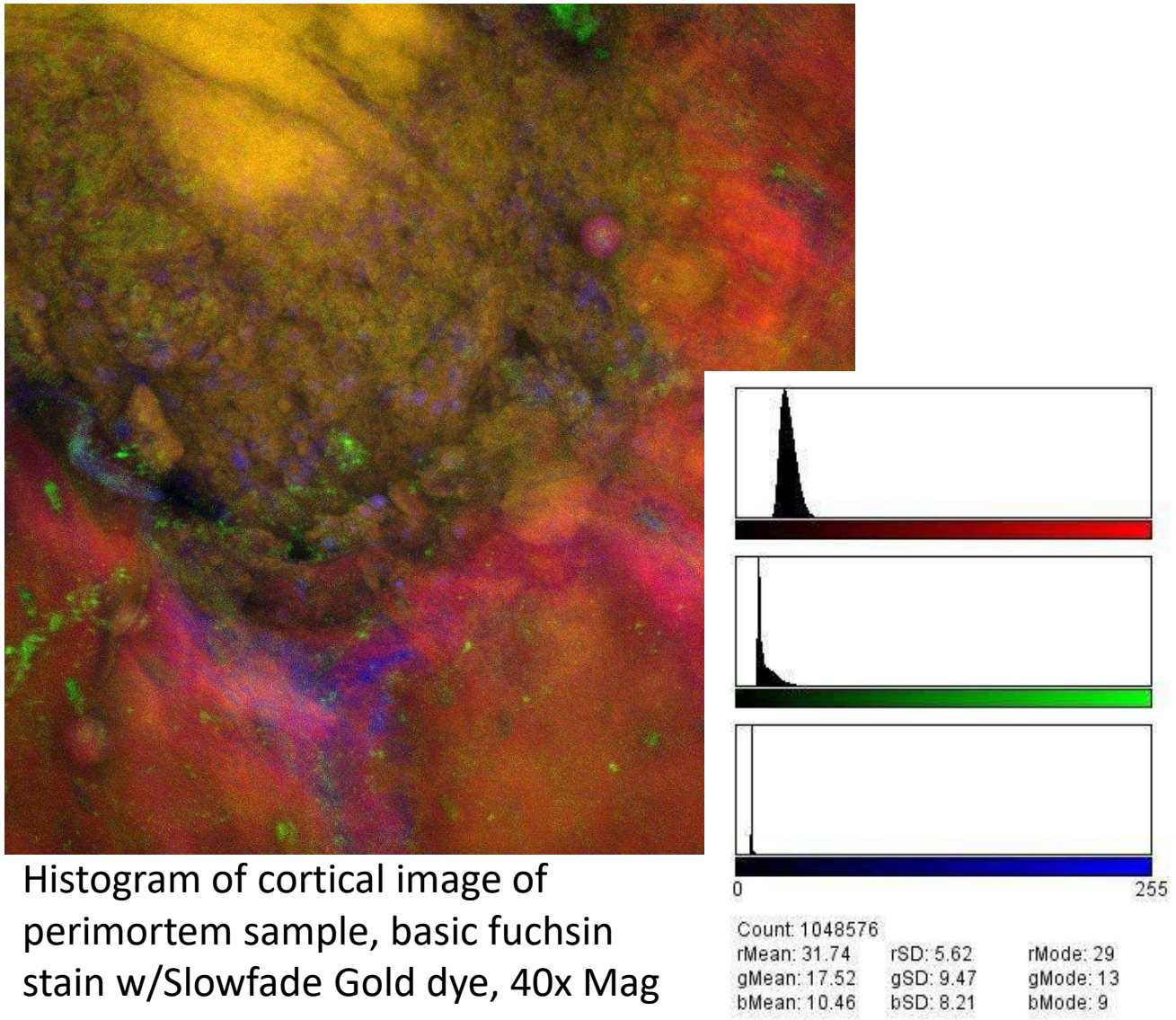
- 12 pieces pig bone
  - 6 “Fresh” <48hrs.
  - 6 Dry 5 years
  - 6 Pre-stained with basic fuchsin
- Methyl Methacrylate
- Sectioned to 10µm
- Alexafluor 488 + SlowFade Gold, TRAP 87, H&E
- LSM 510 Meta LSCM
- DAPI-GFP 4 Ch. Multitrack
- 405nm, 488nm, 543nm, 633nm

Screenshot of Zen 2009© LSCM Imaging Set-Up



Data Analysis

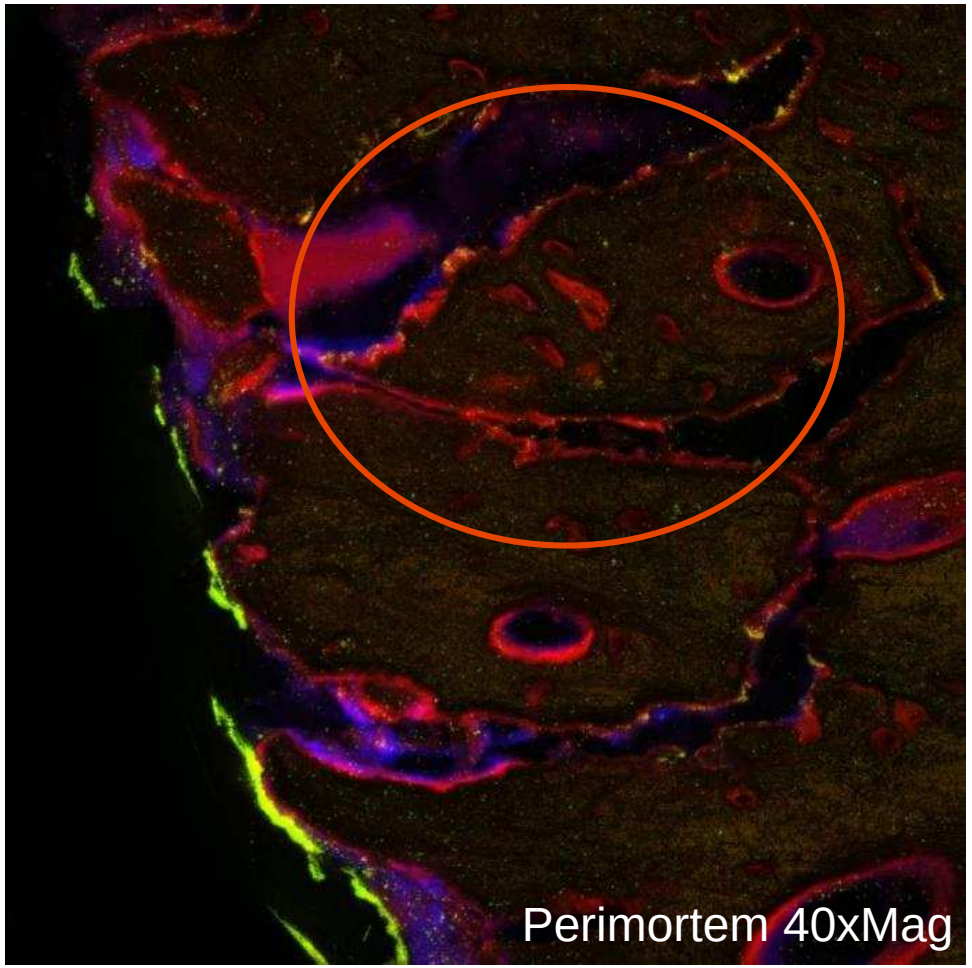
- Qualitative
  - 3D Imaging of edge
  - Microstructure
- Quantitative
  - Protein/Cell Count
  - Mean Hue/Std Dev.
  - RGB Count
- Statistics
  - One-way ANOVA
  - Pearson r^2 Correlation
    - Mean Measure and PMI



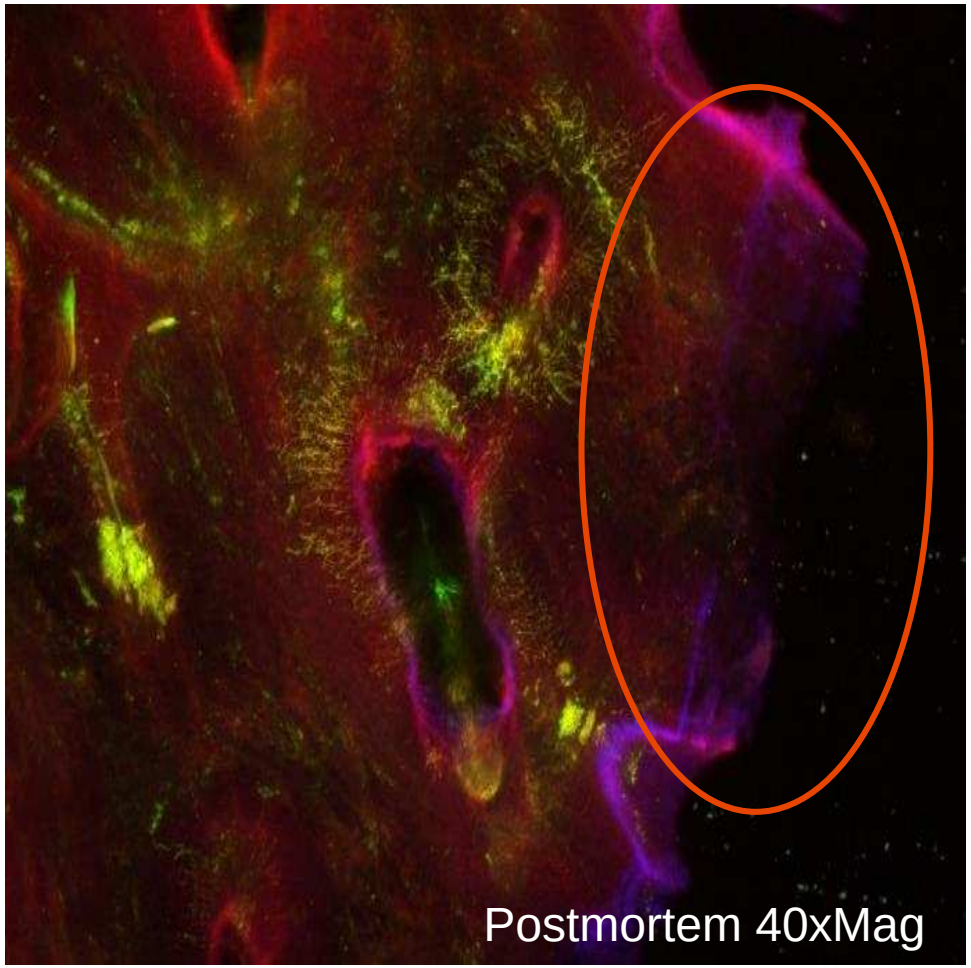
Histogram of cortical image of perimortem sample, basic fuchsin stain w/Slowfade Gold dye, 40x Mag

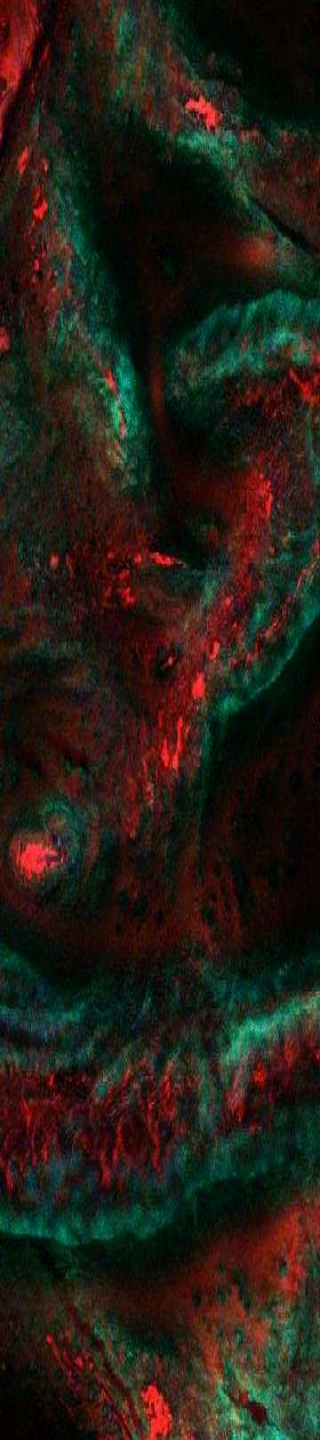
MICROSTRUCTURAL EDGE RESULTS
40 X MAGNIFICATION

Perimortem jagged break edge



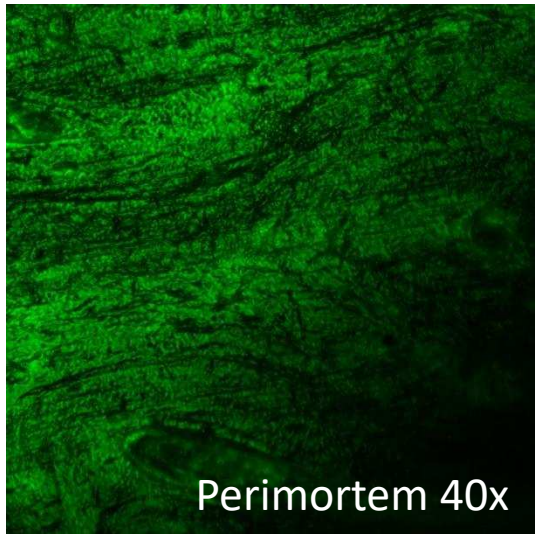
Postmortem smooth break edge

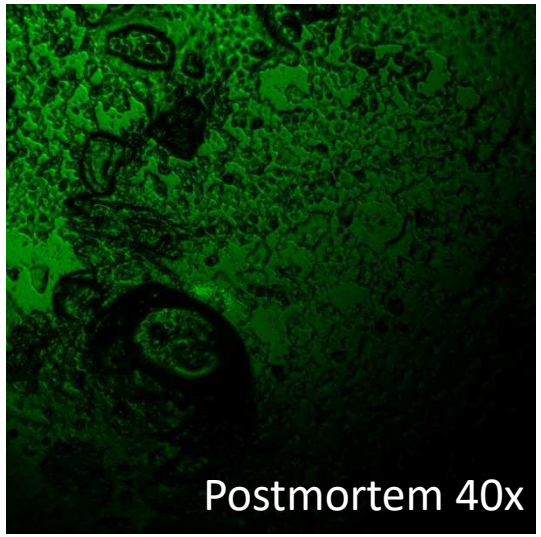


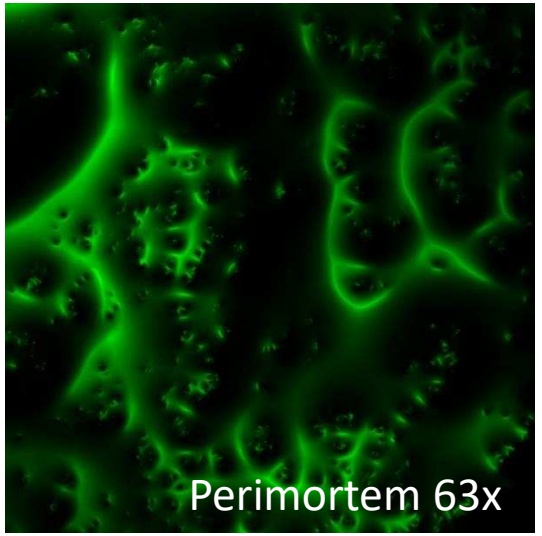


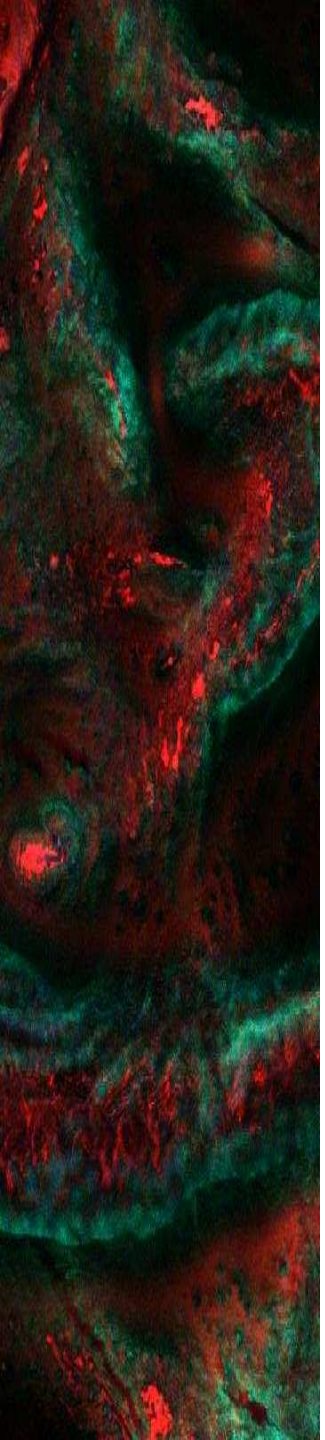
Qualitative Microstructural Results

- Perimortem
  - Contiuous
  - Smooth
  - Natural pitting
- Postmortem
  - “Dissolved”
  - Liquid-appearance
  - Acidic etching



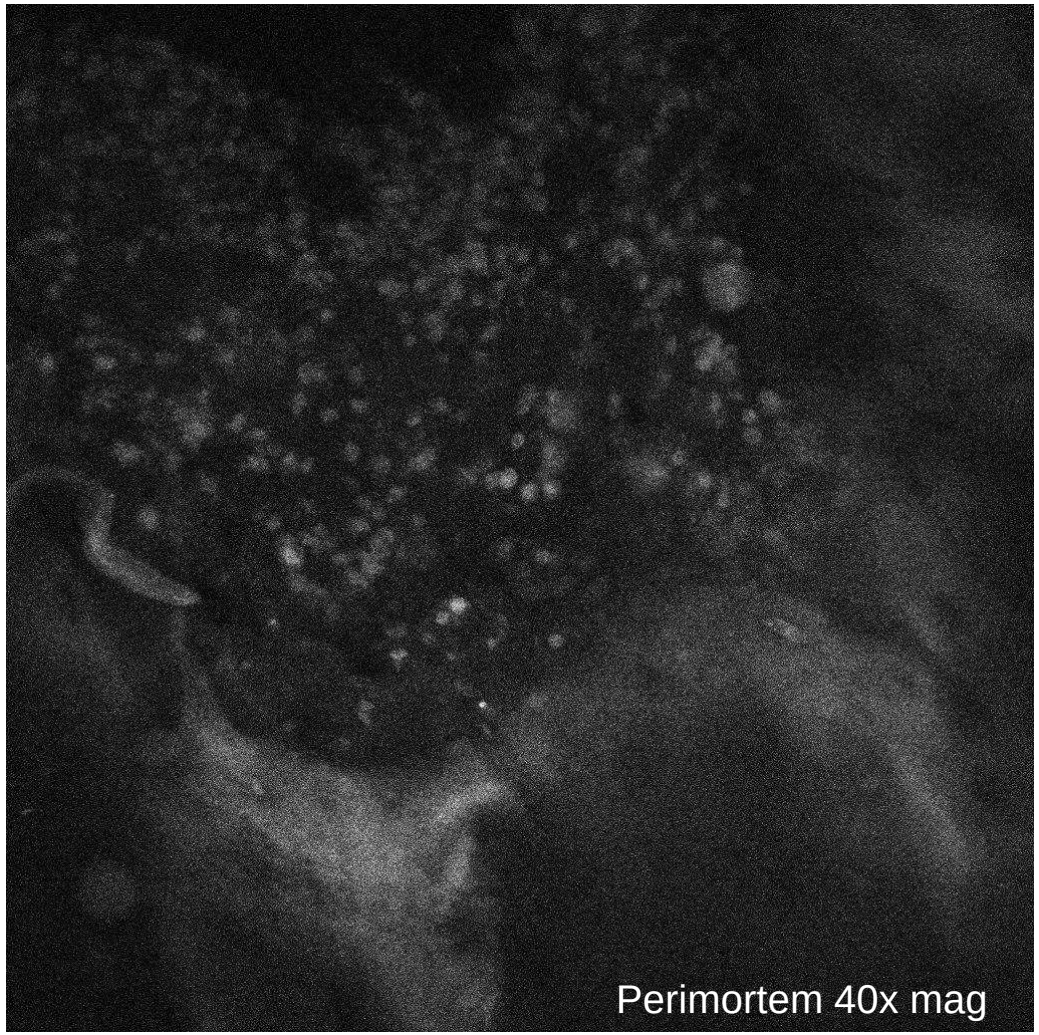




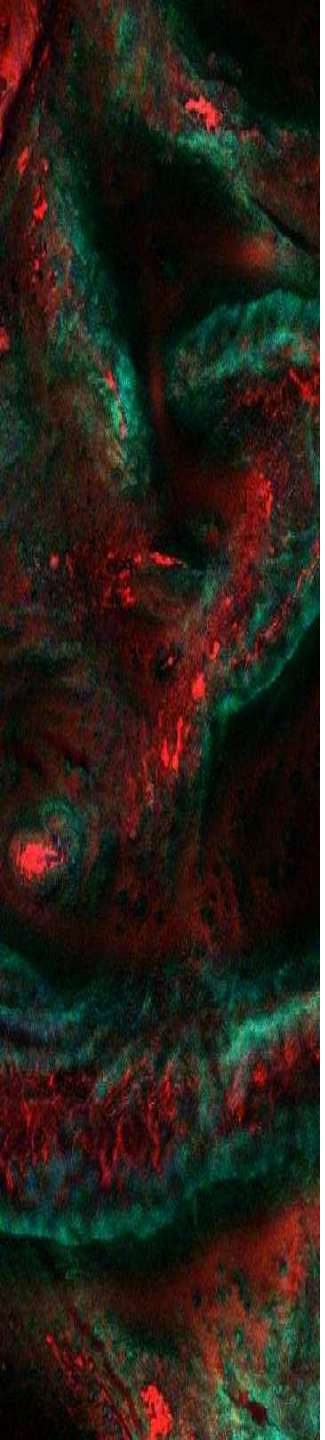


Qualitative Histochemcial Results

- Differences with laser wavelengths
  - 405nm Proteins/Cells
  - 633nm Collagen
  - 488nm Mineral Content
- Protein and Collagen
  - Perimortem
    - Higher hue counts
    - Presence of proteins
    - More collagen present
    - Higher mineral intensity
  - Postmortem
    - Lower hue count
    - No proteins present
    - Less Collagen

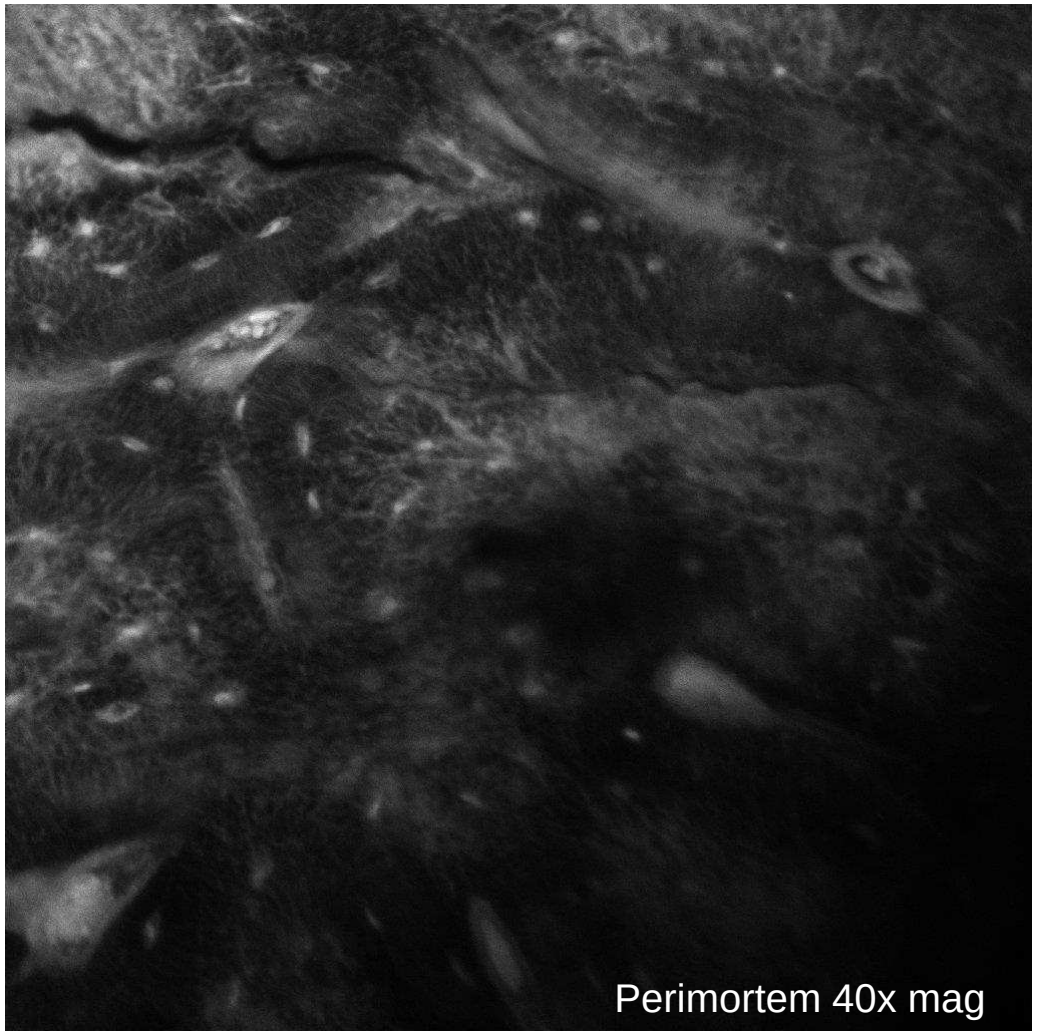


Greyscale image of proteins in cortical bone, taken with 405nm Diode laser (blue channel).

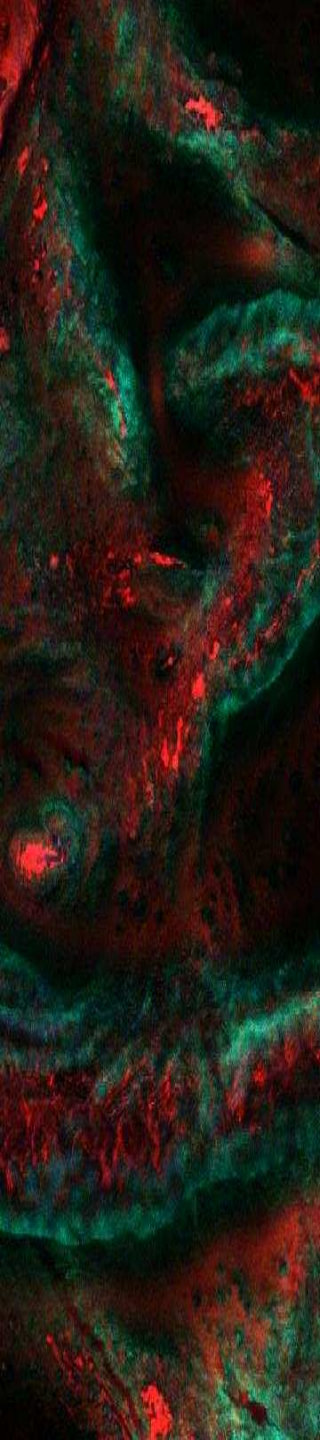


Statistical Results

- Perimortem
  - Significantly higher protein count
    - p=0.000
    - r^2=0.962
  - Tighter hue-value ranges
  - Lower hue-value means, std. dev., modes
  - Higher pixel count at mode
- Postmortem
  - No protein/cells registered
  - Wider hue-value ranges
  - Higher hue-value means, std. dev., modes
  - Lower pixel count at mode
- Blue channel (405nm)
  - Significant difference between peri- & postmortem
    - p=0.004
  - Significant correlation
    - Hue-value mean r^2=0.564
    - Hue-value mode r^2=0.609
  - Due to presence of proteins



Greyscale image of cells in cortical bone taken with a 633nm HeNe 2 laser (orange channel)



Discussion & Conclusion

- Issues raised
  - H&E bleaches with natural autofluorescence
  - Undecalcified samples
    - Basic fuchsin
- Perimortem
  - Jagged edge
  - Higher protein/cell counts
  - Collagen/mineral fluorescence more intense
- Postmortem
  - Smooth edge
  - No protein/cells
  - Wider hue-value ranges
  - Collagen/mineral fluorescence weaker in intensity

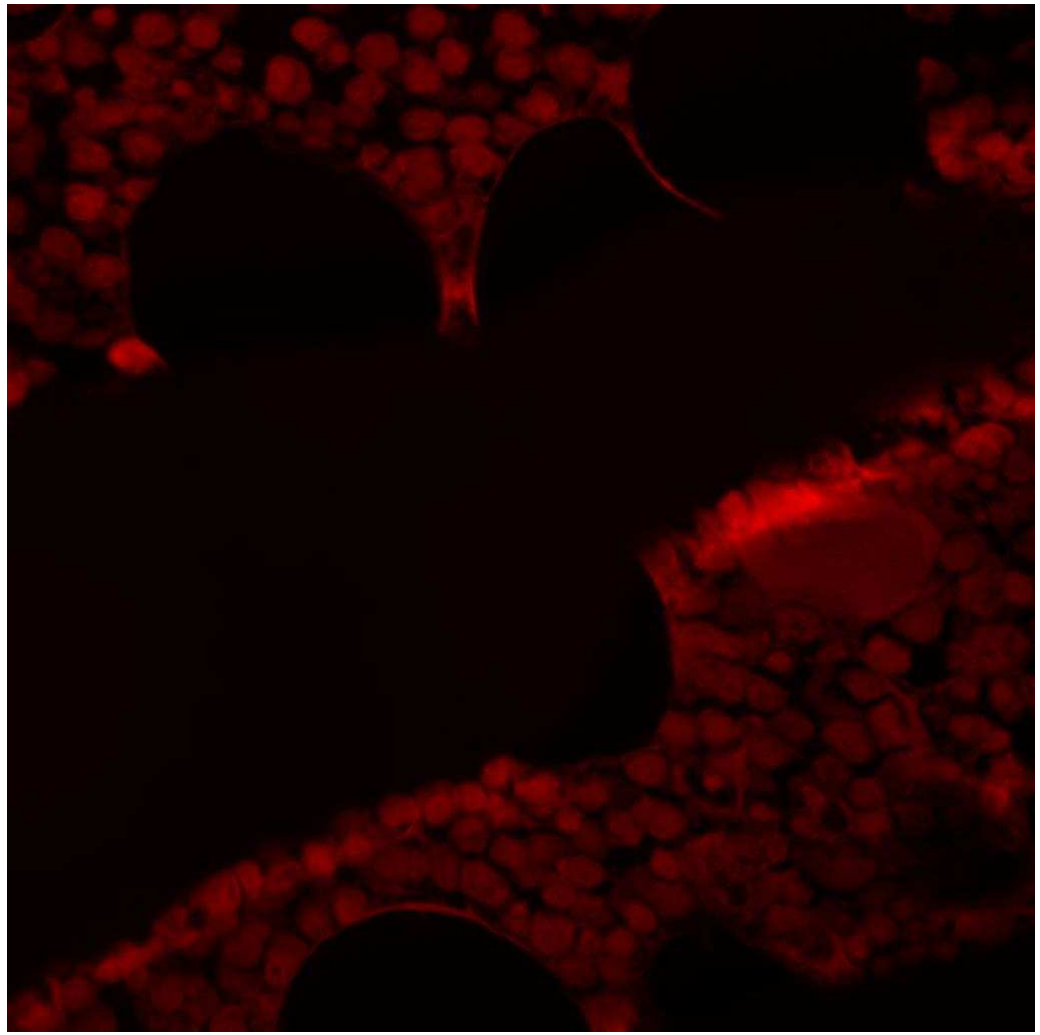
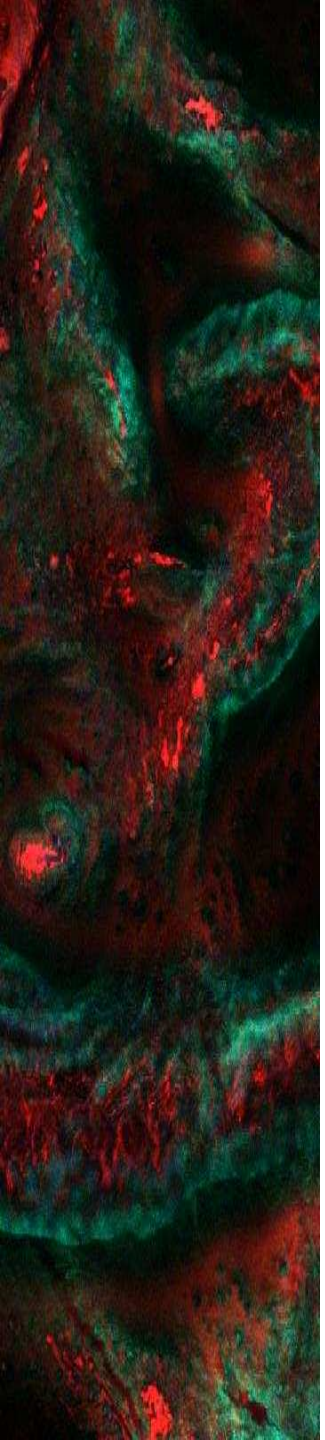


Image of human trabecular bone proteins taken with a 543 nm HeNe 1 laser



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