A33 Automated Techniques for Cortical Bone Histological Variable Segmentation and Image Enhancement

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Learning Overview: The goal of this presentation is to introduce a collection of automated image processing techniques for high-resolution imaging of cortical bone tissue. The objectives of the image processing suite include: (1) noise and artifact reduction to aid automated feature extraction; (2) streamlining researcher training by standardizing variable analysis; (3) consistent sampling techniques for reduction of inter-observer error; and (4) targeting accessible, open-source software for development of macros and standard operating procedures.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing novel image processing toolkits that correct imaging artifacts and automatically extract histological variables relevant to forensic anthropology. 3D imaging techniques, including micro-Computed Tomography (μCT), Synchrotron Radiation micro-CT (SRμCT), and confocal microscopy visualize 3D spaces and soft tissues within bone. Analyses focus on cortical pore and osteocyte lacunar-canalicular networks, which experience morphological change in response to aging, pathology, and drug abuse.

These image processing macros and workflows are primarily developed for ImageJ (National Institutes of Health [NIH]), Dragonfly (Object Research Systems), and DataViewer (Bruker), which are currently freely available for non-commercial use. Certain workflows also incorporate CTAnalyzer (Bruker).

The image processing automation focuses on four main objectives: (1) Artifact Correction—μCT and SRμCT images commonly include artifacts derived from defects in the imaging system. Workflows differentiate artifacts from real tissue spaces using pixel brightness and morphological filtering. Additionally, prior to image binarization, this study corrected for 3D tilt of the scanned bone or bone core by extracting a binary mask of the total area and aligning its skeletonized centerline; (2) Structure Segmentation—Morphological characterization of 3D tissue spaces, such as cortical pore networks and osteocyte lacunar-canalicular networks, requires conversion of the grayscale image stack into a binary image stack with pixels designated as "bone" or "space." For μCT, SRμCT, or confocal images, this study used a combination of smoothing filters, low-contrast local thresholds for pixel brightness, despeckling, and morphological closing operations to extract cross-sectional tissue spaces as 3D objects for subsequent morphological measurements. High-resolution SRμCT datasets may pose the additional challenge of removing soft tissue visible within cortical pore canals. This study employed a cascade of morphological operations to seal pore systems that are perforated by soft tissue inclusions or by intersection with the region of interest boundaries. Confocal microscopy images, which visualize canalicular networks between osteocyte lacunae, are similarly confounded by fluorescing microcracks and soft tissue. The object analysis feature of Dragonfly uses morphometric filtering and Boolean operations for semi-automatic removal of these artifacts and further separation of osteocyte lacunae from canalicular networks; (3) Regional Analysis—Mechanical loading variation within bone cross-sections is known to alter the local distribution of cortical pore networks and trabecular bone. For μCT scans of femora and tibiae, this study extracted masks of anterior, posterior, medial, and lateral quadrants of both the cortical shell and the marrow cavity through automated angular rotation of the major axis. These quadrant masks facilitate regional analysis of cortical pore systems and trabecular architecture; and (4) Histological Enhancement—2D imaging with light microscopy has long been used for histological age-at-death estimation and can be further employed to interpret mechanical loading history and longitudinal changes in bone remodeling. This study modified the light microscopy setup to visualize changes in the bone tissue of rabbits following prolonged opioid exposure. The low-contrast cement lines that circumscribe rabbit secondary osteons—essential for histological age-at-death estimation—are indistinguishable on brightfield images but become visible with Differential Interference Contrast (DIC). Widefield fluorescence microscopy identifies the calcein labels administered at set timepoints during drug dosing. By combining fluorescence and DIC images, this study can isolate the secondary osteons and bands of cortical thickness that formed specifically during experimental drug dosing. This study also built a circular polarizer and analyzer, using commercially available polarizing film, to visualize Collagen Fiber Orientation (CFO). CFO is a proxy of mechanical loading in bone tissue, which may be altered by individual life history or disorganized by pathology. Regional patterns in CFO can be quantified using the Weighted Mean Gray Level (WMGL) of circularly polarized light images.

Automated image processing reduces imaging noise and inter-observer error, while improving the time efficiency of researcher training and histological analysis. Future microscopic techniques should seek automated alternatives to manual image cleaning and variable segmentation.

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