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Introduction

In bioanthropological research, Computed Tomography (CT) scanning has revolutionized the analysis of bone morphology by offering high-resolution, three-dimensional imaging at various scales (micro, macro and nano). Despite its cost, the technique of scanning multiple bones simultaneously has been identified as both efficient and cost-effective, particularly for large-scale studies or when under tight deadlines. This approach not only accelerates data acquisition but also maximizes resource utilization, proving essential for comparative morphological analyses (Figure 1).

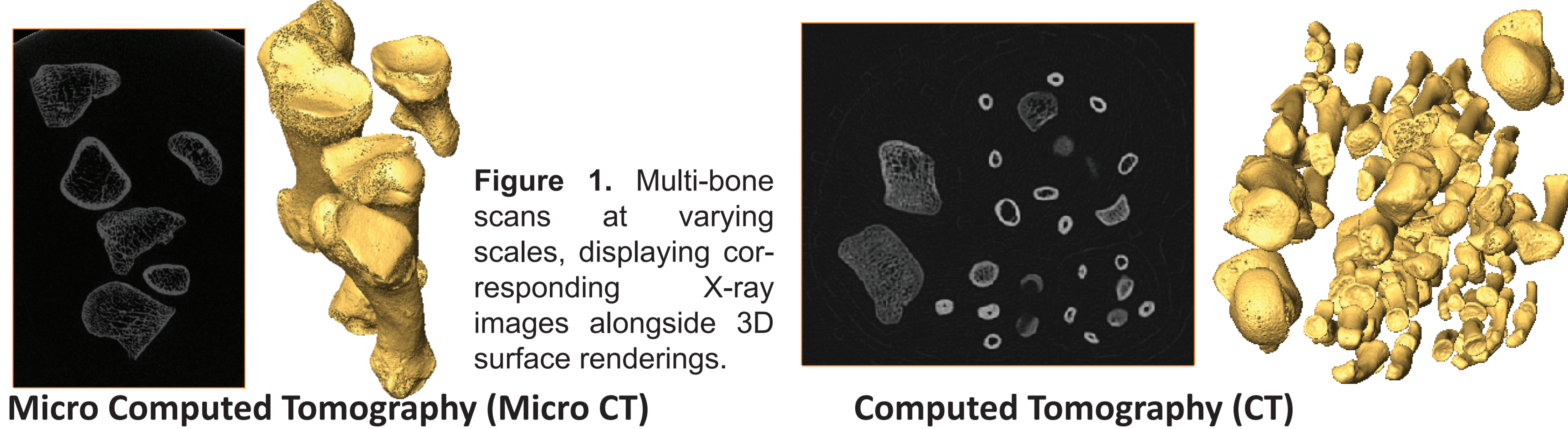


Figure 1. Multi-bone scans at varying scales, displaying corresponding X-ray images alongside 3D surface renderings.

However, the subsequent challenge of virtually separating these bones for individual analysis highlights the need for automated segmentation methods to maintain morphological integrity while reducing manual effort. Additionally, accurate bone positioning is critical for ensuring the reliability of morphometric analyses (e.g., cross-sectional geometry, leaner measurement) in the complex 3D environments provided by current software platforms (e.g., Amira, VG Studio Max) (Figure 2).

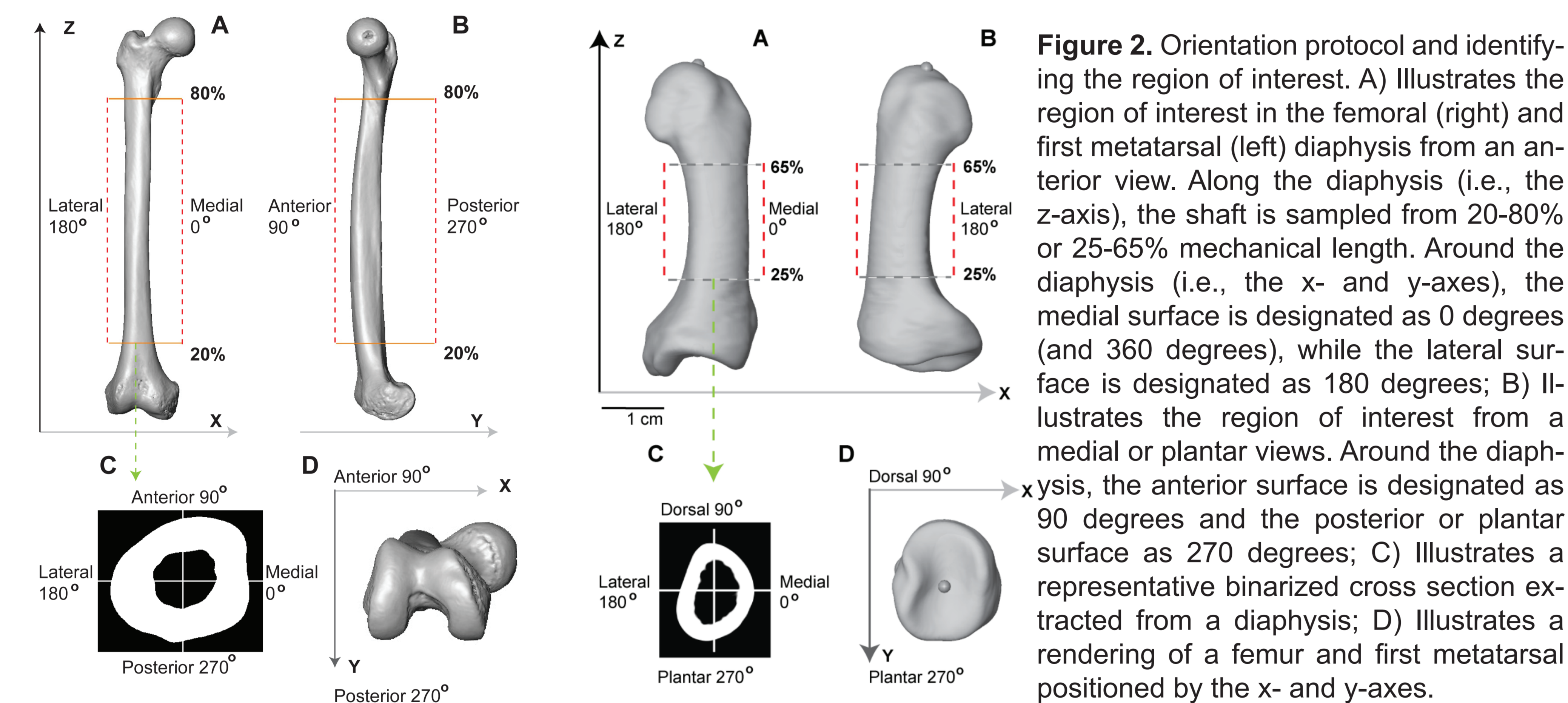


Figure 2. Orientation protocol and identifying the region of interest. A) Illustrates the region of interest in the femoral (right) and first metatarsal (left) diaphysis from an anterior view. Along the diaphysis (i.e., the z-axis), the shaft is sampled from 20-80% or 25-65% mechanical length. Around the diaphysis (i.e., the x- and y-axes), the medial surface is designated as 0 degrees (and 360 degrees), while the lateral surface is designated as 180 degrees; B) Illustrates the region of interest from a medial or plantar views. Around the diaphysis, the anterior surface is designated as 90 degrees and the posterior or plantar surface as 270 degrees; C) Illustrates a representative binarized cross section extracted from a diaphysis; D) Illustrates a rendering of a femur and first metatarsal positioned by the x- and y-axes.

This study leverages modern technology and innovative methodologies to enhance the efficiency and accuracy of multi-bone scanning within paleoscience and anthropology. By addressing the challenges of virtual bone separation and ensuring precise bone positioning, this research aims to advance the field, enhance data integrity, minimize operational costs, and refine the analysis process for future studies.

Results

Separation of Bone - AT Part 1: The integration of the Automation Tool (AT) in bone separation processes has markedly increased both speed and precision. The traditional manual separation of bones, which could take 1.5 to 2 hours for 20 bones, has been reduced to a mere 25 to 30 minutes with the AT, including the renaming of bone volumes. This not only represents a significant leap in efficiency but also minimizes the need for extensive manual intervention and 3D software expertise, requiring only basic knowledge of bone morphology.

Positioning of Bone - AT Part 2: The Automated Tool (AT) significantly enhanced bone positioning, ensuring standardized and reproducible alignment. The Bland-Altman analysis revealed a mean bias of 349.1 seconds favoring the automated method over the manual process, with a lower limit of agreement at 8.12 seconds and an upper limit at 690.08 seconds. The paired t-test demonstrated a significant time efficiency of the automated process (T-statistic: 6.35, P-value: 0.00013), with automated alignment averaging 145 seconds compared to 900 seconds for manual alignment. This efficiency establishes the automated method as the preferred choice for optimizing time in bone positioning tasks. This statistical confirmation supports the AT's utility in providing reliable and efficient enhancements to bone analysis through Amira3D software.

Extracting Cross-Sections for Cross-Sectional Properties Studies - AT Part 3: The Automation Tool (AT) greatly optimized the extraction of cross-sectional data by automating the slicing process, resulting in a uniform and efficient analysis of cross-sectional properties like cortical thickness and moment of inertia. The tool's precision substantially diminished the variability typically associated with manual slicing. High Intraclass Correlation Coefficient (ICC) values, close to or above 0.9, reflect the consistent measurements among observers using the AT for automatic alignment and slicing of the bone's longitudinal shaft. These values indicate a noteworthy decrease in variability due to observer differences. However, for manual alignment and slicing, the ICC values dropped to 0.??, with paired T-test results showing no significant statistical difference, suggesting less consistency than the automated method.

Acknowledgements

Material and Methods

Scanning Protocol Overview: Specimens were meticulously prepared with foam paper for enhanced stability and clarity before being placed in an X-ray opaque container to prevent artifacts. High-resolution micro-CT or CT scans were tailored to each specimen's specific size and detail needs. Scanning parameters were finely tuned to ensure optimal image quality while minimizing radiation exposure (Figures 3A-B). **Image Processing and Segmentation:** Utilizing Amira software, complemented by our in-house developed tools, we automated the segmentation process. This began with thresholding to distinguish bone from surrounding tissues, followed by region growing and manual adjustments for overlapping bones, ensuring precise segmentation. Initial steps involved creating a mask for all bones, assigning labels to each, and refining bone boundaries to include every part of the bone in the separated volume (Figures 3C-D).

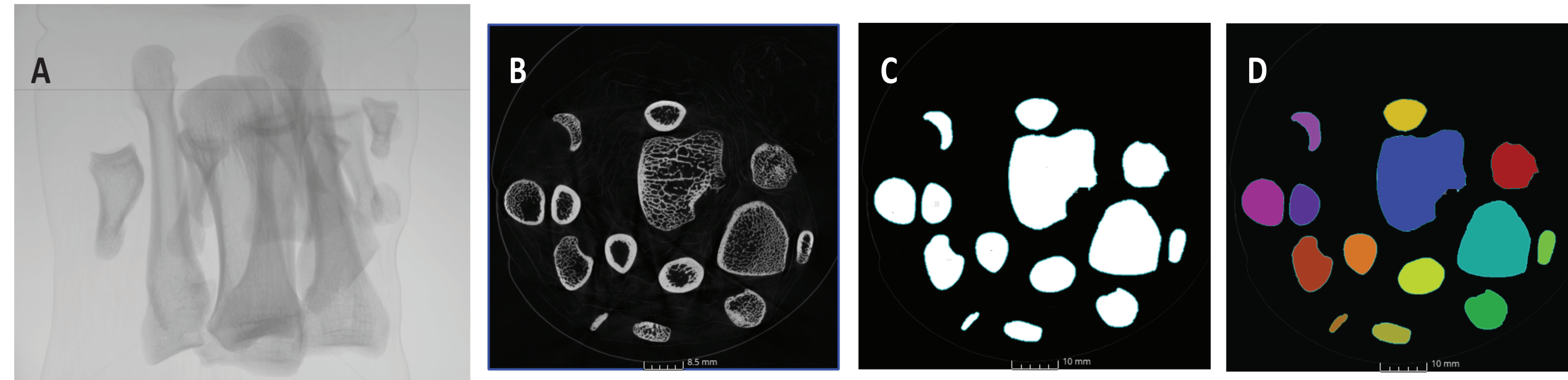


Figure 3. Multi-bone Scans: (A) X-ray Frame, (B) Transversal Section of X-ray Volume, (C) Applied Mask to Each Bone at Given Threshold After Filling Holes, (D) Labeling and Extending Labels for Each Bone.

Bone Virtual Separation and Labeling: After automatic segmentation, bones were separated into components corresponding to the bones of interest. These components were then manually labeled and automatically saved in a predefined folder, significantly streamlining the cataloging and analysis process. This meticulous separation and labeling were key for accurate identification and further analysis of each bone (Figure 4A). **Bone Positioning for Analysis:** We introduced a standardized protocol within the Amira Software Platform, enhanced by our custom automated tools, for consistent bone orientation across various bone types, ensuring uniformity. This protocol facilitated direct measurements and cross-sectional analysis from 3D surface meshes, reducing manual input while maintaining precise alignment (Figure 4B).

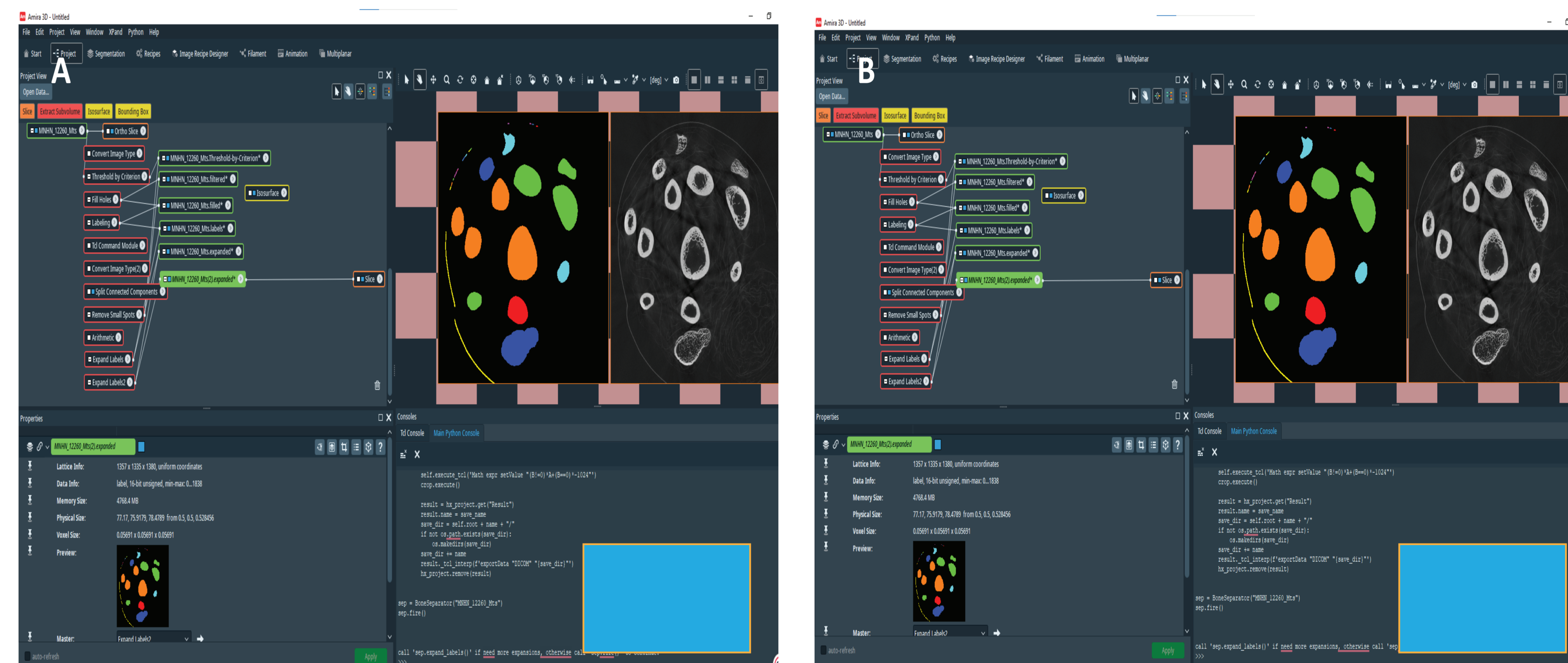


Figure 3. Multi-Bone Scans: (A) Bone Separation and Saving, (B) Bone Positioning for Cross-Sectional Analysis.

Validation and Reproducibility: We validated the efficiency of our protocol by comparing manual versus automated processes in terms of separation time, alignment, and data acquisition. Reproducibility was evaluated by contrasting cross-sectional properties with those obtained through traditional manual measurements. Tools such as Bland-Altman Analysis, Intraclass Correlation Coefficient, and t-tests were employed to verify accuracy and consistency across multiple observers.

Discussion

The integration of the Automated Tool (AT) into Amira3D software represents:

- **Pivotal Advancement in Research:** The integration of the Automated Tool (AT) into Amira3D marks a significant leap in bioanthropological research, especially for multi-bone micro-CT scanning, streamlining data processing and reducing manual errors.
- **Autonomy and Efficiency:** The AT's autonomy in identifying, segregating, and preparing bones enhances efficiency and precision, critical for extensive bone studies.
- **Sophisticated incorporation of Amira modules:** Its support precise bone separation and alignment, crucial for comparative studies and ensuring high-level detail and accuracy in analyses based on specific morphological needs.
- **Minimizing Variability and Errors:** Empirical validation shows the AT's role in reducing variability and discrepancies between observers, addressing major challenges in the field and promoting methodological innovation.
- **Simplifying Workflow:** Designed to minimize user intervention, the AT simplifies research workflows and effectively addresses the traditionally time-consuming nature of post-processing methods.
- **Comprehensive Bone Analysis:** The AT impacts the analysis of both cortical and trabecular bones, providing deep insights into their microarchitecture and the biological processes they undergo.

These findings collectively highlight the significant advancements by the AT in the key areas of bone separation, positioning, and cross-sectional analysis. The tool not only improves efficiency and accuracy but also ensures greater consistency and reliability in bone studies, setting a new standard for automated bone analysis in paleoscience and anthropology.