



Accelerating the analysis of complex bone structures using semi-automated segmentation

Introduction

Micro-computed tomography (microCT) has become a highly valuable tool for the study of bone health in pre-clinical models of diseases, and is particularly well suited for imaging and longitudinal quantitative evaluations.¹ However, data analysis of micro CT imaging is limited in sample size due to the significant effort required for segmentation.²

Current segmentation approaches are often reliant on manual contouring of individual bones or density-based thresholding approaches. Both of these can lead to inaccuracies, and manual segmentation methods can result in inter-user variability.³ As a result, researchers may restrict themselves to analyzing only a single bone or subset of bones, reducing their ability to identify biomarkers of bone health for further studies.²⁻³

In an attempt to overcome these limitations, researchers led by Prof. Hani Awad at the University of Rochester Medical Center have developed a high-throughput, semi-automated method for the segmentation of murine hindpaw microCT data.³ The team hopes that their findings will facilitate the identification of bone-specific biomarkers with the potential for future clinical applications.

Developing a workflow using Amira Software

To establish this workflow, *in vivo* microCT datasets from a large number of mice hindpaws were collected at monthly intervals (from 2–8 month-old males and 2–5 month-old females). Additionally, the hindpaws of a 9.5 month-old male were imaged by *ex vivo* micro-CT. The data was then exported to Thermo Scientific™ Amira™ Software, where watershed-based algorithms were used to perform automated segmentation, followed by user correction. Along with filtered data and a binary mask, these inputs were also used to generate a fully segmented dataset. Overall, the automated portion of the watershed seed placement had an accuracy of 79.2% for the *in vivo* bone segmentation and 91.1% for the analysis of the *ex vivo* data. The difference can be related to slight movements *in vivo*, which have an impact on the segmentation accuracy. Although error rates for some bones were higher than others, most bones were correctly segmented without the need for user correction. Additional methods were subsequently developed to correct for these errors. It is worth noting that, once the segmentation process is complete, each individual bone volume can be extracted as its own data table within Amira Software.

No experience required – validating the workflow's ease of use

To validate the approachability of this workflow, an experienced user and three novice users (who had not previously worked with Amira Software) were asked to perform the same analysis. Novice users were first trained using a series of videos along with six practice datasets from the *in vivo* scans. After this, they analyzed the data, and their segmentation time was recorded. A strong correlation was seen between the results of the novices using the semi-automated method and those achieved by an experienced person performing conventional segmentation analysis. The semi-automated workflow was also significantly faster than the conventional method. Additionally, results were internally consistent when the experienced user carried out the semi-automated segmentation twice.

Overall, the experienced user was able to complete the segmentation nearly 10 times faster with the semi-automated method compared to the conventional approach. Even novice users were able to complete their work significantly faster with this novel approach when compared to conventional segmentation carried out by the experienced user. Excellent inter-user reliability was observed between the results of the novice and experienced users.

This indicates that new users can perform high-quality data segmentation after only 1–2 days of training, and are prepared to carry out high-throughput and reliable data analysis.³

A promising approach for accelerated performance

By adopting the semi-automated method outlined here, a standardized protocol could be established for bone volume measurements across users and institutions. These results could also be used to train machine learning models, producing fully automated methods that require little to no user input. Potentially, this approach could even be applied to other complex skeletal structures across a variety of species, as well as other tissues and imaging modalities, like magnetic resonance (MRI).

This model offers significant advantages for various pre-clinical bone and joint studies, ultimately accelerating clinical translation.³ Additionally, such extensive analysis of microCT data has the potential to reveal novel bone-specific biomarkers, which is a vital application that was not previously possible.

Thermo Scientific technology

Amira Software provided the Awad team with a powerful platform for 2D–5D image visualization, analysis, and processing. Amira Software is designed to accelerate the drug discovery pathway across a wide range of imaging modalities, including CT, MRI, and 3D microscopy. Automated image processing in Amira Software allowed the team to produce custom detection workflows, facilitating faster image analysis and reproducibility while also lowering the learning curve for new users. Amira Software is a flexible solution that provides both the convenience of ready-to-use recipes with AI-powered automation as well as a range of customization options that can be adapted to your needs and incorporate your own intellectual property.

References

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