Auto separation of metaphyseal trabecular from cortical bone using a morphological escalator

Method note
MCT-124

1. Introduction

The rodent long bone metaphysis, at the proximal tibia and distal femur either side of the knee, are standard sites for the analysis of trabecular and cortical bone. This can be for studies of how to mitigate gonadectomy related bone loss (osteopenia) or the analysis of phenotypes in genetic research.

A previous method note (MCT-94) describes the method of selecting the volumes of interest for trabecular and cortical analysis, defined by distance from the growth plate in the axial direction toward the mid-shaft or diaphysis. A metaphyseal region is selected for trabecular analysis and a near diaphyseal one for cortical bone.

This article shows a methodology for automatic separation by CTAn software of metaphyseal trabecular from cortical bone. That is, the delineation of a volume of interest whose boundary runs closely parallel with the endosteal or "endocortical" boundary between medullary trabecular bone and the surrounding cortical bone. This selected volume is illustrated in figure 1 below. Note that it is also possible to delineate this boundary by manual ROI drawing in CTAn. The options of manual and automated trabecular delineation are both available.

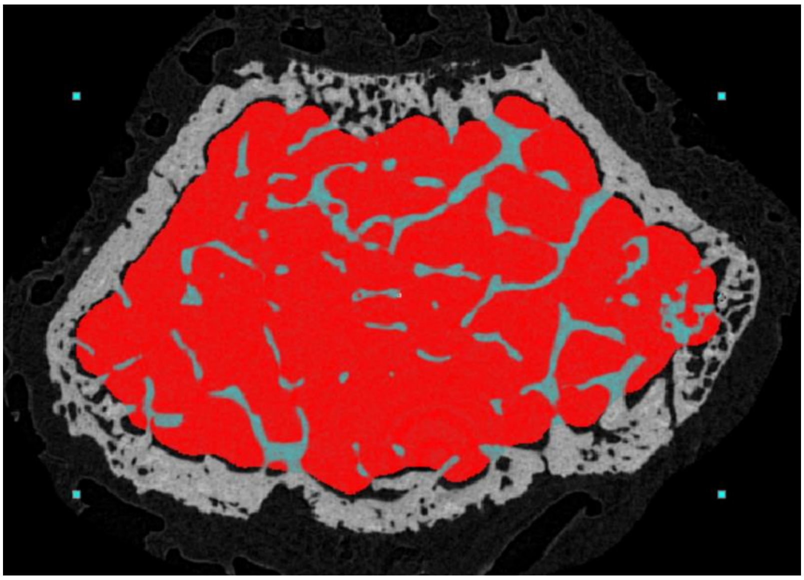


Figure 1. The red region is a trabecular volume of interest delineated by an automated task list in CTAn custom processing.

2. How do I determine a rotation step?

The method description here will not repeat the first steps of standardised volume of interest selection for trabecular bone that are set out in the earlier method note on that subject (MN 94). These first steps standardise the orientation of the bone and establish the range of cross-sectional slices to be analysed with respect to distance from the growth plate. This note will focus on the automated procedure for separating trabecular from cortical bone using a custom processing task list.

Once the growth plate reference level is identified, and the range of slices for trabecular analysis – relative to the growth plate reference – is decided, then you should create an ROI dataset consisting of the full metaphyseal cross-section, of both cortical and trabecular bone, over this selected range of slices (see figure 2). It helps the speed and data management if this range of metaphyseal slices is saved as a new smaller dataset. This is done in CTAn using the "Save New Dataset From ROI" function at the second, ROI page.

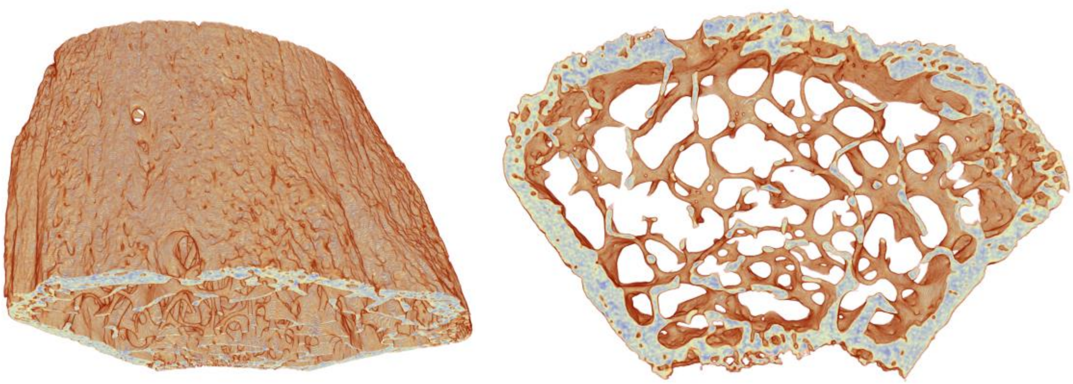


Figure 2. The growth plate-referenced metaphyseal segment for trabecular analysis should be prepared with both trabecular and cortical bone present – the complete bone envelope – for subsequent automated trabecular-cortical separation.

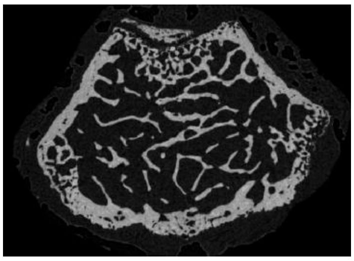
It should be noted that there are several approaches to separating trabecular from cortical bone by automated image processing, that can be effective. Some of these have been published [i, ii]. The method for trabecular delineation at the rodent knee metaphysis, that is described here, is carried out in a task list in the custom processing page in CTAn. This task list is set out in full in table 1 below. This method consists of three parts: first a "shrink-wrap" to set the bone boundary, second an inverse thresholding to binarise the medullary space, and thirdly a series of morphological operations called the "morphological escalator".

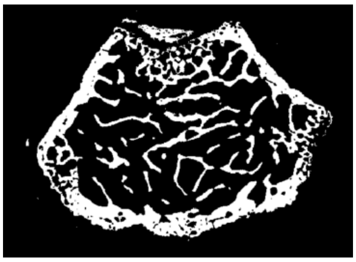
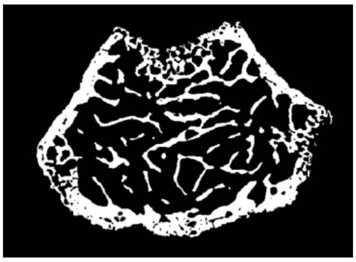
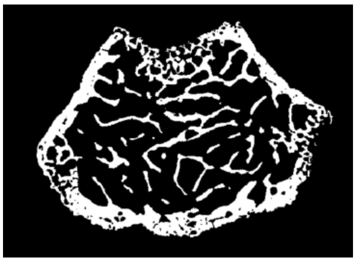
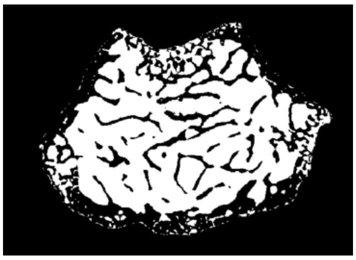
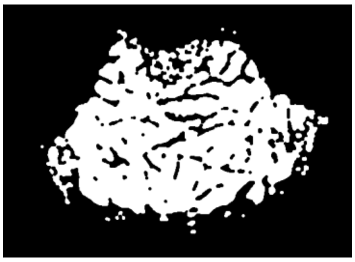
The three stages in the process of creating the trabecular VOI automatically are:

1. A "shrink-wrap" distance transform operation to wrap a boundary around the periphery of the bone outline at the periosteum or outer cortical structures;
2. Inverse binarisation of the medulla cavity and cortical porosity, while excluding the surrounding space;
3. The "morphological escalator" – a series of opening-closing operations with a stepwise increasing pixel radius that consolidate the binarised medullary space into a continuous volume with both trabecular structures closed out and cortical pores and peripheral artefacts removed. The shape corresponds to the medulla and can be used as the trabecular volume of interest or "VOI".

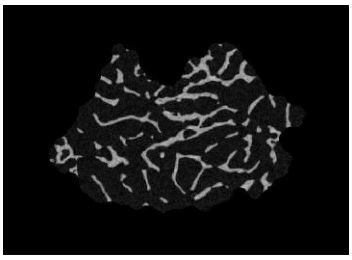
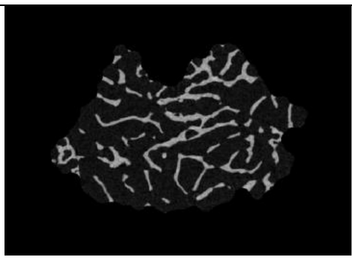
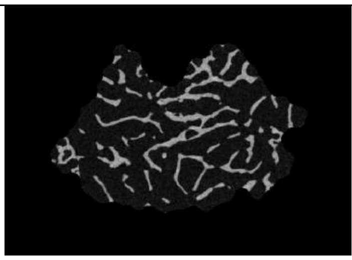
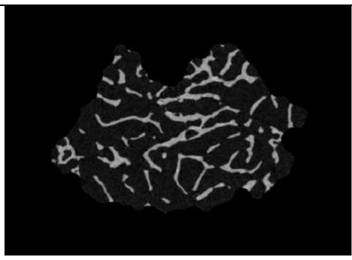
Table 1 shows the series of steps in CTAn custom processing that delineate the trabecular VOI. For each step, both the current image within the ROI, and the ROI, are shown as they currently exist at that stage of the task list, after the described step is carried out. The custom processing plugin that is used is briefly described, with some accompanying explanation in blue text. In a further section after table 1, images of the configured plugins run at each stage are shown in full.

Table 1. Custom processing task list to delineate the boundary of medullary trabecular bone from surrounding cortical bone in the mouse distal femur.

	IMAGE INSIDE ROI	ROI
1. Open the metaphysis dataset (both trabecular and cortical bone present)		

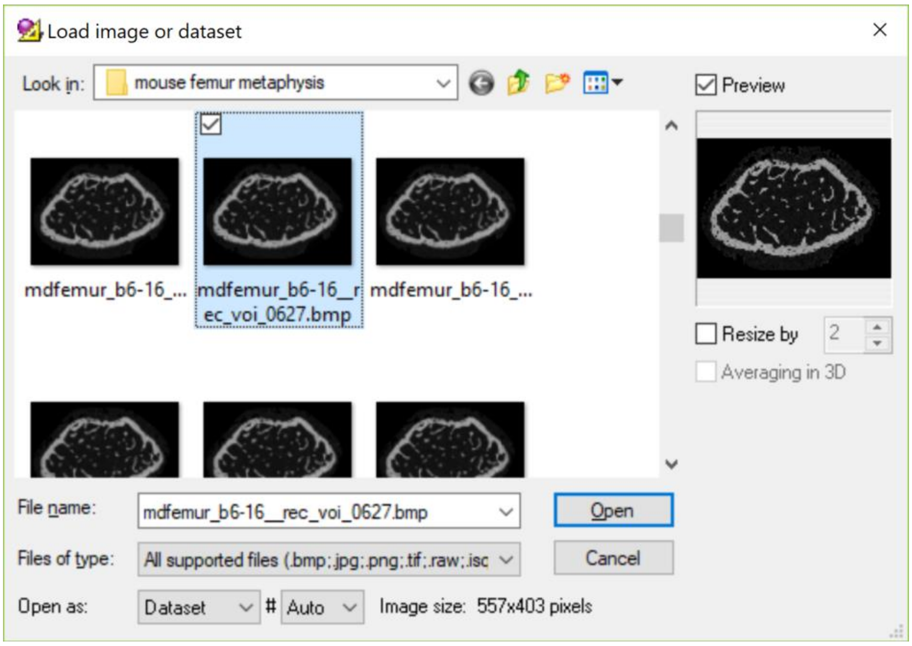
2. Thresholding of trabecular and cortical bone (global or adaptive)		
3. Despeckle: remove all but the largest object, 3D (apply to image) Removes disconnected objects such as around the edge of the bone		
4. Shrink-wrap ROI, 2D, stretch over holes with radius 32 pixels. Makes a VOI wrapped around the outer surface of the bone		
5. BITWISE operations: image = image XOR ROI (apply to image) Inversely binarises the porous space of the metaphysis to start the trabecular VOI selection		
6. Morphological operations, opening then closing, 3D, radius 2 pixels (apply to image) The first step of the *morphological escalator*: 2 pixel radius		

7. Morphological operations, opening then closing, 3D, radius 4 pixels (apply to image) The second step of the *morphological escalator*: 4 pixel radius		
8. Morphological operations, opening then closing, 3D, radius 6 pixels (apply to image) The third step of the *morphological escalator*: 6 pixel radius		
9. Morphological operations, opening then closing, 3D, radius 8 pixels (apply to image) The fourth step of the *morphological escalator*: 8 pixel radius		
10. Morphological operations, opening then closing, 3D, radius 10 pixels (apply to image) The fifth step of the *morphological escalator*: 10 pix. radius		
11. Morphological operations, erode, 2D, 3 pixels (apply to image) A small erosion separates the trabecular boundary from the endosteum		

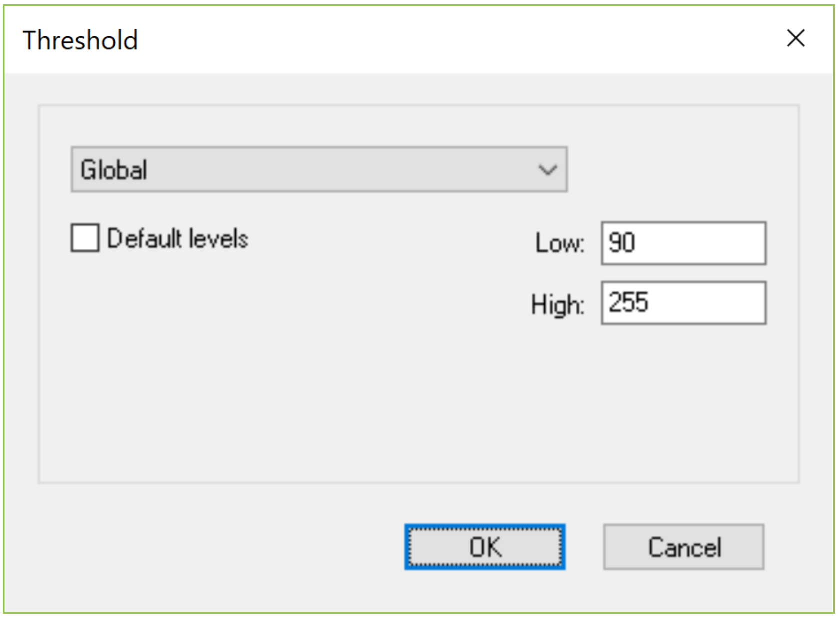
12. BITWISE: ROI = COPY image The consolidated binary medulla image is copied to the ROI channel to be the trabecular VOI		
13. RELOAD: image The original grey scale image is reloaded into the image channel – while the trabecular VOI remains in place		
14. DESPECKLE: Sweep, remove all except largest object, 3D. (Apply to ROI) Deletes any fully disconnected parts of the VOI space.		
15. DESPECKLE: Remove pores, 3D. (Apply to ROI) Deletes any fully enclosed pore spaces in the VOI space.		
16. SAVE BITMAPS: ROI, BMP format, monochrome The trabecular VOI is complete and is saved as a binary image stack in 1-bit format.		

2.1. An image of the configured window for each custom processing operation in table 1 above, is given below.

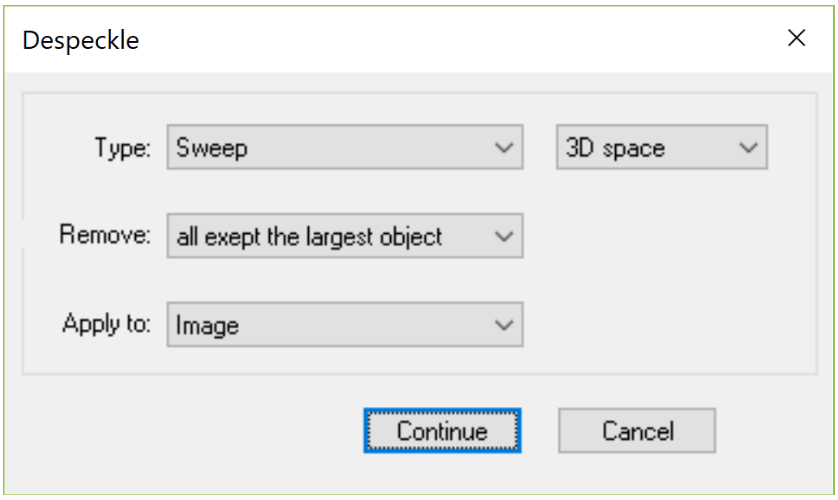
Step 1: Load image



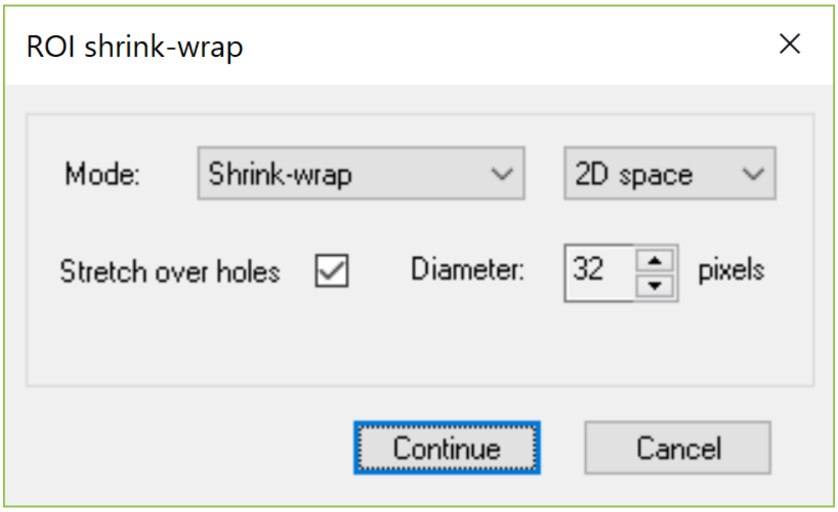
Step 2: Thresholding



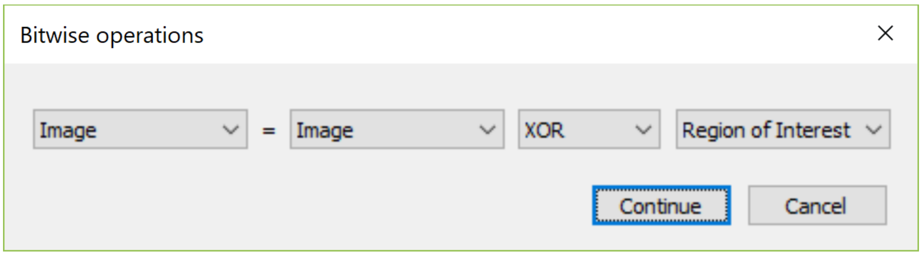
Step 3: Despeckle: remove all but the largest object, 3D (apply to image)



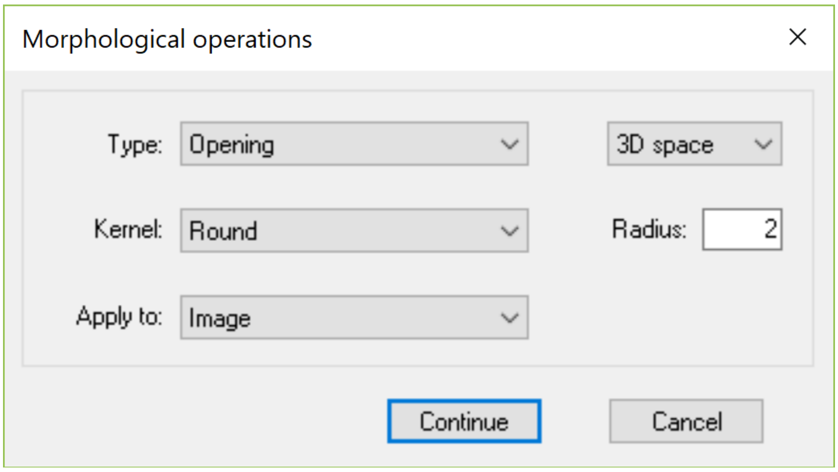
Step 4: Shrink-wrap ROI, 2D, stretch over holes with radius 32 pixels



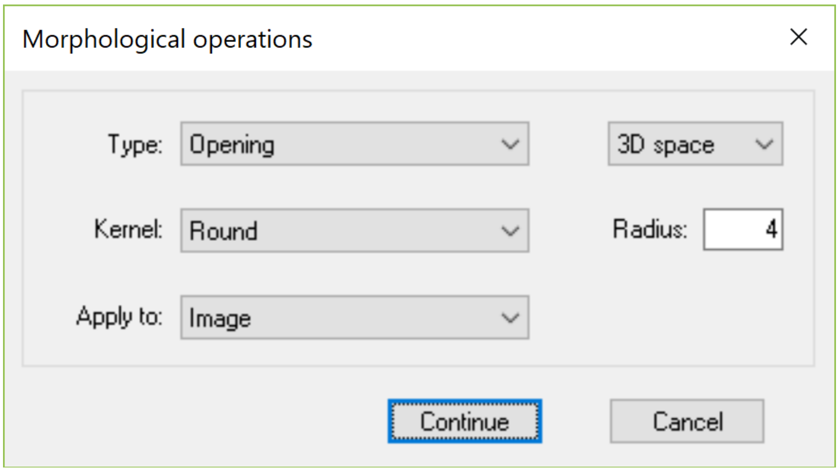
Step 5: BITWISE operations: image = image XOR ROI (apply to image)



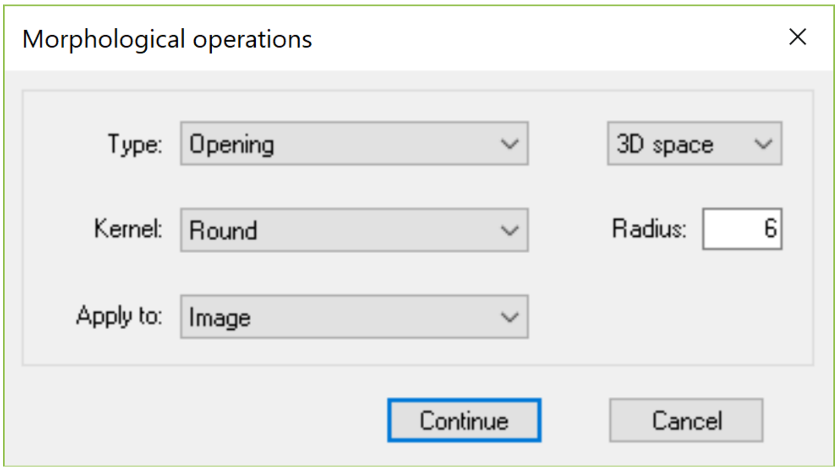
Step 6: Morphological operations, opening then closing, 3D, radius 2 pixels (apply to image)



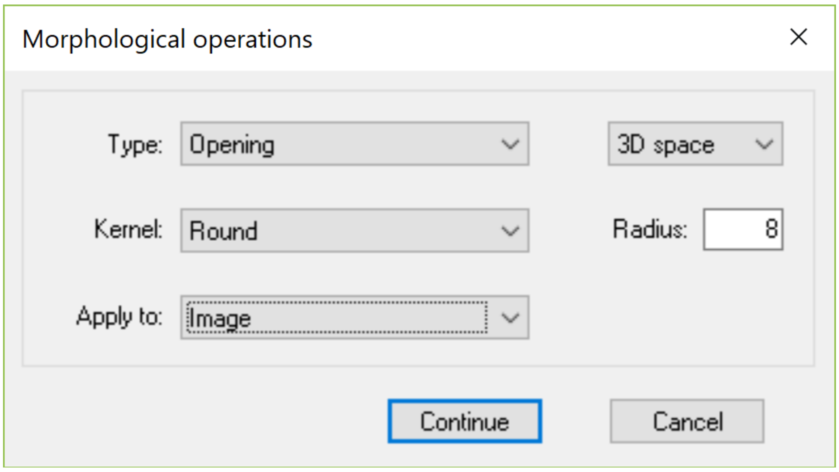
Step 7: Morphological operations, opening then closing, 3D, radius 4 pixels (apply to image)



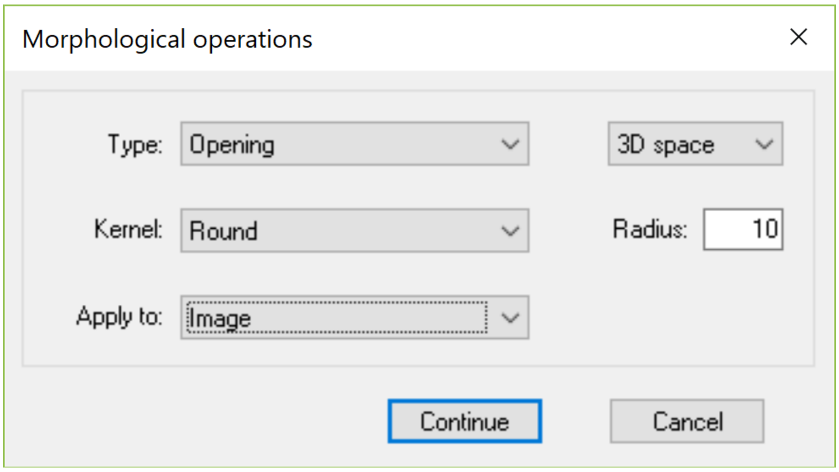
Step 8: Morphological operations, opening then closing, 3D, radius 6 pixels (apply to image)



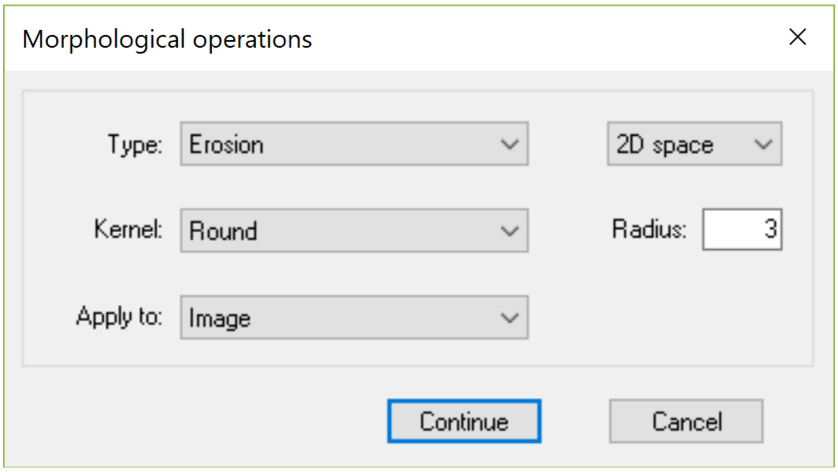
Step 9: Morphological operations, opening then closing, 3D, radius 8 pixels (apply to image)



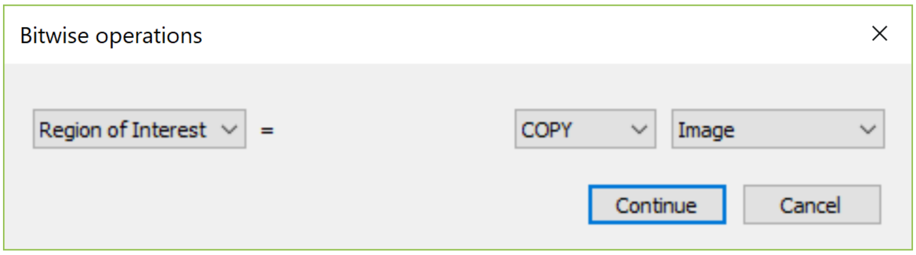
Step 10: Morphological operations, opening then closing, 3D, radius 10 pixels (apply to image)



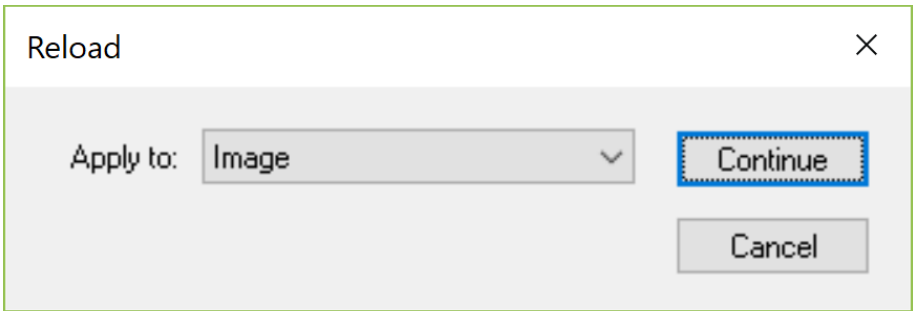
Step 11: Morphological operations, erode, 2D, 3 pixels (apply to image)



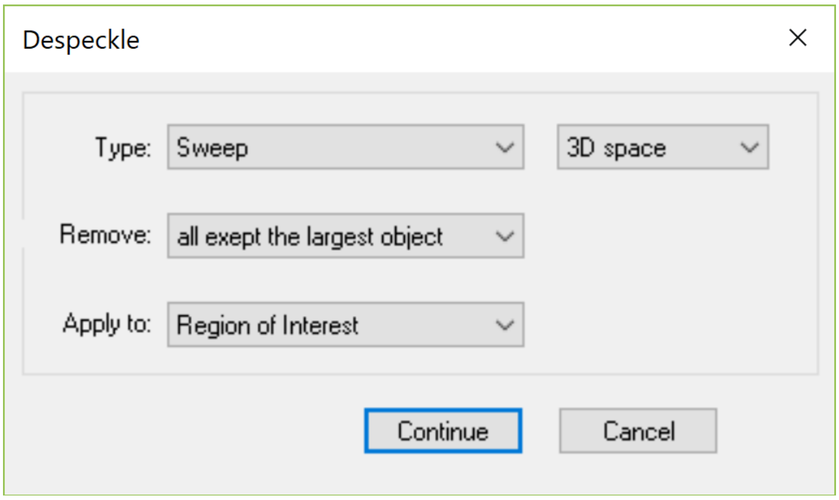
Step 12: BITWISE: ROI = COPY image



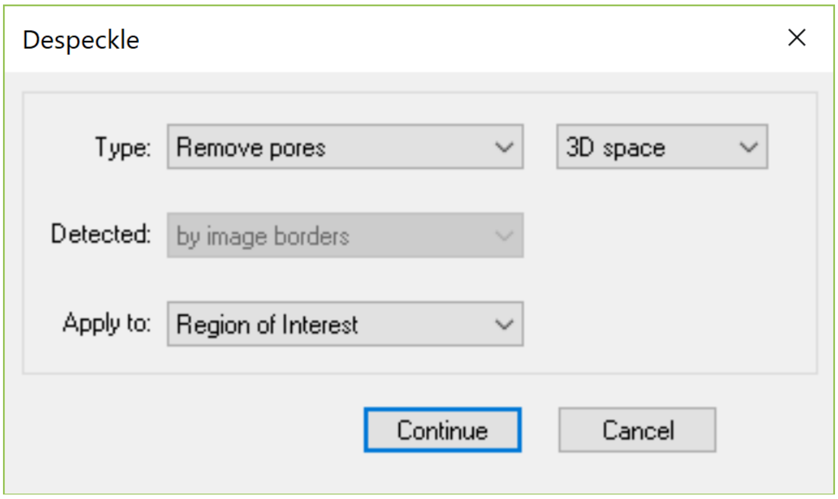
Step 13: Reload image



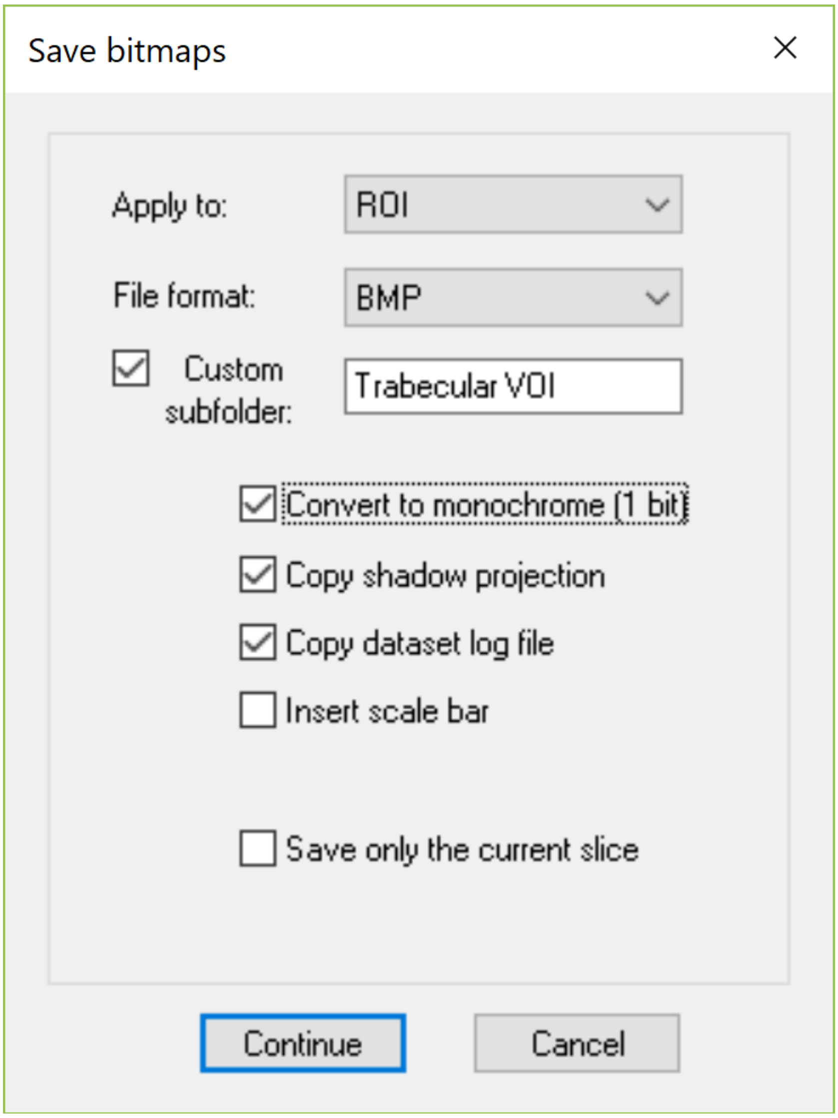
Step 14: DESPECKLE: Sweep, remove all except largest object, 3D. (Apply to ROI)



Step 15: DESPECKLE: Remove pores, 3D. (Apply to ROI)



Step 16: SAVE BITMAPS: ROI, BMP format, monochrome



3. *Results and discussion*

The results of trabecular – cortical separation carried out by the above method using the morphological escalator, are shown below in figure 3. Note that near the growth plate, top image, peripheral vestiges of the growth plate visible as highly porous cortical regions are successfully excluded from the trabecular VOI which thus differentiates between primary and secondary spongiosa.

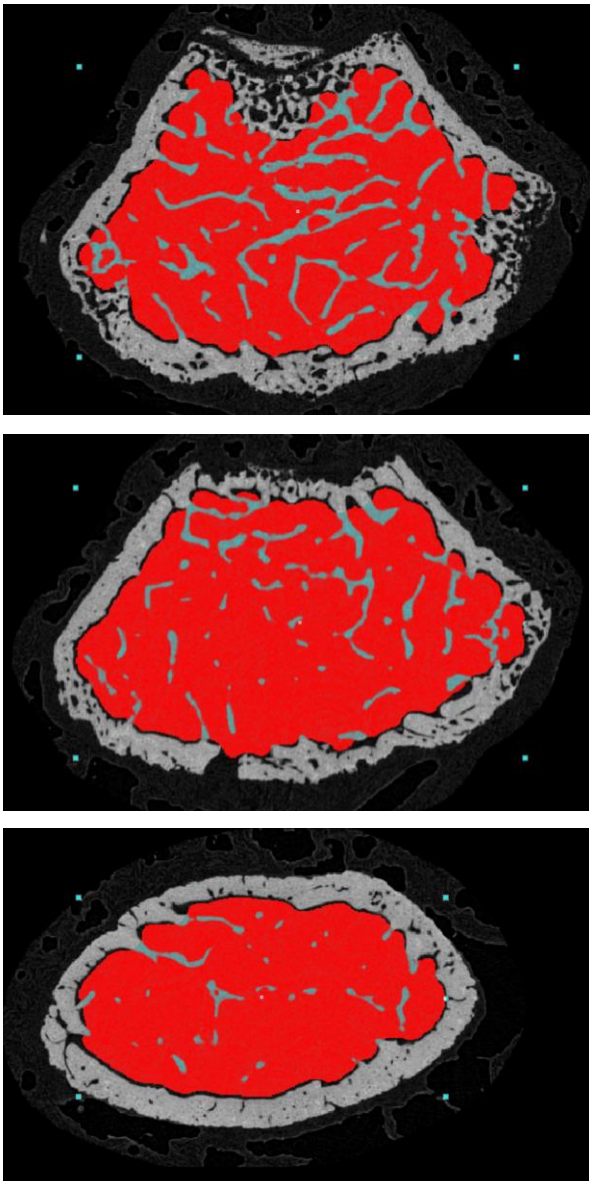


Figure 3. Cross-section of the metaphyseal region with trabecular bone automatically delineated, starting from the end nearest the growth plate (top) to the diaphyseal end (bottom).

Note also in the middle image in figure 3 that the trabecular ROI stretches over a hole in the cortical boundary at about the 6-7 o'clock position.

The point where endocortical bases of trabecular structures are cut by the VOI boundary is defined as a local thickness of the trabecular structure. This is determined by the maximum diameter of the open-close operations in the task list. In the example shown this diameter is 10 pixels; please note however that the maximum radius can be adjusted to suit the bone images that are being analysed. Likewise the threshold value in the task list is not fixed and can also be optimised for the images being analysed.

4. Glossary of terms

Metaphysis	The segment of the long bone such as femur and tibia near the growth plate that contains trabecular bone. It begins at the growth plate and extends in the direction of the mid shaft (diaphysis) for as far as trabecular bone is present. Only at the metaphysis is there both a cortical boundary and significant trabecular bone in the medulla.
Diaphysis	The central shaft of a long bone composed of a cortical bone ring. There is little or no trabecular bone inside the diaphysis.
Medulla	The space inside the cortical bone tube (the metaphysis and diaphysis) in the long bone. It contains bone marrow throughout, and at the metaphysis only contains also trabecular bone.
Epiphysis	The end of the long bone at the joint, such as the knee, between the articulating condyles and the growth plate. The epiphysis and metaphysis are on opposite sides of the growth plate.
Condyles	The articulating surfaces at a joint such as the knee.
Distal femur	Part of the femur furthest from the connection with the body at the hip, at the knee.
Proximal tibia	The part of the tibia closest to the connection with the body at the hip, at the knee.
Growth plate	The transverse structure at the knee end of the femur (distal) and tibia (proximal) where new bone is formed and which is the central location where longitudinal bone growth occurs. At the center of the growth plate is a non-mineralised seam of chondrocyte cells arranged in a columnar pattern which synthesis new bone, outputting mineralised bone on the metaphyseal side (furthest from the knee end of the bone) of the growth plate.
Primary spongiosa	The very fine textured bone that emerges from the growth plate on the metaphyseal side. This is "fresh" bone that has only recently been made.

Secondary spongiosa	Trabecular bone that began as primary spongiosa, but with time after being formed, has undergone several cycles of bone remodelling and become thicker and with a smoother labyrinthine architecture.

5. References

[i] Buie HR, Campbell GM, Klinck RJ, MacNeil JA, Boyd SK. Automatic segmentation of cortical and trabecular compartments based on a dual threshold technique for in vivo micro-CT bone analysis. Bone. 2007 Oct 1;41(4):505-15.

[ii] Valentinitsch A, Patsch JM, Deutschmann J, Schueller-Weidekamm C, Resch H, Kainberger F, Langs G. Automated threshold-independent cortex segmentation by 3D-texture analysis of HR-pQCT scans. Bone. 2012 Sep 1;51(3):480-7.