Video tutorial for cortical bone analysis (mouse tibia midshaft)

By PCMD MicroCT Imaging Core

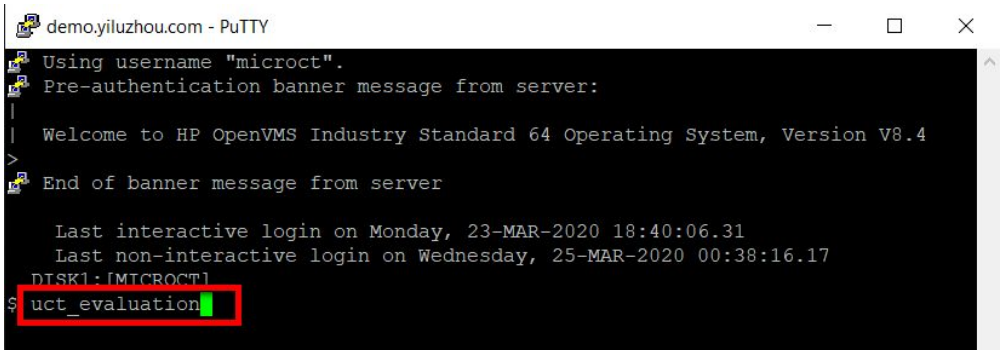
Youtube link: https://www.youtube.com/watch?v=B4OE9X8Bkwg
All our video tutorials are listed on our website: https://www.med.upenn.edu/orl/uct/user-tutorials.html

This is the video tutorial for cortical bone analysis, using mouse tibia midshaft (on μCT35, Sample#: 7972, Measurement#: 19450) as an example.

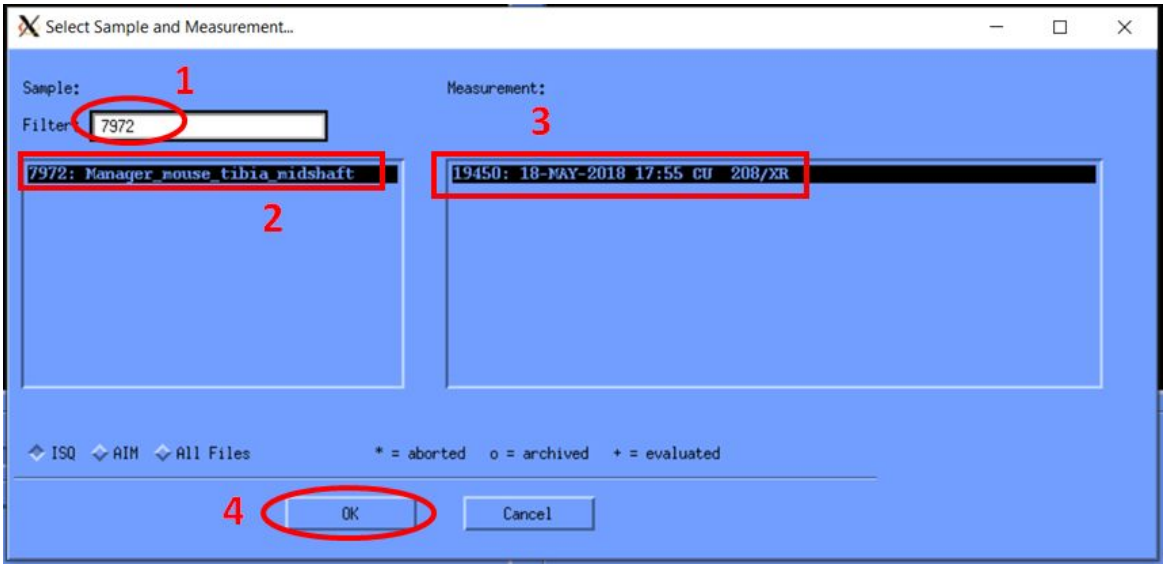
1. Open the sample images and locate the beginning slice:

Note: Make sure you turn off CapsLock and Numlock on the keyboard!
Double click "uCT 35" icon:

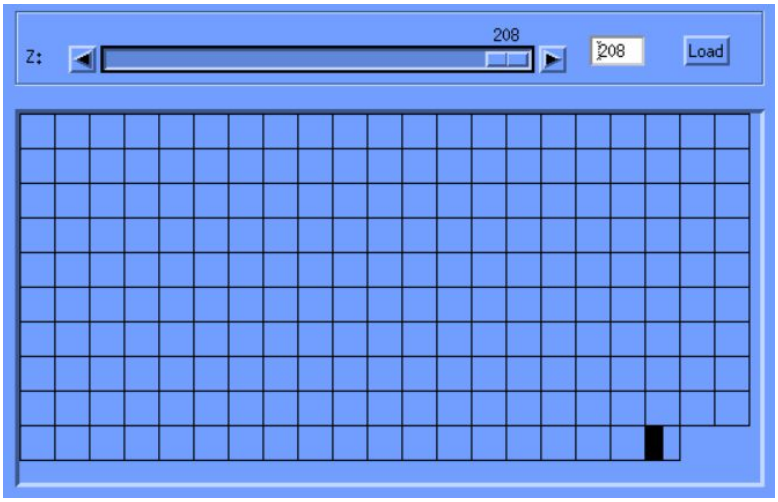
Type uct_evaluation (Right click to paste), Press Enter



You will see "Select Sample and Measurement..." window.
Select the Sample#: 7972, Measurement#: 19450, and click "OK".



In this example (mouse tibia midshaft), a 2.2 mm region consisting of 208 slices at the center of the mouse tibia were scanned at 10.5 μm nominal voxel size.



To determine which slices for your analysis, we recommend you to read the related publications with similar research purpose and animal species. For all past publications from users of our core facility, please visit https://www.med.upenn.edu/orl/uct/publications.html

In this example, we will analyze the middle 50 slices (0.525 mm) in the midshaft region of mice tibia. There are 208 slices in this sample, so we will contour from the 79th to 129th slice.

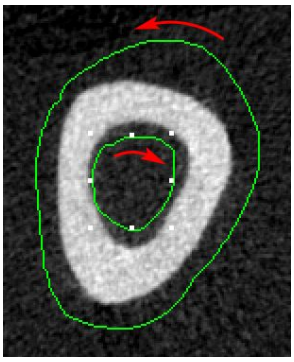
2. Draw the contours of cortical bone:

Locate the 79th slice.

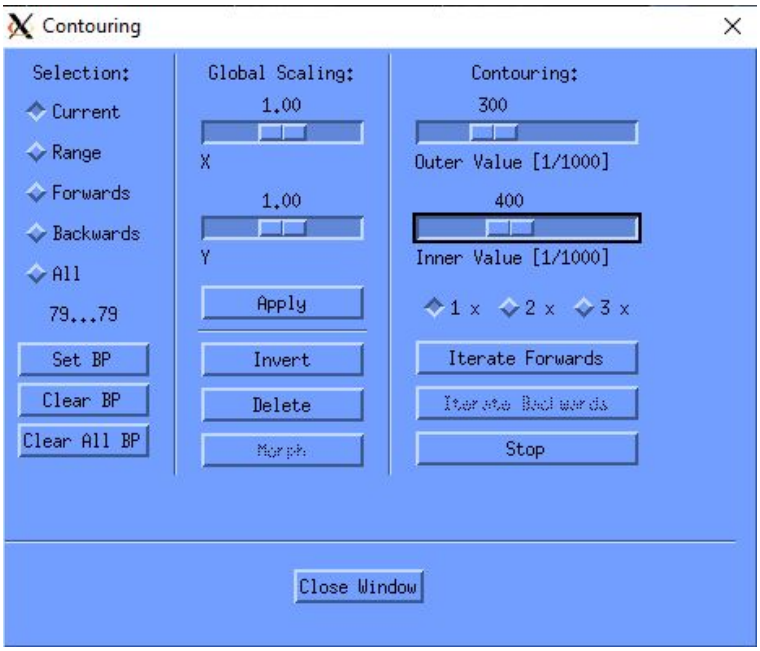
Click the draw contour button (Contours will be save as GOBJ file). 

Draw the contour in the COUNTER CLOCKWISE direction around the outer cortical bone perimeter.

Click the draw contour button again. 
Draw the contour in the CLOCKWISE direction around the inner cortical bone perimeter. You don't need to draw these contours perfectly, as they could be automatically corrected later.

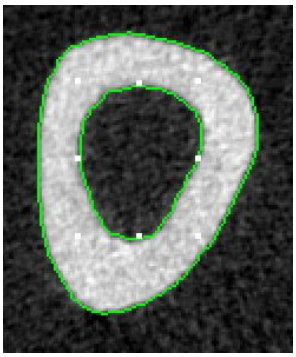


Click the “C...” button. Set contouring parameters:
Outer: 300, Inner: 400. If these parameters don’t work well, you need to play around with it.

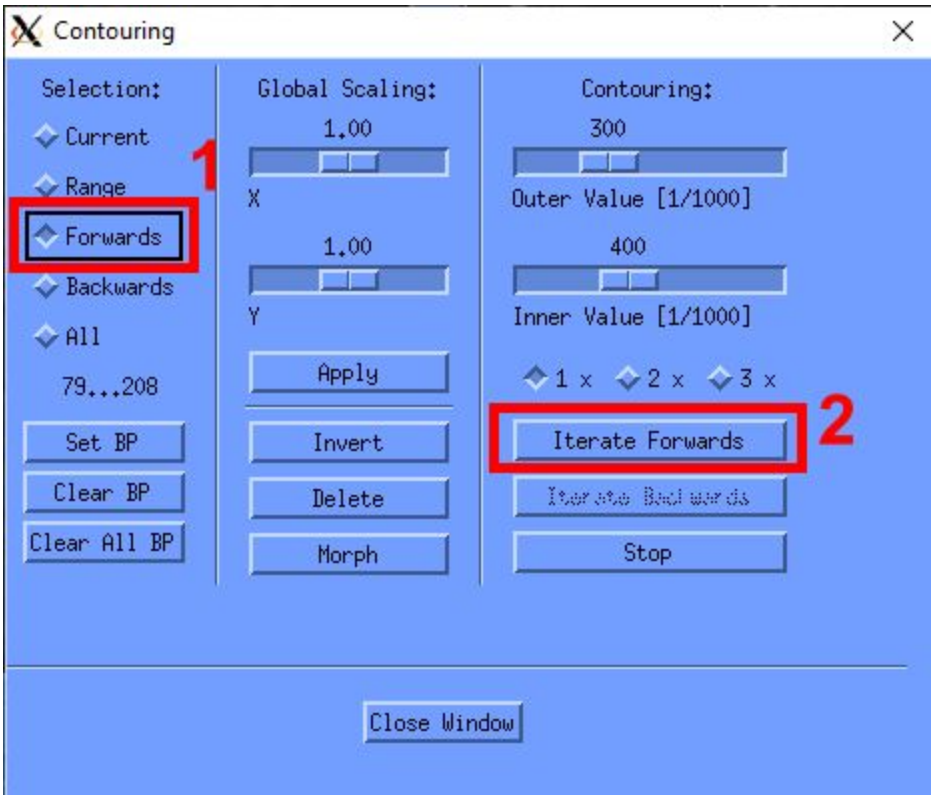


Note 1: If the contours are drawn too far away from the actual cortical bone, the software might crash.
Note 2: Sometimes the automatic iterations don't work too seamlessly, especially in aged mice where the cortical bone begins to trabecularize. You might need to go back in to manually adjust the contours. It's important to check after the 50 slices are contoured.

Double click the regions between the cortical bone edges and the contour you just drawed imperfectly. It will automatically re-adjust your contour. Repeat double clicking until the contour is close enough to all cortical edges.



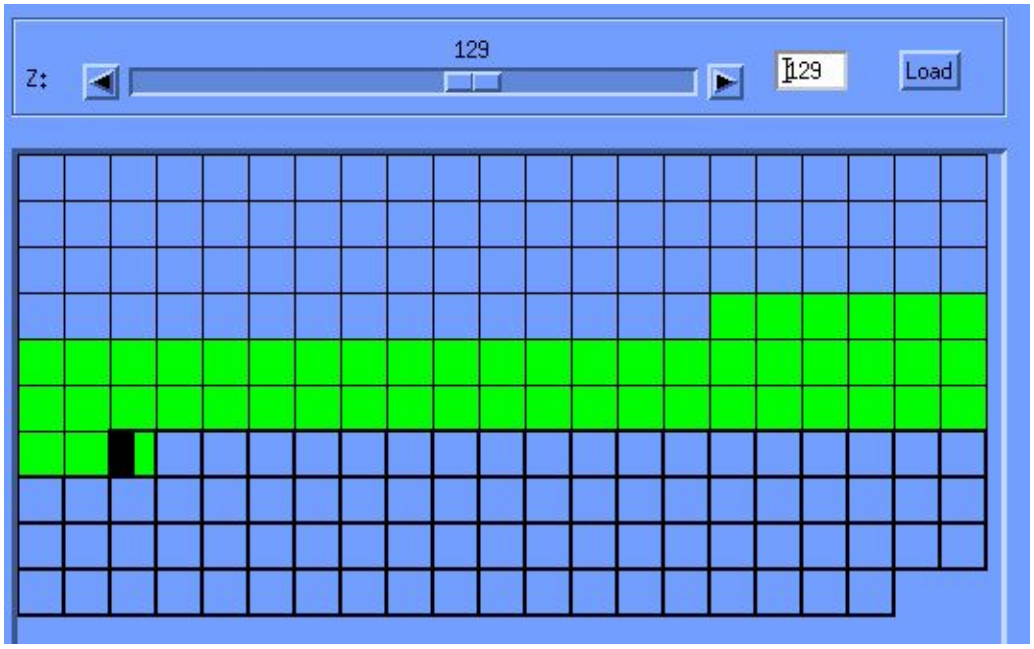
In the “Contouring” window, click the "Forward" button, then click "Iterate Forwards" button.



Then, the software will automatically draw contours on the slices forward.

After it completes 50 slices, you can click the “Stop” button.

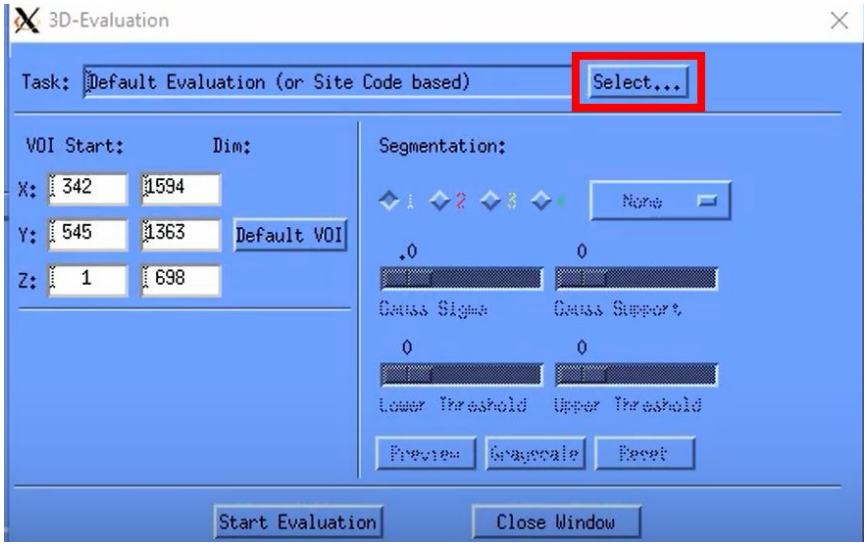
The green slices indicate the slices where contours have been drawn



3. Start cortical bone analysis.

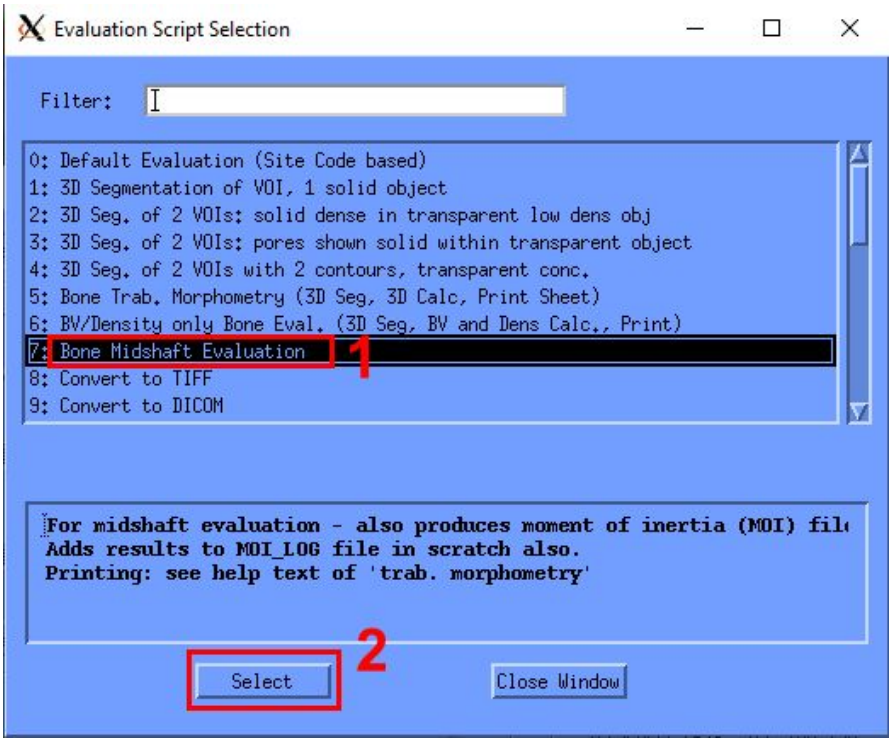
Click 'T...' 

Click "Select..."



Select Task: 'Bone Midshaft Evaluation',

Click "Select"

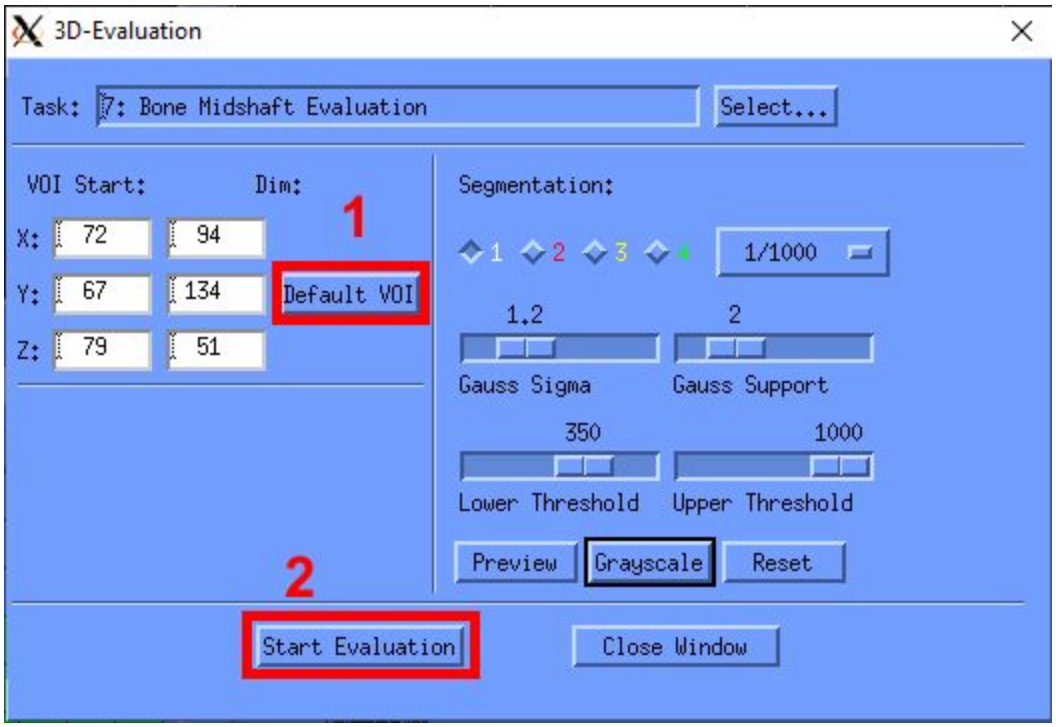


Pay attention to these parameters (Sigma, Support, Lower Threshold)!

Important! In the same project, keep these parameters the same!

You may adjust the “Lower Threshold” to an appropriate level. You may either move the bar by dragging your mouse or use the keyboard arrow key. Alternate clicking “Preview” and “Grayscale” to visualize the images. In this demo of mice tibia cortical bone, we set it: Sigma=1.2, Support=2, Lower Threshold=350.
(Note: Users should modify these parameters based on what they are analyzing.)

After the Lower Threshold is selected, click "Default VOI" (**VERY IMPORTANT!**)
Click "Start Evaluation" (Save contours when it asks you)



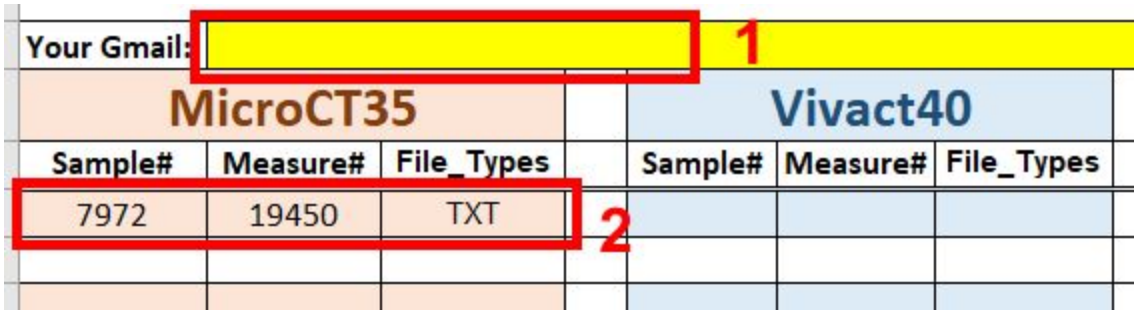
(This process will run in the background and can take up to 1 hour depending on your image size.)
You may proceed to analyze other samples.

4. Request the analysis result file.

Visit our website: https://www.med.upenn.edu/orl/uct/user-tutorials.html
Click to download "MicroCT File Request Form (Excel download)"

Open this Excel spreadsheet,

1) Enter your Gmail. (Analysis result files will be later shared to the Google Drive associated with this Google account.)
2) Make sure you enter the Sample# and Measure# under the correct scanner!
   In this demo, you would like to request files from the MicroCT 35 (Sample#: 7972, Measurement#: 19450).
   Enter 7972 at the Sample# column;
   Enter 19450 at the Measure# column;
   Enter TXT at the File_Types column.



3) Save this Excel spreadsheet, and send it to pcmd.microct@gmail.com
4) Our system will automatically process your request. You will receive a notification email from Google Drive with a shared folder containing the files you have requested. That's it!

Explanation for the related parameters (adapted from Scanco technical document):

- Ixx ~ Sum(cm.y - pos.y)^2: MOI around x-axis, determined by extent in Y direction.
- pMOI = polar MOI = Ixx + Iyy
- Long object axis has cw-angle 'Angle' towards old x-Axis
- Imax is MOI around shorter axis
- BArea is Cross Sectional Area of segmented object (of 'bone' only): cortical area.
- TArea is the total area (everything within contour).
- Ixx/Cy: MOI around x-axis divided by max.extent in y direction
- Imax/Cmax: MOI around new y-axis divided by max.extent in x direction relates to 'Breaking Strength'
- Imin/Cmin: MOI around new x-axis divided by max.extent in y direction relates to other 'Breaking Strength' (you may also call Cmin the 'max radius perpendicular to Imin direction')
- Mean1: BMD, 'Mean of TV' (mean of everything within volume of interest)
- Mean2: TMD, 'Mean of BV' (mean of segmented region)