

Mouse Bone Analysis

Workflow

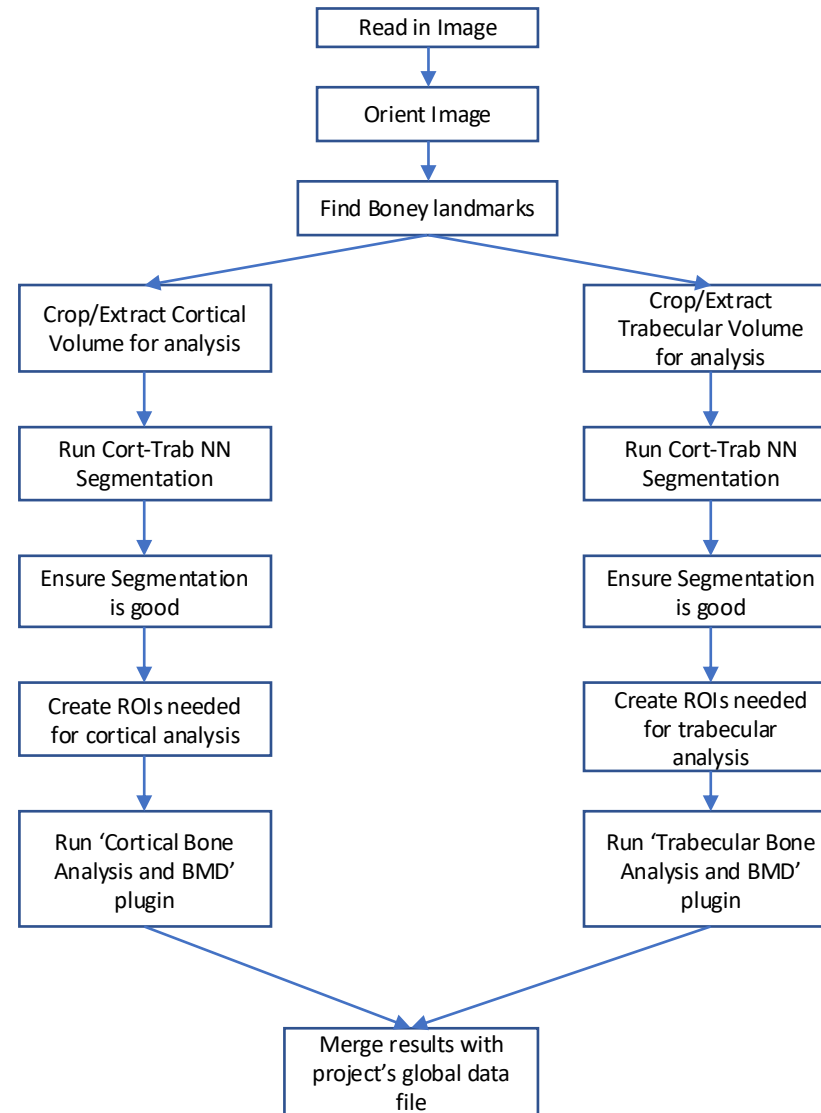
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Tips, tricks and hints

- Object is visible 
- Object is not visible 
- When aligning femur vertically, use the 'T' key for a quick check on alignment progress

Mouse Bone Analysis Process Steps



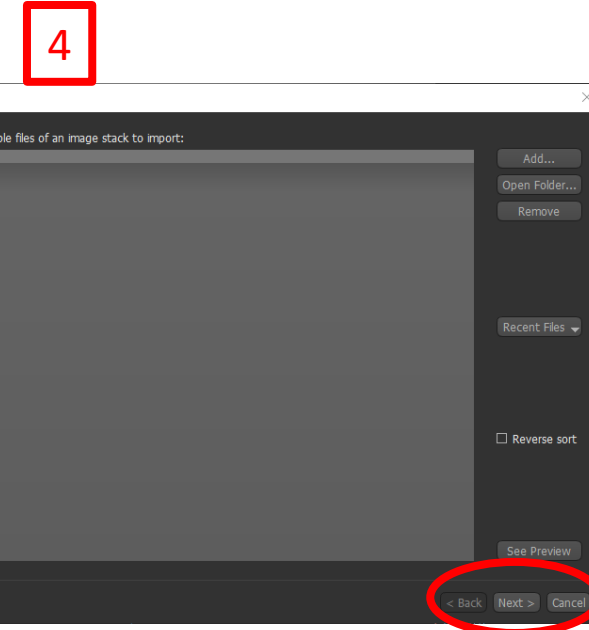
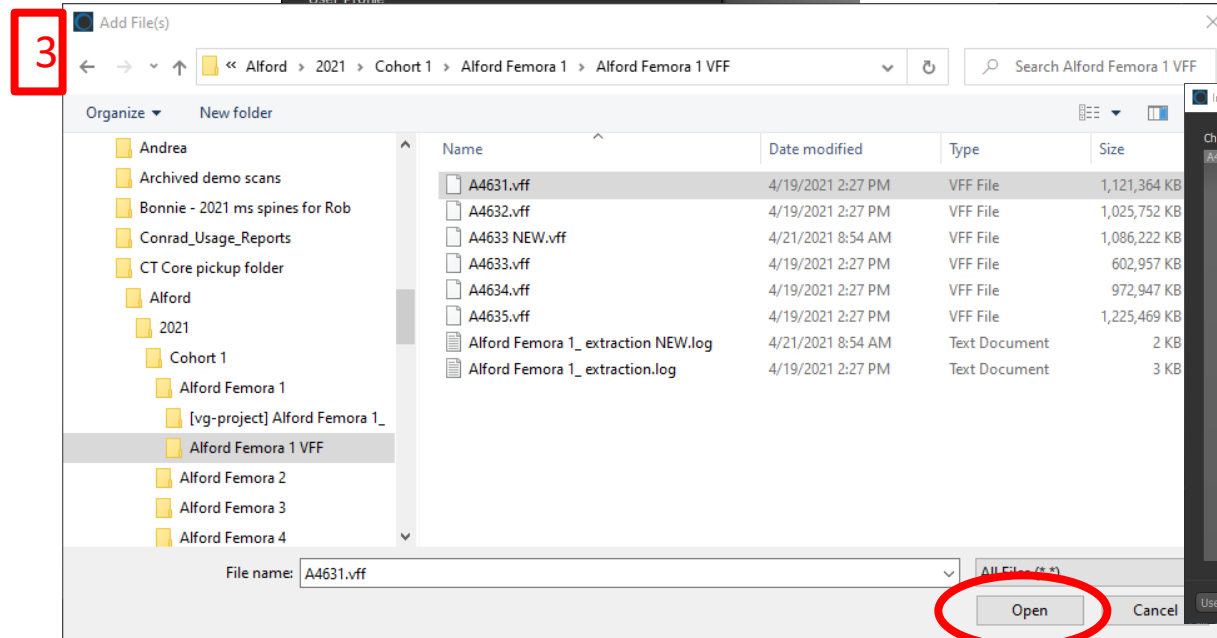
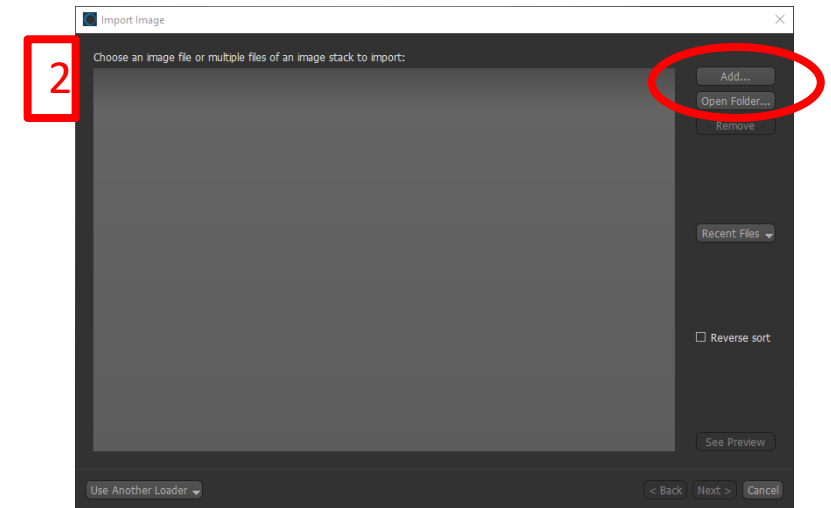
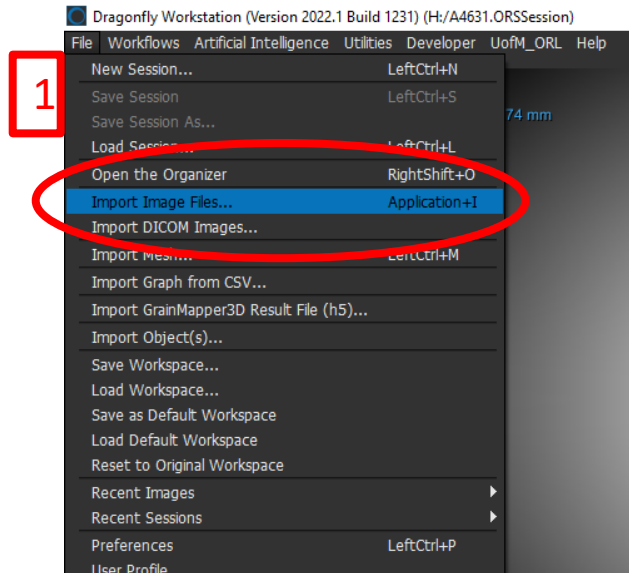
Read Image into Dragonfly

Dragonfly allows Users to read in micro/nano-Ct images in the following file formats:

- vff – usually micro/nano CT images that have been converted into Hounsfield Units (HU); Scanco images can be converted to this format but will not be in HU.
- vgl/vol – for nano CT images that are a file combination of .vgl and .vol, you will need to select the .vgl file for import.
- tiff – usually files from the Bruker or Skyscan μ CT scanner (and sometimes Scanco images), you will need to select all images for the reconstruction image.
- Dicom – images from the Scanco scanner can be converted to this format for importing into Dragonfly (NOTE: Scanco images in this format are in 8-bit format though).
- ISQ – Scanco format that can be imported if reader installed – Contact Rob Goulet rgoulet@med.umich.edu.
- For other formats refer to the Dragonfly documentation.

Read Image into Dragonfly

1. Go to “File->Import Image File...”
2. In the Import Image file window, click the “Add” button.
3. In the Add File(s) window scroll to the folder containing your image file(s), select it and then “Open”. Screen shot shows selection of a vff file, for a sequence of tiff files you would select all tiff files for the reconstruction and then “Open”.
4. In Import Image file window select “Next”



Read Image into Dragonfly

5. Ensure image properties are correct in each dimension (image spacing should be $\sim 8\text{ }\mu\text{m}$ for nanoCT images, $\sim 18\text{ }\mu\text{m}$ for GE μCT images, ~ 9 or $18\text{ }\mu\text{m}$ for Skyscan images, and $\sim 12\text{ }\mu\text{m}$ for Scanco images)

NOTE: Sometimes the Z-spacing may be off in the 6th or more decimal place, if it is copy the X or Y spacing value and paste in Z-spacing.

6. Select “Finish”

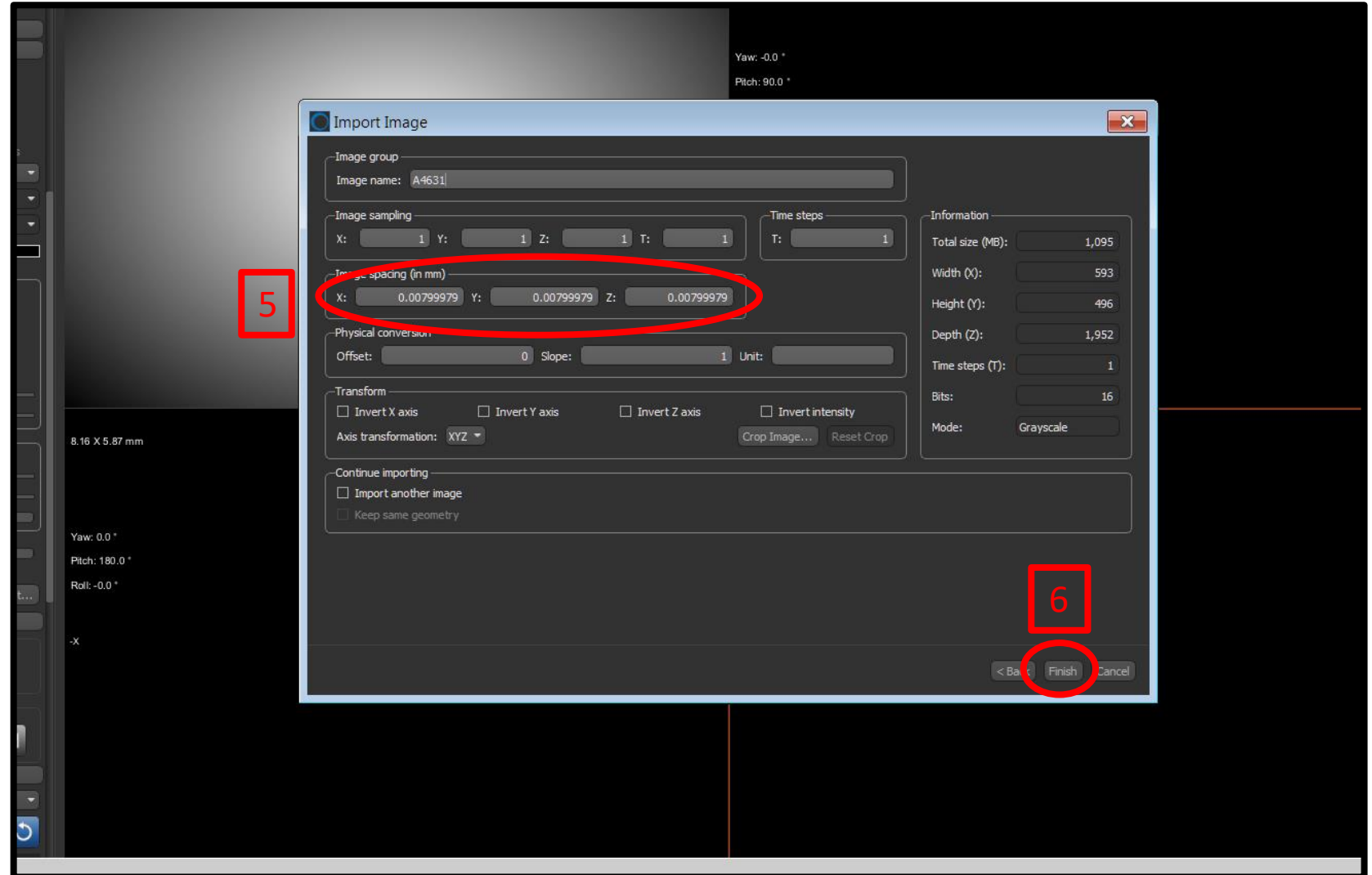


Image Orientation Steps

Your next step is to invert the femoral head to the positive Z position

Steps to invert image in Z-axis:

1. Right click on image name under 'Data Properties and Settings'
2. Select 'Modify and Transform'
3. Select 'Invert'
4. Check box 'Invert Z Axis'
5. Ensure 'Create new' box checked
6. Select 'Apply'
7. Click 'Close'

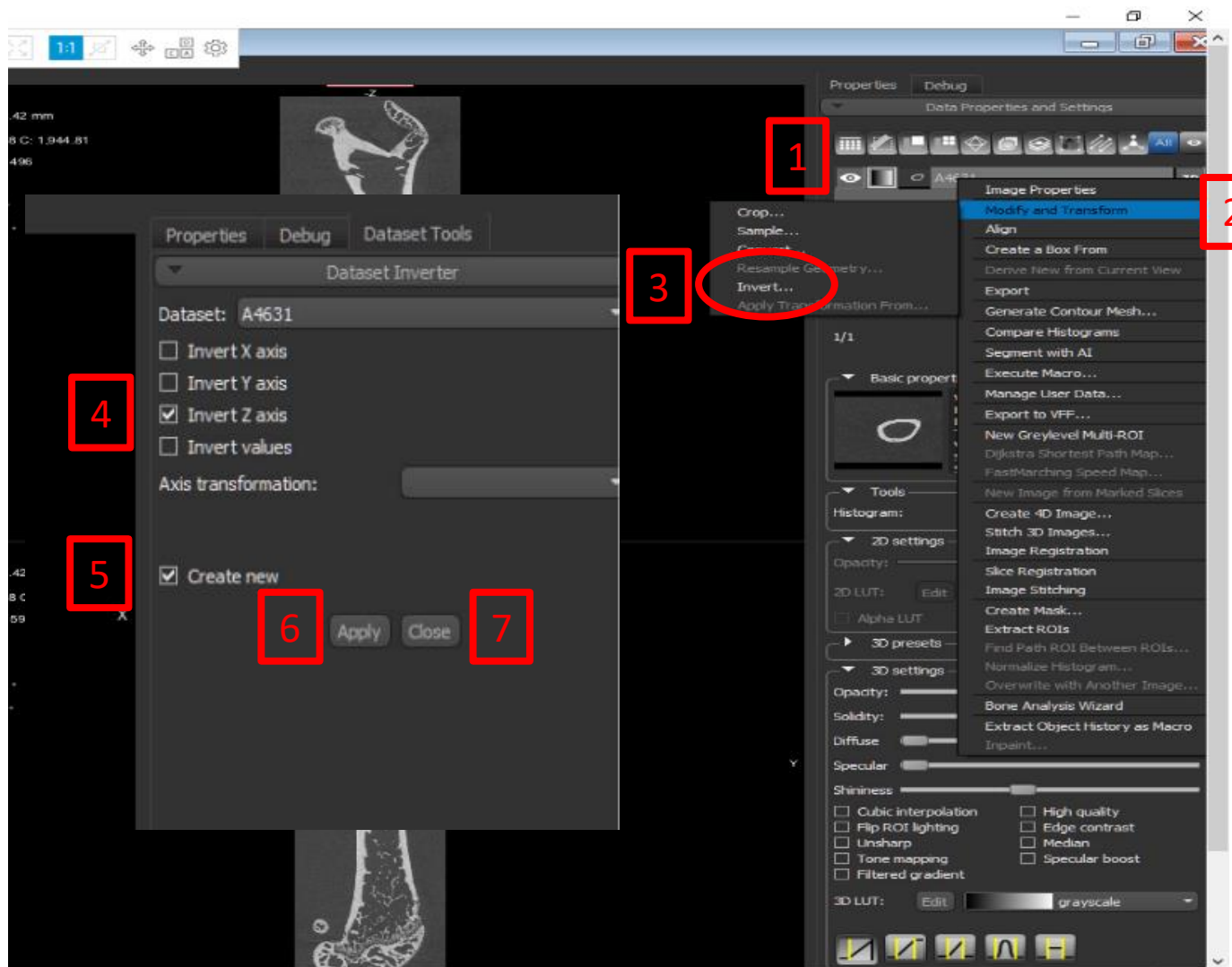


Image Orientation Steps

Change image viewing:

- Turn off original image by clicking the visibility icon



- Select new inverted image and make visible



- The femoral head is now in the positive **Z** position



Image Orientation Steps

1. Under 'Main' tab scroll down to 'Shapes' and select the 'Box' shape.
 - This will create a new object in the stack of images on right side.
2. Turn off the 'Box'
3. Re-select your inverted image.

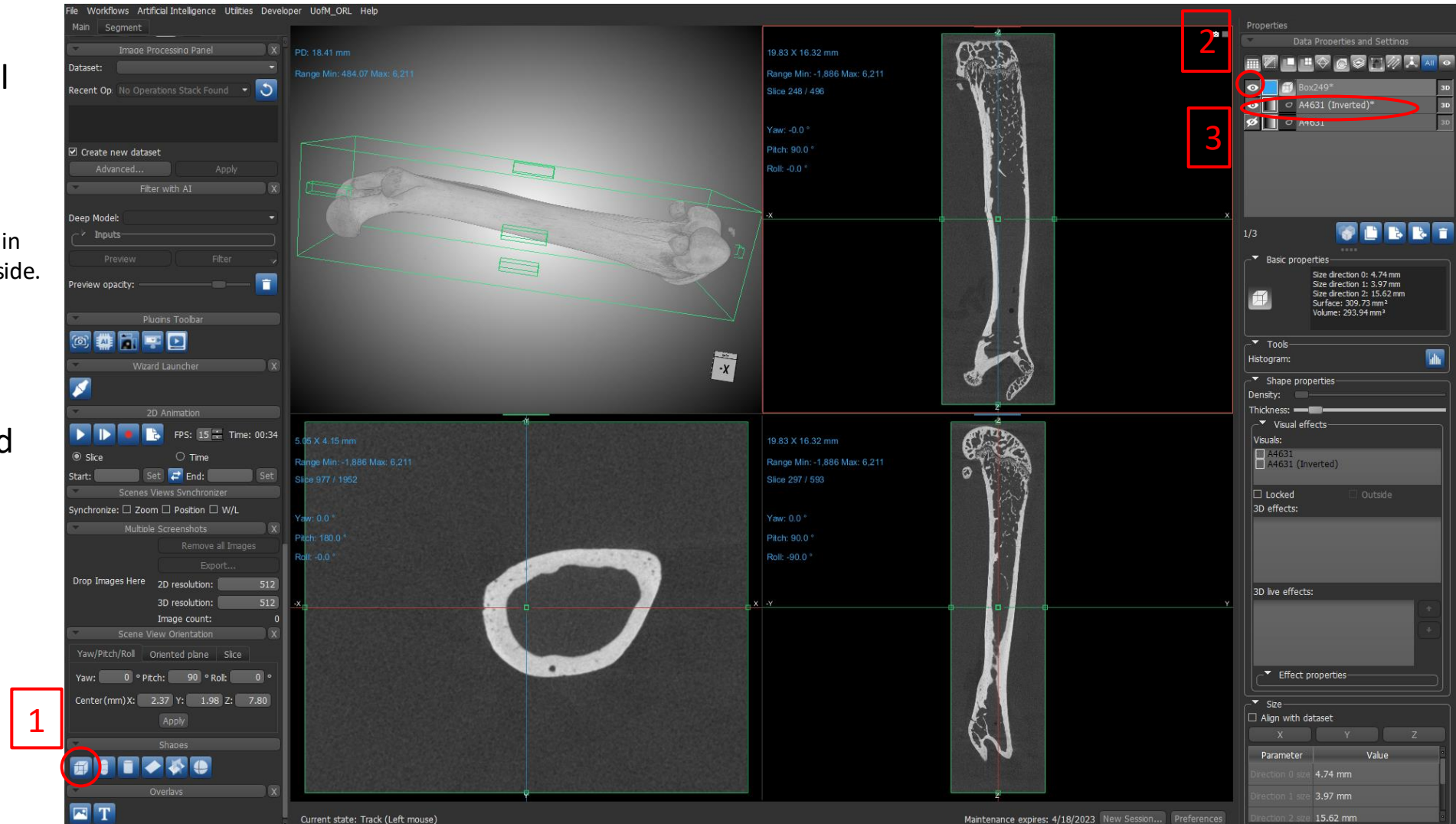


Image Orientation Steps

It is critical to align your femoral axis in the 'Z' axis, so your cortical analysis gives meaningful results

1. Click in any of the 2D views
2. Under 'Main', go to 'Translate/Rotate' and select 'Displace'
3. In the Z-X and Z-Y views, rotate the bone so that cortical region of interest (volume just distal to the 3rd trochanter) is aligned with the Z axis
4. In the Y-X view, align the 'point' of the 3rd trochanter along the +X axis

(NOTE: You can align with the +Y axis but you must align all specimens for your studies in the same manner!)



Image Orientation Steps

1. Now re-select the Box you created and make visible
2. You will now need to adjust the Box in the X, Y, Z directions to include just the femur.
 - a. Use the 3D view to ensure you are not **excluding** any part of the femur
 - b. When adjusting box, do not select the small 'boxes' along edge as this will rotate Box



Image Orientation Steps

- Once your box is the appropriate size right click on it in the Data Properties and Settings table to bring up menu and select 'Extract Structured Grid...'
- In dialogue window, make sure the 'Object' is set to your inverted, rotated image and set the 'Name' to indicate the specimen id and distinction that it has been inverted/flipped, rotated, and cropped(e.g., *specimenID_IRC*)
- Click 'OK'

(NOTE: It's best practice to save your work at this point)

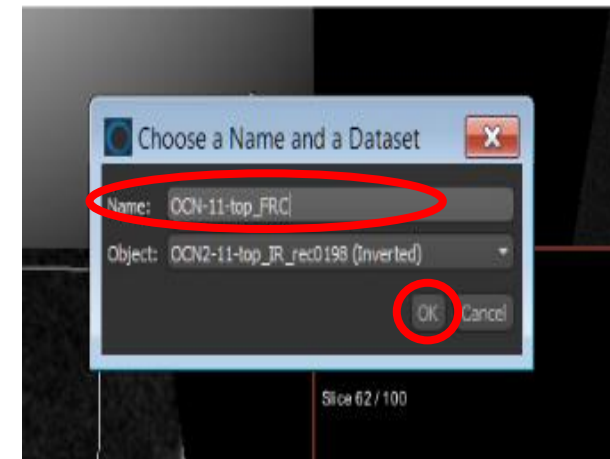
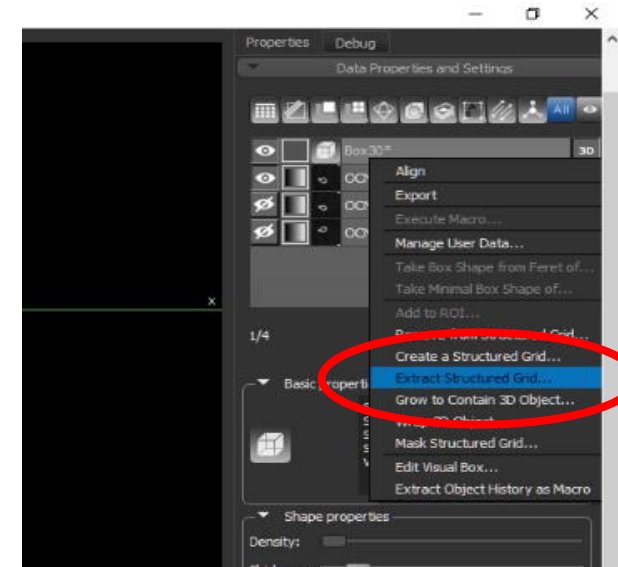


Image Orientation Steps

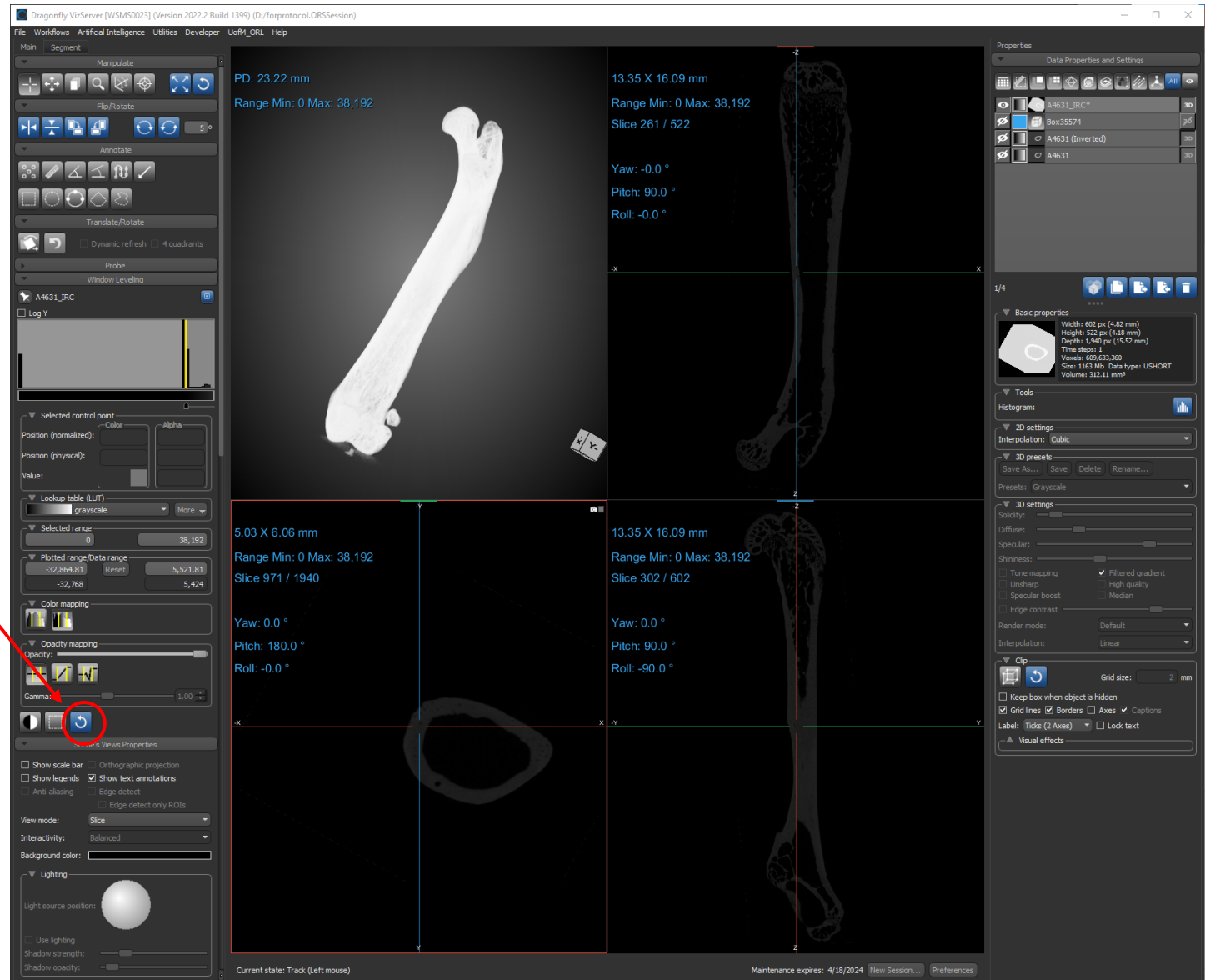
-Select a 2D window, under the Window Leveling menu, click the blue “re-set” icon

Repeat this for the 3D window

After resetting the Window Level for all views, image will appear bright again.

The next 5 slides show 2 methods on how to reset the ‘corner’ voxels to the background greyscale value and have the bone/background appear when first read in

NOTE: *there are 2 methods, read thru both to decide which you prefer*



Resetting 'Corners' to Background Greyscale values

Resetting the 'corner' voxels grayscale values to be that of the background values will make the image appear normal as well as help with the 3D display not appearing 'washed' out but is not mandatory to do for the cortical or trabecular bone analysis.

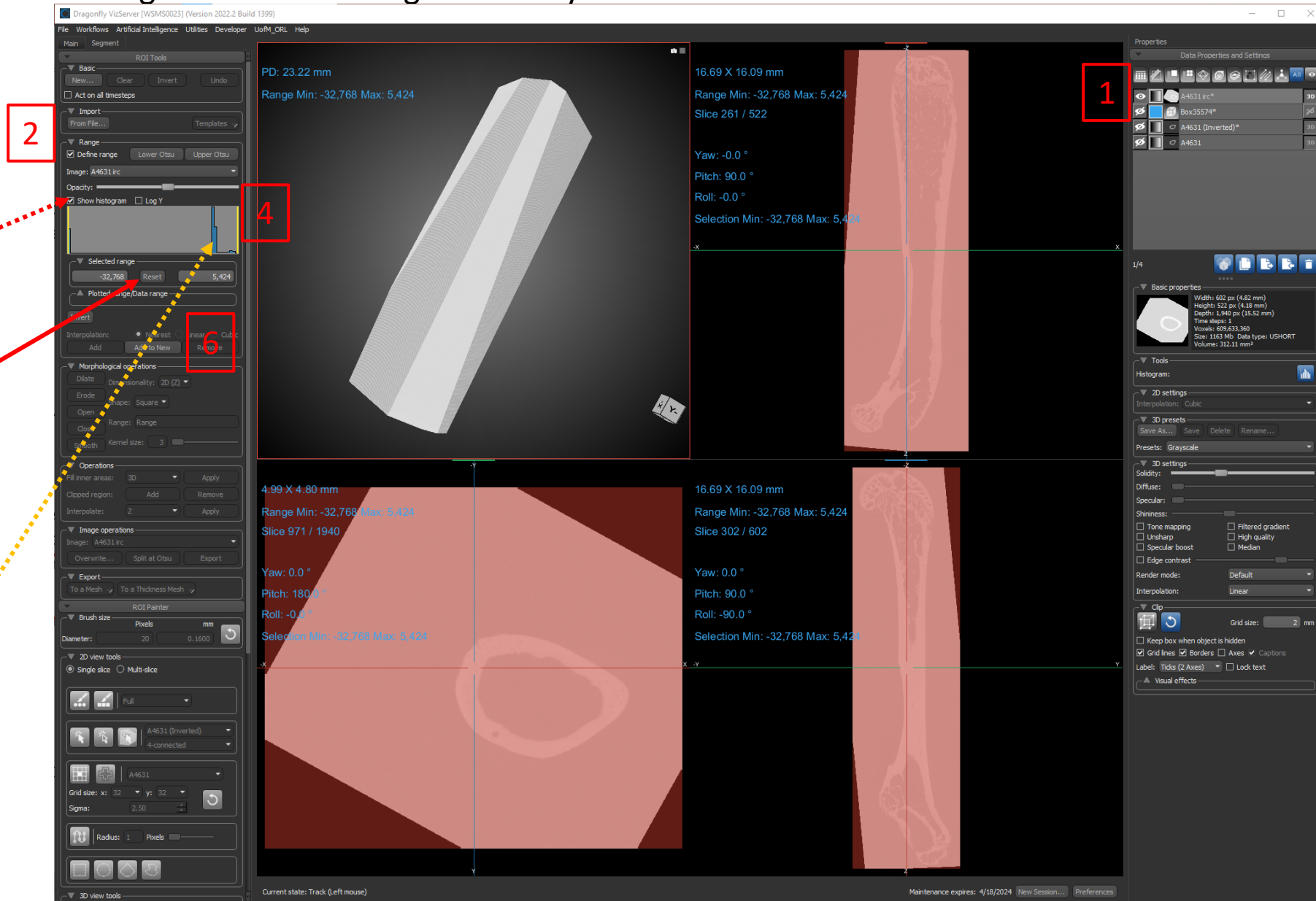
The steps outlined in the next several slides are optional and recommended if you plan on making screenshots or movies using the image.

The steps to reset the corners can add 5-10 minutes to the overall analysis time and thus are why we show them but are completely optional.

Resetting 'Corners' to Background Greyscale values

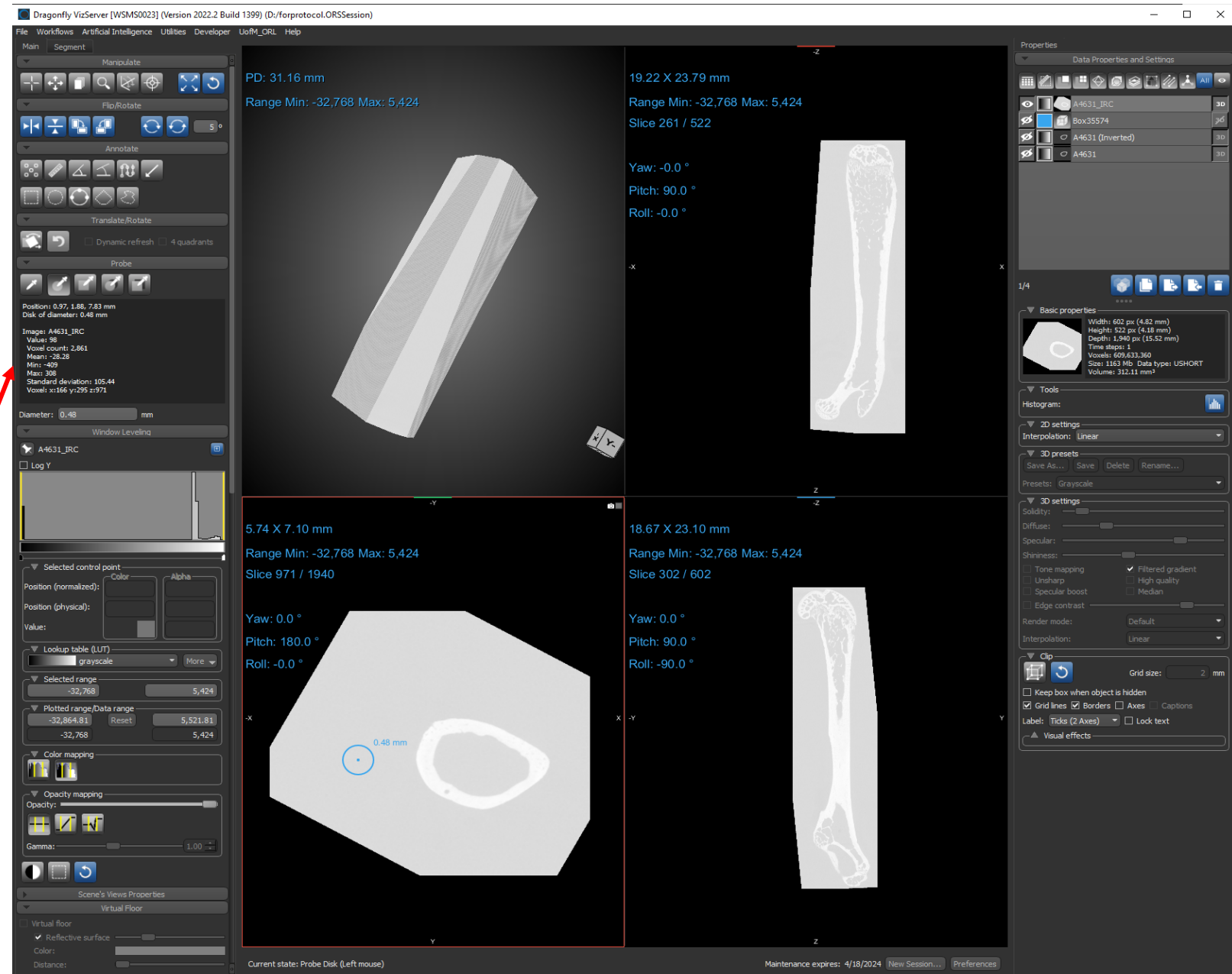
To correct the 'corners' that were set to '0' from the rotation step, the voxels can be set to the mean (average) background grayscale value.

1. Select the rotated/cropped image
2. Go to the 'Segment' tab, under 'Range' check 'Define Range'
 - It is helpful to then click the 'Show Histogram' option.
3. The "Selected range" will be that of the original exported image that had the Offset set to 0. Click the "Reset" button to have the Selected range on the new image min – max values.
4. Leave the lower range value set to -32768 and move the upper range (yellow line will switch to red indicating you can adjust upper value) to a value just below the min background grayscale value. Or to 1000.
5. As you move the upper range value down, note the value when you see just a few voxels left on in the background (this will be near the peak histogram for background values), use this value when overwriting the corner voxels (described in two slides)
6. Then click the 'Add to New' button
 - Doing this will give you a 'New ROI' image on your stack to the right.



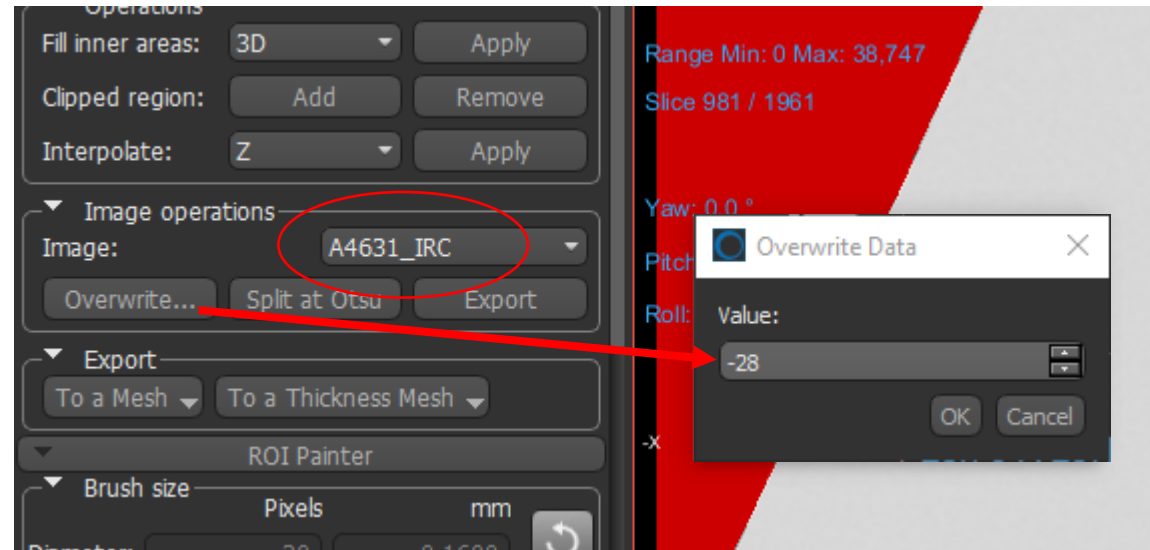
Resetting 'Corners' to Background Greyscale values

An alternative method to get the value used for overwriting the corner voxels, would be to uncheck the 'Define Range' segmentation option. Go to the 'Main' tab, open the 'Probe' tool and select the probe with a 'circle' and then pan around in the background area (avoiding any bone & ROI) and note the 'mean' value found; this value will be used to reset the 'corner' regions.



Resetting 'Corners' to Background Greyscale values

-Now that you have the value, you'll use to correct the corners, make sure the 'New ROI' created for the 'corner regions' is selected in the 'Data Properties and Settings' table, go to 'Image operations' under the 'Segment' tab, select the inverted/rotated/cropped image, click the 'Overwrite...' button and enter the average 'mean grayscale' value found using the 'Probe' tool. Click 'OK', then turn off 'New ROI' and reselect your image.



Note: Make sure the correct "Image:" is selected before clicking the 'Overwrite...' button. When the correct image is selected, the 'Split at Otsu' button will be "Active" (that is, clickable).

Resetting 'Corners' to Background Greyscale values

After 'correcting' the 'corner' grayscale values, the image Window Leveling can be reset so that the background is no longer washed out.

Click in one of the views, then go to 'Main' and then 'Window Leveling' and click on the blue reset arrow (will need to do both 3D & 2D views).



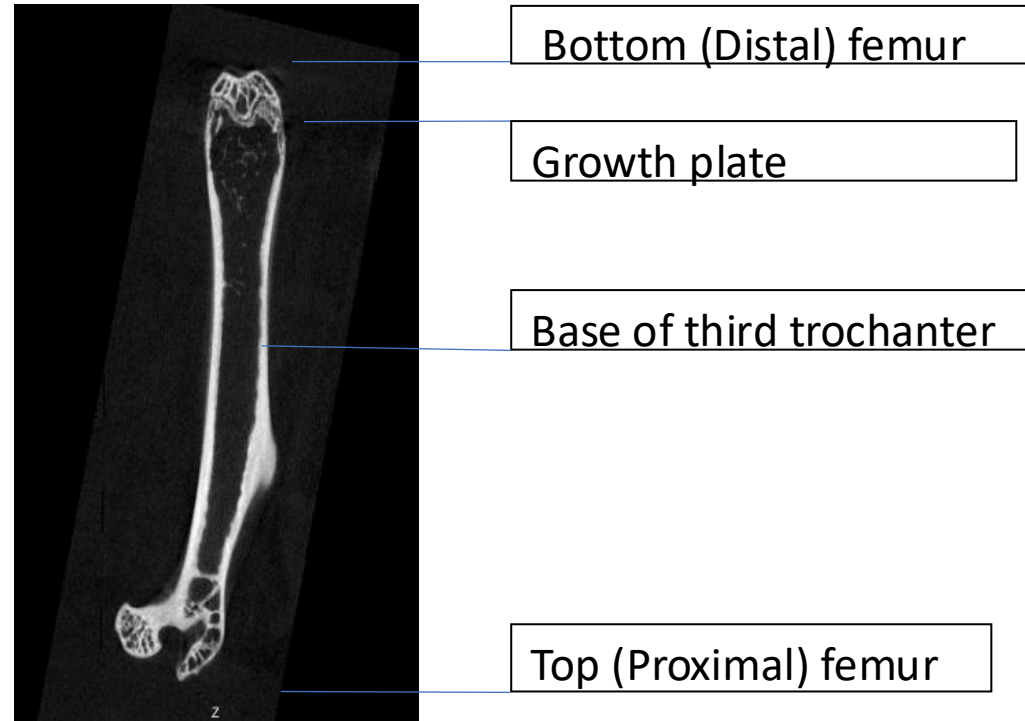
**Determining the volumes of
interest for cortical and
trabeculae bone analysis;
AKA: finding bone landmarks**

Determine the volumes of interest for cortical and trabeculae bone analysis

-There are 4 boney landmarks needed to enter from which the Z dimensions are determined for the trabeculae and cortical regions. These four landmarks are *top (proximal) of the bone*, *base of the third trochanter*, the *distal metaphyseal growth plate*, and *bottom (distal) of the bone*. One also needs to specify the percent of the total bone length to define the height/Z dimension of the region of interest (ROI). Common values of the percent are 10-15%, with default being 10%.

-This can be done using the linked Excel workbook as a template:

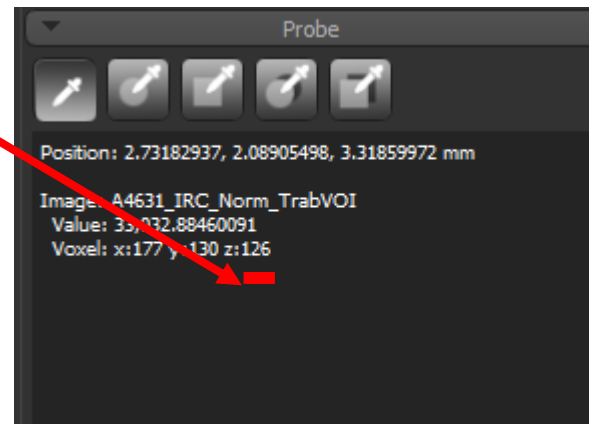
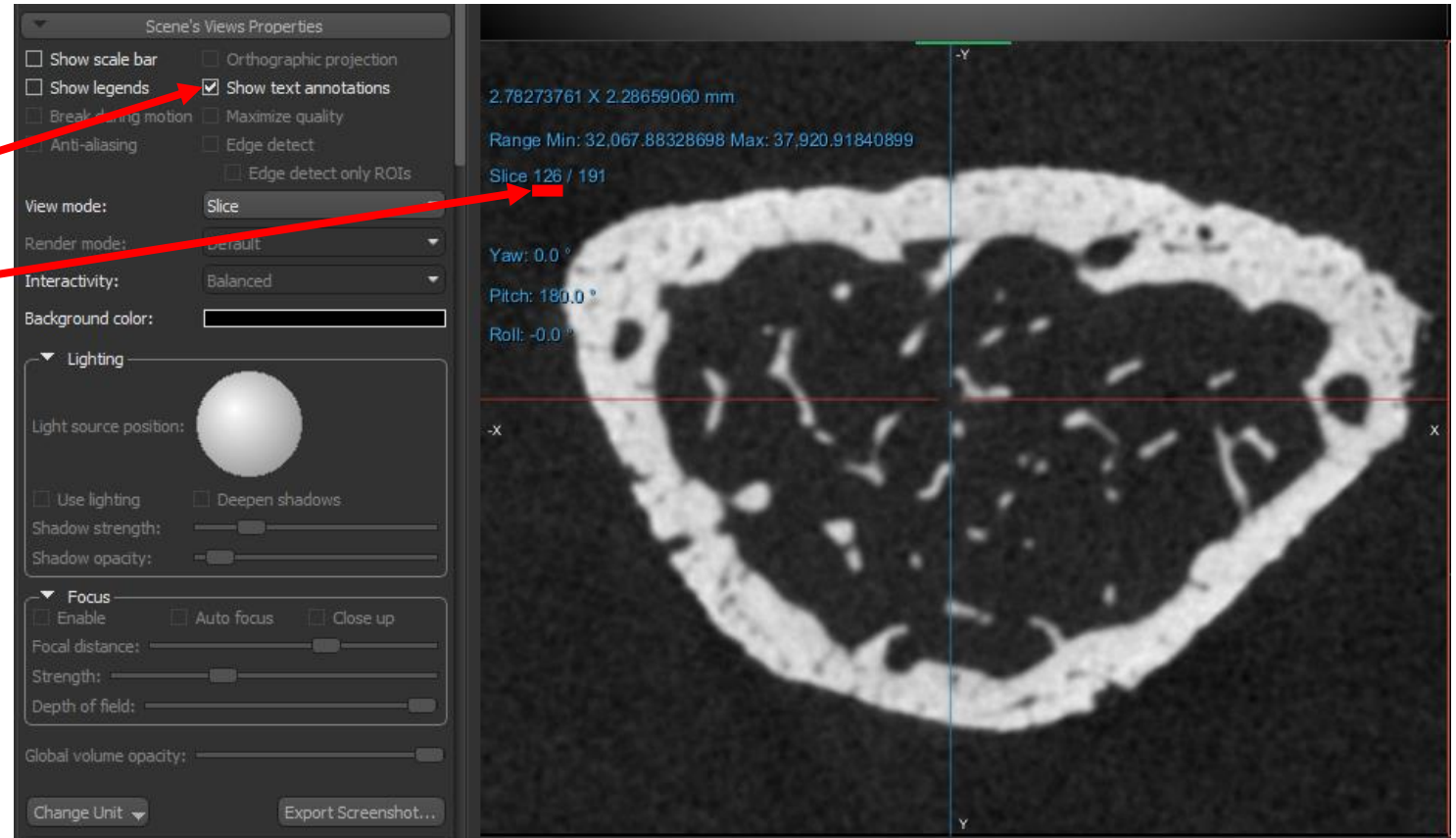
[BoneAnalysis_workbook.xlsx](#)



Determine the volumes of interest for cortical and trabeculae bone analysis

To get the Z-slice value for the various landmarks, it is easiest to leave the 'Show Text annotations' on and then in the XY View (Z slice), the Z-Slice will be the first number of the "Slice: " line.

NOTE: When using Dragonfly during a remote login session, the "Slice" number may not increment/change as you transverse through the slices. If this happens, you will need to use the 'Probe' tool. When you move the probe over the XY View, you will see the X & Y values change but the Z value will remain constant, this is the value you will use for the Z-Slice.



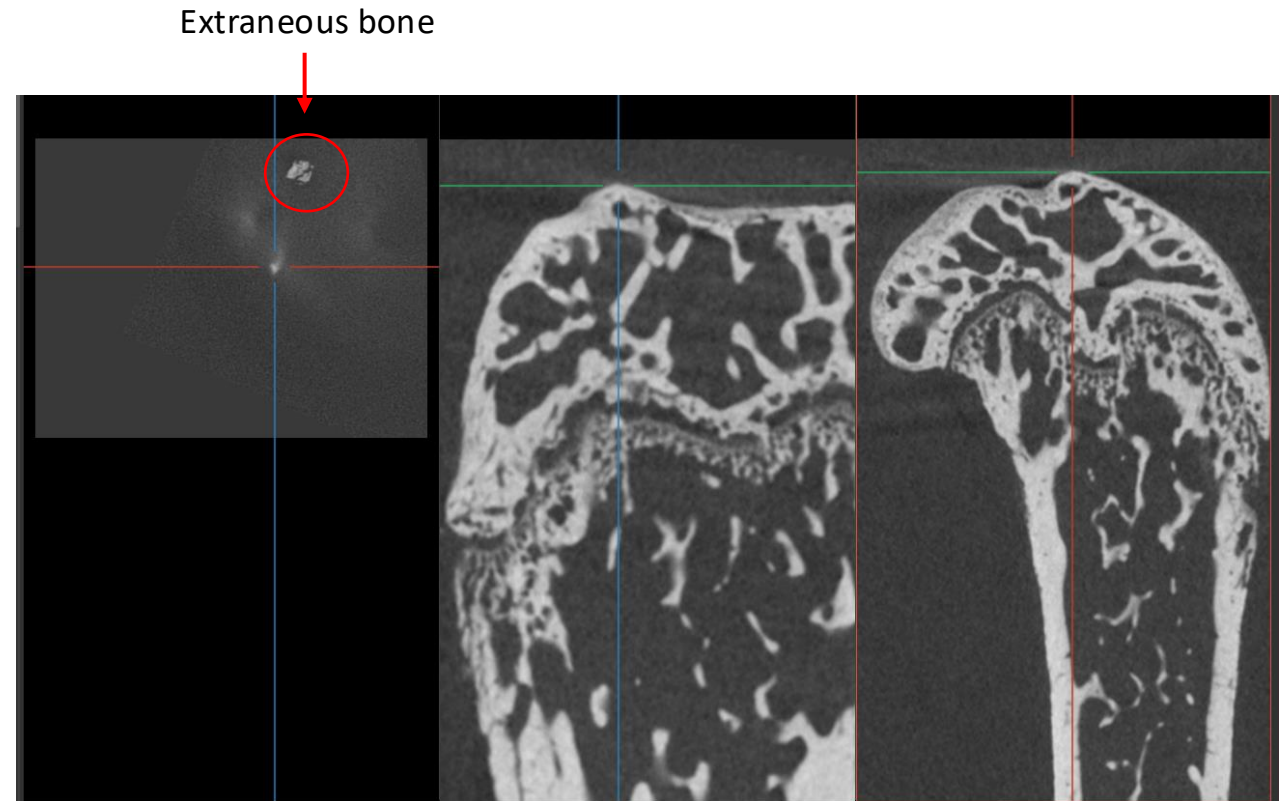
Determine the volumes of interest for cortical and trabeculae bone analysis

The **Bottom**, or most distal end, of the bone is where just a few voxels are showing of the condyle on the Z axis.

note: This image has extraneous bone that can be ignored in determining the bottom of the bone

Enter this value (Z slice) into column E if using the Bone Analysis Template excel file.

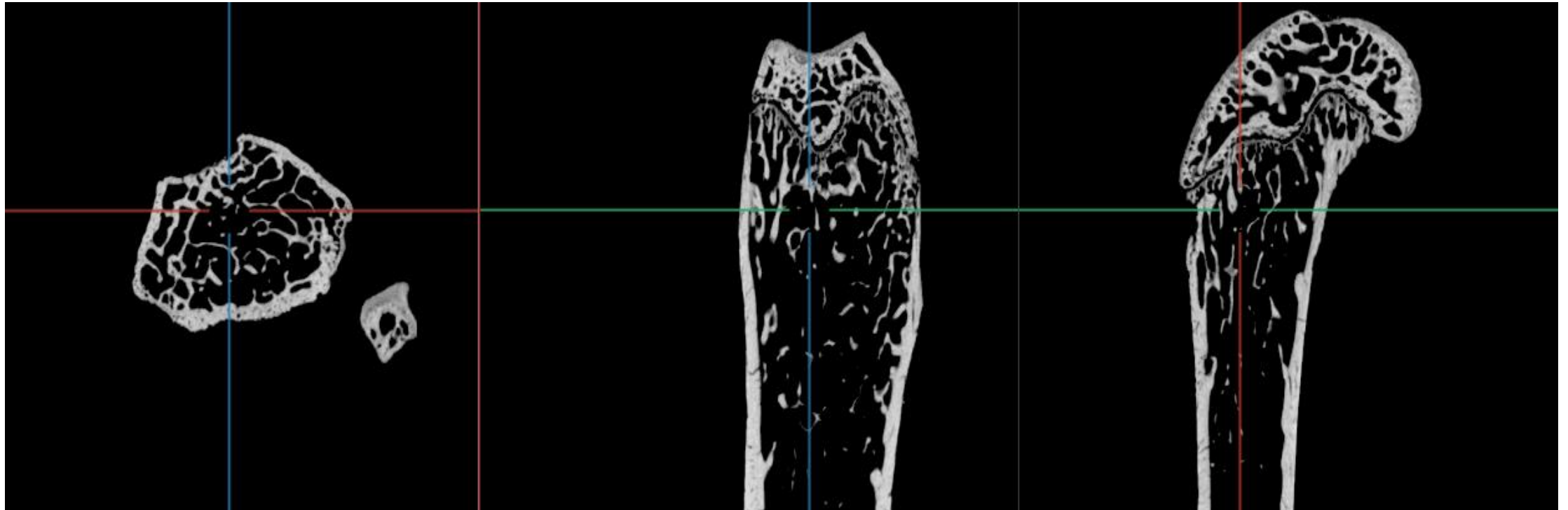
This position, in conjunction with top of the bone, is used for determining the length of the bone.



Determine the volumes of interest for cortical and trabeculae bone analysis

The **Growth Plate**

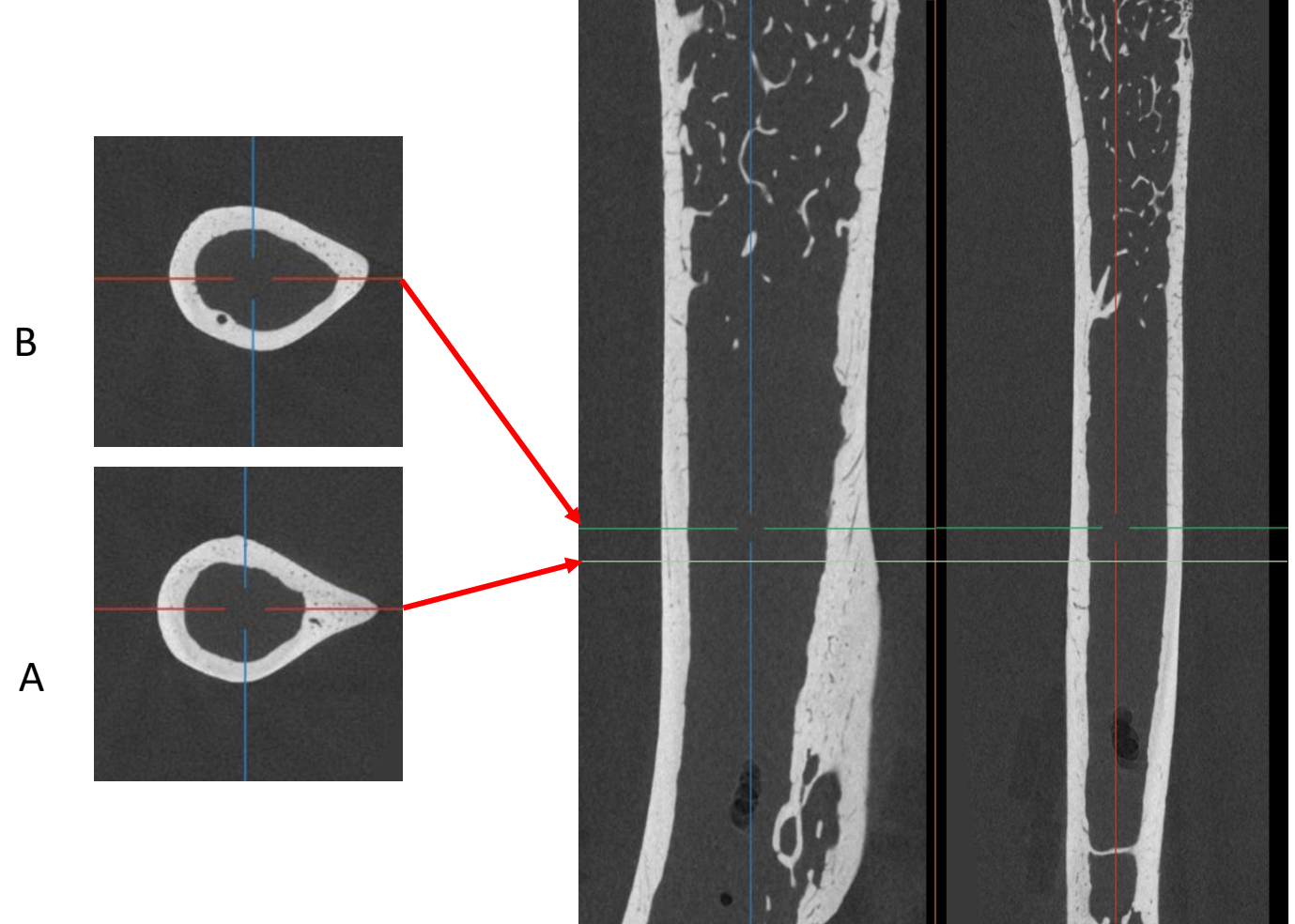
(Z slice) location is north of where the cortical shell has minimum remnants of the growth plate still visible; from there move up 15 slices and enter this value in column F if using the Bone Analysis Template excel file.



Determine the volumes of interest for cortical and trabeculae bone analysis

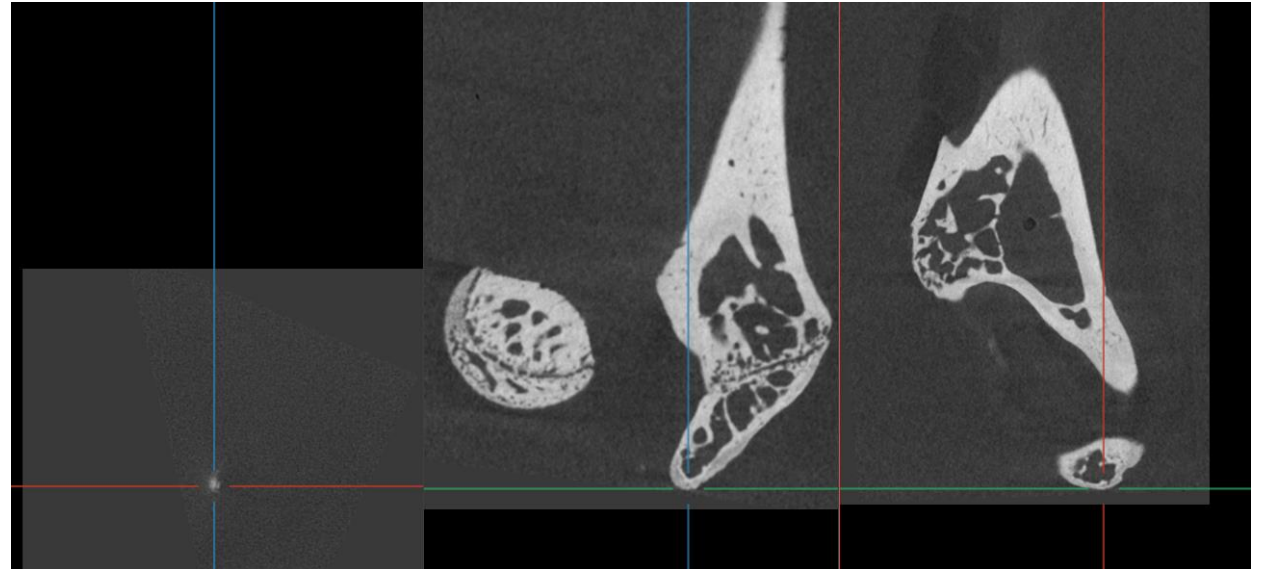
3rd Trochanter

Z position is found by locating the slice where the trochanter is more rounded (B image) than elongated (image A). Enter this value (Z slice of image B) in column G if using the Bone Analysis Template excel file.



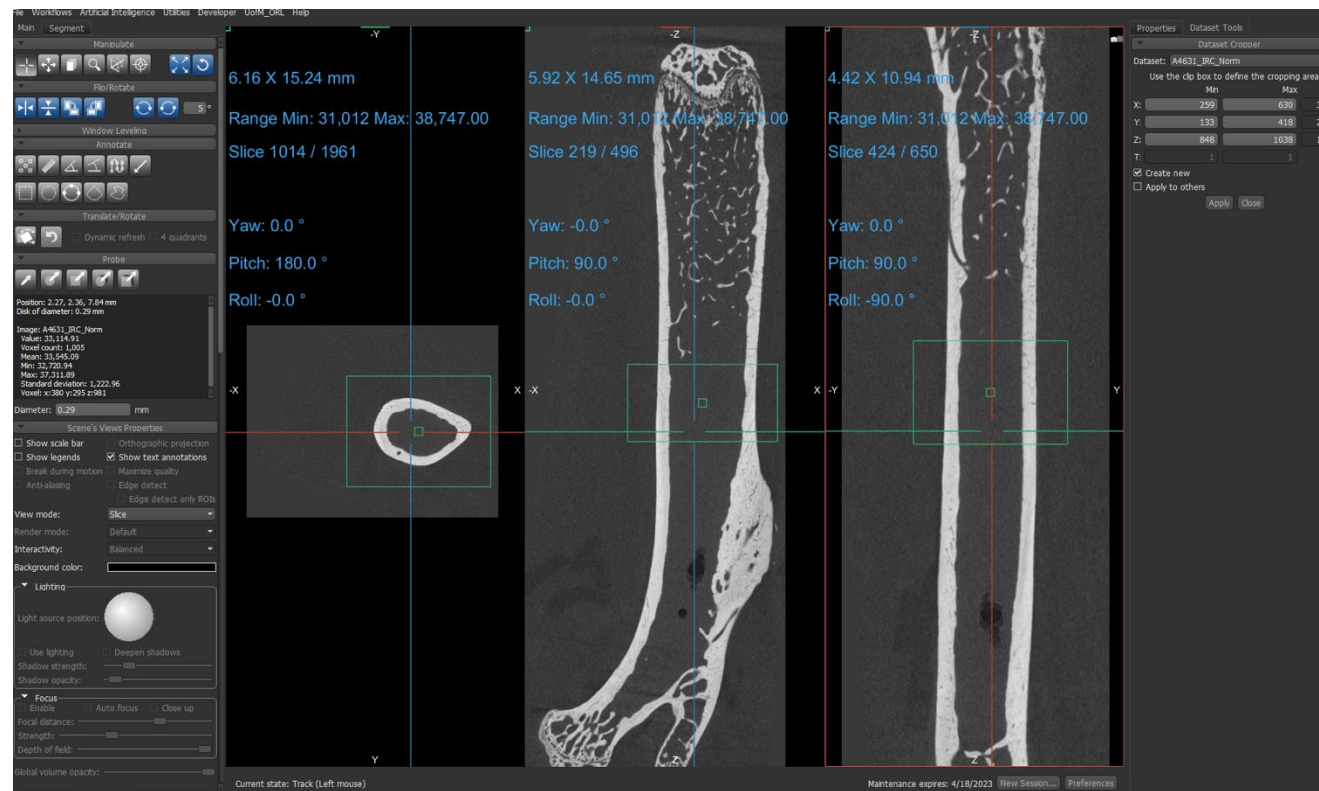
Determine the volumes of interest for cortical and trabeculae bone analysis

The **Top** of the bone is where just a few voxels are showing at the tip of the Greater Trochanter. Enter this value in column H if using the Bone Analysis Template excel file.



Creating Cortical VOI and ROI's

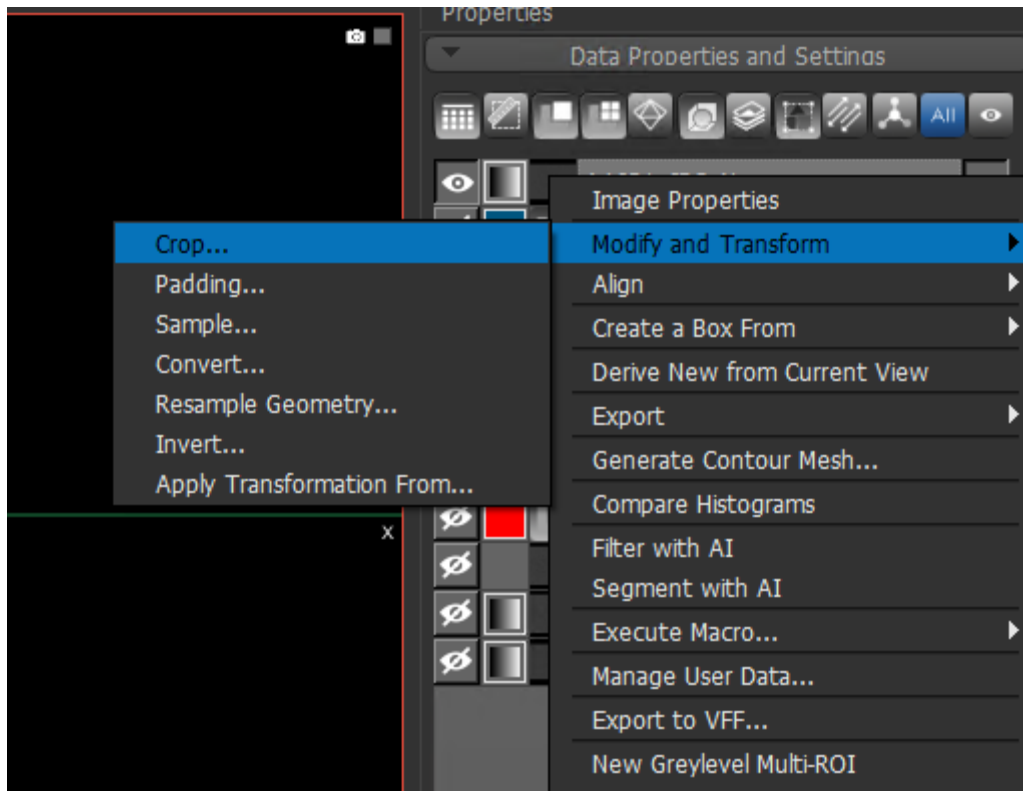
-After aligning the femur so 3rd Trochanter is aligned with the X or Y axis and the long axis of the bone is aligned with the Z axis and having found the boney landmarks, crop the cortical volume(s) to be analyzed and save as new image object(s).



Creating Cortical VOI and ROI's

- Right click on your image and select menu option 'Modify and Transform -> Crop'.

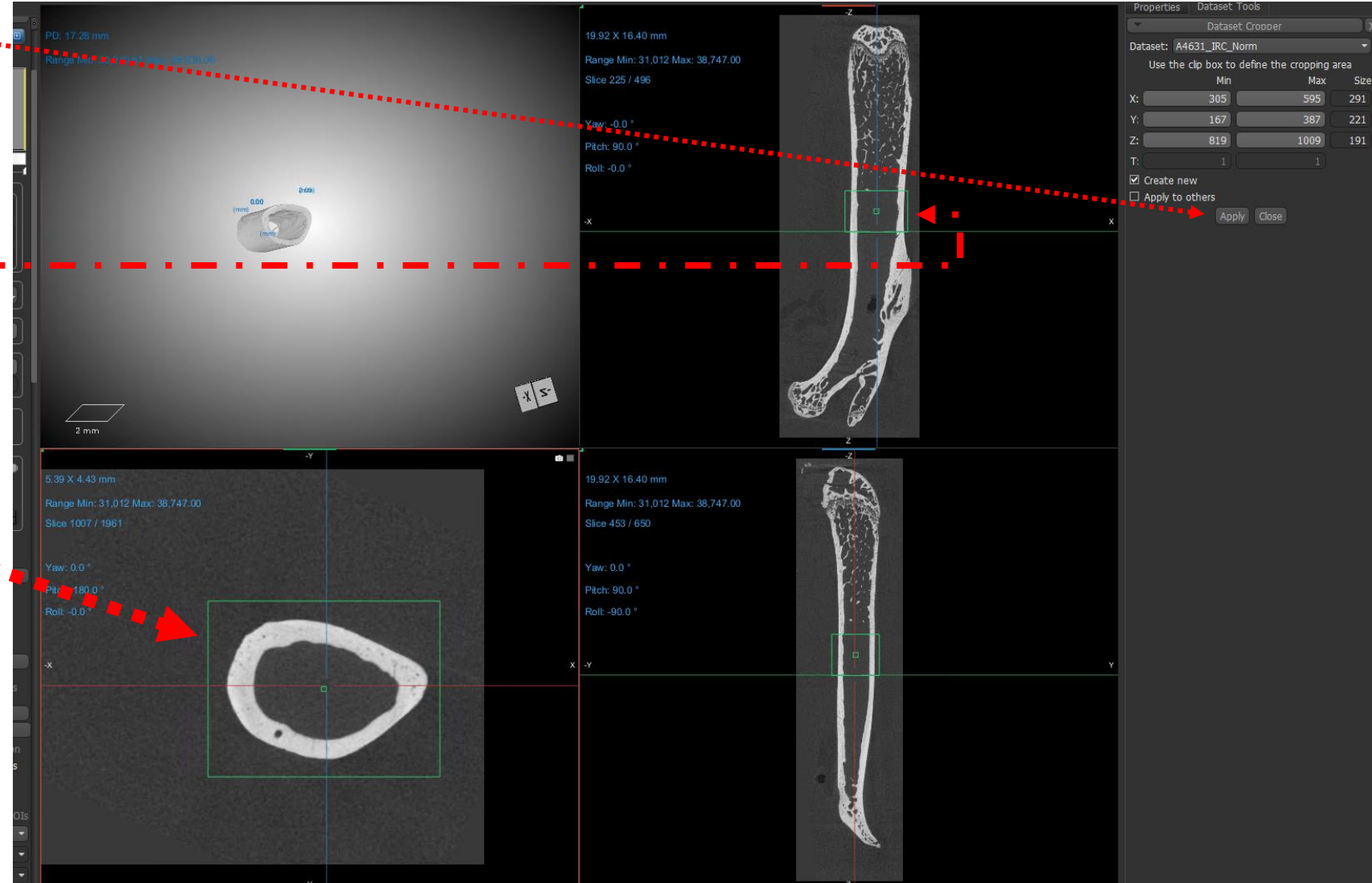
- From the Excel spreadsheet, use the values in columns P & Q for the min & max Z to define the cropped region's Z limits. See next slide for adjusting X, Y limits.



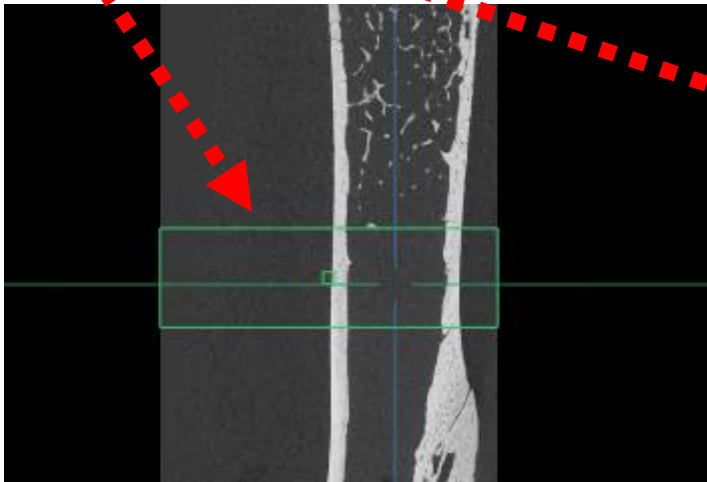
	M	P	C	R
h for				
ROI		Cort ROI Z-	Cort Z	% Length fo
nt		Height (voxels; used)	min slice	Trab ROI Height
)	191	819	1009	0.10
)	1	0	0	0.10
)	1	0	0	0.10
)	1	0	0	0.10
)	1	0	0	0.10

Creating Cortical VOI and ROI's

PRIOR to hitting 'Apply' and then 'Close'; the X & Y limits should be adjusted (green box) so that it encompasses the cortical bone with ~2mm's of background at the widest part, this is easily done on the Z slice that is closest to the 3rd Trochanter.



PRE & POST ADJUSTING:



Creating Cortical VOI and ROI's

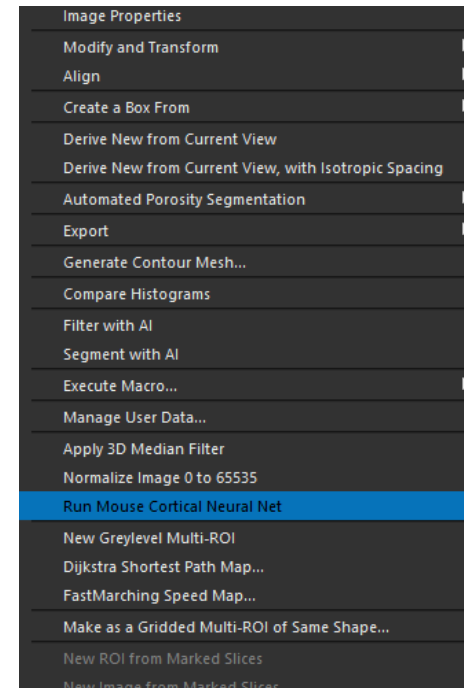
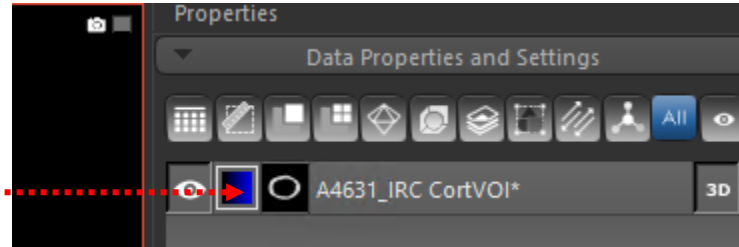
Running Cort-Trab neural network

- Rename new image to

A4631_IRC CortVOI

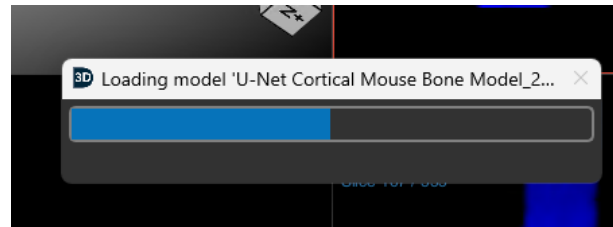
(NOTE: It's best practice to save your work at this point)

Right click on image and select



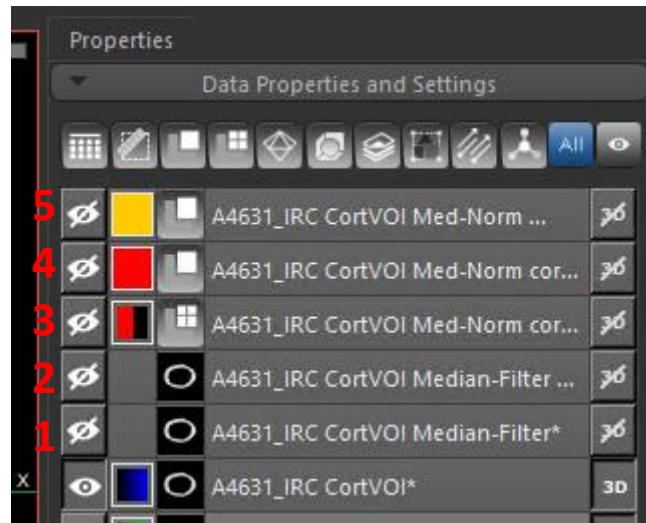
- Run Mouse Cortical Neural Net

- Status dialogue shows the model running



- When finished this will create:

1. Median filtered image
2. Normalized image
3. Multi ROI of cortical bone from the neural net
4. ROI of just cortical bone with minimal clean up
5. A copy of the cortical ROI with marrow region filled in



Creating Cortical VOI and ROI's

Check ROI's needed for analysis

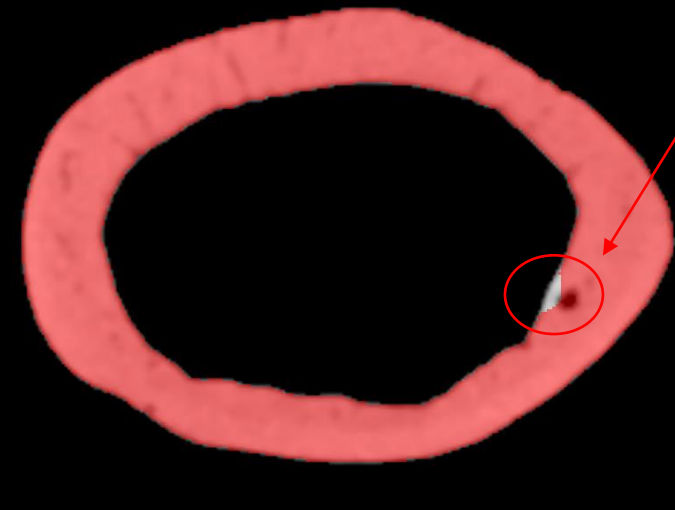
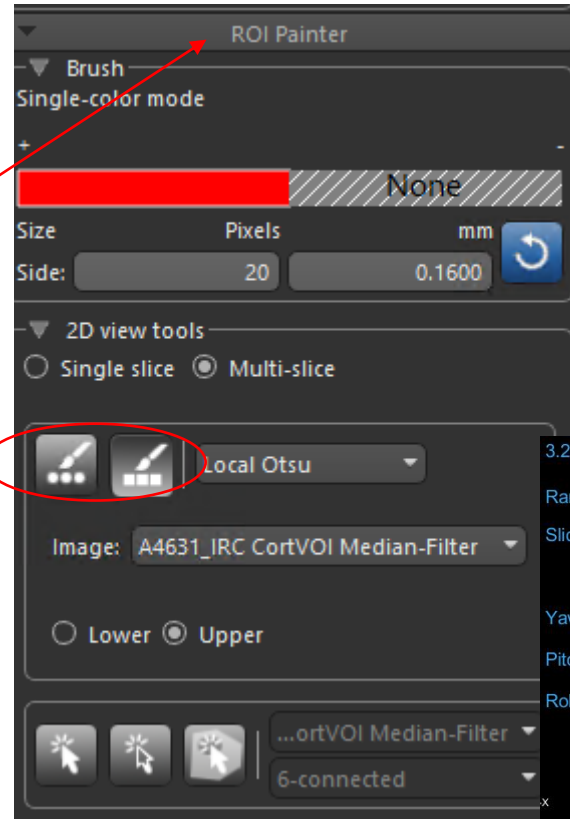
Clean up all needed slices:

- Go to 'Segment'
- Scroll down to 'ROI Painter'
- 2D view tools
- Choose either 'Single slice' or 'Multi-slice'
- Select either paintbrush.

Use left mouse & either 'Shift' or 'Ctrl' to increase/decrease brush size as well as to remove/add.

Double check all slices of your ROI and address as needed.

Note: The filled in cort-marrows ROI should not need any clean up. The only time it might is if there was a gap in the cortical roi which caused a slice(s) to not be filled in. If this occurred, then you would need to fill in those slices.



Areas
Needing
Addressing

Steps to Run 'Cortical Bone Analysis with BMD' Plugin

After the ROIs are created, run the cortical slice analysis plugin found under the menu 'UofM_ORL -> Cortical Bone Analysis with BMD'

As the analysis needs to run along the long axis of the bone, make sure the 2D view that is perpendicular to this axis is the selected 2D view. For example, we used the X-Y slice (or Z-slice) which you can see is highlighted by the red box in the Dragonfly display window.

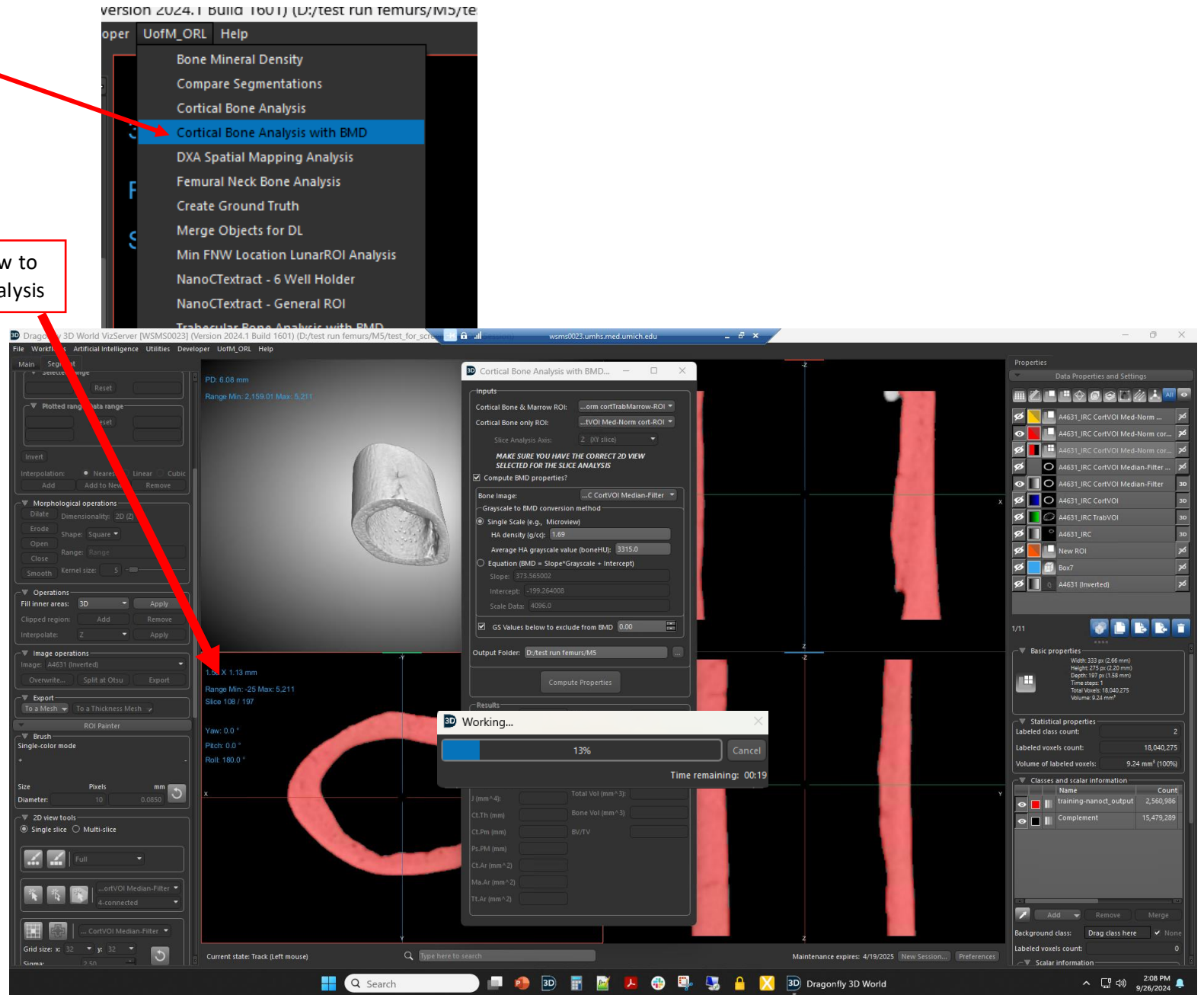
2D View to run analysis

In the dialog window that opens, select the:

- The Cortical bone and Marrow filled in ROI
- The Cortical Bone only ROI
- Click box to do the BMD calculation (*optional*)
 - Select calibration method
 - Enter appropriate values
 - Check box to exclude grayscale values below set value (e.g., 0.0) from BMD calculation
- The folder to save the results to
 - If no output folder is specified, the default location will be used, C:\ORSTemp.

Once all the inputs are entered and the 2D View selected to run the analysis along, click the 'Compute Properties' button.

A status dialog will be displayed giving estimated time to run analysis. Analysis should take at most two to three minutes to run.



Steps to Run 'Cortical Bone Analysis with BMD' Plugin

NOTES on inputs used for the BMD analysis within the bone analysis plugin:

Image:

- *Bone image* – this will be the bone image (can be in HU or xray attenuation grayscale value) to calculate the BMD/TMD on

ROI's:

- *BoneMarrow ROI* – this ROI will contain all the bone voxels and marrow/void/gaps filled in voxels in region want to determine bmd/tmd
- *Bone only ROI* – this ROI will contain just the bone voxels in the '*BoneMarrow ROI*'
 - *NOTE: For the cortical bone analysis, only the Bone Only ROI is used; thus the BMD and TMD results will be the same.*

Conversion method/parameters:

- *Single Scale* – this method uses a single HA phantom for conversion; this method is generally used for nanoCT scans or those done in old GE Microview scanner
 - *HA Density* – mineral density of phantom in g/cc (1.69 g/cc is value for HA phantom used in nanoCT scans)
 - *Average HA grayscale value* – this is the average grayscale value found for an ROI in the HA phantom (usually scanned with specimen); and is usually in HU values but doesn't have to be
- *Equation* – this method is used when have equation to convert for grayscale to density measures, usually when have multiple HA phantoms at different densities scanned (such as used in the Skyscan micro-ct scanner); the values for the slope and intercept need to be determined such that BMD/TMD will be found in mg/cc.
 - *Slope* – slope for equation to convert from grayscale to density values
 - *Intercept* – intercept for equation to convert from grayscale to density values
 - **Scale – for scanco images you will enter scale value (usually 4096) for most other images scale value of 1 is fine**

Grayscale values to exclude from BMD calculation

- Set the grayscale value to exclude with summing values to determine BMD/TMD. Values below 0.0 are using noise or air voxels. The voxel count will be the same for the selected ROI, only a value of 0.0 will be used for the summation.

'Cortical Bone Analysis with BMD' Plugin Results

When the analysis is finished, various cortical and BMD properties will be displayed; and 3 files will be created for the cortical analysis and 2 files for the BMD analysis in the designated 'Output' folder as shown in next slide.

3D Cortical Bone Analysis with BMD...

Inputs

Cortical Bone & Marrow ROI: ...orm cortTrabMarrow-ROI
 Cortical Bone only ROI: ...tVOI Med-Norm cort-ROI
 Slice Analysis Axis: Z (XY slice)
 MAKE SURE YOU HAVE THE CORRECT 2D VIEW SELECTED FOR THE SLICE ANALYSIS
☒ Compute BMD properties?
 Bone Image: ...C CortVOI Median-Filter
 - Grayscale to BMD conversion method
☒ Single Scale (e.g., Microview)
 HA density (g/cc): 1.69
 Average HA grayscale value (boneHU): 3315.0
☐ Equation (BMD = Slope*Grayscale + Intercept)
 Slope: 373.565002
 Intercept: -199.264008
 Scale Data: 4096.0
☒ GS Values below to exclude from BMD 0.00
 Output Folder: D:\test run femurs\M5
 Compute Properties

Results

# Slices	BMC (mg)
197	2.8431 1.8051
box (mm^3): 0.1255	BMD (mg/cc): 2166.52 1375.55
lyy (mm^4): 0.2545	TMC (mg): 2.8431 1.8051
imax (mm^4): 0.2545	TMD (mg/cc): 2166.52 1375.55
imin (mm^4): 0.1255	Total Vol (mm^3): 1.3123
J (mm^4): 0.38	Bone Vol (mm^3): 1.3123
Ct.Th (mm): 0.2155	BV/TV: 1.0
Ct.Pm (mm): 8.4653	
Ps.PM (mm): 4.8652	
Ct.Ar (mm^2): 0.8327	
Ma.Ar (mm^2): 0.9347	
Tt.Ar (mm^2): 1.7674	

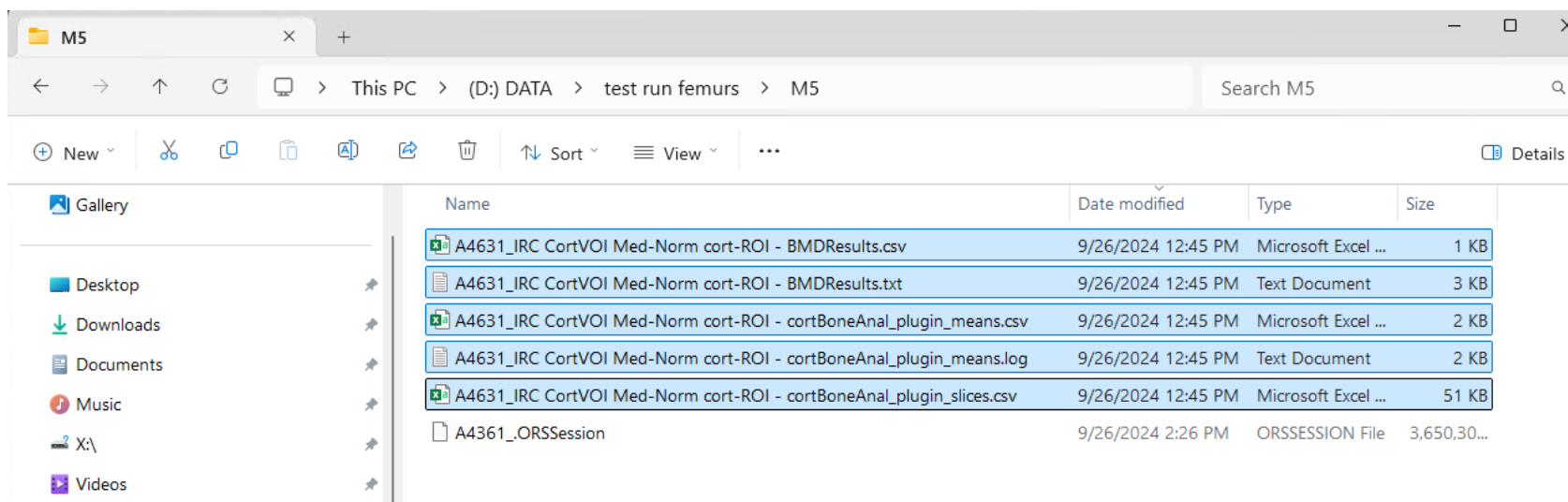
When using the 'Single Scale' conversion method:

- First set of results are for using ratio of HA/BoneHU value
- Second set of results are done using extra calibration factor found in Microview program

For the 'Equation' method, will only have one set of results

*confirm results table "Number of Slices" count matches Depth dimension; if they don't, rerun the plugin analysis ensuring the correct 2D view is selected

Steps to Run ‘Cortical Bone Analysis with BMD’ Plugin



The `<output_rootfilename>` - BMDResults.csv file will have the variable names in the first row and results for each in the second row separated by commas.

The `<output_rootfilename>` - BMDResults.txt file will contain the results written to file in a more descriptive format showing the inputs used for the analysis.

The `<output_rootfilename>` - cortBoneAnal_plugin_means.csv file contains the results averaged over all slices analyzed, as well as the standard deviation and variance for each property.

The `<output_rootfilename>` - cortBoneAnal_plugin_means.txt file contains the results (means, std, var) in a readable format and has the names of the ROI's used and time it took to run the analysis.

The `<output_rootfilename>` - cortBoneAnal_plugin_slices.csv file contains the results for the properties calculate on each slice analyzed.

Steps to Run 'Cortical Bone Analysis with BMD' Plugin

The following properties are calculated:

Ixx - Moment of Inertia XX (mm⁴)
Iyy - Moment of Inertia YY (mm⁴)
Imax - Maximum Moment of Inertia (mm⁴)
Imin - Minimum Moment of Inertia (mm⁴)
J - Polar Moment of Inertia (mm⁴)
Ct.Th - Cortical Thickness (mm)
Ct.Pm - Perimeter of all cortical bone (mm)
Es.Pm - Endosteal Perimeter (mm)
Ps.Pm - Periosteal Perimeter (mm)
Ct.Ar - Cortical Area (mm²)
Ma.Ar - Marrow Area (mm²)
Tt.Ar - Total Area (mm²)
2D Area (mm²)
2D Eccentricity
2D Equivalent Diameter (mm)
2D Filled Area (mm²)
2D Major Axis Length (mm)
2D Minor Axis Length (mm)
2D Orientation (degrees)
2D Perimeter (mm)
2D Roundness
2D Aspect Ratio
2D Area/2D Perimeter (mm²/mm)
2D Max Feret (mm)
2D Min Feret (mm)
2D Feret Angle (degrees)
Centroid X (mm)
Centroid Y (mm)
Centroid Z (mm)
Centroid_index X
Centroid_index Y
Centroid_index Z
Theta (degrees)

These calculations are made using the "All Bone & Void filled ROI"

These are the properties calculated as shown in the *.txt file:

```
capsule_ring_ROI - cortBoneAnal_ X +
File Edit View

Cortical Bone Analysis Plugin results
Plugin ver 1.0.0
Dragonfly (https://dragonfly.comet.tech/), ver 2024.1.0.1627

Results written in the folder: C:/ORSTemp/sliceAnalysis_propsOSU

Analysis done for the following ROI and Data files
All Bone & Void filled in ROI: capsule_ROI
Cortical Bone & Void filled in ROI: capsule_ring_ROI

Calculations for Slice analysis done along the Z axis

RESULTS:
NumberSlices = 10

Ixx (mm^4) = 0.190331 (stDev=0.000316; var=0.0)
Iyy (mm^4) = 0.689581 (stDev=0.000768; var=1e-06)
Imax (mm^4) = 0.695029 (stDev=0.000768; var=1e-06)
Imin (mm^4) = 0.184882 (stDev=0.000317; var=0.0)
J (mm^4) = 0.879911 (stDev=0.001075; var=1e-06)

Ct.Th (mm) = 0.151646 (stDev=2.4e-05; var=0.0)

Ct.Pm (mm) = 12.500709 (stDev=0.022668; var=0.000514)
Es.Pm (mm) = 6.71918 (stDev=0.014158; var=0.0002)
Ps.Pm (mm) = 5.78153 (stDev=0.009377; var=8.8e-05)

Ct.Ar (mm^2) = 0.911 (stDev=0.000289; var=0.0)
Ma.Ar (mm^2) = 2.020882 (stDev=0.002523; var=6e-06)
Tt.Ar (mm^2) = 2.931883 (stDev=0.002804; var=8e-06)

2D Area (mm^2) = 2.928728 (stDev=0.0; var=0.0)
2D Eccentricity = 0.888963 (stDev=0.0; var=0.0)
2D Equivalent Diameter (mm) = 1.931055 (stDev=0.0; var=0.0)
2D Filled Area (mm^2) = 2.928728 (stDev=0.0; var=0.0)
2D Major Axis Length (mm) = 2.871273 (stDev=0.0; var=0.0)
2D Minor Axis Length (mm) = 1.314981 (stDev=0.0; var=0.0)
2D Orientation (degrees) = -83.839598 (stDev=0.0; var=0.0)
2D Perimeter (mm) = 7.054303 (stDev=0.0; var=0.0)
2D Roundness = 0.452314 (stDev=0.0; var=0.0)
2D Aspect Ratio = 0.457978 (stDev=0.0; var=0.0)
2D Area/2D Perimeter (mm^2/mm) = 0.415169 (stDev=0.0; var=0.0)
2D Max Feret (mm) = 2.700903 (stDev=0.0; var=0.0)
2D Min Feret (mm) = 1.200373 (stDev=0.0; var=0.0)
2D Feret Angle (degrees) = 172.853935 (stDev=0.0; var=0.0)

Centroid X (mm) = 2.920801 (stDev=3.4e-05; var=0.0)
Centroid Y (mm) = 2.91042 (stDev=5.2e-05; var=0.0)
Centroid Z (mm) = 7.049233 (stDev=0.024221; var=0.000587)
Centroid_index X = 182.2 (stDev=0.421637; var=0.177778)
Centroid_index Y = 129.0 (stDev=0.0; var=0.0)
Centroid_index Z = 0.0 (stDev=0.0; var=0.0)
Theta (degrees) = 173.837961 (stDev=0.005036; var=2.5e-05)

Analysis started at: 04/06/2025 16:09:54
and finished at: 04/06/2025 16:09:56
```

Steps to Run 'Cortical Bone Analysis with BMD' Plugin

This screenshot of the *.txt file shows results from BMD analysis.

```
A5054_frc_trabVOI_croppedROI - BMDResults.txt - Notepad
File Edit Format View Help
BMD Plugin results
  Plugin version 1.0.0
  Dragonfly (www.theobjects.com), ver 2022.1.0.1259

Results are for input image: A5054_frc_trabVOI_croppedROI
  using BoneMarrow ROI: TrabMarrow_ROI
  and BoneOnly ROI: Trab_ROI

Results written folder: C:/ORSTemp/Test_BMD

Converted image grayscale values to BMD using BoneHU and Scaling value
  BoneHU = 3160.0
  HA Density = 1.69 (g/cc)
  NOTE: Set slope and intercept for equation to 1 and 0

Image voxel sizes and Voxel counts for Total and Bone volumes
  image spacing X (mm): 0.00799992
  image spacing Y (mm): 0.00799992
  image spacing Z (mm): 0.00799992

  number of voxels in boneMarrow ROI: 7625762
  number of voxels in bone only ROI: 805314
    totalVolume (mm^3): 3.9042730134669936
    boneVolume (mm^3): 0.4123083985006559
    boneVolume/totalVolume (bv_tv): 0.10560439730482017

Min, Max, Mean values of image for boneMarrow_ROI
  min = -578.0
  max = 4490.0
  mean = 395.4293844995409
  stdev = 771.888588103408
And from these, the BMD-BMC values
  BMD: 211.47963917855193
  BMC: 0.8256742481425575
values using Microview's scaling properties
  BMD_mv: 134.27080049620488
  BMC_mv: 0.5242298628739434

Min, Max, Mean values of image for boneOnly_ROI
  min = 1294.0
  max = 4490.0
  mean = 2432.8622934656523
  stdev = 724.4283445315023
And from these, the TMD-TMC values
  TMD: 1301.1193911256178
  TMC: 0.536462452413152
values using Microview's scaling properties
  TMD_mv: 826.0953293951408
  TMC_mv: 0.3406060422717823

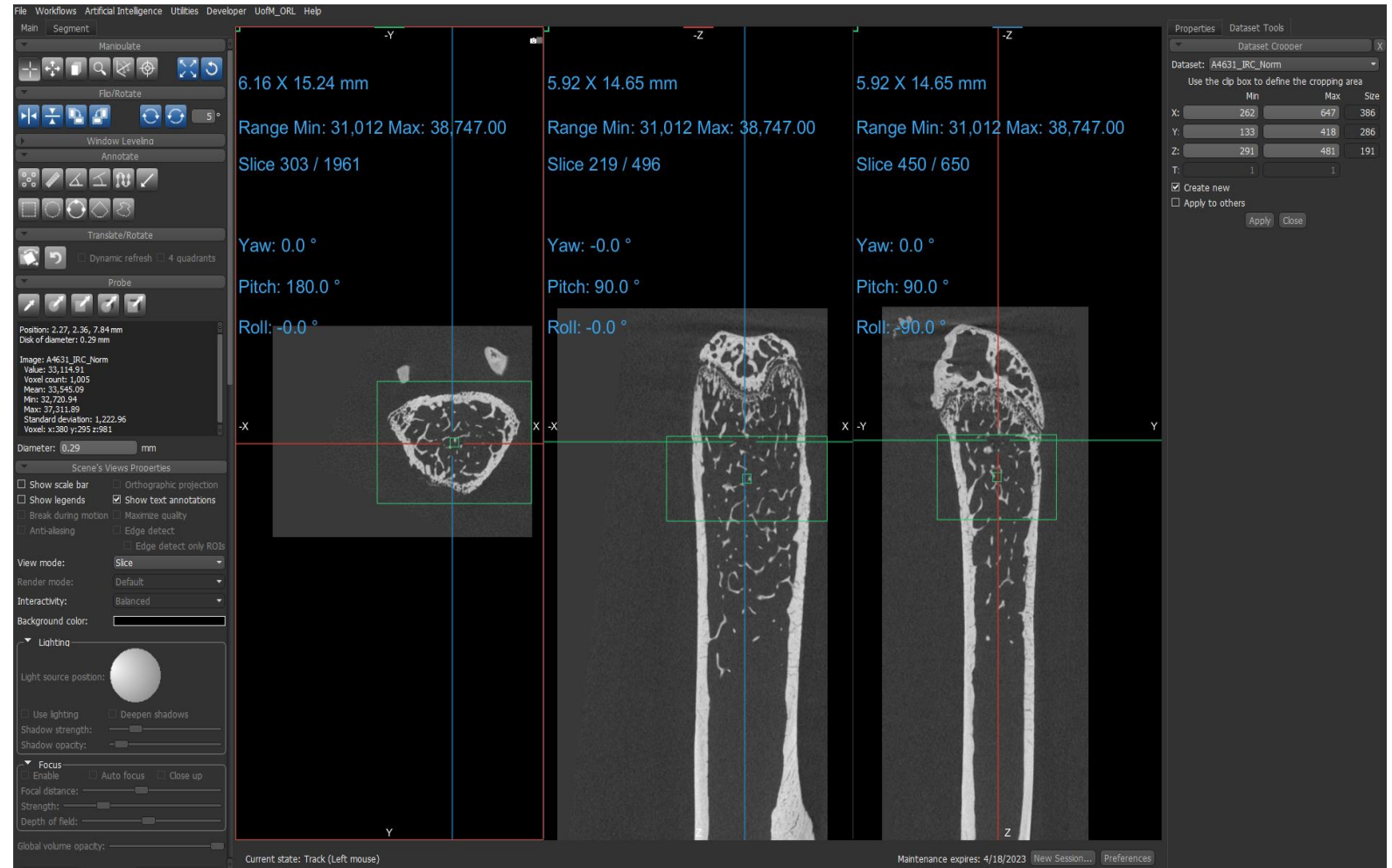
Analysis started at: 08/07/2022 10:16:40
and finished at: 08/07/2022 10:16:40
```

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

Creating Trabeculae VOI and ROI's

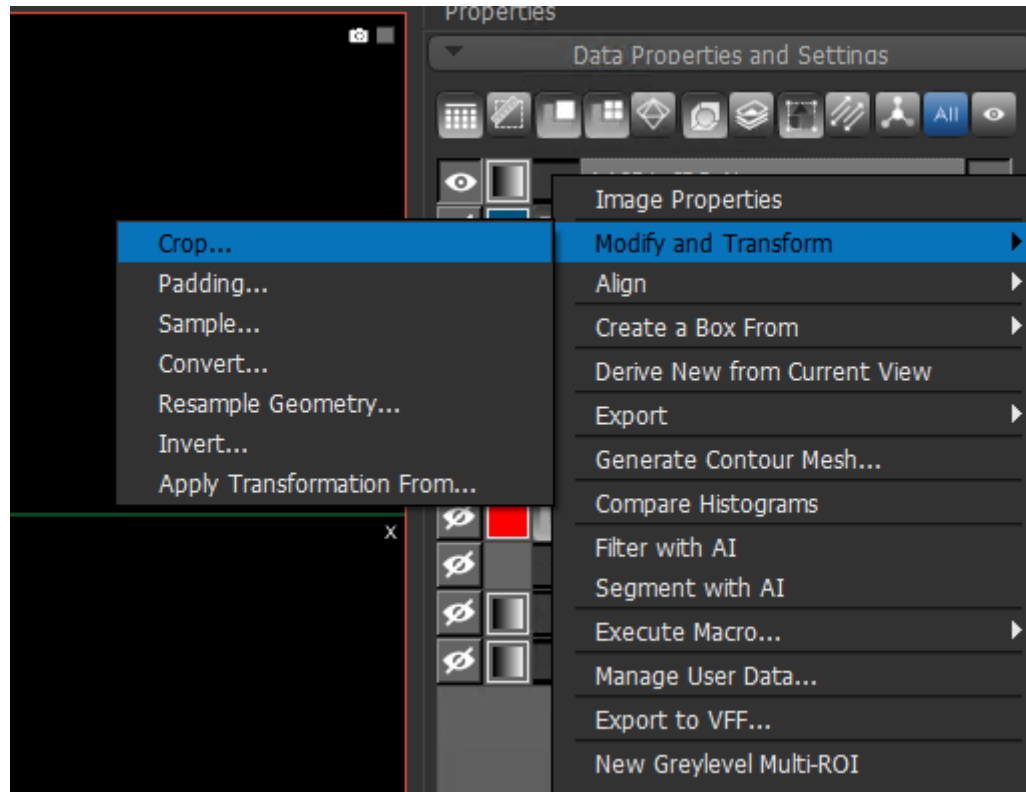
After aligning the femur so 3rd Trochanter is aligned with the X (or Y) axis and the long axis of the bone is aligned with the Z axis, crop the trabecular volume(s) to be analyzed and save as new image object(s).



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

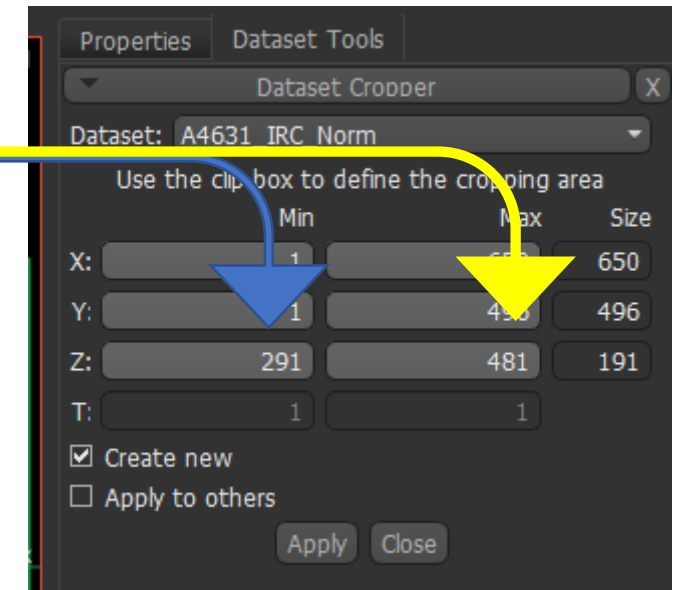
Creating Trabeculae VOI and ROI's

- Right click on your 'IRC' image and select menu option 'Modify and Transform -> Crop'.



- From the Excel spreadsheet, use the values in columns W & X for the min & max Z to define the cropped region's Z limits. See next slide for adjusting X, Y limits.

	T	W	X	
Trab ROI Z-Height (voxels; used)		Trab Z min slice	Trab Z max slice	B
191		291	481	3:
1		0	0	3:
1		0	0	3:



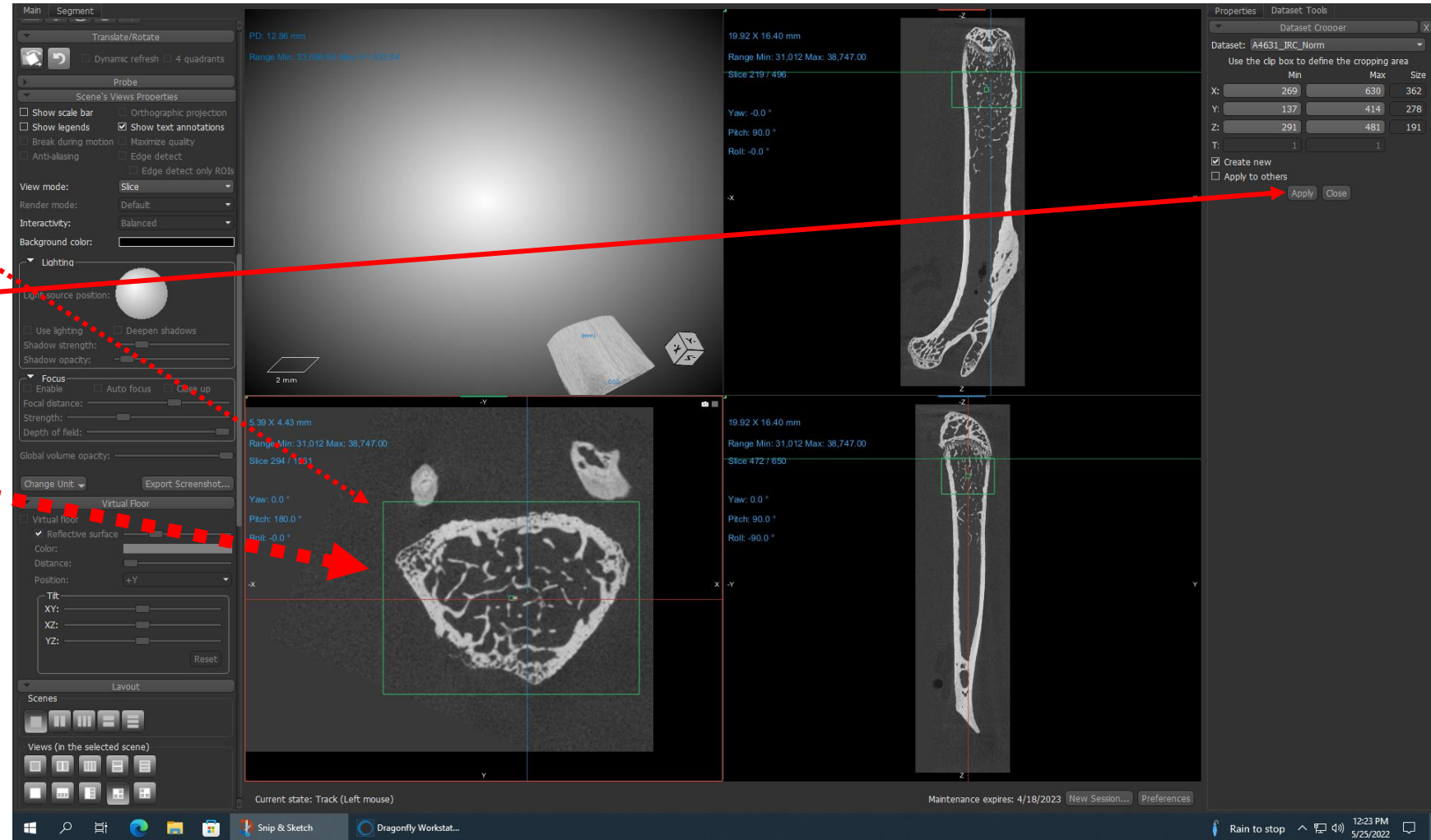
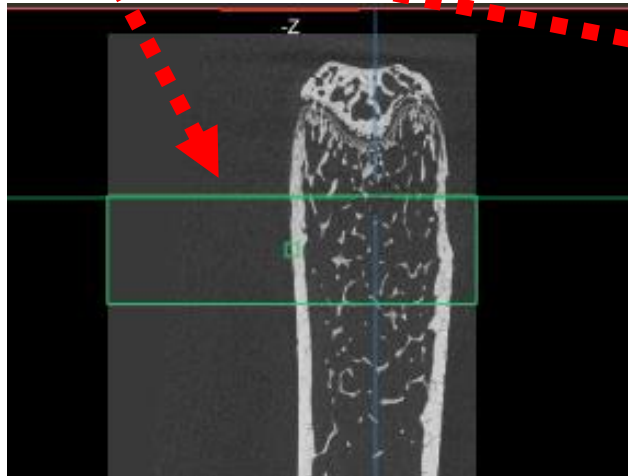
Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

Creating Trabeculae VOI and ROI's

In the 2D views adjust the X & Y limits so that it encompasses the cortical bone with ~2mm's of background at the widest part, this is best done on slice that is closest to the Growth Plate.

Hit 'Apply' and then 'Close'

PRE & POST ADJUSTING:



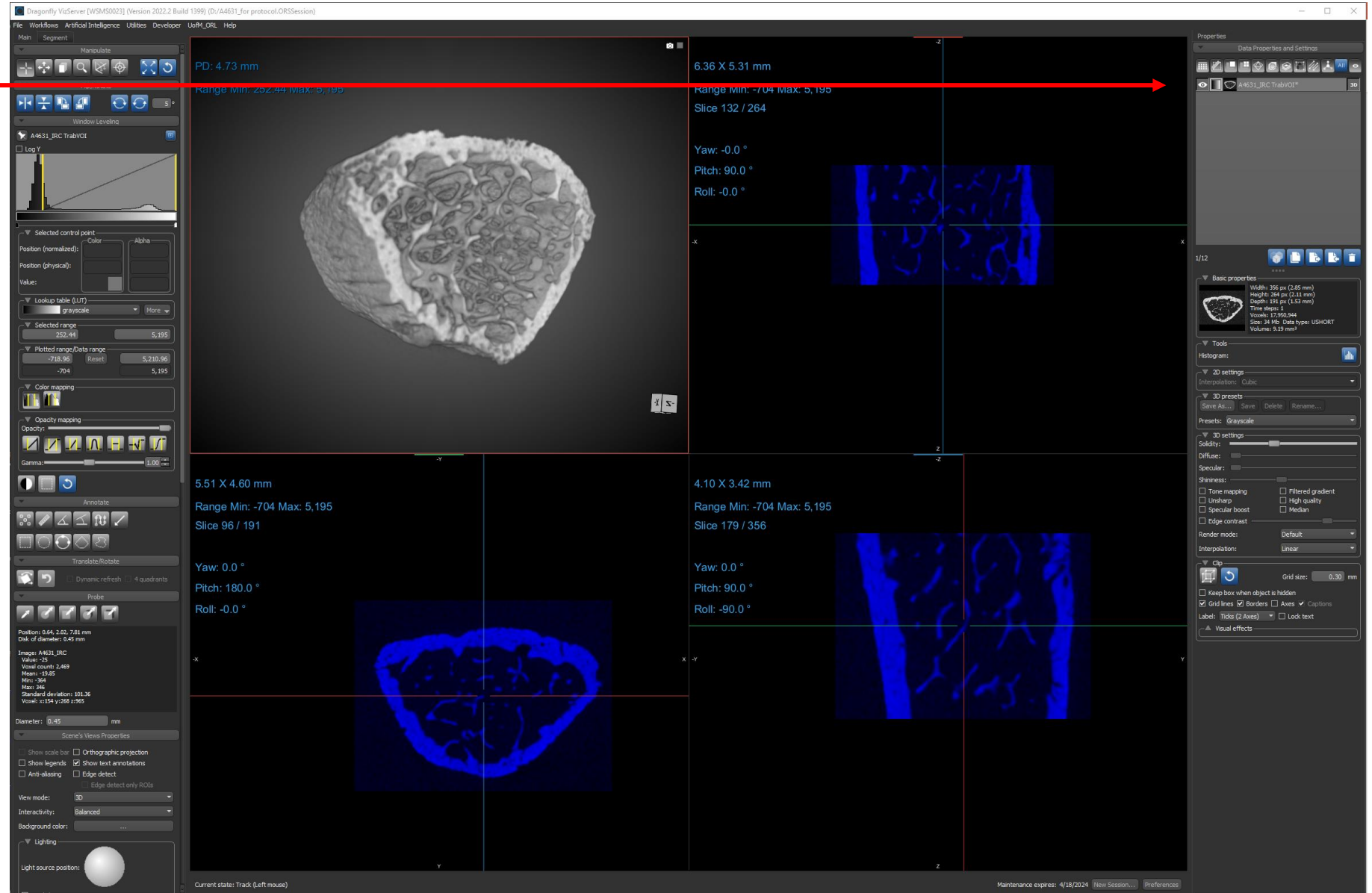
Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

Creating Trabeculae VOI and ROI's

The resulting Trabecular VOI is highlighted here with the darker background and blue highlighted bone.

Rename your cropped image to indicate that it's the Trabecular VOI, such as:

A4631_IRC(Cropped)
to
A4631_IRC_TrabVOI



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

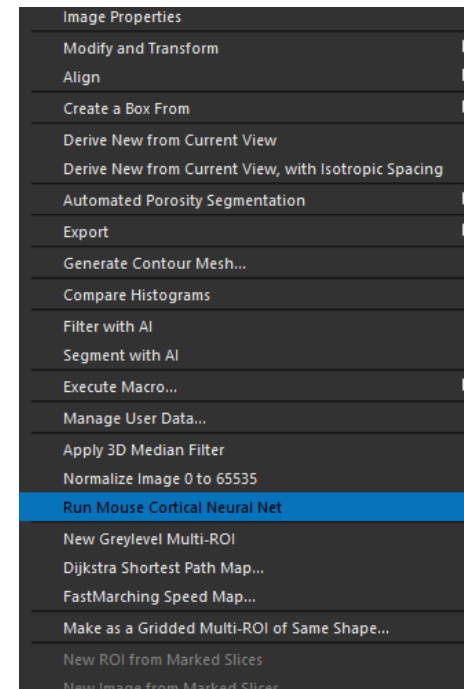
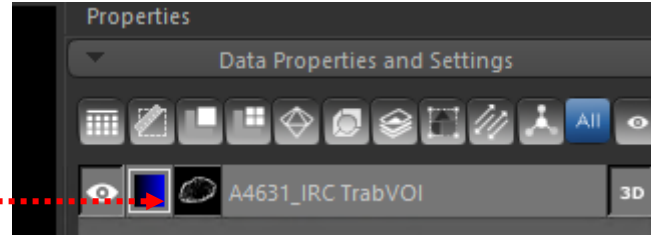
Creating Trabecular VOI and ROI's

- Rename new image to

A4631_IRC TrabVOI

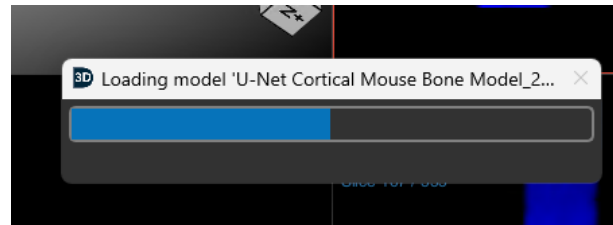
(NOTE: It's best practice to save your work at this point)

Right click on image and select



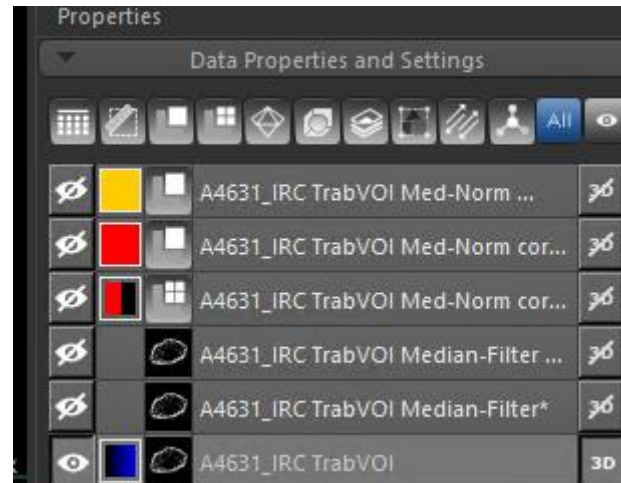
- Run Mouse Cortical Neural Net

- Status dialogue shows the model running



- When finished this will create:

1. Median filtered image
2. Normalized image
3. Multi ROI of cortical bone from the neural net
4. ROI of just cortical bone with minimal clean up
5. A copy of the cortical ROI with marrow region filled in



(NOTE: It's best practice to save your work at this point)

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

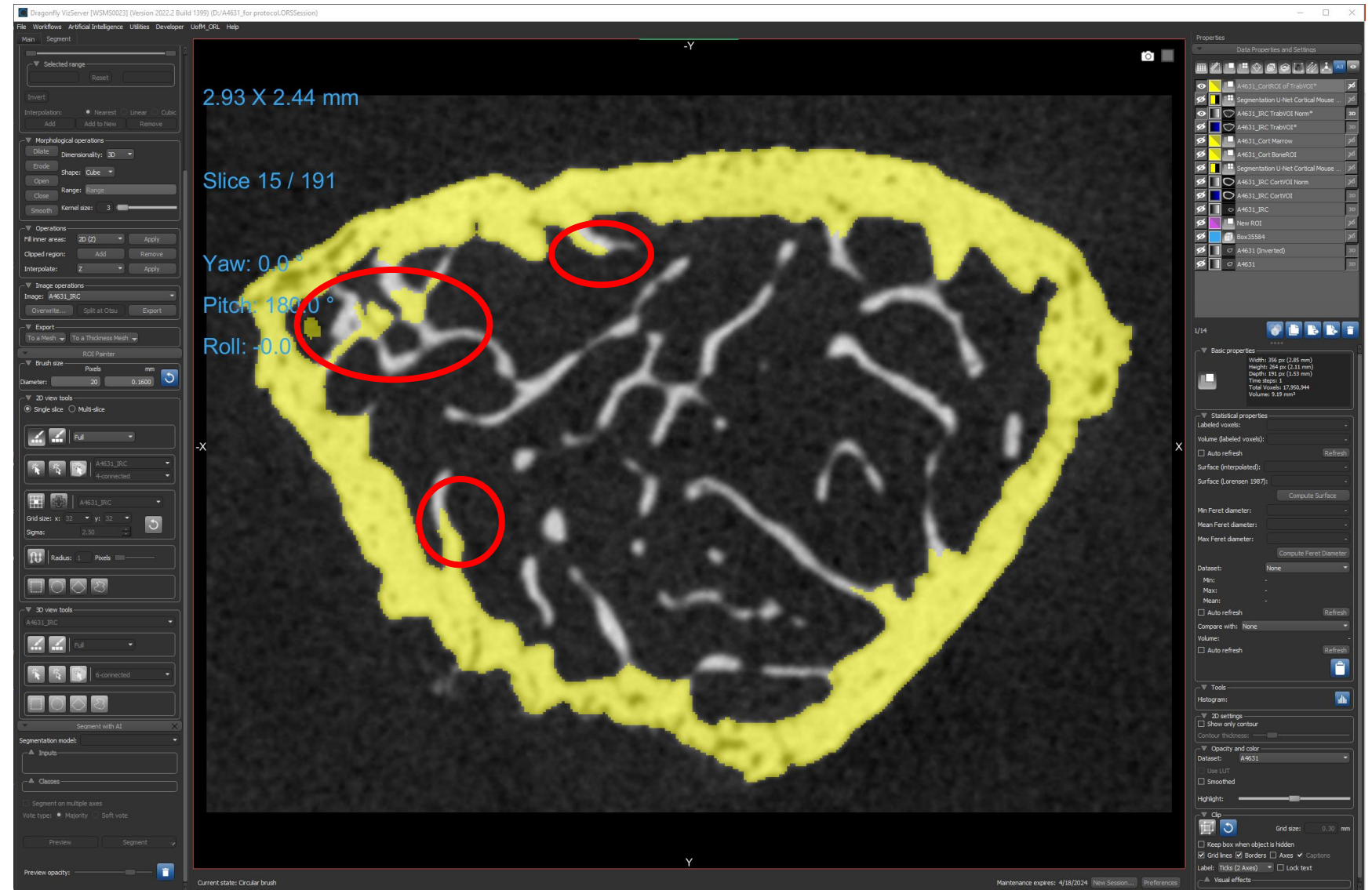
The menu option to run the neural net and extract the cortical ROI will do a minimum clean up, but you will need to check the ROI and clean up all needed slices:

- Go to 'Segment'
- Scroll down to 'ROI Painter'
- Ensure 'Single slice' is checked
- Select either paintbrush.

Use left mouse & either 'Shift' or 'Ctrl' to increase/decrease brush size as well as to remove/add.

Double check all slices of your ROI and address as needed.

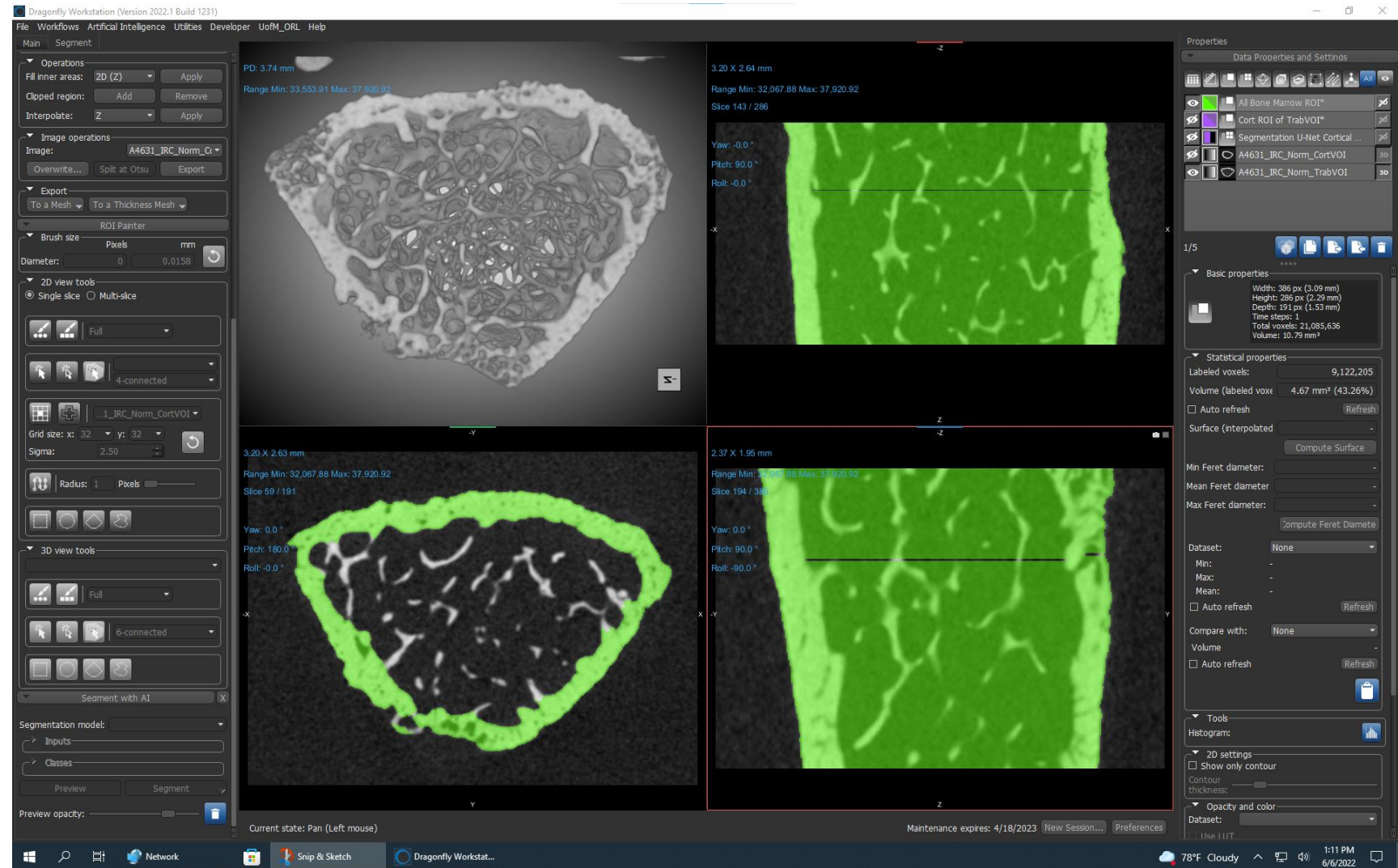
Examples
of areas
needing
attention



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

For the 'filled in' ROI, you will also need to look for slices that have gaps due to cortical not being connected. If there are gaps:

- Delete the "All Bone Marrow ROI"
- Using the paint brush tool, fill/connect cortical shell on the "Cort ROI of TrabVOI" ROI
- Duplicate "Cort ROI of TrabVOI" naming it "All Bone Marrow ROI"
- Re-run the 'Fill inner area' operation
- Repeat this until no gaps are present.

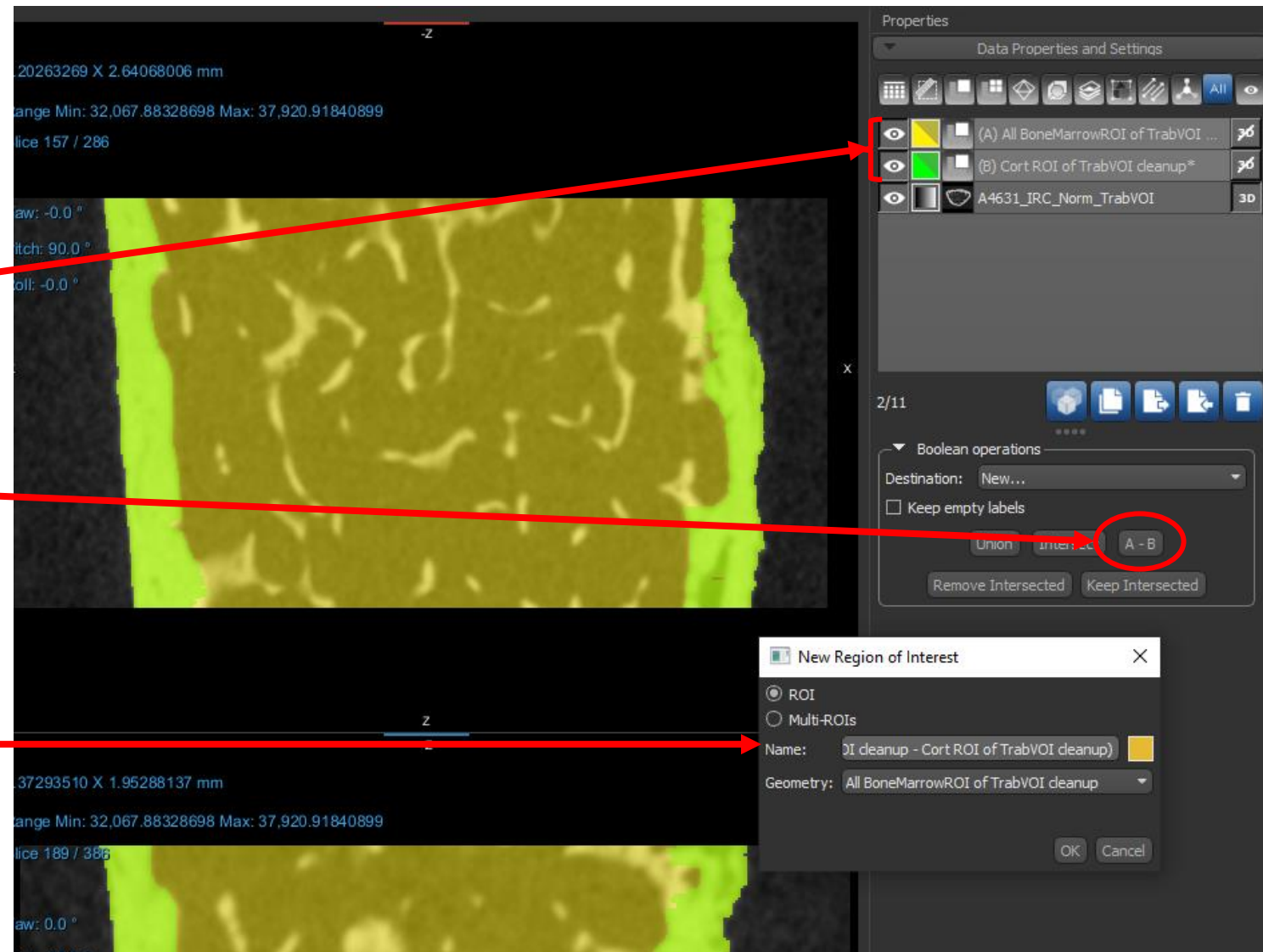


Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

Subtract the “Cort ROI of TrabVOI” from the “All BoneMarrow ROI of TrabVOI”

- Select the “All Bone Marrow ROI of TrabVOI” and while holding the <Left><Ctrl> key select the “Cort ROI of TrabVOI” (*NOTE: Need to select them in proper order for subtraction to work properly*)
- Then in the ‘Boolean operations’ menu that is displayed, select the “A-B” option
- In the Dialog window that opens change the “Name:” field to “TrabMarrow ROI of TrabVOI”, and hit the ‘OK’ button

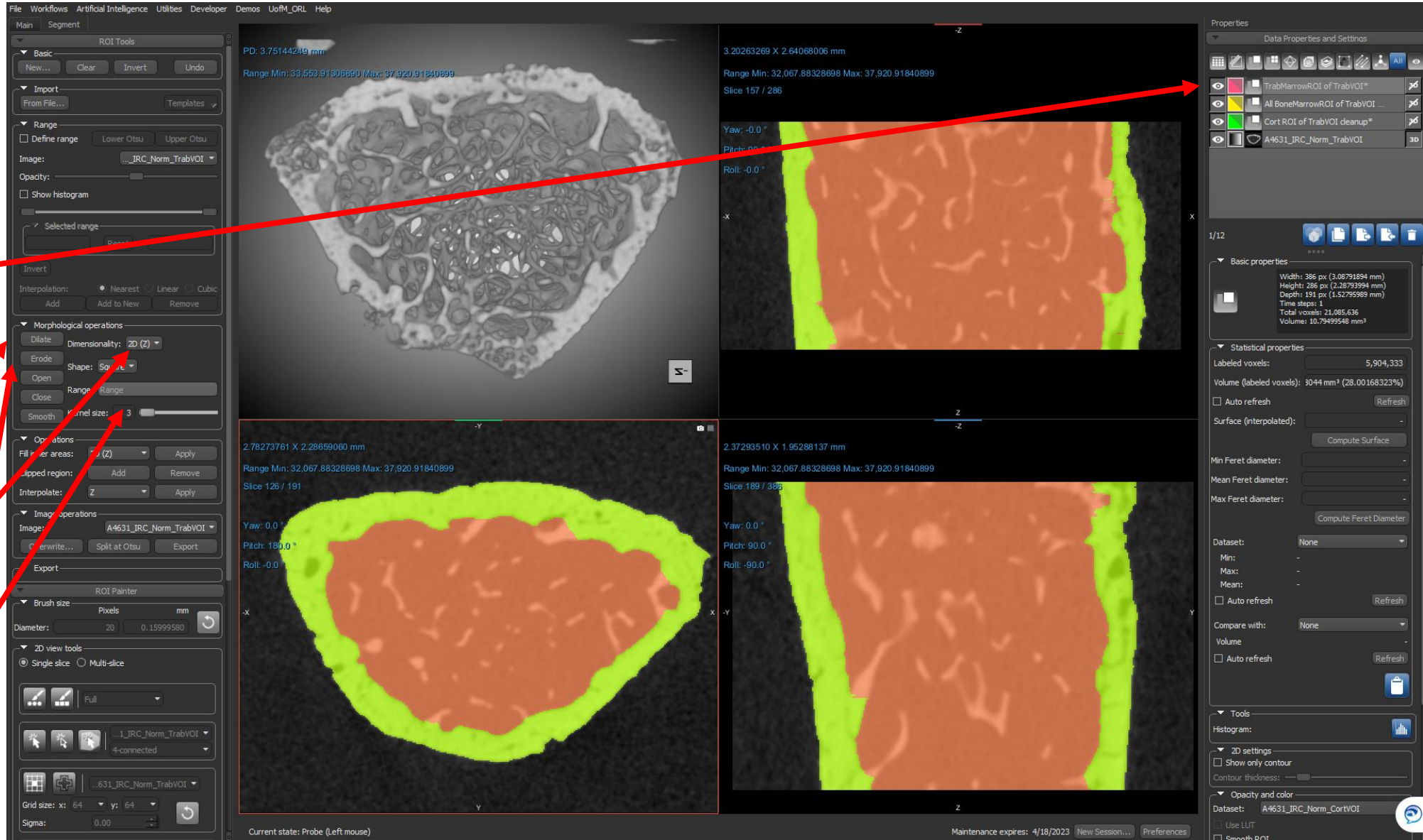
(NOTE: You can change the “Geometry:” to be that of the “...TrabVOI” image or the color of the ROI but it is not mandatory)



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

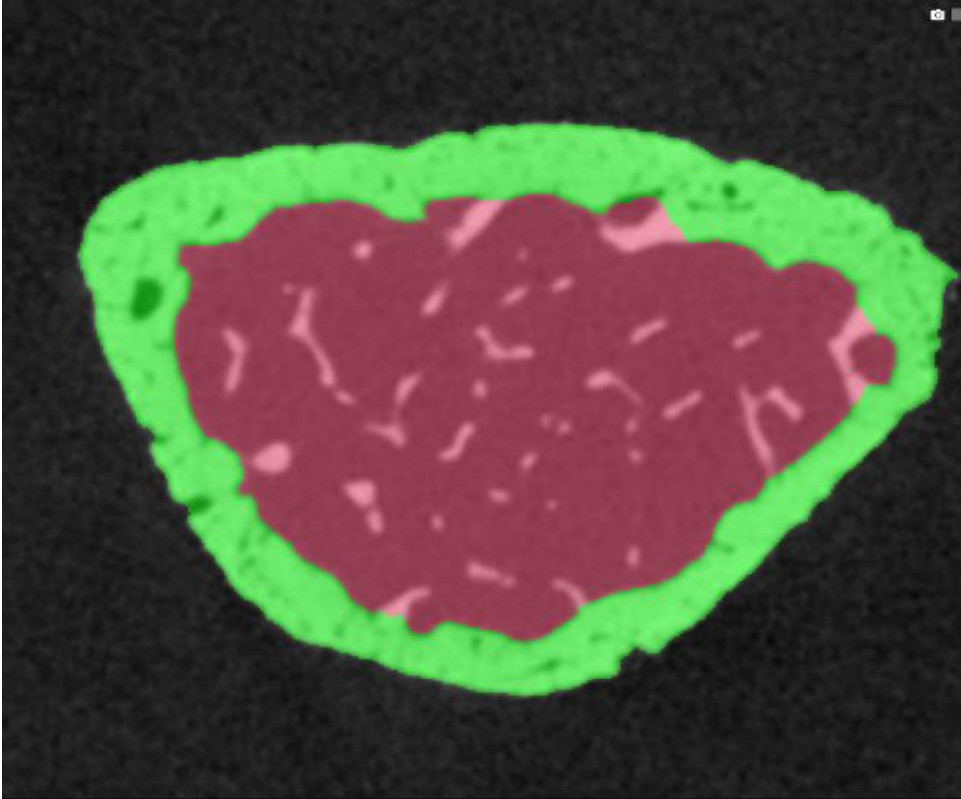
Do 2 Erosions on the "TrabMarrow ROI of TrabVOI"

- Select the "TrabMarrow ROI of TrabVOI"
- Under the 'Segment' tab, go to the "Morphological operations" menu
 - Set the "Dimensionality" to the '2D(Z)' direction
 - Set the "Kernel size" to 3
 - Click the "Erode" button

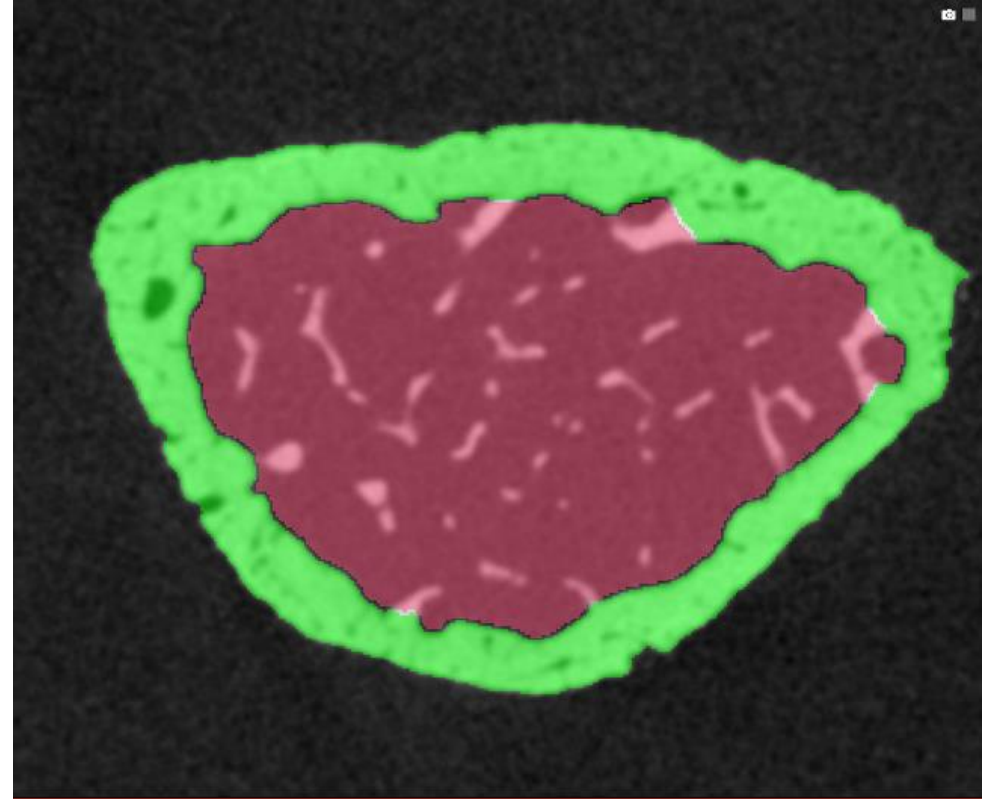


Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

This will
decrease the
TrabMarrowROI
by one to two
voxels around
the edge in the
X-Y direction



Before applying 'Erosion'



After applying 'Erosion'

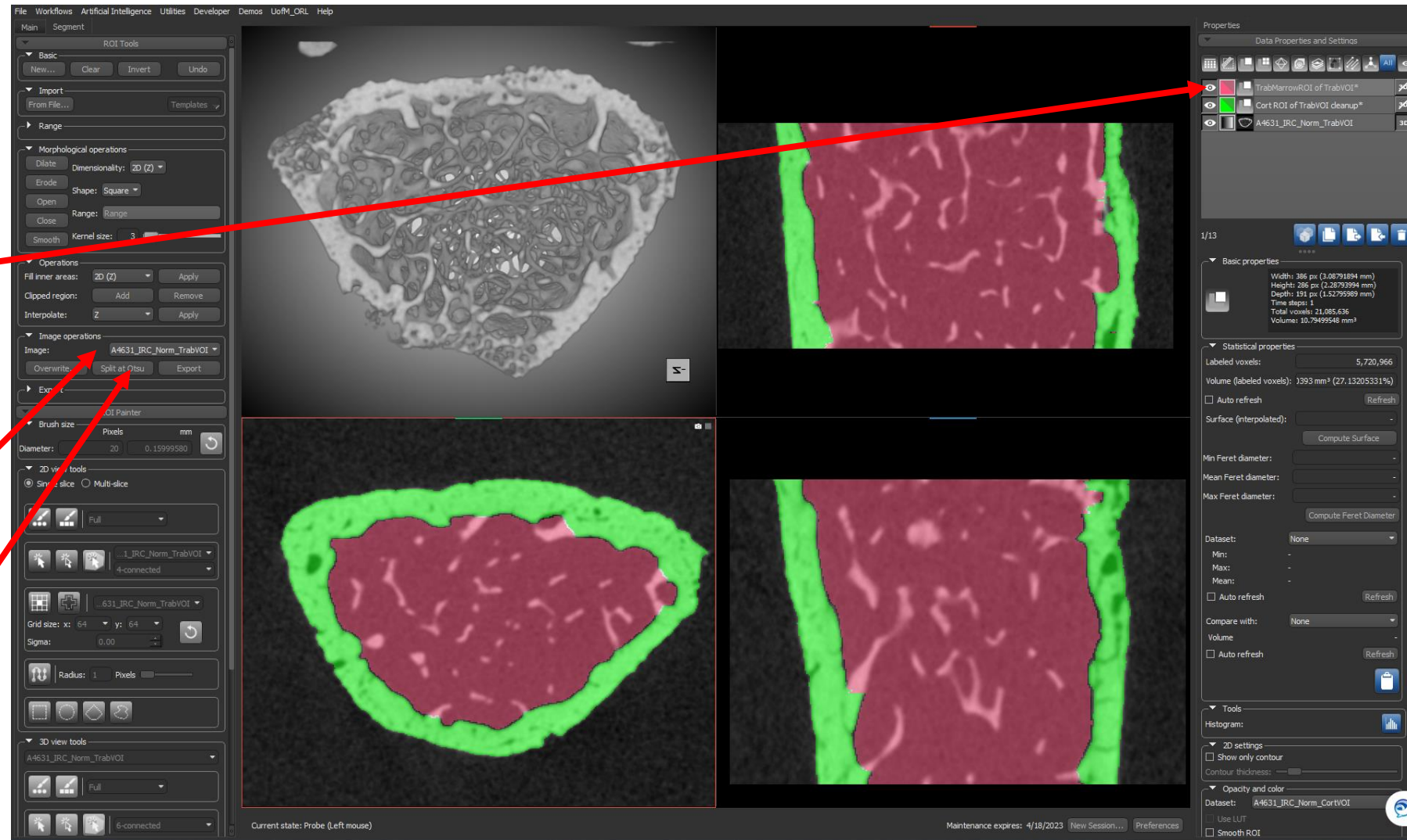
*NOTE: There will be an approximately
1-2 voxel gap between ROI's*

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

The “TrabMarrowROI of TrabVOI” needs to be split into ROI's of just the trabecular bone and marrow voxels. This is done by using the ‘Split at Otsu’ operation on the “TrabMarrow ROI of TrabVOI”

- Select the “TrabMarrowROI of TrabVOI”
- Under the ‘Segment’ tab, go to the “Image operations” menu
 - Make sure the “Image” is set to the Original (non-normalized) ‘specimenID_TrabVOI’ image
 - Click the “Split at Otsu” button

NOTE: This button will only be active if the correct image is select to do the split at otsu on



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

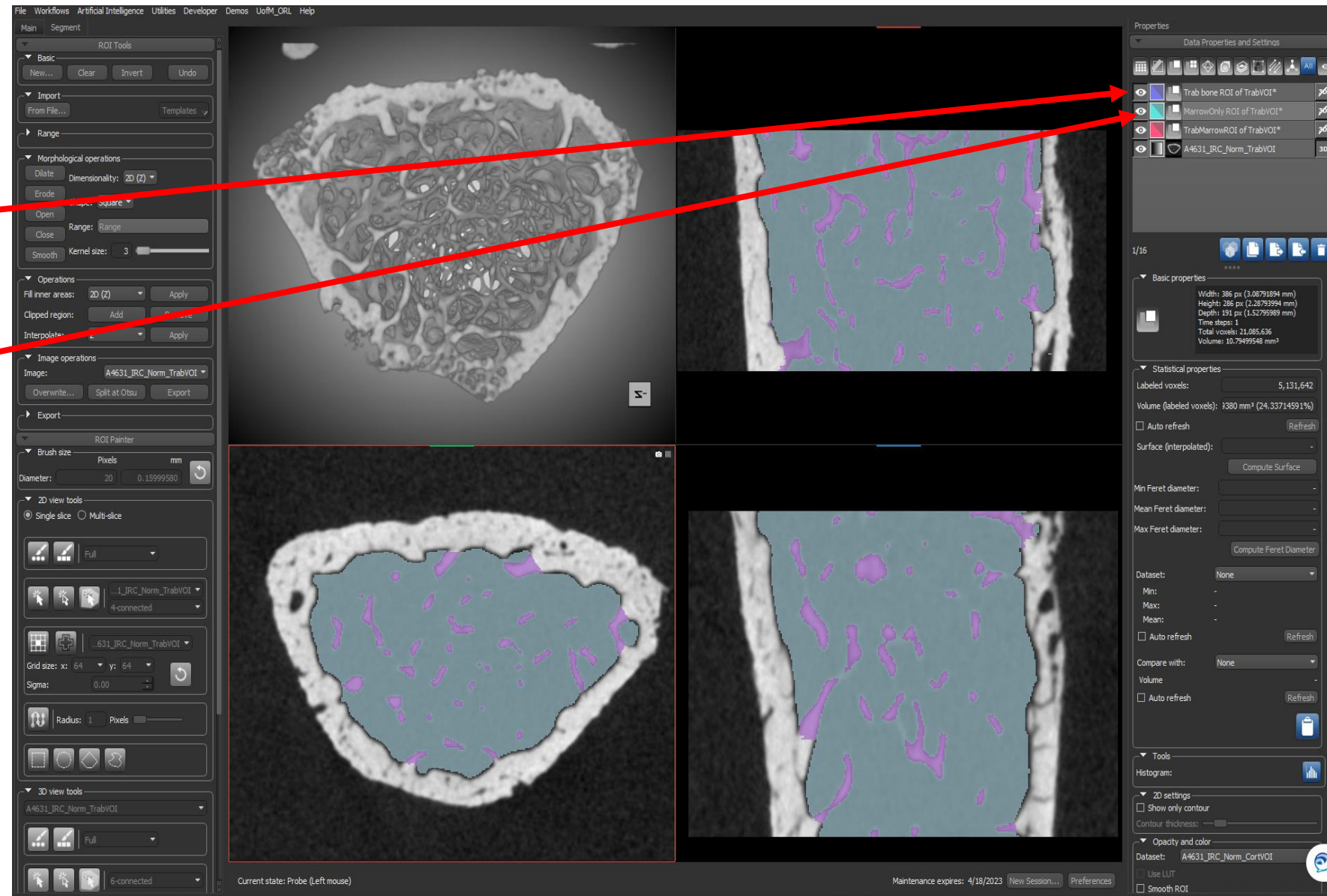
The two resulting ROI's will be named

TrabMarrowROI of TrabVOI Foreground
TrabMarrowROI of TrabVOI Background

Rename the *Foreground ROI* as "Trab bone ROI of TrabVOI"

Rename the *Background ROI* as "MarrowOnly ROI of TrabVOI"

NOTE: This is probably a good time to Save Session file



Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

To run the “*Trabecular Bone Analysis with BMD*” Plugin, go to the menu “UofM_ORL-> Trabecular Bone Analysis with BMD”

In the dialog window that opens, select the:

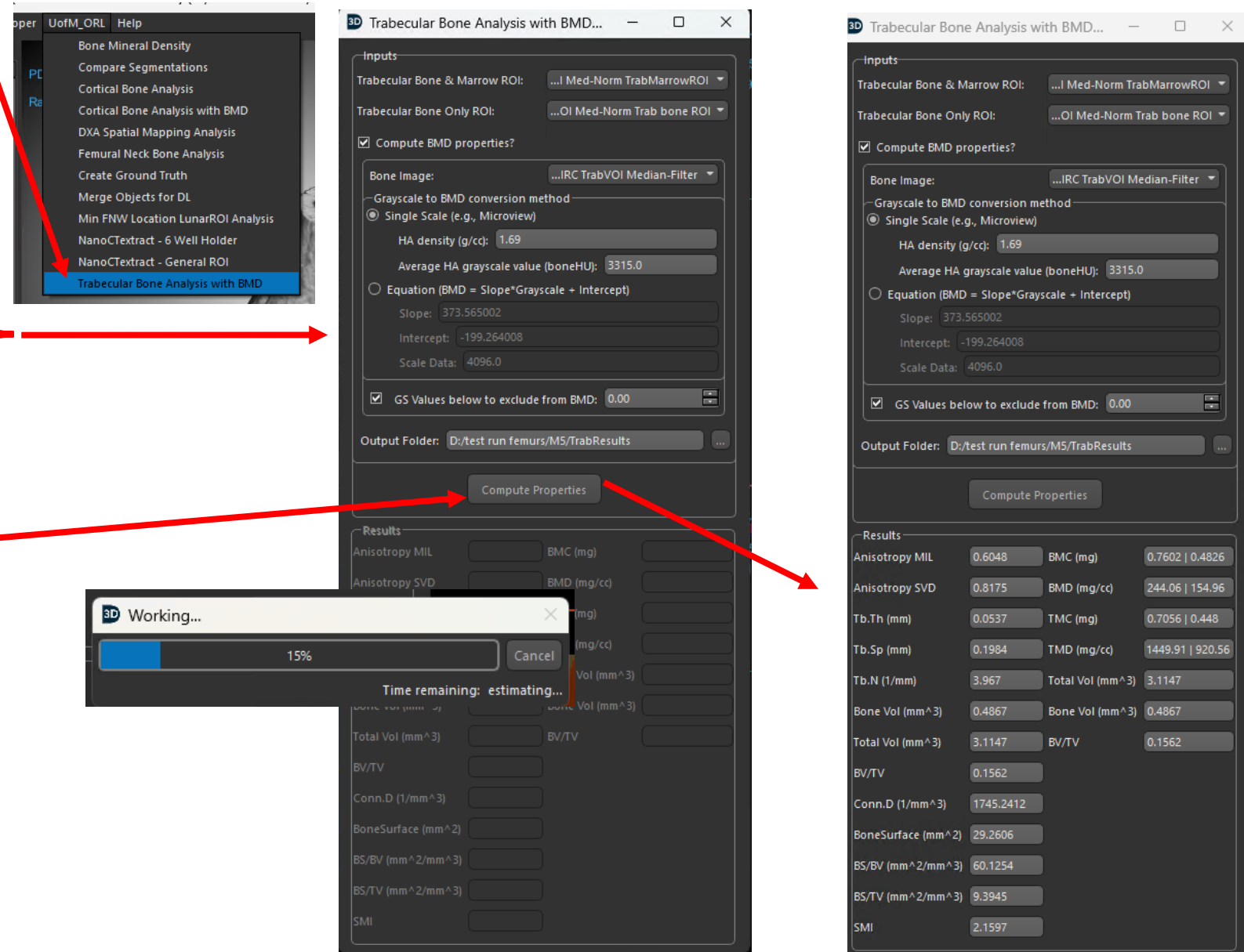
- The Trabecular bone and Marrow filled in ROI
- The Trabecular Bone only ROI
- Click box to do the BMD calculation (*optional*)
 - Select calibration method
 - Enter appropriate values
 - Check box to exclude grayscale values below set value (e.g., 0.0) from BMD calculation
- The folder to save the results to
 - If no output folder is specified, the default location will be used, C:\ORSTemp.

Once all the inputs are entered, click the ‘Compute Properties’ button.

A status dialog will be displayed giving estimated time to run analysis. Analysis should take at most one to two minutes to run.

When done, close the “Plugin window” (click X in upper right corner)

Once the analysis is finished, 4 files will be created in the designated ‘Output’ folder (as shown in the next slide).



Steps to Run ‘Trabecular Bone Analysis with BMD’ Plugin

This PC > (D:) DATA > test run femurs > M5 > TrabResults

Search TrabResult

Sort

View

Name	Date modified	Type	Size
 A4631_IRC TrabVOI Med-Norm TrabMarrowROI - BMDResults.csv	10/8/2024 1:32 PM	Microsoft Excel ...	1 KB
 A4631_IRC TrabVOI Med-Norm TrabMarrowROI - BMDResults.txt	10/8/2024 1:32 PM	Text Document	3 KB
 A4631_IRC TrabVOI Med-Norm TrabMarrowROI - trabBoneResultsGlobal.csv	10/8/2024 1:32 PM	Microsoft Excel ...	2 KB
 A4631_IRC TrabVOI Med-Norm TrabMarrowROI - trabBoneResultsGlobal.log	10/8/2024 1:32 PM	Text Document	2 KB

The <output_rootfilename> - BMDResults.csv file contains the results for properties calculated in CSV file with first line being header line with variable names.

The <output_rootfilename> - BMDResults.txt file contains the results in a readable format and has the names of the ROI’s used and time it took to run the analysis.

The <output_rootfilename> - trabBoneResultsGlobal.csv file contains the results for properties calculated in CSV file with first line being header line with variable names.

The <output_rootfilename> - trabBoneResultsGlobal.log file contains the results in a readable format and has the names of the ROI’s used and time it took to run the analysis.

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

NOTES on inputs used for the BMD analysis within the bone analysis plugin:

Image:

- *Bone image* – this will be the bone image (can be in HU or xray attenuation grayscale value) to calculate the BMD/TMD on

ROI's:

- *BoneMarrow ROI* – this ROI will contain all the bone voxels and marrow/void/gaps filled in voxels in region want to determine bmd/tmd
- *Bone only ROI* – this ROI will contain just the bone voxels in the '*BoneMarrow ROI*'

Conversion method/parameters:

- *Single Scale* – this method uses a single HA phantom for conversion; this method is generally used for nanoCT scans or those done in old GE Microview scanner
 - *HA Density* – mineral density of phantom in g/cc (1.69 g/cc is value for HA phantom used in nanoCT scans)
 - *Average HA grayscale value* – this is the average grayscale value found for an ROI in the HA phantom (usually scanned with specimen); and is usually in HU values but doesn't have to be
- *Equation* – this method is used when have equation to convert for grayscale to density measures, usually when have multiple HA phantoms at different densities scanned (such as used in the Skyscan micro-ct scanner); the values for the slope and intercept need to be determined such that BMD/TMD will be found in mg/cc.
 - *Slope* – slope for equation to convert from grayscale to density values
 - *Intercept* – intercept for equation to convert from grayscale to density values
 - **Scale – for scanco images you will enter scale value (usually 4096) for most other images scale value of 1 is fine**

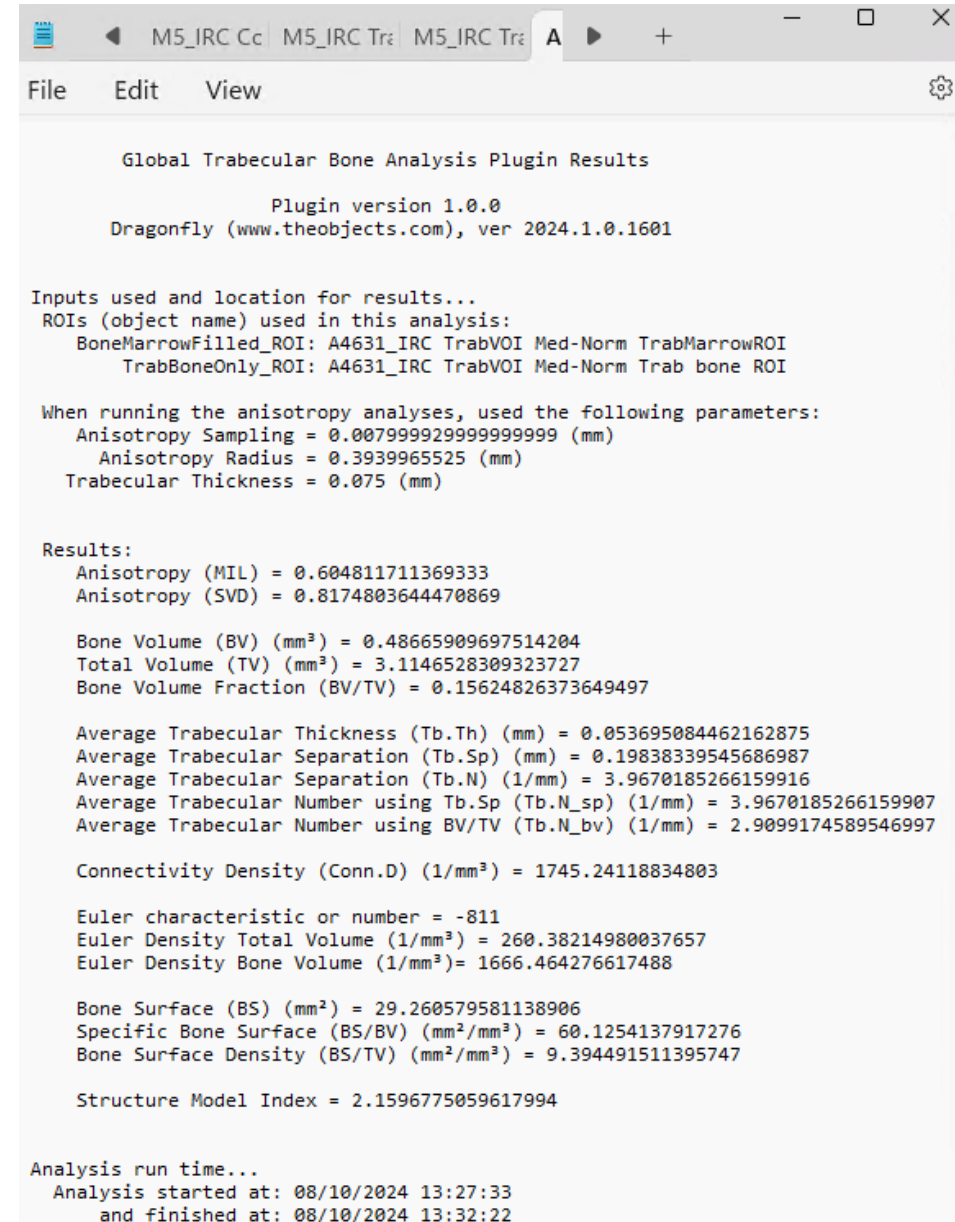
Grayscale values to exclude from BMD calculation

- Set the grayscale value to exclude with summing values to determine BMD/TMD. Values below 0.0 are using noise or air voxels. The voxel count will be the same for the selected ROI, only a value of 0.0 will be used for the summation.

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

The following trabecular bone properties are calculated:

Anisotropy-MIL	- Measure of Anisotropy using MIL
Anisotropy-SVD	- Measure of Anisotropy using SVD
BV	- Trabecular Bone Volume (mm^3)
TV	- Total Volume in ROI (mm^3)
BV/TV	- Trabecular Bone Volume Fraction
Tb.Th	- Trabecular Thickness (mm)
Tb.Sp	- Trabecular Separation (mm)
Tb.N	- Trabecular Number (1/mm)
Tb.N_sp	- Trabecular Number based on using Tb&Sp(1/mm)
Tb.N_bv	- Trabecular Number based on using BV/TV(1/mm)
Conn.D	- Connectivity Density (1/ mm^3)
Euler #	- Euler number
Euler Density_TV	- Euler Total density (mm^{-3}); = -Euler # / TV
Euler Density_BV	- Euler Bone density (mm^{-3}); = -Euler # / BV
BS	- Bone Surface (mm^2)
BS/BV	- Specific Bone Surface (mm^2/mm^3)
BS/TV	- Bone Surface Density (mm^2/mm^3)
SMI	- Structure Model Index



```
Global Trabecular Bone Analysis Plugin Results

Plugin version 1.0.0
Dragonfly (www.theobjects.com), ver 2024.1.0.1601

Inputs used and location for results...
ROIs (object name) used in this analysis:
  BoneMarrowFilled_ROI: A4631_IRC TrabVOI Med-Norm TrabMarrowROI
  TrabBoneOnly_ROI: A4631_IRC TrabVOI Med-Norm Trab bone ROI

When running the anisotropy analyses, used the following parameters:
  Anisotropy Sampling = 0.007999929999999999 (mm)
  Anisotropy Radius = 0.3939965525 (mm)
  Trabecular Thickness = 0.075 (mm)

Results:
  Anisotropy (MIL) = 0.604811711369333
  Anisotropy (SVD) = 0.8174803644470869

  Bone Volume (BV) ( $\text{mm}^3$ ) = 0.48665909697514204
  Total Volume (TV) ( $\text{mm}^3$ ) = 3.1146528309323727
  Bone Volume Fraction (BV/TV) = 0.15624826373649497

  Average Trabecular Thickness (Tb.Th) (mm) = 0.053695084462162875
  Average Trabecular Separation (Tb.Sp) (mm) = 0.19838339545686987
  Average Trabecular Separation (Tb.N) (1/mm) = 3.9670185266159916
  Average Trabecular Number using Tb.Sp (Tb.N_sp) (1/mm) = 3.9670185266159907
  Average Trabecular Number using BV/TV (Tb.N_bv) (1/mm) = 2.9099174589546997

  Connectivity Density (Conn.D) (1/ $\text{mm}^3$ ) = 1745.24118834803

  Euler characteristic or number = -811
  Euler Density Total Volume (1/ $\text{mm}^3$ ) = 260.38214980037657
  Euler Density Bone Volume (1/ $\text{mm}^3$ ) = 1666.464276617488

  Bone Surface (BS) ( $\text{mm}^2$ ) = 29.260579581138906
  Specific Bone Surface (BS/BV) ( $\text{mm}^2/\text{mm}^3$ ) = 60.1254137917276
  Bone Surface Density (BS/TV) ( $\text{mm}^2/\text{mm}^3$ ) = 9.394491511395747

  Structure Model Index = 2.1596775059617994

Analysis run time...
Analysis started at: 08/10/2024 13:27:33
and finished at: 08/10/2024 13:32:22
```

Steps to Run 'Trabecular Bone Analysis with BMD' Plugin

This screenshot of the *.txt file shows results from BMD analysis.

```
Dragonfly (https://dragonfly.comet.tech/), ver 2024.1.0.1601

Inputs used and location for results...
Results are for input image: A4631_IRC TrabVOI Median-Filter
using BoneArrow ROI: A4631_IRC TrabVOI Med-Norm TrabArrowROI
and BoneOnly ROI: A4631_IRC TrabVOI Med-Norm Trab bone ROI

Results written folder: D:/test run femurs/M5/TrabResults

|
Image voxel sizes and Voxel counts for Total and Bone volumes
image spacing X (mm): 0.007999929999999999
image spacing Y (mm): 0.007999993
image spacing Z (mm): 0.007999993

Grayscale conversion information and values excluded from mineral density calculation...
Converted image grayscale values to BMD using BoneTV and Scaling value
BoneTV = 3315.0
HA Density = 1.69 (g/cc)
NOTE: Set slope, intercept & scale for equation method to 1, 0, 1

Voxels with grayscale values below threshold excluded from BMD calculation
threshold = 0.0

Bone Volume Fraction results...
number of voxels in BoneArrow ROI: 6083466
number of voxels in bone only ROI: 950531
totalVolume (mm^3): 3.1146528309323736
boneVolume (mm^3): 0.4866590969751421
boneVolume/totalVolume (bv.tv): 0.15624826373649495

BMD/BMC results...
Min, Max, Mean values of image for BoneArrow ROI
min = -25.0
max = 4715.0
mean = 478.7345853498644
And from these, the BMD-BMC values
BMD: 244.06876900189169
BMC: 0.7681645650912741
values using Microview's scaling properties
BMD_mv: 154.95692611776911
BMC_mv: 0.48263702860528823

TMD/THC results...
Min, Max, Mean values of image for BoneOnly ROI
min = 1430.0
max = 4715.0
mean = 2844.04850519546
And from these, the TMD-THC values
TMD: 1449.9070612452587
THC: 0.7056104708566816
values using Microview's scaling properties
TMD_mv: 920.5623066131143
THC_mv: 0.44800002064569207

Analysis run time...
Analysis started at: 08/10/2024 13:32:22
and finished at: 08/10/2024 13:32:22
```

Merge Results

Now that you have finished Cortical and Trabecular VOI bone analyses, merge results for this bone with your projects global results file(s).

Save Session file and proceed to next Bone

