

Subadult Virtual Anthropology Database (SVAD)

Data Collection Protocol: Amira

K.E. Stull^{1,2} and L.K. Corron¹

¹Department of Anthropology, University of Nevada, Reno, United States; ²University of Pretoria, South Africa

*Corresponding author contact information:

Dr. Kyra E. Stull

Department of Anthropology

University of Nevada, Reno

1664 N Virginia St MS 0096

Reno, NV 89557 USA

Phone: 775-784-4834

kstull@unr.edu

This document presents the protocols developed to visualize skeletal and dental elements on 2D computed tomography scan (CT) slices and 3D renderings of these elements, as well as the segmentation protocol to extract element surfaces (Stock et al. 2020). The protocols were developed as part of National Institute of Justice Awards 2015 DN-BX-K009 and 2017 DN-BX-0144.

If the methodology discussed herein is used in a presentation or publication, then this document needs to be cited. We strongly encourage all research using this protocol to be submitted to the Subadult Virtual Anthropology Database Zenodo Community to facilitate sharing and discovery of information (<https://zenodo.org/communities/svad/?page=1&size=20>).

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Isosurface & Surface Generation Protocol in Amira™ (v. 2020.2)

First Steps

1. Open Amira and choose [Open Data...] (green button in the “Project View” pane in the upper left corner).
2. Find the individual you are interested in and select all of the DICOMs in that folder (not the DICOM directory). Click [Open].

If you get a warning about the file size being too large, choose the radio button for “Load complete volume into memory” and click [OK].

A dialog box called “DICOM Loader” will prompt you to check the parameters of the DICOM stack. Scroll through the window and if everything looks good (no out of order numbering, gaps in the data, etc.), press [OK].

3. The file appears as a green lozenge in the “Project View” pane. Before selecting anything else, click the perspective button. The button is located in a bar of other buttons just above the viewing

panes and looks like an eye with this symbol in front:  Clicking the button will change the

symbol in front of the eye to parallel lines:  Use the parallel line setting for all specimen editing and measuring.

4. Save the project.

Click File > Save Project As. Save the project as the **Case Number** in that corresponding Individual’s folder. Saving the project enables you to pull up all of the objects, commands and settings at the time you save, preserving your workspace just how you left it.

A dialog box titled “Save Project policy” may appear asking how if you want to save only necessary data to the disk and compute it later, or save all data. Unless inappropriate for your project, Select “Minimize project computation” to save all data, and click [OK].

5. Save the data.

While the green data lozenge is selected, click File > Save Data As. Save the data as the “**Patient name**” with the file extension automatically chosen by Amira. This links the loaded DICOM stack to the Amira project. Save the data file every time you load in a new set of DICOMs.

Occasionally, Amira will automatically create an orange lozenge titled “Ortho Slice” that shows a single slice of the CT data. You can choose to keep this on until the Isosurface is created, or should you choose to turn it off for a cleaner work space, simply click the little red box within the orange lozenge. The Ortho Slice should now be removed from the workspace until you wish to turn it back on. Through this process it might be useful to turn different surfaces on and off to compare,

or keep a clean work space. Just remember to click the red box inside the lozenge to switch it on or off.

If your individual is comprised of two or more stacks of DICOMs, load and save all of the data using Steps 1 & 3. Because of UNM's scanning procedures, some of the z-coordinates of the patient's location may be off, resulting in misplacement of one set of scans relative to the others along the body's long axis. To fix this we can reset the coordinates of the segmented and smoothed surfaces or Generated Surface (but it won't fundamentally change your data file). Select the green data lozenge that has the scan you want to move, look in the "Properties" pane in the lower left corner, and click the Transform Editor button. It looks like a bunch of green balls arranged in a hexagon with lines connecting them. You may have to increase the size of the property box for it to appear. In the "Manipulator" section of the area that appears, click [Dialog]. A new window pops up with several different options, select the "Relative to Global Axes" tab. Adjust the z-axis in small positive and/or negative increments until the scan is properly placed relative to its counterpart. **Warning:** Once you move a scan there is no reset or CTRL-Z to put the image back where it started. Also, note that this procedure should be followed both for Isosurfaces and Generated Surfaces (instructions in the next several Steps).

6. Selecting the bone, excluding the surrounding soft tissue (bone segmentation)

This presents the steps for semi-automatic segmentation of bone elements, with some manual adjustments.

Navigate to the "Segmentation" pane. Make sure the data file you are working on appears in the "Image:" area. If not, select your file from the pull-down menu.

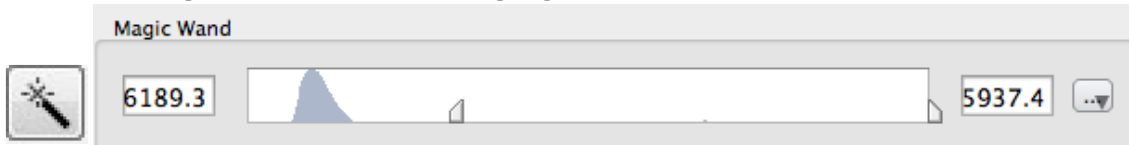
Create a new material by clicking [Add] at the bottom of the Material window. You can rename your material something pertinent (like "Diaphyses") as well as change its color in this pane.

Scroll through the CT slices until the bone you would like to select is at least partially visible within the slice. Below, select the "Magic Wand" tool in the lower left hand pane.

Using the histogram sliders, encompass the range of values that you think belong to bone. You can type an exact value within the right number box to expedite the process of finding a beginning threshold. Usually, begin with 160 as a default threshold value to and then roll to higher or lower values using the cursor until you reach a satisfactory level of segmentation (only the bone is highlighted and the edges are smooth). Once you have attained an appropriate threshold level, make sure to record the value in a separate excel file or notebook.

To check your selection, next to "Preview" below the histogram, check the "2D" and "3D". Ensure the 3D viewing window is open by clicking a pane viewer box that has more than one pane, which would be the three buttons farthest to the right on the bar above the viewing panes. An example of the viewing pane options are shown below with the series of 5 white boxes with varying lines in them. The voxels whose grayscale values are encompassed within the histogram range that you set will then glow blue in the viewing window to the right, and a 3D representation of the selected

material will appear in the 3D viewing pane. Then click on part of the bone in the pictures shown in the viewing window. This should highlight the bone in red.



A red line will appear on the masking slider at the value of the point you have just selected on the bone. Adjust the slider, or by typing in the numbers in the box to the left of the histogram, until all of the bone is selected, but no other materials are selected in the slice in which you are currently viewing the scan. Once you feel happy with your selection, click the “All Slices” check box above the histogram. This now applies your selection to all of the slices, and if you navigate to the 3-D view in the Segmentation pane, you should see a red, 3D image of the specimen. Rotate the 3-D image and see if it looks complete, and scroll through the sliders in the various plane viewing windows.

You can change orientation by going to Segmentation → Orientation → (whichever orientation you haven’t done yet). An easier way to toggle between views of the orientations is using the toolbar above the viewing pane that looks like this:



In this toolbar, clicking on the different rectangular boxes gives you from one up to four (x-, y-, z-planes and 3D) planes/surfaces open simultaneously in your viewer window. To toggle between different views in the one-view option, just click on the single rectangle repeatedly.

If you notice parts of the skeleton not in the selection or extra bits of soft tissue material that aren’t bone in the selection, you may want to adjust the number to the left of the histogram slider.

Once you have selected the skeleton (or part of the skeleton), highlight the Material that you want to add your selection too, navigate to “Selection”, and click “Interpolate” (or just ctrl + i). Then click the Add [+] button under Selection in the bottom left hand pane. Multiple, non-contiguous areas can be selected with consecutive clicks if the Shift key is held down during clicking. You can also subtract selected areas from a Material as well by clicking the Subtract [-] button.

There are other tools that can be used for segmenting as well. These include the paint brush tool, the lasso and the blow tool (buttons as they appear below). Voxels selected using all of these tools can be added to, as well as removed from, a material.



The paint brush tool lets you select a wider area, and you can change the size and shape of the brush strokes. You can select some helpful options for this tool, like ‘Select only current material’ to only allow the brush to select the Material that is currently highlighted.



The lasso tool operates just like in Photoshop. It is one of the only tools that can be used in the 3-D option of the Segmentation viewer, but be forewarned that it will then select

everything behind (from your perspective) whatever you are lassoing. Also, this is can be a very time intensive process.



The blow tool works like blowing up a really irregular balloon, whose edges conform to the boundaries of different grayscale values. Click and drag around an area of interest to grow the balloon. The higher the 'Tolerance' value, the easier it will be for your balloon to jump over boundaries.

Remember that CTRL-Z always works to undo a mistake, and you can zoom in and out using the +/- magnifying glass buttons (just don't mistake them for the [+] or [-] buttons to add/subtract your selection from the materials!!)

7. Smoothing the slices

Re-select all of the area that you just segmented into your new Material above by clicking the 'Select' box next to the Material you are working with.

From the menu bar, choose Selection → Grow → Volume.

Next choose Selection → Smooth → All Slices. Smooth all slices in this manner in all three orientations (XY, YZ, and XZ). See Step 5 above for help toggling between views.

Choose Selection → Shrink → Volume.

Click the [+] button to add the selection to your material

8. Saving the raw file. Return to the Project View pane by clicking the tab in the upper left. Right click on the green lozenge that ends in "labels" navigate to the "Compute" folder, and click on the red gear labeled "Generate Surface" from the options that appear, click [Create]. Within the surface generating dialog that appears in the lower left, **change the Smoothing option to "None"** then click the green [Apply] button at the bottom.

A dialog box may appear, warning you that the image is rather large. Click [Continue].

A new green lozenge is generated (ending in .surf). Select it, then in the menu bar go to File → Save Data As... and save the generated surface as "(Record number).surf.am". This is ".am" is the file type to save surfaces for viewing in Amira.

Select the newly created green lozenge again, then in the menu bar go to File → Export Data As...

Choose .ply as the file type, and name the file so that it reads "**(Record number) raw.ply**". Be sure you are in the folder for the correct individual and click [Save].

If you would like, click the yellow [Surface View] button to see what the specimen looks like at this stage in the main Amira viewing window. To manipulate the Surface, the left-click rotates the view, the center-click transposes the view and the center scroll ball zooms in and out.

9. Filling in all the holes in the specimen. Return to the Materials Selection Area (upper left corner) in the “Segmentation” pane. You want the same selection that you had when you created the raw file, so click the ‘Select’ box next to the Material into which the bone is segmented. This will make all the voxels belonging to this material glow red.

There are two ways to fill holes in the specimen:

1) Go to Selection → Fill → All Slices. This method will indiscriminately fill every hole in every slice. So be careful! It is easy to accidentally fill up unintended areas, especially if your surface isn’t watertight.

2) Fill in slice by slice using the paintbrush tool (see explanation in Step 5). This method is painstaking but highly accurate. You will probably need to use it to fill up any holes that communicate with the air outside the specimen (such as tooth roots with holes at the bottom of them). If you do need to do manual paintbrush filling to block holes that communicate with the ‘outside world’ as in step #2 above, unless you completely fill the empty space manually, you will need to go back and run step #1 above. This is probably a good idea after manually filling holes anyway, just to double-check as erroneously missing voxels are hard to catch.

Once you have selected the full specimen, with no internal bubbles, click the Add [+] button to add your selection to the Material.

10. Saving the filled surface. Once you are satisfied with your highlighted selection, return to the Project View tab, located in the upper left. Highlight the .labels lozenge again and click the red [Generate Surface] above. Once the surface is generated click the red lozenge that is created and change the smoothing drop down bar to ‘None’. Once that is changed click ‘Apply’. A message may appear warning you that your surface will be large, click ‘Continue’. A new green lozenge will appear ending in “record number.surf”.

Click the red “Generate Surface” lozenge to create a new surface (green lozenge) and save your work. Use File → Export Data As... to save the newly generated surface as a .ply file with the name “**(Record number) fill.ply**” to the same file folder as before. Create a new Surface View if you desire.

11. Smoothing the surface.

Click the green lozenge ending in ‘.surf’ that corresponds to the filled surface. Now that you have segmented the bone, you can smooth the surface by pressing the red [Smooth Surface] button at the top of the Project View pane. If the button is not there, smoothing can be accessed by right-clicking the green lozenge, going to Surface Transforms → Smooth Surface, and pressing [Create].

Once the Smooth Surface dialog has opened in the lower left corner of the screen, change the iterations to 20 (**but do not change the lambda value—should always = 0.6**) and press [Apply] in the bottom right corner. This will create a new lozenge in the Project View pane.

12. Saving the segmented surface

Use File → Export Data As... to save the final green lozenge created as “(record number)_smooth.ply” in the same folder as the other .ply files.

Create a surface view by clicking the yellow [Surface View] button. To manipulate the Surface, the left-click rotates the view, the center-click transposes the view and the center scroll ball zooms in and out. **This is the surface view from which all diaphyseal measurements will be taken!**

13. Final steps

Before closing out the window or moving on to the next scan, make sure to save the project and its contents. Go to File > Save Project. Since you already saved the project in Step # 3, the .hx file should already exist. When saving, a Dialog box may pop up that notes if you haven't saved aspects of the project that would need to get saved in order to re-open the project. Save the files using the same Record number and into the same folder where the rest of the data for this specimen are stored.

CT Scan Visualization and Measurement Protocol in Amira™ (v. 2020.2)

1. Opening Amira and loading CT scan stacks

Open Amira™ by double clicking the Desktop icon and choose [Open Data...] (green button in the [Project View] pane in the upper left corner).

Find the individual you are interested in and select all of the DICOMs in that folder (NB: not the DICOM directory). **Click [Open]**. Always select the folder that has “ST” in the name. This stands for “Soft Tissue” and has a better resolution than “Bone”. You can choose folders with names including “Head, Head-Neck”, or “Whole Body” as they all include the upper body and skull.


If you get a warning about the file size being too large, choose the radio button for “Load complete volume into memory” and **click [OK]**.

A dialog box called “DICOM Loader” will prompt you to check the parameters of the DICOM stack. Scroll through the window and if everything looks good (not out of order numbering, gaps in the data, etc.), **click [OK]**. Any errors will be shown as red numbers in the list of DICOMs. If that is the case, only select the DICOM files that appear in black by pressing shift and clicking on the first and last DICOM of the stack that you want to open.

2. Visualizing the CT slices.

The file appears as a green lozenge in the “Project View” pane. *Before you do anything, make sure*

the ‘Perspective’ tool is set to orthogonal. The button looks like this:  *and you want the lines in*

front of the eye to be parallel, not angled, like this:  The button is located in a bar of other buttons just above the viewing panes. Use the parallel line (orthographic) setting for all specimen editing and measuring.

Save the project. Click File > Save Project As. Save the project as the Case Number in that corresponding Individual’s folder. Saving the project enables you to pull up all of the objects, commands and settings at the time you save, preserving your workspace just how you left it.

A dialog box titled [Save Project policy] may appear asking how if you want to save only necessary data to the disk and compute it later or save all data. Unless inappropriate for your project, Select “Minimize project computation” to save all data, and click [OK].

Select the orange [**Orthoslice**] tab in the “Project View” pane. The CT slice should appear in the viewing console on the right. You can change the plane of the slice view by selecting xy, xz, or yz in the console header. If the hand is selected in that header, you can use it to move the exam around. You can also scroll through the slices by clicking the **box next to the Slice Number line** in the “Properties” pane and using the mouse to scroll, or moving the cursor between the two triangles.

Save the data. While the green data lozenge is selected, click File > Save Data As. Save the data as the [Patient name] with the file extension automatically chosen by Amira™. This links the loaded DICOM stack to the Amira™ project. Save the data file every time you load in a new set of DICOMs.

Occasionally, Amira™ will automatically create an orange lozenge titles [Ortho Slice] that shows a single slice of the CT data.

If your individual is comprised of two or more stacks of DICOMs, load and save all of the data using Steps 1 & 3. Because of some facilities' scanning procedures, some of the z-coordinates of the patient's location may be off, resulting in misplacement of one set of scans relative to the others along the body's long axis. To fix this we can reset the coordinates of the segmented and smoothed surfaces or Generated Surface (but it will not fundamentally change your data file). Select the green data lozenge that has the scan you want to move, look in the [**Properties**] pane in the lower left corner, and click the Transform Editor button. It looks like a bunch of green balls arranged in a hexagon with lines connecting them. You may have to increase the size of the property box for it to appear. In the [Manipulator] section of the area that appears, click [Dialog]. A new window pops up with several different options, select the [Relative to Global Axes] tab. Adjust the z-axis in small positive and/or negative increments until the scan is properly placed relative to its counterpart. Warning: Once you move a scan there is no reset or CTRL-Z to put the image back where it started.

3. Navigating through the CT slices

To scroll through the slices, select the orange [Orthoslice] lozenge. Go to the Properties pane below and select one of the three planes you want to visualize the images in. Scroll through the slices by selecting the little triangle and moving it up and down. If you want to zoom in the slice, place the cursor on the slice, press the roller on the mouse and roll it to zoom in.

4.a. Creating a Volume Rendering of an element

Select the green lozenge. Click on the yellow [**Volume Rendering**] button. The soft tissue of the scanned body should appear in the viewing console on the right. You can use the mouse on hand mode to turn the body around at different angles.

In the "Properties" pane, move the left cursor of the "Colormap" higher to adjust the masking value (the number on the left). This allows you to hide soft tissue and only visualize bones or teeth, the further you adjust the value. Higher values will only allow to visualize the densest elements (metal, teeth, etc.). You can also type a value in that left box and lower the right slider as well. If you cannot see the teeth, you can move the body around and/or unselect the orange square in the [**Orthoslice**] lozenge to hide the contents of the CT slice. A good range of values should encompass part of the grey peaks you can see in the "Colormap".

To adjust the transparency, in the "Properties pane", click on the [**Edit box**] of the Colormap line. You can scroll down the menu and choose one of the colormap options. You can always revert back to the original setting if necessary by selecting it in the "Edit" box.

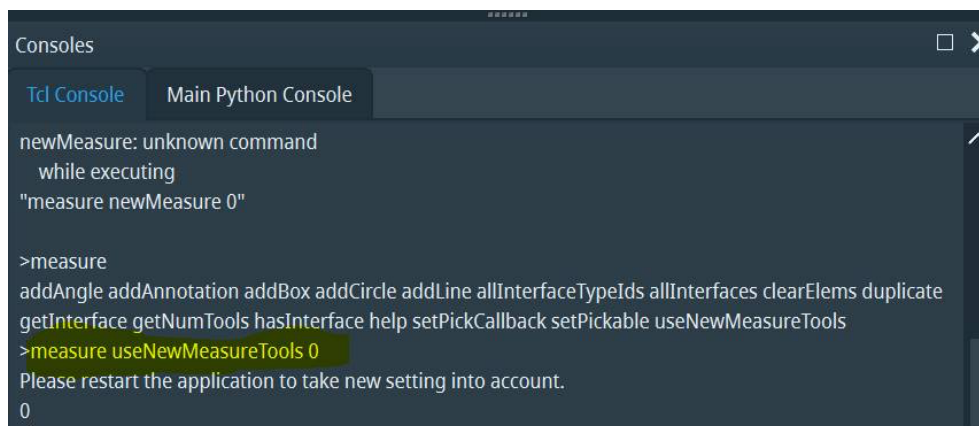
4.b. Creating an Isosurface/segmented surface of an element

See protocol for segmentation/volume rendering of bones and teeth to obtain virtual surfaces of these elements ("*Amira Isosurface & Surface Generation Protocol*").

5. Measurements

To install the Amira plug-in for exporting measurement sets, follow these steps:

- Open Amira, click on "Blank project"
- In the upper banner, click on "Project" and then on "Consoles" below that. A set of consoles should appear in the bottom right: "Tcl console" and "Main Python console".
- Make sure you are in the Tcl console (the name is highlighted in blue) and enter **measure useNewMeasureTools 0** (see image below), then hit Enter. It should say "Please restart the application to take new setting into account".



```
Consoles
Tcl Console Main Python Console
newMeasure: unknown command
  while executing
  "measure newMeasure 0"

>measure
addAngle addAnnotation addBox addCircle addLine allInterfaceTypeIds allInterfaces clearElms duplicate
getInterface getNumTools hasInterface help setPickCallback setPickable useNewMeasureTools
>measure useNewMeasureTools 0
Please restart the application to take new setting into account.
0
```

- Restart Amira.
- When you select the measuring tool now, an "Export" button should appear at the bottom of the Properties pane now (see second image attached).
- When you click on that "Export" button, a green lozenge appears in the "Project View" pane, called "Measurement.SpreadSheet". You can now save or export that as a .csv file.
- Re=open the stack of CT scans/segmented surfaces to take your measurements

5.a. Measurements on CT slices

Once you have the slice you want to take the measurement on, navigate to the header to View and scroll down to select [Measuring]. Or go to the panel above the slice and select the ruler image. This will make a new yellow lozenge pop up. When you have the yellow [Measurement] lozenge selected in the Project View pane, various options will pop up in the [Properties] pane in the lower left-hand corner of the screen.

Then click [Line] in the [Properties] pane/the ruler icon, and a new measurement line will pop up in the box below. Navigate the cursor into the viewer window and click and drag between the points to take the measurement. The distance will be displayed both next to the line in the viewer window as well as on the line in the [Properties] pane. Scroll through the slices to see if your measurement is ok. Remember that left clicking will rotate the image, center clicking and dragging will transpose the image and scrolling will zoom in/out. If you wish to edit the placement of a point, click on the line of that measurement in the [Properties] pane, make in the line in question become highlighted with little red balls on its endpoints. Left click on the point you wish to move and drag it to its appropriate location. When you are satisfied with the measurement, click on [Line] to start a new measurement. In order to preserve already-taken measurements, you can click on the lock on the left side of each measurement so that you cannot change them accidentally. Clicking on the eye next to the lock removes that measurement from your view.

5.b. Measurements on virtual surfaces

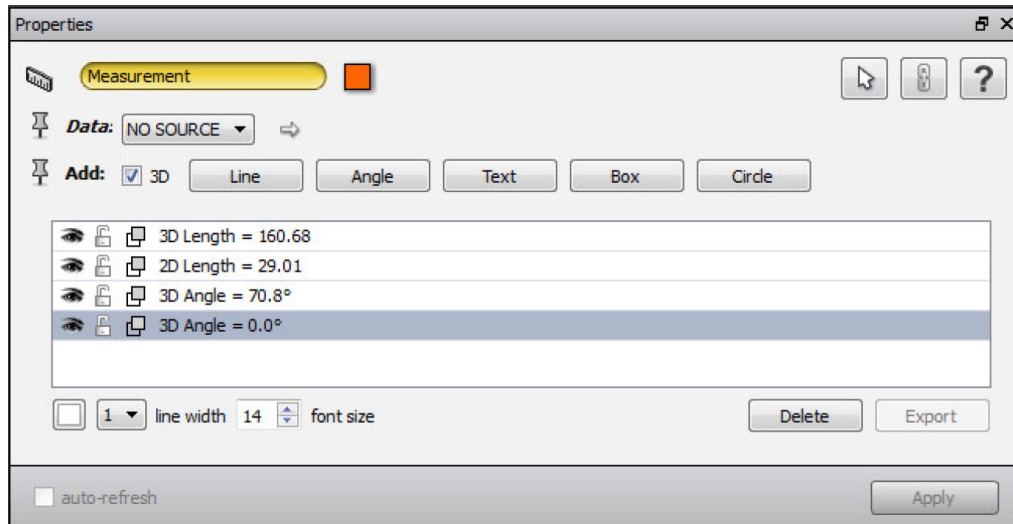
Diaphyseal and pelvic measurements are taken in Amira from volume renderings (see step 4.a. above) the filled smoothed surfaces generated after following the segmentation protocol (see *“Isosurface & Surface Generation Protocol in Amira”*) using the 3D measurement tool to the nearest hundredth of a millimeter. Before you do anything, if you have not done this before in the first steps, make sure the ‘Perspective’ tool is set to orthogonal. You want the lines in front of the eye to be parallel, not angled.

While the generated Surface is open in the viewer window in the “Project View” pane, navigate to View > Measuring. This will make a new yellow lozenge pop up. When you have the “Measurement” lozenge selected, various options will pop up in the “Properties” pane in the lower left-hand corner of the screen. Check the 3-D box—this makes sure that i) the measurements you are taking will stick to the Surface when you move it around, and ii) you must place the endpoints of a linear measurement on the Surface (i.e., it will not allow you to click into thin air).

Then click [Line] in the “Properties” pane, and a new measurement line will pop up in the box below. Navigate the cursor into the viewer window and left click on the two end points (or click and drag between the points). The distance will be displayed both next to the line in the viewer window as well as on the line in the “Properties” pane. In order to check the placement of your points, switch to the Hand selector tool (in the toolbar above the viewer window) to manipulate the surface to get a better view. Remember that left clicking will rotate the image, center clicking and dragging will transpose the image and scrolling will zoom in/out. If you wish to edit the placement of a point, click on the line of that measurement in the “Properties” pane, make in the line in question become highlighted with little red balls on its endpoints. Left click on the point you wish to move and drag it to its appropriate location. When you are satisfied with the measurement, click on [Line] to start a new measurement. In order to preserve already-taken measurements, you can click on the lock on the left side of each measurement so that you cannot change them accidentally. Clicking on the eye next to the lock removes that measurement from your view.

6. Saving your measurements

To save your measurements, go to File>Save Project as and save your project.



You can also export your measurement set as an Excel spreadsheet by clicking on the “Export” button, at the bottom right of the Properties box of the measuring tool.

7. Saving the Data and Saving the Project

Go to File>Save Data and enter the name you wish to save your data and the path to where your data will be located.

References

Stock, MK., HM. Garvin, LK. Corron, CN. Hulse, LE. Cirillo, AR. Klaes, KL. Colman, and KE. Stull. 2020. "The Importance of Processing Procedures and Threshold Values in CT Scan Segmentation of Skeletal Elements: An Example Using the Immature Os Coxa." *Forensic Science International* 309.