BOUNTI USER MANUAL

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Avizo/Amira Add-on Windows Executable Python Library

BounTI

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Introduction

BounTI is a boundary-preserving segmentation tool based on threshold iteration. The paper on it is available (here/insert hyperlink). With the success we have been able to achieve in the segmentation of various vertebrate craniofacial regions, it is crucial that the tool is made as accessible as possible. To achieve this a comprehensive guide is provided here.

We would like to thank Dr Anastasiia Maliuk and Prof Susan Evan's Lab for the lizard scan used in these demonstrations.

Preprocessing

Here the preprocessing step by step preprocessing is shown in Avizo. However, other tools can achieve the same preprocessing results. These preprocessing steps need to be completed for all of the processing methods.

The tool takes in 16bit unsigned data as input. Therefore 32bit, 16bit signed and 8bit data needs to be converted. In Avizo your data type can be found here:
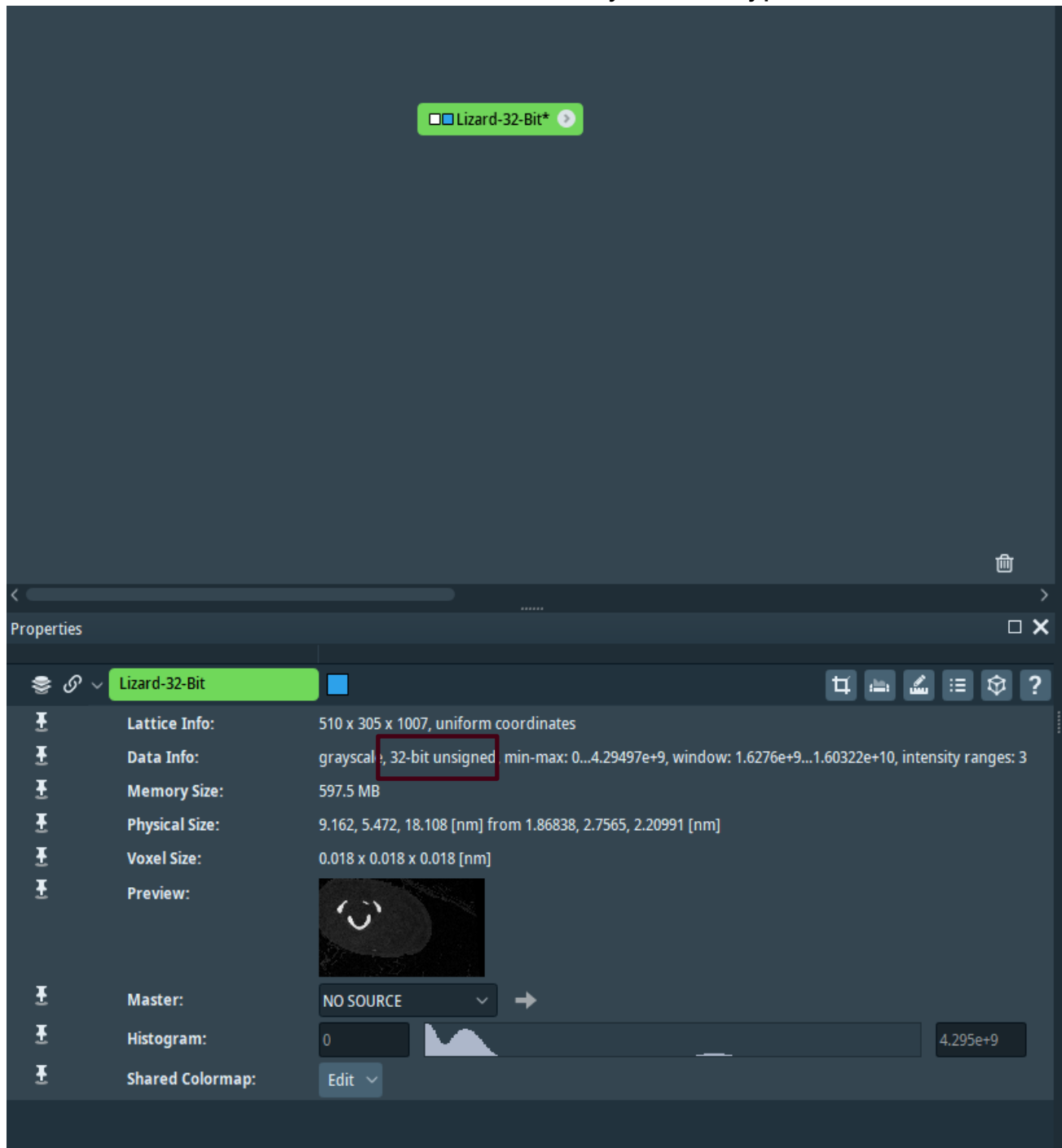
It can be converted to 16bit unsigned data using the *Convert Image Type* module:
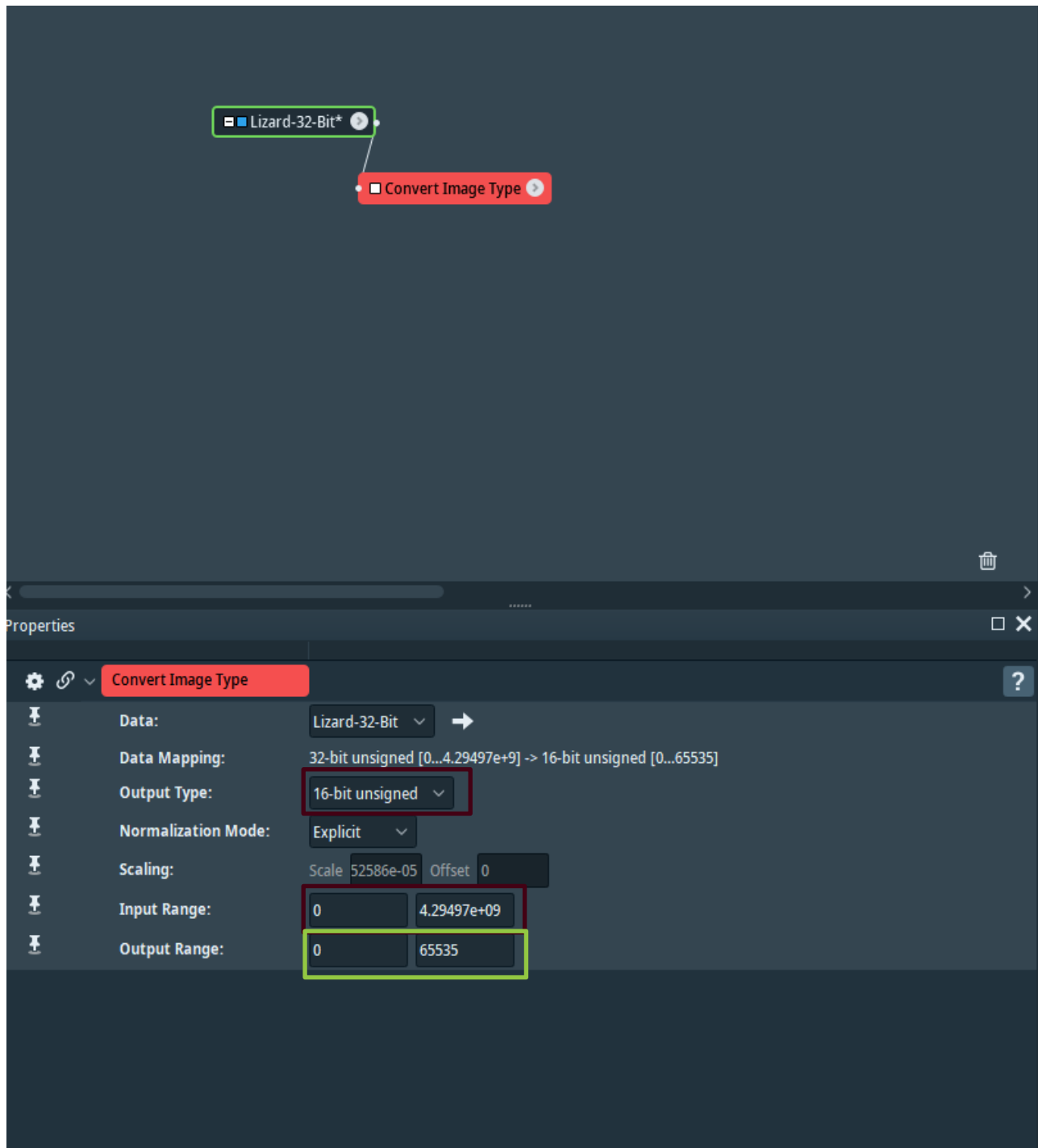
Output type should be set as 16-bit unsigned and the input range should be adjusted to lose as little grey value information as possible. For 8bit data the output range should be adjusted to 0 and 255. (green)
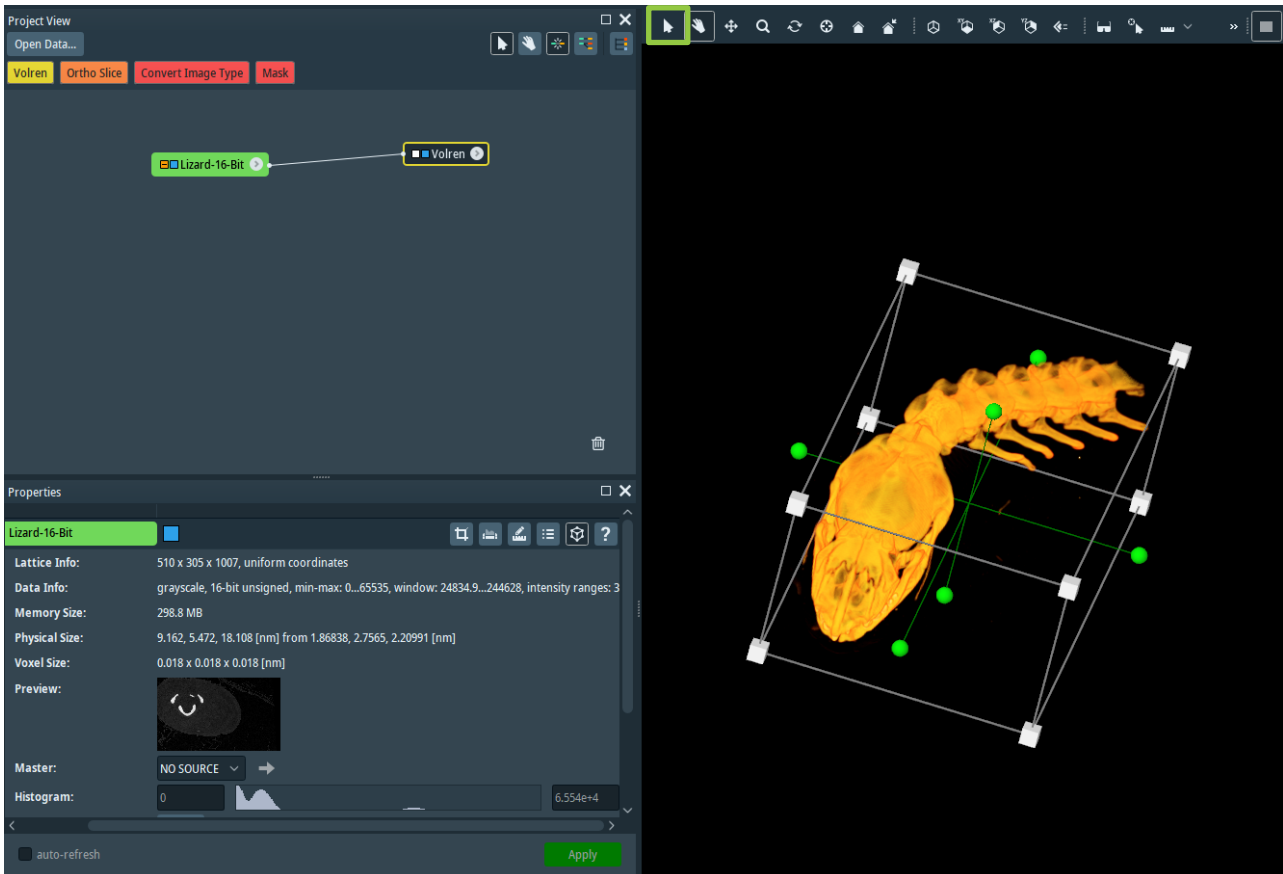
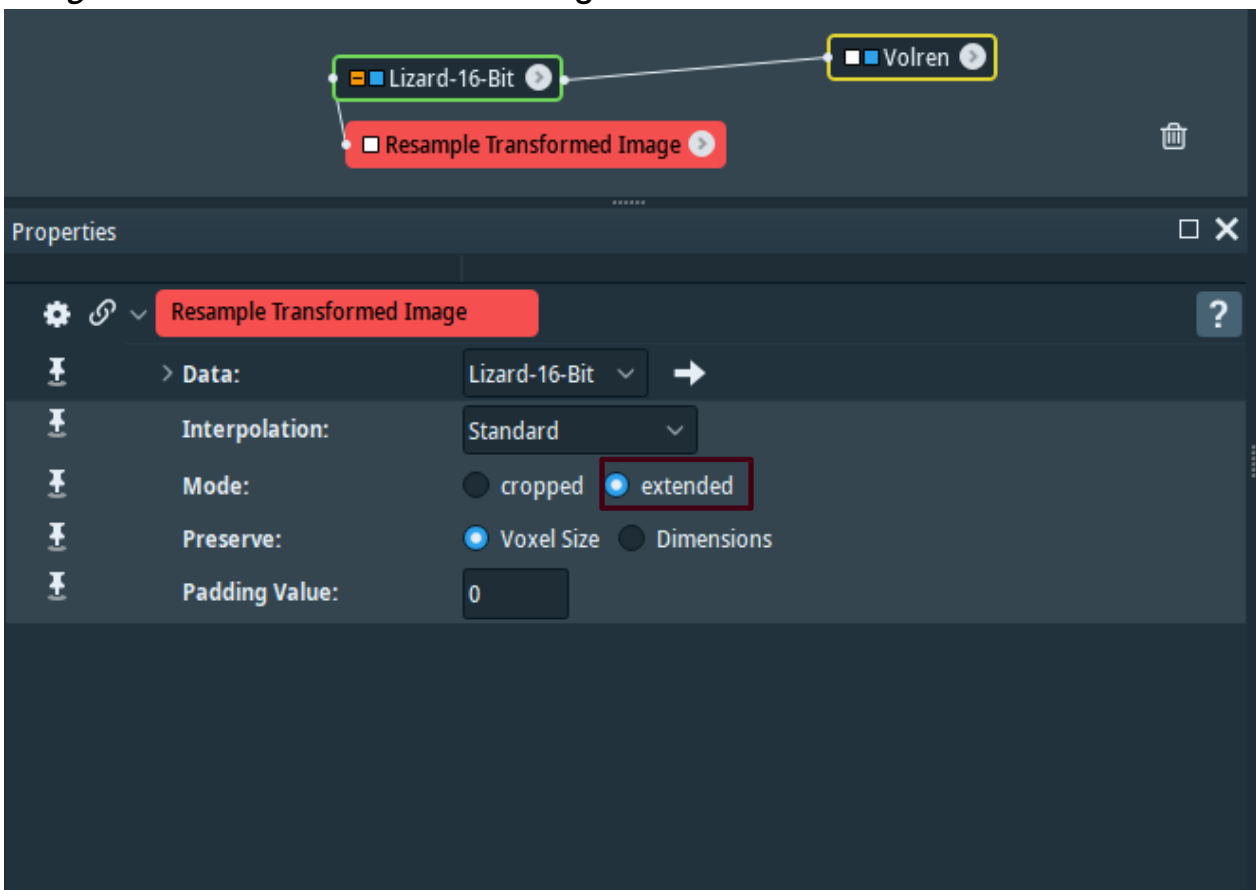Using the *Volren* module the scan can be visualised in 3D by adjusting the color sliders:
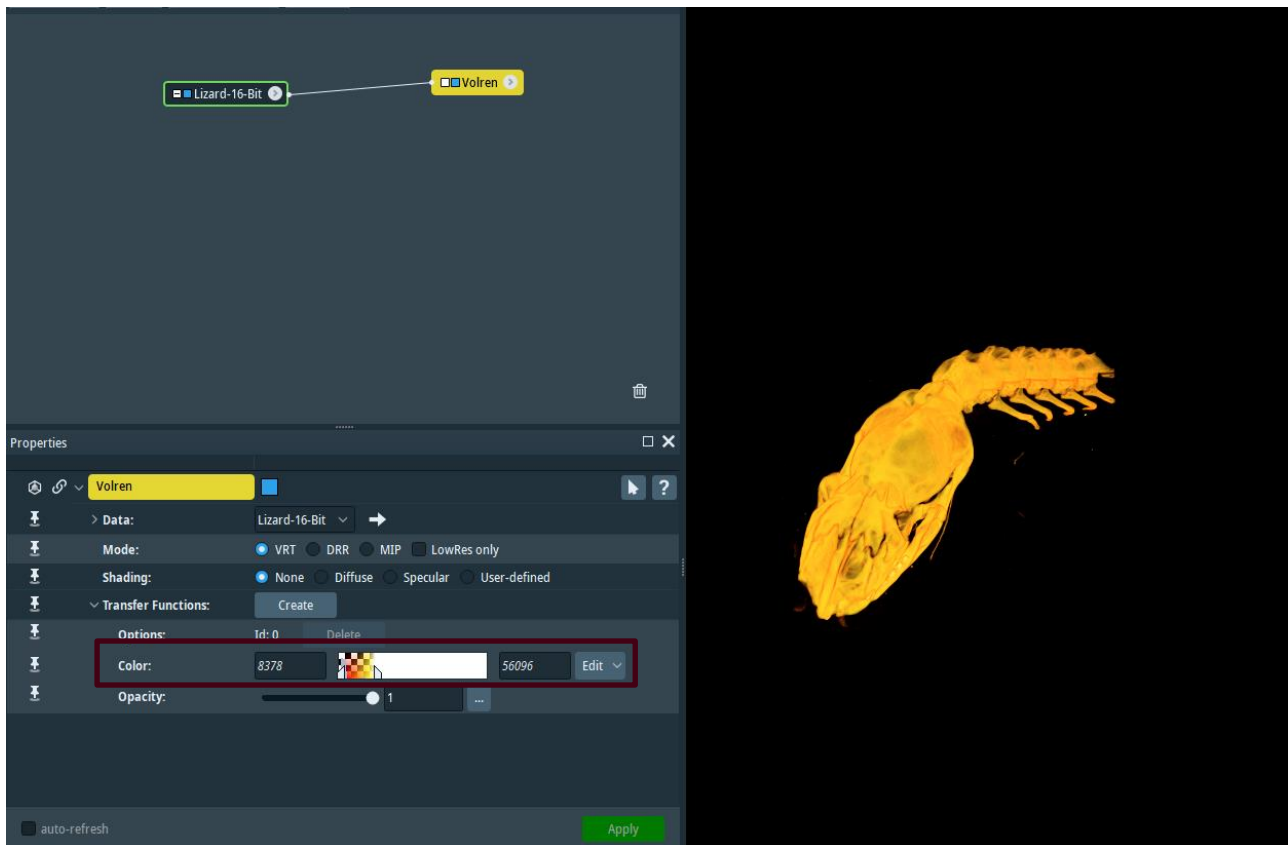
The data should be rotated to the desired position using the *Transform Editor*.

Using *Interact* (green) the green draggers can be used to position the scan:
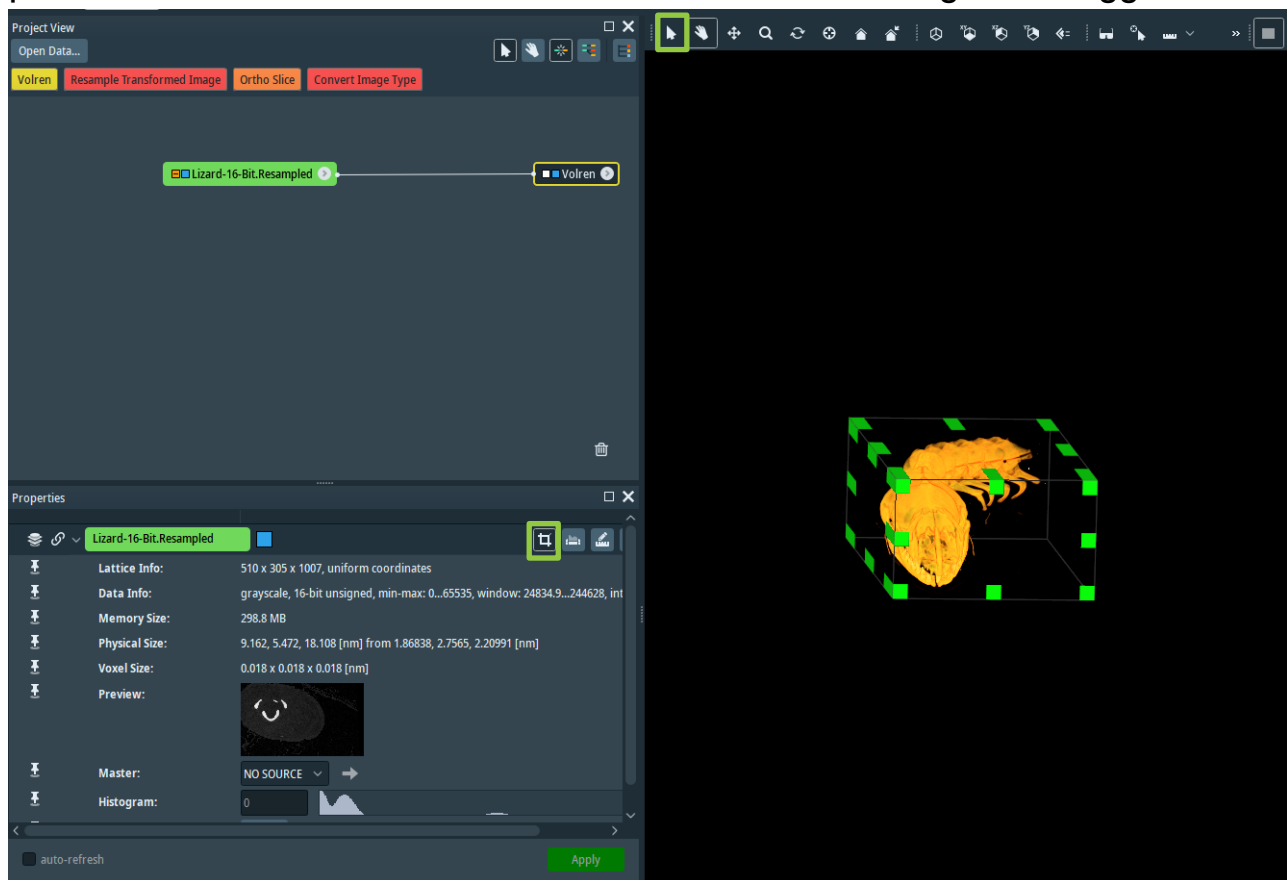

The modified scan should be resampled using the *Resample Transformed Image* module with the mode changed to extender:

The resampled scan can then be cropped using the *Crop Editor* in a similar process to the transform editor with the *Interact* tool and green draggers:

Resampling is not needed after cropping and pressing OK in the dialog will save the results to the scan.

With this, your data is ready for BounTI. However, experienced users may want to further filter, transform or combine their data before passing it to BounTI.

All of the steps conducted here in Avizo can be completed in other software and are the most basic operations of image processing which should be included in all CT image processing software packages. Thus, not having access to Avizo/Amira should not discourage you from using the tool.

NOTE: If the cropped 16-bit volume is more than 1GB it should be resampled by reducing the voxel size to be close/under 1GB larger volumes provide little increase in segmentation quality but will make the segmentation process significantly longer.

Processing

Input Parameters

With the volume data ready for processing the four numeric input parameters need to be determined. These are the same across the three different processing options, here it will be shown how to determine each parameter from the data using Avizo, however, other tools can be used.

INITIAL THRESHOLD

The Initial threshold is a high threshold at which the segmentation results in poor definition but adequate separation. Navigate to the Segmentation tab (green box) and use the threshold tool (burgundy box):
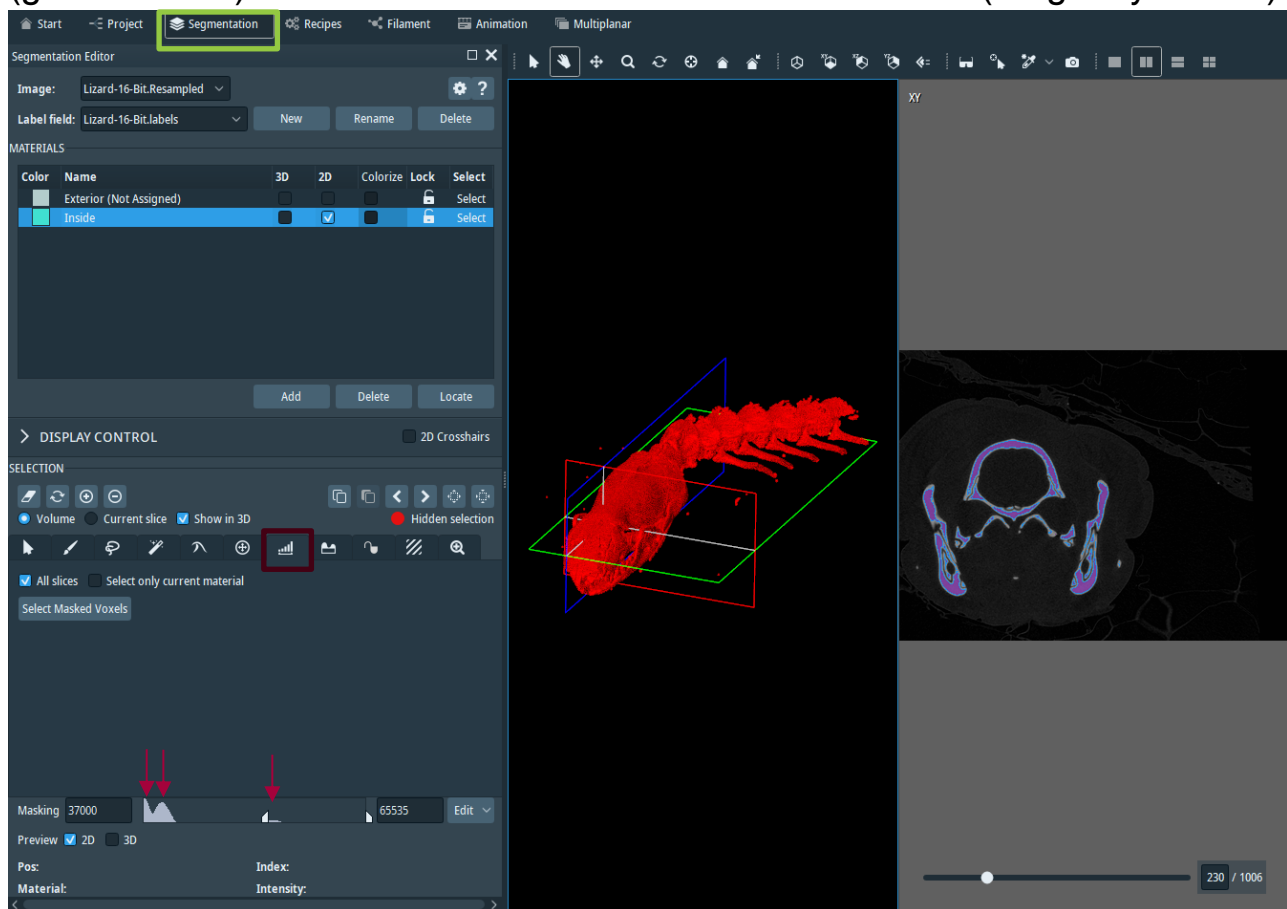
The threshold should be selected in the masking box. In the case of this lizard, three peaks are visible in the grey value histogram. The first one is the background/air, the second is the soft tissues and the third is the bone. However, the bone peak will often not be visible as the bone volume usually is relatively small compared to the soft tissues and air. If the bone peak is visible the initial threshold is usually at around the start of the peak with a slight offset to the right, however, the scan quality will dictate how high the threshold is required to be to obtain separated segments. More sharpness in the images will allow the threshold to be lower, while more blurry images will require a higher initial threshold.

The bone peak may not be visible then the threshold should be chosen to the right of the soft tissue peak and the results checked using the pick tool:
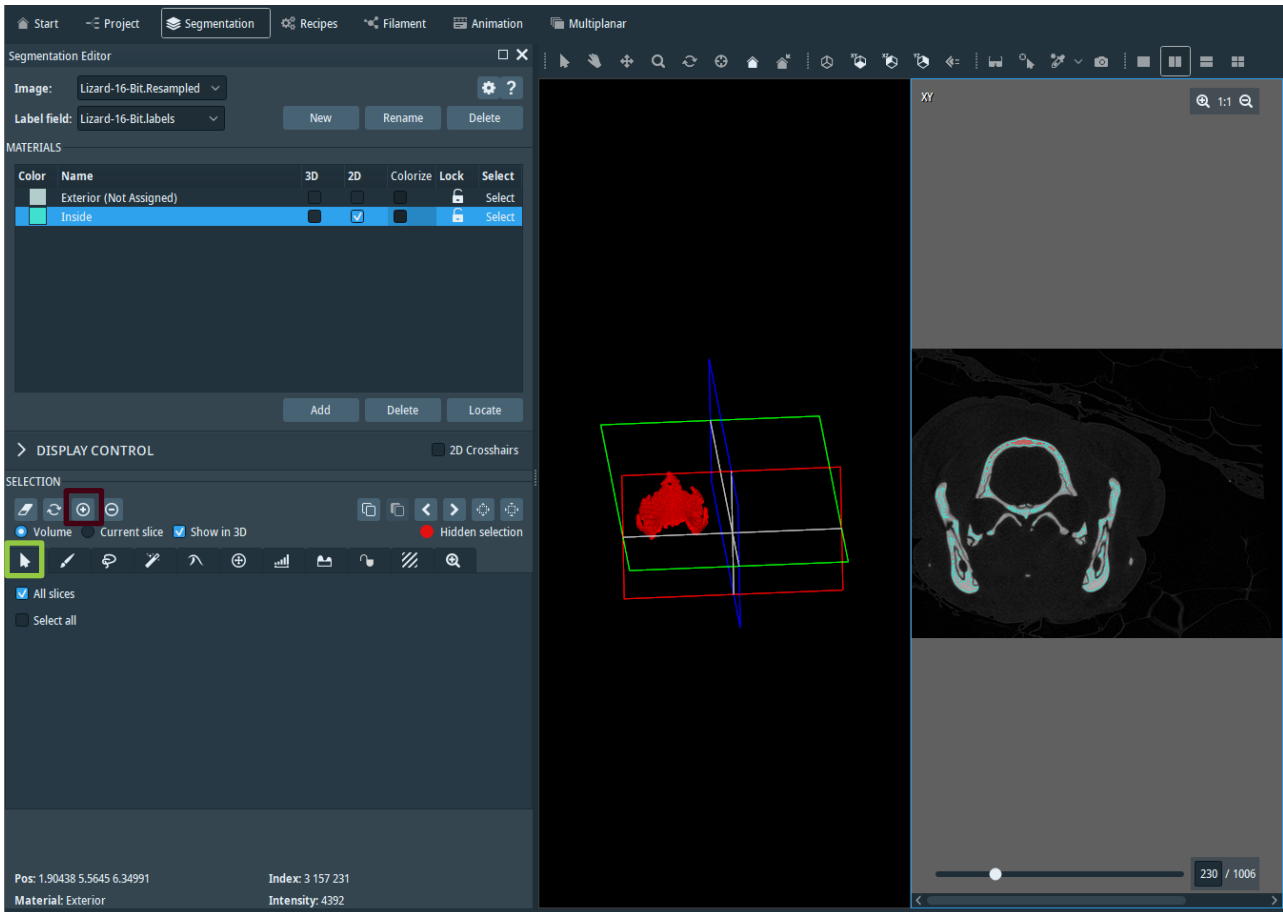
First, the masked voxels should be added to a material by using the add function (burgundy). Using the pick tool (green) by clicking on the bone segments in the slices, you should obtain poorly defined but separate bone parts. If the selection yields more than one bone piece increase the initial threshold, if you notice that some of the bones are completely missing, decrease the initial threshold. In the case of this lizard scan a sufficient initial threshold of 40000 was found.

TARGET THRESHOLD

The same segmentation tab can be used to obtain the target threshold. This is a low threshold where the desired bone definition is achieved. No consideration for connectivity is required:
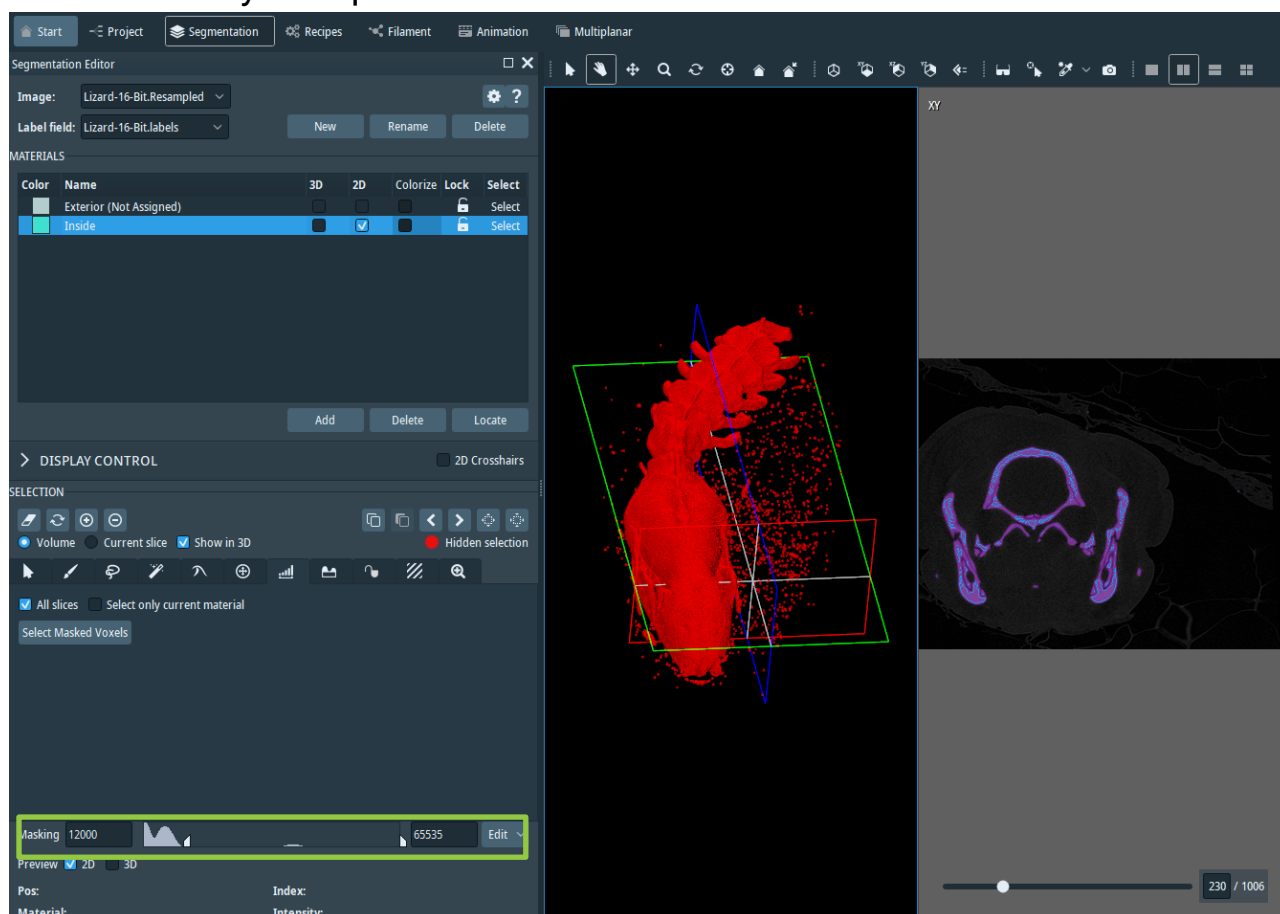
The target threshold is usually where the maximum bone definition is achieved without introducing significant noise or soft tissues. This tends to be directly to the right of the soft tissue peak in the histogram. The small disconnected pieces can be disregarded here as they will be removed during processing. The selection seen here represents the final segmentation without the separation. The target threshold of 12000 will be selected for this scan.

NUMBER OF SEGMENTS

The number of segments defines how many separate pieces will be segmented. The largest unconnected components from the initial threshold segmentation are assigned separate materials and the number of the components used is equal to the number of segments. Usually, the number of segments should be slightly larger than the number of disconnected anatomical components as some bones may be separated into two separate pieces which can be joined afterwards manually. For the snake scan, 100 segments will be used as the ribs and vertebrae are included.

NUMBER OF ITERATIONS

The number of iterations defines how many times the threshold iteration process will be conducted. It is best to run a quick segmentation using a small number of iterations. After investigating the results(the number of disconnected pieces is sufficient and the different bones of interest are separated) the number of iterations can be increased and the segmentation re-run to obtain cleaner boundaries, as this parameter primarily affects the interface between two separate segments. A good starting number is 20 and can be increased to 100 or 200 depending on the computational capabilities, file size and urgency.

Tools

After the input parameters have been determined and the input data has been prepared BounTI can be used on one of the three different platforms. While the data and input parameter preparation has been shown here on Avizo other methods are acceptable as long as the parameters required can be obtained.

AVIZO/AMIRA ADD-ON

The add-on can be accessed by dragging the BounTI.pyscro file into the Avizo project view:
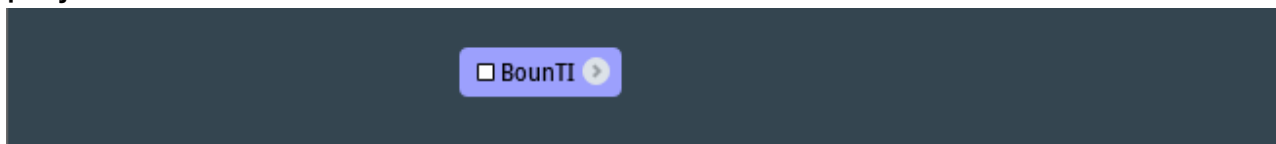
The input data and parameters can be inputted here:
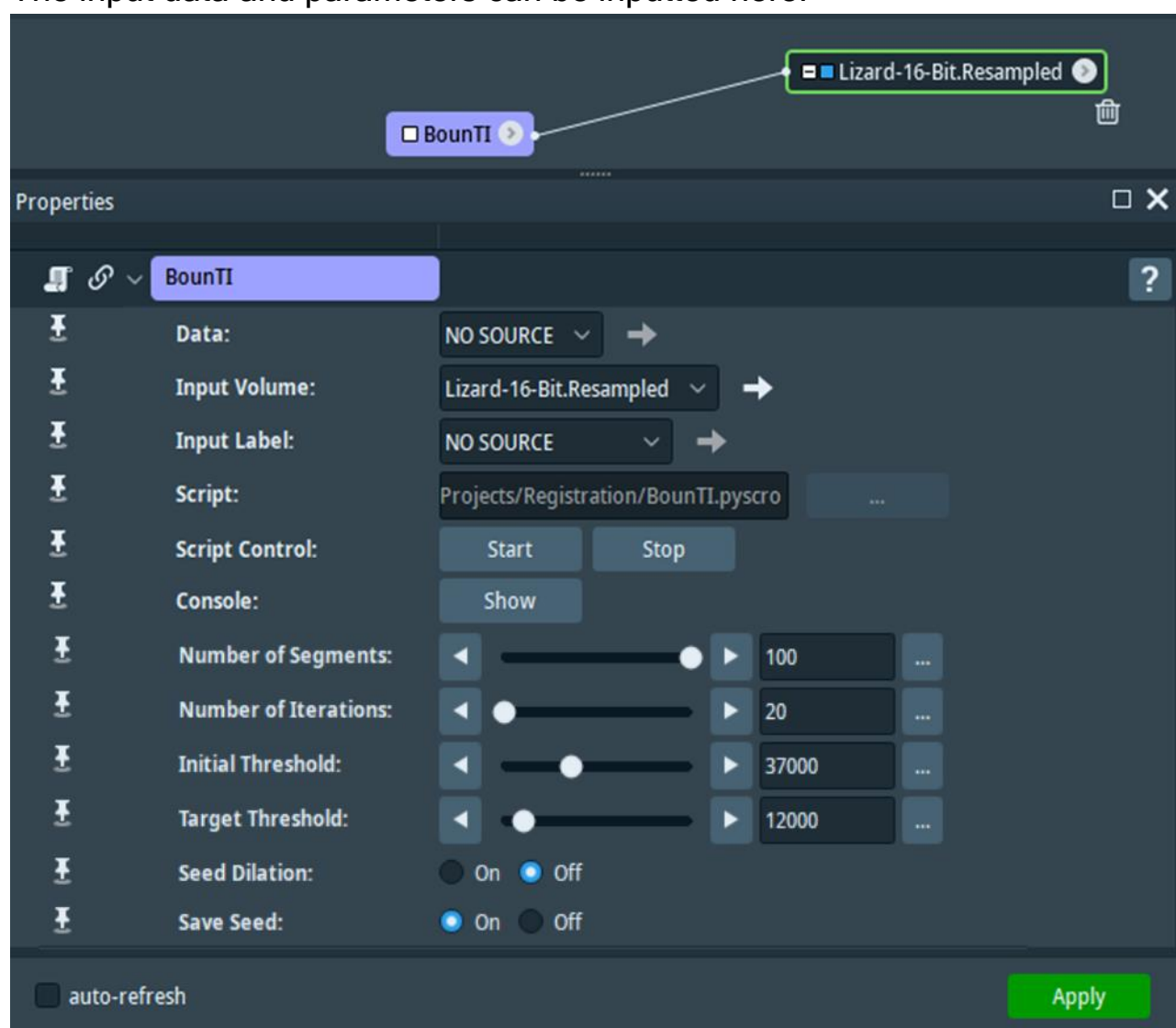
The input volume should be connected to the prepared 16-bit unsigned volume data, while the input parameters should be input into their corresponding boxes. Additionally, seed dilation can be turned on, which dilates the initial segmentation by 1 voxel, this can result in cleaner segmentation but is usually

not required. Save seed allows the initial segmentation to be saved after the segmentation. It should be turned on when attempting to find the optimal parameters as it can be a helpful tool in investigating whether the number of segments is sufficient and whether the initial threshold produces the required separated segments. Apply needs to be pressed to run the segmentation.

Clicking on the resulting segmentation and accessing the Segmentation Tab allows us to investigate the results:
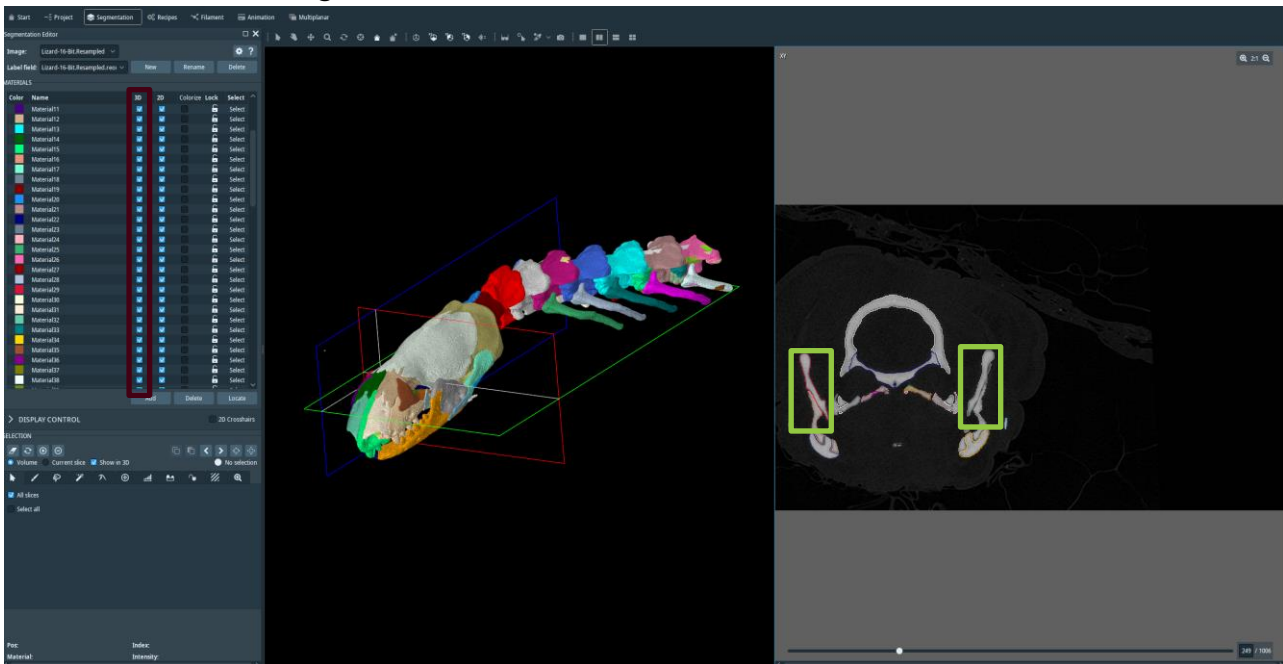

The 3D results can be visualised using the 3D checkboxes (burgundy). While the segmentation is in general accurate, there are some minor areas for improvement. Namely, the mandibular bones on the right have not been separated while on the left they have. To fix this while it may be possible to manual separate the two bones, in the final segmentation, the connection between them is continues and it would take some time to manually disconnect the two bones.

Instead, the seed can be investigated for what went wrong:
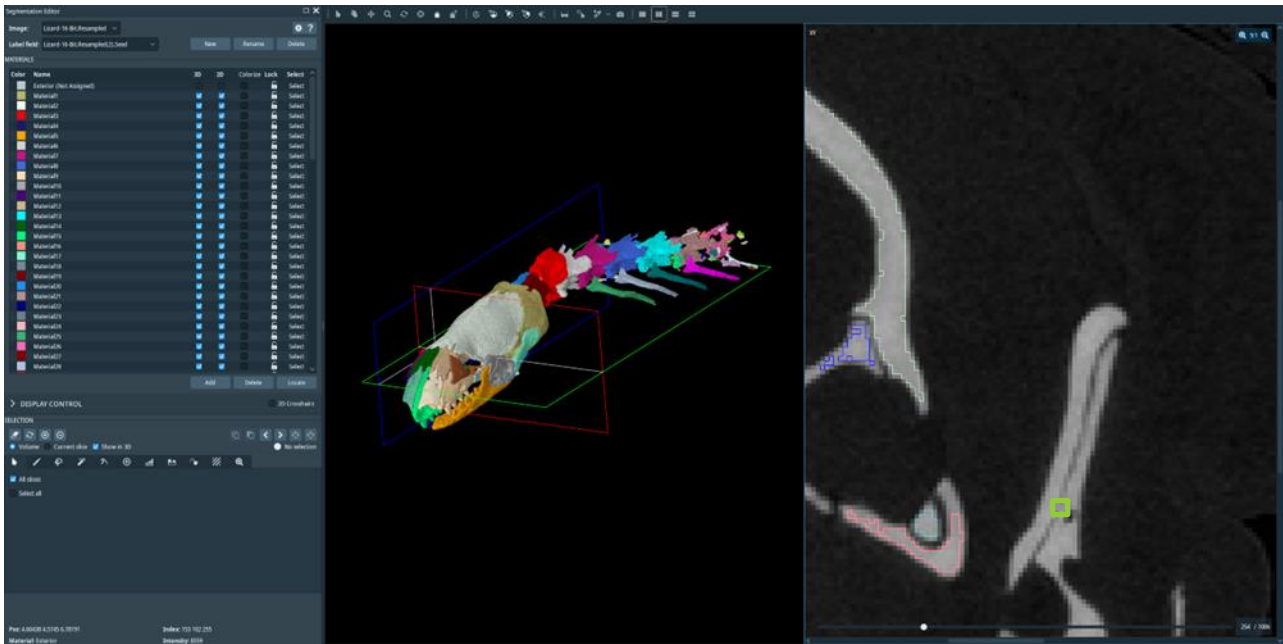
The culprit of this error is a single voxel bridge, while it could be possible to amend this by adjusting the initial threshold, we are already starting to lose some of the final vertebrae. Thus instead we can manually adjust the seed using the brush tool (green) and subtracting the selection from the material (burgundy):


https://github.com/Didziokas/BounTI
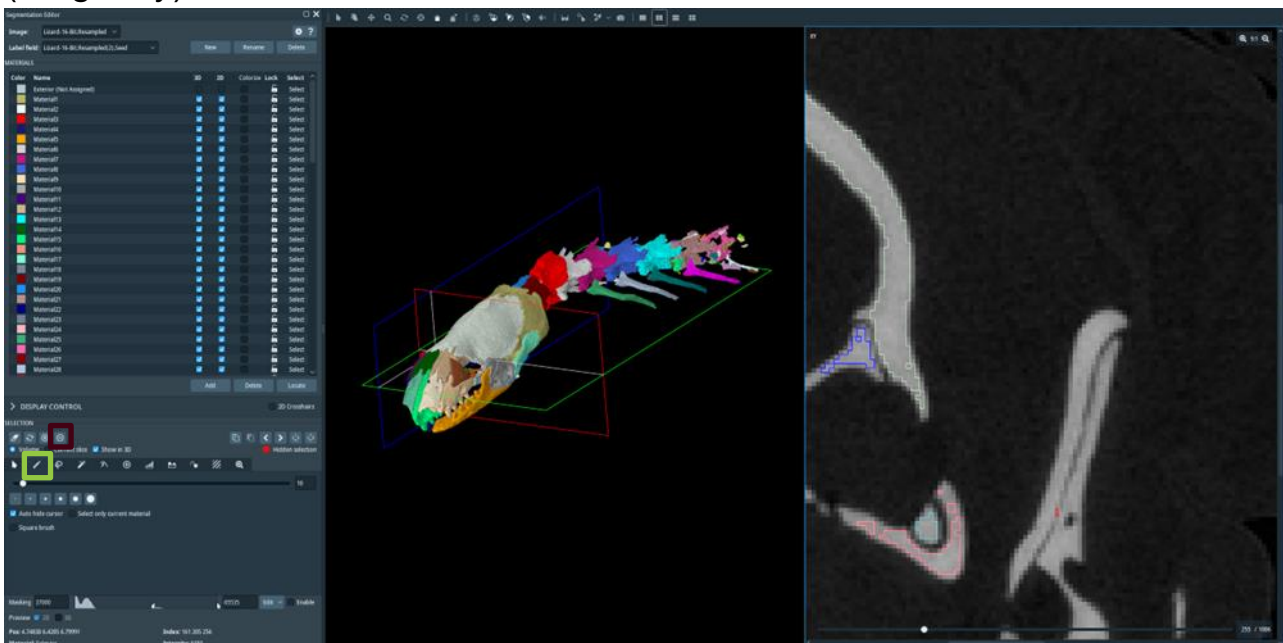
Using the pick tool (green) we can select the newly disconnected segment, add a new material (burgundy) and add it (pink) to that material:
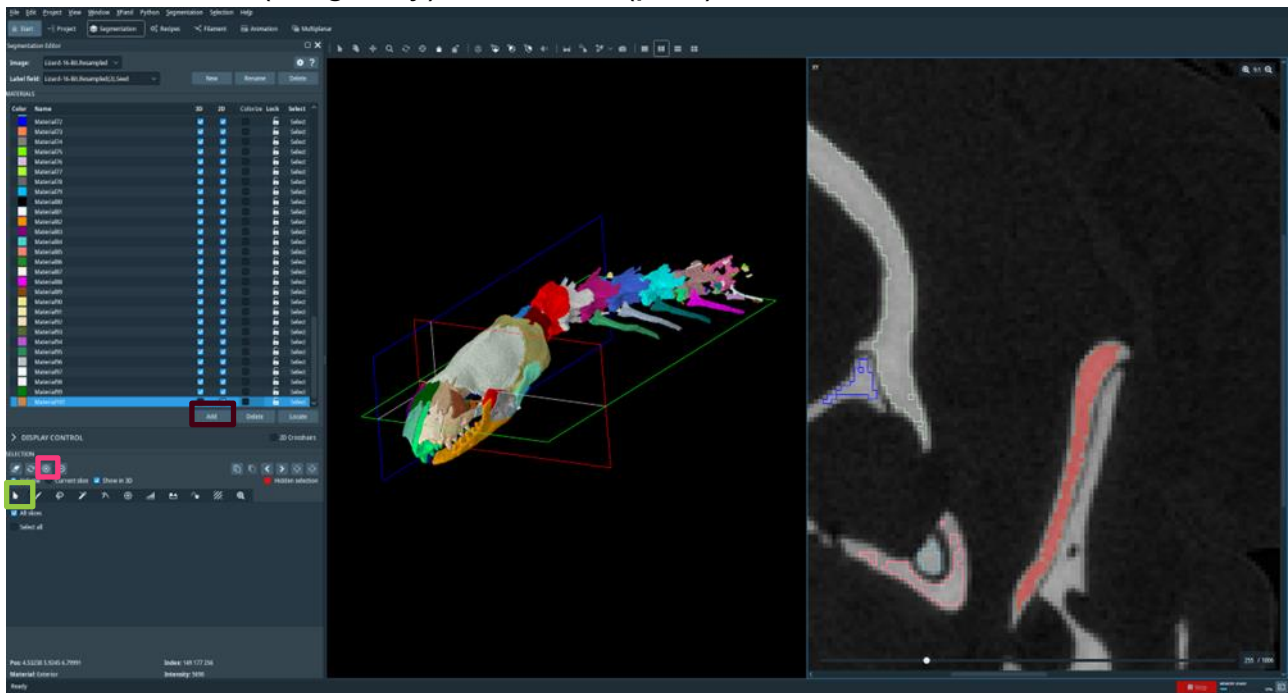
With this, the seed can be used as an input label (green) for BounTI:
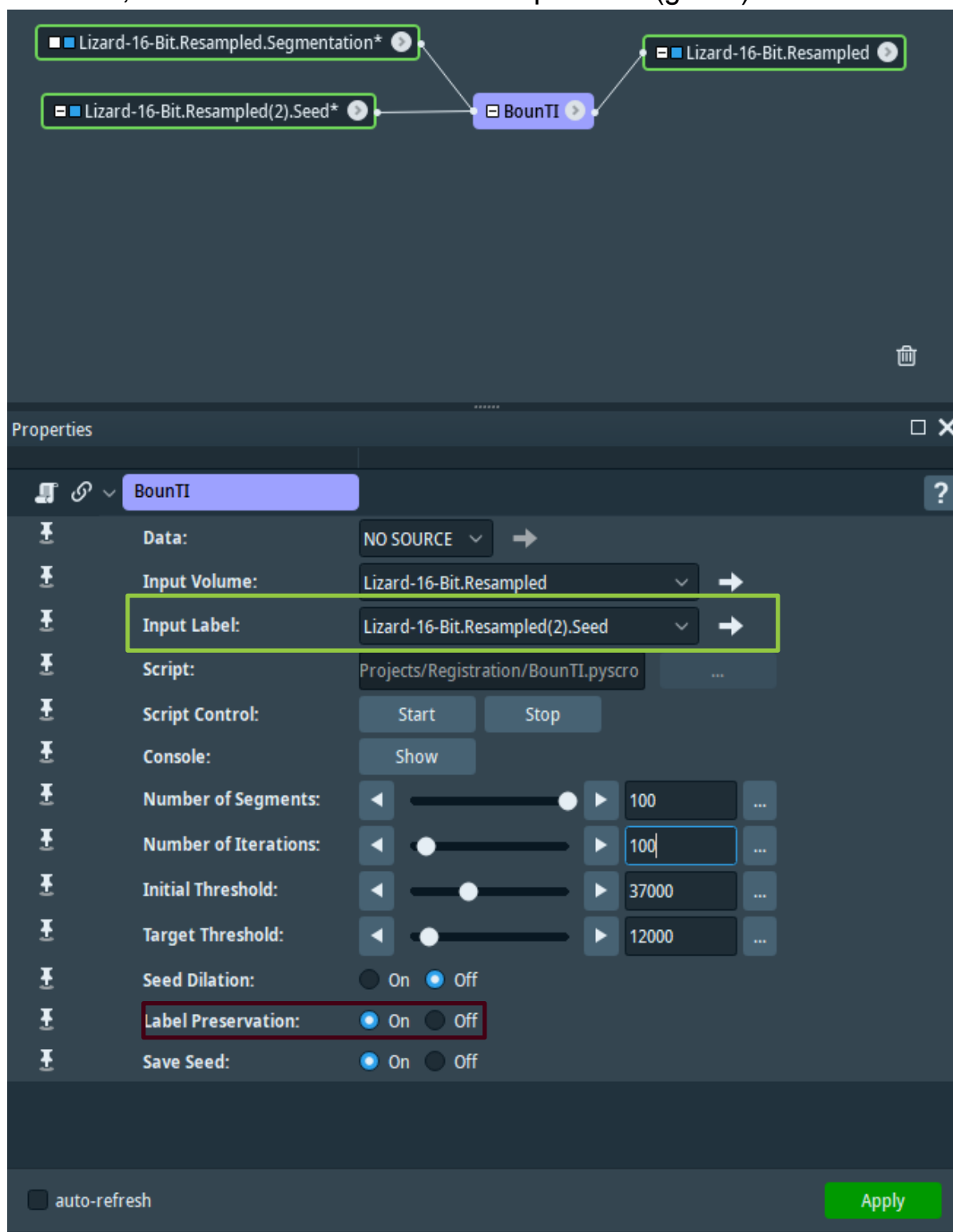
A new option appears – label preservation (burgundy). If the option is on. The segments will remain the same, if it's off the segment will again be obtained by getting the largest separate segments from the seed. Here it is left on. The number of iterations has now been increased to 100 as apart from that one erroneous connection we are happy with the results.

https://github.com/Didziokas/BounTI
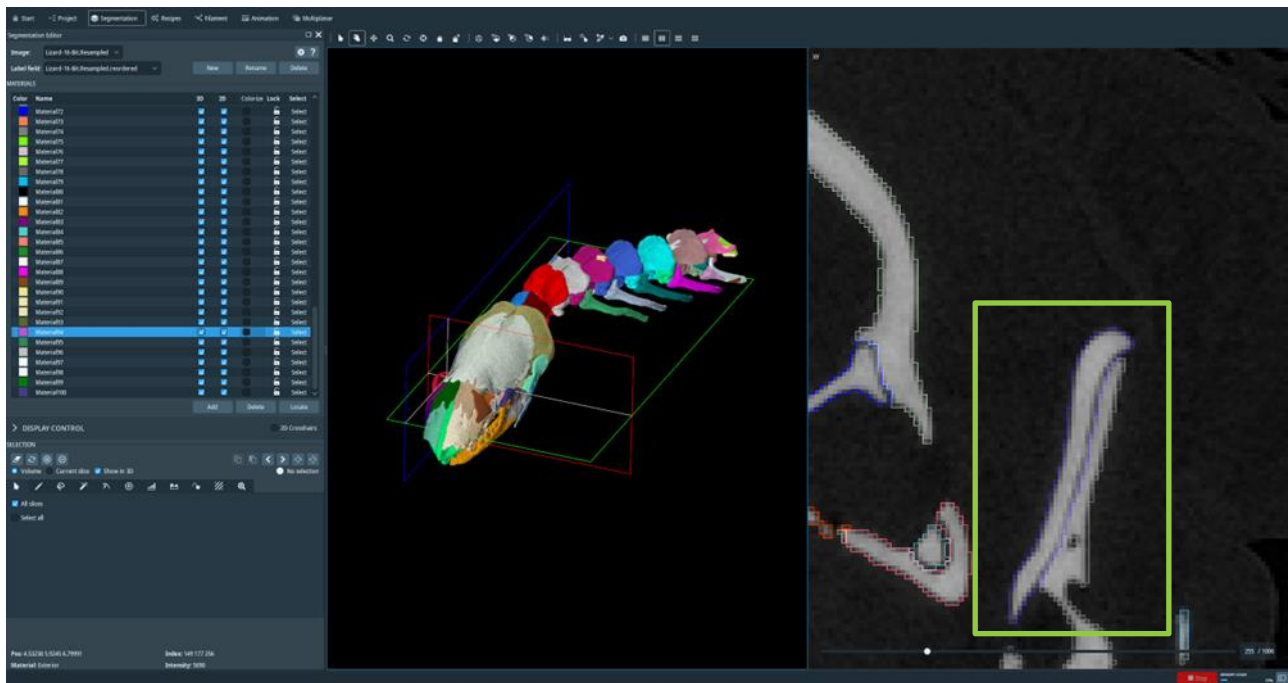
The result now produced the desired separation of the mandible bones without significant manual effort. However, some segments are now erroneously disconnected, namely in the vertebrae.
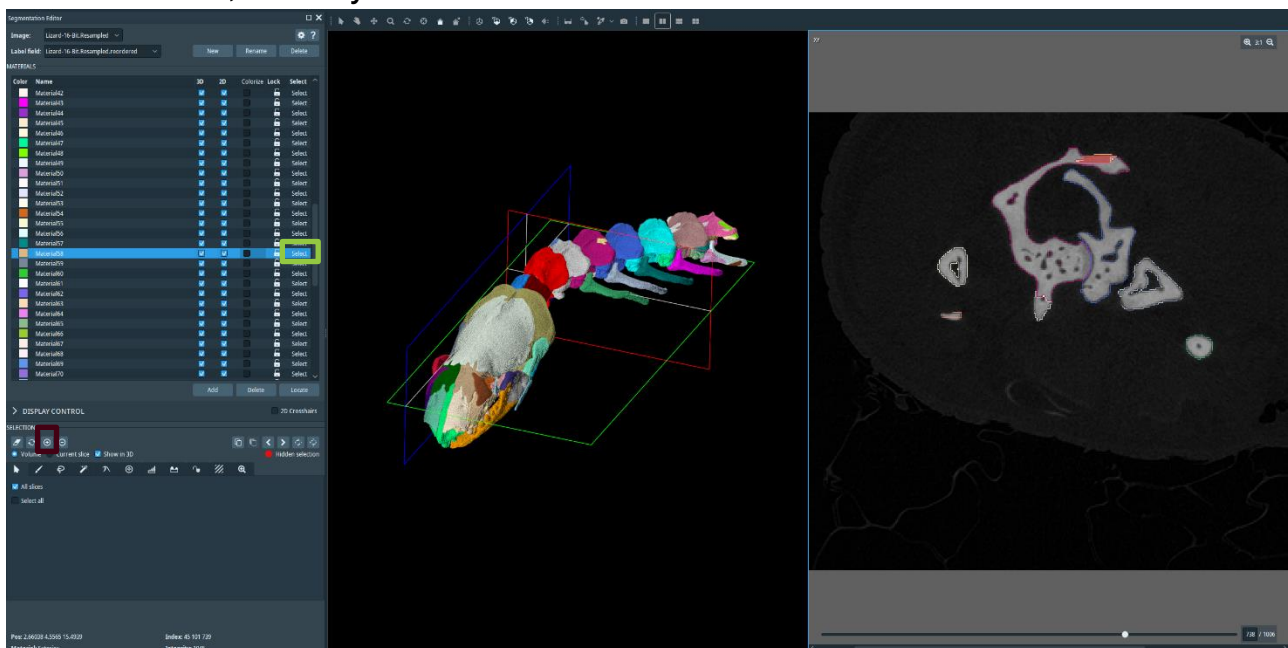
The offending segments can be either manually selected (green) and added (burgundy) to the correct material or the number of segments can be reduced. However, this would require additional computational time, thus often it is more convenient to quickly join the erroneously disconnected elements.

https://github.com/Didziokas/BounTI

Optionally smooth labels can be used:
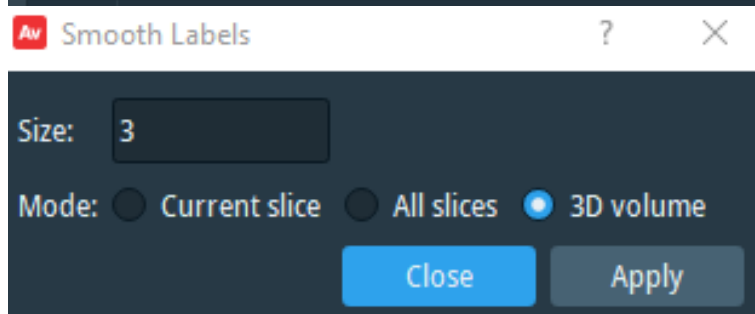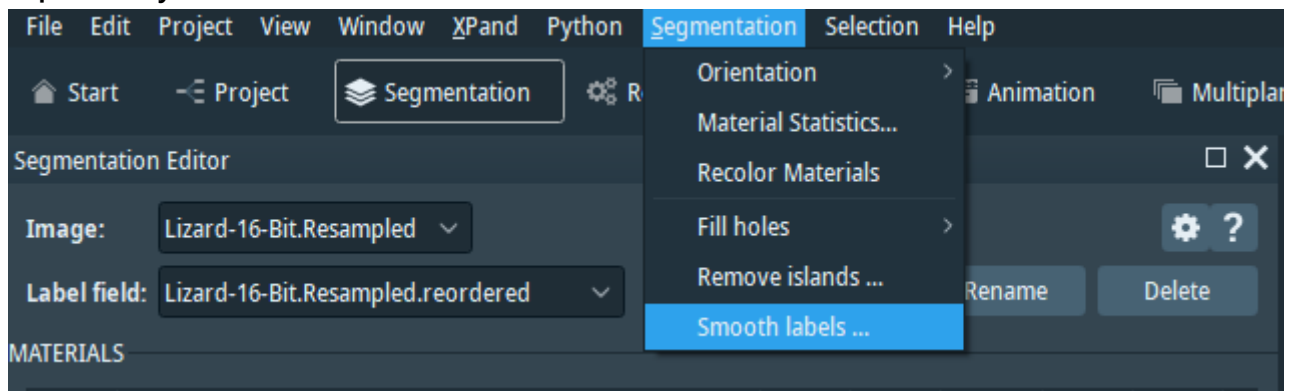
Before	After

This operation in addition to smoothing the labels, more importantly, tends to clean any inaccuracies in the boundaries. (See before and after smoothing)

With this, your segmentation is finished it can be investigated directly, surfaces can be generated or further manipulation can be done which will be discussed in the Further Analysis Suggestions section/

https://github.com/Didziokas/BounTI

WINDOWS EXECUTABLE

See Avizo add-on guide for an in-depth explanation as all of the parameters are the same. The only difference is that the volume data should be exported as a 3D tif from your preferred image processing software after the determination of the initial parameters.

All of the parameters required here are the same as the ones in the Avizo add-on.

PYTHON LIBRARY

The Python library can be installed through pip or on [GitHub](#). With the pip install bounti command.

```
bounti.volume_import(volume_path, dtype = np.uint16)
```

volume path – path to the volume (string)
dtype – NumPy data type default = np.uint16 (np.dtype)

Is a built-in function to import volumes and convert them to a 16-bit unsigned NumPy array. However, other methods can be used as long as the final output is the NumPy array. This can allow experienced users to manipulate the data and determine input parameters computationally.

```
bounti.segmentation(volume_array, initial_threshold, target_threshold, segments,
iterations, label = False, label_preserve = False, seed_dilation = False)
```

volume_array – 3D NumPy array of the volume data (np.uint16)
initial_threshold – value used as the initial threshold (int)
target_threshold – value used as the target threshold (int)
segments – number of segments to be created (int)
iterations – number of iterations (int)
label – label array default = False (bool(if unused)\np.uint16)
label_preserve - whether the label numbering and boundaries should be preserved if False the label connectivity will be recomputed default = False (bool)
seed_dilation - whether the seed segments should be expanded by one voxel default = False (bool)

Further Analysis Suggestions

Manual segmentation producing comparable results to BounTI can be prohibitively time-consuming and is usually done as a one-off. However, with the functionality of the tool, it is possible to segment much greater numbers of volumes than previously. This section aims to introduce some further processing that can be done with the obtained segmentation. These include visualisation, soft tissue joint introduction and bone volume analysis in Avizo. This is not meant to be a comprehensive list of what can be done with the segmentation instead some suggestions which may be of use to some users.
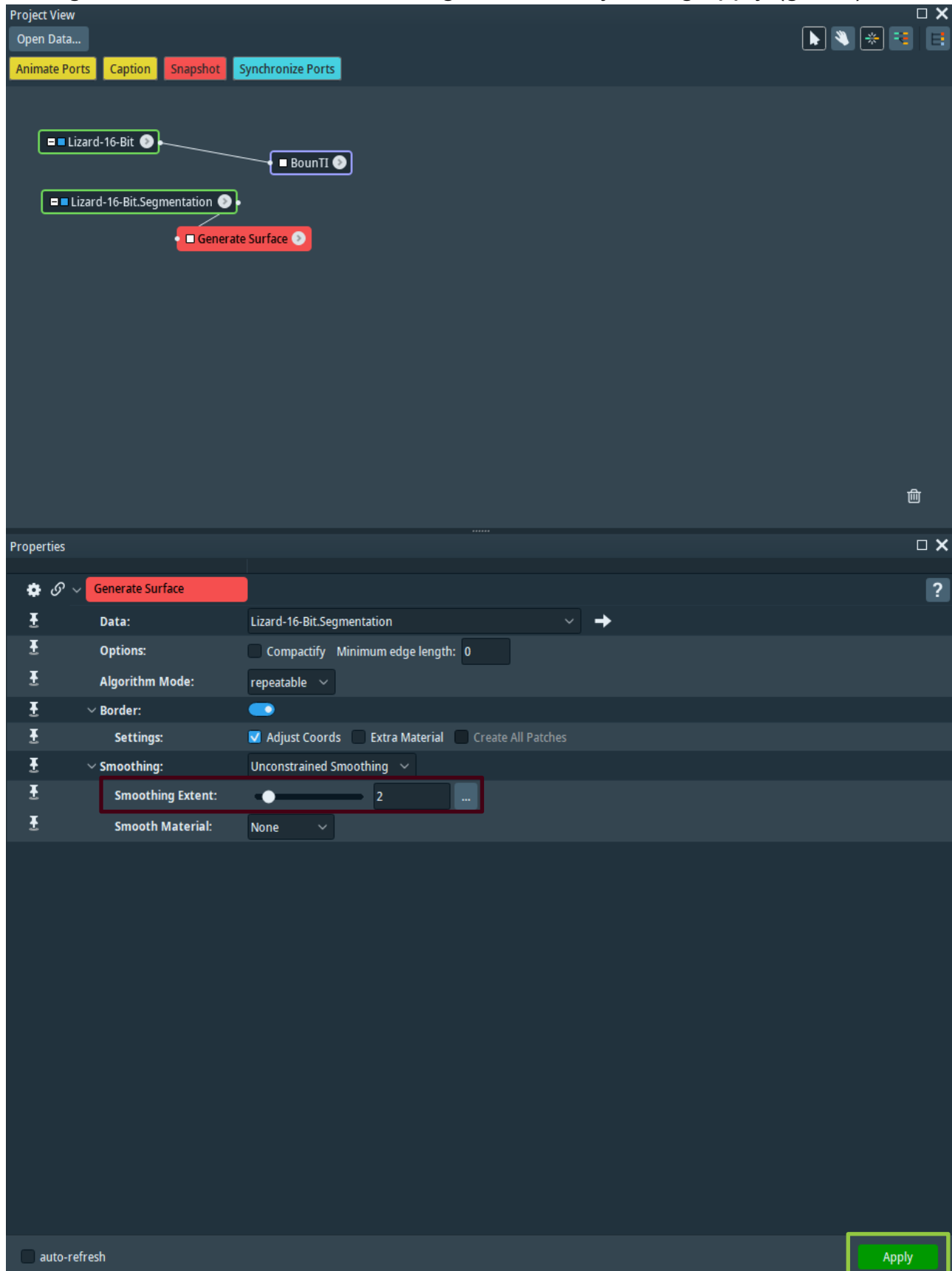
Visualisation

While many different ways of visualisation can be used with the obtained segmentation to show different aspects of interest. The generated surfaces
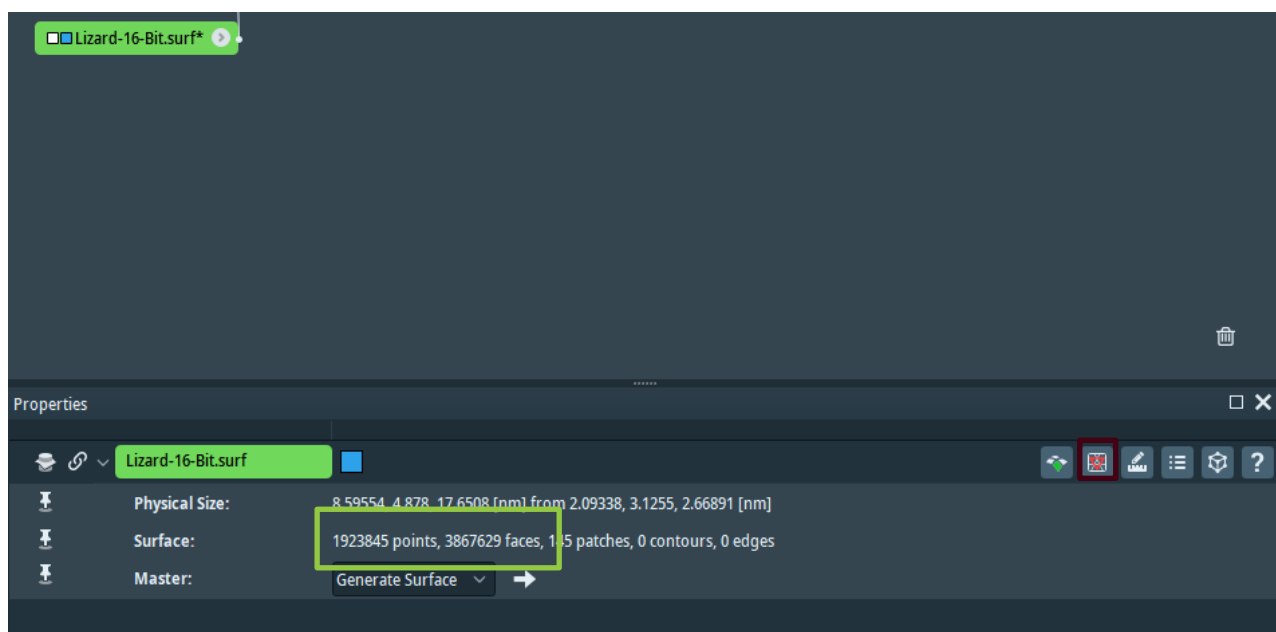
pair particularly well with [Explode Surface Animation Script](#) Avizo Extras addon developed by .

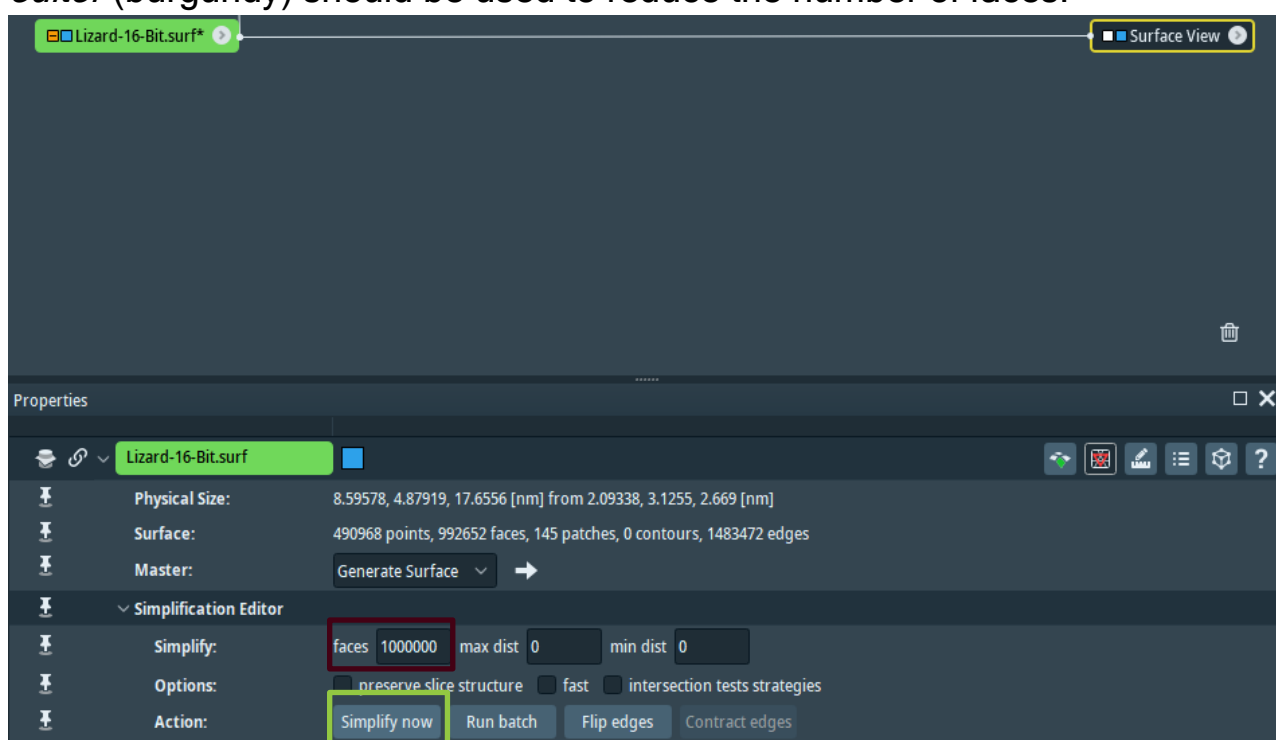First generate a surface from the segmentation by hitting apply (green):

The default *Smoothing Extent* (5) tends to be too much and values of 2 or 3 should be selected if smoothing is desired.

The generated surface in Avizo will be extremely large (green). *Simplification editor* (burgundy) should be used to reduce the number of faces:


The number of faces should be reduced. For this type of model 1000000 faces (burgundy) usually are sufficient to capture the surface while at the same time reducing the mesh to a usable size. Apply the simplification (green). The mesh can be further regularised using the *Remesh* module.

The explode surface script object can be imported by dragging and dropping it into the Project View:
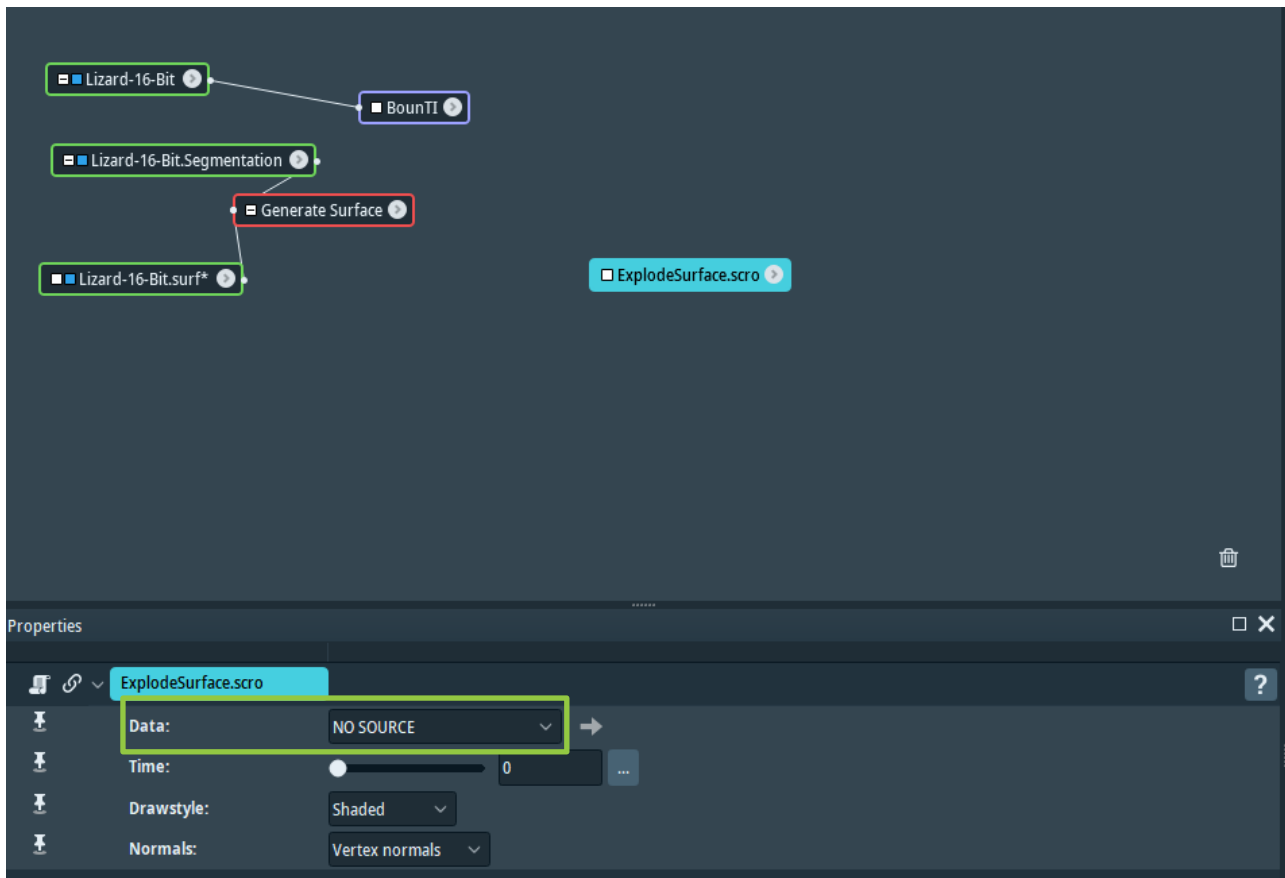
Set Data (green) to the generated surface:
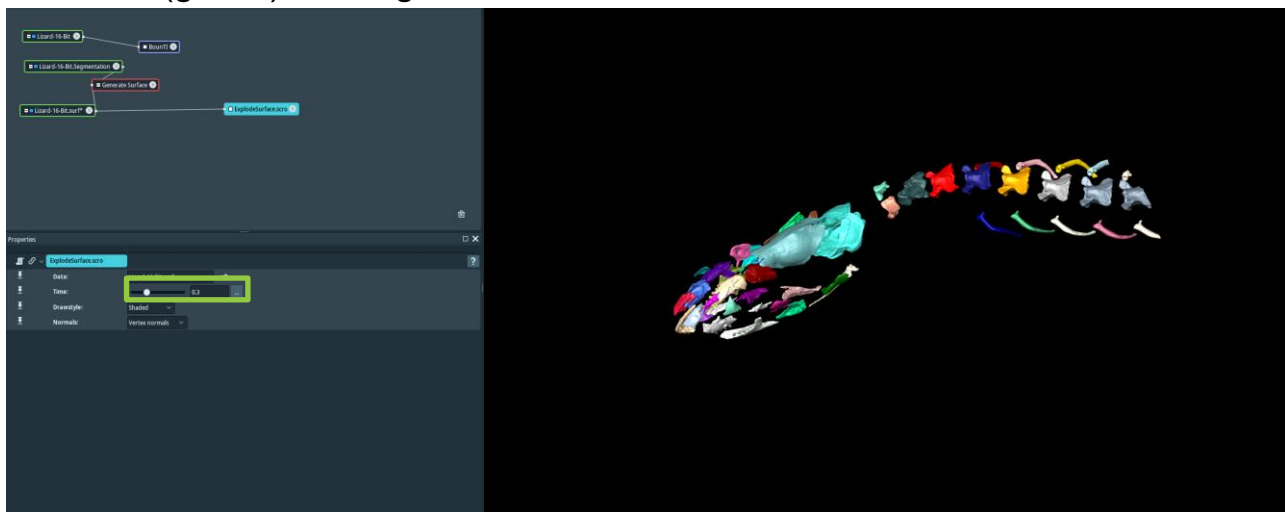
By adjusting the Time parameter (green) the degree of explosion can be defined. This add-on works particularly well with animations.

Soft tissue joint generation

Soft tissue joints can be introduced to the segmentation by determining the contact voxels of different bone segments and setting those as sutures. This has severe limitations as it means that the segments need to be in direct contact to detect a suture. Hence, the approach laid out here will only work on well on adult specimens, where the suture gaps are relatively small.

https://github.com/Didziokas/BounTI
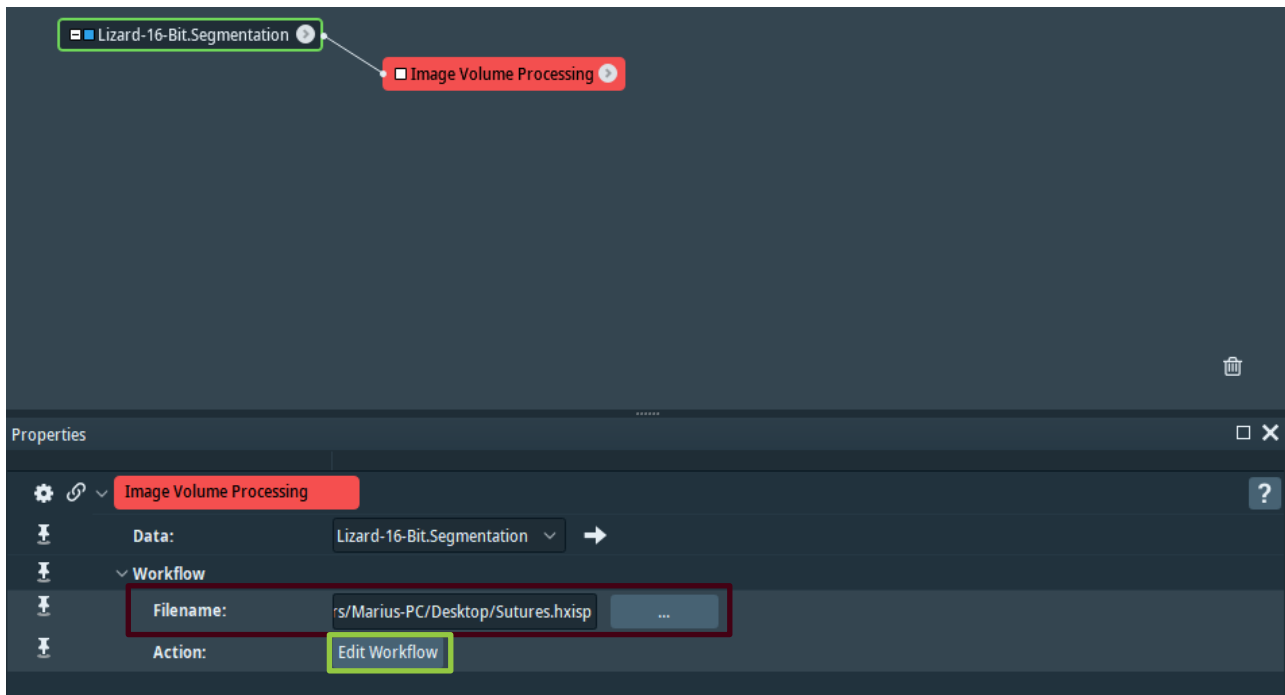
Image Volume Processing module should be used on the segmentation. A workflow file has been prepared and can be obtained from the GitHub repository. The steps to obtain the sutures can be seen by editing the workflow (green).
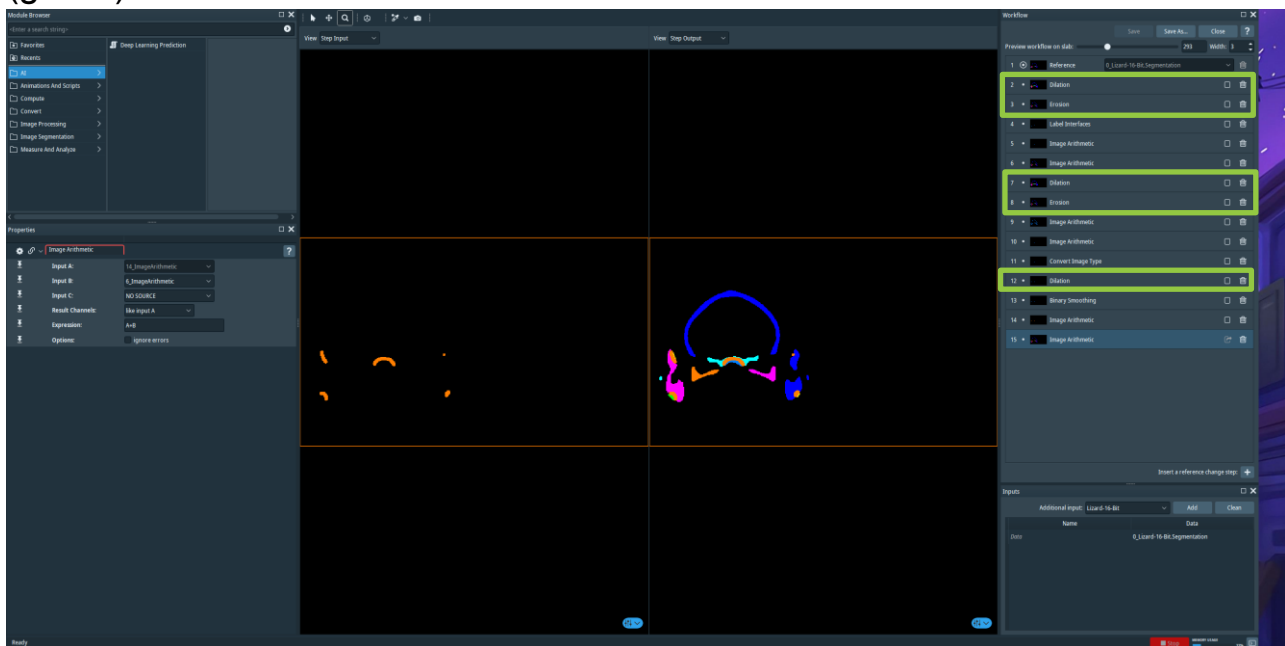

To adjust the aggressiveness of the sutures, I.E how large are they and how large of a gap should be considered a suture. The dilation and erosion parameters can be adjusted (green). All the steps done here are purely arithmetic and should be recreatable outside Avizo. The results of suture extraction workflow:
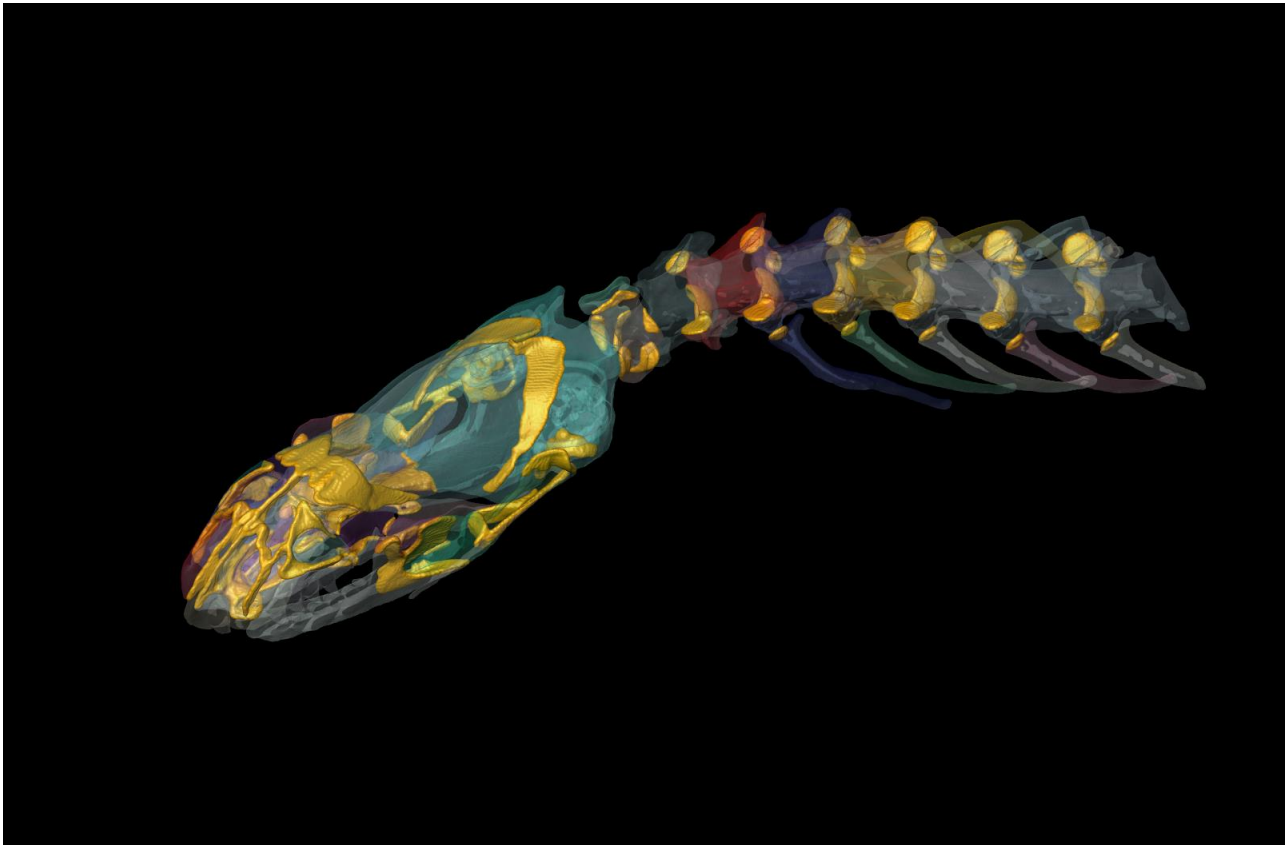
While some manual adjustments to the soft tissue joints may still be required the workflow at the very least significantly reduces the manual input.

Bone Volume Analysis

Volume Fraction module in avizo can be used to directly investigate the volume of each segment:
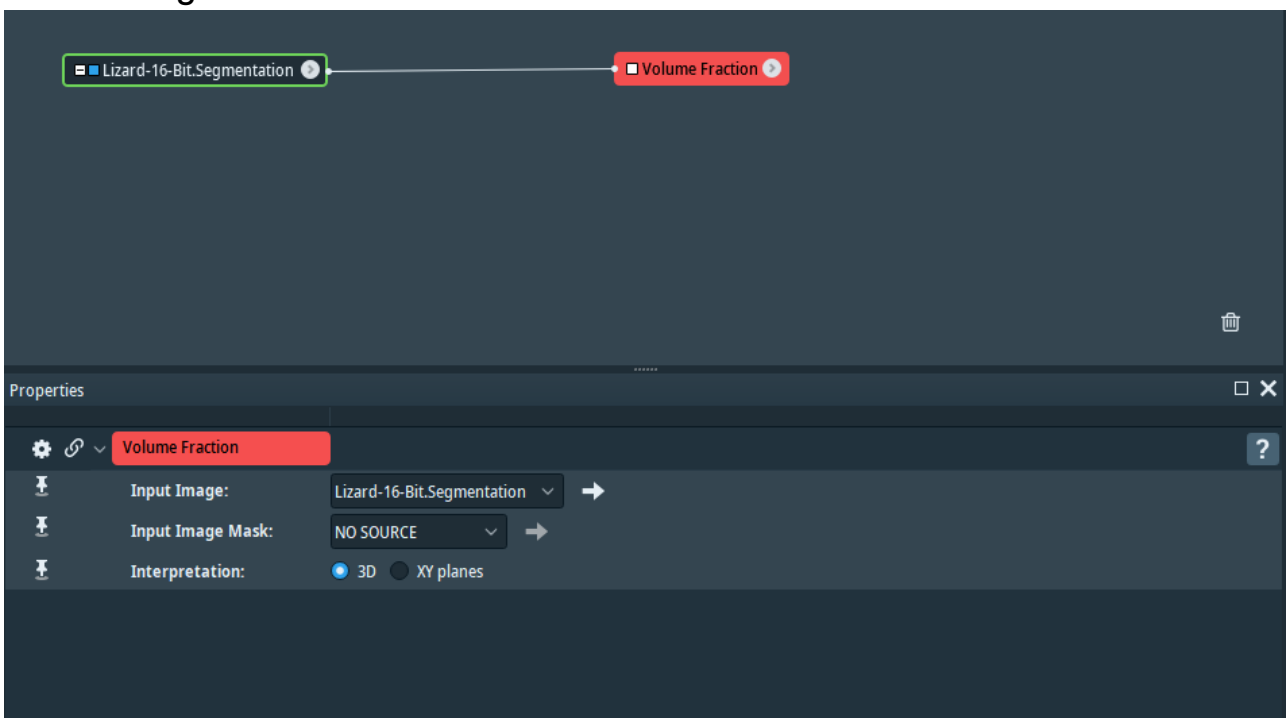
This can be done in other software and with code directly by calculating the number of voxels in each segment.

https://github.com/Didziokas/BounTI