FACHHOCHSCHULE
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MicroCT Segmentation Guide for Mice

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Houndsfield unit (HU) A mapping of the linear attenuation coefficient of the X-ray beam on to an arbitrary scale called Houndsfield scale[1]. 9

MRB Medical Reality Bundle (.mrb, .zip): MRB is a binary format encapsulating all scene data (bulk data and metadata). Internally it uses zip format. [2]. 13

OpenVR "[...] an API and runtime that allows access to virtual reality hardware from multiple vendors without requiring that applications have specific knowledge of the hardware they are targeting"[3]. 4

ROI Region of Interest. 51

VRAM GPU memory. 4

1 Introduction

This guide is intended to help newcomers get started with segmentation tasks in a software called "3D Slicer". 3D Slicer is a comprehensive and fully featured program for visualization, processing, segmentation, registration and analysis of 3D datasets. Unfortunately, this also means that it can be overwhelming for someone who lacks experience working with such tools. This guide aims to alleviate this issue at least for segmentation workflows. It tries to explain tools and possible workflows in an easy-to-follow manner and enable the reader to complete their segmentation without needing to watch multiple tutorials or read bits of documentation scattered around the internet.

1.1 Using the guide

1. **Jump around using links:** The guide makes extensive use of hyperlinks, which enable the reader to easily jump to important information. Make use of this feature to save yourself some time. Note that most but not all PDF readers will support this feature.

2. **Jump to information you require:** This guide covers many tools and functionalities of 3D Slicer. You will most certainly not use every tool. Use the table of contents to jump to tools that are important for the task at hand.

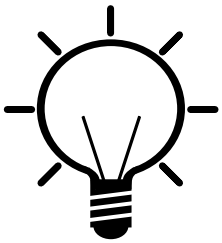
3. **Make a backup before experimenting:** Sometimes 3D Slicer makes it difficult to undo an operation. If you wish to experiment with a tool you have never used before it is advisable to duplicate your dataset and save it to a separate file. So that in case something does not turn out the way you intended, and you do not know how to undo the unwanted change, you can always load a known good copy of your dataset.

1.2 Conventions used in this Guide

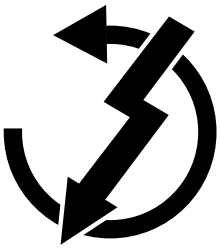
(a) 2D symbol (b) 3D symbol

Figure 1: 2D (figure 1a) and 3D (figure 1b) symbols signifying if a tool can be used in 2D or 3D

If you are already familiar with 3D Slicer and want to start with the segmentation right away, skip to page 47 for the Quick Start Guide.

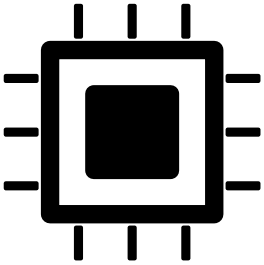


(a) Hint symbol

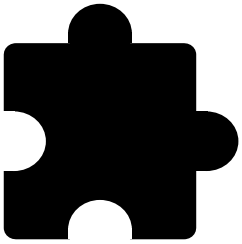


(b) Irreversible symbol

Figure 2: Hint (figure 2a) symbol signifying tip or hint and Irreversible(figure 2b) symbol signifying irreversible operation



(a) Performance symbol



(b) Plugin symbol

Figure 3: Performance(figure 3a) symbol signifying resource intensive operation and Plugin (figure 3b) symbol signifying 3rd party plugin tool

1.3 Installation

Basic installation instructions for 3D Slicer:

1. Browse to: `https://download.slicer.org`
2. Download the installer of the latest stable release[1] for your operating system
3. Run the installer and follow its instructions

1.3.1 Installation on macOS

On macOS, 3D Slicer can be installed in two different ways:

Either via the provided package on the downloads page, or via Homebrew: `https://formulae.brew.sh/cask/slicer`
For the conventional installation refer to the 3D Slicer wiki: `https://slicer.readthedocs.io/en/latest/user_guide/getting_started.html#mac`

[1] Version 5.6.2 at the time of writing this document

1.3.2 Installation on Linux

3D Slicer ships with all its dependencies on Windows and macOS.
On Linux it is required to install some dependencies via your distributions package manager.
The 3D Slicer wiki gives information about required packages and their respective names on different distributions: `https://slicer.readthedocs.io/en/latest/user_guide/getting_started.html#linux`.
To make this process easier, this guide provides a distrobox manifest file (see page: 50), which allows the user to create a Linux container holding all necessary dependencies without polluting the host system.

To run 3D Slicer via the container run:

```
distrobox assemble create --file /path/to/manifest_file.ini
distrobox enter slicerbox -- ./path/to/Slicer/binary/Slicer
```

1.4 System Requirements[4]

Note: as of November 19, 2024

1.4.1 Official supported operating systems

- Windows: 10 or 11
- macOS: 11 or later
- Linux:
  - Ubuntu 20.04 or later
  - Debian 10 or later
  - Fedora 35 or later
  - CentOS 7 or later

1.4.2 Hardware requirements

- Memory: At least 4 Gigabytes, more is strongly recommended.
  The 3D Slicer Wiki mentions needing about 10 times more memory than the amount of data you plan to load.
  3D Slicer on its own uses about 456 Megabytes of RAM. After loading a 26.17MiB Dataset it consumes about 614 MB.
- CPU: 3D Slicer uses multi-threading for some calculation and will thus benefit from a multicore CPU.
- GPU: A dedicated GPU is recommended but not required as 3D Slicer only uses it for interactive volume rendering. If you restrict your usage to the 2D views, you will hardly ever use your graphics card.

1.4.3 Hardware recommendations

The following points are not strictly needed in order to use 3D Slicer, but they make working with the software a lot easier.

- Input devices: 3D Slicer supports mice, touchpads, pens, graphic tablets and OpenVR headsets. The easiest input method though is a 3 button Mouse with a mouse wheel.
- Internet connection: for downloading extensions, online documentation and sample data sets.
- VRAM: for interactive volume rendering it is recommended to have more GPU texture memory than the data set you plan to load.
- A large display or monitor with a decent screen resolution. A 14-inch screen or larger is strongly advised. Screen resolution does not need to exceed 1920x1080, but going below 720p is also not advisable.

1.5 Interface and Usage

Upon first launch you will be greeted by the 3D Slicers welcome screen (figure 4) The welcome screen has some quick links to useful modules like load data and

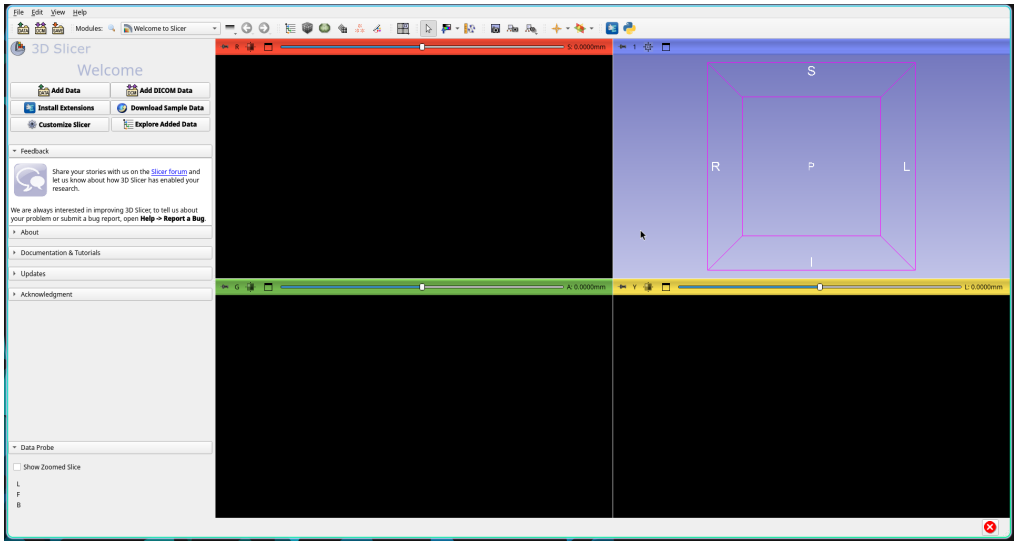


Figure 4: 3D Slicer welcome screen

download datasets.

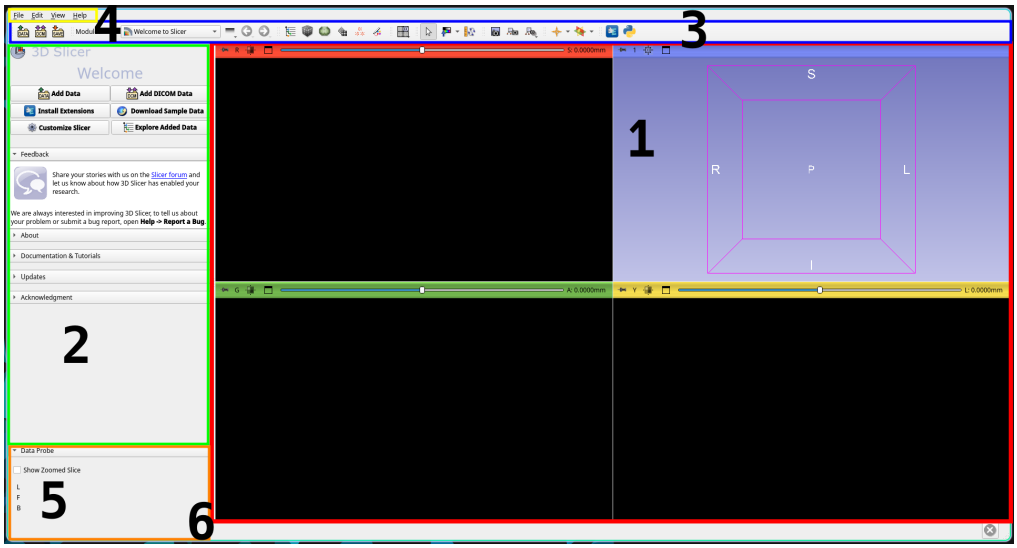


Figure 5: 3D Slicer window

The 3D Slicer window consists of several areas, which provide different tools to interact with the program. A quick overview of these areas is provided by figure 5:

1. View area
2. Module panel
3. Toolbar
4. Application menu
5. Data probe
6. Status bar

1.5.1 The view area

By default 3D Slicer shows the view area (figure 6) with three 2D slice views and the interactive 3D view at the top right.

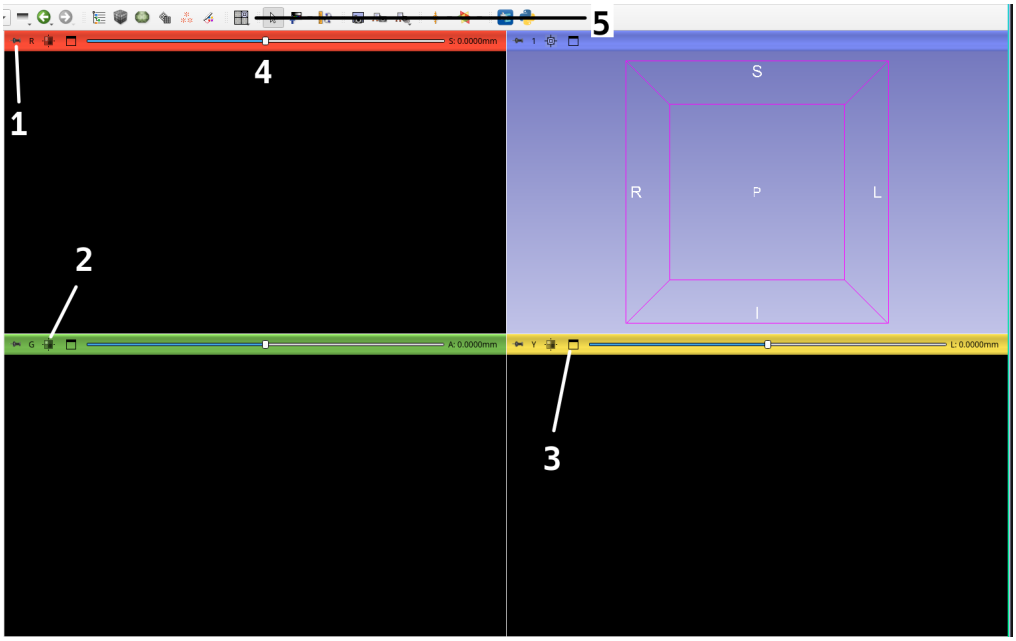


Figure 6: 3D Slicer view area

3D Slicer calls this the `Four-up` view.

There is a numerous amount of view presets to choose from by clicking on the `change view` option in the toolbar: figure 6:5.

Each slice view has its own scroll bar (figure 6:4). The number on the right end of the scroll bar shows by how much a single tick scrolls through the dataset. In other words: it shows the slice thickness of the view.
Scrollbar controls:

- left click and drag the scrollbar
- mouse wheel
- `B` and `F` Keys
- up, down, left and right arrow keys

To maximize a single slice view, click on the `Maximize view` button (figure 6:3). Click it again to return to the previous layout. Double left-clicking on the slice has the same effect.

'Reset FOV' can be used to reset the zoom level and shift the slice into its isocenter (figure 6:2). The `R` key has the same effect.

The pin icon (figure 7:1) brings up a submenu.
Here (figure 7:2) it is possible to quickly change the displayed dataset if more than one has been loaded.
Change the displayed slice plane by clicking on figure 7:3.
Toggle slice visibility in 3D views: figure 7:4.
Clicking on the `double arrow` button (figure 7:5) will reveal more configuration options, which this guide will not cover, as they are not usually needed for segmentation tasks. However, the 3D Slicer documentation provides more information about this submenu: `https://slicer.readthedocs.io/en/latest/user_guide/user_interface.html#slice-view`

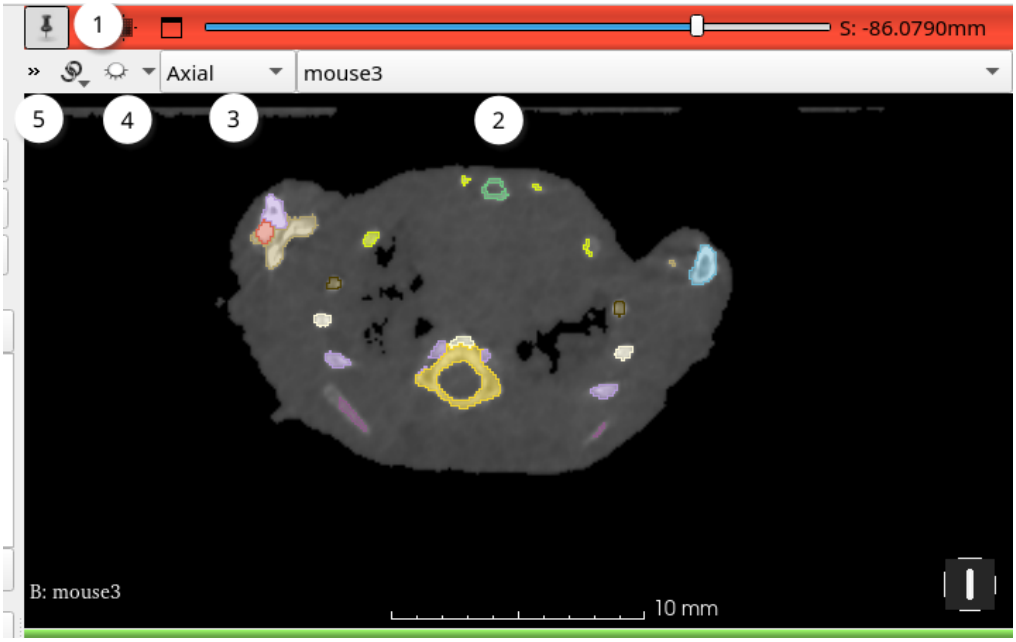


Figure 7: 3D Slicer pin menu

1.5.2 The module panel

Figure 5:2 the module panel displays options, tools and information of the currently loaded module.

1.5.3 The Toolbar

Figure 8: 3D Slicer toolbar

The far left end of 3D Slicers toolbar holds buttons for loading and saving data. Loading data from a file or directory can be done via the `Add Data` button' figure 8:1.
Keyboard shortcut: `Ctrl + o`
The next button (figure 8:2) loads the `Add DICOM Data` module, which can also load data from files or directories and save it to 3D Slicers on disk DICOM database.
Saving data can be done via clicking on the `Save Data` button (figure 8:3).
Keyboard shortcut: `Ctrl + s`
Figure 8:4 marks the module toolbar. Clicking on the lens button opens a full text search for all installed modules.
Keyboard shortcut: `Ctrl + f`
Loadable modules can also be found via the dropdown list right next to the lens icon. 3D Slicer keeps track of the recently used modules and allows to quickly return to the most recent eight[2] modules. Either via the `Modules history` dropdown list or the forward and back buttons (figure 8:4). The toolbar has a designated part for `Favorite Modules` (figure 8:5), which can be fully customized via the settings menu.
Figure 8:6 can be used to change the view layout, like mentioned in section 1.5.1.
3D Slicer has different mouse modes, where depending on the selected mode, the mouse has different functionalities. By default, `translate/rotate view` is selected (figure 8:7).
Keyboard shortcuts:
`right click + mouse drag` to zoom in and out.
`Ctrl + mouse wheel` to zoom in and out.
`Shift + left mouse click + drag` pan view.
`Shift + move mouse` display ROI under mouse in all views, 3D Slicer calls this the crosshair.
The icon to the right is called `adjust window/level`.
`Left click` and drag to create a ROI, 3D Slicer then tries to find the optimal window and center for that ROI.
Alternatively hold `Ctrl` and `left mouse`, then drag the mouse to adjust the window manually.
The final button of this part of the toolbar toggles an additional markups toolbar on and off, this guide will not go into further detail as this is not needed for segmentation.

[2]the amount can be configured in the settings menu

3D Slicer has a build in screenshot and screen recording module as can be seen in figure 8:8.
To toggle visibility of the crosshair, that has been mentioned above the left button in figure 8:9 can be used. The button to the right of that toggles slice intersection lines on and off.
Finally, on the right end (figure 8:10) of the toolbar 3D Slicer has buttons to open a Python console window and opening the `Extension Manager`.

1.5.4 The Application Menu

See figure 5:4.

- `File`: Loading and saving data, download sample data, unloading data and close 3D Slicer
- `Edit`: Cut, Copy, Paste and the `Settings Menu`
- `View`: Show or hide additional windows and toolbars. Open the `Extension Manager`, `Python console`, `Error Log` and change the view layout.
- `Help`: Access online documentation.

1.5.5 The Data Probe

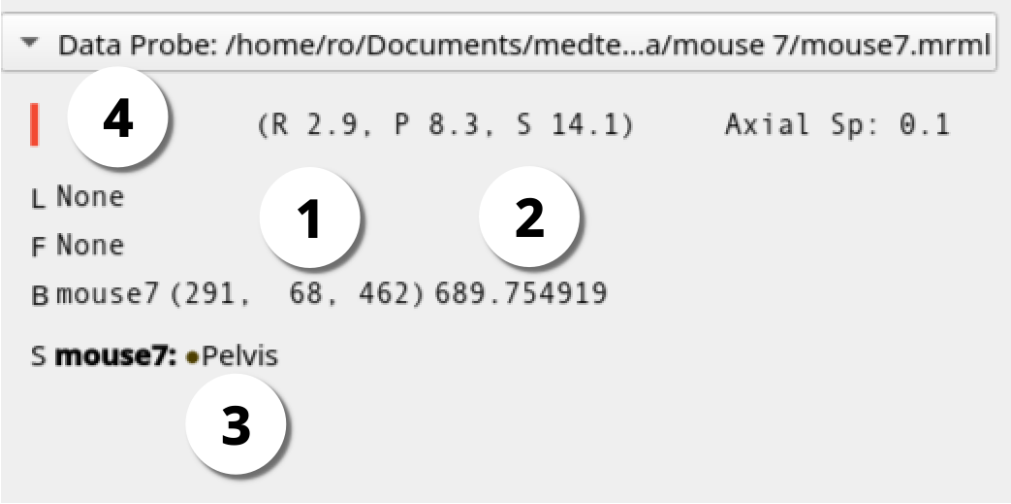


Figure 9: 3D Slicer Dataprobe

The `Data Probe` shows useful information about the data currently being displayed. More specifically it informs the user about the ROI under the cursor position.
To start using it, just hover the mouse cursor over a ROI, if the cursor is moved, the data probe will update in real time.
Most notable information shown:
The numbers in brackets in figure 9:1 display the x-, y- and z-Position of the ROI. After that, outside the brackets, it displays the Houndsfield unit (HU)

value of that ROI (figure 9:2).
In figure 9:3 the data probe shows which segmentation ROI belongs to if any.
Figure 9:4 displays the currently loaded dataset.

1.5.6 The Status Bar

The status bar located on the bottom of the 3D Slicer window, is of no great importance during normal operation. Notably the `X` icon on the far right opens the error log, which may be useful for debugging.

2 Basic usage

From this point onwards, this guide makes the following assumptions:

1. 3D Slicer has been successfully installed
2. The data you wish to work on has already been acquired
3. The computer running 3D Slicer has access to the data

2.1 Loading Data

3D Slicer can load data in two different ways. Loading data from file or folder or load data from folder and save to database (8:2). The database option is advisable if you are dealing with a larger number of studies, as it enables quick switching between studies. If only a single study needs to be loaded, usage of the database is not necessary. Clicking on the `Add Data` Button (8:2) or using the keyboard shortcut `Ctrl + o` brings up a file picker dialogue.

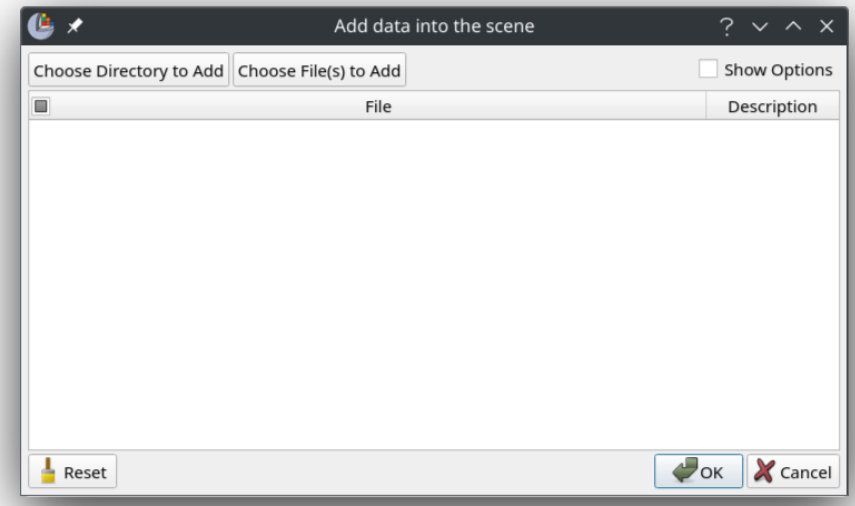


Figure 10: 3D Slicer file picker

Depending on whether the data is contained in a single or multiple files, click either `Choose Directory to Add` or `Choose File(s) to Add`. Browse to your data, select it and confirm your selection by clicking `Choose`. Show additional import options by setting a checkmark at `Show Options`. Here it is possible to load the dataset as a Label map, force 3D Slicer to ignore similar files, automatically center the volume, ignore orientation information in the DICOM header, show or hide the volume and set the color table. After confirming the import, depending on size of the dataset, some patience may be required. If

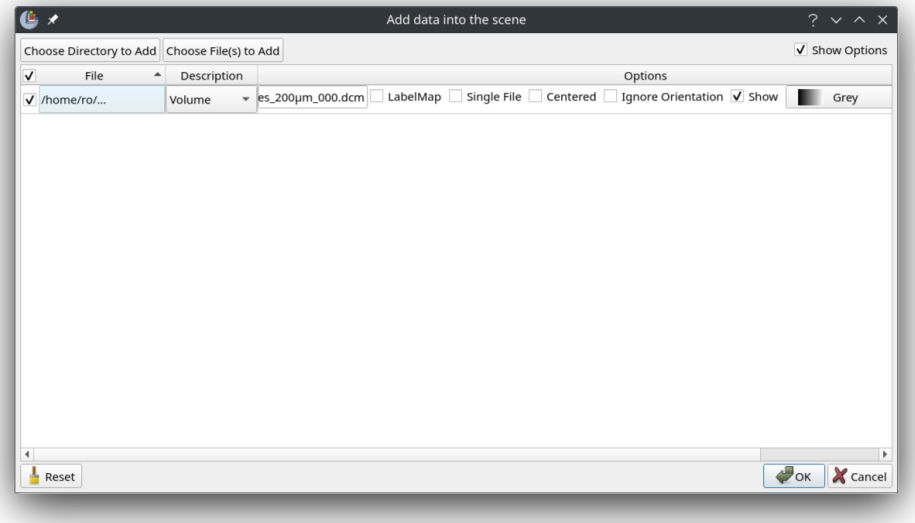


Figure 11: 3D Slicer file picker options

the import was successful and the dataset has not been set to be hidden after import, 3D Slicer will automatically populate the view area.

2.2 Saving Data

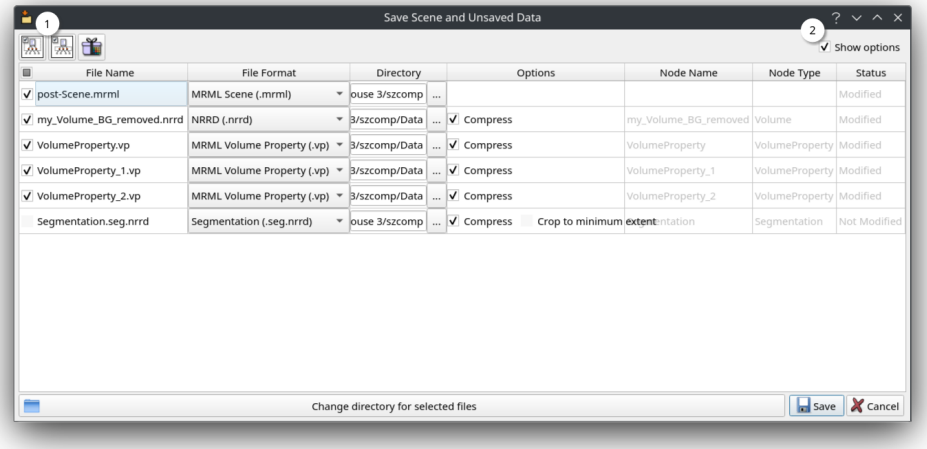


Figure 12: 3D Slicer file save menu

Save your work in 3D Slicer by clicking on the `Save Data` button in the figure 8:3. This will open a popup window which holds information about what

data will be saved and the format it will be saved to. 3D Slicer defaults to bundling up your data into a single file. Change this by clicking on the bundle icon (figure 12:1) and set a checkmark at `Show options` (figure 12:2). The window should now look like Figure 12. The first column shows the file names, which are derived from their names in the `Data` module and their file format.

Let us take a look at what 3D Slicer is going to save:

post-Scene.mrml "MRML file is a xml-formatted text file with scene metadata and pointers to externally stored data files"[2] (Metadata file)

my_Volume_BG_removed.nrrd volume data in a non-DICOM general purpose multidimensional format

VolumeProperty.vp volume property file storing information on volume rendering (Metadata file)

Segmentation.seg.nrrd "Segmentation labelmap representation"[2] storing the segmentation as a multidimensional volume

3D Slicer defaults to compressing all data before saving and there should be no reason to turn this behavior off. The second column is dedicated to the file format of the mentioned files. 3D Slicer supports a number of different file formats (see: `https://slicer.readthedocs.io/en/latest/user_guide/data_loading_and_saving.html#supported-data-formats`). However, exporting for example a volume or segmentation in a different format, say STL for 3D printing, I recommend choosing the `Data` module over changing file formats in the save dialogue. Compression can be turned off or on per file in the option's column. And somewhat importantly, the last column, the status column, shows if a file has been modified since the last save.

Clicking on the "Bundle" icon (Figure 12:1) collapses the file view, as this instructs 3D Slicer to not save individual files, but bundle them in a MRB file.

Pick a directory by clicking on `Change directory for selected files` and confirm via the save icon. Depending on the file size this operation may require some patience.

2.3 Addressing Performance issues

If loading the data took a long time or scrolling in the 2D views is sluggish, it might be worth considering reducing the size of your dataset. Make sure to save after every operation to reduce the loss of progress in case of a crash.

2.3.1 Cropping

Locate the `Crop Volume` module either via the dropdown menu (figure 8) `Converters -> Crop Volume` switch to it. Or click the lens icon to use the text search (figure 13) Before any cropping can happen it is required to do some setup in the newly opened module panel.

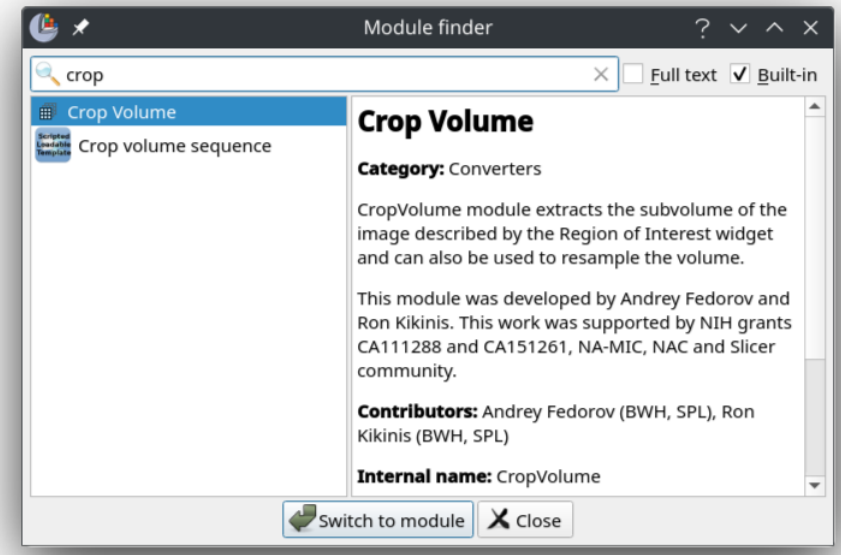


Figure 13: 3D Slicer module switcher

See figure 14 for reference. Under `IO -> Input Volume` make sure the dataset you loaded is selected. Create a new ROI under `IO -> Input ROI` choose `Create new ROI as…` and give it a distinctive name. Also make sure it is not hidden by looking for an open eye next to `Display ROI`.

To get a better visualization of the volume that is going to remain define the ROI in the `Volume rendering` module.

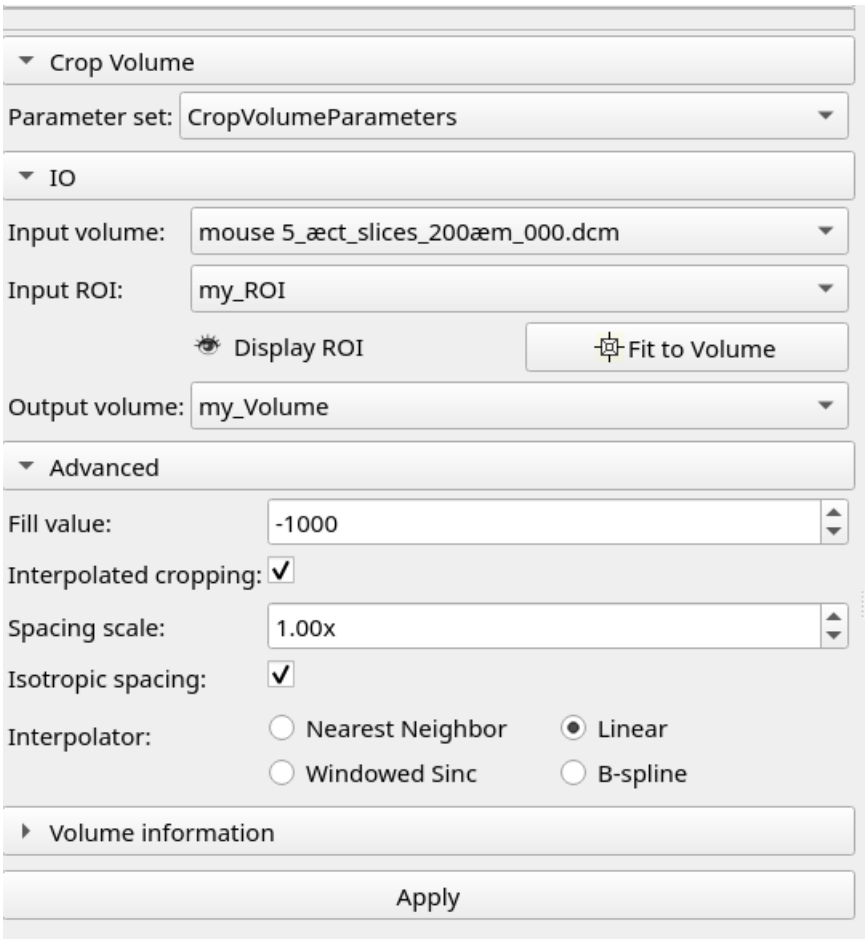


Figure 14: 3D Slicer cropping module panel

Next choose a name for your output volume under `IO -> Output volume -> Create new volume as…` and give it a distinctive name. Under `Advanced -> Fill value` choose a HU value for everything outside your ROI. It should be easily distinguishable from the structure or tissue you are segmenting. For bone segmentation I recommend choosing -1000 HU[3]. Checking the tick box `Advanced -> Interpolated cropping` will ensure that the output volume has the same dimensions as the input volume. Also check the box: `Advanced -> Isotropic spacing` with a `Spacing scale` of 1x to ensure the voxels stay isotropic. Now adjust your ROI in the 2D view areas by clicking and dragging the colored dots at the edges and corners of the ROI. Crop out as much excess volume as possible without affecting the anatomy you wish to segment. Check positioning and size of your ROI by scrolling through the 2D view on all three planes, after that click `Apply`. This operation might require some patience depending on the size of the dataset. Note that there will be no visual confirmation the cropping operation is finished. However, you can check by switching to the `Data` module. If 3D

[3]The HU value of air

Slicer is unresponsive, the operation is not done yet.

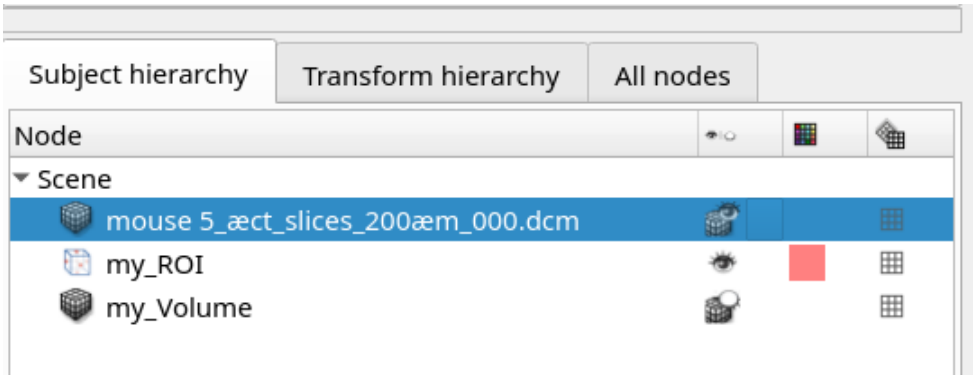


Figure 15: 3D Slicer data module

Under `Subject hierarchy -> Node -> Scene` (figure 15) you should see:

1. the loaded dataset
2. the newly created ROI
3. the newly created volume

Show your cropped volume by clicking on the closed eye on the same line as its name. Hide the ROI by clicking on the open eye next to its name. 3D slicer will then automatically hide the original dataset and populate the view area with the cropped volume dataset.

2.3.2 Masking

In the last step we cropped out the part of the source volume which does not contain any material or tissue of interest. This step is dedicated to homogenizing the volume which was not cropped out in the last step. Switch to the `Segment Editor` module (figure 16:1). Make sure your cropped volume is selected as the `Source volume` (figure 16:2). Click on the `+ Add` button (figure 16:3). You should see a new segment appear in the segments list below. Double click to rename it to "Background" or leave its default name. Make sure your "Background"segment is select and then activate the `Threshold` tool (figure 16:4). Shift the lower threshold limit until the background is covered by the segment color in the 2D views. Afterward shift the upper threshold limit until your tissue of interest is definitely no longer covered by the segment color. Before applying (figure 16:7) the threshold segmentation make sure that 3D Slicer is allowed to edit `Everywhere` and overwrite all other segments (figure 16:6).

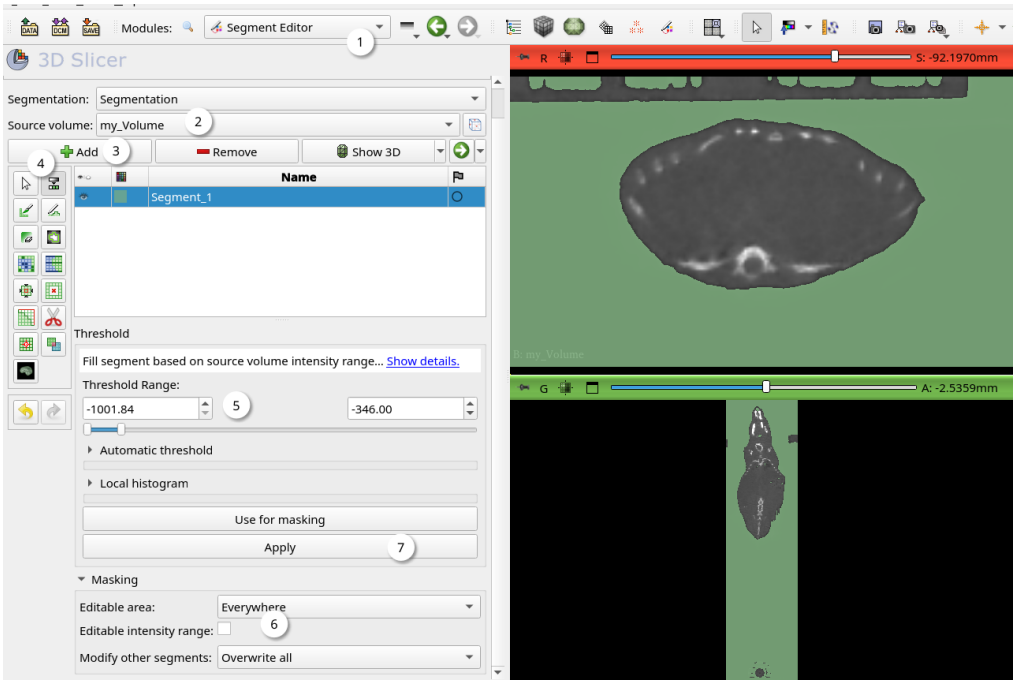


Figure 16: Threshold background

Next, activate the `Scissors` tool (figure 17:1) with your "Background" segment selected. This tool provides a simple way of selecting materials and tissue that are not easily distinguished by HU value, like the MicroCT couch and positioning tools. Change its operation mode to `Fill inside` (figure 17:2) and its shape to `Rectangle` or `Free-form` (figure 17:3). In the 2D views you can now click and drag to create a shape, upon releasing the left mouse button, the tool will add the volume inside the shape to your "Background" segment.

The scissors tool "punches" through the volume, this makes it very easy to accidentally select something intentionally. Always make sure there is no ROI in the area on the previous or next slices and check your positioning on the other planes. In most cases it will be easier to work on small sections of your volume, rather than selecting a large volume in one single step.

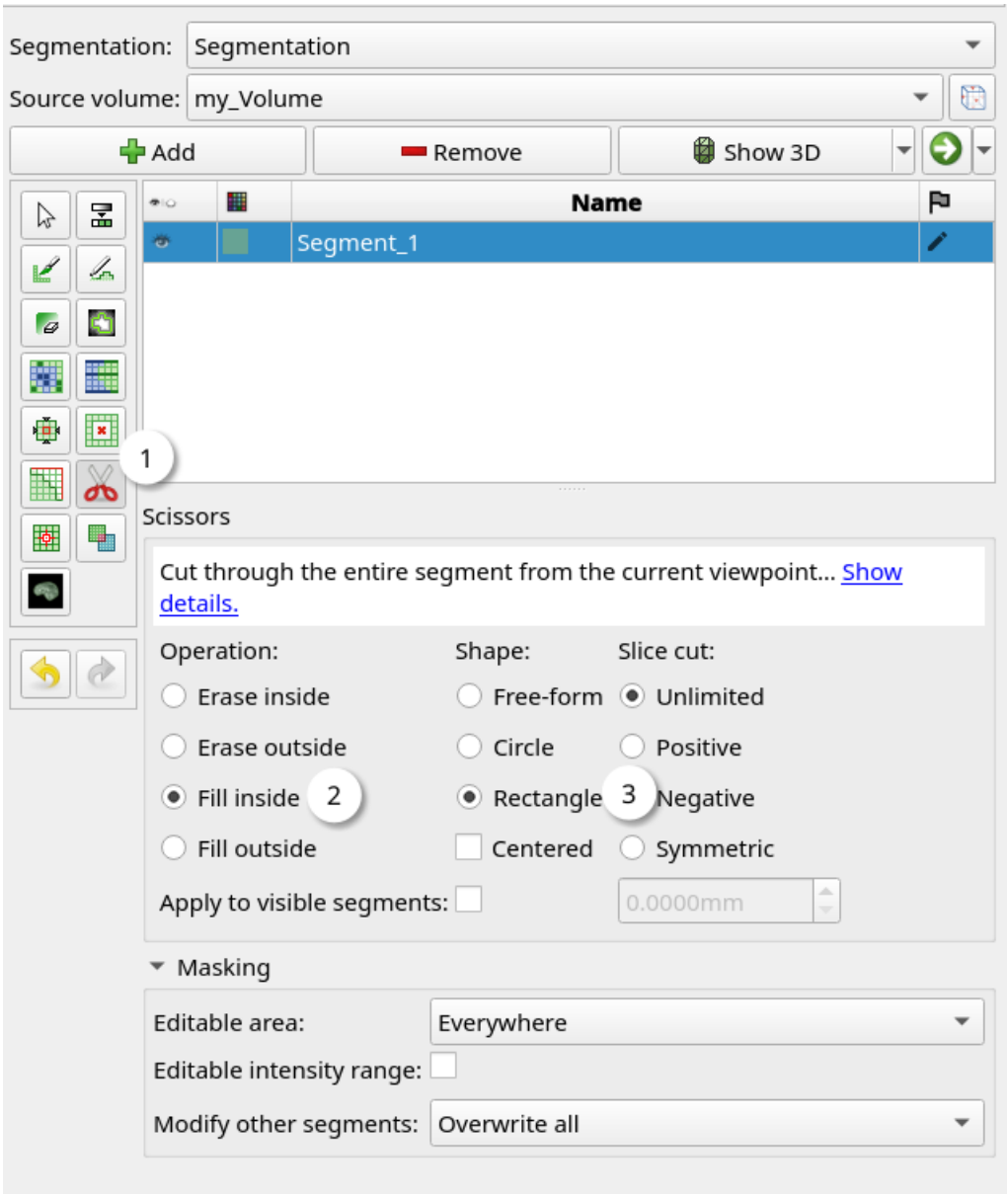


Figure 17: Scissors background

Activate the `Mask volume` tool (figure 18:2) and make sure your "Background" segment is selected (figure 18:1). As for the operation mode, choose `Fill inside` (figure 18:3). The fill value (figure 18:4) should be the same as in the cropping step (section 2.3.1). Select your cropped source volume as the `Input Volume` (figure 18:5). For the result, create a new volume by clicking on `Output Volume -> Create new Volume as…` (figure 18:6) and give it a distinctive name.

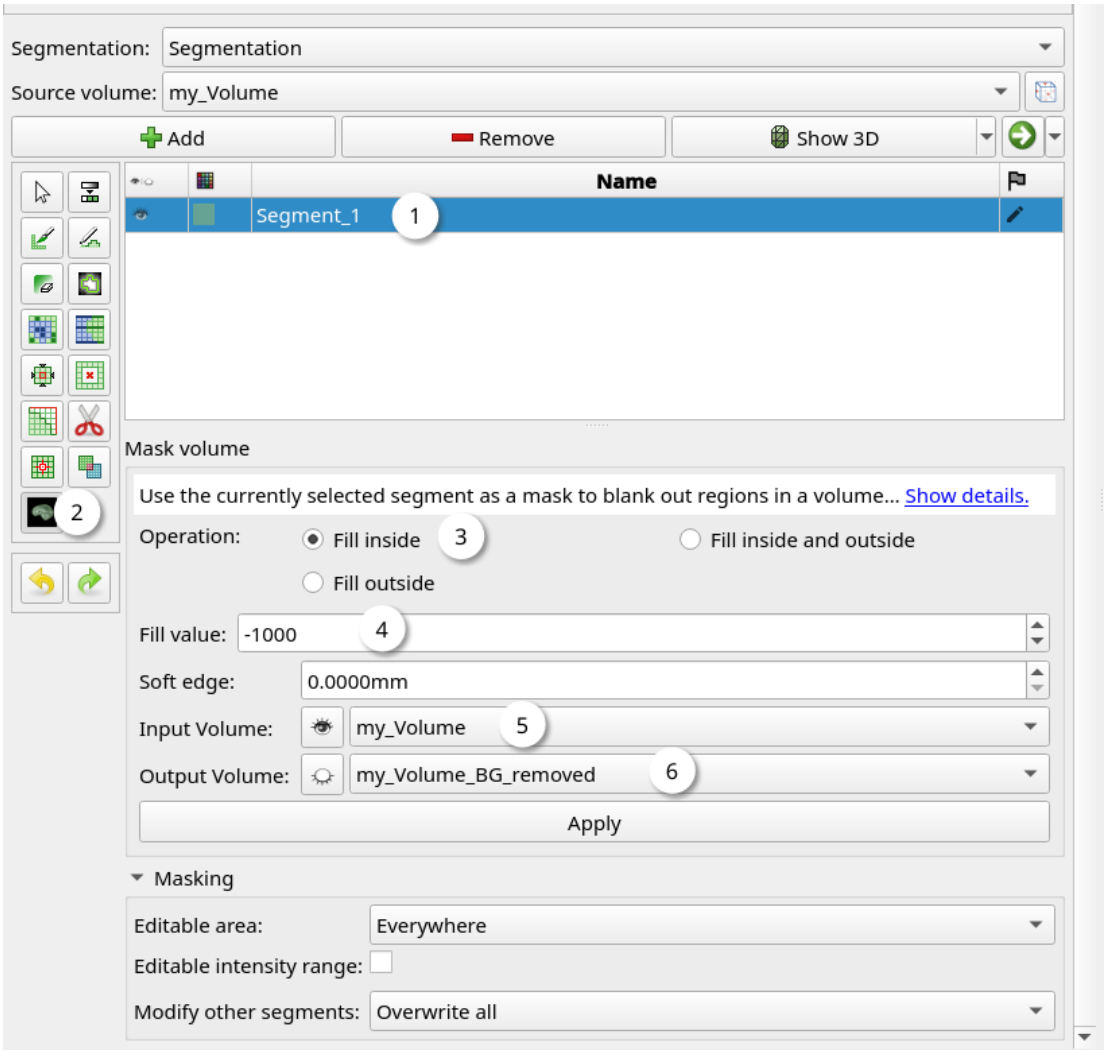
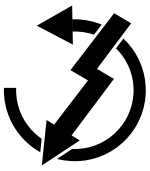


Figure 18: Mask background



Always create a new volume when working with the `Mask volume` tool. It overrides the individual voxel intensity values, this *can not be undone* as 3D Slicer does not store the original intensity values in its undo history.

2.3.3 Cleanup

Switch to the `Data` module. Here we see all the items we have created so far.

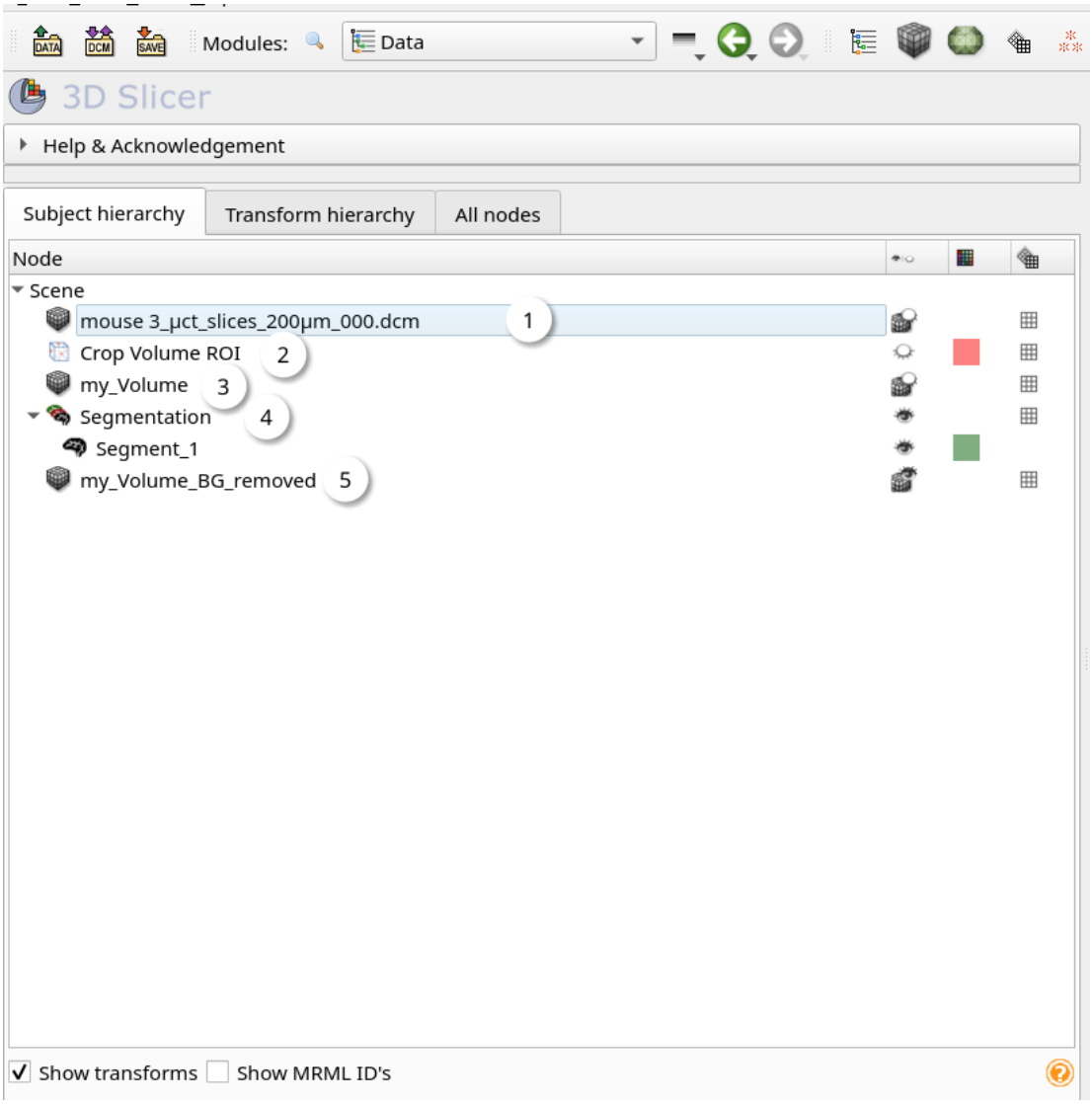


Figure 19: Data cleanup

Figure 19:1 is the DICOM dataset from the MicroCT scanner. Figure 19:2 is the cropping ROI created in section 2.3.1 and figure 19:3 is the volume that resulted from the cropping operation. The segmentation item (figure 19:4) holds all segmentations created in the `Segment Editor` module. So far it only holds the "Background" segment created in Section 2.3.2. And the final item (figure 19:5) is the most recent volume resulting from the `Mask volume` operation. Items figure 19:1-4 can be deleted to save harddrive space and RAM while working in 3D Slicer. In this example the data volume on disk could be decreased from 309 megabytes to (source DICOM data) to 15 megabytes. Which equates to

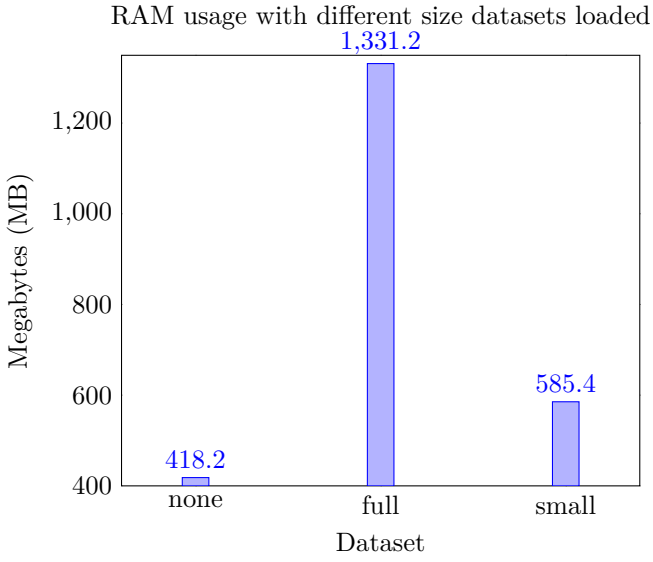


Figure 20: RAM usage comparison

approximately a 95% reduction in file size. As a result of this, 3D Slicers RAM consumption has dropped from 1.3 gigabytes to 585 megabytes, which equates to approximately 55% reduction. See Appendix A.2 for more information.

Figure 20 is a RAM usage comparison of 3D Slicer under different workloads, with the intention of showing the user what amount of resource consumption to expect.
`none` refers to 3D Slicers base RAM usage without any dataset loaded.
`full` refers to the RAM usage with a raw dataset of 309 megabytes file size.
`small` refers to the RAM usage with the same dataset as above but reduced down to 15 megabytes file size using the method explained in section 2.3.

3 Segmentation tools

With your dataset loaded and prepared switch to the `Segmentation Editor` module. Before you start to segment *ALWAYS* make sure the masking options are set correctly.

Editable area which areas are affected by your tool

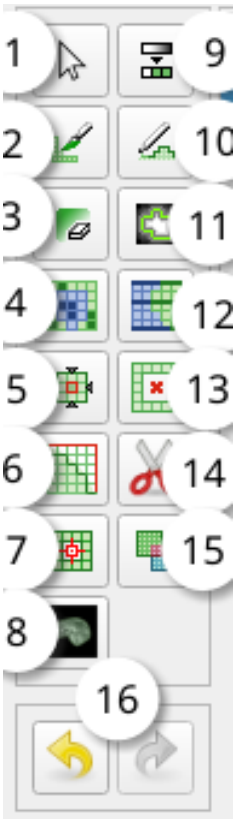
- **Everywhere** no restrictions, tool is usable anywhere in the view areas
- **Inside all segments** tool can overwrite any existing segment, does not work outside of segments
- **Inside all visible segments** tool can overwrite any existing segment that is not hidden, does not work outside of segments
- **Outside all segments** inverse of `Inside all segments`, tool works only outside existing segments
- **Outside all visible segments** inverse of `Inside all visible segments`, tool works only outside existing non-hidden segments
- **Inside `user created segments`** tool can override only selected existing segment, does not work outside of segment

Editable intensity range restrict tool to affect only specific HU value range

Modify other segments define interaction with other existing segments

- **Overwrite all** tool can overwrite all existing segments
- **Overwrite visible** tool can overwrite all existing non-hidden segments
- **Allow overlap** tool does not overwrite existing segments, instead segmented areas are shared between the affected segments

3.1 Built in segmentation tools



1. No editing
2. Paint
3. Erase
4. Grow from seeds
5. Margin
6. Smoothing
7. Islands
8. Mask volume
9. Threshold
10. Draw
11. Level tracing
12. Fill between slices
13. Hollow
14. Scissors
15. Logical operators
16. Undo and Redo

Figure 21: Segmentation tool

3.1.1 No editing

Selected by default. Enables inspection of 2D and 3D views without accidentally modifying a segmentation.

3.1.2 Paint

2D 3D

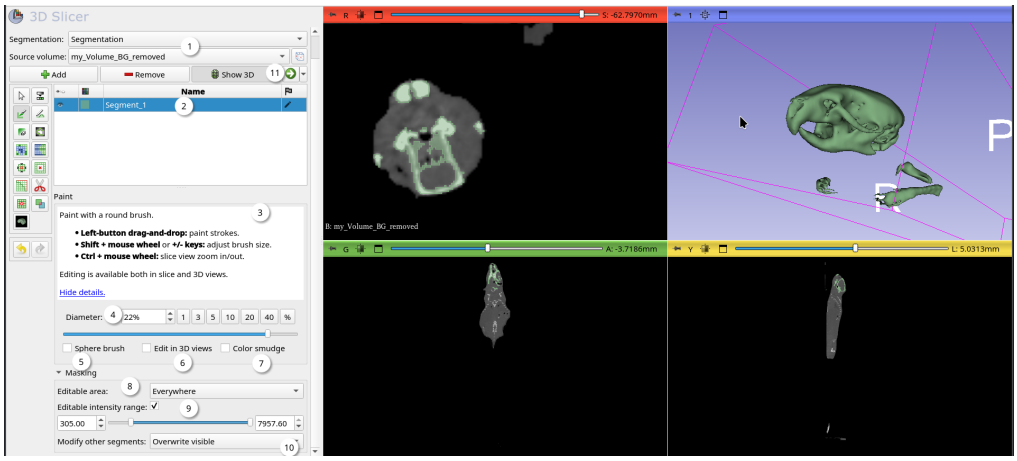


Figure 23: Paint tool

The `Paint` tool is the most widely applicable manual segmentation tool. It is also the basis for most semi-automatic segmentation tools. To get started, first make sure the correct volume dataset and segmentation dataset are loaded (figure 23:1). Create a new segment to segment a new object or organ. Or select an existing segment to continue working on it. Navigate to the region you wish to work on in the 2D view areas and activate the `Paint` tool. Check your masking options before making any modifications (figure 23:10). 3D Slicer displays the most important keyboard options in the help area (figure 23:4). Left-click and drag to draw, Shift+Mouse wheel to change the brush size, Ctrl+mouse wheel to zoom, middle mouse drag and move to pan. Figure 23:4 displays the brush size modification options, click and drag the slider to increase the brush size, alternatively click one of the size presets. In most cases setting the brush size via this menu will be not necessary, instead use the keyboard shortcuts. Keeping the mouse in the view area during segmentation enforces a non-disruptive workflow. Make the `Paint` tool affect more than one slice by making the brush spherical (figure 23:5). The sphere brush can also be used in the 3D view if enabled (figure 23:6). If you wish to allow overlapping segments, enable `Color smudge` (figure 23:7).

3.1.3 Erase

Erase segmentation with brush. Inverse of Paint tool, for usage see section 3.1.2.

3.1.4 Grow from seeds

2D 

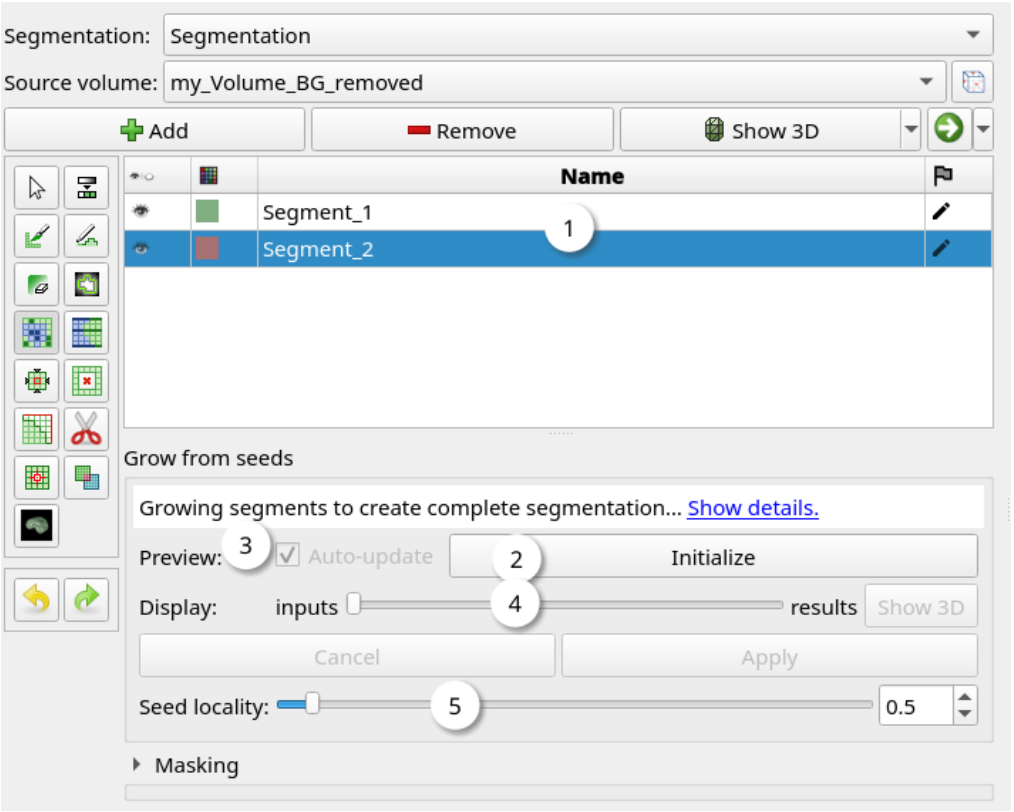


Figure 25: Grow from seeds tool

Semi-automatic segmentation tool based on seeds and distinctive HU changes. To get started create at least two segments (figure 25:1), one for the "Background" and one for the actual segmentation. Use the paint tool to create seeds in those segments. Try to evenly distribute seeds throughout your segmentation area in all three planes. Additionally, try to define the border between your segments manually. Click `Initialize` and wait for the 2D views to display the segmentation preview. Change the opacity of the preview via the slider (figure 25:4) or display it in the 3D view. The first iteration will most likely be not satisfactory. Turn of `Auto-update` (figure 25:3) and switch back to the `Paint` tool. Add seeds in all areas `Grow from seeds` segmented incorrectly. Switch back to `Grow from seeds` and click `update`. If the segmentation does

not grow enough or grows disproportionate, change the seed location modifier (figure 25:5). Increasing the modifier forces the segmentation to be more localized and vice versa. Repeat this cycle of adding seeds and letting the segmentation update until the segmentation is satisfactory. Click apply to confirm the segmentation, delete the "Background" segment if its no longer useful.

3.1.5 Margin

2D

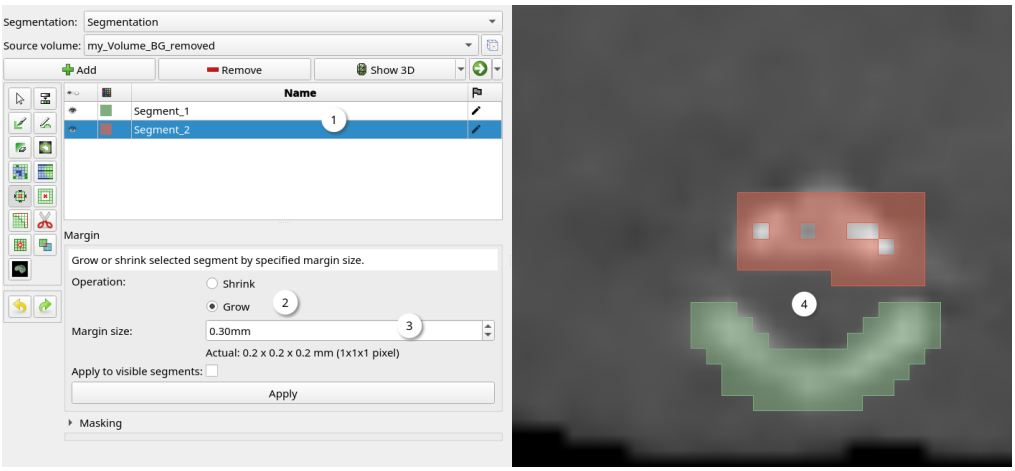


Figure 27: Margin tool

Grow or shrink a segmentation by specific amount. Select the segment you wish to modify (figure 27:1). Pick `Grow` or `Shrink` depending on your needs. Set the size to grow or shrink by (figure 27:3) and click `Apply` to confirm the operation.

This tool can be used to fill small holes by first growing a segment slightly and then immediately shrinking it by the same amount. Or it can be used to separate two segments which have some overlap by shrinking both.

3.1.6 Smoothing

2D

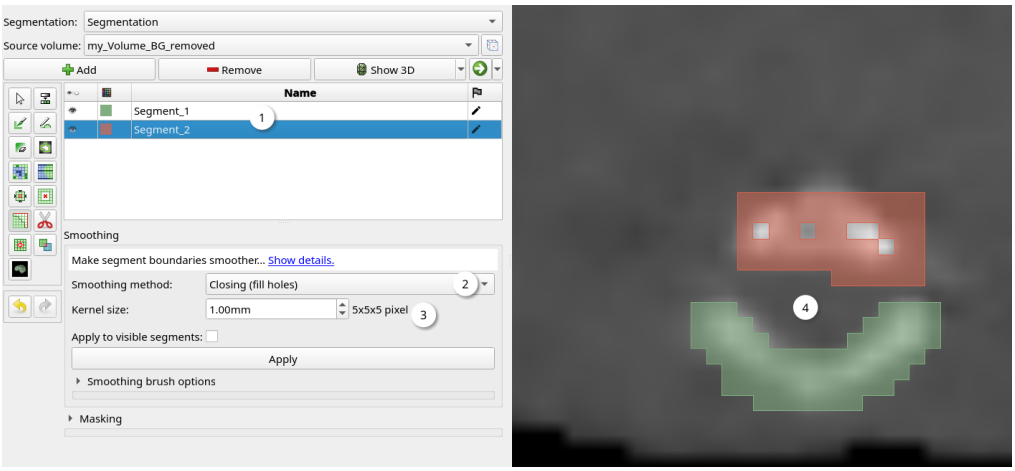


Figure 29: Smoothing tool

Smooth segmentation or fill small holes. Select the segment you wish to modify (figure 29:1). Select a smoothing method (figure 29:2) and kernel size (figure 29:3). If you are not sure which kernel size is best for your dataset, set the kernel size as small as possible in order to avoid large modifications. Undo if the result is not satisfactory, increase the kernel by a small amount and try again.

Median removes small extrusion while leaving smooth areas mostly unchanged

Opening removes extrusions smaller than the kernel size

Closing fills holes and sharp corners smaller than the kernel size

Gaussian smooths all contours and shrinks the segment

Joint smoothing affects all visible segments, smooths multiple segments at once

3.1.7 Islands

2D 3D

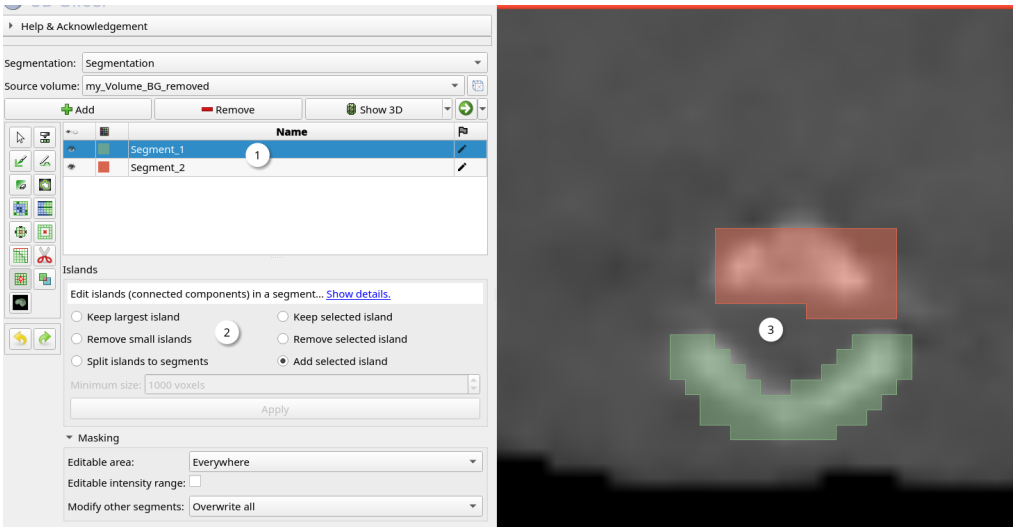


Figure 31: Islands tool

Multitool operating on disconnected segmentation areas or connected segmentation areas with different labels.

Keep largest island deletes all islands but the largest one

Remove small islands deletes all islands smaller than the specified minimum size

Split islands to segments deletes all islands smaller than the specified minimum size, the remaining islands will be automatically assigned a new segment each

Keep selected island click on an island you wish to keep, all other islands will be deleted

Remove selected island click on an island you wish to delete, all other islands will be unaffected

Add selected island click on an island you wish to join with the selected segment, it will inherit the label of the segment

3.1.8 Mask volume

2D 

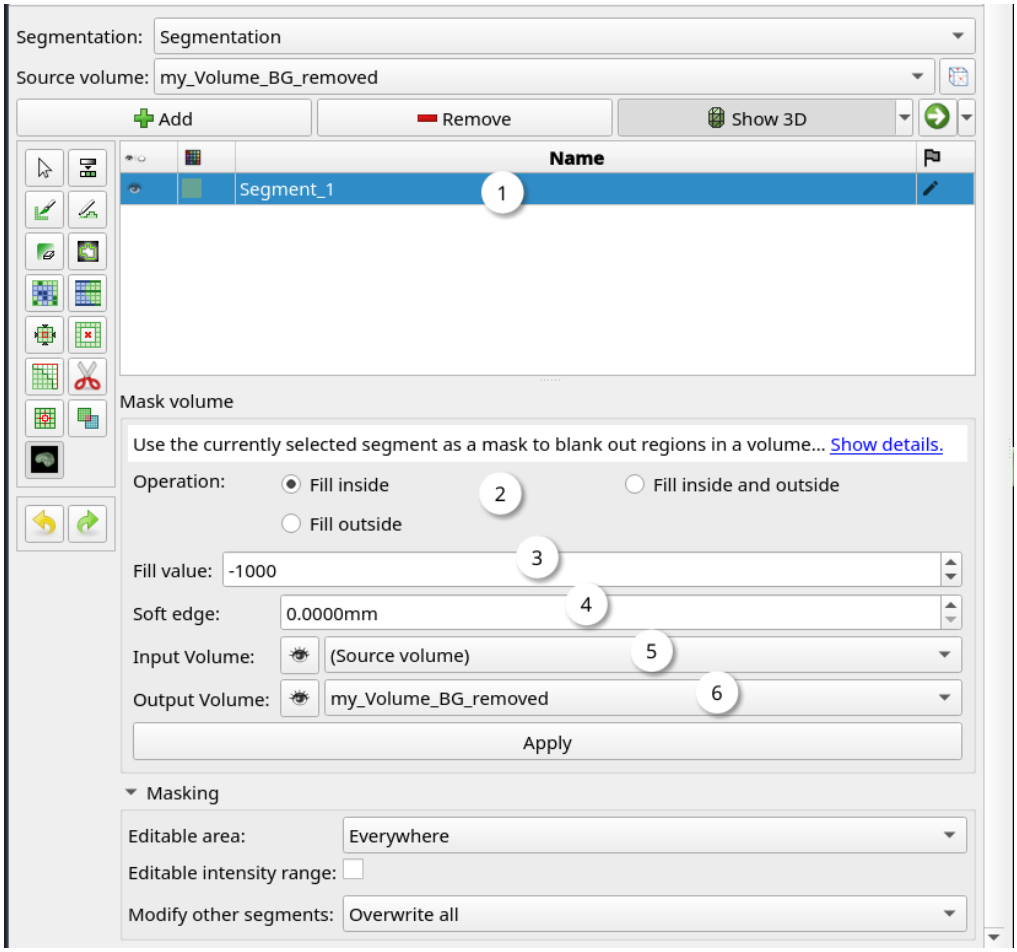
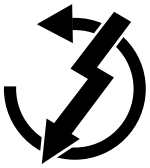


Figure 33: Mask volume tool

Tool to overwrite parts of a volume.



`Mask volume` overwrites HU values in your dataset and 3D Slicer does not save the previous values in its undo history. Thus, if you accidentally overwrite something unintentionally you will not be able to undo your action. When using this tool *always* save your progress and make a backup copy of your save file.

To get started, create and select a segment you wish to modify (figure 33:1).

The segment can for example be the background you wish to blank out. Or it can be an object or organ you wish to blank out or preserve. In the example Figure 33 `Mask volume` was used to blank out the background, some support structures and just preserve the scanned animal. This was achieved by using the `Threshold` tool (section 3.1.9) to segment the air. Then the `Scissors` tool (section 3.1.14) was used to segment the positioning and support structures. Next, select an operation mode (figure 33:2) and fill value (figure 33:3).

Fill inside fill volume inside selected segment with `Fill value`

Fill outside fill volume outside selected segment with `Fill value`

Fill inside and outside takes two fill values (inside and outside), fill volume inside and outside with these values respectively

The tool can also smooth the edge between segments with a blur. Increase the `Soft edge` (figure 33:4) value above zero. Before applying the operation, make sure the correct `Input Volume` is selected and create an `Output Volume`.

3.1.9 Threshold

2D

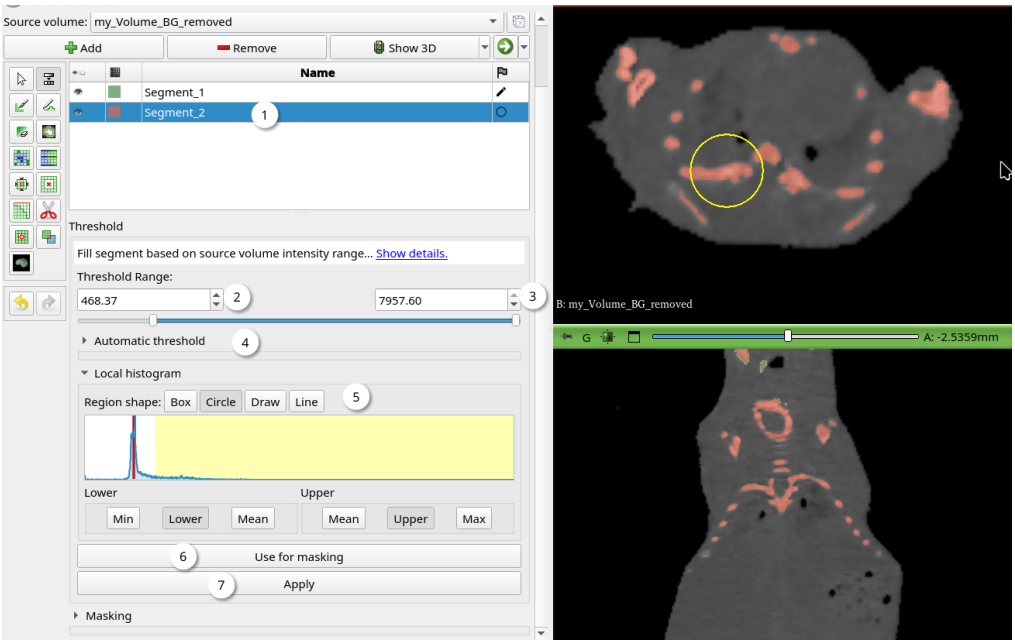


Figure 35: Threshold tool

Segment by HU value range.
First, select the segment to threshold (figure 35:1). Next, pick a lower (figure 35:2) and upper (figure 35:3) limit either by clicking and dragging the sliders

or by typing in the HU values manually. A dynamically updated preview will be available in the 2D views. Automatic threshold algorithms (figure 35:4) can be used to determine the limits but in most cases clicking and dragging the slider will be faster. You may want to see a histogram of a ROI and pick your lower and upper limit from the histogram (figure 35:5). In order to do that, pick a ROI shape and click and drag it in a 2D view area. Click on the histogram to choose your limits. If you do not want to save your threshold to a segment but intend to use it for masking with the `Paint` tool, click on `Use for masking` (figure 35:6). This will activate the `Paint` tool with your threshold limits set as the masking option.

3.1.10 Draw

2D

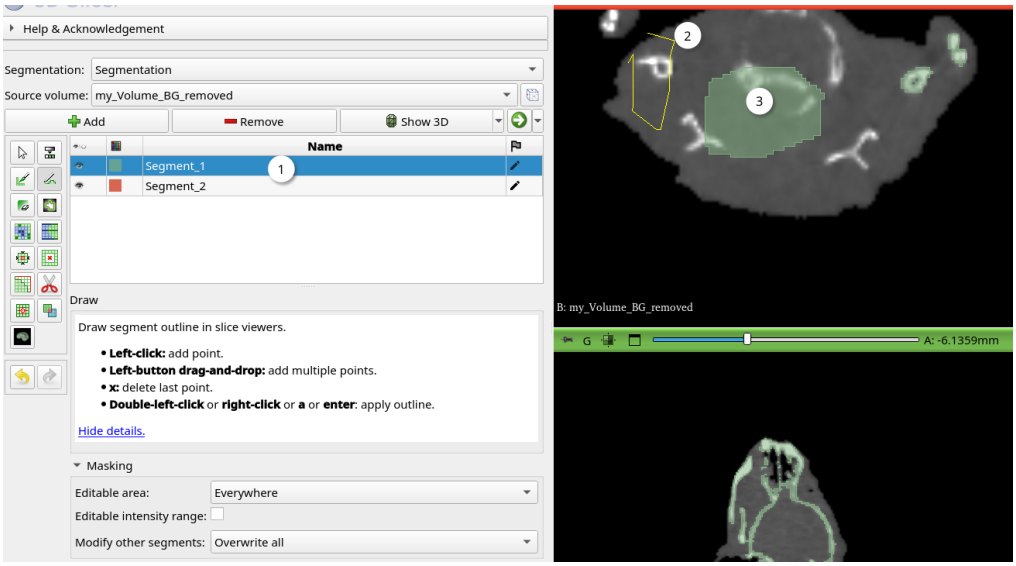


Figure 37: Draw tool

Simple drawing tool. Make sure the correct segment is selected (figure 37:1). Click on a 2D view to create points, double click to connect the last point with the first point. The encompassed area will be filled in (figure 37:3).

3.1.11 Level tracing

2D

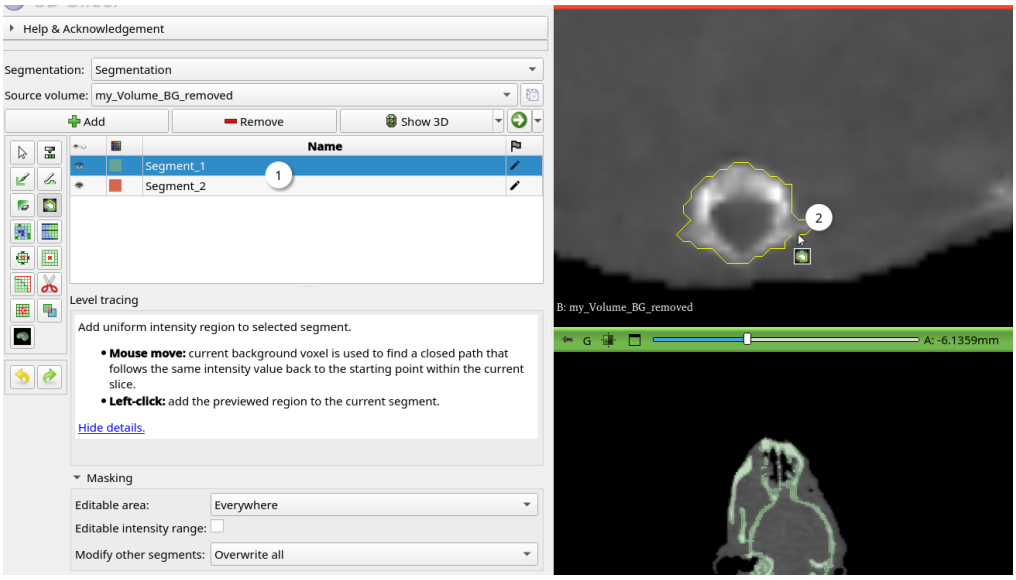


Figure 39: Level tracing tool

`Level tracing` has a similar role as the `Draw` tool (section 3.1.10). But instead of manually defining points and connecting them, the tool automatically tries to find areas of similar intensities. Hover your mouse over a 2D view to see the outline of an area, click to confirm (figure 37:2).

3.1.12 Fill between slices

2D 

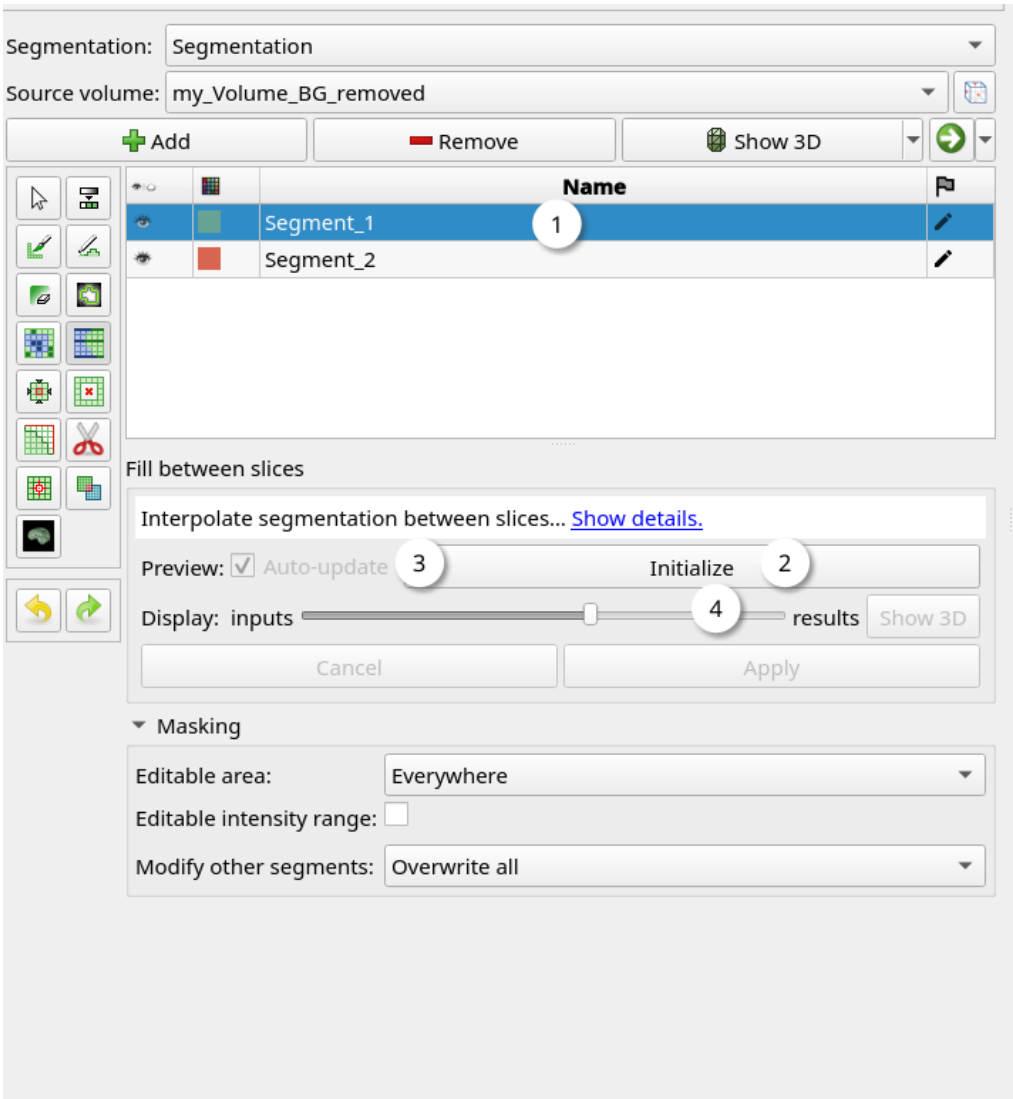


Figure 41: Fill between slices tool

`Fill between slices` is used similar to `Grow from seeds` (section 3.1.4). Start by creating at least one segment and segment a slice with a manual tool. Skip at least one slice and segment the next slice. Repeat the last 2 steps until you have covered the desired segmentation volume. Click on `Initialize` (figure 41:2) and wait for the interpolation preview to appear in the 2D view area. The first segmentation will most likely not satisfactory. Deactivate `Auto-update` (figure 41:3), switch to the `Paint` tool and manually paint over the slices with

inaccuracies. Switch back to the `Fill between slices` tool and click `Update`. Repeat until the segmentation is satisfactory.

3.1.13 Hollow

2D

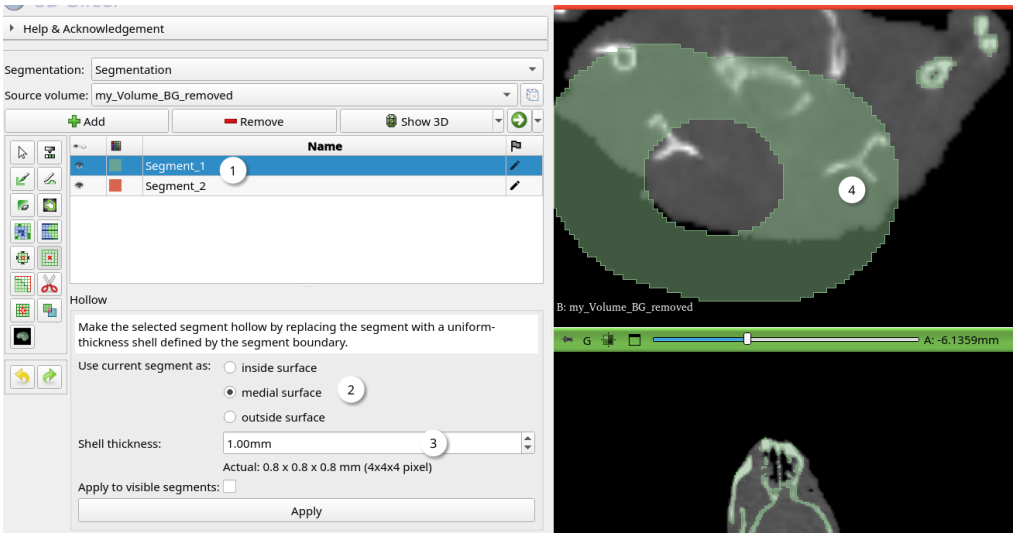


Figure 43: Hollow tool

Hollows out a segment (figure 43:4) by replacing it with a border of a specified thickness (figure 43:3). To use this tool, select the desired segment (figure 43:1), select if the segment should represent the `inside`, `medial` or `outside` border. Finally set the border thickness (figure 43:3) and click apply.

3.1.14 Scissors

2D 3D

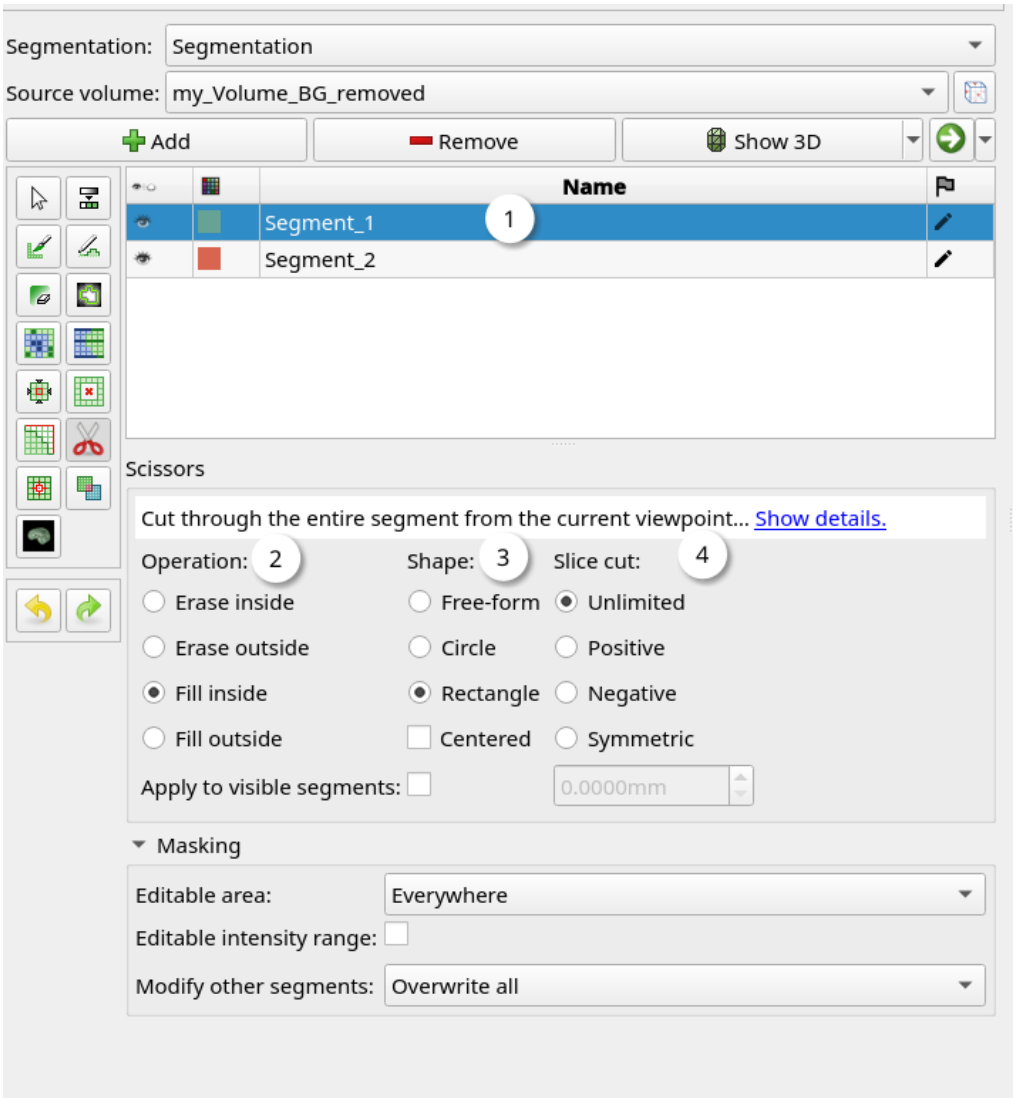


Figure 45: Scissors tool

`Scissors` can be used to cut or fill through a segment. Select a segment to cut (figure 45:1) and pick a mode of operation (figure 45:2).

Erase inside erases already segmented area inside ROI

Erase outside erases already segmented area outside ROI

Fill inside fills ROI with segment label

Fill outside fills everything but the ROI with segment label

Pick a ROI shape (figure 45:3). `Centered` means that the ROI will not be drawn from its edge but the center, this may be useful for drawing circular ROIs. Finally, choose which slices should be affected by the `Scissors` tool (figure 45:4).

Unlimited affect all slices

Positive only affect slices with a higher slice number than the current one

Negative only affect slices with a lower slice number than the current one

Symmetric affect both higher and lower slices numbers but only a specific distance

3.1.15 Logical operators

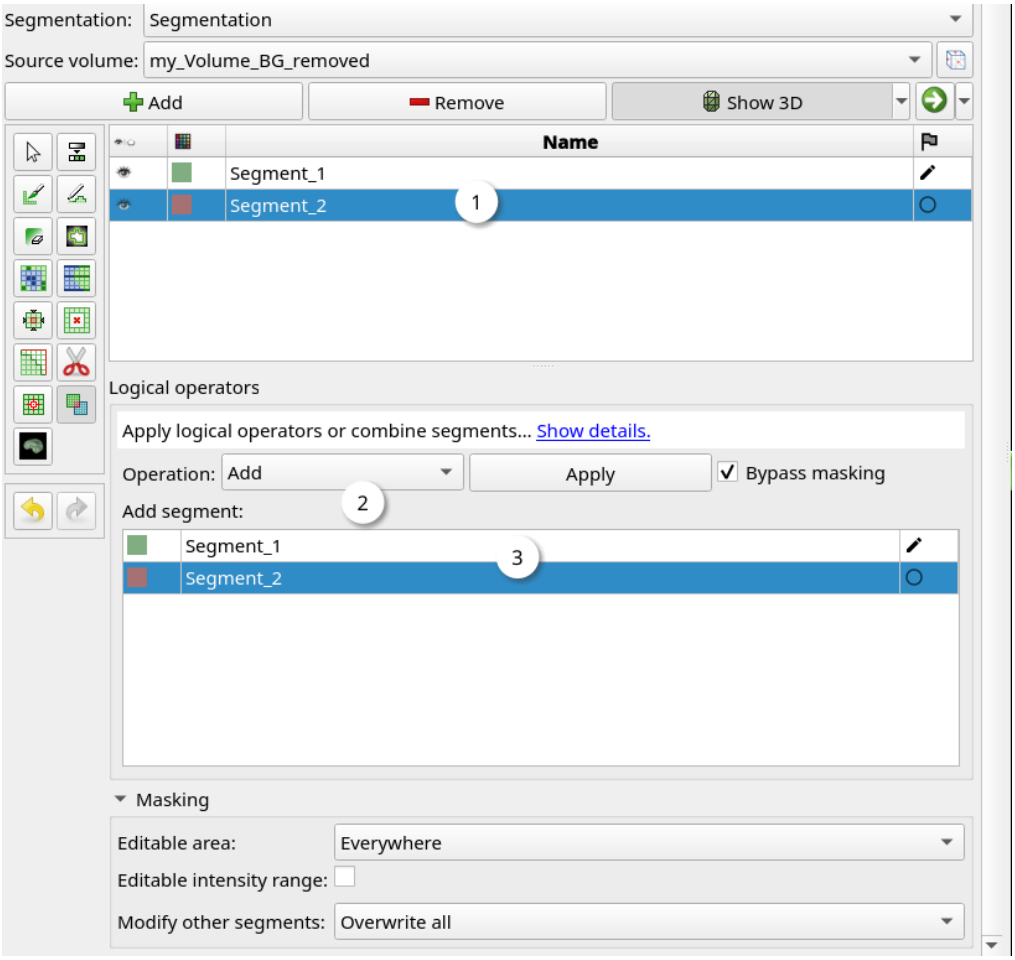


Figure 46: Logical operators tool

Boolean operators processing up to two segments at a time. First, make sure the correct segment is selected (figure 46:1). Second, pick an operation (figure 46:2).

Copy copy the segmentation of the modifier segment to the active segment, effectively replacing its segmentation

Add combine active and modifier segment

Subtract remove overlapping parts of the modifier segment from the active segment

Intersect only keep overlapping parts of the modifier segment and the active segment

Invert invert the active segment

Clear clear the active segment, the same can be achieved via the right click menu and selecting `Clear selected segments`

Fill fill the whole volume with the active segment

Finally, if applicable, pick the modifier segment (figure 46:3) and click apply.

3.1.16 Undo and Redo

Undo and Redo work the same as just about in any other software. The only caveats are that 3D slicer allows only 9 undo operations and that some operations cannot be undone (section 3.1.8)

3.2 Additional segmentation tools

Some tools are not included with the download of 3D Slicer. There can be several reasons for this. Some may not be considered stable enough yet. Some may have considerable overlap in their use case with a built-in tool. And some may just not fit 3D Slicer's development direction. Fortunately, 3D Slicer provides an API which enables the development of extensions. One such extension is "SlicerSegmentEditorExtraEffects"[5] by Andras Lasso. This extension expands the tool section in the `Segmentation Editor` module with the following tools:

1. `Draw Tube`
2. `Engrave`
3. `Fast Marching`
4. `Flood Filling`
5. `Split Volume`
6. `Surface Cut`
7. `Watershed`
8. `Local Threshold`

Figure 47: SlicerSegmentEditorExtraEffects

Install the extension by clicking on the `Extensions Manger` in the toolbar (figure 8:10). In the newly opened window, click on `Install Extensions`, then use the search bar to find "SegmentEditorExtraEffects". Click install and wait for 3D Slicer to ask you for a restart. The extra tools will be available after restarting 3D Slicer.

3.2.1 Draw tube

2D 3D

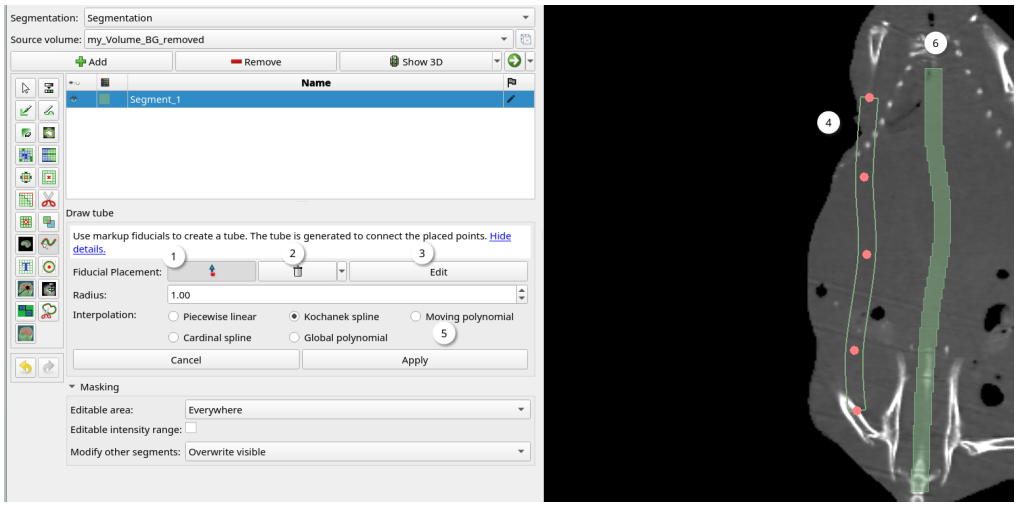


Figure 49: Draw tube tool

Draws a tube of fixed radius along a path of points. First, make sure the desired segment is selected. Click on `Place a control point` (figure 49:1) to start drawing. Create as many control points as you need by clicking on your volume in the 2D or 3D views. Exit the point creation mode by double left-clicking on the last point or right-clicking anywhere in the view area. The points can now still be moved freely by clicking and dragging them around. If you wish to delete the last added point, click on the trashcan icon (figure 49:2). Pick a tube radius in millimeters and an interpolation algorithm (figure 49:5) by experimenting with the available options and reviewing changes in the preview (figure 49:4). Click `Apply` to confirm your changes (figure 49:6). The result however can still be changed by clicking `Edit` (figure 49:3).

3.2.2 Engrave

2D 3D

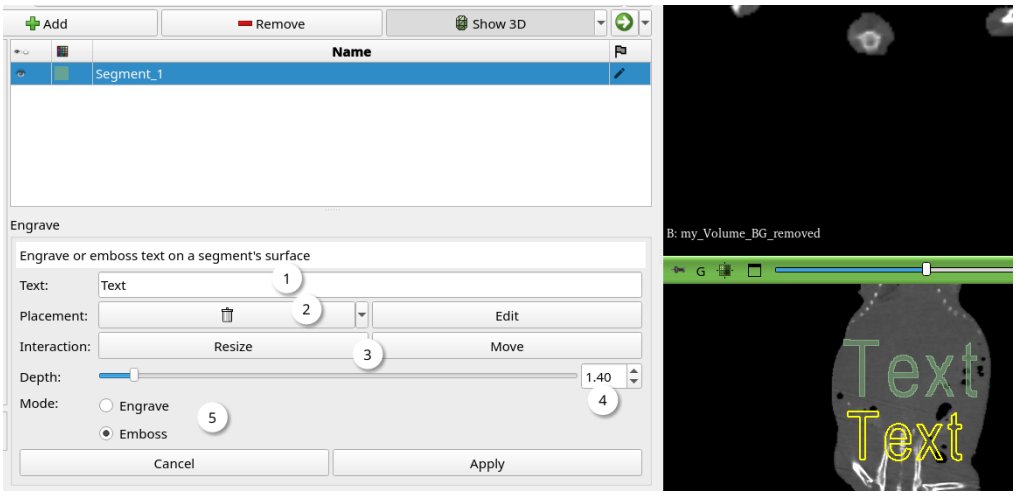


Figure 51: Engrave tool

Draws or carves out text on a segments surface. First insert your desired text string (figure 51:1). Next, click on `Place a control point` (figure 51:2) and place your text by clicking on the desired position in either the 2D or 3D view area. Change size and location by selecting either (figure 51:3) and manipulating the text in the view area. Change the depth of penetration in millimeters by entering a value or dragging the slider (figure 51:4). Finally choose an operation mode (figure 51:5):

Engrave carve the text out of an existing segment, requires prior segmentation

Emboss stamp the text on a segment, requires no prior segmentation

3.2.3 Fast Marching

2D 

Works similar to `Grow from seeds` (Section 3.1.4) with the big difference that it does not need a "Background" segment and can be used on a single segment at a time. `Fast Marching` is also considerably faster than `Grow from seeds` `Maximum volume` refers to relative amount of your dataset `Fast Marching` will try to segment. Control segmentation leakage via the `Segment volume` slider.

3.2.4 Flood Filling

"Add to the current segment all similar intensity voxels near the clicked position. Generally "Local threshold" effect is recommended instead of this effect because this effect often either cannot prevent leaking into other structures or provides incomplete segmentation."[5] This tool has been found to be unreliable and tends to crash often.

3.2.5 Split Volume

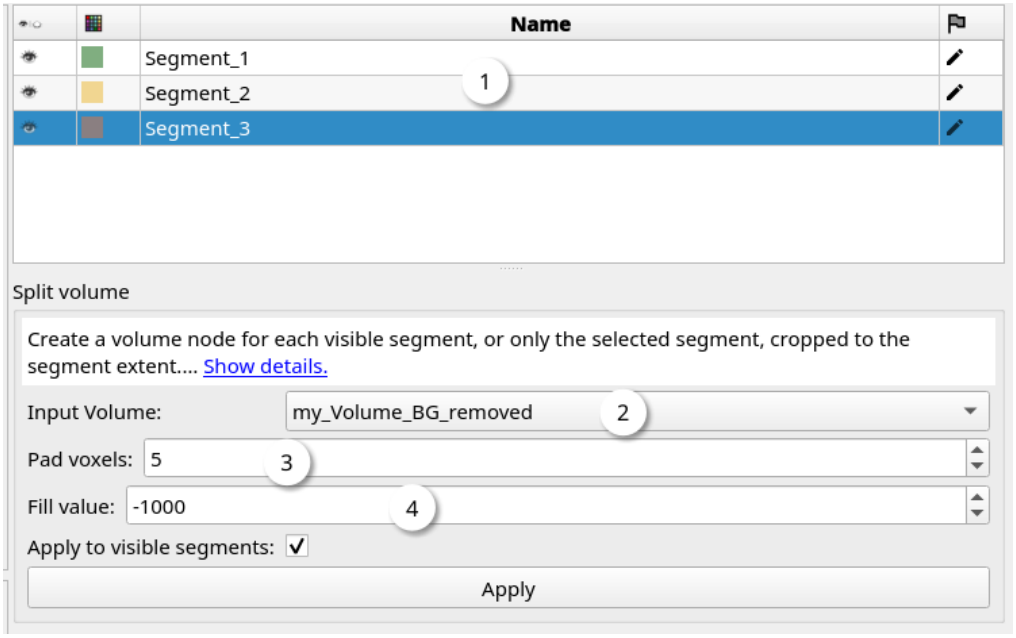


Figure 55: Split volume tool

Creates a new volume for each segment and requires at least two existing segments. Start by segmenting parts of your dataset that you wish to split (figure 55:1). Make sure to select the correct input volume (figure 55:2). Set the amount of padding voxels around each segment (figure 55:3) and the fill value outside the segmentation (figure 55:4). After clicking `Apply` the `Data` module will show some new volumes according to the number of segments you split.

3.2.6 Surface Cut

2D 3D

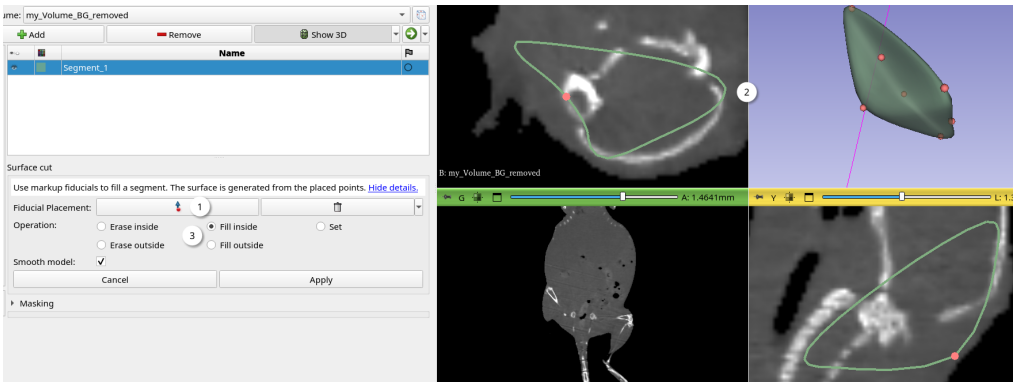


Figure 57: Surface cut tool

Works similar to `Draw tube` (section 3.2.1) and `Scissors` (section 3.1.14). Start by creating fiducial markers (figure 57:1) in the 2D or 3D views (figure 57:2). Exit fiducial creation mode by either double-clicking on the last fiducial or right-clicking anywhere in the view area. Modify fidicual placement by clicking and dragging them to the desired position. Choose an operation mode:

Erase inside erases already segmented area inside fiducial volume

Erase outside erases already segmented area outside fiducial volume

Fill inside fills fiducial volume with segment label

Fill outside fills everything but the fiducial volume with segment label

Set fills fiducial volume with segment label

3.2.7 Watershed

2D 

Works almost identical to `Grow from seeds`, with the exception that segmentation smoothing factor can be customized via the `Object scale` setting. For usage see: Section 3.1.4.

3.2.8 Local Threshold

2D 

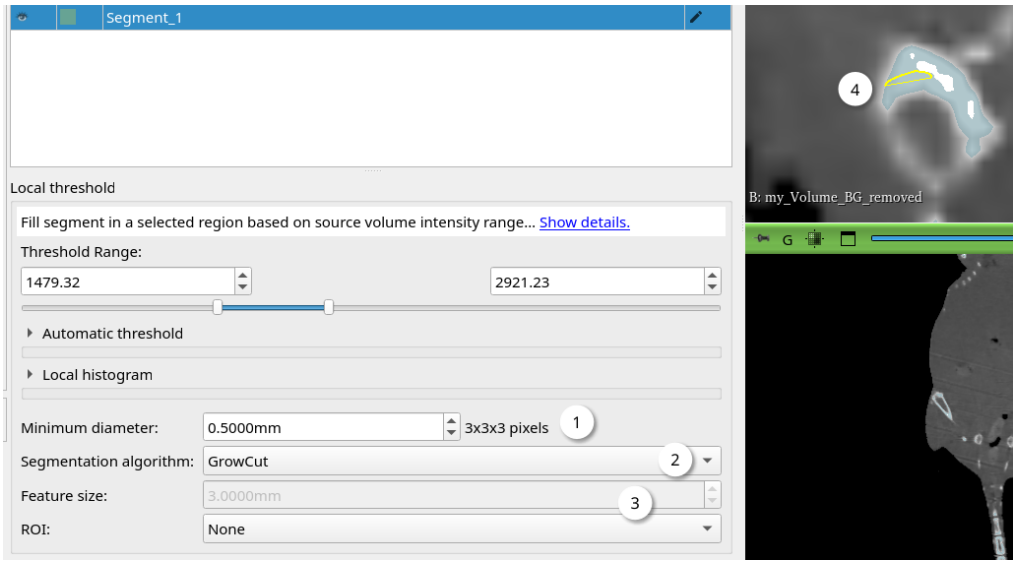


Figure 60: Local threshold tool

ROI based threshold tool. Start by drawing a ROI in the 2D view area (figure 60:4). The segmentation algorithm (figure 60:2) will automatically set the lower and upper threshold limit. If the segmentation is leaky, reduce the `Minimum diameter`. This will prevent the segmentation algorithm from considering bridged areas with bridges smaller than the specified amount. If the watershed algorithm is selected (figure 60:2), `Feature size` can be used to control the amount of smoothing the algorithm performs. If you wish to locally restrict the threshold, create and draw a ROI (figure 60:3) before using the tool.

4 Performing segmentations

Segmenting is now a matter of applying a selection of the tools mentioned above in a meaningful order. This guide will focus on bone segmentation as this was the authors use case, but the general workflow should apply to most anatomical structures. The next two sections will show you possible workflows. If you already face performance issues when loading the dataset, start by following the instructions in section 2.3.

4.1 Manual

1. **Identify the target structure** Navigate to your target structure using the 2D views or visualize it in the `Volume Rendering` module.

2. **Isolate the target structure** Crop out as much unneeded volume as possible using the method described in section 2.3.1. This will not only improve performance, it will also make navigation easier.

3. **Generate a mask for the structure** Using the threshold tool (section 3.1.9) generate a mask for your target structure, in case of this guide bone structures. This will make it almost impossible to accidentally segment unwanted tissue.

4. **(Optional) close holes and smooth edges** If the threshold tool left some holes throughout your target structure it is possible to fix this using a `Closing` operation (section 3.1.5).

5. **Cut away unwanted parts** Use the `Scissors` tool (section 3.1.14) in 2D or 3D view to cut away unwanted structures from the mask segment.

6. **Create segments** Create a segment for each structure that is going to be segmented and name them accordingly (figure 61:1).

7. **Manually separate structures** Use manual tools like `Draw, Paint, Draw tube` to separate elements. Overwriting the mask segment created in the third step. This can be achieved by selecting the mask segment as the `Editable area` in the `Masking` options (figure 16:6, figure 61:3). Make sure to separate your target structure carefully in all planar views (figure 61:4).

8. **Connect remaining islands** Use the `Islands` tool (figure 61:2) to connect the middle segment of the last step, effectively finishing the segmentation.

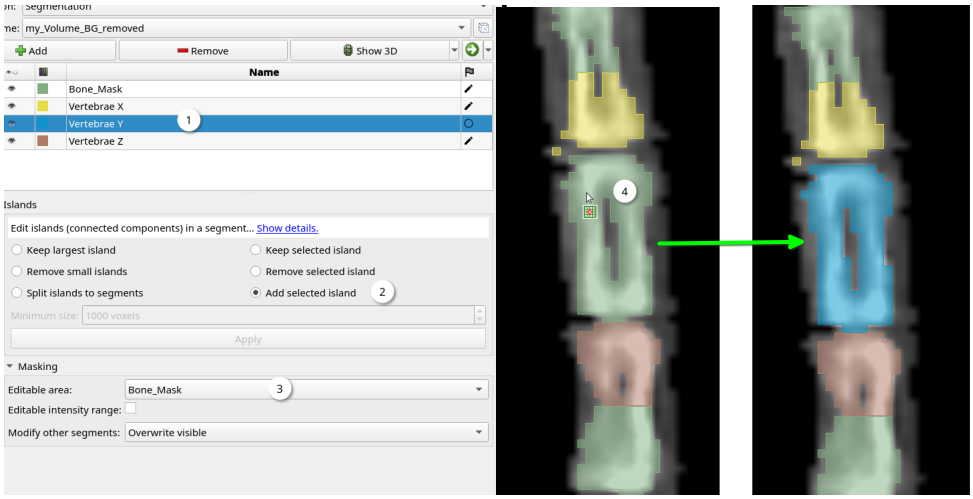


Figure 61: Manual segmentation via islands

4.2 Semi-Automatic

1. **Identify the target structure** Navigate to your target structure using the 2D views or visualize it in the `Volume Rendering` module.

2. **Isolate your target structure** Crop out as much unneeded volume as possible using the method described in section 2.3.1. This will not only improve performance, it will also make navigation easier.

3. **Determine threshold for masking** Using the threshold tool (section 3.1.9) generate a mask for your target structure, in case of this guide bone structures. Alternatively just use the threshold tool to determine the upper and lower limits and use them in the masking tab of the chosen semi-automatic segmentation tool.

4. **Create segments** Create a segment for each structure that is going to be segmented and name them accordingly. If you plan to use `Grow from seeds` (section 3.1.4) also create a background label.

5. **Paint seeds** Manually paint seeds inside the structures you wish to segment. Make the seeds evenly spread throughout the structure and add extra seeds where structures border. When using `Grow from seeds` also do this for the background segment.

6. **Perform semi-automatic segmentation** Let the tool calculate a segmentation based on your seeds. Improve the segmentation by going back to step 5 and adding more seeds. Repeat until the result is sufficiently accurate.

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A Appendix: Code

A.1 distrobox manifest file

```
[slicerbox]
image=docker.io/library/fedora:latest
# Xorg/GUI
additional_packages="itk mesa-libGLU libglvnd alsa-lib alsa-utils freetype libSM
↪  pulseaudio-libs-glib2 libXcomposite libGL libXdamage libXrandr libXrender
↪  libXft libxkbcommon-x11 libXxf86vm libXext libXcursor libXi libXtst libX11"
# data manipulation
additional_packages="dcmtk libxcrypt vtk teem"
# QT5
additional_packages="qt5-qtbase qt5-qtbase-common qt5-qtbase-gui
↪  qt5-qtdeclarative qt5-qttranslations qt5-qtwayland qt5-qtxmlpatterns"
# Plugin Support
additional_packages="libffi libnsl nss"
# AI support
additional_packages="conda python3-tinygrad"
#export=" "
init=false
nvidia=false
pull=true
root=false
replace=true
start_now=false
```

A.2 Resource usage comparison

```
# output of: ps_mem -S -p $(pidof SlicerApp-real)


# 3D Slicer without any dataset loaded
Private  +   Shared  =  RAM used   Swap used        Program

416.0 MiB +   2.2 MiB = 418.2 MiB     0.0 KiB        SlicerApp-real
-------------------------------------------------
                        418.2 MiB     0.0 KiB
=================================================

# 3D Slicer with the full dataset loaded
Dataset: 309M    ./pre-Scene.mrb

Private  +   Shared  =  RAM used   Swap used        Program

1.3 GiB +   6.0 MiB =   1.3 GiB     0.0 KiB        SlicerApp-real
-------------------------------------------------
                          1.3 GiB     0.0 KiB
=================================================


# 3D Slicer with the reduced dataset loaded
Dataset: 15M     ./post-Scene.mrb

Private  +   Shared  =  RAM used   Swap used        Program

579.4 MiB +   6.0 MiB = 585.4 MiB     0.0 KiB        SlicerApp-real
-------------------------------------------------
                        585.4 MiB     0.0 KiB
=================================================
```

B Appendix: Shortcuts used in this Guide

Table 1: 3D Slicer shortcuts

`right click + mouse drag` (up/down)	zoom in and out
`Ctrl + mouse wheel`	zoom in and out
`middle click + mouse drag`	pan/translate view
`left arrow/right arrow`	show previous/next slice
`up arrow/down arrow`	show previous/next slice
`b/f`	show previous/next slice
`Shift + mouse move`	move crosshair in all views/ show ROI in all views
`left click + v`	toggle slice visibility in 3D view
`b`	reset zoom and pan
`g`	toggle segmentation visibility
`Ctrl + o`	add/load data
`Ctrl + s`	save data to file
`Ctrl + w`	close scene
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view
middle click and mouse drag	pan/translate view