

Manually segmenting volumes from 3D images using Imaris

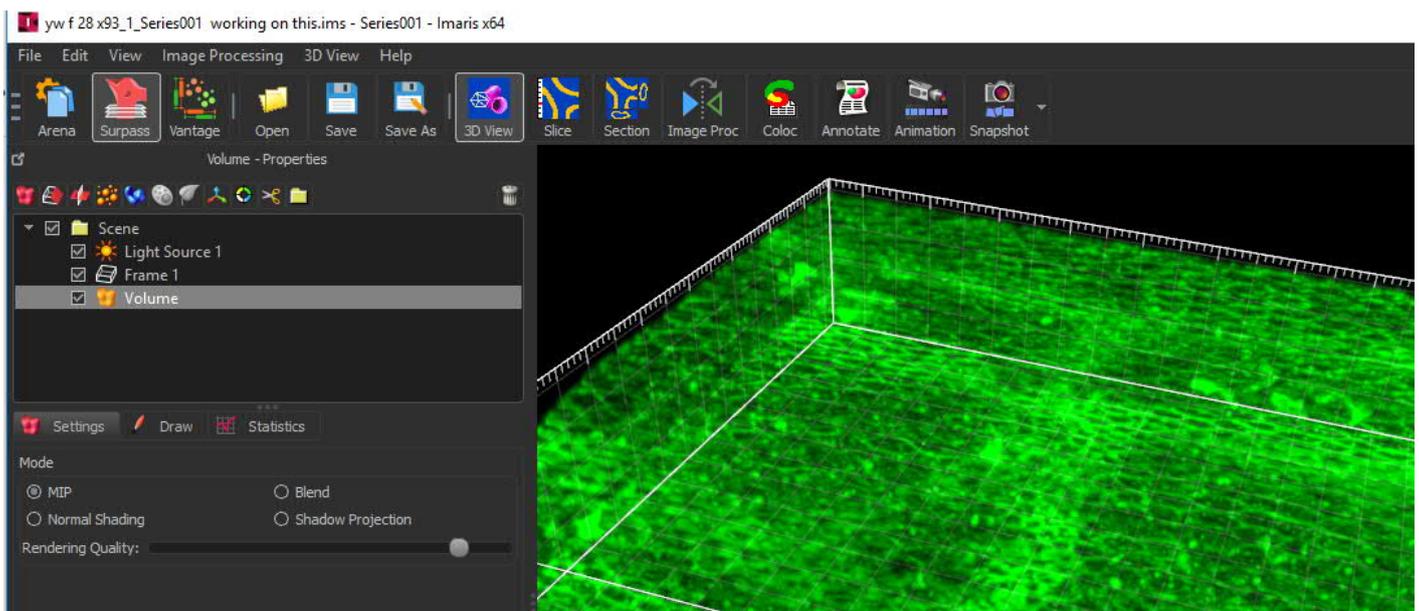
To use Imaris to analyse your data, your data has to be in the Imaris proprietary format (.ims) if you are working with a version newer than 9.2. Luckily, Imaris provides us with a simple to use file-converter that can be used to convert all microscope image data into Imaris format.

The converter is installed on all the computers with Imaris and as of Imaris 9.5.1 the icon looks like this

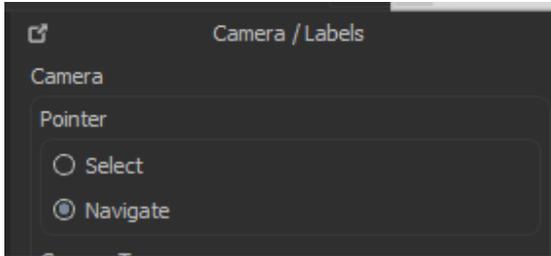


Needless to say, as Imaris get updated the version number might change. However the file name should say ImarisFileConverter. As this manual is meant for users with some experience with Imaris, it is assumed that the user can convert their files.

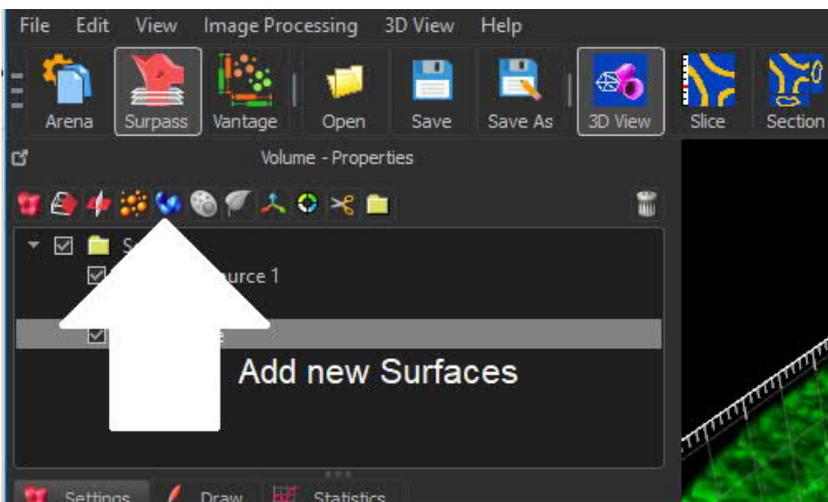
To open a file in Imaris you should be in the Surpass mode. To activate the surpass mode, click the icon 'Surpass'. Now you can File/Load your .ims file into Imaris.



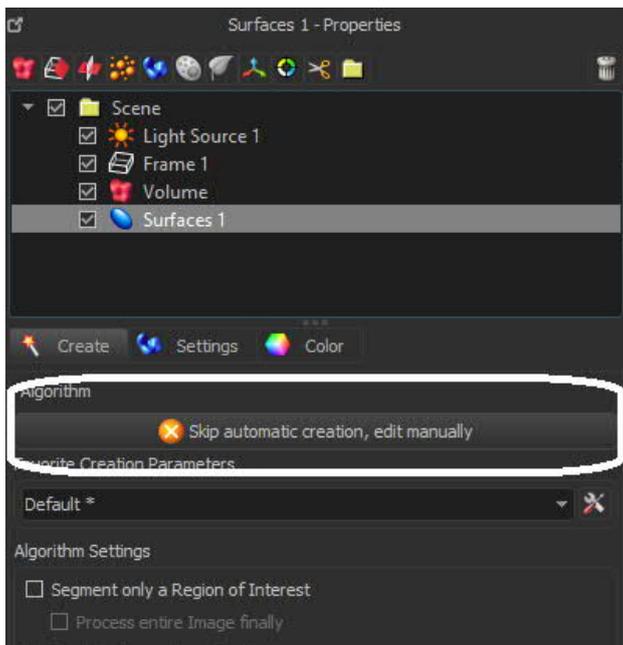
While Imaris can usually 'easily' segment objects with color IN the object, the software handles membrane stained cells less well. While Imaris Cell alleviates some of the problems, the issues with membrane stains – particularly in tissue samples often mean that automatic segmentation is not feasible.



You should familiarize yourself with the menu on the top right. When 'navigate' is selected, the mouse can be used to turn and rotate your image. When 'Select' is selected, you can draw and select objects. This becomes important later.

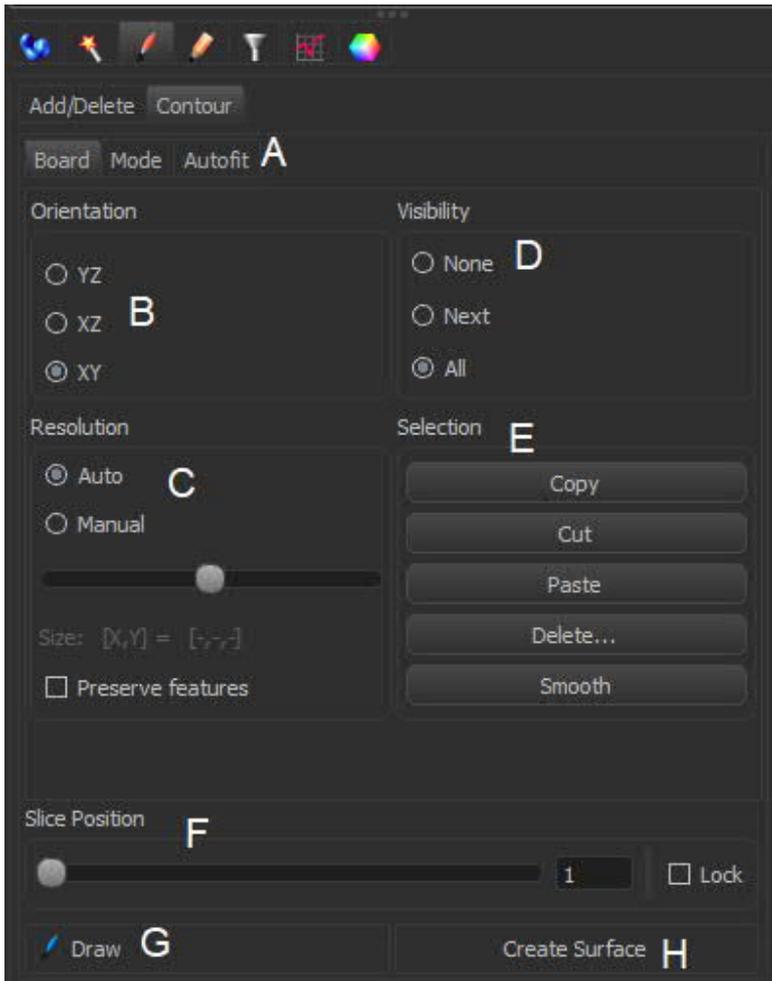


To manually segment a volume select 'Add new Surfaces'. This opens volume creation wizard on the bottom left of the screen.



From this wizard locate button saying 'Skip automatic creation, edit manually'. This is shown in image below.

This will open the following set of controls.

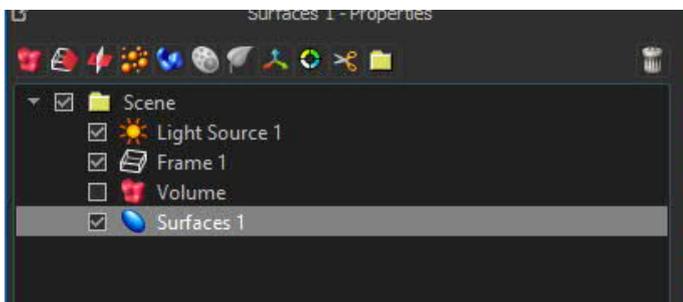


The menu A lets you navigate between 'board' as shown here, 'mode' where we chose the segmentation mode and 'autofit' that can be used to automatically fit contours to signal.

Autofit does not work well when we have cells touching each other so this manual will not detail that particular tool.

For the time being we should remain in the 'board' menu. Now you should navigate the sample into a 'good' orientation where you think you can see a structure you want to segment.

Next you should 'turn off' the volume from the top right menu.

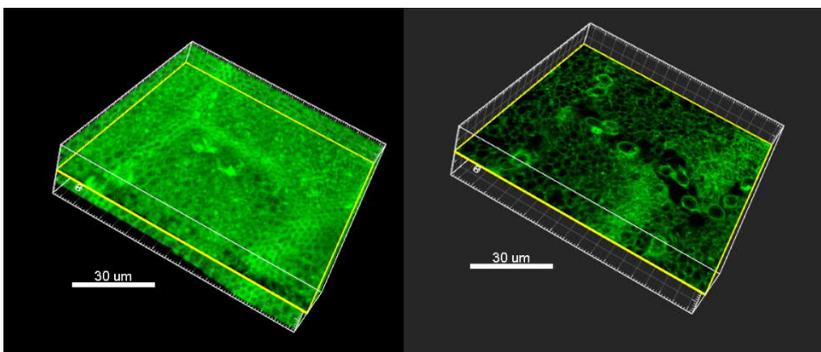


Simply untick the box next to the text 'Volume'.

Now your image should go from...

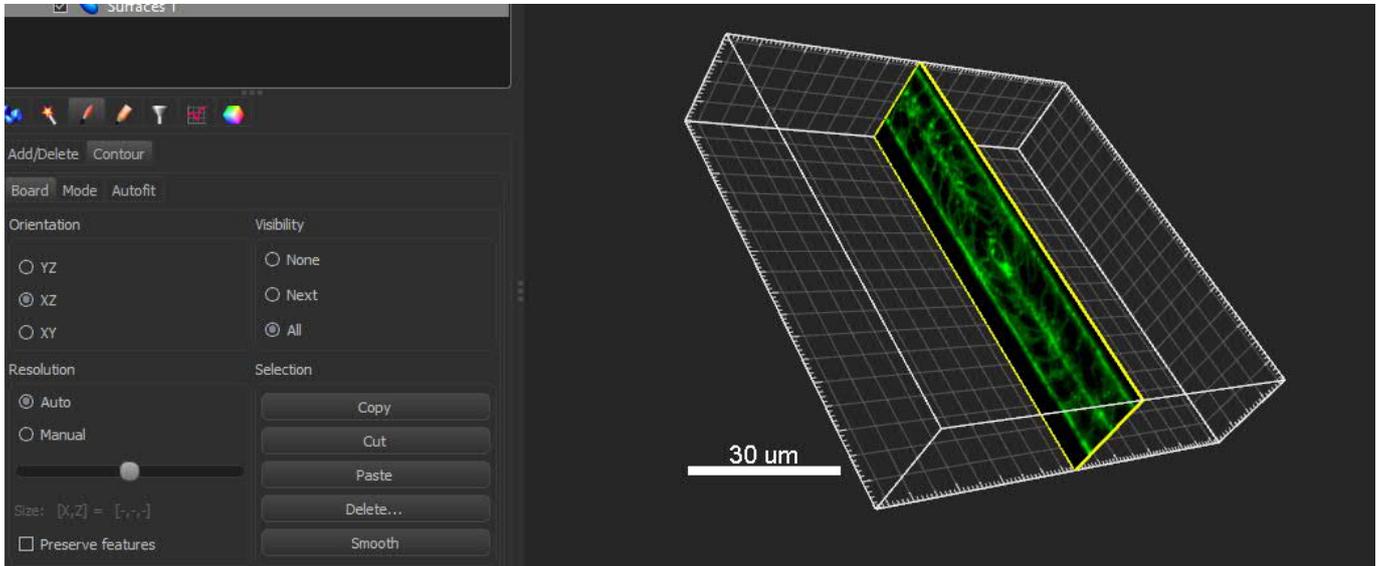
This...

to this.

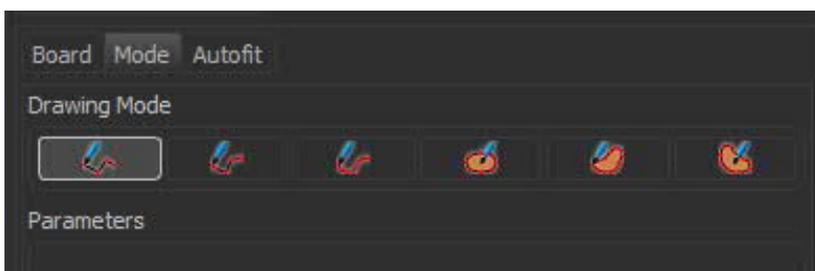


If you cannot see anything on your slice, move the slice position (F) towards the middle until you can see signal. Use IMAVIS Display Adjustment to make sure your signal is visible.

The slice position slider (F) allows you to move the selected slice up and down. You should now eyeball your object of interest to get an idea where it starts and where it ends and if you can reliably trace its borders. If you have no idea where the object starts and ends, Imaris is not going to be able to help you. The orientation of the slide is selected from the 'Orientation' (B). For example XZ orientation looks like this.

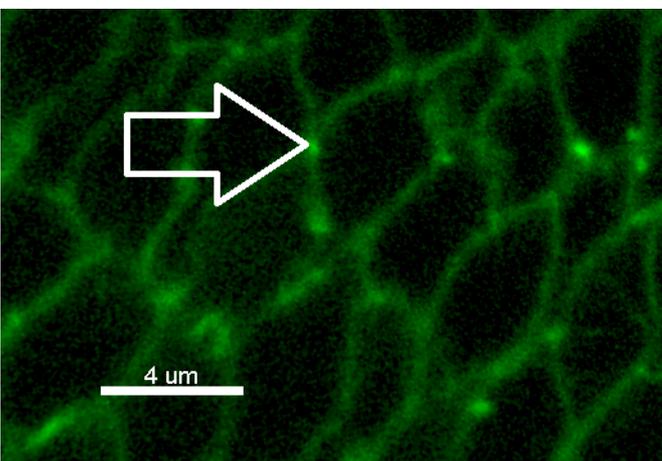


Considering how confocals work you should have no reason to use anything but XY orientation but the option is available. You should have the 'navigate' selected when you are trying to figure out which object to segment. Once you have picked a cell/ object you should chose 'mode' from the menu 'A'.



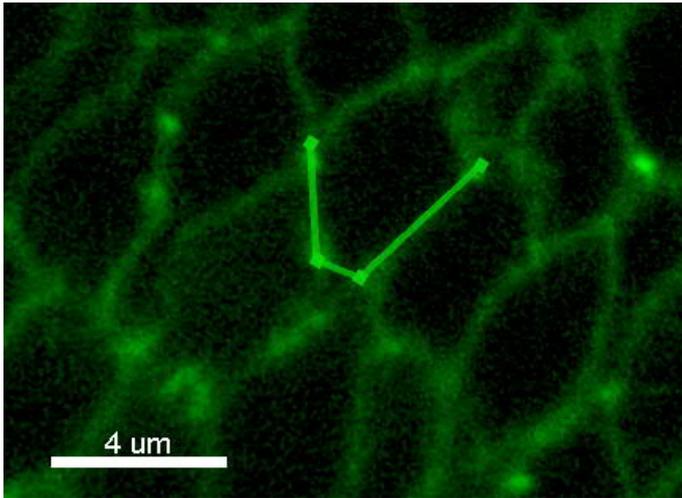
Here you should chose the leftmost drawing mode (Click).

Now we go back to the 'Board' and zoom / turn the image until we have the object in sight.

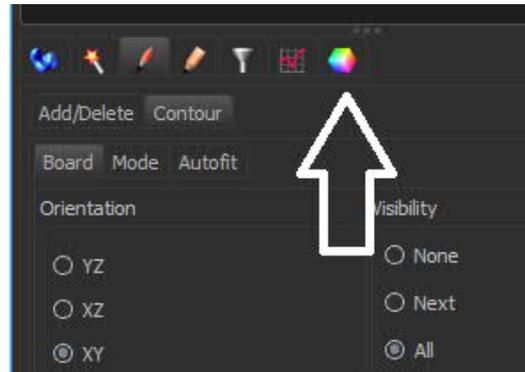


Now we chose 'select' from the select/navigate menu and then click 'Draw' (G) on the menu on the bottom left.

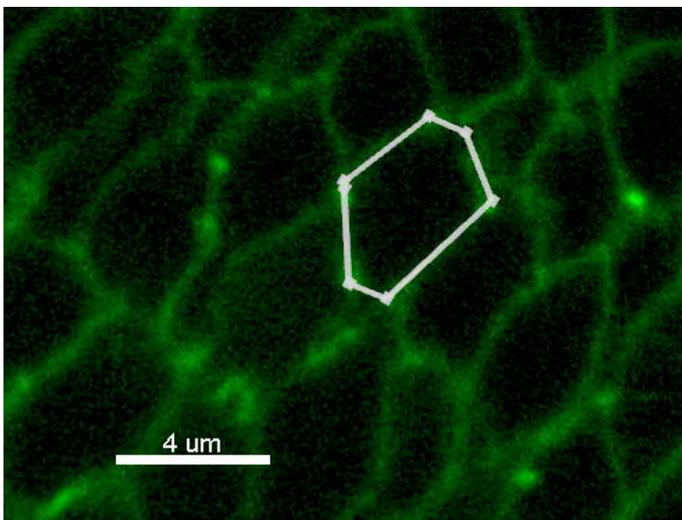
Now we can start adding points that will form the contour for this given plane.



The line colour can be adjusted from this menu.

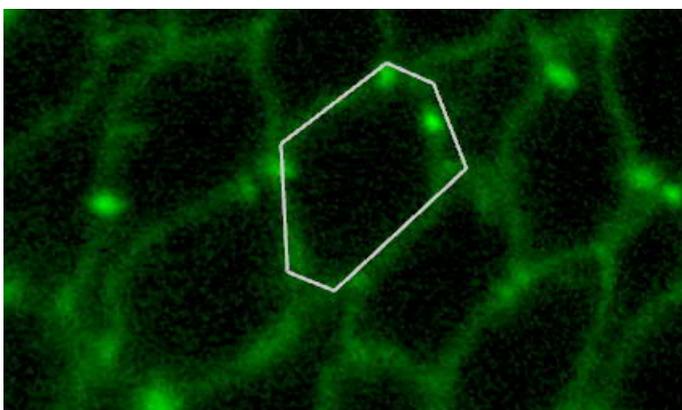


Now we can just finish tracing our object on this slice.



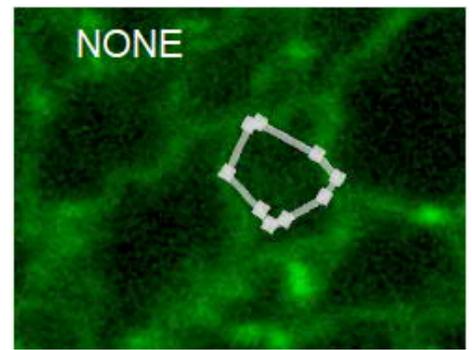
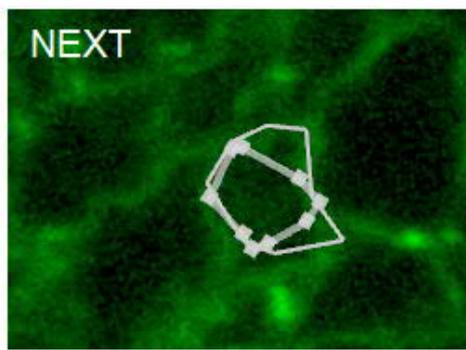
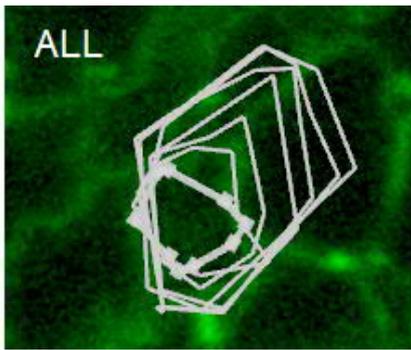
Once we have traced our object we can move to the next slice. If we are unhappy with our handiwork we can delete our contour on THIS slice using the 'Delete' command from the 'selection menu' E.

Assuming we are happy with our contour, we can move to the next slide using the slider (F). If we move to the slice above, the contour disappears from sight. If we move to the slice below we can see a phantom of our contour on the slice above.



The visibility of these 'ghost' contours can be adjusted from the 'Visibility menu' (D).

'All' shows all contours above the current plane using a thin line. The active slice has a thick line.



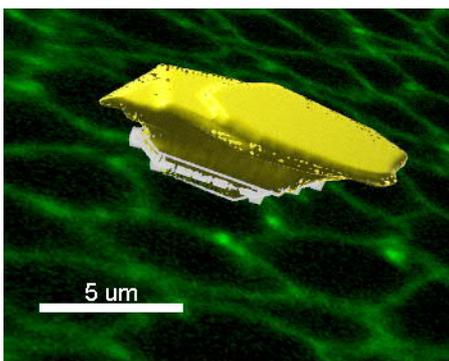
'Next' shows the contour on the slice above (and only the slice above) using a thin line. 'None' shows only the contour on the current slice.



Once you have drawn all of your contours, we can create the volume. Do note that you do not have to trace contour on every plane, you should decide the level of accuracy you want.

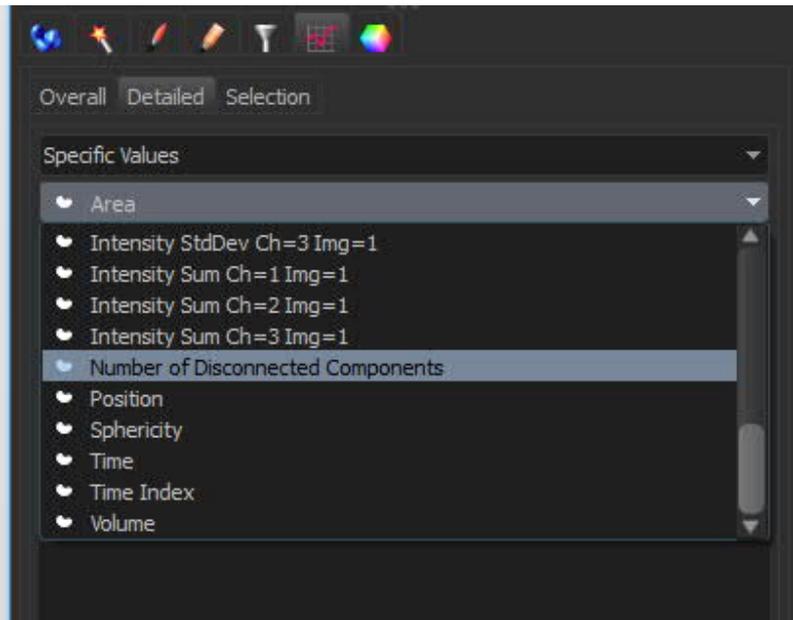
Before we can hit the 'Create Surface' button we need to hit the 'Manual' in the 'Resolution' menu (C). This lets us use the slider to determine how accurately the volume will follow the contours. At the extreme right, the volume will hug the contours. At the extreme left, the volume will roughly try to fill the space between the lines.

If you are using high resolution, you cannot skip planes, i.e. you cannot have planes without contours, or you will end with multiple objects as Imaris interprets that the slice without contours means that the object ends there.



Once you have set your resolution, you can press 'Create Surface' (H). If you chose 'high' resolution for your volume you should see something like this.

Statistics



This newly created volume behaves exactly like any other volume in Icaris meaning that you can use it to extract statistics and mask channels if you so desire.

To fine the volume (or any other value of interest) navigate to the 'statistics' tab (red squiggly line) and click 'detailed' and chose the parameter of your choice from the drop down menu.