LaserVox

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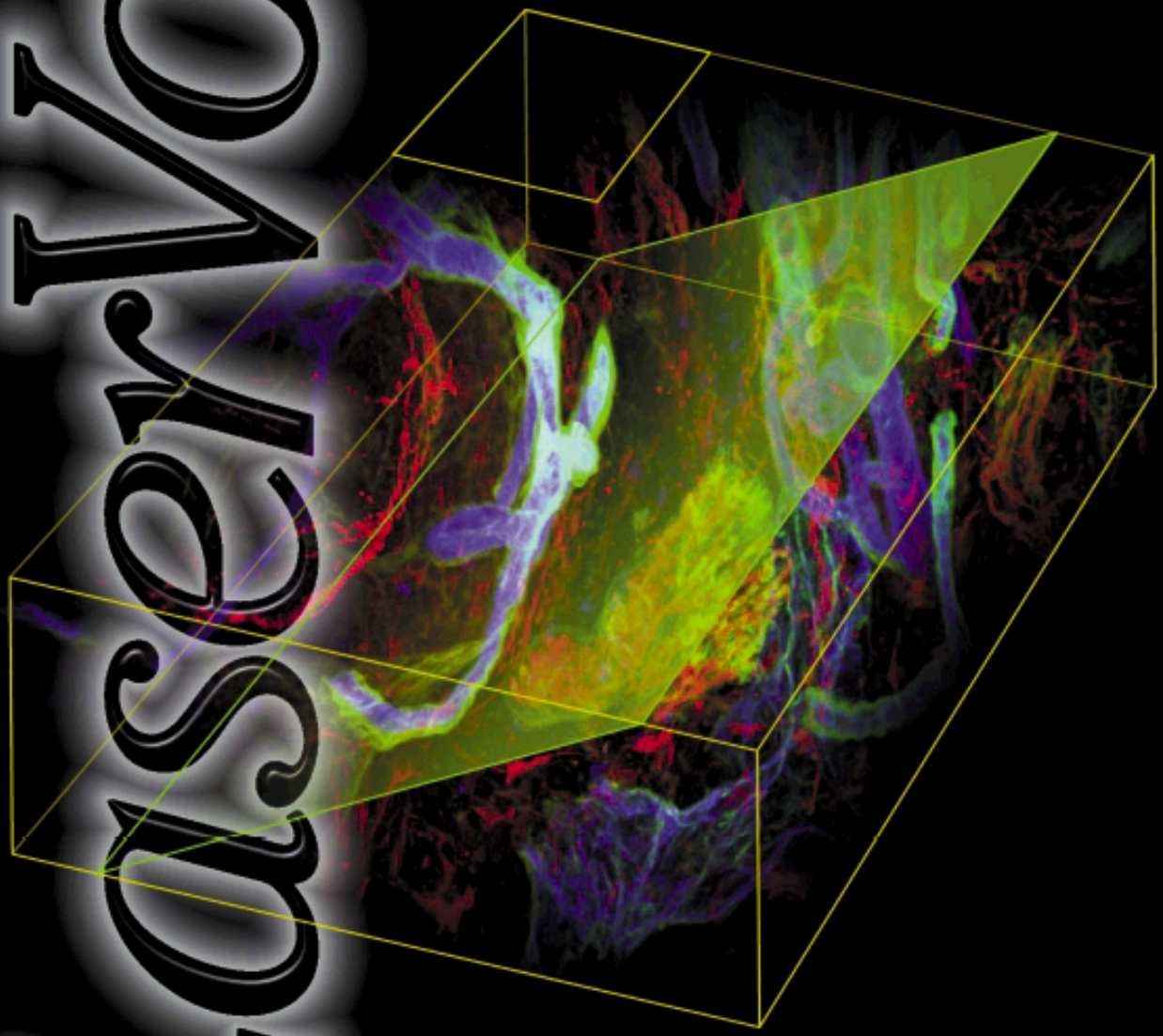
Users

Quick Guide



BIO-RAD

9MRC60UM08

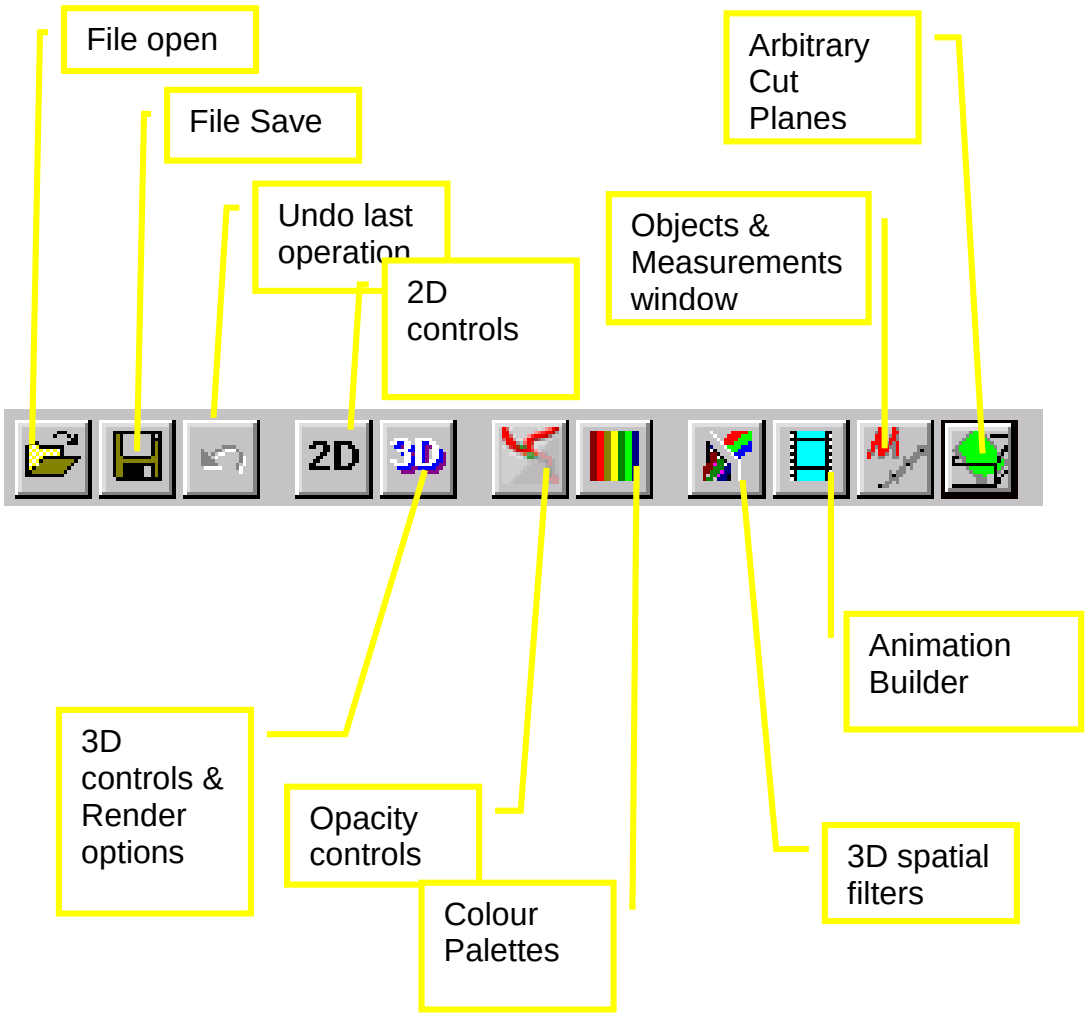
1 Quick Guide

1.1 Introduction

This quick guide is intended to introduce you to a selected range of LaserVox functions. It is NOT A COMPREHENSIVE tutorial or manual.

1.2 The Tool Bar

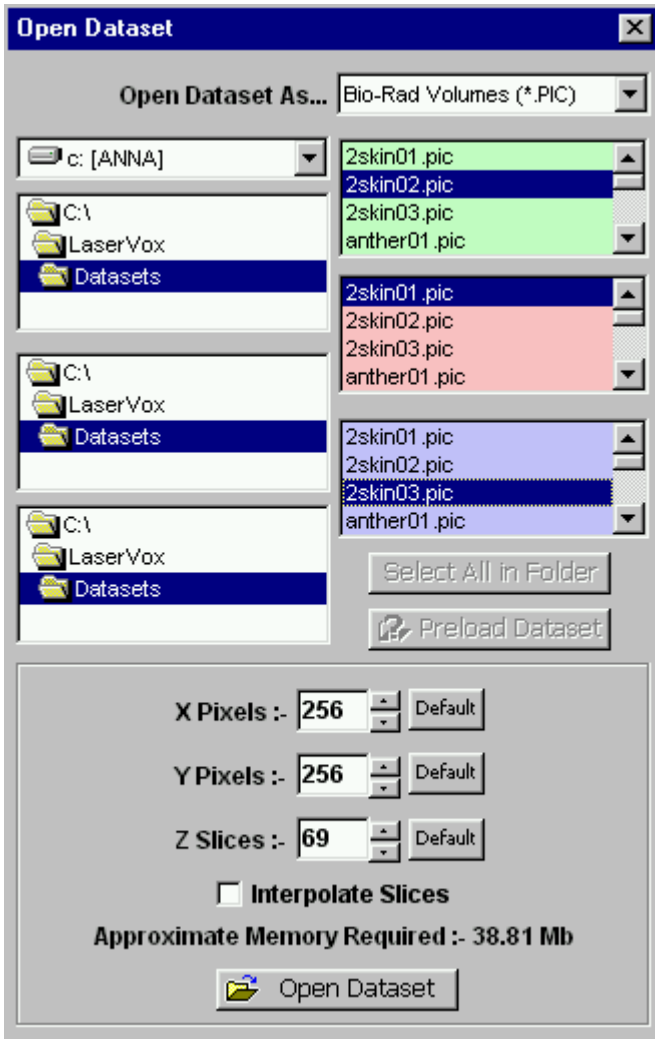
The Tool Bar along the top of the LaserVox Application window displays the following buttons.



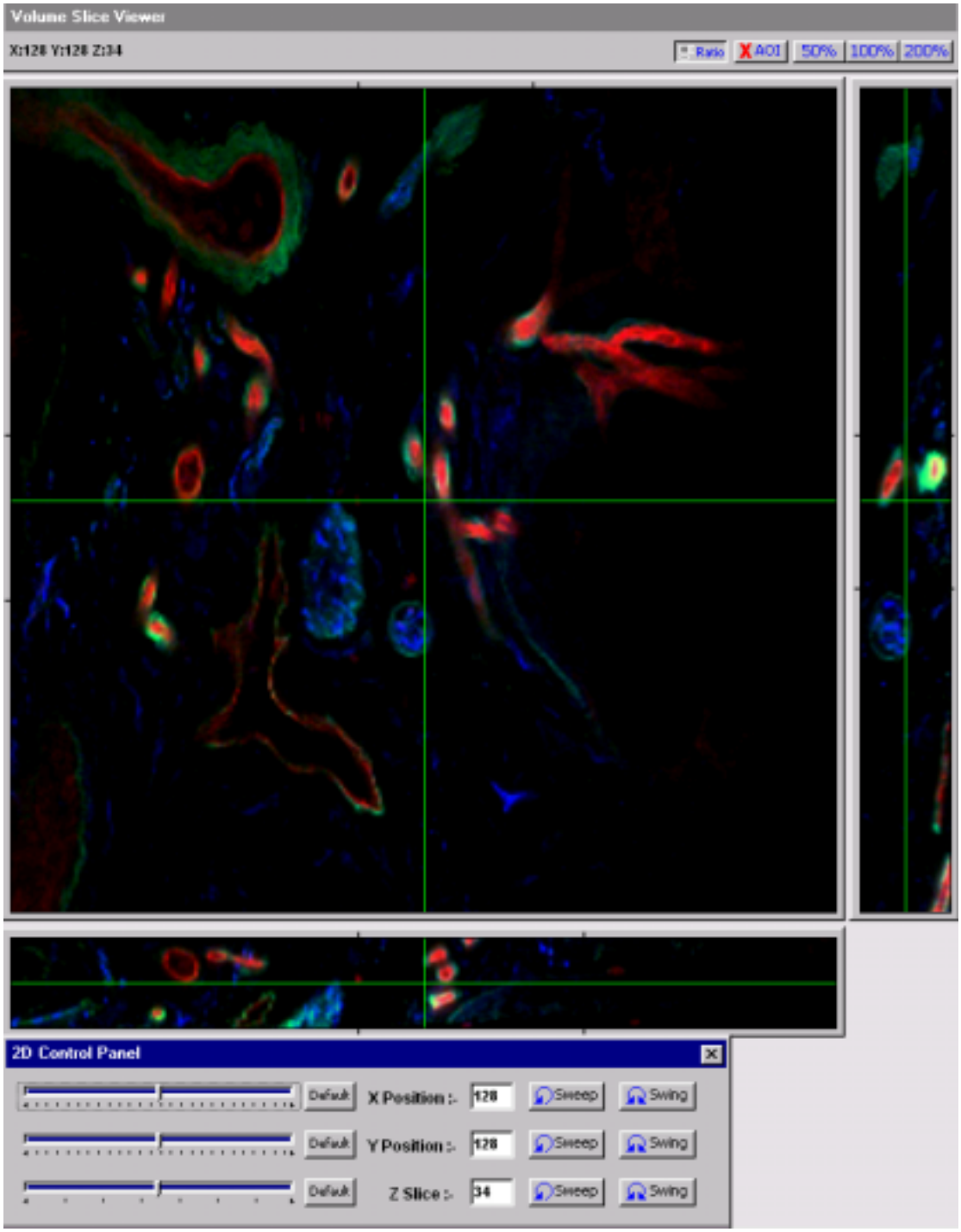
 These two buttons allow settings relating to colour palette, render mode and opacity to be saved and reapplied to different data sets.

1.3 Exercise 1 Opening and Navigating a Dataset

Start LaserVox from the desktop . Open the demo dataset 2SKIN01.PIC from the Open Dataset dialogue box by clicking once on the filename in the green upper panel of the dialogue.



Note that 2SKIN02 and 2SKIN03 (ie the red and blue channels) are automatically selected and that these could be deselected by clicking on the filename in the appropriate panel. Note that the dataset pixel dimensions are 256 X 256 X 69. Click on OPEN DATASET progress bar at the top of the LaserVox screen shows as the file is being opened.
You will see the 2D Volume slice viewer and the 2D control panel.



The 2D volume slice viewer shows the XY, YZ and XZ orthogonal views. Animate these by clicking on the LOOP button for the Z slice on the 2D control panel. Click again to stop the animation.

1.3.1 Zooming images

LaserVox has 2 types of zoom. The image window can be zoomed to set percentages of its original size (see at top of image window). Alternatively,

parts of the image can be zoomed up within the image window by using the zoom tool from the Mouse Mode bar. When zoom is allocated to one mouse button, negative zoom is automatically allocated to the other. The zoom mag glass icon does not disappear if zoom is currently allocated to either button, so it is necessary to allocate both mouse buttons to other functions when not using zoom.

1.3.2 Saving PIC files

When the context menu is allocated to a mouse button, a drop down menu appears which has options to save current image, current group of 3 images (XY,XZ and YZ) or save the dataset. Save current image will save just the visible view as a TIF file. Save the dataset will save the entire dataset or a subsampled dataset (if an AOI has been drawn) as a Bio-Rad PIC file.

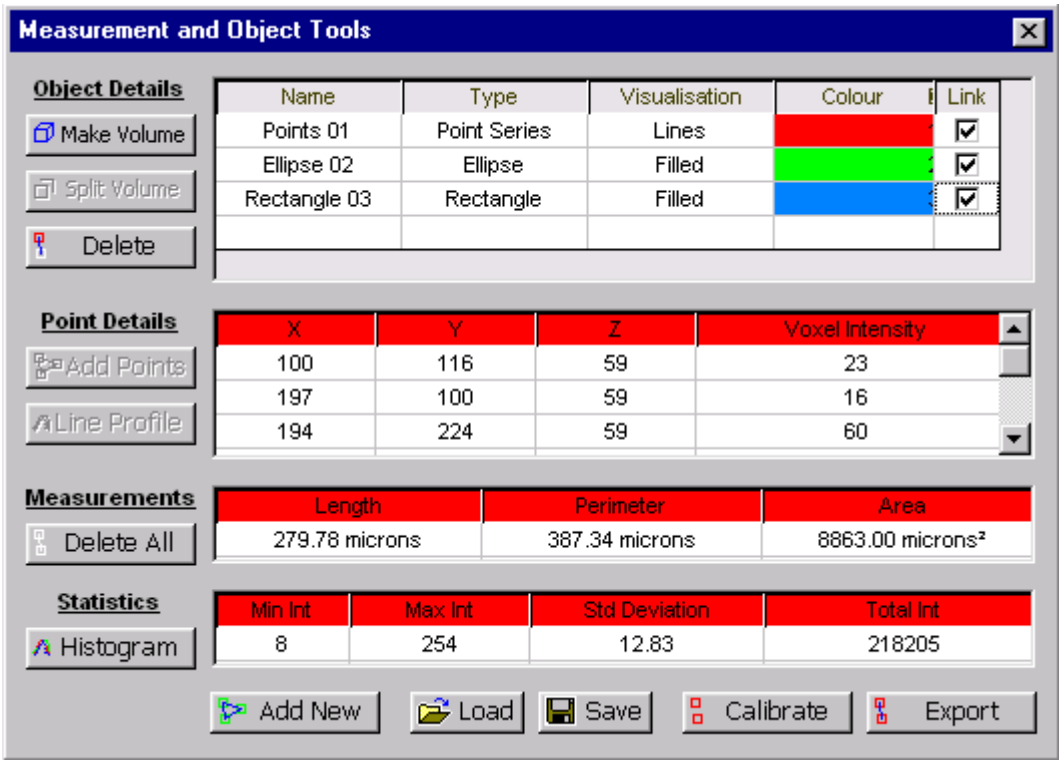
1.4 Exercise 2 Using the Mouse Mode Bar

Move the 2D volume slice viewer window to the right to reveal the Mouse mode bar.

Using the left mouse button, click on the cross-hairs  . The icon will display a red border signifying that the cross-hairs function is assigned to your left mouse button. When you place your mouse onto any of the 3 views in the 2D volume slice viewer, you will see green cross-hairs. Using your left mouse button, move the cross-hairs around in each of the 3 orthogonal views. Note that the cross-hairs automatically move to the same voxel in the other 2 orthogonal views. As you move the cross-hairs, note that the current X,Y,Z position of the voxel under the cross-hair is displayed at the top of the window. Note also, that the current voxel intensity is displayed in the 4th section of the status bar at the bottom of the LaserVox Application window.

1.5 Exercise 3 Creating and measuring a line object

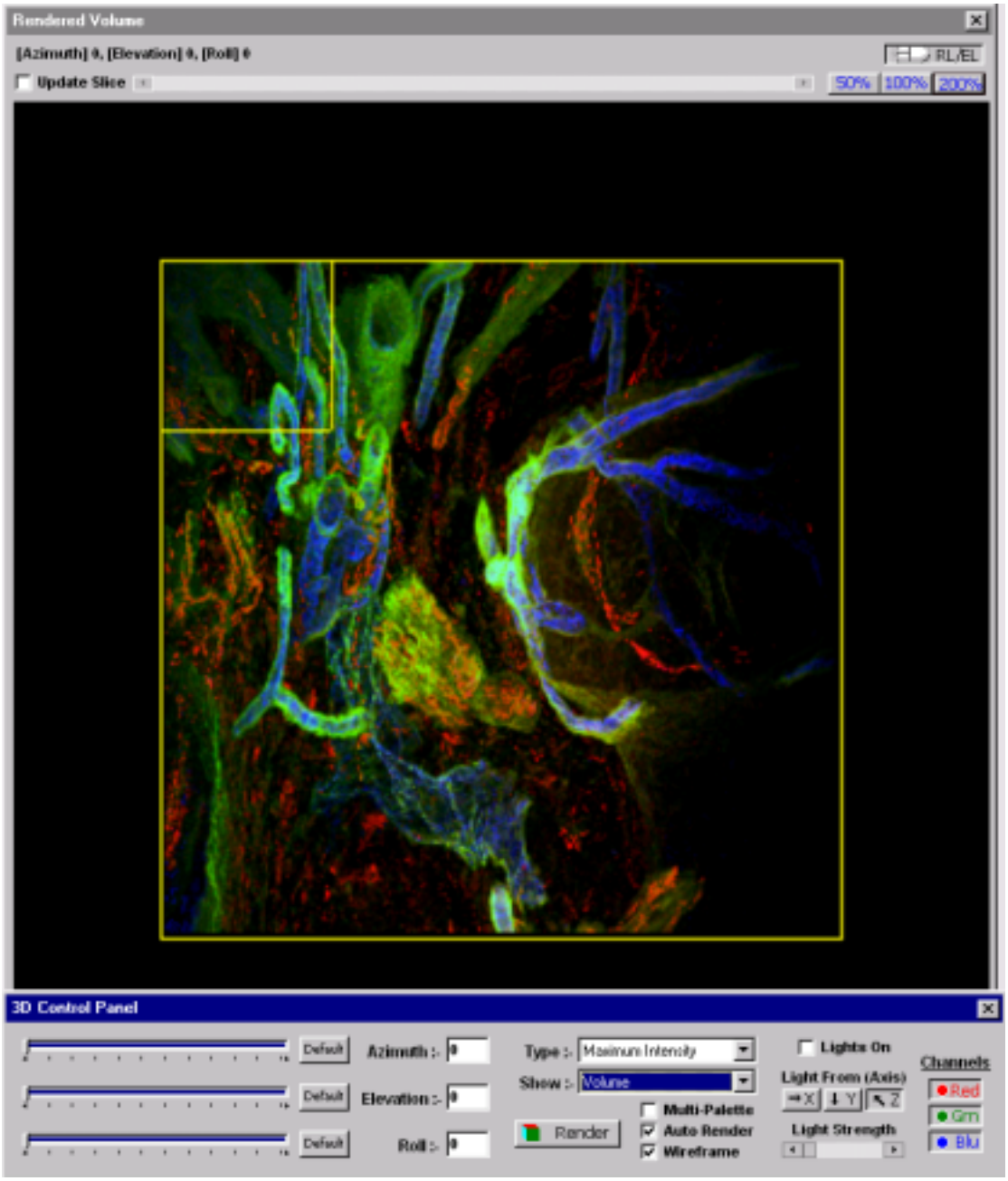
Now, create a line object within the data which can be used to measure or follow the path of structures in the dataset. Do this by clicking the right mouse button on the Line icon on the mouse mode bar  . The icon will display a blue border signifying it is assigned to the right mouse button. In the 2D volume slice viewer, use the left mouse button in one of the smaller XZ or YZ windows and move it so that the current z slice = 0. Using the right mouse button, click 2 or 3 points which form a line along a structure. Use the PgDn keyboard key to move to the next z slice and click some more points. Repeat this until you have moved right through the data set ie until you have reached the last z slice. You can see the measurements associated with your line in the objects and measurements window .



You can define as many objects as you like by clicking ADD NEW and continuing to define points - each will automatically be assigned a different colour. Please note that several line objects, several ellipses or several rectangular objects can be linked together to MAKE A VOLUME. Line objects have a LINE PROFILE available as a graph and ellipse and rectangle objects have a histogram.

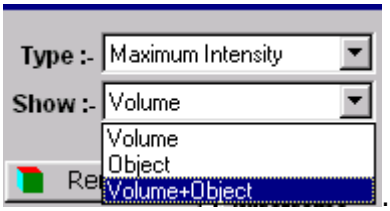
1.6 Exercise 4 3D rendering

Now make a 3D rendered view by selecting the render window  from the tool bar along the top of the screen. You will now see the render window and the 3D control panel.

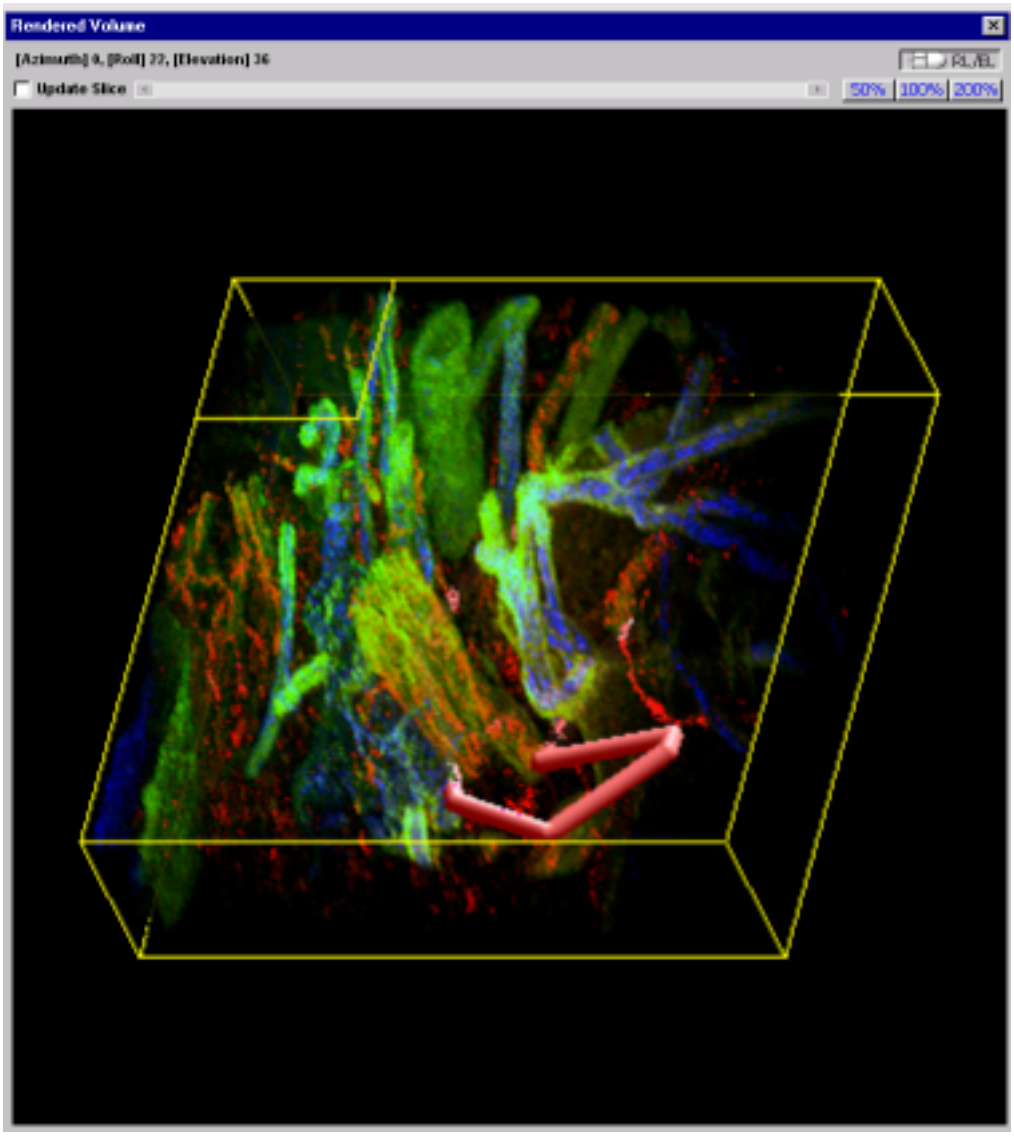


From the 3D control panel, select the Maximum Intensity option from the TYPE drop-down menu and the Object + Volume option from the SHOW

drop-down menu Click the RENDER icon if the Auto Render option is not checked. Read details about other Render modes.



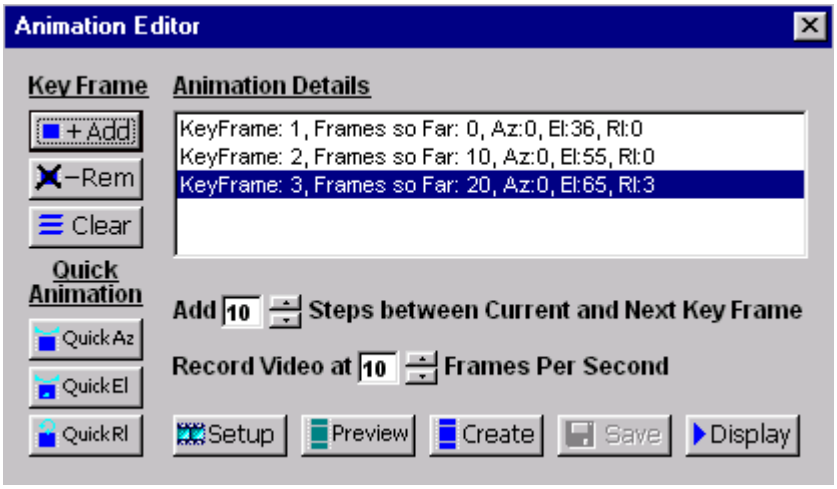
This will render both the original dataset volume as well as the line object that you have defined. From the mouse mode bar, left-click on the wire frame icon  and in the render window, use the left mouse button to drag around the yellow wire frame to a desired view. When you release the mouse button, the new view will automatically be rendered.



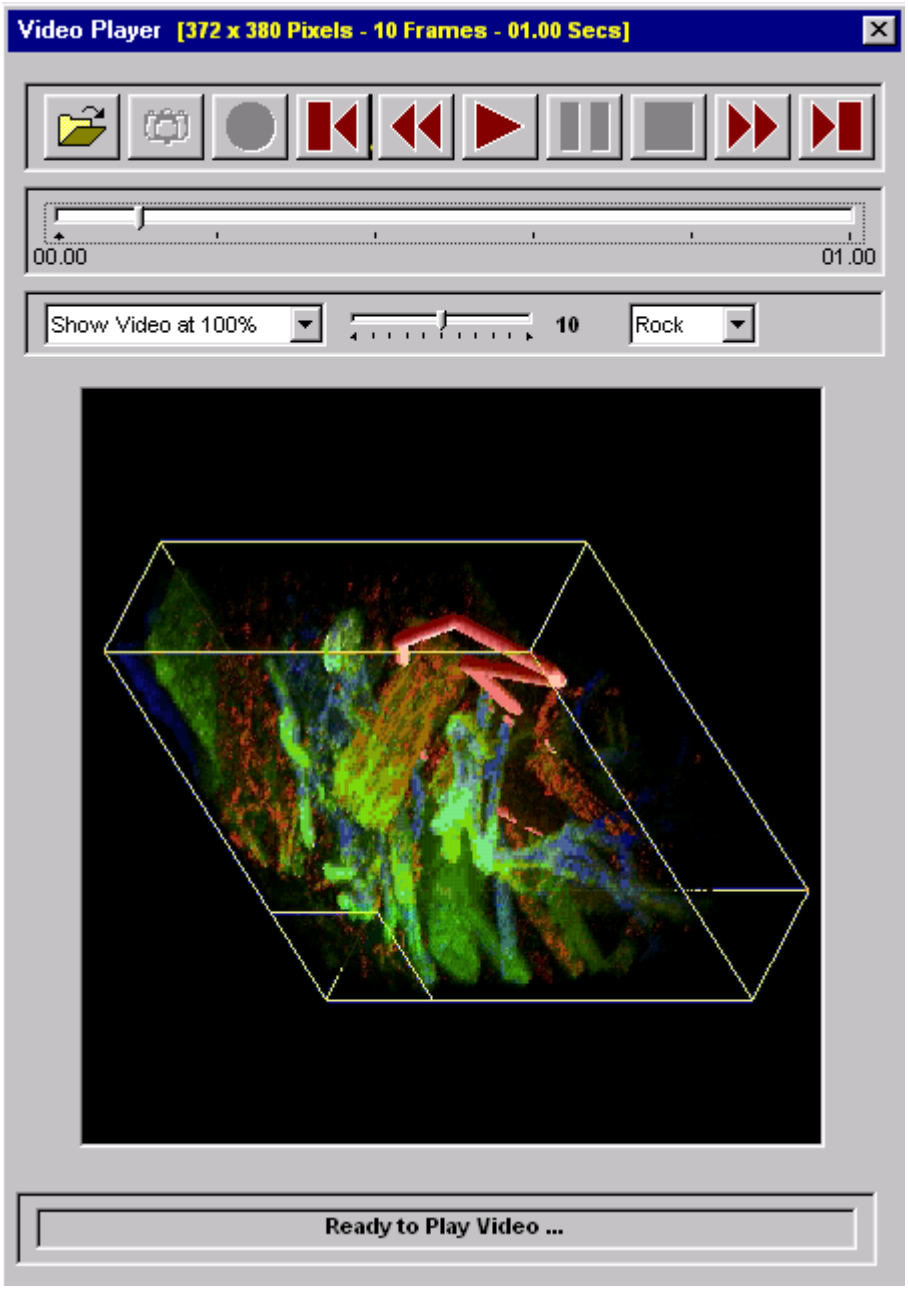
Try this several times at different angles.

1.7 Exercise 5 Animations

Now select the Animation window . Initially, click one of the QUICK buttons on the bottom left of the animation dialogue. When the build is complete, click DISPLAY. Now close the Video player and CLEAR the keyframes in the Animation Window.

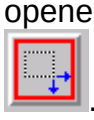


Back in the render window, move the wire frame to a position where you would like to start a movie, then click ADD in the animation window. Then in the render window, move the wire frame to a position where you would like to end the movie and click ADD again. In the animation window, click the CREATE button. The render window will then show each view of the movie being built – the status of the build is displayed in the status bar at the bottom left of the LaserVox screen. When the build is complete, click DISPLAY. A video player now opens and shows your movie. Please note that it is possible to animate colour palettes, opacities and different AOIs. Simply adjust the current view before ADDING it to the animation.

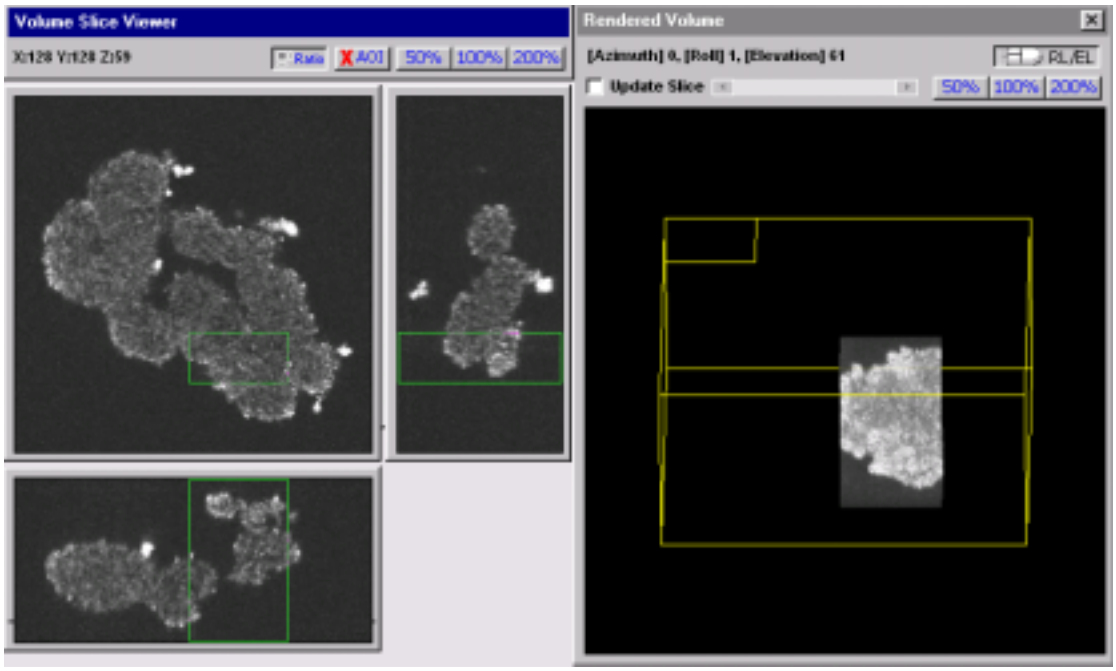


This can be stopped by clicking the square video stop button. Close the video player and click the SAVE button in the animation window to save your movie as an AVI file.

1.8 Exercise 6 Subsampling Volumes

Now open the single channel dataset STARCH.PIC. When the file has opened, left-click the mouse button on the AOI icon on the mouse mode bar  . In the 2D volume slice viewer, go to the XY main window and using

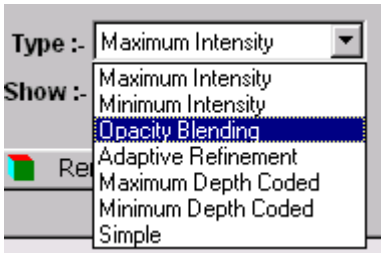
the AOI rectangle tool, define a subvolume of the data to render. Click the 3D render icon from the tool bar.



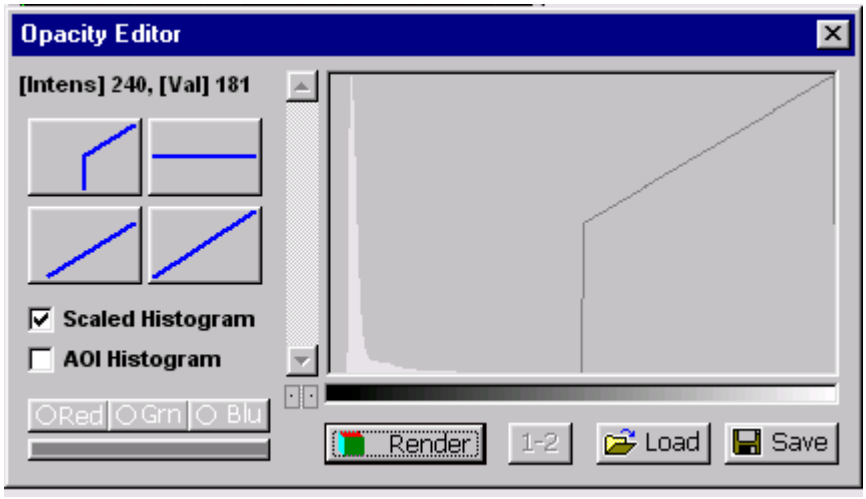
Note that only the subvolume has been rendered. This allows you to render selected parts of the data and ignore parts which would only slow down the processing rate. Using the AOI tool in either of the smaller orthogonal view windows allows you to only render a selected range of z slices, perhaps choosing to ignore the first and last few slices from your rendered volume. To remove the subvolume restriction, click the REMOVE AOI button in the 2D Volume slicer 

1.9 Exercise 7 Opacity blending

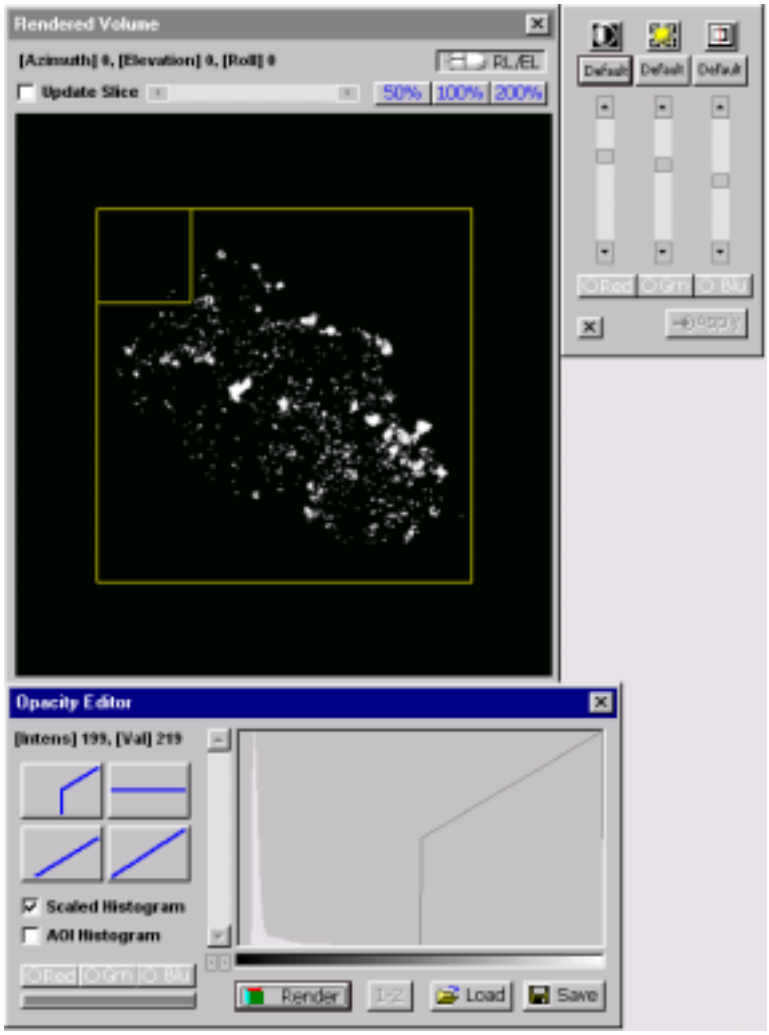
Using the same dataset, select the Opacity blending render mode from the 3D control panel and click once inside the render window.



The dataset may now look very different indeed. Select the opacity window  There are 4 default opacity settings at the left hand side of the opacity window.

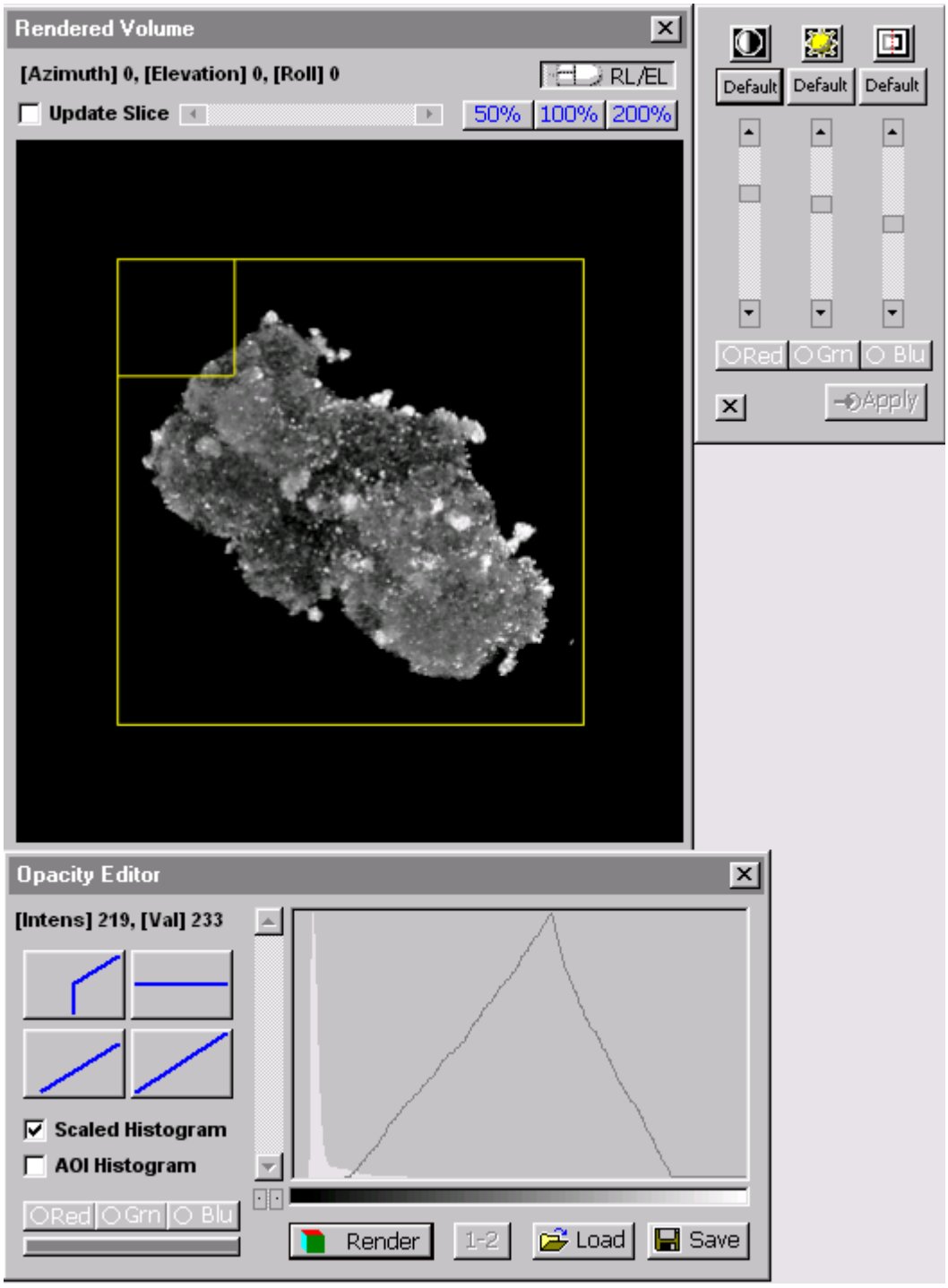


Select one of them and click the render button.



Do this in turn for each of the default settings and observe the changes each setting makes to the appearance of the rendered data. Now, you can try to create and save your own opacity setting to make some of the lower intensity

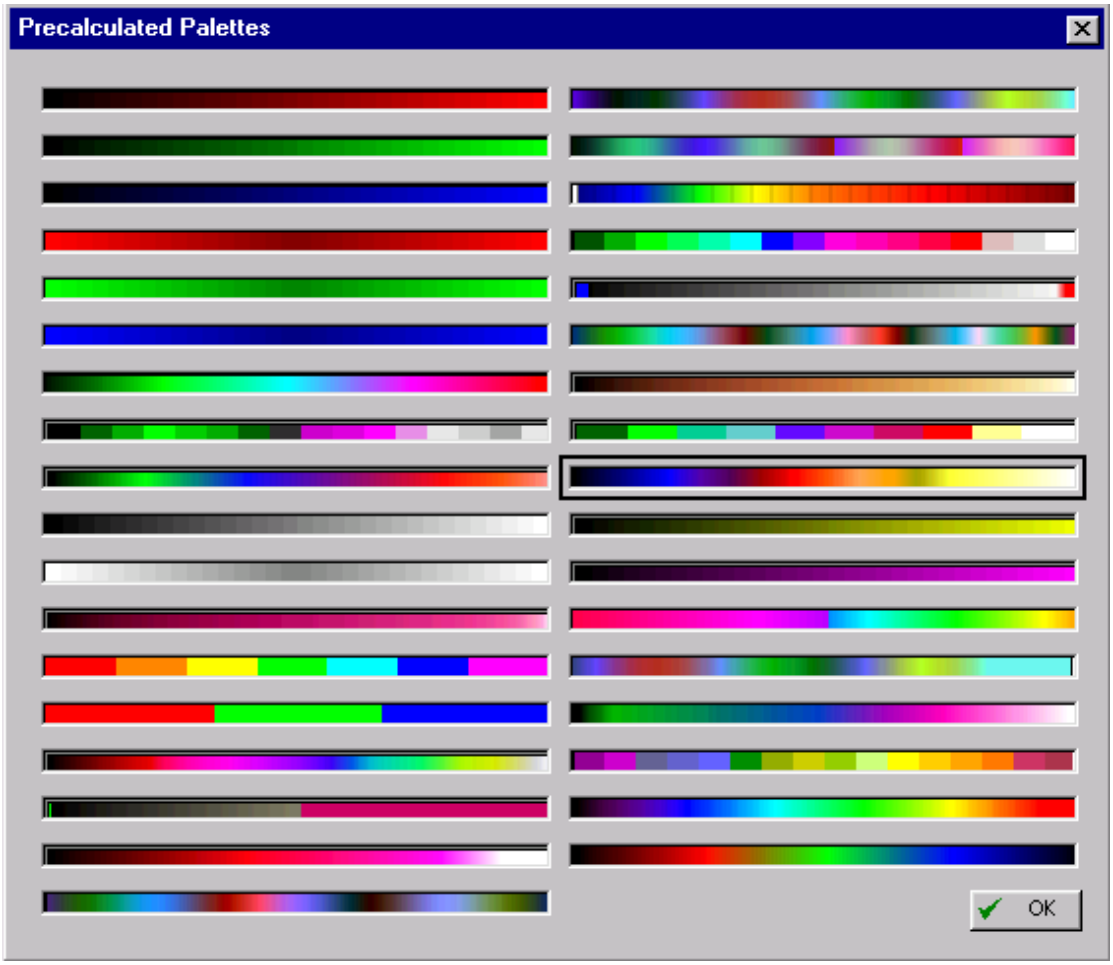
parts of the data become more opaque, but without completely obscuring the high intensity structures. Note, that with the example below, one is able to make the medium intensities appear more ‘solid’ such that they partially obscure the higher intensities. Do not forget that low (even black) intensity voxels can be made to obscure high intensity voxels and will be displayed with their original voxel intensities. This means that if black voxels are given a higher opacity than brighter voxels – the rendered view will appear black!



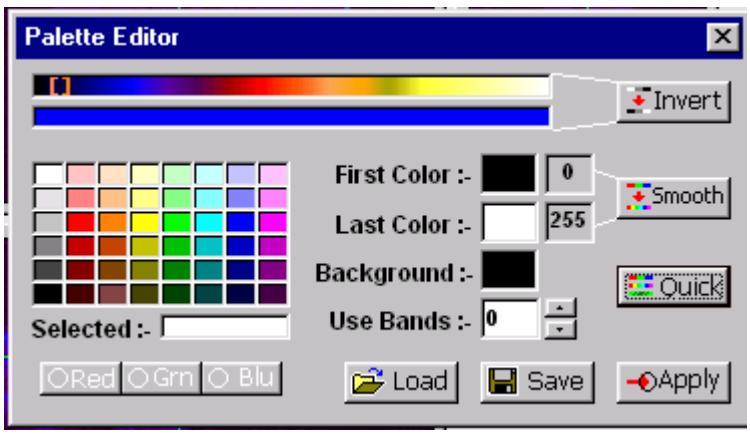
Save this opacity setting as a OPC file for future use.

1.10 Exercise 8 Colour Palettes

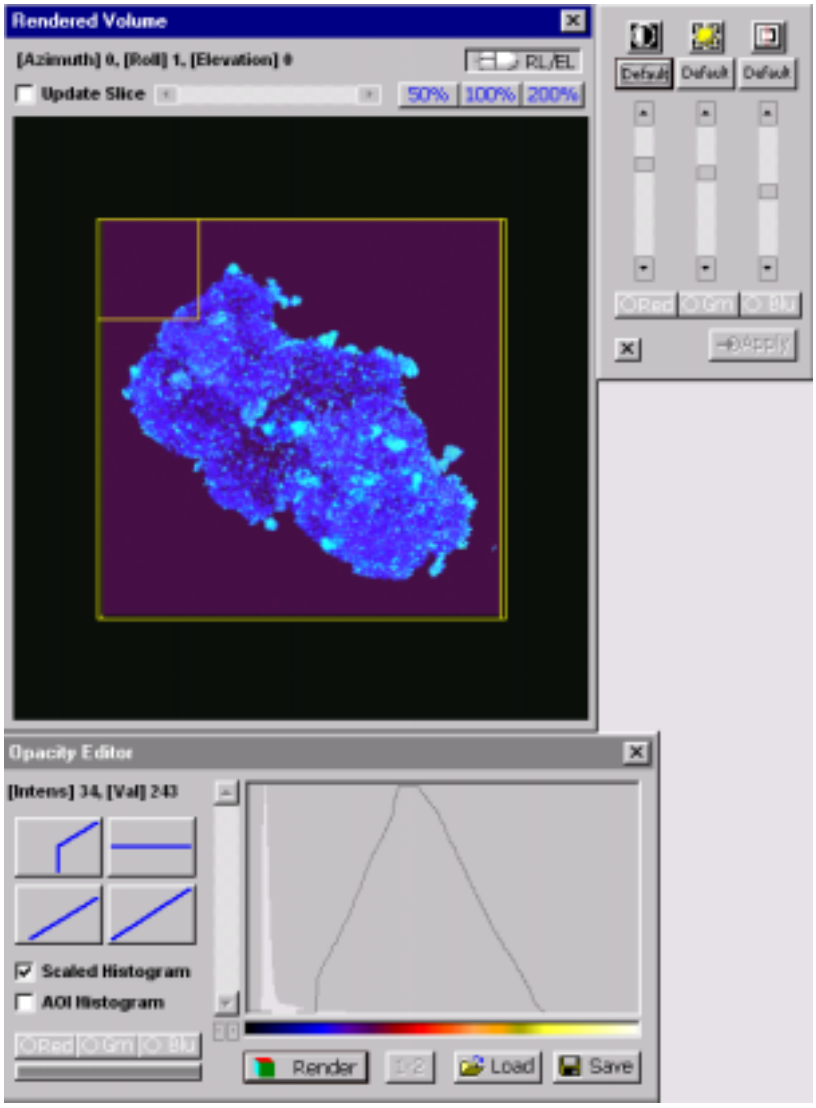
Now try changing the colour palette of your dataset by selecting the palette window Firstly, click the QUICK button in the palette window, select a palette, click OK and then APPLY the palette to your dataset.



Do this several times with different palettes.

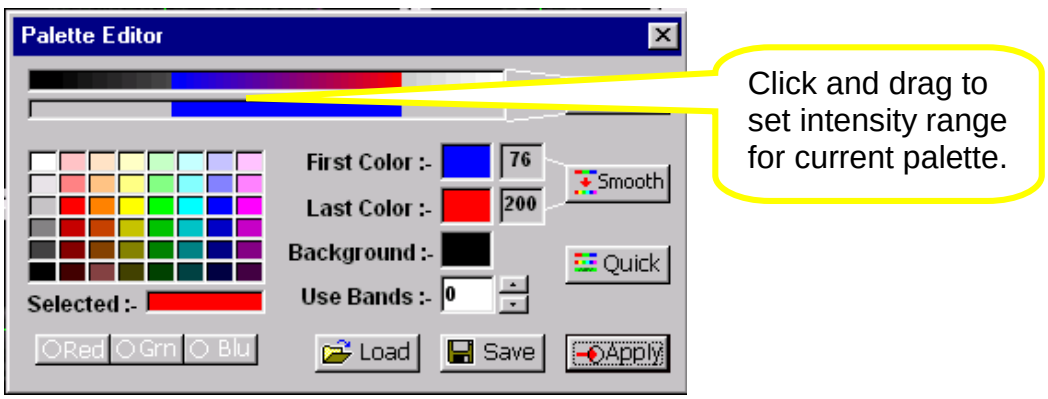


Note that this is now applied to the rendered view



Also note, that since the palette is shown in the opacity editor, it is possible to set specific opacities to be applied to features in the image with particular colours.

Now create and save a customised colour palette. Point the mouse to the upper of the 2 horizontal bars in the palette window.

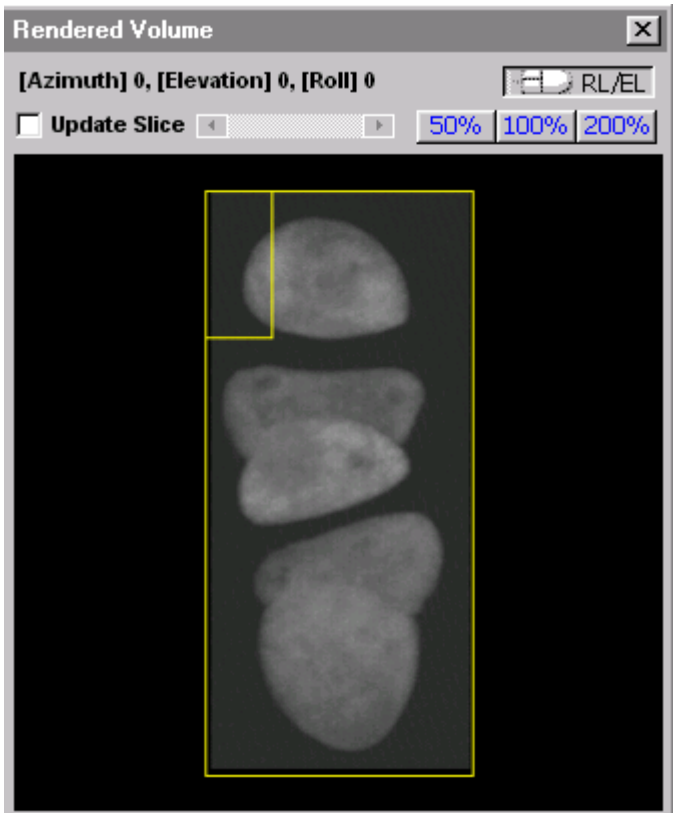


This displays all the intensities from 0 to 255 to which you can assign colour. Move your mouse (with no button depressed) leftwards from the 0 postion until a value of about 75 is displayed. Then click the left mouse button and drag the mouse to the right until the value reads 200. In order to make this intensity range be displayed as a range of colours between blue and red (only for example), you need to select the blue colour from the palette displayed and click the First colour box, then select red from the palette and click the Last colour box. Then click the Apply palette button. Now, when your image is rendered, intensities between 75 and 200 will be displayed in a range of colour from blue to red. Now add another palette between 200 and 255.

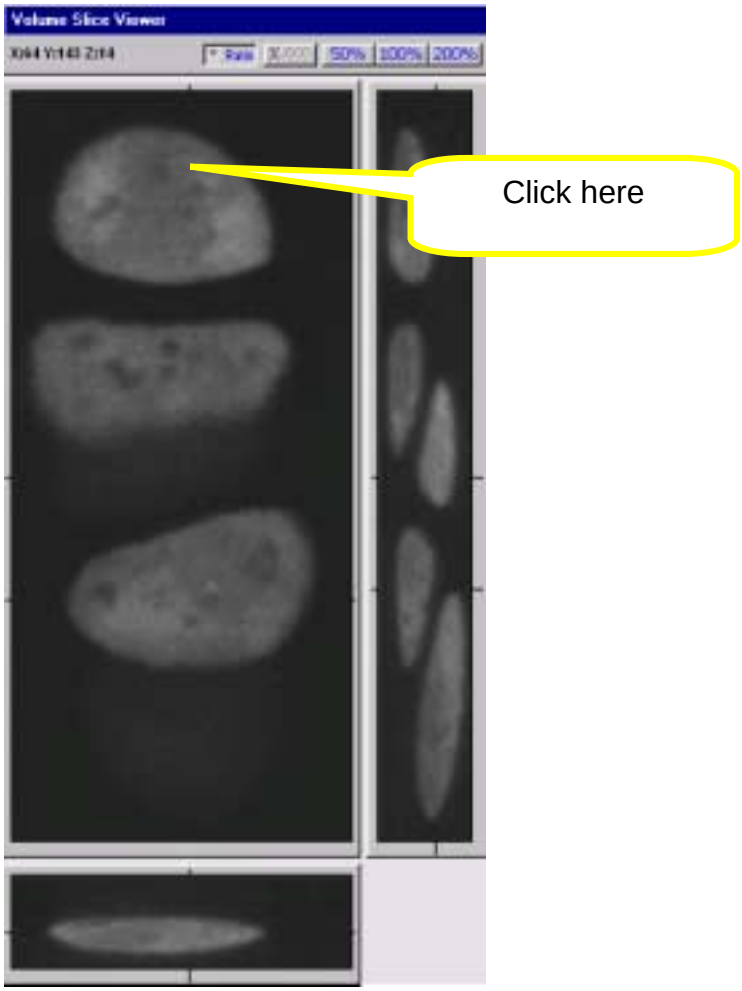
Save the palette as a .PAL file.

1.11 Exercise 9 Object Segmentation

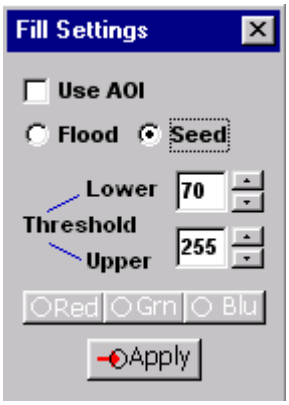
Open Chondro.pic. This dataset consists of 5 chondrocytes which were imaged live with a Bio-Rad confocal system. Although the cells appear quite dim, this is a very good dataset to demonstrate the use of seed-filling. Animate the dataset by clicking the LOOP or ROCK button on the 2D control panel. Move to Z slice 14. Here, you can see three out of the five cells. Click the 3D tool on the tool bar and render the volume.



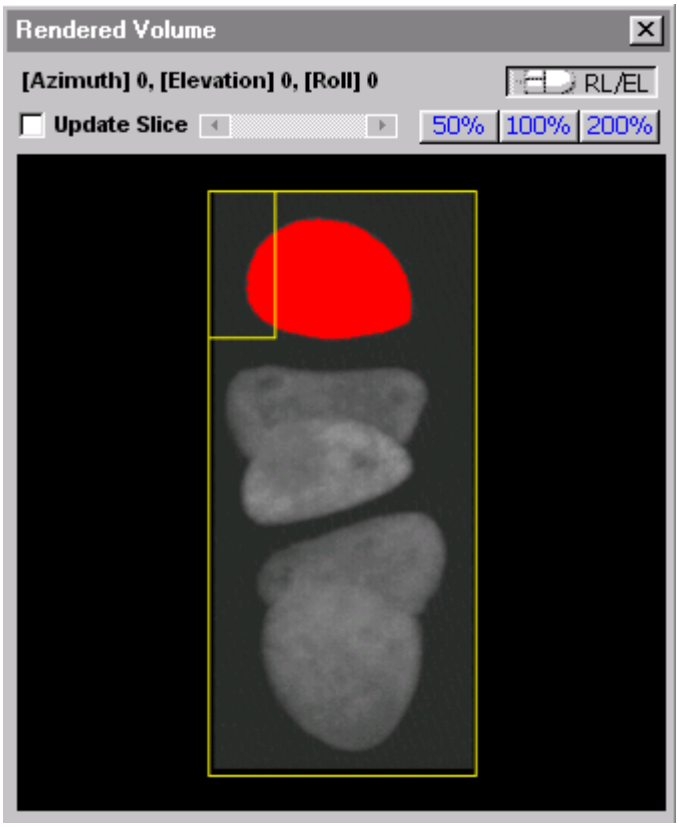
Go back to the 2D volume slicer window. Select the Seed/Flood-fill option from the Mouse Mode Bar with the left mouse button. It will display a red border  . Click somewhere inside the uppermost cell in the image



Now, the Flood/Seed-fill dialogue appears. Select the seed-fill radio button and leave the intensity range between 70 and 255 (default setting) and click APPLY.

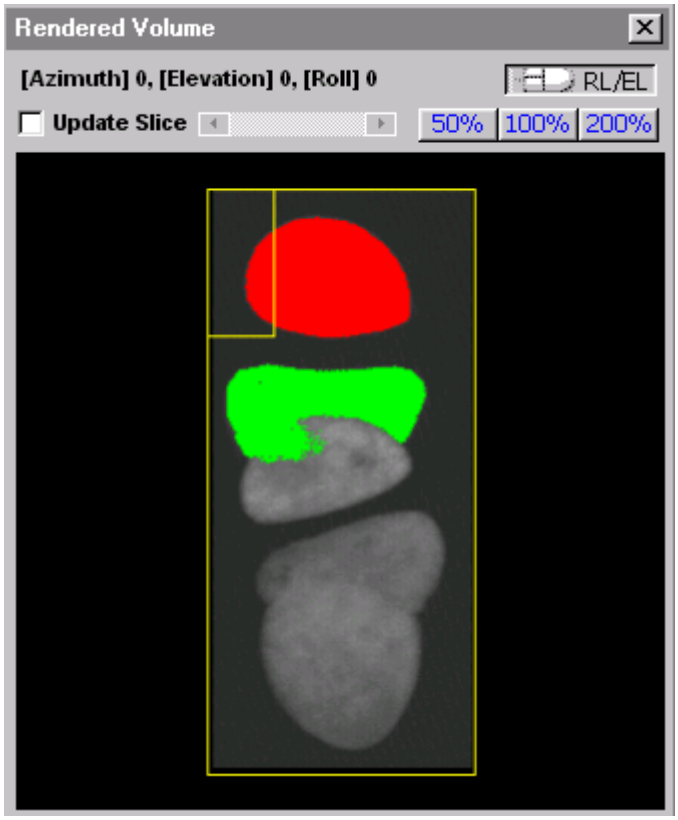


Now, select SHOW volume + objects from the 3D control panel and click RENDER.

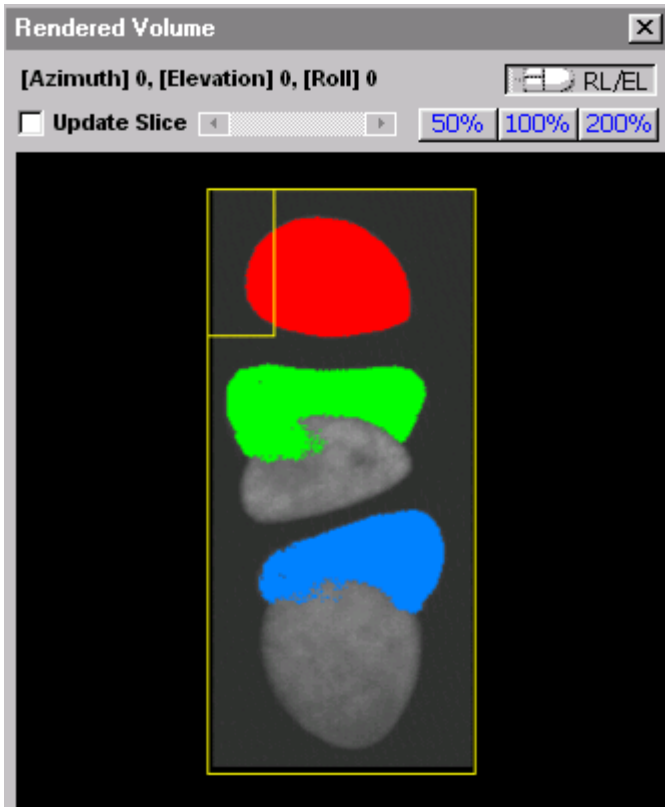


Now, the top cell has filled to make an object which can be measured. Go to the measurements window, click on the word VOLUME in the object TYPE column and read the volume in cubic microns.

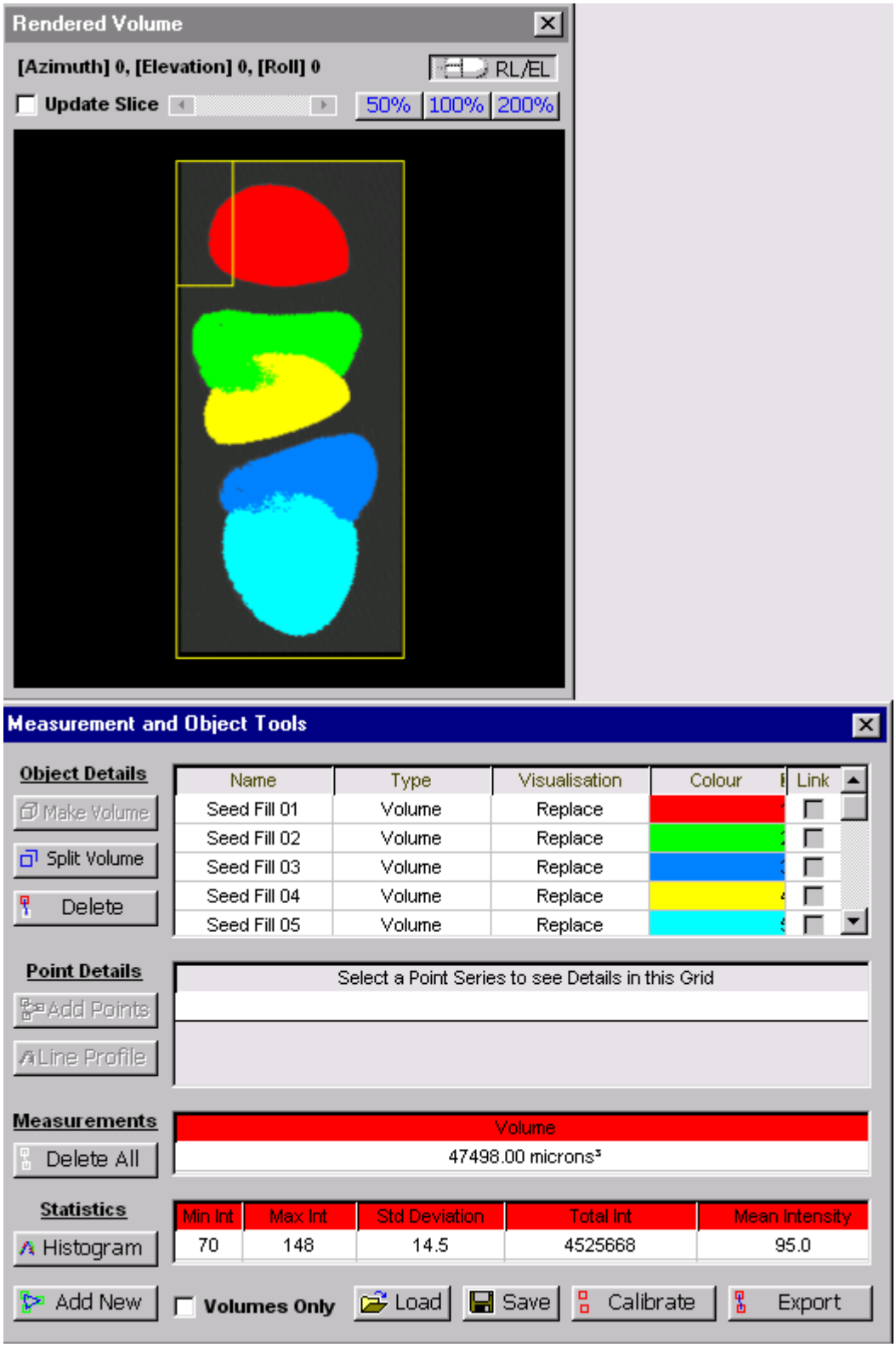
Now, go back to the 2D volume slicer and click somewhere in the second cell down. Do another seed-fill and render as before.



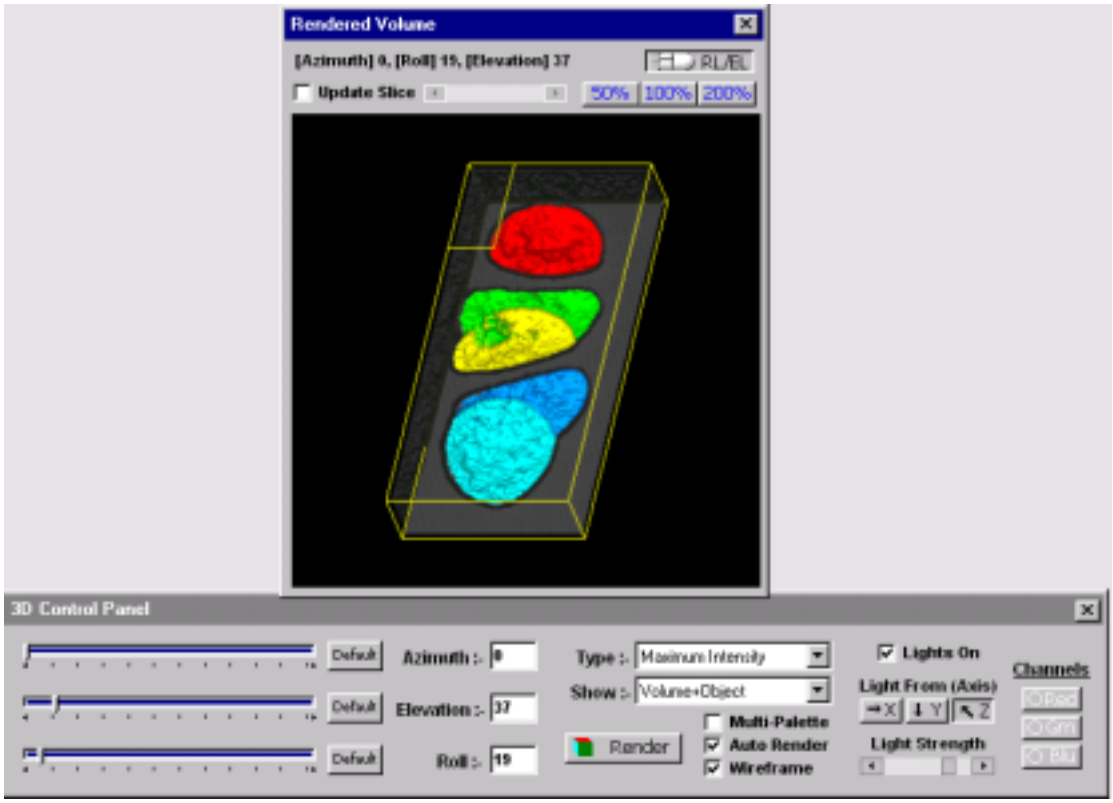
Now, 2 cells are filled. Each fill is a different object and has different measurements associated with it. Now click inside the lower oof the visible cells.



Now, 3 cells are filled. Before filling the other two, move the z slice position to 27 using the 2D control panel. Now, the other 2 cells are visible. Seed-fill each of these as before to attain a rendered view of the 5 cells, each in a different colour.

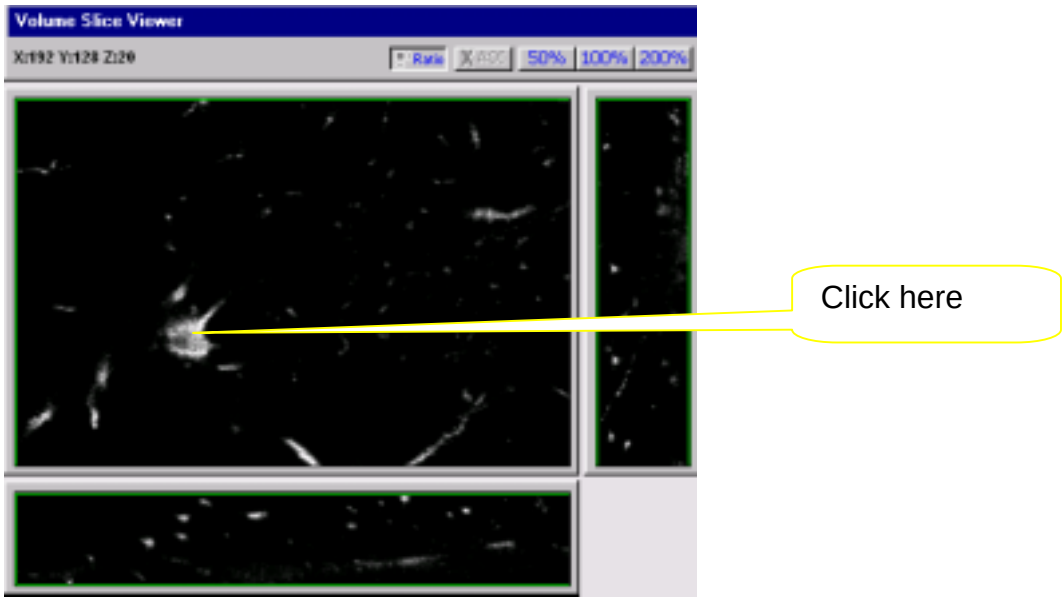


It is also possible to select the LIGHTS option from the 3D control panel and increase the strength of the lights to produce a textured surface effect on the 5 cells.

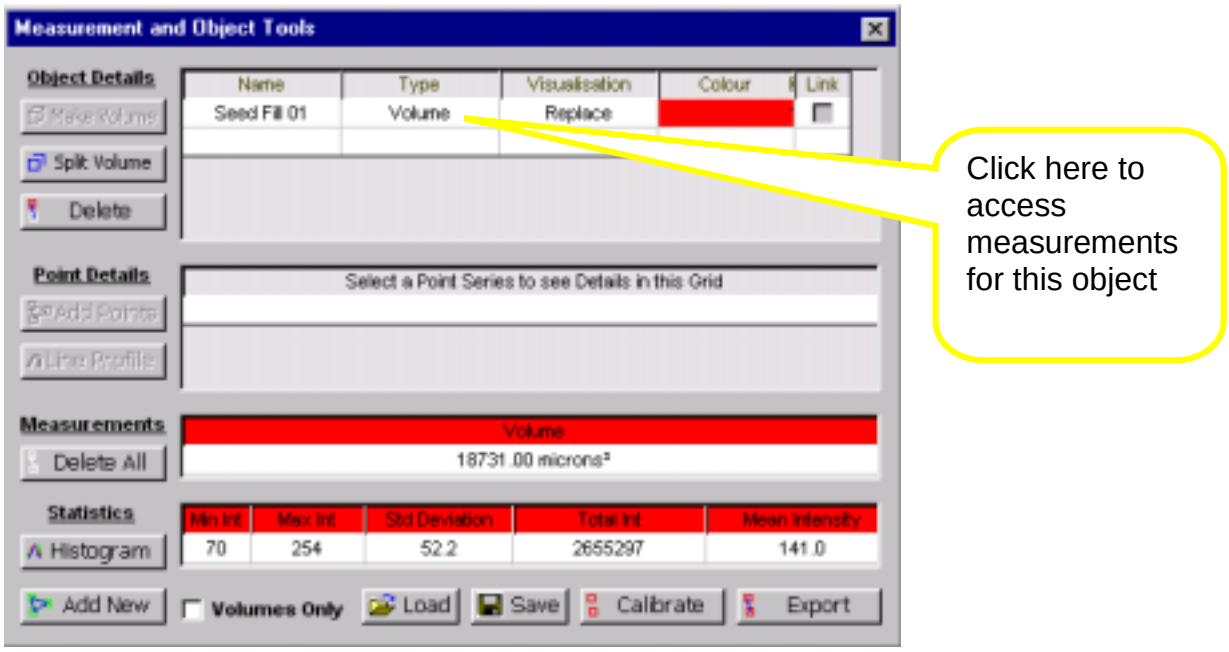


1.12 Exercise 10 Changing the appearance of an object.

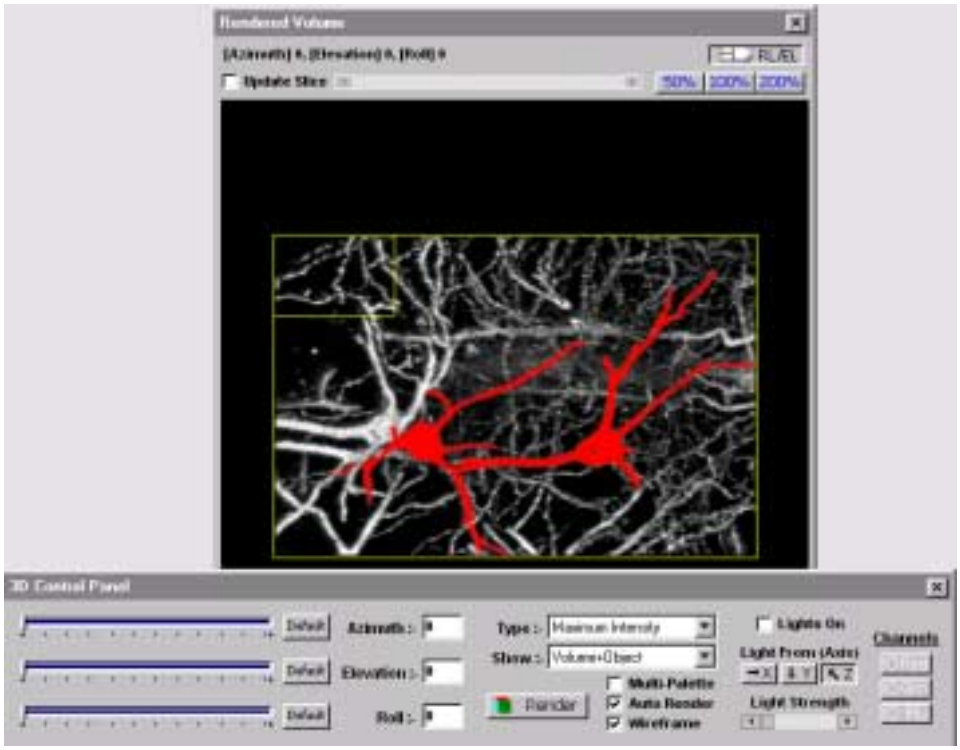
Open Neuron.PIC and use the 2D control panel to move to z slice 20. Select the Seed/Flood fill option from the mouse mode bar and click inside the partial cell body which is visible.



The Seed/Flood-fill dialogue box will apear. Select the Seed option, leave the default intensity values and click APPLY. The Object/Measurements dialogue will appear. Under the TYPE column, double-click on the VOLUME label to access the measurements for the object.

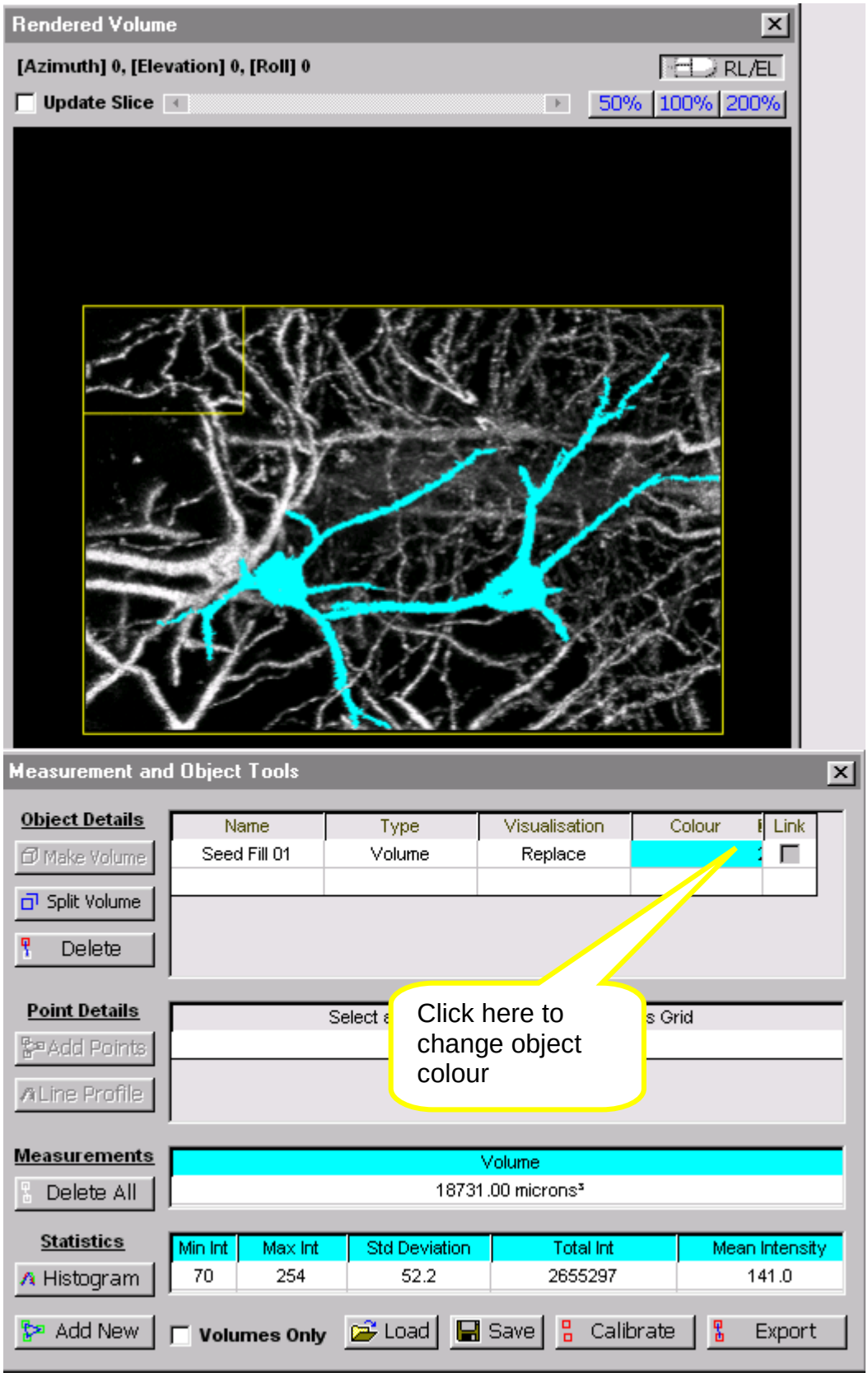


Now, click the 3D button on the toolbar to render the volume and the seed-filled neurons (appear in red).



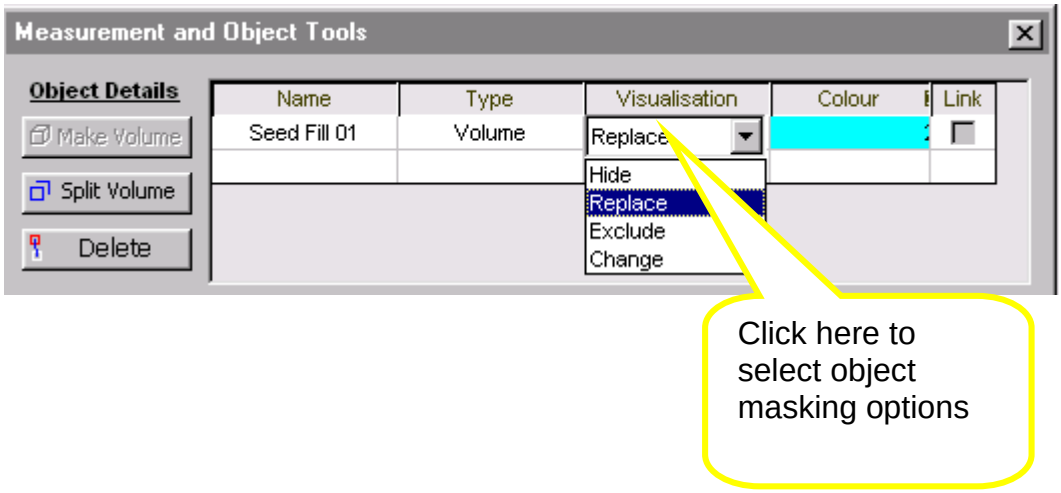
Two neurons have been seed-filled to make one object and are displayed as a binary red colour. This means that the object is of uniform intensity. The

colour of the object can be changed by double-clicking on the colour in the objects/measurements dialogue and selecting a different colour from the colour palette.

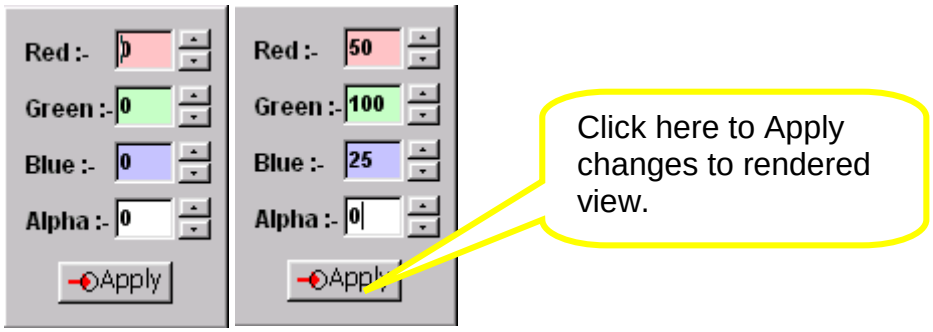


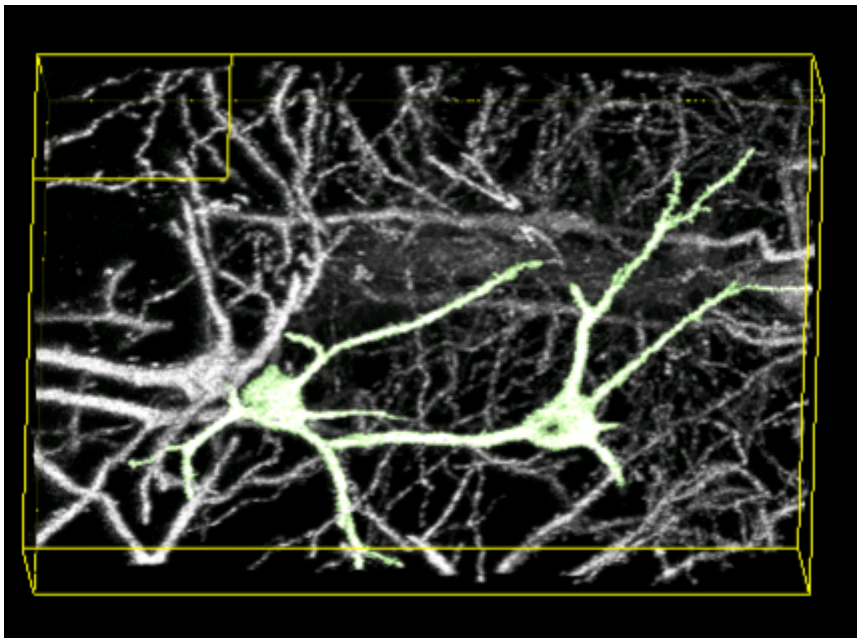
Objects can be used as MASKS. This means that once an object is defined (as a seed or flood-filled structure), it can be used to MASK or reveal the original data. The default option is to REPLACE the original data with the object as illustrated in the previous example. Other available options are to HIDE the object (so that the original data is again visible), to EXCLUDE the object (so that the original data and object are removed from the data) and to CHANGE the object (so that it is rendered as original data but can have different colours and opacities applied to it for visualisation purposes).

To experiment with these options, use the seed-filled neurons coloured red from the previous example.



Experiment with HIDE, REPLACE and EXCLUDE and lastly, select CHANGE. This will bring up a small dialogue box allowing the segmented data to be rendered with different colours and a different OPACITY or ALPHA value.





By making the ALPHA or opacity value negative or positive, it is possible to make the segmented structure more transparent or more opaque. It is useful to remember that since LaserVox allows animation of any view from the render window, objects and data can be rendered differently in consecutive views to highlight different structures within the data during the animation.

1.13 Exercise 11 Linking, combining and converting objects

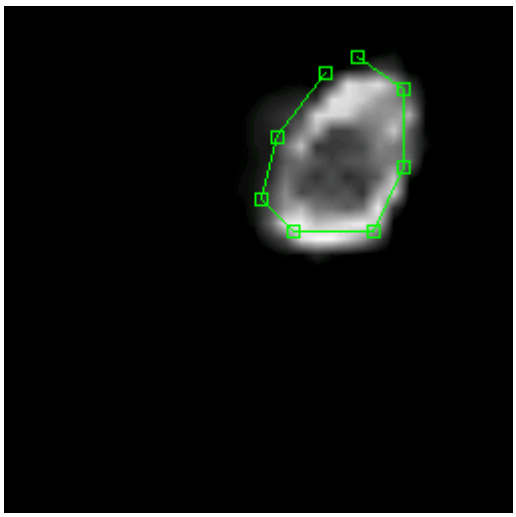
LaserVox is designed to enable the user to draw a series of polygons (either using lines, ellipses or rectangles and to either LINK them together into one volume or CONVERT them into a volume described by each shape area X the z step. This enables (by LINKING) eg to determine the volume of a structure where its cross-section does not vary very much from one optical section to another or (by CONVERTING) eg to determine the volume of a structure or multiple structures defined by several polygons per optical section. Finally. LaserVox also allows any objects defined by seed- or flood-filling or by LINKING or CONVERTING to be COMBINED into a single volume for visualisation and measurement.

1.13.1 Linking shapes.

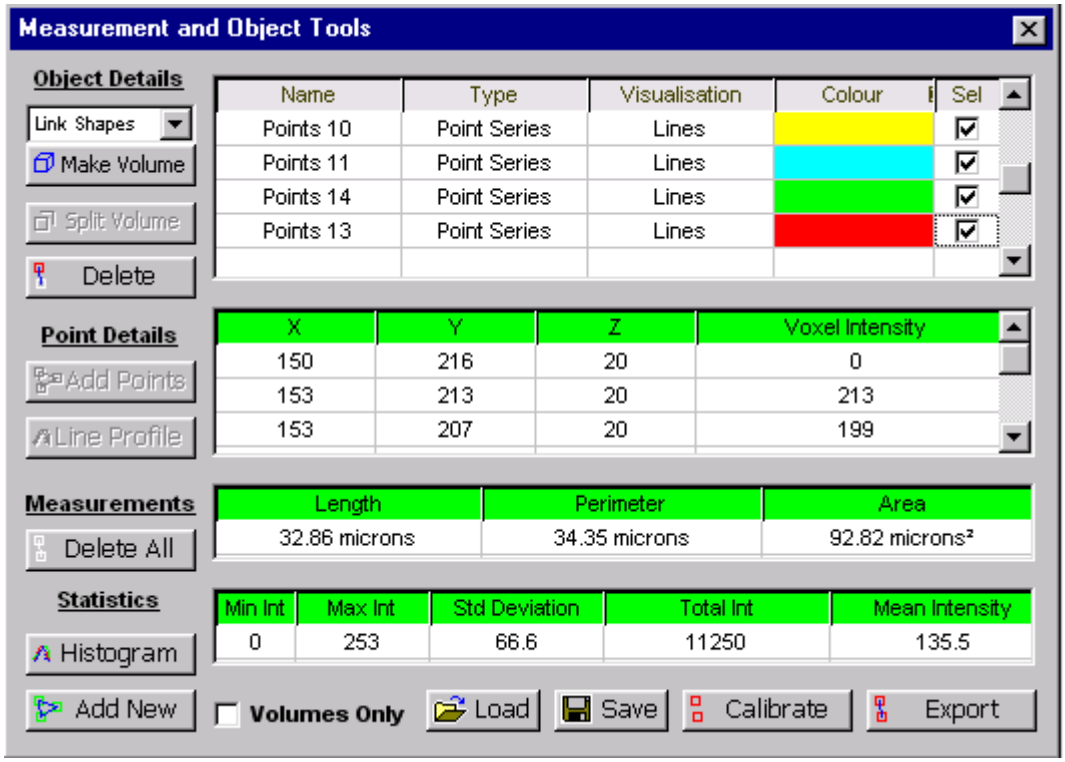
Linking shapes is designed to link polygons drawn around cross-sections which change gradually throughout a z series. **Only one polygon** may be drawn per optical section. If the structure of interest is perfectly cylindrical, it would only be necessary to draw one circle around the structure in the bottom optical section and one circle around the structure in the top optical section. However, if there are continuous changes, the more polygons which

you describe throughout the z series, the better the object can be rendered and measured. If you define polygons using the points/line icon, you need to ADD NEW to draw a new polygon on the next optical section. However, if you use ellipses or rectangles, ADD NEW is not necessary. The example below uses

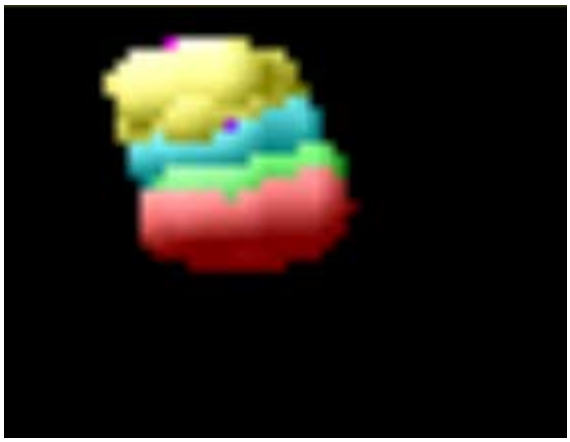
Open Skino3.PIC . Move to Zslice 12 and using the zoom from the Mouse Mode Bar, Zoom up 3 times with the mag glass over the small circular blood vessel in the bottom left quandrant of section 12. From the Mouse Mode Bar, select the Points/line icon. Click several (about 8) points around the circumference of the blood vessel. Note that the point appears a few pixles above the mouse pointer, so aim slightly ‘low’.



Use the PgDn Key twice to move to section 14. From the Measure window, click ADD NEW and draw a second series of points around the new section of the blood vessel. Use Pg Dn twice again , This time, select the ellipse tool from the Mouse Mode Bar and draw an ellipse around new section of the blood vessel. With ellipses, you do not need to click the ADD NEW function from the measurements window. Repeat this by adding 2 more ellipses on two new z slices using PgDn twice each time. Note that the measurements window now displays a series of measurements and colours, each associated with one of the polygons you drew.

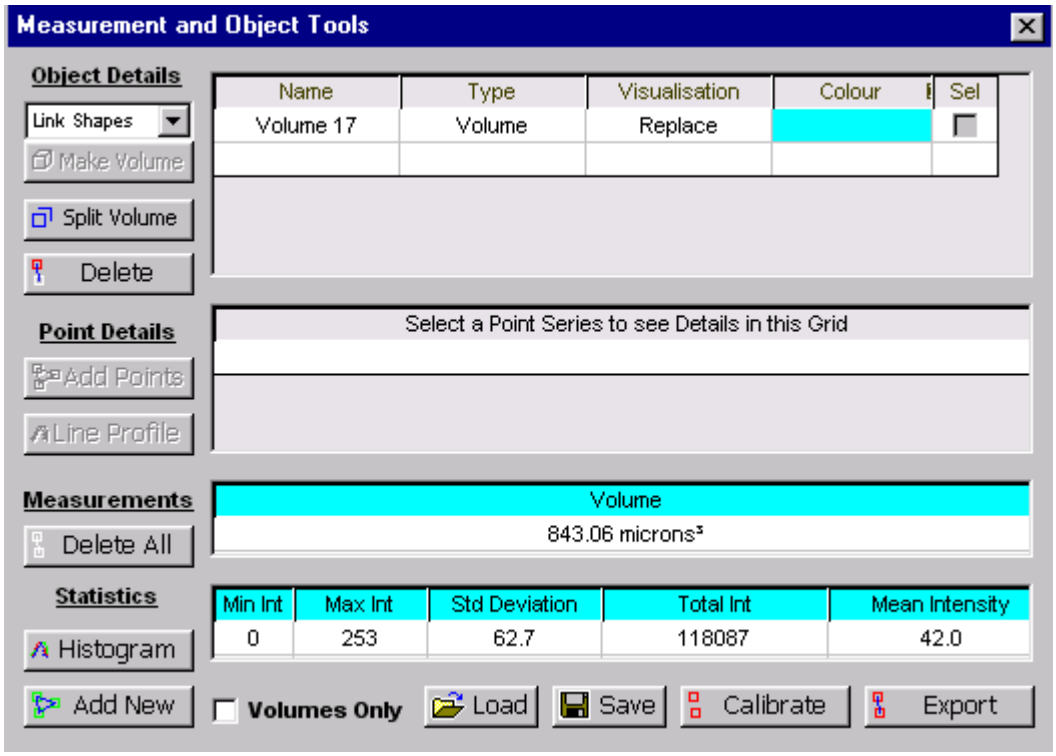


Now, click the 3D button from the tool bar, select SHOW OBJECTS from the 3D control panel and click the RENDER button. Zoom up 3 times using the zoom tool from the Mouse Mode Bar. Note that you have rendered several objects, each displayed in a different colour. These objects describe the shapes you have drawn around the blood vessel.



In the Measurements window, in the right hand column (SEL – select) , click in each of the boxes beside each colour object. Then, with the LINK SHAPES option selected from the left hand drop down menu, click the MAKE VOLUME button. The shapes which you drew have now been linked together to form a single volume.

The measurements window now only displays a single colour and a single volume measurement.

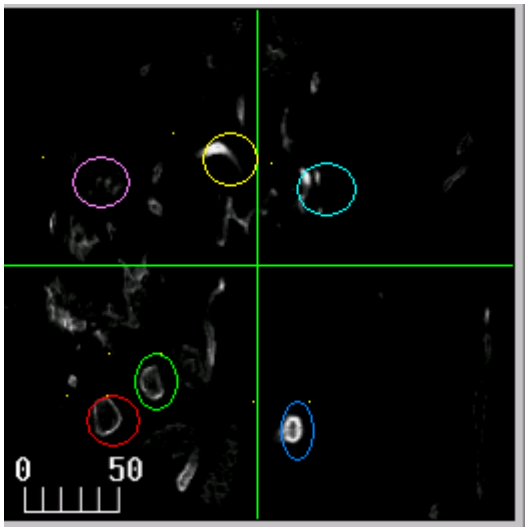


You can split the volumes again by clicking SPLIT VOLUME.

1.13.2 Converting shapes

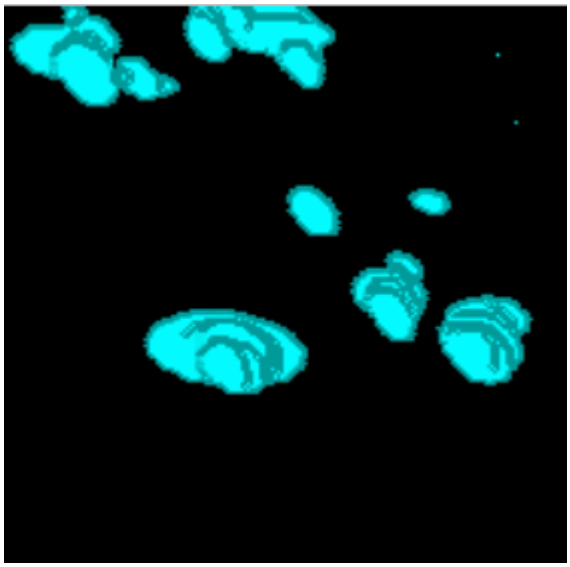
The CONVERT SHAPES option is designed to enable many polygons to be drawn in each optical section in order to determine the shape and volume of either a series of separate structues or a reticular structure such as a neuron which may display many parts of branches within one optical section. You MAY NOT DRAW ellipses in more than ONE of the windows in the 2D volume slice viewer ie draw ALL in the XY, YZ orXZ window.

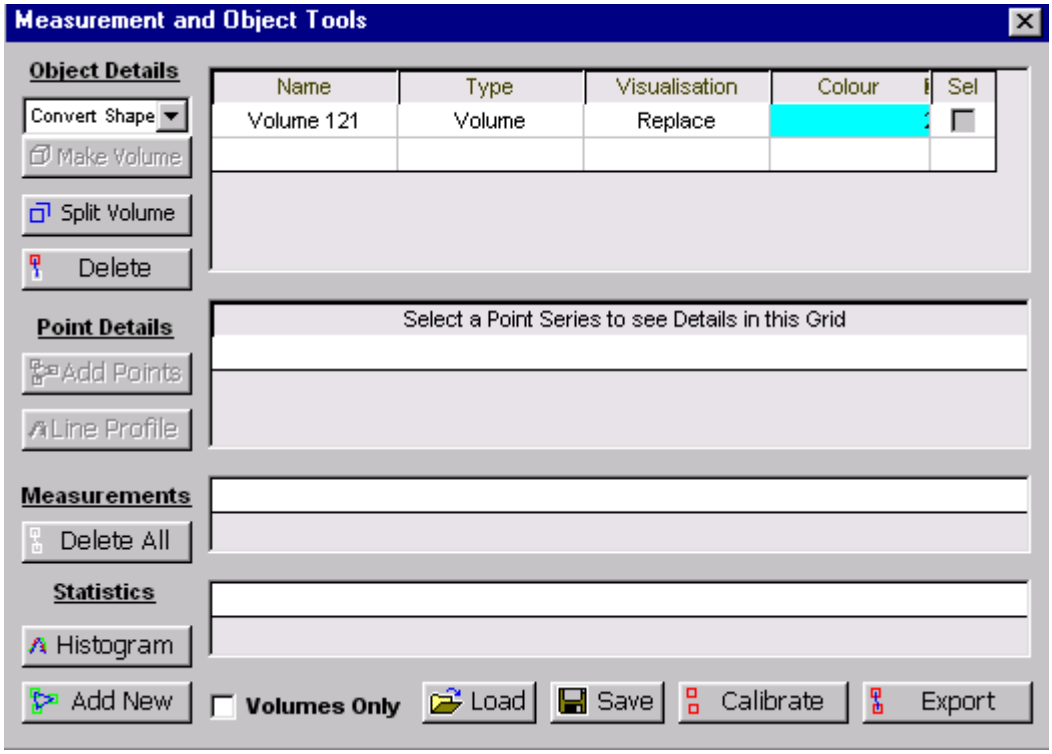
Open Skin03.pic z slice 35. You will see several small blood vessels. On Z slice 35, draw several ellipses around some of these. Use PgDn key 3 times, and draw several more ellipses on z slice 38. Repeat this several times until you have about 30 or 40 ellipses drawn over 5 or so z slices.



Click the 3D button on the toll bar, select SHOW OBJECTS from the 3D control panel and hit the RENDER button. You will now see a series of objects in the render window and the measurements window will display a different colour and area for each ellipse. Now select CONVERT SHAPES from the left hand drop down menu in the Measuremnets window, then click in the SEL column to check all the ellipses oyu have drawn (do this from the first or top object in the list and it will automatically scroll down to the next one). Now click the MAKE VOLUME button.

The render window will now render each ellipse as an object with a depth of exactly one optical section, and all ellipses converted become part of the same volume for both visualisation and for measurements.



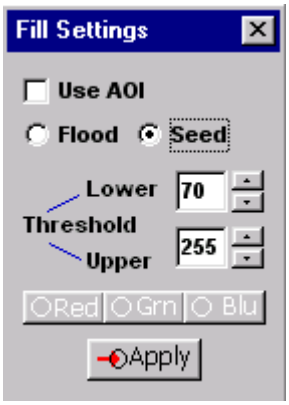


Note: A good effect can be achieved by using the LIGHTS ON option on the 3D control panel to make the objects appear less flat.

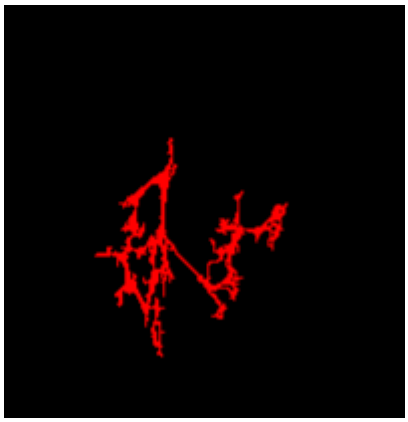
1.13.3 Combining volumes

LaesrVox is designed to enable objects generated from seed-filling or flood-filling and from linking or converting shapes to be combined into a single volume. Seed-fills do not always pick out the entire object of interest, so it may be necessary to perform more than one seed-fill to achieve this. Since these two fills may represent different branches of the same object, it is beneficial to be able to COMBINE the two volumes into one.

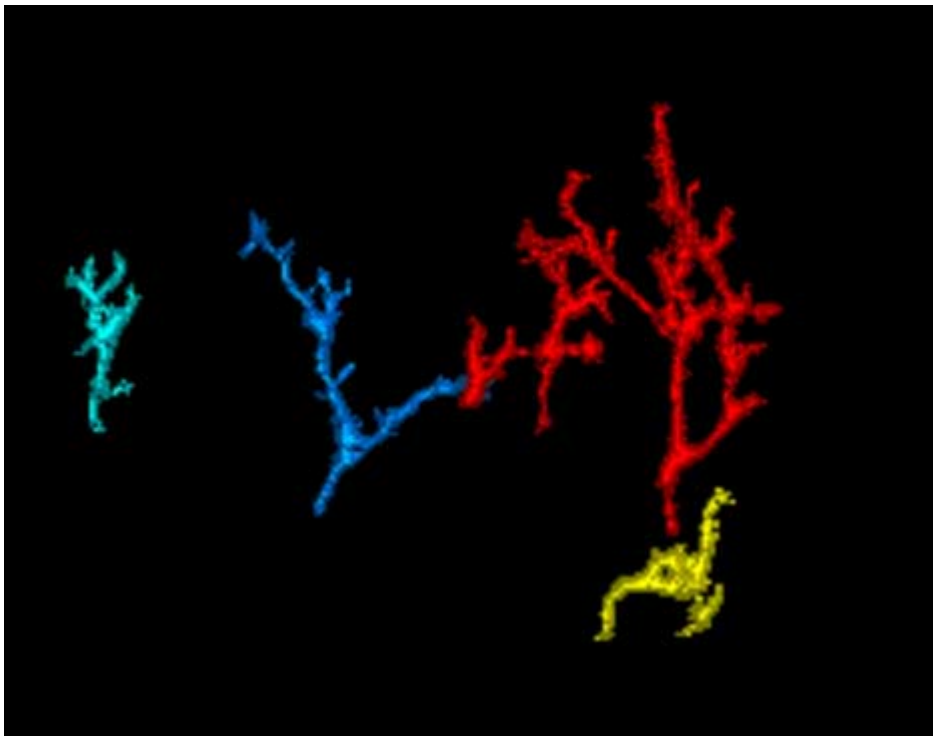
Open Retic.PIC. Click the 3D button to get an idea of what the rendered view looks like. Now select the SHOW OBJECTS option from the 3D control panel. In the 2D volume slicer, move to about z slice 17. Select the Seed-fill icon from the Mouse Mode Bar, click on a bright pixel somewhere in part of the network of fibers (you can see the pixel intensity under the mouse pointer at the bottom of the LaserVox screen as you move the mouse). The Seed-fill dialogue will appear.



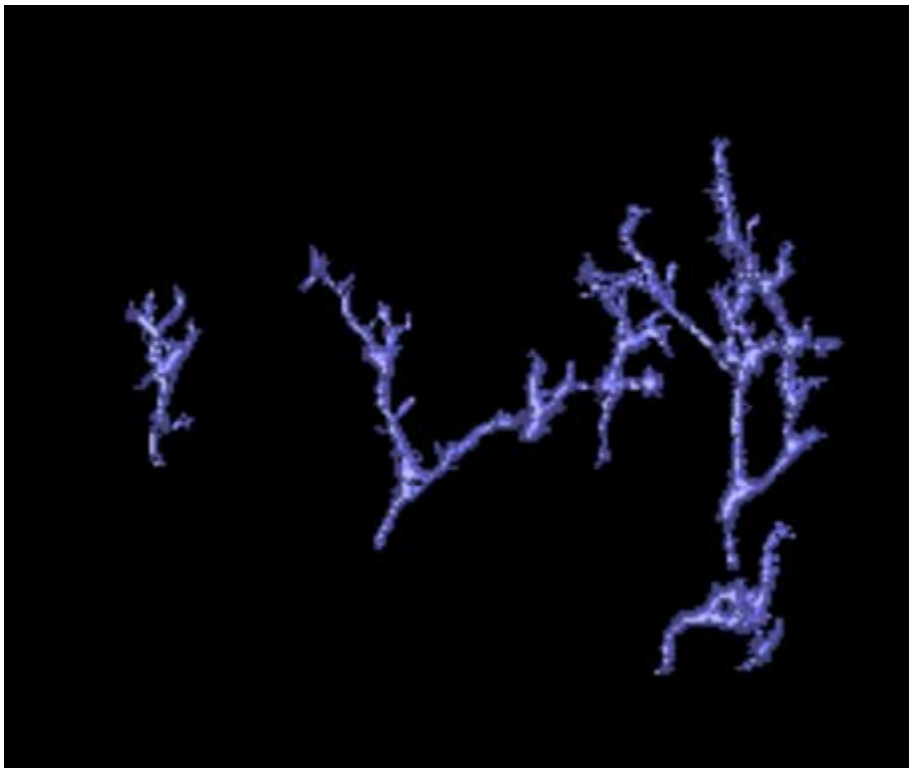
Select the SEED option, leave the default intensity settings and click APPLY. Now RENDER from the 3D control panel. The render window should now show part of the network of fibers filled with a colour.



Move to another z slice and fill a second part of the structure and then a third and fourth. You should now see four different parts of the network filled each with a different colour.



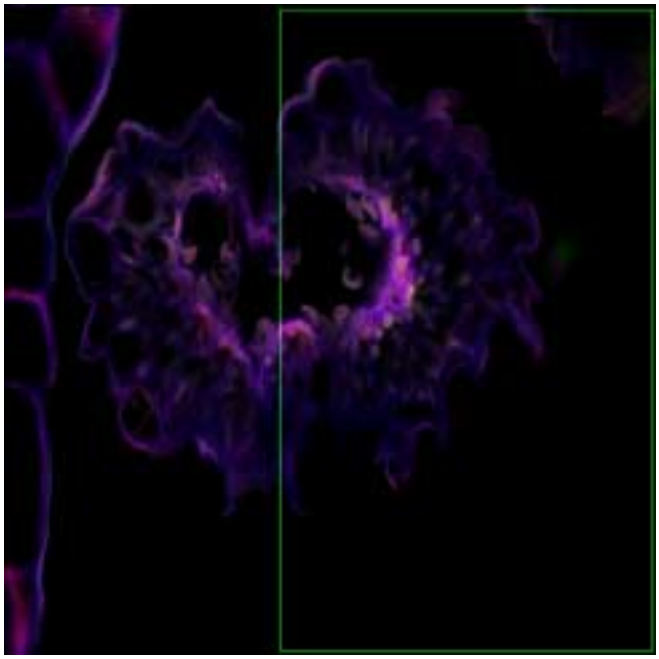
From the measurements window select the COMBINE VOLUMES option from the left hand drop down menu. Click in the SEL column to the right of the object colours and check all four boxes. Click the MAKE VOLUME button. The render window should now show all four objects in one colour and should display a single volume measurement.



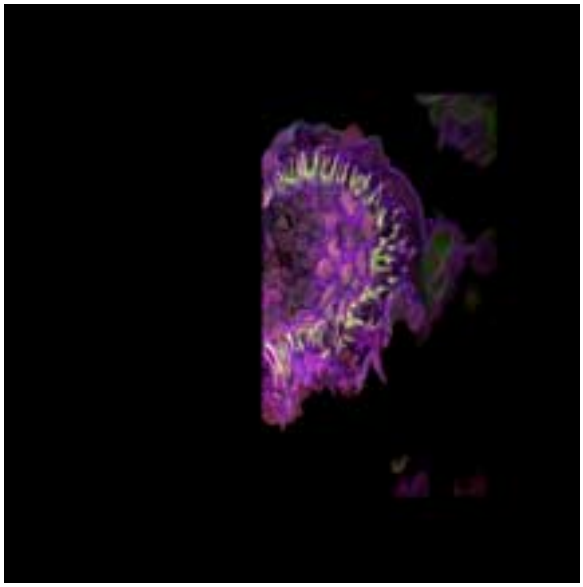
1.14 Exercise 12 Layer Peeling

LaserVox allows definition of part of the dataset using an AOI which contains structures which the user wishes to Protrude from the dataset. This allows the entire dataset to be rendered, but in the selected volume, data will only be projected in channels chosen by the user. So with a RGB dataset, the user can use an AOI to select the volume containing the structures to protrude from the rest of the data, then render the whole volume but with 2 of the channels ‘switched off’ in that part of the volume.

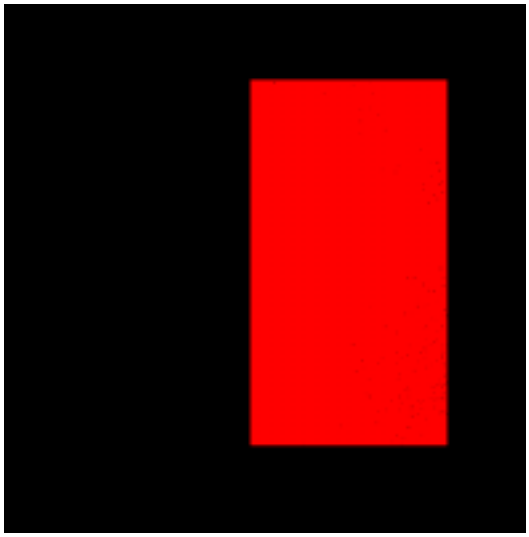
Open anther01, 02, 03.PIC as a 3 channel dataset. From the Mouse Mode bar, select the AOI optin with the left mouse button and the Seed-flood-fill option with the left mouse button. Draw an AOI around the right hand side of the visble z slice.



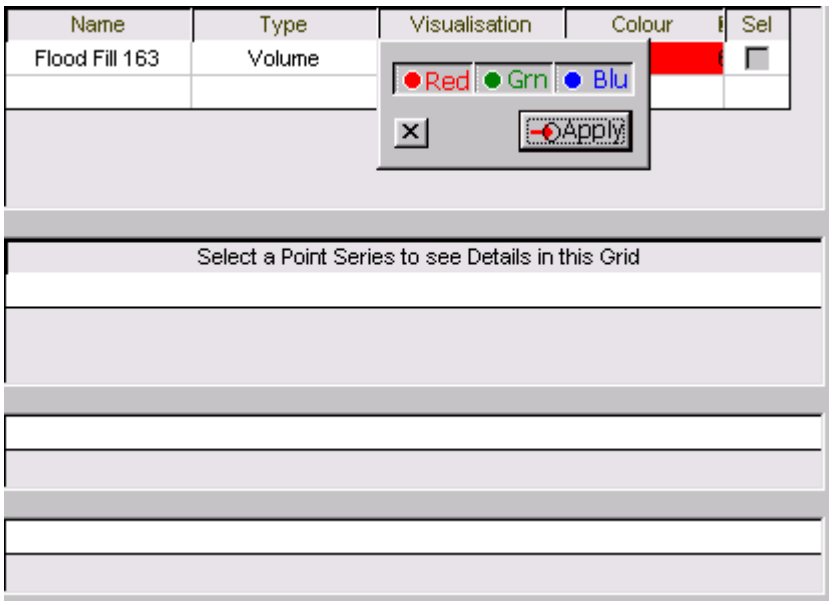
Click the 3D button on the tool bar and ensure that SHOW VOLUME + OBJECT is selected from the 3D control panel. The right hand side of the data only should now appear in the render window.



Click in the 2D volume window and the flood-fill dialogue will appear. Select the FLOOD option (with AOI checked) and set the lower intensity value to zero. Click APPLY. You should now see the entire right side of the dataset rendered as a solid coloured object.



Click on the measurements tool on the tool bar. In the measurements window, select the EXCLUDE option from the menu in the column headed ‘visualisation’.



Click the green button (to exclude the green channel from the AOI region) and click APPLY. Then click the little cross to close this dialogue. From the 2D slice viewer, click the Close AOI button (red cross above image window). The volume will now render with all 3 channels on the left hand side and only 2 on the right.