Renishaw plc
Spectroscopy Products Division
New Mills, Wotton-under-Edge,
Gloucestershire GL12 8JR
United Kingdom

Tel +44 (0) 1453 524524
Email raman@renishaw.com

www.renishaw.com

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TM011 – 3D imaging WiRE™ 5

This document aims to show the WiRE™ 5 users how to collect, process, analyse and view 3D volume data.

In order to use this capability ensure the StreamLineHR (optionally with *Rapide*) and 3D passwords have been purchased and entered into the WiRE Feature permissions.

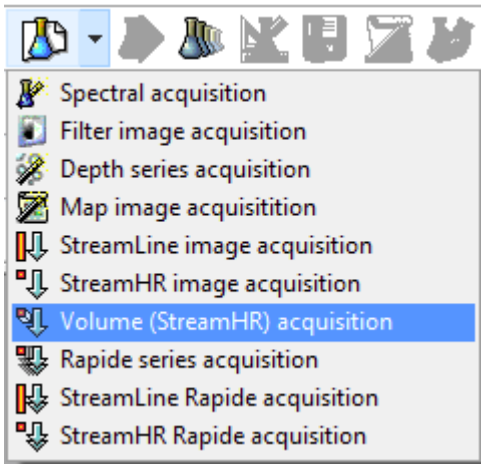
Collection of data

3D data collection can be achieved using StreamLineHR (TM009) or StreamLineHR *Rapide* (TM029) measurement types. Prior to the set-up and collection of data, it is recommended that the Z distance of the volume be separately investigated to ensure values used within the volume are appropriate for the analysis.

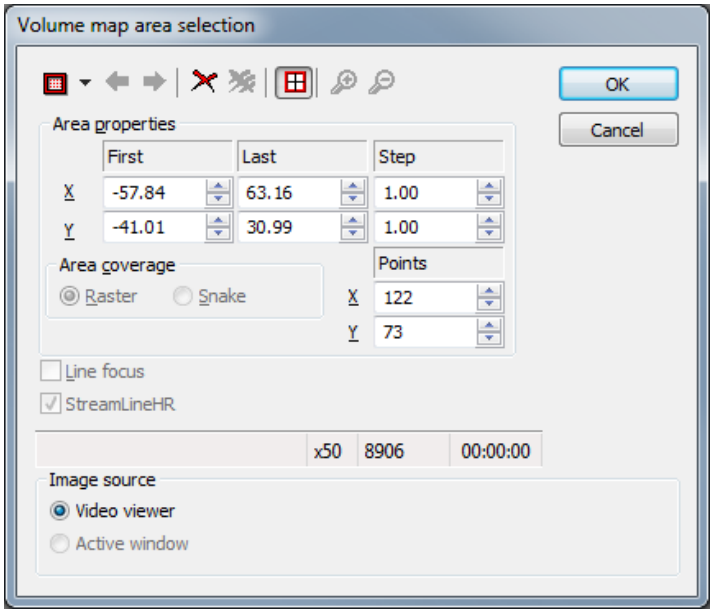
The set-up of the volume measurement combines the collection of StreamLineHR or StreamLineHR *Rapide* data in XY with an additional Z control for each collected XY plane. The resulting spectra within the dataset, covering the 3 spatial dimensions, are analysed together to produce a single hypercube of spectra. This enables spectral information to be analysed in all dimensions simultaneously.

Refer to the StreamLineHR *Rapide* training module (TM029) for further information.

1. Select **Volume acquisition** from the new measurement options.



2. Define the collection area on the live video image (video viewer), or snap / montage image (still image viewer).



The StreamLineHR box is automatically ticked, and cannot be un-ticked.

The XY first and last values, and the step size (in µm) can be adjusted both within this dialogue and within the Map measurement set-up dialogue.

The number of points can be changed. This shows the image size in pixels, each pixel consisting of a Raman spectrum.

3. When the map is defined, and **OK** is pressed, the Map Measurement Setup window is activated. The Range, Acquisition, File, and Advanced tabs are identical to those that appear in the general Spectral Acquisition setup.

   The Depth series and Area setup tabs allow the user to review and adjust the settings for the XYZ spatial values

4. Set the **Depth** information by entering the Start position (µm) and the number of acquisitions in the Z dimension. The interval value (µm) then defines the final position (the interval value can be set to be positive or negative to ensure the appropriate volume is imaged).

   See TM5 for detailed information on using the depth series measurement tab.

   The total number of spectra to be collected is shown in the Area setup tab and includes the Z depth number.

5. Go to the ‘Range’ tab and set the centre position of the scan (the spectral range and spectral resolution will depend on the laser wavelength, grating groove density and detector type).

6. Go to the ‘Acquisition’ tab and select the time and laser power to be used with the scan. StreamLineHR has a minimum exposure time of 0.05 s (50 ms). StreamLineHR Rapide can use less than 0.001 s (1 ms)

7. Go to the 'File' tab and define the filename and location. Select the 'Auto increment' option to ensure the data cannot be overwritten.

8. Once **OK** is pressed, the Volume acquisition measurement can be started using the  button.

Data will be collected in XY planes at the specified Z depths.

Data processing

The following processing options are available to be applied to the 3D dataset within WiRE:

- Baseline subtraction
- Cosmic ray removal
- Noise filtering
- Truncate
- Differentiate
- Normalise
- Smooth
- Zap

Data analysis

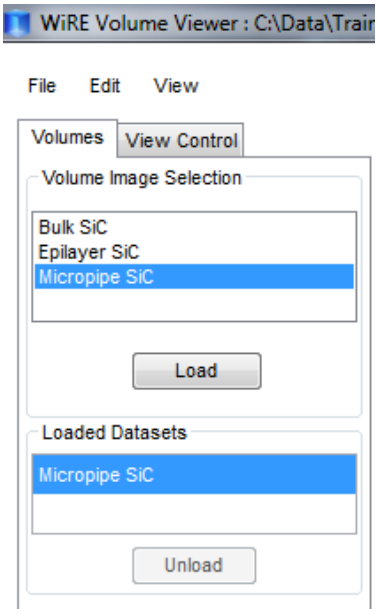
All image generation options are available for the 3D dataset. (See TM14 and TM15)

Viewing and manipulating the volume image

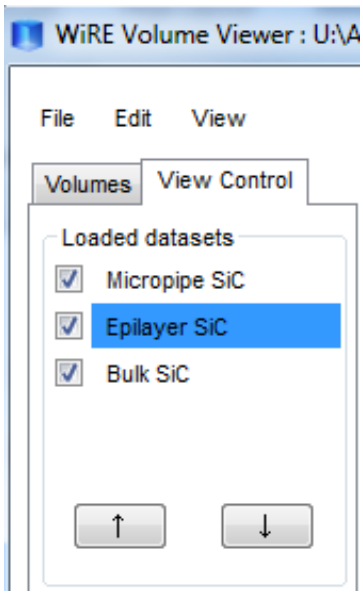
Loading volume images

Once the volume images have been generated from the mapping data they can be viewed by selecting the appropriate single image within the map review dialogue. This will launch the data into the volume viewer where all generated volume images are accessible.

More than 10 volume images can be viewed simultaneously within the volume viewer. Additional volumes can be added to the initially loaded image. Select the ‘Volumes’ tab, highlight the image to be loaded and select ‘Load’.



The 'View Control' tab houses options to enable loaded images to be temporarily hidden (un-tick box) or made active (click on image name). The look-up table is shown for the active image and layers this image on top of any other loaded images.



Once loaded, the order of the layers can be adjusted using the up and down arrows.

Controlling the volume image

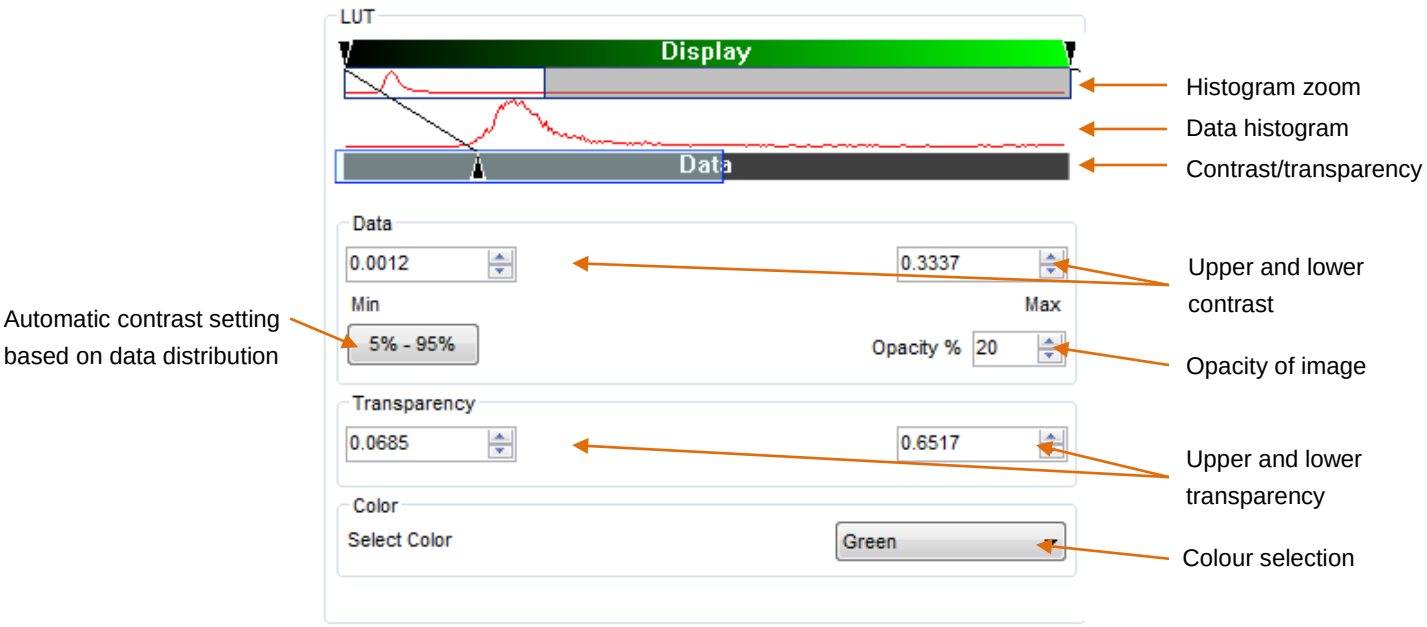
The volume image can be spatially manipulated using the following options:

- Free rotate (hold down left mouse button and move mouse)
- Constrained rotate (hold down Ctrl key on keyboard to restrict axis of rotation)
- Zoom (use mouse wheel)
- Translate (click background of image view, hold down mouse wheel button and move mouse)

The colour, contrast, and transparency of volume pixels (voxels) within the volume image are controlled using the look up table (LUT). The volume viewer LUT is similar to that within WiRE.

The LUT consists of

- Data histogram
- Black triangle drag bars on the data to control mapped colour values (also controllable by entering numbers and using the spin boxes). This controls the image contrast.
- White box drag bars on the data to control mapped transparency values (also controllable by entering numbers and using the spin boxes)
- A 5% - 95% auto button



Colour control

To change the colour scheme, use the dropdown. It is worth noting that the simple colours vary on a linear scale, whilst the more complex schemes vary non-linearly.

Data (image value) histogram

The bottom red trace shows a histogram of image values. The left number indicates image values to be represented as black and the right number image values to be represented as white (for grey colour selection). The data between these points are viewed in the image as a linear variation between these extremes.

Data triangular grab handles (contrast control)

Adjusting the 'Data' triangular grab handles limits the range of values used to create the image. This therefore affects the contrast of the image. You can cross the triangles over to get an 'inverse' image. This may be useful to highlight differences over the mapped region. When 'Dynamic' is checked, any changes made occur in real time. If preferred, LUT values can be entered manually by adjusting the upper and lower limits.

The '5% - 95%' sets the data values to best represent this proportion of the data, i.e. offer no contrast for any data in the lower and upper 5% extreme. This is useful if erroneous values have been created in the image, due to cosmic ray features for example.

Data triangular grab handles

Adjusting the 'Display' triangular grab handles limits the range of colours used in that scheme.

Data histogram zoom

Grab the far left or far right part of the upper red trace to zoom into the lower red trace.

Transparency control

Dragging the left or right sides of the 'Data' part of the LUT produces darkened bars. Values within the range of these bars are made transparent in the viewed image.

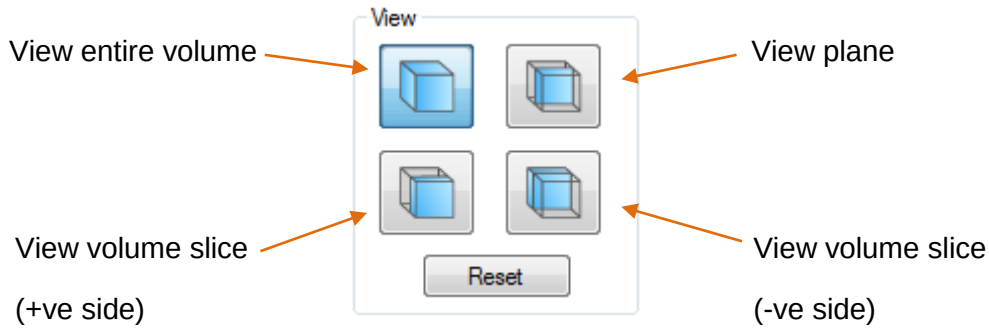
If preferred, transparency values can be entered manually by adjusting the upper and lower limits.

Opacity control

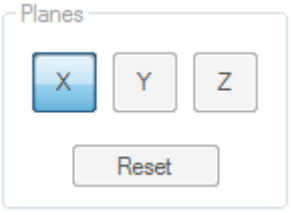
Reducing the percentage value from 100 makes the selected volume image semi-transparent. This is useful where multi-volumes are shown at the same time, and volumes are contained within other volumes.

Controlling the volume view mode

There are three different view modes; volume, slice, and plane. These modes are individually selected using the buttons indicated below.



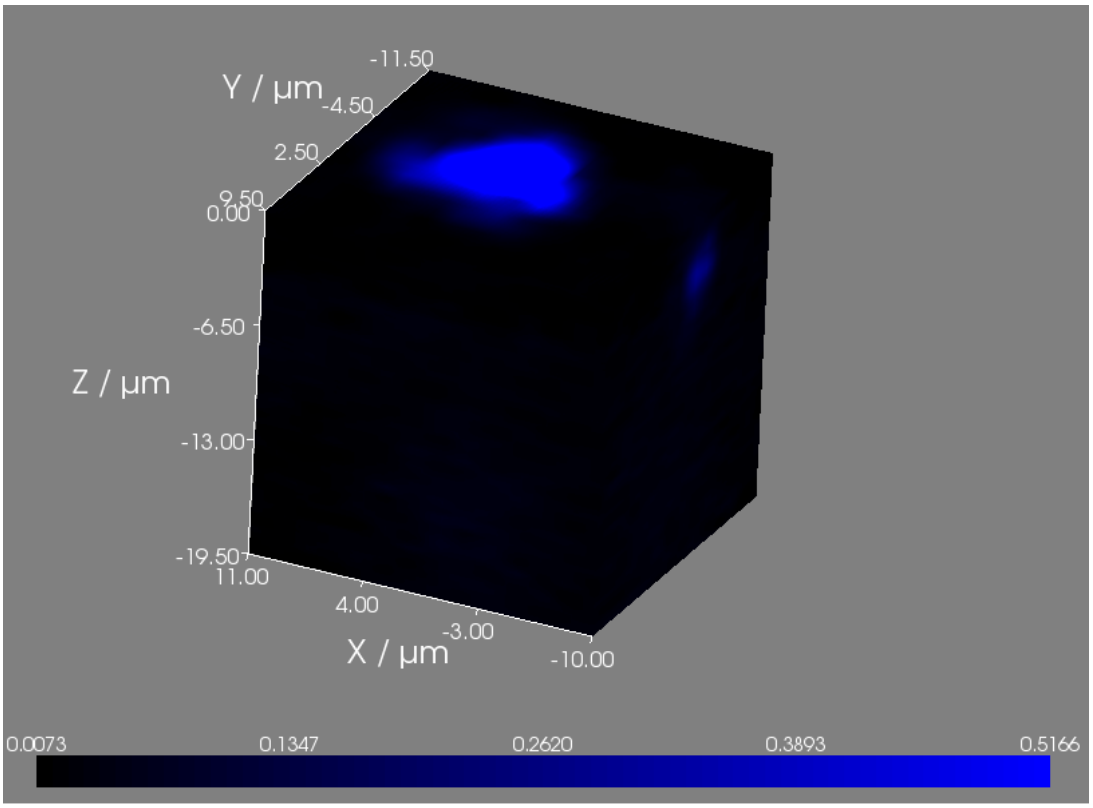
For the slice and plane view modes the displayed plane or plane combinations (for plane view mode) can be specified.

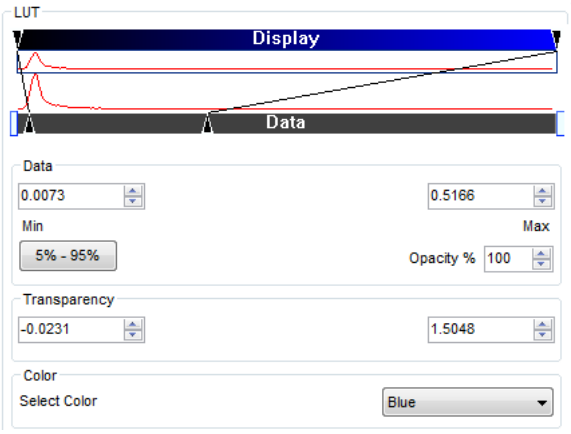


The practical use of these options is shown below for a core inclusion in silicon carbide

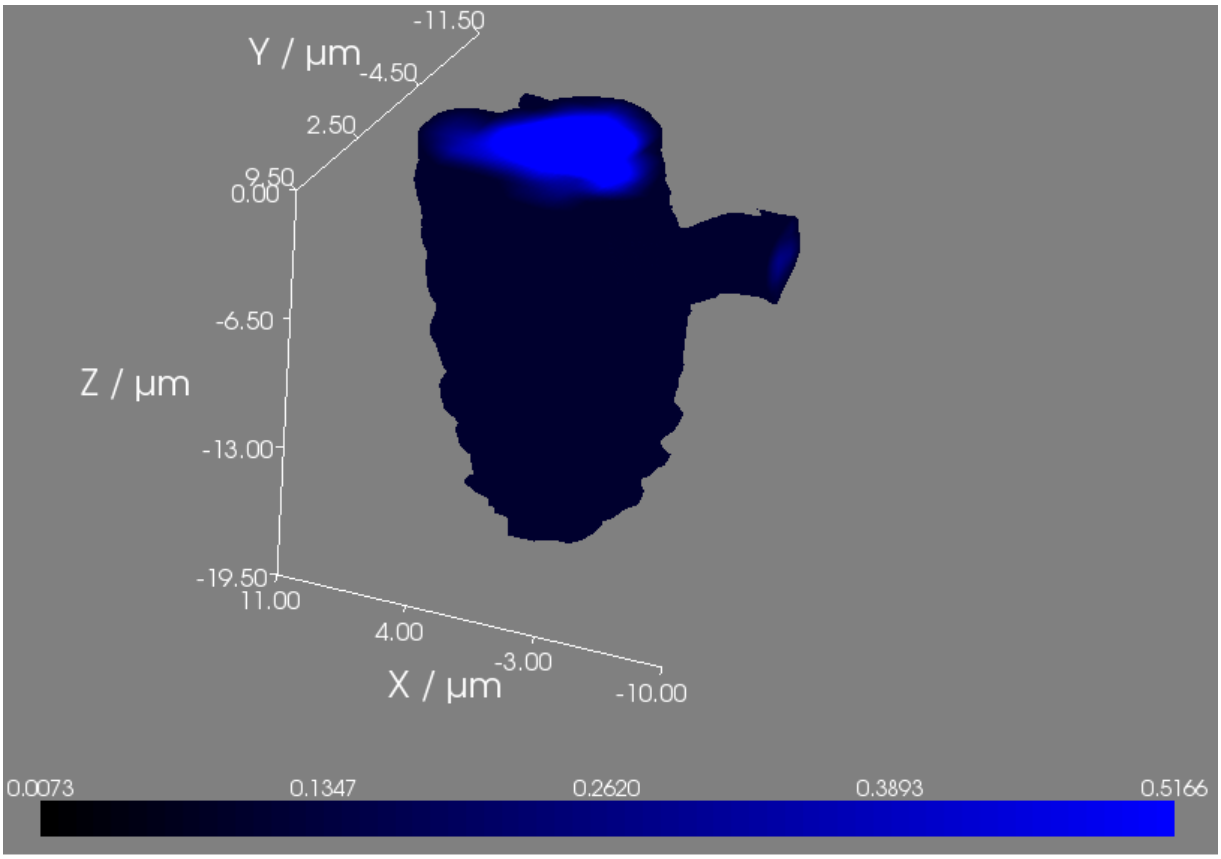
- Volume

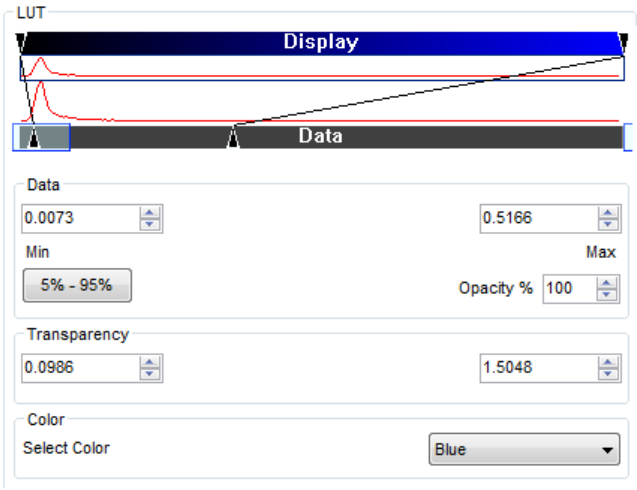
  - The entire volume is displayed





- The entire volume is displayed with transparency. Here the first transparency value has been increased from -0.0231 to 0.0986. Making all the values below this transparent.



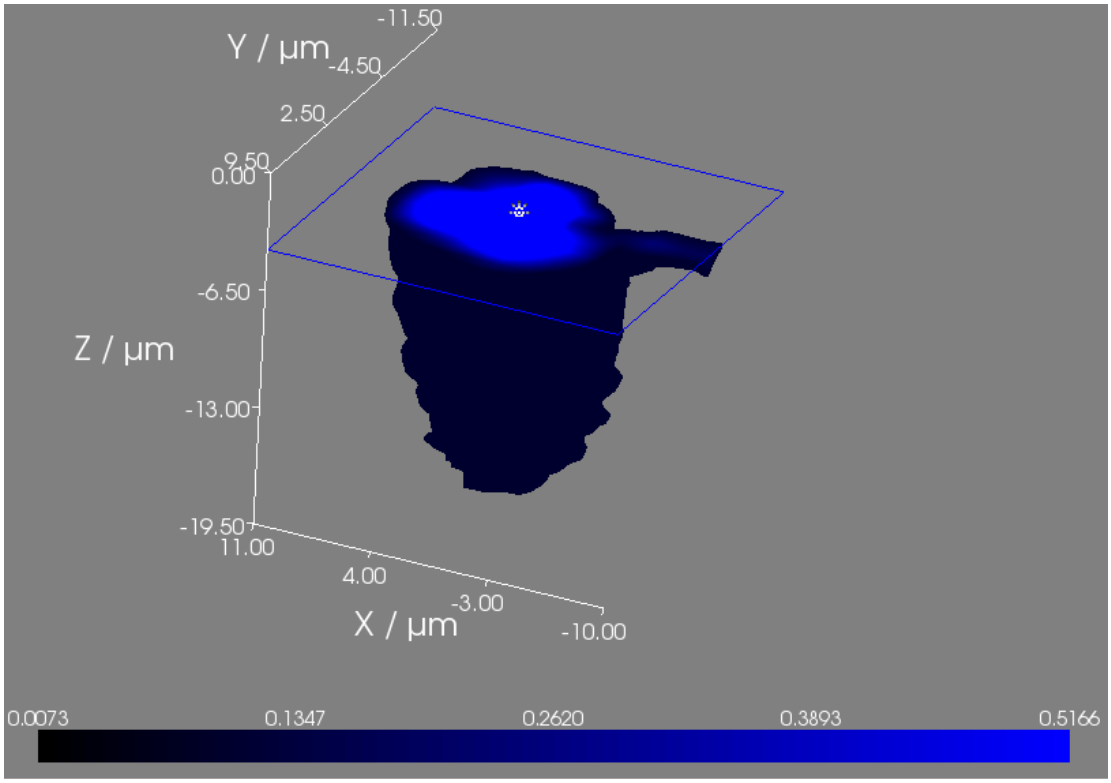


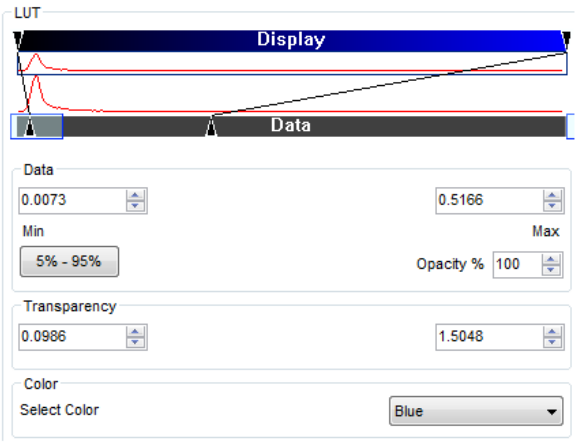
It is not possible to select different planes or individual spectra in Volume mode.

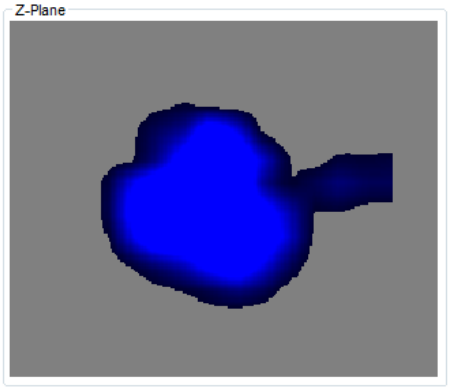
- Slice

  - The slice option can be shown for X, Y, and Z planes separately
  - For each plane the +ve or –ve side can be shown
  - Hold down the mouse wheel button and select the face of the slice, move the mouse to drag the slice

The 2D plane image changes instantly as the slice is changed



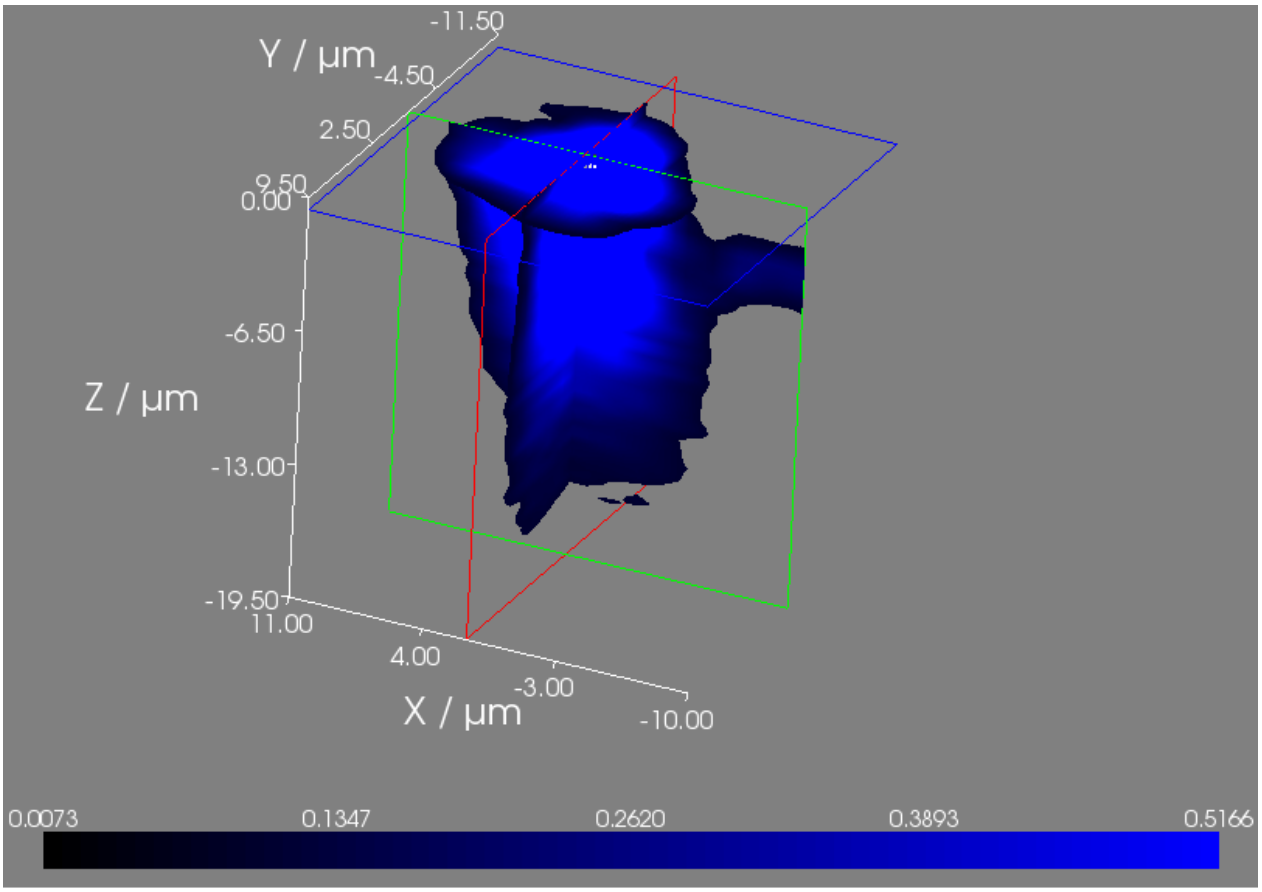


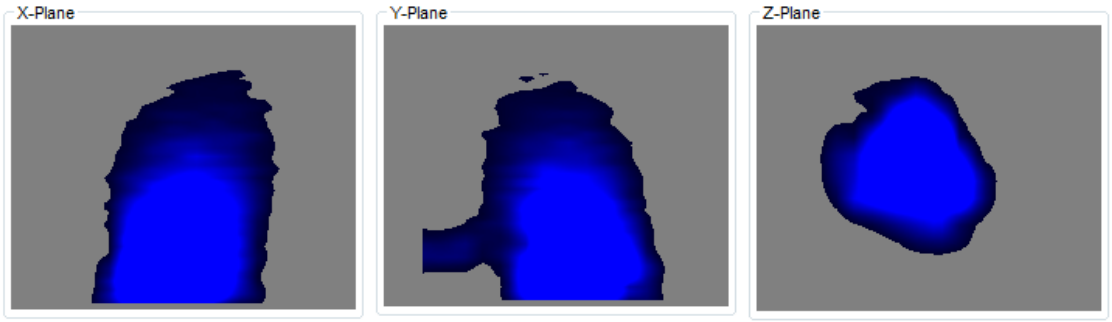


- Plane

  - The plane can be shown individually or together for X, Y, and Z
  - Hold down the mouse wheel button and select the face of the plane, move the mouse to drag the plane

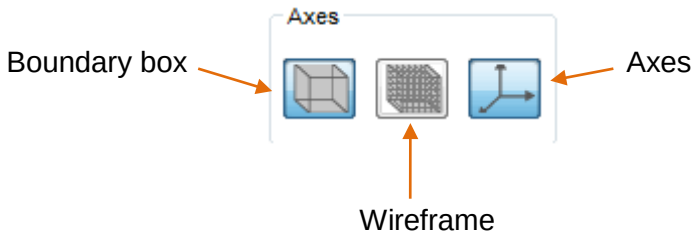
The 2D plane image changes instantly for each selected plane



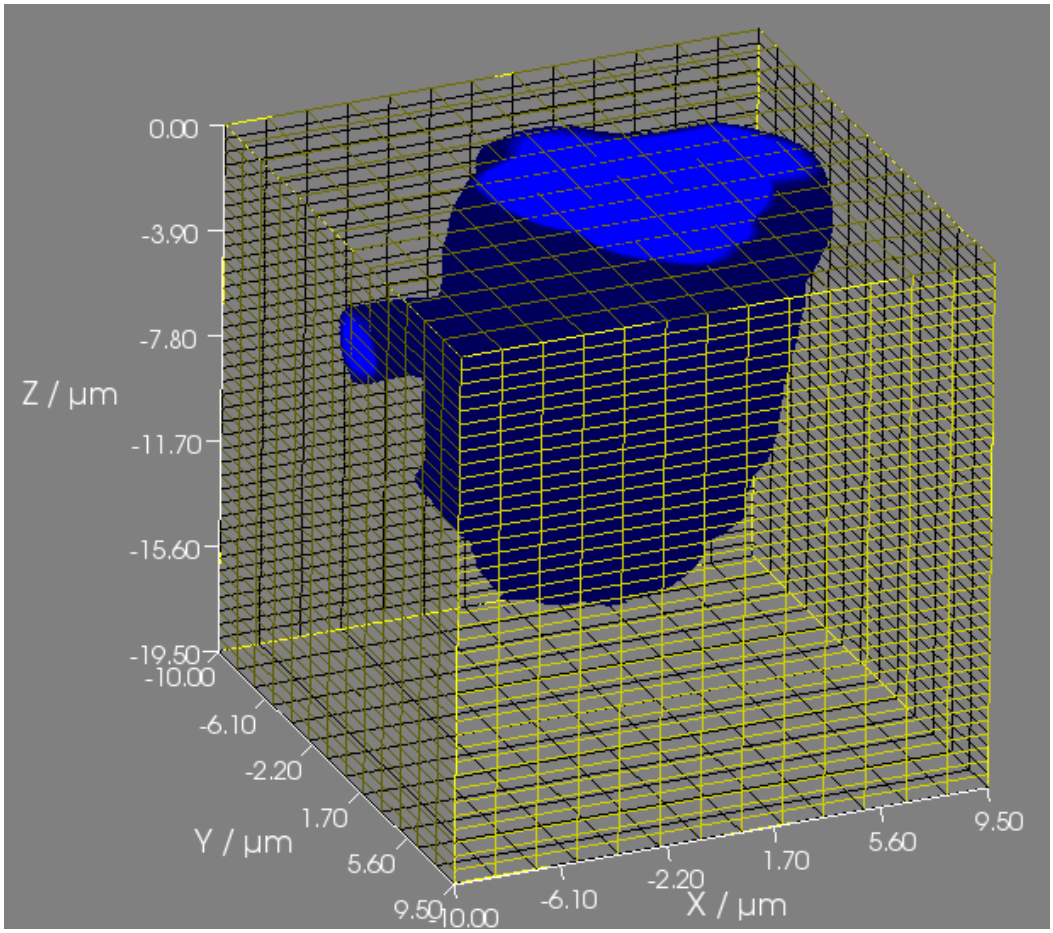


Controlling the axes view mode

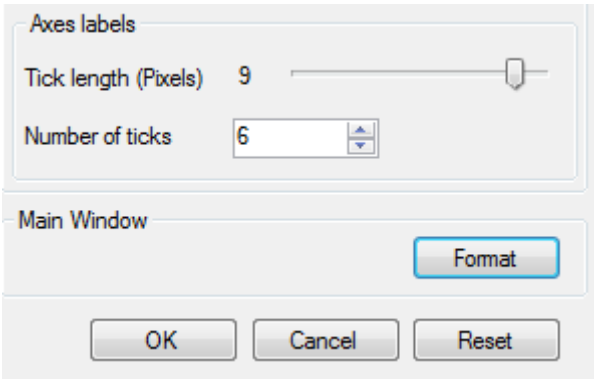
There are three axes modes that can be viewed individually, in combination, or not at all. These modes are selected using the buttons indicated below.



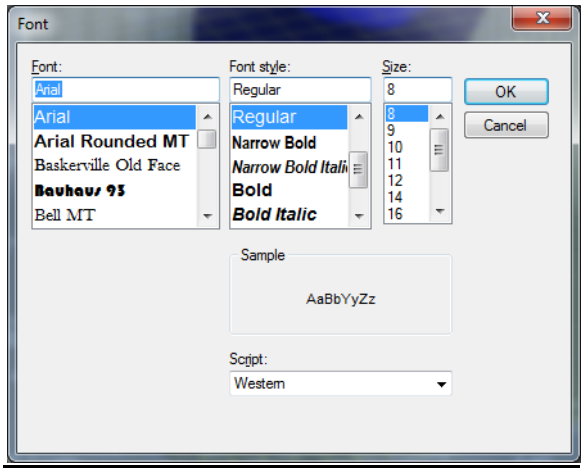
The boundary box and axes buttons are used to display boundary limits and XYZ axes, respectively. The wireframe button places a wireframe view around the boundary limits, offering the user the capability to easily size small particles.



The axes labels tick length and frequency can be altered by selecting **Edit...Format Settings**



Clicking the 'Format' button within the 'Main Window' section allows the user to set the main window font.



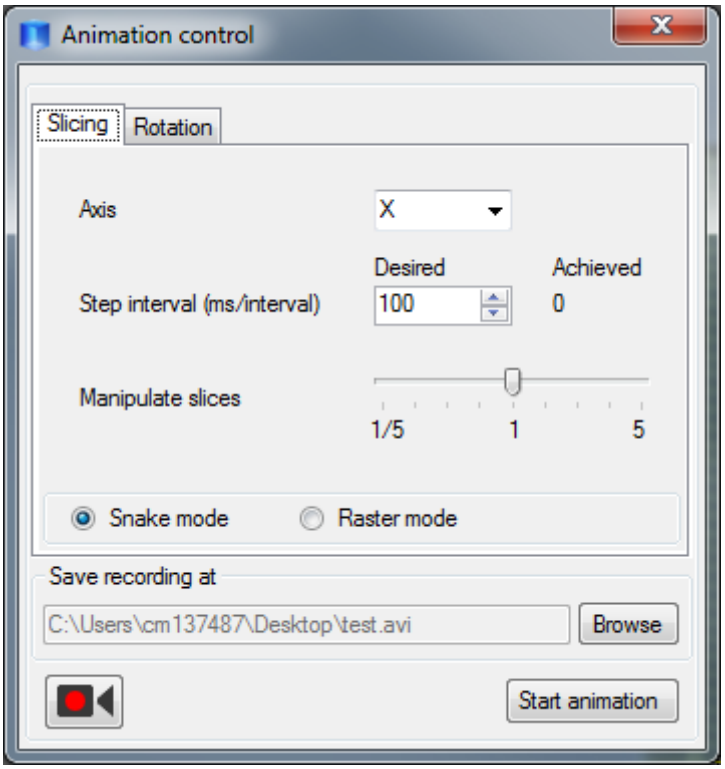
Creating an animation

Animations can be created within the volume viewer under **View.....Animation**. Two animation modes are available; slicing and rotation.

- Slicing
  The animation runs through volume slices. The view mode prior to entering the animation control window is irrelevant here; the animation will run through entire slices over the full volume of the image regardless of which view mode the image was in. The axes mode will remain as set within the volume viewer excepting the case where the wireframe is turned on; upon entering the slicing animation, the wireframe will automatically turn off.

  To set up a slicing animation:
  - The user must select the desired axis through which to step the slices, along with the step interval (ms/interval). Note that the desired step interval is input by the user. Upon exiting the animation, the achieved step interval is output by the software.

- The manipulate slices slider controls the frequency of slices that will be present within the animation; a higher manipulate slices value will result in an increased number of slices, whereas a lower value will result in fewer slices being stepped through within the animation.
- If snake mode is selected, the animation will run through the slices starting from the lower value of the selected axis, running up to the top slice, before running back down to the bottom slice, and repeating – i.e. a multi-directional animation will be produced. If raster mode is selected, the animation will run from the bottom of the axis to the top and will then immediately start from the bottom again – i.e. the animation will run in one direction only.



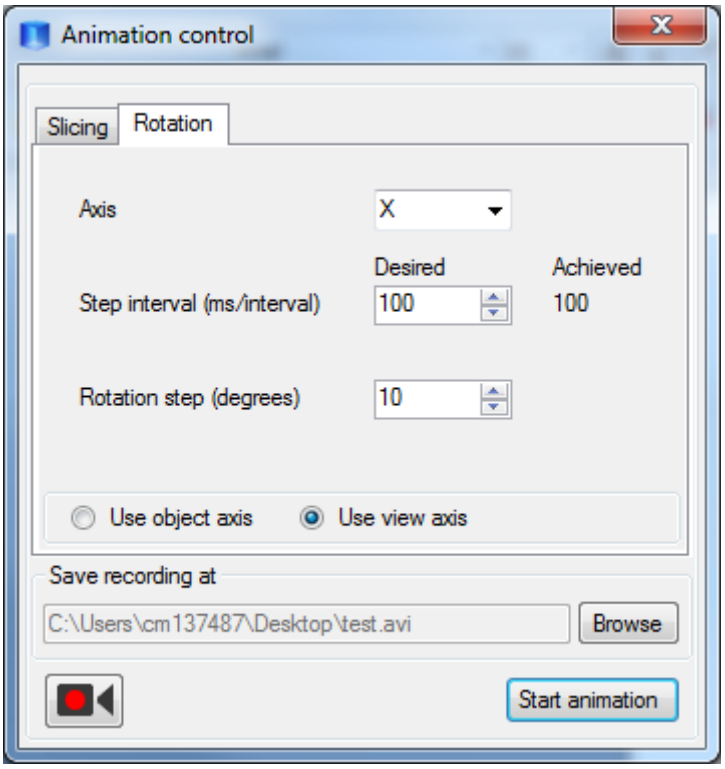
- Rotation

The animation rotates the image around the selected axis. The image view mode set prior to entering the animation control is relevant here; the image will remain in its' user set state throughout the animation. This is inclusive of all view control and axes modes. If the wireframe is turned on prior to opening the animation control dialogue, it will remain on throughout the rotation animation (this differs to the axes modes available within the slicing animation). The exception to the above rules is the case where a slicing animation is first performed. In such a case, the rotating image will appear as upon exiting out of the slicing animation.

To set up a rotation animation:

- The user must select the desired axis around which to rotate the image, along with the step interval (ms/interval) and the rotation step per interval (degrees). Note that the desired step interval is input by the user. Upon exiting the animation, the achieved step interval is output by the software.

- If 'Use object axis' is selected, the object will be rotated about its centre (and around the previously selected axis). If 'Use view axis' is selected, the object will be rotated about the volume boundary axes.



For both slicing and rotation animations, the animation is started by clicking the 'Start animation' button. If the user wishes to save a recording of the animation, the file name and location can be specified under **Browse** prior to starting the animation. Pressing the record button will record and save the animation as an 'AVI' format to the specified location. The record button can be pressed at any point during the animation, so that the whole or a part of the animation may be saved. If the record button is pressed prior to the 'Start animation' button, the animation will be recorded from the start of the animation. The recording can be ended either by again pressing the record button, or by clicking the stop button which will also end the animation. Note the 'Start animation' turns to 'Stop' upon beginning the animation. While recording, the record button is highlighted green  and when not recording, it is red  .

Viewing spectra from the volume

Spectra can be viewed in Slice or Plane mode. Use the left mouse button to click on the plane to see the spectrum. In these modes the spectrum will update as the plane or slice is changed.

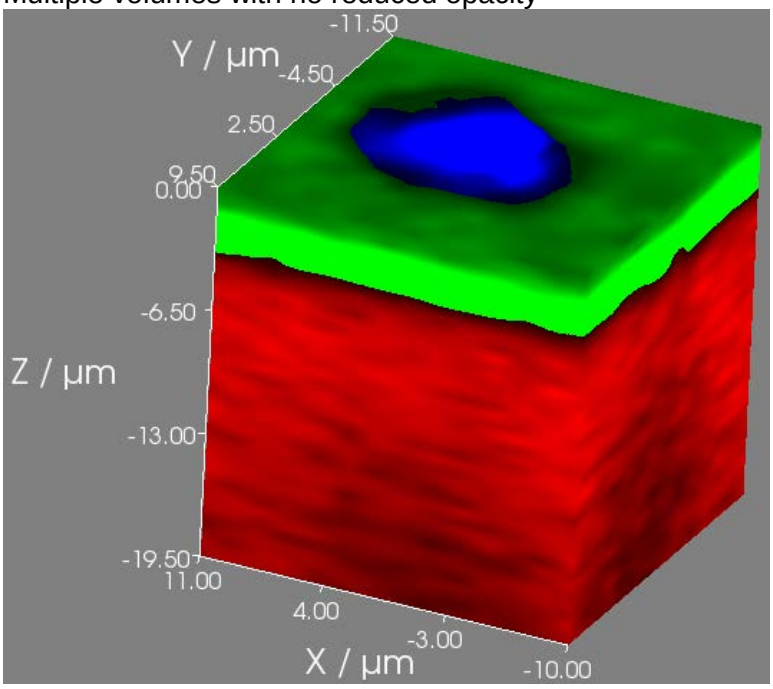
The X, Y, and Z keyboard keys can be used to select different spectra within the plane. Used in conjunction with the shift key, the direction of travel is reversed. A small delay may be observed as the individual spectrum is displayed from the large multifile.

A cursor indicates on the image the location from which the spectrum is displayed. The cursor can be removed from the view by unselecting View....Selected point.

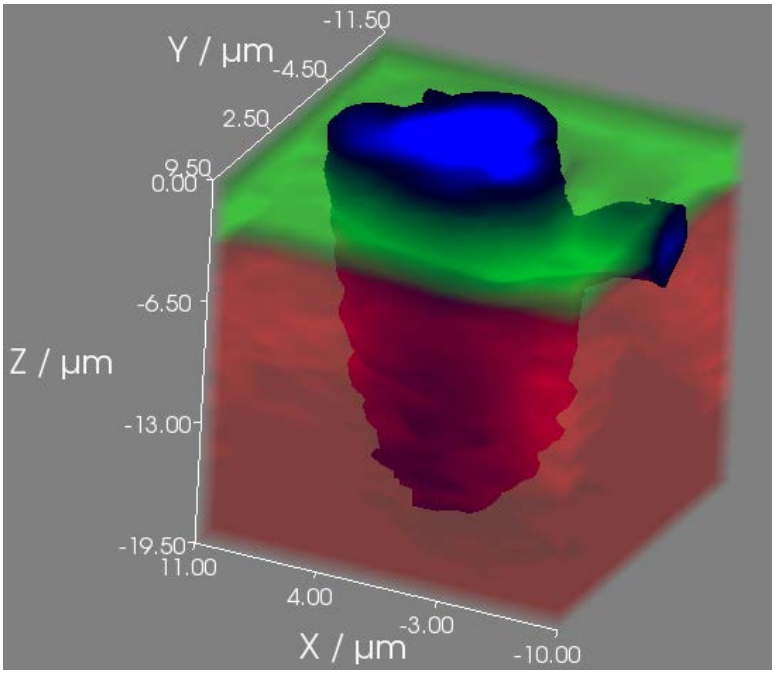
Multiple volumes and opacity

Up to three volumes can be shown together. In such cases it is often convenient to make one or more of the individual images semi-transparent. This can be done by reducing the opacity value.

- Multiple volumes with no reduced opacity

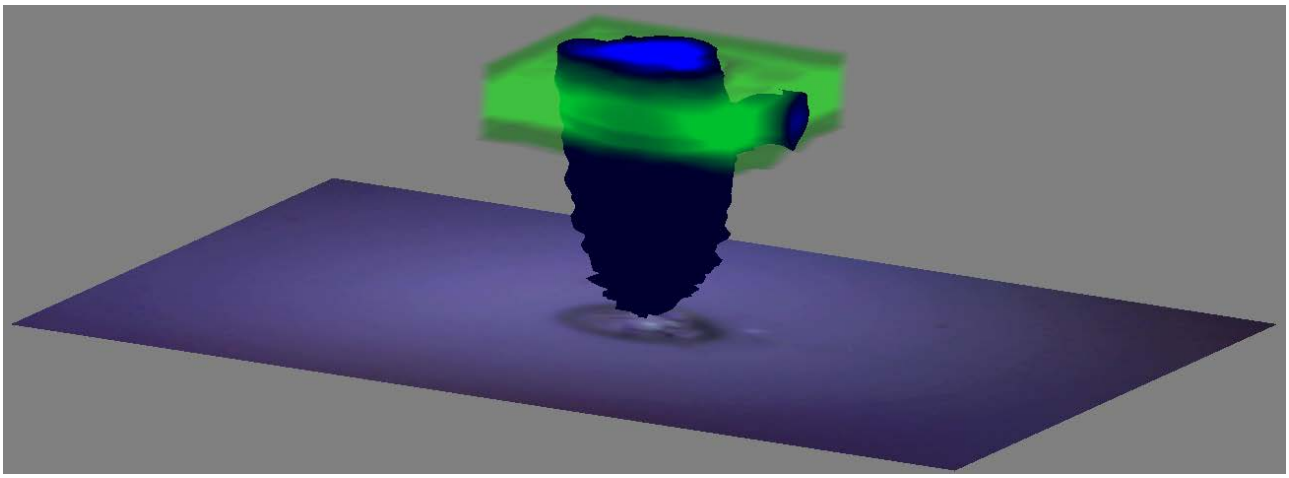


- Multiple volumes with reduced opacity (20%) for red and green images



Adding the white light image

By selecting **Edit...Add white light image** we can project the white light image under the volume.



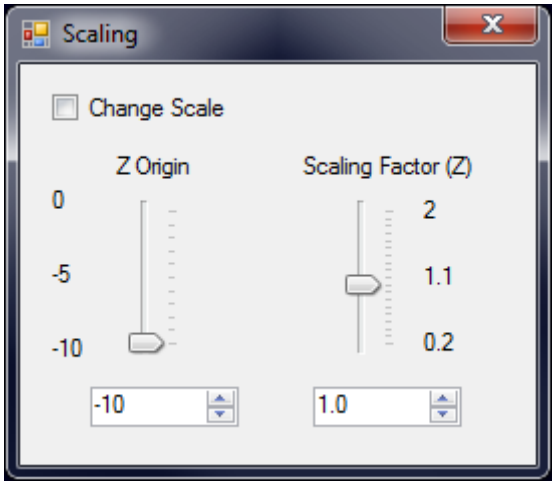
Controlling the Z scale of the volume image

The Z scale has additional control to account for refractive index (RI) effects as the laser light travels from air into the material.

This process has several affects on our data:

- The depth analysed is deeper by a factor of the RI of the material compared to the distance the sample has moved in Z
- The confocal collection volume becomes larger as we collect at deeper depths within the sample

Select **View.....Scaling adjustments** to access adjustment to the Z scaling within the image.

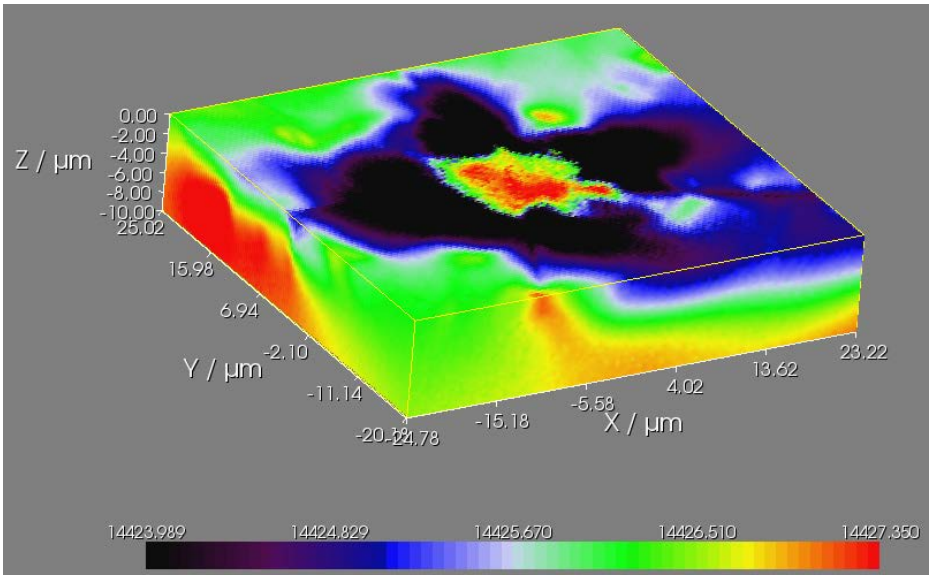


Adjusting the scaling factor will stretch the image in Z compared to XY. The Z origin defines from where in Z the scaling factor is applied.

If the **Change scale** box is ticked the Z values increase as the image is stretched in Z.

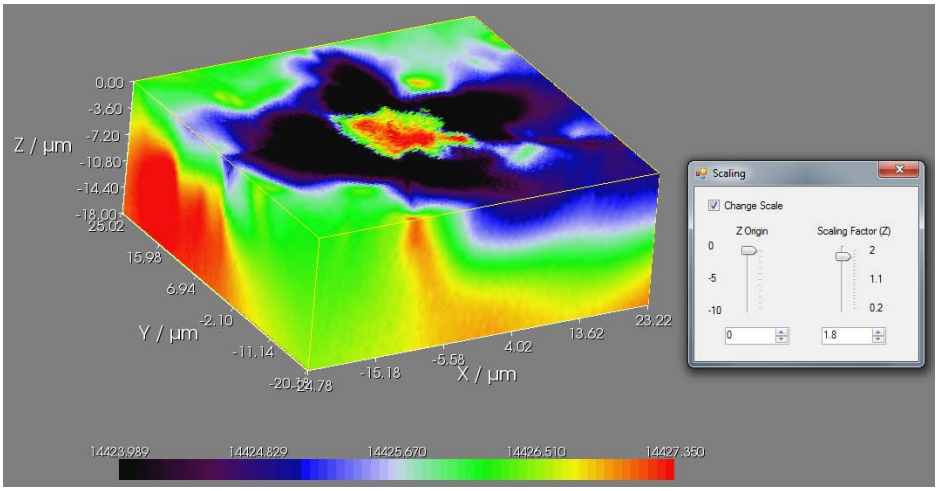
For a volume image of stressed ruby, this feature would be used in the following way:

Initially the scaling factor is set to 1. The Z scale therefore represents the distance the sample has travelled in Z. 0 µm is the sample surface, -10 µm is 10 µm into the sample.



The refractive index of the ruby is approximately 1.77 at 532 nm.

Now the scaling factor has been set to 1.8 with the change scale box ticked. The Z origin is set to 0 (the sample surface).



Now we can see the Z axis values have been stretched by a factor of 1.8 such that the bottom of the image is now at -18.00 µm. The Z scale represents the analysis depth within the sample (to a first approximation) and shows a more realistic representation of the volume in X, Y, and Z.
This correction procedure can be avoided through the use of index matching immersion objectives. Here the refractive index difference is minimised by using oil which has an RI much closer to that of the sample compared with air. In the case where the RI's are identical the distance the sample has travelled in Z is the same as the analysis depth.

Exporting the volume image

The volume image can be copied using **Edit.....Copy to Clipboard** or directly saved as a Jpeg using **Edit.....Save Image**.

Exporting slices from the volume image

In either plane or slice mode, right click on the X, Y, or Z plane image on the right hand side of the window.

Select:

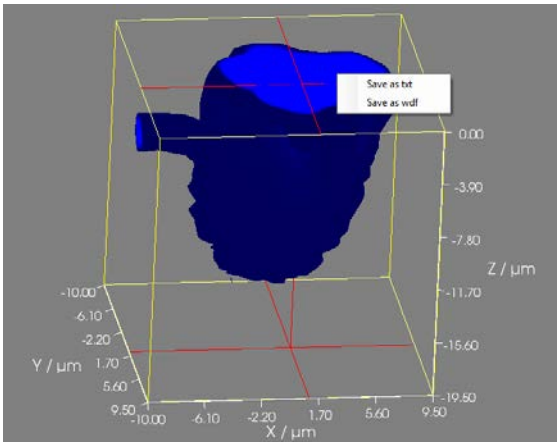
- 'Export nearest slice to 2D wdf file' – enables a new wdf file to be generated, consisting of just that XY plane.

  If the plane shown in the image is interpolated between real spectral slices the nearest data slice will be exported. In this case the image observed in WiRE from the new file may not be identical to that seen in the volume viewer.

  The new wdf file can be processed and analysed in WiRE in exactly the same way as one collected natively in that way.

- 'Export slice as image' – enables the exact shown image to be saved as a .jpg.

In volume mode only, the z profile can be directly exported from the volume image using **Edit... Z-Profile**. A tick box next to **Z-Profile** indicates this mode is turned on. This option restricts access to the **View** tab and **View Control** options. Left click on the volume image to select the desired z-profile location to be exported, then right-click & **Save as** the desired format (txt or wdf).



To exit out of this mode, again select **Edit....Z-Profile** so that the tick box next to 'Z-Profile' disappears and the **View** tab and **View Control** options are accessible to the user.