

# arivis | **Vision4D**

The Basics

arivis AG  
Einsteinstr. 10  
85716 Unterschleißheim, Germany  
info@arivis.com  
www.arivis.com

arivis AG – Imaging Science –  
Kröpeliner Str. 54  
18055 Rostock, Germany

Effective from: 02 / 2017

©2017 by arivis AG – all rights reserved

This document or any part of it must not be translated, reproduced, or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or by any information or retrieval system.

arivis reserves the right to make modifications to this document without notice.



# Content

Introduction .....	6
The SIS file format and ImageCore technology .....	7
1. The arivis Vision4D interface.....	8
1.1. Access to Palettes.....	9
1.2. Handling Viewers.....	9
2. Opening Image Files .....	11
2.1 Opening Native (SIS) Files .....	11
2.2 Importing non-native formats .....	12
2.2.1 Single file import .....	12
2.2.2. Multiple file import – simple image series.....	15
2.2.3. Multiple file import – complex image series.....	17
2.2.4. Pattern Matching.....	19
2.2.5. Apply Dimension Order.....	21
2.3. Tile Sorter.....	23
2.4. Editing metadata .....	28
3. Image visualisation .....	29
3.1. Switching view modes .....	30
3.2. 2D Viewer.....	31
3.2.1. The Navigator palette.....	32
3.2.2. Zooming and panning.....	33
3.2.3. Navigating through planes .....	34
3.2.4. Navigating through frames .....	35
3.2.4. Color scale and LUT adjustments .....	36
3.2.5. The Color Bar .....	37
3.2.6. Color Sliders Palette.....	38
3.2.7. Channel Visibility .....	39
3.3. Gallery view .....	41
3.3.1. Loupe tool .....	43

3.4. 4D Viewer.....	44
3.4.1. Volumetric/Max intensity rendering modes .....	44
3.4.2. Adjusting render setting .....	46
3.4.3. Volume manipulation tools .....	48
3.4.4. Rotating, zooming and panning.....	49
3.4.4. Jump to orientation.....	50
3.4.5. Bookmarks .....	51
3.4.6. Bookmarks Palette .....	52
3.4.7. Fly through mode .....	53
3.4.8. Clipping planes .....	54
3.4.9. 4D Clipping palette.....	55
3.4.10. Clipping regions .....	57
3.5. Projection Viewer.....	59
3.5.1. Projection .....	60
3.5.2. Ortho Slicer.....	61
3.5.3. Sectional Plane.....	63
3.5.4. Extracting projections.....	63
3.6. Split view .....	64
3.6.1. Changing the number of viewers in the Viewing Area .....	64
3.6.2. Linking viewers .....	65
3.7. Sharing images.....	67
3.7.1. Sharing viewer content.....	67
3.7.2. Sharing High Resolution Snapshots.....	67
3.7.3. Sharing Movies – The Storyboard.....	70
3.7.4. Key frames and sequences.....	71
3.7.5. Exporting movies .....	74
4. Image processing and analysis.....	76
4.1. The Filter Gallery .....	77
5. Saving and exporting images .....	79
5.1. Saving images for use in arivis Vision4D .....	79

5.2. Saving images for use in other imaging packages .....	79
6. Example workflows .....	82
6.1. Import a time series .....	82
6.2. Import and rearrange a dataset with a complex file structure using pattern matching .....	82
6.3. Import and rearrange a complete set with tiles .....	83
6.4. Create an animation to reveal structures of interest .....	84
Glossary .....	86
FAQs .....	89
A note on large images: .....	92
Acknowledgements .....	94

## Introduction

arivis Vision4D is a modular software for working with multi-channel 2D, 3D and 4D images of almost unlimited size independent of available RAM. Vision4D is designed around the central concept that file size should never be the issue.

With the arivis ImageCore technology at its heart, this software is capable of opening and rendering multi-terabyte sets almost instantly, without the need for expensive hardware of extraordinary specification.

This **Quick Start Guide** is intended as a first point of contact for novice users with the software and not as a replacement for the full manual and help files that are included with all installations.

As such, many intricacies and subtiles of the operation of the software are omitted from this guide and users are encouraged to refer to the help file and manual if this guide does not fully answer their questions.

---

*Remember that as with other Windows software packages, pressing the F1 key on your keyboard calls up the Help files.*

*In arivis Vision4D, this shortcut is context sensitive and will load the help page for the current tool or window.*

---

Users are also encouraged to work through the examples and exercises with the available demonstration data that can be downloaded from <http://demodata.arivis.com> before attempting to process their own images to gain some familiarity with the tools and functions prior to handling complex problems.

Because many of the terms used in this guide are technical and some are unique to the arivis Vision4D workflow, users are encouraged to familiarise themselves with these terms. We have included a glossary at the end of this guide with links throughout the document to definitions of these terms.

## The SIS file format and ImageCore technology

The arivis ImageCore is a powerful software library which provides fast and smooth retrieval of stored images independent of size and zoom level.

The concept of the arivis ImageCore aims at transparently providing applications with highly performant memory. For this, different caching and pre-fetching strategies are combined with an optimized data format and efficient methods for accessing the data. Because ImageCore is essential to the way that arivis Vision4D handles image data, all non-native files that a user wishes to open in the software for processing and analysis must first be converted to the SIS format.

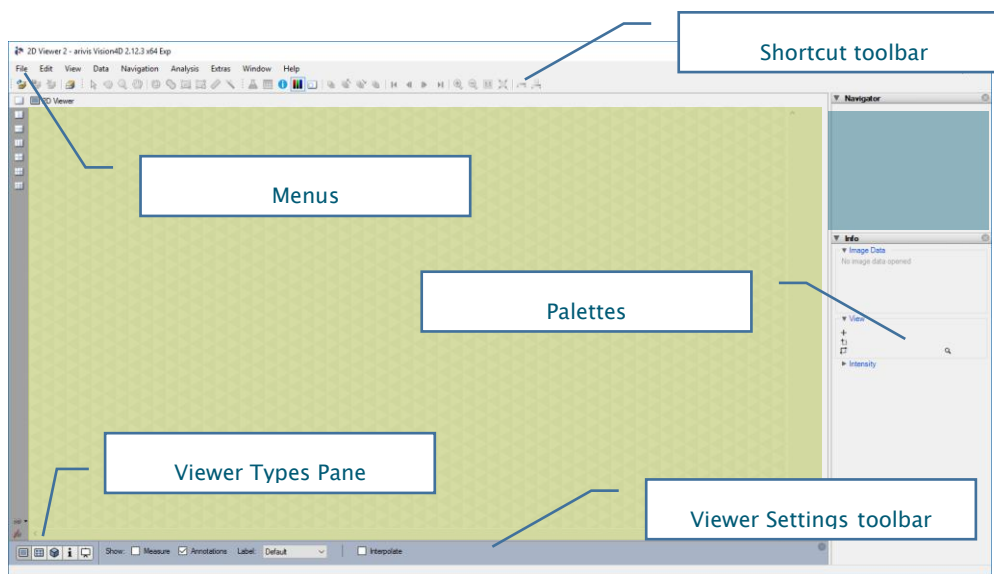
For [native files](#) (\*.SIS files), this process is not necessary and users can simply select to open a file to display and process it almost instantaneously.

Please see the section below on [opening and importing](#) for the practical implications of this aspect of the software.

## 1. The arivis Vision4D interface

The arivis Vision4D interface consists of one or more Viewers. Each Viewer can display one SIS file and is constructed from several individual elements that are assembled to give users access to all the tools and functions required to process and analyse images as well as providing a rendering space for visualising image data.

In the snapshot below, you will find the main components of this interface.



**Menus** – provide access to all the interface elements, tools and functions.

**The Shortcut toolbar** – provides access to commonly used tools and functions through icon buttons. The Shortcut Toolbar is context sensitive and will offer a different selection of shortcut buttons depending on the image rendering mode (e.g. view orientation in the 4D viewer).

**The Viewing Area** – where images are rendered for the user to interact with. The Viewing Area can be split to display multiple sets or image views

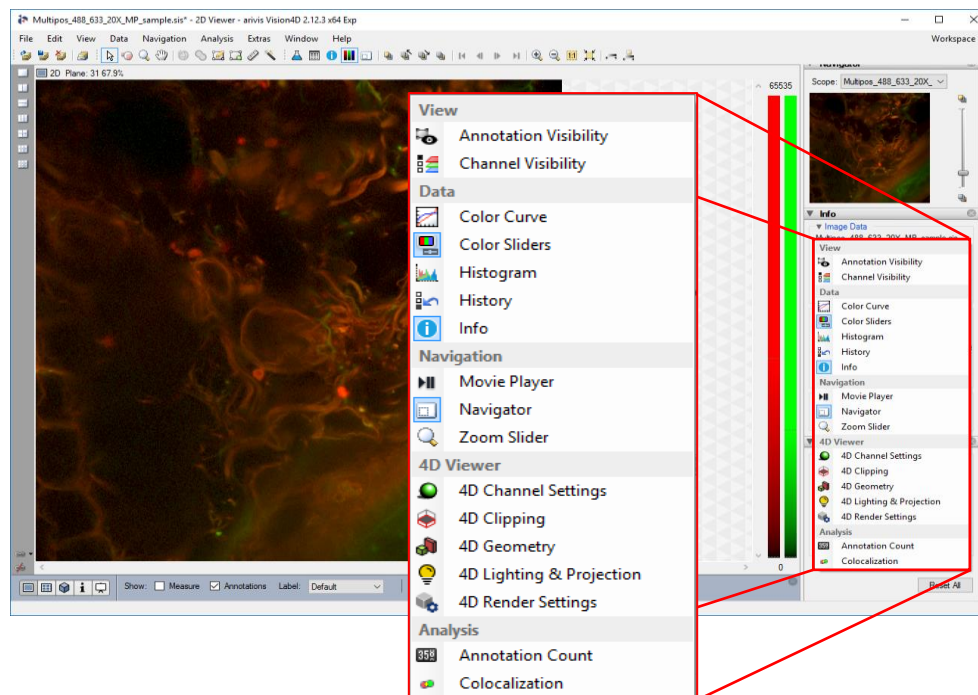
**The Viewer Settings toolbar** – positioned at the bottom of the Viewing Area, it provides tools specific to the specific viewer type currently being used as well as shortcuts to commonly used elements (e.g. enable or disable display of annotations and window decorations), and also include the **Viewer Types Pane** to quickly switch between viewer types.

**Palettes Sidebar** – Positioned to the right of the Viewing Area, the palettes sidebar displays individual [palettes](#). Palettes provide groups of information and settings elements relating to certain aspects of rendering and display.



Palettes can be undocked and moved to other screens to provide more rendering space for the images. Palettes are context sensitive and some palettes are displayed automatically when changing rendering modes. Other palettes can be accessed from the Window menu or by right-clicking with the mouse on empty palette space.

## 1.1. Access to Palettes



**Right-click** on empty palette space to access the **Palettes** menu where you can enable or disable all available palettes.

*Note that some interface elements are only available depending on your license configuration (e.g. the Colocalization panel is only available if you have purchased this specific option)*

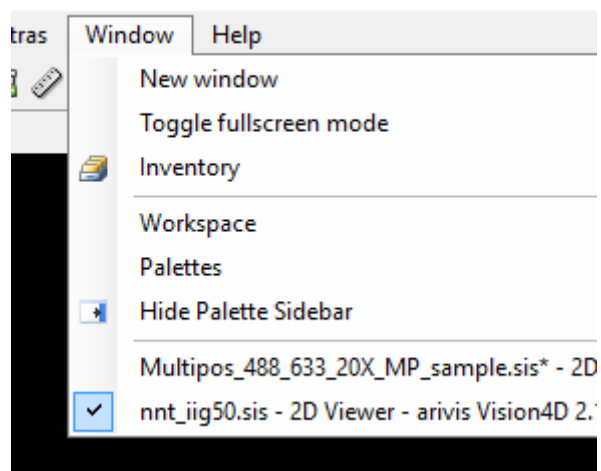
## 1.2. Handling Viewers

Viewers allow users to display their images, They offer various options in terms of rendering mode and multiple viewers can be opened simultaneously.

To switch rendering modes within a viewer, or to view the files information, users can simply select the type of viewer they wish to use either by clicking on its icon in the bottom left of the Viewer Settings Toolbar, or by selecting it from the View menu.



Switching between multiple viewers can be done by clicking anywhere within the viewer window that you want to activate, selecting it from the taskbar, or by selecting the desired viewer from the Window menu.



## 2. Opening Image Files

The simplest way to open an image file in arivis Vision4D is to simply drag and drop the file you want to open from the Windows Explorer into the arivis Vision4D Viewer window. Native files will open immediately and non-native files will automatically be imported. For more complex imports and more options in opening and importing files, see below.

### 2.1 Opening Native (SIS) Files

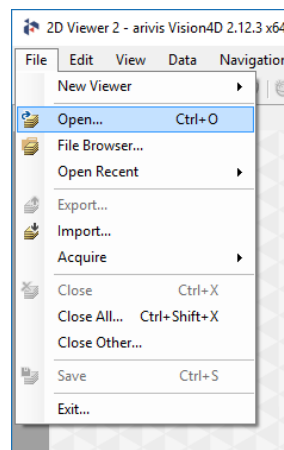
As explained in the section concerning the arivis ImageCore technology above, only [native files](#) can be opened, visualised and processed in arivis Vision4D. [Non-native files](#) need to be converted through the [import](#) process to be opened.

To open an existing SIS file, you can drag and drop it into a viewer as described above

Alternatively, go to the File menu and select the Open-option, or select the

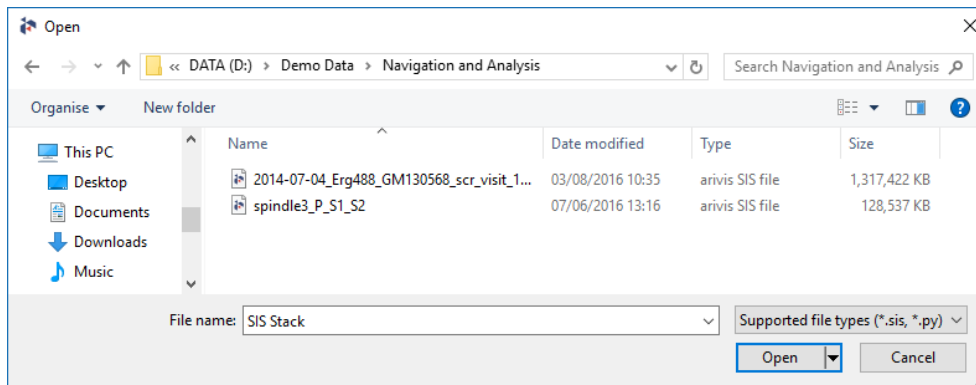


icon from the shortcut toolbar.



1. In the Open dialog, navigate to the file you wish to open and select it by clicking on it.

- Then, either double click the file or click on the Open button to open it.



*Note that only files with the .sis extension, for image files, or .py for scripts, will be displayed in this window by default. If the file you are looking for is not shown, you may need to use the Import function instead.*

## 2.2 Importing non-native formats

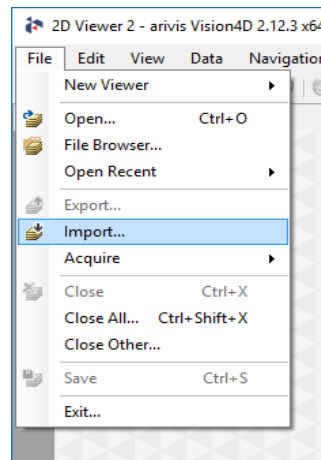
Depending on the complexity of the structure of the imported [files](#) and whether you are importing a series of files or a single document, the importing process can offer different options and levels of complexity. Example files for each of the following types of import can be found in the demo data at <http://demodata.arivis.com>.

For practice, see [exercises](#) at the end of the guide.

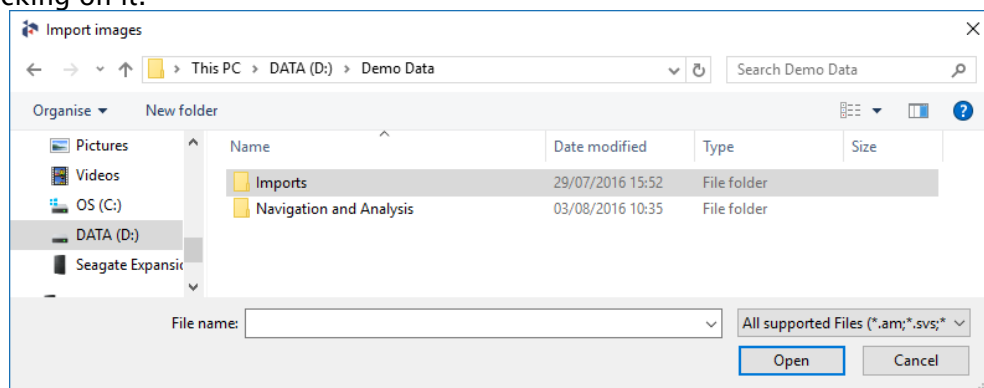
### 2.2.1 Single file import

Supported set file formats (CZI, LIF, ND2, OIF etc.) and single images can be imported very quickly and easily.

Either click and drag the selected file from the Windows Explorer into the arivis Vision4D workspace or go to the File menu and select the **Import...** option.



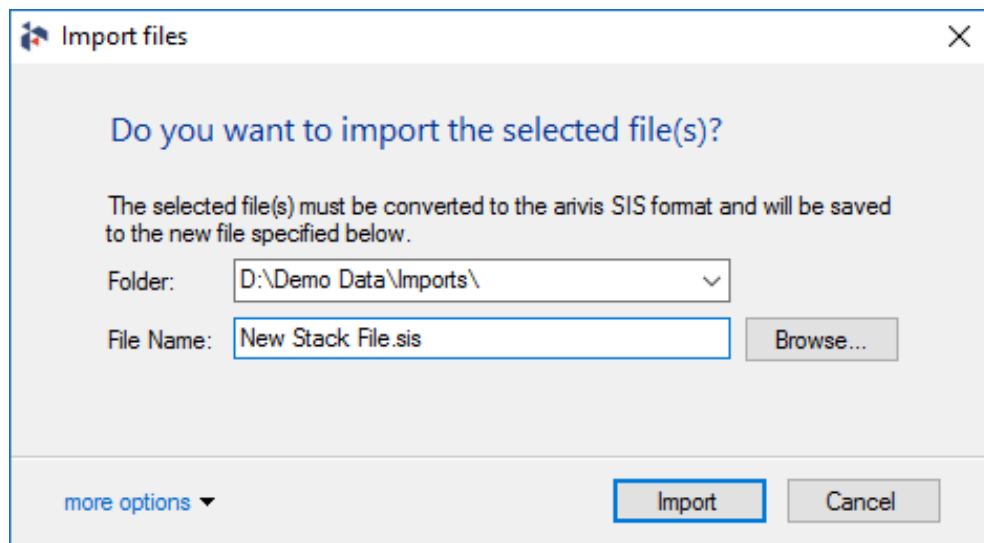
In the **Open dialog**, navigate to the file you wish to open and select it by clicking on it.



Then, either double click the file or click on the Open button to open it.

*Note that all supported image format files will be displayed in this window. You can restrict the type of image files to display by selecting them from the drop-down list immediately above the Open button.*

You will then be presented with the Import dialog where you can choose name and location for the resulting SIS file.



1. Select the desired save location by clicking on the Folder drop-down selection box and choosing your preferred save location.

---

*Note that this selection box defaults to the last selected save location. Other save locations can also be selected by clicking the Browse button. The current location of the files to be imported can also be selected from this list.*

---

2. Select the desired name for the output file.

---

*Note that the import will propose the original name by default but will replace the original file extension with .sis.*

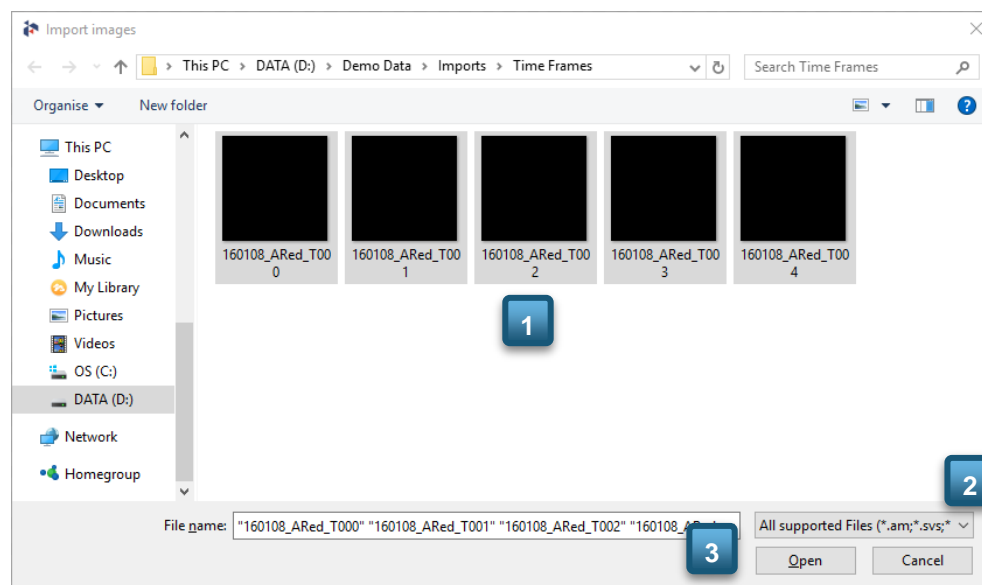
---

3. Then click **Import** to import the files and create a new SIS file from the selected image. The imported file will automatically open.

## 2.2.2. Multiple file import – simple image series

When opening multiple image files that are part of a series, or files that contain multiple images, some further configuration will be required to define the [dimension](#) order.

To import a series of images, go to the **File** menu and select the **Import** option.



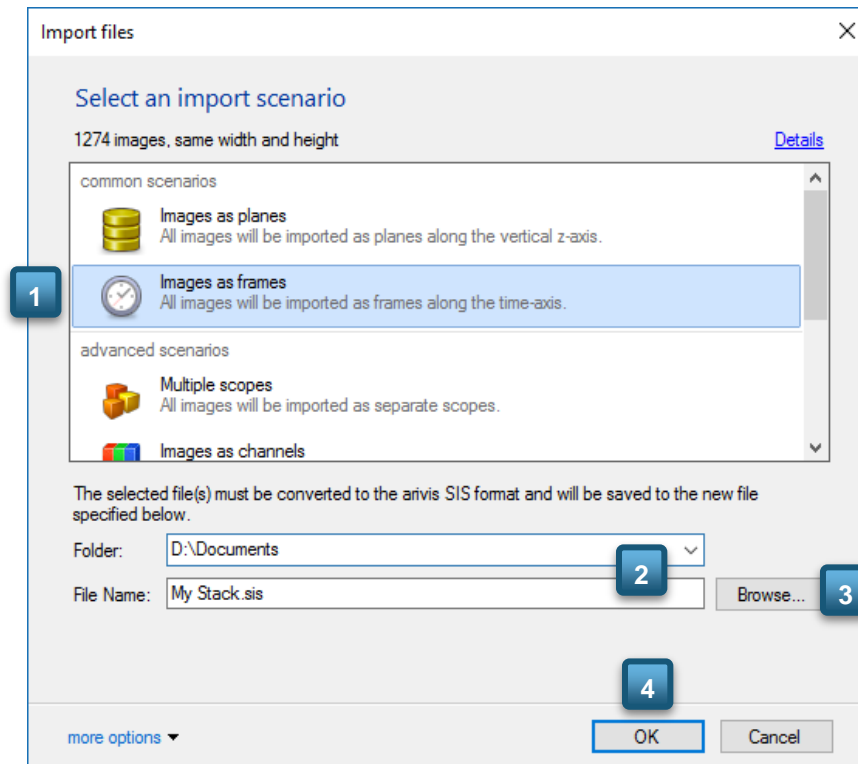
1. In the Open dialog, navigate to the files you wish to open and select them by clicking on them. (1)

*You can use the keyboard shortcut **Ctrl-A** to select all the files in the current folder. You can also use the **Shift** and **Ctrl** keys while clicking to select multiple contiguous or non-contiguous files respectively.*

*Note that all supported image format files will be displayed in this window.*

2. You can restrict the type of image files to display by selecting them from the drop-down list immediately above the **Open** button. (2)
3. Then, click on the **Open** button to open the series. (3)

You will then be presented with the Import dialog where you can choose the name and location for the resulting SIS file, and the structure of the set.



1. For simple time series or Z-stacks, you can select the appropriate option from the **common scenarios** list. (1)
2. Select the desired save location by clicking on the **Folder drop-down selection box** and choosing your preferred save location. (2)

*Note that this selection box defaults to the last selected save location.*

3. Other save locations can also be selected by clicking the **Browse** button.  
Select the desired name for the output file. (3)

*Note that the import will propose the original name by default but will replace the original file extension with .sis.*

4. Then click **OK** to create a new SIS file from the selected images and open it. (4)



### 2.2.3. Multiple file import – complex image series

arivis Vision4D supports importing files with multiple dimensional elements.

In most cases, when opening set files from supported providers (LSM, LIF, CZI etc.), multiple dimensions are automatically processed into their recipient dimensions in arivis Vision4D.

---

*Please refer to single file imports for these cases.*

---

However, if importing sets with multiple dimensions from image series, manual configuration of the dimensional arrangement will be required.

1. To import a complex series of images, go to the **File** menu and select the **Import...** option.
2. In the **Open** dialog, navigate to the files you wish to open and select them by clicking on them

---

*You can use the keyboard shortcut **Ctrl-A** to select all the files in the current folder. You can also use the **Shift** and **Ctrl** keys while clicking to select multiple contiguous or non-contiguous files respectively.*

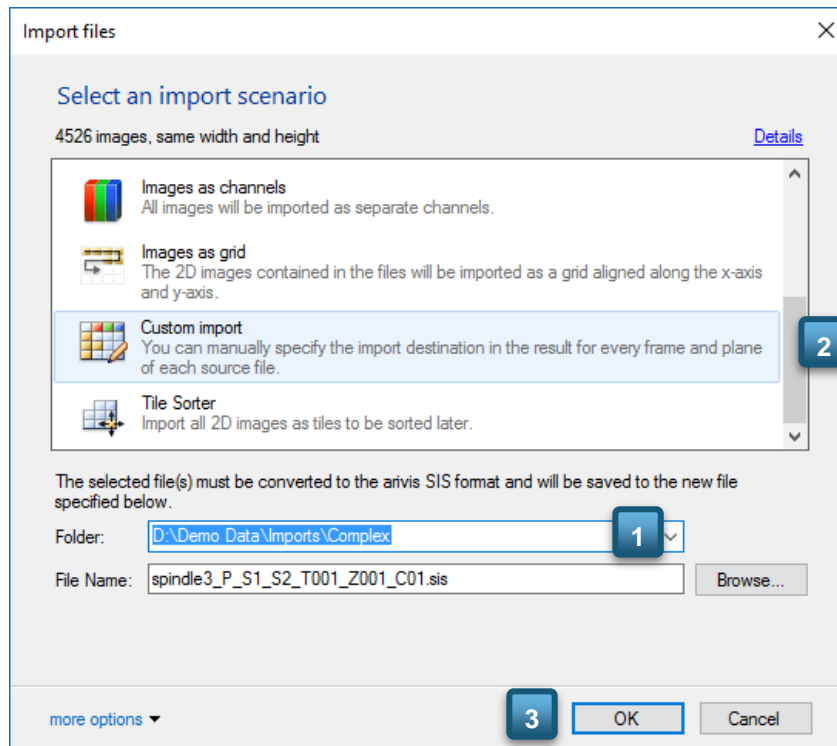
---

*Note that all supported image format files will be displayed in this window. You can restrict the type of image files to display by selecting them from the drop-down list immediately above the **Open** button.*

---

3. Then, click on the **Open** button to open the series.
4. If importing more than 100 files, you will then be prompted to choose whether you wish to assume the same structure for all files.
5. Select **Yes** if appropriate.

You will then be presented with the **Import dialog** where you can choose the name and location for the resulting SIS file, and the structure of the set.



1. Select the desired save location by clicking on the **Folder drop-down selection box** and choosing your preferred save location. (1)

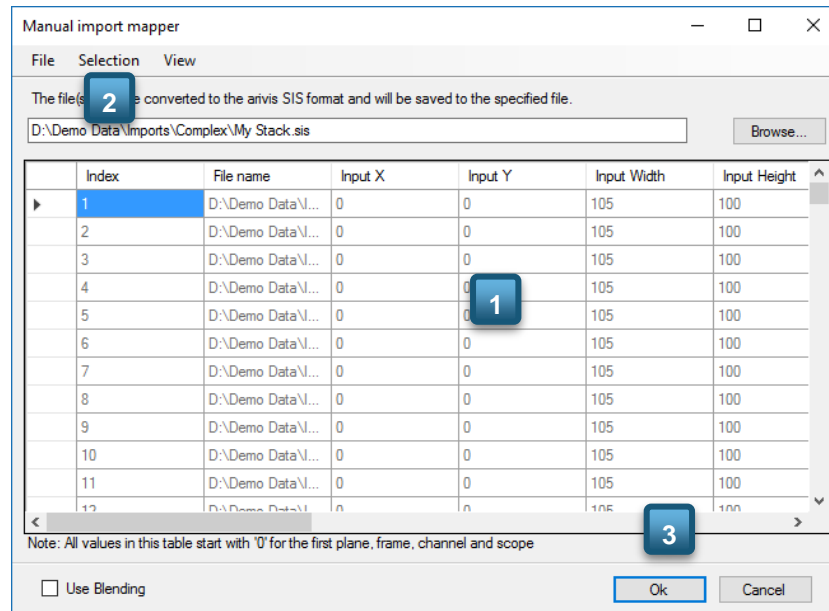
*Note that this selection box defaults to the last selected save location. Other save locations can also be selected by clicking the Browse button.*

2. Select the desired name for the output file.

*Note that the import will propose the original name by default but will replace the original file extension with .sis.*

3. For multiple tiles of an image mosaic, select the **Tile sorter** scenario and see section 5.c for details of the Tile sorter. (2)
4. For multidimensional sets, scroll down the import scenario list and select the **Custom import** option and click **OK**. (3)
5. For simple time series or Z-stacks, see section on importing simple series above.

You will then be presented with the **Manual import mapper** dialog.



In the **Manual import mapper** dialog users are presented with a grid of the image indices along the Y axis and their input and output coordinates along the X axis.(1)

#### 2.2.4. Pattern Matching

If the image names include details of their respective dimensional coordinates (e.g. ImageSet\_C001\_T002\_Z005 where the number following the dimensional identifier characters defines its position along this dimension), you can use the **Pattern matching** function to allow the software to arrange all the images into their correct positions.

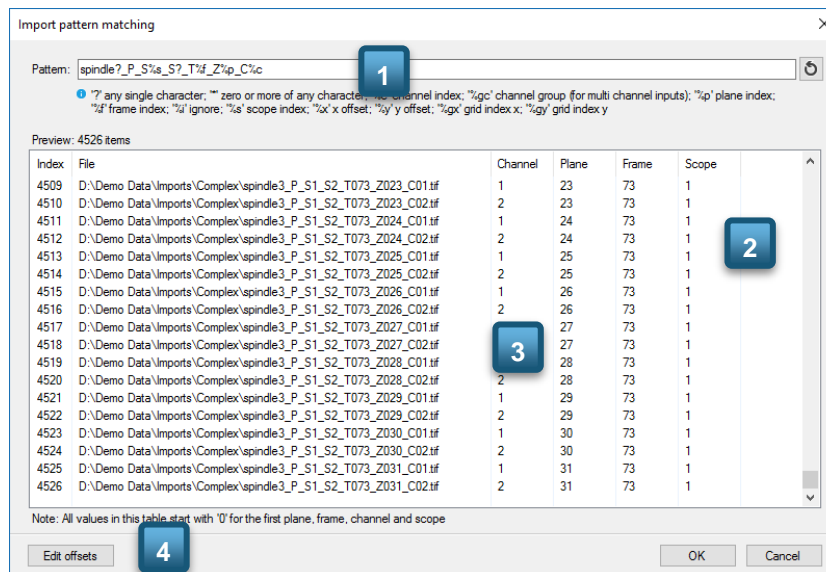
To do this, go to the **Selection** menu and select the **Pattern matching...** option. (2)

---

*Note that the operation will apply to all images unless a sub-selection has been made. In that case, the operation will be applied only to the sub-selection*

---

You will then be presented with the **Pattern matching dialog** below.



1. Use the pattern definition nomenclature at the top of the dialog to update the **Pattern** matching string (1)
2. Then click the **Refresh** button to update the **Preview** pane (2), (3).
3. If necessary, it is possible to edit offsets using the **Edit offsets** button (4)
4. When satisfied with the results, click **OK** to confirm.

Please refer to the help file for a further details.

---

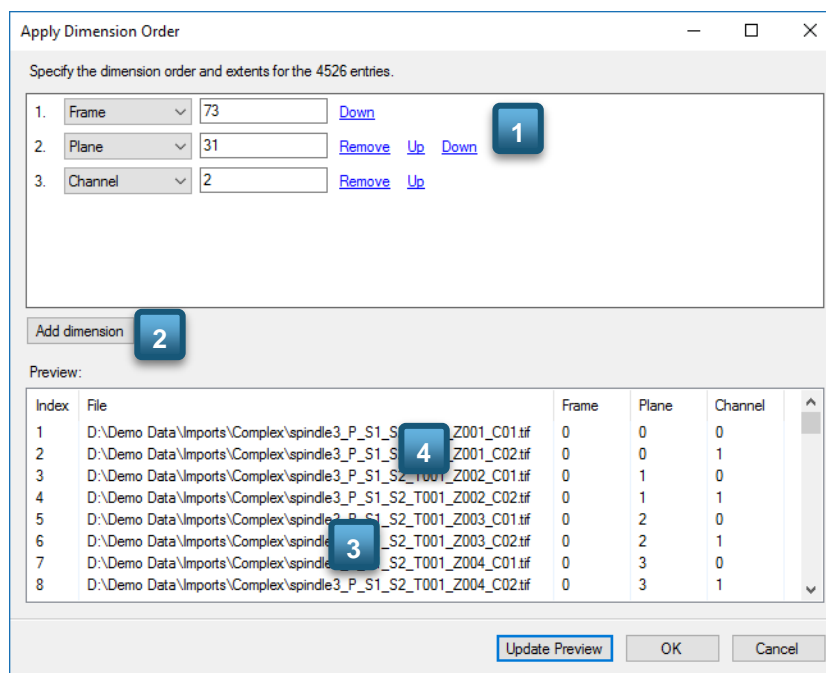
*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

### 2.2.5. Apply Dimension Order

If the file name gives no indication of the image coordinates, or if the images are extracted from a multiple image document (e.g. OME TIFF), you will need to manually select and define the dimension and their order.

- To do this, go to the **Selection** menu and select the **Apply Dimension Order...** option. You will then be presented with the **Apply Dimension Order** dialog below.



The Apply Dimension Order is split into 2 sections.

- The upper section lists the currently selected dimensions and their extent, the lower part previews the image coordinates. The most common two dimensions are automatically added to the initial dimension list.(1)
- To add dimensions, click on the **Add dimensions** button.(2)
    - To modify the dimension type, select a new type from the **drop-list box** on the left of the dimension you want to modify.
    - To modify the dimension extent, type desired extent in the **textbox** in the centre of the dimension you want to modify.
    - To modify the dimension order, click the **Up / Down** button on the right of the dimension you want to modify.
    - To remove a dimension, click the **Remove** button next to dimension you wish to remove.

---

*Note that at least one dimension must be present and by default the first dimension cannot be removed. If the first dimension is incorrect, you should modify it rather than remove it.*

---

*Some trial and error may be required unless you already know the dimension order.*

---

2. Update the image coordinate preview by clicking the **Update Preview** button at the bottom of the dialog to verify your input. (3); (4)
3. Adjust each dimension as needed, then click **OK** to confirm your selections and return to the Manual import mapper.
4. Click **Open** to complete the set definition and import the selected images into your new SIS file.

---

*Note that it is also possible to manually edit all these parameters within the Manual Import Mapper. Please refer to the help files for more details.*

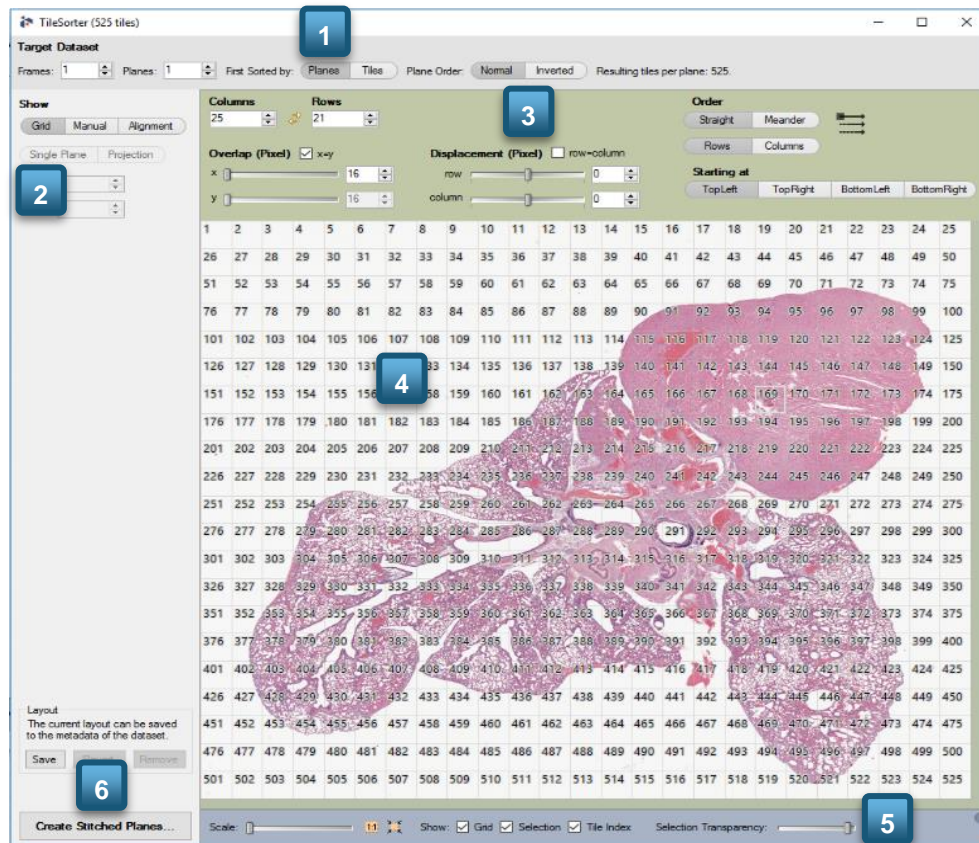
---

## 2.3. Tile Sorter

arivis Vision4D includes tools to rearrange tiled images into a large mosaic and adjust the alignment of images tiles/stacks. The image [import](#) described above allows you to import images directly into the Tile Sorter tool, but this tool can also be accessed after the image import has been completed as a separate step from the **Data** menu.

*Note that the Tile Sorter takes a stack of images as input. If the chosen images are not imported directly into the tile sorter from the import scenario dialogs they must first have been imported as a single Z-stack or organized as tile stacks in the frames or scopes of the dataset.*

The Tile Sorter dialog is composed of several discreet elements:



**Target Dataset definition** – where users define the number of [frames](#) and [planes](#) expected in the output [scope](#) and their order.(1)

**Sort Mode** – where users choose how to sort the tiles, whether in a grid pattern, manually or by applying an alignment algorithm. (2)

**Columns and Row definition** – where users select the number of rows and columns, and defines their spacing and orientation. This section is dependent on the type of operation chosen. It is hidden in the manual alignment and offers different tools for automatic alignment. (3)

**Tile Sorter Preview** – where users can see in real time the effect of tiling adjustments. (4)

**Tile Sorter Viewer Settings** toolbar – which functions similarly to the main Viewing Area Settings Toolbar in giving the user the option to change how the tiles are displayed. (5)

To access the Tile Sorter during import, simply select the **Tile Sorter** scenario from the import scenarios.

---

*If you already have your mosaic tiles opened as an SIS file in arivis Vision4D you can access the Tile sorter by going to the **Data menu** and selecting the **Tile Sorter** option.*

---

In general, the tile sorting process is as follows:

1. Users align the images using basic sorting by grid.
2. Manually refine the alignment (if needed or if the tiles are not arranged by grid).
3. If necessary, users can then further refine the alignment using pattern matching to correct for slight errors in positioning from tile to tile.
4. Once the alignment is finalised, the stitched image can then be generated and saved.

### Grid tile positioning

As most mosaic tiles are acquired using automated tiling systems with motorised stages, the simplest method for aligning the image correctly for stitching is to use the grid sort mode. The grid mode works by defining the number of frames, planes, rows and columns and then rearranging the images into their order of acquisition.

1. First, select the number of [frames](#) and [planes](#) in the resulting [scope](#). (1)
2. Then, select the grid option from the **Sort Mode** column. (2)
3. Adjust the **Columns** and **Rows** values until the preview image resembles the expected results. (3)
4. You may also need to adjust the **Order** and **Starting at** options to get the desired results. (3)



5. Once the tiles are sorted into the correct order, you may need to adjust the overlap and displacement options to get a perfect match between images. (3)
6. To do this, it is recommended that you should zoom to the intersection between four tiles at 1:1 zoom level using the **Viewing Area settings toolbar**. (5)
7. Then, adjust the **overlap** and **displacement** values until the tile overlap becomes seamless. Hiding the grid display may help at this stage, this can be done by deselecting the **Grid checkbox** in the **Viewing Area settings toolbar**.

---

*You can also toggle the visibility of the currently selected tile with the Space key*

---

### Manual tile positioning

In case where the tiles to be merged were acquired using manual positioning of the stage, using a manual placement of the images on the screen may be more effective. This positioning does not need to be perfectly accurate as it can be finished off using automatic alignment.

1. First, select the number of [frames](#) and [planes](#) in the resulting [scope](#). (1)
2. Then, select the **Manual** option from the **sort mode** column. (2)
3. Click and drag individual frames in the tiling workspace to their approximate locations relative to each other.
4. Then, adjust the overlap and displacement of the images by clicking on the tile whose position you are looking to adjust and using the arrow keys on your keyboard to move it up, down, left or right until the tile overlap appears seamless. Changing the selection transparency may help at this stage, this can be done by adjusting the **Selection Transparency** slider in the **Viewing Area settings toolbar**.

### Alignment calculation

Having set up the tile positions, either manually or using the grid alignment, some slight misalignment of frames may still be present. Aravis Vision4D includes automatic matching tools to get the alignment pixel perfect prior to blending the tiles.

1. Select the **alignment** option from the **Sort mode** column.
2. Choose a maximum horizontal and vertical alignment limit. This will limit the margin of error in case the overlap pattern is weak. Setting

a larger alignment margin also means the alignment process can take longer.

3. Choose an alignment algorithm and your preferred optimiser
4. When ready, you can choose to **Align Current Plane** and assume that each stack is well aligned with the next, or use the **Batch Alignment** to calculate the alignment for some or each plane in the stack.

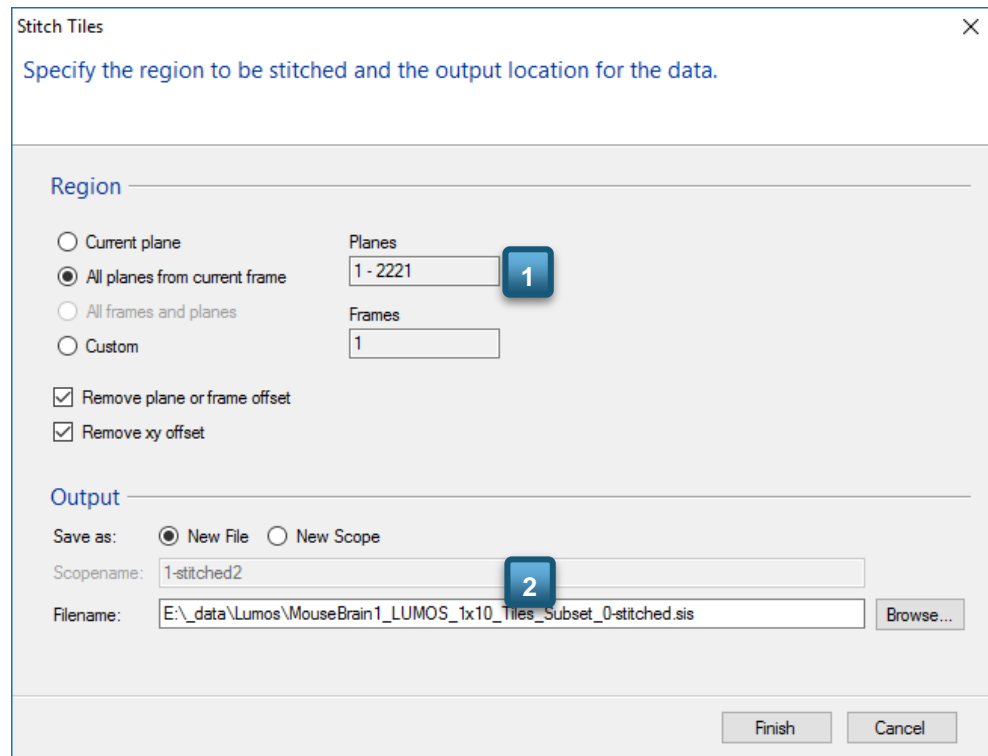
Consult the help files for a more details on the alignment options.

---

*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

Once the tiles are sorted and aligned, you can complete the process by clicking the **Create Stitched Planes...** button.(6)



1. You will then be prompted to choose the **Region** to process (if you do not wish to process every plane in a stack, for example) (1)
2. Choose the desired **Output**. (2)

---

*The stitched image can be saved to a new scope within the current file, or as a new separate file altogether. If selecting a new file, you will also need to select the output file's name and location.*

---

3. Select your options as desired and click **Finish** to produce your tiled sorted scope.

For full details, please refer to the Help files.

---

*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

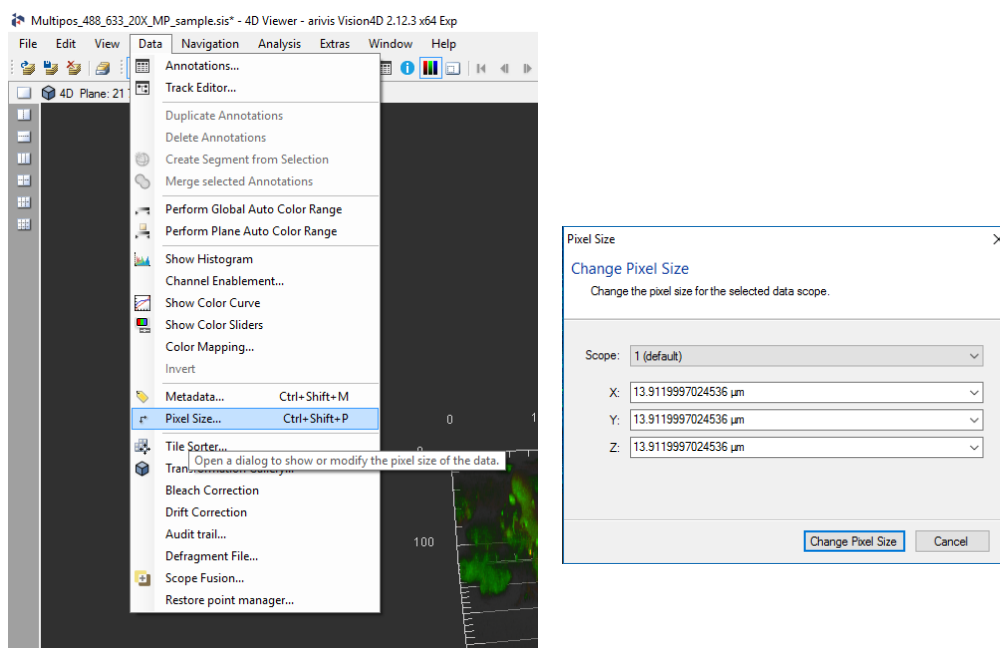
---

## 2.4. Editing metadata

In some instances, the metadata relating to an image may be incorrect or missing.

While in many cases missing metadata does not affect the visualisation or analysis of the images, it is particularly important that the pixel spacing information is correct in every case as this is used to calibrate the rendering of the image and measurements of annotations.

- To edit pixel dimension values, go to the **Data menu** and select the **Pixel size...** option.



1. In the **Pixel size** window, select the required value for each dimension, making sure that you select the correct units.
2. Click **Change Pixel Size** when ready to apply changes.

---

*Hint: units can be selected simply by typing out the full value including units. In that case " $\mu\text{m}$ " can also be written out as "um"*

---



---

*Remember to save your file after applying these changes to avoid having to make these changes next time you open this file.*

---

### 3. Image visualisation

Because large datasets typically have more information than can be displayed on the screen at any one time, choosing the most appropriate rendering mode is an essential part of the workflow.

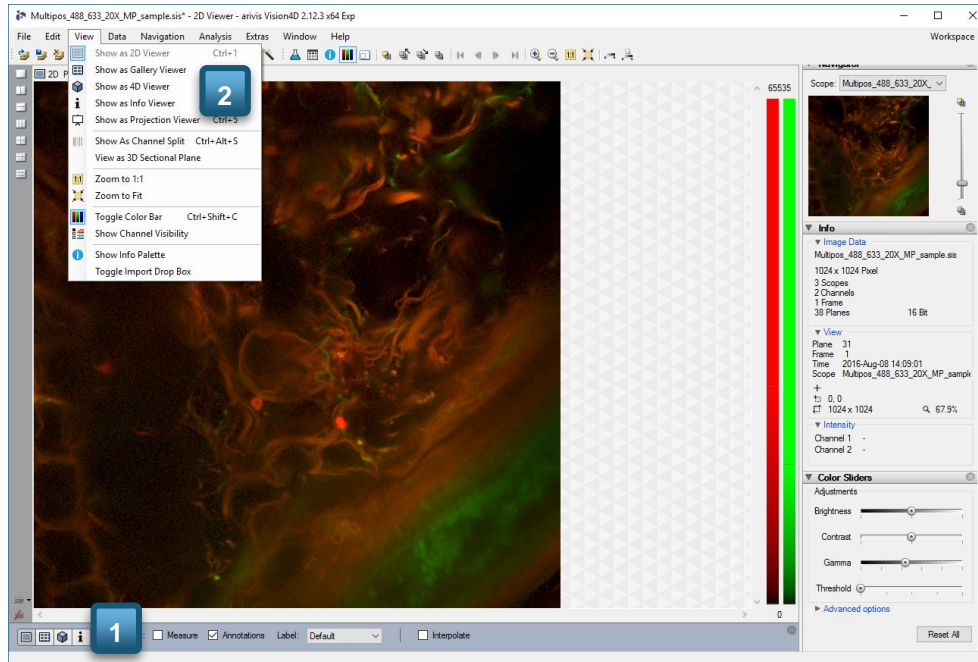
The viewer types available in arivis Vision4D are 2D, Gallery, 4D, Info and Projections.

Each viewer type is particularly suited to different kinds of images and workflows.

- The **2D view** is ideally suited to sets that do not include depth information or to processing images within an analysis workflow where users need to interact with annotation and see the results of operations at full resolution.
- The **Gallery view** allows users to see all plane images and/or frames from a scope in a grid arrangement and can be useful for navigation in conjunction with the Split View.
- The **4D viewer** is best suited to visualising sets with volume information and looking at the structures within them, but is not well suited to the manual definition of regions of interest.
- The **Info viewer** offers information about the data set, and allows the user to change the dataset structure (e.g. remove planes, remove results scopes or edit scope offsets).

### 3.1. Switching view modes

Switching viewer types is fast and easy in arivis Vision4D.

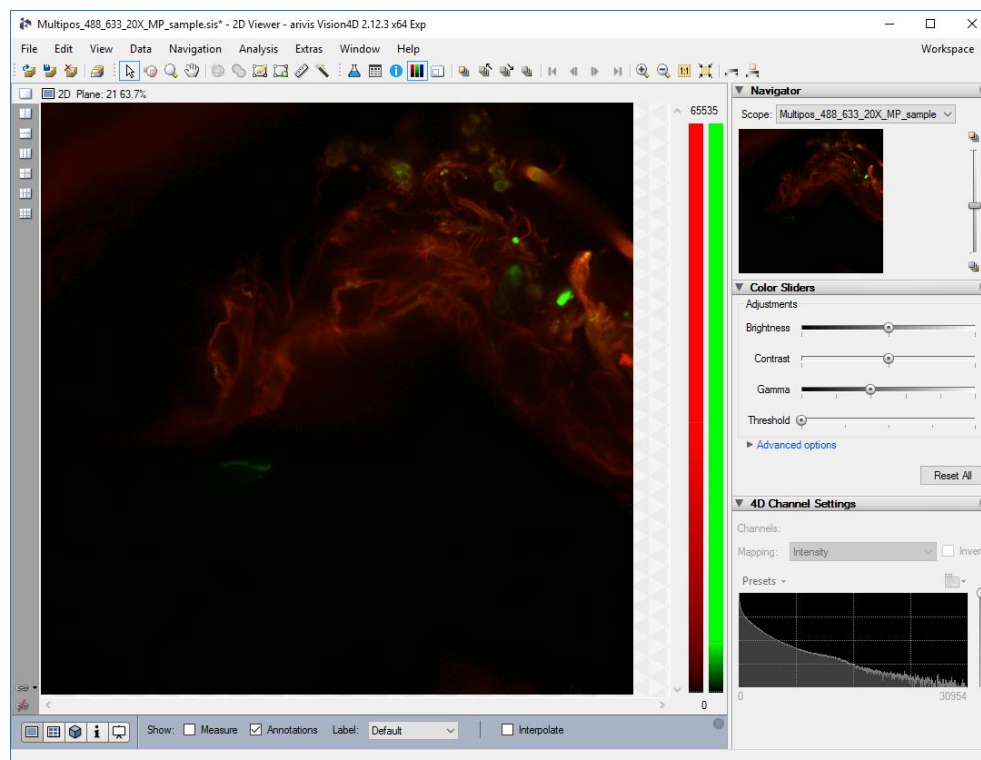


1. To do this, simply click on the icon referring to your preferred mode in the **Viewer Settings toolbar**. (1)
2. These options are also available from the **View menu**. (2)

### 3.2. 2D Viewer

The 2D view is a great rendering for examining 2D data or looking at individual portions of the set with the highest degree of detail by giving direct access to the raw pixel data. It is also a very useful view for testing out data analysis operations prior to executing them on complete stacks.

- To activate the 2D viewer, simply click its icon in the **Viewer Types** pane **Viewer Settings** toolbar.

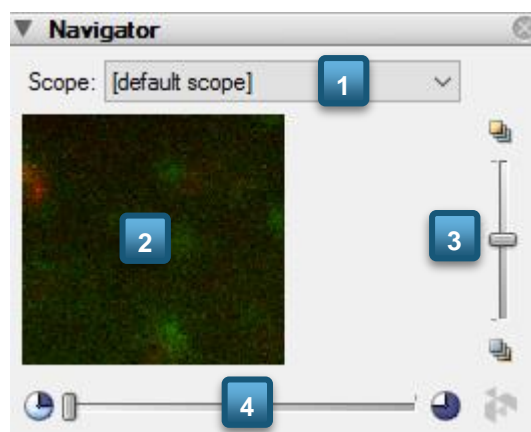


Because by its nature the 2D viewer restricts the amount of information that can be displayed at any one time to a single plane, arivis Vision4D offers several tools to allow the user to navigate through the set to focus on specific portions of the image data.

### 3.2.1. The Navigator palette

The Navigator Palette is a useful tool for quickly navigating through the set's [dimensions](#).

It is context sensitive and displays navigation tools for each of the available dimensions, except for [channel](#). Tools are only displayed if the current set has an extent greater than 1 for that dimension. This means that if your file only contains 1 scope, the scope selection list will not be displayed.



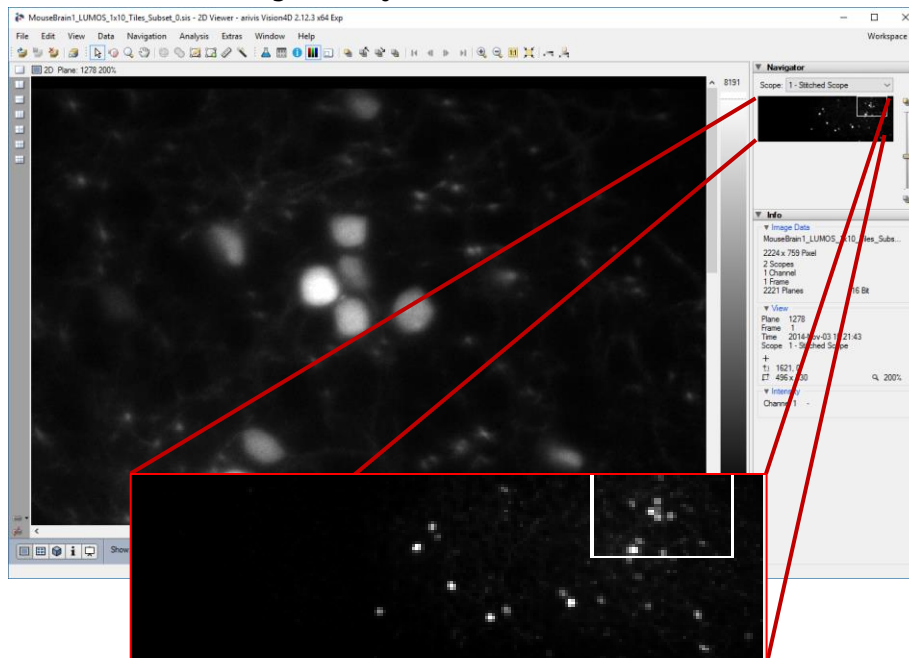
The droplist box at the top allows the user to navigate through scopes (1). Simply select the scope you wish to view from the list to activate it.

The image **Navigator preview** shows the current view area in the context of the current [plane](#). (2)

The sliders on the right and bottom allow you to scrub through planes and frames respectively (3)&(4)



Users can also drag the current view area on the **Navigator preview** to navigate to this portion of the image in the viewer, or click and drag outside of the current view rectangle to adjust the viewer to the selected area.



### 3.2.2. Zooming and panning





- To zoom in on an image in the 2D view, you can use your mouse scroll wheel or the various zoom tool from the **shortcut toolbar**.
- With the magnifying glass tool, **left-click** on the image to zoom in, or hold down the **Shift** key when clicking to zoom out.
- You can also drag a rectangle on the image to zoom to that specific area.
- To pan across the image, you can use the scroll bars on the right and bottom of the Viewing Area, or the Hand tool from the **shortcut toolbar**.

### 3.2.3. Navigating through planes

Navigating through [planes](#) can be done in several ways.

Users can use the Navigator palette as described above, or use the dedicated icons from the shortcut toolbar. The icons are greyed out if it is not possible to move further in the button's navigation direction.



-  The **Go To Top Plane** button display the first plane in the stack.
-  The **Plane Up** button display the plane immediately above the current plane.
-  The **Plane Down** button displays the plane immediately below the current plane.
-  The **Go To Bottom Plane** button displays the last plane in the stack.

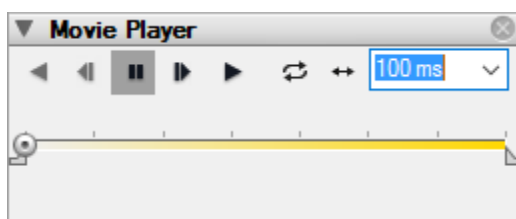
### 3.2.4. Navigating through frames

Navigating through [frames](#) can also be done in several ways.

As with navigating through depth, the most accessible way is to use the Frame slider at the bottom of the **Navigator palette**.

The **Movie Player palette** is another great tool for navigating through time frames.

- To activate it, right-click in an empty space of an existing palette and select it from the contextual menu. Alternatively, you can activate it by going to the **Window menu** and selecting it from the **Palette submenu**.



The buttons along the top allow you to:

- ◀ Play all frames from the current frame in reverse order.
- ◀◀ Step to the previous frame.
- ⏸ Pause the navigation.
- ▶▶ Step to the next frame.
- ▶ Play all frames from the current frame in forward order.
- ↺ Toggle looping through the sequence. When active the player jumps back to the first frame after the last frame is displayed.
- ↻ Toggle automatic play directions reversal. When active, the player reverses play order after reaching either extremity.

The **combo box** on the top right allows you to change the frame display time.

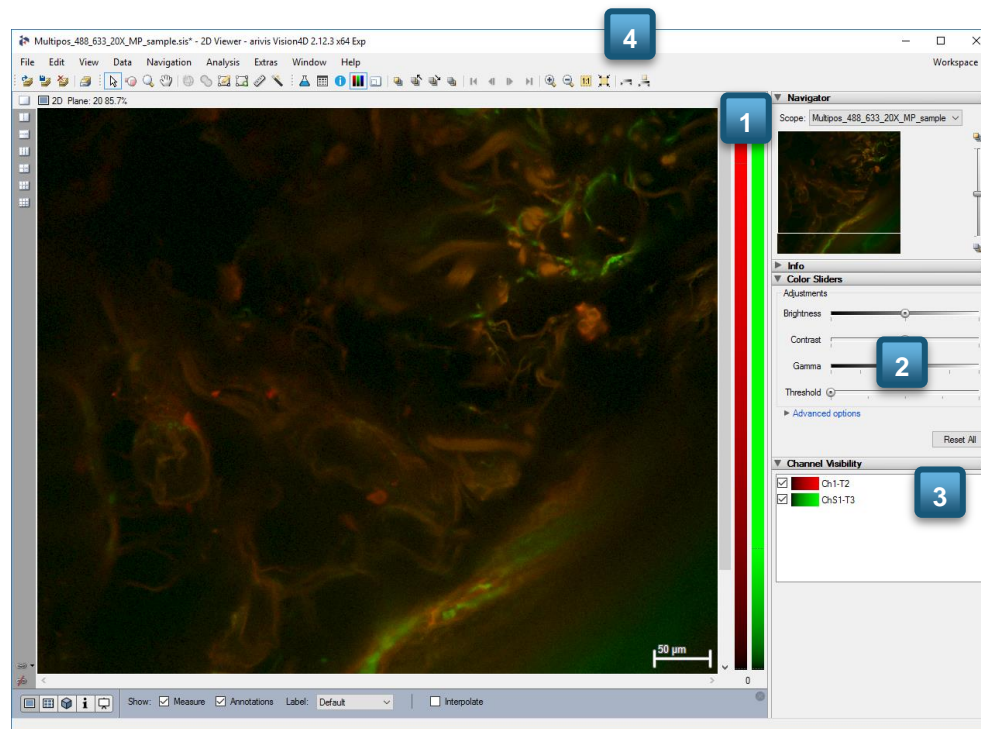
Users can either select a pre-set from the list or manually type their desired display time.

- To play the sequence at 10 frames per second, you would need to set the display time to be  $1\text{s}/10 = 0.1\text{s}$  (or 100ms)

### 3.2.4. Color scale and LUT adjustments

arivis Vision4D offers a range of tools for color and contrast adjustment to help users best show their data.

Several tools for image adjustments are displayed in the workspace by default, while others can be accessed through the palettes.



The main tools for image adjustments are the [Color Bar \(1\)](#), [Color Sliders \(2\)](#) and [Channel Visibility \(3\)](#).

When opening images for the first time in arivis Vision4D, the display range from the source will automatically be applied if it can be read, otherwise it will automatically be set to the dynamic range of the image. If a different display range is required, it can be adjusted.

The fastest and most efficient way to get a good approximation of the best display range for the image is to use an automatic display range selection.

The Shortcut toolbar has two buttons for automatic display range calculation. (4)

- **Perform Plane Auto Color Range** uses the brightest and darkest pixels from the current plane in the stack to calculate the upper and lower saturation points.
- **Perform Global Auto Color Range** uses pixel values from the complete stack to make the same calculation. Because the Plane auto colour range uses a much more restrained data set it is much faster, but also prone to inadequate results if the selected plane is not representative of the stack.

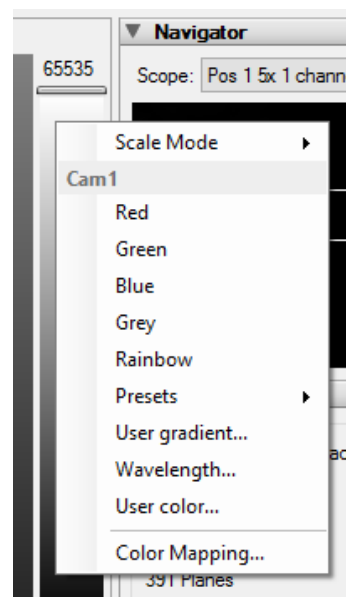
---

*Note: Global auto color range is slower, and more so if the stack is larger, but generally performs better. In either case, the calculation may not fit your specific data set and further adjustments may be necessary.*

---

### 3.2.5. The Color Bar

The color bar scale is the most accessible and often most useful tool to adjust intensity rendering in images and is shown automatically on the right of the **Viewing Area**.



It can be shown or hidden by selecting the **Show Color Bar** option from the **View** menu or by using the **Toggle Colour Bar** button from the **Shortcut toolbar**.

1. To adjust the high or low saturation points (black and white levels), click and drag the **sliders** at either end of the scale.

- To adjust the colour tint, right click on the colour scale and select the preferred colour from the available pre-sets.

*The pre-set colour ranges included cover the three primary colours of Red, Green and Blue, along with the back to white greyscale and rainbow colouring.*

Where possible, arivis Vision4D will use the available image metadata to automatically display the images in the colour corresponding to what was set in the acquisition software.

If unavailable or incorrect, users can also choose to manually select the tint colour to match the wavelength by selecting the Wavelength pre-set and entering the wavelength of the selected channel.

arivis Vision4D will automatically adjust the colour bar to the images' data range (from 8 to 32bit).

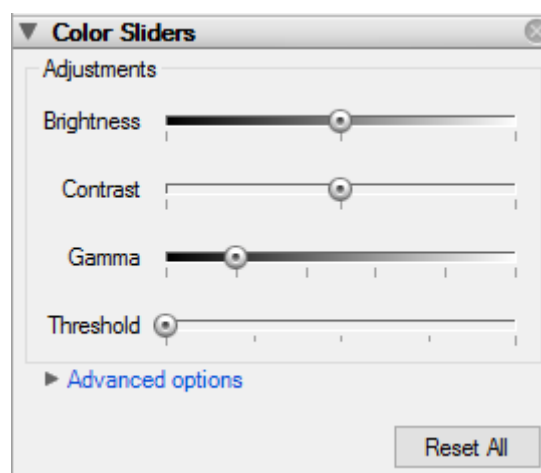
For images taken with a 10, 12, or 14bit camera, you can select these [bit depth](#) automatically from the Scale Mode sub menu.

### 3.2.6. Color Sliders Palette

While display range adjustments are often all that is required to enhance an image for visualisation, other tools can also be useful.

The Color Sliders palette offers additional tools for brightness, contrast and gamma adjustments.

To activate the Color Sliders palette, right-click on empty palette space and select it from the contextual menu or select it from the **Window > Palettes** menu.



1. As with other slider based tools, simply click and drag each slider to make changes. Changes are applied live on the image.
2. Click on the **Advanced Options** dropdown for the ability to adjust each channel individually if needed and to apply changes to the current plane or complete stack.

---

*Note that these settings affect the rendering of the images in all the different viewer types , so changes made in the 2D Viewer will also appear in the 4D Viewer and vice versa.*

---

Please refer to the help files for a more complete breakdown of the color slider palette functionality.

---

*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

The 4D Viewer also has rendering settings that pertain specifically to this type of rendering.

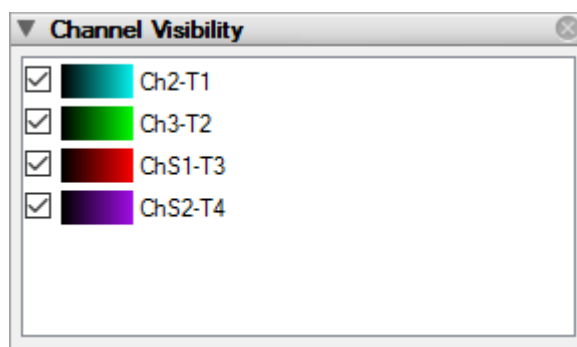
---

*For more info, see below in the 4D Viewer section*

---

### 3.2.7. Channel Visibility

While showing multiple image [channels](#) overlaid on top of each other is a useful tool, sometimes visualisation and analysis is best done with fewer or just one channel displayed.



- To show or hide individual imaging channels, use the Channel Visibility palette.
- To show or hide a channel simply check or uncheck the check box next to the channel you want to affect.

---

*Note that most of these adjustments affect the rendering of the image in other viewing modes such as the 4D Viewer and Gallery view but are specific to one viewer only. With multiple viewer opened, adjusting color range and visibility will affect that window only.*

---

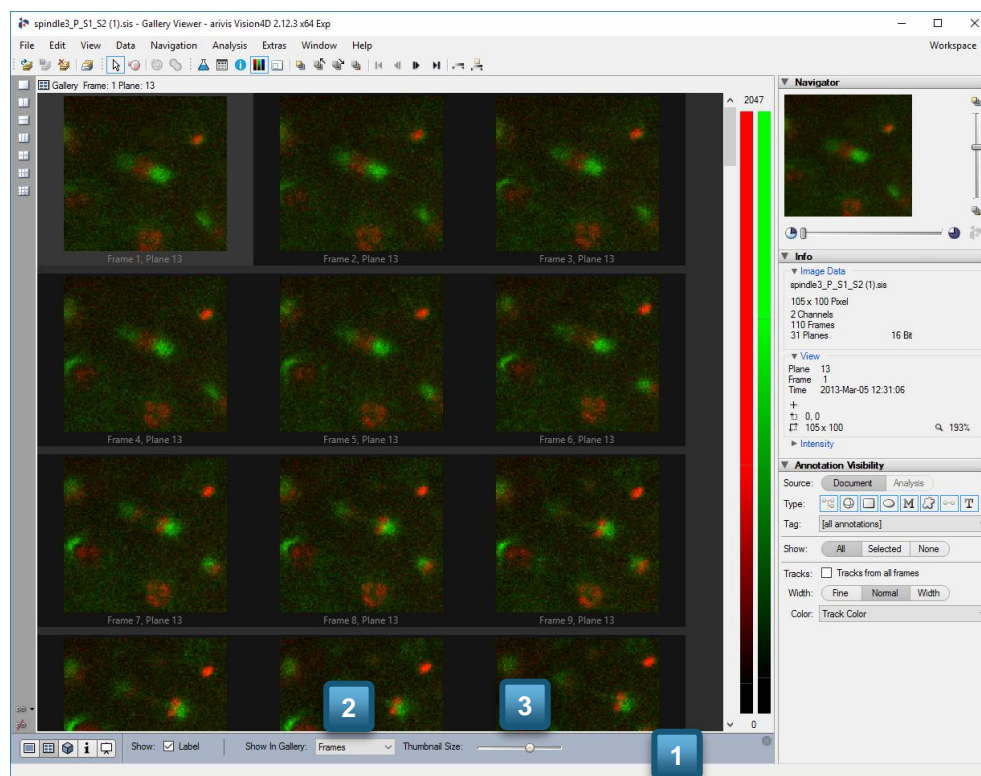


### 3.3. Gallery view

The Gallery view allows you to display all images in a stack side by side. In conjunction with the split view, it can be a useful tool for navigating through a set. To activate the Gallery view, simply click its icon in the Viewing Area toolbar.



Once activated, the Viewing Area should look like this:



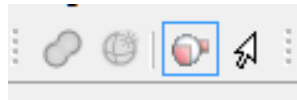
The **Viewing Area toolbar** will automatically update to show you tools and options relevant to the Gallery view. (1) Here you can choose whether each panel in the gallery corresponds to a plane at the current frame or a frame at the current plane. You can also select to view every frame and plane in a multidimensional stack.

1. Simply click on the **Show in Gallery** drop list box and select your preferred viewing option. (2)

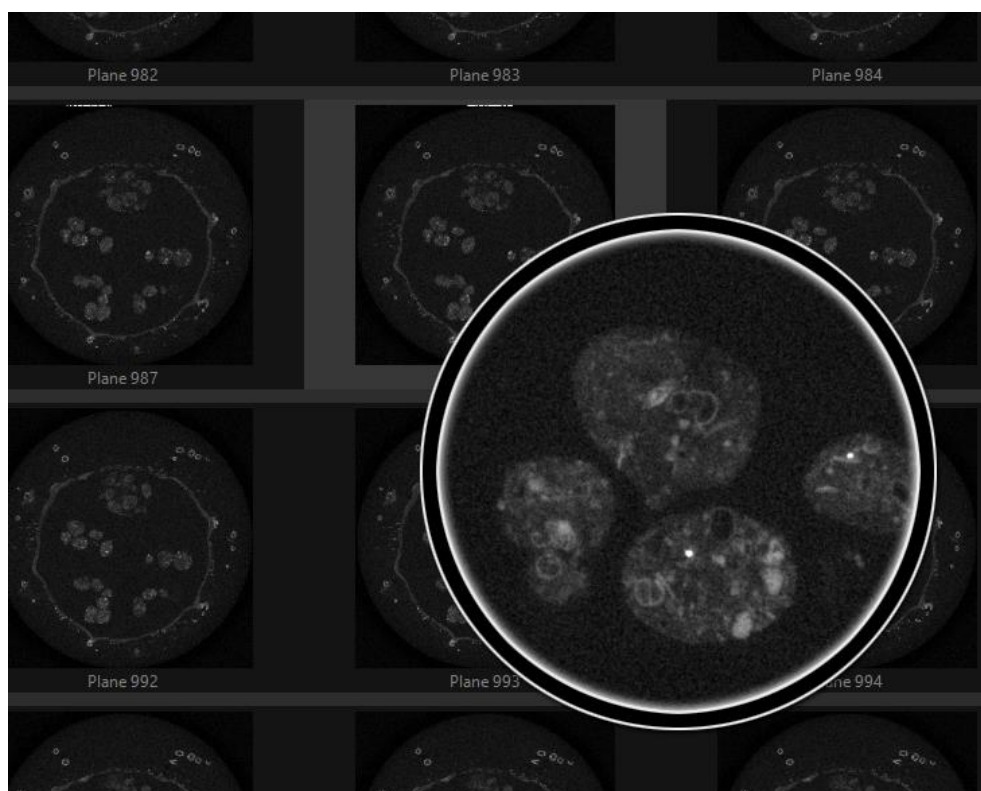
2. Use the **Thumbnail Size** slider to change the thumbnail magnification. (3)
3. To activate a frame or plane, simply click on it in the viewer. If using this with [linked viewers](#) in the split view, this plane will also be activated in any linked viewer.

### 3.3.1. Loupe tool

You can use the **Loupe tool** to see a portion of the thumbnail at full magnification.



- To activate the loupe tool, click on the Loupe icon in the **shortcut toolbar**.



Then, drag your cursor over the panel you want to examine to see a 1:1 zoom of the section of the image under your cursor.

You may use the mouse scroll wheel to zoom the loupe view.

### 3.4. 4D Viewer

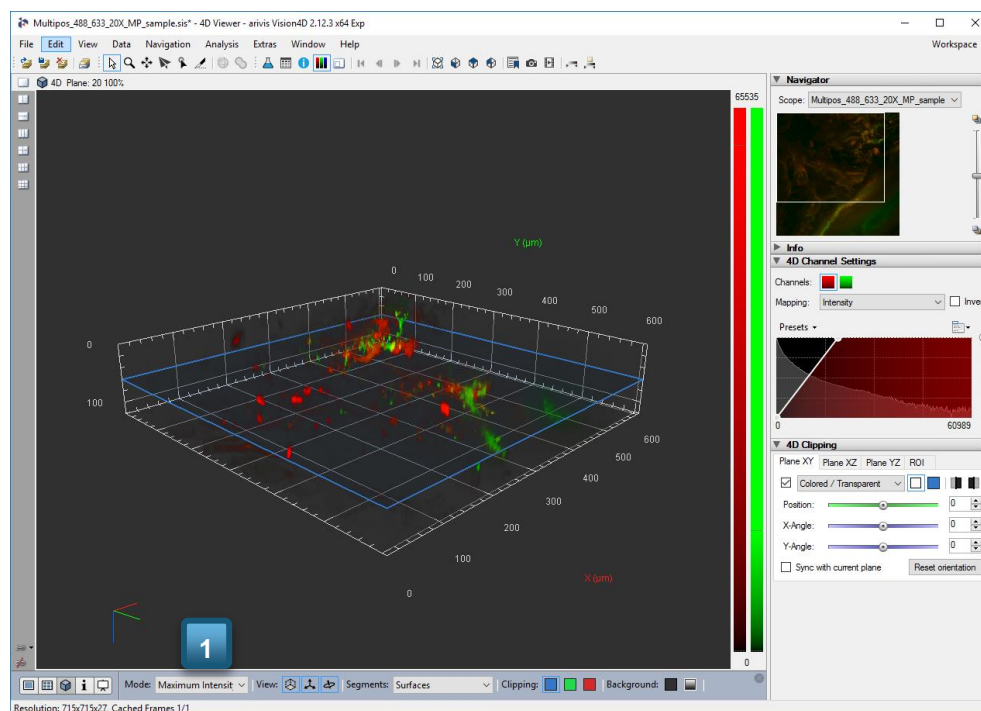
The 4D Viewer is an interactive environment for getting visual insights into 3D data sets.

It is called the 4D Viewer because it also allows the visualisation of time dependent data in 3D. Because the visualisation perspectives are essentially infinite, the 4D Viewer offer several tools to manipulate 3D sets.

To activate the 4D Viewer, simply click its icon in the Viewer Types Pane or select it from the View menu options.



Once activated, the viewing area should look something like this:



#### 3.4.1. Volumetric/Max intensity rendering modes

The 4D Viewer has two main rendering modes available, the so called **Maximum Intensity** and **Volumetric** options.

It is also possible to hide the volume data to only show elements such as annotations and clipping planes. The rendering mode can be selected from the **Viewing Settings Toolbar (1)**

- The **Maximum Intensity** rendering mode works by displaying the brightest pixel in the axis of observation of the 3D volume.
- The **Volumetric** rendering mode integrates the color and transparency of the visible data. Using a virtual light source, volumetric rendering provides a shaded and semi-transparent data representation.

Because each rendering mode has a significant impact on the visibility of the intensity information in the image, each is more suited to certain types of visualisation requirements.

---

*The **Maximum Intensity** mode is better suited for the observation of the inner structures of objects (e.g. cells in tissue or organelles in cells), while the **volumetric** rendering is somewhat better suited to the visualisation of surfaces (e.g. organs).*

---

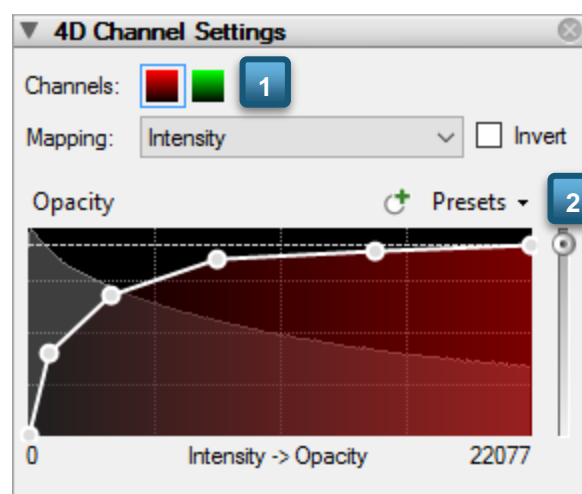
However, the rendering mode is usually a matter of personal preference. In either case, further adjustment of the rendering parameters may be useful or necessary.

### 3.4.2. Adjusting render setting

As seen in the **2D Viewer**, [adjusting LUT settings](#) can have a big effect on the appearance of the image in the viewer.

In the 4D Viewer, additional settings are available that are specific to this rendering mode to further allow you to tune the visualisation to best match your needs.

The **4D Channel Settings** palette opens automatically with the switch to the **4D Viewer**, but can be opened like any other palettes if it has been closed.



1. In the 4D Channel Settings palette, first select which [channel](#) to adjust. By default the mapping is based on the pixel intensities, but it can be changed to map the 3D dimension axis and can also be inverted. In most cases, Intensity mapping is the preferred option. (1)
2. Below the mapping options you'll find the **opacity curve**. The opacity curve is used to map the opacity (Y axis) against the intensity (X axis) of the pixels. (2)

---

*By default, the curve is set match the rendering mode. For Volumetric renderings it is a straight line, whereas for Maximum Intensity Projection rendering it is set to a curve.*

---

- The **opacity curve** can also be manually adjusted by
  - Clicking and dragging on the line anchor points (grey circles)
- **Additional anchor points** can be added to the opacity curve for more flexibility by

- Holding down the Shift key and clicking away from existing anchor points
- **Extraneous anchor** points can be removed by
  - Holding down the Shift key and clicking on the point to be removed.
- The entire curve can be shifted left or right by clicking and dragging in the curve window outside of existing anchor points.
- The opacity of the entire volume can be adjusted by using the slider to the right of the curve window.

For more details on the 4D Channel Setting palette, please consult the help files.

---







*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

### 3.4.3. Volume manipulation tools

The 4D Viewer makes use of a variety of tools to allow the user to interact with the volume rendering, such as zooming, panning, flying in and out of the object, etc.

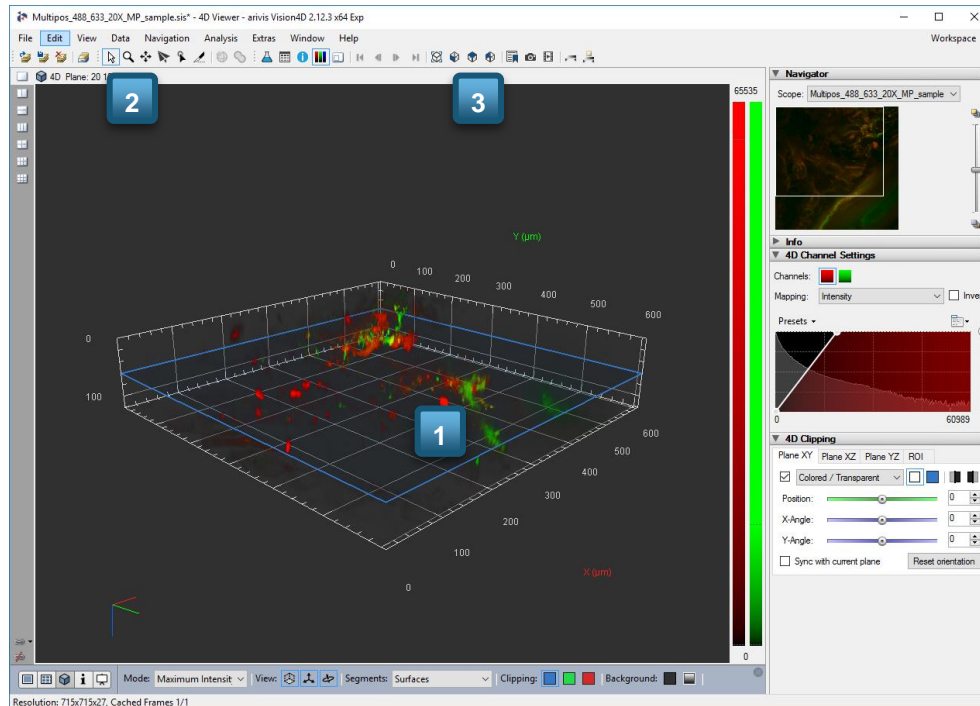
The main 4D Viewer navigation tools are:

- |   |             |  |
|---|-------------|--|
|    | Rotate      | The Rotation Tool is the default navigation tool of the 4D Viewer. Its primary mode is rotating the dataset by moving the mouse while holding down the left mouse button.  |
|    | Zoom        | The primary mode of the tool is zooming in and out of the dataset by moving the mouse up and down, or left and right while holding down the left mouse button.   |
|   | Panning     | The primary mode of the tool is moving the dataset in a lateral fashion by moving the mouse while holding down the left mouse button.  |
|  | Fly-Through | Used in conjunction with the keyboard, this tool allows the user to virtually fly through the object in any direction and orientation.   |
|  | Selection   | The purpose of the Selection Tool is two-fold: <ul style="list-style-type: none"> <li>• Left-clicking on Annotations or Geometry Objects allows you to select these objects. Right-clicking anywhere within the viewer will deselect all objects.</li> <li>• Holding down the Left Mouse Button or Right Mouse Button while pointing at a Clipping Plane allows you to rotate and move that plane respectively.</li> </ul> |
|  | Split tool  | This tool helps you split annotations that should be two instead of one.   |



### 3.4.4. Rotating, zooming and panning

As soon as you switch to the 4D Viewer the images will be rendered in three dimensions and you can immediately start interacting with the image.







1. Click and drag on the image in the **Viewer** to change the orientation. Dragging the set up and down rotates the object along the Y axis (horizontal rotation), while dragging left or right rotates the object along the X axis (vertical rotation). (1)
  - To zoom in or out, use the scroll wheel on your mouse or scroll action on your trackpad.
  - Press the **Shift** key on your keyboard while zooming or changing the orientation for more delicate manipulation.
2. Switch to the **Panning** tool to drag the 3D view across the screen without rotating. The **Panning** tool can be selected from the **Shortcut toolbar**. (2)
  - As with the zooming and rotation, use the **Shift** key while dragging for fine adjustments.

### 3.4.4. Jump to orientation

Because of the almost infinite degree of variation in orientation and zoom level, the 4D Viewer has several pre-set orientations to choose from in the **Shortcut toolbar**. (3)



These buttons allow you to quickly switch to:

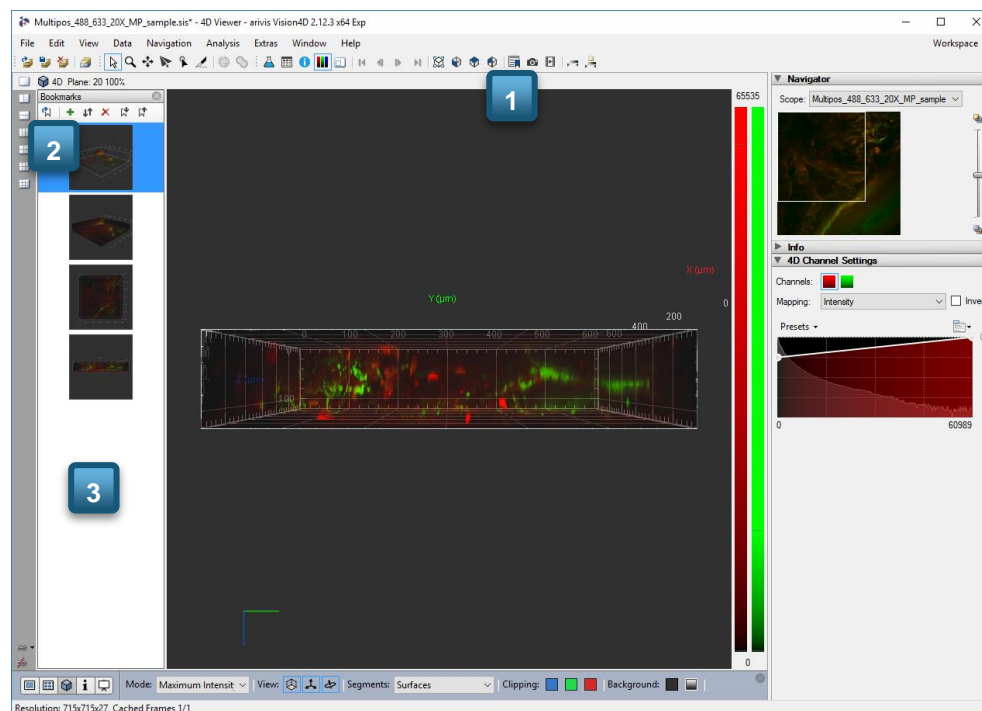
-  **Home View** – the default orientation and zoom when images are first rendered in the 4D Viewer.
-  **Front View** – looking at the object along the Y axis
-  **Top View** – looking at the object along the Z axis
-  **Left View** – looking at the object along the X axis


From these pre-set views, users can proceed to change the orientation as usual

### 3.4.5. Bookmarks

Additionally, arivis Vision4D allows the saving and restoring of [Bookmarks](#). Bookmarks help you to record interesting views of your data and to save or share them as needed. They capture the current scene settings (e.g. render mode and view properties) and store them for later use.

Bookmarks are stored in the metadata of the document and can be shared by exporting them to a proprietary file format (\*.bookmarks) for use with other sets if needed.



To open the **Bookmarks** palette, click on the bookmark icon  in the **Shortcut toolbar** or select it from the **View** menu. (1)







Once activated, the Bookmarks palette will appear on the left of the viewer.

1. Add bookmarks by clicking on the green + icon in the bookmarks toolbar. (2)
2. Each new bookmark will appear within the Bookmarks palette space. (3)

### 3.4.6. Bookmarks Palette

The bookmarks palette toolbar offer the following functions:




-  Restores the bookmark that is currently selected in the list below. Alternatively, you can double-click on any given bookmark in the list to restore it.
-  Creates a new bookmark from the current view and scene settings.
-  Updates the selected bookmark with the current view and scene settings.
-  Removes the selected bookmark.
-  Allows you to import a .bookmark file, adding all bookmarks contained in the selected file to the bookmark list.
-  Exports the current list of bookmarks into a .bookmarks file.

### 3.4.7. Fly through mode

When trying to visualise the inner structure of objects, the region of interest may be obscured by the object envelope. One way to visualise these obscured structures is to virtually fly through the sample for a closer look at the structures of interest.

This tool is the most versatile navigation tool of the 4D Viewer, offering six degrees of freedom in motion using a set of keys as well as the mouse.

- To begin using the Fly-Through Tool, select its icon  from the Shortcut toolbar and click once in the centre of the viewer area using the left mouse button.
- The mouse is now 'caught' by the viewer and contrary to the other tools, it is not required to hold down the mouse button for interaction, simply click the mouse again to release it.
- Moving the mouse while it is caught allows you to look around.
- To move around your dataset you can use the arrows keys on your keyboard. Alternatively you can utilize the more advanced key setup which offers the full functionality of the Fly-Through Tool as below.
- Use the **Shift** key together with the movement keys for faster movements.
- Click the mouse again to release it

These are the keys to using the Fly-Through Tool depending on the Input Mapping.

For **QWERTZ** or **QWERTY** keyboards:

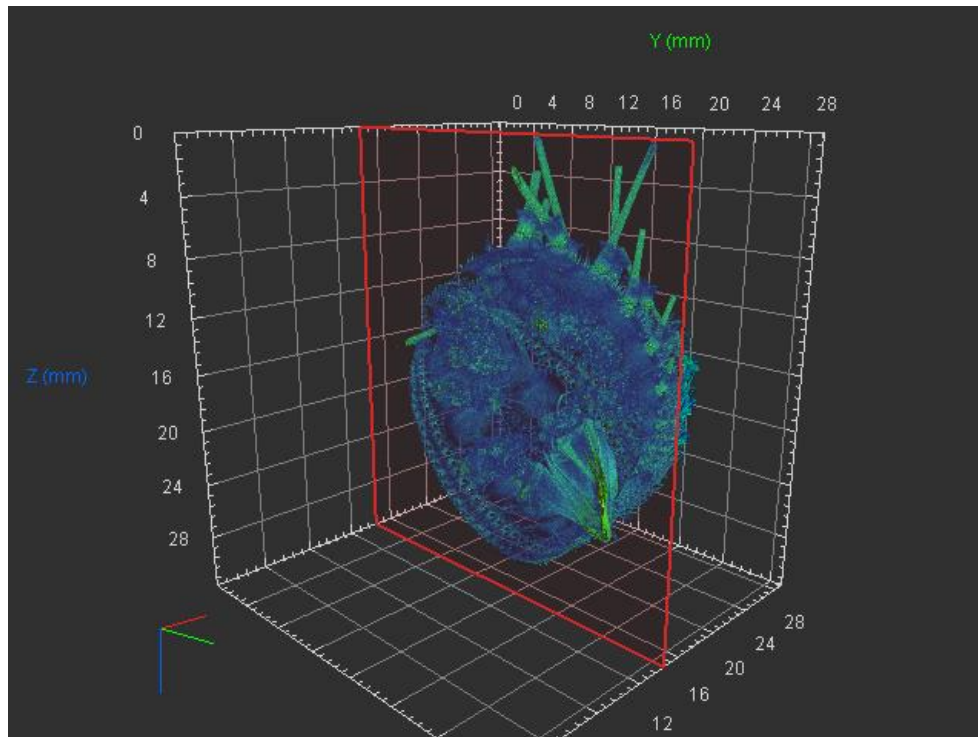
- By using the W, A, S, and D keys you can move directionally (similar to the arrow keys).
- The Space and C keys allow you to move up and down respectively.
- Use the Q and E keys for rolling around the view axis.

For **AZERTY** keyboards:

- By using the Z, Q, S, and D keys you can move directionally (similar to the arrow keys).
- The Space and C keys allow you to move up and down respectively.
- Use the A and E keys for rolling around the view axis.

### 3.4.8. Clipping planes

Clipping planes are a great tool to reveal the inner structures of objects. By hiding all the image information on one side of a clipping plane, it's possible to reveal objects that might otherwise be obscured by their surroundings, without losing the context that the surrounding provides.



Clipping planes can be added or removed with a single click from within the 4D Viewer by using the dedicated buttons in the **Viewer Settings** toolbar.

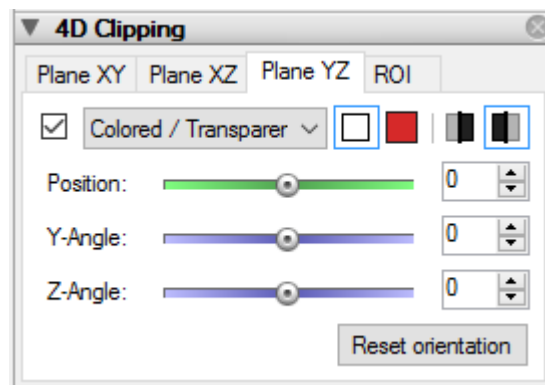


The angle of the clipping plane can then be adjusting by clicking and dragging on it using the **Selection** tool, or by clicking the **Alt** key while using the **Rotation** tool to temporarily switch to the **Selection** tool.

### 3.4.9. 4D Clipping palette

The 4D Clipping palette allows users to change each plane's visualisation options independently of the others and to adjust the clipping settings of ROIs as well.

As with other palettes, right-click in an empty palette space or select it from the **Window > Palette** menu options to activate it.



The following additional options are available for clipping planes in the 4D Clipping palette:

<p>Show/Hide Plane check box</p>	<p>Check this to show the clipping plane and un-check it to hide it. This corresponds to the toggles found in the <b>Viewer Settings toolbar</b></p>
<p>Visibility / Rendering</p>	<p>Changes how the plane handles the image data.</p> <p><b>Invisible:</b> show no information about the voxels on the plane but are transparent to allow visualisation of everything behind it from the point of view of the observer.</p> <p><b>Textured / Opaque:</b> show the pixel intensity along the plane as measured in the image but does not allow the visualisation of anything directly behind the plane from the point of view of the observer.</p> <p><b>Binary / Opaque:</b> as with the textured/opaque plane, but pixel intensities on the plane are shown in binary (either black or white).</p>

	<p><b>Textured / Transparent:</b> Pixel intensities are rendered with the same opacity settings as the 3D volume.</p> <p><b>Coloured / Transparent:</b> as with the Textured settings, but the plane itself is tinted according to the plane outline colour.</p>
Show/Hide Outline check box	Click to show or hide the edge outline for the current plane
Plane Colour	Changes the colour of the outline, and plane tint when using the Coloured / Transparent rendering.
Clipping direction	Shows or hides volume data either side of the plane front and back faces.
Position/Angle sliders	Adjusts the position and angle of the plane relative to the plane's axis.
Reset Orientation	Resets all options relating to the current plane to factory defaults.



### 3.4.10. Clipping regions

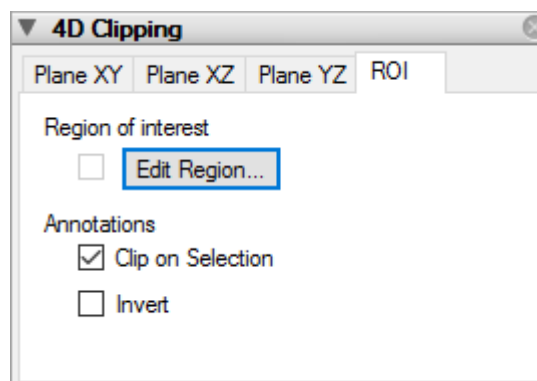
The Clipping palette also offers the possibility of clipping around regions of interest, which can be useful for analysis or to load a higher resolution rendering for a smaller portion of a set.

Clipping regions can be based on a pre-existing 3D segment, or can be manually selected from within the clipping palette.

To clip around a region, first open the 4D Clipping palette and go to the ROI tab.

#### 3.4.10.1. Clipping from Annotations

- To clip from an existing annotation, select it in the 4D Viewer or annotations window and check the option to **Clip on Selection**.




---

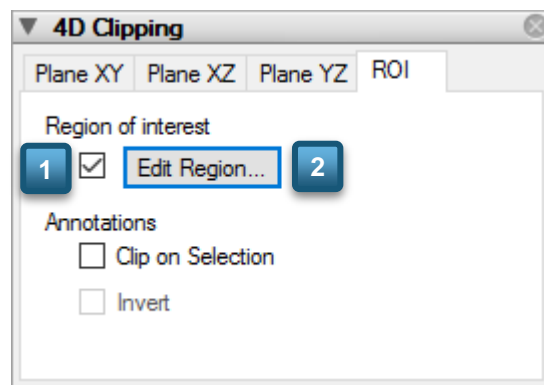
*You can also clip hide everything within the region by using the Invert option.*

---

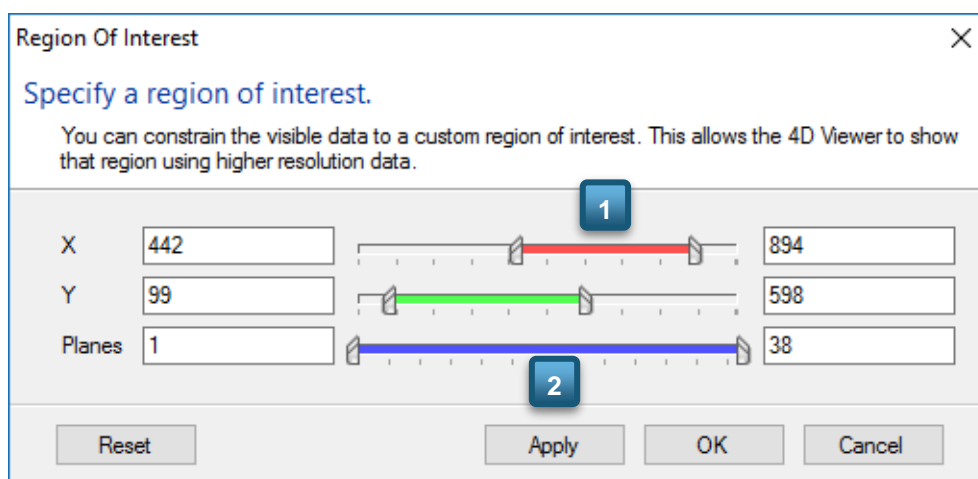
*Note that you may also need to hide the annotation to show the result either by removing the display or annotations or by switching annotation display to bounding box only.*

---

### 3.4.10.2. Clipping from a region of interest



1. To clip to a region, check the Region of Interest option. (1)
2. Click on Edit Region to open the region of interest selection window. (2)



1. Then, either type in the start and end coordinate for the region in each axis or adjust the sliders to get a visual feedback in the viewer of the current selection dimension. (1)
2. Once happy with the selection, click the **Apply** button then click OK to close the window. (2)

---

*Note that when using the clipping the software will automatically recalculate the rendering resolution for the selected sub-volume based 4D Viewer settings preferences.*

---

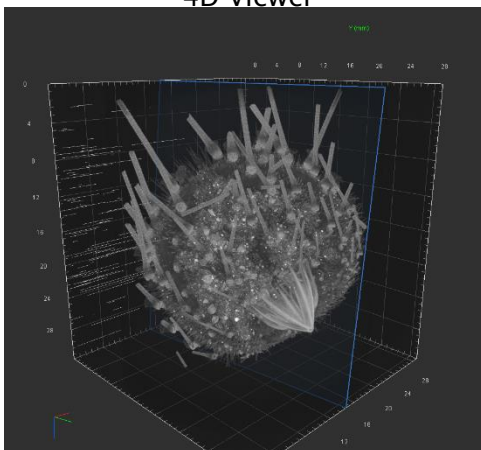
### 3.5. Projection Viewer

The **Projection viewer** allows users to see information from a 3D model in 2 dimensional flat section by compressing or sub sampling the data. It can also be used to reorient a set based on the rotation of clipping planes.

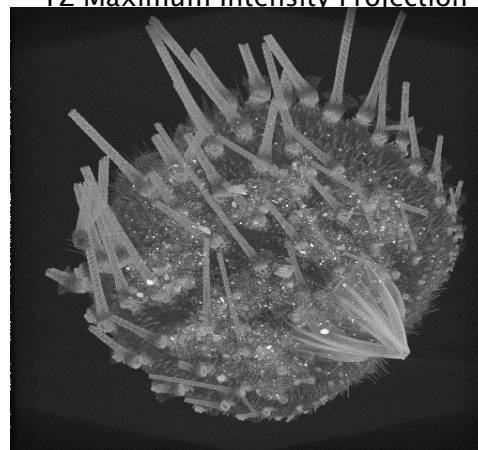
The Projection viewer has three modes:

- Projection – Compresses the data from multiple planes into a single plane
- Orthoslicer – Renders a single slice through the dataset along one of the three main axes
- Sectional plane – Renders a single slice through the dataset according to clipping planes or through manual definition of orientation angles

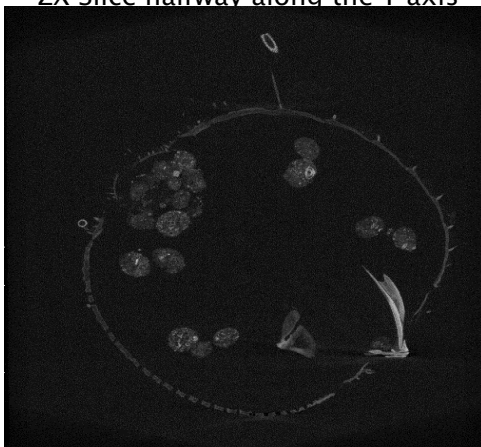
4D Viewer



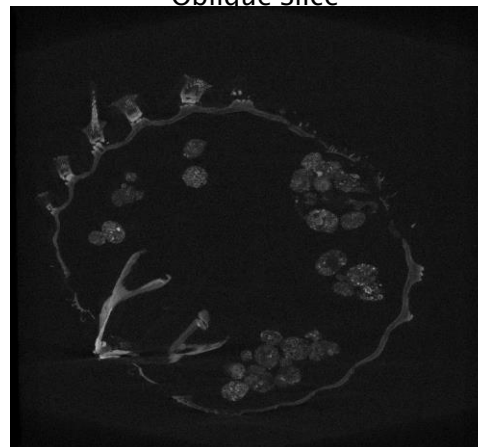
YZ Maximum Intensity Projection



ZX Slice halfway along the Y axis



Oblique Slice

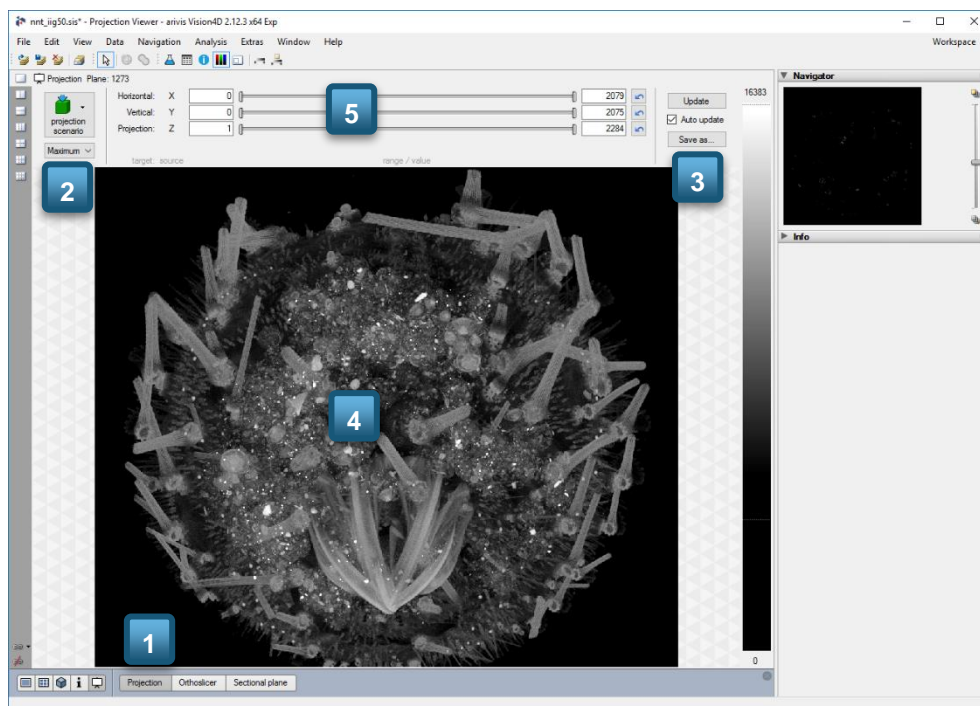


The images above show the same sample using the 4D Viewer on the left an XY projection over Z on the right, a ZX slice along the Y axis and an obliquely oriented sectional plane.

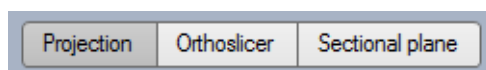
To activate the **Projection Viewer**, simply click its icon in the **Viewer Types** pane or select it from the **View** menu options.



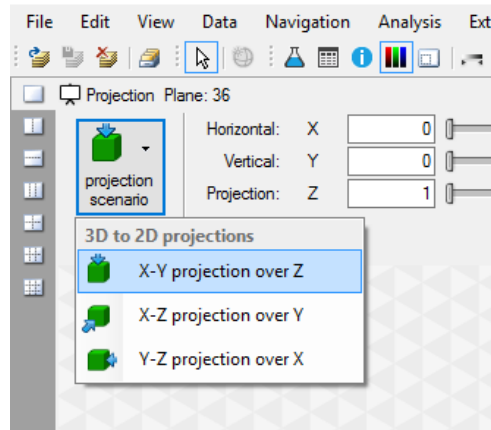
### 3.5.1. Projection



1. To create a projection, first select the **Projection** option from the **Viewer Settings Toolbar**. (1)



- Then, select the axis of projection from the **Projection scenario** dropdown, and the projection mode (Maximum/Minimum). (2)



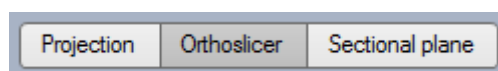
*The projection mode defines how the data is compressed along the projection axis. Projection can be done by either displaying the brightest pixel along the projection axis (maximum intensity) or the darkest (minimum intensity). Different projection modes are more suited to different types of datasets. For example, fluorescence images are typically best displayed using a maximum intensity projection.*

- Click the **Update** button to render using the current settings into the viewing area. (3), (4)
- To crop the projection to a smaller proportion of the stack, use the **sliders** to adjust the bounds in each dimension as needed. (5)
- Check the **Auto-Update** box to see the changes applied as soon as you let go of each slider. (2)

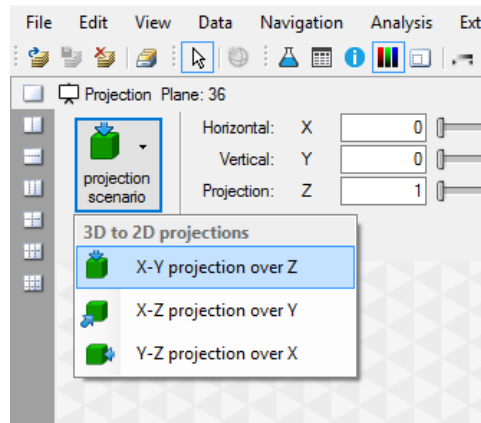
*Turn off the auto update if the projection calculation takes too long for very large stacks or regions.*

### 3.5.2. Ortho Slicer

- To create an orthogonal slice projection, first select the **Orthoslicer** option from the **Viewer Settings** toolbar.



1. Select the axis of projection from the **Projection scenario** dropdown, then click the **Update** button to render.



2. Use the projection **sliders** to select the slice to display and the brackets on the Horizontal and Vertical axes to crop to a smaller section of the image.
3. Check the **Auto-Update** box to see the changes applied as soon as you let go of each slider.

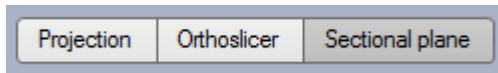
---

*As with the Projection mode, avoid using this option for very large stacks as the rendering could take a long time.*

---

### 3.5.3. Sectional Plane

1. To create a sectional plane projection, first select the **Sectional plane** option from the **Viewer Settings toolbar**.



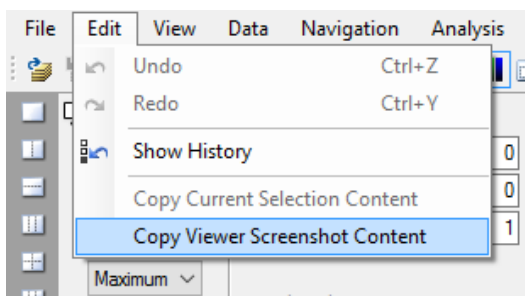
2. Then, select from the available sectional planes already defined within the 4D Viewer, or select the manual option to manually define the position and orientation of the sectional plane.

### 3.5.4. Extracting projections

In any projection mode, you can use the **Save As ...** button (below the **Update** button), to save the resulting 2D plane as a new SIS file or a new [scope](#) within the current file.

When used in conjunction with sectional planes the **Save** function can also be used to reorient the set by saving a complete stack rotated according to the sectional plane.

You can also use create a snapshot of the current view by going to the **Edit** menu and selecting the option to **Copy Viewer Screenshot content**.



This will copy the current rendering to the clipboard and allow you to paste it into another application (e.g. word processing, presentation).

---

*Note that the option to copy viewer screenshot content is available in all rendering modes.*

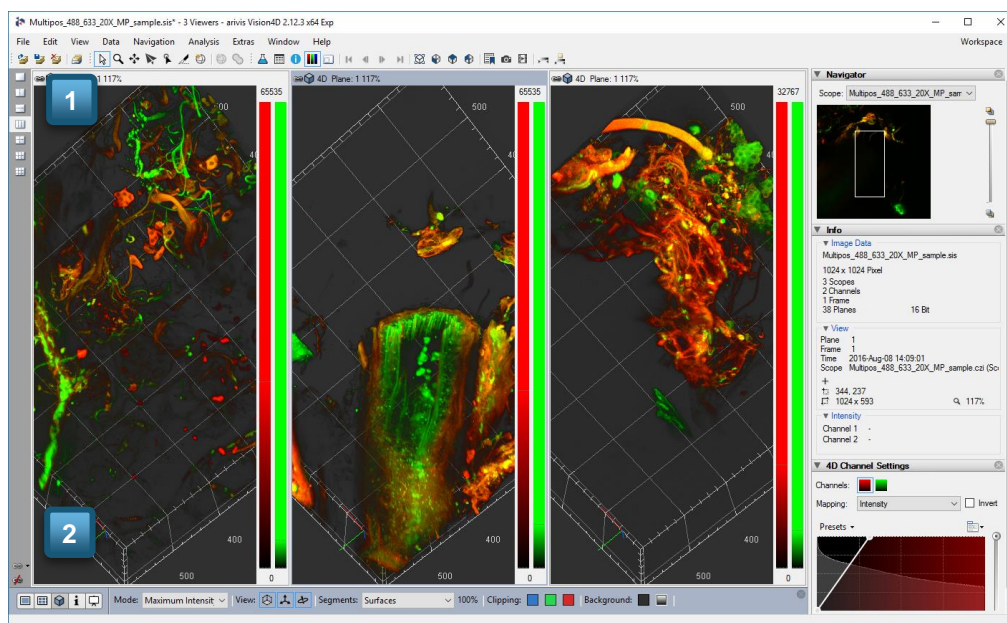
---

### 3.6. Split view

The split view allows users to visualise multiple images simultaneously, whether these are different renders of the same image or different scopes from the same set.

*Note that the split view can only work with different scopes or image regions of the same document opened with the same Viewer window.*

Visualising images from different documents will require that they be imported into the same SIS file first.










#### 3.6.1. Changing the number of viewers in the Viewing Area

1. To change the numbers of viewers in the Viewing Area, click on the split view button corresponding to the number and layout of viewers you want. (1)



Users can switch back and forth between any viewer configurations at will.

-  Single viewer
-  Two viewers, split left/right
-  Two viewers, split top/bottom
-  Three viewers side by side
-  Four viewers rectangular grid (2x2)
-  Six viewers rectangular grid (3x2)
-  Nine viewer rectangular grid (3x3)

Once you have multiple viewers displayed you can set the view for each individually.



Simply click within on the viewer's title bar or body to activate it then change any of the parameters as required (change view style, scope etc.).

### 3.6.2. Linking viewers

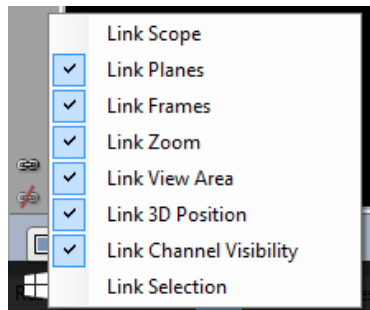
Linking viewers is a great way to compare between scopes within a file or between rendering modes for a single scope.

To ensure that the views match each other in terms of orientation and position, it is possible to link all the viewers together and arivis Vision4D will automatically adjust each viewer to match changes made by the user in any one viewer.

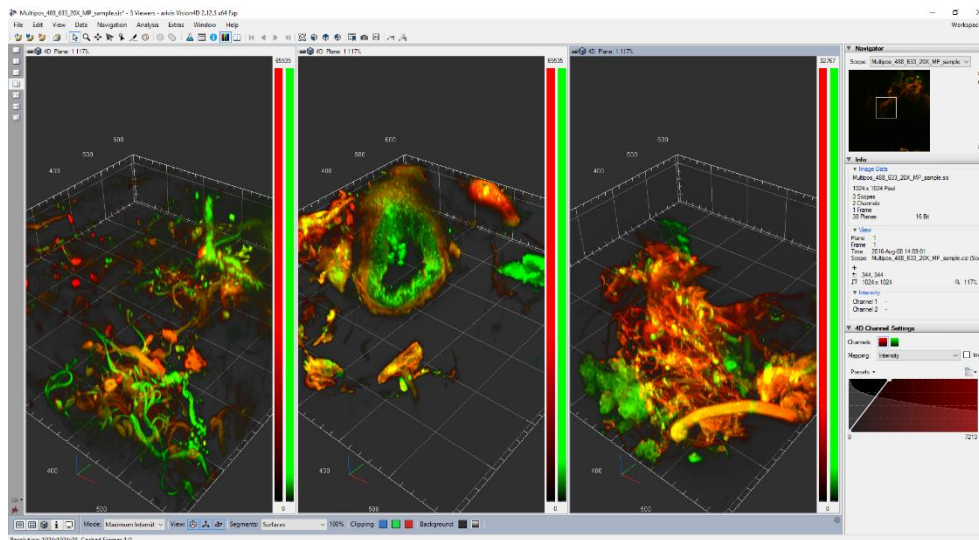
- To enable linking of viewers, click the chain-link icon at the bottom left of the Viewing Area. (2)
- To disable linking, click on the crossed out chain-link icon in the bottom left of the Viewing Area.

-  Link subviewers
-  Unlink subviewers

Clicking on the **drop-down arrow** to the right of the linking button gives additional options regarding what aspects of the subviewers should be synchronised.



The example below shows three different scopes from the same SIS file, each in the 4D Viewer with linked 3D positions.



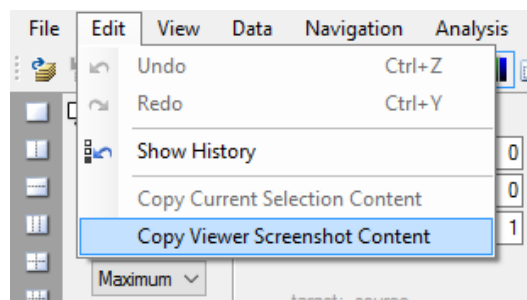
### 3.7. Sharing images

arivis Vision4D provides a range of tools to share the visual insights that can be gained from manipulating the data outside of the software by enabling the export of high resolution animation movies and snapshots.

Animations can render changes in viewing angle, zoom level, position and visibility of clipping planes and various rendering options (e.g. transparency, black/white levels).

#### 3.7.1. Sharing viewer content

In any most viewer types (2D/4D/Projection), you can create a snapshot of the current view by going to the **Edit** menu and selecting the option to **Copy Viewer Screenshot content**. This will copy the current rendering to the clipboard and allow you to paste it into another application (e.g. word processing, presentation).



This tool is effectively a screen copy that uses the current screen display mode and resolution and can only be used for a single view and time point.


*For more flexibility and higher resolution options, please see the snapshots and movie export options below.*

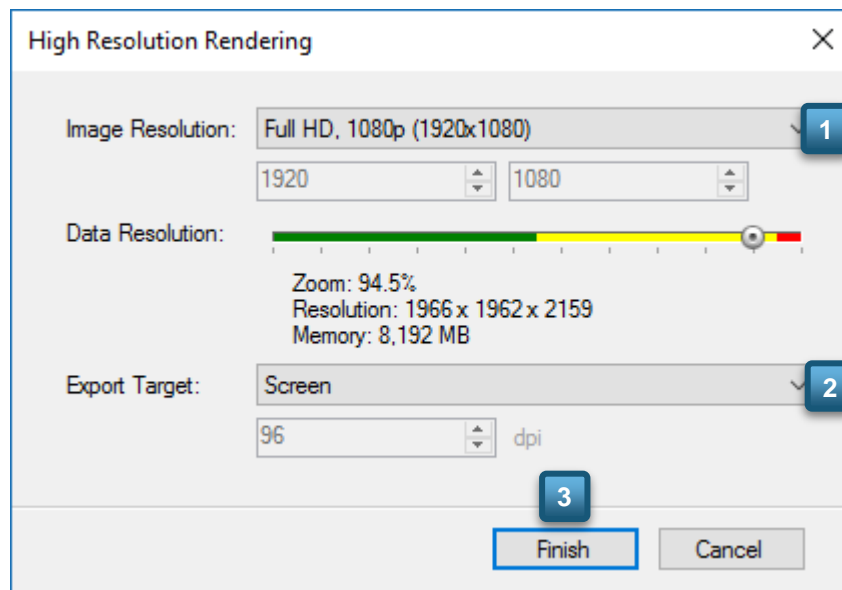
#### 3.7.2. Sharing High Resolution Snapshots

To export a single point of view with a specific rendering, including decorations (annotations, clipping planes), you can use the **High Resolution** dialog.

Whereas the arivis Vision4D rendering engine may load lower resolutions to enable the software to render 3D objects quickly enough to be interactive, the snapshot tool can load as much image information as can be displayed at the user's selected output resolution, resulting in much higher resolution, publication quality images. Because the snapshot tool loads a lot

more information, the process of rendering these high resolution images is comparatively much more time consuming.

- To create a high resolution snapshot, go to the **View** menu and select the **High Resolution Image** option, or click on the Camera icon  in the shortcut toolbar, this will open the **High Resolution Rendering** window.



1. Choose an image and data resolution (1)

---

*The **image resolution** defines the **size** of the output image.*

*The **data resolution** slider affects the **quality** of the rendering.*

*Data resolutions within the **green** range of the sliders can be loaded into the video memory for **very fast rendering**.*

*Data resolutions within the **yellow** segment will fit within the system memory for **fast rendering**.*

*Data resolutions in the **red** segment will not fit in either video or system memory requiring occasional reloads and causing **slow renderings**.*

---

2. Choose an export target. (2) The **Export Target** allows you to select the intended target for the rendering. You can choose between the predefined options **Screen (96 dpi)**, **Low Resolution Printer (150 dpi)** and **High Resolution Printer (600 dpi)** or specify a custom dpi value.

3. Click **Finish** to confirm your selections and the software will render the image as selected. You can then save the image if satisfied with the results. (3)

---

*Note that the higher the data resolution the longer the rendering takes.*


---

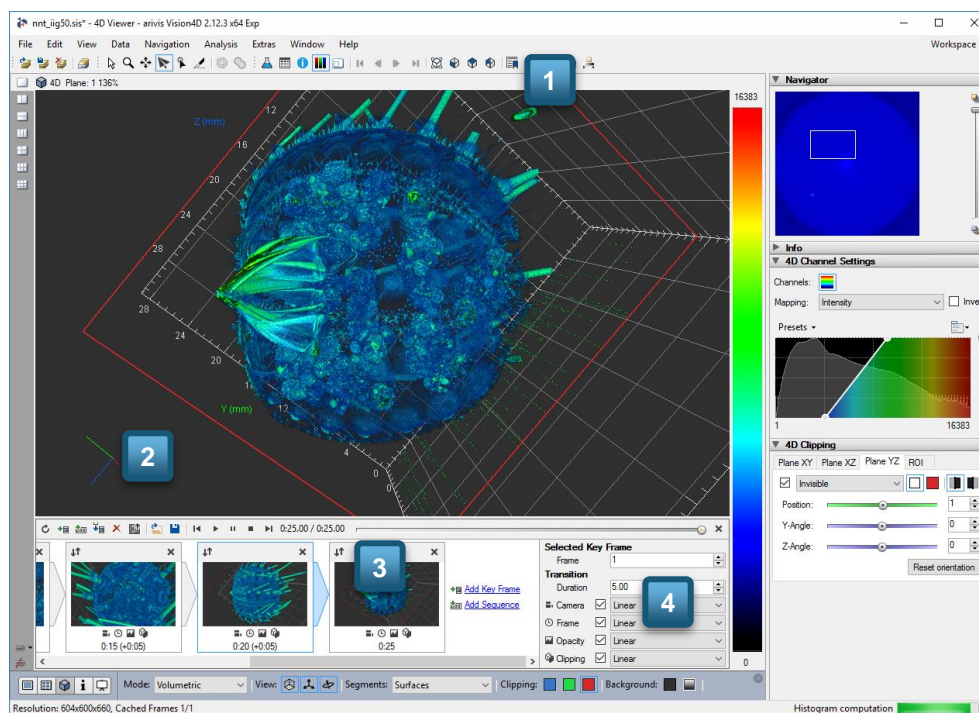
### 3.7.3. Sharing Movies - The Storyboard

The storyboard is an extension of the 4D Viewer through which users configure animations to be rendered as a movie for exports. It works through the concept of adding, removing and arranging “key frames” that are specific rendering of the 3D model, and then interpolates between those positions to create an animation.

Like a [Bookmark](#), a key frame contains the following properties from the current scene:

- Time point from dataset
- Camera position and rotation
- Channel visibility and opacity
- Clipping planes and their various properties

To open the storyboard, click on the Storyboard icon  in the **Shortcut toolbar**. (1)

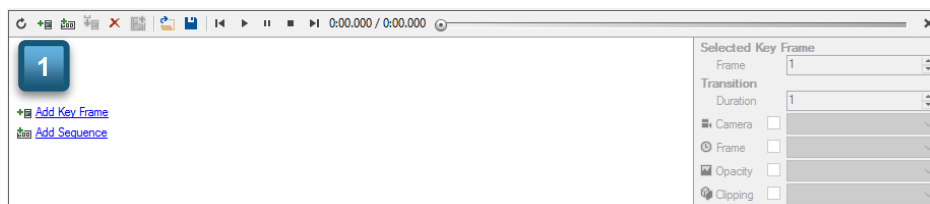


The Storyboard palette will then open at the bottom of the workspace and is initially empty.

Once the Storyboard is opened, simply add, remove or otherwise rearrange key frames to define the animation trajectory.

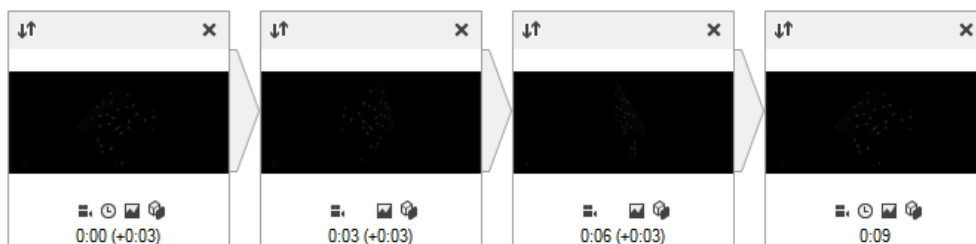
### 3.7.4. Key frames and sequences

1. To add key frame to the Storyboard, start in the **4D Viewer** by setting up the details of the orientation and view that you want to display in your animation sequence.
2. Then, click the **Add Key Frame** link in the Storyboard, or click the **Add Key Frame** icon from the **Storyboard toolbar**. (1)




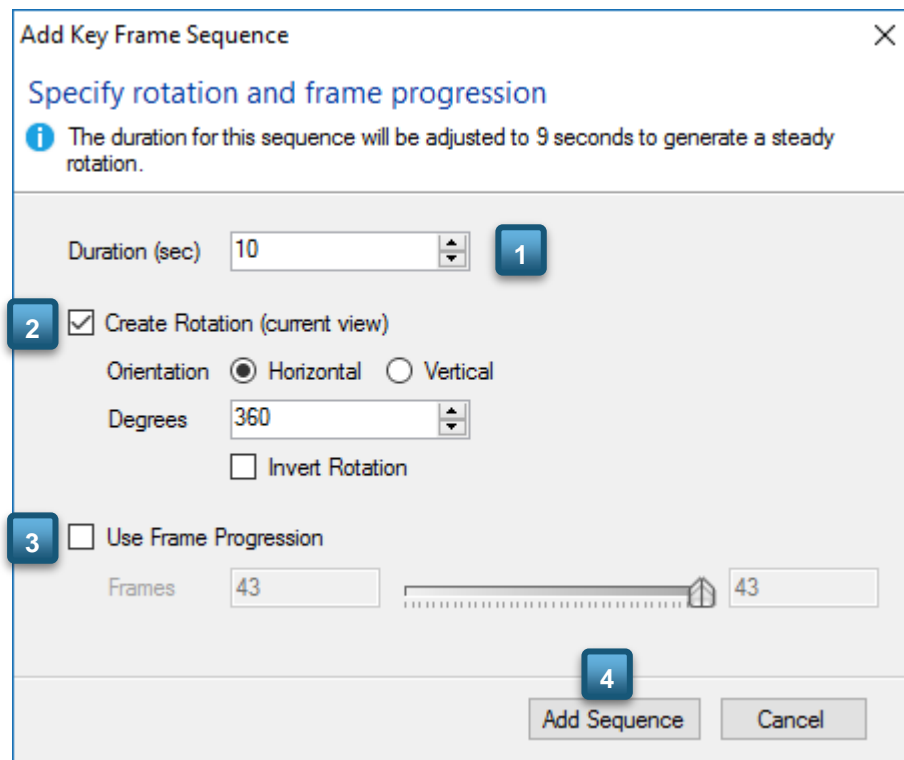
3. Modify the rendering of your 3D volume (orientation, zoom, mapping...) and add more key frames as needed. Each key frame will be added to the end of the existing key frames in the storyboard. Snapshots of the Key Frames renders will appear in the **Storyboard**. (3)

arivis Vision4D can automatically generate sequences of motion, like rotat-



ing the 3D model through 360 degrees.

- To add a sequence, click the Add Sequence link in the storyboard or the add sequence icon  in the storyboard toolbar. This will open the sequence definition window.



1. Select the sequence **Duration**; this is the time it will take in the rendered movie to progress through the sequence. (1)
2. If you want to rotate the 3D view, click to check the **Create Rotation** check box and define the rotation options. (2)
3. If you want to add frame progression (playback through time [frames](#)), also check the **Use Frame Progression** check box.
4. Use the sliders to select the first and last frame in the frame progression. (3)

---

*Note that the frame progression option is only available for scopes that have multiple frames.*


---

5. Click the **Add Sequence** button and the software will automatically create all the interstitial frames required to render the sequence as selected. (4)


---

*Note that for a rotation through 360 degrees, four key frames will be created.*

---

If a key frame has been added in error, or is no longer needed, you can delete them by clicking on the cross icon  on the top right of the key frame in the storyboard.



6. To modify a key frame, select it by double clicking on it in the storyboard, then adjust the rendering as needed in the viewer.
7. Finally, click on the replace key frame icon  on the top left of the key frame in the storyboard.
8. To change the order of the animation sequence, click and drag the **key frame** you want to move to the position you prefer within the **storyboard**.

---

*Every key frame (except the last one) will have a transition to the next key frame. You can specify how the individual key frame properties will be animated during the transition to the next key frame.*

---

9. To edit animations presets, adjust the options as required by selecting the **key frame** you want to modify and changing the parameters in the **key frame options**.(4)

---


*Please refer to the manual for the complete run down of the frame transition properties.*

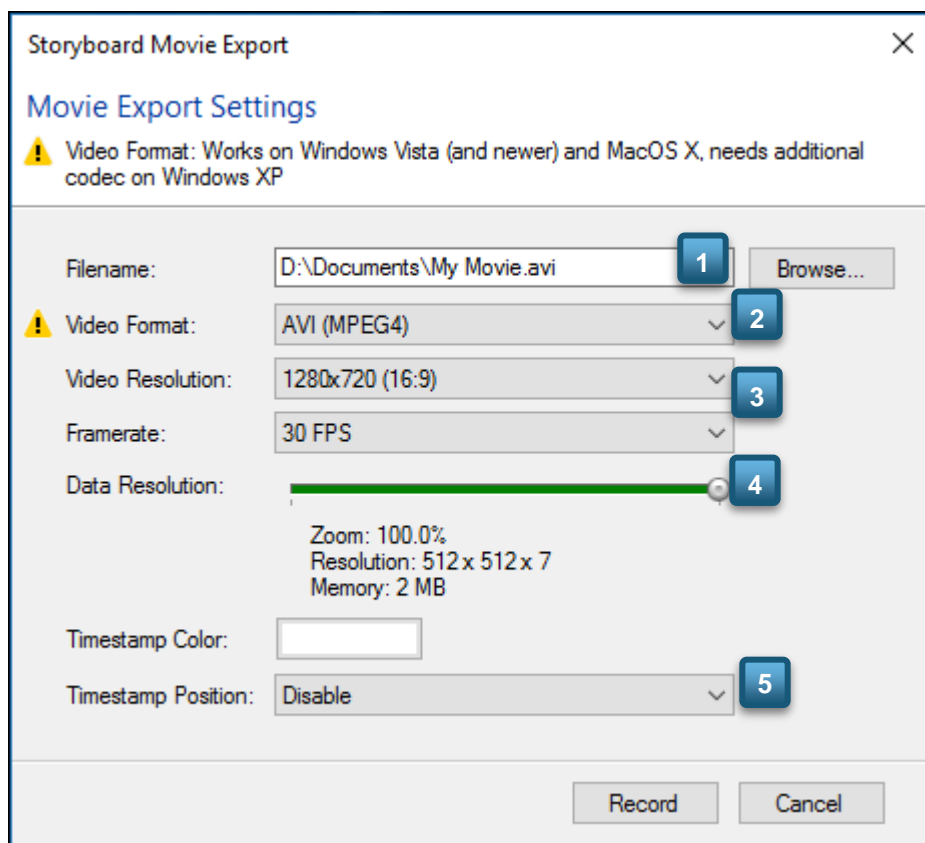
---

### 3.7.5. Exporting movies

Throughout the process of creating and editing your animation sequence, you can use the **playback buttons** at the top of the **storyboard** to review your work.

The software will execute a fast render of the key frames and transition to play through the sequence in “real time”. Once you are satisfied with your animation sequence, you can create a high resolution rendering of your movie as a movie file that can be imported in most media players and presentation software.

- To export your animation, click on the **Export movie** icon  in the **storyboard toolbar**, which will open the **export window**.



- Set up your export options as needed by choosing a name and export location, video format, resolution and frame rate, and the render quality.(1), (2), (3), (4)

---

*As with the high resolutions snapshot tool, the data resolution slider's color reflects the amount of memory required to render individual frames in the animation.*

*When the slider is **Green**, individual frames can be processed in the video memory for very fast rendering. When **Yellow** it will use system memory for fast rendering (though slower than when using video memory), and when **Red** will not fit in either causing occasional reloads and slow renderings.*

*Avoid unnecessarily high render quality settings to save time in the rendering process.*

---

2. If so desired, you can choose to display a time stamp in your exported movie file by selecting your preferred option in the **Timestamp Position** droplist box. (5)
3. When ready, click **Record** to create and save your movie to the previously selected location.

## 4. Image processing and analysis

Image processing and analysis allows the user to reveal the information contained within an image. This includes a range of tools and techniques to produce qualitative and quantitative data and generally falls under two categories with significant overlap: image enhancements and data analysis.

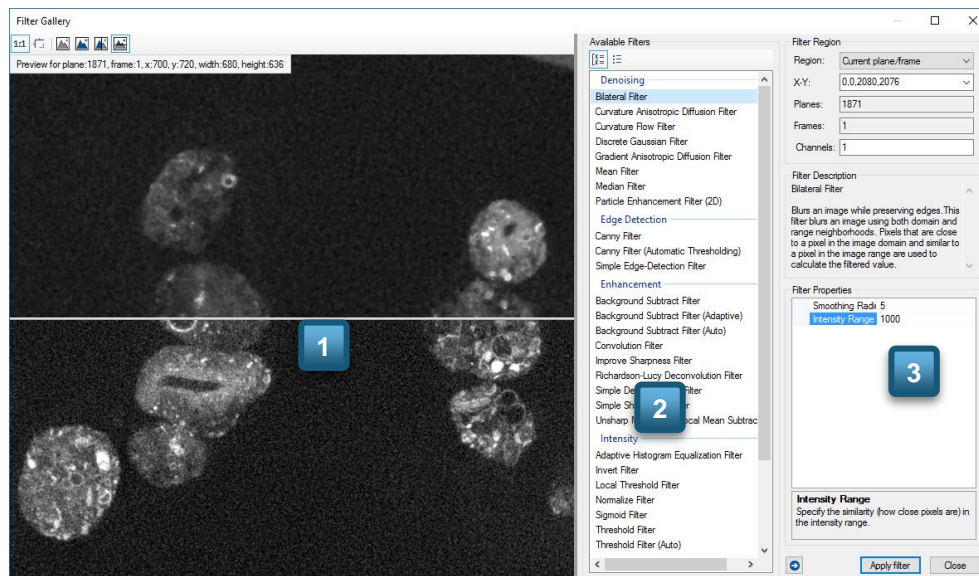
Image enhancement tools generally have two intended results, to make specific parts of the image information more easily visible and to facilitate the analysis.

arivis Vision4D offers a range of tools, some of which are optional, for image analysis and processing. Details of the use of these optional features are not covered in this guide.

## 4.1. The Filter Gallery

The Filter Gallery provides an interface for selecting, previewing and applying filters to an image.

- To open it, go to the Analysis menu and select the Filter Gallery–option.



The Filter gallery is divided into three sections:

- The Preview area** – where users can preview the effect of filters on the image. (1)
- The Available Filters list** – where users can choose what type of filter to apply to the image. (2)
- The Filter Parameters** – where users can adjust the region of the image to apply the filter to and the various parameters related to the chosen filter. The filter parameters area also includes a filter description for the selected filter, and an explanation of the effect of the selected filter parameter. (3)
- To preview a filter, first select it from the list of available filters. The available filters are sorted into the following categories:
  - Denoising** – these generally work by blurring small features. Some specifically avoid affecting edges while others use local variance to affect those pixels only within a certain threshold of the local intensities.
  - Edge Detection** – these detect gradients in the image and highlight their locations.

- **Enhancements** – these generally attempt to process the image so that the results look closer to the assumed real data assuming perfect conditions. For example, background correction filters estimate what the image would look like without a sample in the optical system to calculate what an image of the sample would look like with perfectly even illumination.
- **Intensity** – mostly affect pixels based on their intensity within the general context of the image rather than their immediate neighbourhood. For example, threshold filters analyse the image to identify a threshold to differentiate between what some consider objects and background.
- **Morphology** – Filters that look to enhance the image by using the shape of structures within the image.

Once you've selected a filter, its description will appear on the right, with the parameters available to tune its effects, and a preview will appear on the left.

The preview can be modified to show the results on the whole image zoomed to fit, or on the central region at 1:1 screen pixel to image pixel ratio.

The preview window can also be split left/right top/bottom with before/after renditions of the image.

---

*For a full run down of each filter, please refer to the help files.*

---

*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

## 5. Saving and exporting images

Once you have made changes to an image, whether these only affect the visualisation or include more significant changes like masking, annotation or filters, it is necessary to save your images to preserve your work.

---

*The import process automatically creates a SIS file, but the user must choose whether to keep or discard this file when closing. Note that if you do not save your file when closing, the entire import process will be necessary and all changes will be lost the next time you want to open these images.*

---

Several options are available depending on your future intended usage of the image data.

### 5.1. Saving images for use in arivis Vision4D

If the images are to be reopened within arivis Vision4D then images should always be saved in the native SIS format.

Files saved in this format will maintain all aspects of the visualisation (e.g. LUT adjustments, colour scales), any changes to the metadata (e.g. pixel size), but can also include analysis pipelines and annotations.

- To save an image in the SIS format, simply go to the **File menu** and select the **Save option** or press the Ctrl-S key combination on your keyboard.

---

*Note that the option to save images is only available if changes have been made to the image since it was opened or created and will be greyed out in the file menu otherwise.*

---

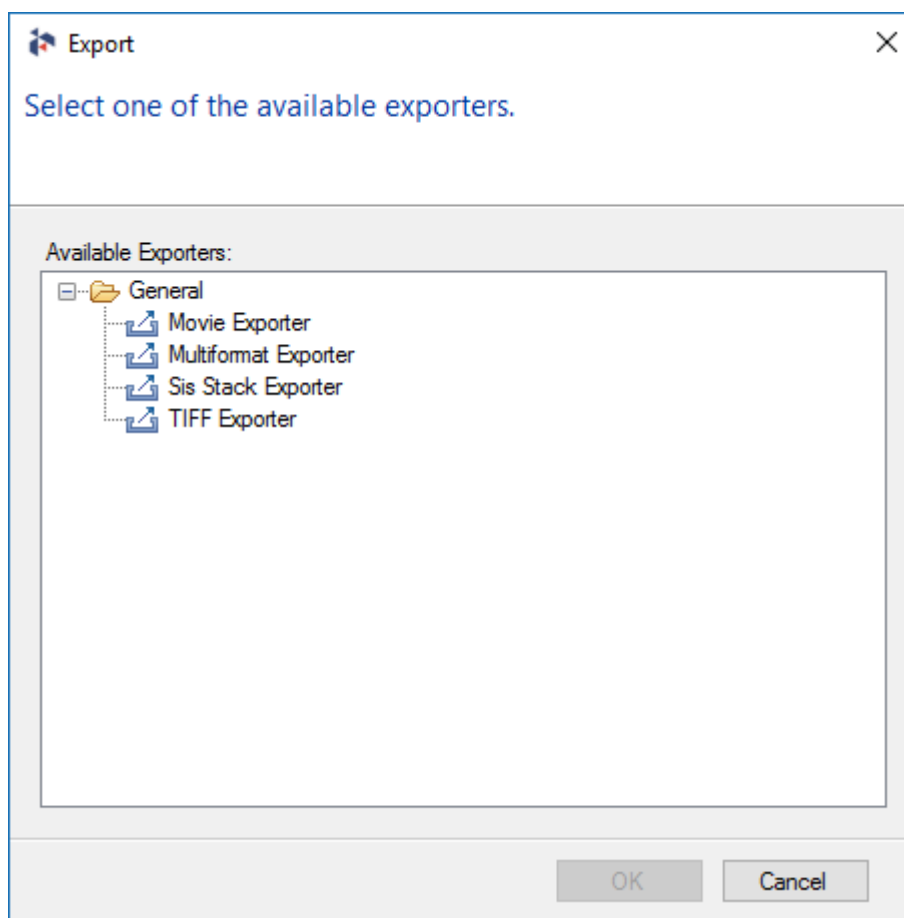
### 5.2. Saving images for use in other imaging packages

Having processed an image set within arivis Vision4D, users may wish to export those images to other imaging packages that do not have support for the SIS file format.

If that is the case, you should use the export function to save the image to a standard image file.

arivis Vision4D can export files to a variety of formats, including TIFF, PNG, JPEG, AVI and more depending on the needs.

- To export an image to another format, go to the **File menu** and select the **Export option**. You will be presented with the Export window where you can select what type of export you want to use.
- If you wish to export the images for inclusion into text documents, posters or presentations, you should use the Movie or Multi-format Exporters.
- If you want to export the images for analysis in another package, you should use the SIS Stack or TIFF exporters.



- The Movie and Multi-format Exporters both offer the option to save the images with annotations written to the pixel data so that they can be visible in any software package that can display the images.
- The SIS File Exporter and TIFF Exporter allows to export images at their native bit depth with the metadata to ensure data integrity, though bear in mind that not all imaging packages that open TIFFs look for the same metadata in the same structure which may cause some slight problems in the case of calibrations for example.



---

*For full details of each exporter, please refer to the help files.*

---

*Remember you can use the F1 key on your keyboard for quick access to the help page on any topic.*

---

## 6. Example workflows

### 6.1. Import a time series

Use the images in Import/Time Frames from <http://demodata.arivis.com>.

1. Go to **File> Import** and navigate to the **Time Frames** folder. Select all the image files contained within and then click **Open**.
2. Select the source folder as the destination and select the **Images as Frames** import scenario and click **OK**.

### 6.2. Import and rearrange a dataset with a complex file structure using pattern matching

Use the images in Import/Complex from <http://demodata.arivis.com>.

1. Go to **File> Import** and navigate to the **Complex** folder. Select all the image files contained within and then click **Open**.
2. Select the source folder as the destination and rename the file to Spindle, then, select the **Custom Import** option from the **import scenarios** and click **OK**.
3. Go to **Selection> Pattern** matching to open the **Import Pattern Matching** window.
4. Change the **Pattern** to `spindle%c_P_S%i_S%i_T%f_Z%p_C%c`, this will ensure that first two numbers in the file name are ignored, the number next to T corresponds to the frame, the number next to Z corresponds to the plane, and the number next to the C corresponds to the channel. Click the Refresh button next to the pattern name to check that the allocations are correct.
5. Click **OK** to confirm and close this window and return to the **Manual Import Mapper** window.
6. Check that the save location and image allocation is correct then click **OK** to complete the import process.


### 6.3. Import and rearrange a complete set with tiles

Use the images in Import/Mosaic Tiles from <http://demodata.arivis.com>.

1. Go to **File> Import** and navigate to the **Mosaic Tiles** folder. Select all the image files contained within and then click **Open**.
2. Select the source folder as the destination and rename the file to **Tile**, then, select the **Tile Sorter** option from the **import scenarios** and click **OK**.
3. Once the set is imported the tile sorter will open. Set the order to **Straight** and **Rows**. Make sure that the starting position is set to **Top Left**.
4. Set the number of **Frames** to 1, **Planes** to 1, **Columns** to 25 and **Rows** to 26.
5. Zoom in to 100% at the boundary between four tiles within the tissue section and adjust the displacement and overlap until satisfied that the images match.
6. Click **Create Stitched Planes...** to open the Stitch Tiles Dialog.
7. Set the output to **New Scope** and leave the rest of the default options unchanged.
8. Click **Finish** to confirm and stitch the tiles according to the previously selected parameters.

## 6.4. Create an animation to reveal structures of interest

Use demo set “Sea Urchin” from <http://demodata.arivis.com>.

1. Go to File> Open and select the Sea Urchin data set into arivis Vision4D.
2. Use the Viewer Types switch on the left of the Viewer Settings toolbar to switch to the 4D viewer.
3. Right-click on the colour scale to switch the color to the rainbow scale.
4. Switch the rendering mode to Volumetric from the workspace toolbar.
5. Use the sliders on the color scale to adjust the transparency of the black level and the threshold of the volume surface.
6. Use the Home View icon from the Shortcut toolbar to reset the orientation to the default orientation.
7. Click on the Storyboard icon  in the Shortcut toolbar to open the Storyboard.
8. Add the current orientation as a key frame to your storyboard.
9. Use the fly-through mode to navigate towards the sample so that you are facing the “mouth” of the object, then add this orientation as a key frame in the Storyboard.
10. Use the fly-through mode to navigate to the centre of the object and add this view as a key frame in the Storyboard.
11. Use the fly-through mode to rotate the view through 180 degrees and reverse out of the object then add that view as a key frame in the Storyboard.
12. Rotate the object so that you are viewing it ‘side on’ and add another key frame to the storyboard.
13. Add a clipping plane to the 4D Viewer and orient it so that it bisects the object. Then, adjust the position so that it no longer cuts the objects and add another key frame.
14. Move the clipping plane back to its bisecting position and add a final clipping plane.
15. Click the Export Movie button.
16. Set the movie resolution to 1280x720 and the data resolution to the last green setting before it turns yellow.
17. Set your export location then click Export.
18. Try importing your movie into a presentation or playing it back on another computer.



## Glossary

- ANNOTATION:** Any additional element on an image that is not pixel data. Annotations are used both for illustration and measurements
- BIT DEPTH:** This defines the degrees of variation between the minimum and maximum intensity values in the image. Typically, this is dictated by the acquisition device used to generate the images and is stored in computer systems as a number of 'bit'. Higher bit depths have more degrees of variation but require more computer memory for processing and storage. An 8bit image stores values between 0 and 255 for each pixel and each pixel requires 1 byte of memory for storage and processing. A 16bit image stores values between 0 and 65535 for each pixel and requires 2 bytes of memory. Note that when talking about colour images the usage of "24bit colour" is common, but what is usually meant is 8bit per channel.
- BOOKMARK:** Bookmarks are a record of the current visualisation parameters for your data. They capture the current scene settings (e.g. render mode and view properties) and save them for later use. Bookmarks are stored in the metadata of the document and can be shared by exporting them to a proprietary file format (\*.bookmarks). Bookmarks can be transferred between different data sets and installations of the software.
- DIMENSION:** The coordinates required to define the location of each pixel in a set. The SIS file supports the following dimensions:
- SCOPE:** This dimension is used to contain all the other dimensions relating to a discrete sample. Multiple scope can be used to contain different wells in a plate, or different states of a scope (e.g. as the result of a filtering operation), or different components belonging to the same data set.
- CHANNEL:** The channel dimensions stores the different modalities or colours of a set. It is most useful in fluorescence microscopy where each fluorochrome is stored as a discrete monochrome channel and can be displayed independently of the others or overlayed to show the correspondence between channels.
- FRAME:** The time element of a series. Each time point is a different frame. The frame information is important for tracking operations and monitoring changes over time.
- PLANE:** A 2-dimensional matrix of intensity values for all points at a specific depth position in the sample.
- FEATURE (of segments):** Features are calculated characteristics for Segments/Tracks/Annotations (they are calculated and are only available

when you do analysis operations), often referred to as measurements.

**FILE:** A document containing data that can be shared, copied or deleted from the Windows File Explorer. Files can typically be opened and edited within dedicated applications. Files that can be opened in arivis Vision4D can be classified in two categories:

**NATIVE FILES:** Files that are created and read by arivis Vision4D. These files are labelled with the extension .SIS in the case of image files, for 'Single Image Stack'. This type of file enables the arivis ImageCore technology at the heart of arivis Vision4D. Non-native files must first be imported into an SIS file to be opened into arivis Vision4D.

**NON-NATIVE FILES:** Supported files can be imported into an SIS file for processing. Supported files include most common image files, including TIFF, BMP, JPEG, DICOM, and many proprietary microscopy file formats including CZI (Zeiss), LIF (Leica), OIB (Olympus), ND2(Nikon), and many others besides. Users can check the complete [list of supported file formats](#) from the Help documentation and from the Import File selection window.

**IMPORT:** The process by which images are converted to the SIS format. It is the first and necessary step for any non-native file supported in arivis Vision4D (anything other than SIS). This enables the use of the Image Core technology at the heart of Vision4D.

**OPEN:** Only available for SIS files. This makes the image data in a file available in the software workspace.

**PALETTE:** A user interface element that contains information and adjustments relating to a specific aspect of an image or image object (e.g. all properties relating to a clipping plane).

**PIXEL / VOXEL:** Pixel is short for Picture Element. It is the smallest individual component of an image. Each pixel represents an intensity value / measurement within the context of the 2 dimensional matrix that is the plane of an image. The dimension of a pixel extends from the edge of a pixel to the edge of the next. Voxels are the pixel equivalent of a 3D volume and extends in Z as well as X and Y.

**PIXEL DIMENSION:** A calibration value that allows the software to convert measurements from an image from a number of pixels to real world units. Typically defined as the distance from the centre of one pixel or voxel to the centre of the next.

**PROPERTIES:** Properties are characteristics of annotations or image data. In contrast to features, they are always there and part of the model and saved with the image data.

**RENDER:** How the image pixel intensity data is displayed on the screen. The rendering modes are dependent on the selected viewer

type and affect what portion of the image data can be visualized. Vision4D supports various rendering modes implemented in viewers including 2D plane, 2D projection and 3D views.

**RESTORE POINT:** The arivis ImageCore file format provides versioning capabilities that are used for a comfortable undo/redo handling as well as for the specific creation of long-lasting versions of your image data within one .SIS file. A single restore point stores your entire image data, the available metadata and the created annotations. Therefore, it can easily support different filter and analysis experiments.

**SEGMENT:** An annotation or objects derived from analysis steps resulting in segmentation of an image or the manual annotation of an image.

**STACK** (also Z-stack): A series of images planes representing a 3D object data.

**VIEWING AREA:** The part of the user interface that displays the set data. The viewing area can display the set in various modalities (2D, 3D, Info viewer) and also displays previews of the effect of analysis or filtering operations as well as the visual representation of the results of such operation.

**Viewer:** A single window in which a single SIS file can be displayed and processed. It contains all the tools required to render and interact with a SIS file. Viewers can be set to display the content of the SIS file in any of several rendering modes, including 2D, 4D, Projection etc.

**WORKSPACE:** The layout of the tools and palettes within the application window. Workspaces can be saved to quickly change between different combinations of tools and palettes related to a specific workflow.



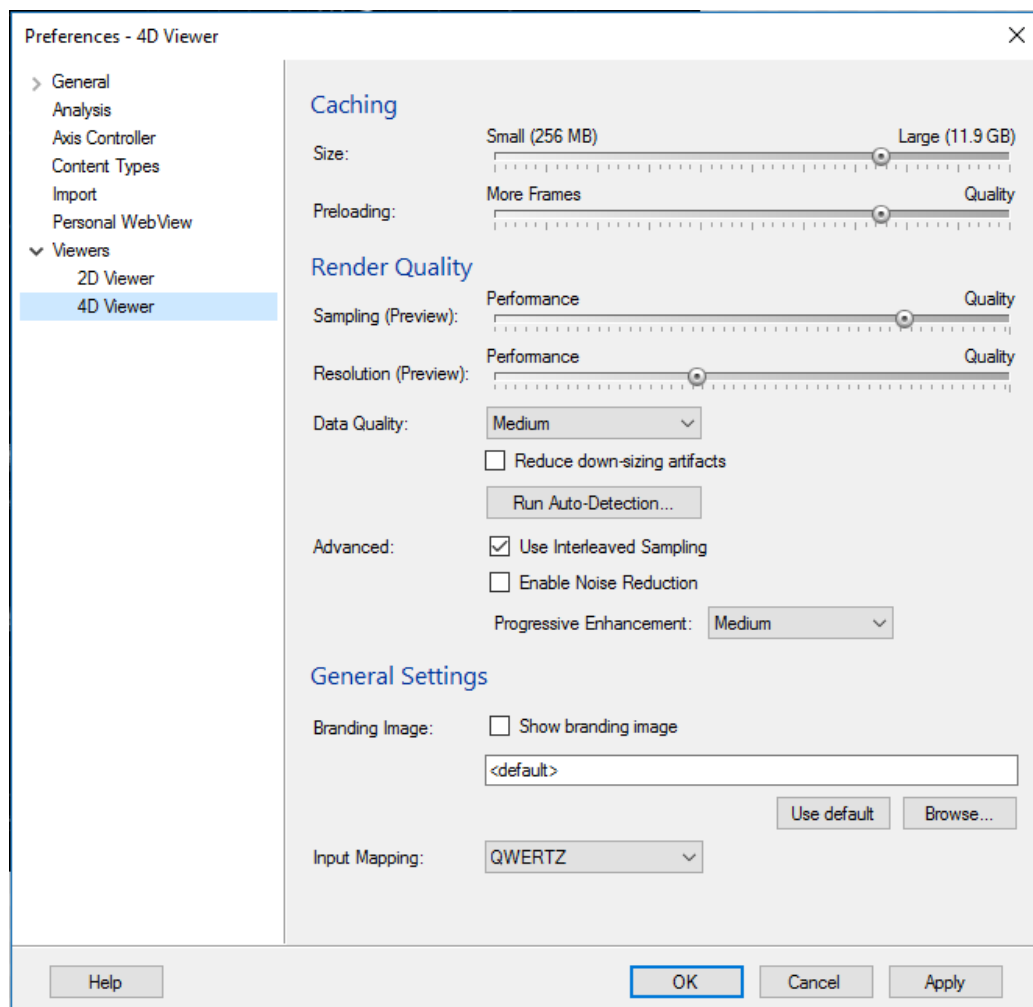
## FAQs

*How can I improve the render quality for my images?:*

The quality of the 3D renderings is highly dependent of your graphics card, your PC configuration and software configuration.

When the 4D Viewer first runs after the installation of the software it will perform an automatic hardware check and optimise settings according to the results. However, users may choose to sacrifice some speed to improve the render quality while interacting with the data. To do this, you will need to change the 4D Viewer preferences.

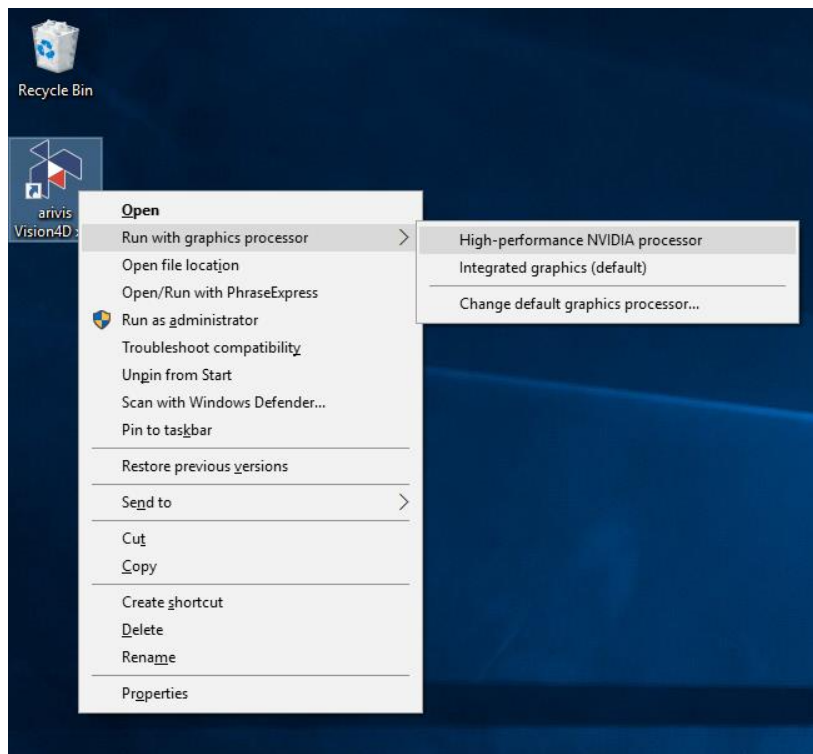
To access the 4D Viewer preferences, go to the **Extras** menu and select the **Preferences** option. In the Preferences window, expand the Viewers tab and select the 4D Viewer tab.



Adjusting the Sampling and Resolution sliders will Improve or reduce the quality of the render. Higher quality will normally result in slower rendering speeds.

*I have a high spec graphics card but the renders appear to be of low resolution. How can I make sure the software is making the most of my hardware?*

When running arivis Vision4D on most laptops and some desktops, Windows attempts to reduce the power requirements by only enabling the use of dedicated graphics hardware if specifically requested to do so by the user. User can force the use of the graphics card by right-clicking on the program icon or shortcut and selecting which graphics processor to use the application from the contextual menu.



It is also possible to change the default for specific programs, although the interface for this will depend on the device manufacturer.

If you've run the 4D Viewer for the first time and the auto detect was run without the use of your preferred graphics hardware, you can rerun the automatic detection from the 4D Viewer preferences.

In the 4D Viewer tab of the preferences window, click the Run Auto-Detection... button and wait for the process to complete.

Any images displayed in the 4D Viewer should automatically reload, but you may need to change out of the 4D Viewer and back in for change to take effect.

---

*If this does not improve the quality of the render, or if the 3D render simply fails to appear, you should also check your hardware configuration. Make sure that your drivers are up to date and also make sure that your monitor is connected to your graphics card and not to your motherboard graphics connector.*

---

*Where can I change the default directory for data export?*

By default arivis Vision4D will save new documents (SIS files, bookmarks, movies etc) to the user's Documents folder. To change this default, go to the Extras menu and select the Preferences option.

In the Preferences window, select the General tab and you'll find the default directories for new documents and scripts.

To change the default new document location, either click the Select... button and navigate to your preferred location, or simply type the path of your save folder in the text box.

## A note on large images:

Throughout this guide we make mention of “large data sets”, with many implications regarding processing speeds and rendering quality. The definition of what constitute as “large set” is, however, somewhat dependent on the configurations of your workstation and the operation that is required.

The ImageCore technology is designed to enable the fast visualisation of sets of almost any size. This is done by enabling the software to access any part of the image at will without the need to access the full image data. This process is particularly well suited to the rendering of images where in any case most systems do not have the capability to display the complete document data, whether this is because the screen itself is two-dimensional whereas arivis Vision4D can handle up to 5 dimensions, or simply because most computer displays only have in the region of 2 to 8 million pixels whereas some sets can be several orders of magnitude larger.

The 4D Viewer will automatically analyse the configurations of the PC (graphics card, available memory) to calculate an optimal scaling resolution for fast rendering of the data and is therefore relatively immune to the overall size of the data. However, rendering of segments within a 3D set can be quite slow in comparison, especially if there are many small objects or the surface geometry is very complex. Again, what is meant by “many objects” and “complex geometry” is somewhat dependent on the computer specification.

Analysis operations, especially those concerned with filtering or segment generation, usually require the software to access and process each and every single pixel concerned within the selected region. In 8bit images, each pixel represents one byte of memory usage and in 16bit, each pixel needs two bytes. A 1 megapixel image will therefore require 1 megabyte of memory. If a stack contains multiple planes, the amount of memory required to process the entire stack will be equivalent to the number of pixel in each frame multiplied by the number of planes in the stack. Some operations can process each plane individually (e.g. 2D filters and threshold based segmentation), while others will require multiple planes (e.g. blob finder and 3D filters). Typically, operations that require less than 25% of the available system memory can be executed relatively quickly, while larger tasks may be slowed down by the use of virtual memory and the need to cache data.

Also, while where possible arivis Vision4D will make use of multiple processors to process more of the data at any one time, not all processes can be parallelised.

Discussing the exact requirements of each type of operation and the effect of various hardware choices would take much too long for this guide. So, while there is no hard and fast definition of what constitutes a “large set”, considering the amount of physical memory necessary to process a plane, frame or scope compared to the amount of memory available from the system can be a good indication. Generally, expect sets that represent more than 25% of the available system memory to take significantly longer to process.

## Acknowledgements

The images used in the screenshots for this manual are used with kind permission from their respective owners.

The spinning disc time lapse of male meiotic spindle in *c.elegans* courtesy of:

Gunar Fabig, TU Dresden, Müller-Reichert Lab

Lightsheet recording of a Lumos cleared muse brain courtesy of:

Olga Efimova, Kurchatov Institute Moscow.

The  $\mu$ CT data set of a sea urchin courtesy of:

Dr. Alexander Ziegler, Institute for Immunogenetics, Charite Berlin

Tile Scan of mouse lung courtesy of:

Objective Imaging Ltd, Cambridge, United Kingdom

Laser scanning confocal of Epidermis courtesy of:

Dr Marco Marcello, Institute of Integrative Biology, University of Liverpool, Liverpool, United Kingdom