arivis

Vision4D

Imaging Science

arivisVision4D

Analysis

Contents

Introduction

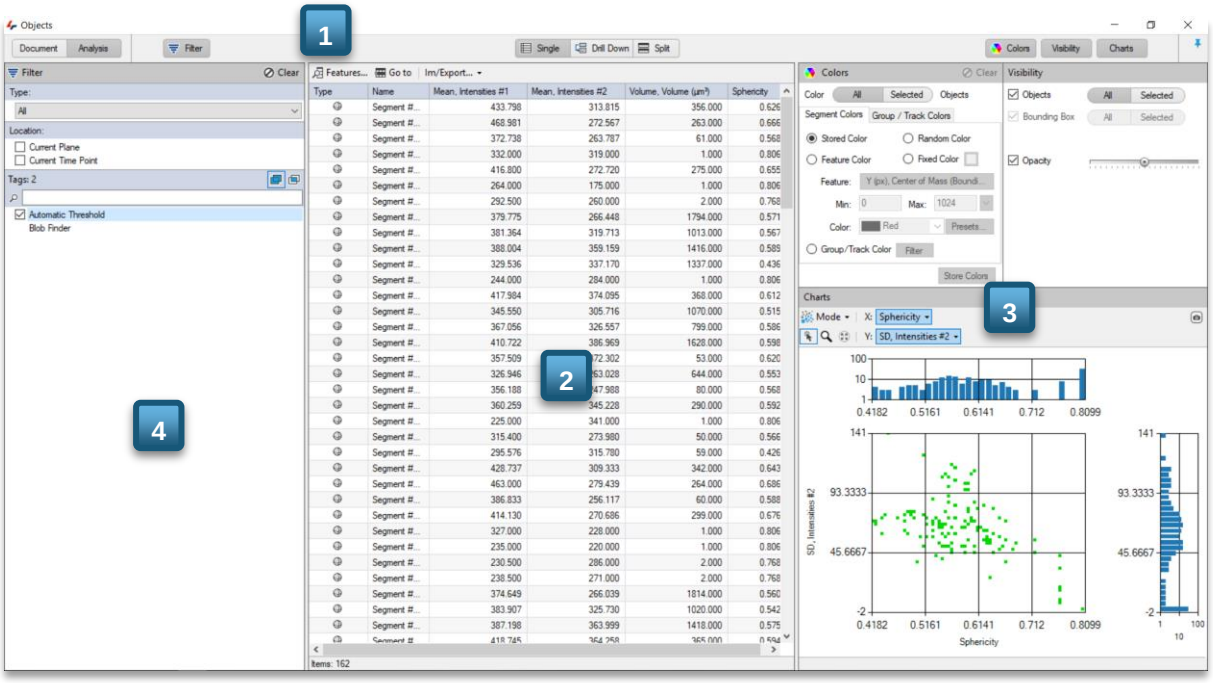
With arivis ImageCore at its heart, arivis Vision4D is made to visualise, process and analyse images of any size with support for 5 dimensions (X, Y, Z, time, channel). Because such large images can rarely be held entirely in the memory of most computers the software is designed to handle such images by using techniques such as sub volume selection, blocking, and parallelisation. Most of this is built into the core of the software and requires no direct input from the user to take full advantage of these capabilities.

Analysis in arivis Vision4D revolves around the concept of objects. Objects are defined on the image and their properties or feature serve to characterise them. Objects can be create manually or automatically depending on the image, and examined directly within the software, both as displayed objects on top of the image data, and within tables and graphs generated by the software. Objects can be stored with the image data, but the results of analysis operations can also be exported for further work in other software packages.

Most image analysis will be done through the analysis panel by creating, adjusting and running pipelines, though manual object definition may sometimes be necessary.

Objects window

Objects are what arivis Vision4D uses to characterise features of an image. They can be single points or 2D/3D polygons and can be created manually or through pipeline operations. As objects are added, they can be reviewed and modified on the image in the viewer and in the Objects window.



The Objects window is set to open by default after a segmentation operation in the analysis pipeline, though this setting can be modified from the Preferences window. It can also be opened manually from the Objects menu and shortcut toolbar.

The objects window is made up of multiple distinct elements, some of which can be shown or hidden as required:

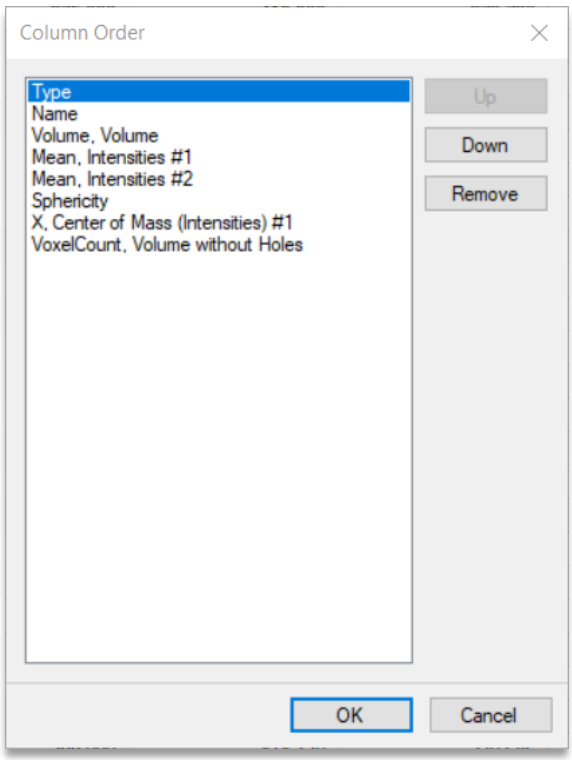
1) Toolbar – Where users generally enable or disable various elements of the Objects window, such as the Visibility filters, Colors and Visibility options, and Charts
2) The Objects Table – The table displays the features of objects created during analysis of the image (intensity and geometric measurements).
3) The Colors, Visibility and Charts panels – These panels can be shown or hidden individually by clicking their respective buttons in the Toolbar
4) Filter panel – Where users can select what objects to display in the table and the image based on tags, type and/or location.

Object Table

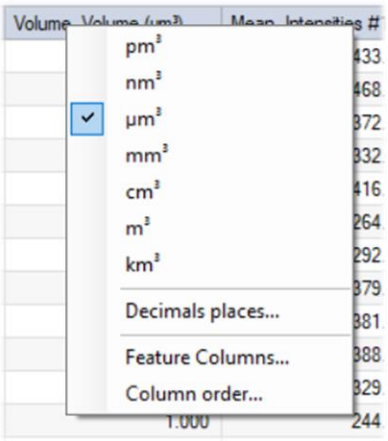
The Objects table part of the Objects window display the features of the object currently shown. The features of objects are displayed as alphanumerical values in columns aligned in rows with the objects they belong. By default, objects are ordered according to their ID, which is generated when the object is created.

A single click on a column title will reorder the object table in ascending or descending order according to the selected feature. A further click on the column title will reverse the order.

Columns appear in the order in which they were added to the table but can be reordered by right-clicking on any column title and selecting the Column order… option.

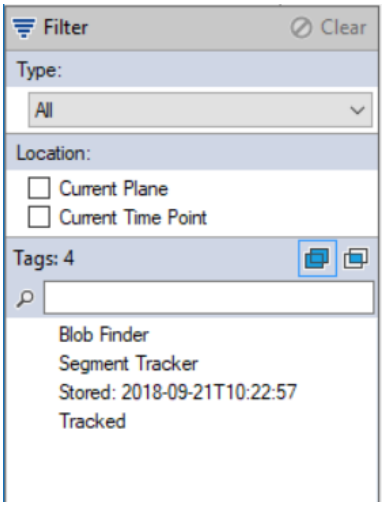


For columns with calibrated units, right-clicking on the column title will also allow the user to change the units and number of decimal places as preferred.


Volume
Mean Intensities #
pm³
nm³
µm³
mm³
cm³
m³
km³
Decimals places...
Feature Columns...
Column order...
433
468
372
332
416
264
292
379
381
388
329
1.000
244

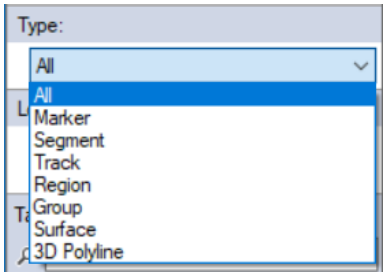
Object Filter Panel

When objects are added to the image and object table, they are usually assigned various tags to can be used to group them with other objects that were also subject to the same specific operation. The tags are also used to allow the user to quickly switch the visibility of whole groups of objects to facilitate visualisation and provide clarity.



The Filter Panel can be shown or hidden by clicking the Filter button in the Objects window toolbar. The Filter acts on the Objects window, as well as the viewers and is indicated by at the top of the Viewer Window as well as in the Objects Settings Panel.

First, the user can select to filter objects by type so that only objects of a certain type are displayed in the table or the image.



Then, users can filter visibility based on the location of the objects. This can be particularly useful when dealing with large stacks with multiple time points where a large number of objects might have been identified but only a small fraction of those are present in any single time point or plane. These selections are nonexclusive so that the user can choose to show objects from the current plane or the current time point or both.

Finally, object visibility can be filtered by tag. By default, as each operation in a pipeline is run the objects affected by that operation are tagged and the visibility is automatically set to show only objects with the tag from that operation. Selecting a different tag will switch the visibility to those

objects, though the switch can also be done by clicking on the operation in the pipeline. The display of objects from multiple tags can be set to be exclusive, meaning only objects with all the selected tags are shown, or inclusive so that objects with any of the selected tags are shown.

Please note that you can also show only selected objects if you choose select objects  in the Object Settings panel or in the Visibility panel in the Objects window.

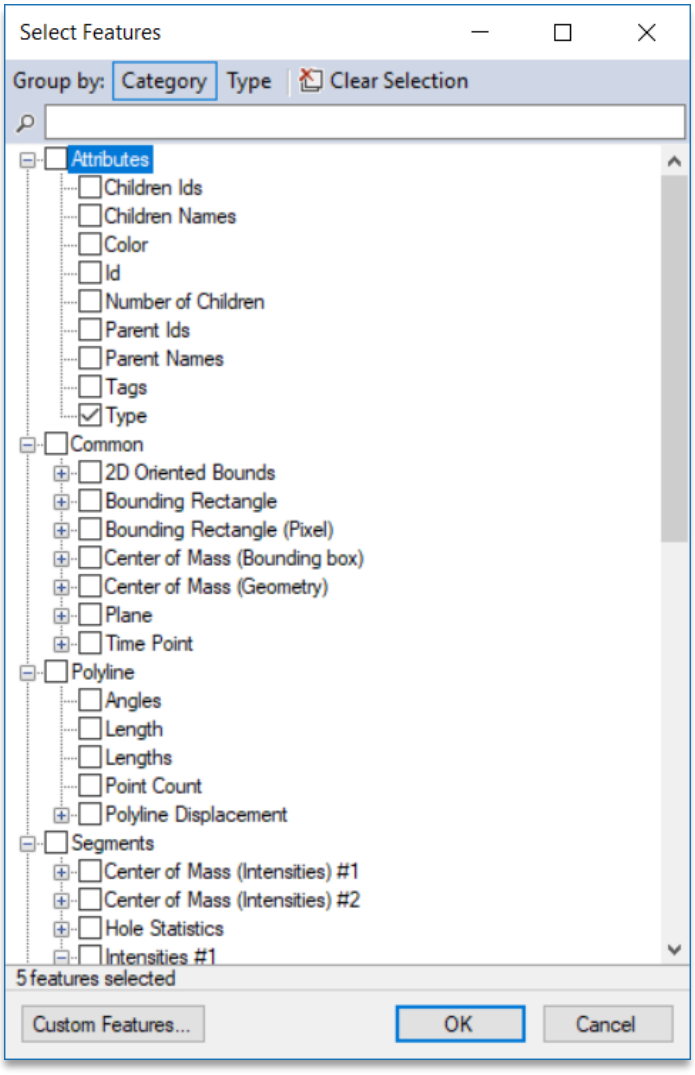
Please see the section on the analysis pipeline below for information about object tags.

Object Features

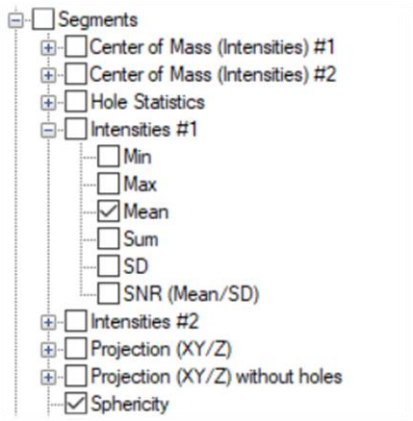
Features are characteristics of an object that can be displayed in the Objects table and generally stored or exported as needed. They can be enabled or disabled in the table by clicking the Features button to open the Features window and then selecting them from the Features window.

Object features can be grouped either by category or type. By default, features are grouped by category. These categories are:

- Attributes: features that are generally independent of object type and position
- Common: measurement features which are common to all object types
- Polyline: features specific to polyline objects
- Segments: features specific to segmented volumes or surfaces
- Tracks: Features specific to track objects



In the Features window, various features can be selected by checking the box to the left of individual features or groups. Some, such as intensities, are grouped and checking the box for the group enables or disables all the features in that group, though individual features from the group can be enabled or disabled through expanding the group to list its individual features. Click on the [+] sign to the left of a group to expand it, or the [-] to collapse it.



In the snapshot above, the Intensities group for channel 1 has been expanded and the Mean value alone has been selected.

Generally, the features are sorted into groups depending on whether they are attributes of objects or groups, features that are common to all object types, or features that are only relevant to specific object types, like segments, polylines or tracks.

Please check the Help files for more details of the features available to collect in arivis Vision4D.

Remember you can always access the Help files by pressing the F1 key on your keyboard.

The help is context sensitive and, when called, will load the page relevant to the currently selected tool.

Additional features can be defined through the Custom Features dialog.

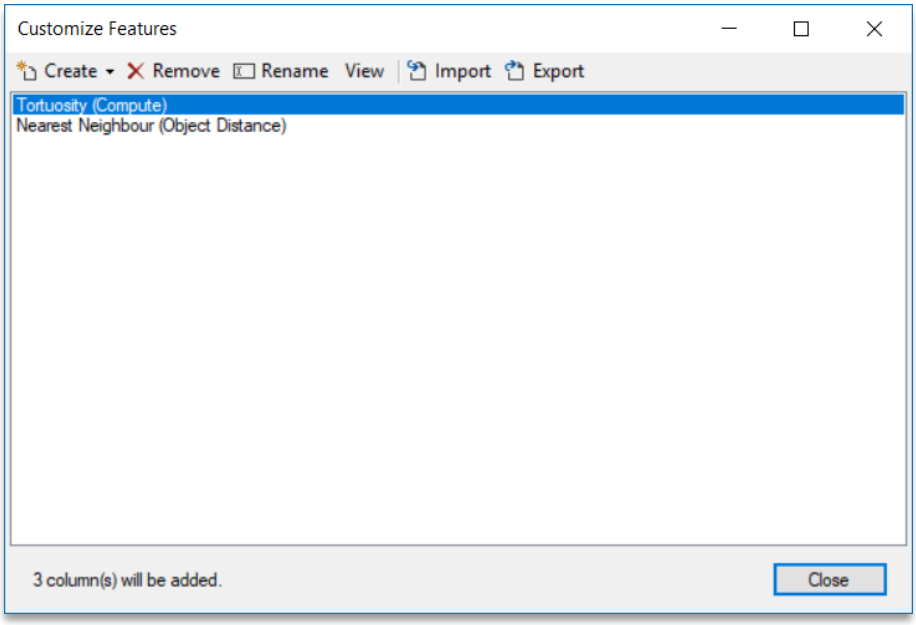
Custom Features

Custom features are user defined features of an object or group that can be calculated from other features or feature statistics. These can be distance between objects within an image, or the ratio of two other features for example.

New custom features can be created through the Customize Features window. This can be opened from the Object menu or by clicking on the Custom Features button in the Features window.

Custom features can be based on intensities whiting segments, group statistics or distances to neighbours.

Once created, the features can be automatically added as new columns in the Objects table, selected for inclusion in the Export pipeline operation or exported as a feature to other installations of arivis Vision4D.



To create a new custom feature, start by clicking on the Create drop-down button and selecting the type of feature you want to add. See below for examples of custom features.

Other buttons are available to remove, rename or view the details of existing feature. For any of those, start by selecting the feature from the table and then click on the relevant button.

Using the Export button, users can export the complete list of current custom features for import on another arivis Vision4D installation.

Here are a couple of cases of custom features and how the user would define them.

Example Custom Feature: Tortuosity of a polyline

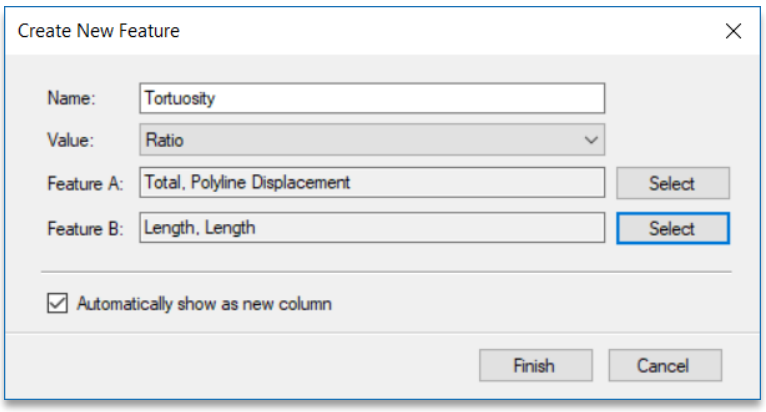
The linearity of a polyline can be defined as the ratio of the distance between the end points and the length of the complete line so that a perfect line will have a linearity of 1 and anything else would have a lower value. To add this new feature, start by clicking on the Create drop-down button and select the Compute option.

In the Create New Feature window, start by naming the new feature, then select what type of computation to use. In this case the computation is a simple ratio.

From the Polyline features, select the Polyline Displacement> Total feature, this will be the numerator.

From the Polyline features, select the Length feature, this will be denominator.

By clicking Finish the new feature will now be created and be available to display in the Objects table or add to object feature exports. Selecting the option to Automatically show as new column will add the feature to the objects table.

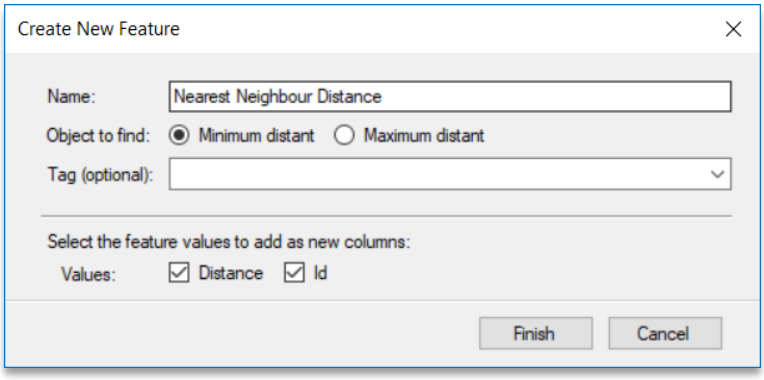


Example Custom Feature: Distance to Nearest Neighbour

When looking at complex systems, often the individual characteristics of objects are complementary to their proximity to secondary structures. The Segment colocalization filter can give useful indications of these spatial relationships, but a simple distance to the nearest neighbour can be a great starting point.

As before, to add this feature start by clicking on the Create drop-down button and select the Object Distance option.

In the Create New Feature window, start by naming the new feature. Then, select the Minimum distant option. If the user is interested merely in the distance to the nearest object, whatever the type of that object might be, the Tag field can be left empty, but if the user wishes to measure the distance to the nearest object of a specific type then selecting the corresponding tag from the list is required.



By clicking Finish the new feature will now be created and be available to display in the Objects table or add to object feature exports. Selecting the option to Automatically show as new column will add the feature to the objects table.

Note that the distance calculated in this case is that from the centroid of the object to the centroid of the nearest neighbour. For more complex distance measurement, please check the Segment Colocalization operation.

Manually adding Objects

While arivis Vision4D has some powerful tools in the analysis panel to automatically create and characterise millions of objects, some are most easily segmented manually by the user. Arivis Vision4D includes a range of tools to facilitate the creation of such objects.

- **Place Objects** is ideally suited to simple geometric shapes, points or polylines length measurements
- **Draw Objects** is better suited to identify complex 3D objects that can't easily be segmented using threshold based techniques
- The **Magic Wand** is a great starting point for object editing or identify a handful of significant objects from a larger selection of similar features
- The **Measure** tool is a quick and easy way to measure simple distances or areas to inform parametrisation of other operations

All of these, aside from the Measure tool, can be used to create objects that are collected in the Objects window and whose features can be used to evaluate statistics, create charts or otherwise be used in further workflows.

Place Objects Tool

Defining simple three-dimensional objects can be done by placing them on an image. To access this tool, click on the Place objects icon in the Shortcut toolbar, then select what type of object you want to add to the image.

These objects are relatively simple and can be one of the following types:

Spheres	A 3D object. The width and height of the sphere is defined by click and dragging on the image, the depth of the object is calculated accordingly.
Marker	A single pixel object. Click to add marker points. Markers have no width, height or depth a define a single pixel.
Region	A 2D object. Click and drag on the image to define the width and height of the region.
Polyline	A 3D object. Click on the image to add point. Go up and down through the planes as you go to follow features through the depth of the image.

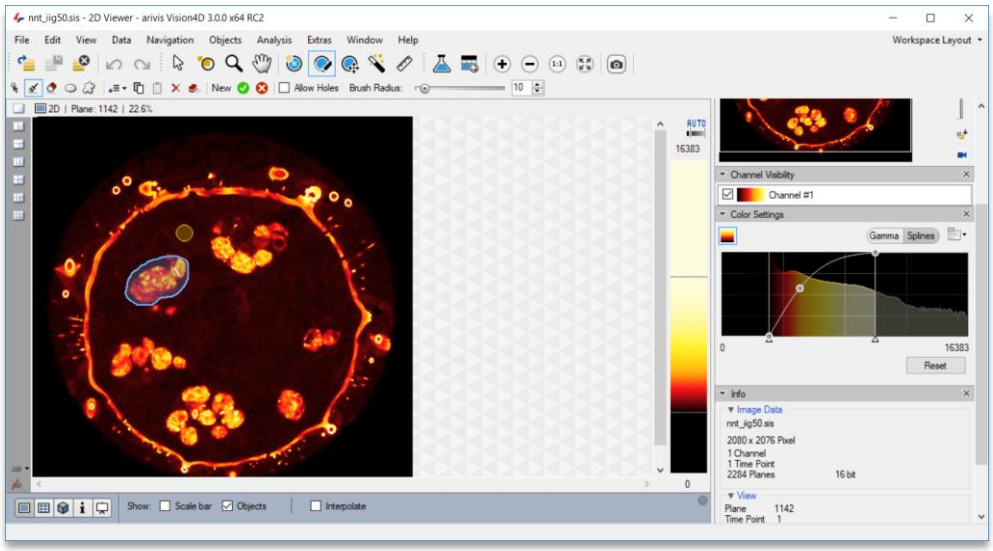
Please check the help page for additional information.

Remember you can always access the help files for the active tool by pressing the F1 key on your keyboard.

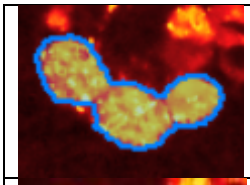
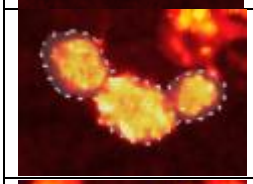
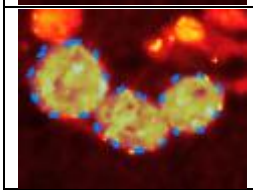
Draw Objects Tool

While placing objects on an image is relatively, the complexity of the objects that can be defined that way is somewhat limited. Drawing objects on the image can be used to create more complex 2- and 3D objects. This tool can be used to define completely new objects or modify existing ones as needed. Generally speaking drawing objects works by defining the outline of objects on a 2D plane then going up and down through the stack as needed, adjusting the outline of the object as needed until the complete object is outlined.

To activate it, click on the Draw objects icon in the shortcut toolbar and the Draw Objects toolbar will appear.



1. By default, the brush tool will be activated and users can simply drag and drop the brush on the image to define the outline of the object in the current plane.
2. To remove unnecessary parts of the objects, use the right mouse button to erase portion of the outline using the brush, or select the erase tool from the toolbar.
3. As the user navigates up or down through the stack a shadow of the object outline will appear shaded white. Using the brush or erase tool on this shadow will confirm the presence of the object in the current plane and the outline will turn blue. Correct the outline as required.
4. Outlines of the same object define over non-contiguous planes will automatically be interpolated through interstitial planes and appear as blue dashed regions. These can be corrected using the brush or erase tool as needed.

	Outline of an object defined in the current plane.
	Outline shadow in a plane currently not included as part of the object.
	Outline of an object interpolated from outlines defined both above and below

5. Proceed up and down through the whole depth of the object, adding or removing from the outline as needed until the object outline is complete, then validate your results by clicking the Finish icon, or click the Cancel button to discard changes.

As mentioned previously, rather than define a new object, the Draw Objects tool can also be used to correct existing objects. In that case the user should start by using the Select Object button to first activate an object for editing and then the brush and erase tools can be used as described above.

Along with using the brush and erase tools to define the object as described above, users can also use an ellipse of polygon to define the outline on the active plane. In either case the brush and erase tools can still be used to further amend the outline if needed.

Please check the help files for more information on the draw object tool and additional options available.

Remember you can always access the help files for the active tool by pressing the F1 key on your keyboard.

5.4. The Magic Wand

While all the object definition tools above are purely user defined, meaning that the actual content of the image is irrelevant, only the user's interpretation and interaction defines the size and shape of the object, some objects can be defined relatively quickly using a simple threshold and the Magic Wand. The Magic Wand works by defining a search region within which to detect objects and then performing a simple threshold based segmentation to identify objects from groups of pixels within a certain range of intensities. Unlike the Place Objects and Draw Objects tools, the Magic Wand can work both in the 2D and the 4D viewer.

To activate it, click on the Magic Wand icon in the shortcut toolbar.

In either case, whether working in the 2D or 4D viewer, once activate the user must set up the tolerance and type of objects to be identified. The tolerance sets the intensity threshold tolerance based on the object type. When the user clicks on the image to detect an object the magic wand will take the intensity of the pixel selected and, depending on the object type, set the inclusion threshold as follows:

- Bright Objects: All pixels with **the same intensity value - tolerance** and higher than the selection are added to the object.
- Dark Objects: All pixels with **the same intensity value + tolerance** and lower than the selection are added to the object.
- Tolerance Range: All pixels with the **same intensity value ± tolerance** are added to the object.

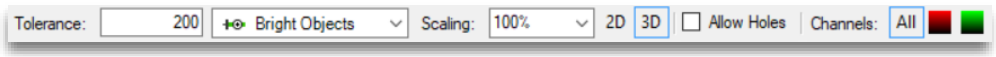
This means that in the case of a user looking for bright objects with a tolerance of 50, if the user were to click with a pixel with an intensity of 128, the object would include all pixels within the region with an intensity of 78 or more.

The magic wand can be set up to work on all channels of a multichannel image, or on individual channels as needed by clicking on the appropriate button in the Magic Wand toolbar.

Details of how the Magic Wand works within the context of the 2D and 4D viewer are below. Please check the help files for additional details.

Remember you can always access the help files for the active tool by pressing the F1 key on your keyboard.

5.4.1 Magic Wand in 2D Viewer



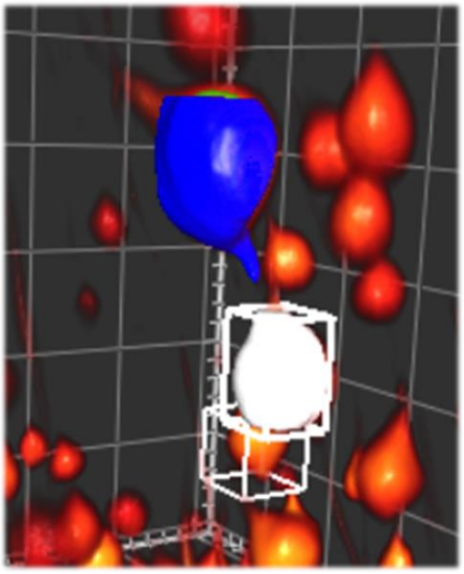
When used in the 2D Viewer, the magic wand works by clicking and dragging a selection box around the structure the user wants to identify. The bounding box restricts the area of processing. The depth of the box is calculated automatically based on either the width or height of the selection, whichever is highest, and extends evenly above and below the current plane. The width, height and depth of the search box are displayed underneath it (units are pixels). With the selection box drawn around the object, the user then clicks on a pixel within the object and the magic wand will identify the complete object based on the intensity of the pixel and the tolerance.

Additional objects can be identified by clicking on them without the need to draw a new selection box each time as the last dimensions will be used. If a new smaller or larger selection box is required, the user can click and drag a new region around the object and proceed as before.

Magic Wand in 4D Viewer

When used in the 4D Viewer, the magic wand is used in much the same way except for the definition of the selection box. To selection box is positioned in the depth of the volume according to the opacity settings set in the 4D Channel Settings and the first opaque pixel in the axis of the mouse cursor position. To adjust the size of the selection box the user can use the mouse scroll wheel or trackpad scroll gesture.

Once the selection box is defined, the user clicks on the image as before and the segmentation is carried out.



In the snapshot above, the object in blue has already been detected. The object in white is the last detected object, which is also the currently selected object. The empty box behind them is the current selection box centred around the pixels the mouse cursor is pointing to.

The Analysis panel

Image analysis and automatic object segmentation in arivis Vision4D is managed through the analysis pipeline. The Analysis panel can be accessed through the Analysis menu or by selecting its icon from the shortcut toolbar.

The analysis panel uses a linear pipeline concept where the user chooses which operations to apply to the image for enhancement, segmentation, tracking, etc, and then runs them consecutively.

The analysis panel is split into three sections. At the top is the analysis panel toolbar where users control the pipeline operations and access tools relevant to the analysis, such as eporting or importing pipeline or access to the analysis options. Immediately below that is the analysis pipeline, where the user add and rearrange operations and define their parameters. The bottom part holds all the operations that are available in the software, sorted by type (voxel operations, segment generation etc). Users select actions by double clicking them in the Analysis Operations section or dragging and dropping them from the Analysis Operations section and into the Pipeline.

As each operation is added, the user can adjust the parameters as required, preview the results where possible, and run the operation. Pipelines can be run either partially or in full, individual actions can be done and undone, and complete pipelines can be saved and run on additional images as necessary.

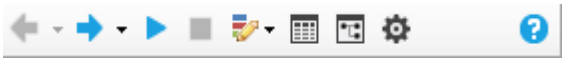
The Analysis Pipeline

The pipeline is where the user defines the operations to execute and their parameters. The pipeline works in a linear fashion with each operation being run only once the previous operation is complete.

A general strategy for pipeline building it to start by selecting a manageable portion of the image to test parameters, add, configure and test operations in turn, and when satisfied with the results of the pipeline, run it on the complete image without further user interactions until it is complete.

Because running operations on the complete file can take considerable time and resources, the first step of the pipeline is always the selection where users can choose what portion of the image to run the pipeline on. See the Selection section below for more information.

At the top of the pipeline is the Pipeline toolbar where users can interact with the pipeline as a whole.



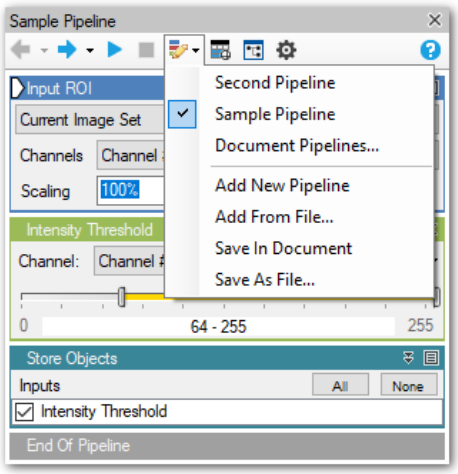
The first two icons allow you to step forward and back through the pipeline. These are particularly useful during the designing stages of the pipeline where a user may wish to run a single operation to test the parameters, and undo that operations to change it if necessary. A single click on the icon executes or undoes the next or previous operation. Clicking the drop-down arrow to the right of each of those buttons allows to run or undo multiple operations up to a certain step.

The Play icon executes the complete pipeline from the current point to the end.

As each operation runs a progress status will appear in the title bar for that operation, allowing the user to get some indication of the time required for completion. Note that the completion progression may not be linear as the operation may include various calculation steps that require varying degrees of computing time.

The Stop icon can be used to terminate any uncomplete operation. This can be useful in cases where it is obvious that the operation is not producing the required results or if the current execution of the pipeline is preventing other operations from running.

The Pipeline management menu can be used to select, store, retrieve and otherwise manage pipelines.



Storing pipelines in the Document can be useful if the pipeline still needs modifications before it can be run reliably. Saving the pipeline as a File may be preferable if it is to be used with multiple documents.

For batch processing we also offer a separate Batch Analysis option in the Analysis menu to analyse multiple images automatically.

All of the pipeline operations have settings that can be defined within each operation, but some settings can be set as default for all pipeline operations. These can be accessed and modified through the Analysis settings pane of the Preferences window (see details in the help file or press F1 to access the help file).

Analysis operations

Generally, the process for analysing an image follows a relatively linear workflow. The user may start with pre-processing the image outside of the analysis panel (e.g. applying bleach, background or drift correction), before proceeding. Typically the analysis pipeline will include the following types of operations:

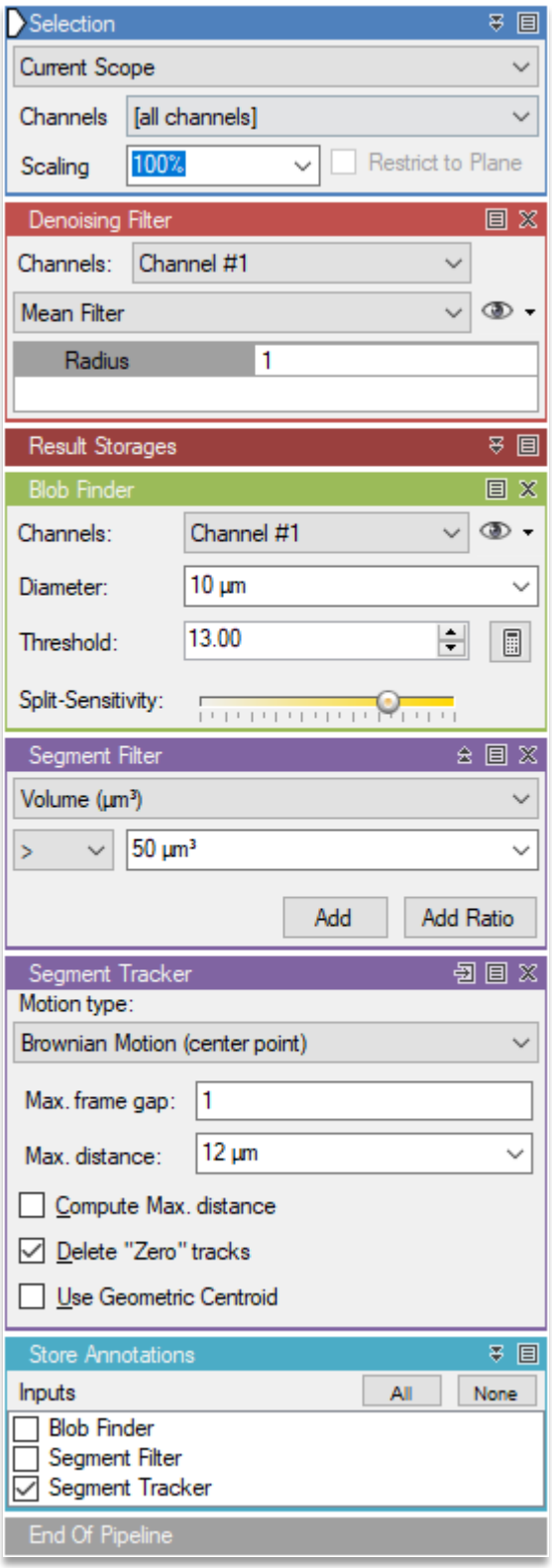
1) Applying voxel operations to enhance aspects of the image that the user is interested in
2) Segmenting objects from the image
3) Processing segments to remove spurious objects or analyse the relationships between segments (tracking, colocalization)
4) Reviewing and storing the results of the analysis operations

Analysis operations are sorted by type and must be run by type order. The user can add as many voxel operations as needed, but these but all be run prior to running any segment generation operation. If the workflow requires the generation of segments followed by a voxel operation (e.g. masking), this will require multiple pipelines run consecutively. For examples of this, please see the Use Cases section below.

Some operations, such as Input ROI and Result storage, are necessary and added automatically. The Store Objects operation is recommended and is also added automatically to the end of the pipeline since the results of a pipeline operation are properties of the pipeline and not the document. If the objects are not stored at the end of the pipeline the user will need to run the pipeline again the next time they open the document if they wish to see the results. Otherwise, without storing the objects in the document, the user can play around with the analysis pipeline safely and will not modify the document accidentally.

To add operations to the pipeline, simply double click on the desired operation, or drag and drop it to the desired location in the pipeline. Since operations are run sequentially from the top down, the order in which operations appear in the pipeline is particularly important. Applying a denoising filter before an edge detection filter will have a different result than if the operations were run in the reverse order.

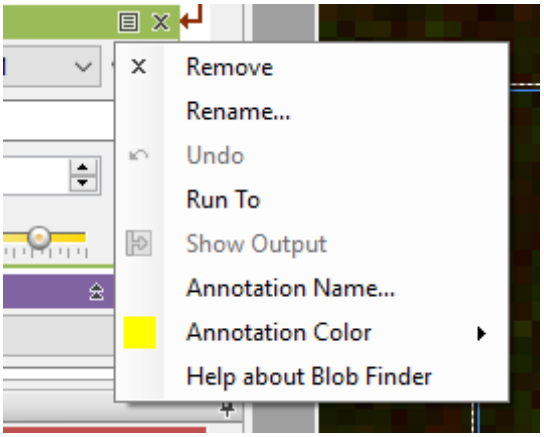
Also, the operation type order must be respected. This means that all voxel operations must be completed prior to any segment generation operation, and voxels operation cannot be added to the pipeline below segment generation operations.


Selection
Current Scope
Channels
[all channels]
Scaling
100%
Restrict to Plane
Denoising Filter
Channels:
Channel #1
Mean Filter
Radius
1
Result Storages
Blob Finder
Channels:
Channel #1
Diameter:
10 µm
Threshold:
13.00
Split-Sensitivity:
Segment Filter
Volume (µm³)
>
50 µm³
Add
Add Ratio
Segment Tracker
Motion type:
Brownian Motion (center point)
Max. frame gap:
1
Max. distance:
12 µm
Compute Max. distance
Delete "Zero" tracks
Use Geometric Centroid
Store Annotations
Inputs
All
None
Blob Finder
Segment Filter
Segment Tracker
End Of Pipeline

As each operation appears in the pipeline the user will modify the parameters of that operation as needed. Users can then run each operation in turn. Or the entire pipeline as needed.

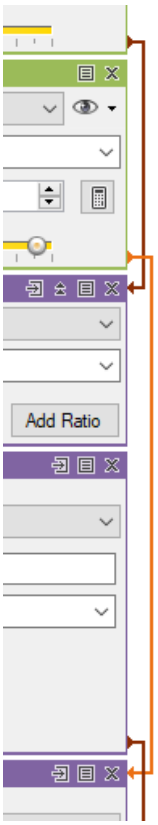
Each operation appears with a title bar which can include up to 4 additional buttons.

- The Close icon ✕ can be used to remove an operation from the pipeline if it is deemed unnecessary.
- The Menu icon to the top right of an operation gives the user access to several options relating to that operation, including renaming the operation or accessing the help files. This is particulalrly important as it relates to the tags that are applied to segments. Please see the Segment generation section below for more details.



- Clicking the dropdown icon displays parameters additional to the defaults. In the case of voxel operations it can be used to set specific store locations for the output of the filter. In some segmentation operation it can be used to set additional parameters.

- The Input icon can be used to select the input for an operation where more than one input is available. For example, the segment colocalization needs the segment generation operations as input.
  By default, the pipeline assumes that operations take the previous operation as input for the next and follows a linear workflows. However, it may be useful under several circumstances to have multiple operations running in parallel to generate objects and tracks that can be combined at a later point. For example, a user may choose to segment and process objects from multiple channels, but combine those into a single list. Since segment generation operations typically only operate on one channel, this process would require two separate segment generation operations.
  Having generated those segments the user may choose to further apply a segment filter to eliminate out of range objects, but to use a different threshold for each object type. Since all segment generation operations must be complete prior to executing any segment process operations a linear workflow is not possible and the input icon can be used to specify which segment generation operation is to be used for which filter.
  To select an inputs, click the input icon in the operation title bar and select the necessary input from available options.
  See the use cases section below for more details of where these options can be important and/or useful.



As mentioned above, the analysis operations are arranged by type. Five types of operations are currently available in the analysis panel:

1) Voxel operations

Voxel operations modify the image in preparation for the operations to follow. This can include masking to black out unnecessary parts of the image, denoising and morphological filters to enhance specific features of the image etc.

2) Segment generation

These operations generate segments. These segments have various properties generated from their geometry, location and the values of the pixels within them. They can be generated by importing them from the document, by using automatic or manual threshold based methods, or using advanced segmentation techniques such as the Blob finder.

3) Segment operations

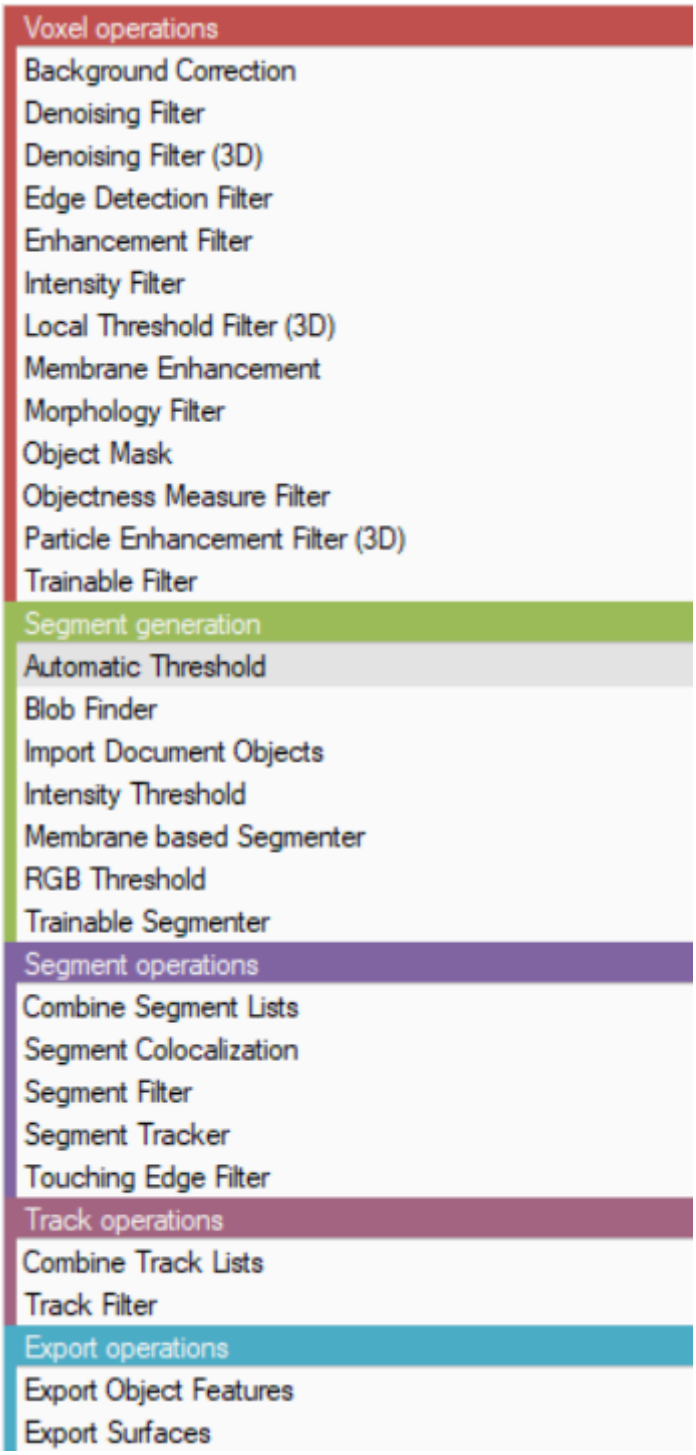
Segment operations process existing segments to refine or enhance the results of the segmentation. This can include filtering segments to keep or discard them based on any of their measurable properties, of processing them to extract some properties of the segments based on other segments in the image set (e.g. tracking, distance measurements)

4) Track operations

If tracks have been generated, these operations can be used to filter tracks based on any measurable track property or to combine multiple track lists (e.g. tracks made from different channels)

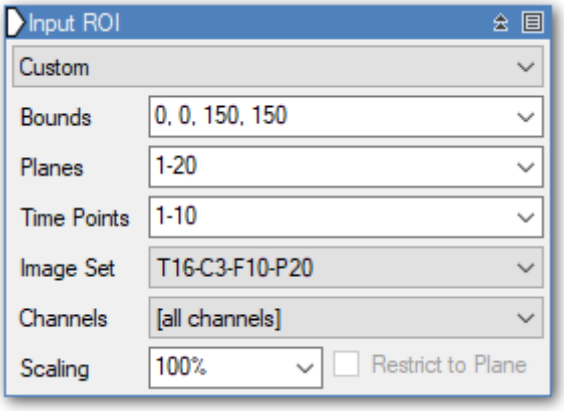
5) Store operations

Because objects belong to the pipeline they must be stored if the user wants to review them at a later date without the need to run the pipeline again. Segments and tracks can be stored within an SIS file for ease of use, or the features or mesh can be exported to external documents for use in other packages (e.g. XLS files for data mining or OBJ files for visualisation in 3rd party 3D viewers)


Voxel operations
Background Correction
Denoising Filter
Denoising Filter (3D)
Edge Detection Filter
Enhancement Filter
Intensity Filter
Local Threshold Filter (3D)
Membrane Enhancement
Morphology Filter
Object Mask
Objectness Measure Filter
Particle Enhancement Filter (3D)
Trainable Filter
Segment generation
Automatic Threshold
Blob Finder
Import Document Objects
Intensity Threshold
Membrane based Segmenter
RGB Threshold
Trainable Segmenter
Segment operations
Combine Segment Lists
Segment Colocalization
Segment Filter
Segment Tracker
Touching Edge Filter
Track operations
Combine Track Lists
Track Filter
Export operations
Export Object Features
Export Surfaces

Input ROI

The selection operation is automatically added to every pipeline and cannot be removed. It is used to define what portion of the image file is to be processed. This is particularly important when dealing with very large images (i.e. larger than the available RAM), as setting up an analysis pipeline may require some trial and error. Generally, when building and testing pipelines it is best to start by working on a small selection of the volume to test out the parameters quickly, and only once the pipeline is complete to then change the selection to run it on the complete image if necessary.



In the selection section, users can select from several options:

- Current view – to process the portion of the image that is visible at the current zoom level in the 2D Viewer
- Current plane – to process only the current plane
- Current time point – to process every plane in the current time point
- Current image set – to process every plane and time point in the image set
- Custom – to manually select from a region of interest from the current planes and time point

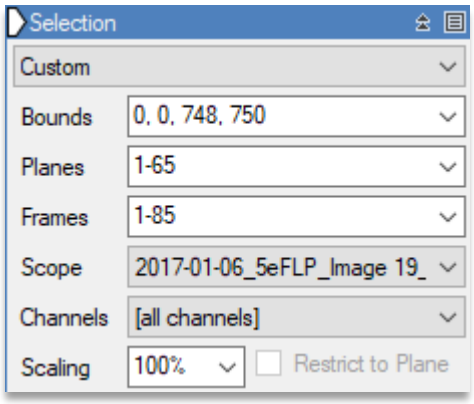
Additionally, users may choose to work on only a portion of the available channel or at a scaled resolution.

Generally it is best to create and edit the pipeline with the image displayed in the 2D Viewer as this will give the user better visibility of the results of operations with the added possibility of previewing many operations, which can't be done in the 4D Viewer. The 4D Viewer will display objects and filtered images, but only after the operations are run with no option for previewing.

Custom selection

When using the custom option, the user defines a region from a selected image set, using the following parameters:

- **Bounds**: Specify a rectangular region of a plane as follows: X and Y of the top left corner, width, height.
  - All, the default option, selects the whole plane.
  - View uses the bounds of the current 2D Viewer.
  - Object uses the bounding box of any selected objects.
- **Planes**: Specify the planes to use in the analysis. This can be a range "10-20", individual planes separated by a semicolon, or a combination of both.
  - Current, the default option, selects the current plane.
  - All uses all planes of the current time point.
  - Object uses the plane extent of the bounding box of any selected objects.
- **Time points**: Specify the time points to use in the analysis. This can be a range "10-20", individual planes separated by a semicolon, or a combination of both.
  - Current, the default option, selects the current time point.
  - All uses all time points of the current image set.
  - Object uses the time point extent of the bounding box of any selected objects.
- **Image set**: Select the scope to use in the analysis.



Channels selection

When working with multichannel images, not all channels may be necessary for processing and segmentation and removing them from the selection can save time.

To select only one or all channels, select the applicable option from the Channels drop list.

To select only some of the channels, select the Custom option and then Ctrl-click the channels to select. Only the highlighted channels will be available for processing in the pipeline.

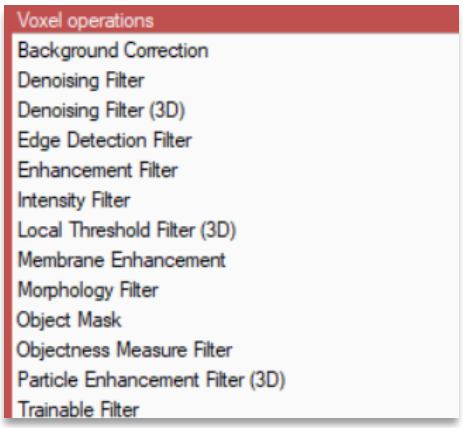
Scaling

Working with the image at full 1:1 resolution can mean more precision and better accuracy, but depending on the size of the volume can require a lot of processing time. Reducing the volume selection can help, but in many cases the precision and accuracy may not be required (e.g. if the purpose it to identify a masking region or to identify a track for drift correction). By scaling the

resolution of the image down in the pipeline it is possible to significantly reduce the computing time. Each reduction of 50% of the resolution can reduce computing time by as much as 75%.

Because in many cases images have higher resolution in XY than in the Z axis, it may be preferable to only apply the scaling to the planes. Use the “Restrict to plane” option to restrict the scaling only to the XY dimensions.

Voxel operations



Voxel operations are those that change pixel intensities in the selected channels. This can be used to enhance the image (e.g. denoising), highlight specific structures (e.g. morphological filters), and masking. Each voxel operator will generate a new image that the pipeline can use for further operations. The location for these new images is set to be a temporary document in the location set as default in the preferences, but this can be changed if the resulting image needs to be saved for use beyond the current pipeline. Because each voxel operation creates a new version of the image that is additional to the original image data it is recommend that the user ensure their working directories have plenty of space to store these. It is generally recommended that users preserve at least 5 times as much spare disk space as is required to store the raw data, though off course more space may be required if more operations are run.

To add a voxel operation to your pipeline, select it by double clicking on the entry in the Analysis operations section, or drag it from the Analysis operations into the desired position in the pipeline.

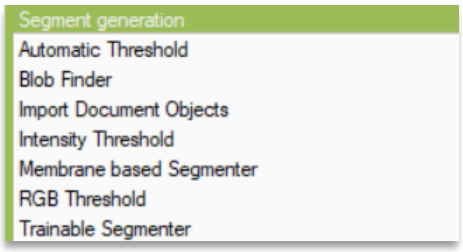
Multiple voxels operations can be applied consecutively on the same channels if required. The operations will be executed in the order in which they appear in the list. To modify this order, simply drag and drop the title bar for a selected operation in the pipeline from its current position into its desired location.

Several operations include an option to preview the results. If this option is available, an Eye icon will appear. Clicking the icon will enable the preview. If changes are made to the parameters these changes will automatically apply to the preview when the user presses the Enter key on their keyboard or the focus of the dialog changes.

Use the Menu icon in the title bar of each operation to rename it, remove it, set the output location or review the output after the operation has run.

Please see the use cases and tips and tricks sections below for advice on which operations to use in which cases. Consult the help files by clicking the F1 key on your keyboard for details of specific operations.

Segment Generation



Segment generation operations create the geometries that define the bounds of objects. These geometries can be generated through segmentation operations in Vision4D, imported from the document or through scripting via MATLAB. Segments in the pipeline can be further refined or processed in additional steps of the pipeline, visualised in the 2D or 4D viewers, and can be used to measure or otherwise characterise objects. Segment objects are necessary for operations like tracking and can also be used in masking operations in subsequent pipelines. Segments already in the document can be imported by using the “Import Document Object” operation.

There are three types of segment generation operations available in Vision4D:

- Import based, where the user already has segments and imports them into the pipeline for further processing
- Threshold based, where Vision4D will use some form of intensity threshold operation to generate the outlines of objects
- MATLAB segmentation means using a script to send the image data to MATLAB for processing and returning the outlines of objects as generated by MATLAB

As with voxel operations, segment generation operations can be added to the pipeline by double clicking on them or dragging and dropping them into the pipeline to their desired location.

As each segement generation operation is run, segments will appear on the image and in the data table. Each segment generation operation or segment operation adds a tag to the segments it affects. These tags can be used to display objects in the data table according to which operations have impacted them. For example, a segment that is part of a track will have at least two tags, one relating to the segment generations and the other relating to the tracking. Tags are generated from the name of the operation, so renaming the operation changes the tag. For example, renaming an segment generation operation from “Intensity Threshold” to “Cell Type 1” will apply the tag “Cell Type 1” for all segments generated by that operation.

Usng a different colour for different operation can help to differentiate visually on the image from various object types.

Importing objects

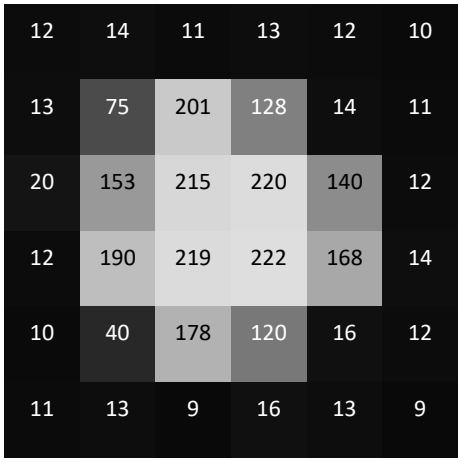
The best import method will depend on the type of image the user is working from and the intended workflow. Importing the objects may sometimes be the only option if the segments can't be generated automatically in other fashions, but can also be useful in other cases.

Objects must have a tag to be imported into a pipeline. Tags are applied automatically to objects as they are created by the tool used to create them. In the case of manually defined segments and objects the tag is usually the name of the tool used to create it and/or the name of the Object for markers. To define a custom set of objects the user can add custom tags by using the "Add Tag.." entry in the context menu of the Object table.

Threshold based segmentation

Threshold based segmentation methods use either the pixel intensities or the intensities of filtered versions of the image to identify groups of pixels and generate their bounding geometries.

In the following figure you can see a small kernel of pixels with their values shown on the image.



In this case, the object of interest is made up of bright/high intensity pixels relative to the background. By selecting only those pixels above a certain intensity threshold it is possible for the software to analyse this image and identify contiguous groups of pixels within the selected intensity range as a single object.

12	14	11	13	12	10
13	20	201	128	14	11
20	177	215	220	190	12
12	190	219	222	168	14
10	40	178	120	16	12
11	13	9	16	13	9

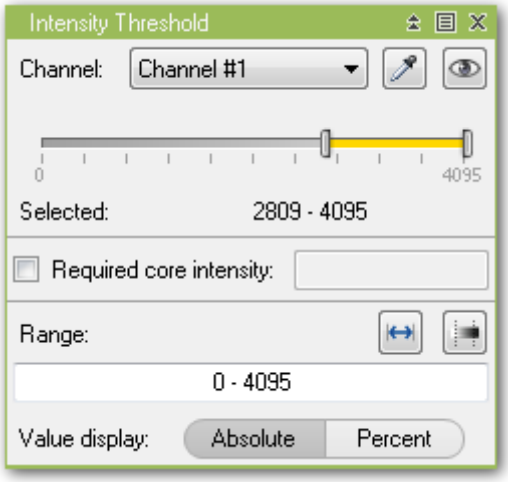
In this case, only those pixels whose value is above the 100 greyscales intensity threshold are considered to be part of objects. This segments out a contiguous group of 16 pixels. Those pixels

that share a side with pixels that are not part of the group are considered to be edge pixels which the software then uses to measure circumference. If the pixels are over multiple planes they are considered to have a thickness and are called voxels. If the image is calibrated, the pixel size information can be used to calculate a range of geometric values corresponding to the group.

The exact threshold value will clearly have an effect on the measure values as fewer or more pixels/voxel will be included in the group. In this case, a threshold of 20 or above would mean two additional pixels would be included and the measures size of the object would increase 12.5%. Therefore, some care should be taken when setting thresholds to ensure reliability and repeatability in the results. This will, off course, be also dependent on the quality of the image data.

When working with threshold based segmentation the software selects voxels from a range of intensities, from lowest to highest values allowed. The threshold levels can be set manually or automatically by choosing either an automatic segmentation method.

Intensity Threshold operation

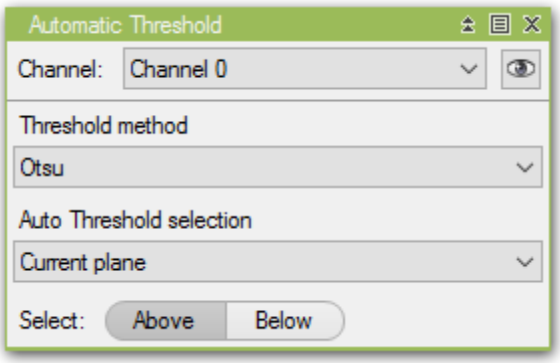


The Intensity Threshold operation identifies objects based on the intensity and proximity of their voxels, as described above. In this case the threshold values are set by the user either by adjusting the sliders, picking them from the image using the picker, or manually typing the selected values.

To manually pick the colours from the image, click on the colour picker icon in the operation window, then click on the image to select a voxel of the minimum required intensity. All pixels with the same intensity or above that of the selected pixel will also be included in the selection.

Please consult the help files for further details.

Automatic Threshold operation



The Automatic Threshold operation works in much the same way as the Intensity Threshold, but uses algorithms based on the range of intensities in the image to automatically set the upper and lower levels. This can provide a more systematically unbiased to set the threshold across multiple datasets of varying intensity.

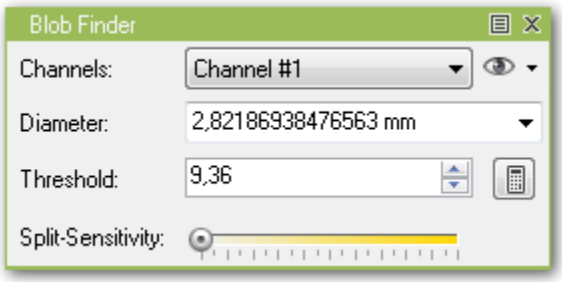
Please consult the help files for further details.

RGB Threshold

The RGB Threshold operation works by setting multiple thresholds based on the RGB display on the image. It is not based on the channel intensity values, but on the display colours. A single channel displayed in Cyan will require a threshold in both green and blue.

As with the Intensity Threshold, the threshold values can be set either by adjusting the sliders, using the picker and clicking on the image or by manually typing the selected values.

The Blob Finder

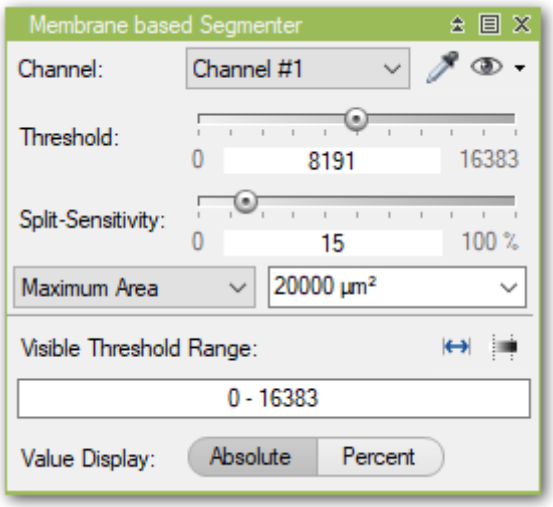


This operation is used to find rounded 2D and 3D segments close to sphere-like shapes in a noisy image.

The Blob finder is a special kind of threshold based segmentation method that includes a combination of automatic seed finding based on structural information of an object map, and a watershed algorithm.

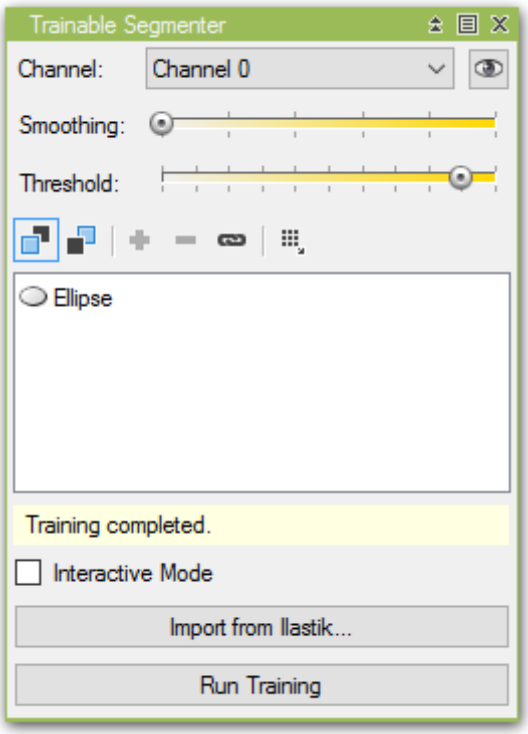
This operator uses the Gaussian scale to find the object seeds and a watershed algorithm to identify object boundaries, and therefore needs no additional filtering. Best results are found with no filtering or some enhancement filtering (such as background subtraction).

The Membrane Based Segmenter



The Membrane Based Segmenter finds objects by their bounding outline. It can be particularly useful in finding objects such as living cells that are bound by a membrane. It is based on first thresholding the membranes and then applying a watershed algorithm. This operation may work well on it's own in images with good signal to noise ratio and an even background, but is often best combined with the Membrane Enhancement voxel operation as a pre-processing step.

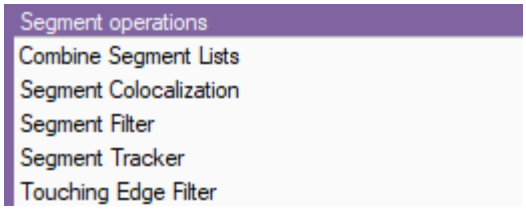
The Trainable Segmenter



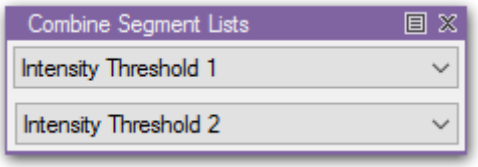
The trainable segmenter generates segments based on a classification results. By using training regions for background and objects and various filters to classify pixels the software can identify regions that could not be identified on threshold alone.

Segment Process operations

Segment process operations take existing segments as an input and apply tags to these based on a set of conditions defined by the chosen operation. This can be used to filter segments out based on their properties, combined segments from multiple sources into one list, identify segments based on their proximity to others, and track them.

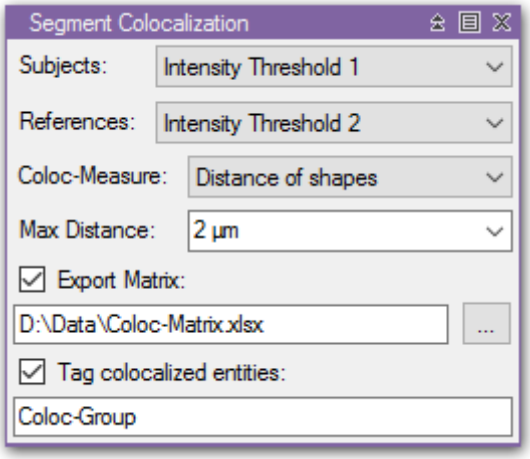


Combine Segment Lists



This operation can be used to merge multiple segment lists into one. This can be useful to display multiple segment types after filtering, thereby keeping spurious objects out of the display.

Segment Colocalisation



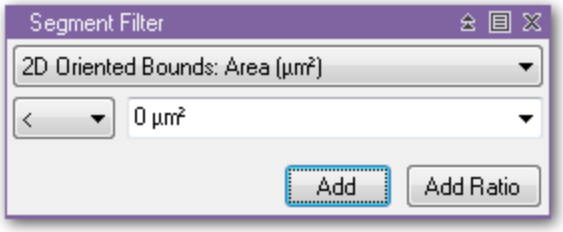
This operation can be used to tag segments based on their proximity to other segments of a different type. The operation requires two input:

- Subjects are the segments the user is looking to filter or characterise
- Reference objects are the segments whose proximity define the status of the subjects

The operation works by calculating a geometrical distance between the references and subjects, either based on the object's geometries or their bounding boxes. The level of interaction allowed between subjects and references can be limited by setting up a maximum distance radius, or by specifically looking for subjects that are either partially or fully covered by the references.

The calculated distance cannot be displayed in the Object window. If the user needs such measurements, those values must be exported to an export matrix which lists all references and subjects and their measured distance from each other as defined in the Coloc-Measure field.

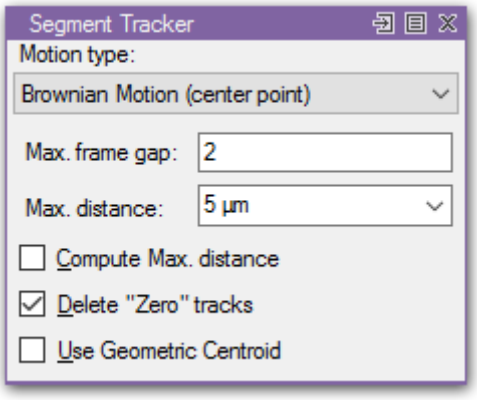
Segment Filter



The Segment filter works by tagging objects based on whether one or more of its features meets certain criteria. This can be particularly useful when trying to refine analysis to remove spurious objects. In many cases, the objects that the user seeks to characterise fit within a certain range of expected shapes and sizes. Using the Segment filter can be used to only highlight those segments whose features fit within those expected values.

The segment filter can be used to filter multiple based on multiple features simultaneously, simply click the dropdwon arrows and click Add to select additional features and conditions. Users can also use the Add Ratio option to filter objects based on the ratio of two object features together with an inclusion condition.

Segment Tracker



This operation groups multiple segments over multiple time points into a track group which can be displayed as an Object. This segment Object can also be used to calculate features that characterise the movement of the objects. Tracks are not a geometry in the same way that segments are and

have different features. Segment features do not apply to tracks and track features do not apply to the segments in the tracks.

The segment tracker works generally by attempting to match objects in one time point with objects from the preceding time point based on a range of conditions.

The Motion type condition is used to define what type of motion is expected of the objects.

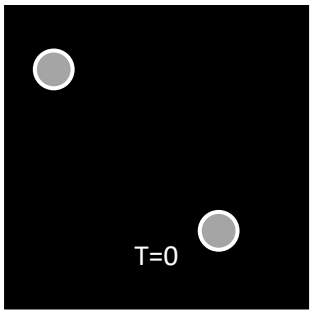
If objects move in a generally linear direction, a linear regression algorithm can be used. It will work generally better if objects are in close proximity within a time point, but will most likely fail to correctly recognise tracks if the objects suddenly change direction or move back on themselves.

If the motion of objects is more chaotic and objects can move in any direction at any time, then a Brownian Motion method may be more suitable.

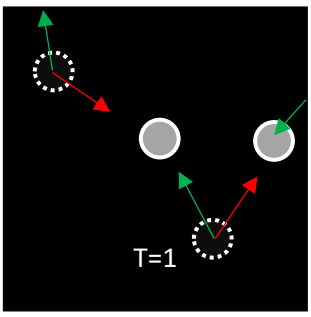
The Max time gap option can be used to compensate to some extent for case where objects disappear in one time point and reappear later. The disappearance can be caused by noise in the image or the overlapping of one object by another, but in either case it is expected that the object will reappear.

The Max distance option is used to set the maximum distance an object is expected to move from one time point to the next.

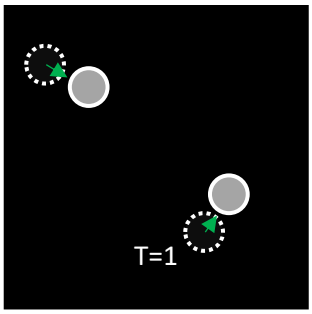
In many cases the correct identification of tracks can be challenging due to various factors, especially if multiple objects are present in each time point. In general, to facilitate the correct identification of tracks and reduce errors it is recommended that experimental setup is such that interframe object movement is no more than 20% of the intraframe object separation.



Time point 1 – two objects have been identified



Interframe movement ≈50% of intraframe object separation, two previously identified objects are no longer in their original position, but multiple scenarios are equally likely



Interframe movement ≈20% of intraframe object separation, two previously identified objects are no longer in their original position, but only one likely candidate in each case

The segment tacker operation is best used after a segment filter operation as this step will remove false positives and facilitate the correct identification of tracks.

Once the tracking operation is run, the track editor can be used to refine results if needed. The track editor gives the user the option to split, merge and link tracks in cases where the calculated track may not match the user interpretation. Please see the Track Editor section below for more details.

Touching Edge Filter

The touching edge filter is used to specifically tag objects only if their boundary is not in contact with any of the image set borders. This option is particularly important depending on the type of measurements that are required from the object and deals with the fact that random distribution of objects means some will inevitably appear to be cut off by the border of the image frame.

When considering objects that are touching the edges of the images there are essentially three types of scenarios likely.

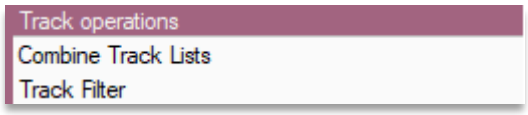
Object count – in this case the user is interested in measuring the total number of objects, however, in most cases the absolute count is an unrealistic figure to obtain and values such as numerical density (objects per unit volume), are more informative. When dealing with a rectangular image frame and assuming a random object distribution, objects are equally likely to touch either opposite borders. Also, this random distribution means that over multiple samples the typical object size for an object that touches the border is 50% that of a whole object. Likewise, with objects that touch the corners, the typical corner object size will be 25% of a whole object. Therefore, it is common in this type of application to only count objects if they do not touch any of two adjacent borders and to count all objects that do not touch those two borders as whole objects for this purpose.

Object size and shape characterisation – in this case users are interesting in measuring the individual characteristics of objects. Since by definition it is impossible to measure what can't me be sampled, objects whose boundaries cross the border of the image cannot typically be included in those measurements. In this case it is typical to include objects only if they are fully within the image frame.

Percentage volume/area covered – in this case the user is typically unable to measure individual objects are is more concerned with the ratio of the total volume of interest covered by one type of objects or another. In this case, individual object characteristics are irrelevant and all objects identified are included so this filter is not required.

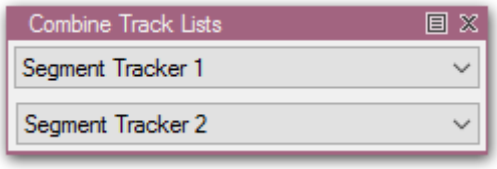
Note that if all three types of characterisations are required, the operation may be added multiple times to the pipeline with different parameters to add the required tags to each group as needed.

Track Process Operation



Track process operations, much like segment process operations, apply tags to track groups. These can be used to combine multiple track types into a single group, or filter tracks based on features of those tracks.

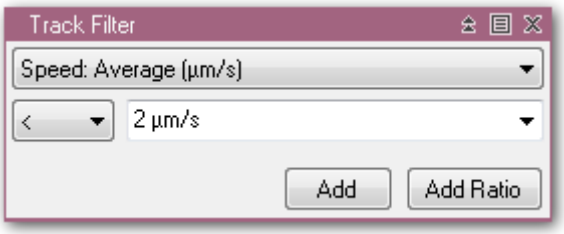
Combine Track Lists



This operation can be used to merge multiple track lists into one. This can be useful to display multiple track types after filtering, thereby keeping spurious tracks out of the display. Simply select which track lists to combine using the dropdown lists.

A least two track operations are necessary to use this operation. Only two track lists can be combined per operation. If more lists need to be combined, more Combine Track Lists operations can be added.

Track Filter



The track filter operation works much the same way as the segment filter by adding tags to tracks based on their features or combination of features.

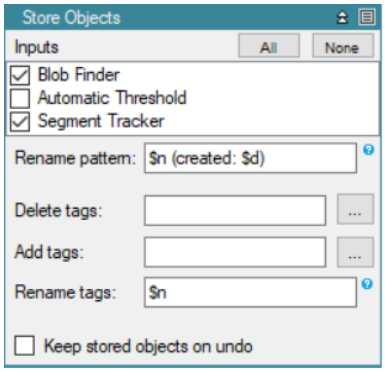
Select the feature type and rule from the dropdown lists, then select the threshold value(s) for your exception rule to define your filter.

Click Add to add another filter to filter by. Objects will only be tagged if they match all of the filter rules. As with the Segment Filter operation, a Ratio of features can be added by using the Add Ratio button. Expanding the dialog using the expand icon in the operation menu bar may be necessary to reveal this option.

Store Operations

As mentioned above, objects can belong to either the document or the analysis pipeline. Objects in the pipeline are temporary objects that are discarded once the application is closed. However, the results of an analysis pipeline can be stored in a variety of ways depending on future requirements.

Store Objects

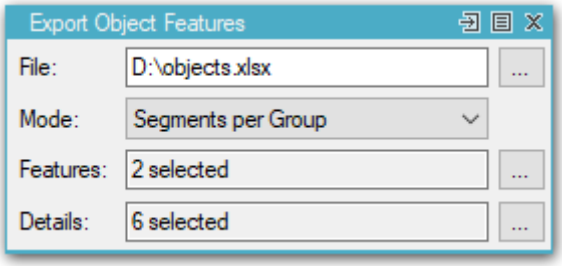


This operation stores the objects from the pipeline into the document. Saving the objects in the document means they can be reviewed in the objects table, with all of the options available in the Object table, on any system with arivis Vision4D as long as the user keep the metadata file together with the SIS file.

The Store Objects operation is added to the pipeline by default, but the user must select which objects to store by using the check box for each segment tag as required. All object tags can be added by clicking on the All button, and can all be deselected by clicking the None button. Note that as mentioned above, if this operation is not run, or is run without having selected any objects to save the objects will not be discarded when the pipeline is completed.

Please check the help file for more details of additional options relating to this operation.

Export Objects Features

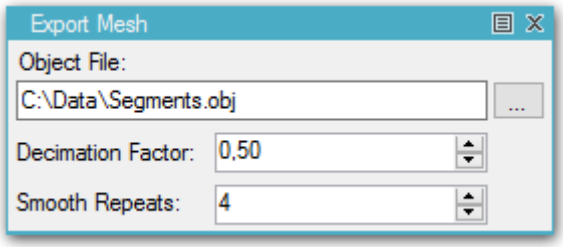


The Export Objects Features operation is used to create a Microsoft Excel file containing the features of the objects only. Outlines of objects are not included in this export since this is not supported by Excel.

Once added to the pipeline, the user must select a save location, either by typing the path in the Export File field, or by clicking the … button to the right to browse to the save location and selecting a name.

Only selected features are included in the export, so the user must use the check boxes next to the feature or group of features to select or deselect which features to include as required.

Export Mesh



The Export Mesh operation is used to export the outline of objects to a Wavefront .obj file for use in other compatible software packages. OBJ files can be opened by most 3D visualisation and computer aided design software packages.

Displaying Analysis Results

Once analysis operations are completed, the viewer will update automatically to display the results of that operation. Voxel operations will display the process image, segment generation and segment or track filter operations will display the segments in the viewer and also the objects table. However, additional options are available to adjust the display options for objects within the viewer and to sort or graphically plot the results in the Object table.

Object Visibility

The main visibility options for the display of objects in the viewer are found in the Objects Window. Click on the Visibility button to open the Visibility panel.

The options affect both the 2D and 4D viewer, though the options regarding the bounding box only apply to the 4D viewer. In the 2D viewer the objects are tinted and outlined and changing the opacity affects the tint only, while in the 4D viewer the objects are rendered as surfaces and the opacity affects the opacity of the surface. These changes in opacity in the 4D viewer are part of the parameters stored in keyframes in the storyboard. Note that the opacity of objects in the 4D viewer may slow down rendering speed in the 4D viewer as transparent objects have a higher computational requirement.

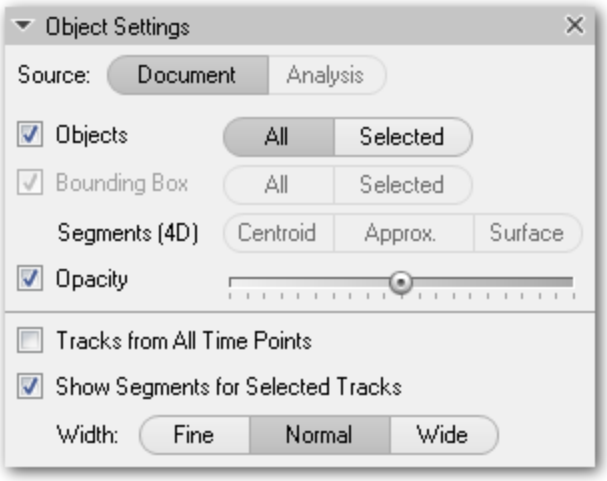
Once the surfaces have been displayed they will be saved within the document when the sis file is saved and will be displayed much faster when opening the file again.

In the 2D and 4D viewer workspace toolbars are options to display or not the objects by checking or unchecking the box.

In the 4D viewer, the option to show objects is part of a list of segment display options:

In this case the user can choose to display the object centroids, centroids scaled to the object bounding box or polygonal surfaces. Clicking on one or multiple object(s) using the selection tool in either the 2D or 4D viewer, or by selecting them in the Object table will activate those objects and they will be displayed with a bounding rectangle in the 2D viewer, or highlighted in white in the 4D viewer. The option to display the bounding box for selected object in the 4D viewer can be particularly useful in identifying an object in the viewer if there are a large number of objects.

Additionally, the Objects Settings palette provides further visibility settings. To open the Object Settings palette right-click in the Palette space or go to the Window> Palettes menu to open the Palette Selection menu, and select it from the list:



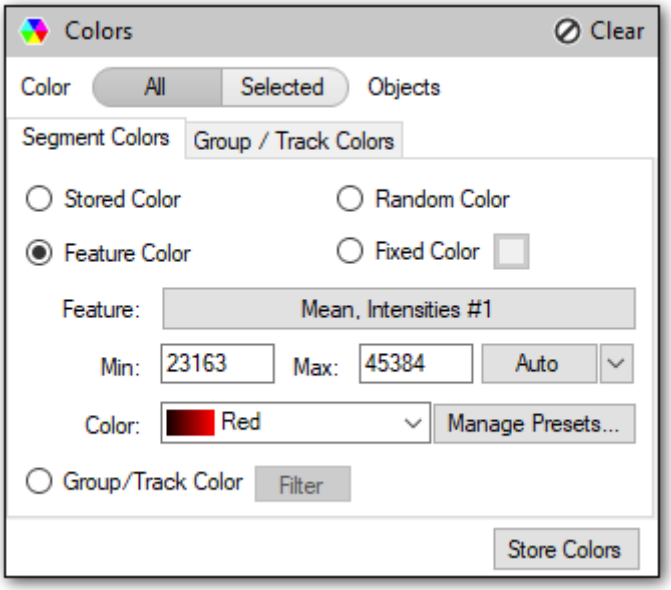
Please consult the help files for full details of the Objects Settings palette options.

Object Colors

Along with the various visibility settings described above, advanced colouring options can be a great way to facilitate a visual interpretation of the rendered image and objects.

As seen above, objects are colored through the pipeline operation. Object creation steps typically assign a colour to the objects created and object operations can also modify those assigned colours. However, more coloring options are available through the Colors panel of the Objects window.

As with the object Visibility panel, this panel can be opened by clicking the Colors in the Objects window.



Object colours can be adjusted differently depending on whether the objects are segments or groups or tracks. It can also be used to apply a different color to all objects in the table, or only to the selected objects. To switch between segment colors and group/track colors, select the correct tab from the top to the Colors panel.

Most coloring options are the same for group/tracks and segments, except for the following:

- Objects can be colored according to the track colour and vice versa
- Tracks can be color using a time gradient with the color indicating where in the sequence a track finishes or ends.

When objects are created or tagged in the pipeline, the default is to display those objects using the Stored color as assigned by the operation. However, in many cases changing the color can be useful to further differentiate objects in the viewer.

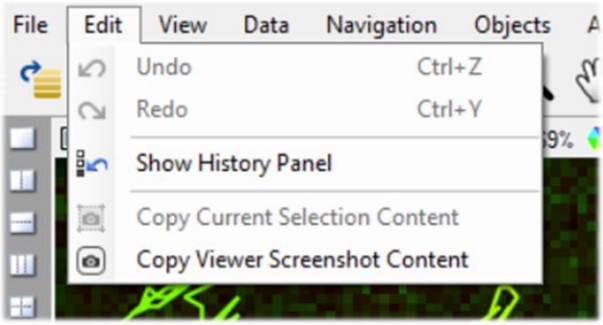
When transient coloring (without storing) is applied the color indicator at the top of the Viewer Window as well as in the Objects Settings Panel is visible.

When dealing with a multitude of closely packed objects it can be difficult to quickly identify where one object ends and the next one begins. Using random coloring makes it easier to see this delineation.

When looking at the distribution or range of certain features of objects, the graphing tools are useful, but changing the colour of objects according to a specific feature makes it easier to see this information in context. Feature coloring is available for any object and any feature, whether it is a custom or standard feature.

Exporting Image Views

Having adjusted the display options as needed, users can export the content of the viewer for display in other media (presentations, posters, papers, etc.). The simplest way to export images with the analysis result is to copy the viewer content. Simply go to the Edit menu and select the option to Copy Viewer Screenshot Content to add it to the clipboard. Then, simply paste it into a word processing, presentation or desktop publishing software.



However, copying the viewer screenshot will produce an image of the same resolution as the display. To create higher resolution images, users can use the High Resolution Image or Storyboard tool in the 4D Viewer to produce high resolution snapshot or animation that can include Object display, changes in transparency and clipping etc.

Please consult the Basics guide or help files for further details on these tools and how to use them.

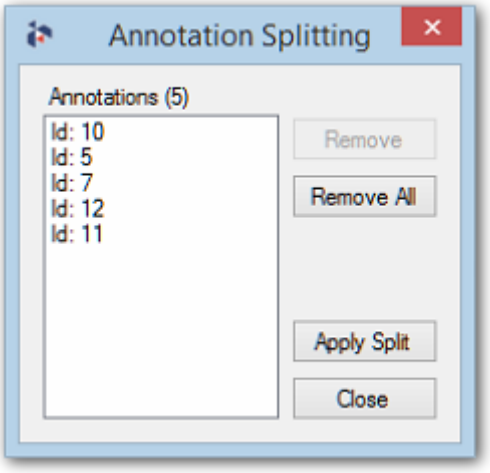
Editing Analysis Results

Having used the pipeline to create tracks or objects, it may, on occasion, be necessary to further manually refine the results, and arivis Vision4D provides tools to manually split incorrectly connected objects, merge or rearrange tracks.

Splitting Objects

The best way to split objects is within the 4D Viewer. This allows the user both to better see the shape of a segment in 3 dimensions, but also to better position the object so that the split line corresponds to the object orientation.

Activate the splitting tool by clicking the  icon in the shortcut toolbar. This will also open the splitting window.



Click and drag across the object you want to split and the selected object will appear purple. Click **Apply Split** in the Object Splitting window to actuate the split and the objects table and viewer will automatically update.

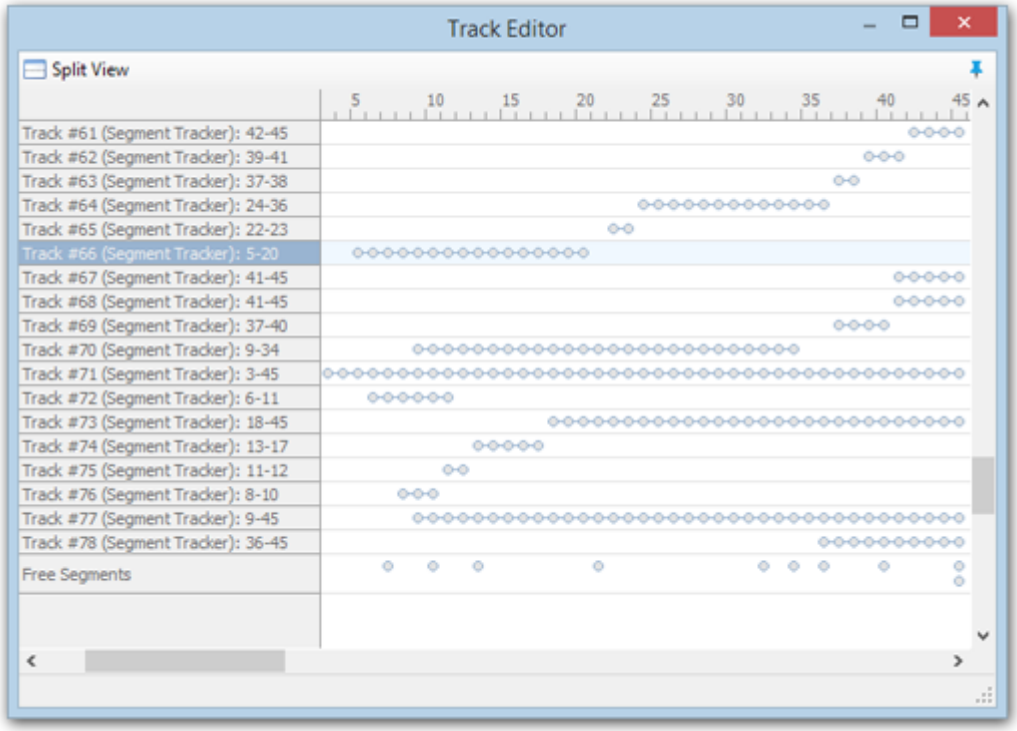
Sculpting Objects

Along with splitting objects, it may be sometimes necessary to sculpt objects by adding or removing pixels from the objects. Use the Draw Objects tool to do this if necessary.

Track Editor

Depending on the quality of the segmentation and tracking operation, it may be necessary to correct some tracks for accurate results.

Tracks can be split or merged from the track editor window. To open the track editor, click on the  icon in the analysis panel toolbar.



Identify tracks by clicking on a track in either the viewer or the Track Editor window. The tracks appear in the Track Editor window as a series of interconnected segments. Each dot represents a segment with the line connecting them representing the track. Free segments appear at the bottom of the table.

Use Cases

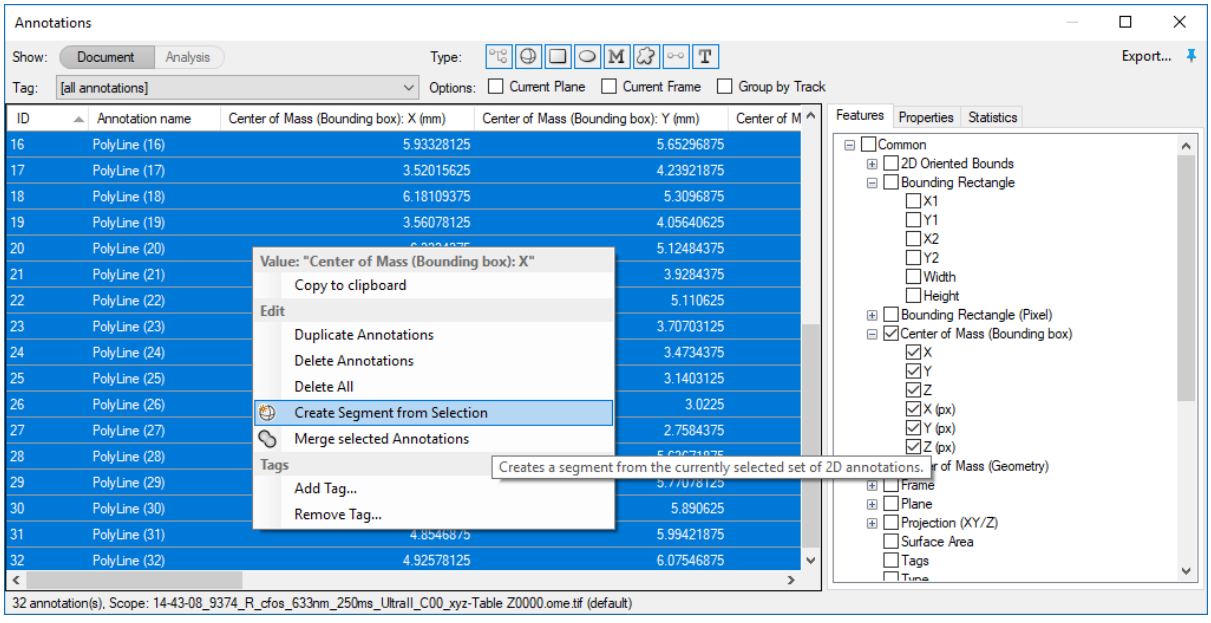
For all the following use cases we recommend you start by opening the demo image provided and have the analysis panel open.
Please download the demo images from here:

http://demodata.arivis.com

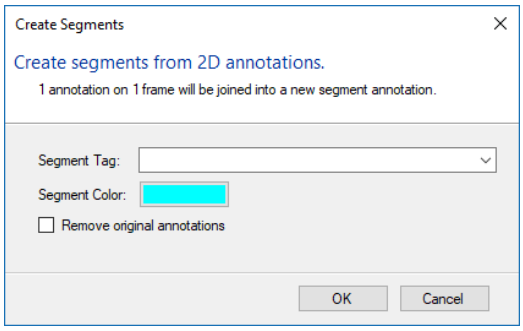
Analysing data from a manually defined region of interest

Example Image:

1) Find an approximately central plane to the region you are interested in.
2) Use the manual objects tool to define the outline of the region in the current plane.
3) Scroll up and down through the planes and define additional regions as needed. It is not necessary to have objects on every plane, but you should aim to have enough plane for a correct interpolation. More globular objects will require fewer outlines, more irregular objects may require more. Make sure you have objects at the very top and bottom of your region.
4) Open the objects table and select the first Object.
5) Use shift+click or ctrl+A to select all the objects that define your objects.
6) Right-click on your selection and select the “Create Segment from Selection” option



7) Select a tag for your Object. For the sake of clarity you may want to also remove the original objects from which the segment is generated.



8) Open the analysis panel and create a new pipeline if necessary.
9) Set the Selection operation to custom and adjust the bounds and planes to match the region dimension.
10) Add the Object Mask operation to your pipeline and select the tag created in step 7.
11)

Using selection scaling with an intensity threshold to identify a region of interest