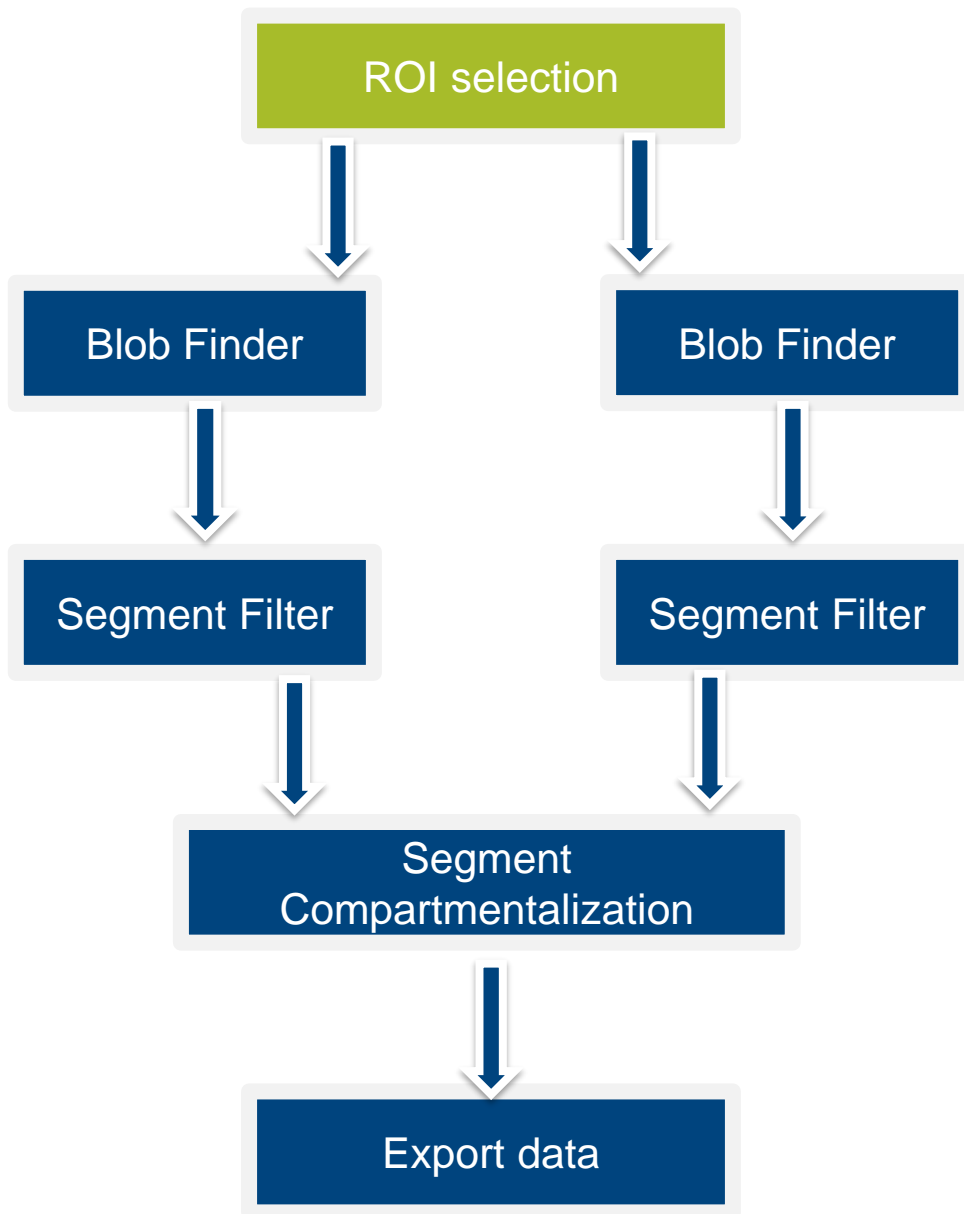


## How to guide - Sample Pipeline «COLOCALIZE PARTICLES»

The pipeline purpose is to detect objects, falling in two different classes, using Blob Finder operators. The detected objects are compared to find partial or total overlap conditions. It can be applied to Cells, Nuclei or any kind of small particles.

## Working Flowchart



## Index

1. Download the demo dataset
2. Open the demo dataset
3. Select and activate the sample pipeline
4. Pipeline operators layout
5. Execute the pipeline step by step
6. Execute the pipeline in a single run
7. View the results
8. Modify the pipeline

# 1. Download the demo dataset

## Step 1

In order to run the pipeline described here below, please download the demo dataset according to the following instruction.

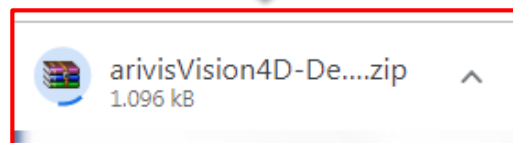
### Step 1.1

Click on the below link to access to the Arivis downloading demo dataset's area.



[Demo Dataset](#)

*arivisVision4D-DemoData-SamplePipelines-DetectStructures.zip* file is saved on the download folder.




### Step 1.2

Create a new folder on your local disk.  
Move the ZIP file from the download folder inside it.

### Step 1.3




UnZip the file:

*arivisVision4D-DemoData-SamplePipelines-DetectStructures.zip*

 arivisVision4D-DemoData-SamplePipelin... 10/12/2018 07:39



Three files are now available in the folder.

	4Channels_3DStack.metadata	06/12/2018 10:12	File METADATA	4 KB
	4Channels_3DStack.objects	06/12/2018 10:12	File OBJECTS	108 KB
	4Channels_3DStack	06/12/2018 10:12	arivis SIS file	4,190 KB

## 2. Open the demo dataset

### Step 2

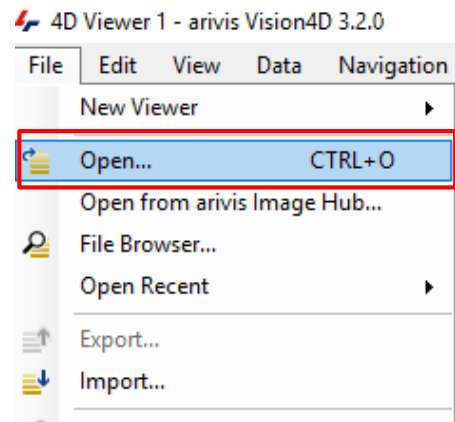
Open the Dataset on Vision4D.

#### Step 2.1

Select the *Open..* item from the file menu.

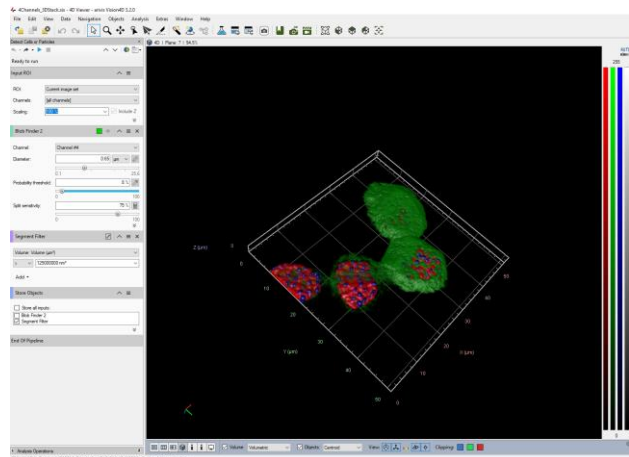
#### Step 2.2

Select the dataset from the file browser.



### TIPS :

The dataset is visualized according to the current rendering setting parameters. Please refer to the (*arivis Vision4D Help*) for further details.



### DETAILS:

The dataset is a multi dimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (Optical sectioning) of multiple channels (dyes) in a temporal sequence of time points (located in several spatial positions).

Usually the dataset shows a single experimental situation ( a complete experiment can be composed by several datasets). The datasets are available as graphic files saved in plenty of file formats (standard formats as well as proprietary formats )

## 3. Select and activate the sample pipeline

### Step 3

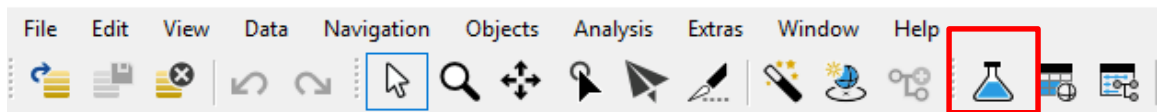
Select and activate the «*Colocalize Particles*» pipeline.

#### Step 3.1

Open the Analysis panel

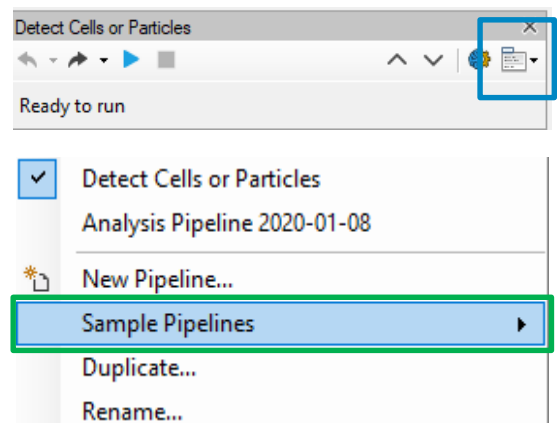


4Channels\_3DStack.sis - 4D Viewer - arivis Vision4D 3.2.0



#### Step 3.2

Drop down the analysis panel menu.



#### Step 3.3

Select *Sample Pipeline*.

#### Step 3.4

Click on the *Colocalize Particles* item.

Colocalize Particles

#### TIPS :

The active Pipeline, if any, will be replaced by the new one. Please refer to the (*arivis Vision4D Help*) for further details.

## 4. Pipeline operators layout

### Step 4

Pipeline operators layout.

#### Step 4.1 *Region Of Interest:*

This operator allows the region of interest (ROI) selection. ROI defines the dataset subarea that will be processed and analyzed by the pipeline.

#### Step 4.2 *Denoising Filter:*

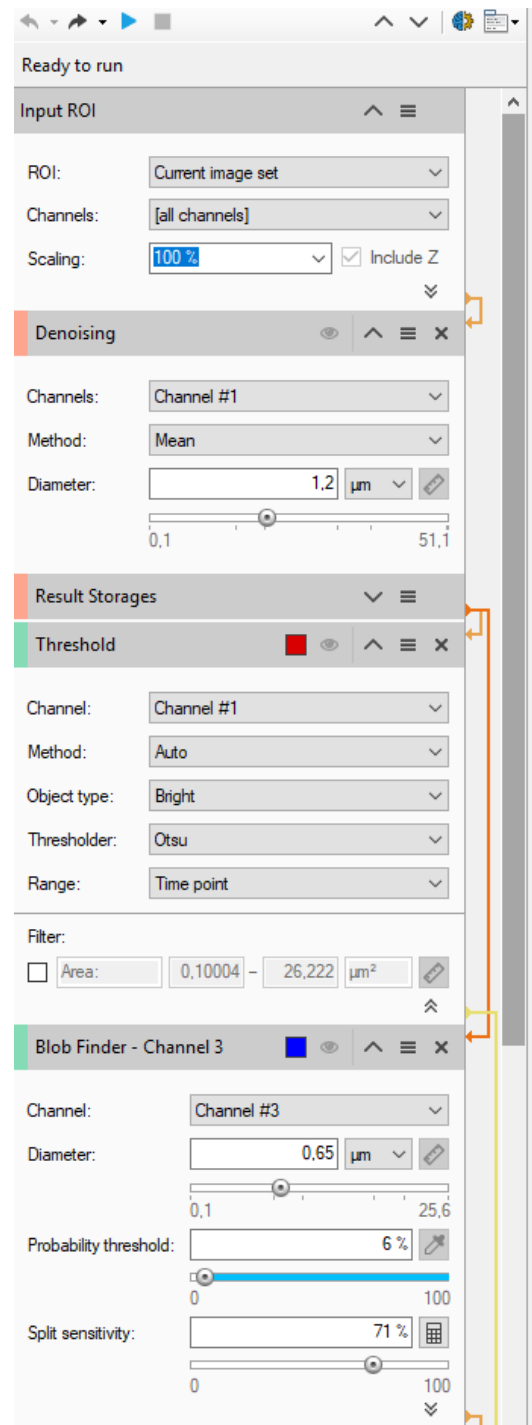
Set of operators performing noise reduction. The «Discrete Gaussian Filter» with diameter of 1.2  $\mu\text{m}$  is used.

#### Step 4.3 *Threshold*

Allows the segments detection using one of the available automatic threshold algorithm (Otsu method is used).

#### Step 4.4 *Blobs Finder:*

Automatic small objects detection algorithm. It uses a local threshold method.



## 4. Pipeline operators layout

### Step 4.5 *Segment Filter*

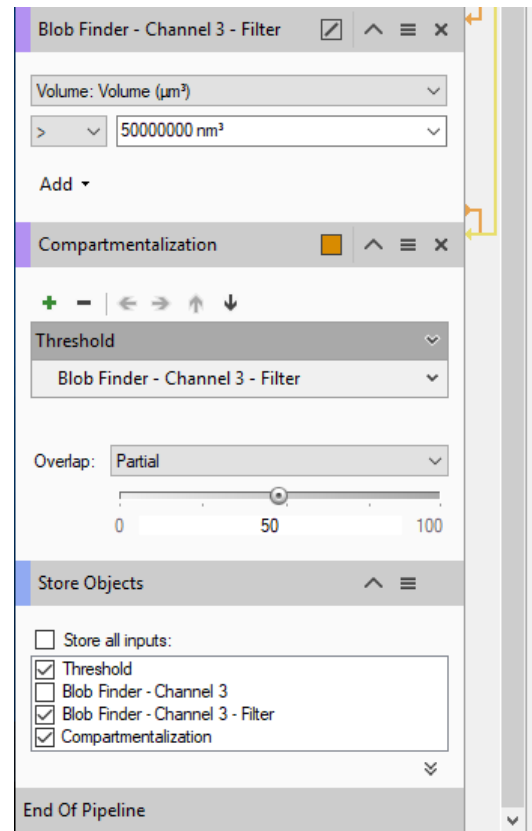
Allows the blob filtering based on multiple parameters selection.

### Step 4.6 *Compartmentalization*

The detected objects' positions are compared to verify its partial or total overlap conditions.

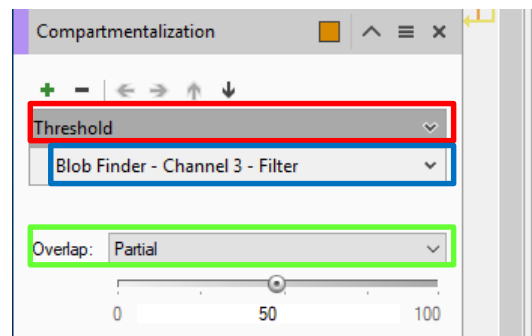
### Step 4.7 *Store Objects*

Store the detected segments (TAG) in the active dataset.



### DETAILS :

Compartmentalization requires the definition of a **reference Tag** and a **subject Tag** as well as the **Overlap** method (Partial/Full) selection. The Subject segments are compared to the Reference segments to verify if they matches the criteria of compartmentalization





## 5. Execute the pipeline step by step

### Step 5

Execute the pipeline step by step.

#### DETAILS :

The pipeline can be executed step by step (back and forth). This method allows to run and undo a single Operation. Either the arrow buttons or the Operation list can be used to go through the operators list.

#### Step 5.1

Run the single operator



#### Step 5.2 (optional)

Undo the single operator



#### TIPS :

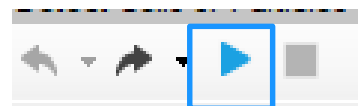
Undo the last operator executed if you need to change the operator settings.

## 6 - Execute the pipeline in a single run.

Execute the pipeline in a single run.

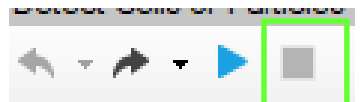
#### Step 6.1

Run the whole pipeline



#### Step 6.2 (optional)

Stop the pipeline execution



#### DETAILS :

This icon, located on the right side of the operator title bar, shows the operator status.

Task running



Task completed



## 7. View the results

### Step 7

View the results.

#### TIPS :

Results (segments and measurements) will be stored in the dataset only if the Store Objects operator has been correctly set.

Please tick appropriately the option as shown below before complete the pipeline execution.



### Step 7.1

Open the data table (if not already visible)



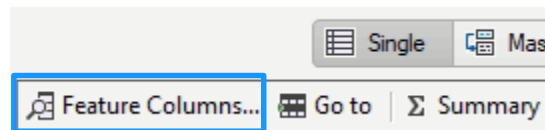
Measurements are now visible in the data table

Segment	Volume, Volume (µm³)	Sphericity
Segment #004 (Blob Finder)	0.164	0.641
Segment #012 (Blob Finder)	0.496	0.546
Segment #013 (Blob Finder)	0.579	0.584
Segment #014 (Blob Finder)	0.308	0.569
Segment #015 (Blob Finder)	0.213	0.575
Segment #016 (Blob Finder)	0.386	0.620
Segment #017 (Blob Finder)	0.430	0.580
Segment #019 (Blob Finder)	0.345	0.585
Segment #020 (Blob Finder)	0.425	0.571
Segment #025 (Blob Finder)	0.237	0.611
Segment #028 (Blob Finder)	0.418	0.643
Segment #030 (Blob Finder)	0.518	0.544
Segment #032 (Blob Finder)	0.318	0.659
Segment #035 (Blob Finder)	0.276	0.525
Segment #036 (Blob Finder)	0.222	0.576
Segment #038 (Blob Finder)	0.562	0.550
Segment #039 (Blob Finder)	0.310	0.559
Segment #040 (Blob Finder)	0.450	0.565
Segment #042 (Blob Finder)	0.279	0.624
Segment #046 (Blob Finder)	0.305	0.601
Segment #047 (Blob Finder)	0.315	0.615
Segment #048 (Blob Finder)	0.225	0.588

#### TIPS :

Features can be added or removed from the data table using the *Feature Column* command.

Please refer to the (*arivis Vision4D Help*) for more details



## 8. Modify the pipeline

### Step 8

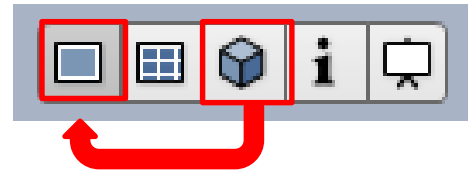
Modify the current pipeline.

#### DETAILS :

The pipeline can be modified to be adapted to another datasets.  
Therefore, all the pipeline parameters should be set according to the new dataset features.

#### Step 8.1

Switch the Viewing area from 4D to 2D view mode.



#### TIPS :

Before starting to modify the Pipeline layout, switch the Viewing area from 4D to 2D view mode.

During analysis setup, the Operator preview mode is only available in 2D mode. Once the pipeline has been executed, you can switch back to 4D view mode to display the segments. Please refer to the **(User Guide)** for more details

#### DETAILS :

Almost all the operators have the preview icon located on the panel header (title bar).



Click on this icon to preview the operator result on the current plane / time point

#### TIPS :

Use the Navigator Panel, located on the workspace area, to select the preview Z plane and/or Time Points (if any). Please refer to the **(arivis Vision4D Help)** for more details



## 8. Modify the pipeline

### Step 8.2

Change the Input ROI' operator parameters

#### Step 8.2.1 – ROI:

Sets the processing and analysis target space.

Current View : The selected Z plane and the viewer area are processed.

Current Plane : The selected Z plane is processed (XY) .

Current Time Point : The selected time point is processed (XYZ) .

Current Image Set : The complete dataset (XYZ and time) is processed.

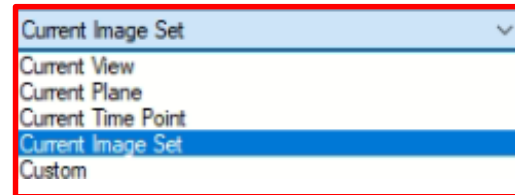
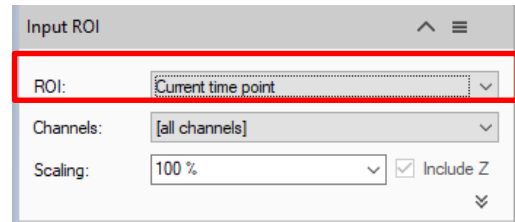
Custom : Allows to mix the previous methods.

Expand the Input Roi dialog.

Bounds : Sets the analysis area edges. The whole XY bounds, the viewing area or a custom space can be applied.

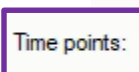
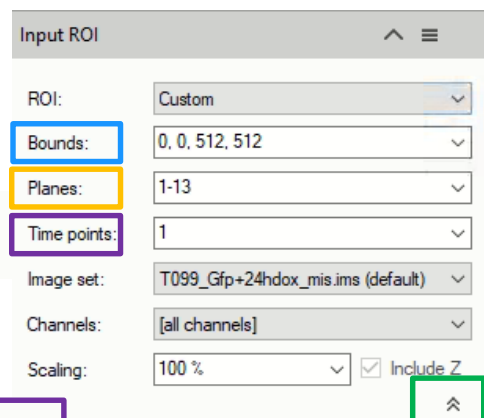
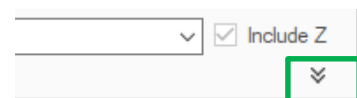
Planes : Sets the analysis planes range. A single plane, a range of planes or the whole stack can be selected.

Time Points : Sets the analysis time points range. A single TP, a range of TPs or the whole movie can be selected.



#### DETAILS :

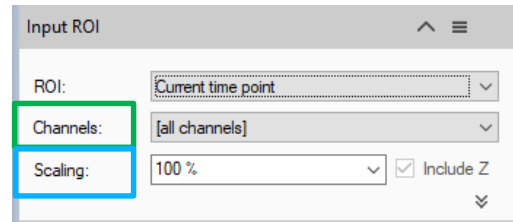
Use the Custom option during the pipeline setting and testing . Set a sub volume (XY, Planes, Time Points, channels) of your dataset on which perform the trial. This will speed up the setting process.



## 8. Modify the pipeline

### Step 8.2.2 – Channels:

Sets the processing and analysis target channels. Selecting a single channel, all the operators in the pipeline will be forced to use it.



### Step 8.2.3 – Scaling:

It scale the dataset reducing the size. The measurements will not be modified by the scaling factor.

#### TIPS :

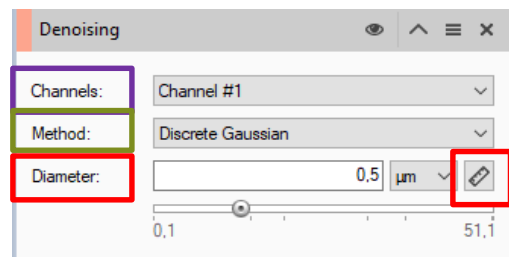
Please refer to the (*arivis Vision4D Help*) for more details

## Step 8.3

Change the Denoising' operator parameters

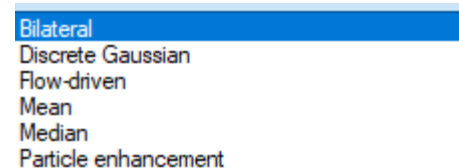
### Step 8.3.1 – Channels:

Sets the processing and analysis target channel(s).



### Step 8.3.2 – Method:

Set the denoising algorithm



### Step 8.3.3 – Diameter:

Set the reference objects diameter.

#### TIPS :

The filter size is expressed as the smaller objects diameter of the structures that you want to preserve or enhance. This parameter must be expressed in metric unit.



The structure diameter can be measured directly from the dataset. Please refer to the (*arivis Vision4D Help*) for more details

## 8. Modify the pipeline

### Step 8.4

Change the Threshold' operator parameters

#### Step 8.4.1 – Channels:

Sets the processing and analysis target channel(s).

#### Step 8.4.2 – Method:

Set the Threshold algorithm.

2 groups are available:

1. Global – include manual and auto method
2. Local – include local threshold method

#### Step 8.4.3 – Object type:

Set Bright objects (Dark Background) or Dark objects (Bright Background) scenario.

#### Step 8.4.4 – Threshold:

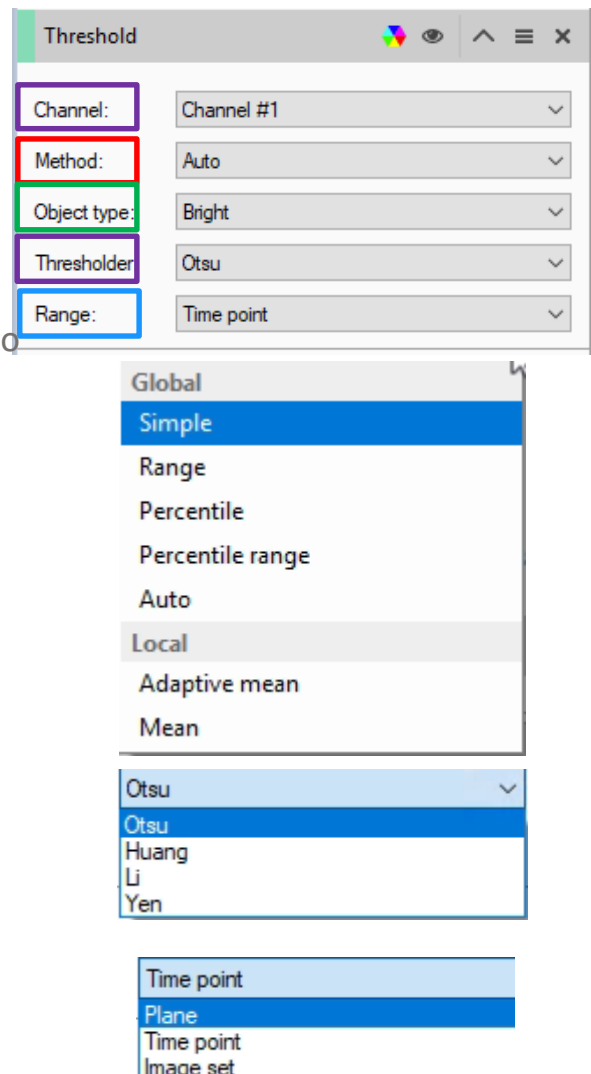
Available with Method = Auto

Set the auto algorithm to be used

#### Step 8.4.5 – Range:

Available with Method = Auto

Set the auto algorithm to be used



## 8. Modify the pipeline

### Step 8.5

Change the Blob Finder' operator parameters

#### Step 8.5.1 – Channels:

Sets the processing and analysis target channel(s).

#### Step 8.5.2 – Diameter:

Set the reference objects diameter (Max).

#### TIPS :



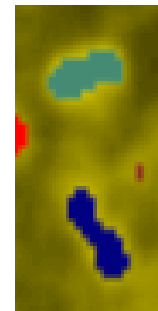
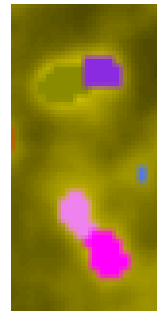
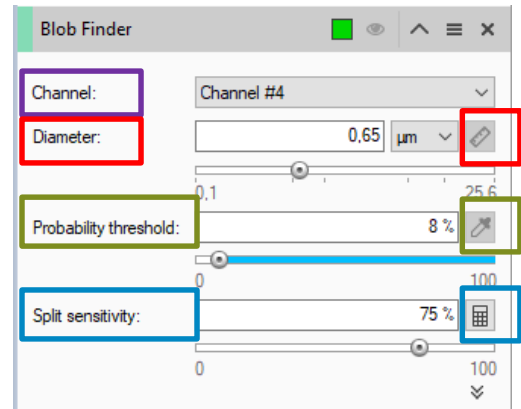
Object Diameter can be measured directly from the dataset. Please refer to the (*arivis Vision4D Help*) for more details

#### Step 8.5.3 – Probability threshold:

Set the probability threshold coefficient.

#### Step 8.5.4 – Split sensitivity:

Set the Split-Sensitivity coefficient.

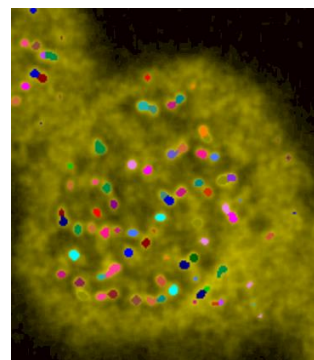


#### DETAILS :



Blob Finder has the preview icon located on the panel header (title bar).

Click on this icon to preview the operator result on the current plane / time point



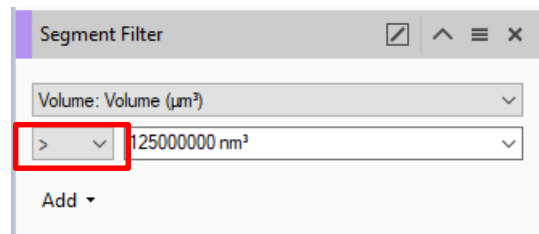
## 8. Modify the pipeline

### Step 8.6

Change the Segment Filter ' operator parameters

#### Step 8.6.1

Sets the feature to be used to filter the segments. Drop down the feature list and select the feature.

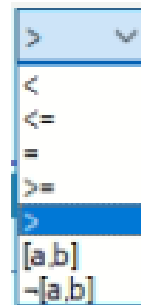


#### Step 8.6.2



Sets the Filter action.

Segments smaller than, bigger than, in the range of, outside the range filter criterias are available.



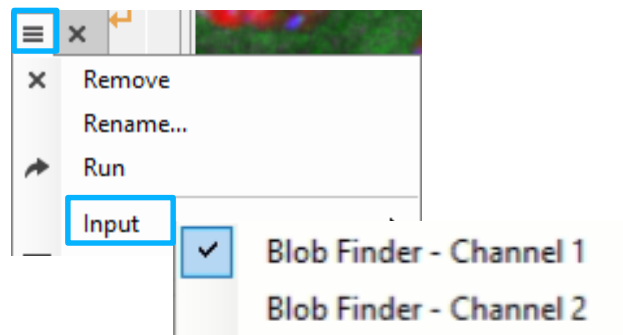
#### Step 8.6.3



Input

Sets the Filter input.

If more then one Segment Operator is present in your pipeline, the correct input source have to be set.

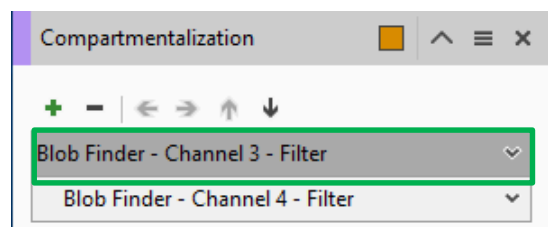


### Step 8.7

Change the Segment compartmentalization' operator parameters

#### Step 8.7.1

Sets the reference Tag






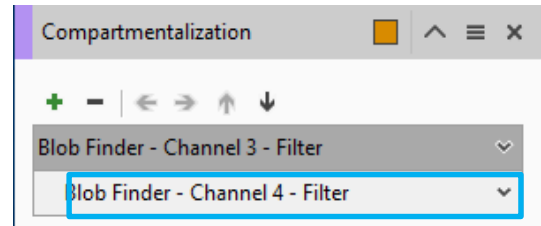
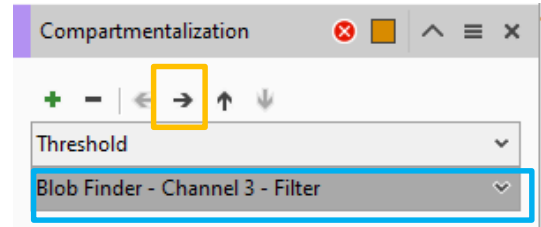
## 8. Modify the pipeline

### Step 8.7.1

Sets the [subject](#) Tag

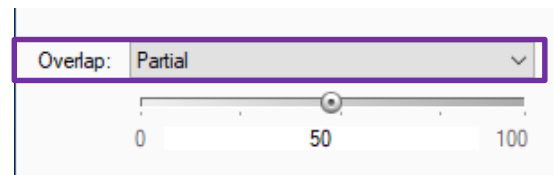
The subject Tag must be right shifted.

Use the Right Arrow Icon  to move the subject definition on right



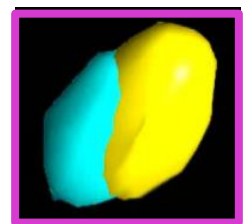
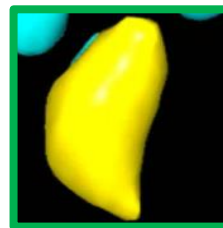
### Step 8.7.2

Sets the [Overlap method](#)



[Partial Overlap](#)

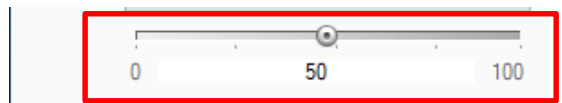
[Full Overlap](#)



### Step 8.7.3

Sets the [Overlap percentage](#)

(Available only with the Partial overlap method)



### DETAILS :

The segment compartmentalization operator compares the objects set as Subjects (child) with the objects set as References (Parent).

The Operator find overlapped objects according to the overlap method (partially or totally) .

## 8. Modify the pipeline

### Step 8.5

Add or remove operators from the pipeline

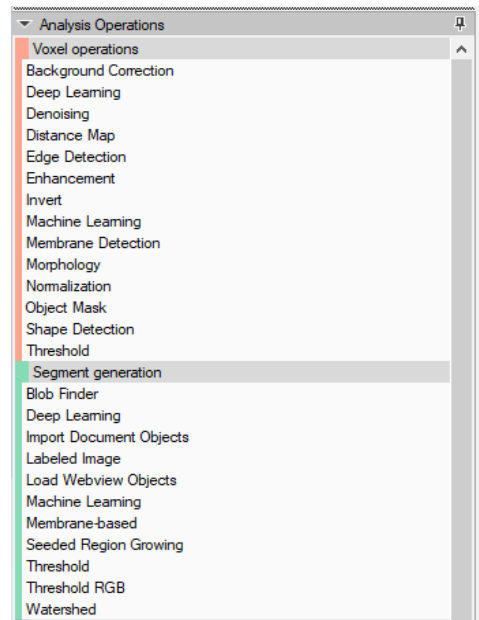
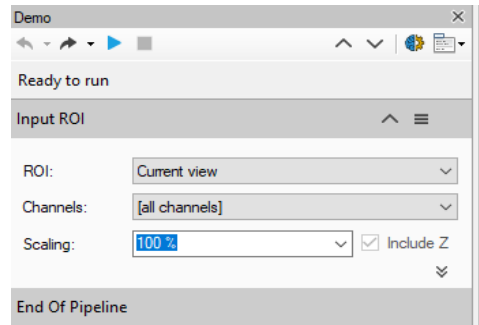
#### DETAILS :

The Analysis Pipeline panel consists of two main areas. The Pipeline area and the analysis operations list area .

The **Operators** can be added to Pipeline in two ways

1. Double click on the **Operator** you wish to add to the current Pipeline. The **Operator** will be inserted at the end of the group of operations to which it belongs. Voxel Operations are positioned before the Segment generation meanwhile Store operations are put always at the end of the Pipeline.
2. Drag and drop the **Operator** you wish to add to the current Pipeline. The **Operator** will be automatically inserted in any place within the group of operations to which it belongs.

The **Operator** cannot be added during the Pipeline execution.



To remove an Operator from the Pipeline, press the X button located in the right side of the operator title bar.



#### TIPS :

Please refer to the (*arivis Vision4D Help*) for more details



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