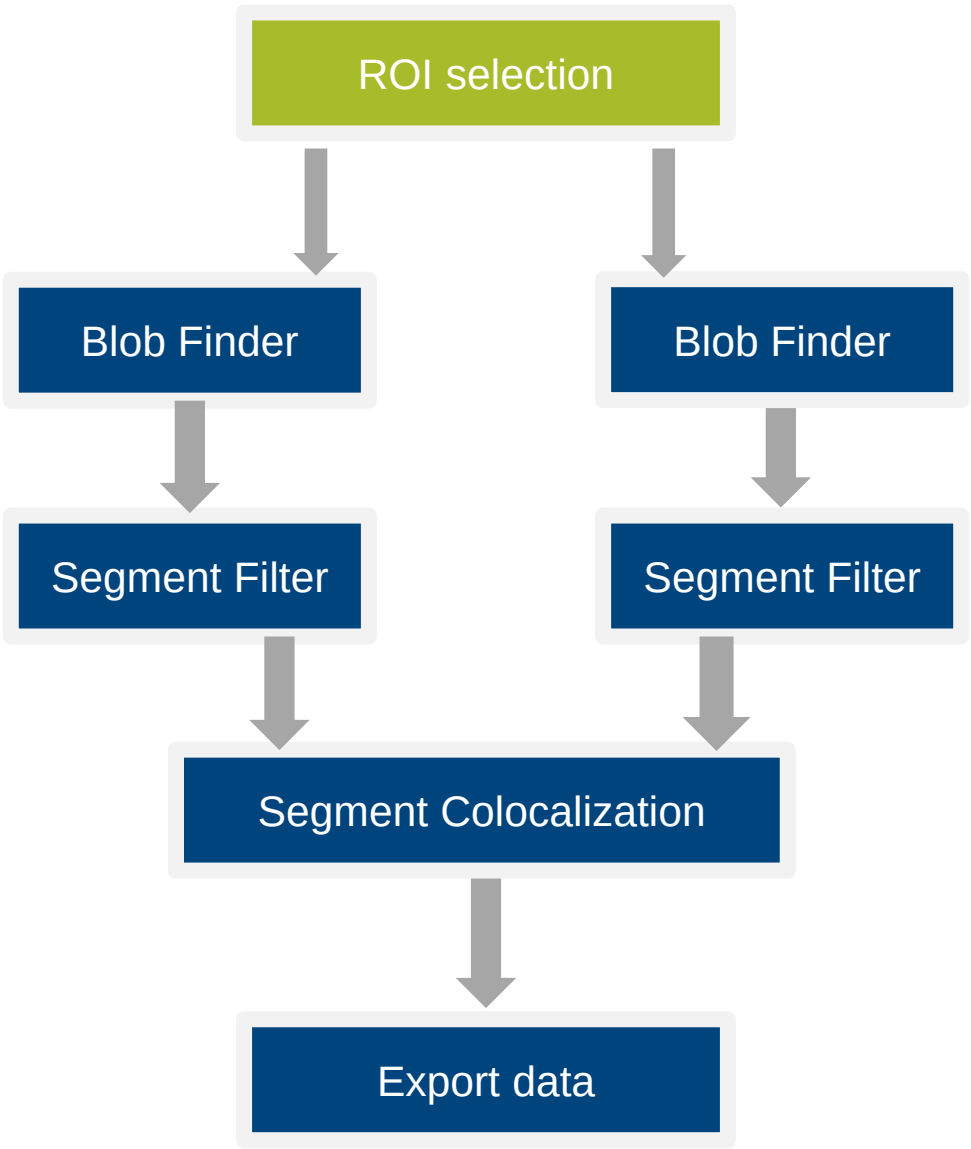
CELLS/PARTICLES COLOCALIZATION

«*Cells or Particles colocalization*»

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.

Arivis Vision4D Pipeline example «*Cells or Particles colocalization*» Working Flowchart :



Arivis Vision4D Pipeline example

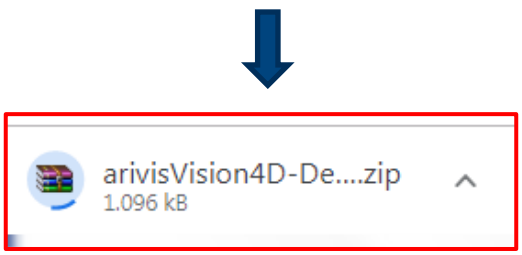
In order to run the pipeline described here below, please download the demo dataset according to the following instruction.

Step 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 2.

Create a new folder on your local disk.

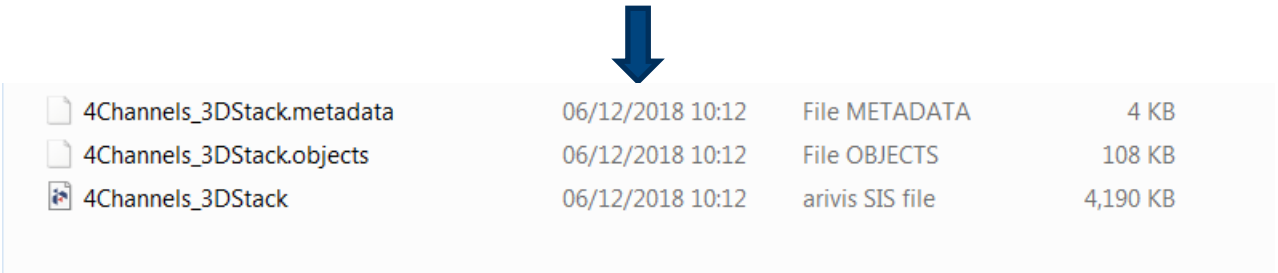
Move the ZIP file from the download folder inside it.

Step 3.

UnZip the file:

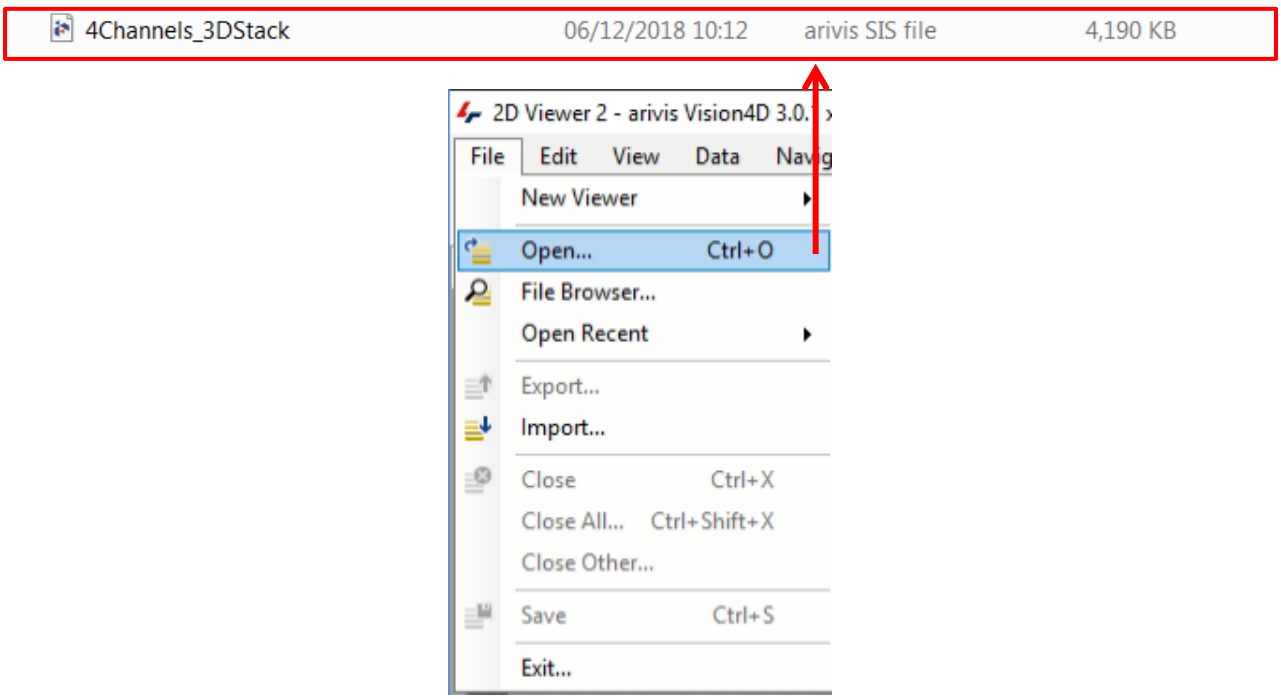
***arivisVision4D-DemoData-SamplePipelines-DetectStructures.zip*.**

Three files are now available in the folder.



Step 4.

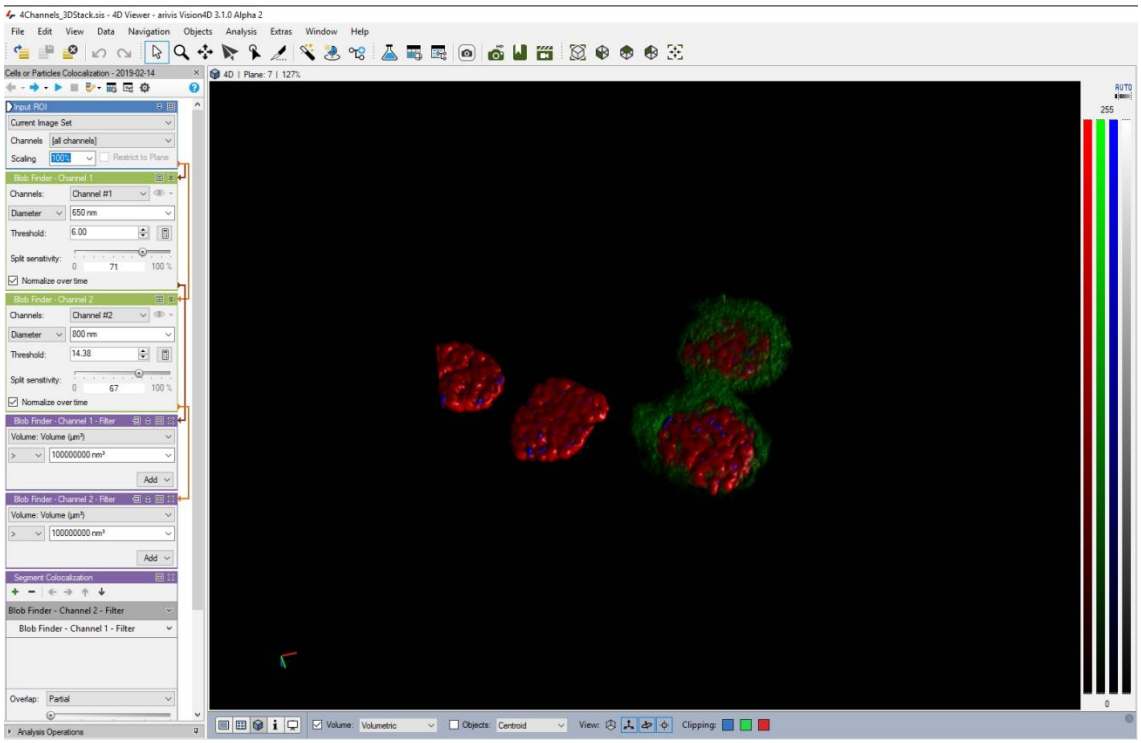
Open the SIS file on Vision4D.



The dataset is visualized in the V4D viewing area.

TIPS :

The dataset is visualized according to the current rendering setting parameters. Please refer to the ***User Manual*** for more details about how to set or modify the rendering options.



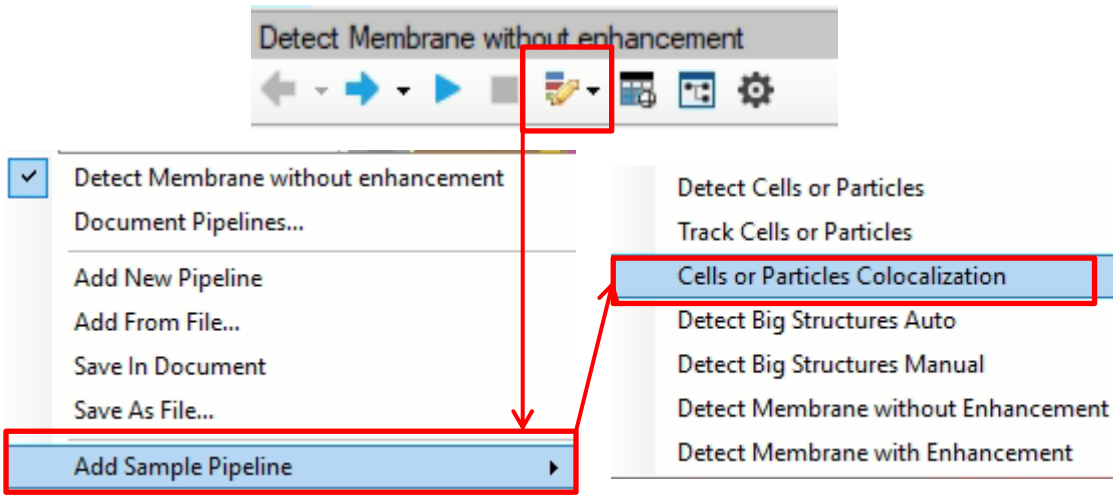
DETAILS:

The dataset is a multidimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (eg 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 dataset).
The datasets are available as graphic files saved in plenty of graphic formats (standard formats as well as proprietary formats)

Step 5.

Activate the «***Cells or Particles colocalization*** » *pipeline.*

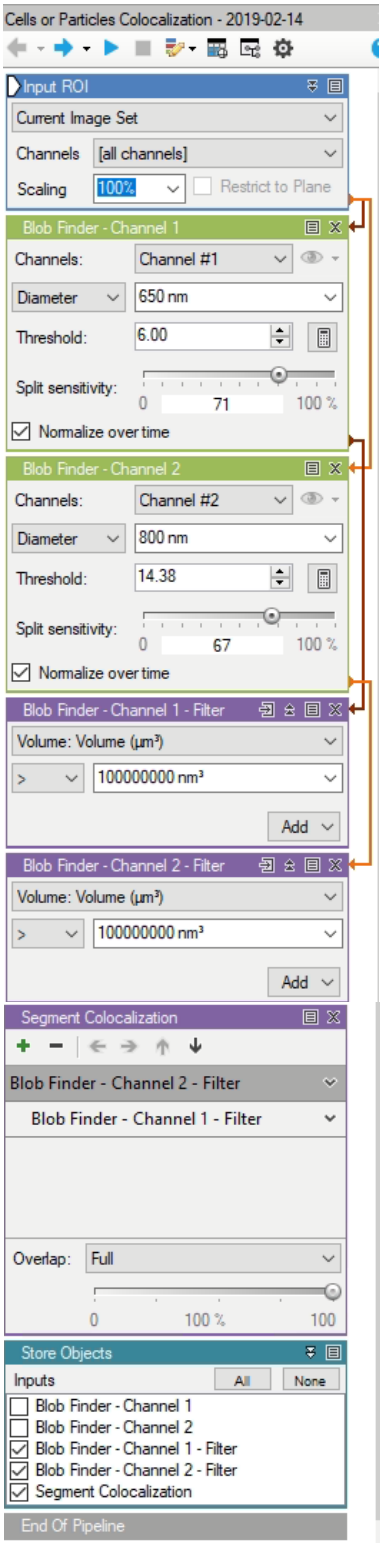
If not already done, open the Analysis panel



Select “Add Sample pipeline”
Then click on the «***Cells or Particles colocalization***» *item.*

TIPS:
The active Pipeline, if any, will be replaced by the new one.
Please refer to the ***User Manual*** for more details about how to export a pipeline.

The «*Pipeline Template* - ***Cells or Particles colocalization*** » *pipeline operators layout.*

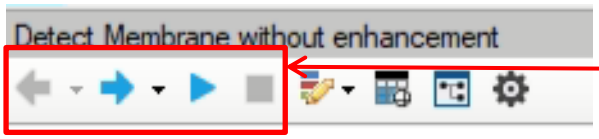


1. *Region Of Interest:*
   This operator allows the region of interest (ROI) selection. ROI defines the dataset subarea that will be processed and analyzed by the pipeline.
2. *Blobs Finder:*
   Automatic small objects detection algorithm. It uses a local treshold method.
3. *Segment Filter*
   Allows the blob filtering based on multiple parameters selection.
4. *Segment Colocalization*
   The detected objects are compared to find partial or total overlap conditions.
5. *Store Objects*
   Store the detected segments (TAG) in the active dataset.

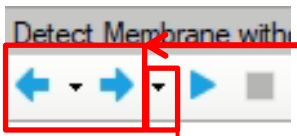
Step 6.

Execute the «***Cells or Particles colocalization***» *pipeline.*

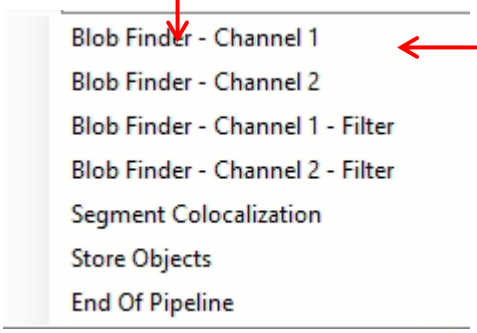
TIPS :
The pipeline can be executed as single shot or step by step.
Step by step method allows to run and undo a single ***Operation.***
Single shot method runs all the pipeline in one task (no stop until the pipeline execution ends).
Either the arrow buttons or the ***Operation*** list can be used to run both methods.



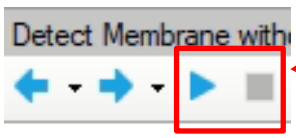
Pipeline commands tools



Step by step tools (back and forth).

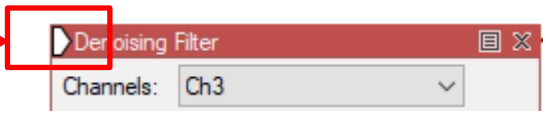


Step by step list



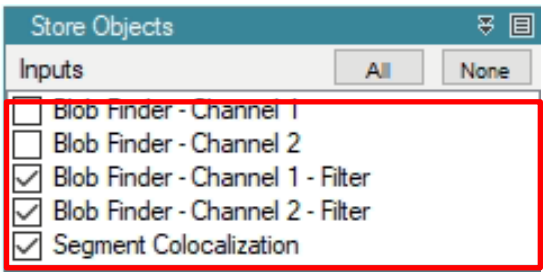
One shot tools (run and stop).

The white arrow on the operator title bar shows the next step that will be executed.

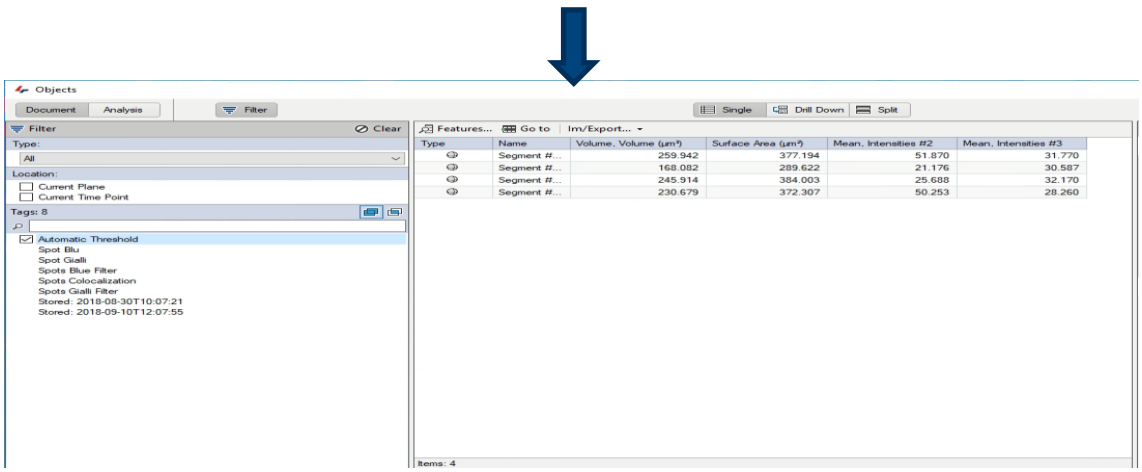


Step 7.
View the results

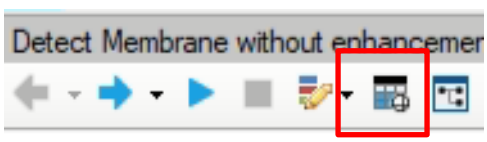
TIPS :
Results (segments and measurements) will be stored in the dataset only if the ***Store Objects*** operator has been correctly set.
Please tick appropriately the option as shown below before complete the pipeline execution.



Selected TAGs measurements are now visible in the data table

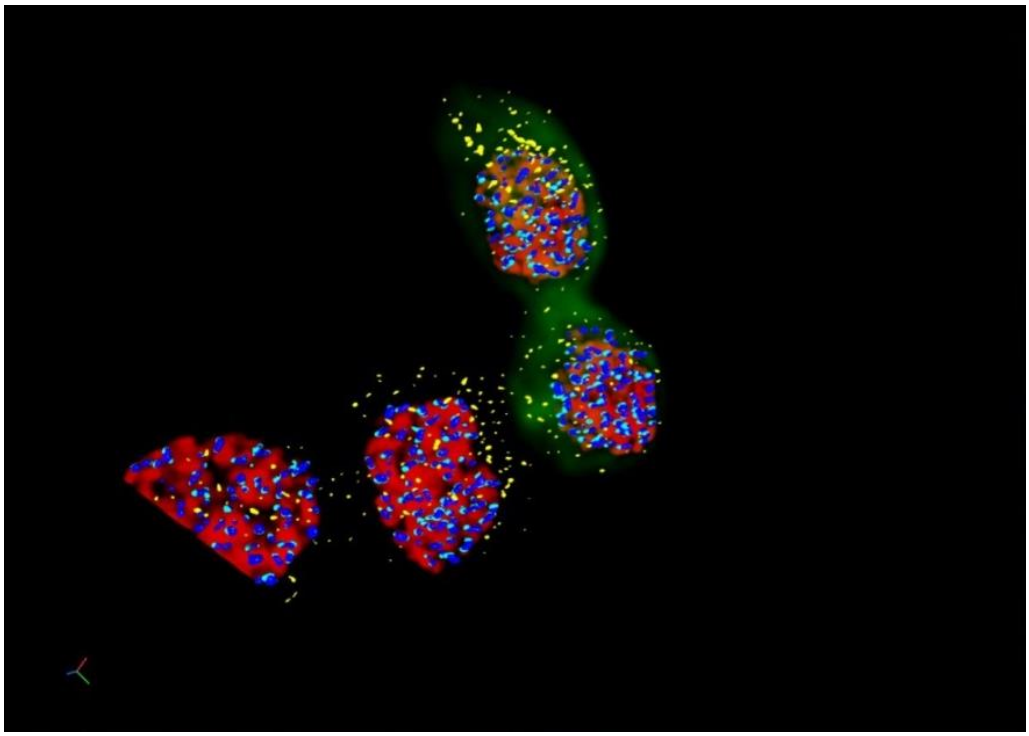


TIPS :
If the data table is not already visible, please click on the related icon to open it.



View the results.

Segments can be visualized either in 2D as well as 4D according to the currently set options.

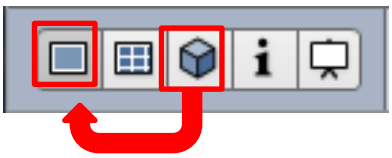


TIPS :

Please refer to the ***User Manual*** for more details about how to visualize segments on the dataset.

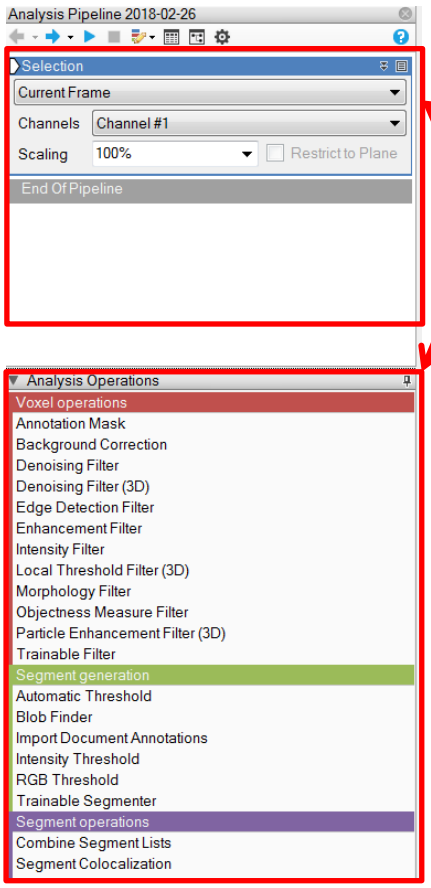
The «***Cells or Particles colocalization***» pipeline can be modified to be adapted to your datasets. All the pipeline parameters must be set according to your dataset features.

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 segments have been generated, you can switch back to 4D view mode.



TIPS :
Please refer to the ***User Manual*** for more details about how to switch the Viewing Area from 4D to 2D view mode.

Arivis Vision4D Pipeline setup



DETAILS:

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

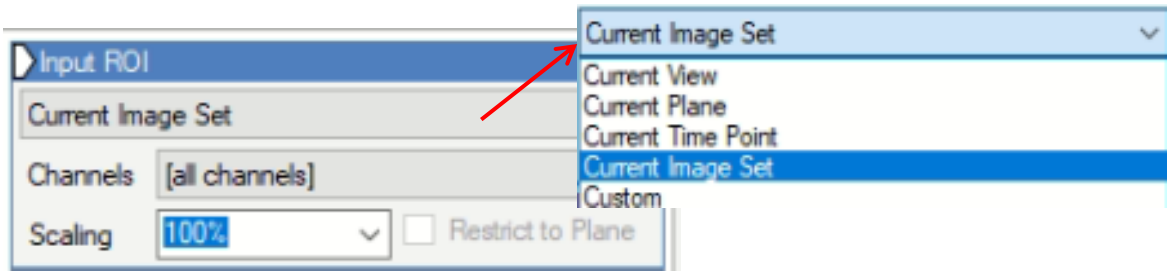
TIPS :

Please refer to **Addendum A** for more details about how to add or remove an ***Operator*** to the current Pipeline

DETAILS :

Analysis Pipeline protocol is executed from top to bottom of the pipeline. The Operations must be added to the Pipeline in the correct order.

Step A.
How to set the ROI operator



Processing & Analysis target options:

a. *Current View*
   Only the selected Z plane and the visualized area in the viewer are processed.
b. *Current Plane*
   Only the selected Z plane is processed regardless to the visualized area (real XY pixel size).
c. *Current Time Point*
   The selected time point is entirely processed (all Z planes and the real XY pixel size)
d. *Current Image Set*
   The complete dataset (XYZ and time) is processed.
e. *Custom*
   Allows a detailed selection of each parameters.

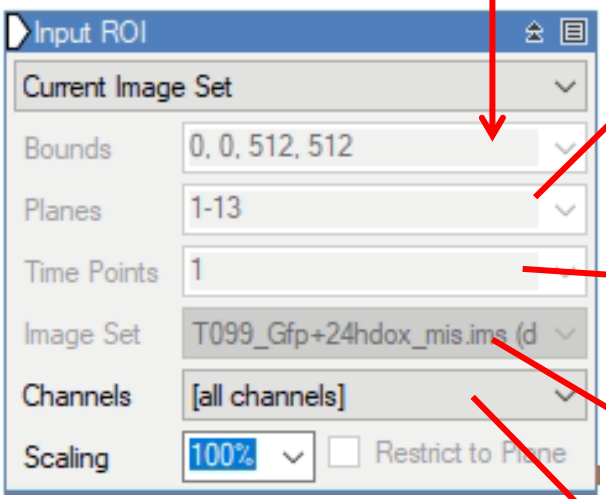
DETAILS :
Use the Custom option during the pipeline setting and testing . Set a sub volume (XY, Planes, Time Points, channels) of your dataset on which perform the trial. This will speedup the setting process.

TIPS :
Please refer to the ***User Manual*** for more details about how to select the active Z plane and/or the active Time Point.

Step A.

How to set the **Input ROI** operator
Custom option

The full XY size, the viewing area or a free area setting (by coordinates) can be applied



Single Z plane, a range of Z planes or the full Z planes can be selected

Single Time point (TP), a range of TP or the full TP can be selected

Select the source Image Set

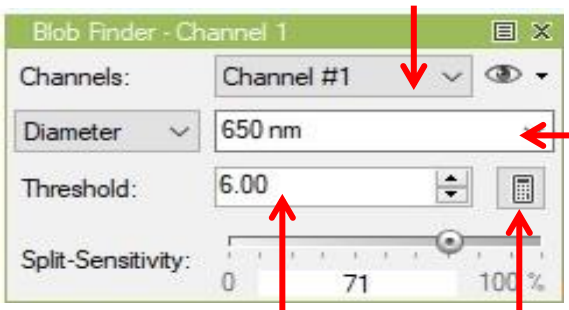
One or more of the available channels can be selected. Be careful, only the selected channel(s) can be used in the pipeline

The dataset volume can be downsized by 50 % or 25 %. This option is used to speed up analysis pipeline when the dataset is very large.

Step B.

How to set the **Blob Finder** operator

Select working Channel(s).

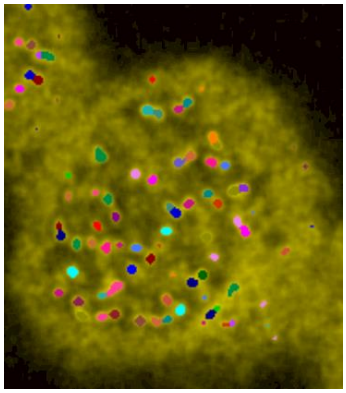


Set the reference objects diameter.

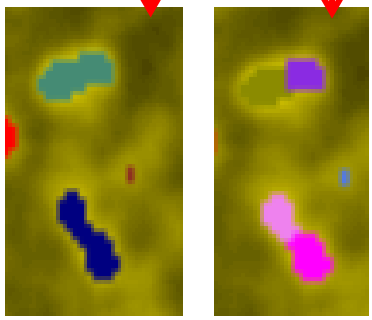
Set the object threshold coefficient.

Use the calculator to automatically set the threshold coefficient.

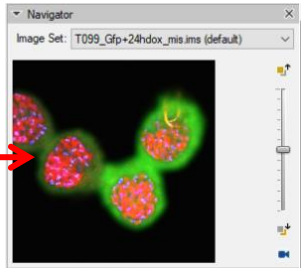
TIPS :
Object Diameter can be measured directly from the dataset.
Please refer to **Addendum B** for more details about how to measure object diameter.



Move the Split-Sensitivity slider to decrease/increase the splits objects sensitivity.

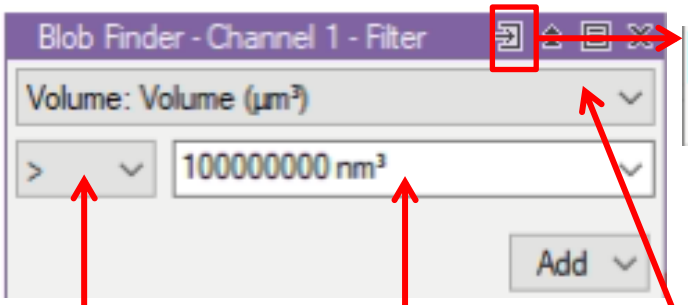


TIPS :
Use the ***Navigator Panel*** to select the preview Z plane and/or Time Points (if any)

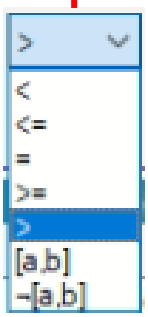


Step C.

How to set the **Segment Filter** operator

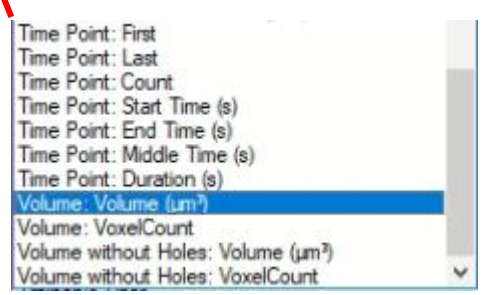


Select the Blob Finder operator source.



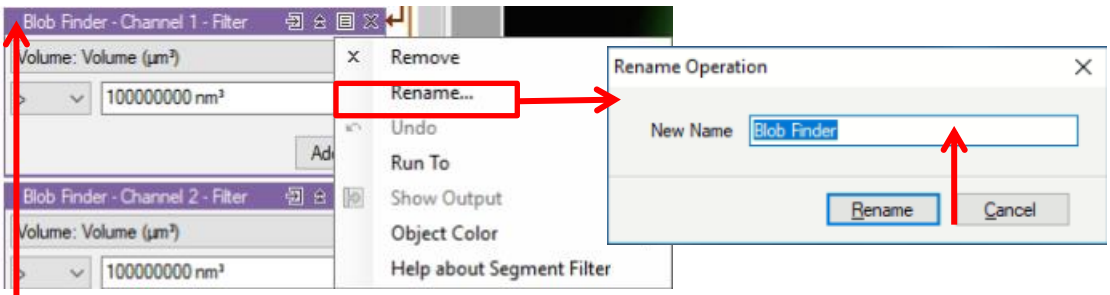
Set the limit used to filter the Blob Finder results according to the set filter rule.
The values are expressed in metric unit

Select the filter rule.



Select the Feature to be used for filtering the blobs

TIPS :
Segment Filter Tag can be renamed



Right mouse click on the title bar

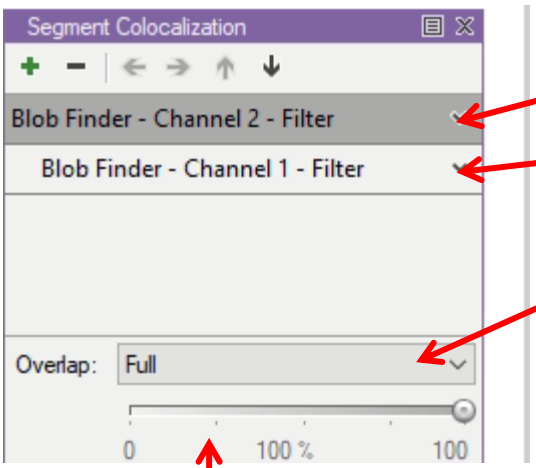
Digit the new Tag name and press ***Rename***

Step D.

How to set the **Segment Colocalization** operator

DETAILS :

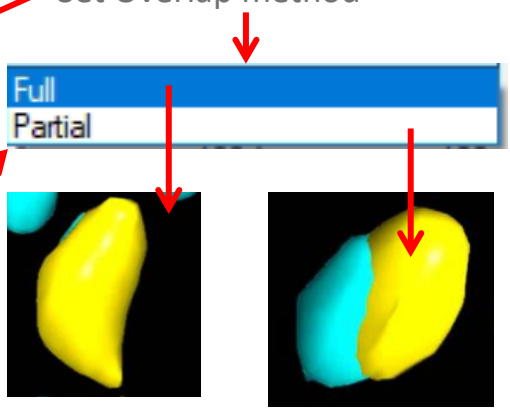
The **segment colocalization** operator compares the objects set as Subjects with the objects set as References.
The Operator find overlapped objects (partially or totally) according to the Coloc-Measure method.



Set References source (Parent)

Set Subjects source (Child)

Set Overlap method

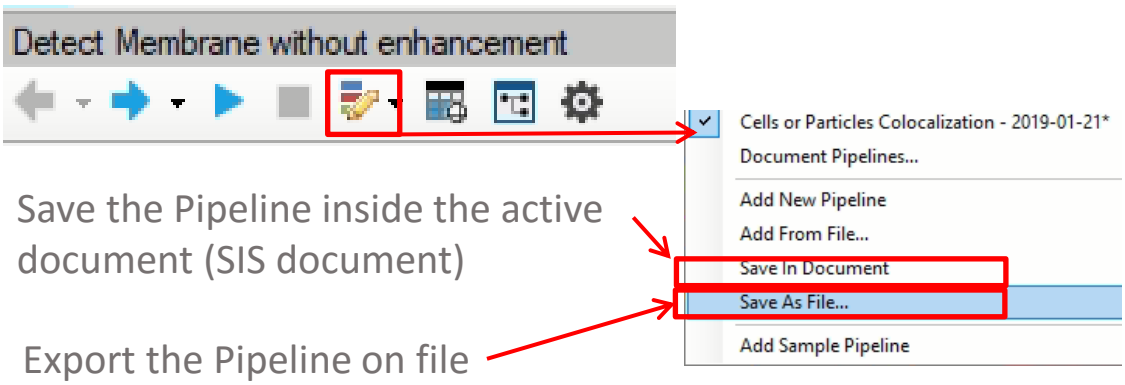


Set the **Overlap percentage**.
The option is enabled when the Partial Overlap method is selected

Step E.

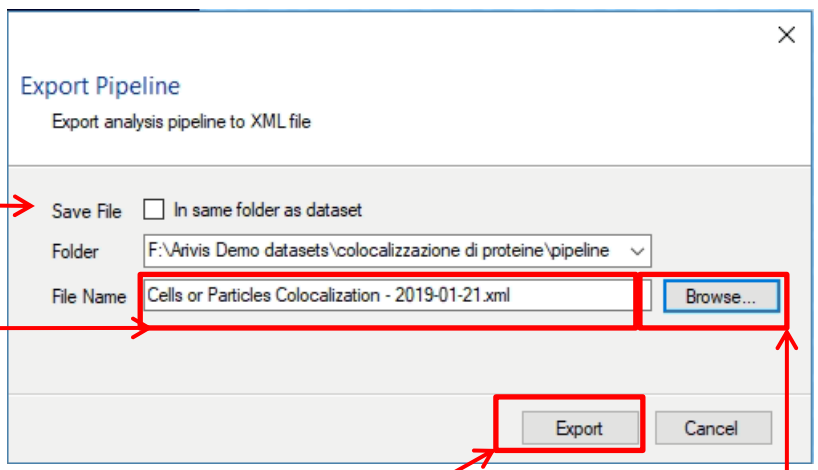
How to export the modified Pipeline

Once you have finished your pipeline settings, according to your needs, the pipeline can be exported on disk.
Exporting the pipeline on file allows you to run it with different datasets.



Save the Pipeline inside the active document (SIS document)

Export the Pipeline on file



Store in the dataset source folder

Set a new Pipeline name (optional)

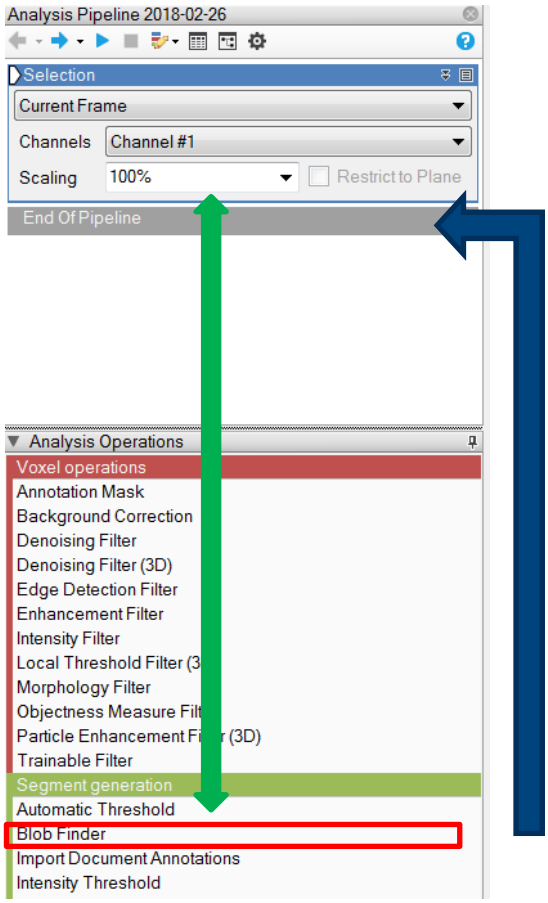
Export the Pipeline

Browse the destination folder

Arivis Vision4D Pipeline setup

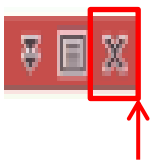
Addendum A:

How to add or remove an *Operator* from the pipeline.



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 :

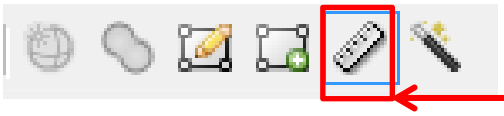
Please refer to the ***User Manual*** for more details about how to add a new ***Operator*** to the current Pipeline.

Arivis Vision4D Pipeline setup

Addendum B:

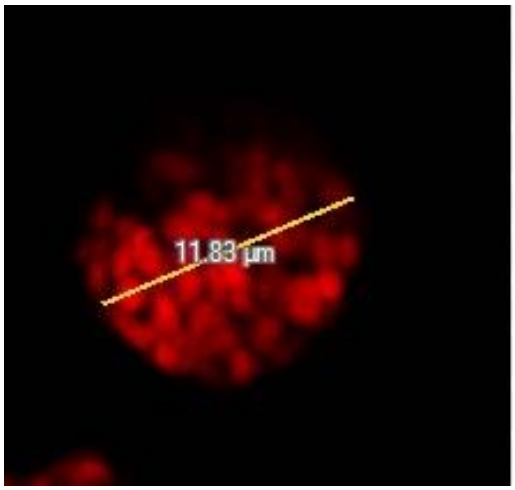
How to measure Object diameter

Switch to 2D view mode.

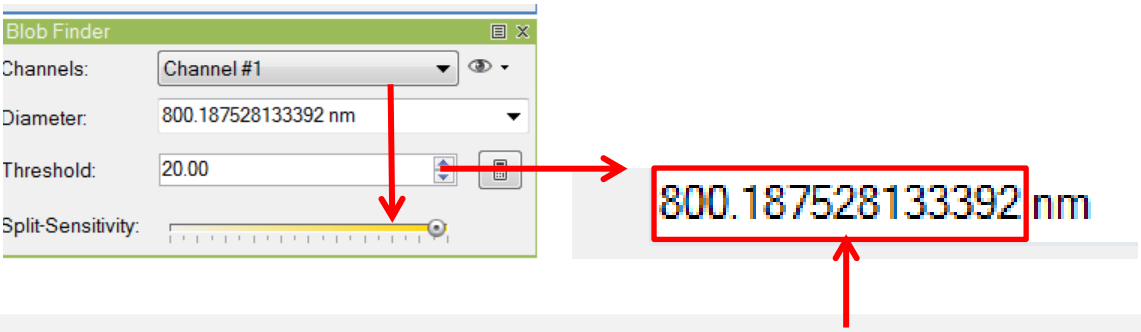


Press the Ruler icon in the ***Shortcut toolbar panel***.

1. Move the mouse cursor (it shows a little ruler instead of the standard arrow) on one side of the structure you want to measure.
2. Keeping the left mouse button down, draw a line over the structure diameter.
Once the mouse button is released, the distance measured is shown over the image.



3. Take note of this number and digit it in the ***Diameter Box***



TIPS :

Before write down the diameter in the Box, select from the list the right metric unit you want to use.

Then digit the measure without delete the unit in the box