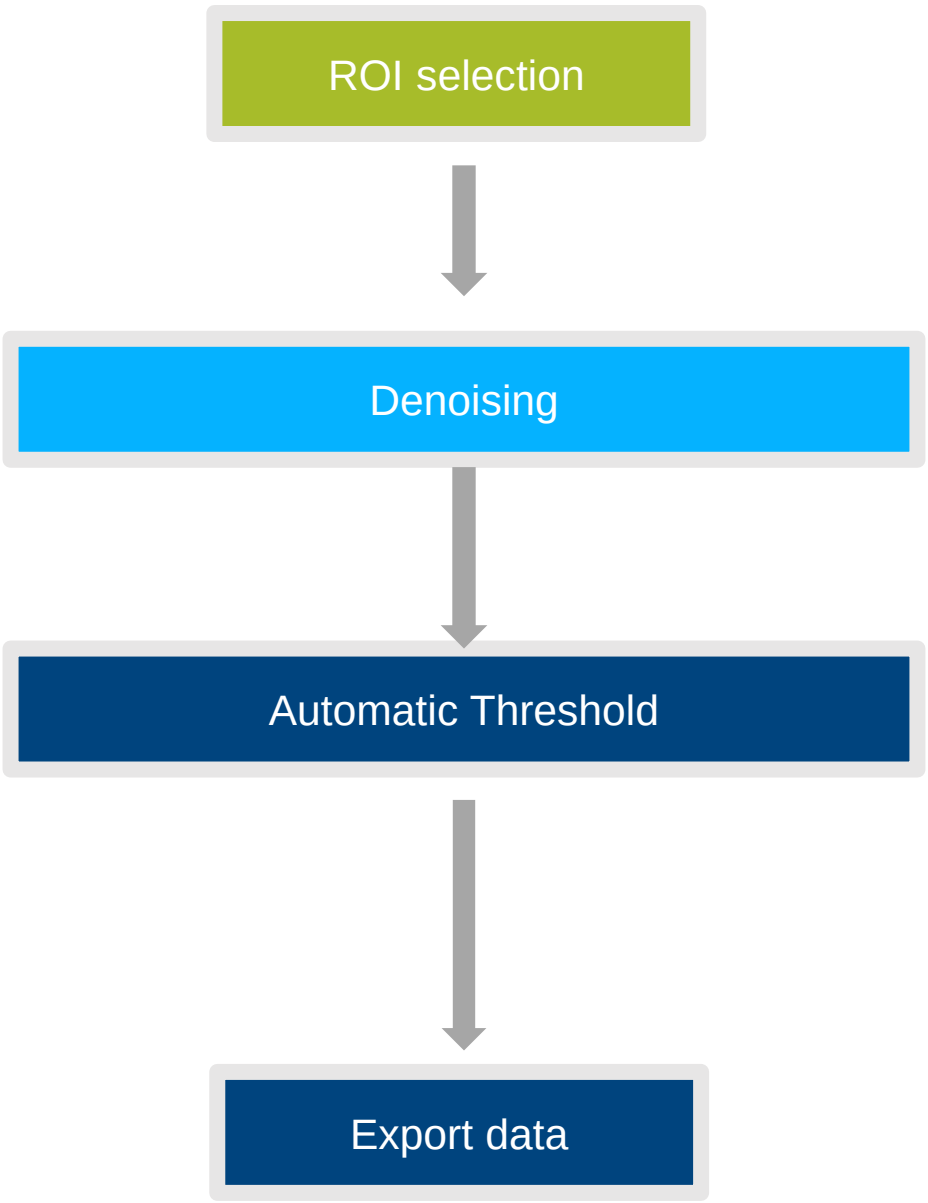
BIG STRUCTURES – AUTO

«*Detect Big Structures - Auto*»

The pipeline purpose is to detect objects having a random shape and not well defined borders. It can be applied to any cellular compartments or biological structures.

Arivis Vision4D Pipeline example «*Detect Big Structures - Auto*» Working Flowchart :



Arivis Vision4D Pipeline example

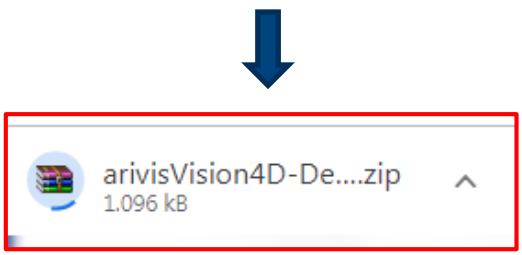
In order to run the pipeline described here below, please download the demo dataset according to the following instruction.

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.

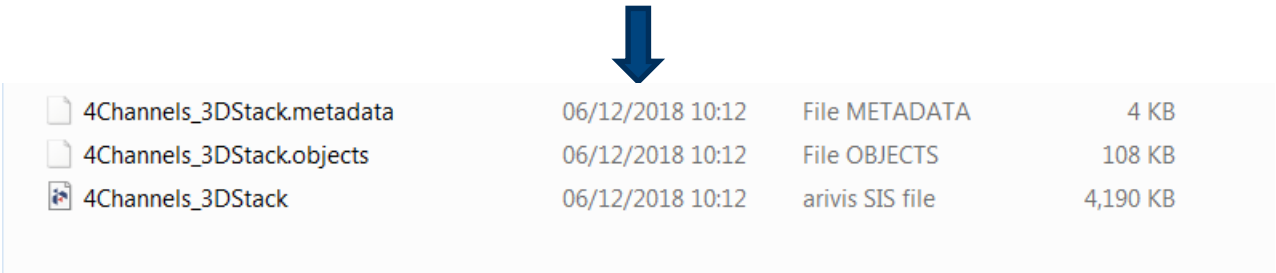
Move the ZIP file from the download folder inside it.

Step 3.

UnZip the file:

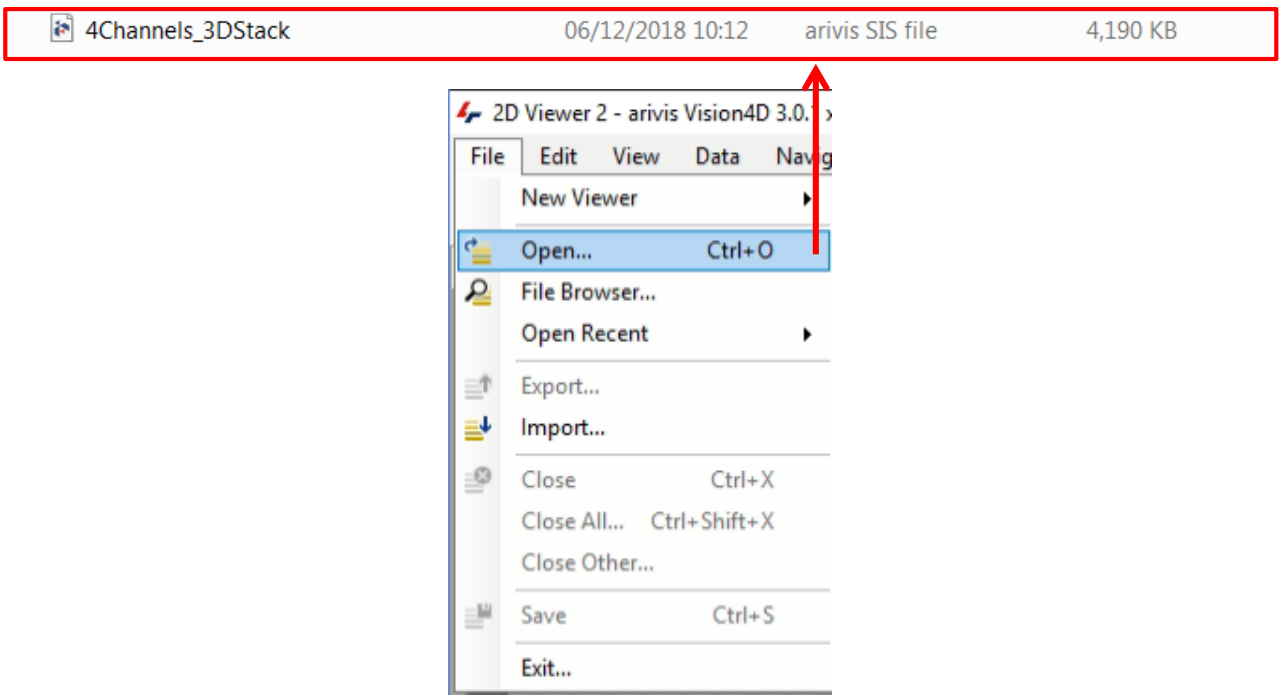
***arivisVision4D-DemoData-SamplePipelines-DetectStructures.zip*.**

Three files are now available in the folder.



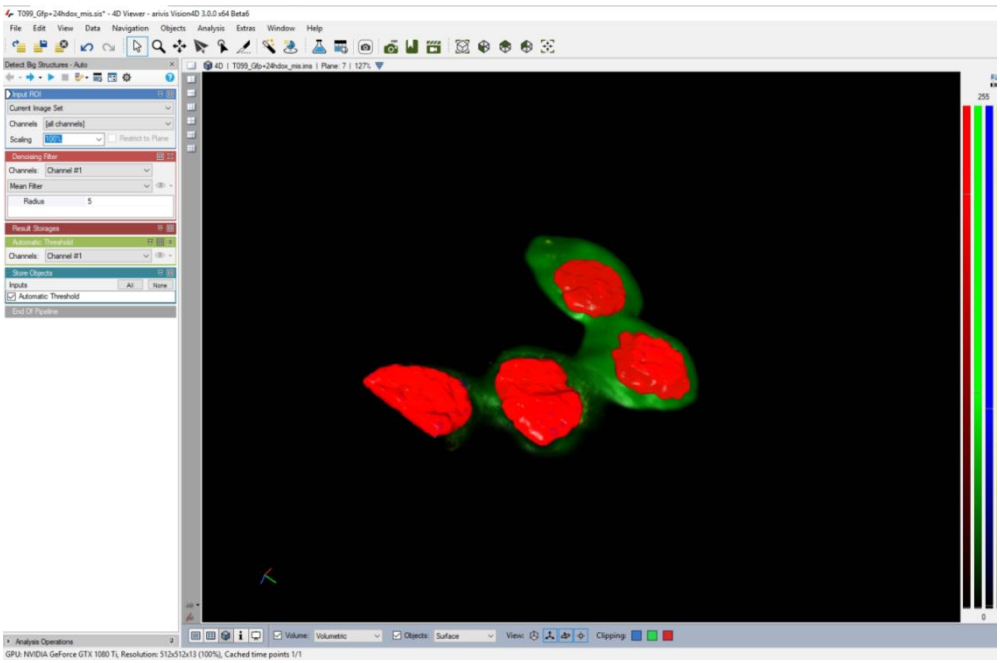
Step 4.

Open the SIS file on Vision4D.



The dataset is visualized in the V4D viewing area.

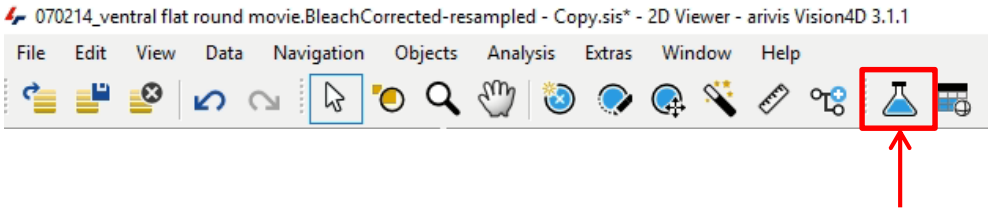
TIPS :
The dataset is visualized according to the current rendering setting parameters. Please refer to the ***User Manual*** for more details about how to set or modify the rendering options.



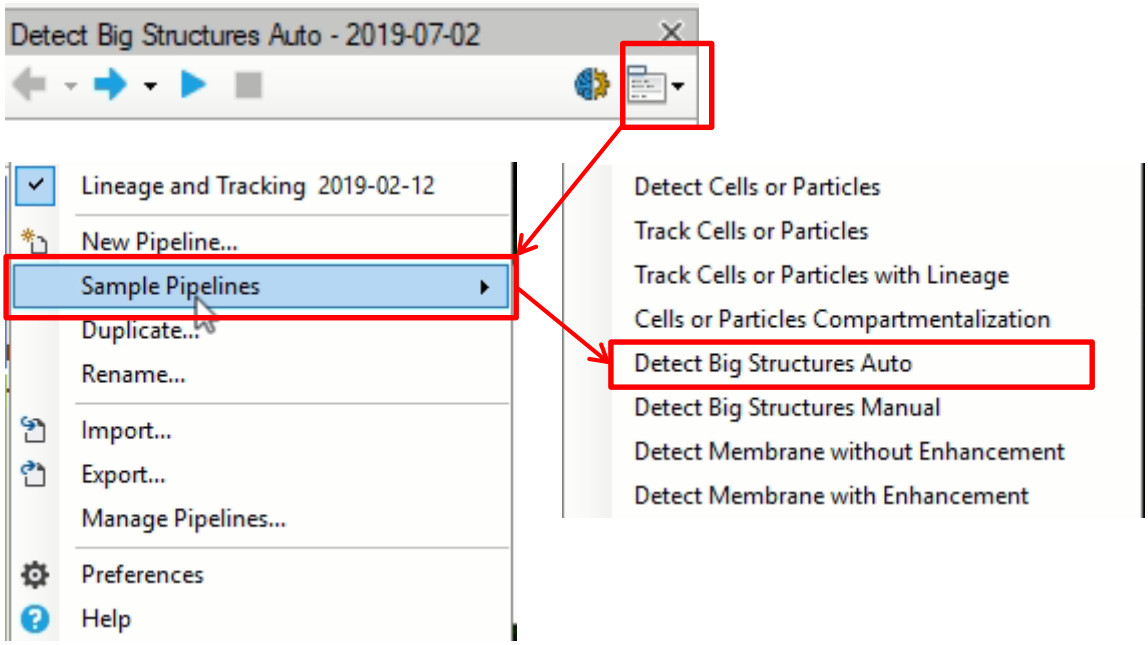
DETAILS:
The dataset is a multi dimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (eg Optical sectioning) of multiple channels (dyes) in a temporal sequence of time points (located in several spatial positions).
Usually the dataset shows a 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 4.

Activate the «***Detect Big Structures – Auto***» *pipeline.*



If not already done, open the Analysis panel



Select “Add Sample pipeline”

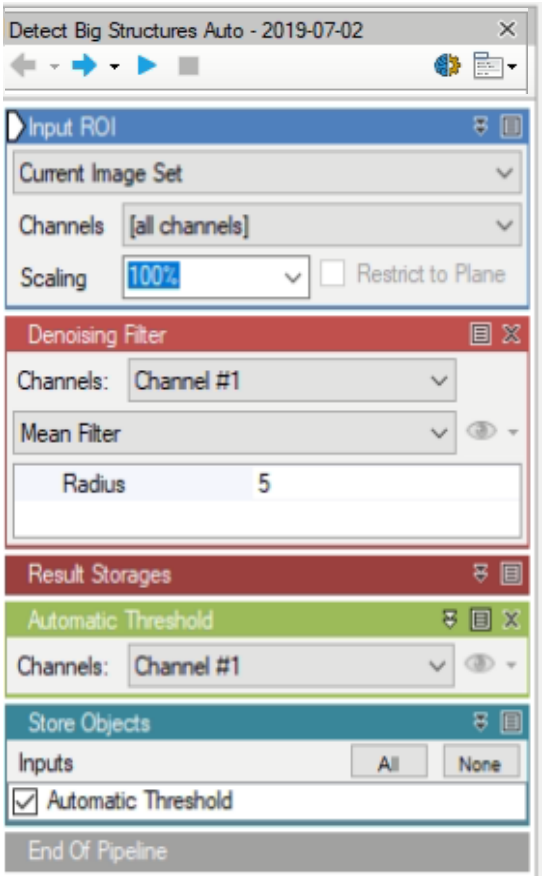
Then click on the «***Detect Big Structures – Auto***» *item.*

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 «***Detect Big Structures - Auto***» *pipeline operators layout.*

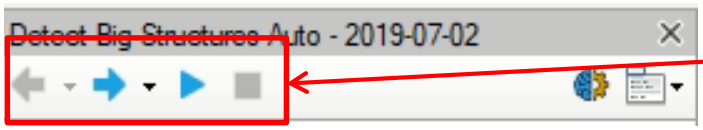


1. *Region Of Interest:*
   This operator allows the region of interest (ROI) selection. ROI defines the dataset subarea that will be processed and analyzed by the pipeline.
2. *Denoising Filter*
   Set of operators performing noise reduction. The «Discrete Gaussian Filter» with radius 5 is used. Several other filters are available.
3. *Result storages:*
   The processed dataset results can be store in different ways.
4. *Automatic Threshold:*
   Allows the Objects detection using one of the available automatic threshold algorithm.
5. *Store Objects*
   Store the detected segments (TAG) in the active dataset.

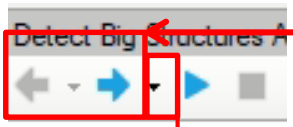
Step 5.

Execute the «***Detect Big Structures - Auto***» *pipeline.*

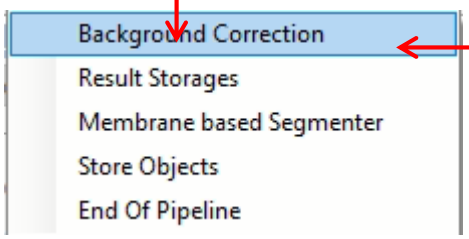
TIPS :
The pipeline can be executed as single shot or step by step.
Step by step method allows to run and undo a single ***Operation.***
Single shot method runs all the pipeline in one task (no stop until the pipeline execution ends).
Either the arrow buttons or the ***Operation*** list can be used to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



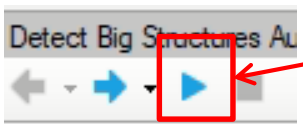
Pipeline commands tools



Step by step tools (back and forth).

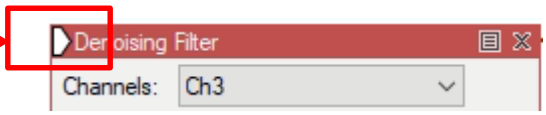


Step by step list



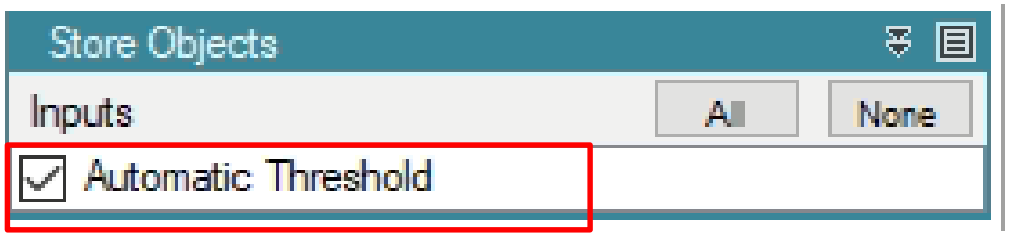
One shot tools (run and stop).

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

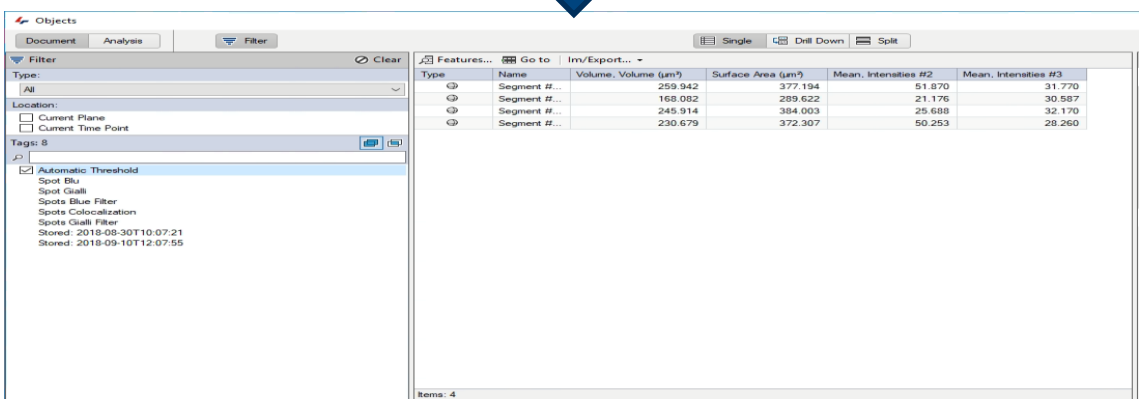


Step 6.
View the results

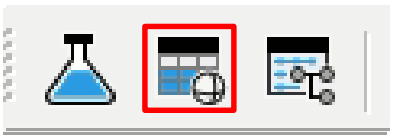
TIPS :
Results (segments and measurements) will be stored in the dataset only if the ***Store Objects*** operator has been correctly set.
Please tick appropriately the option as shown below before complete the pipeline execution.



Measurements are visible in the data table

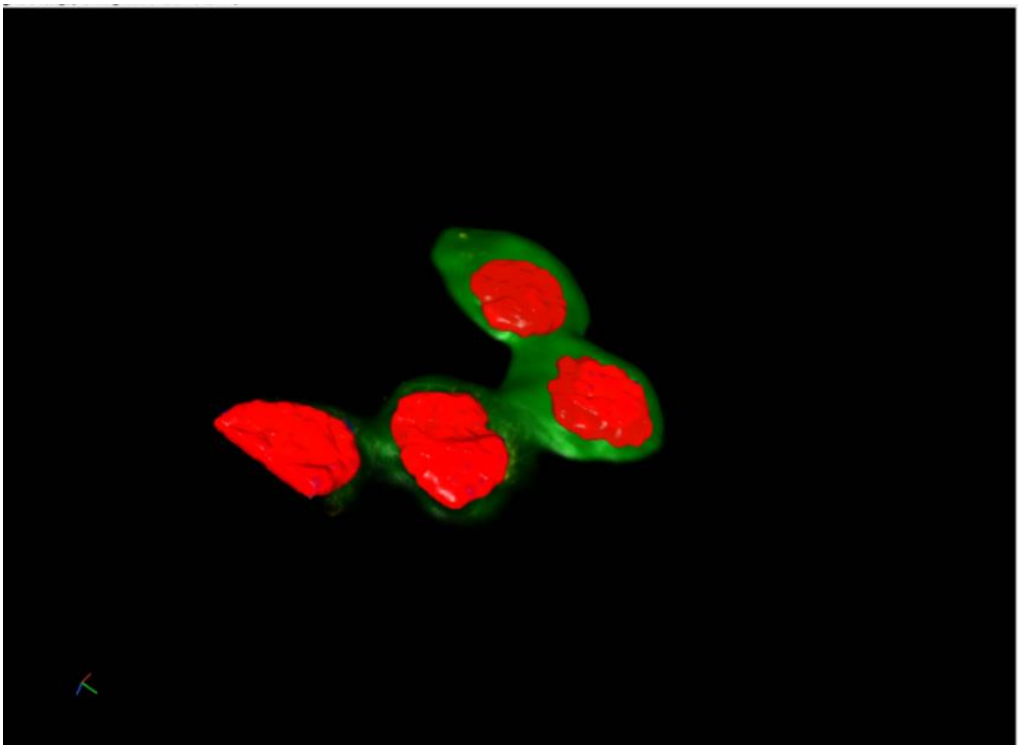


TIPS :
If the data table is not already visible, please click on the related icon to open it.



View the results.

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



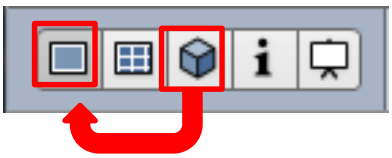
TIPS :

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

Arivis Vision4D Pipeline example

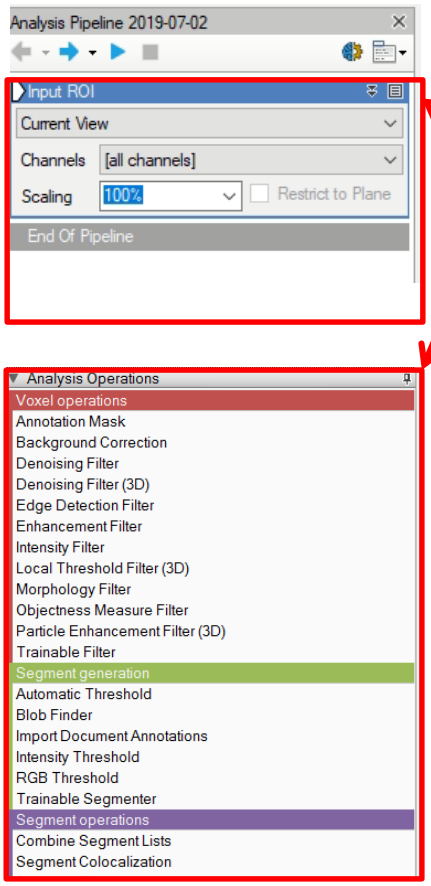
The «***Detect Big Structures - Auto***» pipeline can be modified to be adapted to your datasets.
All the pipeline parameters must be set according to your dataset features.

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



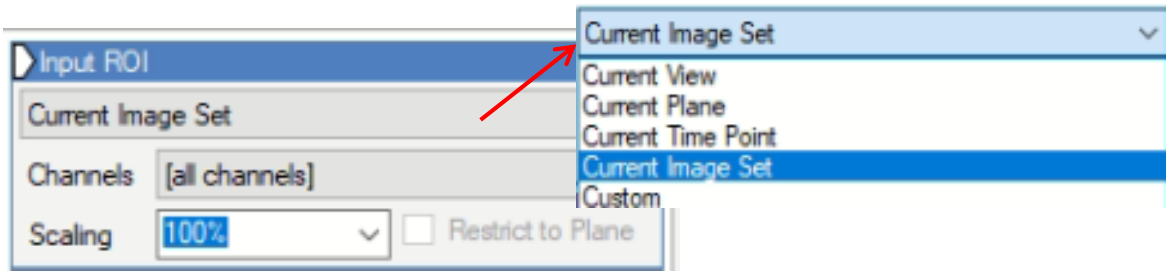
DETAILS:
The Analysis Pipeline panel consists of two main areas. The Pipeline sequence area and the analysis operations list area .

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 operation must be added to the Pipeline in the correct order.

Step A.

How to set the **Input 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.

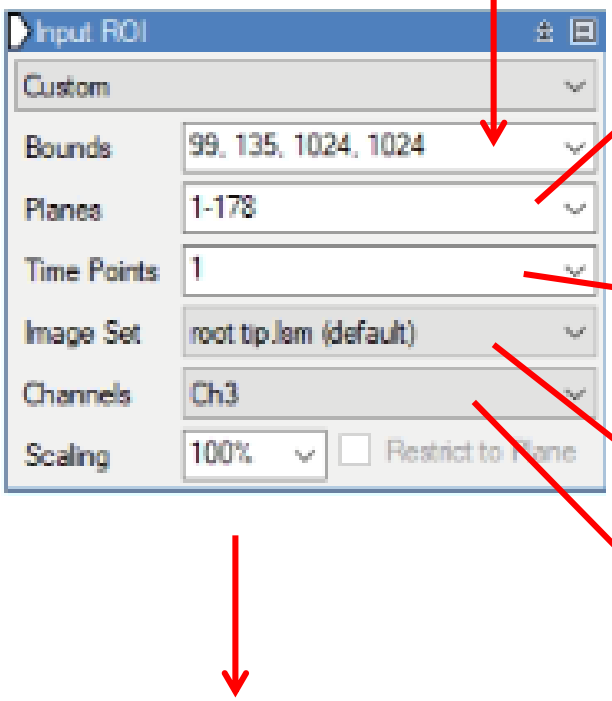
DETAILS :
Use the Custom option during the pipeline setting and testing . Set a sub volume (XY, Planes, Time Points, channels) of your dataset on which perform the trial. This will speedup the setting process.

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

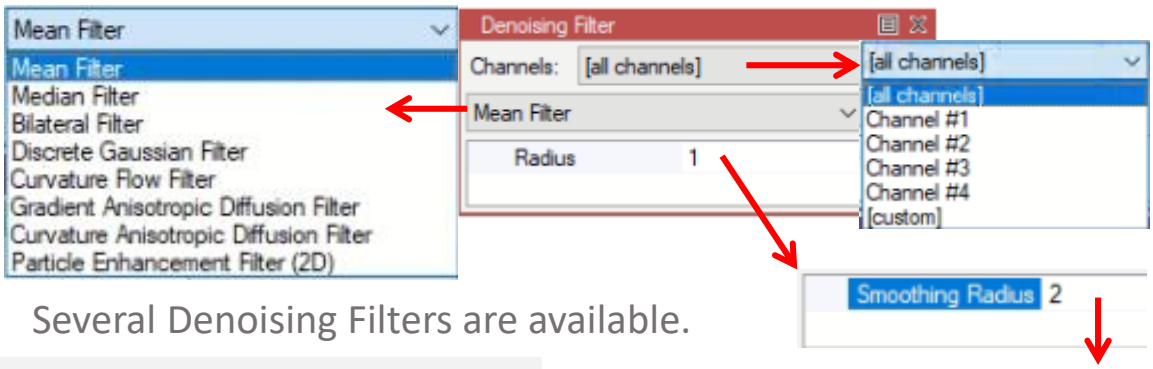
The dataset volume can be downsized by 50 % or 25 %. This option is used to speed up analysis pipeline when the dataset is very large.

Step B.

How to set the **Denoising** operator

DETAILS :
The Denoising operator technique is used to remove noise from an image. Digital Images are affected by noise derived from a variety of sources. Further use of these images will often require that the noise be (partially) removed.

Select the channel(s) on which the operator will be applied. Only the channel(s) defined in the ROI operator are listed here.



Several Denoising Filters are available.

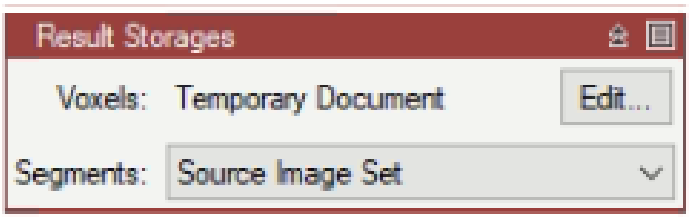
TIPS :
Please refer to Addendum B for more details about how to measure Object diameter

The filter size must be set. Bigger the size stronger the noise reduction effect. Please consider that the blur effect is also increasing with the filter size.

TIPS :
The filter parameter is expressed as the smaller objects radius must be kept by the denoising operator. This means the half of the object diameter must be used. Mean, Median or Gaussian filters are the most used suggested choices.

Step C.

How to set the **Result Storages** operator



Voxels (set to temporary document)
Segments : (set to Source Image Set)

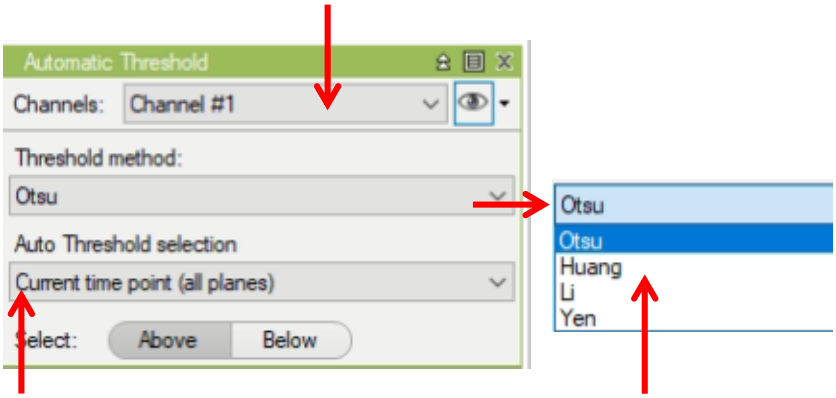
These default can be modified.

TIPS :
Please refer to the ***User Manual*** for more details about How to modify the ***Result Storages*** operator.

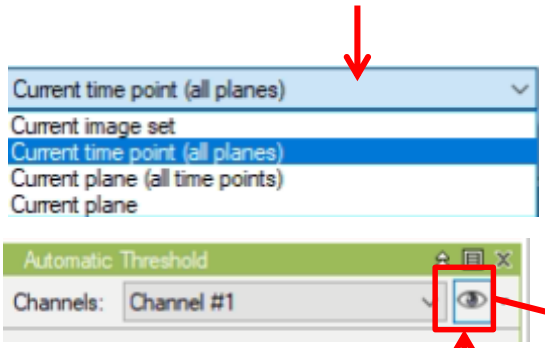
Step D.

How to set the **Automatic Threshold** operator

Select working Channel(s).

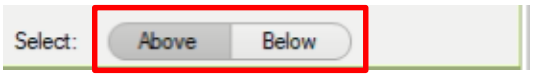


Threshold value calculated according to the selected method

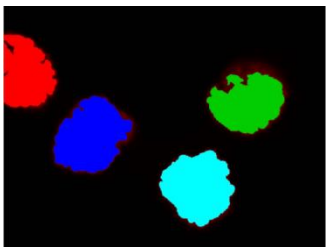


Press the “Eye” icon to show the objects detection preview.

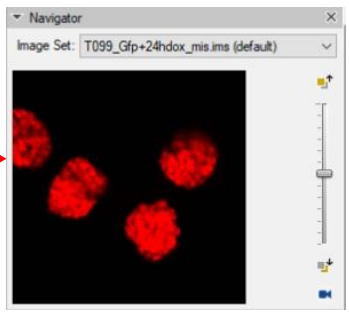
Set the object threshold algorithm from the list.



Set “Above “ for bright objects (Dark Background).
Set “Below” for dark objects (Bright Background).



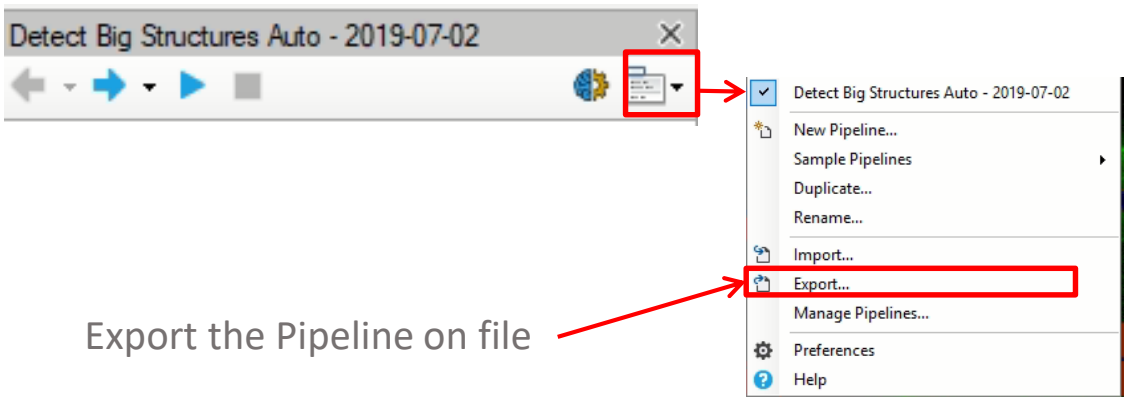
TIPS :
Use the ***Navigator Panel*** to select the preview Z plane and/or Time Points (if any)

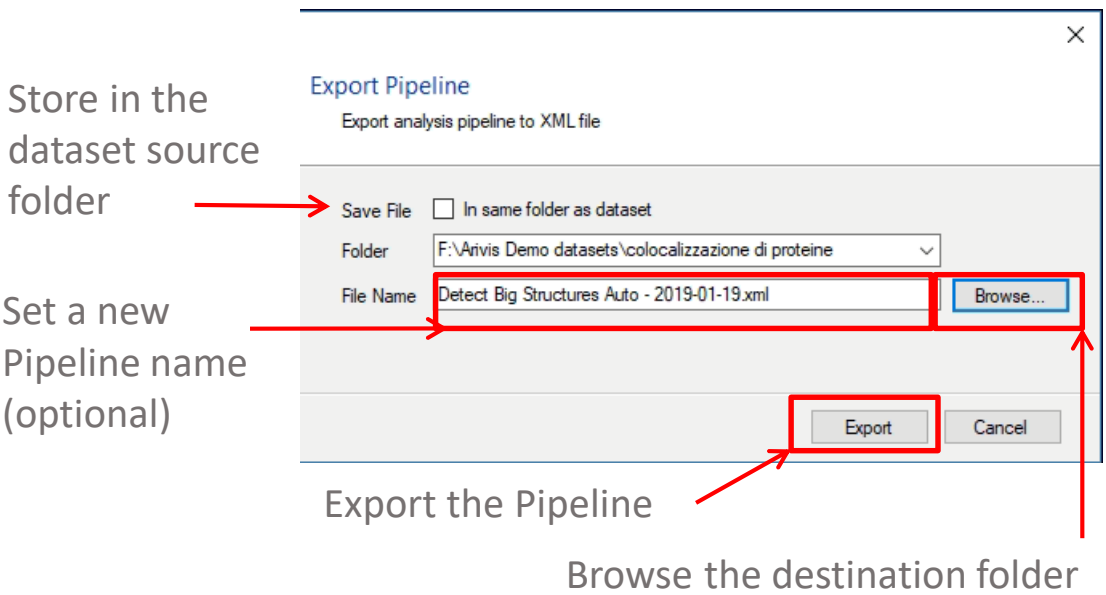


Step E.

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.

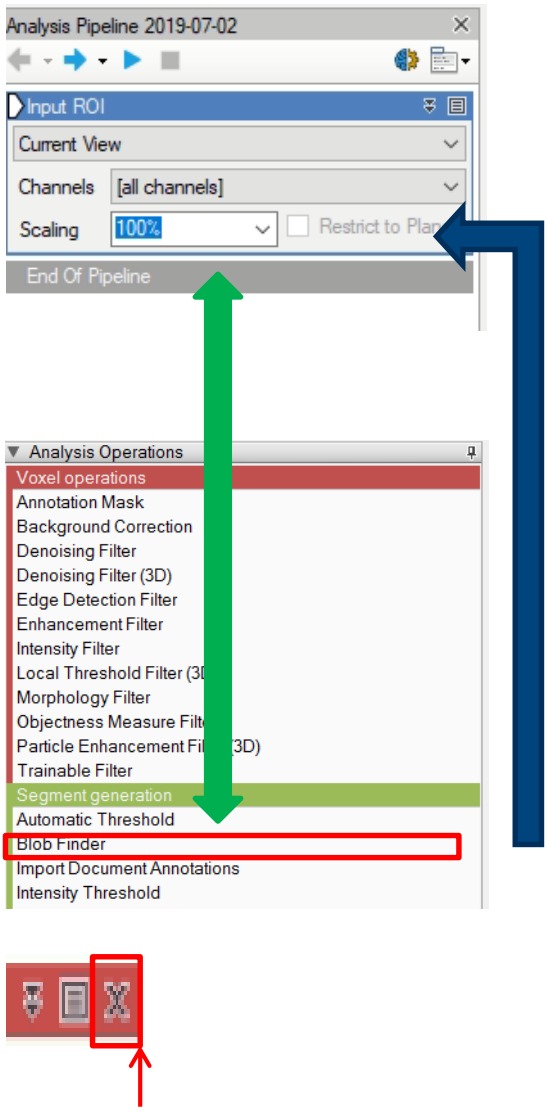




Arivis Vision4D Pipeline setup

Addendum A:

How to add or remove the *Operators* 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

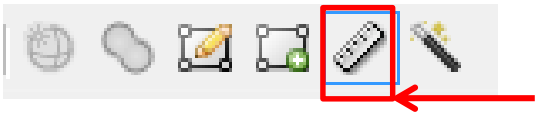
To remove an Operator from the Pipeline, press the X button located in the right side of the operator title bar.

TIPS :

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

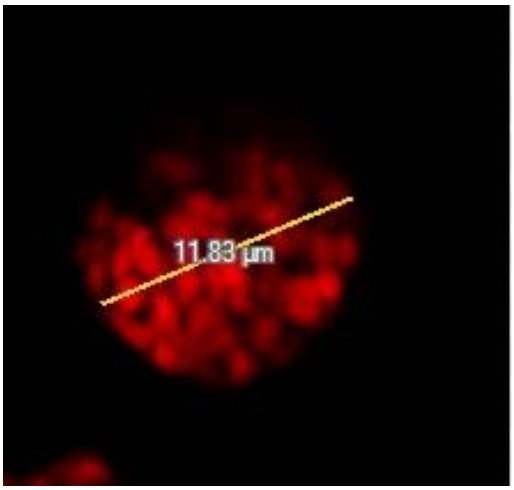
Arivis Vision4D Pipeline setup
Addendum B:
How to measure Object diameter

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 destination ***Diameter*** text box.

TIPS :
Before write down the diameter in the text box, select from the list the right metric unit you want to use.
Then digit the measure without delete the unit in the box

Arivis Vision4D Pipeline setup

Addendum C:

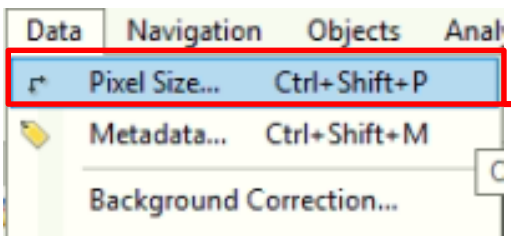
How to convert Object diameter to pixel

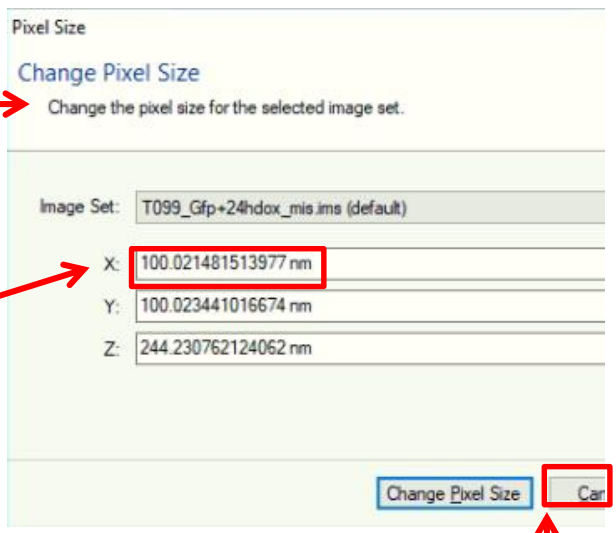
DETAILS :

Almost all the ***Voxels Operator*** require a reference size of the structures that you want to preserve or enhance. This parameter must be expressed in pixels rather than metric unit.

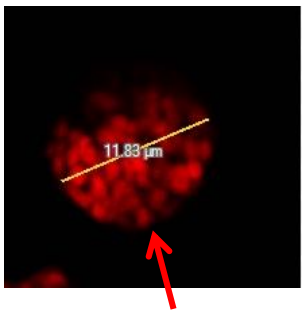
A metric unit diameter of the structure can be easily obtained as shown on the **addendum B**. Once the diameter is available, it can be converted to pixels following the next steps.

From Data menu, select the ***Pixel Size*** item.





Unit/pixel *value reported in the X field*



Measured Diameter

Exit without changing the parameters

Calculate the diameter in pixels as (rounded - no decimals) :
Measured Diameter *(metric unit) /* ***Unit/pixel*** *value*
Example - > 800.32 / 100.021 = 8 pixels