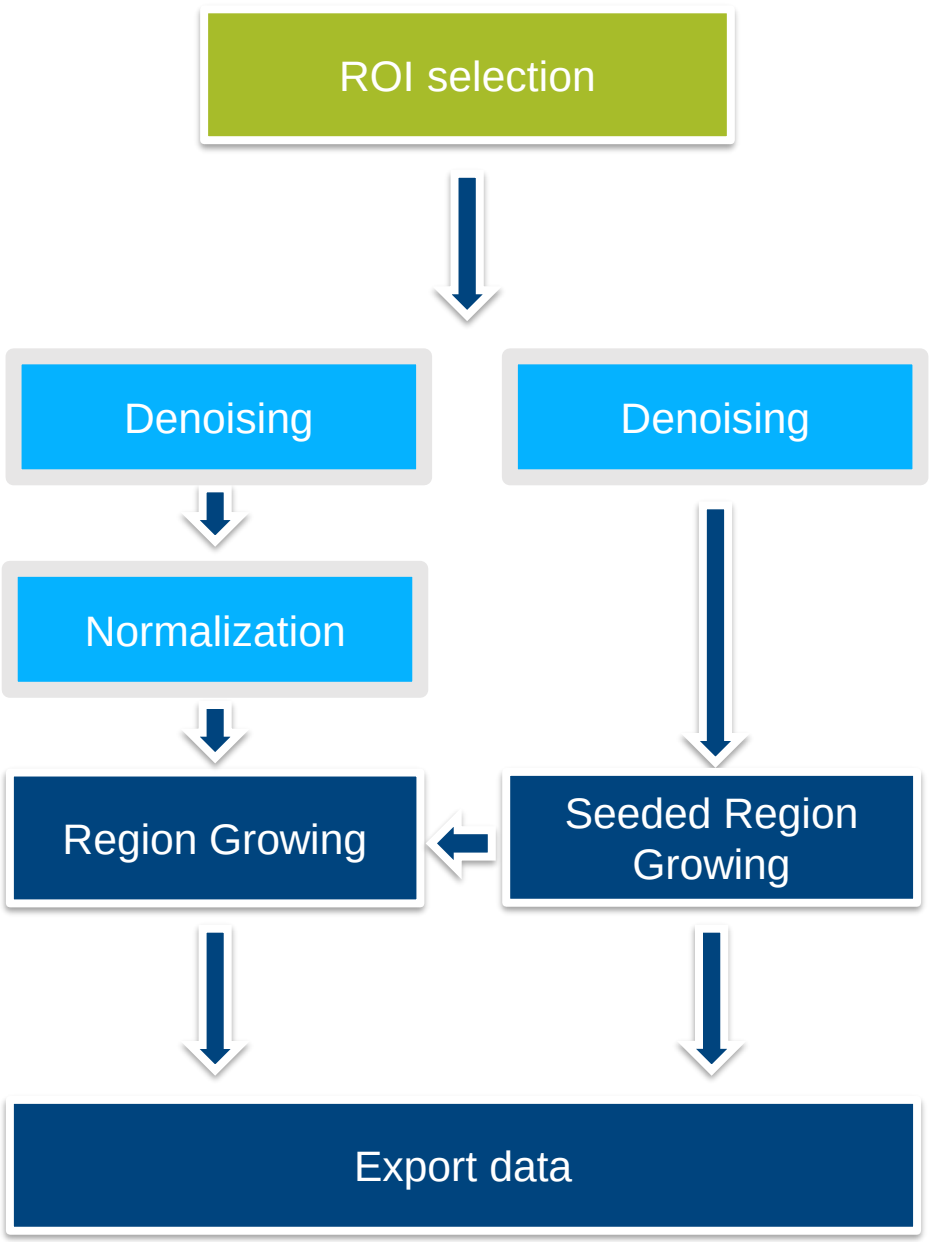
How to guide - Sample Pipeline «DETECT CELLS OR PARTICLES USING SEEDED REGION GROWING»

The pipeline purpose is to detect objects edges starting from an internal seed.
The seed is grown till it reached the main structures borders according to the defined criterias.
The pipeline can be applied to Cells, Nuclei or any kind of small particles

Working Flowchart



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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-Seed.zip file is saved on the download folder.

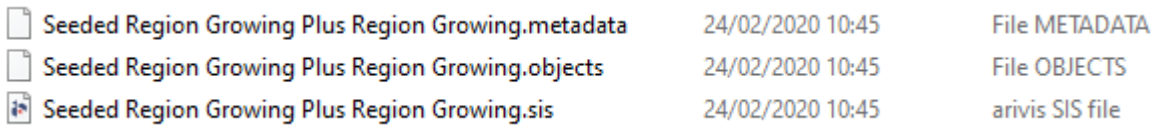
Step 1.2

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-Seed.zip

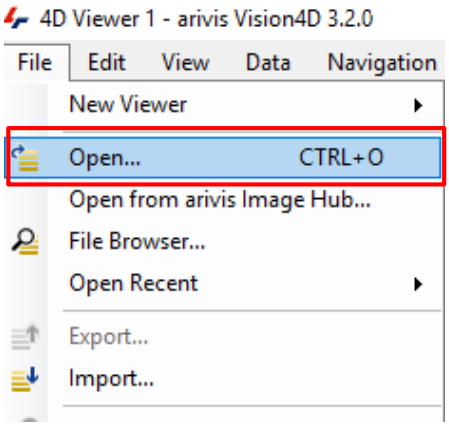
Three files are now available in the folder.



2. Open the demo dataset

Step 2

Open the Dataset on Vision4D.



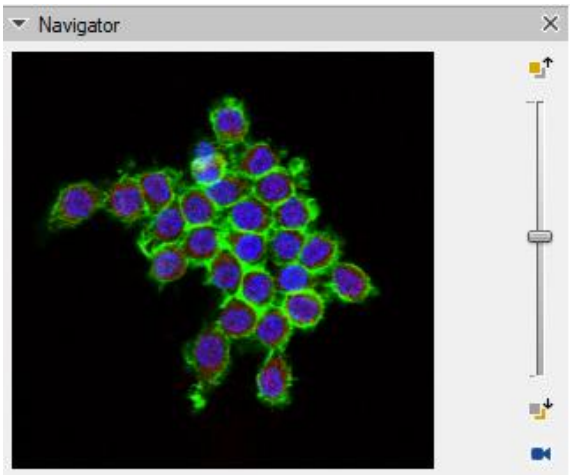
Step 2.1

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)

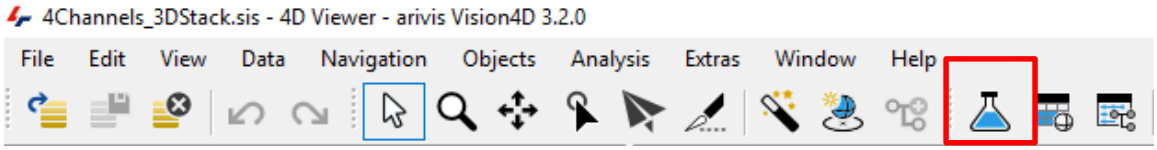
3. Select and activate the sample pipeline

Step 3

Select and activate the ***«Detect Cells or Particles Using Seeded Region Growing»*** *pipeline.*

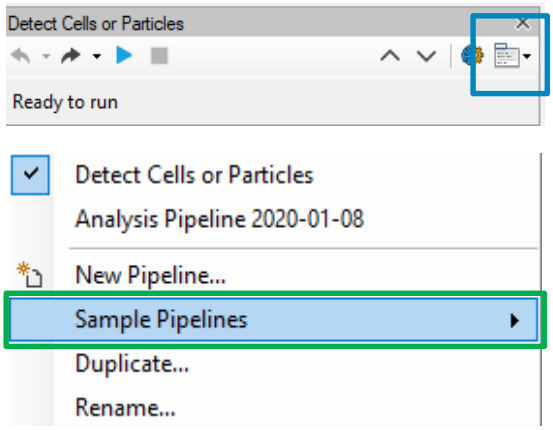
Step 3.1

Open the Analysis panel



Step 3.2

Drop down the analysis panel menu.



Step 3.3

Select *Sample Pipeline*.

Step 3.4

Click on the ***Detect Cells or Particles Using Seeded Region Growing*** item.

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

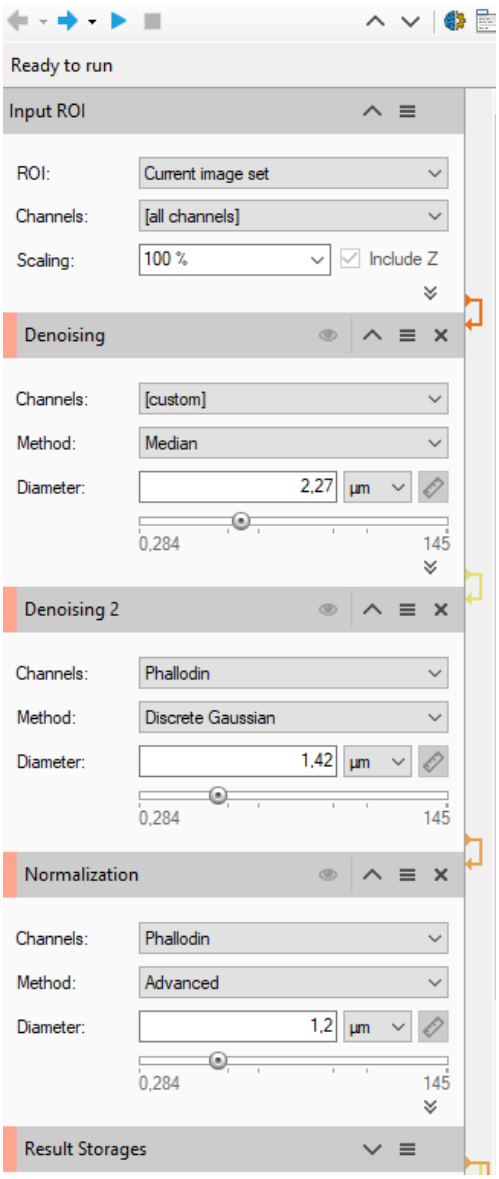
Step 4.1 *Region Of Interest:*
This operator allows the region of interest (ROI) selection. ROI defines the dataset subarea that will be processed and analyzed by the pipeline.

Step 4.2 *Denoising:*
Set of operators performing noise reduction. The «Median» with diameter of 2.27 um is used on 2 channels (Ch1 & Ch3).

Step 4.3 *Denoising:*
Set of operators performing noise reduction. The «Discrete Gaussian» with diameter of 1.41 um is used on channel 2.

Step 4.4 *Normalization*
The filter enhances and normalizes the intensity range distribution. Applied on channel 2 with a diameter of 1.2 um

Step 4.5 *Store Objects*
Store the detected segments (TAG) in the active dataset.



4. Pipeline operators layout

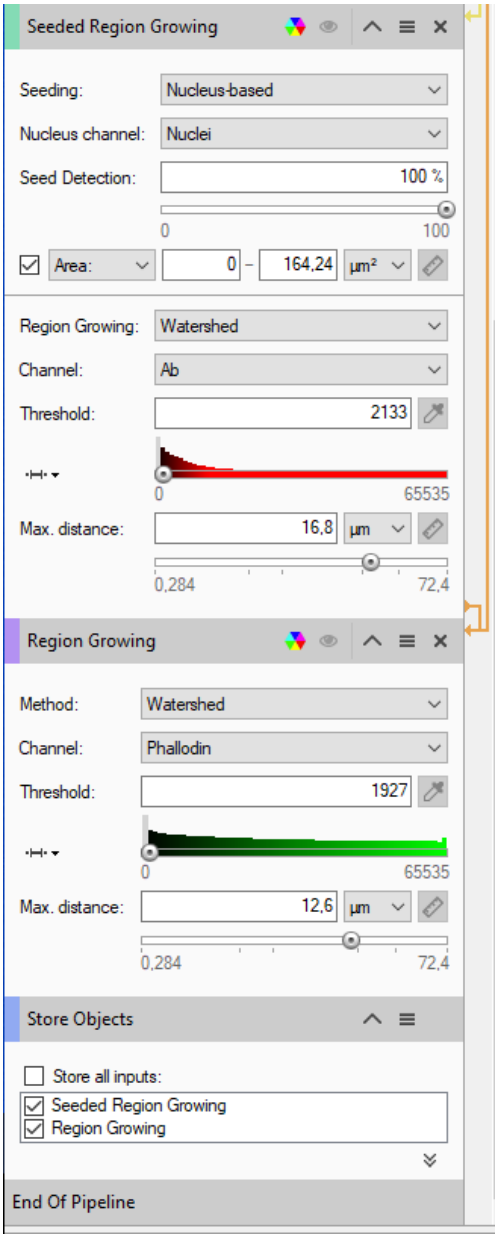
Step 4

Pipeline operators layout…..

Step 4.6 *Seeded region growing:* Automatic objects detection algorithm based on region growing approach. It uses seeds as starting point for the growing task.

Step 4.7 *Region Growing :* Automatic objects detection algorithm based on region growing approach. It uses seeds created by other threshold algorithms. Here, the seeds created by the previous Seeded region growing' operator (Nuclei- based seeding) were used.

Step 4.8 *Store Objects* Store the detected segments (TAG) in the active dataset.



5. Execute the pipeline step by step

Step 5

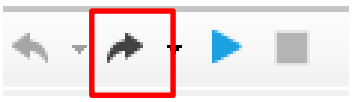
Execute the pipeline step by step.

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.

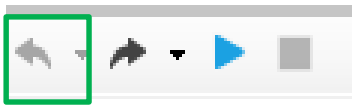
Step 5.1

Run the single operator



Step 5.2 (optional)

Undo the single operator



TIPS :

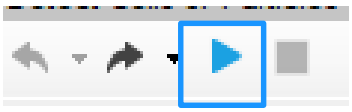
Undo the last operator executed if you need to change the operator settings.

6 - Execute the pipeline in a single run.

Execute the pipeline in a single run.

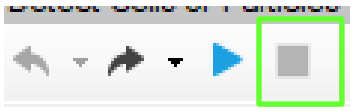
Step 6.1

Run the whole pipeline



Step 6.2 (optional)

Stop the pipeline execution

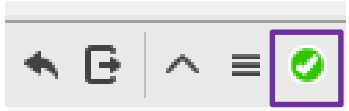


DETAILS :

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

Task running

Task completed



7. View the results

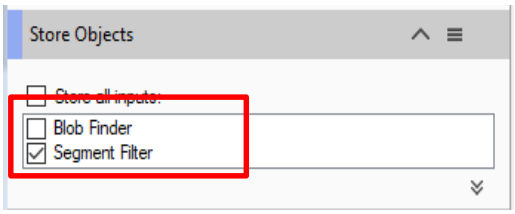
Step 7

View the results.

TIPS :

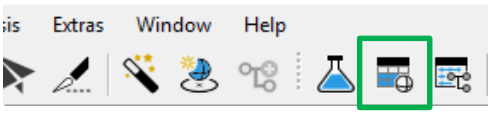
Results (segments and measurements) will be stored in the dataset only if the Store Objects operator has been correctly set.

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

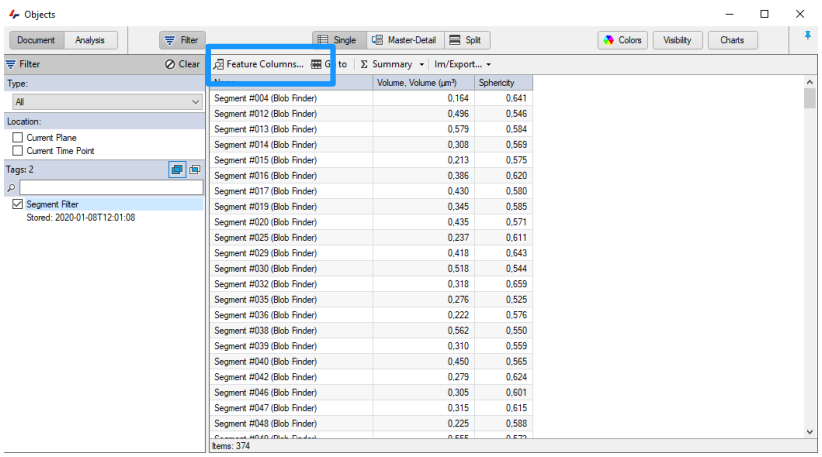


Step 7.1

Open the data table (if not already visible)



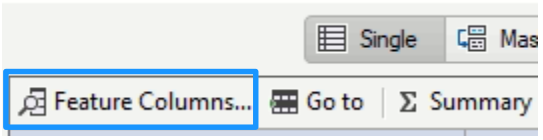
Measurements are now visible in the data table



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

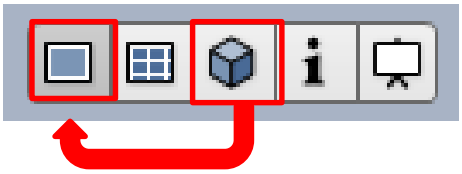
Modify the current pipeline.

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

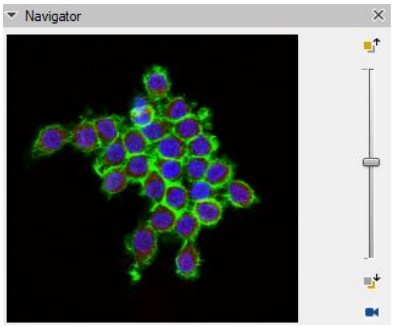
DETAILS :

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 :

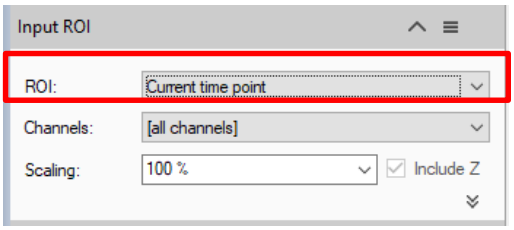
Use the Navigator Panel, located on the workspace area, to select the preview Z plane and/or Time Points (if any). Please refer to the (***arivis Vision4D Help***) for more details



8. Modify the pipeline

Step 8.2

Change the Input ROI' operator parameters



Step 8.2.1 – ROI:

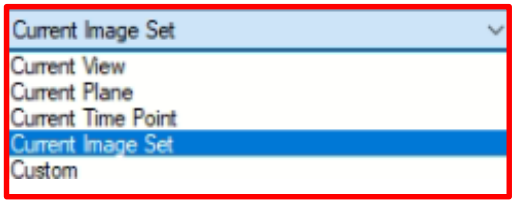
Sets the processing and analysis target space.

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.

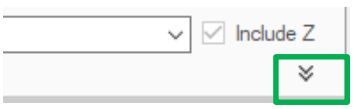


DETAILS :

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.

Custom : Allows to mix the previous methods.

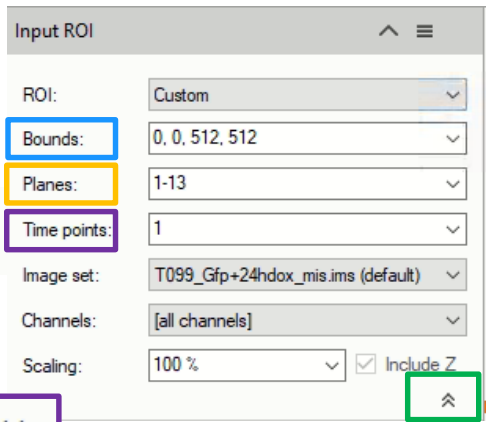
Expand the Input Roi dialog.



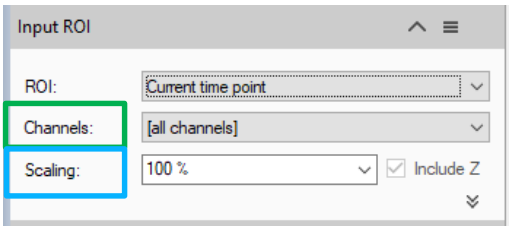
Bounds : Sets the analysis area edges. The whole XY bounds, the viewing area or a custom space can be applied.

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.



8. Modify the pipeline



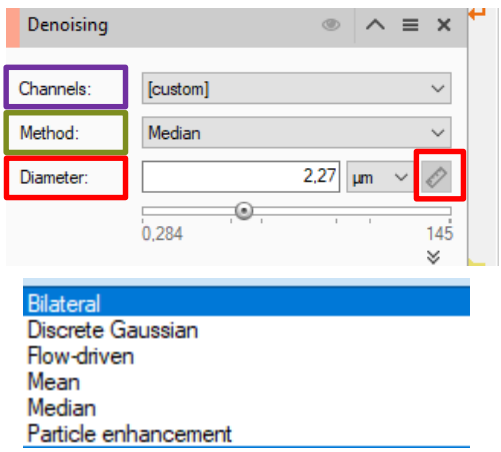
Step 8.2.2 – Channels:
Sets the processing and analysis target channels. Selecting a single channel, all the operators in the pipeline will be forced to use it.

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 Normalization' operator parameters

Step 8.4.1 – Channels:

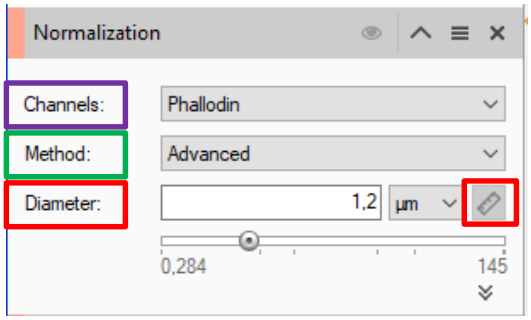
Set the processing target channel(s).

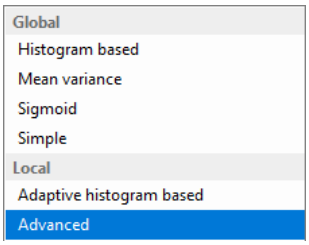
Step 8.4.2 – Method:

Set normalization method.

2 groups are available:

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





Step 8.4.3 – Diameter:

Set the reference filter 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.

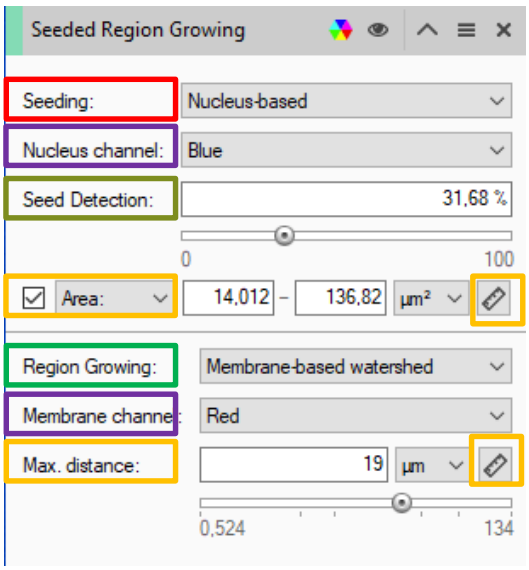
The structure diameter can be measured directly from the dataset.

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

8. Modify the pipeline

Step 8.5

Change the Seeded region growing ' operator parameters

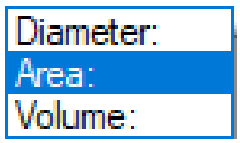


Step 8.5.1 – Seeding:
Set the seeding method.

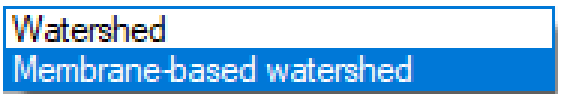
Step 8.5.2 – Channels:
Sets the analysis target channel.

Step 8.5.3 – Seed Detection:
Set the seed threshold percentage.

Step 8.5.4 – Area:
Set the Area (Volume/Diameter) seeds range. Only the segments that matches the range are accepted as seeds.
The filter can be enabled/disabled.



Step 8.5.5 – Region Growing:
Set the Region Growing method.

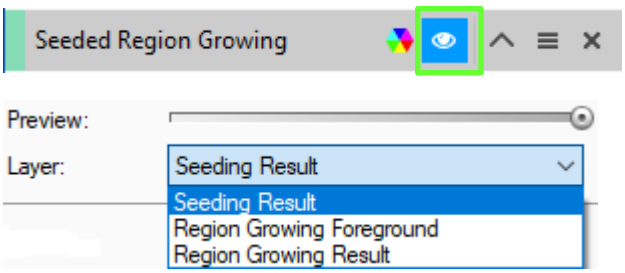


Step 8.5.6 – Membrane channel:
Sets the Membrane target channel.

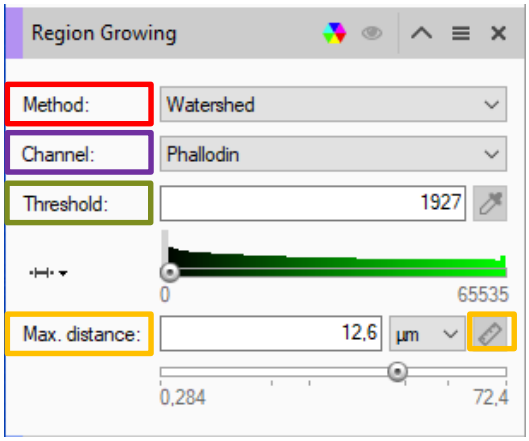
Step 8.5.7 – Max. distance:
Set the Maximum distance on which the region growing will act.

DETAILS :
The Seeded region growing 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 (if any).
The preview can show the seeding result as well as the Region Growing result.

TIPS :
Object Area (Volume/Diameter) and the Maximum distance features can be measured directly from the dataset. Please refer to the (***arivis Vision4D Help***) for more details



8. Modify the pipeline



Step 8.6

Change the Region Growing ' operator parameters

Step 8.4.1 – Method:
Set the seeding method.

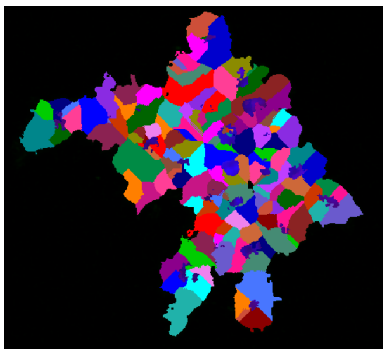
Step 8.4.2 – Channel:
Sets the analysis target channel.

Step 8.4.3 – Threshold:
Set the Region Growing threshold.

Step 8.4.7 – Max. distance:
Set the Maximum distance on which (within) the region growing will act.

TIPS :
Object Area (Volume/Diameter) and the Maximum distance features can be measured directly from the dataset. Please refer to the (***arivis Vision4D Help***) for more details

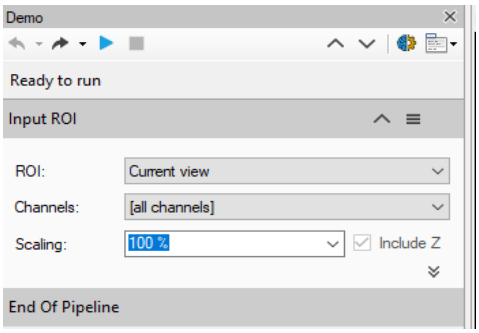
DETAILS :
The Seeded region growing has the preview icon located on the panel header (title bar).
Click on this icon to preview the operator result on the current plane / time point (if any).



8. Modify the pipeline

Step 8.5

Add or remove operators from the pipeline



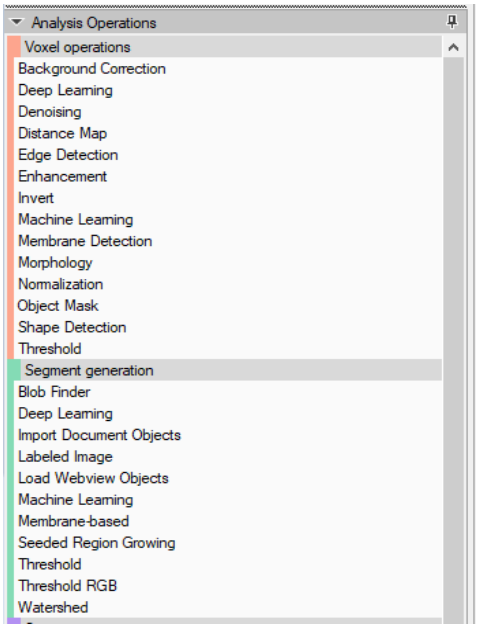
DETAILS :

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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