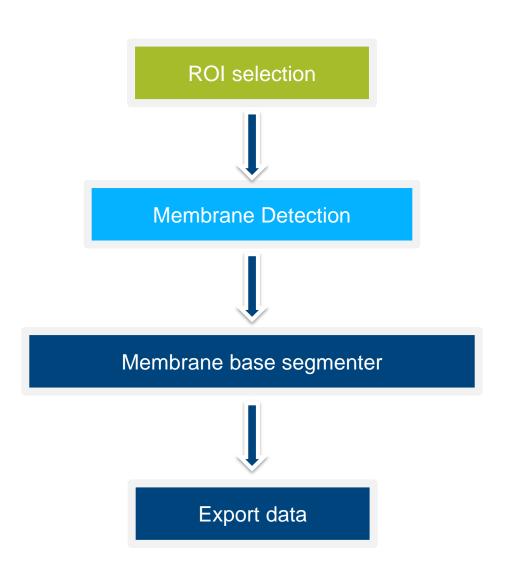




# How to guide - Sample Pipeline «DETECT CELLS BASED ON MEMBRANES WITH ENHANCEMENT »

The pipeline purpose is to identify cell body starting from the membrane markers.

### **Working Flowchart**





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- 1. Download the demo dataset
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- 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.



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



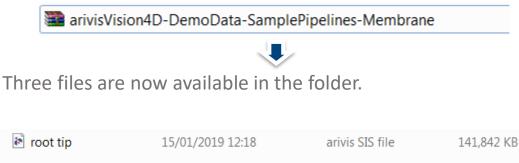
#### **Step 1.2**

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-Membrane.zip





### 2. Open the demo dataset

#### Step 2

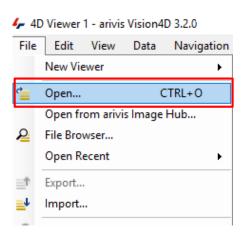
Open the Dataset on Vision4D.

#### Step 2.1

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

#### Step 2.2

Select the dataset from the file browser.



ir i	root tip

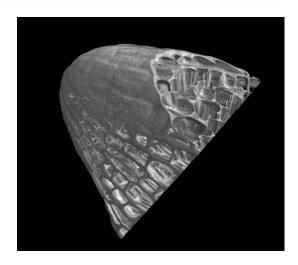
15/01/2019 12:18

arivis SIS file

141,842 KB

#### 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).

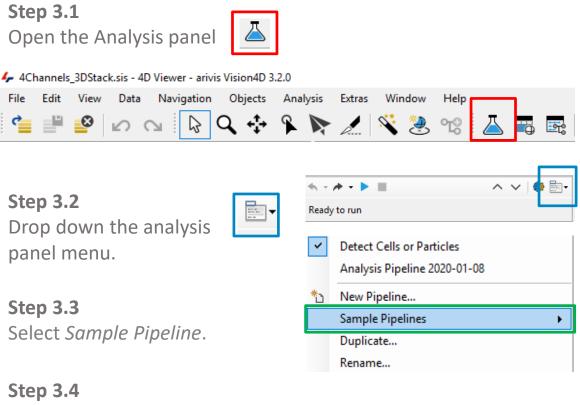
Usually the dataset shows a single experimental situation ( a complete experiment can be composed by several datasets). The datasets are available as graphic files saved in plenty of file formats (standard formats as well as proprietary formats )



### 3. Select and activate the sample pipeline

### Step 3

Select and activate the **«Detect Cells Based on** Membranes with Enhancement » pipeline.



Click on the Detect Cells Based on Membranes with Enhancement item.

#### **TIPS:**

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

Detect Cells Based on Membranes with Enhancement



### 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** *Membrane Detection:* The filter is used to enhance the membrane edges.

Step 4.3 Membrane based segmenter: Detects the cell body starting from the membrane.

Step 4.4 Store Objects Store the detected segments (TAG) in the active dataset.

Detect Membrane with Enhancement - 2020-01-14 (2) $ imes$			
<ul> <li></li> <li></li> <li></li> <li></li> <li></li></ul>		^ ~   🚸 🗄	•
Ready to run			
Input ROI		^ ≡	
ROI:	Current image set	~	
Channels:	Ch3	~	
Scaling:	100 %	✓ ✓ Include Z	
Membrane De	tection	● ^ = ×	7
Channels:	Ch3	~	
Membrane width	:	0,674 µm 🗸 🖉	
	0,0872	4,36	
Max gap size:		0,59 µm 🗸 🔗	
	0,0872	4,36	
Result Storage	25	~ ≡	Ļ
Membrane ba	sed Segmenter		4
Channel:	Ch3	~	
Min intensity: (membrane):		8 🌽	
·H· <del>•</del>	3	255	
Split sensitivity:		29,62 %	
	0	100	
Max diameter:	~	20 µm ~ 🖉	
Store Objects		^ ≡	
Store all inpu	uts:		
Membrane b	ased Segmenter		
		*	
End Of Pipeline			



### 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

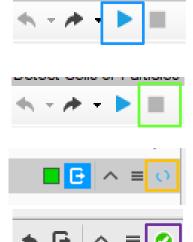
#### **DETAILS** :

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

Task running

Task completed







Vision4D 3.2

### 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.

Store Objects	^ ≡
☐ <del>Store all inpute:</del> ☐ Blob Finder ☑ Segment Filter	
	*

**Step 7.1** 

Open the data table (if not already visible)



Measurements are now visible in the data table

Document Analysis	🐺 Filter	目 Single	🖼 Master-Detail 🗮 Sp	let.	Colors Visibility Charts	
Filter	Ø Clear	🖉 Feature Columns 🛲 G 🛛 to 🕸 Σ	Summary • Im/Export	•		_
ype:			Volume, Volume (µm³)	Sphericity		
All	~	Segment #004 (Blob Finder)	0.164	0.641		
ocation:		Segment #012 (Blob Finder)	0,496	0.546		
Current Plane		Segment #013 (Blob Finder)	0.579	0.584		
Current Plane		Segment #014 (Blob Finder)	0,308	0,569		
		Segment #015 (Blob Finder)	0,213	0.575		
igs: 2		Segment #016 (Blob Finder)	0,386	0.620		
		Segment #017 (Blob Finder)	0,430	0.580		
Segment Filter		Segment #019 (Blob Finder)	0,345	0.585		
Stored: 2020-01-08T12:01:08		Segment #020 (Blob Finder)	0,435	0.571		
		Segment #025 (Blob Finder)	0,237	0.611		
		Segment #029 (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

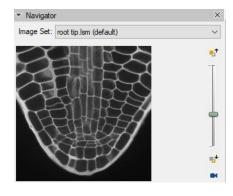
#### **DETAILS** :

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

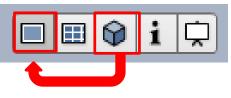
Click on this icon to preview the operator result on the current plane / time point

#### 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



Sets the processing and analysis target space.

<u>Current View</u>: The selected Z plane and the viewer area are processed. <u>Current Plane</u>: The selected Z plane is processed (XY).

<u>Current Time Point :</u> The selected time point is processed (XYZ) . <u>Current Image Set :</u> The complete dataset (XYZ and time) is processed.

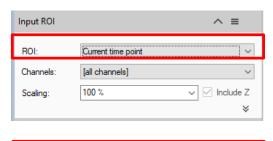
<u>Custom :</u> Allows to mix the previous methods.

Expand the Input Roi dialog.

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

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

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



Current Image Set	~
Current View	
Current Plane	
Current Time Point	
Current Image Set	
Custom	

#### **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.

	✓ Include Z
	*
Input ROI	^ ≡
ROI:	Custom
Bounds:	0, 0, 512, 512 ~
Planes:	1-13 ~
Time points:	1 ~
Image set:	T099_Gfp+24hdox_mis.ims (default) $\sim$
Channels:	[all channels] ~
Scaling:	100 % 🗸 🗸 Include Z
nts:	*



### 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.

Input ROI	^ ≡
ROI:	Current time point ~
Channels:	[all channels] ~
Scaling:	100 % 🗸 🗸 Include Z
	*

#### 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 Membrane Detection ' operator parameters

Step 8.3.1 –	Channels:	
Sets the proce	essing and	analysis
target channel(s).		

Step 8.3.2 – Membrane width:

Set the Minimum membrane width

Set the probability threshold coefficient.

TIPS :

The Minimum width & the Max gap can be measured directly from the dataset. Please refer to the (arivis Vision4D Help) for more details

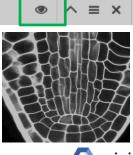
### **DETAILS**:



Membrane Detection

Membrane Detection 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



Membrane Dete	ection	● ^ = ×
Channels:	Ch3	~
Membrane width:		0,674 µm 🗸 🖉
	0,0872	4,36
Max gap size:		0,59 µm 🗸 🖉
	0,0872	4,36

## 8. Modify the pipeline

#### Step 8.4

Change the Membrane based segmenter' operator parameters

Step 8.4.1 - Channels: 🐴 📀 Membrane based Segmenter  $\equiv x$ Sets the processing and analysis Preview: target channel(s). Ch3 Channel: Min intensity: 100 🍼 Min intensity: Step 8.4.2 (membrane) (membrane): Set the Minimum membrane 255 intensity (GL). 29 % Split sensitivity 100 Step 8.4.3 - Split sensitivity: 18,485 µm Max diameter  $\sim$ Ø Set the Split-Sensitivity coefficient. Decrising the Split sensitivity will fuse more objects. Increasing the Split sensitivity will split more objects. Max diameter: Step 8.4.4 – Max diameter Max diameter: Set the membrane maximum diameter Max area: (Area/Volume). Drop down the list to Max volume: select the feature TIPS : Membrane Maximum Diameter (Area/Volume) can be measured directly from the dataset. Please refer to the (arivis Vision4D Help) for more details Membrane based Segmenter  $^{\sim} \equiv \times$ **DETAILS**: Membrane based segmenter 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 8.5

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.

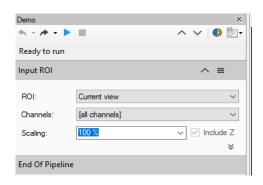
To remove an Operator from the Pipeline, press the X button located in the right side of the operator title bar.

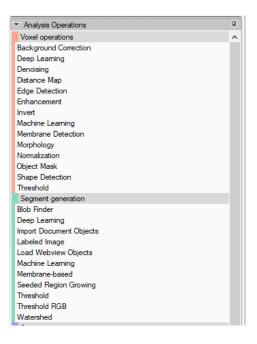
×



#### TIPS :

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









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