



# How to guide - Sample Pipeline «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.



### **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.



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.

🗲 4D Viewer 1 - arivis Vision4D 3.2.0					
File	Edit	View	Data	Navigation	
	New Vie	wer		×	
¢	Open		C	TRL+O	
	Open fr	om arivi	s Image	Hub	
R	File Browser				
	Open Re	ecent		•	
<u> </u>	Export				
∎•	Import				
-					

4Channels\_3DStack

#### 06/12/2018 10:12 arivis SIS file

### 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 Big Structures Auto» pipeline.





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 0.5um is used.

#### Step 4.3 Threshold

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

Step 4.4 Store Objects

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

Detect Big Structu	ures Auto	)
<, - / <b>&gt; - ▶</b>	■ ^ V   <b>(</b> )	11 X.
Ready to run		
Input ROI	^ ≡	
ROI:	Current image set	
Channels:	[all channels] ~	1
Scaling:	100 % ✓ Include Z	
Denoising	<ul> <li>● </li> <li>∧ = &gt;</li> </ul>	ĸ
Channels:	Channel #1 ~	-
Method:	Discrete Gaussian 🗸	-
Diameter:	0.1 51.	1
Result Storage	es ∨ ≡	
Threshold		ĸ
Channel:	Channel #1 ~	
Method:	Auto	1
Object type:	Bright ~	1
Thresholder:	Otsu ~	7
Range:	Time point ~	
Filter:		
Area:	0,10004 - 26,222 µm²	>
Store Objects	^ ≡	
Store all inp	uts: ×	
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

	1

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.

#### Step 7.1

Open the data table (if not already visible)



^ ≡

×

Measurements are now visible in the data table

👍 Objects						- 0	×
Document Analysis	₹ Filter	🗐 Single	🕼 Master-Detail 🗮 Spl	it	Scolors Visibility C	harts	Ŧ
<del>Ţ</del> Filter	⊘ Clear	🕫 Feature Columns 📟 G 🛛 to 🕸 Σ	Summary + Im/Export.	•			
Type:		**	Volume, Volume (µm³)	Sphericity			^
Al	~	Segment #004 (Blob Finder)	0,164	0.641			
Landing.		Segment #012 (Blob Finder)	0,496	0.546			
		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			
Tags: 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			
		Removal 4040 (Disk Disks)	0.555	0.670			~

Store Objects

Threshold

Store all inputs:

#### 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





#### 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 (if any).

#### 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









#### 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.

Custom : 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 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) ∨
Channels:	[all channels] $\checkmark$
Scaling:	100 % 🗸 🗸 Include Z
nts:	*



#### How to guide - Sample Pipeline «Detect Big Structures Auto»

## 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 Denoising' operator parameters

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

Step 8.3.2 – Method: Set the denoising algorithm

Channels: Channel #1 Method: Discrete Gaussian  $\sim$ 0.5 µm Ø Diameter 0,1 51, İ Discrete Gaussian Flow-driven Mean Median

Denoising

Particle enhancement

Step 8.3.3 -Diameter: Set the reference objects diameter.



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



≡  $\sim$ 

#### Step 8.4

Change the Threshold' operator parameters

Step 8.4.1 – Channels: Sets the processing and analysis target channel(s).	Threshold     •     •     ×       Channel:     Channel #1     ~       Method:     Auto     ~
Step 8.4.2 – Method: Set the Threshold algorithm. 2 groups are available:	Object type:     Bright     ✓       Thresholder     Otsu     ✓       Range:     Time point     ✓
<ul> <li>method</li> <li>Local – include local threshold method</li> <li>Step 8.4.3 – Object type:</li> <li>Set Bright objects (Dark Background) or Dark objects (Bright Background)</li> <li>scenario.</li> </ul>	Global Simple Range Percentile Percentile range Auto Local Adaptive mean Mean
Step 8.4.4 – Thresholder:         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	Otsu ~ Otsu Huang Li Yen Time point Plane Time point



#### **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

in the right side of the operator title

X Pipeline, press the X button located



#### **TIPS:**

bar.

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



<ul> <li>Analysis Operations</li> </ul>	<b>₽</b>
Voxel operations	~
Background Correction	
Deep Learning	
Denoising	
Distance Map	
Edge Detection	
Enhancement	
Invert	
Machine Learning	
Membrane Detection	
Morphology	
Nomalization	
Object Mask	
Shape Detection	
Threshold	
Segment generation	
Blob Finder	
Deep Learning	
Import Document Objects	
Labeled Image	
Load Webview Objects	
Machine Learning	
Membrane-based	
Seeded Region Growing	
Threshold	
Threshold RGB	
Watershed	







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