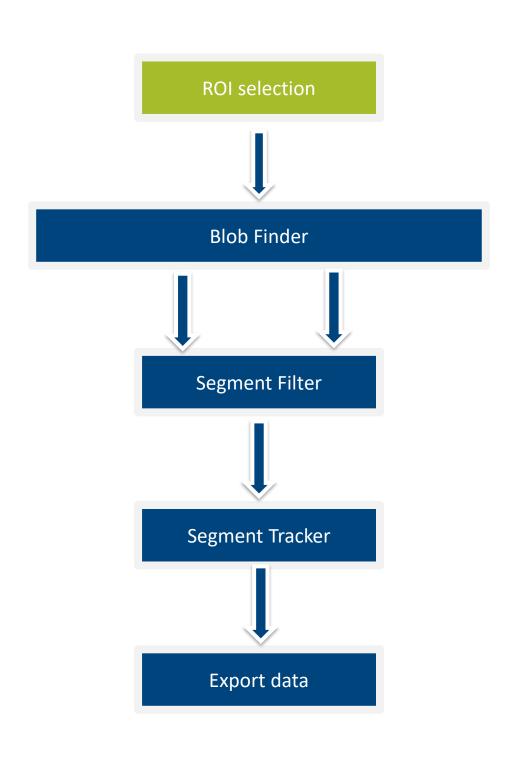




How to guide - Sample Pipeline «TRACK CELLS OR PARTICLES»

 The pipeline purpose is to track objects, detected using the Blob Finder operator, gathering its motion features (direction, speed, linearity, ect).
 It can be applied to Cells, Nuclei or any detected structures. How to guide - Sample Pipeline «Track Cells or Particles »

Working Flowchart





Index

- 1. Download the demo dataset
- 2. Open the demo dataset
- 3. Select and activate the sample pipeline
- 4. Pipeline operators layout
- 5. Execute the pipeline step by step
- 6. Execute the pipeline in a single run
- 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-Tracking.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-Tracking.zip*

arivisVision4D-DemoData-SamplePipelin... 10/12/2018 07:39

Three files are now available in the folder.

2Channels_3D_Tracking

06/12/2018 10:14

arivis SIS file



2. Open the demo dataset

Step 2

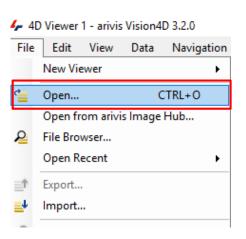
Open the Dataset on Vision4D.

Step 2.1

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

Step 2.2

Select the dataset from the file browser.



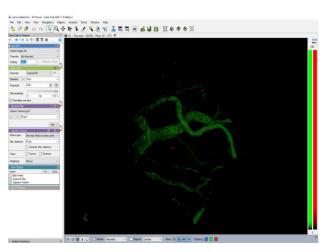
2Channels_3D_Tracking

06/12/2018 10:14

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).

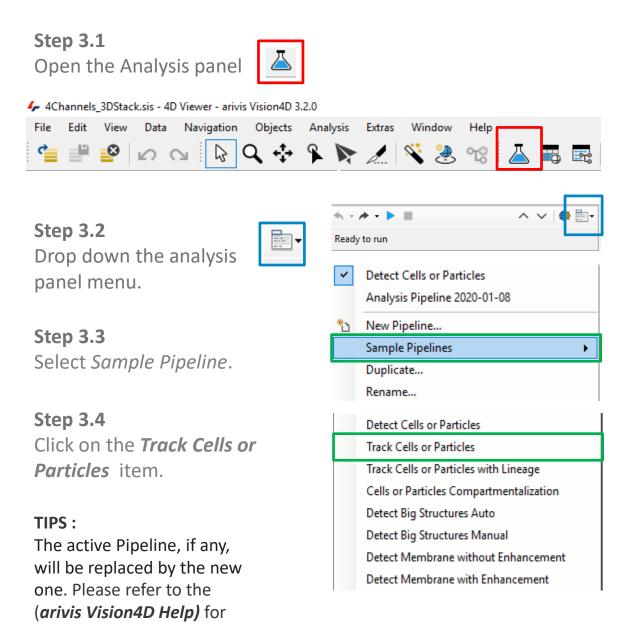
Usually the dataset shows a single experimental situation (a complete experiment can be composed by several datasets). The datasets are available as graphic files saved in plenty of file formats (standard formats as well as proprietary formats)



3. Select and activate the sample pipeline

Step 3

Select and activate the «Track Cells or Particles» 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 Blobs Finder:

Automatic small objects detection algorithm. It uses a local threshold method.

Step 4.3 Segment Filter Allows the blob filtering based on multiple parameters selection.

Step 4.4 Segment Tracker Track objects overt time according to the motion algorithm selected.

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

★ -	ticles	×
Ready to run	_	<u></u>
Input ROI	^ ≡	^
ROI:	Current image set \checkmark	
Channels:	[all channels] ~	
Scaling:	100 % V Include Z	
Blob Finder	▼	
Channel:	Channel #2 ~	
Diameter:	6 µm ~ 🖉	
	0,85 218	
Probability thresh		
	0 100	
Split sensitivity:	94 %	
	0 100	
Segment Filte		
Segment me		
Volume: Volume		
> ~ 30	htua 🔨	
Add 🝷		
Segment Trac	ker $\square \land \equiv \times$	لم ا
Motion type:	Brownian Motion (Centroid) $\qquad \qquad \lor$	
Max. distance:	20 µm ~	
	Compute Max. distance	
Centroid:	Compute Max. distance	
Centroid: Track:		-
	Center of Geometry ~	-
Track: Continue tracks: Max. time gap:	Center of Geometry Fusions Divisions [None]	-
Track: Continue tracks: Max. time gap: Delete "Zero	Center of Geometry Fusions Divisions [None] 'tracks	-
Track: Continue tracks: Max. time gap: Delete "Zero Color segme	Center of Geometry Fusions Divisions [None] 'tracks nts by track	-
Track: Continue tracks: Max. time gap: Delete "Zero	Center of Geometry Fusions Divisions [None] 'tracks	
Track: Continue tracks: Max. time gap: Delete "Zero Color segme	Center of Geometry Fusions Divisions [None] 'tracks nts by track [None]	
Track: Continue tracks: Max. time gap: Delete "Zero Color segme Weighting:	Center of Geometry ✓ Fusions Divisions [None] ✓ 1 ✓ 1 ✓ "tracks ✓ nts by track ✓ [None] ✓ ★ ★	
Track: Continue tracks: Max. time gap: Delete "Zerc Color segme Weighting: Store Objects Store all inp Blob Finder	Center of Geometry ~ Fusions Divisions [None] ~ 1 ~ "tracks	-
Track: Continue tracks: Max. time gap: Delete "Zerc Color segme Weighting: Store Objects	Center of Geometry ✓ Fusions Divisions [None] ✓ 1 ✓ "tracks ✓ nts by track ✓ [None] ✓ ∴ ✓ ⊥ ✓ ↓ ✓	
Track: Continue tracks: Max. time gap: Delete "Zero Color segme Weighting: Store Objects Store Objects Diob Finder Segment Fil	Center of Geometry ✓ Fusions Divisions [None] ✓ 1 ✓ or tracks ✓ nts by track ✓ [None] ✓ ∞ ✓ acker ✓ × ×	



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









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	^ ≡
☐ Store all inpute: ☐ 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	🗏 Single	🕼 Master-Detail 🗮 Sp	dit	Second Colors Visibility Charts	
Filter	⊘ Clear	🖉 Feature Columns ⊞ G 🛛 to 🕸 Σ	Summary • Im/Export			
Type:		21	Volume, Volume (µm²)	Sphericity		
AI	~	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		
ags: 2		Segment #016 (Blob Finder)	0,386	0,620		
D		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





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

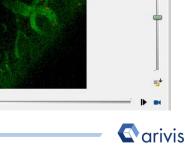
DETAILS :

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

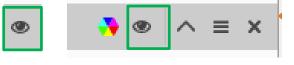
Click on this icon to preview the operator result on the current plane / time point

TIPS :

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







Navigator

. 0

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Step 8.2

Change the Input ROI' operator parameters



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.

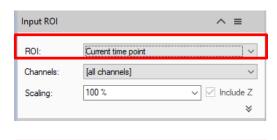
Custom : Allows to mix the previous methods.

Expand the Input Roi dialog.

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

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

Time Points : Sets the analysis time Time poir 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)
Channels:	[all channels]
Scaling:	100 % 🗸 🗸 Include Z
nts:	*



How to guide - Sample Pipeline «Track Cells or Particles »

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.

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

TIPS :

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

Step 8.3

Change the Blob Finder' operator parameters

Step 8.3.1 – Channels:

Sets the processing and analysis target channel(s).

Step 8.5.2 – Diameter:

Set the reference objects diameter (Max).

TIPS :

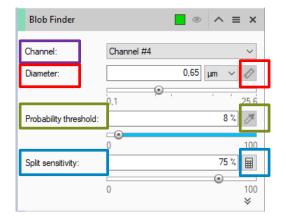
Object Diameter can be measured directly from the dataset. Please refer to the (*arivis Vision4D Help*) for more details

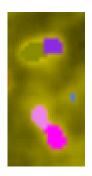
Step 8.3.3 – **Probability threshold**: Set the probability threshold coefficient.

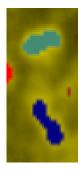
Step 8.3.4 - Split sensitivity:

Set the Split-Sensitivity coefficient.

Decrising the Split sensitivity will fuse more objects. Increasing the Split sensitivity will split more objects.







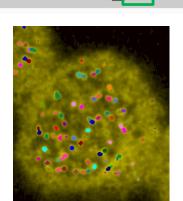


DETAILS :

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Blob Finder

Blob Finder 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



● ^ = ×

Step 8.4

Change the Segment Filter ' operator parameters

Step 8.4.1

Sets the feature to be used to filter the segments. Drop down the feature list and select the feature.

Step 8.4.2

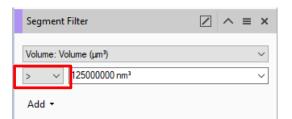


Sets the Filter action. Segments smaller than, bigger than, in the range of, outside the range filter criterias are available.

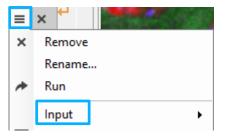
Step 8.4.3



Sets the Filter input. If more then one Segment Operator is present in your pipeline, the correct input source have to be set.









Step 8.5

Change the Segment Tracker ' operator parameters

Motion type: Step 8.5.1 -Set the Motion algorithm

DETAILS:

Three Motion type algorithm are available:

Brownian Motion (center point): Segments move more or less randomly in any direction Linear Regression (center point): Segments move in a somewhat straight direction.

Conal Angle (center point): Segments move in a more restricted straight direction.

Step 8.5.2 - Max. distance:

Enter the maximum distance that a segment can travel between time

points. If Compute Max. Distance is **ON**, the distance is automatically

computed.

Step 8.5.3 -Track:

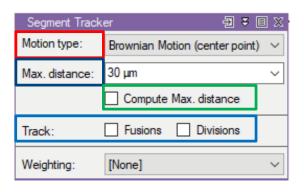
Set the Track option.

Fusions: Enables the tracking of two or more segments fusing into a single segment over time.

Divisions: Enables the tracking of one segment dividing into a two or more segments over time.

TIPS :

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



Brownian Motion (center point) Linear Regression (center point) Conal Angle (center point)

Centroid:	Center of Geometry \sim		
Track:	✓ Fusions	Divisions	
🔽 Limit	2		-
🔽 Limit	2		-
Min. fusion time	: 0		

Limit : Restricts the number of segments that can fuse into a single segment simultaneously. Limit : Restricts the number of segments into which a single segment can divide simultaneously.



Step 8.6

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.

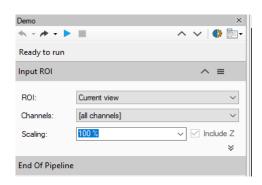
To remove an Operator from the Pipeline, press the X button located in the right side of the operator title bar.

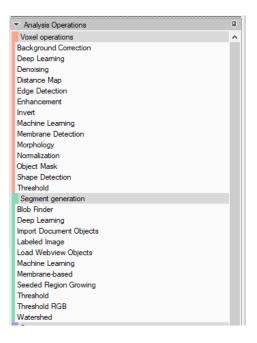
×



TIPS :

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









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