



# TRACK CELLS OR PARTICLES PIPELINE (USING BLOB FINDER)

«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.

### Arivis Vision4D Pipeline example «*Track Cells or Particles* » Working Flowchart :





arivis AG . Imaging Science . Erika-Mann-Straße 23 . Munich

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-Tracking.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.



#### <u>Step 3.</u> UnZip the file: *arivisVision4D-DemoData-SamplePipelines-Tracking.zip*



#### <u>Step 4.</u> Open the SIS file on Vision4D.

2Channels_3D_Tracking	06/12/2018 10:14 arivis SIS file
Fi Pi	2D Viewer 2 - arivis Vision4D 3.0. > e Edit View Data Nav g New Viewer • Open Ctrl+O File Browser Open Recent • Export
	Import Close Ctrl+X Close All Ctrl+Shift+X Close Other Save Ctrl+S Exit

Carivis

### 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 datasets). The datasets are available as graphic files saved in plenty of graphic formats (standard formats as well as proprietary formats )



### <u>Step 4.</u> Activate the «**Track Cells or Particles** » pipeline.



Select "Add Sample pipeline" Then click on the «*Track Cells or Particles»* 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 «**Track Cells or Particles**» pipeline operators layout.

Track Cells or Par	ticles - 2019-07-02	×	ς		
<b>← - → - </b> ►		- 📑 🌗	)		
Input ROI		₹ 🗉			
Current Image S	et	~			
Channels [all o	channels]	~			
Scaling 100	🖌 🧹 🗌 Restrict f	o Plane	Ъ		
Blob Finder			┙		
Channels:	Channel #2	v 🛈 -			
Diameter 🗸 🗸	6 µm	~			
Threshold:	5.00	-			
Split sensitivity:	0 04	100 %			
Normalize ov	ertime	100 %			
Comment Dillor					
Volume: Volume	(um <sup>3</sup> )				
	un-				
		Add ${\scriptstyle\checkmark}$	5		
Segment Track	er Đ	₹ 🗉 🛛	┙		
Motion type:	Brownian Motion (center p	oint) 🗸			
Max. distance:	30 µm	~			
	Compute Max. distance	•			
Track:	Fusions Division	8			
Weighting:	[None]	~			
Store Objects		₹ 🗉			
Inputs	A	None			
Blob Finder					
Segment Filte	er cker				
End Of Pipeline					

- <u>Region Of Interest:</u> This operator allows the region of interest (ROI) selection. ROI defines the dataset subarea that will be processed and analyzed by the pipeline.
- <u>Blobs Finder:</u> Automatic small objects detection algorithm. It uses a local treshold method.
- 3. <u>Segment Filter</u> Allows the blob filtering based on multiple parameters selection.
- 4. <u>Segment Tracker</u> Track objects overt time according to the motion algorithm setting.
- 5. <u>Store Objects</u> Store the detected segments (TAG) in the active dataset.



#### Step 5.

Execute the «*Track Cells or Particles*» 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.



### <u>Step 6.</u> 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		₹ 🗉
Inputs	A	None
Blob Finder ✓ Segment Filter ✓ Segment Tracker		

Measurements are now visible in the data table

						_	
Document Analysis Filter					Single Q Drill Dri	own Split	
Filter	Ø Clear	호텔 Feature	s ⊞ Goto	Im/Export			
ype:		Туре	Name	Volume, Volume (µm²)	Surface Area (µm <sup>2</sup> )	Mean, Intensities #2	Mean, Intensities #3
All	$\sim$	0	Segment #	259.942	377.194	51.870	31.770
ocation:		9	Segment #	168.082	289.622	21.176	30.587
Current Plane		0	Segment #	245.314	384.003	25.668	32.170
Current Time Point			Segment #	230.873	372.307	50.253	20.200
Spots Colocalization Spots Gall Reir Stored: 2018-08-30710-07-21 Stored: 2018-09-10712-07:55							

#### TIPS :

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





#### Arivis Vision4D Pipeline example

The «*Track Cells or Particles*» 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.





#### **DETAILS**:

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

#### TIPS :

Please refer to <u>Addendum A</u> 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 Operations must be added to the Pipeline in the correct order.

#### Step A.

### How to set the Input ROI operator

nput ROI		Surrent Image Set
Current Ima	age Set	Current View
Channels	[all channels]	Current Plane Current Time Point
Scaling	100% V Restrict to	Current Image Set Custom

Processing & Analysis target options:

a. <u>Current View</u>

Only the selected Z plane and the visualized area in the viewer are processed.

b. <u>Current Plane</u>

Only the selected Z plane is processed regardless to the visualized area (real XY pixel size).

- <u>Current Time Point</u>
   The selected time point is entirely processed (all Z planes and the real XY pixel size)
- *d.* <u>*Current Image Set*</u> The complete dataset (XYZ and time) is processed.
- *e.* <u>*Custom*</u> 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

Input ROI		≙ 🗉	Single Z plane, a range of Z
Custom		~	planes or the full Z planes can
Bounds	0, 0, 457, 504		be selected
Planes	1-46	~	
Time Points	1-30		of TP or the full TP can be
Image Set	piovra elabo4.ims (default)	~	selected
Channels	[all channels]	~	
Scaling	100% ~ Restric	ct to Plane	Select the source Image Set
The datas downsized option is pipeline v large.	et volume can be d by 50 % or 25 %. Thi used to speed up anal vhen the dataset is ve	is ysis ry	One or more of the available channels can be selected. Be careful, only the selected channel(s) can be used in the pipeline



#### Step B.

### How to set the Blob Finder operator

Select working Channel(s).



arivis AG . Imaging Science . Erika-Mann-Straße 23 . Munich

### Step C.

### How to set the Segment Filter operator



Select the filter rule.



### Step D. How to set the **Segment Tracker** operator

Set the required parameters according to the sample's motion behaviour.



**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. 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.

Enter the maximum distance that a segment can travel between time points. If **Compute Max. Distance** is ON, the distance is automatically computed

#### TIPS :

Please refer to Addendum C for more details about how to track objects



### Arivis Vision4D Pipeline setup Addendum A:

How to add or remove an *Operator* 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.



1875281

3. Take note of this number and digit it in the *Diameter Box* 

Blob Finder	
Channels:	Channel #1 v 👁 🗸
Diameter 🗸 🗸	8μm · Cum
Threshold:	4.00
<b>TIPS :</b> Before writ right metri	te down the diameter in the Box, select from the list the c unit you want to use.

C arivis

### Arivis Vision4D Pipeline setup <u>Addendum C</u>: How to Track objects.

Objects tracking operator provides a way to trace multiple segments (Annotations) progress as they move about in a time points to the next. Objects can move completely random or following a pseudo linear motion.

To face the objects motion behavior, three tracking algorithms are available



The appropriate tracking algorithms must be selected according to the user experience and knowledge of the sample features.



### Arivis Vision4D Pipeline setup <u>Addendum C</u>: How to Track objects.

The available Segment Tracker options.

Click on the double arrow icon on the Segment tracker operator top bar to expand the dialog box.

Segment Track	ter	휜 원	7 8 X
Motion type:	Brow	nian Motion (center po	nt) 🗸
Max. distance:	30 µr	n	~
		ompute Max. distance	
Track:	🗌 Fi	usions 🗌 Divisions	
Weighting:	[Non	e]	~
Comment Trees	1	5	
Motion type:	ĸer	Brownian Motion (ce	ente 🗸
Max. distance:		30 µm	~
		Compute Max.	
Centroid:		Center of Geometry	~
Centroid: Track:		Center of Geometry	visions
Centroid: Track: Imit fusions		Compute Max. Center of Geometry Fusions Div 2	∨ visions
Centroid: Track: Limit fusions Limit divisior	): 15:	Center of Geometry Fusions Div 2	visions
Centroid: Track: Limit fusions Limit division Min. time for fus	a: ns: sion:	Compute Max. Center of Geometry Fusions Div 2 2 0	visions
Centroid: Track: Limit fusions Limit division Min. time for fus Continue tracks	a: ns: sion:	Compute Max. Center of Geometry Fusions Div 2 2 0 [None]	visions
Centroid: Track: Limit fusions Limit division Min. time for fus Continue tracks Max. time gap:	): ns: sion: s:	Compute Max. Center of Geometry Fusions Div 2 2 0 [None] 1	visions
Centroid: Track: Limit fusions Limit division Min. time for fus Continue tracks Max. time gap:	e: ns: sion: s:	Compute Max. Center of Geometry Fusions Div 2 2 0 [None] 1 Delete "Zero" tra	visions
Centroid: Track: Limit fusions Limit division Min. time for fus Continue tracks Max. time gap:	s: ns: sion: s:	Center of Geometry  Fusions Div  Center of Geometry  Fusions Div  Center of Geometry  Center of Geometry  Center of Geometry  Div  Center of Geometry  Div  Center of Geometry  Center of	visions vis

If the **Conal Angle** Motion type is selected, the Max Angle text box is also shown.

Segment Tracker	, Ð ± ⊟ ×
Motion type:	Conal Angle (center poi $ \smallsetminus $
Max. angle:	180.00
Max. distance:	30 µm 🔨 🗸 🗸
Max. distance:	30 µm → ✓ Compute Max.

Enter maximum angle a segment can deviate from its previous straight direction.

Enter the maximum distance that a segment can travel between time points. If **Compute Max. Distance** is ON, the distance is automatically computed

**Geometric Centroid** option uses the real object center of mass as the position reference. 3 options are available.

Center of Geometry	$\sim$
Center of Bounding Box	
Center of Geometry	
Center of Mass (Intensity)	



# Arivis Vision4D Pipeline setup Addendum C:

How to Track objects.

Fusions	Divisions
2	<b>÷</b>
2	l\$
0	
	2 2 0

Min. time for fusion: Using this option the tracker will look for invalid fusions. A fusion is invalid if a division occurs within the given number of time points after the fusion.



Delete "Zero" tracks: Remove tracks that have no length. Color segments by track: Recolors segments to the color of the tracks they have been assigned to. 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.

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

 Continue tracks: Enables the tracking of a given set of start objects. These objects can be segments, marker, or even tracks.

Max. time gap: Enter the maximum number of time points that a segment may absent



## Arivis Vision4D Pipeline setup Addendum C:

How to Track objects.



#### TIPS :

As a Max Deviation value , you can use both absolute values and relative values digitizing the suffix "%" after the number.

#### **DETAILS**:

Weighting feature is used to define if the segment under evaluation should be considered as part of the current track.

If it doesn't match the requirement, the segment will be discharged and not added to the track path.



# Arivis Vision4D Pipeline setup Addendum C:

How to Track objects.



Weight: Defines the importance of a feature regarding the similarity from one time point to the next. The higher the weight the more important it is during the tracking that the feature value remains similar. The lower the weight the more important the motion direction is during the tracking.



#### **DETAILS:**

If one feature value is already outside of the allowed deviation range, two segments of different time points will be considered unequal and not be connected in a track regardless the setting of this option.

Using a **Weight** of 0 (slide in a central position), allows you to define specific constraints without influencing the rest of the matching.



### Arivis Vision4D Pipeline setup <u>Addendum C (con't)</u>: How to Track objects.

Object can disappear from the dataset for some time points and reappear again after a while. The motion algorithm must take in consideration this gap in order to establish the correct link between objects and build the track.

If the maximum number of missed time points overcome the set limit, the object under evaluation will be considered as part of a new a track. Use the *Max. frame gap* parameter to set the maximum number of time points that can be missed.



arivis

### Arivis Vision4D Pipeline setup <u>Addendum C (con't)</u>: How to Track objects.

Moving distance of an object in contiguous time points in is also used to establish the correct link and build the track.

If object maximum distance overcome Max. Distance limit, the object under evaluation will be considered as part of a new a track. Use the *Max. distance* parameter to set the maximum distance between contiguous objects in consecutive time points.



Carivis