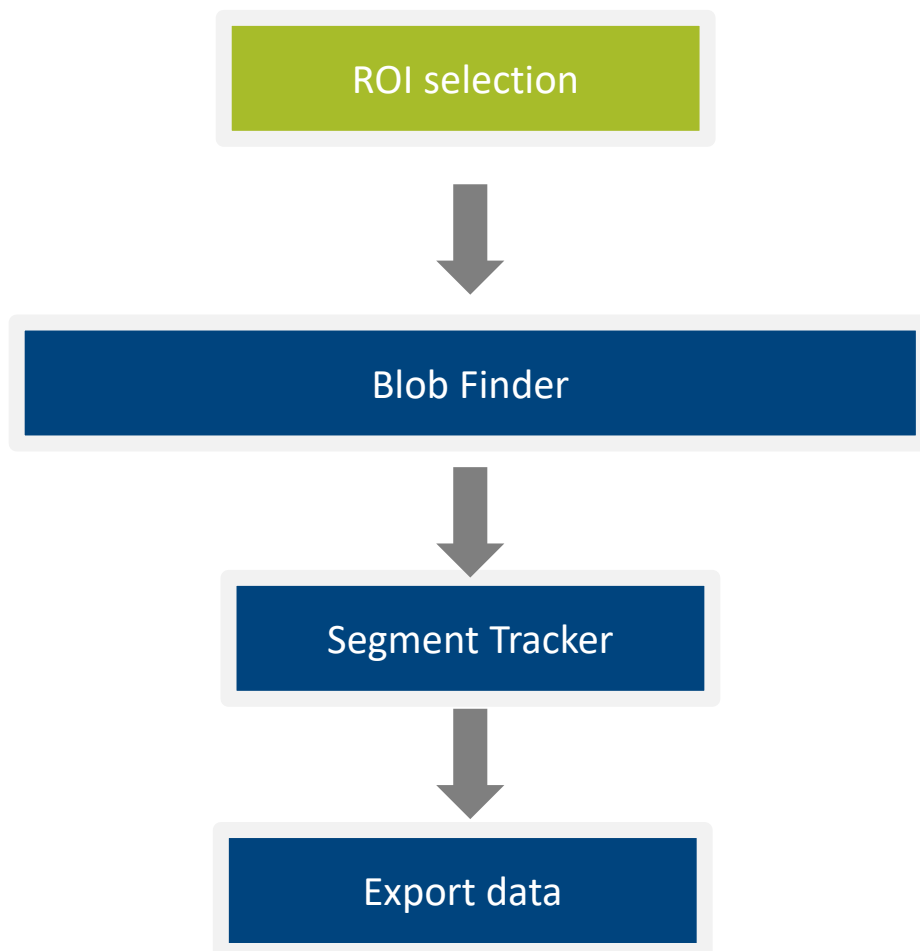


TRACK CELLS OR PARTICLES PIPELINE (USING BLOB FINDER)

«*Track Cells or Particles (Lineage)*»

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 Vision4D Pipeline example

In order to run the pipeline described here below, please download the demo dataset according to the following instruction.

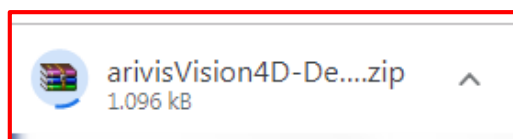
Step 1.

Click on the below link to access to the Arivis downloading demo dataset's area.



[Demo Dataset](#)

arivisVision4D-DemoData-SamplePipelines-Lineage.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.

Step 3.


UnZip the file:

arivisVision4D-DemoData-SamplePipelines-Lineage.zip

 arivisVision4D-DemoData-SamplePipelines-Lineage 15/02/2019 13:30


This file is now available in the folder.

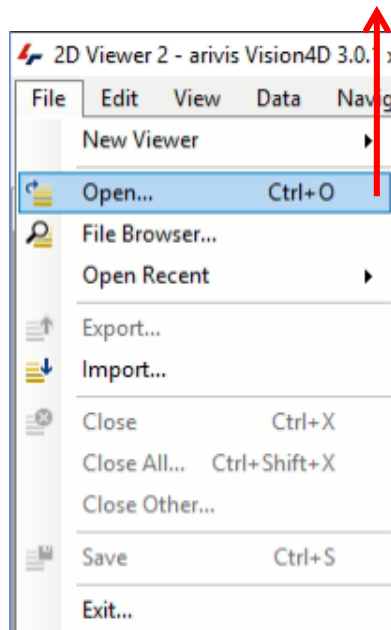


 2Channels_3D_Tracking_with_Lineage 15/02/2019 10:17 arivis SIS file

Step 4.

Open the SIS file on Vision4D.

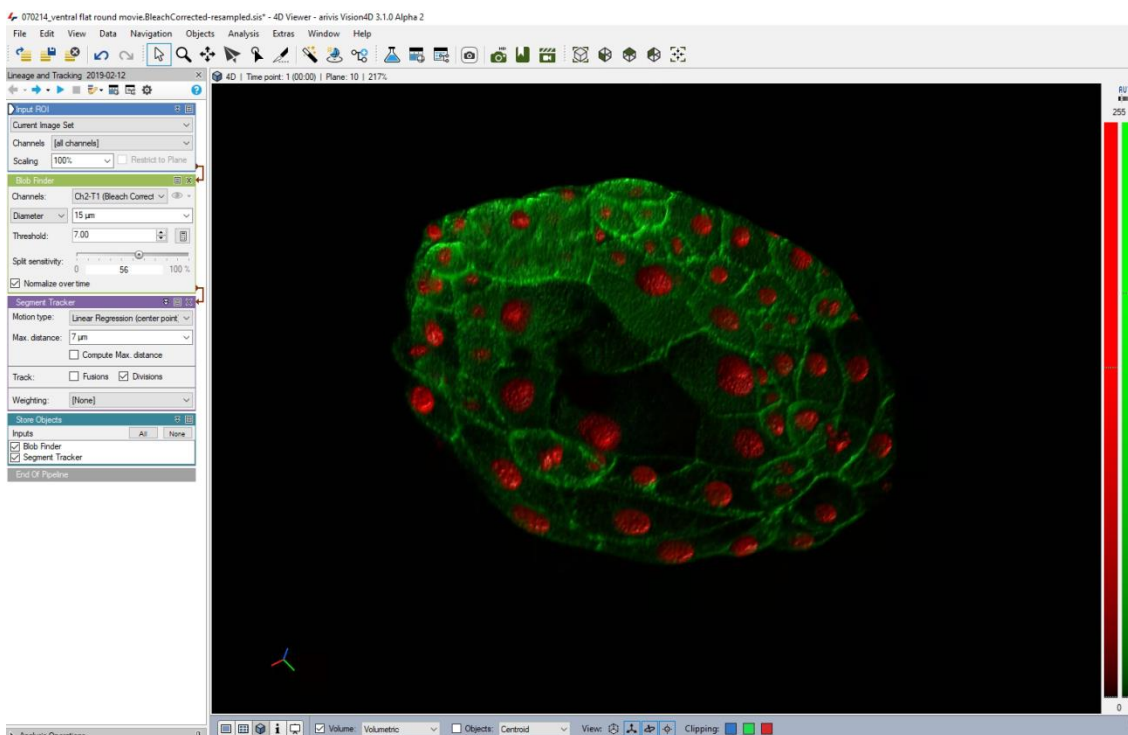
 2Channels_3D_Tracking_with_Lineage 15/02/2019 10:17 arivis SIS file



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.

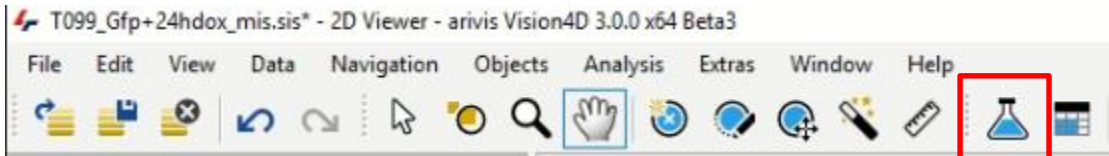


DETAILS:

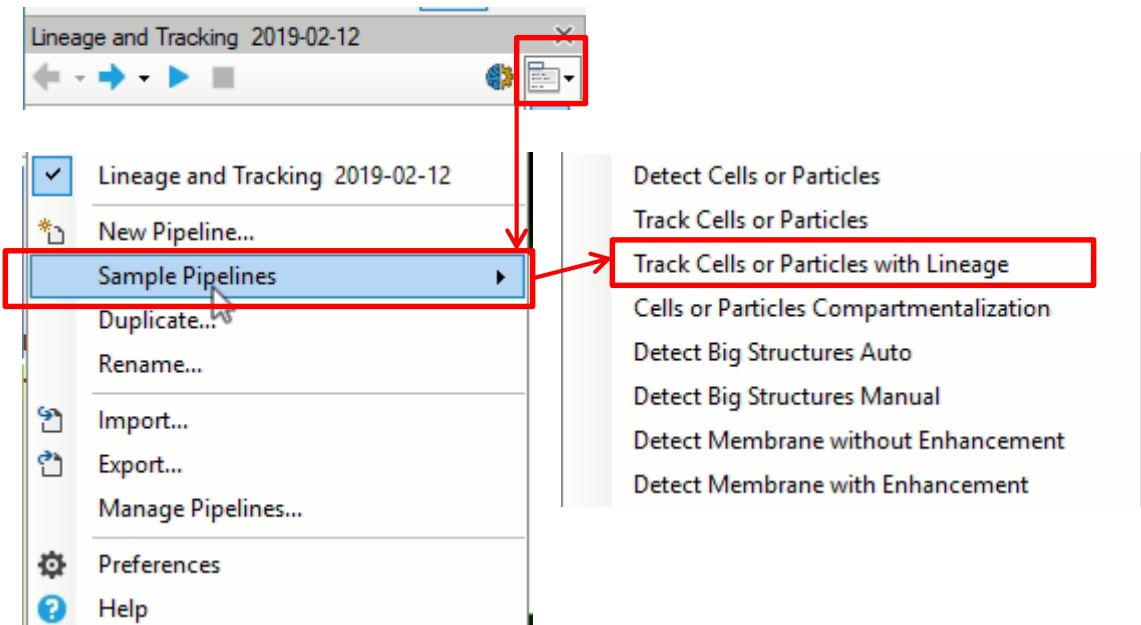
The dataset is a multi dimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (eg Optical sectioning) of multiple channels (dyes) in a temporal sequence of time points (located in several spatial positions). Usually the dataset shows a single experimental 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)

Step 4.

Activate the «*Track Cells or Particles with Lineage*» pipeline.



If not already done, open the Analysis panel



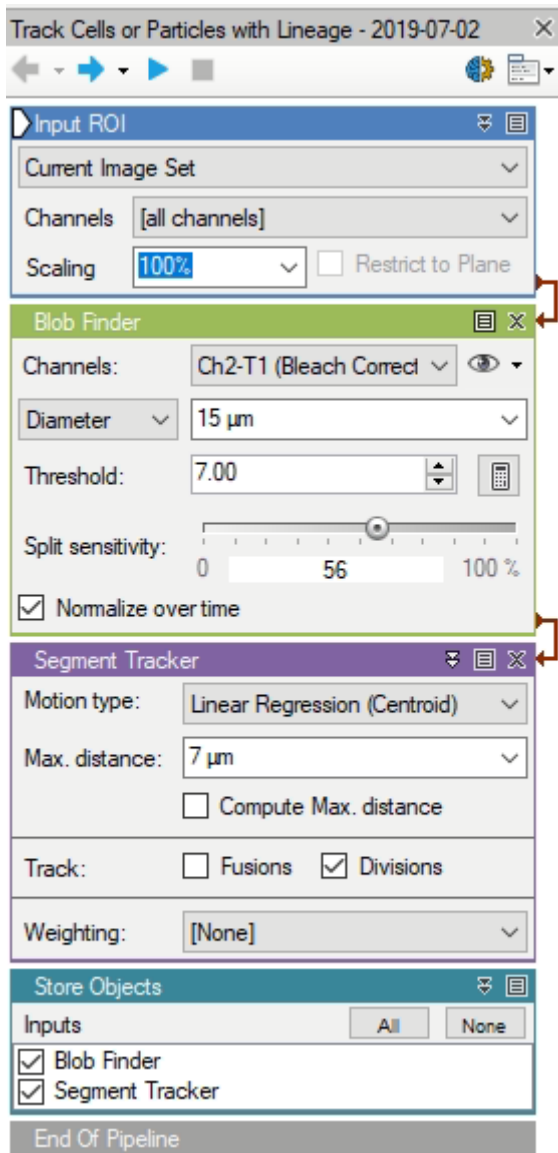
Select “Add Sample pipeline”

Then click on the «*Track Cells or Particles (Lineage)*» 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 (Lineage)*» pipeline operators layout.



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.
2. *Blobs Finder:*
Automatic small objects detection algorithm. It uses a local threshold method.
3. *Segment Filter*
Allows the blob filtering based on multiple parameters selection.
4. *Segment Tracker*
Track objects overt time according to the motion algorithm setting.
5. *Store Objects*
Store the detected segments (TAG) in the active dataset.

Step 5.

Execute the «**Track Cells or Particles (Lineage)**» pipeline.

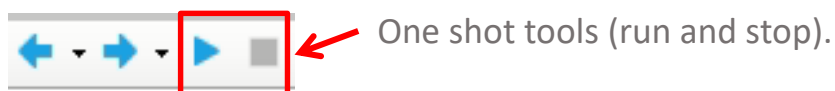
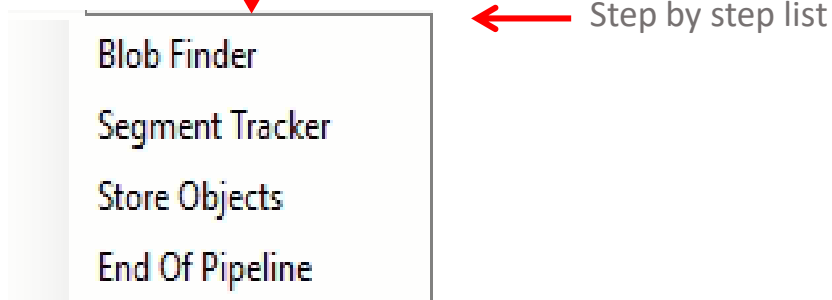
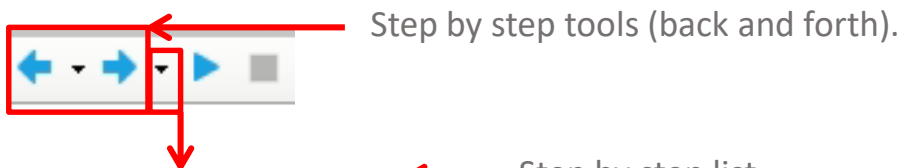
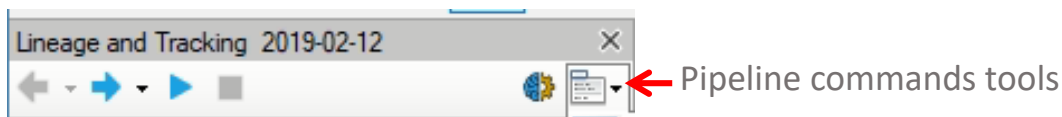
TIPS :

The pipeline can be executed as single shot or step by step.

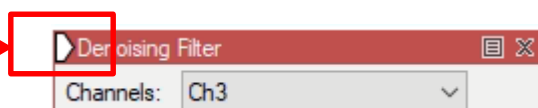
Step by step method allows to run and undo a single **Operation**.

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.



The white arrow on the operator title bar shows the next step that will be executed.

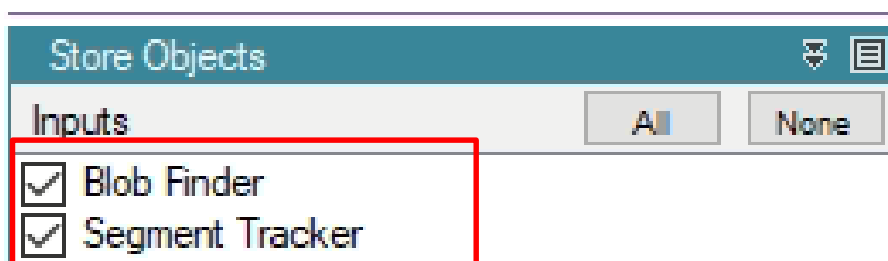


Step 6.

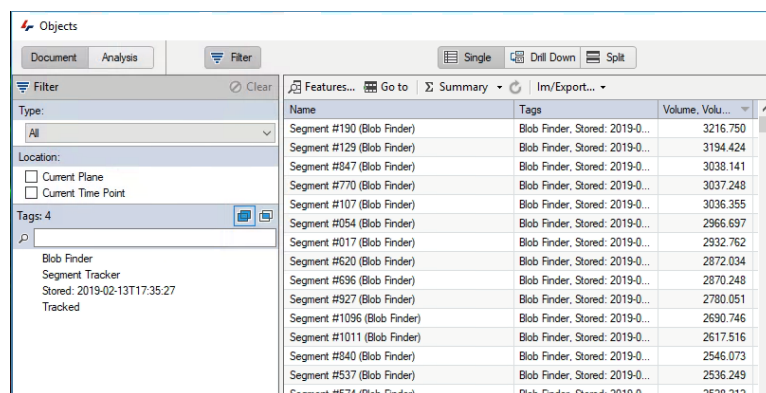
View the results

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.



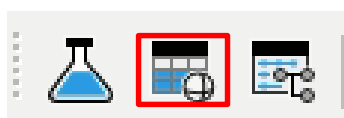
Measurements are now visible in the data table



Name	Tags	Volume, Volu...
Segment #190 (Blob Finder)	Blob Finder, Stored: 2019-0...	3216.750
Segment #129 (Blob Finder)	Blob Finder, Stored: 2019-0...	3194.424
Segment #847 (Blob Finder)	Blob Finder, Stored: 2019-0...	3038.141
Segment #770 (Blob Finder)	Blob Finder, Stored: 2019-0...	3037.248
Segment #107 (Blob Finder)	Blob Finder, Stored: 2019-0...	3036.355
Segment #054 (Blob Finder)	Blob Finder, Stored: 2019-0...	2966.637
Segment #017 (Blob Finder)	Blob Finder, Stored: 2019-0...	2932.762
Segment #620 (Blob Finder)	Blob Finder, Stored: 2019-0...	2872.034
Segment #696 (Blob Finder)	Blob Finder, Stored: 2019-0...	2870.248
Segment #927 (Blob Finder)	Blob Finder, Stored: 2019-0...	2780.051
Segment #1096 (Blob Finder)	Blob Finder, Stored: 2019-0...	2690.746
Segment #1011 (Blob Finder)	Blob Finder, Stored: 2019-0...	2617.516
Segment #840 (Blob Finder)	Blob Finder, Stored: 2019-0...	2546.073
Segment #537 (Blob Finder)	Blob Finder, Stored: 2019-0...	2536.249
Segment #574 (Blob Finder)	Blob Finder, Stored: 2019-0...	2528.212

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 (Lineage)***» 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.

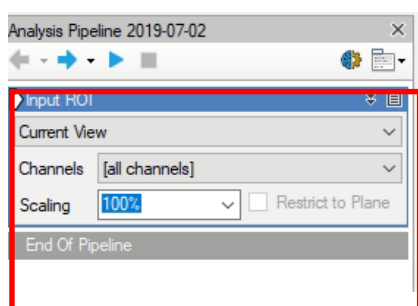
During analysis setup, the Operator preview mode is only available in 2D mode. Once the segments have been generated, you can switch back to 4D view mode.



TIPS :

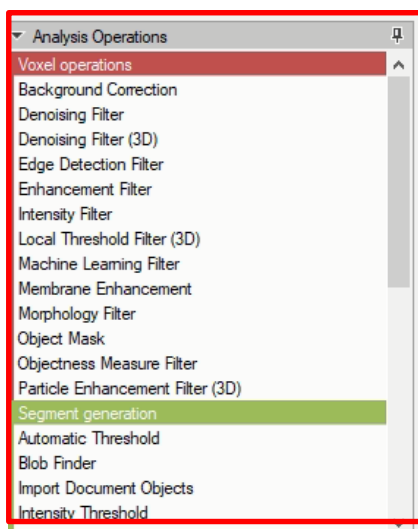
Please refer to the ***User Manual*** for more details about how to switch the Viewing Area from 4D to 2D view mode.

Arivis Vision4D Pipeline setup



DETAILS:

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



TIPS :

Please refer to [Addendum A](#) for more details about how to add or remove an **Operator** to the current Pipeline

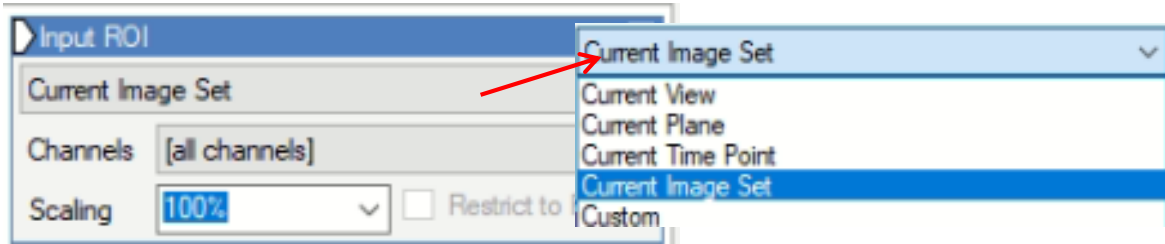
DETAILS :

Analysis Pipeline protocol is executed from top to bottom of the pipeline. The Operations must be added to the Pipeline in the correct order.

Arivis Vision4D Pipeline setup

Step A.

How to set the **Input ROI** operator



Processing & Analysis target options:

- a. Current View
Only the selected Z plane and the visualized area in the viewer are processed.
- b. Current Plane
Only the selected Z plane is processed regardless to the visualized area (real XY pixel size).
- c. Current Time Point
The selected time point is entirely processed (all Z planes and the real XY pixel size)
- d. Current Image Set
The complete dataset (XYZ and time) is processed.
- e. Custom
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 :

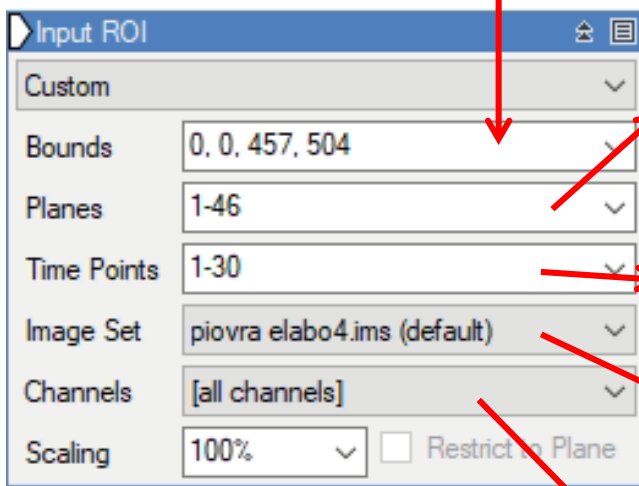
Please refer to the **User Manual** for more details about how to select the active Z plane and/or the active Time Point.

Arivis Vision4D Pipeline setup

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



Single Z plane, a range of Z planes or the full Z planes can be selected

Single Time point (TP), a range of TP or the full TP can be selected

Select the source Image Set

One or more of the available channels can be selected. Be careful, only the selected channel(s) can be used in the pipeline

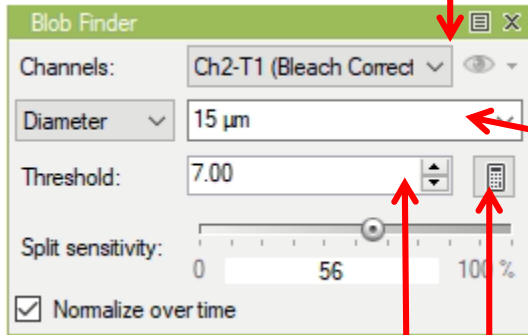
The dataset volume can be downsized by 50 % or 25 %. This option is used to speed up analysis pipeline when the dataset is very large.

Arivis Vision4D Pipeline setup

Step B.

How to set the **Blob Finder** operator

Select working Channel(s).



Set the reference objects diameter.

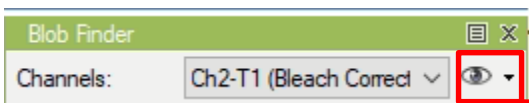
Set the object threshold coefficient.

Use the calculator to automatically set the threshold coefficient.

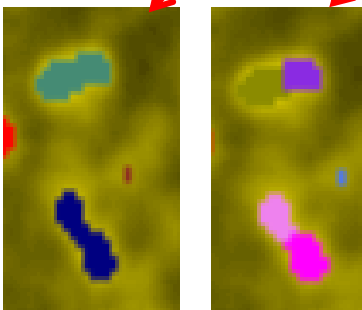
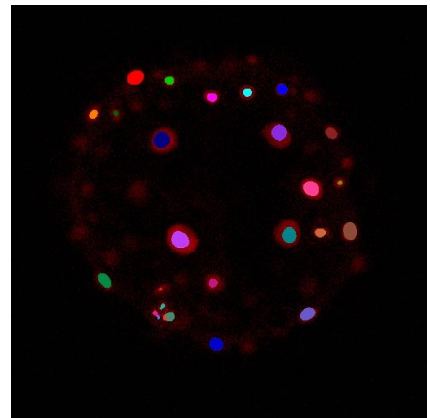
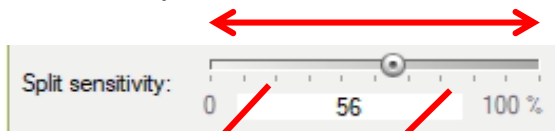
TIPS :

Object Diameter can be measured directly from the dataset.

Please refer to **Addendum B** for more details about how to measure object diameter.

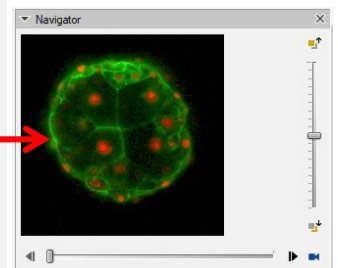


Move the Split-Sensitivity slider to decrease/increase the splits objects sensitivity.



TIPS :

Use the **Navigator Panel** to select the preview Z plane and/or Time Points (if any)

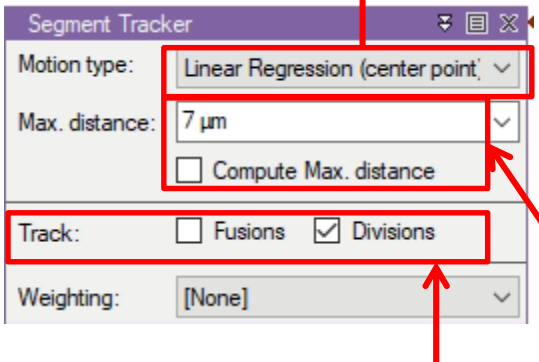
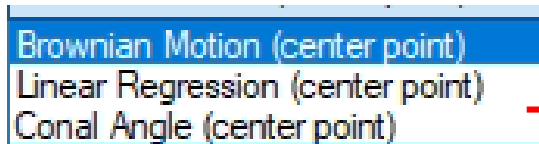


Arivis Vision4D Pipeline setup

Step C.

How to set the **Segment Tracker** operator

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



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.

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.

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

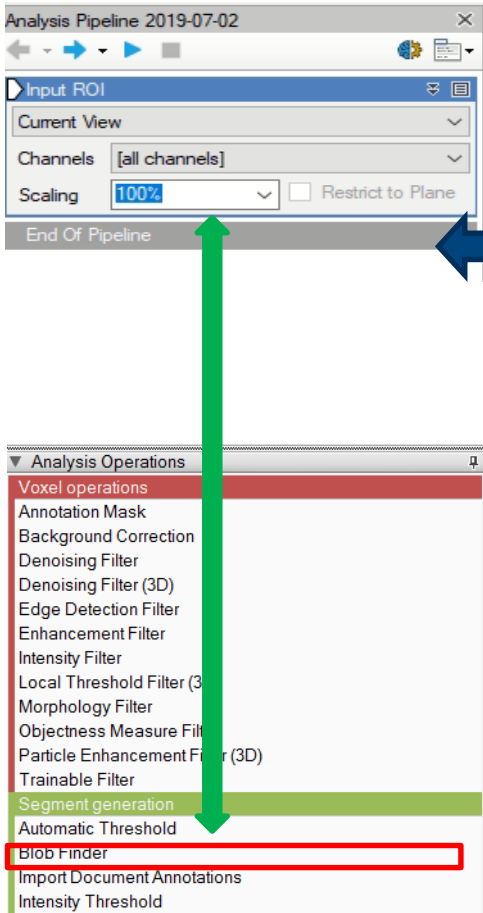
TIPS :

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.



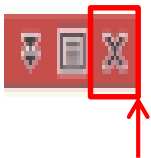
The **Operators** can be added to Pipeline in two ways

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*.

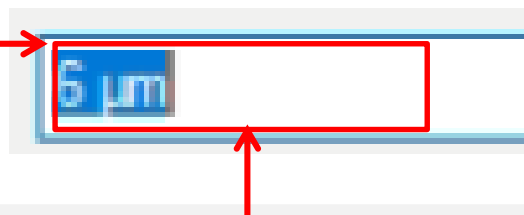
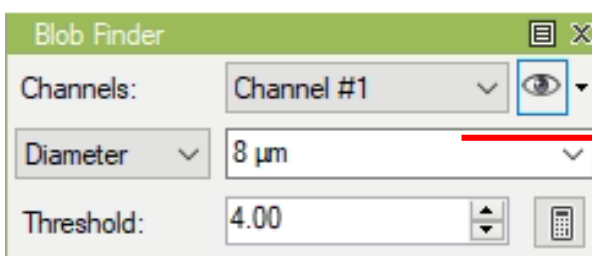
1. Move the mouse cursor (it shows a little ruler instead of the standard arrow) on one side of the structure you want to measure.

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.



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



TIPS :

Before write down the diameter in the Box, select from the list the right metric unit you want to use.

Then digit the measure without delete the unit in the box

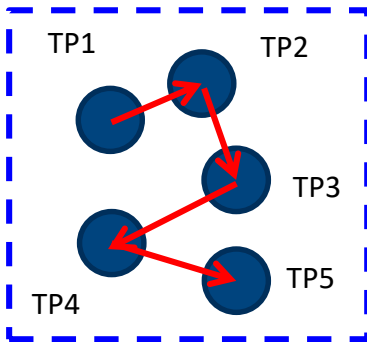
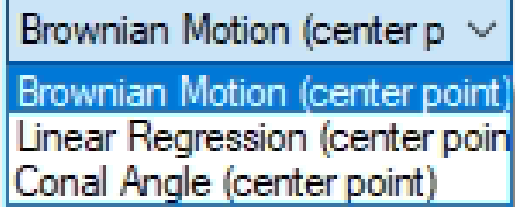
Arivis Vision4D Pipeline setup

Addendum C:

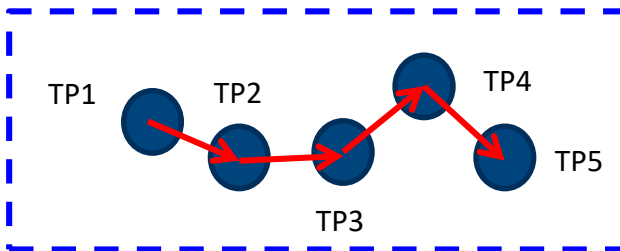
How to Track objects.

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.

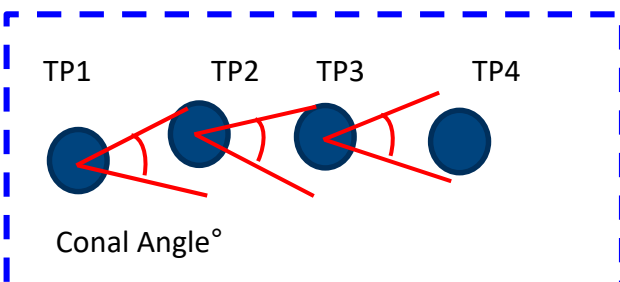
To face the objects motion behavior, three tracking algorithms are available



Brownian Motion



Linear Regression



Conal Angle

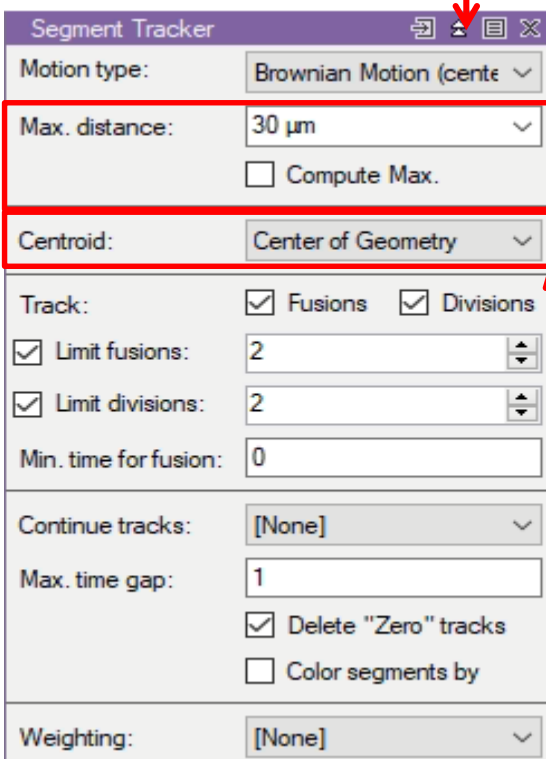
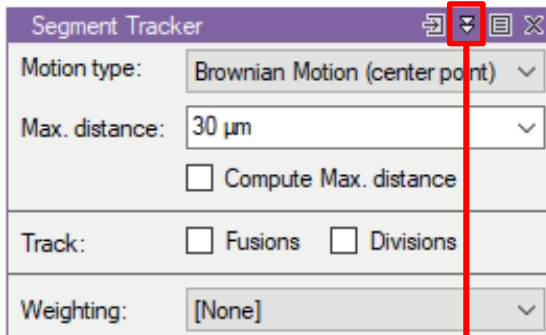
The appropriate tracking algorithms must be selected according to the user experience and knowledge of the sample features.

Arivis Vision4D Pipeline setup

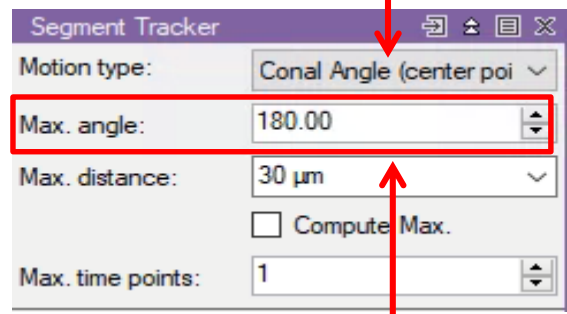
Addendum C: How to Track objects.

The available **Segment Tracker** options.

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



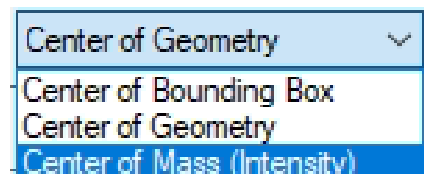
If the **Conal Angle** Motion type is selected, the Max Angle text box is also shown.



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



Arivis Vision4D Pipeline setup

Addendum C:

How to Track objects.

Track:	<input checked="" type="checkbox"/> Fusions	<input checked="" type="checkbox"/> Divisions
<input checked="" type="checkbox"/> Limit fusions:	2	
<input checked="" type="checkbox"/> Limit divisions:	2	
Min. time for fusion:	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.

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:	[None]
Max. time gap:	1
<input checked="" type="checkbox"/> Delete "Zero" tracks	
<input type="checkbox"/> Color segments by	

Delete "Zero" tracks: Remove tracks that have no length.

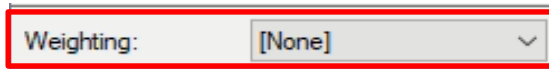
Color segments by track: Recolors segments to the color of the tracks they have been assigned to.

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

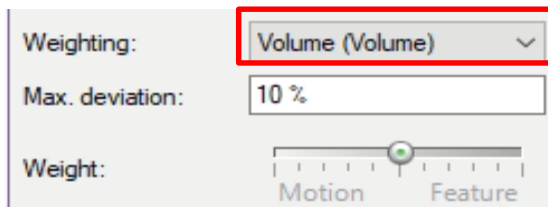
Max. time gap: Enter the maximum number of time points that a segment may absent

Arivis Vision4D Pipeline setup

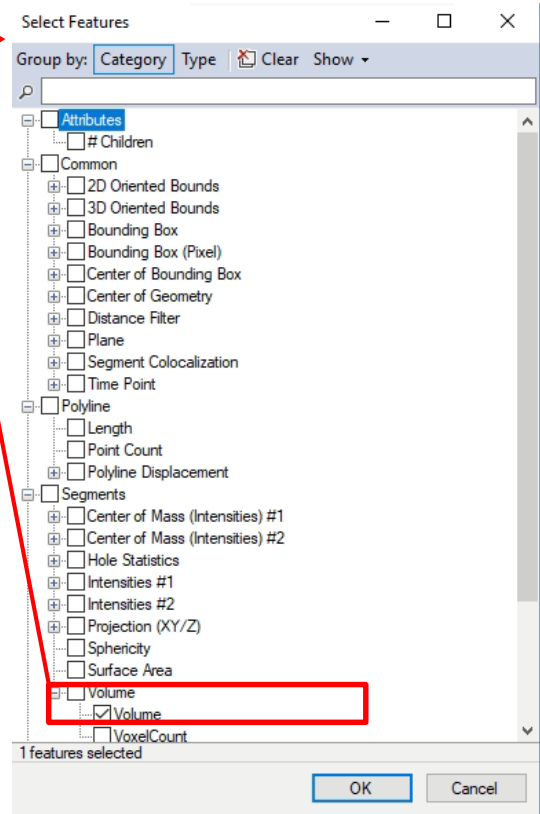
Addendum C: How to Track objects.



Select the features which should be considered during the tracking.



Digit the **Max Deviation** value.
This option defines the tolerance range of the feature.



TIPS :

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

DETAILS:

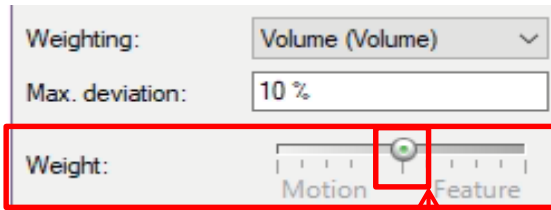
Weighting feature is used to define if the segment under evaluation
should be considered as part of the current track.

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:

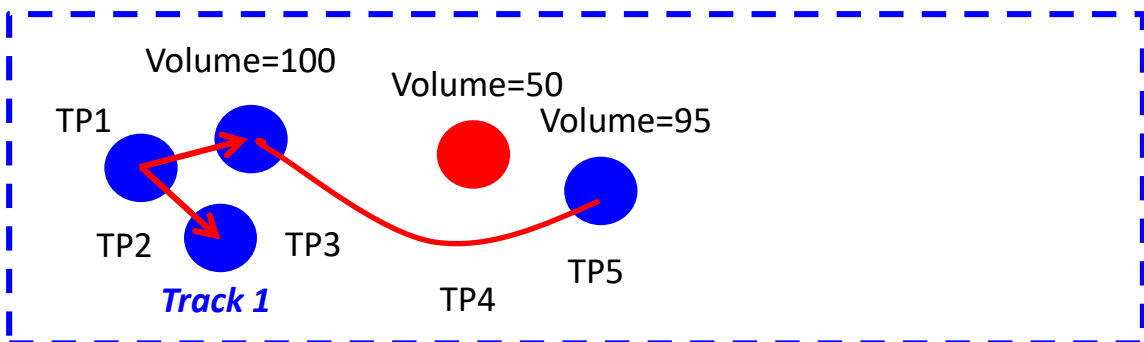
How to Track objects.



Weight value = 0
(slide in a central position)

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.

Max Deviation value = 10%



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

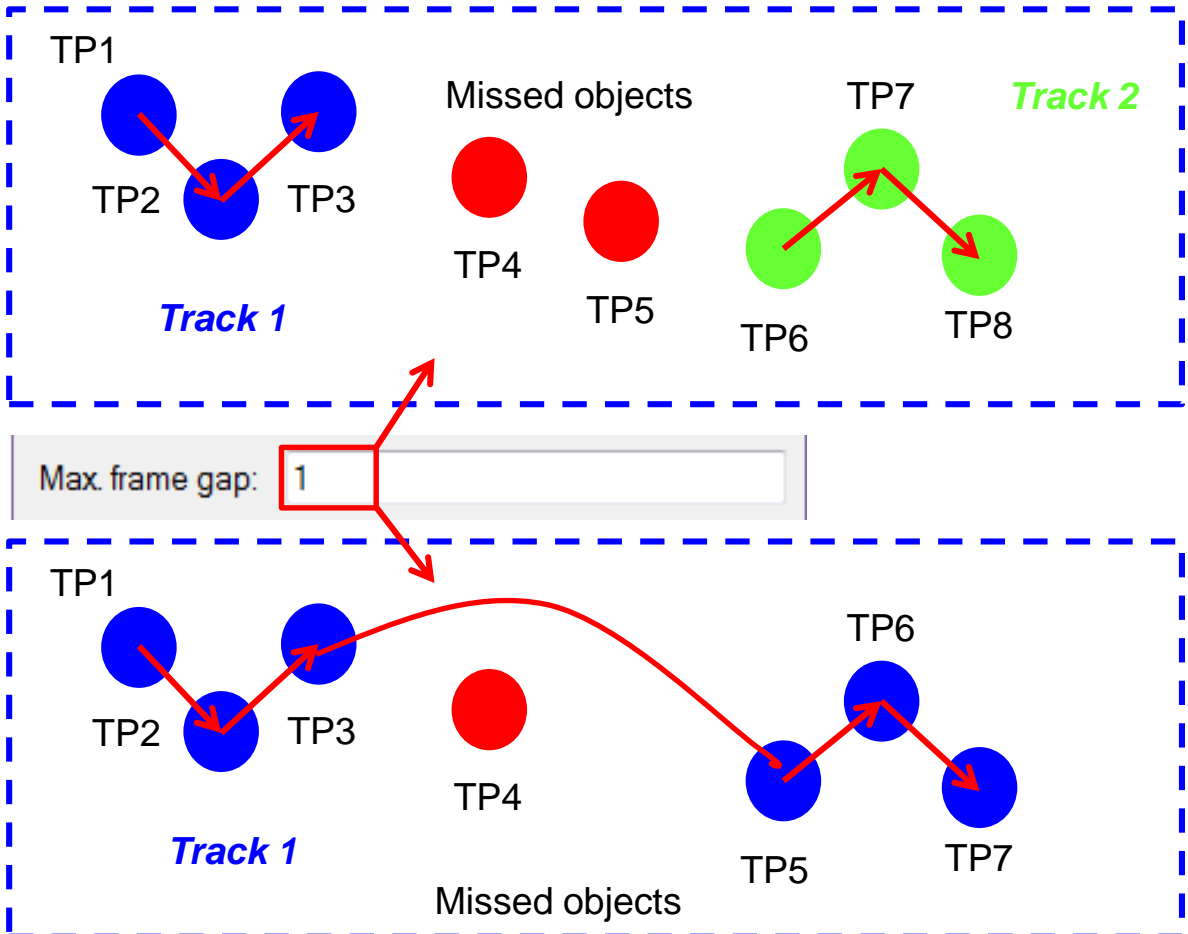
Addendum C (con't):

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 Vision4D Pipeline setup

Addendum C (con't):

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.

