Application Note #6

How to create :
«Spiral Oriented Sub Volume»

The application-note purpose is to guide the user in creating a spiral based boxes. The boxes can be used as ROIs for further analysis (Compartmentalization, gradient distribution, heat map, etcetera)

Application Flowchart

Draw a 3D Polyline

- The center of the spiral, the radius and the direction must be defined manually drawing a 3D polyline on a single plane (any)

Run the script

- The script creates a series of concentric boxes distributed over a spiral path.

Pipeline execution (optional)

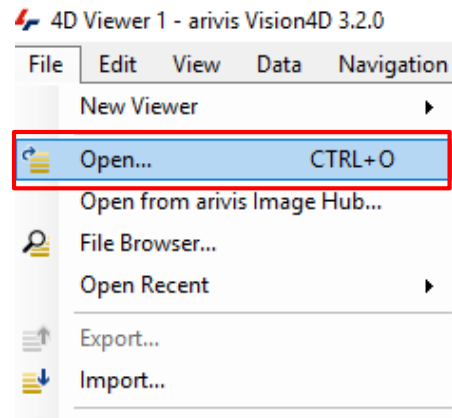- The Pipeline uses the boxes as ROIs to compute complex objects distribution analysis

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1. Open the working dataset on Vision4D

Step 1.1
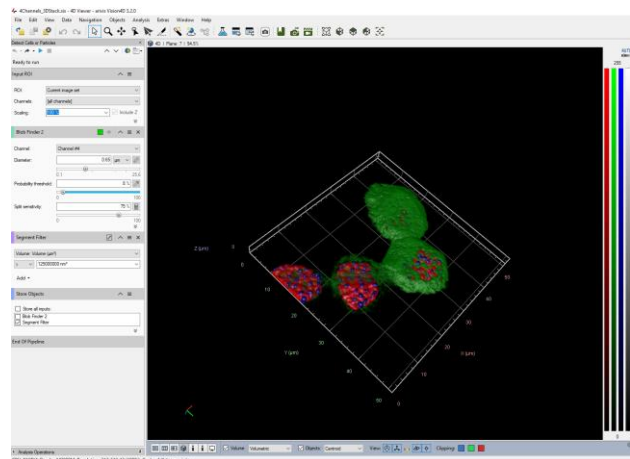Select the *Open..* item from the file menu.

Step 1.2
Select the dataset from the file browser.

TIPS :
The dataset is visualized according to the current rendering setting parameters. Please refer to the (arivis Vision4D Help) for more 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)
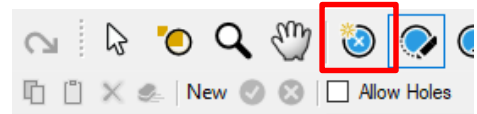
2. Draw the reference 3D polyline

Step 2.1

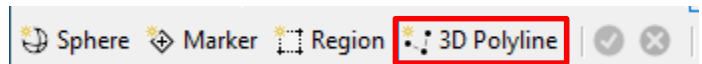Switch the Viewing area from 4D to 2D view mode.
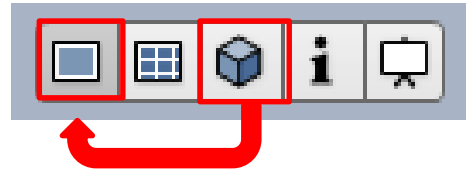
Step 2.2
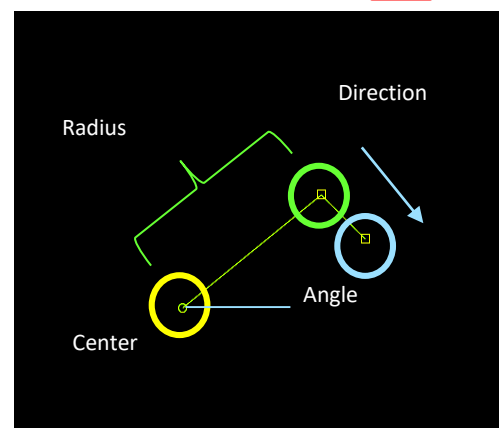
Select the "Place a new object" Tool

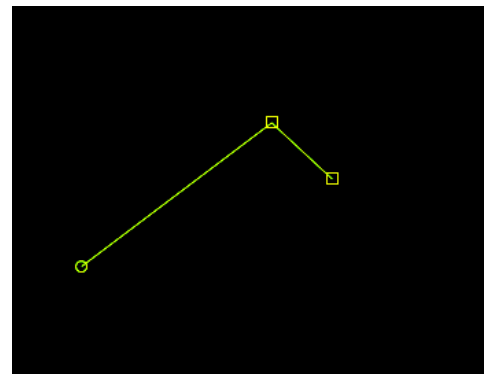**Step 2.3**

Select the "3D Polyline" tool

Step 2.4

Draw the 3D Polyline over any Z plane. The 1st point represent the center of the spiral, the 2nd point defines the radius and the starting angle, the 3rd defines the direction accordingly to the follow schema:
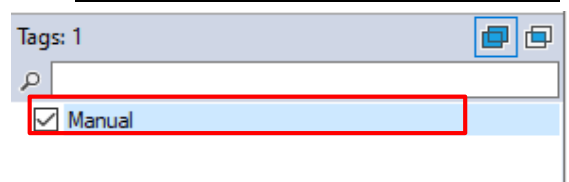
Step 2.5

Press the green icon to confirm the 3D Polyline
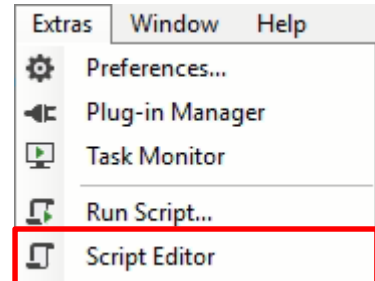
DETAILS :

The TAG «Manual» is now available in the data table

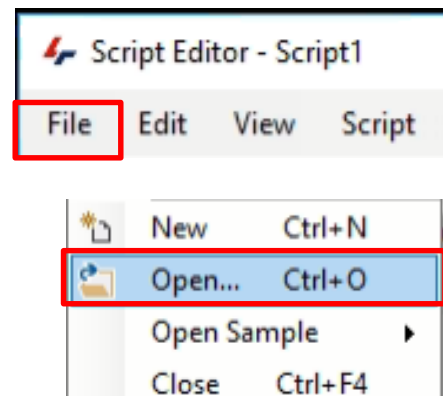# 3. Load the Python Script

Step 3.1
Open Python Script Editor.
From the «*Extra*» menu, select the «*Script Editor*» item

Step 3.2
Load the "*Spiral Oriented Sub Volume*" Python Script.
Browse the folder on which the file has been saved

Python script code overview

```
1    # coding: utf-8
2    # SCRIPT description
3    # NAME : Spiral-Oriented Sub-volume
4    # CREATION DATA : 21/03/2020
5    # WRITTEN BY : Maurizio Abbate
6    # RELEASE 1.0.4
7    # RELEASE DATA : 22/03/2020
8    # PURPOSE : Drawing a spiral ROIs
9    #
10   # Tested for V4d Release : 3.1.4 ->
11   #
12   #       NOTE: The boxes are not yet divided on the Z axis
13   #
14   # ---------------------------- External Package Import ----------------------------
15   import math as Math
16   import time
17   import arivis
18   import arivis_core as core
19   import arivis_objects as objects
20   # ---------------------------- End of external Package Import ----------------------------
21   #
22   # @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
23   FIRST_PLANE = -1        # -1 == bottom plane
24   LAST_PLANE = -1         # -1 == top plane
25   STEP_ANGLE = 20.0       # <----- STEPS IN DEGREES BETWEEN THE BOXES
26   SPIRAL_LOOP = 1         # <----- Numbers of concentric spiral -> 0.5 = 180° 1 == 360° 2 = 2* 360° .....
27   COEFFICENT_B = 10       # <----- Coeffcient used to define the distance between
28   #                                the concentric rings
29   BOX_HEIGHT = 40         # <----- PIXELS
30   BOX_WIDTH = 20          # <----- PIXELS
31   # @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ END USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
32   #
```

NOTE :
Only the parameters located in the "*USER SETTING*" area can be modified.
Don't change any other number, definition or text in the code outside this dedicated area.

4. Set the Script features

In order to define the spiral distributed sub-regions (sampling volume) features, some parameters of the script should be adjusted to match your analysis needs. These parameters are located in the code area labeled as "**USER SETTING**"

```
22    # @@@@@@@@@@@@@@@@@@@@@@@@@@@@ USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
23    FIRST_PLANE = -1          # -1 == bottom plane
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30    BOX_WIDTH = 20            # <----- PIXELS
31    # @@@@@@@@@@@@@@@@@@@@@@@@@@@@ END USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
32    #
```

Step 4.1

Set the Z planes range.
FIRST_PLANE defines the lower Z plane of the sub-regions ROI.
LAST_PLANE defines the higher Z plane of the sub-regions ROI.
The values of -1 set the Z planes range equal to the whole volume depth (total number of Z Planes available.

```
FIRST_PLANE = -1
LAST_PLANE = -1
```

Step 4.2

STEP_ANGLE = Defines the step size in degrees. The boxes will be positioned on the spiral curve every STEP_ANGLE.
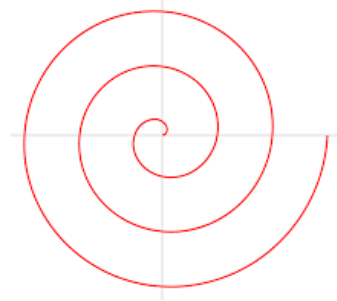
```
STEP_ANGLE = 20.0
```

Set the Script features (continue)

Step 4.3

SPIRAL LOOPS defines the number of loops (concentric spirals). Any single loop is a complete 360° spiral. Values less than 1.0 draw a partial spiral.
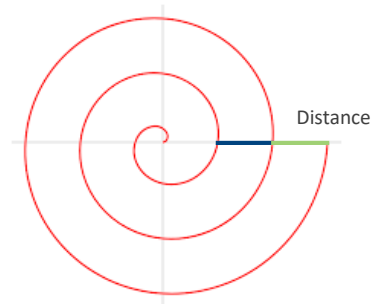
`SPIRAL_LOOP = 1`

Step 4.4

COEFFICENT_B Coefficient used to define the distance between the concentric spiral.

`COEFFICENT_B = 10`

Distance

Step 4.5

BOX_HEIGHT Sets the height of the boxes (Y)
BOX_WIDTH Sets the width of the boxes (X)
Both are expressed in pixels

`BOX_HEIGHT = 40`
`BOX_WIDTH = 20`

TIPS :

Set the distance of the loops and their numbers accordingly to the size of the boxes. Wrong parameters setting can overlap the boxes, especially in the inner part of the spiral.
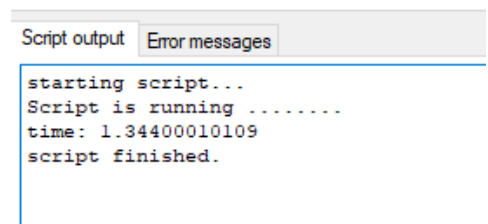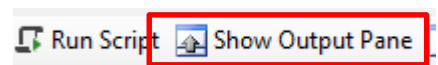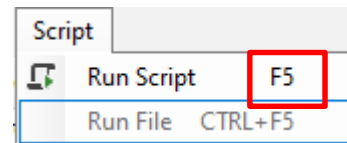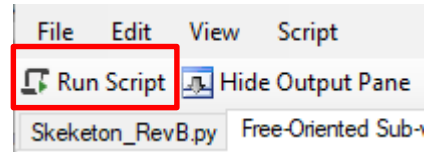
5. Run the Python Script

Step 5.1
Run the "***Spiral Oriented Sub Volume***" Python Script pressing the "Run Script" button or pressing the F5 key.

File	Edit	View	Script

🖵 Run Script 🖵 Hide Output Pane

| Skeketon_RevB.py | Free-Oriented Sub-v |

Script
🖵 Run Script   F5
Run File   CTRL+F5

🖵 Run Script 🔼 Show Output Pane

TIPS :
Activate, if not already displayed, the "Output Panel". The status of the script execution (errors including) will be visualized here

| Script output | Error messages |

```
starting script...
Script is running ........
time: 1.34400010109
script finished.
```

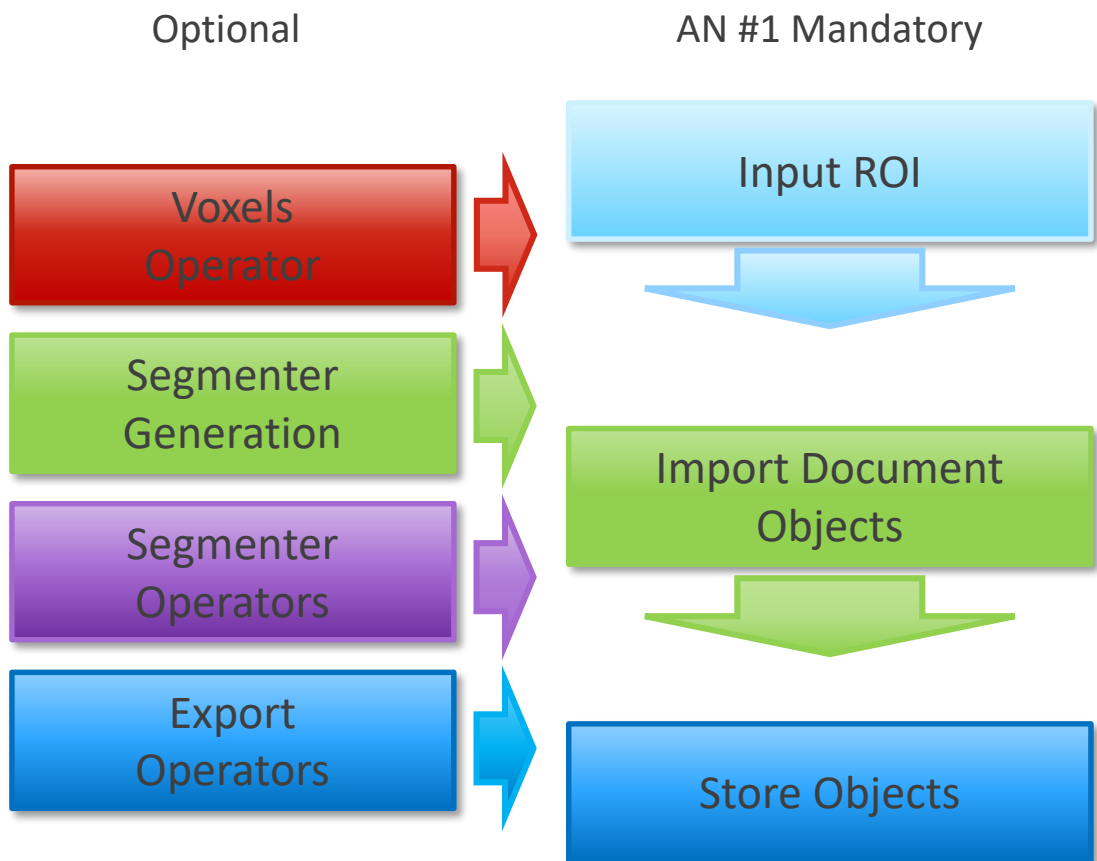# 6. Build the analysis Pipeline

DETAILS :

The pipeline have to be created according to the user analysis requirements as well as the sample typology.

The sample labeling, the imaging technique (Fluorescence, EM, Tomography, bright-field ...) and the image characteristics are important to drive the pipeline setup.

The knowledge of the biological structures under evaluation, it's behavior and the expected features' trend are also important as well. All the above information should be used to build a target driven pipeline.

The achieve the application note goals, only a couple of operators are mandatory. As described here below.

Typical Pipeline workflow

Optional	AN #1 Mandatory
Voxels Operator	Input ROI
Segmenter Generation	Import Document Objects
Segmenter Operators	
Export Operators	Store Objects

Build the analysis Pipeline (continue)

Step 6.2
Change the "***Input ROI***" operator parameters

Step 6.2.1 – ROI:
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) .
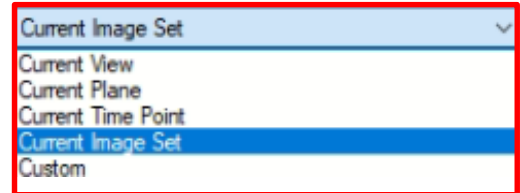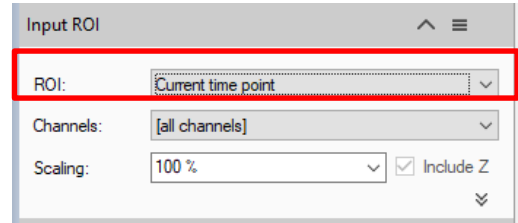Current Image Set : The complete dataset (XYZ and time) is processed.

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.

Custom : Allows to mix the previous methods.
Expand the Input ROI dialog.

Bounds : Sets the analysis area edges. The whole XY bounds, the viewing area or a custom space can be applied.
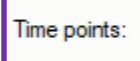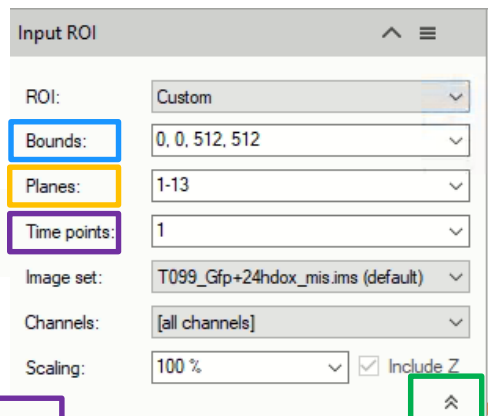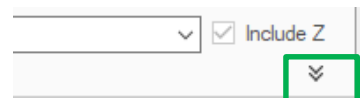
Planes : Sets the analysis planes range. A single plane, a range of planes or the whole stack can be selected.

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

Build the analysis Pipeline (continue)

Step 6.2.2 – Channels:
Sets the processing and analysis target channels. Selecting a single channel, all the operators in the pipeline will be forced to use it.
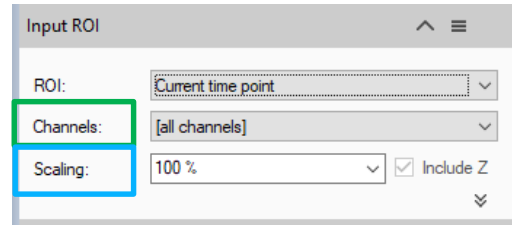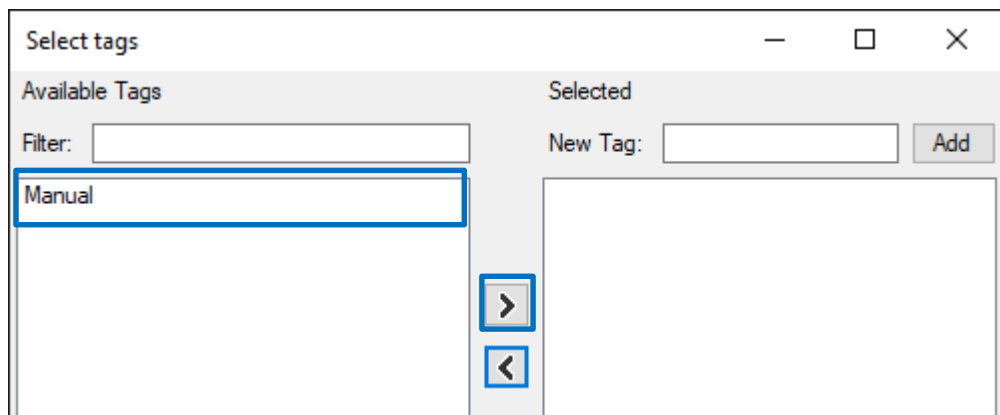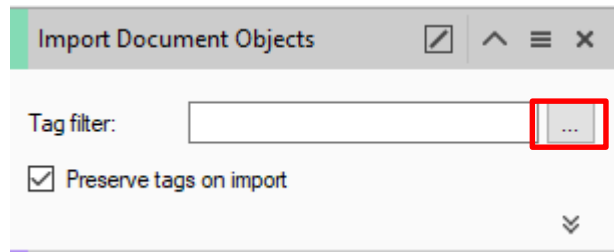
Step 6.2.3 – Scaling:
It scale the dataset reducing the size. The measurements will not be modified by the scaling factor.

Step 6.3
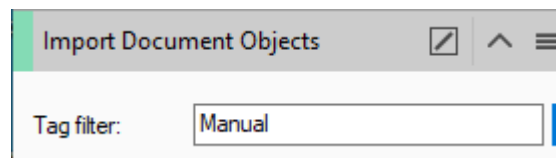Set the «*Import Document Objects*» operator

Step 6.3.1 – ...
Select the TAG filter

Step 6.3.2 – >
Click on the "*Manual*" TAG.
Use the right arrow button to move the TAG on the right table

Build the analysis Pipeline (continue)

Step 6.5
Add or remove optional operators inside the pipeline
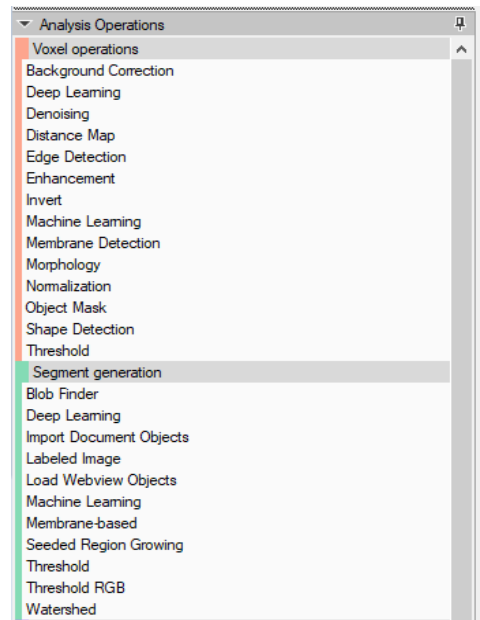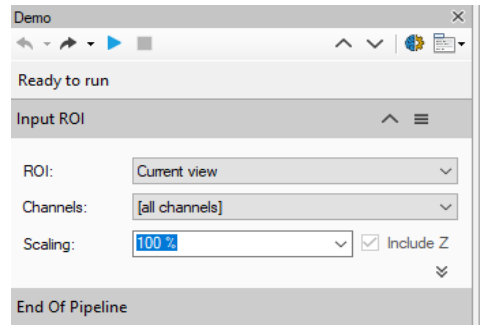
DETAILS :
The Analysis Pipeline panel consists of two main areas. The Pipeline area and the analysis operations list area .

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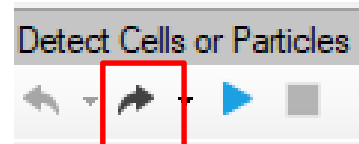Please refer to the (***arivis Vision4D Help***) for more details

7. Run the analysis Pipeline

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 7.1
Run the single operator

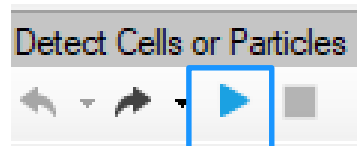**Step 7.2 (optional)**
Undo the single operator

TIPS :

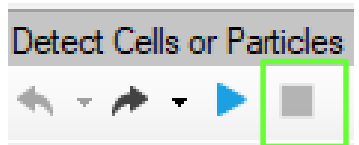Undo the last operator executed if you need to change the operator settings.

Step 7.3
Run the whole pipeline
(no pauses)

Step 7.4 (optional)
Stop the pipeline execution

DETAILS :

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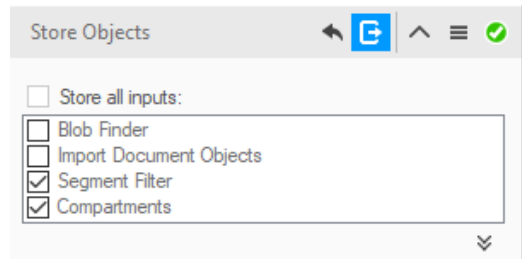
Task running

Task completed

8. View the results

DETAILS :

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 8.1

Open the data table (if not already visible)

Measurements are now visible in the data table

| Name	Count, volume stats	Sum, volume stats (µm³)	Mi
Segment #014	20	9,356	
Segment #015	33	14,137	
Segment #016	11	5,204	
Segment #018	21	9,026	
Segment #019	17	8,090	
Segment #020	5	1,532	

NOTE :

The spots count in the single sub-region is shown in the data table. The empty sub-regions are not listed. To get the total spots count the group statistic feature must be used.

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
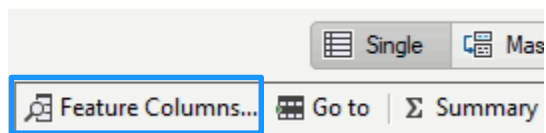
Contact the arivis local area sales manager to get more information about how to get the python script mentioned here.

Contact the arivis application support to receive additional technical details about the topic described in the application note, or how to adapt the application workflow to your requirements.

"The quantitative analysis of the images represents the art of transforming a visual sensation into its schematic and discrete form allowing its univocal description, classification and mathematical and logical interpretation of its spatial and temporal components"

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