Application Note #1

How to perform : «Objects density (heat-map) and object distribution gradient plotting (profile)»

The application-note purpose is to guide the user in performing the Objects density distribution analysis per volume unit (heat-map).
The same workflow can be used to evaluate the Objects count' distribution gradient along a specific direction (gradient profile). The application uses a Python script to create the contiguous sub-regions (sampling volume) and a pipeline to count the objects and to compute its density per volume unit (single sub-region) or the distribution along the longest direction of the sub-regions structure.
The evaluated Objects can be any biological structures like Cells, Nuclei, Neurons, as well as any small structures like Vesicles, Protein clusters, Mitochondria, and etcetera. The sampling volume can describe and limiting any anatomical regions inside the sample.
The unique limits is related to the sampling volume shape, only regular 3D boxes are available.

Vision4D 3.4

Application Flowchart

Manual drawing of the reference ROI

- A 2D free hands ROI is drawn to define the orientation and the size of the sampling volume

Python Script (sub-regions creation)

- The Python script creates the contiguous sub-regions (sampling volume) freely oriented in the dataset volume

Pipeline execution

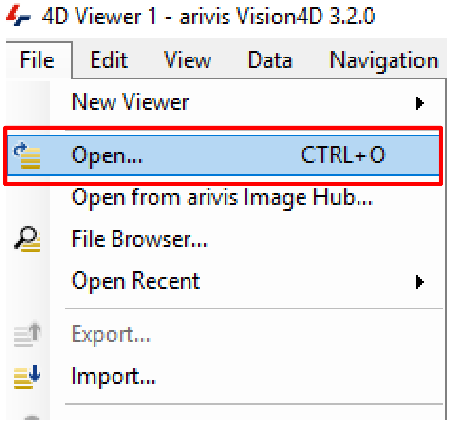
- The Pipeline will segment the objects and, using the sampling volume, it define the objects distribution in the volume unit.

Index

1. Open the working dataset on Vision4D

Step 1.1

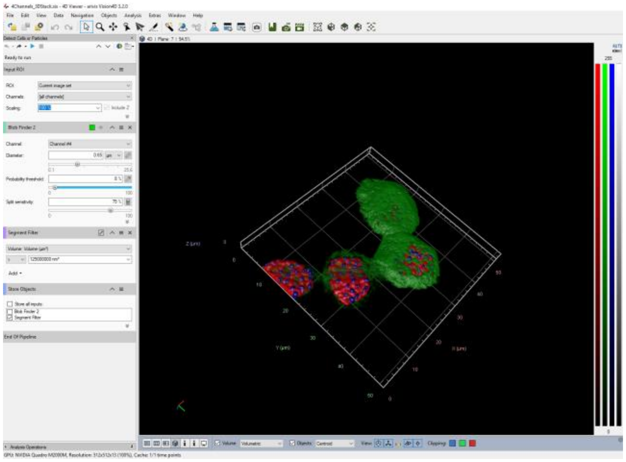
Select the *Open..* item from the file menu.



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

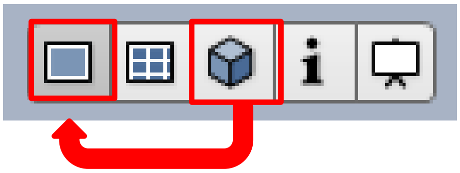


DETAILS:
The dataset is a multi dimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (Optical sectioning) of multiple channels (dyes) in a temporal sequence of time points (located in several spatial positions).
Usually the dataset shows a single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

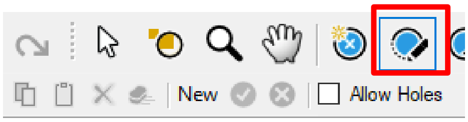
Step 2.1

Switch the Viewing area from 4D to 2D view mode.



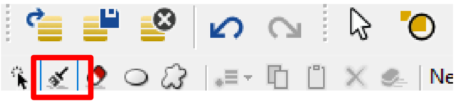
Step 2.2

Select the “Draw Objects Tool”



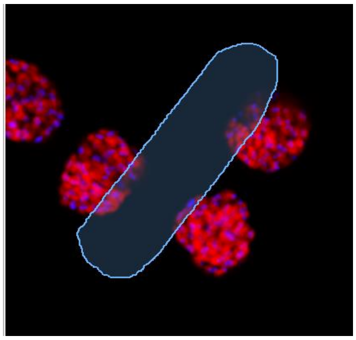
Step 2.3

Select the “Brush” tool



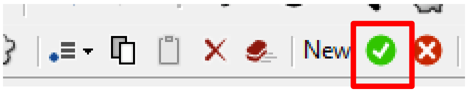
Step 2.4

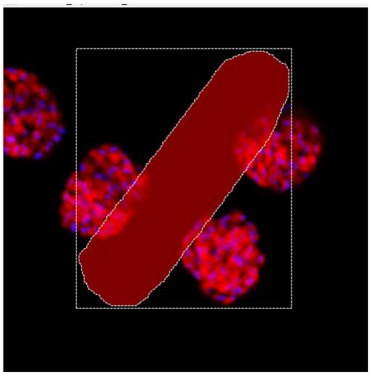
Draw the 2D ROI over any Z plane.
Use the «Erase Brush» to correct the ROI if necessary



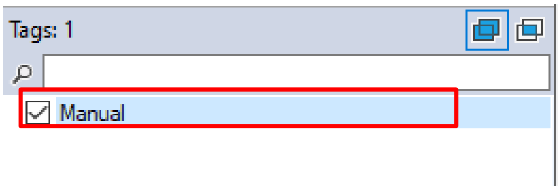
Step 2.5 ✓

Press the green icon to confirm the ROI





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

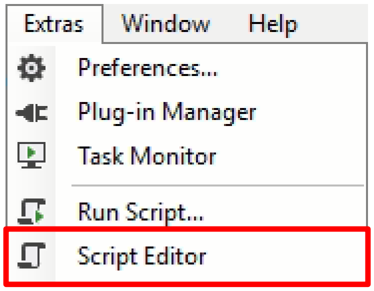


Tags: 1

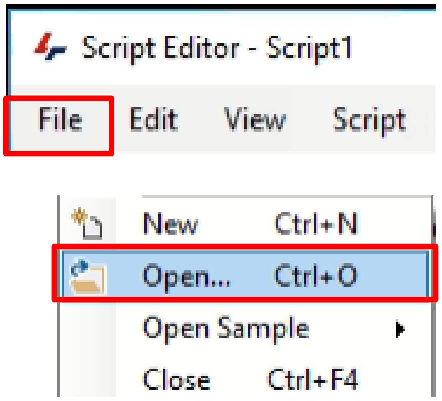
☑ Manual

3. Load the Python Script

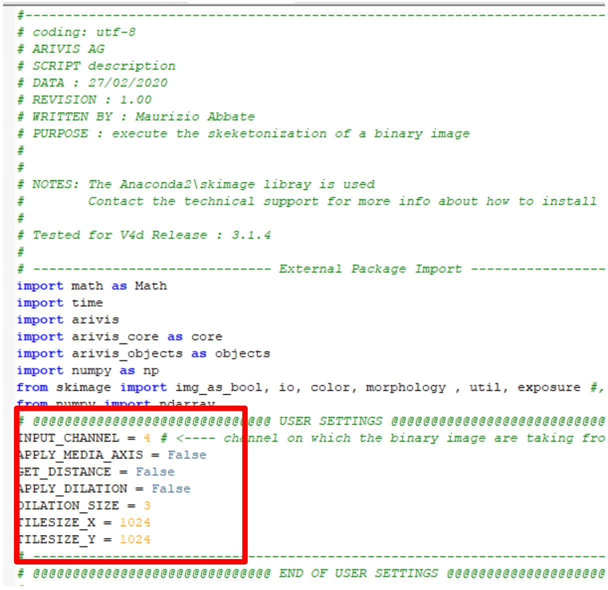
Step 3.1
Open Python Script Editor.
From the «***Extra***» menu, select the «***Script Editor***» item



Step 3.2
Load the "***Free-Oriented Sub-volume***" Python Script.
Browse the folder on which the file has been saved



Python script code overview

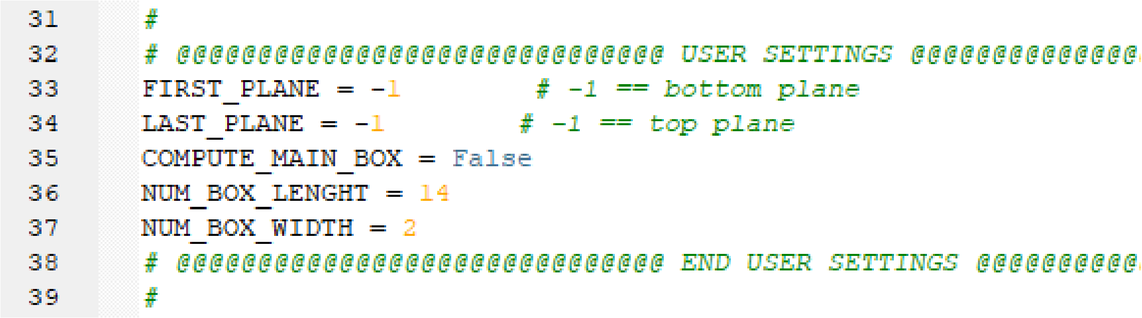


```
#---------------------------------------------------------------------------
# coding: utf-8
# ARIVIS AG
# SCRIPT description
# DATA : 27/02/2020
# REVISION : 1.00
# WRITTEN BY : Maurizio Abbate
# PURPOSE : execute the skeketonization of a binary image
#
#
# NOTES: The Anaconda2\skimage libray is used
#        Contact the technical support for more info about how to install
#
# Tested for V4d Release : 3.1.4
#
# ------------------------------ External Package Import -----------------
import math as Math
import time
import arivis
import arivis_core as core
import arivis_objects as objects
import numpy as np
from skimage import img_as_bool, io, color, morphology , util, exposure #,
from numpy import ndarray
# @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@
INPUT_CHANNEL = 4 # <---- channel on which the binary image are taking fro
APPLY_MEDIA_AXIS = False
GET_DISTANCE = False
APPLY_DILATION = False
DILATION_SIZE = 3
TILESIZE_X = 1024
TILESIZE_Y = 1024
# -------------------------------------------------------------------------
# @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ END OF USER SETTINGS @@@@@@@@@@@@@@@@@@@@@
```

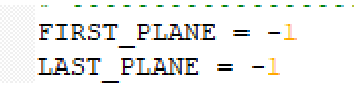
NOTE :
Only the parameters located in the "***USER SETTING***" area can be modified.
Don't change any other number, definition or text in the code outside this dedicated area.

4. Set the Script features

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



```
#
# @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ USER SETTINGS @@@@@@@@@@@@@@
FIRST_PLANE = -1        # -1 == bottom plane
LAST_PLANE = -1        # -1 == top plane
COMPUTE_MAIN_BOX = False
NUM_BOX_LENGHT = 14
NUM_BOX_WIDTH = 2
# @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ END USER SETTINGS @@@@@@@@@@
#
```



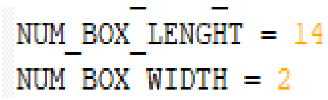
```
FIRST_PLANE = -1
LAST_PLANE = -1
```

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.

```
COMPUTE_MAIN_BOX = False
```

Step 4.2
COMPUTE_MAIN_BOX = True enables the creation of an additional ROI having the same sizes of the total sub-regions ROI size.

Set the Script features (continue)



```
NUM_BOX_LENGHT = 14
NUM_BOX_WIDTH = 2
```

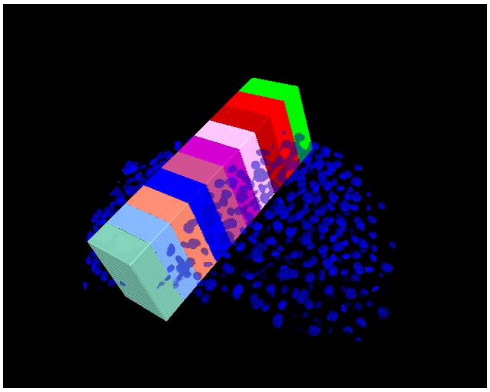
Step 4.3

NUM_BOX_LENGHT defines the number of sub-regions along the main axis (the longest one)
NUM_BOX_WIDTH defines the number of sub-regions along the minor axis (the shortest one).

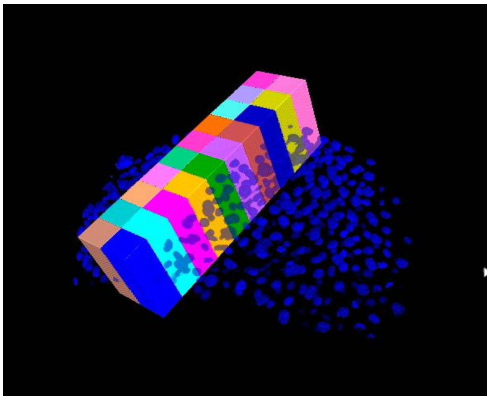
TIPS :
Set the number of sub-regions accordingly to the total size of the reference ROI . Don’t create boxes too small.

Examples:

NUM_BOX_LENGHT = 10
NUM_BOX_WIDTH = 1



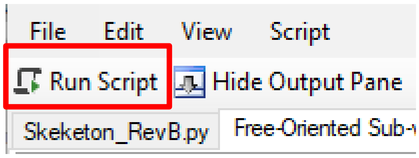
NUM_BOX_LENGHT = 10
NUM_BOX_WIDTH = 2

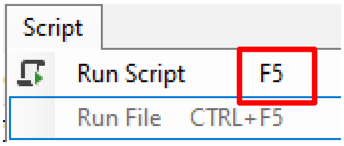


5. Run the Python Script

Step 5.1

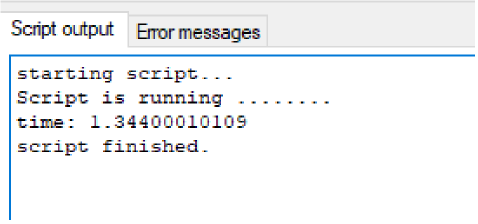
Run the "***Free-Oriented Sub-volume***" Python Script pressing the "Run Script" button or pressing the F5 key.





TIPS :

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

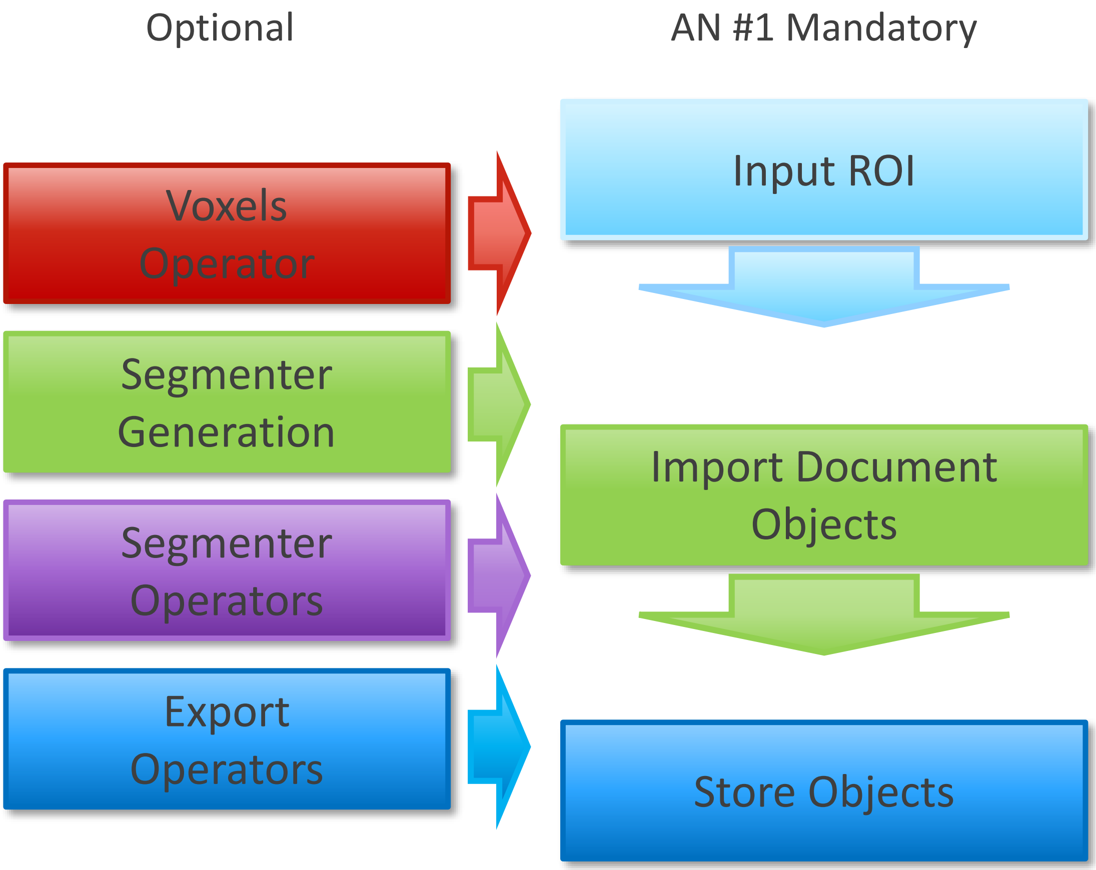


```
starting script...
Script is running ........
time: 1.34400010109
script finished.
```

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



Build the analysis Pipeline (continue)

DETAILS :
The Operators are grouped in several categories.
Each category includes the functions that perform specific tasks, ordered by their usage. Here below a short summary.

Voxels Operator

PURPOSE :
The group includes the operators that interact directly with the voxels. Typically, spatial filters, Intensity normalization, intensity range equalization and so on.

APPLICATION :
Sample Noise removal, background subtraction, Intensity disomogenity correction, details enhancement, edges detection (eg membrane), morphological math.

Segmenter Generation

PURPOSE :
The group includes the threshold operators used to detect and create the segments. Automatic and manual method, machine learning approach, watershed and proprietary algorithms are available

APPLICATION :
Structures identification. Nuclei, Neurons, small spots (Blob Finder), Cells, Vessels, Filaments (Watershed, Region Growing) or microstructures (Machine learning) can be detected using the appropriate method or a combination of them.

Segmenter Operators

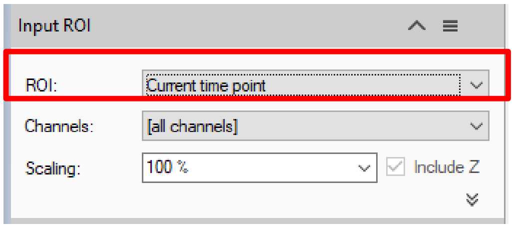
PURPOSE :
The group includes the operators that interact directly with the segments. Feature filtering, morphology, Compartmentalization, distance measurements and more can be applied.

APPLICATION :
Objects colocalization, object classification by features, objects merging or splitting, objects grouping, objects parent/child relationship and even more

Build the analysis Pipeline (continue)

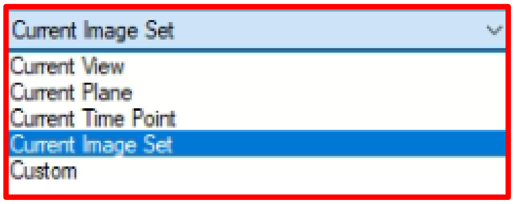
Step 6.2

Change the “***Input ROI***” operator parameters



ROI: Current time point
Channels: [all channels]
Scaling: 100 %
☑ Include Z

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.

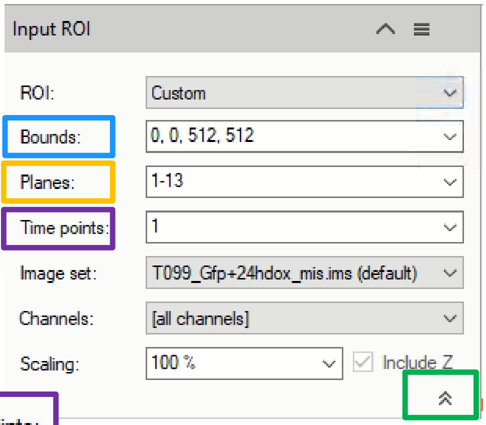
DETAILS :
Use the Custom option during the pipeline setting and testing . Set a sub volume (XY, Planes, Time Points, channels) of your dataset on which perform the trial. This will speed up the setting process.

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. Bounds:

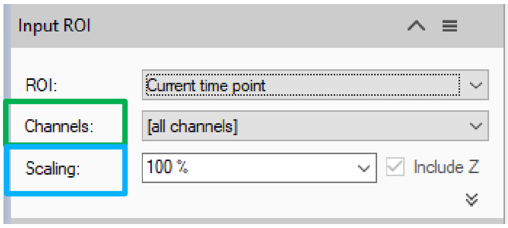
Planes : Sets the analysis planes range. A single plane, a range of planes or the whole stack can be selected. Planes:

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



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

Build the analysis Pipeline (continue)



Input ROI

ROI: Current time point

Channels: [all channels]

Scaling: 100 %

☑ Include Z

Step 6.2.2 – Channels:

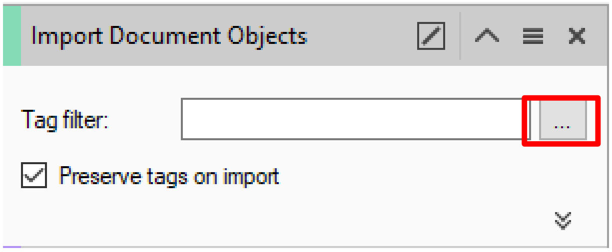
Sets the processing and analysis target channels. Selecting a single channel, all the operators in the pipeline will be forced to use it.

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



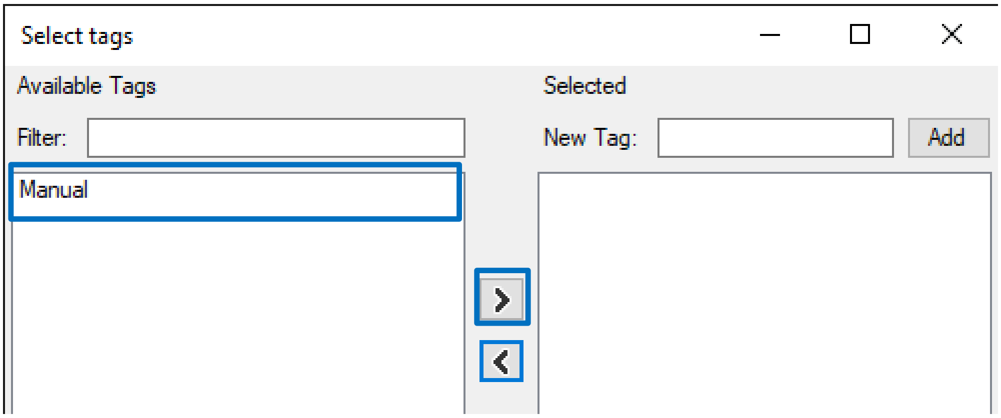
Import Document Objects

Tag filter:

☑ Preserve tags on import

Step 6.3.1 – ...

Select the TAG filter



Select tags

Available Tags

Filter:

Manual

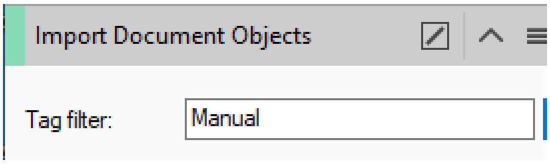
Selected

New Tag:

Step 6.3.2 – >

Click on the "***Manual***" TAG.

Use the right arrow button to move the TAG on the right table



Import Document Objects

Tag filter: Manual

Build the analysis Pipeline (continue)

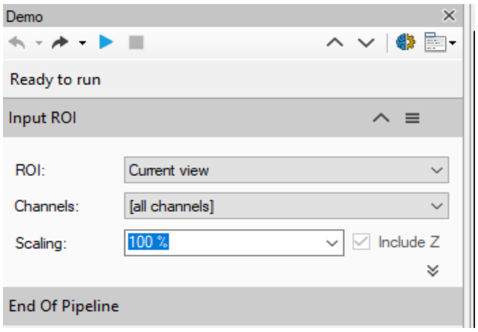
Step 6.5

Add or remove optional operators inside the pipeline

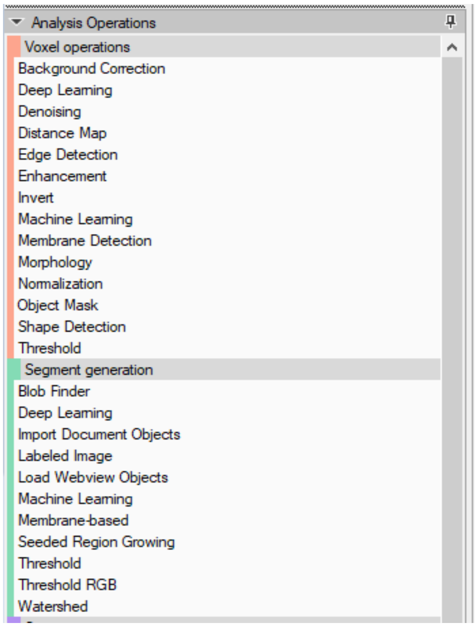
DETAILS :

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

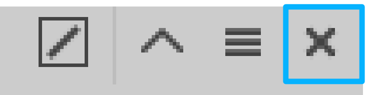
The ***Operators*** can be added to Pipeline in two ways
1. Double click on the ***Operator*** you wish to add to the current Pipeline. The ***Operator*** will be inserted at the end of the group of operations to which it belongs. Voxel Operations are positioned before the Segment generation meanwhile 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.



ROI: Current view
Channels: [all channels]
Scaling: 100 %
☑ Include Z



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



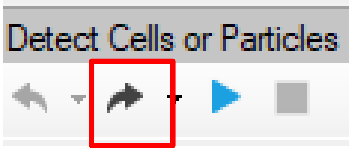
TIPS :

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

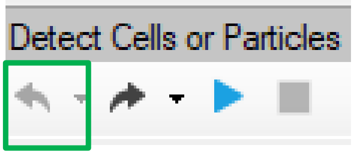
7. Run the analysis Pipeline

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 ↷

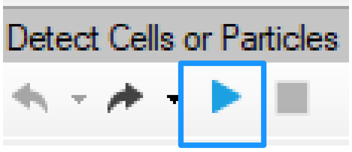


Step 7.2 (optional)
Undo the single operator ↶

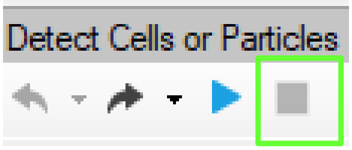


TIPS :
Undo the last operator executed if you need to change the operator settings.

Step 7.3
Run the whole pipeline (no pauses) ▶



Step 7.4 (optional)
Stop the pipeline execution ■



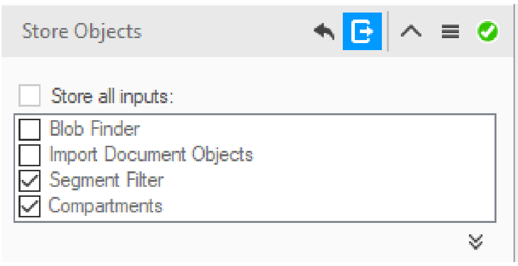
DETAILS :
This icon, located on the right side of the operator title bar, shows the operator status.

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.



Store Objects

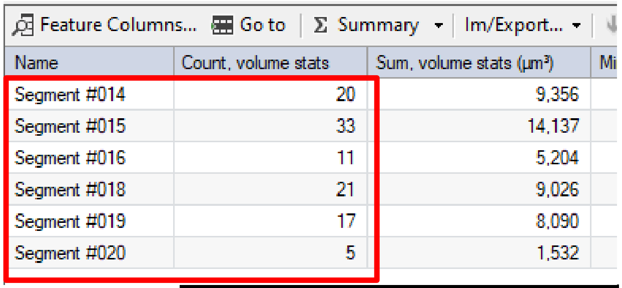
☐ Store all inputs:

☐ Blob Finder
☐ Import Document Objects
☑ Segment Filter
☑ Compartments

Step 8.1

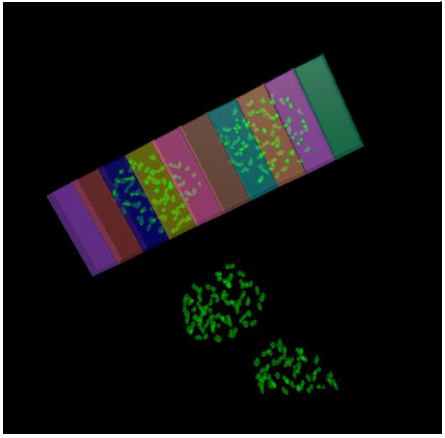
Open the data table (if not already visible)

Measurements are now visible in the data table

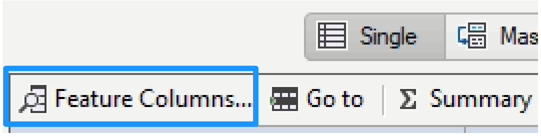


Name	Count, volume stats	Sum, volume stats (µm³)
Segment #014	20	9,356
Segment #015	33	14,137
Segment #016	11	5,204
Segment #018	21	9,026
Segment #019	17	8,090
Segment #020	5	1,532

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



arivis

Vision4D

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”

arivis AG, Am Kabutzenhof 21,
18057 Rostock, Germany

Email : support@arivis.com