

## Application Note #27

### How to create : «Heat-Map – density distribution»

The application-note purpose is to guide the user in creating a density distribution map, also called "Heat-Map". The script generates contiguous boxes in X,Y and Z directions that can be used as ROIs for further analysis (Compartmentalization, gradient distribution, etcetera)

## Application Flowchart

### 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

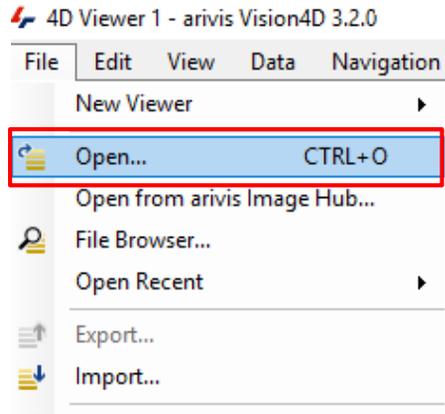
## Index

1. Open the working dataset
2. Load the Python Script
3. Set the Script features
4. Run the Python Script
5. Analysis options overview
6. Build the analysis Pipeline
7. Run the analysis Pipeline
8. View the results

# 1. Open the working dataset on Vision4D

## Step 1.1

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

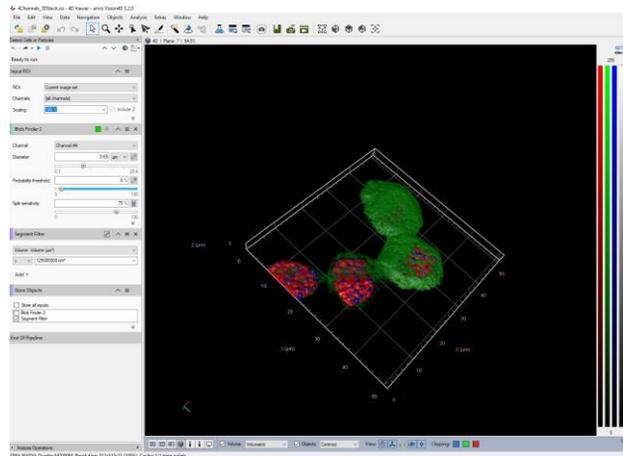


## Step 1.2

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).

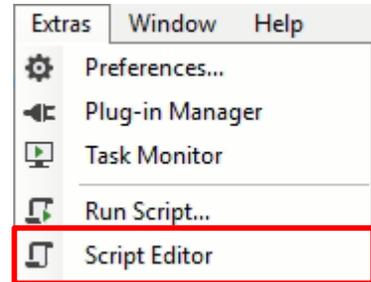
Usually the dataset shows a single experimental situation ( a complete experiment can be composed by several datasets). The datasets are available as graphic files saved in plenty of file formats (standard formats as well as proprietary formats )

## 2. Load the Python Script

### Step 2.1

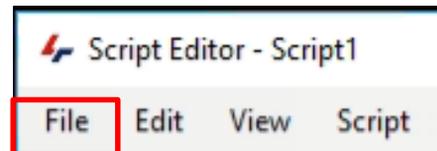
Open Python Script Editor.

From the «*Extra*» menu, select the «*Script Editor*» item



### Step 2.2

Load the “*Divide\_Scope*” Python Script.  
Browse the folder on which the file has been saved

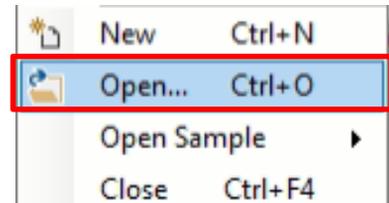


### NOTE:

The script name can change accordingly to the new version released.

The latest script is:

«*DivideScope RevE (3\_4).py*»



Python script code usage rights.

```
#  
# NAME: Divide Scope  
# FILE: Divide_Scope_RevF (3_4)  
# REVISION : 1.1.0 - 20/04/2021  
# AUTHOR : Maurizio Abbate  
# Copyright(c) 2021 arivis AG, Germany. All Rights Reserved.  
#  
# Permission is granted to use, modify and distribute this code,  
# as long as this copyright notice remains part of the code.  
#  
# PURPOSE : the whole volume is divided in contiguous boxes  
#           the segments volume are divided in contiguous boxes  
#  
# PARAMETERS: f TAG_SEGMENTS is set, the boxes are created inside the segments  
#              bounds (each object is divided in boxes)  
#              TAG_SEGMENTS == "" the whole Imageset volume is used to compute  
#              the boxes  
#              TAG_BOXES sets the output boxes TAG  
#              TAG_BOXES == "" - the default tag "Script" is used  
# NOTE: Keep the object visualization OFF and the objects table closed during the script  
#       run  
# Tested for V4d Release : 3.4  
#
```

The user has the permission to use, modify and distribute this code, as long as this copyright notice remains part of the code itself.

**Copyright(c) 2021 arivis AG, Germany. All Rights Reserved.**

### 3. Set the Script features

In order to define the contiguous 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**”

```
# @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@ USER SETTINGS @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
#
#-----
# SIZE_BOX_X,SIZE_BOX_Y,SIZE_BOX_Z expressed in um
# SIZE_BOX_? must be calibration factor * MINIMUM_SIZE_BOX_COEFF (at least)
#-----
SIZE_BOX_X = 10
SIZE_BOX_Y = 10
SIZE_BOX_Z = 3
```

#### Step 3.1

SIZE\_BOX\_X : Set the X sub-volume size.

SIZE\_BOX\_Y : Set the Y sub-volume size.

SIZE\_BOX\_Z : Set the Z sub-volume size.

All the values are expressed in metric units (um)

#### NOTE:

If one of the box dimension is bigger than the correspondent volume size, the script will not be executed and an error message is issued.

#### 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.

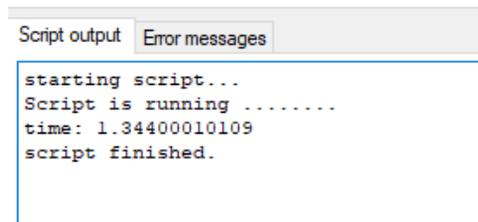
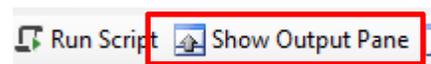
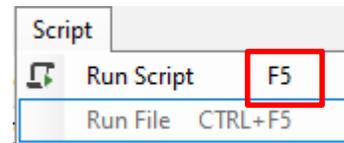
## 4. Run the Python Script

### Step 4.1

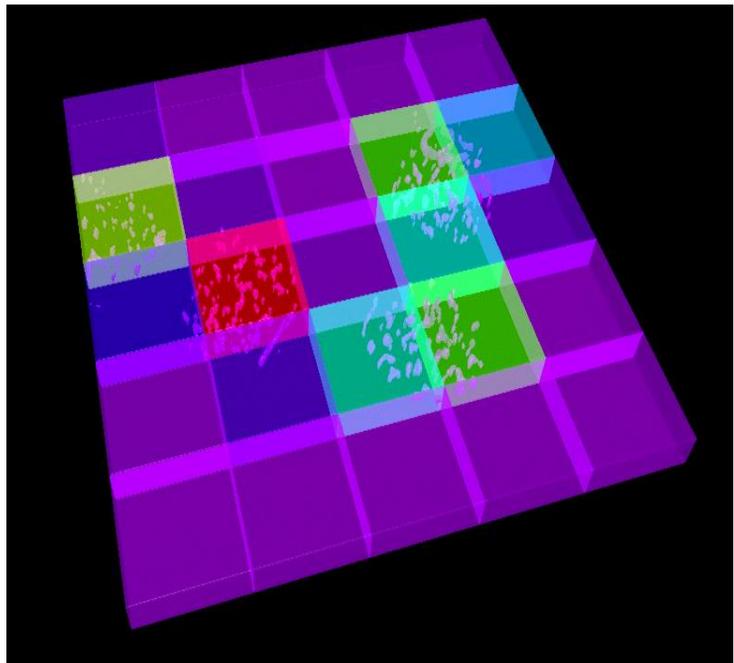
Run the “*DivideScope RevE (3\_4)*” Python Script pressing the “Run Script” button or pressing the F5 key.

### TIPS :

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



Here the script result, a 3D matrix of sub-volumes.



### NOTE :

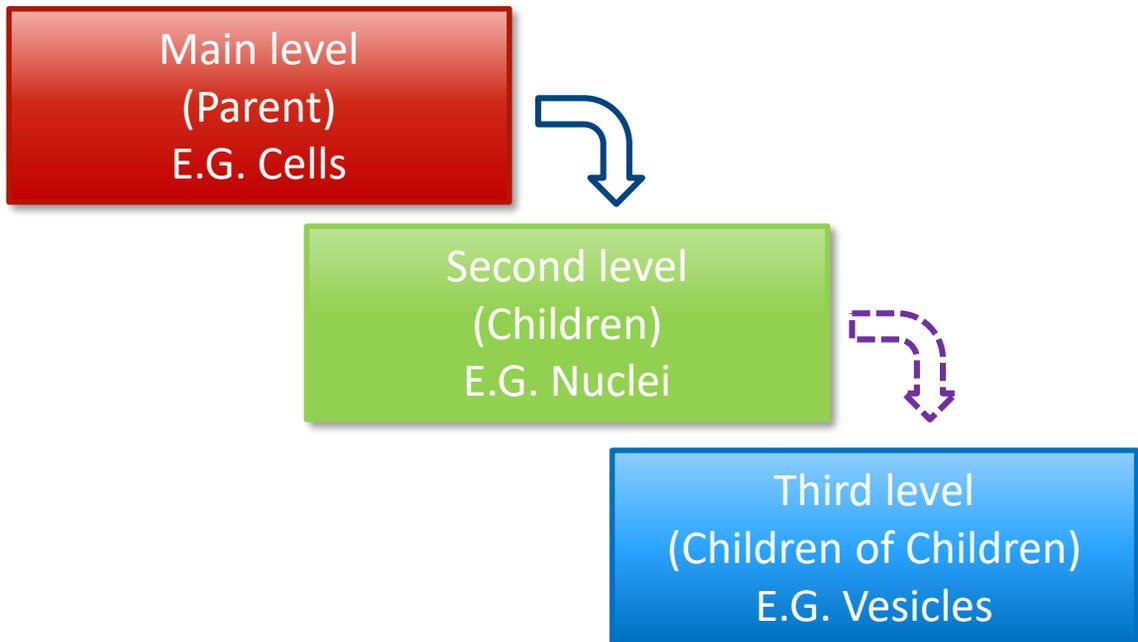
The sub-volumes segments are shown in the objects table using the TAG “*Script*”

## 5. Analysis options overview

Once the script is executed and the sub-volumes have been created, several density distribution analysis can be performed on the sample. The simplest one is to get the single/multiple channel(s) intensity features inside the boxes. More sophisticated approaches can include a compartment analysis.

The COMPARTMENTALIZATION' concept is strictly related to the studies of the interactions and of the relationship between the structure' compartments.

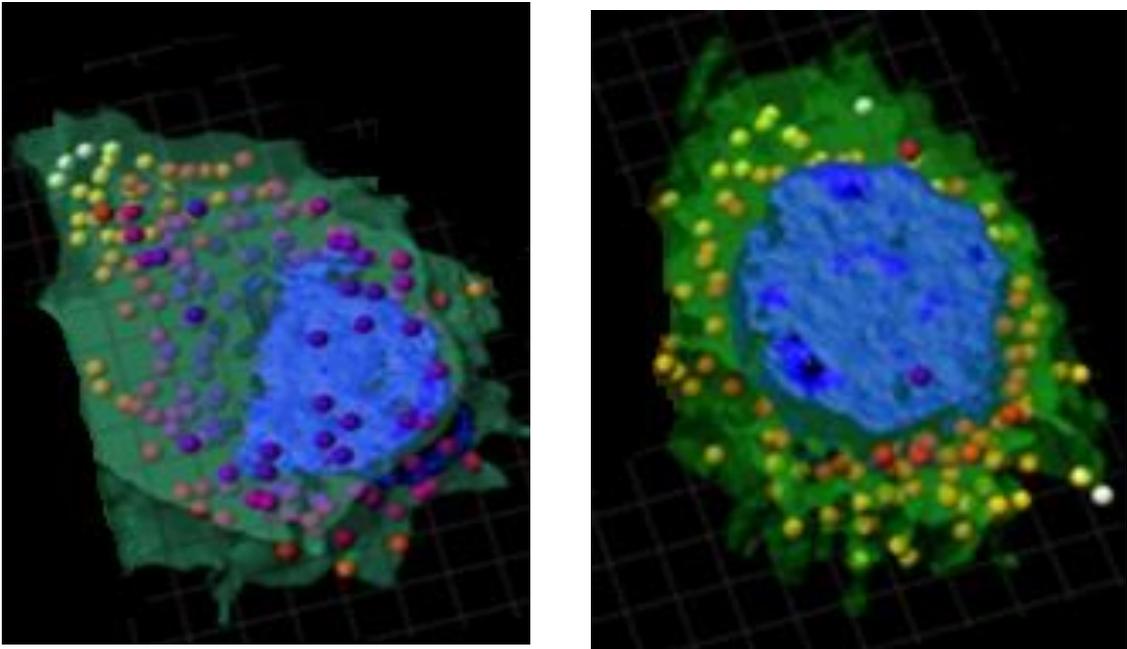
Complex hierarchies between the structures can be established and evaluated using this operator. Objects inside a parent structure can be selected, their position inside the main structure, as well as their distribution (clustering), and other features, can be evaluated. A child object can be a parent for other objects. The number of the available nested levels are, theoretically, unlimited.



In the example here above, The COMPARTMENTALIZATION is extended on 3 levels. The result is a hierarchical link between the Cell (Reference) and its nucleus (subject) . The nucleus is, in turn, related to the vesicles it contains. Finally, the vesicles count per cell is obtained.

## 5. Analysis options overview

The COMPARTMENTALIZATION' analysis is not limited to the biological samples, even if this is the most common situation. Any structure located inside of a defined surrounding volume can be evaluated.



It is not mandatory that the parent object is a defined structure (E.G. Cell or Nucleus), it can be an anatomical region or, generally speaking, a sub-region of interest of the sample volume.

These regions can be drawn both manually or using the interactive method.

The COMPARTMENTALIZATION' approach is the base on which more complex and sophisticated evaluations can be performed.

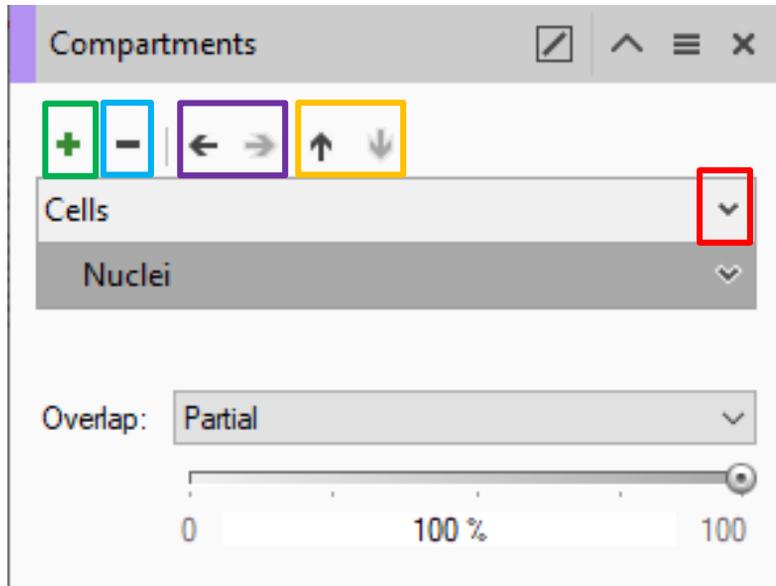
*On the next pages the compartment concept will be detailed.*

### NOTE :

Refer to the **Application Note #8** for detailed information about «**How to Draw objects interactively**»

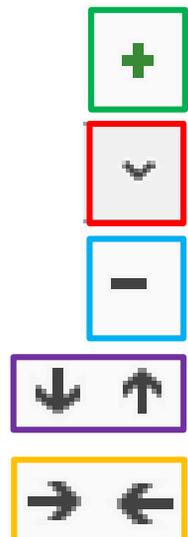
## 5a. The Compartments Operator

**5a.1** The Compartments Operator allows to set the structures hierarchy to be used to evaluate their levels of relationships. Several nested levels are possible as well as two or more compartments at the same hierarchical basis.



### 5a.2 Settings for Compartments:

- Add the **TAG** of the structures of interest as a source entry.
- Use the input selection button to select a different operation from the preceding pipeline.
- Remove the selected TAG from the entry.
- Move the selected TAG up & down in the hierarchical levels.
- Move the selected TAG as a "child" of the "parent" above it and push it up or down in the hierarchy.



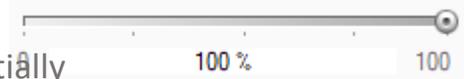
## 5a. The Compartments Operator (continue)

- Select the required amount of overlap.

Overlap:

1. Partial Overlap.

Check whether a "child" segment is partially covered by a "parent" structure. You can set the necessary amount of overlap with the slider or the text input.



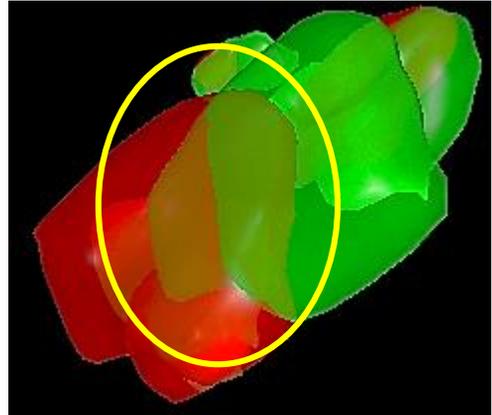
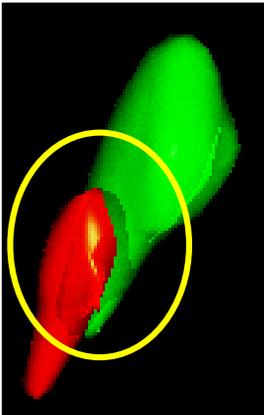
2. Full Overlap.

Check whether a "child" structure is fully covered by a "Parent" volume.

### NOTE :

The Partial Overlap defined the percentage of the subject structure volume that must be covered by the Reference volume to be considered as compartmentalized.

*If you choose 0%, it still checks whether there is at least 1 voxel of coverage.*

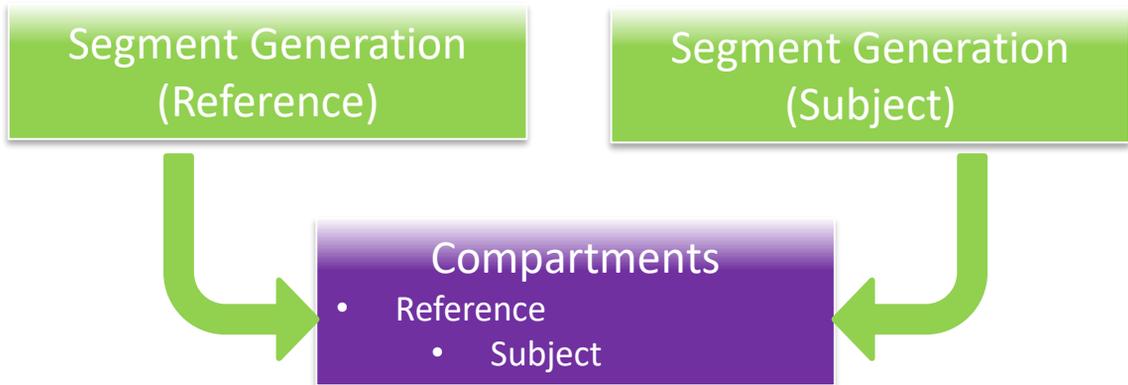


### COMMENT :

The selected parent **TAG**, as well as the child **TAG** can label more objects. For any object in the Parent **TAG**, all the child objects are compared to it in order to establish their belonging.

## 5b. Simple Compartments (2 levels)

**5b.1** The Simple Compartments' schema is organized on two levels of hierarchy, the Reference (Parent) and the Subject (Child). The child objects belonging to the Parent spaces are labelled (TAG) as compartmentalized.

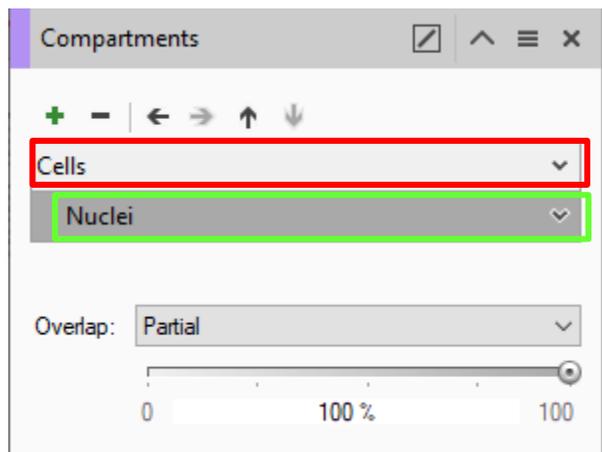


**NOTE :**

These Reference structure can also be drawn both manually or using the interactive method. Refer to the **Application Note #8** for detailed information about «**How to Draw objects interactively**»

**Compartments setup:**

The left most item is a reference  
The items below the left most item is the subject. It must be shifted one position on right compared to the reference.



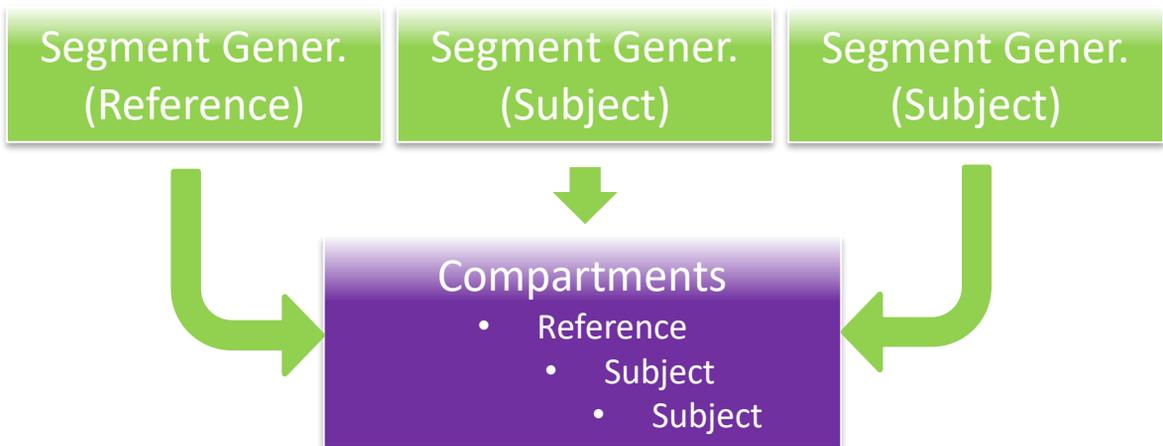
**TIPS :**

The commands on top of the dialog are used to set the hierarchy. (refer to Page 6)



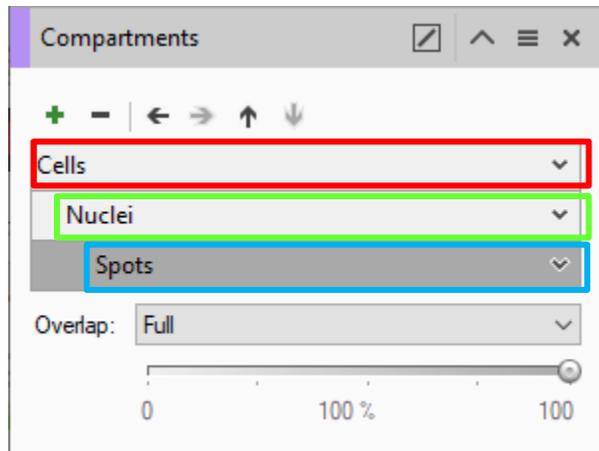
## 5c. Multiple Compartments (multiple levels).

**5c.1** The Multiple Compartments' schema uses 3 levels or more of hierarchy. Starting from the first child on the top, each of the level is checked with the previous one. The child objects belonging to its Parent spaces are labelled (TAG) as compartmentalized.



### **Compartments setup:**

The left most item is a reference  
The items below the left most item is the subject. It must be shifted one position on right compared to the reference.  
The 3rd levels is set shifting the TAG one position on right compared to the subject on top of it.

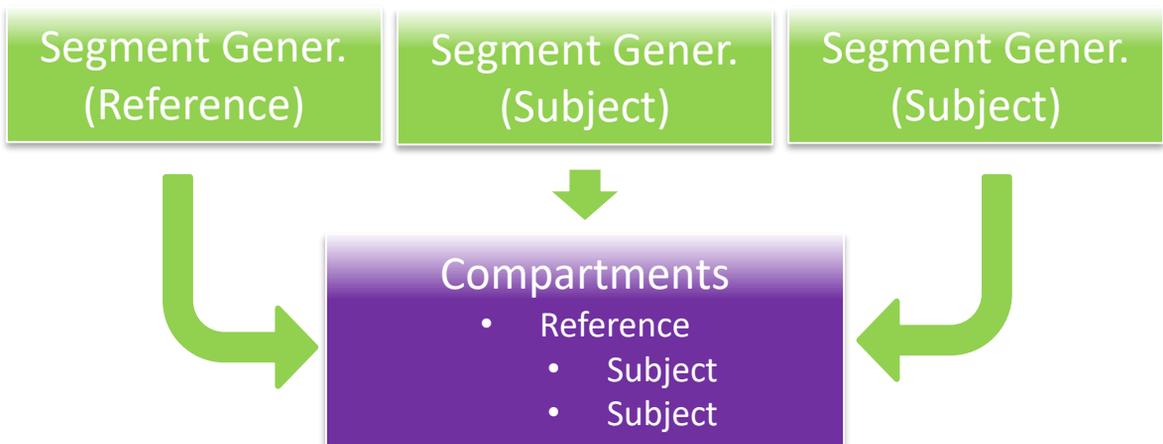


### **TIPS :**

The commands on top of the dialog are used to set the hierarchy. (refer to Page 6)

## 5d. Single Compartments (multiple subjects).

**5d.1** The Simple Compartments' schema (multiple subjects) is organized on two levels of hierarchy, but the child level can have more entries. The child objects of each entry, belonging to the Parent spaces, are labelled (TAG) as compartmentalized.

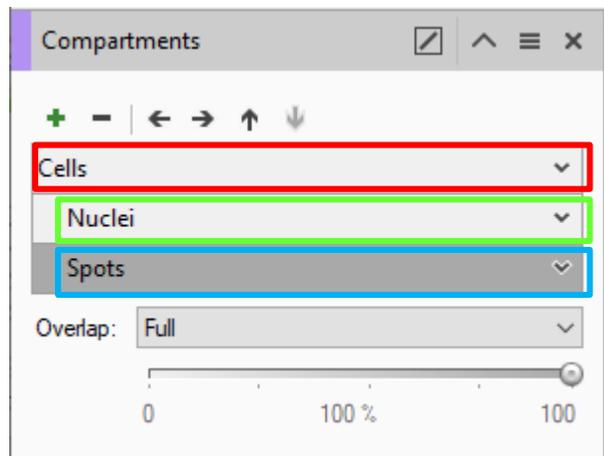


### Compartments setup:

The left most item is a reference

The items below the left most item is the subject. It must be shifted one position on right compared to the reference.

The 2nd subject is set to the same level of the



### NOTE :

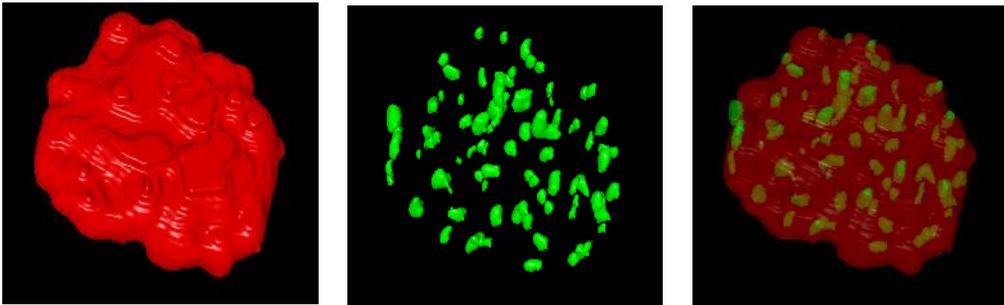
The Compartments results are shown in the data table. The TAG «**Compartments**» is used to label the classified objects.

### TIPS :

The commands on top of the dialog are used to set the hierarchy. (refer to Page 6)

## 5e. Compartments example

### 5e.1 Simple Compartments (2 levels)

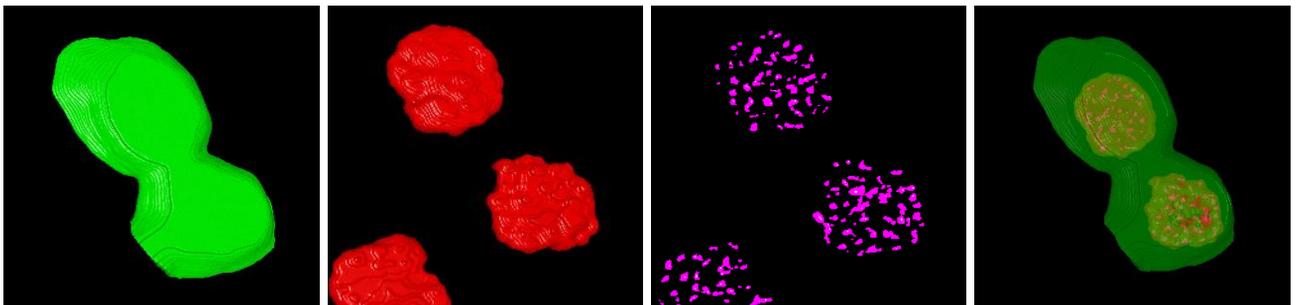


Reference

Subject

Result

### 5e.2 Multiple Compartments (multiple levels).



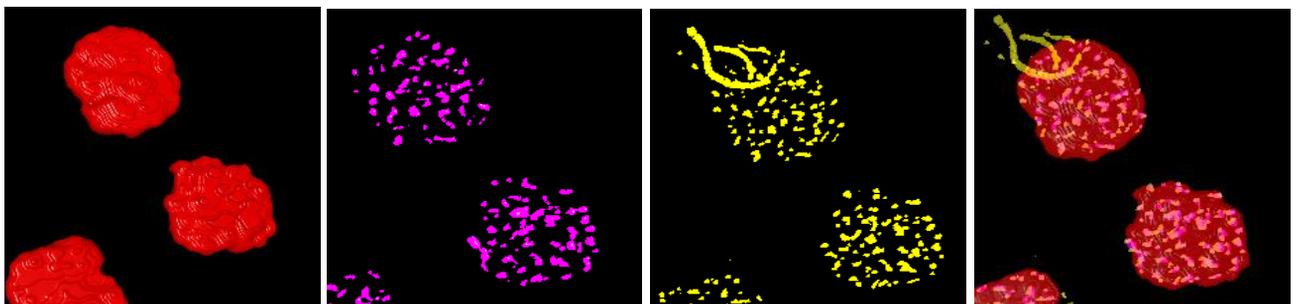
Reference

Subject #1

Subject #2

Result

### 5e.3 Single Compartments (multiple subjects).



Reference

Subject #1

Subject #2

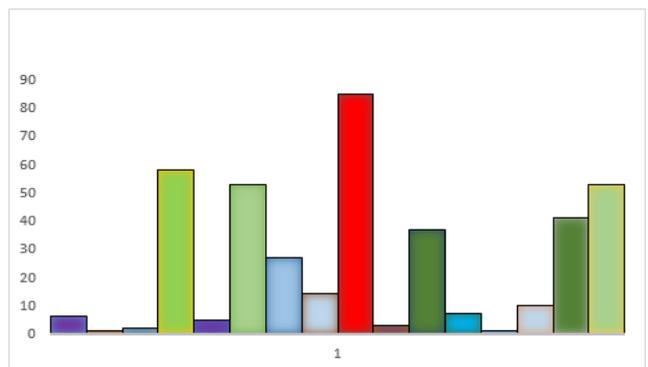
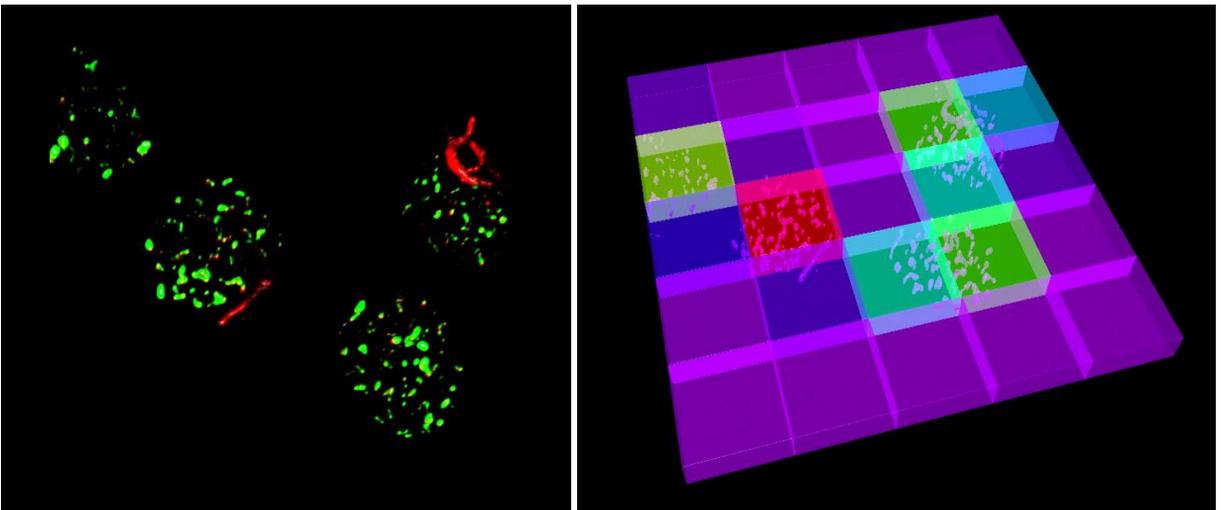
Result

## 5f. Heat-Map Compartments application

### 5f.1 Objects density distribution (heat-map).

The purpose of the density distribution Heat-Map, is to evaluate, through a colored map (heat-map), the objects population concentration per volume' unit inside the whole sample.

These boxes are used as Parent (compartment reference) to establish the compartmentalization of the counted objects (child) in the sampled volume. The different colors shows the density (or concentration) in the specific sub space. Usually, the cold colors (E.G. Blue hues) show a low concentration while the hot colors (E.G. red hues) show high concentration values.



Objects count per sub volume

## 5f. Heat-Map Compartments application

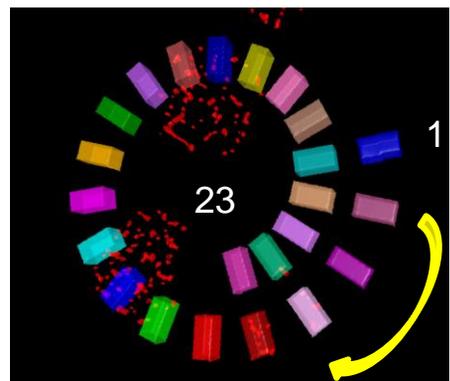
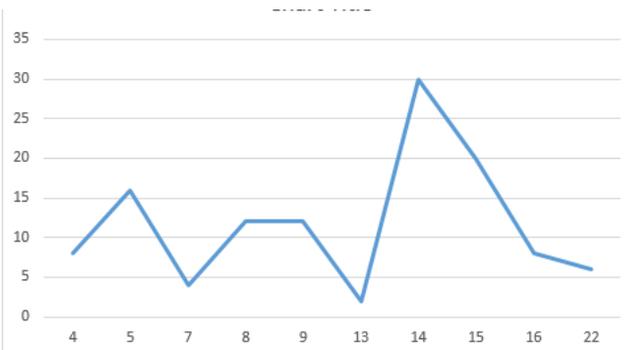
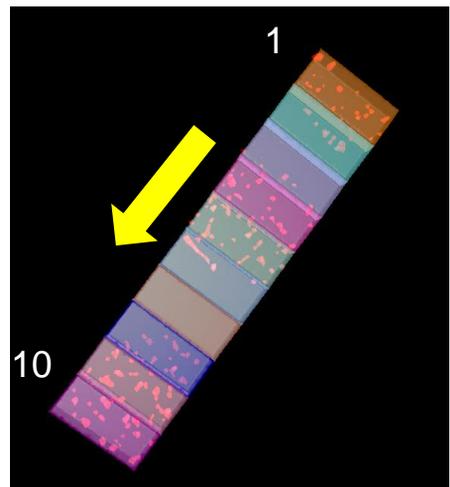
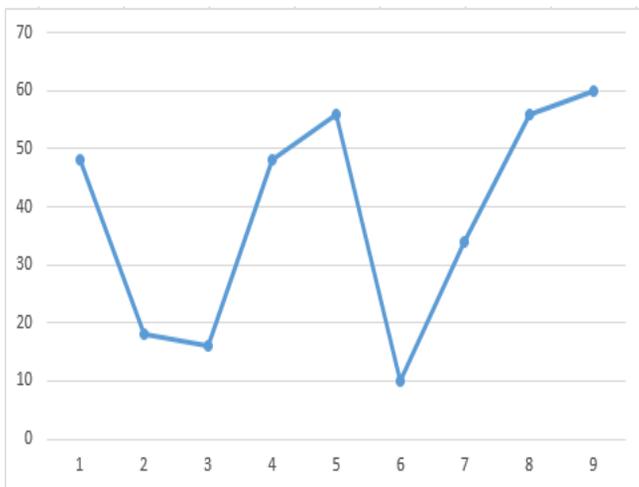
Using different scripts, special boxes layout can be created.  
*Here below a quick examples of the Gradient profile and the Spiral distribution map are shown.*

### 5f.2 Object gradient profile.

The Object gradient profile task, computes the concentration' gradient of the child objects along a specific direction.

Regular sized boxes are created to cover the selected part of the volume' sample with contiguous subspaces.

These boxes are used as Parent to establish the compartmentalization of the counted objects (child) in the sampled volume. The boxes can be created following a linear progression, or covering a more complex paths (E.G. Spiral path)



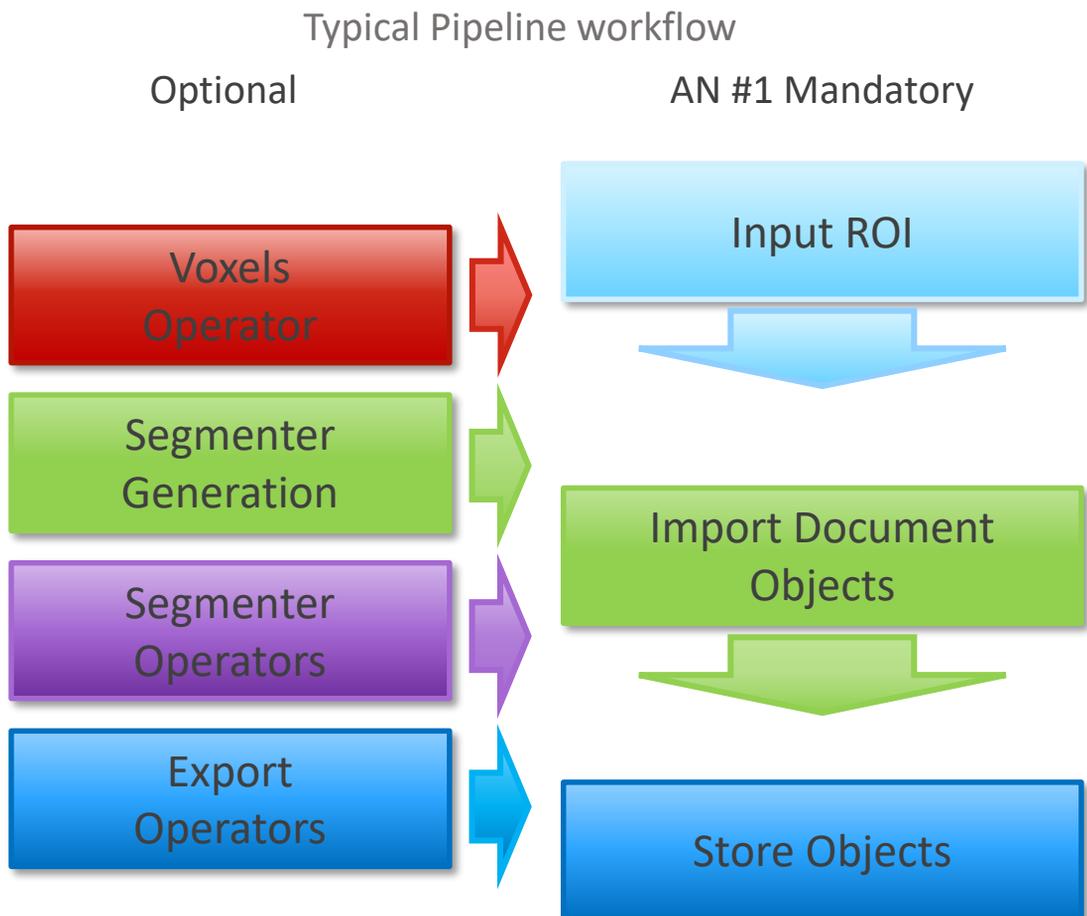
## 6. Build the analysis Pipeline

### DETAILS :

In order to apply the compartment operator, a pipeline must be created according to the user analysis requirements as well as the sample expected behavior.

Pre-processing tasks, as well as segment generation and manipulation procedures can be freely used.

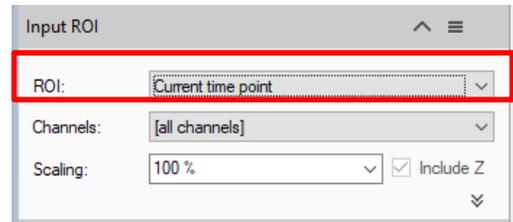
However, some operators usage is mandatory. For example, the **Import Document Objects** operator is used to import the boxes created by the script.



## 6. Build the analysis Pipeline (continue)

### Step 6.2

Change the “*Input ROI*” operator parameters



#### Step 6.2.1 – ROI:

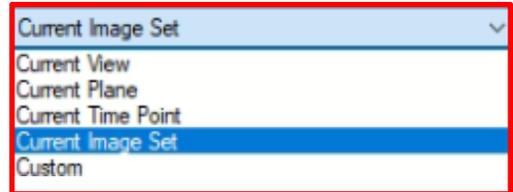
Sets the processing and analysis target space.

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.

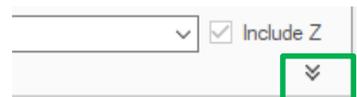


#### DETAILS :

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.



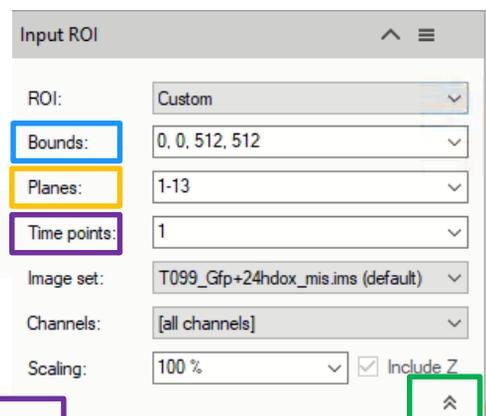
Bounds : Sets the analysis area edges. The whole XY bounds, the viewing area or a custom space can be applied.



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



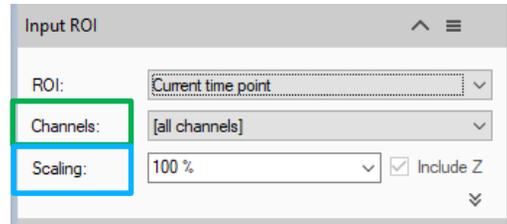
Time Points : Sets the analysis time points range. A single TP, a range of TPs or the whole movie can be selected.



## 6. 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.

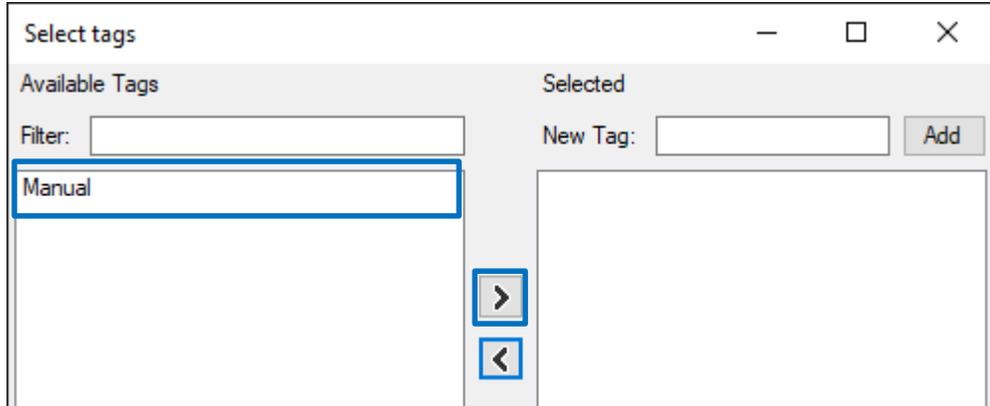
### Step 6.3

Set the «*Import Document Objects*» operator



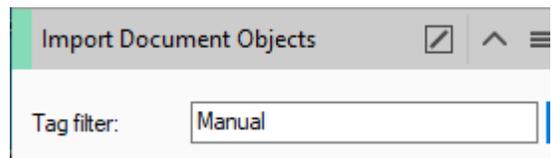
### Step 6.3.1 – ...

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



### NOTE :

The boxes segments have by default, the TAG “*Script*”

## 6. 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 .

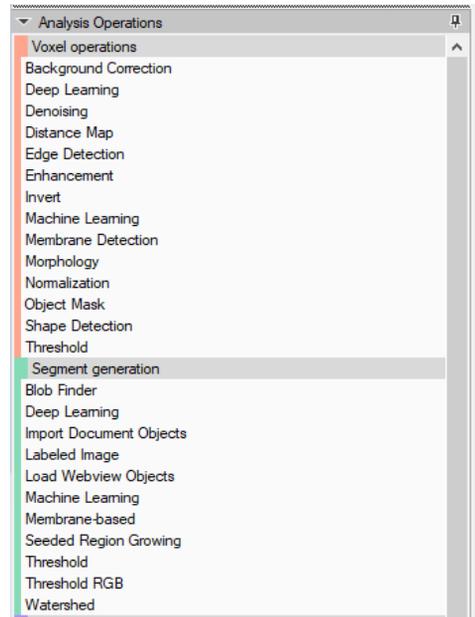
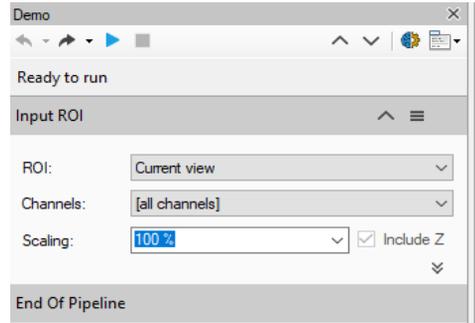
The **Operators** can be added to Pipeline in two ways

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

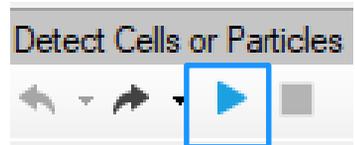


### TIPS :

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

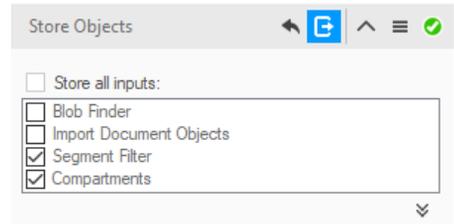


## 8. View the results

### 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)

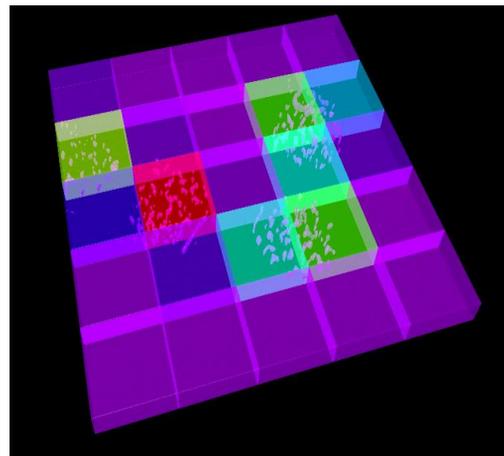


Measurements are now visible in the data table

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 :

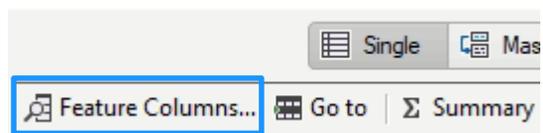
The spots count in the single sub-region is shown in the data table. The empty sub-regions are not listed. To get the total spots count the group statistic feature must be used.



### 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”*

arivis AG, Am Kabutzenhof 21,  
18057 Rostock, Germany

Email : [support@arivis.com](mailto:support@arivis.com)