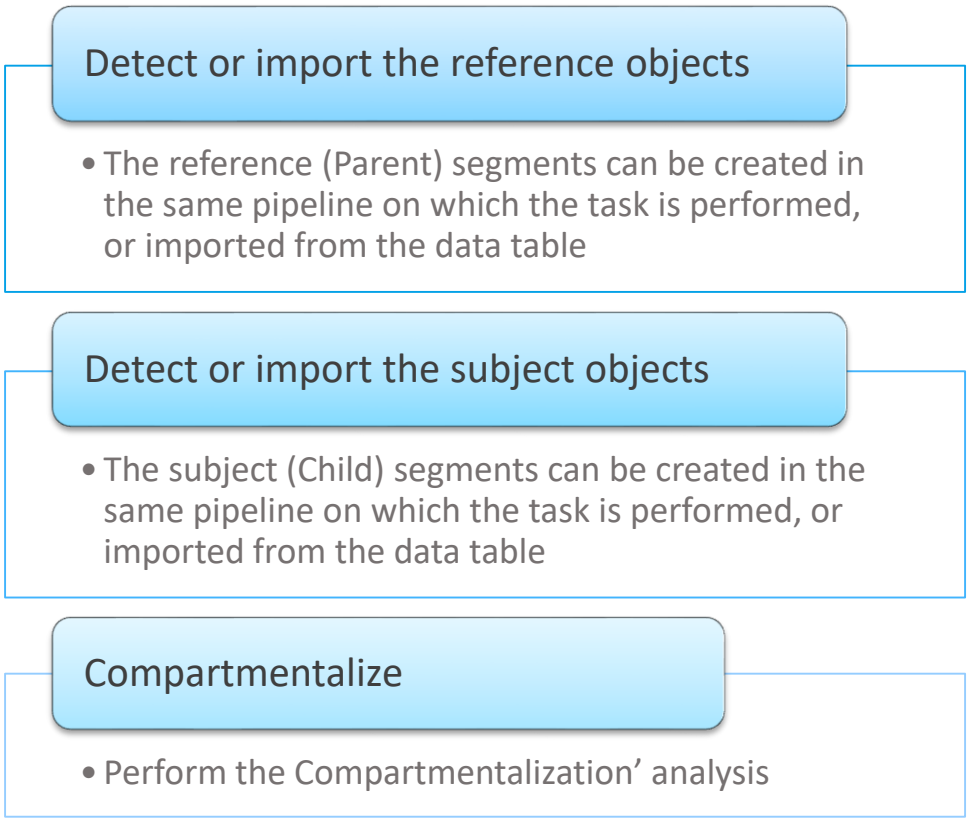
Application Note #9A

How to perform :
«the Compartmentalization analysis»

The application-note purpose is to guide the user in performing a Compartmentalization analysis. It explains how to setup a study focused on the interactions and of the relationships between compartments of the structure under evaluation.

Application Flowchart



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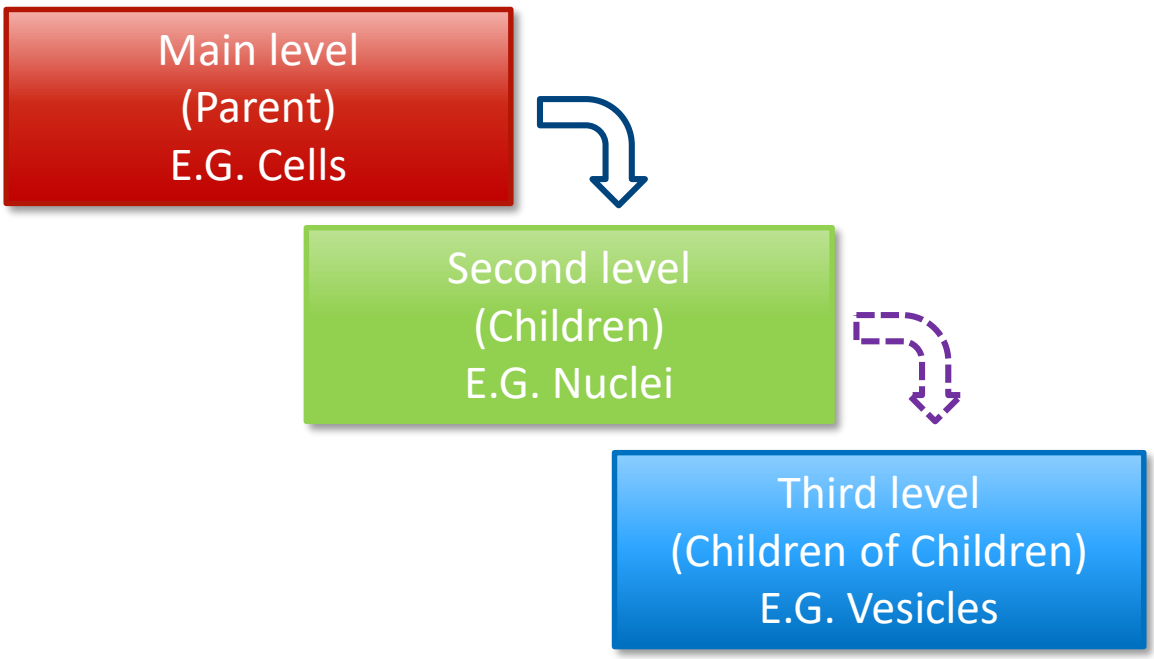
1. Overview

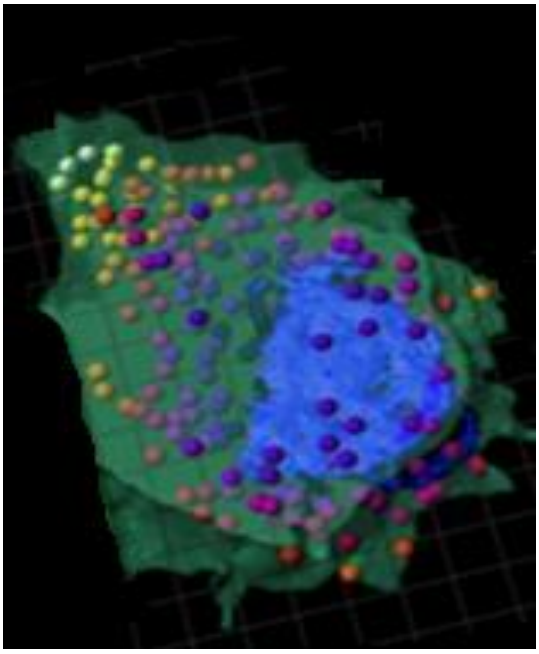
The COMPARTMENTALIZATION' concept is strictly related to the studies of the interactions and of the relationship between the structure' compartments.
Complex hierarchies between the structures can be established and evaluated using this operator. Objects inside a parent structure can be selected, their 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.

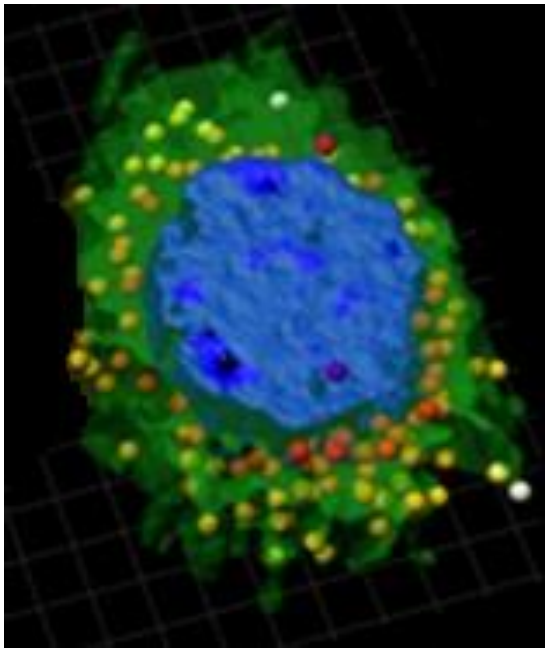


In the example here above, The COMPARTMENTALIZATION is extended on 3 levels. The result is a hierarchical link between the Cell (Reference) and its nucleus (subject) . The nucleus is, in turn, related to the vesicles it contains. Finally, the vesicles count per cell is obtained.

1. Overview (continue)

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.





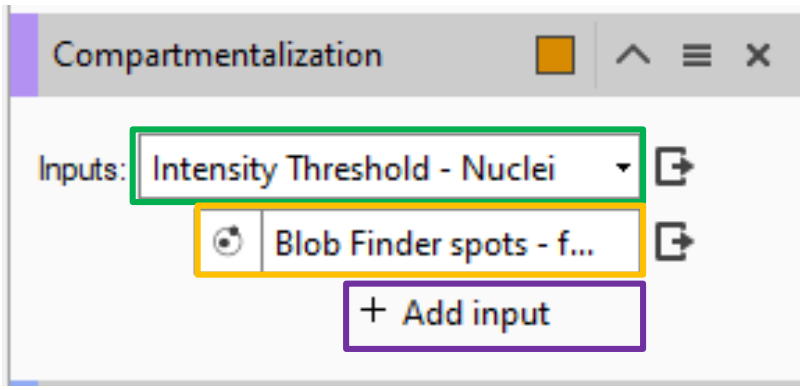
It is not mandatory that the parent object is a defined structure (E.G. Cell or Nucleus), it can be an anatomical region or, generally speaking, a sub-region of interest of the sample volume.
These regions can be drawn both manually or using the interactive method.

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

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

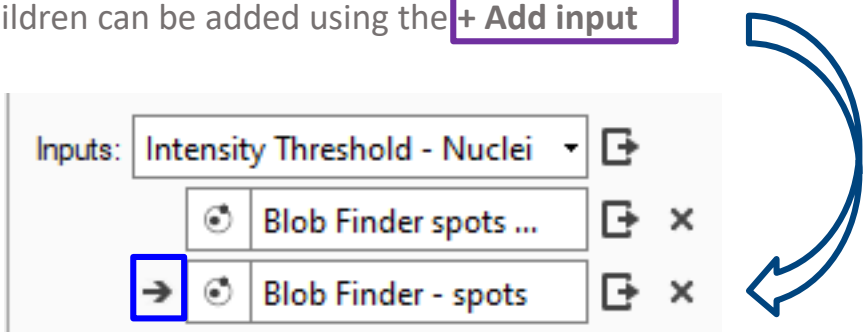
2. The Compartments Operator

2.1 The Compartments Operator allows to set the structures hierarchy to be used to evaluate the relationships between levels.
Several nested levels are possible as well as two or more compartments at the same hierarchical basis.

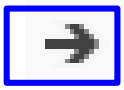


2.2 Compartment hierarchy settings :

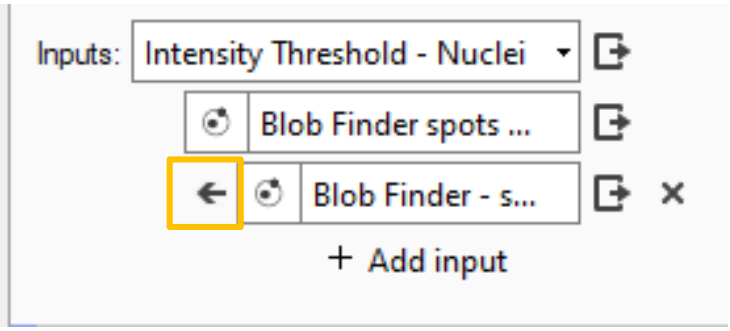
- ***Inputs*** : Select the **TAGs** of the structures to be compartmentalize. The top list shows the reference **TAG**, while the other lists show the children **TAGs**. By default, 2 hierarchical levels are provided. Additional children can be added using the **+ Add input**



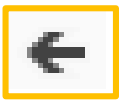
- Move the selected TAG as a "child" of the above **TAG** and push it down in the hierarchy.



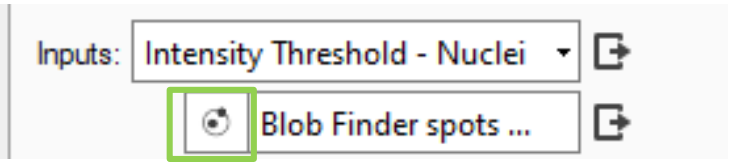
2. The Compartments Operator (continue)



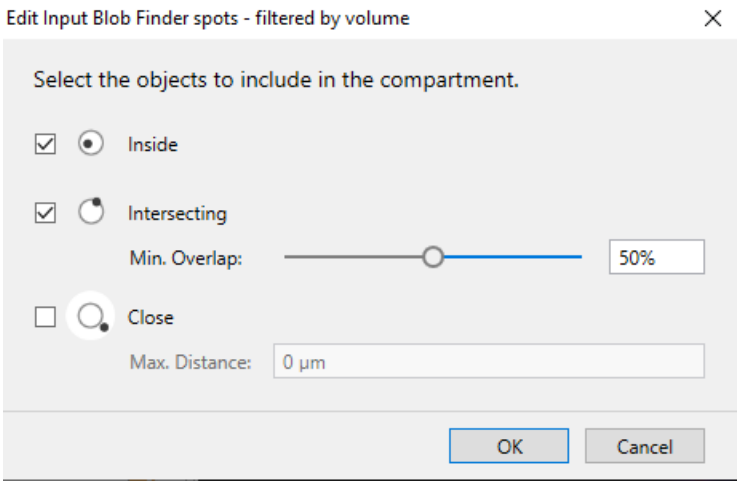
- Move the selected **TAG** to the same level of the above **TAG** and push it up in the hierarchy.



2.3 Structures relationship settings :

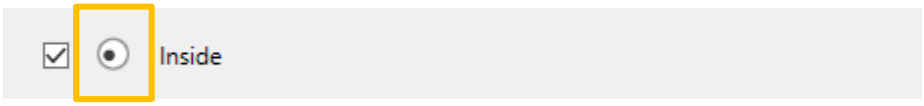


- Open the Tag relationship panel.
  From this panel, various options can be defined.



2. The Compartments Operator (continue)

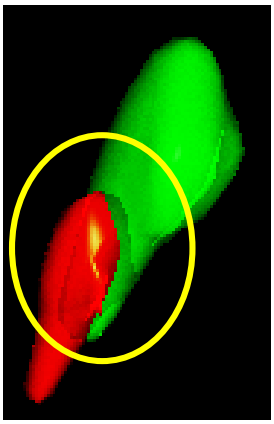
- The following options (rules) are available:

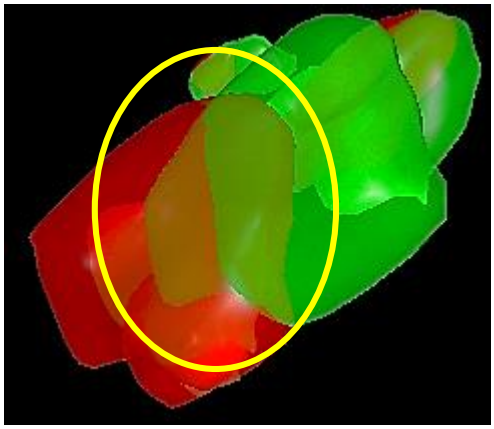


- Child object must be completely inside the parent structure

- Check whether a "child" object is partially covered by a "parent" structure. You can set the minimum amount of overlap using either the slider or the text box.

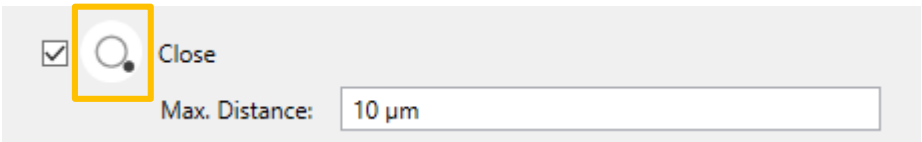
NOTE :
The Min. Overlap value defines the percentage of the child object volume that must be covered by the parent structure to be considered as compartmentalzed.
If you choose 0%, it still checks whether there is at least 1 voxel of coverage.





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.

2. The Compartments Operator (continue)



- Child object must be located within the max. distance value (The children distances are taken outside the parent structure only)

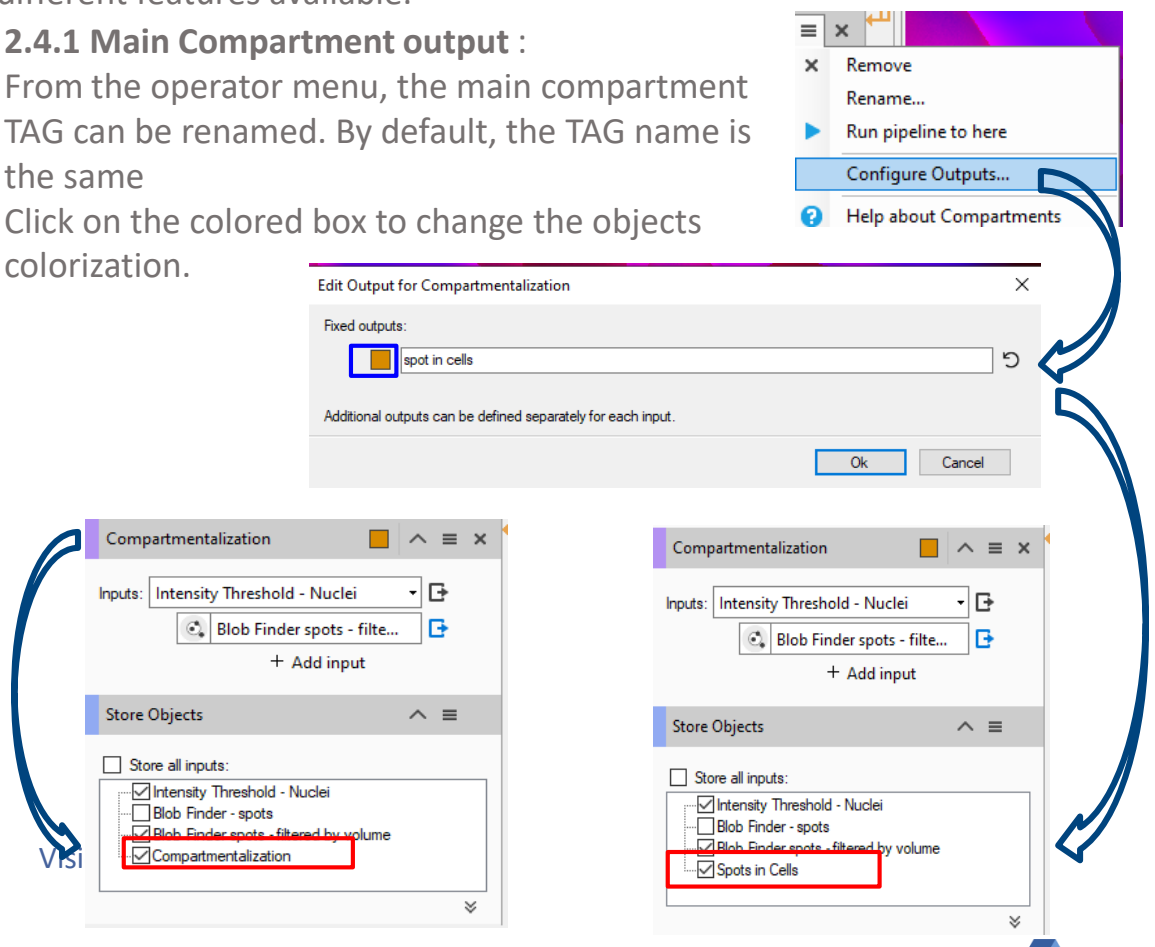
These 3 options can be set independently or in combination between them.

2.4 Compartment output settings :

The compartment operator generates different outputs. Each of them can be enabled and named freely by the user.
Multiple outputs allow to better group and distinguish between the different features available.

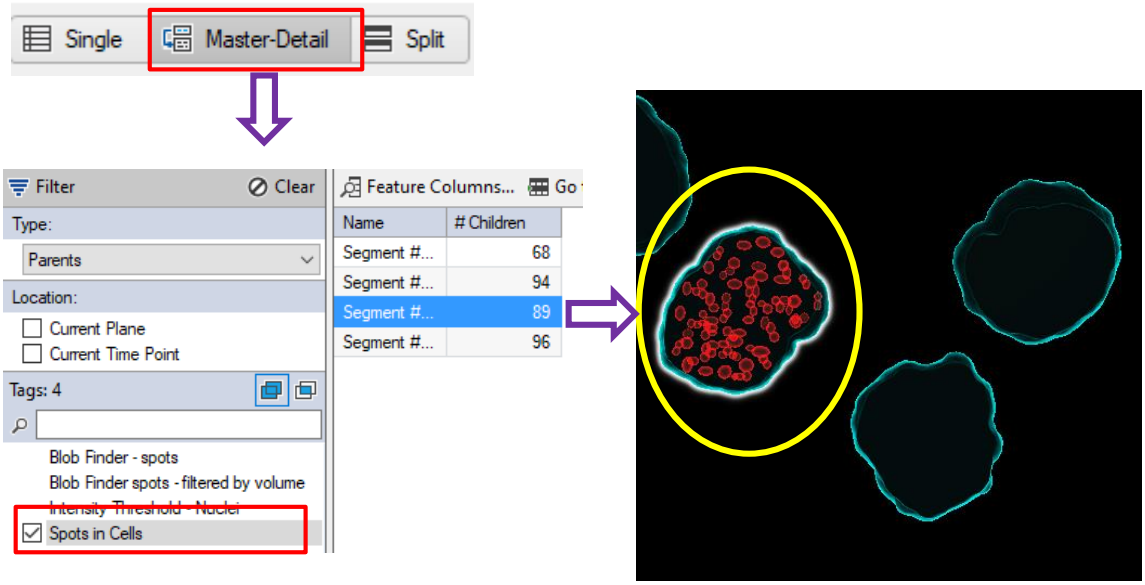
2.4.1 Main Compartment output :

From the operator menu, the main compartment TAG can be renamed. By default, the TAG name is the same
Click on the colored box to change the objects colorization.



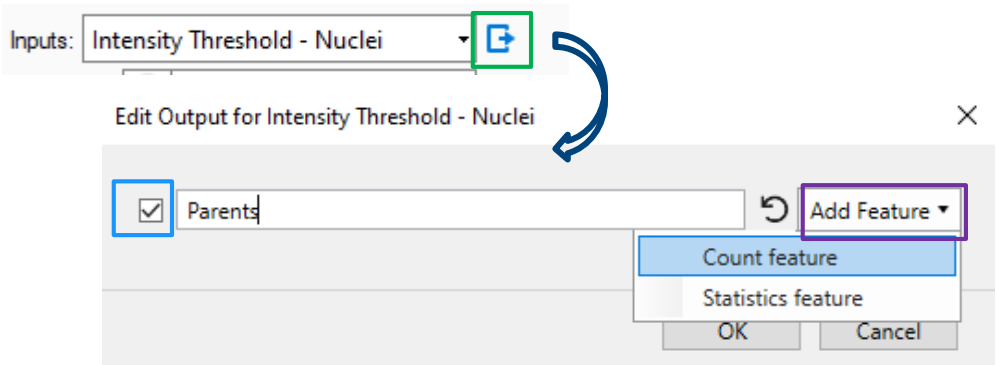
2. The Compartments Operator (continue)

Main Compartment TAG in the objects table



Each parent structure reports the children belonging to it accordingly to the Compartmentalization settings.

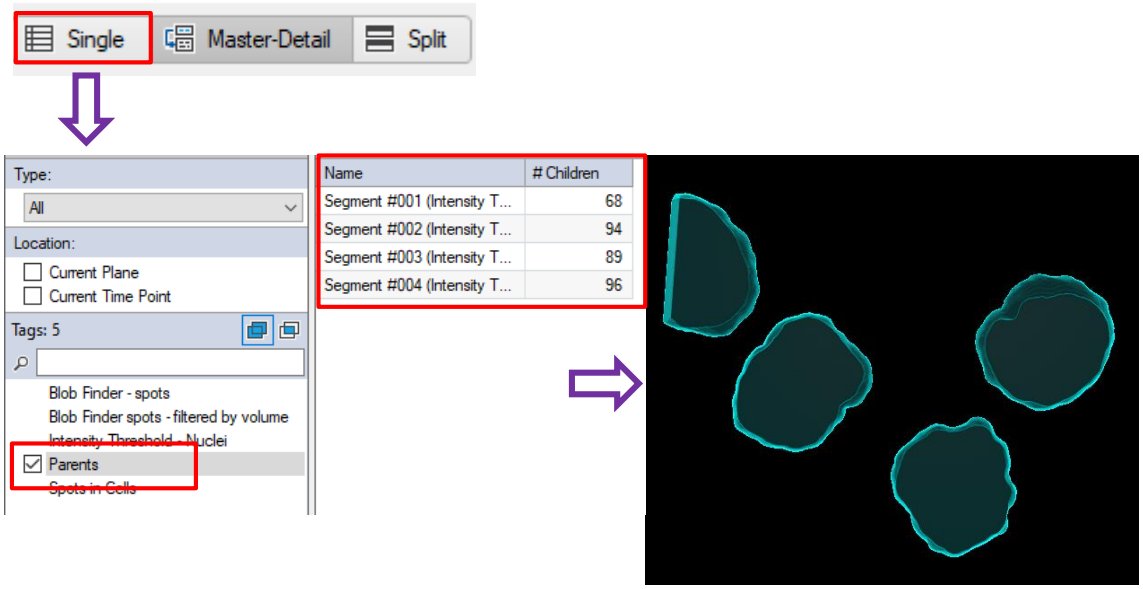
2.4.2 Parents Compartment output :
A new TAG collecting the parents structures can be created.
Press the «Configure output» icon on the right of the parent selection.



Check ON to activate the option and type the Tag name in the text box.
Pull down the list of additional Features on the right in order to create group statistics.

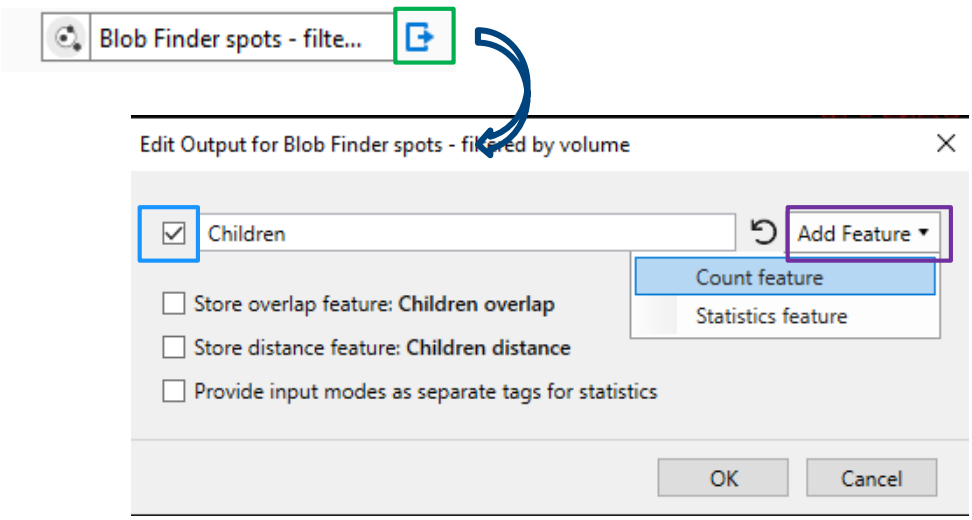
2. The Compartments Operator (continue)

- Parent Compartment TAG in the objects table



The parent structures are shown with the related teatures.

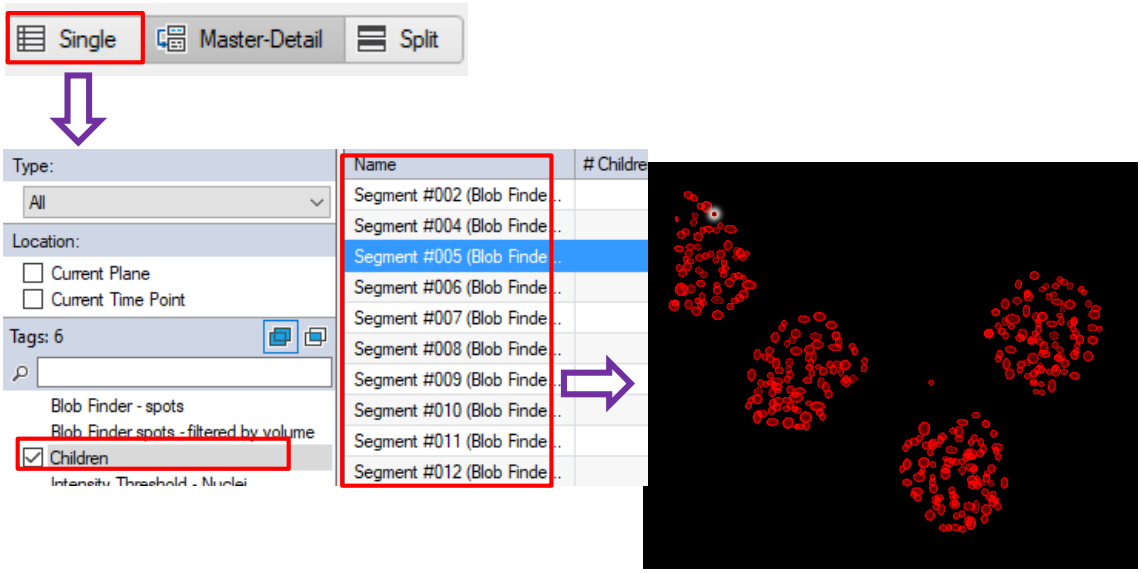
2.4.3 Children Compartment output :
A new TAG collecting the parents structures can be created.
Press the «Configure output» icon on the right of the parent selection.



Check ON to activate the option and type the Tag name in the text box.
Pull down the list of additional Features on the right in order to create group statistics.

2. The Compartments Operator (continue)

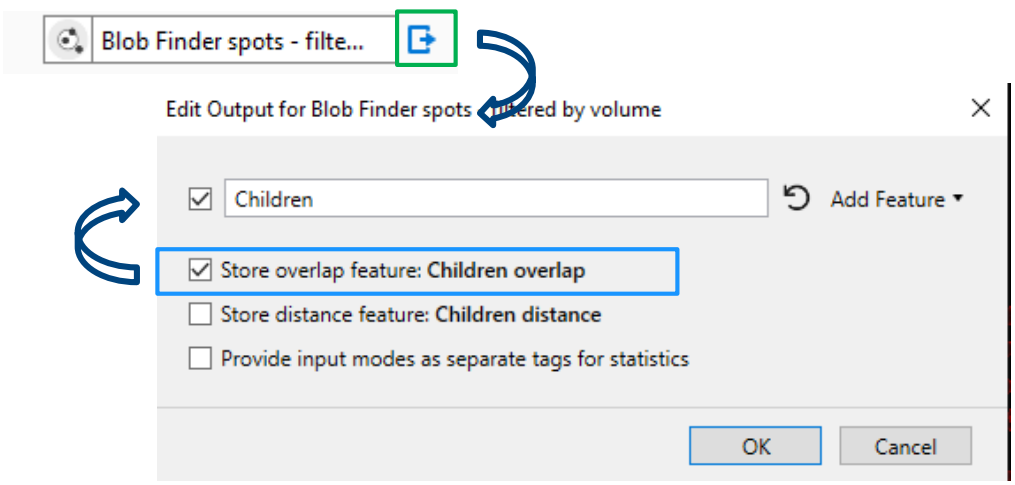
- Children Compartment TAG in the objects table



All the children objects are shown with the related teatures.

2.4.4 Additional Children Compartment output :
Additional features related to a specific relationship criteria, can be set.

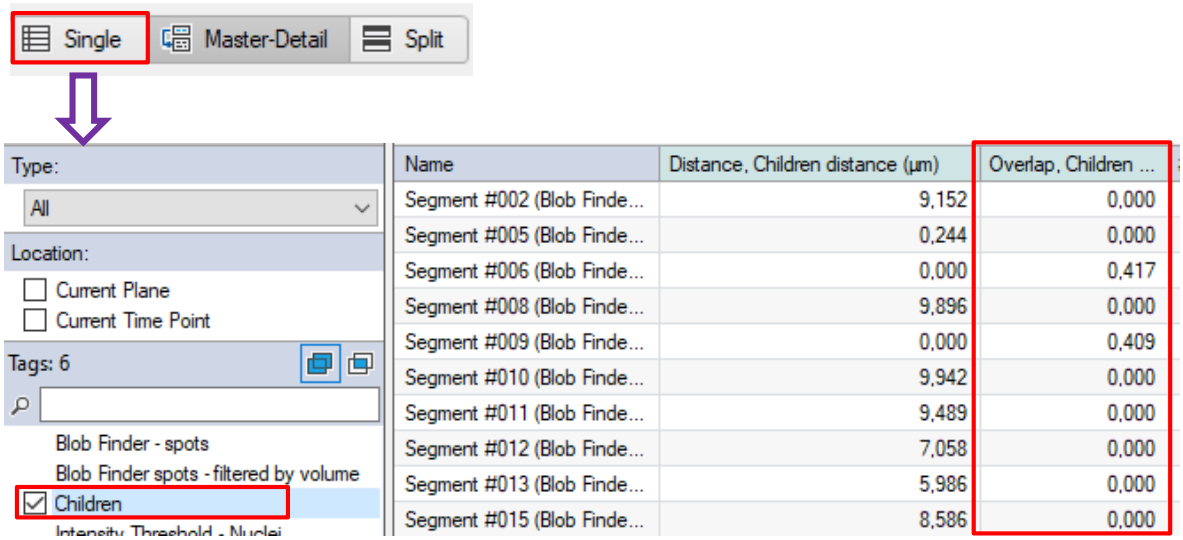
Store overlap feature : Children Overlap



Store overlap feature saves in the objects table a custom feature reporting the children overlap ratio. The feature is available for the objects included in the «Children» compartment TAG.

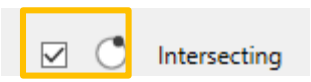
2. The Compartments Operator (continue)

- Additional «Store overlap feature» in the objects table.



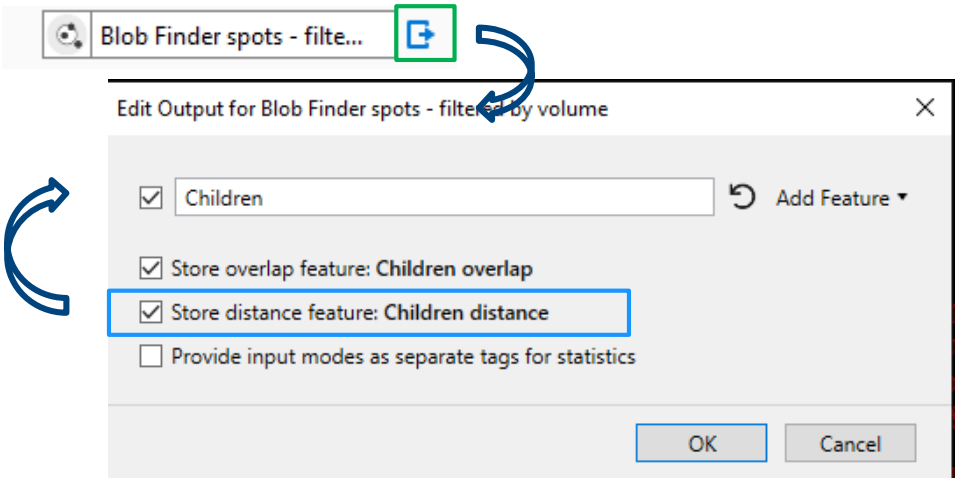
Values in a range of 0.0 to 1.0.
Value equal 1.0 means object fully compartimentalized.

NOTE :
The **Store overlap feature** option is available in the Edit Output dialog box only if the related criteria is ON.



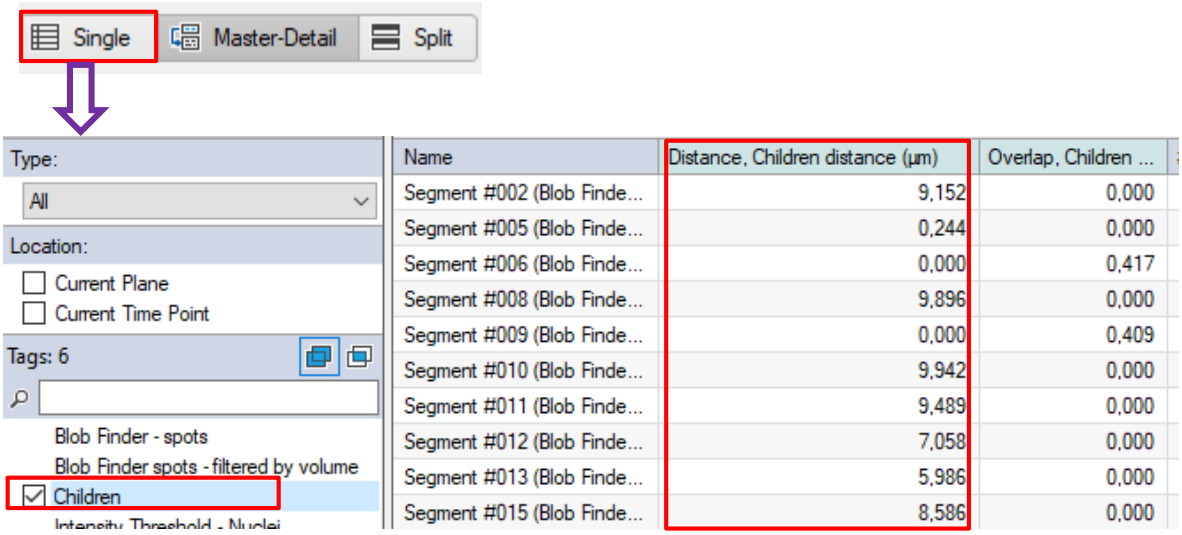
2. The Compartments Operator (continue)

Store distance feature : Children Distance



Store distance feature saves in the objects table a custom feature reporting the children distance from the parent surface. The feature is available for the objects included in the «Children» compartment TAG.

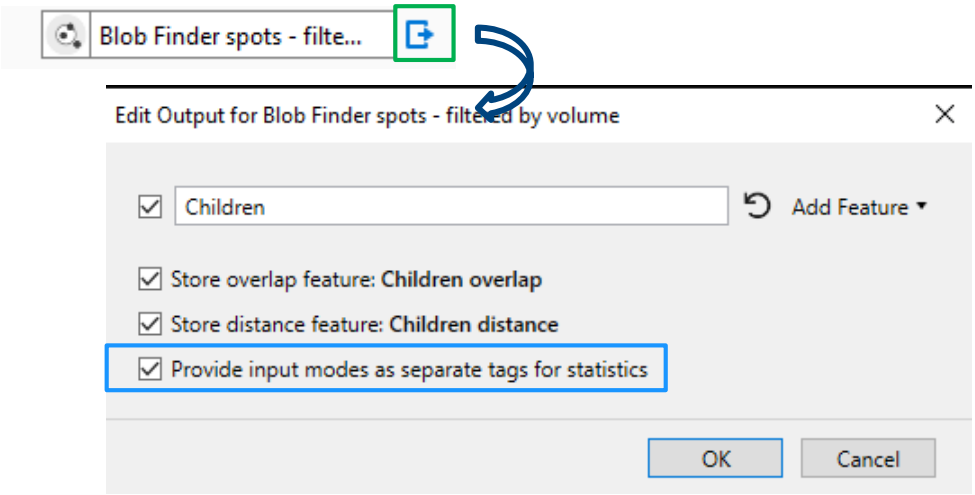
- Additional «Store distance feature» in the objects table.



NOTE :
The **Store distance feature** option is available in the Edit Output dialog box only if the related criteria is ON.

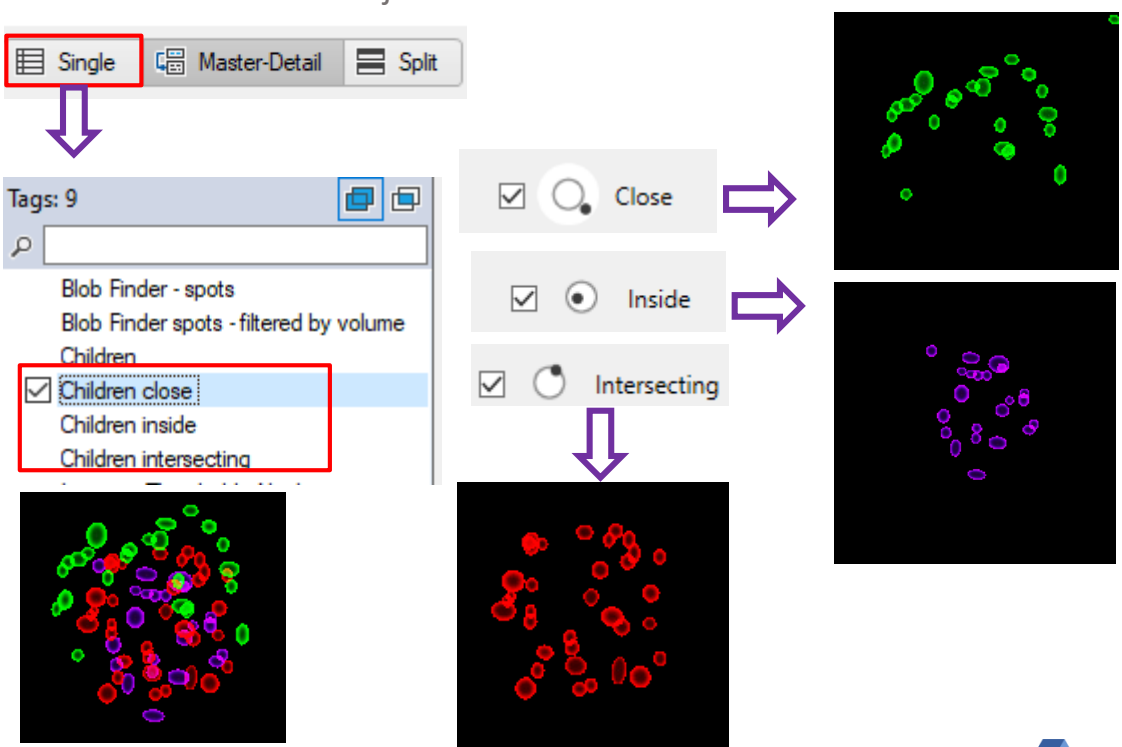
2. The Compartments Operator (continue)

Provide input modes as separate Tags for statistics



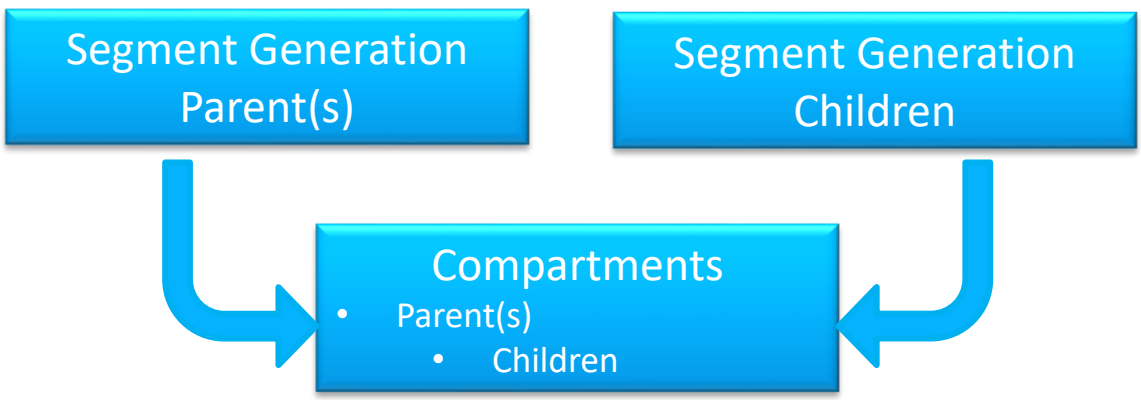
Provide input modes as separate Tags for statistics creates new Tags grouping the children objects belonging to each of the active option described here above.

- Additional «Provide input modes as separate Tags for statistics» in the objects table.



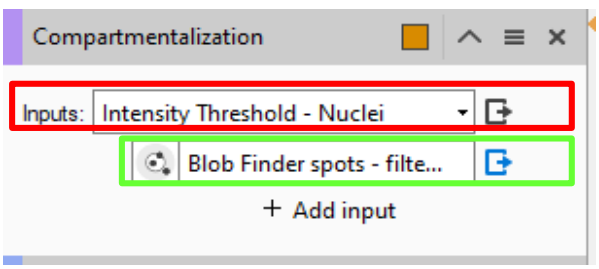
3. Simple Compartments (single children level)

3.1 The Simple Compartments' schema is organized on two levels of hierarchy, the Parent(s) and the potential Children. The children objects belonging to the Parent spaces, accordingly to the criteria setup, are labelled (TAG) as compartmentalized.



Compartments setup:

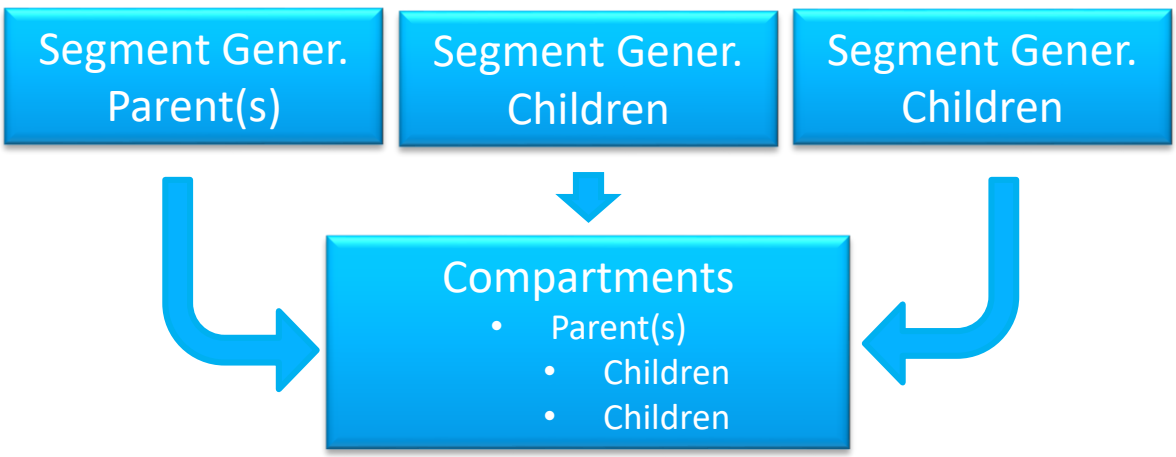
The top input defines the parent structure(s) while the row below the parent input, represent the children objects



NOTE :
These parent(s) / children structures can also be drawn manually.
Refer to the ***Application Note #8*** for detailed information about ***«How to Draw objects interactively»***

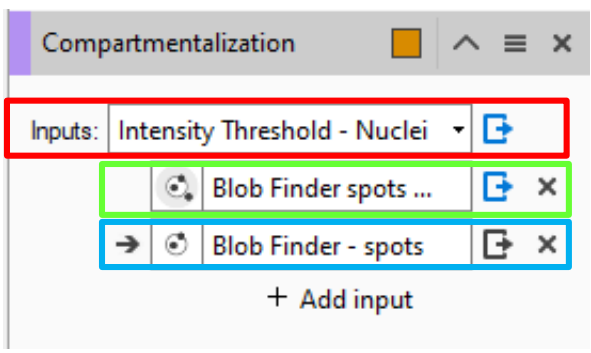
4. Single Compartments (multiple children levels).

4.1 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:

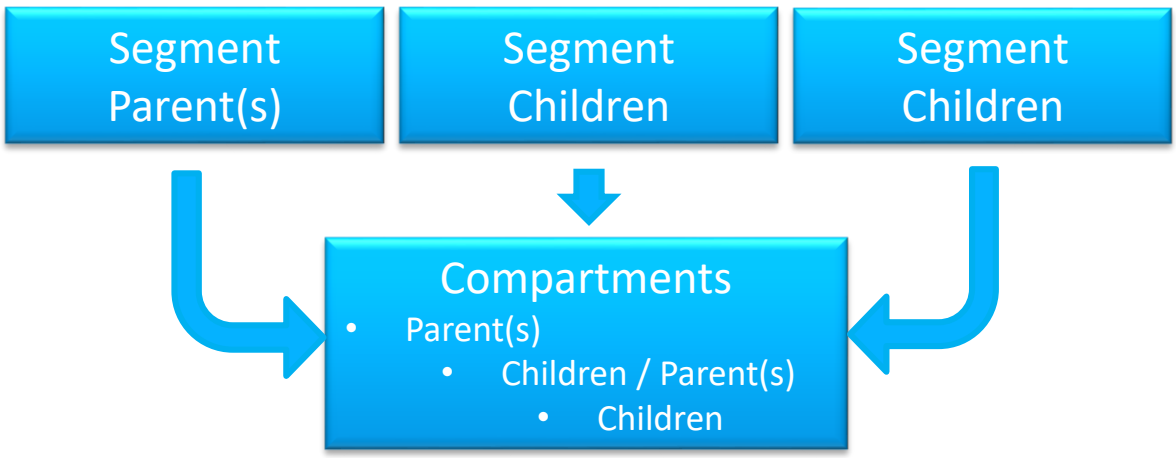
The top input defines the parent structure(s) while the rows below the parent input, represent the children objects. The children inputs are both evaluated using the same parent(s)



5. Multiple Compartments

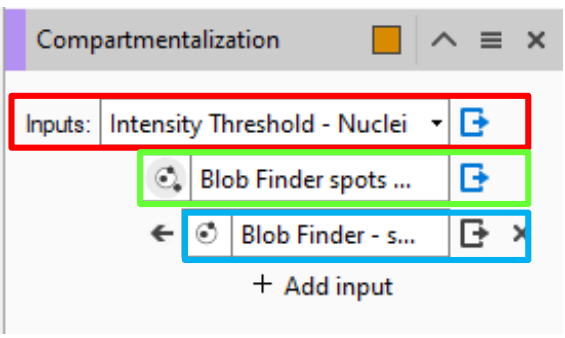
(multiple Parent(s) / Children levels).

5.1 The Multiple Compartments' schema uses 3 or more levels 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:

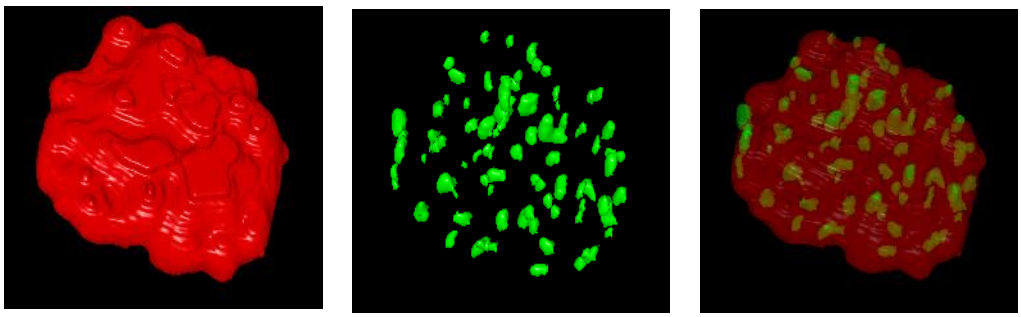
The top input defines the parent structure(s). The second input, just below the parent input, represent the children objects to be compared to the main parent(s).
The last input represent the children objects candidate to be children of the level above.



NOTE :
The **Compartment relationship settings** and the **Compartment output settings** rules are the same described here above for all the hierarchical levels present in the compartment operator.

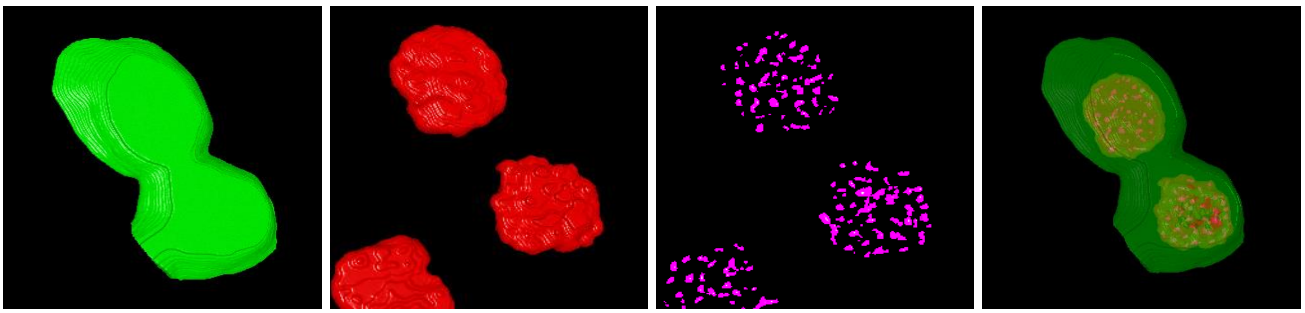
6. Compartments example

6.1 Simple Compartments (2 levels)



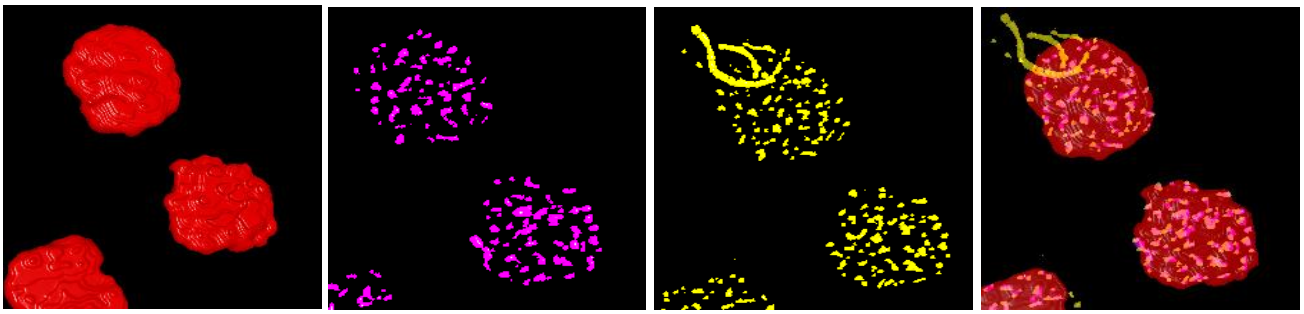
Reference Subject Result

6.2 Multiple Compartments (multiple levels).



Reference Subject #1 Subject #2 Result

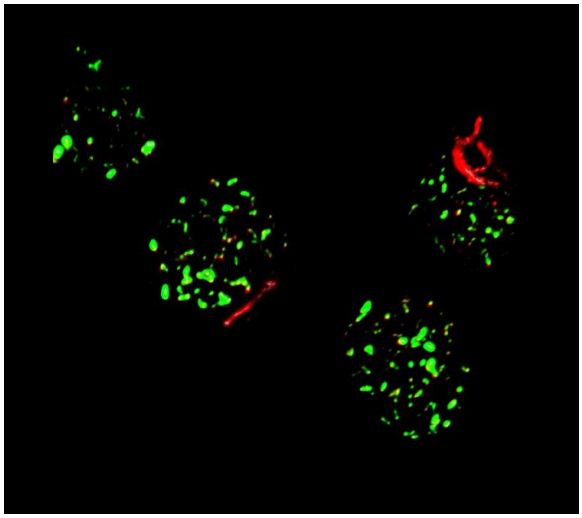
6.3 Single Compartments (multiple subjects).

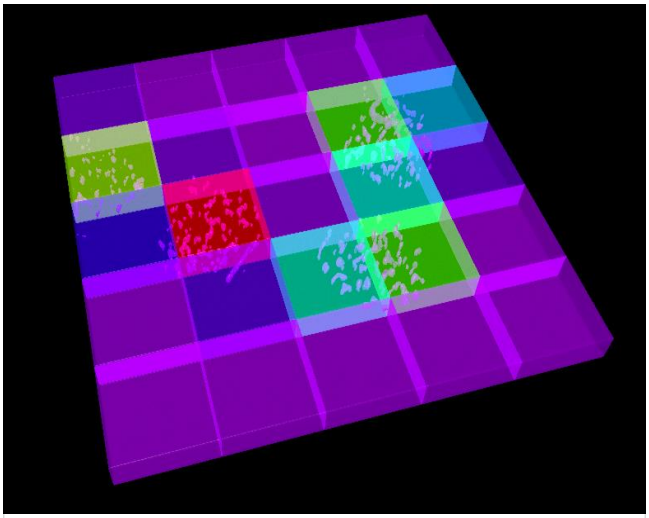


Reference Subject #1 Subject #2 Result

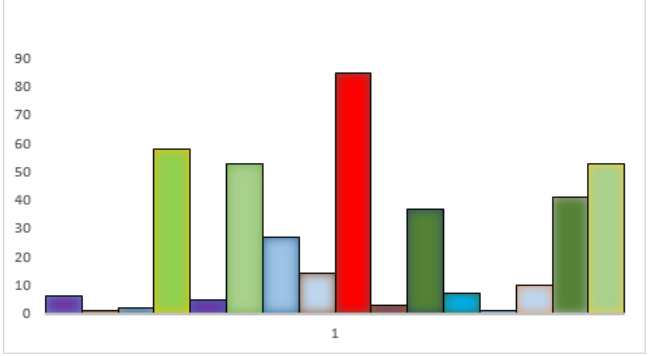
7. Special Compartments application

7.1 Objects density distribution (heat-map).
The purpose of the density distribution task, is to evaluate, through a colored map (heat-map), the objects population concentration per volume' unit inside the whole sample.
Contiguous, regularly sized, boxes are created to divide the whole volume sample in sub spaces.
These boxes are used as Parent 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.





TIPS :
A Python script (***Divide Scope***) is available to create the sub-volumes boxes matrix (Parent objects).



Objects count per sub volume

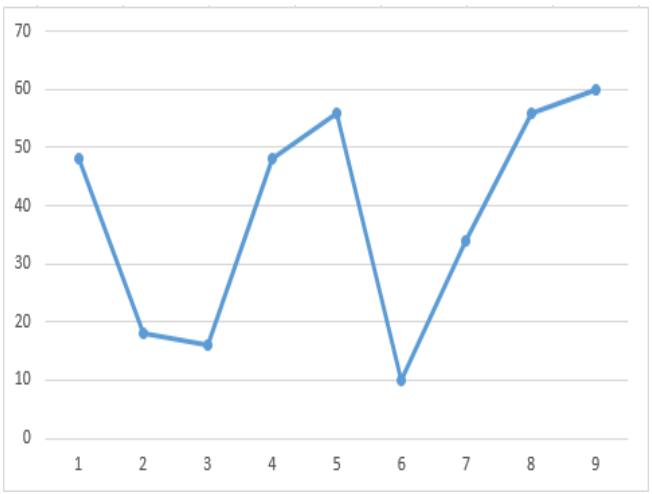
7. Special Compartments application (continue)

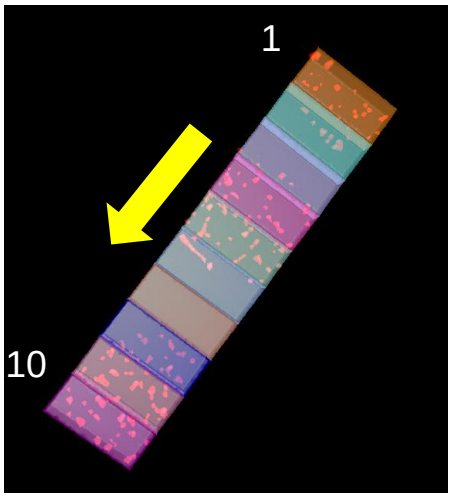
7.2 Object gradient profile.

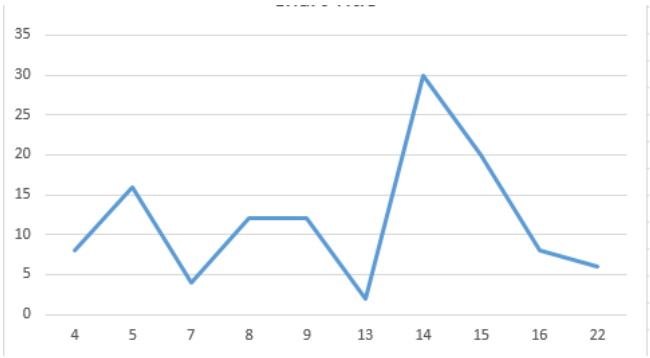
The Object gradient profile task, computes the concentration' gradient of the child objects along a specific direction.

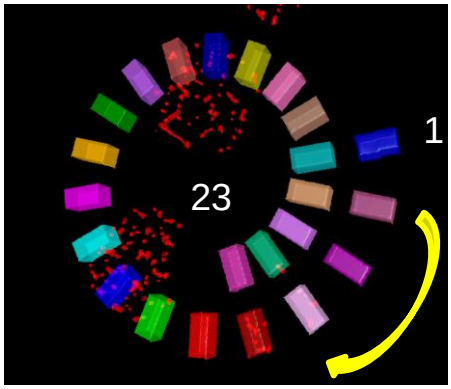
Regular sized boxes are created to cover the selected part of the volume' sample with contiguous subspaces.

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)









8. Tips

8.1 The **Application Note #1** (Gradient) describes the steps to achieve the linear gradient' profile (objects count along a specific path), as well as the map of the concentrations ("Heat Map" - objects count per volume unit).

8.2 The **Application Note #6** (Spiral Oriented Sub Volume) describes the steps to achieve the spiral gradient' profile (objects count along a specific concentric path) .

8.3 The **Application Note #8** (Draw Manual Objects) describes the steps to draw objects interactively.

8.4 A Python scripts (**Segments rotation, Spiral Oriented Sub Volume**) are available to create the sub-volumes boxes matrix (Parent objects) described in the chapter 7.

Contact the arivis local area sales manager to get more information about how to get the python scripts 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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