

# Presentation of GcoPS Icy plugin

GcoPS is a fully automatic and non parametric colocalization algorithm with four possible outputs:

- *Statistical test*: GcoPS computes a statistical test to evaluate the **global (anti-)colocalization** between two 2D/3D binary images, where the background is encoded by the value 0 (see the caution below),
- *Random geo-copositioning*: GcoPS computes a statistical test on randomly located small windows from a pair of binary 2D/3D images to identify **regions of colocalization**,
- *Dense geo-copositioning*: GcoPS computes a statistical test on a regular grid of small windows from a pair of binary 2D/3D images to extract a **dense map of colocalization**,
- *Temporal interaction profile*: GcoPS computes a statistical test between image pairs extracted from two binary image sequences by considering different temporal shifts between the sequences. It enables to **evaluate if colocalization is driven by one of the two channels**.

## Caution:

- GcoPS analyzes binary images, which typically means that a preliminary segmentation algorithm must have been applied. In Icy, this preliminary step can for instance be performed by the *Spot Detector* device in 2D or the C-CRAFT plugin in 3D. Specifically, each pixel for which the intensity is superior to 0 is considered as part of the object(s) by GcoPS. This means that the background of the images must be encoded by the value 0.
- Make sure that you have the latest version of Java and Icy.

## 1 Statistical test

When you open GcoPS plugin, you obtain the interface shown in Fig. 1. By default, *Statistical test* is selected in the field *Mode for GcoPS*.

### 1.1 Inputs

- Input image 1  
This input corresponds to one of the two binary images for which you want to analyze colocalization. It is also possible to select an image sequence, then a colocalization test is independently performed at each time step. Moreover, GcoPS allows the input of a multi-channel image, in which case each channel must be a binary image where the background is encoded by 0. In this situation, you must choose the channel you want to analyse by GcoPS with the next input. A sequence of multi-channel images can also be selected.

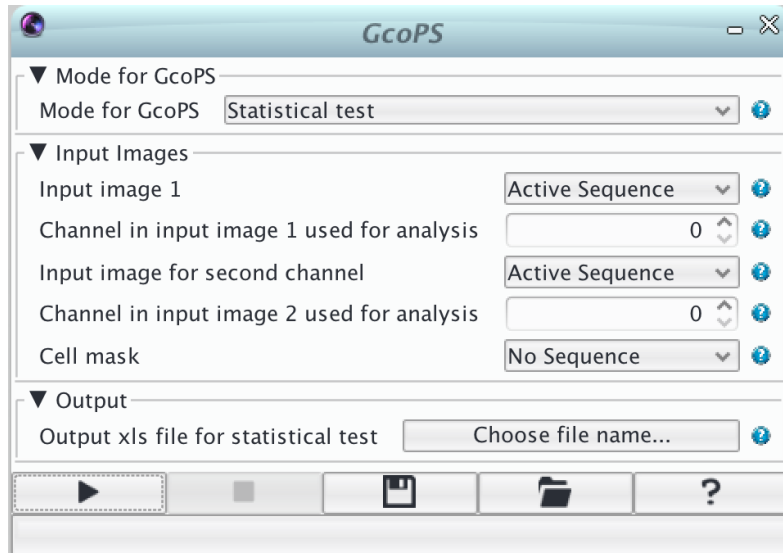


Figure 1: Screenshot of *Statistical test*.

- **Channel in Input image 1 used for analysis**  
This input allows you to choose the channel of the **Input image 1** for which you want to analyze the colocalization. If the **Input image 1** is just a binary image, or a sequence of binary images, the only possible choice is the default choice "Channel 0".
- **Input image 2**  
This input corresponds to the second binary image for which you want to analyze colocalization. Similarly as for **Input image 1**, it is possible to select an image sequence or a multi-channel image or a multi-channel image sequence. Note that in the two latter cases, the same input as **Input image 1** can be selected, a typical example being the situation where you want to analyse colocalization between two channels of the same image.
- **Channel in Input image 2 used for analysis**  
This input allows you to choose the channel of the **Input image 2** for which you want to analyze the colocalization. If the **Input image 2** is just a binary image, or a sequence of binary images, the only possible choice is the default choice "Channel 0".
- **Cell mask**  
The cell mask enables to consider a specific region in the colocalization analysis. In this mask, you have to define an intensity superior to 0 inside the region and equal to 0 outside. This input is optional.
- **Output xls file for statistical test**  
The output Excel file name has to be defined to export the results. If the file does not exist, it is created. If it already exists, the new output is recorded in a new sheet.

## 1.2 Output

The output of *Statistical test* is a xls file containing the p-value(s), the colocalization score(s) and a label to specify if colocalization or anti-colocalization was tested. This test depends on the correlation: a positive correlation implies a colocalization test while a negative correlation implies an anti-colocalization test.

## 2 Random geo-copositioning

When you select *Random geo-copositioning* in the field *Mode for GcoPS*, you obtain the interface shown in Fig. 2.

### 2.1 Inputs

- Input image 1  
Same as in Section 1.
- Channel in Input image 1 used for analysis  
Same as in Section 1.
- Input image 2  
Same as in Section 1.
- Cell mask  
Same as in Section 1.
- Number of tests  
By changing this input, the number of randomly tested windows is modified:
  - Very high number of tests: total number of pixels/voxels in the cell mask (if defined) or the image (if not) divided by 20 in 2D and 200 in 3D,
  - High number of tests: total number of pixels/voxels in the cell mask (if defined) or the image (if not) divided by 100 in 2D and 1000 in 3D,
  - Moderate number of tests: total number of pixels/voxels in the cell mask (if defined) or the image (if not) divided by 500 in 2D and 4000 in 3D,
  - Small number of tests: total number of pixels/voxels in the cell mask (if defined) or the image (if not) divided by 2000 in 2D and 5000 in 3D.
- Window width for random geo-copositioning  
Window height for random geo-copositioning  
Window depth for random geo-copositioning  
Windows sizes used for statistical tests. If Window width for random geo-copositioning is superior to half the image width, it is then defined as half of the image width. The same remark stands for Window height for random geo-copositioning and Window depth for random geo-copositioning. The latter specification only applies to 3D images.  
For a safe detection, we recommend choosing a windows size that is at least five times larger than the average size of the objects.
- Output xls file for randomization study  
The output Excel file name has to be defined to export the results. If the file does not exist, it is created. If it already exists, the new output is recorded in a new sheet.

### 2.2 Output

The output xls file contains the proportion of windows for which the  $p$ -value obtained with the statistical test is inferior to 0.05. In the output image, the colocalization hits (resp. anti-colocalization hits) are overlaid on the input image as white circles (resp. blue circles). Specifically, these hits are the centres of the tested windows where colocalization (resp. anti-colocalization) have been detected. Take care that if the windows used for detection are large, these centres may be not so close to the co-localized objects, which may be located at the boundary of the windows. For this reason, the *Random geo-copositioning* device is mainly relevant when small windows have been chosen for the detection, which is possible if the initial images are sufficiently large, to ensure a minimal fluorescence information in each windows.

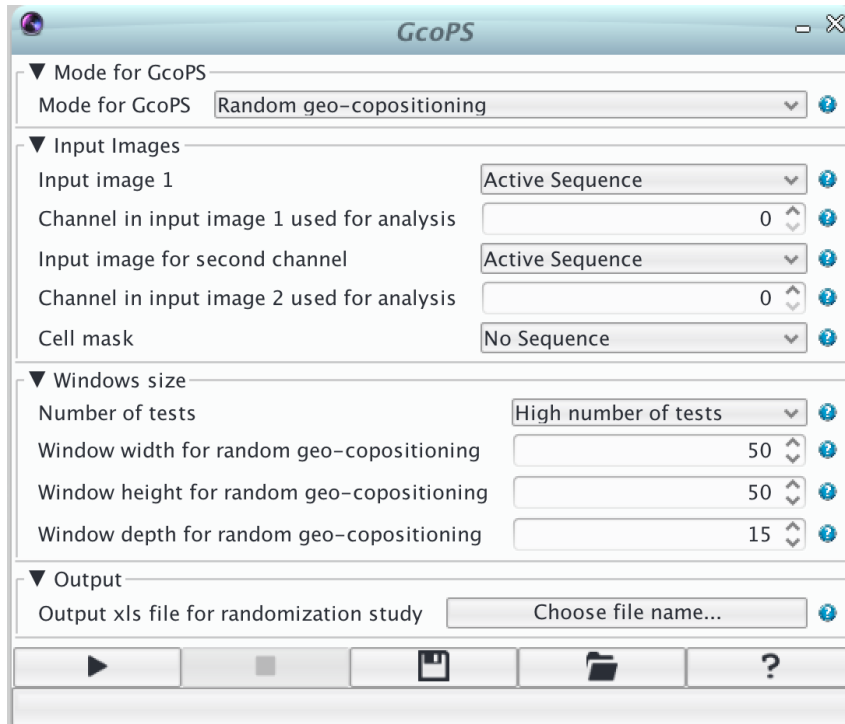


Figure 2: Screenshot of *Random geo-copositioning*.

### 3 Dense geo-copositioning

When you select *Dense geo-copositioning* in the field *Mode for GcoPS*, you obtain the interface shown in Fig. 3.

#### 3.1 Inputs

- Input image 1  
Same as in Section 1.
- Channel in Input image 1 used for analysis  
Same as in Section 1.
- Input image 2  
Same as in Section 1.
- Cell mask  
Same as in Section 1.
- Window width for dense geo-copositioning  
Window height for dense geo-copositioning  
Window depth for dense geo-copositioning  
Windows sizes used for statistical tests. If Window width for dense geo-copositioning is superior to half the image width, it is then defined as half of the image width. The same remark stands for Window height for dense geo-copositioning and Window depth for dense geo-copositioning. The latter specification only applies to 3D images.

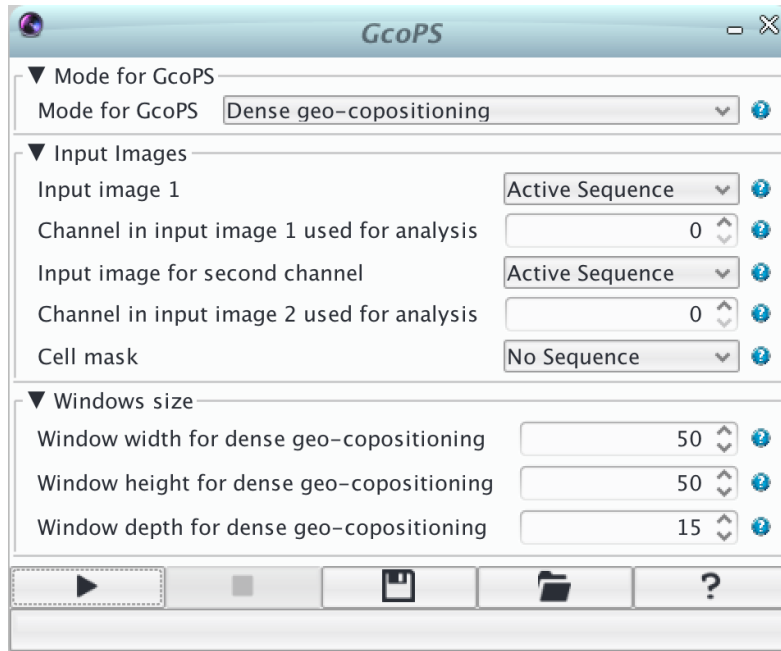


Figure 3: Screenshot of *Dense geo-copositioning*.

## 3.2 Output

An output image corresponding to a dense colocalization score map is generated in Icy. We advice you to customize this map with the *colormap* device of Icy.

## 4 Temporal interaction profile

When you select *Temporal interaction profile* in the field *Mode for GcoPS*, you obtain the interface shown in Fig. 4.

### 4.1 Inputs

- Input image 1  
Same as in Section 1, but the input must be a sequence.
- Channel in Input image 1 used for analysis  
Same as in Section 1.
- Input image 2  
Same as in Section 1, but the input must be a sequence.
- Cell mask  
Same as in Section 1.
- Maximum shift between the two channels  
This input corresponds to the maximum temporal shift you want to consider between the two channels (from -Maximum shift between the two channels to +Maximum shift between the two channels). If the Maximum shift between the two channels is superior to the number of frames, it is defined as the number of frames.
- Output xls file for temporal interaction profile  
The output Excel file name has to be defined to export the results. If the file does not exist, it is created. If it already exists, the new output is recorded in a new sheet.

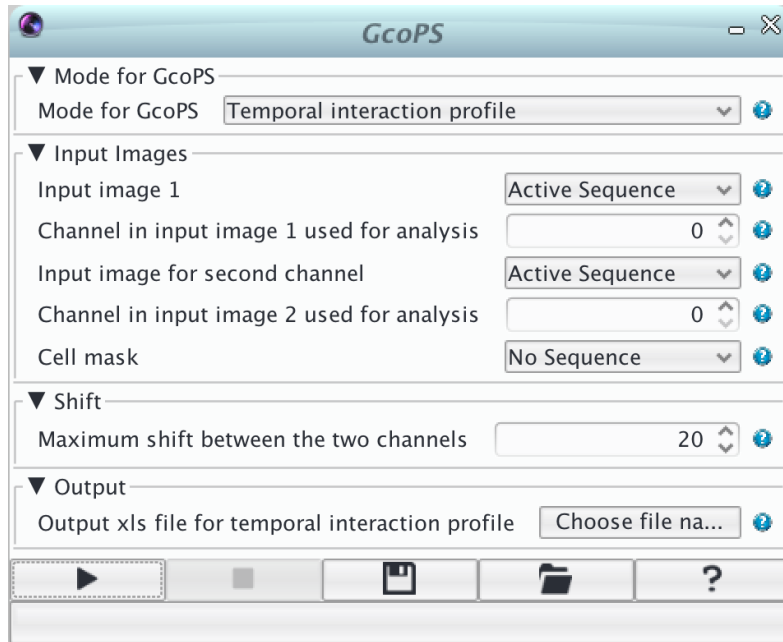


Figure 4: Screenshot of *Temporal interaction profile*.

## 4.2 Output

The output of *Temporal interaction profile* is a xls file containing for each temporal shift the colocalization score(s) between the shifted frames. The box plots and/or the average colocalization score for each shift can then be displayed to analyze which channel is driving colocalization.