# Explanations Gelatin\_DegradationIndex.ijm

This macro treats a folder and computes a degradation index, which is the ratio between the area of gelatin degradation and the number of nuclei in the image. The gelatin degradation is revealed by the absence of fluorescence in the fluorescently-labeled gelatin while the nuclei are detected with the DAPI channel.

1/ The macro asks the folder to treat; all images with the extension specified line 31 by ext\_file:

In our case, ext\_file = ".tif". The files with other extensions will be ignored

Une image contenant texte

Description générée automatiquement

2/ The macro:

* asks for the channel representation: the nucleus and gelatin,
* offers the possibility, if the image is a stack, to choose the slices for the maximum z-projection (if checked, for each Z-stack image, a maximum z-projection on **all slices** is performed)

Une image contenant texte, capture d’écran, affichage, Police

Description générée automatiquement

Remark: this means that all images treated together (*i.e.* in the same folder) should have the same order for the channel acquisitions (or at least for nucleus and gelatin).

3/ For **each image** which name ends by the specified extension, the code checks if a result already exists for this image (from a previous analysis). If yes, the result is directly loaded, and the macro **loads the following image**. This result file should therefore be manually deleted from the Result folder if the user needs to re-analyze previously analyzed images.

If the image was not treated before:

a/ The nucleus channel is extracted, and nuclei are counted on a maximum z-projection, thresholded (default threshold) after a blur of 2 pixels. Too small nuclei or nuclei on the border are removed from the analysis. The user is asked to validate this number of nuclei:

Une image contenant capture d’écran, Graphique, Système d’exploitation, graphisme

Description générée automatiquement

b/ 3 possibilities for the image studied in step c/:

* If the gelatin image is a z-stack AND the user unchecked the option "If z-stack, Max Projection on the entire z-stack?", he is asked to choose the plans on which this image should be analyzed:

Une image contenant texte, capture d’écran, affichage, logiciel

Description générée automatiquement

**Remark:** in case the user makes a mistake choosing the number for first and last slices (below 1 or above the real number of slices), the projection will be performed on all the slices; this is written in the Log:

If more than 1 plan are chosen, the maximum z-projection is performed on the selected slices.

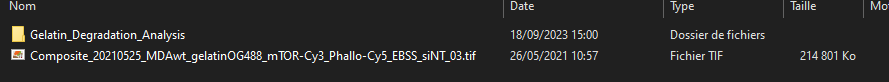
* If the gelatin image is a z-stack AND the user checked the option "If z-stack, Max Projection on the entire z-stack?", the maximum z-projection on all slices is performed
* If the gelatin image is not a stack, the image is not changed and used as it is.

c/ On the image from step b/, the user is asked to choose the threshold to define what is degradation; the user should modify the sliders and click ok once all regions they consider to be degradation are highlighted in red:

Une image contenant texte, capture d’écran, carte, Logiciel multimédia

Description générée automatiquement

d/ The result table associated to this this threshold, for this image is saved in a folder created within the original folder.



4/ Once all the images are treated, the result table summarizing the degradation index which is the area of degradation (**in pixels^2**) divided by the number of nuclei (column *Area/Nuclei*) per image is created and saved in the same folder as the intermediate results:

Une image contenant texte, capture d’écran, Police, ligne

Description générée automatiquement