# Explanations macro quantif\_DNAdamage\_nbPigments\_v5.ijm

This macro is written to quantify signal of one or several channels in the cell and/or in nucleus. The quantification is made on a maximum z-projection on slices chosen by the user

1/ Choose the image to treat: should be a multi-tiff, Z-stack, several colors, 1 time point; it is expected that the last channel is the nucleus (at least put one where the nucleus is visible to manually draw it), and the one before is the transmitted light channel (especially a minimum projection is applied on this channel).

* If you want the pigments to be counted (and that it appears in the final Results table), you should first launch the macro detectPigment\_generalisation\_v3 to detect the pigments

2/ if the ***image was treated by detectPigment\_generalisation\_v3***, there is (at least) one ROI zip; if several are present, the user is asked to choose the one to use for this analysis.   
If the image ***was not treated by this macro***, the user is asked to choose the slices of analysis (remove the slices with out of focus signal) and to draw the cells for analysis.

3/ For each channel, choose the quantification to compute and the label to appear in the Result table:

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

Le contenu généré par l’IA peut être incorrect.Une image contenant texte, Police, nombre, capture d’écran

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4/ For each cell, sequentially, an automatic method is applied to detect the nucleus in the last channel in the current cell; this uses the MaxEntropy Threshold method [1]. If only one ROI is detected, it is shown to the user for validation, if 0 or more than 1, the user is asked to draw the nucleus (you can check the cell ROI in the ROI Manager), do not forget to add to the Manager:

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

Le contenu généré par l’IA peut être incorrect.

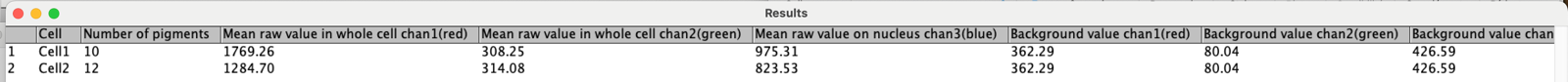
5/ Draw a square representing background (same ROI used for **all** channels):

Une image contenant texte, capture d’écran, logiciel, Système d’exploitation

Le contenu généré par l’IA peut être incorrect.

[1] KAPUR, Jagat Narain, SAHOO, Prasanna K., et WONG, Andrew KC. A new method for gray-level picture thresholding using the entropy of the histogram. *Computer vision, graphics, and image processing*, 1985, vol. 29, no 3, p. 273-285.

6/ The macro computes the Results table according to your choice in step 3/; the quantification is made on the Maximum z-projection and the “Mean” value is extracted; the raw value as well as the subtraction with the background value estimated in step 5/ is displayed:



This result table as well as the ROIs cells/nuclei are saved at the end of the macro.