

CounZoox Manual

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I. Goal

Quantify the concentration of Zooxanthellae automatically, assuming we have a fluorescent staining (here we used chlorophyll autofluorescence) and that the sample is in a Mallassez cell (having a depth of 200µm).

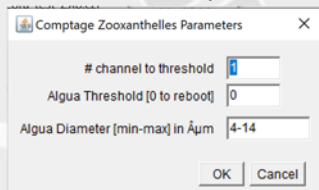
II. Download

Program, tutorial and demo image are available here :

<https://github.com/SebastienSchaub/CounZoox>

III. How to Use

1. Open ImageJ
2. Plugins > Macros > Install... and select the CounZoox.ijm
3. Open an image.
4. [9] to init the variables and the result table "MySummary"
5. [0] to define the parameters



- The channel number has to be used to detect algae (or nucleus, etc...)
 - Fluorescence threshold to use for the set of images. If "0" is selected, the macro will propose automatically a value (see...)
 - Alga diameter is a range ValMin-ValMax. It's important to keep the "-" between the 2 values.
6. [1] to start the measure.
If the threshold is set at "0", then the program let you see the suggested threshold and the effect with the possibility to change it:



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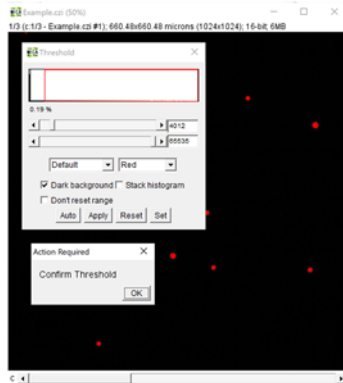
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Plateforme d'Imagerie par Microscopie (PIM)



"Confirm Threshold" when you get the good value.

7. The macro provides:

- table "Results" provide data per alga (or nucleus, etc...) in particular Area (in μm^2)
- it adds a line to the table "MySummary" with :
 - **Directory:** directory of the image
 - **Filename:** of the image
 - **N.Alga :** the absolute number of algae
 - **<Alga Area>:** the mean surface (in μm^2) of the algae
 - **AA+/-:** the standard deviation of surface (in μm^2) of the algae
 - **<Chlorophyll>:** mean of intensity (integration) per algae. Measured in fluorescence intensity.
 - **Chl +/-:** the standard deviation of chlorophyll intensity
 - **C.Alga per mm^2 :** Concentration of algae per mm^2 . Useful if the depth of the sample is unknown
 - **C.Alga per mm^3 :** Concentration of algae per mm^3 based on the ChamberThickness parameter (0.2 for Mallassez cell)
 - **Est Vol mm^3 :** the total volume analyzed.
 - **ThreshAlga:** the threshold value for chlorophyll signal. Useful to reapply the same value.
 - **CamSize:** the number of pixel in the image (Width x Height)
 - **PxlSize:** the pixel size read from the images

8. When the parameters are correctly defined, start "[2] Batch Measure"

- The macro requires the heir folder of the images.
- Then the macro will analyze all images of all subfolders (if any) and concate the results in the table "MySummary".
- At the end, the macro saves the table "MySummary" in the heir folder root as "MySummary.csv"



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