AdipoQ User Guide - QuickStart

Input – fluorescently labeled cultured cells

You may have used different methods to acquire your images. AdipoQ Preparator accepts the following files:

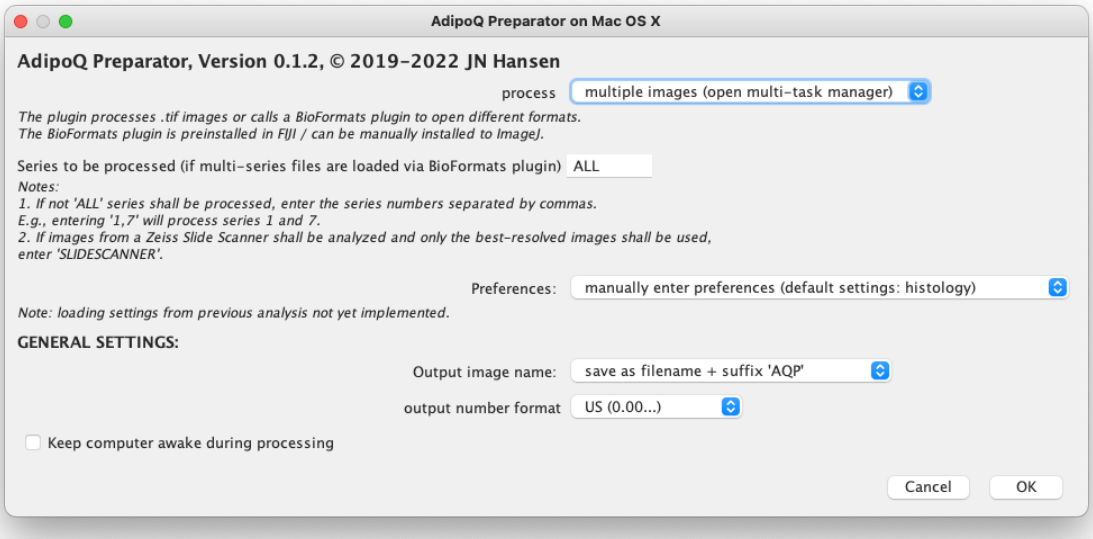
- a **microscope image data file** containing several images, image-stacks, tile images, time-lapse, etc.
- or a single **tiff image file**

Of note: AdipoQ allows to process 2D images, but not 3D image stacks. However, you can convert a 3D image into a 2D image. For example, if you would like to extract the sharpest plane of a stack in a fully automated manner, see https://github.com/hansenjn/ExtractSharpestPlane_JNH.

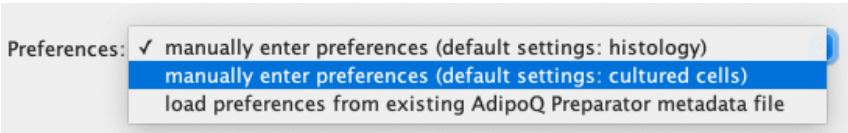
1. AdipoQ Preparator: Preprocess images to segment individual channels

AdipoQ Preparator is used to prepare individual channels for analysis with AdipoQ by applying an intensity threshold to specific channels (a process called "segmentation").

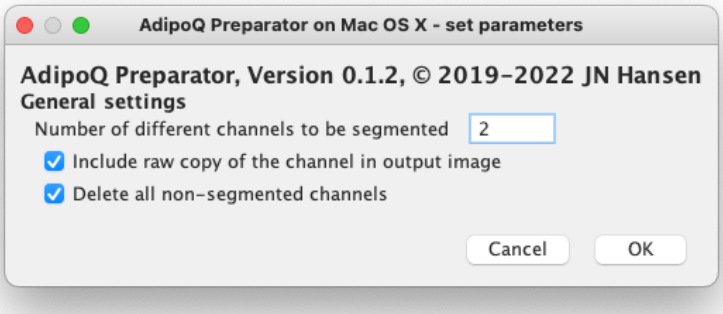
Launch it via **PLUGINS > ADIPOQ > 1. ADIPOQ PREPARATOR V...**



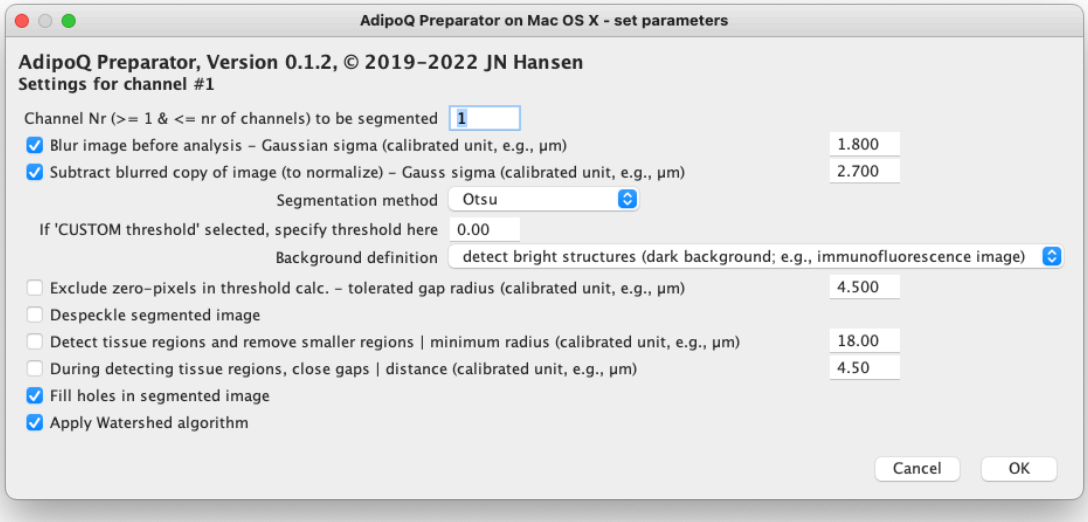
a) Select default settings for cultured cells



b) Select how many channels shall be segmented. If your image file contains both the nucleus and the lipid droplet image, you can analyze them simultaneously by entering in AdipoQ preparator that you want to segment 2 different channels.



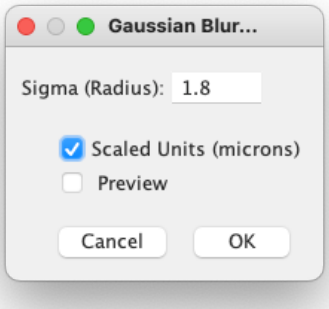
c) A dialog will pop up where you can change the parameters for analysis for your first channel (labeled *Settings for channel #1*), e.g., the channel representing the nuclei. If you want to work with the default settings, you can just press okay.



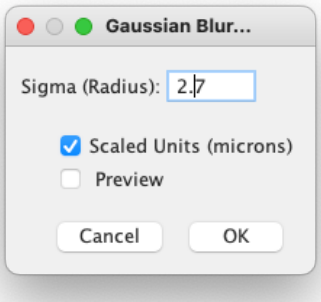
d) *If you have selected to segment two channels, there will be a second dialog popping up labeled with "Settings for channel #2".*

Before analyzing a whole data set, it is recommended that you manually apply the settings to an exemplary image to see whether you may need to optimize the parameters for your data set. For example, changing and optimizing the parameters is necessary if you use a different input file format, a different dye, or a different imaging setup (a different microscope or the same microscope but a different magnification). See the example for a more in-depth workflow.

1. Duplicate image of the channel of interest so you have **two** copies of your channel of interest: IMAGE > DUPLICATE
2. Blur **image 1** PROCESS > FILTERS > GAUSSIAN BLUR…; e.g., 1.8 (and select "Scaled units")



3. Blur **image 2** (pick a sigma that is larger than the first) e.g., 2.7



4. **Subtract** Blurred copy of **image 2** from **image 1:** PROCESS > IMAGE CALCULATOR: Operation: subtract; Create new window; 32-bit (float) result
5. Apply a **threshold**. IMAGE > ADJUST > THRESHOLD
    - Nuclei: Li, Mean, Otsu
    - Lipid droplets: Otsu, Moments, or calculate a 'CUSTOM threshold' in a region of the image without cells

Now: check the image. Does the intensity threshold allow to accurately detect the structure of interest (e.g., nuclei, or droplets)? If yes, test this for at least one other image of your data set. If no, change settings until you observe a satisfactory detection.

After identifying the optimal settings, adjust the parameters accordingly in AdipoQ preparator and run the analysis for your whole data set.

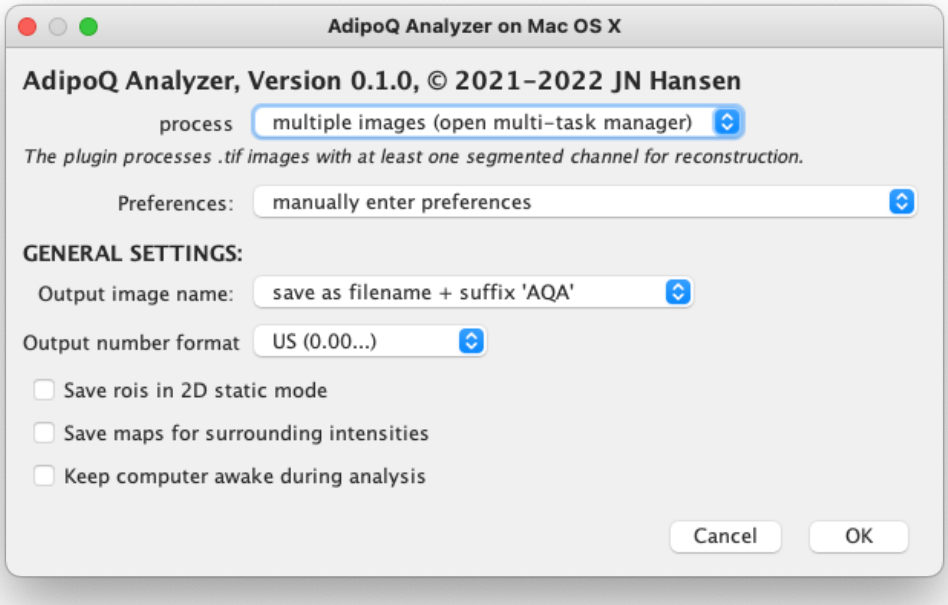
Output File:	_AQP.tif	New file that contains the original and the segmented image
	_AQP.txt	Metadata file that describes the input settings

2. AdipoQ: Quantification of images

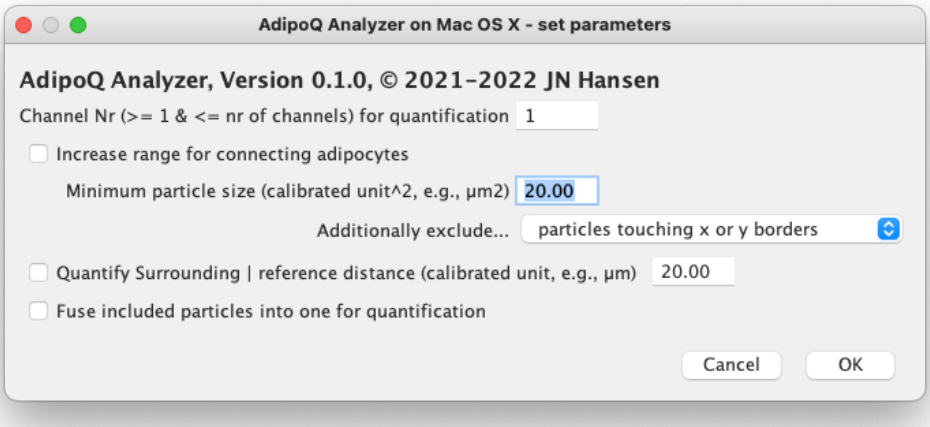
AdipoQ is used to quantify the segmented images and output results tables

a) If you have segmented two channels (e.g., nuclei and droplets): Let the analyzer run separately for each segmented channel (the best option is to duplicate the AQP.tif and split them into two folders, e.g., nuclei and lipid droplet image).

b) Launch AdipoQ Analyzer via PLUGINS > ADIPOQ > 2. ADIPOQ ANALYZER.



c) Press OK to manually enter your preferences.



d) Chose a minimum particle size to exclude small objects like dirt from your analysis (e.g., 4 µm^2).

e) Important: For Additionally exclude… choose “nothing”

Output files:	_AQP_AQA_IDs.zip	IDs of objects (ROIs) in a zip file (can be loaded to ImageJ to overlay on an image by drag and drop)
	_AQP_AQA_RP.tif	A copy of the input image also containing labels of the detected objects.
	_AQP_AQA.txt	A text file containing the analysis settings and results.
	_AQP_AQAs.txt	A text file containing only the results lines from the file, including the table caption.

3. Explore data sets in a post-hoc analysis

To batch-process data sets created by AdipoQ, have a look at the R scripts that we provide in the AdipoQ GitHub repository.
The tab-delimited text file containing only the results lines, including the table caption (_AQAs.txt), can be readily read in for a programming-based post-hoc analysis (Python, R, MATLAB) or into table calculation software for a manual post-hoc analysis (e.g., Microsoft Excel).

Further information:
Please refer to the AdipoQ User Guide for a detailed and in-depth explanation of the AdipoQ Preparator and Analyzer tool.

Please refer to the exemplary guide for a walk-through guide with images.