AdipoQ User Guide - QuickStart

Input – for histological stainings using hematoxylin and eosin

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

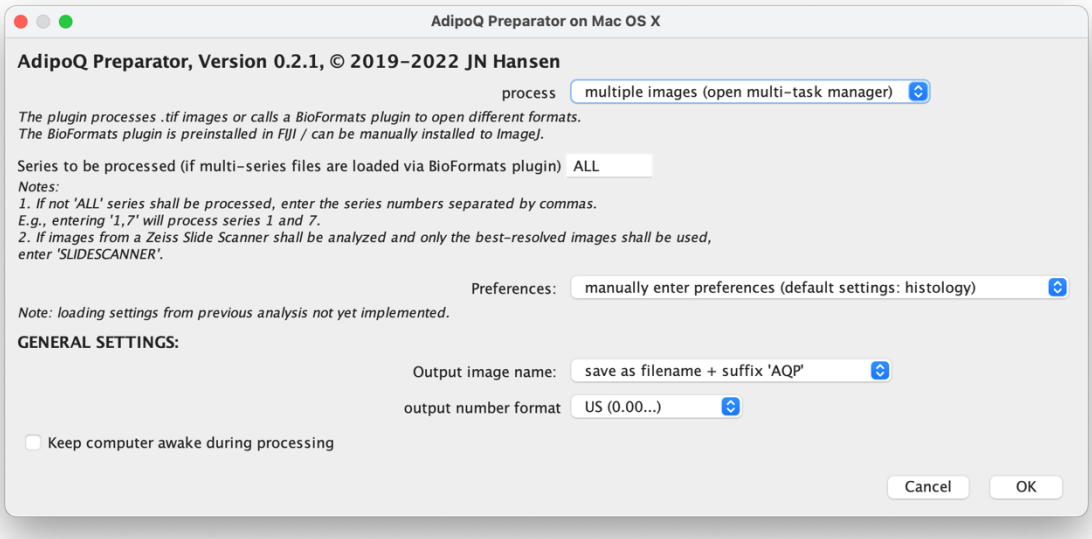
- a **microscope image data file** containing several images, image-stacks, tile images, time-lapse, etc.
- or a single **tiff image file**

Of note: 8-bit or 16-bit single-channel tiff files are sufficient. An RGB image can also be analyzed – here, we recommend using the green channel (the channel number for a green channel in an RGB image is number 2).
AdipoQ allows analysis of 2D images, but not 3D stack images.

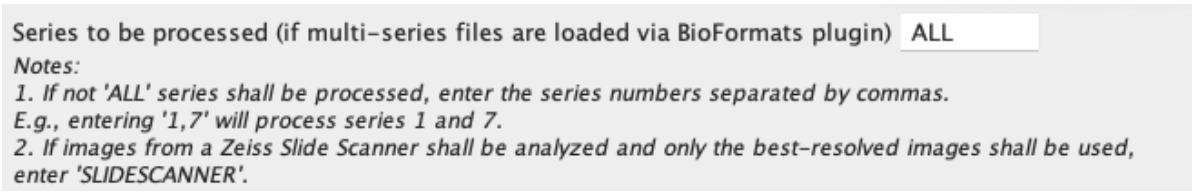
1. AdipoQ Preparator: Preprocess images to segment individual channels

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**

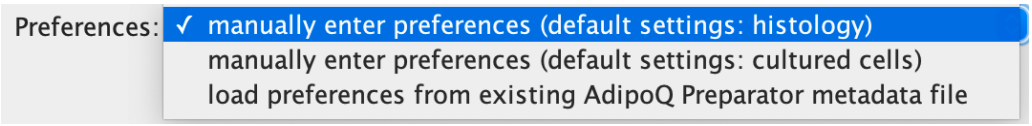


a) Select series to be processed (this option is only important analyzing a microscope image data file containing several images)

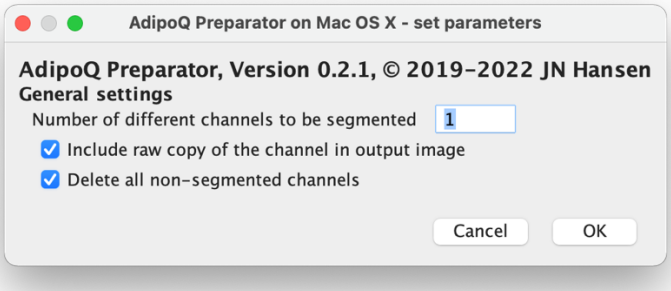


If not 'ALL' series shall be processed you can specify which series to be analyzed. Slidescanner: In the "czi" file format generated by Zeiss Microscopes (e.g., a Zeiss slidescanners), the images are stored at multiple resolutions. If you would like to extract the highest resolution image of a ".czi-file", select SLIDESCANNER.

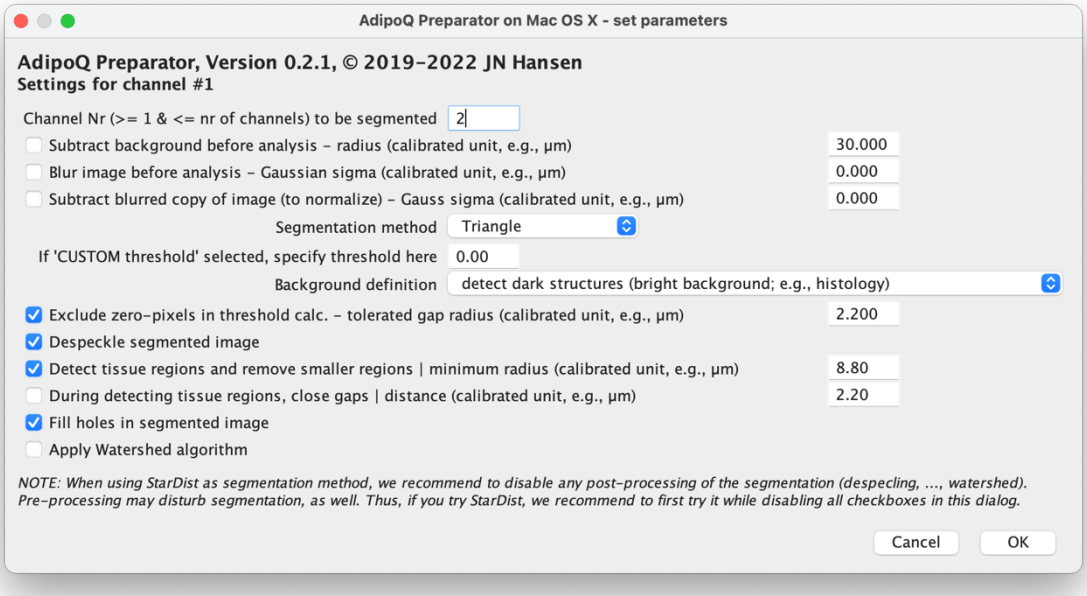
b) Select default settings for histology



c) Select how many channels shall be segmented. For histology, this is usually just 1 channel.



d) A dialog will pop up, in which you can change the parameters to analyze the first channel (labeled *Settings for channel #1*). Select the channel to be segmented: The green channel of an RGB image is the channel number 2, if you use a single-channel image, enter channel number 1.



e) If you have selected to segment two channels, there will be another dialog popping up after this one labeled with "Settings for channel #2" (For histological stainings, segmenting other channels usually only makes sense if you have additional fluorescent channels that you plan to analyze in addition).

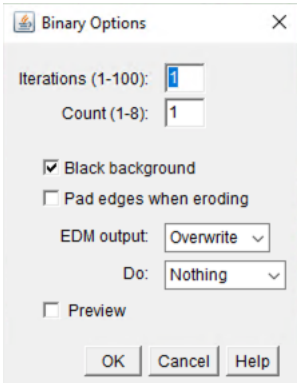
f) Before analyzing a whole data set, it is recommended that you test the settings using an exemplary image to test whether you may need to optimize the parameters for your data set. For example, changing and optimizing the parameters is necessary if you use a different input file format or a different dye. You may test different settings on the exemplary image, e.g., different threshold algorithms or introducing a blur to smooth the image.

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

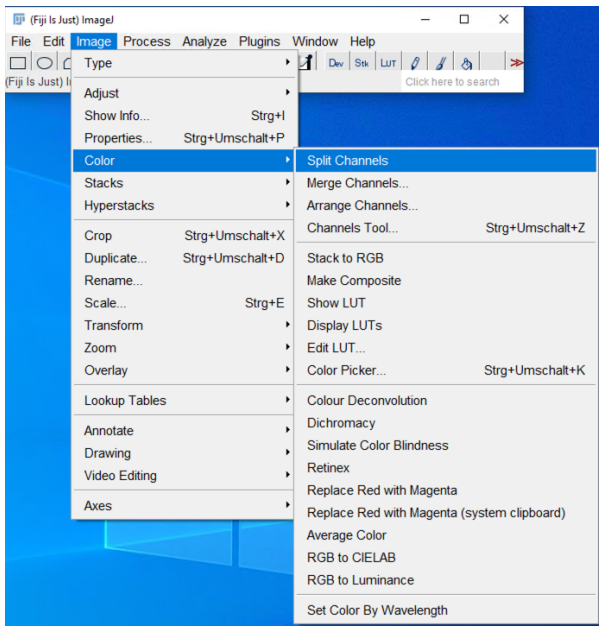
Output File: _AQP.tif New file that contains the original and the segmented image.
_AQP.txt Metadata file that describes the input settings.

h) Open the new segmented file _AQP.tif to manually scrutinize the success of the segmentation and correct errors.

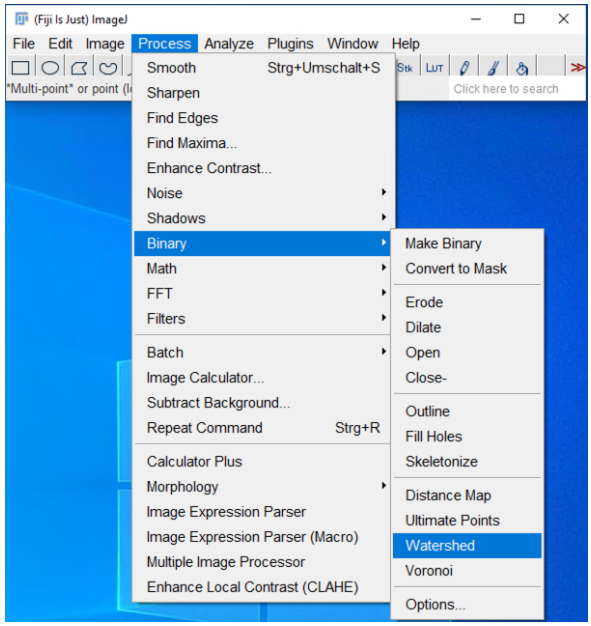
i) Manually delete areas that are not adipocytes, such as empty spaces between tissue areas or vessels. This can be done by using the common ROI tools in ImageJ to draw a region of interest that you want to delete (one of these tools  or the wand tool  , which allows to automatically select a whole detected region) and then press "Delete" on the key board. For more information, see the Walk-Through-Guide.
Note: This correction approach will only succeed if the following setting is set as follows: Check via **PROCESS -> BINARY -> OPTIONS** that Black Background is selected) (in case foreground and background are switched, reverse them)



j) It may be helpful to additionally add a watershed step to better separate neighbored adipocytes:
First, split channels: Go to **IMAGE -> COLOR -> SPLIT CHANNELS** and select the C1 image (segmented channel, shows only black and green if created from an RGB image)



Second, go to **PROCESS -> BINARY -> WATERSHED**



Third, merge channels back together by going to **IMAGE -> COLOR -> MERGE CHANNELS** and save the resulting image as a new file: e.g. _AQP1.tiff.

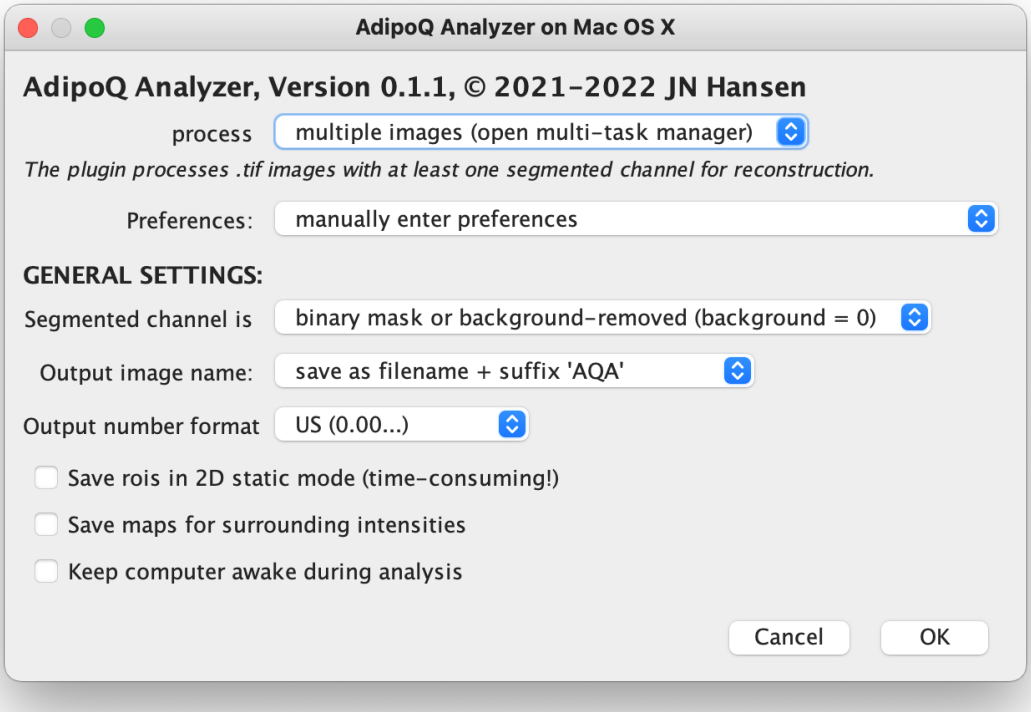
Note 1: The Watershed step can also be automatically done by AdipoQ Preparator (see Watershed option in the settings dialog). We however noticed that for images with a lot of none adipocyte structures (e.g., large blood vessels), it is easier to first remove the non-adipocyte structures manually and then, run the Watershed algorithm, since before the Watershed algorithm such regions can be much easier selected automatically with the wand tool than after running the Watershed algorithm, which splits such regions into many small particles.

Note 2: If you want to run a more automated analysis, you may consider to omit this step and instead later filter the analysis results of AdipoQ Analyzer, from which you could remove detected objects with a high Asphericity index and/or unusual area.

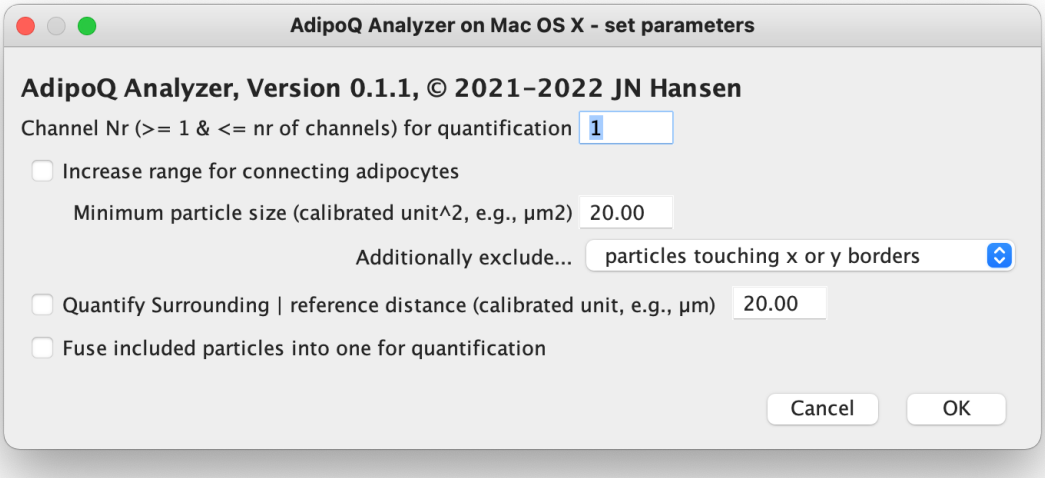
2. AdipoQ: Quantification of images

AdipoQ is used to quantify the area of the individual objects in segmented images.

a) Launch AdipoQ Analyzer via **PLUGINS > ADIPOQ > 2. ADIPOQ ANALYZER**.



b) Press OK to manually enter your preferences.



c) Chose a minimum particle size to exclude small objects like dirt (e.g., the default 20 µm^2)) from your analysis.

Output files:	_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
_AQP_AQA_IDs.zip	IDs of objects (ROIs) in a zip file (can be loaded to ImageJ by drag and drop into the ImageJ window – the ROIs will then overlay the opened image and will be accessible in the ROI Manager)

3. Explore data sets in a post-hoc analysis

Import _AQP_AQAs.txt to excel: Column J “Area [micron^2]” displays the area of each detected particle in your image.

Further information:
Please refer to the AdipoQ User Guide for a detailed and in-depth explanation of the AdipoQ Preparator and Analyzer tool, as well as more sophisticated analysis functions.

Please refer to the example file for a walk-through guide using an exemplary image.