

# AdipoQ - walk-through guide for an exemplary image

## Input – For histological staining with hemalum and eosin

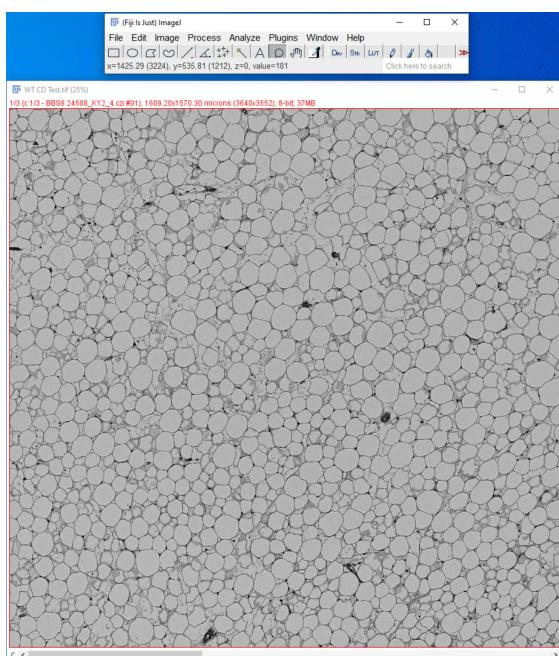
The exemplary image is an HE stained paraffin section of white adipose tissue from a wildtype mouse on normal diet. Image acquisition was performed with a Zeiss Axio Scan.Z1 Slide Scanner. Data are generated as .czi files in which the images are stored at multiple resolutions. AdipoQ Preparator has the option to directly handle this data and extract the highest quality image for analysis.

For simplicity, I manually selected and extracted the .tiff image and only selected a part to reduce the size of the image for quick analysis.

See “WT CD Test” as exemplary image:

This is an RGB image (three channels: red, green, and blue)

Open the image in ImageJ:

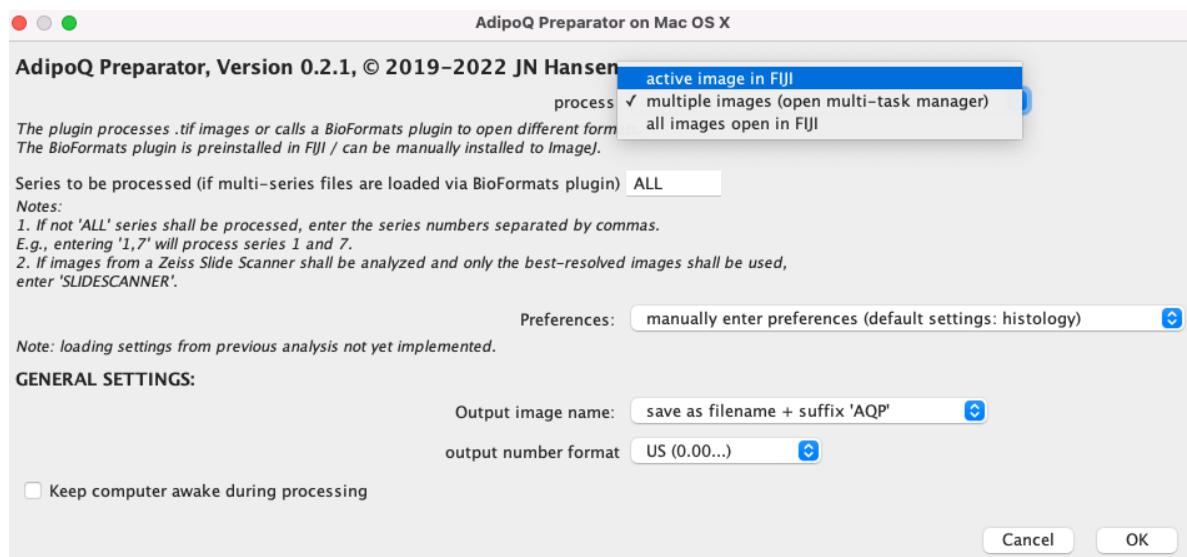


## 1. AdipoQ Preparator: Preprocess images to segment individual channels

*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**

Select process “active image in FIJI”.



a) Select series to be processed:

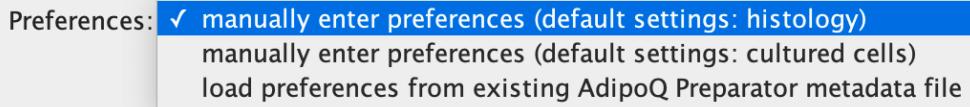
The exemplary image is a **single** tiff image file. The default setting 'ALL' is correct, since there are no other series to choose from.

Series to be processed (if multi-series files are loaded via BioFormats plugin) **ALL**

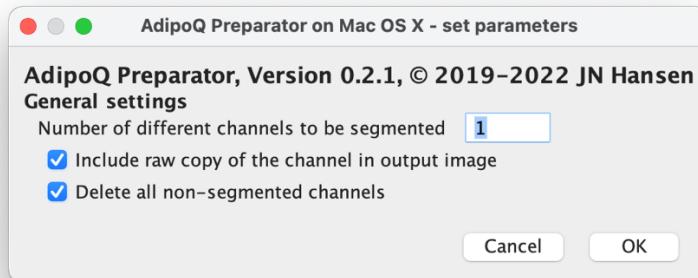
**Notes:**

1. If not 'ALL' series shall be processed, enter the series numbers separated by commas. E.g., entering '1,7' will process series 1 and 7.
2. If images from a Zeiss Slide Scanner shall be analyzed and only the best-resolved images shall be used, enter 'SLIDESCANNER'.

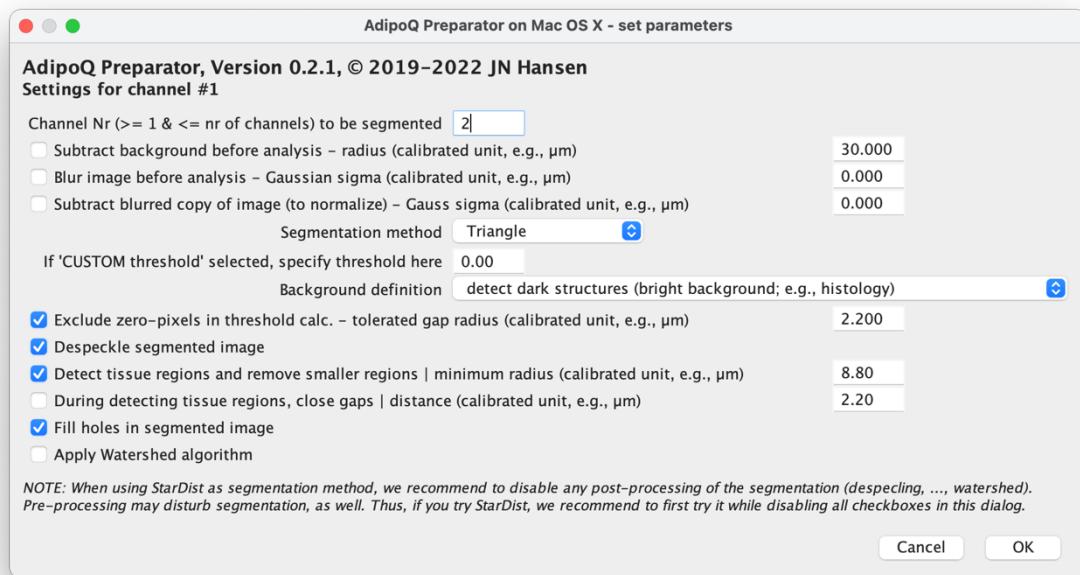
b) Select default settings for histology



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

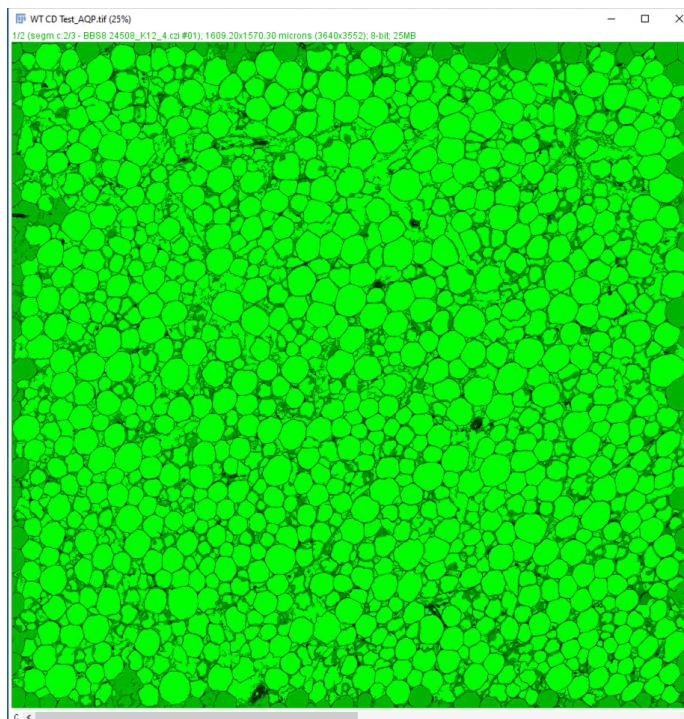


d) A dialog will pop up where you can change the parameters for analysis for your first channel (labeled *Settings for channel #1*). Select the channel to be segmented: In this exemplary image we want to choose the green channel of this RGB image, which is the channel number 2.



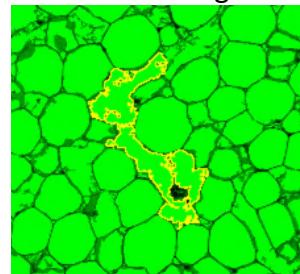
**Output File:** \_AQP.tif New file that contains the original and the segmented image  
\_AQP.txt Metadata file that describes the input settings

**Open \_AQP.tif file:** It is composed of two channels: 1 channel is the segmented channel, channel 2 is the original green channel  
All cells at the edge of the image are already excluded in the segmented channel indicated by the darker green color. So only complete cells will be analyzed.

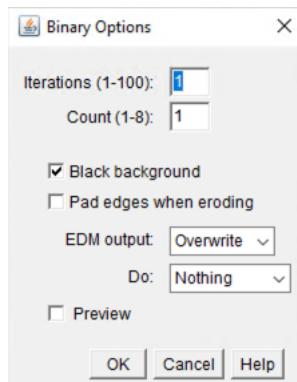


- e) Manually delete areas that are not adipocytes, such as empty spaces between tissue areas or vessels or other artifacts: Just select areas with the magic wand tool by

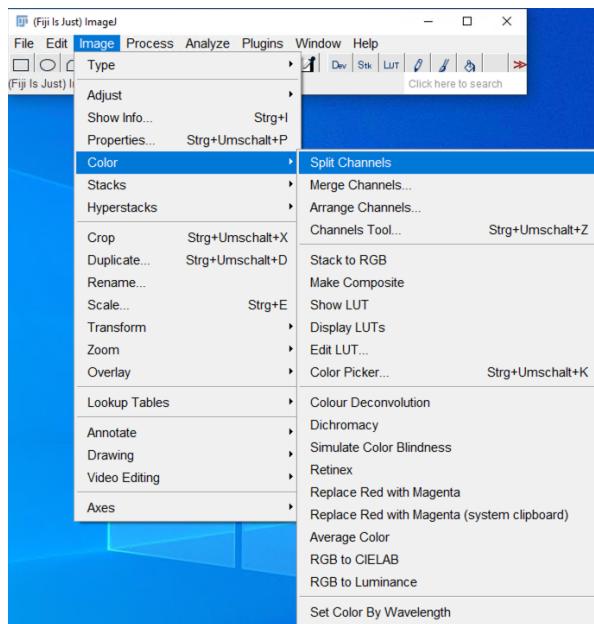
clicking on them (note: this only works if channel 1 is selected in your image – this is done by moving the channel bar below the image to the left) and delete.

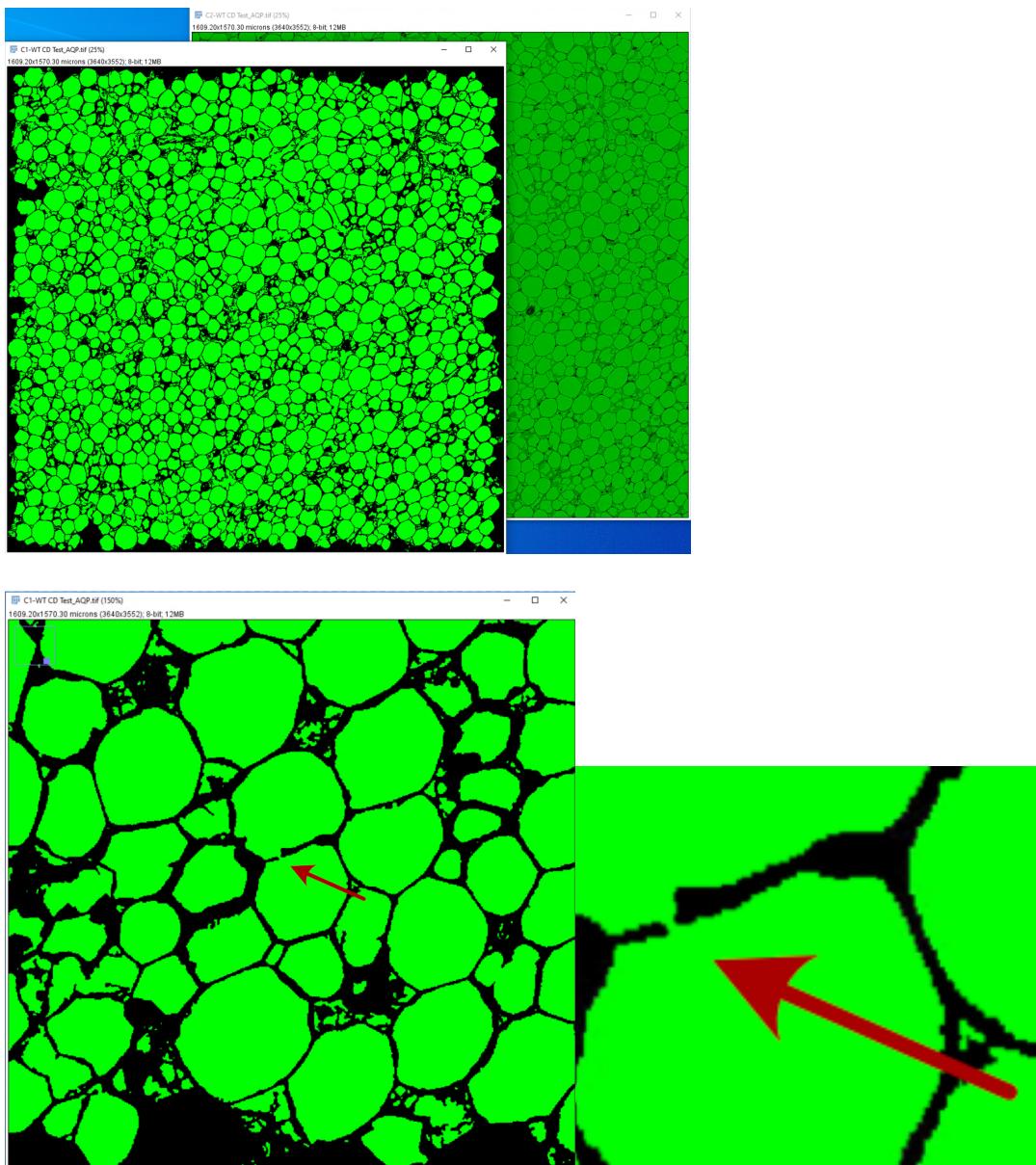


Note: This correction approach will only succeed if the following setting is set as follows: Check via **PROCESS -> BINARY -> OPTIONS** that Black Background is selected (in case foreground and background are switched, reverse them)



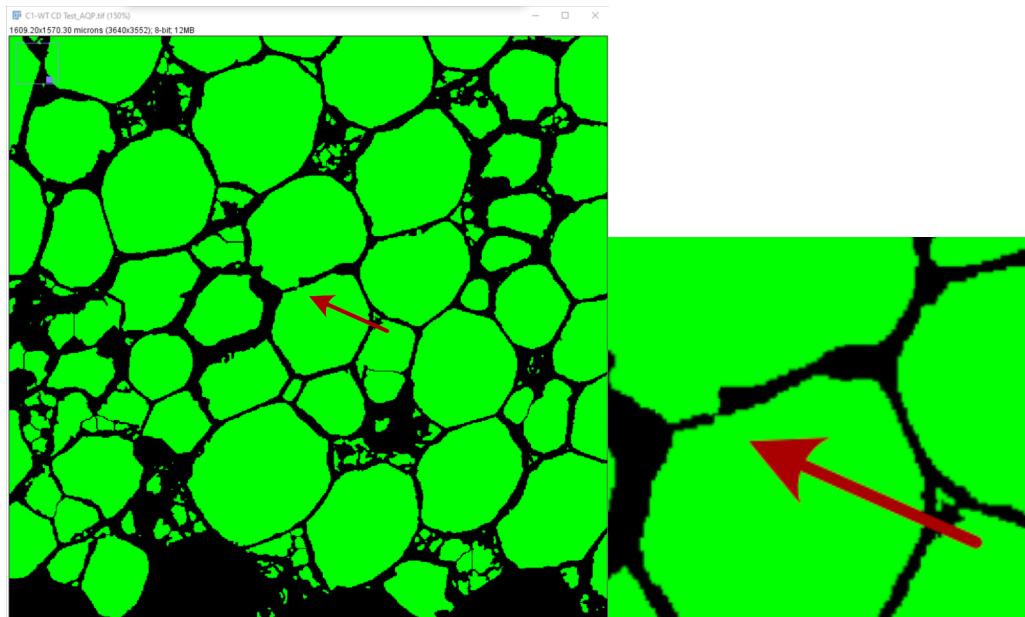
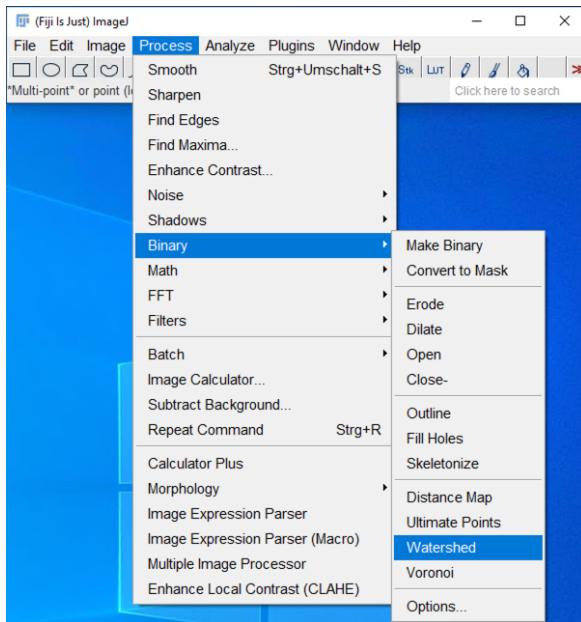
- f) We recommend to add a watershed step to better separate neighbored adipocytes: First, split channels: Go to **IMAGE -> COLOR -> SPLIT CHANNELS** and select the C1 image (segmented channel, shows only black and green if created from an RGB image)





Some cells might not be completely separated: Such an example is indicated by the red arrow. These gaps are hard to detect by a quick visual inspection. To fill these gaps and to correctly separate all cells add a watershed step. Images with a weak staining will more often display such non-separated cells.

Second, go to **PROCESS -> BINARY -> WATERSHED** This ensures complete separation of each cell.

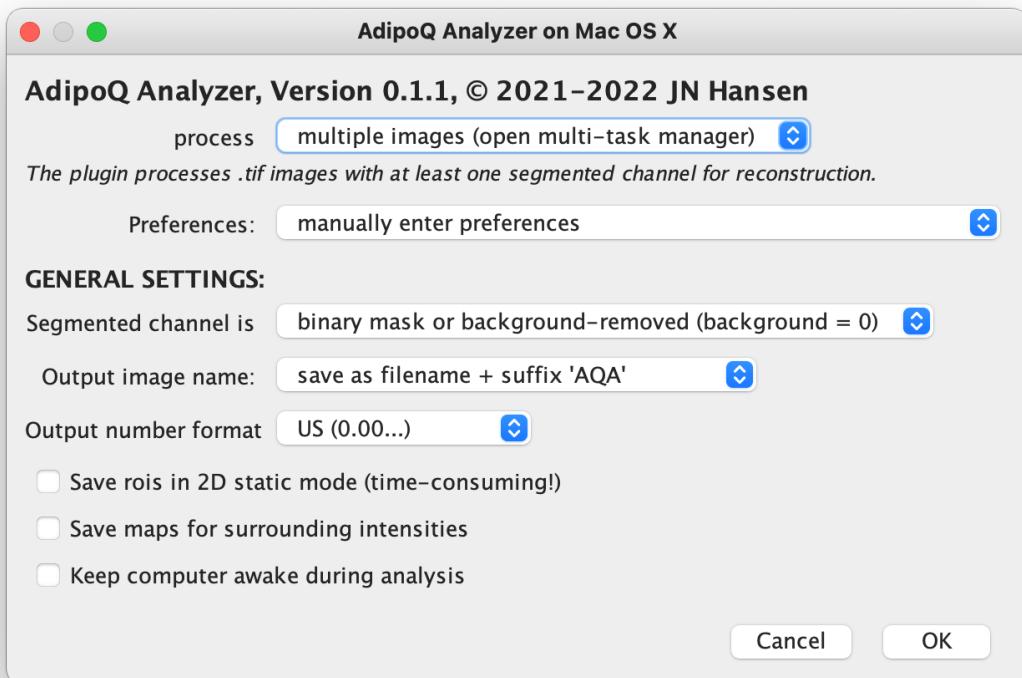


Third, merge channels back together by going to [IMAGE -> COLOR -> MERGE CHANNELS](#) and save the resulting image as a new file: e.g. \_AQP1.tiff.

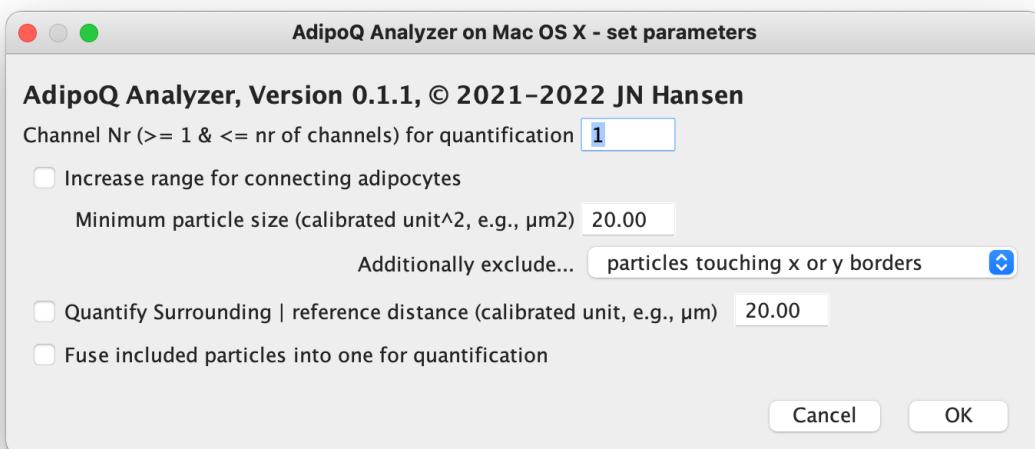
## 2. AdipoQ: Quantification of images

*AdipoQ is used to quantify the area of segmented images*

- a) Launch AdipoQ Analyzer via [PLUGINS > ADIPOQ > 2. ADIPOQ ANALYZER](#).



- b) Press OK to manually enter your preferences.



- c) Choose a minimum particle size to exclude small objects like dirt (e.g., 20  $\mu\text{m}^2$  (default)) from your analysis.

Output files:	<a href="#">_AQP_AQA_RP.tif</a>	A copy of the input image also containing labels of the detected objects
	<a href="#">_AQP_AQA.txt</a>	A text file containing the analysis settings and results
	<a href="#">_AQP_AQAs.txt</a>	A text file containing only the results lines from the file, including the table caption
	<a href="#">_AQP_AQA_IDs.zip</a>	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<sup>2</sup>]” displays the area of each detected particle in your image.

To explore data sets analyzed with AdipoQ programmatically, have a look at the R scripts that we provide in the AdipoQ GitHub repository.

#### **Further information:**

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