

# 3D Accounting and Measuring Plugin

v. 1.0.1

## Manual

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May 2016

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# 1 3D-AMP Manual

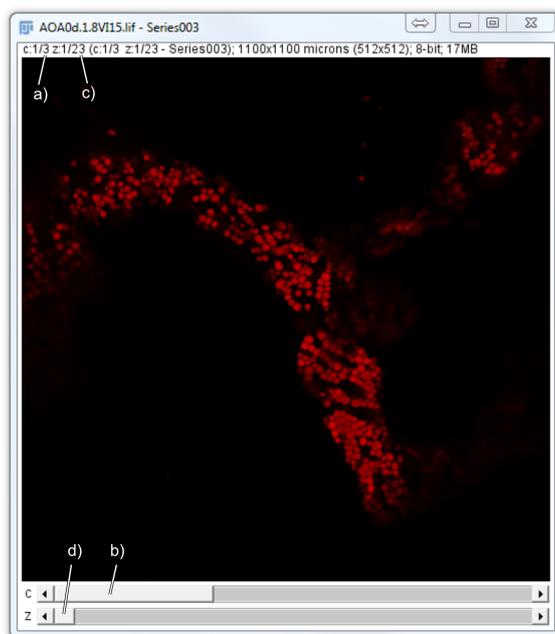
This plugin works with ImageJ (Abràmoff et al. 2004; <http://imagej.net>) and Fiji (Schindelin et al. 2012; <http://fiji.sc>). The manual refers to Fiji hereafter, but all steps are identical in ImageJ. You can use most kinds of (hyper)stacks with one or more channels in the common image formats.

## 1.1 Installation of 3D-AMP

Paste the file 3D-AMP\_1-0-0.ijm to the `\plugins\Macros` folder of your Fiji directory.

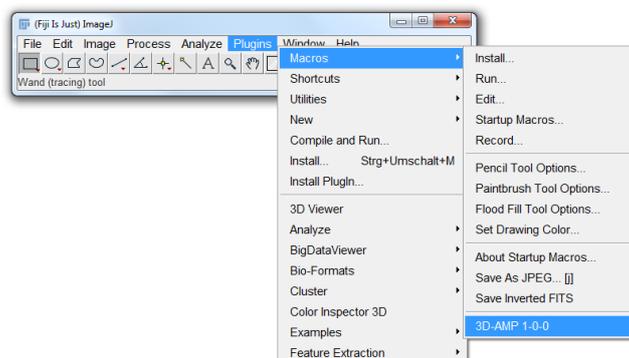
## 1.2 Working with 3D-AMP

- Start Fiji.
- Open the stack you want to analyze. The example in *Fig. 1.1* shows a hyperstack with 3 channels (*Fig.1.1a*) through which we can scroll with the upper scroll bar (*Fig.1.1b*) and 23 images per channel (*Fig.1.1c*) through which we can scroll with the lower scroll bar (*Fig.1.1d*).



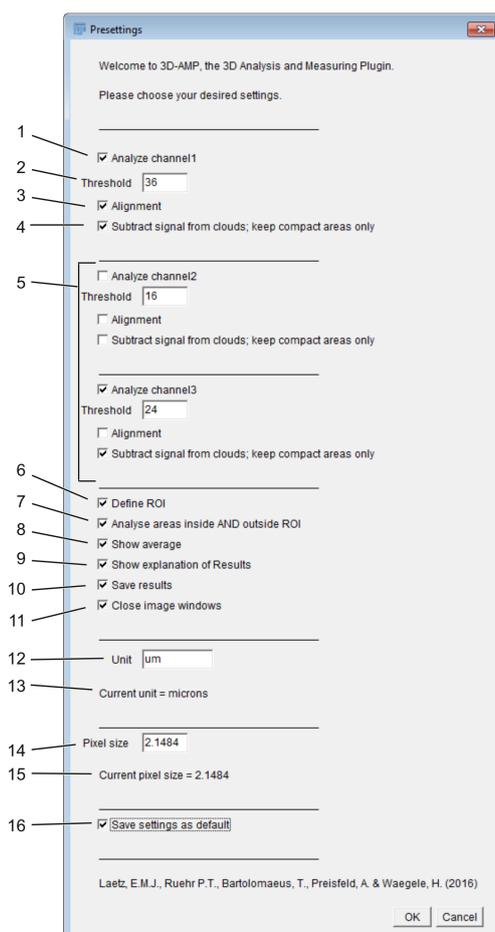
**Figure 1.1:** Stack window with **a)** number of channels, **b)** slide bar for switching between channels, **c)** number of images in each channel, and **d)** slide bar to scroll through images of current channel.

- To start the script, go to *Plugins* → *Macros* → *3D-AMP 1-0-0* in the ImageJ menu (*Fig.1.2*).



**Figure 1.2:** How to access 3D-AMP from the Fiji menu.

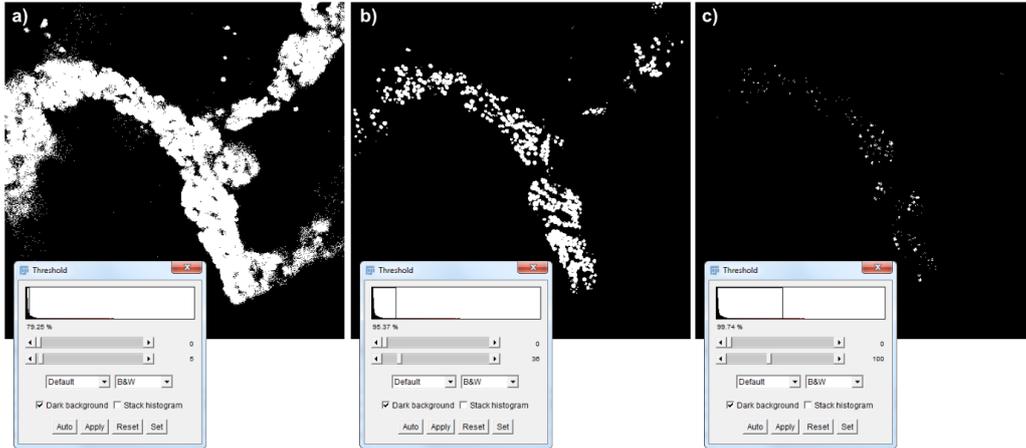
- In the first dialogue, choose your desired presettings (*Fig.1.3*) for each channel as follows:



**Figure 1.3:** Presettings of 3D-AMP. For further information refer to main text.

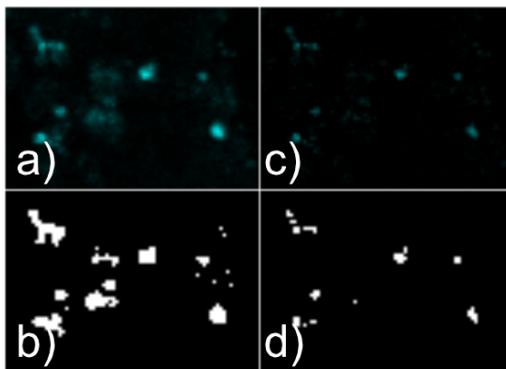
- 1) Check to analyze this channel, uncheck to skip this channel during analysis.

- 2) Set the desired threshold to filter the pixels that you want to be counted. *Fig.1.4b*) shows a suitable threshold for analyzing chloroplasts, while the threshold in *Fig.1.4a*) is set too low and in *Fig.1.4c*) too high. You have to manually find a good threshold for each channel you want to analyze by using the preview of the threshold function at *Image* → *Adjust* → *Threshold...* in the Fiji menu.



**Figure 1.4:** Different thresholds applied to the same image: **a)** 5, **b)** 36, **c)** 100.

- 3) If the stack needs alignment, check this box and the StackReg algorithm (transformation=Affine; Thévanaz et al. 1998) will be applied.
- 4) The background subtraction can be used for filtering out signal from blurry data points, such as in the lysosome image in Fig.1.5. The exemplary result of this process (background subtraction, rolling ball radius = 1 px) is shown in *c*). *b*) and *d*) show the thresholded versions of *a*) and *d*), respectively. Please note that this process differs from the ordinary function of the background subtraction process (i.e., removing smooth continuous backgrounds from images) due to the small rolling ball radius.

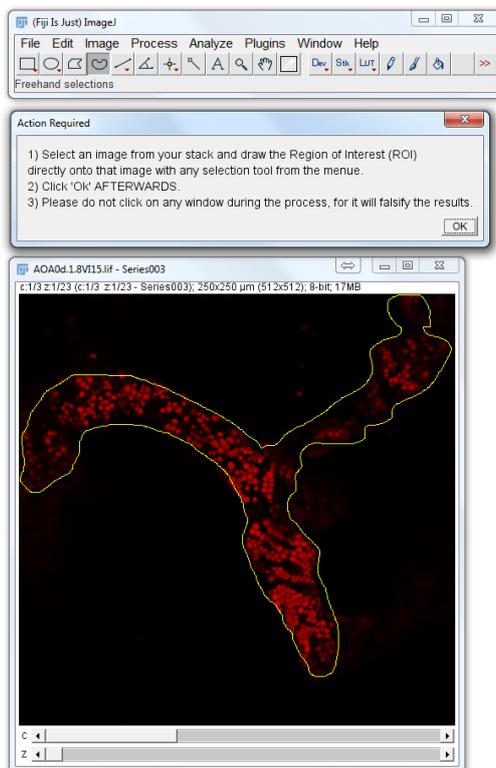


**Figure 1.5:** Subtraction of background for separating data from blurry background. **a)** and **b)** show data before background removal, **c)** and **d)** show data after background removal. **b)** and **d)** are thresholded (threshold = 24).

- 5) Change the settings for the remaining channels. If you have only one channel in your stack, you will not see any options here.

- 6) Check this box in order to not analyze the full image dimensions, but only a region of interest (ROI) which you can define later.
  - 7) Check this box if you would like to measure areas above threshold outside the ROI and compare them with those areas within the ROI. Leave unchecked to only measure data within ROI.
  - 8) Check this box to add a row in the results table showing the mean percentage of the area covered by pixels above the threshold and the sum of this area in unit<sup>2</sup>.
  - 9) Leave checked to show a dialogue after analysis that explains the results table.
  - 10) Check to open a dialogue that allows you to save the results table as an Excel chart (\*.xls). If you leave the box unchecked, you can still copy and paste the results table into another program after analysis.
  - 11) Leave this box checked to allow Fiji to close all windows that were created during the analysis process. This is highly recommended since it saves a lot of time.
  - 12) Define your desired unit here (ümför for  $\mu$ /microns).
  - 13) Shows the current unit of the images loaded.
  - 14) Type in the pixel size in the previously defined unit.
  - 15) Shows the current pixel size of the images loaded.
  - 16) Check to save the current settings of this dialogue as default settings. The settings will be saved as `3D-AMP_settings.txt` inside the `\plugins\Macros` folder. It is recommended to keep a backup of this file. At first launch of 3D or when `3D-AMP_settings.txt` has been deleted, the plugin will automatically create a new settings-file with empty settings.
- Click Ok.
  - Now select your ROI on an image of your choice from the stack. Use one of the selection tools from the Fiji menu, such as the Rectangular, Oval, Polygon, or Freehand (as in Fig.1.6) selection tool.
  - Click Ok in the Action required window to start the analysis. Avoid using Fiji and clicking any Fiji windows during the analysis. Feel free to use other software.
  - In the Log window you will find some information on your image stack and settings.
  - If you checked the Save results checkbox before (*Fig.1.3: 10*), you will be asked to type a name for the results file and then to choose the directory where you want to save the file.

- If you checked the Show average box, a dialogue will show you an explanation of the results table.



**Figure 1.6:** Select region of interest (ROI, yellow) with Freehand selection tool *before* clicking Ok.

- After the process, the ROI will still be visible on your original stack. Thus, if you want to restart the analysis with different settings, you can use the same ROI as before. If you want to draw a new ROI, use any selection tool, left click on your image next to the existing ROI to deselect it, and draw a new ROI.
- Make sure to save the results. Running 3D-AMP again will irretrievably close the results table.

### 1.3 References

ABRÀMOFF, M.D., MAGALHAES, P.J., RAM, S.J. (2004): Image Processing with ImageJ. *Biophotonics International* **11**(7): 36-42.

SCHINDELIN, J.; ARGANDA-CARRERAS, I.; FRISE, E.; KAYNIG, V.; LONGAIR, M.; PIETZSCH, T.; PREIBISCH, S.; RUEDEN, C.; SAALFELD, S.; SCHMID, B.; TINEVEZ, J.Y.; WHITE, D.J.; HARTENSTEIN, V.; ELICEIRI, K.; TOMANCAK, P.; CARDONA, A. (2012): Fiji: An Open-Source Platform for Biological-Image Analysis. *Nature Methods* **9**: 676-682.

THÉVENAZ, P.; RUTTIMANN, U.E.; UNSER, M. (1998): A Pyramid Approach to Subpixel Registration Based on Intensity. *IEEE Transactions on Image Processing* **7**(1): 27-41.