

AuTOCellQuant: Automated Tool for Objective Cell Quantification

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1 Introduction

AuTOCellQuant is an image analysis tool enabling objective cell quantification by blinded selection of Regions of Interest (ROIs) in one channel (e.g. showing blood vessels) while counting cells in a different channel (e.g. showing the cells of interest only). The macro therefore minimizes the risk of user-dependent selection bias. Quantification results are saved in terms of numerical values as well as images outlining the counted particles in the sample. Parameters calculated by AuTOCellQuant include cells per ROI, selected area size, ROI position and sample size marked by the borders of the concentric zones. The AuTOCellQuant macro is useful in fields such as ocular disease, infarct zone remodeling or any other setting where particles are counted and ROIs blindly selected in a different channel.

2 Installation

Download AuTOCellQuant to the plugins folder of ImageJ. Then, restart ImageJ. Start AuTOCellQuant by selecting Plugins -> AuTOCellQuant. AuTOCellQuant needs ImageJ 1.44o or later. In order to use the automated cell quantification method, the ImageJ plugin ITCN- 1_6 has to be downloaded to the plugin folder (<http://www.bioimage.ucsb.edu/downloads/automatic-nuclei-counter-plug-in-for-imagej>, 05/03/2012).

3 Usage

3.1 *Image- Requirements*

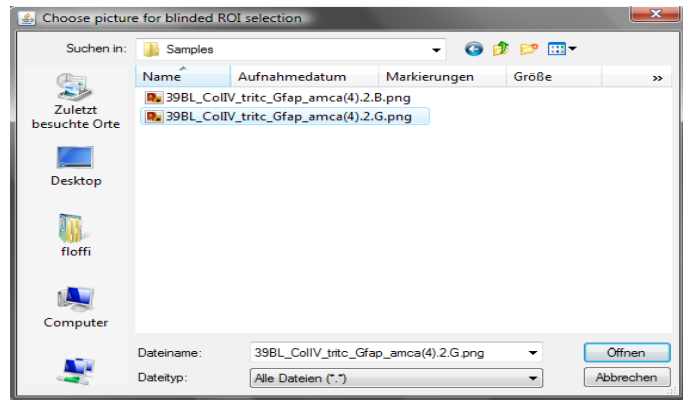
AuTOCellQuant is compatible with any of ImageJ's image file formats including JPEG, TIF (tagged image file format), PNG (portable network graphics) or BMP (Windows bitmap). Sample images though have to be 8 bit grey-scale images. If images are of other types (16 bit or 32 bit), they have to be converted to 8 bit grey-scale format before loading to the AuTOCellQuant. Color images such as

RGB must be split into separate channels (Image -> Color -> Split channels) to get 8 bit grey-scale images.

3.2 Processing an image

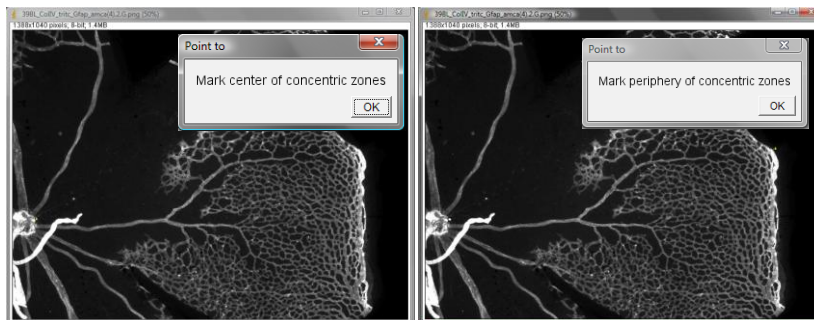
After starting the macro under Plugins -> AuTOCellQuant, two windows appear to choose the images for ROI selection and cell quantification, respectively.

The macro will create a subfolder called “Results” in which the evaluated images and numerical counting results are saved. In the subsequent



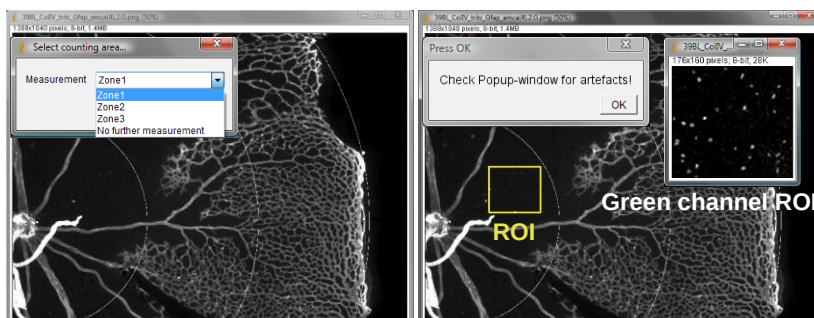
window, parameters such as quantification method or ROI size may be adjusted (for further details see 3.3).

Next, the image for ROI selection is presented. Popup-windows guide the user to mark the center and the border of the evaluation area. Based on these marks,



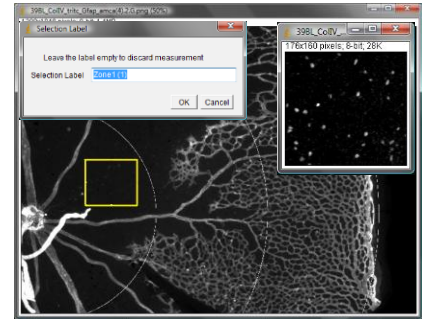
concentric lines are drawn dividing the sample into multiple zones. The number of zones may be adjusted (see

paragraph 3.3). The user chooses the zone in which the first ROI will be selected (this step is important for labeling purpose in the result image), and plots a ROI in

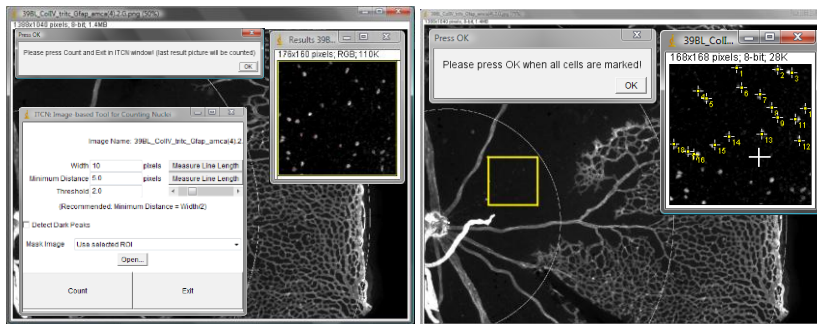


that area. The ROI is going to be adjusted to a standardized size (in pixels) predefined in the setup window

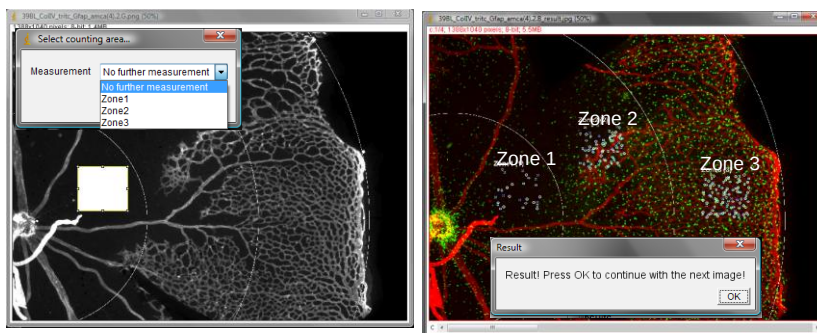
(for adaption see paragraph 3.3). Then, the ROI is projected onto the quantification image. This part of the quantification image is then presented to the user in order to screen for sample artefacts that could distort the quantification process.



In the subsequent steps, the cell quantification process is carried out in the selected area. The AuTOCellQuant macro offers multiple cell counting methods (for further details, see paragraph 3.3).



After the counting process is finished, the user is taken back to the dialogue window to select the next ROI. The ROI selection process can be repeated as often as necessary. In order to avoid double quantification, the areas already



evaluated are marked by a color. If the evaluation of one image is finished, “No further measurement” is chosen. The result

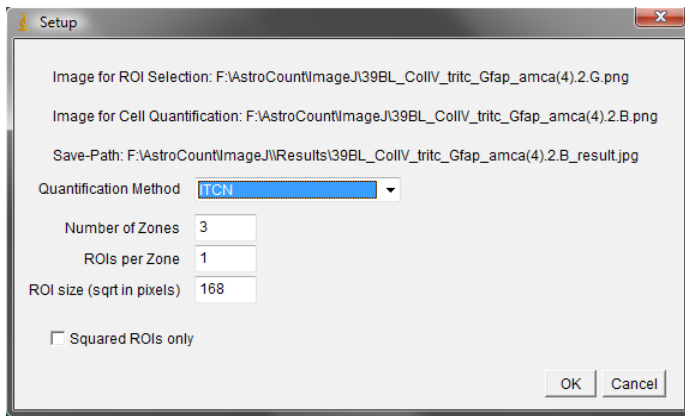
image with the outlined cells appears and after approval the macro returns to the dialogue window for selection of the next sample images.

The numerical data are provided in table format in a .csv file that is easily imported into a data analysis program. The labels of the columns are: name of image evaluated, zone, count, 3 columns to be removed, size of selected area,

distance from center to ROI, radius of evaluation area. Please note that these columns are only valid in the case a newly downloaded ImageJ is used. Other use of this ImageJ may alter the column settings.

3.3 *Setting parameters*

Several parameters may be adapted in the setup window:



Quantification method:

The AuTOCellQuant Macro offers multiple cell counting methods. The user may choose between an automated cell selection process based on the ITCN plugin or manual cell selection.

If images of good quality, that is high solution and contrast, are to be processed, automated cell quantification is recommended. In ITCN, the user adjusts the width, minimum distance of the cells of interest and threshold in order to optimize the specificity of the quantification process. If the cell selection process under the chosen parameters is insufficient, the parameters may immediately be readjusted and the selection process repeated by pressing “Count” in the ITCN-window. Only the last quantification result will be saved. If the user prefers to use the Auto-Threshold method of ImageJ, the ITCN (with AutoTreshold) method may be chosen. In this case, the AutoThreshold-function is applied to generate a binary image which is used for cell counting. The manual cell counting process is recommended for images of low quality or highly complex cell selection criteria.

Number of zones:

The user may choose the number of zones the sample is divided into.

Number of ROIs per zone:

The user may determine the number of ROIs per zone.

Size of ROIs:

The size of ROIs can be adjusted by the user. The value to be chosen is the square root of the ROI area in pixels. If all ROIs are supposed to be quadratic, the box “Squared ROIs only” has to be checked. Otherwise ROIs will be of rectangular shape.