User Manual for:

Granule Analyzer

by Markus Pieth

1. Starting the program

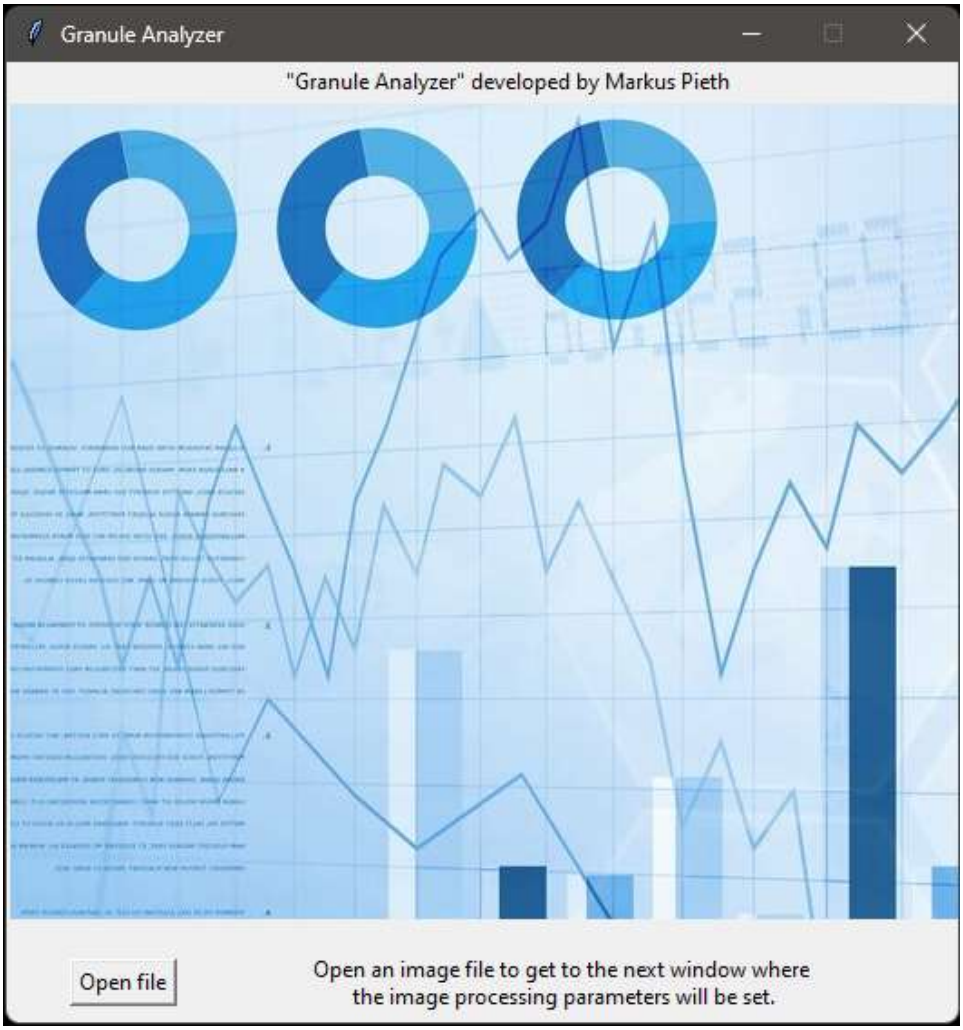


Fig. 1: Window after starting the program for opening a file

After having the program opened and started, this window (Fig.1) will appear demanding to open an image file to be processed and analysed in following steps.
By clicking on “Open file” a dialogue window pops up which allows to browse the local storages, open and load the image file searched for in the program’s environment.

2. Put in image associated information

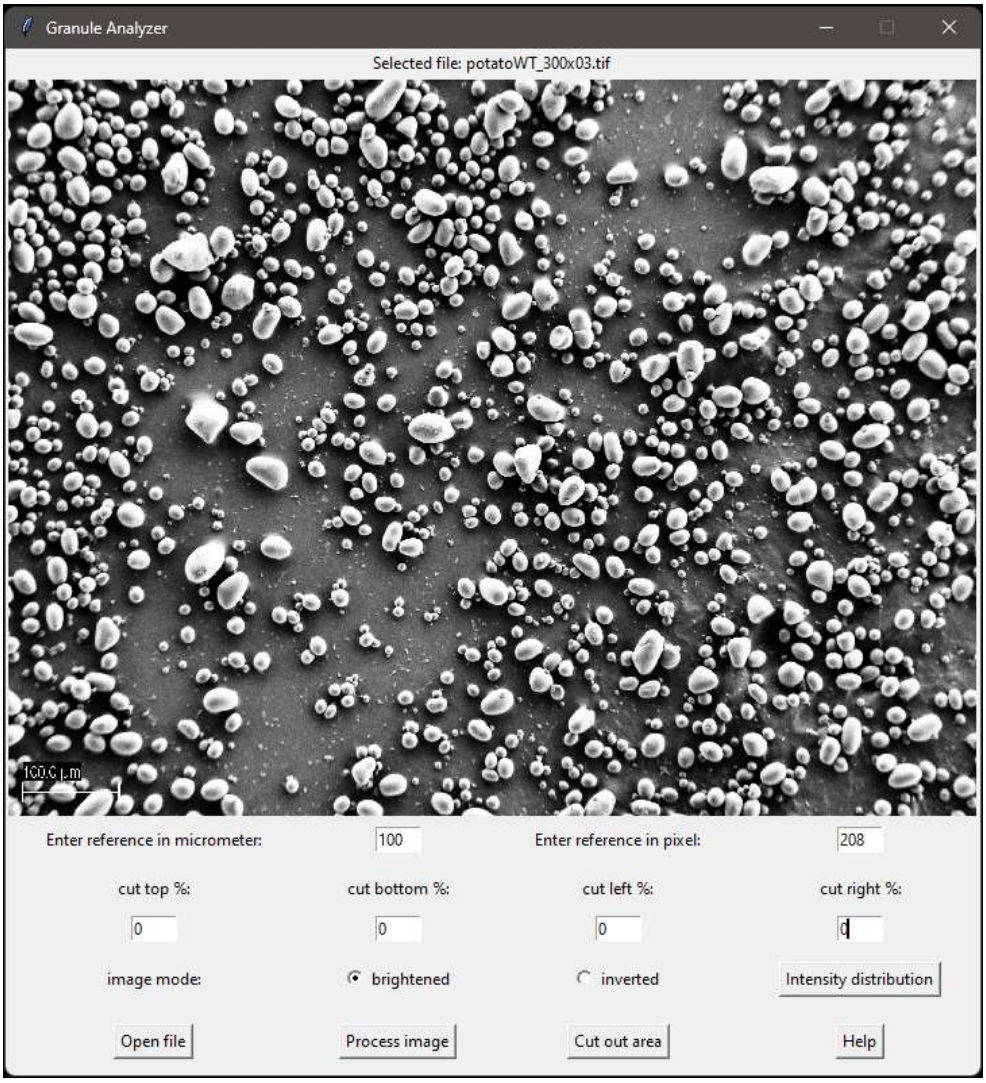
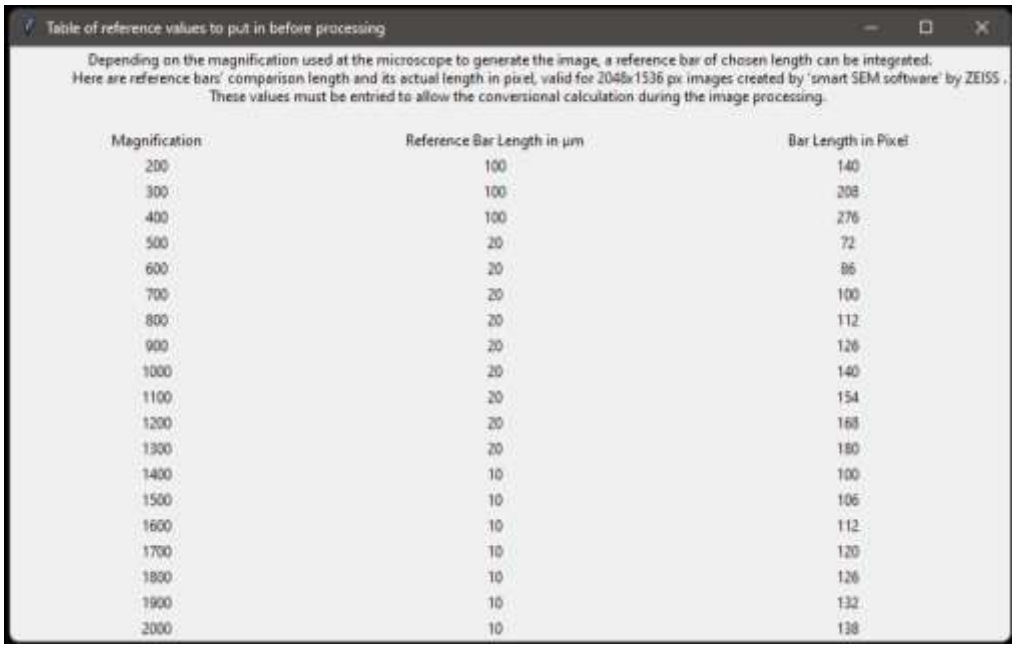


Fig. 2: Window with preview of chosen image (brightened mode), entry fields for size references, image mode and cutting parameters and buttons for calling different functions

At the top of the new window (Fig. 2) the name of the opened file is written to check whether the right file was opened. Right below the chosen image is shown in a reduced size. Under the image, two compulsory entry fields are given: the left one must be filled with a reference length (should be available in the image scale information) in µm, in this case 100 µm, and the right one must get a number of pixels that correspond to the reference length in the image. Here 100 µm in the image correspond to 208 pixels.
If only a part of the image is needed to be processed and analysed the next four entry fields can be used to cut a percentage off the top, bottom, left or right side respectively. When using this cut-out-function all four entries need to be filled, just typing a 0 for not cutting on one side. After the values for cutting are set “Cut out area” must be clicked and the image will be cut and overwritten, so the cut image can be checked. Just in case it was cut off too much, the initial state can be restored by typing four times 0 into the entry fields before clicking “Cut out area” again. For reducing the part to be cut away on only one side the respective value can be reduced and the difference to the former setting is added back to the image after having clicked “Cut out area”.

The next important information is the image mode, “brightened” like the one above (see Fig. 2) or “inverted” like further below, by marking the associated radio button.
In the last row “Open file” will open a dialogue window for opening another image file again and loading it into the program.
“Process image” leads to the next step and initiated the first processing of the image, cut or not, where refinements in the analysis settings can be done.
Clicking on “Help” opens a separate table (Fig. 3) with reference values in µm and pixels for images taken with a resolution of 2048 x 1536 pixel depending on the magnification used at the electron microscope. The magnification is divided into steps of 100 units, so it is recommended to take images using these magnification values to ease the use of the program.



Magnification	Reference Bar Length in µm	Bar Length in Pixel
200	100	140
300	100	208
400	100	276
500	20	72
600	20	86
700	20	100
800	20	112
900	20	126
1000	20	140
1100	20	154
1200	20	168
1300	20	180
1400	10	100
1500	10	106
1600	10	112
1700	10	120
1800	10	126
1900	10	132
2000	10	138

Fig. 3: Table with reference values necessary for the algorithmic conversion from pixel into micrometers and statistic calculations

“Intensity distribution” leads to another new window (Fig.4) showing a histogram of the pixel intensities’ distributions.

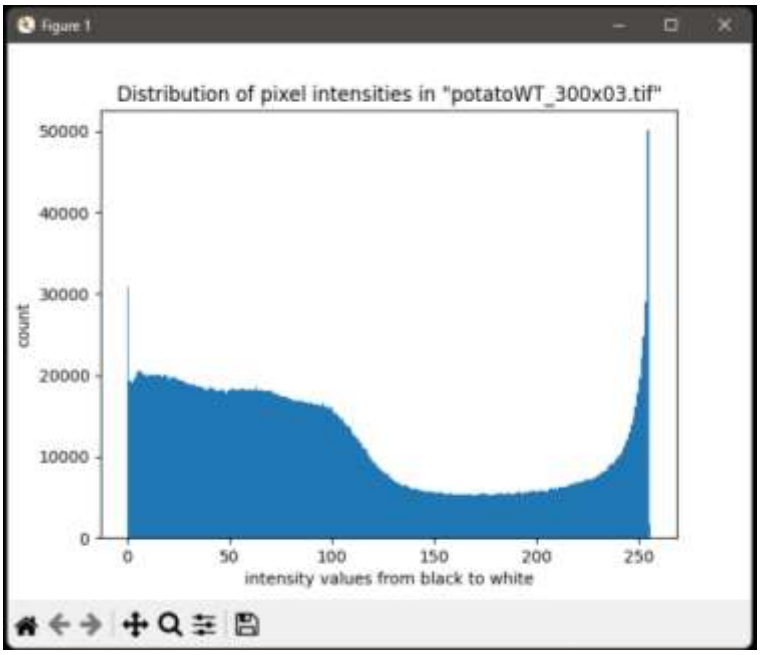


Fig. 4: Histogram showing the distribution of pixel intensities within the opened gray-scaled image (brightened mode)

The diagram depicts the count of pixels per intensity from 0 to 255 and is meant to help getting an idea of how well the algorithm can analyse the image. A clearly distinguishable bimodal distribution with a clear valley between the two maxima is desired as it confirms an apparent contrast between fore- and background.

Below (Fig. 5 and Fig. 6) is an example for an inverted electron microscope image shown.

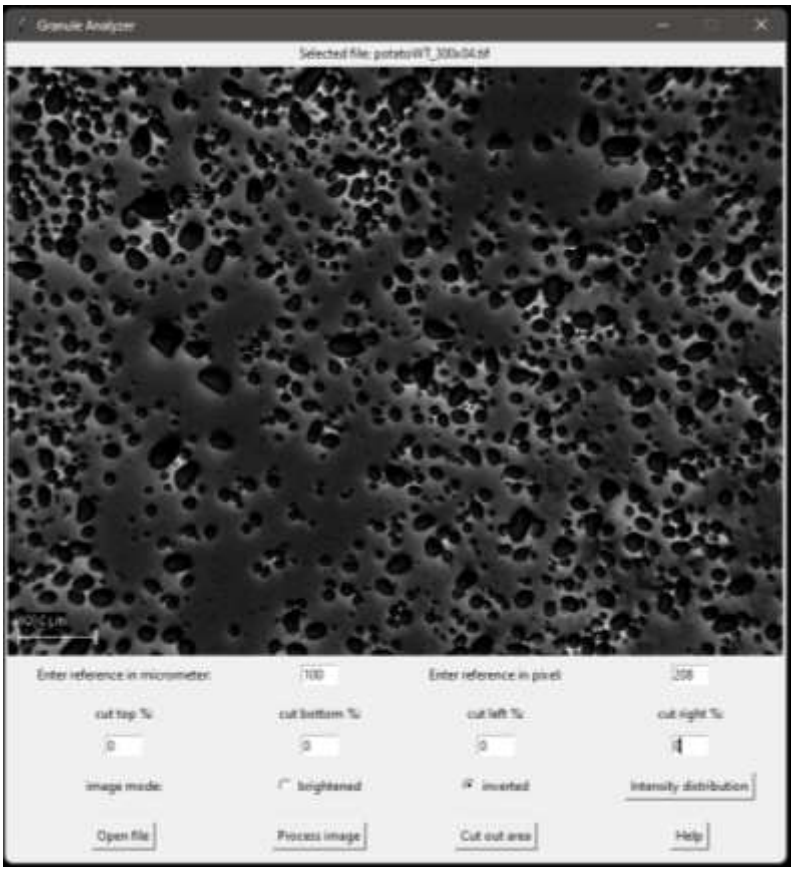


Fig. 5: Window with preview of chosen image (inverted mode), entry fields for size references, image mode and cutting parameters and buttons for calling different functions

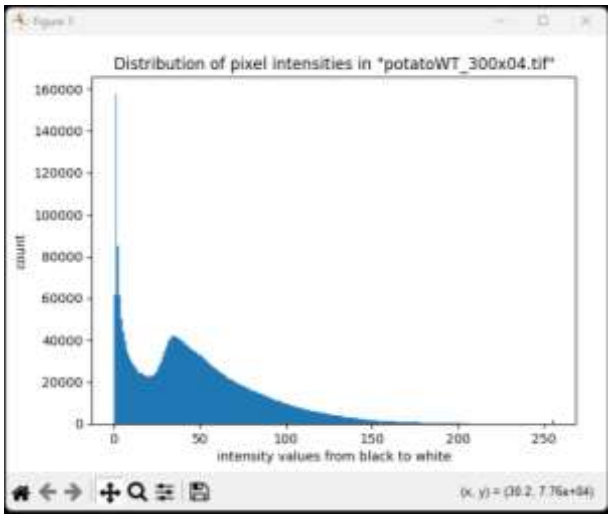


Fig. 6: Histogram showing the distribution of pixel intensities within the opened grey-scaled image (inverted mode)

3. Refinement of processing parameters

At next, after clicking “Process image” (Fig. 2 or Fig. 5) two new windows appear showing the results of the first rough image processing (Fig. 7 and Fig. 8) and a third with button and entry fields for refining the processing parameters (Fig. 9).

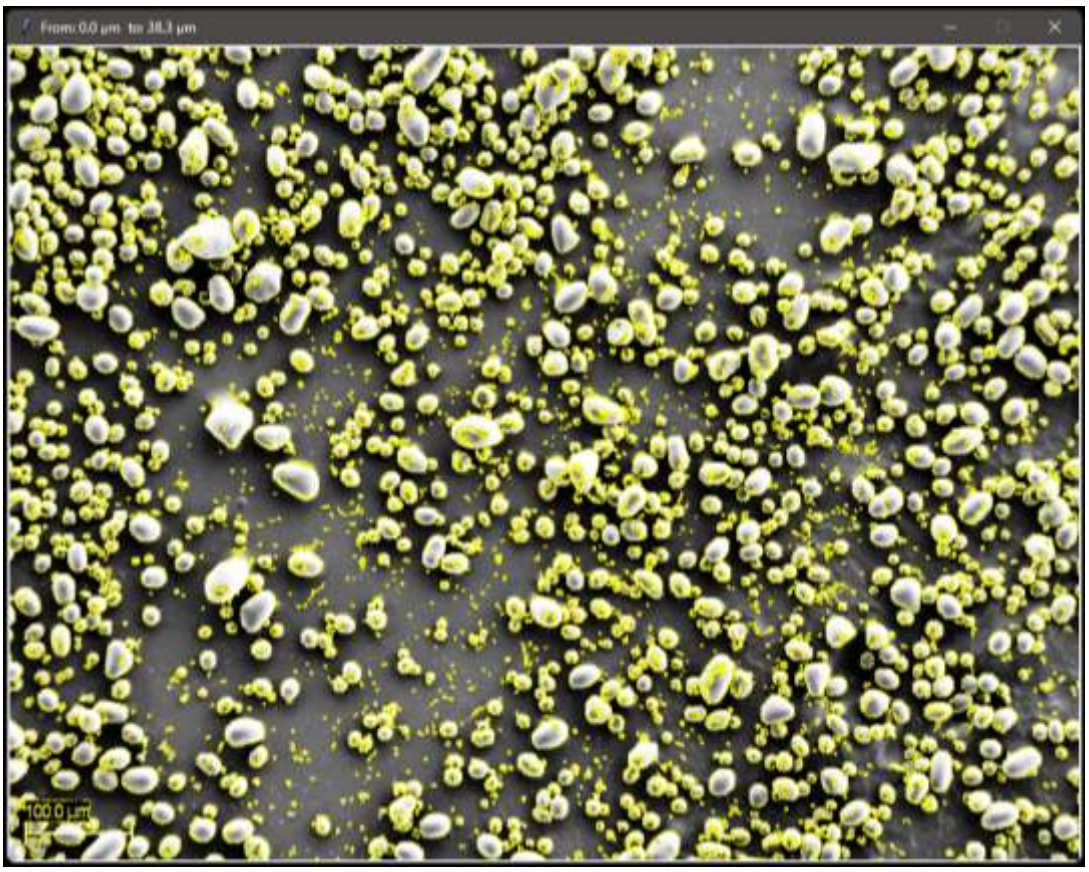


Fig. 7: Feedback window depicting the detected granule outlines matching the preset processing parameters in yellow

This window (Fig. 7) gives an impression of contours detected using the preset parameters. All outlines of structures that meet the parameter’s requirements are drawn in yellow. The second window will show diagrams of the distributions of average granule diameter, granule length and width as histograms with counted values and probability curve along with two vertical lines indicating median and mean of the distributions.

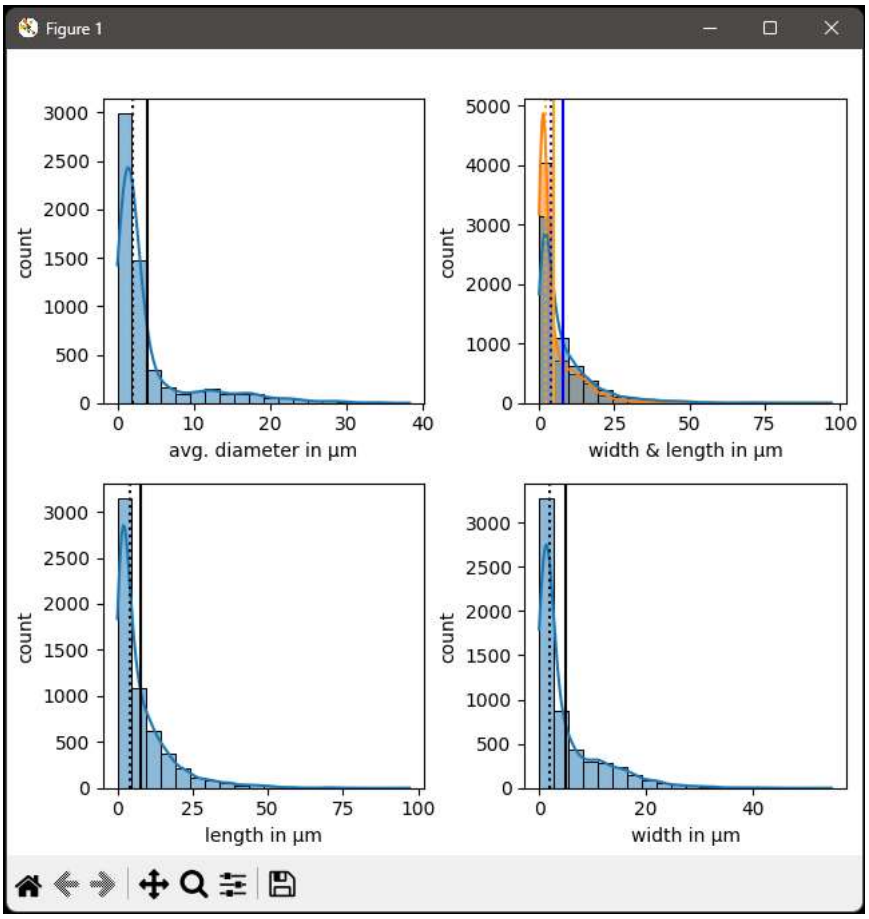


Fig. 8: Window with Histograms showing the distributions of the granule measurements after initial image processing and analysis

Initially, the histograms (Fig. 8) are depicted with a bin number of 20. At the bottom there are some buttons for resetting the depiction after altering something (house), moving through the zoomed in diagram (cross arrows), zooming into a diagram area (magnifying glass), edit the arrangement of diagrams in the window (controllers) and saving the edited diagrams to the computer storage (floppy disk) for later usage or publication.

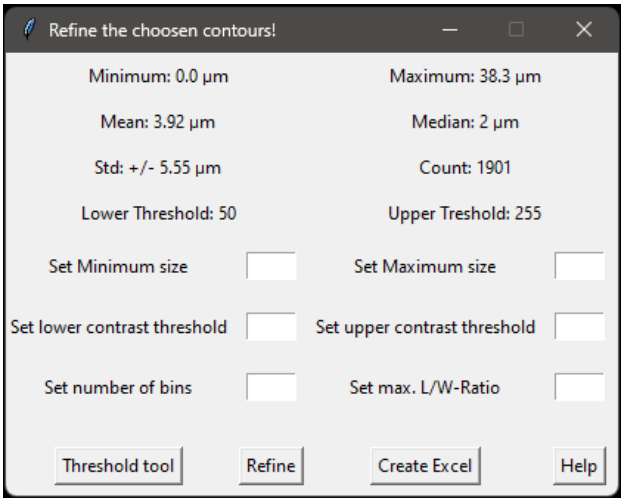


Fig. 9: Window for refining the image processing and analysing parameters

The third window (Fig. 9) appearing after the first rough processing shows at the top part the descriptive statistics of the initial analysis to give information about the photographed granule sample. The preset threshold values for the pixel intensities are chosen in a way that the image containing the yellow contours drawn onto is not to overcrowded by contours most likely not intended to be taken into consideration.
For filtering and refining the initially found contours six entry fields must be filled in. Both upper entry fields, at the middle of the refinement window, are used for setting the minimal and maximal granule size that shall be considered and sorted by the algorithm. The number of bins is important for the histograms and in how many columns the size range chosen shall be divided. As the algorithm measures the longest possible diameter of a contour or granule outline as its length and orthogonally to that its width a ratio approximating the granules ellipsoidal nature is calculated. The lowest possible length to width ratio is 1 meaning perfect circular shape. And the higher the value for “L/W-Ratio” is set the more elongated the filtered contours are allowed to be for taken into analysis later on.
After having filled in the desired values “Refine” must be clicked to reprocess the image and therefore causing new statistics according to the chosen filters. Clicking “Help” will open a window providing short information about the parameter setting and the histograms.
In the second and third entry fields threshold values for the pixel intensities must be given. To ease this, an auxiliary tool can be opened by clicking on “Threshold tool” (Fig. 10 and Fig. 11).

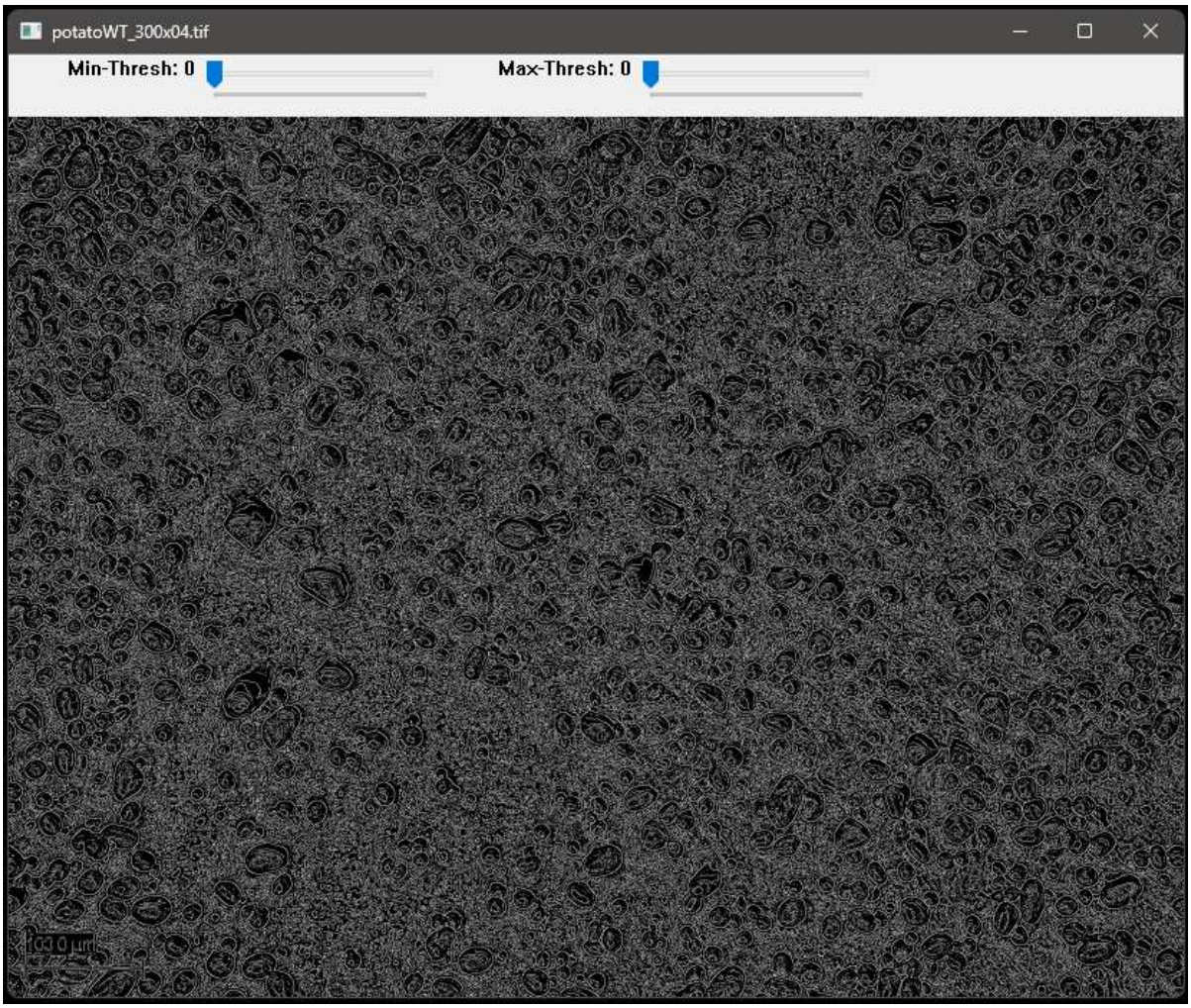


Fig. 10: Auxiliary “Threshold tool” for finding lower and upper threshold values (pixel intensities) of granule outlines to be considered in algorithmic analysis before having thresholds set

This “Threshold tool” (Fig. 10 and Fig. 11) can help to find a well matching pair of threshold values to begin with and being entried into the refinement window’s respective fields. Two controllers at the top of the window allowing to set the lower and upper threshold values and the real time feedback panel below will adapt automatically to the chosen values and showing which detected outlines after the processing of the image using the thresholds set are left to be considered in the following analysis of sizes and their statistics.

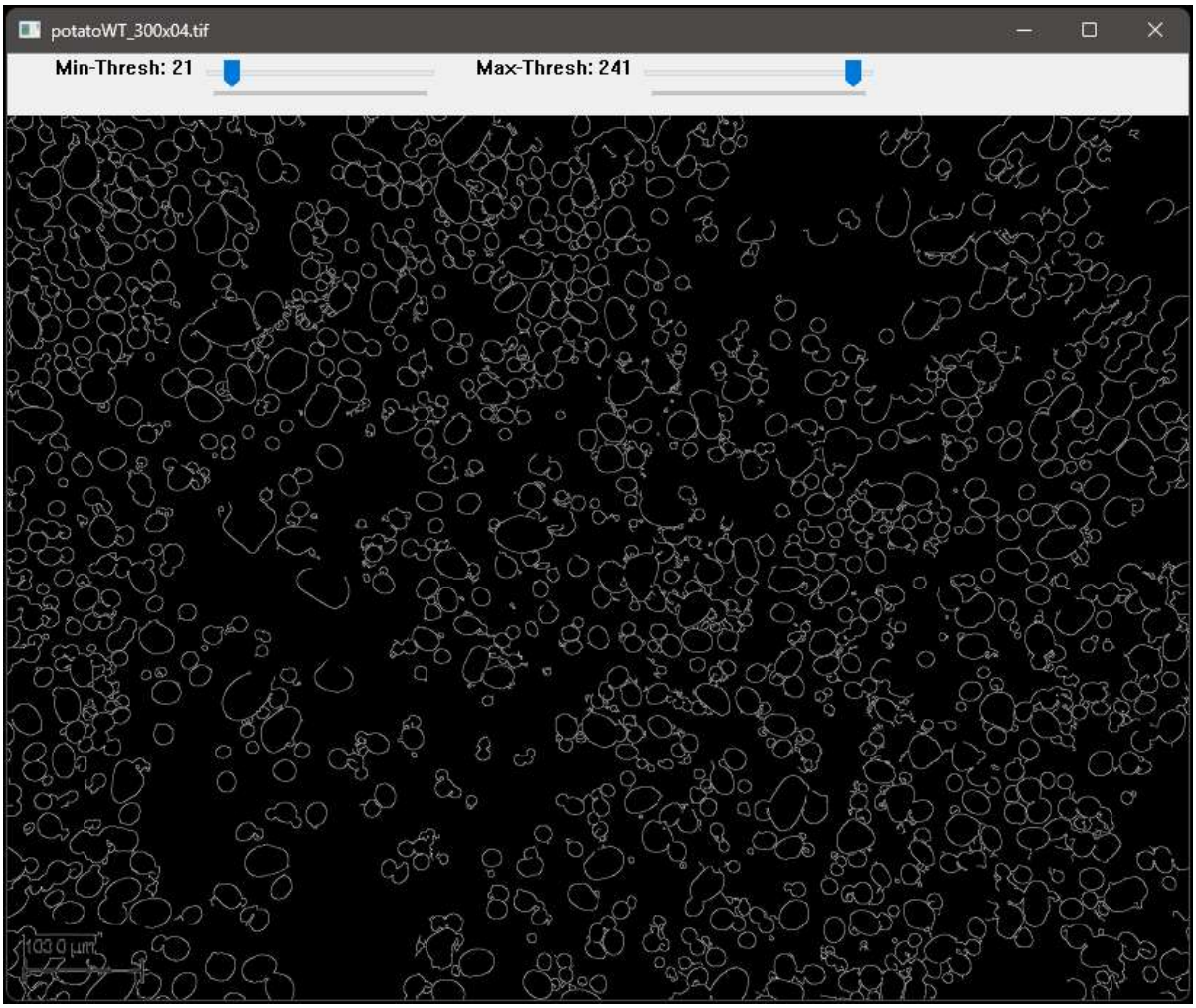


Fig. 11: Auxiliary “Threshold tool” showing contours left after having thresholds set in real time

When the threshold values are chosen carefully, the vast amount of small edges and fragments, usually on granule surfaces and from irregularities in the background or surface of the sample carrier, can be excluded, leaving an image of contours and outlines which are under the hood will be detected by the algorithm during processing and used for the analysis and generation of statistics.
The values found need to be typed into the threshold value fields in the refinement window.
When using the “Threshold tool” the other windows are blocked due to the real time feedback loop running behind, so it must be closed before typing the threshold values to begin with into the refinement window’s fields.

4. Further refinement and saving Excel file

Having found the wanted parameters and clicked on “Refine”, the three windows from before (Fig. 7, Fig. 8 and Fig. 9) are overwritten looking quite similar but containing now different information.

Fig. 12: Feedback window depicting the detected granule outlines matching the refined processing and analysis parameters using a brightened mode image

Because of the processing and analysis refinement the feedback window (Fig. 12) is now showing the alteration of contours detected and whether they are considered in analysis or not. Like before yellow contours and outlines were used in the analysis of the granule sizes. Contours in dark blue are excluded from being analysed.
As consequence the statistics calculated by the algorithm changed as well (Fig. 14) and the histograms in the diagram window got adapted and overwritten by new ones (Fig. 13).

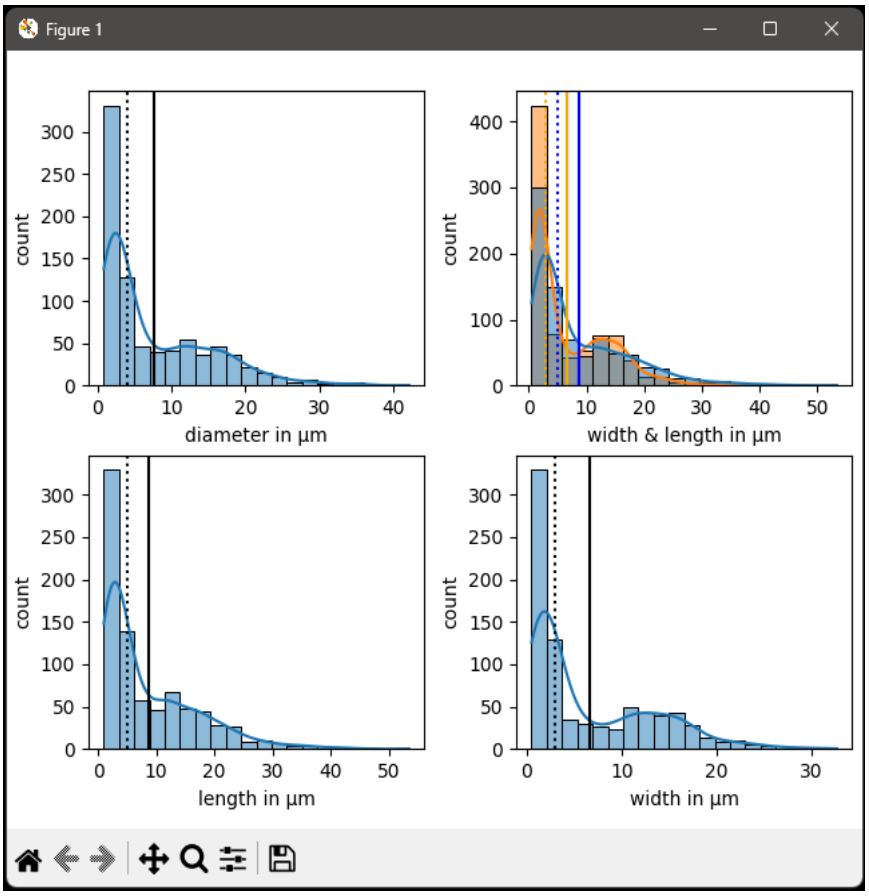


Fig. 13: Window with Histograms showing the distributions of the granule measurements after refined processing and analysis of the brightened image

In the overwritten refinement window (Fig. 14) the newly calculated statistics are shown and the chosen parameters for the refinement step are still available within the entry fields.

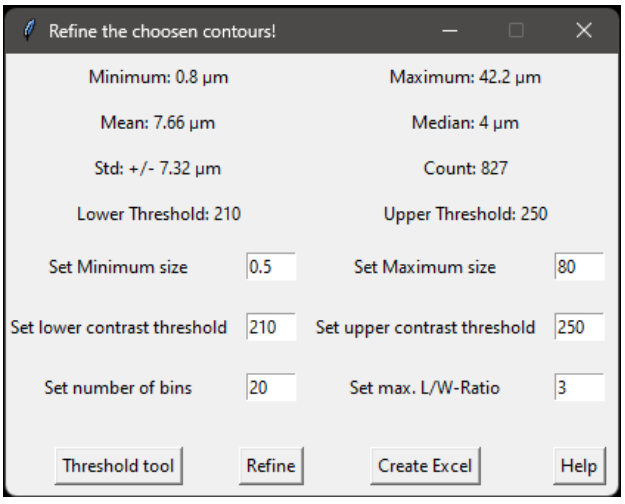


Fig. 14: Window for refining the image processing and analysing parameters showing new statistics after refinement step for a brightened image

In case of further refinements, the values can be altered and “Refine” clicked again until the included and excluded contours, the statistics and diagrams as well are satisfying. The three windows are overwritten every time.

On the other hand, if the results of processing and analysis output are satisfactorily clicking “Create Excel” (works only after a first refinement) will automatically store the raw data, image with drawn contours and descriptive statistics in an Excel workbook. Furthermore, histograms with added probability distribution curves are created within excel according to those shown in the diagram window (Fig. 13). A dialogue window will appear and ask for where to save the file. A name of including the file type suffix “.xlsx” at the end must be entered before saving the workbook.

For comparison, here are the windows using an inverted image for granule size analysis. At first the feedback image window (Fig. 15) showing the included and excluded contours detected in yellow and dark blue.
The refinement window (Fig. 16) looks quite the same, but showing other parameter values depending on the inversion of the microscopic image.

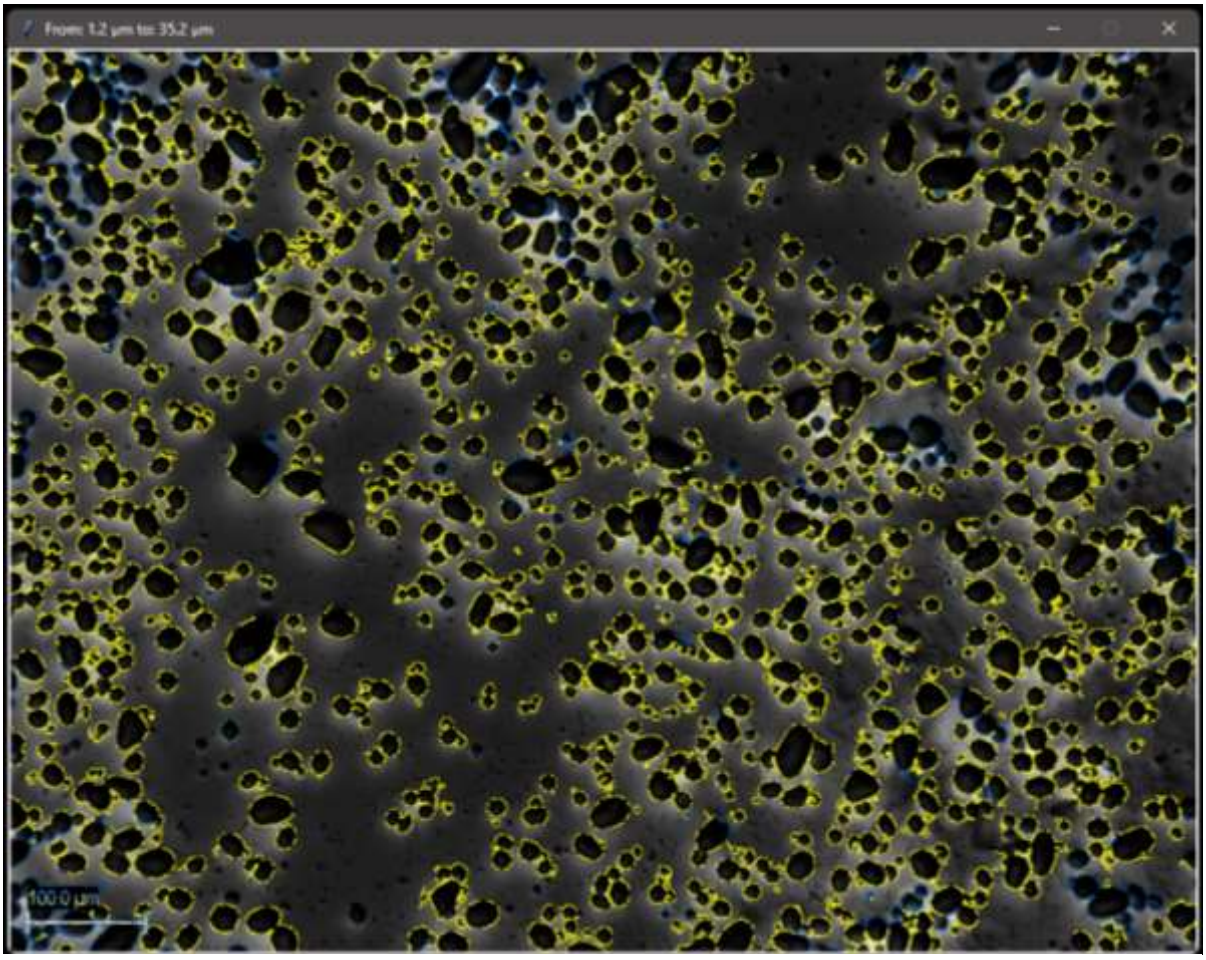


Fig. 15: Feedback window depicting the detected granule outlines matching the refined processing and analysis parameters using an inverted mode image

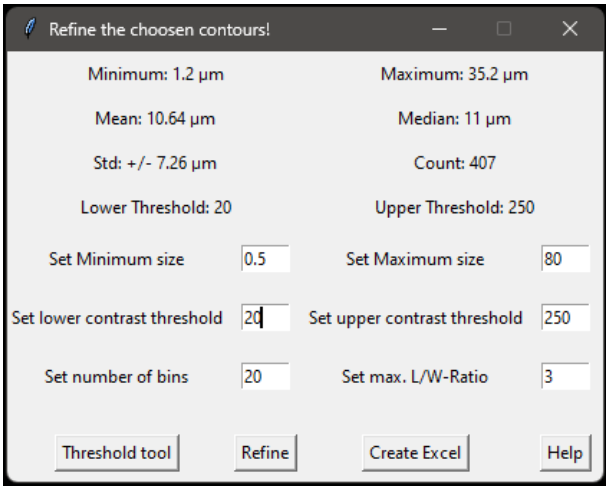


Fig. 14: Window for refining the image processing and analysing parameters showing new statistics after refinement step for an inverted image

The histograms (Fig. 17) are adapted to the parameters as well and as contours detectable structures.

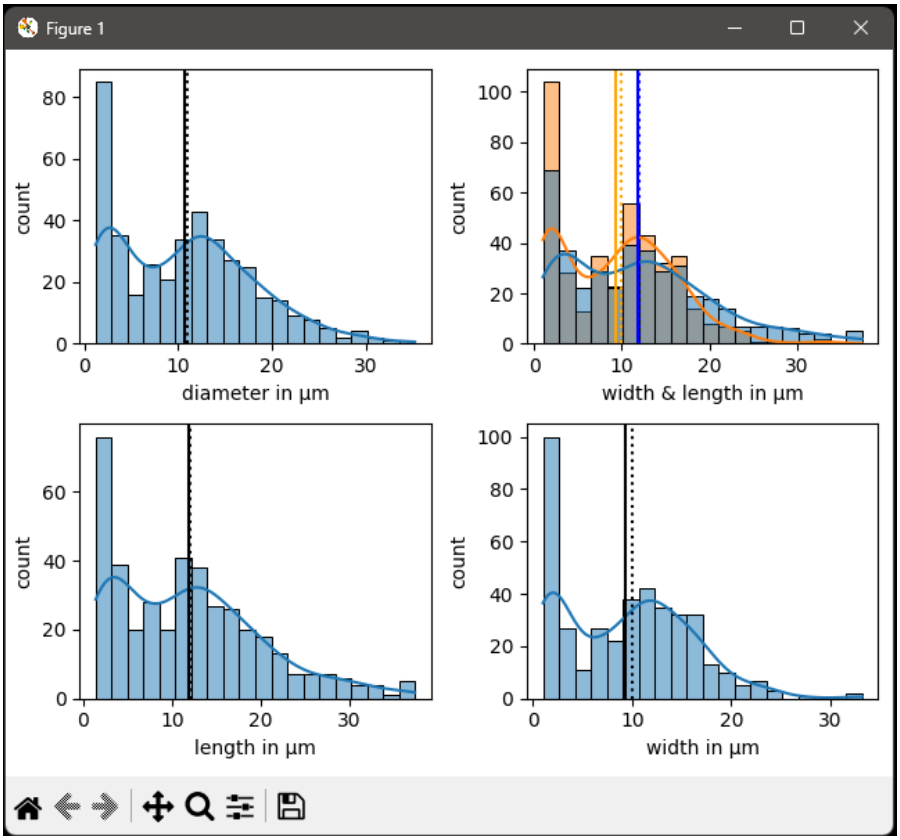


Fig. 17: Window with Histograms showing the distributions of the granule measurements after refined processing and analysis of the inverted image

5. New image and closing program

For opening, processing and analysing another electron microscope image of starch granules the diagram window, refinement window and feedback image window must be closed by clicking the “X” at the top. So, only the window under point 2 (Fig. 2 or Fig. 5) that is showing the chosen file, demands reference measures and allows to cut out an area of the image loaded into the program, is left. By clicking “Open file” at the bottom left, a new dialogue window for opening an image file pops up and a new image can be loaded into the program.
After finishing work with the program, all windows must be closed clicking “X” in order to completely shut all functions down and ending the program running.