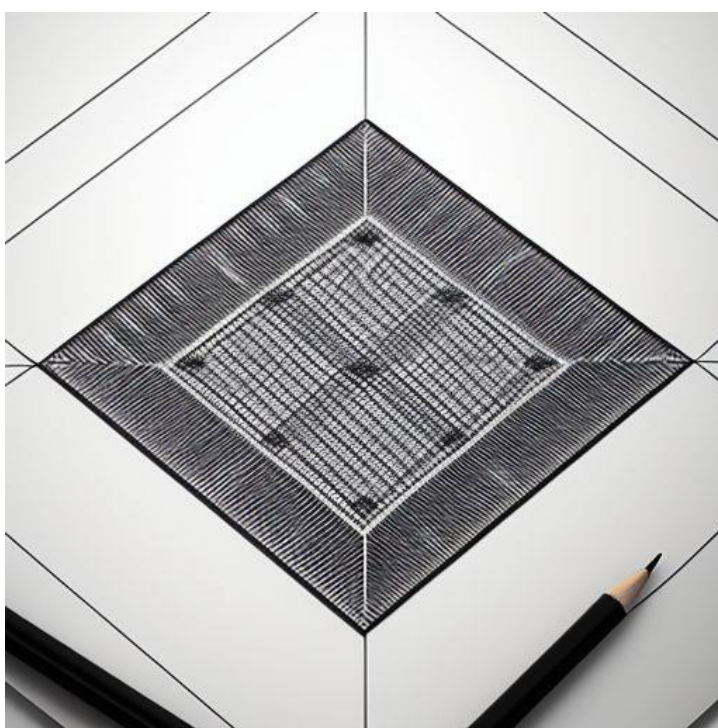


Algorithm description of Grid_Ruler plugin for ImageJ



Štěpán Helmer

Crop Research Institute, Prague



**Crop Research
Institute**

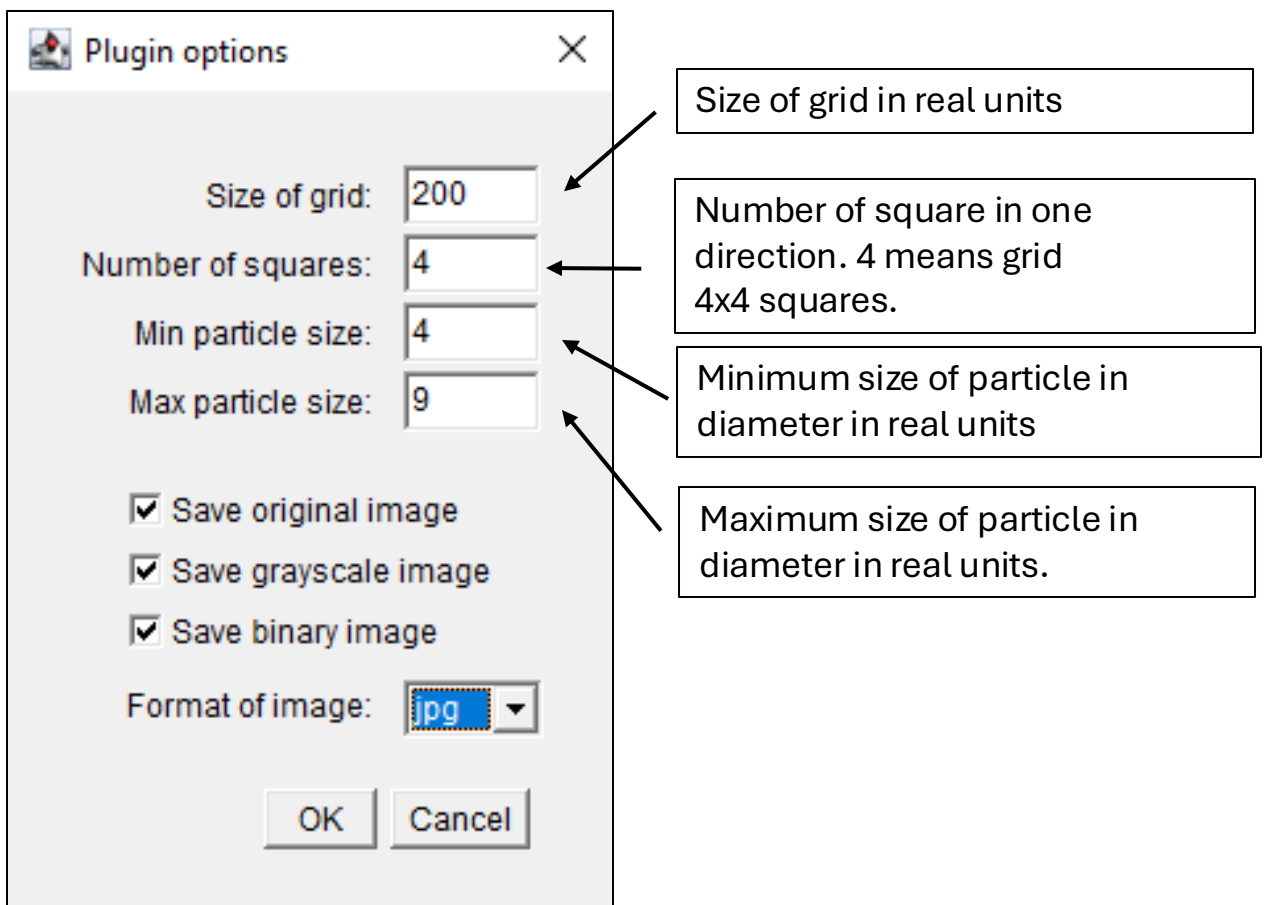
Steps of algorythm

The Grid_Ruler plugin for ImageJ follows a series of steps to analyze images with a grid structure and identify particles within the grid. Here are the detailed steps of the algorithm:

- Setting image properties
- Conversion to Grayscale
- Calculation of average Line Color
- Gridline detection
- Detection of squares
- Recognition of grid
- Gridline masking
- Size calibration
- Tresholding-binarization
- Objects pre-processing
- Particle analysis

Setting image properties

- In setting - in dialog window is chosen number of squares of grid in both directions and the real size of grid. Also the minimum and maximum diameter of searched particles is chosen.

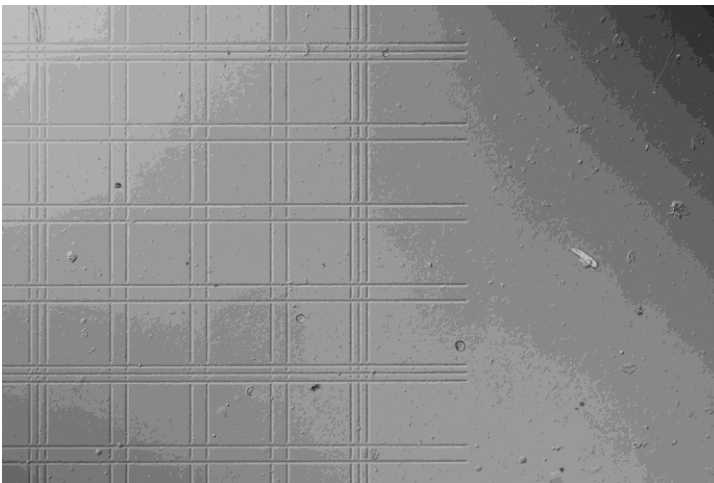


Conversion to Grayscale

- The Image is converted to an 8-bit (grayscale) format.
- 8-bit format has 256 values, with each pixel ranges from 0 (black) to 255 (white).



Color image



Grayscale (8-bit) image

Calculation of Average Line Color

- The image is analyzed row by row and column by column to calculate the average pixel value for each row and column.
- These average values are then stored in separate lists: one for rows and one for columns.
- The formula used for calculating the average pixel value for each row and column is as follows:

- For rows:

$$XY_1 = (x_1y + x_2y + \dots + x_ny) / n$$

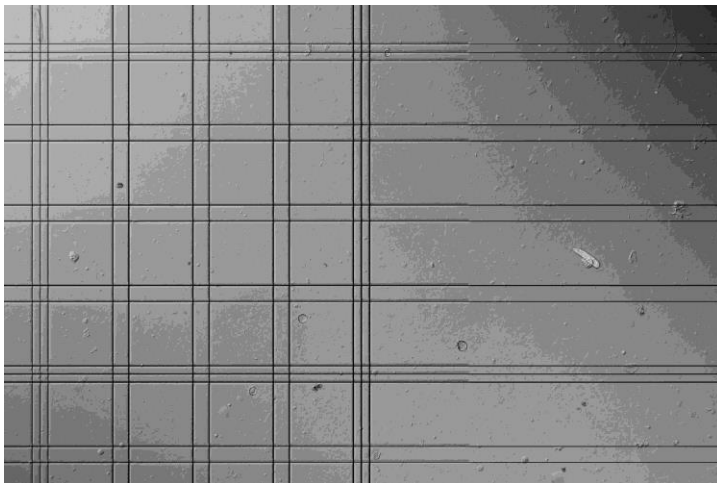
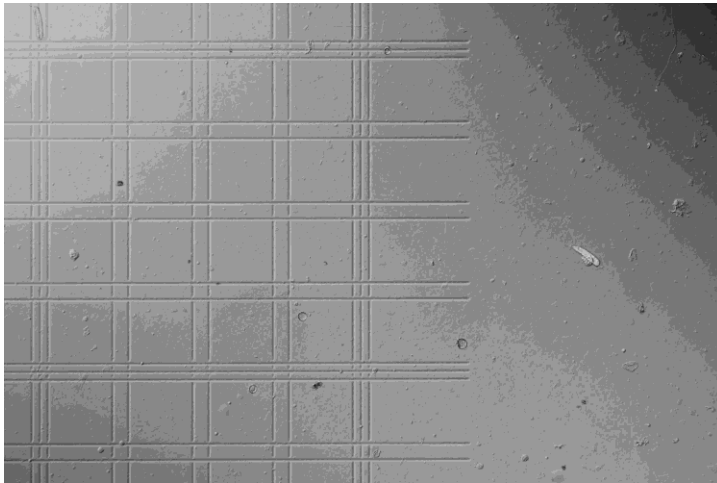
- For columns

- $X_1Y = (xy_1 + xy_2 + \dots + xy_m) / m$

x_1y_1	x_2y_1						x_ny_1

Grid line detection

- Lists of average color values for rows and columns of pixels are analysed.
- If the average value between two rows or two columns differs by a certain value, the respective row or column number is stored to the list of detected rows or columns. These detected lines are lines of grid.



Lines of grid are detected

Square detection

- Square lines are selected from the detected gridlines.
- The lines forming the largest square are identified.
- Distances of gridlines within the largest square are used to search for pairs of lines in both horizontal and vertical directions.
- Detected pairs of lines are written into separate lists for horizontal and vertical lines.

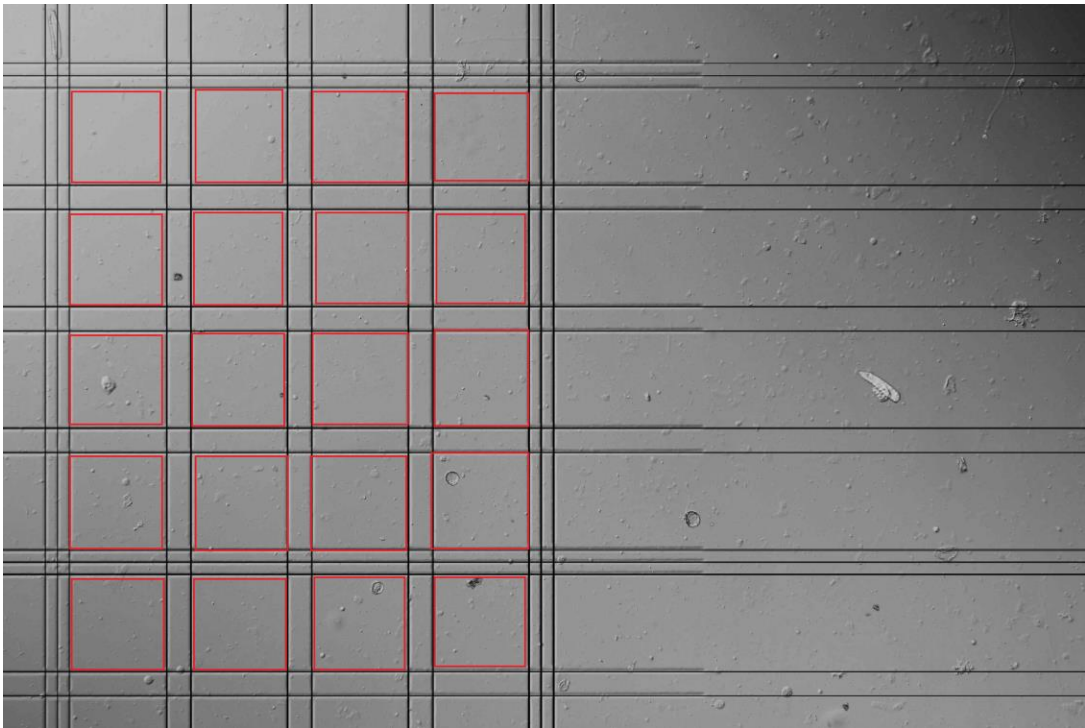
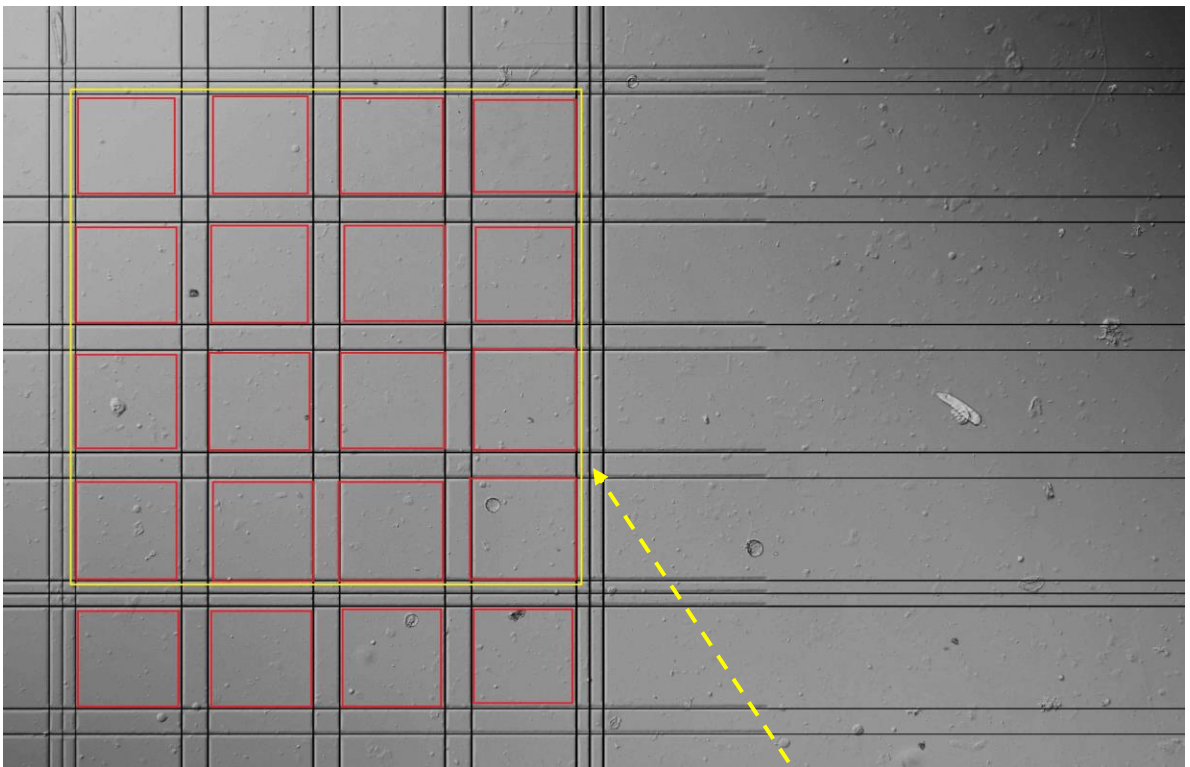



Figure 5 - The lines bounding the squares are selected from the grid lines

Recognition of grid

- The grid structure is recognized based on the selected number of squares and the detected pairs of line forming squares.
- Using the input from the Dialog window, the grid is defined according to the specified number of squares in each direction.



 Plugin options ×

Size of grid:

200

Number of squares:

4

Min particle size:

4

Max particle size:

9

Number of square in one direction. 4 means grid 4x4 squares.

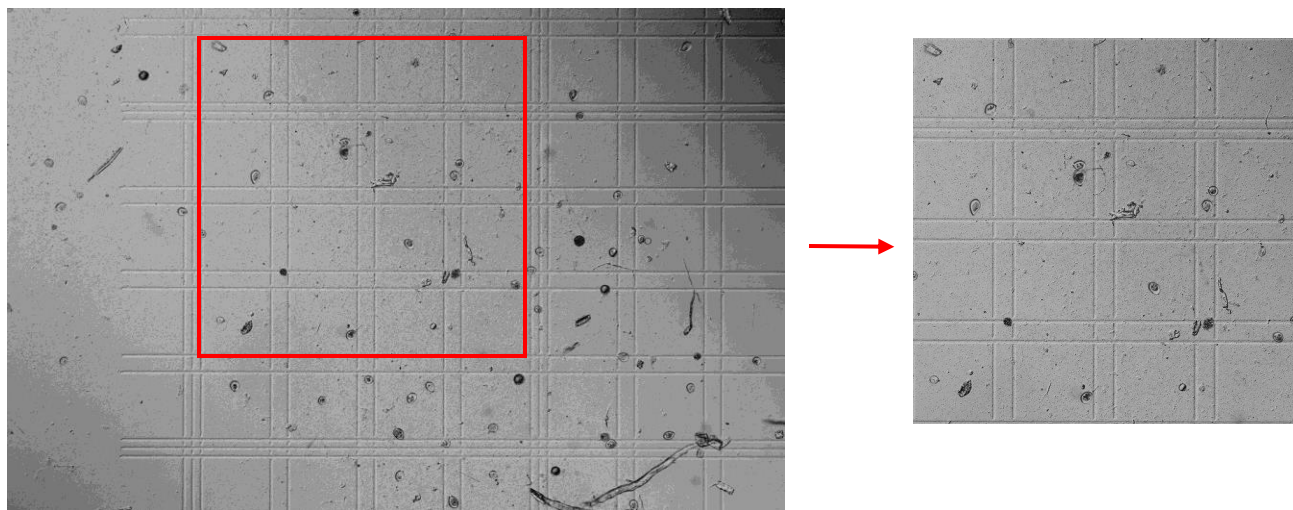
The square is selected from the squares based on the number of squares, that are chosen in Dialog window

Gridline masking

- Pixelvalue of color is set to value 255. This step is important for the Tresholding step, because gridlines will not be detected as particles and they will not disturb tresholding.

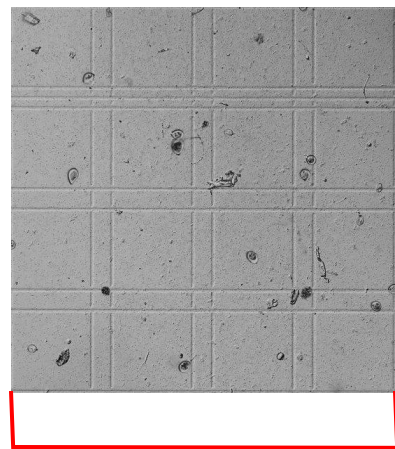
Grid selection

- Recognized square of grid is cropped



Size calibration

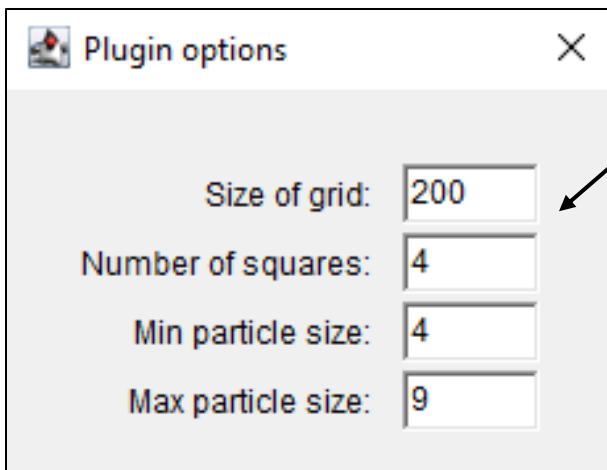
- Based on information about the real size of the grid, units are assigned appropriate to the image dimensions.



200 μm

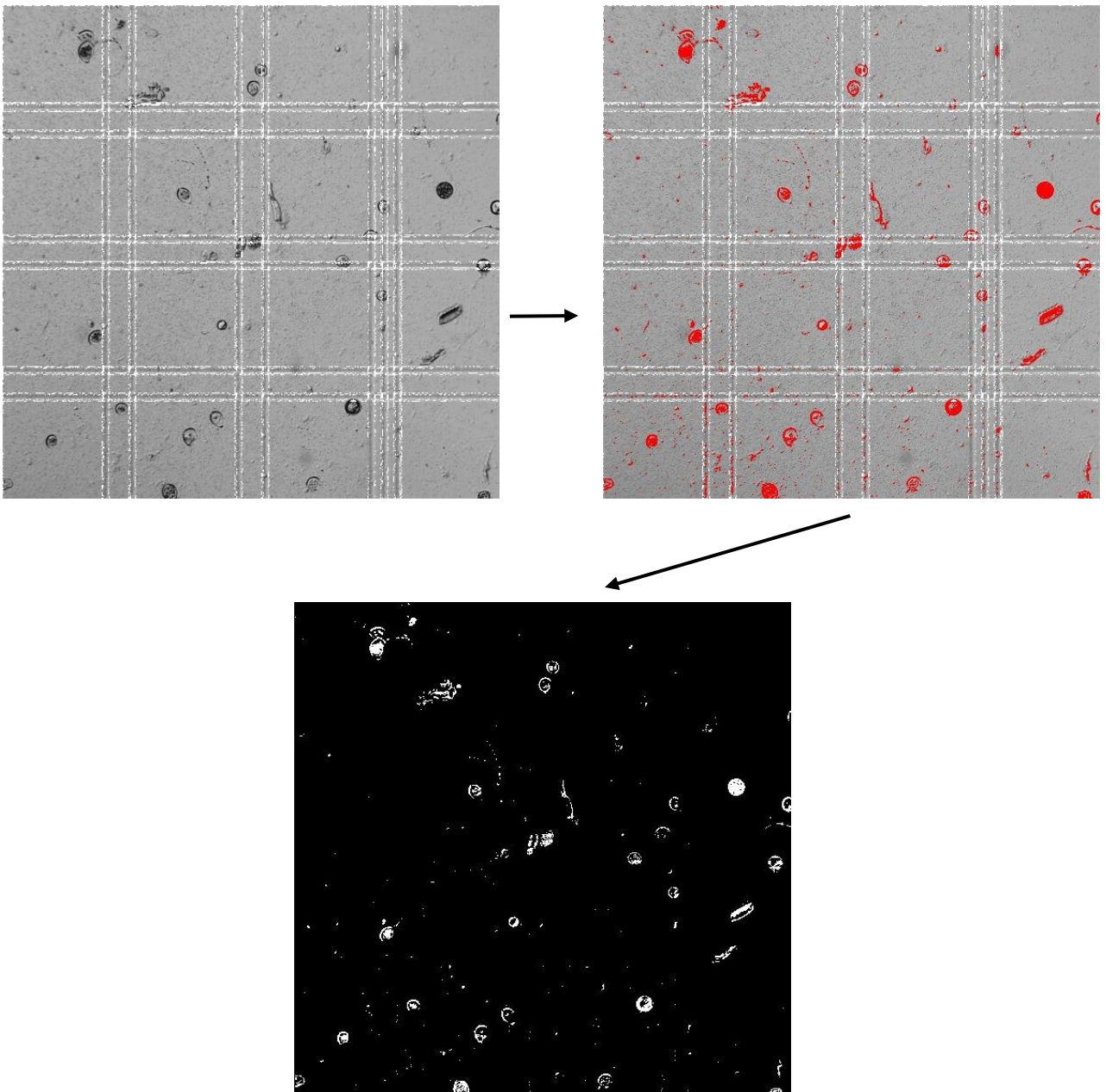


Size of grid in real units



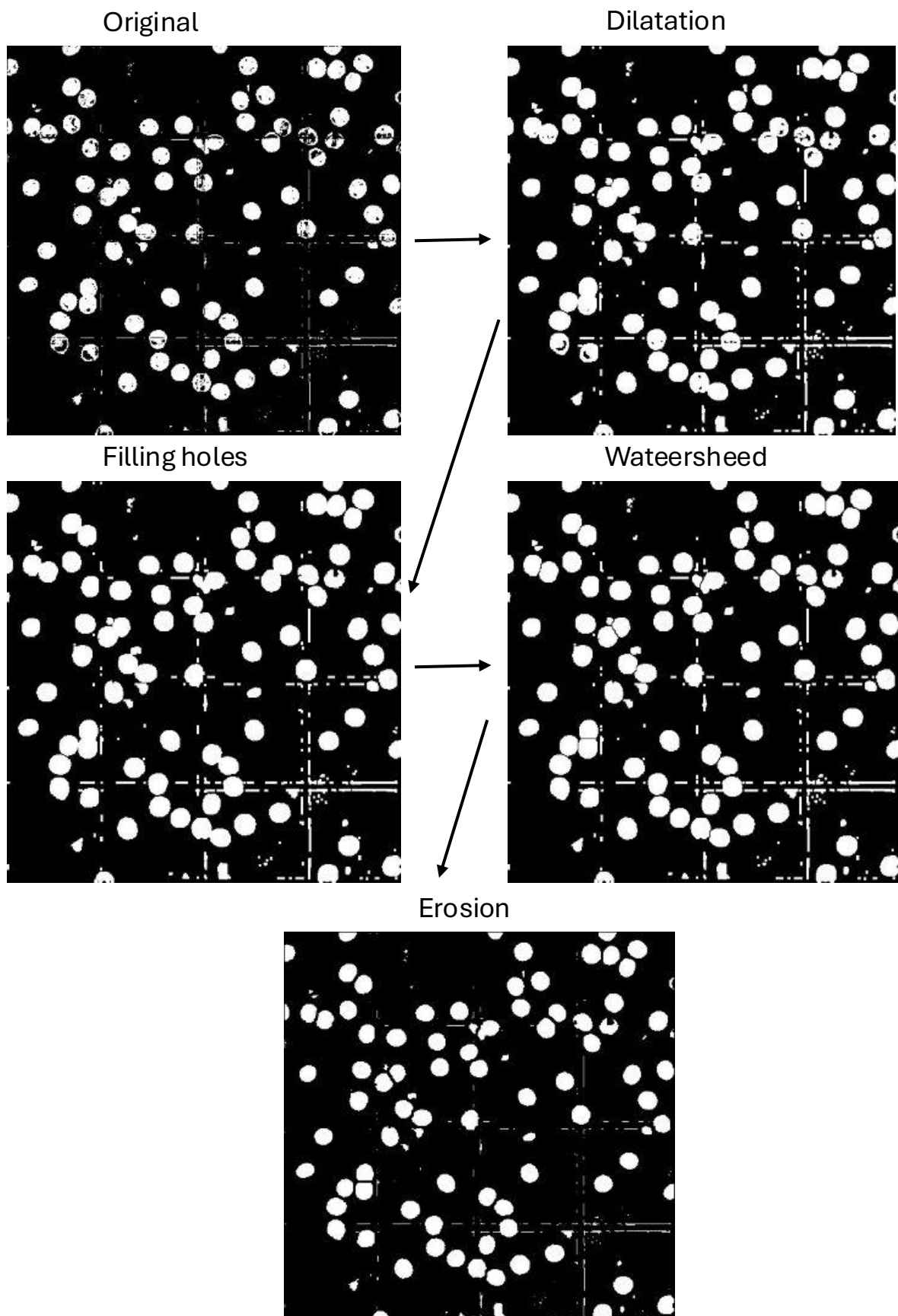
Tresholding

- This step converts a grayscale image to a binary format where pixels are divided into two categories – usually (black) background and (white) objects of interest.
- Thresholding is the process of setting an intensity threshold that distinguishes pixels that representing objects of interest from the background.
- The maxEntropy method is used for thresholding.



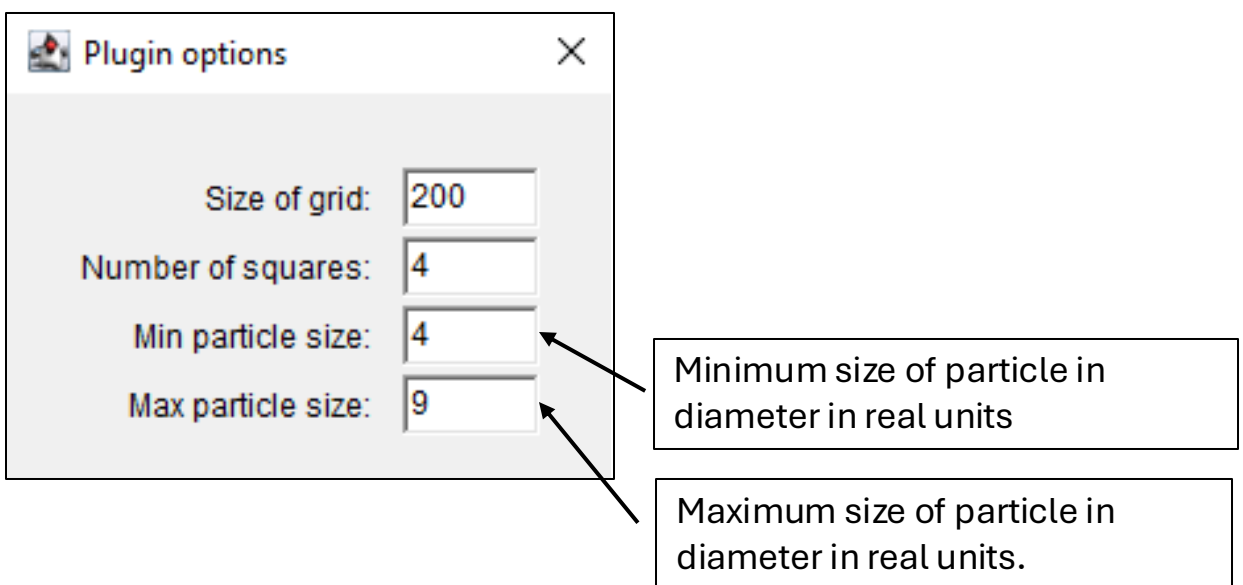
Object pre-processing

- Objects are pre-processed for size-based counting.
- **Dilation:** Enlarges objects to combine nearby objects into a single unit and removes small inaccuracies.
- **Fill Holes:** Fills small gaps or holes inside objects.
- **Watershed:** Segments overlapping objects.
- **Erosion:** Reduces or narrows the objects in the image, reversing dilation.



Particle analysis

- Identifies for contiguous areas of white pixels on a black background based on their size. The parametres for size of particles are choosen by Dialog window.
- Particles are searched as objects with content equal to $(\pi * (\text{diameter}/2))^2$
- Each particle characteristics such as size, shape and position are analyzed
- The obtained data are processed and exported as tables.



Diameters of particles in real units are used for searching particles between this sizes.