Supplementary Material for qCMA: a desktop application for Quantitative Collective cell Migration Analysis

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1   Installation and download

Information about download and installing the software is available at:
`http://www.weizmann.ac.il/complex/compphys/software/qcma/`.

2   The Graphical user interface (GUI)

The format of the GUI appear as Figure S1, with a number next to each item which we explain below.

1. This parameter sets the threshold used when transforming the images from gray-scale to binary. High values of the parameter corresponds to more stringent binarization and hence will not be sensitive enough to detect weak objects. On the other hand, a low value will be very sensitive but might detect many background pixels as positive. For images with very good contrast a low threshold is recommended. The suggested default values are 0.75 for phase-contrast and 0.35 for fluorescent images. Setting this parameter is the most important task of the training session, since the biggest variation between images is due to the light conditions and background intensity.

2. This parameter sets the size for a few of the smoothing steps, which are used in spatial median filter, morphological closing, and Wiener filter (value in pixels). This parameter will mostly affect the smoothness of the boundary curve; a low value will produce more sensitive and less smooth lines, while large values will produce smoother but less sensitive boundaries.

3. This parameter sets the threshold for the first round of small objects removal (value in pixels).

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4. This parameter sets the threshold for the second round of small objects' removal, and hence can be bigger (value in pixels).

5. This parameter sets the threshold for the irregularity of the gap (standard-deviation, in pixels). The variance of the gap width is calculated by sampling the width at several places along the gap, and if the variance is too large (larger than this parameter) the value assigned to the gap width is NaN.

6. This parameter sets the width of the frame where the analysis is done (value in pixels). It is important to set it to be smaller than the image width to eliminate confounding effect caused by the boundaries. The left/right position of the frame is set to be optimal such that the gap is in the center of the frame. While analyzing movies, the left/right position is set by the first image and used for the rest of the images.

7. This field contains the input folder, where your images/movies are located. The gray button enables browsing in the file system.

8. This field sets the output folder, where the resulting images/movies will be saved. The gray button enables browsing the file system.

9. Set the type of output files you want, choose between "png" or "pdf" file formats.

10. Set the type of input files you have; we allow two types: "jpg" and "TIFF". When using the "Decompose movie" option, each frame will be saved as a "jpg" file.

11. Mark this check-box if you are analyzing a movie. When analyzing movies, we treat the images as a sequence and hence assume that the cells are moving only forward, which makes the results more smooth.

12. Set the algorithm to be applied according to the type of microscopy used.

13. Press this button to start the processing of all the images in the data-set (input folder).

14. Use this option when you want to analyze movie. The first step should be decomposing the movie to a set of images. Pressing this button will decompose the "avi" file selected in the "input file" (#16) field.

15. If the movie you want to analyze was taken such that the gap is horizontal, the images need to be rotated by 90 degrees. Marking this check-box will rotate each frame of the movie while decomposing it.

16. Select here the input file. This is relevant when performing movie decomposition (file should be "avi"), and when training the parameters (file should be "TIFF" or "jpg").

17. Press this button to run a training session on the input file selected in the "input file" (#16) field. The results of the training run will appear in on the right (item #18).

18. This item presents the results of the training run.
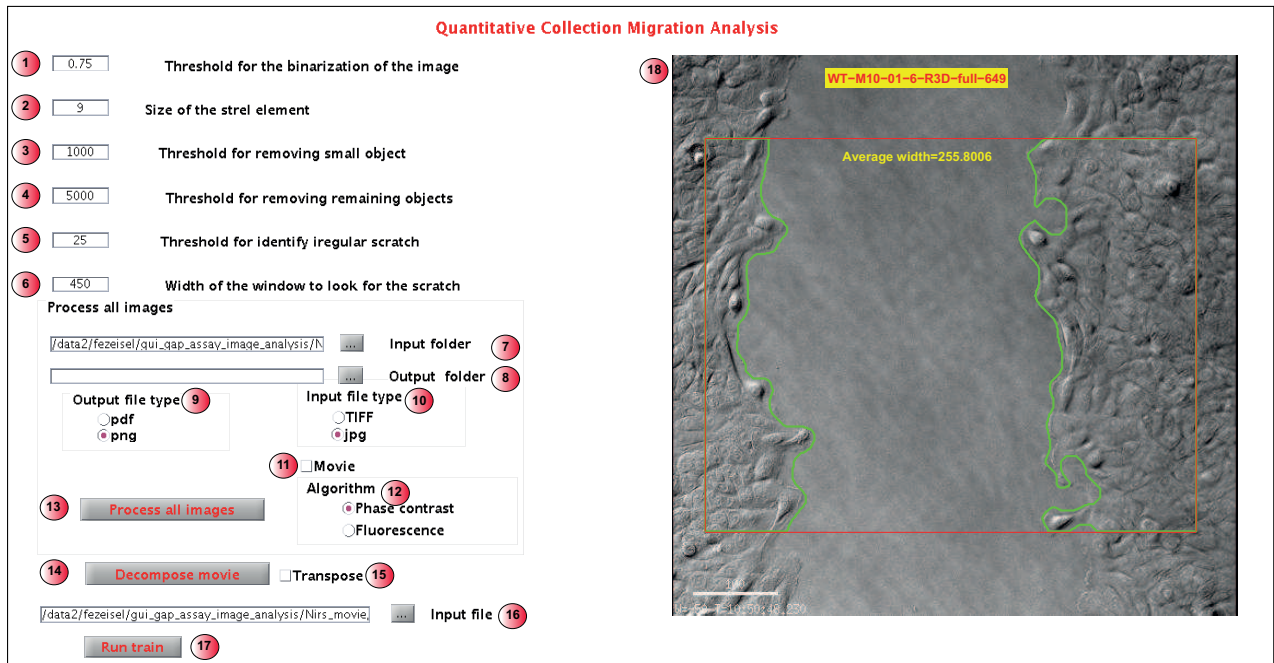
Supplementary Figure S1: Screenshot of the graphical user interface

3 Processing flow

We provide below an example of the steps taken in order to get the segmented image. This is shown separately for a fluorescent image (Figure S2) and for phase contrast image (Figure S3).

Detailed description for steps in processing a fluorescent image (Figure S2):

1. Raw gray-scale image.

2. Binary image, binarization taken with a threshold equal to the level calculated by the "graythresh" MATLAB function, multiplied by the user selected threshold (item #1 in the GUI).

3. Same as 2, but after discarding the upper and lower 15% of the image.

4. Filling the holes using the "imfill" MATLAB function.

5. 2D median filter with a square bin defined by the user (item #2 in the GUI).

6. Performs "morphological closing" with a disk structuring element where the size is defined by the user (item #2 in the GUI).

7. Filling the holes using the "imfill" MATLAB function.

8. Perform "removing small objects" operation, the threshold for the small objects defined by the user (item #3 in the GUI)

9. 2D median filter with a square bin defined by the user (item #2 in the GUI).

10. Performs "morphological closing" with a disk structuring element where the size is defined by the user (item #2 in the GUI).

11. Filling the holes using the "imfill" MATLAB function.

12. Perform "removing small objects" operation, the threshold for removing remaining objects is defined by the user (item #4 in the GUI).

13. The resulting binary image.

14. Overlay of the segmentation and the raw image.

Detailed description for steps in phase contrast image (Figure S3):

1. Raw gray-scale image.

2. Invert the image (black to white) and adjust intensities (contrast enhancement).

3. Gray scale image of the local standard-deviation (using "stdfilt" function) after adjusting the intensities (contrast enhancement).

4. Perform noise removal Wiener filter (using "weiner2" function).

5. Binary image, binarization taken with a threshold equal to the level calculated by the "graythresh" MATLAB function, multiplied by the user selected threshold (item #1 in the GUI).

6. Filling the holes using the "imfill" MATLAB function.

7. Performs "morphological closing" with a disk structuring element where the size is defined by the user (item #2 in the GUI).

8. Filling the holes using the "imfill" MATLAB function.

9. 2D median filter with a square bin defined by the user (item #2 in the GUI).

10. Perform "removing small objects" operation, the threshold for the small objects defined by the user (item #3 in the GUI)

11. Performs "morphological closing" with a disk structuring element where the size is defined by the user (item #2 in the GUI).

12. Perform "removing small objects" operation, the threshold for removing remaining objects is defined by the user (item #4 in the GUI).

13. The resulting binary image.

14. Overlay of the segmentation and the raw image.
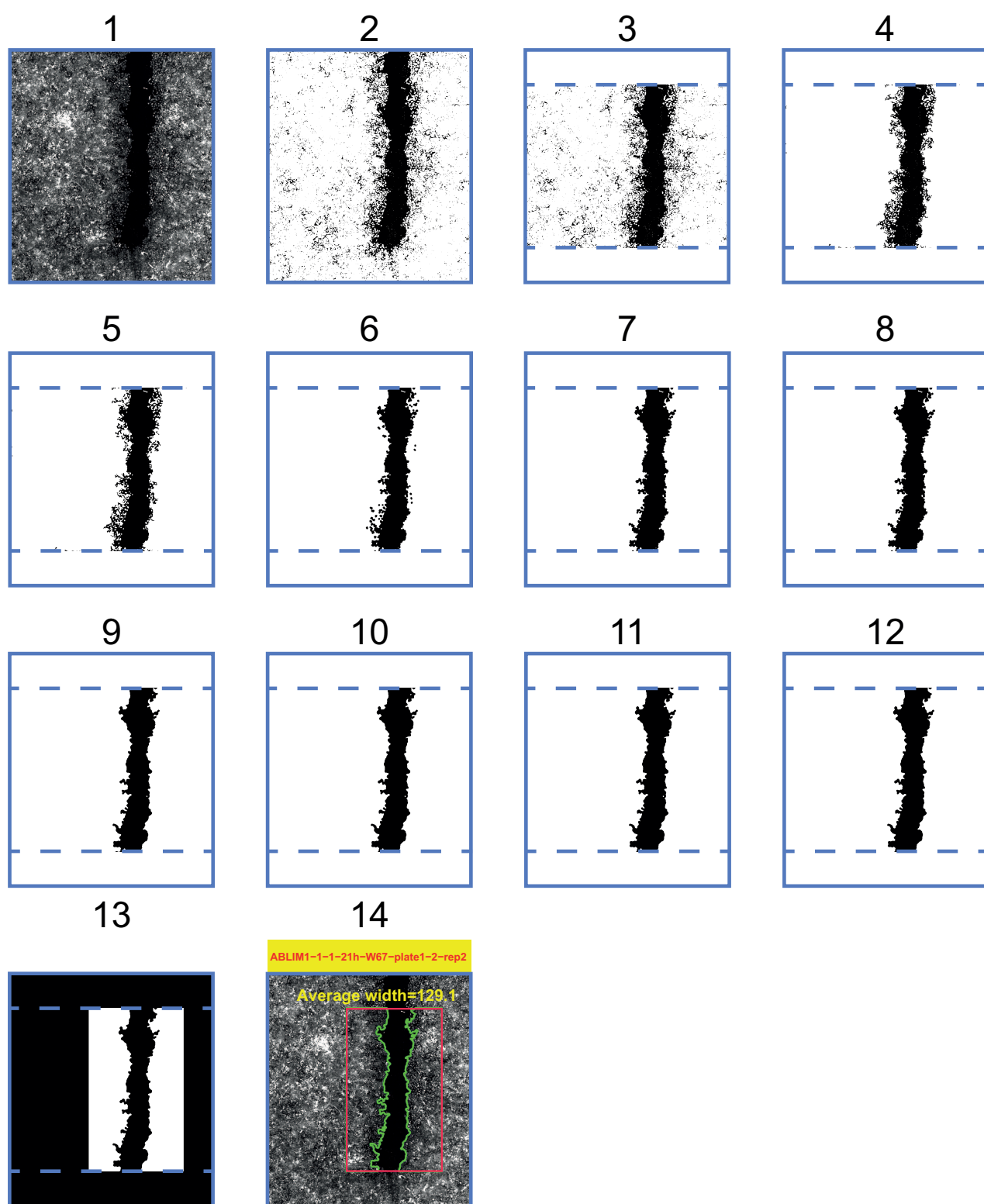
Supplementary Figure S2: Example for processing steps taken while analyzing a fluorescent image, see text for details.
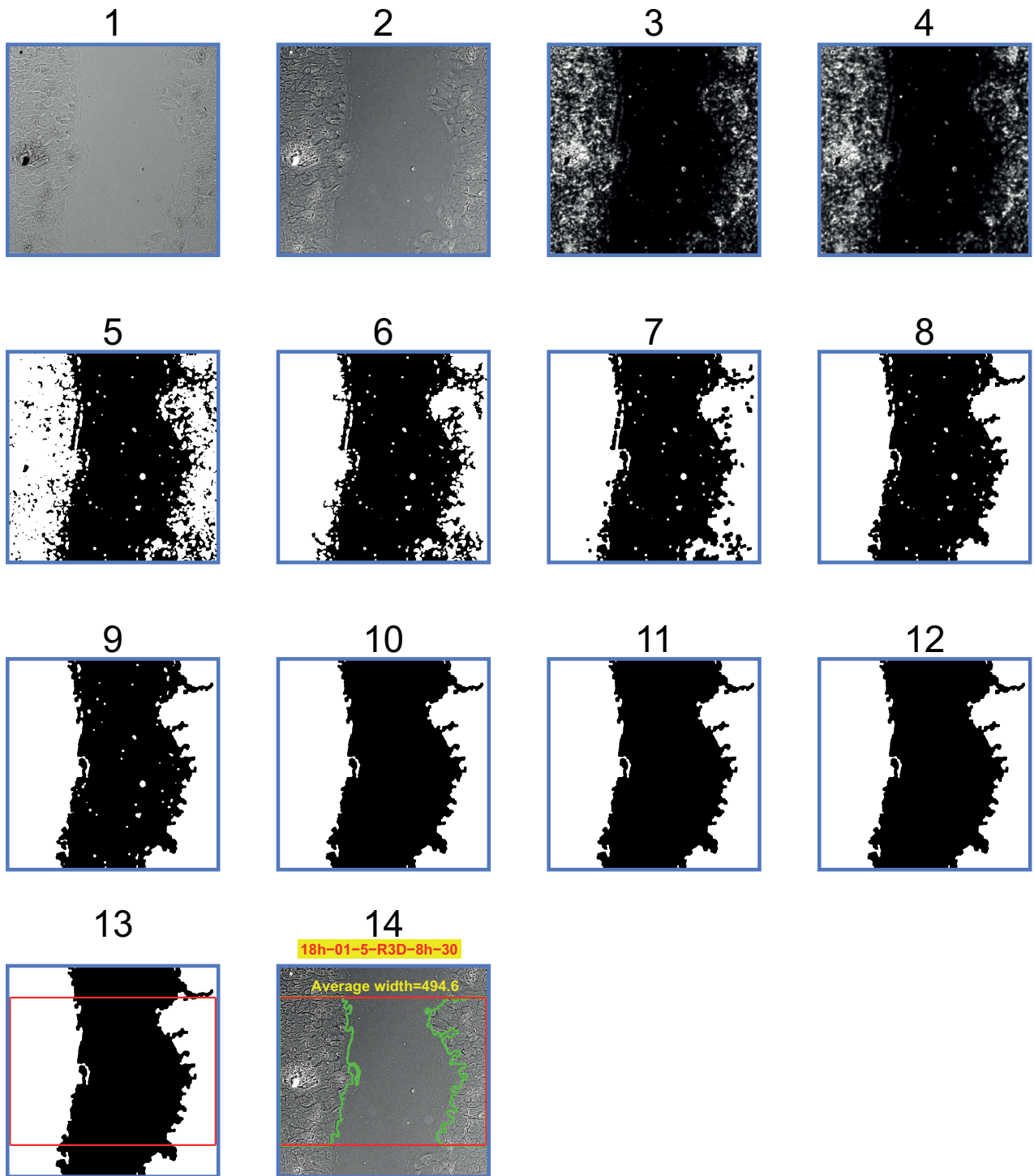
Supplementary Figure S3: Example for processing steps taken while analyzing a phase-contrast image, see text for details.