RMPJ USER MANUAL

Version 1.0

Yositaka Kimori

Project web: https://github.com/ykimori/rmpj

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1. General description

RMPJ is an ImageJ plugin that implements operations based on rotational morphological processing (RMP)[1], a variation of mathematical morphology. This plugin is primarily developed to process biomedical images and can be used to: enhance the delicate structures of biological objects with low contrast, remove noise and artifacts while preserving the delicate structures of biological samples, and separate and extract aggregated particulate structures.

The plugin comprises seven RMP-based operations: opening, closing, white top-hat, black top-hat, morphological smoothing, and two morphological contrast enhancements.

2. Installation

ImageJ can be downloaded from the ImageJ website: https://imagej.nih.gov/ij/download.html. Download RMPJ.jar to the plugin folder of ImageJ and then restart ImageJ.

3. Rotational morphological processing (RMP)

Mathematical morphology[2-4] is a theory used in image processing and analysis. When morphological operations are applied to an image to be processed, structural elements (SEs) that can be defined as arbitrary shapes and sizes are used. Even when the same morphological operation is applied, different SE sizes and shapes yield different processing results.

In RMP, the image to be processed is rotated by multiple arbitrary angles, and morphological operations are applied each time. After applying the operations, images are combined to produce a single image.

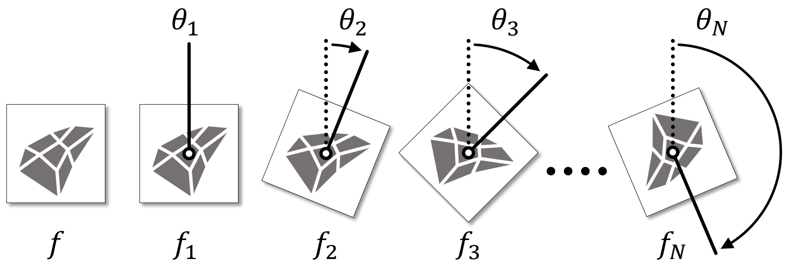


Fig. 1. Rotational process of original image f in RMP.

Figure 1 shows the process of rotating the original image f to be processed. The image is rotated, and a morphological operation is applied to the rotated image each time. Let N be the number of iterations of the morphological operation, and $\theta_\alpha = 180/N$. The rotation angle of image f for the i-th operation is expressed as follows:

$\theta_i = \theta_\alpha(i-1)$.

Here, $i = 1, 2, \cdots, N$ and $\theta_i \in [0,180)°$. The clockwise direction of rotation is positive. The image rotated by an angle θ_i is denoted as f_i. f_1 is the image at $\theta_1 = 0°$ and is equal to the original image *f*.

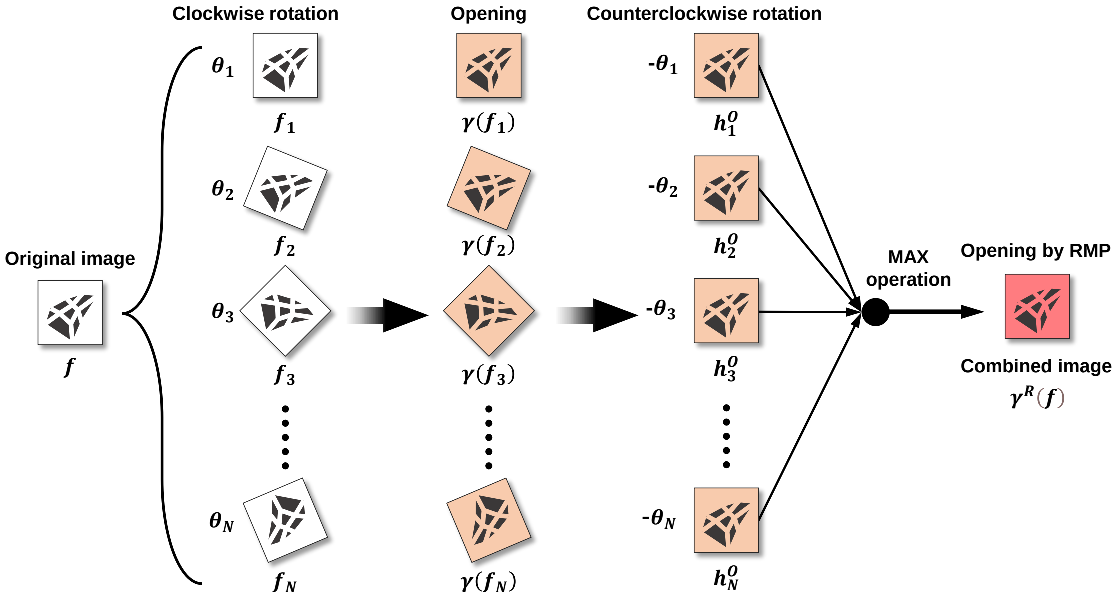


Fig. 2. Process flow for opening by RMP.

Figure 2 illustrates the flow of opening via RMP. The opening operation is performed *N* times. First, the original image *f* is rotated clockwise from θ_1 to θ_N and an opening operation is performed on each of the rotated images. The result of opening the rotated image f_i is denoted as $\gamma(f_i)$.

Subsequently, the opened images are rotated counterclockwise from θ_1 to θ_N, i.e., the opened image of the image rotated by θ_i degrees ($\gamma(f_i)$) is rotated counterclockwise by θ_i degrees and aligned in the same orientation as the original image. The rotated image is denoted as h_i^O.

Finally, these counterclockwise-rotated images are combined and produced as a single image. In the case of opening by RMP, the maximum operation is performed among the images. In other words, the intensity value of the pixel at position (*x*, *y*) in the produced image is the maximum pixel value at the same pixel position (*x*, *y*) for images from h_1^O to h_N^O.

Closing by RMP involves a minimum operation on counterclockwise-rotated closed images (from h_1^C to h_N^C) and their combination into a single image.

Opening and closing by RMP are expressed as follows:

$$\text{Opening by RMP:} \quad [\gamma_B^R(f)](x,y) = \max_{i\in(1,2,\ldots,N)} h_i^O(x,y) \tag{1}$$

$$\text{Closing by RMP:} \quad [\phi_B^R(f)](x,y) = \min_{i\in(1,2,\ldots,N)} h_i^C(x,y) \tag{2}$$

The operations implemented in this plugin were defined based on the opening and closing by RMP. These operations are white top-hat and black top-hat by RMP; morphological smoothing, which is a LOCO filter (linear combination of open-closing and close-opening)[5] based on RMP; morphological contrast enhancement type 1[6]; and morphological contrast enhancement type 2, which is a simple neighborhood-based morphological contrast operator[4] based on RMP.

White top-hat by RMP: $WTH^R(f) = f - \gamma^R(f)$ (3)

Black top-hat by RMP: $BTH^R(f) = \phi^R - f$ (4)

Morphological smoothing: $MS^R(f) = \frac{1}{2}[\gamma^R(\phi^R(f))] + \frac{1}{2}[\phi^R(\gamma^R(f))]$ (5)

Morphological contrast enhancement type 1: $MCE_1^R(f) = f - MS^R(f)$ (6)

Morphological contrast enhancement type 2: $MCE_2^R(f) = f + WTH^R(f) - BTH^R(f)$ (7)

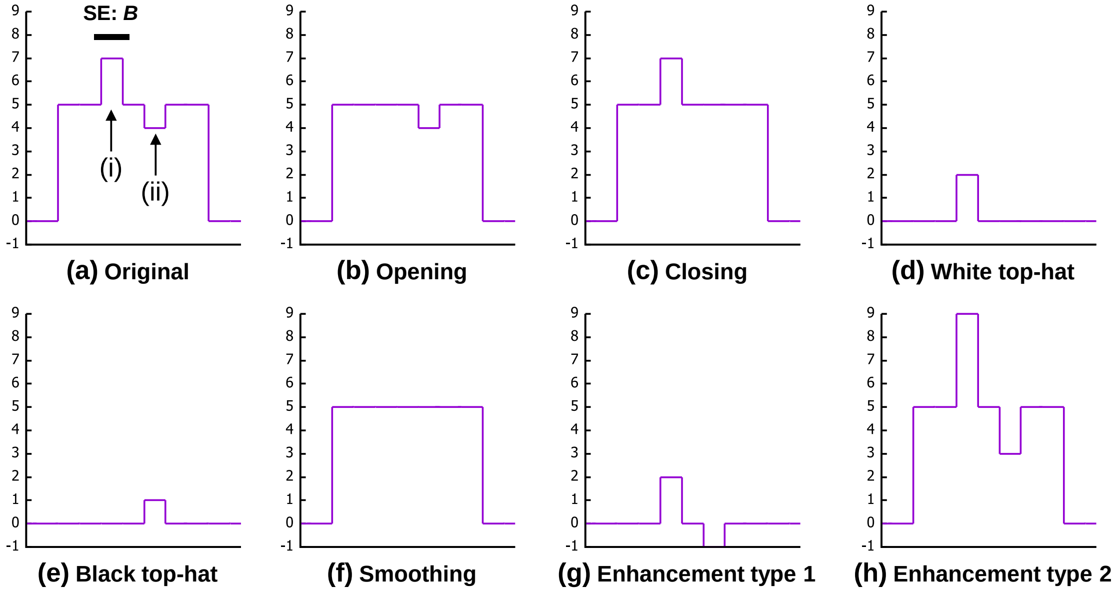


Fig. 3. Effects of the seven morphological operations implemented in the plugin. (a) Intensity profile of partial region of original image. Results of morphological operations performed on original image using SE *B* based on (b) opening, (c) closing, (d) white top-hat, (e) black top-hat, (f) morphological smoothing, (g) morphological contrast enhancement type 1, and (h) morphological contrast enhancement type 2.

Figure 3 illustrates the processing effects of the aforementioned operators. Figure 3a represents a portion of the intensity profile of the original image *f*. This profile contains two structures: (i) and (ii). Structure (i) is constructed with a higher intensity value than its surroundings, and structure (ii) is constructed with a lower intensity value than its surroundings. The size of SE *B* used for morphological operations is larger than the width of these structures.

`Opening` removes structure (i) and preserves structure (ii) (Figure 3b). It removes structures (peaks, ridges, etc.) with higher intensity values than their surroundings in regions smaller than the size of the SE.
`Closing` preserves structure (i) and fills structure (ii) (Figure 3c). It fills structures (valleys, grooves, etc.) with lower intensity values than their surroundings in regions smaller than the size of the SE.
`White top-hat` is defined as the difference between the original image and its opening. White top-hat extracts structures (i.e., peaks, ridges, etc.) that were removed by the opening (Figure 3d).
`Black top-hat` is defined as the difference between the closing of the original image and the original image. The Black top-hat extracts the structures (i.e., valleys and grooves) that were filled by closing (Figure 3e).
`Morphological smoothing` involves the combination of both opening and closing operations. The removal of structure (i) and the filling of structure (ii) can be achieved using this smoothing technique (Figure 3f).
`Morphological contrast enhancement type 1` is defined as the difference between the original image and the smoothed image obtained using Eq. (5). The structures removed by morphological smoothing (i.e., structures (i) and (ii)) are extracted.
`Morphological contrast enhancement type 2` consists of first adding the white top-hat to the original image and then subtracting the black top-hat from the resulting image.

In general, the resulting images processed by the top-hats and two morphological contrast enhancements have a small dynamic range. Negative intensity values may occur in the results obtained using these image-enhancement filters. Therefore, the software first shifts the intensity distribution such that the minimum intensity corresponds to zero. Subsequently, it performs two image histogram modifications, i.e., histogram equalization and histogram linear contrast stretching, resulting in an image with a wide dynamic range. These processes allow the top-hats and the morphological contrast enhancements to enhance the extracted structures.

4. Software description

4.1 Graphical user interface (GUI)

Figure 4 shows the graphical user interface (GUI) of the plugin. The plugin operates on 8- and 16-bit grayscale images. The maximum input image size is limited to 2048 × 2048 pixels.

- **Operation type:** From this drop-down list, the following seven morphological operations can be selected:
  `Opening` implements Eq. (1). Opening removes structures that are smaller than the size of the SE and have higher intensity values than their surroundings.
  `Closing` implements Eq. (2). Closing fill structures that are smaller than the size of the SE and have lower intensity values than their surroundings.
  `White top-hat` implements Eq. (3). White top-hat extracts and enhances structures that are

smaller than the size of the SE and have higher intensity values than their surroundings.
`Black top-hat` implements Eq. (4). Black top-hat extracts and enhances structures that are smaller than the size of the SE and have lower intensity values than their surroundings.
`Smoothing` is a morphological smoothing method that implements Eq. (5). This operation can be used as a smoothing filter that utilizes the effects of the opening and closing operations.
`Enhance-type 1` is a morphological contrast enhancement type 1 that implements Eq. (6). This operation emphasizes the structures removed by the morphological smoothing.
`Enhance-type 2` is a morphological contrast enhancement type 2 that implements Eq. (7). This operation emphasizes the structures in the original image extracted by the white top-hat and black top-hat.

- **Shape of structuring element:** This drop-down menu allows three types of SE shapes to be selected: `Disk`, `Line`, and `Square`.
- **Size of structuring element:** The size of the SE corresponding to the diameter in the case of a disk-shaped SE, the length of a line in the case of a line-shaped SE, or the length of a side in the case of a square SE are determined. The possible inputs are odd values with a minimum value of 3 pixels and a maximum value of 99 pixels.
- **Number of iterations:** The number of iterations of the morphological operations (N) related to the number of times the image has been rotated is determined. For operations using disk- and square-shaped SEs, $N = 8$ is the optimal value. For operations using the line-shaped SE, $N = 36$ is the optimal value, and setting $N = 1$ is consistent with the typical morphological operations. The greater the value of N, the higher the computational cost.
- **Stack options:** If the checkbox has been checked, a dialog box is opened to process a stack. The dialog box allows the user to decide whether to process all the images in the stack, only a specific range of images in the stack, or only specific images in the stack (Figure 5).

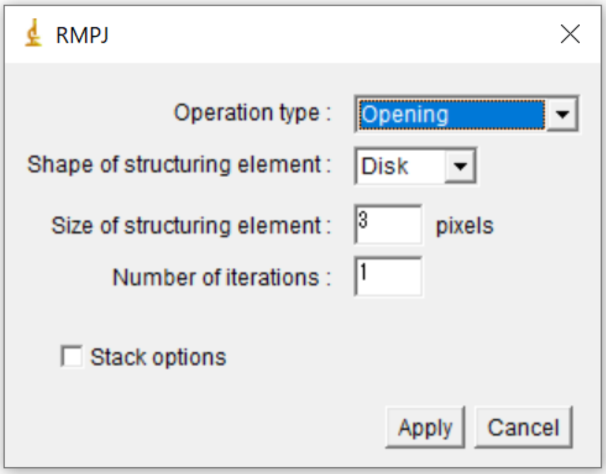


Fig. 4. Graphical user interface of RMPJ.

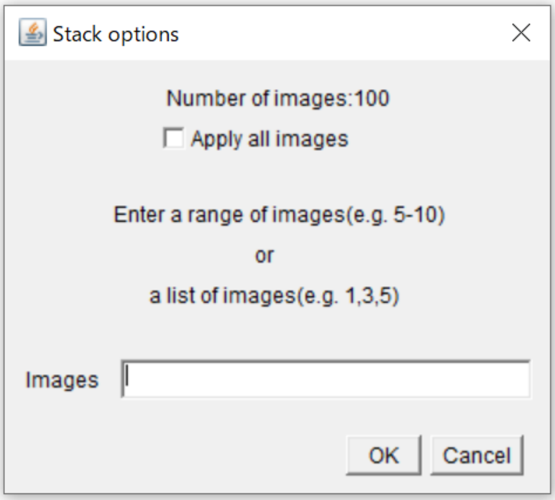


Fig. 5. Dialog box for stack options.

4.2 Interpolation when rotating an image

In this plugin, the bilinear method is used for interpolation when rotating an image. Note that if RMP is applied to a binary image, the output image shows grayscale values due to this interpolation.

4.3 Output file name description

The SE type, size, and *N* values are automatically displayed in the image file name after various operations are applied, thus allowing the processing conditions to be recorded. The names of the operations and SEs are abbreviated as follows:

- Opening: Opn
- Closing: Clos
- White top-hat: WTH
- Black top-hat: BTH
- Morphological smoothing: MS
- Morphological contrast enhancement type 1: MCE1
- Morphological contrast enhancement type 2: MCE2
- Disk-shaped SE: D
- Line-shaped SE: L
- Square-shaped SE: S

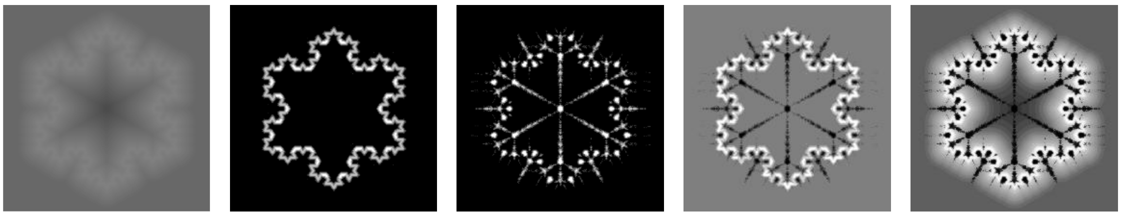
For example, if an opening by RMP ($N = 8$) is applied to the original image named **`sample.tif`** using a disk-shaped SE with a diameter of 3 pixels, the output file name is **`sample-Opn_D3N8.tif`**. If a white top-hat by RMP ($N = 36$) using a line-shaped SE with a length of 11 pixels is applied to this opened image, the output file name is **`sample-Opn_D3N8-WTH_L11N36.tif`**.

5. Illustrative examples

5.1 Extraction and enhancement of low-contrast fine structures in image

Figure 6 shows the results of applying the white top-hat, black top-hat, morphological contrast enhancement type 1, and morphological contrast enhancement type 2 operations to the original image (sample1.tif, 300×300 pixels, Figure 6a). A disk-shaped SE with a diameter of 9 pixels was used for all operations, which were performed under $N = 8$.

Figure 6b presents the results for white top-hat. The ridge structure in the original image (constituting the Koch curve) was extracted. Figure 6c shows the results for black top-hat. The groove structures were extracted. Figure 6d shows the results of the morphological contrast enhancement type 1, in which both the ridge and groove structures were extracted and emphasized. Figure 6e shows the results of the morphological contrast enhancement type 2. The extracted ridge structures were added to the original image, and the groove structures were subtracted from the results. The intensity values of the original images were enhanced along with those of the structures.

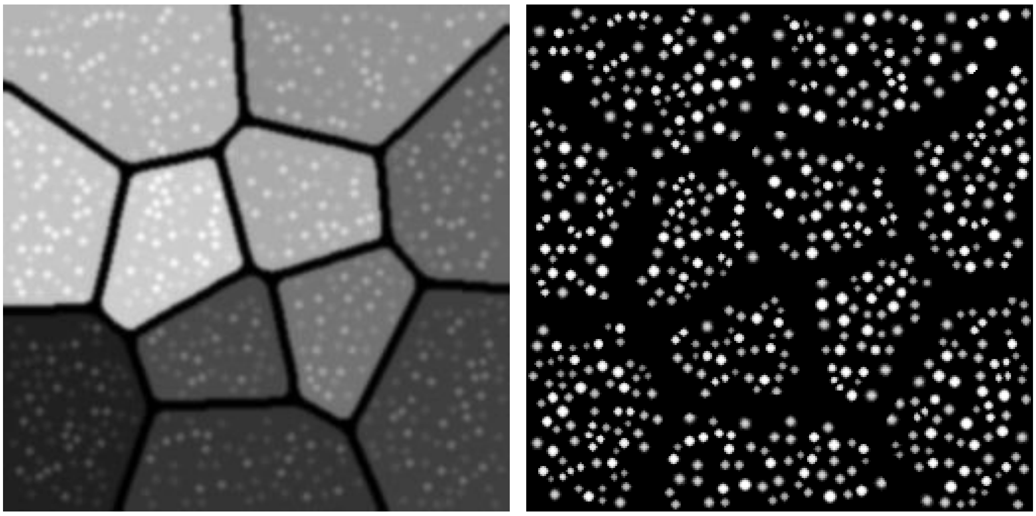


(a) Original (b) White top-hat (c) Black top-hat (d) Enhancement type 1 (e) Enhancement type 2

Fig. 6. Examples of morphological operations for extracting and enhancing low-contrast structures. (a) Original image (sample1.tif). Four morphological operations, i.e., white top-hat, black top-hat, morphological contrast enhancement type 1, morphological contrast enhancement type 2, were performed under the following conditions: **`“Shape of structuring element: Disk”`**, **`“Size of structuring element: 11 pixels”`** and **`“Number of iterations: 8”`**. Figures (b) to (e) show the result of applying these operations to the original image.

5.2 Extraction of aggregated particulate structures

RMP-based operations with line-shaped SE can be used to extract aggregated particulate structures. Figure 7a shows the original image (sample2.tif, 256×256 pixels). Figure 7b shows the result of applying a white top-hat ($N = 36$) with a line-shaped SE of 11 pixels in length to the original image. The result shows that it is possible to separate and extract each of the adjacent particle structures.



(a) (b)

Fig. 7. Examples of extraction of aggregated particulate structures. (a) Original image (sample2.tif). (b) Result of applying white top-hat to original image under the following conditions: **"Shape of structuring element: Line"**, **"Size of structuring element: 9 pixels"** and **"Number of iterations: 36"**.

References

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[4] Soille, P., *Morphological Image Analysis*. Springer, 2nd edition, 2003.

[5] Schulze, M. A. and Pearce, J. A., "Linear combinations of morphological operators: the midrange, pseudomedian, and LOCO filters," *1993 IEEE International Conference on Acoustics, Speech, and Signal Processing*, Minneapolis, MN, USA, **5**, 57-60,1993.

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