Segmentation of nearly isotropic overlapped tracks in photomicrographs using successive erosions as watershed markers – Supplementary Material

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Abstract

This supplementary material contains the methods used to present WUSEM, the complementary results, and the code published with this study.

Keywords: Automatic counting, Diallyl phthalate, Digital image processing, Fission track dating

1. Supplementary Methods

WUSEM (Watershed Using Successive Erosions as Markers) is based on the following techniques: 1. ISODATA threshold; 2. morphological erosion; 3. watershed transform, and 4. labeling. We explain these topics below.

1.1. The ISODATA threshold

The Iterative Self-Organizing Data Analysis Technique (A) (ISODATA) threshold [1, 2] is an histogram-based method. It returns thresholds that separate the image into two pixel classes, where the threshold intensity is halfway between their mean intensities.

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When applied for two classes, ISODATA is always convergent [3]; in our case, these classes are the regions of interest (ROI) and the background. We used the algorithm `filters.threshold_isodata`, implemented in scikit-image.

1.2. Morphological erosion

Erosion is a basic operation in morphological image processing. It acts shrinking the border of all ROI in a binary image by the radius of a chosen structuring element. We used disks as structuring elements for processing the test photomicrographs. The algorithms used were `morphology.disk` and `morphology.erosion`, contained in scikit-image.

1.3. Watershed transform

The watershed algorithm is a non-parametric method which defines the contours as the watershed of the gradient modulus of the gray levels of the input image, considered as a relief surface detection method [4].

In this algorithm, the input image is seen in a three-dimensional perspective. Two dimensions correspond to spatial coordinates, and the third represents the gray levels. In this interpretation, we consider three kinds of points [5]:

1. Points in regional minima.
2. Points where a drop of water would flow to a common minimum.
3. Points where a drop of water would flow to different minima. The set of these points is named *watershed* line.

The aim of watershed algorithms is to find the watershed lines. The method used in this paper is implemented in the function `morphology.watershed`, from scikit-image.

1.4. Labeling algorithm

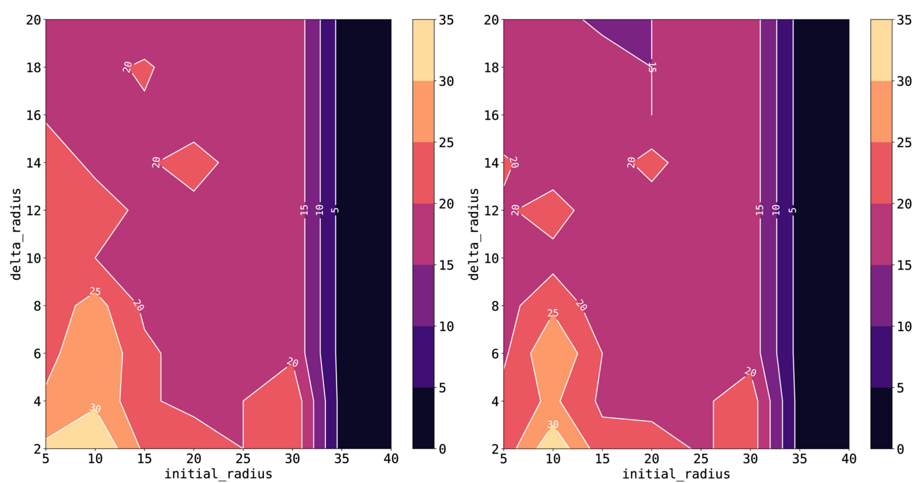
In image processing, the labeling algorithm labels connected regions of a binary input image, according to the 2-connectivity sense: all eight pixels surrounding the reference pixel. Pixels receive the same label when they are connected and have the same value. We used the algorithm `measure.label` from scikit-image, implemented as described in Fiorio et al. [6].

The WUSEM algorithm and the image processing tools developed for separating tracks in overlapping track images are contained in the Python packages available in Section 3.

2. Supplementary Results

2.1. Processing photomicrographs of ^{78}Kr tracks

To observe the counting variation of the WUSEM algorithm, we attributed several values to `initial_radius` (between 5 and 40, step 5) and `delta_radius` (between 2 and 20, step 2). Counting results tend to zero when `initial_radius` is large (Figure 1). Increasing `delta_radius` decreases the number of counted tracks, but its influence is not as significant as the presented by `initial_radius`.



(a) (b)

Figure 1: Contour map representing tracks counted in `orig_figures/dataset_01/Kr-78_4,5min/K90_incid/K90_incid4,5min_1.bmp`, according to the variation of `initial_radius` and `delta_radius`. The number of counted tracks decreases as `initial_radius` increases because the initial structuring element becomes larger than the ROI within the image. The erosion using these larger structuring elements removes ROI smaller than them, hence decreasing the track number. Increasing `delta_radius` decreases the number of counted tracks, but the difference is not significant when compared to `initial_radius`. (a) Considering borders. (b) Ignoring borders. Colormap: `magma`.

According to the comparison between manual and automatic counting, the best parameters for WUSEM are `initial_radius = 5` and `delta_radius = 4` when considering border tracks. This candidate appears for eight of ten samples for 4.5 min and seven of ten samples for 8.5 min.

Number of samples	**Candidates: 4.5 min** (`initial_radius`, `delta_radius`)	**Candidates: 8.5 min** (`initial_radius`, `delta_radius`)
8	**(5, 4)**	–
7	–	**(5, 4)**
6	(5, 6)	(5, 6); (10, 4)
5	(10, 2); (10, 4); (15, 2)	(20, 2)
4	–	(15, 2)
3	(5, 8); (5, 12)	(5, 8); (10, 6); (15, 4)
2	(10, 6)	(5, 10)
1	(5, 10); (5, 14); (10, 10); (10, 20); (15, 4); (15, 8); (20, 2)	(5, 2); (10, 2); (5, 14); (5, 16); (25, 2)

Table 1: Candidates within the established manual tolerance. The best parameters when considering border tracks are `initial_radius = 5` and `delta_radius = 4` (bold), since this candidate appears for eight of ten samples for 4.5 min and seven of ten samples for 8.5 min.

Also according to the comparison between manual and automatic counting, the best parameters for WUSEM are `initial_radius = 25` and `delta_radius = 2` when ignoring border tracks. This candidate appears for nine of ten samples for 4.5 min and eight of ten samples for 8.5 min.

After separating each track, we can obtain their shades of gray (Figure 2) and diameters.

2.2. Processing photomicrographs of fission tracks in DAP

Repeating the previous processes for photomicrographs in dataset 2, we first use the ISODATA threshold. The binarized image is generated for two scenarios: considering and ignoring border tracks (Figure 3). Here, regions in the binary image are also filled using the function `ndimage.morphology.binary_fill_holes()` from scipy.

We can study the automatic counting variation of the WUSEM algorithm attributing different values to `initial_radius` (between 5 and 40, step 5)

Number of samples	Candidates: 4.5 min (`initial_radius`, `delta_radius`)	Candidates: 8.5 min (`initial_radius`, `delta_radius`)
9	(5, 6); (10, 4); (10, 6); (15, 4); (20, 4); **(25, 2)**	–
8	(5, 4); (25, 4)	(15, 2); (20, 2); **(25, 2)**; (30, 2)
7	(15, 8)	(10, 2); (15, 4); (5, 2)
6	(15, 6); (20, 2); (20, 6); (30, 2)	(5, 4)
5	(10, 10); (15, 14); (30, 4)	(10, 4); (20, 4)
4	(5, 12); (10, 8); (10, 18); (10, 20); (15, 16); (20, 8); (20, 10); (25, 6)	(30, 4)
3	(15, 12); (20, 12); (25, 8)	(5, 6); (5, 8); (20, 6); (25, 4); (25, 6)
2	(5, 6); (5, 8); (20, 6); (25, 4); (25, 6)	(10, 6); (30, 8); (35, 2)
1	(5, 14); (10, 16)	(5, 10); (5, 12); (5, 14); (10, 8); (10, 10); (10, 12); (10, 18); (10, 20); (15, 6); (15, 8); (15, 10); (15, 12); (15, 14); (15, 16); (15, 18); (15, 20); (20, 8); (20, 10); (20, 12); (20, 14); (20, 16); (25, 8); (25, 10); (30, 6); (30, 10); (30, 12); (30, 14); (30, 16); (30, 18); (30, 20)

Table 2: Candidates within the established manual tolerance. The best parameters when ignoring border tracks are `initial_radius = 25` and `delta_radius = 2` (bold), since this candidate appears for nine of ten samples for 4.5 min and eight of ten samples for 8.5 min.

and `delta_radius` (between 2 and 20, step 2). Counting results tend to zero when `initial_radius` is large; for the test photomicrograph, the value of `delta_radius` is not significant when `initial_radius` is higher than 10 (Figure 4).

According to the stated comparison, the best parameters are `initial_radius = 10` and `delta_radius = 8` for both scenarios (Table 3).

3. Supplementary Code

The supplementary code and instructions on how to use it are available at `https://github.com/alexandrejaguar/publications/tree/master/2017/dap_segmentation`.

Number of samples	Candidates: 4.5 min (`initial_radius`, `delta_radius`)	Candidates: 8.5 min (`initial_radius`, `delta_radius`)
2	(5, 20); **(10, 8)**	(5, 12); (5, 14); (5, 16); (5, 18); (5, 20); **(10, 8)**
1	(5, 14); (5, 16); (5, 18); (10, 4); (10, 6); (10, 10); (10, 12); (10, 14); (10, 16); (10, 18); (10, 20); (15, 4); (15, 6); (15, 8); (15, 10); (15, 12); (15, 14); (15, 16); (15, 18); (15, 20); (20, 2); (20, 4); (20, 6); (20, 8); (20, 10); (20, 12); (20, 14); (20, 16); (20, 18); (20, 20); (25, 2)	(5, 8); (5, 10); (10, 2); (10, 4); (10, 6); (10, 10); (10, 12); (10, 14); (10, 16); (10, 18); (10, 20); (15, 6); (15, 8); (15, 10); (15, 12); (15, 14); (15, 16); (15, 18); (15, 20); (20, 4); (20, 6); (20, 8); (20, 10); (20, 12); (20, 14); (20, 16); (20, 18); (20, 20); (25, 2); (25, 4); (25, 6); (25, 8); (25, 10); (25, 12); (25, 14); (25, 16); (25, 18); (25, 20); (30, 2); (30, 6)

Table 3: Candidates within the manual established tolerance. The best parameters are `initial_radius = 10` and `delta_radius = 8` (bold), since this candidate appears for both scenarios (considering and ignoring borders).

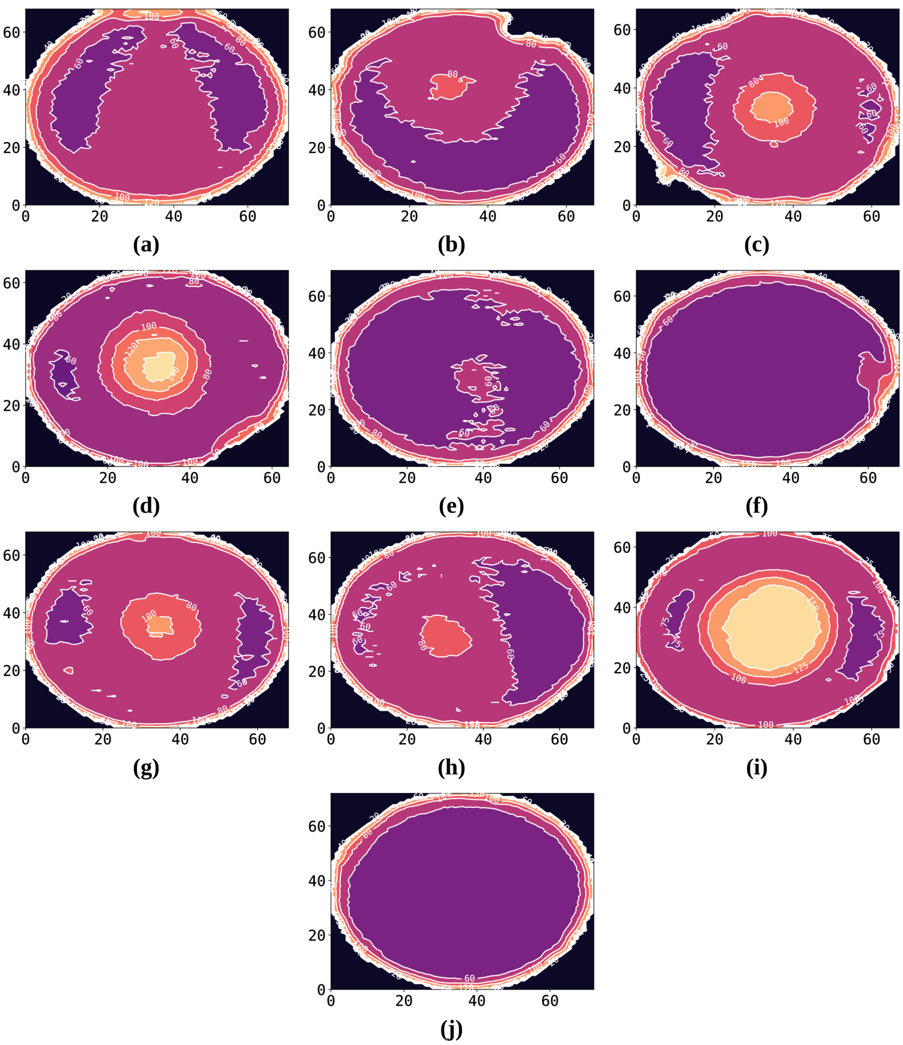


Figure 2: Analysis of the gray shade variation of each track from `orig_figures/dataset_01/Kr-78_4,5min/K90_incid/K90_incid4,5min_1.bmp` using contour maps. Colormap: `magma`.

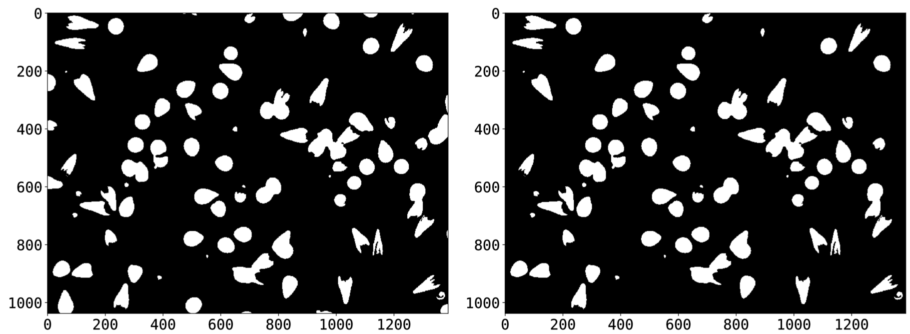
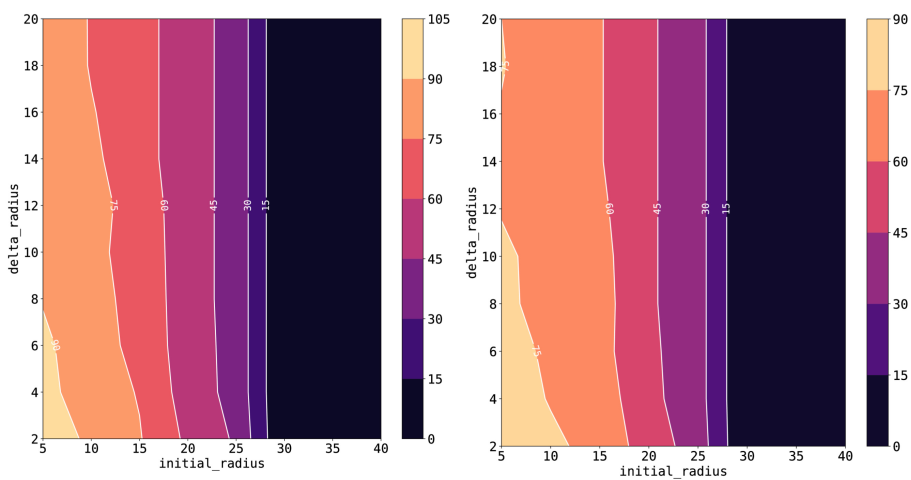
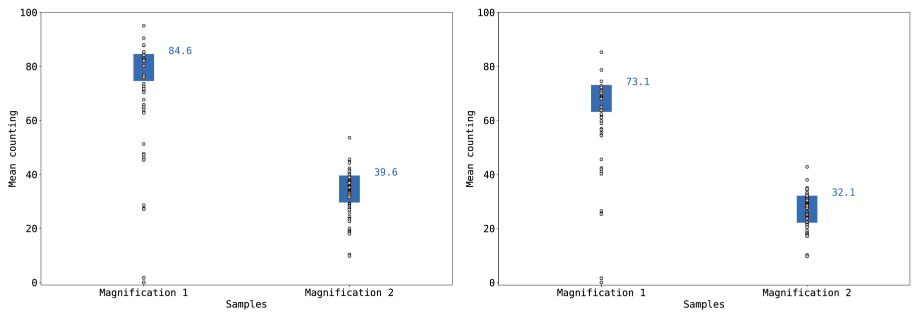

(a) (b)

Figure 3: Input photomicrograph `orig_figures/dataset_02/FT-Lab_19.07.390.MAG1.jpg` binarized using the ISODATA threshold (threshold = 0.5933) and region filling. (a) Considering border tracks. (b) Ignoring border tracks. Colormap: `gray`.



(a) (b)

Figure 4: Contour map representing tracks counted according to the variation of `initial_radius` and `delta_radius` for tracks in `orig_figures/dataset_02/FT-Lab_19.07.390.MAG1.jpg`. The number of counted tracks also decreases as `initial_radius` increases. Increasing `delta_radius` makes no difference in the number of counted tracks when `initial_radius` is higher than 10. (a) Considering borders. (b) Ignoring borders. Colormap: `magma`.



(a) (b)

Figure 5: Manual counting mean (top of the blue bar; values on the right) for each sample and automatic counting results with mean within (yellow points) and outside (gray points) the tolerance interval (blue bar) for the second dataset. (a) Considering borders. (b) Ignoring borders.

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