Segmentation of Partially Overlapping Nanoparticles Using Concave Points

Sahar Zafari[1], Tuomas Eerola[1], Jouni Sampo[2], Heikki Kälviäinen[1,3], and Heikki Haario[2]

[1] Machine Vision and Pattern Recognition Laboratory,School of Engineering Science, Lappeenranta University of Technology, Lappeenranta Finland
[2] Mathematics Laboratory, School of Engineering Science, Lappeenranta University of Technology,, Lappeenranta Finland
Firstname.Lastname@lut.fi
[3] Monash University Malaysia, School of Information Technology, Selangor, Malaysia
heikki.kalviainen@monash.edu

Abstract. This paper presents a novel method for the segmentation of partially overlapping nanoparticles with a convex shape in silhouette images. The proposed method involves two main steps: contour evidence extraction and contour estimation. Contour evidence extraction starts with contour segmentation where contour segments are recovered from a binarized image by detecting concave points. After this, contour segments which belong to the same object are grouped by utilizing properties of fitted ellipses. Finally, the contour estimation is implemented through a non-linear ellipse fitting problem in which partially observed objects are modeled in the form of ellipse-shape objects. The experiments on a dataset consisting of nanoparticles demonstrate that the proposed method outperforms two current state-of-art approaches in overlapping nanoparticles segmentation. The method relies only on edge information and can be applied to any segmentation problems where the objects are partially overlapping and have an approximately elliptical shape, such as cell segmentation.

1 Introduction

Segmentation of overlapping objects aims to address the issue of representation of multiple objects with partial views. Overlapping or occluded objects occur in various applications, such as morphology analysis of molecular or cellular objects in biomedical and industrial imagery where quantitative analysis of individual objects by their size and shape is desired [1–3]. In many such applications, the objects can often be assumed to have approximately elliptical shape. For example, the most commonly measured properties of nanoparticles are their length and width, which can correspond to the major and minor axis of an ellipse fitted over the particle contour [4].

Even with rather strong shape priors, segmentation of overlapping objects remains a challenging task. Deficient information from the objects with occluded or overlapping parts introduces considerable complexity into the segmentation process. For example, in the context of contour estimation, the contours of objects intersecting with each other do not usually contain enough visible geometrical evidence, which can make contour estimation problematic and challenging. Frequently, the segmentation method has to rely purely on edges between the background and foreground, which makes the processed image essentially a silhouette image (see Fig. 1). Furthermore, the task involves simultaneous segmentation of multiple objects. A large number of objects in the image causes a large number of variations in pose, size and shape of the objects, and leads to a more complex segmentation problem.

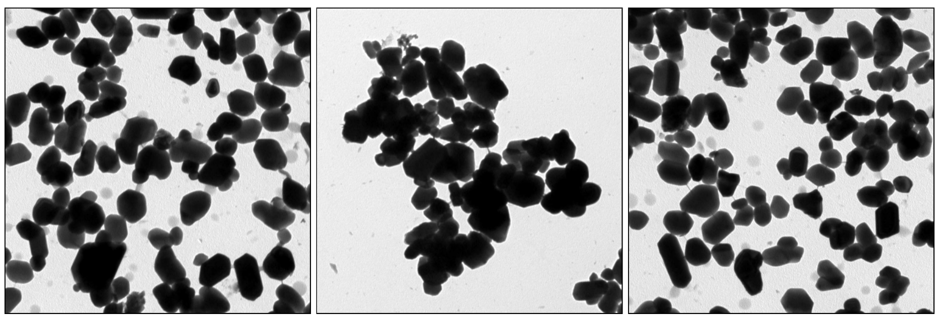


Fig. 1: Overlapping nanoparticles.

Several approaches have been proposed for the segmentation of overlapping objects in various applications. The watershed transform is one of the commonly used approaches in overlapping cell segmentation [5–7]. However, methods based on the watershed transform suffer from a poor or inadequate initialization and may experience difficulties with segmentation of highly overlapped objects in which a strong gradient is not present.

Several approaches have resolved the segmentation of overlapping objects within the variational framework through the use of active contours [8, 9]. The efficiency of the active contour based methods depends highly on the accuracy of the model initialization. Active contour based methods are also computationally heavy when the amount of objects is large.

Also morphological operations have been used for overlapping object segmentation. In [1], an automated morphology analysis coupled with a statistical model for contour inference to segment partially overlapping nanoparticles was proposed. The method is prone to under-segmentation with highly overlapped objects.

In [2], the problem of overlapping objects segmentation was approached using concave points extraction through the polygonal approximation and ellipse fitting. Although this approach is efficient for regular shaped objects, such as bubbles, objects with a shape that deviates from elliptical shape, such as nanoparticles, cause problems for the method. In [10], the problem of incomplete cells with non-elliptical shapes was addressed by proposing a method combining certain heuristics with concave point extraction. In [11], a modified generalized hough transform for recognizing partially occluded objects was proposed. The method is, however, computationally expensive if the number of objects is large.

In this paper, a novel and efficient method is proposed for the segmentation of partially overlapping nanoparticles with a convex shape. The nanoparticles are assumed to be clearly distinguishable from the background of the image and their contours form approximately elliptical shapes. The proposed method relies on two sequential steps of contour evidence extraction and contour estimation. The contour evidence extraction step is further divided into two sub-steps: contour segmentation and segment grouping. In the contour segmentation step, object contours are divided into separate contour segments. In the segment grouping step, contour evidences are built by joining the contour segments that belong to the same object. Once the contour evidence is obtained, contour estimation is performed using numerically stable direct ellipse fitting. The proposed method relies only on edge information and can be applied also to other segmentation problems where the objects are partially overlapping and have approximate elliptical shapes, such as cell segmentation.

The main contribution of this work is a novel combined model for contour evidence extraction that relies on detection of concave points through curvature analysis, the concavity test and an efficient search procedure. The proposed method is shown to outperform earlier methods in the task of overlapping nanoparticles segmentation based on detection rate and segmentation accuracy.

2 Overlapping Object Segmentation

The proposed method consist of two consecutive main step: contour evidence extraction and contour estimation. Fig. 2 summarizes the method. Given a gray-scale image as input, the segmentation process starts with pre-processing to build an image silhouette and the corresponding edge map. The binarization of the image is obtained by background suppression based on the Otsu's method [12] along with morphological opening to smooth the object boundaries. The edge map is constructed using the Canny edge detector [13]. In the contour evidence extraction steps, edge points that belonged to each object are grouped using concave points and properties of fitted ellipses. Once the contour evidence has been obtained, contour estimation is carried out to infer the missing parts of the overlapping objects.

2.1 Contour Evidence Extraction

The first step of the proposed method is to extract the contour evidence containing the visible parts of the objects boundaries that can be used to inference the

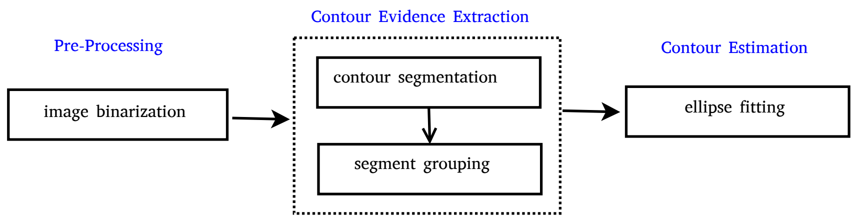


Fig. 2: Proposed method.

occluded parts of overlapped objects. The contour evidence extraction involves two separate tasks: contour segmentation and segment grouping.

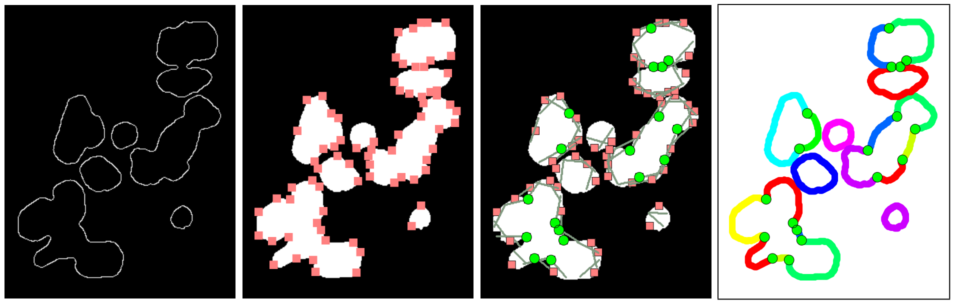
Contour Segmentation. A partial overlap between two or more elliptic-shape objects leads to a concave shape with concave edge points that correspond to the intersections of the object boundaries. It is a common practice to utilize these concave points to segment the contour of overlapping objects. Different methods such as polygonal approximation [2, 10], curvature [14], and angle [15, 10] have been applied to determine the location of concave points in the image.

In this work, after extracting the image edge by canny edge detector [13], the concave points are obtained through the detection of corner points followed by the concavity test. The corner points are detected using the modified curvature scale space (CSS) method based on curvature analysis [16]. The output of the corner detector includes the points with the maximum curvature lying on both concave and convex regions of object contours. Since being only interested in the concave points joining the contours of overlapping objects, the detected corner points are examined if they lie on concave regions.

Let us denote a detected corner point by p_i, and its two kth adjacent contour points by p_{i-k} and p_{i+k}. The corner point p_i is qualified as concave if the line connecting p_{i-k} to p_{i+k} does not reside inside the object. The obtained concave points are used to split the contours into contour segments.

Fig. 3 shows an example of concave point extraction and contour segmentation.

Segment Grouping. Due to the overlap between the objects and the irregularities in the object shapes, a single object may produce multiple contour segments. Segment grouping is needed to merge all the contour segments belonging to the same object. The basic idea behind the proposed method for segment grouping is to find a group of contour segments that together form an object with



(a) (b) (c) (d)

Fig. 3: Contour segmentation: (a) Edge map; (b) Corner detection by [16]; (c) Concavity test to extract concave corners (green circle) and removed convex corners (pink sqaure); (d) Contour segmentation by concave points (the colors are used only for illustrative purpose to visualize the segmented contour by concave points).

elliptical shape. Segment grouping in its naive form, iterates over each pair of contour segment, examining if they can be combined. In this work, to optimize the grouping process, a limited search space is applied and the contour segment under grouping process is only examined with the neighbouring segments. Two segments are neighbour if the Euclidean distance between their center points is less than the predefine threshold value.

The contour segment grouping is carried out through the process of ellipse fitting. Given a pair of contour segments, s_i and s_j, and a function measuring the goodness of ellipse fitting, the segment s_i is grouped to s_j if the goodness of ellipse fitted to the joint segments is higher compared to the goodness of ellipses fitted to each individual contour segments separately.

The goodness of fit is described as average distance deviation (ADD) [2] which measures the discrepancy between the fitted curve and the candidate contour points. The lower value of ADD indicates higher goodness of fit and, therefore the joint rule to perform segment grouping in terms of ADD is defined as

$$\begin{aligned} \mathrm{ADD}_{s_i \cup s_j} &\leq \mathrm{ADD}_{s_i}, \\ \mathrm{ADD}_{s_i \cup s_j} &\leq \mathrm{ADD}_{s_j}, \end{aligned} \tag{1}$$

where the definition of ADD is as follows:

Given the contour segment s_i consisting of n points, $s_i = \{p_k(x_k, y_k)\}_{k=1}^{n}$, and the corresponding fitted ellipse points, $s_{f,i} = \{p_{f,k}(x_{f,k}, y_{f,k})\}_{k=1}^{n}$, ADD_{s_i} is defined as

$$\mathrm{ADD}_{s_i} = \frac{1}{n} \sum_{k=1}^{n} \sqrt{(x_k - x_{f,k})^2 + (y_k - y_{f,k})^2}. \tag{2}$$

Within the transformed coordinate system

$$\begin{bmatrix} x'_k \\ y'_k \end{bmatrix} = \begin{bmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} x_k - x_{eo} \\ y_k - y_{eo} \end{bmatrix}, \tag{3}$$

Eq. (2) can be simplified to

$$\mathrm{ADD}_{s_i} = \frac{1}{n}\Big[\sum_{k=1}^{n}\sqrt{x'^2_k + y'^2_k}(1 - \frac{1}{|D_k|})\Big], \tag{4}$$

where D_k is given by

$$D_k^2 = \frac{x'^2_k}{a^2} + \frac{y'^2_k}{b^2}, \tag{5}$$

and a, b, (x_{eo}, y_{eo}) and θ are the ellipse parameters, the semi-major axis length, the semi-minor axis length, the ellipse center point, and the ellipse orientation angle with respect to x axis, respectively.

The plain ADD criterion for segment grouping often leads to undesired results if the contour points do not strictly fit to the ellipse model. In order to address this issue, additional rules are needed.

Given a pair of contour segments to be processed for grouping, the segment with longer length usually provides a more reliable clue to the object than the shorter one. Based on this assumption, a weighing scheme using the length of contour segments is added to the grouping process where the ADD of contour segment with longer length is down-weighted by the ratio of its length with respect to the total length of contour segments to be grouped. Assuming the contour segment s_i is longer than contour segment s_j, Eq. (1) is replaced by

$$\begin{aligned} \mathrm{ADD}_{s_i \cup s_j} &\leq w_i \mathrm{ADD}_{s_i}, \\ \mathrm{ADD}_{s_i \cup s_j} &\leq \mathrm{ADD}_{s_j}. \end{aligned} \tag{6}$$

where

$$w_i = \frac{l_i}{l_i + l_j},$$

and l_i and l_j are the lengths of contour segments s_i and s_j, respectively.

The contour segments in far proximity are less likely to represent a single object and should not be merged. As the result, the two contour segments whose ellipse models are at very far distance from each other should not grouped. Either, ellipse fitted to the combined contour segments should not be at far distance from the ellipses fitted to each individual contour segments. Following these conventions and being interested in grouping of close contour segments, two additional rules are applied similarly to [10].

Let us denote the centroids of the fitted ellipse for the contour segments s_i, s_j and $s_{i\cup j}$ by e_i, e_j and $e_{i\cup j}$, respectively. The contour segments s_i and s_j

should not be grouped as a single segment provided that, first, the distance from the ellipse centroid of the combined contour segments $e_{i \cup j}$ to the center of its members,e_i and e_j, is larger than the preset threshold t_1:

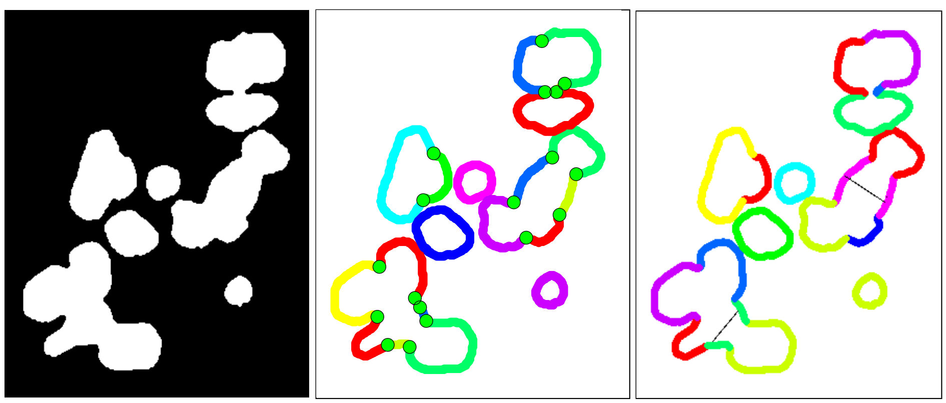
$$\begin{aligned} d(e_i, e_{ij}) &> t_1 \\ d(e_j, e_{ij}) &> t_1, \end{aligned} \tag{7}$$

and second, the distance between their corresponding ellipse centroids is larger than the predefined threshold t_2

$$d(e_i, e_j) > t_2, \tag{8}$$

where $d(p_1, p_2)$ is the Euclidean distance between points p_1 and p_2.

The value of t_1 can be determined using the object properties [10] and is usually close to the length of the minor axis of fitted ellipses to the smallest object in the image. The value of t_2 should be set in such way that prevents the grouping of the contour segment belong to different objects or as [10] proposed 2.5 to 4 times higher than the threshold t_1. Fig 4 shows an example of segment grouping.



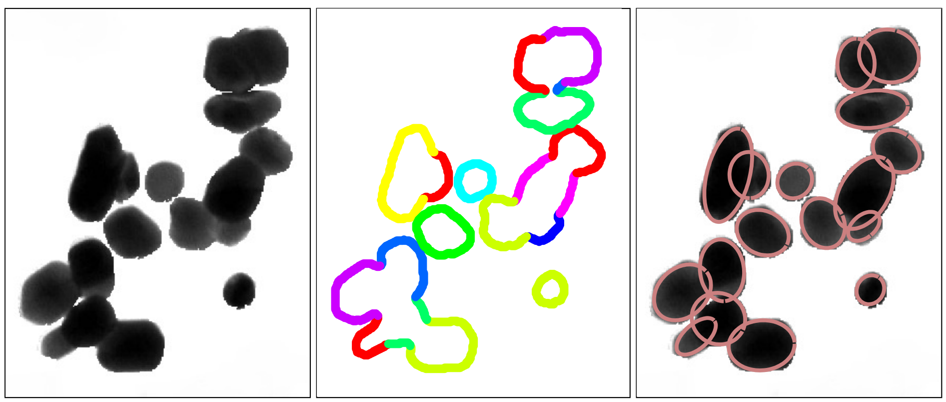
(a) (b) (c)

Fig. 4: Segment grouping: (a) Original binary image; (b) Contour segmentation; (c) Segment grouping (the thin gray lines are added to illustrate the grouping of non-adjacent segments).

2.2 Contour Estimation

The last step of proposed method is the contour estimation, where, by means of the visual information produced from the previous step, the missing parts of the object contours are estimated. Ellipse fitting is a very common approach in overlapping object segmentation, especially in the medical and industrial applications.

The most efficient recent ellipse fitting methods based on shape boundary points are generally addressed through the classic least square fitting problem. In this work, the contour estimation is addressed through a stable direct least square fitting method [17] where the partially observed objects are modeled in the form of ellipse-shape objects. Fig. 5 shows an example of contour estimation applied to contour evidences.



(a) (b) (c)

Fig. 5: Contour estimation: (a) Original image; (b) Contour evidence extraction; (c) Contour estimation.

3 Experiments

3.1 Data

The experiments were carried out using a dataset consisting of nanoparticles images captured by transmission electron microscopy. In total, the dataset contains 11 images of 4008×2672 pixels. Around 200 particles were marked manually in each image by an expert. The annotations consist of manually drawn contours of the objects. Since not all the objects are marked, a pre-processing step was applied to eliminate the unmarked objects from the images. It should be noted that the images consist of dark objects on a white background and, therefore, pixels outside the marked objects could be colored white without making the images considerably easier to analyze.

3.2 Results

To evaluate the method performance and to compare the methods, two specific performance measures, True Positive Rate (TPR) and Positive Predictive Value (PPV), were used:

$$TPR = \frac{TP}{TP + FN} \tag{9}$$

$$PPV = \frac{TP}{TP + FP} \tag{10}$$

where True Positive (TP) is the number of correctly segmented objects, False Positive (FP) is the number of incorrectly segmentation results and False Negative (FN) is the number of missed objects.

To decide whether the segmentation result was correct or incorrect, Jaccard Similarity coefficient (JSC) [18] was used. Given a binary map of the segmented object O_s and the ground truth particle O_g, JSC is computed as

$$JSC = \frac{O_s \cap Og}{O_s \cup Og}. \tag{11}$$

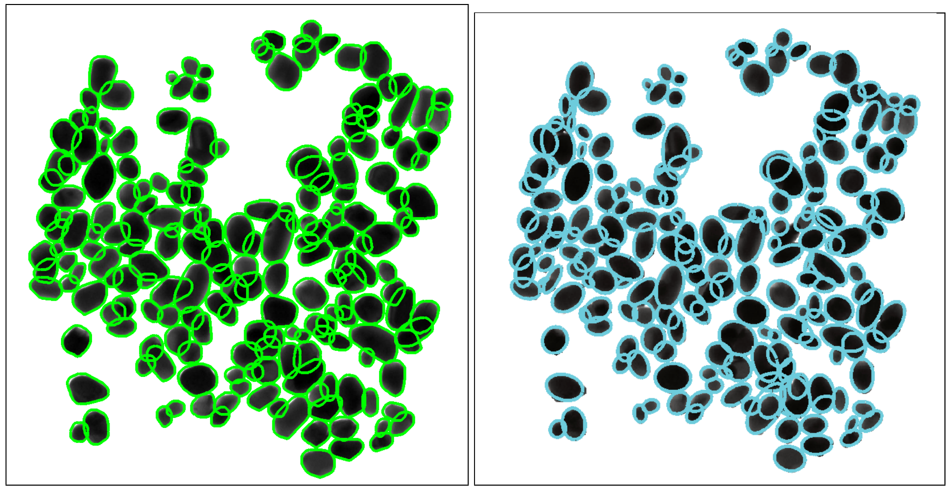
The threshold value for the ratio of overlap in the JSC was set to 0.5. The average JSC (AJSC) value was used as the third measure to evaluate the segmentation performance.

The method parameters, k, t_1 and t_2 were set experimentally to 10, 23 and 45, respectively. Fig. 6 shows an examples of proposed method segmentation result in the nanoparticles dataset. The performance of the proposed segmentation method was compared to two existing state-of-the-art methods, Nanoparticles Segmentation (NPA) [1] and Concave-point Extraction and Contour Segmentation (CECS) [2]. The NPA and CECS methods are particularly chosen as previously applied for segmentation of overlapping convex and elliptical shape objects, respectively. The implementation made by the corresponding authors was used for NPA [1]. CECS was implemented by ourselves based on [2]. Examples of typical segmentation results are presented in Fig. 7. NPA suffers from under-segmentation while CECS tends to over-segment the objects. The proposed method neither under- or over segments the objects.

The corresponding performance statistics of the competing methods applied to the dataset are shown in Table 1. As it can be seen, the proposed method outperforms the other two with respect to the TPR and JSC and achieves a comparable performance with NPA in terms of PPV. The high JSC value of the proposed method indicates its superiority with respect to the resolved overlap ratio.

3.3 Computation Time

The proposed method was implemented in MATLAB, using a PC with a 3.20 GHz CPU and 8 GB of RAM. With the selected combination of parameters the



(a) (b)

Fig. 6: An example of the proposed method segmentation result on nanoparticles dataset: (a) Ground truth; (b) Proposed method;

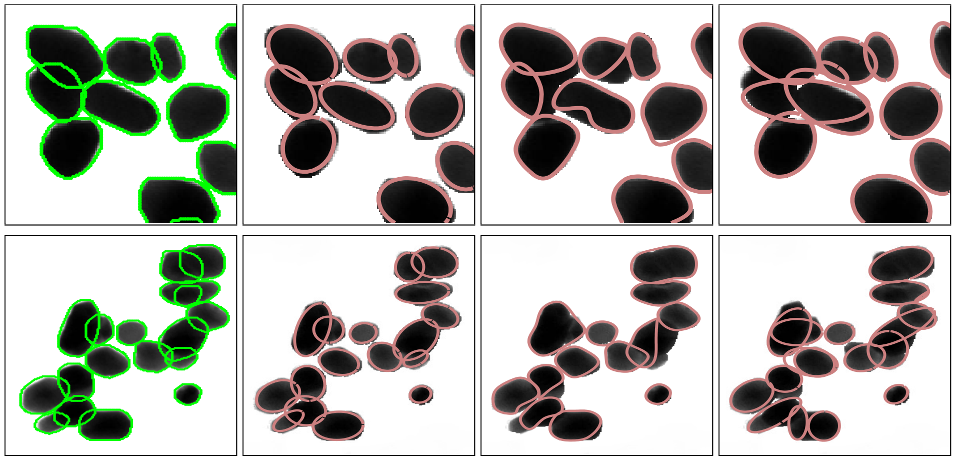
Table 1: Comparison of the performance of the proposed method for the nanoparticles dataset.

Methods	TPR [%]	PPV [%]	AJSC [%]
Proposed	**85**	84	**73**
NPA	62	**90**	58
CECS	66	73	53

computational time was 21 seconds per image, while NPA demanded 200 seconds and CECS 77 seconds. The computational time breakdown was as follows: contour segmentation 3%, segment grouping 95%, and ellipse fitting 2%. However, it should be noted that the method performance was not optimized and the computation time could be improved.

4 Conclusions

This paper presented a method to segment multiple partially overlapping convex shaped nanoparticles in silhouette images using concave points and ellipse properties. The proposed method consisted of two main steps: contour evidence extraction to detected the visible part of each object and contour estimation to estimate the final objects contours. The experiments showed that the proposed method achieved high detection and segmentation accuracies and outperformed two competing methods on a dataset of nanoparticles images. The proposed



(a) (b) (c) (d)

Fig. 7: Example segmentation results on slice of nanoparticles dataset: (a) Ground truth; (b) Proposed method; (c) NPA; (d) CECS.

method relies only on edge information and can be applied also to other segmentation problems where the objects are partially overlapping and have an approximately elliptical shape, such as cell segmentation.

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