Segmenting overlapped cell clusters in biomedical images by concave point detection

Miquel Miró-Nicolau\textsuperscript{a}, Gabriel Moyà-Alcover\textsuperscript{a,\ast}, Manuel González-Hidalgo\textsuperscript{b,c}, Antoni Jaume-i-Capó\textsuperscript{a}

\textsuperscript{a}UGiVIA Research Group, University of the Balearic Islands, Dpt. of Mathematics and ComputerScience, 07122 Palma, Spain
\textsuperscript{b}SCOPIA Research Group, University of the Balearic Islands, Dpt. of Mathematics and ComputerScience, 07122 Palma, Spain
\textsuperscript{c}Health Research Institute of the Balearic Islands (IdISBa), E-07010, Palma, Spain

Abstract

In this paper we propose a method to detect concave points as a first step to segment overlapped objects on images. Given an image of an object cluster we compute the curvature on each point of its contour. Then, we select regions with the highest probability to contain an interest point, that is, regions with higher curvature. Finally we obtain an interest point from each region and we classify them between convex and concave. In order to evaluate the quality of the concave point detection algorithm we constructed a synthetic dataset to simulate overlapping objects, providing the position of the concave points as a ground truth. As a case study, the performance of a well-known application is evaluated, such as the splitting of overlapped cells in images of peripheral blood smears samples of patients with sickle cell anaemia. We used the proposed method to detect the concave points in clusters of cells and then we separate this clusters by ellipse fitting. Experimentally we demonstrate that our proposal has a better performance than the state-of-the-art.

Keywords: Image processing, Computer vision, Overlapped objects, Segmentation, Concave points, sickle-cell disease.

1. Introduction

Segmenting overlapped objects on images is a process that can be used as a first step in many biomedical and industrial applications, from blood cells study \cite{9} to grain analysis \cite{25}.

In these applications an analysis of individual objects by their singular features is typically required. The existence of image areas with overlapping objects reduce the available

\ast Corresponding author

Email addresses: miquel.miro@uib.es (Miquel Miró-Nicolau), gabriel.moya@uib.es (Gabriel Moyà-Alcover), manuel.gonzalez@uib.es (Manuel González-Hidalgo), antoni.jaume@uib.es (Antoni Jaume-i-Capó)
information for some of the objects in the image, the so-called occlusion zones. These occlusion zones introduce an enormous complexity into the segmentation process and makes it a challenging problem that can be solved from multiple points of view and is still open.

In this paper we propose a new method to detect concave points that increases the precision with respect to state-of-the-art methods. We constructed a synthetic dataset to simulate overlapping objects and providing the position of the concave points as a ground-truth. We used this dataset to compare the detection capacity and the spatial precision of the proposed method with the state-of-the-art. As a case study, we evaluated the proposed concave point detector with a well-known application, such as the splitting of overlapped cells in microscopic images of peripheral blood smear samples of red blood cells (RBC) of patients with sickle-cell disease (SCD). The purpose of the case study was to check if the spacial precision of the concave points detector method affects the results of a classification algorithm of the morphology of RBC in a real world scenario. It is important to point out that this algorithm is not limited to cell segmentation, it can be used for other applications where separation between overlapping objects is required.

Multiple strategies have been used to address this problem. Watershed algorithm [22, 19, 16] and level sets [3] are widely used on scenarios with well-defined boundaries between the overlapped objects. However, these methods are unable to separate the overlapped objects with an homogeneous intensity values because the detection of initial markers is not accurate, provoking a diffuse boundary.

To avoid these problems, another type of segmentation methods that can be used are those based on the detection of concave points. These points denote the positions where the contours of the different objects overlap and, at the same time, are the locations where the overlapped object changes from one of its sub-objects to another one. Once the concave points are detected, multiple techniques can be used to divide these objects. The advantages of the detection of concave points is that those are invariant to scale, color and orientation.

Furthermore, the methods based on the detection of concave points can perform a good segmentation without using a big dataset and without input image size constraints unlike deep learning methods. Moreover, the detection of concave points for segmenting overlapped objects can be considered to be transparent because presents simulatability, decomposability and algorithmic transparency [1].

The remainder of this paper is organized as follows. In the next section we describe the related work. In Section 2 we explain the proposed method for the efficient detection of concave points. In Section 4 we specify the experimental environment and we describe the datasets used for experimentation. Section 5 is devoted to discuss the results and comparison experiments obtained after applying the proposed method to synthetic and real images of clusters of objects. Finally, in Section 6 we give the conclusions of our work.

2. Related Work

In the state-of-the-art we can find multiple approaches based on concave point detection. Following the taxonomy proposed by Zafari et. al. [28], but adding a category for other methods, we classified these methods in four categories: skeleton, chord, polygon approximation,
curvature and others.

2.1. Skeleton

The approaches based on skeleton use the information of the boundary and its medial axis to detect concave points. Song and Wang \cite{21} identified the concave points as local minimums of the distance between the boundary of the object and its medial axis. The medial axis was obtained by an iterative algorithm based on the binary thinning algorithm followed by a pruning method. Samma \textit{et al.} \cite{20} found the concave points by intersecting the boundary of the object, obtained by applying the morphological gradient and the skeleton of the dilated background image.

These methods need a big change in the curvature to detect the existing concave points, so, skeleton-based methods tends to fail on contours objects with smooth curvatures.

2.2. Chord

Chord methods use the boundary of the convex hull area of the overlapped objects. This boundary consists of a finite union of curve segments, each of one is an arc of the object’s contour $C$, or it is a line with its end points on $C$, named a chord. If $L_i$ is one of those lines (a chord) and $C_i$ is the segment of $C$ with its same end points, the union of $L_i$ with $C_i$ generate a simple closed curve, which determines a convexity defect. Chord methods use the convex hull contour and the convexity defects to detect concavities. The main idea of these approaches is to identify the furthest points between the contour and the convexity defect.

Multiple solutions used the chord analysis to extract convex points. Farhan \textit{et al.} \cite{7} proposed a method to obtain the concave points by evaluating the line fitted to the contour points, a concave point is detected if the line that joins the two contour points does not reside inside the object. The proposal of Kumar \textit{et al.} \cite{13} used the boundaries of concave regions and their corresponding convex hull chords. The concave points were defined as the points on the boundaries of concave regions that maximize perpendicular distance from the convex hull chord. Similarly, Yeo \textit{et al.} \cite{26} and LaTorre \textit{et al.} \cite{14} proposals applied multiple constraints to the area between the convexity defect and the contour to determine its quality.

Chord methods consider that only exists one concave point for each convexity defect, in clusters with more than two objects this assumption is not always true, therefore some concave points are missdetected.

2.3. Polygon Approximation

Polygon approximation is a set of methods that represents the contours of the objects through a sequence of dominant points. These methods aims to remove noise approximating the contour to a simpler object.

Bai \textit{et al.} \cite{2} developed a brand new algorithm to follow this approximation. Their algorithm analysed the difference between a set of contour points and the straight line that connected their extremes. The points with a big distance to this previously defined line were considered dominant points. Chaves \textit{et al.} \cite{4} used the well-known algorithm of
Ramer–Douglas–Peucker (RDP) \cite{6} to approximate the contour and the concave points were detected using conditions on the gradient direction at the contour. Another similar approach to detect these points was presented in \cite{29} by Zafari et al. where the authors proposed a parameter free concave point detection to extract dominant points. They selected the concave ones using a condition based on the scalar product of consecutive dominant points described in \cite{32}.

Same authors \cite{27}, used a modified version of curvature scale space proposed in \cite{11} to find interest points. Finally they discriminated them between concave and convex points.

Zhang et al. \cite{30}, similarly to \cite{27}, used the modified version of curvature scale space to make the object approximation. From the application of the previously described object approximation algorithms they obtained a set of dominant points, among them the concave points were found. The concave points were detected by evaluating angular change on these dominant points, and these angular changes were evaluated using the \texttt{arctangent} criteria. The points with an angular change higher than a threshold were classified as concave points.

These methods are highly parametric and are not robust to object size changes. Another weakness of these methods is that they deform the original silhouette to simplify it. The approximation is a trade-off between the lack of precision in the position of the concave points and the smooth applied to the contour. This trade-off affects the final results.

2.4. Curvature

The methods that falls in this category identifies the concave points as the local extreme of the curvature. The curvature, $\kappa$, at each point $q_i = (x_i, y_i)$ of the contour is computed as:

$$\kappa(q_i) = \frac{x_i' \cdot y_i'' - y_i' \cdot x_i''}{(x_i'^2 + y_i'^2)^{3/2}},$$

where $x_i$ and $y_i$ are the coordinates of the contour points.

Wen et al. \cite{24} calculated the derivative by convolving the boundary with Gaussian derivatives. González-Hidalgo et al. \cite{9} used the $k$-curvature and the $k$-slope to approximate the value of the curvature. The dominant points, the ones with most curvature, can be located in both concave and convex regions of the contours. In \cite{28} three different heuristics are described to detect the concave ones.

These methods tend to fail when multiple concave points are located in small areas, the main reason for this error is the loss of precision by the approximation of the curvature value. Another source of problems is the existence of noise, these algorithms tend to identify the noise on the contour as changes on the curvature, one way to fix this problem is to use a more coarse approximation.

2.5. Other methods

Despite the taxonomy proposed by Zafari et al. there are other techniques to find concave points that do not fall into any of the previous categories. We describe some of these works below.

Fernández et al. \cite{8} defined a sliding window for the contour and calculates the proportion of pixels that belongs to the object and the pixels that belong to the background on this
window. This proportion determined the likelihood of an existing concavity on the evaluated point. He et al. [12] adapted this method to use it in three dimensions. Best results were obtained in scenarios with high concavity. This method is very sensitive to changes on the size of the objects and its accuracy decreased with the existence of noise. These two problems are a consequence of the lack of generalization of the method.

Wang et al. [23] proposed a bottleneck detector. They defined a bottleneck as a set of two points that minimize its euclidean distance and maximize the distance on the contour. The set of points that defined a bottleneck were the concave points. A cluster can contain multiple bottlenecks. This algorithm was unable to discover how many elements belong to a cluster, the number of elements is an hyperparameter of the algorithm. Another limitation was that they did not considered clusters with an odd number of concave points.

Zhang and Li [31] proposed a method to find the concave points with a two step algorithm. First, they detected a set of candidates points with the well known Harris corner detector [10]. Second, they selected the concave points with two different algorithms, one for obvious concave points and another for uncertain concave points. Their algorithm have a high number of parameters. This higher number of parameters have two different consequences: on one hand the method is highly adjustable to the features of the overlapped objects, on the other hand this amount of parameters provoke a high complexity of the algorithm.

Motivated by the state-of-the-art performance and with the aim of improving the results on the challenging task of separating overlapped objects, we developed a new algorithm inspired on the works of Chaves et al using the RDP algorithm and modifying the detection of the points of interest and the selection of the concave points proposed in González-Hidalgo et al.

3. Methodology

We detected the concave points by analyzing the value of the curvature in every contour point. A contour is a set of n ordered points with orientation that defines the shape of an object, $c = \{(x_0, y_0), (x_1, y_1), \ldots, (x_{n-1}, y_{n-1})\}$. The whole process is summarized in Figure 1.

We divided the proposed method into four consecutive steps. Given an object cluster, we simplified its contour using the RDP algorithm [6] as the first step. In the second step, we computed the curvature on each point of the contour and we selected subsets of adjacent points with the highest curvature values, that is, the subsets with the highest probability to contain an interest point. As third step we obtained an interest point from each one of the subsets from the previous step. Finally, we identified the concave points.

3.1. Curvature

We approximated the value of the curvature through a well-known technique, the $k$-curvature [18]. This technique considers the curvature of every point as the difference of its slope. The $k$-curvature is separable, it allowed us to made the calculation independently for each direction. The Eq. (2) and Eq. (3) define the $k$-slope for the horizontal ($m_x$) and
vertical \( (m_y) \) axis, while the \( k \)-curvature in each direction, \( z_x \) and \( z_y \), is defined by the Eq. (4) and Eq. (5).

\[
m_x(i, k) = \frac{y_i - y_{i+k}}{x_i - x_{i+k}},
\]

\[
m_y(i, k) = \frac{1}{m_x(i, k)} = \frac{x_i - x_{i+k}}{y_i - y_{i+k}},
\]

\[
z_x(i, k) = m_x(i, k) - m_x(i - k, k),
\]

\[
z_y(i, k) = m_y(i, k) - m_y(i - k, k),
\]

where \( x_i \) and \( y_i \) are the horizontal and vertical coordinates of the \( ith \) point and \( k \) is the amount of displacement between the points used to calculate the curvature.

The \( k \)-curvature at each point is the product of the absolute values of the \( k \)-curvature values in each direction, see Eq. (6).

\[
z(i, k) = |z_x(i, k)| \cdot |z_y(i, k)|.
\]

As a result of this first step, we assigned a curvature value to each contour point.

### 3.2. Points of interest

In this section we define a methodology to find points of interest by analyzing the curvature of each contour point. A point of interest can be a concave or convex point. Therefore, the input of this step is the list of contour points with the values of its curvatures.

The first step to obtain the interest points was to determine subsets of contiguous points that included those with highest curvature. We called these subsets, regions of interest. All points that belong to a region of interest have a curvature greater than a certain threshold, it is defined by its start and end points. The process to determine the regions of interest
is a recursive procedure that we describe below, which provided a list of regions of interest and ensured the presence of a point of interest within each of them.

Let $C$ and $t$ be the set of contour points and an initial threshold for the curvature value, respectively. We also defined two other thresholds to control the length of the regions of interest, namely $l_{\text{min}}$ and $l_{\text{max}}$. The $l_{\text{min}}$ value aims to avoid having an excessive number of regions and reduce the effect of noise on the object contour. The $l_{\text{max}}$ value is useful to prevent that the point of interest is located in an excessively large region, as we were interested to extract one concave point from each region. The recursive procedure that allowed us to detect the regions of interest is as follow:

**Step 1:** Taking the contour points $C$, we constructed a list of regions of interest $\text{l\_regions}$ selecting all non-overlapping sets of adjacent points which curvature was greater than $t$. If necessary we updated $t$. The original image is displayed in Figure 2a. Figure 2b depicts the list of regions of interest detected, different regions of interest are marked with different colors.

**Step 2:** Let $r$ be a region of interest in $\text{l\_regions}$, we denote by $\ell(r)$ its length. We considered three possible cases:

- **Case 1:** If $l_{\text{min}} \leq \ell(r) \leq l_{\text{max}}$, we continued with the next region of interest.
- **Case 2:** If $\ell(r) > l_{\text{max}}$, we returned to Step 1 with $r$ and $t + \delta t$. We updated $\text{l\_regions}$ with the regions into which $r$ is divided, and $r$ is removed from the list.
- **Case 3:** If $\ell(r) < l_{\text{min}}$, we combined the region $r$ with its closest region. That is, we looked for $r_{\text{closest}}$ such that $d(r, r_{\text{closest}}) < k$, where $k$ was the displacement allowed to calculate the $k$-curvature. Let $r_{\text{new}} = r \cup r_{\text{closest}}$ be the new region.

  a) If $\ell(r_{\text{new}}) > l_{\text{min}}$, we returned to Step 1 with $r_{\text{new}}$ and $t + \delta t$. If necessary updated $\text{l\_regions}$.

  b) If $\ell(r_{\text{new}}) < l_{\text{min}}$, we moved to Case 3 with $r_{\text{new}}$ instead $r$.

The process ended when all the regions of interest in $\text{l\_regions}$ had a length between $l_{\text{min}}$ and $l_{\text{max}}$. In Figure 2d we display the final list of regions of interest we detected. As above, different regions of interest are marked with different colors. Moreover, in Figure 2e we show a zoom with an initial region of interest, and in Figure 2f we depict its final state. As we can see, the initial region of interest is divided into two new regions, each containing a point of interest.

After this recursive procedure, we obtained a list of regions of interest, where each of the regions contained a point of interest, that is a concave or a convex point. Finally, we identified one interest point inside each region of interest. We used the weighted median of the curvature to locate them, because it is a well-known technique that assume that the point of interest is located near the center of the region but this central position is not a perfect location and it can be improved.
Figure 2: Successive steps to identify regions of interest with a point of interest within each of them. (a) Original image. (b) Regions of interest detected by Step 1. Different regions of interest are marked with different colors. (c) Regions of interest finally detected by the proposed recursive procedure. Different regions of interest are marked with different colors. (d) and (e) zooming of an initial region of interest and its final state, respectively, showing how the initial region is divided into two regions, each one containing a point of interest.
3.3. Concave points

Once we determined the set of points of interest we needed to identify the concave ones. This part of the algorithm was based on the analysis of the relative position of the neighbourhood of each point. The classification step followed the next three steps:

1. **Determine two \( k \)-neighbour points**: We selected two points on the contour, they were located at \( k \) and \(-k\) distance relative to the interest point. This step is described in Figure 3a.

2. **Definition of a line between the \( k \)-neighbours**: We built a straight line between the points selected in the previous step, see Figure 3b.

3. **Middle point of the line**: We classified the point as concave if the middle point of the previously defined line was outside the object, otherwise was classified as convex. See Figure 3c.

4. Experimental Setup

In this section we present an experimental framework to analyse the proposed method and to compare it with other methods of the state-of-the-art. In particular, we describe the image datasets, the metrics we used to measure the performance and the methods selected to be compared. Finally, we describe the two experiments that we designed in order to evaluate the performance of the algorithm with the state-of-the-art.

4.1. Datasets

We used two different sets of images. On the one hand, we created a set of synthetic images, that we called OverArt dataset. It contains 2000 images, each with 3 overlapping objects. On the other hand, we used the *ErythrocytesIDB2* dataset of real images from González-Hidalgo et al. [9], it contains 50 images of peripheral blood smears samples of patients with sickle cell anaemia.
4.1.1. OverArt Dataset

We generated the OverArt dataset in order to obtain a ground truth of the concave points on overlapped objects. Each image of the dataset contains a cluster with three overlapped ellipses. The use of three ellipses is a good trade-off between complexity and reality. The code is available at [https://github.com/expainingAI/overArt](https://github.com/expainingAI/overArt). The same set of images we used in this paper can be created choosing number 42 as the random seed. Figure 4 depicts three different examples of this dataset.

The ellipses of each image were defined by three parameters: the rotation, the feret diameter size and its center. We randomly generated these values by the set of constraints described in Table 1.

To construct each cluster we located the first ellipse in the center of the image. The positions of the other two ellipses are related to this first one. We randomly selected the location of the second ellipse inside the area defined by the minimum and maximum distance to the center of the first ellipse. Finally, we followed the same process with the third ellipse, it was randomly placed inside the area defined by the minimum and maximum distance to the center of the first and second ellipses.

| Parameter                      | Value  |
|-------------------------------|--------|
| Minimum feret                 | 45 px  |
| Maximum feret                 | 100 px |
| Minimum distance between centers | 45 px  |
| Maximum distance between centers | 85 px  |
| Minimum rotation              | 0 °    |
| Maximum rotation              | 360 °  |

Table 1: Parameters we used to generate the OverArt dataset. The table detail the range of the values that defines each ellipse. The distance metrics are defined in pixels (px). Angles are defined in degrees.

To compare the performance of the precision of the different methods to find the con-

Figure 4: Three examples from the OverArt dataset. In blue the concave points, in white the clusters defined by three overlapped ellipses.
cave points we needed a ground truth of its location. We calculated it and we added this information to the dataset. A concave point is defined by the position where two or more ellipses intersects and must be located over the contour that defines the overlapping region.

The overlapped objects are defined by the equation of the ellipse, see Eq. (7) and Eq. (8). For each image of the dataset we obtained the position of all of its concave points by analytically solving Eq. (9).

\[
\lambda_1 = \frac{((x - center_x) \cos(\phi) + (y - center_y) \sin(\phi))^2}{(a^2)},
\]

\[
\lambda_2 = \frac{((x - center_x) \sin(\phi) - (y - center_y) \cos(\phi))^2}{(b^2)},
\]

\[
\lambda_1 + \lambda_2 = 1,
\]

where \(x\) and \(y\) are the unknown variables, \(center_x, center_y\) defines the central point of the ellipse, \(\phi\) the angle between the horizontal axis and the ellipse feret. Finally \(a\) and \(b\) represents each semi-axis, respectively.

4.1.2. ErythrocitesIDB2 Dataset

In this work we used microscopic images of blood smears, collected from ErythrocitesIDB2 [9], available at [http://erythrocytesidb.uib.es/](http://erythrocytesidb.uib.es/). The images consist of peripheral blood smears samples of patients with sickle cell anaemia classified by a specialist from “Dr. Juan Bruno Zayas” Hospital General in Santiago de Cuba, Cuba. The specialist’s criteria was used as an expert approach to validate the results of the classification methods.

The patients with sickle-cell disease (SCD), are characterized by red blood cells (RBCs) with the shape of a sickle or half-moon instead of the smooth, circular shape as normal cells have. In order to confirm the SCD diagnose, peripheral blood smear samples are analyzed by microscopy to check for the presence of the sickle-shaped erythrocytes and compare their frequency to normal red blood cells. The peripheral blood smear samples always include overlapped or clustered cells, and the sample preparation process can affect the quantity of overlapping erythrocytes in the images studied. Clinical laboratories typically prepare blood samples for microscopy analysis using the dragging technique, using this technique, more cell groups are apparent in the samples due to the spreading process [9].

Each image were labeled by the medical expert. There are 50 images with different number of cells (see Figure 5). The 50 images contains in total 2748 cells. These cells belongs to three classes defined by the medical experts. Those are circular, elongated and others as can be seen in figure 6.

4.2. Performance measures

The results of our algorithm should be measured with multiple numerical and well defined metrics in order to ensure its quality. The objective of these metrics should be to evaluate the precision on the prediction of the position of a concave point and how this change in precision affects on the split of overlapped objects. We used five different metrics: MED, F1-Score, SDS_Score, MCC and CBA.
• **Mean of the Euclidean distance (MED).** Let \( f = \{ C_i \}_{i=1}^p \) be the detected concave points for the proposed method for a given image, and \( GT = \{ GC_i \}_{i=1}^l \) be the ground truth concave points of that image, we know that it may be that \( l \neq p \). However, for each point \( GC_j \) exists \( C_{i_j} \in f \) such that \( d(GC_j, C_{i_j}) \) is minimum, then we define the MED performance measure by Eq (10).

• **F1-Score.** It is a standard and widely used measure. It is the harmonic mean of precision and recall, see Eq. (13). The precision and the recall depends on the number of false positives (FP), true positives (TP) and false negatives (FN). We also included the precision and the recall to the results in order to explain the F1-Score.

• **Sickle cell disease diagnosis support score (SDS_Score).** Proposed by Delgado-Font et al. [5], the SDS-Score indicates the usefulness of the method for the diagnosis of patients with sickle cell disease. This metric does not consider as a mistake a misclassification between elongated and other cells (or vice versa), due to the nature of the disease. See Eq. (14).

![Example of image of the ErythrocitesIDB2 dataset.](image1)

![Detail of the image containing a cluster.](image2)

**Figure 5:** Sample of patient with sickle cell anemia from ErythrocitesIDB2 dataset.

![Example of healthy circular cell.](image3)

![Example of other cell.](image4)

![Example of elongated cell.](image5)

**Figure 6:** Examples of the three types of cells present in the ErythrocitesIDB2 dataset. *Elongated* cells are also known as sickle cells.
• **Matthew’s Correlation Coefficient (MCC).** Introduced in [15], is a correlation measure between the prediction and observation. We used the adaptation proposed by Mosley et al. [17] for multi-class problems, see Eq. (15). This metric lies in [-1,1] range where -1 represents perfect misclassification, 1 a perfect classification and 0 a random classification. It is designed to deal with unbalanced data.

• **Class Balance Accuracy (CBA).** Introduced by Mosley et al. [17]. Represents the overall accuracy measure built from an aggregation of individual class metrics. This measure is designed to deal with unbalanced data. See Eq. (16).

\[
MED = \frac{\sum_{j=1}^{l} d(GC_j, C_{ij})}{l},
\]

\[
Precision = \frac{TP}{TP + FP},
\]

\[
Recall = \frac{TP}{TP + FN},
\]

\[
F1 = 2 \cdot \frac{Precision \cdot Recall}{Precision + Recall},
\]

\[
SDS\_Score = \frac{\sum_{i=1}^{3} c_{ii} + c_{23} + c_{32}}{\sum_{i=1}^{3} \sum_{j=1}^{3} c_{ij}},
\]

\[
MCC = \frac{\sum_{i,m=1}^{n} c_{ii} \cdot c_{ml} - c_{li} \cdot c_{im}}{\sqrt{\sum_{z=1}^{n} (\sum_{l=1}^{n} c_{lz})(\sum_{f,g=1}^{z} c_{gf})} \sqrt{\sum_{l=1}^{z} (\sum_{i=1}^{z} c_{il})(\sum_{f,g=1}^{z} c_{fg})}},
\]

\[
CBA = \frac{\sum_{i=1}^{z} c_{ii}}{\max(\sum_{j=1}^{3} c_{ij}, \sum_{j=1}^{3} c_{ji})},
\]

where \(c_{ij}\) is the number of elements of class \(i\) predicted as the class \(j\) and \(z\) the number of classes. In particular, \(c_{23}\) represents the cells predicted as other when they are elongated and \(c_{32}\) are the other cells predicted as elongated.

We used the paired t-test to check the difference between the F1-Score of our results and the state-of-the-art methods. The null hypothesis was that our results were greater. Previously, the normality of the data distribution was checked, by using the Shapiro-Wilk test.
4.3. State-of-the-art Methods

In the introduction section we made an study of the methods of the state-of-the-art that separate overlapped objects by finding concave points. To perform our experiments, we selected a representative subset of them. We excluded the methods we could not reproduce due to the absence of information on the original paper and the lack of access to the source code.

We had access to the original code of Zafari et al. [27] and González-Hidalgo et al. [9]. In addition to the two previously named methods, we considered the following methods in the comparative study: LaTorre et al. [14], Fernández et al. [8], Song and Wang [21], Chaves et al. [4], Bai et al. [2], Wang et al. [23] and Zafari et al. [29]. As we stated before, we implemented these methods using the information available on their respective papers.

The main issue we found with the methods we developed is that we did not have the values of the hyperparameters of all of them. In order to make a fair comparison, we performed an exhaustive search to obtain the hyperparameters of each method for each experiment, even if we had their original values.

Table 2: First column summarize the original parameters of each method. Second and third column summarize the set of hyperparameters for Experiment 1 and Experiment 2 respectively.

4.4. Experiments

In order to evaluate the quality of the proposed method and compare it to the state-of-the-art we designed two experiments. The first one was designed to detect the spatial...
precision for the concave point prediction algorithms. The second one was designed to evaluate the proposed concave point detector with a well-known application, such as the splitting of overlapped cells in microscopic images of peripheral blood smear samples of red blood cells.

4.4.1. Experiment 1

This experiment aimed to compare the detection capacity and the spatial precision of the proposed method with the state-of-the-art. We used the OverArt dataset that we generated, because it contains the position of each concave point. Training and test sets were constructed by randomly selecting 1000 images, it is important to notice that intersection between both sets is empty.

In order to evaluate the performance of each method we used two different performance measures from section 4.2: the Mean Euclidean Distance (MED) and the F1-Score. To compute the F1-Score we matched each detected concave point with a ground truth point. We matched two points if its distance was smaller than 15 pixels. Furthermore, if there were more than one candidate we selected the nearest one. We considered a false positive the predicted points that had not been matched with a ground truth point. We considered a false negative when there was not a candidate for a ground truth point.

4.4.2. Experiment 2

This experiment was designed to determine how the precision of concave point detection affect the division of overlapped objects in a real world scenario. We used the ellipse fitting method proposed by González et al. [9] to divide the overlapped objects from the detected concave points. After this division we compared the ground truth with the predicted objects. In this experiment we used the ErythrocytesIDB2 dataset. As a training set we selected 34 images randomly, that was 70\% of the images in the dataset.

The problem addressed on this experiment was a multi-class problem, for this reason we used the CBA, MCC, and SDS-Score and the adapted version of the F1-Score averaging the results for each class. We considered the prediction of a non existing cell in the ground truth as false positive, and the omission to predict an existing cell in the ground truth as a false negative. Figure 7 depicts some examples of these false positives and false negatives detections.

5. Results and discussion

In this section we analyze the results of the experiments. We developed two experiments in order to study two different characteristics of the proposed method. First, the precision of the concave points detection. Second, how the precision of the detection affects the posterior segmentation of the overlapped objects.

As we pointed previously, see Section 4.3 in order to make a fair comparison of the multiple proposals, we fine tuned the hyperparameters of each method for each experiment, its values are summarized on Table 2.
Tables 3 and 4 outline the results obtained for the detection of concave points in Experiment 1. They summarize the precision, recall, F1-Score and MED values of the concave point detection methods. We also added the standard deviation (STD) of the MED measure in order to provide complementary information. In our evaluation it is important to obtain an small value in MED but it was also important to ensure that this measure was not scattered.

Table 3 summarize the results obtained for the training set. We can observe that the proposed method achieved the best value for the F1-Score and it is almost tied with González-Hidalgo et al. method. The methods of Chaves et al. and Zafari et al. [27] also had good results for this measure. The other methods had an unbalance between results from the precision and recall, usually a higher precision value provokes a lower recall value and vice-versa. When this situation occur the methods obtain a low F1-Score performance. Regarding to the MED measure, the proposed method obtained the second best result with the lowest standard deviation. This indicates that the values tend to be close to the mean and less scattered than the others.

Table 4 sum up the results obtained for the test set. We can observe that the results were very similar than the ones we obtained using the training set. Also, the proposed method achieved the best F1-Score and it was the third best approach regarding MED measure with a very low STD. In this case, the differences between the three best values of the MED measure were small.

From Table 3 and Table 4 we can observe that Zafari et al. [29] obtained the best results in both train and test set regarding the MED measure with a low STD. This is due the high concave point detection rate. This method detected too many concave points, some of them close to the ones on the ground truth, for that reason this method obtained a high recall but a very low precision of 0.124 and 0.122 for train and test set respectively.

From the previous analysis we can state that the proposed method find the concave points with the highest balance between the precision and the recall, that means lower rates of false positives and false negatives. The lower values on MED and STD metrics denote that the detected points are close to the ground truth ones, that means a high degree of spatial precision.

The results for Experiment 2 are shown in tables 5, 6 and 7. Tables 5 and 6 summarize
the results obtained with the images from ErythrocitesIDB2 dataset. As in the description of the previous experiment, we separated the results in two different tables, for the training set and the test set, respectively.

The results for the training set are shown in Table 5. We can see that the proposed method was surpassed in the CBA measure by the method described in Bai et al. but had the best results in all the other metrics. Table 6 shows the results obtained for the test set. It should be noted that when the proposed method has been faced with unseen data it has achieved the best values in all metrics.

Figure 8 depicts some results of the Experiment 2 for an image in the test set. In the original image we can observe that there are two different clusters of cells. The proposed method and the one by González-Hidalgo et al. were the only ones capable to segment both clusters correctly, that shows the difficulty to solve this problem as we had the overlapping zones that did not provide any information. In that figure we can observe that the application of different concave point detection methods led to different object segmentation results, it is necessary to point out that we used the same algorithm to segment the clusters for all the approaches.

To compare the methods and to determine if the proposed one outperforms the other ones significantly, we used the paired t-test. The numerical results of the comparison of the algorithms for the F1-score performance measure are summarised in Table 7. Using the F1-score, we compared our method with each one of the other methods using the t-test with a confidence level of 95%. The proposed method outperformed significantly most of the other proposals, except the ones based on curvature estimation as González-Hidalgo et al., Chaves et al. and Bai et al., where the improvement was not statistically significant.

From the analysis of the results obtained with Experiment 2, we can determine that the increase on the precision in the detection of the concave points implies an improvement in the results in the segmentation of the overlapping objects.

| Method            | Precision | Recall | F1-Score | MED  | STD  |
|-------------------|-----------|--------|----------|------|------|
| Proposed method   | 0.998     | 0.943  | 0.969    | 1.777| 1.551|
| LaTorre et al. [14]| 0.445     | 0.928  | 0.604    | 9.116| 20.226|
| Fernández et al. [8]| 0.846    | 0.437  | 0.576    | 16.348| 31.593|
| González-Hidalgo et al. [9]| 0.986    | 0.949  | 0.967    | 4.896| 3.729|
| Song and Wang [21]| 0.483     | 0.421  | 0.449    | 41.201| 51.772|
| Zafari et al. [27]| 0.993     | 0.916  | 0.953    | 2.375| 9.577|
| Chaves et al. [4]| 0.953     | 0.919  | 0.936    | 5.821| 11.174|
| Bai et al. [2]    | 0.837     | 0.501  | 0.626    | 9.479| 9.412|
| Wang et al. [23]  | 0.279     | 0.255  | 0.266    | 83.752| 62.325|
| Zafari et al. [29]| 0.124     | 0.987  | 0.221    | 1.350| 3.015|

Table 3: Results of the Experiment 1 using 1000 images of the training set from synthetic dataset. MED is the mean of the euclidean distance from a detected point to the closest ground truth point, STD is its standard deviation.
Figure 8: Example of results obtained from the test set of ErythrocytesIDB2 dataset for each method. Colors used in this figure: blue for the elongated cells, green for the circular cells, yellow for the other cells. The proposed method and the one by González-Hidalgo et al. are the only ones capable segment both clusters correctly.
| Method                     | Precision | Recall  | F1-Score | MED   | STD  |
|----------------------------|-----------|---------|----------|-------|------|
| **Proposed method**        | 0.995     | 0.948   | 0.971    | 1.762 | 2.023|
| LaTorre et al. [14]        | 0.551     | 0.955   | 0.698    | 4.187 | 17.751|
| Fernández et al. [8]       | 0.976     | 0.888   | 0.930    | 1.756 | 10.206|
| González-Hidalgo et al. [9]| 0.983     | 0.936   | 0.959    | 3.115 | 4.816|
| Song and Wang [21]         | 0.511     | 0.461   | 0.484    | 38.156| 51.613|
| Zafari et al. [27]         | 0.990     | 0.919   | 0.952    | 2.730 | 11.914|
| Chaves et al. [4]          | 0.953     | 0.922   | 0.937    | 5.645 | 9.921 |
| Bai et al. [2]             | 0.823     | 0.308   | 0.448    | 7.436 | 10.321|
| Wang et al. [23]           | 0.239     | 0.221   | 0.229    | 96.446| 70.646|
| Zafari et al. [29]         | 0.122     | 0.991   | 0.218    | 1.199 | 2.535|

Table 4: Results of the Experiment 1 using 1000 images of the test set from synthetic dataset. MED is the mean of the euclidean distance from a detected point to the closest ground truth point, STD is its standard deviation.

| Method                     | Precision | Recall  | F1-Score | SDS-Score | CBA   | MCC |
|----------------------------|-----------|---------|----------|-----------|-------|-----|
| **Proposed method**        | 0.862     | 0.882   | 0.872    | 0.908     | 0.659 | 0.739|
| LaTorre et al. [14]        | 0.813     | 0.806   | 0.808    | 0.873     | 0.637 | 0.642|
| Fernández et al. [8]       | 0.777     | 0.789   | 0.781    | 0.846     | 0.569 | 0.587|
| González-Hidalgo et al. [9]| 0.847     | 0.852   | 0.850    | 0.902     | 0.649 | 0.703|
| Song and Wang [21]         | 0.669     | 0.849   | 0.746    | 0.842     | 0.545 | 0.595|
| Zafari et al. [27]         | 0.787     | 0.871   | 0.826    | 0.881     | 0.636 | 0.687|
| Chaves et al. [4]          | 0.848     | 0.873   | 0.860    | 0.901     | 0.648 | 0.723|
| Bai et al. [2]             | 0.871     | 0.870   | 0.870    | 0.907     | 0.667 | 0.731|
| Wang et al. [23]           | 0.729     | 0.842   | 0.781    | 0.856     | 0.594 | 0.621|
| Zafari et al. [29]         | 0.704     | 0.783   | 0.724    | 0.822     | 0.509 | 0.563|

Table 5: Results of the Experiment 2 using the 34 images of the train set from ErythrocytesIDB2. SDS is the sickle cell diagnosis support score. MCC is the Matthew’s Correlation Coefficient and CBA is the Class Balance Accuracy.

Finally, we want to remark that the proposed method can be considered transparent [1], because has the ability of simulatatability (being simulated or thought about strictly by a human), decomposability (explaining each of the parts of the method), and algorithmic transparency (the user can understand the process followed by the method to produce any given output from its input data). This is especially important in health, to trust the behavior of intelligent systems.

6. Conclusions

The concave point detection is a first step to segment overlapped objects in images, the existence of this clusters reduces the information available in some areas of the image, maintaining it an still challenging problem.

19
| Method                  | Precision | Recall | F1-Score | SDS-Score | CBA   | MCC   |
|------------------------|-----------|--------|----------|-----------|-------|-------|
| Proposed method        | 0.856     | 0.861  | 0.858    | 0.888     | 0.665 | 0.724 |
| LaTorre et al. [13]    | 0.813     | 0.783  | 0.798    | 0.865     | 0.602 | 0.631 |
| Fernández et al. [8]   | 0.766     | 0.752  | 0.758    | 0.843     | 0.598 | 0.601 |
| González-Hidalgo et al. [9] | 0.838   | 0.821  | 0.829    | 0.877     | 0.625 | 0.677 |
| Song and Wang [21]     | 0.665     | 0.825  | 0.735    | 0.829     | 0.592 | 0.595 |
| Zafari et al. [27]     | 0.766     | 0.842  | 0.801    | 0.845     | 0.650 | 0.655 |
| Chaves et al. [1]      | 0.826     | 0.846  | 0.836    | 0.873     | 0.634 | 0.684 |
| Bai et al. [2]         | 0.842     | 0.836  | 0.838    | 0.879     | 0.638 | 0.693 |
| Wang et al. [23]       | 0.711     | 0.821  | 0.762    | 0.829     | 0.618 | 0.592 |
| Zafari et al. [29]     | 0.696     | 0.728  | 0.697    | 0.806     | 0.532 | 0.536 |

Table 6: Results of the Experiment 2 using the 16 images of the test set from ErythrocytesIDB2. SDS is the sickle cell diagnosis support score. MCC is the Matthew’s Correlation Coefficient and CBA is the Class Balance Accuracy.

| Methods          | p-value    | t statistic |
|------------------|------------|-------------|
| LaTorre et al.   | $2.46 \times 10^{-3}$ | 3.051       |
| Fernández et al. | $3.62 \times 10^{-5}$ | 4.682       |
| González-Hidalgo et al. | $6.41 \times 10^{-2}$ | 1.565       |
| Song and Wang    | $1.11 \times 10^{-4}$ | 4.378       |
| Zafari et al.    | 0.01546    | 2.297       |
| Chaves et al.    | 0.1693     | 0.973       |
| Bai et al.       | 0.1824     | 0.922       |
| Wang et al.      | $5.42 \times 10^{-4}$ | 3.707       |
| Zafari et al.    | $7.15 \times 10^{-7}$ | 5.516       |

Table 7: Results of applying the t-test between our proposed method and the rest of state-of-the-art methods. The alternative hypothesis is: the mean of our method is greater than the others.

The methodology we proposed in this paper is based on the curvature approximation on each point of the contour of the overlapped objects. First, we identified the concave points as the local extreme of the curvature. As a second step, we selected regions with the highest probability to contain an interest point, that is, regions with higher curvature. Finally we obtained an interest point from each region by applying a recursive algorithm and we selected the concave ones.

We included as results the implemented code, the confusion matrices with the raw data of all the studied methods and the images used as training and test sets to allow researchers to more easily compute other metrics, see [https://github.com/expainingAI/overlapped-objects](https://github.com/expainingAI/overlapped-objects). As an additional contribution we constructed and opened to the scientific community a synthetic dataset to simulate overlapping objects, we provided the position of the concave points as a ground-truth, as far as we know, is the first public dataset containing overlapping objects with annotated concave points. We used this dataset to compare the capacity of detection and the spatial precision of the proposed method with the
state-of-the-art, see https://github.com/expainingAI/overArt. For the sake of scientific progress, it would be beneficial if authors published their raw data, code and the image datasets that they used.

Finally, as a case study, we evaluated the proposed concave point detector and the state-of-the-art methods with a well-known application, such as the splitting of overlapped cells in microscopic images of peripheral blood smear samples of RBC of patients with sickle-cell disease. The goal of the case study was to check if the spatial precision of the concave points detector method affected the results of a classification algorithm of the morphology of RBC in a real world scenario.

Results from experimentation proved that the proposed method had better results in both synthetic and real datasets, to ensure its quality we used multiple standard metrics. We can conclude that the proposed methodology detects concave points with the highest exactitude as it obtain lower values in the MED metric and a small standard deviation. Regarding the same experiment it have obtained the best F1-Score, that means a good balance between its precision and recall detecting concave points. We designed a second experiment to determine how the precision of concave point detection affect the division of overlapped objects in a real world scenario. From its results we can conclude that a method with higher precision to find concave points, as the proposed one in this paper, help to achieve a better cell classification. Finally, it is important to notice this method is not limited to the case study we performed in this paper, it can be used for other applications where separation between overlapping objects is required.

Author Statement

Miquel Miró-Nicolau: Software, Visualization, Formal analysis, Writing - Original Draft. Gabriel Moyà Alcover: Conceptualization, Validation, Project administration. Manuel González-Hidalgo: Methodology, Formal analysis, Writing - Review & Editing. Antoni Jaume-i-Capó: Methodology, Writing - Review & Editing, Resources.

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