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CNN-Based Approach for Overlapping Erythrocyte Counting and Cell Type Classification in Peripheral Blood Images

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ABSTRACT Classification and counting of cells in the blood is crucial for diagnosing and treating diseases in the clinic. A peripheral blood smear method is a fast, reliable, robust diagnostic tool for examining blood samples. However, cell overlap during the peripheral smear process may cause incorrectly predicted results in counting blood cells and classifying cell types. The overlapping problem can occur in automated systems and manual inspections by experts. Convolutional neural networks (CNN) provide reliable results for the segmentation and classification of many problems in the medical field. However, creating ground truth labels in the data during the segmentation process is time-consuming and error-prone. This study proposes a new CNN-based strategy to eliminate the overlap-induced counting problem in peripheral smear blood samples and accurately determine the blood cell type. In the proposed method, images of the peripheral blood were divided into sub-images, block by block, using adaptive image processing techniques to identify the overlapping cells and cell types. CNN was used to classify cell types and overlapping cell numbers in sub-images. The proposed method successfully counts overlapping erythrocytes and determines the cell type with an accuracy rate of 99.73%. The results of the proposed method have shown that it can be used efficiently in various fields.

KEYWORDS
Blood cells
Deep learning
Microscopy
Machine learning
Classification

INTRODUCTION

Developing fast and reliable methodologies and equipment that solve problems in the field of health provides various advantages to clinical staff and positively affects the quality of life of societies. Eliminating this developed equipment's cost and negative aspects is as essential as the development phase. Microscopes used in the laboratory are the most common example of this type of equipment. However, detecting and counting objects in complex images or videos from microscopes is a challenging and time-consuming task that is encountered in many traditional applications (Aliyu 2017). The reliability of the applications, especially in the medical field, is critical in the accurate and successful implementation of the diagnosis and treatment processes of the specialists. For this reason, systems that provide decision-support mechanisms to experts in the field are critical (Alimadadi *et al.* 2020).

Today, hematological tests, the standard procedure of every laboratory, are significant for the clinical diagnosis of cancer, anemia, and other blood diseases. However, analyzing the obtained data is a susceptible process (Ahn *et al.* 2018). Although there are various automated systems for performing these tests, one of the most used methods today is the examination of blood samples with peripheral smears under the microscope. Even today, this examination method is performed manually by trained hematol-

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ogists. Experts obtain results by evaluating the morphological characteristics of blood cells, such as size and shape, by examining blood cells under a light microscope. This method, which has a high potential for obtaining erroneous results, requires effort (Bain 2005). One of the primary problems is the increase in morphological diversity due to intracellular and intercellular variations producing false results. The cells can be spread on the slide with concentric or edge overlapping in the peripheral spreading process. In addition, the variable properties of the image, such as color and contrast caused by the imaging system, cause differences between the same samples. In addition to all these physical factors, the skill and experience of the hematologist examining the sample also creates a subjective evaluation of the results. Quantitative analysis methods are gaining importance in overcoming these problems (Mohammed *et al.* 2014).

Blood consists of two separate components: plasma and blood cells. In the samples prepared by peripheral smear, three different types of cells can be observed: red blood cells (RBC), white blood cells (WBC), and platelets. Healthy adult individuals typically have $4,500 \times 10^3/\mu L$ RBCs, $8 \times 10^3/\mu L$ WBCs, and $300 \times 10^3/\mu L$ platelets. Platelets are the smallest cells of the blood, with an average diameter of $2-4\mu m$, disc-shaped morphology, and no nuclei. Granules in platelets contain substances that will instigate clot formation activity in case of bleeding. Therefore, the main task of platelets are hemostasis and prevention and control of bleeding. White blood cells, also known as leukocytes, have significant morphological differences compared to other blood cells and have a diameter of $10-20\mu m$. White blood cells do not contain hemoglobin, and the cytoplasm density is low, while the nuclear densities are high. An essential part of the immune system, Leukocytes move from the blood fluid to the tissues and protect the body. It counteracts damage by deactivating bacteria, viruses, or other foreign organisms and provides a defense mechanism by producing antibodies (Beydoun *et al.* 2016). Therefore, the total leukocyte concentration in the blood is a vital indicator of the human immune system, and many diseases can be detected only by calculating the leukocyte count. There are five different types of leukocytes: eosinophils, lymphocytes, neutrophils, monocytes, and basophils. Red blood cells, also known as erythrocytes, are the most common cell type in the blood, with a diameter of $7-10\mu m$ and a biconcave disc-shaped erythrocyte with a thickness of $2.2\mu m$ (Aliyu 2017). Their main functions in the body are to transmit oxygen to the tissues in circulation and to remove wastes and carbon dioxide from the tissues. Their color is red because they contain hemoglobin protein.

Researchers are making significant efforts to count the cells in the images obtained from a peripheral smear. As a result of these efforts, systems that produce results with remarkable accuracy have emerged. These traditional systems produce results by applying the steps of pre-processing, segmentation, feature extraction, and classification steps to the obtained images, respectively (Gonzalez *et al.* 2004). Pre-processing steps are used to remove the images' noise and the colors' distortions. In general, images are corrected by applying spatial or frequency plane operations. Examples are pre-processing algorithms' histogram correction, average receiver, and median filter. The most optimal images should be obtained for the segmentation step. The next step, segmentation, plays the most crucial role in the defined system and significantly affects its accuracy. There are various traditional or hybrid algorithms for segmentation and many methods developed for pre-processing (Kibunja 2021; Gould *et al.* 2009; Li *et al.* 2017; Çimen *et al.* 2019). In the feature extraction steps, the image segments' morphology, color coefficient, or other descriptive properties are obtained from the segmentation process (Zhang *et al.* 2019). The defining features obtained in this step are essential as they reveal the success of the classification. The primary purposes of feature extraction are to ensure that the images taken as input are defined as fingerprints and to identify the numerical or vector quantities obtained as output (Nixon and Aguado 2019). Various feature extraction methods that are flexible and adaptively may be preferred to improve performance rates. Various algorithms such as artificial neural networks, support vector machines, Naive Bayes networks, linear discriminant analysis, and multilayer networks are used in order to use the property values obtained from blood cells for counting and classification (Bayat *et al.* 2018; Ye *et al.* 2004).

This study proposes a new CNN-based strategy to eliminate the overlap-induced counting problem in blood samples prepared with peripheral smear and determine the blood cell type. In the proposed method, the images of the entire peripheral blood slide were divided into sub-images using adaptive image processing techniques to identify the overlapping cells and cell types. Each cropped image was labeled by the number of overlapping cells with hematology expert opinion, thus providing ground truth data. In addition, white blood cells are labeled as a separate class. CNN was used to classify cells divided into sub-images as blocks from the original images. The proposed method achieved 99.73% accuracy in counting overlapping red blood cells and separating RBC-WBC blood cell types. The results show that the proposed method can be adapted to areas where high-resolution images are found and reliable results.

PROPOSED METHOD

With the increased processing capacity of graphics processor units in recent years, deep learning methods have started to be used frequently in classification, recognition, and detection tasks, especially in the medical field (McLeod and Ozcan 2016; Chiroma *et al.* 2019; Jang and Cho 2019). Considering the problems related to blood count, using deep learning methods in blood count and classification steps can bring many advantages. The first of these advantages is that high image quality input is not required for deep learning methods, so problems arising from physical conditions are avoided (Wang *et al.* 2015). Secondly, it does not need to perform feature extraction and segmentation operations outside the system due to its convolutional layers. It produces results by applying straightforward approaches to solving complex problems that require high processing power (Dodge and Karam 2016). A Convolutional Neural Network (CNN), which is defined in literature as a unique structure of deep learning methods, is widely used to classify blood images and solve problems in other fields (Rere *et al.* 2016; Xue *et al.* 2016; Pala *et al.* 2022).

Convolutional neural networks are a learning architecture inspired by the visual perception mechanism of living things. CNN methods follow end-to-end training metrology, eliminating the pre-processing steps of complex images. CNN techniques are more similar to biological neural networks than other machine learning methods due to the layers – and it works very effectively on displaced, scaled, bent, or deformed images (Choi *et al.* 2017; Sun *et al.* 2019). The convolutional layer in the CNN architecture aims to learn the basic parameters that represent the features in the input images. Convolutional layers consist of various filters that allow the learning of different properties. These filters have various magnitudes and shift coefficients and are convoluted with the input image. As a result, each image taken as input is processed and given as output as a new feature image. By applying more than one convolution operation to the input layer, the depth of the network is increased, and with the network, more accurate results can be produced. The new feature layers obtained as the output of the convolutional layer can be high-valued compared to the inputs due to the multiplication process (Xue *et al.* 2016). This situation can cause overfitting in the network structure. In order to prevent this situation and increase the training performance, normalization layers can be added to the network structure (Huang *et al.* 2019). The main task of the normalization layer is to bring the values formed as a result of multiplications to a specific range and transmit the appropriate values to the next layer. Since images have a static structure, distinctive features found at one point in the image can also be found in other areas. This feature makes it possible to express the defining features of images with smaller areas. The pooling layers in the CNN structure enable these features that spread over large areas to be expressed in small areas. Pooling layers filter the input images with a specific size, like convolution layers. The pooling layer prevents situations such as memorization that will occur in the network structure. The outputs of the pooling layer are smaller than the input image, with the size depending on the filter size (Barbastathis *et al.* 2019; Liu *et al.* 2019; Strumberger *et al.* 2019).

Many high-performance methods have been proposed to classify non-overlapping blood cells. However, the overlap problem in blood cells counted using the peripheral smear method is widespread, and these processes are ignored when creating data sets. This is most commonly seen in the count of erythrocytes. Counting problems occur when at least two or more erythrocytes overlap. In this study, CNN was used to count the erythrocytes with overlapping observed in the samples prepared by the peripheral smear method and simultaneously make the RBC-WBC classification. The steps in this study are shown in Fig. 1.

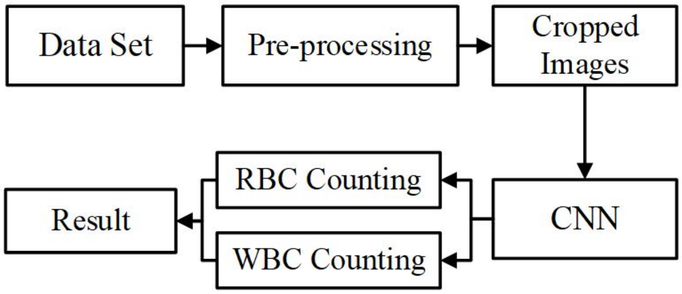


Figure 1 A brief schema of the proposed method

Data Set

LISC dataset was used in this study (Rezatofighi and Soltanian-Zadeh 2011). The LISC database was digitized in the hematology laboratory by preparing the blood samples collected from healthy individuals by the peripheral smear method. Different peripheral smear slides were prepared from 8 individuals, and 117 whole slide images were collected. The Gismo-Wright staining technique was used in the peripheral smear, and a microscope with a 100x optical lens was used to collect images. The obtained images were transferred to digital media using a camera. The images in the dataset are 720 × 576 resolution.

Proposed Cell Localization Method

A simple and adaptive pre-processing algorithm is intended to separate peripheral slide images into sub-images. Pre-processing step aims to divide the overlapping cells into sub-images and input them into the deep learning model more effectively. This way, the deep learning-based segmentation problem, which requires high computational operations, has been transferred to the classification problem. All the applied pre-processing steps are adaptive and can be used in applications such as real-time and various other datasets. Applying this pre-processing step to images allows for higher resolution blood image classification. In addition, giving the data as input to the CNN model by dividing it into sub-images provides computational efficiency during training and testing. First, the images in the RBC color space in the data set were converted to gray-level images. Gray-level 256-bit images were converted to binary images adaptively using the Otsu method (Otsu 1979). In binary-level images, the centers of erythrocytes and leukocytes resemble the background due to the cytoplasm structure. Therefore, the holes-filled method was applied to the centers of the obtained binary level images. While determining the center of the images at the binary level, the morphological erosion operator is applied to ensure the clarity of the edges. Finally, the centers of the blocks in the binary images were found. The bounding boxes' positions were mapped onto the RGB images in the original dataset. Bounding boxes in blocks were cropped from the original images, and a window size of 128 × 128 was transferred. The applied pre-processing steps are shown in Figure 2.

In Figure 3, the results of the applied pre-processing steps used to separate images into sub-images are given. RGB images were used during the training and testing times, and the pre-processing steps were used only to divide into sub-images.

Figure 4 shows images of randomly selected overlapping erythrocytes from the cropped sub-images.

Different numbers of erythrocytes overlap in the sub-images recorded due to pre-processing from the original dataset. The cropped sub-images were counted and labeled with the opinion of the hematologist. The number of overlapping cells in each image is labeled, and all WBC cells are labeled as a separate class. 117 RGB images with 720 × 576 resolution were found from the dataset, and 13345 RGB images with 128x128 sub-images were cropped and labeled. All sub-images were used during CNN training and testing. The distribution of the labels resulting from sub-images is given in Table 1.

Proposed CNN Model

In the proposed model, the images taken as input data during the training phase are forwarded to each layer, and then the model produces a result for each image. The loss function calculated the difference between the results obtained as the model output and the actual results. As a result of the loss obtained from each image, the model updates its internal weights to increase the learning rate. In the training phase of the model, the gradient descent optimization algorithms were used to update the optimum internal weights. After the training phase was completed, test images were used to test the model's success.

The general structure of the proposed CNN model is shown in Figure 5. In our proposed CNN model, we use four convolutional layers, three max-pooling layers, and two fully connected layers, which means two hidden layers and one output layer. 128 × 128 × 3 sub-image from the data set normalized and applied to the model as input. To construct the 1st, 2nd, 3rd, and 4th convolutional layers, we used filter sizes of 3x3, 2x2, 3 × 3, and 3 × 3 and stride sizes of all layers are 1 × 1. The ReLU activation function and the same padding technique are used in all convolution layers. 2x2 kernel max-pooling with 2 × 2 stride is applied for the mapping

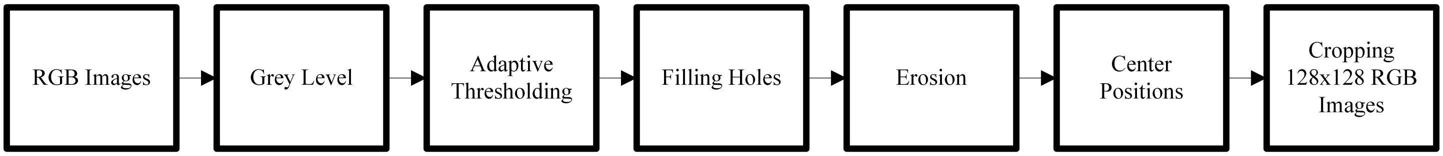


Figure 2 Applied pre-processing steps

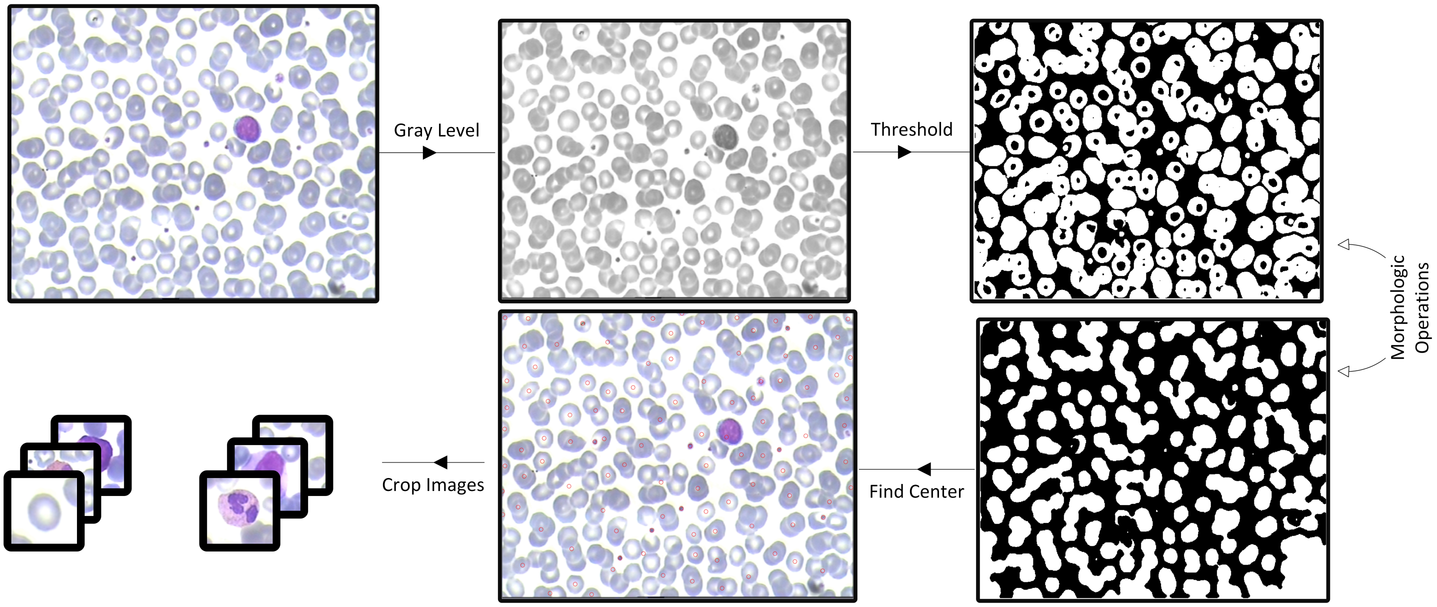


Figure 3 Cropping images into sub-images with pre-processing steps

Table 1 Data distribution

	Number of overlapped RBCs								WBC	
Class	1	2	3	4	5	6	7	8	9	10	Total
Number of samples	2545	1041	2040	2779	2553	1346	422	113	155	351	13345

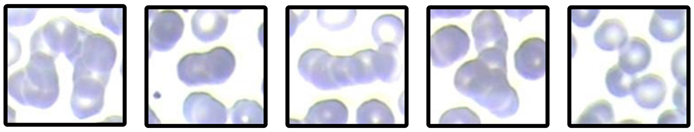


Figure 4 Examples of erythrocytes with different numbers overlapping

feature. A dropout layer of 0.25 was used at the output of all pooling layers. ReLU activation function is used in all convolution layers. The maximum-pooling layer's output matrix in the second block is flattened and transferred to the dense block. There is a neural network in the last layer for classifying the number and type of blood samples. The fully connected layer consists of two dense layers of 512 and 256 neurons and an output layer of 10. In order to prevent overfitting, 0.2 dropout was used in the second dense layer. The dropout rate is chosen experimentally. We use the softmax activation function in the output layer. The Adam optimization function is used in the training steps, and the batch size is 64. Early stopping was used depending on the accuracy rate during the proposed CNN model training. The best weights obtained during the training process were saved.

Experimental Results

Various performance criteria were used to test the performance of the proposed CNN model. Early stopping was used depending on the accuracy during the proposed CNN model training. The training was stopped at the 50 epochs when the accuracy and loss stabilized. The weights with the highest accuracy and lowest loss value were recorded. Figure 6 shows the accuracy and loss graphs of the training and validation results. The proposed CNN model reached the highest accuracy value of 99.73% in 50 epochs. The hardware environment includes an Intel Core i7-7700 HQ CPU, 16 GB of RAM, and the Windows 10 operating system (64-bit mode) to implement the model. In order to accelerate the computations and improve efficiency, GPU-accelerated computing with NVIDIA GTX 1050 is also utilized.

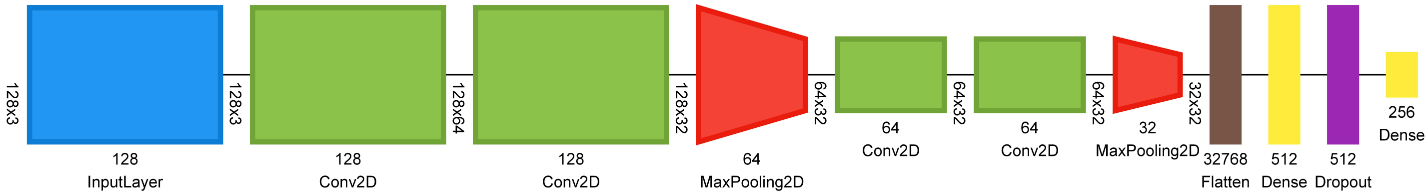
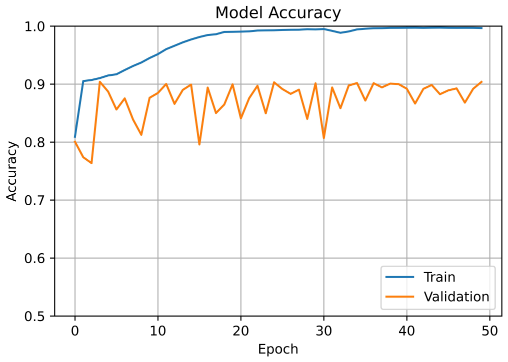
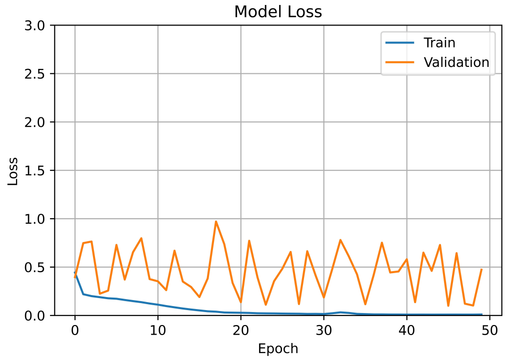


Figure 5 Proposed CNN model



(a) Accuracy of train and validation



(b) Loss of train and validation

Figure 6 Results of the proposed CNN model performance results

Classification results were evaluated using other performance criteria such as Precision, Recall, and F1-Score. Precision, Recall, and F1-Score in the proposed CNN model were 93.42%, 96.27%, and 94.73%, respectively. In addition, the confusion matrix was used to show the success of the proposed method in the test data. The confusion matrix shows the correct and incorrect classification of the test data of the proposed method in detail. The confusion matrix of the proposed model is shown in Figure 7.

CONCLUSION

Accurate counting and classifying of blood samples are critical in diagnosing diseases and following treatments. The peripheral smear method is the most commonly used method in laboratories and clinics for cell counting and determining type. However, overlapping erythrocytes is one of the biggest obstacles to a reliable counting process. This study proposes a new CNN-based strategy to eliminate the overlap-induced counting problem in peripheral smear blood samples and accurately determine the blood cell type.

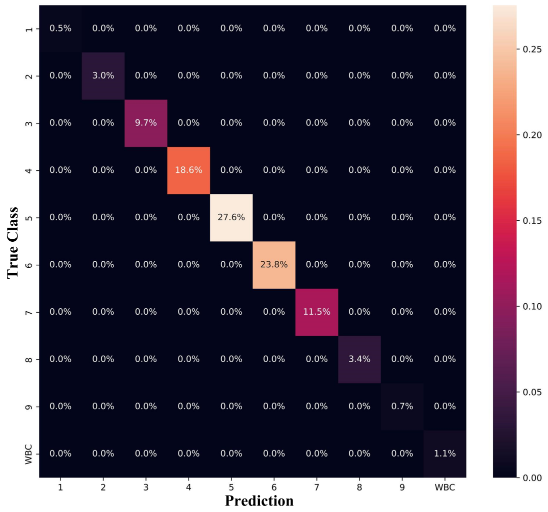


Figure 7 The confusion matrix of the proposed CNN model

In order to count the overlapping cells, the segmentation problem was transformed into a classification problem. In addition, giving the data as input to the CNN model by dividing it into sub-images provides computational efficiency during training and testing. In the proposed method, peripheral blood images were divided into sub-images, block by block, using adaptive image processing techniques to identify overlapping cells and cell types. Each image was labeled with an expert opinion. Each overlap number in red blood cells is labeled as a separate class. In addition, white blood cells are labeled as a separate class. Data augmentation methods were applied to ensure data distribution. Convolutional neural networks were used for classification. Convolutional neural networks can learn the properties of erythrocytes and leukocytes with more than one overlap in the image, thus producing more successful results than the methods used. The proposed CNN model consists of three blocks and has significantly less computational complexity. Classifier performances are measured using accuracy, precision, recall, and F1-Score metrics. Validation of results has been carried out by tenfold cross-validation. CNN-based approach counts overlapping cells and determines cell type with 99.73% accuracy. Precision, Recall, and F1-Score in the proposed CNN model were 93.42%, 96.27%, and 94.73%, respectively. It is seen that the proposed method performs multitasking with higher accuracy compared to other methods.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Availability of data and material

Previously reported data were used this study and are available at (Rezatofighi and Soltanian-Zadeh 2011). These prior studies (and datasets) are cited as references at relevant places within the text.

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