



BRB U-Net for Instance Segmentation of Cells in Microscopy

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Abstract: Biological cell analysis like cell segmentation, cell classification, cell tracking etc. aid in quantitative analysis of cells which is useful for cellular level knowledge of biological activity. Microscopy imaging allows for the generation of cell images and are used for cell studies in computational biology research and clinical disease diagnosis. In this article we explore instance segmentation of DIC-C2DH-HeLa cells in microscopy images. Specifically, a deep learning model Bottleneck residual blocks U-Net (BRB U-Net) is utilized for the task of separating individual cell instances from the background. This method achieved a Dice Index of 88% for DIC-C2DH-HeLa cells.

Keywords: Instance segmentation, Deep Learning, Microscopy, Marker controlled Watershed algorithm.

I. INTRODUCTION

Microscopy image analysis of cells and nuclei play a crucial role in the study of cell activities which may be useful for drug discovery studies [1], angiogenesis [2], wound healing [3], etc. Microscopy images are captured using microscopes equipped with image acquisition systems. These experiments produce humongous data consisting of hundreds or thousands of cells in microscopy images. Often microscopy imaging faces several challenges like low contrast, low illumination, occluded cells to mention a few. Traditionally these images were analysed manually by human experts. However, with the advancement of microscopy technology and high-through-put experiments, manual analysis has not only become difficult but impossible. In addition, manual analysis is prone to inter-observer variability and intra-observer variability. Due to these reasons, automated or semi-automated analysis of microscopy cell images has become essential. To this end there are plenty of computerized algorithms which are designed for these applications like cell segmentation [4], cell detection and counting [5], cell tracking [6] and cell classification [7].

In this article we utilize BRB U-Net [8] for instance segmentation of cells in microscopy. Segmentation is of two types: Semantic segmentation and instance segmentation. In semantic segmentation every pixel in an image is categorized into background or foreground. In instance segmentation every pixel is assigned an object instance. For images with occluded cells semantic segmentation generates a prediction segmentation map in which segmented cells may be combined and individual cells may not be separated. Whereas, in instance segmentation the task is to segment each individual cell instance. For example, if more than two cells are touching instance segmentation map should separate these touching cells into individual cell entities.

In the article [9] they combined the benefits of Convolutional Neural Network (CNN) and the watershed method. The mask corresponding to the input images was learned by a CNN using the Euclidean distance transform (EDT) (deep distance estimator). Then, faster R-CNN is employed to identify individual cells in the EDT image. Using the results of the first two steps the final segmentation is carried out. In [10] they trained the deep network using a novel embedding loss based on cosine similarities, so that it predicts distinct embeddings for each instance. In Usiigachi [11] Mask R-CNN is utilized for instance segmentation of cells. In [12], they perform instance segmentation by utilizing marker- controlled watershed and CNN. In this article we explore the use of BRB U-Net in [8].

The rest of the article is presented as follows: Section II discusses materials and methods, and section III discusses the results followed by conclusions and then references.



II. MATERIALS AND METHODS

A. Dataset and Data Pre-processing

The dataset for the current research is DIC-C2DH-HeLa cells and are taken from cell tracking challenge [13]. This dataset consists of a total of 168 time-lapse microscopy images. The sample images along with the ground truth are shown in Fig. 1. As can be seen from the figure the contrast of the image is very low, making it challenging for good segmentation performance. In data pre-processing image normalization is done prior to Histogram equalization for each image. Marker images and weight-maps for each image are calculated.

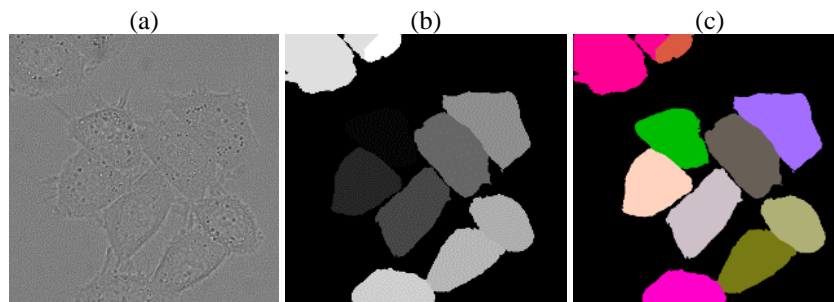


Fig 1: (a) Raw Input Image, (b) Instance Segmentation Ground truth, (c) Instance Segmentation Ground Truth with different colours for each instance

B. Methodology for Instance Segmentation

Instance segmentation method from [12] is adapted in this article utilizing BRB U-Net as the CNN model. The block diagram of the methodology is depicted in Fig. 2. The pre-processed images along with the mask, marker and weight map is given to the BRB U-Net for model training. For training, the entire dataset is splitted into 80% train, 10% validation and 10% test images. The model is trained with weighted cross entropy loss for 300 epochs with initial learning rate of 0.0003. Two models, each for mask and marker are generated. Predicted mask and markers are utilized for marker-controlled watershed algorithm to generate the final predicted result. The final predicted result consists of cell instances. The model is implemented using Python programming and Pytorch framework using Google Colab Pro. The training loss graphs for mask and marker models from tensorboard are shown in Fig. 3.

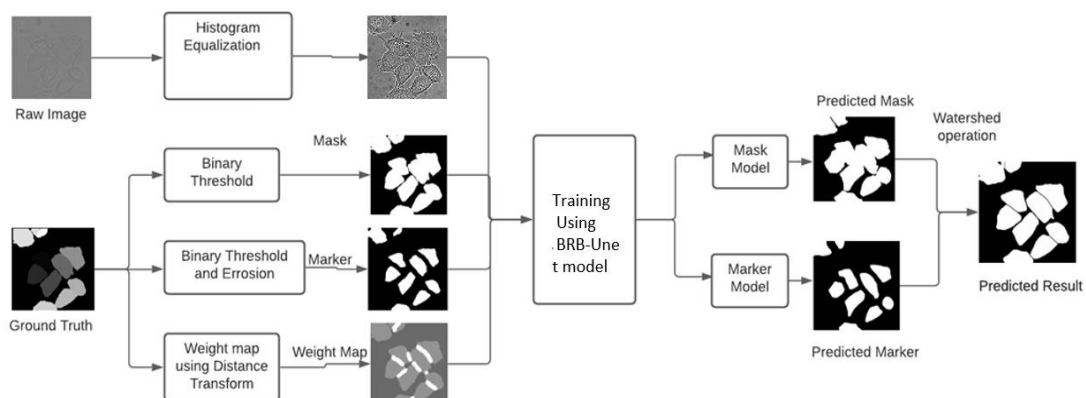


Fig 2: Block diagram

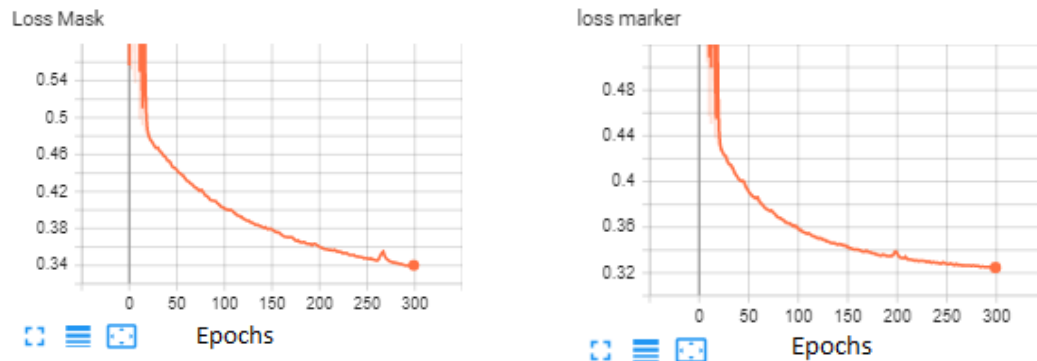


Fig 3: Training curves

III. RESULTS

Dice Index (DI) is utilized to measure the segmentation performance metrics. Dice Index is given by Eq. 1

$$DI = \frac{2 * (GT \cap PM)}{|GT| + |PM|} \quad (1)$$

where, GT stands for Ground Truth, PM stands for Predicted mask. DI value lies between 0 and 1, higher value of DI indicates that the ground truth and the prediction mask are more similar. For the discussed method the value of DI is 0.88. Visual output of the current method for a specific image is given in Fig. 3.

This clearly shows that BRB U-Net combined with marker-controlled watershed has given appreciable instance segmentation performance.

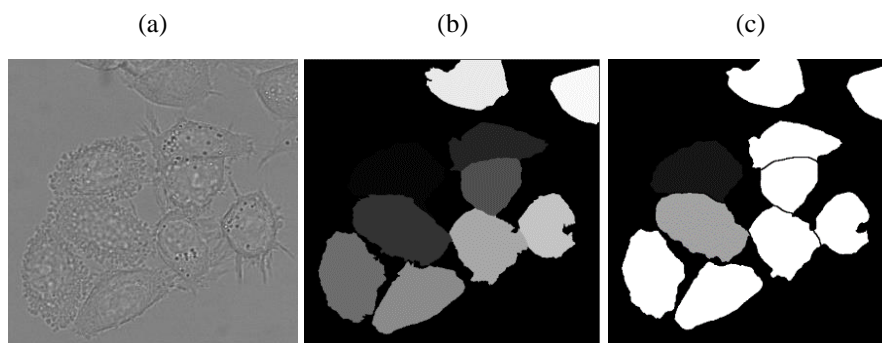


Fig 3: (a) Raw input image (b) Ground Truth Mask (c) Predicted Mask

IV. CONCLUSION

In this method BRB U-Net in combination with marker-controlled watershed algorithm is utilized for the instance segmentation of DIC-C2DH-HeLa microscopy cells.

This method gave Dice Index of 0.88. In future instance segmentation of various other microscopy cell types may be studied and evaluated. Also this method may be explored for different CNN models to improve the segmentation performance.



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