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A framework for nucleus and overlapping cytoplasm segmentation in cervical cytology extended depth of field and volume images

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ABSTRACT

We propose a framework to detect and segment nuclei and segment overlapping cytoplasm in cervical cytology images. This is a challenging task due to folded cervical cells with spurious edges, poor contrast of cytoplasm and presence of neutrophils and artifacts. The algorithm segments nuclei and cell clumps in extended depth of field (EDF) images and uses volume images to segment overlapping cytoplasm. The boundaries are first approximated by a defined *similarity metric* and are refined in two steps by reducing concavity, iterative smoothing and outliers removal. We evaluated our framework on two public datasets provided in the first and second overlapping cervical cell segmentation challenges (ISBI 2014 and 2015). The results show that our method outperforms other state-of-the-art algorithms on both datasets. The results on the ISBI 2014 dataset show that our method missed less than 5% of cells when the pairwise cell overlapping degree was not higher than 0.3 and it missed only 7% of cells on average in a dataset of 810 synthetic images with 4860 (overlapping) cells. On the same dataset, it outperforms other state-of-the-art methods in nucleus detection with precision 0.961 and recall 0.933. The results on the ISBI 2015 dataset containing real cervical EDF images show that our method misses around 20% of cells in EDF images where a segmentation is considered a miss if it has dice similarity coefficient not greater than 0.7. The 20% miss rate is around half of the miss rate of two other recent methods.

1. Introduction

It is estimated that 12,990 new cases of invasive cervical cancer will be diagnosed in 2016 and it will result in 4120 deaths in the U.S., according to American Cancer Society (American Cancer Society, 2015). The Pap test is one of the main screening tests used to diagnose cervical cancer in its early or precancerous stages. Conventionally, the slides containing the Pap smear are examined under a microscope by a cytologist or pathologist and cytopathology grading is based on a system such as The Bethesda System (Solomon et al., 2002). However, this manual process is time consuming and prone to errors and intraobserver variability. False negative rates are as high as 20–30% (Burd, 2003). These errors can occur because of clumping of cells, blood, bacteria or yeast contamination (Burd, 2003). To reduce such errors and workloads on pathologists and produce more consistent results computer systems are proposed to automate the slide analysis. Although newer preparation techniques (such as ThinPrep, SurePath, etc.) remove most of the mucus and inflammatory cells and decrease the number of overlapping cells, automating analysis of slides is still a very challenging task due to (1) folded cervical cells with spurious edges, (2) poor contrast of the cytoplasm area, (3) presence of bacteria, neutrophils, mucus and inflammatory cells and (4) overlapping cells. Also, the prepared *monolayer* slides can still range upwards of 30 μm from glass to coverslip (Lee et al., 2011). Therefore, it is impossible to have all cervical cells in-focus simultaneously in a single focal plane. One solution is to capture images at different focal planes in a way that each cell is in focus in at least one of them. This can be done by approximating the thickness of the slide by finding the top and bottom focal planes with cells and capturing images at intervals in between (Fan and Bradley, 2016). All the images in such stacks need to be checked for abnormal cells. Alternatively, with extended depth of field (EDF) algorithms (Bradley and Bamford, 2004; Forster et al., 2004), a single image can be created from the whole stack of images to have all cells in focus. These EDF images can subsequently be used by automated systems to be segmented and searched for abnormal cells.

Most of the current automated systems mainly aim to segment (overlapping) nuclei and cell clumps (cellular masses) (Jung et al., 2010; Gençtav et al., 2012; Plissiti et al., 2011; Zhang et al., 2014;

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Bergmeir et al., 2012; Plissiti and Nikou, 2012). These methods focus on nucleus segmentation and/or cell clumps and do not address the individual cytoplasm segmentation. Some methods address the segmentation of free lying or partially overlapping cells cytoplasm (Yang-Mao et al., 2008; Harandi et al., 2010; Béliz-Osorio et al., 2011). These methods assume that either the cells are not overlapping or the overlapping degree is very low. However, this is not realistic as cells usually have higher overlapping degree in real cervical cytology images. Cell clumps have a median of three nuclei and they contain more than four nuclei on average (Fan and Bradley, 2016).

Recently, there was an increase in the number of methods proposed for complete segmentation of overlapping cervical cells. This increase is mostly due to the first and second overlapping cervical cell segmentation challenges held in International Symposium on Biomedical Imaging (ISBI) 2014 and 2015 (Lu et al., 2016, 2015). The datasets in both challenges are publicly available and contain training (with ground truth) and test sets. This made the evaluation and comparison of different methods possible. Therefore, recent methods are mostly addressing the more challenging task of overlapping cytoplasm segmentation (Lu et al., 2015, 2016; Tareef et al., 2014; Lee and Kim, 2016; Ahmady Phoulady et al., 2016).

Lu et al. (2015) segmented cell clumps by applying the Quick Shift method (Vedaldi and Soatto, 2008) that results in a map of super pixels, applying an edge detector and learning an unsupervised binary classifier. They detected and segmented nuclei by finding the Maximally Stable Extremal Regions (MSER) (Matas et al., 2004) and segmented overlapping cytoplasms by joint optimization of multiple level set functions, where each function (representing a cell) has both unary (intra-cell) and pairwise (inter-cell) constraints. Ushizima et al. (2014) created a map of super pixels by merging regions based on pixel adjacency and intensity similarity using a graph-based linear-time algorithm (Nock and Nielsen, 2004) and followed it by a global search cut-off algorithm (Zack et al., 1977) to segment cell clumps. To segment nuclei inside cell clumps they used a local thresholding method (Phansalkar et al., 2011) and segmented overlapping cytoplasm by partitioning the image into convex polygons through Voronoi diagrams. Lee and Kim (2016) also generated superpixels to segment cell clumps: the SLIC superpixel method (Achanta et al., 2012) was used to generate a superpixel map and the map was thresholded using adaptive thresholding algorithm (Zack et al., 1977) to obtain the final cell clump regions. To segment nuclei they performed a local thresholding and removed outliers based on features such as mean intensity, circularity and size. They finally segmented overlapping cytoplasm by superpixel partitioning and assigning each superpixel region to the nearest nucleus. Then, the boundaries were refined by a cell-wise contour refinement with graph cuts (Boykov and Jolly, 2001) for each cell separately.

Most of these methods rely on superpixel and/or level set methods. Due to the high resolution of cervical cytology images, superpixel methods usually are only used as a preprocessing step (e.g. to extract edges (Lu et al., 2015) or to segment cell clumps (Lee and Kim, 2016; Ushizima et al., 2014)) or need to be followed by extra processes (e.g. cell-wise contour refinement (Lee and Kim, 2016)) to give accurate segmentations at the cell level. On the other hand, level set methods are computationally expensive and are sensitive to parameter initializations. Therefore, we propose a method that sets most of its parameters adaptively and is substantially faster than other recent methods. In the previous version of our algorithm (Ahmady Phoulady et al., 2016) we segmented nuclei by iterative thresholding. Regions were grown naturally by subsequent thresholdings and were filtered based on their features such as mean intensity, solidity, standard deviation, circularity and size. Cell clump segmentation was done by thresholding based on a learned GMM followed by morphological operations. To segment overlapping cells we defined a focus measure for subimages in the images and assigned subimages to nuclei based on their location and focus similarity. The contours were then refined in two coarse (subimage level) and fine steps (pixel level). Current work is an improved version of the previous work (Ahmady Phoulady et al., 2016). The nucleus segmentation process has been replaced by an algorithm that considers solidity of regions as their main feature. This simplified the algorithm, made it more resistant to brightness changes in images and also increased the method's nucleus detection accuracy in the experiments. The cell clump segmentation step is also improved by adding a filtering step. The main modification was done to the cytoplasm segmentation step, replacing the previous refinement step. For the refinement, we defined a weight vector and used it in the process of finding the new boundary points candidates. A smoothing filter was applied to the candidate points and the outliers were found based on the distance of each candidate point to its corresponding new location. After removing outliers, we applied the smoothing filter again and obtained the new estimated boundary. The whole process was repeated until the boundaries converged.

The major contribution of this work is the proposed novel method for the boundary approximation of overlapping cells that utilizes the information in the image stacks efficiently to approximate the boundary for the subsequent refinements (the method is discussed in 2.3.1). Two other contributions are: (1) the proposed nucleus detection and segmentation method that achieves superior results compared to other state-of-the-art methods in terms of *F*-measure (the method is discussed in Section 2.1 and the results are presented in Section 4.3.1), and (2) the fine refinement step (discussed in Section 2.3.3) with the defined weight vector (Eq. (7)) that can refine the boundaries effectively if only a small ratio of useful edge pixels exist (note the refined boundaries in Fig. 1(d)).

Other than the contributions mentioned above the methods run very fast (as discussed at the end of Section 4.3) and achieve substantially better results compared to other state-of-the-art algorithms in terms of the rate of missed cells. Moreover, our algorithm has fewer parameters to tune (two parameters, as discussed in Section 4) than other state-of-the-art algorithms. Finally, we propose to report a new metric (in Section 4.1), False Discovery Rate at object level, that helps to capture the cell detection accuracy more effectively.

2. Methods

Each cell contains a nucleus and segmenting nuclei is generally easier than segmenting individual cytoplasms. We start the segmentation process by segmenting nuclei and then segment corresponding cytoplasm for each nucleus. The framework contains three main steps: (1) nucleus detection and segmentation (Section 2.1), (2) cell clump segmentation (Section 2.2) and (3) overlapping cytoplasm segmentation (Section 2.3). The first two steps use the EDF image and the last step uses a stack of images to segment cytoplasm (in the case of the ISBI 2014 dataset that does not contain stack images, the algorithm was modified to only use the location of subimages as discussed in 4.3.2). Fig. 1 shows the steps on a real cervical cytology EDF image.

2.1. Nucleus detection and segmentation

The goal of this step is to detect and segment nuclei.

Nuclei are commonly represented by small uniform relatively dark and convex regions. Therefore, the three most visually distinctive and important features of nuclei are size, average intensity and solidity. We used these three features to design an iterative algorithm to detect and segment nuclei. Because each segmented

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