# SEGMENTATION OF SUBCELLULAR COMPARTIMENTS COMBINING SUPERPIXEL REPRESENTATION WITH VORONOI DIAGRAMS

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## ABSTRACT

We propose and implement a computer vision algorithm for detecting individual cells, including the ability to distinguish subcellular compartments, such as nucleus and cytoplasm. Our approach consists of three main steps: (a) cellular mass estimation, and (b) nuclei detection through superpixel representation and, (c) cytoplasm detection through nuclear narrow-band seeding, graph-based region growing and Voronoi diagrams. We test our implementation on both real and simulated cervical cell images, containing an assortment of cells and configurations that often present occlusion and/or poor contrast. Our results show both qualitative and quantitative assessment of the datasets, using a completely automated computer program. The quantitative performance presents average Dice Coefficient equals to 0.85.

*Index Terms*— Segmentation of occluded cells, Cervical Image Analysis, Superpixel with Voronoi

#### **1. INTRODUCTION**

While the ability to collect and record image data has increased drastically, available tools for Pap smears image analysis and quantification are not precise enough to deliver relevant information about the smears content [1]. Automated cell analysis from digitized Pap smear slides requires segmentation of subcellular compartments, such as nucleus and cytoplasm. According to Lu et al [2], there are three main approaches to cervical cell segmentation: (i) threshold-based, (ii) mathematical morphology-based and (iii) others based on front propagation. We combine these three concepts to deliver an efficient algorithm for automated nucleus and cytoplasm segmentation of overlapping cervical cells from 2D image datasets [2].

This paper proposes a new unsupervised method, implemented using Fiji [3] that fuses key methods such as superpixel representation with Voronoi diagrams to detect cells, and split cellular mass into individual nuclei and cytoplasms. Our algorithm also includes other support processing functions, such as image enhancement, noise minimization, segmentation of cellular mass, followed by nuclei and cytoplasm detection tested on a database with both real and simulated cervical cell images.

### 2. MATERIALS/METHODS

The "Overlapping Cervical Cytology Image Segmentation Challenge" provided a dataset consisting of 961 images, which are 512x512 pixels, and they contain 2 or more cells with different degrees of overlap, contrast, and texture. Among these images, only 16 are from real Pap smear slides, while the others are synthetically generated, as described in [2]. One of the advantages of considering this simulated dataset is the presence of two subsets: images with and without ground-truth (GT), which are an essential part of the quantitative analysis of segmentation procedures.

In order to detect and segment cells into nuclei and cytoplasms, our algorithm relies on three main steps: (i) segmentation of the cellular mass (a.k.a. cell clumps in [2]) by defining superpixels [4]; (ii) segmentation of nuclei using Phansalkar's local search from low contrast images [5]; (iii) detection and segmentation of cytoplasm by calculating the Voronoi diagram of the previously detected nuclei as constrains to the cytoplasm boundary. The top left of Fig.1 shows a diagram of the proposed image processing workflow. The other pictures in Fig.1 present an example of a Pap smear slide analysis, illustrating partial results during processing up to final segmentation (Fig.1. bottom right)

The following paragraphs explain the three main steps of our algorithm to segment overlapping cervical cells: (i) Superpixels: Instead of using the pixel-grid as the underlying representation of cells, we calculate low-level pixel clusters that partition the image into a much smaller number of segments (superpixels). First, we reduce textural information from small intensity variations, and preserve borders by applying the bilateral filter [6]. Second, we perform contrast limited adaptive histogram equalization [7] to enhance nuclei with poor contrast, which often happens due to cytoplasm overlap. Third, we merge regions statistically regarding both pixel adjacency and intensity similarity using the graph-based linear-time algorithm from [8], which generates the image oversegmentation or superpixels. We benefit from the oversegmentation output by extracting the cellular mass after applying the triangle algorithm [9], which determines the global search cut-off by normalizing the height and dynamic range of the intensity histogram. Finally, we add a halo to the cellular mass using morphological dilations to increase searchable target area.



Fig. 1. Cervical cell detection workflow (top-left), and partial results for image 23 from training dataset before nucleus/cytoplasm individualization through superpixel representation, Phansalkar's local search algorithm and Voronoi diagrams.

(ii) Nucleus detection with local search: Phansalkar et al [5] designed an algorithm for local thresholding (*t*), suitable for cytology images with low contrast. This algorithm enables us to determine the threshold for each pixel according to the image properties within a window of radius r=15, and weighting parameters k=0.25, s=1, p=2, q=10, such as:  $t = \mu^*(1 + p^* \exp(-q^*\mu) + k^*((\sigma/s) - 1))$ 

(iii) Cytoplasm detection through Voronoi diagrams: After removing the nuclei from the cellular mass, we calculate a narrow-band around the nuclei to find seeds inside the cytoplasm – they are fundamental to recover the cytoplasm mass through the region growing. In order to split this cytoplasmic mass into individual cytoplasms, we consider the previously detected nuclei as input to calculate the lines having equal distance to the nearest nuclei. In other words, we partition the image into convex polygons such that each Voronoi polygon contains one nucleus.

#### 4. RESULTS/CONCLUSION

We summarize our results in Table I, using the outputs from running the provided code evaluateCvtoSegmentation.m. In order to show the influence of changing the main parameters in our algorithm, we compared our results varying: (i) the bilateral filter, (ii) the number of segments for oversegmentation, and (iii) the radius for local search, which are listed in this order in column 1 of Table I. Table I also shows that the proposed algorithm is insensitive to small variations of the parameters, with Dice coefficient (DC) around 0.88 in all cases. The average running time of our algorithm on the synthetic dataset was 12 seconds per image using an unoptimized Fiji script on a Cray XC30 supercomputer with a 12-core Intel "Ivy Bridge" processor at 2.4 GHz with 64GB RAM. Our algorithm recovers a reasonably accurate approximation of the nucleus and cvtoplasm. Improvements should consider cvtoplasm boundaries to be perturbed and attracted to the real boundaries using level set optimization, similarly to [2].

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Table	E	Results	for	train/test	set	with	different	parameters.
	••							parative verb.

Database	DC	FNo	ТРр	FPp
Train45	0.872	0.277	0.817	0.0026
30_25_30	±0.075	±0.263	±0.119	±0.0043
Train45	0.880	0.155	0.825	0.0019
30_12_15	±0.074	$\pm 0.208$	±0.120	±0.0026
Train45	0.881	0.203	0.827	0.0019
60 25 15	±0.075	±0.272	±0.122	±0.0027
Train45	0.881	0.155	0.826	0.0019
(30_25_15)*	±0.0741	±0.208	±0.119	$\pm 0.0026$
Test90	0.867	0.174	0.826	0.0015
(30_25_15)*	±0.0832	±0.21	±0.013	$\pm 0.0018$

\*same parameters used for test set with 810 images

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