

Iterative H-minima Based Marker-Controlled Watershed for Cell Nucleus Segmentation: Supplementary Material

Can Fahrettin Koyuncu, Ece Akhan, Rengul Cetin-Atalay, and Cigdem Gunduz-Demir

Abstract—Automated microscopy imaging systems facilitate high-throughput screening in molecular cellular biology research. The first step of these systems is cell nucleus segmentation, which has a great impact on the success of the overall system. This technical report contains the supplementary material for the iterative h-minima based marker-controlled watershed algorithm that we developed for the purpose of segmenting the nuclei of cells in fluorescence microscopy images [1].

Index Terms—Nucleus segmentation, h-minima transform, watershed, fluorescence microscopy imaging

I. INTRODUCTION

WE recently developed a new marker-controlled watershed algorithm for cell nucleus segmentation [1]. We tested this algorithm on the images of cultured human hepatocellular carcinoma (Huh7 and HepG2) cell lines as well as on the images of liver tissue sections from mouse liver. In [1], we reported the nucleus based F-score measures for quantitative evaluation. This technical report presents the detailed quantitative results.

II. EXPERIMENTS ON CELL LINE IMAGES

The first dataset includes the images of cultured human hepatocellular carcinoma (Huh7 and HepG2) cell lines. It contains 785 training nuclei taken from 10 randomly selected images. These training nuclei are used to select the model parameters. For this type of images, we use three test sets that contain cells of different confluency levels. The first test set includes 891 nuclei taken from 11 images of the Huh7 cell line. The second one contains 985 nuclei taken from 16 images of the HepG2 cell line. The last set consists of 1065 nuclei taken from 4 images of the HepG2 cell line; these images contain more confluent cells. We referred them as the *Huh7 test set*, the *HepG2 test set*, and the *dense HepG2 test set*, respectively. The cell nucleus boundaries in these images were annotated by our biologist collaborators.

For the quantitative evaluation of our algorithm, we first determined the correctly identified nuclei. For this purpose, we matched each nucleus that an algorithm segmented with

C. F. Koyuncu and C. Gunduz-Demir are with the Department of Computer Engineering, Bilkent University, Ankara TR-06800, Turkey (e-mail: {koyuncu, gunduz}@cs.bilkent.edu.tr).

E. Akhan is with the Department of Molecular Biology and Genetics, Bilkent University, Ankara TR-06800, Turkey (e-mail: akhan@bilkent.edu.tr).

R. Cetin-Atalay is with the Graduate School of Informatics, Middle East Technical University, Ankara TR-06800, Turkey (e-mail: rengul@metu.edu.tr).

TABLE III

FOR THE EXPERIMENTS ON TISSUE SECTION IMAGES, COMPARISON OF THE ALGORITHMS IN TERMS OF THE PRECISION, RECALL, AND F-SCORE MEASURES. THESE RESULTS ARE OBTAINED ON THE TEST SET IMAGES.

	Precision	Recall	F-score
Iterative h-minima [1]	84.78	87.96	86.34
Adaptive h-minima [2]	84.30	73.71	78.65
Conditional erosion [3]	86.77	75.50	80.75
Iterative voting [4]	81.60	75.61	78.49
ARGraphs [5]	85.46	77.88	81.49

an annotated nucleus in the gold standard. Then, we identified *one-to-one* matches, which would be considered as correctly identified nuclei, as well as oversegmentations, undersegmentations, false detections, and misses. After finding the correctly identified nuclei, we calculated the nucleus-based *precision*, *recall* and *F-score* measures. Additionally, we calculated the same performance measures on pixels by considering the correctly segmented pixels of only the correctly identified nuclei as correct segmentation.

In our experiments, we compared the results of our proposed algorithm with those of four nucleus segmentation methods: adaptive h-minima [2], conditional erosion [3], iterative voting [4], and ARGraphs [5]. We also calculated the same measures on the segmentation results of these methods. The comparison results obtained on the Huh7, HepG2, and dense HepG2 test sets are given in Tables I and II.

III. EXPERIMENTS ON TISSUE SECTION IMAGES

The next dataset consists of 13 images of tissue sections from mouse liver. We randomly separated these images into the training and test sets. The training set includes 766 cell nuclei from four images, on which the model parameters are selected. The test set contains 1894 nuclei from the remaining nine images. Our biologist collaborators annotated these images by marking the cell nuclei without drawing their boundaries.

For quantitative evaluation, we considered *one-to-one* matches and computed the *precision*, *recall* and *F-score* measures. However, since the annotations do not include the nucleus boundaries but a marker for each nucleus, we defined one-to-one match as follows. A segmented nucleus was considered as one-to-one match if this nucleus contained only a single marker, which indicated a gold standard nucleus, inside. The comparison results obtained on the test set by our proposed algorithm and the comparison methods are given in Table III.

TABLE I

FOR THE EXPERIMENTS ON CELL LINE IMAGES, COMPARISON OF THE ALGORITHMS IN TERMS OF SEGMENTED-ANNOTATED NUCLEUS MATCHES ON THE (A) HUH7, (B) HEPG2, AND (C) DENSE HEPG2 TEST SETS.

	One-to-one	Overseg	Underseg	False	Miss
Iterative h-minima [1]	796	31	22	17	42
Adaptive h-minima [2]	745	11	120	28	15
Conditional erosion [3]	738	27	77	34	49
Iterative voting [4]	721	51	60	59	34
ARGraphs [5]	788	35	51	17	20

(a)

	One-to-one	Overseg	Underseg	False	Miss
Iterative h-minima [1]	791	31	48	37	115
Adaptive h-minima [2]	684	4	280	43	17
Conditional erosion [3]	617	15	297	57	56
Iterative voting [4]	721	58	131	75	45
ARGraphs [5]	780	37	116	44	52

(b)

	One-to-one	Overseg	Underseg	False	Miss
Iterative h-minima [1]	746	22	126	32	171
Adaptive h-minima [2]	506	12	467	49	80
Conditional erosion [3]	473	27	395	91	170
Iterative voting [4]	528	57	287	122	193
ARGraphs [5]	681	39	197	62	148

(c)

TABLE II

FOR THE EXPERIMENTS ON CELL LINE IMAGES, COMPARISON OF THE ALGORITHMS IN TERMS OF NUCLEUS-BASED AND PIXEL-BASED PRECISION, RECALL, AND F-SCORE MEASURES ON THE (A) HUH7, (B) HEPG2, AND (C) DENSE HEPG2 TEST SETS.

	Nucleus-based			Pixel-based		
	Precision	Recall	F-score	Precision	Recall	F-score
Iterative h-minima [1]	89.24	89.34	89.29	83.58	73.93	78.46
Adaptive h-minima [2]	88.27	83.61	85.87	82.57	79.47	80.99
Conditional erosion [3]	85.21	82.82	84.01	86.11	72.61	78.78
Iterative voting [4]	81.28	80.92	81.10	81.26	68.48	74.33
ARGraphs [5]	88.14	88.44	88.29	78.28	85.51	81.74

(a)

	Nucleus-based			Pixel-based		
	Precision	Recall	F-score	Precision	Recall	F-score
Iterative h-minima [1]	86.35	80.30	83.22	80.09	65.02	71.77
Adaptive h-minima [2]	80.37	69.44	74.50	67.16	66.33	66.74
Conditional erosion [3]	73.89	62.63	67.80	64.24	55.86	59.76
Iterative voting [4]	75.89	73.19	74.52	70.67	61.12	65.55
ARGraphs [5]	81.41	79.19	80.28	65.75	75.37	70.24

(b)

	Nucleus-based			Pixel-based		
	Precision	Recall	F-score	Precision	Recall	F-score
Iterative h-minima [1]	84.48	70.05	76.59	74.94	59.89	66.58
Adaptive h-minima [2]	71.37	47.51	57.05	48.89	44.08	46.36
Conditional erosion [3]	61.67	44.41	51.64	49.22	39.82	44.02
Iterative voting [4]	58.41	49.58	53.63	52.52	42.32	46.87
ARGraphs [5]	74.34	63.94	68.75	59.69	62.87	61.24

(c)

REFERENCES

- [1] C. F. Koyuncu, E. Akhan, T. Ersahin, R. Cetin-Atalay, and C. Gunduz-Demir, "Iterative h-minima based marker-controlled watershed for cell nucleus segmentation," submitted, 2015.
- [2] J. Cheng and J. C. Rajapakse, "Segmentation of clustered nuclei with shape markers and marking function," *IEEE Trans. Biomed. Eng.*, vol. 56, no. 3, pp. 741-748, Mar. 2009.
- [3] X. Yang, H. Li, and X. Zhou, "Nuclei segmentation using marker controlled watershed, tracking using mean-shift, and Kalman filter in time-lapse microscopy," *IEEE Trans. Circuits Syst. I*, vol. 53, no. 11, pp. 2405-2414, 2006.
- [4] B. Parvin, Q. Yang, J. Han, H. Chang, B. Rydberg, and M. H. Barcellos-Hoff, "Iterative voting for inference of structural saliency and characterization of subcellular events," *IEEE Trans. Med. Imag.*, vol. 16, no. 3, pp. 615-623, Mar. 2007.
- [5] S. Arslan, T. Ersahin, R. Cetin-Atalay, C. Gunduz-Demir, "Attributed relational graphs for cell nucleus segmentation in fluorescence microscopy images," *IEEE Trans. Med. Imaging*, vol. 32, no. 6, pp. 1121-1131, Jun. 2013.