

SEGMENTATION OF LIVING, FLUORESCENTLY LABELED CELLS USING GRAPH CUTS

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Abstract

Accurate cell segmentation is an essential initial step for most detailed automatic quantitative analysis. When the images are captured sequentially from the 3D culture containing living, proliferating and moving cells the incidences of cell-cell interactions and collisions increase, which makes cell segmentation very challenging. Here we present a method which utilizes the edge probability map and graph cut method to segment individual cells from within a cluster.

Overview

Our cell segmentation method consists of three (for 2D images) and four (for 3D images) separate stages. In the first stage, cells are separated from background. In the second stage, seeds for individual cells are found. This is our main contribution as we propose a new method for locating seeds for individual cells within a cell cluster without making any assumption about cell shape. In the third stage, watershed is used to find boundaries of individual cells. In case of 3D images, an additional (fourth) stage is required to obtain 3D cell segmentation from individually segmented slices. For 3D stacks, first all slices are processed in 2D, then individual objects from each slice in 3D stack are connected in axial direction by considering their overlap with objects in those slices.

Graph Structure

2D Image is represented by a grid based graph. Every pixel is represented by a grid node, which is connected to its 4-neighboring nodes and two terminal nodes (cell/foreground and background). The cost of edges between neighboring nodes, p and q are set using the difference of edge probability value [1] at their location according to:

$$edge(p, q) = C * e^{-\frac{|E_d(p) - E_d(q)|}{\tau_1}}$$

d is the direction of edge between the pixels and E_d is the edge probability in d direction. GridCut [2] is used to find the pixel labeling with minimum cost.

FG/BG Separation

First step in the processing chain is to detect all the pixels belonging to any cell. The terminal edge costs for this step are computed using:

$$edge(FG, p) = e^{-\frac{I(p)}{\tau_2}}$$

$$edge(BG, p) = 1 - e^{-\frac{I(p)}{\tau_2}}$$

Min-cut of this graph separates cell pixels from background.

Cell Seed Detection

Cost of edges connecting grid nodes to terminal nodes in graph are modified to force boundary pixels within cell clusters to belong to background using:

$$edge(FG, p) = e^{-\frac{I(p)}{\tau_2}} + w * E(p)$$

$$edge(BG, p) = 1 - e^{-\frac{I(p)}{\tau_2}} - w * E(p)$$

Each connected components in the min cut of this graph is a seed for a cell, which is utilized by watershed to find the cell boundaries within a cell cluster.

3D Slice merging

Due to anisotropic nature of 3D stacks, direct application of this method in 3D does not result in good performance. Therefore we apply this method slice by slice to whole stack and the maximum intensity projection. Then we find the best matching (based on overlap) object in 3D stack for each segmented object in maximum intensity projection. This object is expanded in axial direction by checking overlap with objects in adjacent slices.

Results

Table 1 compares the performance of our method with some common segmentation methods on a simple sequence (a frame is shown in Fig.1) and shows that it outperforms these basic segmentation methods. Fig. 2 show the segmentation results for more challenging image stacks which include cells with highly flexible shape. The main contribution of our method is that it does not make any assumption about cell shape and can cope with wide variety of cell shapes. Our segmentation method performs quite well on some challenging cell clusters in which the intensity and shape information can not easily be utilized. However it sometimes fails to separate touching cells if they happen to have very similar intensity and texture on both sides of the boundary, it also can not take advantage of cell shape when cells have a somewhat rigid shape.

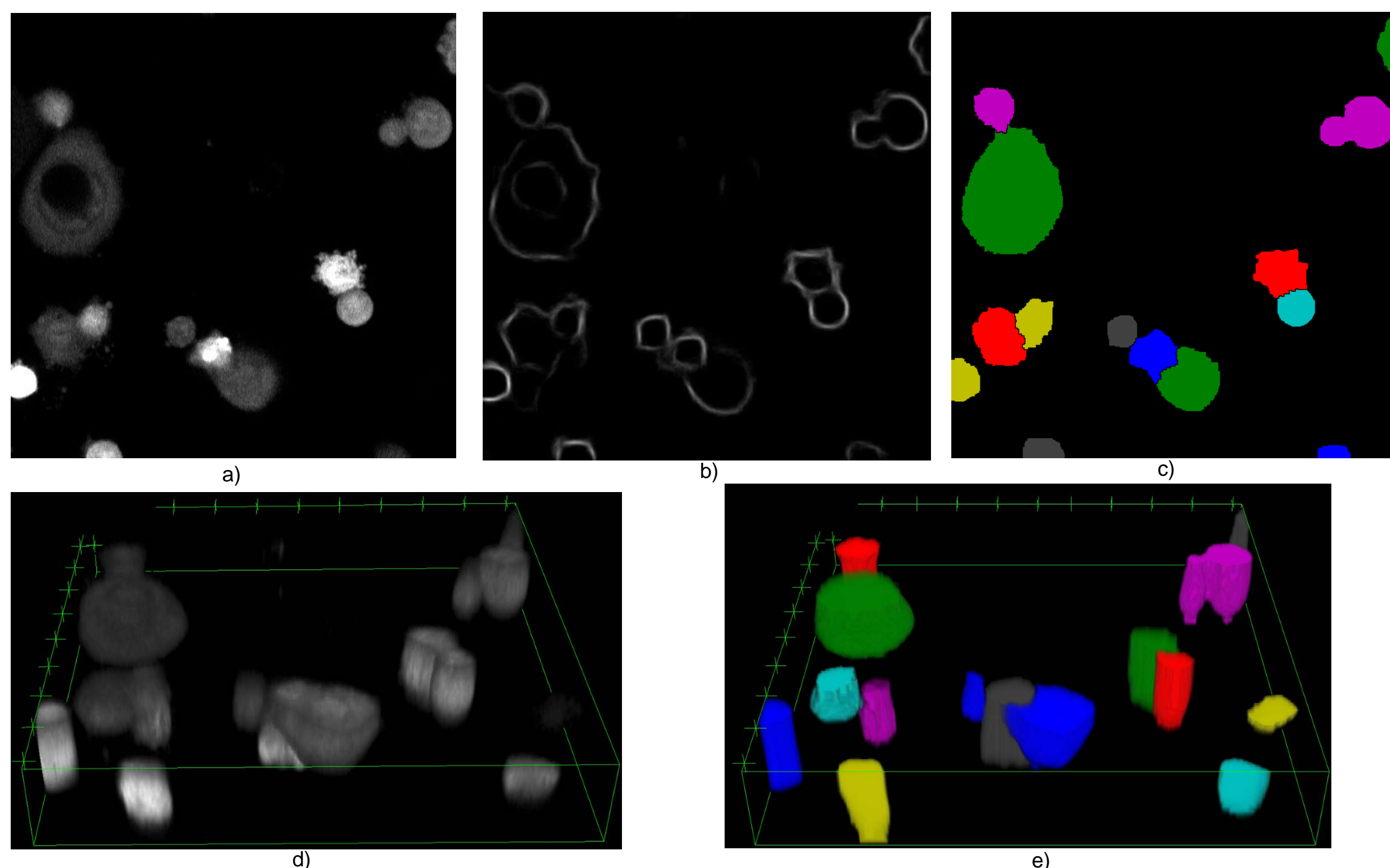


Fig. 1. a) Slice # 15 of 3D stack shown in (d), b) Edge Probability Map of (a), c) Segmentation of (a), d) 3D Stack, e) 3D Segmented stack.

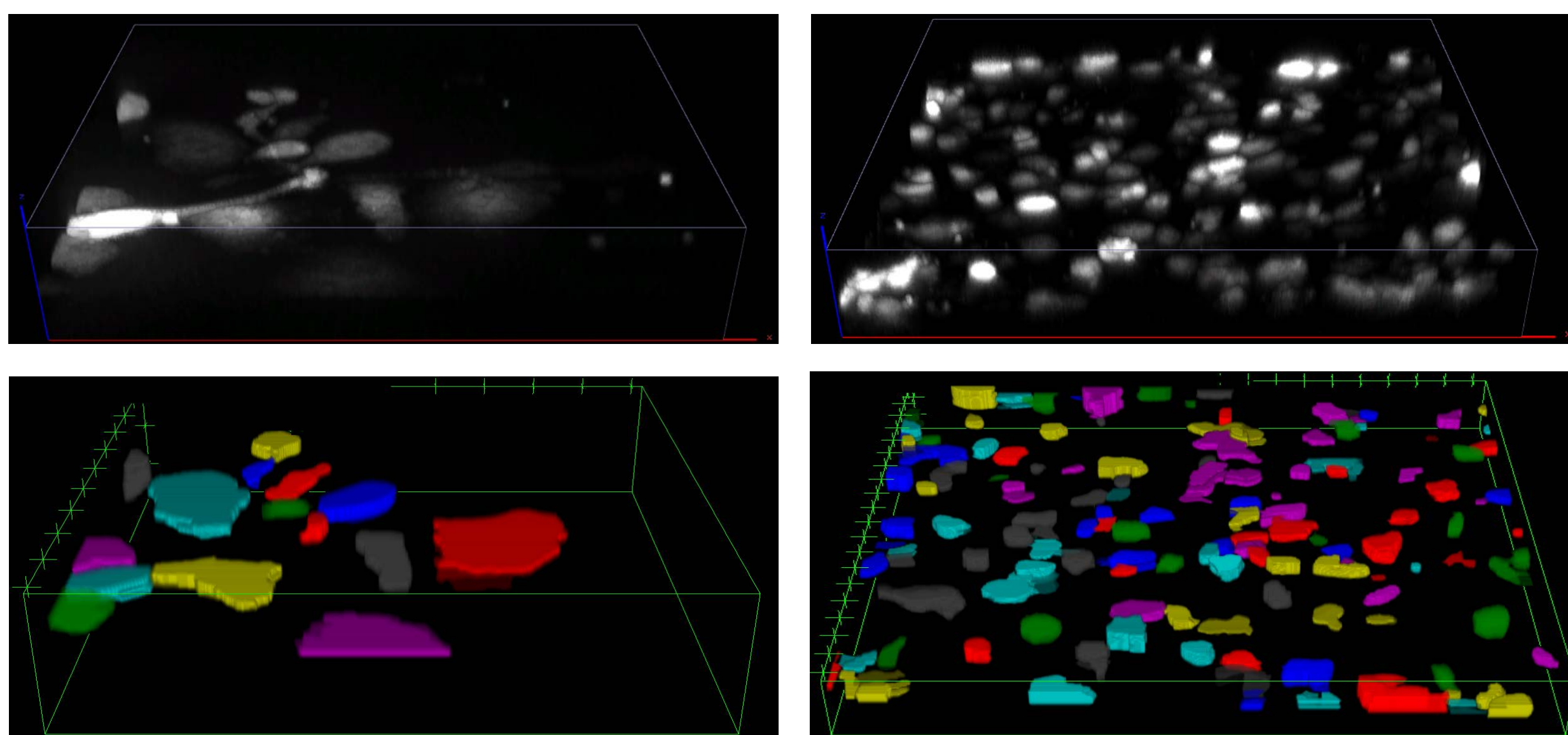


Fig. 2. Two more challenging stacks along with their segmentations

	Precision	Recall	F1-Score
GC (Our Method)	92.2	91.4	0.92
GC-Blob [3]	66.4	76.2	0.71
MSER [4]	88.7	51.2	0.65
OTSU	71.6	54.6	0.62

Table 1. Comparison of our method with few common segmentation methods.

References

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For more Information

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