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A validated active contour method driven by parabolic arc model for detection and segmentation of mitochondria

Serdar F. Tasel [a,b,*], Erkan U. Mumcuoglu [a], Reza Z. Hassanpour [b], Guy Perkins [c]

[a] Department of Health Informatics, Graduate School of Informatics, Middle East Technical University, 06531 Ankara, Turkey
[b] Department of Computer Engineering, Cankaya University, 06810 Ankara, Turkey
[c] National Center for Microscopy and Imaging Research, University of California, San Diego, CA 92093-0608, USA

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ABSTRACT

Recent studies reveal that mitochondria take substantial responsibility in cellular functions that are closely related to aging diseases caused by degeneration of neurons. These studies emphasize that the membrane and crista morphology of a mitochondrion should receive attention in order to investigate the link between mitochondrial function and its physical structure. Electron microscope tomography (EMT) allows analysis of the inner structures of mitochondria by providing highly detailed visual data from large volumes. Computerized segmentation of mitochondria with minimum manual effort is essential to accelerate the study of mitochondrial structure/function relationships. In this work, we improved and extended our previous attempts to detect and segment mitochondria from transmission electron microcopy (TEM) images. A parabolic arc model was utilized to extract membrane structures. Then, curve energy based active contours were employed to obtain roughly outlined candidate mitochondrial regions. Finally, a validation process was applied to obtain the final segmentation data. 3D extension of the algorithm is also presented in this paper. Our method achieved an average *F*-score performance of 0.84. Average Dice Similarity Coefficient and boundary error were measured as 0.87 and 14 nm respectively.

1. Introduction

Mitochondrial structural correlates to function are attracting increased attention as the relation between mitochondrial function and degenerative disorders related to aging such as Alzheimer's and Parkinson's diseases is becoming empowered by recent studies (Burté et al., 2014; Franco-Iborra et al., 2015; Wang et al., 2014). Three dimensional (3D) visualization of mitochondria is coming into prominence because these studies expose the need for detailed analysis of high-resolution physical alterations in mitochondria.

Investigations of subcellular structures are being made more effective by means of advances in electron microscopy. The physical formation of mitochondria emerges in detail as new imaging techniques have been developed. Serial block-face scanning electron microscopy (SBEM) is one of the emerging methods for volumetric mitochondrial imaging that provides 3D datasets consisting of voxels with a size typically of 5–10 nm in *x*- and *y*-axes and 20–80 nm in *z*-axis (Chavan et al., 2015; Wanner et al., 2015). Transmission electron microscope (TEM) tomography is another powerful technique that renders possible the visualization of structures down to a few nm resolution (Davies et al., 2014; Harapin et al., 2013). Such a resolution offers a clear separation of the double membrane structure of mitochondria and distinctive arrangement of cristae as well as the visualization of other subcellular structures such as synaptic vesicles (SV) and the endoplasmic reticulum (ER). Considering that the size of mitochondria varies between 0.3 and 10 μm (Scheffler, 2007), large 3D tomographic volumes are often required to study the structure of whole mitochondria.

In a study by Perkins et al. (2003) various structural features, such as the width of the peripheral inner and outer membranes of mitochondria and cristae, the number of crista segments, crista junctions and contact site diameters were measured and hypothesized to have potential effect on mitochondrial function. Visualization details depend on the resolution of the tomographic volume as well as the structural preservation of the sample. To perform a detailed mitochondria segmentation in such volumes, both peripheral and cristae membranes are required to have high contrast with respect to the background. By heavy-metal staining, TEM tomography highlights the two mitochondrial membrane systems. Cristae segmentation provides a basis for our motivation to develop an

* Corresponding author at: Department of Health Informatics, Graduate School of Informatics, Middle East Technical University, 06531 Ankara, Turkey.
E-mail address: fst@cankaya.edu.tr (S.F. Tasel).

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automatized segmentation method for mitochondrial boundary to be used in this imaging modality.

Visual investigation of tomographic volumes of mitochondria are currently carried out by manual segmentation utilizing specialized software tools such as IMOD (Kremer et al., 1996) and Amira. However, hand segmentation of features of a volume may produce flawed results because of human error even with highly trained segmenters. Because mitochondria are pleomorphic and appear in many different forms based on cell type, respiration or disease state, and sample preparation, a perfect automated segmentation is still an unsolved problem.

Various attempts have been made to automatically segment membranes and mitochondria from transmission electron microscope (TEM) tomography images. A confidence-connected and level-set based segmentation scheme was realized to extract contours of membranes of mitochondria (Bazán et al., 2009). The method produced successful segmentation results. However, it requires a manual connected-component removal and clear visualization of membranes.

Mitochondria segmentation based on classification of random forest patches and contour pairs (Giuly et al., 2012), utilization of supervoxel segmentation (Ghita et al., 2014; Lucchi et al., 2012) and spectral clustering techniques (Dietlmeier et al., 2013) which basically use intensity distribution of mitochondria and differentiation from background were also asserted. However, these methods are not suitable for specimens specially prepared for cristae analysis as mentioned earlier since the differentiation of the intensity distribution is lost in the preparation process.

Several generic methods to segment membrane-like thin structures from electron tomography images were developed as well. A membrane segmentation method based on Hessian-based ridge detection was developed for extraction of membrane-like structures such as mitochondria boundary, SV and ER (Martinez-Sanchez et al., 2014, 2013, 2011). Bartesaghi et al. (2005) proposed a semi-automated 3D segmentation method based on minimal surface of closed geodesic curves. Another approach was introduced by Sandberg and Brega (2007) in which a contour tracing mechanism utilized local features extracted by line and orientation filter transforms. A bilateral edge filtering method was presented by Pantelic et al. (2007) for 2D segmentation of noisy electron microscopy and cryo-electron microscopy images. A 3D extension of this method was later proposed by Ali et al. (2012). A cascaded hierarchical model (CHM) by Seyedhosseini et al. (2013b) was used for membrane segmentation in SBEM and TEM images and provided promising results. Another intriguing semi-automated procedure was established by Page et al. (2015) for cell segmentation which is based on watersheds exploiting the differentiation of structures inside and outside of the cell. Although notable results have been obtained, these proposed methods have not been capable of separating mitochondria from each other or from membranes of other subcellular structures, or segmenting the more morphologically complex mitochondrial inner membrane.

In our previous attempt (Mumcuoglu et al., 2012), we employed a double membrane detection based on kernel pairs and an ellipse fitting approach for 2D detection and separation of mitochondria followed by active contours and a modified livewire method for 2D accurate segmentation of mitochondria. This algorithm depends on the successful removal of cristae in one of the intermediate steps to locate the peripheral mitochondrial membranes. Our current work describes a better mechanism to separate peripheral membranes from cristae membranes.

In another study (Seyedhosseini et al., 2013a), algebraic curve based segmentation was applied to 2D mitochondrion images which is reasonable to obtain a curve which tracks the boundary of a mitochondrion. However, it can easily be attracted by cristae and further, it is not sufficient to separate mitochondria from each other.

In our preliminary study (Tasel et al., 2014), a 2D detection method for mitochondria performed by active contours using a parabolic arc based membrane detection process was proposed. Although the method was tested on a limited dataset, the study revealed that the curve based membrane detection approach is useful to extract mid-level features which are relatively easy to manage and capable to differentiate mitochondria peripheral membranes and cristae membranes.

In this study, we have improved the aforementioned parabolic arc model fitting algorithm and presented the separation of the two peripheral membranes and cristae membranes. In our new scheme, an active contour model driven by a curve energy image is utilized to obtain candidate mitochondrial regions. Finally, a validation process in order to filter false shapes is applied by using features extracted from continuity, curvature and signature characteristics of boundary and additional curve energy of cristae. Instead of removing cristae (as in our previous attempt), we adopted a mechanism which uses existence of cristae as a sign of the presence of a mitochondrion, because mitochondria are the only structures inside a cell that possess cristae. We have tested our method on a much larger volume of datasets that were used in our preliminary study. The major contribution of the work is 3D extension of the algorithms. Detection and segmentation performances of 2D and 3D method are also compared and discussed.

The subsequent sections of this paper present information about the datasets used, the acquisition of ground truth, the proposed methods, experiments and results.

2. Datasets and ground truth

The datasets used for the experiments in this work were collected from the Cell Centered Database (CCDB) supported by the National Center for Microscopy and Imaging Research (NCMIR) (Martone et al., 2008, 2003, 2002). We chose eight TEM datasets which include tomograms having diversity in image contrast and variety in mitochondrial membrane and crista characteristics. The collection comprises mitochondria appearing in various shapes and sizes. Some properties of the datasets such as image size or voxel size are given in Table 1.

Table 1
Properties of datasets used in the experiments.

Dataset basename	Accession number	Image size (width × height)	Number of slices used	Voxel size (nm) ($X \times Y \times Z$)
6_22.sub	5,274,878	1960 × 2560	91	1.1 × 1.1 × 1.1
bclpb-d.sub	5,274,930	720 × 878	61	2.4 × 2.4 × 2.4
cone.sub	54	736 × 1010	97	2.4 × 2.4 × 2.4
gap18_sub	8747	350 × 600	54	2.2 × 2.2 × 2.2
mac_serial_sub	5,274,996	907 × 1172	111	2.4 × 2.4 × 2.4
od.sub	8752	1960 × 2560	91	1.1 × 1.1 × 1.1
pedicle	5,274,970	950 × 1280	31	2.4 × 2.4 × 2.4
spherule24mos1	8495	1996 × 1996	86	1.67 × 1.67 × 1.67