

STRUCTURAL METHODS IN SUBCELLULAR IMAGE ANALYSIS

CENTRE FOR IMAGE PROCESSING AND ANALYSIS

Julia Dietlmeier and Paul F. Whelan, Vision Systems Group, Dublin City University, Dublin, Ireland



Centre for Image Processing & Analysis



THE NATIONAL BIOPHOTONICS & IMAGING PLATFORM IRELAND



INTRODUCTION

Mitochondria play a central role in cellular bioenergetics and in the regulation of apoptotic cell death. Mitochondrial morphology (shape and *cristae* architecture) is crucial to the understanding of apoptosis mechanisms and the subsequent development of therapies targeting age- and cancer-related diseases^[1,2].

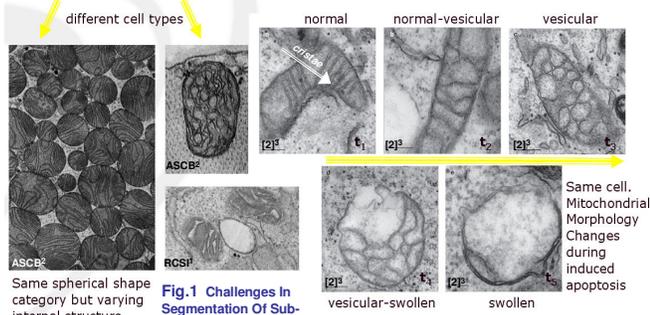


Fig.1 Challenges In Segmentation Of Sub-cellular Domains.

There is a high demand in automated segmentation tools which can provide an objective quantitative information in a reasonable time frame^[2]. The state of the art however is still dominated by manual segmentation tools^[3]. Early attempts to address the challenges shown above are based on the machine learning framework^[4].

THEORY AND METHODS

The variety of available methods^[5] reflects the complexity of the subcellular domain. We adopt a multi-stage segmentation process which consists of the removal of noise artifacts, salient feature enhancement, localization and shape extraction. Some theoretical ingredients are shown below:

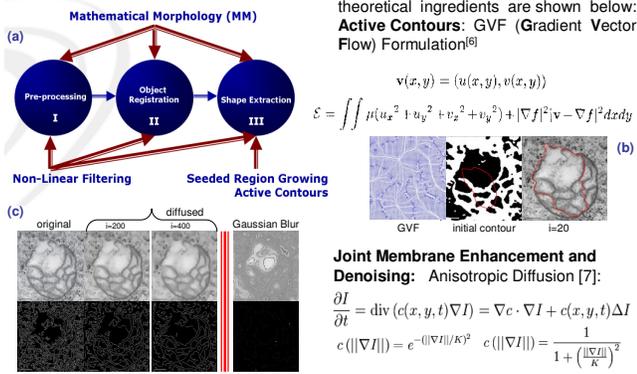


Fig.2 (a) Proposed approach. (b) GVF framework. (c) Anisotropic Diffusion (Perona-Malik)^[7].

RESULTS I: SINGLE MITOCHONDRION

Here, the segmentation of a mitochondrion is entirely performed by means of Mathematical Morphology (MM). The nonuniform illumination is corrected in step II. Contrast Enhancement in step III, Filtering in step IV, Binary mapping in step V. Particle size analysis filters the components with area $A < A_{th}$ in step VI. The gaps present in the outer membrane are sealed in step VII. Flood fill and shape refinement operations are given in VIII-IX and the extracted mitochondrial contour in X.

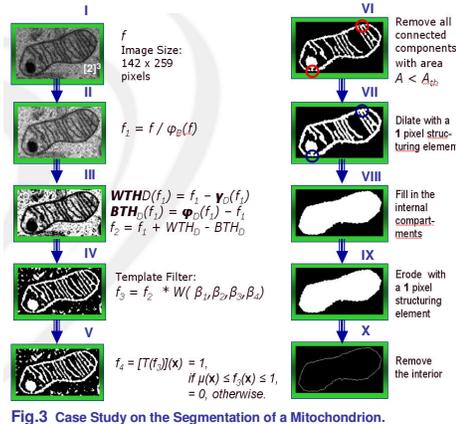


Fig.3 Case Study on the Segmentation of a Mitochondrion.

RESULTS II: SEPARATED ORGANELLES

In the case of well separated organelles we apply MM tools to segment distinct objects. Here *reconstruction-by-dilation*, also frequently referred to as *opening-by-reconstruction* on gray-scale images is being considered.

The notion of a *mask* and a *marker* along with the structuring element provide the basis for this approach.

The objective is to process the grayscale image such as to fill in the inner region of a mitochondrion. Granularity and texture analysis are used in the classification of objects.

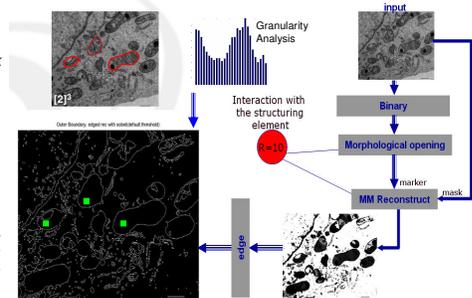


Fig.4 Segmentation of Separated Organelles with MM Methods.

RESULTS III: DEFORMED AND CLUSTERED

We apply a joint edge- and region-based segmentation approach which is based on the selection of appropriate localization markers and either the contour propagation or the marker-controlled region-grow. Low SNR of acquired TEM images results in disconnected membrane. Initialization and stopping criteria determine the yield.

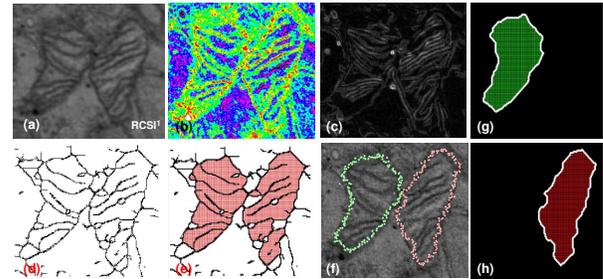
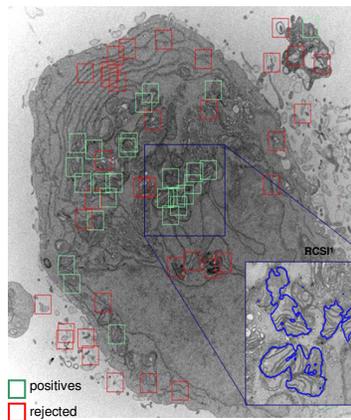


Fig.5 (a) Original image. (b) Contour map. (c) Gradient map. (d) Binary skeleton. (e) Region grow results. (f) Extracted mitochondrial boundaries. (g-h) Segmentation results.

DISCUSSION AND OUTLOOK

TOWARDS THE 'WHOLE CELL' AUTOSEGMENTATION



At present, our work is focused on the analysis of mammalian cells under the apoptosis treatment (present preparation by RCS1¹). The present segmentation bottle-neck poses a considerable problem for Electron Tomography imaging. The imaging of a cell's interior typically consists of more than 100 slices and more than 30 distinct objects to be segmented^[1].

Need for AUTOSEGMENTATION

The figure on the left shows our results for the STS treated cancer cell DU-145 as compared to the ground truth data. Our current and future work focuses on the development of auto-segmentation algorithms and tools.

Fig.6 STS treated DU-145 Localization and Segmentation Results.

ACKNOWLEDGMENT

This research is funded as part of the HEA-PRTL I V National Biophotonics and Imaging Platform Ireland (NBPI). We would like to thank the Royal College of Surgeons in Ireland¹ (RCSI) and American Society for Cell Biology² (ASCB) for the provision of TEM images of mitochondria. Special thanks go to the Nature Publishing Group³ for the permission to use selected micrographs from^[2].

REFERENCES:

[1] G.A. Perkins, M.G. Sun, and T.G. Frey, Correlated light and electron microscopy/electron tomography of mitochondria *In Situ. Methods in Enzymology*, 456:29–52 (2009). [2] M.G. Sun, J. Williams, C. Munoz-Pinedo, P.G. A. J.M. Brown, M. H. Ellisman, D.R. Green, and T.G. Frey, Correlated three-dimensional light and electron microscopy reveals transformation of mitochondria during apoptosis. *Nature Cell Biology*, 9(9):1057–1065 (2007). [3] J.R. Kremer, D.N. Mastrorade, and J.R. McIntosh, Computer visualization of three-dimensional image data using IMOD. *Journal of Structural Biology*, 116:71–76 (1996). [4] R. Narasimha, H. Ouyang, A. Gray, S.W. McLaughlin, and S. Subramaniam, Automatic joint classification and segmentation of whole cell 3D images. *Pattern Recognition*, 42:1067–1079 (2009). [5] Kristian Sandberg, Methods for Image Segmentation in Cellular Tomography, chapter 20 in *Methods in Cell Biology*, 79:769–798 (2007), Elsevier Inc. [6] C. Xu and J.L. Prince, Gradient Vector Flow: A New External Force For Snakes, in *IEEE Proc. On Comp. Vis. Patt. Recog. (CVPR'97)*, pp 66-71 (1997). [7] P. Perona and J. Malik, Scale-Space and Edge Detection Using Anisotropic Diffusion, *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 12(7):629–639 (1990).