Computer-Guided Recognition of Mitochondria in Densely Cluttered Subcellular Environments

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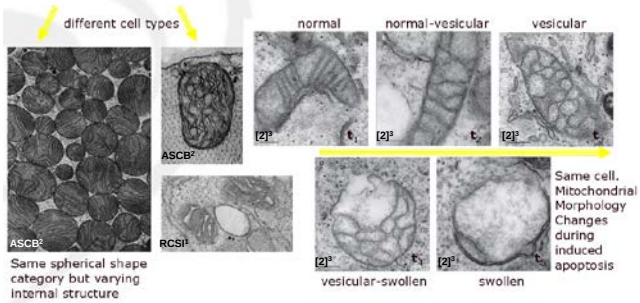
NBIPI THE NATIONAL BIOPHOTONICS & IMAGING PLATFORM IRELAND

HEA NDP

Introduction

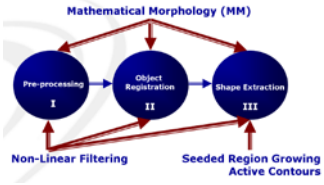
Mitochondria play a central role in cellular bioenergetics and in the regulation of apoptotic cell death. Mitochondrial morphology (shape and *cristae* architecture) is crucial to the understanding of apoptosis mechanisms and the subsequent development of therapies targeting age- and cancer-related diseases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



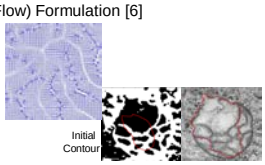
There is a high demand in automated segmentation tools which can provide an objective quantitative information in a reasonable time frame [2]. The state-of-art however is still dominated by manual segmentation tools [3]. Early attempts to address the challenges shown above are based on the machine learning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

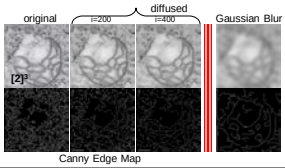
Theory and Methods

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:



Active Contours: GVF (Gradient Vector Flow) Formulation [6]





Joint Membrane Enhancement and Denoising: Anisotropic Diffusion:

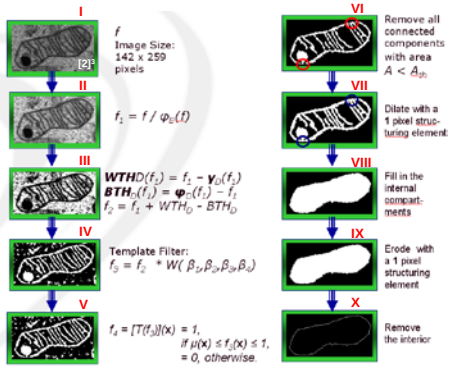
$$\frac{\partial I}{\partial t} = \operatorname{div}\left(c(x,y,t)\nabla I\right) = \nabla c \cdot \nabla I + c(x,y,t)\Delta I$$

Perona-Malik (PM) Solution [7]

$$c(\|\nabla I\|) = e^{-(\|\nabla I\|/K)^2} \qquad c(\|\nabla I\|) = \frac{1}{1+\left(\frac{\|\nabla I\|}{K}\right)^2}$$

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 < Ath in step **VI**. The gaps present in the outer membrane are sealed in the step **VII**. Flood fill and shape refinement operations



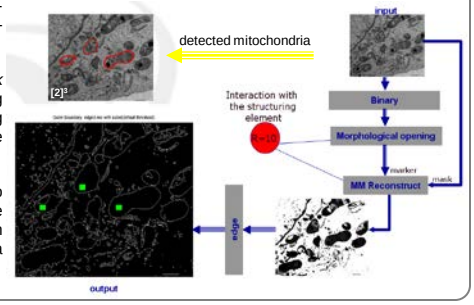
are given in **VIII** and the extracted mitochondrial contour in **X**.

Results II: Pool of separated organelles

In 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.

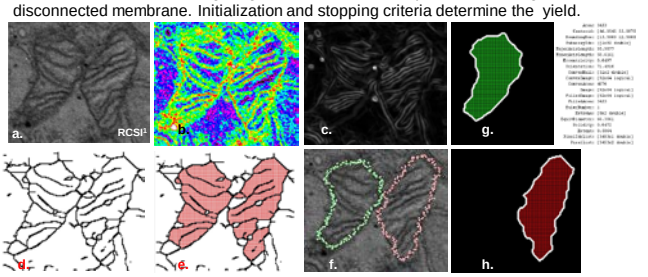
The notion of a *mask* and a *marker* along with the structuring element provide the basis for this approach.

The objective is to process the grayscale image such as to fill in the inner region of a mitochondrion.



Results III: Deformed and Clustered

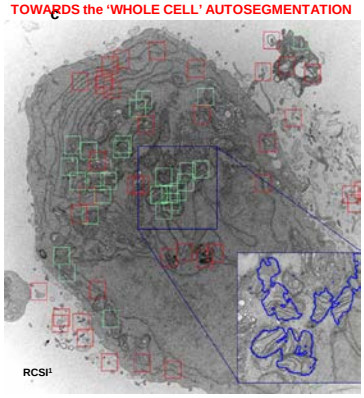
We apply joint edge- and region-based segmentation approach which is based on the selection of appropriated localization marker and further either the contour propagation or the marker-controlled region-grow. Low SNR of acquired TEM images results in disconnected membrane. Initialization and stopping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



The above figure shows in a: original image, b: contour map, c: gradient map, d: binary skeleton, e: region grow results, f: approximated mitochondrial boundaries, g-i: segmentation results with shape statistics.

Discussion and Outlook

TOWARDS the 'WHOLE CELL' AUTOSEGMENTATION



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

■ Need for **AUTOSEGMENTATION**

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

Acknowledgements

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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. Mastronarde, 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**.