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

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].
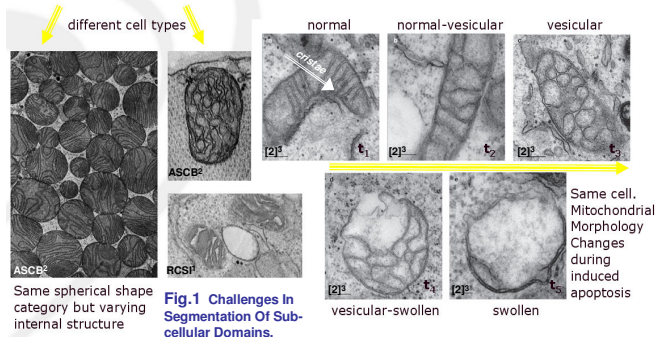

Fig.1 Challenges In Segmentation Of Sub-cellular Domains.

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:
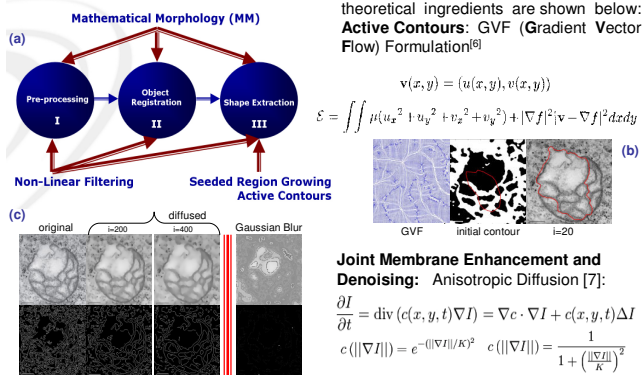
Active Contours: GVF (**G**radient **V**ector **F**low) Formulation[6]

$$\mathbf{v}(x,y) = (u(x,y), v(x,y))$$

$$\mathcal{E} = \int\int \mu(u_x^2 + u_y^2 + v_x^2 + v_y^2) + |\nabla f|^2 |\mathbf{v} - \nabla f|^2 dx dy$$

Joint Membrane Enhancement and Denoising: Anisotropic Diffusion [7]:

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

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

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**.

f
Image Size: 142 x 259 pixels

$f_1 = f / \varphi_B(f)$

$WTHD(f_1) = f_1 - \gamma_D(f_1)$
$BTHD(f_1) = \varphi_D(f_1) - f_1$
$f_2 = f_1 + WTHD - BTHD$

Template Filter:
$f_3 = f_2 * W(\beta_1, \beta_2, \beta_3, \beta_4)$

$f_4 = [T(f_3)](\mathbf{x}) = 1,$
if $\mu(\mathbf{x}) \le f_3(\mathbf{x}) \le 1,$
= 0, otherwise.

VI Remove all connected components with area $A < A_{th}$

VII Dilate with a **1** pixel structuring element

VIII Fill in the internal compartments

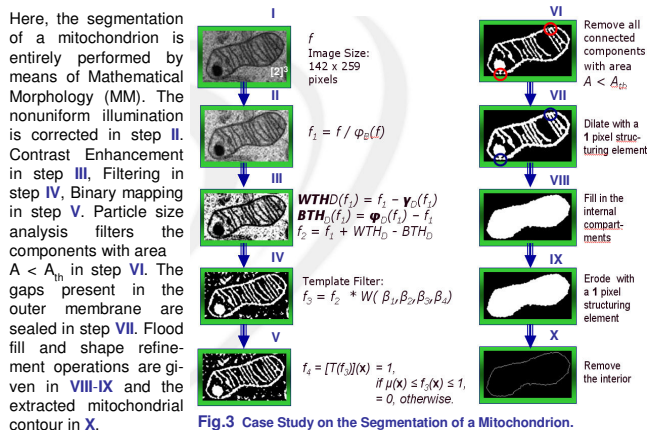IX Erode with a **1** pixel structuring element

X Remove the interior

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.
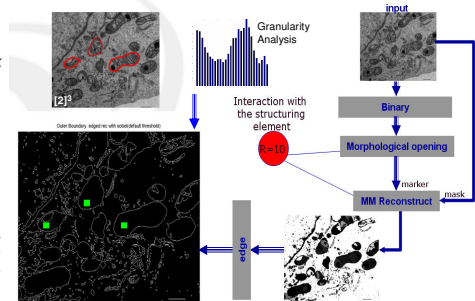

Fig.4 Segmentation of Separated Organelles with MM Methods.

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.
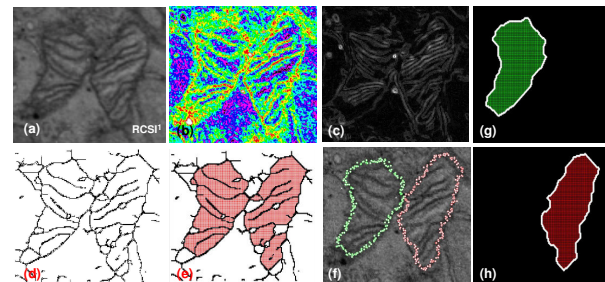

Fig.5 (a) Original image. (b) Contour map. (c) Gradient map. (d) Binary skeleton. (e) Region grow results. (f) Extracted mitochondrial boundaries. (g)-(h) Segmentation results.

DISCUSSION AND OUTLOOK

TOWARDS the 'WHOLE CELL' AUTOSEGMENTATION

At present, our work is focused on the analysis of mammalian cells under the apoptosis treatment (sample preparation by RCSI[1]). 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.

☐ positives
☐ rejected

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

ACKNOWLEDGMENT

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**).