Automatic Cell Segmentation Using Level Set Methods

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Abstract — High-throughput RNA interference screening can result in a large number of images, thereby necessitating the use of a computer-based automatic analysis system. The first part of the analysis involves segmenting the cells. This research centres on the use of level set methods to perform this segmentation, particularly investigating the inclusion of texture-based features in the level set based segmentation.

Index Terms — Fluorescent microscopy, image segmentation, level set methods, texture.

I. INTRODUCTION

This research centres on the segmentation of biological cellular images. Biologists use RNA interference to determine the functions of certain genes by observing the changes in the biological processes when gene specific double stranded RNA are added [1]. Because many studies of this nature are performed on a high-throughput basis, manual analysis is not feasible and thus a computer-based automated analysis is required. A prerequisite for further biological analysis is the accurate segmentation of the cells in the image. The key challenges to this problem are that the cells in the image do not present a clear border, they are often touching or even overlapping and the intensity can vary significantly within individual cells.

Because of the imaging technology, different parts of the cell are shown under different illumination; these are shown as separate channels where there will be (at a minimum) a channel that illuminates only the nucleus and a channel that illuminates the entire cell. Figure 1 shows a pair of sample images of the nucleus and cytoplasm.

Figure 1. Sample images of the channels showing (a) the nucleus and (b) the cytoplasm

II. BACKGROUND

a. Image Segmentation

Image segmentation involves separating an image into homogenous regions that ideally represent separate objects or phenomena. Recently, the characterisation and use of texture information in image segmentation has been a fruitful area of research [2].

Because the nuclei regions (shown in the nuclei channel) are generally separate and have lower intensity variation within the cell, nuclei segmentation can be performed using a simple dynamic threshold technique, with a Watershed algorithm to separate touching nuclei [1]. The nuclei channel typically contains some interference from the other channels and thus methods of removing this interference are being pursued.

b. Level Set Methods

The level set method is a powerful technique used to track moving interfaces [3] [4] and has been successfully applied in many varied scientific fields [5]. One of the distinguishing properties of the level set method is its ability to represent a curve, \( c_{\text{interface}} \), by an isocontour of a surface. \( \phi \): \( c_{\text{interface}} = \{(x,y) | \phi(x,y) = 0 \} \). Figure 2 illustrates this representation.

Figure 2. Diagram illustrating how implicit functions help represent topological changes in contours.

The key issue is how the entire level set function should be evolved such that the interface wraps around the boundary of the objects that are being segmented. Classical level set methods address this issue by defining a force, \( F \), and evolving \( \phi \) according to the level set equation defined in [6] as:

\[
\phi_t + F |\nabla \phi| = 0
\]

\( F \) is a function of external forces (such as the characteristics and features of the underlying image) and internal forces (such as the local curvature of the interface). Variational level set methods address this issue by formulating an energy functional and seeking to minimise it. One of the most popular energy functionals is the Mumford-Shah functional given in [7] and defined as:

\[
E = \lambda_1 \sum_{i=1}^{M} \int_{\text{in}(c_i)} |I - c_i|^2 dx dy + \lambda_2 \int_{\text{out}} |I - c_{\text{out}}|^2 dx dy + \mu \cdot \text{length}(\phi = 0) + \nu \cdot \text{area}(\phi \geq 0)
\]

\[1\] Assuming that, according to the convention, \( \phi \geq 0 \) is inside the contour.
The first two terms penalise intra-region variation, the third term penalises interface length and the fourth term penalises area within the region. Terms that penalise high intra-region texture variation [8] and terms that tend to draw the interface toward the high gradients along object boundaries have also been developed. The minimisation is typically performed using the Euler-Lagrange equations of the energy functional.

Variational level set methods are preferred in this research because of the ease with which the models can be developed and manipulated to consider not only intensity and gradient but also texture.

c. Texture Extraction

There is no formal definition of image texture [2] [9]. Essentially, image texture concerns itself with repetitive patterns in the image, the scale and direction over which these repetitions occur and the frequency with which they occur. Textural characteristics are concerned with spatial arrangement. No single method has been shown to be superior in quantifying and extracting textural features from an image although there are several successful methods.

Two of the most popular feature methods used to characterize textures are Gray Level Co-occurrence Matrices [10] and Gabor Filters [9]. A comprehensive survey on texture analysis can be found in [9].

III. PROPOSED RESEARCH

The aim of this research will be to segment fluorescent microscopy images using level set methods that exploit the texture information of the image [1]. Texture-based segmentation is typically done using some form of clustering algorithm to group areas with similar texture. However, here the texture information will be a part of a larger model that will include image intensity and gradient information. Part of this research will also be concerned with improving the quality of the images by attempting to remove interference from the other channels by using some form of background subtraction technique [11]. We will also investigate ways of improving the inclusion of texture information in the level set model [1] [8]. Furthermore, an evaluation of different texture extraction methods will be carried out to determine their effectiveness in discriminating and extracting patterns in cell images.

IV. METHODOLOGY

Literature review: A thorough review of the theory and literature around level set methods and texture extraction, the inclusion of texture information in the level set model, elementary background subtraction algorithms and other methods of cell segmentation will be done.

Model development: a mathematical model of the segmentation problem will be developed. This will be centred on the background subtraction technique, the weighting of the various terms of the energy functional and the choice and design the texture extraction technique.

Algorithm implementation: algorithms will be prototyped in MATLAB and the final algorithms will be coded in C++ using the OpenCV libraries.

Interpretation of results: The segmentation results will be assessed in terms of accuracy by comparison with the results given by available published cell segmentation software and in terms of computation time, due to the large data set.

V. CONCLUSION

We have presented the central ideas behind the segmentation of cellular images by briefly introducing level set methods in the context of image segmentation. The methodology for this research has been given. The result of this research will be a better understanding of the use of texture in level set methods, particularly but not exclusively in the area of cell segmentation, through a set of cell segmentation algorithms.

REFERENCES


Jonathan Parker received a BScEng (Electronic) (summa cum laude) in 2010 from the University of KwaZulu-Natal. He is currently pursuing an MSc, also at UKZN. His research interests include image processing and computer vision.

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