ANALYSIS AND ENHANCEMENT OF BIOMEDICAL CELLS USING GRAPHIC EDITOR

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Abstract

A tool is designed and tested to provide histopathologists with a powerful mean for studying and analyzing cells and tissue sections. Image analysis techniques are described for automatic detection, segmentation, feature description, and classification of the biomedical cell within the specified organ. The results obtained represent a quantitative description of the tested cells, based on statistics of the component. Combining newly advanced algorithms with other techniques has developed a software package for analysis of these images. Cells were sampled and each was digitized into 512x512 pixels. The pixel gray values have an 8-bit resolution, allowing 256 discrete values. Two features were extracted from each cell: *form features* and *gray level features*. Finally, a comparative study is made between the produced images and the Electron Microscope image results found in literature. The results are quite comparative.

Keywords: Pattern recognition, Image analysis, and edge detection algorithms

1. Introduction

The Optical Laser Microscope (OLM) has been designed to overcome the limitation of conventional microscope in biological studies as well as material microscopy and semiconductor device assessment. The image contrast is achieved using a beam of GaAlAs laser diode to scan over the sample surface. The diode is of a small size, light weight, low power consumption, high efficiency, large spectral width, long life time, low noise and low cost features¹. The use of laser as a light source produces images of exceptional clarity and contrast. Its preferred characteristic from the conventional light microscope is that image formation is contrast².

Microscope image processing algorithm is a broad term that covers the use of digital image processing technique to process, analyze, and present image obtained from OLM³. Such processing is now commonplace in a number of diverse fields such as medicine, material surface testing, biological research, cancer research, metallurgy ⁴.

OLM enables high quality images of variety of biostructure samples in transmission mode to be produced without the possible beam damage associated with electron microscopes. This makes it a very promising technique for the examination of sensitive materials. Two kinds of electron microscope (EM) can provide morphologic and analytic data on cells and tissues: Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM). The primary improvement in EM⁵ is that EM's beam wavelength is approximately (1/2000)th that of light microscope, thereby increasing resolution by a factor of 1000.

The present paper describes one of the applications developed using MATLAB and comparing between images of OLM and EM.

2. Material and Methods

The liver cell is the largest mass of glandular tissue in the body and the largest internal organ. The body depends on it to perform a number of vital functions such as regulation, synthesis, and secretion of many substances important in maintaining body's normal state⁶.

Liver cell, which was magnified by 300 times in the proposed system, was imaged by the computer with the aid of a digital camera of CMOS sensor.

Image analysis technique is described for automatic detection, segmentation, and feature description of Liver cell or any biomedical component, the result of the analysis is a quantitative description of the cells, based on statistics of the components³.

Various cell features where extracted in the first stage and shipped to the second for the final statistical analysis, performed with the aid of scientific programming using MATLAB.

After producing the image by the digital camera, it can be displayed by the monitor; image processing program allows the user to zoom and pan the displayed microscope image, and to control the processed part.

The algorithm presented here involves a series of preprocessing steps to remove noise from the image followed by image segmentation, contour encoding.

Image Enhancement techniques are the first algorithms that was used to improve an image such as increasing the signal to noise ratio and sometimes subjectively by making certain features easier to be seen by modifying the colors or intensities⁸. Two algorithms of image enhancement⁹ are used: Noise Removal [detect and remove unwanted noises from a digital image], and Contrast Enhancement [process images to increase the visibility of the features of interest]. The most common type of image enhancement that was used in this work is the *median filter*. It is a non-linear filter used to remove the noise⁹. Using this filter, the value of an

output pixel is determined by the median of the neighborhood pixel. The median is much sensitive to extreme value, therefore, median filters better able to remove these outliers without reducing the sharpness of the image^{8, 9}. Contrast enhancement method used in this work is a histogram equalizer. The histogram of an image records the frequency distribution of gray scale in that image. For contrast enhancement, histogram equalization is used to redistribute gray levels in an attempt to flatten the frequency distribution^{9, 10}. Edge detection can be considered as the dual of image segmentation⁹. This implies that the goal of edge detection is to mark the points of an image at which the intensity changes rapidly¹¹. Edge detection algorithms generally calculate the derivative of this intensity change. Canny edge detection technique may be considered as a standard algorithm³. To know the properties of surface topography or acute pins that exist in the image; Sobel operator^{12,13}, Wenty contour⁸ and calculation of object properties are used. Before displaying image as 3D representation the operation of image quantization (mapping groups of pixels into one pixel⁷) must be applied.

3. Results

The slides shown Fig.'s 1 through 8 illustrate all processes of the block diagram shown in Fig.1 from capturing the image to the detection of boundaries of liver cell image 3D projections for that image. The first step in algorithm is to apply enhancement process as shown in Fig.1 after defining the Region of Interest. Fig.1a illustrates selection of that region from the original image. Fig.1b shows extracting of the image and displaying it in another window, thereby magnify the area.



Figure 1 Region-of-interest image Geometry, (a) Original Image. (b) Applying image geometry operations

Fig.2 represents the adjustment of the image by redistributing the intensity along the pixels by Histogram Equalizer. This process will distinguish the object from its surroundings. The effect of Median filter on the specified object is shown in Fig.3 taking a thresholding level of 150. After smoothing the object data, it is necessary to detect the boundary of object using Canny edge detection algorithm; Fig.4 shows the border of an object showing one of five different values of adaptive thresholding method. Large number of data was provided for labelling the object to extract the features of interest. These features provide information about the location and the shape of the object.







Figure (3) Effect of applying Median filter to the image

The image was transformed into a binary form in order to estimate the perimeter of the object as shown in Fig.5. That is, a certain pixel intensity level is chosen as the threshold, and pixels on either side of the threshold area changed either to black or white depending on which side of the threshold they fall¹⁴.

Fig. 6 and 7 displays the OLM images of liver cell surface. These would normally be displayed in pseudo colour, which can present more information to the viewer by assigning colours to ranges of data values¹⁵.



Figure (4) Border-Finding Algorithm for five different values using the Adaptive Thresholding method



Figure (5) Determining the perimeter of the object in a binary image after applying Canny edge detection.

Fig. 6 shows a contour display calculated from the OLM data. Two different contour levels display (4 and 8) were used.



Figure (6) Wenty Contours of peaks of the image

Fig. 7 represent different pixel Gray values in the original image.



Figure (7) Combined 2D and 3D map of the image

Fig. 8 shows the image of a human liver cell produced by electron microscope which is magnified 6000 times⁵. When comparing this image with Fig.1a, it shows that more detailed analysis of liver structure organization is provided by electron microscope, but after applying the algorithms of image processing on OLM image; more comparable results were gained over that of electron microscope image, especially with contour and 3D displays of the image. In Wenty contour, we can know each part of the cell, its place and its level very clear in 3D contour; e.g., for biologists, the blue area in Fig.6 represent the nucleus and the sky blue around this area represent the Rough Endoplasmic Reticular. Also we can recognize Smooth Endoplasmic Reticular. In OLM image processing algorithm the image is displayed as 3D representation. In 3D representation of image; gray level value is displayed for each element but this cannot be represented by electron microscope.



Figure (8) Liver cell represented by the electron microscope

4. Discussion

The use of image vision technique in OLM will produce an image in a digital form, which is a well suited technique for digital image storage and processing for restoration, enhancement and analysis. This system may be used for processing operations such as histogram equalization, edge enhancement or any digital image processing algorithm.

Biologists are able to know the Nucleus/Cytoplasm ratio from the Histogram of the image. The shadowy part of the Histogram, shown in Fig.3, represents the Nucleus and the rest represents the Cytoplasm. When the density of Nucleus is higher than that of the Cytoplasm, i.e. Nucleus/Cytoplasm ratio is high; it indicates the activity of the Nucleus; which means that the Cytoplasm is energetic. Inversely it refers to inactivity of the Nucleus (normal cell).

The surface data map is a part of a broad class of maps, whose purpose is to describe topology concepts such as the distribution of densities, relative magnitude, gradients, spatial relationships and movements^{15,16}. Contour maps of the image indicate the organism of the cell and its location. More intensity portrayal of a surface can be obtained by mapping it in 3D rather than as a Contour map.

It seems from the comparison between OLM and EM that EM is clearer. But when comparing image outcomes, especially various image features provided by OLM, we can realise the advantage over EM. The limitations of using EM is related to its large expenses and to its technical requirements and maintainance. EM is sensitive to vibration and external magnetic fields, samples have to be viewed in vacuum. Real EM images are of gray scale form only.

An important limitation of using EM concerns material samples. The observation is restricted to the surface (or at least a thin layer of about 100 nm thickness). EM obtains images as a product of electrons that are *reflected* by the surface of the object. A transmission EM will only *shine through* about 100 nm of material. Using OLM image processing algorithms can be represented in a 3D form. Also OLM has the advantage of simplicity and it does not need the complexity required by EM.

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