



## Original Research

## Auto Segmentation of Lymph Node Microscopy Images

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### ABSTRACT

The manual histology assessment on the biopsy tissue sample still remains the gold standard procedure for cancer and its progression in human body. Auto nuclei segmentation is an important method to measure cellularity but often suffered an issue due to the present of overlapping nuclei. The implementation of auto segmentation of cells could speed up the process of histology assessment for cancer cases. The first step to implement, a wide data profile of normal and cancerous need to be compile and analyze further as a reference guide. Tissue data profile can be collected based on cellularity property of the tissue which can be automatically segmented using MATLAB software. The objective of the study is to develop an auto nuclei segmentation using MATLAB software to measure cellularity between normal and cancerous cells of lymph node tissue. Histological images of the tissue were analyzed using MATLAB software by using thresholding method and the result was compared with ImageJ. The pre-processing part of the image processing includes converting the image into 8-bit grayscale image. The segmentation parts include adaptive filtering to remove the noise using Wiener filter and the thresholding Otsu method. Results from the ImageJ and manual counting on the cellularity shows a comparable result to the automated cellularity measured using MATLAB. The cellularity of the cancerous lymph nodes was found lower than normal lymph nodes.

### INTRODUCTION

Lymph nodes are made up of multiple lymphoid lobules circle around by lymph-filled sinuses and enclosed by a capsule. The main component of the lymph node is the lymphocytes that act as the primary site for immune system interactions between the body's host cells and foreign elements such as bacteria, viruses, antigens, and toxins (Willad et al., 2006). Normally, the evaluation of lymph nodes' microscopic images is tedious and depends on the assessment of images stained with multiple types of dyes (Gomolka et al., 2017).

The existing cross-sectional imaging modalities such as MR imaging, computed tomography, and ultrasonography are

lacking the desired accuracy for characterizing lymph nodes as it relies on the insensitive size and morphologic criteria of lymph nodes (Junping et al., 2012). This is because metastases can also be seen as non-enlarged lymph nodes and not all enlarged nodes considered malignancies (Torabi et al., 2004). Overall, it may be said that the lymph node size is not a reliable parameter for tissue discrimination. Thus, the analysis of cellularity which represents an indirect indicator of the complexity of intercellular spaces and junctions can be a better prognostic indicator of histological architecture (Junping et al., 2012).

In general, there are two main methods for cellularity measurement, manual counting and auto segmentation. The manual segmentation method is usually conducted by a histologist or a pathologist. The cons of this method are very time-consuming, have a large variability and are prone to intra and interobserver variability. The slowness of the analysis and non-standard precision depending on the operator's abilities are

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also the drawback for manual segmentation (Putzu et al., 2013). Therefore, the aim of the study is to develop an auto segmentation of lymph node microscopic images using MATLAB software and measure the cellularity in normal and cancerous lymph node tissue using the developed auto segmentation MATLAB software. In addition, ImageJ software will be used to validate the cellularity measurement.

In cases where the person has cancerous tumour that managed to break off from the main tissue site and some of cancerous cells managed to travel through the lymph vessels, some might end up at the lymph nodes around the original tumour area. This escaped cell is called as metastasis cell which is cancerous cell that broke off from the main cancer tissue to ended up in the lymph node (Cox et al., 2008).

Auto segmentation of cells is important in this context to help the pathologist to assess the condition and diagnose the severity stage of the cancer. Cells auto segmentation itself brought the meaning of separating the individual cells from the background and is the first most important step in image processing for cells (Yang et al., 2013). Manual counting is not only prone to human error, but also not efficient. The workload of pathologist has increases compared to the old days where manual counting is still doable.

One of the methods of selecting segmentation and measurement for cell and tissue was using an algorithm in ImageJ to analyse the data (Prodanov et al., 2012). While another method was introduced for quantification of cell size using temporal diffusion spectroscopy, in this method the cell size and intracellular volume fractions was quantified using temporal diffusion with different weight acquisition and the combining measurements was made using oscillating gradient spin echo (OGSE) and a conventional pulsed gradient spin echo (PGSE) acquisition with a single, relatively long diffusion time (Jiang et al., 2016)

Another popular and frequently used method for image segmentation in image processing is thresholding. Thresholding is one of the most direct and simple way of image segmenting. It works based on the principle of selecting (or clipping) threshold value to create binary image from grayscale image (Ragne et al., 2020).

In this research, thresholding image processing method using MATLAB were done and the result was compared with result obtained from using existing image segmentation software, ImageJ. The pre-processing part of the image processing incudes converting the image into 8-bit grayscale image. Grayscale image were image rendered as grids of black dots on a white background with the size of the black dots determining the degree of darkness or lightness of the image (Ragne et al., 2020). The segmentation parts include adaptive filtering to remove the noise using Wiener filter and the thresholding Otsu method.

A new coding has been written and tested using MATLAB. In the programming, included an adaptive filter which is the Wiener filter. The Wiener filter is a space-varying filter where the noise is assumed to be white or background (Kumar et al., 2010). By including the Wiener filter which is a low pass filter for areas where image variance is lower than the noise variance, the boundaries of the image are treated as zero scale value which will put the value as background (Kumar et al., 2010). By doing this, we can minimize the error in a magnified view where areas between cells can be mistaken as cells.

The Wiener filter is to filter out noise that has corrupted a signal. It functions by estimating the desired underlying signal

by using linear time invariant filtering. One is assumed to have knowledge of the spectral properties of the original signal and the noise, and one seeks the LTI filter whose output would come as close to the original signal as possible (Kumar et al., 2010).

The method of image processing used is the Otsu thresholding. The Otsu threshold method begins by creating a histogram of the grayscale image. The histogram is used to obtain the weight, mean and variance of the pixel levels of the image. The variance is used to create the threshold by determining whether the pixel is included in the foreground (cell) or background (empty space) (Sezgin et al., 2001).

Otsu threshold method is used as a separation of pixels level for the image into a foreground and background. Threshold method is also used in ImageJ and becomes a simple but effective tool to separate objects from the background (Sezgin et al., 2001).

The 8-bit image type is the most commonly used to turn the image into grey scale image. 8-bit means that there are 256 grey levels in the turned image with each pixel can have 0 as black to 255 as white (Kumar and Verna, 2010). This method is used to make sure the nuclei of each cell is extracted from the background including the overlapping cell.

Auto nuclei segmentation is an important method to measure cellularity and more accurately than manual nuclei counting. But often there is always a problem while using auto nuclei segmentation as some of the images contain noise or the image has an overlap nuclei and different morphology. The aim for this project was to develop an auto segmentation method that can extract the nuclei from the background.

In this study, the objectives were to develop an auto segmentation of lymph node microscopic images using MATLAB software, measuring the cellularity in normal and cancerous lymph node tissue using the developed auto segmentation MATLAB software and also to validate the cellularity measured using ImageJ software.

## MATERIALS AND METHOD

### Cellularity measurement using MATLAB

The method of image processing used can be classified into two which are pre-processing and segmentation. The image pre-processing is composed of colour space transformation where the colours of the image is converted into grayscale by using the code below:

```
grayscale = rgb2gray
```

For the magnified images, an adaptive filtering is used which is the Wiener filter which is a 2D noise removal filter for grayscale images.

```
grayscale=wiener2(grayscale)
```

After filtering, the image will undergo histogram equalization in order to create a histogram on the measure of spread of the pixel level.

```
hist_eq = imadjust(grayscale)
```

The histogram is used in order to use the Otsu threshold. The threshold of the picture is then obtained after processing. The image is then converted into a binary image and is complemented so that the colour white will represent as cell and black is represented as the background.

`bw=im2bw( grayscale, threshold/255)`

The boundary is then drawn on the image and is counted. The value counted represents the number of cells in the picture. In order to calculate the cellularity, the area that contains the cells must first be obtained by using code below:

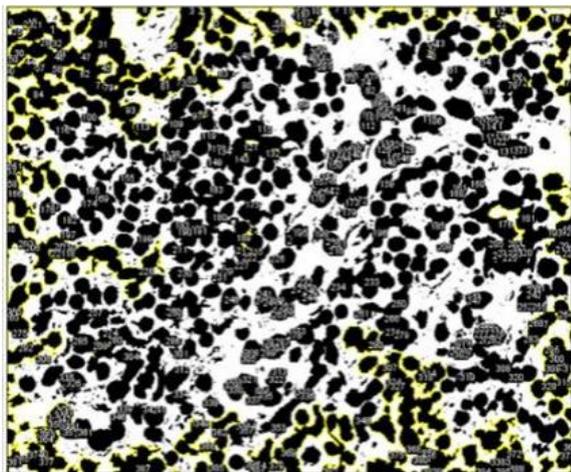
`Area = bwarea(bw)`

The number of cells will then be divided by the area to obtain the cellularity of the picture.

**Cellularity measurement using thresholding ImageJ**

The method of cell counting using the ImageJ is by threshold method. Threshold method is a method of separating the image into many classes of pixel or grey level of the pixel. This method is also a tool for separating the object from the background. Before adjusting thresholding level, there are several steps that need to be done.

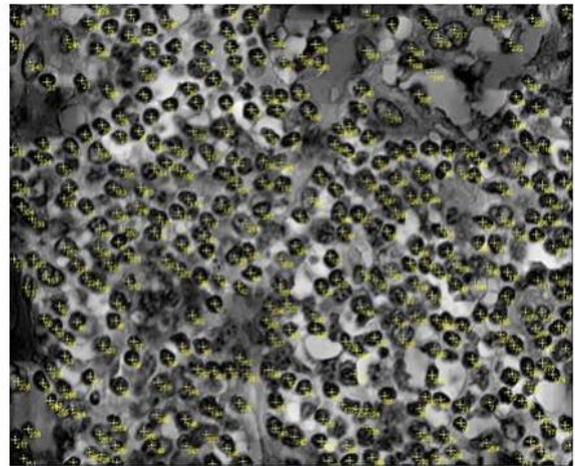
First step is inverting the chosen image. After that change the resolution of the inverted image into an 8-bit type. Then adjust the threshold value between 150 to 258 by comparing it with the original image. On top of that, before final steps are taken which is analyse the particles, the picture is changed into black and white and also with a dark background. This is to make sure all the cells are included to be counted. Lastly analyse the image using analyse particle features in ImageJ to see the summary of the number of cells. Result from auto-segmentation using ImageJ as shown in Figure 1.



**Fig. 1** Automatic cellularity measurement using thresholding ImageJ on tissue sample.

**Manual cellularity measurement using ImageJ**

Apart from doing auto-counting in the ImageJ software, the method of manual counting is also used to compare the number of cells found for a magnified part of the image. Before doing any adjusting on the cell's image, the pictures resolution was changed to an 8-bit to show the cell boundaries clearer. The threshold also adjusted in the desired range (between 40-50); varies the value to get a clearer image of cells. The picture was changed into black and white, darker in background to differ cells and surroundings. By using features named 'multi-point', manually ticked, and counted the number of cells in the region. The result from the manual counting is shown in Figure 2.



**Fig. 2** Manual cellularity measurement using ImageJ on tissue sample.

**RESULT AND DISCUSSION**

According to Table 1, it shows the result of cell count done using three different methods; automated using the coding written in MATLAB, automated using threshold method in ImageJ and manual counting using ImageJ done for two types of samples obtained from lymph node; normal healthy cells and metastatic cells. Using manual counting as the reference, percentage of error from using both MATLAB and ImageJ were calculated. Cell counts done using both MATLAB and ImageJ show the same pattern despite having different error rate. The low error rate in ImageJ allows it to be the reference value for the full experimental simulation.

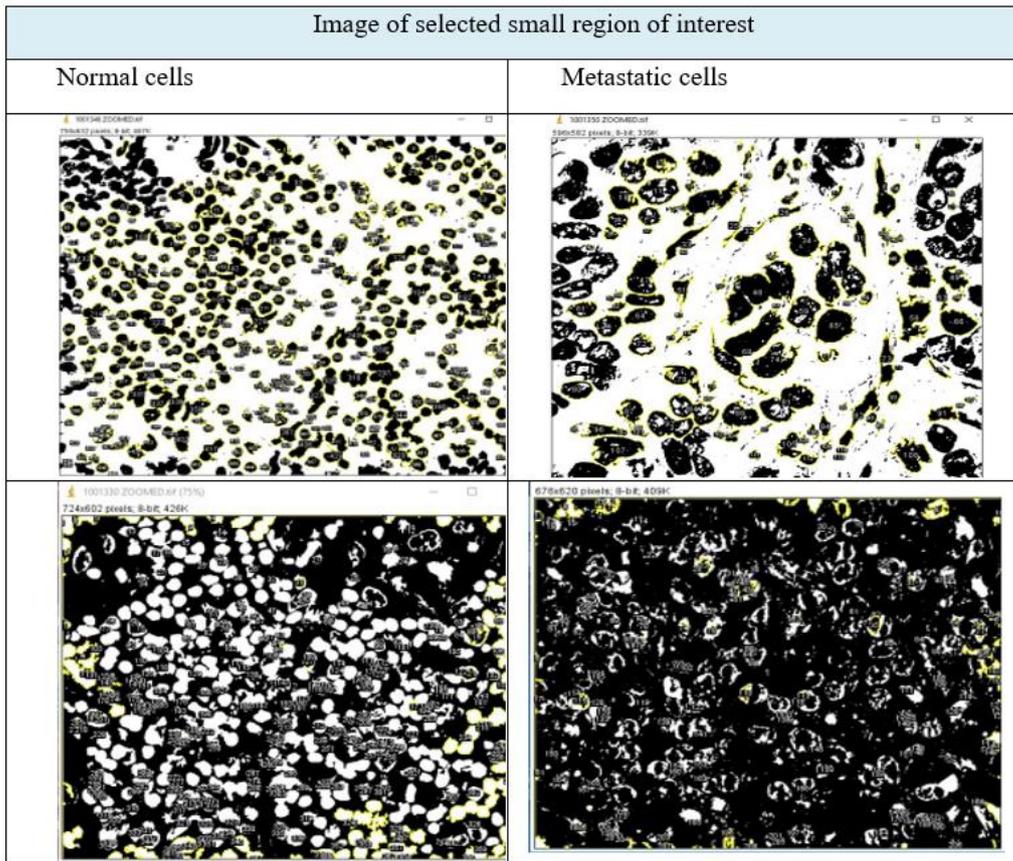
**Table 1** Cell count by using MATLAB, ImageJ, and manual counting for selected small region of interest.

Cell count for selected small region of interest (ROI) with ± Percentage of error (%)		
MATLAB	ImageJ	Manual Counting
Normal cells		
385 ± 13 %	448 ± 2 %	441
306 ± 14 %	368 ± 4 %	354
Metastatic cells		
183 ± 7 %	113 ± 9%	104
201 ± 1 %	208 ± 3 %	201

Table 2 shows the image of selected small region of interest for both normal and metastatic cells. While the result for cell count, cellularity, area, and percentage of error for the full-size sample were shown in Table 3. It shows consistent pattern for both the cell counts and cellularity compared to the result for the smaller area of interest. The cellularity is obtained from the area and cell counts as cellularity is defined as the number of cells found in a given area of interest (Junping et al., 2012).

The experimental simulation was done for two types of cells, normal healthy cells and metastatic cells found in lymph nodes. As observed in Table 4, the morphology of normal and

**Table 2** Image of selected small region of interest for both normal and metastatic cells.



metastatic cells are different, and it is one of the markers to differentiate between the two types of cells. From the image of the cell provided for Table 4, normal cells have uniform size and shape of cell while the metastasis cell shape is often abnormal or irregular and has larger size than normal cell shape. Other than the nucleus of the normal cell is spheroid shaped while the metastasis nucleus has irregular shape and is also larger in size (Coet al., 2018).

The experimental simulation approach using MATLAB uses the image extracted in .tiff file instead of the normally used .png or .jpg. It was found that the assessment of morphological details was higher when TIFF images were considered, indicating a better image quality. There was no significant difference in the diagnostic accuracy between uncompressed images and compressed ones, although the intraobserver reproducibility in the diagnostic judgement was higher for uncompressed images.

Despite loss in image details, factor 30 compressed video microscopic images enable a good diagnostic accuracy (Seidenari et al., 2004).

Since the report's main purpose is to develop an auto segmentation method using MATLAB, the team decided to use ImageJ, an existing auto segmentation software to become the reference in this experimental simulation. In order to verify the efficiency of both the proposed method of using MATLAB and ImageJ, manual counting of selected small region of interest (ROI) was also done. As shown in Table 1, the difference in manually counting the cells and by using ImageJ was low. This can be easily understood from the calculation of percentage of error between the manually counted cells and ImageJ counted cells in Table 2. The error for ImageJ is consistently less than 10% which supports the software to be used as reference in this experimental simulation. The percentage of error for using the

**Table 3** Comparison on cell counts cellularity, area, and percentage of error for full size sample.

Sample id	Cell count		Cellularity (No. of cells / mm <sup>2</sup> )		Area (mm <sup>2</sup> )	Percentage of error (%)
	MATLAB	ImageJ	MATLAB	ImageJ		
Normal cells						
346	12798	10387	1061.19	861.28	12.06	23
330	14156	12038	735.376	625.35	19.25	17
Metastatic cells						
350	6290	5056	547.13	459.22	11.01	24
336	5123	4043	554.43	437.55	9.24	26

proposed MATLAB method on the other hand showed inconsistency. This is due to the MATLAB method is more efficient and suitable in segmenting the full-size image and has some limitation for the magnified view of a region of interest. In a magnified view, the spaces in between cells may be mistaken as cells, therefore there is a need to include the Wiener filter to remove the noise.

Despite the team using manual counting for the early verification of both MATLAB and ImageJ cell counts, it must also be taken into consideration that manual counting is becoming more irrelevant and inefficient in real life hospital environment. According to a study it was found that the developed, clustering-based, fully automated segmentation approach segments complete lung nodule cross-sectional histology slides in less than 1 minute, compared to manual segmentation which requires multiple hours to complete. They found the accuracy of the automated approach to be comparable to that of the manual segmentation with the added advantages of improved time efficiency, removal of susceptibility to human error, and 100% repeatability (Sieren et al., 2010).

In Table 3, area of the cells and cellularity was also listed. This is due to normal practice of cellularity being the main function in determining normal and abnormal cells instead of cell counts. Cellularity is defined as the state of tissue or other mass with regard to the degree, quality, or condition of cells (Kumar and Verna, 2010). Based on Rakhlin et al. (2019), correct cellularity assessment increases the chances of getting an appropriate treatment and facilitates the patient's survival. During anticancer therapy, the size of the tumour may remain the same, but overall cellularity may be drastically reduced. As a result, it makes the residual tumour cellularity an important factor in assessing the response treatment (Rakhlin et al., 2019).

Cellularity is obtained by dividing the cell count with the region of interest. Metastasis cells are different to cancer cells as cancerous cells have higher cellularity compared to normal healthy cells. The study found that cancerous cells have larger size and high cellularity (Prodanov and Verstreke, 2012). However, metastasis cells have lower cellularity compared to normal cells as they are larger in size, so the cell count in a given area would be lower compared to normal cells. As can be seen from Table 3, the cellularity of metastasis cells has lower cellularity compared to normal cells. This shows that the coding used for this simulation is able to be used to differentiate normal cells and metastasis cells by the value of cellularity.

The proposed MATLAB method is found to be a good alternative in cells auto segmentation as the percentage of error it gives is in the range of 17% to 26% which is an acceptable range for early development of this method.

## CONCLUSION

In conclusion, the cellularity of the metastasis lymph nodes is lower than normal lymph nodes due to the cells having larger size. By using MATLAB software, we are able to differentiate normal cells and metastatic cells by calculating the cellularity. Even though there is a difference between the value of cellularity between the result obtained from ImageJ and MATLAB, the error percentage is not high and the result also shows the same pattern. Although there is a difference between the cell count obtained by using manual counting and MATLAB, however the time needed to obtain the cell count greatly differs. Auto segmentation is one of the promising methods in cellularity measurement. With the help of auto segmentation, human error from manual cell counting can be

reduced. In addition, the amount of cellularity helps specialists such as pathologists and medical doctors in determining the stages or phases of the cancer cell. Therefore, we are able to achieve our goal by using MATLAB to perform auto segmentation on samples that will enable us to differentiate normal cells and metastatic cells.

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