

Application program for wound healing measurement based on the region growing technique: A trial version

Phadngam S^{*1} Ruthiang J¹ Ina N² Ferraresi A³ Isidoro C³

¹ Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai Province, Thailand

² Department of Radiology, Faculty of Medicine, Prince of Songkla University, Hat Yai Province, Thailand

³ Laboratory of Molecular Pathology, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy

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ABSTRACT

Background: The study of cell migration is one of the most interesting topics in cancer research. Wound healing assay is a basic 2D technique used to follow cell migration. The area of a wound's images are measured and calculated to demonstrate the percentage of moving cells. Normally, the measurements are estimated by direct scaling of observers, which unavoidably involves human error. Moreover, there is yet to be a gold standard technique for measurement. Recently, image processing tools have been provided to use and easily carry out measurement, such as ImageJ software and others.

Objectives: This study proposed to take the advantage of the region growing algorithm for wound healing measurement.

Materials and methods: Basic algorithm was used for segmentation of the low contrast area of the wound area from the background in each image. Moreover, contrast limited adaptive histogram equalisation and edge detection techniques were applied in order to improve the quality of images. Program codes were generated and conformed to be the finalised program, named the wound healing measurement program for the trial version.

Results: The program was compared to ImageJ, with the results showing that there were no statistically significant differences ($p > 0.05$, $n = 3$). However, time for all processes of this program was fast and not dependent on the shape of the wound. Intra observer and inter-observer reproducibility of this study found a correlation coefficient within groups close to 1. The average satisfaction of observers for using the program was 4.45.

Conclusion: Thus, it was concluded that this study could apply the region growing technique for the measurement of cell migration program. However, there were problems that occurred during the proceeding in this version. Therefore, we aim to resolve and improve the efficiency of this program in future work.

* Corresponding author.

Author's Address: Department of Radiologic Technology,
Faculty of Associated Medical Sciences, Chiang Mai University,
Chiang Mai Province, Thailand

** E-mail address: suratchanee.phadngam@cmu.ac.th

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Introduction

Cell migration is one of main subjects that biology researchers have been studying, especially those cells associated with cancer. For in vitro 2D-study, the wound healing assay was performed by scratching the monolayer cell with 80-100% confluence. Then, the study followed the movement of the healing of wound and calculated in percentage of movement. Generally, measurement comprises the area of the wound each time by using a ruler or other measurement instrument.¹⁻³ Therefore, it is a manual technique and the values are approximate, with human error definitely possible. Moreover, the captured images in the study have appeared in low contrast properties by microscopy camera. Somehow, it was difficult to distinguish between cells and background, which caused more errors in their results. Avoiding errors will provide more accurate results. Ideally, it would be nice to have some kind of technique or program to measure an area easily and accurately, with acceptable error.⁴ Nowadays, ImageJ software is used globally, whether for image adjustment or measurement.⁵⁻⁶ The ROI function of ImageJ allows manual drawing along the wound area and gives a result in pixel units. However, some observers have been unable to get precise results due to the low contrast of images, as mentioned before. Moreover, in the case of low ability to use a hands-free function to draw an area of observes could be one problem for uncertainty in the area of the wound. Even though most images are low contrast, the wound area is apparently different in texture. The wound is a background of the image and almost uniform in intensity, while the cell layer part is individual but not uniform. To easily measure the wound area, each part must be separated from others. The one basic segmentation technique in image processing that could be applied for this purpose is region growing.⁷⁻⁹ This algorithm can separate the uniform and communicated areas. Therefore, this study proposes to apply the basic region growing technique to measure the wound area in wound healing images. This study aims to develop a basic program for measuring the migration area in wound healing images to get precise, fast and convenient results. Moreover, we will develop a more complete version in the future.

Materials and methods

Materials

In this study, wound healing images were taken by a microscope, (Leica DMI6000B, Leica Microsystem AG) provided by the laboratory for Molecular Pathology, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Novara, Italy. All experiments were performed on a PC with HP Inter® Core™i3 CPU 3.07 GHz and 2.00 GB RAM using MATLAB version R2013a and ImageJ Software (freely available at <http://imagej.nih.gov/ij/>). Thirty observers were students in the Faculty of Associated Medical Sciences, Chiang Mai University, during the period of study. They were 13 females and 17 males with an average age of 20.3 years.

Algorithm description

Region growing technique for image segmentation

Region growing is a region or pixel-based image segmentation. Its work involves the initial seed point, which is selected in the first step. The algorithm will then expand to the neighbouring pixels of its initial seed points and determine whether the pixel neighbours should be added to the region or ignored by using the threshold value. This approach to segmentation is used to find data clustering the same as general data clustering algorithms. A general discussion of the region growing algorithm includes:

- 1) For image R, let p denote the initial seed, after region growing, the region R1 generated from p ($p \in R1$) is connected.
- 2) Assume that R1 is unconnected, then there are at least two subsets $R1,1 \subset R1$, $R1,2 \subset R1$, and $R1,1 \cap R1,2 = \emptyset$. Setting the seed $p \in R1,1$, as $R1,1 \cap R1,2 = \emptyset$, the algorithm ends after finishing the search of the subset R1,1, we get the result $R1,2 \subset R1$. However, the result is paradoxical with the assumption. So, the proposition is correct, namely, R1 is connected.

We follow the algorithm to generate the basic software to measure the wound area image for users. The basic steps are described as below.

Step 1. Generate the matrix 1x5 as RG data collection to collect the data of only 5 areas in one image. Therefore, this software allows a user to select the seed point in 5 unconnected ROI. Each seed point is determined as the centre of each ROI as the queue Q.

Step 2. Check all the pixels around the Q, one by one, to determine whether they are similar to the seed. Let p denote the current seed, and q is one element of p's neighbouring pixel set, I(p) and I(q) denote the intensities of p and q, respectively. If $|I(p) - I(q)| < 1$, p and q belong to the same region. Otherwise, p and q belong to different regions. The segmentation result is denoted by RG after this region growing. The region that includes the initial seed is marked as "1", while other regions are marked as "0".

Step 3. Pop the first pixels and add to R1 set. Then check whether it is empty set. If not, take the first element from the current Q as the seed and repeat Step 2. Otherwise, push the elements that are not yet added to R1 to set R2. Find the total area of 1 which is connected and add as RG data to the RG data collection matrix. The final result of region growing is summarised from the RG data. Fig. 1 shows an example of region growing based image segmentation.

The wound healing measurement program developed and Algorithm framework

The code was generated and compiled by MATLAB program. Considering that wound healing images are often taken with low-contrast properties and weak edges (shown in Figure 2), it affects traditional region segmentation algorithms. Therefore, we added the improvement algorithms, the contrast-limited adaptive histogram equalisation^{10, 11} and edge detection¹²⁻¹⁴ to be a qualified image for the region growing algorithm. The entire procedure of the algorithm follows certain steps.

Step 1. Select image I and conduct the contrast-limited adaptive histogram equalisation on the image I to be the

higher contrast image.

Step 2. To avoid taking a long time for processing, select only the wound area with 10-20 % of cell layer by cropping to obtain Image I selected.

Step 3. Obtain the edge image of image I selected by the Sobel edge detection algorithm. Then dilate the edge to fill and connect all edges together using the dilated gradient mask technique.

Step 4. Enter the final image in Step 3 to the traditional region segmentation algorithms. The result will appear in a pop-up window in pixel units.

Results and discussion

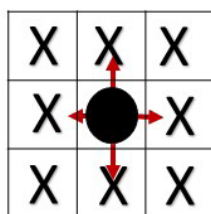
The image results by the wound healing measurement program

This version of the wound healing measurement program enables true and grey scale imaging. We tested the proposed program on two set images with different format types. The results show the successful measuring in wound area for both types (data not shown). Somehow, the algorithm in the program is liable to produce isolated regions in some images epically, which are not clear or have artefacts in the area, as shown in Figure 3. However, this program provides the summation connected area according to the region growing technique. Therefore, the results depend on the appearance of an image. The segmentation results and area measurement shown in Figure 4 indicate that our propose program obtains satisfactory

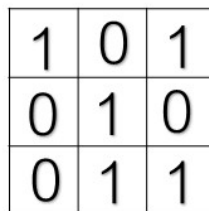
measurement results based on the region segmentation and improved image algorithms. Moreover, this program is useful for the maximum unconnected area in 5 areas, as shown in Figure 2C.

Image evaluation measure for the program results

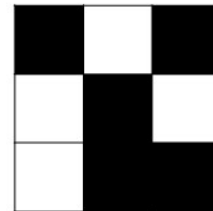
In this section, we consider the performance and accuracy of the measurement algorithms, which are the important aspects of this program. We test the ability of the program for 3 different images with different manner of wound area, as shown in Figure 2, and compare the results using ImageJ software. The results are shown in Table 1. We observed that the results from both programs are similar and not statistically significant difference, ($p>0.05$, $n=3$). Also, both programs show the area measurement related to the area. In contrast, the time cost of both programs is different. These results indicate that Image J's proceeding time depends on the shape and complexity of the area. In the other hand, an uncertainty image, such as in Figure 6C, takes longer than others. However, our program shows the time cost depends on the size of the wound only. Moreover, 3 observers independently measured the 19 images for both programs two times. The results were analysed for intra observer and inter-observer reproducibility. The results are shown in Table 2. We found the correlation coefficient within groups to be close to 1 for both the intra observer and inter-observer reproducibility. These results indicated that this study program is reliable.



1. Select a seed point



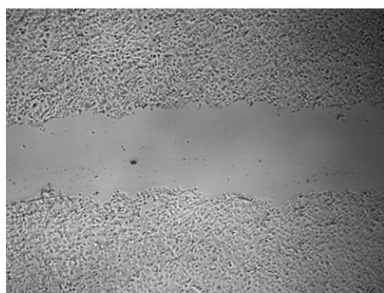
2. Define a growth criteria



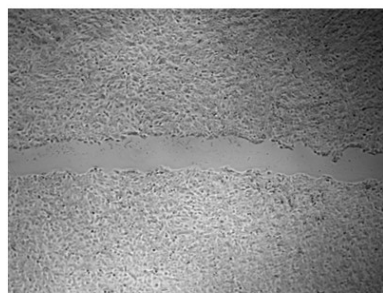
3. Joint all pixel connected to the seed that follow the growth criteria

4. Stop when no adjacent pixel agree with the growth criteria

Figure 1. The region growing algorithm.



a.



b.



c.

Figure 2. Wound healing image with low contrast and weak edges.

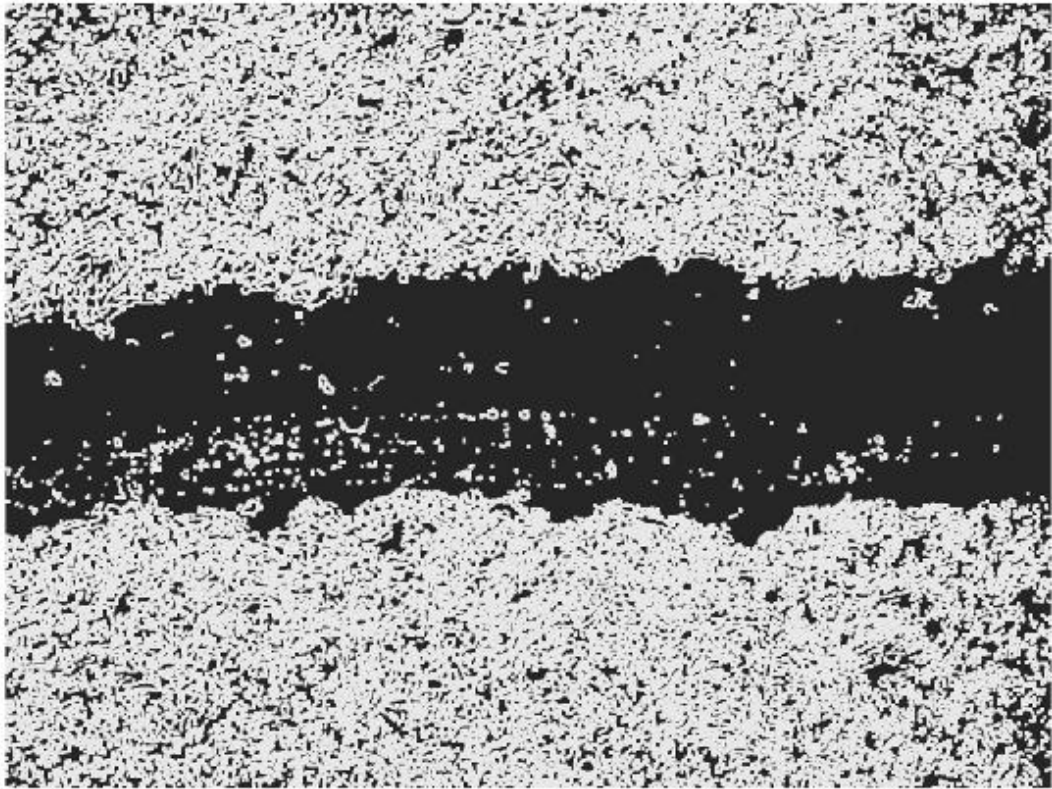


Figure 3. Isolated point in the image.

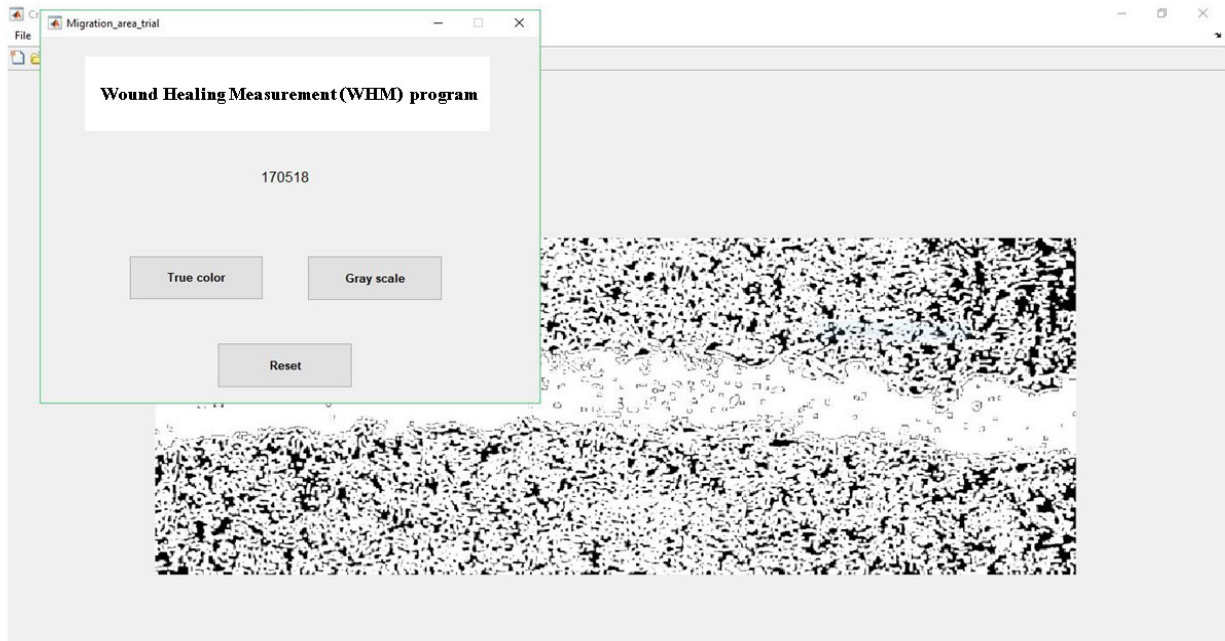


Figure 4. The result of measurement area by program.

Table 1 Results of measurement for both programs.

Image	ImageJ program		This study program	
	Area (pixels)	Time(s)	Area (pixels)	Time(s)
A	552435±4211	47±6	554137±2746	68±2
B	192371±4008	38±8	189636±1524	17±2
C	81966±2154	41±18	72792±2895	12±2

Table 2 Intra-class correlation coefficient in this study.

Intra observer	ICC (intra-class correlation coefficient)
Observer 1	0.998
Observer 2	0.995
Observer 3	0.997
Inter observer	ICC (intra-class correlation coefficient)
All 3 observers	0.998

Satisfaction of users for the program

Finally, we provided the program to 30 users. All users had different performance using the image processing program and computer. The users were advised of the proceeding for the wound healing measurement (WHM) program. The program included the manual measurement by ruler and ImageJ program. Later, we asked all users to measure 3 times independently for each image in 3 different techniques, as mentioned. At the end, the satisfaction for this program

was observed. Table 3 shows the results of satisfaction for this program. We were pleased that our program had a good average of satisfaction for users at 4.45. Moreover, we observed the SD data from 3 measurement techniques as shown in Table 4. The results showed that the highest SD ($SD \geq \pm 2.0$) and lowest SD ($SD \leq \pm 1.0$) were the manual techniques and this work, respectively. SD resulted can verify that our program has less human error, high accuracy and is similar to ImageJ.

Table 3 Satisfaction of 30 users for WHAM program.

Title	Mark
1. Time for proceeding	4.2
2. Steps of use	4.6
3. User interface	4.6
4. Reliability of the program compared to other technique	4.0
5. Reasonable wound healing image compared to other technique	4.8
6. Application for other images compared to other technique	4.5
Average	4.45

Table 4 Standard deviation of the percentage of migration from three techniques.

	Average Migration (%)					
	Manual technique		ImageJ		WHM	
	T1	T2	T1	T2	T1	T2
30 users	±2.04	±2.63	±1.12	±1.32	±0.72	±0.55

Conclusion

In this work, we proposed a beginning program based on the region growing algorithm for measuring the area in a wound healing assay. We named this program the wound healing measurement (WHM): the trial version program 2018. The program also introduced another algorithm to improve the image, such as the Contrast limited adaptive histogram equalisation and Edge detection techniques for improving the quality of images. The program is able to measure in true scale and grey scale without any problem. Moreover, we designed an easier user interface for application. Briefly, the image needs to be loaded into the program from the data storage in a computer. The result is presented in a common window of the user interface in pixel units after selecting the area for 5 seed points. From our experience, we have not seen any wound image that had more than 5 unconnected areas, unless a region is small enough to ignore. That is the reason to set the seed point only 5 times. However, our program has a limitation of the maximum unconnected area, which has to be resolved in the next version. The experimental results show that our work is similar and not statistically significant difference to ImageJ, while the manual was quite different from both techniques. Moreover, the results strongly indicate that the WHM program can obtain reliable, satisfactory and reasonable measurement for wound images. However, this program might depend on whether the image has high contrast and a high level of noise included in the image. Nevertheless, the program has a relatively high time cost depending on the size of the wound and the efficiency of the computer. Therefore, improving the efficiency of the program is the aim of future work.

Conflicts of interests

The authors reported no potential conflict of interest

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