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Automatic image analysis system to measure wound area in vitro

Abstract: In-vitro wound area measurement tracks the rate of wound healing. This project develops and validates an automatic image analysis system to calculate wound area from digital images of an in-vitro 3D tissue model wounded with a biopsy punch. The algorithms were evaluated for repeatability, reliability, and reproducibility, and validated against a known area. Repeatability was checked through repeated measurements under repeated conditions. Reproducibility was evaluated using a Bland Altman plot and paired t-test. Reliability was validated using an image of a known pixel area as a control. The validated image analysis system then calculated wound area from the digital camera and microscope images obtained from an in vitro photo biomodulation treatment experiment. A total of 48 wounded tissues were grouped into red and blue light treatment groups and untreated controls. All daily images were fed into the image analysis system to calculate wound area. The wound area (normalized by day 0) is plotted along the 2-week treatment experiment period to observe wound area in time. The normalised wound area plotted across treatment days show no change in wound area during the treatment period. Future work will adapt the imaging system for visualizing the reepithelialisation cell front marked by live dyes.

Keywords: automation, image analysis, in vitro, wound area, wound healing, image processing.

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1 Introduction

Wounds that are chronic in nature need external energy to ease healing. We use photobiomodulation [1] to stimulate healing of 3D organotypic tissues, injured using a 2 mm biopsy punch. Wound area addresses the healing achieved during the wound hearing treatment experiment.

Our first work on the wound area measurement was made on digital images of the wounded agar model, through tolerance (contrast) edge detection followed by filtering the images [2]. The tolerance edge detection method was later compared with a contour edge detection method, which was performed on the digital images of a biological experimental setup. A method comparison study was done between the tolerance and contour methods, with results showing lesser variability of the contour method on the 'true' wound area [3]. Though the contour method is less variable, it requires the user to manually mark the wounded area.

Wound area measurements can be carried out manually using ImageJ software (National Institutes of Health), where all the stages of wound area measurements are done manually. Therefore, it is important to develop an automated image analysis system without user-input need, capable of calculating the wound area along the days of wound healing treatment experiment. This motivates us to develop two MATLAB algorithms for the automatic calculation of wound area for in-vitro biological wounds, for digital images from a camera and microscope. This work involves the automatic wound area measurement using MATLAB algorithms and comparing them with those calculated manually using ImageJ and plotting the wound areas along the days of treatment, after evaluating the proposed MATLAB algorithms [4].

2 Method

The input for the wound area measurement consists of two sets (camera and microscope) of 48 digital images over 16 days. The images were grouped into groups of 16 images depending upon light of treatment, as red and blue and the untreated control. Initially, images from day 0 are taken for analysis, assuming them to be the true initial wound size.

The digital images from the camera consists of the wounded tissue present within a cup in a tray and therefore requires an algorithm to focus the tissue. The digital wound images from the camera are fed into the MATLAB algorithm and are cropped to select the region of interest. Cropping is

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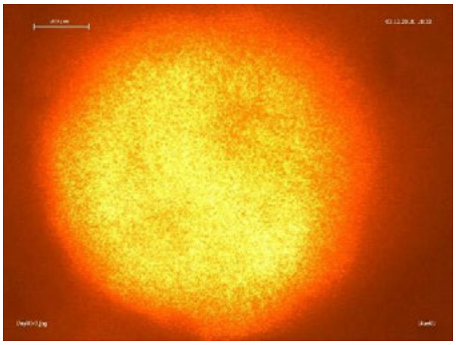

Figure 1: Digital image example of an in-vitro tissue wounded with a biopsy punch, acquired using microscope at 40x magnification.

done after checking the images for scaling to ensure all the images fed into the system are normalised. Here, the diameter of the cup within which the wounded tissue is present is chosen as a scaling factor. The cropped RGB wound image is then binarized to calculate the wound area in pixel.

The microscope images, as shown in Figure 1, have wounded tissues imaged at fixed 40x magnification. Therefore, the microscope images do not require the scaling and cropping stages required for camera images, and follow the remaining stages involved in the wound area measurement. The wound areas for camera and microscope images were also calculated manually by feeding them into ImageJ, for evaluation. Using ImageJ, stages of wound area calculation such as scaling, cropping, and binarization are manually performed.

The reproducibility of the MATLAB algorithms is checked in terms of agreement and the absence of significant differences between the measurement methods. Here, Bland Altman plot checks the agreement between the wound areas measured using ImageJ and MATLAB algorithms. The paired t-test checks the significant difference present between the measurement systems. Repeatability and reliability of the algorithms are checked in terms of Standard Deviation (SD) as follows [3]:

$$\boldsymbol{Repeatability = 1.96 \times \sqrt{2} \times within\ subject\ SD}$$

$$\boldsymbol{Reliability = BSSD^2 \div (BSSD^2 + WSSD^2)}$$

where, BSSD is between subject SD and WSSD is within subject SD. The developed MATLAB algorithms are evaluated by feeding in an image of a known pixel area and comparing the output.

The evaluated MATLAB algorithms are used for measuring the wound areas of wounds along a 16-day wound healing process. The wound areas obtained are normalized as,

$$\boldsymbol{Normalised\ area = Area\ at\ day\ n \div Area\ at\ day\ 0}$$

were, n is the number of days. The resulting normalized wound areas are plotted along the treatment days according to their light of treatment for tracking wound healing.

3 Results

The wound areas were calculated for day 0 digital images from camera and microscope, automatically using the MATLAB algorithms and manually using ImageJ. The resulting wound areas were plotted in box plots, separately for camera and microscope images. Figure 2 shows that the Interquartile range (IQR) for the areas measured using MATLAB algorithm is greater than those measured using ImageJ for the camera images and vice versa for microscope images. Table 1 shows the median, interquartile range, minimum and maximum of the Box plots for camera and microscope images in pixels.

The wound areas obtained for day 0 images are used for comparing the image acquisition systems by plotting areas

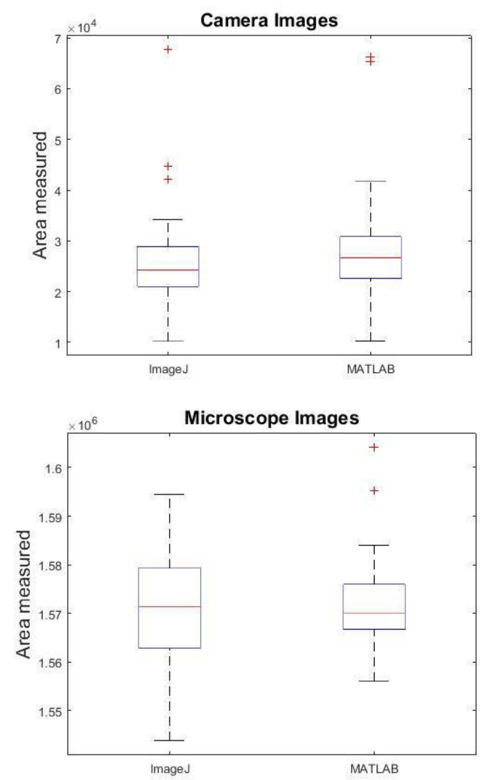

Figure 2: Box plots of initial (day 0) wound area measured using ImageJ and MATLAB for Camera and Microscope images.

Table 1: Boxplot statistics of initial (day 0) wound area in pixels

Images	**Camera Images ($x10^4$)**		**Microscope images ($x10^6$)**	
Algorithm	ImageJ	MATLAB	ImageJ	MATLAB
Median	2.5	2.6	1.572	1.57
IQR	0.75	0.95	0.016	0.011
Minimum	1.25	1	1.544	1.557
Maximum	3.45	4.15	1.595	1.584

obtained using ImageJ against MATLAB algorithms. The scatter plot for camera images in Figure 3, show that most wound areas are present around the line of equality, with wounds of smaller area lying along the line of equality and a wound of larger wound area deviating from the line of equality, thereby having a wider range of distribution. Also, it is seen from the plot that overestimation of wound areas occurs in the case of the MATLAB algorithm for camera images. The scatter plot for the microscope images, on the other hand, shows that all the wound areas are present around the line of equality with maximum points lying on the line of equality within a shorter distribution range. The observations show that overestimation of wound areas occur for some wounds of larger size and underestimation occurs for some wounds of smaller size.

The wound areas calculated using ImageJ and MATLAB algorithms for camera and microscope images were plotted in a Bland-Altman plot with differences between wound areas calculated using ImageJ and MATLAB, plotted against their corresponding means in Figure 4. The Bland-Altman plot for the camera images shows that all the points except one, are present within the limits of agreement and those for microscope images shows that all the plots lie within the limits of agreement with two points falling out of the 95% confidence

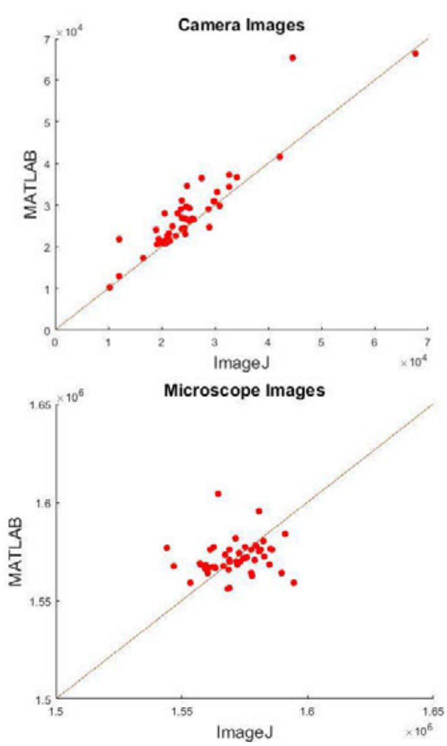

Figure 3: Scatter plot of wound area measures (MATLAB method against ImageJ method) with the line of equality for camera and microscope images.

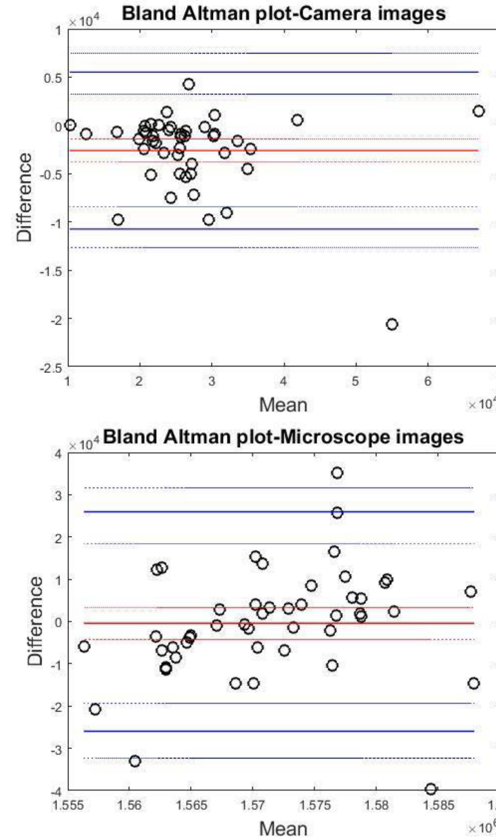

Figure 4: Bland-Altman plot of wound areas for camera and microscope images. Here, the red lines indicate the mean and the blue lines indicate the upper and lower limits of agreement, and the dotted light lines indicate the 95% confidence levels.

interval. This shows the agreement between the ImageJ and MATLAB algorithms for camera and microscope images.

The paired t-test performed for the camera images gives $p = 0.0009$ (for $\alpha = 0.05$), showing that the difference between the methods of measurement is significant and those performed for the microscope images gives $p = 0.82$ (for $\alpha = 0.05$), showing the absence of significant differences between the measurement methods. Hence reproducibility of the MATLAB algorithm for microscope images is proved in terms of agreement and the absence of significant differences between the methods of measurement. Repeatability of the algorithms checked by making repeated measurements using the same sample shows that the absolute difference between the measurements is zero, proving the repeatability of the system. The reliability of both the algorithms are checked, where the reliability coefficient is calculated to be 0.476 for camera images and 0.567 for microscope images, showing the presence of measurement errors.

The normalised wound areas obtained after carrying out wound area measurements using the evaluated MATLAB algorithms for 16 days, were plotted according to the light treatment as shown in Figure 5. Here, value 1 representing the

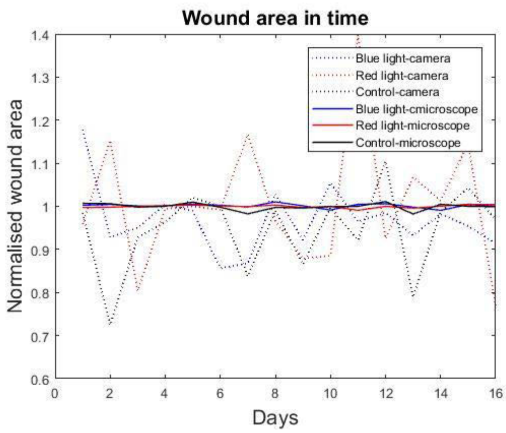

Figure 5: Normalised wound area for camera and microscope images plotted along days, according to the light treatment.

100% initial wound size and a value less than 1 representing decrease and a value greater than 1 representing an increase in the wound structure. The wound therefore shows no healing.

4 Discussion

Two MATLAB algorithms are developed for the automatic measurement of wound area in-vitro and comparisons were made with the manual ImageJ measurements, assuming them to be true wound areas. The observations made show that the MATLAB algorithms overestimate or underestimate the wound area values which were made using ImageJ. It is also seen that the wound areas plotted along the days of treatment do not show healing and stay around the inital wound area.

The previous work on wound area measurement was majorly focused on the image acquisition and planimetry system for developing wounding techniques, which was done in an agar model [2]. This work was followed by a method comparison study performed between the existing tolerance method and the proposed contour method for an in-vitro tissue model [3]. The contour method developed only for the digital camera images required a user input to mark the wounded region manually and did not deal with the scaling issues. The wound area measurements were made for 64 wound samples and were evaluated for wound healing in time during a treatment experiment.

It is notable that the manual measurements using ImageJ are not exact, as the threshold is set manually for all the wound images. Despite wounding the tissues with a 2 mm biopsy punch, the wound areas in pixels for the camera images have a wider range of distribution because of variation in the initial wounding area, thereby impacting the contrast of the wound area. Another limitation in the camera images is the presence of bright spots above the wound due to the lightning environment during image acquisition, which greatly affects the thresholding stages in the algorithm. Therefore, attention must be given to the wounding assay and lighting environment. Future work will aim at adapting the imaging system for visualizing the reepithelialisation cell front marked by live dyes.

5 Conclusion

This work presents automatic image analysis systems for the in-vitro wound area measurement. The MATLAB algorithms measure the wound areas of digital wound images obtained from a camera and microscope during a 16-day wound healing experiment. The results show that the wound areas measured from microscope images have greater accuracy to the 'true' wound area in comparison with those measured from the camera images. This may be improved through adopting new imaging techniques at a finer, cellular scale. Therefore, we suggest the further adaptation of the proposed MATLAB algorithms for the wound area measurements in the ongoing wound healing treatment-control experiments.

Author Statement

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