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Measurement of Mouse Wound Area Using Image Processing and Comparison with Manual Methods

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Abstract

Accurate wound area measurement is essential for evaluating wound healing and treatment outcomes. This study aimed to develop and assess an automated image-processing method for wound area measurement in mice and to compare its accuracy with the conventional manual method using ImageJ software. Twelve mice with excisional wounds were photographed on days 14and 21post-injury. A blue diamond-shaped frame of known area (4cm²) was placed around each wound as a reference scale. The images were processed using OpenCV algorithms, including color space conversion, image blurring, edge detection, contour extraction, and pixel counting. The wound area was calculated based on the ratio between wound pixels and the reference frame. The results were statistically compared with manual ImageJ measurements using an independent t-test. The automated image-processing method demonstrated a strong correlation ($R = 0.96$) with the manual method. The mean wound areas obtained by the manual and automated methods were 0.3248cm² and 0.3159cm², respectively. The difference between the two methods was not statistically significant ($P = 0.5471$). The absolute and relative differences were 0.0089cm² and approximately 2.78%, respectively, indicating high accuracy and consistency .

The proposed automated method provides accurate, rapid, and non-invasive wound area measurements comparable to manual ImageJ results. By eliminating the need for precise camera distance calibration and manual tracing, it offers an efficient alternative for laboratory wound assessment. Future improvements could further optimize this method and enable its implementation as a user-friendly mobile application for clinical and research use.

Keywords: wound area, image processing, ImageJ, measurement

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Introduction

An important parameter for studying wound healing is the assessment of wound size, which is considered the primary metric in wound evaluation [1,2]. Numerous studies have highlighted the significance of wound measurement, including monitoring healing progression, evaluating treatment efficacy, and identifying stagnant wounds [1–3]. Measuring wound area provides a general, objective, and direct method for tracking healing progress and assessing therapeutic outcomes. Various measurement techniques have been developed and applied in both clinical and experimental studies [4].

Conventional methods can track wound healing in a simple and cost-effective manner. However, they often require considerable time and sometimes multiple personnel to complete, especially when large datasets from multiple experimental groups need to be analyzed. Moreover, several manual adjustment steps may reduce reliability and introduce computational bias [5–9]. Therefore, developing a more accurate and efficient method for measuring wound healing is essential for clinical research and translational applications.

Several commercial wound-measuring cameras are available that utilize built-in algorithms for wound size estimation. However, due to their high cost, not all clinics or research laboratories have access to them. Since digital cameras and smartphones are more widely available, capturing wound images has become easier and more popular, making them increasingly used as tools to record and monitor wound healing. Developing methods that facilitate their use could also benefit clinical settings. Often, researchers or clinicians manually assess these images to observe wound changes, a process that is slow, labor-intensive, and requires trained personnel. This challenge becomes a bottleneck as study size increases. Furthermore, wound images are not always captured with future computer-based image processing in mind, so care may not be taken to produce clean, clear, and consistent data. While images can provide additional information (e.g., tissue type, skin condition), ensuring consistency in imaging is critical [1–3,10,11].

Currently, proper imaging for wound analysis generally requires:
(a) Inclusion of a ruler in wound images,
(b) Consistent distance between the wound and the camera,
(c) A fixed relative angle between the wound and the camera lens [10].

Since meeting all these requirements is not always possible, particularly in time-limited clinical settings, it is important to develop a process robust to such variations. Traditional preclinical methods for assessing cutaneous wound healing include caliper measurement, in situ wound tracing, and histological analysis [12].

Caliper measurement typically assumes specific wound shapes and may inaccurately represent irregularly shaped wounds [13]. In situ wound tracing, where wound edges are traced onto a transparent film, may suit irregular wounds but can be time-consuming for large or complex wounds and less reliable for smaller wounds. It may also cause discomfort or damage to the wound [14]. Both methods require animal anesthesia for accurate measurement, which raises ethical concerns if repeated. Histological analysis provides detailed information about wound and skin structure but requires euthanasia and

larger sample sizes for temporal analysis. Overall, these methods present various challenges that must be addressed for reliable cutaneous wound closure analysis [15].
Digital imaging has emerged as a popular alternative that can overcome some of these challenges. Digital planimetry involves photographing a wound alongside a ruler for calibration, positioning the camera lens perpendicular to the wound plane, and digitally identifying the wound area in the image using manual or automated tools [16]. This method is relatively inexpensive and reasonably accurate [17].
However, definitions of wound closure vary across the literature, with limited consensus. Some studies consider wounds closed when re-epithelialization occurs without visible moist granulation tissue [18], while others measure open wounds using the outermost edge [19]. Thus, reliable, consistent, and comparable digital wound assessment methods are needed to capture healing complexity and complement histological analysis.
Wound area measurement using planimetry software (or a graphical software with suitable functions) and digital photographs is straightforward and cost-effective. A photograph is taken of the wound with a ruler or size marker placed near the wound edge. The image is transferred to a computer and opened in the planimetry software. The ruler or marker is used to calibrate linear dimensions in the image. After manually tracing the wound boundary with a mouse, the wound area is calculated and displayed. Free software for wound area measurement can be downloaded from the National Institutes of Health website [20].
When a camera is not positioned perpendicular to the wound plane, the reproduced wound area in the image is not the same as that obtained at 90 degrees; it is proportional to the cosine of the angle. If this angle is known, compensation is straightforward, but typically it is not. Another challenge concerns the ruler placement, which should be close to the wound edge and visible in the image. For example, if the camera is at an 85-degree angle relative to the perpendicular axis, the top-center angle of the image is 95 degrees and the right/left angles are 90 degrees. Placing the ruler below the wound in such a scenario results in underestimation of wound area using digital planimetry. Conversely, placing the ruler above the wound overestimates the area. The angle, if not 90 degrees, always reduces the reproduced wound area compared to a 90-degree view, while the ruler may appear larger or smaller depending on its position. If the ruler is reproduced smaller, the calculated wound area is likely overestimated relative to the true area, and because ruler and wound reproduction differ, the measurement result is inaccurate.
For proper linear calibration, it has been suggested to use two rulers: intersecting at 90 degrees directly over the wound, producing an average linear reproduction. An alternative is four rulers placed around the wound forming a rectangle; the average linear dimensions of all rulers can then be used for accurate wound area measurement, though this significantly increases measurement time [21].
In the current method, a blue cloth is used to define the area around the wound in pixels, which is then compared with the number of colored pixels using color range detection to determine the wound size.

Literature Review

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Various methods exist for wound imaging and analysis to study wound morphology, generally including 2D and 3D imaging techniques. Some of these methods are reviewed below:

In 1998, Langemo DK and colleagues compared methods of wound measurement using a ruler, calculating wound length and width, manual perimeter measurement, and stereophotogrammetry. They concluded that although stereophotogrammetry provided higher accuracy, it was more time-consuming and costly. Manual perimeter measurement was accurate but invasive, while the ruler method was less accurate and also invasive [22].

In 1999, Rajbhandari SM and colleagues measured wounds using two methods: perimeter measurement with graph paper and image processing using DesignCAD 97. They found that image processing was faster, easier, and non-invasive, although it still required manual adjustments. Both methods required expertise for proper execution (43).

In 2002, Oien and colleagues compared digital planimetry, mechanical planimetry, manual perimeter measurement, and ruler-based measurement for wound evaluation. They concluded that although all methods were significantly correlated, digital planimetry was faster and easier than the others despite its invasiveness (44).

Throughout the 2000s, many studies focused on wound measurement using digital planimetry. While non-invasive, these methods generally had lower accuracy. In 2010, Van Poucke and colleagues compared manual tracing of wounds in images and a closed-corner algorithm to establish a standard clinical measurement. They found a high correlation between the two methods ($r = 0.99$), although accessibility and ease of use were not compared [23].

In 2011, Chang AC and colleagues compared digital planimetry with digital photography and image analysis using ImageJ. They reported no significant difference in wound measurement between the two methods, noting that digital photography is non-contact and avoids contamination or patient discomfort [24].

In 2013, Bilgin M and colleagues evaluated wound size in 40 patients using a ruler, manual planimetry, and digital photography with ImageJ. They found that ruler measurements significantly differed from the other two methods, and digital photography was non-invasive and faster [25].

In 2015, Stockton KA and colleagues compared digital planimetry and 3D imaging for wound size assessment using the LifeViz system. A high correlation was observed between the methods, with 3D imaging considered faster and easier [26].

Also in 2015, Wang C and colleagues applied a CNN-ConvNet model to 650 wound images for automatic segmentation, achieving 95% accuracy in wound area measurement [27].

In 2017, Gupta A and colleagues processed wound photographs using HSV color space, median filtering, dilation, and measured wound area with Otsu's threshold method and Suzuki85 algorithm, achieving 70% accuracy in pixel-based wound area measurement [28].

In 2019, Ohura N and colleagues compared four segmentation methods—SegNet, LinkNet, U-Net, and U-Net with VGG16 encoder—on 396 wound images. They found that U-Net with VGG16 encoder provided the highest accuracy [28].

Also in 2019, Wu W and colleagues used color-based segmentation along with a coin for scale measurement in a mobile software application [29].
In 2022, Dymarek R and colleagues compared digital planimetry and AutoCAD software with manual segmentation using a ruler as a reference. They concluded that AutoCAD provided higher accuracy in wound area measurement [30].

Materials and Methods

To obtain wound images, 12 mice were used. Initially, a portion of their skin was removed, and photographs of the wounds were taken on days 14 and 21 after wound induction. Before photographing, the mice were anesthetized, and a fixed diamond-shaped blue frame was placed around each wound. All images were captured at a constant distance and perpendicular angle to ensure the wound was centered in the image, under identical lighting conditions, and then stored.
To measure the wound size using a new image processing method, the following steps were performed:

1. **Color Space Conversion**
   First, one image from the target group was selected, and the original colors were converted from RGB to grayscale [20].

2. **Image Blurring**
   In order to measure the wound area, some parts of the image had to be ignored [21,31]. The wound area was blurred to help its identification. Suitable parameters were applied for blurring. After blurring, thresholding methods were used for image segmentation.

3. **Edge Detection**
   A combination of different operators, including erosion, dilation, and the Canny edge detector, was used to identify the edges. This method improves the accuracy of edge detection in different directions.

4. **Contour Extraction**
   All segmented regions were separated, and the region closest to the image center was selected as the wound area.

5. **Connecting Discontinuous Borders**
   The edges were connected to form a complete shape of the wound area.

6. **Pixel Count of the Wound Area**
   Using a binary mask (white background and black wound area), the number of wound pixels was extracted. However, because the distance and scale of the image alone were insufficient to calculate the real wound size, the number of pixels within the blue diamond-shaped frame was also determined.

7. **Pixel Count of the Diamond Frame**
   The blue color range was isolated, and the number of pixels within the inner area of the blue frame was calculated.

8. **Calculation of Actual Wound Size**
   Since both the number of pixels corresponding to the wound area and the frame area were known, and the physical area of the frame had been previously measured as 4 cm^2, the actual wound area was determined by ratio.

9. **Statistical Comparison**
   The initial wound size measurements were compared with those obtained from the new method using the t-test (Figure 1).

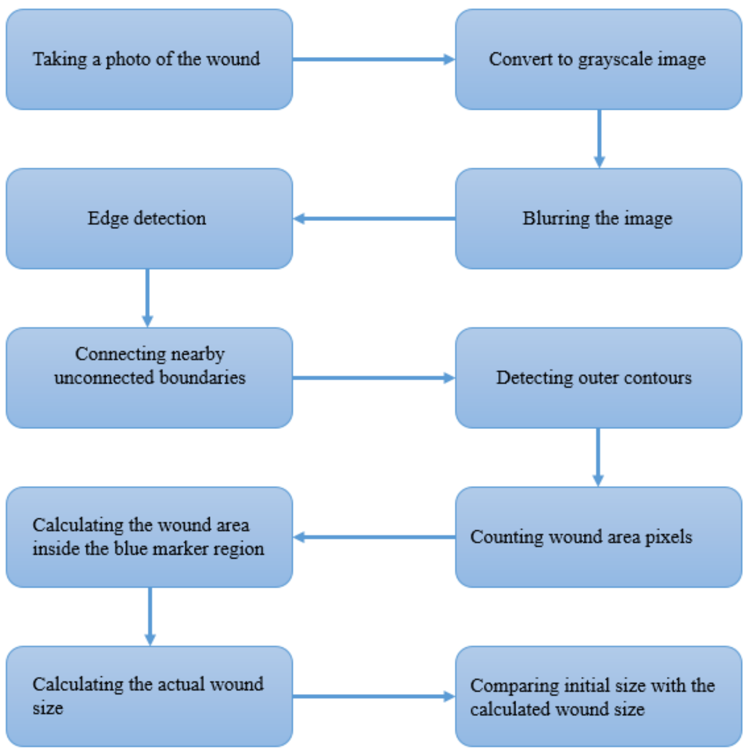

Figure 1 - The steps of the working method can be seen.

3. Results

The proposed method in this study utilizes algorithms available in OpenCV to measure the wound area. The implementation is available at the following link: https://github.com/amir19906/image-proccessing.
The suggested approach consists of the following steps:

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4-1 First, the images are converted to grayscale.
4-2 The image is then blurred and adjusted based on contrast and brightness.
4-3 In the next step, image noise is removed.
4-4 Boundary detection and wound area segmentation are performed, and disconnected edge egments are joined.
4-5 In the final step, the inner section of the detected boundary is filled with black, and the remaining area is set to white. The number of black pixels is then calculated (Figure 2).
This systematic process enables accurate measurement of the wound area by isolating and quantifying the wound region based on pixel count.

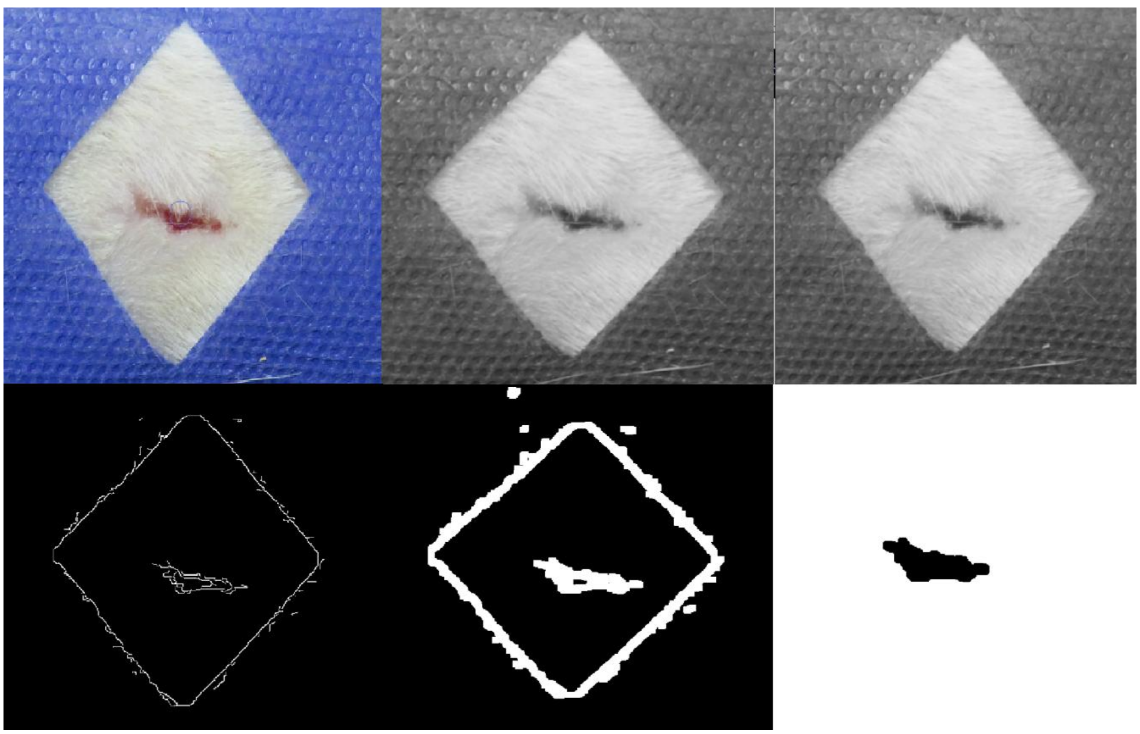

Figure 2. Wound segmentation steps from stage 4-1 to 4-5.

Figure 3 illustrates how the blue-colored area is identified and then traced. After detection, this area is converted into a white mask, while the remaining region inside is shown in black. The number of pixels in this region is then calculated (Figure 2). Considering that the area of the inner blue fabric is 4 cm², the actual wound size is determined by applying a proportional calculation. The results of this process are presented in Table 1.

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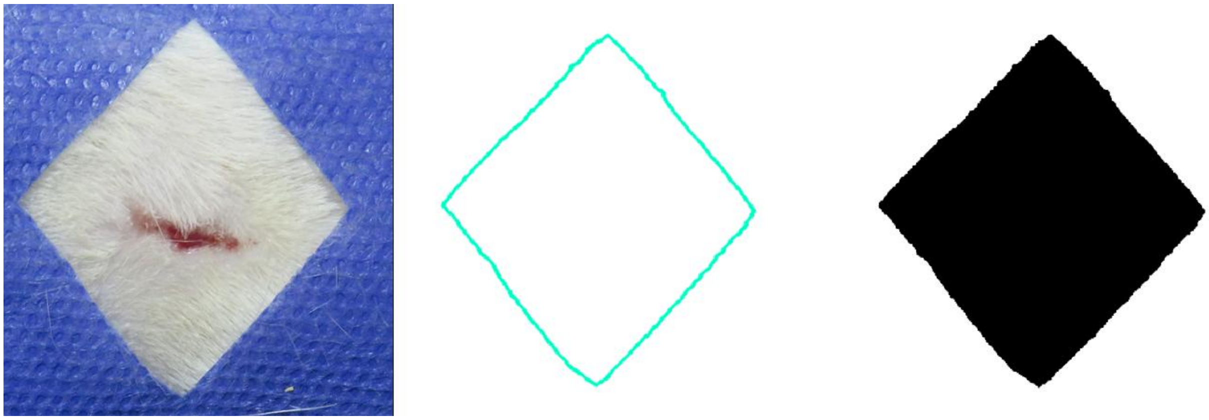

Figure 3: Blue color range image

In this study, wound size measurements were compared between two measurement methods using a t-test. The obtained P value was 0.5471, which is greater than the 0.05 significance level. Therefore, the observed difference between the two methods is not statistically significant, indicating a considerable similarity in wound measurement results. This finding suggests that both methods demonstrate similar performance in estimating wound area. In addition, the absolute difference between the two methods was approximately 0.008896, and the relative or percentage difference was about 2.78% (around 3%). This small difference indicates that the proposed method can be considered a reliable and accurate alternative to the standard method. Overall, these results support the **precision and reliability** of the new method in wound area assessment.

Table 1 - Size of original images and measured images

Size with the method discussed	Original image size	Image name
0.71	0.82	W0-1-day14
0.21	0.16	W0-1-day21
0.67	0.64	W0-2-day14
0.11	0.10	W0-2-day21
0.27	0.24	W0-3-day14
0.04	0.04	W0-3-day21
0.084	0.083	W1-1-day14
0.052	0.053	W1-1-day21
0.36	0.043	W1-2-day14
0.033	0.038	W1-3-day14
0.025	0.035	W1-3-day21

0.051	0.09	W2-1-day14
0.0255	0.026	W2-1-day21
0.19	0.14	W2-2 day14
0.014	0.021	W2-2-day21
0.33	0.27	W2-3-day14
0.034	0.030	W2-3-day21
0.97	0.88	W3-1-day14
0.125	0.116	W3-1-day21
0.88	0.86	W3-2-day14
0.83	0.94	W3-2-day21
0.63	0.88	W3-3-day14
0.057	0.03	W3-3-day21
0.590	0.53	WC-1-day21

Table 2 presents the descriptive statistics for the Main and Test groups, including mean, standard deviation, standard error of the mean, and sample size. The mean values of 0.324750 for the Main group and 0.315854 for the Test group indicate a small numerical difference between the two groups. To further evaluate the significance of this difference, an independent t-test was conducted. As summarized in Table 3, the obtained P value of 0.5471 is greater than the conventional significance threshold of 0.05, indicating that the observed difference is not statistically significant. The mean difference between groups was 0.008896, with a 95% confidence interval ranging from −0.021217 to 0.039009. The t value was 0.6111 with 23 degrees of freedom, and the standard error of difference was 0.015. The R value of 0.9604 reflects a strong linear relationship. The absolute difference was 0.008896, and the percentage difference was approximately 2.78%, supporting the conclusion that the difference between the two groups is minimal.

Table 2- Statistical results related to the comparison of two groups

Group	Mean	SD	SEM	N
Main	0.324750	0.333349	0.068045	24
Test	0.315854	0.313132	0.063918	24

Table 3- Statistical Analysis Summary

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Item	Value
P value	0.5471
Statistical Significance	Not statistically significant
Mean Difference (Main - Test)	0.008896
95% Confidence Interval	-0.021217 to 0.039009
t	0.6111
df	23
Standard Error of Difference	0.015
R value	0.9604
Absolute Difference	0.008896
Percentage Difference	≈ 2.78 %

Discussion

Measuring the actual wound area is not straightforward, as it requires considering image quality, total image pixel count, and the number of pixels calculated for the wound in order to convert these values into metric units. Digital measurement methods have certain limitations that can affect accuracy. The wound area may not be measured precisely due to factors such as image quality or DPI (dots per inch), which can reduce measurement precision. Another challenge is the distance between the camera and the wound surface, since most commonly used camera sensors lack sufficient accuracy for distance detection. The statistical results indicate that the algorithm proposed in this study produces wound measurements comparable to those obtained with current manual methods. By using a blue fabric reference in the proposed approach, the issue of camera-to-wound distance was effectively addressed. Additionally, manual calculations are no longer required, significantly increasing processing speed.

However, repeated testing on different images revealed several limitations that can be addressed to enhance the algorithm and extend its use to wounds with more diverse characteristics. One limitation is wound morphology. Because the current algorithm focuses on the wound area at the center of the image, accuracy decreases significantly if the wound is divided into multiple separate regions. This issue can be resolved by modifying the algorithm to detect multiple wound areas simultaneously.

Another limitation is the presence of hair or noise in the image, which can reduce accuracy—a challenge shared by many automatic image analysis methods. This problem can be mitigated by ensuring clean, high-quality imaging conditions. Additionally, the curvature of the mouse body can cause slight discrepancies between the measured and actual wound area. The proposed method requires images to be taken perpendicularly to the wound surface, which is also a common requirement in other imaging methods. Despite these constraints, the method remains highly useful for monitoring wound healing progression.

Future improvements can overcome these limitations and enable the development of a user-friendly mobile application, making the method a comprehensive and fast wound measurement tool.

Conclusion

This study presents a method for measuring wound area using image processing techniques. The developed method was tested on images of mouse wounds taken in a laboratory setting. The case study showed a significant similarity between the proposed method and the current manual method using ImageJ and manual boundary tracing.
Given its higher speed and independence from strict camera distance requirements, the proposed approach has the potential to offer a more efficient alternative for wound measurement. Future work should involve testing the method with a larger number of images and varying distances. Ultimately, after addressing the identified limitations, this method can be integrated into a mobile application and made accessible to non-specialists, enabling quick and practical wound measurements.

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Authors' Contribution

Amir Fallah-Sohy: Conceptualization, methodology, data collection, analysis, writing – original draft and editing.
Mitra Heidari Nasr Abadi: Data curation, experimental investigation, writing – review and editing.
Simzar Hosseinzadeh: Supervision, validation, analysis, and writing – review and editing.
Roya Rad: Visualization, data management, statistical analysis, and manuscript formatting.

Conflict of Interest

None declared.

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