

ORIGINAL ARTICLE

Evaluation of the scars' vascularization using computer processing of the digital images

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Abstract

Background: The application of new techniques of the scars' correction requires the objective evaluation of their vascularization.

Objective: To evaluate the effectiveness of digital program ImageJ in assessing neo-vascularization of pathologic scars.

Material and methods: In this cross-sectional study, a total of 25 patients with pathologic scars were enrolled. Vessel selection in the first set of digital images of their scars was performed by computer processing started from thresholding with subsequent manual correction. In the second set of the same pictures, Vessel Analysis plugin was used. Comparison of both approaches was performed by three independent investigators. The time required for images processing was measured.

Results: The average time that image processing and calculation have taken in the first group (753.3 ± 88.02 seconds) was statistically longer ($P < 0.0001$) than in the second one (358.1 ± 105.91 seconds). Independent investigators scored the precision of vessel selection in the first group as 80.4 ± 9.82 , in the second group as 72.6 ± 10.53 ($P < 0.0001$). Kolmogorov-Smirnov test demonstrated higher precision of vessel selection by method that involves manual correction ($P < 0.001$). The results of Vascular Density measurements were obviously overestimated in the second group. More expedient looks calculation of the Vascular Length Density: ratio of skeletonized vasculature area to total area. Skeletonization avoids overestimation of Vascular Density, but the density of the vessel mesh can be determined.

Conclusions: Computer processing of the scars' digital photographs using ImageJ software gives cheap, technically easy and not cumbersome way of superficial scars' vascularization objectifying. Vessel selection with subsequent manual correction has advantage of higher precision.

KEYWORDS

computerized evaluation, digital imaging, ImageJ, neovascularization, scar, telangiectasia

1 | INTRODUCTION

It is declared that some of the scar assessment scales for evaluating scars (Vancouver Scar Scale [VSS], Visual Analog Scale [VAS],

Patient and Observer Scar Assessment Scale [POSAS]) can calculate the vascularity among other characteristics. But really they permit to score the grade of the scar redness, are user dependent and show considerable variability between raters. They do not provide any information about neovascularization of the scars. It is necessary to keep in mind that some treatment approaches, for example, topical

Prior Presentations: No.

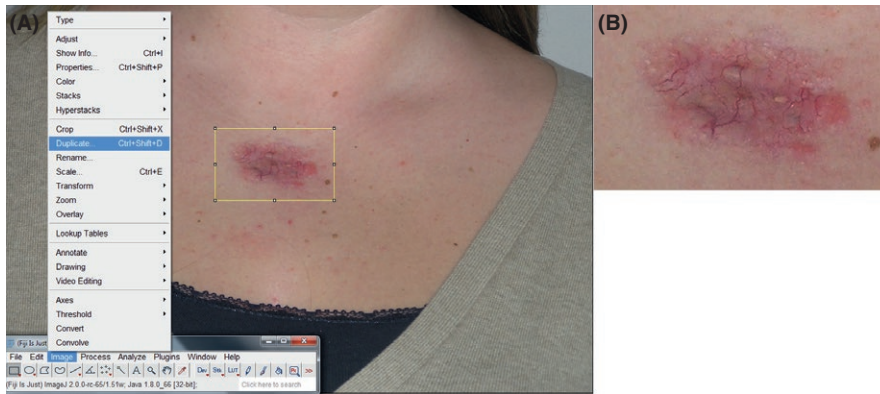


FIGURE 1 Selection, duplication, and cropping of the scar bearing area. (A) Rectangular selection of the scar bearing area. (B) A new window containing a copy of rectangular selection

glucocorticoid injections, may be responsible for the telangiectasia formation. Steroid-induced telangiectasia occurs due to stimulation of nitric oxide release from a dermal vessel endothelial cells leading to abnormal dilatation of capillaries.¹ This effect essentially worsens esthetic results of conservative scar treatment. Thus, relevant appears the need for using the not burdensome, fast and objective method for estimating the amount of telangiectasia in the scar. The application of new techniques and therapies requires the objective evaluation of a number of well-defined parameters. This enables a valuable scar evolution assessment and comparison of the effectiveness of treatments by noninvasive means to be completed.²⁻⁵ The growing use of digital photography with their subsequent computer processing in medicine suggests a more objective approach to scars evaluation.

The objective of investigation was to evaluate the effectiveness of digital program ImageJ in assessing neovascularization of pathologic scars.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

This cross-sectional study was conducted in the Department of Surgery #2 of Bogomolets National Medical University and 1st Surgical Department of City Clinical Hospital #4, Kyiv, Ukraine. Data were collected from case histories and ambulatory charts. A total of 25 patients were enrolled into the study between May 2016 and March 2018. Inclusion criteria—the presence of pathologic scars. Exclusion criteria—narrow normotrophic scars with the color similar to the color of surrounding skin.

2.2 | Image processing and digital analysis

Pictures of the scar bearing areas were taken by commercially available digital camera Panasonic DMX LC 15. Vessel selection in the first set of these photographs was performed by their computer processing started from thresholding with subsequent manual correction. In the second set of the same pictures, vascular selection was done using Vessel Analysis plugin.

We used downloadable distribution of ImageJ/Fiji 1.46r program^{6,7}—a public domain Java image processing and analysis program inspired by NIH Image. This software can display, edit, analyze, process, save, and print 8-bit, 16-bit, and 32-bit images.⁸ Its latest version can always be obtained from <http://imagej.nih.gov/ij/docs/guide>.

Spatial calibration is available to provide real world dimensional measurements in units such as millimeters. To easier setting of the scale, the pictures were taken with the tape measure fixed to the skin surface. Density or gray scale calibration is also available.

The first group of digital images of the scars of all 25 patients were processed using thresholding, followed by the manual correction:

1. After opening of the original image, rectangular selection of the area with the scar defect was duplicated (Figure 1A). This permitted simultaneously duplicate and crop the image (Figure 1B). For future processing, RGB color image was converted into 8-bit grayscale using Menu command: Image ▶ Type ▶ 8-bit (Figure 2).

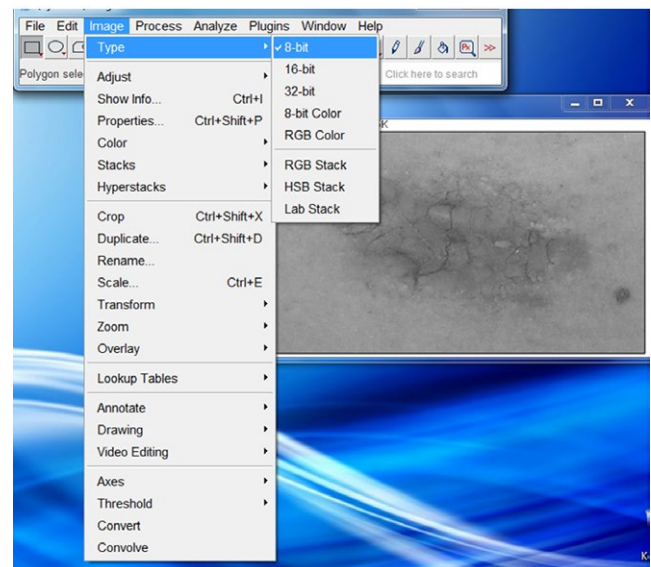


FIGURE 2 Converting image from RGB color format to grayscale

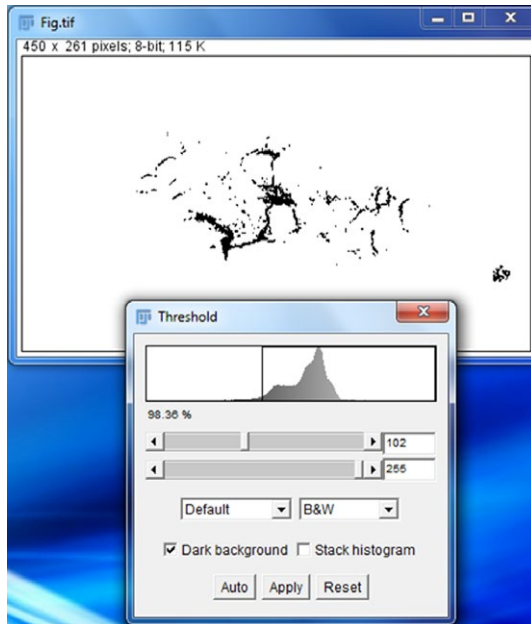
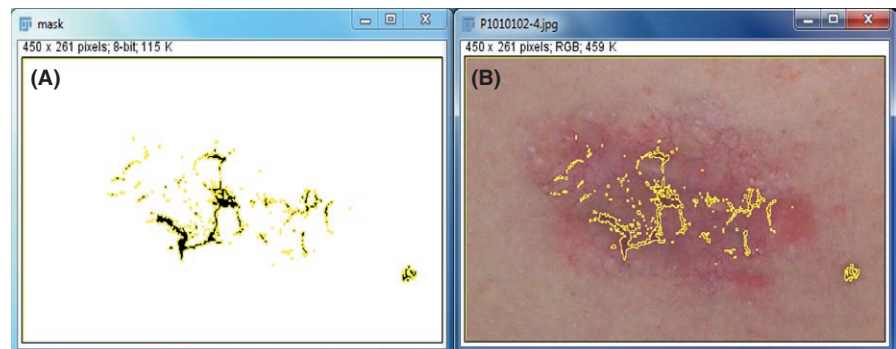


FIGURE 3 Thresholding of the image

2. Then adjustment of threshold was performed by Menu command: Image ▶ Adjust ▶ Threshold (Figure 3). It should be specified whether or not to make the background dark or light. Adjustments of the minimum and maximum sliders are needed until receiving satisfactory saturation level of the image.
3. On the next step, masks were created using Menu command: Edit ▶ Selection ▶ Create Mask. Based on the image and set threshold, some portions of the image may be over/under saturated. Thus the portion of the image that needs to be adjusted was selected. The tool Dilate was used to grow the included pixels to further saturate this portion of the image. The tool Erode permitted to remove saturation (from Menu command: Process ▶ Binary ▶ Dilate/Erode).
4. Selection of the objects within the mask was created using Menu command: Edit ▶ Selection ▶ Create Selection.
5. From the mask, selections were transferred to the given image. Before transferring the mask's selections, the image was reverted to its original form. At first the mask was selected (Figure 4a), then the original image, and command \uparrow Shift+E transferred the mask's selections (Figure 4b). From this picture,

FIGURE 4 Transferring the mask's selections to the given image. (A) Mask selections (B) Mask selections were transferred to the original image. Yellow outlines—selection of the vessels



it is easily seen that selection needs manual correction. Not all small vessels, mainly at the upper part of the photograph were automatically selected.

6. If a correct segmentation of the blood vessels from the background is not obtained, manual correction should be done. Using hierarchy: Image ▶ Adjust ▶ Brightness/Contrast command can be opened. This helps to improve the visualization and facilitates the manual selection. Drawing outside current selection while pressing Shift creates new content. The next Figure 5 demonstrates the results of manual correction of automated selection.
7. Scar contour may be outlined by hand (Figure 6). This approach gives more precise results than using Color Threshold tool. All selections including scar outline were added to regions of interest (ROI) Manager.
8. To present the results of measurement in calibrated units, such as millimeters, the spatial scale of the active image was defined. Before using this command, the straight line selection was activated to make a line selection that corresponds to a known distance. Then, bring up the Set Scale dialog from Menu command Analyse, the Known Distance and unit of measurement were entered. After clicking "OK," the Distance in Pixels field automatically filled in based on the length of the line selection (Figure 7). Then the area of the scar, total area of the selected vessels and part that last one takes from the scar area in percent were calculated.

The alternative variant of vascular selection was studied in the second group of same photographs of all enrolled patients. It was done by the Vessel Analysis plugin. The "complete" program guides us through preprocessing steps to prepare the multi-channel image for vascular density and diameter measurements:

1. First of all, after opening of multi-channel image in Fiji, the Vessel Analysis plugin was executed (Plugins ▶ Vessel ▶ Analysis ▶ Vessel Analysis [complete]).
2. Using the rectangular selection tool, we were asked to select the image area to analyze. Once completing and clicking OK, a cropped image was generated, followed by its binary version. The contour of the scar was transferred as an overlay from the multi-channel image by the help of ROI manager (Figure 8).

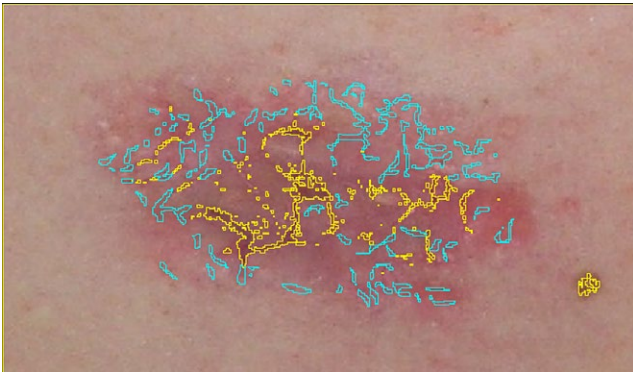


FIGURE 5 The results of manual correction of automated selection of the vessels in the scar. Yellow outlines—automated vessel selection, Blue outlines—manual vessel selection

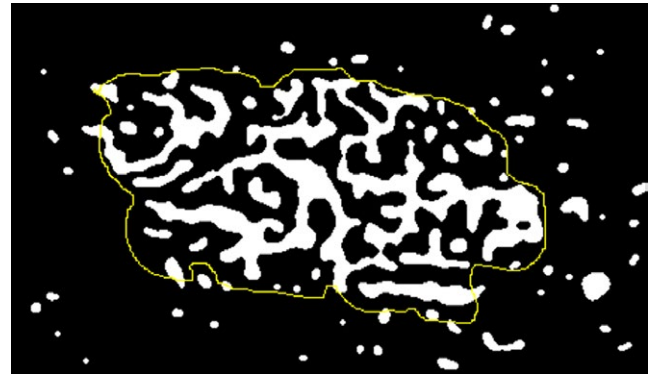


FIGURE 8 Binary-cropped image version of the scar. Yellow line—outline of scar contour



FIGURE 6 Outlining the contour of the scar by Freehand selection tool. Blue line - outline of scar contour

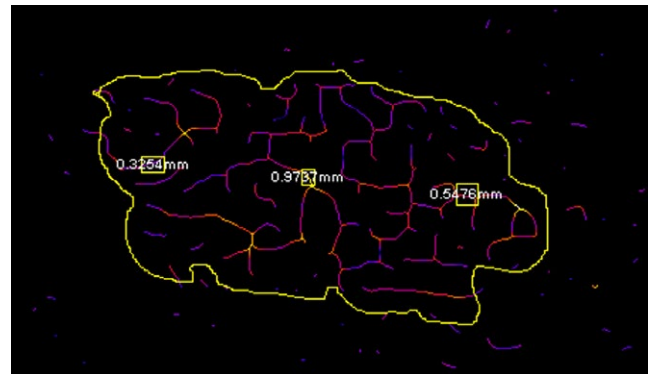


FIGURE 9 Measurement of the vessels' diameter. Yellow line—outline of scar contour. The samples of the vessels' diameter measurement are presented in the squares

skeletonized image is generated (Figure 9). The relative thickness of the vessel in the selected area corresponds to some color, which ranges from white (thick) to purple (thin). When scaling is performed the average diameter of the vasculature in selected area (rectangle selection) may be measured in millimeters.

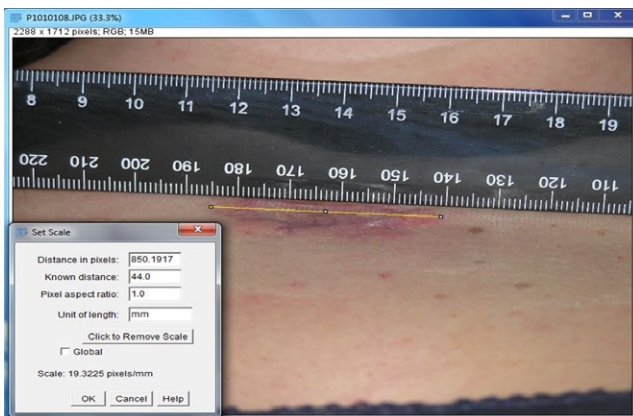


FIGURE 7 Setting Scale

Then the following measurements were collected using this binary image:

- Vascular Density: ratio of vasculature area to total selection area;
 - Vascular Length Density: ratio of skeletonized vasculature area to total area.
3. This plugin permits also measurement vessels' diameter. After selection of a region of interest and clicking OK a colored,

2.3 | Outcome measures

The adequacy of vessel selection was assessed by three independent investigators using Visual Analog Scale (VAS)—straight horizontal line of 100 mm length. The left end corresponds to the worst adequacy, the right end—to the best adequacy. Term “adequacy” means that number of selected vessels and accuracy of selection maximally corresponds to reality. The result was obtained by measuring the distance from the left edge of the line. The time required for computer image processing and measurement was also assessed.

2.4 | Data analysis

Statistica 8.0 (Statsoft Inc., Tulsa, OK) software was used for the data processing. Graphical assessment of normality was supplemented

by Shapiro-Wilk test. Some researchers recommend it as the best choice for testing the normality of data in not large samples.⁹ Data were presented as “mean \pm SD”. The means for two groups were compared by an independent samples *t*-test. Kolmogorov-Smirnov test was used for comparing two not normally distributed samples. Differences between the samples were considered statistically significant when *P*-value < 0.05.

2.5 | Ethics

This cross-sectional study was approved by the local Ethics Committee (Bogomolets National Medical University, Kyiv, Ukraine on 30th May 2016, Ref. Nr. 96).

3 | RESULTS

The average time that image processing and calculation has taken in the first group (753.3 \pm 88.02 seconds) was statistically longer (*P* < 0.0001) than in the second one (358.1 \pm 105.91 seconds).

Independent investigators scored the precision of vessel determination in the first group as 80.4 \pm 9.82, in the second group, where Vessel Analysis plugin was used, as 72.6 \pm 10.53, respectively. This showed that the first method is more consistent with the accuracy (*P* < 0.0001). However, in 14 cases from 150 at least one expert gave preference to the second method. Kolmogorov-Smirnov test demonstrated statistically confident higher precision of vessel selection by method that involves thresholding followed by manual correction comparing to above-mentioned vessel analysis plugin (*P* < 0.001). The following picture (Figure 10) gives possibility visually compare the accuracy of both methods. It is obvious that automatic vessel selection is not completely correct.

The results of Vascular Density measurement (part of the scar area that selected vessels' area takes in percent) were clearly overestimated in the second group. For example, in presented case first method of vessel selection revealed that their total area takes 15.8% of scar area and second method—39.0%. More expedient looks the

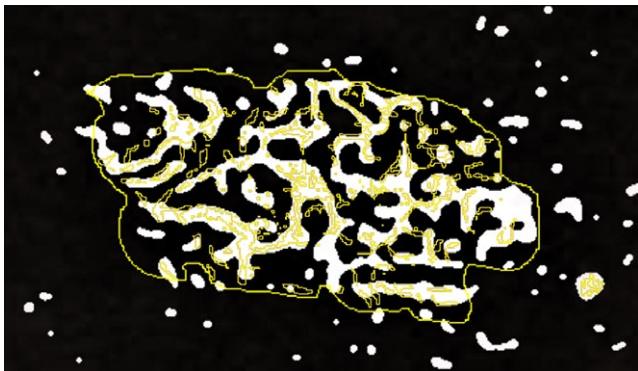


FIGURE 10 Comparison of accuracy of two methods of vessel selection. Yellow color—outline of vessel selection made by thresholding followed by hand correction that overlays binary-cropped image of the scar produced by the Vessel Analysis plugin

measurement of the Vascular Length Density: ratio of skeletonized vasculature area to total area. Skeletonized vessels are represented by outlines of 1-pixel width. This does not allow the calculation part of scar area taken by vessels. At the same time, it is possible to avoid overestimation of the results and the density of the vessel mesh can be determined.

4 | DISCUSSION

Analysis of literature made by S.H. Bae and Y.C. Bae in 2014¹⁰ demonstrated that five scar assessment scales were used most frequently: POSAS, VSS, VAS, Manchester Scar Scale, and Stony Brook Scar Evaluation Scale. Using this instruments vascularization of the scars may be assessed only indirectly—by evaluation of scar tissue color. Despite the large number of studies published in scientific literature, no ideal scar scale exists to effectively assess the vascularization of scars.¹⁰⁻¹²

Some treatment strategies, such as intralesional glucocorticoids injections, may have as a side effect the telangiectasia formation.¹³

Objective methods have advantages over scar scales.² Their efficacy is based on the use of validated tools, fundamental to ensure reliable outcome measurements.¹⁴ Scar vascularity have already been assessed using dermoscopy¹⁵ and Intravital video-capillaroscopy.⁵

In this investigation not expensive, easily available methods of computer processing of digital images were compared. Program ImageJ is in the free access. The use of open source tools is especially important due to their transparency and inherent ability for sharing and extensibility.⁶

Following approach may be used not only for scars assessment but also for evaluation of different methods of telangiectasia treatment.

The obtained results have demonstrated that both presented variants of vessels selection do not take too much time, although it is much faster to use the Vessel Analysis plugin. We give the preference to vessel selection by thresholding followed by manual correction because of its higher precision. It is wise to refine it and make less time-consuming.

Evaluation of Vascular Length Density from Vessel Analysis plugin before and after a specific treatment can be used to confirm the possibility of therapy to cause telangiectasias formation. On the other hand, this approach can provide an opportunity to evaluate the effectiveness of approaches to eliminate telangiectasias: laser therapy, radiofrequency thermal ablation, sclerotherapy.

5 | CONCLUSION

1. Computer processing of the scars' digital photographs using ImageJ free software gives cheap, technically easy, and not cumbersome way of objectifying of superficial vascularization of the scars.

2. Method of vessel selection by thresholding with subsequent manual correction has advantage of higher precision comparing with Vessel Analysis plugin, however, is more time-consuming. This method gives possibility to calculate the part of the scar's area that telangiectasias take, which depends both of the number of vessels and their width.
3. Measurement of Vascular Length Density using Vessel Analysis plugin gives information about vascular net density and its change after different treatments, but does not give possibility to calculate the part of the scar area covered by telangiectasias.
4. Proposed approach may be used in all situations when it is necessary objectively characterize telangiectasias.

CONFLICT OF INTEREST

None.

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