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Image Processing in the Study of Wound Healing

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Wound healing is an important field in dermatology. At least in European countries, dermatologists routinely treat chronic nonhealing wounds, including venous leg ulcers, pressure sores, vasculitis ulcers, diabetic ulcers, and ulcers caused by inoperable vascular diseases.[1] In general, treatment is focused on improving the patient's condition, treating the underlying disorder whenever possible, and wound care. Wound care has become a specialty in itself. In the last 15 years, an enormous amount of wound care materials have been introduced on the market. Many of these products have not been properly tested in randomized, double-blind, placebo-controlled clinical trials. Such trials are desperately needed to supply clinicians with information to guide them in their choice of wound care products. One of the problems in performing clinical trials on wound healing is the lack of objective evaluation methods. The evaluation method should be adapted to the wound type and the wound healing phase (debridement phase, granulation tissue formation, epithelialization phase, or remodeling phase). If a product is designed for use in the epithelialization phase, then the time required for complete wound healing, or the reduction in wound size within a certain time limit, can be used as a primary outcome parameter. If a product is designed for wound cleaning, the wound size is a useless parameter, because wound size can remain exactly the same or increase slightly during the debridement phase, while important qualitative changes occur: the yellow or greenish black layers of fibrin and necrosis are removed and gradually replaced by healthy red granulation tissue. This article describes a digital image analysis (DIA) system designed to measure the shift from black/yellow necrosis to red granulation tissue objectively.

Measuring Wound Surface

The simplest method is to trace the wound margins on a transparent sheet and to redraw the line with a computer pen on a digitizing tablet later. The advantage is that the clinician decides where the wound ends and epithelialization begins. The disadvantage is that on two occasions, errors may occur in tracing the ulcer outline. If the clinical observer and the computer operator are experienced, these errors are low (<2%). Another method is to obtain a digital image of the wound or a photograph of the wound (including a centimeter scale in two directions) and trace its outline directly on the screen, by using a mouse. In some wounds digital image analysis (see below) can be used to detect the wound margins automatically; however, the color differences between granulation tissue, surrounding skin, and the thin, partly transparent layer of the newly formed epithelium can be too small to allow automatic detection.

Wound contraction is not easy to quantify in human studies, because orientation marks are needed to measure the approximation of the wound edges. In animal studies, tattoos can be used.

Measuring Wound Volume

In deep wounds like decubitus ulcers one may want to measure how fast the wound fills up with granulation tissue. In this case, volume is an important parameter. Stereophotogrammetry can be used,[2] but it is less suitable if the wound has undermined borders. Calculating the volume by casting, or filling it up with water, is an alternative.[3–6] These volumetric methods may be painful and unreliable, as the volume of some wounds change considerably depending on the position of the patient.[7] Some promising new noninvasive volumetric techniques using ultrasonic imaging, laser profilometry, or color-coded structured light have been described.[8–10]

Measuring Wound Debridement

Wound cleaning (debridement) is an essential first phase in wound healing.[11] Wound debridement can be achieved by surgical methods, by the repeated application of moistened hydrophillic gauzes, or by the use of special products designed for debridement such as proteolytic enzymes, dextranomers, polysaccharide granulates, hydrocolloid dressings, and intracavity gels.[12–14] These products dissolve and absorb necrotic

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tissue, or provide an optimal climate for wound debridement by phagocytic cells and the natural proteolytic enzymes of the host. Either way, the result is reduction of necrotic tissue and increased formation of healthy red granulation tissue during treatment. As mentioned before, the wound size or the time until wound closure cannot be used as an endpoint for debridement, as these parameters may remain unchanged during debridement and are influenced by a cascade of events taking place after the actual debridement phase. Therefore, efforts should be made to measure the absolute amount or percentages of healthy tissue (granulation tissue) and nonhealthy tissue (yellow slough and necrosis) during treatment.

The Black–Yellow–Red Model

The debriding effect is usually quantified using the black/yellow/red model, in which black is black necrosis, yellow is yellow necrosis (slough), and red is granulation tissue.[15] This model has been generally accepted by clinicians as a tool to classify wounds on the basis of color. Some pharmaceutical companies have grouped their wound care products according to the same classification model. This three-color visual evaluation model has been used in most clinical trials on wound debridement until now. The computer system described below is based on the same classification principle. One should realize of course that there are thousands of different hues of red in granulation tissue and that the color of necrotic tissue varies from white, yellow, greenish, hemorrhagic brown to deep black, including all colors inbetween.

Consistency of Visual Estimations

The differences between necrotic tissue and granulation tissue are clearly distinguishable by the human eye but, because of the complex two-dimensional structures, difficult to estimate exactly. To investigate the interobserver and intraobserver consistency in estimating surface percentages, 15 observers (5 dermatologists, 5 dermatology residents with experience in clinical judgment of venous leg ulcers, and 5 medical students in their last year of clinical training) were asked to assess the amount of granulation tissue in three drawings of a venous leg ulcer during treatment. To avoid differences in the interpretation of colors, schematic but realistic drawings were used, consisting of irregularly shaped but homogeneously colored yellow, red, and black areas. Because only three basic colors were used, the real surfaces could be measured simply and exactly by the computer system described below. The observers were not informed about the accuracy of their estimations. Approximately a half-year later, the same persons were asked unexpectedly to do the same test. The results are shown in table 1. At first sight, digital image analysis seems to be unnecessary, because the average visual estimations are very near the computer measurements. But if we look at the ranges, differences from 15 to 36% were found between two observers, 28 to 720 times higher than the range in 15 repeated computer analyses (ranges: 0.05, 0.25, and 0.53%). Also the intraobserver variability is considerable (Fig 1). Differences up to 30% were found between the first visual estimation and the second estimation 6 months later. In conclusion, the intraobserver and interobserver consistency is low, especially if we realize that these were schematic representations of ulcers, in which there was no disagreement about the classification of the colors. There was no relationship between the clinical experience of the observer and the accuracy of the visual estimation. About half of the observers used only round figures (steps of 5%) in their estimation.

Morphometric Analysis

Morphometric methods have been used in electron microscopy to estimate the number of cells, cell organelles, or other structures on a photograph. A trans-

Table 1. Comparison Between Visual Estimations (by 15 Observers on Two Occasions with a 6-Month Interval) and Digital Image Analysis (15 Measurements)

	Average (%)	*Min–Max (%)*	*Range (%)*	*SD*
Yellow surface, day 0				
First visual estimation	77.3	61.00–86.00	25	6.945
Second visual estimation	79.5	65.00–93.00	28	7.189
Digital image analysis	77.9	77.73–77.98	0.25	0.064
Yellow surface, day 4				
First visual estimation	68.3	54.00–81.00	27	8.762
Second visual estimation	68.6	53.00–89.00	36	9.030
Digital image analysis	66.5	66.48–66.53	0.05	0.015
Yellow surface, day 7				
First visual estimation	21.5	15.00–31.00	16	6.642
Second visual estimation	20.5	15.00–30.00	15	5.343
Digital image analysis	21.6	21.35–21.88	0.53	0.149

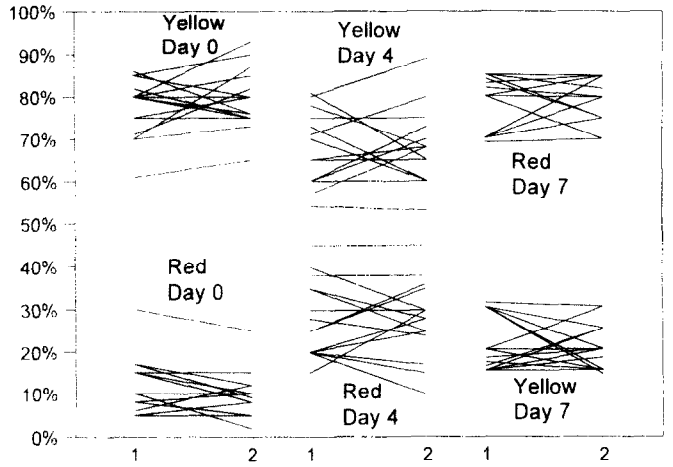


Figure 1. *Interobserver and intraobserver variability of visual estimations of the amount of red and yellow tissue in three ulcers. Fifteen observers were asked to estimate the red, yellow, and black surfaces in a schematic representation of a wound, on two occasions (1 and 2), with a 6-month interval.*

parency with a sample raster is used. Only cells or structures within a sample field are counted. We used this method to evaluate the amount of red, yellow, or black tissue in a wound. A sample raster composed of 3 × 3-mm squares was placed on standard Polaroid pictures of venous leg ulcers. The area within the sample squares was compared with a color palette of yellow, red, and black colors. For each square it was decided whether the area was red, yellow, or black. In some squares it was impossible to decide between red and yellow, and the square was counted as half yellow and half red. The method gives a more reliable result than visual estimations, and correlates well with the computer image analysis method described below (Fig 2). The disadvantages are that a photographic step is required and the method is extremely time consuming.

Colorimetric Analysis

Surface colors can be measured very accurately with colorimeters such as the Minolta Chromameter CR 200. The equipment is being used throughout the world for commercial, medical, and scientific purposes. It can be used to measure skin color, including erythema, as well as the color shift from yellow to red in an ulcer.[16] The color differences are measured using coordinate geometry. A color is determined by three values (x, y, and z, or L^*, a^*, and b^* in the Cielab color space system).[17] Apart from practical problems regarding hygiene and sample size, the method is less suitable for measuring the shift from yellow to red in wounds, because if we move from yellow to red in the three-dimensional colorimetric color space, only a minor change in one of the coordinates is observed. This minor numeric change does not correlate well to the enormous difference in the perception of yellow and red by the human eye. In addition, the method cannot measure surfaces precisely, and the data are not numeric and therefore not accessible to routine statistics.

Digital Image Analysis

Methods

A video image of a wound is obtained by positioning a video camera and a light source, both mounted with Polaroid filters to prevent reflection, in a standardized way to the wound. All variables such as height, distance, angle, light, and diaphragm are recorded. The zoom lens adapts to any wound size. Ink marks are made on the adjacent skin to facilitate positioning. The distances between three of these marks are recorded for calibration purposes. The video image can be projected on the image stored the day before, to obtain exactly the same closeup. A color scale and gray scale can be positioned near the wound for calibration purposes.

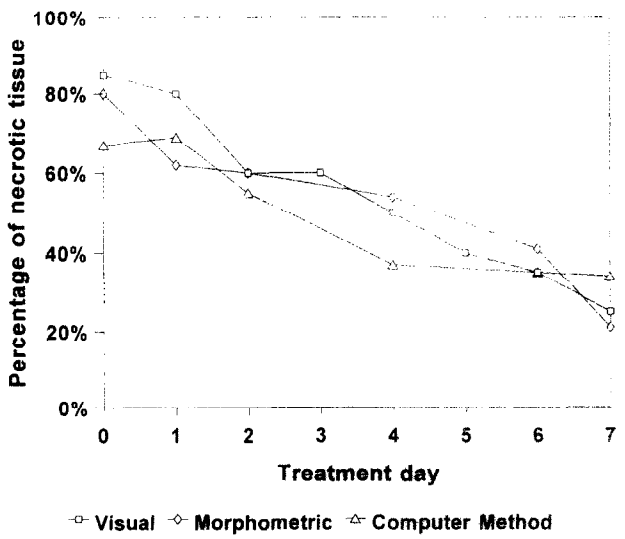
Hardware

The computer system consists of an IBM-compatible AT-386 personal computer, a frame grabber, VGA monitor and RGB monitor, and a digitizing tablet (Fig 3). As each stored image requires 500 kB, two large hard disks and a tape streamer are installed.

Software

The software was specially written by the Department of Medical Physics of the Academical Medical Centre, University of Amsterdam, and consists of a mixture of the original software provided with the frame grabber

Figure 2. *Estimation of the amount of necrotic tissue in an ulcer, during 7 days of treatment with proteolytic enzymes, by three different methods: visual estimation, morphometric estimation, and computer image analysis.*



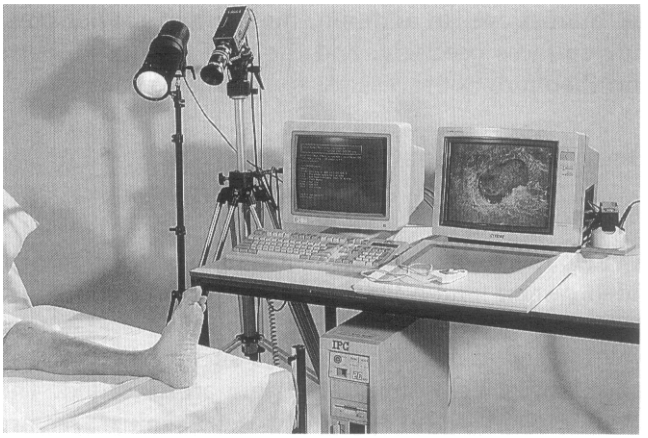


Figure 3. *The digital image analysis system consists of an AT-386 personal computer provided with a frame grabber, VGA and RGB monitors, a digitizing tablet, a video camera mounted with a zoom lens and Polaroid filter, and a light source with Polaroid filter.*

(Vision), programs written in the language C, and a menu structure composed of MS-DOS batchfiles.

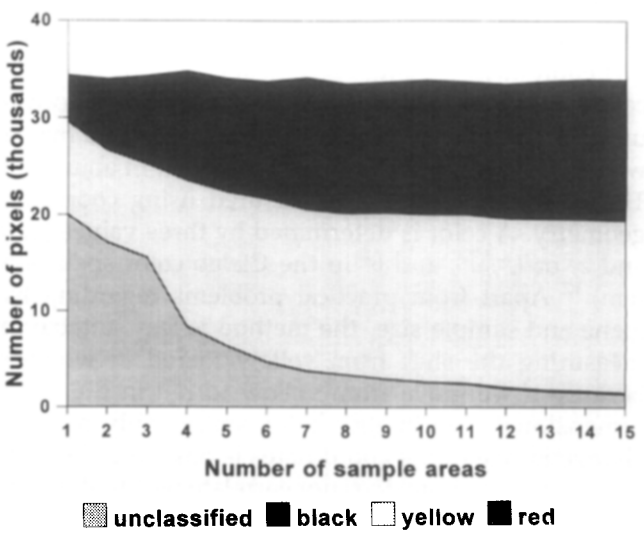
Calibration Procedures

In the video system, each color is composed of a certain amount of red, green, and blue light. The Vision 16 frame grabber in our computer system divides each basic color (red, green, and blue) into 32 intensity steps. As a result, $32^3 = 32{,}768$ different colors can be recognized. As the color of a pixel is determined by three values between 0 and 31, a three-dimensional mathematical model can be used (a color cube). The position and color of each point in this color cube are determined by three coordinates. Point 0,0,0 is black, point 31,31,31 is white. The computer has to be told which colors can be encountered in the granulating area or necrotic area of a wound. One method to accomplish this is to give the minimum and maximum acceptable values of red, green, and blue for each tissue component (coordinate classification method). This method cannot be used, because of the complex distribution of the colors of interest, in the three-dimensional color cube. The red hues found in granulation tissue vary in intensity from dark red to light red, and in color from red to brown, purple, orange, or pink. They form an irregularly shaped three-dimensional cloud in the color cube, which cannot be described with three-coordinate mathematics.

It is therefore necessary to create large classification tables for each area of interest. These tables list which colors are present or absent in, for instance, necrotic tissue. They consist of 32 pages of 32 × 32 tables, and are generated semiautomatically by the computer. To construct a classification subtable for necrosis, the clinician selects a large number of small representative necrotic wound areas from the computer screen. The color found in these sample areas are combined, sorted by frequency, and stored in a classification subtable for necrosis. In the end several subtables are combined and analyzed for overlapping colors. If there is no overlap, the resulting color definition table can be used for analysis. One single color definition table is used throughout a study for all wounds. Separate tables are used for human and animal studies, to compensate for different recording conditions. The flexibility of the software and the way in which the color definition tables are generated make digital image analysis suitable for several other purposes, like measuring epithelialization in a wound, evaluating laser treatment of pigmented lesions, calculating the percentage of skin covered with psoriasis plaques, and measuring repigmentation in vitiligo spots[18] or monoclonal stained cells in histologic sections.[19]

The system can measure color deviations and light conditions. Every part of the computer image can be enlarged if necessary for calibration purposes. The color of each individual pixel in an image can be determined. Analyzed images and measurement results can be stored. Depending on the quality of the image (reflections) and contamination (textile fibers, zinc ointment, etc) in the ulcer, a certain amount (0.01–5%) of the surface remains unclassified. The proportion of unclassified wound area is also determined by the number and quality of the samples used to construct the classification table. To get an impression of the sufficient amount of representative samples needed, one ulcer was evaluated with 15 subsequent classification tables, constructed from 1 to 15 samples (Fig 4). If only one sample area is selected for each color, and combined into a classification table, then this table

Figure 4. *Correlation between the amount of sample areas selected for calibration procedures and the total unclassified area. If more representative wound areas are added to the color definition file, the total unclassified area diminishes.*



leaves 58% of the wound area unclassified. After 15 samples of each of the three colors are used, the unclassified wound area is 4.19%. The next step is to analyze which region of the wound remains unclassified, to zoom in on this area, and to select more samples until the unclassified area is below 2%. This percentage can be further improved to 0.01% by additional semiautomatic checking and editing functions, but for routine use we think that an unclassified area less than 5% is already acceptable.

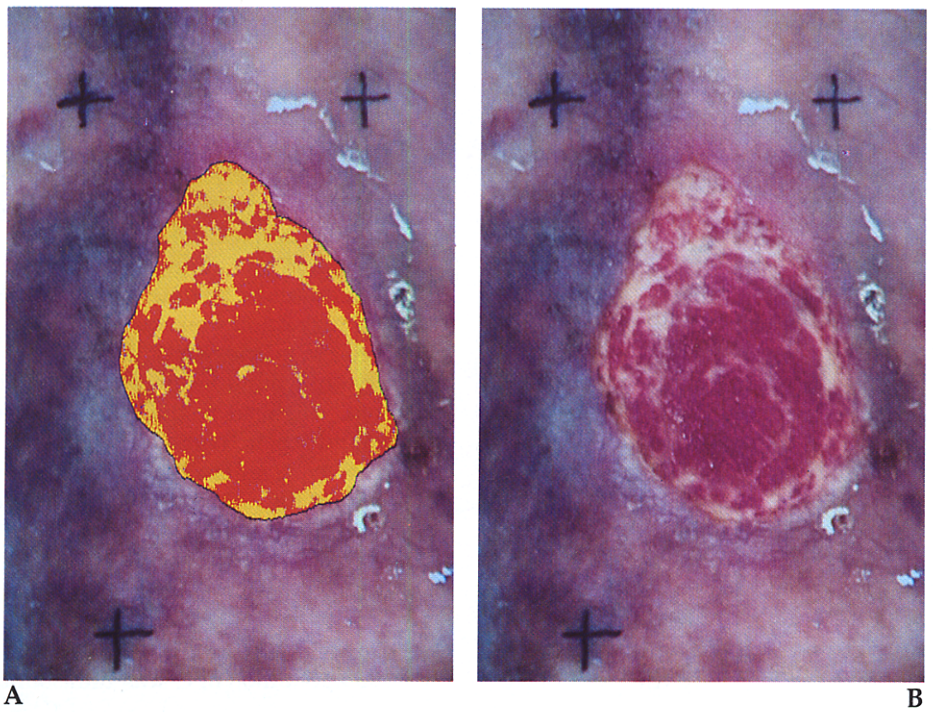
Measurement Procedures

After restoring the image from disk or tape, the ulcer outline is traced on the screen, using the digitizing tablet. Three orientation points around the wound are clicked on with the mouse. After the previously recorded horizontal and vertical distances between these orientation marks are entered into the computer, the red, yellow, black, and total wound areas are calculated in pixels, percentages, and square millimeters. It takes about 2 minutes to fully measure one wound. Figure 5A, photographed directly from the computer monitor, shows a typical venous leg ulcer. The red granulating wound area is clearly distinguishable but, because of the complex two-dimensional structure, difficult to estimate. Figure 5B shows how the areas are recognized by the computer system.

Accuracy, Objectivity, and Consistency

Important questions for the validity assessment of this computer method are whether the measurements are accurate, objective, and precise (reproducible).[20] The digital image analysis method is certainly precise: 15 repeated measurements of three wounds by two independent operators showed maximum differences of 1.83% in total wound surface and 0.53% for one of the individual components (yellow necrotic tissue). These differences were caused by slightly different line tracings. The accuracy is difficult to assess, as no other objective methods are available that can serve as a gold standard (in fact, this is the reason why this computer system was developed, to become a gold standard). Histologic examination of the entire wound is possible in animal studies, but is not helpful, because it is not proper recognition of the structures that is the issue, but their correct estimation in percentages. The only date to compare with are the visual assessments of trained clinical observers; however, it is unlikely that the results of visual assessments are accurate (correct) or consistent, as we have shown above. The first step in evaluating ulcers is to recognize which area should be called "necrotic" and which area "granulating." This is a matter of experience, but relatively easy to learn. The second step, to estimate the surface in per-

Figure 5. **(A)** *Computer image of a venous leg ulcer, photographed directly from the computer monitor. The red granulating wound area is clearly distinguishable but, because of the complex two-dimensional structure, difficult to estimate.* **(B)** *Same ulcer analyzed by the digital image analysis system. Total wound surface, 777 mm^2; granulating wound area, 75.5%; yellow necrotic area, 24.4%; black necrosis, 0%; unclassified area, 0.1%.*



A B

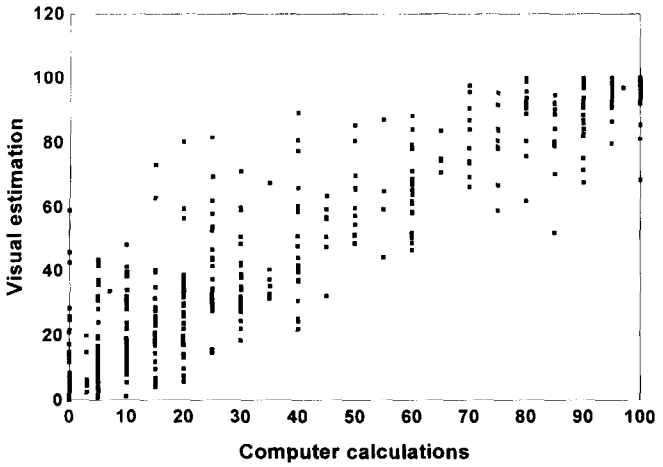


Figure 6. Correlation between the amounts of granulation tissue estimated visually and by computer image analysis in 532 experimental animal wounds. Correlation coefficient r = .945.

centages, is more difficult, because often there is a complex distribution of necrotic and granulating areas.

To investigate the correlation between visual estimation and computer analysis, 96 different experimental wounds were judged by one experienced investigator at regular intervals during treatment and later analyzed by the computer system. In total, 532 wounds were evaluated with both methods. The investigator (J.R. Mekkes) had 4 years of experience in estimating wound surfaces for clinical trials, in both human and animal studies. The correlation between the two methods was good ($r = .945$), but it appeared that small amounts of granulation tissue (below 20%) in an ulcer are underestimated by the human observer (Fig 6).

The method of creating the color definition tables could in theory be subject to observer bias. This can be minimized by asking other clinicians to select representative wound areas and by increasing the sample frequency. But even if there would be any bias, this does not influence the outcomes of clinical trials, because all wounds, including the placebo or control treated wound, are evaluated with the same definition table throughout the study. The actual measurements are fully objective, because they cannot be influenced by human observers. They can be carried out during or after the study. The study design is usually randomized, double-blind, and placebo controlled, but if blind application of study materials is impossible, digital image analysis makes observer-blind evaluation possible.

Discussion

The digital image analysis system described above is a precise and objective method for quantifying the debriding properties of wound care products.[21] The advantages of this system, compared with similar wound analysis systems described earlier,[22] are the possibility of checking the quality of the computer image immediately and of performing bedside measurements, if necessary; the absence of a photographic step; the possibility of obtaining exactly the same closeup as the day before; the flexibility regarding wound size; and, most important, the complete elimination of reflection by means of two Polaroid filters.

For a good interpretation of the results one should realize that removal of necrotic tissue during treatment does not instantly reveal granulation tissue, but it allows for the formation of granulation tissue. Once granulation tissue has been formed near the margin of an ulcer, it will be overgrown with epithelium. As a result, the total wound size will be reduced. This means that the relative amount of granulation tissue first increases, but then diminishes. Therefore, the best method for evaluating debridement is to measure the surface covered with necrotic tissue in square millimeters. During the first days of treatment the total wound size may increase as a result of the moisturizing effect of most debriding products.

The good correlation with the visual estimations of an experienced observer may suggest that computer measurements are not necessary; however, we have shown that human observations are subjective, imprecise, and inconsistent. With the digital image analysis method, double-blind or observer-blind trials, multicenter if necessary, are feasible.

Conclusions

All wound care products that are said to be suitable for wound cleaning should preferably be evaluated with an observer-blind, quantitative system, as described above.

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