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Original article

Computer-assisted visualization and quantitation of experimental gastric lesions in rats

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Abstract

Introduction: Photography is commonly used to evaluate macroscopic morphology of experimental gastric lesions. The conventional methods for quantitation of these lesions tend to be highly subjective, error-prone, and tedious. This study reports a simple and novel method for visualization of experimental gastric lesions by direct scanning of stomach samples and their quantitation by using computer-assisted image analysis. **Methods:** Gastric mucosal lesions were produced by indomethacin or ethanol in male Wistar rats. The stomachs were removed, opened along the greater curvature, and washed. The flattened stomach samples were sandwiched within a transparent plastic folder and scanned. The captured image was saved and subjected to lesion quantitation using a freely accessible image analysis program, Scion Image. A macro subroutine was optimized for automation of the entire procedure for ease in frequent applications. **Results:** The images obtained by direct scanning of stomach samples showed excellent morphologic visualization of gastric lesions. The standard spots having variable areas of same density, or same areas of variable densities, showed a direct relation with signal intensity (pixels). A linear correlation was observed between measured and actual values of the known standard areas. The representative samples of indomethacin- and ethanol-induced gastric ulcers showed the total lesion areas of 4.62 ± 0.74 and 169.26 ± 13.23 mm^2, respectively. **Discussion:** Direct scanning of stomach samples is a simple, cost-effective, rapid, and efficient procedure for morphologic evaluation of experimental gastric lesions. The output images of scanned stomach samples are superior to scanned photographs, and can be instantly used for quantitative assessment of mucosal injury using the image analysis program.
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Keywords: Computer application; Gastric ulcers; Imaging; Methods; Morphology; Quantitation; Rat; Scanning; Visualization

1. Introduction

The quantitative assessment of experimentally induced gastric lesions is a problematic and error-prone task due to their predominantly multiple and irregularly shaped occurrence (Parmar & Desai, 1993; Szabo et al., 1985). The simplest type of lesion index for quantification of stress- or chemically induced ulcers has been described as the cumulative length (mm) of all hemorrhagic erosions (Liu et al., 1998; Santucci, Fiorucci, Di Matteo, & Morelli, 1995; Trapkov, Boudunova, Burova, Filimonov, & Poroikov, 1997). For comparatively better approximation of total ulceration, the width of lesion has also been taken into account (ulcer index = length × width) (Watanabe et al., 2000). Several investigators have measured the area (mm^2) of gastric lesions with the help of squared grid under dissecting microscope, summed per stomach and used as lesion score (Naito et al., 1998; Suzuki, Araki, Komoike, & Takeuchi, 2000; Takeuchi, Okada, Ueshima, Ohuchi, & Okabe, 1993). Tracing of the outermost lining of acetic acid- or ethanol-induced ulcerated tissue on a transparency or glass plate and its copying on squared grid or graph paper has also been used to calculate the ulcer area (Shen, Koo, & Cho, 2001; Shin et al., 2002). Photographs of lesioned stomachs have helped in the usage of digital calipers (Santucci et al., 1995) and their enlargements for better visibility of indomethacin-induced tiny lesions (Joseph, Varela, Kanji, Subramony, & Mihas, 1999). A technical advancement based on microprocessor-linked planimetry with a stereomicroscope was introduced about two decades ago (Szabo et al., 1985) and was successfully applied for sensitive and accurate quantification of gastric lesions (Sun et al., 2000; Tepperman,

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Table 1
Sequential steps for quantitation of gastric lesions using the Scion Image program

Step	Menu	Task
1	File	Open
2	Process	Convert to gray scale
3	Process	Arithmetic, subtract
4	Options	Threshold
5[a]	Analyze	Set scale, 12 pixels = 1 mm
6	Analyze	Measure

[a] Refer to the Results section for details.

Vozzolo, & Soper, 1993; Wallace, Keenan, & Granger, 1990). However, this technique remained beyond the reach of small laboratories due to its high cost and instrumental sophistication, while many investigators still rely on conservative grading system for the quantitation of gastric lesions (Al Moutaery & Tariq, 1997; Joseph et al., 1999; Lee, Aldred, Lee, & Feldman, 1992; Schiantarelli, Cadel, & Folco, 1984; Valcavi et al., 1982).

In this investigation, an attempt was made to develop a simple, inexpensive, and rapid method for visualization and quantitation of experimental gastric lesions in rats. The approach is primarily based on direct scanning of stomach samples instead of conventional photography, whereas a freely available image analysis program was used for lesion quantification.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (six animals per group), fed on standard chow diet, were maintained in a temperature- and humidity-controlled room at 12-h light/dark cycles. All the animals were kept on 12 h fasting (with free access to drinking water) before administering the ulcerogens.

2.2. Induction of gastric lesions

Two types of commonly used ulcerogens including indomethacin (for tiny circular lesions) and ethanol (patchy bandlike lesions) were used for induction of experimental gastric ulcers. Indomethacin was suspended in 1% carboxy methylcellulose in water and administered by gavage at the dosage of 30 mg/kg body weight (Bhargava, Gupta, & Tangri, 1973). The animals were sacrificed 4 h after indomethacin administration. For producing ethanol-induced lesions, the animals were administered with 1 ml of 100% ethanol by gavage (Natale et al., 2001). The animals were sacrificed 1 h after ethanol administration. The stomachs were removed and opened along the greater curvature. After washing with saline, the specimens were subjected to morphologic visualization and lesion quantification procedures.

2.3. Image capturing for morphologic visualization

The stomach samples were flattened and carefully sandwiched between the two layers of a transparent plastic folder of A4 size (a commonly used stationary item). The specimens within the plastic folder were scanned using a scanner and the captured image was saved (TIFF format) in the computer hard drive. The plastic folder can hold several stomach samples that can be scanned at a time and stored as a single image. This master image can subsequently be partitioned into individual samples. The scanning procedure involves no additional setting or configuration, and can be performed on any scanner type. Five spots of known areas (1, 4, 9, 16, and 25 mm^2) drawn on a white paper were also scanned for calibration.

2.4. Quantitation of gastric lesions using Scion Image menu commands

The scanned image was subjected to analysis for the quantification of gastric lesions using the public domain image processing and analysis program developed at the National Institute of Health, USA. The PC version of this program (2.15 MB), known as Scion Image, is available on the Internet for free download from Scion (http://www.scioncorp.com) (Scion Image for Windows, Release Beta 4.0.2). The step-by-step procedure for quantitation of gastric lesions is given in Table 1. Briefly, the protocol is composed of six tasks that are sequentially performed as follows: opening of image file, image conversion to gray scale, subtraction of unlesioned area, thresholding, scale setting, and area measurement. The subtraction units of 125 for indomethacin-induced lesions and 150 for ethanol-induced lesions (Table 1) are not arbitrary and should be

Table 2
Macro programming for automation of steps involved in lesion quantitation

Macro subroutine

```
macro 'Compute Total Area [T]';
 begin
  ApplyLUT;
  AddConstant( – 125);
  AutoThreshold;
  SetOptions('Area');
  SetScale(12, 'mm');
  Measure;
  ShowResults;
 end;
macro 'Reset [R]';
 begin
  ResetCounter;
 end;
```

In line 4, 125 was used for indomethacin-induced gastric lesions, and 150 for ethanol-induced gastric lesions. A more appropriate value can be obtained by conducting a few trials for a particular lesion type. In line 7, the value of 12 is applicable only to images with × 4 magnification (refer to the Results section for details).

carefully used as they might change depending on sample type, image quality, and equipment used. An optimum subtraction unit can be simply obtained by conducting a few trials using a range of 100 to 150 with the increments of 10 units (or 5 for fine tuning) until the best-filtered image is obtained showing all the lesions without any background. While thresholding the image, watch the resulting output carefully to ensure the perfect retention of gastric lesions without any background noise. For quantitation of standard areas, the same procedure was adopted except the skipping of background subtraction as the standards were drawn on a white paper and there was no background color. It is also important to note that Scion Image only supports TIFF and BMP files, whereas compressed or JPEG files cannot be used with this program.

2.5. Quantitation of gastric lesions using macro programming

Scion Image offers the facility of 'macro' language for the automation of complex and repetitive tasks by just pressing an assigned key. Macros are extremely useful for execution of the same procedure (a set of commands) repeatedly, for example, quantitation of gastric lesions in large number of

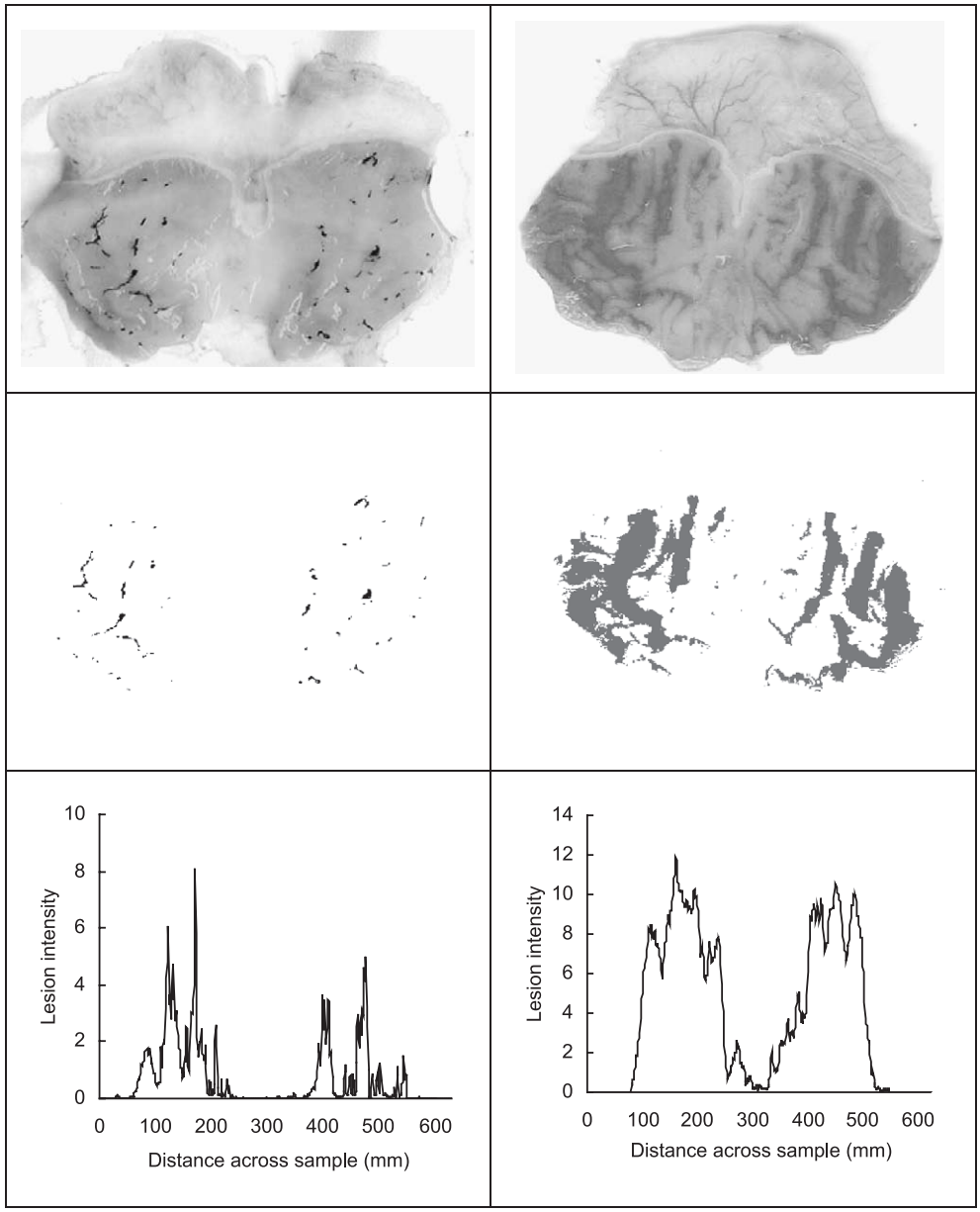

Fig. 1. Scanned images of indomethacin-induced (top left) and ethanol-induced (top right) gastric lesions in rats. Middle panel shows the same images after optimized background subtraction (125 for indomethacin- and 150 for ethanol-induced lesions). The line graphs (lower panel) show the profiles of gastric lesions along the planar axis.

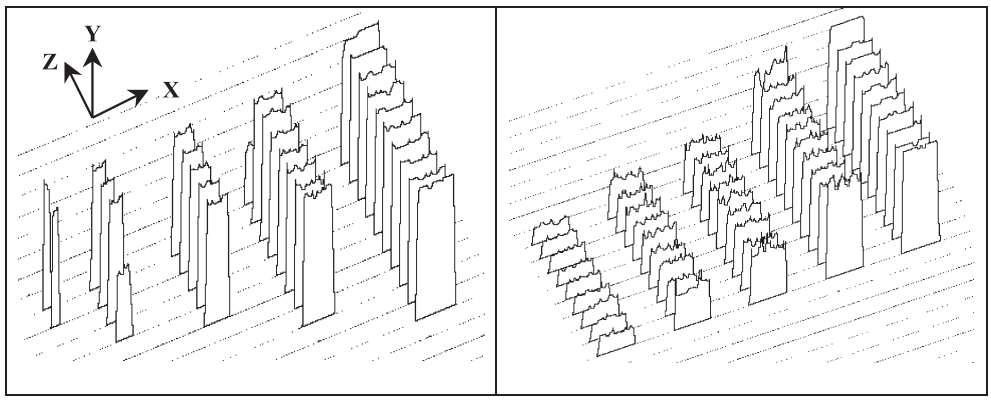

Fig. 2. Three-dimensional surface plots of known standards showing the effects of variable areas of same density (left: increasing number of stacks of same height) or same areas of variable densities (right: same number of stacks of increasing height). The *x*- and *z*-axes constitute the sample surface and the *y*-axis shows the optical density.

samples. An optimized macro (Table 2) for quantitation of indomethacin- and ethanol-induced gastric lesions can be used by exactly typing all the 14 lines on a notepad; saving on a computer drive and assigning to image analysis program. To activate the macro, it has to be loaded on Scion Image program from 'Load Macros' command within 'Special' menu; the appearance of macro's name in the list bar indicates its proper loading. Once a macro has been loaded, it will remain functional until replaced by another macro. Now open the image file and press 'T' to compute the total lesioned area in the stomach sample. For a precautionary measure, keep an eye on the filtered image to ensure an optimum background subtraction. Repeat these two steps (opening the file and pressing 'T') for other specimens, whereas pressing 'R' will reset the counter in the results window.

2.6. Manual quantitation of gastric lesions using grading system

For comparing the results of computer-assisted ulcer quantitation with those of commonly used grading system

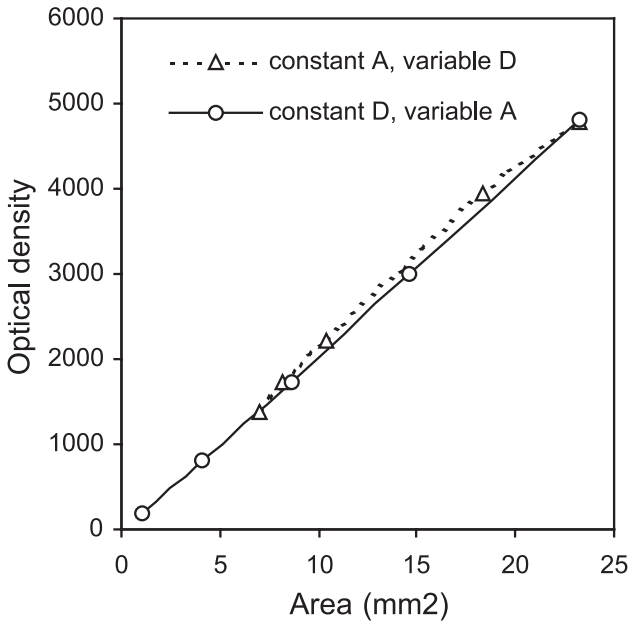

Fig. 3. Relationship between measured lesion area and optical density signal. Smoothed line shows the pattern of spots of constant density (D) and variable areas (A), whereas the dotted line shows the pattern of spots having same areas but variable densities.

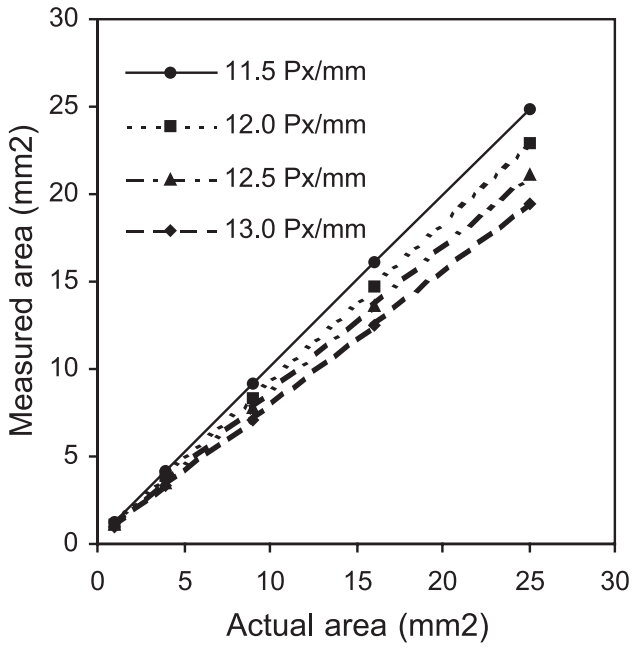

Fig. 4. Effect of pixel to millimeter ratio (Px/mm) on accuracy of area measurement. Usually, the Scion Image program automatically sets the Px/mm value according to variation between original sample size and the displayed image size (≈ 12 for × 4 and ≈ 6 for × 2 magnification). For a manual setting, the range of 11.5–12.0 is optimum for quantitation of gastric ulcers in × 4 magnified images (original sample to computer screen display) or half of these values for × 2 magnified images.

Table 3
Quantitation accuracy of the procedure for indomethacin- and ethanol-induced gastric lesions in rats

Gastric ulcer model	Lesion area (mm^2)					
	Zone 1	Zone 2	Zone 3	Zone 4	Total of all zones	Total measured area
Indomethacin	2.02	1.48	1.19	2.08	6.77	6.76
Ethanol	91.78	17.10	36.40	53.65	198.93	198.99

The areas of images shown in Fig. 1 were divided into four zones and the lesioned areas were quantified for each zone as well as the whole image to determine the accuracy of the procedure.

for indomethacin-induced (Valcavi et al., 1982) and ethanol-induced (Schiantarelli et al., 1984) ulcers, all the specimens were quantified by an experienced observer unaware of the sample identity. Each sample was graded triplicate in a blinded fashion and the mean value was reported.

3. Results

3.1. Morphologic visualization of gastric lesions

The images obtained by direct scanning of stomach samples are shown in the upper panel of Fig. 1. The original images were colored (not shown), while the clarity of lesions was perfectly retained even after their conversion to grayscale images. The morphological differentiation between indomethacin- and ethanol-induced gastric lesions can easily be observed by these images (Fig. 1). The gastric lesions shown in the original images have been filtered using an optimized background subtraction, and the resultant output corresponding to the lesioned area is presented in the middle panel of Fig. 1. Whereas the graphical profiles of both indomethacin- and ethanol-induced gastric lesions are given in the lower panel of Fig. 1.

3.2. Quantitation of gastric lesions

The association of signal intensity with the variable areas of same density and the same areas of variable densities is shown with the help of three-dimensional surface plots of known standards (Fig. 2). The effect of these two important determinants (lesion area and density) on optical density signal observed by the Scion Image program is shown in Fig. 3. A correlation between the observed and actual values of known areas is given in Fig. 4. Although Scion Image software can autodetect an optimum pixel to millimeter ratio (Px/mm ≈ 12 for $\times 4$, and ≈ 6 for $\times 2$ magnification from original sample size to image display on computer screen) for a particular image, this parameter can also be set manually for greater accuracy and/or in the event of unassigned Px/mm (Fig. 4). Table 3 shows the quantitation accuracy of this procedure for the measurement of indomethacin- and ethanol-induced gastric lesions in rat. A comparison between computer-aided quantitation of lesioned areas and the commonly used grading systems for measuring ulcer indices resulted in the correlation coefficients of .789 for indomethacin and .838 for ethanol-induced ulcers (Fig. 5).

4. Discussion

The results of direct scanning of stomach samples have clearly demonstrated its excellence in visualization of gastric images and presentation of lesion morphology (Fig. 1). This novel methodology not only significantly

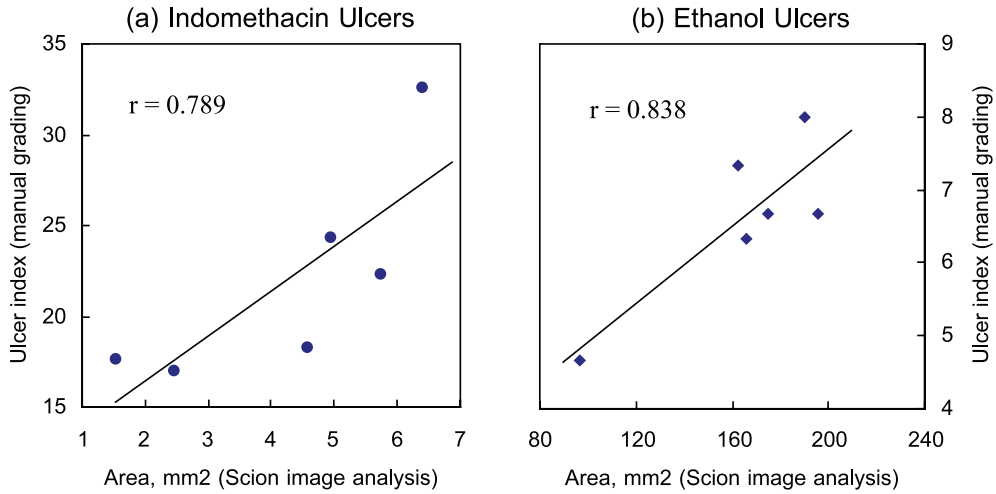

Fig. 5. Correlation between the procedure described in this study and the commonly used grading system for quantitation of experimental gastric ulcers in rats (six animals per group). Different grading systems were used for indomethacin- and ethanol-induced ulcers as described previously (Al Moutaery & Tariq, 1997; Schiantarelli et al., 1984; Valcavi et al., 1982). *r*, correlation coefficient.

shortens the time and efforts involved in lengthy photography steps, but is also cost effective as no additional equipment is required except the scanner which is an inexpensive device and available in almost all the laboratories. Even the scanner is also a prime requirement while working with photographed images, as they need to be converted into digital format prior to computer-assisted image analysis. The procedure of direct scanning of stomach samples does not involve any technical complexity or necessitates any special expertise, as the case of photomicrography. Nonetheless, the clarity of gastric lesions on directly scanned stomach samples (Fig. 1) appeared to be far better than photographed stomachs that we experienced earlier (not shown) and reported elsewhere (Liu et al., 1998; Naito et al., 1998; Tuncel, Erkasap, Sahinturk, Ak, & Tuncel, 1998). In fact, satisfactory photography of indomethacin-induced ulcers is difficult due to tiny spotlike morphology of such lesions, and an added help of microscopy is usually required to photograph these samples. Whereas direct scanning has been shown to possess a greater potential of capturing even minutely sized lesions. This study also enacts two additional models for presenting lesion morphology based on either lesion-only display that results after an optimum background subtraction (Fig. 1, middle panel) or its graphical presentation along the plainer axis (Fig. 1, lower panel).

For quantitation of gastric lesions, a public domain (freely accessible) image analysis program, Scion Image (Scion Image for Windows, Release Beta 4.0.2) was used to fulfill the need of small laboratories that cannot afford the cost of commercial software. The application of computer-assisted image analysis has long been utilized for the assessment of gastric mucosal damage (Arroyo, Lanas, & Sainz, 2000; Rainsford, 1989; Suzuki, Kagoshima, & Shimada, 1991; Umemoto et al., 1989; Vincze, Kiraly, Suto, & Mozsik, 1992). Whereas the alternate methodology based on conventional grading system tends to be highly strenuous due to inherent complexities of such scaling systems. Moreover, such types of lesion scoring are highly subjective, error-prone, and must be conducted in a blinded fashion by well-trained observers. On the other hand, computerized quantitation of gastric lesions has numerous advantages including time efficiency, sensitivity, accuracy, and reproducibility (Parmar & Desai, 1993; Umemoto et al., 1989).

However, it is critically important to standardize computer-based image analysis prior to its real application for quantitation of gastric lesions to ensure the accuracy of results. Both the area and the density (darkness) of lesions are directly related to signal intensity (Fig. 2). Scion Image program is capable of measuring both, area as well as its density. Whereas, the threshold command of the software automatically adjusts for the density and only the black pixels are integrated for area measurement resulting in nearly consistent relationship between measured areas and optical density (Fig. 3). The observed values of standard areas also showed a good correlation with the actual values (Fig. 4). The accuracy of this procedure was verified by computing the same areas repeatedly (data not shown) as well as by comparing the total lesion area with the sum of individual lesion areas of several partitions within the same image (Table 3). After getting acquainted with the methodology, the users can also apply one-step automation of entire procedure with the support of macro language (Table 2).

In conclusion, direct scanning of stomach samples provides a simple, rapid, and efficient methodology that might replace the costly and time-consuming photography for visualization of experimental gastric lesions. The scanned images can instantly be used for morphologic evaluation as well as for lesion quantification.

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