FULL TITLE *Histamine iontophoresis as in vivo model to study human skin inflammation with minimal barrier impairment: pilot study results of application of the model to a sensitive skin panel.*

SHORT TITLE *Skin responses to histamine*

AUTHORS

1. Denise Falcone, MSc[1]
2. Natallia Uzunbajakava, PhD[2]
3. Renée Richters, MD[1]
4. Peter C.M. van de Kerkhof, Prof, PhD, MD[1]
5. Piet E.J. van Erp, PhD[1]

CITY and COUNTRY

[1]Department of Dermatology, Radboud University Medical Center
Address: René Descarstesdreef 1
6500 HB
Nijmegen, The Netherlands
[2]Philips Research, Eindhoven, The Netherlands
Address: High Tech Campus 11
5656 AE
Eindhoven, The Nethelrands

CORRESPONDING AUTHOR: D. Falcone, MSc
denise.falcone@radboudumc.nl

JOURNAL Skin Pharmacology and Physiology

ABBREVIATIONS

CIE	Commission Internationale de l'Éclairage
CLAHE	Contrast-limited adaptive histogram equalization
CMOS	Complementary metal-oxide semiconductor
CV	Coefficient of variation
ROI	Region of Interest

MATERIALS AND METHODS

Acquisition set-up

Skin photographs were acquired using a commercially available single-lens reflex digital camera (Nikon D3200), equipped with a complementary metal-oxide semiconductor (CMOS) sensor (23.2 mm x 15.4 mm, 24.2 megapixels) and with a 18-55 mm zoom lens. To provide a shadow-free illumination, a 32-white light LED ring flash (Polaroid PLMRFN) with a color temperature of 5500 K was mounted on the camera. All experiments were performed under the same ambient light conditions (neon-lighting from the ceiling, curtains closed to avoid daylight). A circular sticker of known area was attached to the skin and used to estimate the area of the wheal-and-flare (in cm^2) by a mathematical proportion between the pixels belonging to the sticker and to the erythema [1]:

$$Area_{erythema} = \frac{\left(Pixel_{sticker} * Pixel_{erythema}\right)}{Area_{sticker}} \quad (1)$$

Skin photographs were taken while holding manually the camera perpendicular to the skin and the sticker, at a distant sufficient to image the wheal-and-flare reaction and the sticker. The following settings were used to take photographs:

a. Manual focus and mode (M) – aperture and shutting speed adjusted to match optimal exposure;
b. Focal length 55 mm;
c. ISO 200;
d. Image quality "FINE" – corresponding to JPEG images with a low (1:4) compression ratio;
e. Color space sRGB;
f. Ring flash in continuous ("Light") mode;
g. Preset manual white balance – obtained by acquiring an image of a white A4-sized sheet in the experimental conditions (ambient lighting and ring flash in continuous mode).

Contrast enhancement: histogram stretching and equalization

The histogram of a grey-level image X represents the number of pixels n_m for each grey level $m = 0, 1, \dots, L-1$ belonging to the image. In histogram equalization, the histogram is flattened across the dynamic range $[0, L-1]$ so that the cumulative histogram, or cumulative density function, approximates a linear ramp. Each pixel of the contrast-enhanced image Y can be expressed with the following formula:

$$Y_i = floor[(L-1)\sum_{m=0}^{X_i} P(X_m)] \quad (2)$$

Where n represents the total number of pixels in the image, $P(X_m) = \frac{n_m}{n}$ is the normalized histogram and $floor[\,]$ rounds down to the nearest integer.

In histogram stretching, the histogram is stretched between a minimum and maximum grey level, mostly the dynamic range $[0, 255]$. Each pixel of the contrast-enhanced image Y can be expressed with the following formula:

$$Y_i = 255 * \frac{[X_i - \min(X)]^\gamma}{[\max(X) - \min(X)]} \quad (3)$$

Where $\min(X)$ and $\max(X)$ represent the maximum and minimum grey level in X, respectively. If $\gamma = 1$ the histogram is linearly stretched across the predefined dynamic range, while if $\gamma > 1$ the histogram is non-linearly stretched with greater emphasis on enhancing contrast of high grey levels.

Thresholding: Otzu's method and Isodata algorithm

Thresholding is the process of reducing a grey-level image into a binary image by selecting a grey level M so that all pixels with grey level $m \geq M$ are classified as belonging to the object of interest and all pixels with grey level $m < M$ are classified as belonging to the background. The selection of the most suitable threshold M can be improved by increasing the separation between peaks in histogram stretching or equalization. Two widely used approaches for thresholding are the Otzu's method and the Isodata algorithm. In Otzu's method [2], the threshold M is chosen by maximizing the variance (distance) between the object and the background. In the Isodata method [3], the threshold M is iteratively computed as the average between the average grey level of the background and of the object. The algorithm stops when the absolute difference between the thresholds M obtained in iterations k and $k - 1$ is lower than a predefined value ϑ.

Algorithm for segmentation of histamine-induced wheal-and-flare

The algorithm for erythema segmentation is based on the use of built-in functions implemented in Matlab (version R2013a, The MathWorks, Inc., USA). The first step consists in manually choosing a rectangular Region of Interest (ROI) containing the wheal-and-flare reaction. The ROI is converted from the RGB to the CIE Lab color space, in which color is expressed as a three-dimensional quantity defined by an L*-axis (brightness), a*-axis (red-green chromaticity) and b*-axis (yellow-blue chromaticity) [4, 5]. All subsequent steps are performed on the ROI representing a*, since erythema induces a significant increase in this component making it particularly suited for its evaluation [4-6]. The white point corresponding to the CIE standard illuminant "d55" is used in the conversion between RGB and CIE Lab [6]. As a pre-processing step, contrast-limited adaptive histogram equalization (CLAHE) is applied on small ROI regions (10 x 10 pixels) in order to decrease the occurence of

inhomogeneities in the background of the ROI. Subsequently, global contrast enhancement in the range $0-255$ and thresholding are performed. Six segmented binary images are obtained by combining the three histogram-based contrast enhancement techniques (histogram equalization and histogram stretching with $\gamma = 1$ and $\gamma = 2$) with the two thresholding methods (Otzu's and Isodata). The aim was to compare these combinations to find the most suitable for segmenting the histamine-induced wheal-and-flare. As a fourth step, post-processing on the binary images is performed: firstly, a median filter of dimension 10 x 10 pixels is applied to eliminate spurious pixels and smoothen the boundary of the segmented wheal-and-flare; secondly, empty regions inside the segmented erythema are "filled", to include pixels belonging to the centrally-developed edema to the total area interested by the histamine reaction. Because this segmentation approach is based only on the pixel intensities and is independent of the shape and configuration of the object of interest, pixels belonging to the borders or to outer regions of the wheal-and-flare could be segmented as well. This issue is solved in the last step by automatically selecting the biggest segmented area in the ROI, which corresponds to the total area interested by the histamine reaction.

In addition to manually select the ROI containing the histamine-induced wheal-and-flare, the user is asked to manually select another ROI containing the sticker. The segmentation of the sticker is performed to calculate the pixels belonging to it in order to apply (1) to the segmented wheal-and-flare. The steps of the algorithm are summarized in Figure 1.

Of note, the application of the algorithm is suitable when the flare surrounds the wheal in the characteristic wheal-and-flare reaction. In our experiments we noticed, however, that when the histamine reaction decreased (usually starting between 30 and 60 minutes after application), the flare decreased in extent and intensity, not necessarily symmetrically around the wheal. In addition, in most volunteers an irregularly dotted erythema pattern appeared in correspondence of the wheal. We propose therefore a variant of the algorithm in which no application of CLAHE in the pre-processing step nor filling and selection of the biggest area in the post-processing step are performed, the reasons being the irregularity in shape and the limited extention of the flare in later time points, not involving background inhomogeneities in skin color. Although the lack of selection of the biggest segmented area may result in the possible inclusion of spurious pixels belonging to outer regions, we noticed that the selection of a small ROI close to the flare could partially overcome this issue resulting in satisfactory results.

Data analysis

The algorithm was used to segment the wheal-and-flare reaction at 5 and 30 minutes after histamine iontophoresis. Since in most volunteers a clear decrease in the reaction was visible at 60 minutes after histamine iontophoresis, the variant of the algorithm was applied at this time point.

In order to test the accuracy of the algorithm, the wheal-and-flare reaction on the original ROI was segmented manually by pointing as many points as needed on its border [7]. The area of this polygon was calculated using (1) and used as reference to evaluate the accuracy using a border detection error coefficient, or XOR [8]:

$$XOR = \frac{\left(Pixel_{manual} \oplus Pixel_{erythema}\right)}{Pixel_{manual}} * 100 \quad (4)$$

Where $\oplus$ is the exclusive-OR operation giving the pixels for which the semiautomatically and manually segmented areas disagree. To test reproducibility, the algorithm and the manual selection were repeated three times for each image and the coefficient of variation (CV) was computed. Results are presented as mean ± SD.

Also the variant of the algorithm was tested three times on each image and the CV was computed. In this case, no manual segmentation was performed and results are qualitatively presented.

RESULTS

The algorithm was tested on all 18 volunteers at 5 and 30 minutes after histamine iontophoresis. The area, CV and XOR obtained with the six combinations are presented in Table 1.

The method based on histogram equalization ("Histeq") provided satisfactory results but was in general slightly conservative (i.e. it tended to segment a smaller area belonging to the wheal-and-flare reaction compared to the manual segmentation). In addition, at 5 minutes and in three volunteers, one to two repetitions of the algorithm provided a poor segmentation (i.e. the area belonging to the wheal-and-flare reaction was only partially segmented). At 30 minutes the performance of the algorithm improved, since the poor segmentation occurred in two volunteers and for just one repetition. The thresholding with Otsu's and Isodata methods yielded the same outcome. The method based on linear histogram stretching ("Imadjust$_{\gamma=1}$") provided the best results in terms of accuracy (hence the lowest XOR). However, at 5 minutes after stimulus, two to three repetitions in three volunteers for the Otsu's method and in one volunteer for the Isodata method erroneously segmented a part of the background of the ROI due to strong skin color differences between the sun exposed and non-exposed areas. At 30 minutes, this was the case in the same volunteers but occurred only in one to two repetitions. The method based on non-linear histogram stretching ("Imadjust$_{\gamma=2}$") yielded the worst performance at 5 minutes, in which poor segmentation occurred in five (Otsu's method) and three (Isodata method) volunteers (hence the highest XOR). The method improved at 30 minutes but, especially using Otsu's thresholding, it remained conservative and poor segmentation occurred in two volunteers. Representative segmentation results are shown in Figure 2.

The variant of the algorithm was tested on all 18 volunteers at 60 minutes after histamine iontophoresis. The area and CV obtained with the six combinations are presented in Table 2.

The method based on histogram equalization was the least robust to low-contrast erythema borders and tended to include spurious pixels belonging to the background, whereas the method based on non-linear histogram stretching was the most conservative and the most reproducible as demonstrated by the lowest CV. The method based on linear contrast enhancement had an intermediate behaviour. Thresholding with Otsu's or Isodata methods yielded equal results for the histogram equalization and similar results for the linear histogram stretching approaches, while for the non-linear histogram stretching approach, Otsu's method was more conservative than the Isodata. Representative segmentation results are shown in Figure 3.

TABLES

Table 1. Results of the algorithm for segmentation of histamine-induced wheal-and-flare at 5 minutes (n=18) and 30 minutes (n=18) after histamine iontophoresis						
Method	Area T_{5min} [cm^2]	CV T_{5min} [%]	XOR T_{5min} [%]	Area T_{30min} [cm^2]	CV T_{30min} [%]	XOR T_{30min} [%]
Manual	23.7 ± 5.7	3.5 ± 1.7	-	22.3 ± 5.1	4.5 ± 1.6	-
Histeq T_{otsu}	21.9 ± 5.5	7.0 ± 6.9	24.1 ± 8.5	20.3 ± 4.4	6.1 ± 3.2	21.3 ± 5.4
Histeq T_{iso}	21.9 ± 5.5	7.0 ± 6.9	24.1 ± 8.5	20.3 ± 4.4	6.1 ± 3.2	21.3 ± 5.4
$Imadjust_{\gamma=1}$ T_{otsu}	25.3 ± 6.9	4.5 ± 3.8	21.6 ± 4.7	23.7 ± 6.4	3.5 ± 3.4	20.2 ± 5.0
$Imadjust_{\gamma=1}$ T_{iso}	24.0 ± 6.3	3.3 ± 3.0	20.8 ± 5.5	22.8 ± 5.9	3.6 ± 2.5	19.2 ± 4.0
$Imadjust_{\gamma=2}$ T_{otsu}	20.1 ± 6.3	5.5 ± 5.2	28.0 ± 11.5	20.8 ± 6.0	5.2 ± 6.2	21.9 ± 7.3
$Imadjust_{\gamma=2}$ T_{iso}	21.1 ± 6.4	4.7 ± 4.1	26.4 ± 10.8	20.6 ± 5.0	4.2 ± 3.5	20.3 ± 5.3
CV = coefficient of variation; Histeq = histogram equalization; Imadjust = histogram stretching; Iso = Isodata method; Otsu = Otsu's method.						

Table 2. Results of the variant of the algorithm for segmentation of histamine-induced wheal-and-flare at 60 minutes (n=18) after histamine iontophoresis		
	Lower back	
Method	Area T_{60min} [cm^2]	CV T_{60min} [%]
Histeq T_{otsu}	9.9 ± 4.3	10.4 ± 9.6
Histeq T_{iso}	9.9 ± 4.3	10.4 ± 9.6
$Imadjust_{\gamma=1}$ T_{otsu}	9.0 ± 5.4	8.8 ± 10.1
$Imadjust_{\gamma=1}$ T_{iso}	9.3 ± 4.7	8.3 ± 8.0
$Imadjust_{\gamma=2}$ T_{otsu}	7.1 ± 5.1	4.7 ± 6.3
$Imadjust_{\gamma=2}$ T_{iso}	8.0 ± 4.8	5.0 ± 7.5
CV = coefficient of variation; Histeq = histogram equalization; Imadjust = histogram stretching; Iso = Isodata method; Otsu = Otsu's method.		

FIGURES

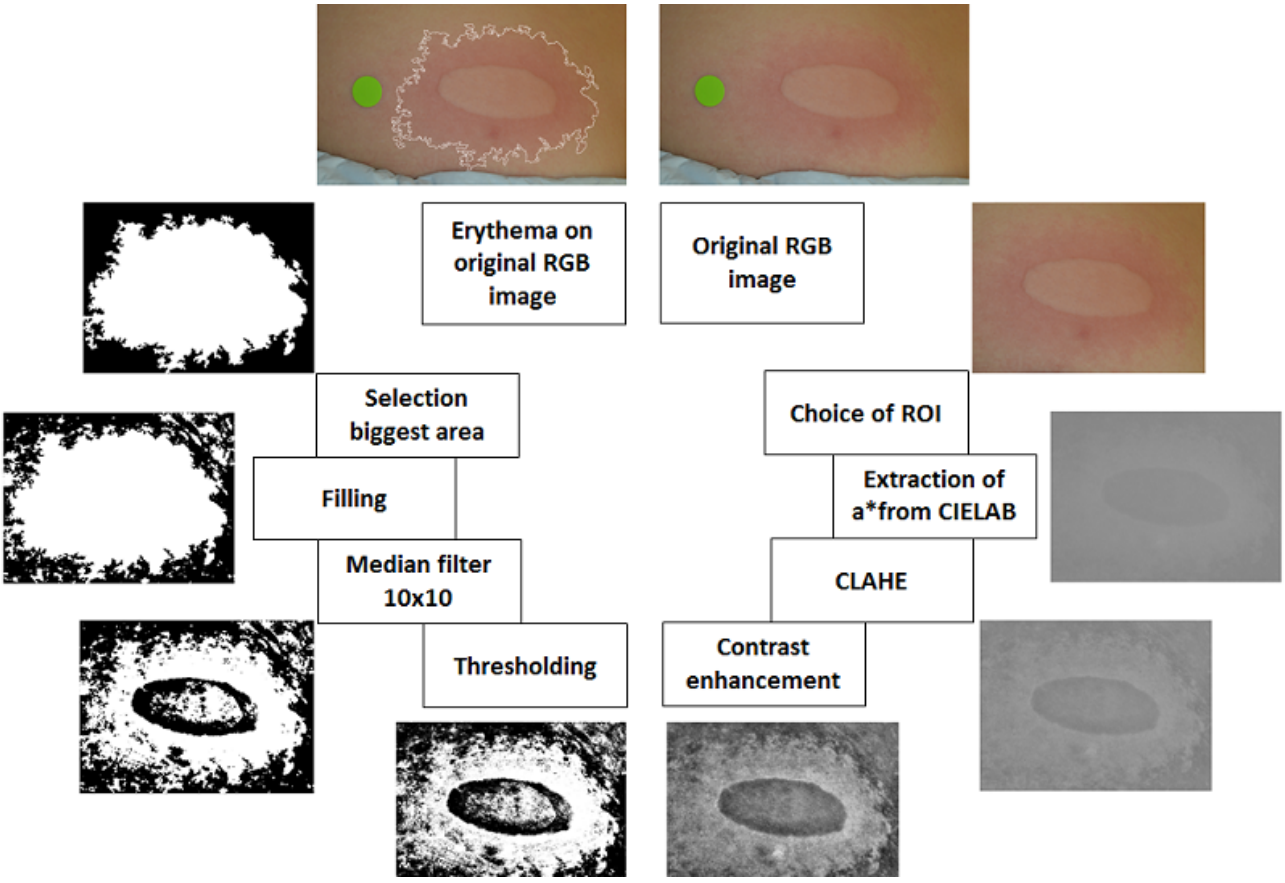

Figure 1. Algorithm for histamine-induced wheal-and-flare segmentation consisting in histogram-based contrast enhancement and thresholding. All steps are implemented in Matlab (The Mathworks, Inc., USA)

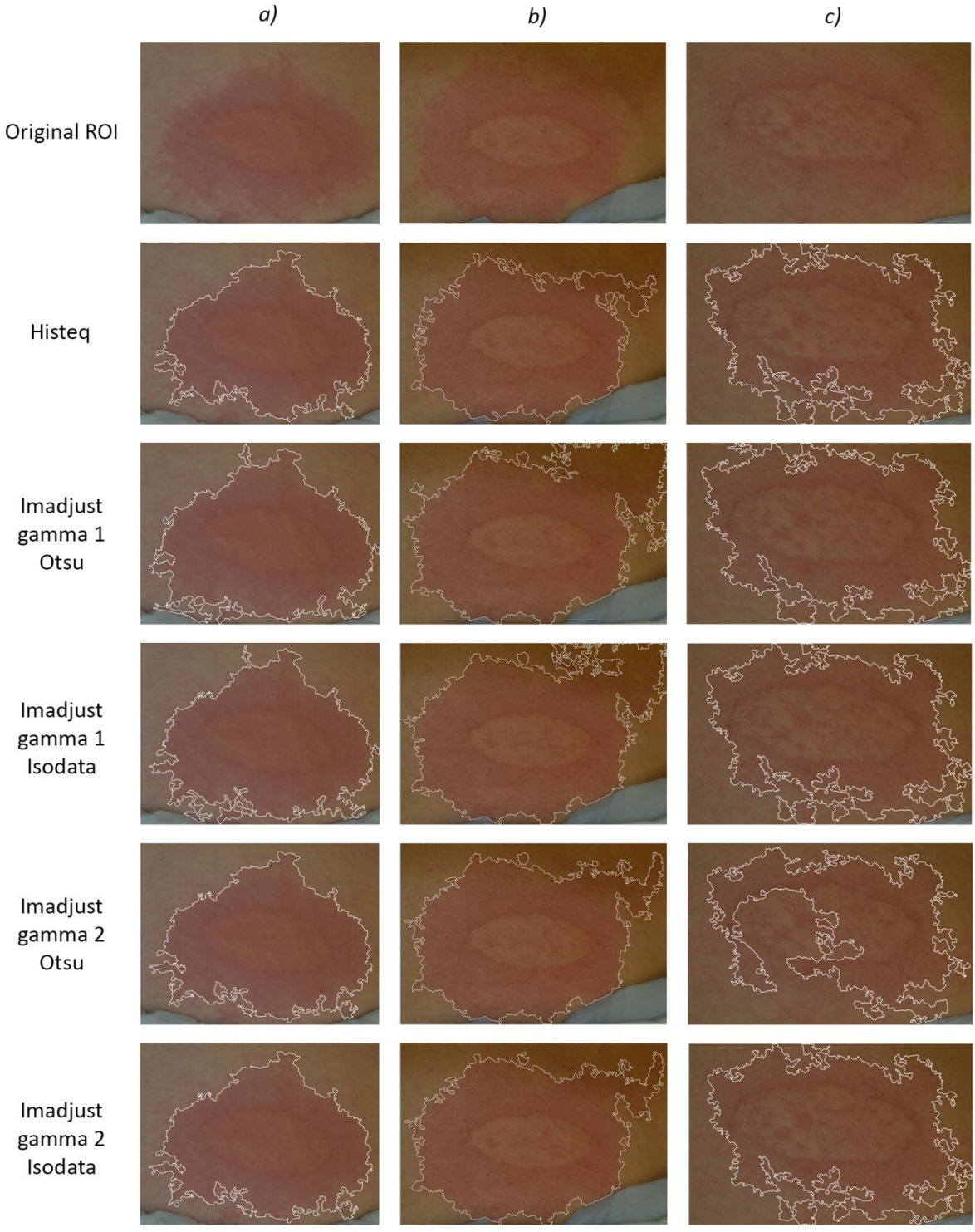

Figure 2. Representative results of the algorithm for histamine-induced wheal-and-flare segmentation obtained in images taken on the buttock of three volunteers (a-c) after histamine iontophoresis. a) Good segmentation results obtained in all six combinations of histogram-based contrast enhancement and thresholding. b) The linear histogram stretching methods erroneously selected an area belonging to the background because of strong inhomogeneity in skin color. c) Poor segmentation results obtained by the non-linear histogram stretching method with Otsu's thresholding

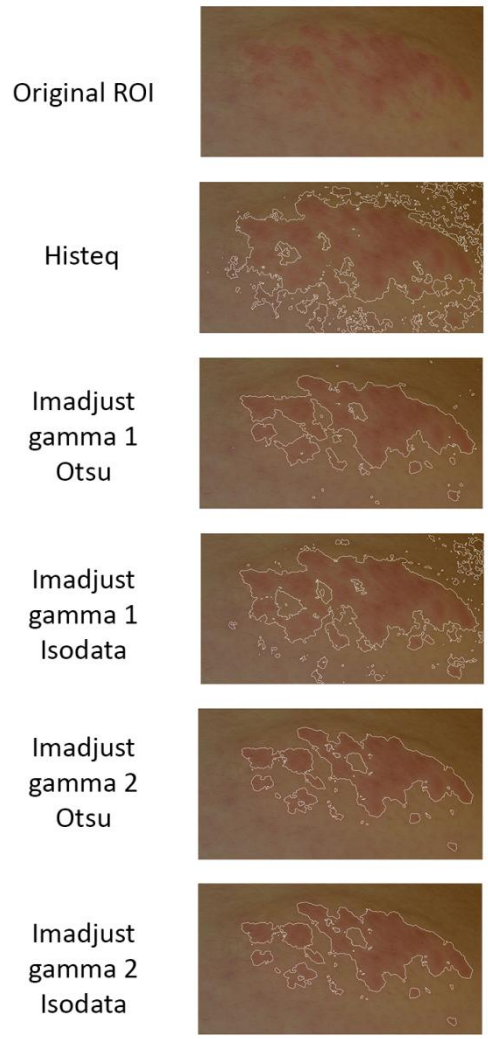

Figure 3. Representative results of the variant of the algorithm for histamine-induced wheal-and-flare segmentation obtained in images taken on the buttock at 60 minutes after histamine iontophoresis. The surrounding flare has faded and there is the appearance of an irregularly dotted erythema pattern in correspondence of the wheal. The non-linear histogram stretching methods show the most conservative results with the least influence of spuriois pixels belonging to the background

REFERENCES

1. dos Santos RV, Titus RG and Lima HC, *Objective evaluation of skin prick test reactions using digital photography.* Skin Res Technol, 2007. **13**(2): p. 148-53.
2. Otsu N, *A threshold selection method from gray-level histograms* IEEE Trans SMC, 1979. **9**: p. 62-66.
3. Ridler TW and Calvard S, *Picture thresholding using an iterative selection method.* IEEE Trans SMC, 1978. **8**: p. 630-632.
4. Takiwaki H, *Measurement of skin color : practical application and theoretical considerations.* J Med Invest, 1998. **44**(3-4): p. 121-6.
5. Pierard GE, *EEMCO guidance for the assessment of skin colour.* J Eur Acad Dermatol Venereol, 1998. **10**(1): p. 1-11.
6. Nischik M and Forster C, *Analysis of skin erythema using true-color images.* IEEE Trans Med Imaging, 1997. **16**(6): p. 711-6.
7. Roullot E, Autegarden J-E, Devriendt P and Leynadier F, *Segmentation of erythema from skin photographs for assisted diagnosis in allergology*, in *Pattern Recognition and Image Analysis*, S. Singh, Editor. 2005. p. 754-763.
8. Celebi ME, Iyatomi H and Schaefer G, *Contrast enhancement in dermoscopy images by maximizing a histogram bimodality measure*, in *Proceedings of the 16th IEEE international conference on Image processing*. 2009: Cairo, Egypt. p. 2601-2604.