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# Skin prick test digital imaging system with manual, semiautomatic, and automatic wheal edge detection and area measurement

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**Abstract.** A novel biomedical instrument for supporting the physician in Skin Prick Test analysis was designed, developed, characterized, and is now ready for clinical trials. Skin Prick Test is the gold standard front-end analysis for diagnosis of allergies in human subjects. The forearm skin is punctured with different allergens and the resulting reaction wheals are analyzed and compared to standard reaction, with larger areas corresponding to stronger allergy to the specific allergen. The wheals inspection and allergy diagnosis are performed, visually and subjectively, by the Medical Doctor. This procedure is laborious and somehow unreliable, being subject to variability both intra- and inter-operator because the doctor subjectivity in detecting and measuring the wheals is significant. Registration of the exam result is rarely available in a digital format, useful for data saving, transmission, retrieval and comparative analyses. Many of the above criticalities of the actual Prick Test manual practice are addressed and resolved by the proposed biomedical instrumentation that makes use of digital image-processing and data storage. In this work, we present a prototype of wheal measurement system, designed, developed and characterized to specifically measure geometry

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and areas of the allergic reaction wheals in Prick Test clinical exams. After software developments from previous version of the instrument, the wheal-meter now allows manual, semi-automatic, automatic, and fully-automatic operating conditions always providing digital exams output.

**Keywords** Biomedical image analysis · Skin Prick Test · Digital exams, wheal measurement · Image reconstruction · Allergy

## 1 Introduction

The Skin Prick Test (SPT) [4, 5, 8] is one of the most common clinical methods to diagnose allergies and it has been carried out in a similar and laborious manner over many decades. Despite its wide spread, relative simplicity, and low cost, the reading of SPTs is not yet analytically standardized and can not reach high repeatability in its clinical results. The lack in repeatable measurements is inherent in the actual analysis of the wheals geometry and size, which is performed manually by the physician and thus with a significant amount of subjectivity. In fact, the allergist needs to locate reaction spots on the skin of the patient and has to determine their geometry by visual inspection and personal interpretation of their geometrical characteristics (*i.e.*, diameters and areas). The basic idea of this work is to provide a measurement method and an engineered instrument for automated detection and dimensional analysis of the wheals, by digital imaging of the skin surface. The output of the proposed machine and image processing algorithms will provide for better standardization, practical save/retrieve/exchange of clinical data, and finally comparisons of medical results from SPTs.

Previous research, conducted by other groups, addressed different methods for process automation in measuring the allergic reactions in an SPT. To this purpose, different systems and algorithms based on multiple technologies were studied and developed. An excellent review of different solutions proposed for automatic wheal measurements in Prick Tests is found in [6]. Depending on the technology used for wheal measurements, we can distinguish in methods and systems based on: 1) 2D scanners; 2) blood flow analysis; 3) skin impedance measurements; 4) thermal imaging; 5) digital photography; 6) 3D scanners (see Ref. [6] for more details on these techniques).

Our work is aimed at the development and testing of a simple and inexpensive biomedical instrumentation (http://forimages.com/ponfometro.html) named "digital wheal-meter". The procedure is based on the following steps: a) uniform lighting and direct photography of the treated area, b) digital image pre-processing and storage, c) wheals detection and contouring; d) quantitative geometrical analysis of the wheals; e) creation and saving of a digital test report (Prick Test result). The novelty of the method and machine here proposed is that wheals contouring can be performed both by manual intervention from the allergist -in order to preserve the possibility of human operator contouring specific wheals based on the "subjective" Medical Doctor experience- and also by automatic image detection and analysis, in order to achieve repeatable and much faster "objective" measurements. This is a novel and versatile approach when compared to standard manual SPT analysis [1] or to fully automatic measurement of skin wheals [10]. It is the Author's opinion, also confirmed by interviewed clinicians, that the availability of such mixed-mode-operating machine can be of great use to Medical Doctors performing SPTs.

## 2 Traditional wheal-measurement proceudure

In an allergy test based on the Prick Test method [2, 7, 9], the patient forearm is exposed to a set of allergens. Each allergen is first deposited as a drop onto the skin, which is then lightly carved with a pricker by the doctor performing the test. A typical waiting time of 15 min follows and then the skin reaction to the different allergens is evaluated and “rated” by the physician [7], usually noting on a paper sheet the “values” of different wheals observed by visual inspection. See Fig. 1 for a typical test condition, patient forearm preparation, and results (wheals) after puncturing/pricking with different allergens. Noted values can be geometrical parameters of the wheals —*e.g.* areas or diameters— to be compared to the corresponding values for the “standard” skin reaction to histamine. They can be directly


**Fig. 1** **a** patient forearm preparation for an SPT on the forearm: different pen-written names note the different allergenes and refer to different regions of the skin exposed to different allergens. The skin reactions after pricking with 9 different allergens can be seen. The top part of the figure refers to “Neg”ative amd “Pos”itive control values. **b** another example of patient forearm showing 8 wheals pen-numbered by the physician

expressed as subjective evaluations, personally given by the physician, in a “plus-levels scale” with values [null (−), +, ++, +++, ++++] indicating from a negative to a very positive reaction of the skin to the specific allergen. Modern research activity is moving toward digital imaging for precise and repeatable wheal size measurement [3, 12].

## 3 Novel wheal-measurement instrumentation and imaging procedures

The basic idea presented in this paper is the development of a versatile machine for wheals detection and measurement in a Prick Test, capable of different operating conditions ranging from manual to fully-automatic. We propose a relatively simple and inexpensive instrument (http://forimages.com/ponfometro.html, [11]) based on an ergonomic plastic enclosure, to fit the patience forearm and uniformly light the skin surface. A commercial tablet is integrated in the top part of the machine, for digital image acquisition and processing as well as digital exams storage and retrieval. Our goal is reducing the influence of visual judgment from the physician in the wheals measurement procedure and hence increasing repeatability and standardization of the SPT results. Using digital storage and processing of the forearm image after exposing to allergens, the proposed method also allows for objective data digital-archiving and simple transmission and comparison of results from different exams performed on the same patient (*e.g.* very useful to evaluate the effect of a therapy) or on different subjects (to compare results between different sets of population or between subjects having specific histories or living conditions). A photograph of the novel digital wheal-meter is shown in Fig. 2. The plastic box consists of a cylindrical enclosure, to precisely host the patient forearm, which is internally lit by halogen lamps providing uniform illumination of the skin. On top of the box a tablet PC is used for imaging and data analysis and exams storage.

Before insertion in the machine, the patient forearm is prepared by sticking on it an adhesive paper “ruler” (see Fig. 3) which is used to guide the physician in correctly placing the allergens in the correct zones –center of each semicircle of the ruler corresponding to a different allergen– before the skin puncturing. The calibrated ruler is also used to provide dimensional calibration, from pixel measurements into millimeter measurements, to the images taken by the machine. The different test areas, see Fig. 3, are spaced 25 mm apart one from the other on two parallel lines along the forearm direction and the total horizontal length of the ruler is 150 mm from the centers of the 1st to 7th test area (both on the upper and lower test lines). The vertical distance between correspondent spots on the two horizontal lines is again 25 mm. The total width of the paper ruler, from left to right external borders (black areas) is 250 mm, whereas its height is 30 mm. Image correction for the forearm curvature is performed by proprietary software using the rules corners and black/white calibration squares as fixed points for a theoretically flat-surface image. The two righter most puncturing areas are dedicated to the histamine (“i”) and negative control (“cn”) tests. After removal of the adhesive center part of the ruler, see Fig. 2b, only the ruler borders -left and right calibrated square boxes- will remain attached on the patient skin for the digital photo and data processing.

The proposed procedure for wheals detection by digital imaging is divided into 4 steps:

1) arm *preparing* (cleaning, ruler placement, allergens disposal, puncturing, inner-ruler removal, 15 mins waiting time);
2) arm insertion in the machine, uniform lighting, and digital *photography* (see Fig. 2b);

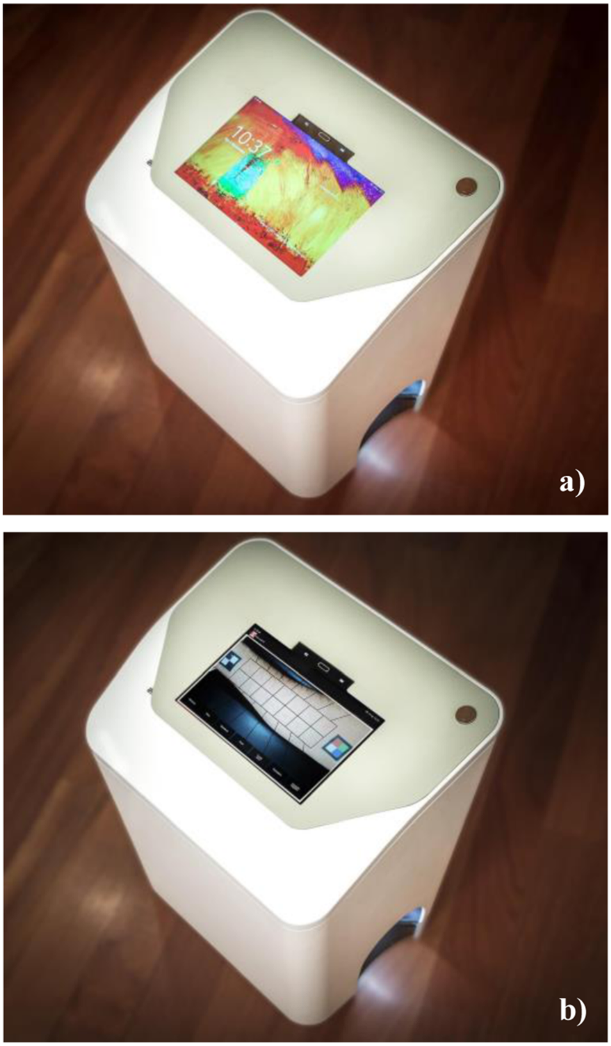


**Fig. 2** The wheal-metering machine and side view of the lit cylindrical enclosure: **a** tablet at start up of the procedure; **b** tablet showing the photograp of a patient forearm and the retcule used for wheals measurements

3) mapping of the ruler corners and *identification* of different wheals contours on the digital image;
4) digital *processing* of different wheals geometry and extraction/recording of digital test results.

The first step does not differ from the *standard procedure for patience preparing and puncturing* commonly performed by the physician, except for the placement of the paper ruler on the patient forearm to guide doctor's puncturing in predefined areas and to allow for image curvature correction in the next processing phase.

The second step is specific of the proposed method and machine: it allows for *repeatable digital imaging and storage* of the patient forearm skin photography, showing allergic

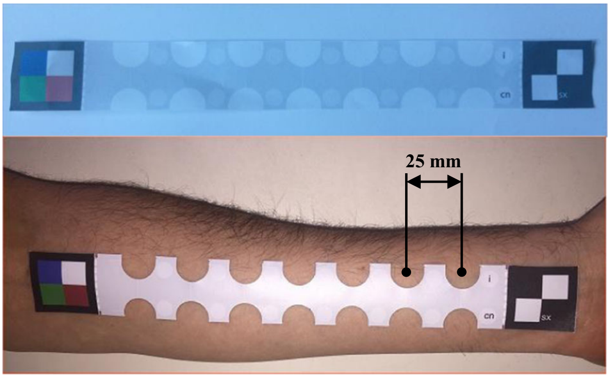


**Fig. 3** Top: adhesive-paper ruler of overall dimensions 250 mm × 30 mm between the ruler's corners to be soft-glued to the forearm of the patient. Bottom: photo of a patient forearm after removing the center part of the ruler and pricking the different test areas

reactions after the Prick-Test puncturing. Forearm curvature correction is performed by proprietary software embedded in the tablet computer.

The third step includes different possible procedures and algorithms for *wheals identification and contouring*. The significant novelty of the proposed wheal-meter is that this very delicate operation can be performed in a variety of different ways with different levels of human intervention. The wheal detection and measurement, and thus the final digital result of the exam, can be performed either with some manual intervention by the medical doctor in the wheals location and contouring (manual and semi-automatic procedures) or by automatic software detection of the wheals (automatic and fully-automatic procedures). All of these different procedures will be described in the following but we can start noticing that the final fully-automatic procedure works without any human intervention.

The fourth step performs *digital analysis of wheal areas* and it is in any case performed by the machine software, independently from the specific method (manual, semi-automatic, automatic, or fully-automatic) by which the wheals identification and contouring has been performed. In the simpler case of manual reconstruction, the digital wheals analysis gets as input the digital contours provided at the end of step three and provides as output the measured values of wheals areas, their ratios to the negative histamine area, and the corresponding SPT results. The same operations are performed also by the other three more automatic wheal identification and reconstruction and metering procedures but with less and less human intervention. All allergy test results produced by the machine are available as full-digital exam outputs (original image, processed image, wheals areas and areas ratios). The traditional exam results, on a plus-levels scale, are also provided. All data and photos are saved in the patient exam file (under patient name and date of the exam), available for future retrieval, analyses, and intra- or inter-comparisons.

In the Author's opinion, also shared with the interviewed clinicians, the possibility of performing different kinds of wheal measurements (manual, semi-automatic, automatic, and fully-automatic) using the same machine is an important novelty. This novel biomedical imaging machine will anyway provide for full-digital test results, offering the significant advantage that, from time to time and depending on the test conditions and allergic results, the physician can decide which of the four available measurement procedures should be used (if not more than one together).

The manual wheal detection and measurement allows the physician to fully exploit his decisional ability -based on his medical experience- in contouring each of the wheals that appear in the digital image of the patience forearm. The result of each hand-made contouring –by pinpointing the wheal corners on the screen using the tablet pen- is promptly visible on the screen and it is superimposed to the original wheal image. In this way, the doctor can decide whether to accept his contouring as just drawn or better refining it by repeating the manual contouring operation. Only when all the wheals appearing in the digital photography have been contoured by polygons, in a way that is satisfactory for the doctor, the tablet PC software will process the contoured wheals and to extract their individual areas. The same software will also compute the percentage ratio of each wheal area divided by the area of the reference wheal corresponding to the histamine chlorhydrate exposure, used as a standard positive reaction. The "histamine" puncturing area is kept in the upper-right part of the puncturing zones, as can be seen in Fig. 3, and the corresponding reaction wheal is used as a reference standard for the specific patient and test performed. Based on the calculated areas ratios, expressed in percentage to the histamine area, the software will real-time produce and display the test result also using the common plus-levels scale (see Table 1). This manual procedure for wheals measurements trades between the technical disadvantage of subjective analysis and results -operator-dependent and less repeatable- and the clinical advantage of exploiting the physician experience for the evaluation of wheals extensions and allergic reactions. The proposed manual procedure is yet obtained relying significantly on the physician experience but, compared to a fully-manual-and-analog traditional exam, our method provides for rapid, efficient, accurate, and repeatable digital reconstruction of the wheals areas. Of course the manual contouring initially performed by the operator is not fully repeatable. This novel manual procedure with digital output allows for much more inter- and intra-operator repeatability, when compared to a typical Prick Test analysis and its old-style paper results. The proposed instrumentation always provides for promptly available test results in a fully digital format.

The importance of offering more and more automatic wheal measurements procedures (from semi-automatic to fully-automatic) clearly lies in allowing much more repeatable measurements on different wheals, leaving the possibility of choosing how much human intervention is used. By increasing automation in the procedure, each wheal is detected and measured automatically by the machine software, directly starting from the original digital image. Yet, more or less human intervention can be used in the software reconstruction of different wheals. The development and testing of automatic wheals detection algorithms was recently concluded. Accuracy of digital wheals detection and of their geometrical metering yet needs to be quantitatively evaluated. This will be the content of a future work where also comparisons will be carried out with traditional clinical results, obtained from expert allergists manually contouring the same wheals processed by the novel digital wheal-meter.

**Table 1** Dependance of the plus-levels scale for the tested allergen, with the corresponding test result (Neg. = Negative, Pos. = Positive), on the allergen wheal area ratioed to the histamine wheal area standar

| | | | | | |
|---|---|---|---|---|---|
| Area ratio | <25% | 25% to 50% | 50% to 100% | 100% to 200 | >200% |
| Plus-levels scale | null (−) | + | ++ | +++ | ++++ |
| Result | Neg. | Neg. | Pos. | Pos. | Pos. |

## 4 Manual image reconstruction

In the manual wheal measurement mode, the physician is asked by the machine to pinpoint on the tablet (typically using an high-precision sharp-pin tablet pen) the four corners of the calibration ruler which are shown in the photo retaining the ruler external black parts (see Fig. 2b). This allows for image curvature correction, due to human harm non-flatness and inclination with respect to the camera plane, and it also provides dimensional calibration of the scale, from knowing the geometrical size of the ruler and relating it to its pixel size in the image. After this image pre-processing, the manual mode of image contouring asks the physician to draw the contour of each wheal on the tablet touchscreen displaying the digital photo of the patient forearm. One by one for the different wheals, the allergist can decide for a simple contouring, using "circle" or "ellipsis" contour, where he has to pinpoint a circle radius or two ellipsis axes, respectively. More complex contouring is also possible in the form of a closed regular or irregular polygonal shape, and in this case the physician has to pinpoint the irregular borders of the wheal. After contouring of each of the wheals is done, and finally accepted (since non-satisfactory contouring can be repeated for further improvement), the operator will confirm on the tablet touchscreen that the manual contouring operation is concluded. The machine can then calculate for each contoured shape the corresponding area, being evaluated as pixels numbers, but also converted into $mm^2$ values thanks to the image calibration initially performed by pinpointing the ruler corners.

## 5 Manual reconstruction validation

In this Section we present the validation of the manual wheal reconstruction algorithm and image calibration by performing repeated (manual) area measurements on the same standard figures, of known geometry, including circles, ellipses, and squares. The figures used in the tests were chosen with different dimensions providing for "big", "medium", and "small" synthetic-wheal sizes, as indicated in Table 2.

Each of the figure dimensions was measured with a caliper of resolution 50 μm and hence with a quantization uncertainty ~15 μm. This uncertainty level is absolutely negligible compared to the required image reconstruction dimensional accuracy and repeatability. The different standard figures were positioned along the ruler inside the digital wheal-meter and, on the corresponding digital photo (see Fig. 4), each synthetic wheal was pen-pointing manually contoured by the operator and then measured by the tablet software.

**Table 2** Geometry and dimensions of the standard test figures

| Figure/size | Circle<br>diameter and area | Ellipsis<br>axes and area | Square<br>side and area |
|---|---|---|---|
| "Small" | 5 mm<br>19.63 $mm^2$ | 5&2.5 mm<br>9.82 $mm^2$ | 5 mm<br>25.00 $mm^2$ |
| "Medium" | 10 mm<br>78.54 $mm^2$ | 10&5 mm<br>39.27 $mm^2$ | 10 mm<br>100.00 $mm^2$ |
| "Big" | 20 mm<br>314.16 $mm^2$ | 20&10 mm<br>157.08 $mm^2$ | 20 mm<br>400.00 $mm^2$ |


**Fig. 4** Tablet display showing a photo of different standard geometrical figures placed within different predefined allergenes areas. At the leftmost and rightmost part of the picture the ruler borders are shown (left and right calibrated square boxes)

The tests performed with manual area reconstruction provided for error values limited in a range of ±10% from the true area value. This happens for all the test figures of Fig. 4 except for the small ellipsis where the error was significantly larger, up to 20%. However, this happens when considering a very small wheal, of $5 \times 2.5\ mm^2$ area in our case, which is not of clinical interest. In typical clinical practice, only wheals with diameter > 3 mm are considered. We must keep in mind that the error due to measurement procedure —figures geometry calibration and software area reconstruction after operator contouring— is limited to less than 1%, so the remaining contribution of the error is due to the human intervention in manual contouring of the synthetic wheals. The achieved results are repeatable, for the same geometrical figure manual contouring and reconstruction, keeping errors in most of the cases below ±10%. This repeatability capability is positively comparable with the one obtainable by visual inspection by the allergist of the wheals (estimated in the range of ±20% even for an experienced doctor).

## 6 Digital image pre-processing

In the automatic wheal measurement modes, the physician only has to take the photo of the forearm inserted in the machine whereas the tablet will perform image calibration and automatic detection of the wheals and their geometry. In particular, wheals are identified by digital image processing and then rapidly contoured and measured by the machine in a repeatable way. The area of each wheal is evaluated, in number of pixel units, and its percentage ratio to the histamine area is calculated and displayed in the test report together with the traditional plus-levels-scale evaluation of reactions to different allergens. The final Prick Test report, as it was happening with the manual procedure, will be available in fully digital form, including wheals areas, ratios, plus-levels-scale values, and full digital images (the original and its processing).

In order to perform the automatic wheal measurements, by means of semi-automatic or fully-automatic wheal reconstruction and analysis, some additional image pre-processing is required. This pre-processing is used to enhance the wheal borders and the wheals-to-background contrast. To explain how the proposed image pre-processing works we will refer to a specific original image of the whole patient forearm, showing different wheals (of different shapes, sizes, and color features) in the 2 × 7 puncturing grid of Fig. 2b. The original image of punctured forearm is shown in Fig. 5 where the highlighted top-right block contains the histamine reaction region and the corresponding standard positive wheal.

This particular region of the forearm image is also shown enlarged in Fig. 6 and it is the original image used for the pre-processing aimed at wheal detection and reconstruction. From the poor contrast in the original photo of Fig. 6 it is evident that direct manual/visual contouring of the wheal, without the aid of some image pre-preprocessing, is quite difficult. The result of fully-manual contouring can be ambiguous and non-repeatable, depending on the subjectivity of the human operator.

In order to pre-process the image of Fig. 6, pixel thresholding and decomposition in RGB (Red, Green, Blue) components were first attempted. However, this simple procedure was not effective in enhancing the wheal area and so image decomposition in the HSV (Hue, Saturation, and Value) color space was more effectively used. Figure 7 shows the result of this HSV image analysis on the same original image of Fig. 6. By direct comparison of the H, S, and V channels with the original image, it can be seen how the Hue channel is particularly representative of the wheal reaction area. Manual processing of the hue image, or other possible digital processing output, can be now left to the physician hand and expertise resulting in the so-called *semi-automatic* wheal reconstruction that will be described in Sect. VII. We can also decide to perform automatic processing of the pre-processed image of Fig. 7 (in its H channel), so that the wheal contouring and area extraction are performed by the software without "manual" intervention from the physician.

Automatic image reconstruction can be performed yet with some minimal human interaction, to asses, for the H channel, first an intensity threshold (below which the pixels are considered background) and then an area threshold (minimum area for wheal detection and contouring), and we will call it an *automatic* reconstruction, as described in Sect. VIII. Otherwise, we can let the software decide for proper values of the aforementioned parameters (intensity and area thresholds) so that the machine is providing *fully-automatic* detection and measurement of the different wheals and their areas, as described in Sect. IX.

A first alternative to the manual analysis of the wheals already presented in Sect. IV is to allow a semi-automatic processing of the forearm image. This semi-automatic procedure is done in three steps: 1. digital processing of the tablet-acquired image to provide the physician with a picture easier to understand; 2. intervention of the operator and software to identify the wheals borders; 3.

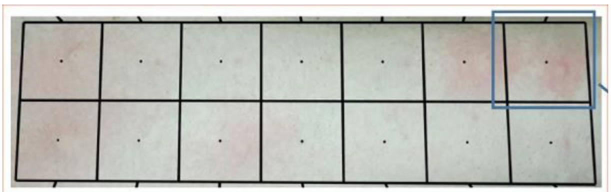

**Fig. 5** Forearm exposure grid and skin reactions in different puncturing regions. The highlied top-right block containins the hystamine reaction wheal

**Fig. 6** Zoomed original color image of the region with the histamine reaction and the corresponding "reference" wheal

calculation of the different wheals areas, areas ratios, and digital exam output. In the first step the original digital image is thresholded and processed to provide more isolated and clear-to-identify regions where the wheals are located. In the second step the pre-processed image is presented to the physician who can choose between three different options: a) manually identify and set the borders of the wheals on the pre-processed digital image (the semi-automatic procedure of Sect. VII); b) choose intensity threshold and area threshold unique for the whole forearm image or identify and mark on the image the regions where specific thresholds can be applied, always chosen by the operator, but potentially different for different regions of the image (this is the so-called automatic procedure of Sect. VIII); c) use unique and predefined values of intensity threshold and area threshold to extract the wheals borders from the whole forearm image and then evaluate the amounts of wheals areas falling in each of the 14 test zones, as referred (or normalized) to the reference histamine area, this latter assuming a normalized area value of 1 (fully-automatic procedure of Sect. IX). Since the new instrument is offering a variety of options of digital image reconstruction for step 2. of the wheal detection and metering -ranging from manual wheals analysis with no image preprocessing to fully-automatic image and wheals processing with digital exam output without any intervention from the physician- we mostly focused our attention on these two extreme working conditions leaving the intermediate options and refinements mostly to future studies, if necessary. Thus, the semi-automatic method, briefly described in the next paragraph, is here only proposed and it will be implemented in the digital wheal-meter only if the physician manual intervention (choice of wheals zones, contours, thresholds…) is really required. This could happen for special wheal detection and metering where the other more automatic procedures would result ineffective.

Instead, the automatic and fully-automatic procedures for image reconstruction and analysis -corresponding to choices b) and c) in step 2. of the wheal analysis- were successfully implemented and tested as described in the next Sections VIII and IX.

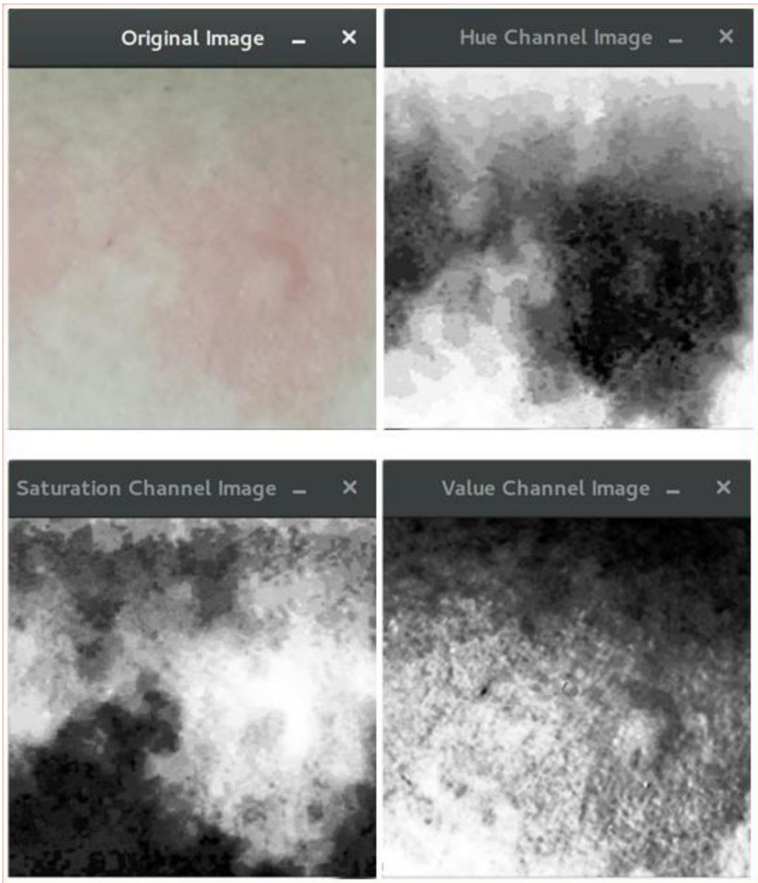


**Fig. 7** Original image and its Hue, Saturation, and Value channel decomposition

## 7 Semi-automatic wheal detection

The semi-automatic procedure is done in three steps:

1. digital processing, with some human intervention, of the original tablet-acquired image to provide the physician with a picture easier to understand. The operator must select the different zone of the image where the wheals are located. Digital thresholding is applied separately for each zone, with threshold values and cutoff area values properly chosen, time by time, by the operator. The output of this step is an image with much more clearly identified wheal borders now easier to be detected from the physician;
2. intervention of the physician to identify and contour the wheals borders on the digitally processed image available at the output of step 1.;
3. software calculation of areas and areas ratios for the wheals, selected and contoured by the physician in step 2., and finally digital exam output.

This semi-automatic procedure, apart for the deeper image processing involved, is not far from the manual procedure of Sect. IV and, compared to it, offers little improvements in terms of repeatability and accuracy of the detected and measured wheals, since the whole procedure is yet strongly affected by human decisions and subjectivity.

## 8 Automatic wheal detection

We start from the *Original Image* of Fig. 8a, representing the whole forearm (the same digital photo of Fig. 5), and we extract the hue channel for all the 14 areas of interest. To the resulting *Hue channel image* we then apply histogram normalization, scaling the grey-scale original to a full range 0–255 dynamic, using the min and max values detected in the hue image itself. The result of this normalization of the hue channel provides the *Hue Channel Image* depicted in Fig. 8b.

The next step is to apply an intensity thresholding to the previous hue channel image (here we chose an intensity threshold value of 97 out of 256 grey levels). This threshold level needs to be set manually by the operator, since its previously saved value is not necessary optimized for the specific image. Applying this threshold, we obtain a Black-and-White new image (see Fig. 8c, *Thresholded Image*) where the pixels within the wheals areas are given in White and the background pixels are given in Black. Digital contouring of the white-pixel areas in this last image is now performed by standard contour-tool software based on topological structural analysis [11]. The output of contouring algorithm gives the final *Contours Image* of Fig. 8d where only the contours having an internal area above a specific Area-threshold are shown. In Fig. 8d) the adopted area threshold value is 190, out of 256 grey levels, but this threshold level needs to be chosen by the operator. The resulting wheal areas (measured number of pixel counts within each wheal contour) is numerically given inside the contour of each wheal, being *de facto* digitally extracted from the Hue image. As it can be seen from this reconstruction and measurement method, also in comparison to the Hue (Fig. 8b) and Original (Fig. 8a) images, the wheal area is detected in a specific and objective way without significant manual intervention from the physician, apart from choosing two threshold values. As clearly visible from the results of Fig. 8, when some of the wheals are larger than one pre-defined square zone, two or more wheals can physically overlap one-on-the-other in the same image. In this case, a single larger area is calculated as the superposition of more than one wheal (see the left part of Fig. 8d).

Since we want each extracted contour and area value to correspond to a single wheal, some refinement analysis was added to measure individual wheal areas also in the case of large wheals. Instead of choosing unique thresholds (for intensity and area) valid for the whole image, in this case the operator can select different relevant areas, starting from the histamine region and moving to the other wheals regions. Each relevant rectangular area can be processed with its specific threshold, different from one region to the other, used to detect and contour and finally meter a single wheal inside each region. Figure 9a) shows the case of 5 specifically selected relevant areas, applied to the Original Image of Fig. 8a) without the black grid of 14-squares, in order to extract 5 non-overlapping wheals and the corresponding wheal areas. The areas are then normalized to the histamine wheal area and for each of them a simple numerical value is given (Fig. 9b) showing, how much larger/smaller each wheal is in comparison to the histamine standard reaction (in the top-right part of Fig. 9b).

The just described automatic wheal detection and metering is much less dependent on the manual intervention of the operator as compared to the manual or semi-automatic procedures where the operator needs to manually contour each wheal perimeter. However, also in this automatic procedure the operator intervention is required to "subjectively" choose two general threshold levels or different relevant areas with specific threshold levels valid for each area. This procedure is called

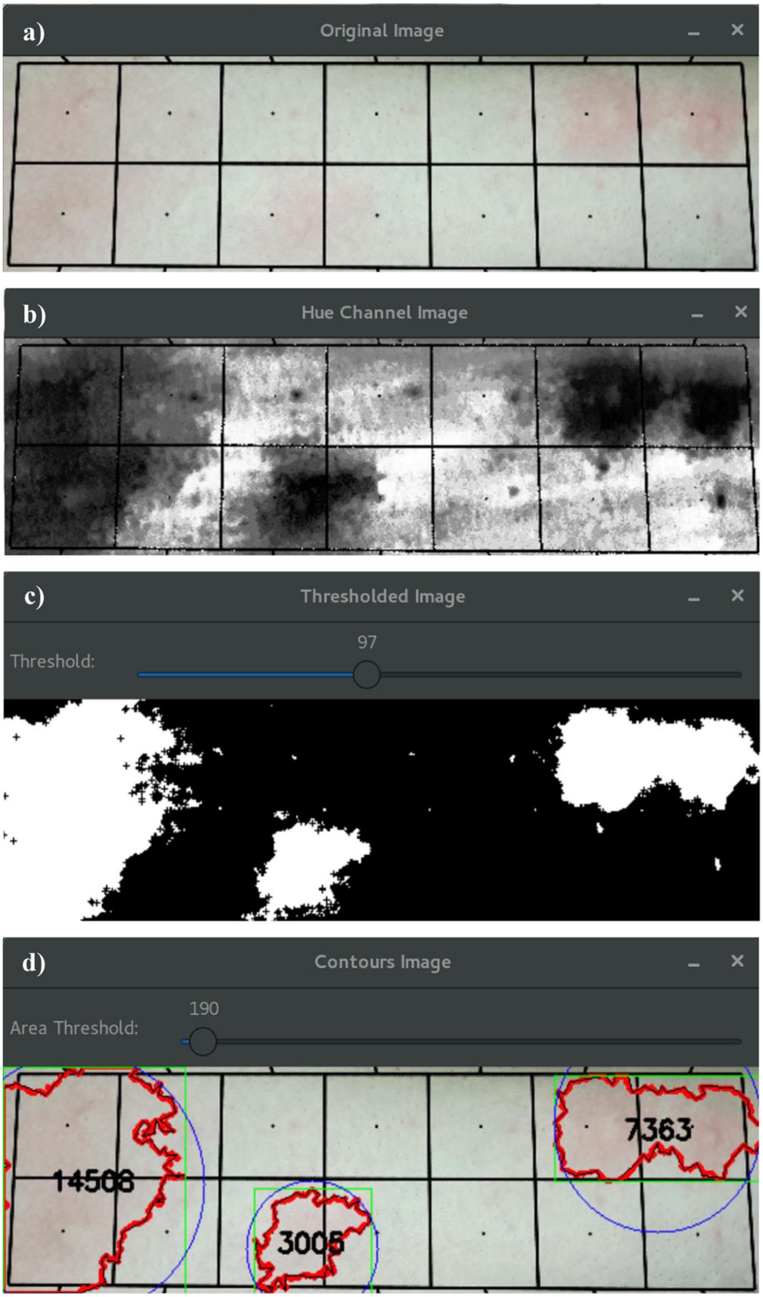


**Fig. 8** Images obtained during the automatic procedure for wheal detection: from *Original Image* (a) to the final *Contours Image* (d)

automatic since the operator does not have to manually pinpoint the borders of each wheal but still the residual human intervention in wheals detection and metering can provide for subjectivity in the measurements of wheal areas, *e.g*. when choosing different thresholds or different relevant areas.


**Fig. 9** Image processing of 5 different relevant areas (chosen by the operator) for individual wheals extraction and measurement

## 9 Fully-automatic wheal detection

The final option available for the image reconstruction is to achieve fully-automatic wheal detection and area metering with absolutely no intervention from the human operator. In this case, named as fully-automatic, fixed reading areas are used instead of the operator-defined relevant areas of Section VIII. The fixed reading areas are the rectangular regions of the ruler allowed for each puncturing and wheal development. Of course when the wheal reaction exceeds the boundaries of the rectangular region some errors result in the reconstruction. However, this is not a critical case since when a wheal size is exceeding its pre-defined region it means that a very positive reaction occurs and no precise detection of its area is needed. Using pre-defined reading areas makes the analysis much faster and extremely repeatable. Now, each of the 14 square puncturing areas of Fig. 8a is used as a fixed relevant area for measuring the wheal extension within the specific allergen relevant area.

The image processing algorithm can exploit the knowledge about the presence (histamine area) and absence (control area) of skin reaction to automatically determine a proper hue intensity threshold, independently from the human operator. To do this, the distribution of all the pixels in the two squares (*i.e.*, with histamine and with no allergen) is computed, and the software searches, as in the Otsu's method, for the threshold value that minimizes the intra-class variance (*i.e.*, the variance within the class) assuming only two classes exist (*i.e.*, standard reaction and no reaction). This hue threshold selection procedure has been proven to be simple and effective the for Caucasian skin color in the preliminary tests performed. However, it must be noted that shadows and uneven lighting conditions affect significantly this automatic procedure. Indeed, experiments on images captured outside the wheal-meter machine of Fig. 2 did not result in satisfactory outputs and repeatable measurements. For the area threshold a fixed value is used for the whole forearm image and this area was selected by considering the number of pixels in a square region and it has proven being quite discriminative rejecting spurious returns due to noise or skin defects. It might happen that more than one wheal gets segmented by the method: in this case the software selects the biggest one.

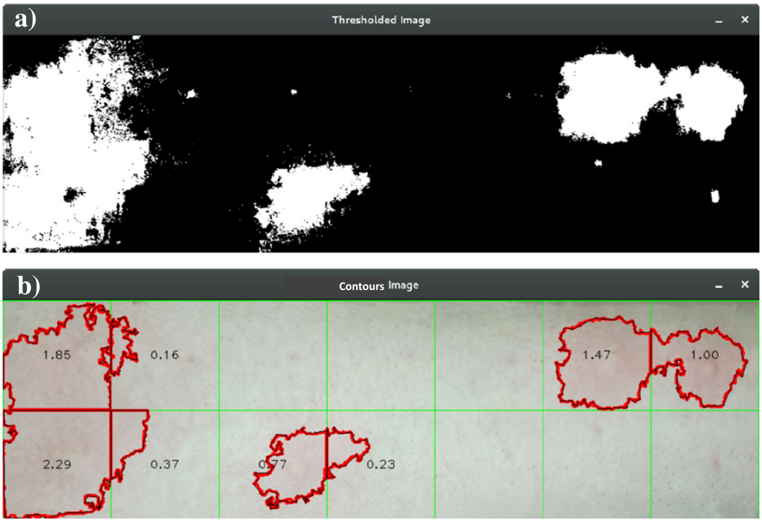


**Fig. 10** Image processing of 5 different relevant areas (chosen by the operator) for individual wheals extraction and measurement

The problem of wheals overlapping is now inherently solved since each rectangular area defines how much of a wheal (even for wheals larger than a single predefined square) is falling in that specific region where the wheal is being measured. Starting from the *Hue Channel Image* of Fig. 8b, the software performs automatic thresholding as described above and obtains the *Thresholded Image* of Fig. 10a. Using the predefined 14 square regions for the wheals the software automatically counts above-threshold pixels in each region. So, from the *Thresholded Image* (Fig. 10a) the fully-automatic procedure provides the *Contours Image* (Fig. 10b). This happens with no human intervention, and time, in deciding optimal threshold values or detection areas. In the resulting image and measurement of Fig. 10b, the contoured wheals are shown in red lines together with the corresponding numerical values of areas ratios to the histamine standard. Giving a look back to Table 1 and to the normalized area values displayed in Fig. 10b, one can clearly see each of the examined wheals as Neg(ative) or Pos(itive) reactions on the plus-levels scale. This is done for each of the 14 investigated, *i.e.* punctured, square regions of the patient forearm. For this image, the resulting Prick Test exam from the digital wheal-meter agrees very well with the results obtained by physicians. In fact, the novel machine detects each wheal properly and assigns to the wheals their correct values in the standard plus-levels scale.

This last procedure is obtained with no subjective intervention from the operator and provides full-automatic Skin Prick Test results in a rapid and very repeatable way.

## 10 Conclusion

A novel biomedical instrumentation (http://forimages.com/ponfometro.html, [11]) was developed for more repeatable wheal measurements in Skin Prick Tests. The instrument works with different degrees of automations (in a manual, semi-automatic, automatic or fully-automatic way) on digital images and provides for digital exam results. The proposed machine is called “digital wheal-meter” and it allows for effective allergic wheal detection, contour reconstruction, and metering resulting in a final Prick Test result on the usual plus-levels scale to evaluate allergy reactions. Compared to the work described in [11], now the automatic and fully-automatic image reconstruction procedures have been added providing for much less human intervention and subjectivity in the analysis.

The dependence of different image thresholding levels on skin types and colors needs yet to be experimentally investigated and taken into account for more generalized use of the machine developed. Direct comparisons will be performed between the classical Prick Tests, fully-manual being visually performed by the physician, and the different available machine outputs. This comparative analysis will serve as a calibration tool for the new machine and in the end it will validate the digital wheal-meter results with respect to the ones obtained by the physicians. A throughout validation campaign in clinical environment will be performed before setting the new machine into the market, where it will initially support the physician work and then finally perform the tests autonomously. The goal is to provide for equivalent results in terms of diagnostic accuracy but with the speed and repeatability of a fully-automated and machine-performed digital analysis. In the end, this digital wheal-meter will provide, depending on the choice of the physician, for wide options of wheal analysis as shown in this paper. The operating procedures allow now ranging from manual to fully-automatic wheal detection and measurements, always resulting in rapid or very-rapid digital outputs for the Prick-Test results.

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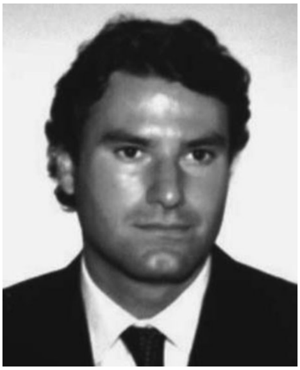

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