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Morphometry in Clinical Dermatology*

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Rapid and simple methods are presented which allow the estimation of areas, area fraction and contour lengths in clinical dermatology. They are based on point counting and intercept measurements using simple grids made on transparent film or on overhead foils. Because of their ease of use, these grids allow the determination of the size as well as the irregularity of skin lesions even during daily clinical work. The applications presented here include the area determination of naevi, leg ulcers, wheal and flare reactions and migration areas in lymphocyte and macrophage migration assays. Additionally, the determination of area fraction in psoriasis and sebutape evaluation is described. Other possible applications such as estimation or contour length, and determination of irregularity and estimation of the volume of tumours are discussed. ***Key words: Area determination; Area fractions; Contour length; Point counting; Skin lesions; Leg ulcers; Psoriasis; Sebutape.***

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In dermatology, the kind, form, size and distribution of lesions is an indispensable part of any description of skin diseases. Very often however, instead of stating the dimensions of a lesion in inches or in centimetres, a comparison to daily used items – such as coins – is made, despite the fact that few persons are able to spontaneously state the diameter of even the most commonly used coins.

Still more irritating, the size of two-dimensional structures is not infrequently compared to three-dimensional objects, especially to those of botanical origin, such as nuts. Even when nodular lesions are compared to such objects, the size of the latter is usually not defined. For example, walnuts from California differ considerably in size from those of Central Europe, as shown in Fig. 1.Clearly, there is a need for accurate and simple methods to determine the size of lesions or the area they occupy, exemplified by the discussion on the size of congenital and acquired naevi (1, 2), as well as on the extent of psoriatic lesions (3), respectively.

As outlined in this text, methods exist which allow rapid and simple area determination, at least for macular or plaque-like lesions.

MATERIALS AND METHODS

Methods

The basis of morphometry, i.e. the determination of size by different methods, is quite old. Mainly used in geology and metallurgy, these methods have made an impact in biology and medicine in recent decades, after the pioneering work of Weibel and others (4), as witnessed by a number of textbooks and reviews on this subject (5–9).

Further progress has been made recently with the advent of other stereological tools such as the disector and the nucleator, to name just two (10). In medicine, however, the main applications for the latter methods are in histology, where quantification is of particular interest, e.g. for discriminating between malignant and benign lesions.

For clinical dermatological purposes, the most important tools for estimating the size of lesions and their contour length are point counting and intercept measurements, respectively. To some, these methods might seem old-fashioned in view of the fact that semiautomatic and automatic devices exist. However, these devices cannot be used in routine clinical practice, because they require and intermediate step of outlining the lesion on a transparent sheet before evaluation. Moreover, they are not as effective (in the sense of rapid, cheap, easy to use) as the 'old' point counting methods. This has been demonstrated convincingly (11).

Point-counting procedures are based on the fact that points of a grid can be considered as 'representative' of a certain area associated with each point (4). The value of this 'point area', also called 'unit cell', depends on the geometric properties of the grid spacing (4, 5). In fact, points distributed randomly on an area could be used, but grids with regularly spaced points are usually employed, whether represented by points, crosses, or by crossover-points of lines. A detailed discussion of the properties of grids with other geometric patterns is provided elsewhere (4, 5, 12).

It must be emphasized that determination of an area by point counting is not a measure in a strict mathematical sense, but an estimation. This estimation is quite accurate, however, if an appropriately spaced grid is used. Then, the number of points times the 'unit cell area' yields a rather precise estimate of the area (Fig. 2). The area A to be estimated is calculated by the formula

$$A = n^* a \quad (1)$$

where n is the number of points falling onto the structure, and a is the area of the 'unit cell' of the grid used.

Fig. 1. Walnuts from Europe and California, demonstrating considerable volume differences.

*Dedicated to Prof. Dr. H. Zaun (Homburg) on the occasion of his 60th birthday.

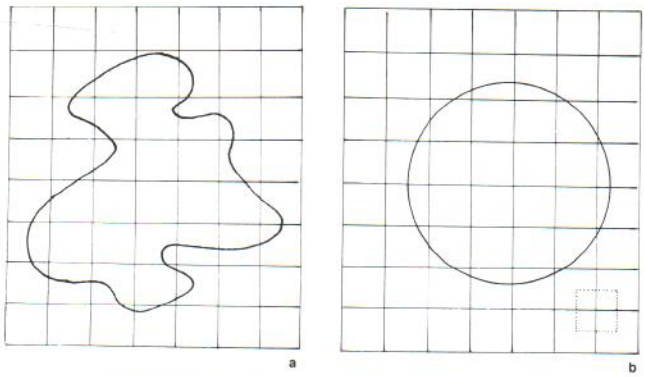

Fig. 2. Two areas of about the same size, but of different shape. Given a grid with grid lines 0.5 cm apart, the 20 points on *a* give an area of 5.0 cm^2, the 21 points on *b* an area of 5.25 cm^2 (expr. 1). Boundary length (expr. 3) in *a* is 11.85 cm, in *b* 7.9 cm. The form factors (expr. 5) are 0.45 in *a* and 1.07 in *b*, respectively.

The accuracy of such an area estimation mainly depends on the number of points falling onto the structure. For clinical dermatological work it is not necessary to use grids with a large number of points, however. For area determination, a few points (up to 10) are appropriate. Detailed information on this topic and on the calculations for determining the appropriate grid spacing (i.e. number of points) as well as on the non-trivial determination of the coefficient of error is found in Gundersen & Jensen (12).

For clinical dermatological purposes, square lattice grids or grids with points arranged in a square fashion are to be preferred because their geometric pattern allows rapid counting. These grids can be made either on transparent photographic high-contrast film or from drawings photocopied onto overhead foils. Especially the latter method is useful if the grids have to be discarded after use as is the case with infected leg ulcers.

The area of flat and slightly elevated skin lesions can be determined with a set of such grids even during normal clinical work, because they can be carried in the pocket. This seems to be especially important in the case of naevi, whether congenital or acquired (13), to provide a sound basis for estimating the size-related risk of the development of malignancy. Furthermore, naevus size could be related to total body area (14).

In the so-called 'dysplastic naevus syndrome' not only the area, but also the irregularity of such a lesion is of interest. Determination of irregularity is possible by using intercept measurements, a procedure whose basis can be traced back to the almost 300-year-old needle problem of Buffon (5).

If parallel lines, a known distance d apart, are placed onto a lesion, the length of its contour L can be determined by the formula

$$L = \pi/4^* \, d^* \, N \qquad (2)$$

simplified

$$L = 0.79^* \, d^* \, N \qquad (3)$$

where N is the number of intersections of these lines with the boundaries of the lesion. To assess the relation of the area and the contour length as a measure of irregularity, a form factor can be used, calculated according to the formula

$$FF = \frac{4^* \, \pi^* \, n^* \, d^2}{\pi/16^* \, N^2 \, d^2} \qquad (4)$$

simplified

$$FF = 20.4^* \, n/N^2 \qquad (5)$$

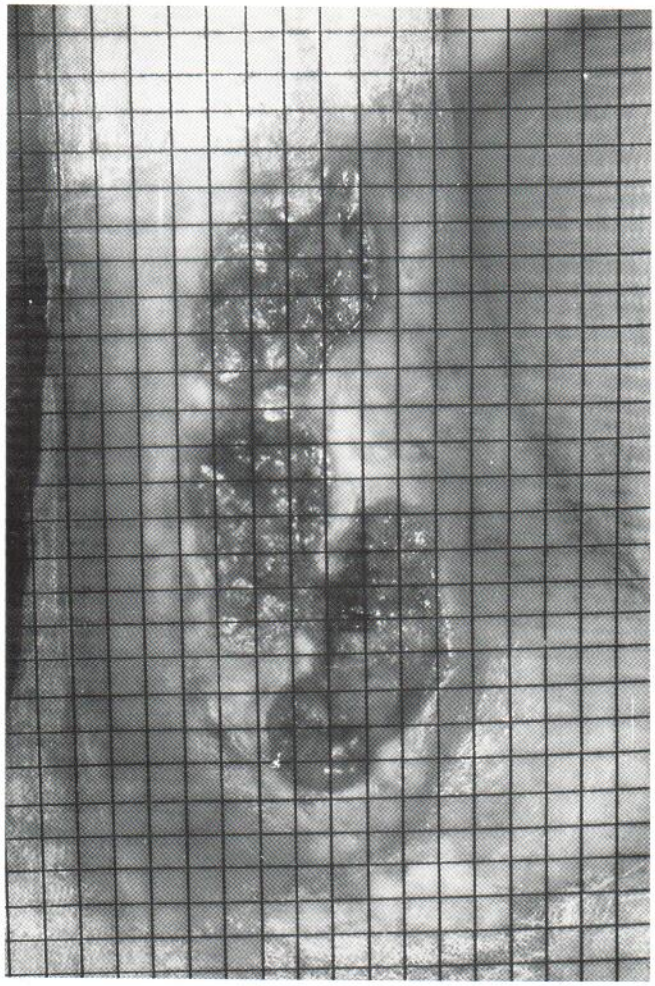

Fig. 3. Bizarre-shaped leg ulcer with an area of 15 cm^2, because 60 points, each with a 'unit cell' value of 0.25 cm^2, are counted over the ulcer.

where n denotes the number of points falling onto the structure, and N the number of intersections of the grid lines with the boundary of the structure.

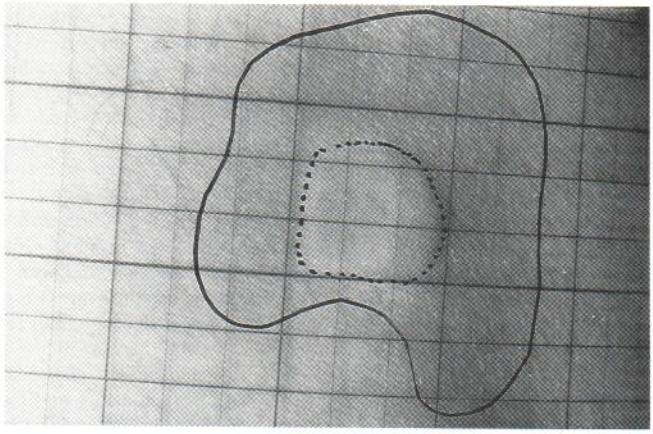

Fig. 4. Prick test reaction. The outlines of the wheal and flare are marked for better visibility. Area of the wheal is 6.5 cm^2 (26 × 0.25) and of the flare, 1.5 cm^2 (6 × 0.25).

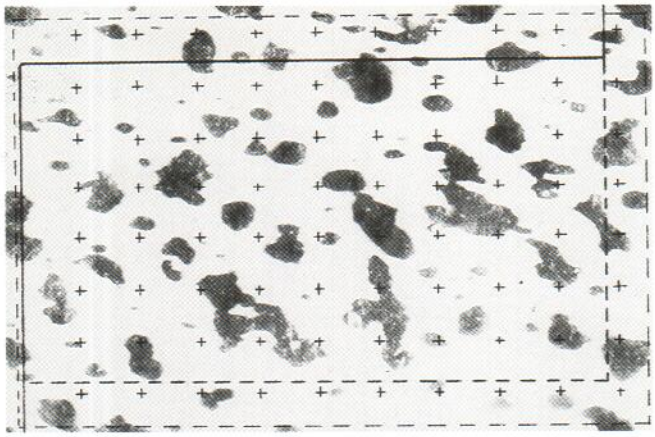

Fig. 5. Original Sebutape with frame and grid. Reference area contains 80 points, sebum areas account for 15 points. Relative area of sebum thus 15/80 points (= 18.8%).

Fields of application

Leg ulcers are very often irregular in shape. With point counting grids, copied onto overhead foils, their area can be determined independently of shape (Fig. 3). Here, the disposable grids make superfluous the tedious process of outlining the ulcer border on transparent plastic sheets, with subsequent area evaluation (15).

Wheal and flare prick test reactions often are not round as they should be for the usual scoring by the sum of the diameters a and b in two opposite directions, divided by 2 (i.e. $(a + b)/2$). With a grid (Fig. 4), the area of the wheal as well as that of the flare can be determined simultaneously and accurately (16). For routine clinical work this rather precise method is not necessary, however, but for research work it could be of value.

Therapeutic studies in skin diseases with disseminated lesions such as psoriasis also require accurate determination of the extent of the disease, as well as of its severity (3). Here, the proportion of the area involved is of interest rather than the area of an individual lesion. With point counting applied to photos taken in a standardized manner, such a determination is simple (17). The optimal number of points at a given coefficient of error can be taken from the nomogram in Weibel (4). On this basis, we have developed a simple scoring system for the assessment of disease severity in atopic dermatitis (18).

Another interesting application of point counting is the determination of lymphocyte and macrophage migration areas in the same manner as described for naevi, but with small grids placed onto the migration areas under a stereomicroscope or under an enlarging lens (19).

There are several other possible applications for poing counting. For example, quantification of teleangiectasia and newly formed vessels as in the case of corticosteroid assays and in angiogenesis experiments, respectively, is simple (in preparation). Because the "human eye is notoriously poor as an estimator of area fractions" (7), the rather crude 'semi-quantitative' methods could be avoided.

Another application could be the quantification of the involved nail plate in onychomycosis, e.g. in studies on the effects of antimycotics. Since the nailplate area changes by growth it must be ensured that always the same area of the nail plate (reference area) is taken.

In the case of systemic sclerosis, measurement of the mouth orifice by calipers has been used for the assessment of disease progress (20). If the mouth is photographed in a standardized manner and with a ruler to guarantee that the same magnification is used for the assessment, determination of the orifice area by point counting could replace the use of calipers (in preparation).

With the sebutape, sebum excretion can be determined, based on the fact that the structure of the tape changes in such a way that the sebum drops are visible as spots when viewed against a black background (21). Usually, the assessement of the amount of sebum excreted is made visually by means of reference patterns supplied with the tapes. This method is rather crude because the individual spots are of different size, are irregularly shaped and might show considerable confluence. It has been shown that semiautomatic morphometric devices allow a fairly exact quantification (22). Since this method is time consuming, it has not gained wide acceptance. With the use of small grids, a good magnifying lens or a stereomicroscope quantification is a matter of minutes (23). By adhering to the rule of 'forbidden lines' (9) even the unbiased number of excretion spots, as well as their average size, corresponding to the follicle orifices, can be determined (Fig. 5). Here, only those particles are counted which are inside – or partly inside – the dashed counting frame, except those which intersect the full-drawn 'forbidden lines'. Documentation can easily be performed by high-power photography (24) of the tape, thus allowing retrospective measurements.

However, not only flat or nearly flat lesions can be quantified. If a body can be sliced into slabs of known thickness, its volume can be estimated by simple multiplication of the total area of the slabs by the average distance of the sections, according to the principle of Cavalieri (9). With this rapid and simple method we have found a rather good correlation between tumour volume and maximal vertical tumour thickness in different skin tumours (25). For clinical purposes, it should be possible to estimate the volume of tumours by high-frequency ultrasound in vivo. Naturally, the accuracy of this method mainly will depend on the ability to distinguish between neoplastic and surrounding tissue, using ultrasound (26).

DISCUSSION

With the use of simple grids made on high-contrast photographic film or on transparent overhead foils, it is possible to proceed from the semiquantitative assay of areas and area fractions to true quantification. As pointed out, the old principle of point counting and intercept measurements is highly topical in view of the demands of clinical dermatology for quantification.

Morphometry in this rather limited sense can thus provide the basis for the solution of problems where the determination of the size of lesions, as in the case of naevi, or the relative area of skin affected by lesions as in psoriasis, is important.

In addition, the length of structures such as teleangiectasia can be estimated, e.g. in corticosteroid assays.

It should be stressed that these estimations can be carried out without any bulky or expensive equipment. Furthermore, they do not require time-consuming intermediate steps such as drawing outlines before measuring. Thereby, quantification in clinical dermatology could reach a basically simple dimension.

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