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Quantification of dry (xerotic) skin by image analysis of scales removed by adhesive discs (D-Squames)

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Synopsis

Clinical grading of dry (xerotic) skin is unreliable, being influenced by several variables, especially ambient humidity. We utilized commercially available adhesive-coated discs (D-Squames) to sample the outer portion of the stratum corneum. A procedure was then developed using image analysis to quantify the degree of scaling, using two parameters: 1) the percentage area covered by scales and 2) the distribution of scales according to five thickness levels. There two values were mathematically integrated into one final value, the desquamation index.

We showed that image analysis accurately distinguished the intensity of scaling among individuals whose legs were, respectively, non-dry, moderately dry, and severely dry. The method was especially valuable for quantifying the differing efficacies of three moisturizers evaluated by the regression procedure.

INTRODUCTION

The subjective assessment of dry xerotic skin by sight and touch is fraught with errors. Grading is strongly influenced by ambient meteorological conditions. For example, in Philadelphia, Pennsylvania, one day of wet, warm weather in winter hydrates the surface and sharply reduces the signs of dry skin, though no real change to the desquamating portion of the horny layer has occurred. The limitations of subjective grading are numerous, well known, and a source of frustration to clinicians and manufacturers of moisturizing products (1).

Collecting corneocytes on adhesive tape is one approach toward an objective assessment of scaly, rough, xerotic skin. The stripping technique was first described by Wolf over 50 years ago but has never come into fashion (2). Later, workers in our laboratory utilized adhesive-coated glass slides (sticky slides) to obtain a sample of the loose desquamating portion of the outer horny layer (3,4). When appropriately stained, scales, which are really clumps of corneocytes, develop an intense color. However, the

results are variable, depending on the nature of the adhesive and the thickness of the coating on the slide.

Recently Serup *et al.* collected scales on adhesive discs (D-Squames), and estimated the quantity of scales by the attenuation of transmitted light (5).

The commercial availability of these adhesive discs prompted us to develop a method that could quantify accurately the degree of scaling.

MATERIALS AND METHODS

SAMPLING DESQUAMATING HORNY CELLS ON ADHESIVE DISCS

The sampling device is a 22-cm clear-adhesive-coated disc (D-Squames, CuDerm Corporation, Dallas, TX). Its properties have already been described (5). After peeling off the protective seal, the D-Squames disc is briefly pressed to the skin surface. The disc is peeled from the skin with tweezers and placed on a black storage card included in the D-Squames kit.

ILLUMINATION

We built a white light box to insure homogeneous illumination of the sample. The box was illuminated from two sides by means of two fiber-optic light carriers. The light was diffused through white translucent glass. Constant illumination was provided by two halogen lamps with a highly regulated power supply. This setup greatly enhances the contrast of the corneocyte clusters on the discs. Because the scales scatter and reflect light, they appear white against the black background. Brightness is proportional to the thickness of the scales, providing an index for quantification.

CALIBRATION

The lamps were calibrated by adjusting the power supply, based on a reference gray card. The intensity of the image was evaluated with the histogram function of the image analysis program.

VIDEO IMAGING

The image analysis system consists of four components: 1) the live image source through a video camera, 2) display of the image on a video screen, 3) a video digitizing board frame grabber, and 4) a computer to run the software.

The images were obtained by a high-resolution black-and-white CCD video camera (Dage-MTI CCD72, Michigan City, IN) connected to a stereo-microscope (OPMI 1-FC, Zeiss, Germany). A separate video control panel with manual gain and black level controls guaranteed consistent video processing under identical conditions.

The image was captured by an image analysis program (Java, Jandel Scientific, CA) using a frame grabber board (Truesvision Targa-M8 Frame Grabber), both installed in a Unisys personal computer. The frame grabber translates the image into 512 × 480

picture elements (pixels). Each pixel is given a numerical value according to its intensity on a gray scale from 0 to 255.

IMAGE ANALYSIS

An electronic mask was used to define a measurement area of 200 mm^3. No contrast enhancement was necessary. We then used a look-up table to rate gray levels on a 1 to 5 scale. Each pixel was assigned to one of these levels, corresponding to five arbitrary thickness levels of the corneocyte clusters. We calculated the number of pixels in each thickness group as a percent value. Additionally, we determined the percentage area occupied by the scales. These two functions were integrated to yield the desquamation index (D.I.) according to the following formula:

$$\text{D.I.} = \frac{2\,A + \sum_{n=1}^{5} T_n \times (n - 1)}{6}$$

A = percent area covered by scales.
T_n = percentage of scales in relation to thickness.
n = thickness level (1–5).

The macro-facility of the program was used to record all of the measurement functions and transformations, performing these automatically with one keystroke.

RESULTS

ASSESSMENT OF LEG DRYNESS BY THE D-SQUAMES METHOD

D-Squames from non-dry, moderately dry, and severely dry legs of adult women were captured by the video camera and processed by image analysis.

Figure 1 illustrates the gross appearance of D-Squames against a black background for non-dry, moderately dry, and severely dry leg skin. The differences are very striking to the naked eye.

Figure 2 illustrates the gross appearance of pseudo-colored D-Squames for the three levels of dryness. The reference color bar enables the eye to estimate quickly the approximate thickness of the scales.

Figure 3 illustrates the scale distribution generated by image analysis. The differences between non-dry and dry skin are dramatic both in regard to the area covered by scales and the larger proportion of thick scales.

Figure 4 shows the desquamation indexes in relation to the distribution of scales for the three levels of clinical dryness and the abbreviated treatment period; the usual period is three weeks.

The post-treatment evaluations (regression phase) were made on Monday, three days after the last application, to avoid interference from residues of the moisturizers, and on the following Monday, 10 days after the last treatment.

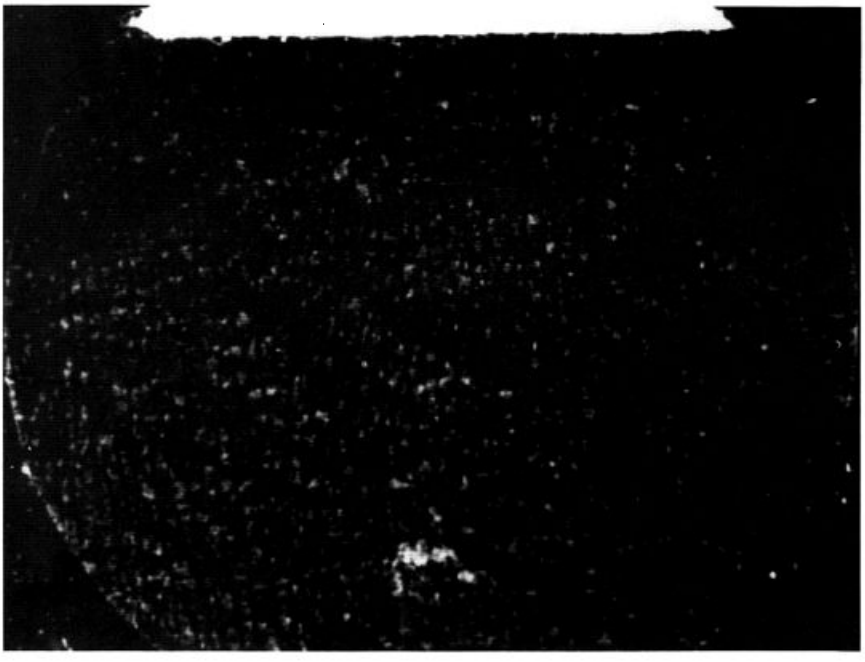


Figure 1a. D-Squames sample from a non-dry leg. The scales cover only 15% of the surface and are very thin.

At the same times, trained monitors graded dryness on the following scale:

0 = no dryness.
1+ = slightly dry, powdery, ashy appearance.
2+ = moderately dry, small uplifting scales.
3+ = severely dry, large scales, with marked flaking.
4+ = extremely dry, fissured, severe scaling.

Figure 5 is a bar representation showing the desquamation indexes for the three test creams at days 3 and 10 of the regression phase. Statistical significance was assessed by the paired t-test. Product 3 was the most effective, being significantly different from both the untreated control and the other two creams at days 3 and 10 ($p < 0.005$). Product 1 was next most effective and was statistically different from the control and product 3 at days 3 and 10 ($p < 0.005$). Product 2 was ineffective, with values very similar to those of the untreated control.

However, by clinical grades, product 2 was estimated to be statistically more effective than the untreated control at day 3 but not at day 10 (Mann-Whitney U Test). This discrepancy is discussed below. Otherwise, there was a good correspondence between the clinical grades and the data generated by image analysis.

DISCUSSION

The assessment of dry (xerotic) skin, whether for purposes of classification, diagnosis, or therapeutic evaluation, has been handicapped by grading systems based on visual and

Figure 1b. D-Squames sample from subject with moderately dry leg skin. Scales cover most of the surface, and are thicker and larger.

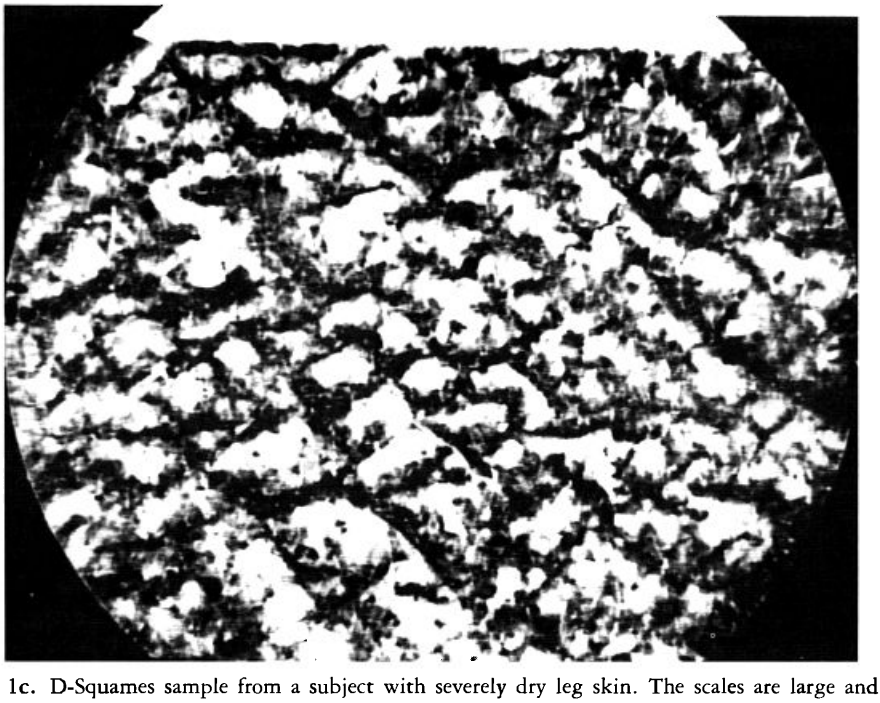


Figure 1c. D-Squames sample from a subject with severely dry leg skin. The scales are large and very thick, covering the entire surface except where interrupted by furrows.

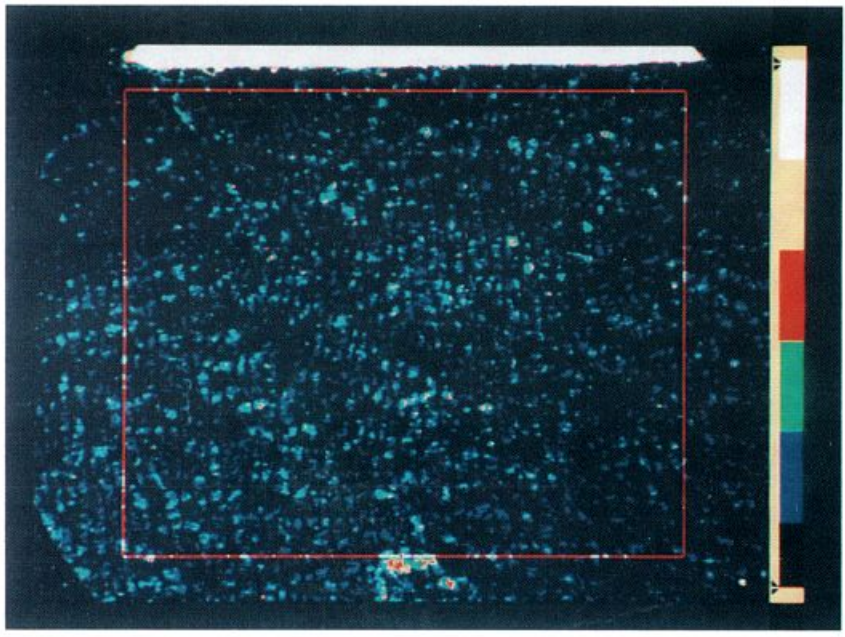


Figure 2a. Pseudo-colored D-Squames image of a non-dry leg. Blue-green indicates thin scales.

tactile scoring. These are highly subjective and suffer from excessive variability from grader to grader, and also from poor reproducibility from day to day. A day or two of humid, warm weather ruins all such subjective grading systems since hydration swells the outer horny layer, masking scaling.

D-Squames are a technical advance that enable a standardized way of collecting scales from the outermost portion of the horny layer, which we have termed the "presumptive desquamating layer," where the loosened horny cells are ready to be shed.

To demonstrate the usefulness of the D-Squames technique, we developed procedures based on computerized image analysis enabling quantification of the degree of scaling. We measured both the percentage area covered by scales, and the distribution of scales according to five thickness levels. From these, we calculated the desquamation index, which expresses the degree of xerosis in one intergrated value.

We validated the method by comparing the efficacy of three moisturizers that we had previously determined to be different by the regression procedure.

The three products clearly differed in efficacy. Product 3 was most efficacious while product 2 performed rather poorly. As expected, in each case greater efficacy was demonstrable at three days post-treatment in comparison to day 10.

We call attention to an interpretation problem with product 2. By clinical examination, this agent scored better than the control on day 3 (Wilcoxon means). However, based on the desquamation index, product 2 was equal to the control only on day 3 and definitely worse on day 10.

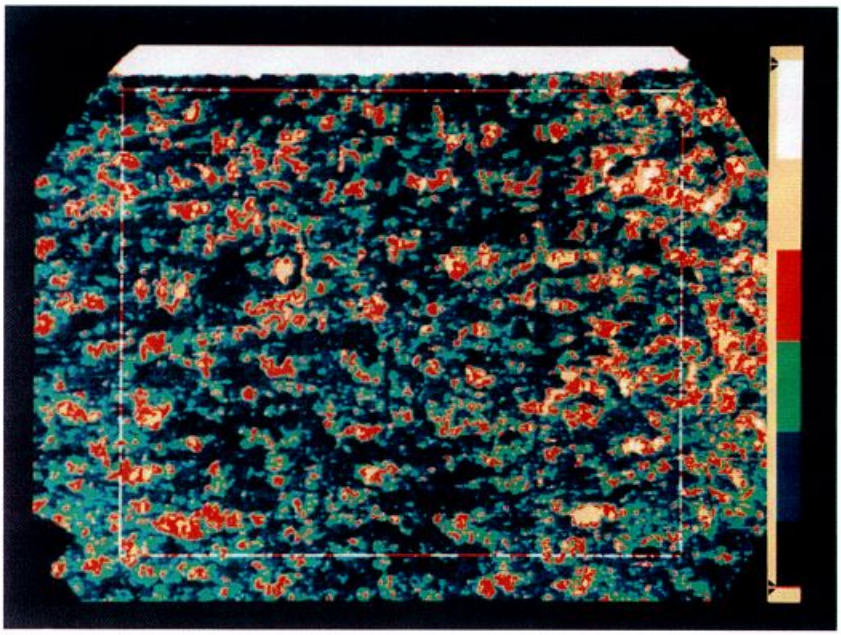


Figure 2b. Pseudo-colored D-Squames image of a moderately dry leg. Red indicates larger scales intermingled with thin scales (green).

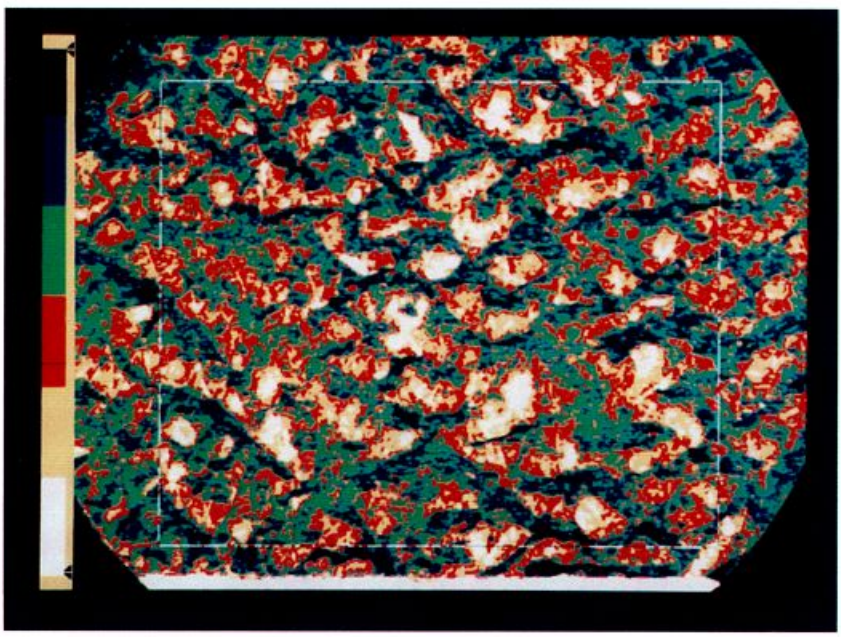


Figure 2c. Pseudo-colored D-Squames image of a severely dry leg. Yellow signifies very thick scales and white even more so. Clearly, each field is heterogeneous in regard to size of the scales.

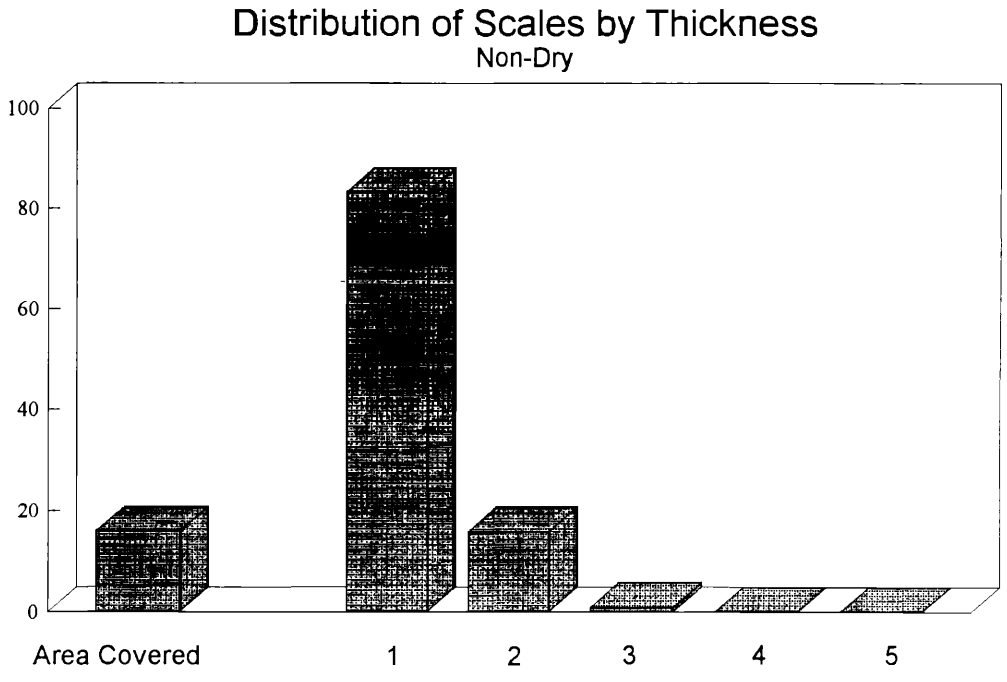


Figure 3a. Non-dry. 16% of the surface is covered with scales, and almost all of the scales are very thin (thickness level = 1).

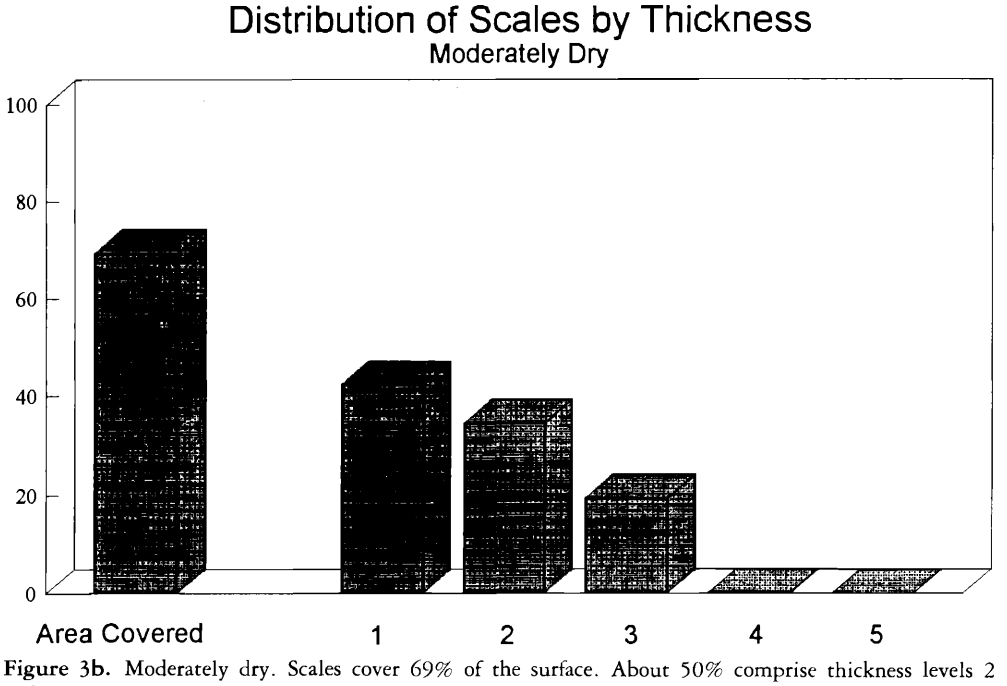


Figure 3b. Moderately dry. Scales cover 69% of the surface. About 50% comprise thickness levels 2 and 3.

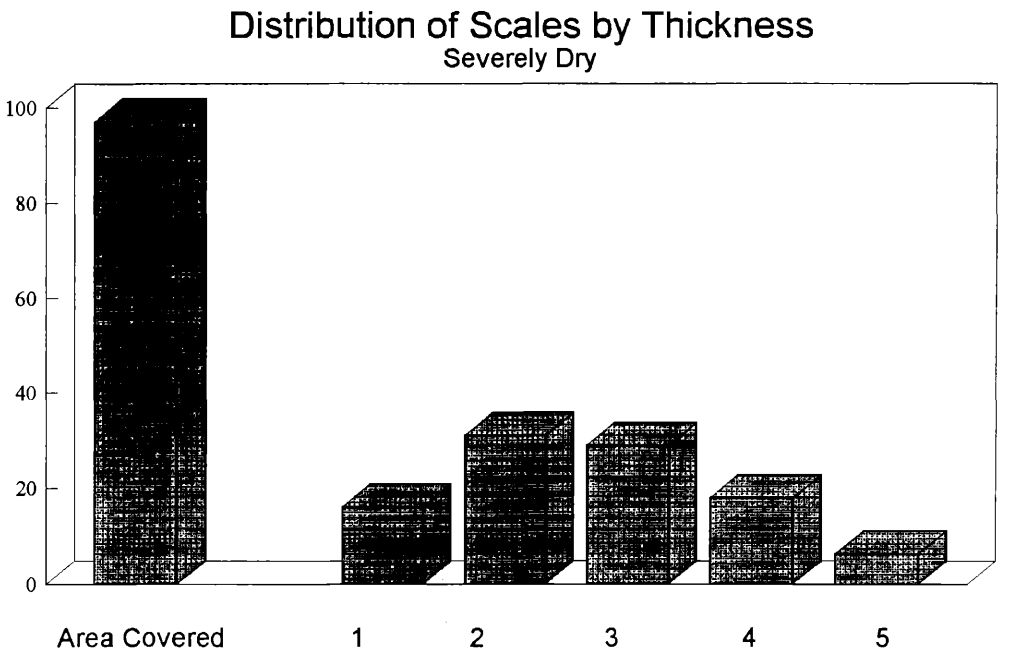


Figure 3c. Severely dry. The scales cover the entire surface. 85% compromise thickness levels of 2 or greater.

Our explanation for this seeming paradox relates to the relatively high viscosity of this particular moisturizer, which thickly coats the surface without spreading. The net effect is that scattering and reflection of light are reduced, creating the illusion of a high therapeutic effect. As it turns out, this experience strongly reinforces the greater reliability of the D-Squames method, which samples the desquamating layer without regard to appearance.

We make note here of a recent refinement that enhances the visualization of scales as well as loosening them for more uniform sampling by D-Squames. This is accomplished by exposing the skin to 2 ml of ether:acetone (1:1) or ether alone in a glass cylinder for one minute. After evaporation, the surface becomes increasingly whiter and flakier in proportion to the amount of scaling. This delipidization procedure often reveals scales that are not evident clinically and always enhances preexisting scaling. Wet weather conceals scaling. This variable is completely overcome by delipidization prior to sampling.

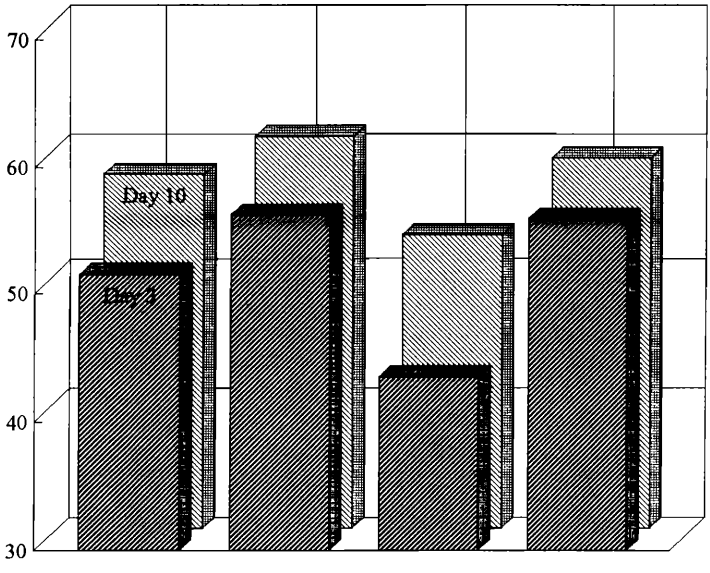
Image analysis requires sophisticated and expensive equipment. We mention here a simpler alternative that is reliable but less precise. This involves sonicating the D-Squames forcefully in water, which not only floats off the scales but disperses them into a unicellular suspension of corneocytes. The latter can then be counted in a hemocytometer from which one can calculate the number of corneocytes per square millimeter of surface.

For experimental purposes, one can also use critical point drying of the D-Squames and examine the microtopography of individual corneocytes by scanning electron microscopy after metallic shadowing. D-Squames can also be directly stained with methylene blue, mounted on a glass slide, and examined by stereomicroscopy. The intensity of staining

Thickness, Distribution of Scales and Desqumation Index for Non-Dry, Moderately Dry and Severely Dry Skin

		Non-Dry	Moderately Dry	Severely Dry
% Area occupied by scales		16%	69%	97%
Thickness Levels	1	83.2%	42.2%	16.0%
	2	15.9%	34.4%	30.9%
	3	0.9%	19.4%	28.8%
	4	0.0%	0.1%	17.9%
	5	0.0%	0.0%	6.4%
Desquamation Index		8.1	37.2	60.3

Figure 4. In the progression from non-dry to severely dry skin, the scales cover more of the surface, they increase in thickness, and they are accompanied by a substantial increase in the desquamation index.



	Product 1	Product 2	Product 3	Control
Day 10	57.7	60.7	52.7	59.0
Day 3	51.5	56.2	43.5	55.8

Figure 5. Desquamation indexes for the three moisturizers on days 3 and 10 of the regression phase. Product 2 is ineffective and does not differ from the control. Product 3 is clearly the most effective product. Product 1 is slightly effective compared to the control.

increases with the level of scaling, allowing reasonably reliable semiquantitative estimates.

We recognize that determining the desquamation index by image analysis is something of a technological luxury. Simpler methods may be adequate for routine work, especially for screening moisturizers. In this regard, the optical attenuation method of Serup *et al.* has merit even though information on the distribution of thickness levels is not retrievable (5). Our modification is to place the D-Squames disc in an ordinary 35-mm slide mount that is viewed by a slide file projector (Model H-I, Slidex, Slidex Corp., Tokyo, Japan). Optical transmission can then be measured from the projected image using a Mavolux digital light meter (Gossen GMBH, Erlangen, Germany) mounted to a shield fitted to the screen.

Finally, it is appropriate to mention a new modification developed by Pierard *et al.* that they call 'Squamometry." D-Squames are obtained under standardized pressure. They are then stained and examined by quantitative colorimetry using the Minolta chromameter. A value of chromaticity is then derived that extracts still more information than the calculation of the desquamation index. Obviously, squamometry is not for everyday use but may be useful to the meticulous experimenter with specialized needs.

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