Special article

Analytic morphology in clinical and experimental dermatology

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During the past several years, quantitative morphology has gained increasing attention in diagnostic pathology and in certain research applications. In the field of dermatopathology, quantitative morphology has been applied to numerous problems, ranging from the interactive measurement of nuclear contours to fully automated, high-resolution image analysis of ultrastructural micrographs. Dermatologic applications are reviewed, and potential developments in the future are briefly outlined. (J AM ACAD DERMATOL 1993;29:86-97.)

Analytic morphology denotes a set of techniques that provide quantitative and statistically evaluable data from morphologic images. In most quantitative techniques measurements are interactively or automatically performed, and the results are expressed on a linear scale.

Depending on the method used, different hardware systems are required. For basic stereology, an ocular grid or a ruler may be sufficient, but high-resolution image analysis and computer simulations need sophisticated computer technology with a computational speed markedly exceeding the capabilities of a conventional personal computer.

The challenge of quantitative morphology is to describe image features that are beyond the perception of the human eye. In dermatology, with its sound morphologic background, there are numerous potential applications for quantitative techniques. A broad range of them is reviewed herein.

TECHNOLOGICAL ASPECTS

In analytic morphology, many different terms are used. Some are synonymous, whereas others describe well-defined specialized techniques. In general, *analytic morphology* and *quantitative morphology* can be regarded as synonyms and include all techniques that provide quantitative (or semiquantitative or binary) data from morphologic images. The quantitative features can be gained by objective measurements based on different hardware devices. *Morphometry* includes all techniques for the measurement of two-dimensional features from structural elements or particles by geometric criteria, be it by point counting, measuring distances with a ruler, or tracing contours on a digitizer board. *Stereology* is a body of methods that derive estimates referring to the three-dimensional space based on measurements obtained in fewer dimensions. *Image analysis* includes all methods that deal with completely digitized images in computer systems; this includes digital processing and enhancement of images before the measurements. These procedures require a substantial degree of hardware and software. *Three-dimensional reconstruction* and *image modeling* by computer simulations may be regarded as specialized image analysis methods.

STEREOLOGIC METHODS FOR DERMATOPATHOLOGY

Histologic slides provide flat images from spatial structures. Particles (cells, nuclei) appear as flat areas; spatial surfaces (basement membranes, vessel walls) appear as flat curves; spatial curves (collagen, fibers) appear as points. Moreover, in oriented tissues like skin and muscle, the properties of the

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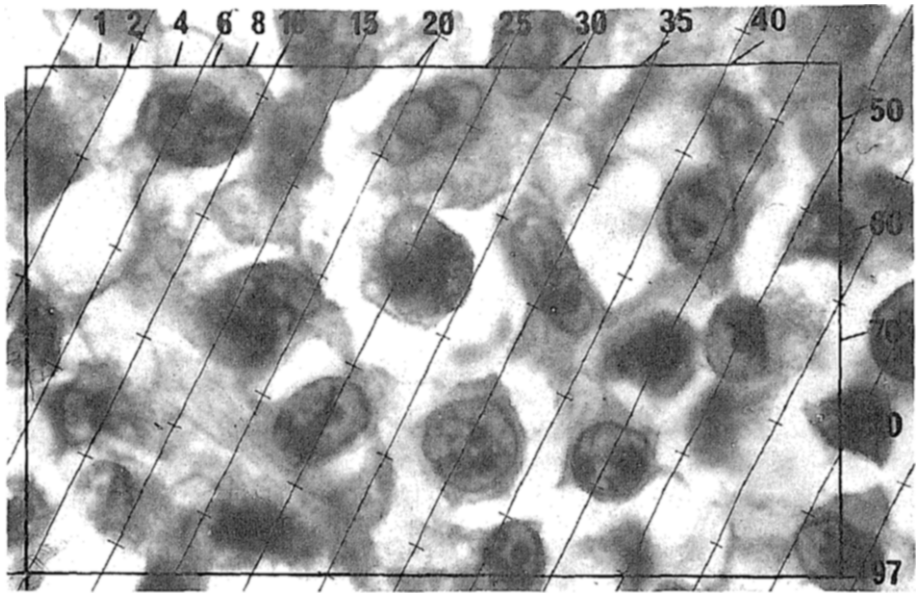

Fig. 1. Estimation of tumor cell nuclei from vertical section through a nodular malignant melanoma. Paraffin section. Linear intercepts through all those tumor cell nuclear profiles that have been hit by the evenly spaced points are measured. (Hematoxylin-eosin stain; ×555.)

microscopic image depend crucially on the direction of cutting. To draw meaningful conclusions about a biologic tissue from measurements on histologic slides, all these facts must be taken into consideration. Fortunately, many mathematical relations between the original structure and its image are known, and these are often surprisingly simple. Stereology is the method that allows extrapolation from measurements on sections to real three-dimensional structures.[1] In skin sections many important stereologic quantities can be determined from vertical sections.[2] Thus various planar parameters can be measured in routine skin specimens and unbiased estimates of three-dimensional quantities obtained even in retrospective studies. Volume fractions (percentage of tissue volume occupied by a particular structure) can easily be determined by point counting. For the estimation of surface areas (e.g., total surface of all nuclei per tissue volume), so-called cycloid test systems are necessary as a special measuring device. For the calculation of the size of cell nuclei, the method of the "volume-weighted mean nuclear volume" can be applied. This procedure requires a set of parallel lines with evenly spaced reference points. The parallel lines must yield randomly selected, weighted angles with the vertical axis. The measurements can then be obtained by rulers, or the whole procedure can be implemented in an image analysis system. In the context of dermatopathology, the volume-weighted average of nuclear volume (vv) has been studied extensively in pigmented tumors of the skin, particularly in melanocytic nevi, lentigo maligna, and malignant melanoma[3-5] (Fig. 1). The results of multivariate analysis have shown that vv is in fact an independent indicator of prognosis.

DNA ANALYSIS OF MICROSCOPIC SLIDES

Nuclear DNA measurements can be performed with a cytophotometer (DNA cytophotometry), an image analysis system (DNA image cytometry), or a flow cytometer. Although image cytometry and image cytophotometry are more time-consuming than flow cytometry, these methods have the advantage that each individual measurement can be interactively controlled and that the information on DNA content can be combined with morphologic data of the particular cell. Furthermore, DNA measurements on histologic sections do not require the preparation of single cell suspensions, which is often difficult to perform.

In contrast to pure morphometric methods, DNA analysis directly reflects the functional status of the particular cell with regard to ploidy and cell cycle

phase. Whereas most normal tissues are composed of a majority of diploid cells in the G_0 or G_1 phase of the cell cycle, along with a small proportion of tetraploid cells in the G_2 phase and some cells between the diploid and tetraploid values (S phase), hyperproliferative tissues show an increase of S and G_2 phase cells. Particularly in malignant tumors, cell clones with DNA values other than diploid or tetraploid may occur (aneuploidy).

Image cytometry has repeatedly been applied to facilitate a differential diagnosis between benign and malignant melanocytic lesions. Coefficient of variation of DNA content,[6] mean optical density, nuclear area,[7] and differences in these features between the superficial and deep parts of the lesions[8] proved to provide sensitive discrimination between common melanocytic nevi and Spitz nevi, on the one hand, and of malignant melanoma on the other. Stolz et al.[9] found the mean value of DNA content, standard deviation of nuclear area, and 95th percentile of DNA distribution as the most efficient criteria. An effective discrimination between benign and malignant melanocytic lesions could also be obtained by quantifying not only nuclear size and density, but also chromatin distribution and architecture by high-resolution image and multivariate analysis, even when only intraepidermal melanocytic nuclei were taken into account.[10] Efficiency was found to be 100% at the ultrastructural level[11] and 96% in light microscopic semithin sections.[12]

The results concerning prognosis of malignant melanomas have been less encouraging because tumor thickness usually turned out to be more significant than DNA content.[13] However, significant correlations between tumor thickness, on the one hand, and mean nuclear area, 80th percentile of DNA distribution and the number of nuclei with a relative DNA value higher than 5c (relative DNA value compared with chicken erythrocytes as DNA standard), on the other, could be demonstrated in 34 cases of malignant melanoma.[14]

In premalignant melanocytic lesions, a relation between morphologic features of atypia and of abnormalities in the DNA histograms could be demonstrated.[15]

QUANTITATIVE NUCLEAR FEATURES IN MALIGNANT CUTANEOUS LYMPHOMAS AND PSEUDOLYMPHOMAS

The differential diagnosis between malignant cutaneous lymphomas and pseudolymphomas (e.g., lymphocytic infiltration of Jessner-Kanof, different types of cutaneous lymphoid hyperplasia, actinic reticuloid, and lymphomatoid papulosis) can be difficult. Early stages of cutaneous T-cell lymphomas (mycosis fungoides, Sézary syndrome) often resemble benign inflammatory dermatoses such as chronic contact dermatitis, atopic dermatitis, and psoriasis, both clinically and histologically. There is evidence that quantitative morphology may be helpful for differential diagnosis.

Cutaneous malignant T-cell lymphomas are characterized by lymphoid cells with deep cerebriform indentations in their nucleus. Qualitatively similar cells, however, may also occur in chronic eczema, lichen planus, contact dermatitis, and peripheral blood of healthy donors. Nevertheless, detailed ultrastructural analysis of the nuclear contour index (NCI; defined as the ratio of the nuclear perimeter and the square root of the nuclear area) by a graphic tablet interfaced with a small computer revealed striking differences between the cerebriform nuclei of malignant lymphoid infiltrates and reactive inflammatory dermatoses,[16] with higher NCI values in the malignant cases. These differences were also found in blood cells.[17] The assessment of nuclear indentations revealed a sensitivity of 62% with a specificity of 100%.[18]

Because most neoplasms are characterized by an increased number of polyploid and aneuploid cells, the DNA content of cutaneous lymphomas and pseudolymphomas was investigated by image analysis. It was shown that the majority of the cells in pseudolymphoma are diploid and have relative DNA values of 2c. In contrast, malignant lymphomas showed a variable increase of cells with DNA values in the tetraploid region. In addition, an increased rate of cells in the region between 2.25c and 3.5c and cells with DNA values higher than 5c were observed. With the 2c deviation index (2cDI), which reflects the increased variation of the DNA values around the normal value of 2c in malignant lymphomas, a differentiation between pseudolymphomas and malignant lymphomas was achieved with a sensitivity and specificity of 95%. Further insights into the cytology of cutaneous lymphoproliferative diseases can be expected from analysis of the chromatin structure[19] and the combined application of DNA cytometry and immunolabeling.[20]

NUCLEOLAR ORGANIZER REGIONS

An interesting, simple, and more common additional method for the histologic evaluation of tumors is the determination of nucleolar organizer regions

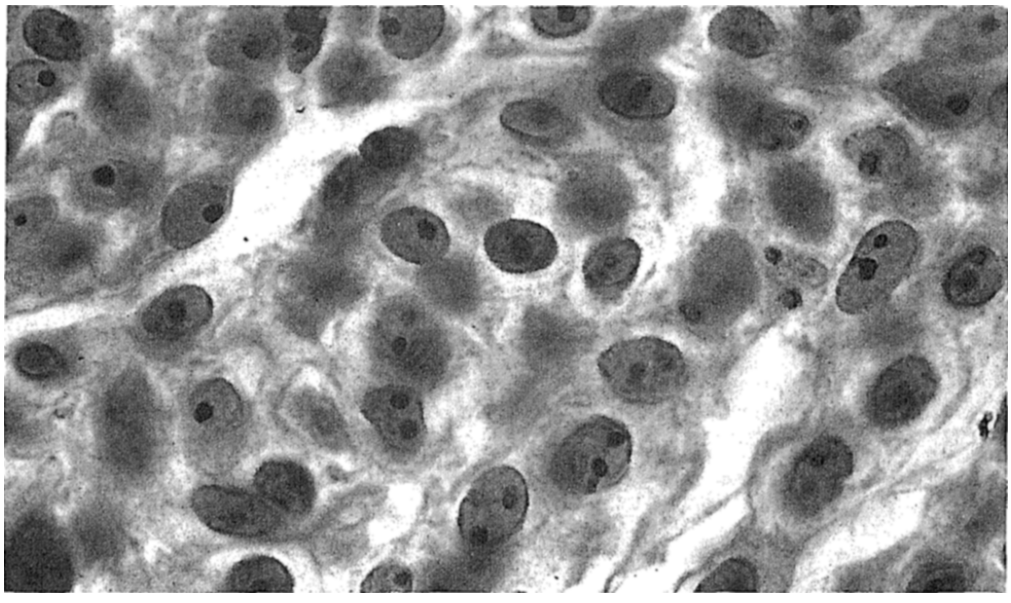

Fig. 2. Nucleolar organizer regions (NORs) in melanocytic nevus. Most cells contain one or two small NORs per nucleus. (Silver staining technique; ×400.)

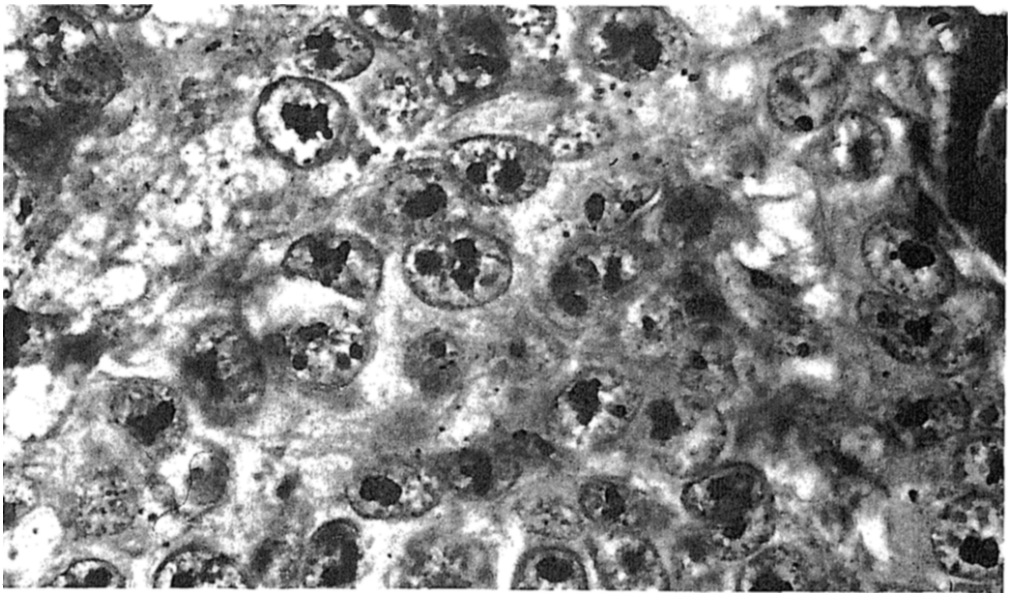

Fig. 3. Nucleolar organizer regions (NORs) in malignant melanoma. Most cells show multiple large NORs. (Silver staining technique; ×400.)

(NORs). NORs are segments of DNA that contain coding genes for ribosomal RNA and contribute to the regulation of cellular protein synthesis. Their association with argyrophilic proteins makes it possible to visualize NORs in conventional histologic sections by a modification of a silver staining technique.[21-23] Morphologic changes in NORs are signs of rapidly dividing cells with high metabolic activity. Usually NORs are larger and more numerous in malignant than in benign cells, as has been demonstrated in tumors of the breast, endometrium, brain, kidney, prostate, colon and rectum, lymphomas and others.[21, 23, 24]

In dermatopathology NORs have been investigated mainly in melanocytic lesions.[22, 23, 25-28] Significantly higher NOR rates have been found in melanomas than in melanocytic nevi[29] (Figs. 2 and 3). Because melanomas and melanocytic nevi also differ with respect to the immunohistologic assessment of actively cycling cells,[30] the NOR results in

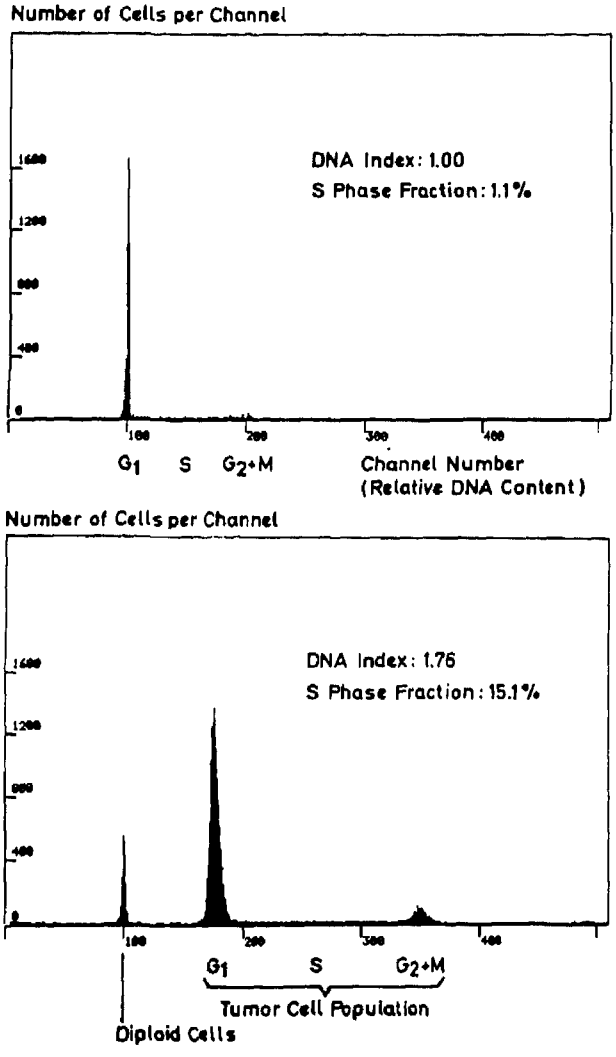

Fig. 4. *Top*, Flow cytometric DNA histogram of normal human skin. The S and G_2+M phase region has been enlarged 8-fold *(dotted line)* to become visible. *Bottom*, Flow cytometric DNA histogram of melanoma metastasis shows small peak of normal diploid cells at channel 100 and a population of aneuploid tumor cells (DNA index = 1.76) with increased S phase fraction (15.1%).

melanocytic lesions may, in fact, indicate differences in proliferative activity. Recent studies indicate that morphometric analysis of NOR areas (e.g., NOR area/nucleus) may be of greater value than simple NOR counting.[29]

Besides melanocytic skin tumors, NOR determination also shows significant differences between keratoacanthoma and squamous cell carcinoma,[31] although the diagnostic accuracy remains to be determined. Remarkable NOR findings have been described in basal cell carcinomas. These tumors seem to have more NORs than other (benign or malignant) epidermal tumors.[32] This may be because basal cells of the normal epidermis possess more NORs than cells of the stratum spinosum.[23]

FLOW CYTOMETRY

Like other cytometric methods, flow cytometry in dermatology is mainly aimed at the diagnostic and prognostic characterization of skin tumors. Flow cytometric measurements of the cellular DNA content provide three types of information (Fig. 4):

- The ploidy state, i.e., the existence or not of abnormal (aneuploid) cell lines
- The DNA index (DI), i.e., the mean cellular DNA content of a cell population relative to the diploid (euploid) DNA value
- The portions of cells in the cell cycle phases, particularly the S phase fraction as an indirect measure of proliferative activity

The flow cytometric histogram of normal human skin (Fig. 4, *top*) shows a predominant narrow peak representing the cells in the G_1 phase of the cell cycle. The portions of cells in the S and G_2+M phase are small, reflecting the relatively slow but continuous proliferation of epidermal cells. The S phase fraction is normally 1% to 2%. In contrast, Fig. 4 *(bottom)* shows the histogram of a tumor sample (a melanoma metastasis). In addition to a peak of diploid cells from normal epidermal cells or lymphocytes in the specimen that can be used as an internal standard to define the position of the diploid value, the histogram exhibits a population of aneuploid (DNA index = 1.76), fast-proliferating (S phase fraction = 15.1%) tumor cells. The clinical usefulness of these flow cytometric data has been proved in many malignancies.[33, 34] The main advantage of flow cytometry is the ability to measure large quantities of cells in a short time. However, problems may arise by preparing single cell suspensions from solid tumor tissues.

In primary malignant melanoma the existence of aneuploid tumor cells is correlated with tumor thickness and is associated with an unfavorable prognosis. Aneuploid tumors with hypertetraploid cell lines and/or more than one aneuploid cell line (so-called multiclonal tumors) exhibit an increased risk of recurrence and metastasis.[35] S-phase fractions above 15% indicate a low survival probability. In the metastatic stage of the disease sequential measurements show that an increased genetic insta-

bility of the tumor as detected by changes in the ploidy pattern is associated with short patient survival time.[36]

In squamous cell carcinomas of the skin aneuploid cell lines and particularly multiclonal cell populations are found more frequently in advanced stages. The proliferative activity is higher in aneuploid tumors than in diploid ones. Thus ploidy state and S phase fraction can be used as prognostic criteria in addition to clinical and histopathologic findings.

Measurements of the cellular DNA and protein content[37] or immunocytologic surface markers may also yield additional information in certain tumor systems.

QUANTITATIVE IMMUNOHISTOLOGY

Immunohistology facilitates the selective staining of defined molecules or antigenic epitopes in histologic sections. Although the intensity of the staining reaction is usually not stoichiometrically correlated with the amount of antigen present, the relative differences in staining intensity are considered to represent differences in the amount of antigen, if the staining procedures are kept identical. Immunohistology has been used to characterize certain cell types in histologic slides, but also for the detection of particular cellular activities (e.g., the expression of oncogene products).

There are two basic approaches for quantifying immunohistologic slides by image analysis. The number, size, and distribution of stained structures can be assessed either by manual, interactive, or automated image analysis. Second, the intensity of the reaction products can be quantified by densitometry with automated image analysis systems.

The analysis of natural killer (NK) cells in malignant skin tumors may serve as an example for quantifying positively stained structures. NK cells are cytotoxic to certain tumor cell clones in vitro and are considered to play a role in host-tumor interaction in vivo. However, most studies implicating host resistance to tumor growth by NK cells have been performed with NK cells isolated from peripheral blood mononuclear cells. In vitro, the experiments have been carried out with an NK cell/tumor cell ratio of 1:1 up to 100:1. However, stereologic estimation of the amount of NK cells and tumor cells in melanomas[38] revealed that only a minority of infiltrating cells belonged to the NK cell population, whereas most of the inflammatory cells were lymphocytes and macrophages. The NK cell/tumor cell ratio in vivo was found to be about 10^3 times lower than that used experimentally in vitro. On the basis of these quantitative immunohistologic results it became obvious that the results obtained in vitro may be highly artificial and cannot be related to the actual situation in vivo.

Immunoelectron microscopy has been used to enhance the accuracy of morphometric measurements in the diagnosis of cutaneous T-cell lymphoma. Limiting the assessment of the nuclear contour index to infiltrating cells expressing a pan-T cell marker excludes potential errors arising from histiocyte-like cells with a cerebriform nucleus.[39]

The other approach to quantitative immunohistology is to measure the staining intensity of the immunohistologic reaction product. S-100 protein is known to be expressed in melanocytic skin lesions. However, there is no qualitative difference between benign and malignant tumors with regard to S-100 protein expression. With automated image analysis, the staining intensity of the immunohistologic reaction to S-100 protein was assessed in benign melanocytic nevi and in malignant melanomas.[40] It was found that the overall staining intensity did not differ between benign and malignant lesions. However, malignant lesions were characterized by an increased staining intensity in the depth of the lesion, which was not the case in benign nevi.

PROLIFERATION IN MELANOCYTIC SKIN TUMORS

A useful candidate for quantitative immunohistology in tumors is the monoclonal antibody Ki-67 that enables the determination of the proliferative pool (G_1, S, G_2, M phase) of a given tissue component on frozen sections in situ.[30]

In a recent study in 145 melanocytic skin tumors mean numeric density (NV mean) and maximum numeric density (NV max) of Ki-67–positive nuclei (number per cubic millimeter) were quantitatively evaluated by interactive image analysis. There was a strong correlation between mean and maximum numeric density ($r = 0.815$; $p < 0.05$) indicating that each of the two factors may be regarded as representative of proliferative activity. Maximum numeric densities in melanocytic nevi, primary malignant melanoma, and metastatic melanoma revealed highly significant differences. In primary malignant melanoma mean and maximum numeric density

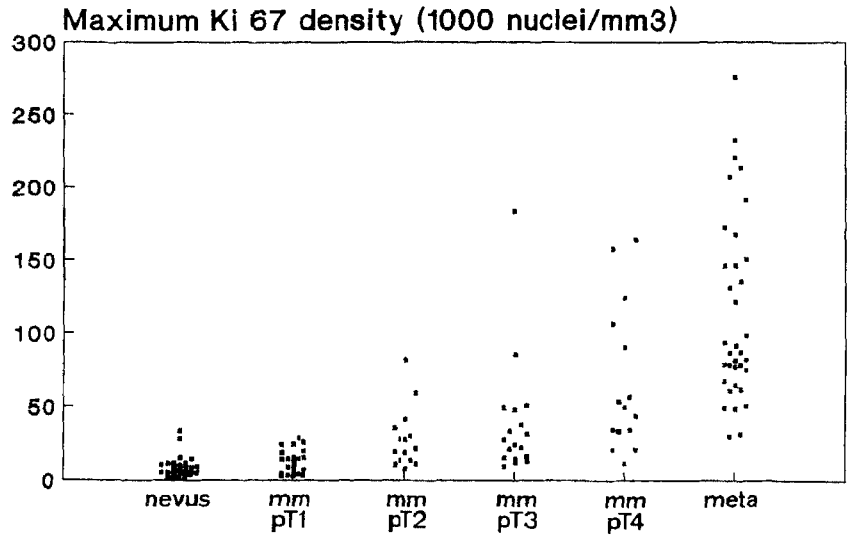

Fig. 5. Maximum numeric density of Ki-67–positive nuclei in melanocytic skin lesions ($N = 145$). The different lanes refer to benign nevi, primary malignant melanoma with tumor thickness ≤ 0.75 mm *(pT1)*, primary malignant melanoma with tumor thickness between 0.76 and 1.50 mm *(pT2)*, primary malignant melanoma with tumor thickness between 1.51 and 3.50 mm *(pT3)*, primary malignant melanoma with tumor thickness ≥ 3.51 mm *(pT4)*, and metastatic malignant melanoma *(meta)*.

values showed a noticeable variation ranging from low values as observed in benign nevi to high values as observed in metastatic melanoma with a considerable overlap between the diagnostic groups (Fig. 5). Within the group of primary malignant melanomas, there was a significant correlation between proliferative activity (NV max) and maximal tumor thickness according to the Breslow or Clark level of invasion and mitotic rate.

A prospective short-term evaluation of the patients with primary malignant melanoma showed a significantly shorter disease-free interval in patients with high NV max. Furthermore, all five patients who died of malignant melanoma were in the group with a high NV max.

The use of quantitative microscopic systems obviously enables more precise data from Ki-67–immunostained preparations to be derived.[41] Further studies will reveal whether the estimation of the maximum numeric density of Ki-67–positive nuclei represents a superior prognostic indicator compared with the well-established Breslow index.

IN SITU HYBRIDIZATION

Similar to immunohistology, image analysis may also be of value in objectively defining the reaction product in in situ DNA or RNA hybridization. Global differences in optical density between labeled and control regions after in situ hybridization were evaluated by Uhl et al.[42] More precisely, image analysis was applied at the cellular level to quantify the grains either interactively[43] or automatically taking background labeling also into account.[44] Reliability and objectivity of this method were evaluated by comparing the values of grains per cell obtained by conventional and automatic techniques after in situ hybridization with α-collagen DNA probes in various specimens.[45] Correlation coefficients were 0.97 for fibroblasts embedded into a three-dimensional collagen gel, 0.94 for dermal fibroblasts in skin obtained from a patient with progressive systemic scleroderma, and 0.90 for fibroblasts in a 2-week-old scar.[44] Because the automatic analysis technique allows a more rapid and reliable quantitative evaluation of in situ hybridization, similar procedures are also being applied in various nondermatologic research projects.[46, 47]

MORPHOMETRY IN EXPERIMENTAL DERMATOLOGY IN VIVO

All aforementioned studies are largely observational. However, quantitative morphology can also be of particular value in experimental investigations. Besides in vitro application of morphometric meth-

ods, many studies have been performed in vivo with image processing to investigate the morphology of cells[48] and tissues (above all vascular network[49-52]). As in other applications, adequate sampling, testing of reproducibility, and critical interpretation are mandatory.

Quantification of cells or their morphologic changes is one of the great advantages of image analysis procedures in comparison to visual assessment. For example, morphometric methods demonstrated by image processing that only contact sensitizers, but not tolerogens (DNTB) or nonsensitizers (DCNB), induced migration of Langerhans cells to draining lymph nodes in mice (manuscript in preparation). Therefore important questions not only in clinical dermatology but also in experimental dermatology concerning immunology and oncology that need quantitative assessment might be answered by morphometry.

Quantitative morphology may also serve as a biologic alternative to classical physical dosimetry in the study of radiation damage.[53] The skin seems to be a preferential organ for the development of biologic indicators because it is constituted of proliferating cells and is always involved if a patient is exposed to ionizing radiation. Short-term organ cultures of human skin were irradiated in vitro with varying doses (1 to 6 Gy, x-rays 240 kV) and kept in culture for different time periods after irradiation (6 to 72 hours). At different time intervals, the specimens were fixed, processed, and analyzed with a fluorescent microscope connected to an image-analyzing device. Whereas no significant change in nuclear area of the basal cells was found after irradiation with 1 Gy, a remarkable reduction of the nuclear area was detected after single-dose irradiation with 6 Gy. In addition, the individual response to identical radiation doses at defined time intervals appeared to vary considerably. Thus quantitative morphology may be a valuable adjunct in assessing the individual biologic effect of ionizing radiation on human skin.

QUANTITATIVE MORPHOLOGY IN VITRO

Numerous aspects of dermatologic research are carried out in vitro. In many cases, nonmorphologic quantification (biochemical, radiochemical, immunochemical assays) is sufficient. In some instances (e.g., tumor cell invasion) morphologic quantification has to be performed.

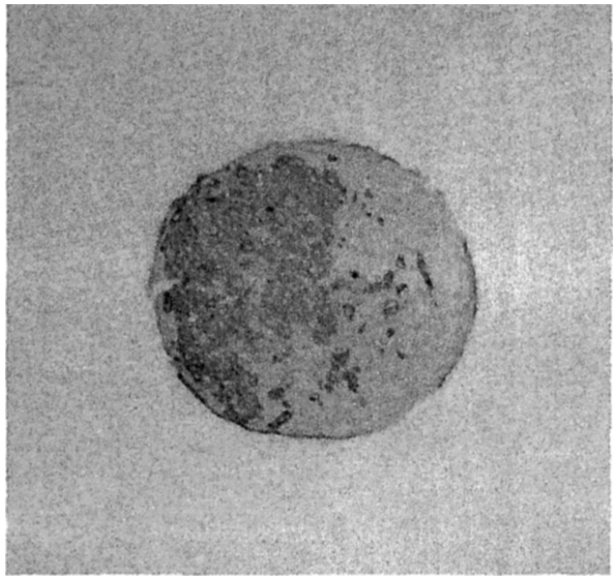

Fig. 6. Confrontation culture of multicellular melanoma spheroid *(right)* with fragment of embryonic chick heart *(left)* after 72 hours. Heart fragment becomes invaded and partially disintegrated by melanoma cells. (Anti–chick heart antiserum; ×100.)

To elucidate the mechanisms involved in tumor cell invasion and to search for antiinvasive strategies, the embryonic chick heart model has been introduced.[54] Tumor cells are cultured as multicellular spheroids of about 200 μm in diameter and are subsequently confronted with fragments of embryonic chick heart, which serve as a stroma analog. After 1 to 7 days, confrontation pairs are harvested, sectioned, and stained immunohistochemically for distinction of the tumor and the stroma component. For quantification, immunohistologically stained sections are fed into an image analyzing computer (Fig. 6). A program has been developed that facilitates the measurement of nine quantitative features simultaneously referring to tumor and stroma proliferation and to several aspects of invasion.[55, 56] These features are created by algorithms of image operations based on mathematical morphology. The statistical evaluation program finally compares various experiments and provides a full text interpretation of the results, thereby providing an objective base for the evaluation of potentially antiinvasive strategies.

THREE-DIMENSIONAL RECONSTRUCTIONS IN DERMATOLOGY

Besides measuring specified features in single images, comparing several different images of the same

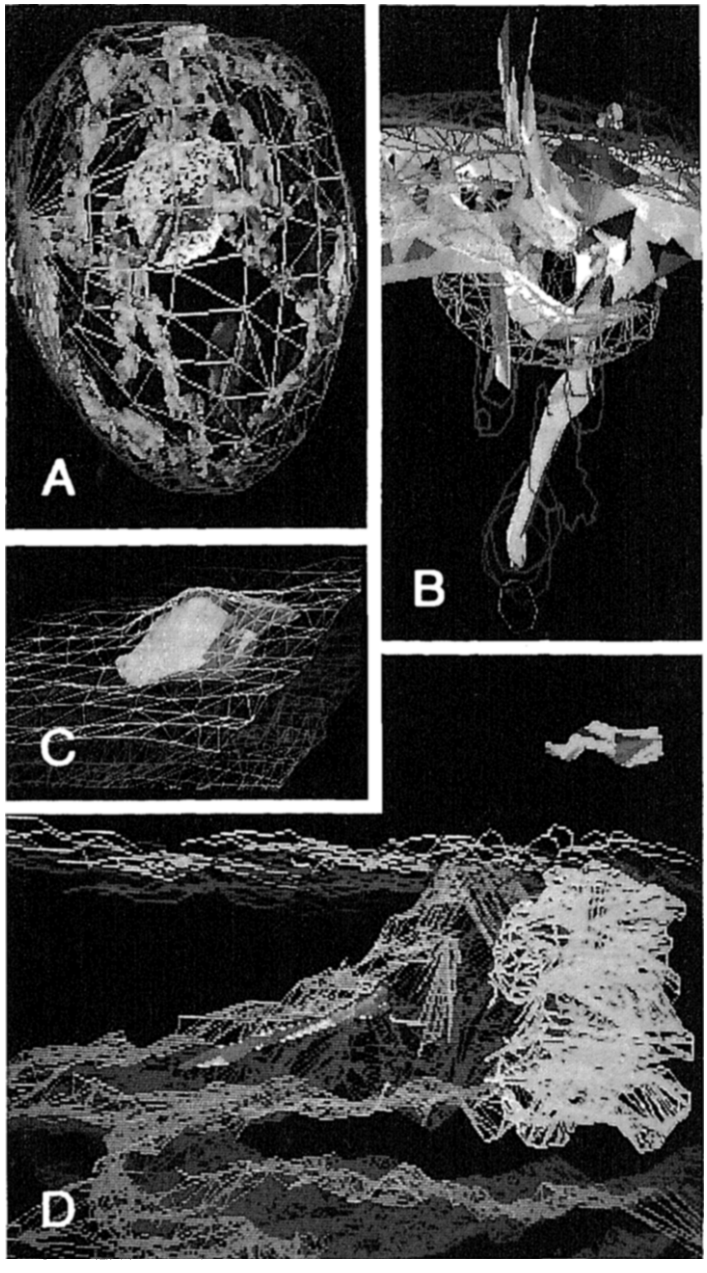

Fig. 7. Three-dimensional reconstruction. Examples of reconstruction of mitochondrion from electron micrographs (**A**), hair follicle structure in keratosis pilaris derived from light microscopy (**B**), senile angioma studied with 20 MHz sonography (**C**), and normal human hair follicle reconstructed from high-frequency ultrasound B-scan sections of skin (**D**).

structure may provide qualitative and quantitative new information beyond the capability of immediate visual examination. This is particularly true when the three-dimensional morphology of particular structures has to be derived from two-dimensional sections. In the past, the structures had to be copied section by section on wax or polystyrol plates, which were aligned to form a section pile. Finally this pile was trimmed to the structure contour, a task that was very labor-intensive.

Computer-aided techniques can reduce considerably the time necessary for such a three-dimensional reconstruction, especially when the interface between the user and the computer has been well de-

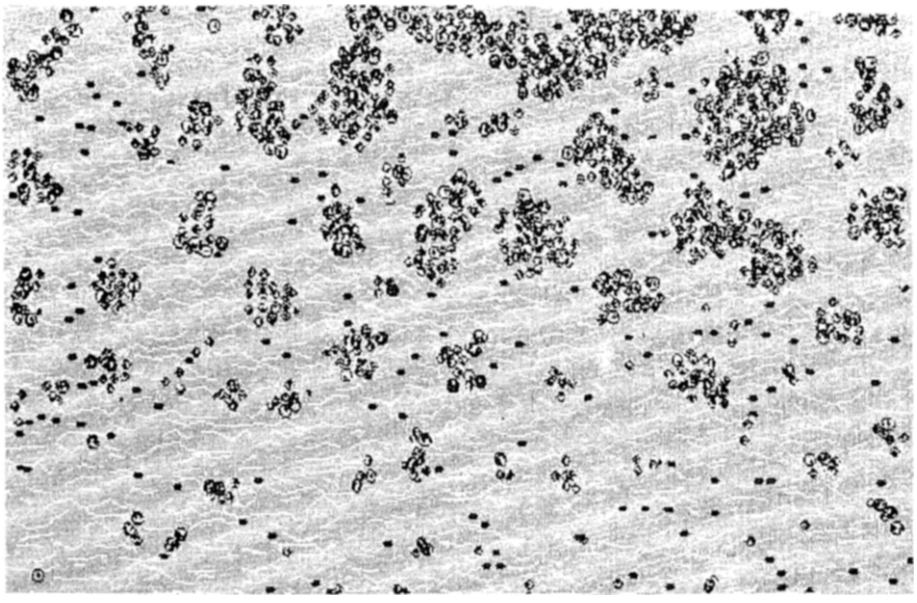

Fig. 8. Tissue modeling. Example of tumor pattern simulated by computer shows morphologic result of moderate degrees of tumor cell proliferation and motility, expansive growth property and strong tumor cell cohesion.

signed. Such reconstruction programs may facilitate different viewing modes for every structure, real-time rotation, scaling, realistic perspective surface modeling with a virtual light source to improve in-depth illusion, and volume and surface calculations.[57-59]

For data input, light or electron micrographs, traced on a digitizer board, automated discriminated structures in on-line images or even ultrasound images can be used. For display, a line-contour model, a wire-frame model, or a surface-area model can be chosen. Combining these different presentation modes, structures within structures can easily be studied.

Fig. 7 shows examples of the reconstruction of a mitochondrion from electron micrographs (Fig. 7, *A*), the hair follicle structure in keratosis pilaris derived from light microscopy (Fig. 7, *B*), a senile angioma studied with 20 MHz sonography (Fig. 7, *C*), and a normal human hair follicle reconstructed from high frequency ultrasound B-scan sections of the skin (Fig. 7, *D*).

In addition to three-dimensional reconstruction as just described, the measurement of features corresponding to the real three-dimensional tissue may be of substantial value. Tumor volume measurements obtained from serial sections of cutaneous melanomas proved superior to thickness as a prognostic indicator.[60]

TISSUE MODELING AND TISSUE INTERPRETATION

Although the descriptive analysis of tumor morphology provides useful hints for diagnostic and prognostic assessment,[61, 62] the relation between biologic properties of the tumor cells and the evolving morphologic patterns is not yet clear. To elucidate this relation, tissue interpretation through tissue modeling has been introduced.[63]

The method is based on the development of a mathematical model of the dynamic process underlying a particular morphologic situation, for example, the process of tumor cell growth, motility, decay, and stroma interaction in the case of morphologic tumor patterns. After formulation and implementation of the model, a large reference set of simulations is created, with virtually all possible variations of the simulation factors. From a comparison of the simulation factors, which are set at the beginning of each simulation, with the resulting morphologic pattern, it can be learned in which way specific biologic properties influence the morphologic appearance[64-66] (Fig. 8). Thus, for example, the importance of both tumor cell proliferation and motility for

morphologic patterning can be demonstrated. The assumptions of the simulation model are supported by observations of melanocytic skin tumors[64, 66, 67] and of animal experimental systems.[65] As soon as the relation between biologic properties (i.e., simulation factors) and morphology has been quantitatively established in computer simulations, this information can be used to estimate these biologic properties from static histologic images of real tumors. In melanocytic skin tumors, biologic tumor cell properties estimated by this procedure proved to have prognostic significance.[67] Thus tissue modeling by computer simulation may lead to tissue interpretation beyond the capabilities of pure heuristic description.

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