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SHAPE DESCRIPTORS IN DIAGNOSIS

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ABSTRACT

The aim of the study was to analyze the behaviour of the shape descriptors of cell nuclei comparing them with other parameters normally used in digital image analysis. The study was carried out in two steps: 1) Parameters of several different parameter sets (shape descriptors, invariant moments, parameters derived from the histogram and the co-occurrence matrix of the extinction values of the pixels and partially densitometric parameters) were compared with one another in five patient groups (colon carcinoma, colon adenoma, urothelial papilloma, prostatic carcinoma and macrophages in broncho-alveolar lavages). 2) The most important parameters of each set, detected by a factor analysis, were matched in a new general data base and newly analyzed. According to the results of these analyses, two main groups of shape descriptors can be postulated: The "key parameter" of the first group is the axial ratio (B/A), either calculated by the nonlinear least squares fit method or by a Fourier analysis. The "key parameter" of the second group is the bending energy. Additionally, a close correlation between the two parameters, axial ratio (B/A) and the second invariant moment (PHI 2), describing the nuclear texture, could be observed in each of the five patient groups.

Key words: form factor, shape descriptor, carcinoma, adenoma, AIDS, macrophages.

INTRODUCTION

The term "form" has played an important role in the metaphysics of Aristoteles (384 to 322 before Christ). He distinguished between form and material. According to Aristoteles, objects become more real if they assume more defined forms; or, of two objects, the one with more form is also more real (Russell, 1946). Does this statement still hold true after more than 2300 years and does it serve as a basis for our continuous efforts to describe morphological alterations using the most up-to-date methods of quantitation?

When investigating the significance of form alterations in pathology, the subcellular (e.g. nuclear) or cellular form and the form of tissue structures should be distinguished. The term "form of tissue structures" is not yet widely used. The form of nuclei and cells is easy to recognize by the examination of one single object. The analysis of the form of a tissue structure requires, however, the assessment of the interrelationship with other structures, in addition to the assessment of single objects (Rodenacker and Bischoff, 1990).

Nuclear pleomorphism, increased mitotic activity and alterations in chromatin structure have long been recognized as hallmarks of neoplasia, and have been integrated into most of the currently used grading systems for dysplasia or malignant tumours. The morphometric equivalent of nuclear pleomorphism is an irregularity as well as variability of the nuclear shape. Therefore, quantitative measurement of nuclear shape could be expected to show some correlation with tumor biology, perhaps even with prognosis in a given patient (Umbricht et al., 1989).

The circle-related form factor, which is often used as a shape descriptor is not highly discriminative. It measures any deviation from a circle, including perfectly elliptical shapes, which are not necessarily an expression of a pleomorphism of the structure (Gschwind et al., 1986). Therefore, we began to look for further shape descriptors. Some were found in the scientific literature (Young et al., 1974; Bowie and Young, 1977; Gschwind et al., 1986), others were developed by our group. In morphometrical and digital image analysis, the following parameters were routinely calculated in addition to the shape descriptors: The profile area of the objects of interest (mostly nuclei); parameters derived from both the histogram and the co-occurrence matrix (Haralick et al., 1973) of the extinction values of the pixels; four of the invariant moments; as well as densitometric parameters in a narrower sense (e.g. DNA index, relative integrated optical density). These parameters are fully described and explained by Christen et al. (1989) and Oberholzer et al. (1991 a).

When using many of parameters for quantifying morphological structures two questions arise. What is the value of the parameters for discriminating diagnostic or prognostic groups? Which relationships or correlations exist among the parameters used? In the present paper, we have focused mainly on the second question as applied to shape descriptors.

MATERIAL AND METHODS

Patient groups

Five different patient groups were examined. The characteristics of each group are shown in table 1. The patients with colon carcinoma (group A) were selected prospectively and at random. In this group, the influence of various method for preparing measurable specimens on the several parameters was studied. Three types of specimens were differentiated: Histological sections, routinely prepared; cytological monolayer smears made

Table 1. The analyzed patient groups and subgroups. A: colon carcinoma, B: colon adenoma, C: urothelial papilloma, D: prostatic carcinoma, E: macrophages in broncho-alveolar lavages. H: histology, C: cytology. Feul: Feulgen, Papa: Papanicolau. (For further abbreviations see the text.)

Group	Sub-group	Number of patients	Number of nuclei	Preparation	Staining
A 1	--	9	60	H	Feul
2	--		60	C (fresh)	Feul
3	--		60	C (tissue)	Feul
B	Mild	10	30 - 35	H	Feul
	moderate	9	31 - 36	H	Feul
	severe dysplasia	10	31 - 39	H	Feul
C	Pap	10	57 - 69	H	Feul
	Pap --> Pap	8	60 - 70	H	Feul
	Pap --> Carc	8	42 - 76	H	Feul
	Carc	8	61 - 64	H	Feul
D	Short surviving	8	76 - 99	C	Papa
	long surviving	11	56 - 108	C	Papa
E	Control	11	47 - 49	C	Papa
	AIDS	13	40 - 50	C	Papa
	AIDS with pneumocystis carinii	6	44 - 50	C	Papa
	Carcinoma	6	34 - 49	C	Papa

from fresh tumour material; and specimens from embedded tissue blocks by dissolving 25 µm thick sections (Hubler et al., 1992, in preparation).

The patients in the "urothelial papilloma" study (group C) were observed during 15 years. The first subgroup (Pap) contained cases without recurrence in the observation period, the second cases, which showed 2 - 10 recurrencies (Pap --> Pap), the third, cases which developed a carcinoma in the observation period (Pap --> Carc) and the fourth consisted of carcinomas (2 cases with the staging Ta N0 M0 and 6 cases with the staging T2 N0 M0) (Carc). In all subgroups the first biopsy were evaluated.

The 19 cases with prostatic carcinoma (group D) had the following common characteristics: Staging T2-3 Nx M0, over 50 years old at the time of diagnosis and treated with a palliative therapy. The subgroup "short surviving" died of the carcinoma within a period of 53 months; the subgroup "long surviving" was still living 70 months after diagnosis.

The analyses were carried out on a VIDAS image analysis system of the KONTRON company, Eching near Munich (Federal Republic of Germany). VIDAS is a system working with 256 gray values and is based on an IBM compatible computer (386 microprocessor). The system was connected with a photomicroscope (ZEISS, Oberkochen, Federal Republic of Germany) by a CCD camera. For the illumination of the test field, a voltage of 3.0 to 4.5 volts was used. The measurements were carried out on shading corrected images and at a magnification of 1:787. The software was

developed at the Department of Pathology, University of Basel. The whole image analysis system consists of two parts: The tool for interactively or semiautomatically measuring the raw (primary) data (extinction values of the pixels) and the tool for calculating the image analytical parameters (secondary data) from the raw data. The coordinates of the object contours needed for calculating the shape descriptors were calculated from the primary data by using a special algorithm. The fundamental concept for managing all the data obtained from the measuring system is described by Oberholzer et al. (1991 a).

Shape descriptors

Table 2 gives an overview of the shape descriptors used in this study and in our image analysis system. The individual shape descriptor is extensively described by Gschwind et al. (1986).

The roundness factor (RNF, circle-related shape descriptor) is defined by the formula (eq.1):

$$RNF = C^2 / 4 \pi A \quad (1)$$

C: Circumference of the object
A: Profile area of the object.

The fundamental formula of the ellipticity factors is:

$$EL = RNF_{orig} / RNF_{ell} \quad (2)$$

Table 2. View of the shape descriptors used.

Shape descriptors	Abbreviation	Determination of the axial ratio of the contour	Key elements
Roundness factor	RNF	--	--
Ellipticity factors	EL (Pro)	Projection method	--
	EL (Fit)	Nonlinear least squares fit method	--
	EL (Mom)	Principal moments of inertia	--
	EL (Four)	Fourier analysis	--
Percent difference area between the original contour and the fitted ellipse	PDAF	Nonlinear least squares fit method	--
Concavity factors	PCAF	--	Convex hull
	PARIS	--	Concave contour segments
Bending energy	BEND	--	--
Axial ratio	B/A (Fit)	Nonlinear least squares fit method	
	B/A (Four)	Fourier analysis	

RNF_{orig}: Roundness factor of the original contour

RNF_{ell}: Roundness factor of the corresponding ellipse determined by computing the axial ratio.

To obtain the axial ratio, it is necessary to find the best "fitting" ellipse. To obtain the best "fitting" ellipse we developed the following approaches: Projection, nonlinear least square fit, principal moments of inertia, Fourier analysis. With the projection method, the length of the projections of the contour on the x-axis (i.e. the Feret diameters) is measured. The contour is rotated, and the Feret diameter of the projections at every rotation angle is calculated.

The nonlinear least squares fit method consits of iteratively fitting an ellipse through the contour, until an ellipse with a minimal "difference area" is obtained. This form factor is influenced by the size of the profile area of the structure under investigation.

The following two approaches were used for the determination of the concavity factors: The first method consists of determining the convex hull of the contour, calculating the area of the contour given by the new, partly original and partly convexe hull, and subtracting this area from the profile area of the original contour. The corresponding concavitiy factor is defined as:

$$PCAF = [(A_{cont\ new} - A_{cont\ orig}) / A_{cont\ orig}] \cdot 100 \quad (3)$$

The second method is based on the calculation of the Feret diameters of the objects and by the projection of m small straight segments of the contour of the objects onto the x-axis, while the objects are rotated in the x- / y-plane. (For details see Gschwind et al., 1986.)

If a circle made on a homogenous elastic material is deformed, energy is needed. This picture illustrates the term "bending energy". It should be kept in mind that the bending energy of an object is dependent of the profile size of the object. The bending energy of a circle, for instance, is inversely proportional to the area (Bowie and Young, 1977).

For calculating the parameters identifying the DNA content, histological or cytological standard preparations of human lymphocytes or of erythrocytes of chickens were used. In addition to these parameters, other parameters quantifying the deviation of the integrated optical density (IOD) in the subgroups of the group "colon adenoma" from the IOD of human lymphocytes were calculated. These calculations are based on the upper 90% conficence limit of the median value of IOD [$IOD_{(2c)}$] of human lymphocytes [$IOD_{(2c,90\%)}$]. The following formulae are applicable for the class width (CW) and the offset point (OP) of the histogram:

$$CW = [IOD_{(2c,90\%)} - IOD_{(2c)}] \cdot 2 \quad (4)$$

$$OP = IOD_{(2c)} - 1.5\ CW \quad (5)$$

It is of great interest to compare the shape descriptors with the other textural or densitometric parameters. These comparisons were made by applying the Kayser image factor analysis (Feldman et al., 1987). From each of the four parameter groups (shape descriptors, invariant moments, parameters derived from the histogram and the co-occurrence matrix of the extinction values of the pixels and partially densitometric parameters) the factor analysis was carried out in a first step. The parameters demonstrating the highest loading value on each resulting factor were extracted and integrated in a general, but reduced data base for each patient group. In a second step, the factor analysis was re-applied to this new data base.

The data resulting from discriminant analyses additionally carried out were not taken into consideration in this paper because of space; they will be prepared for presentation in a complementary paper.

STATISTICAL ANALYSIS

For every variable discussed below the following statistical parameters were calculated for each patient from 30-108 nuclei: median and mean value, standard error of the mean, variation coefficient (standard deviation / mean) and relative variation coefficient (standard error of the mean / mean) (Sachs, 1978). In the statistical test procedures, the median values of the variables were used.

The relationships among the single parameters were tested by calculating the Spearman rank correlation coefficients. Additionally, the above mentioned multivariate analyses were carried out. For analyzing the relationship among the shape descriptors, principal component analysis was used. The relationship among all parameters was tested by the Kayser image factor analysis. In both methods, the loading values on the resulting factors were considered to be relevant if their values were $\geq 2\ [1 / \sqrt{n}]$. The number of factors to extract depends upon the eigenvalues (eigenvalue > 1) (Feldman et al., 1987). As a transformation method, we selected the oblique solution.

For comparisons between two subgroups , the Mann-Whitney test was used; for comparisons among three subgroups the Kruskal-Wallis test. When the subgroups were paired, the Wilcoxon test and the Friedman test were applied respectively. The results of the tests were considered to be statistically significant if the two-sided error probability was equal to or lower than 5% ($2P \leq 0.05$).

RESULTS

The results can be seen from three different perspectives: 1) The influence of the preparation methods on the values of the shape descriptors; 2) the interdependence between the various

shape descriptors; 3) the correlations among all the parameters, usually calculated in digital image analysis.

Influence of the preparation methods on the values of shape descriptors

The influence of the methods for preparing specimens for digital image analysis was studied on nine patients with colon carcinoma. All shape descriptors used showed significant differences among the three methods (cytological smears from fresh material, cytological smears from embedded material and histological sections). The two-sided probability error was between 0.001 and 0.020. However, when taking the mean profile area into consideration, no statistical differences could be observed (fig. 1). The values of B/A (Fit) and RNF resulting from the evaluation of histological sections were similar to those resulting from the evaluation of cytological smears made from embedded material (fig. 2). Considering the values of the bending energy and of PARIS derived from histological sections, similarity to the values obtained on cytological smears made from fresh material could be established (fig. 3).

Interdependence between the various shape descriptors

The results of the comparisons among the different shape descriptors in the five patient groups analyzed are presented in table 3a-3b. Viewing these results, one discovers that in all groups the parameters B/A (Fit) or B/A (Four) respectively both lie on the identical factor as the parameters RNF, EL (Mom) and EL (Four), except for the group "macrophages in broncho-alveolar lavages". The loading values of all these parameters were high. The parameters were all independent from both the mean profile

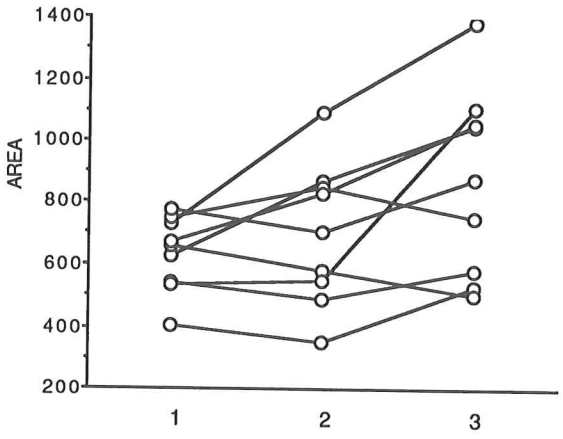

Fig. 1. Patient group "colon carcinoma". Profile area (pixels) = f(preparation method). 1st column: cytological preparation from fresh material; 2nd column: cytological preparation from embedded material (25 µm thick tissue sections); 3d column: histological section.

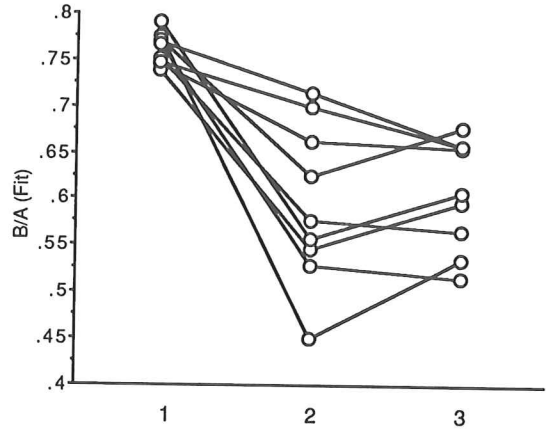

Fig. 2. Patient group "colon carcinoma". B/A (Fit) = f(preparation method). 1st column: cytological preparation from fresh material; 2nd column: cytological preparation from embedded material (25 µm thick tissue sections); 3d column: histological section.

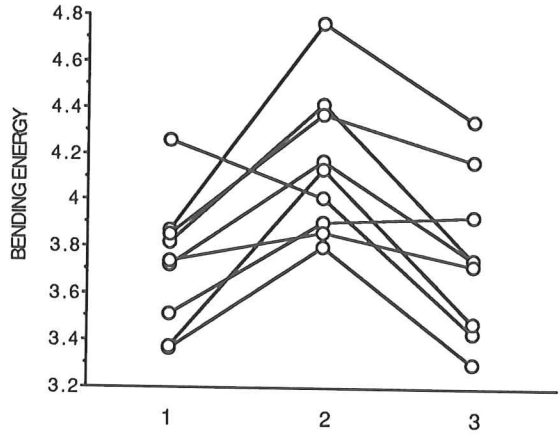

Fig. 3. Patient group "colon carcinoma". Bending energy = f(preparation method). 1st column: cytological preparation from fresh material; 2nd column: cytological preparation from embedded material (25 µm thick tissue sections); 3d column: histological section.

area and the bending energy (tbl. 3a). In all five groups, the bending energy lay on the identical factor as the parameters EL (Pro), EL (Fit) and PARIS (except for group A2, tbl. 3b) and also showed high loading values. The theoretically known correlations between the bending energy and the mean profile area could be empirically observed in all groups or subgroups, except in the subgroup "colon carcinoma, histological sections" (group A1, tbl. 3b). These findings permit one to propose that the presented shape descriptors can be divided in two different

Table 3a. Results of the principal component analysis in the set of the shape descriptors: Loading values of the extracted factors, on which the parameter B/A (Pro or Fit) was sited. In () loading values below the relevant limit are indicated (see also tbl. 1). --: not significant.

Group	B/A	AREA	RNF	EL (Mom)	EL (Four)	PDAF	PARIS
A 1	-.991	--	.919	.666	.682	--	--
2	.952	--	(-.426)	--	--	--	--
3	-.985	--	.943	.663	.730	.787	--
B	-.954	--	.983	.848	.849	.576	.670
C	-.902	--	.830	.695	.658	.729	--
D	-.939	--	.941	.679	.603	--	--
E	.975	--	-.588	--	--	--	--

Table 3b. Results of the principal component analysis in the set of the shape descriptors: Loading values of the extracted factors, in which the parameter bending energy was sited (see also tbl. 1 and tbl. 3a).

Group	BENDING	B/A	AREA	EL (Pro)	EL (Fit)	PDAF	PARIS
A 1	.773	--	--	.778	.786	.910	.946
2	.678	--	-.939	--	--	--	--
3	.705	--	(-.628)	.978	(.633)	--	.807
B	.805	--	-.807	.932	.951	.649	.694
C	.812	--	-.584	.888	.944	--	.513
D	.912	--	(-.464)	.965	.937	.729	.933
E	.925	--	-.837	.978	.945	.536	.922

groups (tbl. 4) describing two different aspects of shape alterations.

Correlations among all the parameters, usually calculated in digital image analysis

Analyzing the results of the factor analyses carried out on the general data base of each patient group (tbl. 5a-e), the following observations can be made: The shape descriptors demonstrate the highest loading value nearly always on the second factor (Fact 2), never on the first factor (Fact 1). In all five patient groups, B/A come out as one of the most important shape descriptor, because this parameter is extracted

Table 4. Two main groups of shape descriptors can be distinguished. (For details see the text.)

Group	"Key parameter"	Correlated parameters
# 1	B/A (Fit) or B/A (Four)	El (Mom), El (Four), RNF
# 2	BENDING ENERGY	El (Pro), El (Fit), PARIS

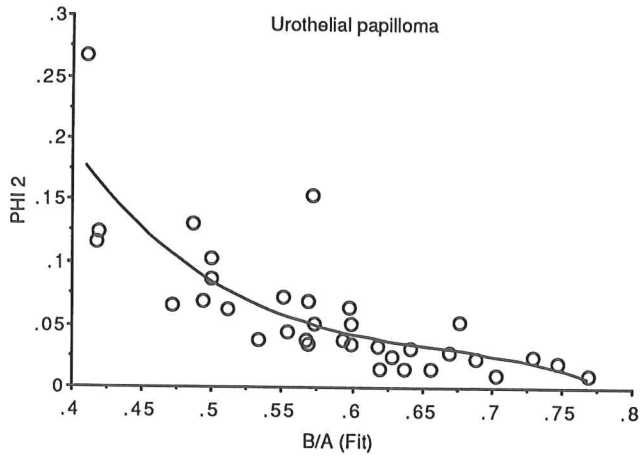

Fig. 4. Patient group "urothelial papilloma". PHI 2 (second invariant moment) = f[axial ratio (B/A (Fit))] in the patient group "urothelial papilloma" [rho = -0.844, 2P < 0.001)].

in the first step in each patient group. A narrow correlation with a negative sign of one of the two loading values can be observed between B/A and PHI 2 in each patient group (fig. 4).

Table 5a. Patient group "colon carcinoma". Results of the Kayser image factor analysis on the general data base composed with the most important parameters of the single parameter set. From the sets, the parameters with the highest loading value on the extracted factors (also determined by a Kayser image factor analysis) were introduced in the general data base. PHI: invariant moments. ANISO/ENTRO: quotient of anistropy and entropy calculated from the distributions of the extinction values. OD MEAN: mean of optical density. Hist: derived from the histogram. Cooc: derived from the co-occurrence matrix. Fact 1: extracted factor #1 (and so on).

		Fact 1	Fact 2	Fact 3	Fact 4	Fact 5
EL	(Pro)	-.022	**.699**	.057	-.017	.134
B/A	(Four)	-.799	-.175	.061	-.004	.042
PHI 2		**.892**	-.040	-.180	.053	.276
PHI 3		.017	.001	.192	-.088	**.624**
ENTROPY	(Hist)	.013	-.021	**.802**	.305	.094
ANISO/ENTRO		-.114	-.447	-.415	.264	.156
OD MEAN		.689	-.175	.133	-.081	-.093
ENTROPY	(Cooc)	-.050	.004	.181	**.939**	-.036
VARIANCE		-.478	.044	-.293	.066	.298
CONTRAST		-.031	.548	-.235	.065	-.053
Total explained variance		.322	.245	.087	.192	.154

Table 5b. Patient group "colon adenoma". (See explanations of tbl. 5a.) IOD: integrated optical density. ASM: angular second moment. DI_MEDIAN: quotient of the median value of IOD in the test group and in the calibrating group.

		Fact 1	Fact 2	Fact 3	Fact 4	Fact 5
AREA		.693	.018	.062	.046	.021
PDAF		-.119	-.321	-.071	.001	.147
PARIS		-.018	-.419	-.005	-.260	.175
B/A	(Four)	-.014	**.865**	.001	.068	.248
PHI 2		-.024	-.828	.136	.230	-.045
PHI 3		.084	-.006	.228	.087	**.767**
ANISO/ENTRO		-.019	.047	.029	**.691**	-.045
ENTROPY	(Hist)	-.077	.070	**-.939**	-.050	-.150
IOD		.819	-.005	.003	-.090	-.022
CORRELATION		-.057	.021	.038	-.602	-.100
CONTRAST		-.221	.012	.742	-.090	-.001
ASM		.226	-.170	-.092	.317	.173
DI_MEDIAN		**.833**	.012	.011	-.106	-.019
Total explained variance		.259	.196	.117	.173	.082

Table 5c. Patient group "uorthelial papilloma". (See explanations of tbl. 5a.) INV DIF MOM: inverse difference moment.

		Fact 1	Fact 2	Fact 3	Fact 4	Fact 5
B/A	(Fit)	.305	-.777	.022	.026	.224
EL	(Fit)	-.098	.006	-.225	**.886**	-.082
PDAF		.038	.622	.090	.293	**-.603**
PHI 1		.421	.452	.640	-.001	.108
PHI 2		.064	**.892**	.230	-.049	.136
ENTROPY	(Hist)	**.873**	-.138	.031	-.001	-.068
ANISO/ENTRO		-.160	-.001	.851	-.212	-.032
CONTRAST		-.506	.005	.102	.524	.556
ENTROPY	(Cooc)	.698	-.287	.286	.108	.124
INV DIF MOM		-.225	.804	-.048	-.017	.003
MEAN OF SUMS		.012	-.015	**.905**	.002	-.003
Total explained variance		.248	.278	.223	.136	.075

DISCUSSION

The shape descriptors were introduced to complete the usually applied set of parameters for quantifying morphological alterations (Pesce Delfino et al., 1990). The probability of finding relevant differences among various diseases increases when many parameters are used. Many parameters grasp more aspects than only few. As a reminder, the term "parameter" in the context of this paper means the median value of all the

Table 5d. Patient group "prostatic carcinoma". (See also explanations of tbl. 5a, 5b.)

		Fact 1	Fact 2	Fact 3	Fact 4	Fact 5
PCAF		-.149	-.276	**.860**	-.103	.278
B/A	(Fit)	.034	**-.891**	-.025	.085	.046
PDAF		.137	.227	.795	.048	-.004
PHI 4		.306	.303	-.139	.340	.565
PHI 2		.145	.889	-.037	-.038	.097
ENTROPY	(Hist)	-.006	-.024	-.568	.054	.128
IOD		-.121	.108	-.248	.315	**-.690**
ANISO/ENTRO		**.860**	.090	.101	.001	-.023
ASM		.217	-.150	.036	**.843**	-.021
CONTRAST		.145	-.002	.071	-.792	-.001
VARIANCE		-.832	.001	.059	.020	-.014
Total explained variance		.220	.228	.181	.166	.146

Table 5e. Patient group "Macrophages in broncho-alveolar lavages". (See also explanations of tbl. 5a, 5b, 5d.)

		Fact 1	Fact 2	Fact 3	Fact 4	Fact 5
PDAF		-.007	.389	.054	-.266	-.003
PARIS		.009	**.863**	-.063	.121	.055
B/A	(Fit)	-.067	.049	**-.869**	.055	.220
PHI 4		-.044	-.001	-.025	.007	**.717**
PHI 2		-.083	-.017	.818	.074	.233
OD MEAN		-.025	.059	-.045	**-.798**	.007
AREA		.007	-.335	.016	.270	.033
ENTROPY	(Hist)	-.752	.171	.071	.115	-.059
CORRELATION		-.178	-.100	.004	.667	.022
ASM		**.784**	-.013	-.053	.080	.034
CONTRAST		-.001	.308	-.028	.555	-.034
INV DIF MOM		.605	.389	.252	.001	-.033
Total explained variance		.219	.192	.149	.280	.094

parameters in discussion. Disponing of many parameters, the problem is to know relationships which may exist among the single parameters. If this is the case, one wishes to find out the most powerful parameters and to correctly reduce the total, partly redundant, information in the whole data base by reducing the number of variables (parameters).

According to the appropriate statistical tests, the existence of two groups of shape descriptors can be postulated (tbl. 4). The "key parameter" of the first group is given by the axial ratio (B/A), either calculated by the nonlinear least squares fit method or by a Fourier analysis. This theory is based on the following facts: When B/A could be observed as lying on a factor

resulting from a factor analysis, then its loading values were always very high. This observation was made in every analyzed patient group and can be interpreted as sign of a great contribution of B/A to the mathematically found factors.

The bending energy turned out to be the "key parameter" of the second group. It was the sole shape descriptor in each of the five patient groups being absolutely independent from B/A. The same yields for EL (Pro) and EL (Fit). However, because these two parameters belong to the ellipticity factors and because the two other ellipticity factors [EL (Mom) and EL (Four)] showed strong correlations with B/A, the parameters EL (Fit) and EL (Pro) were not designated as "key parameter" of the second group.

The relationships between the ellipticity factors and RNF on the one hand and B/A on the other hand were additionally tested in a theoretical model. In a first step, 16 different descending values for B/A were defined. Then the values of the four ellipticity factors, as well as the value of RNF, were calculated for each above mentioned axial ratio. Finally, the results of the shape descriptors were compared with B/A. In this partial study, it could be demonstrated, that the values of EL (Pro) and EL (Fit) decreased only to a very small extent combined with ascending axial ratios. Whereas at the same time, the values of EL (Mom) and EL (Four) as well as those of RNF significantly fall up (fig. 5). This observation suggests again a separation of the ellipticity factors into two different groups as demonstrated in table 4. Both parameters, EL (Pro) and EL (Fit) allow formal recognition of an elliptic object (profile area) as a true ellipse, but nothing more. EL (Mom) and EL (Four), however, not only indicate that the analyzed object must be an ellipse but additionally also contain information concerning the shape of the ellipse. These findings derived from the theoretical model agree very well with the results empirically found on our material by multivariate factor analyses.

A very surprising observation made during the systematically carried out analyses was the correlation between both the two parameters B/A and PHI 2 (second invariant moment) ($n = 34$, $rho = -.844$, $2P < 0.001$, fig. 4), observed in each of the five patient groups. From analyses on cell nuclei of pleural effusions (Oberholzer et al., 1991 b) and on model images (Oberholzer et al., 1992, in preparation), it is well known that the invariant moments, calculated according to Christen et al. (1989), describe the geometrical distribution of the chromatin on the one hand and are influenced by the mean optical density on the other. Therefore, the correlation between B/A and PHI 2 can be interpreted as expression of complex morphological alterations occurring in biological processes. Such processes seem to be reflected not only in densitometric parameters or in invariant moments, for instance, but also in shape descriptors. These findings emphasize the importance of multivariate measurements for adequately quantifying morphological alterations.

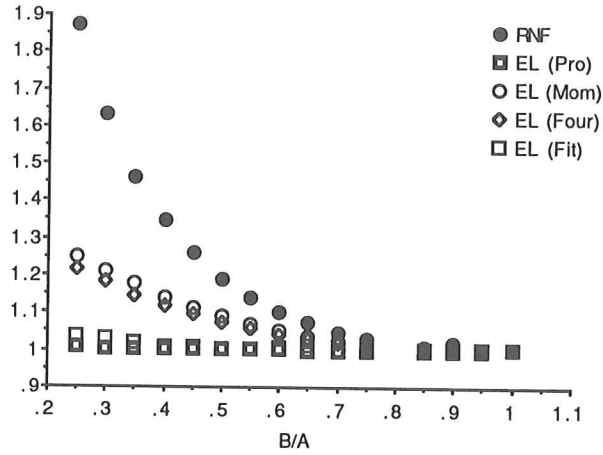

Fig. 5. Results of the analysis of a theoretical model: Correlations among the axial ratio (B/A), the roundness factor (RNF) and the ellipticity factors [EL (Pro), EL (Mom), EL (Four), EL (Fit)].

It could expected that the preparation methods for measurable specimens influence the values of the shape descriptors, because the cells are very differently treated in transit from fresh tissue to the glass slide. The values of the shape descriptors showed statistically significant differences among all the three groups of preparation methods. The error probability of the observed differences was higher for the three parameters: bending energy; EL (Pro); and EL (Fit) ($0.01 < 2P < 0.05$) than for the other shape descriptors ($2P < 0.01$). To what extent this finding may indirectly support the thesis that the shape descriptors can really be subdivided in two groups, cannot be definitively answered. One reason for this can be the small size of the examined collective ($n = 9$). In contrast to the shape descriptors, the mean profile area seems not to be significantly influenced by the preparation method, because no significant differences could be found (fig. 1).

Aside from the preparation method, the methods for object segmentation must be taken into consideration as also affecting the values of the shape descriptors (Gil et al., 1986; Marchevsky et al., 1987). In the present paper, the cell nuclei were exclusively interactively segmented by the same person. Therefore, an additional methodological influence beside of a systematic bias on the above discussed results can be excluded.

The analysis of the shape of an object should not be limited to the assessment of the roundness factor, as is often still the case. Ellipticity, concavity factors and bending energy should be assessed as well. The bending energy and/or the shape descriptor PARIS must be considered as the two most important and discriminatory shape descriptors. This statement can be supported not only from the results of the present paper but also from papers published earlier (Oberholzer et al., 1991 b).

Using shape descriptors, however, it must be taken into account, that the absolute value of form factors as well as the individual contribution of each factor to a better discrimination between normal and disease, depends on the tissue or organ investigated, as well as on the method for preparing measurable histological or cytological specimens.

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