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MORPHOLOGICAL MARKERS IN CYTOLOGY

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ABSTRACT

Morphological markers in cytology concern cytometric features of diagnostic relevance. These may be global size or shape descriptors, global photometric features, such as mean or total extinction of cellular constituents, and finally features describing the fine structure of the chromatin distribution in the nuclei. The role of cellular morphological markers and their validity with respect to different cytological tasks, such as diagnosis of cervical intraepithelial lesions, tumor diagnosis or typing and prognostic grading of cancer was investigated in gynecological smears and fine needle aspirates of the thyroid and the breast.
In all cases it was evident that chromatin pattern features, which can only reasonably be derived by local resolutions at the physical limits of light microscopy, are more powerful than global karyometric or photometric features.

Keywords: Breast, cervix, chromatin feature, morphological markers, thyroid.

INTRODUCTION

Visual cytology and cytopathology is based to a great extent on morphological features of single cells and tissues. The measurement of such features is called cytometry. Low resolution cytometry concerns flow-through techniques or karyometry. The measureable features of cell nuclei, when applying these techniques, concern typical size and shape and only in a few cases are additional parameters derived as e.g. from light scattering in flow-through, displaying to some extent the internal nuclear structure. The explicit and direct measure of this nuclear chromatin structure, in addition to all other morphological and photometric features, is only possible by high resolution scanning techniques.

MATERIAL AND METHODS

We investigated morphological features of isolated cells by means of TV-image analysis with a local resolution defined by 0.25 µm pixel distance and a nominal grey level resolution of 8 bits at a wavelength of 550 nm. The material varied greatly from exfoliative gynecological specimens (monodis-

perse, wet fixed, Papanicolaou stained), to fine needle aspirates of the thyroid and breast (air dried, Pappenheim stained).
The features extracted from the original digitized nuclear images can be grouped as follows:

morphological: -global: size, shape
-structural: number, size, shape of hyper- and hypochromatic regions
-textural ≡ structural after twodimensional linear and nonlinear filtering displaying local correlations

photometric: -grey level histograms of unfiltered and filtered nuclei and subregions of it and moments of these histograms.

In total more than 100 features may be defined, with generally less than 10 being used for cytopathological correlation analysis and classification, which were suitably selected by statistical tests.

GOALS

The goals in the cases presented here were
- detection and grading of cancer and its precursors in gynecological smears (monodisperse specimens)
- tumor malignancy diagnosis and cancer typing in thyroid aspirates
- prognostic cancer grading in breast aspirates

RESULTS

- Gynecological Cancer and Precursors

An automated screening machine must be able to recognize accurately enough all benign cell types and corresponding clusters, constituting the vast majority of cells in a smear, so that the false positive single cell decision rate does not cover the true positive event rate. A possible approach is to train a classifier to distinguish stepwise benign cell classes from the rest and end up with a suspicious object pool consisting of true and false positive single cells and other objects. Their relative amount, may then define an atypia index for the specimen. To improve the results, an analysis of the suspicious objects with respect to their position in the feature space, may then lead to an atypia index for the specimen.

An example for a decision tree classifier is given in fig. 1. At each node the most relevant features (F-value selected) are given. It can be seen that global features, as well as chromatin pattern features (for explanation of features see Burger et al. 1981, Rodenacker et al. 1981) are important at different nodes. The chromatin features depend sensitively on the local resolution. This is demonstrated by the F-values for such features as a function of local resolution as derived for a 2 class discrimination task (Table 1).The number of features selected above certain F-value thresholds decreases considerably with local resolution.

Summing up the existing know how on automated diagnosis of CIN-lesions from cervical smears, the measure of which provides also the basis for prescreening machines, it is obvious that chromatin features will play a decisive role.

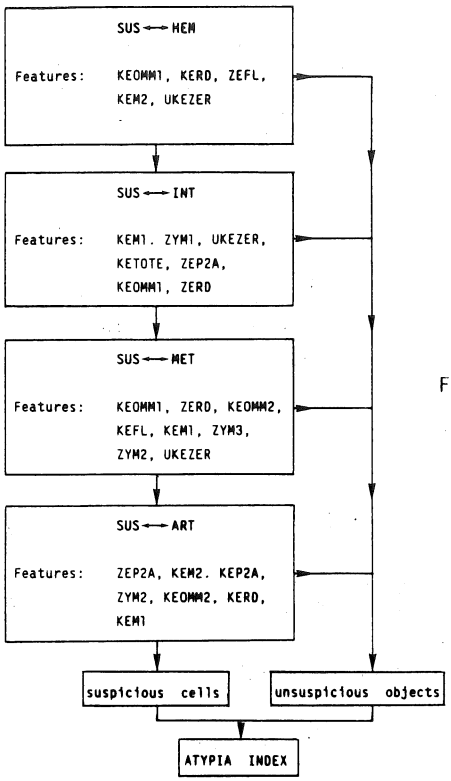

Fig. 1. Decision tree of the cervix
(SUS = suspicious cells,
HEM = blood cells, histiocytes,
INT = epithelial cells,
MET = metaplasia cells,
ART = artefacts)

Table 1. Chromatin pattern features as selected by F-statistics in stepwise linear discriminant analysis for the discrimination of two pooled classes (benign-malignant) of cervical cells.

local resolution (pixel size)	F-value ≥ 300	≥ 200	≥ 100
0.5 micron	KEOMAN KEOMLF KEOMM1	KEG1MX KEOMM2	KEOMNA KEOMLF KEOMAN KELXM1 KEOMFL
1.0 micron		KEG1MX KEOMM1	KEOMNA KEOMLF KEOMAN KEOM
2.0 micron		KEG1MX	KEG1M3 KELXM1

- Thyroid Cancer Typing and Malignancy Diagnosis

Visual discrimination of cancer from benign lesions is difficult if not impossible for thyroid nodes with follicular proliferation or of oncocytic type. The problem was tackled by investigation of 111 specimens, mostly diagnosed histologically, consisting of 40 adenomas and 58 carcinomas, (both of different types), and 13 negative cases, as given in the last column of table 2.

From each aspirate 100 isolated randomly located cells were selected interactively and pooled according to the desired classification problem. Applying linear discriminant analysis delivers 'a posteriori' probability distributions for the cells per specimen belonging to either class (Burger and Jütting 1985). Based upon these distributions a specimen classification was performed. Figure 2 shows the decision tree and table 2 the classification results.

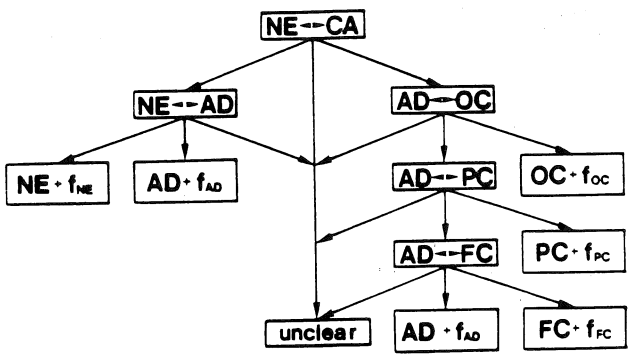

Fig. 2. Scheme of the decision tree for the thyroid
(AD = Adenoma, CA = Carcinoma, FC = follicular CA,
PC = papillary CA, OC = oncocytic CA, NE = negatives)

Table 2. Classification result of the decision tree

	NEG/ FUNCT.	FOLLIC. AD	FOLLIC. CA	PAPILL. CA	ONCOCYT. CA	UNCL.	TOTAL
NEGATIVE	10	2	-	-	-	1	13
ADENOMA	-	18	3	2	-	10	33
FOLLIC. CA	2	3	6	6	2	9	28
PAPILL. CA	-	-	-	12	1	3	16
ONCOCYT. CA	-	-	-	-	8	-	8

Only the first moment of the 'a posteriori' probability distributions (mean value p) was used for classification. All specimens with values of p in the region $.47 \leq p \leq .53$ were sorted into an unclear class. The results are surprisingly good. They are even better when only certain binary classification problems are considered (Schenck et al. 1985a). From this one can conclude that it is possible, with a very high validity, to differentiate

- negative cases versus functional diseases
- follicular adenomas versus follicular carcinomas
- oncocytic adenomas versus oncocytic carcinomas
- follicular carcinomas versus papillary carcinomas

The cellular features selected were predominantly chromatin features. Global morphological or photometric features do not play any significant role. This is shown in table 3 for specimen classification results with chromatin features (left side) and with global features (right side).

Table 3.

Specimen classification results of follicular adenoma and carcinoma

	with chromatin features					with global features only			
	AD	CA	UNCL	TOTAL		AD	CA	UNCL	TOTAL
Adenoma	18	4	12	34	Adenoma	9	3	22	34
Carcinoma	4	12	4	20	Carcinoma	5	6	9	20

Breast Cancer

An important goal in breast cancer cytology is prognostic grading. This could only be investigated straight forward on the basis of survival of untreated patients. As this is not possible, and hence corresponding cytological material is not available, other correlations have to be studied. In a first experiment we measured 100 interactively selected cells from 26 patients and correlated the cytometric findings with the receptor status, which is said to be of some prognostic significance (Schenck et al. 1985b). The cell data bank consisted of 1441 nuclei from 15 receptor negative cases and 965 nuclei from 11 receptor positive cases. Again, first the 'a posteriori' distributions of the cells per specimen belonging to either group (receptor positive or negative), were determined. The mean values define an index of atypia, which was compared with the visual ranking of the specimens according to the degree of cellular differentiation. Fig. 3 shows the results.
The following results were derived for the correct reclassifications: 12 out of 15 receptor negatives with two misclassified, and 10 out of 11 receptor positives with 3 specimens classifications unclear, according to a similar criterium as mentioned in case of thyroid analysis. The features selected have again been predominantly chromatin pattern features. If only global features are offered the results come down to 9 negatives correct with 5 false positives and one unclear and 6 positives correct with three false negatives and two unclear decisions.

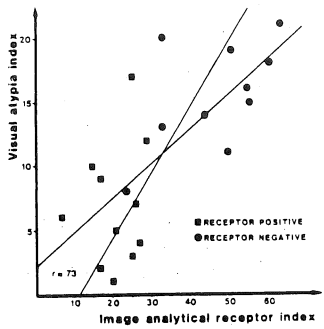

Fig. 3. Correlation of the image analytical receptor index with a visual atypia index

The results could be confirmed recently by an independent experiment. In this case 29 imprints were available, which had been wet fixed and Feulgen stained, 20 specimens were receptor positive and 9 receptor negative*. Again 100 cells had been selected interactively and measured. The specimen classification was done as described, the results are as follows:

Receptor	-	+	unclear
-	7	1	4
+	3	13	2

In this experiment additional cytological data were available, as e.g. the average mitotic index for each specimen. It could be shown that this index also highly correlates with chromatin pattern features. The 95% confidence interval for the multivariate correlation coefficient is $.69 \leq \rho \leq .92$.

CONCLUSIONS

High resolution imaging cytometry has shown to contribute essentially to the solution of numerous problems in clinical and diagnostic cytology, such as diagnosis of cervical intraepithelial neoplasias, malignancy diagnosis and tumor typing in thyroid aspirates and a correlation of cellular features with receptor status in case of breast aspirates. In all cases it was proven that independent of staining and preparatory conditions, the structural and textural features of the nuclei were of a high discriminating power.

*Preliminary results from a project with IST, Genova (Drs. Nicolao and Giaretti).

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