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CYTO- AND HISTOMETRY IN HISTOLOGICAL SECTIONS OF *COLON CARCINOMA*: METHOD

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ABSTRACT

Histological sections from *colon carcinoma* were evaluated by quantitative TV-microskopy. As a first step the nuclear profiles were digitized, segmented and measured. Besides classical cytometrical parameters like several global textural features, the fine structure of eu- and heterochromatin distribution was measured in terms of arrangement and neighbourhood relationship.

The resulting features are related to corresponding pathological diagnosis. As an example it is shown, that the existence of metastases may be correlated with features of the nuclear chromatin distribution.

Keywords: cytometry, graph theory, histometry, image analysis

INTRODUCTION

General goals in analytical cytology applied to oncology are the improvement of malignancy diagnosis with emphasis on patients prognosis. The final goal is to provide quantitative information relevant for therapy management.

Tumor characteristics known to determine prognosis are the degree of invasive growth and the potential of cellular dissemination. These features seem to be the result of specific genetic outfit of certain tumor cell populations. It is the basic hypothesis in cytometry that the involved cell clones express phenotypic changes especially in chromatin distribution, which may be measurable by high resolution imaging methods.

At the same time total nuclear extinction is measured, but the lack of resolution due to the problem of nuclear sectioning may prevent any reasonable DNA-analysis.

MATERIAL

From the Kantonsspital Basel, histological sections from more than 145 patients with *colon carcinoma* have been collected since 1978. From each patient three consecutive sections exist, stained with PAS, HE and the Feulgen reaction. All clinico-pathological data are available and the patients are followed up until now. On these sections clearly

invasive tumor tissue, the tumor front, defined by the deepest infiltration of tumor cells into the neighbouring tissue, and the tumor centre were located. From each area about 150 tumor cells were selected at random and the nuclear profiles were stored as digital images on optical disk. For additional standardization purposes ten lymphocytes per specimen were measured. This was done primarily with Feulgen stained specimens and will be done with the other available stains for comparison of textural features.

METHOD

Sampling

Single tumor cell nuclei are selected and their digitized image acquired. This so-called single cell sampling provides focal adjustment and background evaluation for each nuclear profile which may lay in different depths and partly cover each other. If measured in depth of focus it can be hoped that the chromatin pattern of a nucleus is not too much affected by the underlying background of neighbouring nuclei. This is demonstrated in Fig. 1 where two neighbouring cells where taken at different focal depth (no. 1 right and

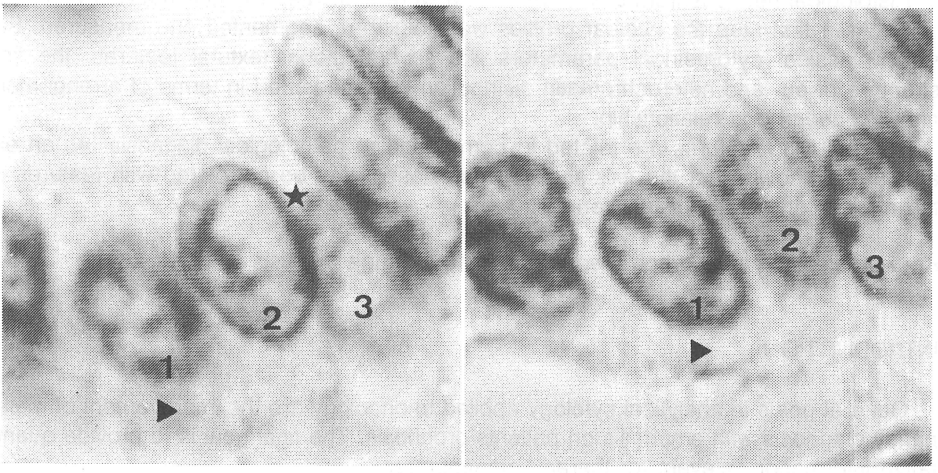

Figure 1. Three neighbouring cell nuclei displayed at different focal depth.

no. 2 left) with no. 2 and 3 partially overlapping. The selection of tumor cells can then be done in a random manner, without any restrictions concerning the final choice of cells. Depending on the specimen, especially on the nuclear contrast and amount of disorder in the region, often only a few isolated, non-overlapping nuclei can be found in sufficient number.

The digitization is performed via a TV measuring camera (Bosch, T1VK9B1, FRG) attached to a frame grabber (microPicture, VTE, Braunschweig) at a VAX 750 (Digital, Maynards, Mass., USA) with 100x/1.3 ap objective (oil immersion) and narrow band filter (547 nm) on an Axiomat microscope (Zeiss, Oberkochen, FRG). Each digital image consists of 128^2 pixels with pixel size of about 0.25 µm. The true resolution of grey values is about 64.

Segmentation

In sections with densely distributed cell profiles, the segmentation is often the most difficult task of the whole evaluation. Especially in *colon* cancer sections the nuclei are often not isolated and not segmentable by a single grey value threshold. In these cases a manual delineation is performed with a mouse, either corrective or complete. On the screen a menu of possible choices is displayed with the most likely one proposed, e.g. if an automatical threshold detection algorithm (Kunze et al., 1989) was successful, the command *next object* is offered.

Thresholding eventually succeeded by an interactive delineation results in a binary mask with value **true** for each pixel of the object of interest and **false** otherwise. This mask is stored in the digitized image using the property *odd grey value* for **true** and *even grey value* for **false**. This reduces the possible range of grey values of digitized images from 0–255 to 0–254 in steps of 2 (0, 2, 4,...). However the true resolution of grey values of the digitized image was only into 64 intervals, hence no loss of information takes place. This method allows an easy and efficient storage of the digitized image combined with the segmentation.

Featuring Method

The following three classes of features can be distinguished:

i) extinction features of different regions of the nuclear profile,
ii) textural features inside the nuclear profile and
iii) features of the arrangement of chromatin particles

i) Extinction Features

As different regions the original mask of the nucleus, defining the **whole object**, a so-called (inside) **border zone** and **dark** and **bright particles** resulting from two different automatically performed segmentation methods are used (Fig. 2). The border zone is

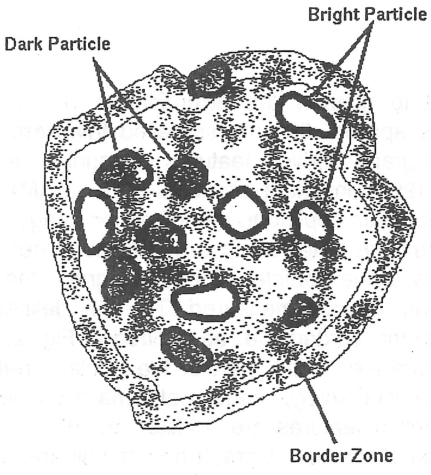

Figure 2. Regions for extinction features

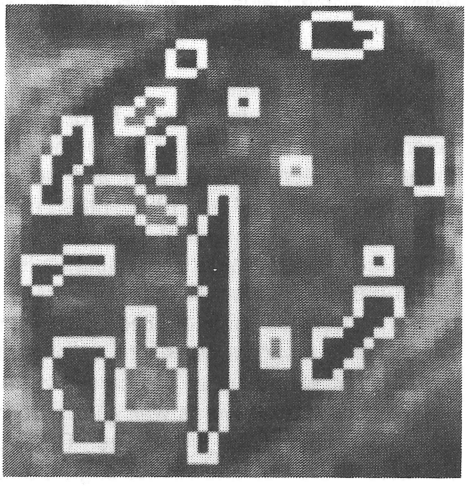

Figure 3. Segmentation of particles

generated as a rim inside the whole object with given thickness. The dark and bright particles are segmented by the application of either a median filter (Method 1) or a

combination of an opening and closing (Method 2, Rodenacker et al., 1981) succeeded by a threshold application to the difference of the original extinction image and the filtered one. The particles can be considered as contrasted regions disappearing, when the smoothing transformations mentioned above are applied onto the original image (Fig. 3). Hence the difference image contains the particle regions.

The digitized image, originally in grey values of transmitted light, is pixelwise transformed to extinction values by the formula given in Eq. 1.

$$E = -150 * \log_{10}((T - B) / (W - B)) \quad (1)$$

T = measured transmission value (between 0 and W)
B = transmission value of dark field (depending from camera offset)
W = transmission value of white field (background)

The extinction features shown in Tbl. 1 are calculated. They are selfexplaining. Additionally the morphometric features *area*, *zones of influence around each particle (ZOI*, see Fig. 4) and *number of particles* inside a nucleus are listed there.

ii) Textural features

Different methods for the generation of features are used. The principle is to apply a certain image transformation, which leads either again to an image or to any other 1- or 2-dimensional array. The transformations were **gradient**, **laplacian**, **flat texture** leading to transformed images and the random model based transformations **run length** and **grey value co-occurence**, leading to 2-dimensional frequency distributions. From the transformed images for the slightly eroded whole object the statistical parameters area, mean, SD, skewness, kurtosis and mode (A, M1, ..., MD) were calculated. From the random model based transformations the features listed in Table 2 and 3 are computed using all pixels inside the whole object. Feature names are prefixed then by G, L, F, RL and CO.

iii) Arrangement features

Beside textural features it is of interest to quantify the arrangement of eu- and heterochromatin particles. Two methods are applied, first the so-called **invariant moments** (Christen et al., 1989) and second graph derived features (Chaudhuri et al., 1988; Rodenacker et al., 1987, 1988, 1989, 1990). Invariant moments (**IM1**, ..., **IM7**) are derived from the concept of 2-dimensional moments applied to the extinction image. With graph theoretical methods the segmented particles (method 2) are used to generate a graph describing the neighbourhood relations of the particles. From this graph the rim-standing particles are selected and extinction features measured. The hull defined by rim-standing particles represent a pendant to the borderzone, as defined in Fig. 2. The deviation of extinction in the border zone from the central part of the nucleus reflects pathological properties as well known from visual cytology, namely chromatin condensation near the nuclear membrane. The extinction features are prefixed by **HD** for dark particles at the hull and **HH** for bright particles at the hull. A graph and its hull are shown in Fig. 4.

Evaluation

The resulting set of features per image or cell nucleus, respectively and per specimen was analyzed with the statistical analysis system SAS (SAS/STAT, 1990) using primarily

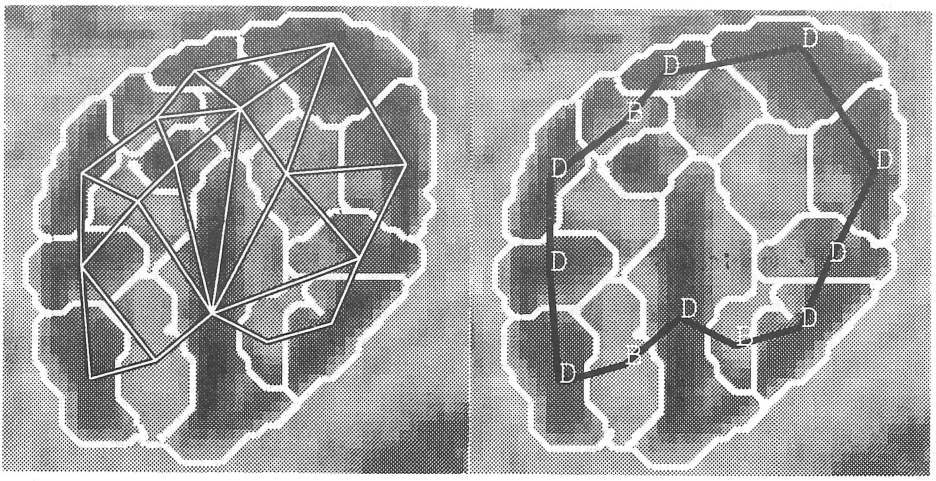

Figure 4. Graphs of eu- and heterochromatin (left: base graph, right hull graph)

linear disciminant analysis methods. With growing amount of available data other methods like logistic analysis and survival time analysis will be applied to relate clinical follow up data with quantitative features.

PRELIMINARY RESULTS

In a first step of the study *carcinoma* with (5 cases) and without metastases (5 cases) (Tbl. 5) were selected; 150 cells in each specimen were measured, pooled and classified. The first seven specimens were used to train a test classifier. A single cell classification was performed by means of a stepwise linear discriminant analysis with the SAS procedure STEPDISC (SAS/STAT, 1990). From all extracted features only those were offered for the classification which had an univariate F value with a significance level $p<0.01$. These features built the feature data set. On the basis of F-statistics the feature with the highest univariate discrimination was used in the first selection step. In the next steps the features providing the best combined discrimination were chosen. The procedure stopped when either no new feature was significant to improve the result or the maximum number of features was reached.

Using only the pooled cells from 7 specimens (4 pM_0, 3 pM_+) following features are chosen: BITOTE, FNM3, BIM2, HHANZ, CO11, RL2, CO7. The cell classification result is shown in Table 4a. Applying this classifier to the next new three specimens (1 pM_0, 2 pM_+) resulted in the confusion matrix of table 4b. Using the whole set of ten specimens to train a classifier nearly the same features were chosen (DTOTE, FNM3, BIM2, HHANZ, IM1, RL2, CO6). Although we have not yet been able to prove statistical significance on the specimen level, the results let us suggest that there is a difference in the chromatin pattern in tumor cells of tumors with and without metastases. The *area* and the *perimeter* were offered but not used in this classification task.

SUMMARY

A short description of a project of histometry of *colon carcinoma* is given with emphasis to the methodical aspects. Problems occuring with specimens of histological sections are mentioned (focus, overlap, segmentation) and possible strategies of cytometric measurements are described. The main effort concerns selection of tumor cells and the segmentation in cases of densely distributed and overlapping nuclei. All other subsequent processing steps are performed fully automatically.

The resulting quantitative features are stored together with the original images on optical disk for further examinations and reexaminations, allowing the direct relation of feature values with visual image content. The evaluation of the features as well as their interpretation is only at the beginning. Especially the problem of relating section information with true three-dimensional information is not described here and necessitates further research.

In a preliminary trial only tumors without and with metastases were analyzed. The preliminary results correspond with findings of Kunze et al. (1989).

TABLES

Table 1: List of extinction features

Regions:	Whole Object	Border Zone	Particles Dark Method 1	Particles Dark Method 2	Particles Bright Method 1	Particles Bright Method 2
Area	A	BIA	DA	FPA	HA	FNA
Mean	M1	BIM1	DM1	PM1	HM1	NM1
SD	M2	BIM2	DM2	PM2	HM2	NM2
Skewness	M3	BIM3		PM3		NM3
Kurtosis	M4	BIM4				
Minimum	MIN	BIMIN				
Maximum	MAX	BIMAX				
Mode	MD	BIMD				
IOD	TOTE	BITOTE	DTOTE	PTOTE	HTOTE	NTOTE
Number			DANZ	FPANZ	HANZ	FNANZ
Mean ZOI			DM1Z		HM1Z	
SD ZOI			DM2Z		HM2Z	
IOD ZOI			DTOTEZ		HTOTEZ	

Table 2: List of features from grey value CoOccurrence (Spider, 1983)

CO1	Angular 2nd moment	CO8	Sum entropy
CO2	Contrast	CO9	Entropy
CO3	Correlation	CO10	Difference variance
CO4	Sum of squares: Variance	CO11	Difference entropy
CO5	Inverse difference moment	CO12	Measure of correlation 1
CO6	Sum average	CO13	Measure of correlation 2
CO7	Sum variance		

Table 3: List of features from RunLength (Spider, 1983)

RL1	Short runs emphasis	RL4	Run length non uniformity
RL2	Long run emphasis	RL5	Run percentage
RL3	Grey level non uniformity		

Table 4a: Confusion matrix reclassification (7 specimens, hold one out for nuclei)

		Number of nuclei into pM_0	pM_+	% correct
From	pM_0	494	142	77.7
	pM_+	219	260	54.3

Table 4b: Confusion matrix classification (3 new specimens)

		Number of nuclei into pM_0	pM_+	% correct
From	pM_0	90	58	60.8
	pM_+	166	256	60.7

Table 5: pTNM status of the selected specimens

B78/13198	$pT_3N_0M_0$
B80/3037	$pT_3N_1M_0$
B80/8258	$pT_3N_1M_0$
B81/3508	$pT_3N_2M_0$
B81/7556	$pT_3N_1M_0$
B77/15945	$pT_3N_1M_1$
B79/377	$pT_3N_0M_1$
B78/22518	$pT_3N_1M_1$
B82/29252	$pT_4N_2M_1$
B84/31550	$pT_4N_1M_1$

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