ACTA STEREOL 1983; 2: 312-318
STEREOLOGY AND MORPHOMETRY IN PATHOLOGY
Proc 2nd Symp Morphometry in Morphol Diagnosis
Collan Y. et al., eds.
Kuopio University Press, Kuopio 1983

KARYOMETRIC DATA BY IMAGE ANALYSIS AND THEIR USE IN PATHOLOGY

Klaus Voss, Peter Hufnagl, Hubert Martin and Karl Roth

Institute of Pathology, Humboldt University
DDR - 1040 Berlin, Schumannstr. 20-21

ABSTRACT

An overview is given on image analysis applications in histopathology. In comparison to automated cytology, there are only few practical efforts in this field. But they have shown that karyometric data yielded by image analysis give valuable informations for description of histological specimens. Further in the paper, general principles of object segmentation, feature generation, object classification, and characterization of histological specimens by karyometric data are shortly discussed. These principles are realized in the author's image analysis system. Finally, a practical example is presented (brain tumours). The mean effective time for evaluating one specimen (out of 346 cases) is 5-10 minutes (300-1000 investigated nuclei).

INTRODUCTION

Many efforts have been made to use image analysis for cytological investigations. But only few research groups have dealt with automation in histology. The reason for that is the very complex nature of histological images which needs complex scene analysis methods for a detailed description (Stenkvist et al.,1979). A way to overcome these difficulties is to concentrate the investigations on the cell nuclei. Especially with Feulgen staining, only the nuclei are visible. They can be located, measured, classified, and counted. Classification is necessary if there are different types of nuclei as in liver tissue (Kunze et al., Simon et al.,1980, Preston and Dekker,1980, Voss et al., 1981).

Very few authors have investigated tissue structure on the base of locations and orientations of nuclei. But such an approach is very similar to the thinking of pathologists. Therefore neighborhood relations and graph structures could be a valuable tool in automated histology (Craciun and Tasca,1976, Prewitt et al.,1978, Stenkvist et al.,1979, Kayser and Schlegel,1982).

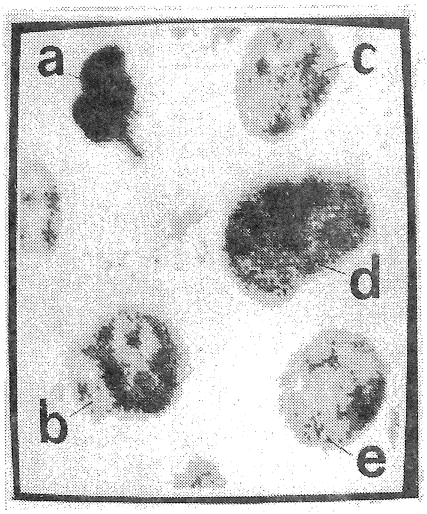
In most cases known up to now, karyometric data as feature values of nuclei without respect of their sites and orientations are used in quantitative histopathology. The reason for that is the relative simplicity of this approach. We refer here to investigations of liver tissue (Kunze et al.,1978, Kranz et al.,1980, Simon et al.,1980, Wenzelides et al.,1981), thyroid gland (Christov et al.,1974, Schuh et al.,1980, Boon et al.,1982), brain tumours (Martin et al.,1981, Martin and Voss, 1982, Gottschalk et al.,1983), and other tissues (Rigaut et al.,1982, Enchev and Raichev,1982). For further informations on histopathological applications of image analysis see Preston and Dekker (1980), Rigaut et al. (1982), and Simon et al. (1983).

Measurement values and count results in respect to the nuclear structure are also essential for histopathology. Especially the chromatin distribution (Christov et al.,1974) and nucleolar data (Kranz et al.,1980, Wenzelides et al.,1981) should be investigated.

In comparison to time effort of about one minute per nuclei using usual morphometrical methods (Boon et al.,1982), the automated methods of image analysis need per nuclei an effective time of 8 seconds (Kunze et al.,1978), 3.5 seconds (Rigaut et al.,1982) or 1.1 seconds (Martin et al.,1981). Additionally to this advantage in time, image analysis allows to determine essentially more karyometric data (size, shape, extinction, texture etc.) than usual morphometrical methods.

KARYOMETRIC DATA

For object segmentation, we use the contour tracing method for its high effectiveness and applicability. A major advantage offered by this method is the possibility of using local operators (Voss and Klette, 1981). To find approximately all objects of an image, we use a stepwise segmentation technique with increasing thresholds (Voss and Roth,1984). Contour features of an object are yielded in a single pass of contour tracing. We get primary features as area A, perimeter P or data of circumscribing rectangle and octogon. From these parameters, secondary features are derivable as form factor $F1 = P^2/A$, area AC of the convex hull of the object, form factor $F2=A/AC$ or form factor $F3 = P^2_{convex}/AC$.



	KOFL	FOFK	EXTS	EXTM
a	541	166	3816	705
b	926	141	3376	364
c	770	144	2384	309
d	1248	154	5432	435
e	817	140	2434	297

Fig.1. Feature values of some nuclei (KOFL=area in picture points,FOFK=form factor F3 of the convex contour, EXTS = extinction sum, EXTM = mean extinction).

For some problems, diameter ratio Dmin/Dmax and orientation angle of an object are valuable parameters. We use a simple but effective approach to determine these quantities by means of the widths WX,WY,WP,WM of the circumscribing octogon. Here WP is the width in direction P=X+Y, and WM is the width in direction M=X-Y (Idesawa,1982).

From the grey values of picture points within the object contour, primary extinction parameters are derivable as extinction sum E or maximal extinction EA. Secondary features follow as mean extinction EM=E/A or the socalled compactness C=EM/EA. For special applications also other parameters are used as p.e. nucleolar data (Voss and Roth,1983). In Fig.1 some examples of nuclei and a subset of feature values are presented.

Using the determined object features, nuclei can be grouped by classifiers (Voss et al.,1981). For each interesting object class, we determine statistical data (number of nuclei per unit area, mean extinction sum, standard deviation of form factor, skewness of size distribution etc.). Such statistical parameters describe quantitatively the ensemble of nuclear sections visible in the histological specimen (Fig.2).

IMAGE ANALYSIS IN PATHOLOGY - AN EXAMPLE

We have investigated many histological specimens, both from human material (autopsies, biopsies) and from animal material (Simon et al.,1983). Especially our studies of 346 gliomas (Martin and Voss,1983) and of 131 pituitary adenomas (Gottschalk et al.,1983) can be considered as first experiments in computer aided diagnosis of brain tumours.

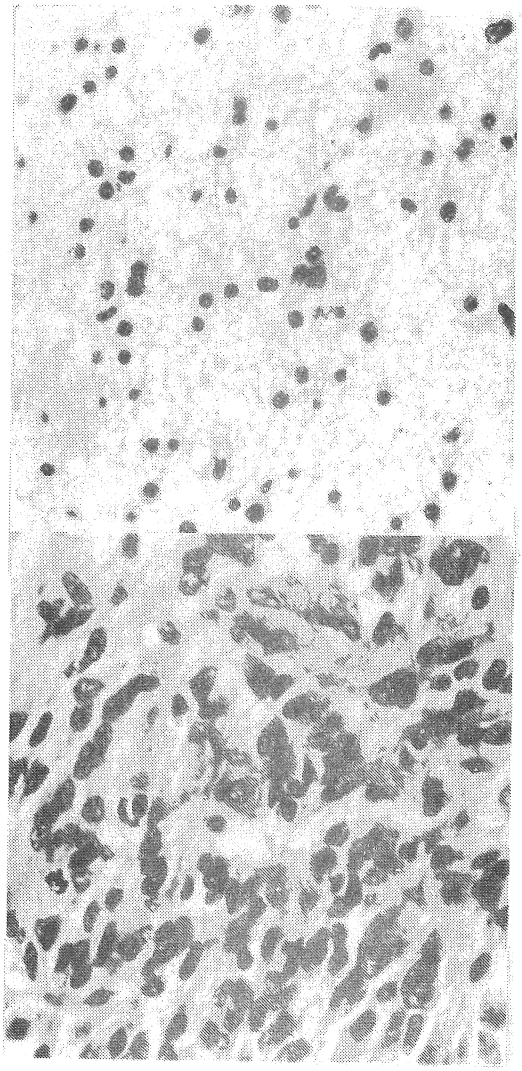


Fig.2. Astrocytomas (grade one of malignancy - top, grade four - bottom) and some histological feature values (AREA = part of area covered by nuclei, KRNZ = number of nuclei per unit area, FOFK = mean convex form factor F3, KOFL = mean nuclear area in picture points).

Proliferative properties of gliomas are expressed in numerical density of tumour nuclei, relative standard deviations (of nuclear areas, extinction sums, convex form factors etc.), and part of area covered by nuclei. Glioma grading based on karyometric data is practicable, as may be seen from our results (Martin 1983, see Fig.3). By automatic grading, grading, the computer results compared with results of three pathologists are better in respect to reproducibility and accuracy (80% correct results). The effective time for evalu-

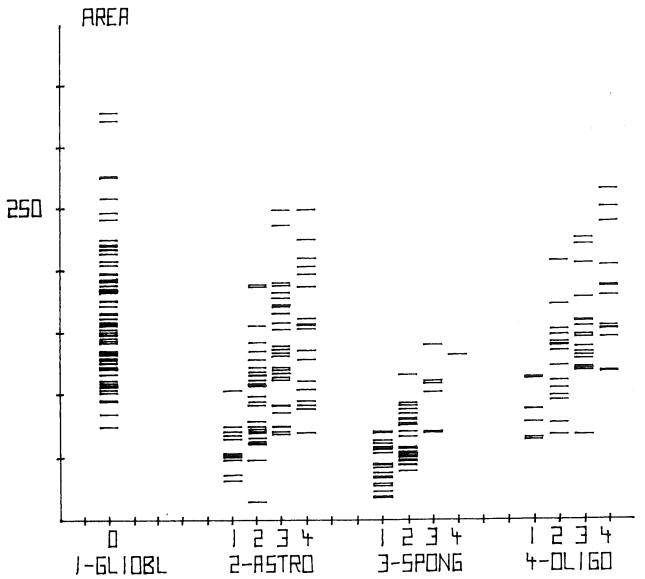


Fig.3 - Values of parameter AREA (part of area covered by nuclei) for glioblastomas (GLIO), astrocytomas (ASTRO), piloid astrocytomas (SPONG), and oligodendrogliomas (OLIGO). Each bar represents one case. The numbers characterize the grade of malignancy in a four-grade scheme.

ating one specimen (300-1000 nuclei) was 5-10 minutes. Our conclusion is that image analysis in histopathology can be a valuable tool for differential diagnosis, diagnosis of borderline cases, and gradually changed tissue quantitation.

REFERENCES

Boon ME, Loewhagen T, Cardozo PL, Blonk DI, Kurver PJH, Baak JPA. Computation of preoperative diagnosis probability for follicular adenoma and carcinoma of the thyroid on aspiration smears. Anal Quant Cytol 1982; 4: 1-5

Craciun EC, Tasca C. Karyo-interkaryometry as a periodic control for clinical prognosis. In: Underwood EE, De Wit R, Moore GA, eds. Proc 4th Int Congr Stereology, Washington 1976; 419-422

Christov K, Kiefer G, Kiefer R, Sandritter W. Changes in the nuclear structure during thyroid carcinogenesis in rats - an image analysis study. Beitr Path 1974; 152: 19-36

Enchev VG, Raichev RD. Zytomorphometrische Untersuchungen bei normaler Magenschleimhaut, präkanzerösen Krankheiten und Magenkrebs. Arch Geschwulstforsch 1982; 52: 641-647

Gottschalk J, Hufnagl P, Rohde W, Knappe G. Automatisierte morphometrische und densitometrische Untersuchung von Hypophysenadenomen. Zbl allg Pathol 1983; 127: in press

Idesawa M. A quantitative analysis method of orientation for fibrous pattern. Proc 6th ICPR, Munich 1982; 810-815

Kayser K, Schlegel W. Pattern recognition in histopathology - basic considerations. Meth Inform Med 1982; 21: 15-22

Kranz D, Voss K, Wenzelides K, Simon H. Quantitativmorphologische Untersuchungen an Lebern und Lebertumoren nach Einnahme von Kontrazeptiva. Zbl allg Pathol 1980; 124: 441-447

Kunze KD, Herrmann WR, Voss K, Image processing in pathology. VII. Computer assisted differential diagnosis of liver diseases in biopsies by automatic recognition and morphometric analysis of liver cell nuclei. Exp Path 1978; 16: 186-193

Martin H, Schmidt D, Voss K. Automatische morphometrische und densitometrische Untersuchung und mathematische Klassifizierung von Gliomen. Zbl allg Pathol 1981; 125: 414-428

Martin H, Voss K. Automated image analysis of glioblastomas and other gliomas. Acta Neuropathol (Berl) 1982; 58: 9-16

Martin H, Voss K. Computerized classification of gliomas by automated microscope picture analysis. Acta Neuropathol (Berl) 1982; 58: 261-168

Martin H. Morphometrische und densitometrische Untersuchungen an Gliomen mit Hilfe der automatischen Mikroskopbildanalyse als Basis einer objektivierten Klassifizierung und Gradierung. Promotion B, Humboldt-Universität Berlin 1983

Preston K, Dekker A. Differentiation of cells in abnormal human liver tissue by computer image processing. Anal Quant Cytol 1980; 2: 203-220

Prewitt JMS, Barber A, Wu SC. An application of pattern recognition to histology. Proc PRIP Conf, Chicago 1978; 499-506

Rigaut JP, Margules S, Boysen M, Chalumeau MT, Reith A. Karyometry of pseudostratified, metaplastic and dysplastic nasal epithelium by morphometry and stereology. Path Res Pract 1982; 174: 342-356

Schuh D, Steidl R, Voss K. Unterscheidung von follikulären Adenomen und Karzinomen in der Schilddrusenfeinnadelbiopsie durch computergestutzte zytomorphometrische Untersuchungen. Zbl allg Pathol 190; 124: 557-560

Simon H, Kranz D, Voss K, Wenzelides K. Automated data sampling in sections from selected liver diseases. Path Res Pract 1980; 170: 388-401

Simon H, Kunze KD, Voss K, Herrmann WR, Hrsg. Automatische Bildverarbeitung in Medizin und biologie. Dresden: Steinkopff Verlag 1975

Simon H, Voss K, Wenzelides K. Automated Microscopic Image Analysis. Jena: Fischer Verlag 1983

Stenkvist B, Pressman NJ, Bartels PH, Fu KS, Granlund GH, McClure DE, Preston K, Prewitt JMS, Zajicek G. Cells and tissues - group report. In: Fu KS, Pavlidies T, eds. Biometrical Pattern Recognition and Image Analysis. Weinheim: Verlag Chemie 1979

Voss K, Simon H, Wenzelides K. Logical classifiers for image analysis in medicine. Anal Quant Cytol 1981; 3: 39-43

Voss K, Klette R. Zeiteffektive Algorithmen zur Objektisolierung mittels lokaler Operatoren. Elektron Inform Verarb EIK 1981; 17: 539-553

Voss K, Roth K. The problem of nucleolar number in cell nuclei and in sections of cell nuclei. Biom J 1983; 25: in press

Voss K, Roth K. Objektisolierung in der automatisierten Mikroskopbildanalyse. Bild und Ton 1984; 37: in press

Wenzelides K, Kranz D, Simon H, Voss K, Anwendung der automatischen Mikroskopbildanalyse in der Praxis am Beispiel verschiedener Lebererkrankungen. Zbl allg Pathol 1981; 125: 532-538