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HIERARCHICAL TEXTURE ANALYSIS OF SOFT TISSUE TUMORS USING ATTRIBUTED GRAPHS

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ABSTRACT
Histological slides of six soft tissue tumors of various cell type were Feulgen stained. The cell types include malignant fibrous histiocytoma, fibrosarcoma, and osteosarcoma. The two dimensional texture of the tumors was analyzed as follows: The centers of gravity of tumor cells were defined as vertices. Tumor cells fulfilling the neighborhood condition of O`Callaghan were connected by edges. Features of tumor cells such as nuclear area, integrated optical density, maximum and minimum nuclear diameter were associated to the vertices and the difference of features of neighboring vertices were associated to the edges. The result is an edge and vertex attributed graph. The minimum spanning tree (MST) was calculated from the attributed graph for various attributes. Decomposition of the MST was performed by rejecting those edges from the MST exceeding the mean value added by twice of the standard deviation of the corresponding attribute. The procedure decomposes the MST into several clusters which contain characteristic structural properties. The center of gravity of the clusters was defined as vertex of a new -higher order- graph, and the procedure for constructing a new MST was repeated. First results of the analyzed soft tissue tumors reveal characteristic structural properties of the soft tissue tumors being preserved in the MST of different order.

Keywords: Graph theory, attributed minimum spanning tree, cluster tree, soft tissue tumors.

INTRODUCTION

The diagnostic procedure in histopathology is usually performed by the analysis two-dimensional structures being visible in a histological slide. The diagnostic procedure includes a variety of algorithms such as screening for the area containing the appropriate information called the area of interest, the analysis of the texture patterns performed at a low microscope magnification, the search for special staining behaviour such as mucus or expression of antigens, the analysis of nuclear textures such as identification of mitotic

figures or nucleoli. Morphometric measurements are usually not included in the diagnostic algorithms, they may, however, contribute to certain diagnostic properties such as staging of melanomas, and grading of breast carcinomas.
It could be shown by various authors that the analysis of two dimensional structures being present in histological slides contributes to various aspects of tumor biology and diagnosis: Rodenacker and Bischoff (1989) developed a graph theory algorithm for analyzing the changes in textures of epidermoid growth in preneoplastic and neoplastic tissue. Kayser and Höffgen (1984) presented an algorithm of analysis of textural patterns by use of the graph theory approach. The authors described an algorithm for texture analysis using a hierarchic order of the constructed graphs. The hierarchy was defined by regularities (symmetries) being present in the basic graph (first order graph). This concept was appropriate for application in various difficult diagnoses and could be successfully applied for distinguishing mesothelioma from metastatic adenocarcinoma (Kayser et al, 1987).
This approach is different and combines structural properties with morphometric features. Kayser et al (1989) tried to analyze the amount of DNA in lung carcinoma cells in relation to the number of neighboring tumor cells and found remarkable differences in the integrated opitical density depending upon the number of neighboring cells and upon the cell type. Tumor cells of epidermoid carcinoma had a higher DNA content in branching points of the MST opposite to tumor cells of large cell anaplastic carcinoma. It can be concluded from these data that combined morphometrical and structure analysis is a useful tool for histomorphological insight into human lung carcinoma growth. However, the question remains still open whether this approach may be adequate for simulation of diagnostic algorithms and for computerized aids in difficult diagnostic cases. The actual paper will describe the basic ideas and first results in application of hierarchic ordered MST in a difficult histomorphological problem such as classification of soft tissue tumors.

MATERIAL AND METHODS

Technical procedure: Histological slides, 4-6 µm thick were obtained from formalin fixed, paraffin embedded tissue, and were Feulgen stained according to the technique as described by Mikel et al (1985). The characteristic area was chosen in the HE stained slides and was interactively marked on the corresponding Feulgen stained slide. An automated image analysing system (Quantimet 570) was used for image transfer and for segmentation of the tumor cell nuclei. The matrix of digitized images was set to 512 x 512 pixel at 8 bit. The automated feed back of the CCD camera was disabled in order to preserve the stochiometry of the Feulgen stain. No filtering procedure was used in the seqmentation algorithm due to the same reasons. After segmentation the center of gravity of each tumor cell was computed, and the nuclear features were measured. These comprise nuclear area, maximum and minimum diameter of best fitting ellipsis, orientation of major nuclear axis, integrated optical density. A low magnification objective (x20) was used in order to obtain a sufficient

number of tumor cell nuclei per image being useful for texture analysis. 150 - 300 tumor cell nuclei were analyzed within one tumor area. Six different soft tissue tumors were measured including osteosarcoma, fibrous histiocytoma, and fibrosarcoma.

GRAPH THEORY APPROACH

The presentation of the planar, non-directed graphs was performed similar to measurements already published: The center of gravity of tumor cell nuclei was considered as node, and the coordinates were stored in a corresponding vector. This vector was associated with the features measured during the segmentation procedure. In order to maintain the geometrical information in the basic graph (called C-graph) a neighborhood analysis was applied for the vertices. The neighborhood condition given by O`Callaghan (1975) was chosen due to its proven practicability in texture analysis of histological slides (Kayser et al,1985, 1987; Kayser,1988). Neighboring vertices were connected by edges, and the C-graph was constructed. In a second step the feature depending distances between neighboring vertices were associated with the corresponding edge and an edge attributed vector was computed on each edge of the C-graph. The edge attributed vectors were used as basis for computation of the different minimum spanning trees (MST). The procedure allows the visualisation and corresponding interpretion of local distances between the measured features.

DECOMPOSITION OF THE MST AND CONSTRUCTION OF THE CLUSTER TREE

An attributed MST may contain certain edges with extraordinary large distances in respect to a certain feature. Rejecting these edges from the MST results in a partition of the MST into several connected subsets of the MST called tree clusters. These consist of areas with homogenous values (limited distances) of the corresponding feature. Various constraints may be applied for definition of the "homogeneity". In our approach a decomposition function was chosen according to the attempt of Zahn (1971) using the mean value and twice of the standard deviation of the measured feature, i.e. all edges were removed with an associated feature exceeding this distance. An example showing the mean orientation of the main nuclear axis of tumor cells in the obtained tree clusters is given in Fig. 1.

After decomposition, a higher order C-graph was constructed as follows: the center of gravity of the obtained tree clusters was computed by weighting the coordinates of the vertices with the corresponding feature. A different approach could be the definition of the centers of the rejected edges as new vertices. A vertex associated vextor of the tree cluster was computed by calculating the mean value of the associated features. After computation of the vertices and their attributes of the "new higher order graph" the edges were computed using again O`Callaghans neighborhood condition. Neighboring vertices were connected by edges. The procedure results in a new "Higher ordered" C-graph called cluster C-graph. Based upon this cluster C-graph different cluster MST were calculated.

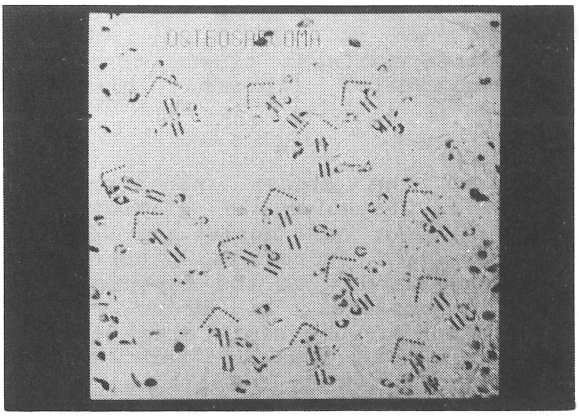

Figure 1: Osteosarcoma, Clusters in orientation of the main-nuclear axis, as indicated by arrows

DISCUSSION

The graph theory approach for texture analysis of histomorphological images seems to preserve certain structural properties of the image being of importance for the diagnosis of the pathologists (Kayser et al,1985, 1987, Kayser and Höffgen, 1984). Although the tumor growth occurs in the three dimensional space an artificial two dimensional plane will be of some representation of the underlying texture. Thus, certain distances between structural parameters of different tumors may reflect to the two dimensional space being the basis for the histomorphological diagnosis. Several authors have proven the consistancy of this theory and the application of graph theory in histopathological slides for aids in tumor classification. Prewitt (1978) used the graph theory for classification of preneoplastic changes of bladder mucosa. The basic idea was the measurement of distances in the obtained complete graphs of normal and preneoplastic bladder mucosa defined by a cost/benefit function. A similar concept was used by Sanfeliu (1980) for distinguishing the patterns of enzymatic changes in the muscle fiber arrangement of neuromuscular diseases from primarily muscular disorders. Kayser et al.(1987) introduced the concept of hierarchic orders of graphs based upon different definition of vertices in histomorphological slides. The vertices can be defined in context to single cells (first order structure), to regular arrangements of cells such as intima of vessels, nerve sheets, etc (second order structure), to functional units such as glands, vessels, nerves, etc (third order structures). The authors were able to discriminate between healthy, adenomatous and carcinomatous colon mucosa by analysis of the complete graphs based upon the formed glandular textures. A difficult diagnostic problem to discriminate between epithelial mesothelioma and metastatic adenocarcinoma into the pleura could be successfully solved by graph theory representations at glandular and cellular level (Kayser et al.,1987). Rodenacker and Bischoff (1989) introduced a concept of hierarchy on histometric measurements of epithelial textures by use of image operators such as union, intersection, and

joining in complete graphs. Other authors introduced the minimum spanning tree (MST) for determination of textural structures. Dussert et al. (1989) described the tumor cell heterogeneity by the variance of obtained MST in tissue cultures. Kayser et al. (1988) analyzed the minimum distance of edges in the MST of small cell anaplastic carcinoma of lung in relation to survival of the patients. The authors reported a poor survival of patients if the average length of the edges measured below 8 µm.

To combine structural properties with morphometric measurements seems an appropriate technique for studying the biology of human tumors. Kayser et al.(1989) could demonstrate that in human lung carcinoma the existence of well defined structures reflects to the amount of DNA content in tumor cells in relation to the number of neighboring tumor cells as defined by the MST. After computation of a vertex attributed MST tumor cells of epidermoid carcinoma representing branching points in the MST showed a higher DNA-content compared to tumor cells in non-branching points. The opposite was found for large cell anaplastic carcinoma being a tumor with undifferentiated growth. These data could be obtained by creating a vertex attributed MST. The "natural next step" is the construction of a vertex and edge attributed MST. The attributes can be chosen in relation to the questionaire and have not necessarily to be metric properties. The soft tissue tumors are frequently composed of spindle cells, i.e. tumor cells with a main and minor nuclear axis. The topical arrangement of tumor cells in relation to the orientation of the main nuclear axis reflects to a growth pattern called "palisading". Especially neurogenous tumors such as neurinoma may present the "palisading". The decomposition technique related to the orientation of the main nuclear axis as shown in this paper reflects to the "palisading". It is, however, more sensitive. Several tree clusters could be detected in all analysed tumors not being visible at the first view. The distances and the number of the tree clusters varies within the different tumor types as shown in Tbl. 1.

Table 1. Cell type, number of calculated tree clusters, and DNA properties in malignant soft tissue tumors

Case	Cell type	No of clusters	Cluster Distance (µm)	DNA content
1	MFH*	24	23	diploid
2	MFH	13	26	diploid
3	MFH	17	25	diploid
4	Fibrosarcoma	3	30	aneuploid
5	Fibrosarcoma	6	28	diploid
6	Osteosarcoma	14	35	aneuploid

*MFH : Malignant Fibrous Histiocytoma

Whether the obtained data are of diagnostic value has still to be proven with a more numerous set of cases.

The construction of a C-graph of next "higher" order is especially useful if several neighboring "fields of interest"

can be analyzed, and composed to a combined area of interest. The procedure allows the analysis of textural patterns in large fields of interest. It may reflect to an "upside down" procedure used by pathologists in search for areas of interest. The decomposition of the obtained cluster graph can be performed similar to the procedure as described above if it contains a sufficient number of clusters. The described algorithm has the advantage that the "magnification steps" between the hierachic ordered graphs are depending upon the geometric arrangement of the textures. If numerous clusters are observed in a special field of interest the next higher order cluster graph has a smaller "difference in magnification" compared to a cluster graph with only a limited number of clusters in the same field of interest.

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