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ANALYSIS OF DISTRIBUTED OBJECTS: An Application in Quantitative Histology

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ABSTRACT

A goal of quantitative digital image analysis is the measurement of object properties. The object as well as the measurent have to be defined algorithmically. Often an object consists of several sub-objects or -structures, which are the very constituents. For these cases of natural hierarchy methods are described to define order structures on object classes at different levels. They allow computer oriented processing of objects in terms of definition, merging, sequencing etc., and, based on formerly defined objects, the automatical measurement. An example in histometry is illustrated.

Keywords: image analysis, graph theory, histometry, modelling.

INTRODUCTION

In histometry, as well as in many other areas, objects of interest, e.g. cell nuclei, are locally distributed. Their overall resemblance or arrangement respectively, as well as their properties are the base of any diagnosis or qualification (Oberholzer et al. 1988; Gössner and Oberholzer, 1988; Rodenacker et al., 1988). We call the estimation of quantitative properties of cellular objects the *cytometrical* approach, which is already done in our lab since several years, and the quantification of so called *arrangement* properties the *histometrical* approach.

For the latter a program has been developed which allows on the base of a certain *graph arithmetic* (Klette and Voss, 1987; Harary, 1969)

i) the definition of object groups,
ii) the processing of neighborhood or more generalized of relational operations,
iii) the construction of object hierarchy levels (Kayser, 1988; Kayser and Höffgen, 1984),
iv) the computation of quantitative features of objects at every hierarchy level and
v) the display of graphs.

The program is used to analyse histological sections from different origins.

In a preceding step the image processing is performed on a VAXstation GPX/II (Digital, Maynards, USA) with an image processing system (IPS, Kontron, Eching, FRG) attached, resulting in the base object features and their relationships. In an interactively controlled step the modelling concept, mostly heuristic derived from the knowledge about the material, is developed. This modelling concept leads to a procedure, which is automatically applied to every set of section image data.

A complete tissue section evaluation consists of

i) the segmentation of the objects of interest,
ii) the evaluation of object specific quantitative features (cytometrical approach),
iii) the estimation of the relation R_2 (see DEFINITION OF HIERARCHIES),
iv) the construction of the objects of the necessary hierarchy levels (see DEFINITIONS FOR GRAPHS and EXAMPLES) and
v) the evaluation of the level specific quantitative object features.

DEFINITION OF HIERARCHIES

A hierarchical representation of structures is a quite wellknown and widely used method (Mesarovic, 1970). In Fig. 1 a concept of tissue is displayed, as it is used for the

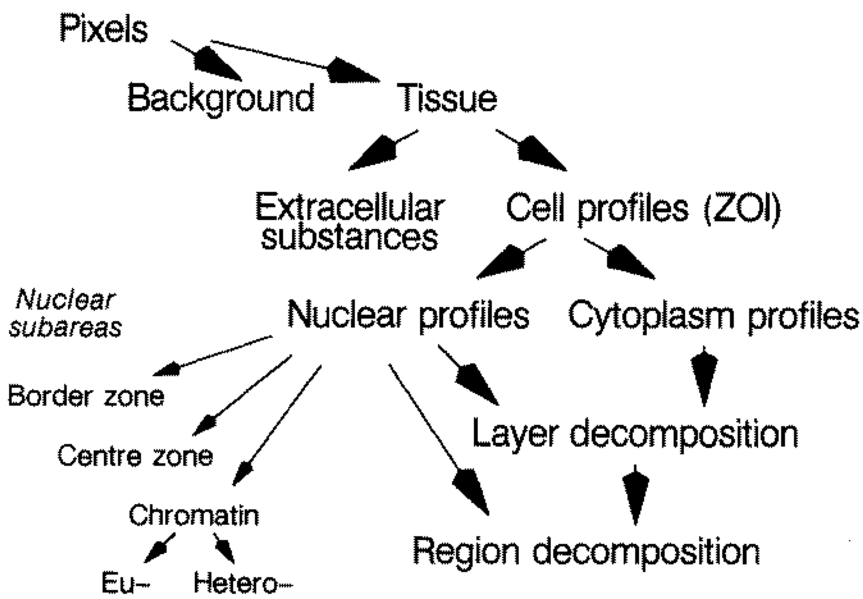

Fig. 1. Jllustration of *one* hierarchical concept of a tissue section

qualitative and quantitative analysis of sections. Of course, this is only one of a wide variety of possibilities. The construction of a hierarchy or the application of an hierarchical concept depends always and strongly from the modelling concept (Thompson, 1961).

Def.: A *hierarchy* consists of a number of *levels*. Each hierarchy level n is built by a set of *objects* O_n and a *relationship* $R_n \subseteq O_n \times O_n$ defined above the objects O_n. The objects O_{n+1} of hierarchy level n+1 are constructed using a *selection* criterion for objects from all lower levels under application of the corresponding relations.

– Pixel Hierarchy

Def.: For image processing, the set of objects O_1 of the lowest hierarchy are represented by the *pixels* of an image. The relation R_1 is any pixel neighborhood.

– Object Hierarchies

Def.: For the histological analysis of tissue sections the next hierarchy level consists of the the objects *tissue areas* and *background areas* (O_2).

The selection criterion of pixels (objects O_2) might base on certain stain and textural properties and/or the background (*Granlund*, 1978) or, more simple, by interactive choice, e.g. contour tracing (Oberholzer, 1983). The relation is for this level without importance.

For every higher level, there exist an increasing number of possibilities to define the objects as well as relations. To guarantee interpretability, we follow some concepts of pathologists, stating that *tissue* is built by *cells* and *intercellular substances*. The cells might be represented by the nuclear profiles of a tissue section (Fig. 1).

Def.: Let the objects O_3 of HE_3 be the *nuclear profiles*. A nuclear profile is *part* of the tissue, it is *simply connected*, *without holes*, and, for simplicity, let the *pixels* of nuclear profiles *be selectable* by a certain algorithm.

To define a feasible relation R_3 some more effort is necessary. In Rodenacker et al. (1987) it is described how the relation R_1 is inherited to nuclear profiles (relation R_3) by the zones of influence (ZOI). This method results in a nicely, even from human observers acceptable, neighborhood relation (Meyer, 1989).

In the meantime, some other methods are examined, especially the combination of local relations based on ZOI, Delaunay or Voronoi (Toussaint, 1988), with other non-locally based relations as

- being in a certain size class (small, medium, big),
- having a certain celluar type (mitotic, non-differentiated) or
- being not too far from a certain other object (near the basal layer, at the surface).

Of course some of the terms in brackets have to be defined as hierarchy levels. In general terms, this leads to some sort of small scale expert system, since this is an accepted way of knowledge representation.

The existence of a relation between two objects can be considered as a connection. Hence the type of connectivity of objects depends totally from the relationship defined. This can be quite complicated in cases of non-locally based relations. At least, a relation inherited from the neighborhood of ZOI can be represented as a subset of a Delaunay triangulation (Fig. 3c).

DEFINITIONS FOR GRAPHS

Starting from HE_3, a graph representation of the objects as *nodes* and the relationship as *edges* allows a formal operating as well as an easy display. In the following chapter some definitions of graphs and graph operations are exhibited. The mathematical morphology (Serra, 1982) has been proven as a valuable tool. The definition of graph operations follow this concept in a certain extent.

– General Premises

Def.: A *graph* is a 2-tuple $G := (V,E)$ with $V=\{\text{nodes}\}$ and $V \times V \supseteq E=\{\text{edges}\}$.

In all definitions the operators $\cap$, $\cup$ and $\setminus$ are used as symbols for *set union*, *intersection* and *difference*. Additionally pr_i denotes the *projection*

$$pr_i : X_1 \times \ldots \times X_n \rightarrow X_i \ , \quad 1 \leq i \leq n, \quad n \in \mathbb{N}.$$

Def.: A graph $G_1 = (V_1,E_1)$ is *contained* in another graph $G = (V,E)$ if

$$G_1 \subseteq G \ :\Leftrightarrow V_1 \subseteq V,\ E_1 \subseteq E\ .$$

Def.: Let $G_i = (V_i,E_i)$, $i=1,\ldots,m \in \mathbb{N}$ a set of graphs. A graph $G = (V,E)$ is called *base graph*, if $\forall\, i=1,\ldots,m \in \mathbb{N}\ \ G_i \subseteq G$.

In the following let $G = (V, E)$ be a base graph for $G_i = (V_i,E_i)$, $i \in \mathbb{N}$ if not otherwise mentioned.

– Boolean graph operations (Fig. 2)

Def.: *Union* $G_1 \cup G_2 := (V_1 \cup V_2, E_1 \cup E_2)$ (Fig. 2c).

Def.: *Intersection* $G_1 \cap G_2 := (V_1 \cap V_2, E_1 \cap E_2)$ (Fig. 2d).

Def.: *Join* $G_1 +_G G_2 := (V_1 \cup V_2, E \cap [(V_1 \times V_2) \cup (V_2 \times V_1)] \cup E_1 \cup E_2)$ (Fig 2c).

This operation can be considered as an extension of the union of graphs, where additionally the edges are added from the base graph G, whose corresponding nodes are both in $V_1 \cup V_2$ (Harary, 1969).

Def.: *Complement* $C_G(G_1) := (V \setminus V_1, E \cap [(V \setminus V_1) \times (V \setminus V_1)])$ (Fig 2d).

This definition avoids the existence of graphs with edges, whose corresponding nodes are not in the set of nodes. It differs considerably from the usual complement.

Def.: *G-reachable* nodes

$$N_G(G_1) := pr_2 ([V_1 \times (V \setminus V_1)] \cap E) \cup \cup pr_2 ([(V \setminus V_1) \times V_1] \cap E)$$ (Fig 2a).

$N_G(G_1)$ is the set of nodes reachable via *one* edge starting from G_1 with endpoint not in G_1. It is considered as a graph $(N_G(G_1), \emptyset)$ with *empty* set of edges.

Def.: *Closure* $A_G(G_1) := (V_1, E \cap (V_1 \times V_1))$,

or, using the join operation $+_G$,

$$A_G(G_1) := G_1 +_G G_1 .$$

A graph G_1 is called *closed* if $A_G(G_1)=G_1$.

– Derived graph operations

Based on the basic boolean graph operations defined above, the following operations oriented at the mathematical morphology (Serra, 1982) can be defined.

Def.: *Dilation* $D_G(G_1) := A_G(G_1) +_G N_G(G_1))$ (Fig 2e).

The graph G_1 is thus *dilated* by the *G-reachable* nodes N_G and *joined* with the corresponding edges (Klette and Voss, 1985).

Def.: *Erosion* $ER_G(G_1) := C_G(D_G(C_G(G_1)))$ (Fig 2e).

Def.: *Opening* $O_G(G_1) := D_G(ER_G(G_1))$ (Fig 2f).

Def.: *Closing* $F_G(G_1) := ER_G(D_G(G_1))$ (Fig 2f).

The clos*ing* operation F_G should not erroneously be taken for the clos*ure* A_G. It is necessary to remark the relativity of the operations to the base graph G.

EXAMPLES OF OBJECTS IN HISTOLOGY

For a tissue example the objects O_3, the nuclear profiles, are shown in fig. 3a. The relation R_3 is derived from zones of influences (Fig. 3a) as shown in fig. 3b. Two different hierarchy levels above O_3 are shown.

– Growth layers in epithelial tissue

The decomposition of epithelial tissue into certain growth layers, earlier shown by Chaudhuri et al. (1988), can be performed as an iteration:

n=1: Selection of objects of O_3 as first layer L_1 := basal layer (Fig.3b).

n=n+1: $L_{n+1} := A_G(N_G(\cup O_3 \in L_i, i=1..n))$ (Fig. 3c).

The resulting sequence of layers reflects the depth architecture of the tissue from the basal layer up to the surface.

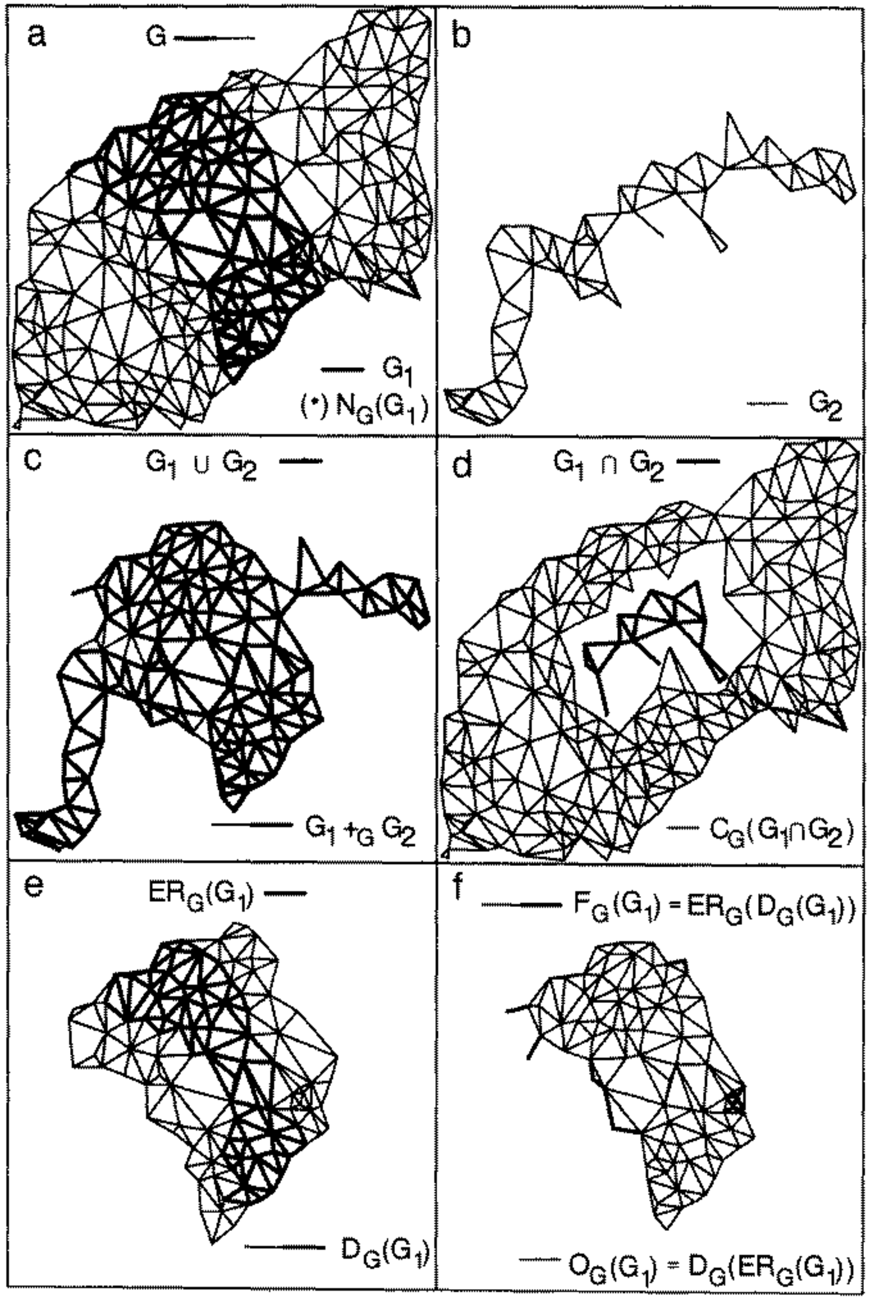

Fig. 2. Some graph operations illustrated (see text)

This sequence, a possible relation $\mathbf{R}_2$, can be used to create an order relation $\mathbf{R}_3'$ on the nuclear profiles $\mathbf{O}_3$, with respect to the layering. E.g. the nuclear profiles are layer-wise numbered. This allows it to create tissue regions.

- Tissue regions

Compared with growth layers, a coarser tissue region can be defined using feature values of the objects $\mathbf{O}_3$ applying the relation $\mathbf{R}_3'$ mentioned above. As an example let us use the feature *area of ZOI* and certain thresholds, e.g. 1/3, 2/3 of the whole tissue area A. Again an iteration is performed with

n=1: Selection of the first object, $i = 1$, of $\mathbf{O}_3$ under relation $\mathbf{R}_3'$,
while Σ area of $ZOI(O_i) < A/3$, $O_i \in R_n$ do $i = i + 1$, $R_n = R_n \cup O_i$;

n=2,3: $i = i + 1$, $R_n = O_i$.
while Σ area of $ZOI(O_i) < A/3$, $O_i \in R_n$ do $i = i + 1$, $R_n = R_n \cup O_i$.

The results are the objects R_1, R_2 and R_3, which represent a rough partition of the tissue (Fig. 3d). Such partition is used by pathologists for diagnostic purposes, e.g. to estimate the appearance of mitotic cells (specific objects) in each region or to compare the degree of differentiation (specific object arrangement and shape) in the different regions. This approach leads to the extraction of features for the objects defined above.

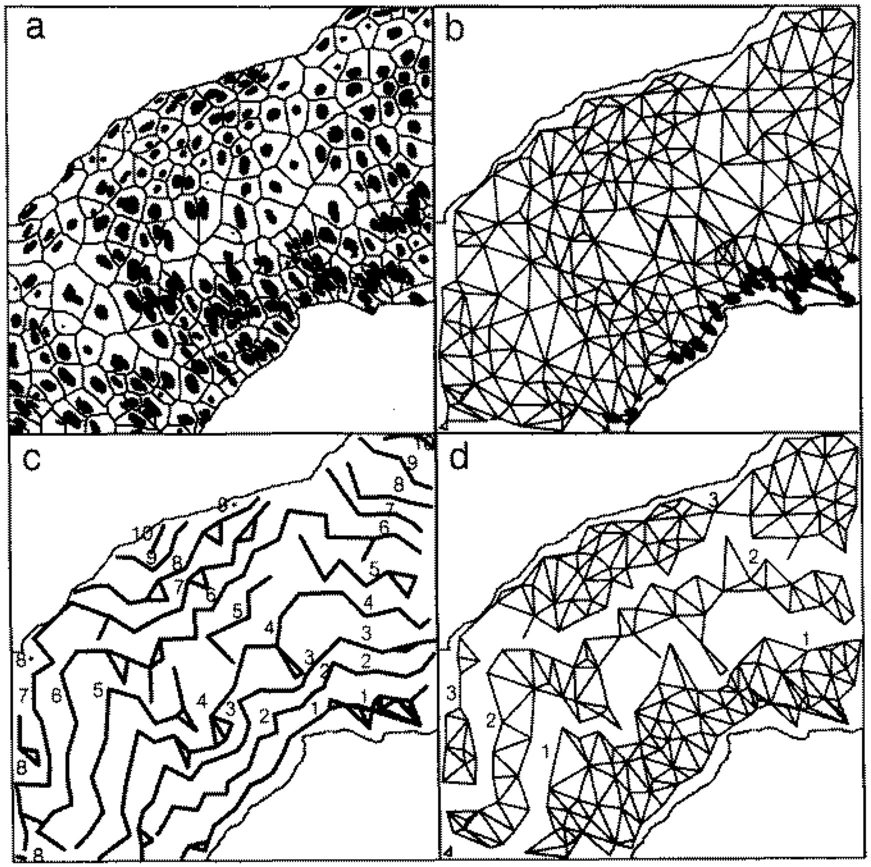

Fig. 3. Tissue section in different hierarchy levels (see text)

FEATURING OF OBJECTS

The features of pixels (objects of O_1) are at least the pixel value and the location. The features of objects of higher levels can be the statistical parameters of the pixel value distributions of every object. This method leads, for nuclear profiles O_3, to the so called cytometrical approach with several features quantifying size, shape and extinction of the objects. The latter can directly be applied to objects of other hierarchy levels.

In addition to cytometrical features other types, e.g. quantifications of the local or relational arrangement respectively, of objects can be computed. A simple example is the mean distance of objects O_3 which are part of an object of another hierarchy level. Another more complicated one is the maximum free path length. This type of features is called the *histometrical* approach. The combination of both sets of features can be used for a classification sceem comparable with those applied in classical pathology.

SUMMARY

A method and a concept is shown to describe in a quite formal way *objects* in a *hierarchical system*. This is, in principal, the usual way defining objects, however it is rarely consequently performed. For an example from pathology the methods are illustrated. Starting from the concepts of mathematical morphology for the description of shapes, this approach can be used to describe arrangements or architectures of objects. This might lead to a concept of shape in a more abstract but formal fashion.

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