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The Analysis of 3-D Organ Microstructure Assisted by Computers. Its Application to Histopathological Studies

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1. Stereology vs. 3-D Reconstruction

Expectations are growing to obtain 3-D images of the human body interior with the aid of CT or other techniques for non-invasive imaging. In histopathology, visualization of 3-D structure from serial sections has long been used in studies of tissue microstructure. This has been necessary because the materials available for pathologists only consist of 2-D sectional pictures such as microscopic slides, electron micrographs and so on, while actual structures to be analyzed are always 3-dimensional, forming a continuous parenchymal mass behind or in the foreground of section. With only a single sectional picture at hand, one is often unable to answer an essential question of histopathology: in what an architectural change an organ is involved in what disease. It appears that whenever observing a 2-D sectional picture, pathologists are attempting to construct an imaginary 3-D picture from it, though, in most cases, not being aware. However, this attempt often ends in confusion particularly when a complicated structure is encountered. In fact, a number of controversial problems of pathology which have long been studied and still are unsettled appear to harbor this sort of difficulty.

Stereology should be the first resort, of course, for one who seeks some quantified information about 3-D morphology. In fact, new principles of stereology

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have been developed also in the domain of histopathology and anatomy by, among others, Weibel (1963a, 1963b) and Suwa (Suwa *et al.*, 1976; Suwa, 1978) who contributed much in correlating the form of tissues with their function. An example may be found in the determination of the alveolar surface area of human lung and its changes in emphysema, which provided quite an important understanding in respiratory pathophysiology. However, we know today that the use of stereology cannot be so far-reaching as previously believed, as shown in the list of DeHoff (1983) (Table 1) who classified quantities according to whether or not they are accessible to stereological estimation. Thus, it is only in rather special problems of histopathology and under limited conditions that one can rely on stereology. We are often in a situation in which we have to deal with the spatial morphology in a direct way, i.e., by 3-D reconstruction from serial sections.

Table 1. Classification of geometric properties of three-dimensional features (DeHoff, 1983).

Class I: Standard stereological properties, estimated without geometric assumptions.
Volume fraction
Area of surfaces or interfaces
Length of lines, edges, or triple lines
Integral mean curvature of surfaces
Integral curvature of lines
Integral torsion of lines
Class II: Properties that require geometric assumptions for their estimation
Feature size distribution
Number of features (simple shapes)
Feature averages (volume, area, size)
Degree of anisotropy
Class III: Properties that cannot be estimated stereologically.
Number of features (general)
Connectivity of features (general)
Size distributions (by volume, area, diameter)
Spatial distribution information
Real feature shape

There is a technical difficulty inherent in 3-D reconstruction: when performed manually, it demands a grinding work of a performer. The senior author has long been engaged in reconstruction of human organs or tissues, since his final goal has always been to establish the principle of tissue architecture; this also allows to understand how, in diseases, the structure of an organ can deviate from the norm.

Recently, we attempted with the cooperation of Olympus Co. Ltd. to develop a 3-D reconstruction-aiding system. Based on these experiences, we describe in the present article in what aspects of histopathology a 3-D analysis is unavoidable, and, what more future assistance we can expect in extending the application of computers to studies in this domain.

2. Some Problems Requiring 3-D Analysis

2.1 Spatial distribution information

Figure1A is a sketch of a 3-D image integrated in a computer display. Here reconstructed are bile ducts draining the left liver lobe which was surgically removed because of bile duct carcinoma arising in the hilar region. In patients who have a bile duct carcinoma but in whom the tumor appears localized in the left or right lobe of the liver, surgeons attempt to remove the extrahepatic bile ducts and a liver lobe that are considered to harbor the tumer. So far, however, the postoperative prognosis has generally been poor, reflecting the difficulty in establishing an adequate design for surgical therapy in such patients. This is due to lack of accurate knowledge of pathology, i.e., about the way cancer develops and extends along the bile ducts. However, the biliary system is a tree spreading in the 3-D space. In a routine examination of surgical material taken at an operation, a pathologist observes at most several 2-D sectional pictures of the material, from which he cannot integrate 3-D distribution of tumor. These circumstances urged us to undertake a serial section analysis in a series of cases. In the case reconstructed in Fig. 1A, the surgical material was reduced into 139 sequential slices using a ham slicer, with a single slice being 1 mm thick, and the contours of bile ducts appearing on the slices were inputted into a computer by digitizing. Ducts with carcinoma in site (CIS), those with dysplastic epithelia (premalignant changes) and those with ordinary epithelia were inputted as separate files in order to reproduce in different colors. Tumor visualization in such a 3-D mapping may correspond to obtaining what in Table 1 was designated as "spatial distribution information", a task apparently beyond the scope of stereology.

It may be clear in the reconstruction of Fig. 1A that the operation was at least successful in totally removing the ductal segments with cancer growing in the wall. However, the cancer is shown surrounded by a zone of segments having dysplastic epithelia, i.e., precancerous cells. This dysplastic zone has apparently reached the surgical stump of the right hepatic duct draining the right liver lobe which was not removed. Consequently, such precancerous cells have to be considered remaining in the patient. In view of the very high susceptibility of dysplastic epithelia to cancerous changes, there appears to be a high risk of tumor recurrence in this patient. Fig. 1B is from another liver that was taken at autopsy and also has bile duct carcinoma, where reconstruction involved 235 serial slices. Here, carcinoma is shown to arise as multiple foci. Again there are zones of dysplasia surrounding the foci of carcinoma.

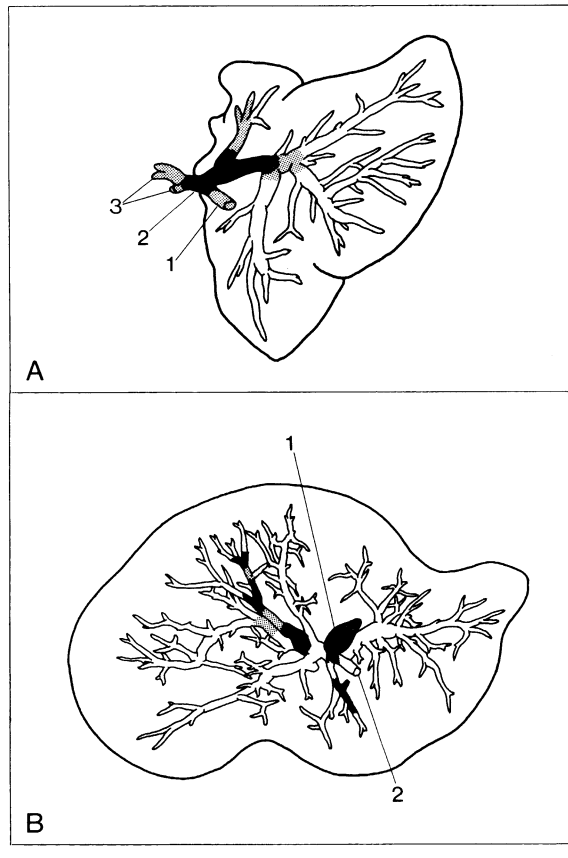

Fig. 1. Sketches from computer-integrated 3-D reconstruction in two livers showing extension of carcinoma along bile ducts. A: Left liver lobe removed by operation, in which ducts with carcionoma (darkly hatched) are wholly contained, but dysplasia (lightly hatched) has reached the stumps. 1: the common bile duct, 2: the left hepatic duct, 3: the right hepatic duct. B: An autopsy liver harboring bile duct carcinomas (dark) forming multiple foci. 1: the gallbladder, 2: the common bile duct. Note that carcinoma (dark) is forming multiple foci. The lightly hatched areas denote the presence of dysplasia.

One of the authors (M.S.) performed such an analysis on materials from eleven patients operated for carcinoma of hilar bile ducts (Suzuki *et al.*, 1989). In as many as seven of the eleven patients, carcinoma proved to have a surrounding zone of dysplasia, and in five, it arose as multiple foci. The unexpectedly wide spread of precancerous lesion and frequent multiplicity all account for the surgical incurability—A sour reality, which, once disclosed, will help surgeons select the most adequate strategy against this intractable disease.

2.2 Topology

Figure 2 presents a macroscopic appearance of liver cirrhosis. Cirrhosis, in a word, is a common terminal state of various chronic liver diseases such as chronic (viral) hepatitis, alcoholic injuries and so on. Usually, a cirrhotic liver contains a vast number of spherical "nodules" and internodular "interstitium". The interstitial tissues are scar zones created by damages of liver cells due to viral hepatitis or alcohol; nodules are the masses of liver cells that survived damages and remain gradually swelling by regeneration of cells. Serial section analysis from various cirrhotic livers demonstrates that 3-dimensionally, various types of cirrhosis have a common architectural skeleton; nodules are not a separate structure but are united with the adjacent ones to form a network in the space with fine meshes (Fig. 3). Significantly, precirrhotic lesions of the liver such as chronic hepatitis or alcoholic injuries all have a similar skeleton, where the parenchymal tissues that survived necrosis are forming a 3-D network as well. Thus, there is an aspect in the morphogenesis of cirrhosis where topological analysis of the structural framework is required. Assume, for example, that the network established at the very initiation of disease is maintained through the cirrhogenetic stages until the cirrhotic changes have been completed many years later. If so, the whole structure would remain unchanged from a topological point of view, with invariables of connectivity like the 1st Betti number (p_1) maintained constant throughout the process. This would imply that cirrhogenesis simply comprizes a continuous nodular transformation of parenchymal tissues that are swelling due to liver cell regeneration. What if, on the contrary, the network skeleton is susceptible to frequent alteration of its connectivity due to recurrent injuries to hepatic parenchyma, which gradually coarsen the network so that the 1st Betti number is reduced stepwise. Then, one has good reason to assume that a precirrhotic liver is subjected to a radical reformation of skeleton before it reaches a final cirrhotic state. Such injuries, if they can happen at all, may correspond to the bout of liver cell necrosis that is assumed to recur in patients with chronic liver disease. A necrotic bout can be so extensive as to sever internodular

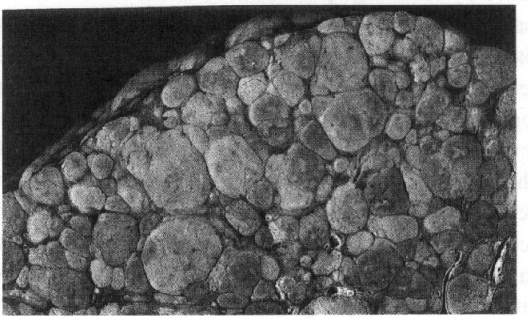

Fig. 2. A typical appearance of liver cirrhosis (posthepatitic type) with spherical regenerative nodules.

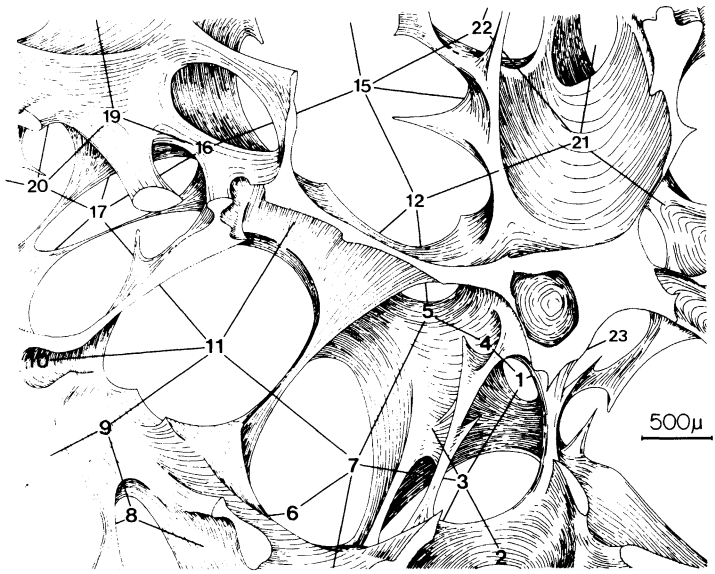

Fig. 3. A graphic reconstruction (manually performed) of interstitial septa from a cirrhotic liver. The spherical spaces correspond to nodules. Note that the nodules are all united to the adjacent ones, forming a 3-D network as illustrated by a linear system.

connections, resulting in a coarsened network and correspondingly, a reduction of its 1st Betti number. Along which of these possible courses the actual event unfolds, a problem of basic importance in clinical hepatology, is apparently beyond the reach of stereology since it deals with connectivity of 3-D structures (Table 1).

One of the authors (T.T.) coped with this task by performing a manual analysis of topology (Takahashi and Suwa, 1978). In livers of six patients with various types of chronic disease, the 1st Betti number (p_1) of parenchymal network in a whole organ was estimated by counting upon serial sections the internodular connections contained in a sample volume. Calculation of p_1 was made based on the Euler-Poincaré formula (Fig. 4). In the result shown in Fig. 4, let us pay attention only to the difference between the case of chronic hepatitis and that of Type B cirrhosis, a type generally regarded as typical post-chronic hepatitis variety. It is shown that the 1st Betti number that is estimated at about 6 million in chronic hepatitis steeply falls to one hundred thousand at a stage of completed cirrhosis. Thus, the morphogenesis of cirrhosis is likely to involve a process of what we call a skeleton reorganization (Fig. 5). The mechanism underlying the process is considered to be the bouts of liver cell necrosis, an assumption consistent with clinical experiences and offering a feasible explanation for the morphogenesis of cirrhosis from chronic hepatitis.

The above are some tasks of histopathological research where one cannot rely

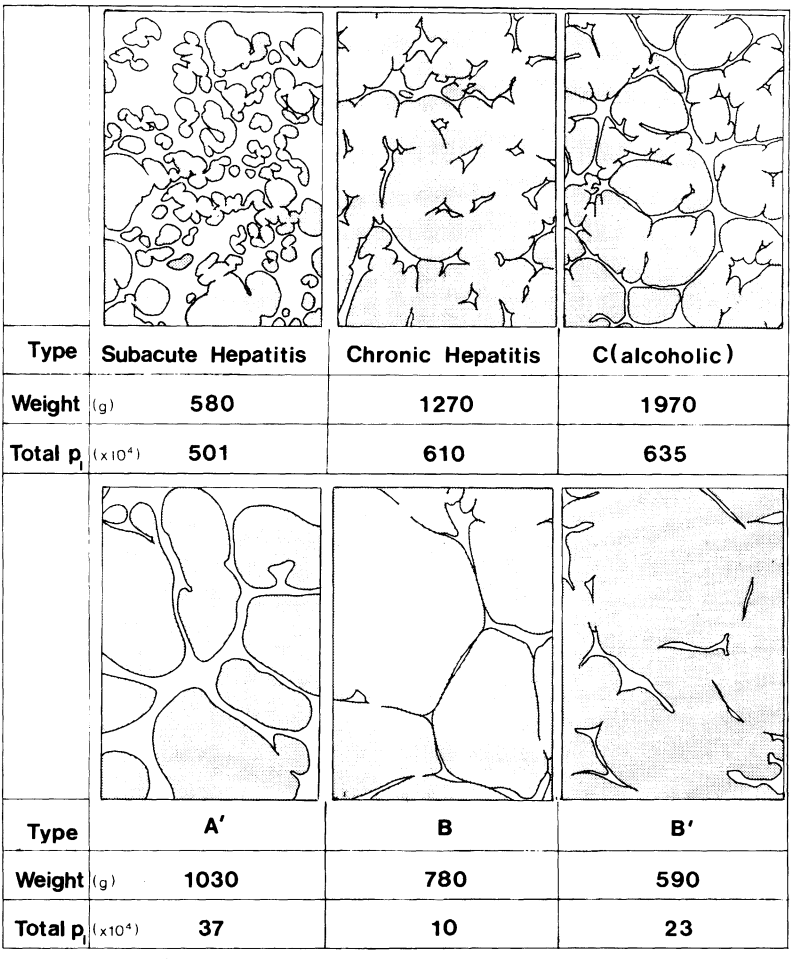

Fig. 4. Topological properties of chronic liver diseases expressed by 1st Betti number of the nodular (parenchymal) network. A, B and B' are types of cirrhosis, the latter two being the posthepatitic type. p_1: estimates of 1st Betti number for the whole organ.

on principles of stereology: the spatial distribution of morbid changes and their topological character. In fact, a review of literature discloses that also historically, it has always been in one or the other of these problems that researchers resorted to 3-D reconstruction. In the next place some considerations follow on the aspects of 3-D structural research where a computer-assist is expected to be most helpful.

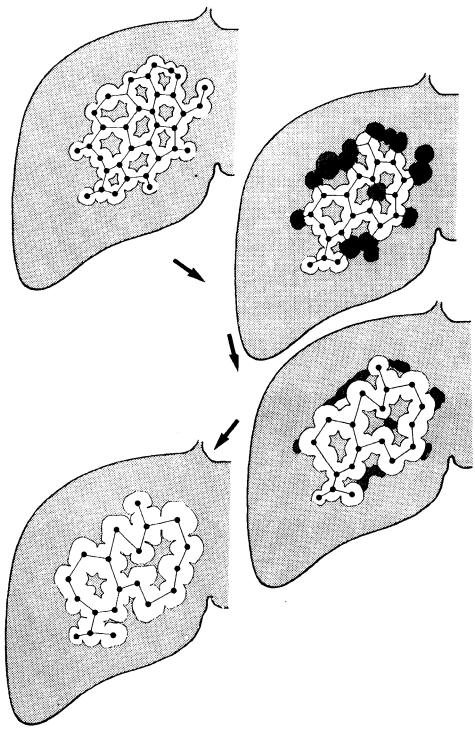

Fig. 5. A schema illustrating the morphogenesis of cirrhosis. The fine network at an early stage of development is transformed into an coarsened skeleton through repeated parenchymal necrosis (hatched).

3. The Computer-Assisted Reconstruction

Prior to reconstruction, one has to prepare a set of serial microscopic sections from a tissue block, and this requires a fully manual work (Table 2). How many sections are necessary depends on the purpose of study. A 3-D reconstruction begins with the contour extraction from structures of interest in a microscopic picture of section which usually comprizes a mixture of various components, of which, reconstruction always involves only a part. Reproduction of the entire components is meaningless; if performed, the result would be little more than creating a solid mass, where one cannot gain any insight into its internal structure. Suppose, for instance, that a lung tissue is to be reconstructed to visualize the architecture of intrapulmonary airways. A microscopic picture of lung contains, besides the airways, arteries, veins, connective tissues and alveoli; in morbid

Table 2. Procedures for 3-D reconstruction.

Step 1	Preparation of serial microscopic sections
Step 2	Abstraction of profile contours from structures of interest
Step 3	Inputting of profiles by digitizing
Step 4	Integration and visualization of a 3-D image
Step 5	Computation of 3-D quantities

conditions, inflammatory exudate, granulomas or other lesions can add, further complicating the picture. Therefore, serial sectional pictures of lung tissue have to be purified prior to reconstruction so as to eliminate all components except the airways. As yet, however, we are not in a state in which we can enjoy a full computer-aid in the purification process. A microscopic picture of tissue contains so much noise that even with the most developed technique of image analysis one does not appear capable of abstracting any desired structure from a tissue section. Manual abstraction of contours still remains the sole option. Thus, it is only in Steps 4 and 5 of Table 2 that we can expect a computer-aid, i.e., the graphic integration of 3-D images and the calculation of 3-D quantities.

Figure 6 is an example of 3-D image from a small part of a cirrhotic liver integrated from serial sections in various ways, using a system we constructed based on a Hewlett-Packard model 310. Figure 6A was produced as what we call a "stack of slices". The picture, though giving a 3-dimensional impression, becomes stuffed up already at a stack of some 10 slices, keeping one from gaining insight into the connecting relation of nodules. Apparently, the purpose of reconstruction that is to study the topological features of the nodular network is far from being satisfied. In Fig. 6B, the nodules were expressed by wire-framing. Here, one can at least see the nodules intertwining with small blood vessels. This is an information essential in understanding that the nodular network is coined, somehow but closely, by the spatial arrangement of small vessles. However, one still remains unable to see the connectivity among the nodules. Thus, we attempted to depict the skeleton of nodular network with a linear diagram of Fig. 6C. Here, each profile in a 2-D picture was represented with a point corresponding to its center of gravity. A pair of points in two adjacent section were connected by a line when the two profiles proved to be united. Although the linear diagram may still be far from being geometrically similar to the original structure, it offers at least a faithful topological description of the nodular network, allowing the computer to enumerate the 1st Betti number of nodules. In Fig. 6D, surface smoothing was performed by creating triangular "tiles" with which to cover the terraced surface. However, this takes ten hours or more and appears to be a task beyond the performance of a microcomputer.

Three-D reconstruction is a method of study demanding much time and energy of the performer. We cannot afford to pay such a grinding work simply to enjoy a

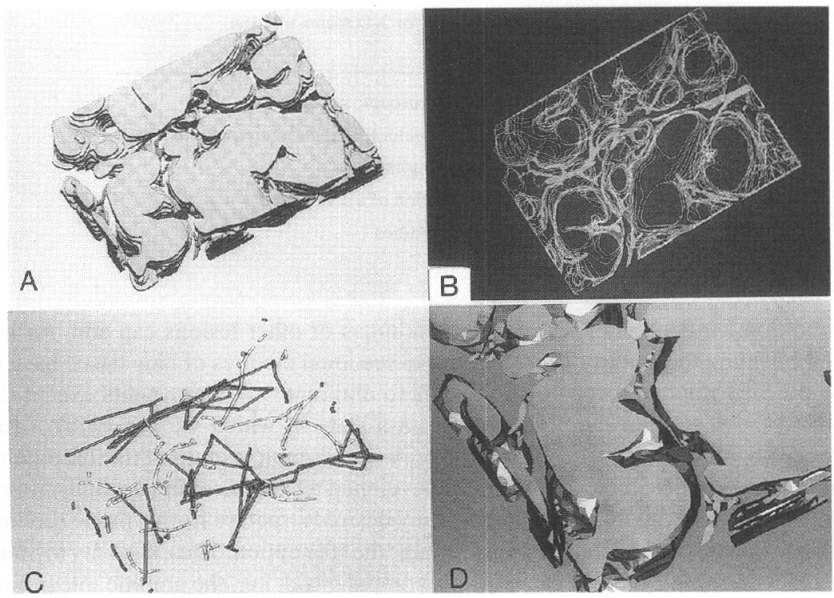

Fig. 6. An example of computer-aided reconstruction in a cirrhotic liver. A: "stack of slices". B: wire-framing of nodules. C: skeleton of nodular network. D: surface smoothing with triangular patches. Originally shown in color.

novel, spectacular graphics. When one decides to undertake reconstruction, one should do it as the last resort, being fully aware of what information he is going to obtain and whether it deserves the labor he is going to pay. Whoever embarks for reconstruction without having a purpose more definite than commanding a 3-D view would be liable to disappointment at a picture integrated in a computer display, which very often is little more than a sort of surface image. For example, the cirrhotic nodules reproduced in Fig. 6A present their surfaces, while we are left uninformed of the basic 3-D morphological qualities: the internodular connectivity, the nodulo-vascular interrelationship, and so on. Advancing steps of reconstruction only add renewed surfaces. This causes the same sort of frustration we often have while observing a scanning electron micrograph, where we wish to have a look into the "inner" structures by breaking the surface; however, even if it were done, the result would be that we only find a renewed surface through the hole, not the inner structure. Thus, what we expect to have in the future is a technique by which to deal with a 3-D structure in such a way as to allow us not only to have images but to help study its 3-D properties; connectivity, distribution, and so on. Essential will be the development of a technique that looks like the depiction of 3-D skeleton, which, if realized, will satisfy nearly all of what we require in 3-D analysis.

After all, we recur to the question: What do we expect to obtain by performing 3-D reconstruction? The answer should be to obtain information inaccessible to stereology. For that purpose, integration of a beautiful image is not necessary at all. A study of 3-D morphology may even be designed so that, the goal is to obtain quantified data, not a picture.

4. Conclusion

Here we outlined why we need to study 3-D tissue structures and what computer-aid is and will be available in such a study. In a computer-assisted reconstruction, the form data of 3-D tissue component are totally stored in a computer memory as a set of coordinates. The data allow one to obtain by computation various three-D quantities that cannot be estimated stereologically. We are making some such attempts, in other words, 3-D computational geometry (Takahashi and Chiba, 1990), but a detailed report will be found elsewhere.

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