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PRACTICAL GUIDELINES FOR A MORPHOMETRIC STUDY

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ABSTRACT

The article gives practical advice for those planning to perform a morphometric study. The following aspects are handled: the definitions of histological parameters, the morphometric model, the selection of stereologic and morphometric parameters, the methods for stereologic and morphometric measurements, the apparatus for point counting methods, microscopic magnifications, grids, sampling problems, tissue processing, the relevance of stereologic formulas, and the reproducibility of measurements. An exercise on point counting methods is included.

INTRODUCTION

Stereology and morphometry are fields in which mathematical background theory and practical measurements meet and are applied in a fashion which does not allow the separation of these two aspects. Earlier experience in morphometric measurements greatly helps in application of mathematical principles. In this article we try to give the reader some hints which could be valuable in a starting situation, and help the investigator in finding suitable ways to apply morphometry in practice. Several aspects involved are handled and each is given its own subtitle in the text.

DEFINITIONS

In histopathology tissue changes are often described by collective terms. Examples of such entities are interstitial

fibrosis, dysplasia, atypia, hyperplasia, and hypertrophy. The terms are more or less well defined and as such often sufficiently characterize the tissue changes to a well educated person. Sometimes these terms are insufficient, however. In a morphometric study it would be best to handle terms which are based on tissue characteristics such as they are present in a section, e.g. on the staining qualities of a specific structure. One practical example is the quantitation of the amount of connective tissue ("grade of fibrosis") in the myocardium (Romppanen et al. 1982, Jantunen et al. 1983). If the sections are stained with van Gieson and hematoxylin stains, the connective tissue can be defined as red-stained fibrillar intercellular areas. In other words, the definition should be such that a person not familiar with minute histological details could distinguish the tissue component under study. One thing is important: definitions of different concepts, parameters, or structures should not have overlap, or the possible overlap must be kept on minimal level.

MORPHOMETRIC MODEL

After having defined the histologic structures one can proceed to a morphometric model. The morphometric model includes two things:

1. A compartmental model which is a graphic presentation of different tissue compartments and their relationships to each other.

2. A geometric model containing geometric definitions of tissue structures. These definitions are important when 2-dimensional findings are used to reveal size distribution in 3 dimensions.

The compartmental morphometric model can be constructed in several ways. The way depends on what structures are to be measured and what measuring methods are used. For example suppose we measure fat in fatty metamorphosis of the liver. The amount of fat should be measured by estimating the volume density (volume fraction) of the fat droplets, which are seen as circular empty spaces inside the hepatocytes.

Figure 1 shows three alternatives for a compartmental morphometric model. The first alternative (A) is quite simple. Liver tissue is divided into two tissue compartments - fat and other structures. In the second model (B) the tissue is first separated into the portal fields and parenchyma, and the compartment parenchyma again into two compartments - fat and other structures. In the third model (C) the parenchymal compartment of the liver is divided into hepatocytes and other structures, and fat is presented as a part of the hepatocytes.

Our choice of the appropriate morphometric model among these three alternatives depends on how we select the

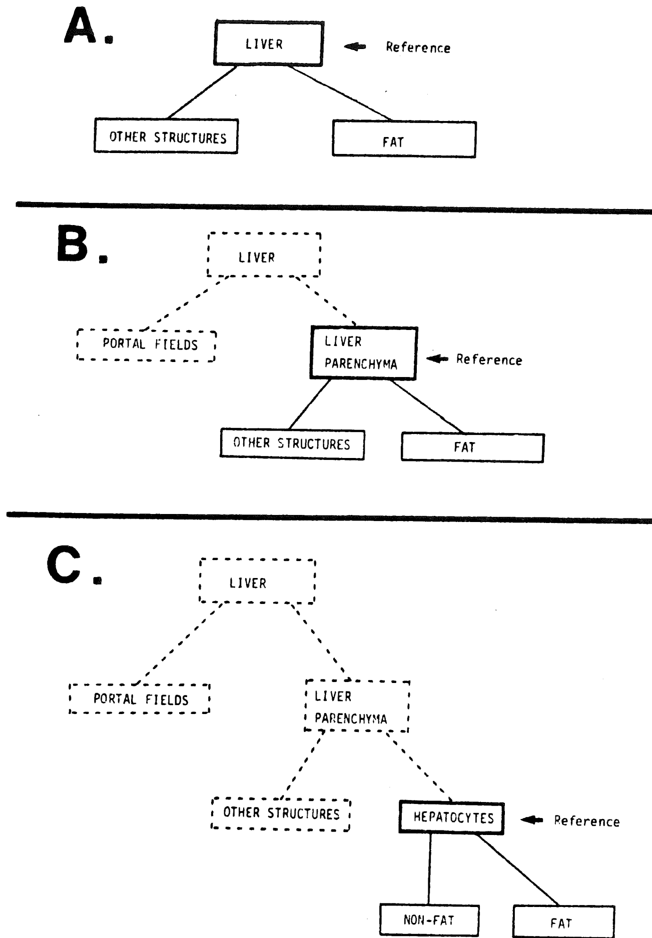


Fig. 1. Three alternatives for the compartmental model of the liver. Each could be applied in studies on the volume density of fat droplets in liver cells (fatty metamorphosis). For further details see the text.

appropriate reference volume (or the reference area) for the fat droplets. In A the total liver tissue is the reference volume, in graph B the reference volume is the liver parenchyma, and in graph C it is the hepatocytes. It is a good general rule that the reference area or reference volume should not change at all or that changes if present should not be from reasons independent of the changes we want to measure. In model A the portal fields are included in the reference, but they may be enlarged independently of the amount of fat, for example, in cirrhosis of the liver. Comparison of graphs A

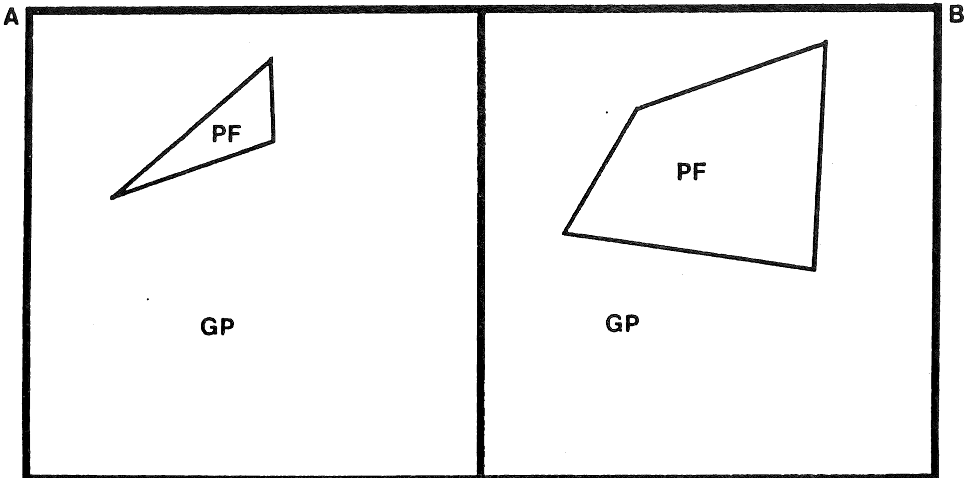


Fig. 2. Influence of the size of the portal fields on the volume density of fat droplets in the liver. For details see the text. PF = portal field, GP = glandular parenchyma of the liver.

and B in Fig. 2 demonstrates this. Due to the relatively large portal fields in liver cirrhosis (graph B, Fig. 2), the maximal amount of fat possibly present would never exceed a certain level - in graph B about 80%, but in graph A more than 95%.

For the above reasons model A (Fig. 1) should be rejected. In models B and C the portal fields are not included in the reference volume. In model B, however, liver parenchyma includes hepatocytes and sinusoids; the latter do not contain fat droplets. If the sinusoids are not altered significantly, this model will be the most practical one. Theoretically, model C would be the best, but measurement of the sinusoidal areas - included in compartment other structures - would require more precision and take more time than is needed in model B.

Choosing an adequate reference volume may also be difficult because the size of the organ studied may differ significantly among the individuals to be investigated. This occurs in investigations of growing organs, for example, in developmental studies. The organ may grow so rapidly that relative morphometric parameters (such as the volume density of a tissue structure) diminish, although the absolute volume of the tissue structure increases. In this situation one has to decide whether one is interested in relative volumes or whether the absolute volumes are aimed at. If the absolute volumes are specially interesting the volume densities will not characterize them sufficiently well (Romppanen 1981) and one should try to measure the absolute volumes directly.

The morphometric model should also include geometric

definitions of the structures. In cases in which the numerical densities of corpuscle-like structures are the objects of interest, the shape of the structures must also be defined. An assumption of shape is usually necessary. Cells and their nuclei can be considered spheres or ellipsoids; tubular structures can be considered cylinders. After the assumptions have been made, established geometrical laws can be used in calculating the appropriate parameters (Romppanen et al. 1980).

3. SELECTION OF APPROPRIATE PARAMETERS

Table 1 gives a list of parameters most often evaluated in stereologic or morphometric studies (for details see Collan et al. 1983, Elias et al. 1971, Underwood 1970, Weibel 1979). In a randomized situation the first three pairs of parameters (V_V-A_A, S_V-B_A, L_V-Q_A), presented two- or three-dimensionally, are directly proportional to each other. The selection of a two- or three-dimensional expression depends on the nature of the investigation and the investigator. Relationships concerning the numerical density of particle-like structures in sectional plane (N_A) or in space (N_V), however, are more complicated.

The selection of appropriate parameters depends on the nature of the investigation. In diagnostic or descriptive histopathology the two-dimensional approach is often satisfactory, because the diagnosis is based on two-dimensional sections. In cases where the morphometric parameters are compared with biochemical or other laboratory findings the three-dimensional approach is more useful. In many cases absolute volumes, surfaces, lengths, and numbers are most suitable because a change in the reference volume does not influence the results on these parameters (Romppanen 1981).

4. METHODS FOR MEASUREMENTS

Three types of methods are commonly used for morphometric measurements:

1. Point counting and intersection counting methods.
2. Semiautomatic optomanual tracing.
3. Automatic image analysis.

Often we are interested in having average values for different morphometric parameters. In this case the point counting or intersection counting methods are most suitable. The registration of point hits or intersections is relatively rapid, and the number of microscopic fields can be large enough to cover the tissue in a representative fashion. The measurements in each single microscopic field need not be very

TABLE 1. A list of some stereologic and morphometric parameters

3-dimensional	2-dimensional
1. V_V Volume density (mm^3/mm^3) (volume fraction)	A_A Area density (mm^2/mm^2) (area fraction)
2. S_V Surface density (mm^2/mm^3)	B_A Length density (boundaries of two tissue phases) (mm/mm^2)
3. L_V Length density (mm/mm^3)	Q_A Numerical density (cross sections of linear or tubular structures(mm^{-2})
4. N_V Numerical density (mm^{-3})	N_A Numerical density (cross sections of particles) (mm^{-2})
5. Special measurements (diameters, size distribution of diameters, distances etc.)	Special measurements (diameters, size distribution of diameters, distances etc.)
6. Total numbers of tissue structures	Total numbers of sectioned tissue structures

Equations

1. $V_V = A_A = P_P$ — P_P = point fraction

2. $S_V = \frac{4}{\pi} B_A = 2 I_L$ — I_L = number of intersections of tissue phase boundaries and test line, per unit length of test line

3. $L_V = 2 Q_A$

4. a) $N_V = N_A/D$ — D = mean caliper diameter

   b) $N_V = \frac{1}{\beta} K \frac{N_A^3}{V_V}$ — β = shape factor; K = size distribution factor

accurate if we are interested in average values. The variation within a single field is compensated by the large number of fields available for analysis (Mathieu et al. 1981)

On the other hand, if the object of interest is the size variation of a defined tissue structure, for example the nucleus, the semiautomatic tracing method is most suitable (Rohr 1977, Weibel 1979).This method applies a magnetic graphic table. The histologic pictures are projected or the micrographs laid on this table. The contours of the structures are followed with a cursor which results in a digitized "image" in computer memory. The impulses from the cursor or from the system in general can be transferred to a printer or into a computer file. This system is best suited for analyzing variations of nuclear shapes and sizes in malignant tumours (Baak et al. 1981, Ooms et al. 1981). The pathologist can select the area to be analyzed. The values for each single structure are relatively exact, much more so than when the point counting method is used (Gundersen et al. 1981, Gundersen et al. 1981, Mathieu et al. 1981). The time required for tracing may, however, be a limiting factor.

Automatic image analysis can also be used for nuclear measurements. However, the staining methods may not be discriminative enough for totally automatic operation. Different structures behave differently in this connection and special staining methods are often necessary. The standard staining methods (e.g. hematoxylin and eosin stain) are not always suitable and different cellular structures have different needs. If a discriminating staining method is found automatic operation will usually not be successful because most methods are based on differences in shades of color, and not only on differences in shades of grey tones. The image analysers of today operate on grey levels, not on colors. If they could operate on colors, microspectrophotometers could soon start to be old-fashioned. Some programs are available which are said to aid in color detection, but lots of work has to be done before one can do completely automatic analysis of microscope image. So there is no automatic image analysis in the sense that the instrument could take the job of the diagnostic physician (On the other hand, the whole idea that the instrument could take the place of a physician is absurd, and the point is that the instrument will help him to be more accurate and reproducible in his diagnostic work). So, we can only speak of interactive computer assisted image analysis, because in most if not all programs, the computer is unable to distinguish between structures with same grey levels but different colors, or with structures with roughly similar shapes. This means that the operator (read: pathologist) has to be able to tell the computer which of the structures the computer selected should be included in the measurements. This decision loop is part of most of the program packages. There are also special problems in analyzing electron microscopic pictures.

By applying "automatic image analysis" the size and shape variation of nuclei in various conditions have been analyzed (Baak 1981, Rigaut et al. 1981, Rigaut et al. 1982, Voss 1983). Here again the selection of the microscopical fields is done best by a pathologist. Automatic image analysis has not made the pathologist unnecessary.

Finally, selection of the method depends on the apparatus the investigator has available in his institute. Point counting and intersection counting methods are simple and inexpensive. They can be used by any pathologists. Therefore, some problems in point counting method are discussed in detail in the following.

5. APPARATUS FOR POINT COUNTING METHODS

Point counting and intersection counting are applied in various ways:

1. Photography of the microscopic fields. After photography point and intersection counting are performed on the photographic (micrographic) image. A transparent grid with points and lines is laid on the photograph and the number of hits registered.
2. Projection microscope. The microscopic image is projected on a projection head. A lattice can be attached on the projection head of the microscope and the hits estimated on this projection head. A motor for moving the specimen in a systematic or random manner can be connected with the microscope. The registration of point hits occurs with a hemocounter. The counts are then collected for further processing. One can also connect the counter and a computer and feed the results of pointcounting into computer memory for further processing (Rätz et al. 1983).
3. Drawing tube for projecting a lattice from the table on the microscopic image. The image of the lattice is seen on the microscopic image. Point hits and intersections can be registered with a hemocounter as explained above.
4. Ocular lattice. A special grid (line grid or point grid) is introduced into the eye piece of the microscope. The grid lattice is seen on the microscope image allowing point hits and intersections to be registered as above.

The two last systems (3 and 4) have the advantage that the focusing level can be continuously controlled and microscopic details kept sharp which allows unequivocal registration of point hits. Corresponding control of focusing is not available with projection microscopes in which minor blur is usually not detected. The drawing tube and the ocular lattice have the additional advantage that their use is possible without a dark room.

6. MICROSCOPIC MAGNIFICATIONS

When selecting the magnification, two aspects need be considered:

1. The magnification should allow distinction of the tissue structures without difficulties.

2. The magnification should be as low as possible, within the limitations given above, so that the section can be analyzed in a representative fashion.

In measuring surfaces, for example the surface of the mitochondrial inner membrane or the surface of the endoplasmic reticulum, great variations between different laboratories have been reported. One reason for the differences is the use of different magnifications (Paumgartner et al. 1981).

7. GRIDS

Grids (lattices) are used for counting point hits (P), line intersections (I), and numbers (N) of tissue structures within the grid frame (Weibel 1979). To calculate the secondary morphometric parameters from these primary counts one has to pay special attention to test points, test lines and test area.

1. Test points. A point has no dimension. It is defined as a point formed by the sides of two crossing lines. When the number of points hit on a given tissue compartment (P_i) and the number of points on the reference area (P_T) has been registered, the areal density (A_{Ai}) and the volume density (V_{Vi}) of the tissue compartment i can be calculated:

$$A_{Ai} = V_{Vi} = P_i/P_T.$$

2. Test lines. The number of intersections (I_i) of the boundaries of a tissue phase with the test line is registered. The number of intersections per unit length of the test line system (I_L) can be derived by dividing the intersection number I_i by the total length of test line on the reference area (L_T): $I_L = I_i / L_T$. The total length of test line on the reference area can be calculated from the number of points (P_T), if the test system is coherent (Weibel 1979). A coherent test system means that in a given grid the point number, length of the test line, and the test area have a certain fixed relationship to each other (Weibel 1979). In the case of a square lattice (Fig. 3), where the distance of two neighbouring lines is d, the total length of test line L_T can be calculated as follows: $L_T = 2\ Pd$. In Weibel's MP-lattice (Fig. 4) the corresponding relationship is: $L_T = 1/2\ Pd$, where d is the distance between two neighbouring points on a line. For square

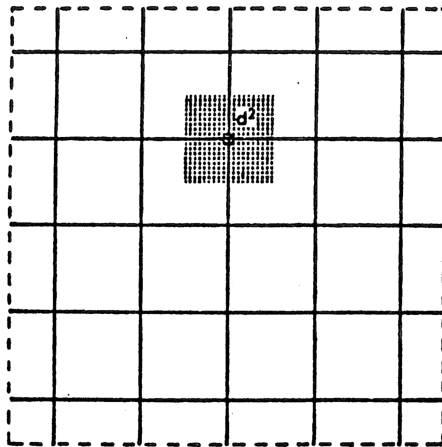


Fig. 3. Square lattice of 25 points. Distance between lines is d. Each point is responsible for an area of d .

lattice the following equation can be used for calculating the length density of the tissue phase boundaries in section (B) and the corresponding surface density in space (S):

$$S_V = \frac{4}{\pi} B_A = 2 I_L = \frac{I_i}{Pd}$$

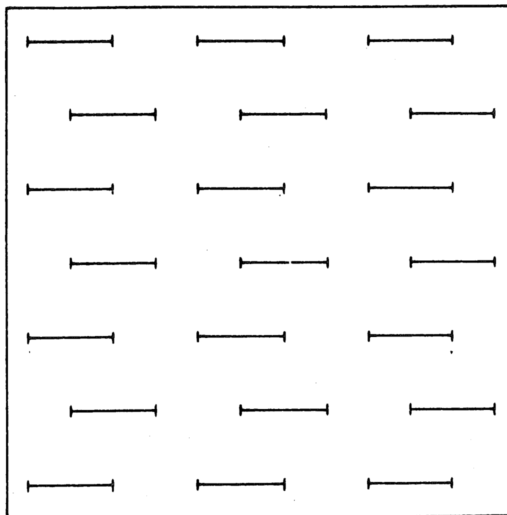


Fig. 4. Weibel's multipurpose lattice with 42 points (MP-42).

3. Test area. The test area is the area surrounded by the frames of the grid. The size of the test area (A_T) is needed when the numbers of structure profiles per unit area (N_A) are estimated. The number of features inside the frame (N) is counted and this is divided by the test area (A_T): $N_A = N/A$. If the test system is coherent, the test area can be calculated from the number of points on the reference area (P_T):

$$A_T = P_T\ d^2 \qquad \text{and} \qquad N_A = N / Pd^2$$

In counting the number of features inside the test area, features touching the test frame should be counted or excluded according to the unbiased counting rule (Gundersen 1977) (Fig. 5).

Figures 4, 5, 6 and 7 present some commonly used grid types:

1. Square lattice (Fig. 3). This lattice has vertical and horizontal lines and points at the intersections of these lines.

2. Weibel's multipurpose lattice (Fig. 4). In Weibel's multipurpose lattice (MP-lattice) (Weibel 1979) an excess of lines is avoided by using short interrupted test lines.

3. The Merz lattice (Fig. 6). In this type of lattice the lines are composed of semicircles (Merz 1968). This kind of lattice is advantageous for measuring surfaces which are not randomly oriented in plane section. Merz lattice is frequently

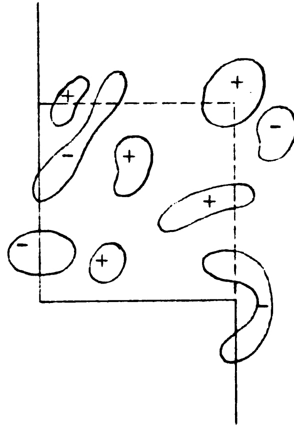


Fig. 5. The unbiased counting rule as presented by Gundersen (1977). All profiles of tissue structures completely outside the frame or touching the solid line are excluded and not registered by the hemocounter (-). Those profiles should be registered which are completely enclosed by the frame or touch the interrupted lines (+).

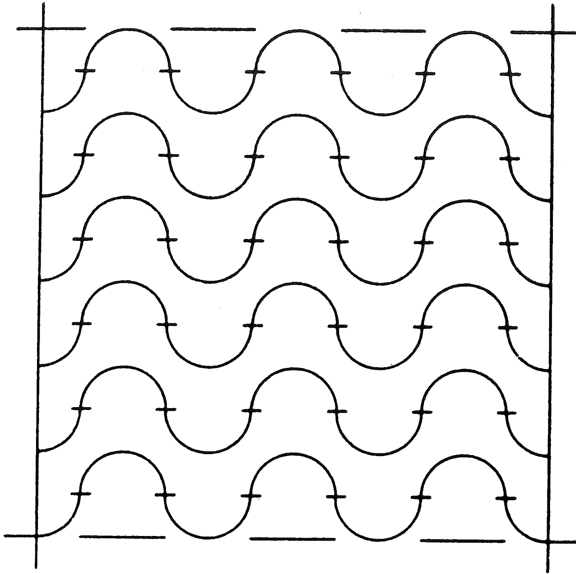


Fig. 6. The Merz lattice.

used in bone morphometry (Hoikka 1981, Merz 1968, Arnala et al. 1981). It has, however, one disadvantage: the observer rapidly gets tired of looking at the wavy lines.

4. Lattices with two sets of points (Fig. 7). These lattices have coarsely arranged points (heavy points, point distance large) and tightly arranged points (light points, point distance small). Lattices with several sets of points can be used if the volume density of one tissue compartment is small in relation another compartments. The light points are used for registration of hits on the smaller tissue compartment and the heavy points for registration of hits on the larger tissue compartment (for example, on the reference area). As the relation of the heavy points to the light points is known (in Fig. 7: one to four), the correspondence of area or volume densities can easily be calculated. This approach can save time by avoiding the counting of unnecessarily great numbers of hits on a large tissue compartment. But there will be enough hits on the smaller tissue compartment.

8. SAMPLING PROBLEMS

In the clinical practice of biopsy interpretation the decision about whether or not a specimen is representative for the patient's disease depends on many factors. Co-operation of the clinician and the pathologist, experience of the pathologist, organ studied, and nature of the disease

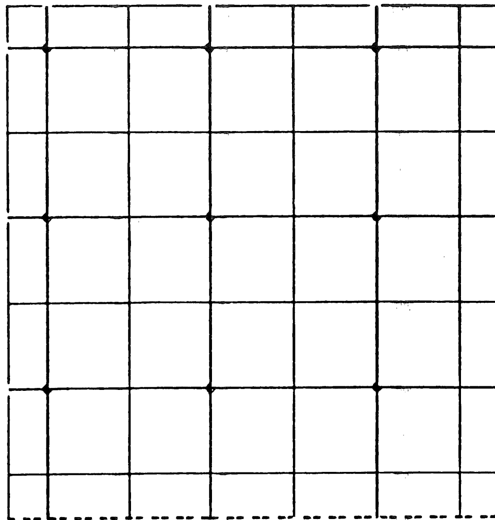


Fig. 7. Square lattice with two sets of points. The number of heavy points is 9, the number of light points is 36.

influence the decision (Collan 1981, Collan 1982, Collan 1984). In most cases the decision of the representativeness is made, usually on intuitive basis (Collan 1981, Collan 1982). There are ways to evaluate the representativeness of a biopsy and an example is given in the article by Collan et al. in this volume (Collan et al. 1983).

In a group investigation (group morphometry; see Selkäinaho and Collan 1983) values for individual patients are allowed to vary relatively widely (small precision), because one is interested in average values for a certain group of patients with the same disease. The need for detailed results for each tissue specimen can be compensated by increasing the number of blocks, or patients, or animals (Gundersen and Osterby 1981).

In contrast, in diagnostic histopathology we often have only a small sample of tissue for the investigation, the results of which should help the clinician to decide about the diagnosis, prognosis, and treatment. In this case one might like to have the most precise results from the sections. Of course these results then must be compared with results obtained earlier for a given group of patients. After the comparison the diagnostic group of the patient can be determined in the classification. But to get an idea about how accurate the results of measurement are, one can try to estimate the possible error in measurements. So we ask: How many counts are necessary to keep the limits of error within a given range?

The total number of points to be counted can be estimated empirically with cumulative mean plots (Fig. 8). First we count a large number of fields to get an unbiased estimate of

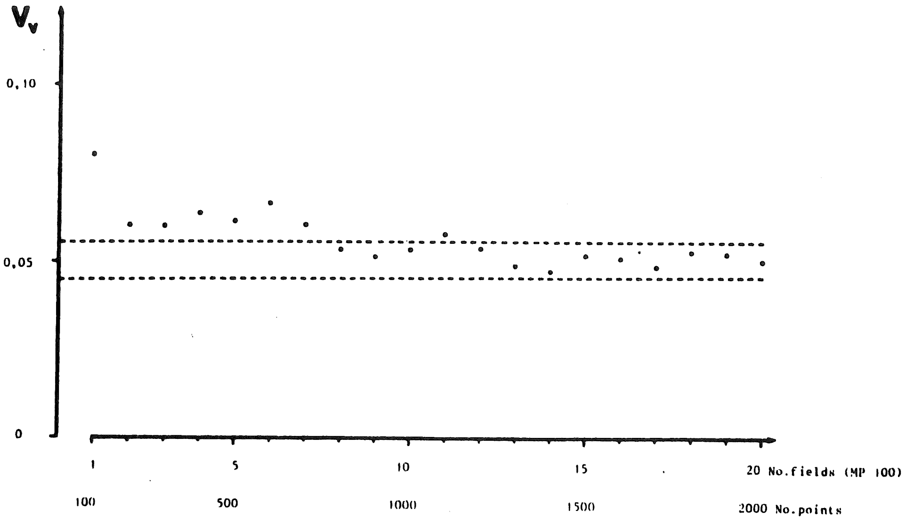


Fig. 8. Empirical determination of the needed number of points with cumulative mean plots. After 12 microscopic fields (1200 points) the cumulative mean estimate of the volume density in question remains constantly within error limits of 10% of the unbiased estimate.

of the volume density in question. The number of fields should be so large that the result would be identical or almost identical if we repeated the estimation. If we write down the results of each individual field we can then calculate the mean of these results. We can take any number of fields and so base our estimation on different numbers of points used for the estimation. If we start with estimating the volume fraction from one field, then take two fields, and thereafter three and more fields, we get a list of estimates which can be plotted on a graph. Because these estimates are means of estimates from several fields and are based on an increasing number of points, they are called cumulative means. The more fields we accept for our estimation the closer we get the unbiased reference estimate. At the point where the cumulative mean constantly remains inside a certain accepted variation range of the unbiased mean, the number of microscopic fields (number of points) can be assumed to be sufficient. One should realise that the researcher has to make the decision about the acceptable variation range and that there are no absolute rules about what size this range should be in each type of investigation. The needs of the research situation are most important here (Selkäinaho and Collan 1984). If one would like to make a large number of cumulative mean plots, one could estimate the probability that with a certain number of points the result would fall within the above variation range. In fact this is what one should aim at. However, this could be

quite laborious, and usually one is satisfied with determining the number of points after the level of which the cumulative mean will constantly stay within the limits of the accepted variation.

Because point counting measurements are in fact probabilistic experiments, the number of points necessary for a certain accuracy can also be estimated by calculations. Weibel (1979) has given a useful equation:

$$E(V_V) = 0.6745 \sqrt{\frac{1 - V_V}{V_V \times P}}$$

Here the accuracy is estimated by the relative error of the mean $E(V_V)$, which depends on two factors:

1. (Expected) volume density of the tissue compartment (V_V).
2. Total number of points counted on the reference area (P).

The equation is presented graphically in Fig. 9. By counting a total of 400 points with a volume density of 0.1 the relative error is about 10%. If one would not like to accept the relative error of 10% but instead would like have a smaller relative error - 5% - the number of points needed would be about 1600. In practice the number of points should be a little larger than the number given by these theoretical calculations. This is because the tissue structures are seldom distributed homogeneously. As a rough estimate one could say that for an expected volume density of 0.1, 500-600 points should be counted for a 10% limit of error. Accordingly, a minimum of about 50 point hits on the tissue compartment should be registered.

One can see from the graph (Fig. 9) that when the volume density of the tissue compartment decreases, the total number of points to be counted should increase, if the relative error of mean should be kept constant. Finally, the number of point hits on the smallest tissue compartment determines the accuracy of the method. This is why coarser lattices can be used for measurements of larger tissue compartments. For example, if the size ratio of the larger and the smaller tissue compartment is 4, a lattice with two sets of points presented in Fig. 7 is suitable.

But one should also estimate the optimal level of time consumption. To make the investigation possible both the time consumption and accuracy have to be within acceptable limits.

Diagnostic histopathologist wants to tell the difference between normal, and abnormal or pathological sample. The normal values for a certain parameter might be much smaller than abnormal values and the distinction between these two alternatives could be made with crude and fast measurements. So the nature of classifications should influence the decision on the

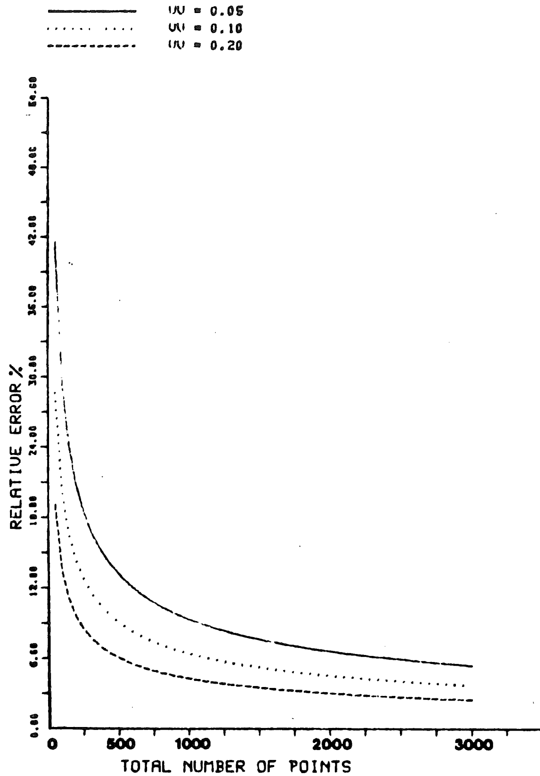


Fig. 9. Relative error of volume density measurement. The results are given for three volume densities (0.05, 0.10, 0.20). The x axis shows the total number of points applied in the test system.

acceptable limits of error in measurements (Selkäinaho and Collan 1983). Estimation of reproducibility can also serve as a useful guide for the acceptability of the method (Selkäinaho 1983, Selkäinaho and, Collan 1983, Kosma et al. 1983).

9. TISSUE PROCESSING

The study situation is not always ideal in light microscopic histopathology. There are at least two complicating factors in a morphometric study:

1. Effect of section thickness.
2. Effect of tissue shrinkage.

Owing to the Holmes effect, we see more stained tissue compartments in thicker sections than in thinner sections, and thus the morphometric results are different. For particles of

defined geometrical shape (Romppanen 1972, Weibel 1979) or for irregular particles (Weibel and Paumgartner 1978) equations have been derived for correcting this effect. In many cases, however, especially in comparative studies, the Holmes-effect can be ignored if we are not interested in the absolute values of the results. Certain general recommendations can be given (Gundersen 1979): If the section is thicker than one tenth of the diameter of a particle-like structure, the Holmes effect has a significant effect on the morphometric results. For tubular structures the limit lies at about one fifth of the tubular diameter. For determination of sheath thickness, the Holmes effect does not influence the results significantly if the section is not thicker than the sheath.

In comparative studies, however, we are not interested in the absolute values of the morphometric parameters. In these cases the effect of section thickness can be ignored. A prerequisite, however, is that the sections are of equal thickness in the groups to be compared. The variation of thickness may cause problems in diagnostic histopathology and in diagnostic morphometric measurements. However, the influence of section thickness variation on the results can be estimated (Collan et al. 1983).

Another factor that influences the values of the morphometric parameters is tissue shrinkage. Usually tissue shrinkage can be ignored, because again the researcher is not usually interested in absolute values in living tissues. This approach is justified in comparative studies, when the tissue processing is strictly standardized. The fixative must be the same and at a constant pH and osmolality (Mathieu et al. 1978); fixation time should be constant; the embedding procedure should be standardized. Attention should also be given to careful cutting of the sections.

In some rare situations, however, it may be necassary to measure shrinkage. A stepwise measurement of the effects of fixation, embedding, and cutting is recommended (Romppanen 1981). The shrinkage of formalin-fixed and paraffin-embedded chicken spleen is about 40% (initial tissue volume is reduced from 100% to 60%) (Romppanen 1981).

10. RELEVANCE OF EQUATIONS

Before the equations connecting the primary parameters (P, Q, I, N) with the secondary parameters (A_A, V_V, B_A, S_V, L_V, N_A, N_V, diameters etc.) are applied the following two questions should be answered:

1. Is it necessary to know something about the orientation of the tissue structures?
2. Is it necessary to know something about the shape of the tissue structures?

For measurements of volume or area density (V_V ,A_A) these aspects need not be considered, provided that the sample is representative.

When determining surface density (S_V) and length density (L_V), however, one needs information about the orientation of the surfaces or tubular structures. The general equations ($S_V = 2I_L$, $L_V = 2Q_A$) are valid only if the surfaces or tubules are randomly oriented in space. For other cases where the surfaces (planar orientation) or tubules (linear orientation) are oriented, the equations must be modified (Mathieu et al. 1981). This, however, requires that one determines the degree of orientation. This can be roughly determined using grids with test lines in only one direction. After counting the intersections, the position of the grid is turned 90 degrees and the intersections are registered again. If the number of intersections in both grid positions are about equal, the structures can be considered randomly oriented. By cutting the block in three planes perpendicular to each other the degree of orientation can be tested in three dimensions. For detailed determinations of planar or linear orientations equations have been derived (Underwood 1970).

For determination of the numerical density of particle-like tissue structures knowledge about the orientation as well as the shape of the particles is needed. Usually assumptions have to be made about the shape of particles. The same is true when structural measures (e.g. diameters etc.) of particles are are used to estimate the size distribution of particles.

11. REPRODUCIBILITY

One of the main advantages of the morphometric method is that it is usually (but not necessarily always; see Kosma et al. 1983) more easily reproduced than more subjective estimations. However, the morphometric method is not free from variation, but usually the various sources of variation can be estimated (Collan et al. 1982, Collan 1983). Morphometric investigations should also consider the following types of variation:

1. Intraobserver variation.
2. Interobserver variation.
3. Variation between sections.

Because the estimation of these sources of variation may be problematic - and this also sometimes applies to the estimation of the necessary number of points in point counting studies - estimation of the reproducibility of the applied method may be helpful (Selkäinaho 1983, Selkäinaho and Collan 1983).

12. AN EXERCISE ON MORPHOMETRY

Two transparent sheets of lattices follow this volume. Find them and you are ready to start.

Fig. 10 is a picture of a cell in an electron microscope section. You see a nucleus, Golgi vesicles, mitochondria and lysosomes.

1. Construct a compartmental morphometric model for a study on the area fraction (estimate of volume fraction) of mitochondria a) the whole cell, and b) the cytoplasm with organelles as the reference area. In this exercise no other information is available of the cell type under study.

2. Estimate without measurements the area fractions of the nucleus, mitochondria, Golgi vesicles and lysosomes in

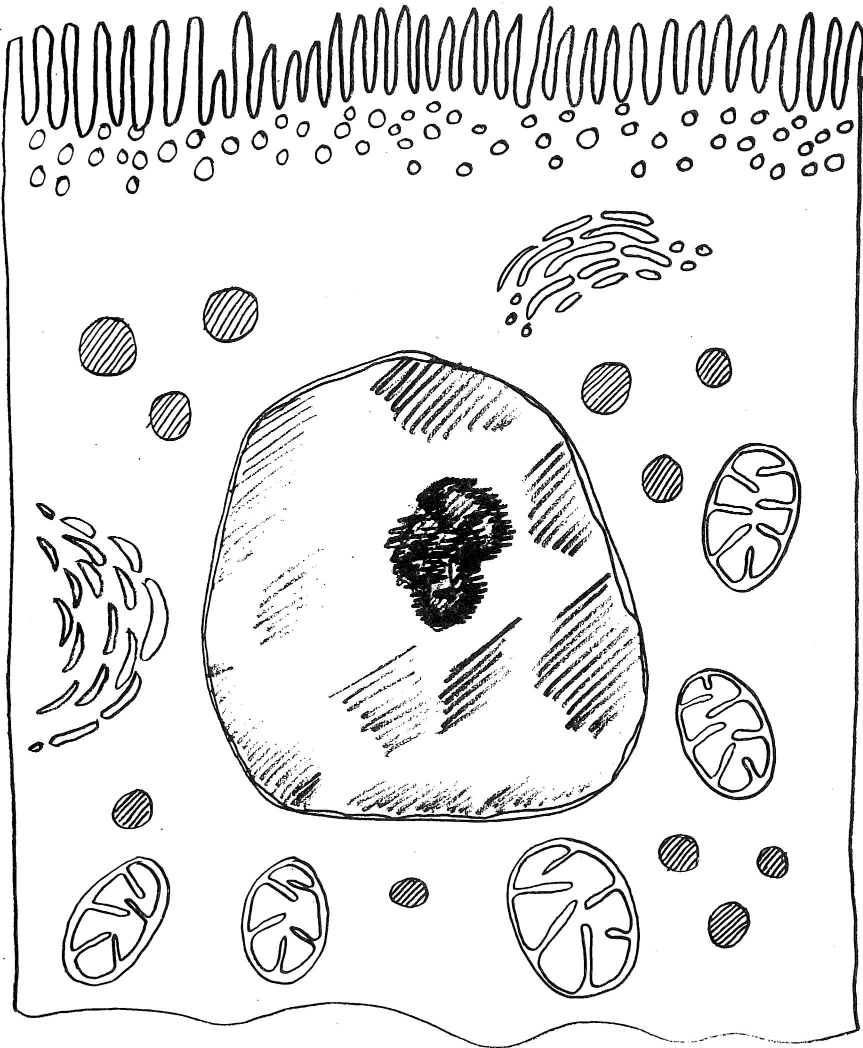


Fig. 10

A.

B.

C.

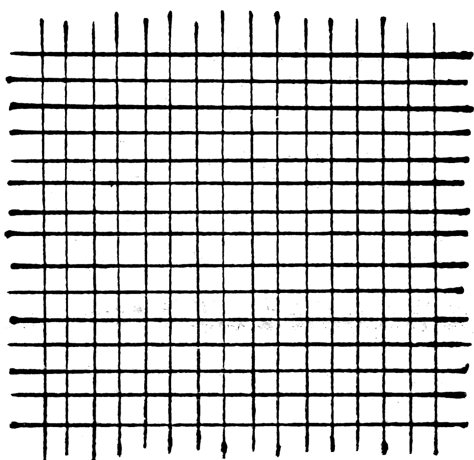


Table 1.

	Area fraction in the whole cell x 100		Area fraction in the cytoplasm including organelles x 100	
	Subjective estimate	Point counting	Subjective estimate	Point counting
Nucleus				
Mitochondria				
Golgi vesicles				
Lysosomes				
Others				

Fig. 10. Enter your results in Table 1.

3. Estimate the area fraction of the nucleus by point counting (reference: the whole cell). Use lattice A. Enter the results in Table 1 and compare them with your subjective estimate.

4. Select an appropriate lattice for measurement of the area fractions of mitochondria, Golgi vesicles and lysosomes (lattice A, B, or C) so that the accuracy of the results is about the same as for nuclear area fraction measurements. The numbers of points in the lattices A, B and C relate to each other as 1:4:16.

5. Measure the area fractions of mitochondria, Golgi vesicles (note: not Golgi area) and lysosomes. Enter the results in Table 1. Use both the whole cell and the cytoplasm with organelles as reference. Enter the results in Table 1 and compare them with your subjective estimates.

SOLUTION

1. Compartmental morphometric model; the whole cells as the reference:

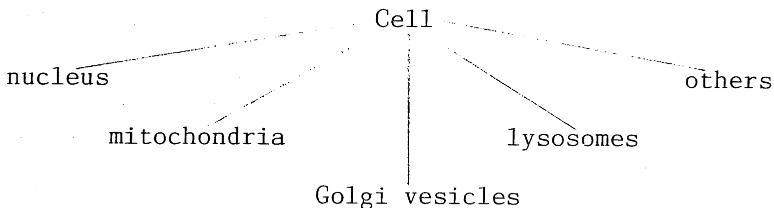
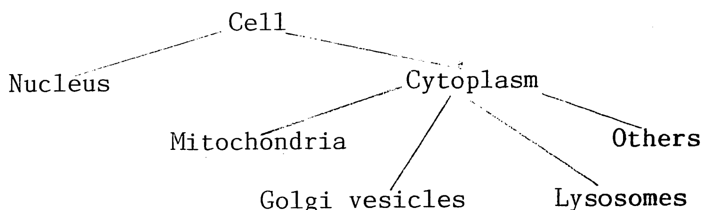


Table 2. Results of area fraction (volume density) measurements by pointcounting on Fig. 10.

Number of points	Area fraction
Number of points on the nucleus ________	Area fraction of the nucleus (the whole cell as reference)________
Number of points on the whole cell________	
Number of points on mitochondria________	Area fraction of mitochondria (reference: the whole cell) ________
	Area fraction of mitochondria (reference: the cytoplasm) ________
Number of points on Golgi vesicles________	Area fraction of Golgi vesicles (reference: the whole cell) ________
	Area fraction of Golgi vesicles (reference: the cytoplasm) ________
Number of points on lysosomes ________	Area fraction of lysosomes (reference: the whole cell) ________
	Area fraction of lysosomes (reference: the cytoplasm) ________

Compartmental morphometric model; the cytoplasm with organelles as reference:



2. You should make the subjective estimates yourself. The subjective estimates of different individuals may vary considerably. You can test this by asking your colleagues to make the same subjective estimates.

3, 5. Enter your results on morphometric measurements in Table 2. First enter the pointcounts on the left side. Then divide the counts on individual organelles with the count on the whole cell or the cytoplasm. This gives you the area fractions. Try to estimate how the number of points counted affects your results. See pages 14 - 16 on this point.

4. Mitochondria should be measured with lattice B, Golgi vesicles and lysosomes with lattice C. The precision of the measurements is determined by the number of point hits, which should be about equal on different compartments. On this point see pages 14 - 16.

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