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QUANTITATIVE HISTOPATHOLOGY
DISCOURSE

STEREOLOGY IN HUMAN MORPHOLOGY

Miroslav Kališnik

Institute of Histology and Embryology, University
Medical Faculty, Korytkova 2, 61105 Ljubljana,
Slovenia

ABSTRACT

The image is a 2-dimensional representation of the form and inner structure of the body or parts thereof. It can be obtained either by physical or optical sectioning or by projection from the mostly uneven surface or different levels from the interior of the object. Quantitative image analysis data obtained from sections may be interpreted stereologically in 3-dimensional space, though the data obtained on projections can be taken as semiquantitative astereological information of the inner structure. The crucial point for obtaining valuable stereological results is an appropriate sampling strategy as well as a sufficient number of sample units. The task of analytical statistics is often to decide whether two samples are from the same or from different populations. The decision is sometimes possible after observation of data from one variable, but more accurate after consideration of two or more variables. These data and appropriate statistical procedures enable exact description of morphological processes, correlation of morphological data with biochemical data, discrimination between normal and pathological conditions, and between pathological conditions with various levels of deviation from the normal.

KEY WORDS: physical sectioning, optical sectioning, projection, quantitative image analysis, stereology, astereology, sampling strategy, statistical analysis.

Under human morphology we understand laboratory and clinical branches of medical science dealing with the forms and inner structure of the human body and parts thereof. In addition to normal and pathological cytology, histology, anatomy and embryology, also medical radiology is included in human morphology. Morphometry investigates quantitative aspects of form and inner structure of the human body and its parts. It comprises all modes of quantitative image analysis, e.g. stereology, astereology, microdensitometry, flow cytometry. The final goal of all these procedures is the recognition of patterns characteristic for normal or pathological conditions, and specially in diagnostic pathology differentiation between them.

The image is a flat, 2-dimensional representation of the form and inner structure of the body or its parts. It can be obtained either by sectioning the body or by projection from it. Object sectioning may be physical or optical. Projection is possible from the surface, which in most cases is uneven, or from usually different levels of the interior of the body. Quantitative image analysis is possible in all these cases, but the interpretations are different (Fig. 1).

Quantitative data obtained from the image of 2-dimensional sections enable data interpretation in 3-dimensional space, taking into account the principles of geometric probability of integral geometry. This type of analysis is the most often used in research and routine diagnostic work and is called stereology (Weibel, 1992).

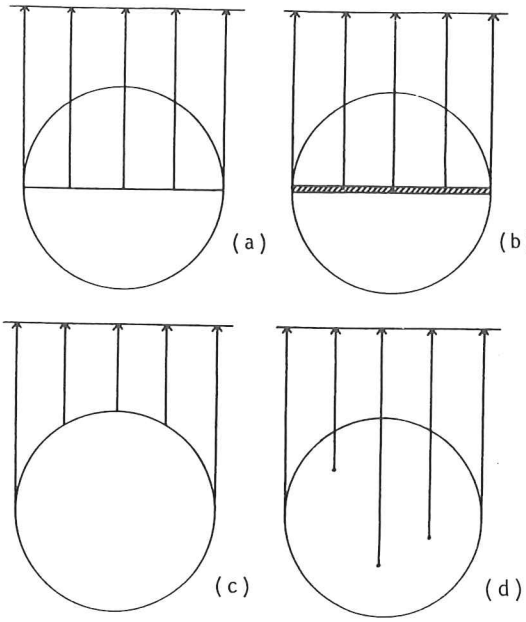

Fig. 1. Possibilities for obtaining an image of an object in the (a) optical or (b) physical sectioning, (c) projection from the surface, (d) projection from the interior (after Kališnik and Obrez, 1987).

Physical sectioning of microscopical biological objects produces no real sections but thin slices of finite thickness. This may cause the effect of underprojection or overprojection (Holmes effect). This effect depends on the ratio between the slice thickness and average particle diameter on the one hand, and on the difference in densities of opaque and translucent object components on the other hand.

In microscopical observations additional optical sectioning is performed besides physical sectioning. It depends on the optical properties of the microscopical objective as well as on the accommodation capabilities of the observer's eye. The principle of optical sectioning has been fully developed in the tandem-scanning reflected light microscope or confocal microscope from Petran et al. (1968).The variant of it using laser light is especially promising (Carlsson, 1991).

In medical radiology several methods of macroscopical optical sectioning of the living human body have been developed, where the images are observable without magnification, e.g. computer tomography, ultrasonographic tomography, nuclear magnetic resonance imaging, positron emission tomography. All these methods offer quantitative data, which can be interpreted stereologically in 3-dimensional space.

If the data are obtained by projection from an uneven surface (as from a scanning electron micrograph or from a cytological slide) or from various levels of the body interior (e.g. in scintigram), a straightforward interpretation of these data in 3-dimensional space is not possible. Such data enable only a semiquantitative analysis of the object inner structure. This method has been named astereology (Kališnik et al., 1980). This type of semiquantitative analysis is often used in basic research as well as in routine diagnostic work of cytologists, hematologists and pathologists, although it is not designated with this name. In some cases data obtained from flat sections through the body are not interpreted in 3-dimensional space (e.g. number of nuclei per area), because of methodological difficulties; such data nevertheless offer precise information about the inner structure of the body, although they are not stereological but remain at the astereological, i.e. 2-dimensional level.

Stereological image analysis is based on dealing with the data interpreted as binary. It can be extended using microdensitometry (scanning photometry). By measuring the extent of light absorption at a defined wave length the mass of a coloured substance in an area unit is evaluated. In the computerized representation of the image (Oja and Collan, 1983) microdensitometry is reduced to the grey level analysis of pixels over a defined area. This "in situ" cytometry has an "extra situm" counterpart in flow cytophotometry, offering information about various physical properties of isolated cells dispersed in a liquid medium.

All the above morphometric methods enable pattern recognition based on mathematical interpretation of one or more morphometric variables. In other words, quantitative description of one or many characteristics of the structural components of the studied body or its parts offers the possibility of exact identification of them, also offering certain diagnostic conclusions.

The crucial point for obtaining valuable stereological results is appropriate sampling strategy. This implies the rule that all units have an equal chance of being selected into the sample, which assures that the sample is representative. Moreover, a sufficient number of units (fields, sections, blocks, individuals) should be sampled. This keeps the relative standard error (RSE) reasonably small, which assures that the results are sufficiently accurate. In the life sciences a general rule is that the relative standard error RSE < 0.05. The necessary number of units to be sampled can be determined "a posteriori", i.e. after performing pilot observations in which averages and standard deviations of the orientation samples have been estimated. Possible anisotropy of the objects requires special sampling strategies; they have been developed for estimating a surface area from vertical sections (Baddeley et al., 1986) and for estimating a curve length from vertical slices (Gokhale, 1989).

If a variable is normally distributed, the sample or the population may be described by determining the average, the standard deviation and the coefficient of variation or the relative standard error.

The task of analytical statistics is often to decide whether two samples are taken from the same or from two different populations. In other words, we have to test the zero hypothesis, that both samples are from the same population. This decision is sometimes possible already after the comparison of one variable in two samples. In the case that sample averages are close and relative standard deviations are relatively high, it is sometimes possible to sharpen the decision power of hypothesis testing by increasing the number of units in both samples (Fig. 2).

The decision power of hypothesis testing is increased if we take into consideration two or more variables. An example of bivariate analysis is presented in Fig. 3. From a large data set the variables most important in prognostication can be selected with multivariate analysis methods. Usually these methods advice us about the best combination of predictors for finding the most powerful prognosticators.

Such procedures of analytical statistics enable morphologists to describe a process (ontogenetic, experimental or pathological) exactly in time, to correlate the morphological and biochemical data, to decide whether an experimental condition has a significant influence on a studied variable, to discriminate between normal and pathological condition, or between two pathological conditions with various levels of deviation from the normal (meaning different prognoses of the disease). For many examples of application in tumour and non-tumour pathology see Baak and Oort (1983).

Morphometry and specially stereology are today an indispensable tool in morphological basic research. It is estimated that in up to 4% of the total material entering an average department of pathology, quantitative microscopical analysis is useful. In a teaching institute or a laboratory with a specialized function, e.g. in tumour pathology, this percentage may be higher (Baak, Oort, 1983). It is often claimed that in practice there is not enough time for morphometric or stereological analysis. But the essential factor for applying these methods in departments of pathology is knowledge. Therefore education in morphometry and stereology should be introduced at undergraduate as well as at postgraduate level in medical schools (Collan, 1983).

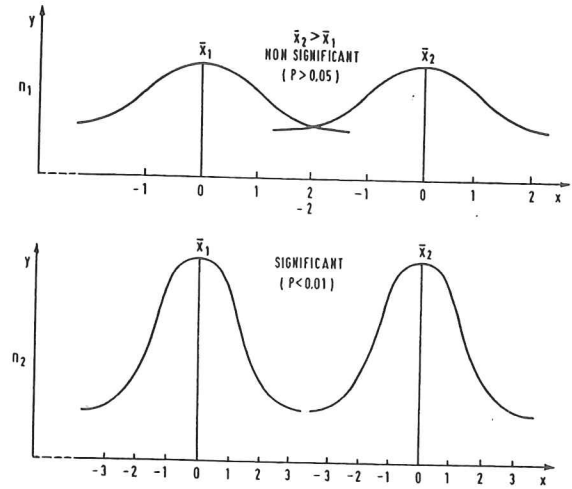

Fig. 2. Increasing the number of sample units can sharpen the decision power of hypothesis testing by lowering RSE ($n_1 < n_2$). Standardized deviations Z on the abscissas (after Kališnik, 1988).

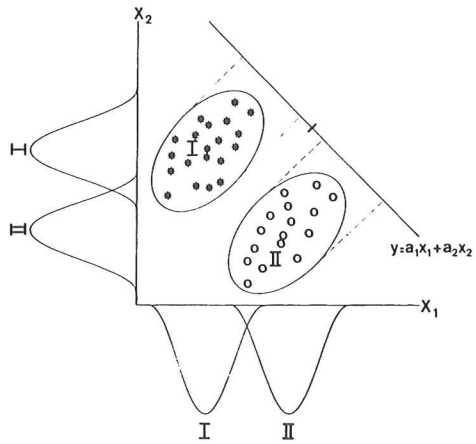

Fig. 3. Bivariate analysis of two samples I and II (which produce overlapping in separate analysis of variables X_1 and X_2), enabling complete separation of both samples (with permission after Baak and Oort, 1983).

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