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IMAGE ANALYSIS AND STEREOLOGY

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ABSTRACT

The detailed quantitative description of geometric properties of examined objects is the main task of stereology and belongs to the objectives of image analysis too. The following overview of similarities and differences in approaches, characteristics and driving forces of the both disciplines is concluded by a recommendation for their closer cooperation in order to yield better results in a more efficient way.

Key words: image analysis, stereology, volume analysis

COMMON OBJECTS OF STUDY

Stereology (Underwood, 1970; Weibel, 1979, 1980, 1982; Saxl, 1989) and image analysis (Rosenfeld, 1969; Rosenfeld and Kak, 1973; Pratt, 1979; Castelman, 1979; Coster and Chermant, 1989) deal with similar objects, namely either with singular objects of complicated shape, or, more frequently, with numerous populations of structural elements of stochastic nature manifested by variations in their size, shape, spatial arrangement and physical properties. The common objective is the detailed description of the geometrical properties of examined objects based on a pictorial input. The goal of image analysis is somewhat broader and includes the visualization and recognition of the examined objects and of their features (components) and the evaluation of their selected physical properties (spectral transmission, reflection etc.). Beside the classical geometric quantities like volume, surface area, length etc. occurring in the global description, and their distributions encountered in the particle analysis and widely accomplished by the both disciplines, various new functions (covariance, second order moment function, K- and L-functions, pair correlation function - Matheron, 1975; Stoyan et al., 1987), set functions (star, skeleton, exoskeleton, Steiner compact - Matheron, 1975; Serra, 1982) and concepts (fractality, distances of different kind, dual representation of point and particle arrangement by induced tessellations - Falconer, 1990; Stoyan and Stoyan, 1992; Saxl, 1993; Moller, 1989) have been introduced.

DEVELOPMENT AND FEATURES

Despite the development of stereology and image analysis was more or less independent, their common features and trends can be recognized and underlined. The parallel can be traced in the growing range of dimension of the working space (the space in which the measurement is carried out) with respect to the object space (the space in which the object is embedded), in objectives moving from a global view to the characteristics of the feature interaction, in a fundamental importance of correct sampling. On the other

hand, the driving forces of the development constitute the main distinction.

*Dimensionality.* The difference between the dimensions of the working and object spaces is in the both disciplines essential, as it determines both the picture acquisition and interpretation. In stereology, the steps 2D→3D, 1D→3D and 0D→3D are marked by the names of Delesse, Rosiwal and Glagoleff, resp. (Underwood, 1970; Weibel, 1979, 1980) whereas the recognition of stereology as an art of using the statistical inference in order to obtain a  $d$ -dimensional information from a partial  $k$ -dimensional information,  $k \leq d$ , is mainly due to Miles (1973). The consequences of the loss of information caused by sampling in a lower dimension are well known; the difference in dimensions limits proportionally the range of quantities that can be estimated as well as the accuracy of the estimates.

The situation is more complex in image analysis, since any point of the object can be imaged in a different way depending on the illumination as well as on the local physical conditions (spectral properties, reflection conditions). Consequently, the analysed picture is a complex information on the object, in which the information needed for estimation of its geometrical properties is partly distorted. The development of image acquisition, proceeding from binary image to grey tone, multi-spectral and multi-modal images, reduces the total loss of information but can increase at the same time the noise. Consequently, the segmentation of profiles of studied objects on available pictures, i. e. their transformation into binary ones suitable for geometrical measurements, is a considerable problem in practice (especially when considering a biological tissue - Simon, 1975; Brugal, 1984; Liang, 1993;). It is a permanent challenge teasing engineers, computer scientists and specialists in artificial intelligence and it is responsible for the enduring dependence of SW on HW of contemporary image analysers. Fortunately, a manual segmentation as a practical solution of this problem in those cases, when a reliable automatic segmentation is too expensive as yet (Moss et al., 1989; Krekule and Gundersen, 1989), brought stereological methods into the menu of contemporary image analysers thus increasing their efficacy (Zhao et al., 1992).

Leaving aside the "image analysis by mathematical morphology" (Serra, 1982; Coster and Chermant, 1989) as a special branch for which an image is rather a tool of spatial analysis than the main intention, the central stereological problem, namely the inference concerning 3D space and based on lower dimensional information, emerges in image analysis with the advent of 3D non-invasive image technologies (e.g. tomography) - Herman (1990). However, the attention was paid mainly to a 3D-object visualization exploiting computer graphics, hence to the progress in imaging, and only later to a quantitative characterization of the object (volume analysis). The voxel instead of the pixel comes into the centre of interest and the evaluation of geometric parameters proceeds directly on a 3D reconstruction (Udupa and Herman, 1991; Barillot, 1993). Unfortunately, such a reconstruction is only an unstable estimate of the imaged body; it may be important for qualitative considerations (e. g. in medical sciences), but its parameters can differ considerably from that ones of the original body and a prediction of the bias is hardly possible. Hence, such an approach does not lead to reliable quantitative estimates; they can be obtained at lower costs and with a greater accuracy directly from the lower dimensional sections and projections using the stereological principles (Cruz-Orive and Howard, 1991).

*Objectives.* The historical sequence of objectives in stereology → global properties → particle properties → spatial arrangement (including also the orientation and feature interaction) - is well known as well as the accompanying increase in the size of the sample, in the complexity of data treatment and in the effort required. The characteristics of the spatial arrangement are predominantly responsible for the majority the new concepts mentioned

above (second order analysis, distance analysis etc. Diggle, 1983; Ripley, 1982; Stoyan et al., 1987). Likewise complicated are the techniques of handling the anisotropy (of volume, boundary, position - Weibel, 1980; Stoyan and Beneš, 1992; Weil, 1993) and shape (in interwoven "shapeless" systems somewhat loosely replaced by the homeomorphic connectivity characteristics) - Goodey and Weil (1992), Weil (1993), Stoyan and Stoyan (1992), Small (1991). The primary problem here is to find suitable functions and/or set functions for the description of complicated spatial systems and that is where the random set theory (stochastic geometry) and the contemporary convex geometry come into the play. Stereology then closely follows and develops the procedures for an estimation of such quantities from various sections. As the most recent examples, the concepts of stereological estimation of the shape of convex bodies from planar sections (Goodey and Weil, 1992), of the description of the line anisotropy by means of zonotopes (Weil, 1993; Campi et al., 1993) and of the estimation of the second order quantities using 3D probes (Jensen and Kieu, 1991; Kieu and Jensen, 1993) can be mentioned.

In image analysis, the evaluation of geometrical characteristics of object constituents (features, particles) from 2D sections or projections is usually called the particle analysis. It is accomplished by digital computers by processing the matrix of elements (pixels) representing the analysed picture (Duff and Levialdi, 1981; Kittler and Duff, 1985; Jarvis, 1988). Its device "measurement, not estimation" regardless of the effect of discrete processing is nearly antithetical to stereological principles and the bias of thus obtained "particle size distributions" is completely unknown. Here as well as in the description of the spatial arrangement, the absence of close connections between image analysis, geometry and statistics is the main cause of the delay in implementation of new concepts including 3D probes. Fortunately, the recent volume analysis recognized the importance of the sequence theoretical study → implementation → experimentation, so that image analysis is under pressure simultaneously from two sides.

*Sampling.* The correct sampling is crucial for the both disciplines, but its importance has been properly recognized only in stereology. The systematic attention to this problem called for appropriate models of investigated situations (model based and design based stereology - Miles, 1978; Serra, 1982) and led to the main sampling schemes: arbitrary sections for isotropic stationary structures, isotropic uniform random sections for arbitrary structures, weighted sections for ratio estimators in a global analysis (Miles and Davy, 1976) and for weighted distributions in the stereological particle analysis (Jensen and Gundersen, 1989). In replicated sampling, a definite preference is given to the systematic sampling as the danger (usually discoverable) of an interference between the spatial patterns of test elements and of examined features is outweighed by smaller variances of estimators (Gundersen and Jensen, 1987; Cruz-Orive 1989; Mattfeldt, 1989). Another important result is the rule of a decreasing sequence of variance values when passing from the object (individual) to sample, section, observed field and probe section (Miles and Davy, 1977). It leads to decreasing demands on the accuracy of the final measurement expressed by the popular Weibel's maxim "Do more less well" (Gundersen and Osterby, 1981). Finally, a proper attention must be paid to the correct treatment of the edge effects (Miles, 1974; Serra 1982; Ripley 1988).

In image analysis, the main sampling step is accomplished by the discretization of the pictorial data and concerns geometry (pixel size and location) as well as radiometry (intensity) - Cole (1971), Pratt (1978). Its consequence is a loss of information, which may result in a significant distortion of the picture data in the digital form; various correction algorithms (Andrews and Hunt, 1977; Gonzales and Wintz, 1977) are available but to estimate the resulting bias of data is, in general, impossible (it depends also on the properties of the imaged object). The sampling rules

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valid for stereology apply here as well but are not used. Instead of it, the aforementioned elusive credo of image analysis leads to the processing of all available pictorial information without concerning its possible bias and without improving it by replication. The SW statistical packages offer comprehensive estimates of the size distributions of observed profiles, but their accurate relation to the feature size distributions remains unclear. Thus one of the main conceptual differences between commercial image analysis and stereology consists in different attitudes to sampling; whereas a processing of numerous data of ill-defined provenance is common in the former case, a sophisticated treatment of sparse data of known origin prevails in the latter one.

*Driving forces.* Despite the deep affinity in the area of interest, objectives, approaches and their rules and laws, the development of the both discussed disciplines is driven by different forces. Contemporary stereology develops in a close alliance with mathematics; convex and integral geometry as well as random set theory are to a high extent stimulated by the needs of stereology, which concurrently changes its face. This feedback is beneficial for the perpetual development of the involved parties, however, it also limits the number of customers waiting for new stereological methods and addresses a rather small circle of those not intimidated by pretentious demands of contemporary mathematics.

On the other hand, image analysis as an off-spring of computer science and technology (Leviaidi, 1985) is closely tied to its engineering aspects. It profits from a well developed technology and commercial background, has a huge number of customers and a vast support of general public believing in the "computer omnipotent which measures". Among the drawbacks following naturally from this situation are the constraints (e.g. a limited resolution of cameras and displays) imposed by the commercially efficient and thus cheap TV technology (Cole, 1971) as well as the preference for experiments and the omission of tedious theoretical studies, which result in many short-lived products blurring the domain. Of course, the interaction with mathematics proceeds too, selected concepts of random set theory and discrete geometry have already been implemented and tested in several laboratories, but successful popularization, general acceptance and inclusion into the standard SW are lacking as yet.

### CONCLUSIONS

Though the problems that have to be solved by stereology and image analysis are much the same, their cooperation is far from being close nowadays. An interdisciplinary enlightenment is missing and the boundaries are constructed and defended rather than destroyed. A serious obstacle to further development and wider cooperation of the both disciplines is an insufficient education in geometry, geometric probability and statistics as well as in image formation and perception, especially at the universities orientated towards natural sciences.

The development should proceed in a way of a mutually beneficial cooperation. The powerful impact of mathematical morphology, unifying the tools and approaches of the both disciplines and introducing set algebra into image analysis in the seventies, must be mentioned here as a positive example (Lay and Lantuejeul, 1983). Also the implementation by contemporary image analysts of various stereological tools, like are the coherent test systems, confirms further the viability of such tendencies. On the other hand, the second order analysis and distance analysis with appropriate edge corrections, correct assessment of serial sections with a reliable profile identification etc. are far from being a part of the standard image analysis SW.

Although the visualization of examined objects as accomplished by the top of the available computer technology (e.g. computerized confocal microscopy) is

spectacular and attractive (Kaufman et al., 1990), it provides only a qualitative information (Vannier et al., 1984; Barillot, 1993). However, technology, clinics and research require also a quantitative knowledge expressed in terms of meaningful and well-defined characteristics and only a concerted application of stereology and image analysis supported by a well-tailored HW in all stages of measurement can meet such demands. The advance of the volume analysis as a new part of image analysis can provide a cue to predict the future progress: by a growing application of computers in stereology directed to data processing and especially to modelling and by a growing effort to implement in image analysis mathematical concepts already domesticated in stereology, the both disciplines will be driven together and will amalgamate at least partly for the benefit of the large community of users.

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