Ann. Inst. Statist. Math.
Vol. 43, No. 3, 455–468 (1991)

RECENT DEVELOPMENTS IN THE STEREOLOGICAL ANALYSIS OF PARTICLES*

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(Received November 14, 1989; revised March 26, 1991)

Abstract. Recent developments in the stereological analysis of particles are reviewed. The trend has been towards methods which are applicable without specific assumptions about particle shape. Geometric samples of a local 3-d character are used. Stereological estimators of particle intensity, particle size distribution and particle interaction are presented and discussed.

Key words and phrases: Intensity, K-function, mark distribution, marked point processes, measure decomposition, nucleator, second-order properties, stereology, stochastic geometry.

1. Introduction

Stereology is the science of making statistical inference about spatial structures from samples of a geometric nature. Such methods are used in the study of the different components of spatial materials such as metals, minerals, synthetic materials or biological tissues. The physical size of the components of interest may be of the order of μm and are then studied by some microscopic technique.

Until recently, the geometric samples used in a stereological analysis have consisted of line or plane sections of the structure. The step from spatial structures to their sections involves a great loss of information and so traditional stereological methods commonly yield only "global" information of a statistical character. A further consequence of this loss of information is that, in order to give a spatial interpretation to size data collected on sections, the solutions of ill-posed problems are required: numerical solutions in which small deviations due to measurement error can lead to large discrepancies in the final solution (cf. Stoyan *et al.* (1987), Coleman (1989) and references therein).

In the present paper, we will demonstrate that, with samples of a local 3-d (three-dimensional) character, it is possible to get sound statistical information

* An earlier version of this article was presented at the Symposium on the Analysis of Statistical Information held in the Institute of Statistical Mathematics, Tokyo during December 5–8, 1989.

of a local character, e.g. about the local architecture of biological tissues. The simplest sample of this type is a disector (cf. Sterio (1984) and Gundersen (1986)), which consists of two parallel plane sections, a known distance apart. This sample can be obtained by taking physically two plane sections or one can use two focal planes created by a non-destructive technique like light microscopy (cf. Gundersen (1986)) or confocal microscopy (cf. Petran *et al.* (1968) and Howard *et al.* (1985)). Reviews and introductions to these developments can be found in Cruz-Orive (1987*a*), Gundersen *et al.* (1988*a*, 1988*b*) and Weibel (1989).

We will concentrate on the stereological analysis of particle systems. From a statistical point of view, such systems are interesting because we have an inherent replication in the system. The particles are described by means of a marked point process. We shall draw heavily on the solid framework for such processes laid down by the East German School of Stochastic Geometry (cf. Stoyan *et al.* (1987)).

In Section 2, we define the particle model. The particles are sampled by a so-called nucleator (cf. Gundersen (1988)) resulting in a central section through each of a sample of particles (cf. Section 3). In Sections 4, 5 and 6, stereological estimators of the intensity, the mark distribution and the K-function are presented and discussed. In Section 7, some ideas for future work are outlined.

2. The particle model

The particles are regarded as a realization of a so-called germ-grain model (cf. Hanisch (1981) and Stoyan *et al.* (1987), p. 186). Below, we define this model and discuss first- and second-order characteristics. A similar exposition can be found in Penttinen and Stoyan (1989).

Let $\Psi = \{[x_i; \Xi_i]\}$ be a marked point process where the x_i's are points in $\mathbb{R}^3$ and the Ξ_i's are elements of the set $\boldsymbol{k}$ of compact subsets of $\mathbb{R}^3$. The set $x_i + \Xi_i$ is the i-th particle of the particle process, x_i will be called the nucleus of the i-th particle and Ξ_i the primary particle. We assume that $O \in \Xi_i$. Furthermore, we assume that Ξ_i has non-void interior and finite surface area. The particles may overlap, provided this does not interfere with their identifiability. The process of nuclei is denoted by $\Psi_n = \{x_i\}$.

The Borel σ-algebra in $\mathbb{R}^3$ is denoted $\mathcal{B}^3$. On the set $\boldsymbol{k}$ of compact subsets of $\mathbb{R}^3$, we use the σ-algebra $\mathcal{K}$, defined as the restriction to $\boldsymbol{k}$ of Matheron's σ-algebra on the closed subsets of $\mathbb{R}^3$ (cf. Matheron (1975), p. 27).

It will be assumed that the marked point process is stationary, i.e. Ψ_x has the same distribution as Ψ for all $x \in \mathbb{R}^3$, where

$$\Psi_x = \{[x_i + x; \Xi_i]\}. \tag{2.1}$$

Furthermore, the model is assumed to be isotropic, i.e. $A\Psi$ has the same distribution as Ψ for all rotations $A \in SO(3)$ where

$$A\Psi = \{[Ax_i; A\Xi_i]\}. \tag{2.2}$$

The stationarity implies that the intensity measure of the marked point process is of the form

$$\begin{aligned} \Lambda(B \times K) &\overset{\mathcal{D}}{=} E\#\{i : x_i \in B, \Xi_i \in K\} \\ &= \lambda V(B) P_m(K) \end{aligned} \tag{2.3}$$

where $B \in \mathcal{B}^3$, $K \in \mathcal{K}$, λ is the intensity of Ψ_n, V is volume and P_m is the mark distribution. We always assume that $0 < \lambda < \infty$. Below, Ξ_0 is a random compact set with distribution P_m. Note that $O \in \Xi_0$ with probability 1. Often, Ξ_0 is called a typical primary particle. The isotropy of Ψ implies that P_m is isotropic, i.e. $P_m(AK) = P_m(K)$ for all $K \in \mathcal{K}$ and $A \in SO(3)$.

The second-order properties of Ψ can be described by the factorial moment measure α, defined by

$$\alpha(B_1 \times K_1 \times B_2 \times K_2) = E \sum_{\substack{[x_1;\Xi_1]\in\Psi \\ [x_2;\Xi_2]\in\Psi}}^{\neq} 1_{B_1}(x_1)1_{B_2}(x_2)1_{K_1}(\Xi_1)1_{K_2}(\Xi_2), \tag{2.4}$$

$B_1, B_2 \in \mathcal{B}^3$ and $K_1, K_2 \in \mathcal{K}$. The summation $\sum^{\neq}$ goes over all ordered pairs of marked points with $x_1 \neq x_2$. The factorial moment measure α_n of the process of nuclei is

$$\alpha_n(B_1 \times B_2) = \alpha(B_1 \times \boldsymbol{k} \times B_2 \times \boldsymbol{k}). \tag{2.5}$$

We will base the second-order analysis of the process of nuclei on the so-called K-function (cf. Stoyan *et al.* (1987), p. 120). The K-function has been primarily used in the theory of liquids as a "cumulative radial distribution function". The K-function can be expressed as

$$\lambda K(r) = E_0 \sum_i 1(0 < \|x_i\| \leq r), \qquad r > 0, \tag{2.6}$$

where E_0 is the mean value operator for the Palm distribution P_0 of the nuclei process. The Palm distribution can be interpreted as the distribution of Ψ_n when the origin of $\mathbb{R}^3$ is chosen as a typical nucleus. Therefore, $\lambda K(r)$ can be interpreted as the mean number of further points in a ball of radius r centered at a typical nucleus. For the Poisson process, $K(r) = (4/3)\pi r^3$, the volume of a ball with radius r.

The isotropy of Ψ implies isotropy of Ψ_n and P_0. The Palm distribution is related to the original distribution of Ψ_n by the refined Campbell theorem

$$E \sum_i h(x_i, \Psi_n) = \lambda \int_{\mathbb{R}^3} E_0 h(x, \Psi_{nx}) dx \tag{2.7}$$

where h is any non-negative measurable function.

We will assume that $E\Psi_n(B_1)\Psi_n(B_2) < \infty$ for any pairs of bounded Borel sets, where $\Psi_n(B) = \#\{i : x_i \in B\}$. This assumption ensures that both $\alpha(\cdot \times K_1 \times \cdot \times K_2)$ and $\alpha_n(\cdot \times \cdot)$ are σ-finite measures on $\mathcal{B}^3 \times \mathcal{B}^3$. Obviously $\alpha(\cdot \times K_1 \times \cdot \times K_2) \ll \alpha_n(\cdot \times \cdot)$ for all $K_1, K_2 \in \mathcal{K}$ and so there exists a Radon-Nikodym density $M_{x_1x_2}(K_1 \times K_2)$ satisfying

$$\alpha(B_1 \times K_1 \times B_2 \times K_2) = \int_{B_1 \times B_2} M_{x_1x_2}(K_1 \times K_2)\alpha_n(dx_1, dx_2). \tag{2.8}$$

Under the assumption of stationarity and isotropy of Ψ, $M_{x_1 x_2}$ depends only on the distance $r = \|x_1 - x_2\|$ and we use the abbreviation M_r. The distribution of M_r is called the two-point mark distribution. If the marks are independent of the point process and mutually independent, then

$$M_r(K_1 \times K_2) = P_m(K_1)P_m(K_2). \tag{2.9}$$

The value $M_r(K_1 \times K_2)$ may, in general, be interpreted as the probability that the marks of the points in O and x ($\|x\| = r$) are in K_1 and K_2, respectively, under the condition that in O and x there are indeed points from Ψ_n. For a more detailed treatment, see Stoyan (1984*a*).

Usually, only some part of the two-point mark distribution is studied, e.g.

$$E_{0,r}V(\Xi_1)V(\Xi_2) = \int_k \int_k V(\Xi_1)V(\Xi_2)M_r(d\Xi_1, d\Xi_2), \qquad r > 0. \tag{2.10}$$

It can be shown, using the two-point Campbell theorem (cf. Stoyan (1984*a*)) that the empirical counterpart below is closely related to this quantity. Thus, with $B \in \mathcal{B}^3$,

$$E \sum_{x_1 \in B} \sum_{\substack{x_2: \\ r_1 < \|x_2 - x_1\| \le r_2}} V(\Xi_1)V(\Xi_2) = \lambda^2 V(B) \int_{r_1}^{r_2} E_{0,r}V(\Xi_1)V(\Xi_2)K(dr). \tag{2.11}$$

3. Nucleator sampling

Nucleator sampling (cf. Gundersen (1988)) of a particle process is a special type of local 3-d sampling. The information available is a collection of parallel planar sections. The sections are central sections through particles in a sampling box B.

More formally, let $L_{2(0)}$ be a plane through the origin parallel to one of the faces of the box. The set of planar sections is then $\{x_i + L_{2(0)} : x_i \in B\}$ (cf. Fig. 1). This type of sampling is possible if the x_i's are clearly identifiable.

A practical way of obtaining this type of information is by optical sectioning. The idea is to start by making only one, relatively thick section B containing the particles and then make thin optical sections inside the thick one by moving the plane of focus up and down. Such a thin optical section is made through each nucleus in B. Optical sectioning will be at its best when used on a confocal microscope (see Petran *et al.* (1968) and Howard *et al.* (1985)). An alternative is to use thick plastic sections on a conventional light microscope which in various ways has been modified (cf. Gundersen *et al.* (1988*b*)).

We assume below that we have unlimited information on the sections. This is the case in the applications we have in mind. Here, the height of the box is equal to the thickness of the initial thick section while the horizontal extension of the box is much smaller than that of the thick section. In other applications where

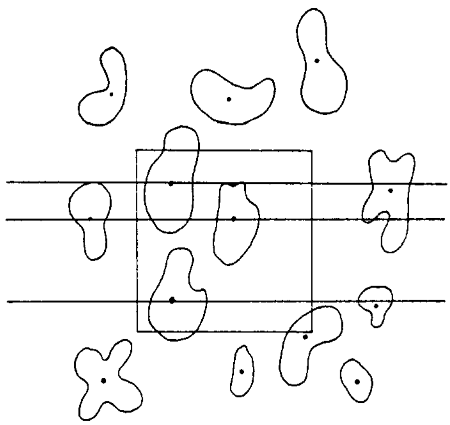

Fig. 1. 2-d illustration of nucleator sampling. A collection of parallel line sections are sampled, viz. the line sections through nuclei inside the square B. In 3-dimentional space, the line sections are replaced by plane sections parallel to one of the faces of a sampling box B. All plane sections through nuclei in B are sampled.

information is available only inside the box B, edge effects corrections are needed of a similar type as those described in Stoyan *et al.* ((1987), p. 125).

4. The intensity

The stereological estimation of the intensity λ of the particles has been a long-standing problem in stereology. Many indirect methods have been invented. The basic stereological estimation method has until recently been based on the identity $\lambda = \lambda_A / D$, where λ_A is the intensity of particle sections on a plane section and D is the mean particle height in the direction perpendicular to the plane. In order to estimate λ_A correctly, it is necessary to treat the edge effects carefully (cf. Fig. 2). Good reviews of these methods can be found in Gundersen (1978) and Cruz-Orive (1980). The estimate of D is either based on a model of particle shape or direct locally serial sectioning.

A by-product of the nucleator sampling is a direct observation of the number of nuclei in the sampling box B. The intensity λ can therefore be estimated directly.

It is also possible to estimate the intensity in a direct way, without using the nuclei. For this purpose, a disector can be used which consists of two parallel sections a distance h apart which is smaller than the minimal particle height (cf. Fig. 3); one section L_2 has a counting frame T and is hence the counting plane whereas the other section L_{2h} is a "look-up" plane. The procedure is to count all the particles that lie within the frame of the counting plane and do not intersect the look-up plane, thus "end" in the space between the planes (cf. Sterio (1984) and Weibel (1989)). The resulting count

$$C = \sum_i 1_A(x_i + \Xi_i), \tag{4.1}$$

where

$$A = \{y \in \boldsymbol{k} \mid Y \cap L_2 \neq \emptyset, Y \cap L_2 \text{ inside frame } T, Y \cap L_{2h} = \emptyset\}, \tag{4.2}$$

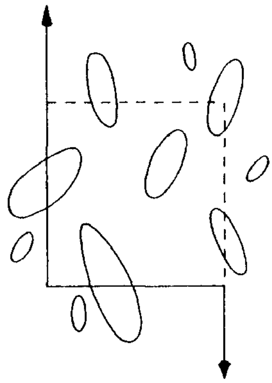

Fig. 2. Estimation of the intensity λ_A of particle sections; here illustrated for convex particles which always give rise to connected particle sections. All particle sections which are inside the counting frame T are counted (cf. Gundersen (1977)). A particle section is said to be inside the frame if it hits the square but does not hit the full-drawn lines. If a particle is non-convex, a section may consist of separate connected components which are then treated as a whole.

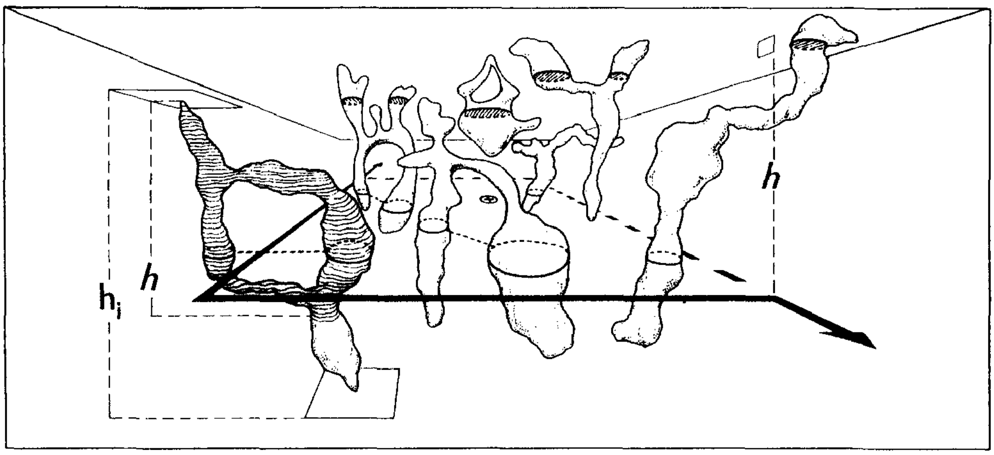

Fig. 3. A disector consisting of two parallel sections a distance h apart which is smaller than the minimal value of the particle heights h_i (from Sterio (1984), with permission).

has mean $\lambda \cdot \text{area}(T) \cdot h$ and $C/\text{area}(T) \cdot h$ is therefore an unbiased estimator of λ. Modifications exist which do not require a known and positive lower limit of particle height. Another variant of this concept is the so-called "selector" (cf. Cruz-Orive (1987*b*)).

The estimation procedures described above are valid without the isotropy assumption.

5. The mark distribution

Until recently, information about the size distribution of the particles has been sought under assumptions about the shape of the particles. The most classical example is the sphere model (cf. Wicksell (1925, 1926)). For spherical particles, the problem reduces to estimating the diameter distribution. The classical approach is to consider a plane section through the particles and determine the size distribution of the circular disks on the plane section. This size distribution is related to the distribution of sphere diameters by a known integral equation of Abel type. By some kind of inversion, the sphere diameter distribution can be determined from the observed distribution of diameters of circular disks.

This approach is not applicable very often because real particles are seldom spheres. Apart from that the inversion is numerically unstable. For a recent review, see Coleman (1989). See also Watson (1971).

For this reason, the recent developments have been towards getting information about the mark distribution without specific assumptions about the particle shape. Until now, the interest has been in the volume distribution and the surface area distribution, i.e. the distribution of $V(\Xi_0)$ and $S(\Xi_0)$, respectively. The first and second moments of these two distributions can be estimated, using nucleator sampling (cf. Jensen and Gundersen (1989) and Jensen *et al.* (1990*b*)). These results are special cases of a new integral geometric formula (cf. Zähle (1990) and Jensen and Kiêu (1991)).

The parameters which can be estimated stereologically without assumptions about particle shape are $EV(\Xi_0)^q$ and $ES(\Xi_0)^q$, $q = 1, 2$. The procedure for deriving these estimators follows standard methodology. First, we consider a fixed set Y, which is written in the form $Y = x_0 + \psi_0$ where $x_0 \in Y$ and $\psi_0 \in \boldsymbol{k}$. The quantities $\gamma(Y)$, $\gamma = V, S, V^2, S^2$, are expressed as an integral with respect to the rotation invariant probability mearure on planes through the origin. Such integral geometric formulae can be derived, using geometric measure theory (cf. Federer (1969)). Let $L_{2(0)}$ be a plane through the origin, let $\mathcal{L}_{2(0)}$ be the set of such planes and let $\overline{dL}_{2(0)}$ be the element of the rotation invariant probability on $\mathcal{L}_{2(0)}$. Then, the integral geometric formulae are of the form

$$\gamma(Y) = \int_{\mathcal{L}_{2(0)}} \hat{\gamma}(Y, x_0, L_{2(0)}) \overline{dL}_{2(0)}. \tag{5.1}$$

The integrand $\hat{\gamma}(Y, x_0, L_{2(0)})$ is a non-negative function of the plane section $Y \cap (x_0 + L_{2(0)})$ which may depend on the point x_0 and also on a spatial neighbourhood of the particle section. Thus, extended information might be needed. The integrand satisfies

$$\hat{\gamma}(x_0 + \psi_0, x_0, L_{2(0)}) = \hat{\gamma}(x_0 + A\psi_0, x_0, AL_{2(0)}), \qquad A \in SO(3), \tag{5.2}$$

i.e. invariant under simultaneous rotation of particle and plane around x_0.

The next step in the development is to notice that the invariant probability measure on $\mathcal{L}_{2(0)}$ defines a special type of random planes $L_{2(0)}$, called isotropic planes. According to (5.1), the mean of $\hat{\gamma}(Y, x_0, L_{2(0)})$ with respect to an isotropic random plane $L_{2(0)}$ is $\gamma(Y)$. Now, let us interchange the randomness and consider a random particle $x_0 + \Xi_0$, where the distribution of Ξ_0 is P_m. The plane $L_{2(0)}$ and the point x_0 are regarded as fixed. Because of the isotropy of P_m, the mean of

$$\hat{\gamma}(x_0 + \Xi_0, x_0, L_{2(0)})$$

does not depend on $L_{2(0)}$ and, using (5.1), the common mean is $E\gamma(x_0 + \Xi_0) = E\gamma(\Xi_0)$. Applying this result to each particle in the box B, we find that the mean of

$$\sum_{x_i \in B} \hat{\gamma}(x_i + \Xi_i, x_i, L_{2(0)}) \tag{5.3}$$

is $\lambda V(B) E\gamma(\Xi_0)$. The resulting estimator of $E\gamma(\Xi_0)$ becomes

$$\sum_{x_i \in B} \hat{\gamma}(x_i + \Xi_i, x_i, L_{2(0)}) / \#\{i : x_i \in B\}. \tag{5.4}$$

The integrand of (5.1) is of the form

$$\gamma = V : \int_{X_2} 2\|x - x_0\| dx^2 \tag{5.5}$$

$$= S : \int_{X_1} 2\|x - x_0\| / \sin\alpha(x) dx^1 \tag{5.6}$$

$$= V^2 : \int_{X_2} \int_{X_2} 4\pi \operatorname{area}(x_0, x_1, x_2) dx_1^2 dx_2^2 \tag{5.7}$$

$$= S^2 : \int_{X_1} \int_{X_1} 4\pi \operatorname{area}(x_0, x_1, x_2) / \sin\alpha(x_1) \sin\alpha(x_2) dx_1^1 dx_2^1 \tag{5.8}$$

where $X_2 = Y \cap (x_0 + L_{2(0)})$, $X_1 = \partial Y \cap (x_0 + L_{2(0)})$, dx^q is the element of q-dimensional volume measure, $\alpha(x)$ is the angle between the tangent plane to ∂Y at $x \in \partial Y$ and $L_{2(0)}$ and $\operatorname{area}(x_0, x_1, x_2)$ is the area of the triangle with vertices x_0, x_1 and x_2. Regularity conditions for (5.1) to hold with $\hat{\gamma}$ equal to (5.5)–(5.8) can be found in Jensen and Kiêu (1991).

The variance of the estimator (5.4) depends, among other things, on particle shape and the choice of nuclei inside the particles. If the nuclei are centrally positioned in the particles and the particles are not too irregularly shaped, the variance due to stereology will be low. In particular, $\hat{\gamma}(x_i + \Xi_i, x_i, L_{2(0)}) \equiv \gamma(\Xi_i)$, for all $L_{2(0)}$, if $x_i + \Xi_i$ is a ball and x_i its centre. For this reason,

$$\{\hat{\gamma}(x_i + \Xi_i, x_i, L_{2(0)}) : x_i \in B\} \tag{5.9}$$

will be close to the direct sample of the mark distribution:

$$\{\gamma(\Xi_i) : x_i \in B\}, \tag{5.10}$$

under the circumstances mentioned. As a curiosity, nucleator sampling gives directly the size distribution of diameters of spherical particles and therefore offers

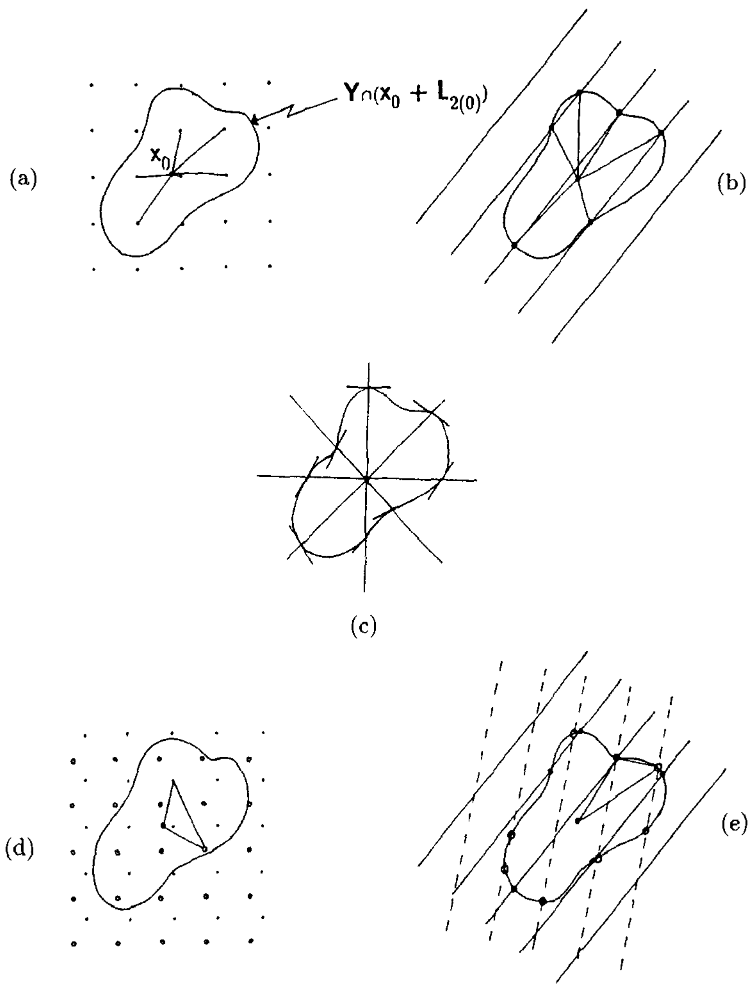

Fig. 4. Illustration of the geometric measurements needed on a particle section $Y \cap (x_0 + L_{2(0)})$ through the nucleus x_0 for estimating (a) mean particle volume, (b) mean particle surface area, (c) the mean particle volume and surface area, based on line information, (d) mean squared particle volume and (e) mean squared particle surface area.

a direct solution to Wicksell's problem, provided that the (observable) nucleus is at the centre.

Apart from the mentioned low stereological variance, the estimators are robust against overprojection because the boundary of a centrally sectioned particle is very often nearly perpendicular to the section plane. Besides, nucleator sampling is sometimes the only possibility, if a particle can only be recognized on a central section.

The actual determination of $\hat{\gamma}(Y, x_0, L_{2(0)})$ (cf. (5.5) to (5.8)) presents various degrees of difficulty. For an illustration, see Fig. 4. For $\gamma = V$, both manual and automatic determination can be used. Manual determination is based on a plane grid of points with uniform position in relation to x_0 (cf. Fig. 4(a)). If automatic determination is used then $Y \cap (x_0 + L_{2(0)})$ is represented in the computer as a set of pixels and the distance from each of these to x_0 is determined. For $\gamma = S$ (cf. Fig. 4(b)) only manual determination is possible at the moment. Uniform points on the boundary can be found as intersection points with a grid of lines with uniform orientation and uniform position in relation to x_0. At each of the intersection points, a distance and a spatial angle must be determined. If the boundary is sufficiently sharp, the angle can be measured by moving the focal plane up and down and observing the travelling distance of the intersection boundary. External information is therefore needed.

The estimators presented in Fig. 4(a)–(b) depend on planar information. Analogous versions based on line information are also available. In Fig. 4(c), an example with a systematic set of 4 lines is shown. The estimators depend again on distances and angles. Spatial angles can in this case be replaced by planar angles, as indicated on Fig. 4(c) (cf. Jensen and Gundersen (1987)).

The estimators of the second moments require two grids of points or two grids of lines if determined manually (cf. Fig. 4(d)–(e)).

Alternative methods of estimating particle size without shape assumptions exist which do not require the existence of nuclei. First of all, the selector which combines disector sampling of particles with the use of a spatial grid of points hitting each sampled particle instead of a nucleus (cf. Cruz-Orive (1987*b*)). First-order moments such as $EV(\Xi_0)$ and $ES(\Xi_0)$ can also be estimated by calculating the ratio between an estimate of the total particle volume or surface area per unit volume and an estimate of the intensity of particles (cf. Sterio (1984)). If the volume mark distribution is of main interest, local serial sectioning, using Cavalieri's principle, can give a precise estimate of this distribution (cf. Marcussen *et al.* (1989)). The workload in doing this type of estimation is however large. Volumeweighted moments can be estimated on a single section (cf. Jensen and Gundersen (1985)). Such moments have been useful in cancer grading (cf. Nielsen *et al.* (1989)).

6. The K-function

As for the mark distribution, stereological analysis of second-order properties of the particle nuclei has been investigated under specific assumptions about particle shape. The spherical model has been studied in e.g. Hanisch and Stoyan (1981), Hanisch (1983) (see also Tanemura (1986)). Here, inversion of integral equations is again involved. Using local 3-d sampling, it is, however, possible to estimate the K-function of the nuclei process without specific assumptions about particle shape.

The idea of this method of estimating the K-function is due to S. Evans (cf. Gundersen *et al.* (1988*b*) and Evans and Gundersen (1989)). In order to present the method, we will proceed along the same lines as in the last section. Thus, we start by considering a series of fixed points $\{x_i\}$ in space. Let $L_{2(0)}$ be an isotropic plane through O and let $\underline{h} + L_{2(0)}$ be a parallel plane, a distance $h = \|\underline{h}\|$ from $L_{2(0)}$. We sample a point $x \neq O$ if it lies between the two planes. It is easy

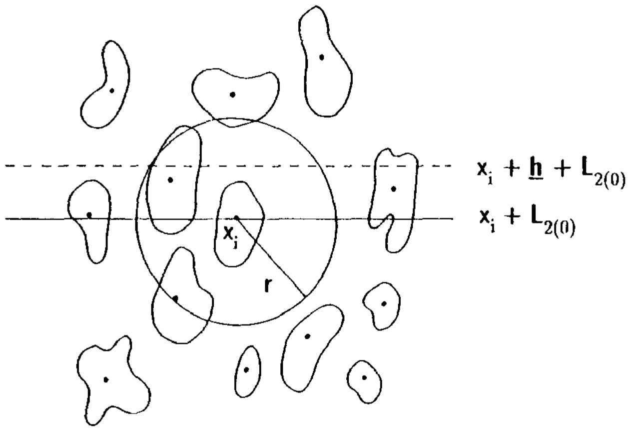

Fig. 5. 2-d illustration of the estimation of $K(r)$, $r > 0$. Each nucleus x_i in the sampling box is chosen as origin. Nuclei at a distance at most r, which lie between $x_i + L_{2(0)}$ and $x_i + \underline{h} + L_{2(0)}$, must be identified.

to see that the probability of being sampled is

$$p_{x,h} = \begin{cases} h/[2\|x\|], & \text{if} \quad \|x\| \geq h \\ 1/2, & \text{otherwise.} \end{cases} \tag{6.1}$$

We now revert the roles of randomness. The points $\Psi_n = \{x_i\}$ thus constitute a stationary and isotropic point process while the planes $L_{2(0)}$ and $\underline{h} + L_{2(0)}$ are fixed and chosen parallel to one of the faces of the sampling box B. The above sampling rule is applied, using each of the points in the sampling box B as origin. In order to estimate $K(r)$, the above sampling rule is applied to all points at a distance at most r (cf. Fig. 5) and the inverse sampling probabilities are summed. The result

$$\sum_{x_i \in B} \sum_{x_j \in S(x_i)} 1_{]0,r]}(\|x_j - x_i\|) p^{-1}_{x_j - x_i, h}, \tag{6.2}$$

where

$$S(x) = \{x_j \in \Psi_n \mid x_j \text{ between } x + L_{2(0)} \text{ and } x + \underline{h} + L_{2(0)}\}, \tag{6.3}$$

has mean $\lambda^2 V(B) K(r)$. This can be shown easily, using the isotropy of the Palm distribution of Ψ_n and Campbell theorem. Therefore,

$$\sum_{x_i \in B} \sum_{x_j \in S(x_i)} 1_{]0,r]}(\|x_j - x_i\|) p^{-1}_{x_j - x_i, h} / \#\{i : x_i \in B\} \tag{6.4}$$

is an estimate of $\lambda K(r)$. We can therefore also estimate the local numerical density at a distance between r_1 and r_2, say, which is

$$\{\lambda K(r_2) - \lambda K(r_1)\} \Big/ \left(\frac{4}{3}\pi r_2^3 - \frac{4}{3}\pi r_1^3 \right). \tag{6.5}$$

In practice, we need for each $x_j \in S(x_i)$ to determine whether the condition $\|x_j - x_i\| \leq r$ is fulfilled. This question can be determined by combining knowledge of the distance between x_i and the projection of x_j on $x_i + L_{2(0)}$ and the distance of x_j to $x_i + L_{2(0)}$. Each of these distances can be determined using the optical sectioning technique. In case of non-spherical particles, the x_i's are identifiable "natural" points, like the nucleolus of a neuron (cf. below).

An application of these methods from the brain is shown in Fig. 6. The situation is slightly more general, because two types of cells are involved, viz. neurons and glia cells. The graph clearly illustrates that the glia cells are clustered around the neurons. See also Gundersen *et al.* (1988*b*).

Stereological second-order analysis of spatial structures of dimension 1 or more can also be performed without specific assumptions (cf. e.g. Stoyan (1984*b*, 1985), Cruz-Orive (1989) and Jensen *et al.* (1990*a*, 1990*b*)).

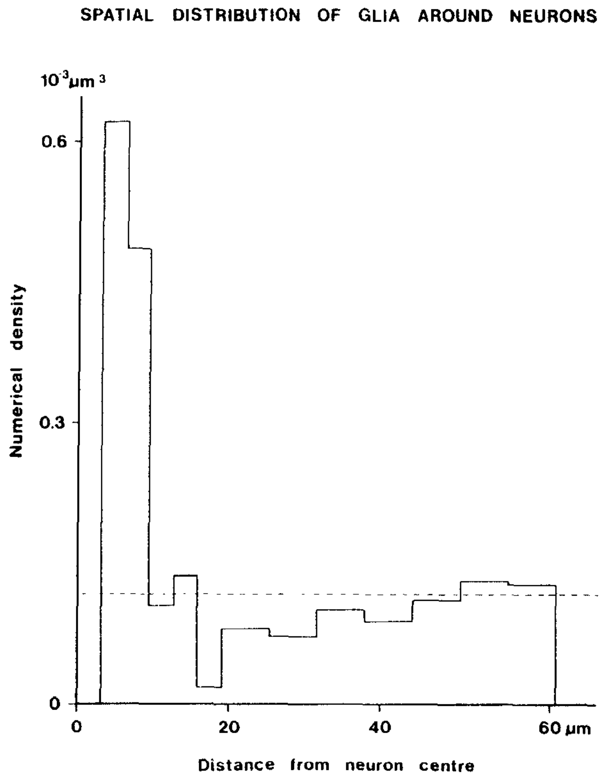

Fig. 6. The variation in the local numerical density of glia cells as a function of the distance from a neuron nucleolus (from Gundersen *et al.* (1988*b*), with permission).

7. Discussion and future work

It still remains to construct stereological estimates of the two-point mark distribution, e.g. of $E_{0,r}V(\Xi_1)V(\Xi_2)$, $r > 0$. One possibility is to concentrate on a cumulative version like the one presented in Section 2 and use a measure decomposition analogous to the one presented in Section 5 for squared volume. The details still remain to be worked out.

It would also be of interest to study the case of one-dimensional particles. There do exist measure decompositions for length like the ones described for volume and surface area, but the statistical properties of the resulting estimators are not nice in this case. For instance, if the particle is a line segment with the mid-point as nucleus, the measure decomposition is not well-defined. Another example: let a particle be a plane circle in space with nucleus equal to the centre of the circle. The measure decomposition gives an unbiased estimator of the length, but the estimator has infinite variance.

The estimation of first- and second-order properties is for many biological applications the final objective. But from a methodological point of view, it is also of interest to study the stereological analysis of a parametric model. The stereological estimate of the K-function can prove to be a useful tool in such an analysis.

References

Coleman, R. (1989). Inverse problems, *Journal of Microscopy*, **153**, 233–248.

Cruz-Orive, L. M. (1980). On the estimation of particle number, *Journal of Microscopy*, **120**, 15–27.

Cruz-Orive, L. M. (1987*a*). Stereology: recent solutions to old problems and a glimpse into the future, *Acta Stereologica*, **6**, 3–18.5.

Cruz-Orive, L. M. (1987*b*). Particle number can be estimated using a disector of unknowm thickness: The selector, *Journal of Microscopy*, **145**, 121–142.

Cruz-Orive, L. M. (1989). Second-order stereology: Estimation of second moment volume measures, *Acta Stereologica*, **8**, 641–646.

Evans, S. M. and Gundersen, H. J. G. (1989). Estimation of spatial distributions using the nucleator, *Acta Stereologica*, **8**, 395–400.

Federer, H. (1969). *Geometric Measure Theory*, Springer, Berlin.

Gundersen, H. J. G. (1977). Notes on the estimation of the numerical density of arbitrary profiles: The edge effect, *Journal of Microscopy*, **111**, 219–223.

Gundersen, H. J. G. (1978). Estimators of the number of objects per unit area unbiased by edge effects, *Microscopica Acta*, **81**, 107–117.

Gundersen, H. J. G. (1986). Stereology of arbitrary particles: A review of unbiased number and size estimators and the presentation of some new ones (in memory of William R. Thompson), *Journal of Microscopy*, **143**, 3–45.

Gundersen, H. J. G. (1988). The nucleator, *Journal of Microscopy*, **151**, 3–21.

Gundersen, H. J. G., Bendtsen, T. F., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sørensen, F. B., Vesterby, A. and West, M. J. (1988*a*). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis, *Acta Pathologica Microbiologica et Immunologica Scandinavica*, **96**, 379–394.

Gundersen, H. J. G., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sørensen, F. B., Vesterby, A. and West, M. J. (1988*b*). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis, *Acta Pathologica Microbiologica et Immunologica Scandinavia*, **96**, 857–881.

Hanisch, K.-H. (1981). On classes of random sets and point processes, *Serdica*, **7**, 160–166.

Hanisch, K.-H. (1983). On stereological estimation of second-order characteristics and of hard-core distances of systems of sphere-centres, *Biometrical J.*, **25**, 731–743.

Hanisch, K.-H. and Stoyan, D. (1981). Stereological estimation of the radial distribution function of centres of spheres, *Journal of Microscopy*, **122**, 131–141.

Howard, V., Reid, S., Baddeley, A. and Boyde, A. (1985). Unbiased estimation of particle density in the tanden scanning reflected light microscope, *Journal of Microscopy*, **138**, 203–212.

Jensen, E. B. and Gundersen, H. J. G. (1985). The stereological estimation of moments of particle volume, *J. Appl. Probab.*, **22**, 82–98.

Jensen, E. B. and Gundersen, H. J. G. (1987). Stereological estimation of surface area of arbitrary particles, *Acta Stereologica*, **6**, 25–30.

Jensen, E. B. and Gundersen, H. J. G. (1989). Fundamental stereological formulae based on isotropically orientated probes through fixed points with applications to particle analysis, *Journal of Microscopy*, **153**, 249–267.

Jensen, E. B. and Kiêu, K. (1991). A new integral geometric formula of Blaschke-Petkantschin type, Research report, No. 180, Department of Theoretical Statistics, Institute of Mathematics, University of Aarhus (submitted to *Math. Nachr.*).

Jensen, E. B., Kiêu, K. and Gundersen, H. J. G. (1990*a*). On the stereological estimation of reduced moment measures, *Ann. Inst. Statist. Math.*, **42**, 445–461.

Jensen, E. B., Kiêu, K. and Gundersen, H. J. G. (1990*b*). Second-order stereology, *Acta Stereologica*, **9**, 15–35.

Marcussen, N., Ottosen, P. D., Christensen, S. and Olsen, T. S. (1989). Atubular glomeruli in lithium-induced cronic nephropathy in rats, *Laboratory Investigation*, **61**, 295–302.

Matheron, G. (1975). *Random Sets and Integral Geometry*, Wiley, New York.

Nielsen, K., Ørntoft, T. and Wolf, H. (1989). Stereologic estimates of the nuclear volume in non-invasive bladder tumors (Ta) correlated with the recurrence pattern, *Cancer*, **64**, 2269–2274.

Penttinen, A. and Stoyan, D. (1989). Statistical analysis for a class of line segment processes, *Scand. J. Statist.*, **16**, 153–168.

Petran, M., Hadravsky, M., Egger, M. D. and Galambos, R. (1968). Tandem scanning reflected light microscope, *J. Opt. Soc. Amer. A*, **58**, 661–664.

Sterio, D. C. (1984). The unbiased estimation of number and sizes of arbitrary particles using the disector, *Journal of Microscopy*, **134**, 127–136.

Stoyan, D. (1984*a*). On correlations of marked point precesses, *Math. Nachr.*, **116**, 197–207.

Stoyan, D. (1984*b*). Further stereological formulae for spatial fibre processes, *Mathematische Operationsforschung und Statistik, Series Statistics*, **15**, 421–428.

Stoyan, D. (1985). Stereological determination of orientations, second-order quantities and correlations for random fibre systems, *Biometrical J.*, **27**, 411–425.

Stoyan, D., Kendall, W. S. and Mecke, J. (1987). *Stochastic Geometry and Its Applications*, Akademie, Berlin.

Tanemura, M. (1986). On the stereology of the radial distribution function of hard-sphere systems, *Science on Form: Proceedings of the First International Symposium for Science on Form* (eds. Y. Kato, R. Takaki and J. Toriwaki), 157–165, KTK Scientific Publishers, Tokyo.

Watson, G. S. (1971). Estimating functionals of particle size distributions, *Biometrika*, **58**, 483–490.

Weibel, E. R. (1989). Measuring through the microscope: Development and evolution of stereological methods, *Journal of Microscopy*, **155**, 393–403.

Wicksell, S. D. (1925). The corpuscle problem I, *Biometrika*, **17**, 84–89.

Wicksell, S. D. (1926). The corpuscle problem II, *Biometrika*, **18**, 152–172.

Zähle, M. (1990). A kinematic formula and moment measures of random sets, *Math. Nachr.*, **149**, 325–340.