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STEREOLOGICAL DETERMINATION OF THE PAIR CORRELATION FUNCTION OF THE CENTRES OF THE NUCLEI IN A RAT LIVER USING A THIN SECTION TECHNIQUE

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ABSTRACT

The use of the pair correlation function for the analysis of a configuration of cell nuclei in a rat liver is demonstrated. A procedure is suggested which enables the stereological determination of the pair correlation function of the nuclei centres using related quantities of a thin section of the rat liver.

INTRODUCTION

The stereological methods for the determination of spatial mean values as N_V, V_V, S_V or K_V and of spatial size distribution functions from planar, thin and linear sections give no information on the variabliity of the particle system considered, on relationships between the particle positions.

A step towards getting those quantities consists in the consideration of second-order quantities. Let us assume that A is a random spatial sphere configuration which is homogeneous (= stationary) and isotropic, i.e. the distribution of A is invariant under translations and rotations. The first second-order quantity of A of interest is then the covariance $C_V(h)$, $h \geq 0$, see Serra

(1982), given by

$$C_V(h) = P(0 \in A, \underline{h} \in A), \qquad (1)$$

where $\underline{h}$ is a point with distance h to the origin O. Hence, $C_V(h)$ gives the probability that two fixed points a distance h apart are covered by A.

In the following we are concerned with thin sections of sphere configurations. Exact formulae for the stereological determination of the covariance from related quantities of thin sections are not known. Weibel (1980) and Gerlach & Stoyan (1984) gave some approximate formulae.

Further second-order quantities for the description of a sphere configuration are related to the positions of the sphere centres. From our experience, the pair correlation function $g_V(r)$ of the sphere centres is a suitable quantity for the description of the "inner structure" of a sphere configuration. It has the following meaning. Let N_V denote the mean number of spheres per unit volume, dV_1 and dV_2, two volume elements a distance r apart. Then $N_V^2\, g_V(r)\, dV_1\, dV_2$ gives the probability that in dV_1 and dV_2 there is each one sphere centre. In our opinion, the pair correlation function $g_V(r)$ is in many cases more instructive than the covariance $C_V(h)$ for the description of the variability of the sphere configuration, for example in case of known sphere radii distribution function R_V, particularly for constant radii. The reason for this is the fact that C_V is a quantity for general geometrical structures, whereas g_V is especially designed to random sphere configurations.

Hence, in the following we will give a method for the stereological determination of the pair correlation function g_V of sphere centres from thin sections with known section thickness.

PAIR CORRELATION FUNCTIONS OF RANDOM SPATIAL POINT SYSTEMS

In the following it is assumed that the sphere centres form a random spatial point system Φ_V which is homogeneous and isotropic (see Ripley (1981)). Then the pair correlation function $g_V(r)$ of Φ_V is given by $N_V^2\, g_V(r)\, dV_1\, dV_2$ = probability that in the volume elements dV_1, dV_2 a distance r apart there is each of one point of Φ_V.

For the case of a Poisson process Φ_V (purely random distribution of points) $g_V(r) \equiv 1$ is obtained, see fig. 1. Values $g_V(r) < 1$ for a distance r indicate that there are fewer point pairs in the point process with a distance approximately equal to r than in a Poisson process, i.e. there is a repulsion of points within this distance. Vice versa, $g_V(r) > 1$ indicates an attraction of points with distance approximately equal to r in the sense that there exist more point pairs with such a distance than in a Poisson process. For hard-core processes with hard-core distance $\tau = 2R$ (case of non-overlapping spheres with fixed radius R), $g_V(r) = 0$ for $r < \tau$ is obtained.

Often the form of g_V gives rise to the assumption of a special model of Φ_V. Fig. 1 shows pair correlation functions of various typical models of random spatial point systems. There are treated two hard-core processes (Matern's second hard-core process, see Hanisch & Stoyan, 1981, and the centres Φ_V of a dense random packing of hard spheres), the Poisson process and a cluster process (Matern cluster process). Furthermore, from fig. 1 two properties of g_V can be seen.
For a wide class of random point systems (mixing random point systems, see e.g. Franken, König,

Arndt & Schmidt (1982)) $g(r) \rightarrow 1$ for $r \rightarrow \infty$ is obtained. For $r \rightarrow 0$ in the most cases $g(r) \rightarrow c < \infty$ holds.

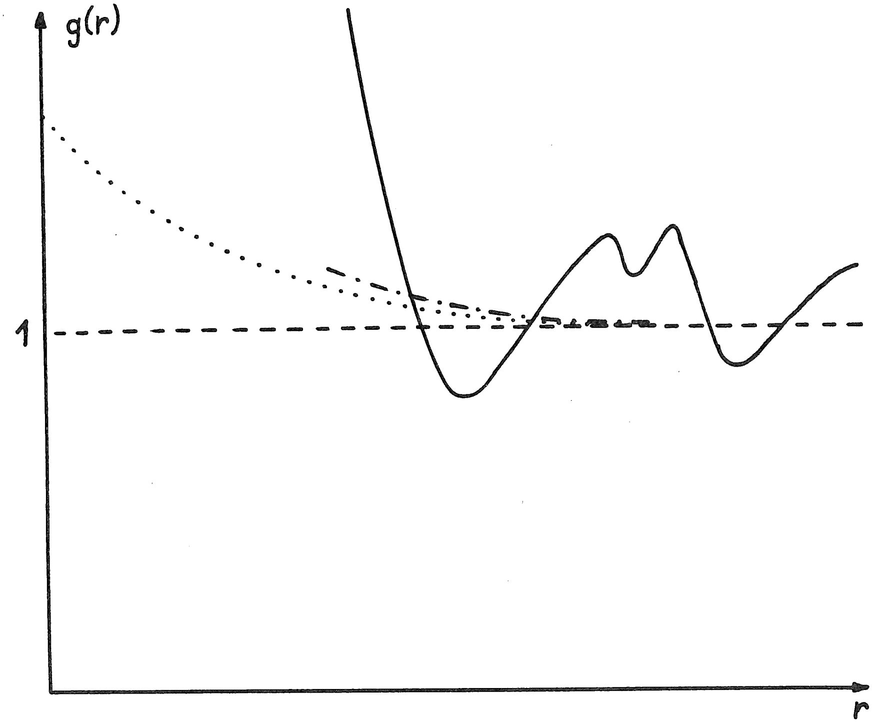

Fig. 1. Pair correlation functions for various models of spatial point systems

--- Poisson process

—— Dense random packing of hard spheres

-.-.. Matern's second hard-core process

.... Matern cluster process

STEREOLOGICAL DETERMINATION OF THE PAIR CORRELATION FUNCTION FOR THE CELL NUCLEI OF A RAT LIVER

Fig. 2 shows a diagram of a thin section (section thickness 2 t = 4 μm) of a rat liver. The objects of interest are the spherical nuclei. The aim of this section is to describe - at least partially - the spatial configuration of the cell

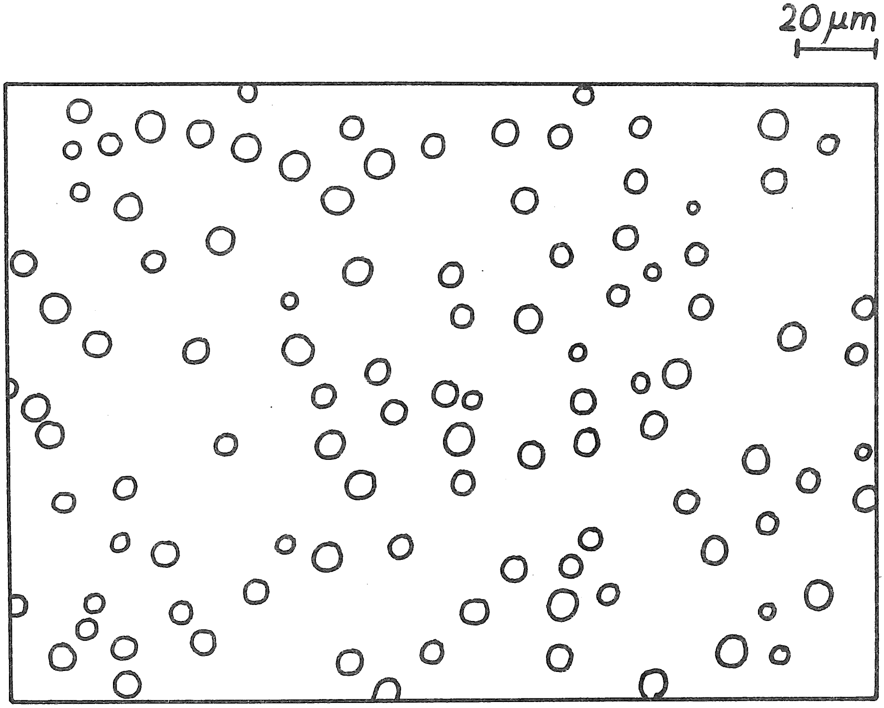

Fig. 2. Thin section of a rat liver

nuclei for this single thin section. Therefore, suitable quantities have to be determined stereologically from the thin section.

For the description of the configuration of the cell nuclei the following model is used. The nuclei are assumed to be spheres with radii distributed according to a distribution function R_V. The centres of the nuclei form a random spatial point system Φ_V. The sphere radii are mutually independent and independent of the point system Φ_V. Then in the thin section of the rat liver the projections of the sectioned nuclei can be observed as circular profiles. Their centres form again a random planar point system $\bar{\Phi}_A$.

Obviously, from fig. 2 it can be seen that the configuration of observed circular profiles can be assumed to be - at least locally - homogeneous and isotropic. Hence, we assume that the centres of the observed circular profiles form a part of a realization of a homogeneous and isotropic point system Φ_A and, furthermore, that Φ_V is homogeneous and isotropic, too.

A measurement of the radii of the circular profiles shows, that radii approximately equal to 5 μm occur very often. From this the conjecture results that the spherical nuclei approximately have constant radii 5 μm. This is supported by the following. For the mean radius of the circular profiles m_A and the corresponding variance σ_A^2 we obtained $m_A = 4.2$ μm and $\sigma_A^2 = 18.52$ μm^2. Theoretically, under the condition of constant sphere radii R,

$$m_A = \frac{\pi R^2 + 4Rt}{4(t+R)}, \qquad (2)$$

$$\sigma_A^2 = \frac{R^4(32-3\pi^2) + R^3 t(80-24\pi)}{48(R+t)^2}, \qquad (3)$$

where 2t is the section thickness. Then equation (2) yields R = 4.8 μm, equation (3) R = 4,9 μm. Because of the good correspondence of these two numbers in order of magnitude of the measuring precision, also from this point of view the assumption of constant sphere radii seems to be justified.

Then the mean number N_V of nuclei per unit volume was determined using the well-known formula (see Weibel, 1980),

$$N_V = \frac{N_A}{2\{m_V + t\}}, \qquad (4)$$

where N_A is the mean number of intersection circles per unit area and m_V is the mean sphere radius (nuclei radius). For the nuclei configuration $N_V = 1.56 \cdot 10^{-4} \mu m^{-3}$ was computed. Using this, other mean values of the sphere configuration such as V_V, S_V or K_V can be determined. For the aim of more insight into the inner structure of the sphere configuration then the pair correlation function $g_V(r)$ was determined stereologically.

For this Hanisch (1983) gave the integral equation

$$g_A(r) = \frac{1}{(m_V + t)^2} \int_0^\infty f_V(u,t) g_V(\sqrt{r^2 + u^2})\,du \qquad (5)$$

with

$$f_V(u,t) = \begin{cases} t - \frac{u}{2} + \int_0^u [1-R_V(y)]\,dy \\ + \int_u^\infty [1-R_V(u-y)][1-R_V(y)]\,dy; \quad u \leq 2t \\ \int_{\frac{u}{2}-t}^{u-2t} [1-R_V(u-y-2t)][1-R_V(y)]\,dy \\ + \int_{u-2t}^{u} [1-R_V(y)]\,dy \\ + \int_u^\infty [1-R_V(u-y)][1-R_V(y)]\,dy; \quad u > 2t. \end{cases} \qquad (6)$$

In this connection $g_A(r)$ is the pair correlation function of the random point system Φ_A of intersection circle centres. It has an interpretation analogous as g_V.

For the case of constant sphere radii R, (5) takes the form

$$g_A(r) = \frac{2}{d^2} \int_0^d (d-x)\, g_V(\sqrt{r^2 + x^2})\,dx, \qquad (7)$$

where $d = 2R+2t$.

Hence, $g_V(r)$ can be obtained by estimating $g_A(r)$ from the configuration of intersection circle centres and by solving the integral equation (7).

The pair correlation function $g_A(r)$ can be estimated as follows. Assume that in a bounded sampling window W (in our case W is a rectangle) the intersection circle centres $x_1, \ldots, x_N$ are given. Then the integrated quantity $G_A(r)$,

$$G_A(r) = N_A^2 \int_0^r 2\pi x \, g_A(x) \, dx \tag{8}$$

is considered. It can be interpreted as the mean number of points of Φ_A in a circle with radius r centred at a randomly chosen point of Φ_A multiplied with N_A. Hence, there occur edge effects in the estimation of $G_A(r)$, because the mentioned point number can not be computed for points of Φ_A near the boundary of W exactly. However, there exist various unbiased and strongly consistent estimators using the homogeneity of Φ_A for correcting these edge effects (Ripley (1981) and Ohser & Stoyan (1981)). One of them is

$$\hat{G}_A(r) = \sum_{\substack{i,j=1 \\ 0<d(x_i,x_j)<r}}^{N} (A((W-x_i) \cap (W-x_j)))^{-1}, \tag{9}$$

where $d(x_i,x_j)$ denotes the distance of x_i and x_j, $W-x_i = \{x \in \mathbb{R}^2 : x+x_i \in W\}$ denotes the set W shifted by $-x_i$ and A(B) denotes the area of a set B. Hence, $\hat{G}_A(r)$ is obtained by summing up over all point pairs in the window with distance less than r, where each point pair gets - in order to correct edge effects - the weight $w_{ij} = A((W-x_i) \cap$

$(W - x_j))^{-1}$, see fig. 3.

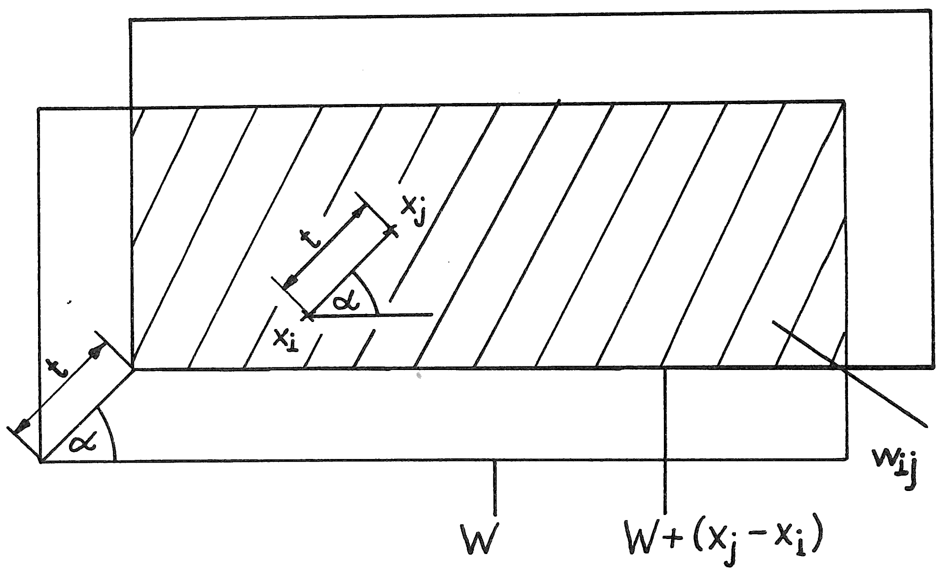

Fig. 3. Determination of the weight w_{ij} used in (9)

This estimator $\hat{G}_A(r)$ is asymptotically normal, see Heinrich (1984). From this an estimator $\hat{g}_A(r)$ of $g_A(r)$ can be obtained by numerical differentiation. Of course, it seems also to be appropiate to use a kernel estimator analogous to (9) for obtaining an estimate of $g_A(r)$ directly, see e.g. Jolivet (1984) and Taylor (1983).

The pair correlation function $\hat{g}_A(r)$ for the cell nuclei obtained in the described manner is given in fig. 4.

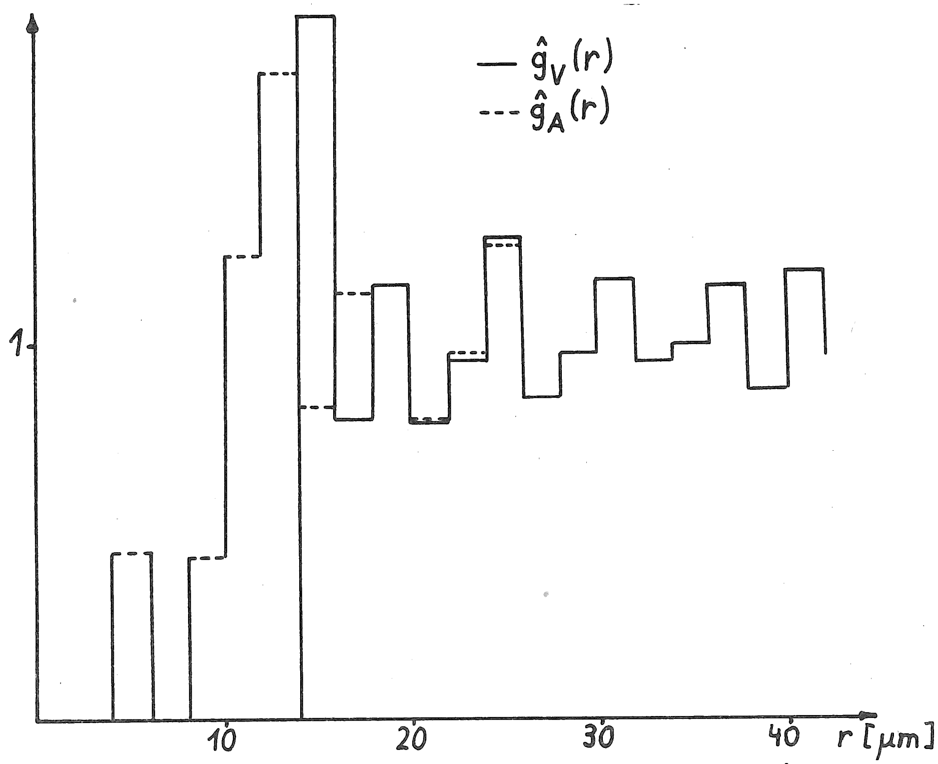

Fig. 4. The pair correlation functions $\hat{g}_A$ of the intersection circle centres and $\hat{g}_V$ of the nuclei centres

Using this function integral equation (7) was solved numerically. For doing this we have transformed the problem into a linear fitting problem with a side condition which results from the non-negativity of $g_V(r)$. The transformed problem can be solved with standard methods. Examples show that this procedure is relatively stable in the sense that fluctuations of the function $g_A(r)$ cause fluctuations of $g_V(r)$ in the same order of magnitude. Details will be the subject of a further paper.

The so obtained pair correlation function $\hat{g}_V(r)$ of nuclei centres is also shown in fig. 4. The hard-core distance seen there ($\hat{g}_V(r) = 0$ for

$r < 14\ \mu m$) is due to the fact that the cell nuclei are non-overlapping and that there is a further repulsion. Furthermore, it can be seen that the first peak of $\hat{g}_V$ is somewhat higher than that of $\hat{g}_A$; it is also shifted somewhat to the right. Up to these two exceptions $\hat{g}_V$ is very similar to $\hat{g}_A$. The reason for this is that integral equation (7) yields $g_A(r) \approx g_V(r)$ for r not too small in comparison with $d = 2R + 2t$. The peak of $\hat{g}_V$ in $r = 18\ \mu m$ indicates that pairs of cell nuclei with approximately this distance are favoured. and, hence, that the 'conventional' cell radius is about $9\ \mu m$. Clearly, this analysis of the nuclei is only a further tool for the stereological description of such a cell structure and it has to be connected with common methods for analyzing the cells themselves. The frequency of pairs of nuclei with other distances $r > 18\ \mu m$ corresponds to that for a Poisson process.

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