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UNBIASED LINEAR PROPERTY ESTIMATION FOR SPHERICAL PARTICLES, FROM 'THIN' SECTIONS

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ABSTRACT

Formulae are derived for unbiased estimation of volume fraction - and other linear properties - for 'thin' section sampling of spherical particles, the resulting circular profiles being subject to a simple lower resolution limit. Their computation is illustrated on lysosomal data.

INTRODUCTION

In the 'Holmes' effect problem of stereology, a 'thin' section of thickness t - from a medium containing randomly distributed spherical particles - is viewed by transmission microscopy. The resulting projected profiles are circular; let the number of them with centres in the fixed section area, A, be denoted by N and their diameters by $\{y_1, y_2, \ldots, y_N\}$. It is assumed that diameters less than a known limit (q) are not measurable and that overlap effects are negligible.

Literature on the Holmes effect for spheres includes Bach (1959), Goldsmith (1967), Keidins et al. (1972), Coleman (1979, 1980) and Piefke (1976). The first two papers consider only the case $q = 0$ and, with the exception of Piefke (1976), all concentrate on inversion of the relation between circle diameter and sphere diameter distributions rather than unbiased estimation of specific properties.

The distribution function (d.f.) of the diameters (x) of all spheres in the medium for which $x >= q$ is denoted by $G(x|q)$, and the number of such spheres per unit volume by $N_V(q)$. Making no parametric assumptions about $G(.)$, unbiased estimation is sought of the linear properties

$$\theta(q) = N_V(q) \int_{x=q}^{\infty} \ell(x)\,dG(x|q) \qquad (1).$$

Here $\ell(x) = \pi x^3/6$, πx^2, x and 1 define $\theta(q) = V_V(q)$, $S_V(q)$, $J_V(q)$ and $N_V(q)$, respectively the total volume, surface area, diameter and number of particles of diameter $>=q$ in a unit volume of the medium. The general formulation of Nicholson (1970) applies in this case. It follows (e.g. Clarke, 1975) that there exists an unbiased estimator of the form

$$\hat{\theta}(q) = A^{-1} \sum_{i=1}^{N} h(y_i) \qquad (2)$$

if a function $h(y)$ can be found to satisfy

$$\int_q^x yh(y)/(x^2-y^2)^{.5}dy + th(y) = \ell(x) \qquad (3),$$

for all x in (q,∞).

METHODS

The Volterra integral equation (3) is very similar to that solved by Bach (1959) and Goldsmith (1967), relating circle and sphere diameter distributions; see also Coleman (1979) or Clarke (1975) for a solution involving only elementary mathematics. An analogous approach, followed by substantial manipulation, yields the convenient computational form

$$h(y) = h_0(y) - \exp(cY^2) \int_0^Y h_1(z)\exp(-cz^2)\,dz \qquad (4),$$

where for

$$N_V(q):\ h_0(y) = t^{-1}E, \qquad h_1(z) = t^{-2} \qquad (5),$$

$$J_V(q):\ h_0(y) = 1 + qt^{-1}E - 2\pi^{-1}\sin^{-1}(D)$$

$$h_1(z) = 2q\pi^{-1}F + qt^{-2} - zt^{-1}F^{.5} \qquad (6),$$

$$S_V(q):\ h_0(y) = 4Y - 4t + 4t(1 + cq^2)E$$

$$h_1(z) = 4(1 + cq^2) \qquad (7),$$

$$V_V(q):\ h_0(y) = (4^{-1}\pi y^2 - ty + t^2) + qt(1 + 2cq^2/3)E$$

$$+ 2^{-1}(y^2 + c^{-1})\{D(1-D^2)^{.5}(1+2D^2/3) - \sin^{-1}(D)\},$$

$$h_1(z) = (2/3)q^5(cF + 2F^2 + 2c^{-1}F^3) - tzF^{.5} \qquad (8)$$

and $D = q/y$, $E = \exp(c(y^2-q^2))$, $F = (q^2+z^2)^{-1}$, $Y = (y^2-q^2)^{.5}$, $c = \pi/(4t^2)$.

Assuming that $q = y_0 < y_1 < y_2 < \ldots < y_N$, the absence of y from the integrand of the numerical integral in (4) may be exploited to give the computationally efficient form

$$\hat{\theta}(q) = \sum_{i=1}^N h_0(y_i) - \sum_{j=1}^N a_j I_j \qquad (9).$$

Here

$$I_j = \int_z h_1(z)\exp(-cz^2)\,dz, \qquad a_j = \sum_{i=j}^N \exp(c(y_i^2-q^2)) \qquad (10),$$

where the integration for z in I_j is over $(y_{j-1}^2-q^2)^{.5}$ to $(y_j^2-q^2)^{.5}$; simple 'Simpson rule' evaluation is adequate. For grouped frequency data, y_i having frequency f_i, the factor f_i should be inserted in the summation defining a_j in (10) and the summation of $h_0(.)$ in (9).

It follows from Nicholson (1970) that, if the sphere centres form a Poisson process, an unbiased estimator of the variance of $\hat{\theta}(q)$ is $A^{-2}\Sigma h^2(y_i)$. A consequence of Mecke and Stoyan (1980) is that (4)-(10) are valid for a general stationary point process of sphere centres; however, validity of the variance estimate requires the stronger assumption of a Poisson distribution for N.

RESULTS AND DISCUSSION

The data in Table 1 are extracted from a study by Lowe et al. (1981) of digestive cell lysosomes from the digestive diverticula of the common mussel M. edulis. Secondary lysosomes were distinguished in cryostat sections by their azo-dye reaction product for lysosomal β-N-acetylhexo-saminidase. The present data are just that from a single digestive tubule from each of two animals; row 1 is a control and row 2 an animal exposed for 103 days to the water accommodated fraction of North Sea crude oil (30 $\mu g \ell^{-1}$ total oil derived hydrocarbons). Table 2 gives the relevant linear property estimates. This example has been included primarily to facilitate the checking of any computer software that the reader may produce for equations (4)-(10); its toxicological implications will not be discussed here beyond noting that the pattern of increased lysosomal volume fraction and reduced numerical density, under such oil exposure, is properly established by the full designed experiment.

In the current computing environment, the calculations are neither complex nor expensive. Their use is advocated for small samples, insufficient for an 'unfolding' approach.

In particular, when many such point estimates are to be input to a second-stage analysis, unbiasedness becomes an important property.

<u>Table 1</u>: Frequency (f_i) of lysosome profile diameters (y_i), for epithelial area A = 400 μm^2, resolution q = 1.2μm.

y_i (μm)	1.2	1.5	1.8	2.1	2.4	2.7	3.0	3.3	3.6	3.9	4.2	4.5
Control f_i	27	15	8	8	8	2	3	1	7	0	2	0
Oil f_i	4	4	7	6	1	0	1	1	1	0	1	2

y_i (μm)	4.8	5.1	5.4	5.7	6.0	6.3	6.6	6.9	7.2	7.5	7.8	N
Control f_i	0	0	0	0	1	0	0	0	0	0	0	82
Oil f_i	1	0	2	0	0	1	2	0	0	1	2	37

<u>Table 2</u>: Linear property estimates and their standard deviation estimates, for the data of Table 1.

Property	(dimension)	Control	Oil
$N_V(q)$	(μm^{-3})	.00365(±.00041)	.00147(±.00025)
$J_V(q)$	(μm^{-2})	.00705(±.00083)	.00448(±.00081)
$S_V(q)$	(μm^{-1})	.0524(±.0080)	.0624(±.0152)
$V_V(q)$	(1)	.0256(±.0057)	.0592(±.0178)

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