THIELE CENTRE
FOR APPLIED MATHEMATICS IN NATURAL SCIENCE

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Research Report No. 01 | January 2007

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This Thiele Research Report is also Research Report number 488 in the Stochastics Series at Department of Mathematical Sciences, University of Aarhus, Denmark.

Particle sizes from sectional data

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SUMMARY. We propose a new statistical method for obtaining information about particle size distributions from sectional data without specific assumptions about particle shape. The method utilizes recent advances in local stereology. We show how to estimate separately from sectional data the variance due to the local stereological estimation procedure and the variance due to the variability of particle sizes in the population. Methods for judging the difference between the distribution of estimated particle sizes and the distribution of true particle sizes are also provided.

KEY WORDS: Local stereology; Particle size distribution; Star-shaped sets; Variance estimation.

1. Introduction

Most statistical consultants have at least once in their carreer been asked to find the cell size distribution from a histological section showing plane sections of individual cells. One of the principal difficulties in answering this question is that a plane section of the cells cannot be regarded as a representative sample from the cell population in the usual statistical sense. Planes sample cells with a probability proportional to cell height. The involved sampling problem may perhaps be understood more clearly if the analogy of a tomato salad is used. A uniform random tomato slice picked from the salad has a higher probability of originating from a large tomato than from a small tomato.

Swedish mathematical statistician S. D. Wicksell first treated the problem of estimating the size distribution of spherical particles from plane sections, cf. Wicksell (1923, 1925). His results were used to study cancer of the spleen where the size distribution of small focal tumours were to be estimated from observations in plane sections of tissue. Wicksell's main results were later derived independently by Scheil (1935, 1938), Fullmann (1953), Reid (1955) and Santaló (1955).

The mathematical solution of the problem of finding the size distribution of spherical particles from the size distribution of circular sections is fascinating but its practical relevance is limited because particles are in most applications not spherical and the methods developed for spherical particles are very sensitive to departures

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from the assumption of spherical shape. Nevertheless the main part of the statistical literature on estimating particle size distribution from sectional data concerns spherical particles. Reviews can be found in Cruz-Orive (1983), Stoyan et al. (1995, Section 11.4, pp. 353–369) and Ohser and Mücklich (2000, Chapter 6).

The problem of estimating the sphere size distribution from the distribution of circular sections is ill-posed. Techniques proposed for its solution include numerical analysis (Anderssen and Jakeman, 1975a; Anderssen and Jakeman, 1975b), Gauss-Chebyshev quadrature methods (Mase, 1995), kernel density estimation (Taylor, 1983; Hall and Smith, 1988), smoothing splines (Nychka et al., 1984), singular value decomposition (O'Sullivan, 1986) and wavelets (Antoniadis et al., 2001).

This paper provides new statistical tools for obtaining information about particle size distributions from sectional data without specific assumptions about particle shape. These tools utilize methods from local stereology, a branch of stereology that has been developed in the eighties and nineties in close collaboration with users through publications in non-statistical journals such as *Journal of Microscopy*, cf. Sterio (1984), Miles (1985), Cruz-Orive (1987), Gundersen (1988), Jensen and Gundersen (1989), Jensen and Sorensen (1991), Jensen and Gundersen (1993), Tandrup et al. (1997). It is now clear that local stereological methods are very powerful in biological and medical application areas. Recent examples are Andersen et al. (2003), Hundahl et al. (2006) and Hosseini-sharifabad and Nyengaard (2007). The theory of local stereology has been unified in the monograph Jensen (1998), see also the related concept of second-order stereology described in Jensen et al. (1990). An introduction to stereological particle analysis, written for statisticians, may be found in the recent monograph Baddeley and Jensen (2005, Chapter 11).

In the present paper, we show how these local methods can be developed into practical statistical procedures for analyzing particle size distributions without specific assumptions about particle shape. The new tools require that the sampling of particles is changed, see Section 2 below. The benefits obtained by changing the sampling are, however, clear. First of all, the mean and variance of the particle volume distribution can be estimated from sectional data without specific shape assumptions and in some cases even the whole particle volume distribution. It is also possible to get information about other types of size parameters without specific shape assumptions.

The organization of the paper is as follows. In Section 2, we describe the principles behind local sampling and local estimation of particle sizes. In particular, for a size parameter φ, we discuss how to estimate, from sectional data, (i) the variance σ_φ^2 of the size parameter in the particle population and (ii) the total estimator variance $\sigma_{\hat{\varphi}}^2 = \sigma_\varphi^2 + \tau_\varphi^2$, where τ_φ^2 is the extra variance due to the local stereological estimation procedure. Specific examples of local particle size estimators are given in Section 3. In Section 4, we study the distribution of the variance estimators $\hat{\sigma}_{\hat{\varphi}}^2$ and $\hat{\sigma}_\varphi^2$ and give practical guidelines for how many particles we need to sample in order to get reliable variance estimates. The answer depends on the mixture of shapes represented in the particle population. In Section 5, we give a simple procedure for judging how close the distribution of estimated particle sizes is to the distribution of true particle sizes. In Section 6, the developed tools are tried out on a data set consisting of sections through neurons from the human hippocampus. Section 7 concludes.

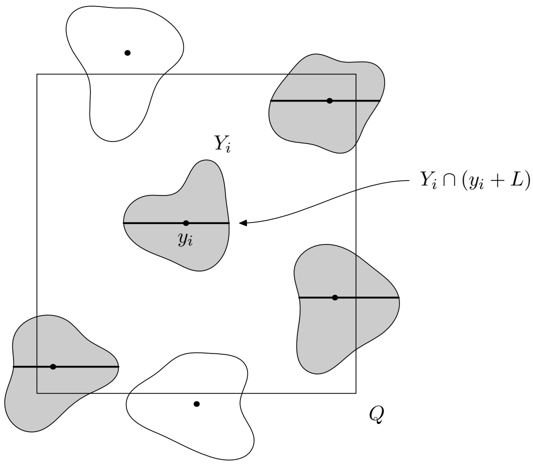

Figure 1. Two-dimensional illustration of sampling of particles, using their reference points. All particles with reference points in Q are sampled (shown hatched). The sections $Y_i \cap (y_i + L)$ can be observed completely for particles with $y_i \in Q$.

2. Local sampling and local estimation of particle sizes

Let Y be a randomly chosen particle from the particle population $\{Y_i\}$ under study. We aim at making inference about the distribution of $\varphi(Y)$ where φ is a size parameter such as volume, surface area etc. We let μ_φ and σ_φ^2 be the mean and variance of $\varphi(Y)$.

The basic idea is to collect a sample of particles and for each sampled particle estimate its size. The sampling and estimation of sizes are based on methods from local stereology. These methods require that we can associate a reference point $y \in Y$ to each particle Y. In bioscience, this is a mild requirement. If, for instance, the particles are cells, we may choose the centres of the nuclei of the cells as reference points.

In contrast to traditional sampling with planes, the sampling is performed such that the resulting sample is representative for the particle population. Such sampling can be implemented in a number of ways, cf. e.g. Baddeley and Jensen (2005, Chapter 11), depending on the type of microscope available, the staining techniques, the magnification needed for identifying the particles, etc. If optical sectioning of a transparent tissue block Q is performed, then our sample $\mathcal{S}$ may consist of all particles with reference points in Q, i.e.

$$\mathcal{S} = \{i : y_i \in Q\}.$$

This type of sampling is illustrated in Figure 1.

For each sampled particle Y_i, we estimate its size $\varphi(Y_i)$, using a local probe passing through the associated point y_i of the sampled particle Y_i. In this paper, we will as local probe mainly use a plane $y_i + L$ where L is the parallel plane through the origin. The size estimator is a function g of the intersection between the particle and the section plane

$$\hat{\varphi}(Y_i) = g(Y_i \cap (y_i + L)), \tag{1}$$

where g is chosen such that under the assumption of uniform randomly oriented (isotropic) particles

$$E[\hat{\varphi}(Y)] = \mu_\varphi. \tag{2}$$

If the particles cannot be regarded as uniform randomly oriented, we may instead randomize the orientation of the section plane and still use the estimator $\hat{\varphi}$.

Based on the sampled particles, a (ratio-)unbiased estimator of μ_φ is

$$\hat{\mu}_\varphi = \sum_{i \in \mathcal{S}} \hat{\varphi}(Y_i)/N, \tag{3}$$

where $N = N(\mathcal{S})$ is the number of sampled particles. This result holds for an arbitrarily positioned sampling cube Q if the particles can be regarded as homogeneously distributed in space. If this assumption is unrealistic we may instead choose the sampling cube Q with uniform random position.

In what follows, we let $\mu_{\hat{\varphi}}$ and $\sigma^2_{\hat{\varphi}}$ be the mean and variance of $\hat{\varphi}(Y)$. Note that because of (2), $\mu_{\hat{\varphi}} = \mu_\varphi$.

The mean particle size μ_φ may also be estimated by dividing an estimate of the total population value $\sum_i \varphi(Y_i)$ with an estimate of the number of particles in the population, cf. e.g. Baddeley and Jensen (2005, Chapter 11, p. 262). This approach is, however, not appropriate if more information about the particle size distribution is needed like the variance.

If we are able to determine $\varphi(Y_i)$ with high precision, then we can regard

$$\hat{\varphi}(Y_i), \quad i \in \mathcal{S}, \tag{4}$$

as a sample from the size distribution. This is the case for optical sectioning of spherical particles where the size can be measured directly from a central section. However, the general situation is that the particles are of unknown varying shape. In such situations, it is important to be able to estimate from sectional data the two components of the total estimator variance $\sigma^2_{\hat{\varphi}}$, consisting of the variance τ^2_φ due to the stereological estimation procedure and the variance σ^2_φ due to the variability of particle sizes in the population. We have

$$\sigma^2_{\hat{\varphi}} = \sigma^2_\varphi + \tau^2_\varphi. \tag{5}$$

In order to estimate the variance components from sectional data, we can utilize that the second moment in the particle size distribution can be estimated, using a procedure similar to the one used for the estimation of the first moment. Let $\psi = \varphi^2$ and

$$\hat{\psi}(Y_i) = h(Y_i \cap (y_i + L))$$

such that

$$E[\hat{\psi}(Y)] = \mu_\psi.$$

Then,

$$\hat{\mu}_\psi = \sum_{i \in \mathcal{S}} \hat{\psi}(Y_i)/N \tag{6}$$

is a (ratio-)unbiased estimator of μ_ψ.

Irrespectively of the interaction structure of the particles, we can always estimate τ_φ^2 by

$$\hat{\tau}_\varphi^2 = \frac{1}{N} \sum_i \hat{\varphi}(Y_i)^2 - \hat{\mu}_\psi.$$

We have here utilized that $\mu_{\hat{\varphi}} = \mu_\varphi$ and

$$\begin{aligned} \tau_\varphi^2 &= \sigma_{\hat{\varphi}}^2 - \sigma_\varphi^2 \\ &= \left[E(\hat{\varphi}(Y)^2) - E(\hat{\varphi}(Y))^2\right] - \left[E(\varphi(Y)^2) - E(\varphi(Y))^2\right] \\ &= E(\hat{\varphi}(Y)^2) - E(\varphi(Y)^2). \end{aligned}$$

Using (5), the variance in the particle size distribution can be estimated by

$$\hat{\sigma}_\varphi^2 = \hat{\sigma}_{\hat{\varphi}}^2 - \hat{\tau}_\varphi^2, \tag{7}$$

where $\hat{\sigma}_{\hat{\varphi}}^2$ is an estimator of the total variance. The actual form of $\hat{\sigma}_{\hat{\varphi}}^2$ will depend on the interaction structure. If we can assume that $\{y_i\}$ and $\{Y_i - y_i\}$ are independent sequencies and $\{Y_i - y_i\}$ consists of independent and identically distributed random sets, then the standard estimator

$$\hat{\sigma}_{\hat{\varphi}}^2 = \frac{1}{N-1} \sum_i (\hat{\varphi}(Y_i) - \hat{\mu}_\varphi)^2 \tag{8}$$

is unbiased for $\sigma_{\hat{\varphi}}^2$. The case of interacting particles will be considered in a future paper, cf. Pawlas and Nyengaard (2007).

A mathematical justification of the results presented in this section can be found in the Appendix where particles are modelled by a stationary and isotropic marked point process (Mecke et al., 1990; Stoyan et al., 1995; Stoyan and Stoyan, 1995), see also Pawlas and Jensen (2006). If this model is not appropriate, the methods can be justified in a design-based sense using appropriately randomized sampling devices. For details, see Baddeley and Jensen (2005, Chapter 11, 257–269).

3. Local particle size estimators

Estimators $\hat{\varphi}$ of the form (1), satisfying (2), have been constructed in local stereology for a number of size parameters φ, including volume and surface area. For volume, there are at least three possibilities of which we will mainly concentrate on the following local estimator

$$\hat{V}(Y) = 2 \int_{Y \cap (y+L)} \|x - y\| \, \mathrm{d}x,$$

see also the related estimator discussed in Cabo and Baddeley (2003). If Y is star-shaped relative to y such that any ray starting from y hits the boundary of Y exactly once, then

$$\hat{V}(Y) = \frac{2}{3} \int_0^{2\pi} \rho_{Y \cap (y+L)}(\alpha)^3 \mathrm{d}\alpha, \tag{9}$$

where $\rho_{Y \cap (y+L)}(\alpha)$ is the distance from y to the boundary of Y in the direction determined by the angle α in the plane $y + L$. We can rewrite (9) as

$$\hat{V}(Y) = \frac{4\pi}{3} R^3,$$

where

$$R = \left[\int_0^{2\pi} \rho_{Y\cap(y+L)}(\alpha)^3 \frac{\mathrm{d}\alpha}{2\pi} \right]^{1/3}.$$

Note that if Y is a ball centred at y, then $\rho_{Y\cap(y+L)}$ is constant and the estimator $\hat{V}(Y)$ determines $V(Y)$ without error. In Jensen and Petersen (1999), the class of particles, having an exact volume estimator (called quasi-spherical bodies), is studied. The general situation is, however, that the particles are not spherical (or quasi-spherical). Indeed, in biological particle populations, a mixture of shapes will very often be present.

Stochastic simulation for specific shapes can help to get an impression of how much extra variability the distribution of estimated sizes exhibits compared to the distribution of true sizes. Let us consider a particle population with particles distributed as $\rho B Y_0$ where ρ is a random positive variable, B is a uniform random rotation and Y_0 is a fixed particle. Then, the variance in the particle volume distribution is

$$\sigma_\varphi^2 = \mathrm{Var}(V(\rho B Y_0)) = V(Y_0)^2 \mathrm{Var}(\rho^3),$$

while, due to the homogeneity of the volume estimator ($\hat{V}(\rho B Y_0) = \rho^3 \hat{V}(B Y_0)$), the extra variance due to the estimation of volumes becomes

$$\tau_\varphi^2 = \mathrm{Var}(\hat{V}(\rho B Y_0)) - \mathrm{Var}(V(\rho B Y_0)) = E(\rho^6) V(Y_0)^2 \kappa, \tag{10}$$

where

$$\kappa = \frac{\mathrm{Var}(\hat{V}(B Y_0))}{V(Y_0)^2}$$

depends on Y_0. If we let $k = \sigma_{\hat{\varphi}}/\sigma_\varphi$ be the increased variability due to the estimation procedure and $\gamma = \sigma_\varphi/\mu_\varphi$ the variability in the particle size distribution, we have under this simplified model that

$$k = \sqrt{\frac{(\gamma^2+1)\kappa}{\gamma^2} + 1}. \tag{11}$$

The value of κ is given in Table 1 for an ellipsoid Y_0 with semi-axes a, b and c. (Ellipsoidal particles have earlier been discussed in, e.g., Wicksell (1926), Cruz-Orive (1976) and Cruz-Orive (1978).) The value of κ is only dependent on a/b and b/c. Note that κ is larger for prolate ellipsoids ($b = c$) than for the corresponding oblate ellipsoids ($a = b$). Using Table 1, we can for instance conclude that for a particle population of ellipsoids with $a/b = 2$ and $b/c = 1$, the increased variability due to the estimation procedure will be

$$k = \sqrt{\frac{(\gamma^2+1)0.11835}{\gamma^2} + 1}.$$

For $\gamma = 0.5$ we get $k = 1.26$ while for $\gamma = 1$ we get $k = 1.11$.

There is a total of 14 estimators of the same type as $\hat{V}(Y)$ for volume, surface area, length and number, cf. Jensen (1998). Except for the estimators of volume, certain angular measurements are needed which may be difficult to implement. Very

Table 1

The values of κ from (10) for a particle population of ellipsoids with semi-axes of lengths a, b, c.

a/b	b/c	κ
1.0	1.0	0
1.0	1.5	0.02937
1.0	2.0	0.07945
1.5	1.0	0.03704
1.5	1.5	0.10154
1.5	2.0	0.17238
2.0	1.0	0.11835
2.0	1.5	0.21857
2.0	2.0	0.31307

recently, Cruz-Orive (2005) has constructed an alternative estimator of surface area that avoids angular measurements. It is also possible to avoid angular measurements by sampling in thick sections, see Kiêu and Jensen (1993) and Tandrup et al. (1997). Length estimation without angular measurements has been discussed in Mouton et al. (2002).

For the estimation of the variance in the particle size distribution it is needed to construct an unbiased estimator of $E\varphi^2(Y)$, as explained in Section 2. If $\varphi = V$, such an estimator can be constructed in a canonical fashion. The resulting estimator has the following form, cf. Jensen (1998),

$$\hat{\psi}(Y) = 2\pi \int_{Y\cap(y+L)} \int_{Y\cap(y+L)} \nabla_2(x_1 - y, x_2 - y)\, \mathrm{d}x_2\, \mathrm{d}x_1, \tag{12}$$

where $\nabla_2(z_1, z_2)$ is twice the area of the triangle with vertices O, z_1 and z_2. If Y is star-shaped relative to y, then

$$\hat{\psi}(Y) = \frac{2\pi}{9} \int_0^{2\pi} \int_0^{2\pi} \rho_{Y\cap(y+L)}(\alpha)^3 \rho_{Y\cap(y+L)}(\beta)^3 |\sin(\alpha - \beta)|\, \mathrm{d}\alpha\, \mathrm{d}\beta. \tag{13}$$

4. The distribution of $\hat{\sigma}^2_{\hat{\varphi}}$ and $\hat{\sigma}^2_{\varphi}$

The statistical properties of the variance estimators $\hat{\sigma}^2_{\hat{\varphi}}$ and $\hat{\sigma}^2_{\varphi}$ will depend on the mixture of particle shapes represented in the particle population, the size variability of the particles, the number of sampled particles and the interaction between particles. A question of great practical importance is to decide on the number N of particles to be sampled in order to get a desired precision of the estimators. Such questions can be evaluated by stochastic simulation.

In Figure 2, we report the results of such a simulation for a particle population consisting of triaxial ellipsoids with semi-axes of lengths A, B and C, where $(\ln A, \ln B, \ln C)$ has a normal distribution with parameters $((\ln a, \ln b, \ln c), \Sigma)$ and $\Sigma = \sigma^2 I_3$ is a 3×3 diagonal matrix with σ^2 in all diagonal elements. Figure 2 shows histograms based on 1000 simulated observations of $\hat{\sigma}^2_{\hat{\varphi}}/\sigma^2_{\varphi}$ (white) and $\hat{\sigma}^2_{\varphi}/\sigma^2_{\varphi}$ (grey) for different choices of particle shape (a, b and c), variability in the particle volume distribution ($\gamma = \sigma_\varphi/\mu_\varphi$) and number of particles (N). The expected values $E(\hat{\sigma}^2_{\hat{\varphi}}/\sigma^2_\varphi)$ and $E(\hat{\sigma}^2_\varphi/\sigma^2_\varphi) = 1$, respectively, are indicated by vertical lines.

The left column in Figure 2 corresponds to the case $a = b = c = 1$ where the particles do not have a systematic deviation from spherical shape but the actual shapes may vary considerably. The right column is based on very elongated ellipsoids $a = 4, b = 1, c = 0.25$. In this case we have quite high variability in the estimation of the variances. To avoid that the estimator $\hat{\sigma}^2_\varphi$ of the variance in the distribution of particle volume is negative a large number (N=1000, say) of observations are needed. For the more realistic and moderately elongated ellipsoids in the middle column ($a = 2, b = 1, c = 0.5$), negative variance estimates are seldom observed.

It may tentatively be concluded from Figure 2 that for a population of nearly spherical particles, sampling and measurement of $N = 100$ particles or less provide variance estimates of a reliable order of magnitude while for a population with marked nonspherical particle shapes $N = 1000$ or more are needed.

5. Assessing the difference between true and estimated sizes

In some applied papers, see e.g. Tandrup and Braendgaard (1994) and Vestergaard et al. (1997), the distribution of estimated sizes has been used as an estimate of the distribution of true sizes. In this section, we provide tools for evaluating the difference between these two distributions. We suppose that estimates of μ_φ, σ^2_φ and $\sigma^2_{\hat{\varphi}}$ are available from the sections. Furthermore, we will utilize that it is generally known that

$$\hat{\varphi}(Y) = \varphi(Y) \cdot Z,$$

where $EZ = 1$ and, consequently, the size variable $\varphi(Y)$ and the error variable Z are uncorrelated. It does, however, not seem to be possible to derive exact results about the difference between the distribution of $\hat{\varphi}(Y)$ and $\varphi(Y)$ without further assumptions. One possibility is to assume that the size variable and the error variable are independent (they are known to be uncorrelated) and both log normally distributed. Then, it is easy to derive the following result:

$$\begin{aligned} D &= \sup_{t>0} |P(\hat{\varphi}(Y) \le t) - P(\varphi(Y) \le t)| \\ &= \sup_{t>0} \left| \Phi\left(\frac{\ln t \sqrt{1+\gamma^2 k^2}}{\sqrt{\ln(1+\gamma^2 k^2)}} \right) - \Phi\left(\frac{\ln t \sqrt{1+\gamma^2}}{\sqrt{\ln(1+\gamma^2)}} \right) \right|, \end{aligned} \tag{14}$$

where $\gamma = \sigma_\varphi/\mu_\varphi$, $k = \sigma_{\hat{\varphi}}/\sigma_\varphi$ and Φ is the distribution function of the standard normal distribution.

In Figure 3, we directly compare the distribution of estimated volumes with the distribution of true volumes. There is good agreement between the size of D and the apparent similarity of the two distributions.

6. Example

As an example, we will use the methods developed in the present paper in a study of the volume distribution of neurons in the Granular layer of the hippocampus of the human brain. The hippocampi came from autopsies of brains from five Danish

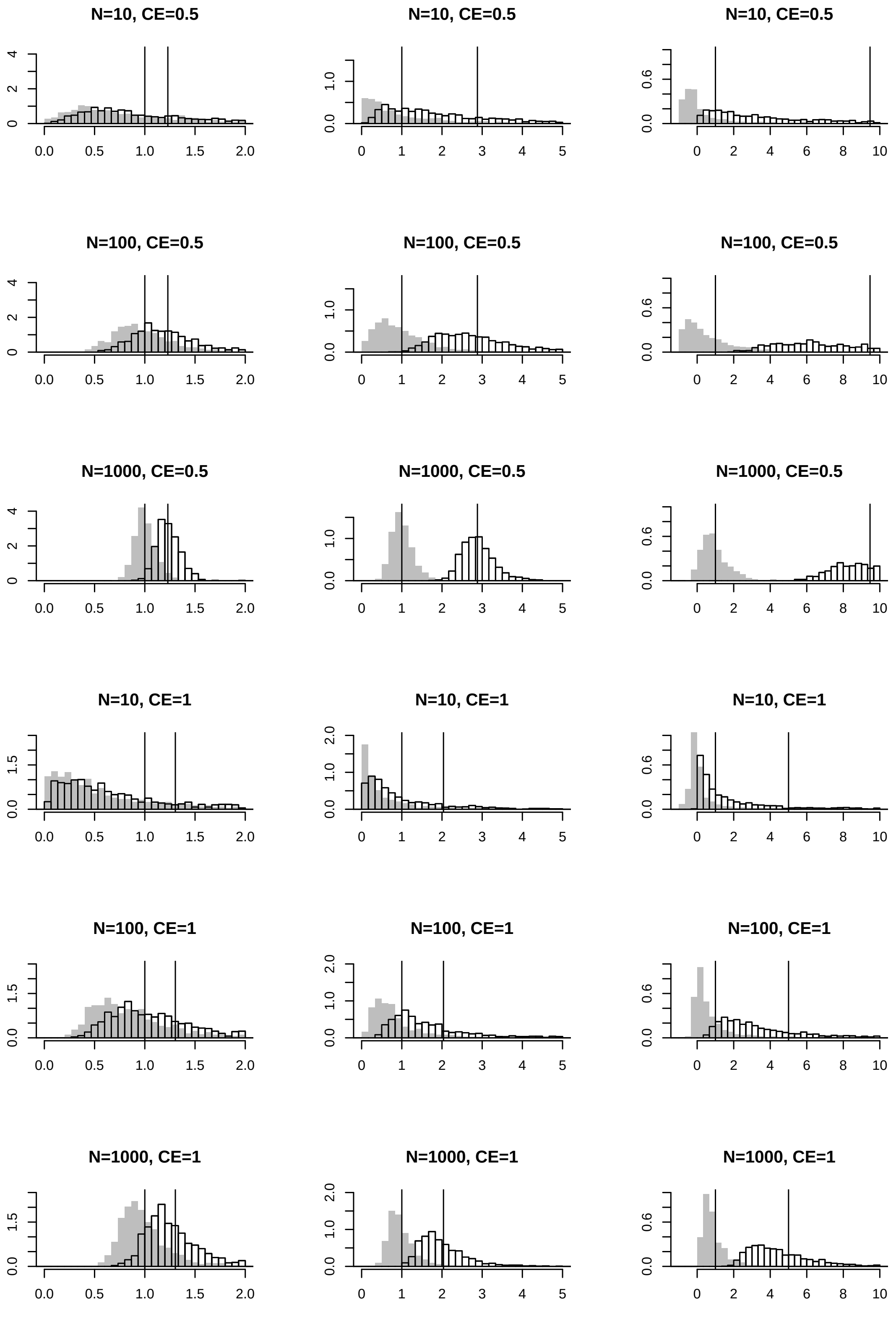

Figure 2. Distributions of $\hat{\sigma}^2_{\hat{\varphi}}/\sigma^2_{\varphi}$ (white) and $\hat{\sigma}^2_{\varphi}/\sigma^2_{\varphi}$ (grey) for $a = 1$, $b = 1$, $c = 1$ (left column), $a = 2$, $b = 1$, $c = 0.5$ (middle column), $a = 4$, $b = 1$, $c = 0.25$ (right column). For further details, see text.

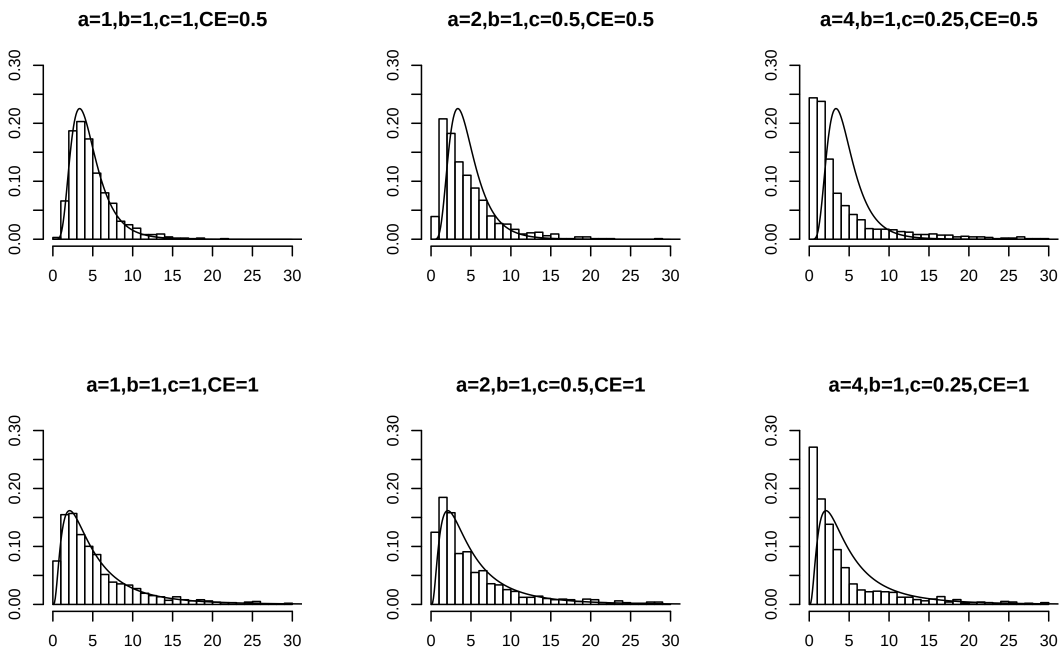

Figure 3. The distribution of the estimated volumes (histograms) and the distribution of true volumes. The simulated particle populations are the same as in Figure 2. The value of the index D is (from top left to bottom right) 0.034, 0.177, 0.360, 0.046, 0.120 and 0.258, respectively.

Table 2
The estimates of μ_φ (10^3 μm^3), σ^2_φ (10^6 μm^6), τ^2_φ (10^6 μm^6), γ^2, k^2 and D of neurons from the Granular layer of the human hippocampus from 5 subjects.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
μ_φ	0.87993	0.65658	0.99920	0.59748	0.63681
σ^2_φ	0.05327	0.09129	0.14895	0.02204	0.03423
τ^2_φ	0.02345	0.02539	0.10257	0.01538	0.01601
γ^2	0.06880	0.21176	0.14919	0.06174	0.08441
k^2	1.44036	1.27814	1.68856	1.69777	1.46779
D	0.05507	0.04037	0.08452	0.07952	0.05891

males, aged 52–84 years. A total of $N = 25$ neurons was sampled in the Granular layer of each subject. The neurons could be regarded as star-shaped with respect to the centres of their nuclei which were chosen as reference points. A digital image was taken when the nucleus of a sampled neuron was in focus. The boundary of the neuron profile was traced by an expert. The resulting 25 central sections of neurons from the Granular layer are shown in Figure 4 for each of the five subjects.

For each subject, we used the methods described in detail in Sections 2 and 3 to estimate the mean particle volume μ_φ, the variance σ^2_φ in the particle volume distribution and the variance τ^2_φ due to the stereological estimation procedure. Neurons in the Granular layer may be regarded as uncorrelated in position and size. Accordingly, the total variance $\sigma^2_{\hat\varphi}$ was estimated, using (8).

In Table 2, the estimates of μ_φ, σ^2_φ and τ^2_φ are shown for each subject. The estimates of the squared coefficient of variation $\gamma^2 = \sigma^2_\varphi/\mu^2_\varphi$ in the particle volume distribution and the increased variability $k^2 = \sigma^2_{\hat\varphi}/\sigma^2_\varphi$ due to the stereological estimation procedure are also given. The values of the index D have been calculated from formula (14) in Section 5, using the values of γ^2 and k^2 estimated from the sectional data. Note that D is quite small for all subjects.

In order to get a rough idea about particle shapes in 3D, we have used the relation (11) and the estimates of k and γ to calculate an estimate of the shape factor κ. For the 5 subjects, we get the following estimates of κ

$$0.0283, 0.0486, 0.0894, 0.0406, 0.0364.$$

Comparing with Table 1, the deviations from spherical shape seem to be quite modest. This particle population resembles mostly the simulated population in the upper left corner in Figure 2. This is in accordance with the fact that sampling of $N = 25$ particles was enough to give well-behaved estimators.

We also analyzed a similar data set collected from the CA1 layer of the human hippocampus. The same five subjects were examined. For each subject $N = 25$ neurons were sampled from the CA1 layer. It turned out that the number of neurons sampled in this case was too small to give valid estimates of the variance components because the neurons in this layer of the hippocampus exhibited a much more marked discrepancy from spherical shape. Actually, all the estimates of the variance in the

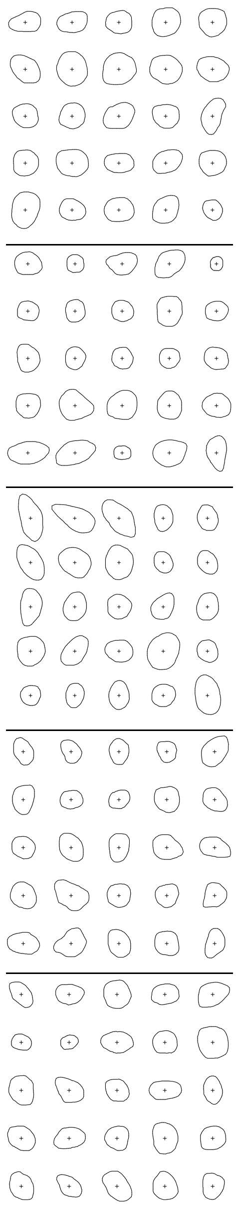

Figure 4. Sections through reference points of 25 neurons from the Granular layer of the human hippocampus in five subjects.

particle volume distribution were negative presumably due to a heavy left tail of the distribution of the estimator of the variance in the particle volume distribution, see the grey histograms in the upper right corner of Figure 2.

The shape variability of the neurons has earlier been studied in Hannila et al. (2004), see also Hobolth (2003).

7. Conclusion

In the present paper, we have developed new statistical tools for obtaining information about particle size distributions from sectional data without specific assumptions about particle shape. It has been shown how the mean and variance in the particle size distribution can be estimated, using methods from local stereology. The methods have been exemplified by choosing volume as size parameter. An index, describing the difference between the distribution of true sizes and the distribution of estimated sizes, has been constructed. The value of this index can be estimated from sectional data.

Acknowledgements

The authors want to thank Adrian Baddeley for fruitful discussions about the state of art of stereological particle analysis. We also want to thank Lars Madsen for excellent graphical assistance. The research was supported by a Marie Curie Fellowship of the European Commission, the Danish Natural Science Research Council, the Grant Agency of the Czech Republic, project 201/06/0302, and the Czech Ministry of Education, project MSM 0021620839. The MIND Center is supported by the Lundbeck Foundation.

References

Andersen, B. B., Gundersen, H. J. G. and Pakkenberg, B. (2003). Aging of the human cerebellum: a stereological study. *Journal of Comparative Neurology* **466**, 356–365.

Anderssen, R. S. and Jakeman, A. J. (1975a). Abel type integral equations in stereology. I. General discussion. *Journal of Microscopy* **105**, 121–133.

Anderssen, R. S. and Jakeman, A. J. (1975b). Abel type integral equations in stereology. II. Computational methods of solution and the random spheres approximation. *Journal of Microscopy* **105**, 135–154.

Antoniadis, A., Fan, J. and Gijbels, I. (2001). A wavelet method for unfolding sphere size distributions. *Canadian Journal of Statistics* **29**, 251–268.

Baddeley, A. and Jensen, E. B. V. (2005). *Stereology for Statisticians.* Monographs on Statistics and Applied Probability 103. Chapman & Hall/CRC, Boca Raton.

Cabo, A. and Baddeley, A. (2003). Estimation of mean particle volume using the set covariance function. *Advances in Applied Probability* **35**, 27–46.

Cruz-Orive, L. M. (1976). Particle size distributions: the general spheroid problem. I. Mathematical model. *Journal of Microscopy* **107**, 235–253.

Cruz-Orive, L. M. (1978). Particle size distributions: the general spheroid problem. II. Stochastic model and practical guide. *Journal of Microscopy* **112**, 153–167.

Cruz-Orive, L. M. (1983). Distribution-free estimation of sphere size distributions from slabs showing overprojection and truncation, with a review of previous methods. *Journal of Microscopy* **131**, 265–290.

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

Cruz-Orive, L. M. (2005). A new stereological principle for test lines in three-dimensional space. *Journal of Microscopy* **219**, 18–28.

Fullmann, R. (1953). Measurement of particle sizes in opaque bodies. *Journal of Metals* **5**, 447–452.

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

Hall, P. and Smith, R. L. (1988). The kernel method for unfolding sphere size distributions. *Journal of Computational Physics* **74**, 409–421.

Hannila, M. L., Jensen, E. B. V., Hobolth, A. and Nyengaard, J. R. (2004). Shape modelling of spatial particles from planar central sections - a case study. *Journal of Microscopy* **215**, 183–190.

Hobolth, A. (2003). The spherical deformation model. *Biostatistics* **4**, 583–595.

Hosseini-sharifabad, M. and Nyengaard, J. R. (2007). Design-based estimation of neuronal number and individual neuronal volume in the rat hippocampus. *J. Neurosci. Meth.* .

Hundahl, C., Kelsen, J., Kjaer, K., Ronn, L. C., Weber, R. E., Geuens, E., Hay-Schmidt, A. and Nyengaard, J. R. (2006). Does neuroglobin protect neurons from ischemic insult? a quantitative investigation of neuroglobin expression following transient MCAo in spontaneously hypertensive rats. *Brain Research* **1085**, 19–27.

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., Kiêu, K. and Gundersen, H. J. G. (1990). Second-order stereology. *Acta Stereologica* **9, Suppl. I**, 15–35.

Jensen, E. B. V. (1998). *Local Stereology.* World Scientific, Singapore.

Jensen, E. B. V. and Gundersen, H. J. G. (1993). The rotator. *Journal of Microscopy* **170**, 35–44.

Jensen, E. B. V. and Petersen, L. (1999). When are local stereological volume estimators exact? Technical Report 4, Laboratory for Computational Stochastics, University of Aarhus.

Jensen, E. B. V. and Sorensen, F. B. (1991). A note on stereological estimation of the volume-weighted second moment of particle volume. *Journal of Microscopy* **164**, 21–27.

Kiêu, K. and Jensen, E. B. V. (1993). Stereological estimation based on isotropic slices through fixed points. *Journal of Microscopy* **170**, 45–51.

Krumbein, W. C. and Pettijohn, F. J. (1938). *Manual on Sedimentary Petrography.* D. Appleton-Century, New York.

Mase, S. (1995). Stereological estimation of particle size distributions. *Advances in Applied Probability* **27**, 350–366.

Mecke, J., Schneider, R. G., Stoyan, D. and Weil, W. (1990). *Stochastische Geometrie.* DMV Seminar Band 16. Birkhäuser, Basel.

Miles, R. E. (1985). A comprehensive set of stereological formulae for embedded aggregates of not-necessarily-convex particles. *Journal of Microscopy* **138**, 115–125.

Mouton, P. R., Gokhale, A. M., Ward, N. L. and West, M. J. (2002). Stereological length estimation using spherical probes. *Journal of Microscopy* **206**, 54–64.

Nychka, D., Wahba, G., Goldfarb, S. and Pugh, T. (1984). Cross-validated spline methods for the estimation of three-dimensional tumor size distributions from observations on two-dimensional cross sections. *Journal of the American Statistical Association* **79**, 832–846.

Ohser, J. and Mücklich, F. (2000). *Statistical Analysis of Microstructures in Materials Science.* Wiley, Chichester.

O'Sullivan, F. (1986). A statistical perspective on ill-posed problems. *Statistical Science* **1**, 502–518.

Pawlas, Z. and Jensen, E. B. V. (2006). Further results on variances of local stereological estimators. *Image Analysis and Stereology* **25**, 155–163.

Pawlas, Z. and Nyengaard, J. R. (2007). Estimation of variance components of local stereological volume estimators. In preparation.

Reid, W. P. (1955). Distribution of sizes of spheres in a solid from a study of slices of the solid. *J. Math. Phys.* **34**, 95–102.

Santaló, L. A. (1955). Sobre la distribución de las tamaños de corpúsculas contenidos en un cuerpo a partir de la distribución en sus secciones a projecciones. *Trabajos de Estadística* **6**, 191–196.

Scheil, E. (1931). Die Berechnung der Anzahl und Grossenverteilung kugelformiger Kristalle in undurchsichtigen Körpern mit Hilfe der durch einen ebenen Schnitt erhalten Schnittkreise. *Zeitschr. anorg. allgem. Chem.* **201**, 259–264.

Scheil, E. (1935). Statistische Gefügeuntersuchungen I. *Z. Metallk.* **27**, 199–.

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

Stoyan, D., Kendall, W. S. and Mecke, J. (1995). *Stochastic Geometry and its Applications.* Wiley, Chichester, second edition.

Stoyan, D. and Stoyan, H. (1995). *Fractals, Random Shapes and Point Fields.* Wiley, Chichester.

Tandrup, T. and Braendgaard, H. (1994). Number and volume of rat dorsal root ganglion cells in acrylamide intoxication. *J. Neurocytol.* **23**, 242–248.

Tandrup, T., Gundersen, H. J. G. and Jensen, E. B. V. (1997). The optical rotator. *Journal of Microscopy* **186**, 108–120.

Taylor, C. C. (1983). A new method for unfolding sphere size distributions. *Journal of Microscopy* **132**, 57–66.

Vestergaard, S., Tandrup, T. and Jakobsen, J. (1997). Effect of permanent axotomy on number and volume of dorsal root ganglion cell bodies. *J. Comp. Neurol.* **388**, 307–312.

Wicksell, S. D. (1923). Et iagttagelsesteoretisk problem (in Danish). *Assurandoeren* **24**, 243.

Wicksell, S. D. (1925). The corpuscle problem. A mathematical study of a biometric problem. *Biometrika* **17**, 84–89.

Wicksell, S. D. (1926). The corpuscle problem. Second memoir. Case of ellipsoidal corpuscles. *Biometrika* **18**, 152–172.

Appendix

Marked point processes

The particles are assumed to be non-empty compact subsets of $\mathbb{R}^3$. Together with the reference points they constitute a marked point process $\Xi = \{[y_i; Y_i]\}$ which is assumed to be stationary and isotropic. To such a marked point process we can associate a mark distribution P_m which is a probability distribution on the set of non-empty compact subsets of $\mathbb{R}^3$. The particle size distribution corresponding to a size parameter φ is then the distribution of $\varphi(Y)$ where Y is a random particle distributed according to P_m. The mean and variance in the particle size distribution are

$$\mu_\varphi = \int \varphi(Y) P_m(\mathrm{d}Y)$$

and

$$\sigma_\varphi^2 = \int (\varphi(Y) - \mu_\varphi)^2 P_m(\mathrm{d}Y).$$

The function g in the size estimator $\hat{\varphi}$ in (1) is chosen such that

$$\int_{S_+^2} g(Y \cap L_\omega) \frac{\mathrm{d}\omega}{2\pi} = \varphi(Y),$$

where Y is any fixed particle, L_ω is a plane through the origin with normal vector ω and S_+^2 is the positive hemisphere. Utilizing that P_m is isotropic, it can be shown that

$$\int \hat{\varphi}(Y) P_m(\mathrm{d}Y) = \mu_\varphi,$$

which is the precise formulation of (2) in the main text.

Using that φ as a size parameter is invariant under translations and rotations, and the Campbell-Mecke theorem for marked point processes, we furthermore have

$$E \sum_{i \in \mathcal{S}} \hat{\varphi}(Y_i) = \lambda V(Q) \mu_\varphi,$$

where λ is the intensity of particles. In particular,

$$EN(\mathcal{S}) = \lambda V(Q),$$

and it follows that

$$\frac{E \sum_{i \in \mathcal{S}} \hat{\varphi}(Y_i)}{EN(\mathcal{S})} = \mu_\varphi.$$

Accordingly, the estimator $\hat{\mu}_\varphi$ in (3) of the main text is ratio-unbiased.