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# THE CORPUSCLE PROBLEM: REEVALUATION USING THE DISECTOR

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ABSTRACT

No other stereological problem but that of estimating the sphere size distribution from the profile distribution observed in a single section has been shown on so many occasions to be statistically ill-posed. In this paper, we reconsider the corpuscle problem and suggest a direct solution based on local spatial information. The key tool is the disector which uses information from two parallel sections simultaneously.

Keywords: Corpuscle problem, disector, particles, spheres, systematic sampling.

## 1. INTRODUCTION

Wicksell's classical corpuscle problem (1925) concerns the estimation of sphere diameter distribution from measurements of diameters of circular profiles on a plane section through the aggregate of spherical particles. The relation between the observed distribution of profile diameters and the sphere diameter distribution is a well-known integral equation of Abel type (if section thickness is zero). Let F be the distribution function of sphere diameters and G the distribution function of diameters of circular profiles. Then

$$G(y) = \int_0^y \frac{z}{\mu} \int_z^\infty (x^2-z^2)^{-\frac{1}{2}} \, dF(x)\,dz \qquad (1)$$

where $\mu$ is the first moment of F. A whole range of methods have been suggested for estimating F from G, including finite difference algorithms, product integration methods and parametric methods. For a critical review, see Cruz-Orive (1983). Most of these methods have been extensively criticized on statistical and numerical grounds, cf. e.g. Watson (1971) and Anderssen and Jakeman (1975). A main reason for these short-comings is that the integral equation constituting the analytical solution of the problem contains a singularity in the integrand.

Instead of adding yet another partial solution to an apparently ill-posed problem, we suggest that one should take the consequence of recent developments in stereological particle analysis and solve the corpuscle problem by entering the third dimension locally, cf. Sterio (1984), Cruz-Orive (1986), Gundersen (1986). The suggestion involves a change in the geometric sampling of particles and more measurements than just a single diameter in one plane section through each sampled particle.

The sampling is based on information from two parallel sections simultaneously. The idea is to sample all particles hitting one of the planes (the reference plane), but not the other plane (look-up plane). Such a pair of parallel planes is called a <u>disector</u>. Using this type of sampling, each particle has, in contrast to usual plane sampling, a <u>constant chance</u> (not dependent on size) of being sampled. Until now, the power of this idea has been demonstrated by the development of number and mean size estimators based on disector sampling, which are applicable to arbitrarily shaped particles. In a further development of the disector - dubbed 'the fractionator' (Gundersen, 1986) - it is not even necessary to know the distance between section planes, the reference volume or assume anything about shrinkage/swelling. Also on a single section, one may obtain an unbiased estimate of a mean size valid for arbitrarily shaped particles (Jensen and Gundersen, 1985).

Depending on how close the particles are to spherical shape, different procedures with varying degrees of robustness against departures from the shape assumption can be used for determining the size of each sampled particle. All the described procedures are based on measurements from two or more parallel sections through a sampled particle.

## 2. DISECTOR SAMPLING

The disector is a geometric sampling device by means of which it is possible to obtain a uniform random sample of particles from a spatial aggregate of particles. The disector can be applied to arbitrarily shaped particles.

A disector consists of two parallel sections of thickness $t \geq 0$ and with mutual distance $h \geq t$. Typically, $h$ will be a multiple $k$ of $t$ $(k \geq 1)$; for instance, when a spatial block containing particles is serially sectioned and every k'th section is analysed. A particle is sampled if some part of it is contained in one of the sections (reference section) but not in the other section (look-up section), cf. Fig. 1. If the particles are actually spherical, it will be particularly easy to identify particles present in both sections since the positions of the centers of the circular profiles are identical on the two sections.

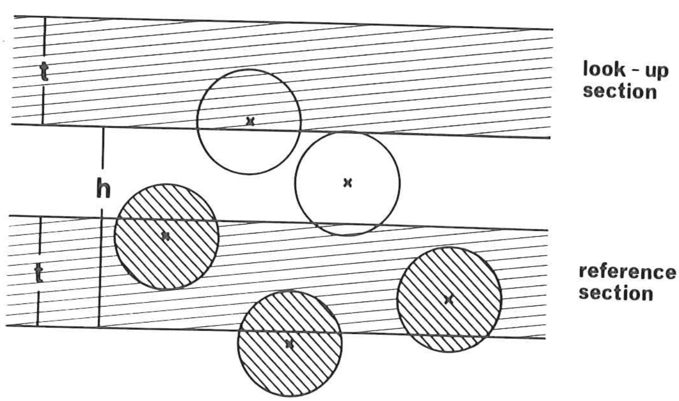



Fig. 1. A disector consisting of two parallel sections. Particles hitting the reference section but not the look-up section are sampled (hatched particles).

It is assumed that the height of any particle is larger than $h-t$, such that any particle can hit both sections simultaneously. In a large number of practical situations, two consecutive sections of thickness $t$ are used, wherefore $h = t$. This procedure has the advantage that no assumptions are necessary regarding the minimal particle height.

With uniform random position (but arbitrary orientation) of the disector, it is easy to show that each particle is sampled with a constant probability, cf. Sterio (1984). This is also true, if sphere caps with a height below a given limit $h(cap)$, say, cannot be observed.

An alternative sampling procedure would be to sample all particles hit by both sections. This would result in a sample of particles from a weighted version of the diameter distribution

$$\frac{x-h+t-2h(cap)}{\mu-h+t-2h(cap)}\, dF(x) \qquad (2)$$

where $\mu$ is the mean diameter calculated in the distribution $F$. Apart from the basic fact that the sampling is no longer from $F$ directly, this procedure would require knowledge of $h(cap)$.

## 3. MEASUREMENTS FROM TWO PARALLEL SECTIONS

The size of a perfect sphere is uniquely determined by the information provided by two parallel sections through the sphere. Thus, as shown below, the diameter $x$ of each sampled sphere is a simple function of the diameters $y_1$ and $y_2$ observed on two parallel sections with known distance $h$. The distance between the two sections may depend on the size of the sampled sphere and does not need to be equal to the distance between the look-up and reference sections in the disector. One of the sections used in the diameter determination will typically be the reference section of the disector.

If $t = 0$, the two diameters $y_1$ and $y_2$ can be expressed as

$$[x^2-(2z\pm h)^2]^{\frac{1}{2}},$$

where $z \geq 0$ is the distance from the sphere center to the 'mid-plane', that is the parallel plane having equal distance to the two planes. From (2), it easily follows that the diameter of the sphere can be calculated as

$$x = \left(h^2 + \left(\frac{y_1+y_2}{2}\right)^2\right)^{\frac{1}{2}} \cdot \left(h^2 + \left(\frac{y_1-y_2}{2}\right)^2\right)^{\frac{1}{2}} /h. \qquad (3)$$

In the general case of possibly positive section thickness, $y_1$ and $y_2$ are diameters of the circular profiles obtained after projection of the sphere fragments contained in the two sections. The sampled sphere can be in one of three positions with respect to the two sections, see Fig. 2: The center is outside the two sections (a), inside one of the sections (b) or in the space between the two sections, if such a space exists (c).

The significant difference between these three cases is the distance $h'$ between the two planes of zero thickness from which the two observed, projected diameters $y_1$ and $y_2$ actually come. As shown in the Appendix, it is possible to develop an algorithm for deciding between the three cases, based on knowledge of $y_1$ and $y_2$, together with the constants $h$ and $t$. The algorithm is also presented in Fig. 2. In case a, (3) is used unmodified, in case b $x = y_1$, simply, and in case c, (3) is used after substitution of $h$ with $h-t$ in the formula.

The results of this section are evidently not affected by the phenomenon of lost caps.

## 4. MEASUREMENTS FROM A SERIES OF PARALLEL SECTIONS

The method described in the preceding section is satisfactory from a mathematical viewpoint but entirely unsatisfactory from a statistical viewpoint. The main reason for this is that the method is heavily dependent on the sphere assumption and therefore not robust at all against deviations from spherical shape. Particles from "the real world" are seldom perfect spheres.

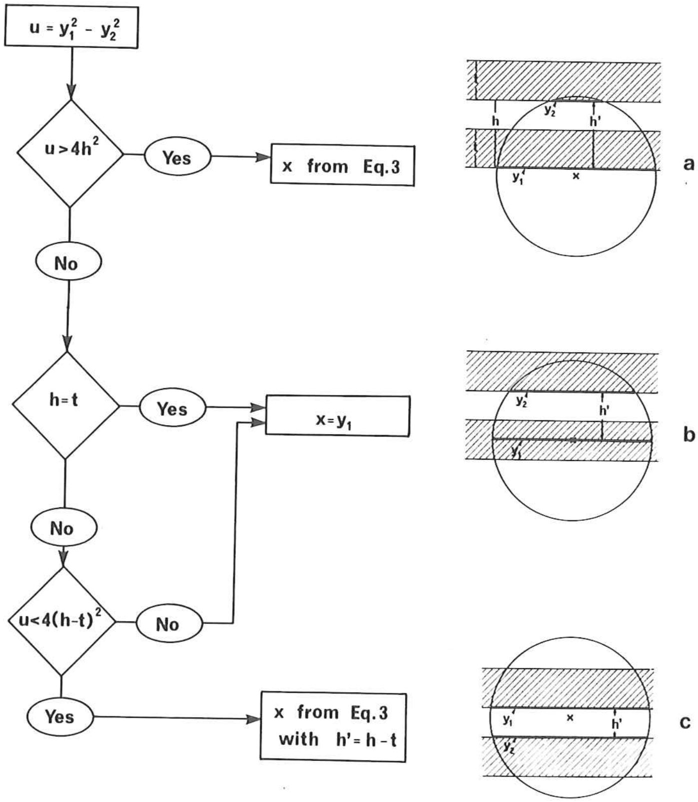



Fig. 2. Algorithm for calculating the diameter $x$ of a sphere from measurements of the diameters $y_1$ and $y_2$ of the circular profiles obtained after projection of the sphere fragments contained in the two sections of distance $h$. To the right, the distance $h'$ between the two section planes of zero thickness, from which $y_1$ and $y_2$ actually come, is shown. The algorithm is derived in the Appendix.

In this section, we discuss robust methods of determining the size of a disector sampled spherical particle from more complete data. The data are the projected areas $a'_1, \ldots a'_m$ measured from $m$ consecutive sections of thickness $t$. More precisely, the particle is exhaustively sectioned by parallel section planes with constant separation $t$, and $a'_i$ is the area of the projection of the sphere fragment contained in the i'th section, $i = 1,...,m$. The set of section planes must be positioned uniform randomly on the particle, i.e. the first section to hit the particle must be uniform random in an interval of length $t$. The number $m$ of sections is thereby usually a random number.

The diameter of the largest projected area is a direct estimator of the sphere diameter. In Gundersen (1986), it is shown for a particle of general shape that $t$ times the sum of the remaining areas is an approximately unbiased estimator of the volume $v$ of the particle. For a spherical particle, this so-called Archimedes-Cavalieri estimator is exactly unbiased, since

$$\hat{v} = t\left(\sum_{i=1}^{m} a'_i - \max(a')\right) = t \sum_{i=1}^{n} a_i, \tag{4}$$

where $a_1,...,a_n$ are the $n = m-1$ areas originating from the $n$ section planes of zero thickness, cf. Fig. 3.

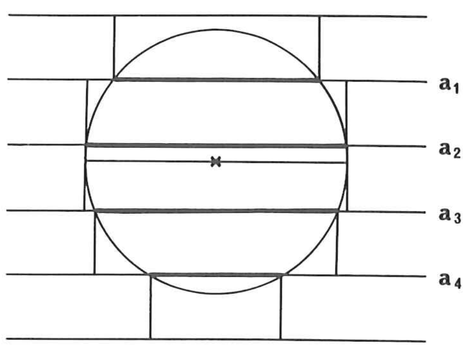



Fig. 3. A sphere sectioned by $n = 4$ section planes. The projected areas are, after removal of the maximal one, in one-to-one correspondence with the areas $a_1$, $a_2$, $a_3$, $a_4$ observed in the section planes.

The extraordinary precision of this type of estimator based on systematic geometric sampling has recently been studied by Gundersen and Jensen (1986), Serra (1986) and L.M. Cruz-Orive, this issue. The variance of $\hat{v}$ is an oscillating function of the average number $\bar{n}$ of section planes hitting the particle. Following the rather deep analytical methods described in Matheron (1965, 1971), an approximation to the variance can be derived using the covariogram of the section area as a function of the position of the section plane. For a sphere, the approximation is

$$CE^2(\hat{v}) \approx \frac{1}{10}\,\bar{n}^{-4}. \qquad (5)$$

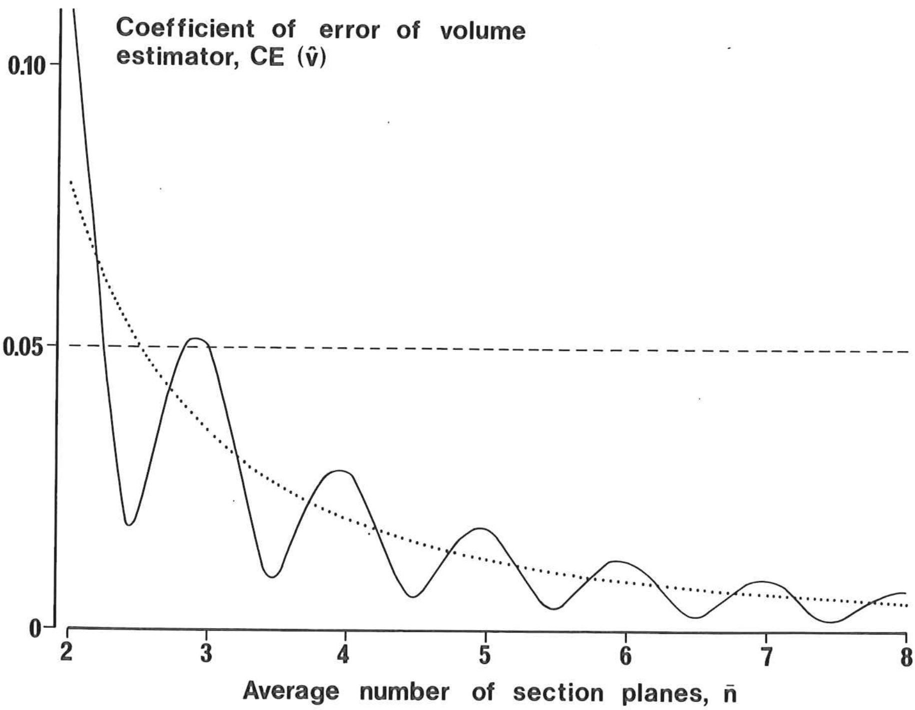



Fig. 4. The coefficient of error of the Archimedes-Cavalieri estimator $\hat{v}$, determined by simulation, is shown for a sphere, as a function of the average number of section planes (fulldrawn). The dotted curve is the approximation. With $\bar{n} \geq 3$, $CE(\hat{v}) \leq 0.05$, as illustrated by the horizontal stippled line.

The exact $CE^2(\hat{v})$ obtained by simulation (Gundersen and Jensen (1986, Section 7)) is shown in Fig. 4 together with the approximation. Notice that $CE^2(\hat{v})$ is of order $1/\bar{n}^4$ compared to order $1/\bar{n}$ for independent and identically distributed observations, reflecting the very high precision of estimators based on systematic sampling. With $n = 3$ or more section planes, $CE(\hat{v}) \leq 0.05$, cf. Fig. 4. It is evidently important that the variation of $\hat{v}$ is very small compared to the variation of volume in the sphere population, otherwise the distribution of estimated volumes of disector sampled particles do not represent the distribution of volume in the sphere population. A coefficient of error of at most 0.05 will, however, in many cases be satisfactory.

If it is not convenient to measure the areas directly, they can be estimated unbiasedly without shape assumptions and with high precision using a point counting method

$$\hat{a}_i = d^2 \cdot P_i, \qquad (6)$$

where $P_i$ is the number of points from a square grid with point density $d^{-2}$, hitting the circular profile from the i'th section, $i = 1,\ldots,n$. It is assumed that one of the grid points is uniform random in a $d \times d$ square. The variance of $\hat{a}_i$ is an oscillating function of the average number of points hitting the particle section with area $a_i$, cf. e.g. Matérn (1985). Following Matheron, an approximation to the variance can be derived. For the resulting estimator

$$\hat{\hat{v}} = t \sum_{i=1}^{n} \hat{a}_i \qquad (7)$$

the result is, cf. Gundersen and Jensen (1986, formula (A42)),

$$CE^2(\hat{\hat{v}}) \approx \frac{0.0569\ td^3 s}{v^2} + CE^2(\hat{v}) \qquad (8)$$

where $s$ is the surface area of the spherical particle. Using the approximation (5) of $CE(\hat{v})$ and recalling that $\bar{n} = x/t$ for a sphere of diameter $x$, we find

$$CE^2(\hat{\hat{v}}) \approx (0.2750\ td^3 + 0.0422\ t^4)v^{-4/3}. \qquad (9)$$

The total number of points counted for a sphere of volume $v$ is on the average

$$\bar{P} = \frac{v}{td^2} . \qquad (10)$$

Parametric approaches are dependent on the spherical shape assumption, but provide more information about the particles than just size. For an ideal sphere of diameter $x$, we have up to measurement error

$$a_i \approx \pi\left[\left(\frac{x}{2}\right)^2 - \left(\frac{x}{2} - (y+(i-1)t)\right)^2\right], \qquad (11)$$

$i = 1,\ldots,n$, where $y$ is the distance from the lower extreme point of the sphere to the first section plane. Regarding $x$ and $y$ as unknown parameters the relation (11) can be used to determine estimates of these parameters by some (weighted) non-linear regression method. The number $n$ of section planes should be so high that the variance of the estimate of $x$ (and $y$) is very small compared to the variation in the sphere size distribution. Note that $y$ is the extra information needed for determining the center of the sphere.

The actual number of section planes $n$ times the section thickness $t$ is an extra, unbiased estimator of $x$.

## 5. VERY SMALL SPHERES

There are a number of cases where the sphere diameters are smaller than the thickness of even the thinnest section which can be cut. Taking three consecutive sections, using the middle one as the reference section and the other two as look-up sections, diameters of all spheres having their largest (or only) diameters in the reference section constitute a uniform random sample of sphere diameters, cf. Fig. 5. Equivalently, spheres are sampled, if their centers are in the reference section of known thickness $t$ and the sample thereby also provides a direct estimate of particle number. Note that the validity of this sampling procedure is not affected by lost caps.

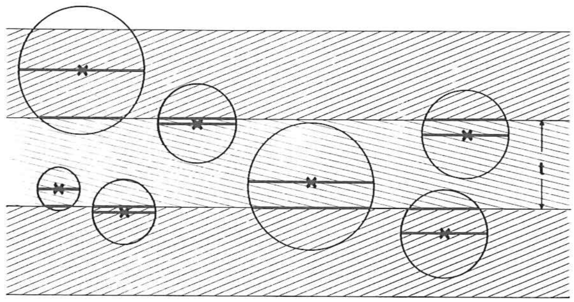
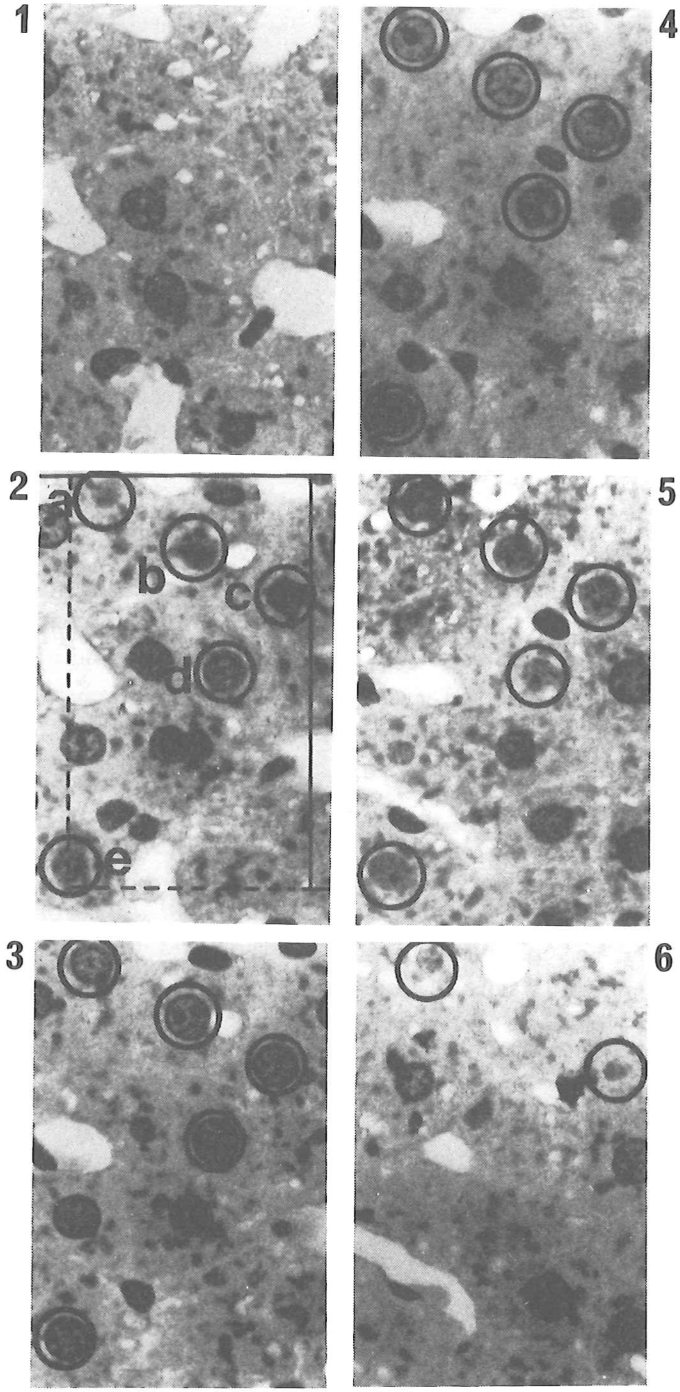



Fig. 5. Sampling of spheres using three sections. All spheres having their largest (or only) diameters in the middle section are sampled.

There will most likely be an observational problem in knowing precisely whether the diameter of a sphere in the reference section is actually larger than that in a neighbouring section, see Fig. 5. The problem is very easily solved by sampling only such spheres where the ambiguity is present between the reference section and the upper look-up section. Spheres which have their seemingly equally large diameters in the reference section and the lower look-up section are ignored.

## 6. EXAMPLE

In order to illustrate how the discussed techniques may look like in a real case, a series of micrographs of 2 μm thick methylmetacrylate sections of liver tissue from an adult Wistar rat have been analysed, cf. Fig. 6. The problem was to estimate the size of hepatocyte nuclei which are very close to spherical shape. The disector used in the sampling consisted of two consecutive sections. The circular profiles of a sampled nucleus in the reference section and all following sections were identified. The diameter of each profile was determined directly using class-intervals of length $\Delta$ = 0.59 μm and the area was estimated by


1
4
2
a
b
c
d
e
5
3
6

point counting. The distance between vertical or horizontal neighbouring grid points was chosen to be $d = 2.35$ µm. The expected standard deviation of the volume estimate $\hat{\hat{v}}$ µm$^3$ is thereby, cf. (9),

$$SD(\hat{\hat{v}}) \approx 2.80 \ \mu m^2 \cdot v^{1/3} \ \mu m.$$

The estimate of the sphere diameter $\hat{\hat{x}} = (6\ \hat{\hat{v}}/\pi)^{1/3}$ has then an approximate standard deviation of

$$SD(\hat{\hat{x}}) \approx 1.44 \ \mu m^2 \cdot x^{-1} \ \mu m^{-1}$$

For spherical particles of diameter $x = 6$ µm and 10 µm, say, the expected standard deviation is 0.24 µm and 0.14 µm, respectively. The diameter estimates obtained by this point counting design are, as we shall see, just as precise as those obtained by directly classifying the maximal diameters in intervals of length $\Delta = 0.59$ µm which yields an SD of 0.17 µm for all diameters.

If each observed circular profile is the projection of the total sphere fragment contained in the section (overprojection, as discussed in the previous sections), then both the maximal diameter $x_{max}$ observed in the total series of $m$ sections <u>and</u> $(m-1)\cdot t$ are unbiased estimates of the sphere diameter $x$. For the liver data, $(m-1)\cdot t$ was systematically smaller than $x_{max}$, so overprojection is not a satisfactory description of these data. An alternative is that each observed circular profile is the projection of the part of the sphere which is

Fig. 6. Part of the series of micrographs of 2 µm thick sections. The micrographs were analysed at magnification 850 which is identical to the one shown here. The first and second sections are the look-up and reference sections of the disector, respectively. Within the unbiased sampling frame used in the reference section (Gundersen, 1977), a total of 5 hepatocyte nuclei, a-e, was sampled and identified in the following sections as indicated. The nuclei a, b, c, d, e can be found in Table 1 as Nos. 13, 3, 5, 6, 4, respectively. At the magnification shown, the distance between vertical or horizontal grid points used in the point counting was 2 mm.

present in the whole section (underprojection, the spheres are 'cheese holes'). Then, $(m+1)\cdot t$ is an unbiased estimate of the diameter $x$, but this estimate turned out to overestimate the diameter. An intermediate description, where the observed circular profile originates from the mid-plane of the section, was satisfactory. Curiously enough, this corresponds to observing from $m$ sections of zero thickness.

The results are shown in Table 1 for the 18 sampled nuclei. For each nucleus, $x_{max}$ is shown together with the diameter estimate $\hat{x}$, calculated from $\hat{v} = t\Sigma a_i$, cf. (4). The point counting was performed twice, resulting in two realizations of $\hat{\hat{x}}$, which are also shown in Table 1. The number of points counted was approximately 25 and 50 for small and large spheres, respectively. The diameter was also estimated using (3) and the two diameters $y_1$ and $y_2$ measured in the reference section and in the section with distance $h = 4$ µm from the reference section. Apart from a few exceptions the different procedures give very similar results, as expected for spherical particles.

## 7. DISCUSSION

The method of estimating sphere sizes from data in two sections given in Section 3 is heavily dependent on the sphere shape assumption. A more robust alternative would be to use the fact that an arbitrary ellipsoid (sphere, oblate, prolate or triaxial) is uniquely determined by the ellipses seen in three parallel section planes of known separation, cf. Møller (1986). The parametric method in Section 4 is also dependent on roughly spherical particles. This method provides, however, in addition estimates of the positions of the sphere centers whereby it is possible to study directly the point process of sphere centers in 3-dimensional space. This is the topic of a forthcoming report.

In addition to these shape specific procedures, stereological methods of estimating particle number, mean sizes and size distributions are now available (Cruz-Orive, 1980; Sterio, 1984; Gundersen, 1986) without shape assumptions. Apart from estimates

| Nucleus | $x_{max}$ | $\hat{x}$ | $\hat{\hat{x}}$ | | x(Eq.3) |
|---|---|---|---|---|---|
| 1 | 9.4 | 9.8 | 9.5 | 10.0 | 10.0 |
| 2 | 7.6 | 7.7 | 8.0 | 7.9 | 7.9 |
| 3 | 10.0 | 10.2 | 10.4 | 10.2 | 10.3 |
| 4 | 9.7 | 9.6 | 9.9 | 10.0 | 9.5 |
| 5 | 10.0 | 10.3 | 10.4 | 10.3 | 10.1 |
| 6 | 10.3 | 10.0 | 10.0 | 10.0 | 10.2 |
| 7 | 7.9 | 7.9 | 8.0 | 7.6 | 8.1 |
| 8 | 9.4 | 9.8 | 10.0 | 10.3 | 9.5 |
| 9 | 10.6 | 10.0 | 10.0 | 10.1 | 10.6 |
| 10 | 7.9 | 7.9 | 7.9 | 7.6 | 8.1 |
| 11 | 9.7 | 10.1 | 10.0 | 10.0 | 9.7 |
| 12 | 6.5 | 6.6 | 6.5 | 6.3 | 6.8 |
| 13 | 10.0 | 10.1 | 10.1 | 10.1 | 10.0 |
| 14 | 9.7 | 9.9 | 10.0 | 9.9 | 9.7 |
| 15 | 10.0 | 10.1 | 10.2 | 10.4 | 9.4 |
| 16 | 10.0 | 10.3 | 10.5 | 10.1 | 8.8 |
| 17 | 8.5 | 7.7 | 7.6 | 7.9 | 7.8 |
| 18 | 8.2 | 8.5 | 8.5 | 8.5 | 8.2 |

Table 1. The diameter estimates of the 18 sampled hepatocyte nuclei: $x_{max}$ is the maximal observed diameter, $\hat{x}$ and $\hat{\hat{x}}$ are diameter estimates calculated from the volume estimates $\hat{v}$ and $\hat{\hat{v}}$ given in (4) and (7), respectively, and x(Eq.3) is the diameter estimate obtained using (3). For more details, see the text.

of usual mean size parameters, an estimate of the volume-weighted particle mean volume $\bar{v}_V$ can be determined for arbitrarily shaped particles from just a single random section, using point-sampled, cubed linear intercept lengths, $\bar{l}_0^3$ (Gundersen and Jensen, 1985). The particular weighting in $\bar{v}_V$ is actually a significant advantage in many biological applications (Nielsen et al., 1986; Howard, 1986).

All the applications of the disector principle for number estimation requires that the distance h is known. The disector is, however, also used to obtain a uniform random sample of particles, cf. Section 2, and for that use precise knowledge of h is not necessary. Cruz-Orive (1986) has recently combined this property of the disector with the estimator of particle volume based on point-sampled intercepts to a so-called selector. It is thereby possible to estimate (unweighted) particle mean volume $\bar{v}_N$ without knowing h.

If the aim of a stereological study cannot be fulfilled by estimating particle quantities like particle number, total volume, total surface, and mean sizes, it may be necessary to consider the particle size distribution. The principle of estimating particle volume distribution by disector sampling and accurate determination of volume of sampled particles by the Archimedes-Cavalieri method is valid without shape assumptions. The number of sections through a particle needed for a precise volume determination is however shape dependent. In Gundersen and Jensen (1986), practical methods of determining the precision of this volume estimator are reviewed.

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APPENDIX

The quantity $y_1^2 - y_2^2$ depending on the two measured diameters $y_1 \geq y_2$ is in each of the three cases a, b and c a simple function of the distance $z \geq 0$ from the center of the sphere to the 'mid-plane' with equal distance to the two sections, cf. Fig. 2:

$$y_1^2 - y_2^2 = 8h\left(z - \frac{t}{2}\right), \qquad \text{Case a} \qquad \text{(A1)}$$

$$= 4\left(z + \frac{h-t}{2}\right)^2, \qquad \text{Case b} \qquad \text{(A2)}$$

$$= 8(h-t)z, \qquad \text{Case c} \qquad \text{(A3)}$$

In Case a, the distance $z$ is thereby

$$\frac{(y_1^2 - y_2^2)}{8h} + \frac{t}{2} > \frac{h+t}{2} \qquad \text{(A4)}$$

whereby

$$y_1^2 - y_2^2 > 4h^2. \qquad \text{(A5)}$$

Furthermore, it is easy to see from (A2) and (A3) that (A5) does not hold in Case b or c. It remains to decide between Case b and c, if Case c is actually possible, i.e. $h > t$. In Case c, the distance $z$ is

$$\frac{y_1^2 - y_2^2}{8(h-t)} < \frac{h-t}{2} \qquad \text{(A6)}$$

whereby

$$y_1^2 - y_2^2 < 4(h-t)^2 \qquad \text{(A7)}$$

In Case b, (A7) does not hold, as easily seen from (A2).