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THEORETICAL ESTIMATION OF ERRORS IN EVALUATING SPHERE SIZE DISTRIBUTION AND POPULATION DENSITY DUE TO VARIATIONS IN SECTION THICKNESS

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ABSTRACT

A method is presented for the theoretical estimation of the magnitude of error in the experimental determination of sphere size distribution and population density by trans mission microscopy arising as a consequence of deviations in the thicknesses of the sections used from the putative value,i.e. the thickness which the sections are purported or believed to possess.

If, in an experimental situation the distribution of section thicknesses about the mean value is known, the treatment enables the range of error in determining the sphere size distribution and population density arising from variations in section thickness to be estimated.

INTRODUCTION

In the experimental determination of the size distribution and population density of opaque spheres randomly dispersed throughout a transparent matrix from the frequency distribution of image diameters obtained by transmission microscopy account has necessarily to be taken of section thickness, and an accurate knowledge of section thickness is a prerequisite in an ideal experiment. Nevertheless, in cutting sections it is often difficult to control section thickness with the desired accuracy. The frequency distribution of the thickness of a large batch of sections may, however, usually be determined without difficulty, and it is therefore possible to estimate the probability that the thickness of any field randomly selected from the batch lies within a specified range of some specific value.

Since the size distribution and population density is usually estimated on the basis of a putative mean section thickness, it is desirable for the purpose of experimental design and in the interpretation of results to be able to estimate the magnitudes and probabilities of the errors in the size distribution and population density which arise as a consequency of deviations in the section thickness from the putative value.

This paper presents a theoretical basis of estimating these errors using a matrix method for evaluating sphere size distribution (Rose,1980).

GENERAL TREATMENT

It is assumed that the population of spheres of any diameter is a Poisson random variable about a position-invariant mean. It can then be shown (Rose,1980) that

$$n_k = A\Delta R_{km} N_m \tag{1}$$

where n_k is the mean number of projection figures in a field of area A for which the diameters lie between the limits $(k-1)\Delta$ and $k\Delta$, N_m is the mean population density of spheres of diameter $(m - \frac{1}{2})\Delta$, Δ is the frequency distribution class interval, and

$$R_{km} = (m - \tfrac{1}{2}) \left\{ \left[1 - \frac{(k-1)^2}{(m-\frac{1}{2})^2}\right]^{\frac{1}{2}} - \left[1 - \frac{k^2}{(m-\frac{1}{2})^2}\right]^{\frac{1}{2}} \right\}; \quad k < m$$

$$= \frac{T}{\Delta} + (m-\tfrac{1}{2}) \left[1 - \frac{(k-1)^2}{(m-\frac{1}{2})^2}\right]^{\frac{1}{2}} \quad ; \quad k = m$$

$$= 0 \quad ; \quad k > m$$

where T is section thickness.

The sphere size distribution and population density are then

$$N_m = \frac{1}{A\Delta} P_{mk} n_k \tag{2}$$

and

$$N_V = \frac{1}{A\Delta} Q_k n_k \tag{3}$$

respectively, where P_{mk} is derived from R_{km} by matrix inversion, and

$$Q_k = \sum_{m=1}^{M} P_{mk}.$$

Using the bracketed subscripts (a) and (p) to denote terms evaluated on the basis of the actual and the putative section thicknesses respectively, the frequency distribution of image diameters is related to the sphere size distribution by the equation

$$n_m = A\Delta R_{km(a)} N_{m(a)}, \tag{4}$$

whereas evaluating the sphere size distribution from the frequency distribution of image diameter on the basis of a putative section thickness gives

$$N_{j(p)} = \frac{1}{A\Delta} P_{jk(p)} n_k. \tag{5}$$

It follows that

$$N_{j(p)} = P_{jk(p)} R_{km(a)} N_{m(a)}; \tag{6}$$

the coefficients of $N_{m(a)}$ specifying the manner in which the sphere size distribution is artificially distorted by evaluation using a putative section thickness differing from the actual value.

From equation (6) it follows that

$$N_{V(p)} = Q_{k(p)} R_{km(a)} N_{m(a)}. \tag{7}$$

The factor by which each N_m is under- or over-estimated as a consequence of a deviation in section thickness from the putative value is given by the corresponding element of $Q_{k(p)} R_{km(a)}$, i.e.:

$$\frac{N_{V(p)(Nm)}}{N_{m(a)}} = Q_{k(p)} R_{km(a)}. \tag{8}$$

Since it is perhaps more convenient to consider the factors by which $N_{V(p)(Nm)}$ and T_a deviate from $N_{m(a)}$ and T_p respectively, the error factors $E(N_m)$ and $E(T)$ are defined as

$$E(N_m) = \frac{N_{V(p)(Nm)} - N_{m(a)}}{N_{m(a)}}$$

and

$$E(T) = \frac{T_a - T_p}{T_p}.$$

Hence,

$$E(N_m) = Q_{k(p)} R_{km(a)} - 1. \tag{9}$$

The manner in which $E(N_m)$ varies over a range of putative section thicknesses often encountered in morphometric experiments is illustrated for various values of E(T) in Figure 1.

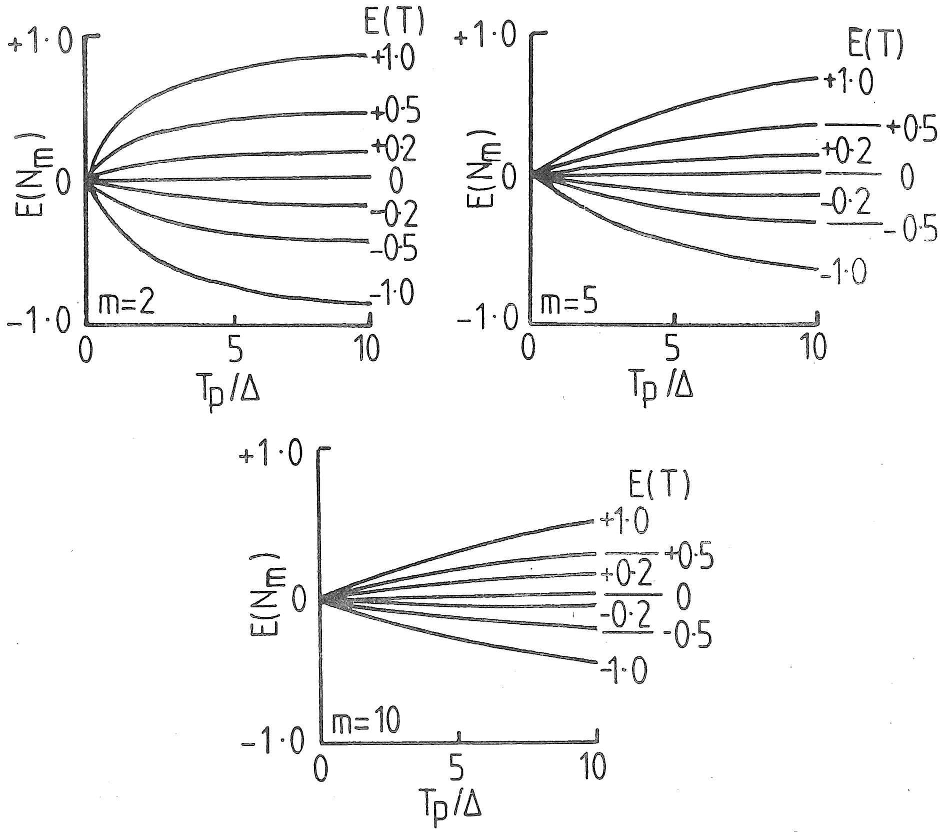

Figure 1. Variation of $E(N_m)$ with Tp/Δ for selected m and E(T)

To determine the morphometrically important limiting case in which Ta/Δ and Tp/Δ approximate to infinity, we note that

$$\mathop{Lt}_{T/\Delta\to\infty} R_{km} = \frac{T}{\Delta}\delta_{km}, \tag{10}$$

whence it can be shown that

$$\mathop{Lt}_{T/\Delta\to\infty} N_{j(p)} = \frac{Ta}{Tp}\delta_{jm} N_{m(a)}, \tag{11}$$

and therefore

$$\mathop{Lt}_{T/\Delta\to\infty} E(N_m) = E(T). \tag{12}$$

CONCLUSION

If T_l and T_u are the lower and upper limits of a range of section thicknesses about T_p, the limits between which $N_{m(a)}$, $N_{V(a)}$ and $E(N_m)$ lie are

$$N_{m(l)} = P_{mk(l)}n_k$$
$$= P_{mk(l)}R_{kj(p)}N_{j(p)} \quad (13a)$$

$$N_{m(u)} = P_{mk(u)}n_k$$
$$= P_{mk(u)}R_{kj(p)}N_{j(p)}, \quad (13b)$$

$$N_{V(l)} = Q_{k(l)}n_k$$
$$= Q_{k(l)}R_{kj(p)}N_{j(p)} \quad (14a)$$

$$N_{V(u)} = Q_{k(u)}n_k$$
$$= Q_{k(u)}R_{kj(p)}N_{j(p)}, \quad (14b)$$

and

$$E(N_m)_{(l)} = Q_{k(p)}R_{km(l)} - 1 \quad (15a)$$
$$E(N_m)_{(u)} = Q_{k(p)}R_{km(u)} - 1. \quad (15b)$$

It follows that if $Pr\{T_a : T_l] T_a [T_u\}$ is the probability that the thickness of a randomly selected section lies between T_l and T_u,

$$Pr\{N_{m(a)} : N_{m(l)}] N_{m(a)} [N_{m(u)}\} = Pr\{T_a : T_l] Ta [Tu\} \quad (16)$$

$$Pr\{N_{V(a)} : N_{V(l)}] N_{V(a)} [N_{V(u)}\} = Pr\{T_a : T_l] T_a [T_u\} \quad (17)$$

and,

$$Pr\{E(N_m) : E(N_m)_{(l)}] E(N_m) [E(N_m)_{(u)}\}$$
$$= Pr\{T_a : T_l] T_a [T_u\} \quad (18)$$

The treatment presented in this paper therefore provides a model for the estimation of limits between which the size distribution, population density and error factors lie in a given experimental situation. Moreover, by considering the limits of a range of section thicknesses about the putative value and the probability that a section thickness falls within these limits, the treatment may be used to determine the probability that the sphere size distribution, population density and associated error factors lie within certain bounds defined by the range of section thicknesses used.

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REFERENCES

Rose PE. Improved tables for the evaluation of sphere size distributions including the effect of section thickness. J Microsc 1980;118:135-41.