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# AUTOMATIC DETECTION OF SPHERE DISTRIBUTION IN THICK SECTION

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## ABSTRACT

The size distribution of somatotroph secretory granules on electronmicrographes of rat adenohypophysis are automatically detected by two methods; 1) 'Erosion-chord method' and 2) 'distance-maximum method'. In the later method, the diameter of each disc of the granules is automatically detected in a way which depends upon Rosenfeld´s distance function. In the former method, the Serra´s erosion is employed prior to the detection of the chord length to separate the profile of the granules on the electron micrographs. The two results are compared with the results using the manual methods. In these anlyses, the thickness of the section is stereologically introduced.

## INTRODUCTION

The size distribution of spherical particles can be determined instrumentally from the chords of disk profiles using Specter´s equation. This is simpler to instrument than methods based on diameter detection, however, Spector´s equation has not been extended to correct for section thickness. In this paper the authors´ corrected form of the equation is utilized to obtain size distributions of sectretory granules of rat hypophysis from chords of disk profiles separated by Serra's erosion technique. The results of this approach are compared with those obtained from a purely manual method and an instrumented one in which Rosenfeld's distance function was used for the automatic determination of the radii of sliced granules.

## MATERIALS AND METHODS

Specimens of adenohypophysis of Kyoto-Wister rat were fixed in 2.5% glutaraldehyde and then in osmuim tetraoxide, embedded in Epon 812, ultrathin sections were prepared using a LKB Ultratome, and stained with uranyl acetate and lead citrate according to the manner of Pease (1964). The electron micrographs were taken by JEM-100 CX electron microscope (JEOL, Tokyo). The two employed procedures for automatic analysis and the procedure for purely manual method are described below.

### 1. Computer Method:

For the two computer analyses, eleven photographs which were printed at a final magnification of 7,000 were used. The pictorial information in 5x5cm photograph was transformed into a digital matrix composed of 512x512 pixel and fed into a minicomputer (PDP-11-40/DEC) having 64K bytes of core memory using a mechanical picture reader (JCR-80/JEOL,Tokyo) and an analog-to-digital converter. This process translates the pictorial information into digital information of 256 gray levels. The pixel spacing corresponds to 13nm in the original material. A 5-gray-level representaion of the digitized pictures in the computer can be visually exhibited on a Versatec dot printer. More than one thousand disks of the sectioned hormone granules were detected by following two methods; 1) the erosion-chord method and 2) the distance-maximum method. Further details on these approaches are provided by Baba et al. (1980). The thickness of the ultrathin section (t) was assumed to be 50nm in this study.

**1.1 Erosion-chord method:**

The "black or white" pictures which had been stored on the magnetic disk were also processed by the erosion-chord method after Baba et al. (1980). The "black or white" picture were eroded twice by a cruciform pictorial operator composed of a plus-sign-like-arrangement of 5 pixels of which the center was assigned as the characteristic point according to Serra's (1969) algorithm. The eroded picture was scanned in core memory of the computer and the lengths of the chords crossing the eroded disks of the sectioned granules determined.

After correcting the distribution of the chord length for the shrinkage of the granular profile due to the erosion procedure, the distribution of the chord length (f(li)) was transformed into the three distribution of spherical size of the granules (F(Dj)) using the following equation.

$$F(D_j) = [\frac{\pi \Delta l^2}{4}[\Lambda_{ij}] + t\,\Delta l\,[\Phi_{ij}]]^{-1} f(l_i) \quad \ldots. (1)$$

where, $\Lambda_{ij}$ is $(2i-1)$ provided than $(i \leq j)$; $\Delta l$ is a class interval of the chord length; f(li) is the linear frequency of the chord length of the ith class; $\Phi_{ij}$ is $\sqrt{((j-1/2)^2-(i-1)^2)}-\sqrt{((j-1/2)^2-(i)^2)}$. The notation $[X]$ expresses the inverse matrix of [X]. When t=0, equation 1 becomes the equation given by Spector (1950). Equation 1 can be estimated by extention of the Sector's (1950) approach according to Figure 1.

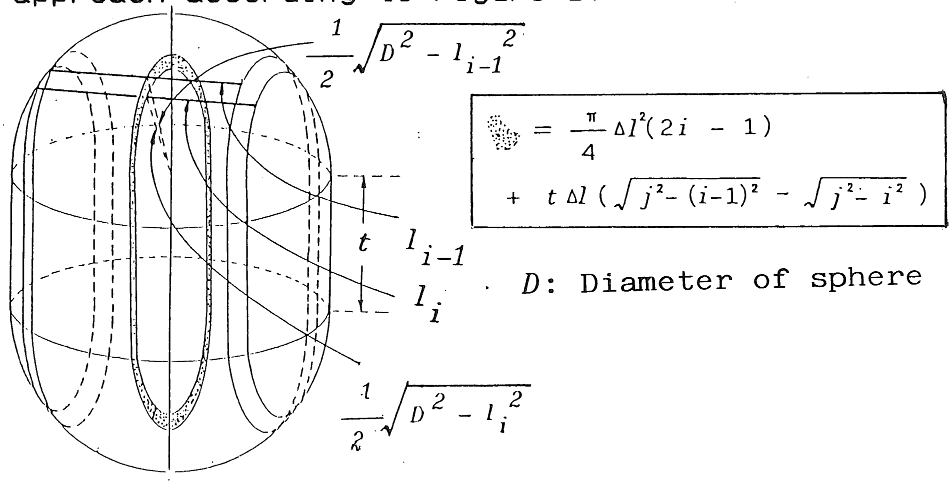


Fig. 1. Pictorial Estimation of Equation 1

**1.2 Distance-maximum method:**

The digitized picture was transformed into a "black or white" picture which was copied onto magnetic disk for later analysis. The black and white picture was converted into a city block distance map according to the method of Rosenfeld et al. (1968). To search the clusters of pixels which were evaluated as local maxima, we designed an algorithm fundamentally based upon the grain-couting algorithm employed by current linear picture analyzers.

From the two dimensional frequency distribution of the values of the local maxima, i.e., tantamount to the radii of the disks representing the sectioned granules, the three dimensional frequency distribution (F(D)) of the spherical diameter of the granules was stereologically computed by the following equation:

$$F(D_j) = [\Delta d[\Phi_{ij}] + t[\delta_{ij}]]^{-1} f(d_i) \quad \ldots . (2)$$

where F(Dj) is the spatial distribution frequancy of the granules belonging to the jth class of the spherical diameter; f(di) is the two-dimensional distribution of the sectioned granules separated into the ith class according to the diameter of their disks; $\Phi_{ij}$ is identical with that of equation 1; $\delta_{ij}$ is Kronecker's delta; $\Delta d$ is a class interval of the diameter of the sliced granule; t is the thickness of the ultrathin section. Equation 2 for t=0 is identical to the equation of Scheil (1935) or Saltikav (1958).

**2. Manual Method:**

Eleven good quality prints of electron mirographs taken at a final magnification of 15,000 were selected for manual determination of the diameter of the sectioned profiles. The detected frequency distribution of the diameter of the profiles (f(d)), is converted to the frequency distribution of the spherical diameter of the granule by equation 2. The influence of the thickness of the ultrathin section on the size distribution of granules is eliminated by the second term of the equation 2.

## RESULTS

Both computerized methods detected numerous small particles or dust. Therefore granules smaller than 100nm were omited from these data. The results obtained using the three different methods are displayed in Figure 2. It is apperent that within the expected data fluctuation. The three methods gave the same frequncy distribution.

Ten minutes were required to digitized and store a binary picture. In the subsequent processing distance mapping requred 5 minutes but erosion followed by chord length measurements was required only 1 minute to process one picture.

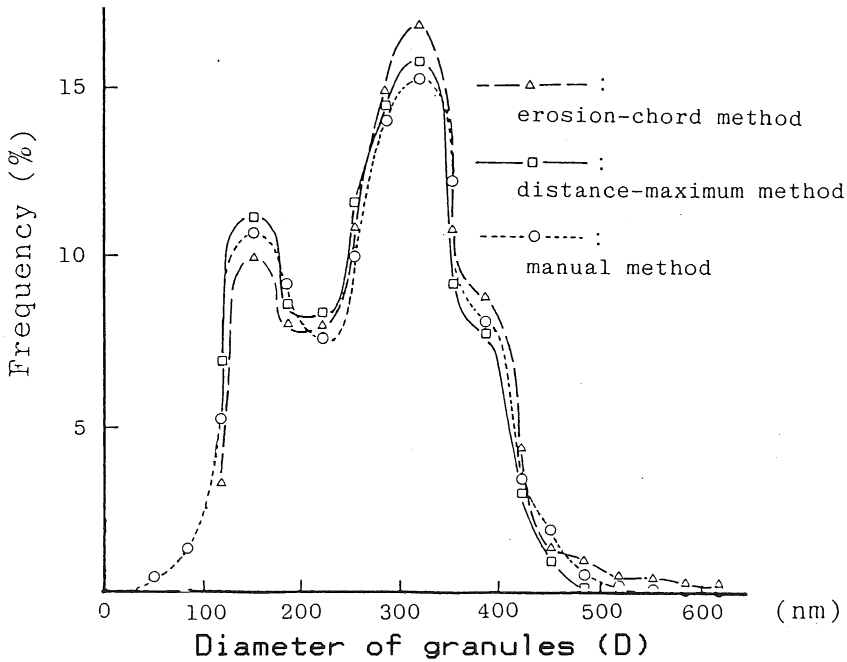


Fig. 2. An example of the distribution of the size of the spherical granule diameter (F(D)).

## DISCUSSION

Equation 2 makes possible information recovery appropriate to the detection of the spatial distribution, both by classical manual means as well as by the distance-maximum method. The distance-maximum method proves faster than manual means.

The equation Spector (1950) and Cahn and Fullman (1956) are useful in machine data processing but are limited to very thin sections. The present thickness correction has improved this situation.

The conversion matrix of the second term (thickness term) of equation 1 (one dimensional chord equation) is identical with the conversion matrix of the main term of equation 2 (one dimensional diameter equation). This cross relationship between the both equation in the thickness term and the main term is conserved when the detection quantity becomes two or three dimensional (Miyamoto & Baba, 1983).

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