# A Stereological Method of Granulometry

In the domain of pathology, we often face spherical bodies of various size dispersed in the organ. Though a classic subject of stereology, the quantitative treatment of particles has been a matter of extreme difficulty, and the estimation of sphere number in a unit volume $N_V$ (the numerical density) still remains a hardest problem of biometry. In the above, Langerhans islets and metastatic nodules in the liver (Chapter 2) and cirrhotic nodules (Chapter 8) were treated applying the stereological method developed by Suwa *et al*. (1976). In the following, the algorithm of this method is to be outlined, taking the study of pancreatic islets as an example.

**Fig. A-1 Dispersed spheres and chord length measurement** (Chapter 2, p. 32)
**Fig. A-2 $N(r)$, the distribution of sphere radius** (Chapter 2, p. 39)

We consider a space in which spheres of different radius $r$ are dispersed as in Fig. A-1. Let the number of spheres in a unit volume (the numerical density) be denoted as $N_V$. Besides, we assume that the sphere radius $r$ follows a distribution function $N(r)$. If the number of spheres in a unit volume with radius between $r_1$ and $r_2$ is denoted as $N_V(r_1, r_2)$,

$$N_V(r_1, r_2) = \int_{r_1}^{r_2} N(r)dr$$

as shown in Fig. A-2.

Any function may be assumed, so long as its integration from 0 to infinity is definite and equal to $N_V$. In this study of islets, Weibull distribution was employed, but logarithmic normal distribution and gamma distribution may also be useful. The Weibull distribution is written as

$$N(r) = N_V m\alpha(\alpha r)^{m-1} \cdot \exp\left[-(\alpha r)^m\right].$$

The advantage of employing this function has been described in Chapter 2 (see Fig. 2-7).

The analysis starts with sampling of islets on microscopic sections, where we employed chord length measurement by line sampling. As shown in Fig. A-1, suppose that on a section we randomly draw a test line, which in the figure is being drawn on the frontal face of the cube. Spheres emerge in the face as circles of various dimen-

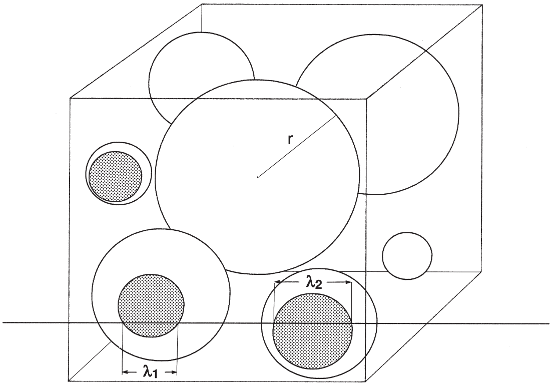


Fig. A-1. The geometric model of dispersed spheres with varying radius $r$. Measurement of chord length $\lambda$ generated by a sampling line randomly drawn on a section.

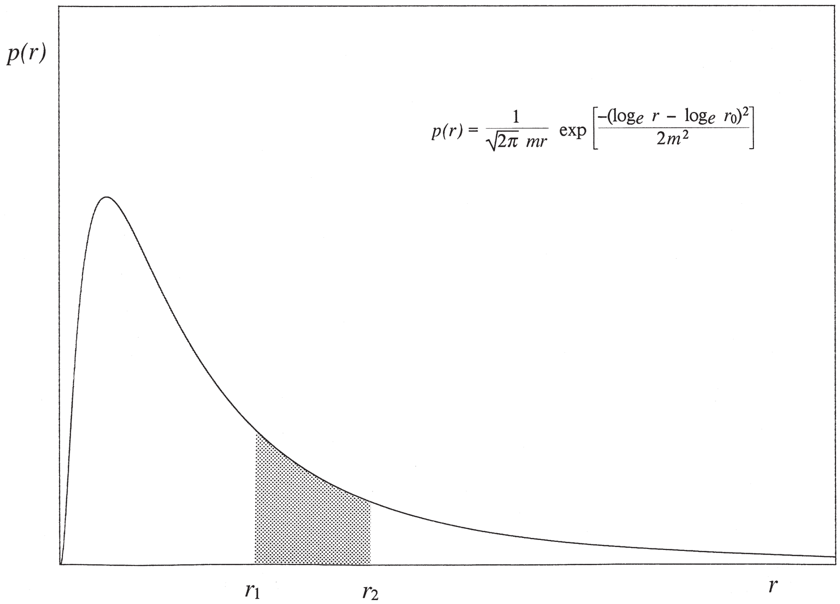


Fig. A-2. $N(r)$, the distribution of sphere radius. The total area below the curve and delimited by $r$-axis corresponds to $N_V$, the number of spheres in a unit volume (numerical density). The number, in a unit volume, of spheres with radius ranging from $r_1$ to $r_2$ is given by the area hatched in the figure.

sion, and if the line is sufficiently long, it comes to intersect a series of circles and at each intersection, generates a chord of length $\lambda$. By measuring the length $\lambda$ for about 200 chords consecutively, we obtain its sample distribution. The problem is how to estimate the distribution of sphere radius $r$ and its parameters (mean, variance, etc.) from this.

**Fig. A-3. Hitting of a single sphere**

Suppose that a single sphere, $r$ in radius, is randomly hit and penetrated by a test line as in Fig. A-3 left. We assume that with this, a chord of $\lambda$ in length is generated at a distance $x$ from the sphere center. Here let us try to find the probability that at a trial of penetration, a chord with length between $\lambda$ and $\lambda + d\lambda$ is generated. If the probability is $dP\ (\lambda, \lambda + d\lambda)$,

$$dP(\lambda, \lambda + d\lambda) = \frac{2\pi x}{\pi r^2} dx. \tag{A1}$$

This would be understood by referring to the schema in the right part of the figure, which expresses the equatorial plane of the sphere from a viewpoint along the penetrating line. The sampling line can hit evenly any corner of this circular plane, and a chord of length $(\lambda\ , \lambda + d\lambda)$ is generated when the narrow concentric belt, $dx$ in breadth, is hit by the line. Therefore $dP\ (\lambda, \lambda + d\lambda)$ corresponds to the area of the belt divided by the total area of the equatorial plane, as in the above.

On the other hand

$$\frac{\lambda^2}{4} + x^2 = r^2. \tag{A2}$$

By differentiating (A2)

$$\frac{\lambda}{2} d\lambda = -2x dx. \tag{A3}$$

From (A1) and (A3) we obtain

$$dP(\lambda, \lambda + d\lambda) = \frac{\lambda}{2r^2} d\lambda. \tag{A4}$$

by discarding the negative sign.

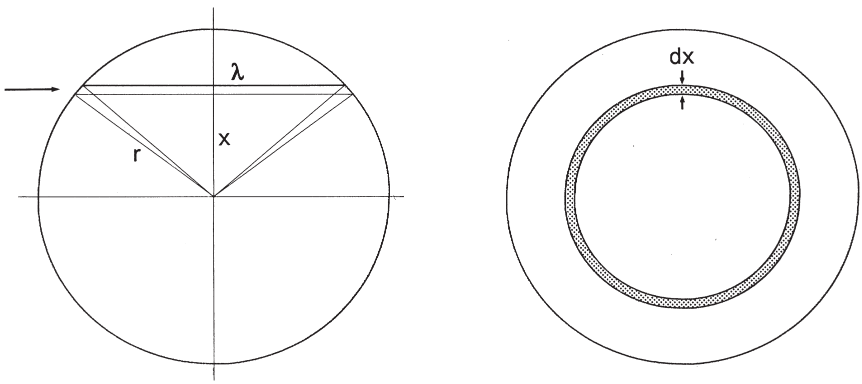


Fig. A-3. (Left) Hitting of a single sphere with a sampling line. (Right) The probability $P(\lambda, \lambda + \mathrm{d}\lambda)$ that the line generates a chord ranging $[\lambda, \lambda + d\lambda]$ is given by the ratio of the hatched area to the whole circle.

**Fig. A-4. Sampling of spheres by penetrating the space with a line**

Now let us extend the discussion to a space in which a number of spheres are dispersed. Suppose a cubic space as in Fig. A-4, where a cube with edges of unit length contains a number of spheres with different radius $r$. Of course we assume that $r$ follows $N(r)$. The number of spheres contained in the cube is $N_V$ because the cube is of a unit volume.

Now we consider that the cube is penetrated by a random test line which is parallel to one of the edges. A number of spheres would be hit by the line as shown in the figure. In this situation, we try to find the expected number $dN(r, r + dr)$ of spheres that are hit by the line and are between $r$ and $r + dr$ in radius. If the number of spheres between $r$ and $r + dr$ contained in the cube is expressed as $dN_V(r, r + dr)$,

$$dN_V(r, r + dr) = N(r)dr.$$

For a sphere $r$ in radius, the area of the equatorial plane is $\pi r^2$, which therefore means that the probability that the sphere is hit by the line is $\pi r^2$ (note that the area of the side of the cube is 1). Accordingly,

$$dN(r, r + dr) = \pi r^2 N(r)dr. \tag{A5}$$

A chord of $\lambda$ can emerge from spheres of various $r$, and a sphere of $r$ can generate various length of chords. Therefore in the next place, we try to define the expected number of chords between $\lambda$ and $\lambda + d\lambda$ in length that are generated from the spheres between $r$ and $r + dr$ in radius. If the expected number of such chords is $dF'[(\lambda, \lambda + d\lambda) \mid (r, r + dr)]$, it is obtained by multiplying $dN(r, r + dr)$ and $dP(\lambda, \lambda + d\lambda)$. Hence, from (A4) and (A5),

$$dF'\left[(\lambda, \lambda + d\lambda) \mid (r, r + dr)\right] = \frac{\pi}{2} \lambda N(r)\ dr d\lambda. \tag{A6}$$

A chord $\lambda$ in length can arise from spheres of any radius equal to or larger than $\lambda/2$.

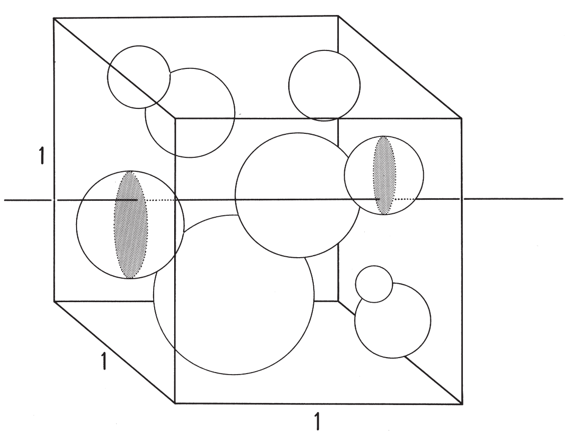


Fig. A-4. Sampling of spheres by penetrating the space with a sampling line. For explanation see the text.

Accordingly, we obtain the total number of chords $dF(\lambda, \lambda + d\lambda)$ that are generated by line sampling if we integrate (A6) with regard to $r$ from $\lambda/2$ to infinity, as

$$dF(\lambda, \lambda + d\lambda) = \left[\frac{\pi}{2}\int_{\frac{\lambda}{2}}^{\infty} \lambda N(r)dr\right]d\lambda.$$

One can find in this equation that $dF(\lambda, \lambda + d\lambda)$ is expressed as a product of $d\lambda$. Thus we obtain

$$F(\lambda) = \frac{\pi}{2}\int_{\frac{\lambda}{2}}^{\infty} \lambda N(r)dr \tag{A7}$$

which relates the distribution function of sphere radius $N(r)$ with that of chord length $F(\lambda)$. Based on this, now we can calculate the parameters of $N(r)$.

We define $I_n(\lambda)$, the $n$-th moment of $\lambda$, as

$$I_n(\lambda) = \int_0^{\infty} \lambda^n F(\lambda)d\lambda. \tag{A8}$$

On account of (A7), the equation contains parameters of $N(r)$. Now consider the 0th, 1st and 2nd moments. According to the definition of $I_n(\lambda)$, the 0th moment $I_0(\lambda)$ corresponds to the expected number of chords, the 1st moment $I_1(\lambda)$ to the sum of $\lambda$, and the 2nd moment $I_2(\lambda)$ to the sum of $\lambda^2$, each per a unit length of sampling line. Thus,

$$I_0(\lambda) = \int_0^{\infty} F(\lambda)d\lambda = N(\lambda)_L$$

$$I_1(\lambda) = \int_0^{\infty} \lambda F(\lambda)d\lambda = \Sigma(\lambda)_L$$

$$I_2(\lambda) = \int_0^{\infty} \lambda^2 F(\lambda)d\lambda = \Sigma(\lambda^2)_L.$$

If $N(\lambda)_L$, $\sum(\lambda)_L$, and $\sum(\lambda^2)_L$ are replaced with the corresponding measurement data, we have a set of simultaneous equations.

The equation (A8) can be re-written by replacing $F(\lambda)$ with (A7), as

$$I_n(\lambda) = \frac{\pi}{2}\int_0^{\infty}\int_{\frac{\lambda}{2}}^{\infty} \lambda^{n+1} N(r)drd\lambda. \tag{A9}$$

By solving the above simultaneous equations, the parameters of $N(r)$ can be obtained. Fortunately, if we assume Weibull distribution, the integration in (A9) is analytically soluble as follows.

In (A9), we exchange the sequence of integration taking in account that $r \geqq \lambda/2$.

Thus,

$$I_n(\lambda) = \frac{\pi}{2}\int_0^\infty N(r)dr\int_0^{2r} \lambda^{n+1} d\lambda$$

$$= \frac{2^{n+1}\pi}{n+2}\int_0^\infty r^{n+2} N(r)\,dr.$$

Here we replace $N(r)$ with Weibull distribution function, as

$$I_n(\lambda) = \frac{2^{n+1}\pi}{n+2} N_V \int_0^\infty m\alpha r^{n+2} (\alpha r)^{m-1} \exp\left[-(\alpha r)^m\right] dr. \tag{A10}$$

Make a substitution of

$$(\alpha r)^m = t$$

and on account of

$$r = \frac{t^{1/m}}{\alpha}$$

and

$$dr = \frac{1}{\alpha m} t^{(1-m)/m} \cdot dt,$$

(A10) can be re-written into

$$I_n(\lambda) = \frac{2^{n+1}\pi N_V}{(n+2)\alpha^{n+2}} \int_0^\infty t^{\frac{m+n+2}{m}-1} \cdot e^{-t} dt. \tag{A11}$$

Now we find that in (A11), the integration has been transformed into the form of gamma function:

$$\Gamma(x) = \int_0^\infty t^{x-1} \cdot e^{-t} dt.$$

With this, (A11) is reduced to:

$$I_n(\lambda) = \frac{2^{n+1}\pi N_V}{(n+2)\alpha^{n+2}} \Gamma\left(\frac{m+n+2}{m}\right). \tag{A12}$$

Further, (A12) can be transformed into

$$I_n(\lambda) = \frac{2^{n+1}\pi N_V}{\alpha^{n+2} m} \Gamma\left(\frac{n+2}{m}\right) \tag{A13}$$

on account of the basic property of gamma function,

$$\Gamma(x) = (x-1)\Gamma(x-1).$$

Thus, the above set of simultaneous equations are reduced to

$$I_0(\lambda) = \frac{2\pi N_V}{\alpha^2 m} \Gamma\left(\frac{2}{m}\right) = N(\lambda)_L$$

$$I_1(\lambda) = \frac{4\pi N_V}{\alpha^3 m} \Gamma\left(\frac{3}{m}\right) = \Sigma(\lambda)_L$$

$$I_2(\lambda) = \frac{8\pi N_V}{\alpha^4 m} \Gamma\left(\frac{4}{m}\right) = \Sigma\left(\lambda^2\right)_L.$$

By substituting the measurement data for $N(\lambda)_L$, $\sum(\lambda)_L$ and $\sum(\lambda^2)_L$, respectively, we can calculate the parameter values $N_V$, $\alpha$ and $m$.