Science on Form: Proceedings of the First International Symposium for Science on Form,
General Editor: S. Ishizaka, Editors: Y. Kato, R. Takaki, and J. Toriwaki, 569-577.

Pictorial Demonstration of Stereological Principles

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Keywords: Stereology, Stereological principle, Pictorial demonstration, Notation system

Principle for determination of both surface density from the perimeter density on the test plates, and spatial length density from the numerical density of the profiles on the test plates are pictorially illustrated by the extended interpretation of Buffon's principle. For the former, distribution of the linear elements (needles) is converted to 3-D in both location and orientation. For the latter, furthermore, dimension of the element shape is converted to 2-D ('platelet'). The author emphasizes that the new notation system (Baba: 1981, 1983) is not only helpful to pictorial demonstration but also fit for physically rational expressions of the test module and any stereological quantities systematically.

INTRODUCTION

The Smith-Guttman principle for determination of surface area from the number of intersections on the test line was beautifully derived with pictorial manner alone by Hans Elias in 1971. His demonstration has nicely contributed to the understanding of the principle among biomedical scientists who are not always familiar with polar integration. The principle for determination of that from the perimeter on the test plates has not been demonstrated pictorially. The principle for the determination of the length of 3-D linear structure from the number of that intersection on the test plates also has not been demonstrated in a pictorial manner.

Extending Buffon's principle, the author pictorially demonstrates the above two 3-D stereological principles with the aid of the previously reported notation system (Baba: 1981, 1983), which makes it easy to express 'effective detection modules' and the other stereological quantities systematically, and then to explain stereological principles with clear physical images. The concept, 'effective detection module', should be one of the most fundamental physical quantities in stereology, never-theless the officially recommended current symbolism is not designed to be able to express 'effective detection modules' by one symbol.

In this paper, the systematized notation system employed is explained first and then the two principles mentioned above are pictorially demonstrated. This paper also to describes this study as an example of interdisciplinary efforts and shows that similar efforts have been continued in the field of stereology.

NOTATION SYSTEM FOR CLEAR PHYSICAL IMAGE

When we use special style characters to denote the four stereological unit quantities (*i.e.* total test space volume, total test plate area, total test line length and total test

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point number), it becomes easy to systematically express other stereologically fundamental quantities such as area of 'pixel'. Then we can give clear physical images onto the derivation process of stereological formulae. The notation system contributes also to demonstrate stereological principles in a pictorial manner.

The four Greek characters (Tab. 1) are introduced to denote the four unit quantities in this paper instead of V_T, A_T, L_T and P_T (see Tab. 1), all of which have been used in the official descriptions for definitions of stereological combined symbols by the International Society for Stereology (ISS). To use Greek characters is not the purpose of this paper, but it is a purpose of this paper to exhibit the benefits of introduction of four special typeface characters into stereological interpretations.

One of the greatest benefits of this notation system is that the below-mentioned 'effective detection module' can be denoted by a combined symbol which is synthesized by two of the four Greek charcters according to the synthesizing manner of ISS for combined symbols. It is authorized in the field of natural science to modify or specify the meaning of the letter by subscript character(s) immediately after that; X_Y, A_{ij} *etc.* The notation system declared by ISS is designed to express both an absolute quantity (Tab. 3) by a single letter symbol and a relative quantity (Tab. 4) by one combined symbol as $X_V = X/V_T$. But the notation system is not fit to express 'effective detection module' such as 'effective volume of a testing point' ($V_T \div$

Table 1. Unit Quantities

Symbol	Definition	(ISS)
υ =::	(total) volume of 3D test space	V_T
σ =::	(total) area of testing plates or 2D test field	A_T
λ =::	(total) length of the testing lines (or 1D test line)	L_T
ν =::	(total) number of the testing points	P_T

Table 2. Effective Test Module Symbols* Synthesized by the Basic Rule

Symbol	Explanation of Synthesized Symbol**	ISS*
υ_ν ...	Effective volume of testing point (cell volume)	V_T/P_T
υ_λ ...	Effective cross-section of testing line (hitting cross-section)	V_T/L_T
υ_σ ...	Effective thickness of the testing plate (cutting distance)	V_T/A_T
σ_ν ...	Effective area of testing point (pixel area)	A_T/P_T
σ_λ ...	Effective width of testing line (scanning width)	A_T/L_T
λ_ν ...	Effective length of test pointing (sampling clearance)	L_T/P_T

* : We can not describe each of these essential modules by one combined symbol without a modification of the traditional notation system (Underwood: 1967)

**: The words for each symbol are not to define the meaning the symbol. Essentially the meaning of each symbol is rationally given by the composing two Greek charactes and their combination form.

P_T) by one symbol. The present notation system makes it easy to express 'effective detection modules' by one combined symbol as shown in Tab. 2, since the unit quantities are denoted by each unique character. Another benefit is that other relative quantities can be denoted by the combined symbols analogous to the form of ISS symbolism as depicted in Tab. 4.

Table 3. Examples of Absolute Quantity

Symbol	Definition in Present Paper	ISS
Structure Quantity:		
V	total volume of particle in the test space	V
S	total area of particle surface in the test space	S
	total area of picture on the test plate	A
L	total length of 3-D linear structures in test space	L
	total length of 2-D linear structures on test plate	L
	or total length of 1-D linear structures on test line	L
Detection Quantity:		
s	total area of profiles on the testing plate	A
l	total length of 2-D linear structures on testing plate	L
	or total length of intercepts on testing line	L
n	total number of profiles in testing plate	N
	or total number of intercepts on testing line*	N
p	total number of the sensed points	P

* : The extremely short intercept is identical with "intersection".

Table 4. Examples of Relative Quantity (also called Density or Ratio)

Symbol	Explanation of Synthesized Symbol**	ISS
Structure Quantity		
V_υ	Total volume per unit (test space) volume	V_V
S_υ	Total surface area per unit (test space) volume	S_V
L_υ	Total length per unit (test space) vòlume	L_V
S_σ	Total area of picture per unit (test field) area	S_V
L_σ	Total perimeter of picture per unit (test field) area	L_V
Detection Quantity:		
s_σ	Total area of profiles per unit (testing plate) area	S_A
l_σ	Total perimeter of profiles per unit (testing plate) area	L_A
n_σ	Total number of profiles per unit (testing plate) area	N_A
l_λ	Total length of intercepts per unit (testing line) length	L_A
n_λ	Total number of intercepts per unit (testing line) length*	N_L
p_ν	Total number of sensed points per number of sensing points	P_P

* : The extremely short intercept is identical with "intersection".

**: The words for each symbol are not to define the meaning of the symbol. Essentially the meaning of each symbol is rationally given by the composing two Greek charactes and their combination form.

GENERAL ASPECT OF PICTORIAL ESTIMATION OF SHAPE-INDEPENDENT PRINCIPLES

The shape independent stereological principles can be calssified into six 'dimensionless' principle and four 'dimension-negative' principle, according to the dimension analysis of the principle equation expressed as $L_v = 2n_\sigma$, *i.e.* relative quantity form. The relational constants shown as 2 in the forementioned equation are always 1 in the case of the dimensionless principles and they range between 1 and 2 in the case of dimension-negative equations. The former relations can be rather easily interpreted using the present notation system which gives clear physical concepts. This paper is concerned with the pictorial demonstration of the latter relations.

The principle, $L_\sigma = (\pi/2)n_\lambda$, and the relationship, $S_v = 2n_\lambda$, were pictorially estimated by Sitte (1967) and Elias et al (1971), respectively. The other two equations in shape-independent principles $S_v = (4/\pi)l_\sigma$ and $L_v = 2n_\sigma$ have been shown to be easily demonstrated using pictorial approachs. This paper completes the studies on the pictorial demonstration of the above two principles. Thereby, we may understand all 'dimension-negative' relations for shape-independent stereology with pictorial demonstration after this paper.

PRINCIPLE FOR DETERMINATION OF SURFACE AREA DENSITY

The equation to estimate the surface density from perimeter density of profile on the test plates:

$$S_v = \frac{4}{\pi} l_\sigma \tag{1}$$

where S_v is the total surface area per unit volume; and l_σ is the total perimeter length of the profile per unit area, can be demonstrated in a pictorial manner as follows. The curved surfaces of the orientation-and-location randomly distributed 3-D structures (Fig. 1a) are divided into k 'platelets' which have the same ΔS in area. Each platelet will be oriented to random direction, and the statistical intersection length of the surface with testing plates will not be influenced by locational movement of platelets unless each platelet changes in its orientation. Then, we may move each platelet to fill completely the hypothetical sphere surface of which the area is S. The total length of perimeter of the profile, *i.e.* the total length of the cut edges of the curved surface, can be calculated as follows. The total length is equal to [lateral area of lantern-like sphere of Fig. 1b] ÷ [cutting distance of testing plate]. The lateral view in Fig. 1b will be like that in Fig. 1c. From Fig. 1b and 1c, we can approximate;

$$\pi(d(1) + d(2) + \cdots) = l$$

and

$$(d(1) + d(2) + \cdots)\, v_\sigma = S/4$$

respectively, where $d(i)$ is diameter of the sliced disk at the i-th level testing plate (Fig. 1c). By the elimination of $(d(1)+$

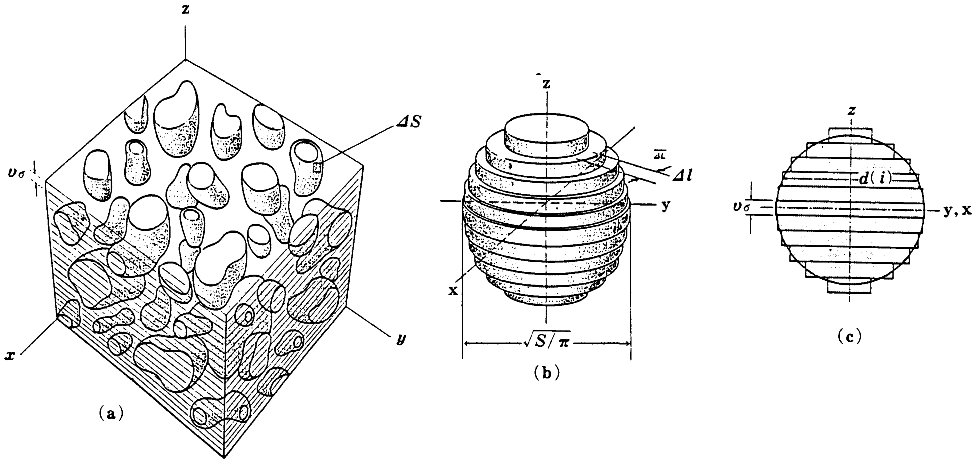

Fig. 1. Model for Pictorial Estimation of Equation 1.

(a): Sketch of test space with testing plates: The profiles behind testing plates are not drawn in (a). Since total area of testing plate σ multiplied by cutting distance is the total volume of the test space v, the cutting distance is given by v_σ. The surface of the granules are area-equally divided into many k platelets of ΔS in area.
(b): Hypothetical sphere after molding: Each platelet ΔS constructs a hypothetical sphere with a diameter of $\sqrt{S/\pi}$ after the translocation without any rotation. The hypothetical sphere is horizontally cut by multiple test plates at equal level distance v_σ. The hypothetical sphere is molded as a step at each level of the testing plate as shown in (b) and (c). The total length of the molded edge corresponds to l.
(c): Lateral view of (b): The lateral view of (b) is useful in estimating the total length of the molded edge shown in (b). The painted area of (c) approximates S/π. [Painted area of (b)] $= \pi \times$ [painted area of (c)]. From this relation, l and then the final equation is obtained.

$d(2)+\cdots$) in the both equations, we obtain $S=(4/\pi)v_\sigma$. When we put $v/\sigma=v_\sigma$, and then $S_v=S/v$ and $l_\sigma=l/\sigma$, we obtain the final equation.

PRINCIPLE FOR DETERMINATION OF SPATIAL LENGTH DENSITY

The equation to estimate the length density of randomly twisted linear structures in a space by the numerical density of the profile on the test plates:

$$L_v = 2n_\sigma \tag{2}$$

where L_v is the total length of the 3-D linear structure per unit volume; and n_σ is the total number of profiles of the linear structure per unit testing plate area, can be derived by a pictorial manner as explained below. The 3-D linear structures are length-equally divided into numerous k needles of which length is ΔL (Fig. 2a). Each needle is oriented randomly. The intersection

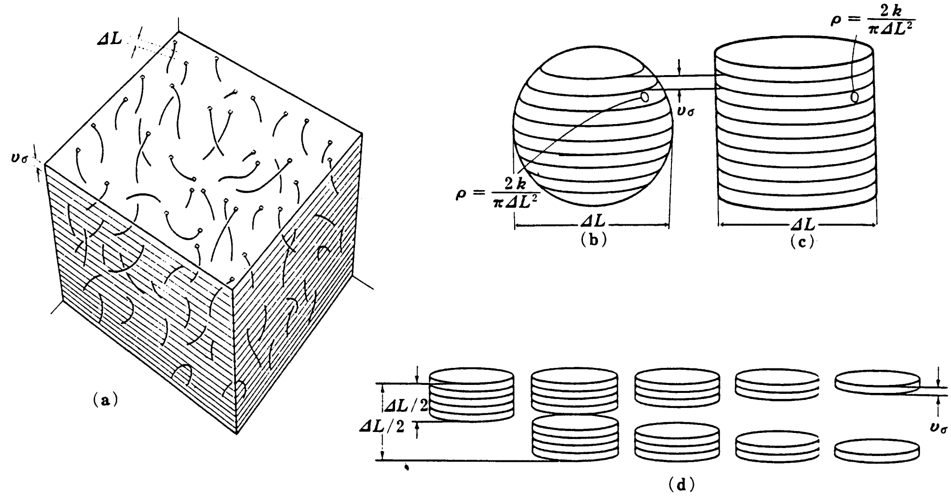

Fig. 2. Mode for Pictorial Estimation of Equation 2.

(a): Sketch of test space with testing plates: The cutting distance by the testing plate is v_σ. The linear structures of L in total length are length-equally divided into numerous k needles of ΔL in length.
(b): Hypothetical sphere: The hypothetical sphere is constructed by an enveloped surface of end edges of the needles which have been translocated to the center of the hypothetical sphere without any rotation. So, the diameter and the surface area of it is ΔL and $\pi(\Delta L)^2$, respectively. The density of the end edges of needles on the sphere surface, ρ, is $2k/(\pi(\Delta L)^2)$.
(c): A circumscribed cylinder of the sphere (b): The sliced lateral surface area of the sphere (b) is equal to that of (c) (the Archimedes' principle).
(d): Calculation diagram: Accumulative sum of the lateral surface area of the sliced cylinder or the sphere is proportional to the number of the inter-section profiles of the needles to the density of end edges on the hypothetical sphere surface, ρ.

probability for each needle with the testing plates is always independent of the translocation, although that probability depends on the change in orientation. Let us imagine, therefore, the translocation of the center of all needles to a defined origin without any rotation. The k needles will construct a pompon like sphere in feature at a certain origin. The envelope surface of the pompon forms a 'hypothetical sphere' of which diameter is equal to the length of the needle, ΔL. The number of the end edges of needles per unit area on the surface of the hypothetical sphere is;

$$[\text{density of edge}] = \frac{2k}{\pi(\Delta L)^2}$$

where $2k$ gives the number of end edges on the hypothetical sphere; and $\pi(\Delta L)^2$ gives the surface area of the hypothetical sphere (see Fig. 2c). Try to horizontally cut the (pompon) hypothetical sphere into a cap and a body at a certain height. The end edge of the needle which is cut at the certain height will be located on the cap produce by the trial cutting at that certain height. As you can understand from the above trial, the number of the inter-section profiles of the needles with the testing plates is proportional to the surface area of the caps, *e.i.* area of the sphere between the testing plate and the nearer pole of the hypothetical sphere. Calculation of the surface area of the cap is very easy, because the sliced lateral surface area of the sphere is equal to that of the circumscribed cylinder (the Archimedes' principle). The lateral area of each sliced sphere is given by;

$$[\text{lateral surface area of a disk}] = \pi \Delta L \cdot \upsilon_\sigma .$$

The double summation of number of the disks between each level and nearer pole and between the two poles can be easily calculated by the aid of Fig. 2c as;

$$[\text{the number of disks}] = \frac{\Delta L}{2\upsilon_\sigma} \times \frac{\Delta L}{2\upsilon_\sigma} .$$

From the sum of the lateral surface areas of the sliced sphere and the density of needles on the sphere, we can obtain the number of the inter-sections with needles;

$$n = [\text{density of end edges of the needles}] \times [\text{lateral surface area of the disks}] \times [\text{number of the disks of Figure 2d}].$$

The final equation can be easily obtained, when we put $L = k\Delta L$, $\upsilon/\sigma = \upsilon_\sigma$, $L_\sigma = L/\sigma$ and $n_\sigma = n/\sigma$, according to the notation rule.

COMMENTS

The present notation system is suitable to describe 'effective detection module'. Not only the concept, 'effective detection module', is very important itself in stereology but it also leads to advanced concepts. For example, effective testing line cross-section (υ_λ) produces an inverse concept, 'testing line density' (λ_υ). The idea of which has been defined by problem after problem in different ways. This concept, λ_υ, is universally essential to study the so-called linear analyses (Baba & Miyamoto: 1980, 1983, 1985: Miyamoto & Baba: 1985).

There are two principles for the determination of surface density from different detection quantities; $S_\upsilon = 2n_\lambda$ and $S_\upsilon = (4/\pi) l_\sigma$. The former principle was pictorially demonstrated by Hans Elias in 1971 for the first time. It may be possible to pictorially demonstrate the latter principle by the combined usage of Elias' demonstration for $S_\upsilon = 2n_\lambda$ and the author's demonstration or modified Sitte's demonstration for $L_\sigma = (\pi/2) n_\lambda$. However, no other study has demonstrated the latter directly in only a pictorial manner, but this study.

All of 'dimensionless' principles irrespective to the shape of the object ($L_\lambda = p_\nu$; $S_\sigma = p_\nu$, $S_\sigma = l_\lambda$; $V_\upsilon = p_\nu$, $V_\upsilon = l_\lambda$, and $V_\upsilon = s_\sigma$) have been pictorially demonstrated with the aid of the author's notation system (Baba: 1981, 1983). So, this paper completed the all studies on pictorial demonstration of shape-independent principles. Author's hypothetical circle (Baba: 1981, 1983) is more competent than Sitte's hypothetical semicircle (1967) to pictorially demonstrate the principle, $L_\sigma = (\pi/2) n_\lambda$, in order to maintain universal connection with the Elias' and the two author's hypothetical spheres mentioned above.

To explain a principle by universal words in a simpler and plainer way should not be set a naught especially in an interdisciplinary science. Stereology esteems the interpretation of principles in a simple way, as well as, the establishment of a new principles. As a result, 'the users of the method' of different fields have correctly and efficiently applied the method to their own problems. Not infrequently they have found and clarified the limitation and some difficulties lying in the method; they have reported the result of the field test of the method to 'makers of method', stereologists, *via* scientific conferences and/or papers; and makers of method have entertain to reply to the users. The science on form functions well as an interdisciplinary science and will display its interdisciplinary outcome. Biomedical scientists expect 'science on form scientists' to make efforts to interpret the subjects difficult for biomedical scientists with universal words and languages, and to entertain problems to be solved in the biomedical fields. Then, a well-functioned interdisciplinary circulation will be established as firmly as it has been among the stereologists and biomedical scientists.

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12-12

Q: I would like to express my appreciation for the work Dr. Baba has been doing in education. It is most anomalous that today in many universities, morphological sciences are taught without any reference to morphometry, stereology, and image analysis. Such education should be associated with all morphology courses, in universities at least. With pleasure I have heard that Prof. Ishizaka includes these aspects in this teaching in Tsukuba. We have also experience in incorporating education on morphometry in pathology courses at the University of Kuopio, Finland. (Collan et al.: Acta Stereol. 1984) (Y. Collan)

A: I am very pleased to know that "symbolism" which I have proposed in 1983 is now widely used. I hope that you would help me to spread this "symbolism" together with our "easy stereology".