

NEW CONCEPTS AND TEST METHODS OF CURVE PROFILE AREA DENSITY IN SURFACE: ESTIMATION OF AREAL DENSITY ON CURVED SPATIAL SURFACE

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ABSTRACT

The concepts of curve profile, curve intercept, curve intercept density, curve profile area density, intersection density in containing intersection (or intersection density relied on intersection reference), curve profile intersection density in surface (or curve intercept intersection density relied on intersection of containing curve), and curve profile area density in surface (A_S) were defined. A_S expressed the amount of curve profile area of Y phase in the unit containing surface area, $S(X)$. The test methods and formulas were deduced, e.g.: $A_S = I_{CI} / I_C = L_{CI} / B_C$. In the formula, I_{CI} and I_C indicated the intersection numbers of test line with curve intercepts (or profiles in 2D) and with the containing curve respectively. B_C denoted the length of containing curve, L_{CI} being the length of curve intercepts. The formula means that A_S are equal to the curve intercept density and equal to the curve profile intersection density in surface. Issues about A_S application and its correction for shrinking tissues were discussed. Example for testing A_S was given.

Keywords: curve profile area density in surface, curve intercept density, stereology, intersection density relied on intersection reference.

INTRODUCTION

As we know that area is a basic parameter important to describe quantitatively a structure. The morphometrical parameters for describing area-related feature include area, area density, surface area, surface density and the ratio of surface to volume. Area (A) is used to describe the absolute size of plane structure. Area density (A_A) denotes the amount of area of interested phase in unit reference area. Surface area (S) is a three dimensional index to describe the absolute surface size of interested phase. Surface density (S_V) means the amount of surface area of interested phase in unit containing space volume. The ratio of surface to volume, $S(X)/V(X)$, denote the ratio of surface area of X phase (or particles) to the volume of the same X phase (or particles) itself (Weibel, 1979; 1980; Collan, 1984; Shen and Shen, 1991; Howard and Reed, 1998). In practice, some biostructures often distribute and take up certain area on or in its containing surface structure, such as desmosomes in cells membrane, nuclear pores in nuclear membrane etc. It is clear that aforesaid area-related parameters can not describe the amount of surface area occupied by particles structure which distribute on or in surface area of membrane or

membrane like structure in unit surface area of correspondent membrane or membrane like structure, for example, the amount of basement membrane surface area occupied by some kind of glandular epithelium cells in unit basement membrane surface area of the gland, the amount of cell membrane surface area occupied by desmosomes in unit surface area of cell membrane, the amount of reticulum surface area occupied by ribosomes in unit surface area of rough endoplasmic reticulum. This kind of problems as aforesaid are generalized in this paper as the problem of seeking curve profile area density in surface, i.e.: $S(Y)/S(X)$. Then, how to solve this kind of problem?

DEFINITION AND FORMULA DEDUCTION

Let A for planar area, S for spatial surface area, A_A for areal density, $S(X)/V(X)$ for the ratio of surface to volume of a set of particles, S_V for surface density, $S(Y)/S(X)$ for the surface density of a spatial curved surface (Y) embedded in a spatial curved surface (X), $S(Y \cap T)$ for profiles of the spatial curved surface Y (T being a probe), $S(Y) = S(Z \cap X)$ for the surface of the intersection of two set (Z, X) (Weibel, 1980).

Definition 1: A spatial curved surface X containing interesting structures in its surface is defined in the paper as containing spatial curved surface or containing surface (or containing curve when in 2D). Let X be a curved spatial surface 3D set containing or intersecting a subset Y of interesting spatial curved structures, the boundary of X (or δX) will be called a containing surface (or a containing curve in case X being a 2D set).

Definition 2: The curved intersection formed by a containing surface intersecting with 3D structure (Fig. 1③,④) is defined in the paper as curve profile (Fig.1⑤,⑥). Let Z be a 3D structure and X a containing surface, then the curved intersection:

$$Y = Z \cap \delta X \quad (1)$$

is called a curve profile. Based on the features of Z , volumic or unvolumic (or shell), which intersect with the containing surface, the curve profiles can be divided into two kinds: the solid curve profile (Fig1⑤) and the empty curve profile (Fig. 1⑥) whether Z is a volumic or unvolumic (or shell) structures. The curve profiles formed by desmosomes or hemidesmosomes intersecting with cell membrane are just a solid curve profiles. And the nucleopores are empty curve profiles.

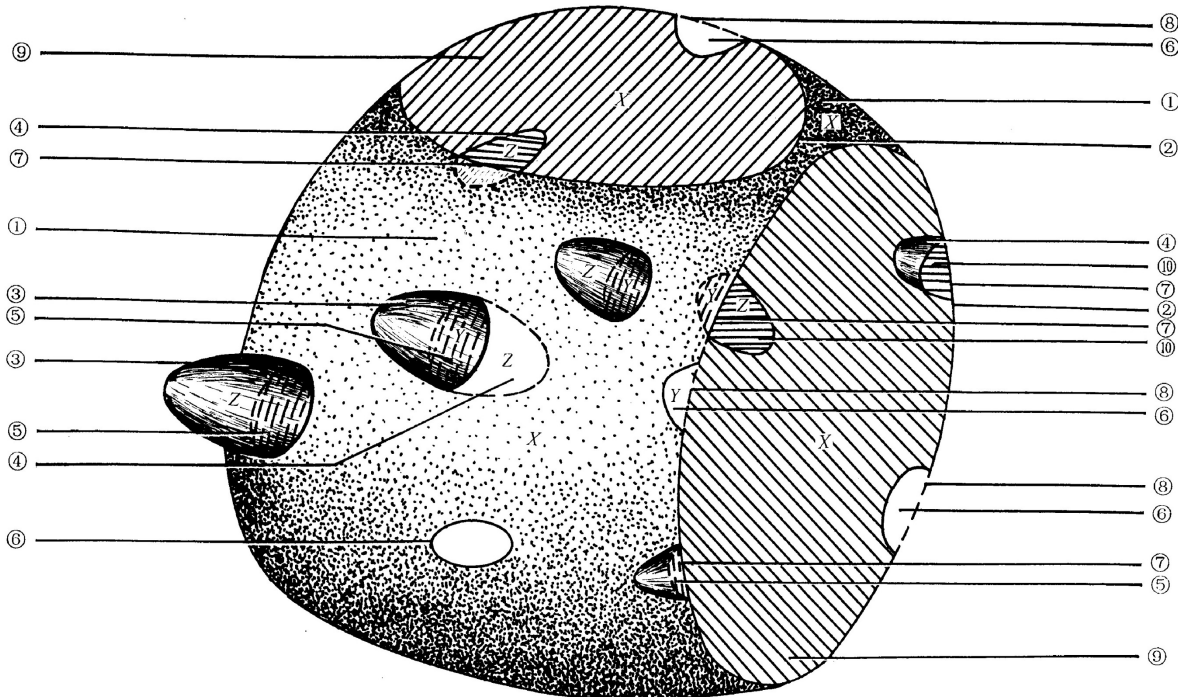


Fig. 1. Show containing surface of X ①, containing curve lines (② formed by containing surface intersecting with cross sections), particles Z (③ for particles towards outside of the containing surface, ④ for particles towards inside of the containing surface), curve profiles Y (⑤ for solid curve profiles formed by curve surface intersecting with solid particles which are towards outside or/and inside of the curve structure or just crossing the curve surface, ⑥ for empty curve profiles formed by breaches or holes in containing surface), curve intercepts (⑦ for solid curve intercepts (black line) formed by a curve line intersecting with solid curve profile, ⑧ for empty curve intercepts (dotted lines) formed by a curve line intersecting with empty curve profiles), cross section in 2D plane (⑨ for the cross section of containing surface structure X and ⑩ for the cross section of 3D particles Z).

Definition 3: The intercept formed by a curve profile intersecting with a 2D plane was defined as curve intercept (Fig. 1Ⓣ,Ⓢ). Check the distribution of section as IUR, and let T_2 be a planar probe (DeHoff RT, 2000), then the intersection

$$Y \cap T_2 = [Z \cap \delta X] \cap T_2 \quad (2)$$

will be called a curve intercept (empty or solid). If a solid curve profile intersecting with a 2D plane, the corresponding curve intercept is named as solid curve intercept (Fig. 1Ⓣ), and if an empty curve profile intersecting with a 2D plane, the corresponding curve intercept, which is invisible in fact, is named as empty curve intercept (Fig. 1Ⓢ).

Definition 4: The length of curve intercepts in unit length of containing curve, when X being a 2D set, is defined as the curve intercept density (CID), i.e.:

$$CID = \frac{\sum \{L[Y \cap T_2]\}}{\sum \{L[\delta X \cap T_2]\}} \quad (3)$$

In Eq. 3, L denotes the length of curves. The formula can rewrite as:

$$CID = \frac{\sum L_{CI}}{\sum B_C} \quad (4)$$

In the formula, L_{CI} is the length of curve intercepts, B_C is the boundary length of containing curve, which can be membrane like structure, in 2D section. It is clear that CID is a parameter with no physical unit.

Definition 5: Let $V(X)$ be the volume of the containing space bounded by a curved spatial surface, then the amount of curve profiles surface area of Y , $S(Y)$ or CPA , in unit volume of containing space is defined as the curve profile area density (CPA_V), that is:

$$CPA_V = \frac{S(V)}{V(X)} \quad (5)$$

The unit of CPA_V may be cm^2/cm^3 , mm^2/mm^3 , $\mu\text{m}^2/\mu\text{m}^3$, etc..

Definition 6: The amount of intersections of intercepts from subset Y in containing curve intersecting with test line in unit intersections of containing curve intersecting with test line is defined as intersection density in containing intersection, or intersection density relied on intersection reference, represented by I_I . Check the distribution of test lines in

3D IUR distribution and let T_1 be an IUR probe line and I denotes the number of intersections. Then:

$$I_I = \frac{\sum \{I[Y \cap T_1]\}}{\sum \{I[X \cap T_1]\}} \quad (6)$$

is intersection density in containing intersection, or intersection density relied on intersection reference. The formula can be rewritten as:

$$I_I = \frac{\sum I_Y}{\sum I_C} \quad (7)$$

In the formula, I_Y is the amount of intersections of intercepts from subset Y in containing curve intersecting with test line; I_C is the amount of intersections of containing curve intersecting with test line.

According to the definition of I_I , the amount of intersections of curve intercepts (or curve profiles) in containing curve (or surface) intersecting with test line in unit intersections of containing curve (Fig. 1Ⓣ, or containing surface of Fig. 1Ⓢ) intersecting with test line, is named as curve intercept intersection density relied on intersection of containing curve (containing intersection), or curve profile intersection density in surface, represented by I_{IS} in the paper. That is:

$$I_{IS} = \frac{\sum I\{[(Y \cap T_2) \cap T_1]\}}{\sum \{I[X \cap T_1]\}} \quad (8)$$

So, I_{IS} can be calculated by:

$$I_{IS} = \frac{\sum I_{CI}}{\sum I_C} \quad (9)$$

In the formula, I_{CI} is the intersections of curve intercepts (or curve profiles in 2D section) intersecting with test line, I_C is the intersections of containing curve intersecting with test line.

Definition 9: The amount of curve profile area ($S(Y)$ or CPA) in unit surface area of containing surface, $S(X)$ or S_C , is defined as curve profile area density in surface, represented by A_S . The definition formula is that:

$$A_S = \frac{S(Y)}{S(X)} \quad (10)$$

Eq. 10 can be rewritten as:

$$A_s = \frac{CPA}{S_C} \quad (11)$$

It is clear by above formula that A_s is a parameter with no physical unit. Because CPA and S_C are hardly to measure or get directly from 3D under microscope, the key question is how to test A_s based on 2D section? The following deduction is to solve this problem.

Dividing separately numerator and denominator of Eq. 11 by V_C , containing space volume, then we can get that:

$$A_s = \frac{CPA/V_C}{S_C/V_C} = \frac{CPA_v}{S_v} \quad (12)$$

In the formula, S_v is the surface density for the containing curve (or containing surface) itself, or $S(X)/V(X)$ for the ratio of surface to volume of a set of X , i.e.: the amount of containing surface area in unit containing space volume, or the ratio of surface area of containing curve to the volume of containing

$$CPA_v = \frac{S(Y)}{V(X)} = 2 \frac{\sum\{I(Y \cap T_1)\}}{\sum\{L(X \cap T_1)\}} = \frac{4 \sum\{B(Y \cap T_2)\}}{\pi \sum\{A(X \cap T_2)\}} \quad (15)$$

or:

$$CPA_v = \frac{2 \sum I_{Cl}}{\sum L_C} = \frac{4 \sum L_{Cl}}{\pi \sum A_C} \quad (16)$$

In the formula, L_{Cl} is the length of curve intercepts of Y phase. By taking formula (13), (15) and (16) into formula (12), we can get that:

$$A_s = \frac{CPA_v}{S_v} = \frac{\frac{S(Y)}{V(X)}}{\frac{S(\delta X)}{V(X)}} = \frac{2 \frac{\sum\{I(Y \cap T_1)\}}{\sum\{L(X \cap T_1)\}}}{2 \frac{\sum\{I(X \cap T_1)\}}{\sum\{L(X \cap T_1)\}}} = \frac{\sum\{I(Y \cap T_1)\}}{\sum\{I(X \cap T_1)\}} = \frac{\frac{4 \sum\{B(Y \cap T_2)\}}{\pi \sum\{A(X \cap T_2)\}}}{\frac{4 \sum\{B(\delta X \cap T_2)\}}{\pi \sum\{A(X \cap T_2)\}}} = \frac{\sum\{B(Y \cap T_2)\}}{\sum\{B(\delta X \cap T_2)\}} \quad (17)$$

The above formula can be rewritten as:

$$A_s = \frac{2 \sum I_{Cl} / \sum L_C}{2 \sum I_C / \sum L_C} = \frac{\sum L_{Cl}}{\sum L_C} \quad (18)$$

$$A_s = \frac{4 \sum L_{Cl} / (\pi \sum A_C)}{4 \sum B_C / (\pi \sum A_C)} = \frac{\sum L_{Cl}}{\sum B_C} \quad (19)$$

It is clear that curve profile area density in surface is equal to the value that the intersections of curve intercepts of subset Y intersecting with test line divided

space. Based on the principle and methods of stereology (Weibel, 1979, 1980; Collan, 1984; Shen and Shen, 1991; Howard and Reed, 1998):

$$S_v = \frac{S(\delta X)}{V(X)} = 2 \frac{\sum\{I(X \cap T_1)\}}{\sum\{L(X \cap T_1)\}} = \frac{4 \sum\{B(\delta X \cap T_2)\}}{\pi \sum\{A(X \cap T_2)\}} \quad (13)$$

or:

$$S_v = \frac{2 \sum I_C}{\sum L_C} = \frac{4 \sum B_C}{\pi \sum A_C} \quad (14)$$

In the formula, X is the containing space and δX its boundary; L_C is the length of test line in containing space; I_C is the numbers of intersections of containing curve (including the part of curve intercepts) intersecting with test line; A_C is the area of containing space in 2D plane; B_C is the length of boundary or curve line of the containing surface in 2D slices. Because CPA , the curve profile area, is a part of surface area in containing surface, S_C , therefore the curve profile area density can be calculated similar as surface density, that is:

by the intersections of containing curve intersecting with test line, and also equal to the value that the length of curve intercepts of subset Y divided by the length of containing boundary (or containing curve line) in 2D plane. So the key formula for A_s is that:

$$A_s = I_{Is} = CID \quad (20)$$

i.e.: curve profile area density in surface is equal to the curve intercept intersection density relied on intersection of containing curve (or curve profile

intersection density in surface), and equal to the curve intercept density.

EXAMPLE FOR TESTING

Fig. 2 is an example for calculating curve profile area density in surface. Now let us use the square lattice test system to test A_S of the particles in the sphere surface on 2D plane. First, counting I_C , the intersections of sphere surface (or the boundary of circle in 2D) intersecting with test line in horizontal and longitudinal directions. It is 56; Secondary, counting I_{Cl} , the intersections of curve profiles (which show as curve intercepts in 2D, and are formed by the shadow particles intersecting with sphere surface), intersecting with test line in horizontal and longitudinal directions, it is 34; Finally calculating A_S by formula (18), i.e.: $A_S = 34/56 = 60.7\%$. If we want to use formula (19) to calculating A_S of the shadow particles in the sphere surface, the entire length of containing curve (or the perimeter of the circle in 2D plane) should be measured or calculated by the diameter of the circle (or by image analysis system) first, it is 18.21 cm. Then, measure the lengths of curve intercepts of the shadow particles intersecting with the circle boundary one by one and sum the lengths, it is 11.28 cm in total. Finally calculating A_S by formula (19), i.e.: $A_S = 11.28/18.21 = 61.8\%$. The results are close each other by aforesaid two methods. If we increase the sample size, the practical testing results will tend to be same by these two kinds of methods.

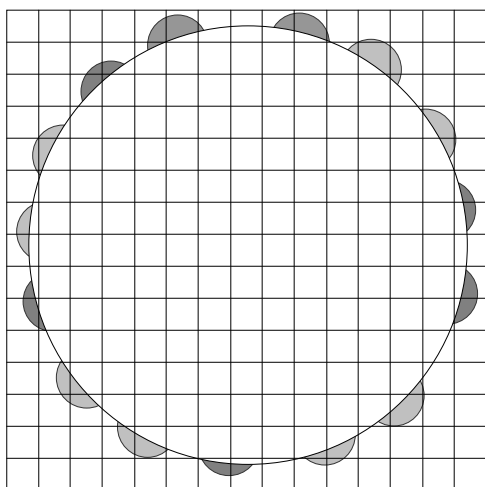


Fig. 2. An example for test of curve profiles area density in surface by square lattice test system.

DISCUSSION

1. *About the correction for tissue shrinkage.* Let the shrinkage coefficient of the curve spatial surface 3D tissue (X) be $f_{S,S}$, the shrinkage coefficient of subset Y , which are formed by a 3D structure Z intersecting with X , be $f_{S,CPA}$, A_S' and A_S be the curve profile area density in surface before and after tissue shrinkage correction. By the general correcting method for tissue shrinkage (Shen 1997, Shen and Shen 1991), we can get that:

$$A_S = \frac{S(Y) \bar{h} f_{S,CPA}^{-2}}{S(X) \bar{h} f_{S,S}^{-2}} = \frac{CPA \bar{h} f_{S,CPA}^{-2}}{S_C \bar{h} f_{S,S}^{-2}} = A_S' \frac{f_{S,S}^2}{f_{S,CPA}^2} \quad (21)$$

If the coefficients of the two intersecting tissues are equal to each other, i.e.: $f_{S,S} = f_{S,CPA}$, then:

$$A_S = A_S' \quad (22)$$

This means that it is not necessary to make correction to the parameter of A_S for the tissue shrinkage if the shrinkage degree is same for these two intersecting tissues. In fact, as we know that curve profiles area is a part of containing surface, so we can reasonably suppose that: $f_{S,S} = f_{S,CPA}$, or $f_{S,S} \approx f_{S,CPA}$. Then we can get A_S directly by formulas (18) or (19). The test method for the coefficient of tissue shrinkage is as same as that in Weibel's book (1979).

2. *About the application for the parameter of A_S .* A_S can be used to quantitatively describe the amount of curve profile area of particles in unit surface area of containing membrane or membrane like structure. In biotissues, it can be used to quantitatively express the amount of mouth area in unit containing surface area, such as the mouth area of adenoduct of sweat gland and sebaceous gland in unit surface area of skin, of intestinal glands in unit surface area of intestine mucosa. It also can be used to quantitatively express the occupied area of some kind of cells on the side of basement membrane of gland in unit surface area of basement membrane. The area occupied by osteoblasts in unit surface area of bone trabecula, by tight junctions or gap junctions, intermediate junctions, desmosomes, microvilli, cilia and synapses etc. in unit surface area of cell membrane, by ribosomes in unit surface area of rough endoplasmic reticulum, by nuclear pores in unit surface area of nuclear membrane, by capillary pores in unit surface area of fenestrated capillary on the side of lumen, by alveolar pores or type II alveolar epithelium in unit surface area of

pulmonary alveoli, or by synaptic vesicle in unit surface area of presynaptic membrane, etc. All above can be quantitatively described with the parameter of A_S .

3. *About empty curve profile.* By the definition of empty curve profile, any holes in membrane or membrane like structures could be considered as empty curve profiles. Glandular duct mouths of all kinds of exocrine glands in surface of mucosa or skin, nuclear pores, alveolar pores and capillary pores etc. are just the examples of empty curve profiles. Empty curve profiles in 2D plane express as a structureless (hole or pore like structures). So we can't see any empty curve intersections actually, but an empty area, holes or pores or mouth, maybe with a boundary. The empty curve intercepts in 2D show as an interrupted or divided intercepts. In fact, it is also invisible. Therefore when a test line passing through this kind of empty area, interrupted or divided intercepts, it should be counted as an intersection with curve intercepts.
4. It is necessary to emphasize that the reference systems of curve profile area density in surface, curve intercept density, curve profile intersection density in surface (or curve intercept intersection density relied on intersections of containing curve), are separately the correspondent surface area of containing surface, the length of containing curve line, and the intersections of containing curve (or curve line) with test lines. The essential difference between the curve profile area density in surface and the curve profile area density depend just on the references system or containing space. The reference for the former is the containing surface, but that for the latter is the volume of containing space. The general used intersection density is different from the intersections density in containing intersection (or intersection density

relied on intersection reference) just in containing system (or reference system) and physical unit. The intersection density, I_L , employ the length of test line in containing space in 2D for its reference system and its physical unit is numbers per mm or μm (Weibel, 1979, 1980; Collan, 1984; Shen and Shen, 1991; Howard and Reed, 1998). But the intersection density in containing intersection, I_I , employ the intersections in containing curve intersecting with test lines for its reference system with no physical unit.

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