Surface Area Estimation: A Brief Review

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ABSTRACT
Surface area and surface density are critical biological parameters, especially in organs such as the lungs, where the total available surface area affects the efficiency of gas exchange. Surface area estimation is widely used in magnetic resonance imaging (MRI) techniques to estimate the surface area of cerebral or other organs. As the applicability of the estimation can be expanded to other biological structures, a brief review of the topic would be useful. This short review therefore summarizes methods of estimating surface area.

Key Words: Surface area, Cavalieri principle, volume, vertical section, isotropic uniform sectioning, stereology

Introduction
Stereology is a methodology that extrapolates two-dimensional planar data taken from images of structures to produce three-dimensional (3D) representations. The main principle of the method is to observe intersections between geometric probes and the object of interest. The term “probe” is used as a geometric question that is asked of the structure of interest in order to obtain correct quantitative data from sections (Gundersen et al., 1988). The geometric probe used to estimate surface area must be a one-dimensional probe, which is a line or curve, in order to obtain correct quantification of the surface area, which is a two-dimensional quantity. As a rule, the probe and the quantity of interest must have a total of 3 dimensions (West, 1993).

One of the most important problems of stereological estimation is “isotropy”, which requires randomness in the slicing or sectioning direction. Isotropy has crucial importance, since it must be ensured that any point of the structure located in a three-dimensional real space must have an equal probability of being sampled, generally in a complex and anisotropic manner. To achieve unbiased estimations, either the object or the probe should be sampled isotropically. There are several ways of overcoming this complex problem, including the use of isotropic random- or vertical sections (Gundersen et al., 1988; Mattfeld et al., 1990). In this brief review, we will first describe the isotropic and vertical sectioning procedures, and then procedures for estimating the surface density and surface density.

Isotropic Uniform Random Sectioning and Vertical Uniform Random Sectioning
An isotropic section is a plane that is perpendicular to an isotropic orientation. For isotropic structures like the liver, isotropic uniform random (IUR) sections are obtained by cutting the block and randomizing the orientation. The generation of IUR sections...
therefore requires random rotation around all axes. This method can be problematic, not only for some biological surfaces (Nyengaard and Gundersen, 1992), but also for some layered structures. In most cases, layered structures can only be identified by prominent tissue landmarks when working on tissue sections, but these landmarks may not be identifiable in IUR sections due to random rotations and displacements (Gokhale et al., 2004). Moreover, if the structure is anisotropic, other methods such as the orientator (Mattfeld et al., 1990) or the isector are needed (Nyengaard and Gundersen, 1992).

As an alternative to IUR sections, vertical uniform random (VUR) sections can also be used (Baddeley et al., 1986). Instead of randomizing all axes, as in IUR sections, one of the axes of the tissue is selected as the ‘vertical’ axis and the tissue is then rotated randomly around this axis to generate VUR sections. The tissue is then sectioned perpendicular to the vertical axis, in the usual systematic random manner (Baddeley et al., 1986).

**Probes Used in Isotropic Uniform Random Sections and Vertical Sections**

Estimation of surface density from isotropic uniform random sections utilizes a linear probe like that in Fig. 1. However, to compensate for the effect of choosing only one axis on randomness, the probe, which will be used in vertical sections, should be a special sine-weighted curve, called the cycloid (that, in 3D, is a curved surface) instead of the linear probes used in IUR sections. A cycloid is a curve that represents the path traced by point on the periphery of a circle as the circle rolls along a straight edge (Fig. 2A). The length of the cycloid is proportional to the sine of its angle with respect to the edge. If the cycloid arc is limited with a rectangle, then the short side of the rectangle represents the minor axis and the long side of the rectangle represents the major axis (Fig. 2B). The length of the cycloid is equal to twice the minor axis. After this brief introduction to surface area estimation and related probes, we will briefly discuss the concept of surface density.

**Surface Density**

Surface density is the surface area of an interface per unit volume of the reference space.

**Estimation of Surface Area in Isotropic Random Sections**

As briefly mentioned above, linear test probes should be used to estimate the surface area on isotropic sections. As in all stereological methods, object–probe intersections are the main basis for estimating the surface area. The relationship between the number of
intersections and the surface area per unit volume (surface density, $S_v$) can be estimated by Eq. 3 (Smith and Gutmann, 1953).

$$S_v = 2I_i$$  \hspace{1cm} \text{Eq. 3}

where $S_v$ is the surface area per unit volume (surface density) and $2I_i$ is twice the number of intersections between the linear surface profiles and the linear probe. This equation is an unbiased estimator of the surface density when isotropy of the object or the probe, or both, is guaranteed (Howard and Reed, 1998).

The number of intersections between a linear probe and the object is proportional to:

1) The surface area of the object (the higher the surface area per unit volume, the larger the number of intersections), 2) The 3-D orientation of the object.

The effect of object orientation on surface area estimation can be demonstrated using the example of a piece of elongated putty and a grid of skewers. The elongated putty shape (object) is placed on a grid of skewers (probes), as shown in Fig. 3A. When the minor axis of the object is aligned perpendicular to the skewers, there is a smaller surface area exposed to the skewers and therefore fewer intersections between the skewers (probes) and the surface area of the putty (object); there will be a greater number of intersections if we place the putty on the skewers with the major axis perpendicular to that of the skewers, as in Fig. 3B, as they now interest the larger surface of the elongated shape.

![Figure 3. A piece of putty (red ball) and many probes (black lines) demonstrate the importance of object orientation on surface area estimation. In A and B, differing orientation affects the number of intersections between skewers (linear probes) and the putty surface (object of interest).](image)

**Estimation of surface area from vertical sections**

In the technique of estimating surface area from vertical sections, the surface is sampled at systematic random positions in three dimensions, and then Cavalieri’s principle is combined with vertical sectioning (Baddeley et al., 1986). Another method is developed to estimate surface area directly from vertical sections rather than the initial measurement of surface density (Michael and Cruz-Orive, 1988). This latter method is especially appropriate for magnetic resonance imagining (MRI).

As mentioned above, in the estimation of surface area from vertical sections, an array of cycloids is used as a probe, instead of the IUR test lines. The arrays of cycloids are superimposed on the sections with random positioning, given that the short axes of the cycloids are parallel to the vertical direction, as shown in Fig. 4.

The formula for estimating surface area from vertical sections (Roberts et al., 2000) is given in Eq. 4

$$S = 2t \cdot \left(\frac{a}{l}\right) \cdot I$$  \hspace{1cm} \text{Eq. 4}

where “$t$” is the section interval, “$a/l$” is area associated with cycloid divided by cycloid length and “$I$” the number of intersections. Estimation of surface area from vertical sections using MRI has several clinical applications, including the assessment of brain atrophy, investigating the structure and function of the cerebral hemispheres and radiotherapy planning (Sonmez et al., 2010). Many studies have used stereology on MRI images (García-Fiñana et al., 2003; Sahin et al., 2003, 2008). In particular, the reader is directed to the study by Henry and Mayhew (1989). They applied the vertical section method to obtain estimates of the boundaries of pial surface area between the cortex and the subcortical matter in whole cerebral hemisphere specimens. For each specimen, an estimate of the cortical volume was obtained using the two surface-area estimates and the mean thickness of the cerebral cortex. Acer et al. (2009) estimated cerebral surface area from MRIs of 13 young subjects that were free of any neurological symptoms and signs. Estimation of surface area from vertical sections is also applicable to biological areas other than medicine. For instance, Dhaliwal et al. (2002) used vertical sections to estimate the surface area of endometrial glands during stages of oestrous cycle in normal cycling maiden heifers and pluriparous cows. Matzl and Schneebeli (2010) also used vertical sections to estimate the specific surface area of...
30 snow samples covering all typical snow types.

Figure 4. Cycloid line-probes are used on vertical sections to estimate surface density. Cycloids are superimposed at random positions with the short axis of the cycloids parallel to the vertical direction of the section.

Conclusion

Surface area is one of the most important and complicated parameters of biological object, but can be efficiently estimated using stereological methods in an unbiased manner. The importance of this methodology is increased by its versatility and application to several fields, including the biological and medical sciences. The current literature suggests that recent stereological methods for estimating surface area from vertical sections provide the most efficient and unbiased technique.

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