Estimating Surface Area in Biological Structures

Mark J. West

Stereological estimates of surface have led to important insights into normal and pathological processes. This article describes the process of estimating surface area in biological structures. It includes a discussion of relationship equations for estimating surface and procedures for estimating surface area of components of organ systems. It also provides an example of estimation of the area of the pial surface of the human brain.

INTRODUCTION

Estimates of total surface area $S$ are of central importance in evaluations of the transfer of substances in and out of organ systems. Two organ systems in which stereological estimates of surface have led to important insights into normal and pathological processes are the lung (Weibel 1963; Michel and Cruz-Orive 1988; Ochs 2006) and the placenta (Mayhew et al. 1984). The number of studies in which the surface features of brain structures has been estimated with design-based stereological techniques is relatively limited (Henery and Mayhew 1989; Acer et al. 2010). Although the functional capacity of neural structures is most often thought of in terms of the number, length, and volume of various structural features in the brain, the cortical surface may reflect changes in the number of radially oriented columns and thereby the input–output capacity of the tissue. At the cellular level, the contact surface between cells is an important aspect of cellular functioning. This is particularly so for the surface area of the contact region of neuronal synapses (Scheff et al. 2007; West et al. 2009), which can be related to the efficacy of synaptic transmission (Ganeshina et al. 2004). Similar to estimates of length, estimators of surface area require isotropic interactions (see Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]) between the probes that are used and the structural features that are of interest. The use of IUR (isotropic uniform random) or VUR (vertical uniform random) sections (see Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]) does ensure isotropic interactions but places some constraints on the orientation of the sections, which may not be desirable. These constraints have been overcome by the design of virtual probes that are applied to thick sections (see the section entitled Virtual 3D Line and Area Probes in Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]).

RELATIONSHIP EQUATIONS FOR ESTIMATING SURFACE

In sectioned material, surfaces appear as lines (Fig. 4 in Introduction to Stereology [West 2012a]), and the length of these lines has a direct relationship to surface area. There are two equations that express this relationship. The first has its origins in the Smith–Guttman equations (Smith and Guttman 1953)
that formed the basis of relationship equations for estimating length. Accordingly, two times the number of intersections $I$ between a linear probe of known length $l$ and the linear features in the sections that represent the surface in three dimensions, is an unbiased estimate of the area of surface per unit volume of tissue, $S_V = 2lI$ (as shown in Fig. 1). The second relationship equation involves the length of the lines $B$ on a section of known area, which, when multiplied by $4/\pi$, is an unbiased estimate of the surface area per unit volume $S_V$, that is, $S_V = 4/\pi B_A$. In the first case, the probe is a line; in the second case, the probe is a section. In both cases, it is necessary to ensure an isotropic interaction between the probe and the surface feature in order for the estimate to be unbiased. Ensuring this interaction places certain constraints with regard to the orientation of the tissue and the orientation of the probe to be used (see Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]). The advent of high-resolution, noninvasive scanning techniques that can be used to obtain virtual sections at any angle through organ systems and with sufficient detail for the analysis of the surface features of tissues has reduced the impact of these constraints, as shown in Figure 4 in Isotropy, iSectors, and Vertical Sections in Stereology (West 2013).

Generally, the preferred approach is to obtain unbiased estimates of the surface through simple counts of the number of intersections $I$ between the linear probe $l$ and lines that represent the surface feature on the sections $I_B$. This is because one can obtain data by simply counting interactions or events, rather than measuring the lengths of lines. In this case, the required isotropic interaction between the linear probes and the surface feature can also be realized through the use of either IUR sections with line probes of arbitrary orientation or the use of VUR sections in combination with either cycloid-shaped linear probes (Figs. 6 and 7 in Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]) or sine-weighted straight-line probes.

The estimator based on data describing $B_A$ requires the use of IUR sections, such as those produced with the iSector or orientator (described in Isotropy, iSectors, and Vertical Sections in Stereology [West 2013]), with all of the ensuing caveats with regard to difficulties orienting oneself in a tissue that is cut at random angles. In addition, it is also necessary to actually measure the length of the boundary.

**FIGURE 1.** Cycloid grid superimposed on a magnetic resonance image through the human brain. The section is a vertical section with the vertical axis corresponding to the side of the image. The system of consecutive cycloid grids is oriented so that the short axis of the cycloids lies along the vertical axis. Intercepts are shown as white circles. The black crosses (points) are used to determine the length of the test lines that lie within the brain (two cycloids per point). The white points denote intersections of the test lines with the cortical surface. (Redrawn, with permission, from Elsevier.)
B with a ruler, digitizer, or planimeter. In general, measuring the length of a line manually is more demanding than counting intercepts of linear probes with surface features. Computerized interactive imaging has, to a certain degree, eliminated this difference.

VIRTUAL CYCLOIDS

Virtual linear probes (Gokhale et al. 2004), in the form of cycloids generated within thick histological sections, eliminate the need for IUR and VUR sections and can be used in sections cut in any plane (Fig. 2). The practical application of the probe is difficult when the surface being estimated runs parallel to the surface of the section, with the result that borders of the surface are not well defined.

PROCEDURES FOR ESTIMATING SURFACE AREA OF COMPONENTS OF ORGAN SYSTEMS

Procedure 1

The surface of a laminar structure could be estimated indirectly from estimates of the volume $V$ and the average thickness $t$ of the structure, $S = V/t$. $V$ can be readily estimated by point counting as described in Estimating Volume in Biological Structures (West 2012b). $t$ is more difficult to estimate, in that one must ensure that $t$ is measured orthogonally to the surface. This is most readily

FIGURE 2. (A) Illustration of a virtual cycloid generated within a thick histological section. The cycloid is generated with software that moves a point to different positions in the $x,y$ plane at different depths of the section. The point moves along a cycloid-shaped path. The short axis of the cycloid is positioned along the vertical axis, indicated by the arrow at left. (B) As one proceeds from one level of the section depth to the next, one determines whether the path passes through a surface, that is, the point is on one side of a surface feature at one level and on the other side at the next level, and counts this event as an intercept of the probe with the surface. With subsequent probing, the plane of the path needs to be rotated at random around the vertical axis of the tissue. (Redrawn, with permission, from John Wiley and Sons.)
accomplished in relatively flat regions of gyrencephalic brains or lysencephalic brains. This approach is further complicated by the need to have an unbiased sample of measurements of $t$ in order for the estimate of $S$ to be unbiased; that is, all positions on the surface have to have the same probability of being sampled when estimating $t$.

**Procedure 2**

The use of noninvasive virtual imaging of the brain (Acer et al. 2010) and other organs (Roberts et al. 2000) provides possibilities for readily generating IUR sections and VUR sections that allow longitudinal studies of surface features within an individual. The area of the pial surface of the human brain can be estimated according to the following relationship, which applies to linear probes on IUR sections, for estimating surface density $S_V$:

$$S_V = 2I_l$$  \hspace{1cm} (1)

and multiplying $S_V$ by the reference volume of the cerebral cortex $V_{REF}$, obtained with Cavalieri’s technique, described in *Estimating Volume in Biological Structures* (West 2012b), as follows:

$$S = V_{REF} \times S_V.$$  \hspace{1cm} (2)

**Procedure 3**

$S$ can also be estimated with a fractionator variant of this formula (Michel and Cruz-Orive 1988; Roberts et al. 2000) (Eq. 3) when using a systematic random series of vertical sections, in which the length of the test line (cycloid) per volume of tissue sampled $T(a/l)$ is used to estimate the total surface $S$:

$$S = 2T \times (a/l) \times \Sigma l.$$  \hspace{1cm} (3)

The derivation of this formula is shown at the end of this article.

**EXAMPLE OF ESTIMATE OF THE AREA OF THE PIAL SURFACE OF A HUMAN BRAIN**

One human brain was cut into 1-cm-thick vertical slabs. The surface of the slabs represented a series of parallel vertical sections, separated by 1 cm and oriented at a random angle around the vertical axis (see Fig. 4 in *Isotropy, iSectors, and Vertical Sections in Stereology* [West 2013]). Using a test grid of cycloids (Acer et al. 2010) with an area-to-length ratio $a/l$ of 2.8 cm and in which the distance between test points was 4.02 cm (see Fig. 3 and Table 1), the number of intersections $I$ of the cycloid probe with the pial surface of each section was recorded.

![Cycloid grid](http://example.com/cycloid.png)

**FIGURE 3.** Cycloid grid after Acer et al. (2010). The grid should be oriented so that the $y$-axis is aligned with the vertical axis. (Redrawn, with permission, from Elsevier.)
An estimate of the total area of the pial surface of the human brain was calculated with Equation 3, in which \( T \), the distance between sections, was 1 cm, the test area associated with each cycloid test line \( a/l \) equaled 2.8, and \( \Sigma I_{1-12} \) was 195.

\[
S = 2T \times a/l \times \Sigma I \\
= 2 \times (1 \text{ cm}) \times (2.8 \text{ cm}) \times 195 \\
= (5.6 \text{ cm}) \times (195 \text{ cm}) = 1092 \text{ cm}^2
\]

The coefficient of error (CE) of the estimate of \( S \) is 0.07 and has been calculated in the same manner as that for estimates of number \( N \) and length \( L \) as described in detail in The Precision of Estimates in Stereological Analyses (West 2012c).

The derivation of Equation 3 is as follows for the grid shown in Figure 4 for IUR sections:

\[
S_V = S/V = 2 \times (p/l) \times \Sigma I/\Sigma p, \\
V = T \times (a/p) \times \Sigma p, \\
S = S_V \times V, \\
S = 2p/l \times \Sigma I/\Sigma p \times T \times a/p \times \Sigma p, \\
S = 2T \times (a/l) \times \Sigma I.
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S = S_V \times V, \\
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S = 2T \times (a/l) \times \Sigma I.
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REFERENCES


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