NUMERICAL DENSITIES FROM SURFACE DENSITIES

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When an estimate for the numerical density of cells is derived from a surface density, two advantages are gained. The effects of artifacts produced by preparation for transmission electron microscopy can be minimized and the coefficents of variation for population estimates are often less than 10% of the mean. This presentation will consider two methods for estimating cell frequencies from surface densities.

The first approach, called the SURFACE AREA RATIO METHOD (1,2,3), is based on a ratio of nuclear surface densities and includes the assumption that the mean surface area of the nucleus in a given population remains constant throughout an experiment. Given this assumption, the ratio of nuclear surface densities (SVn) is equal to the ratio of the number of nuclei (Nn):

$$\frac{SVn\ [E]}{SVn\ [C]} = \frac{Nn\ [E]}{Nn\ [C]},$$

where [E] refers to an experimental time point and [C] to the control. Note that the nuclear surface density is equal to the product of the mean nuclear surface area ($\bar{S}n$) and the number of nuclei divided by a standard unit of cell volume (Vcell):

$$SVn = \frac{Sn}{Vcell} = \frac{\bar{S}n * Nn}{Vcell}.$$

To estimate a relative change in the volume, surface, length, or number of objects in a compartment i in an average cell (abbreviated below as Xi, av.cell), the ratio of the nuclear surface densities is used to keep the number of nuclei constant in a standard unit of reference volume. The general expression is given as:

$$\frac{Xi,av.cell\ [E]}{Xi,av.cell\ [C]} = \frac{XVi\ [E]}{XVi\ [C]} * \frac{SVn\ [C]}{SVn\ [E]}.$$

When the mean nuclear surface area remains constant throughout an experiment, the surface density ratio for two different nuclear types (n1,n2) is expected to equal one. This is a way to check the assumption of the method.

The second approach, called the B-BAR METHOD (4,5,6,7), estimates a cellular numerical density by dividing the surface density of a nuclear compartment by the mean nuclear surface area ($\bar{S}n$):

$$NVn = \frac{SVn}{\bar{S}n}.$$

The mean nuclear surface area is calculated from an estimate for the mean nuclear boundary, and the B-BAR METHOD assumes that all the nuclei within a given population have the same surface area. A spherical transformation factor (derived from serial section reconstructions of nuclei) is used to rearrange the mean surface area of either regular or irregular nuclei into a sphere of equivalent surface area. To estimate the volume, surface, length, or number of objects for a compartment i in an average cell (abbreviated below as Xi, av.cell), the volume density, surface density, length density, or numerical density of a compartment i (abbreviated XVi) is divided by the numerical density of the nuclei:

$$Xi,av.cell = \frac{XVi}{SVn} * \bar{S}n,$$

where XVi is a volume, surface, length, or numerical density.

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