Stereological formulas

AREA FRACTION FRACTIONATOR

Estimated volume fraction ($\widehat{V_v}$)	$\widehat{V_v}(Y, ref) = \frac{\sum_{i=1}^{m} P(Y)_i}{\sum_{i=1}^{m} P(ref)_i}$	*P(ref)* Points hitting reference volume *Y* Sub-region *P(Y)* Points hitting sub-region
Estimated area ($\hat{A}$)	$\hat{A} = \frac{1}{asf}.a(p).P(Y_i)$	*asf* Area sampling fraction *a(p)* Area associated with a point

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CAVALIERI ESTIMATOR

Area associated with a point (A_p)	$A_p = g^2$	g^2 Grid area
Volume associated with a point (V_P)	$V_p = g^2 m\bar{t}$	m Section evaluation interval $\bar{t}$ Mean section cut thickness
Estimated volume ($\hat{V}$)	$\hat{V} = A_p m'\bar{t}\left(\sum_{i=1}^{n} P_i\right)$	A_p Area associated with a point m' Section evaluation interval $\bar{t}$ Mean section cut thickness P_i Points counted on grid
Estimated volume corrected for over-projection ($[v]$)	$[v] = t.\left(k.\sum_{j=1}^{g} a'_j - max(a')\right)$	t Section cut thickness k Correction factor g Grid size a' Projected area
Coefficient of error (CE)	$CE = \frac{\sqrt{TotalVar}}{\sum_{i=1}^{n} P_i}$	$TotalVar$ Total variance of the estimated volume n Number of sections P_i Points counted on grid $TotalVar = s^2 + VAR_{SRS}$

Cavalieri Estimator (2)

Variance of systematic random sampling (VAR_{SRS})	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	m Smoothness class of sampled function s^2 Variance due to noise $A = \sum_{i=1}^{n} {P_i}^2,\ B = \sum_{i=1}^{n-1} P_i P_{i+1},\ C = \sum_{i=1}^{n-2} P_i P_{i+2}$ With: n : number of sections $s^2 = 0.0724 \left(\frac{b}{\sqrt{a}}\right) \sqrt{n \sum_{i=1}^{n} P_i}$ $\frac{b}{\sqrt{a}}$ Shape factor

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CYCLOIDS FOR LV

Area associated with a point (A_p)	$A_p = g^2$	g^2 Grid area
Volume associated with a point (V_p)	$V_p = g^2 m\bar{t}$	g^2 Grid area m Section evaluation interval $\bar{t}$ Mean section cut thickness
Length per unit volume (L_V)	$L_V = 2\frac{[\bar{I}_L^C]_{prj}}{\Delta}$ $L_V = \frac{2}{\Delta}.\frac{(\bar{I}_c^{cyc})_{prj}}{\bar{P}.\left(\frac{l}{p}\right)} = \frac{2}{\Delta}\left(\frac{p}{p}\right)\frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	$[\bar{I}_L^C]_{prj}$ Number of counting frames Δ Section cut thickness I_i Intercepts P_i Test points $[\bar{I}_C^{cyc}]_{prj}$ Average number of intersections of projected images $\frac{p}{l}$ Test points per unit length of cycloid
Estimated volume ($\hat{V}$)	$\hat{V} = m\Delta\left(\frac{a}{p}\right)\sum_{i=1}^{n} P_i$	m Sampling fractions Δ Section cut thickness a Area p Number of test points P_i Test points
Estimated length ($\hat{L}$)	$\hat{L} = 2\left(\frac{a}{l}\right)m\sum_{i=1}^{n} I_i$	a Area l Line length m Sampling fractions I_i Intercepts

Cycloids for Lv (2)

Coefficient of error for line length	$CE(\hat{L}\vert L) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	VAR_{SRS} Variance of systematic random sampling $\hat{L}\vert L$ Estimated length per length I_i Intercepts
Variance of systematic random sampling (VAR_{SRS})	$VAR_{SRS} = \frac{3g_0 - 4g_1 + g_2}{12}$ $g_k = \sum_{i=1}^{n-k} L_i L_{i+k}$	g Grid size L_i Line length at section i

Coefficient of error for length density	$CE(L_V) = \sqrt{\frac{n}{n-1}\left(\frac{\sum_{i=1}^{n} I_i^2}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} I_i} + \frac{\sum_{i=1}^{n} P_i^2}{\sum_{i=1}^{n} P_i \sum_{i=1}^{n} P_i} - 2\frac{\sum_{i=1}^{n} I_i P_i}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} P_i}\right)}$	I_i Intercepts P_i Test points n Number of probes

References

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Gokhale, A. M. (1990). Unbiased estimation of curve length in 3-D using vertical slices. Journal of Microscopy, 159 (2), 133–141.

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IMAGE VOLUME FRACTIONATOR

Estimate of total number of particles (N)	$N = \sum Q^- \cdot \frac{1}{asf} \cdot \frac{1}{zsf}$	Q^- Particles counted asf Area sampling fraction (counting frame/grid size) zsf Section sampling fraction (disector height/virtual section thickness)
Variance due to systematic random sampling – Gundersen (VAR_{SRS})	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} (Q_i^-)^2$ $B = \sum_{i=1}^{n-1} Q_i^- Q_{i+1}^-$ $C = \sum_{i=1}^{n-2} Q_i^- Q_{i+2}^-$ s^2 Variance due to noise
Variance due to noise - Gundersen (s^2)	$s^2 = \sum_{i=1}^{n} Q^-$	Q^- Particles counted n Number of sections used
Total variance – Gundersen ($TotalVar$)	$TotalVar = s^2 + VAR_{SRS}$	VAR_{SRS} Variance due to SRS s^2 Variance due to noise
Coefficient of error – Gundersen (CE)	$CE = \frac{\sqrt{TotalVar}}{s^2}$	$TotalVar$ Total variance s^2 Variance due to noise

Image Volume Fractionator (2)

Coefficient of error – Scheaffer (CE)	$CE = \frac{\sqrt{s^2\left(\frac{1}{f}-\frac{1}{F}\right)}}{\bar{Q}}$	f Number of counting frames F Total possible sampling sites s^2 Estimated variance $\bar{Q}$ Average particles counted
Average number of particles – Scheaffer ($\bar{Q}$)	$\bar{Q} = \frac{\sum_{i=1}^{f} Q_i}{f}$	Q_i Particles counted f Number of counting frames
Estimated variance - Scheaffer (s^2)	$s^2 = \frac{\sum_{i=1}^{f}(Q_i - \bar{Q})^2}{f-1}$	f Number of counting frames Q_i Particles counted $\bar{Q}$ Average particles counted
Estimated variance of estimated cell population - Scheaffer	$\frac{C_{fp}F^2 s^2}{f}$	C_{fp} Finite population correction s^2 Estimated variance f Number of counting frames F Total possible sampling sites
Estimated variance of mean cell count - Scheaffer	$\frac{C_{fp}s^2}{f}$	C_{fp} Finite population correction s^2 Estimated variance f Number of counting frames

Image Volume Fractionator (3)

Estimated mean coefficient of error – Cruz-Orive (*est Mean CE*)	$est\ Mean\ CE\ (est\ N) = \left[\frac{1}{3n} . \sum_{i=1}^{n} \left(\frac{Q_{1i} - Q_{2i}}{Q_{1i} + Q_{2i}}\right)^2\right]^{1/2}$	Q_{1i} Counts in sub-sample 1 Q_{2i} Counts in sub-sample 2 n Size of sub-sample
Predicted coefficient of error for estimated population – Schmitz-Hof (CE_{pred})	$CE_{pred}(n_F) = \sqrt{\frac{Var(Q_r^-)}{R.(Q_r^-)^2}}$ $CE_{pred}(n_F) = \frac{1}{\sqrt{\sum_{r=1}^{R} Q_r^-}} = \frac{1}{\sqrt{\sum_{s=1}^{S} Q_s^-}}$	R Number of counting spaces S Number of sections Q_r^- Counts in the "r"-th counting space Q_s^- Counts in the "s"-th section

References

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Image Volume Fractionator (4)

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Schmitz, C., Hof, P.R. (2000). Recommendations for straightforward and rigorous methods of counting neurons based on a computer simulation approach. Journal of Chemical Neuroanatomy, 20 (1), 93–114.

West, M. J., Slomianka, L., & Gundersen, H.J.G. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. The Anatomical Record, 231 (4), 482–497.

IMAGE VOLUME SPACEBALLS

Length estimate	$$L = 2.\left(\sum_{i=1}^{n} Q_i\right).\frac{v}{a}$$ *This equation does not include the terms F2 (area-fraction) and F3 (thickness-fraction) used by Mouton et al. (equation 2, 2002), but includes that information in v (volume sampled).*	n Number of sections used Q_i Intersection counted v Volume (grid X * grid Y* section thickness) a Surface area of the sphere
Variance due to noise	$$s^2 = \sum_{i=1}^{n} Q_i$$	Q_i Intersection counted
Variance due to systematic random sampling	$$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$$ $$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$$	$A = \sum_{i=1}^{n} (Q_i^-)^2$ $B = \sum_{i=1}^{n-1} Q_i^- Q_{i+1}^-$ $C = \sum_{i=1}^{n-2} Q_i^- Q_{i+2}^-$ s^2 Variance due to noise m Smoothness class of sampled function
Total variance	$$TotalVar = s^2 + VAR_{SRS}$$	VAR_{SRS} Variance due to SRS s^2 Variance due to noise

Image Volume Spaceballs (2)

Coefficient of error	$CE = \frac{\sqrt{TotalVar}}{s^2}$	$TotalVar$ Total variance s^2 Variance due to noise

References

Mouton, P. R., Gokhale, A.M., Ward, N.L., & West, M.J. (2002). Stereological length estimation using spherical probes. *Journal of Microscopy, 206* (1), 54–64.

ISOTROPIC FAKIR

Estimated total surface area	$estS = 2\frac{1}{n}.\sum_{i=1}^{n}\frac{v}{l_i}.I_i$	n Number of line sets (always set to 3) $\frac{v}{l_i}$ Inverse of the probe per unit volume I_i Intercepts with test lines

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NUCLEATOR

Area estimate	$a = \pi \overline{l^2}$	l Length of rays
Volume estimate	$\overline{v_N} = \frac{4\pi}{3} \overline{l_n^3}$	l Length of rays
Estimated coefficient of error	$est\ CV(R) = \frac{\sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (R_i - \bar{R})^2}}{\bar{R}}$	n Number of nucleator estimates R_i Area/volume estimate for each sampling site
Average area/volume estimate	$\bar{R} = \frac{1}{n} \sum_{i=1}^{n} R_i$	n Number of nucleator estimates R_i Area/volume estimate for each sampling site
Relative efficiency	$CE_n(R) = \frac{CV(R)}{\sqrt{n}}$	n Number of nucleator estimates $CV(R)$ Estimated coefficient of variation
Geometric mean of area/volume estimate	$e^{\left(\frac{1}{n} \sum_{i=1}^{n} lnR_i\right)}$	n Number of nucleator estimates R_i Area/volume estimate for each sampling site

References

Gundersen, H.J.G. (1988). The nucleator. *Journal of Microscopy, 151* (1), 3–21.

OPTICAL ROTATOR

Volume of particle	$$\hat{v} = a \sum_{i}^{+/-} g(P_i)$$	a Reciprocal line density $a=k.h$ k Length of slice h Systematic spacing
For vertical slabs and lines parallel to vertical axis	$$g(P) = d_1, if\ d_2 < t$$ $$g(P) = \frac{\frac{\pi}{2} d_1}{arcsin\left(\frac{t}{d_2}\right)}, if\ t \leq d_2$$	d_1 Distance along test line d_2 Distance from origin to test line t ½ thickness of optical slice
For vertical slabs and lines perpendicular to vertical axis	$$g(P) = d_1, if\ \sqrt{d_1^2 + z^2} < t$$ $$g(P) = f\left(\sqrt{t^2 - z^2}\right), if\ \lvert z \rvert < t \leq \sqrt{d_1^2 + z^2}$$ $$g(P) = f(0), if\ t \leq \lvert z \rvert$$ $$f(x) = x + \frac{\pi}{2} \int_x^{d_1} \frac{1}{arcsin\left(\frac{t}{\sqrt{u^2 + z^2}}\right)} du$$	d_1 Distance along test line t ½ thickness of optical slice z Distance in z from intercept to origin

Optical Rotator (2)

For isotropic slabs		$g(P) = d_1, \quad if\ d_3 < t$ $g(P) = \frac{1}{2t}[h(t,d_2) + k(t,d_1,d_2,d_3)], if\ d_2 < t \leq d_3$ $h(t,d) = t^2\sqrt{1-\frac{d^2}{t^2}}$ $k(t,d_1,d_2,d_3) = d_1 d_3 + d_2^2 log\left(\frac{d_1+d_3}{t+\sqrt{t^2-d_2^2}}\right)$	d_1 Distance along test line d_2 Distance from origin to test line d_3 Distance from intercept to origin t ½ thickness of optical slice
Estimated surface area		$\hat{S} = a\sum_j l_j g(l_j)$ $g(l) = 2, \quad if\ d_2 < t$ $g(l) = \pi.\frac{1}{arcsin\left(\frac{t}{d_2}\right)}, \ if\ t \leq d_2$	a Reciprocal line density l_j Number of intersections between grid line and cell boundary d_2 Distance from origin to test line t ½ thickness of optical slice

References

Tandrup, T., Gundersen, H.J.G., & Vedel Jensen, E.B. (1997). The optical rotator *Journal of microscopy, 186* (2), 108–120.

PLANAR ROTATOR

Volume for isotropic planar rotator	$V = 2t\sum_i g_i$	t Separation between test lines g_i Isotropic planar rotator function
Volume for vertical planar rotator	$V = \pi t\sum_i l_i^2$	t Separation between test lines l_i Intercept length along a test line
Isotropic planar rotator function	$g_i(l) = l\sqrt{l^2 + a_i^2} + a_i^2 ln\left[\frac{l}{a_i} + \sqrt{\left(\frac{l}{a_i}\right)^2 + 1}\right]$ $g_{i+} = \sum_{j\ even} g_i(l_{i\ j+}) - \sum_{j\ odd} g_i(l_{i\ j+})$ $g_{i-} = \sum_{j\ even} g_i(l_{i\ j-}) - \sum_{j\ odd} g_i(l_{i\ j-})$ $g_i = \frac{1}{2}(g_{i+} + g_{i-})$	l Intercept length along a test line a_i Distance from origin to test line j Number of grid lines l_{ij} Number of intersections between the j-th grid line and the cell boundary

Planar Rotator (2)

Isotropic planar rotator function (cont'd)	$l_{i+}^2 = \sum_{j\ even} l_{i\ j+}^2 - \sum_{j\ odd} l_{i\ j+}^2$ $l_{i-}^2 = \sum_{j\ even} l_{i\ j-}^2 - \sum_{j\ odd} l_{i\ j-}^2$ $l_i^2 = \frac{1}{2}(l_{i+}^2 + l_{i-}^2)$	l Intercept length along a test line a_i Distance from origin to test line j Number of grid lines l_{ij} Number of intersections between the j-th grid line and the cell boundary

References

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SURFACTOR

Surface area for single-ray designs	$\hat{S} = 4\pi l_0^2 + c(\beta)$	*l* Length of intercept *ß* Angle between test line and surface *c(ß)* Function of the planar angle
Surface area for multi-ray designs	$\hat{\boldsymbol{S}} = \boldsymbol{2\pi} \sum_{\boldsymbol{j=1}}^{\boldsymbol{2r}} \boldsymbol{l_j^2 . c(\beta)}$	*l* Length of intercept *ß* Angle between test line and surface *c(ß)* Function of the planar angle *r* Number of test lines
Function of the planar angle	$c(\beta) = 1 + \left[\frac{1}{2} cot\beta\right] . \left[\frac{\pi}{2} - sin^{-1}\frac{1 - cot^2\beta}{1 + cot^2\beta}\right]$	*ß* Angle between test line and surface

References

Jensen, E.B., Gundersen, H.J.G. (1987). Stereological estimation of surface area of arbitrary particles. *Acta Stereologica, 6* (3).