Stereological formulas

AREA FRACTION FRACTIONATOR

Estimated volume fraction ($\hat{V}_v$)	$\hat{V}_v(Y,ref) = \frac{\sum_{i=1}^{m} P(Y)_i}{\sum_{i=1}^{m} P(ref)_i}$	$P(ref)$ 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

References

Howard, C. V., & Reed, M. G. (1998). *Unbiased Stereology, Three-Dimensional Measurement in Microscopy* (pp. 170–172). Milton Park, England: BIOS Scientific Publishers.

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

References

García-Fiñana, M., Cruz-Orive, L.M., Mackay, C.E., Pakkenberg, B. & Roberts, N. (2003). Comparison of MR imaging against physical sectioning to estimate the volume of human cerebral compartments. *Neuroimage, 18* (2), 505–516.

Gundersen, H. J. G., & Jensen, E.B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy, 147* (3), 229–263.

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COMBINED POINT INTERCEPT

Profile area (a)	$a = a(p).\sum P$	$a(p)$ Area associated with a point $\sum P$ Number of points
Profile boundary (b)	$b = \frac{\pi}{2} d.\sum I$	d Distance between points $\sum I$ Number of intersections

This method is based on the principles described in the following:

Howard, C.V., Reed, M.G. (2010). *Unbiased Stereology* (Second Edition). QTP Publications: Coleraine, UK. See equations 2.5 and 3.2

Miles, R.E., Davy, P. (1976). Precise and general conditions for the validity of a comprehensive set of stereological fundamental formulae. *Journal of Microscopy, 107* (3), 211–226.

CONNECTIVITY ASSAY

Euler number (X_3)	$X_3 = I + H - B$	I Total island markers H Total hole markers B Total bridge markers
Number of alveoli (N_{alv})	$N_{alv} = -X_3$	X_3 Euler number
Sum counting frame volumes (V)	$V = h.n.a$	h Disector height n Number of disectors a Area counting frame
Numerical density of alveoli (N_v)	$N_v = \frac{N_{alv}}{V}$	N_{alv} Number of alveoli V Sum counting frame volumes

References

Ochs, M., Nyengaard, J.R., Jung, A., Knudsen, L., Voigt, M., Wahlers, T., Richter, J., & Gundersen, H.J.G. (2004). The number of alveoli in the human lung. *American journal of respiratory and critical care medicine, 169* (1), 120–124.

CYCLOIDS FOR SV

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 Evaluation interval $\bar{t}$ Section cut thickness
Estimated surface area per unit volume ($est\ S_v$)	$est\ S_v = 2\left(\frac{2p}{l}\right)\frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	p/l Points per unit length of cycloid I_i Intercepts with cycloids P_i Point counts
Estimated volume ($\hat{V}$)	$\hat{V} = m\bar{t}\left(\frac{a}{p}\right)\sum_{i=1}^{m} P_i$	m Evaluation interval $\bar{t}$ Section cut thickness a/p Area associated with each point P_i Point counts
Estimated surface area ($\hat{S}$)	$\hat{S} = 2\left(\frac{a}{l}\right)m\bar{t}\sum_{i=1}^{m} I_i$	m Evaluation interval $\bar{t}$ Section cut thickness a/l Area per unit length I_i Intercepts with cycloids

Cycloids for Sv (2)

Coefficient of error for estimated surface (CE)	$CE(\hat{S} \mid S) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	$\mathrm{Var}_{\mathrm{SRS}}$ Variance due to systematic random sampling $\mathrm{Var}_{\mathrm{SRS}} = \frac{3\mathrm{g}_0 - 4\mathrm{g}_1 + \mathrm{g}_2}{12}$
Coefficient of error for surface density ($CE\ (S_v)$)	$CE(S_v) = \sqrt{\frac{n}{n-1}\left(\frac{\sum_{i=1}^{n} I_i^2}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} I_i} + \frac{\sum_{i=1}^{n} P_i^2}{\sum_{i=1}^{n} P_i \sum_{i=1}^{n} P_i} - 2\frac{\sum_{i=1}^{n} I_i P_i}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} P_i}\right)}$	n Number of measurements I_i Intercepts with cycloids P_i Point counts

References

Baddeley, A. J., Gundersen, H.J.G., & Cruz-Orive, L.M. (1998) Estimation of surface area from vertical sections. *Journal of Microscopy, 142* (3), 259–276.

Howard, C. V., Reed, M.G. (1998). *Unbiased Stereology, Three-Dimensional Measurement in Microscopy*(pp.170–172). BIOS Scientific Publishers.

DISCRETE VERTICAL ROTATOR

Estimated volume **(*Est v*)**	$est\ v = \frac{\pi}{n} . a_p . \sum_{i=1}^{n} P_i\, D_i$	n Number of centriolar sections a_p Area associated with each point P_i Number of points in each class D_i Distance of class from central axis

References

Mironov, A. A. (1998). Estimation of subcellular organelle volume from ultrathin sections through centrioles with a discretized version of the vertical rotator. *Journal of microscopy*, 192(1), 29-36.

FRACTIONATOR

Estimate of total number of particles (*N*)	$N = \sum Q^- . \frac{1}{asf} . \frac{1}{ssf}$	Q^- Particles counted asf Area sampling fraction ssf Section sampling fraction
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
Number-weighted mean section cut thickness ($\overline{t_{Q^-}}$)	$\overline{t_{Q^-}} = \frac{\sum_{i=1}^{m} t_i Q_i^-}{\sum_{i=1}^{m} Q_i^-}$	m Number of sections t_i Section thickness at site i Q_i Particles counted

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	$\frac{C_{fp}F^2s^2}{f}$	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

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

Geiser, M., Cruz-Orive, L.M., Hof, V.I., & Gehr, P. (1990) Assessment of particle retention and clearance in the intrapulmonary conducting airways of hamster lungs with the fractionator. *Journal of Microscopy, 160* (1), 75–88.

Glaser, E. M., Wilson, P.D. (1998). The coefficient of error of optical fractionator population size estimates: a computer simulation comparing three estimators. *Journal of Microscopy, 192* (2), 163–171.

Gundersen, H.J.G., Vedel Jensen, E.B., Kieu, K., & Nielsen, J. (1999). The efficiency of systematic sampling in stereology—reconsidered. *Journal of Microscopy, 193* (3), 199–211.

Gundersen, H. J. G., Jensen, E.B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy, 147* (3), 229–263.

Howard, V., Reed, M. (2005). *Unbiased stereology: three-dimensional measurement in microscopy* (vol. 4, chapter 12). Garland Science/Bios Scientific Publishers.

Fractionator (4)

Scheaffer, R.L., Ott, L., & Mendenhall, W. (1996). *Elementary survey sampling* (chapter 7). Boston: PWS-Kent.

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.

MERZ

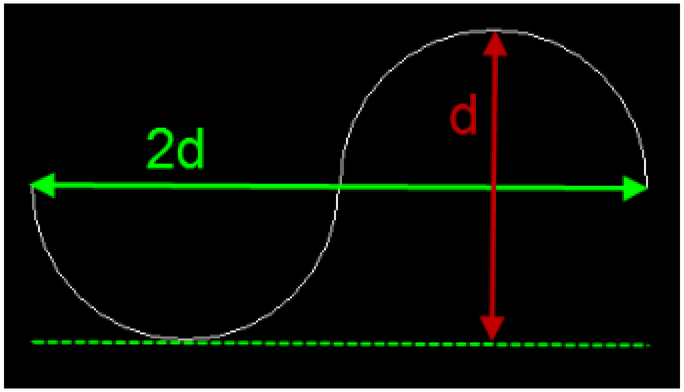
Length of semi-circle (L)	$L = \frac{1}{2}\pi d$	d Circle diameter
Surface area per unit volume (S_v)	$S_v = \frac{2\sum I}{\frac{l}{1}\sum P}$	I Number of intercepts $l/1$ Length of half-circle per point P Number of points Note: *We use $l/1$ for length since there is one point per half-circle*

References

Howard, C. V., Reed, M. G. (2010). *Unbiased stereology*. Liverpool, UK: QTP Publications. {See equation 6.4}

Weibel, E.R. (1979). *Stereological Methods. Vol. 1: Practical methods for biological morphometry*. London, UK: Academic Press.

PETRIMETRICS

Total length ($\hat{L}$)	$\hat{L} = \frac{\pi}{2} \cdot \frac{a}{l} \cdot \frac{1}{asf} \cdot \sum I$ $\hat{L} = d . \frac{1}{asf} \cdot \sum I$	$a/l = 2d/\pi$ Grid constant (2d/π units or ratio of area to length of semi-circle probe) *asf* Area fraction (ratio of area of counting frame to grid-step) *I* Number of intersections counted *d = 2*Merz-radius* where the Merz-radius refers to the radius of the semi-circle used to probe. 

References

Howard, C. V., & Reed, M. G. (2005). *Unbiased stereology*. New York: Garland Science (prev. BIOS Scientific Publishers).

PHYSICAL FRACTIONATOR

Total number of particles (N)	$N = \sum Q^{-} \cdot \frac{1}{asf} \cdot \frac{1}{ssf}$	Q^{-} Particles counted asf Area sampling fraction ssf Section sampling fraction
Variance due to noise ($s2$)	$s^2 = \sum_{i=1}^{n} Q^{-}$	Q^{-} Particles counted n Number of sections used
Variance due to systematic random sampling (VAR_{SRS})	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} (Q_i^{-})^2$ $B = \sum_{i=1}^{n-1} Q_i^{-} Q_{i+1}^{-}$ $C = \sum_{i=1}^{n-2} Q_i^{-} Q_{i+2}^{-}$ s^2 Variance due to noise
Total variance ($TotalVar$)	$TotalVar = s^2 + VAR_{SRS}$	VAR_{SRS} Variance due to SRS s^2 Variance due to noise
Coefficient of error (CE)	$CE = \frac{\sqrt{TotalVar}}{s^2}$	$TotalVar$ Total variance s^2 Variance due to noise

References

Gundersen, Hans-Jørgen G. "Stereology of arbitrary particles*." Journal of Microscopy 143, no. 1 (1986): 3-45.

Sterio, D. C. "The unbiased estimation of number and sizes of arbitrary particles using the disector." Journal of Microscopy 134, no. 2 (1984): 127-136.

POINT SAMPLED INTERCEPT

Volume based on intercept length ($\widehat{V_v}$)	$\hat{\hat{V}}_V = \frac{\pi}{3}\bar{l}_0^3 = \frac{\pi}{3n}\sum_{i=1}^{n} l_{0,i}^3$	n Number of intercepts l Intercept length
Volume-weighted mean volume ($\bar{v}_V$)	$\boldsymbol{\bar{v}_V = \frac{\sum_{i=1}^{n}\bar{l}_0^3}{n}.\frac{\pi}{3}}$	n Number of intercepts l Intercept length
Coefficient of error (CE)	$CE(\bar{l}_0^3) = \sqrt{\frac{\sum_{i=1}^{n}(\bar{l}_0^3)^2}{(\sum_{i=1}^{n}\bar{l}_0^3)^2} - \frac{1}{n}}$	n Number of intercepts l Intercept length
Coefficient of variance (CV)	$CE(\bar{l}_0^3) = CE(\bar{v}_V).\sqrt{n}$	n Number of intercepts l Intercept length $\bar{v}_V$ Volume-weighted mean volume
Variance ($Variance_V$)	$Variance_V(v) = \left[\frac{\pi}{3}.SD(\bar{l}_0^3)\right] = \left[CV(\bar{l}_0^3).\bar{v}_V\right]$	L Intercept length $\bar{v}_V$ Volume-weighted mean volume CV Coefficient of variance

References

Gundersen, H.J.G., Jensen. E.B. (1985). Stereological Estimation of the Volume-Weighted Mean Volume of Arbitrary Particles Observed on Random Sections. *Journal of Microscopy, 138*, 127–142.

Sørensen, F.B. (1991). Stereological estimation of the mean and variance of nuclear volume from vertical sections. *Journal of Microscopy, 162* (2), 203–229.

SURFACE-WEIGHTED STAR VOLUME

Surface-weighted star volume ($\hat{v}_s^*$)	$\boldsymbol{\hat{v}_s^* = \frac{2\pi}{3} . \bar{l}_1^3}$ $\boldsymbol{\hat{v}_s^* = \frac{2\pi}{3} . \frac{\sum_{i=1}^{n} \sum_{j=1}^{m_i} l_{1,(i,j)}^3}{\sum_{i=1}^{n} m_i}}$	n Number of probes l Intercept length m_i Number of intercepts
Sum of cubed intercepts in probe (y_i)	$y_i = \sum_{j=1}^{m_i} l_{1,(i,j)}^3$	m_i Number of intercepts l Intercept length
Coefficient of error (CE)	$CE[\hat{v}_s^*] = \left[\frac{n}{n-1}\left\{\frac{\sum y_i^2}{\sum y_i \sum y_i} + \frac{\sum m_i^2}{\sum m_i \sum m_i} - 2.\frac{\sum m_i y_i}{\sum y_i \sum m_i}\right\}\right]^{1/2}$	n Number of probes y_i Sum of cubed intercepts in probe m_i Number of intercepts

References

Reed, M. G., Howard, C.V. (1998). Surface-weighted star volume: concept and estimation. *Journal of Microscopy, 190* (3), 350–356.

WEIBEL

Surface area per unit volume (S_V)	$$S_v = \frac{2\sum I}{\frac{l}{2}\sum P}$$	I Intersections (triangular markers) P Points (end points circular markers) l Length of each line *Note: We use l/2 for the length represented at each point since there are two end points per line.*

References

Weibel, E.R., Kistler, G.S., & Scherle, W.F. (1966). Practical stereological methods for morphometric cytology. *The Journal of cell biology, 30* (1), 23–38.