Stereo Investigator Stereological Formulas

Area Fraction Fractionator

Estimated volume fraction	$\hat{V}_v(y, ref) = \frac{\sum_{i=1}^{m} P(Y)_i}{\sum_{i=1}^{m} P(ref)_i}$	*P(ref)* Points hitting reference volume *Y* Sub-region *P(Y)* Points hitting sub-region
Estimated area	$\hat{A} = \frac{1}{asf} * a(p) * P(Y_i)$	*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	$A_p = g^2$	g^2: Grid area
Volume associated with a point	$V_p = g^2 m\bar{t}$	g^2: Grid area m: Section evaluation interval $\bar{t}$: Mean section cut thickness
Estimated volume	$\hat{V} = A_p m'\bar{t}\left(\sum_{i=1}^{n} P_i\right)$	A_p: Area associated with a point g: Grid size m': Section evaluation interval $\bar{t}$: Mean section cut thickness P_i: Points counted on grid
Estimated volume corrected for over-projection	$[v] = t \cdot \left(k \cdot \sum_{j=1}^{g} a'_j - \max(a')\right)$	t: Section cut thickness k: Correction factor g: Grid size a': Projected area
Variance of systematic random sampling	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} {P_i}^2$, $B = \sum_{i=1}^{n-1} P_i P_{i+1}$, $C = \sum_{i=1}^{n-2} P_i P_{i+2}$ n: Number of sections s^2: Variance due to noise $s^2 = 0.0724(\frac{b}{\sqrt{a}})\sqrt{n\sum_{i=1}^{n} P_i}$ where $\frac{b}{\sqrt{a}}$ is the shape factor m: Smoothness class of sampled function

Cavalieri Estimator

Coefficient of Error	$CE = \frac{\sqrt{TotalVar}}{\sum_{i=1}^{n} P_i}$	$TotalVar$: Total variance of the estimated volume $TotalVar = s^2 + VAR_{SRS}$ n: Number of sections P_i: Points counted on grid

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.

Howard, C. V., & Reed, M.G. (2005). *Unbiased Stereology, Three-Dimensional Measurement in Microscopy* (Chapter 3). New York: Garland Science/BIOS Scientific Publishers.

Combined Slope Intercept

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

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

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 Lv

Area associated with a point	$A_p = g^2$	g^2: Grid area
Volume associated with a point	$V_p = g^2 m\bar{t}$	m: Section evaluation interval $\bar{t}$: Mean section cut thickness
Length per unit volume	$L_V = 2\frac{[\bar{I}_L^c]_{prj}}{\Delta}$ $L_V = \frac{2}{\Delta}\cdot\frac{(\bar{I}_c^{cyc})_{prj}}{\bar{P}\cdot\left(\frac{l}{p}\right)} = \frac{2}{\Delta}\left(\frac{p}{l}\right)\frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	$[\bar{I}_L^C]_{prj}$: 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 p/l: Test points per unit length of cycloid
Estimated volume	$\hat{V} = m\Delta\left(\frac{a}{p}\right)\sum_{i=1}^{n} P_i$	m: Sampling fractions Δ: Section cut thickness a: Area p: Number of test points
Estimated length	$\hat{L} = 2\left(\frac{a}{l}\right)m\sum_{i=1}^{n} I_i$	a: Area l: Line length m: Sampling fractions I_i: Intercepts
Coefficient of Error for line length	$CE(\hat{L}\|L) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	VAR_{SRS}: Variance of systematic random sampling $\hat{L}\|L$: Estimated length per length I_i: Intercepts

Cycloids for Lv

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
Variance of systematic random sampling (VAR_{SRS}):	$VAR_{SRS} = \frac{3g_0 - 4g_1 + g_2}{12}$	g: grid size $g_k = \sum_{i=1}^{n-k} L_i L_{i+k}$ L_i: Line length at section i

References

Artacho-Pérula, E., & Roldán-Villalobos, R. (1995). Estimation of capillary length density in skeletal muscle by unbiased stereological methods: I. Use of vertical slices of known thickness. *The Anatomical Record, 241* (3), 337–344.

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

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

Cycloids for Sv

Area associated with a point	$A_p = g^2$	g^2: Grid area
Volume associated with a point	$V_p = g^2 m\bar{t}$	m: Section evaluation interval $\bar{t}$: Mean section cut thickness
Estimated surface area per unit volume	$est\ S_v = 2 * \left(\frac{2p}{l}\right) * \frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	$\frac{p}{l}$: Points per unit length of cycloid I_i : Intercepts with cycloids P_i: Point counts
Estimated volume	$\hat{V} = m\bar{t} * \frac{a}{p} * \sum_{i=1}^{m} P_i$	m: Section evaluation interval $\bar{t}$: Mean section cut thickness $\frac{a}{p}$: Area associated with each point P_i: Point counts
Estimated surface area	$\hat{S} = 2 * \frac{a}{l} * m\bar{t} * \sum_{i=1}^{m} I_i$	$\frac{a}{l}$: Area per unit length m: Section evaluation interval $\bar{t}$: Mean section cut thickness I_i : Intercepts with cycloids
Coefficient of Error for estimated surface	$CE(\hat{S}\|S) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	I_i: Intercepts with cycloids
Variance due to systematic random sampling	$VAR_{SRS} = \frac{3g_0 - 4g_1 + g_2}{12}$ $g_k = \sum_{i=1}^{n-k} S_i * S_{i+k}$	g_0, g_1, g_2: covariogram values S_i: Surface area at section i

Coefficient of Error for surface density	$CE(S_V) = \sqrt{\frac{n}{n-1}\left(\frac{\sum_{i=1}^{n} I_i^2}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} I_i} + \frac{\sum_{i=1}^{n} P_i^2}{\sum_{i=1}^{n} P_i \sum_{i=1}^{n} P_i} - 2\frac{\sum_{i=1}^{n} I_i P_i}{\sum_{i=1}^{n} I_i \sum_{i=1}^{n} P_i}\right.}$	n: Number of measurements I_i : Intercepts 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* (170–172). Milton Park, England: BIOS Scientific Publishers.

Discrete Vertical Rotator

Estimated volume	$$est\ v = \frac{\pi}{n} * a_p * \sum_{i=1}^{n} P_i . D_i$$	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

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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 & Optical Fractionator

Estimate of total number of particles	$Optical\ Fractionator\ (3D): N = \sum Q^- * \frac{t}{h} * \frac{1}{asf} * \frac{1}{ssf}$ $Fractionator\ (2D): N = \sum Q^- * \frac{1}{asf} * \frac{1}{ssf}$	Q-: Particles counted t: Section mounted thickness h: Counting frame height asf: Area sampling fraction ssf: Section sampling fraction
Variance due to systematic random sampling (Gundersen)	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} (Q_i^-)^2$ $B = \sum_{i=1}^{n-1} Q_i^- * Q_{i+1}^-$ $C = \sum_{i=1}^{n-2} Q_i^- * Q_{i+2}^-$
Variance due to noise (Gundersen)	$s^2 = \sum_{i=1}^{n} Q^-$	Q-: Particles counted
Total variance (Gundersen)	$TotalVar = s^2 + VAR_{SRS}$	VAR_{SRS}: Variance due to systematic random sampling s^2: Variance due to noise
Coefficient of Error (Gundersen)	$CE = \frac{\sqrt{TotalVar}}{s^2}$	$TotalVar$: Total variance s^2: Variance due to noise

Fractionator & Optical Fractionator

Number weighted mean section cut thickness	$\overline{t_{Q^-}} = \frac{\sum_{i=1}^{m} t_i Q_i^-}{\sum_{i=1}^{m} Q_i^-}$	m: Number of sections t_i : Section thickness at site i Q_i : Particles counted
Coefficient of error (Sheaffer)	$CE = \frac{\sqrt{\left(\frac{1}{f} - \frac{1}{F}\right) * s^2}}{\bar{Q}}$	f: Number of counting frames F: Total possible sampling sites s^2 : Estimated variance $\bar{Q}$: Average particles counted
Average number of particles (Scheaffer)	$\bar{Q} = \frac{\sum_{i=1}^{f} Q_i}{f}$	Q_i : Particles counted f: Number of counting frames
Estimated variance (Sheaffer)	$s^2 = \frac{\sum_{i=1}^{f} (Q_i - \bar{Q})^2}{f - 1}$	f: Number of counting frames Q_i : Particles counted $\bar{Q}$: Average particles counted
Estimated variance of estimated cell population (Sheaffer)	$\frac{C_{fp} F^2 s^2}{f}$	C_{fp}: Finite population correction F: Total possible sampling sites f: Number of counting frames
Estimated variance of mean cell count (Sheaffer)	$\frac{C_{fp} s^2}{f}$	C_{fp} : Finite population correction f: Number of counting frames
Estimated mean coefficient of error (Cruz-Orive)	$est\ Mean\ CE\ (est\ N) = \left[\frac{1}{3n} * \sum_{i=1}^{n} \left(\frac{Q_{1i} - Q_{2i}}{Q_{1i} Q_{2i}}\right)^2\right]^{1/2}$	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)	$$CE_{pred}(n_F) = \sqrt{\frac{Var(Q_r^-)}{R * (\overline{Q_r^-})^2}}$$ $$CE_{pred}(n_F) = \frac{1}{\sqrt{\sum_{r=1}^{R} Q_r^-}} = \frac{1}{\sqrt{\sum_{s=1}^{S} Q_s^-}}$$	R: Number of counting spaces S: Number of sections Q_S^- : Counts in the "s"-th section Q_R^- : Counts in the "r"-th counting space

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

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Scheaffer, R., Mendenhall, W., Lyman Ott, B., & Gerow, K. (1996). *Elementary survey sampling* (chapter 7). Boston: PWS-Kent Publishing.

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.

Isotropic Fakir

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

References

Kubínová, L., & Janacek, J. (1998). Estimating surface area by the isotropic fakir method from thick slices cut in an arbitrary direction. *Journal of Microscopy, 191* (2), 201–211.

Isotropic Virtual Planes

Length per unit volume	$$L_v = \frac{2p(box)}{a(plane)} * \frac{\sum Q}{\sum p(ref)}$$	*p(box)*: Number of corners considered *a(plane)*: Exact sampling area *p(ref)*: Number of corners in region $\sum Q$: Total number of transects
Estimated total length	$$L_2 = \frac{1}{ssf} * \frac{1}{asf} * \frac{1}{hsf} * \frac{1}{psd} * 2\sum Q$$ or $$L_2 = \frac{1}{ssf} * \frac{dx * dy}{a(box)} * \frac{\bar{t}}{h(box)} * d * 2\sum Q$$ $$asf = \frac{a(box)}{dx * dy}$$ $$hsf = \frac{h(box)}{\bar{t}}$$ $$psd = \frac{E[a(plane)]}{v(box)}$$	*ssf*: Section sampling fraction *asf*: Area sampling fraction *hsf*: Height sampling fraction *psd*: Probe sampling density $\sum Q$: Total number of transects *a(box)*: Area of sampling box *h(box)*: Depth of sampling box *d*: Sampling plane separation *dx,dy*: Distances in x,y directions between systematically sampled fields $\bar{t}$: Average section thickness *a(plane)*: Sampling plane area *E*: Expected value *v(box)*: Volume of sampling box
Total plane area	$$A = \sum_{i=1}^{l} \sum_{j=1}^{s} A_{i,j}$$	*l*: Number of layouts *s*: Number of sampling sites $A_{i,j}$: Plane area inside of each sampling box for each layout

Isotropic Virtual Planes

Coefficient of error (Gundersen)	$$CE = \frac{\sqrt{\frac{3(A - Poisson\ noise) - 4B + C}{12} + Poisson\ noise}}{\sum Q}, m = 0$$ $$CE = \frac{\sqrt{\frac{3(A - Poisson\ noise) - 4B + C}{240} + Poisson\ noise}}{\sum Q}, m = 1$$	$$Poisson\ noise = \sum Q_i$$ $$A = \sum Q_i^2$$ $$B = \sum (Q_i * Q_{i+1})$$ $$C = \sum (Q_i * Q_{i+2})$$ A, B, C: Covariogram values Q_i : Particles counted
Plane areas	$$\vec{V} = (A, B, C)$$ $$Planes: Ax + By + Cz + D_i$$ $$D_i = D + (d * i)$$ $$Box: \left\{ \left((x, y, z) \middle\| \begin{matrix} x_0 \leq x \leq x_0 + b_x \\ y_0 \leq y \leq y_0 + b_y \\ z_0 \leq z \leq z_0 + b_z \end{matrix} \right) \right\}$$ $$Area: (box \bigcap planes)$$	A, B, C, D: Given constants d: Distance between planes i: Integer x_0, y_0, z_0: Vertex of a sampling box b_x, b_y, b_z: Dimensions of a sampling box
Average number of counts	$$\bar{Q} = \frac{\sum_{i=1}^{p} \sum_{j=1}^{l_j} Q_{ij}}{\sum_{j=1}^{p} l_j}$$	p: Number of probes l_j: Number of layouts in each probe Q_{ij}: Number of counts in each probe and layout

Total corners of sampling boxes inside the region of interest	$$C = \sum_{i=1}^{p} C_i$$	p: Number of probes C_i : Number of sampling boxes inside the region of interest

References

Larsen, J. O., Gundersen, H. J. G., & Nielsen, J. (1998). Global spatial sampling with isotropic virtual planes: estimators of length density and total length in thick, arbitrarily orientated sections. *Journal of Microscopy, 191* (3), 238-248.

IUR Planes Optical Fractionator

Estimated length	$$est\ L = 2 * \frac{a}{l} \sum I * \frac{1}{ssf} * \frac{1}{asf} * \frac{t}{h}$$	$\frac{a}{l}$: Area per unit length of test line $\sum I$: Number of intersections *ssf*: Section sampling fraction *asf*: Area sampling fraction *t*: Section cut thickness *h*: Height of counting frame
Area sampling fraction	$$asf = \frac{area(Frame)}{area(x, y\ step)} = \frac{x_{CF} * Y_{CF}}{x_{step} * y_{step}}$$	X_{CF}, Y_{CF}: X,Y dimensions of counting frame X_{step}, Y_{step}: X,Y dimensions of grid *area(Frame)*: Area of counting frame *area(x,y,step)*: Area of grid

L-Cycloid Optical Fractionator

Estimated length of lineal structure	$est\ L = 2 * \frac{a}{l} * \sum I * \frac{1}{ssf} * \frac{1}{asf} * \frac{t}{h}$	$\frac{a}{l}$: Area per unit cycloid length $\sum I$: Total intercepts *ssf*: Section sampling fraction *asf*: Area sampling fraction *t*: Section cut thickness *h*: Height of counting frame
Area sampling fraction	$asf = \frac{area(Frame)}{area(x, y\ step)} = \frac{x_{CF} * y_{CF}}{x_{step} * y_{step}}$	*area(Frame)*: Area of counting frame *area(x,y step)*: Area of grid x_{CF}, y_{CF}: x,y dimensions of counting frame x_{step}, y_{step}: x,y dimensions of grid

References

Stocks, E. A, McArthur, ., J. C. , Griffen, J. W. & Mouton, P. R. (1996). An unbiased method for estimation of total epidermal nerve fiber length. *Journal of Neurocytology, 25* (1), 637–644.

Merz

Length of semi-circle	$L = \frac{1}{2}\pi d$	d: Circle diameter
Surface area per unit volume	$S_v = \frac{2\sum I}{\frac{l}{p}\sum P}$	I: Number of intercepts l/p: Length of semi-circle per point P: Number of points

References

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

Fritsch, R. S., Weibel, E.R. (1981). Stereological Methods, Vol. 1: Practical Methods for Biological Morphometry. *Zeitschrift für allgemeine Mikrobiologie, 21*(8), 630–630.

Nucleator

Area estimation	$a = \pi l^2$	l: Mean length of rays
Volume estimation	$V_n = \frac{4\pi}{3} l_n^3$	l: Mean length of rays
Estimated coefficient of error	$estCV(R) = \frac{\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(R_i - \bar{R})^2}}{\bar{R}}$	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	$CE_n(R) = \frac{CV(R)}{\sqrt{n}}$	CV(R): Estimated coefficient of variation n: Number of nucleator estimates
Geometric mean of the area/volume estimate	$e^{(\frac{1}{n}\sum_{i=1}^{n} \ln R_i}$	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 Fractionator & Fractionator

Estimate of total number of particles	$Optical\ Fractionator\ (3D): N = \sum Q^{-} * \frac{t}{h} * \frac{1}{asf} * \frac{1}{ssf}$ $Fractionator\ (2D): N = \sum Q^{-} * \frac{1}{asf} * \frac{1}{ssf}$	Q-: Particles counted t: Section mounted thickness h: Counting frame height asf: Area sampling fraction ssf: Section sampling fraction
Variance due to systematic random sampling (Gundersen)	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} (Q_i^{-})^2$ $B = \sum_{i=1}^{n-1} Q_i^{-} * Q_{i+1}^{-}$ $C = \sum_{i=1}^{n-2} Q_i^{-} * Q_{i+2}^{-}$
Variance due to noise (Gundersen)	$s^2 = \sum_{i=1}^{n} Q^{-}$	Q-: Particles counted
Total variance (Gundersen)	$TotalVar = s^2 + VAR_{SRS}$	VAR_{SRS}: Variance due to systematic random sampling s^2: Variance due to noise
Coefficient of Error (Gundersen)	$CE = \frac{\sqrt{TotalVar}}{s^2}$	$TotalVar$: Total variance s^2: Variance due to noise

Optical Fractionator & Fractionator

Number weighted mean section cut thickness	$$\overline{t_{Q^-}} = \frac{\sum_{i=1}^{m} t_i Q_i^-}{\sum_{i=1}^{m} Q_i^-}$$	m: Number of sections t_i : Section thickness at site i Q_i : Particles counted
Coefficient of error (Sheaffer)	$$CE = \frac{\sqrt{\left(\frac{1}{f} - \frac{1}{F}\right) * s^2}}{\bar{Q}}$$	f: Number of counting frames F: Total possible sampling sites s^2 : Estimated variance $\bar{Q}$: Average particles counted
Average number of particles (Scheaffer)	$$\bar{Q} = \frac{\sum_{i=1}^{f} Q_i}{f}$$	Q_i : Particles counted f: Number of counting frames
Estimated variance (Sheaffer)	$$s^2 = \frac{\sum_{i=1}^{f} (Q_i - \bar{Q})^2}{f - 1}$$	f: Number of counting frames Q_i : Particles counted $\bar{Q}$: Average particles counted
Estimated variance of estimated cell population (Sheaffer)	$$\frac{C_{fp} F^2 s^2}{f}$$	C_{fp}: Finite population correction F: Total possible sampling sites f: Number of counting frames
Estimated variance of mean cell count (Sheaffer)	$$\frac{C_{fp} s^2}{f}$$	C_{fp} : Finite population correction f: Number of counting frames
Estimated mean coefficient of error (Cruz-Orive)	$$est\ Mean\ CE\ (est\ N) = \left[\frac{1}{3n} * \sum_{i=1}^{n} \left(\frac{Q_{1i} - Q_{2i}}{Q_{1i} Q_{2i}}\right)^2\right]^{1/2}$$	Q_{1i} : Counts in sub-sample 1 Q_{2i} : Counts in sub-sample 2 n: Size of sub-sample

Optical Fractionator & Fractionator

Predicted coefficient of error for estimated population (Schmitz-Hof)	$CE_{pred}(n_F) = \sqrt{\frac{Var(Q_r^-)}{R * (\overline{Q_r^-})^2}}$ $CE_{pred}(n_F) = \frac{1}{\sqrt{\sum_{r=1}^{R} Q_r^-}} = \frac{1}{\sqrt{\sum_{s=1}^{S} Q_s^-}}$	R: Number of counting spaces S: Number of sections Q_S^- : Counts in the "s"-th section Q_R^- : Counts in the "r"-th counting space

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.

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

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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.

Optical Rotator

Volume of particle	$\hat{v} = a \sum_{i}^{+/-} g(P_i)$ $a = k * h$	a: Reciprocal line density k : Length of slice h : Systematic spacing
For vertical slabs and lines parallel to vertical axis	$g(p) = \left\{ \begin{array}{c\|c} d_1 & d_2 < t \\ \dfrac{\frac{\pi}{2} d_1}{arcsin\left(\frac{t}{d_2}\right)} & t \leq d_2 \end{array} \right\}$	$d1$: Distance along test line $d2$: Distance from origin to test line t: Thickness of optical slice z: Distance in z from intercept to origin
For vertical slabs and lines perpendicular to vertical axis	$g(P) = \left\{ \begin{array}{c\|c} d_1 & \sqrt{d_1^2 + z^2} < t \\ f\left(\sqrt{t^2 - z^2}\right) & \|z\| < t \leq \sqrt{d_1^2 + z^2} \\ f(0) & t \leq \|z\| \end{array} \right\}$ $f(x) = x + \frac{\pi}{2} \int_x^{d_1} \frac{1}{\arcsin(\frac{t}{\sqrt{u^2 + z^2}})} * du$	$d1$: Distance along test line t: Thickness of optical slice z: Distance in z from intercept to origin

For isotropic slabs	$$g(P) = \begin{cases} d_1 & d_3 < t \\ \frac{1}{2t}[h(t, d_2) + k(t, d_1, d_2, d_3)] & d_2 < t \le d_3 \\ \frac{1}{2t}\left[d_1 d_3 + d_2^2 \log\left(\frac{d_1 + d_3}{t + \sqrt{t^2 - d_2^2}}\right)\right] & t \le d_2 \end{cases}$$ $$h(t, d) = t^2 \sqrt{1 - \frac{d^2}{t^2}}$$ $$k(t, d_1, d_2, d_3) = d_1 d_3 + d_2^2 \log\left(\frac{d_1 + d_3}{t + \sqrt{t^2 - d_2^2}}\right)$$	$d1$: Distance along test line $d2$: Distance from origin to test line $d3$: Distance from intercept to origin t: Thickness of optical slice
Estimated surface area	$$\hat{s} = a \sum_j l_j g(l_l)$$ $$g(l) = \begin{cases} \pi * \frac{2}{\frac{1}{\arcsin(\frac{t}{d_2})}} & d_2 < t \\ & t \le d_2 \end{cases}$$	a: Reciprocal line density l_j: Number of intersections between grid line and cell boundary d_2: Distance from origin to test line t: 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.

Petrimetrics

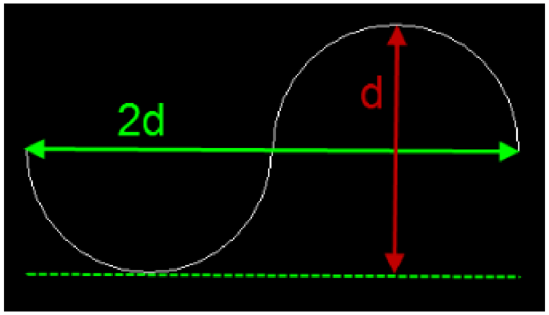
<table>
<tr>
<td>Total length</td>
<td>

$$\hat{L} = \frac{\pi}{2} * \frac{a}{l} * \frac{1}{asf} * \sum I$$

$$\hat{L} = d * \frac{1}{asf} * \sum I$$

</td>
<td>

a/l = 2d/π: 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: The Merz-radius refers to the radius of the semi-circle used to probe.



</td>
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</table>

References

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

Physical Fractionator

Total number of particles	$$N = \sum Q^{-} * \frac{1}{asf} * \frac{1}{ssf}$$	Q^{-}: Particles counted asf: Area sampling fraction ssf: Section sampling fraction
Variance due to noise	$$s^2 = \sum_{i=1}^{n} Q^{-}$$	n: Number of sections used Q^{-}: Particles 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	$TotalVar = s^2 + VAR_{SRS}$	s^2: Variance due to noise VAR_{SRS}: Variance due to systematic random sampling
Coefficient of error	$$CE = \frac{\sqrt{TotalVar}}{s^2}$$	$TotalVar$: Total variance s^2: Variance due to noise

References

Gundersen, H.J.G. (1986). Stereology of arbitrary particles. *Journal of Microscopy, 143* (1), 3–45.

Sterio, D. C. (1984). The unbiased estimation of number and sizes of arbitrary particles using the disector. *Journal of Microscopy 134* (2), 127–136.

Planar Rotator

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

References

Jensen, E.B., Gundersen. H.J.G. (1993). The rotator. *Journal of Microscopy 170* (1), 35–44.

Point Sampled Intercept

Volume based on intercept length	$\hat{V}_V = \frac{\pi}{3}\overline{l_0^3} = \frac{\pi}{3n}\sum_{i=1}^{n} l_{0,i}^3$	n: Number of intercepts l: Intercept length
Volume weighted mean volume	$\bar{v}_V = \frac{\sum_{i=1}^{n}\overline{\overline{l_0^3}}}{n} * \frac{\pi}{3}$	n: Number of intercepts l: Intercept length
Coefficient of error	$CE(\overline{l_0^3}) = \sqrt{\frac{\sum_{i=1}^{n}\left(\overline{l_0^3}\right)^2}{\left(\sum_{i=1}^{n}\overline{l_0^3}\right)^2} - \frac{1}{n}}$	n: Number of intercepts l: Intercept length
Coefficient of variance	$CV\left(\overline{l_0^3}\right) = CE(\bar{v}_V) * \sqrt{n}$	l: Intercept length $\bar{v}_V$: Volume weighted mean volume n: Number of intercepts
Variance	$Variance\ V(v) = \left[\frac{\pi}{3} * SD(\overline{l_0^3})\right] = \left[CV\left(\overline{l_0^3}\right) * \bar{v}_V\right]$	l: Intercept length $CV(\overline{l_0^3})$: Coefficient of variance $\bar{v}_V$: Volume weighted mean volume

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.

Size Distribution

Volume-weighted mean particle volume	$\bar{v}_V = \bar{v}_N * [1 + CV_N^2(v)]$	$\bar{v}_N$: Number-weighted mean volume $CV_N(v)$: Coefficient of variation
Number-weighted mean volume	$\bar{v}_N = \frac{\sum S}{\sum R}$ $S = Q * R$ $T = Q^2 * R$	R: Number of contours Q: Number of points per contour
Variance	$Var_N(v) = \frac{\left[\sum T - \frac{(\sum S)^2}{\sum R}\right] * v(p)^2}{\sum R - 1}$	*v(p)*: Volume associated with a point
Standard deviation	$SD_N(v) = \sqrt{Var_N(v)}$ $SD_N(v) = \sqrt{\bar{v}_N * (\bar{v}_V - \bar{v}_N)}$	$\bar{v}_N$: Number-weighted mean volume $\bar{v}_V$: Volume-weighted particle volume
Coefficient of variation	$CV_N(v) = \frac{SD_N(v)}{\bar{v}_N}$ $CV_N(v) = \sqrt{\frac{\bar{v}_V - \bar{v}_N}{\bar{v}_N}}$	SD_N: Standard deviation $\bar{v}_N$: Number-weighted mean volume $\bar{v}_V$: Volume-weighted particle volume
Coefficient of error	$CE_N(v) = \frac{CV_N(v)}{\sqrt{R}}$	$CV_N(v)$: Coefficient of variation R: Number of contours

References

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

Spaceballs

Length estimate	$$L = 2 * \left(\sum_{i=1}^{n} Q_i\right) * \frac{v}{a} * \frac{1}{ssf}$$ *This equation is equivalent to the equation used by Mouton et al.*	n: Number of sections used Q_i: Intersections counted v: Volume (grid X * grid Y * section thickness) a: Surface area of the sphere ssf: Section sampling fraction
Variance due to noise	$$s^2 = \sum_{i=1}^{n} Q_i$$	Q_i: Intersections 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	$$TotalVar = s^2 + VAR_{SRS}$$	s^2: Variance due to noise VAR_{SRS}: Variance due to systematic random sampling

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

References

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

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.

Surface weighted star volume

Surface-weighted star volume	$\hat{\bar{v}}_S^* = \frac{2\pi}{3} * \overline{l_1^3}$ $\hat{\bar{v}}_S^* = \frac{2\pi}{3} * \frac{\sum_{i=1}^{n}\sum_{j=1}^{m_i} l_{1,(i,j)}^3}{\sum_{i=1}^{n} m_i}$	l: Intercept length m_i: Number of intercepts n: Number of probes
Sum of cubed intercepts in probe	$y_i = \sum_{j=1}^{m_i} l_{1,(i,j)}^3$	l: Intercept length m_i: Number of intercepts
Coefficient of error	$CE[\hat{\bar{v}}_S^*] = \left[\frac{n}{n-1}\left\{\frac{\sum y_i^2}{\sum y_i \sum y_i} + \frac{\sum m_i^2}{\sum m_i \sum m_i} - 2\frac{\sum m_i * y_i}{\sum m_i \sum y_i}\right\}\right]^{1/2}$	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.

Surfactor

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

Sv-Cycloid Fractionator

Estimated surface area per unit volume	$S_V = 2\left(\frac{p}{l}\right) * \frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	p/l: Ratio of test points to curve length $\sum I_i$: Total intercept points on curve $\sum P_i$: Total test points n: Number of micrographs

References

Baddeley, A. J., Gundersen, H.J.G., & Cruz-Orive, L.M. (1986). Estimation of surface area from vertical sections. *Journal of Microscopy, 142* (3), 259–276.

Vertical Spatial Grid

Estimated volume	$\hat{V} = a * d_z * \sum_{i=1}^{m} P_i$	a: Area associated with point d_z: Distance between Z planes m: Number of scanning planes $\sum P_i$: Intersections with points
Area associated with point	$a = \frac{w^2}{2\pi}$	w: Horizontal width
Estimated surface area	$\hat{S} = 2 * \frac{a * d_z}{l + \frac{4}{\pi} * d_z} * (I_{xy} + I_{xz})$	a: Area associated with point d_z: Distance between Z planes l: Length of cycloid I_{xy}: X,Y intersections I_{xz}: X,Z intersections
Length of cycloid	$l = \frac{2w}{\pi}$	w: Horizontal width
X,Y intersections	$I_{xy} = \sum_{i=1}^{m} I_{xy,i}$	m: Number of scanning planes
X,Z intersections	$I_{xz} = 2 * \left(\sum_{i=1}^{m} P_i - \sum_{i=1}^{m-1} P_{i,i+1} \right)$	m: Number of scanning planes $\sum P_i$: Intersections with points

References

Cruz-Orive, L. M., Howard, C.V. (1995). Estimation of individual feature surface area with the vertical spatial grid. *Journal of Microscopy, 178* (2), 146–151.

Weibel

Surface area per unit volume	$S_V = 2N_i = \frac{2N}{d}$	d: Length of test line N_i: Number of intercepts per total length N: Number of intercepts

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.