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

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.

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

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 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)	$L_V = 2\frac{[\bar{I}_L^C]_{prj}}{\Delta}$ $L_V = \frac{2}{\Delta}.\frac{(\bar{I}_c^{cyc})_{prj}}{\bar{P}.\left(\frac{l}{p}\right)} = \frac{2}{\Delta}\left(\frac{p}{p}\right)\frac{\sum_{i=1}^{n} I_i}{\sum_{i=1}^{n} P_i}$	$[\bar{\mathrm{I}}_{\mathrm{L}}^{\mathrm{C}}]_{\mathrm{prj}}$ Number of counting frames Δ Section cut thickness I_i Intercepts P_i Test points $[\bar{\mathrm{I}}_{\mathrm{C}}^{\mathrm{cyc}}]_{\mathrm{prj}}$ Average number of intersections of projected images $\frac{\mathrm{p}}{\mathrm{l}}$ 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	$CE(\hat{L}\|L) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	VAR_{SRS} Variance of systematic random sampling $\hat{L}\|L$ Estimated length per length I_i Intercepts
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

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* (pp. 170–172). BIOS Scientific Publishers.

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)	$CE(\hat{S}\|S) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i}$	$\mathrm{Var}_{\mathrm{SRS}}$ 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)	$N = \sum Q^- \cdot \frac{1}{asf} \cdot \frac{1}{ssf}$	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.

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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.

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

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	$L_V = \frac{2p(box)}{a(plane)} \cdot \frac{\sum Q}{\sum p(ref)}$	$p(box)$ 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	$L = \frac{1}{ssf} \cdot \frac{1}{asf} \cdot \frac{1}{hsf} \cdot \frac{1}{psd} \cdot 2\sum Q$ Or $L = \frac{1}{ssf} \cdot \frac{dx.dy}{a(box)} \cdot \frac{\bar{t}}{h(box)} \cdot d.2\sum Q$ $asf = \frac{a(box)}{dx.dy}$ $hsf = \frac{h(box)}{\bar{t}}$ $psd = \frac{E[a(plane)]}{v(box)} = \frac{1}{d}$	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 XY $\bar{t}$ 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 (2)

Coefficient of error (Gundersen)	$CE = \frac{\sqrt{\frac{3(A - Poisson\ noise)}{12} + Poisson\ noise}}{\sum Q}, m = 0$ $CE = \frac{\sqrt{\frac{3(A - Poisson\ noise) - 4B + C}{240} + Poisson\ noise}}{\sum Q}, m = 1$	Poisson noise is $\sum \mathrm{Q_i}$ $\mathrm{A} = \sum(\mathrm{Q_i}.\mathrm{Q_i})$ $\mathrm{B} = \sum(\mathrm{Q_i}.\mathrm{Q_{i+1}})$ $\mathrm{C} = \sum(\mathrm{Q_i}.\mathrm{Q_{i+2}})$ *A,B,C* Covariogram values $\mathrm{Q_i}$ Particles counted
Plane areas	$\vec{V} = (A, B, C)$ $planes: Ax + By + Cz + D_i = 0, \qquad D_i = D + d.i$ $box: \left(\left\{\left((x, y, z) \middle\vert \begin{matrix} x_0 \le x \le x_0 + b_x \\ y_0 \le y \le y_0 + b_y \\ z_0 \le z \le z_0 + b_z \end{matrix}\right)\right\}\right)$ $area: (box \cap planes)$	*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

Isotropic Virtual Planes (3)

Average number of counts	$\bar{Q} = \frac{\sum_{i=1}^{p} \sum_{j=1}^{l_j} Q_{i\,j}}{\sum_{j=1}^{p} l_j}$	p Number of probes L_j Number of layouts in each probe $Q_{i\,j}$ 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 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*, 238–248.

IUR PLANES OPTICAL FRACTIONATOR

Estimated length	$est\ L = 2.\frac{a}{l}\sum I.\frac{1}{ssf}.\frac{1}{asf}.\frac{t}{h}$	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} XY dimensions of counting frame x_{step}, y_{step} 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	$est\ L = 2.\frac{a}{l}\sum I.\frac{1}{ssf}.\frac{1}{asf}.\frac{t}{h}$	a/l Area per unit cycloid length $\sum I$ Number of intercepts ssf Section sampling fraction asf Area sampling fraction t Section cut thickness h Height of counting frame
Area sampling fraction	$asf = \frac{area(Frame)}{area(x,y\ step)} = \frac{x_{CF}.y_{CF}}{x_{step}.y_{step}}$	x_{CF}, y_{CF} XY dimensions of counting frame x_{step}, y_{step} Dimensions of grid $area(Frame)$ Area of counting frame $area(x,y\ step)$ Area 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)	$L = \frac{1}{2}\pi d$	d Circle diameter
Surface area per unit volume (S_v)	$S_v = \frac{2\sum I}{\frac{l}{p}\sum P}$	I Number of intercepts l/p Length of semi-circle per point P Number of points

References

Howard, C. V., Reed, M. G. (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.

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 FRACTIONATOR

Estimate of total number of particles (N)	$N = \sum Q^- \cdot \frac{t}{h} \cdot \frac{1}{asf} \cdot \frac{1}{ssf}$	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})	$VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{12}, m = 0$ $VAR_{SRS} = \frac{3(A - s^2) - 4B + C}{240}, m = 1$	$A = \sum_{i=1}^{n} (Q_i^-)^2$ $B = \sum_{i=1}^{n-1} Q_i^- Q_{i+1}^-$ $C = \sum_{i=1}^{n-2} Q_i^- Q_{i+2}^-$ 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

Optical 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

Optical 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.

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Optical 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.

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	$g(P) = d_1, if\ d_2 < t$ $g(P) = \dfrac{\frac{\pi}{2} d_1}{arcsin\left(\frac{t}{d_2}\right)}, if\ t \leq d_2$	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	$g(P) = d_1, if\ \sqrt{d_1^2 + z^2} < t$ $g(P) = f\left(\sqrt{t^2 - z^2}\right), if\ \lvert z \rvert < t \leq \sqrt{d_1^2 + z^2}$ $g(P) = f(0), if\ t \leq \lvert z \rvert$ $f(x) = x + \dfrac{\pi}{2} \int_{x}^{d_1} \dfrac{1}{arcsin\left(\frac{t}{\sqrt{u^2 + z^2}}\right)} du$	d_1 Distance along test line t ½ thickness of optical slice z Distance in z from intercept to origin

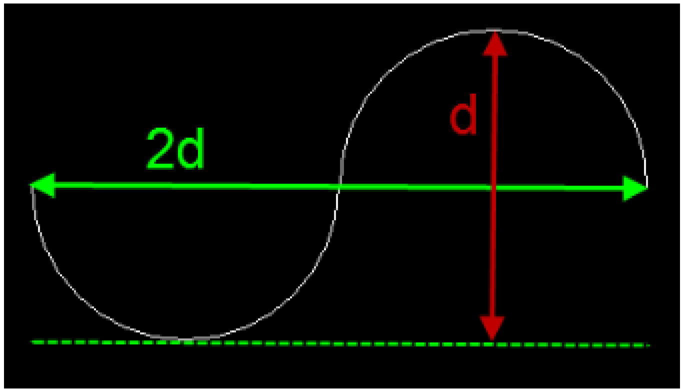
Optical Rotator (2)

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		$\hat{S} = a \sum_j l_j g(l_j)$ $g(l) = 2, \quad if\ d_2 < t$ $g(l) = \pi . \frac{1}{arcsin\left(\frac{t}{d_2}\right)}, \quad if\ t \leq d_2$	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.

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 $d = 2*Merz\text{-}radius$ 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.

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)	$l_{i+}^2 = \sum_{j\ even} l_{i\ j+}^2 - \sum_{j\ odd} l_{i\ j+}^2$ $l_{i-}^2 = \sum_{j\ even} l_{i\ j-}^2 - \sum_{j\ odd} l_{i\ j-}^2$ $l_i^2 = \frac{1}{2}\left(l_{i+}^2 + l_{i-}^2\right)$	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

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

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 ($\bar{v}_V$)	$\boldsymbol{\bar{v}_V = \frac{\sum_{i=1}^{n} \bar{l}_0^3}{n} \cdot \frac{\pi}{3}}$	n Number of intercepts l Intercept length
Coefficient of error (CE)	$CE(\bar{l}_0^3) = \sqrt{\frac{\sum_{i=1}^{n}(\bar{l}_0^3)^2}{\left(\sum_{i=1}^{n}\bar{l}_0^3\right)^2} - \frac{1}{n}}$	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.

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

Size distribution (2)

Coefficient of error	$CE_N(v) = \frac{CV_N(v)}{\sqrt{R}}$	CV_N 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 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 ssf Section sampling fraction
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

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.

SURFACE-WEIGHTED STAR VOLUME

Surface-weighted star volume ($\widehat{\bar{v}}_s^*$)	$\widehat{\bar{v}}_s^* = \frac{2\pi}{3} . \bar{l}_1^3$ $\widehat{\bar{v}}_s^* = \frac{2\pi}{3} . \frac{\sum_{i=1}^{n} \sum_{j=1}^{m_i} l_{1,(i,j)}^3}{\sum_{i=1}^{n} m_i}$	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)	$CE[\widehat{\bar{v}}_s^*] = \left[\frac{n}{n-1}\left\{\frac{\sum y_i^2}{\sum y_i \sum y_i} + \frac{\sum m_i^2}{\sum m_i \sum m_i} - 2.\frac{\sum m_i y_i}{\sum y_i \sum m_i}\right\}\right]^{1/2}$	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.

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	$\boldsymbol{\hat{S} = 2\pi \sum_{j=1}^{2r} l_j^2 . c(\beta)}$	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).

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 n Number of micrographs $\sum \mathrm{I_i}$ Total intercept points on curve $\sum \mathrm{P}_i$ Total test points

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 planes m Number of scanning planes $\sum$ P 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 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 $\sum P$ 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)	$S_v = 2.\left(\frac{I}{\frac{l}{2}.P}\right)$	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.