

Index

A

Abercrombie's method, 33
Archimedes, 19
Area. See also Surface area
 mathematical relationship equations, 4–7
 outlining versus point counting, 28–29
 points for estimation, 19–22
 virtual three-dimensional area probe, 69
 A_{STEP} . See Next sampling point

B

Biological variance ($VAR_{BIOLOGICAL}$),
 137, 144

C

CA1. See Hippocampus
Cavalieri's estimator, 16–18, 101, 110–111,
 166–167
Cavalieri's principle, 15, 19, 24
Cavalieri's Theorem, 16
CE. See Coefficient of error
Clinical significance, 136
Coefficient of error (CE). See also Precision of
 an estimate
 definition, 93
 estimators, 107
 fractionator samples, 104–105
 object number estimate, 104
 observed. See Observed coefficient
 of error
 sectioning interval relationship, 96
 theorem for independent random
 sampling, 110

Coefficient of variation (CV)

 group variances, 93–94
 individual variance effects on group
 variances, 93–94

 precision of an estimate, 91–92
 sampling optimization, 94

Cross section (Q)

 mathematical relationship
 equations, 6–7

 overview, 40

CV. See Coefficient of variation

Cycloid

 sine-weighted, 67–69
 surface area estimation with virtual
 cycloids, 85–86

D

Data reporting. See Publication

Density measures, applications, 8–9

Design-based methods

 object number counting, 33
 unbiased stereology, 11–12

Disector

 object number counting
 counting frame, 42–43
 counting rules, 41
 leading edge counting, 39–40
 optical disector probe, 42–46
 optical fractionator method, 51–54
 overview, 7–8, 38
 physical dissectors, 50
 shrinkage effects on object number
 estimation

- Disector (*continued*)
optical disectors in homogeneous shrinkage, 149–150
physical disector insensitivity, 148–149
three-dimensional reconstruction comparison, 40
total number of objects estimation, 50–52
overview, 4
synapse physical disector counting, 185
- E**
Electron microscopy. See Synapse number and size
Estimate precision. See Precision of an estimate
- F**
Fractionator sampling
coefficient of error for samples, 104–105
spaceballs for length estimation, 79–80
- G**
Geometrical probes. See also specific probes dimensioning, 125–126
isotropic interactions with structure.
See Isotropy
mathematical relationship equations, 4–7
object number estimation with disector, 7–8
selection, 8
spacing, 121–124
types, 3–4
- H**
 H_0 . See Null hypothesis
Hippocampus
CA1 pyramidal cell layer
object number estimation, 37, 44
plotting feature distribution, 118–119
sampling scheme for neuron number estimation, 132–135
spacing between sections, 119–121
volume estimation from points, 26–28
capillary length estimation in CA1 stratum radatum, 80–81
- optical disector for object number estimation, 44–45
optical fractionator method for dentate granule cell neuron number estimation, 52–53
ultrastructure. See Synapse number and size
- I**
Independent random sampling, 109–110
iSector, isotropic interaction attainment, 61–63
Isotropic fractionator, object number counting, 55
Isotropy
definition, 3, 60
interactions between line probe and surface features, 59
probe–structure interactions
iSector technique, 61–63
orientator for achieving isotropic interactions, 61–62
overview, 60–61
vertical sections for achieving isotropic interactions
isotropic direction defining in space, 63–65
overview, 62–63
sine-weighted line probes and cycloids, 67–69
sine-weighted selection of longitude, 65, 67
virtual three-dimensional line and area probes, 69
surface area–length relationship and tissue orientation sensitivity, 60
- J**
Journal article. See Publication
- L**
Length
estimation
density measures, 9, 78
example, 80–81
history of approaches, 72–73
overview, 71

- Smith–Guttman equation, 72, 74, 81
virtual probes
 plane generation within sections, 73–74
 spaceball, 74–80
fractionator sampling for length estimation, 79–80
length density by reference volume
 method, 78–79
shrinkage effects, 153–154
surface area relationship and tissue orientation sensitivity, 60
- Line probe
 isotropic interactions between line probe and surface features, 59
 mathematical relationship equations, 4–7
 sine-weighted line probes, 67–69
 virtual three-dimensional line probe, 69
- Local estimators
 getting started, 127–128
 orientation sensitivity, 157
 overview, 10–11
 volume estimation
 nucleator principle
 overview, 158–163
 variants, 167
 rotator
 overview, 163–165
 variance of estimates, 165–166
 size-weighted samples, 158
- M**
Mathematical relationship equations, 4–7
Mitochondria, reference volume estimation from points, 25–26
- Model-based methods
 object number counting, 32–33
 unbiased stereology, 11–12
- N**
N. See Object number
Next sampling point (A_{STEP}), 122, 124–125
Nucleator principle
 variants, 167
 volume estimation, 158–163
Nucleoli, object counting application, 46–47
- Null hypothesis (H_0), 135
Number of objects. See Object number
- O**
Object number (N)
estimation
 accuracy, 55
 sampling, 31–32
 model-based methods for counting, 32–33
design-based methods for counting, 33
bias sources, 34
direct counts of serially reconstructed objects, 34
indirect counting based on geometrical models
 assumptions, 36–37
 biases, 37–38
 overview of techniques, 34–36
- disector counting
 overview, 7–8, 38
 leading edge counting, 39–40
 three-dimensional reconstruction
 comparison, 40
 counting rules, 41
 counting frame, 42–43
 optical disector probe, 42–46
 physical dissectors, 50
 total number of objects
 estimation, 50–52
 optical fractionator method, 51–54
 isotropic fractionator, 55
 objects for counting, 46
 recognition of object, 47
 section thickness measurement, 47–50
- mathematical relationship equations, 4–7
- number-weighted correction of section thickness, 152–153
- observed coefficient of error calculation for estimate, 103–104
- shrinkage effects on estimators
 homogeneous nonuniform shrinkage, 150–151
 optical dissectors in homogeneous shrinkage, 149–150

- Object number (N) (continued)
optical fractionator estimate
 effects, 150
physical disector insensitivity, 148–149
ultrastructure. See Synapse number
 and size
- Observed coefficient of error (OCE). See also Precision of an estimate
 calculation, 94–96
 calculation for estimates
 object number, 103–104
 volume, 101–103
 contributing variables, 105–106
 factors affecting individual estimate
 variability between sections, 99–100
 variability of counts within individual sections, 97–99
 individual variance effects on group variances, 93–94
 overview, 93
 pilot calculation, 126
 sampling optimization, 94
 single sample approximation formula, 100–101
 t sensitivity improvement strategies, 137–143
- OCE. See Observed coefficient of error
- Optical disector. See Disector
- Optical fractionator study
 data reporting in publication, 194
 dentate granule cell neuron number estimation, 52–53
 overview, 51–52
 rare events and large sampling fractions, 53–54
- Orientator, isotropic interaction attainment, 61–62
- Outlining, point counting comparison, 28–29
- P**
- Physical disector. See Disector
- Point
 area estimation, 19–22
 mathematical relationship equations, 4–7
 probability chart for probe, 21
 systematic grid of test points, 22–23
- volume estimation
 area estimation, 20–22
 hippocampal CA1 pyramidal cell layer
 volume estimation, 26–28
 outlining comparison, 28–29
 overview, 19–20
 point ratio–volume density
 relationship, 24
 systematic grid of test points, 22–23
 tiling, 23–24
 two-tier point counting, 25–26
- Poisson distribution, 124
- Precision of an estimate
 coefficient of error. See Coefficient of error
 definition, 91–92
 observed coefficient of error
 calculation, 94–96
 calculation for estimates
 object number, 103–104
 volume, 101–103
 contributing variables, 105–106
 factors affecting individual estimate
 variability between sections, 99–100
 variability of counts within individual sections, 97–99
 single sample approximation formula, 100–101
 sampling relationship, 92–93
 sectioning interval dependence, 95
 variance measures. See also Coefficient of variation
 individual variance effects on group variances, 93–94
 sampling optimization, 94
- Proportionator sampling, 122
- Publication
 data for reporting by study type, 193–194
- Materials and Methods section
 definitions, 190
 sampling scheme, 190–192
- overview of stereological data reporting, 189
- Results section, 192–193
- shrinkage data handling, 155

- Q**
Q. See Cross section
- R**
Rotator
variance of estimates, 165–166
volume estimation, 163–165
- S**
 S^2 . See Variability of counts within individual sections
Sampling. See also Fractionator sampling
coefficient of variation for optimization, 94
independent random sampling, 109–110
object number estimation, 31–32
observed coefficient of error for optimization, 94
precision of an estimate relationship, 92–93
proportionator sampling, 122
publication of scheme, 190–192
scheme optimization
neuron number estimation example, 132–135
pilot study, 131
power of sampling, 144–145
structure of schemes, 132
t-test utilization
overview, 133–135
sensitivity improvement strategies, 135–144
stereology as, 10
synapse number and size
numerical density estimation
distance between sections in disector stack, 180
number of sections in serial stack, 180
number of stacks needed, 175
position specification for sampling, 171, 173
sampling with stacks, 175–176
section thickness measurement, 180–181
synaptic contact defining and counting, 176–179
ultrathin section thickness, 179
- reference volume estimation, 170–172
region of interest delineation, 171, 173
systematic random sampling, 110–113
Sampling point. See Next sampling point
Schmitz estimator, coefficient of error estimation, 107
SD. See Standard deviation
Sectioning. See also Vertical sections
precision of an estimate dependence on interval, 95
sample representation, 2–3
spacing between sections, 119–121
structural information loss, 1–2
ultrastructure. See Synapse number and size
Section sampling fraction, 51
Section thickness
measurement in object counting, 47–50
recommendations for stereology, 117–118
synapse number and size
measurement of thickness, 180–181
ultrathin section thickness, 179
SEM. See Standard error of the mean
Shrinkage
data handling, 155
number-weighted correction of section thickness, 152–153
object number estimator effects
homogeneous nonuniform shrinkage, 150–151
optical disectors in homogeneous shrinkage, 149–150
optical fractionator estimate effects, 150
physical disector insensitivity, 148–149
object size effects, 153–154
synapse number and size studies, 181–182
types, 147–148
Sine-weighted probes. See Vertical sections
Smith–Guttman equation, 72, 74, 81
Spaceball
data reporting in publication, 194
linear feature interception with surface, 74–76

- Spaceball (*continued*)
size for length estimation, 76–77
Smith–Guttman equation, 74
- Split sample estimator, coefficient of error estimation, 107
- Standard deviation (SD), 91, 134
- Standard error of the mean (SEM), for estimate, 110
- Stereological variance ($\text{VAR}_{\text{STEREOREOLOGICAL}}$), 137
- Stereology
definition, 1–2
tips for getting started
familiarity with material, 116–117
local estimators, 127–128
number of sampling locations, 124–125
observed coefficient of error pilot calculation, 126
plotting feature distribution, 118–119
probe
dimensioning, 125–126
spacing, 121–124
section thickness, 117–118
spacing between sections, 119–121
as sampling, 10
- Surface area
estimation
brain pial surface estimation, 87–89
density measures in estimation, 9
overview of techniques, 85, 87
virtual cycloids, 85–86
length relationship and tissue orientation sensitivity, 60
mathematical relationship equations, 5–7, 83–85
shrinkage effects, 153–154
- Synapse number and size
electron microscopy, 169, 186
hippocampal tissue preparation, 170
physical disector counting, 185
sampling
numerical density estimation
distance between sections in disector stack, 180
- number of sections in serial stack, 180
number of stacks needed, 175
position specification for sampling, 171, 173
sampling with stacks, 175–176
section thickness measurement, 180–181
synaptic contact defining and counting, 176–179
ultrathin section thickness, 179
reference volume estimation, 170–172
region of interest delineation, 171, 173
shrinkage, 181–182
size distribution analysis, 182–183
staining, 186
synapses per neuron, 184–185
total surface of synaptic contacts, global estimate, 183
variance of number of synapse estimate, 184
- Systematic random pattern, 23
- Systematic random sampling, 110–113
- T**
- t*-test
pilot study for sampling scheme, 133–135
sensitivity improvement strategies
negative result documentation, 145
number of sections and probes, 138–144
number of subjects in group, 136
power of sampling, 144–145
work amount per subject, 136
- U**
- Unbiased estimate, 15
Unbiasedness, concept, 18–19
Unbiased stereology, design-based versus model-based methods, 11–12
- V**
- $\text{VAR}_{\text{BIOLOGICAL}}$. See Biological variance
 $\text{VAR}_{\text{GROUP}}$. See Variance of the group mean
Variability between sections (VAR_{SRS})
effects on individual observed coefficient of error, 99–100

- single sample approximation formula, 100–101
- total variance contribution, 103–104
- Variability of counts within individual sections (S^2)
calculation, 102
effects on individual observed coefficient of error, 97–99
- Variance of the group mean (VAR_{GROUP}), 134, 137
- VAR_{SRS} . See Variability between sections
- $VAR_{STEREOREOLOGICAL}$. See Stereological variance
- Vertical sections, isotropic interaction
attainment
isotropic direction defining in space, 63–65
overview, 62–63
- sine-weighted line probes and cycloids, 67–69
- sine-weighted selection of longitude, 65, 67
- Volume
estimation
Cavalieri’s estimator, 16–18
density measures in estimation, 9
points for estimation
area estimation, 20–22
- hippocampal CA1 pyramidal cell
layer volume estimation, 26–28
outlining comparison, 28–29
overview, 19–20
point ratio–volume density
relationship, 24
systematic grid of test points, 22–23
tiling, 23–24
two-tier point counting, 25–26
- principles, 15
unbiasedness concept, 18–19
- local estimators
nucleator principle
overview, 158–163
variants, 167
- rotator
overview, 163–165
variance of estimates, 165–166
- size-weighted samples, 158
- mathematical relationship equations, 4–7
- observed coefficient of error calculation for estimate, 101–103
- shrinkage effects, 153–154
- synapse number and size, reference
volume estimation, 170–172