Article

A Brief Introduction to Stereology and Sampling Strategies: Basic Concepts of Stereology

Berrin Zuhal Altunkaynak*†, Mehmet Emin Önger*, Muhammed Eyup Altunkaynak*, Ebru Ayrancı*, Sinan Canan†

ABSTRACT

Stereology (from the Greek *stereos* = solid) is considered as the spatial interpretation of profiles seen in sections. It is a multidisciplinary science field dealing with the three-dimensional profiles of objects appearing on sectional planes of metallurgical materials or tissues. Stereology supplies practical techniques for obtaining quantitative information about three-dimensional, real-world structures from two-dimensional planar profiles. After acknowledging the fact that the profiles on sections are not true representations of the real objects, the need for stereology in quantitative studies becomes obvious. Stereological methods utilise various specific tools and sampling strategies to provide unbiased and quantitative estimates in order to obtain a wide range of quantitative parameters including the number, size, shape, volume and density. This review aims at to provide some basic knowledge about stereology, stereological terms and sampling strategies.

Key Words: stereology, quantification, unbiased methods, sampling, stereological tools

NeuroQuantology 2012; 1: 31-43

What is the stereology?

Stereology is a branch of morphometry, which is used to obtain three-dimensional data from two-dimensional sections from virtually any type of structure (biological structures, metallurgical samples, etc.) (Baddeley and Jensen, 2004). Sections passing through the building components of any given structure will yield sectional profiles depending on the size, shape, volume, orientation and number of the different components contained in the structure (Gundersen *et al*., 1988). Since it is generally impossible to see those components by any other means, we have only "sectional" profiles, or very thin tissue "slabs", which are practically seen as planar sections, to obtain any quantitative data we seek. Such profiles or projections are generated as a direct product of both the sectioning procedure and the three-dimensional properties of those components. A change in section direction with respect to structure generally results in a wildly different set of projections, especially if the structure has no isotropic orientation in space (as an example, you may visualize different sectioning angles passing through a layered structure, such as skin or the cerebral cortex).

Therefore, quantification attempts based solely on those projections would be very misleading. It is important to note that the sectioning procedure itself will result in a "dimensional reduction" of the objects under investigation (Fig. 1).

Basic terms used in stereology

Computer assisted stereology (CAS)

As you will see in the following review, most of the stereological procedures require repetitive tasks, such as sampling of areas, steps and objects on fairly large sectional areas. Furthermore, in general, huge amounts of data are produced during a few trials in a study, and such data must be logged carefully and reproducibly during the estimation and counting procedures. Such delicate steps need

Corresponding author: Zuhal Altunkaynak, PhD
Address: *Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey and †Department of Physiology, Medical Faculty, Yıldırım Beyazıt University, Ankara, Turkey. Phone: +90 362 312 19 19 ext 2268
Fax: +90 362 312 19 19 ext 2268
berrinzuhal@gmail.com
Received July 17, 2011. Revised Sept 11, 2011. Accepted Jan 2, 2012.
*e*ISSN 1303-5150

to be controlled as precisely as possible in order to obtain a accurate experiment result. The abundance of such steps is also important as they leave plenty of room for human error. Nowadays, computers and additional equipment help researchers greatly in performing such delicate and cumbersome procedures in stereology (**Fig. 2**). Such equipment is now indispensible for a stereology laboratory to achieve the fast and reliable implementation of stereological methods (Kaplan *et al*., 2001; Canan *et al*., 2004; Mandarim-de-Lacerda *et al*., 2010).

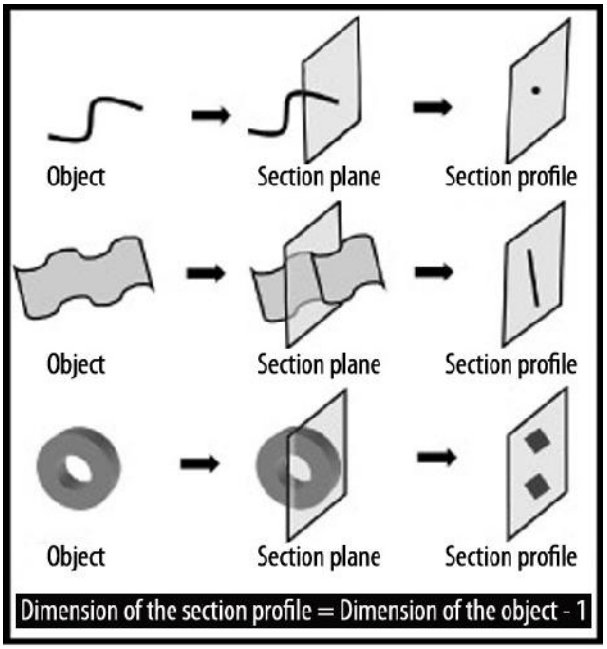

Figure 1. When various objects of one, two or three-dimensions are cut by a two-dimensional section plane, the dimension of the resulting profile (projection) will be one dimension less than its original dimension. A 2-D "surface" gives rise to a 1-D linear profile; while a 3-D object will yield a set of 2-D planar profiles (Howard and Reed, 2005).

Figure 2. A CAS system. This system consists of software, a digital camera, microscope, and monitor. The stage of microscopy is controlled by the software, which also records data during the stereological procedures.

Shrinkage

This term describes biological / pathological changes in the tissue, depending on their volume loss (shrinkage) following or during the histological processes (**Fig. 3**).

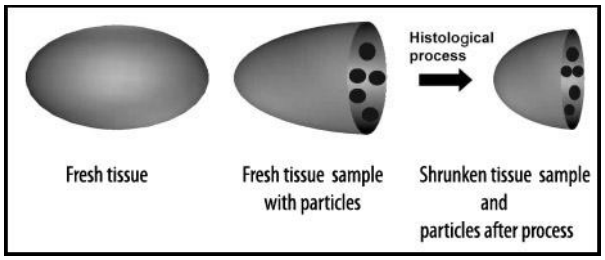

Figure 3. Tissues generally shrink after fixation and other histological processes. Thus, the sizes of particles are decreased and they generally move closer to each other, which directly affects the validity of any biological comment build on "density" estimates.

A Probe

The stereological data is obtained by asking "geometric questions" to the structure of interest. Such special "geometrical questions" are known as "probes" in stereology. Stereological probes can be one, two or three-dimensional. If the geometric question is a surface limiting given area, it is called a "two-dimensional probe". In addition, zero, one and three-dimensional probes are used in stereology, which are used typically in volume, surface area and number estimations, respectively. The dimensional properties of stereological probes are determined by the parameter of interest. As a general rule, the number of dimensions of the parameter and of the probe must add up to 3 (to get the correct information about the actual, three-dimensional structure). For example, if the number of an object is required, one must use a three-dimensional probe, since the number parameter is "zero" dimensional (or dimensionless), due to the fact that the "number" is independent of any dimensional properties (regardless of their size, every particle must be counted as "one" in a counting procedure). We already know that a two-dimensional sectional surface will hit the particles with a chance proportional to their sizes (**Fig. 12**). Similarly, the length parameter, which is one-dimensional by definition, must be measured using a two-dimensional planar probe; while a surface area can only be measured using a one-dimensional linear probe. Finally, if we want to estimate the volume of an object, we must use a "zero-dimensional" point probe (**Fig. 4**). Only those

combinations of parameters and probes can give us accurate and unbiased estimates of the above-mentioned parameters (Gundersen *et al*., 1988a and 1988b; Howard and Reed, 2005; Basoglu *et al*., 2007; Glaser *et al*., 2007, Turgut *et al*., 2009; Elfaki *et al*., 2011).

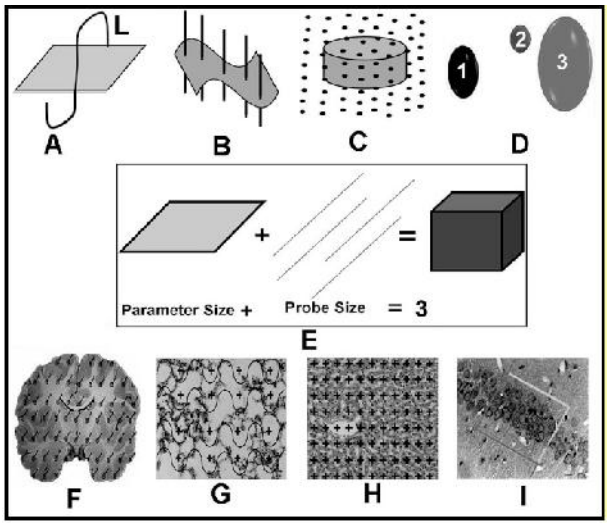

Figure 4. Types of stereological probes (A-D) and their uses (F-I). The sum of the dimensions of the parameter of interest and the dimensions of the probe must be equal to three (E; Howard and Reed, 2005).

Biological variability

This is the range of possible values for any measurable characteristic including number, length, surface area, volume or density among the subjects. It is well known that the biological variability is an important factor affecting the estimation of such parameters (Schmitz *et al*., 1999; Schmitz, 2005).

Cavalieri's Principle

In the 17th Century, Italian mathematician Bonaventura Cavalieri described a method for volume estimation, which is dependent on serial exhaustive slabs from a structure. Today, this method is widely used for volume estimation of objects of varying shapes and sizes. Briefly, the structure of interest is divided into exhaustive serial sections or slabs and then the volume of each slab is estimated by multiplying the surface area of every section multiplied by the section interval or section thickness. Area estimation on sectional surfaces is generally conducted by superimposing a point-grid (which is a "zero-dimensional" probe) on the sectional profiles and counting all the points hitting the area of interest. Since every point in the grid represents a unit area, multiplying this area by the total point count yields the total area of the sectional surfaces. When we multiply the section interval or section thickness with this sectional area estimation, we will have an unbiased estimate of the volume of the structure (**Fig. 5**) (Altunkaynak and Altunkaynak, 2007; Keskin *et al*., 2008; Sahin *et al*., 2008; Uyanik *et al*., 2009; Elfaki *et al*., 2011). This method will be described in more detail in the "*Stereological Volume Estimations*" chapter of this special issue.

Planimetry

This is a widely used approach in morphometry, which relies on manual or automatic boundary delineation of an object in order to estimate its sectional area, generally using software that automatically counts pixels inside the boundary (**Fig. 5A**) (Evans *et al*., 2004). Although it seems very practical at first glance, it is generally more time-consuming with respect to point-counting, since the manual tracing of complex boundaries is generally cumbersome work (Acer *et al*., 2008).

The Nucleator

This is a stereological tool used in particle volume estimations. Briefly, it is applied as a two-step procedure. First, the objects are measured using disector sampling. After that, the volume of each sampled object, such as the nucleolus or nucleus of a cell, is calculated using a special type of sampling that depends on an arbitrary central point that has been determined inside the selected object with a pre-determined nucleator algorithm. This is generally the method of choice if a computer-assisted stereological workstation is available and the obtained value is the mean number-weighted volume (**Fig. 5B**) (Gundersen *et al*., 1988a).

Particle

A particle is the unit of interest in any stereological study design. It bears a different meaning depending on the context of the study. Kidney glomeruli, nerve cells or cell organelles such as mitochondria can be regarded as particles depending on the aim of the study design (**Fig. 6A**) (Gundersen *et al*., 1988a; Altunkaynak *et al*., 2008).

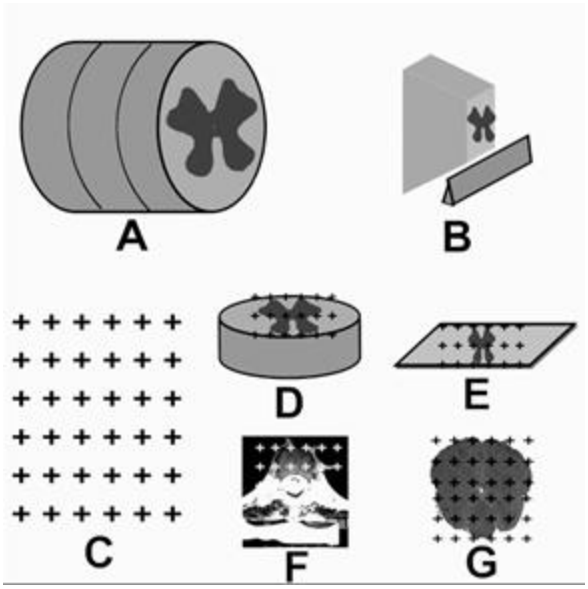

Figure 5. A simple procedure for the estimation of the spinal cord volume using the Cavalieri's principle. This method can be applied to physical slices of tissue (D), as well as histological sections (E), MRI images (F) and microscopic areas (G).

Disector Pair

A section pair composed of two consecutive sections used for particle counting in stereology is known as a disector pair (**Figs. 7B-C**) (Sterio, 1984; Gundersen *et al*., 1988a; Tümkaya *et al*., 2010).

Disector Particles

During the implementation of the disector, it is important to count every particle once, and only once. Such a target can easily be achieved by counting one end of each particle (which can be thought of as analogue to counting heads for a human count). To achieve this, we use two consecutive sections, select using systematic random sampling, and count particle profiles, which are visible in one section while absent in the other. Two sections represent a tiny tissue volume (the disector volume), and particles giving a profile in only one section can be selected as being "trapped" in this disector volume. Using this approach, virtually any type of particle can be counted accurately using disectors. Disector count gives the particle number in the unit volume, which is known as the numerical particle density. This method is described in greater detail in the respective chapter of this issue (**Figs. 7B-C**; particles 3, 5, 6 and 7) (Sterio, 1984; Aslan *et al*., 2006).

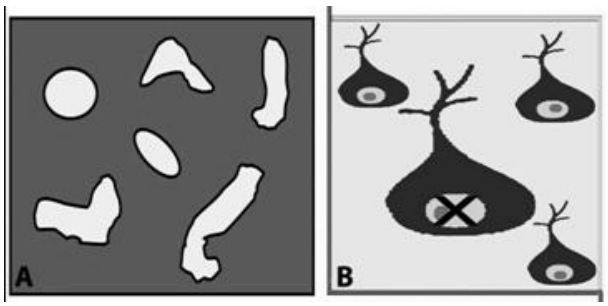

Figure 6. Schematic depiction of planimetry and the nucleator method. A; boundaries (black) of particles are detected and B; using the nucleator, the size of the disector particles are estimated.

The Fractionator

The fractionator is a sampling scheme combined with any type of stereological measurement, resulting in an unbiased estimate of the total quantity of interest. Application of the fractionator in particle counting will be outlined in 'The Fractionator' chapter (**Fig. 9**) (Gundersen *et al*., 1988a and 1988b).

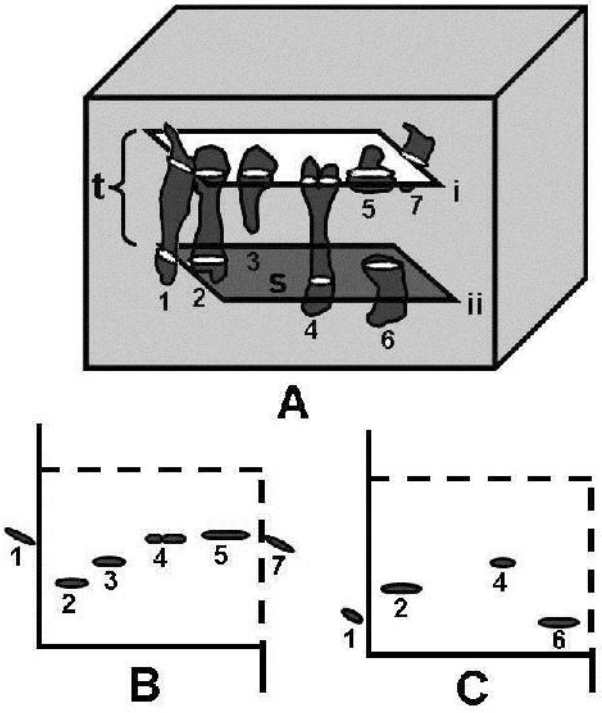

Figure7. Schematic illustration of the application of the disector method. A: A biological object containing particles (1-7). Two consecutive sections (i and ii) were cut from the tissue with an interval of (t). In B and C, two unbiased counting frames are superimposed on the corresponding areas in each section; Particle profiles 1, 2, 3, 4, 5 and 7 are seen in B and 1, 2, 4 and 6 are seen in C. According to disector rules, 3, 5 6 and 7 can be regarded as disector particles, since they are visible only in one particular section.

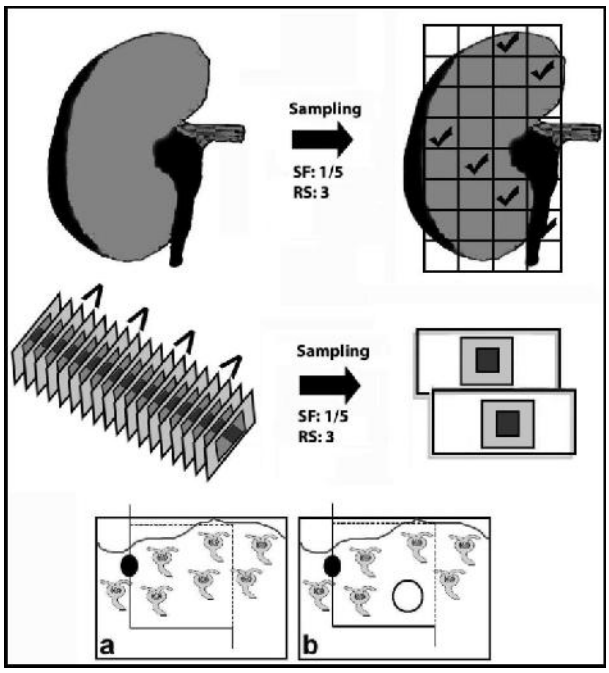

Figure 8. The physical fractionator and disector application are summarised here. For example, we wanted to estimate the number of glomeruli in a rat kidney. Firstly, we removed a whole kidney (A) then we sampled it and selected samples at a standard interval (B). After sampling, the selected samples were cut into serial sections (C). If the serial sections were too much (15-17 sections are generally enough for the disector method) we could sample it. Finally, selected section pairs were used for the physical disector method. So, serial areas of section pairs were evaluated according to disector rules (E and F). In this figure, SF, the sampling fraction is in the step and RS, the randomly selected object; the total sampling faction was obtained by multiplying all SF's with each other. For this study, the total SF=5x5=25 (Gundersen *et al*., 1988a; Altunkaynak *et al*., 2008).

Optical Disector

This is a widely used particle counting and sampling method based on the process of virtual optical sectioning through a thick tissue section and counting particles using an objective with a sufficient numerical aperture (resolution). During the counting procedure, each object is counted once as it appears in the plane of sharp focus, if it is in the boundaries of the counting frame. This is a more suitable method for rounded particles such as the cell nucleus or nucleolus rather than objects with fuzzy boundaries (West *et al*., 1991; Odacı *et al*., 2010; Özyurt *et al*., 2011). The optical disector has almost the same basic criteria as the physical disector with some minor variations in practical applications (**Fig. 9**).

Optical Fractionator

When the optical disector counting and systematic random sampling approach are used in conjunction to obtain the total quantity, it is called the optical fractionator. This relies on multiplying the sampling ratio with the final particle counts obtained from a known fraction (sample) of a tissue. This is generally the method of choice when we are dealing with layered or anisotropic structures, such as nervous tissue (**Fig. 9**) (Turgut *et al*., 2007; Bas *et al*., 2009; Sönmez *et al*., 2010a; Gökçe *et al*., 2011).

Random Sampling

A completely random distribution of selected samples is known as random sampling (**Fig. 10**) (Canan *et al*., 2008; McArt *et al*., 2009).

Systematic Uniform Random Sampling (SURS)

This combines both the unbiasedness of random sampling and the efficiency of a systematic sampling. SURS is based on selecting the final sample with a predetermined interval (systematically) while selecting the first sample of the set randomly within the first sampling interval (**Fig. 10**) (Glaser *et al*., 2007; Canan *et al*., 2008).

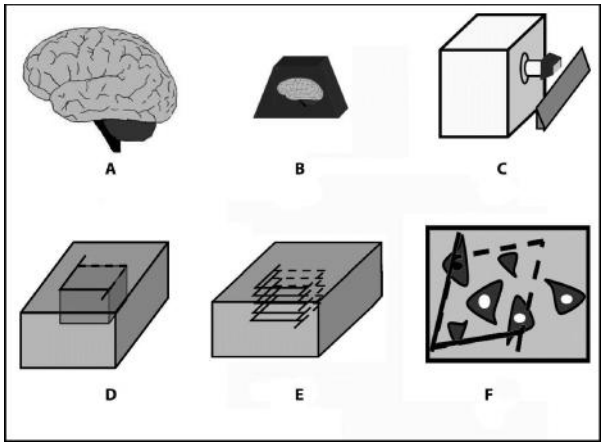

Figure 9. Graphical depiction of the optical fractionator and the disector application. For a study on total neuron number in a given brain region, the whole brain (A) is processed and serially sectioned (B, C). For optical counting, sections should have an average thickness of 20-25 µm. Within the sections, virtual sectional planes are generated using the focal plane of the microscope and particles are counted accordingly (E and F).

Section (Section)

A section is a two-dimensional image taken from a three-dimensional object. This term is used in stereology to describe thin histological slices, which have a certain thickness (Baryshnikova *et al*., 2006) (**Fig. 10**).

Section Sampling Fraction (SSF)

The ratio of systematic-randomly selected sections in all sections obtained from the structure. For example, if 25 sections are selected using systematic-randomly sampling out of 250 sections from the whole structure; then the sampling rate will be 25/250, or 1/10 (**Fig. 10**), (Witgen *et al*., 2006). Section Thickness: The cross-distance between the two cutting edges of a section (**Fig. 10**).

Density (Density)

Many stereological estimators give a ratio of a quantity in a unit volume. This ratio is known as density. The most frequently used ratios are volume density (Vv), surface density (Sv), length density (Lv), and numerical density (Nv) (Aguila *et al*., 1998; Ünal *et al*., 2002; Kiki *et al*., 2007).

Numerical density (N_V)

The number of particles in a unit volume is known as the numerical density. The numerical density can be easily estimated by dividing the number of particles in a set of disectors divided by the total disector volume (Gundersen, 1986).

Total Particle Number (N)

The total number of particles in a given reference volume or structure can be estimated using both the fractionator approach and by multiplying the numerical density estimated using disectors with the total volume of the structure, which could be estimated using several techniques, including Cavalieri's principle (the Cavalieri-Disector junction with the total number of accounts) (Kiki *et al*., 2007). Bias: Methods presenting a systematic variance between the estimation results and the real quantities are considered as biased methods. Such a bias is almost always hidden in real-life studies and cannot be detected without a properly tuned, unbiased technique (Sahin *et al*., 2002; Schmitz and Hof, 2005).

Neutral (Unbiased)

If a method provides estimates, which gradually approach the true value with repetition of the measurements, this means that this method does not cause a systematic deviation from the real value of the parameter of interest and thus is considered as unbiased. Since most of the measurements in the biological studies involve sampling, it is an important feature for a method of choice (**Fig. 11**) (Mühlfeld *et al*., 2010).

Coefficient of error (CE)

In stereological studies, it is important to know the amount of error in a study design. The total amount of error arising from sampling estimation procedures in a stereological study can be expressed as the coefficient of error (CE). The amount of error can be controlled by adjusting the sampling frequency and the measurement intensity (Gundersen, 1987; Schmitz, 1998; Schmitz, 2000; Schmitz *et al*., 1999; Hof and Schmitz, 2000). A preliminary estimate of CE can be obtained by dividing the standard deviation by the sample average, showing the difference between the calculated average of the population and the average variability. Usually, a total CE of less than 0.05 is considered to be adequate for most of the studies to provide accurate quantitative information (Gundersen, 1987).

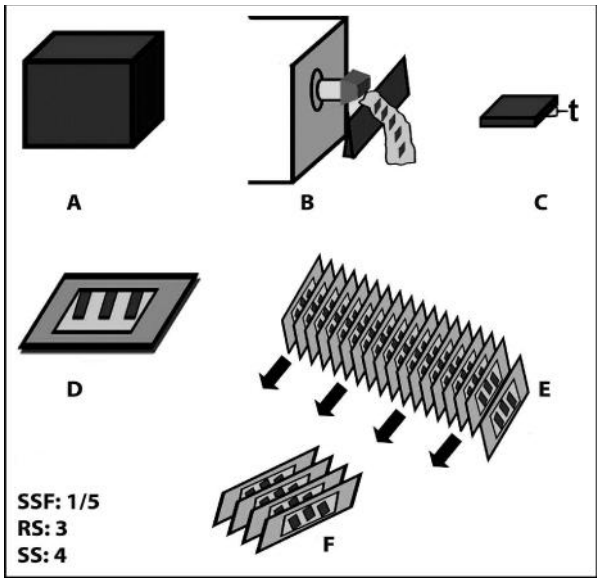

Figure 10. General sampling procedures used in a typical stereological study. Tissue block (A) is serially cut at a thickness of't' (B and C), taken on glass slides (D). Since the whole section set is generally too large to investigate exhaustively, a subset of samples must be chosen (E and F). Here, sections were sampled at a ratio of 1:5 (Section Sampling Fraction-SSF), with a random section (RS) selection from the first five sections (in this case, the 3rd section) and selecting every 5th section after this initial selection. At the end of the sampling, 4 slides containing 3 sections are chosen (selected sections-SS).

Coefficient of variation (CV)

In a stereological application, it is also

important to know the variability between groups of individual. The coefficient of variation (CV) is a measure of the group variability. CV is obtained by dividing the standard deviation of the population by the population means (see also coefficient of error) (Gundersen, 1986).

Variability (Variance)

In stereology, variability appears as a function of multiple factors, including the sampling plan, the estimation methods, selected population, and so on (Russ and DeHoff, 2000).

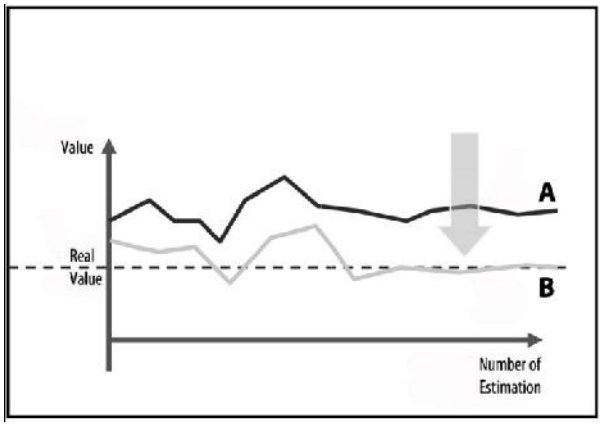

Figure 11. The difference between biased and unbiased methods. Method A presents a systematic deviation from the true value and while the estimation procedures are repeated, the cumulative mean approaches to a steady value which is different from the real (and most of the times, hidden) value. Method B, on the other hand, is an unbiased method, which converges to the real value as the procedure is repeated.

C. Sampling

Vital Criteria of Stereology

Because of the resulting plots of cross-sections of particles and their size, the extent of sectioning is perpendicular to the direction, volume, etc. and are closely related to such factors. Larger particles have a better chance of being cut by the plane, the cross-section probably, than smaller ones (Gundersen and Jensen, 1987), while the small "a" particles have less chance of making the projection (**Fig. 12**). The sectioning direction for the sampling of particles is important. In Figure 12, the direction of sections and the effect of profile number of particles on the chance of sampling are schematised. As shown, with evenly spaced sections, taken in the direction perpendicular to the long axis of any particles will give more profiles than cutting the same size particle in parallel position (**Fig. 13**). However, considering the number of terms, each particle "1" will appear in the census, regardless of the orientation, and whether big or small, all particles should have an equal chance of being sampled. As in this simple example, where the execution of an idea is required regarding two-dimensional projection on the plane and its structure, in almost all cases, researchers have to confront these or similar problems (Gundersen *et al*., 1999). For instance, the size reduction principle is one of them. A scientist, who is working with sections, and tries to understand three-dimensional features from two-dimensional sections, has some important theoretical problems. Samples with two-dimensional plane sections of the "n"-dimensional structures, in section "n-1" in size to be represented by a projection.

That is, a certain volume of a solid element (e.g. any organ) is three-dimensional, the surface is two-dimensional (e.g. a membrane), and a line or curve is one-dimensional (fibres, etc.). However, a point gives the zero-dimension of the projection (Figure 1). Even with this aspect alone, appropriate methods may show just how misleading it can be to carry out morphometric studies and use cross-sections.

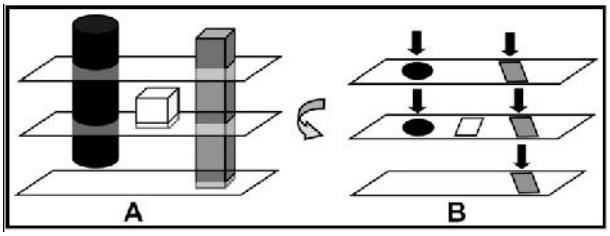

Figure 12. If the particles contained in the tissue have different heights than those normal to the section planes, their probability of being hit by a section plane also differs. This means a big particle has a higher chance of being sampled. In other words, on a section taken arbitrarily from a tissue, we can see bigger particles more frequently then small ones.

Actually, the "cross" concept refers to fact that the plane passes through (t = 0 as) the thickness of any non-rigid structure. As a good example, a mineral sample can be cut and its polished surface could be determined. Metals are not transparent to light, for this reason an observer could not see content of it; in this respect polished surfaces of metals correspond to the two-dimensional plane of real surface (Weibel *et al*., 2007). However, the sections taken from the biological samples, in fact, have a certain thickness, which are slices.

Thickness, regardless of how small, cannot be considered as a direct two-dimensional plane. Hence, thickness of section should be taken into account (Weibel, 1981). As a general rule, if you examine a structure by the naked eye, all details of interest in macro size will be seen and no miss comment could be made about it. However, at the microscopic level there are many factors cause incorrect evaluation. If these measures are not reasonable, the actual values may deviate from the real value of object.

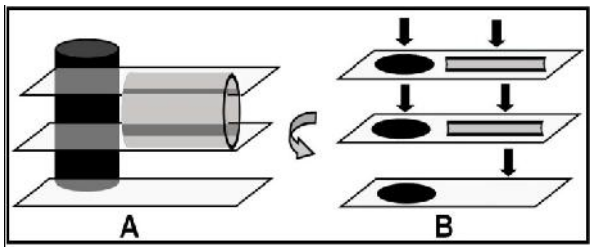

Figure 13. If two particles of the same size have different orientations in the tissue in respect to the section plane, their chance of being hit by a section plane differs due to their orientation. For instance, the upright particle on the left gives more profiles than the horizontally oriented particle, although they both have the same size.

Despite a variety of error sources, in trying to make a pre-adoption of the data that is related to real property of the three-dimensional structure, you can fully understand their relationships with the various cross-sections obtained from the structure. If these and similar sources of error are ignored or overlooked, this may lead to the occurrence of systematic deviations from the true value. With such an approach, exactly the wrong result can be achieved, though it cannot be said that the incidental fact of the results has been obtained in this case. Most of mistakes cannot be determined because of the hidden factors (Odaci *et al*., 2004).

This case is similar to a car with the speedometer that does not working well. For instance, a car's speedometer measures +20 km / hour than the correct speed. The driver reads 80 km / hour speed from the speedometer, and he cannot realise that the car is actually going at a 100 km/hour speed. Here, because it was wrong to refer to any other source that is not available, the rate of errors in the driver's eyes is completely confidential. However, when the driver is caught by a police vehicle, and the speed has been measured from the outside with properly calibrated radar; the driver has to pay a fine. This will make him aware of these errors.

Systematic Random Sampling

Sampling is the most efficient method used in biological studies. This method, as the name implies, is structured and systematic; that is, it is a fixed, predetermined interval in a random manner, but this also makes it possible to carry out the sampling (Jinno and Fukuoka, 2008).

Systematic uniform random

Sampling is carried out as systematic and random components. For example we have a series of 100 sections and after sampling we want to get about ten sections from this series, our systematic sampling interval that would be 10. This is the sampled section interval from the whole sections. A random sampling of section from this collection should be applied so that our sampling being random, from 1 to 10, for example, if we choose 5th section from this collection our sampled series would be 5th, 15th, 25th, 35th, 45th, 55th, 65th, 75th, 85th and 95th (West *et al*., 1991; Gundersen *et al*., 1999) (Figure 15). This is randomness, but at the same time systematicness, which is applied randomly to all samples at intervals of systematic uniform.

Stereological methods are based on the "Systematic Random Sampling" (SRS) strategy. The main feature of this form of sampling will be trying to get samples from the structure where necessary, and the structure of each point ensures an equal chance of sampling. Biological structures usually contain components according to the study (cell, nucleus, vesicles, etc.). Generally, it is impossible that all sections included in studies are obtained from the structure of the evaluation in practice. For instance, in a study aimed at determining the total number of neurons in the human neo-cortex, trying to take histological sections of tens of thousands from the brains is not effective as they cannot all be individually examined. Examples of those obtained (sections) from a selection of specific proportions will be needed.

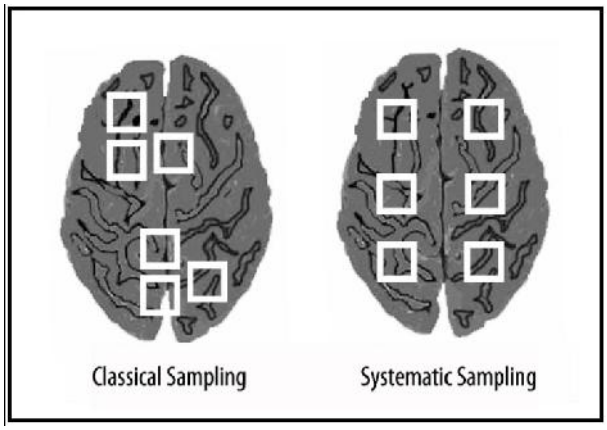

Figure 14. This figure shows the difference between classical and systematic sampling. In classical sampling, sampled areas can be randomly selected whereas there is a known interval between selected areas if systematic sampling is applied.

While making this selection, such structures can be represented in the best shape, and each point of the structure will have an equal chance of providing examples. This is a statistical necessity. Providing these conditions are present, making a random selection will not fully solve the problem. Where the importance of SRS is here, SRS is a fixed and predetermined sampling rate during the first interval, which will start from the entire structure within a random point and go on at the same ratio to interest object exhausted (Howard and Reed, 2005). Predetermined sampling intervals (for example, every tenth section or part decide to choose, the first ten sections of a series), examples of the systematic part, the first interval beginning at a random point (i.e., ten sections from any of those initially selected, or this section after every sample is selected as the tenth section), the features provides the randomness of sampling (**Fig. 12**). Statistical perspectives about this type of sampling show that each point of the structure has an equal sampling chance acquaintance for a homogeneous and efficient sampling (**Fig. 15**) (Gundersen and Jensen, 1987).

Coefficient error
Sign of the quality of the study

One of the most important advantages of stereological studies is that the amount of error in the work is calculable before it has been done. The relationship between the ruler used for measurement, the structure sampling and the calculation is very important in terms of accuracy and precision. Sampling and point or particle counts can be conducted without increasing the workload in accordance with the data obtained as a result of a preliminary study carried out before the original study. In addition, the error coefficient (CE) can be achieved by performing the calculation (Gundersen *et al*., 1987; Cruz-Orive and Weibel, 1990). For an acceptable CE value, we carry out sampling indicating the areas of the cross-sections, and an error value smaller than 5 per cent is regarded as being of sufficient accuracy (Yuen *et al*., 2001; Altunkaynak *et al*., 2009; Yazici *et al*., 2009).

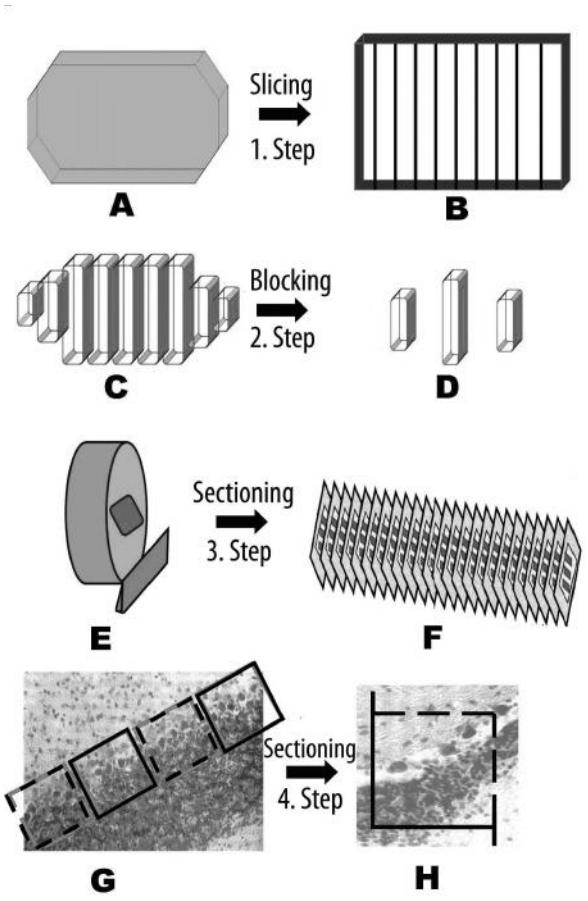

Figure 15. All the possible steps of systematic random sampling are displayed here. Sampling can be made during slicing. For this application, fresh tissue which is so large that it cannot be imbedded in one block is sliced by a slicer with a fixed knife interval (A and B; step I), blocking (C and D; Step II), sectioning (E and F; step III some of all the sections can be selected), microscopically evaluating between areas of vision (G and H step IV; dashed line frames in G were selected to a number estimation using the disector method in H).

In the following samples, CE estimation gives information about the reliability of a stereological study performed with Cavalieri's principle (Example 1) and the

disector method (Example 2).

CE can be calculated using the following table and formulas (the examples given are for display purposes and do not belong to any study. They include the number of random points and particles). For these calculations, an automated MS Excel spreadsheet programme was created and the points of entering the count value of the CE values were calculated automatically.

Example 1

Firstly, the number of points in each section is placed in the following table.

Section Number (i)	P_i	$P_i \times P_i$	$P_i \times P_{i+1}$	$P_i \times P_{i+2}$
1	90	8100	11610	9810
2	129	16641	14061	5031
3	109	11881	4251	3488
4	39	1521	1248	741
5	32	1024	608	704
6	19	361	418	532
7	22	484	616	440
8	28	784	560	448
9	20	400	320	280
10	16	256	224	0
11	14	196	0	0
Total	**ΣP= 518**	**A= 41648**	**B= 33916**	**C= 21474**

Volume=V= t x ΣP_i *x [a(p)] = 0.3 x 518 x 0.13 = 20.20 mm*^{3}

A. Complexity (Noise) to find the value

The first step of the noise calculation is done by using the following formula. For example, if the organ of our interest is the hippocampus, a/b value = 5 should be taken. As n is the number of the total cross-sections (Cruz-Orive and Weibel, 1990; Sönmez *et al*., 2010b).

$$Noise = 0.0724 \times (b/\sqrt{a}) \times \sqrt{n \times \sum P}$$

Noise: complexity of the section surface area

$b/\sqrt{a}$:mean length of the profile boundary / square root of the average of the same profile

n: the number of examined sections

ΣP: the total number of points hitting the section

B. Total area exchange (Var $_{SRS}$):

This step is performed with the help of the following formula. The resulting value of the process is used in the next step.

$$Var_{SRS}(\sum_{i=1}^{n} a) = (3 \cdot (A - Noise) - 4 \cdot B + C)/12$$

$Var_{SRS}(\sum_{i=1}^{n} a)$ Variance of the total area

A: P_i x P_i

B: P_i x P_{i+1}

C: P_i x P_{i+2}

In the formula, the results for the total number of cross-sections represent the area change. Other data are shown in Table 1. This formula is used to calculate the volume with the aid of a table (Table 1) and can be simplified as follows. In the formula, (A, B and C) are written at the end of the columns in the table and the numbers are written in the cells.

C. Total variation:

The following actions are performed, respectively.

$$TotalVar = Noise + Var_{SRS}$$

***Total Var*:** Total variance

Noise: Complexity of the section surface area

Var $_{SRS}$: Variance of the total surface area

D. Coefficient error:

This value is estimated using the following formula.

$$CE(\sum P) = \frac{\sqrt{Total\ Var}}{\sum P}$$

CE: Coefficient error

***Total Var*:** Total variance

ΣP: total number of points hitting the surface of interest.

In this example, CE was obtained as 5.8%. CE values of 5% are less desirable, but a study like this one showed a very acceptable CE measure (Cruz-Orive and Weibel, 1990; Sönmez *et al*., 2010b).

Conclusion

Today, stereological estimations are the gold standard for quantitative studies of volume, length and surface areas such as dimensional parameters or increasing/decreasing particles in clinical and experimental science. SRS is used in the majority of the stereological methods and the principles of the method can be considered as variations of the statistical logic and science composition. SRS is applied in stereological studies on particle count, area calculations and area and volume ratio calculations; the first condition studied in architecture at every stage (the parts selection, cross-section of the sampling, enumeration areas and the field samples.). Knowledge of the basic principles of stereology and sampling strategies will increase the number of quantitative studies and allowing for better quality scientific literature.

About the Authors

B. Zuhal Altunkaynak, PhD, Assistant Professor of Histology and Embryology, Dept. of Histology and Embryology, Ondokuz Mayis University School of Medicine, Samsun, Turkey. **M. Emin Önger**, MSc, Research Assistant of Histology and Embryology, Dept. of Histology and Embryology, Ondokuz Mayis University School of Medicine, Samsun, Turkey. **M. Eyüp Altunkaynak,** MD, Assistant Professor of Histology and Embryology, Dept. of Histology and Embryology, Ondokuz Mayis University School of Medicine, Samsun, Turkey. **Ebru Ayrancı,** MSc, Research Assistant of Histology and Embryology, Dept. of Histology and Embryology, Ondokuz Mayis University School of Medicine, Samsun, Turkey**. Sinan Canan**, PhD, Associate Professor of Physiology, Dept. of Physiology, Medical Faculty, Yıldırım Beyazit University, Ankara, Turkey

References

Acer N, Sahin B, Usanmaz M, Tatoğlu H, Irmak Z. Comparison of point counting and planimetry methods for the assessment of cerebellar volume in human using magnetic resonance imaging: a stereological study. Surg Radiol Anat 2008; 30:335-339

Aguila MB, Mandarim-de-Lacerda CA, Apfel MI. Stereology of the myocardium in young and aged rats. Arq Bras Cardiol 1998;70: 105-109.

Altunkaynak BZ, Altunkaynak ME. Relationship of body weight and volume of liver: A morphometrical and stereological study. Saudi Med J 2007; 28: 891-895.

Altunkaynak ME, Ozbek E, Altunkaynak BZ, Can I, Unal D, Unal B. The effects of high-fat diet on the renal structure and morphometric parametric of kidneys in rats. J Anat 2008; 212: 845-852.

Altunkaynak BZ, Ozbek E. Overweight and structural alterations of the liver in female rats fed a high-fat diet: a stereological and histological study. Turk J Gastroenterol 2009; 20: 93-103.

Aslan H, Altunkaynak BZ, Altunkaynak ME, Vuraler O, Kaplan S, Unal B. Effect of a high fat diet on quantitative features of adipocytes in the omentum: an experimental, stereological and ultrastructural study. Obes Surg 2006; 16: 1526-1534.

Baddeley A, Jensen EB. Stereology for Statisticians. Chapman and Hall/CRC, Boca Raton; 2004.

Baryshnikova LM, Von Bohlen Und Halbach O, Kaplan S, Von Bartheld CS. Two distinct events, section compression and loss of particles ("lost caps"), contribute to z-axis distortion and bias in optical disector counting. Microsc Res Techniq 2006; 69: 738-756.

Bas O, Odaci E, Kaplan S, Acer N, Ucok K, Colakoglu S. 900 megahertz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. Brain Res 2009; 1265: 178-185.

Basoglu A, Buyukkarabacak Y, Sahin B, Kaplan S. Volumetric evaluation of the lung expansion following resection: a stereological study. Eur J Cardio-Thorac 2007; 31: 512-517.

Canan S, Bahadır A, Yıldırım Ş, Odacı E, Şahin B, Baş O, Çolakoğlu S, Bilgiç S, Kaplan S. Stereolojik uygulamalarda kullanılan pratik gereçler ve bilgisayar destekli stereolojik analiz cihazları. Turk Klin Tip Bilim 2004; 24: 672-680.

Canan S, Bozkurt HH, Acar M, Vlamings R, Aktaş A, Sahin B, Temel Y, Kaplan S. An efficient stereological sampling approach for quantitative assessment of nerve regeneration. Neuropath Appl Neuro 2008; 34: 638-649.

Cruz-Orive LM, Weibel ER. Recent stereological methods for cell biology: a brief survey. Am J Physiol 1990; 258: 148-56.

Elfaki A, Osman T, Elsheikh A, Hamdoun A, Şahin B., 2011. Evaluation of the intra-rater variation for the estimation of volume of cerebral structures using the Cavalieri principle on magnetic resonance images. J. Exp. Clin. Med., 28: 22-25.

Evans SM, Janson AM, Nyengaard JR. Quantitative Methods in Neuroscience: A Neuroanatomical Approach Oxford: Oxford University Press; 2004.

Fernández-Viadero C, González-Mandly A, Verduga R, Crespo D, Cruz-Orive LM. Stereology as a tool to estimate brain volume and cortical atrophy in elders with dementia. Rev Esp Geriatr Gerontol 2008; 43: 32-43.

Gardi JE, Nyengaard JR, Gundersen HJ. Automatic sampling for unbiased and efficient stereological estimation using the proportionator in biological studies. J Microsc 2008; 230:108-120.

Glaser JR, Greene G, Hendricks SJ. Stereology for Biological Research. Williston: MBF Press; 2007.

Gökçe MF, Kaplan S, Türkkani A, Kozan R, Ayyildiz M, Emirzeoglu M, Aslan H, Marangoz C. Nitric oxide synthase inhibitors protect cerebellar Purkinje cells from zinc-induced cell loss in adult rat. J Chem Neuroanat 2011; 41, 25–31.

Gundersen HJG. Notes on the estimation of the numerical density of arbitrary particles: the edge effect. J Microsc 1977; 111: 219-223.

Gundersen HJG. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones in memory of William R Thomson. J Microsc 1986; 143: 3-45.

Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-263.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sørensen FB, Vesterby A, *et al*. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988a; 96: 379-94.

Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. The new stereological tools: Disector, fractionator, nucleator, and point sampled intercepts and their use in pathological research and diagnosis. APMIS 1988b; 96: 857-881.

Gundersen HJG, Jensen EBV, Kiêu K, Jensen J. The efficiency of systematic sampling in stereology – reconsidered. J Microsc 1999; 193: 199–211.

Hof PR, Schmitz C. Current trends in neurostereology - introduction to the special issue "Recent advances in neurostereology". J Chem Neuroanat 2000; 20: 3-5.

Howard CV, Reed MG. Unbiased Stereology: Three Dimensional Measurement in Microscopy. 2nd edn. Taylor & Francis, Inc; 2005. Abingdon, UK

Jinno S. Establishment of new research fundamentals in neuroscience by stereology. Fukuoka Igaku Zasshi 2008; 99: 169-74.

Kaplan S, Geuna S, Ronchi G, Ulkay MB, Von Bartheld CS. Calibration of the stereological estimation of the number of myelinated axons in the rat sciatic nerve: A multicenter study. J Neurosci Meth 2010; 187: 90-99.

Kaplan S, Canan S, Aslan H, Unal B, Sahin B. A simple technique to measure the movements of the microscope stage along the x and y axes for stereological methods. J Microsc 2001; 203: 321-325.

Keskin M, Kelly CP, Moreira-Gonzalez A, Lobocki C, Yarim M, Kaplan S, Jackson IT. Repairing critical-sized rat calvarial defects with periosteal cell seeded small intestinal submucosal layer. Plast Reconstr Surg 2008; 122: 400-409.

Kiki I, Altunkaynak BZ, Altunkaynak ME, Vuraler O, Unal D, Kaplan S. Effect of high fat diet on the volume of liver and quantitative feature of Kupffer cells in the female rat: a stereological and ultrastructural study. Obes Surg 2007; 17: 1381-8.

Mandarim-de-Lacerda CA, Fernandes-Santos C, Aguila MB. Image analysis and quantitative morphology. Methods Mol Biol 2010; 611: 211-25.

McArt DG, Wasson GR, McKerr G, Saetzler K, Reed M, Howard CV. Systematic random sampling of the comet assay. Mutagenesis 2009; 24: 373-378.

Mühlfeld C, Nyengaard JR, Mayhew TM. A review of state-of-the-art stereology for better quantitative 3D morphology in cardiac research. Cardiovasc Pathol 2010; 19: 65-82.

Odacı E, Yıldırım Ş, Bahadır A, Canan S, Şahin B, Baş O, Bilgiç S, Kaplan S.Yeni stereolojik yöntemlerin olası hata kaynakları ve çözüm yolları. Turk Klin Tip Bilim 2004; 24: 78-87.

Odaci E, Cihan OF, Aslan H, Ragbetli MC, Kaplan S. Prenatal diclofenac sodium administration increases the number of Purkinje cells in female rats: a stereological study. Int J Dev Neurosci 2010; 28: 145-151.

Ozyurt B, Kesici H, Alıcı SK, Yılmaz S, Odacı E, Aslan H, Rağbetli MÇ, Kaplan S. Prenatal exposure to diclofenac sodium changes the morphology of the male rat cervical spinal cord: A stereological and histopathological study. Neurotoxicol Teratol 2011; 33: 282-287.

Russ JC, DeHoff RT. Practical Stereology, 2nd ed. New York: Kluwer Academic / Plenum Publishers; 2000.

Sahin B, Aslan H, Unal B, Canan S, Colakoğlu S, Bilgiç S, Kaplan S. Deneysel Çalışmalarda Kesitlerdeki Sayısal Yoğunluğun Kullanılmasından Kaynaklanan Hatalar. Türk Hekimler Birliği Vakfı. Stereolojide Temel Kavram ve Yöntemler. Turk Klin Tip Bilim 2002; 22 (Supp 1): 25-29.

Sahin B, Mazonakis M, Akan H, Kaplan S, Bek Y. Dependence of computed tomography volume measurements upon section thickness: an application to human dry skulls. Clin Anat 2008; 21: 479-485.

Schmitz C, Hof PR. Design-based stereology in neuroscience. Neuroscience 2005; 130: 813-31.

Schmitz C. Towards the use of state-of-the-art stereology in experimental gerontology. Exp Gerontol 2000; 35: 429-431.

Schmitz C, Korr H, Heinsen H. Design-based counting techniques: the real problems. Trends Neurosci 1999; 22: 345-346.

Schmitz C. Variation of fractionator estimates and its prediction. Anat Embryol (Berl) 1998; 198:371-97.

Sonmez OF, Odaci E, Bas O, Kaplan S. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900MHz electromagnetic field. Brain Res 2010a; 1356: 95-101.

Sonmez OF, Odaci E, Bas O, Colakoglu S, Sahin B, Bilgic S, Kaplan S. A stereological study of MRI and the Cavalieri principle combined for diagnosis monitoring of brain tumor volume. J Clin Neurosci 2010b; 17: 1499-1502.

Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 1984; 134: 127-136.

Tümkaya L, Kaplan S, Mete UÖ, Ünal B. Light color changes the density and height of synapses in an area of the brain essential for learning. J Exp Clin Med 2010; 27: 109-113.

Turgut M, Tunc AT, Aslan H, Yazici AC, Kaplan S. Effect of pinealectomy on the morphology of the chick cervical spinal cord: A stereological and histopathological study. Brain Res 2007; 1129: 166-173.

Turgut M, Kaplan S, Ünal ZB, Altunkaynak Z, Ünal D, Şahin B, Bozkurt M, Yurtseven ME. Effects of pinealectomy on morphological features of blood vessel in chicken: an electron microscopic and stereological study. J Exp Clin Med 2009; 26: 112-118.

Uyanik A, Unal D, Halici Z, Cetinkaya R, Altunkaynak BZ, Keles ON, Polat B, Topal A, Colak S, Suleyman H, Unal B. Does haloperidol have side effects on histological and stereological structure of the rat kidneys? Renal Failure 2009; 31: 573-581.

Ünal B, Aslan H, Canan S, Şahin B, Kaplan S. Biyolojik Ortamlardaki Objelerin Sayımı Yapılırken Kullanılan Eski (taraflı) Metotların Önemli Hata Kaynakları ve Çözüm Önerileri.Stereolojide Temel Kavram ve Yöntemler. Turk Klin Tip Bilim 2002; 22 (Supp 1): 1-6.

Weibel ER. Stereological methods in cell biology: where are we--where are we going? J Histochem Cytochem 1981; 29:1043-52.

Weibel ER, Hsia CC, Ochs M. How much is there really? Why stereology is essential in lung morphometry. J Appl Physiol 2007; 102: 459-67.

West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991; 231: 482-497.

Witgen BM, Grady MS, Nyengaard JR, Gundersen HJ. A new fractionator principle with varying sampling fractions: exemplified by estimation of synapse number using electron microscopy. J Microsc 2006; 222: 251-255.

Yazici AT, Malkoç I, Altunkaynak BZ, Erdoğan AR, Aydin MD, Dane S, Can S, Gümüştekin K, Unal B. Number of axons in the right and left optic nerves of right-pawed and left-pawed rats: a stereologic study. Anal Quant Cytol Histol 2009; 31: 177-183.

Yuen KH, Wong JW, Yap SP, Billa N. Estimated coefficient of variation values for sample size planning in bioequivalence studies. Int J Clin Pharm Th 2001; 39: 37