Article

Cost–Effective and Efficient Approaches for Applying Stereological Methods

Bülent AYAS, Adnan KORKMAZ, Pınar Naile GÜRGÖR

ABSTRACT

Stereology is an essential tool to drive accurate quantitative information from sectioned material, yet the specialized equipment required for its implementation is not readily accessible to most laboratories or too costly for disciplines where quantification is only a part of their studies. This review deals with the practical aspects of stereology and illustrates our practical experience of applying stereological methods. A description of simple and efficient approaches for applying certain stereological techniques in a rather cost-effective way is presented. They provide economical solutions for critical steps like systematic field sampling, measuring vertical movements of a microscope stage, displaying large reference fields and simultaneous viewing of physical disector pairs. The presented applications may encourage the usage of stereological methods where specialized equipment for performing such steps is not available.

Key Words:stereology, disector, fractionator, number estimation, systematic field sampling

NeuroQuantology 2012; 1: 117-125

1. Introduction

Stereological number and size estimators are the main choice for obtaining accurate quantitative information from two-dimensional images of specimens. Depending on the type of study, a variety of application levels are present. In addition to using radiologic images like CT and MRI scans, stereological methods can also be performed on macroscopic organ slices. But in most cases the application is carried out on histological sections. This is due in part to the fact that the majority of relevant structural information comes mainly from the microscopic level and in part to the wide array of displaying options for the structures of interest. Although the main principles for applying stereological methods are well defined and somewhat uncomplicated, their usage at the microscopic stage introduces some practical constraints in terms of the cost of needed equipment. Apart from common ones, like sampling section fields in a systematic manner, the type of difficulty depends on the used stereological technique and what information is to be extracted. For instance, the sampling strategy in most stereological studies proceeds to the level of generating sections and sampling fields of visions, a task performed at relatively high magnifications. Doing this in a systematic uniformly random fashion demands the usage of a high-precision stepper microscope stage automated in the *X* and *Y* planes. If cells are to be counted, two universally accepted options are available (i.e., one of the physical or optical designs of the disector). Using the any of the optical designs, namely the optical fractionator (West *et al.*, 1991) or optical disector (Gundersen, 1986), requires precise estimation of the vertical movement of the microscope slide, which again necessitates the usage of a programmable motorized stage that is also capable of controlling the Z position. If physical designs (Sterio, 1984; Gundersen, 1986) are preferred (*i.e.,* the physical fractionator or physical disector), one is faced with the problems of sampling and aligning corresponding disector fields on separate slides and then simultaneously viewing them. Several solutions to cope adequately with these problems have evolved and range from using specially designed and equipped microscopes (Howard *et al.*, 1999) to more

Corresponding author: Bülent Ayas, Ph.D.
Address: OndokuzMayısUniversity, Medical Faculty, Department of Histology and Embryology, 55139, Samsun,TURKEY.
Phone: +90 362 312 19 19 Ext: 2266
Fax: +90 362 457 60 41
ayasb@omu.edu.tr
Received July 17, 2011. Revised Sept 11, 2011. Accepted Jan 2, 2012.

sophisticated computer controlled microscope systems. Such equipment however is not readily available in most laboratories interested in quantification, an important cause that limits the widespread use of these invaluable methods.

Here we describe some simple and efficient approaches developed and successfully used in our laboratory. They enable one to apply most of the stereological techniques by utilizing standard tools which laboratories already possess or is within their financial reach. The main principles of these applications are already described in the literature and here we will only summarize them by giving some examples of their usages in an improved form or in different versions.

2. Panoramic views of relatively large reference spaces

This section demonstrates how we have utilized a general purpose flat bed scanner for qualitative and quantitative as well as educational purposes.

Whole images of histological sections provide useful size, shape and arrangement information and are therefore desirable in a number of situations. Studying sections in great detail means that you may lose its context and interpret issues incorrectly. Panoramic views of them are therefore crucial. Apart from being ideal for teaching and consultation purposes, they are invaluable in descriptive and quantitative studies. For educational objectives, the overview image of the entire specimen is an important first step before going into segmental detail and critical focus. Low power images are also very useful in collaborative studies where investigators of different disciplines set out research strategies. In descriptive studies, the whole picture with enough resolution is sometimes necessary for characterizing and demonstrating specimens accurately and for convincing result presentation. Another field that takes advantage of such images is Stereology. Here, they are frequently utilized for delineating reference areas and estimating reference volumes.

Conventionally, overview images are obtained with low power microscope objectives which range between 4x to 1.25x. But even with the smallest magnification, viewing the entire section of moderately sized samples is rarely possible. As for large specimens, conventional microscopy has nothing to do. Developments in digital imaging have provided several ingenious remedies for this problem (cf. 'Coolscope Low-Mag' by Nikon Instruments; 'Mirax Scan' by Carl Zeiss MicroImaging; 'ScanScopeFL' by Aperio Technologies). Instead of using a microscope to examine sections, these solutions offer the usage of novel new microscope systems in which the optics and technology of a microscope is combined with digital imaging. An automated scanner produces high-resolution digital slides of specimens by scanning the histological sections. While their many innovative features may prove invaluable, their usage is currently not a routine practice probably because of their cost. It has been noted however, that a typical flat bed scanner is a form of microscope and can be utilized similarly. Generating stereoscopic pair images (Boyde and Hovell, 2002), reconstructing serial sections (Fiala and Harris, 2002), and collecting data from rock thin sections (Tarquini and Armient, 2003) are some practical examples. We use the HP Precisionscan Pro (hp scanjet 5470c) color scanner at 2400dpi for several purposes. Directly scanning the cover slipped microscope slide provides panoramic views from histological samples with large sectional areas. The resulting pixel size and image quality is reasonable for descriptive purposes and fulfill the needs of many stereological studies.

In **Figure 1** for instance, a scanned digital image from the section of a rat cerebellum is shown. The magnified image in 'c' reveals remarkable details. The borders of the cortical layers, white matter, deep cerebellar nuclei and ventricle can readily be identified. Aside from descriptive and educational purposes, such definitions are also very valuable in stereological studies like volume estimation with the Cavalieri method (Gundersen *et al.*, 1988a) and number estimation with the disector-Cavalieri combination (Pakkenberg and Gundersen, 1988).

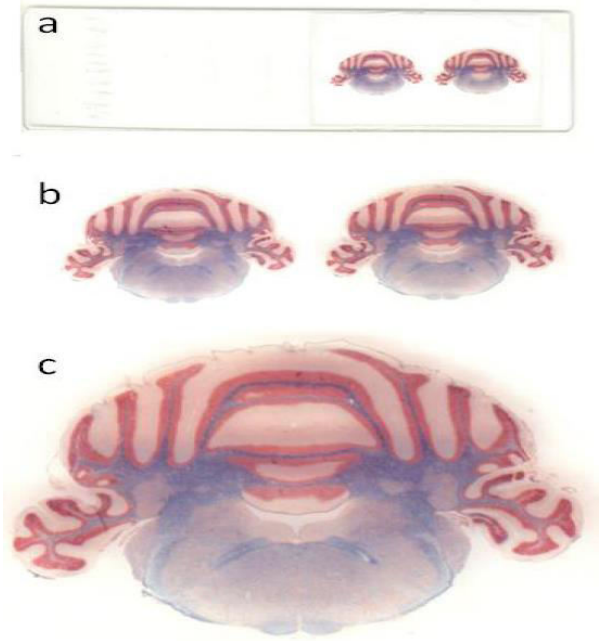


Figure 1.A wholly scanned slide that contains a disector pair taken from the rat cerebellum. The digital image of the consecutive sections in 'a' is magnified in 'b'. The one on the right is shown in 'c' in a bigger scale. Its wide on the slide is about 11 mm and can be fully viewed on a computer monitor with a detail that satisfies many purposes. Luxol fast blue & Neutral red.

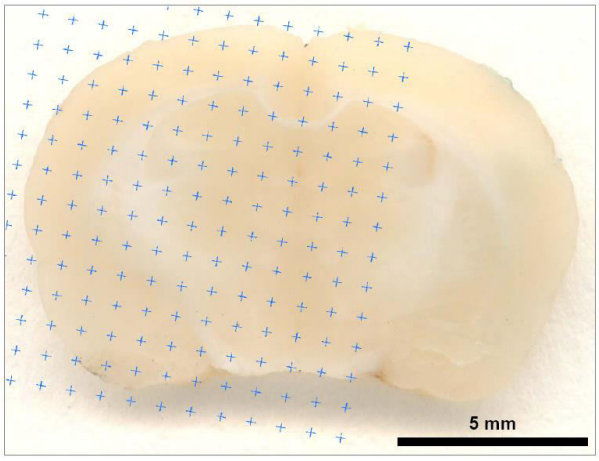


Figure 2. A digital image of a 0.5 mm-thick rat brain slice, obtained by scanning it at 2400 dpi. The brain was cut in a systematic, uniformly random fashion which yielded on average 13 – 14 slices per brain. Acquired Images were displayed in turn on the interface of a standard graphic program(PowerPoint®). The point grid prepared in the same program was placed randomly on each slice image. Points lying over the left and right hemispheres were counted and their volumes were estimated according to the Cavalieri estimator. This stereological volumetric analysis revealed 8 % brain swelling on operated hemispheres compared with opposite ones (unpublished results from our laboratory).

In a study where venous ischemia and brain swelling was evaluated, we have used the same approach on unstained brain slices. Each brain was cut in a systematic random manner into 0.5 mm frontal slabs with a self made razor blade fractionator (a device similar to that shown in Figure 7 of Gundersen *et al.*, 1988b). Scanning slices resulted in 24x magnified images where both hemispheres could be entirely viewed and readily distinguished from the opposite one. Evaluation of brain swelling was performed directly on a PC monitor. The total volume of each hemisphere was estimated with the Cavalieri method using a point probe prepared in the used graphic program (Fig 2).

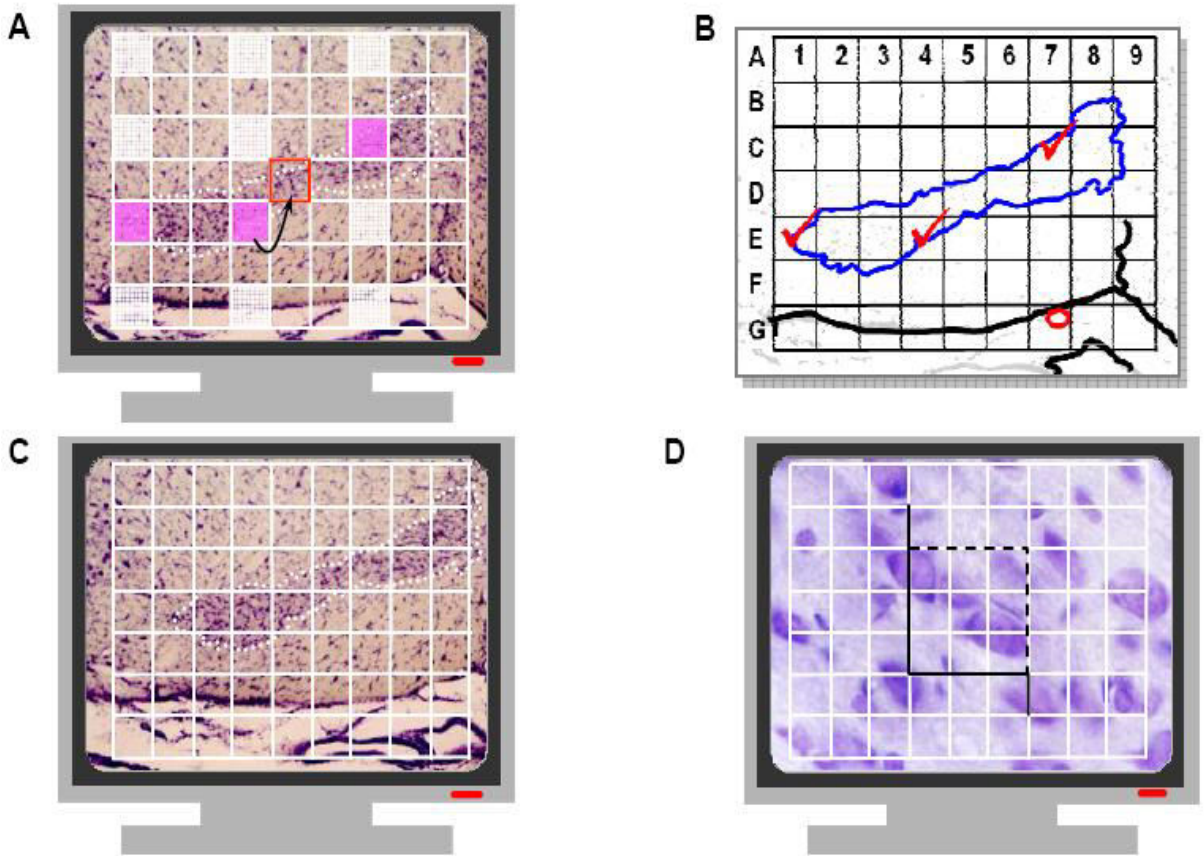
Other cases where we have adopted this image acquisition approach in stereological studies include systematic random area sampling and volume fraction estimations on rat (Çetinkaya *et al.*, 2006b and 2008) and rabbit (Kılıç *et al.*, 2008) mandibles. In these examples scanned areas on the slide went to 30x13 mm^2. Besides, we have used it for illustrative and descriptive purposes. Scanned images of whole rat molars and their surrounding tissues satisfied the needs of showing certain experimental changes (Çetinkaya *et al.*, 2006a). It was also very useful for assessing and illustrating the relationship of several histological features of specimens taken from the nasal dorsum and frontoorbital regions of humans, samples which constituted cross sectional areas of about 25x10 mm^2 (Karacalar *et al.*, 2005a and b).

3. Systematic field sampling

In stereology, drawing conclusions about structural parameters of objects is achieved by superimposing geometrical probes, such as points, lines and volumes on two-dimensional images taken from the object of interest and defining the way in which the probes interact with the image (Gundersen *et al.*, 1988a and b; Howard and Reed, 1988). Some examples of this act carried out with the naked eye are given above. Depending on the type of analysis, however, placement of geometrical probes is in most cases performed at a microscopic level. At this level, the final magnification required for clear-cut definition generally increases considerably and provides an image including only a small portion of the section. This poses several problems for applying the probes. The main hurdle is a general one for almost all stereological studies: the systematic, uniformly random sampling of section fields where the probes are to be applied. Delineating anatomical boundaries of the structure of interest, a critical requirement in volume and many number estimation studies, in such magnified images represents another important problem. The easiest and most efficient way of overcoming this problem is doing this with stereological packages (cf. Prism' in Skoglund *et al.*, 1996; Bioquant' in

Hyman *et al.*, 1998; CAST-grid' in Dorph-Petersen, 1999). Here the sampling is performed automatically with the aid of computer controlled microscopes. The stage moves from one field to the next one within the beforehand-defined boundaries of the working area. But the cost of such set ups is usually very high. We have developed a simple and efficient method for sampling section fields manually (i.e. with no benefit of motorized stages and dedicated software). Using general purpose laboratory facilities allows us to sample fields of visions in a systematic uniformly random manner without compromising its unbiased aspect. The main points of this approach are outlined in **Figure 3.** Detailed illustrations and a practical example are given elsewhere (Korkmaz *et al.*, 2000).

What differs from conventional current applications and what we propose here is simply mimicking the whole scanning and sampling job at a sufficiently low magnification before performing the measurements. Consequently, a two step application: (1) determining the number and positions of fields at a low magnification and then (2) evaluating them in turn at suitable high magnification. So far we have used this approach in a number of doctoral dissertations (Ayas, 2005; Gürgör, 2008) and several studies all related with counting neurons in the central nervous system (Ağar *et al.*, 1999 and 2001; Bostancı *et al.*, 2003). Aside from being economical, these studies proved that the proposed sampling approach is also an effective alternative. After sampling a few fields one becomes acquainted with the application steps.



See next page for figure 3 explanation

Figure 3. The principle and application steps of our field sampling approach. The main point of our approach that distinguishes it from others is that the number and location of fields where assessment will take place are determined in advance at a low magnification. Evaluation in these fields is then performed at a desired suitable higher magnification. Since the number and positions of fields are ascertained in this way, there is no risk to be concerned whether the evaluated field at higher magnifications is within the anatomical borders of the structure of interest. Accordingly, a sampled section is first displayed on a monitor (a requirement of the application) at a magnification where the entire profile area of the structure of interest (a brainstem nucleus outlined with white dots here) is within one field of view (A). Sampling of section fields is overcome with the aid of a transparent indexed sheet (the areas on the sheet represent the scanning movement of the microscope stage in the *x* and *y* axes in conventional current applications). The sizes of the steps (i.e. areas) and sampling intervals are set according to the needs of the study. By superimposing the sheet on the monitor, the profile on the section interacts with the sheet steps in a uniformly random manner. In this example, three steps fall on the structure of interest (highlighted ones) and represent the fields where evaluations should be made. After noting down the locations of these steps along with the boundary of the related structure and certain landmarks (two blood vessels and the inferior border of the brainstem is depicted here) on a paper (B), one proceeds to the next stage of the procedure. In order to evaluate the sampled fields at a suitable higher magnification, each sampled field is moved manually in turn to the center of the monitor. This movement is depicted with the arrow in 'A' (for the sampled field E4) and the final position of the section is shown in 'C'. The field pointed by the arrow-tip represents the area that will occupy the center of the monitor image at the desired higher magnification. The exact position of such a point depends on the used low and high magnifications and should be determined before the procedure. The remaining practice is to evaluate the field by switching to the evaluation magnification at this point (D). Any relevant stereological probe (like the unbiased counting frame shown here) that suits the needs of a particular study is applied at this last stage. After completing the evaluation for one field, the other sampled fields are assessed in turn in the same way. This is accomplished after switching again to the low magnification and locating the section profile to its original position using the records.

4. Estimating the section thickness and optical disector height

A group of stereological applications necessitates the estimation of the final section thickness and/or optical disector height. Estimation of these parameters is carried out with the differential focusing method (West *et al.*, 1991). In this method one simply determines the displacement of the microscope stage in the z axis while focusing from the upper to the lower surface of a section. The height of the sampling unit within a thick section (mounted thickness ≥ 25 µm), i.e. height of the optical disector, is determined in the same way. Here, the section is scanned using optical focal planes and the sampling unit must be placed central to the section. This is carried out by leaving sufficiently large guard areas near the top and bottom surfaces of the section. The measurement of all these distances requires the usage of either a microcator or shaft encoder (Gundersen, 1986; Howard and Reed, 1998). They work by reading the vertical displacement of the microscope stage. Their solely purchase, however, is still neither cheap nor reliable because they will aid to overcome only one stage of the stereological application. As stated above, for the sampling of section fields one will also need a high-end microscope with a fully automated stage and a dedicated software package. A cost-effective alternative way of determining the vertical distance traveled by the microscope stage is developed by Heinsen *et al.* (1994). Their ingenious method is based on the inspiration that the vertical movement of the stage during focusing through a cover glass with a known thickness correlates linearly with the angular displacement of the fine focus knob. They determined that the displacement of the fine focus knob from one gradation mark to the next one represent a stage movement of 3.3 µm. We have further refined this method and made it possible to define more precise (0.28 µm) stage movements (Korkmaz and Tümkaya, 1997). We noticed that the number of gradation marks on a fine focus knob is limited to a few hundreds and are simply too few to make a more precise calibration. But a simple alteration made it possible. Our modification is based on the idea of using a scale with a much longer radius instead of the original scale of the fine focus knob. Since such an enlarged circular scale will have a larger perimeter, much more gradation marks can be visualized on it. Consequently, each gradation mark will represent a much smaller vertical movement of the microscope stage. We have employed a custom-made enlarged circular scale instead of that on the microscope's fine focus knob. A large circular scale was printed on a cardboard and a sufficient piece of it is used by fixing it to the side of a microscope in a position where its center is the same with that of the fine focus knob. The angular displacement on the new scale is read using a long pointer mounted to the fine focus knob (**Fig 4**).

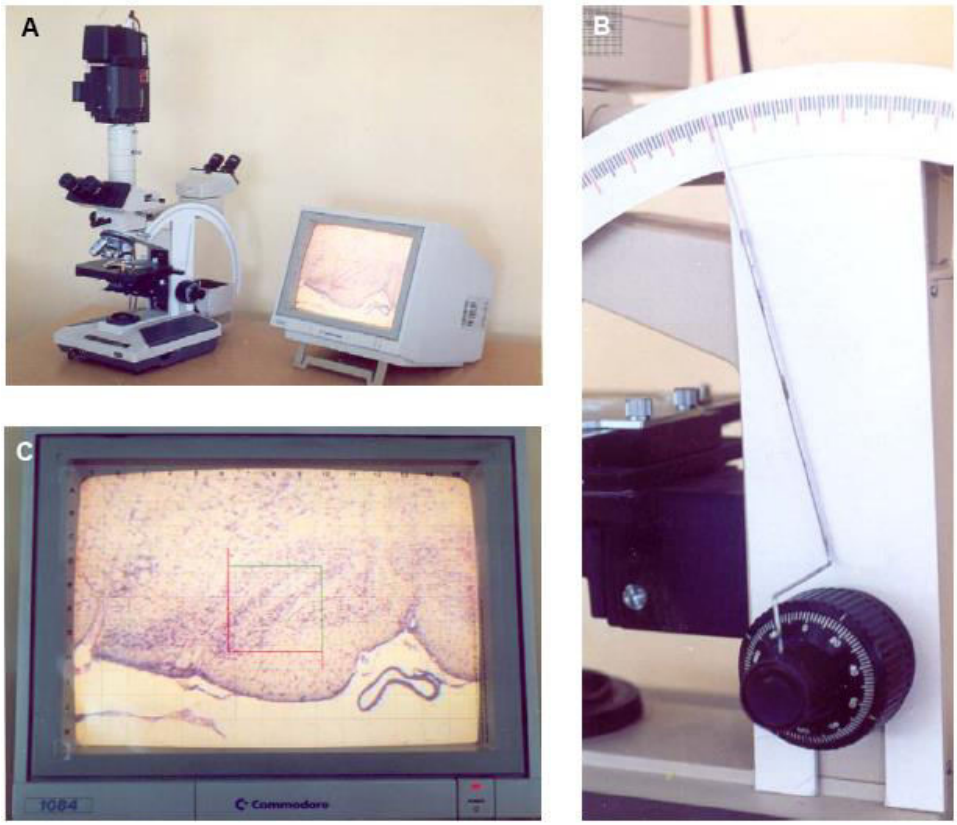


Figure 4. (A) The microscope set-up used to perform optical counting designs. It consists of a microscope (Olympus BH-2) and a color monitor (Commodore 1084). Beside the microscope is a part of a prepared circular scale and a pointer. (B) This part is fitted to the side of the microscope so that it shares the same center with the microscope's fine focus knob. The whole scale has a radius of 20 cm and contains 720 gradation marks. Each mark represents a vertical stage movement of 0.28 µm. The part of the scale used here has 210 gradation marks and represents a vertical traveling distance of about 60 µm. The angular displacement on the scale during the stage movement is read by a pointer which is fixed to the fine focus knob and moves together forward and backwards (C) The transparent indexed sheet superimposed on the monitor is used for field sampling and counting (see text and Figure 3 for its usage). It comprises steps for sampling fields (to be used at low magnification) and an unbiased counting frame (to be used at the evaluation magnification).

Our microscope set-up used to count neurons in optical disectors with this enlarged scale method in conjunction with our area sampling approach is shown in Figure 4. It consists of a microscope and a monitor, tools that are already available in laboratories. It is ready to use after calibrating the scale with cover glasses. Its usage, namely the estimation of the section thickness and optical disector height with this enlarged scale is illustrated in Figure 5. Corresponding events on the section and viewed images that take place during these estimations are also sketched out. Calibration of the enlarged scale and other finer points of how it should operate are detailed in the original article (Korkmaz and Tümkaya, 1997).

We have used this set-up in several doctoral dissertations and studies (Ağar *et al.*, 2001; Bostancı *et al.*, 2003). Besides, it has been utilized in a dozen of research projects (cf. e.g. Yılmaz *et al.*, 2006; Akdoğan *et al.*, 2008; Scroza et al., 2010; Pereira, 2011).

Our own experience has showed that using this approach is quite efficient. Performing the optical fractionator technique in a brainstem nucleus (the rat ventral cochlear nucleus or nucleus olivaris inferior) with our enlarged scale and field sampling approach in ~10 sampled sections took about 3 hours.

An important issue that should be taken into account here is related with the used stereological counting techniques, namely those that use the optical disectors as sampling probes. Regardless of our estimation and sampling approach, the optical designs are open to section deformation and may therefore subsample the sections. For detailed considerations and new estimators for these designs see Dorph-Petersen *et al.* (2001).

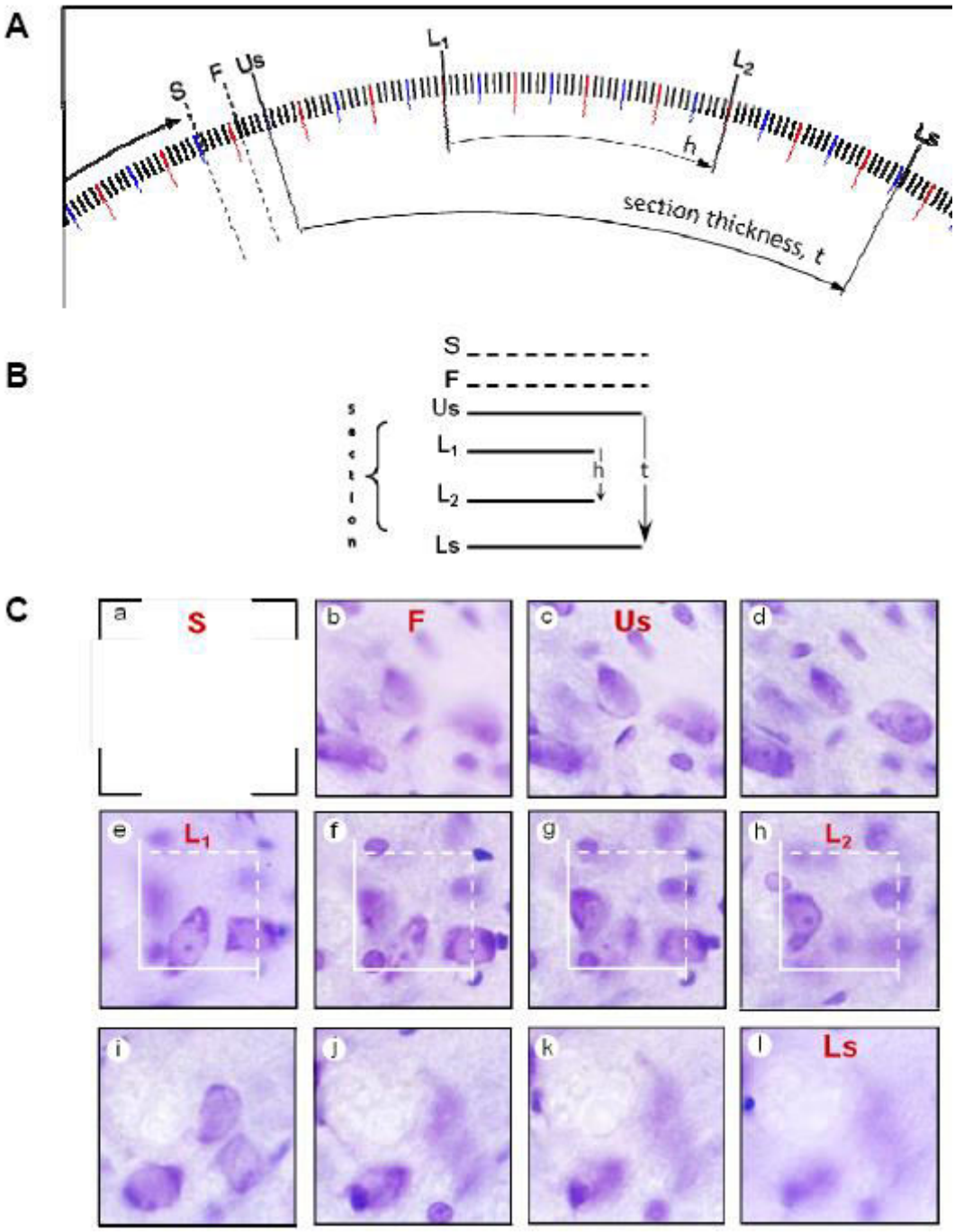


Figure 5. The principle of using the enlarged circular scale to estimate the section thickness (*t*) and optical disector height (*h*). Corresponding events on the enlarged scale, section and monitor image are diagramed separately in A, B and C. Just a part of the scale that represents the working distance is shown in (A). Similarly, only a few optical sections that help to explain the principle are displayed in (C). The upper row represents the optical sections in defining the upper surface. The middle row shows four levels (e – h) of the optical disector, including its upper (L_1) and lower (L_2) levels. The bottom row displays four levels (from 'i' to 'l') where the structural features of a section go out of focus. The positions of the pointer at different focal levels are represented by lettered straight lines in (A). Their corresponding levels on the section are represented by horizontal lines in (B).To estimate *t*, one starts to focus the section at a distance where its upper surface (Us) is not in focus (S). By continuing to rotate the fine focus knob from this point, one approaches the upper surface where its structural features will appear first unclearly (F). This fuzzy appearance is an indication that the stage and fine focus knob moves linearly. Thereafter the upper surface comes into focus (Us). The level where the structural features go out of focus is the lower surface (Ls) of the section. In practice this is one continuous movement from S to Ls and the section thickness is estimated by multiplying the calculated increment of the gradation marks with their total number between Us – Ls. The same is done to determine *h* (the distance between L_1 and L_2). In practice one starts to counts cells within this distance (i.e. when the pointer comes to L_1 from S and stops at L_2). In this example estimates of *t* and *h* are 25.48 µm (91 x 0.28 µm) and 11.2 µm (40 x 0.28 µm), respectively (see original article for details).

5. Aligning disector pairs

The main advantage of optical designs over physical ones is that they do not oblige section pairs and consequently no aligning of corresponding section fields. A series consisting of single thick sections from a block suffices. However, their hurdle of being vulnerable to deformation makes it necessary to fulfill same additional requirements. The physical designs, particularly the physical fractionator, are therefore preferable because their estimate is independent of deformation problems. But, as stated in the Introduction section, they too have an important drawback, the slow and troublesome aligning act of sampling and simultaneous viewing of section fields. This constitutes the main limiting factor to the widespread use of physical designs. The main ways to handle this problem in current practices are photographing section pairs (Pover *et al.*, 1994), employing a specially designed tandem microscope (Howard *et al.*, 1999), utilization of computer systems (Smith & Bedi, 1997) or using more sophisticated computer controlled microscope systems and stereological packages like the CAST® (Olympus Denmark). We have tried a different way by taking advantage of devices available in our laboratory. It consists of two color monitors, a video recorder and a microscope equipped with a video camera (Ağar *et al.*, 1999; Korkmaz *et al.*, 2000). Simultaneous viewing of disector pairs with this set-up does not need any specialized device. It takes advantage of commercial video equipment. But its implementation may seem to be prohibitively tedious and complicated. We have further developed another technique that both accelerates and simplifies the sampling and aligning tasks and brings the implementation within the reach of any researcher. Utilization of a microscope equipped with a camera and an image capturing card is sufficient. Counting is performed on an ordinary personal computer running Microsoft Office®. In our hands, the total time required for the sampling of corresponding section fields (~80 – 90 reference and look-up pairs on about 10 sections), their storage, their simultaneous viewing on the monitor and finally counting neurons (~100 – 150) with the disector principle was usually less than 4 hours. This approach is still in progress. The given data comes from successful trials on the rat ventral cochlear nucleus and nucleus olivaris inferior in two doctoral dissertations.

6. Summary and outlook

The main point in this report is that most of the stereological methods can successfully applied with simple tricks and by taking advantage of general purpose facilities. In the related references our approaches have proven practical and cost-effective. Obviously, their utilization is less general than the above mentioned computerized systems and such stereological packages are more time and cost-efficient in terms of routine usage. The investment of such dedicated systems, on the other hand, may not justifiable for limited needs and for every discipline that is interested in quantifying sectioned material. The approaches suggested here may therefore represent for many potential researchers low-cost alternative options that yield perfect results for many purposes.

Acknowledgments

This review is dedicated to Nusret Çifçi (Emeritus Professor and founder department head), who started us on our research career. His continuous guidance and encouragement of the pursuit of excellence is gratefully appreciated.

References

Akdogan I, Adiguzel E, Yilmaz I, Ozdemir MB, Sahiner M, Tufan AC. Penicillin-induced epilepsy model in rats: Dose-dependant effect on hippocampal volume and neuron number. Brain Res Bull 2008; 77: 172-177

Ağar E, Korkmaz A, Boşnak M, Demir Ş, Ayyıldız M, Marangoz C. Do cochlear nuclei contribute to auditory lateralization? A stereological evaluation of neuron numbers. Ann Oto Rhinol Laryn 1999; 108: 661-665.

Ağar E, Boşnak M, Korkmaz A, Demir Ş, Ayyıldız M. The effect of ethanol on the number of cells in the cochlear nucleus of the male rat: A stereological study. Neurosci Res Commun 2001; 28: 189-200.

Ayas B. Investigation of the postnatal development of the rat cochlear nucleus with stereological methods. PhD thesis, University of Ondokuz Mayis, Institute of Health Science, Turkey 2005

Bostanci MO, Bagirici F, Korkmaz A. The neurotoxic effect of iron on pyramidal cell number in rat hippocampus: A stereological study. Neurosci Res Commun 2003; 32: 151-159

Boyde A, Howell PGT. Using a flap bed reflective scanner to generate stereoscopic pair images. Proc Royal Microsc Society 2002; 37: 39-40

Cetinkaya BO, Acikgoz G, Ayas B, Aliyev E, Sakallioglu EE. Increased expression of vascular endothelial growth factor in cyclosporin A-induced gingival overgrowth in rats. J Periodontol 2006a; 77: 54-60.

Cetinkaya BO, Acikgoz G, Keles GC, Ayas B, Korkmaz A. The effect of cyclosporin A on alveolar bone in rats subjected to experimental periodontal disease. Toxicol Pathol 2006b; 34(6): 716-22.

Cetinkaya BO, Keles GC, Ayas B, Gurgor P. Effects of risedronate on alveolar bone loss and angiogenesis: a stereologic study in rats. J Periodontol 2008; 79: 1950-61.

Dorph-Petersen KA. Stereological estimation using vertical sections in a complex tissue. J Microsc 1999; 195: 79-86.

Dorph-Petersen KA, Nyengaard JR, Gundersen HJG. Tissue shrinkage and unbiased stereological estimation of particle number and size. J Microsc-Oxford 2001; 204: 232-246.

Fiala JC, Harris KM. Computer-based alignment and reconstruction of serial sections. Microsc Analysis 2002; 75: 17-19

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. Thompson. J Microsc-Oxford 1986; 143: 3-45.

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988a; 96: 379-394.

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 and point-sampled intercepts and their use in pathological research. APMIS 1988b; 96: 857-881.

Gürgör PN. Determination of the number of neurons in the rat inferior olivary nucleus with physical disector approaches. PhD thesis, University of Ondokuz Mayis Institute of Health Science, Turkey 2008.

Heinsen H, Henn R, Eisenmenger W, Götz M, Bohl J, Bethke B, Lockemann U, Püschel K. Quantitative investigations on the human entorhinal area: left-right asymmetry and age-related changes. Anat Embryol (Berl) 1994; 190:181-94.

Hyman BT, Gomez-Isla T, Irizarry MC. Stereology: A practical primer for neuropathology. J Neuropath Exp Neur 1998; 57: 305-310

Howard CV, Reed MG. Unbiased Stereology, Three-dimensional measurement in microscopy. First edition, BIOS scientific publishers, UK 1988.

Howard CV, Beech DJ, Reed MG, Moss MC. A double microscope for the efficient application of the physical dissector: the tandem projection microscope. J Cell Pathol 1999; 4: 26-32

Karacalar A, Korkmaz A, Kale A, Kopuz C. Compensatory brow asymmetry: anatomic study and clinical experience. Aesthet Plast Surg 2005a; 29: 119-23.

Karacalar A, Korkmaz A, Içten N. A perichondrial flap for functional purposes in rhinoplasty. Aesthet Plast Surg 2005b; 29: 256-60.

Kiliç E, Ozeç I, Yeler H, Korkmaz A, Ayas B, Gümüş C. Effects of simvastatin on mandibular distraction osteogenesis. J Oral Maxil Surg 2008; 66: 2233-8.

Korkmaz A, Tumkaya L. Estimation of the section thickness and optical disector height with a simple calibration method. J Microsc-Oxford 1997; 187: 104-109.

Korkmaz A, Ciftçi N, Boşnak M, Ağar E. A simplified application of systematic field sampling and low-cost video recording set-up for viewing disector pairs - exemplified in the rat cochlear nucleus. J Microsc-Oxford 2000; 200: 269-76

Pakkenberg B, Gundersen HJG. Total numbers of neurons and glial cells in human brain nuclei estimated by disector and fractionator. J Microsc-Oxford 1988; 150: 1-20.

Pereira MCL, Secco M, Suzuki DE, Janjoppi L, Rodini CO, Torres LB, Araujo BHS, Cavalheiro EA, Zatz M, Okamoto OK. Contamination of mesenchymal stem-cells with fibroblasts accelerates neurodegeneration in an experimental model of parkinson's disease. Stem Cell Rev Rep 2011; 7: 1006-1017

Pover CM, Barnes MC, Coggeshall E. Do primary afferent cell numbers change in relation to increasing weight and surface area in adult rats? Somatosens Mot Res 1994; 11: 163-167.

Scorza CA, Araujo BHS, Arida RM, Scorza FA, Torres LB, Amorim HA, Cavalheiro EA.Distinctive hippocampal CA2 subfield of the Amazon rodent proechimys. Neuroscience 2010; 169: 965-973

Skoglund TS, Pascher R, Berthold CH. Aspects of the quantitative analysis of neurons in the cerebral cortex. J Neurosci Meth 1996; 70(2): 201-10.

Smith SA, Bedi KS. Unilateral eye enucleation in adult rats causes neuronal loss in the contralateral superior colliculus. Journal of Anatomy 1997; 190: 481-90.

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

Tarquini S, Armienti P. Quick determination of crystal size distributions of rocks by means of a color scanner. Image Anal Stereol 2003; 22: 27-34

West M, Slomiank L, Gundersen HJG. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991; 231: 482-497.

Yilmaz I, Adiguzel E, Akdogan I, Kaya E, Hatip Al Khatip. Effects of second generation tetracyclines on penicillin-epilepsy-induced hippocampal neuronal loss and motor incoordination in rats. Life Sci 2006; 79: 784-790