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STEPone - AN INTERACTIVE PROGRAM FOR MANUAL STEREOLOGY

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ABSTRACT

A new software package for stereology, based on the inexpensive MS DOS (PC DOS) computing environment is described. *STEPone* is an interactive program with on-line *HELP* facilities. Raw data (in the form of raw counts obtained with possibly different test systems) can be entered at the keyboard into basic compartments. The user can then define and obtain ratios of linear combinations (with unit coefficients) of the basic compartment variables. The design can be structured into strata, sections and quadrats, and it uses updated statistics. The acquisition of raw data by manual methods is not essential to the program.

Keywords: Image analysis, manual methods, MS DOS, point counting, quadrat, ratio estimation, sampling design, section, software package, stereology, stratification, test system.

1. INTRODUCTION

Since relatively powerful microcomputers have become available at a moderate cost in the early 1980's, there have been a number of reports on computer programs suitable for manual stereology for biological applications, e.g. Hoppeler *et al* (1980), Silage and Gil (1984), Bradbury (1984), de Paz and Barrio (1985), Marchevsky *et al* (1985), Nuñez-Durán (1985), Pentcheff and Bolender (1985); see also the last report of the Software Group of the International Society for Stereology by Howard and Eins (1984). There has also been a considerable interest (commercial or otherwise) in developing software and hardware for fully- or semi-automatic image analysis systems (Clermonts and Birkenhaeger-Frenkel, 1985; Rigney, 1985; Eichler *et al*, 1985; Dallant *et al*, 1986; Gil *et al*, 1986; Curcio and Sloan Jr, 1986; de Paz *et al*, 1986; Poggi *et al*, 1987). Two important facts are worth mentioning, however. First, fully automated image analysis has only rarely contributed successfully and decisively to the solution of stereological problems, at least in biology —this point is clearly made for instance by Moss and Howard (1988). And second, when estimating global stereological quantities such as V, S, L, N, point-counting stereology can be vastly more efficient than semi-automatic image analysis with tracing devices (Mathieu *et al*, 1981, Gundersen *et al*, 1981, Gundersen and Jensen, 1987). There is therefore currently still a need for easy-to-use and comprehensive software packages for manual stereology tapping the power of the now widely available, inexpensive MS-DOS computers.

The purpose of this paper is to describe a new interactive program for manual stereology called *STEPone* (i.e., "*Ste*reology *P*rogram version *I*"). Some of the features which set *STEPone* apart from existing programs are the following: (i) Raw data can be organized according to a standard sampling design involving for instance strata, sections and quadrats, (e.g. Cruz-Orive and Weibel, 1981; see Fig. 1). (ii) Starting from a set of user-defined basic compartments, ratios can be defined of linear combinations of the compartment quantities. (iii) Raw data are immediately corrected for magnification prior to further processing, thus avoiding unnecessary loss of accuracy due to fluctuating magnifications (especially if electron microscopy is used, see Cruz-Orive, 1982).

The system requirements are specified in §2. The scope and limitations of the program are indicated in §3, and its basic structure and mode of operation are described in §4. In §5, details are given on the statistical computations used, and in §6, a case example is illustrated by displaying the corresponding printout. Finally, in §7 a brief account is given of special features and future developments of the program.

2. SYSTEM REQUIREMENTS

STEPone was written for an IBM PC in *Microsoft* PASCAL and *Assembler*. System requirements are an IBM PC, PC XT, PC AT or compatible, equipped with 256 kBytes of RAM. A station may be used with either two floppy disk drives, or with one floppy drive and a hard disk. The operating system required is DOS 2.1 or higher.

3. SCOPE AND LIMITATIONS OF *STEPone*

STEPone is designed as an intermediate tool in a stereological study. The input is raw data consisting of point, intersection or feature counts obtained from planar images observed at a fixed nominal magnification, whereas the output consists of stereological *ratios* (and not absolute quantities) defined by the user for the corresponding spatial structure. Input and output pertain to a single primary sampling unit, e.g. the lungs of one animal. Thus, statistics between animals within one group, and between groups of animals, must be made outside *STEPone*.

If required, *STEPone* will preserve the structure of data coming from a stratified sampling design, see Fig. 1, and will process the data accordingly. For definitions and practical remarks on *stratification* see Michel and Cruz-Orive (1987, §3). A non-stratified design is treated as a particular case with only one stratum. From each stratum, one or more sections may be available, and from each section one or more *quadrats* (namely 'fields of view', 'test windows' or 'micrographs') may be subsampled. On the other hand, one or several compartments or phases may be defined in the structure of interest which must be observable in the quadrat images at the chosen magnification (e.g. lung septa, lung capillaries within the septa) and one or more relevant quantities must be defined for each compartment (e.g. volume, surface area). The target quantities to be computed with *STEPone* are user-defined ratios of linear combinations (with unit coefficients) of the available compartmental quantities, (examples are given in §6). The absolute quantities to be reported at the end of the study must therefore be computed outside *STEPone* by multiplying the relevant ratios with the volumes (or, in general, the absolute measures) of the reference spaces in the denominator of these ratios.

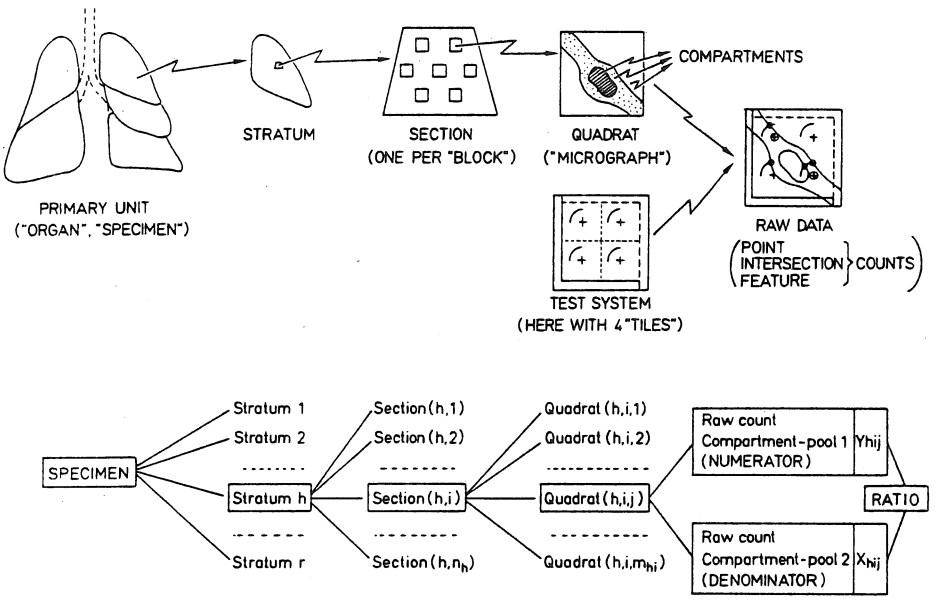

Figure 1. Example of the most general sampling design supported by *STEPone*, see text, §3 and §5.

To analyse each quadrat by point and intersection counting, up to five different test systems of known characteristics may be used (normally, only up to two are used, however). Each raw count is immediately transformed using the final linear magnification at which it was taken and the characteristics of the corresponding test system, as indicated in §5. Raw counts are nevertheless saved as such and can be retrieved at any time. The data can also be expanded or modified at any time.

Apart from the final ratio estimate defined for the whole organ or structure of interest, and its *'Coefficient of Error'* (namely *'Standard Error/Mean'*), the printout also includes the partial ratio estimates for each section and for each stratum, which enables the user to easily assess internal variation. The standard errors of the ratio estimates are computed using the classical approximation of Cochran (1977), see §5. The approach assumes independent sampling; since sampling and subsampling are usually systematic, however, the error estimates computed with *STEPone* may be conservative in practice (see Gundersen and Jensen, 1987, Cruz-Orive, 1989). The issue is a very minor one, however, because the relevant variation is usually that between animals, and this must be assessed outside *STEPone*, as pointed out above.

4. BASIC STRUCTURE AND OPERATION OF *STEPone*

STEPone makes use of a system of two files, namely the *TASK* file and the *DATA* file. The *TASK* file contains information that is essential to produce the *DATA* file and the subsequent computations.

Creating a TASK file.

This is done interactively on successive screen displays, and it consists of the following steps:

(i) *Opening a new TASK file.* The option *CREATE TASK FILE* must be chosen from the main menu. A first screen displays the names of all *TASK* files previously created; on this screen the new name is entered.

(ii) *Definition of the test system (s).* A test system is conceived as a regular 'pavement' of identical regions called *tiles*. Each tile may contain test points and test lines (Fig. 2). As proposed in Cruz-Orive (1982), a test system is characterized by the following four quantities:

p: number of test points in a fundamental tile.
l: total test line length (mm) in a fundamental tile.
a: area (mm^2) of a fundamental tile.
n: total number of fundamental tiles.

Up to five different test systems may be chosen, either from a displayed collection of *standard* test systems (Weibel, 1979) for which p, l, a, n are already given, or by defining a *non-standard* test system with user-chosen characteristics p, l, a, n.

(iii) *Allocation of keyboard keys for the compartments.* Each of the keys 1, 2, ..., 9 of the numerical keypad may be allocated the name of a compartment (e.g. *septum*), the type of counter (i.e., point P, intersection I, or feature count Q or N), and finally the test system (from those defined in step (ii) above) with which the counts will be made. See §6.

(iv) *Definition of the stereological ratios to be estimated.* Up to 15 ratios may be defined in a single *TASK* file. Each ratio is first identified by a name or a set of characters, e.g. *V(cap)/V(septum)*. Then, the keypad numbers of the compartments chosen to make the numerator and the denominator of the ratio are combined in the desired way, for instance $(1+2)/(1+2+3)$. Only '+' and '−' operations are allowed. If desired, a multiplication factor k can be entered; in the preceding example this would yield $k \cdot (1+2)/(1+2+3)$. The default is $k = 1$. The possibility of choosing k is useful whenever the classical stereological equations $S_V = 2I_L$, $L_V = 2Q_A$ have to be replaced with $S_V = (constant) \cdot I_L$, $L_V = (constant) \cdot Q_A$ with a *constant* other than 2, e.g. when analysing anisotropic structures (Mathieu *et al*, 1983, Cruz-Orive *et al*, 1985). When estimating particle number via the equation $N_V = h^{-1}Q_A^-$ with the disector (Sterio, 1984), the multiplication factor is h^{-1}, where h is the thickness of the disector.

An important feature of *STEPone* is the possibility of defining a 'ratio' with the denominator equal to 1, that is, an absolute parameter instead of a ratio. The *Cavalieri estimate* of volume (Gundersen and Jensen, 1987, Michel and Cruz-Orive, 1988) is an obvious example of this. The proper computation of the coefficient of error of such estimate has not yet been implemented in *STEPone*, however.

Creating a DATA file.

(i) *Opening a new DATA file.* A *DATA* file is opened by choosing the *CREATE DATA FILE* option from the main menu. First, the names of the currently available *DATA* files are displayed. After entering the new *DATA* file name, the user has the option to add a comment in words to be stored as part of the new *DATA* file.

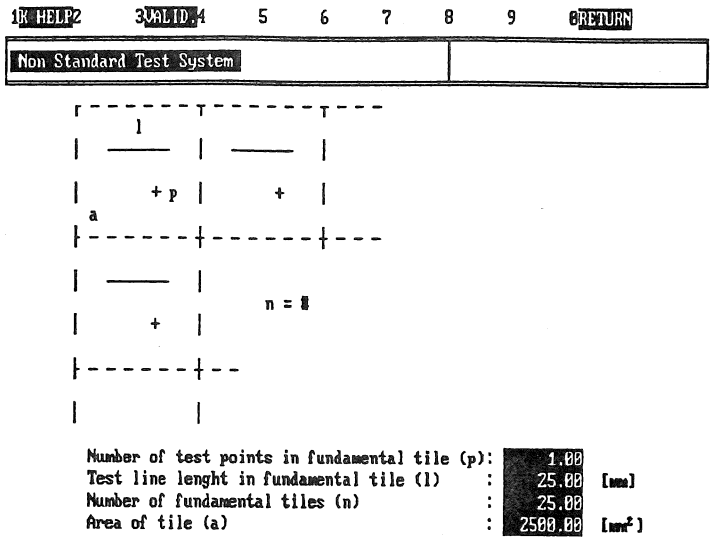

Figure 2. Definition of a non-standard test system for a *TASK* file, see text , §4. The three tiles shown are always displayed only to remind the user of the meaning of the quantitative characteristics *p, l, a, n.* The actual shape of the test system to be used does not enter into the calculations, however, and it does not need to be specified.

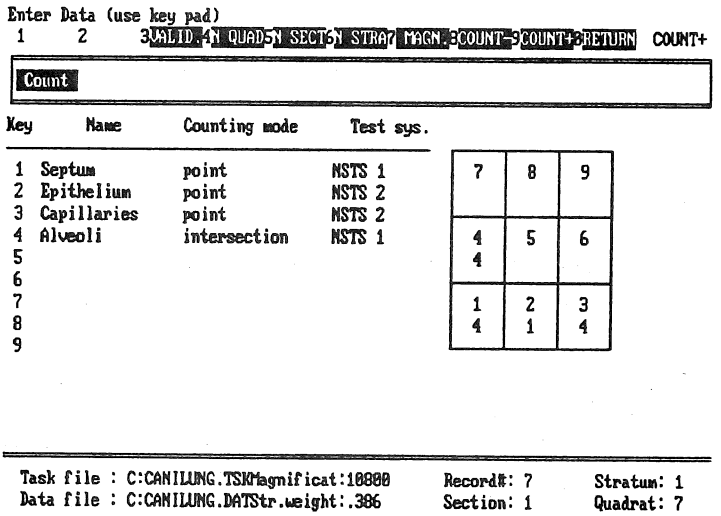

Figure 3. The *counting screen* used to collect raw data for the *DATA* file, see text, §4. The lower figure within each small box represents the current raw count for the corresponding key. Each time a key is pressed, the corresponding count is increased by one unit. An option exists to enter figures in the boxes directly, however. (*NSTS: non-standard test system*).

(ii) *Collecting raw data.* When the *COUNT* mode is chosen from the main menu, the user is prompted to enter the names of the *TASK* and the *DATA* files from the currently available ones. Once both files have been identified, the counting screen appears (Fig. 3).

Before starting to collect raw counts from the first quadrat or micrograph, the user must enter the weight W of the actual stratum where the micrograph comes from. With no stratification, $W = 1$. If there is stratification, however, then

$$W = \frac{Volume\ of\ the\ reference\ space\ in\ the\ stratum}{Volume\ of\ the\ total\ reference\ space}. \quad (1)$$

The definition of the reference space corresponds to that in the denominator of the ratio to be estimated. The corresponding measure does not need to be a 'volume' — it can also be a surface area, for instance. Failure to enter the proper stratum weights will result in biased estimates of the corresponding ratios defined for the whole structure (see §5, equation (12)).

After the stratum weight, the user must enter the final magnification of the first micrograph. The display shows the keyboard allocation; thus, each time a key is pressed, the corresponding count is increased by one. The scores of the counts made for the current micrograph, and the order numbers of the section and of the stratum to which the current micrograph belongs, are also displayed. The function keys of the keyboard (F1–F10) are used for control purposes, such as selecting a new micrograph or a new section, or to go back to the main menu. The main menu has also an option to enter data numerically by hand for any desired compartment.

After a micrograph has been analysed, or when exiting the counting mode, the raw data are saved automatically into the disk.

Modification of DATA- and TASK files

Correction of data in the *DATA* file is possible during counting by either adding or subtracting raw counts in each counter (Fig. 3). If a closed *DATA* file contains incorrect data, this can be mended by means of the modification module (F6) available from the main menu. Likewise, it is possible to modify a *TASK* file, in which case care must be taken not to destroy the logical relationship between the *DATA* and the *TASK* files. Once a *TASK* file has been used to collect raw data, the data acquisition part (e.g. the keypad allocations) must not be changed anymore. However, ratios may be redefined at any time.

Output

Output from *DATA* file can be obtained using the *STATISTICS* module from the main menu. Lists of all *TASK* and *DATA* file names available are first displayed, from which the desired names are chosen. When the results are ready, they can be printed out using appropriate commands, either on the screen or on a matrix printer. The raw data are printed as integer, non-transformed numbers, followed by the ratio estimates and their coefficients of error (see §6).

The *DISPLAY TASK FILE* module of the main menu enables the user to get a hard copy of the *TASK* file for documentation purposes (see §6).

5. COMPUTATION OF THE ESTIMATES

The primary sampling unit of interest (e.g. the lungs of an animal) contains a reference set, denoted *ref*, (e.g. the parenchymal septa of both lungs) which, in turn, contains an object

compartment of interest, denoted by *obj*, (e.g. the capillaries inside the septa). Both *ref* and *obj* are regarded as deterministic bounded sets in $\mathbb{R}^3$, so that the sampling model is design-based. The objective is to estimate $\gamma(obj)$ via the ratio:

$$\mathcal{R} = \frac{\gamma(obj)}{V(ref)}, \tag{2}$$

where γ stands for either volume V, surface area S, curve or tubule length L, integral of mean curvature K or number N. Without loss of generality the denominator of $\mathcal{R}$ is assumed to be a volume.

We consider the general situation in which *ref* is divided into r strata $ref_1, ref_2, ..., ref_r$, (e.g. r lung lobes). Thus, *obj* is also divided into r strata $obj_1, obj_2, ..., obj_r$ and therefore,

$$\begin{aligned}\gamma(obj) &= \gamma(obj_1) + \gamma(obj_2) + ... + \gamma(obj_r) \\ &= V(ref_1)\cdot\mathcal{R}_1 + V(ref_2)\cdot\mathcal{R}_2 + ... + V(ref_r)\cdot\mathcal{R}_r,\end{aligned} \tag{3}$$

where

$$\mathcal{R}_h = \frac{\gamma(obj_h)}{V(ref_h)}, \qquad (h = 1, 2, ..., r) \tag{4}$$

is the ratio defined over the h-th stratum. Dividing both sides of equation (3) by $V(ref)$, we get,

$$\mathcal{R} = W_1\mathcal{R}_1 + W_2\mathcal{R}_2 + ... + W_r\mathcal{R}_r, \tag{5}$$

where

$$W_h = \frac{V(ref_h)}{V(ref)}, \qquad (h = 1, 2, ..., r), \tag{6}$$

is the weight corresponding to the h-th stratum, which must be estimated beforehand. Clearly,

$$W_1 + W_2 + ... + W_r = 1. \tag{7}$$

To estimate $\mathcal{R}_h$, a set of n_h sections are taken at systematic positions from ref_h with either vertical (Baddeley *et al*, 1986) or isotropic orientations (for details see also Michel and Cruz-Orive, 1987, §7). On the *hi*-th section (i.e., the i-th section from the h-th stratum), a set of m_{hi} quadrats are subsampled systematically (see for instance Müller *et al*, 1981, Fig. 3). Thus,

$$\begin{aligned}&\sum_{h=1}^{r} n_h = n && \text{is the total number of sections,} \\ &\sum_{h=1}^{r}\sum_{i=1}^{n_h} m_{hi} = m && \text{is the total number of quadrats.}\end{aligned} \tag{8}$$

The j-th quadrat from the *hi*-th section is analysed at a final linear magnification M_{hij} with a test system of characteristics p, l, a, n, (see §4), and one or more raw counts are made from the set $\left\{P_{hij}, I_{hij}, Q_{hij}, T_{hij}, Q^-_{hij}\right\}$, according to whether γ stands for V, S, L, K, N, respectively. For all $h = 1, 2, ..., r$, $i = 1, 2, ..., n_h$ and $j = 1, 2, ..., m_{hi}$, each raw count is transformed and re-named y_{hij} or x_{hij} according to whether the count is destined to the numerator or the denominator of a ratio, respectively, as follows:

$$\begin{aligned}P_{hij} &\rightarrow \frac{a}{p}\cdot M_{hij}^{-2}\cdot P_{hij} \equiv y_{hij} \text{ or } x_{hij}, \\ I_{hij} &\rightarrow 2\cdot\frac{a}{l}\cdot M_{hij}^{-1}\cdot I_{hij} \equiv y_{hij} \text{ or } x_{hij},\end{aligned} \tag{9}$$

whereas dimensionless feature counts remain unchanged. The following computations are now made. For each section,

$$\begin{aligned} Y_{hi} &= \sum_{j=1}^{m_{hi}} y_{hij} \\ X_{hi} &= \sum_{j=1}^{m_{hi}} x_{hij} \\ R_{hi} &= Y_{hi}/X_{hi}, \quad \text{the estimate of } \mathcal{R}_{hi}. \end{aligned} \tag{10}$$

For each stratum,

$$\begin{aligned} Y_h &= \sum_{i=1}^{n_h} Y_{hi} \\ X_h &= \sum_{i=1}^{n_h} X_{hi} \\ R_h &= Y_h/X_h, \text{ the estimate of } \mathcal{R}_h. \end{aligned} \tag{11}$$

Finally, for the whole object compartment, ,

$$R = W_1 R_1 + W_2 R_2 + \ldots + W_r R_r, \text{ the estimate of } \mathcal{R}. \tag{12}$$

The W's are regarded as known constants; thus, the estimate of the coefficient of error of R, (namely $\mathrm{CE}(R) = \mathrm{SE}(R)/R$) is computed as follows:

$$\mathrm{CE}(R) = R^{-1} \left(\sum_{h=1}^{r} W_h^2 \cdot \mathrm{Var}(R_h) \right)^{1/2} \tag{13}$$

$$\mathrm{Var}(R_h) = R_h^2 \cdot \frac{n_h}{n_h - 1} \cdot \left(\frac{\sum_{i=1}^{n_h} Y_{hi}^2}{Y_h^2} + \frac{\sum_{i=1}^{n_h} X_{hi}^2}{X_h^2} - 2 \cdot \frac{\sum_{i=1}^{n_h} Y_{hi} \cdot X_{hi}}{Y_h \cdot X_h} \right), \qquad (n_h \geq 2). \tag{14}$$

For the ratio estimate within a section,

$$\mathrm{CE}(R_{hi}) = \left\{ \frac{m_{hi}}{m_{hi} - 1} \cdot \left(\frac{\sum_{j=1}^{m_{hi}} y_{hij}^2}{Y_{hi}^2} + \frac{\sum_{j=1}^{m_{hi}} x_{hij}^2}{X_{hi}^2} - 2 \cdot \frac{\sum_{j=1}^{m_{hi}} y_{hij} \cdot x_{hij}}{Y_{hi} \cdot X_{hi}} \right) \right\}^{1/2}, \quad (m_{hi} \geq 2). \tag{15}$$

Remarks

(i) If the number of sections per stratum is less than 2, then *STEPone* does not attempt to compute $\mathrm{Var}(R_h)$ and $\mathrm{CE}(R)$. Similarly, if the number of quadrats per section is less than 2, then the computation of $\mathrm{CE}(R_{hi})$ is not attempted.

(ii) Formulae (14) and (15) are classical error approximations for ratio estimates (Cochran, 1977). As pointed out in §3, they are based on the assumption of independent observations, and therefore they are likely to overestimate error variances whenever sampling is systematic. The corresponding results should therefore be regarded as tentative only.

6. EXAMPLE

The first printout displayed below corresponds to the *TASK* file used to estimate three ratios from the lungs of a canid using electron microscopy. Four compartments of interest were allocated to key numbers 1, 2, 3 and 4, respectively. Two non-standard test systems (*NSTS 1* and *NSTS 2*) were used.

The second printout represents the corresponding *DATA* file. The first two pages contain the raw data, which was structured into $r = 2$ strata with $n_1 = 3$ and $n_2 = 4$ sections, respectively. The numbers of micrographs analysed in each section were $m_{11} = 9$, $m_{12} = 7$, $m_{13} = 8$, $m_{21} = 6$, $m_{22} = 9$, $m_{23} = 8$, and $m_{24} = 10$, namely 57 micrographs in total for the whole animal. The compartment counts (defined in the *TASK* file) are displayed in rows, as well as the section, stratum and animal totals. For instance, the total numbers of test points counted for the whole animal were of 240 in septum, 87 in epithelium and 237 in capillaries, as well as 272 intersections with alveoli. The final magnifications are also displayed: note that they vary from one section to another.

Finally, the results are displayed in the last page of the printout. The notation corresponds fairly closely to that used in §5. Note that the stratum weights are the same for ratios nos. 1 and 3 because their denominators are the same, but this is not the case for ratio no. 2. It is also worth pointing out that the coefficients of error of the final ratio estimates are practically determined by the number of sections used, and to a lesser extent by the number of micrographs. Increasing the number of raw counts on the same set of micrographs by a factor or say 10, or 100, (or, indeed, measuring the relevant compartments in these micrographs exactly with an image analyser), would practically not affect the mentioned coefficients of error.

TASK File :C:CANILUNG.TSK

KEY	NAME	COUNT	CTS
1	Septum	point	NSTS 1
2	Epithelium	point	NSTS 2
3	Capillaries	point	NSTS 2
4	Alveoli	intersection	NSTS 1
5			
6			
7			
8			
9			

ratios : 3

1) V(c)/V(sep)	3/1
2) V(ep)/V(tissue)	2/(1-3)
3) S(alv)/V(sep)	4/1

Test System :
1) NSTS 1: p=1.00 l=50.00[mm] n=25.00 a=2500.00[mm^2]
2) NSTS 2: p=2.00 l=50.00[mm] n=25.00 a=2500.00[mm^2]

Demo:

Sampling: Systematic Quadrats (SQ), EM

DATA File :C:CANILUNG.DAT

-> Record #
-> Stratum (h)
-> Section (i)
-> Quadrat (j)

					1	2	3	4	5	6	7	8	9	Mag.
1	1	1	1	: 6	0	12	3	0	0	0	0	0	1.080E+04	
2	1	1	2	: 5	2	6	5	0	0	0	0	0	1.080E+04	
3	1	1	3	: 3	1	4	3	0	0	0	0	0	1.080E+04	
4	1	1	4	: 8	3	8	2	0	0	0	0	0	1.080E+04	
5	1	1	5	: 1	1	1	0	0	0	0	0	0	1.080E+04	
6	1	1	6	: 4	2	0	5	0	0	0	0	0	1.080E+04	
7	1	1	7	: 4	1	4	4	0	0	0	0	0	1.080E+04	
8	1	1	8	: 2	1	3	3	0	0	0	0	0	1.080E+04	
9	1	1	9	: 1	0	1	2	0	0	0	0	0	1.080E+04	
	1	1	T	:34	11	39	27	0	0	0	0	0		
10	1	2	1	: 5	3	3	8	0	0	0	0	0	1.080E+04	
11	1	2	2	: 4	0	5	4	0	0	0	0	0	1.080E+04	
12	1	2	3	: 4	1	5	3	0	0	0	0	0	1.080E+04	
13	1	2	4	: 4	1	5	6	0	0	0	0	0	1.080E+04	
14	1	2	5	: 8	1	2	1	0	0	0	0	0	1.080E+04	
15	1	2	6	: 3	1	4	3	0	0	0	0	0	1.080E+04	
16	1	2	7	: 0	0	0	1	0	0	0	0	0	1.080E+04	
	1	2	T	:28	7	34	36	0	0	0	0	0		
17	1	3	1	: 5	2	4	5	0	0	0	0	0	1.120E+04	
18	1	3	2	: 1	0	2	6	0	0	0	0	0	1.120E+04	
19	1	3	3	: 2	1	0	1	0	0	0	0	0	1.120E+04	
20	1	3	4	: 2	1	1	4	0	0	0	0	0	1.120E+04	
21	1	3	5	: 3	2	0	1	0	0	0	0	0	1.120E+04	
22	1	3	6	:12	4	2	0	0	0	0	0	0	1.120E+04	
23	1	3	7	: 6	1	8	5	0	0	0	0	0	1.120E+04	
24	1	3	8	: 4	2	4	9	0	0	0	0	0	1.120E+04	
	1	3	T	:35	3	1	1	0	0	0	0	0		
	1	T	T	:97	31	104	114	0	0	0	0	0		

25	2	1	1 : 2	2	0	2	0	0	0	0	0	1.120E+04
26	2	1	2 : 5	3	3	1	0	0	0	0	0	1.120E+04
27	2	1	3 : 0	0	0	0	0	0	0	0	0	1.120E+04
28	2	1	4 : 7	3	4	4	0	0	0	0	0	1.120E+04
29	2	1	5 : 9	1	14	0	0	0	0	0	0	1.120E+04
30	2	1	6 : 8	4	5	7	0	0	0	0	0	1.120E+04
	2	1	T :31	13	26	14	0	0	0	0	0	
31	2	2	1 : 6	3	2	10	0	0	0	0	0	1.120E+04
32	2	2	2 : 9	6	0	8	0	0	0	0	0	1.120E+04
33	2	2	3 : 1	0	2	1	0	0	0	0	0	1.120E+04
34	2	2	4 : 1	0	2	1	0	0	0	0	0	1.120E+04
35	2	2	5 : 1	1	0	3	0	0	0	0	0	1.120E+04
36	2	2	6 : 9	2	12	11	0	0	0	0	0	1.120E+04
37	2	2	7 : 8	3	10	12	0	0	0	0	0	1.120E+04
38	2	2	8 : 3	0	4	7	0	0	0	0	0	1.120E+04
39	2	2	9 : 3	1	5	10	0	0	0	0	0	1.120E+04
	2	2	T :41	16	37	63	0	0	0	0	0	
40	2	3	1 : 7	2	10	4	0	0	0	0	0	1.050E+04
41	2	3	2 : 3	1	3	1	0	0	0	0	0	1.050E+04
42	2	3	3 : 2	1	2	3	0	0	0	0	0	1.050E+04
43	2	3	4 : 3	0	4	1	0	0	0	0	0	1.050E+04
44	2	3	5 : 4	2	2	5	0	0	0	0	0	1.050E+04
45	2	3	6 : 3	3	1	6	0	0	0	0	0	1.050E+04
46	2	3	7 : 5	1	7	6	0	0	0	0	0	1.050E+04
47	2	3	8 : 6	3	5	8	0	0	0	0	0	1.050E+04
	2	3	T :33	13	34	34	0	0	0	0	0	
48	2	4	1 : 1	0	1	0	0	0	0	0	0	1.050E+04
49	2	4	2 : 2	1	1	5	0	0	0	0	0	1.050E+04
50	2	4	3 : 3	1	2	4	0	0	0	0	0	1.050E+04
51	2	4	4 : 6	2	6	6	0	0	0	0	0	1.050E+04
52	2	4	5 : 4	1	5	4	0	0	0	0	0	1.050E+04
53	2	4	6 : 3	1	3	6	0	0	0	0	0	1.050E+04
54	2	4	7 : 3	2	2	4	0	0	0	0	0	1.050E+04
55	2	4	8 :12	4	14	10	0	0	0	0	0	1.050E+04
56	2	4	9 : 0	0	0	0	0	0	0	0	0	1.050E+04
57	2	4	0 : 4	2	2	8	0	0	0	0	0	1.050E+04
	2	4	T :38	14	36	47	0	0	0	0	0	
	2	T	T :143	56	133	158	0	0	0	0	0	
	T	T	T :240	87	237	272	0	0	0	0	0	

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Ratio N° 1. Definition : 3/1

	R(hi)	CE(hi) %	R(h)	CE(h) %	W(h)
1 1 9	0.574	19.21			
1 2 7	0.607	12.24			
1 3 8	0.443	16.00	0.539	9.25	0.386
2 1 6	0.419	30.47			
2 2 9	0.451	30.14			
2 3 8	0.515	14.85			
2 4 10	0.474	10.93	0.467	3.95	0.614

Estimate of V(c)/V(sep) = 0.494 cm^0
Coefficient of error : 4.51 %

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Ratio N° 2. Definition : 2/(1-3)

	R(hi)	CE(hi) %	R(h)	CE(h) %	W(h)
1 1 9	0.379	15.38			
1 2 7	0.318	20.23			
1 3 8	0.333	6.71	0.345	5.01	0.412
2 1 6	0.361	8.85			
2 2 9	0.356	9.93			
2 3 8	0.406	11.88			
2 4 10	0.350	7.22	0.367	3.32	0.588

Estimate of V(ep)/V(tissue) = 0.358 cm^0
Coefficient of error : 2.82 %

-

Ratio N° 3. Definition : 4/1

	R(hi)	CE(hi) %	R(h)	CE(h) %	W(h)
1 1 9	3430.588	22.08			
1 2 7	5554.286	10.11			
1 3 8	6528.000	26.08	5125.742	18.83	0.386
2 1 6	2023.226	43.90			
2 2 9	6883.903	15.07			
2 3 8	4327.273	18.12			
2 4 10	5194.757	15.70	4791.343	19.53	0.614

Estimate of S(alv)/V(sep) = 4920.421 cm^{-1}
Coefficient of error : 13.92 %

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7. SPECIAL FEATURES AND FUTURE DEVELOPMENTS OF *STEPone*

Presently, the stereology program *STEPone* is a functional and tested program. It is generally well behaved and intercepts most user errors without 'crashing'. It runs on IBM PC, PC-AT's or compatible computers. *STEPone* informs the user with on-screen messages about frequent operator errors. An on-line *HELP* module is provided to assist the user. The *COMMUNICATION* module transfers and converts the *DATA* files into ASCII (input/output) or into *SYLK* (output). *STEPone* was conceived as the first member of a family of programs devoted to manual stereology, e.g. to optimize a sampling design using data from several animals or primary sampling units (Gundersen and Østerby, 1981; Cruz-Orive and Weibel, 1981), or to teach how to design a stereological project in an interactive way. Future editions of *STEPone* may incorporate the possibility of accessing raw data not only as keyboard entries, but also as input interactively obtained with other devices.

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