ACTA STEREOL 1982; 187-198 STEREOL 82 SHEFFIELD

### LINE INTERCEPT SAMPLING

Rodney Coleman

Department of Mathématics, Imperial College, London SW7 2BZ, U.K.

### ABSTRACT

A class of estimators for the internal structure of a specimen is introduced. It uses measurements on those features hit by a random line section. The estimators are unbiased and are compared with the usual stereological estimators. Methods of reducing their standard errors through the use of alternative methods of selecting the random line section, through independent repetitions, through serial sectioning and lattice test systems are considered.

The theory applies also to the line intercept sampling of features in a 2-d specimen and to the plane section sampling of features of a 3-d specimen.

### INTRODUCTION

Line intercept sampling is the method of selecting for measurement those features in a photograph or micrograph that are hit by a straight test line. It is a simple extension to include the sampling of the internal features of a 3-d specimen hit by line or plane sections.

Line intercept sampling has been used mainly for ecological investigation (Cormack et al, 1979), for example, to study plant varieties during a country walk. It has also been used to estimate the amount of timber left behind after logging, and to estimate the porosity of rocks by sampling

the lines in sections through the rock caused by fissures.

I seek to adopt the style used by *Miles & Davy* (1977) and *Miles* (1978) in an analysis of quadrat sampling. In any overlap with the content of those papers, this paper will serve as an introduction.

The mathematics used to prove the results of this paper can be found in the booklet (*Coleman*, 1979), though the results given here are new. A full account will eventually be included in a book being written as an expanded version of the booklet.

### UNBIASED ESTIMATION

We shall consider only the situation of trying to determine the internal structure of a specimen.

When the features of the internal structure are small relative to the size of the specimen, and they are evenly distributed throughout its interior, sections of any sort can hardly fail to select a representative sample for measurement. Conversely, if they are not dispersed evenly or if they are large relative to the specimen, sections can look very different one from another, and any particular section may seriously mislead regarding the internal structure. It may entirely miss the features of interest. This is illustrated in Figure 1.



# Figure 1

Specimens showing (a) informative line intercepts, and (b) and (c) uninformative line intercepts.

Nevertheless, by random sectioning we can obtain unbiased estimators of the features of interest regardless of where they are within the specimen. This is when we choose the section uniformly at random from a beam which hits the specimen in a uniformly random direction. This is known as *isotropic uniform randomness*, abbreviated to *IUR*.

As a particular example, let us suppose that an *IUR* line section Q through a specimen hits particles that we label  $Y_1, Y_2, \ldots, Y_n$ , and let those that are missed by Q be labelled  $Y_{n+1}, Y_{n+2}, \ldots, Y_N$ . Those hit by Q are not typical because the larger ones are more likely to be hit. On each particle,  $Y_k$ , hit by Q we take the following measurements:

 $H(Y_k)$  the mean area of projection of  $Y_k$  onto a plane having a uniformly random orientation,

- $V(Y_k)$  the volume of  $Y_k$ ,
- $S(Y_k)$  the surface area of  $Y_k$  .

Then the statistic

 $T = H(Z) \sum_{k=1}^{n} \frac{W(Y_k)}{H(Y_k)}$ 

is an unbiased estimator of

$$ET = \sum_{k=1}^{N} W(Y_k)$$
(2)

where  $W(Y_k)$  is given in Table 1.

The corresponding results for planar specimens sampled by an *IUR* line section are given in Table 2.

For curves  $Y = \{Y_1, \ldots, Y_N\}$  lying in the planar specimen Z, if we interpret L as curve length and C as total curvature, we can take W = 1, L, C in (1) to obtain the estimators of N, L(Y) and C(Y).

(1)

We can similarly obtain unbiased estimators for the internal features of a 3-d specimen hit by a plane section.

Table 1. The line intercept sample estimators T and T/V(Z) given by (1) are unbiased estimators of ET and (ET)/V(Z), when

the particular  $W(Y_{\mathcal{V}})$ s of this table are used.

W(Y <sub>k</sub> )	ET	(ET) / V (Z)
$V(Y_k)$	V(Y)	V <sub>V</sub>
1	N	N <sub>V</sub>
S(Y <sub>k</sub> )	S(Y)	$S_V$

Table 2. The line intercept sample estimators T and T/A(Z) are given by (1), with H = the mean length of projection onto a line having a uniformly random orientation = mean caliper diameter. These are unbiased estimators of ET and (ET)/A(Z) for planar domains  $Y = \{Y_1, \dots, Y_N\}$  when the particular  $W(Y_k)s^T$  of this table are used.

W(Y <sub>k</sub> )	ET	(ET) /A (Z)
А (Y <sub>k</sub> )	A (Y)	$A_A$
1	N	N <sub>A</sub>
$L(Y_{k})$	L (Y)	$L_A$
$C(Y_k)$	C(Y)	$C_A$
 	area	

L boundary length ÷

С total curvature of the boundary ==

### UNBIASED STEREOLOGICAL ESTIMATORS

In stereology our information is generally limited to that in the plane containing the line section or to that in the line section itself. If we consider this latter case, with planar particles embedded in a planar domain, corresponding to the results of Table 2 we have the estimators

 $T' = H(Z) \sum_{k=1}^{n} W_{k}^{\star},$ 

which is unbiased for ET, where  $W_k^*$  and  $ET^*$  are given in Table 3.

### STANDARD ERRORS

In general, none of these estimators will be good, despite their being unbiased. We would expect them all to have large standard errors, which depend also on the unobserved particles, since an *IUR* section is indiscriminately large or small. Small sections will hit so little as to give almost no information about the internal structure. The standard error of an estimate measures its precision and gives due weight to the possibility of small sections.

Sometimes unbiased estimators can be silly as, for example, when the value of the estimator  $T' = H(Z)\Sigma L_{L}$  of

V(Y) is larger than V(Z) itself. It may in fact be better to tolerate a little bias if in doing so we reduce the standard error substantially. The criterion that we generally use is that of minimizing the *mean square error*, where

mean square error =  $(bias)^2$  +  $(standard error)^2$ . (4)

There are various techniques for reducing the mean square error that should be considered.

The first is that of using larger intercepts which would in general hit more of the internal features and so provide more information. One way of obtaining random sections through a specimen that favours those which have

(3)

Table 3. The stereological line intercept estimators T' given by (3) are unbiased when the particular  $W_k^*$ s of this table are used. Some of the measurements are illustrated in Figure 2.

W <sub>k</sub> *	ET
$L_{\mathcal{k}}$	A (Y)
$U_k^{-1}$	Ν
$\frac{\pi}{2} N_k$	L(Y)
$\pi C_k$	C(Y)

 $L_k$  = total intercept length in  $Y_{\mathcal{V}}$ 

- $U_k$  = length of projection of  $Y_k$  onto the perpendicular to the line section Q
- $N_k$  = number of intersections of  $Y_k$ by Q (=6 in Figure 2)
- $C_{k}$  = net tangent count which may be made either parallel or perpendicular to  $Q(C_{k} = 3-5 = -2 \text{ in} Figure 2)$

intercepts larger than the *IUR* ones weights the *IUR* intercept size distribution proportionally to the intercept sizes, with length-weighting for line sections, areaweighting for plane sections. A mechanism for achieving these weighted *IUR* sections is to select a point uniformly at random in the interior of the specimen and then choose the section to pass through that point in a uniformly random direction. The line intercept sample estimators and the stereological estimators will now all be biased. A mechanism which favours line intercepts even longer than the *weighted IUR* ones is that in which the line section

passes through *two points* taken independently and uniformly at random in the interior of the specimen. This also will be considered in the example.



Figure 2. The measurements  $L_k$ ,  $U_k$ ,  $N_k$  and  $C_k$  on a planar particle  $Y_k$  for use with Table 2 in calculating the estimators T' of (3).  $C_k = 3$  (plus tangents) - 5 (minus tangents) = -2.

### RATIO ESTIMATORS

A second method of mean square error reduction is through the use of *ratio estimators*. The ratio of two highly correlated variables will generally vary much less than the variables separately.

If we substitute into (3) the "estimator"  $\widehat{H}$  of H(Z):

$$\hat{H} = \begin{cases} A(Z)/L^{*} & (2-d \text{ specimen, line section}) \\ V(Z)/L^{*} & (3-d \text{ specimen, line section}) \\ V(Z)/A^{*} & (3-d \text{ specimen, plane section}), \end{cases}$$

where  $L^*$  and  $A^*$  are the length and area of intercept made by the section with the specimen Z, then we obtain the usual ratio estimators of stereology

$$T'' = \iint_{k=1}^{n} \bigcup_{k=1}^{n} W_{k}^{*}$$
(5)

As an example, for a 3-d specimen hit by a line section we would estimate V(Y) by

$$T'' = \overset{\wedge}{H} \overset{n}{\sum} L_{k} = V(Z) \frac{\Sigma L_{k}}{L^{\star}} = V(Z) L_{L}$$
(6)

This incidentally cannot take a value in excess of V(Z). If the line section is IUR it will in general be biased, but it will be unbiased if the section is weighted IUR.

### EXAMPLE

We consider a single spherical particle Y at the centre of a spherical specimen Z. We estimate V(Y) from a single line section. The line section is constructed by each of the three randomness mechanisms. Table 4 gives theoretical Values of the relative root mean square error and relative bias (if any):

relative root mean square error

= (mean square error) $\frac{1}{2}/V(Y)$ ,

relative bias = (bias)/V(Y).

This example must however be treated with caution. If we know something about the structure of Y prior to the sampling, we could possibly do better by using an estimator that takes advantage of that knowledge. For example, if we had known that Y is a single particle, we would probably only try to estimate V(Y) if Y were hit, in which case with *line intercept sampling* we can measure it exactly. These techniques are intended for use on irregular structures; there are better ways of sampling regular structures.

We see that none of these estimators is good, that there is an increase in mean square error in using the

194

stereological estimator T' instead of the line intercept estimator T, but not much, and this is more than compensated by using the ratio estimator T''.

Which type of random section to use depends on the size (and in general the structure) of the inclusions. In the example IUR is best when  $V_{V}$  = 0.125, two-point sectioning is

Table 4. The relative root mean square errors in estimating the volume of a sphere at the centre of a spherical specimen when  $V_V = 0.125$  and  $V_V = 0.5$ . A single line section is used. It is *IUR*, lengthweighted *IUR* and taken through two points independently uniform in the specimen. The line intercept estimator *T* of (1) is compared with the stereological estimator *T*' of (3), which uses measurement of the intercept length only, and with the ratio estimator *T*'' of (6). The relative biases, if any, of these estimators are given in brackets.

	$V_{V} = \frac{1}{8}$			$V_{V} = \frac{1}{2}$		
	IUR	WIUR	2 Point	IUR	WIUR	2 Point
Probability that the line section hits Y	0.25	0.35	0.58	0.63	0.78	0.95
Line intercept estimator $T$	1.73	1.95 (0.40)	2.37 (1.31)	0.77	0.70 (0.23)	0.61 (0.51)
Stereological estimator $T$ '	1.87	<b>2.15</b> (0.42)	2.74 (1.44)	0.89	0.89 (0.29)	
Ratio estimator $T''$					0.60	

best when  $V_V = 0.5$ . As a compromise we might choose the weighted IUR section, which is unbiased for the ratio estimator.

195

### INDEPENDENT REPETITIONS

Another technique of mean square error reduction is through repeated sampling. A simple way is to make independent random sections through the specimen. This is perhaps not so easy for particles in a 3-d specimen if those hit are to be extracted for measurement. A decision then has to be made about whether to replace them before taking the next section. Similar problems arise with plane sections, with the specimen having to be restored before each repetition.

After obtaining an estimate based on independent repetitions, we can apply a bias-reducing procedure, for example the so-called *jack-knife(Miller*, 1974). Bias is therefore no longer a serious concern.

With ratio estimators it might be better to take the ratio of the averages rather than the average of the ratios. This is also discussed in *Miller* (1974).

### SERIAL SECTIONS & LATTICE TEST SYSTEMS

A more representative set of features will be sampled if a systematic sampling procedure is used rather than independent repetitions. Each line or plane section parallel to an IUR one (if chosen independently of the internal structure Y) is itself IUR, so, having first made an IUR section, we can continue with serial sections.

However if Y consists of particles or curves which have a particular preferred orientation then even a parallel array of sections can fail to give a representative sample of particles or intercepts.

If we were to choose a section uniformly from a beam which was perpendicular to the *IUR* sections, it too would be *IUR*, and so would any section parallel to it. With the perpendicular test system, if the internal features did have a preferred orientation, an uninformative or misleading parallel system will be compensated for by the perpendicular parallel system.

Similarly we can construct a length-weighted *IUR* lattice system based on a single length-weighted *IUR* section.



Figure 3. With anisotropy a line section may intersect very little and lead to an uninformative sample of features for measurement. In this case even serial sectioning can be uninformative.



Figure 4. (a) An uninformative IUR line intercept compensated by an informative perpendicular IUR line, and (b) an IUR lattice test system.

More complicated lattice systems based for example on 60° angles, can also be made IUR or length-weighted IUR.

There are obvious technical problems in applying 3-d test systems. In practice these would best be avoided by using 2-d test systems on plane sections taken serially through the specimen.

### REFERENCES

Coleman R. An introduction to mathematical stereology. Aarhus, Denmark: Dept of Theoretical Statistics, Inst of Mathematics, 1979: Memoirs No.3: 97 pages.

Cormack R.M., Patil G.P., Robson D.S. (Editors). Sampling biological populations (Statistical Ecology 5). Fairland, Maryland: International Cooperative Publishing House, 1979: 1-192.

Miles R.E. The sampling, by quadrats, of plane aggregates. J Microsc 1978; 113: 257-267.

Miles R.E., Davy P. On the choice of quadrats in stereology. J Microsc 1977; 110: 27-44.

Miller R.G. The jackknife-a review. Biometrika 1974; 61: 1-15.