Standard Test Method for Stereological Evaluation of Porous Coatings on Medical Implants[1]

This standard is issued under the fixed designation F1854; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers stereological test methods for characterizing the coating thickness, void content, and mean intercept length of various porous coatings adhering to nonporous substrates.

1.2 A method to measure void content and intercept length at distinct levels ("Tissue Interface Gradients") through the porous coating thickness is outlined in 9.4.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]
E3 Guide for Preparation of Metallographic Specimens
E883 Guide for Reflected–Light Photomicrography

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *field*—the image of a portion of the working surface upon which measurements are performed.

3.1.2 *intercept*—the point on a measurement grid line projected on a field where the line crosses from solid to void or vice versa.

3.1.3 *measurement grid lines*—an evenly spaced grid of parallel lines all of the same length.

3.1.4 *porous coating*—coating on an implant deliberately applied to contain void regions with the intent of enhancing the fixation of the implant.

3.1.5 *substrate*—the solid material to which the porous coating is attached.

3.1.6 *substrate interface*—the region where the porous coating is attached to the substrate.

3.1.7 *tissue interface*—the surface of the coating that shall have first contact with biological tissue (i.e., the top of the coating).

3.1.8 *working surface*—the ground and polished face of the metallographic mount where the images of the fields are captured.

4. Summary of Test Method

4.1 *Mean Coating Thickness*—Evenly spaced parallel grid lines are oriented perpendicular to the coating-substrate interface on a field. For each gridline, the distance from the coating-substrate interface to the last contact with the porous coating material is measured as the coating thickness. The average of all of the coating thickness measurements obtained from all the measured fields is the mean coating thickness for that coating.

4.2 *Volume Percent Void*—A regular grid of points is superimposed on a field from the working surface. The percentage of points that are in contact with void areas in the coating is the volume percent of void present in that field.

4.3 *Mean Void Intercept Length*—Measurement grid lines are oriented parallel to the substrate interface in a field. The average length of the line segments overlaying the void space is the mean void intercept length for that field. This is a representative measure of the scale, or size, of the pores in a porous structure.

4.4 *Tissue Interface Gradients*—The Volume Percent Void and the Mean Void Intercept Length are characterized in three 200-μm-thick zones below the Tissue Interface in each field.

5. Significance and Use

5.1 All these test methods are recommended for elementary quantification of the morphological properties of porous coatings bonded to solid substrates.

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[1] This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.15 on Material Test Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

5.2 These test methods may be useful for comparative evaluations of different coatings or different lots of the same coating.

5.3 All the methods should be performed on the same set of images of fields.

5.4 A statistical estimate can be made of the distributions of the mean coating thickness and the volume percent void. No estimate can be made of the distribution of intercept lengths.

5.5 There are limits to the accurate characterization of porosity, depending on spacing between the lines in the line grid (or points in the point grid) and the individual and cumulative fields used for the measurements. Increasing the size of the fields, increasing the number of fields, or decreasing the grid spacing will increase the accuracy of the measurements obtained.

5.6 This method may be suitable for ceramic coatings if an accurate coating cross section can be produced. Producing an accurate ceramic coating cross section may require other techniques than standard metallographic techniques.

5.7 For coatings having a mean thickness less than 300 microns, it is not recommended to attempt to determine the volume percent void or the mean intercept length.

6. Apparatus

6.1 The procedures outlined in this test method can be performed manually or using digital image analysis techniques.

6.2 *Microscope,* or other suitable device with a viewing screen, photomicrographic capability, or digital image capture capability should be used to image the sample fields of interest for these test methods.

6.3 For manual measurement, a transparent sheet, with measurement grid lines or points is superimposed on the viewing screen or photomicrograph for the measurements. The line grid (or point grid) should consist of at least five uniformly spaced, parallel lines (or rows).

7. Metallography

7.1 The procedures outlined in this test method for characterizing porous coatings require the preparation of metallographic sections. Good metallographic preparation techniques, in accordance with Guides E3 and E883 shall be used to prevent deformation of the surface of the section or creation of any other artifacts that will alter the morphology of the metallographic section. An example of an unacceptable artifact would be the absence of a portion of the porous coating, caused by its removal, thereby creating an artificial void area.

7.2 Care shall be taken to ensure that the working surface is perpendicular to the substrate interface.

8. Sample Working Surfaces and Fields

8.1 *Sample Orientation:*

8.1.1 *Normal Section Orientation:*

8.1.1.1 For accurate coating thickness measurements, the orientation of sample working surfaces should be approximately perpendicular to the plane of the substrate.

8.1.1.2 If the angle between the tangent to the coating-substrate interface at one edge of a field and the tangent to the substrate interface at the opposite edge of the field is greater than 2º, the substrate curvature is too large.

8.1.1.3 There is a practical limit to the magnification that can be used for measurement of the void content and mean intercept length. As magnification is increased, the number of fields should be increased to obtain a representative sample. If there are too few intercepts in the individual fields, the accuracy of the measurement could decrease.

8.2 *Field Parameters:*

8.2.1 *Resolution:*

8.2.1.1 The magnification used for the field should be high enough to resolve all the features that need to be measured.

8.2.1.2 For most porous coatings, the magnification should be high enough that features as small as 5 µm can be easily distinguished. If digital imaging is used, the pixel size should be less than or equal to 5 µm.

8.2.1.3 For digital images the pixel size in µm and the image field size in pixels shall be included with the images.

8.2.2 *Field Dimensions:*

8.2.2.1 The field height of the images shall be large enough to include all the features of any portion of the porous coating for Mean Coating Thickness determination (section 9.1).

8.2.2.2 A good rule of thumb for an accurate measurement of Mean Void Intercept Length is that the minimum field width should be greater than or equal to 5 times the resulting Mean Void Intercept Length. For example, a Mean Void Intercept Length value of 200 µm should have a measurement field width of at least 1000 µm.

8.2.2.3 It is possible to measure the Mean Void Intercept Length in a field using a series of shorter non-overlapping grid lines. This does not change the requirement for the coating field length required for the calculation. Care should be exercised using multiple short lines in a single field, because it is possible to make the grid lines so short that the accuracy of the result is affected.

8.2.2.4 If the magnification used produces an image with a height or width smaller than that which is required, multiple images may be carefully stitched together to produce a field of sufficient height and width.

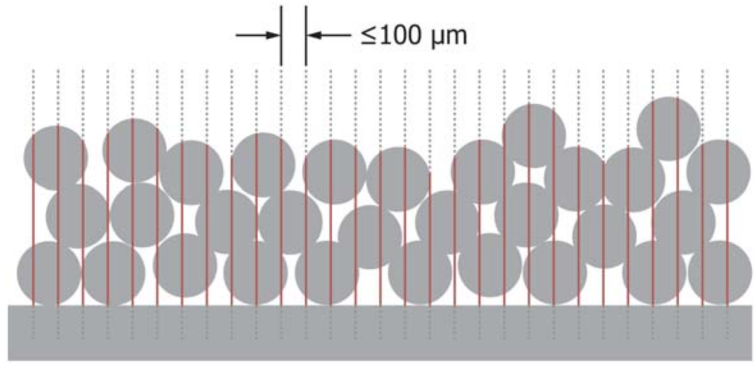
8.2.2.5 All four types of measurements shall be performed over a minimum coating area of 15 mm^2 with no area being measured more than once. For thinner coatings that require higher magnifications to allow reasonable measurements the minimum total measured field length shall be 20 mm with no part of that length being measured more than once.

9. Procedure

9.1 *Mean Coating Thickness:*

9.1.1 An array of equally spaced parallel gridlines should be superimposed on the field perpendicular to the substrate interface, as shown in Fig. 1. The gridlines should be spaced no more than 100 µm apart. Appendix X2 includes two typical sets of gridlines each with ten equally spaced parallel lines.

9.1.2 At each gridline, the distance from the substrate interface to the last contact with a solid coating feature is



NOTE 1—The solid line is the measured distance.

FIG. 1 Illustration of Coating Thickness Measurement

measured. A measurement is only valid if the gridline is oriented 90° ± 2° to the substrate interface.

9.1.2.1 The surface of the substrate is usually rough due to the processing involved in applying the coating. If the surface is too rough, a line indicating a subjective average substrate interface shall be made on the each field. Thickness measurements shall then be made from this line.

9.1.2.2 If a subjective average interface line is used, the same line should be used for the positioning of the other measurements in this standard.

9.1.3 Coating thickness measurements should be obtained over a linear distance of at least 20 mm of porous surface with no overlap between measurement sites.

9.1.4 The average of all the individual coating thickness measurements from all the fields that were measured is the mean coating thickness. The standard deviation estimator and the 95 % confidence interval should be calculated for all the fields. The equations for calculating these values are as follows:

$$\bar{T} = \frac{1}{M \times n} \sum_{i=1}^{n} t_i \quad (1)$$

where:

t_i = the individual magnified thickness line length,
n = the number of thickness measurements,
M = the magnification, and
$\bar{T}$ = the mean coating thickness.

$$\hat{S} = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} \left[\frac{t_i}{M} - \bar{T} \right]^2} \quad (2)$$

where:

$\hat{S}$ = the standard deviation estimator, and
CI = the confidence interval.

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \quad (3)$$

9.1.5 Define the Tissue Interface of the porous coating.

9.1.5.1 The first method to define the location of the Tissue Interface is a physical one. Securely attach a flat metallic surface on the porous interface of the metallographic sample prior to embedding the sample. The attached flat metal surface must show that it has not moved away from the Tissue Interface during the metallographic mounting process and shall have an angle away from the substrate of less than 1º.

NOTE 1—Spring-loaded binder clips have been used to secure the attached flat metal plate to the tissues interface.

9.1.5.2 The second method is to use the average of the longest 5% of the thickness measurements from the Mean Coating Thickness procedure in section 9.1. Use that average to draw a line, in any field examined, that distance from the substrate, parallel within 1º to the substrate, to define the Tissue Interface.

9.1.6 For rough surface coatings, such as plasma spray coatings intended to create a roughened surface if the spacing of the thickness lines is too large it can be affect the repeatability of the thickness measurement. If the mean of the thickness plus the standard deviation of the thickness is more than one half of the standard deviation of the thickness below the value of the tissue interface, then the line spacing should be decreased and the thickness re-measured. For such coatings, it may be better to start with the distance between the thickness measurement lines at 33 µm or less.

9.2 *Volume Percent Void:*

9.2.1 For this measurement, the field should be entirely contained between the Tissue Interface (section 9.4.1) and the substrate interface.

9.2.2 An array containing at least 100 regularly spaced points should be superimposed on the field, as shown in Fig. 2. The points should be spaced no more than 50 µm apart. If the void areas form a regular or periodic pattern, the use of a grid having a similar pattern should be avoided. The height of the array should include the coating from 10% to 90% of the tissue interface value. The same grid positioned at 10% of the tissue interface away from the substrate should be used for all fields. Appendix X2 includes two typical arrays each with at least 100 regularly spaced points.

9.2.3 The number of points overlying void areas (P_α) on the fields shall be counted and recorded. When using the manual method, any points falling on a boundary between a void area and solid features should be counted as one half. Any questionable points should be counted as one half.

9.2.4 The number of contact points in void areas (P_α), divided by the total number of points on the grid (P_T) times 100

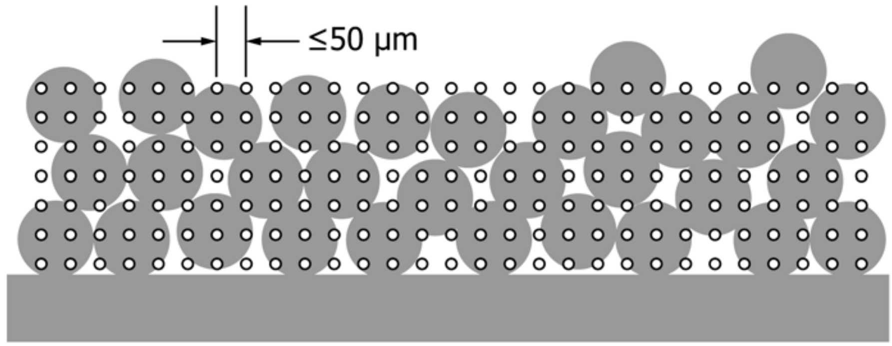


FIG. 2 Illustration of Volume Percent Void Measurement

gives the percentage of grid points on the void for that field. This should be calculated for each grid application.

$$P_v = \frac{P_\alpha}{P_T} \times 100 \tag{4}$$

where:

P_α = the total number of counted points,
P_T = the total number of grid points, and
P_v = the volume percent void.

9.2.5 Fields to be analyzed should include as much of the coating thickness as possible.

9.2.6 These measurements may also be made with an appropriate digital image analysis system. This can be done by considering each pixel as a regularly spaced point in the array. The volume percent void for each field should be the ratio of the number of pixels representing void space to the total number of pixels in the image of the field.

9.2.7 The average percentage of the grid points on the voids from each field provides an unbiased statistical estimator for the void volume percentage in the three-dimensional structure of the coating. The mean void percentage ($\bar{P}_v$), the standard deviation estimator ($\hat{S}$), and the 95 % confidence interval (CI) should be calculated for the volume percent void data from the fields. The equations for calculating these values are as follows:

$$\bar{P}_v = \frac{1}{n}\sum_{i=1}^{n} P_{v_i} \tag{5}$$

$$\hat{S} = \sqrt{\frac{1}{n-1} \times \sum_{i=1}^{n} \left[P_{v_i} - \bar{P}_v\right]^2} \tag{6}$$

$$CI = 2 \times \frac{\hat{S}}{\sqrt{n}} \tag{7}$$

9.2.8 The volume percent void estimate is given by the following relationship:

$$V_v = P_v \tag{8}$$

9.3 *Mean Void Intercept Length:*

9.3.1 For this measurement, the field should be entirely contained between the Tissue Interface (section 9.4.1) and the substrate interface.

9.3.2 An array of equally spaced parallel gridlines should be superimposed on the field parallel to the substrate interface, as shown in Fig. 3. The height of the array should be at least half the distance from the Tissue Interface to the substrate interface, thereby producing a value representative of an average for the entire coating thickness. The gridlines should be spaced no more than 100 µm apart. Appendix X2 includes two typical sets of gridlines, each with ten equally spaced parallel lines.

9.3.3 The number of times that a void region is intercepted by the test lines (N_v) is counted and recorded. There are two methods that can be used for counting.

9.3.3.1 The first method counts the number of intersections along the grid lines. Each time the grid line goes from either solid to void or void to solid is counted as one intersection. The number of intersections (n_i) is twice the number of intercepts (N_v).

$$N_v = \frac{n_1}{2} \tag{9}$$

9.3.3.2 In the second method the crossing direction of any intersection on any line determines if it is counted as an intercept. If the beginning of the line starts on a void, count the transitions from void to solid. If that same line ends on a void, count that as a one more intercept. If the beginning of a line starts on a solid, count the transitions from solid to void. If the

FIG. 3 Illustration of Mean Intercept Length Measurement Field

same line ends on a solid, there is no additional count. In this case the number of counts is the number of intercepts (N_v).

9.3.4 Mean Void Intercept Length should be determined over a cumulative area of at least 15 mm^2 on each working surface for thick coatings and 20 mm of length for thinner coatings.

9.3.5 An estimate of the mean intercept length (L_v) can be calculated from the total length of lines (L_T), the number of intercepts (N_v), the magnification M, and the previously calculated volume percent void (V_v). Since V_v was multiplied by 100 to make it a percentage, it must be divided by 100 in this equation. In addition, in automated systems where the measurements are already calibrated to the magnification, dividing by M is not required. The calculation is as follows:

$$L_v = \frac{\frac{V_v}{100} \times \frac{L_T}{M}}{N_v} \quad (10)$$

9.3.6 For digital measurement using an image analysis system, an alternate measurement method may be used to calculate the Mean Void Intercept Length. The distance from one solid feature to another is measured everywhere a gridline crosses void space. If the region of a grid line crossing a void does not start and end at a solid coating feature (e.g., it starts or ends at the edge of the field or in the middle of a void space), it shall not be included. The average of all the measurements in a field is the mean void intercept length for that field (l_i). For this technique, the field cannot be broken into a set of smaller grids.

$$\bar{L}_v = \frac{1}{n}\sum_{i=1}^{n} l_i \quad (11)$$

9.4 *Tissue Interface Gradient Method:*

9.4.1 Using the Tissue Interface value, divide the region of the porous coating immediately below the Tissue Interface into three zones as shown in Fig. 4. The first zone should be from 0 to 200 µm below the Tissue Interface second zone should be from 200 to 400 µm below the Tissue Interface, and the third zone should be from 400 to 600 µm below the Tissue Interface. If the distance from the Tissue Interface to the substrate is less than 600 µm but more than 500 µm, the third zone should be 400 µm from the Tissue Interface to the substrate. These zone lengths shall be at the same distance from the substrate on every field.

9.4.2 In each of the three zones repeat the measurements in sections 9.2 (Volume Percent Void) and 9.3 (Mean Void Intercept Length) using the zone height as the field height. Appropriate adjustment of the point and line arrays may be necessary to accommodate the decreased field height.

10. Report

10.1 A description of the object from which the sample metallographic sections were obtained and the location of those metallographic sections within that object shall be reported.

10.2 Report the chemical composition of the substrate and the coating materials.

10.3 The original morphological form of the coating material (that is, powder, wire, and so forth) shall be reported.

10.4 The number of fields of view for each measurement, the magnifications used, and the grids and lines used shall be reported.

10.5 The mean coating thickness, its standard deviation, and its confidence interval, the Tissue Interface, the volume percent void, its standard deviation, and its confidence interval, and the mean void intercept length shall be reported.

10.6 If the substrate interface is curved in the plane of the working surface, an estimate of the local radius of curvature shall be reported.

10.7 If the coating Tissue Interface Gradient Method is used, the volume percent void and the mean linear intercept length, together with the distance from the substrate for each gradient zone shall be reported.

10.8 For digital images, the minimum measurement value, (that is, the dimension of a single pixel), at all magnifications used in the measurements and the pixel dimensions of the images shall be reported.

11. Precision and Bias

11.1 The following factors could significantly affect the results obtained using this method:

11.1.1 The presence of structural gradients or inhomogeneities in the section can influence the precision and accuracy of the measurements. If the amount of void in the porous coating changes with thickness, the Tissue Interface Gradient Method may be used to characterize the gradient.

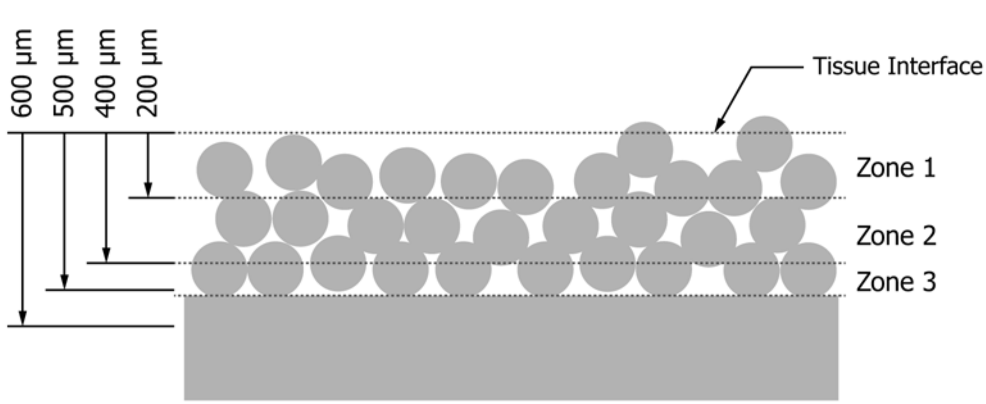


FIG. 4 Illustration of the Gradient Zones

11.1.2 The quality of sample preparation can influence precision and bias.

11.1.3 If the manual method is used to measure volume percent void or the estimation method is used to measure mean void intercept length, the counting of grid points and line ends at void boundaries presents an opportunity for bias in the measure ments. If these techniques are used, the methods outlined in 9.2.6 and 9.3.4, respectively, shall be followed.

11.1.4 The number of fields measured, the method of field selection, and their spacing can influence the precision and accuracy. Random selection of fields and, except where noted, random orientation of grids within the field can help eliminate bias.

11.2 The results of an interlaboratory comparison, although not comprehensive enough to support statistical statements about the precision of this method, are presented in Appendix X4 for information only.

12. Keywords

12.1 coating gradiants; metallography; porosity; porous coatings; stereology; thickness

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 Porous coatings are applied to the surface of medical implants. Standardized techniques to characterize the structure of the porous coatings should be available. These techniques can be used to estimate the uniformity and repeatability of these porous structures. In their book *Practical Stereology*, Russ and Dehoff describe the term mean lineal intercept measurement as a "representative measure of the scale (i.e., size) of the features in the feature set". "Mean void intercept length" is not a specific physical feature such as "pore diameter", but it is a mathematically valid measure of porosity. It is affected by the size of the porosity and its geometry. To a lesser extent it is also affected by pore interconnectivity and surface roughness. There is not another mathematically valid measure that can be made to characterize three-dimensional porosity of a shape that cannot be mathematically defined.

X1.2 Porous coatings with significant gradients in the porosity from the substrate to the surface may not be accurately measurable using the test methods in 9.2 and 9.3.

X1.3 Specimen alignment in the metallographic mount can affect measurement results. Fortunately, for most of the cross section mounts, the error that occurs from not having a perfect perpendicular cross section is minimal. If the sample mounting is not a perpendicular cross section, the coating thickness will tend to increase. However the amount of increase varies with the cosine of the angle off of perpendicular. Even if the sample is 10° off perpendicular the increase of the coating thickness is only about 1.5%. However, the angular tolerance of the substrate is much more stringent in the Tissue Interface Gradient Method. Because of the narrow 200 μ height of the measurement zone, it would be much easier for a measurement to exceed the boundaries of the measurement zone if the Tissue Interface reference plane isn't established with required precision.

X1.4 With the advent of automated image analysis systems, these types of measurements can be made with greater ease. Automated image analysis can be used to make these measurements, but care should be exercised to be sure that the automated techniques are comparable to the hand techniques.

X2. SAMPLE ARRAYS OF LINES FOR LINE INTERCEPT WORK

X2.1 See Fig. X2.1.

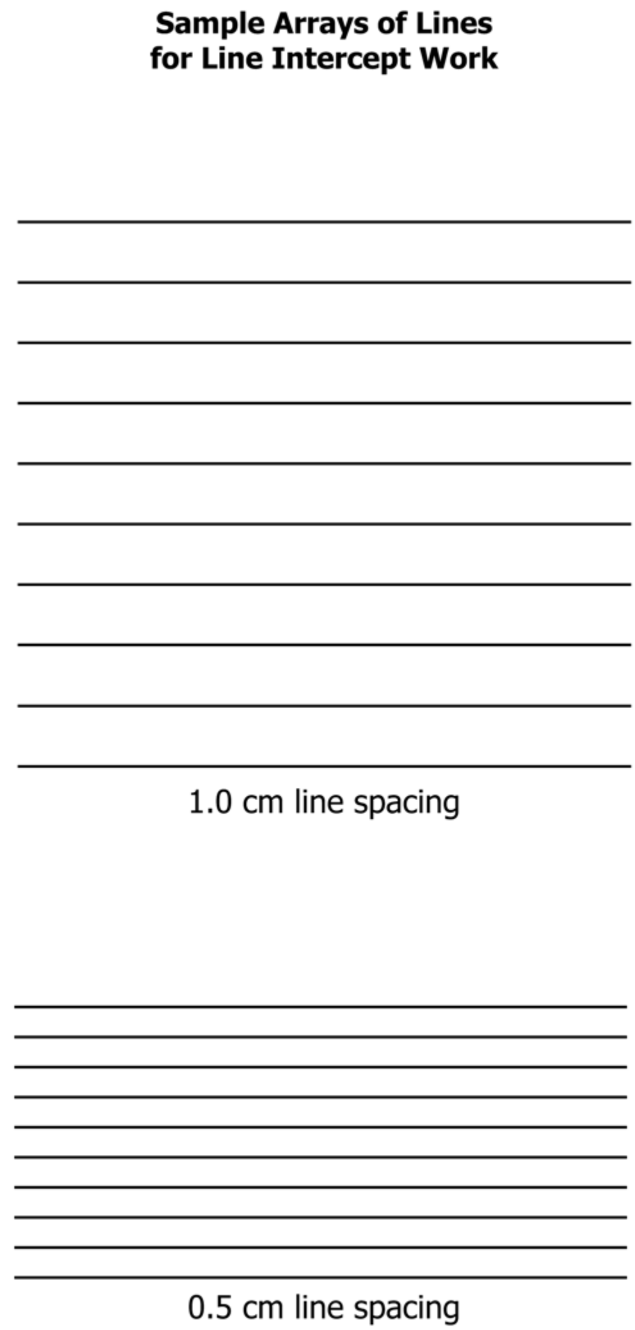


FIG. X2.1 Sample Arrays of Lines for Line Intercept Work

X3. ARRAYS OF POINTS FOR POINT COUNTING METHODS

X3.1 See Fig. X3.1.

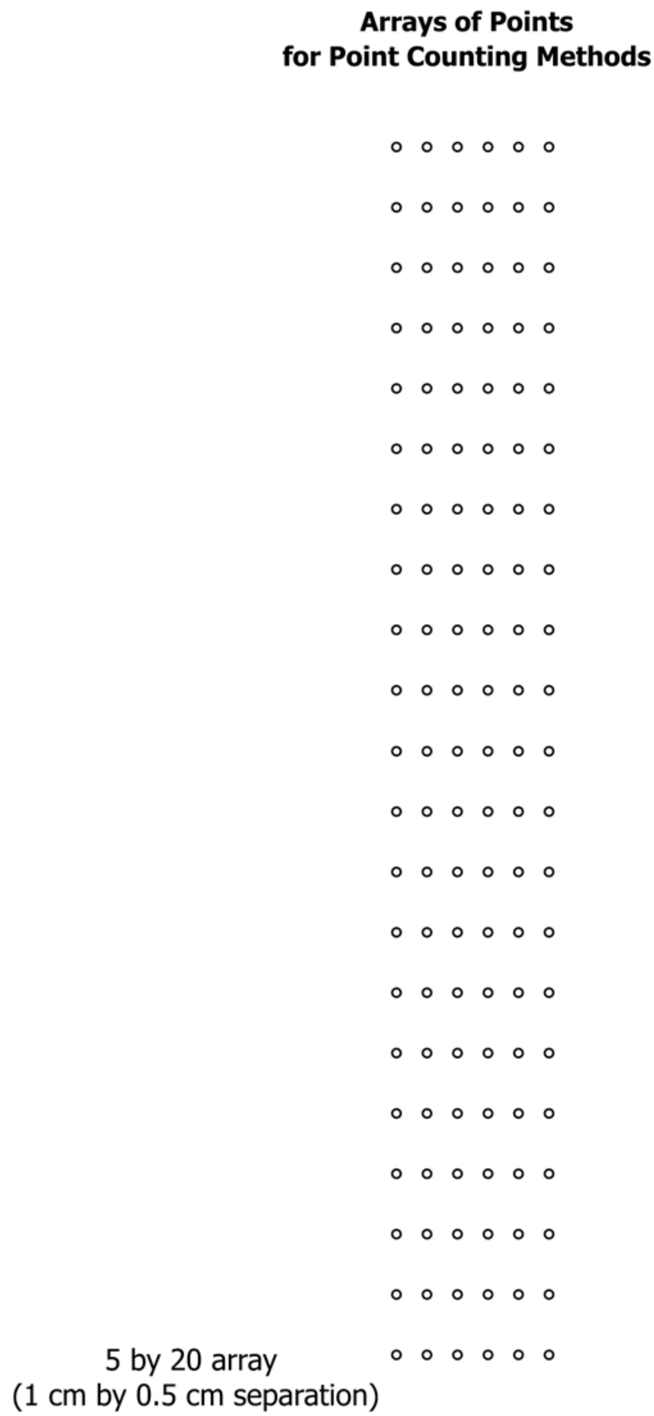
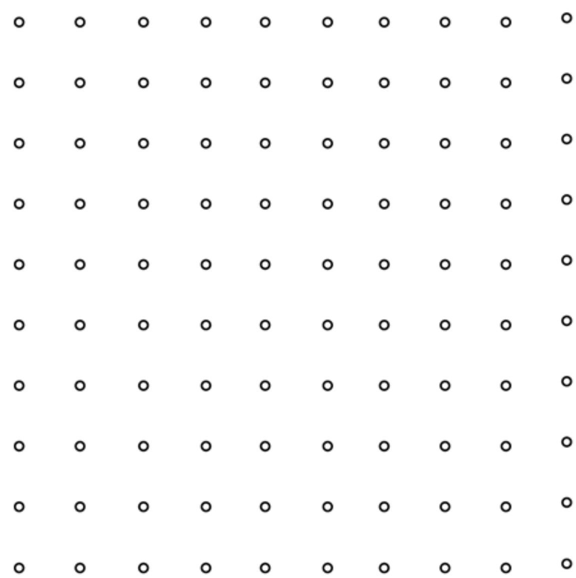


FIG. X3.1 Array of Points for Point Counting Methods

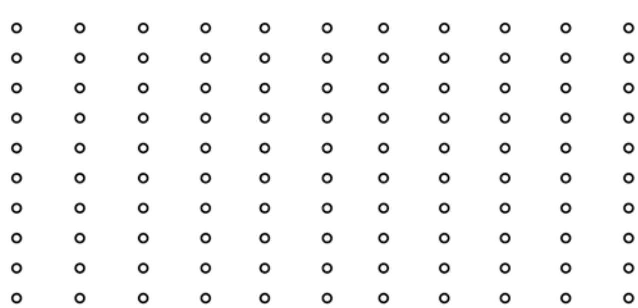
X4. PRECISION AND BIAS

X4.1 *Review of the Round Robin*—Ten laboratories were involved with the round robin. Each laboratory was provided with a copy of six photomicrographs of a sintered titanium bead coating on a Ti-6Al-4V solid substrate. The photomicro graphs were taken, at random, at an angle of 90° to the substrate, from six pocketed disk-shaped samples from a powder-coating vendor. The side walls of the pockets were never included in the micrographs. Each participant was

Arrays of Points for Point Counting Methods



10 by 10 array (1 cm separation)



10 by 10 array
(1 cm by 0.5 cm separation)

FIG. X3.1 Array of Points for Point Counting Methods *(continued)* instructed to evaluate three fields from each photomicrograph. One of the laboratory participants provided only thickness measurement data.

The participants reported only the average result ($N = 18$) for each parameter measured, so that within-laboratory repeatability cannot be determined. The following Table X4.1 may provided some guidance as to the between-laboratory reproducibility.

TABLE X4.1 Results from ASTM Stereology Round Robin

Participant	Thickness	Volume % Void	Mean Intercept Length
A	55.32	36.66	6.50
B	55.28	35.55	6.28
C	54.72	35.30	6.83
D	55.86	33.50	5.31
E	55.50	34.70	7.05
F	56.26	36.25	6.46
G	55.94	34.98	7.92
H	56.40	36.91	7.60
I	54.91	34.49	6.80
J	54.56		
Mean	55.537	35.371	6.929
Standard deviation	0.641	1.102	0.574

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