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Dust Counting Variability

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A dust counting study is described, involving seven people with various amounts of experience, two methods of lighting (light-field and phase contrast), 30 concentrations, and the counting of two types of particles (grains and fibers). The purpose of this study was to determine the effect of these variables on dust count results. An analysis of variance was made by means of a computer on 8400 values comprising the basic data. The biggest single source of variation in results is individual differences of the counters. Methods of keeping this variance to the minimum are discussed. Other methods of improving dust count reliability are also presented.

Introduction

TO QUANTITATIVELY assess the effect of insoluble airborne dust on man, it has been the practice in this country for over 40 years to collect the dust by impingement in liquid and to count the particles trapped in the collection medium by the use of the light-field microscope. These data are then related to the biological reaction of man. This technique, which was proposed to the National Conference of Governmental Industrial Hygienists in 1942, and which today is referred to generally as the Standard Method, is the procedure that will be referred to in this paper.

The validity of the technique and the reproducibility of results obtained from counting of dust have been matters of controversy for a number of years, according to Harris and others.[1-3] The lack of reproducibility of counting results obtained by this method is associated with errors that can be grouped into three major categories. These are: (1) counting errors associated with the inability of one observer to repeat his own count of any given field, or of two observers to obtain identical counts of a given field; (2) errors associated with variations in equipment from that of the Standard Method, such as the use of a phase contrast or dark-field microscope rather than the light-field mircorscope designated in the Standard Method; and (3) errors related to variation in procedure from the Standard Method, when the exact details of the procedure are not elucidated and the counter must make his own interpretation of the technique with respect to the depth of the counting cell, the collecting medium, and the time required for the dust particples of hygienic significance to settle to the bottom of the counting cell prior to counting.

The purpose of this study was to examine by statistical methods some of those variations associated with counting dust that may be considered beyond the control of the counter in comparison with variations ascribable to other sources. In addition, an effort was made to assess the magnitude of these variations, and to determine the conditions that reduce variability to the minimum. The three main sources of counting error considered to be associated with the counting of particles within a given field are: (*a*) between-counter variability, revealed by the lack of agreement in results obtained by different counters when counting the same microscop-

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ic field; (*b*) within-counter variability, demonstrated by the difficulty of one individual in reproducing his count of any one given microscopic field; and (*c*) variability of the random distribution of the particles in the counting cell when only a small portion of the total sample is counted. Preliminary consideration was also given to a variability resulting from one deviation from the Standard Method—the use of phase contrast rather than light-field microscopy, as suggested above.

Statistical techniques—percent standard deviation and analysis of variance—were used to assess the variability in (*a*) and (*b*) above, with counting being done under controlled conditions. A statistical method for predicting the random distribution (*c*) of particles from any one impinger sample, when ten fields are counted from two counting cells, as suggested in the Standard Method, has been reported by Chapman and Ruhf[2] and will not be discussed in this paper.

Experimental Design

One dust sample from an asbestos textile milling operation was collected on a membrane filter and utilized to eliminate the effects of variables related to the dust size distribution.[4] In addition, to preclude the necessity of a subjective evaluation of the size of particulates, all visible particles were counted rather than those in a given size range. It was necessary, however, to differentiate between two types of particles; these were classified either as fibers or as spherical particles (grains). For the purpose of this study a fiber was defined as a particule having a length that was more than five times its width. All other particles were classified as grains. Seven observers counted each of 30 different fields in a slide prepared from the dust sample. The same 30 fields were counted ten times for fibers and ten times for grains by each observer. All counts were made by using light-field and phase contrast microscopy.

Each observer on any given day counted only 15 of the 30 selected fields, and the fields were counted in a predetermined random order to reduce any memory bias. In addition, light-field and phase microscopes were used on alternate days. Results obtained by individual counters were not compared either by themselves or with results of other observers until the experiment was completed

The membrane filter samples were permanently mounted on glass slides by a modification[5] of technique of Hunt *et al.*[6] A section of the filter was placed dust-side up on a glass slide and one or two drops of 0.05% Lucite (methyl methacrylate resin) in chloroform was used to fix the dust particples to the membrane filter. The chloroform was allowed to evaporate. One drop of a solution consisting of 50% dimethyl phthalate and 50% diethyl oxalate was placed on the center of another clean microscope slide. A portion of the membrane filter, with the dust fixed as described above, was placed dust-side down on top of the solution. Another drop of the solution was placed on top of the filter, which was then covered with a cover slip. The membrane filter was cleared (rendered transparent) by this solution because its refractive index was essentially the same as that of the filter. A Loving microslide field finder was superimposed on the sample slides, so specific fields could be relocated for counting.

Thirty fields, with an average number of approximately 10 to 160 particles per field area, were selected for counting. A standard binocular microscope was used, equipped with a 10× objective and a 10× evepiece and a base illuminator. Counting with the phase microscope was accomplished with a 10× phase objective and a 10× eyepiece and a base illuminator The counting field was defined by the left half of a Porton graticule. Adjustments of the microscopes and light intensity were made each day by a trained microscopist, and the counters were required to adjust only the focus and the slide traverse mechanisms of the microscope.

The statistical method utilized to evaluate the 8400 counts was analysis of variance, a mathematical process for separating a total variance into components that can be associated with recognized sources of variation. Analysis was done in two steps. The first step was to determine the concentration at which

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variation appeared to be the least. This determination was based on the counts of grains by the light-field technique and was based on a sample from the 8400 counts, including the last five of the ten counts of seven dust fields, ranging from the lowest to the highest. For direct comparison of results from counting of fibers and for data from observations obtained from counting by phase contrast, the same grouping of results was followed as was found to be most advantageous for grains counted by light-field microscopy.

The following statistics were determined:

1. The arithmetic mean for all counters combined.
2. The variability, expressed as a standard deviation, among seven observers counting the same field.
3. The variability, expressed as a standard deviation, within the five counts for the same observer counting the same fields, with the results for all seven observers averaged together.
4. Each of the above standard deviations expressed as a percentage of the mean.
5. The ratio of the between-counter standard deviation to the within-counter standard deviation.

The dust concentrations found to produce the optimum reproducibility—that is, those fields for which the percentages of the standard deviations for both between-counter and within-counter were the lowest—ranged on the average from 30 to 70 grains per field. Based on these findings, all analysis-of-variance calculations discussed in this paper represent data found in the following three groups of dust densities determined by light-field counting of grains: (1) fields with concentrations ranging from 10 to 30 grains; (2) fields with concentrations ranging from 31 to 70 grains; and (3) fields with concentrations ranging from 100 to 161 grains. These data were calculated for all ten counts by each observer for all 30 fields.

In the second step, computer programs[7] were developed for the several individual analyses of variance required to evaluate the entire 8400 observations, which were divided into the three groups just described.

A three-factor[8,9] analysis of variance, 7 (counters) $\times$ 2 (sequence) $\times$ N (field densities), was performed on the data for each of the three concentrations of particles per field obtained from counting by both light-field and phase contrast microscopy, and for data obtained from counting both grains and fibers.

The number of fields (N) counted was not the same for the three concentrations of dust; 12 fields were counted at the low concentrations, 11 and 7 fields for the intermediate and high densities, respectively. The 10 counts made on each field were pooled for the analysis and considered as the first five and last five sequences of observations. Interaction between combinations of these variables were also calculated.

Review of the data revealed that a single count made by a reader of a given field sometimes deviated considerably from his other observations of that same field. To accurately assess the implications of these wide variations in single field counts, a decision had to be made as to how to handle these deviant (and possibly erroneous) readings.

The mean and standard deviations were calculated for each field count; any value that was ± 3 standard deviations from the calculated mean field count was considered to be invalid and was replaced by the value of the mean field count.

An analysis of variance was done on both the original and the revised data. The analyses indicated that replacing the extremely deviant values by the mean field count made no difference in the final statistical analysis of the data. Therefore, only the original data were used for the computation discussed in this paper.

The statistics used to determine between-counter and within-counter variability for the sample in the first step were repeated for the light-field grain counts for the 30 fields and for all ten counts of each density of dust by the seven observers.

Results

Analysis-of-variance calculations for different components of the total variance associa-

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TABLE I
Analysis of Variance (F Values) Associated with Differences of Repeated Counting of Thirty Fields of Different Densities of Grain-Type Particles by Light-Field Microscopy[a]

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Lowest Concentration (Range of $\bar{x}$: 10 to 30 grains)				
Between counters	6412 8	6	1068.8	24.9[b]
Between sequence	181.1	1	181.1	4.2[b]
Between fields	18677.4	11	1697.9	39.6[b]
Interaction				
Counters–sequence	519.4	6	86.6	2.0
Counters–fields	4786.9	66	72.5	1.7[b]
Sequence–fields	2774.8	11	252.2	5 9[b]
All	3705.9	66	56 1	1.3
Residual	28832.0	672	42.9	
Intermediate Concentration (Range of $\bar{x}$: 31 to 70 grains)				
Between counters	24044.1	6	4007.3	36.0[b]
Between sequence	27.3	1	27.3	0.2
Between fields	145385.0	10	14538.5	130.7[b]
Interaction				
Counters–sequence	2123.2	6	353.9	3.2[b]
Counters–fields	19454.6	60	324.2	2.9[b]
Sequence–fields	604.7	10	60.5	0.5
All	7353.5	60	122.5	1.1
Residual	68507.2	616	111.2	
Highest Concentration (Range of $\bar{x}$: 100 to 161 grains)				
Between counters	300426 4	6	50071.1	102.7[b]
Between sequence	2032.7	1	2032.7	4.2[b]
Between fields	235828.7	6	39304.8	80.6[b]
Interaction				
Counters–sequence	17145.1	6	2857.5	5.8[b]
Counters–fields	55767.9	36	1549.1	3.2[b]
Sequence–fields	3940.0	6	656.7	1.3
All	21570.2	36	599.2	1.2
Residual	191183.6	392	487.7	

[a] The thirty fields were divided by the particle density into three groups (low, intermediate, and high), based on the average mean grain count of the seven observers counting each field ten times by light-field microscopy.

[b] $p < 0.05$.

ated with repeated counts by different observers of the three density groups of grains and of fibers, by two microscope techniques, are presented in Tables I to IV.

The calculated *F* values determined for each of the three density groups for both fibers and grains by both techhniques are significant for the between-counter and the between-field components. These values would be expected to occur less than once in a thousand, by chance alone. However, when the *F* values associated with the differences between the two sequences (first five and last five counts) are considered, some values are not significant, and others are not as highly significant, generally speaking, as for the above-mentioned main effect sources. The differences in the intermediate grain concentration counts by the light-field technique were not statistically significant. The *F* values associated with the low and high ends of the density range by this technique might be expected to occur by chance alone as many as five times in a hundred.

When grains were counted by the phase contrast technique, only the highest density range value was significant. When fibers were counted by the phase contrast technique, the differences between the two sequences of counts showed *F* values that were statistically significant in all groups except the lowest density range.

Other data in Tables I to IV indicate values associated with first-order and second-order interactions to aid in the interpretation of the components of the treatment sums of squares attributable to the main effects, or the three components described above. Further analysis is necessary for a full interpretation of these effects.

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TABLE II
Analysis of Variance (F Values) Associated with Differences of Repeated Counting of Thirty Fields of Different Densities of Fiber-Type Particles by Light-Field Microscopy[a]

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Lowest Concentration (Range of $\bar{x}$: 1 to 4 fibers)				
Between counters	1697.6	6	1282.9	56.5[b]
Between sequence	185.7	1	185.7	37.1[b]
Between fields	1002.1	11	91.1	18.2[b]
Interaction				
Counters–sequence	226.2	6	37.7	7.5[b]
Counters–fields	456.3	66	6.9	1.4[b]
Sequence–fields	143.8	11	13.1	2.6[b]
All	310.4	66	4.7	0.9
Residual	3366 0	672	5.0	
Intermediate Concentration (Range of $\bar{x}$: 3 to 15 fibers)				
Between counters	13276.5	6	2212 7	135.1[b]
Between sequence	371.7	1	371 7	22.7[b]
Between fields	15995.4	10	1599.5	97.7[b]
Interaction				
Counters–sequence	2078.5	6	346.4	21.2[b]
Counters--fields	3735.2	60	62.2	3.8[b]
Sequence–fields	370.5	10	37.0	2.3[b]
All	1565.2	60	26.1	1.6[b]
Residual	10086.0	616	16.4	
Highest Concentration (Range of $\bar{x}$: 6 to 22 fibers)				
Between counters	31420.5	6	5236.7	110.5[b]
Between sequence	458.5	1	458.5	9.7[b]
Between fields	22394.2	6	3732.4	78.8[b]
Interaction				
Counters–sequence	5155.8	6	859.3	18.1[b]
Counters–fields	6871.2	36	190.9	4.0[b]
Sequence–fields	394.4	6	65.7	1.4
All	1230.9	36	34.2	0.7
Residual	18576.0	392	47.4	

[a] The thirty fields were divided by the particle density into three groups (low, intermediate, and high), based on the average mean grain count of the seven observers counting each field ten times by light-field microscopy (Table I).

[b] $p < 0.05$.

In Figure 1 the standard deviation expressed as percentage of the within-counter mean and the between-counter mean is plotted against the total average count. These data indicate the influence of the dust concentration on these two types of variability. The smaller the percentage standard deviation, the better the agreement. The within-counter variability for light-field grains was approximately 22% of the standard deviation at a density of 40 grains per field and was reduced only slightly as the density increased, with about 19% standard deviation at 100 grains per field and approximately 18% standard deviation at 150 grains per field.

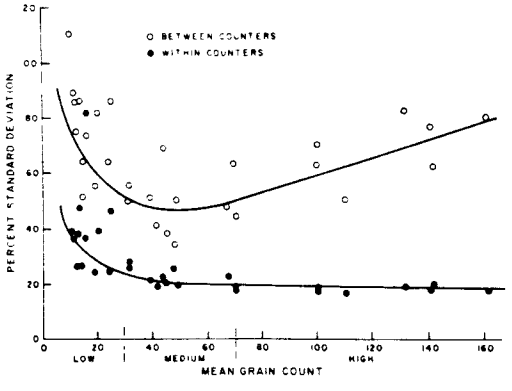


FIGURE 1. Percentage standard deviation versus mean grain count, light-field microscopy.

The data (Figure 1) suggest that the between-counter variability is about 46 to 48% standard deviation between counts of 40 and 60 grains per field. The percentage standard deviation increases rapidly and exceeds 60% at a density of 100 grains per field.

The determination of an optimum dust concentration must take into consideration

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TABLE III
Analysis of Variance (F Values) Associated with Differences of Repeated Counting of Thirty Fields of Different Densities of Grain-Type Particles by Phase Contrast Microscopy[a]

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Lowest Concentration (Range of $\bar{x}$: 14 to 37 grains)				
Between counters	11674 3	6	1945.7	42.4[b]
Between sequence	21.4	1	21.4	0 5
Between fields	43734.2	11	3975.8	86 6[b]
Interaction				
Counters–sequence	404.2	6	67.4	1.5
Counters–fields	5641 9	66	85.5	1 9[b]
Sequence–fields	2374.8	11	215.9	4.7[b]
All	2554 9	66	38.7	0 8
Residual	30845.6	672	45.9	
Intermediate Concentration (Range of $\bar{x}$: 35 + 0 113 grains)				
Between counters	177634.1	6	29605 7	114.2[b]
Between sequence	3.4	1	3 4	0.1
Between fields	373477.9	10	37347.8	144.0[b]
Interaction				
Counters–sequence	14510 7	6	2418.4	9.3[b]
Counters–fields	67736 8	60	1128.9	4.4[b]
Sequence–fields	4506.4	10	450.6	1.7
All	17848 2	60	297 5	1.1
Residual	159732.0	616	259.3	
Highest Concentration (Range of $\bar{x}$: 128 to 206 grains)				
Between counters	773108.9	6	128851.5	158.6[b]
Between sequence	9681 0	1	9681.0	11.9[b]
Between fields	234001.4	6	39000.2	48 0[b]
Interaction				
Counters–sequence	26430.3	6	4405.0	5.4[b]
Counters–fields	64066.5	36	1779 6	2.2[b]
Sequence–fields	7798.5	6	1299.8	1 6
All	35597 4	36	988 8	1.2
Residual	318489.2	392	812.5	

[a]The thirty fields were divided by the particle density into three groups (low, intermediate, and high), based on the average mean grain count of the seven observers counting each field ten times by light-field microscopy (Table I).

[b]$p < 0.05$.

both between-counter and within-counter variability. These combined effects were measured at various densities of dust by the ratio of the between-counter to the within-counter standard deviations.

The optimum dust concentration for reproducibility in counting is the one that produces the lowest ratio, and for which the between-counter and within-counter percentage standard deviations are at a minimum. As shown in Figure 2, the ratio remains at a value of less than 2 4 until the grain mean count (or grain density) exceeds 50 particles per field. At this point the ratio increases to a value of 2.6 at a count of 60 particles.

Discussion and Conclusions

Interpretation of these data has been confined for the most part to the counting of grains as the particle density for grains was higher than that for fibers. Furthermore, evaluation of occupational dust exposure is

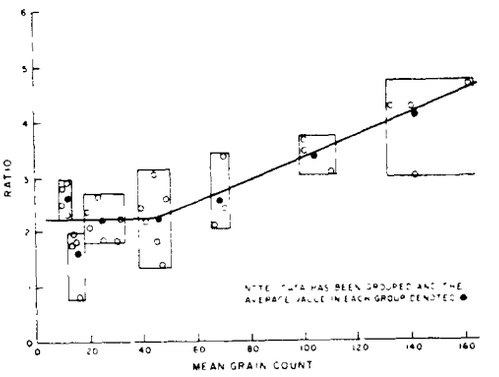


FIGURE 2. Ratio (standard deviation between counters to standard deviation within counters) versus mean grain count, light-field microscopy.

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TABLE IV
Analysis of Variance (F Values) Associated with Differences of Repeated Counting of Thirty Fields of Different Densities of Fiber-Type Particles by Phase Contrast Microscopy[a]

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Lowest Concentration (Range of $\bar{x}$: 3 to 8 fibers)				
Between counters	5314 4	6	885.7	175.1[b]
Between sequence	0 03	1	0 03	0.006
Between fields	2986 0	11	271.4	53.7[b]
Interaction				
Counters–sequence	261 6	6	43.6	8.6[b]
Counters–fields	1245 0	66	18.9	3.7[b]
Sequence–fields	145.8	11	13.2	2.6[b]
All	349.7	66	5.3	1.0
Residual	3398.4	672	5.0	
Intermediate Concentration (Range of $\bar{x}$: 9 to 25 fibers)				
Between counters	43601.4	6	7266.9	266 8[b]
Between sequence	123.2	1	123.2	4.5[b]
Between fields	26114.3	10	2611.4	95.9[b]
Interaction				
Counters–sequence	1794.0	6	299.0	11.0[b]
Counters–fields	6637.0	60	110.6	4.1[b]
Sequence–fields	611.5	10	61.2	2.2[b]
All	2273 8	60	37.9	1.4[b]
Residual	16776.0	616	27.2	
Highest Concentration (Range of x: 19 to 41 fibers)				
Between counters	105122.1	6	17520.4	232.3[b]
Between sequence	1580.4	1	1580.4	21.0[b]
Between fields	23869.6	6	3978.2	52.8[b]
Interaction				
Counters–sequence	3758.4	6	626.4	8.3[b]
Counters–fields	5952.6	36	165.4	2.2[b]
Sequence–fields	629.7	6	105.0	1.4
All	2757 2	36	76.6	1.0
Residual	29562.8	392	75.4	

[a] The thirty fields were divided by the particle density into three groups (low, intermediate, and high), based on the average mean grain count of the seven observers counting each field ten times by light-field microscopy (Table I).

[b] $p < 0.05$

usually concerned with spherical particles. It was assumed that the results obtained from the counting of particles of asbestos would give statistical results similar to those obtained with other mineral dusts. The data suggest that this hypothesis is warranted at least for this type of asbestos and this method of generating the particles.

The method of counting a given field was designed to reduce or, if possible, to eliminate entirely any memory bias in recognizing the configurations of dust particles within any given field. The analysis-of-variance program was designed to determine any detectable deviation from the first to the last count of the ten repeated observations of each given field. The ten counts were divided into two groups, the first five and the last five. The results indicate that at some densities, by both types of microscopy, there is, in fact, little variation between the first five and the second five counts. It should be noted, however, that owning to the statistical technique, it is possible that the true nature of the deviations may not be apparent when as many as ten counts are used for analysis. Consequently, analysis-of-variance computations were also made on the first eight counts, divided into two halves; on the first six counts, divided into halves, and on the first four counts, divided into two halves. It was not possible to detect any memory bias in the sequential observations in this experiment.

The results of within-counter variability are about what would be expected, and the percentage standard deviation for light-field counting of grains is in general agreement with results reported from samples collected with thermal precipitators.[4] However, the

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results of between-counter variability, with a minimum of approximately 46% standard deviation, are about twice the within-counter variability results.

The *F* values associated with differences between counters are all statistically highly significant. When grains are counted, by either microscopic technique, the *F* values are increased with increase in the concentration of the dust. It was observed that the results obtained from individual counters tended to be grouped consistently higher or lower than the grand mean for all counters. It has been suggested that the use of a factor for each counter could reduce this error to the minimum.[4] This, however, does not seem to be a practical solution if the intent is to compare these results to the threshold limit values for various dusts If the basis for making a dust count is to evaluate the effect of engineering control methods where all counts are made by the same counter, it would not be necessary to use such a factor, since an observer can repeat his own counts with greater precision than he can repeat the results of another counter.

A statistical analysis of the relationship between experience and the precision of the results was not attempted, as the number of counters was too few to make a valid conclusion. However, casual observation of the data did not support the hypothesis of less variability in counts with experienced observers.

The data suggest that a minimum variability from between-counters and within-counters, and between-particle densities, of approximately 40 particles is in close agreement with the minimum density for counting suggested in the Standard Method. This assumes a concentration of at least four times the blank count, with a blank count of no more than six particles. The maximum limit of 150 particles in 0.25 mm² of counting area, recommended in the Standard Method, is somewhat more liberal than is the 60 particles per field indicated by the data from this study.

The additional errors associated with deviations from the procedures outlined in the Standard Method are well illustrated (see Tables III and IV) by the variations that occurred with the use of phase microscopy rather than the recommended light-field techniques. Phase contrast microscopy did produce counts that in all cases were higher than the results obtained by light-field microscopy. The first consideration again must be the comparison of the results of dust counts to threshold limit values and to the Standard Method of collection. In this case, higher counts do not necessarily produce results that can be interpreted with increased assurance of their hygienic significance. A detailed examination is being made of results from counting dust by phase contrast microscopy; these results will be published at a later date.

The high variability of the results from counting in all probability could have been caused by a number of contributing factors. Consideration should be given to overlapping of particles as density of dust increases. The decision as to whether an aggregate is actually one or more particles becomes increasingly difficult when the dust particles are in close proximity to each other. Also, the heavier the concentration the more difficult it is for a counter to "keep his place" when attempting to count all particles in a field These effects are exaggerated when one is counting asbestos fibers, where a complicating factor is the necessity for deciding whether several overlapping fibers are actually separate fibers or a bundle of connected fibers. These factors, undoubtedly contribute to the lower between-reader and within-counter variances in the low and medium ranges of dust densities.

Summary

Two basic sampling errors associated with the counting of particles within a given field were considered; these were the between-counters variability, demonstrated by the lack of agreement in results obtained by different counters when counting the same microscopic field, and within-counter variability, revealed by the difficulty of one counter in reproducing his count of any one given microscopic field. In addition, light-field and phase contrast microscopic techniques were used, and

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preliminary comparisons were made of the results and of differences between grains and fibers.

Two statistical techniques — analysis of variance and percentage standard deviation— were used to assess this variability with counting being accomplished under controlled conditions. This analysis indicated that the between-counter variability was about twice the within-counter variability, and the optimum dust density was between 40 and 60 particles per field when counted by light-field microscopy. A preliminary statistical study indicated a higher variance for fibers than the results obtained on the same fields when counting grains.

Phase contrast microscopy produced counts that were higher, in all cases, than the results obtained by light-field microscopy. The possibility of memory bias resulting from repeated counting of the same field was investigated by analysis of variance; none could be detected.

This phase of the analysis points toward several areas that warrant further investigation. A detailed evaluation of some of these variances is currently in progress and will be reported at a later date.

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