AN EVALUATION OF THE COULTER
AUTOMATIC PARTICLE COUNTER

By

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AUTOMATIC PARTICLE COUNTER

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CHAPTER I

INTRODUCTION

With the advent of the servo valve in hydraulic equipment in both military and industrial systems, and the need for increased response characteristics during recent years, the problem of solid matter in hydraulic fluids has become very critical. In order to achieve the response characteristics now specified in the various fields, servo valves are being built smaller, with much closer tolerances. In some cases, valve passages have dimensions in the range of two microns. Solid matter in hydraulic fulids either cause unwanted response characteristics or cause the valve to stop functioning completely, due to clogged passages.

The determination of the contamination level of hydraulic fluids is a major problem in the servo valve field. The present accepted means of evaluating the contamination level, using a microscope, is very slow and tedious. It required a highly-trained operator, and the human error due to changing from one operator to another is very significant.

The Coulter Automatic Particle Counter offers a rapid means of sizing and counting particles in fluids. The range of size of particles that can be counted, with appropriate apertures, is from 0.5 to 500 microns in diameter. Although the Coulter Counter, along with other automatic particle counters, offers a rapid means of sizing and counting particles, the principle differs with each instrument, and a means to evaluate the accuracy is needed. Since there is no standard for counting and sizing particles in fluids, a series of tests must be undertaken to prove the accuracy and precision of the Coulter Counter or any other automatic particle counter.

CHAPTER II

PREVIOUS INVESTIGATION

The automatic particle counter was devised to make it possible to reduce the time for analyzing contamination count and distribution in fluids by the tedious microscope method; and also, to increase the accuracy. Due to the limited number of particles that can be counted by the microscope method, about two-thirds of the counts made, can be considered to be within a random error of four per cent; and one-third of the counts made, will have an error in excess of four per cent, according to Coulter (1)[1].

According to ARP-598, a maximum variation of one to two (± 33 per cent of the average of two runs) in results should be expected when repeating the count on the same sample (2). In tests conducted by the Boeing Airplane Company, and reported by Morris (3), a predicted error of ± 20 per cent on a count by the microscope was made. This count was based on several samples evaluated according to ARP-598.

In another test carried out by the Boeing Airplane Company and reported by Michaelson (4), it was stated that the variation from sample to sample using a Coulter Counter will almost certainly follow a Poisson distribution; that is, the standard deviation (estimate of

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[1] ( ) Refers to Selected Bibliography.

variation) will equal the square root of the average. Michaelson attempted to evaluate the accuracy of the Coulter Counter by adding known amount of contaminate to samples of clean fluid. The contaminant added was pollen of uniform density and nearly monosized. Different amounts were added to the clean samples, and counts were made on each sample. For a 0.4 milligram sample, the reading per cubic centimeter was 930 particles; and for a 0.8 milligram sample, the reading per cubic centimeter was 1865 particles. These results are close enough to a double count to be well within the limits of experimental error.

These tests indicate that the Coulter Counter count is much more accurate than that of the microscope count. The error involved, with a Poisson distribtuion, is much less than the expected 20-per-cent error with the microscope count when the number of particles counted is 100 or more. As the number of particles counted increases beyond 100, the error decreases, percentage-wise, with the Coulter Counter.

According to Coulter, when the operator is counting particles in the same size range, the number of particles counted by the microscope method is limited to about 500 due to the tedious process involved. When particles in several different ranges exist, the number of particles a person can count in each range is considerably reduced. According to ARP-598, paragraph 8.3.4.6, in no case shall the total number of particles in a unit exceed 50 of a specified size range.

Even if a person could count 500 particles, the expected error in repeatability would be ± 100 particles when using a microscope in accordance with ARP-598. The same number of particles counted with the Coulter Counter should yield a maximum error of only ± 22 according to Michaelson.

Michaelson also attempted to make a comparison of the Coulter Counter count with a particle count made by ARP-598. A comparison of 18 samples was made in this evaluation, and a total count above 5 microns was obtained on each of these samples. The results of this test, as presented graphically in Figure 1, indicate that the Coulter Counter and the microscope method agree very well, within the allowable errors of both methods.

One of the main reasons for the use of the automatic counters in evaluating the degree of contamination in fluids is the time required per sample. When evaluating a sample by ARP-598, the minimum time required per sample is one hour, according to Morris. It should be noted that a considerable amount of training and practice is required to be able to make a microscope analysis. Michaelson states that the Coulter Counter has at least a ten-fold advantage in speed over the microscope method; therefore, the average time per sample when using the Coulter Counter is six minutes as opposed to sixty minutes when using the microscope method.

Galloway (5), in tests made by the Douglass Aircraft Company dealing with the evaluation of a HIAC Automatic Particle Counter, suggested the use of colored beads when correlating an automatic count with a microscope count. The advantage of using colored beads is that they are easier to count when using the microscope method of counting. Much of the tedium and error in microscope counting is eliminated when the operator is able to distinguish between various sizes of particles by their color rather than by measuring each particle with a micrometer stage eyepiece. Although each particle need not be measured, error is

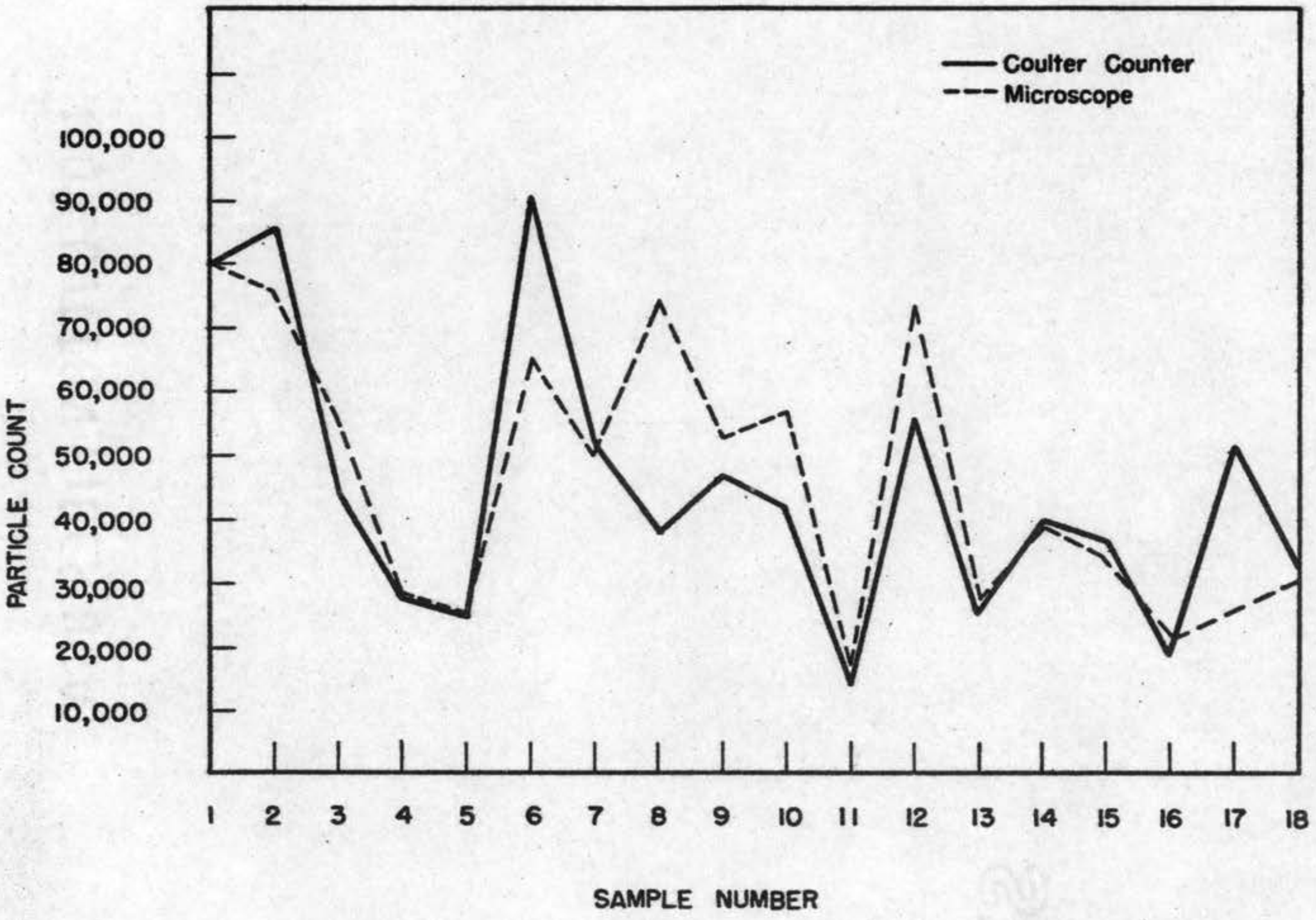

Fig. 1. Correlation of Coulter Counter and Microscope Method by Boeing.

introduced when an operator with very little experience estimates particle diameter by comparison with particles measured.

Ulrich (6) reported various applications of the Coulter Counter, other than the straight particle counts. One of these tests involved dispersal or agglomeration of the sample, causing changes of distribution with time. Other articles containing information of interest on the theory and principles involved in the Coulter Counter include Brecket, et al (7), Grant, et al (8), Kubitschek (9), and Mattern et al (10).

CHAPTER III

STATEMENT OF PROBLEM

In the past when a new measuring instrument was introduced, it was compared with the existing standard in its field to become acceptable. In the field of measuring solid particle contamination in hydraulic fluids, there is no standard. The present accepted means of particle contamination evaluation, the microscope method, leaves much to be desired in both accuracy and speed.

Since the microscope method is the only method now accepted, the object of this study was divided into three parts:

1. Determine the precision of the Coulter Counter.
2. Arrive at a correlation between the microscope method and the Coulter Counter.
3. Outline a procedure for evaluating an automatic particle counter.

Tests made in the past, as stated in Chapter II, indicate the Coulter Counter is more accurate than the microscope method with an advantage in speed. A correlation between the automatic particle counter and the microscope method is needed to validate the use of an automatic particle counter. An outlined procedure for checking the precision of the Coulter Counter or any other automatic particle counter is needed and will be presented in this study.

CHAPTER IV

DETERMINATION OF THE PRECISION OF THE COULTER COUNTER

One of the most important characteristics of the automatic particle counter is the precision or accuracy of the instrument. The precision of this counter is measured by its ability to repeat a count when particles are homogeneously mixed in a solution, and its ability to produce the correct multiple of a count when a certain multiple of particles is added to the counting solution. Unless an automatic particle counter is capable of the precision demanded by the tests being conducted in the laboratory, its advantage of speed is of little value.

The Coulter Counter should repeat counts within 2/3 the square root of the average for three or more counts. Also, when contamination is added in single, double, triple, etc., amounts, the counts should increase by the same amount and fall within the Poisson distribution as stated above.

The following procedure was set up to test the precision of the Coulter Counter:

1. The fluid used in the test was the electrolyte described in Appendix A. The actual fluid used is of little importance to the test, since changing fluid involves only a change in calibration.

   The electrolyte was triple-filtered and checked to see if it met the contamination limits as described in Appendix A; that is, whether it contained no more than 100 particles (above five microns) per two cubic centimeters.

2. The electrolyte was placed in two clean flasks which had been cleaned according to the specifications set forth in Appendix A, section A. A measured amount of contaminant was then added to one flask.

3. Tests were then run using a mixture of the two fluids, such as 10-per-cent contaminated with 90-per-cent clean; 20-per-cent contaminated with 80-per-cent clean; etc., until the last sample was 100-per-cent contaminated fluid. The contaminated fluid should be mixed well by agitating and rolling the fluid in the flask immediately before each mixing of the clean and contaminated fluid. The graduated cylinder used to mix the clean and contaminated fluid should be cleaned according to the specifications set forth in Appendix A, section A.

   More tests should be run in the range from 10-per-cent to 50-per-cent contaminated fluid than above 50-per-cent because the accuracy of the counter, due to the Poisson distribution, is less (percentage-wise) for lower particle counts. This is especially true if only a small number of particles is added to the fluid which makes up the contaminated fluid.

4. The results of the tests described in section 2, when plotted on graph paper (per cent of contaminated fluid vs. particle count) should be represented by a straight line;

that is, it should be possible to draw a straight line through the regions described by the deviation due to the Poisson distribution, since all counts would be expected to fall within this distribution.

In the first attempt to evaluate the Coulter Counter by the above procedure, segregated, colored plastic, and glass beads were used as particles in the contaminated fluid. The count failed to fall within the Poisson distribution, due to the fact that the number of particles decreased constantly with time at any one setting of the counter. It was discovered that the particles were coming out of suspension and settling on the bottom of the beaker. By the manner in which the particles collected in groups around the bottom of the beaker, the settling-out-of-suspension appeared to be caused by the electrical charge on the particles.

By using pollen as the contaminant in the fluid, the problem of settling-out was solved. It was also easier to keep a homogeneous solution in the contaminated flask with pollen, due to its low density.

Two tests were run according to the above procedure. The first test, using ragweed pollen as the contaminant, was run with a relatively high contamination level in the contaminated fluid (over 1500 particles per two cubic centimeters). The results of the first test are presented on the standard Coulter Counter sample data sheet, explained in Appendix B, section E, in Table I. The results are shown graphically in Figure 2. It was possible to draw a straight line, within the Poisson distribution, through all regions, with the possible exception of the data taken at 40-per-cent contaminated fluid.

The second test was run using paper mulberry pollen with fewer particles in the contaminated fluid (less than 300 particles per two cubic centimeters) as compared to the first test. The results of the second test are given in Table II and presented graphically in Figure 3. The results of the second test also allow a straight line to be drawn through the regions described by the Poisson distribution.

Table I. High Contamination Sample Analysis.

PARTICLE CONTAMINATION LABORATORY
OKLAHOMA STATE UNIVERSITY
Coulter Counter Sample Data

Sample ELECTROLYTE Source O. S. U. Electrolyte STANDARD "I" Date 5/7/62

Aperture Diameter 140 μ Manometer Volume 2000 μl Coinc. Factor p = 1.71 Calib. Factor k = 7.35 Dispersant NONE Oper MWZ

Aperture Resistance 120,000 Ω Gain Index 4 NOTES COLUMN 9 (NOTES) INDICATES PERCENT OF CONTAMINATED FLUID

Threshold Dial Expansion Factors	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
	1.00	.504	.255	.1314	.0694	.0386	.0235	.0166	.	.

Threshold Reading (t')	Aperture Current Selector Reading (I)	Particle Contaminant Diameter (d)	RAW COUNTS n'			Average Count $\bar{n}'$	Coincidence Correction $n''=p\left(\frac{\bar{n}'}{1000}\right)^2$	Full Count $\bar{n}''=\bar{n}'+n''$	Actual Count $n=\bar{n}''-$ background	NOTES
			Run 1	Run 2	Run 3					
40	2	20	2	3	3	3	0	3	3	0%
40	2	20	169	178	175	174	0	174	174	10%
40	2	20	320	323	308	317	0	317	317	20%
40	2	20	650	625	648	641	1	642	642	40%
40	2	20	749	780	754	761	1	762	762	50%
40	2	20	1180	1160	1162	1167	2	1169	1169	75%
40	2	20	1550	1565	1572	1556	4	1560	1560	100%

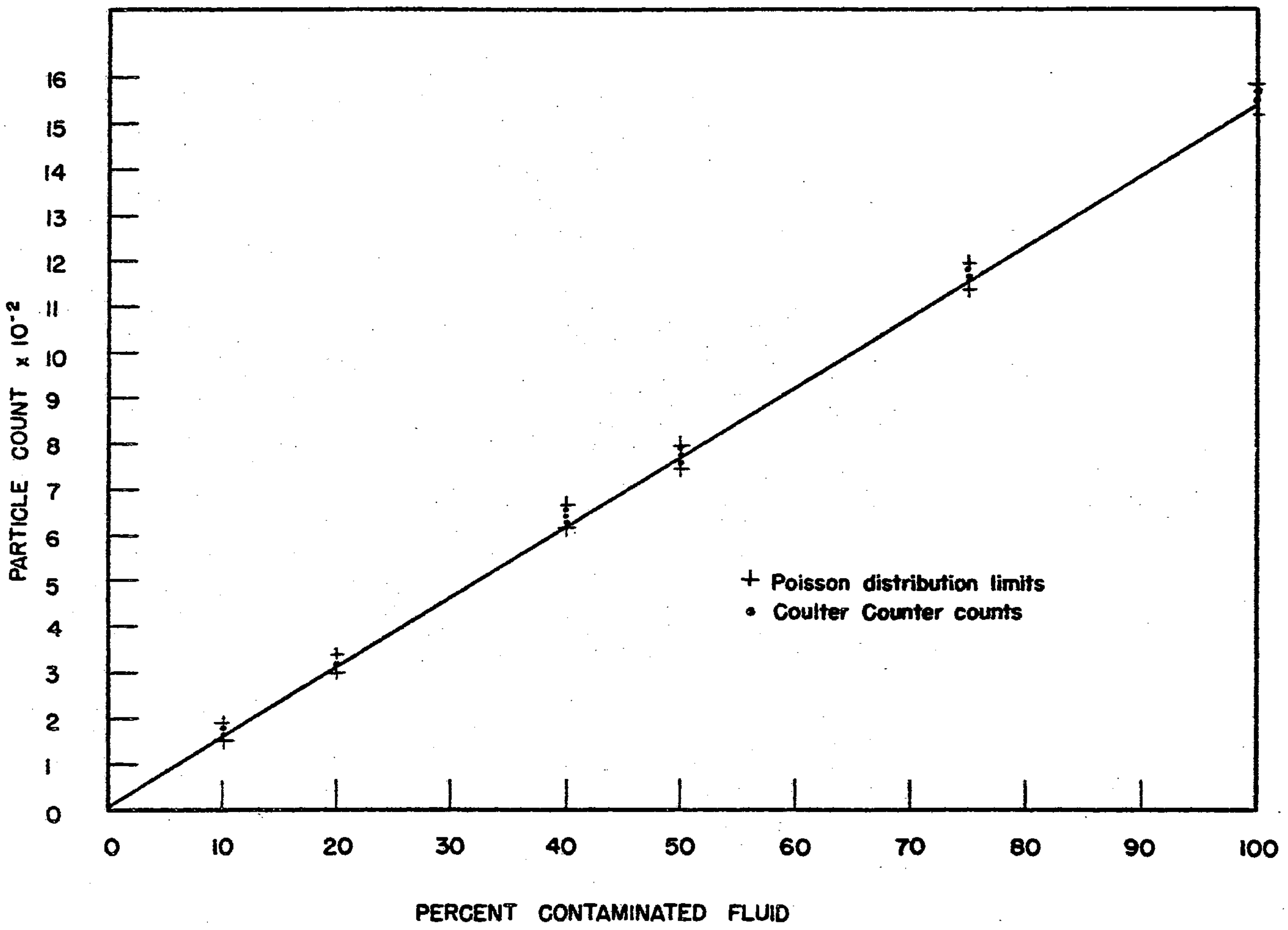

Fig. 2. Determination of the Precision of the Coulter Counter (High Contamination).

Table II. Low Contamination Sample Analysis.

PARTICLE CONTAMINATION LABORATORY
OKLAHOMA STATE UNIVERSITY
Coulter Counter Sample Data

Sample ELECTROLYTE Source O. S. U. Electrolyte STANDARD "I" Date 5/8/62

Aperture Diameter 140 μ Manometer Volume 2000 μl Coinc. Factor p = 1.71 Calib. Factor k = 7.35 Dispersant NONE Oper M.W. Z.

Aperture Resistance 120,000 Ω Gain Index 4 NOTES COLUMN 9 (NOTES) INDICATES PERCENT OF CONTAMINATED FLUID

Threshold Dial Expansion Factors	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
	1.00	.504	.255	.1314	.0694	.0386	.0235	.0166	.	.

Threshold Reading (t')	Aperture Current Selector Reading (I)	Particle Contaminant Diameter (d)	RAW COUNTS n'			Average Count $\bar{n}'$	Coincidence Correction $n''=p\left(\frac{\bar{n}'}{1000}\right)^2$	Full Count $\bar{n}''=\bar{n}'+n''$	Actual Count $n=\bar{n}''-$ background	NOTES
			Run 1	Run 2	Run 3					
36.4	5	10	1	0	1	1	0	1	1	0%
36.4	5	10	27	30	25	27	0	27	27	10%
36.4	5	10	54	51	58	54	0	54	54	20%
36.4	5	10	77	83	77	79	0	79	79	30%
36.4	5	10	105	107	100	104	0	104	104	40%
36.4	5	10	136	140	137	138	0	138	138	50%
36.4	5	10	205	197	213	205	0	205	205	75%
36.4	5	10	286	288	279	284	0	284	284	100%

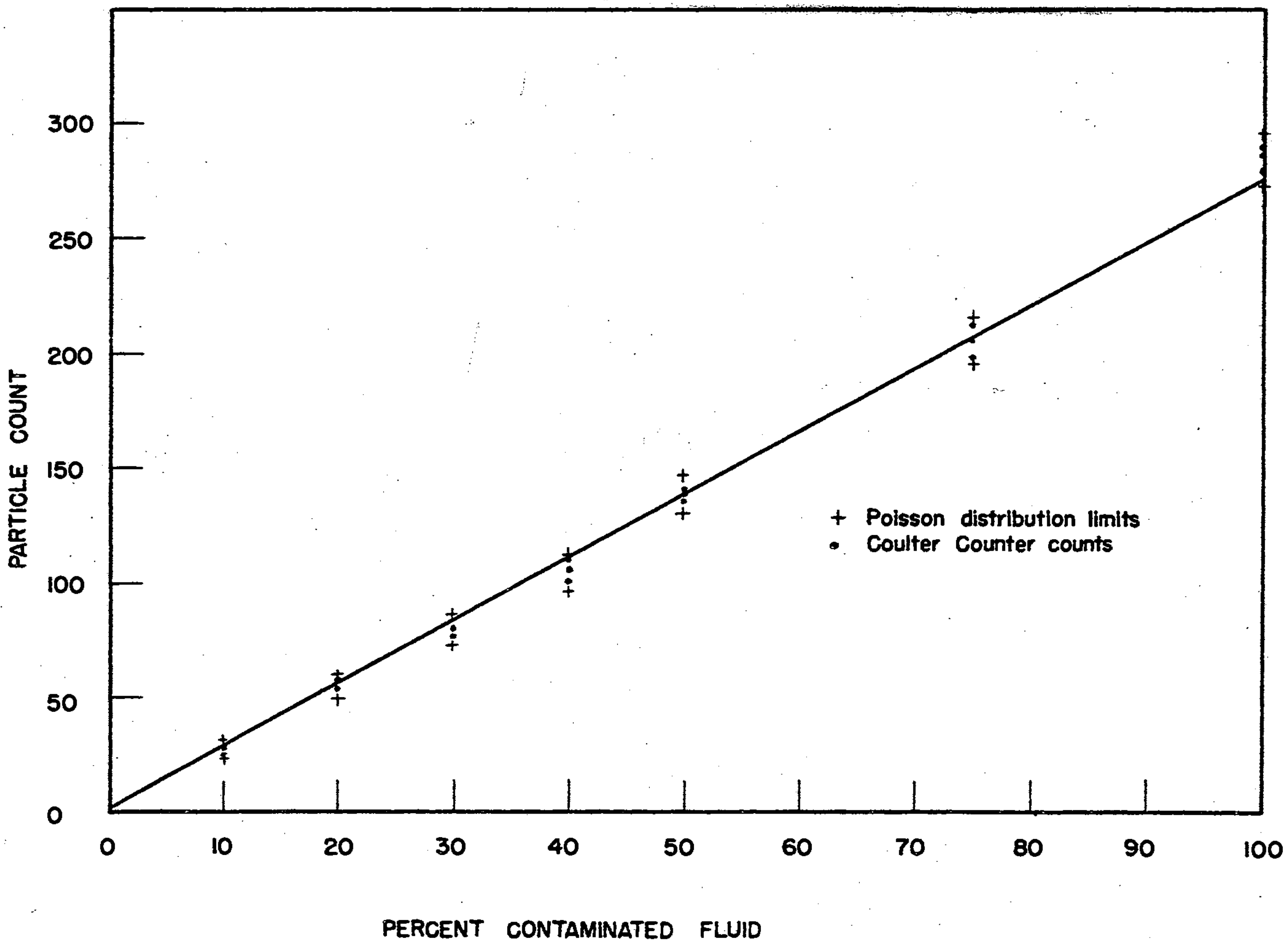

Fig. 3. Determination of the Precision of the Coulter Counter (Low Contamination).

CHAPTER V

CORRELATION OF COULTER COUNTER AND MICROSCOPE COUNTS

Since there is no standard method of evaluating the contamination level in fluids, any automatic particle counter which is to be evaluated must be compared to the present accepted method; that is, a visual count using a microscope. One of the main problems encountered when correlating an automatic particle counter count with a microscope count based on ARP-598 is the fact that the two methods size the particles differently. The size of a particle using ARP-598 is determined by its greatest dimension. This greatest dimension is then recorded as the diameter of the particle. In contrast, a particle passing through the Coulter Counter causes a voltage pulse proportional to the volume of the particle; and the size-setting allows the counter to read out the diameter of a sphere with a volume equal to the volume of the actual particle. (See Appendix C.) Because of the difference in the sizing methods of the Coulter Counter and the microscope counts, identical particles could be recorded in different size ranges.

In order to avoid the problem of different sizing methods, the tests to correlate the two counting methods were conducted by using particles of ragweed pollen. This pollen is essentially spherical; therefore, both the longest dimension and the diameter of an equivalent volume will be the same. It should be noted that the purpose of the test was to correlate the counts by using both the microscope and Coulter Counter as closely as possible, under similar conditions. A greater difference in counts would be expected when using test dust or samples of fluid taken from some system, due to the sizing differences for particle shapes other than spheres; but this greater difference would not necessarily be due to an error in either method.

The fluid used in this test was MIL-H-5606, and the electrolyte used for the counter was the same as that described in Appendix A. Both the hydraulic fluid and the electrolyte were thoroughly filtered to insure that the fluids contained no particles equal to or greater than 20 microns in diameter. The hydraulic fluid is divided into two flasks cleaned according to speficiations set forth in Appendix A, section A. One flask is then contaminated with ragweed pollen. Five samples were prepared by mixing the two flasks of hydraulic fluid in arbitrary proportions. The five samples were then evaluated by the microscope and Coulter Counter methods. The data obtained are presented in Table III. The two right-hand columns give the ± 20 per cent error which is expected in a microscope count. The data taken in this phase of the test are presented graphically in Figure 4. It can be seen from the graph that the results obtained by the Coulter Counter method falls well within the limits of the expected microscope count method error and very close to the actual microscope count.

TABLE III

CORRELATION DATA

Sample No.	Coulter Count	Microscope Count	20% Error	+20% Error	-20% Error
1	8,800	9,250	1,850	11,100	7,400
2	7,800	8,500	1,700	10,200	6,800
3	34,600	38,900	7,780	46,680	31,120
4	8,600	7,420	1,484	8,909	5,936
5	5,300	4,640	927	5,567	3,713

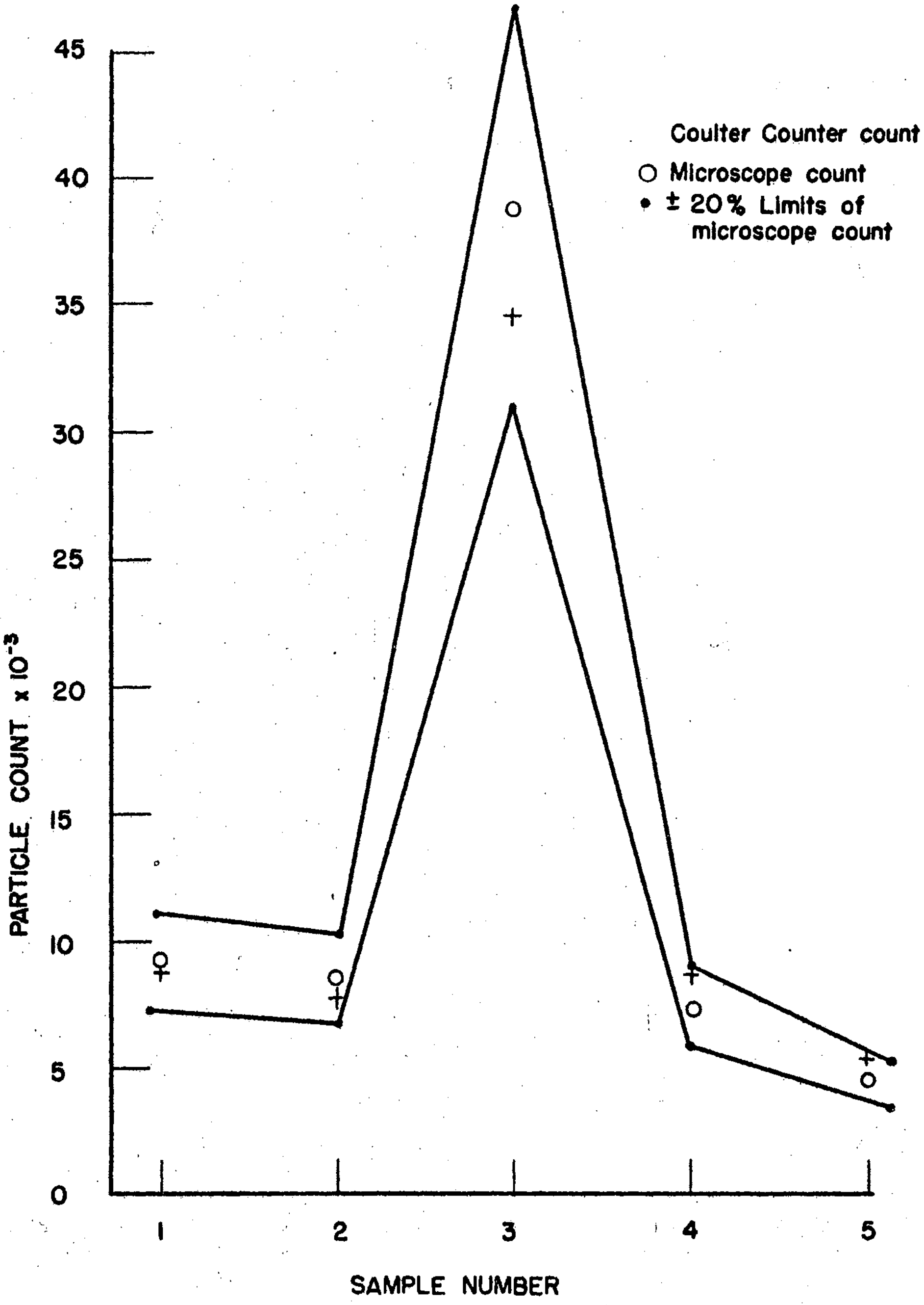

Fig. 4. Experimental Correlation Between the Coulter Counter and the Microscope.

CHAPTER VI

CONCLUSIONS

One of the main objectives of this study was to determine if the predicted precision of the Coulter Counter, ± 2/3 the square root of the average count, could be realized. (See Appendix B) The results of the tests shown in Chapter IV indicate that in every sampled checked, the Coulter Counter did repeat within this Poisson distribtuion. It should be noted that in some cases more than three runs were required to get results that would repeat within the Poisson distribution. In some instances, the aperture would become partially blocked during a run; and in other cases, outside electrical interference would cause an erroneous count. These erroneous readings can easily be detected because the orifice can be seen through the attached microscope, and electrical interferences appear on the oscilloscope screen. These erroneous runs were discarded, and additional runs were made.

The Coulter Counter was also checked to see if the correct multiple of a count was recorded when contaminate was added in multiple amounts. Figures 2 and 3 show that the counter functioned acceptably well in this phase of the test.

The results from the Coulter Counter and microscope method compared very favorably in the correlation tests as described in Chapter IV. In all cases, the two methods were in very close agreement, and the Coulter Counter count was always within the ± 20 per cent error expected of the microscope count. The time required for a Coulter Counter count was about four minutes as compared to about 45 minutes for a microscope count, a ratio of 11.25 to 1.

Although the random number system, (see Appendix E), was used in the microscope count, variations in the number of particles on different area units were observed. When the entire filter was scanned, it was apparent that certain sections were highly contaminated and other sections had a very low contamination level. It seems possible that even when using the random number system, considerable error could be introduced by counting more grids in either the high or low contamination regions. In the case of the Coulter Counter, the fluid is being constantly stirred; and the particles are evenly distributed. This fact is evident since the Coulter Counter will repeat a count within the Poisson distribution.

The tests described in Chapters IV and V can be adapted to any automatic particle counter, although the principle of the instrument may be different than the principle of the Coulter Counter.

Based on the results of this study, the following conclusions can be drawn:

1. The microscope method of evaluating the particle size and distribution should be restricted to: (a) spot checks of samples to determine the particle ranges, and (b) checking samples for largest particles.
2. Automatic particle counters meeting the requirements of this study should be established as the criteria for conducting particle size and distribution studies for the

following reasons:

A. Accuracy. The accuracy of the automatic particle counter (± 2/3 the square root of the average count) exceeds that of the microscope method (± 20 per cent).

B. Speed. The automatic particle counter has a time advantage of 11 to 1 over the microscope method.

C. Operator Decision. The automatic particle counter, regardless of principle, bases its size distribution on volume or area and reads out the count automatically, thus eliminating operator error. Whereas, when using the microscope method, the operator must decide the size of the particle by its longest dimension.

D. Sample Distribution. The sample distribution, in the case of the microscope method, is the consistency by which the particles fall on the grids of the counting filter. In many cases, the particles appear in groups instead of being evenly distributed. In the case of the automatic particle counter, the particles are more evenly distributed since the sample is being stirred constantly. Therefore, the sample counted by the automatic particle counter should be a better representation than that of the microscope method.

E. Training and Experience. Very little training or experience is necessary to operate an automatic particle counter, while a well-trained operator is necessary for the microscope method.

F. Attitude of Operator. Due to the tedium and strain related to the microscope method, operator fatigue, leading to error is possible when a large number of samples must be evaluated. The automatic particle counter, due to its simple operation, relieves this problem.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE STUDY

The main problem involved with the operation of the Coulter Counter is that of finding suitable electrolytes. The one used for MIL-H-5606 hydraulic fluid required a three-to-one mixture of electrolyte to hydraulic fluid. If chemicals could be found that would allow a larger percent of hydraulic fluid to be used per sample, the evaluation of a sample would be more accurate because the chance of introducing contamination in the electrolyte would be reduced since less electrolyte would be required.

There is a possibility that the Coulter Counter could be correlated with a gravimetric analysis of fluid contamination. This could be attempted by using the Coulter Counter to determine the number and volume of the particles, and by using contamination of a known density, the weight of the particle could be calculated and checked against the standard gravimetric analysis.

Research could also be conducted to evaluate the use of the Coulter Counter to study dispersal or agglomeration tendencies of samples by comparing changes of size distribution of particles with time.

APPENDIX A

ELECTROLYTE

Mixing an electrolyte is a very important part of setting up a test on a Coulter Counter. Although the chemicals can vary, depending on the fluid to be analyzed, the procedure for mixing the electrolyte will be the same.

The following procedure for mixing an electrolyte counting solution was suggested by Michaelson (4) for use with MIL-H-5606 hydraulic fluid. This electrolyte is also satisfactory for other hydraulic fluids. Chemicals for use with other hydraulic fluids are suggested by Coulter (11).

Procedure for Mixing Electrolyte

A. Wash all glassware with a laboratory detergent, rinse with clean water, then rinse with isopropyl alcohol which has been filtered three times through a 0.45 micron Millipore filter or its equivalent. Glassware should be covered with Saran Wrap rinsed with triple-filtered isopropyl alcohol or petroleum ether. Do not use stoppers unless covered with rinsed Saran Wrap.

B. Mix four per cent ammonium thiocyanate by weight with isopropyl alcohol. This mixture must be heated to achieve solution, and requires a boiling flask with a water-cooled condenser apparatus to keep the alcohol vapors from escaping. The mixture should be boiled for 15 minutes, then allowed to cool. After cooling, the mixture should be filtered three times through a 0.45 micron Millipore filter or its equivalent.

C. Filter a sufficient volume of 1-2 dichloroethane three times through a 0.45 micron Millipore filter or its equivalent.

D. Just prior to evaluating a sample of hydraulic fluid, mix one part hydraulic fluid to 1.5 parts (B) and 1.5 parts (C). CAUTION, do not mix (B) and (C) until just prior to the evaluation. If these chemicals are mixed for any length of time, the ammonium thiocyanate will precipitate and form crystals in the counting solution. The 1-2 dichloroethane makes the counting solution miscible with the fluid being evaluated. In cases of fluids other than MIL-H-5606, the percentage of 1-2 dichloroethane in the counting solution may have to be increased to achieve a homogeneous solution. Failure to achieve complete miscibility will result in suspended globules of fluid being counted as particles, thus giving an erroneous count. Care should be taken throughout the mixing of the electrolyte to keep glassware and utensils as clean as possible. All glassware used in the mixing and storage of the electrolyte should be covered with rinsed saran wrap, or its equivalent, and stored in a clean atmosphere. A clean room is recommended for mixing and storing of the electrolyte, as well as evaluating the hydraulic fluid samples. After the components, (B) and (C), have been mixed and filtered, a background check of the electrolyte alone, one part (B) and one part (C), should be made. The count at five microns should not exceed 100 for two cubic centimeters of electrolyte. If the count exceeds this maximum, further filtrations of one or both of the components are necessary. Periodic checks, the length of which depends on environmental conditions, should be made to insure a clean counting solution.

APPENDIX B

PRINCIPLE AND OPERATION OF THE COULTER COUNTER

The Coulter Counter is an electrical sensing zone counter. The counter sizes and counts particles in an electrical conductive liquid. The electrical sensing zone is a small aperture with an electrode on either side. (See Figure 5.) The aperture acts as a resistance, the value of which depends on the resistivity of the electrolyte. Particles are forced to pass through the aperture, causing a change in the effective volume of the aperture, resulting in a change in the resistance. The derivation of the equation for the change in resistance is given in Appendix C.

The change in resistance is approximately proportional to the particle volume, as shown in Appendix C, and this change in resistance causes a voltage pulse which is also approximately proportional to the particle volume. For very small particles, those with cross-sectional area 10 per cent or less than the cross-sectional area of the aperture, the change in resistance is directly proportional. In the case of larger particles, there is an increase in current density; and electrical heating occurs in the rest of the aperture. This heating momentarily lowers the resistivity of the electrolyte and the response due to particle passage. This error is compensated for by the last term in the denominator of Equation C-20 as shown in Appendix C.

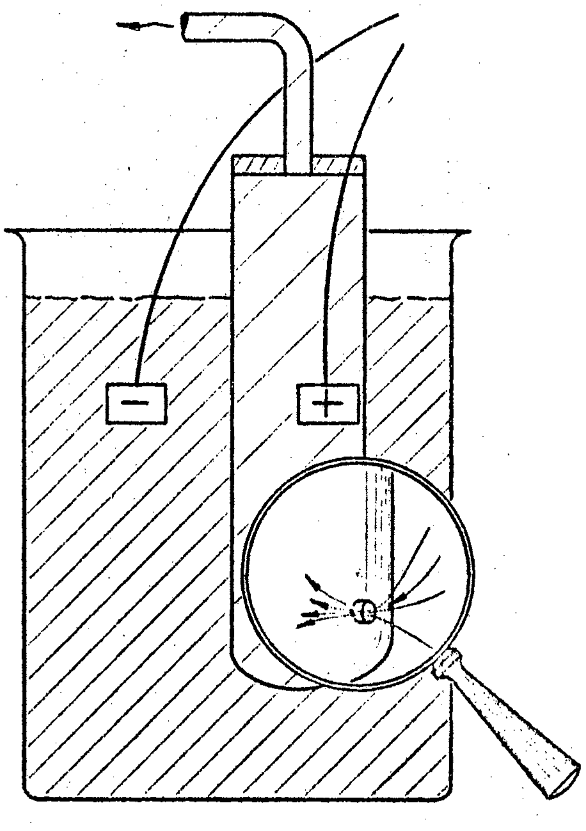

Fig. 5. Electrical Sensing Zone.

This term is negligible for the very small particles; thus the aperture can be used for particles up to a d/D ratio of 0.5, where "d" is the diameter of a sphere with equal volume of the particle passing through the aperture, and "D" is the diameter of the aperture. This limit is placed on the aperture because aperture jamming occurs if particles above this ratio are present. The voltage pulse caused by a particle passing through the aperture is amplified, scaled, and counted.

The operation of the Coulter Counter, referring to Figure 6, is as follows:

A. Select the proper aperture tube for the range of contamination to be counted. The diameter of particles to be counted should fall between one per cent and fifty per cent of the aperture diameter. For best results, particle diameters should be between two per cent and thirty per cent of an aperture diameter. For example, with a 140-micron aperture, particles can be counted in various size ranges between three and forty microns.

It is difficult to distinguish between voltage pulses due to particles and pulses due to noise within the machine when particle size is less than the minimum for a given aperture. If too many particles above the maximum for a given aperture exist, jamming of the aperture will occur. When particles must be counted in wide ranges, it becomes necessary to use two or more aperture sizes, depending on the maximum and minimum limits on the ranges.

B. With the sample in place and the counter set to count the particle size desired, open the stopcock to the external

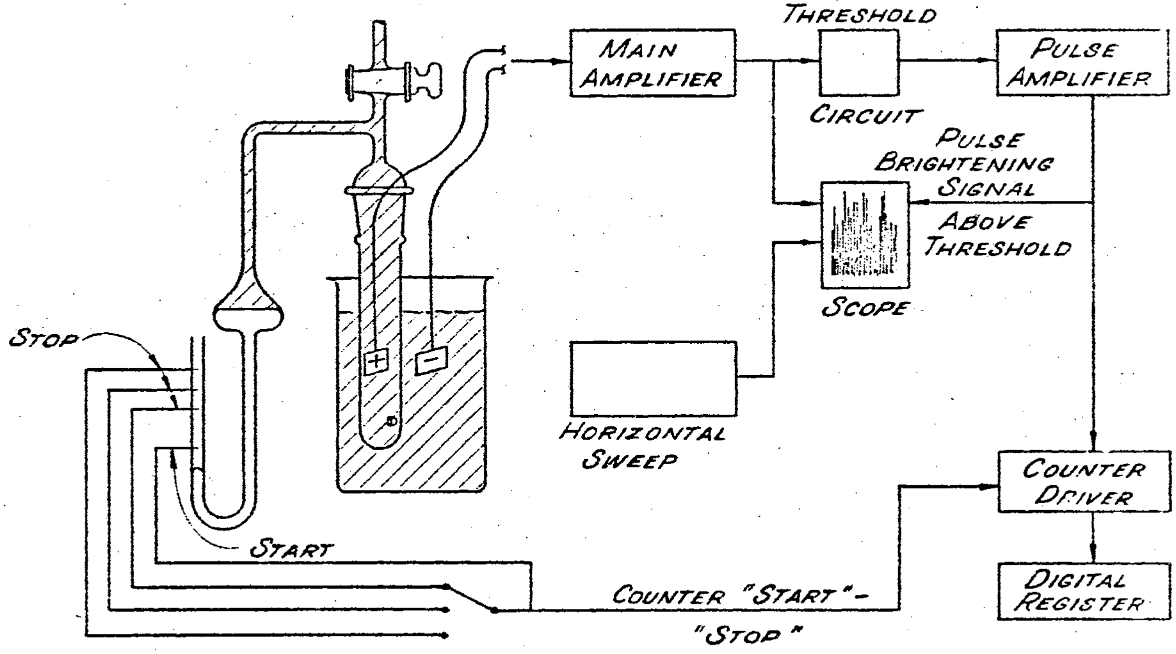

Fig. 6. Schematic of Coulter Counter.

vacuum. This unbalances the mercury column and pulls it down below the counter start probe.

C. Set the three-way switch for the counter stop probe desired. This switch determines the volume of fluid on which the count is based. The three fluid volumes available with the manometer are 0.05, 0.5, and 2.0 cubic centimeters. The selection of the value depends on the contamination level of the fluid. An initial count should be made at one of the manometer positions to determine the degree of contamination of the sample. The stop position chosen for tests should allow a count of at least 100 particles per count.

If the sample is so clean that the 2.0 cubic centimeter stop probe does not allow a count of 100 particles, the alternate timer should be used to start and stop the counter. The timer should be calibrated according to the Timer Control Box Procedure Sheet supplied with the timer.

D. With the stopcock open and the mercury column drawn below the start probe, actuate the counter reset switch and close the stopcock. The system is then isolated from the external vacuum, and the siphoning action of the rebalancing mercury column causes sample flow through the aperture.

As the mercury column advances, it initiates counting as it contacts the start probe and ends counting as it contacts the stop probe. The number of particles will be recorded by the digital register, and this count will be based on the volume described in part C.

E. Data reduction is explained by referring to a sample laboratory data sheet, Table IV. The first two columns, t' and I, are the threshold and aperture current values, respectively. The functions of t' and I are explained in Section F on calibration.

For each particle-diameter setting, the counter counts all particles for that size and above. To get the count in a certain size range, subtract the actual count, n, of the smaller size from the larger size. For example, the number of particles in the range of 30 to 40 microns would be 197-100, or 97 particles. See Table IV.

Column 4, raw counts, is made up of three successive runs. These counts should follow a Poisson distribution, that is, the standard deviation will equal the square root of the average. A method for finding standard deviation is given by Snedecor (12). Finding the standard deviation is a rather complicated procedure, and would require an appreciable amount of time. A close approximation to the standard deviation is 2/3 the square root of the average count, and is the usual method for checking the repeatability of the counter. By making three raw counts, the counter can be checked for each setting to insure that the count is within the Poisson distribution. If the aperture should become momentarily jammed, the erroneous raw count can be located, and a new raw count can be made to correct the error.

Column five is the average of the three raw counts.

Table IV. Example Laboratory Data Sheet.

PARTICLE CONTAMINATION LABORATORY
OKLAHOMA STATE UNIVERSITY
Coulter Counter Sample Data

Sample ELECTROLYTE Source O. S. U. Electrolyte STANDARD "1" Date 3/15/62

Aperture Diameter 140 μ Manometer Volume 2000 μl Coinc. Factor p = 1.71 Calib. Factor k = 8.47 Dispersant NONE Oper MWZ

Aperture Resistance 100,000 Ω Gain Index 2 NOTES EXAMPLE

Threshold Dial Expansion Factors	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}
	1.00	.503	.255	.1307	.0687	.0378	.02259	.01552	.01306	.

Threshold Reading (t')	Aperture Current Selector Reading (I)	Particle Contaminant Diameter (d)	RAW COUNTS n'			Average Count $\bar{n}'$	Coincidence Correction $n''=p\left(\frac{\bar{n}'}{1000}\right)^2$	Full Count $\bar{n}''=\bar{n}'+n''$	Actual Count $n=\bar{n}''-$ background	NOTES
			Run 1	Run 2	Run 3					
104	1	40	102	108	92	101	0	101	100	
44	1	30	210	194	200	201	0	201	197	
13	1	20	563	540	552	552	0	552	540	
23.6	5	10	3410	3396	3418	3408	20	3428	3404	

Column six is the coincidence correction. The primary effect of coincidence is the loss of count when the particle concentration is high, due to more than one particle passing through the aperture at a time. The coincidence factor "P" is obtained by the following formula:

$$P = 2.5\ (D/100)^3\ (500/V)$$

in which D = the aperture diameter in microns,

V = the metering volume in microliters.

The factor 2.5 was obtained experimentally by Coulter using a 100-micron aperture and a 500-microliter metering volume, with successive dilutions of counting, on a monosized system. The count loss, n", is proportional to the square of the observed count and is an approximation of the actual Poisson function. The count loss, n", is obtained by the equation:

$$n'' = P(\bar{n}'/\ 1000)^2$$

Column seven is the full count, $\bar{n}''$, that is, the average count, $\bar{n}'$, plus the coincidence correction, n".

Column eight is the actual count, n, of the sample being evaluated. It is obtained by subtracting the background count in the electrolyte from the full count, $\bar{n}''$ in column seven.

F. The Coulter Counter is calibrated by using monosized particles. The most common particle used is ragweed pollen.

The first step in the calibration process is to add a small amount of pollen to the filtered electrolyte solution. Adjust the gain index and aperture current so that the single height pulses on the oscilloscope screen are in the 10 to 30 range on the threshold dial. Estimate the threshold dial setting that would allow the pulse tops to slightly break above the threshold shadow line. Set the threshold at about 1/2 the estimated position and take several full counts (n_h) on the monosized system. Next, set the threshold dial at 1 1/2 the estimated position and record the oversize count n_o. The actual count, n_a, is obtained by the equation:

$$n_a = n_o + \left(\frac{n_h - n_o}{2}\right)$$

By successive trials, the threshold setting which will give the actual count, n_a, can be found. This is a highly sensitive way to measure the count, so one should not try to get the exact n_a with trial threshold settings. A value with a deviation of 2/3 the square root of the average count is acceptable. Since monosized particles were used, the values of the particle diameter, d, and the threshold reading, t', in Table I, are known.

The next step is to find the aperture resistance with a constant voltage source of 300 volts d.c. The resistance of the aperture can be found by measuring the voltage across the aperture. From the wiring diagram, the resistance in series with the aperture, for an aperture current setting of eight, is 215,000 ohms. The resistance, R, across the aperture is given by:

$$R = \frac{215{,}000\,(v)}{300-v}$$

where v is the voltage across the aperture.

Once the resistance across the aperture is known, the threshold dial expansion factor, F_n, that appears in the heading of the data sheet, Table IV, can be read directly from the manual supplied with the counter. The subscript, n, corresponds to the aperture current selector reading.

The calibration factor, k, is obtained by:

$$k = d/\sqrt[3]{t}$$

where t is given by:

$$t = t'F_n$$

Since in calibration, d is fixed, and the value of t' is found by the method above, the value of t can be calculated and the calibration constant k can be obtained. With the value of k known, the value of t can be calculated, corresponding to any diameter, d, within the limits of the aperture. After t is found, the correct F_n should be chosen to allow t' to fall within the limits of the threshold reading, t', and the aperture current reading, I, corresponding to the subscript n in the threshold dial expansion, F_n, are sufficient to set the counter for a given particle diameter. These values can be calculated for all diameters corresponding to the size ranges needed.

Once the counter has been calibrated, periodic checks should be made to insure that the counter has not drifted from the original calibration. Weekly tests are usually advisable. Any time a new volume of electrolyte is mixed, a new calibration should be made.

Care should be taken at all times to keep the counter as clean as possible. After finishing a series of tests, the aperture tube and sample beaker should be filled with triple-filtered isopropyl alcohol.

When the counter is in active use, the metering manometer section should be cleaned every two months, or when the mercury in the upper reservoir becomes excessively contaminated with oxides and dirt. The oxides will cause current flow from the inner electrode to the mercury, thus weakening the signal, and consequently, giving an erroneous count. Also, as the mercury flows past the start-stop probes, the probes will become coated with contamination and fail to function properly.

The cleaning procedure outlined in Coulter (11) should be followed in cleaning the metering manometer. Special attention should be given to Appendix D before attempting to clean the manometer meter section.

APPENDIX C

DERIVATION OF THE CHANGE IN RESISTANCE OF THE APERTURE DUE TO A PARTICLE PASSING THROUGH THE ELECTRICAL SENSING ZONE

The Coulter Counter operates on the electrical sensing zone principle as described in Appendix A. Since the particle size recorded is a function of the change in resistance of the aperture due to a particle passing through the aperture, the derivation of the equation for change in resistance is very important in understanding the principle of the Coulter Counter. The following assumptions are made in the derivation of the equation by Coulter (11):

1. The aperture contents form a cylindrical resistor in which current density is uniform.
2. Multiplying the aperture length by an appropriate factor covers the electrically effective zones outside the aperture.
3. The passages of individual particles occur at random and are evenly distributed through the aperture cross section.
4. The electrically effective volume of a particle in the aperture may be expressed as a cylinder having the same resistivity as the particle.

The particle which is expressed as a cylinder is (a.d) in length and (b.d) in diameter, where (d) is the diameter of a sphere which would have the same volume as the cylinder. Thus d would be the particle dimension as measured electrically and would not necessarily be the same as a physical dimension of the particle.

Consider the aperture as having a disk segment containing a given particle, having a diameter, D, equal to the diameter of the aperture and a thickness (a.d) equal to the length of the cylindrical particle as in Figure 7.

Let ρ_o be the resistivity of the electrolytic solution and ρ be the resistivity of the cylindrical particle.

The disk segment resistance without the particle is:

$$R_o = \frac{\rho_o a \cdot d}{\left[\pi/4\right] D^2} \qquad \text{C-1}$$

and the segment resistance, of the electrolytic solution alone, with the particle within the segment is:

$$R'_o = \frac{\rho_o a \cdot d}{\pi/4 \left[D^2 - (b \cdot d)^2\right]} \qquad \text{C-2}$$

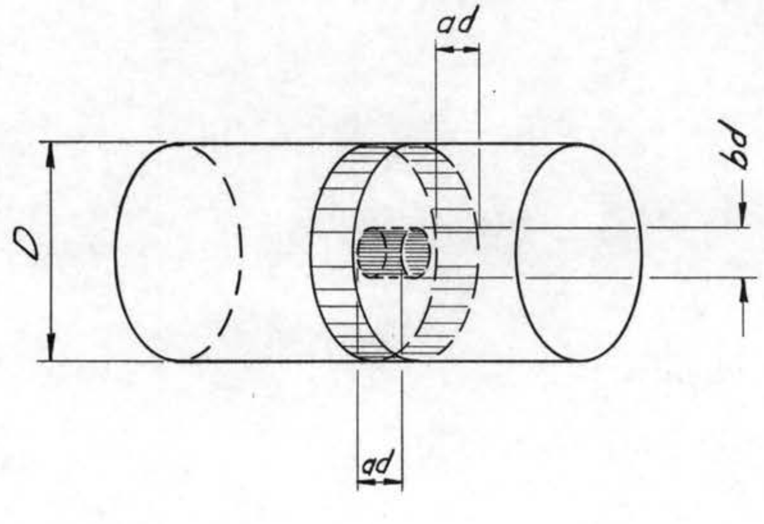

Fig. 7 Aperture

The resistance of the particle within the segment is:

$$R_p = \frac{\rho\, a\cdot d}{\left[\pi/4\right](d\cdot b)^2} \qquad \text{C-3}$$

The total resistance with both the electrolyte and the particle is that of the two resistors (R_o' and R_p) in parallel, or:

$$R = \cfrac{1}{\cfrac{1}{\cfrac{\rho_o a\cdot d}{\left[\pi/4\right]\left[D^2-(b\cdot d)^2\right]}} + \cfrac{1}{\cfrac{\rho\, a\cdot d}{\left[\pi/4\right](b\cdot d)^2}}} \qquad \text{C-4}$$

Therefore, the change in resistance caused by a particle is:

$$\Delta R = R - R_o = \cfrac{1}{\cfrac{\left[\pi/4\right](D^2-\beta\cdot d)^2}{\rho_o\, ad} + \cfrac{\left[\pi/4\right](b\cdot d)^2}{\rho\, a\cdot d}} - \frac{\rho_o\, a\cdot d}{\left[\pi/4\right]D^2} \qquad \text{C-5}$$

By multiplying the first term in the denominator of the first fraction by ρ/ρ and the second term ρ_o/ρ_o and simplifying, we have:

$$\Delta R = \frac{\rho_o\, a\cdot d\, \rho}{\left[\pi/4\right]D^2\rho - \left[\pi/4\right](b\cdot d)^2\rho + \left[\pi/4\right](b\cdot d)^2\rho_o} - \frac{\rho_o\, a\cdot d}{\left[\pi/4\right]D^2} \qquad \text{C-6}$$

By multiplying and dividing each term in the denominator by D to the power which will allow each term to contain D^4, and factoring, we have

$$\Delta R = \frac{\rho_o\, a}{\left[\pi/4\right]D}\left(\frac{d}{\frac{1}{D^2} - \frac{(bd)^2}{D^4} + \frac{(bd)^2\rho_o}{D^4}} - d\, D^2\right) \qquad \text{C-7}$$

Multiplying and dividing the third term in the denominator of the fraction in parenthesis by ρ and factoring, we secure:

$$\Delta R = \frac{\rho_o a}{\left[\pi/4\right] D} \left(\frac{d}{\frac{1}{D^2} - (bd)^2/D^4 + (bd)^2 \ \rho_o/D^4\rho} - dD^2 \right) \qquad \text{C-8}$$

Multiplying and dividing the first term in the denominator of the fraction in parenthesis by (b^2), factoring and simplifying:

$$\Delta R = \frac{\rho_o \ a}{\left[\pi/4\right] D^4} \left[\frac{d}{b^2/D^2 \left[1/b^2 + d^2/D^2 \left(\frac{\rho_o}{\rho} - 1 \right) \right]} \right] - dD^2 \qquad \text{C-9}$$

By finding a common denominator and multiplying and dividing the first term in the numerator of the fraction in brackets by (b^2/D^2), factoring and cancelling like terms:

$$\Delta R = \frac{\rho_o \ a}{\left[\pi/4\right] D^4} \left[\frac{dD^2 \left\{ 1/b^2 - \left[1/b^2 + \frac{d^2}{D^2} \left(\frac{\rho_o}{\rho} - 1 \right) \right] \right\}}{1/b^2 + d^2/D^2 \left(\rho_o/\rho - 1 \right)} \right] \qquad \text{C-10}$$

Removing brackets in the numerator and rearranging the terms in parenthesis:

$$\Delta R = \frac{\rho_o \ a}{\left[\pi/4\right] D^4} \left[\frac{d^3 \left(1 - \rho_o/\rho \right)}{1/b^2 - d^2/D^2 \left(1 - \rho_o/\rho \right)} \right] \qquad \text{C-11}$$

For an equivalent sphere and cylinder in terms of volume:

$$\frac{\pi}{6} d = \left[\pi/4\right] (bd)^2 \ ad \qquad \text{C-12}$$

Cancelling like terms and arranging terms:

$$\frac{1}{b^2} = 1.5 \ a \qquad \text{C-13}$$

From Equations C-11 and C-13:

$$\Delta R = \frac{4\rho_o}{\pi D^4}\left[\frac{ad^3\left(1-\rho_o/\rho\right)}{1.5a - d^2/D^2\left(1-\rho_o/\rho\right)}\right] \qquad \text{C-14}$$

Multiplying and dividing the first term in the denominator of the bracketed fraction by $(1-\rho_o/\rho)$, factoring and cancelling like terms:

$$\Delta R = \frac{4\rho_o}{\pi D^4}\left(\frac{ad^3}{\frac{1.5a}{1-\rho_o/\rho} - \frac{d^2}{D^2}}\right) \qquad \text{C-15}$$

Multiplying the last term in the denominator of the fraction in parenthesis by (a/a), factoring and cancelling yields:

$$\Delta R = \frac{4\rho_o}{\pi D^4}\left(\frac{d^3}{\frac{1.5}{1-\rho_o/\rho} - \frac{d^2}{aD^2}}\right) \qquad \text{C-16}$$

Equation C-16 can be written in the same form as it appears in Coulter (11) by using the following definitions:

A = Aperture area normal to axis

v = particle volume

α = particle area normal to aperture axis

x = particle dimension ratio,

$$= \frac{\text{length parallel to aperture axis}}{\text{diameter of an equivalent volume sphere.}}$$

Multiplying and dividing the last term in the denominator of the fraction in parenthesis of Equation C-16 by 1.5, factoring:

$$\Delta R = \frac{4\rho_o\ d^3}{\pi D^4\ 1.5}\left[\frac{1}{\frac{1}{1-\rho_o/\rho} - \frac{d^2}{1.5a\ D^2}}\right] \qquad \text{C-17}$$

Multiplying and dividing by (a), and substituting Equation C-13:

$$\Delta R = \frac{4\rho_o\ b^2d^3a}{\pi\ D^4}\left(\frac{1}{\frac{1}{1-\rho_o/\rho} - \frac{d^2b^2}{D^2}}\right) \qquad \text{C-18}$$

Multiplying and dividing the equation and the last term in the denominator of the fraction in parenthesis by $(\pi/4)$, and simplifying:

$$\Delta R = \frac{\rho_o v}{A^2}\left(\frac{1}{\frac{1}{1-\rho_o/\rho} - \frac{a}{A}}\right) \qquad \text{C-19}$$

For a right-circular cylinder, the x term which appears in Coulter (11) is equal to one. This term is a shape factor, which compensates for particles other than cylinders, or for a cylindrical particle passing through the aperture with its axis not parallel to the ends of the aperture. With this term in the equation:

$$\Delta R = \frac{\rho_o v}{A^2}\left(\frac{1}{\frac{1}{1-\rho_o/\rho} - \frac{a}{xA}}\right) \qquad \text{C-20}$$

APPENDIX D

SAFETY PRECAUTIONS

When mixing and filtering electrolytes and cleaning fluids, special precautions should be taken to insure proper ventilation. When using a vacuum-type filter apparatus, the exhaust gases from the pump should be forced out of the room. These gases are both a fire hazard and a danger to the health of personnel in the laboratory.

Open flames should be avoided at all times when working with the various chemicals used in conjunction with the Coulter Counter.

Due to the toxicity limits, flammability, and other harmful effects of various chemicals, extreme caution should be taken when using the following: potassium thiocyanate, ammonium thiocyanate, ethylene dichloride, methyl alcohol, butyl alcohol, isopropyl alcohol, and acetone and nitric acid.

Safety precautions, when using nitric acid for cleaning the metering section of the Coulter Counter, cannot be over-emphasized. Always make sure the aperture tube is connected to the counter with rubber bands, and be extremely careful to avoid contact between the nitric acid and any part of the body. Protective eye goggles should be worn at all times when handling nitric acid.

In case of any burns resulting from contact with nitric acid, immediately wash burned area with soap and water.

APPENDIX E

PARTICLE COUNT BY THE MICROSCOPE METHOD

All particle counts using the microscope will be done according to ARP-598 (2), with the following refinements:

1. In paragraph 8.3, of ARP-598, <u>Microscope Analysis Procedure</u>, the size ranges specified will not be be followed. In many cases, the degree of contamination in the sample and the size of particles of interest to the parties involved are limiting factors on the particle-size spread in each range, and the number of ranges which will be counted.
2. In paragraphs 8.3.4 through 8.3.8, of ARP-598, instead of counting particles in randomly-chosen squares, a random numbering system will be used to determine which squares will be counted. (2). Although there are 100 grid squares on the effective filtering surface of the Millipore filter used in this analysis, only about 68 grid squares are whole. The other 32 grid squares are made up of fraction squares cut by the circular shape of the Millipore filter apparatus. Therefore, the random numbering system is based on 68 grid squares, and the numbers are taken from Snedecor (12).

In paragraph 8.3.4.3 the following grid squares will be counted:

54 15 61 05 41

28 17 40 34 06

62 47 24 55 75

16 46 42 68 20

In paragraph 8.3.4.4 the following grid squares will be counted:

54 15 61 05 41

28 17 40 34 06

In paragraph 8.3.4.5, the ten randomly-chosen unit areas will be fractions of the ten grid squares from above.

All grid squares will be numbered from left to right, starting with the first complete grid square in the upper left hand corner of the effective filtering area of the Millipore filter.

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