Comparison of Particle Counting Methods Used for High Purity Water Systems

by

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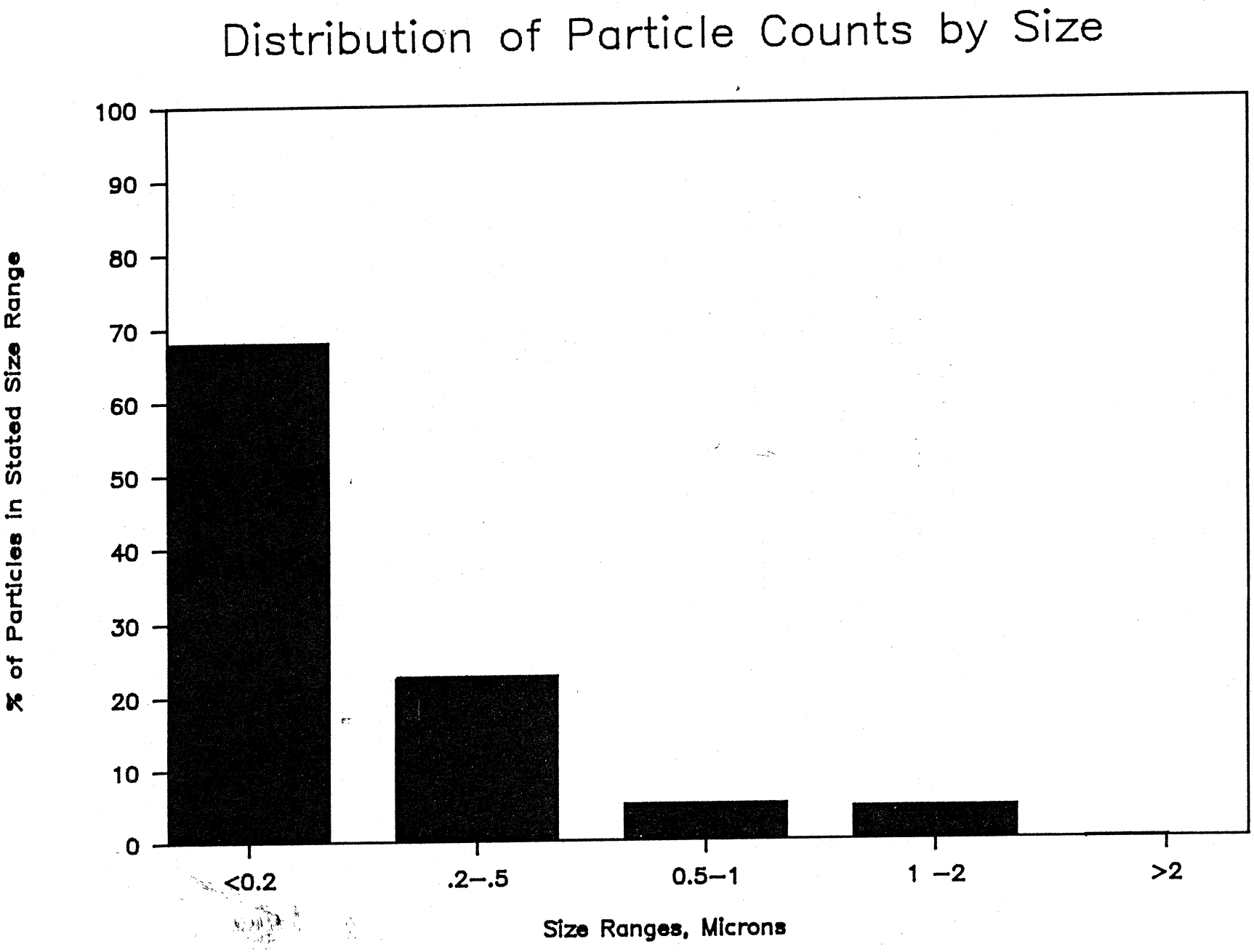
Introduction

The purpose of this paper is to describe our evaluations of particle counting methods. As a private laboratory working with the semiconductor and electronics industries, we perform particle count evaluations of high purity water. Usually, counts are taken routinely, weekly or bi-weekly, for the purpose of routine monitoring. Less frequently, when contaminated water is suspected as the cause of yield loss, testing is done under emergency conditions.

Until several years ago, particles were counted by a method adopted from ASTM, the optical particle counting method (OPCM), which we used routinely. Then, in 1984, the SEM Direct Count method (SEM) was introduced at this conference.[1] The advantages of this method over OPCM were obvious, and we began to use SEM for high purity water samples, after evaluating and practicing the method. Later still, Nomura Micro Science introduced a procedure[2] that had been modified from a drinking water method to apply to high purity water. This method uses light transmission microscopy, and has the advantage of using less expensive equipment than the SEM method. However, in our evaluation and practice of this light transmission microscopy method (LTM) we ran into a number of problems, the major ones being that we could

FIGURE 1


Distribution of Particle Counts by Size
% of Particles in Stated Size Range
100
90
80
70
60
50
40
30
20
10
0
<0.2
.2–.5
0.5–1
1 –2
>2
Size Ranges, Microns

not detect the particles as small as claimed by LTM, nor could we find as many particles as detected by SEM.

Figure 1 is a histogram of the size distribution of particles collected on a 0.1μ pore size Nuclepore filter. As seen, most particles, almost 70%, are in the <0.2μ size range. Also, although it is not obvious from this histogram, most of the particles in the 0.2 to 0.5μ range are closer to the 0.2μ size. Therefore, it is critical that these small particles be detected.

Our experience with all three of these methods, and attempts to practice methods which accurately state the particulate contamination level of processing water have led to this paper.

We would all like to have particle methods which are accurate and which correlate with each other and with on-line monitors. We are far from it, even when we speak of 0.2μ or larger particles. For smaller particles, less than 0.2μ and even less than 0.1μ only the SEM method can detect these particles.

In the following pages I will first describe all three methods--OPCM, SEM, LTM--then will discuss comparative studies of SEM and LTM.

Optical Particle Counting Method

This is a technique that has been used for years, but which is becoming obsolete because only relatively "large" particles are

detected. The method was adapted from an ASTM method, and at best can be considered a coarse technique suitable for detecting gross failures in RO/DI systems. The detection of particles as large or larger than 0.8μm is of little interest in today's environment where most RO/DI systems are filtering to 0.2μm or 0.1μm.

Water is collected in pre-cleaned bottles, from which it is vacuum filtered through a Millipore-MF 0.8μm, pore size 37mm diameter gridded counting filter. In our lab each filter is counted for background counts, it being our experience that there is enough variability in the filter lot to bias the results. Under view of an optical microscope at 100x magnification, ten fields are counted (10% of total).

The total counts are dependent on several factors, other than the actual quantity of particulate on the water:

- Good sampling technique
- Quality of microscope
- Training of operator

Sampling technique: The point is to sample the water stream, and not the sampling valves, etc. Good quality valves, well flushed out, are required. During flush out of up to seven minutes, a stream of water is forced through the valve, then the stream is slowed down to one to two liters per minute, a

rate which will not cause entrapment of airborne particles into the stream.

Quality of Microscope: A good, well maintained scope will make an enormous impact on the number of particles detected.

Training of Operator: The person responsible for counts must be trained in detail, and their results cross-checked with experienced operators. A well-trained operator can distinguish between organic and inorganic particles, and can attain reproducible counts.

At 100x magnification, the size of particles detected will be greater than about 1-2μ, depending on the contrast with filter material, and their shape. Organic particles are very difficult to find against the organic filter background. The 0.8μ pore size membrane collection filter has a mesh-type surface. Some particles less than 0.8μ will penetrate the filter and be "lost". Figure 2a shows the surface of this filter at 800x, upon which can be seen several particles; Figure 2b is a high magnification view of the same filter, showing an approximately 4μ diatom remnant on what can be seen as a fairly porous mesh.

Although this method is becoming obsolete, it does have utility for cases such as filter breakthrough or deterioration, or when checking for resin fines, etc. An experienced operator can detect subtle differences in the filter surface of a high quality water sample. The particle specifications published by SEMI in

1985, are based on the optical particle counting method. Since the particle counts are somewhat subjective, and are heavily dependent on operator training and microscope quality, it is imperative that each facility establish a baseline and monitor changes in counts.

SEM Direct Count Method

This is an excellent method for monitoring for particles and bacteria. It combines the advantages of on-line sampling with the advantage of counting at high magnification. Particles can be identified for elemental composition.

The sampling device (Figure 3) consists of a stainless steel filter holder fitted with a sampling filter, either an 0.1μ or 0.2μ pore size Nuclepore. At the inlet of the filter holder is one-half of a quick-connect. To prepare for sampling, the other half of the quick-connect is attached to the water line, which should have a pressure of at least 20 psig, preferably greater than 40 psig. After thorough sanitization and flushing of the sampling port the flow is set after which the valve is not adjusted. The sampling device is connected by coupling the quick-connect. The sample flow is measured by timing how long it takes to fill a 100-mL graduated cylinder. Typical sampling rate is 100 to 200 mL per minute. For final filter water the minimum sample volume is 200 liters, requiring one to two days of sampling. If the line pressure fluctuates, the flow must be moni-

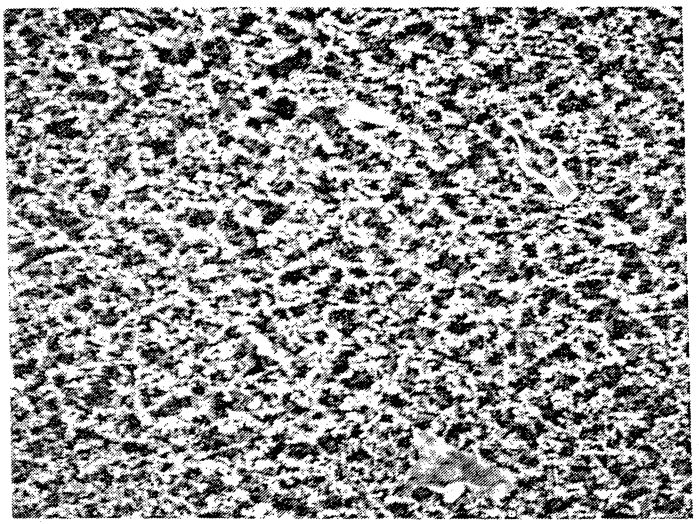


Figure 2a. Millipore MF 0.8u filter 800x

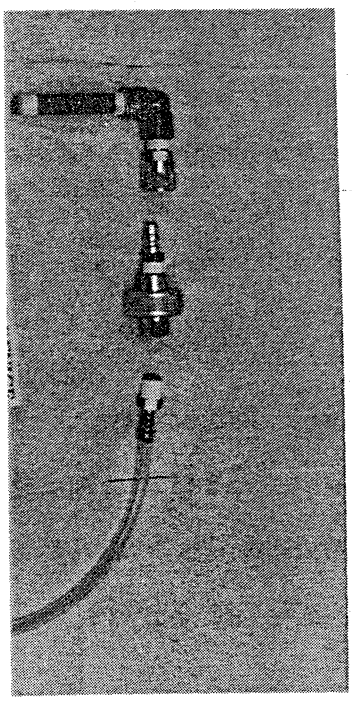


Figure 3. SEM Sampling Device

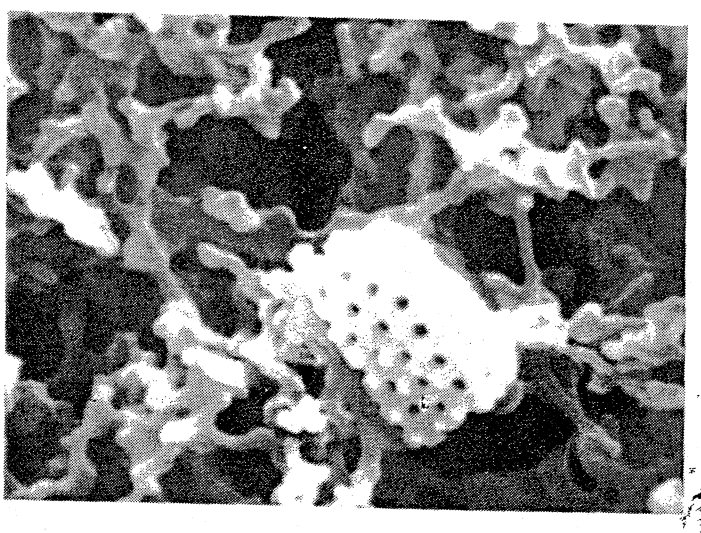


Figure 2b. Millipore MF 0.8u filter 8000x

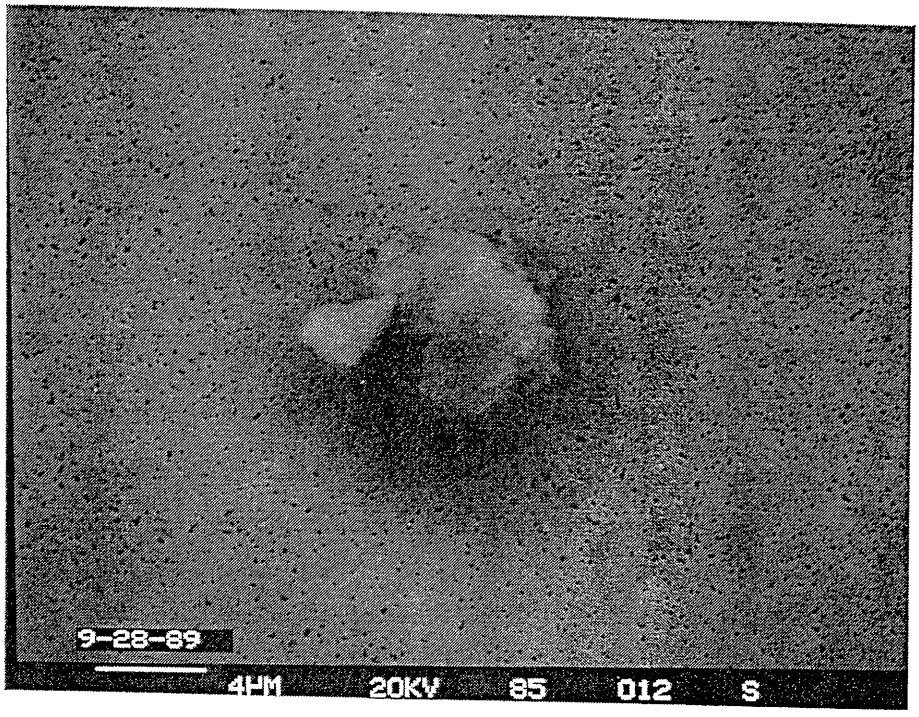
tored frequently during this time in order to accurately determine the sample volume.

After completion of sampling, the filter is removed from the sample holder in a clean area, and a thin coating of gold applied to make it conductive. An area of the filter is identified for counting, which is done at 8,000x magnification with a Scanning Electron Microscope.

At 8,000x there are approximately 1.4 million fields on the filter, of which up to 200 are counted. During counting the operator also sizes the particles, giving a size distribution as well as a total particle count.

Use of the EDX on the SEM can help identify particles. Figure 4 is a picture of a particle and its EDX elemental spectrum, showing it to be similar to silt-type material, probably from the supply water, except the detection of magnesium which was added to treat the water.

The Nuclepore filters trap particles much smaller than the stated pore size, making this method is useful for very fine particles and colloids. Extending the method in this way requires an experienced SEM operator who can spot subtle changes in the filter surface and/or clogging of filter pores, indicative of colloids.



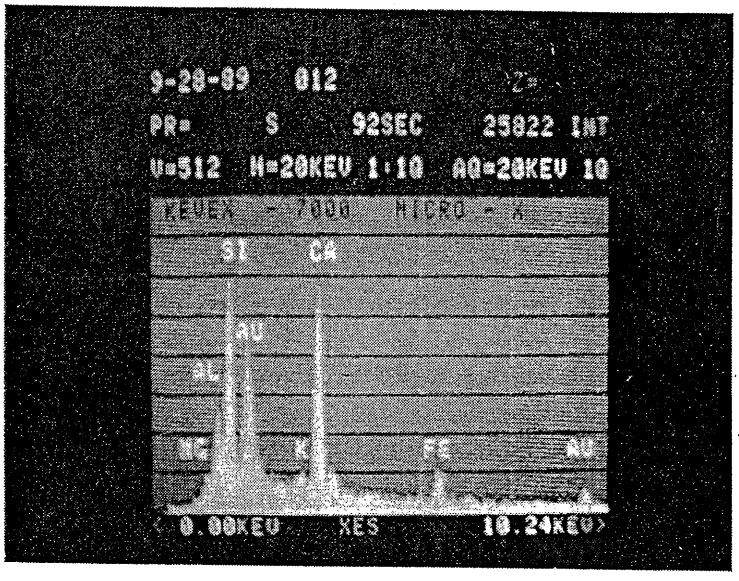


Figure 4. Particle trapped on filter, and EDX spectrum

Light Transmission Microscope

This method makes use of a 0.2μ pore size polycarbonate (Nuclepore) membrane filter, for particle collection. In most cases, sampling is done on-line. The filter is stained with Proca's staining solution, then dried, then observed by high resolution transparent light microscopy, with oil immersion optics. A drop of oil is placed on the filter, to increase the resolution. The Proca stain is made of:

Ziehl's Carbol Fuschin Solution	8 mL
Loffler's Methylene Blue Solution	10 mL
DI water	10 mL

This stain makes the bacteria visible under transparent light microscopy, dyes some particles, and increases the contrast. Sample collection is similar to that for SEM Direct Count Method. A minimum sample volume of 50 liters is necessary when monitoring high purity water. Particulates and bacteria can be identified.

At 1875x magnification there are 58,200 fields on the filter, and a minimum of 50 fields are counted. The actual number of fields counted will depend on the filter loading, with fewer fields counted when the filter is heavily loaded.

The advantage of this method is that the equipment expense is relatively low (less than $12,000 for the microscope) and the counting can be completed in less than an hour. Like the SEM method, it gives integrated rather than real-time results. The

detection of particles, bacteria, and fragments is more subjective than under the SEM.

Comparison of SEM and TLM Methods

Our initial work with the TLM method raised several questions. It did not seem probable that the claimed detection of 0.2μ particles was possible. We found many problems with the background, and we could not get results comparable to the SEM method. In this section, there is a description of work done to evaluate these problems.

A. Detectable Particle Sizes

By SEM, 0.1μ particles are readily detected at 8,000x; increasing the magnification permits detection of even smaller particles. For the LTM method, it is claimed that the lowest detectable particle size is 0.2μ. One of the problems we had with this method was to detect particles against the background, which appears as a multitude of dots. Although it is possible these dots are the 0.2μ pores of the filter, they seemed larger than 0.2μ; also, the Nuclepore filter has many multiplets (pores that are not separated), which can appear as particles. Figures 5, 6, 7, and 8 are pictures (1875x) of stained Nuclepore filter blanks of pore sizes, 0.1μ, 0.2μ, 0.4μ, and 10M, respectively. These pictures illustrate the background problem. As can be seen, each filter type has a background, which could or could not be the pores. On the 10μ filter the large 10μ pores are visible, but there are still a number of small dots. On the 0.4μ filter there

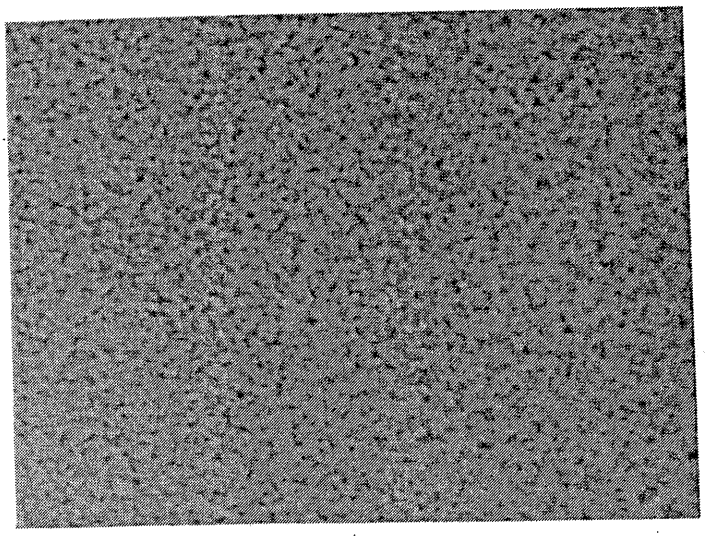


Figure 5. Stained 0.1u Nuclepore blank

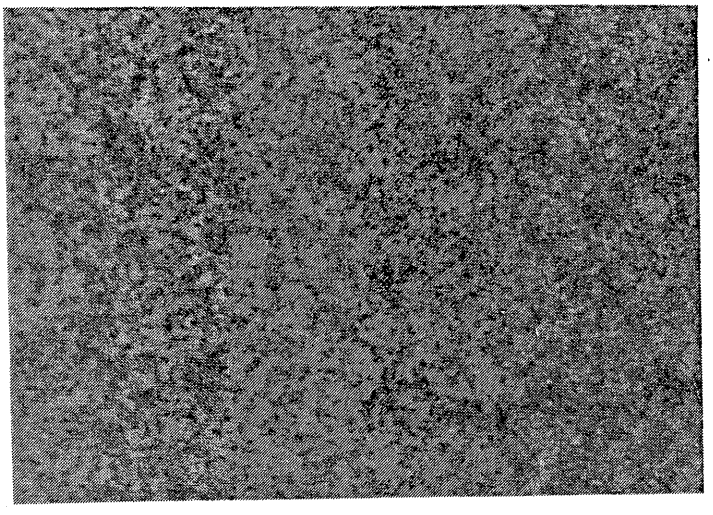


Figure 6. Stained 0.2u Nuclepore blank

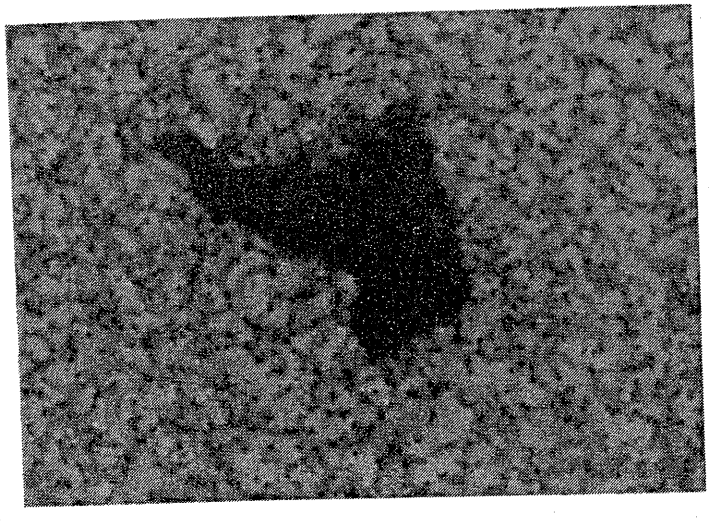


Figure 7. Stained 0.4u Nuclepore blank

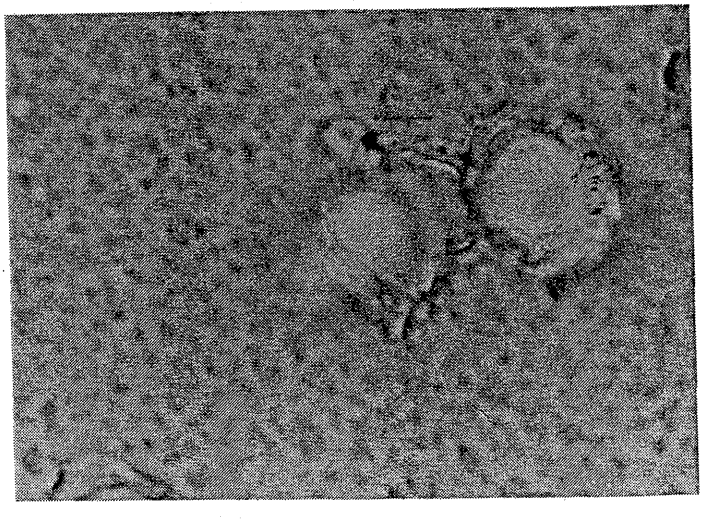


Figure 8. Stained 10u Nuclepore blank

is a large pink particle, and we found many of these of various sizes on the filter blanks and samples. One of the problems in counting was to determine if these particles were background or sample particle.

To understand the ability to detect small particles, we have to look at the resolution of the microscope as well as the background. The resolution of an optical microscope is diffraction limited. The resolving power (R) depends on the wavelength (λ), the quality of the optics and the refractive index (N) of the medium between the sample and the lens.

$$R = \frac{\lambda}{N \sin\theta}$$

For a λ of 426 nm (the shortest visible wavelength) and with the intervening space filled with oil (N greater for oil than for air), the maximum resolution of the light microscope approaches 0.2μ[3].

Figure 9 is an illustration of the concept of resolution, showing the two points on the right that are just resolved[4]. It is obvious that further mangification will not increase the resolution. This concept is important in understanding the particle sizes that can be detected.

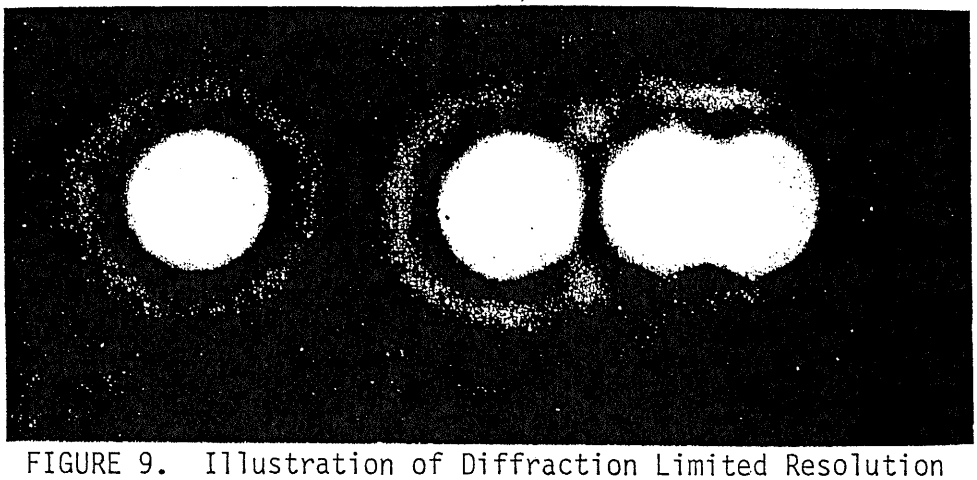


FIGURE 9. Illustration of Diffraction Limited Resolution

An experiment was conducted to find out what sizes were detectable on the stained sample filter. Fluorescent polystyrene latex spheres (covaspheres from Duke Scientific, Menlo Park, CA) were deposited on filters by vacuum filtration of a dilute suspension of the spheres. Some filters were also examined with an epifluorescent microscope. With this scope the filter surface is irradiated with UV light; fluorescent materials re-emit light in the visible region which can be viewed and photographed. No stain was needed because the spheres are fluorescent.

Bead sizes used were 0.5μ, 0.3μ, 0.11μ and 0.086μ. Under white light, these spheres are intense green.

The filters prepared for this experiment contain many more particles than are found in actual field samples, except when there is a catastrophic failure of a system component. Normally, there are a maximum of 3-5 particles per field.

Figure 10 shows the 0.5μ spheres under the EPI microscope, at 1500x. Figure 11 shows the SEM view at 1900x, and Figure 12 is the LTM filter at 1875x. The 0.5μ spheres are visible on all filters; the larger particles are agglomerates.

The views of 0.3μ spheres are in Figures 13, 14, and 15. For the 0.3μ spheres, it is unclear whether individual particles can be detected by LTM. There are many agglomerates which are detected.

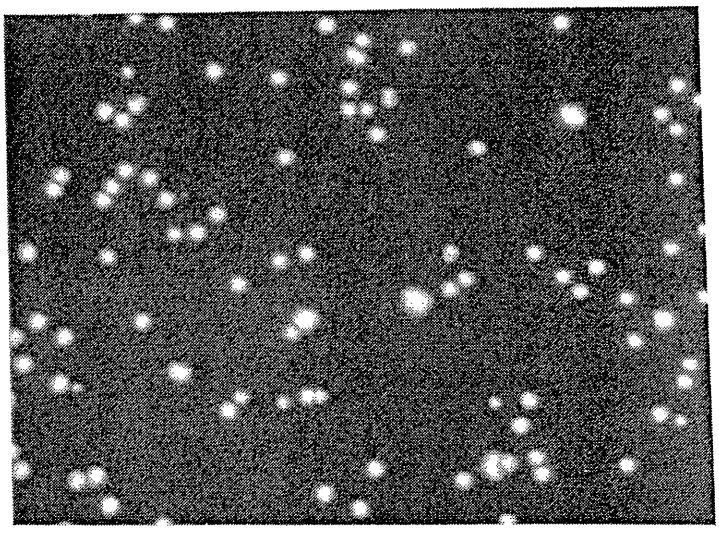


Figure 10. 0.5u Covaspheres, 1500x epifluorescent microscope

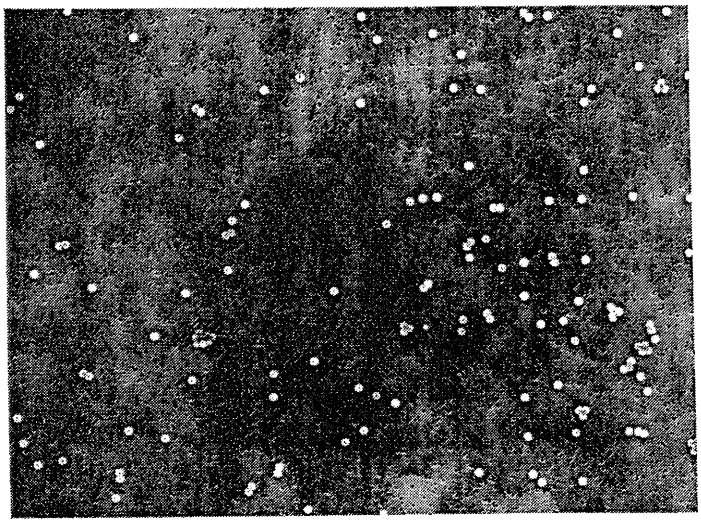


Figure 11. 0.5u Covaspheres, SEM, 1900x

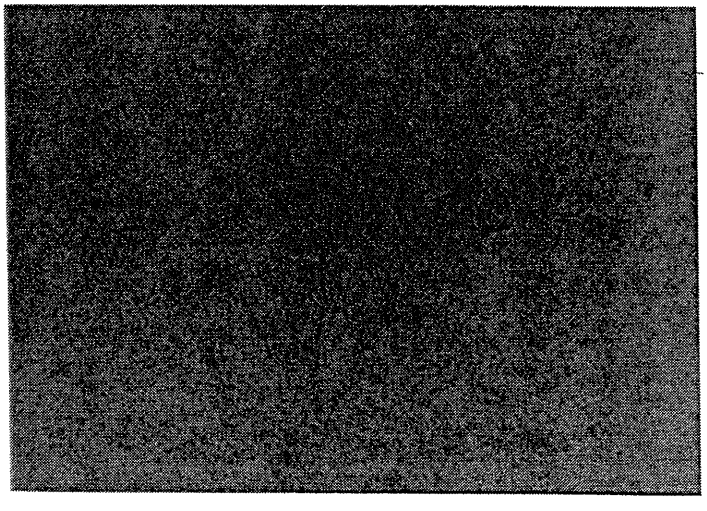


Figure 12. 0.5u Covaspheres, LTM, 1875x

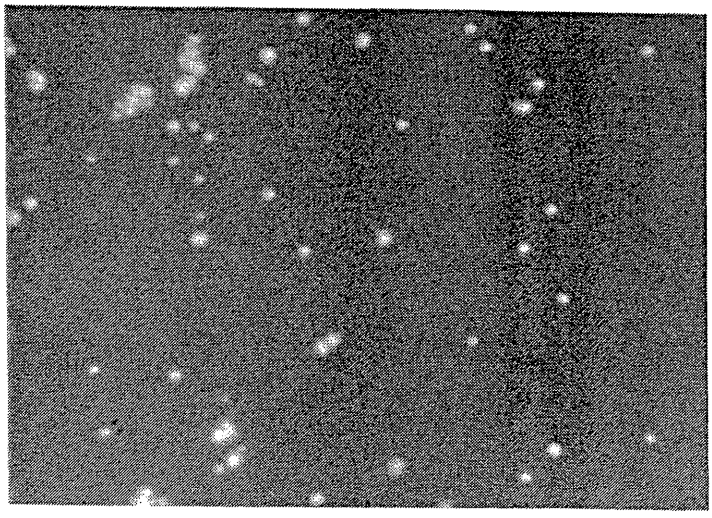


Figure 13. 0.3u Covaspheres, EPI, 1500x

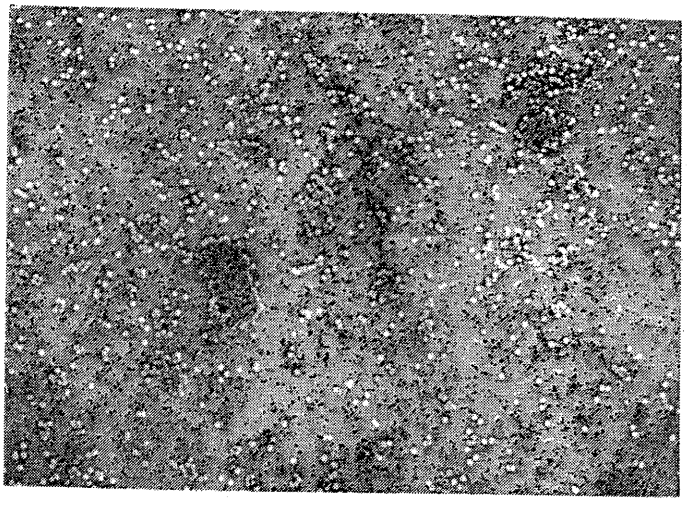


Figure 14. 0.3u Covaspheres, SEM, 1900x

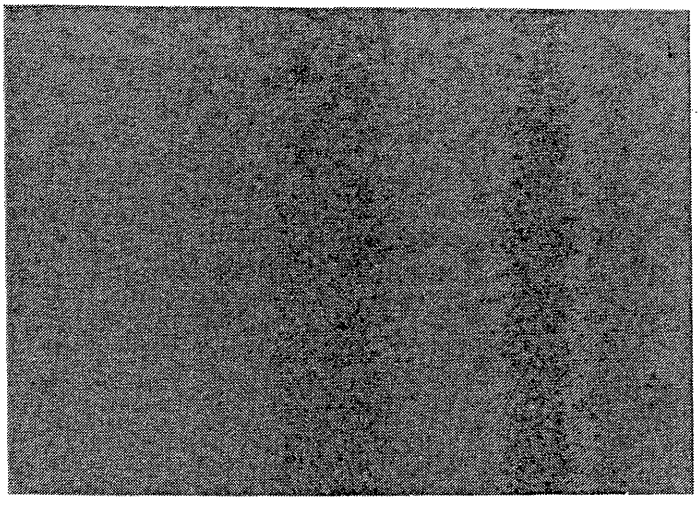


Figure 15. 0.3u Covaspheres, LTM, 1875x

However, it is highly unlikely that 0.3μ or smaller particles will be adequately counted by LTM.

The 0.11μ and 0.086μ spheres on SEM filters are shown in Figures 16, 17, and 18. In these experiments, a 0.1μ pore size Nuclepore was the sample filter. Figure 16 is a 1900x magnification of 0.11μ spheres. Figures 17 and 18 are at the normal counting magnification of 8000x with the 0.11μ and 0.086μ spheres respectively.

We could not locate any of the spheres on the LTM filter, even as agglomerates.

B. Detection of Bacteria and Bacteria Fragments

According to reports of the LTM method, bacteria fragments are detected and counted as particulate matter. Some of the pink stains are evaluated to be bacteria fragments. The SEM method does not differentiate bacteria fragments from other particles. Therefore, in order to learn more about this, an experiment was conducted to attempt to produce identifiable fragments.

A colony of bacteria, obtained on a sample from a high purity water system, was diluted in DI water, a portion of which was filtered through a 0.2μ pore-size Nuclepore filter. The remaining water was sonicated for ten minutes, then filtered through a similar filter. The purpose of sonication was an attempt to break apart or fragment the bacteria. The two filters were

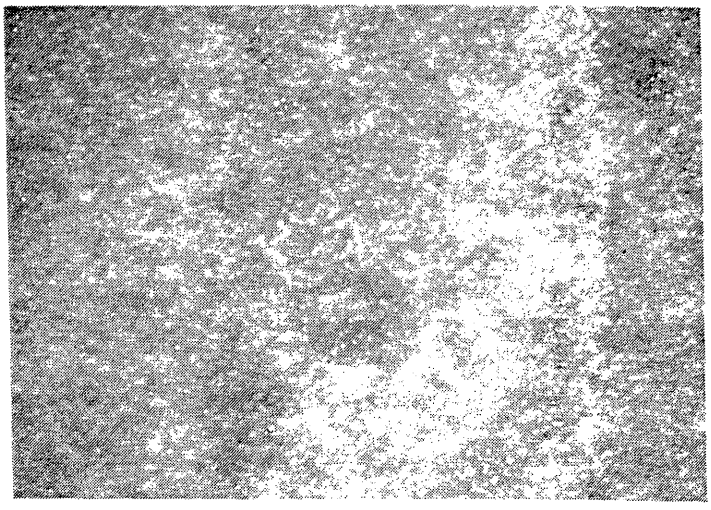


Figure 16. 0.11u Covaspheres, SEM, 1900x

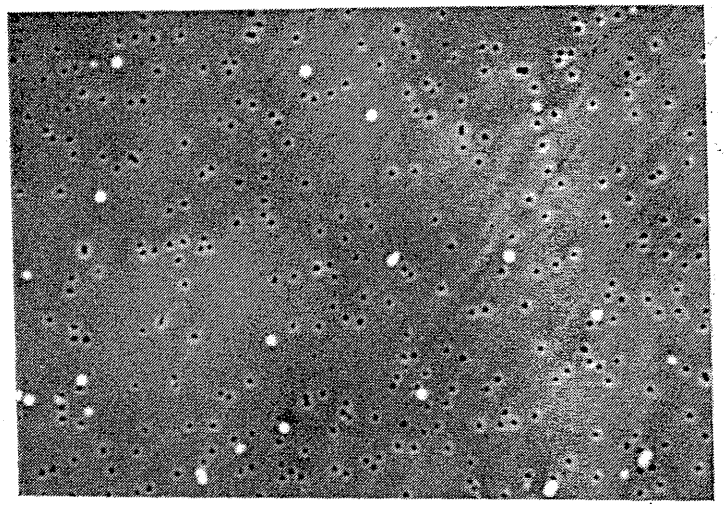


Figure 17. 0.11u Covaspheres, SEM, 8000x

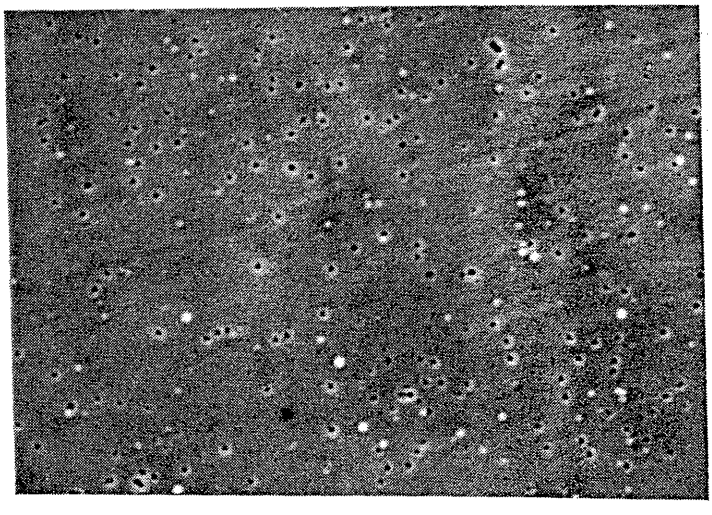


Figure 18. 0.086u Covaspheres, SEM, 8000x

divided in half: one-half was stained and viewed by LTM; the other was gold coated and viewed under the SEM. Photographs are in Figure 19 through 22.

By comparing the same magnification SEM vs. LTM, on non-sonicated samples (Figures 19 and 20), more bacteria are visible on the SEM field than on the LTM field, although both show many, many bacteria, more than would ever be found in a real sample. In comparing the sonicated vs. non-sonicated by LTM (Figures 20 and 22), we still see many whole bacteria, but also some non-defined small pink "stains", about the size of a bacterium.

In the sonicated SEM sample (Figure 21), we can also see evidence of change in that bacteria sizes are smaller, and there are some almost spherical pieces. The most interesting finding, though, is that the filter pores are almost clogged. This is easier to see in the 8000x views of non-sonicated and sonicated samples (Figures 23 and 24). The probable reason for clogging of the pores is that the sonicated water contained viscous or sparingly soluble matter that dried up when the water evaporated. This matter would be bacterial debris, which does not bind the staining material, otherwise we would see an overall pink cast to the LTM field.

Identification of bacteria "fragments", by either LTM or SEM is not very successful, at least with the type of fragments used in this experiment. They were not very visible by LTM, and although

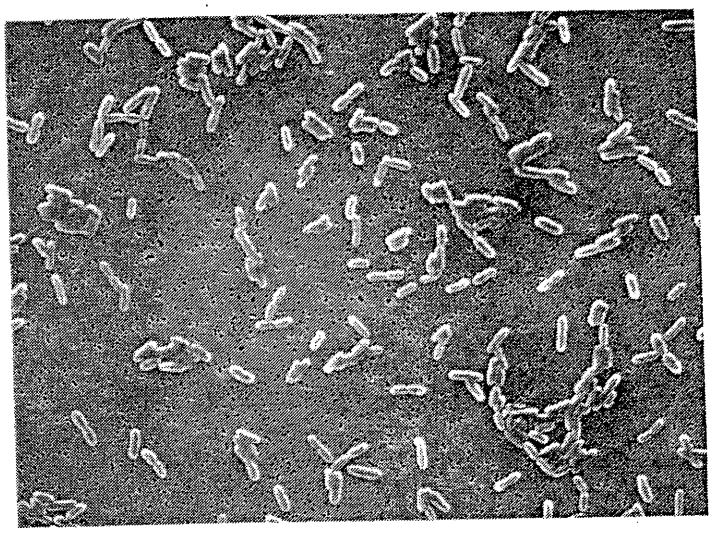


Figure 19. Bacteria, SEM, 1800x

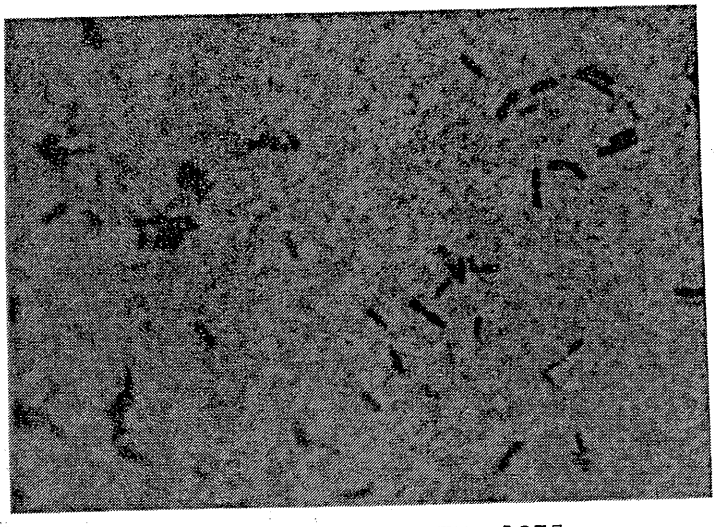


Figure 20. Bacteria, LTM, 1875x

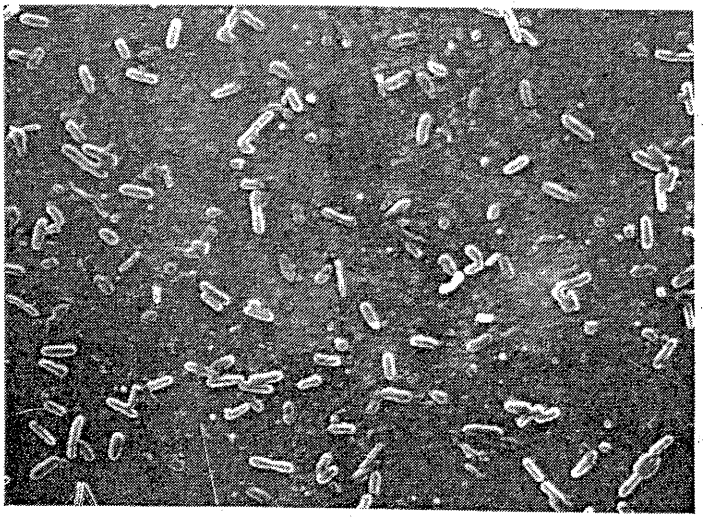


Figure 21. Sonicated bacteria, SEM, 1800x

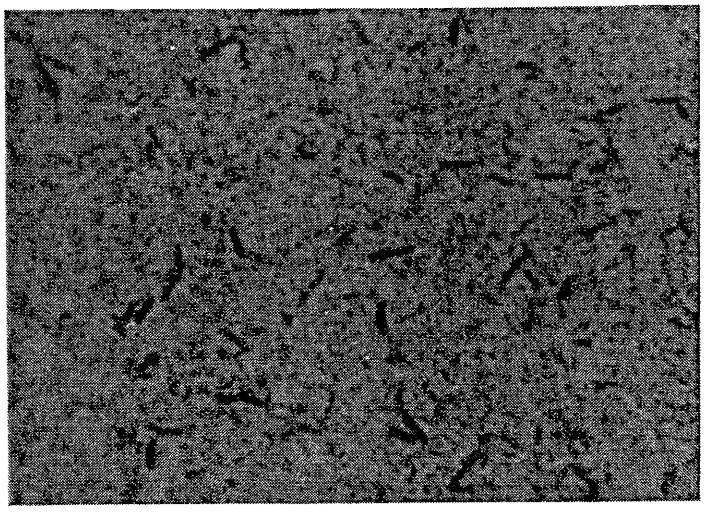


Figure 22. Sonicated bacteria, LTM, 1875x

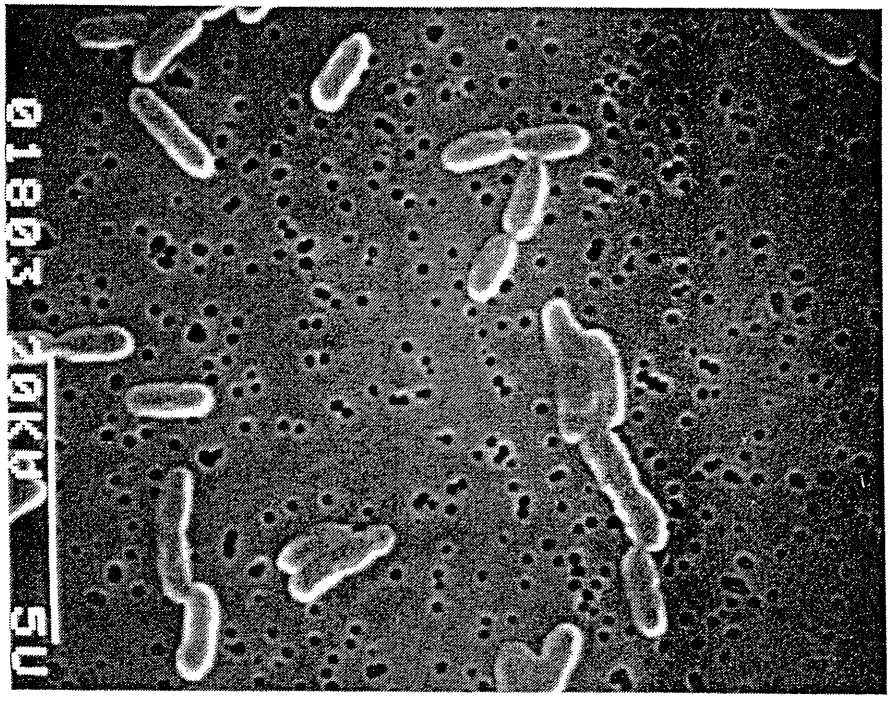


Figure 23. Bacteria, SEM, 8000x

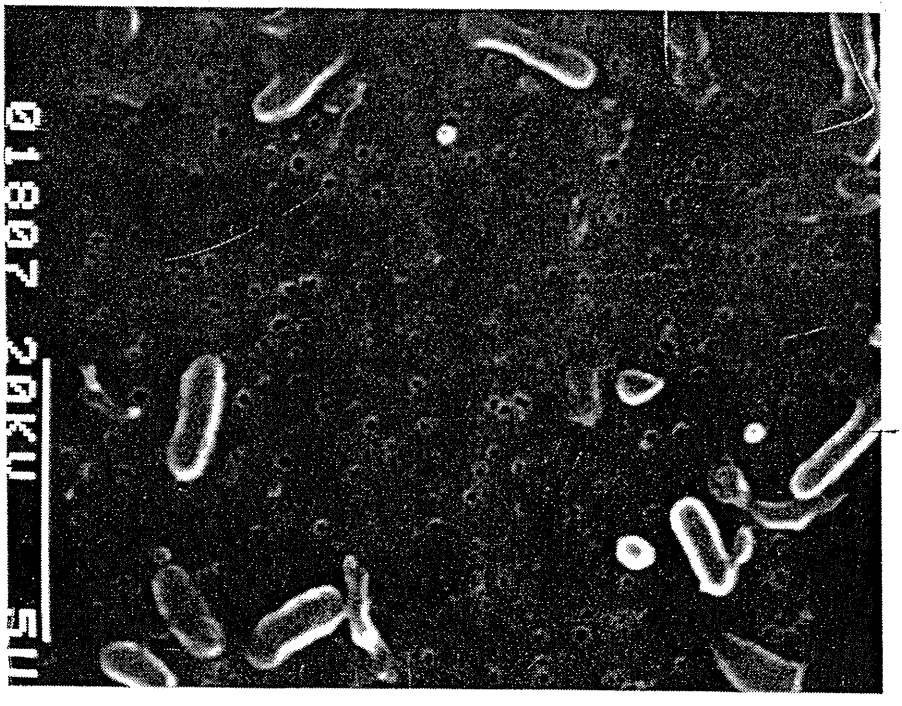


Figure 24. Sonicated bacteria, SEM, 8000x

seen by SEM, they would not be identified as bacteria fragments but as particles. As for the "debris" neither method detected it, except by clogging of the pores. For a normal sample we would undoubtedly never see this much "debris", but could not relate it to bacterial contamination without further testing.

C. Colonies, Clusters, Colloids

By LTM, when relatively large areas of mottled color are found they are counted as deposits of colonies or colloidal material. We had never found this type of area on a SEM filter, but we did note that there were such areas on the LTM filters, including the blanks. We suspected them to be residual staining material. Figure 25 is a SEM view of a stained filter blank, in which some of the large areas can be seen. This can only be the dried staining material, as no water was sampled through this filter.

It would be unlikely to have colloidal material appear on a filter in a localized area. More likely, the evidence of colloids would be the detection of pore clogging, such as seen in the sonicated bacteria samples. The issue of colloids or clusters, however, and the difference in ability to detect them between the two methods was not resolved. Part of the problem is that in order to locate such an area by LTM, the filter surface must be coated with oil, which then interferes with identification of the cluster by other techniques, such as ESCA or SEM/EDX.

D. Comparison of Methods Using Real Samples

The best way to compare methods is with RO/DI samples. Water was filtered from a running stream, by the techniques described earlier. The filters were carefully divided into two sections, one for each method. Total counts were taken from the filters.

1. City Water Sample: The purpose of this test was to load the filters with a large number of particles, some very large, in order to make a comparison of the viewed fields. We wanted to have an example of real particles that were clearly seen on the LTM filter, as can be seen in Figures 26 and 27. Many particles and bacteria are visible. No attempt was made to count these highly loaded filters.

2. Sample downstream of 0.45μ post-polisher filter:
A 3.5 liter sample was taken from water downstream of a 0.45 filter, prior to the UV sterilizer. This sample could be predicted to contain many particles less than 0.45μ, but few greater than 0.45μ provided this filter is functioning well. The sample filter was a 0.2μ Nuclepore.

Figure 28 shows one field of the LTM filter at 1875x and Figure 29 is a SEM field at 1900x. Many particles are visible on both filters, some of which are clearly

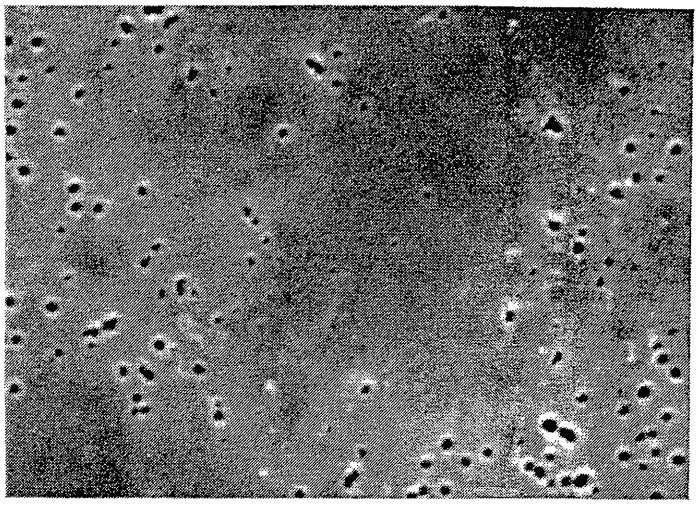


Figure 25. SEM of stained 0.2u filter blank

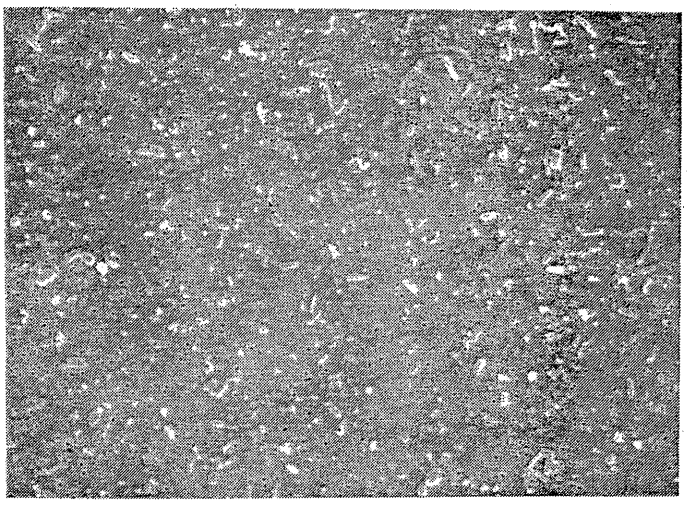


Figure 26. SEM of diluted city water sample, 1800x

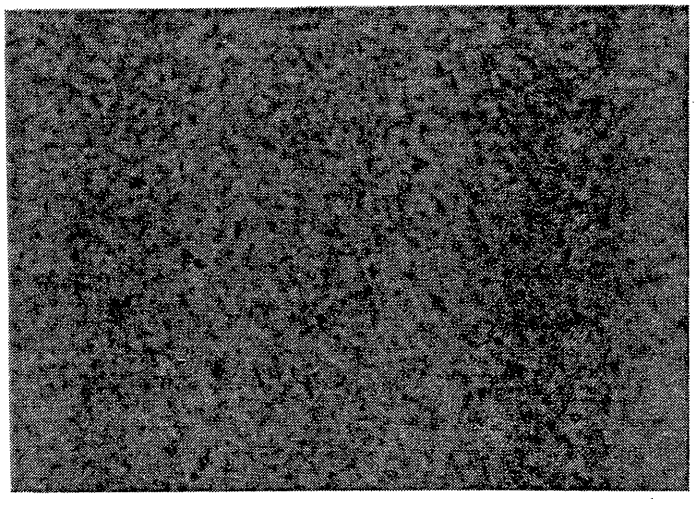


Figure 27. LTM of diluted city water sample, 1875x

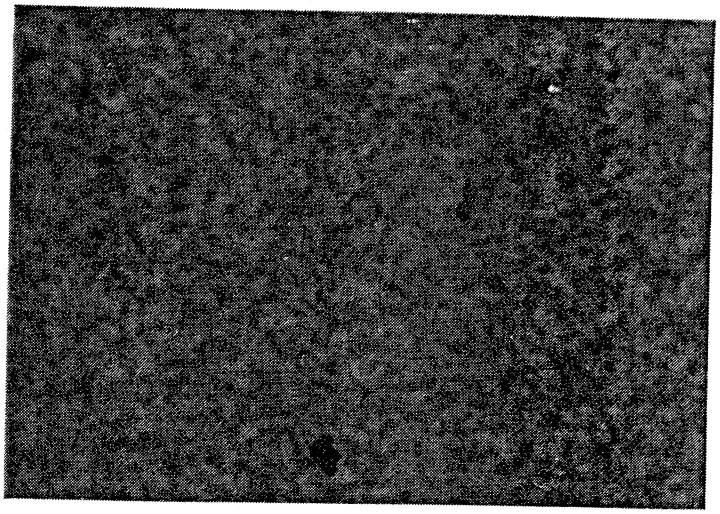


Figure 28. LTM of 0.45u filter effluent, 1875x

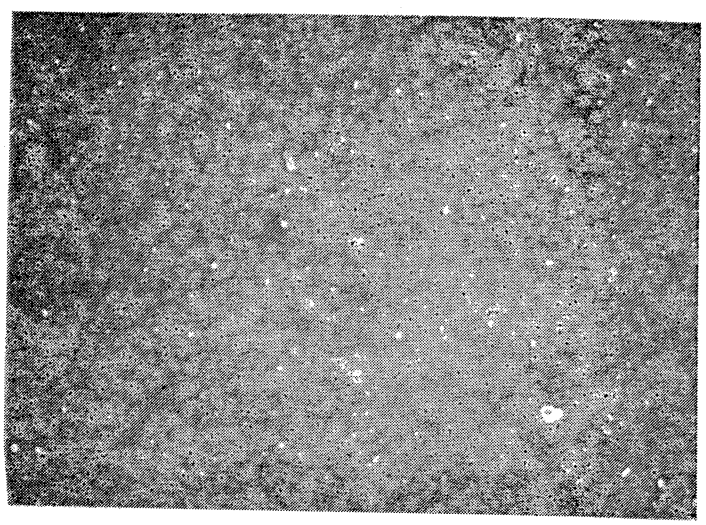


Figure 29. SEM of 0.45u filter effluent, 1900x

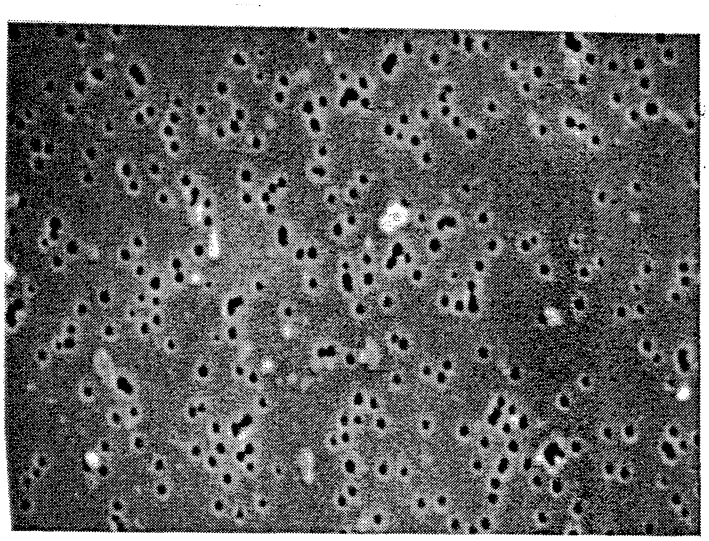


Figure 30. SEM of 0.45u filter effluent, 8000x

larger than 0.45μ. Figure 30 is the SEM view at 8000x, where counting was done.

Table 1 lists the particle counts by SEM and LTM. For LTM, total counts includes bacteria, whereas the particle sizes and bacteria are listed for SEM.

The SEM counts for this water are more than 60 times higher than for LTM, without considering bacteria counts by SEM.

3. Sample of final filter water: A 260 liter sample of final filter water was taken, with the filter being divided for analysis by each method.

Figure 31 is of the LTM filter at 1875x, with Figure 32 of the SEM filter at 1900x. An 8000x view is in Figure 33. In this case, at 1900x magnification the SEM filter looks "clean", but close examination reveals several particles about the same size as the filter pores. The LTM filter looks similar to a blank.

The total counts for this sample are in Table 2. In this case, the SEM is over 185 times higher than the LTM.

TABLE 1. Results of particle and bacteria counts for water sampled downstream of 0.45μ filter

	SEM	LTM
Particles: (pieces/liter)		
Size Range (μ)		
0.2 - 0.5	$480 * 10^4$	
0.5 - 1	$62 * 10^4$	
1 - 2	$2.2 * 10^4$	
>2	$1.1 * 10^4$	
Total >0.2	$545 * 10^4$	$8.7 * 10^4$
Bacteria (counts/100 mL)	29,000	

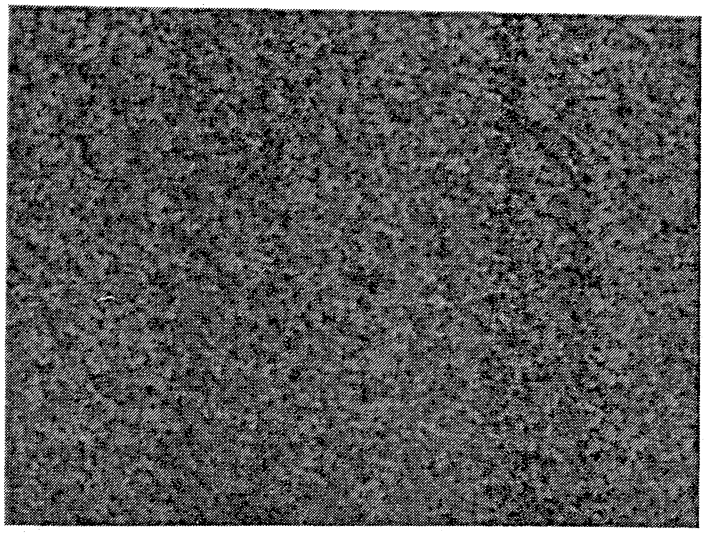


Figure 31. LTM of final filter effluent, 1875x

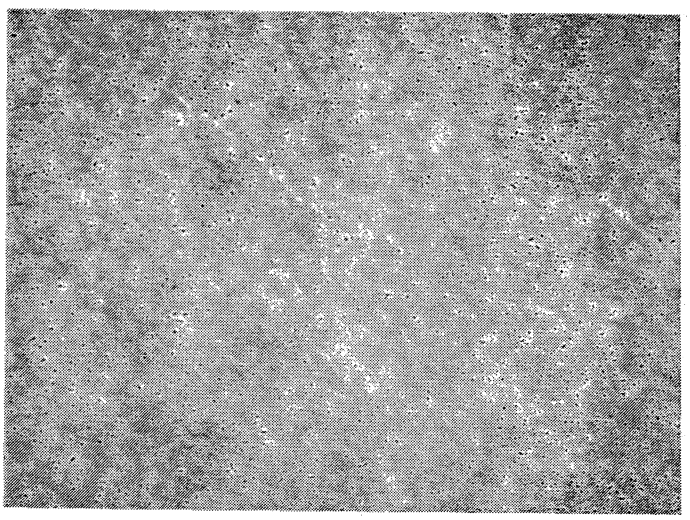
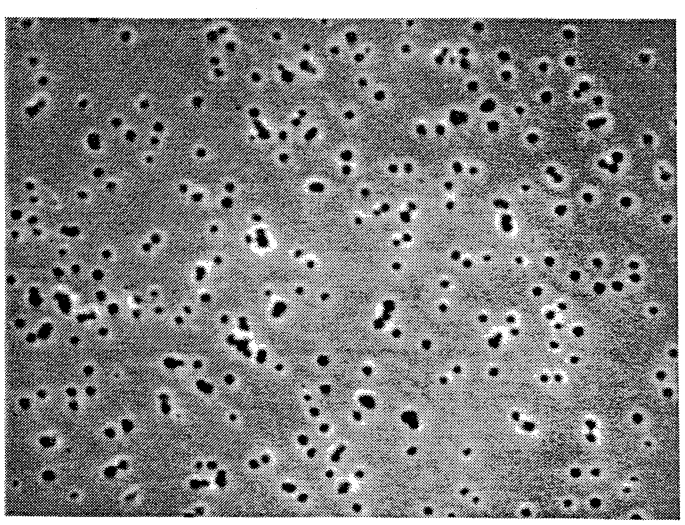


Figure 32. SEM of final filter effluent, 1900x



Filter 33. SEM of final filter effluent, 8000x

TABLE 2. Results of particle and bacteria counts for water sampled downstream of final filter

	SEM	LTM
Particles: (pieces/liter)		
Size Range (μ)		
0.2 - 0.5	860	
0.5 - 1	38	
1 - 2	38	
>2	<1	
Total >0.2	936	5
Bacteria (counts/100 mL)	60	

Summary and Conclusions

The parameters of the three methods are listed in Table 3.

The OPCM is useful for larger particles. Sizing of particles, and assignment between organics and inorganics is possible. It is the fastest way to get particle counts in the higher particle size ranges.

Comparing the SEM with the LTM methods, SEM can detect smaller particles, and it is possible to identify the elemental composition of the particles. The filter background for the gold-coated SEM filter is cleaner and the particles are more clearly identified. The counts are not subjective as they are with the LTM, where the background and limitations of the optical microscope limit the visibility of particles and bacteria above the background of the filter. Residual staining material can be counted and/or misidentified. Because of the comparative ease of counting, operator counting is more consistent with the SEM filters than with the LTM.

For both methods there is a trade off between sample volume, time, and accuracy. The SEM filter has 1.4 million fields at 8000x, of which a minimum of 144 are counted. For very clean water, of, say, less than 2000 particles per liter, a minimum of 200 liters is required so that many of the fields contain one or more particles. The LTM filter has 58,200 fields, of which at

TABLE 3. Parameters of Three Particle Methods

	Optical	SEM	LTM
Particle sizes detected, μ	>1.2	>0.1	>0.2
Detect/count bacteria	No	Yes	Yes
Possible to size particles	Limited	Yes	?
Particle ID	Organic vs. Inorganic	Yes, with EDX	No
Minimum sample volume	1 liter	200 liters	50 liters
Elapsed time for sample collection	<20 minutes	24-48 hours	6-12 hours
Analysis time	15 minutes	30-50 min.	45-60 min.
Equipment cost	$10,000	$40-60,000	$12,000
Comparison with on-line monitors	No	Gives higher counts	?

least 50 are counted. There will be an average of less than three particles per field for 2,000 particles per liter of water.

Large sample volumes are also necessary to overcome any sampling background errors. If any particles are generated during set up of the filtration apparatus, their effect is magnified for smaller sample volumes (5). For the SEM we often take up to 1000 liters or more, or leave the filter in place for a week for routine monitoring.

Neither method gives real time counts; nor does it appear that either method can be adapted for real-time monitoring.

The cost for equipment is less for LTM than for SEM, requiring a good optical transmission microscope rather than the more expensive scanning electron microscope. The sample collection, sample preparation and counting time is less for the SEM procedure, since there is no time required for staining and the particles are easier to detect on the SEM. The relative differences in cost should not be a significant factor in obtaining good particle data, which is so critically important.

At this time a major drawback of both SEM and LTM is that sampling must be done from a pressurized line, in order to collect the volume needed. The best water to sample is water that contacts the product--from dump rinsers, spin rinse dryers, etc. Any time water is transferred to a container, there is sig-

nificant potential for contamination, especially in the small particle sizes. One way to overcome this problem would be to draw water through the filter, using vacuum sources that can handle liquids and that can be brought into the fab area.

The SEM method, or adaptations of it, can yield significantly more detailed data by taking advantage of auto-imaging techniques combined with EDX, to automatically count and size particles down to $<0.1\mu$, while reporting elemental composition of individual particles. With this output, particle sources can be identified, and eliminated.

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