SAMPLING & EVALUATING AIRBORNE ASBESTOS DUST

582

NIOSH

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The student manual for a course in sampling and evaluating airborne asbestos (1332214) dust is presented. The course, conducted by the NIOSH Division of Training and Manpower Development consists of lecture topics, demonstrations, and laboratory work. The lecture topics included asbestosis, sampling and calibration equipment, sampling procedures, asbestos structures as determined by microscopy, microscope use and calibration, counting procedure, and methods for identifying asbestos. Laboratory work includes pump calibration, use of the wet meter bubble buret, microscope preparation and slide mounting, and fiber counting. The manual contains the US Public Health Service/NIOSH method for evaluating airborne asbestos fibers, the Department of Labor and Food and Drug Administration rules and regulations governing asbestos exposure, the physiological aspects of asbestosis, descriptions of sampling and calibration equipment, guidelines for monitoring dust concentrations in the asbestos industry, procedures for use of microscopes and associated equipment, filter mounting procedures, counting procedures, and a calibration work sheet

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SAMPLING AND EVALUATING AIRBORNE ASBESTOS DUST (582)

Conducted by

Division of Training and Manpower Development

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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CONTENTS

ABSTRACT

This course treats the asbestos sampling and evaluating techniques in detail. The theory and explanations given in lectures are applied in a "hands-on" laboratory to reinforce each major concept. At the conclusion of this course, the trainee will be able to properly use the sampling and evaluating equipment and to apply the procedures necessary for the collection and evaluation of asbestos dust samples. The training course manual has been specially prepared for the trainees attending the course and should not be included in reading lists of periodicals as generally available.

ASBESTOSIS: IT'S PHYSIOLOGICAL CONSIDERATIONS

Diseases related to asbestos dust exposure have only recently come into the public's consciousness. The disease asbestosis is not new by any means. Two ancients -- Strabo, a Greek philosopher, and Pliny the Elder, a Roman naturalist -- described a sickness in slaves who worked at weaving asbestos into cloth; but, occupational hazards were of little concern to the ancients.

It was not until the technological era of the twentieth century that the use of asbestos were fully exploited. Its output has increased over a hundred fold in the past sixty years. World production has jumped from five-hundred tons in 1900 to three million tons in 1968. Along with its increased usage came problems of health and safety for workers exposed to the dust.

The first case report of a disease related to asbestos exposure appeared in English Literature in 1907. At that time, H. M. Murray described the post mortem findings on a thirty-four year old carding-machine operator. Murray described the lungs as showing extensive diffuse pulmonary fibrosis and containing spicules of asbestos -- incidently, the other workers who had worked in the same card room succumbed before the age of thirty of pulmonary disease.

The real link between asbestos and pulmonary disease was not made until 1924 when W. D. Cooke, an English physician who gave the disease its name, reported the autopsy findings of a thirty-three year old woman who had started working in an asbestos textile factory at the age of thirteen. The autopsy showed extensive pulmonary fibrosis and some strands of abnormal fibrous tissue connecting the lungs and the pleural membrane surrounding them. In addition, Cooke noted the presence of solid yellowish-brown particles which he called "curious bodies," in the areas of fibrosis. These particles would later be called "asbestos bodies."

Shortly after Cooke's case report in 1924, numerous articles began to appear about asbestos in medical literature. In 1928, Dr. E. R. A. Merewether examined 363 British asbestos textile workers and found that twenty-five percent showed evidence of suffering from pulmonary fibrosis — and that the incidence of fibrosis increased in direct proportion to the number of years of exposure. As a result of Merewether's report, Parliament passed legislation in 1931 that made asbestosis a compensable disease, required improved methods of exhaust ventilation and dust suppression in asbestos textile factories, and instituted periodical medical exams for workers.

The first published suggestion of an association between asbestos and malignant disease was made by Dr. Kenneth M. Lynch, professor of pathology at the medical college of South Carolina. He reported the case of a fifty-seven year old weaver who died of asbestosis and in whose lungs cancer was also found. In 1956 and 1957, 16 cases of a very rare tumor -- mesothelioma -- were diagnosed at the Pneumoconiosis Research Unit in Johannesburg, South Africa by a pathologist named Christopher Wagner.

In the past decade, much attention has been focussed on the relationship between mesothelioma and exposure to asbestos dust. Other cancers, such as those that occur in the gastro-intestinal tract and in blood-forming organs, have also been associated to asbestos exposure by some workers, but the evidence is less conclusive. So what started out as a single disease in the early part of the century has become considerably more complicated and just now are we beginning to understand all the risks involved.

Now I would like to say a few things about the epidemiology of asbestosis, bronchial cancer, and mesothelioma. Up to just recently, it was believed that the finding of asbestos bodies in a worker's sputum or lung tissue was strong evidence

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Prepared by Dr. Howard Raymond.

that a worker had asbestosis. The diagnostic significance of asbestos bodies has fallen into dispute for three reasons:

1 Workers with severe asbestos occasionally are found to have no asbestos bodies in their lungs.

2 Conversely, many workers who are found to have many fibers and bodies have no indication of disease otherwise.

3 By light microscopy, asbestos bodies cannot be differentiated from ferrugenous bodies. (Electron microscopy has proven to be more helpful in this area).

In a study by Raymond Murphy and others reported in the Journal of Medicine, criteria were outlined by which the diagnosis of asbestosis could be made.

1 A shortness of breath on one flight of stairs.

2 The presence of rales.

3 The presence of finger clubbing.

4 The forced vital capacity less than predicted value.

5 X-ray changes of minor to advanced stages.

Any three of these findings would highly suggest the diagnosis of asbestosis. Murphy then compared 101 shipyard pipe covers with controls. The average exposure was five-million particles per cubic foot. The results showed that eleven out of the 101 had the disease and that the disease could be seen in as few as thirteen particles per cubic foot years.

There is very little disagreement that the disease asbestosis is dose related.

Numerous studies have shown that there is higher incidence of lung cancer in asbestos workers. The risk of bronchial cancer (bronchogenic carcinoma) is pretty clearly related to the intensity of past exposure and to the risk of asbestosis. It is not known whether the type of asbestos inhaled affects the risk of bronchial cancer in man. In animals, amosite or crocidolite appears to produce more fibrosis than chrysotile. These tumors are rarely seen in cases having less than fifteen to twenty years of exposure. Although reports differ considerably, the increased risk of bronchogenic carcinoma to asbestos workers is about six to ten times; however, the affect of cigarette smoking greatly confuses the issue.

Several studies have shown that there is no increase in the incidence of lung (bronchial) cancer in non-smoling asbestos workers. It is felt by many, in fact, that cigarette smoking and asbestos exposure act as co-carcinogens.

By 1961, a total of 87 mesotheliomas of the pleura were recognized by Dr. Christopher Wagner in Johannesburg, South Africa. Only twelve of the cases proved to have any industrial exposure to asbestos; but, all the rest came from the arid, windswept regions of the Cape asbestos field where they could have easily obtained exposure simply by living near the dumps. Even among those patients who worked in the mines, the actual exposure appeared to be slight — for in most cases, the lungs showed no evidence of asbestos and only a few fibers and bodies. From this information, Wagner drew the conclusion that mesotheliomas might result from an exposure to asbestos dust that was considerably less than that required to produce asbestosis.

Current studies seem to bear out Dr. Wagner's early conclusions. It appears that the dose relationship is much less certain with mesothelioma than with asbestosis and bronchial cancer; indeed, cases are seen with only slight exposure. Some workers reported as high as a five percent death rate from mesothelioma among asbestos workers. The incidence in the general population being a minuscule one per ten thousand. As with bronchogenic carcinoma, there is a long, latent period from the onset of exposure to the development of the disease — 25 to 35 years. The type of fiber may be important — amosite and crocidolite appear to be the major

offenders. Cigarette smoking does not seem to be an important factor. Both peritoneae and pleural mesotheliomas are seen in about equal proportions.

Several theories have been advanced to account for the pathologic findings in the development of asbestosis.

1 Irritation of the fiber itself leading to fibrosis.

2 Silicic acid and metal ions leached out leading to fibrosis.

3 Autoimmune theory: fiber and phagocyte abn globulin, fibrosis.

4 Stagnation of phagocytes — trapped by fibers — disintegrate and release sclerosing agents.

The major physiological disturbance is a reduced diffusing capacity of the lungs — what is known as an alveolar capillary block. This is the result of interstitial fibrosis involving the alveolar capillary membrane with consequent impairment of oxygen diffusion from the air sacs and into the blood. The defect is non-specific and is seen in any disease that causes interstitial fribrosis — such as sarchoidosis, etc. Other defects seen with interstitial fibrosis include a reduced inspiratory capacity and hyper-ventilation on exertion. Some recent work has shown that airflow obstruction may also be a fact.

The most common symptoms of asbestosis are cough and shortness of breath. The cough associated with the disease is generally of a productive nature. Physical findings seen in asbestosis include pulmonary rales and finger clubbing. Other symptoms and physical findings may be seen, but they are due to complications such as congestive heart failure or cancer.

At this point, I would like to say a few more things about finger clubbing. Finger clubbing (or an increase in the hyponechial angle) may be observed as a familiar variant or as a pathologic entity in such diseases as congenital heart disease and asbestosis. The physiology of this change is not well understood; but, it is thought to be associated with impaired ventilatory function — specifically shunting of oxygenated blood.

In one plant, about 16 out of 500 workers were noted to have clubbing. These 16 workers were matched as to age and sex to 16 other workers with normal hyponechial angles. It was found that both groups had nearly identical years of exposure to asbestos fibers, but the group with clubbing had a significantly higher number of cigarette pack years. This unpublished data supports the generally held contention that cigarette smoking greatly increases the risk to asbestos workers.

There is no specific treatment for asbestosis — other than removal from the environment. Complications (such as congestive heart failure) are treated in the usual manner. I would now like to discuss two of the more important diagnostic procedures — x-ray and pulmonary function.

There are three principle types of x-ray changes seen in asbestosis. The first, and probably the most common, is a parenchymal change (or change within the lung itself). Small opaque areas (also called multiple small opacities) are seen throughout the lung fields. This change is commonly seen in other pneumoconioses such as berylliosis and other diseases such as sarcoidosis and is; therefore, not specific for asbestosis. The second change involves the membranous lining of the lungs, the pleura. This change consists of a thickening of this lining and is seen as a diffuse whitening around the lung tissue. This change is also quite non-specific. A specific type of pleural thickening known as pleural plaques has recently been described. These plaques consist of a thin layer of liquilin tissue deposited on the pleural surface. These shadows may be quite difficult to see on the routine chest film. It is thought that they occur earlier than parenchymal changes and are supposedly specific for asbestosis. The third change involves the membranous lining of the lung and is called pleural calcification. As the name implies, the calcium is deposited within the membranous lining. On an x-ray, this is seen as opaque lines and is most commonly seen on the diaphragm.

In 1964, the bureau (now NIOSH) began a study of asbestos textile workers in the United States. The study involved about eight plants and approximately 2,500 workers. At the present time, we have examined all of them once, and plan to re-examine them three years from now. Our field team consists of one physician, one physiologist, and two technicians. The physician obtains a history by employing a standardized respiratory questionnaire. He listens for rales and measures finger clubbing. The remainder of the team perform and analyze pulmonary function test spirometry for FVC and FEV, and a gas diffusion test at rest and under load. Finally a chest film is taken parallel with the medical study, NIOSH has been taking samples at each of the plants and eventually hopes to correlate the environmental data with the medical data.

DEFINITIONS

Fibrosis - The growth of a white fibrous connective tissue in an organ (or part) in excess of that naturally present.

Pulmonary - Pertaining to, or affecting, the lungs or any anatomic component of the lungs.

Pleura - The membrane that envelops the lung and lines the internal surface of the chest cavity.

Mesothelioma - A primary benign tumor of mesothelial structures (peritoneum, pericardium, or pleura) composed of sheets of cells morphologically resembling mesothelial or endothelial cells — also called endothelioma.

Pneumoconiosis - (Pneumonoconiosis) chronic irritation of the lungs caused by the inhalation of dust — may or may not imply the presence of disease.

Epidemiology - The study of occurrence and distribution of disease.

Bronchi - (Pleural of bronchus) primary branches of the trachea (windpipe) or such of their branches within the lung as contain cartilage in their walls.

Rales - An abnormal sound heard within the lungs or air passages -- non-specific to any disease.

Interstitial - Pertaining to the occupation of the interspaces between an organ's finest connective tissue.

Sarchoisosis - A disease of young adults and sometimes older persons producing among other things, lesions that somewhat resemble true tubercules within the lungs.

Hyperventilation - Forced breathing: an increase in the quantity of air breathed as a result of the rate, depth, or both of respiration.

FVC - Forced vital capacity. The measured quantity of the maximum lung volume of an individual.

FEV_1 - Forced expiratory volume at one second — the volume of air that is forced at maximum effort from an individual's fully inflated lungs.

NIOSH PRESENTS

SAMPLING AND CALIBRATION EQUIPMENT

Here are the objectives for this lecture. They are stated in terms of what you will be able to do after the lecture is completed. The objectives are:

1 List all components of the sampling train.

2 List and define the calibration standards.

3 List the two recommended calibration instruments.

Whenever the part of the lecture that applies to any objective is completed, the objective will be shown. This lecture is divided into two parts — one on sampling equipment and one on calibration equipment.

PART I: THE SAMPLING EQUIPMENT

Any mention of commercial products or concerns does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare.

The backbone of the sampling train is the pump, and you should consider the following guidelines when purchasing a personal sampling pump. These recommendations are the result of many years of field use and laboratory testing.

The pump should not weigh more than about four pounds and should be built of a durable material. The case parts should fit well to prevent dust from entering.

Your pump should have an on/off switch which should be protected from accidental operation during use and airtight to prevent dust from entering.

The M.S.A. has a stiff, push-button switch sealed in rubber. Other pumps such as this Bendix unit has a toggle switch covered by a screw-on cap.

The pump should have an external means of adjusting the flow rate and a rotameter to indicate the flow rate. To prevent accidental adjustment, the adjuster should be concealed (as in the case of this Unico Micronaire) or it should be recessed as in this Model G from M.S.A.

It should have a flow-rate range between one to two-and-a-half liters per minute.

The pump should be able to operate for eight hours at a flow rate of at least 1.7 L/M against a resistance of six inches of water measured at the pump inlet.

Since the pump will often be hanging on a worker, it must have a belt clip. The clip should be designed to prevent slippage from the belt even if its position becomes inverted.

An external connection for battery charging has been found to be most convenient.

Another major part of the sampling train is the sampling head. We have been -- and to a great deal still are -- recommending the 37 millimeter disposable field monitor cassette made by the Millipore Corporation.

As you see, the field monitor has several parts: base plug, pad, filter, center, top cap, and cap plug.

You can buy the pre-loaded and assembled field monitors by the box of 50.

You may purchase these pre-loaded cassettes by ordering #MAWP037AO. Field monitors that are empty require #M00037AO. One hundred filters and pads are ordered by #AAWP03700.

The filters are made of a cellulose ester, having a nominal pore size of .0008 mm. They are tremendously efficient and collect all the dust on the upper 10 to 15 μm of the filter surface. We recommend the white plain filters (designated by W.P. in the order numbers). Gridded filters could be used, but we find the grid lines to be more

Prepared by Stephen G. Bayer, Physical Science Technician, Division of Training, NIOSH, 2/74. Music by Mark Conrad, photographs and visuals by Anne Stirnkorb and Glenda White.

of a hindrance than a help. The filters weigh about .04 grams each. Because they are hygroscopic, variations in humidity cause weight changes; therefore, they must be desiccated for gravimetric analysis.

The pad is a thick fibrous disc that, along with channels in the cassette bottom and the filter porosity, helps to evenly distribute the airflow across the filter.

Although the cassettes are "disposable," they are also re-loadable. Wash all cassettes in warm, soapy water and rinse them in tap water. Drying should be performed in a dust-free place. Upon reassembly, be sure that the center section fits tightly all the way around the filter. This is necessary to prevent air from going around instead of through the filter.

For several years, we have made it our practice to band the filters before use. We use cellulose bands that shrink upon drying. The center and bottom sections are banded by simply slipping the band over the sections. The bands keep out contamination somewhat and provide a convenient, disposable surface for writing on the sample number. Tape may work just as well.

Connecting the pump to the filter is the tubing and related hardware. The tubing should be 1/4 inch inside diameter, non-collapsable tubing. Cut all lengths the same in a range of about 32 to 36 inches.

In one end of the tubing, there is a Leur-slip adapter. This was initially used in the medical field to connect tubing to hypodermic needles. In this case, the adapter inserts very tightly into the base of the cassette. This prevents the cassette from falling while the sample is being collected.

Two clips are needed to securely hold the cassette and tubing to the worker. Alligator clips, clothes pins, or the paper clip shown here all work well.

Many times, there may be no convenient or safe place on which to hang the pump on the worker. An example here is many womens' slacks which have only elastic holding them in place and cannot support the pump. We have found it advantageous to have several "surplus" web-belts on hand to prevent bother to the worker.

There are a couple of final notes that should now be covered. When you insert the Leur-slip adapter into the field monitor, do not crack the plastic. This will cause air leaks and invalidate the sample. When you hang the train on a person, position the clips so that the tubing does not pinch.

At this time you should be able to complete the first objective.

PART II: THE CALIBRATION EQUIPMENT

In sampling an atmosphere for contaminants, our objective is to determine as accurately as possible the concentration of a substance which is usually expressed as weight, number of particulates, or parts per unit volume of the atmosphere being sampled. In the case of asbestos, it is fibers per cubic centimeter or milliliter. We will have to calculate the sampled volume by multiplying the sample time by the flow rate as read from the rotameter. Therefore, we must accurately calibrate the rotameter to know the exact flow rate as indicated by the ball.

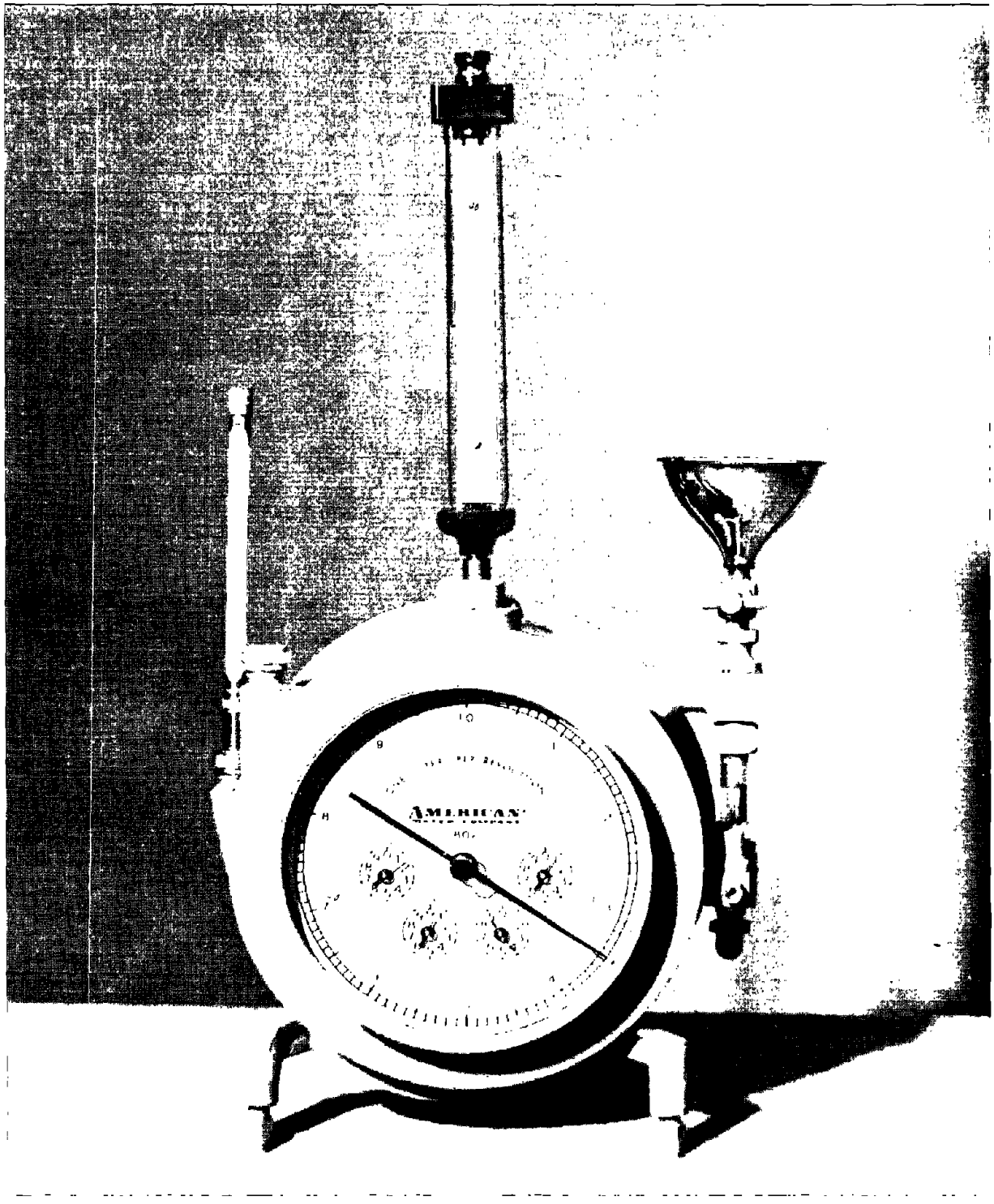
Calibration is defined as the determination, within specified limits, of the true value of the scale reading or indication of an instrument.

There are quite a few instruments that are used for calibration, and they are classified under three standards.

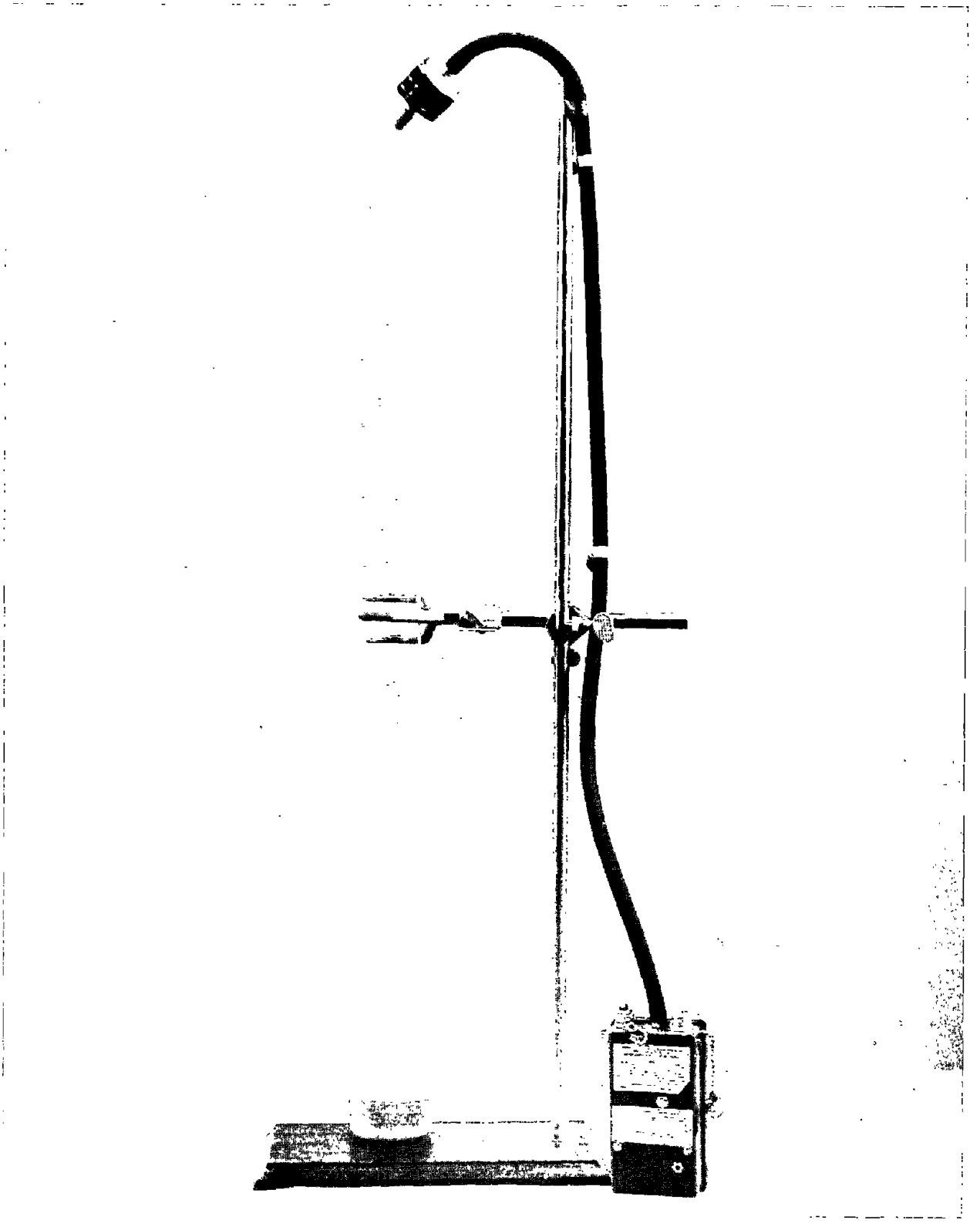
A primary standard is defined as being volumetric in nature. The volume must be measurable by some direct and independent means, independent of the gas or air involved.

An intermediate standard is one that cannot be primary by definition but is just as accurate if maintained and must be calibrated (and periodically recalibrated) by a primary standard.

A secondary standard is any remaining air flow measuring instrument that does not fit



Wet Test Meter



Bubble Burette

the definitions of the other two standards and has been calibrated by a primary standard.

At this point you should be able to complete the second objective.

Let us now examine these three calibration standards and see how we apply them to the calibration of our personal sampling system.

This is a soap-bubble burette calibrator -- a primary standard. It is a 1,000 ml laboratory burette. We could check the volume in question by a direct and independent means -- such as filling it with water and weighing it. Knowing the density of water, the volume could be determined.

The soap bubble or film is generated by simply placing the soap solution (which can either be made or purchased in a toy department of a store) against the mouth of the burette. As soon as the pump is turned on, the bubble will rise in the burette and indicate air volume.

This wet-test meter is a very common example of an intermediate standard. Let's look at a schematic of a wet-test meter to understand how it works and what the various parts are.

Very basically described, a wet-test meter is a drum half filled with water. Inside the drum is a rotor which is attached to an indicating pointer on the outside of the meter. When a pump is connected to the outlet and turned on, air comes through the inlet and rises, causing the rotor to turn. The amount of turning is related to the quantity of air passing through. The indicating needle reads volume.

The wet-test meter you see here indicates 1 liter per each revolution of the needle -- a range which is ideal for calibrating personal sampling systems. At the top center of the wet-test meter is a manometer indicating an internal pressure drop. On the right side is the water level indicator which you now see close-up. When the water is at the proper level, the needle will make a slight depression in the water. On top of the wet-test meter there will be a spirit level which is adjusted by screwing the legs in and out. You may get temperature and pressure corrections with your wet-test meter should you decide to purchase one.

A properly calibrated wet-test meter -- is, one that has been calibrated by a primary standard -- is just as accurate as a primary. But since the drum contains water which can corrode, maintenance and periodic recalibration are necessary. Since the unit has no volume which can be independently and directly measurable, it cannot be considered as a primary calibration standard.

At this time, you should be able to complete the last objective.

Last is secondary standards. The most applicable one to come to mind is the rotameter.

A rotameter is little more than a transparent, tapered tube containing a float. These floats come in various shapes and densities, but the one we'll be using is a round ball. Floats are usually read at the widest point, and the ball is read at the center.

The theory of operation is that air passing up through the tube must push around the ball to get out. As the ball rises, its sides get further from the wall of the tube and eventually the pressure drop cannot cause a further rise, and the ball will remain suspended and give a reasonable indication of the airflow passing through the tube. It must now be said that changes in air density can and do affect how high the ball floats. It is important, then to know that the resistance of the sampling train is what is should be and the barometric pressure will be similar to that under which it was calibrated wherever the pump is used.

At this time, I would like to point out the various deficiencies of the standards.

Since primary standards are volume containers, it takes quite large vessels for high flow rates. For example, to measure 28.7 liters per minute, you would need

about a thirty liter container -- which is quite large. These large units can also get quite costly.

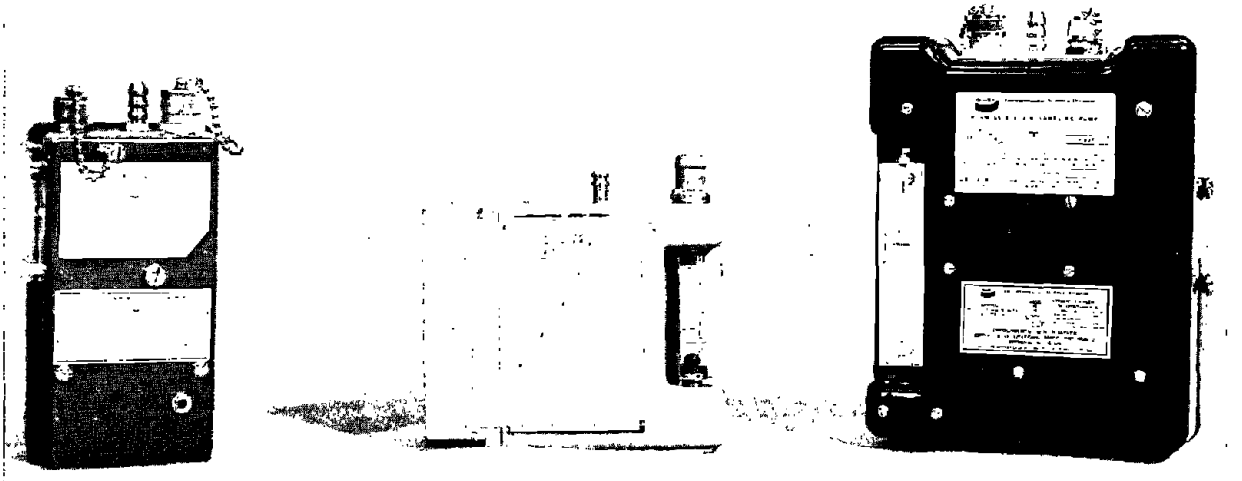
There are several disadvantages to the wet-test meter, for example. As I said earlier, there is the possibility of corrosion. Also, there is friction in the bearings and the mechanical counter. Also, inertia must be overcome at low (< 1 liter per minute for example) flow rates. At high flow rates, the water level surges breaking through the seals at the inlet and outlet.

A rotameter tends to bob up and down as the chugging characteristics of a pump get greater at low flow rates. This makes the ball somewhat hard to read and decreases accuracy. Dirt in the rotameter on walls should be cleaned if noticed because it could change the calibration.

At this time, let's review some of the main points.

There are three standards -- primary, intermediate, and secondary -- and they are classified according to their accuracy.

A primary standard is most accurate because it is volumetric in nature. An intermediate standard may be as accurate, but does require periodic re-calibration. Secondary standards are better to use as an indicating instrument -- such as our pumps rotameter.



Personal Sampling Pump

SAMPLING EQUIPMENT LABORATORY

LABORATORY OBJECTIVE

The trainee will calibrate the rotameter of a personal sampling pump by comparison to a primary standard and an intermediate standard.

BASIC LABORATORY REQUIREMENTS

The trainee will properly adjust the calibration instrument, connect the sampling train, calibrate the rotameter scale and record the results on the enclosed graph paper.

Equipment List

(1) Personal Sampling Pump
(1) Sealed Field Monitor Appropriate Tubing and Connectors
(1) Wet-Test Meter
(1) Soap Film Burette
(1) Stop Watch
(1) Small Screwdriver
(1) Soap-Bubble Solution
(1) Beaker

Formula: $\frac{(\text{liters})(60)}{\text{seconds}}$ = Flow Rate in 1/m

PROCEDURE FOR WET-TEST METER

Intermediate Standard

1. Assemble the sampling train (pump, filter, connectors, tubing, etc.)
2. Be sure the water level in the wet-test meter is correct.
3. Be sure the wet-test meter is level.
4. Connect the sampling train to the meter.
5. Start the pump and adjust the center of the rotameter ball to the lowest number on the rotameter at which the ball is stable.
6. Allow the needle to make one revolution, then:
7. Time the bubble for one revolution — at least (1 liter).
8. Record the time, volume, and rotameter number.
9. Move the rotameter ball up to the next number on the rotameter — proceed from (6).

10. Continue to calibrate the remaining rotameter markings.

11. Using the formula, calculate the flow rates for all rotameter readings.

12. On the wet-test meter graph plot the flow rate versus rotameter number.

PROCEDURE FOR THE BUBBLE BURETTE

Primary Standard

1. Assemble the sampling train (pump, filter, tubing, connectors).

2. *Remove the burette from the ring stand.

3. Dispense some soap-bubble solution down the burette walls and carefully rotate and rock the burette to completely wet the walls.

4. Reclamp the burette.

5. Attach the sampling train.

6. Pour one to two inches of soap solution into the beaker and place the beaker so that the mouth of the burette hangs slightly within the beaker.

7. Start the pump and, using the small screwdriver, adjust the center of the ball to the lowest mark on the rotameter at which the ball is stable.

8. With the pump running, quickly touch the soap-bubble solution to the mouth of the burette.

9. Using the stopwatch, measure the time required for the film to pass from 0 to 1000 (1 liter).

10. Record the volume, time, and rotameter number.

11. Moving to the next highest rotameter number, start at (7).

12. Continue through all rotameter numbers.

13. Using the formula, calculate all rotameter number flow rates.

14. On the appropriate graph-paper plot flow rate versus rotameter number.

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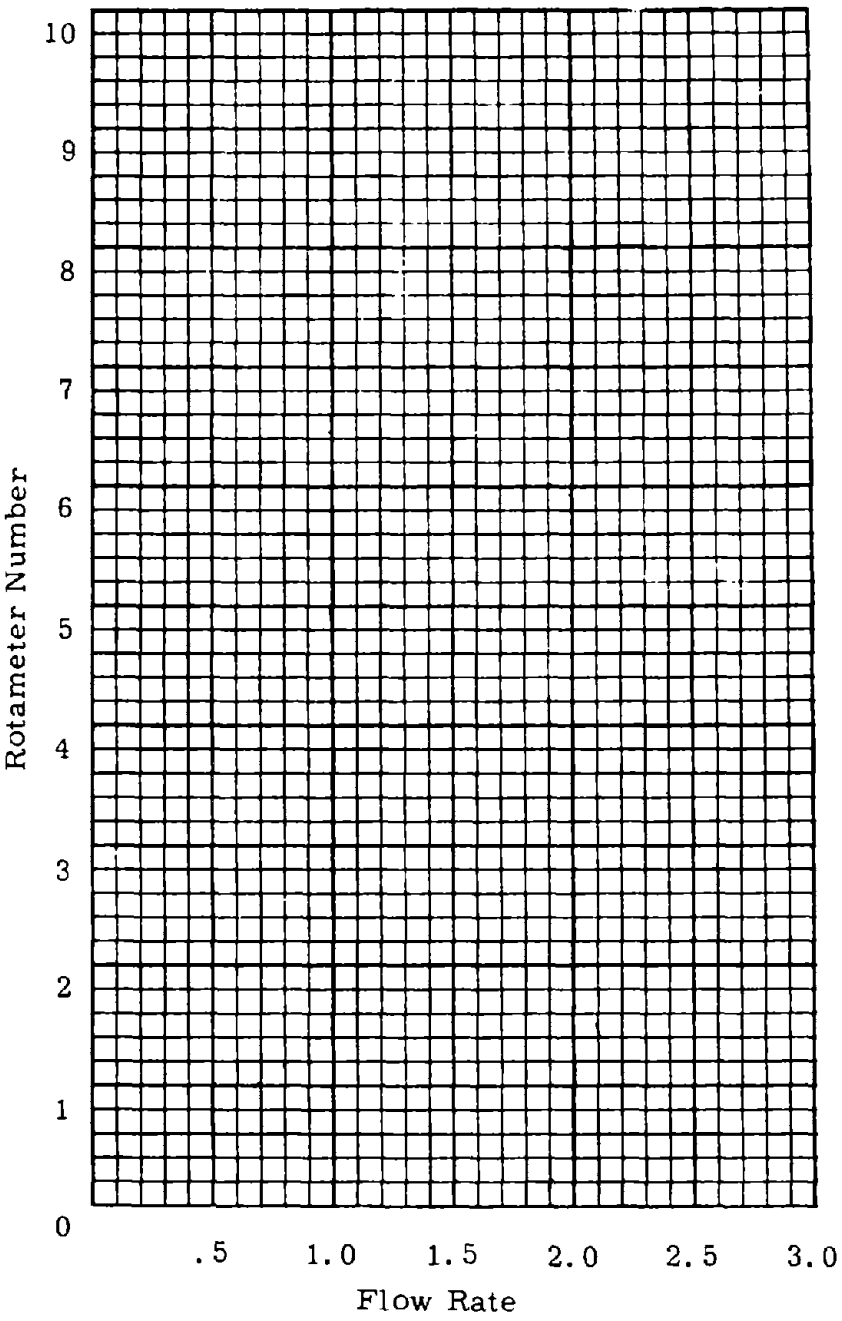
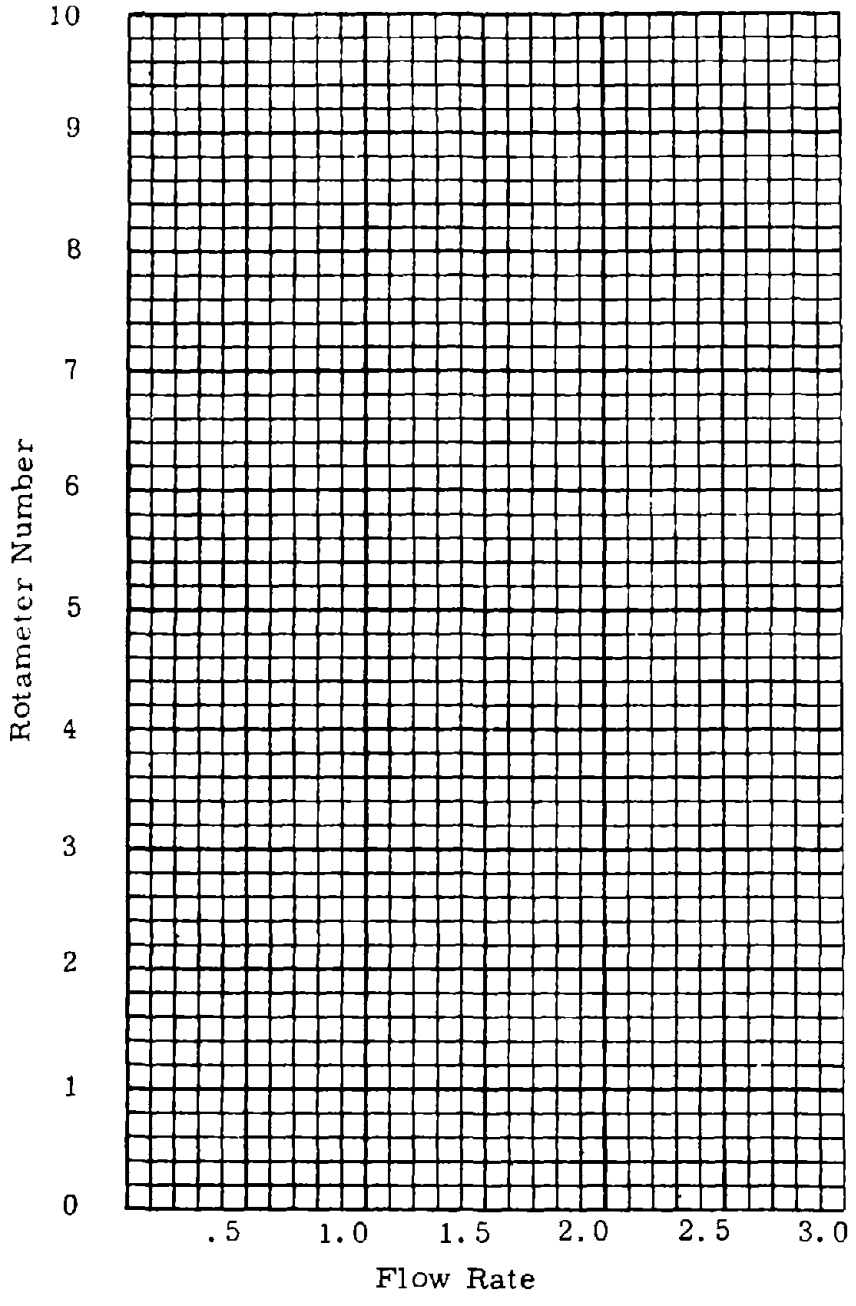
*If the burette walls have already been dampened, go to (5).

Prepared by: Stephen G. Bayer, Physical Science Technician, NIOSH/DTMD, January 1973.

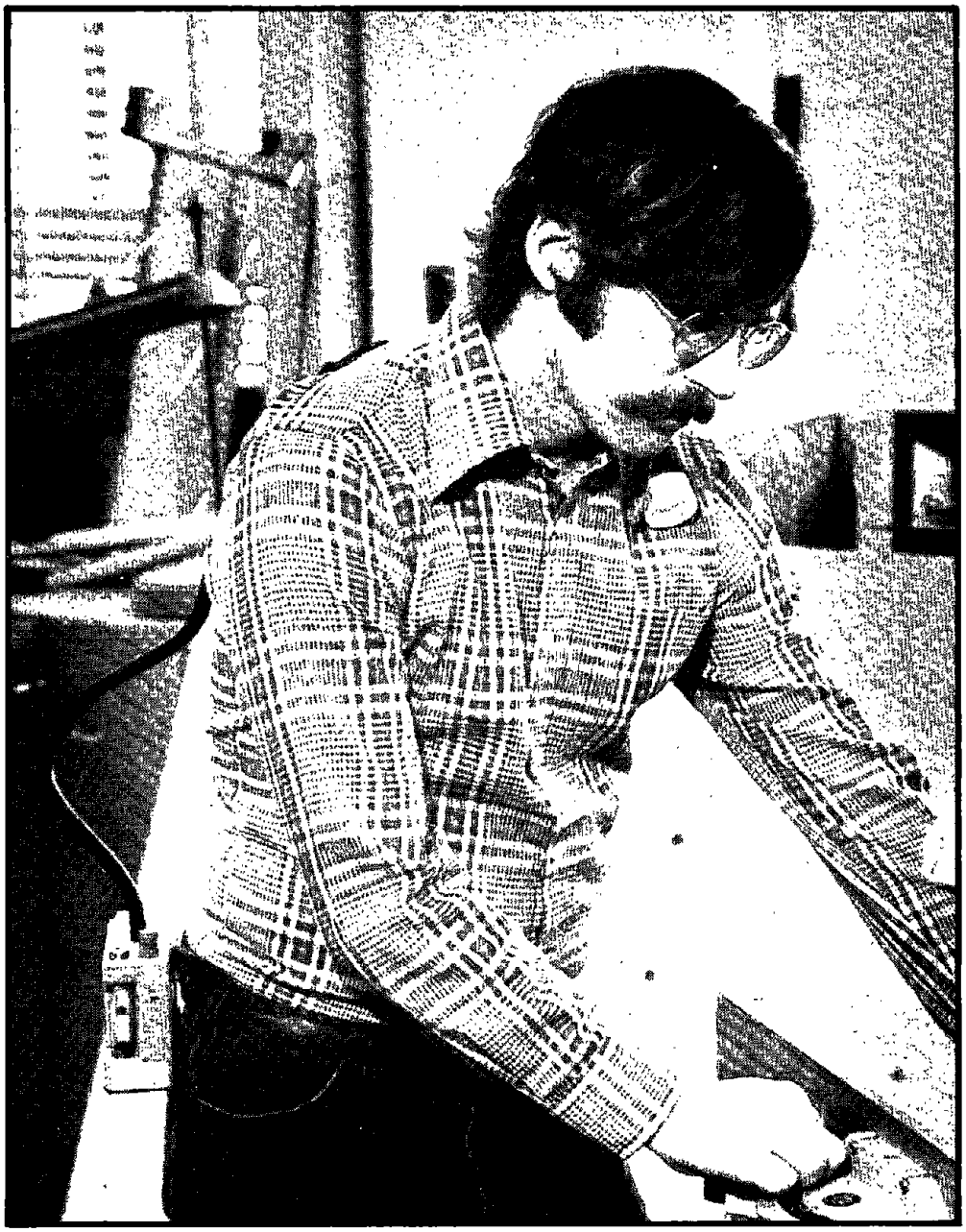


WET TEST METER



BUBBLE BURETTE

ASBESTOS SAMPLING



Prepared by Stephen G. Bayer, Training Instructor, NIOSH/DTMD, 1/79.

ASBESTOS SAMPLING

This lecture describes the basic requirements of an asbestos-sampling program necessary to establish compliance with OSHA regulations covering occupational exposure to airborne asbestos fibers. Here are your performance objectives for this lecture:

List the materials and limits covered by the regulation.

Describe two specific monitoring requirements.

Write the formula for and describe the purpose of an 8-hour time-weighted average.

Given flow rate and field area, use a nomograph to calculate the proper sampling time for an airborne asbestos concentration ranging between 2 to 10 fibers per cubic centimeter.

List the necessary data to be recorded at the time of sampling.

You may be expected to complete these objectives in a quiz at the end of today's instruction.

The OSHA safety and health standards 29 CFR 1910 section 1001 state ". . . every employer shall cause every place of employment where asbestos fibers are released to be monitored in such a way as to determine whether every employee's exposure to asbestos fibers is below the limits. . . . "

The results of the monitoring program are compared to specified limits. If the limits are exceeded, the employer must undertake a compliance program to insure the workers' protection. The federal register defines asbestos fibers as those produced from six different materials: chrysotile, amosite, crocidolite, anthophyllite, actinolite, and tremolite.

Airborne concentrations of asbestos are compared to two types of limits. The first is the current permissible limit of 2 fibers greater than 5 μm in length per cubic centimeter of air. This limit is based upon an 8-hour time-weighted average. The second is a ceiling limit of 10 fibers greater than 5 μm in length per cubic centimeter of air. There is no specific time duration for a ceiling cencentration. In December of 1976, NIOSH published a revised criteria document. In this document, NIOSH suggests lowering the exposures to one-tenth of a fiber per cubic centimeter for the permissible limit and one-half a fiber per cubic centimeter for a peak limit.

Let's look closer now at the difference between the permissible limit and the ceiling limits as they apply to sampling. A permissible limit is the average dust exposure at a location on a specific day. The formula for calculating this average exposure—more properly called a time-weighted average—is as follows: The measured concentration of each sample is multiplied by its sample time expressed in hours. The products are added together for all the samples taken. This sum is then divided by the total sampling time of all the individual samples and should be equal to 8 hours. If the time is other than 8 hours, statistical determinations should be made and these are beyond the scope of this lecture.

Here is one example:

CONC.		TIME		PROD.
0.80	×	2	=	1.6
3.10		1		3.1
1.30		1		1.3
0.57		3		1.71
1.10		1		1.1
		8 hrs.		8.81 fibers/cm^3

$8.81 \div 8 = 1.10$ fibers/cm^3

This conc. of 1.1 is in compliance with the permissible limit of 2 fibers per cc.

Here is another example:

3.1	×	2	=	6.2
2.0	×	2	=	4.0
.03	×	4	=	.12
		8 hrs.		10.32

$$\frac{10.32}{8} = 1.29 \text{ fibers/cc}$$

As you can see, this location is also in compliance with both the permissible limit and the ceiling limit since no sample was over 10 fibers/cc.

Here is a third example:

14.0	×	0.5	=	7.0
2.0	×	3.5	=	7.0
0.5	×	4.0	=	2.0
		8 hrs.		16.0

$$\frac{16}{8} = 2 \text{ fibers/cc}$$

Not only is this location possibly in violation of the permissible limit, one of the samples is well over the ceiling limit.

Please keep in mind that this presentation covers only basics. That is why these calculations are kept simple. However, an employer proving compliance with analytical data and a compliance officer proving noncompliance must apply certain statistical methods which account for the many types of error that makes analytical data questionable.

The federal regulations require industry to collect personal and environmental samples as part of the sampling program. A personal sample is taken by turning the sampler on, adjusting it to the selected flow rate, and then securing it to the worker's belt. The connective tubing is run over the worker's shoulder. Clip the cassette to the lapel as close to the worker's mouth as possible. The open face of the cassette should point the same direction as the worker's mouth and still hang slightly down. The tubing should be secured to the back by clipping it to the shirt. This system will

collect a sample uniquely associated with the person wearing it. In spite of the importance of a personal sample, it is not the only kind required by law. To monitor the extent and magnitude of dust clouds carried by air currents to various areas around the plant it is necessary to collect environmental samples. The equipment and procedures are similar to those necessary for collecting personal samples except that the sampler is placed at a fixed location. Such locations could be at a worktable, attached to a wall, or at a desk in an office area.

Results obtained from both personal and environmental samples are compared to permissible and ceiling limits. The federal register states that the employer has the responsibility of collecting the samples in such a manner that the frequency pattern represents, with reasonable accuracy, employees' levels of exposure. When it can reasonably be foreseen that an employee's exposure level may exceed the legal limit, the period of time between samples cannot exceed six months. Selecting the proper flow rate and sampling time is an important consideration in the sampling process, for these two factors determine the fiber density on the filter.

NIOSH has published in your manual the method's coefficient of variation as a function of total fiber count. Briefly, the coefficient of variation is smallest when a sufficient sample has been taken to produce a total fiber count of 100. A count less than 100 increases the coefficient of variation which is undesirable.

In your course manual, there are several charts which help select a sampling time. These are called optimum-sampling-time nomograms. The one on page 54, Figure 4, is good for general-purpose usage. Please turn to page 54 in your manual. To determine the sampling time, select a flow rate and the field area of the microscope used to count the sample, draw a straight line through the points, and read the time in the center column. This time is called optimum because if the fiber concentration is between 1—10 fibers/cc, then each field area counted should have from 1—5 fibers in it producing field counts of 100 to 20 respectively. Your instructor can explain the details and applications later in the course.

Whenever a sample is taken, it is important to record as much data as possible. Write the sample number on the cassette and the data sheet. Also record the control or blank-filter number. When the pump is activated, record the start time and later, when the sampling is completed, record the finish time. Calculate the duration in minutes and hours. Record the start and finish flow rates, then average them. Identify the type of sample and describe the person or location as completely as possible. These four pieces of information: sample number on the cassette and data sheet, time span, average flow rate, and thorough identification are absolutely essential. There are other pieces of information which prove useful. Describe any control methods such as hoods or respirators. Quickly check the hoods with smoke tubes to estimate their effectiveness. If a respirator is being worn, inspect it, record the make, model, and cannister number. Ask the worker to rate the production activity, low, medium or high. This has an effect on the concentration and may account for extraordinary concentrations. Collect, label, and describe bulk samples which may provide analytical clues to the microscopist when the sample is being evaluated. For example if you think some insulation contains asbestos, send a piece of it along with your air samples.

In summary, a pump and filter is used to collect personal and environmental samples whose analytical results are compared to permissible and ceiling limits. Flow rates and sampling times are selected from nomograms. Record as much data as possible when sampling.

FAMILIARIZATION WITH THE MICROSCOPE: ACCESSORIES, CONTROLS, FUNCTIONS, AND ADJUSTMENTS

The objectives of this paper are to familiarize trainees with the accessories, controls, functions, and adjustments that may be found on phase-contrast microscopes. Since there is no standardization of control placement, and design factors may warrant appearance changes, the novice microscopist may become confused by a phase-contrast microscope. To help remedy the problem three microscopes and illumination systems (American Optical Company; Bausch and Lomb, Inc.; Leitz, Inc.) have been selected as representative examples. The microscope-illumination system will be described by starting at the light source and following the light beam until it exits the system.

I THE ILLUMINATION SYSTEM

It is the function of the illumination system to provide even, glare free illumination of the specimen. The Koehler method provides excellent illumination, and it is specified as a requirement for counting asbestos fibers by phase-contrast microscopy. There are several types of illuminators and each may provide Koehler illumination.

There are three basic configurations of microscope illuminators. The first type is external illuminators (Photo 1). These are in no way attached to the microscope. They shine a beam of light at the microscopes mirror. Substage illuminators are the second type (Photo 2). These may be so compact that they will fit between the legs of the microscope base or so elaborate that the illuminator provides a platform onto which the microscope is clamped. Built-in illumination is the third type (Photo 3). The illuminators are usually built into the base of the microscope and all of the components are permanently attached as an integral part of the microscope system. Nearly all built-in illuminators provide Koehler illumination.

In all three types of illuminators, the light source is usually a light bulb. The filament, the glowing wire inside the bulb, is the origin of the illumination beam and may be found in several configurations. Coil filaments (Photo 4) are least desirable for Koehler illumination. The roundness of the wire and the distance between the coils may provide a very unevenly illuminated microscope viewing field which lessens

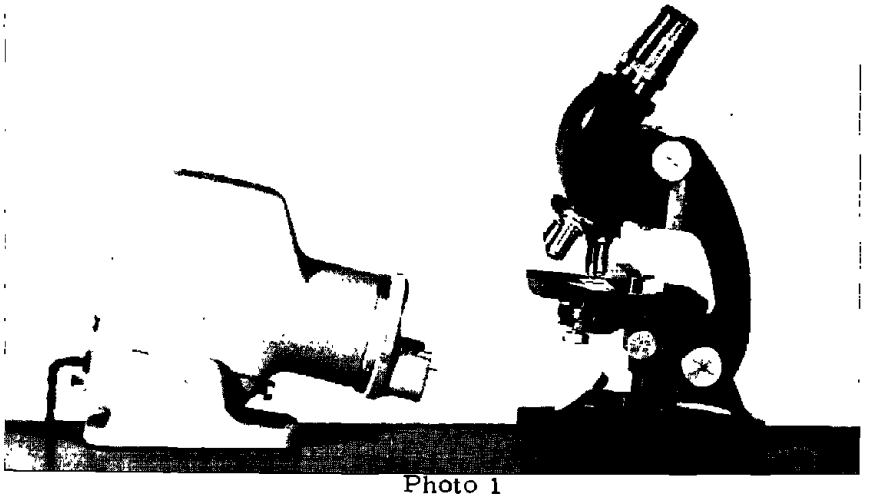


Photo 1

Prepared by Stephen G. Bayer, Physical Science Technician, Division of Training, NIOSH, 10/72.

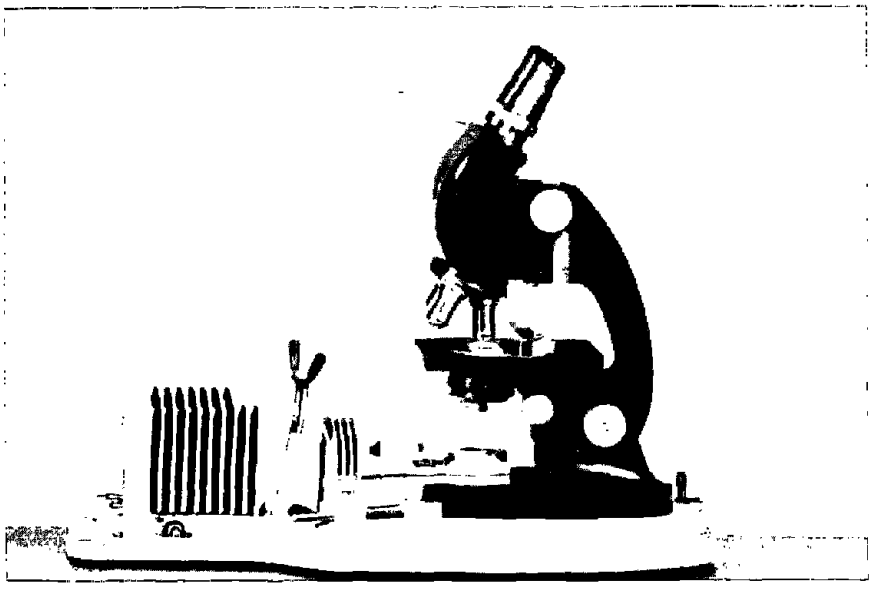


Photo 2

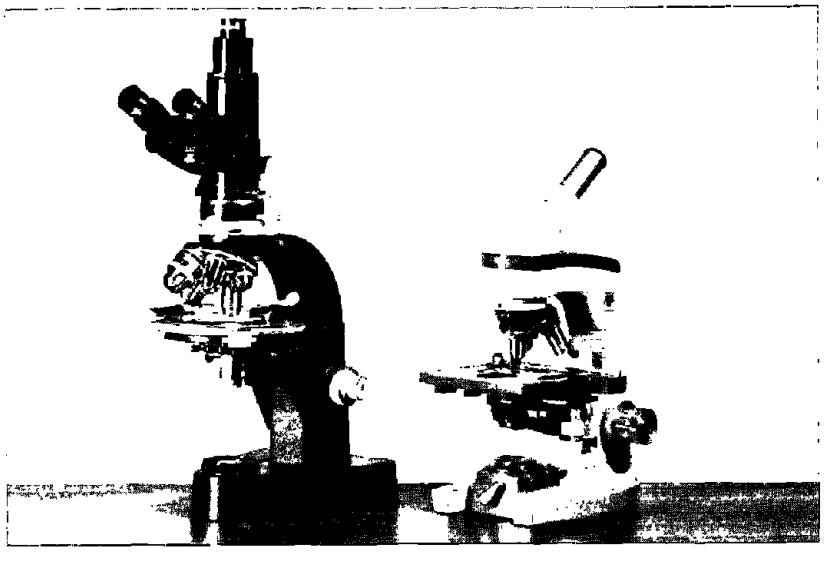


Photo 3

image clarity. Some illuminators use flat-wound coils (Photo 5) of round or flat wire. These are much better because each wire is like a small ribbon and the coils are close together. However, as in the case of most built-in illuminators, the coil filament bulbs may have ground glass between the bulb and the condensing lens. In this case, the ground glass may become the light source and can provide excellent illumination. Last is the ribbon filament bulb (Photo 6). The glowing ribbon filament produces a very even band of light which contributes highly to a superior image.

Often, one may wish to vary the intensity of the illuminating beam. This is accomplished in two ways: transformers and neutral density filters. Most illuminators have a transformer to step down outlet voltage to whatever voltage is required for the bulb. The transformer may have several taps and a

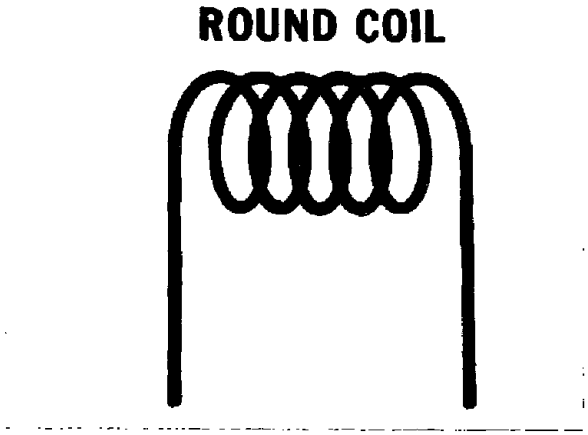


Photo 4

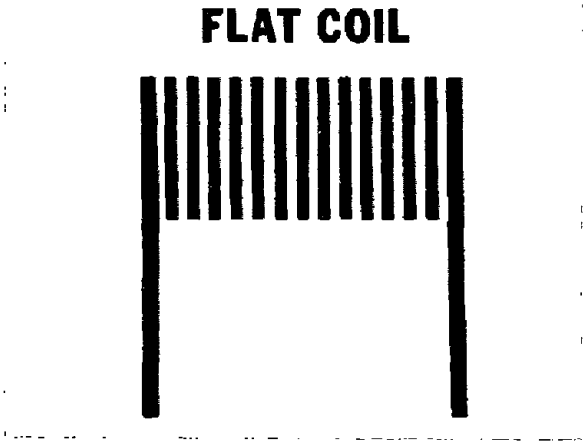


Photo 5

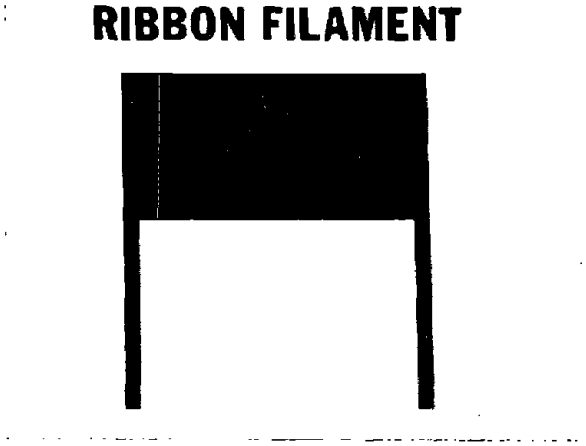


Photo 6

rotary switch so that different voltages (producing different light intensities) can be selected (Photo 7). Other illuminators may have a rheostat which enables the microscopist to select any desired intensity by turning a knob (Photo 8). The rheostat may or may not have a voltage indicating meter. The second form of brightness control one may find is neutral density filters (Photo 9). These may be mounted in a wheel inside the illuminator or simply placed on or in the filter holder. Neutral density filters are silver in color. They are used to dim light intensity and do not impart any coloration to the light beam.

The first optical system through which the illumination beam will go is the illumination condensing lens. This lens is used to project an enlarged image of the illumination source. The lens may or may not be moveable depending upon the type and design of the illuminator.

After the illumination beam has passed through the condenser, its diameter is determined by the field iris (Photo 10). This is used to limit the size of the beam to a slightly larger size than the microscope's field or view.

There are several controls for adjustments on external illuminators. A tilt control knob (Photo 11) will provide proper illumination in the vertical axis. The condensing lens focus knob (Photo 12) controls focus adjustment of the projected illumination source. The outer diameter of the field iris (Photo 13) turns, allowing the microscopist to alter the diameter of the field iris opening.

Since substage and built-in illuminators have similar construction, the controls and adjustments are similar. They usually have a bulb centering control (Photo 14) for proper beam alignment. They may also have controls for centering the field iris (Photo 15). Since the condensing lens is usually fixed, focus may be altered by sliding the light bulb back and forth (Photo 16). The field iris will be similar to that on an external illuminator.

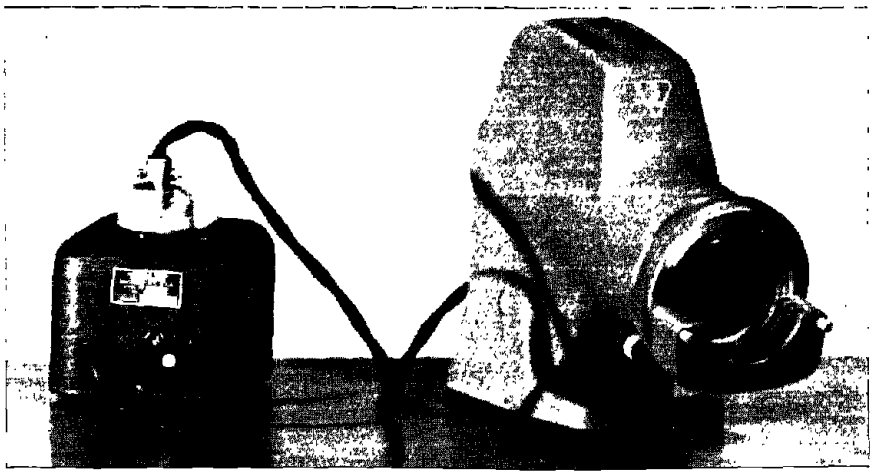


Photo 7

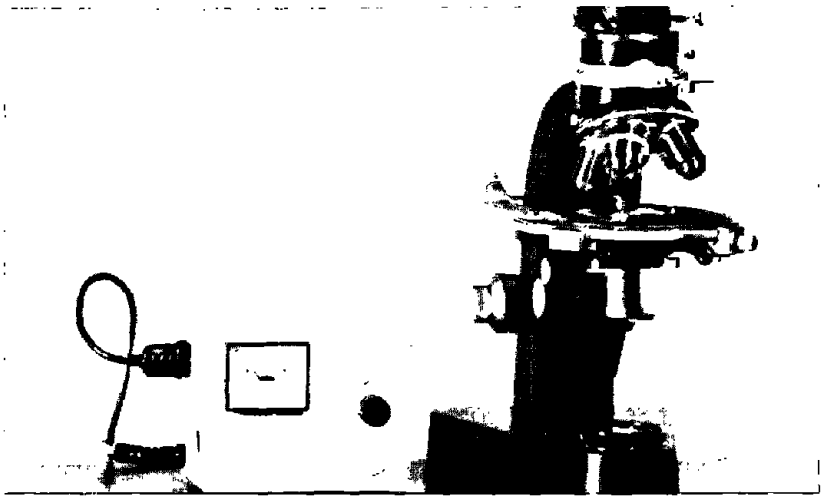


Photo 8

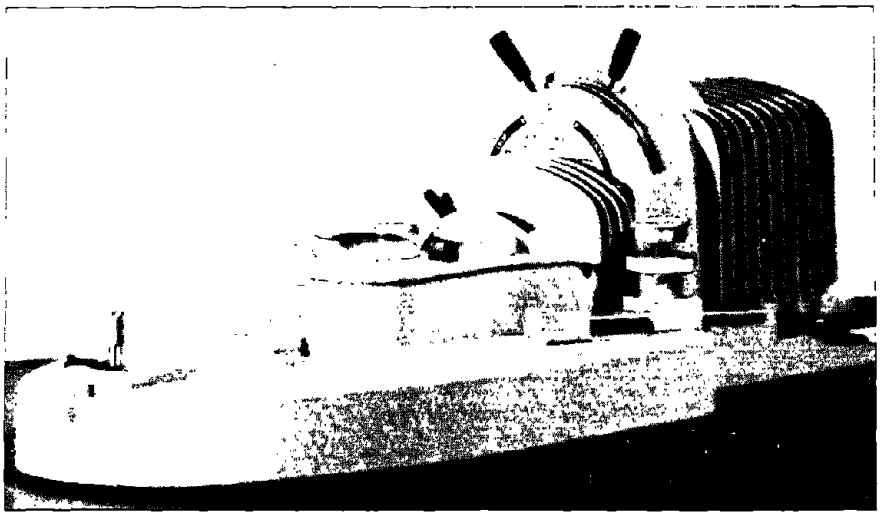


Photo 9

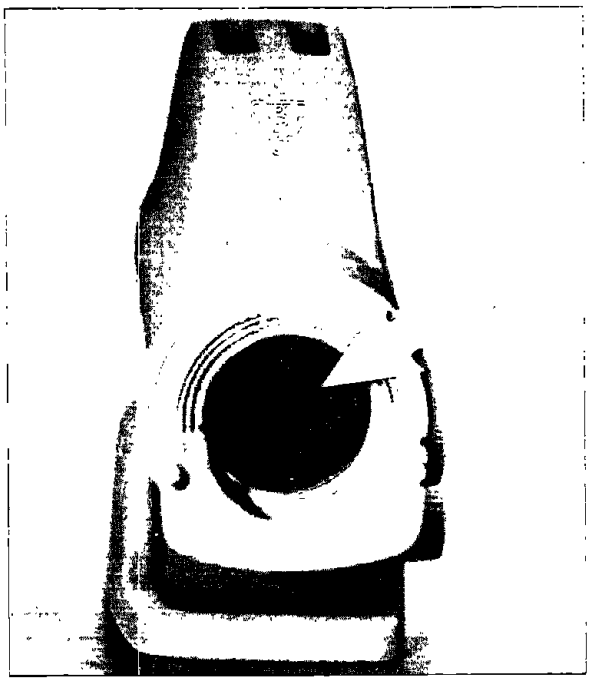


Photo 10

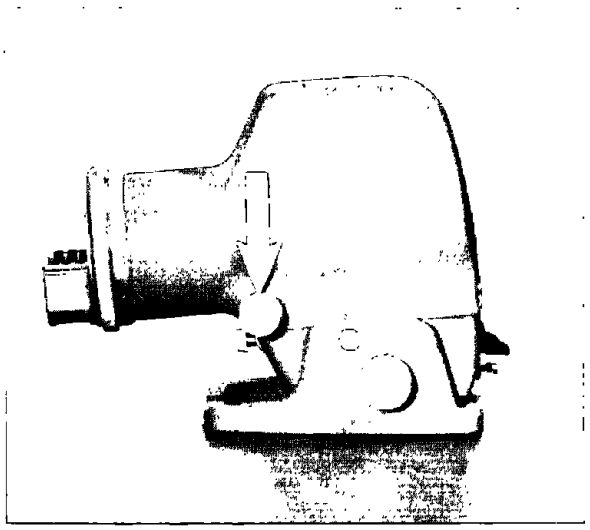


Photo 12

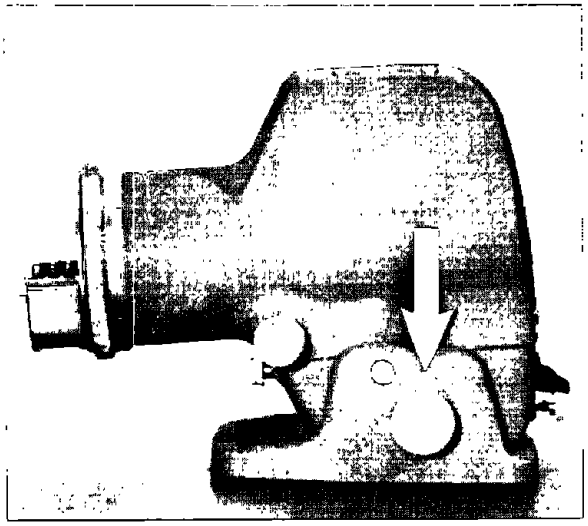


Photo 11

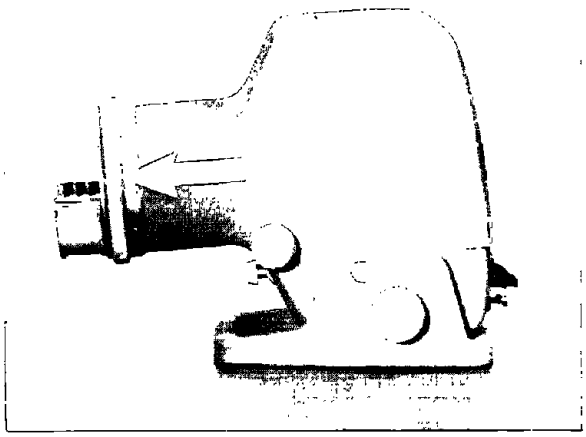


Photo 13

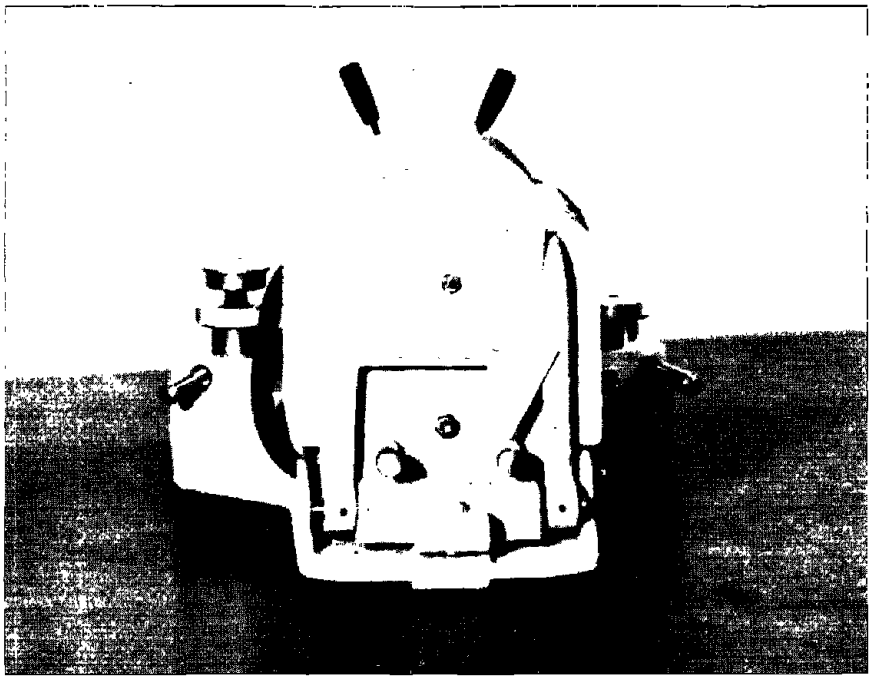


Photo 14

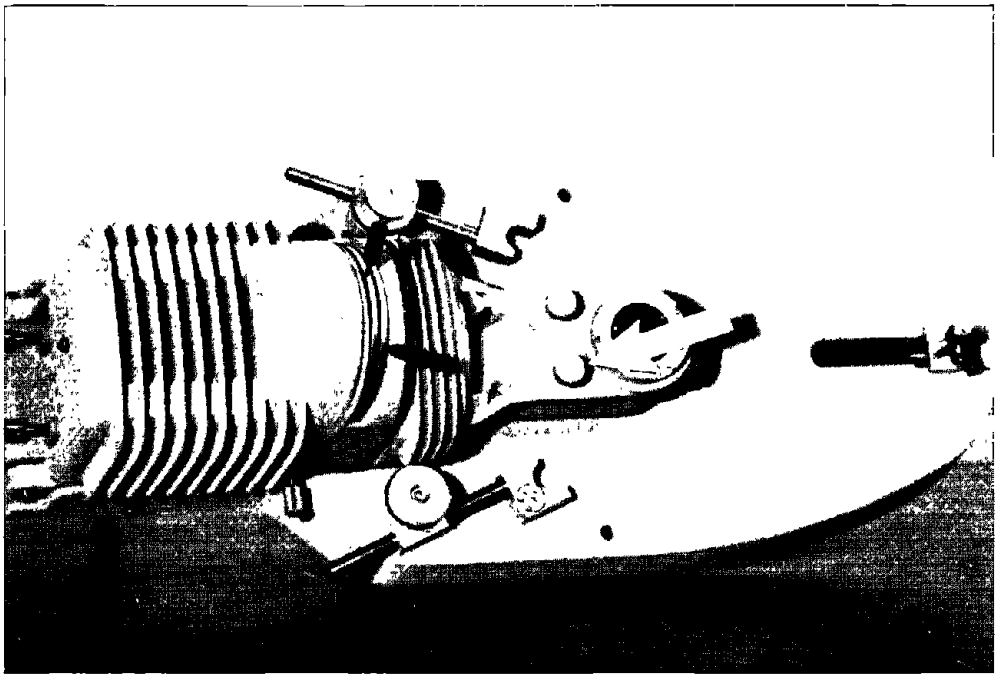


Photo 15

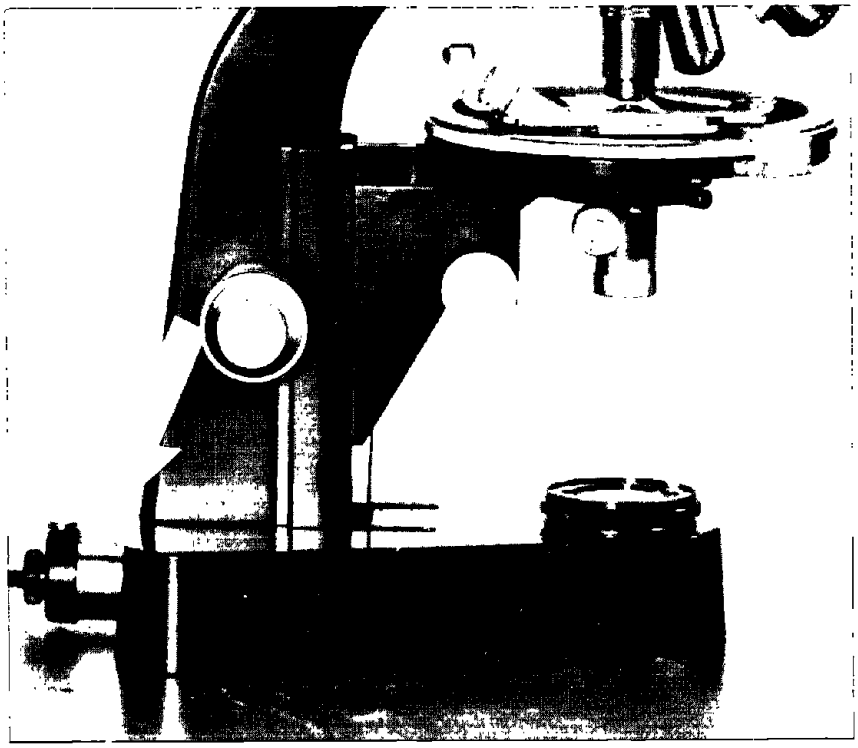


Photo 16

Regardless of the type of illuminator, the system nearly always includes a mirror. There are two types of mirrors (Photo 17), and the microscope's final image can be significantly affected depending on the type which is used. The least desirable mirror is a second-surface mirror. It has the silvering material placed upon the back of the glass. The diagram of the second surface mirror shows that a reflection of two parallel beams of light produces three images. Image one is the original beam reflection. Image two results from a slight reflection from the glass surface. Image three results from reflection and refraction inside the glass. The first-surfaced mirror has the silvering material placed upon the front side of the mirror and they are highly desirable for Koehler illumination. Since no multiple images are formed because of refraction before or after reflection from the silvering, the original light beam will be unaffected.

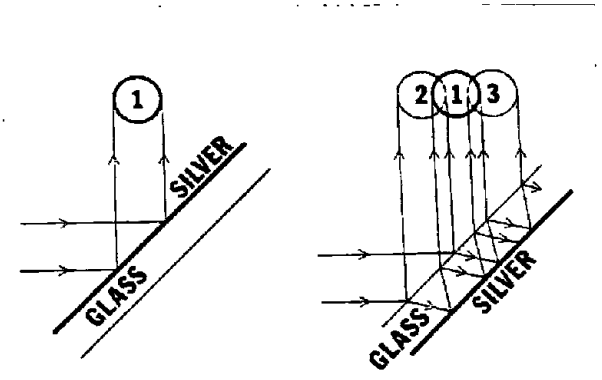


Photo 17

After the light beam strikes the mirror, its light path is diverted into the condenser - the first part of the optical system. It is the condenser's function to change the large beam of light from the illuminator into a tiny spot of intense light.

The Zernike and Abbe condenser (Photo 18) is most commonly used for phase contrast. On or around the condenser, one may find many controls. There will be one large knob on the side of the microscope (Photo 19). This knob is the condenser focus knob and it moves the condenser up and down. This is the adjustment mechanism for focusing the image of the field iris in the plane of the specimen. On the body of the condenser, one may find a small tab (Photo 20) that slides left to right. This tab controls the condenser iris. If one looks through the bottom of the condenser while moving the control, it will be easy to see the diaphragm open and close. This condenser diaphragm or iris is used to control glare. (The substage condenser iris should never be used to control light levels. For phase-contrast viewing, the condenser iris must be fully open. On or around the outside of the condenser, there may be a wheel containing the various annular diaphragms (Photo 21). There will be a window or a pointer to indicate which ring is in place. The number of the annular diaphragm must correspond to the magnification of the objective lens. On the left and right sides of the condenser there may be screws that center the annular diaphragm with the phase-shifting element (Photo 22). On the other hand, some microscopes may have individual adjustments for each annular diaphragm, and other microscopes may have none at all.

The Heine condenser (Photo 23) is another type of phase-contrast condenser. This one contains a mirror element which projects a hollow cone of light. The condenser focus knob (Photo 24) is used to place the condenser at its highest position. A small knob on the side of the condenser body controls the height of the mirror element (which slides up or down within the interior of the condenser body.) At the highest position of the element, dark field viewing can be utilized. By moving the element down, the cone becomes smaller, and, at about the middle, the ring becomes concentric with the phase-shifting element and the result is phase-contrast illumination. Further lowering of the element will produce light field illumination. On each side of the condenser there will be knobs for centering the illumination. The Heine condenser has no condenser iris.

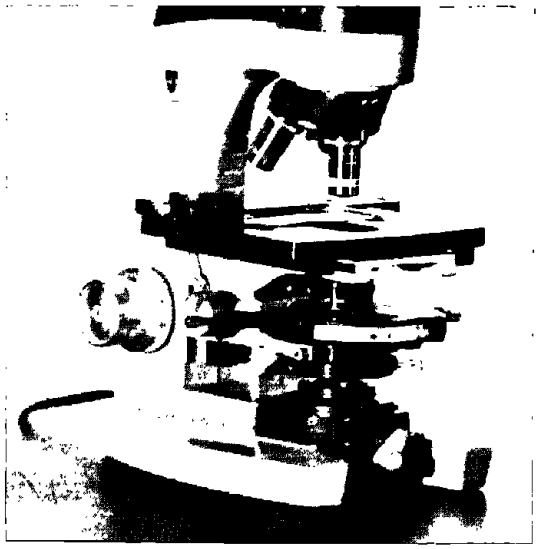


Photo 18

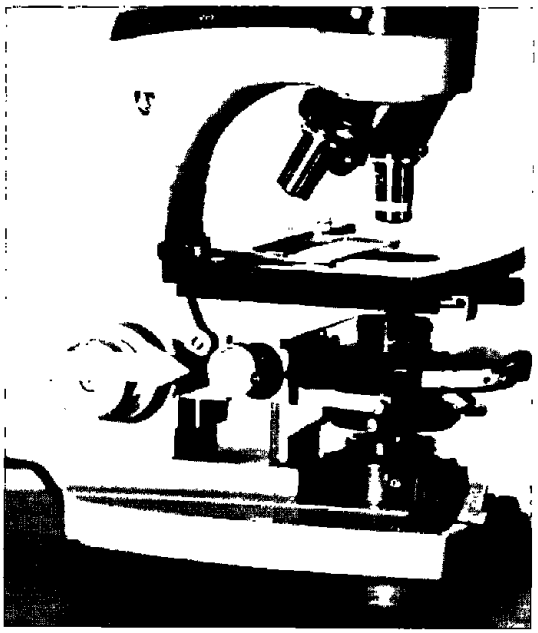


Photo 19

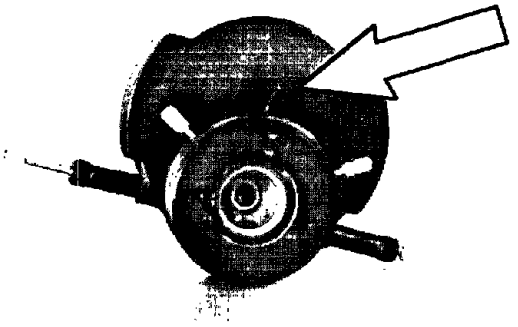


Photo 20

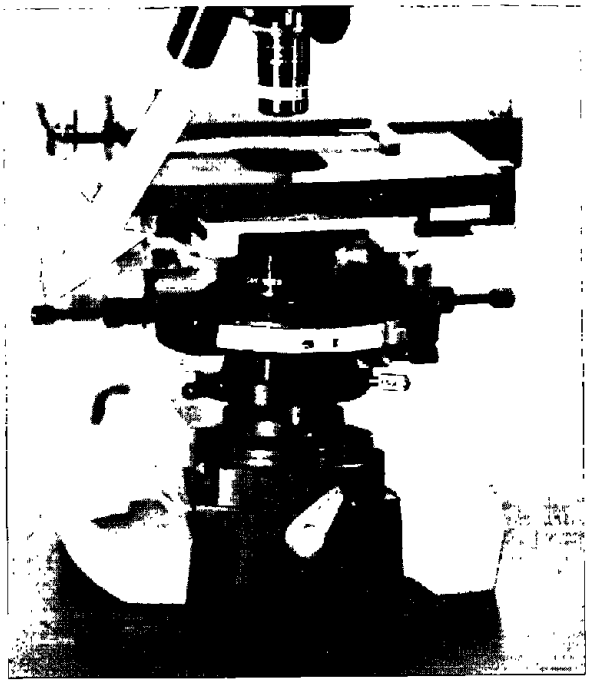


Photo 22

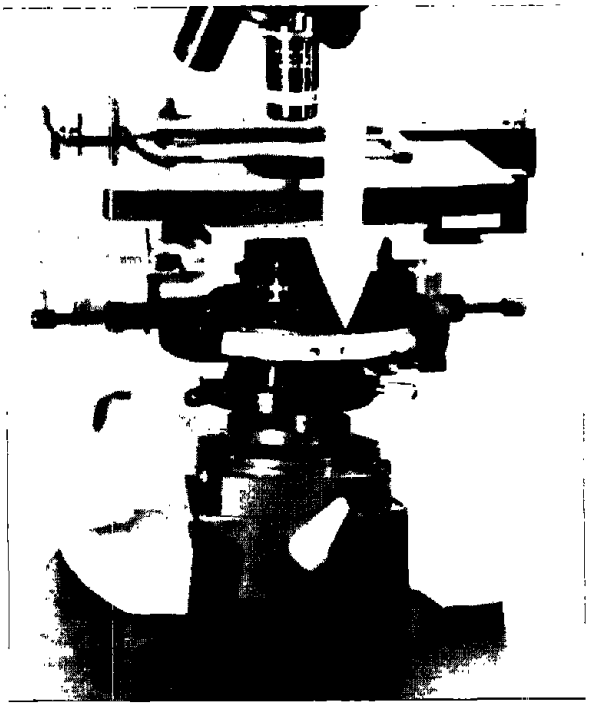


Photo 21

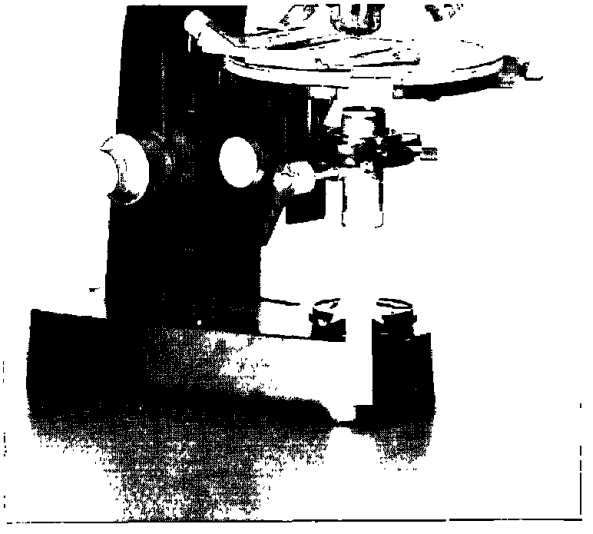


Photo 23

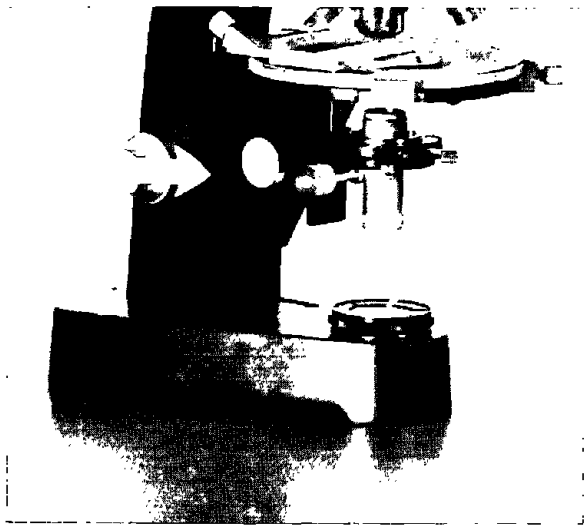


Photo 24

The light path now travels to the stage - the flat part of the microscope body upon which the slide mount is placed. Since asbestos sizing requires a magnification of nearly 450X, one must realize that every movement of the slide is also magnified by 450X. The clips, which are standard equipment, are totally undesirable. One must obtain a mechanical stage (Photo 25). This device, having two knobs, will provide smooth, jerkless travel in the X, Y directions. It also will help the microscopist count in a straight line across the filter. The mechanical stage will accept slides whose dimensions are approximately 1 x 3 inches.

II THE OPTICAL SYSTEM

After the illuminating beam passes through the slide, it is collected and magnified by the objective lens (Photo 26). This lens is the highest magnifying lens on a microscope. They vary from around 3X to about 120 X. Lenses are rated by a number called the numerical aperture. This number represents the light gathering quality of the lens. The higher the numerical aperture is, the finer will be the detail that is produced. One may be offered a selection of the three types of objectives when purchasing a microscope. These are achromats, flourites or semi-apochromats, and the apochromats. Achromats are usually optically corrected for yellow-green light and require the use of a green filter. Flourites are corrected for two colors. Apochromats are corrected for three. Achromats are good for general application. The others are better (but costlier) so the decision may be only a matter of economics. The one adjustment which may be found on

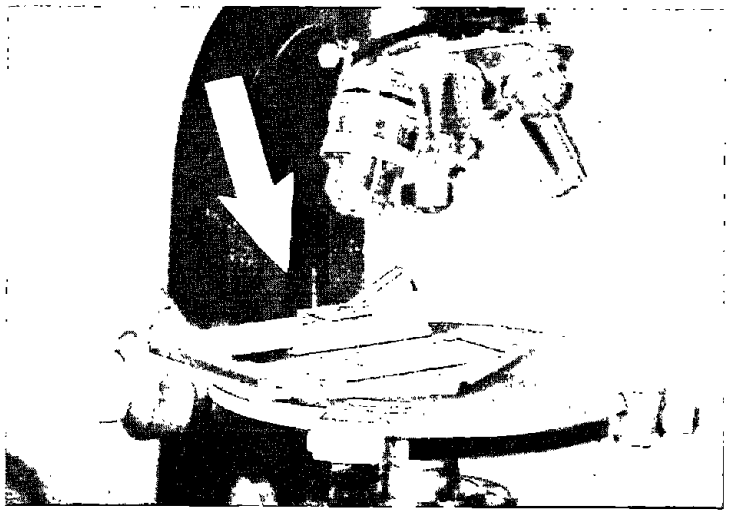


Photo 25

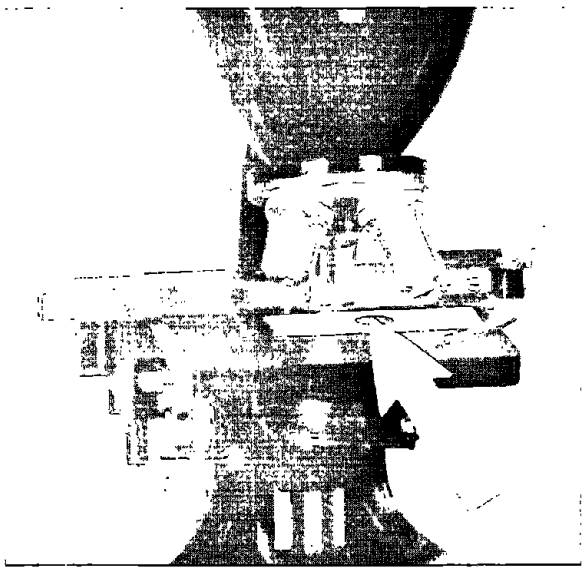


Photo 26

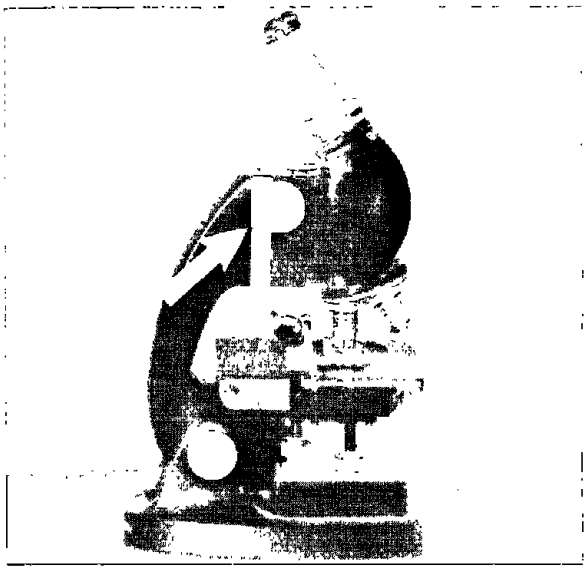


Photo 27

an objective lens body is a cover glass correction collar which corrects the optical system for variations of cover glass thicknesses. Part of the specifications of an objective lens is the working distance. This tells how close the objective lens is to the coverslip when the microscope is in proper focus. Objective lenses with a high numerical aperture usually have a short working distance. (One objective having 40X magnification and a .70 numerical aperture has a .33 mm working distance.) The distance between the objective lens and the coverslip is adjusted by two knobs. The coarse-focus knob and the fine-focus knob are found on the side of the microscope. The coarse-focus knob (Photo 27) changes the distance a great deal with only a small knob movement. The fine-focus knob (Photo 28) may require twenty revolutions to move the objective the same distance as one turn of the coarse-focus knob might. Some microscopes move the stage away from the objective and others move the objective away from the stage. The result is the same. In certain models, the coarse and fine-focus knobs may be built into one knob, or in others they may be separate.

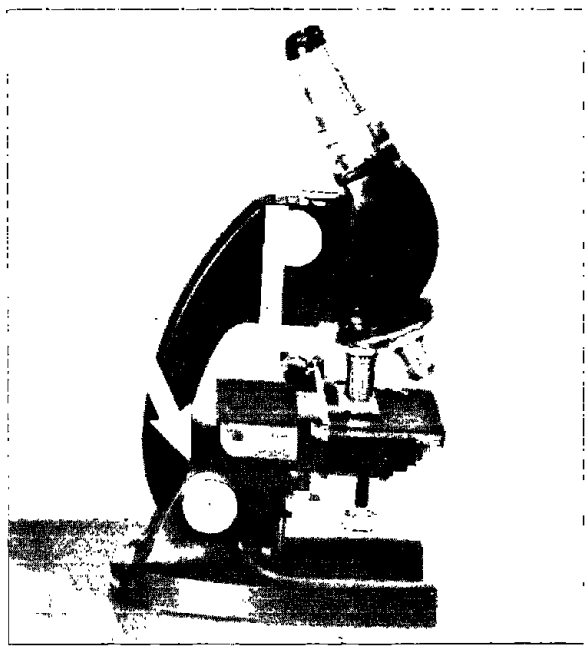


Photo 28

The light beam passes through the objective lens and into the microscope head. The head may be so simple that it contains only one eyepiece or so complex that it contains prisms, analysers, filters, and apertures. There are three heads most commonly found, and the discussion will be limited to these.

The monocular head (Photo 29) is the most simple. It is a single tube either straight up from the objective or at 45 degrees. This head contains only one eyepiece. When the microscopist is examining the specimen, he or she leaves the eye that is not looking into the eyepiece open. After a while one's mind will cancel the vision from the unused eye, and all emphasis will be placed on the eye that is looking with the microscope. It is advisable to avoid using a monocular microscope if the microscopist must examine samples for many consecutive hours per day.

A binocular head (Photo 30) has two eye pieces and it permits a more normal viewing situation. It has a couple of adjustments that are necessary for viewing with both eyes. The first is the interpupillary distance. This is necessary because the centers (pupils) of people's eyes have varying distances between them. This distance is controlled by either turning the barrel of one eyepiece tube, or by moving a lever. The second adjustment

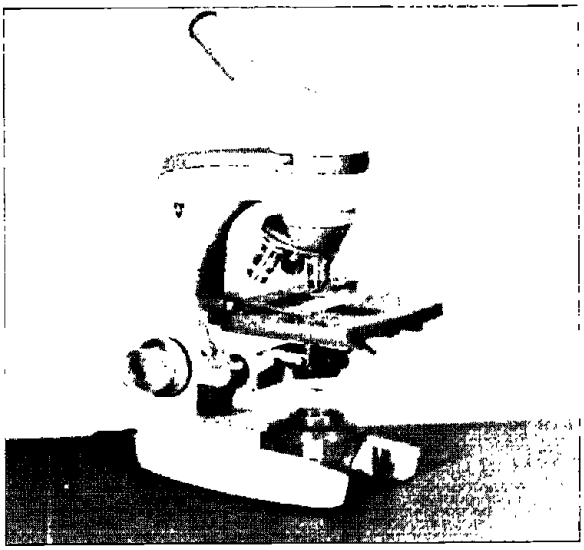


Photo 29

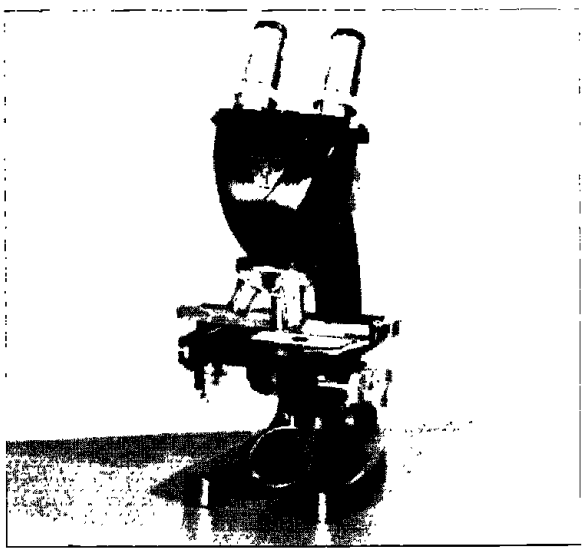


Photo 30

is the eye sight compensator. Every person may have slightly different vision in one of his eyes as compared to the other. Therefore, a binocular microscope has one eyepiece that is stationary and one that can be adjusted for proper focus. The microscopist uses the coarse and fine focus to obtain a sharp image through that eyepiece. The image in the adjustable eyepiece may be focused to clarity by rotating the barrel of that eyepiece.

A trinocular head (Photo 31) is little more than a binocular head with a third, verticle alternative path for the light beam. This verticle tube offers the microscopist the use of a camera. A camera is frequently a highly-desirable accessory because the writer has found that there are frequent requests about a sample's appearance. One may relate this information in terms of size, shape, color, etc., but, truly, a picture is worth a thousand words.

In conclusion, I have touched upon the various components one may expect to find on a phase-contrast microscope. Regardless of your past experience, you should now be familiar with such parts as the condenser focus knob and field iris control. You should be acquainted with operations such as closing the field iris or aligning the annular

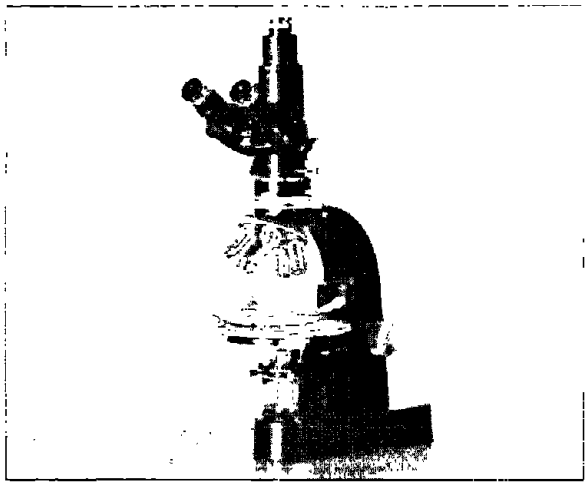


Photo 31

diaphragms. This concludes the lecture about familiarization with the microscope.

DEMONSTRATION: FAMILIARIZATION WITH THE MICROSCOPE

DEMONSTRATION OBJECTIVE

To develop a student's logic about setting up a phase-contrast microscope.

I. There are a number of apparent steps you must perform to see through the microscope.

   A. A slide must be on the stage and centered beneath the objective.

   B. Proper inter-pupillary distance.

   C. Proper eyesight compensation.

   D. Illumination on.

   E. Properly focussed.

II. The basics of the microscope set-up procedure has three (3) steps — usually in the following order:

   A. Focus on the sample.

   B. Focus the field iris on the sample.

   C. Center the phase-contrast elements.

III. How to focus on the sample.

   A. Place a dusty slide onto the stage.

   B. Select the 43x (or that equivalently marked to the 43x objective) annular diaphragm.

   C. Select the 10x objective.

   — This will give dark field illumination at 100x —

   D. Using the coarse-focus knob, move the objective close to the slide while observing the distance from the side of the microscope.

   E. Look into the microscope and widen the objective to slide distance.

   F. The sample will come into focus — white specks on a black background.

   G. Change to the 43x objective.

   H. Sharply focus with the fine-focus knob.

IV. How to focus the field iris.

   A. Remember — focus on the sample.

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B. Close fully the field iris.

C. Use the condenser focus knob to bring the many-sided opening into focus.

D. Open the iris until the field of view is entirely lit — then stop.

E. Remember — this operation takes only a few seconds — check the focus everytime you begin counting a slide.

V. Centering the phase-contrast elements.

A. Remove the eyepiece.

B. Insert the telescope.

C. Focus the telescope on the rings.

D. With the alignment controls, align the rings.

E. Remove telescope.

F. Replace the eyepiece.

VI. Results.

A. It takes about twenty seconds to set up the scope for counting.

B. If, when you look at the phase rings, the rings are not evenly illuminated, call the course director.

C. When you focus up and down, a feature should only get larger and smaller — if the feature moves side to side, etc., malalignment is the cause — call the course director.

D. Keep the eyepieces free of oil film by cleaning them with lens paper wrapped around a stick.

CALIBRATING THE MICROSCOPE

NOTES

This lecture period will be spent by discussing the equipment and procedures involved with setting up a microscope and calibrating a Porton reticle. First, we will discuss the placement of the reticle. Then we will discuss the microscope set-up procedures. Finally, we will discuss the calibration procedure.

I THE PORTON RETICLE

The Porton reticle is a round disc that is made of glass. It is about the same size and thickness as a dime. The reticle has a photographically-produced scale that will be discussed later.

The reticle is placed inside one of the microscope's eyepieces. We make a habit of placing it in the non-adjustable one since the height adjustment may alter calibration.

The eyepiece is called Huygenian or Huygens. It consists of two lenses: the eye lens which is nearest to the eye and the field lens which is farthest from the eye.

Between the two lenses is a field-limiting diaphragm. This limits the field of view to only the flattest part of the primary image coming from the objective.

The eye lens will see anything in the plane of the field-limiting diaphragm in shape focus; therefore, any object placed on this shelf will be sharply focused and superimposed on the field of view. The field-limiting diaphragm is where we set the reticle.

You may think that it is a simple matter to place the reticle on the shelf, but this is not so. You can expect to spend about a half-hour doing it right.

There are several reasons why the reticle mounting is difficult. Since the reticle is in sharp focus, any dirt on the surface of the reticle will also be sharply focused. So, it must be wiped clean prior to insertion in

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Prepared by Stephen Bayer, Physical Science Technician, Division of Training, NIOSH, 1/73.

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the eye piece. The second problem is placing the reticle right side up on the diaphragm. It will usually turn over during its travel down the barrel. If the reticle is inverted prior to its insertion, it will usually land correctly. The last problem is keeping dust off the reticle while screwing on the eye lens. This can be accomplished by holding the eye piece at 45^{o} while you screw in the eye lens. This will cause the metal chippings and paint flakes to fall on the side of the barrel instead of the reticle surface.

When you are finished mounting the reticle, insert the eye piece into the microscope. I'll return to the reticle later; now, we'll turn our attention to setting up our microscopes.

II MICROSCOPE SET-UP

At this stage, our illumination must be set up according to the Koehler method. His method has three requirements: (1) an image of the light source must be in focus at the plane of the microscope condenser iris, (2) the image of the field iris must be in focus at the plane of the specimen, (3) the field iris opening is slightly greater than the microscope's field of view.

Establishing Koehler illumination with an external, ribbon-filament illuminator such as the Bausch and Lomb PR-27 is easy to demonstrate. On the other hand, a microscope such as the Leitz Dialux with a Heine condenser and built-in illumination has its own special set-up procedure and the fact that it has Koehler illumination (and it does) is not easy to demonstrate.

I will limit the set up procedure to the Bausch and Lomb Dynoptic microscope and PR-27 illuminator. The same principles that apply to this particular system will apply to any similar system.

1 The Porton reticle must be mounted in eye piece.

2 Clip the stage micrometer into the mechanical stage.

3 Turn on the illuminator to the high position.

4 Place the front of the filter holder seven inches from the mirror fork.

5 Close the field iris.

6 Center the band of illumination (the projected image of filament) across the mirror fork.

7 Using the microscope's condenser focus knob, move the condenser upward until it nearly touches the slide.

8 Using the illuminator's condenser focus knob, move the lens fully forward.

9 Position yourself so that you can see the reflection of the bottom of the condenser in the mirror.

10 Close the condenser iris.

11 Sharply focus the image of the filament using either the illuminator condenser focus knob or the focus trimming knob at the rear base of the illuminator.

12 Open the microscope condenser iris full.

13 Open the field iris half way.

14 Select the 10X objective.

15 Select the 43X annulus.

16 The first step to finding correct focus is by looking from the side of the microscope while moving the objective to the coverslip until they nearly touch.

17 Look through the eye pieces and widen the objective-coverslip distance with the coarse-focus knob until the image of the micrometer comes into sharp focus.

18 Select the 43X objective and sharply focus the image.

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19 Close the field iris fully.

20 On the field of view there will be a bright spot of light:

a Be sure you are in focus on the stage micrometer.

b Using the microscope condenser knob, focus the spot.

c While looking through the microscope, open the field iris until it just clears the field of view.

After the illumination system is finished, there is one more thing that must be adjusted before you calibrate the microscope; this is centering the cone of illumination with the objective's phase-shifting element. Here are the necessary steps for completion:

1 Insert either the phase telescope (as in the case of Bausch and Lomb) or the Bertrand lens (as in the case of Leitz Dialux) into the optical system. This enables the microscopist to see both rings. One is bright and comes from the condenser; the other is dark and is in the objective.

2 By using the centering knobs on the condenser, make the rings concentric.

3 Remove the telescope or Bertrand lens. (The microscope is now properly set up for phase-contrast viewing and can be used for counting and sizing.)

At this time, we will shift our point of interest back to the Porton reticle.

III PORTON RETICLE CALIBRATION

This is the Porton reticle's image. It has nine circles, a rectangle, and nine globes. Above all this is an equation:

$$D = \sqrt{2^N}.$$

On the left side of the rectangle is a series of markings indicating a height of 100. The rectangle is divided in half: the left is divided into six smaller rectangles and the

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right half is a scale which goes to thirteen. Above the circles and below the globes are identification numbers.

Putting all this together, we have a rectangle that is twice as long as it is high. We see the left-hand markings that tell us the reticle is 100 something high.

This something is a unit of length called L. We now know that the reticle is 100L high and 200L long. We are beginning to gather information about measurements. The next thing we need to do is determine the relationship that the circles share with the rectangle; this is where the formula comes in.

As you may recall, the formula is $D = \sqrt{2^N}$. Deciphered, this means that the diameter (D) of a circle is equal to the square root of two which has an exponent of the circle's number.

For example, the diameter of circle 2 will be $D_2 = \sqrt{2^2}$. The diameter of circle 3 would be $D_3 = \sqrt{2^3}$.

So far, I have been discussing relationships. We need to know numbers that represent length: specifically in terms of millimeters and micrometers (.001 or 1 μm.)

Back to the unit of length, L. If we can relate L to an actual number of length, we are nearly home free. If we alter the equation to read $D = L\sqrt{2^N}$, each circle would represent a true measurement. All we need to do now is find a ruler.

The ruler a microscopist uses to calibrate his reticle is called a stage micrometer. It is the engraved slide we used to set up the microscope. Stage micrometers have a scale that is about 2 millimeters long and is sub-divided into .01 millimeter units.

Momentarily, let's turn our attention once again to the microscope.

1 Find the image of the micrometer with the 10X objective.

2 Focus it sharply.

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3 Change to the 43X objective and do likewise. (Since objectives are parfocal on a turret, the image will need only a slight adjustment with the fine-focus knob.)

4 Using the mechanical stage, relocate the stage micrometer. Align one of the long (.1 mm lines with one end of the reticle, and this will serve as the 0 (zero) reference line.

5 The length of the reticle is 200L - in this case .14 mm. (100L is the height - or .07)

6 Divide .14 by 200 to find L

7 L = .0007

Now we will return to our formula - $D = L\sqrt{2^N}$. We now have everything we need to find the diameter of every circle and even go up to 13 on the reticle's log scale. Now, let's calculate some circle diameters:

$D = L\sqrt{2^N}$

Diameter Circle 1 = $.0007\sqrt{2^1}$

Diameter Circle 1 = .0007 X 1.414

Diameter Circle 1 = .000989 = 1 μm

Diameter Circle 2 = $.0007\sqrt{2^2}$

Diameter Circle 2 = .0007 X 2

Diameter Circle 2 = .0014 = 1.4 μm

Diameter Circle 3 = $.0007\sqrt{2^3}$

Diameter Circle 3 = .0007 2.828

Diameter Circle 3 = .0019 or 2 μm

The diameter of circle three will be two times the diameter of circle 1. Every other circle will double in size; therefore, the diameter of circle 4 will be twice the diameter of circle 2 and so on.

D_4 = 2.8 μm

D_5 = 3.8 μm

D_6 = 5.6 μm

As we can see, the 5 μm size falls between the fifth and sixth circle. This is the reference we will be using when we begin counting and sizing.

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One thing is left; we need to find the value of $(100L)^2$. This is the area of the left side of the reticle - called the field area. This will tell you the size of the filter's area that we are examining.

$$100L \times 100L = .0049 \text{ mm}^2$$

In conclusion, I have shown you the principles of Koehler illumination, how to align the phase-contrast system, how to measure the reticle, and how to calculate the circles and field area.

You are nearly ready to begin counting and sizing.

RETICLE CALIBRATION WORKSHEET

Microscope _____

Eyepiece _____ × _____

Objective _____ × _____

Calibrated by _____ Date ____/____/___

1. 200 L (entire length) = _____mm
2. 100 L = _____
3. 100 L × 100 L = _____ = field area
4. Calculate circles

   Circle #1 = L × 1.414 =

   Circle #2 = L × 2.000 =

   Circle #3 = L × 2.828 =

   Circle #4 = L × 4.000 =

   Circle #5 = L × 5.657 =

   Circle #6 = L × 8.000 =

   Circle #7 = L × 11.314 =

   Circle #8 = L × 16.000 =

   Circle #9 = L × 22.627 =

RETICLE CALIBRATION WORKSHEET

Microscope _____

Eyepiece _____x _____

Objective _____x _____

Calibrated by _____ Date ____/____/___

1. 100 L (entire length) = _____mm
2. 100 L = _____
3. 100 L × 100 L = _____ = field area
4. Calculate circles

   Circle #1 = L × 1.414 =

   Circle #2 = L × 2.000 =

   Circle #3 = L × 2.828 =

   Circle #4 = L × 4.000 =

   Circle #5 = L × 5.657 =

   Circle #6 = L × 8.000 =

   Circle #7 = L × 11.314 =

   Circle #8 = L × 16.000 =

   Circle #9 = L × 22.627 =

FILTER MOUNTING PROCEDURE

This lecture has four objectives, and they are expressed in terms of what you will be able to do after the lecture is completed. The objectives are:

1. List all equipment necessary for sample mounting.

2. Explain how the mounting solution is prepared.

3. Explain how the filter is mounted on a slide.

4. Draw a good slide mount and explain why it is good.

The lecture will be considered successful if, at the end, you are able to correctly answer those four objectives. At the end of each lecture segment that applies to the objective, the appropriate objective will be shown.

For better understanding, the lecture is divided into two segments:

Part I: Preparing the Mounting Solution

Part II: Mounting the Filter

Part I: Preparing the Mounting Solution

The mounting solution that is used for this procedure is specific for mounting cellulose-ester membrane filters made by the Millipore Corporation. At the time of this taping, it is the only certified solution for mounting these filters. Criteria are being developed for other filters and mounting solutions.

This visual is a schematic of the slide mount. Mounting solution is placed on the slide, the filter is placed on the solution dust side up. The cover slip is placed on the sample. Here you see the dissolving filter being immersed in the solution which contacts the cover slip. The solution actually dissolves the filter and becomes an optically-homogeneous plastic mount which holds the sampled particulates in place. Note that I said the solution dissolves the filter. This is contrasted against a technique of refractive-index matching for light-field microscopy. With phase contrast this technique only makes the filter more visible. The mounting solution is a mixture of two reagents containing enough filter material to increase to a suitable level the viscosity of the solution.

Here is the equipment you will need for preparation of your mounting solution:

1. A bottle of dimethyl phthalate.

Prepared by Stephen G. Bayer, Training Instructor, DTMD, NIOSH, Cincinnati, Ohio, 1/79.

2. A bottle of diethyl oxalate.

3. A supply of clean filter material.

4. A 25 ml graduated cylinder.

5. A Wheaton balsam bottle.

6. A chemical balance.

7. Some silicone stopcock grease.

One thing to constantly keep in mind is cleanliness. The work area should be clean — and likewise, all the equipment. Acetone serves as a solvent for cleaning off old mounting solution. This will be necessary when cleaning the Wheaton balsam bottle for re-use in the future.

Your mounting solution is made by dispensing at a 1:1 ratio by volume dimethyl phthalate and diethyl oxalate. I would recommend that you make no more than 25 ml at a time. Under frequent use this will last several months. To prepare the solution, first dispense 12-1/2 ml of dimethyl phthalate into the burette. Now, pour in the diethyl oxalate until it reaches the 25 ml mark. Cap the bottles and set them aside. Remember the criteria? We must thicken the solution by adding .05 grams of clean filter material for each milliliter of solution. You will now need a balance to weigh the filters — in this case 1.25 grams. Generally, the filters weigh about .04 grams; therefore, you will need about 31 filters — but weigh out the quantity. When you remove new filters from the case, you will see that between each filter there is a blue separator. These are discarded. You can use your fingers to manipulate the filter — but be sure your hands are clean. If you drop the filters, throw them out. Take the solution in the cylinder and pour it into the Wheaton balsam bottle — leave the bottle open. Remove the weighed filters from the balance pan and shove them, a few at a time, into the balsam bottle. Use the glass rod to push them down into the bottle if necessary. The filters will now dissolve slowly. You may want to stir the solution every hour or so. The mouth of the lid should be coated with stopcock grease to assure a good seal. Within 24 hours, the solution will be ready to use. As you can see, this pre-made solution is somewhat cloudy — but quite useable. The mounting solution has a normal shelf life of about six months. (There is speculation that refrigeration may extend that.)

So, to summarize, we have prepared the mounting solution by adding dimethyl phthalate to diethyl oxalate in a one-to-one volumetric ratio. Then we added .05 grams of filter material to each milliliter of prepared solution to increase the viscosity. At this point, you should be able to perform these objectives:

1. List all equipment necessary for sample mounting.

2. Explain how the mounting solution is prepared.

Part II: Mounting the Filter

Now that the mess has been cleaned up from part one, we are ready to progress to mounting the sample. Here is the equipment you will need:

1. Your slides will be 25 x 75 mm or 1 x 3 inches with one end frosted.

2. A scalpel with a #10 curved blade works well to cut out a filter segment. A pair of micro-scissors works just as well.

3. You will need a pair of fine-pointed tweezers to lift the filter segment from the cassette.

4. A spatula or fire-polished glass rod is used to spread the mounting solution upon the slide.

5. Buy quality #1-1/2 cover slips which are 25 mm.

6. You'll also need your mounting solution.

7. A supply of lint-free lens tissue is needed for cleaning slides, etc.

8. You'll need a pencil with which to label the slide.

9. Finally, you'll need the sampled filter.

Let's go on to the mounting procedure. Keeping in mind that cleanliness is important, keep open bulk samples — or anything else that is dusty — away from the mounting area. First, lay a couple of sheets of lens paper in front of you on the work surface. The slides will be placed on one, and the mounting utensils on the other. Clean the slides by breathing on them and wiping them with lens tissue. Until you become proficient, mount one slide at a time. Your slides should be cleaned whether or not they are sold to you "precleaned." Cover slips are cleaned in the same manner. Lay the cover slip so that one edge rests upon the non-frosted end of the slide. This eliminates the possibility of lint contaminating the cover slip. Using the glass rod in the Wheaton balsam bottle, apply some mounting solution to the center of the slide. The quantity of solution you use will depend on how large or small you cut your filter segment. Usually, it will be about a drop. Upon withdrawal from the solution, one may note that several drops fall readily from the rod. The size of the second or third drop will be more consistent than the size of the first. Do not touch the Wheaton balsam bottle rod to the slide. Now that we have the drop on the slide, return the glass rod to the bottle and replace the cap. Take the spatula or supplemental glass rod and clean it with lens tissue. Then redistribute the solution into a triangular shape about 1/2 inch on each side. Open the field monitor at the bottom and center section. This reveals the filter. The gridded filter is being used for demonstrative purposes — they are not recommended in the procedure. Clean the scalpel and cut a wedge that is the same size as the mounting solution triangle on the slide. Very little pressure is needed to cut the filter. Cut it with a rocking motion starting from the center and working to the circumference. Clean the tweezers and use them to lift the filter wedge from the cassette. Note how the wedge is held by the filter circumference. Do not touch the wedge with your fingers. The wedge is now placed dust side up on the mounting solution. You may wait about twenty seconds before placing the cover slip on the wedge. If your work area is dusty, cover the filter right away. Once the cover slip is in place, do not re-position it for any reason. After the normal waiting time of ten to fifteen minutes is past, the filter mount should be perfectly transparent. If it isn't, it's because a thin layer of air exists between the solution and the cover glass. This is remedied by pushing lightly on the cover-slip with a clean surface — such as tweezer tips; but pushing on the cover glass is not — I repeat not — a normal part of the procedure. Do not forget to label the slide with the sample number and current date before progressing to the next slide.

In mounting your slides, you will need to strive for an "ideal" filter mount. After the filter is dissolved, the mounting media should extend only slightly beyond the original filter's dimensions. Here you see both extremes of bad filter mounting. The black areas around the filter represents how far the solution has spread. The bottom filter did not completely dissolve because too little solution

was used. The top filter was mounted in too much mounting solution. It has spread to the edges of the cover glass. Here you see that the use of too much mounting solution produces particle migration — which will directly lessen the apparent dust concentration on the filter. Migration will most likely start at the edges — so stay away from the sample's edge while counting. This shows an ideal slide mount. Note that the solution extends only slightly beyond the filter. After your slide is mounted, you should count it within three days. After this time, crystals may grow — and sometimes they form a fibrous shape. The lack of a permanent slide mount worries some people; but, remember, the filter is permanent and another mount can be made at any time. In summary, a proper quantity of mounting solution is applied to a slide. The filter is placed dust side up upon the solution. A number 1-1/2 coverslip is placed on the preparation and the slide is then labeled. At this conclusion of the lecture, let's review the last two objectives:

1. Explain how the filter is mounted on a slide.

2. Draw a good slide mount and explain why it is good.

FILTER MOUNTING LABORATORY

LABORATORY OBJECTIVE:

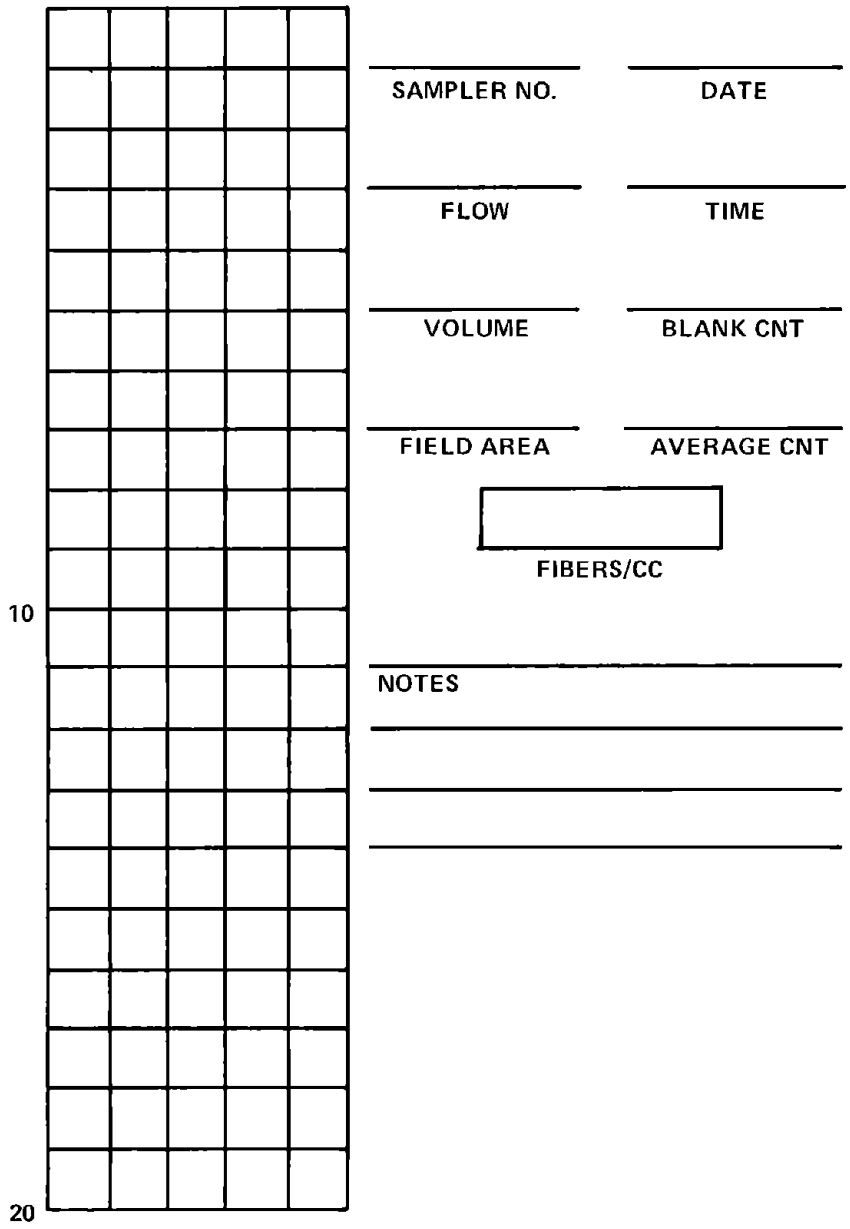
The trainee will properly mount a filter wedge on a slide.

EQUIPMENT:

1. Box of assorted filters
2. Mounting utensils
3. Mounting solution
4. Slides and coverslips
5. Lens tissue

PROCEDURE

Follow the procedure in "Sampling and Evaluating Airborne Asbestos Dust." Mount three (3) samples and initial them. Compare those to the chart to evaluate your progress. Look at them again the following day.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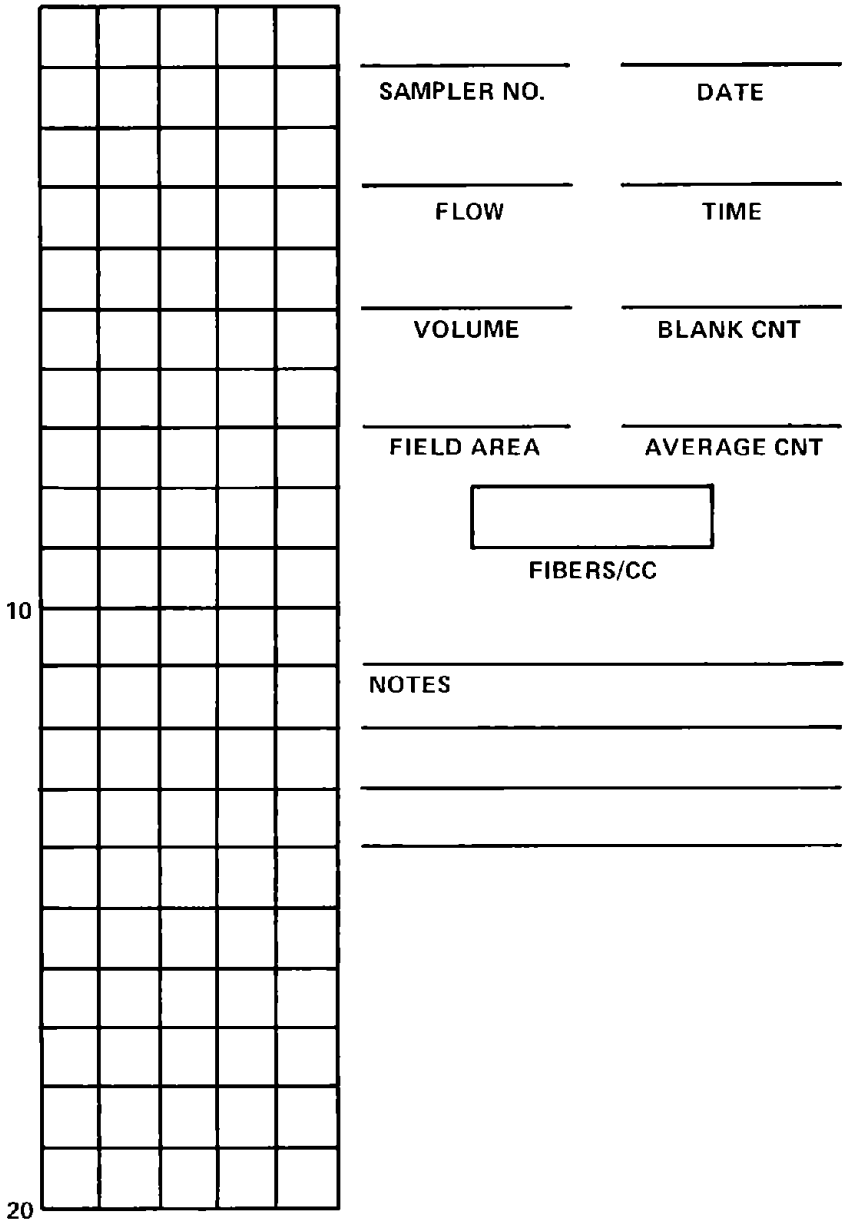
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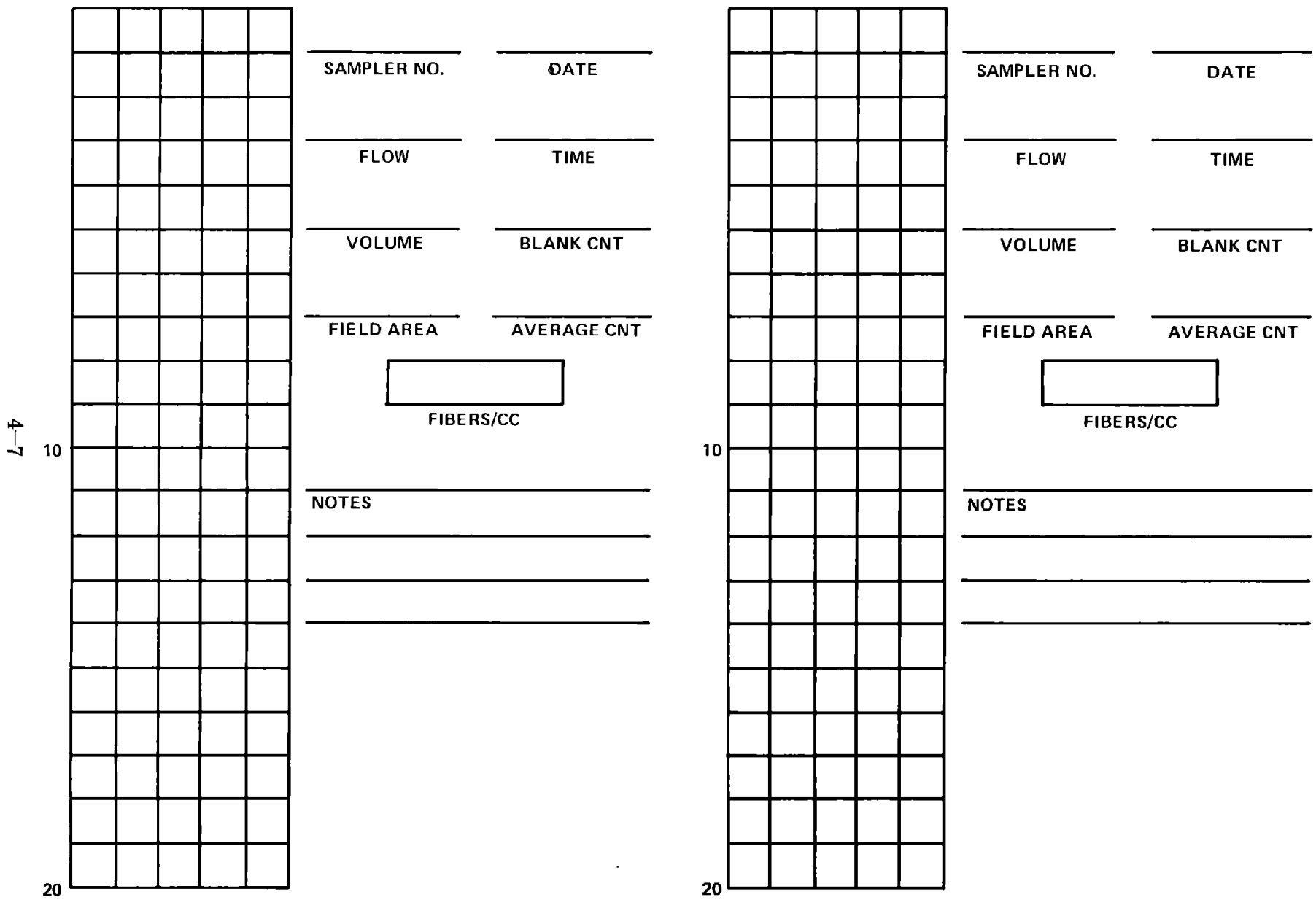
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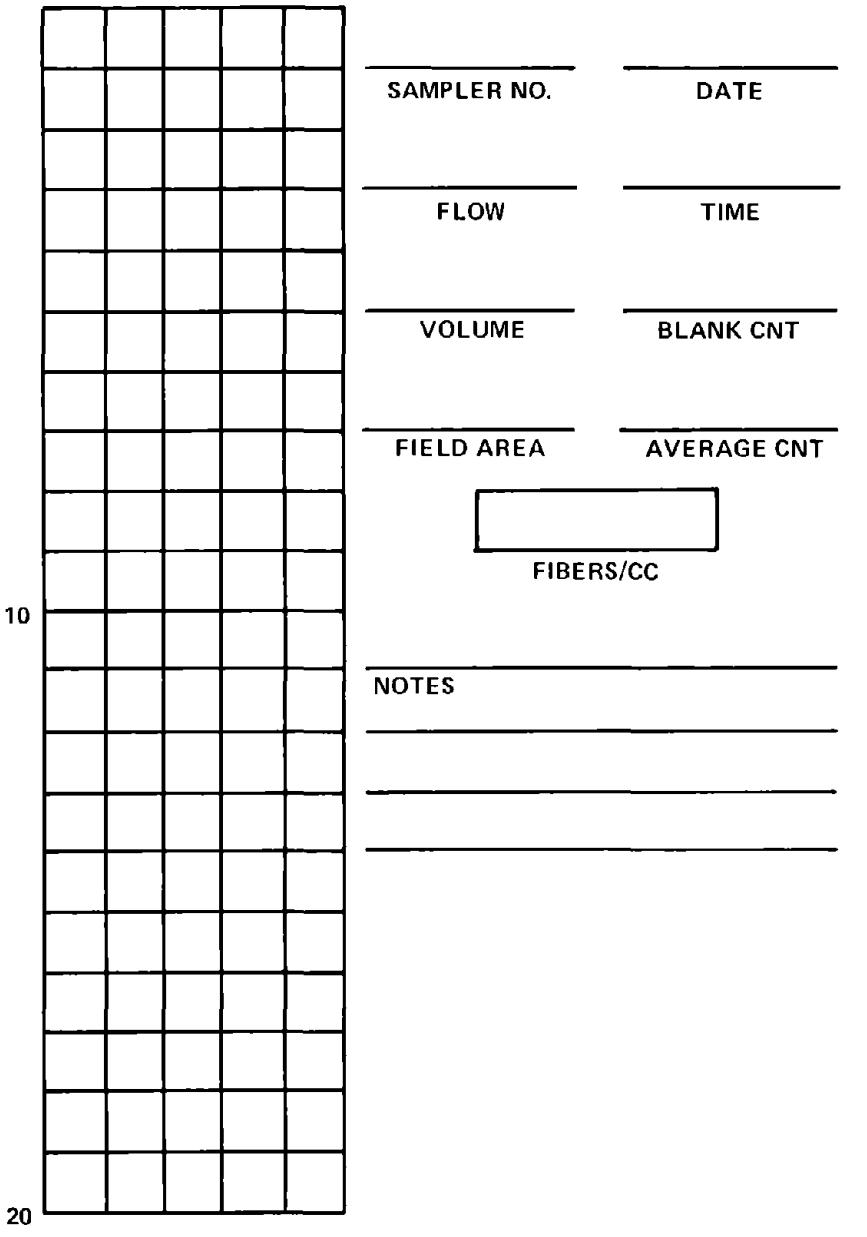
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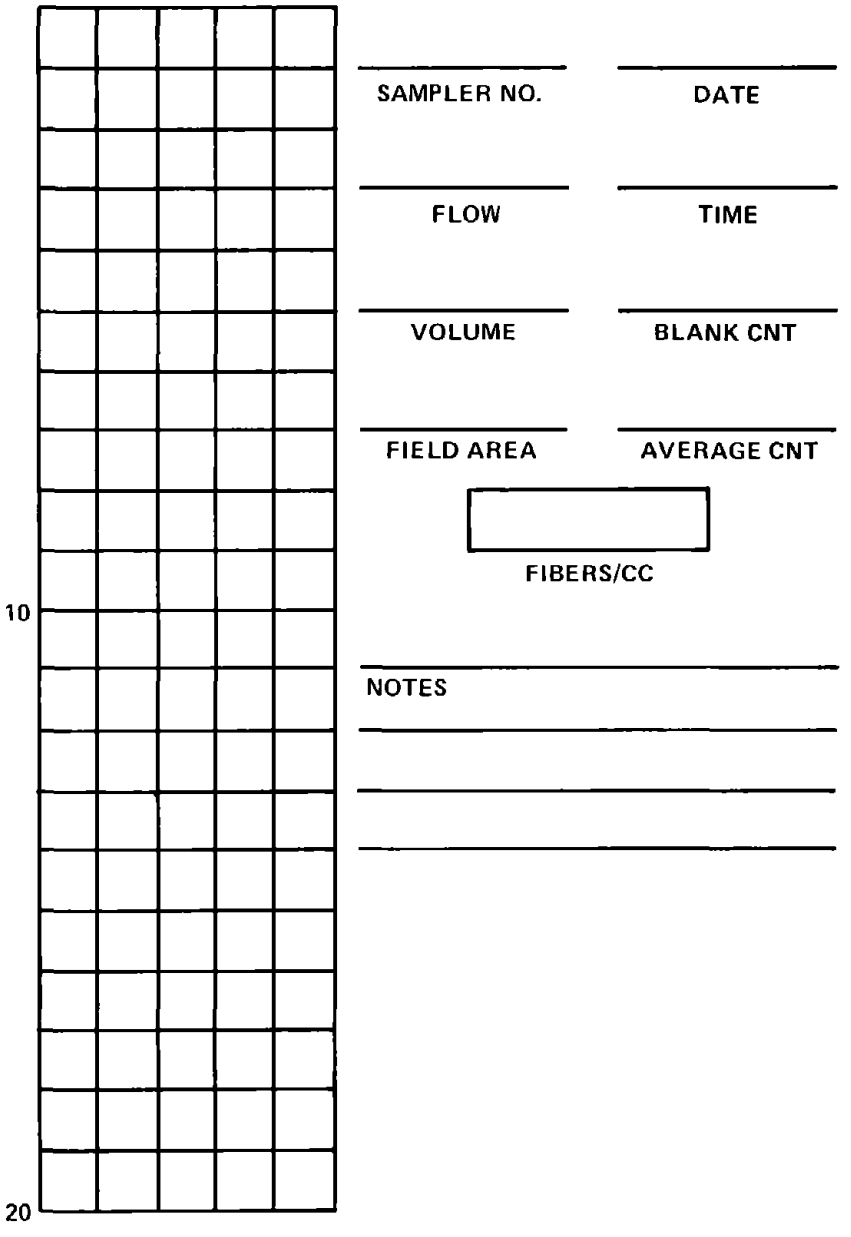
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VOLUME
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FIELD AREA
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FIBERS/CC
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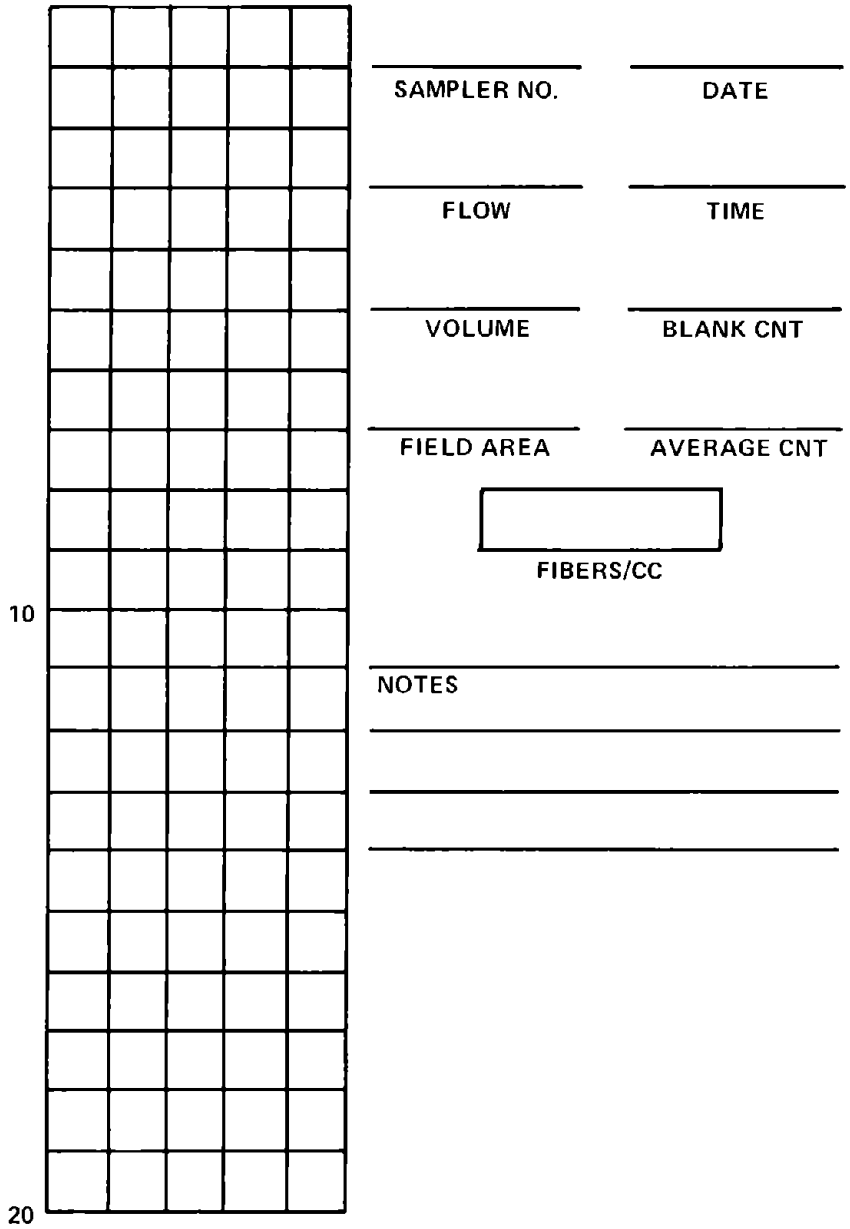
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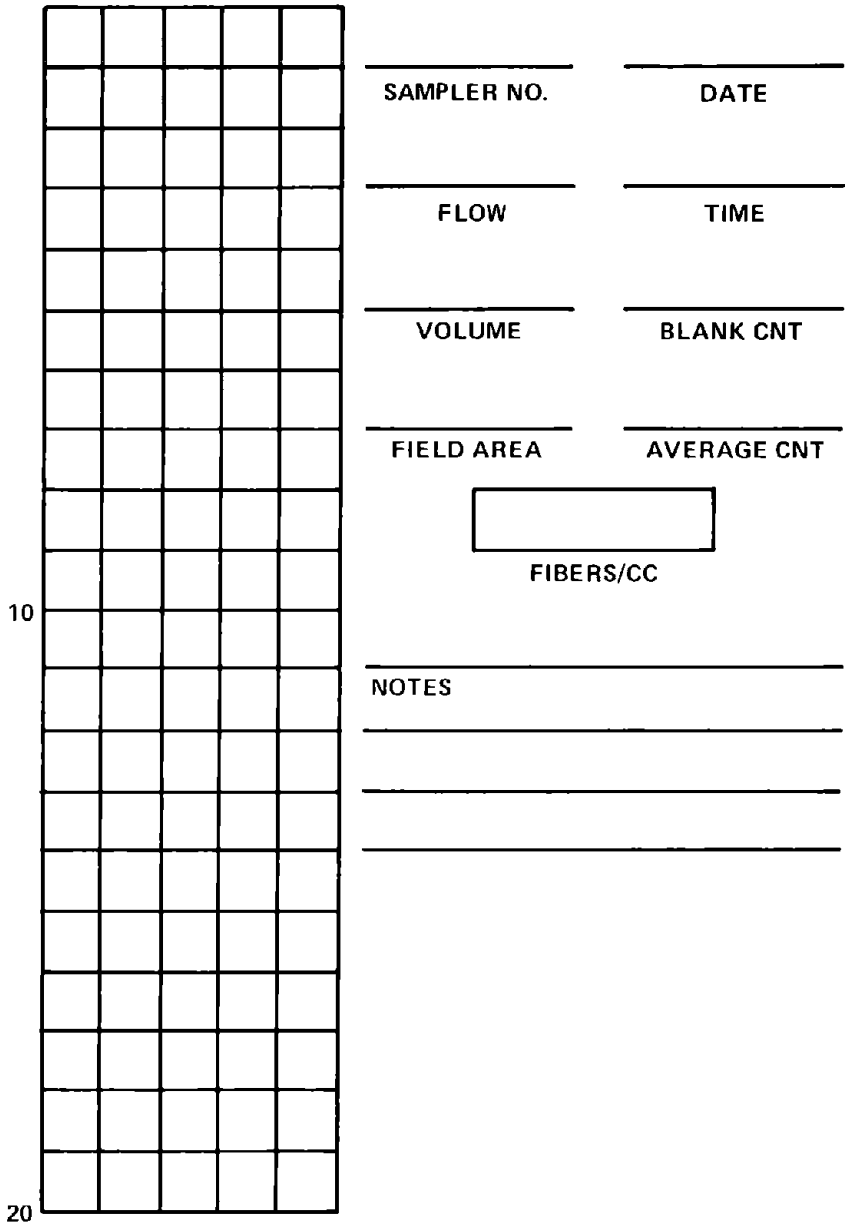
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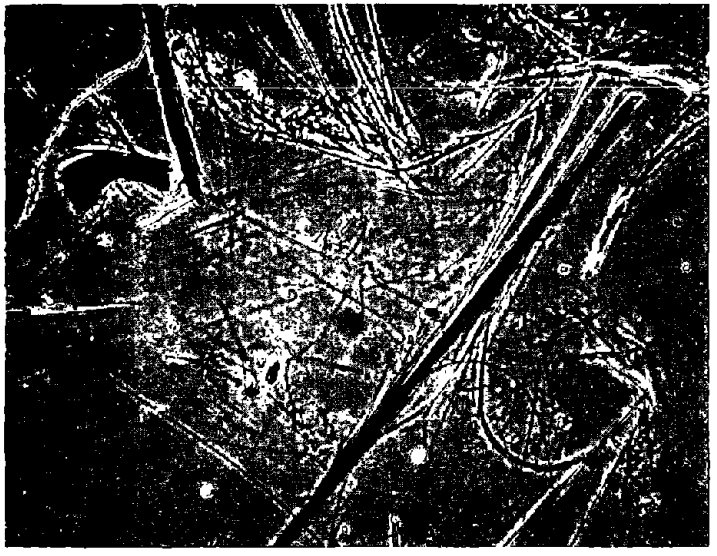
SAMPLER NO.
DATE
FLOW
TIME
VOLUME
BLANK CNT
FIELD AREA
AVERAGE CNT
FIBERS/CC
NOTES

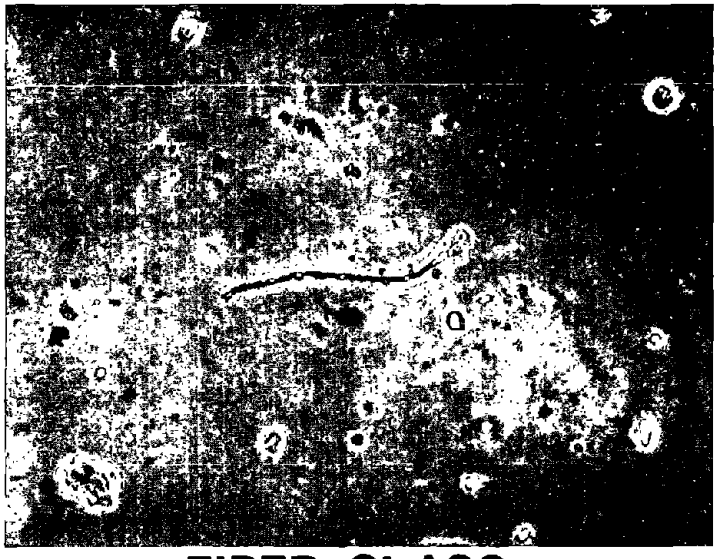
COUNT TABULATION SHEET

Calculate: $\frac{\text{Av. Count}}{\text{f. area}} = \frac{\text{Fibers}}{\text{mm}^2}$

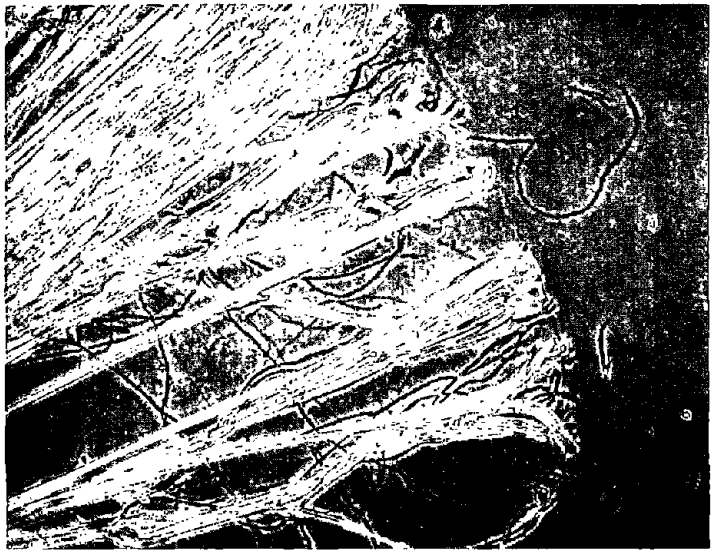
A35–1				A35–2				A33			
1	3	4	6	1	3	5	6	1	2	3	4



CROCIDOLITE

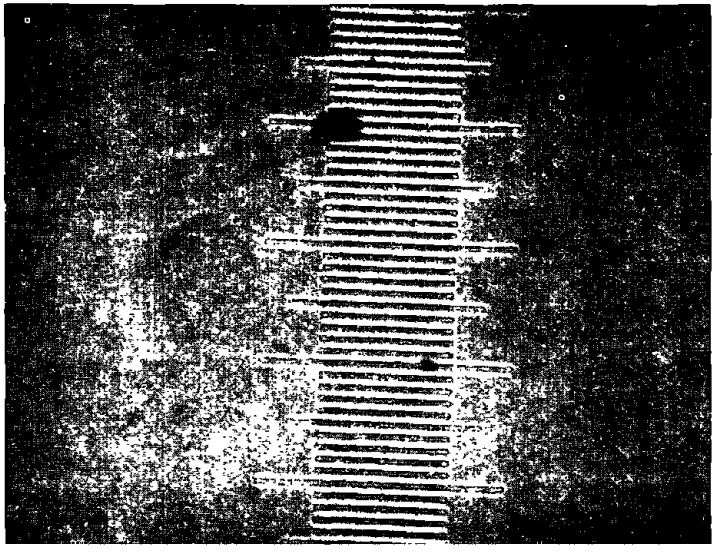


FIBER GLASS



CHRYSOTILE

AMOSITE



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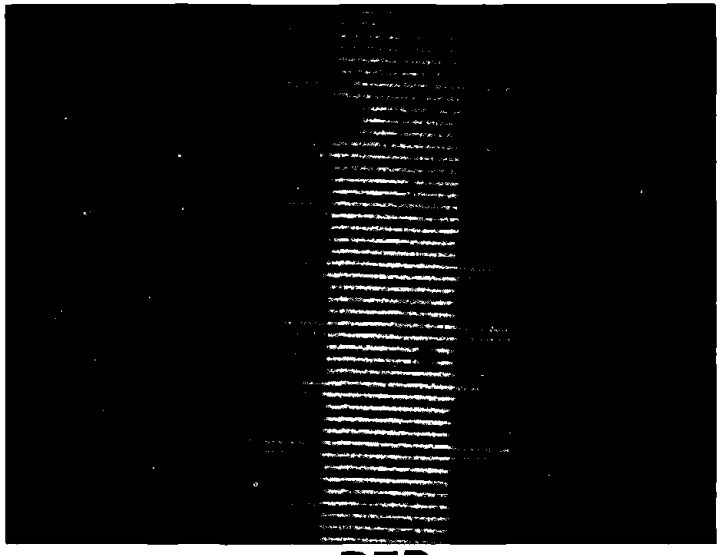

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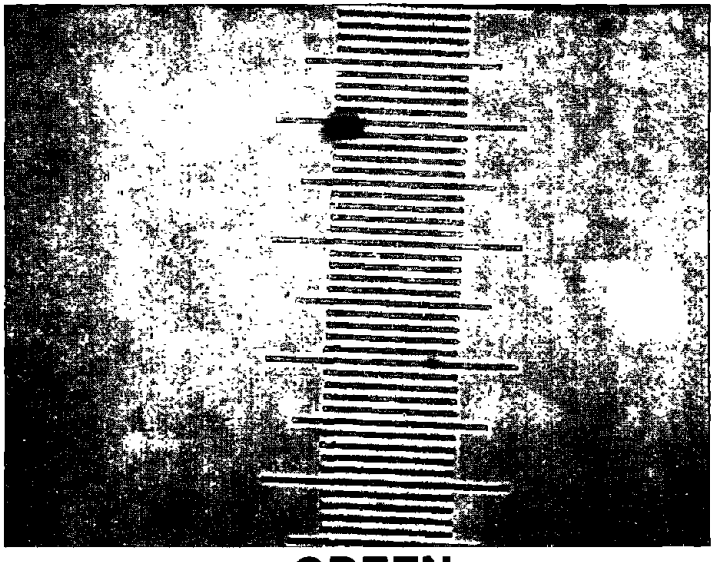

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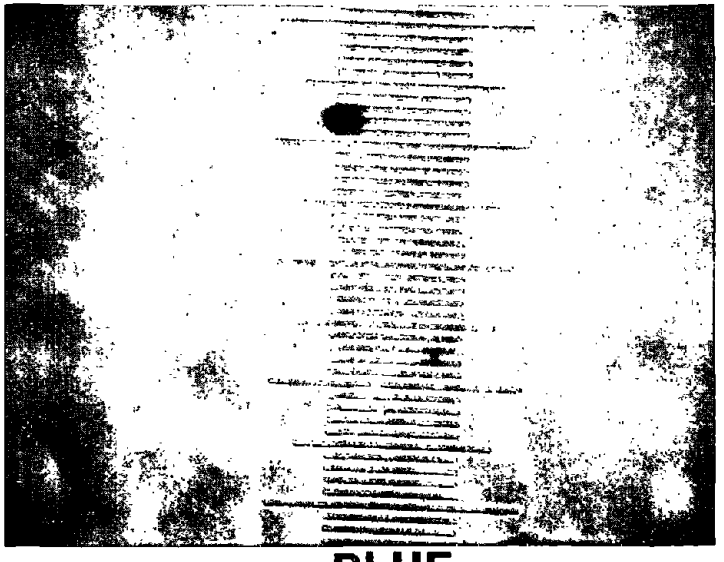
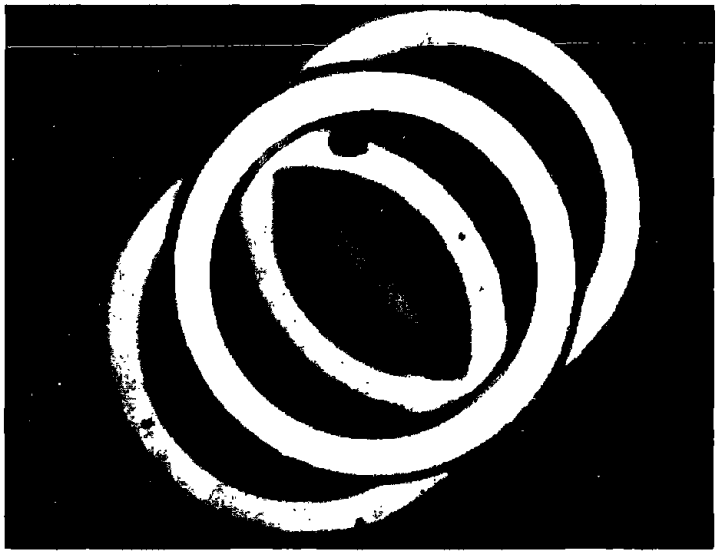


BLUE

WHITE



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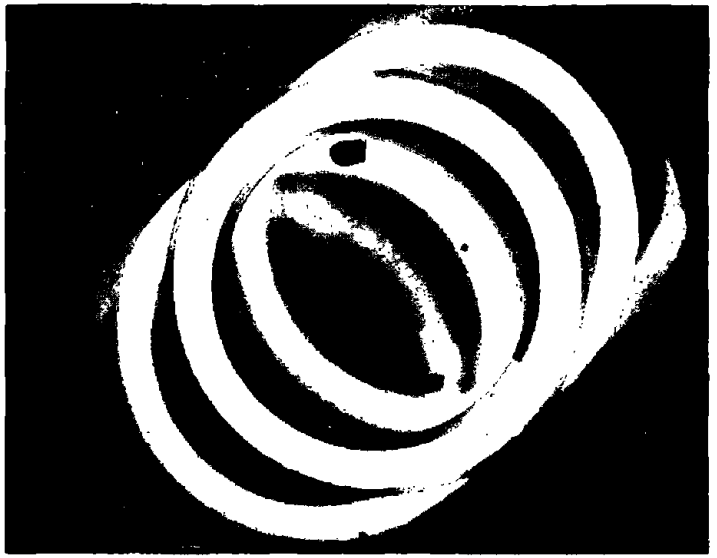

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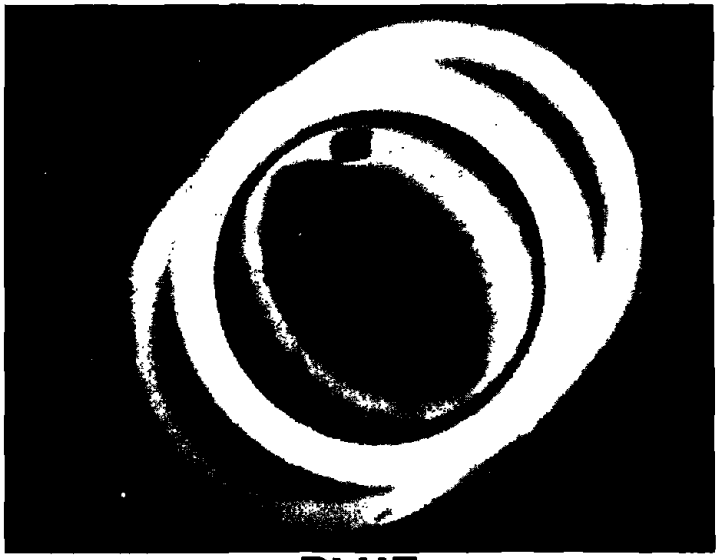

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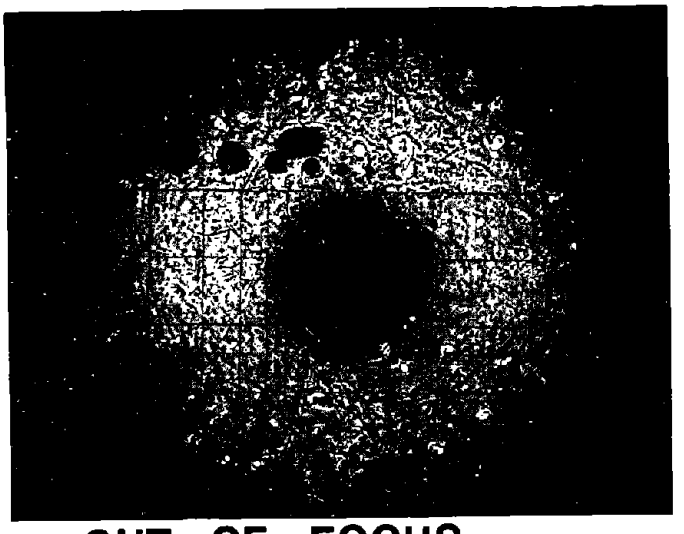

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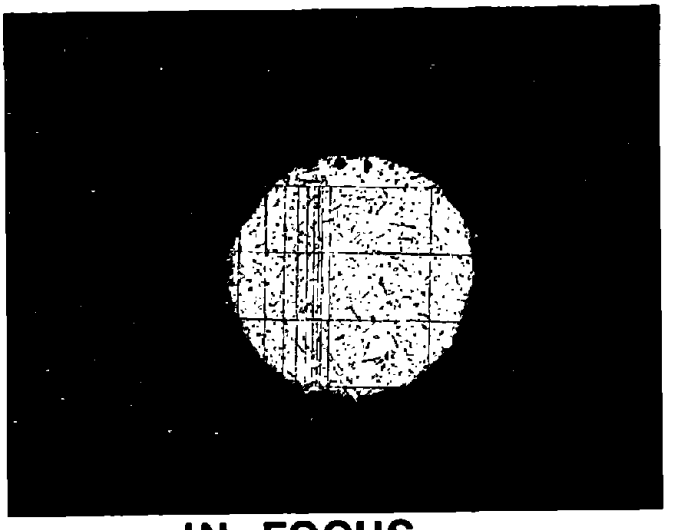

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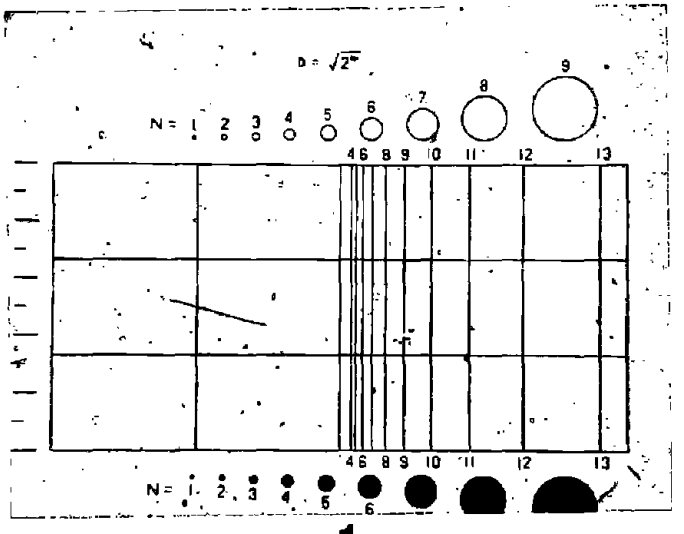

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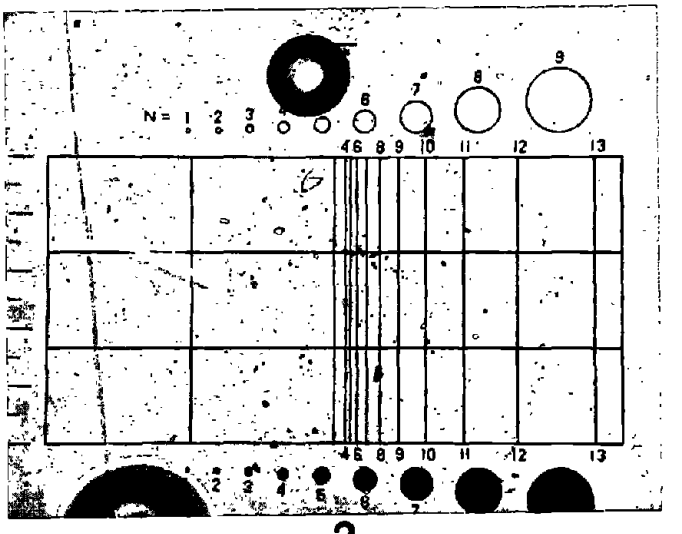

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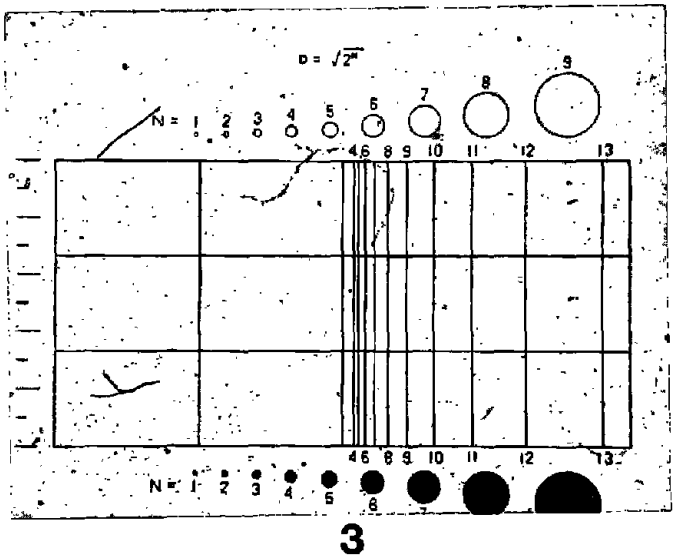

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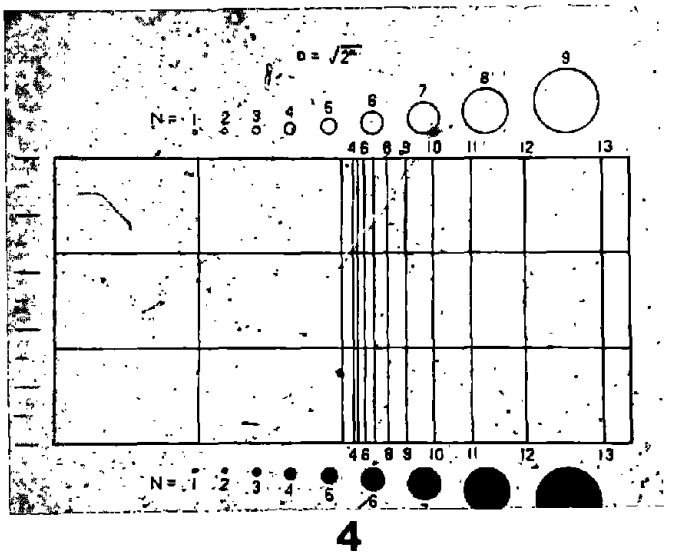

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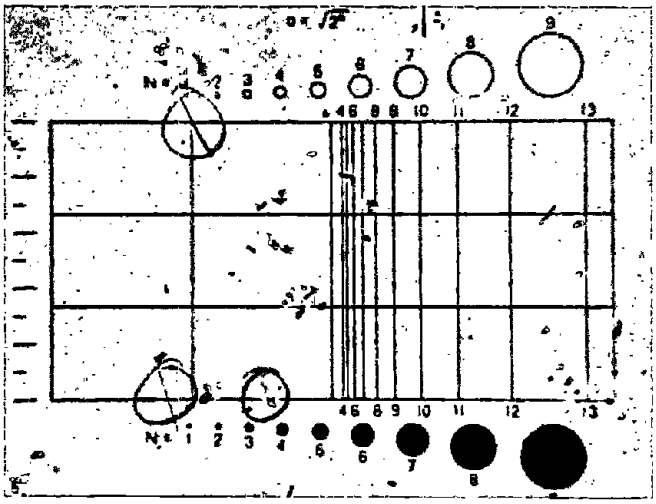

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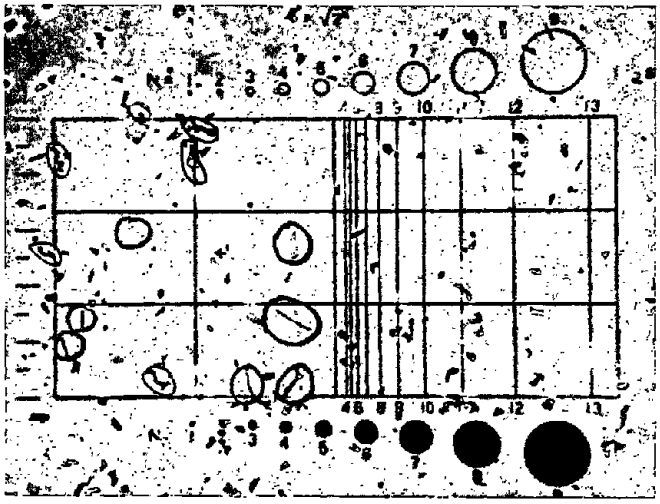

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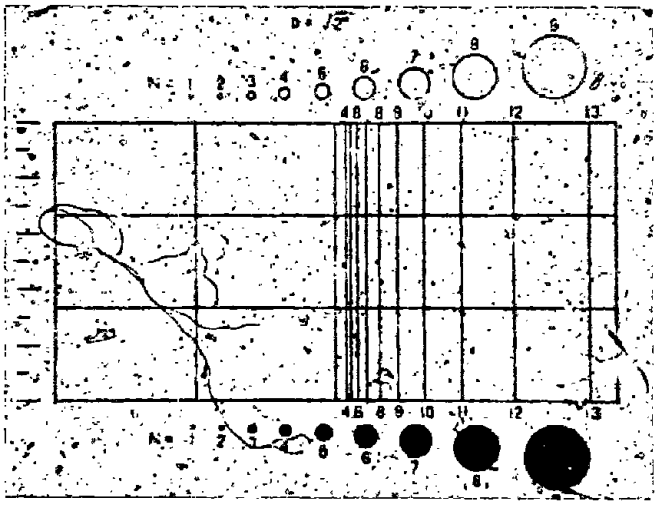

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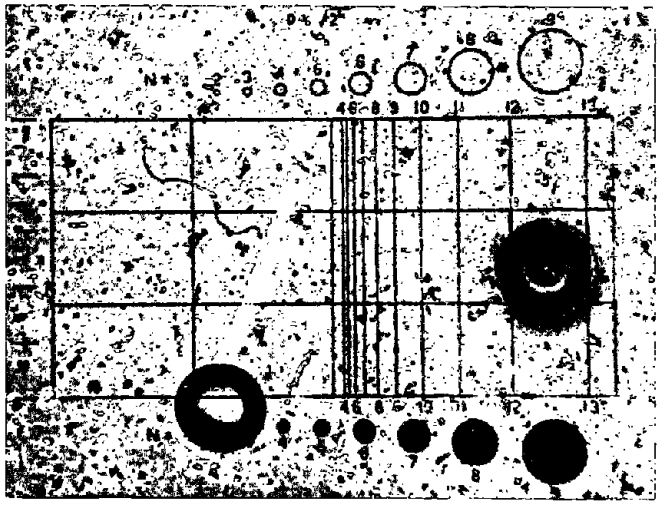

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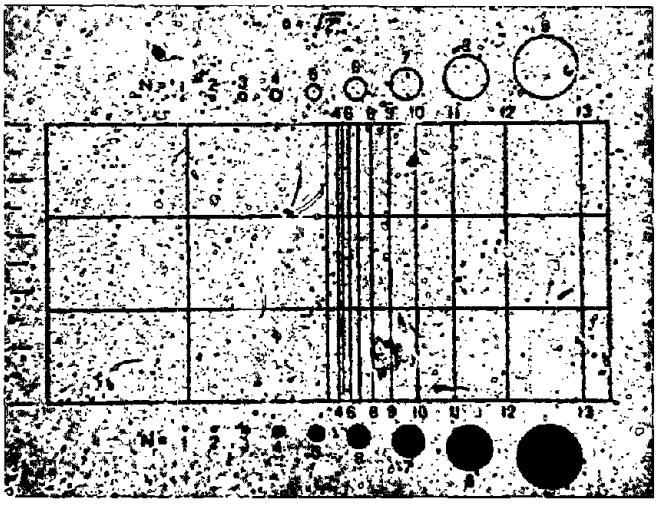

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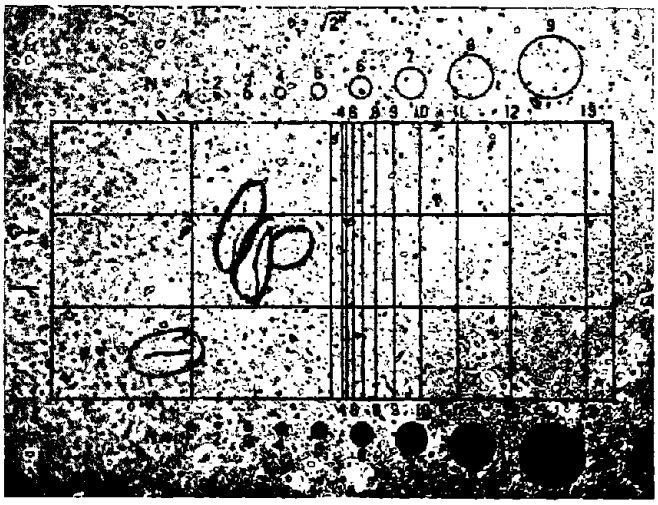

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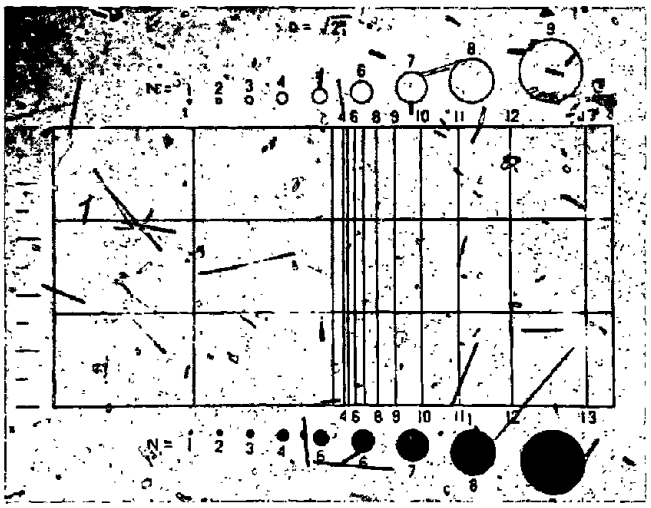

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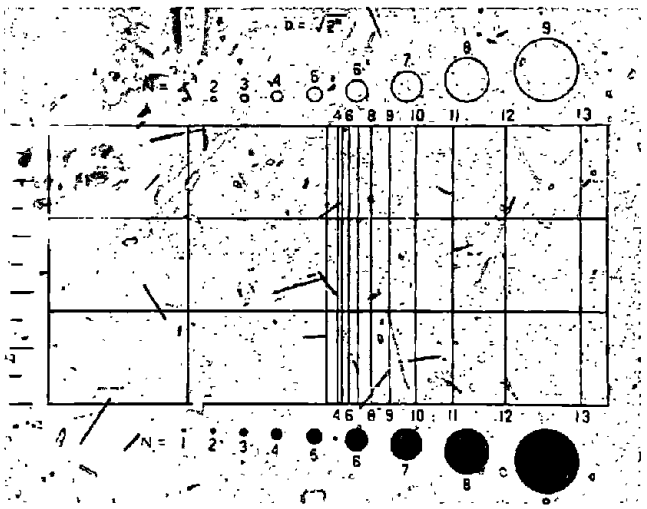

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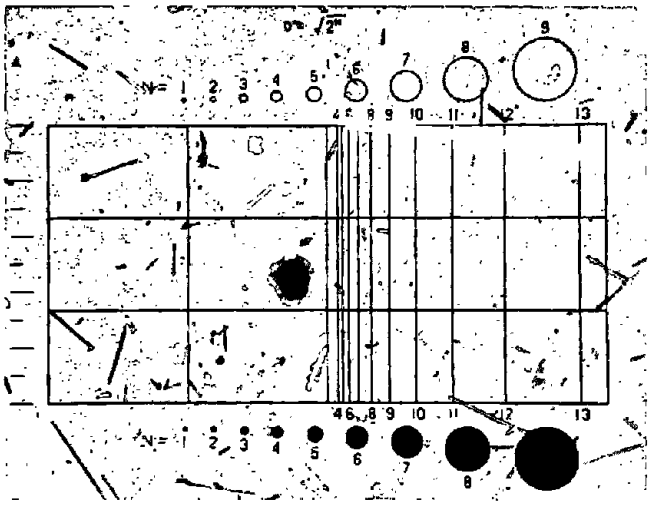

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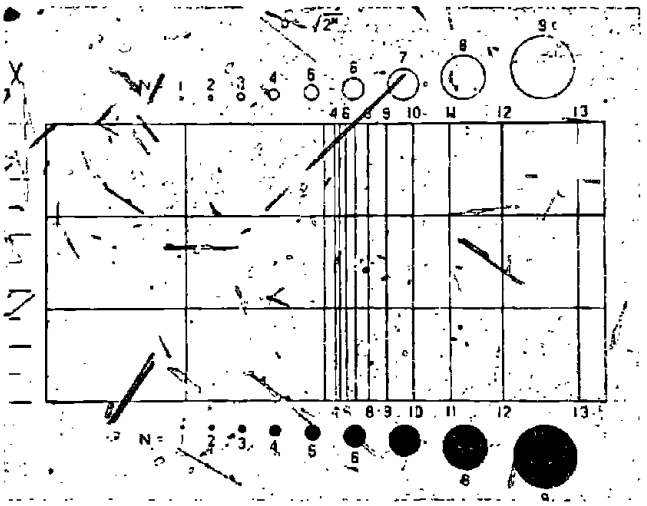

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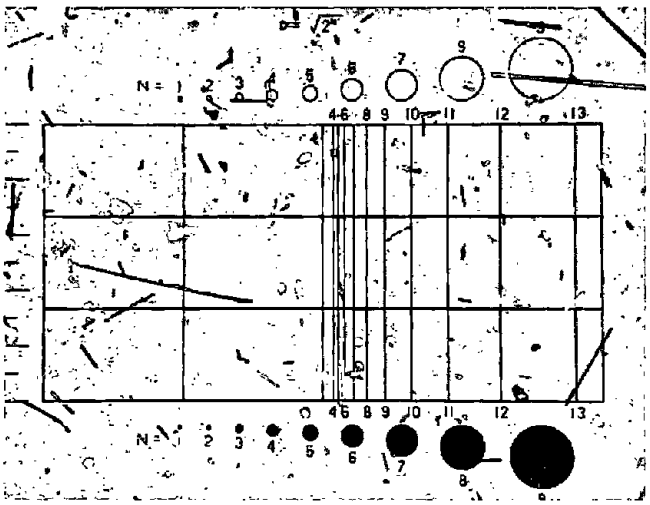

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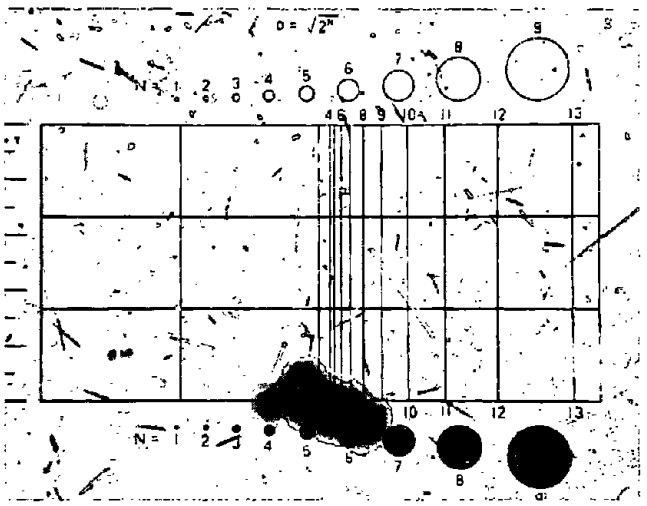

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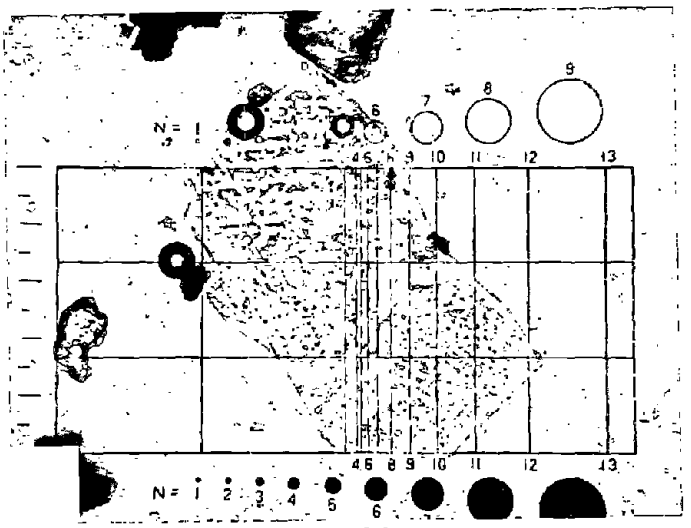

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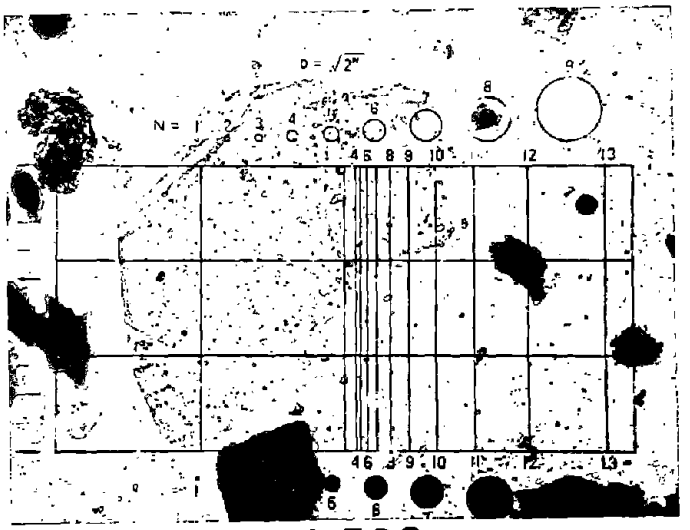

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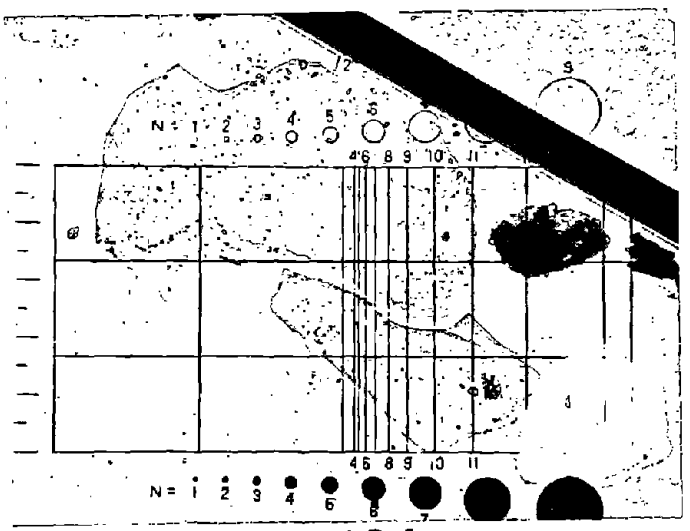

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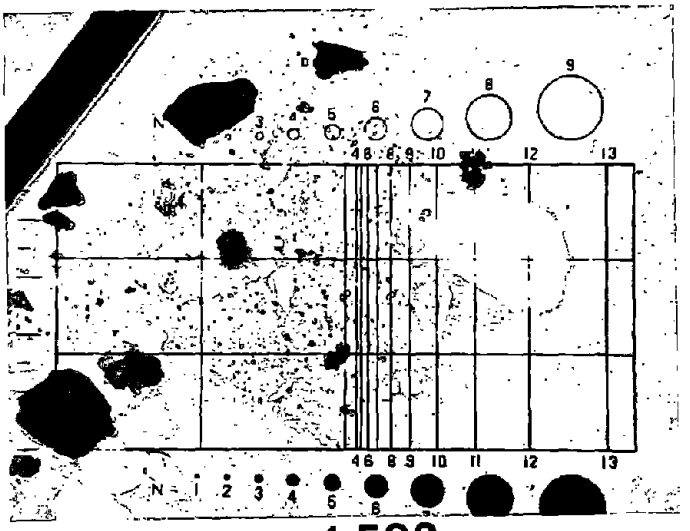

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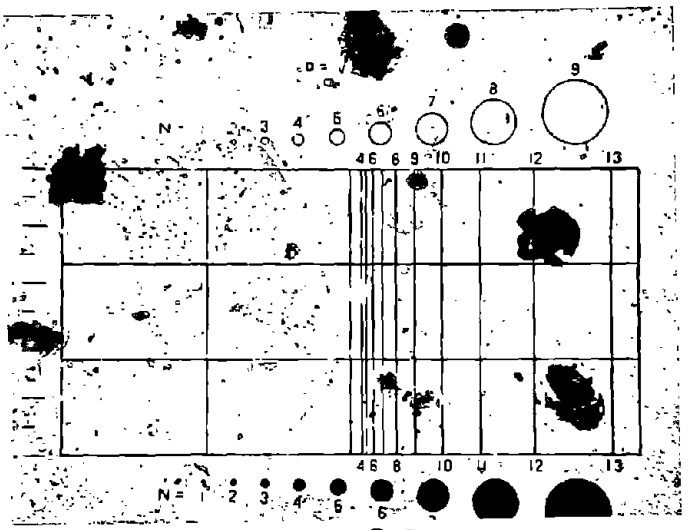

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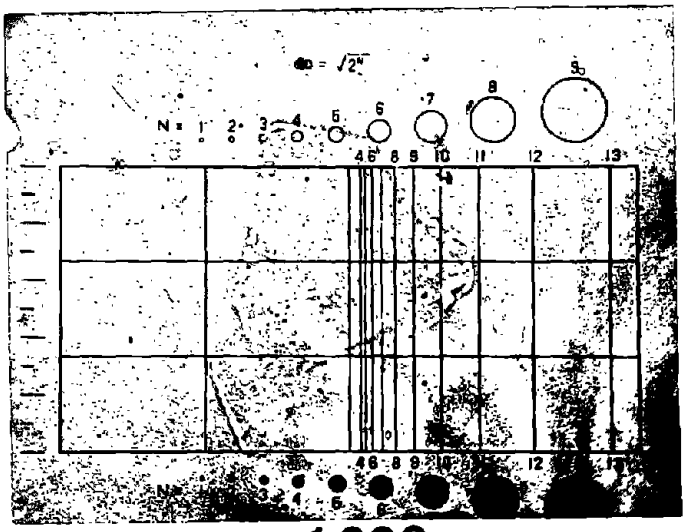

1.600

K^2 TEST FOR SCREENING ASBESTOS

I. SCOPE AND APPLICATION

This colorimetric test is applicable to the detection of magnesium (II) and iron (II) from asbestos in bulk samples. These samples include sprayed on asbestos as well as ceiling tiles. The K^2 test is simple and can be readily used in the field to screen for the presence or absence of asbestos.

II. PRINCIPLE

The K^2 test is based upon the formation of color complexes with Mg^{+2} and Fe^{+2} released from asbestos. The Mg^{+2} from chrysotile is complexed with p-nitrobenzeneazo-α-naphthol. The Fe^{+2} from crocidolite and amosite is complexed with 1, 10-phenanthroline. A positive test is indicated by the formation of colored complex for Mg^{+2} and/or Fe^{+2}, and it indicates possible presence of asbestos.

III. INTERFERENCES

The K^2 test is a colorimetric spot test for Mg^{+2} and Fe^{+2}, and is not specific for asbestos.

The bulk sample may contain Fe compounds other than asbestos. Without treating the sample as in the Procedure, the color forming reagent is added directly to the sample. If red color forms, Fe is present as an interference and these samples can be washed with water prior to the test to eliminate the interference. In the K^2 test, plaster ($CaSO_4$) interference is removed from the sample prior to the Mg test.

IV. SENSITIVITY, PRECISION, AND ACCURACY

With the glycerine treatment the detection limit is about .2 mg of pure chrysotile per test.

From the total of 70 various field samples tested, 52 samples or 74% were correctly identified by the K^2 test as to whether asbestos was present or absent. Only 18 samples or 26% gave false positive reactions. The K^2 test was 100% accurate in identifying 22 samples that did not contain asbestos. The K^2 test results were verified using the transmission electron microscope.

V. APPARATUS

1. Teflon dish
2. Microspatula or glass rod
3. Dropping plastic pipet
4. 15 ml disposable plastic beaker
5. 25 mm size 0.8 μm membrane filter
6. 25 mm Swinnex filter holder and gasket
7. 10 ml disposable plastic syringe

VI. REAGENTS (Reagent Grade)

1. Phosphoric acid, concentrated.
2. 10 N sodium hydroxide — Dissolve 40 g of NaOH in 100 ml of water.
3. Mg Reagent — Dissolve 1 mg of p-nitrobenzeneazo-α-naphthol in 100 ml of 2 N NaOH. Age at least a day. This reagent is stable over a month.
4. Hydrofluoric acid — Dilute 20 ml of HF with 20 ml of water. Add 0.6 ml of concentrated HCl.
5. Fe Reagent — Dissolve 2 g of 1,10-phenanthroline in 50 ml of ethanol. This reagent is stable over a month.

Prepared by Walter S. Kim and Richard E. Kupel, DHEW, PHS, CDC, NIOSH, Division of Physical Sciences and Engineering, Measurement Support Branch. 4/79

6. Glycerine, reagent grade.

7. Double de-ionized water.

VII. PROCEDURE

A. Magnesium Test

1. Place a small portion of sample in a plastic beaker.

2. Add 5 drops of glycerine and mix well with a spatula.

3. Rinse spatula with a small amount of water into the beaker.

4. Filter the sample through the filtration assembly consisting of syringe attached to the Swinnex filter holder loaded with a membrane filter and a gasket.

5. Filter with minimum of 5 washings or about 50 ml of water.

6. Transfer filter to a Teflon dish.

7. Add a drop of H_3PO_4 and mix well by grinding the sample with a spatula.

8. Add 2 drops of 10 N NaOH and mix well.

9. Add 5 drops of Mg Reagent and stir briefly. Note any color change.

10. Add 5 more drops of Mg Reagent and observe the final color.

11. A blue color indicates that chrysotile may be present.

12. No color change with Mg Reagent indicates the absence of chrysotile. Even though amosite contains magnesium, this test does not produce the blue color. The iron test would produce a positive reaction indicating the presence of asbestos.

13. Check for Mg interference as in Section III if positive result is obtained.

B. Iron Test

1. Place a small portion of sample on a Teflon dish.

2. Add a drop of HF solution and mix well.

3. Add 5 drops of Fe Reagent and observe the development of red color.

4. The red color indicates that amosite or crocidolite may be present.

5. Check for iron interference as in Section III.

C. Notes

If both tests give negative responses there is a low probability thay any asbestos is present. Once the presence of magnesium and/or iron is indicated, further analysis if needed is done in the laboratory to confirm, identify the type, and quantitate the percentage of asbestos.

REFERENCES

1. *Caution: Asbestos Dust.* A scriptographic booklet by Channing L. Bete Co., Inc., Greenfield, Mass. Prepared in cooperation with U.S. DHEW, PHS, CDC, National Institute for Occupational Safety and Health, Cincinnati, Ohio (1973).

2. Fritz Feigl: *Spot Tests — In Inorganic Analysis.* Translated by Ralph E. Oesper. 5th Edition. Elsevier Publishing Company, Amsterdam. (1958) p. 225—227.

3. Walter S. Kim, James W. Carter II, and Richard E. Kupel: Quick Screening Test for Asbestos (K^2 Test). U.S. DHEW, PHS, CDC, NIOSH, Cincinnati, Ohio. To be published in IAHAJ.