

ENVR 416
Aerosol Technology - Laboratory Session
Fall 2009

CALIBRATION AND USE OF THE OPTICAL MICROSCOPE

The objective of this lab is for you to become familiar with use of the optical microscope to measure the sizes of particles and other objects.

Before you can use the microscope to measure something, it must be calibrated. Two techniques to measure particle sizes involve use of the Porton graticule, and use of the filar micrometer. The first part of this lab involves calibration of each. Both are calibrated using a stage micrometer. Chapter 20 in your textbook gives good background information.

1. Calibration of the Porton Graticule

Background

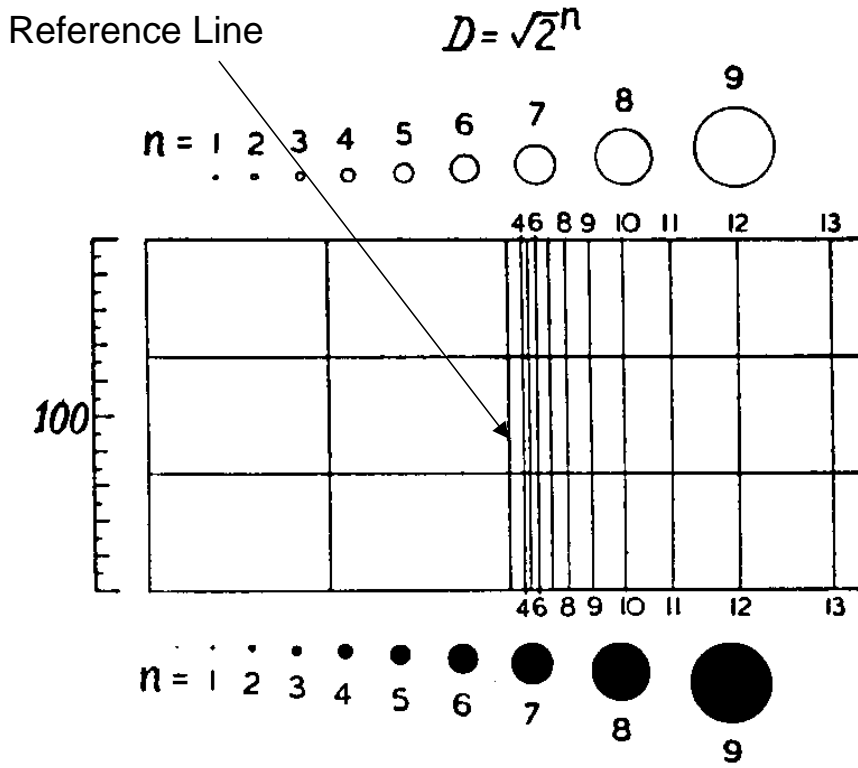
The *Porton graticule* is an etched glass disk placed on a ring within the eyepiece of a microscope. See the top figure on the next page. A similar figure is in your textbook on page 404. The etchings on this disk produce a grid pattern in the field of view. Note the hollow circles above and the solid circles below the numbered, vertical lines. The inside diameter of the hollow circles and the outside diameter of the solid circles are the same as the distance from the reference line to the vertical line of the same number. For example, the diameter of a circle of size 10 is the same as the distance from the reference line to the vertical line numbered 10.

The numbers in the Porton graticule have a $\sqrt{2}$ progression; for example, the diameter of circle size 10 is $\sqrt{2}$ as large as the diameter of circle size 9, and twice as large as the diameter of circle size 8. Similarly, a circle size 12 has twice the diameter of circle size 10, etc. For this reason, the area of each larger circle is twice the area of the circle just below it in the progression; for example, the area of circle size 10 is twice the area of circle size 9, etc.

The *stage micrometer* can be thought of as a tiny ruler mounted on a transparent microscope slide. A picture of the stage micrometer is at the bottom of the next page. The total length of the stage micrometer is generally 1 mm. Stage micrometers are marked in 100 equal divisions, so that the distance between the smallest divisions is 0.010 mm or 10 μm .

Sometimes stage micrometers have two scales, one with divisions of thousandths of an inch and the other with divisions of 10 μm . As a practical matter, these stage micrometers are confusing because the user is never certain which scale he or she is using. We have thrown away all double-scale stage micrometers in our lab and use only those with divisions in micrometers. Stage micrometers are available from scientific supply houses such as Fisher and cost between \$50 and \$100.

Porton Graticule



Stage Micrometer



Figure 19.3 Portion of a stage micrometer as it appears at $400\times$. Actual distance between the smallest lines is $10\ \mu\text{m}$.

Procedure

Place the stage micrometer on the mechanical stage of the Olympus microscope, which is equipped with a 10X eyepiece that contains the Porton graticule. Using 100X magnification, 10X eyepiece and 10X objective, focus on the stage micrometer.

Superimpose the reference line on the graticule with a division on the stage micrometer. Rotate the eyepiece and move the microscope stage to align the scales as necessary. Now measure the distance from the reference line to various numbered lines on the Porton graticule. Construct a semi-log plot of your findings, giving Porton number on the horizontal, arithmetic scale vs. distance on the vertical, log scale. Repeat this procedure using the 40X objective lens, giving 400X total magnification. Label your plots with the name and identifying characteristics of the microscope you used, so that in the future you will know which microscope you calibrated.

2. Calibration of the Filar Micrometer Background

The filar micrometer can be used to make precise measurements of particle dimensions. A knob on the side of this device moves a crosshair in the eyepiece across the field of view. This knob has 100 division markings. Calibration of the filar micrometer is necessary to determine how far the crosshair moves per knob division. Once the filar is calibrated, a particle's dimension can be found by determining how many knob divisions are necessary to move the crosshair across the image of the particle, then multiplying this number by the filar calibration factor. Separate calibrations must be made for each objective lens; however, the microscope need not be recalibrated often unless the objective lenses on the microscope are replaced.

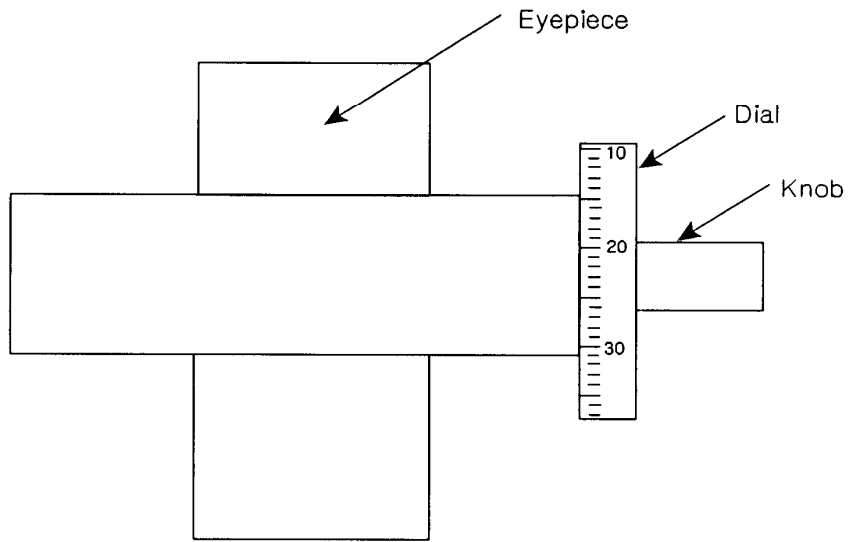
Procedure

See the figure on the next page that shows the parts of the filar micrometer and the view through the filar eyepiece. As you rotate the knob, the vertical line moves across the field of view. The scale attached to the knob contains 100 divisions so that rotating the knob one full turn corresponds to passing 100 divisions. A full rotation of the knob advances the vertical line one unit on the numbered line below the field of view, for example, from "5" to "6".

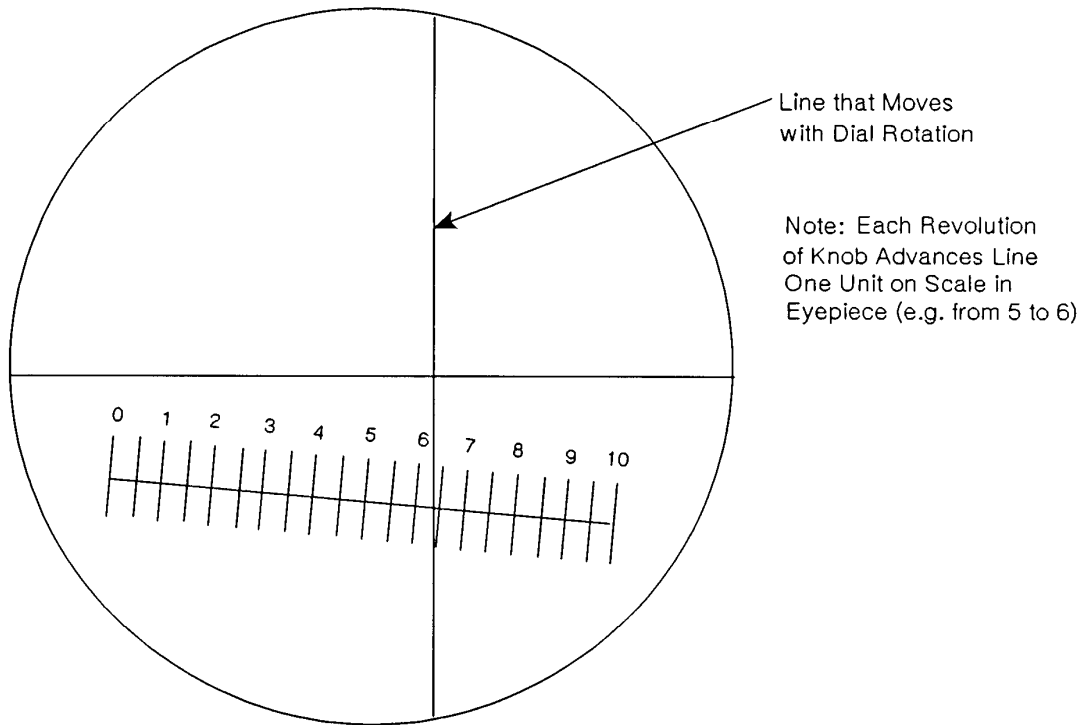
Using the microscope with the filar micrometer as the eyepiece, focus on the stage micrometer. Use the 10X objective lens. The eyepiece with the filar micrometer has a 10X magnification so the total magnification is $10 \times 10 = 100X$. Set the filar micrometer to zero by rotating the larger circular disk with division markings on it until "0" appears at the top. Then adjust the mechanical stage so the filar crosshair is aligned with a major graduation of the stage micrometer. Rotate the smaller, concentric thimble of the filar to move the crosshair at least $100 \mu\text{m}$ across the scale of the stage micrometer, and record the number of filar micrometer divisions required. Repeat, but move the crosshair in the opposite direction. Determine a filar calibration factor by determining the distance the filar crosshair moves per division of the filar scale.

Repeat the filar calibration using the 43X objective lens to give 430X total magnification.

Filar Micrometer



Side View



View Through Eyepiece

3. Use of the Microscope

Hair Diameter

Remove a hair from your head and determine its diameter using the filar micrometer. Measure the diameter of the same hair at several locations to get an idea of how uniform it is. If you have time and can spare the hair (some of us can't) remove another hair or two and make similar measurements. Now make the same measurements for hair from your lab partner. How uniform is your hair diameter? How consistent is diameter from hair to hair? How different is the diameter of your hair from your partner's hair?

Size Distribution of Polydisperse Dust

The size distribution of a polydisperse dust can be measured more rapidly with the Porton graticule than with the filar micrometer. If one must resolve size detail with more precision than is represented by the width of a Porton size range, the filar micrometer is a better choice for measuring size distribution; however, for polydisperse dusts such precision is rarely necessary.

Place a drop of immersion oil on a clean microscope slide. Take a very small sample of dust from the bottle marked "ENVR 416 Class Sample" and using a toothpick or paper clip, mix the oil with the dust until the dust is thoroughly dispersed. Then drop a cover slip over the oil-dust mixture.

Place the slide you have prepared on the microscope stage, and focus on the particle layer. This is done most easily by focusing first with a lower-power objective lens, which has greater depth of field. Then switch to a higher power objective lens by rotating the microscope nosepiece. If the microscope is set up properly, and parfocal lenses are used, the particle layer will be in focus or nearly so at the higher magnification. Parfocal lenses are made so that objects in focus with one lens will also be in focus when another lens on the nosepiece is rotated into position. The lenses supplied with a new microscope will be parfocal, but if lenses from another manufacturer are substituted, the new lens may not be parfocal with the original lenses.

To focus the microscope, first move your head away from the eyepiece and observe the clearance between the objective lens and the slide. Lower the objective lens until it is close to the slide. Then look through the eyepiece and focus by moving the objective lens up, away from the slide. This technique prevents grinding the objective lens into the glass slide; the slide and microscope objective are expensive substitutes for mortar and pestle.

The left part of the Porton graticule contains a grid of rectangles with equal size. Combine one or more rectangles to form a "field" large enough so that about 20 particles are in each. You must keep the same field size throughout your analysis. Field size is chosen so that you can keep track mentally of whether you have sized each particle in it. If you pick a field size that contains too many particles, you may lose track of which particles you have counted. If your field contains too few particles, you will spend too much time focusing on different fields and not enough time counting particles.

Choose a field at random by looking away from the microscope eyepiece and moving the microscope stage. Then focus on the particles in your field. Do not look through the eyepiece while changing fields because the temptation is overwhelming to choose a field where the particle density is about right for counting, or to choose a field that has an interesting particle in it, often a large particle or a particle with an interesting shape or color.

Count and size each particle in your field by comparing mentally its projected area with the projected areas of the circles on the Porton graticule. Do not move the slide to superimpose the Porton circle on each particle as moving the slide would change your field. Make the size comparisons between particles and Porton circle sizes in your mind. Give each particle the size corresponding to the numbered circle whose projected area is just larger than the projected area of the particle. For example, if you decide the projected area of a particle is between that of circles sized seven and eight, call the particle an “eight”.

Recording your data efficiently can be difficult. You can call out the Porton size number for each particle to a partner who writes down your findings, use a mechanical counter, or drop beans into glasses whose numbers correspond to Porton numbers. Whatever method you choose, try not to look away from the microscope while you record your data so you do not lose your place in the microscope field. When you have counted all the particles in a field, look away from the eyepiece, select a new field, and resume counting. *Record data from each field separately*; this procedure is important for stratified counting. Stratified counting is not necessary to obtain a valid size distribution, but it does minimize counting effort necessary to attain a fixed level of precision. An example of stratified counting is given on page 407 of your textbook.

Continue selecting fields and sizing particles until you have a good idea of the particle size distribution. Plot your results on cumulative log-probability paper and determine the count median diameter for this dust. You can download copies of log-probability graph paper from the class website. If the data form a straight line, the dust has a log-normal size distribution. Is this dust log-normally distributed? If so, determine the geometric standard deviation, σ_g , by

$$\sigma_g = d_{50} / d_{16} = d_{84} / d_{50} \quad .$$

where

d_{16} 16% of particles are smaller than this size,

d_{50} 50% of particles are smaller than this size; the median diameter,

d_{84} 84% of particles are smaller than this size.

There is no reason why size distribution data plotted on log-probability paper should form a straight line; that is, there is no theoretical basis for believing the size distribution of any dust should be log-normal.

Some dusts have a log-normal size distribution but most do not. A size distribution plot is always appropriate to present size data. However, if a dust is log-normally distributed, its size

distribution can be expressed accurately without a plot using only two parameters, d_{50} and σ_g . Data presentation by this method is efficient if the size distribution is log-normal; however, the temptation to be efficient should not overcome the need to be accurate. The size distribution of dusts not log-normally distributed should be presented as a curved line on a log-probability plot rather than as a straight line forced through non-linear data.

All dusts, regardless of whether they are log normally distributed, will have a median diameter, d_{50} , as well as a d_{16} and a d_{84} . Thus, one can always speak of the median diameter for a dust regardless of the shape of its size distribution. In the case of a log-normally distributed dust, the relationship between d_{16} , d_{50} and d_{84} will be given by the equation above, whereas the equation will not hold if the dust is not log-normal.

Analysis and Reporting

1. Prepare a semi-log plot for your calibration of the Porton graticule. Put distance in micrometers on the log axis and Porton number on the linear axis. Plot your calibration for the Porton graticule using the 10X objective lens and your calibration using the 40X objective on the same plot.
2. Determine the calibration factor for the filar micrometer using the 10X objective lens. Report this factor as distance in μm per division of the knob scale. Also determine and report the calibration factor for the filar micrometer using the 43X objective lens.
3. Report the diameter of your hair(s) and your lab partner's hair(s) in μm .
4. Plot the cumulative size distribution for the dust you analyzed using the microscope with the Porton graticule. Use log-probability paper. You can download a piece of this paper from the course website. Unfortunately, Excel does not include the option to express axes using a probability scale. Report the count median diameter and discuss whether the dust is log-normally distributed. If it is, report σ_g as well.

You and your lab partner should prepare a single report. Be sure to put both your names on the first page of the report. The report should describe very briefly what you did, then present your results and any conclusions you reached in doing this exercise.

The total length of your report should not exceed two single-sided pages including all figures and tables.

The report should be prepared neatly. Both you and your lab partner will receive the same grade.