

Measurement with a Microscope

Introduction

The aim of this guide is to provide an introduction to the principles and practical methods of measuring, with a compound microscope.

Whatever your particular field of interest, be it Biological, Industrial, Metallurgical or something else, there are many circumstances where measurements are made at the microscopic scale.



Individual applications may require:

- The measurement of small objects
- The need to measure an object to a high degree of resolution
- Measure a particular feature on an object

There are many reasons to make visual measurements using some form of magnification.

Measurement using a microscope is not limited to finding the actual size of a sample, but can also be used to categorize an object into a particular size group or to check that the manufactured shape of a particular feature is within dimensional or angular tolerances.

Some of these tasks are accomplished using general purpose sizing tools such as Horizontal Scales or Protractors, others by using specialised or application specific sizing tools, such as Walton & Beckett Reticles for asbestos fibre sizing and counting. Yet other applications, may benefit from specifically designed sizing tools made to suit an individual task, such as the QA control of hypodermic needle points or the profile of a razor edge.

Measurement with a Microscope

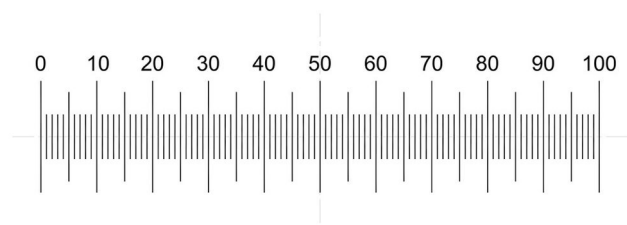
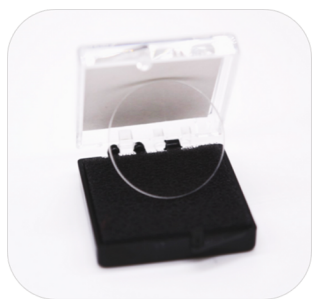
Measurement Methods

So what are the 'sizing tools' used with microscopes. Three methods :

1. Direct Contact. Just like using a normal ruler – but smaller. Normally employed for low magnification / wide field of view(FoV). Where the object being measured and scale ruler are in direct contact and can be magnified together.
2. Movable XY stage. Using a movable XY microscope stage with mechanical/digital vernier gauges, in conjunction with a simple crosshair sighting eyepiece. This method is subject to the mechanical limitations in resolution and accuracy of the mechanical stage and resolution of the gauge.
3. A Scaled Eyepiece Reticle. A patterned reticle mounted in the microscope eyepiece. This introduces a scaled ruler overlay (or another pattern), onto the image viewed from the sample or object at the microscope stage.

Eyepiece Reticles

The eyepiece reticle, is by far the most common and flexible of the solutions above. The basic reticle measurement pattern is a simple ruler like scale. We use the reticle scale in the same manner as a normal ruler, but with the additional step of applying a 'scaling factor' to compensate for the objective magnification.



Scale reticles are available in various sizes, formats and layouts. The most popular simple measurement pattern is a horizontal scale, 10mm long overall divided into 100 divisions (0.1mm each). The scale is marked with a long line at every 5th division and an even longer line at the 10th in addition to the number of divisions.

Measurement with a Microscope

The reason for numbering divisions and not actual dimensions.

When an object is sized with an eyepiece reticle, you count the number of divisions it spans and then apply the 'scaling factor' to convert the number of divisions into an actual measurement. This method enables the scale to be used at any magnification. An alternative would be to have the scale read in 'real' dimensions, but it would only apply to a fixed magnification, such as in fixed magnification of portable microscopes.

Scale and Magnification

Objective magnification not eyepiece magnification.

The scaling factor applied to the reticle scale, to find an actual measurement, depends on the magnification selected on the microscope at that time. This is important when selecting the correct reticle scale size to best suit a particular application. Although this concept is straightforward, for many it proves to be a confusing issue.

Understanding this concept will enable you to:

- Choose the correct scale length for your measurement
- Select the correct division size to enable a good measurement resolution of your sample.
- Enable you to design your own patterns to enable direct sizing and comparison of the sample to specific dimensions.

Firstly the reticle is mounted in the eyepiece. In most cases, this is before any magnification by the eyepiece lenses. So the only 'Magnification of Microscope' we need to consider in the calculation below, is the objective magnification.

In simple terms scaling works like this:

Sample Real Size X Objective Magnification = Size of Sample displayed on reticle

Example

A dot 1mm in diameter at the stage, viewed with a X10 objective, will be the same size as an actual 10mm diameter circle drawn on the reticle.

A real particle with an approximate diameter of 150µm, when placed at the microscope stage will appear on the reticle as 1.5mm using X10 objective or 6mm using X40 objective

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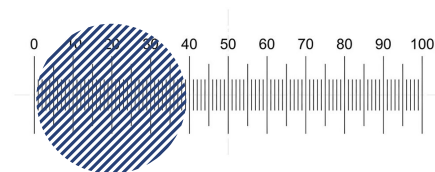
By reversing this process, you can find what size each division represents. Working from the reticle down allows you to judge which reticle scale size will enable you to measure the sample easily with the best resolution.

Size of Sample displayed on reticle / Objective Magnification = Sample Real Size

Example for a measurement at X400 magnification

A microscope with X400 total magnification is commonly made from two elements: X40 from the objective lens & X10 from the eyepiece lens ($40 \times 10 = 400$). As the reticle is mounted before the X10 eyepiece magnification, the magnification we use to work out the scaling is only the X40 objective magnification.

A sample or object 0.1mm in diameter, is magnified 40 times by the objective and would appear 4mm in diameter on the reticle. With a standard NE1 10mm/0.1mm scale reticle, the object would cover 40 small 0.1mm divisions, or from the 0 to the 4mm, marked as '40', the 40th division on the reticle scale.



Estimation and Limits to measurement

Practical size measurement

Working from an image viewed at X40 objective and using a NE1 10mm/0.1mm reticle.

If a sample or object covered from 0 to 3.5mm or '35' on the reticle scale (35 small 0.1mm divisions), the real size would be $3.5 \div 40$ (objective magnification) = 0.0875mm

The figure 0.0875mm from example above is the 'perfect world' answer. However, when measuring with a microscope, actual sizes should only be estimated. So the answer at best should be around 88µm.

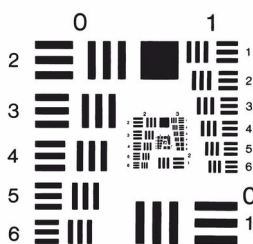
There are a number of reasons for this estimation in measurement. Some we can compensate for, some we can improve by good practice and experience and others are fixed and depend on the quality of the microscope and its resolution limitations.

Measurement with a Microscope

Resolution

The minimum resolution of a normal light microscope is $0.2\mu\text{m}$ (200nm). Although this is instrument quality dependent, there comes a point at which the operator is unable to distinguish between two individual objects. Once two small objects are closer than the limit of resolution ($\approx 0.2\mu\text{m}$) they will appear as one object and not two distinct objects. This is the effect of resolution limiting.

There are a number of resolution test chart products to test the resolution of your optical instruments. Visit our [Optical Resolution Charts Product Category](#) on our website or [Contact us directly](#) for further information.



You may not be concerned with the size of a small gap between objects, but even when sizing larger features, you are making a judgement of where the edge of the object begins. The instruments resolution, quality, optical alignment, as well as set up of any 'phase contrast' or 'condenser' controls and even how the sample is illuminated, can all effect this judgement. Some you can improve/adjust, some you can't and all are subject to the skill and experience of the operator.

One important tip when using a scale reticle. All scale patterns have lines. These drawn lines have a thickness. The thickness of the line can vary a little from reticle to reticle. Sometimes this is by design, but in addition there will also be a manufacturing tolerance variation. For the most accurate measurement, work to the line centre of the reticle scale. The position of the line centre will be the most accurate, and will not be effected by any line width variations that may occur. If working to the scale line edge, then use the same side edge of the division, unless the reticle has been especially designed to measure between inside line edges.

Measurement with a Microscope

Another method to improve your measurement in some critical circumstances, is to take a number of readings and calculate the average. This will help even out any variations. This is one of the methods that NPL used to reduce the uncertainty of measurement that may be due to uncontrollable variations and operator error.

However, in general these 'variations' can be very small and may not significantly vary your measurement.

Microscope Calibration

One important procedure you should carry out with your measurement microscope, is a Calibration Check using a stage micrometer. This is a method of confirming that the scaling factor you are applying to your measurements is correct.

This is especially important if the results are to be used to form the basis of a QA or Safety report, or any other situation, in which you should be able to demonstrate that the measurements recorded are correctly 'known' to controlled provable limits.

To this end, the stage micrometer used for this task should have a traceable certificate of calibration issued from UKAS, or another internationally accredited body.



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