Measurement with a reticle and a stage micrometer

1. Introduction

Whenever there is a need to make measurements with an eyepiece graticule, there is also a need to ensure that the microscope is calibrated. The use of a stage micrometer to check the divisions and measurements on the eyepiece reticle is the best way to achieve this calibration.

There are a number of factors that can influence the true calibration:

- Any inherent inaccuracies of the objective lenses
- Variations in the optical system itself (such as effective tube length)
- Inaccuracies in the reticle scale.

These combined errors need to be taken into account and quantified if measurements taken with the microscope are to be considered accurate within defined limits of uncertainty.

2. What is a Stage Micrometer?

A Stage Micrometer is simply a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and is used for calibration of optical systems with eyepiece graticule patterns. This is particularly important when alternating between objectives on one microscope or when using the same graticule in different microscopes.

3. What is a Eyepiece Graticule?

A microscope may be equipped with a linear measurement scale fitted into one eyepiece. This eyepiece scale is called a reticle or graticule. The terms reticle or graticule are interchangeable. The reticle can be used to measure any planar dimension in a microscope field, since the ocular can be rotated in any direction and the object of interest can be repositioned with the stage manipulators.

Using the procedure explained below a 'conversion factor' can be derived. This will enable the user to convert the apparent size of a subject as seen through the eyepiece scale, into a real world dimension.

An accurately derived conversion factor will compensate for any of the errors discussed earlier. To measure an object length, note the number of divisions spanned by the object then multiply by the conversion factor for the magnification used.

The conversion factor is different at each magnification. Therefore, when using a reticle for the first time it is necessary to calibrate the scale by focusing on a second micrometer scale (Stage Micrometer) placed directly on the stage.

4. Conversion Factor

To get the conversion factor, you are required to calibrate the microscope and its associated reticle with a stage micrometer.

The following is an example procedure:-

1. Select your normal or lowest magnification.
2. Ensure the eyepiece reticle is in sharp focus.
3. Place the stage micrometer on microscope stage.
4. Position and focus so the stage scale is clearly visible.
5. Rotate the eyepiece reticle and position the stage in the field of view so that the two scales appear parallel, one positioned above the other.
6. Adjust the alignment of the scales so that the zero values correspond.

With the zero values aligned, the stage micrometer scale will either appear longer than the eyepiece scale or alternatively it may appear the same length or shorter than the eyepiece scale.

5. If the stage scale appears longer than the eyepiece scale

Count the number of stage micrometer divisions that cover the eyepiece graticule scale.

Calculating the conversion factor

Using a Stage Micrometer with 100 divisions of 0.1mm having a 10mm total length

Example 1 - Selected objective magnification X4.

Suppose the full length of the reticle scale covered 25 divisions of the stage micrometer.
Then the full length of the reticle scale is equivalent to (25 x 0.1mm) = 2.5mm long.
For an eyepiece reticle with 100 divisions, each division will measure 25µm at the stage for this magnification.

Example 2 - Selected objective magnification X10.

Selecting the X10 objective and repeating the exercise above would show that the reticle scale now covers 10 divisions of the stage scale. Then the full length of the reticle scale is equivalent

to (10 x 0.1mm) = 1mm long. For an eyepiece reticle with 100 divisions, each division will measure 10µm at the stage for this magnification.

In summary you can apply these conversion factors to state what each division of the eyepiece reticle is measuring for a selected magnification.

X4 1 division = 25µm
X10 1 division = 10µm

6. If the stage scale appears the same length or shorter than the eyepiece scale

In some cases the stage micrometer scale may appear shorter than the eyepiece scale. In that case note how many eyepiece divisions match the full length of the stage micrometer scale.

Calculating the conversion factor

Using a Stage Micrometer with 50 divisions of 0.002mm having a 0.1mm total length

Example 3 - Selected objective magnification X40.

The full length of the stage scale only spans the first 40 divisions of the reticle scale.
Then the first 40 divisions of the reticle scale is 0.1mm long.
So each reticle division is equivalent to (0.1mm / 40) = 2.5µm.
For an eyepiece reticle with 100 divisions the full length of the reticle scale equals 250µm (0.25mm) at the stage for this magnification.

7. Real case

The examples above give ideal perfect world results. They do not exhibit calibration discrepancies that occur due to any inherent inaccuracies of objectives and the optical system, or the inaccuracies in the eyepiece reticle scale. These errors are more noticeable at higher magnifications using stage micrometers with the finest divisions.

The following is such an example

Using the same Stage Micrometer as example 3 and a X100 Oil immersion objective.

Calculating the conversion factor

Using a Stage Micrometer with 50 divisions of 0.002mm having a 0.1mm total length

Example 4 - Selected objective magnification X100.

The full length of the stage scale only spans the first 95 divisions of the reticle scale.
Then the first 95 divisions of the reticle scale is 0.1mm long.So each reticle division is equivalent to (0.1mm / 95) = 1.05µm. For an eyepiece reticle with 100 divisions the full length of the reticle scale equals 105µm (0.105mm) at the stage for this magnification.
Other factors should be taken into consideration before taking this figure of 1.05µm per division as a clear-cut certainty.

Human error can be introduced in taking the measurement, as alignment of the scales can be a little difficult at high magnifications. It is good practice to reduce the effects of human error by taking a number of measurements and averaging the results.

Mathematically calculating that each division = 1.05µm is OK, but what is the microscopes limit of optical resolution?

Assuming a resolution of 0.5µm or so using oil immersion, how could you report that an object covering 2.5 divisions in the reticle measures exactly 2.625µm?

Additionally, how can you be sure that the Stage Micrometer is in fact 0.1mm long, with divisions of 2µm. Just how precise is it, and how do you prove it? This is of particular importance if the measurements you are taking are critical to your process and required to be part of an ISO quality procedure. This is where a traceable calibration certificate of the Stage Micrometer may be required.