Measuring with a Microscope Reticle

Measuring with the microscope is simple when you understand a few steps to take before using your microscope eyepiece reticle. Prior to making any measurements you will want to calibrate your microscope at the lowest power objective lens using a stage micrometer if one is installed or by using a more crude method of placing a clear plastic "mm" ruler onto the stage. You will use the latter method in this lab.

An eyepiece reticle is a small piece of glass with a linear grid imposed on it that fits into the microscope eyepiece. When looking through the microscope, the reticle image is imposed upon your specimen image. Most often the reticle is used to make measurements or count particles. Don't be confused by any numbers that might be on the reticle. They do not represent any absolute units; they are there to help you count the individual smaller divisions, called reticule units (r.u.)

In this reticle example, there are a total of 50 r.u.

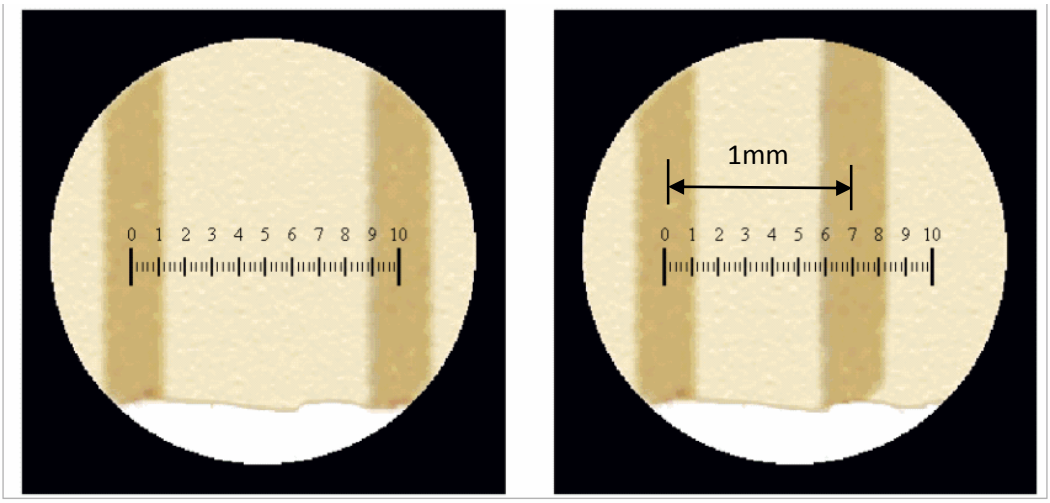
Reticle Ruler

When using a microscope eyepiece reticle with different objective lenses, the distance between the lines, or r.u.'s on the reticle changes as you change magnification. Therefore, you will need to calculate the distance between r.u.'s for each objective lens.

Note: Different types of reticles are available, and individual microscopes may vary slightly in their optics and powers of magnification. Therefore, you must calibrate the reticle for each objective and for each particular microscope that you use. Once you do it the first time and as long as you use the same microscope with the same reticle, you don't have to repeat the calibration.

Steps to Calibrate the Reticle Using Two Examples

To calibrate a reticle, switch to the scanning or low-power objective, place a clear plastic millimeter ruler on the stage, and then focus on the mm lines. What you see will resemble the illustrations below. Notice that microscope A is using an objective with a higher magnification than microscope B. Therefore, the mm lines will look farther apart on microscope A compared to microscope B, even though the size of the reticle units on both microscopes looks the same.



Observe and record the number of reticle units (r.u.'s) which correspond to 1mm as seen through each microscope in the sample illustrations above.

IMPORTANT: You must count the number of reticle units starting from the MIDDLE of the first mm line and ending at the MIDDLE of the second mm line.

In Scope A (5x), 1mm = 50 r.u.

In Scope B (4x), 1 mm = 35 r.u.

Next, determine the length of one r.u., for this magnification by dividing 1mm by the number of r.u.'s counted:

In Scope A (5x), 1 r.u. = 1 mm/50 r.u. = 0.02mm = 20 um (Note: 1mm = 1000um)

In Scope B (4x), 1 r.u. = 1mm/35 r.u. = 0.029mm = 29 um

Both scopes are now calibrated for their respective 4x objective lens.

So, if you were measuring a specimen using Scope B at 4x magnification and the length of the object was 47 r.u., then the actual length would be:

Length = 47 r.u. x 29um/.r.u = 1,363 um (or 1.4mm)

Calculating r.u.'s for Other Magnifications

Once you calibrate the reticle at the lowest power objective, you can calculate the number of um per r.u. for any other magnification on that scope, by the following formula:

µm / r.u. (original objective) x (Magnification of original objective / Magnification of the new objective) = µm / r.u. (using new objective)

So using the 10x objective would be:

Scope A 20um x 4/10 = 8um/.r.u

Scope B 29um x 4/10 = 11.6um/.ru.

And using the 40x objective would be:

Scope A 20um x 4/40 = 2um/r.u

Scope B 29um x 4/40 = 2.9um/r.u.

Record the calibrations for each magnification in your lab notebook for future use. As long as you use the same scope with the same type of reticle, you only have to do this once.