**Introduction:**

A microscope can be used not only to see very small things but also to measure them.  Things seen in microscopes are so small that centimeters or even millimeters are too big.  As a result, micrometers (or microns) are used.    A micrometer, also written µm, is one thousandth of a millimeter - it's 10-6m.

[](http://www.mr-damon.com/experiments/1ib_bio/micrometer_scope_eyepiece3.jpg)For this, a micrometer eyepiece is used in place of the standard eyepiece of the microscope.  This has a series of numbered lines inside of it which make it look like a ruler (see image to the right, click on it to see a bigger version).

The images below show what the eyepiece looks like (with its protective box) and where to put it on the microscope.

   

### Method - How to use it:

1. [](http://www.mr-damon.com/experiments/1ib_bio/micrometer_scope_slide1.jpg)After placing the special eyepiece, it is necessary to calibrate the microscope.  To do this, a calibration slide must be used.  This is a glass slide with one one-hundredth of a millimeter, 0.01mm, engraved on to its top surface (see photo to the right).  Use care when handling this little piece of glass - it costs a lot of money to replace!  Since a hundredth of a millimeter is very small and difficult to see, a circle is drawn around it.  This slide allows us to find out how big things are as we look at them through the microscope at different powers of magnification.  Put the slide on the stage as shown in the photo.  Be sure that the top of the slide (the surface with the microscopic lines engraved on it) is pointing up.



1. Set the microscope to low power and focus on the lines engraved on the surface of the calibration slide.   You should see the following:





1. The number of lines must be counted.  As shown in the figure above, the eyepiece lines have numbers on them whereas the calibration slide's lines do not.  The total number of eyepiece lines (which will be called **X**) are from line 21 to 59.  That's a total of 38 lines.  The number of calibration slide lines (which will be called **Y**) show a total of 10 lines (note that the little lines mark off half spaces and that the first line is not counted because is shows the zero mark).
2. Calculate how much each line of the eyepiece measures.  In other words find out what distance is shown between each line of the eyepiece.  To do so, use this equation:



If we plug in the numbers from the example in step 3, we get 10/38 x 10µm = 2.63 µm.  That means that as you are looking through the microscope at low power, the space between each line on the micrometer eyepiece measures**2.63µm**. Try it on the cell to the right.  The cell goes from line 22 to line 29 on the eyepiece so it measures about 7 lines across.

If a cell is 7 lines wide when observed on low power, that means that in reality, it measures 7 x 2.63µm = 18.4µm.  Since we know that cells are supposed to be between 10 and 30µm, it makes sense that the human cheek cell in the photo above measures just over 18µm.

1. After calibrating for low power observations, medium power should be calibrated.  Switch to the medium power objective lens and focus once again on the lines etched into the top of the calibration slide.  Use the same technique as steps 3 and 4 counting the lines and calculating the measurement between two lines on the eyepiece.  Try with this example (click to see what is viewed in the microscope):



Top of Form

* What is the value for Y?                            (click on the ??? to see the answer)
* What is the value for X?                           
* Using the calculation, what is the value for the measurment between any two lines on the eyepiece?                            
* Now look at the cell below and measure its size in µm.  It is a bit difficult to see where its cell membrane ends because it is stuck together with other cells.  What do you get?  Size =                            Is this close to what we got for the size of a cell in low power?  It should be!



Bottom of Form

1. Do the same for high power.  Here is what the calibration slide looks like:



If you follow the same steps, you should get a value of **0.26µm** for the space between the lines on the eyepiece.  Question:  How many lines would an 18µm-wide cell take up on the eyepiece as you look at it through high power?  If you work it out, you should get about 70 lines.

1. [](http://www.mr-damon.com/experiments/1ib_bio/micrometer_scope_slide3.jpg)Now it is possible to use the microscope to observe and measure things.  Take the calibration slide out and carefully put it back in its protective box so that it does not get damaged.  Now place the slide of what you would like to look at on the stage.

Note that if you change microscopes, the calibration process must be done again for each of the objective lenses that you are using.  Why?  Because the magnification is different on different microscopes.  To save time, only calibrate the eyepiece for the objective lenses that you will be using.

### (Optional) Using a calculation to get other powers:

(If you don't want to do this part, go down to the bottom of the page where there are more examples.)

Another time-saving method is to calibrate one power (low power, for example) and then calculate the proportions rather than measuring the other two.  Note that you do not need to know this for an exam.

Look at our example above:  for 40x (low power), the calibration was 2.63µm.  For 160 power (medium power), the calibration was 1.05µm and for 400 power, it was 0.26µm. A proportion can be set up:



Notice that each time we zoomed in with ojective lens, it increased the value of the proportion by 10 fold.  So, if you have the number for low power (2.63µm for 40x), find out what the magnification power is for the other objective lenses and use the formula below to calculate the calibration for medium power:



In our exapmle this means:



or for medium power to high power...



or...



Concept:

# CALIBRATING A MICROSCOPE

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| --- |
| https://www.microscopeworld.com/images/reticle-grid.jpg |
| To properly calibrate your reticle with a [stage micrometer](https://www.microscopeworld.com/p-652-stage-micrometer-mm-or-inches.aspx), align the zero line (beginning) of the stage micrometer with the zero line (beginning) of the reticle. Now, carefully scan over until you see the lines line up again. You can then use a simple ratio to determine the value that each line represents in your reticle. In the example above, the eyepiece micrometer (reticle image) is on the top and the stage micrometer image is on the bottom. The stage micrometer is 1 mm long with 100 divisions so each division of the stage micrometer is one one-hundredth of a mm (0.01mm or 10 um). Hint, you move the decimal point over three places to the right to change mm to micrometers. The eyepiece micrometer is divided into 100 units. We don't need to know the actual distance between marks on it. When the zero marks are lined up, scan across and look for a convenient point where the lines converge again. If you look at the 30 mark on the reticle, you will see pretty close alignment with the stage micrometer. How many divisions? Did you say 20? You are right! And, if each line is 10um wide, what will 20 lines equal? Answer: 200um. Now it is just a simple math ratio. 30 divisions of the reticle (eyepiece micrometer) equal 200 micrometers. So what does one division on the reticle equal? Let's see, 30 is to 200 as one is to X. Remember how to do a ratio? Two fractions, 30 over 200 equals 1 over X. Cross multiply, you get 30X=200um, solve for X by dividing both sides by 30 and X equals 6.7 um. Notice that they line up again at 60 but alignment is off by one at 90. If we use 90 and 61 (610um) we get 6.8um. The wider the interval, the more accurate your results should be. Remember, this distance between reticle lines is only good for that particular objective lens and it may not come out to be a nice round number. When you switch to a different objective, you must recalibrate. Quiz time: Our [stage micrometer](https://www.microscopeworld.com/p-652-stage-micrometer-mm-or-inches.aspx) has a line 1mm long with 100 divisions. That means that each division is one one-hundredth of a mm (.01mm or 10um). When looking at it with the reticle, you notice that the lines converge at 8 and again at 16. We will choose 16. At the 16 mark on the reticle, we notice 60 lines on the stage micrometer. What does each mark on the reticle represent? If you got 37.5 um, give yourself an A!For further information you may want to view this link about [eyepiece reticle calibration](http://www.microscopyu.com/tutorials/java/reticlecalibration/index.html). |

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