Calibrating a compound light microscope

In order to estimate cell size during microscope investigations, the microscope should be calibrated. This means using a stage micrometer (a microscopic ruler), a calibration grid to measure the distance across each field of view (FOV[1]). As you increase the magnification the FOV decreases. If you are viewing cells under the microscope, at low magnification you may see many cells appearing very small. As you increase magnification you see fewer cells and they appear larger. The cells are not actually changing in size, just appearing larger. You usually get a more accurate estimate of cell size when viewing them at a higher magnification. This procedure describes how to calibrate your microscope to estimate cell size. As every microscope is a bit different, each microscope should be calibrated independently. Once you have established a calibration table for the microscope, you refer to it each time you view different cells.

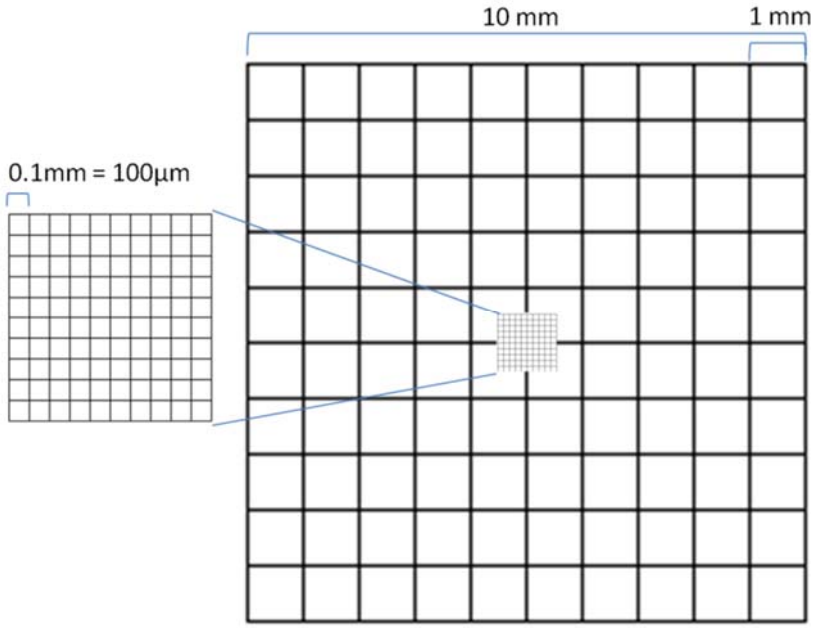
Materials

- ☐ Compound light microscope
- ☐ Stage micrometer or Calibration grid

The following procedure explains calibration of a microscope with 10x eyepiece lenses and 4x, 10x, 40x and possibly 100x objective lenses using a basic grid with smallest division 0.1mm = 100µm.

The calibration grid

Whole grid = 10mm (the grid has 10 squares, 1mm each)
Squares = 1mm
Small inner microgrid: 1 small square= 0.1mm = 100 micrometers



[1] FOV = field of view, the circular area seen through the eyepiece lens at each magnification

Procedure

1. Using the 4x objective lens (40x magnification), place the calibration slide on the stage; focus. At 40x magnification you will see most squares in the grid.
2. Align the left edge of the grid with the left side of the FOV, with a row across the diameter of the FOV.
3. Count the number of 1mm squares across the FOV. Enter that value in Table 1
4. Go to the next highest magnification, 10x objective (100x magnification). Focus.
5. Align the left edge of the grid with the left side of the FOV, with a row across the diameter of the FOV.
6. Count the number of 1mm squares across the FOV. Enter that value in Table 1
7. Go to the next highest magnification, 40x objective (400x magnification). Focus.
8. Now you will need to look at the microgrid in the centre of the large grid. Align the left edge of the microgrid with the left side of the FOV, with a row across the diameter of the FOV.
9. Count the number of 0.1mm squares across the FOV. Enter that value in Table 1.
10. If you have a 100x objective lens (for 1000x magnification), repeat the process.
11. Calculate the diameter and area of each FOV.

Table 1: Calculation diameter and area of FOV at each magnification

Objective lens	Number of squares x Square size	Diameter of FOV (µm)	Radius of FOV µm (and mm)	Area of FOV (mm^2) **area = πr^2**
4x				
10x				
40x				
100x				

When to use Diameter and Area of FOV

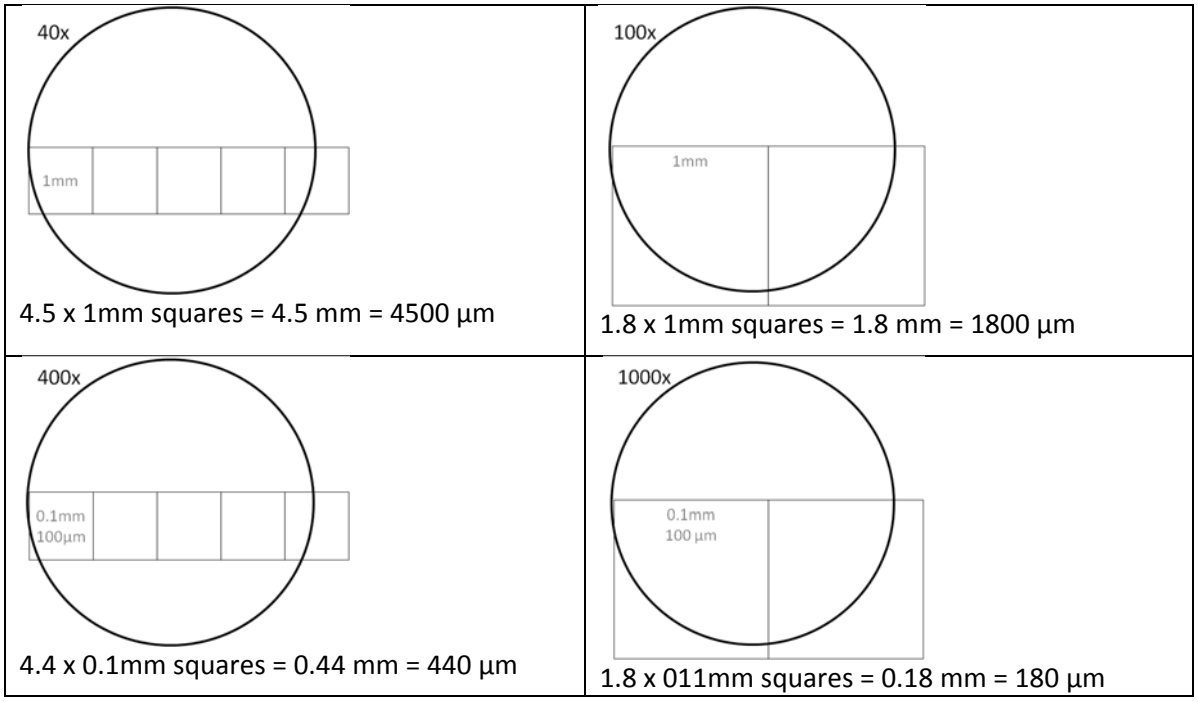
When estimating the size of a cell, use the diameter of the FOV.
Place the sample on the microscope and find the best magnification and light intensity for viewing the cells. Find the maximum magnification in which you can still see whole cells and a number that can reliably be counted. Count the number of cells across the FOV.
Cell size = diameter of FOV ÷ number of cells

When estimating the density of cells or structures such as stomata in a sample, use the area of the FOV.
Place the sample on the microscope and find the best magnification for viewing the cells or structures. Use a magnification that has whole structures clearly visible, and a number that can reliably be counted; if there are too many, you are likely to double count or miss some. Count the number of cells in the entire FOV.
Cell density = number of cells ÷ area of the FOV

Sample calibration

(note that every microscope is a bit different and should be calibrated independently)

Figure 1: Illustration of the procedure for aligning the calibration slide at each magnification. Note that only part of one row of squares is shown here.

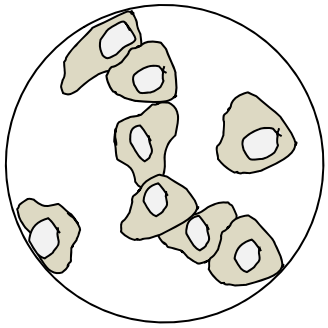


Sample Table 1: Calculate diameter and area of FOV at each magnification

Objective lens	Total magnification	Number of squares x square size	Diameter of FOV (µm)	Radius of FOV µm (mm)	Area of FOV (mm^2 or μm^2) **area = πr^2**
4x	40x	4.5 x 1mm	4500	2250 (2.25)	15.9 mm^2
10x	100x	1.8 x 1mm	1800	900 (0.9)	2.5 mm^2
40x	400x	4.4 x 0.1mm	440	220 (0.22)	0.152 mm^2
100x	1000x	1.8 x 0.1mm	180	90 (0.09)	0.025 mm^2

Examples of application

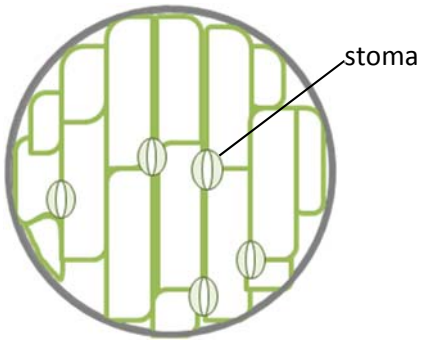
1. *Measuring cell size*



Sample: cheek cells
Magnification = 400x (10x eyepiece, 40x objective)
Diameter of FOV = 440 µm
Number of whole cells across FOV = 6

$$cell\ size = \frac{440\ \mu m}{6} = 73\ \mu m$$

2. *Measuring density of stomata on a leaf surface*



Sample: leaf epidermis
Magnification = 100x (10x ocular, 10x objective)
Area of FOV = 2.5mm^2
Number of stomata across FOV = 5

$$stomatal\ density = \frac{5}{2.5} = 2\ \text{stomata/mm\^2}$$

Sourcing Materials

Item	Supplier	Cost in 2015
Minigrid product # 2210 : A basic grid with smallest division 0.1mm (100µm)	Haines	$19
Minigrid glass slide product # M7.30 : A basic grid with smallest division 0.1mm (100µm)	Southern Biological	$29.50
Same or similar minigrid	Science Supply	
Calibration slide (4 dot): A more complex and precise slide; 4 dots of 0.07, 0.15, 0.6, 1.5mm diameter plus a grid with smallest division = 0.01mm (10µm)	Australian Instrument Services	~$45-50

Note: The method described here used the Haines Minigrid as it is an affordable option for a school laboratory. The grid lines are fairly wide, so measurements were estimated from the middle of each grid line.