

## Digital Microscope

To be able to measure the diameter of a red blood cell, or length of a sperm cell, or the length of an onion cell epidermis, a calibration table must be done first.

To perform the calibration using the software provided with the microscope:

- 1. Connect the cable from the digital camera port to the USB port on your laptop & turn on the microscope.
- 2. Open the ISCapture program that has been loaded onto your desktop.
- 3. Obtain a mini grid slide and place on the stage ready to be viewed.
- 4. Firstly, use the 4x objective lens & use the course & fine focus to observe the grid pattern. This will be seen through the eyepiece AND as a live feed on the computer screen. This is a 40x magnification as the eyepiece is 10x.
- 5. On the top left of the screen on the computer there are three buttons capture, browse, image. Using the mouse locate the arrow cursor & click CAPTURE. This will save the grid image within the program itself & will appear at the bottom of the screen.
- 6. At the top of the screen there is a measure button click that and you will see a drop-down menu appear. Now click on the calibrate icon as indicated below:







- 7. A new screen will appear once the calibrate button is clicked titled Calibration Wizard. Click on the button Load Image and a screen will pop up that will show the captured grid image. Click on the captured image.
- 8. This will then open this image on the screen and the Distance Scaling button will come on. Click on this and move the arrow onto the grid screen this arrow will turn into a + symbol. Click on one of the grid lines (in the centre) & drag the line to another grid line 10 units away (this would be a measurement of 1 mm for instance meaning each square grid is 0.1 mm).
- 9. You must now enter in the scale info in the table that is now active. For example:

Name: 40x – 1mm Length: 1 Pixels: (this is done by the program for you) MeaUnit: mm (this is selected by clicking on the down arrow)

10. Now click OK then Finish

You have successfully calibrated the scaling for the 4x objective lens only.

So now the process must be repeated for the 10x & 40x objective lenses. Unless the mini grid is super fine, the 100x objective lens will be impossible to calibrate.

The idea now is to replace the mini grid slide with a biological slide, whether that be phloem cells, protozoa, red blood cells or onion cells & capture the image of that slide. Always start with the 4x objective lens.

Once the new slide has been captured, go to the measure button again, click it and then click on the following icon:





This will bring up the calibration table that has saved all the calibrations previously done. It is very important that you select the calibration that is the same as the objective lens being used. This is because the software does not recognise which objective lens has been selected.

In the calibration table, you must select the correct calibration and will appear with a blue highlight – once done you must click Apply to Image & the hit close. The slide being observed will come back on the screen. Simply go back to the measure menu and select any of the functions you need to use.

For the diameter of a red blood cell or length of a sperm cell, simply click on the Line icon to measure the linear length of a cell.



If you wanted to measure the diameter, radius, perimeter or area of a red blood cell click on the 2-points or 3-points circle icon.

If you are observing a red blood cell using the 100x objective lens the magnification of that cell is 1000x. However, if your calibration only goes to 400x the measuring will be out by a factor of 2.5. So if the measured red blood cell measures to be 0.02 mm & is being observed at 1000x magnification, 0.02 mm = 0.00002 m  $\div$  2.5 = 8 x 10<sup>-6</sup> m which is 8 µm or 8 microns.