MEASUREMENTS OF THE MICRO WORLD PART 1: CALIBRATING THE CAMERA

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INTRODUCTION:

When we start the venture of microscopy we know that we are going to take a trip into a world that is beyond our sight and that such a world is regularly going to be 1000th the size of a pin head. Obviously we know that if for most of the time we use an optical light microscope, the knowledge of this world will be limited to the wavelength of the part of the spectrum that we are using. It is possible to achieve more resolution with laser based or UV microscopes rather than using "white" light.

Even with the limitations of visible light we can go very, very far into the microscopic world and we can see bacteria that are just a few microns both in width and length.

Today it is possible to accurately measure microscopic subjects, thanks to cameras that have software that enable us to measure selected features within a picture or video frame.

A camera can be helpful to record photos or videos of our microscopic sample without worrying about measurements. But if we do want to take measurements, we need a step that is indispensable i.e. a calibration procedure using subjects of accurately known size and that are within the scale range of subjects that we typically study.

DEVELOPMENT:

For calibrating a camera, most of the packages when they are brand new come with a calibration kit. This consists as in the case of mine of a very small rule, in fact a microscopic rule, drawn on a slide, typically of 1mm.

The use of these so-called micrometer slides is very simple; here are the steps to follow.
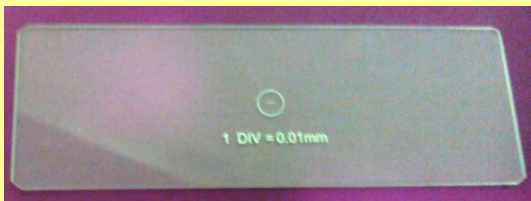

I am going to present the calibration procedure for my camera; an AMSCOPE MU 1000, but more or less it is the same principle for the rest of the brands.

First of all you have to install the software that comes with the camera, once installed you have to plug the camera to the computer and then attach it to the trinocular port or in the place of one of the eyepiece if your microscope does not have a trinocular port.
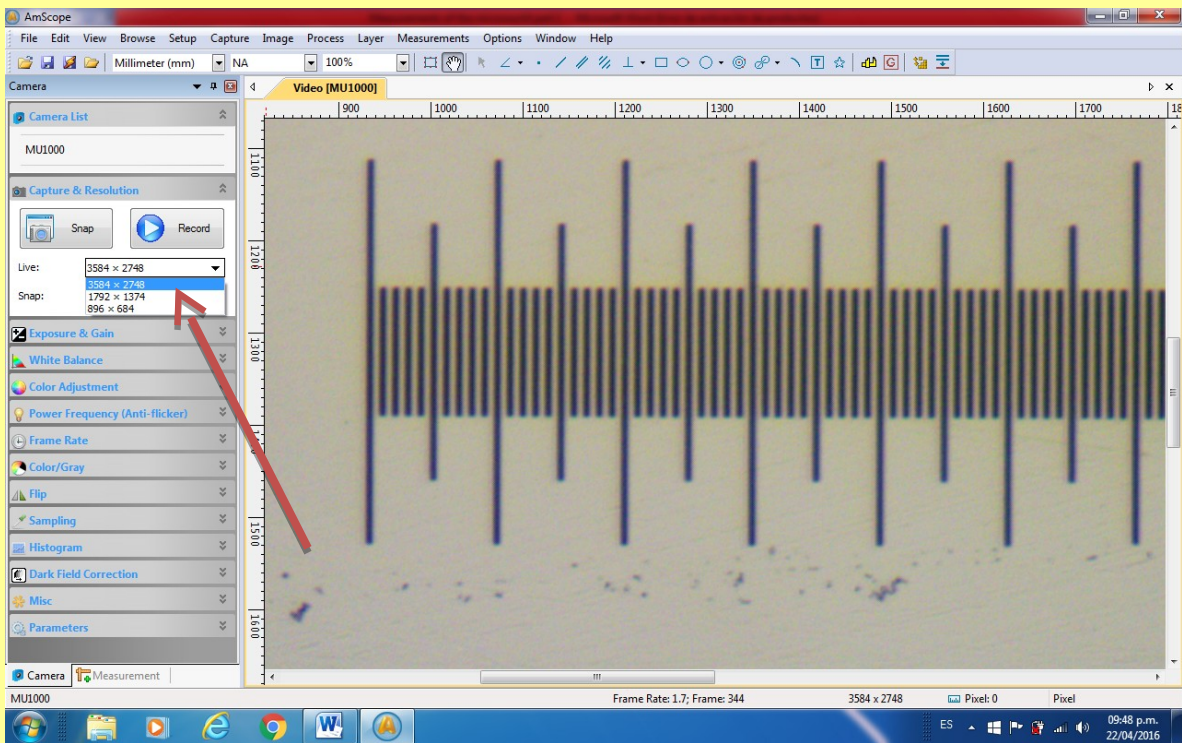
Then go to OPTIONS and to magnification as shown in the following image:
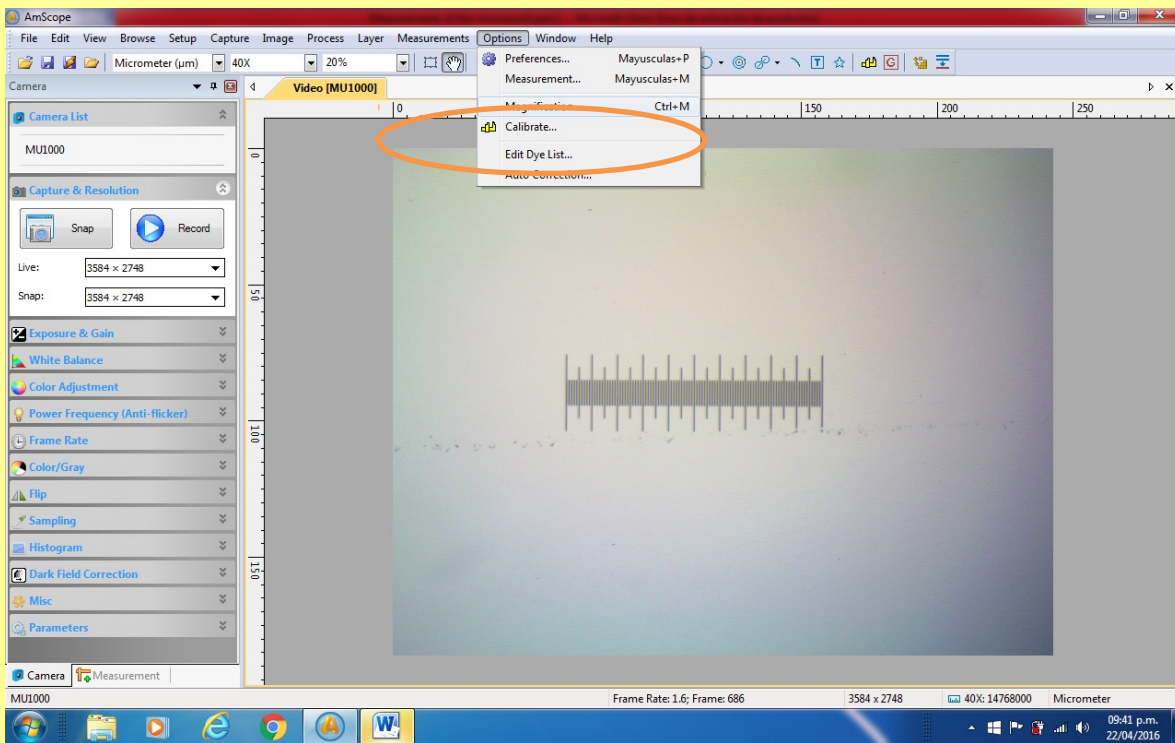
Then if the camera has one magnification recorded by default, delete it.
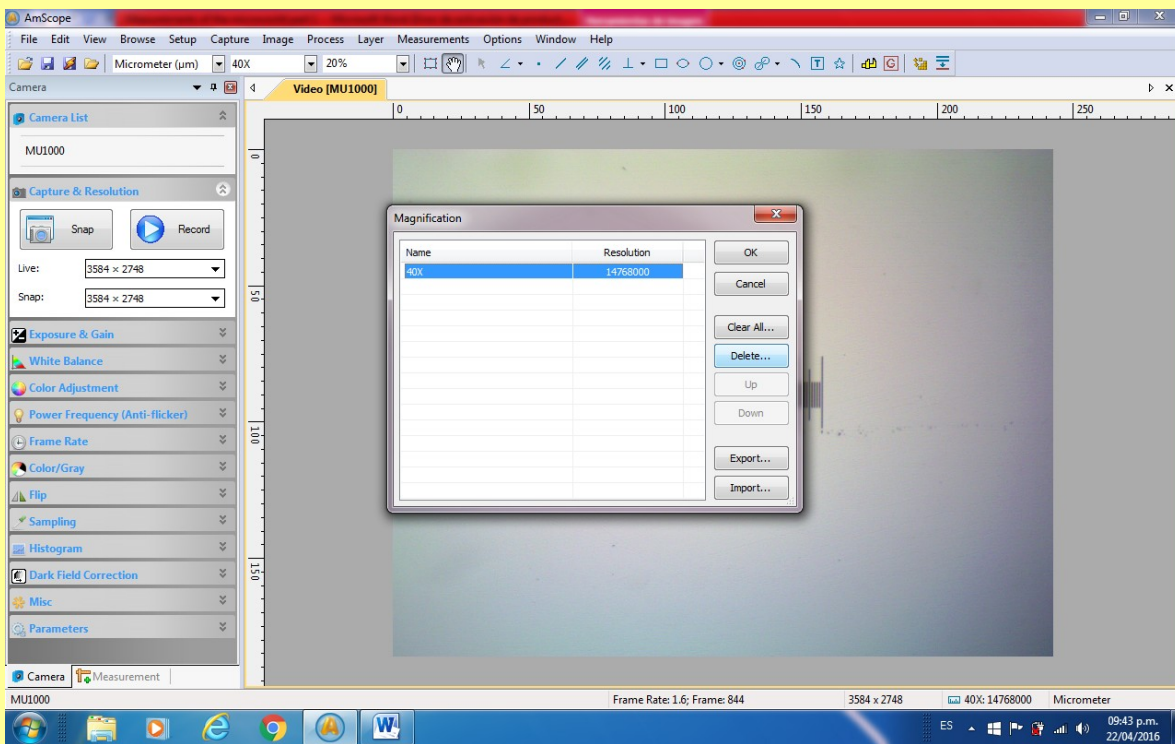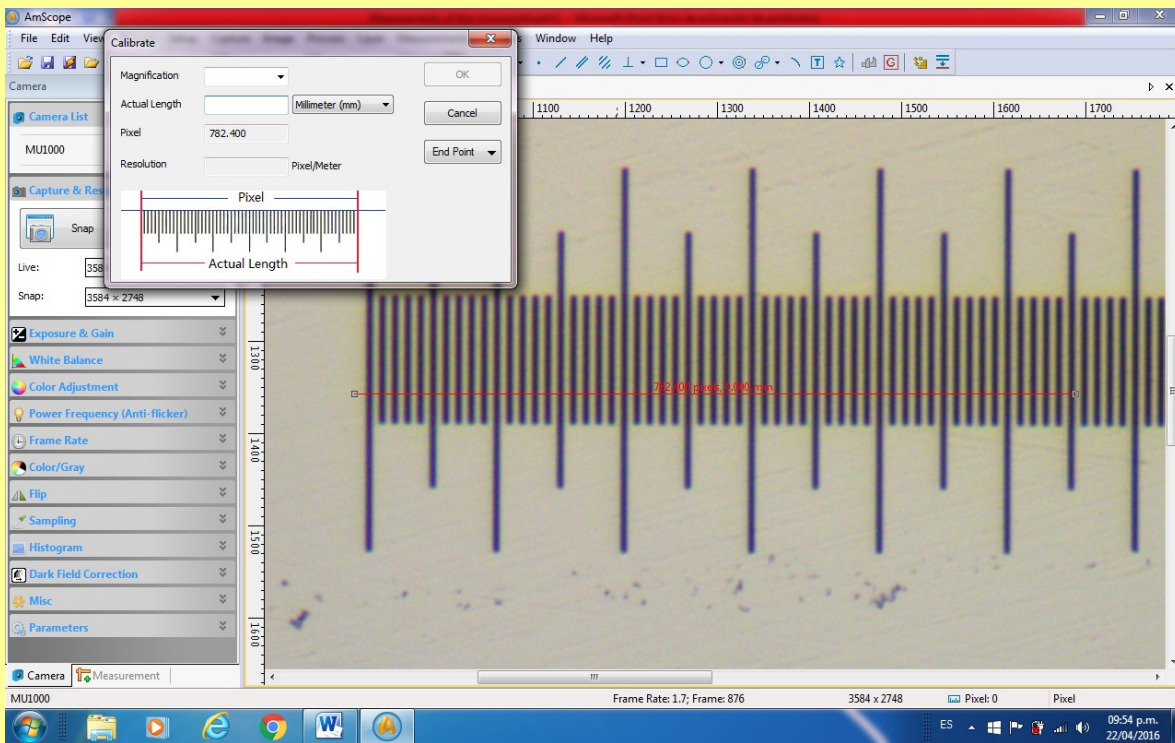

The next step is to place the rule upon the stage of the microscope and adjust the image, but be careful that you have the maximum value of pixels set for your camera and the 100% zoom.
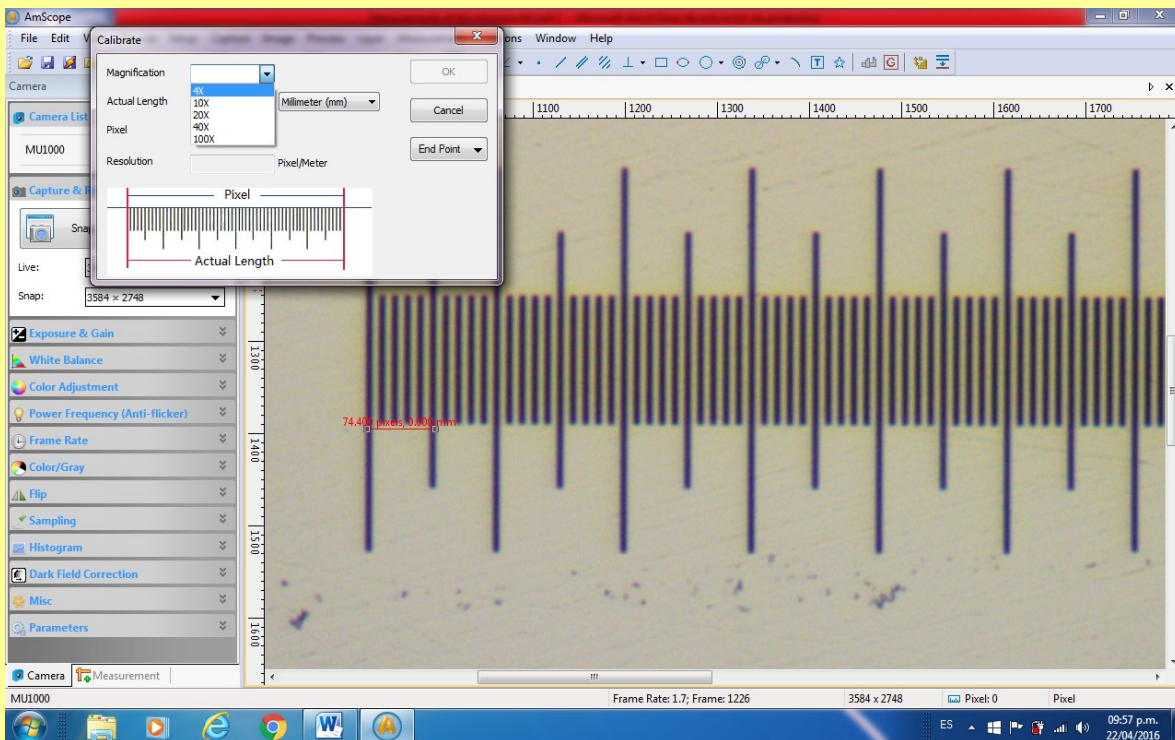
Then you go again to OPTIONS and to calibrate.
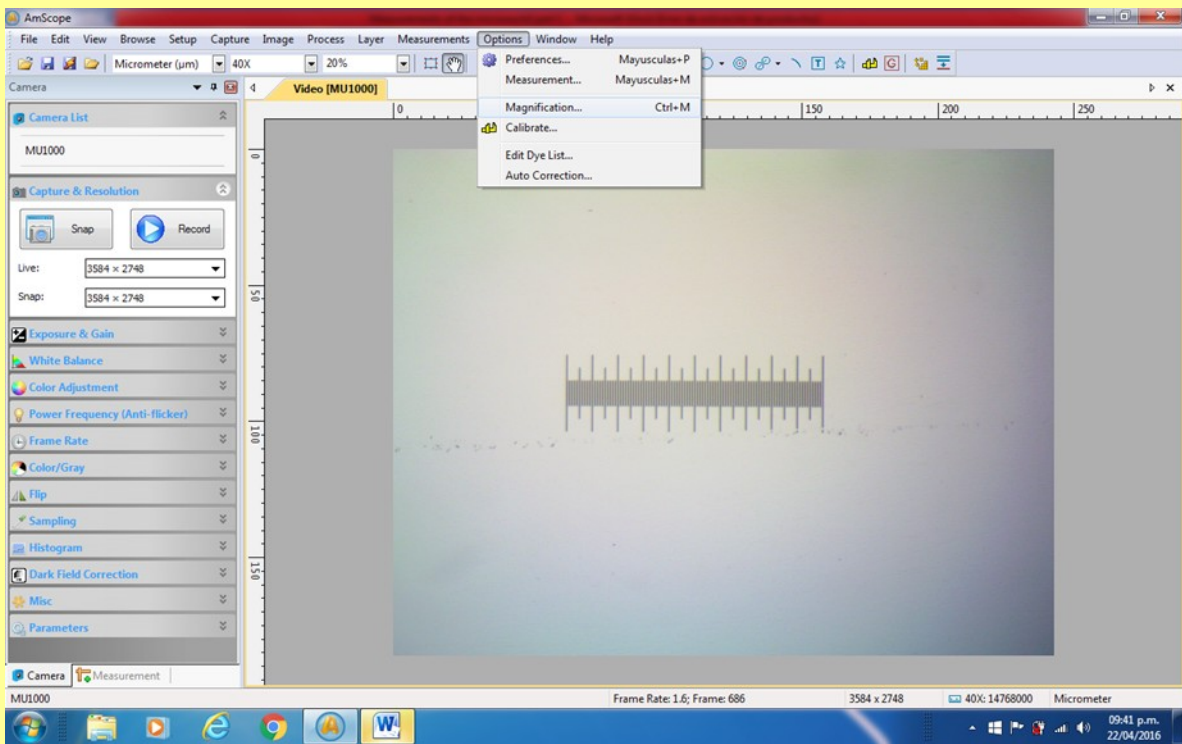
When you open the option and calibrate it, a red line appears upon the image of the rule.
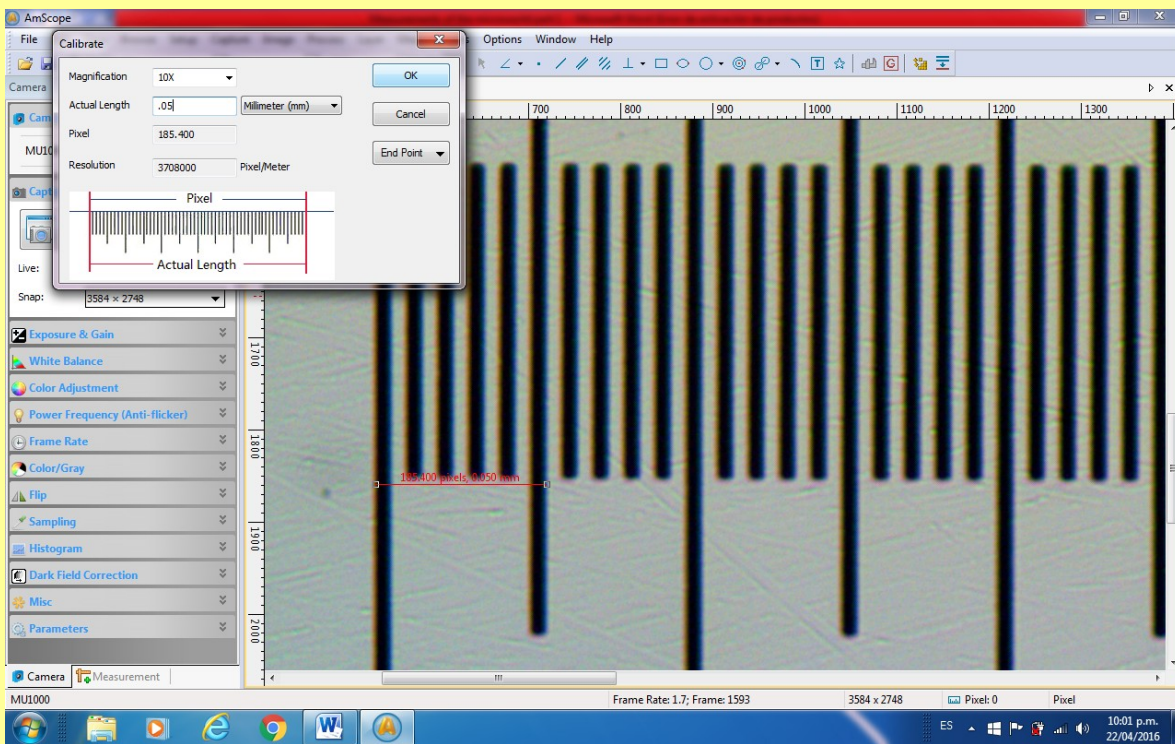
You select the magnification you want to calibrate, in this case 4x and on the rule you adjust - for example to 0.05 mm but remember that each division is 0.01 mm.
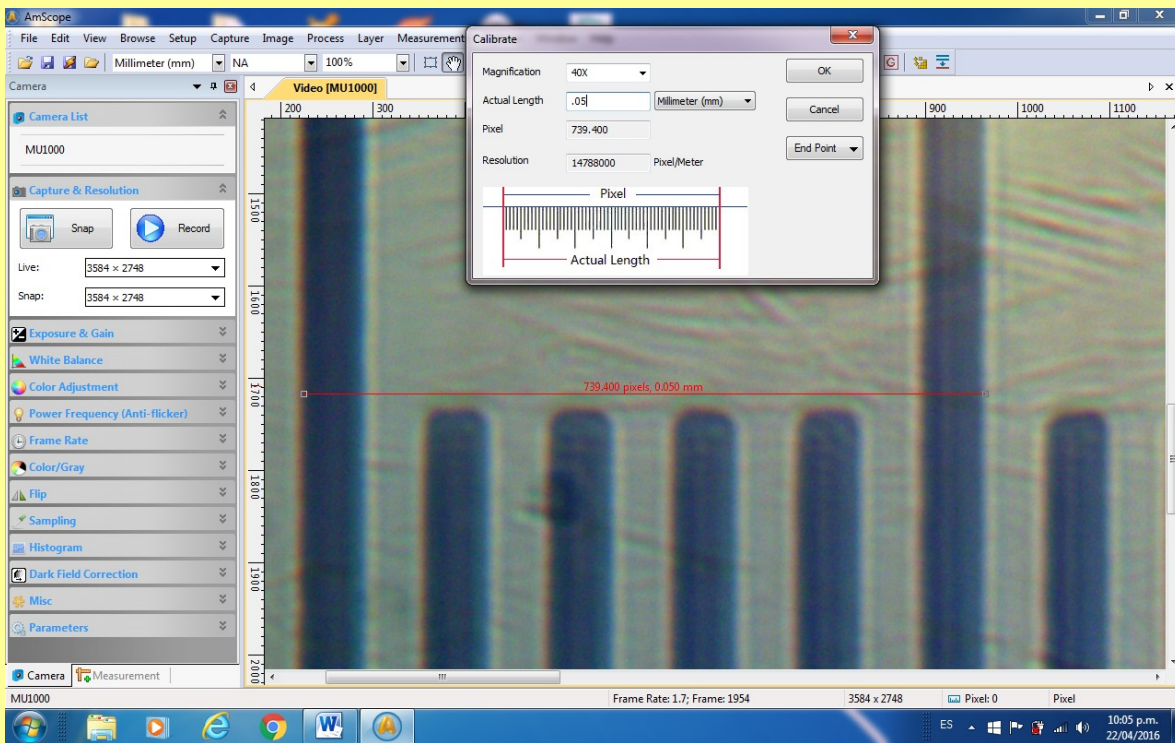

You enter the value of 0.05 mm in the box for the actual length and then you have the first magnification calibrated.
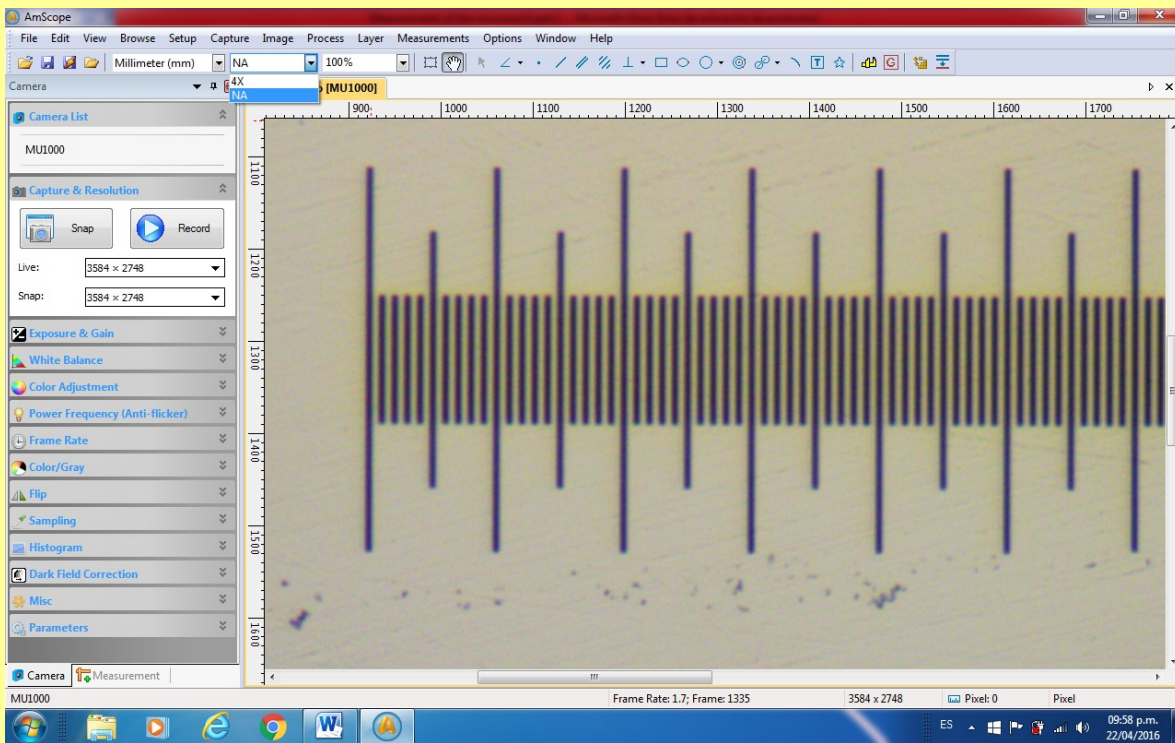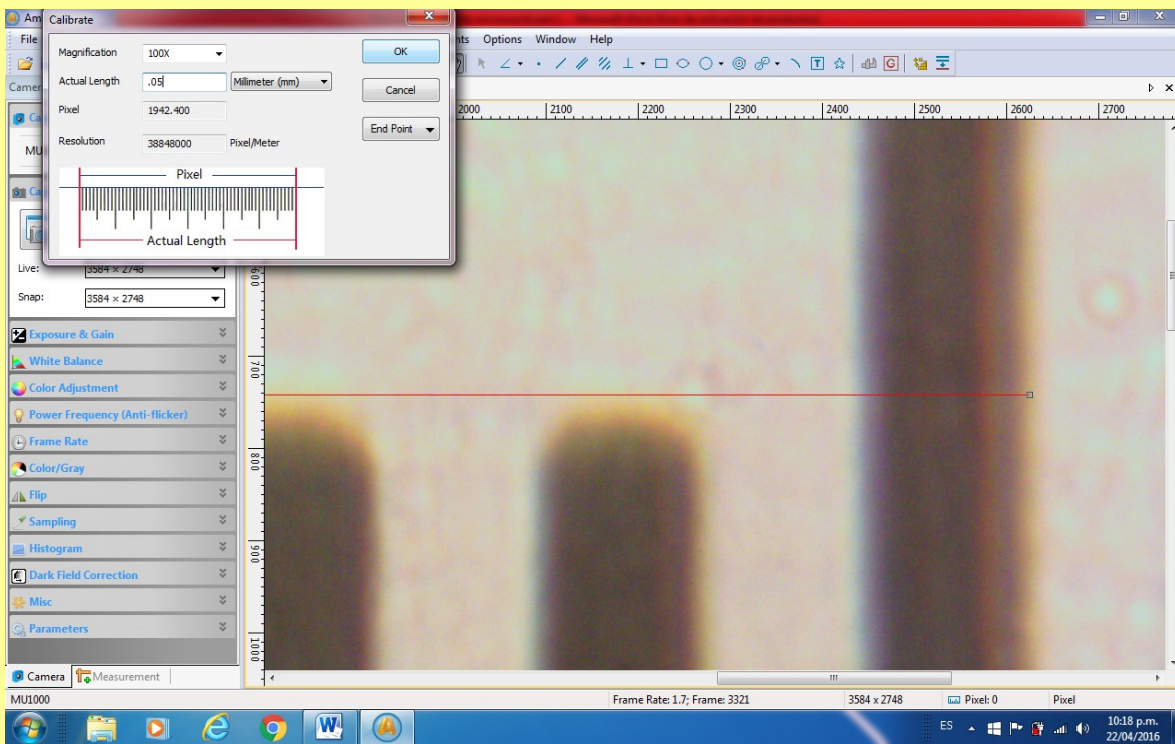
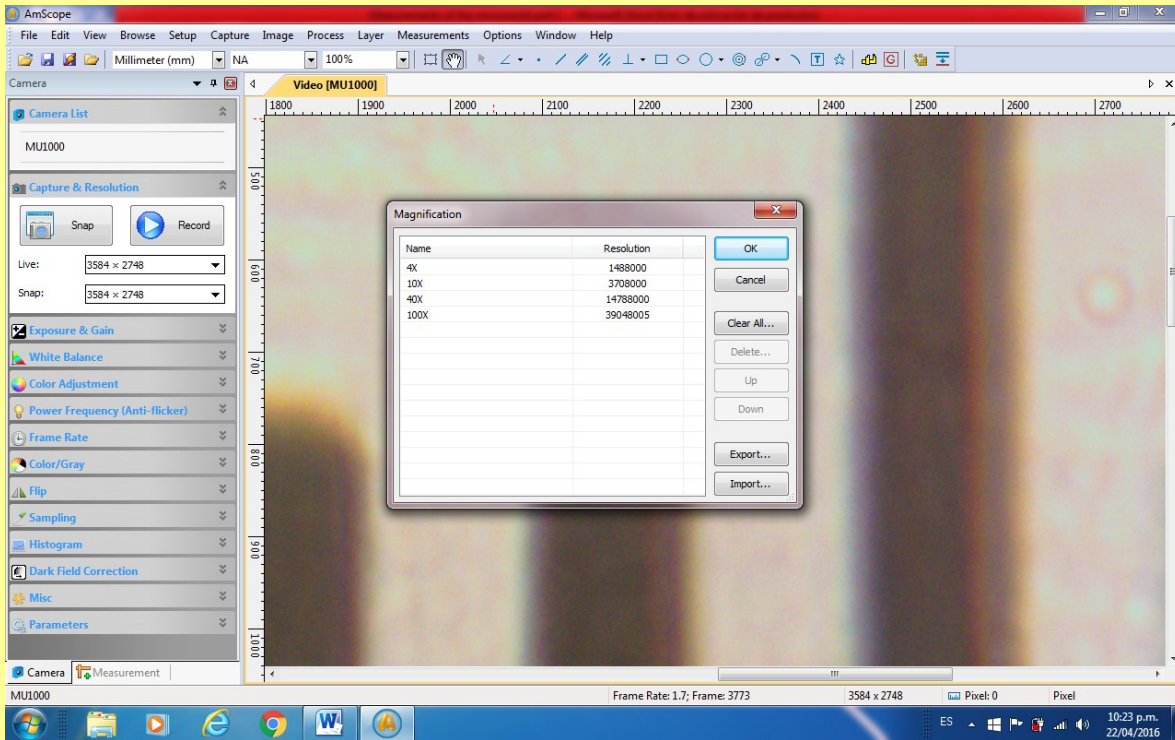The same procedure for 10x.

40x

100x

Then if you open OPTIONS and go to magnification you have there the values you have set with your calibration.

The calibrations are ready to be used to see if they work. To check that they are accurate and trustworthy, you have to try things that you know the size of.
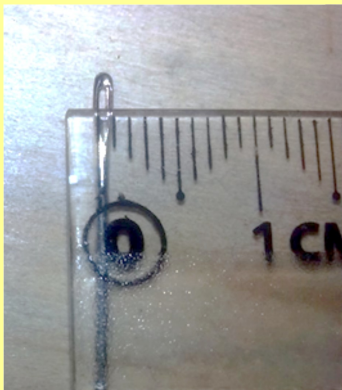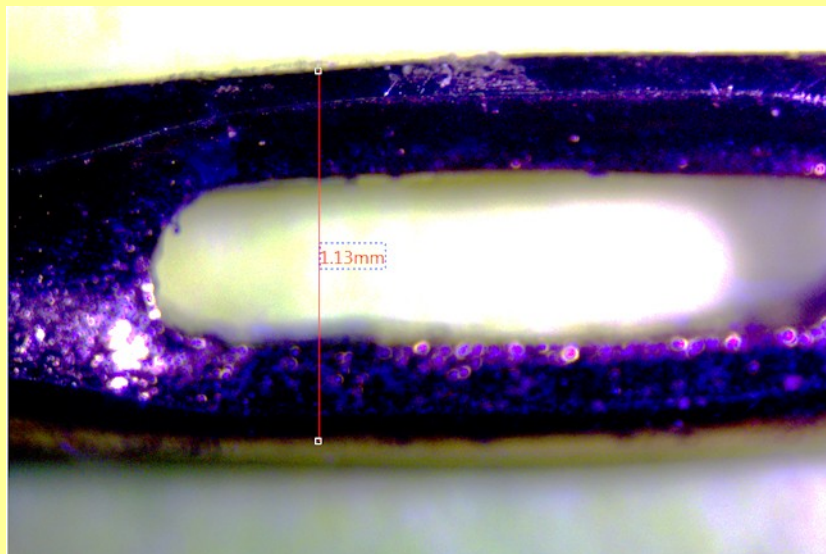
RESULTS:

The mosquito that I have shown in many of my articles can be seen with bare eyes upon the slide so measurement is easy with a rule. It is approximately 1 mm shown in brightfield for the default values of the camera at 4x.
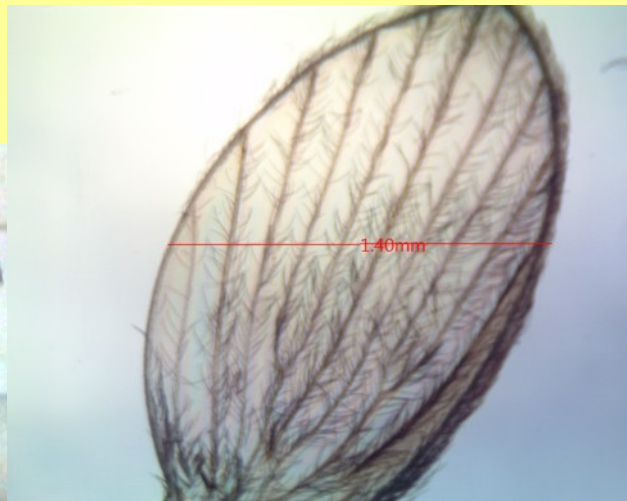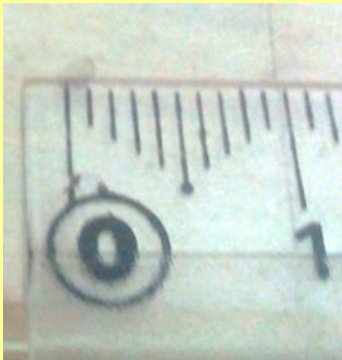

The width of the Mexican one peso coin epi illumination brightfield 4x:
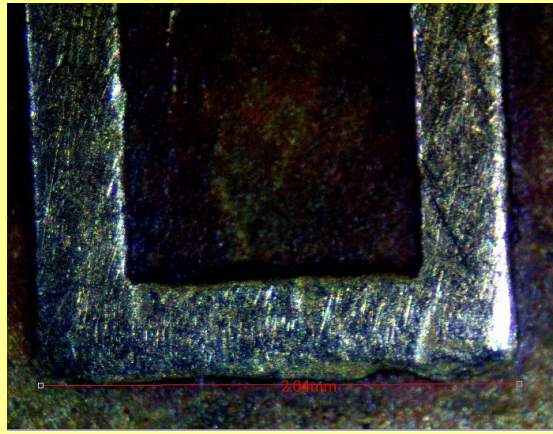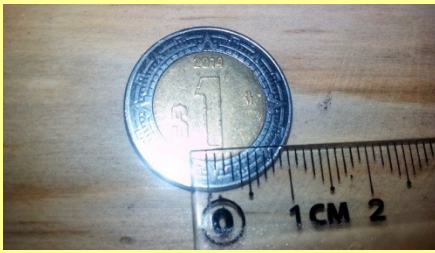
The eye of a needle 4x brightfield epi illumination.

The wing of a mosquito brightfield 4x.

A daphnia brightfield 4x.

For human red blood cells, their size can be found in *Wikipedia*, the free encyclopedia:

Human erythrocytes

A typical human erythrocyte has a disk diameter of approximately 6.2–8.2 µm[31] and a thickness at the thickest point of 2–2.5 µm and a minimum thickness in the centre of 0.8–1 µm, being much smaller than most other human cells. These cells have an average volume of about 90 fL[32] with a surface of about 136 µm², and can swell up to a sphere shape containing 150 fL, without membrane distension.

So we know using this data the value of the diameter of an erythrocyte and we can look for a cell in our sample that meets this size and look we have it.
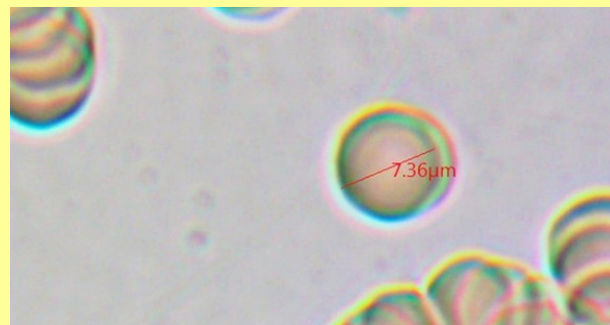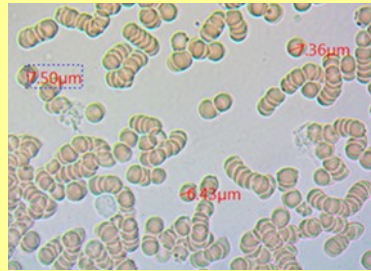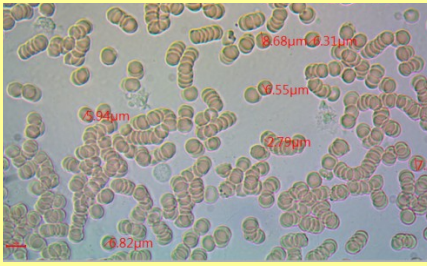
Human red blood cells
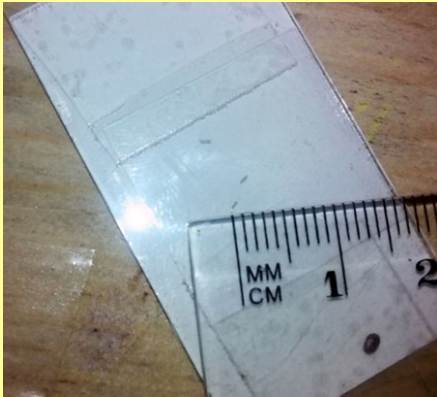
A very small piece of copper wire brightfield epi-illumination

CONCLUSION:

As it can be seen above, calibrating the camera is easy and trustworthy; from now on nobody can miss the opportunity to measure the microscopic world.

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(Above in anti-spam format. Copy string to email software, remove spaces and manually insert the

capitalised characters.)