MEASURING ACCURATELY WITH REFERENCES.

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Introduction:

As I have mentioned in previous articles, one of the things that I enjoy the most is to look for DIY methods to achieve a result related to microscopy. Today I am going to present a form of measuring using calibration references.

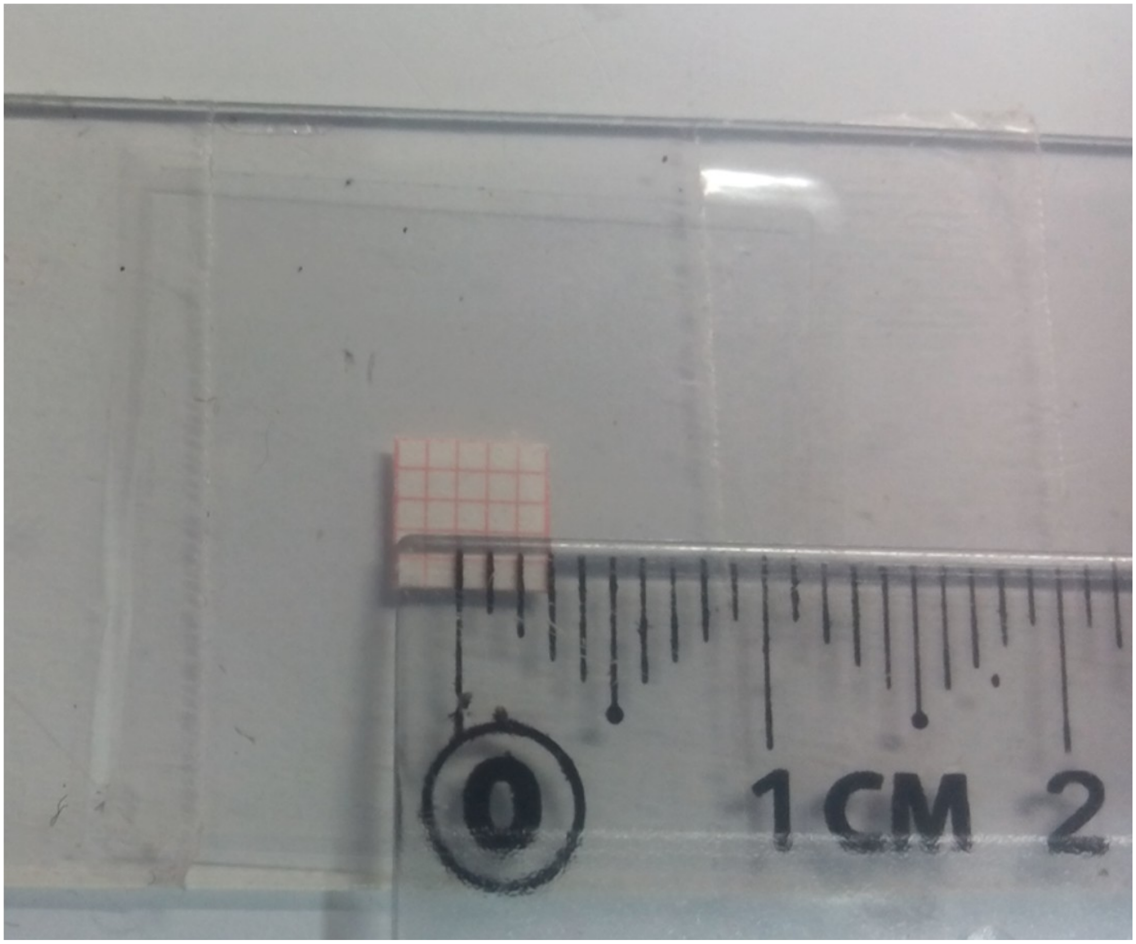
Sometimes we have the camera, but we lack the calibrator, probably because the kit we bought did not include one, or may be it fell down and got broken, or another of a thousand reasons.

Other times we are using a type of camera such as a consumer digital model because we do not have a specialized microscope camera, but we need or want to measure our observations. So the solution for all these problems is here, see below.

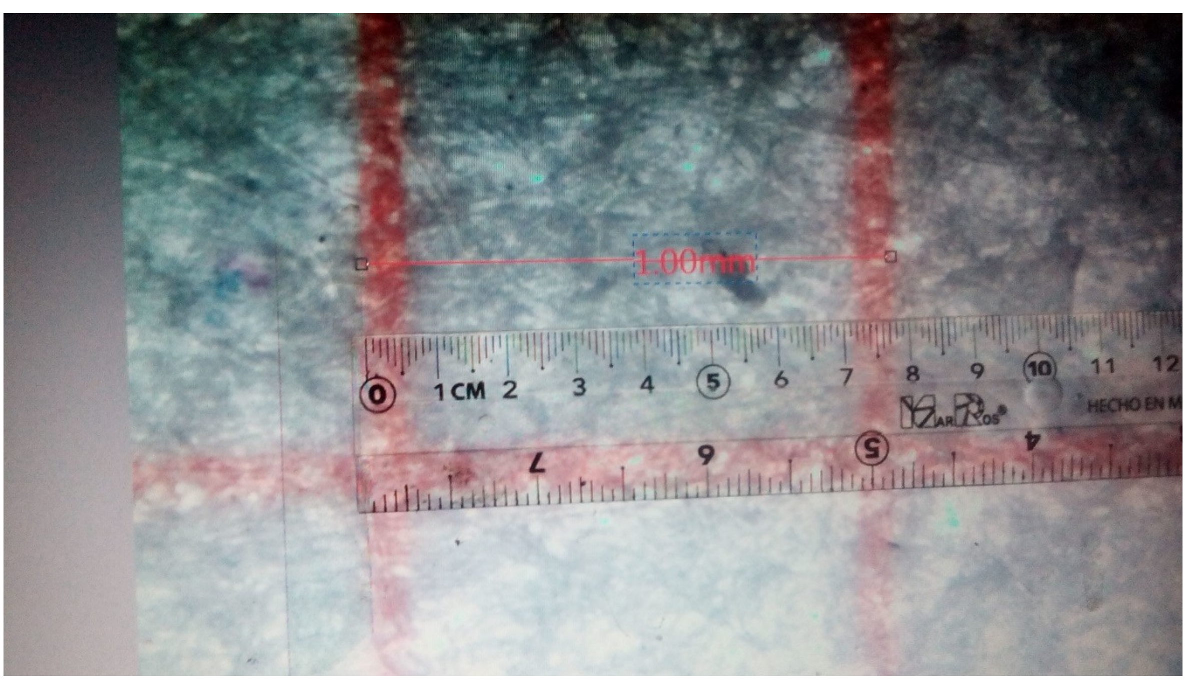
Development:

Last month I presented an article in which I introduced the topic of looking for something that could help establish a baseline for our measurements. I used a laser pointer and/or a caliper to determine the width of several known objects such as a toothbrush bristle, a strand of hair or a bit of copper wire. All of these can be measured accurately to be used as a reference for simply measuring or calibrating the camera of a microscope.

There are many references in the macro world that we can use, it is possible to choose the one we prefer. I selected arbitrarily because it is easy to get graph paper with a 1 mm grid, which can be found at any stationery store. I placed a small piece on a slide, covered it and placed it upon the stage of the microscope.



With the 4x objective on the microscope I open the software of my camera and measured the length of one millimeter in my computer at 20% zoom. Since my camera is already calibrated and with the purpose of showing the accuracy of this method, I demonstrate by measuring with the same camera that the square is one millimeter across.



With a conventional ruler I measure that length that it is going to be the reference value: **1 mm is 7.8cm at 20%** zoom in this software and on this computer.

With this value and with the equation of proportionality:

4x = 7.8

A = B Where x is just the symbol of times for the objective and A,B are the values for any other objective and the size of the image produced, our statements are as follows:

4x objective produces at 20% zoom an image of 7.8 cm regarding a millimeter in any direction of the screen.

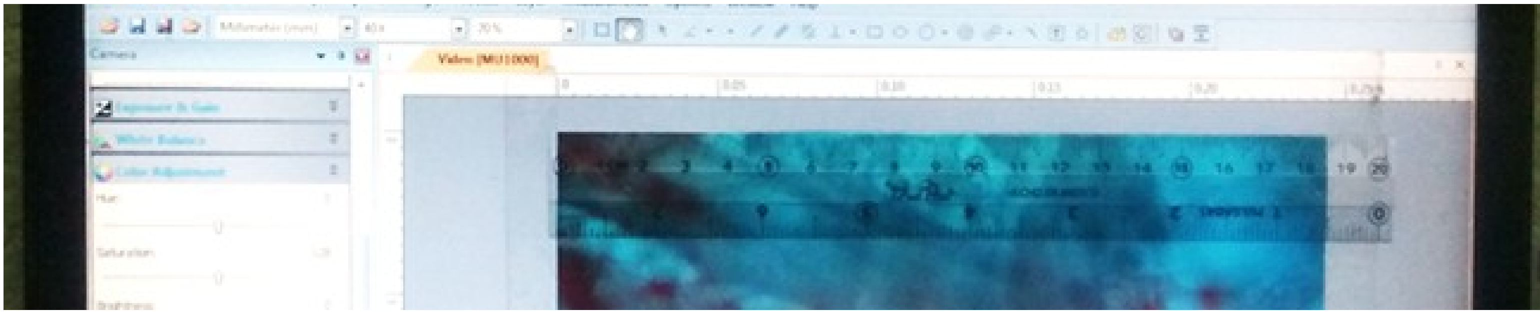
What about the 10x objective?

4x = 7.8   10*7.8 = 78 = **19.5** Following the principle of proportionality, this is the size of an image at 10x, 20% zoom.

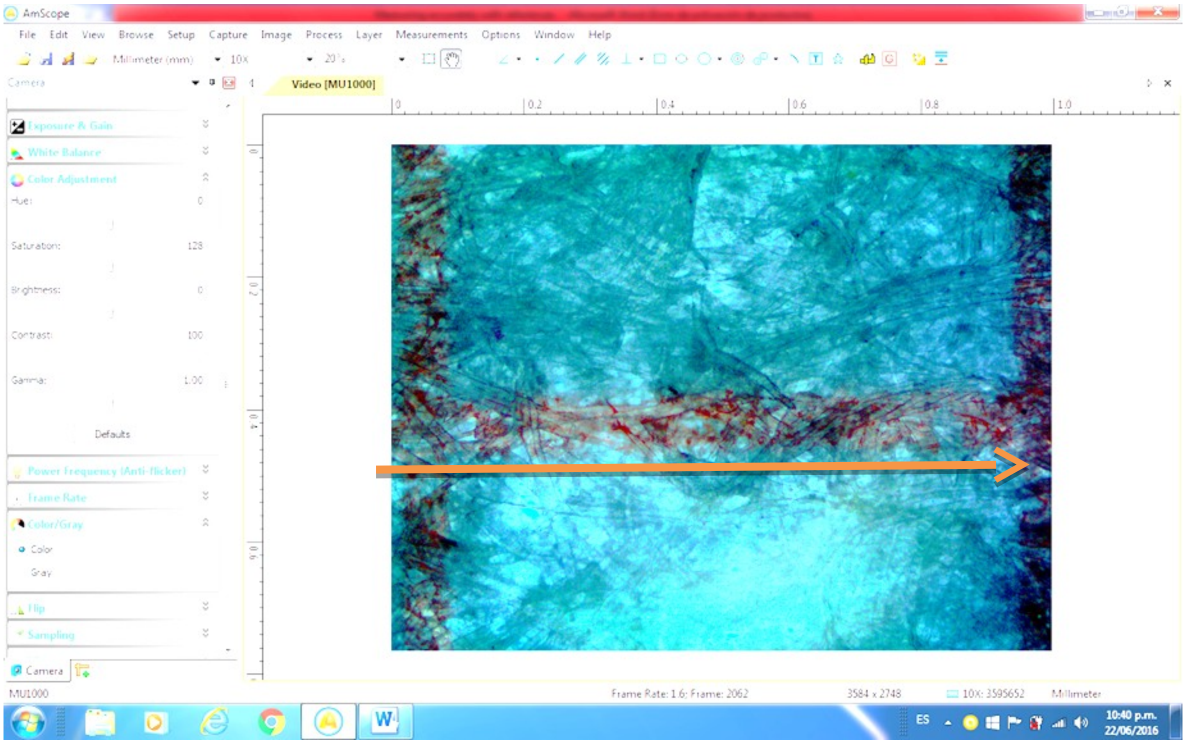
10x B   4

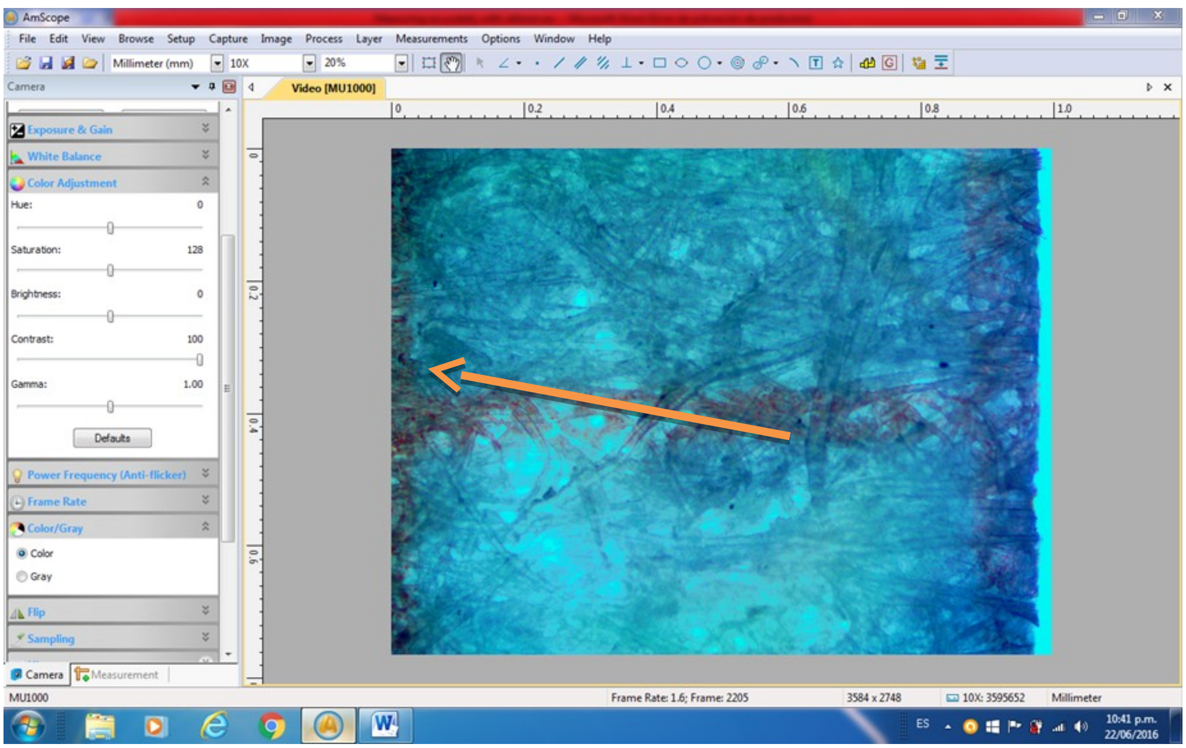
Just for the purpose of demonstrating that this value is accurate and that it corresponds, I took some pictures of this.

Look at the screen at 20% it is 18.5 cm.

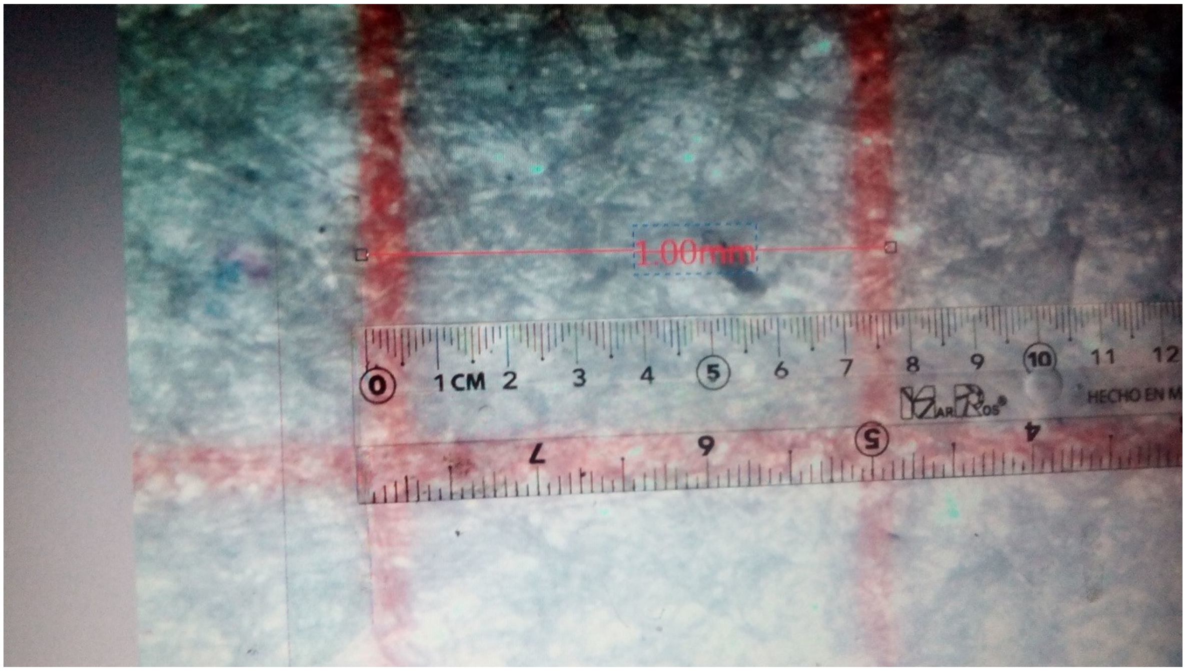


With the 10x objective, the length of the millimetre square uses a whole screen and a bit further.





That is 18.5 cm plus 0.7 cm gives 19.2 which is very close to the value obtained by proportionality. Here it is necessary take into account that for measuring I took some references in the image itself and that a mistake of 0.3 mm is not too much.

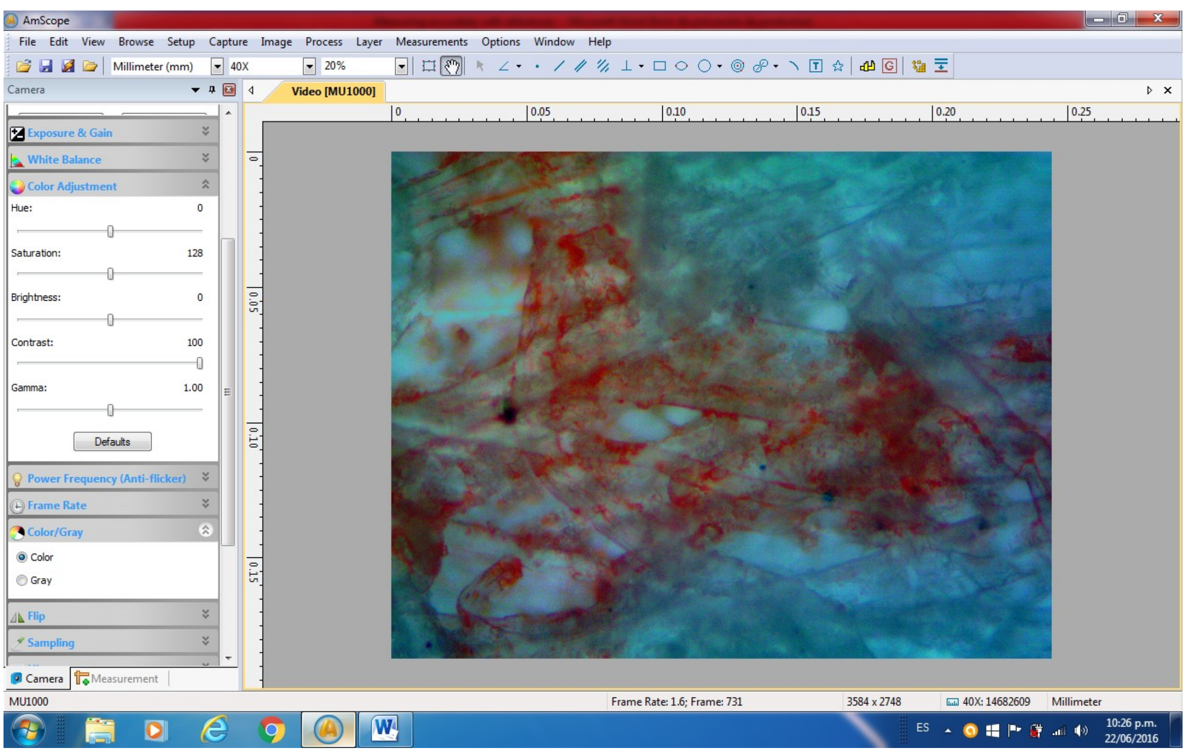


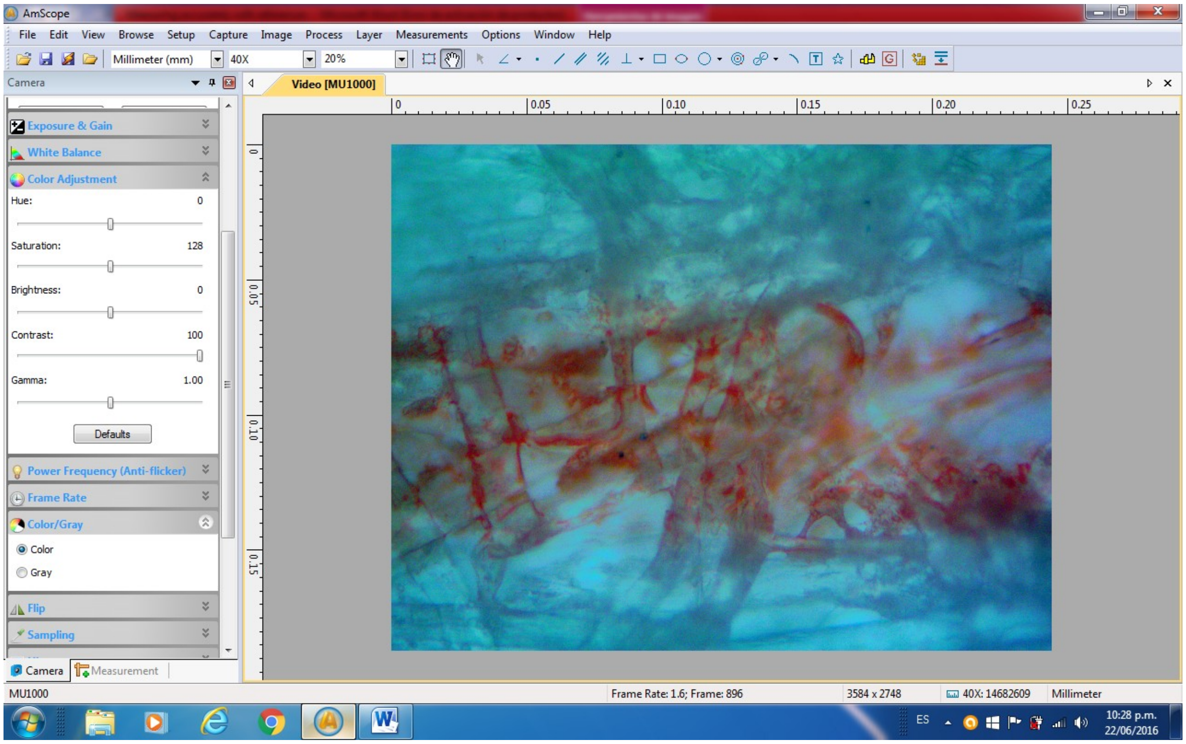
What about the 40x objective? How much does 1 mm represent at 20% zoom?

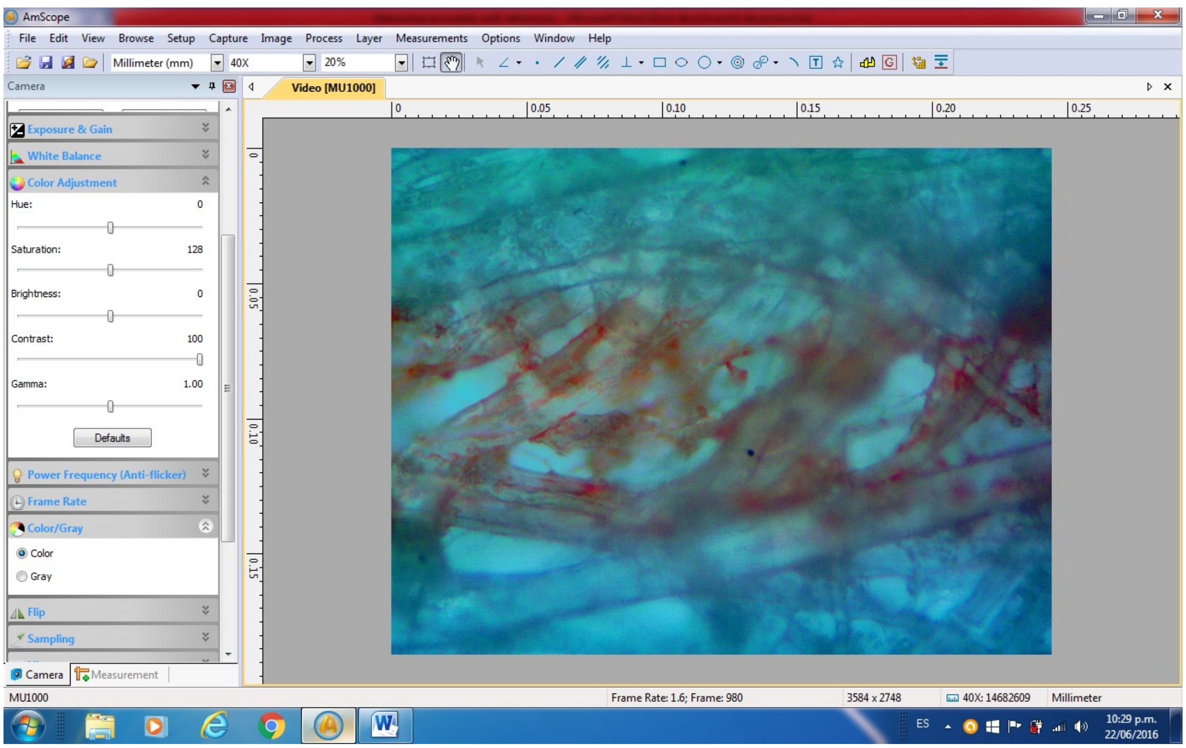
4x = 7.8   40*7.8 cm= 312 cm = 78 cm. Following the principle of proportionality, this is the size of an image at 40x, 20%

40x   B   4

zoom. Again with the purpose of demonstrating accuracy of the method here are the screen captures in the software:




AmScope
File Edit View Browse Setup Capture Image Process Layer Measurements Options Window Help
Millimeter (mm)
40X
20%
Camera
Video [MU1000]
Exposure & Gain
White Balance
Color Adjustment
Hue: 0
Saturation: 128
Brightness: 0
Contrast: 100
Gamma: 1.00
Defaults
Power Frequency (Anti-flicker)
Frame Rate
Color/Gray
Color
Gray
Flip
Sampling
Camera
Measurement
MU1000
Frame Rate: 1.6; Frame: 896
3584 x 2748
40X: 14682609
Millimeter
ES
10:28 p.m.
22/06/2016


AmScope
File Edit View Browse Setup Capture Image Process Layer Measurements Options Window Help
Millimeter (mm)
40X
20%
Camera
Video [MU1000]
Exposure & Gain
White Balance
Color Adjustment
Hue: 0
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Brightness: 0
Contrast: 100
Gamma: 1.00
Defaults
Power Frequency (Anti-flicker)
Frame Rate
Color/Gray
Color
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Flip
Sampling
Camera
Measurement
MU1000
Frame Rate: 1.6; Frame: 980
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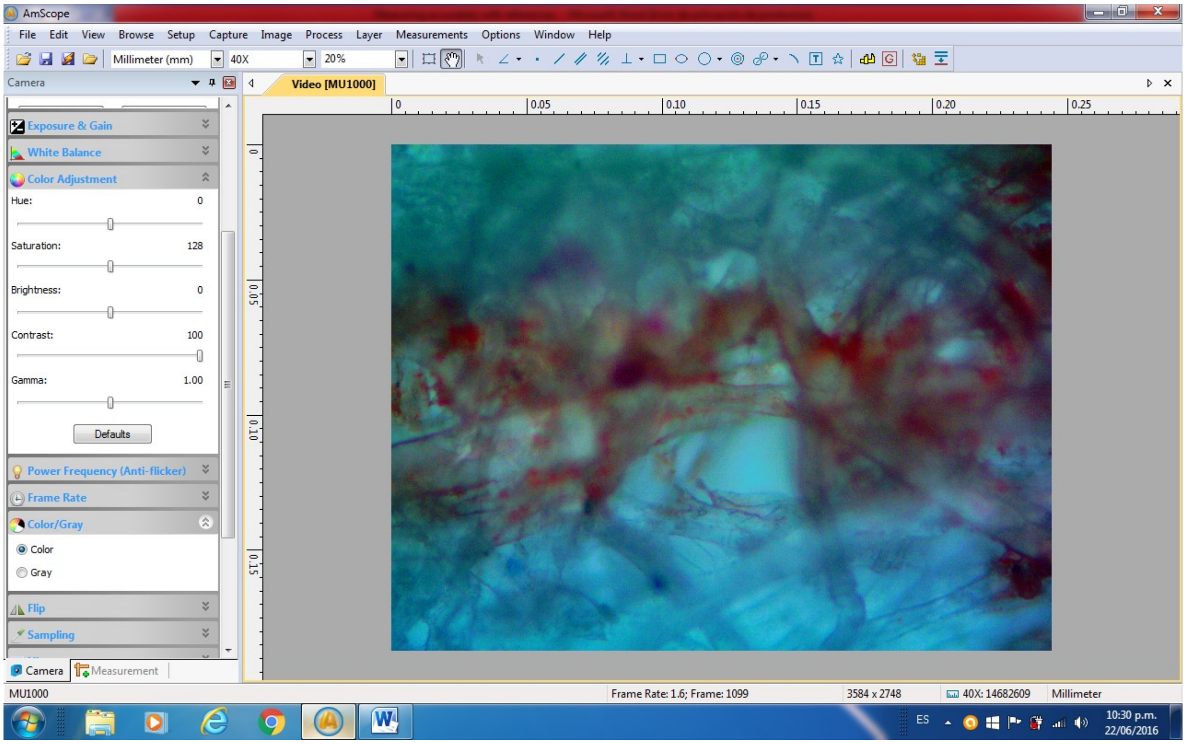
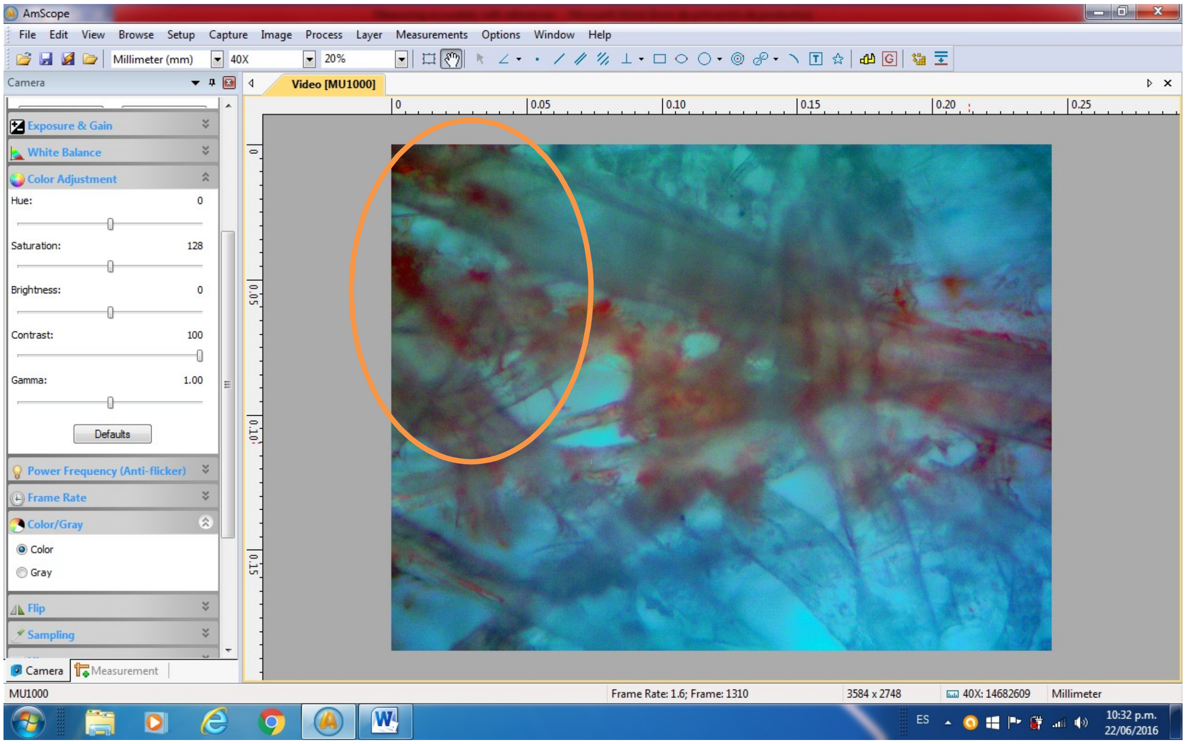
22/06/2016



Four complete screens of 18.5 cm is 74 cm.

Plus one of 3 cm it is 77 cm, very close to the value obtained by the method of proportion.



What about the 100x objective? How much does 1 mm represent at 20% zoom?

Now it is possible to calculate:

<u>4x</u> = <u>7.8</u> So <u>100*7.8 cm</u>= <u>780 cm</u> = 195 cm by proportion, this is the size of an image at 100x, 20% zoom.

100x B 4

<u>The values above are true for my camera, software and computer; in case you try the method, you have to take just the idea and calculate yours.</u>

Since we don't always have images at 20% zoom, it is necessary to use the zoom value in use, let's say 50%.

You follow the same principle of proportion, for example on my computer.

And image of **one** mm at 20% zoom and with the objective of 40x is 78 cm.

<u>20%</u> = <u>78</u> cm where D is the value in centimeters we want for a 50% zoom with the 40x objective

50% D

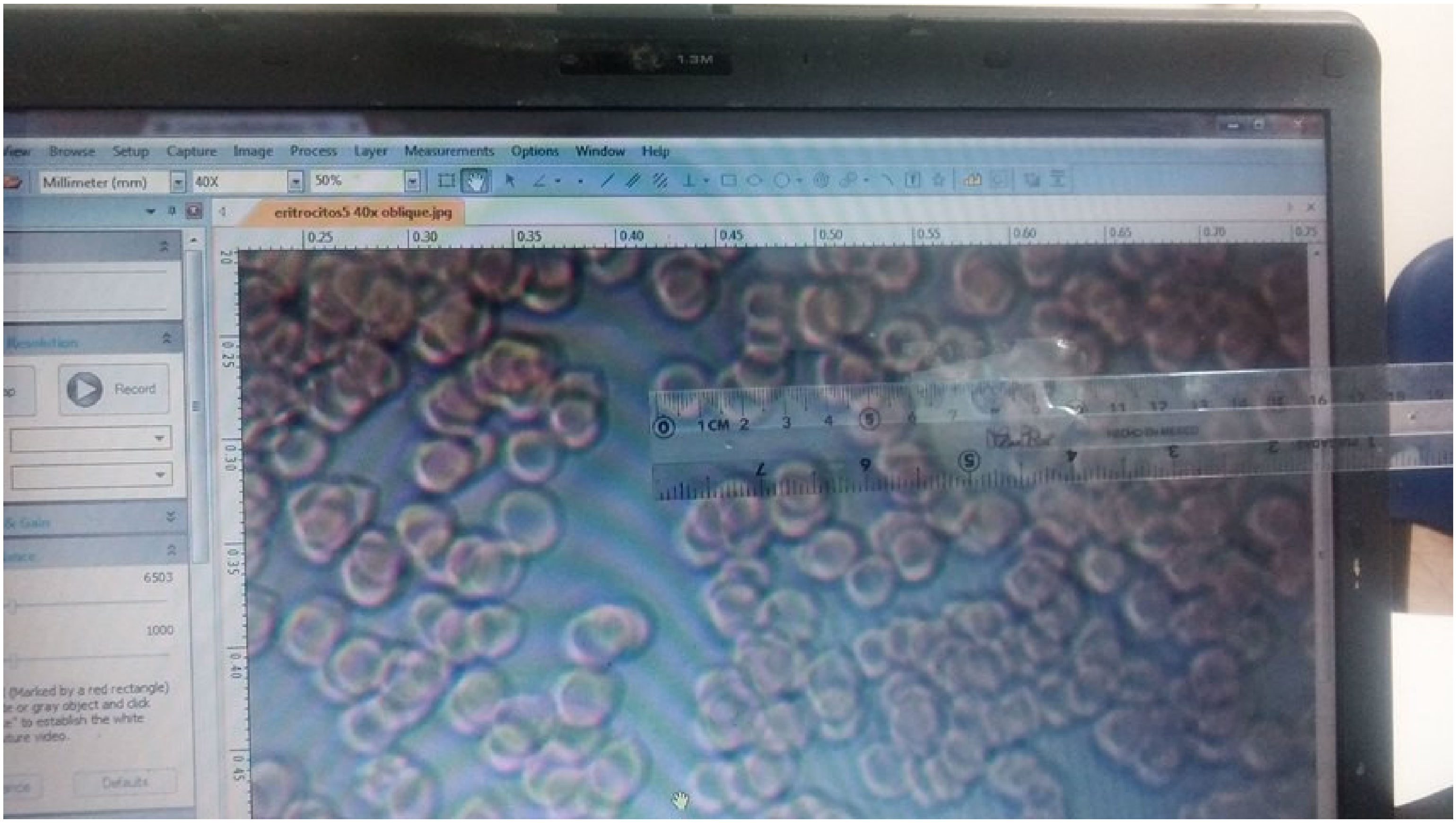
50*78= 3900/20 = 195 this is the size at 50% zoom.

In case you have an image of a sample that is seen better at 50% zoom and that image was taken with the 40x objective, for calculating the size of your sample in the image you have to take into account this as your reference.

Let´s take these values and calculate our first result.

Example of use with very small subjects such as the erythrocytes:

Remember that all the time you have to use a conventional rule for measuring the size in cm of your object on the screen of your computer or any other screen.

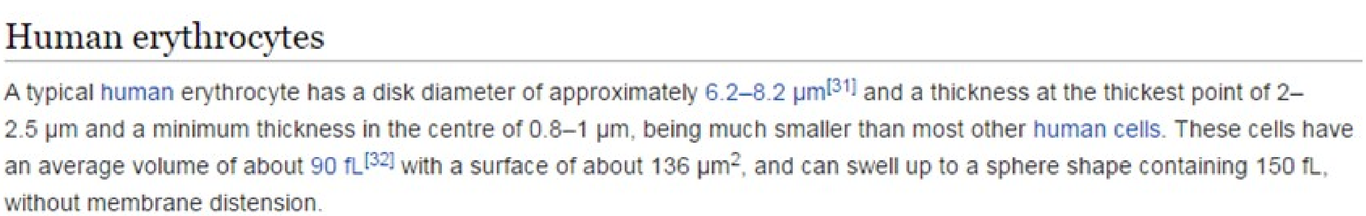


As you can see the erythrocyte in the sample is 1.2 cm long; this image was taken at 40x and zoom at 50%.

We apply the calibration **1 mm at 40x and zoom it 50% is 195 cm long** and the formula:

$\frac{195 \text{ cm}}{1.2 \text{ cm}} = 1 \text{ mm} = \frac{1.2}{195}$ so we obtain 0.00615384615 mm that is 6.15 micrometers.

And according to Wikipedia

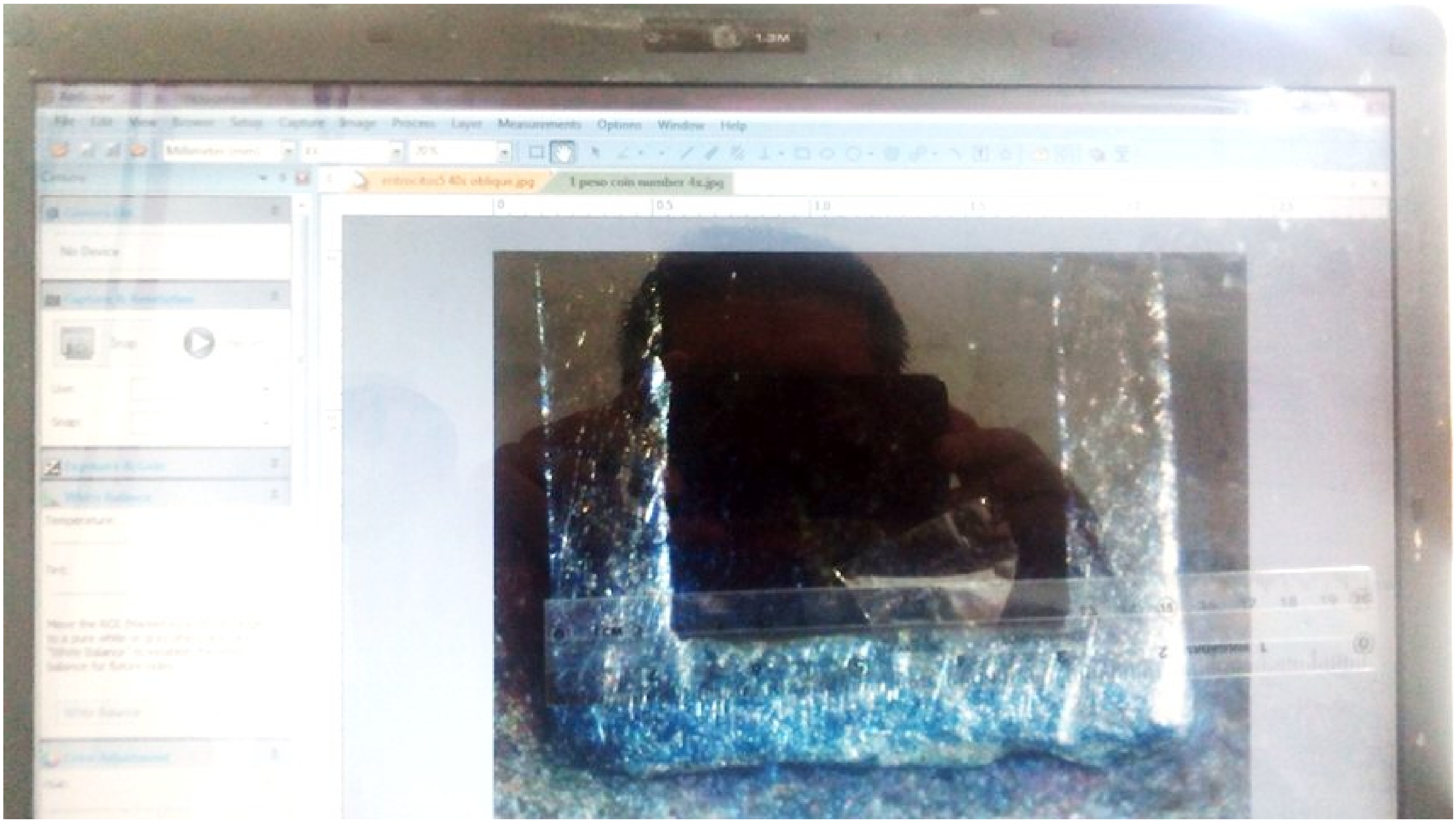


Human erythrocytes

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 µm^2, and can swell up to a sphere shape containing 150 fL, without membrane distension.

We conclude that this result is real.

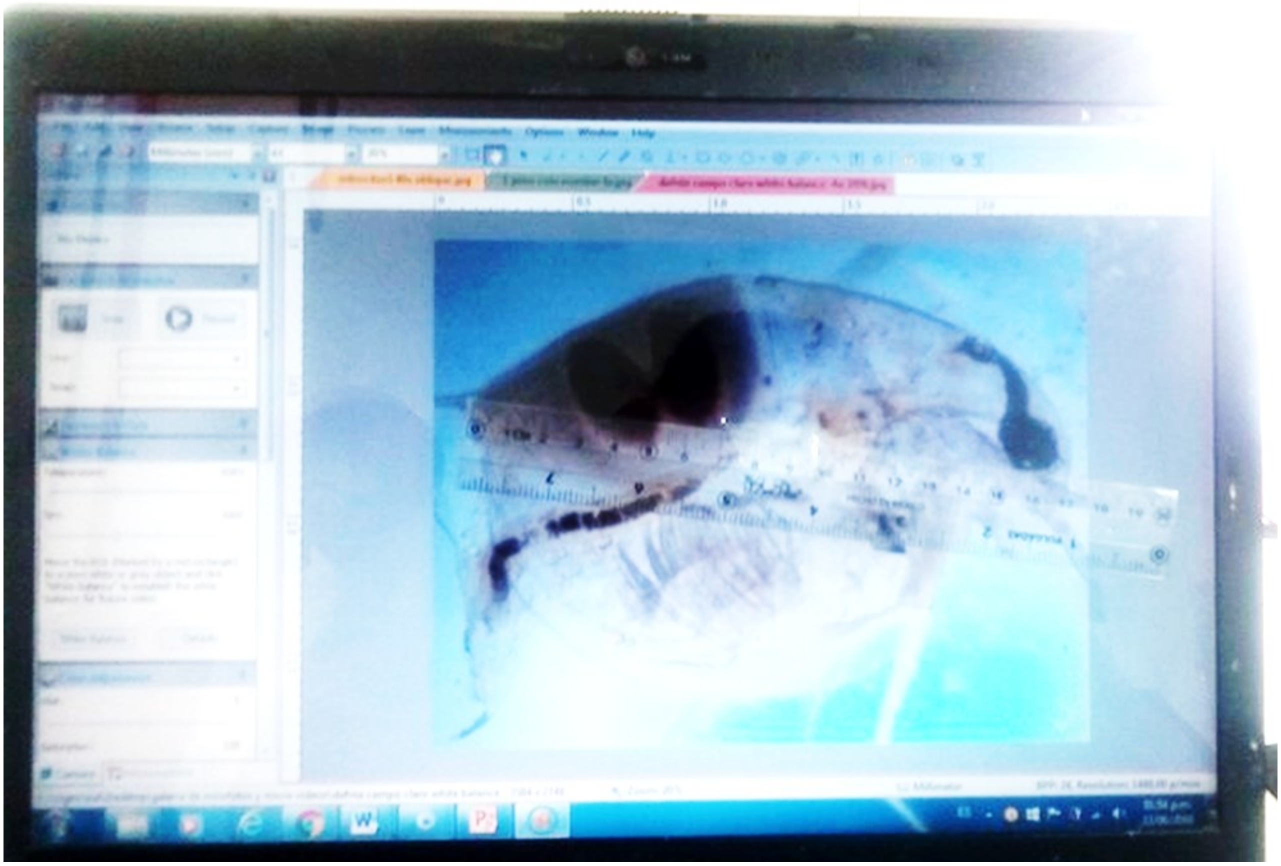
Results:



The one peso symbol of the Mexican one peso coin is 15.4 cm, it was taken at 4x and it is 20% zoom.

The calibration is that **7.8 cm represents 1mm at 20% zoom and with a 4x objective.**

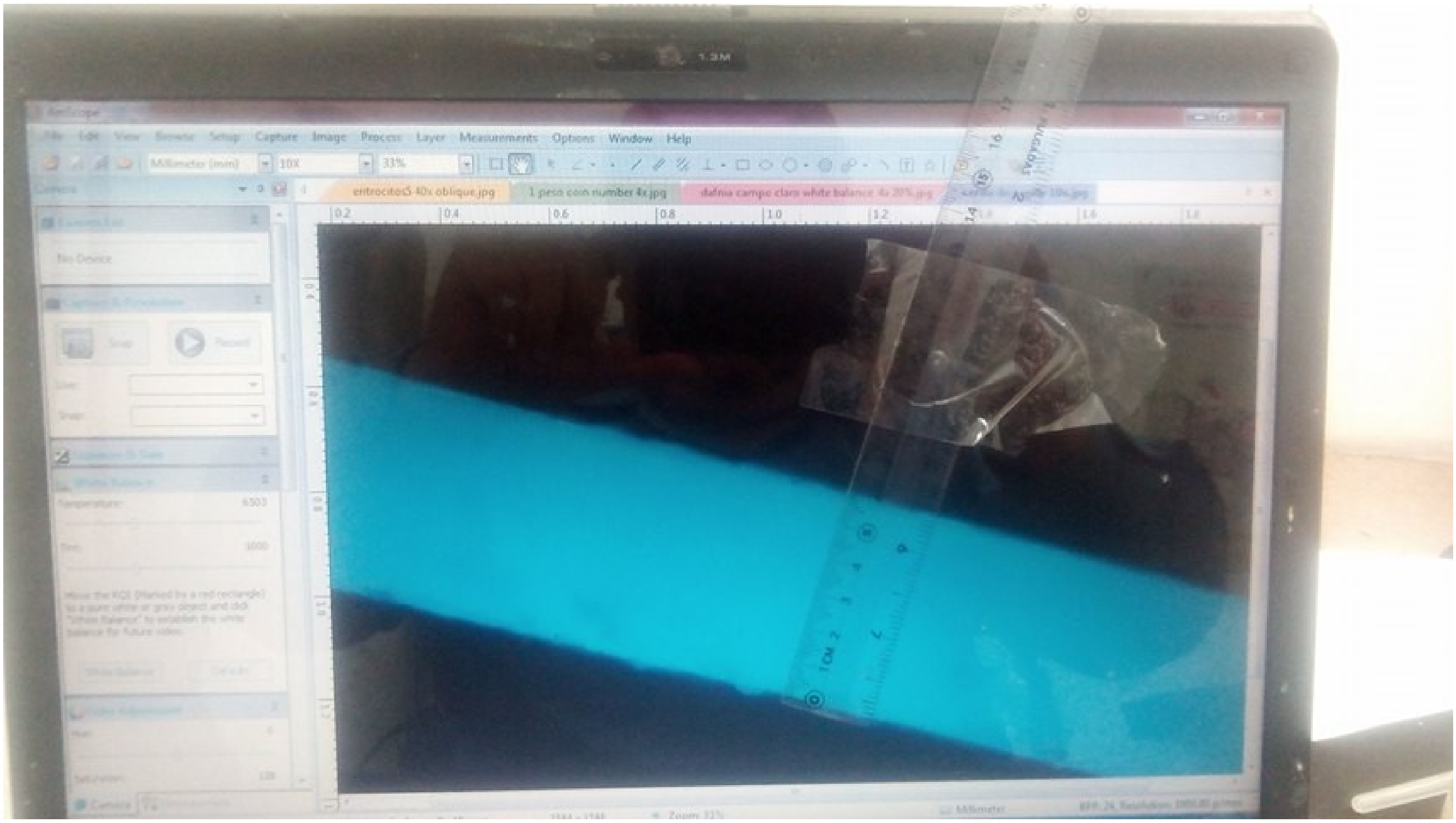
That is: 15.4/7.8 = 1.97 mm that is correct as the ruler shows:



The daphnia is 17cm long on the screen; the calibration again is that **7.8 cm represents 1mm at 20% zoom and with a 4x objective.**

That is: 17/7.8 = 2.17 mm that is correct as shown by the ruler:

Just to try different values now, the calibration is **1 mm at 10x for 33% zoom is 32.175.**



The width of the tooth brush bristle is on the screen 5.9 cm at 10x and 33% zoom.

That is 6/32.175 = 0.1895 mm that is correct as the caliper shows:

Here is a table that I created to make the calculation easy.

ZOOM	MAGNIFICATION OF 1 milimeter			
	4X	10X	40X	100X
20%	7.8 cm	19.5 cm	78 cm	195 cm
33%	12.87 cm	32.175 cm	128.7 cm	321.75 cm
50%	19.5 cm	48.75 cm	195 cm	487.5 cm
75%	29.25 cm	73.125 cm	292.5 cm	731.25 cm
100%	39 cm	97.5 cm	390 cm	975 cm

Note: remember that these values are "true" for my computer and my software - you have to calculate yours for this method to work.

Conclusion:

As a reference we can use whatever we want and not necessarily graph paper; it can be a hair, a wire, a bristle, a point, etc. The only requisite is to know its correct size. The camera must be held in a constant position for the reference to be constant too, no matter if it is a special camera or a consumer model or as in Mol Smith's article with a cellphone

Maybe this looks like a lot of work, but that seems so because I had to show the screens for demonstrating the accuracy of the method, but it is not, in fact once we have the values of the reference it take just a few seconds to do the calculation.

Probably somebody may say that it is better to buy the calibrator and that is all, but If it were not possible, here we have a very accurate method of calculating the size of the microscopic samples.

I can say that if I can have the privilege of knowing how large those marvelous things that I see with my microscope are, no matter how much it takes, it is worth doing.

So let's start to measure the microscopic world.

Email author: doctor2408 AT yahoo DOT com DOT mx

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

capitalised characters.)

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