CALIBRATING A DEDICATED MICROSCOPE CAMERA WITH REFERENCE TO THE LAST LEARNING STEP BEFORE STARTING TO MEASURE THE MICRO WORLD.

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INTRODUCTION:

The great Antoine de Saint-Exupéry says in his master work *The Little Prince* in Chapter 4 that we human beings are like numbers and cyphers and that in this way we believe that we know the world around us.

I believe this is true, and I particularly love this, since I knew that I could measure with my microscope camera software my observations of subject size. It was for me exciting because in the microscopic world there are subjects of different sizes, some of them are extremely small and other are giants within this miniature world. All of this is of course limited for the moment to the resolution that is possible to achieve with light microscopy.

I started this series of articles regarding measurements presenting the calibrating of a special microscope camera,

Then I presented a topic that serendipitously I found while looking for reference objects. I mean the measurement with a laser beam, with the purpose of getting a reference to calibrate my camera.

The third one was using as reference a one millimeter grid on graph paper and with a great level of accuracy, but the probable only problem is that all the time it is necessary to use a conventional rule to measure the object on the screen. It was intended for people who lack a calibrator for the camera or for people using cameras different from the special ones for microscopes.

Today's article intends to be a follow-up of the latter because this time the same reference system is going to be used but this time for calibrating the camera. I mean a dedicated microscope camera and this way again without the need of a calibrator we may assign values to the different magnification powers. See below.

DEVELOPMENT:

When calibrating a camera for the microscope the manufacturers usually sell a slide that has a rule drawn on it, usually it is one millimeter long and can be magnified with the objectives to see the scale. If you know the distance between two divisions you take that as a reference to calibrate regularly. The manufacturer states on the slide the value of each division.

But if we lack such a slide what can we do? Apart from ordering one from the manufacturer we can calibrate by ourselves and for this purpose we use the references that we have created with other objects and which differ from the calibration rule.

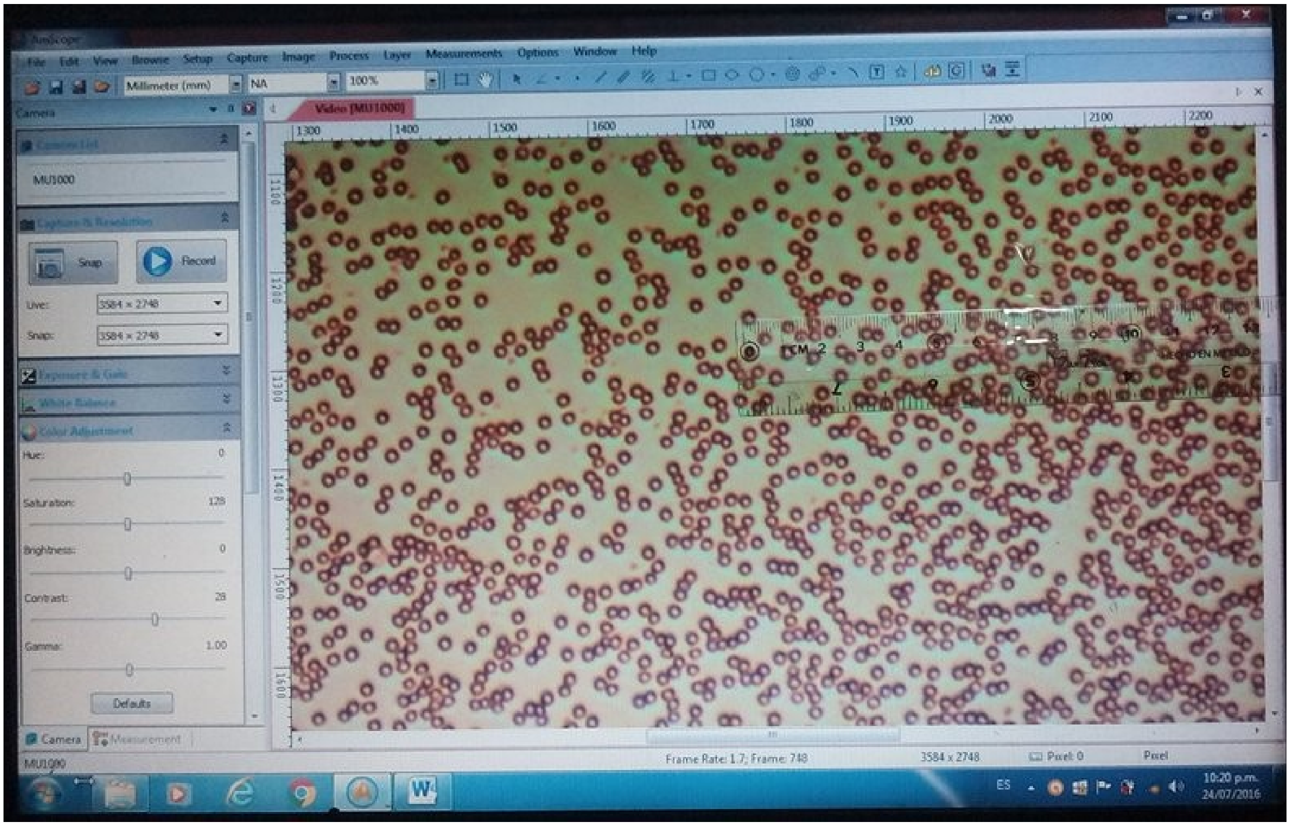
To demonstrate the accuracy of the reference system, I was looking for something to calibrate the camera that were big enough to be seen with the 4x objective at 100% zoom, because when calibrating it is necessary to use the 100% of zoom and the maximum of pixels of the camera, and that were small enough to fit into the 100x objective also at 100%.

I tried a bit of hair, a toothbrush bristle etc but they were too big to be used with 100x objective, so I was wondering what I could employ for calibrating the camera without the rule and finally I got the answer, it was running through my arteries and veins.

I mean blood, **erythrocytes (red blood cells) are big enough to be seen at 4x and small enough to fit under the 100x, both at 100% zoom.**

I then took the reference table from the last month's article, it states that a one millimeter subject viewed under the microscope when shown on my computer screen is as follows:

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



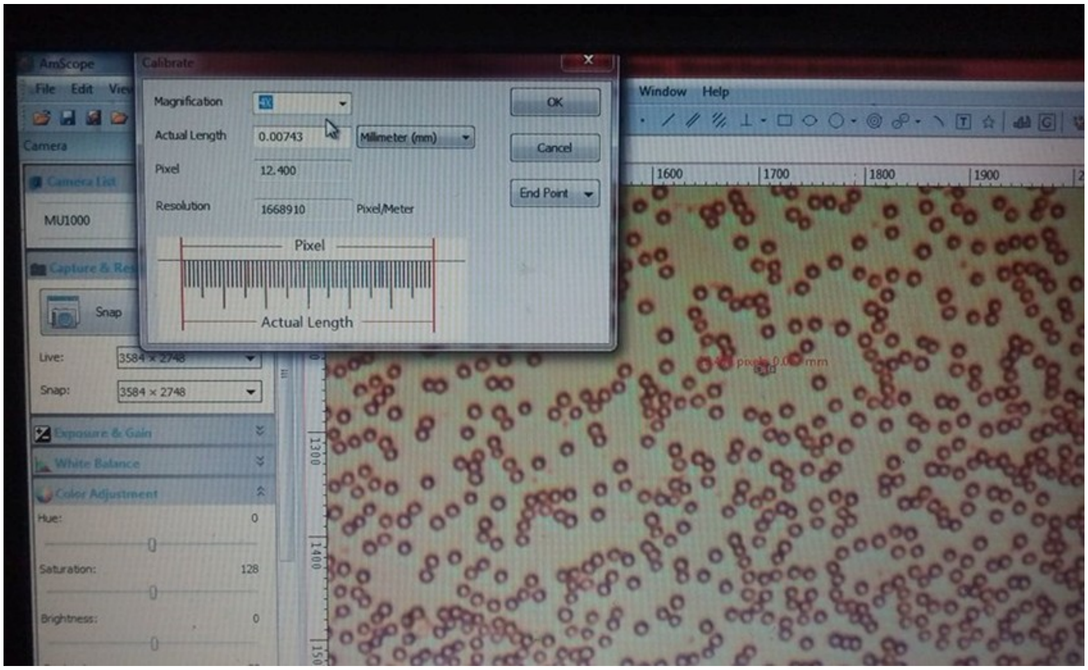
This picture shows erythrocytes with a 4x objective and with the help of the rule we can see that the one taken as a reference is 2.9 mm.

If we use the formula from the table above at 100% zoom, where 'X' is the actual size of the subject, in this case the erythrocyte:

<u>39 cm</u> = <u>1 mm</u> so X = 0.29/39 = 0.00743 mm or 7.43 microns.
0.29 cm X

(Editor's note – the diameter of a human red blood cell is typically in the range 6.2 – 8.2 microns, source Wikipedia.)

We open the calibrating function and adjust to the size of the erythrocyte like this and write the value.

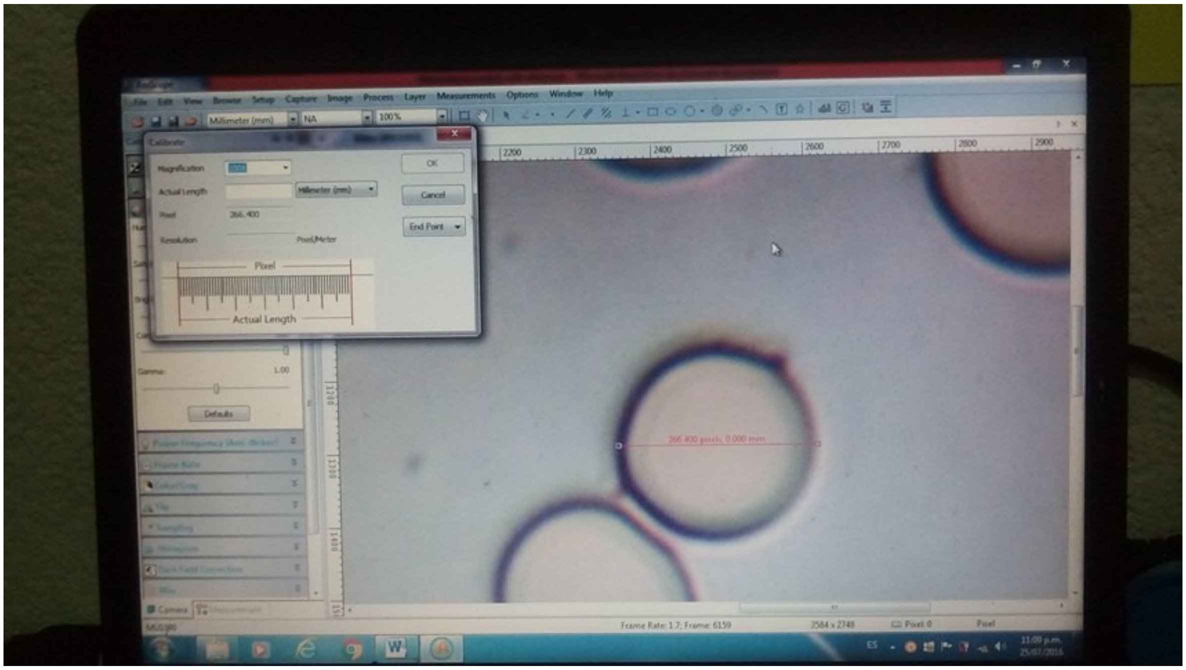


We apply the function and that is all - we have calibrated the camera for the 4x objective with a “household reference”

Just as another example, let’s use the 100x objective for the same subject.

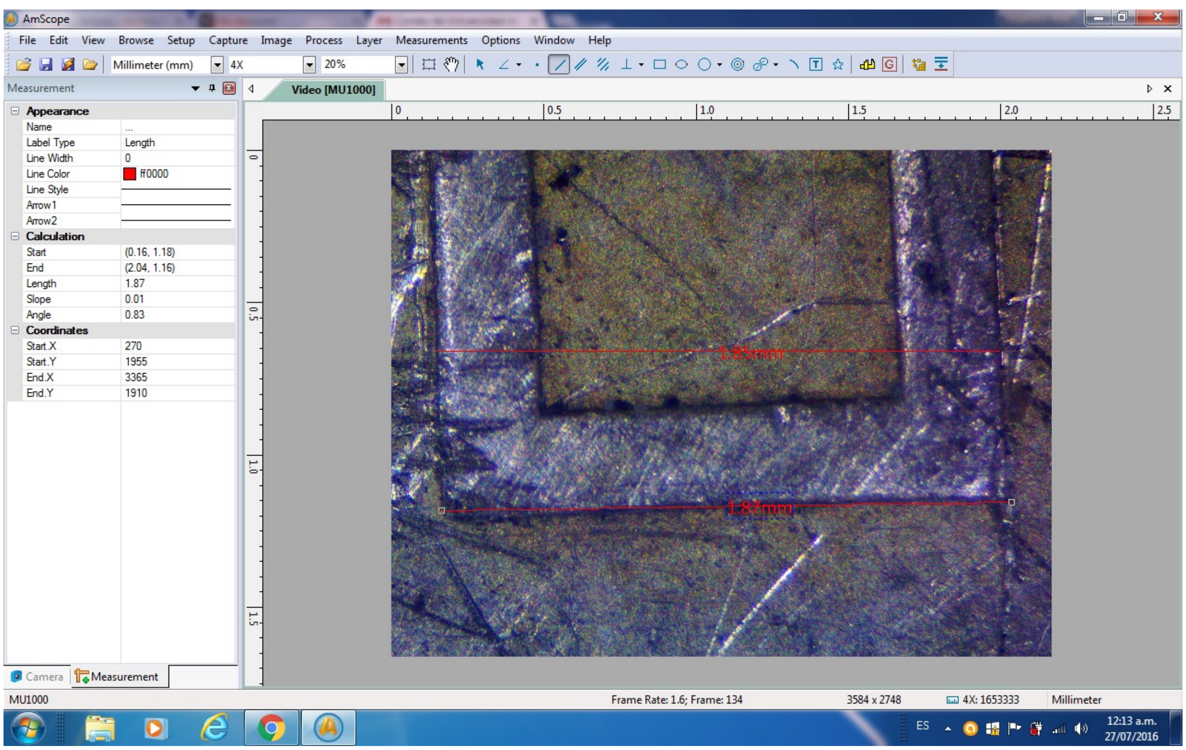
<u>975 cm</u> = <u>1 mm</u> so X = 6.9/975 = 0.0070 mm or 7.0 microns.
6.9 cm X

It is the same procedure for the rest of the objectives using the corresponding value from the table for the objective in use at 100% screen zoom.

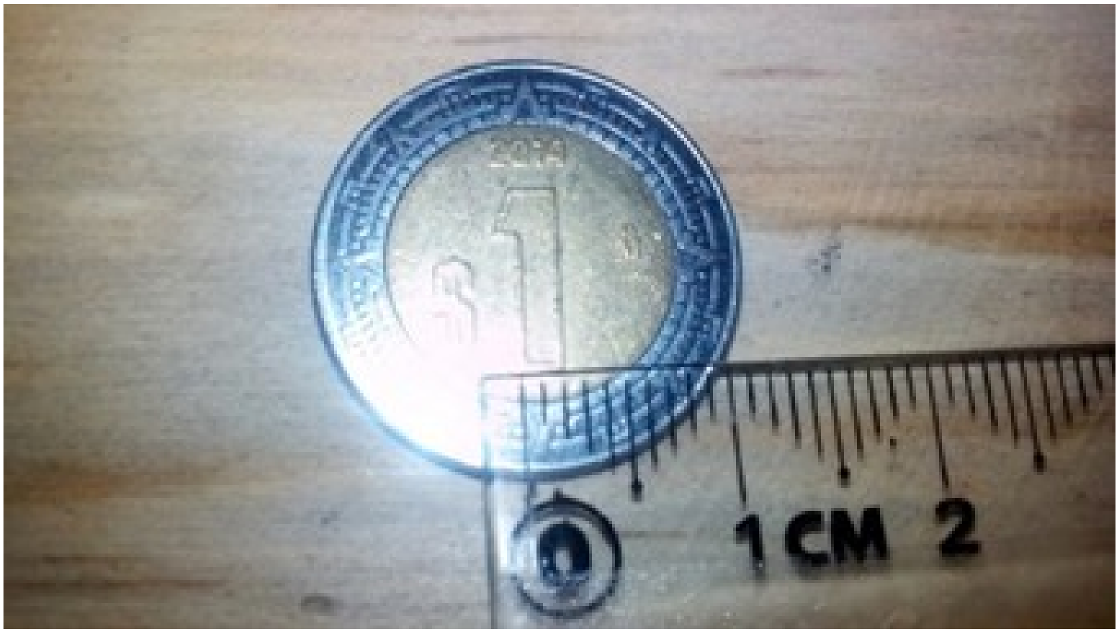


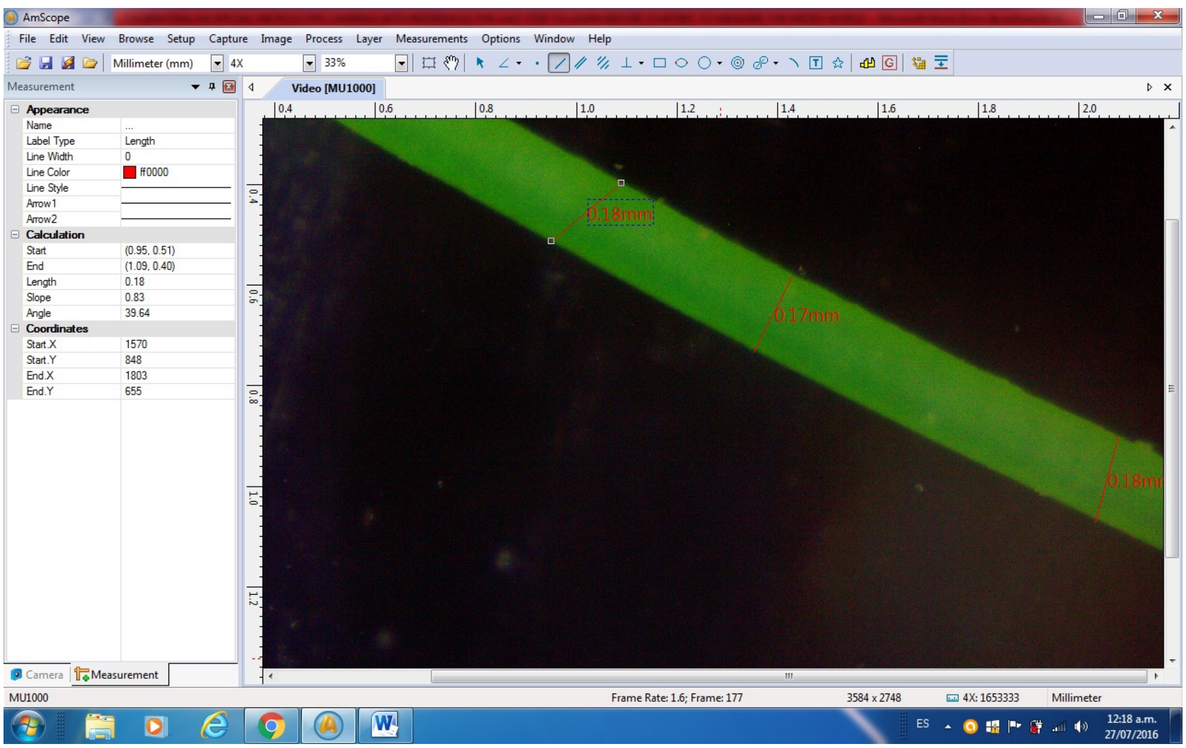
RESULTS:

As in the previous articles about measuring, when I share a method I have to demonstrate the accuracy so the reader may try it with trust and for that reason I use known subjects whose sizes we already know - here are some examples.

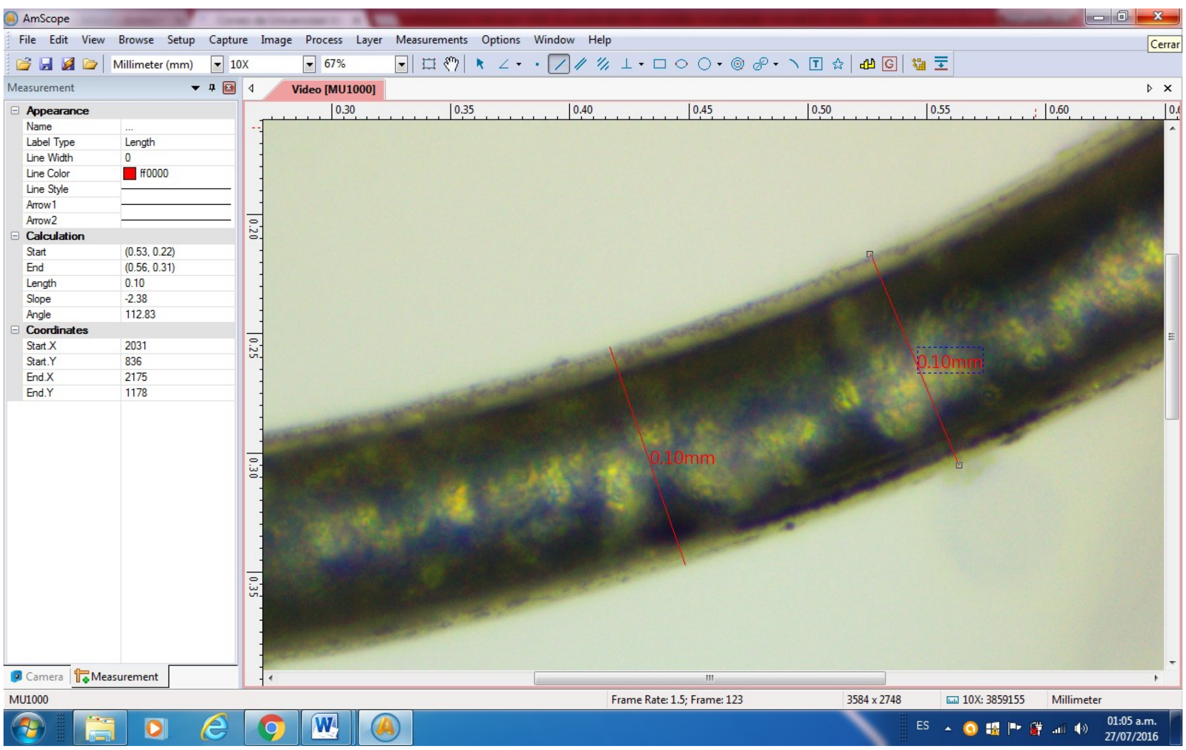


This is the lower part of the one peso symbol of the Mexican one peso coin that is about 2 mm.

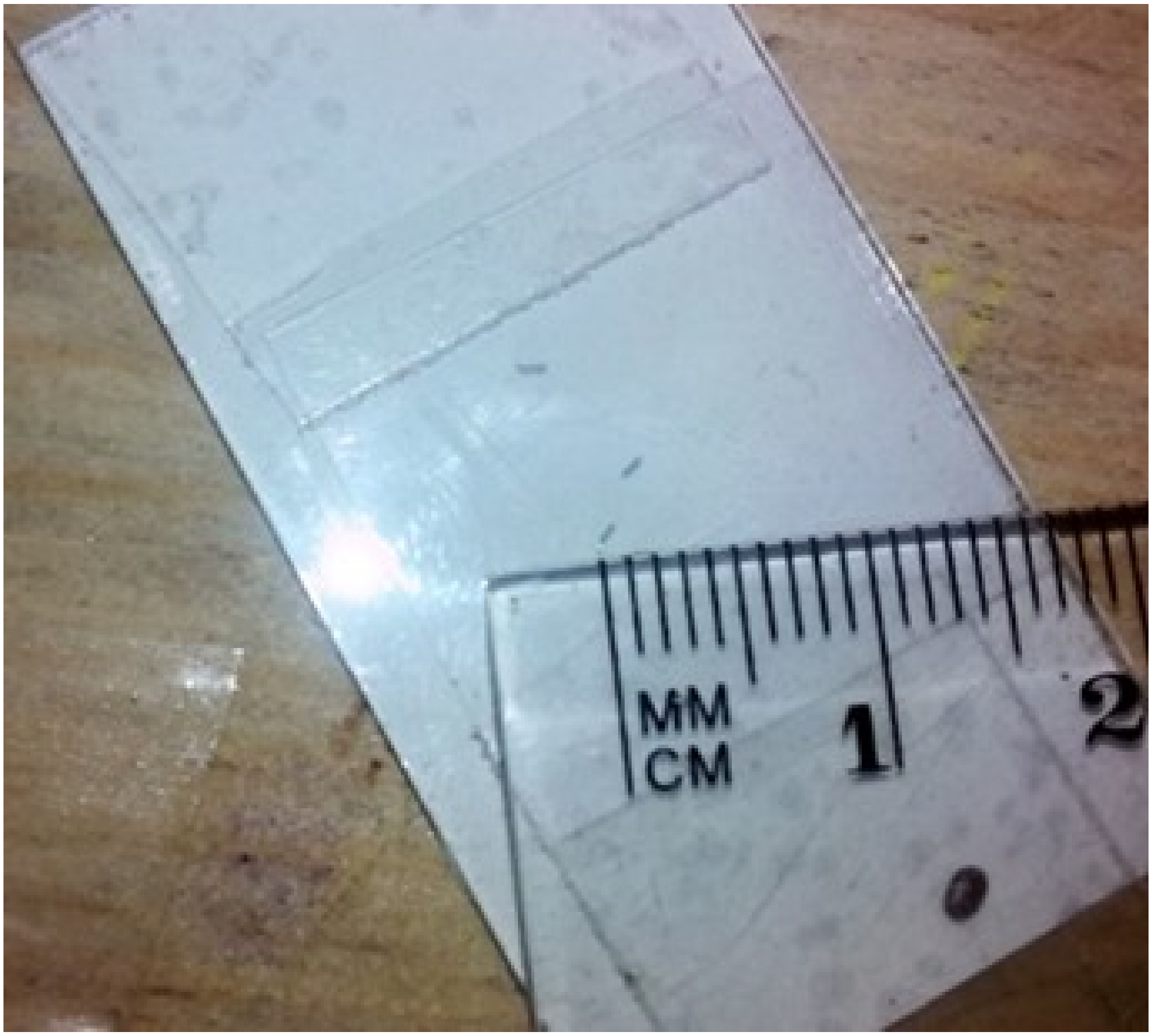




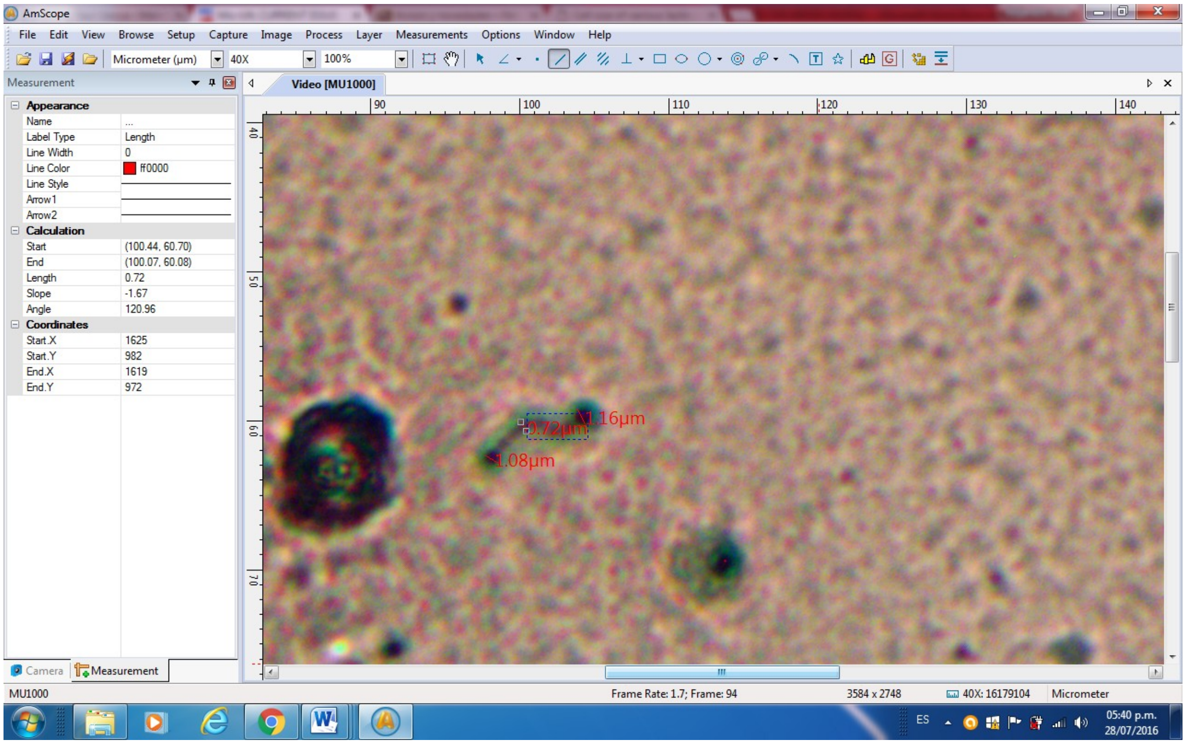
Part of a toothbrush bristle at 4x and 33% zoom it is 0.18 mm on the digital caliper.



This is a very small bit of a copper wire seen with the 10x objective and 67% zoom, it is about 0.1 mm according to the image below.



And finally the test of fire for this kind of calibration, bacteria in this case *Streptococcus* of yogurt at 40x which gives a value on the screen of 0.72 microns in length.



A scientific paper by Kokkinosa *et al,* Section 3, in a description of S*treptococcus thermophilus,* gives a value of between 0.7 – 0.9 microns i.e. the above measurement falls within the range of their values.

CONCLUSION:

The calibration of a microscope camera with a suitable reference is definitely a very good approach. It is as accurate as if it were done with a manufactured calibration slide. Once more I invite readers to try it and starting to measure that marvelous tiny world. We should remember that we have to calculate our own value for the reference.

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capitalised characters.)

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