RESOLUTION PART 1 - THE 4X OBJECTIVE

BY: ALEJANDRO ARIEL GARCIA ARRIAGA, COACALCO DE BERRIOZABAL, ESTADO DE MEXICO, MEXICO

INTRODUCTION:

I have been focused for a while in my recent articles explaining and using some forms of DIY epi-illumination, with the purpose or observing thick samples.

I have used LEDs, a cellphone lamp and lately DIY Lieberkühn mirrors. In all these cases both 4x and 10x objectives were used because of the fact that these have a good space beneath them and allows illuminating the sample from above.

However, the problem is that they are considered low power objectives and obviously with low resolution.

The question here is what resolution is in terms of microscopy:

It is defined as the property of a lens to separate two closed points and make it possible to distinguish them from one another.

The second question is what is the resolution of a low power objective as 4x?

First, it is necessary to define that resolution in optical microscopy is limited by the wavelength of light, which in general terms is approximately 500-550 nm that corresponds to what the eye perceives as green or yellow light, and that is given by the formula:

Resolution = the wavelength of the light used

2 x the numerical aperture*

*this is because both the objective and condenser numerical apertures are considered and when they are the same in general terms that is the acceptance of light in an optical system.

The 4x objective usually has a numerical aperture of 0.1 so if the average of the wavelength of light use in brightfield microscope is 550 nm substituting in the formula it is:

Resolution = 550 nm = 0.00000275 m or 2.75 micrometers

2 x 0.1

It is necessary to remember that other factors also alter positively or negatively the resolution in a sample, such as the contrast of the image, the intensity of the illuminator etc. which for the moment are out of the scope of this article.

However, is this really the full story?

I think it is not, why? Because when you see through the eye pieces with for example the 25x eyepiece and the 4x objective it is possible to see streptococcus of yogurt which I determined its width by my own means in my article HOW BIG IS STREPTOCOCCUS IN YOGURT? Concluding that it is 0.83 micrometers.

DEVELOPMENT:

When most of the time a 4x objective is going to be used for epi-illumination observations, it is a concern how “far” it is possible to go in terms of resolution because we want to see as much as possible.

So trying to define this I took some photomicrographs with the 4x objective of two permanent samples that I made of streptococcus of yogurt and that I stained positively with methylene blue and negatively with candle soot.

It was demonstrated that it is possible to see the width of a streptococcus of yogurt that belongs to this bacteria and that is probably the tiniest specie we can see as amateur microscopists, particularly as I have not found another one.

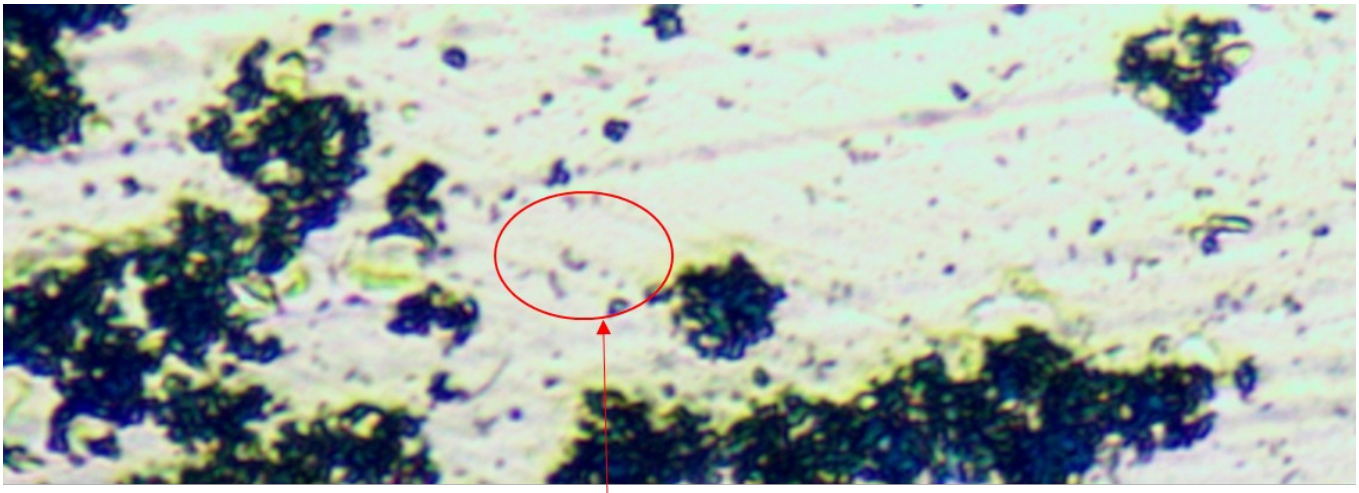
In my article HOW BIG IS STREPTOCOCCUS IN YOGURT? mentioned above I established that 0.83-0.89 micrometers is the average width of a streptococcus.

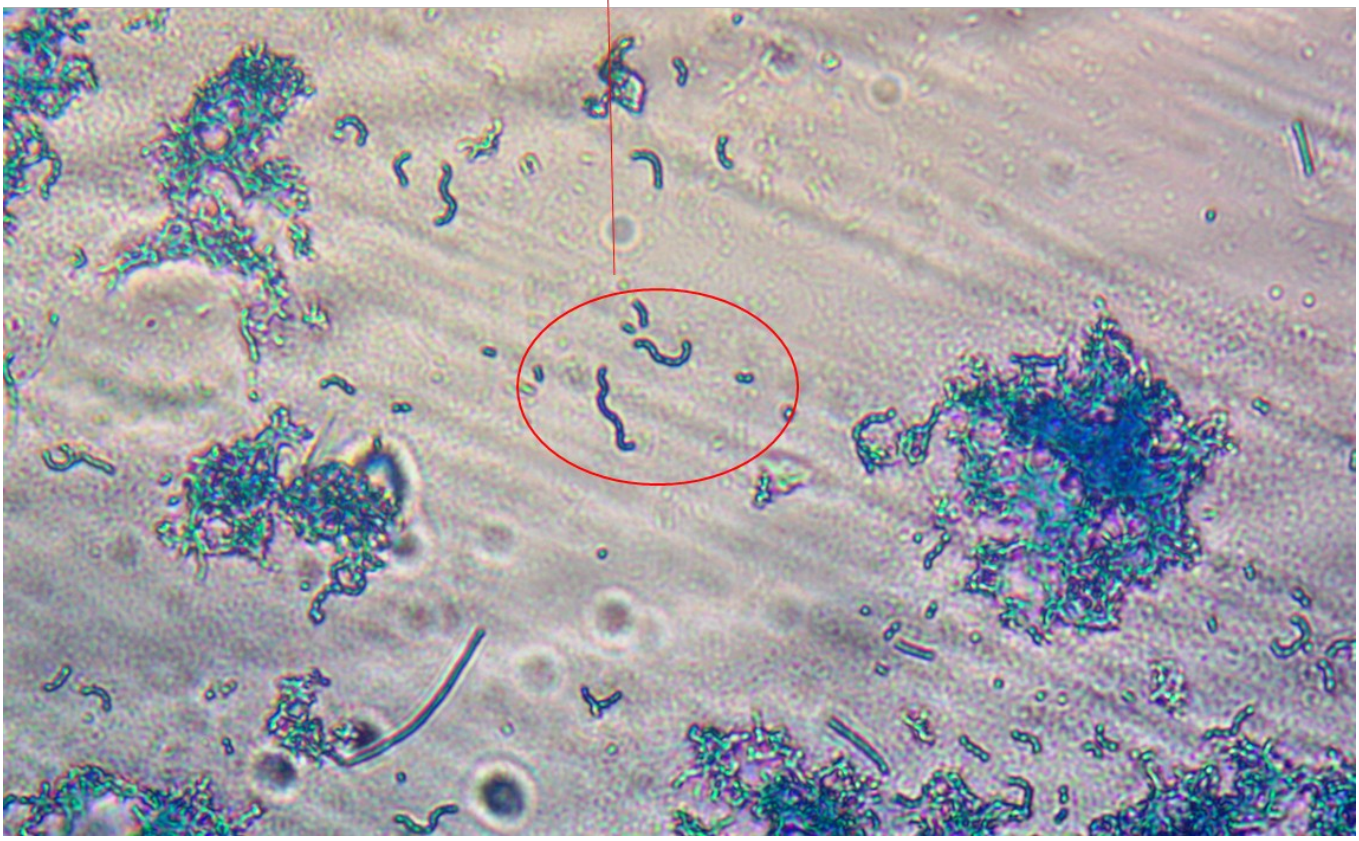
Then that is what is possible to see with the 4x objective, really? Yes it is true.

It is worth clarifying that it is not possible to define the bead-like form of this bacteria as would be possible with other magnifications such as the 40x for example; nevertheless we can conclude that for a point of 0.83 micrometers wide it is possible to see it in the middle of a sample, which is a good level of performance for a “low power objective” and obviously for epi-illumination samples.

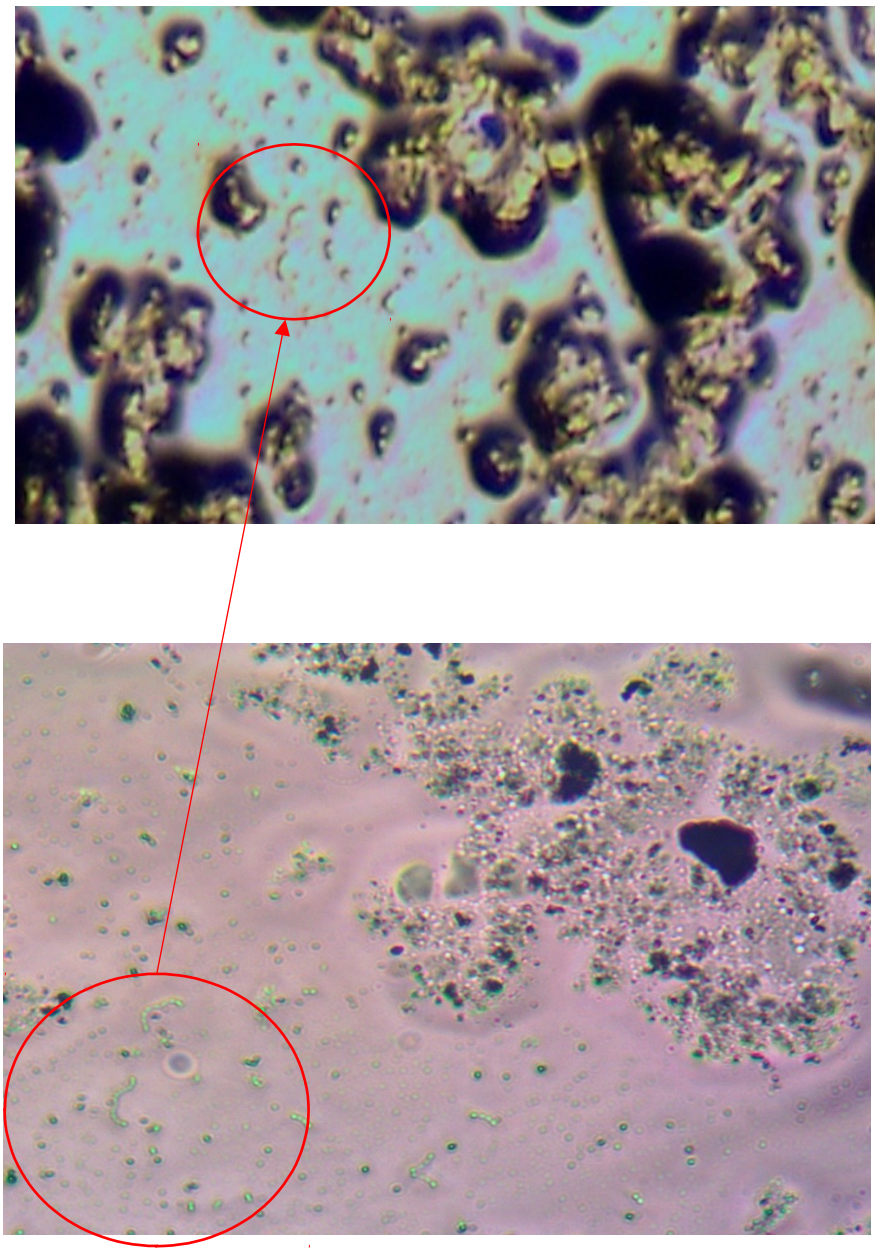
It is possible to see it both through the eyepieces with the 25x eyepiece and through the camera.

RESULTS:





The above image is a crop of the 4x objective photograph of the methylene blue sample, zoomed and compared at the same point within the sample with a 40x image showing the streptococcus clearly.



The above is a crop of the 4x objective photograph of the candle soot stained sample, zoomed and compared in the same point within the sample with a 40x image showing the streptococcus clearly.

CONCLUSION:

4x is not totally a low power objective as it is considered, it can detect isolated fine detail that is useful when seeing thick samples through this objective, and we are not concerned anymore because if necessary we can at least catch the image of a 0.83 micrometers wide object.

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.)

Editor's note: I believe the author is illustrating a principle exploited in the ultramicroscope i.e. especially for self illuminated particles as in epi-illumination, it is possible to detect particles of a size below the resolution limit of an objective as distinct from resolving detail spaced this far apart.

New Editor's note added Oct. 2nd: On further reading I was probably not strictly correct in making the above statement. The Wikipedia entry for the ultramicroscope notes that the term is used for particles with a diameter near or below the wavelength of light, typically 500 nm (green) or 0.5 micron. Streptococci are somewhat larger at 0.8 microns. The author is clearly demonstrating the distinction under normal brightfield conditions between detecting an object of a certain size in visible light but below the resolution of the objective so little or no detail can be resolved in it. In a typical ultramicroscope, strong incident side light is typically used to scatter light from subjects such as tiny particles suspended in colloids and aerosols.

Published in the September 2017 issue of *Micscape* magazine.
www.micscape.org