RESOLUTION PART 2 : THE 10X OBJECTIVE

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INTRODUCTION:

Last month I analyzed the possibility of observing samples less than a micrometer with a 4x objective.

I described the concept of resolution in microscopy: **"… as the property of a lens to separate two closed points and make it possible to distinguish them from one another."**

All this was with the purpose of defining how much resolution it is possible to get with a 4x objective that together with the 10x objective are the two more common to observe thick samples using epi-illumination methods. Nevertheless they are considered as low power objectives but are they?

I think not, because it was shown that a 4x objective may *detect* an object 0.83 micrometer wide that is below the objective's resolution limit.

DEVELOPMENT AND RESULTS:

I should say that according to the formula:

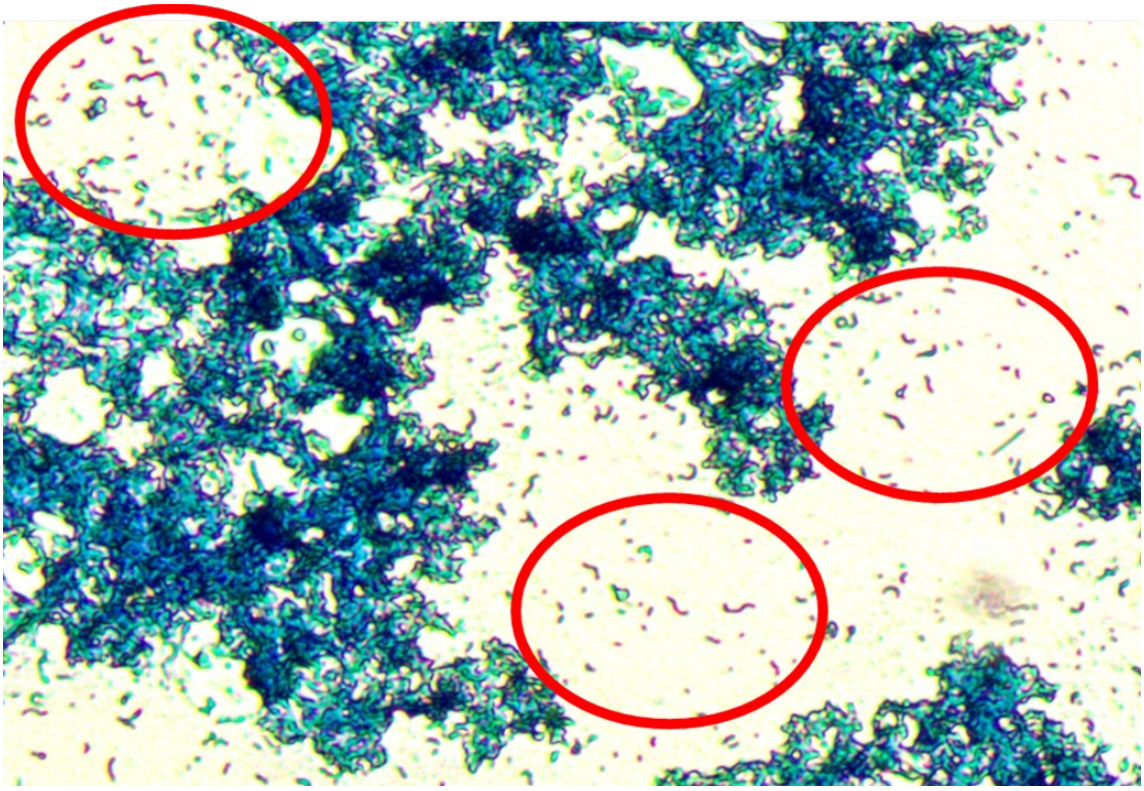
$$\textbf{Resolution} = \frac{\text{the wavelength of the light used}}{2 \times \text{the numerical aperture}}$$

The resolution for a plan achromat 10x NA 0.25 objective that is commonly found in a "typical" microscope is ca. 1.10 micrometers

In the same way as shown for the 4x objective, can a 10x objective *detect* objects below its resolution limit?

Yes, it can because it is possible to observe a 0.83 micrometers wide object such as streptococcus in yogurt.

Look at this a sample of streptococcus stained with methylene blue and zoomed:



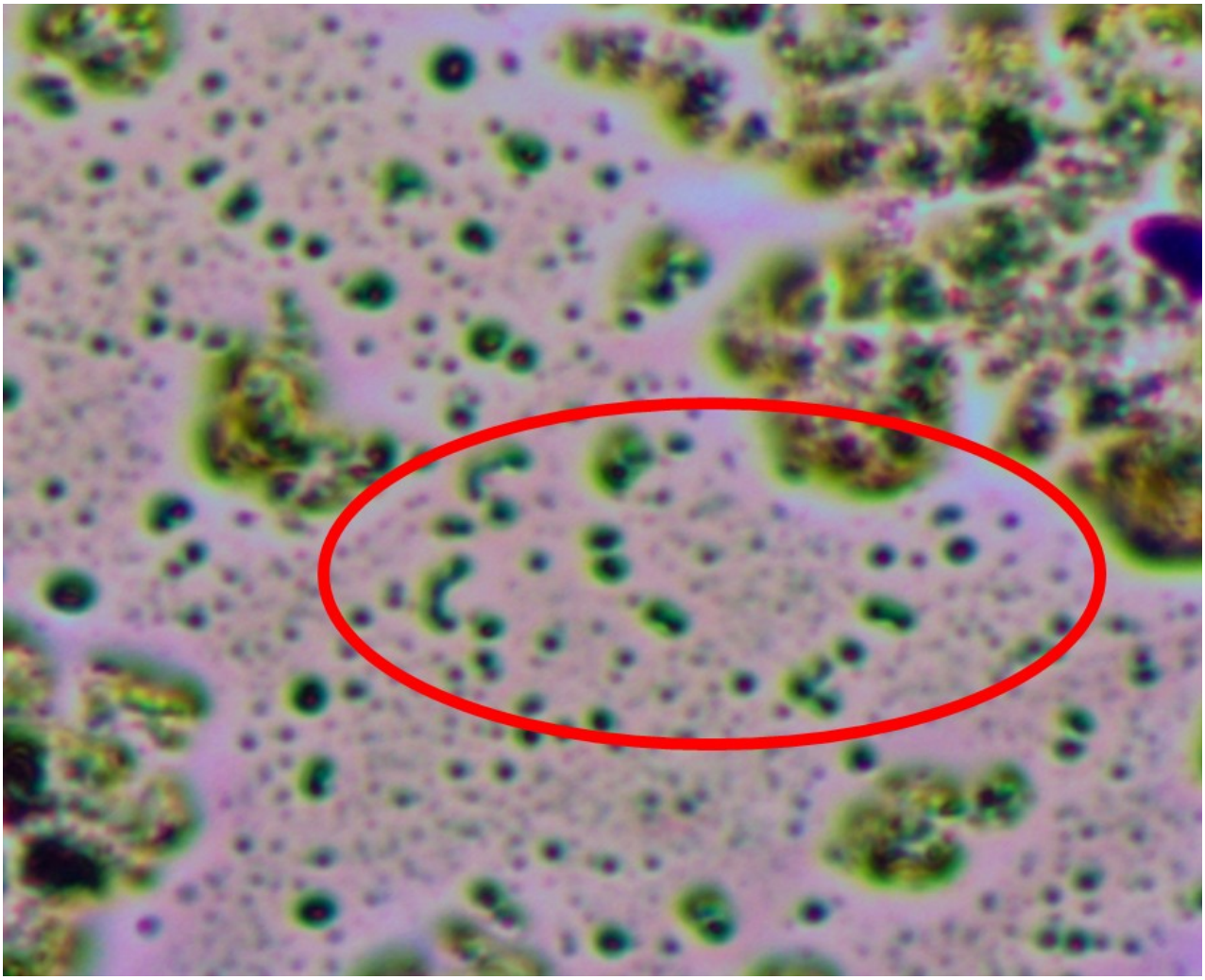
Does it give more resolution than a 4x objective that can also show a 0.83 micrometer wide object?

Not exactly, but why not? Because when observing the streptococcus sample above, the image is augmented in size obviously and easier to observe but not in resolution because it is not possible to distinguish the bead like form of a streptococcus.

However, is it that all?

No, it is not, I found that under certain conditions such as in a sample fixed and stained negatively with candle soot, it is possible to distinguish through the 25x eyepiece and the camera the bead-like form of the streptococcus clearer than with a positively stained sample.

Look at this sample negatively stained with candle soot:



Why cannot we go “further” if we are augmenting the magnification? So-called 'empty magnification' is a factor.

Remember that in optical microscopy the limit of observation is given by the light wavelength.

CONCLUSION:

Probably the resolution of a typical 10x NA 0.25 objective is not able to distinguish completely the separation of the cells of a streptococcus chain as it would be possible with more powerful objectives such as 40x or a 100x:

However, it is possible under some conditions as shown above to distinguish better a less than a micron wide object. So again, as it was stated for the 4x objective, the 10x objective is in some respects not a 'low power' objective at all.

This allows this method to be trustworthy when observing thick tiny objects it is possible to go “very, very far” into the sample.

It was mentioned in an editor’s note of David Walker in the previous article of this series that this sort of experiment is exploring a kind of ultramicroscopy, again this is true for the 10x objective.

New Editor's note: On further reading I was probably not strictly correct in making this 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 the tiny particles suspended in colloids and aerosols.

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Published in the October 2017 issue of *Micscape* magazine.
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