

OPTICAL STAINING PART 2

DARKFIELD ILLUMINATION

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

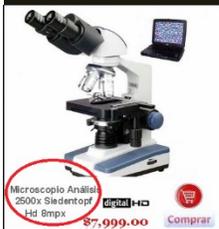
INTRODUCTION:

As it was described in the previous article about optical staining, this concept means that without the help of stains it is possible to create contrast and more resolution in a microscopic sample using different methods of illumination.

In this article I want to “honor” one of the most amazing methods that I have known since I started to gain my knowledge of microscopy, this is for me the most beautiful one and the most incredible, since the principles for this are extremely simple to understand and create. It is DARKFIELD.

DEVELOPMENT:

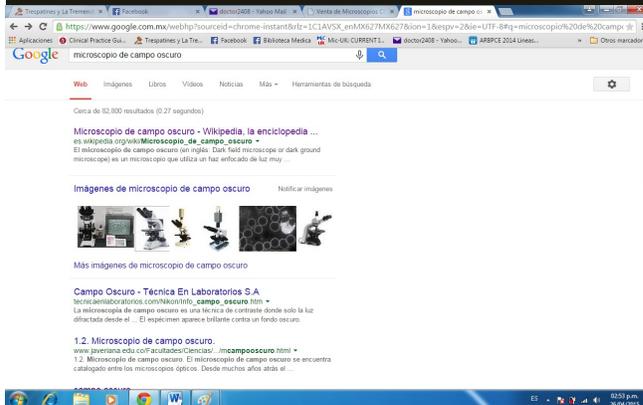
I met DARKFIELD illumination while I was looking for a microscope some days before purchasing mine. I was looking for information about a microscope and I did not know any more than the things that I have learned in high school and university about microscopy - that was just to see some bacteria stained with gram staining and pond life that was taken at that time for the class, obviously observed with a brightfield microscope. In fact I did not know that there existed many different techniques to illuminate a sample, so what I was looking for was a microscope with great magnification - the first I saw in a page here in Mexico was this one that offered 2500x of magnification so I thought of buying it.



But I continued looking at the offers that I could afford so explored the page of the seller and found something that caught my attention - it said in Spanish : “MICROSCOPIO DE CAMPO OSCURO” that means DARKFIELD MICROSCOPE, that term sounded to me interesting and rare at the same time so I wrote it in the search box:



and I found several pages explaining how DARKFIELD works.



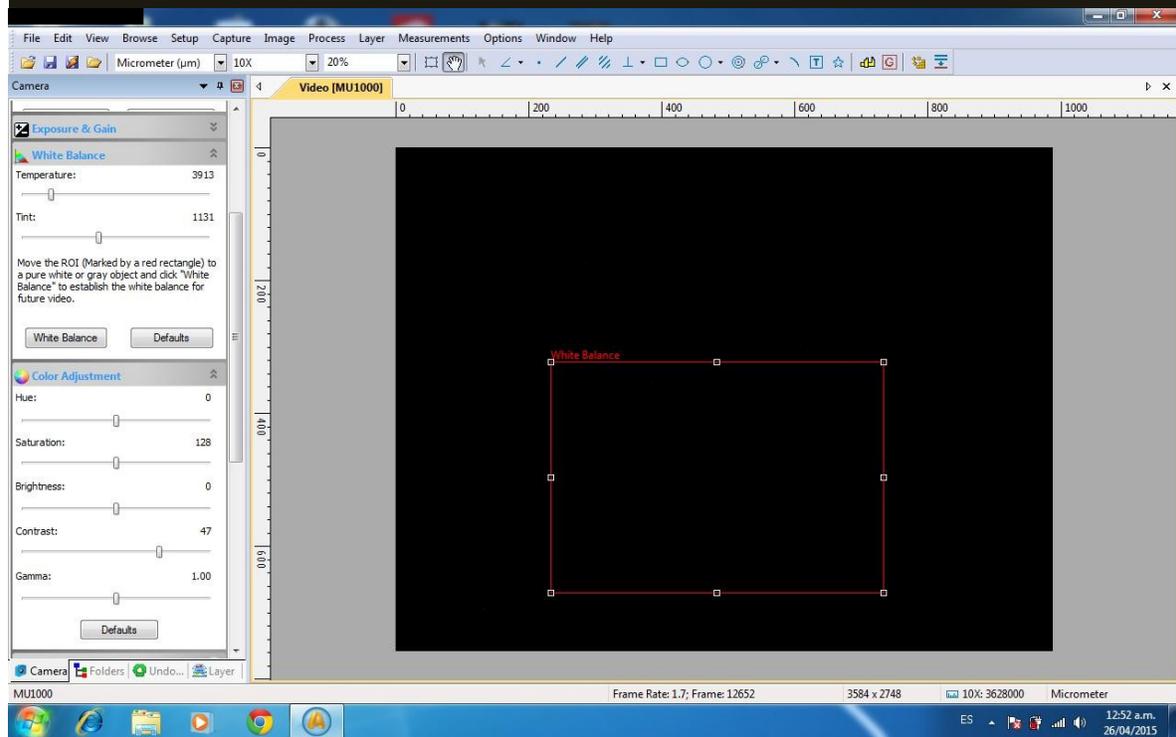
The explanation given was that DARKFIELD is an illumination technique that follows the principle similar to when within a dark room a beam of light enters and illuminates the particles of dust or like when you see in the sky on a clear night where plenty of stars shine against a dark background. Imagine that it is incredible and beautiful isn't it?

I learned of this and decided that I would like to buy a DARKFIELD MICROSCOPE - looking for more information about the manufacturer of these microscopes I found it is established in the United States, so I entered the page of that manufacturer and looked for the options they had and I found a trinocular microscope with both brightfield and darkfield condensers, 2500 x with Köhler illumination too, so I made an international order and it was sent to my address. This way I started to gain the knowledge of the most beautiful microscopy illumination technique, at least this is for me DARKFIELD.

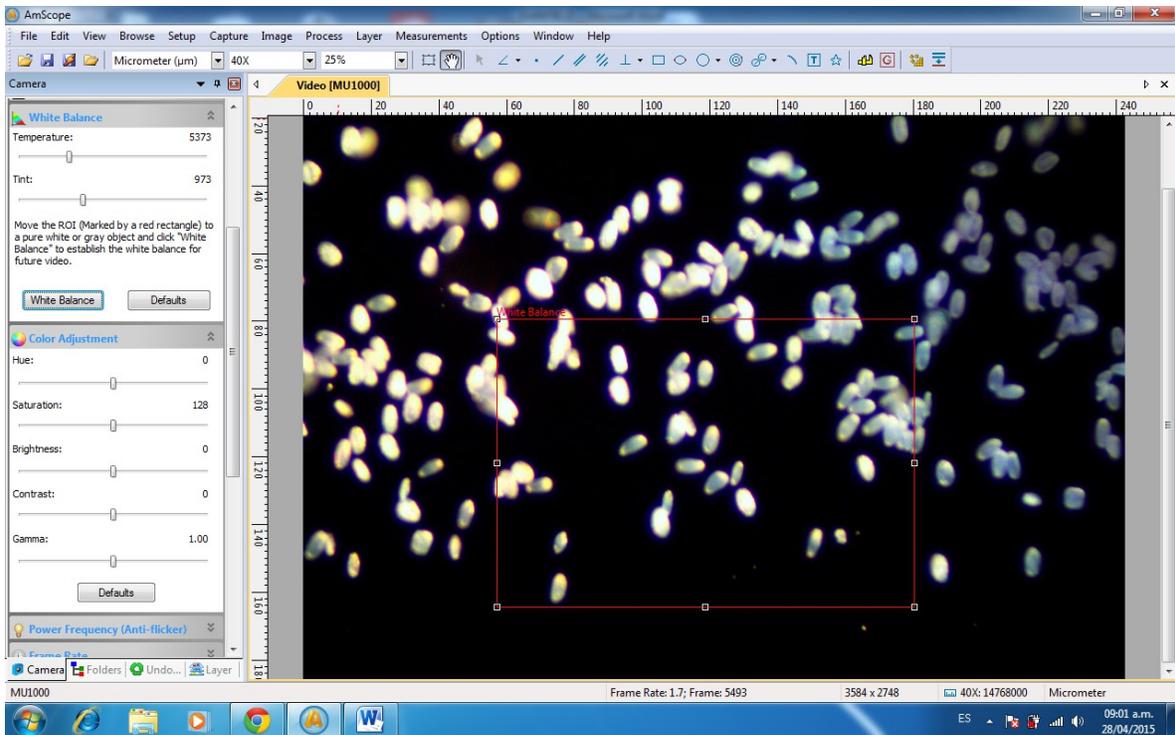


DARKFIELD CONCEPTS:

The first thing that must be understood is that in darkfield you prevent light from entering directly on the sample and in the microscope's objective so the stop must be of an opaque material and must be wide enough to surpass the numerical aperture of the objective, this way when you do not have a sample in the stage of the microscope the only thing which is appreciated is a total dark background,



But when you place a sample upon the stage the same sample produces the effect by diffracting the light that passes around the stop, this diffraction produces the morphology of the sample against a dark background.



A thing to remember here is that diffraction of light is no other thing than the property of light to disperse itself when it meets an obstacle and surround it or finds a hole and passes through it; in the case of darkfield it fulfils these two characteristics because it finds a hole to pass around the opaque stop but it is also dispersed with an obstacle once you place the sample on the stage.

There are microscopes that come equipped with a darkfield condenser like mine that has a dry one, yes I state "dry" because there are dry ones that are used with low magnification powers and oil immersion ones that can be used with high magnification powers.

Here is the darkfield condenser that came with my microscope seen from different aspects.

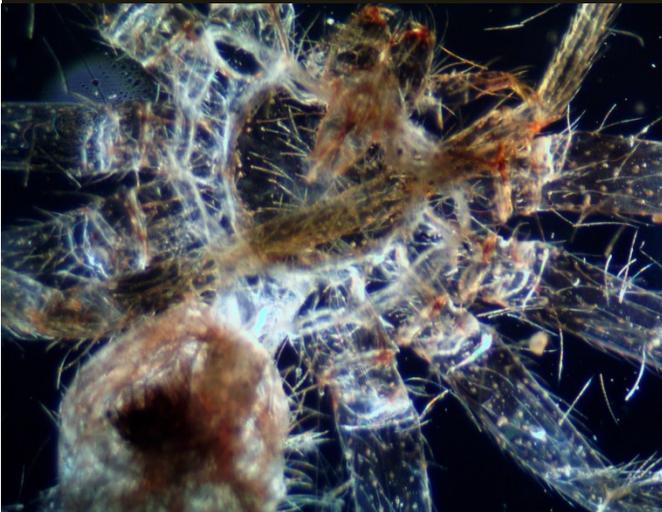


HOW TO MAKE THE DARKFIELD TECHNIQUE?

Since an opaque stop is easy to find everywhere, producing this beautiful technique is also easy in the absence of a real darkfield condenser and having a brightfield one. For the purpose you can use a coin, a circle of black cardboard or black plastic - remember it must be opaque not translucent .

To support it you can use a circle of transparent plastic that fits perfectly on the filter tray of the brightfield condenser or place it, taking care of centering it accurately, upon the light source. In the next article about OPTICAL STAINING I am going to present the results using a DIY-DARKFIELD filter. Today I want to devote my article to presenting just images created with the real DARKFIELD CONDENSER that I have.

RESULTS:



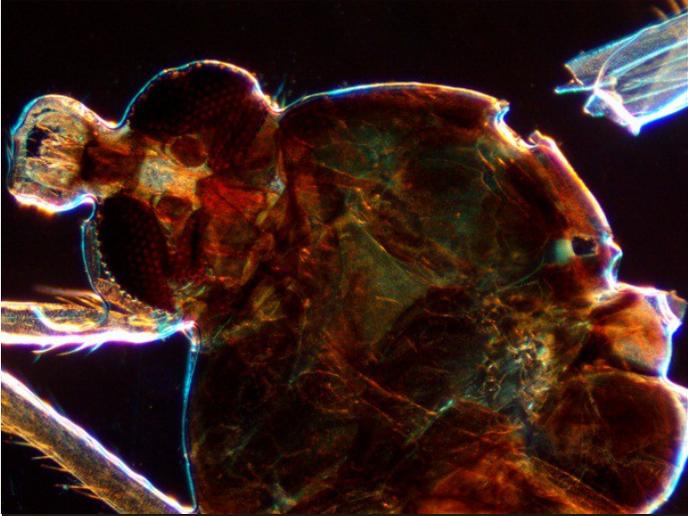
Spider 4x 20%



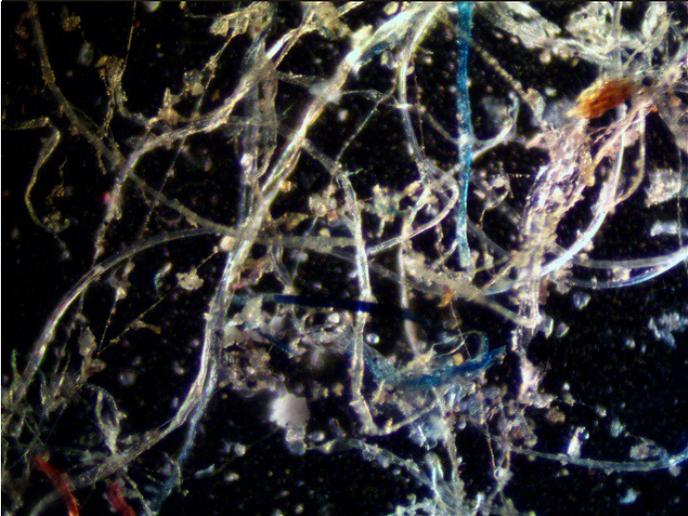
Daphnia 4x 20%



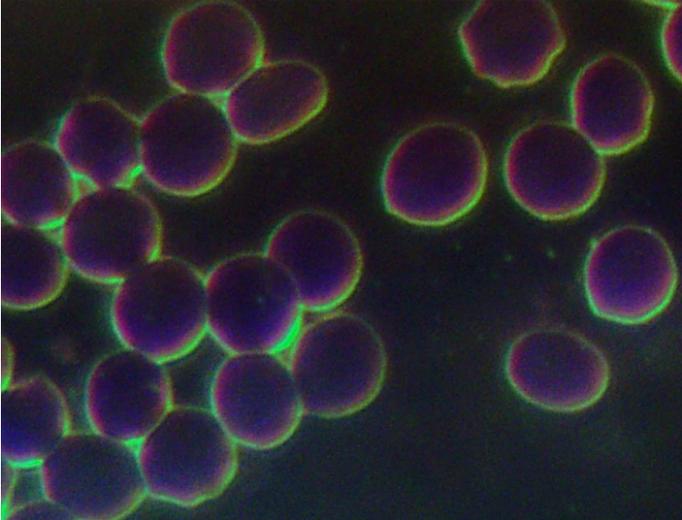
Mosquito 4x20%



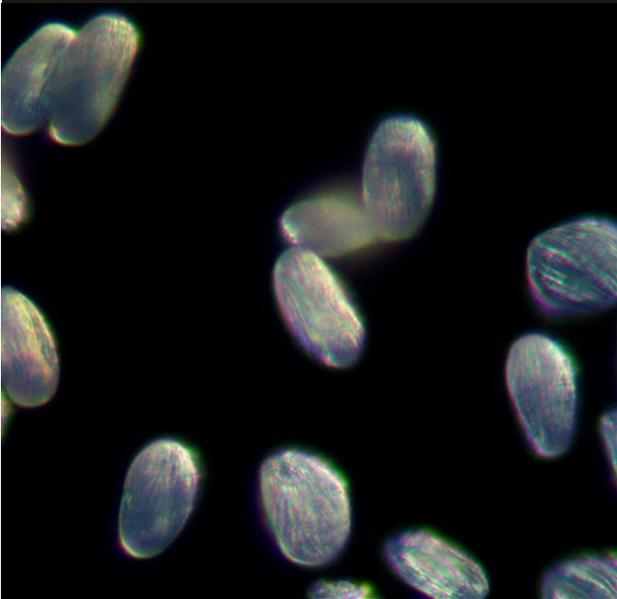
Mosquito 10x 20%



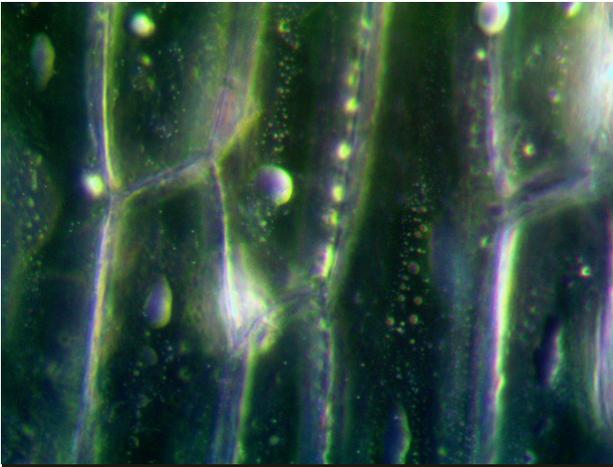
Spider's web 10x 20%



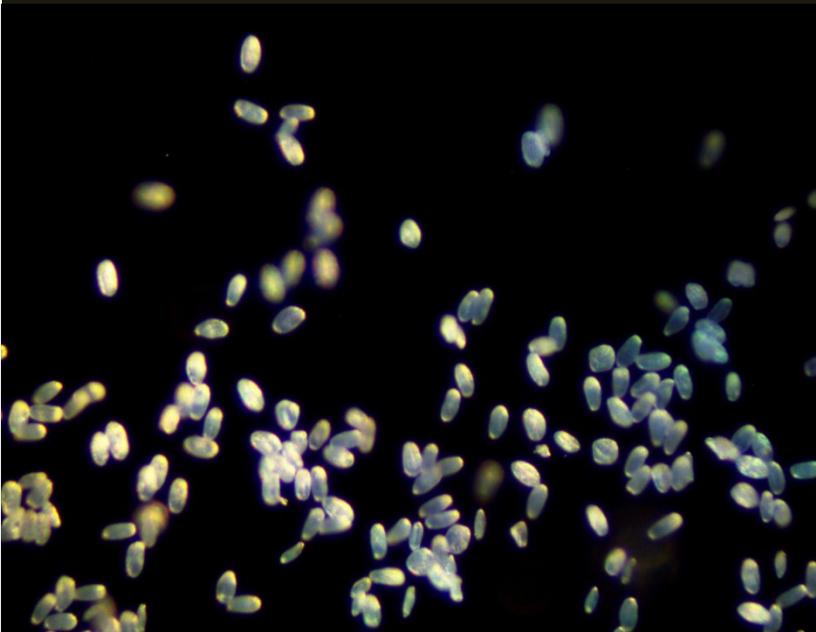
40x 100%



Pollen of *Spathiphyllum wallisii* 40X 25% zoom



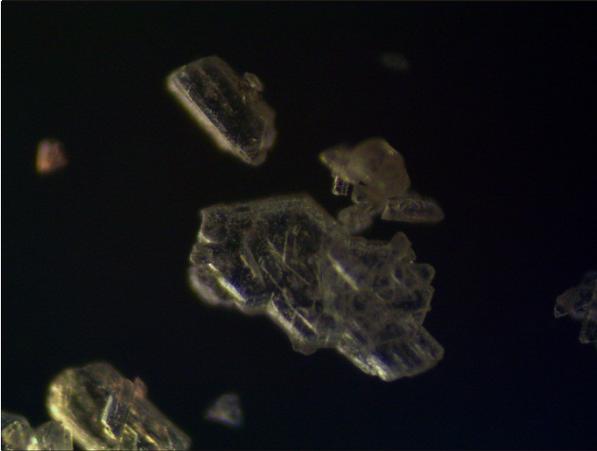
Onion epithelial cells 40x 25% zoom



Pollen of *Spathiphyllum wallisii* 10x 25% zoom



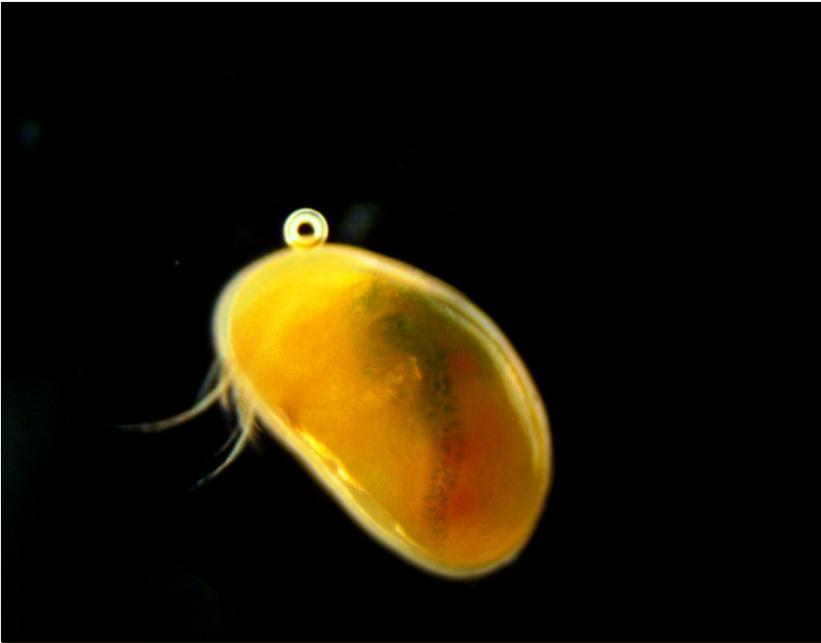
Streptococcus of yogurt 40x 100% zoom



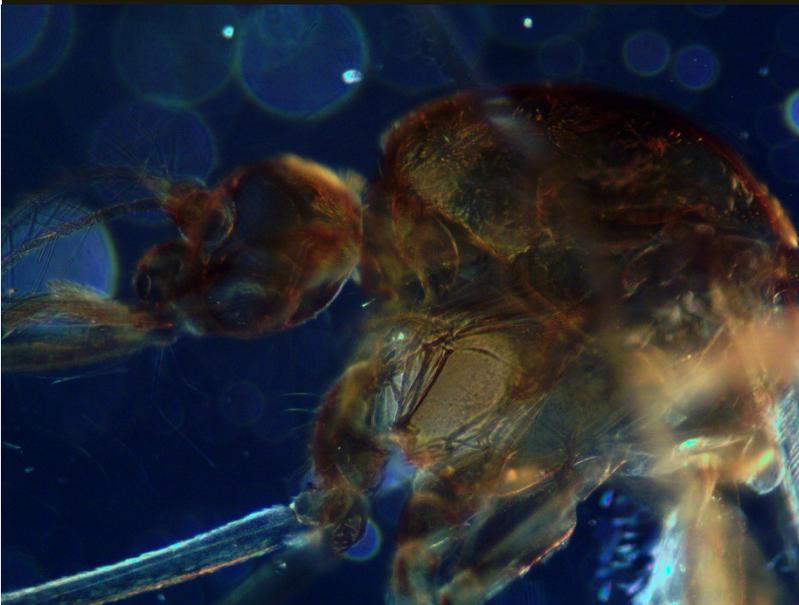
Sodium bicarbonate crystals 10x 100%



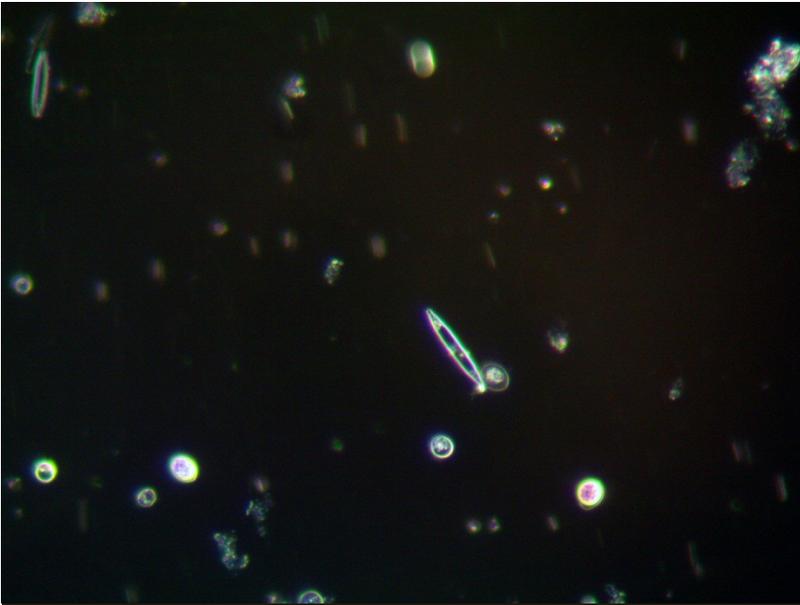
An ostracod shell found in the sediment of the pond where I have the ostracods 4x 20% zoom



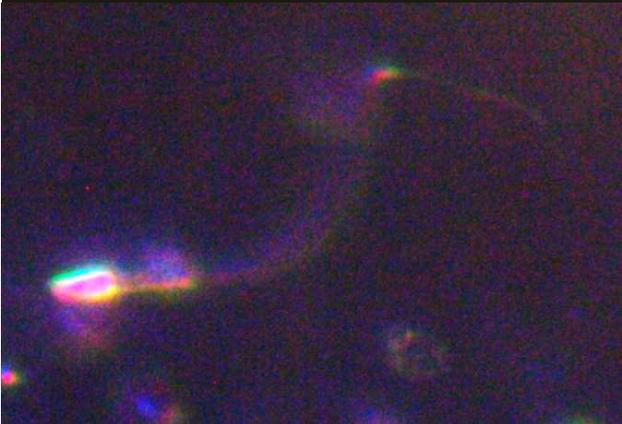
Ostracods probably cypris 4x 20%

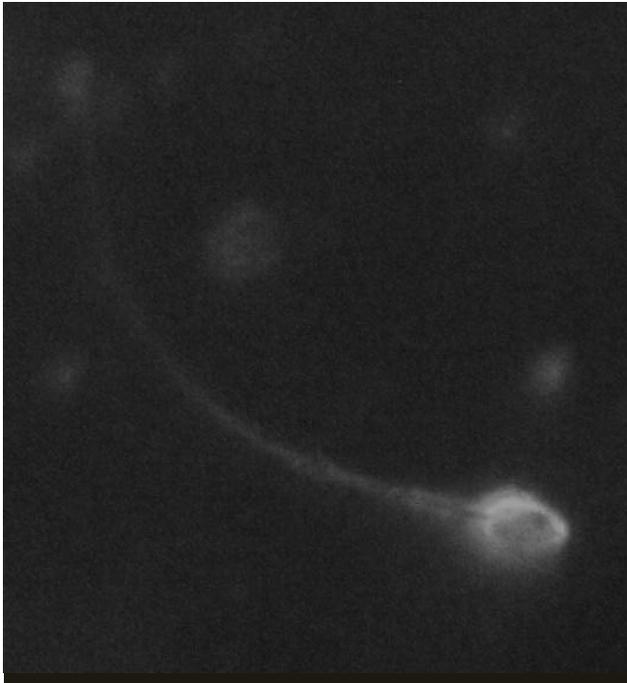


Mosquito 4x 20% zoom

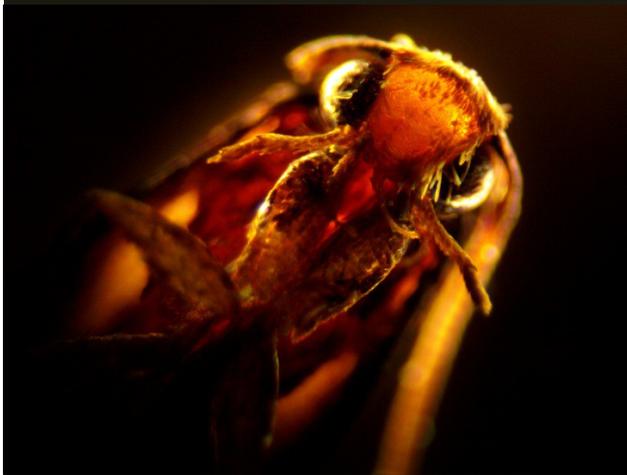


Diatoms and green algae 10x 50%





Sperm 40x 100%



Moth 4x 20%

One of the things to consider when you use DARKFIELD is the effects of a camera. If you observe directly through the oculars invariably the image is perfect with a beautiful darkfield effect. But when you use the camera it is necessary to make some variation in light intensity which you can achieve just by dimming the lamp, especially if you have an halogen bulb like mine. Or the other option and is the better one is to use a blue filter upon the light source, this blue filter is most of the time provided by the manufacturer with the microscope but if not you can make one with some light blue cellophane or with some light blue plastic.



That is the reason why it is perceived in the images a bluish color and in some cases like one of the photos of the sperm that is seen a blue background. For more detailed information please read the article **Darkfield Illumination and the Digital Camera** by Howard Webb, Missouri, USA in MICSCAPE.

CONCLUSION:

What is it possible to see with DARKFIELD? If I answer this question superficially I can say that everything you want to and it is almost true, but DARKFIELD for obvious reasons has some problems of contrast and resolution with dark and/or thick samples, so a good form to get the best of DARKFIELD is with transparent samples like the majority of things that microscopy has and thin ones. NEVERTHELESS TRY IT, IT IS THE BEST.

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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