

BRIGHTFIELD ILLUMINATION: THE BASICS OF MICROSCOPY PART 1

By: ALEJANDRO ARIEL GARCIA ARRIAGA,
COACALCO DE BERRIOZABAL , ESTADO DE MEXICO, MEXICO.

Introduction:

When a “normal” transmitted microscope is used with a standard illuminator (i.e. with either an LED or a halogen bulb), condenser and objectives, an image is obtained of a sample surrounded by a clear or possibly tinted background. This is called BRIGHTFIELD illumination. I said “normal” so as not to include microscopes with the facilities to use e.g. ultraviolet, fluorescence, darkfield, phase contrast, DIC and also using typical standard objectives. Just a compound microscope with a simple source of light.

Development:

Brightfield illumination is the standard type of illumination in a microscope and to observe a subject requires features within the specimen that create a natural contrast. Nevertheless it is possible to study subjects that have very little contrast (or if unstained) if a camera is employed to enhance contrast.

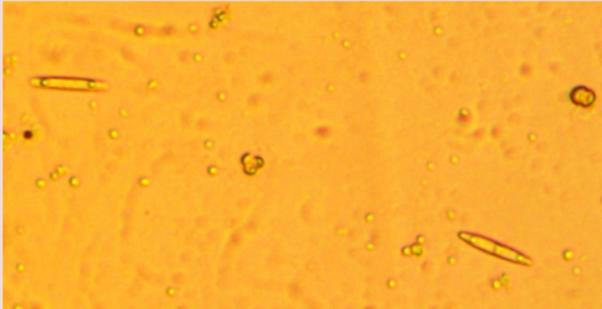
When using a camera you have two possibilities for BRIGHTFIELD. 1) Using the defaults of the white balance which in this case gives you the appearance of a yellowish background with the outline of the object in brown because I have an illuminator with a bulb with a low colour temperature.



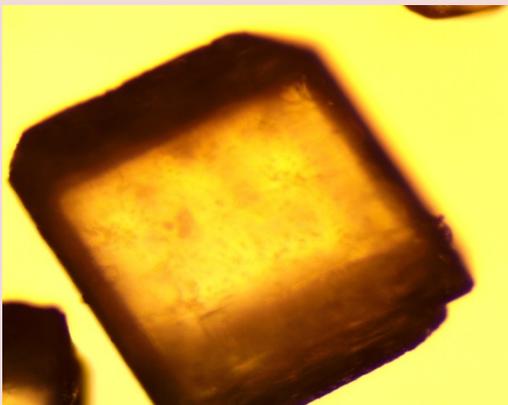
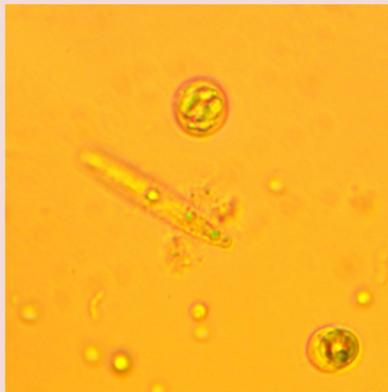
2) Using the white balance menu for each sample, which gives you a better approximation of the real colors of an object in the sample. This is not discussed or shown in this article but I will show this in part 2 of this series on brightfield illumination.

Results using brightfield with uncorrected colour balance:
The mags stated are for the objective used, not the total optical mag.

Diatoms 10x



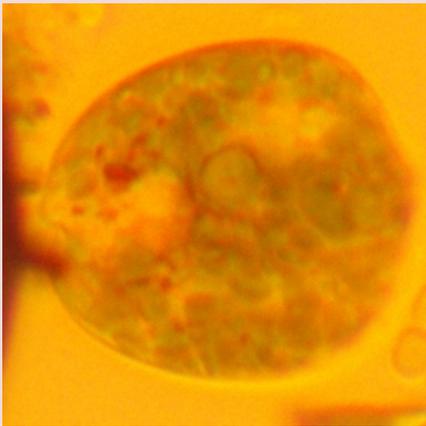
Diatom and green algae 40x



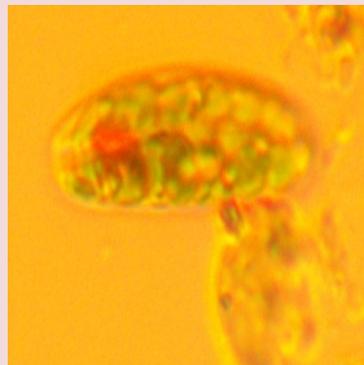
Sugar crystals 10x



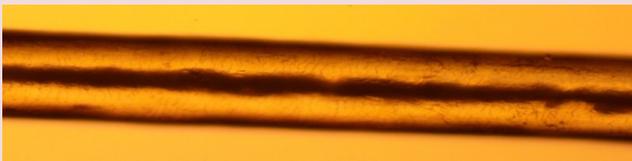
Mosquito 4x



Phacus 40x



Euglena 40x



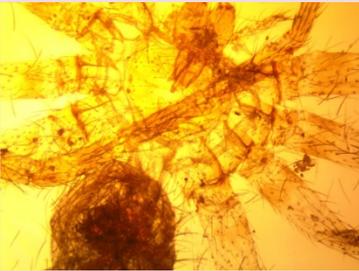
White hair 10x



Onion 10x



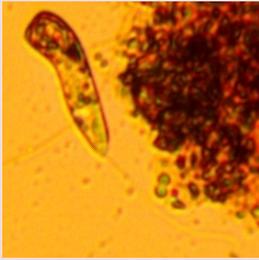
Daphnia 4x



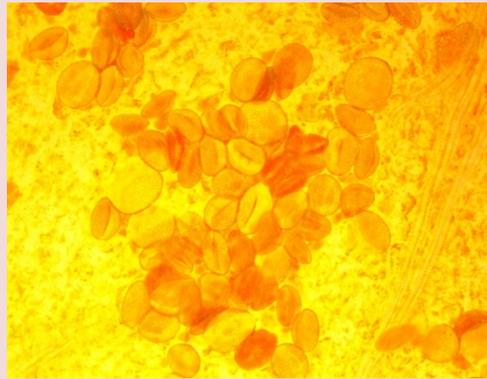
Spider 4x



Habrotrocha 10x



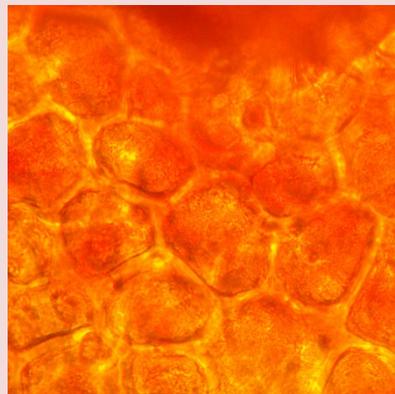
Astasia 4x



Lily pollen 10x

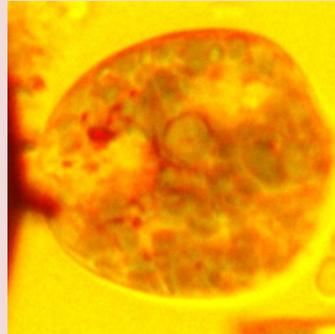


Mouth epithelial cells



Tomato 40x

Obviously it is possible to improve the contrast with MICROSOFT PICTURE MANAGER 2010 and turn on “the lights” for the photo, in the examples above I just wanted to show the effect of pure brightfield without modifications, but as shown below it is possible to obtain a clearer image using brightfield with such software.



Conclusion:

Brightfield is the standard illumination method in microscopy and should not be missed; even if it is true that contrast in a specimen is poor there is something that can be learnt. Besides there are many samples that nature itself has stained e.g. look at a section of tomato, phacus, euglena and green algae which are very worthwhile to study using brightfield.

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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