OPTICAL STAINING PART ONE

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INTRODUCTION:

Observing things that are beyond our sight is amazing, even more so when things on a microscopic scale are most of the time transparent. But sometimes this property of the microscopic world becomes an obstacle mainly for the enthusiast microscopist. Nevertheless, there are many ways we can overcome this inconvenience; within this and a subsequent article I would like to show you how we can make microscopic things visible with beautiful techniques that I have learned in my searches for enhancement methods.

DEVELOPMENT:

When we learn the theory about microscopy we learn something very important i.e. in order to see a microscopic object it must have enough contrast and resolution. These two concepts are very important to understand since they are the basis of all observations.

So I am going to define contrast, as the difference in illumination between an object and the background, I have learned that it is possible to alter contrast by changing the background illumination, or by changing the object illumination.

Resolution can be defined as the capacity of the microscope for making possible the differentiation between two objects. In the case of optical microscopy ca. 0.25 micrometer is the possibility since it is limited by the light wavelength.

When we learn about microscopy most of the time brightfield is the illumination method that we are introduced to at first and we are told that for achieving contrast in an optical microscope we need stains for the transparent samples of the microscopic world. This is partially true because the majority of samples for example in Pathology or Histology are made with stains and observed in bright field. Even more so, the properties of the samples are expressed according to their reaction to the stains. For example, as in the case of bacteria they are classified as gram negative or gram positive.

Nevertheless for the enthusiast microscopist it is difficult sometimes to obtain these kind of stains. If somebody has a microscope, most of the time it is supplied with a brightfield condenser and has no stains supplied at all. Does that mean that we cannot observe some samples? Of course not because it is possible to do optical staining.

Optical staining? Yes, optical staining is a concept that I learned from my searches looking for forms of enhancing my observations and the first technique I found was Rheinberg filter illumination but now I have seen that there are many other beautiful methods.

Optical staining then is the capacity of providing contrast to a transparent microscopic sample without using real stains but instead using filters or stops placed on the light source or in the filter tray of the condenser.

In the present article OPTICAL STAINING is also a variation that you can carry out with the software of a microscope camera.

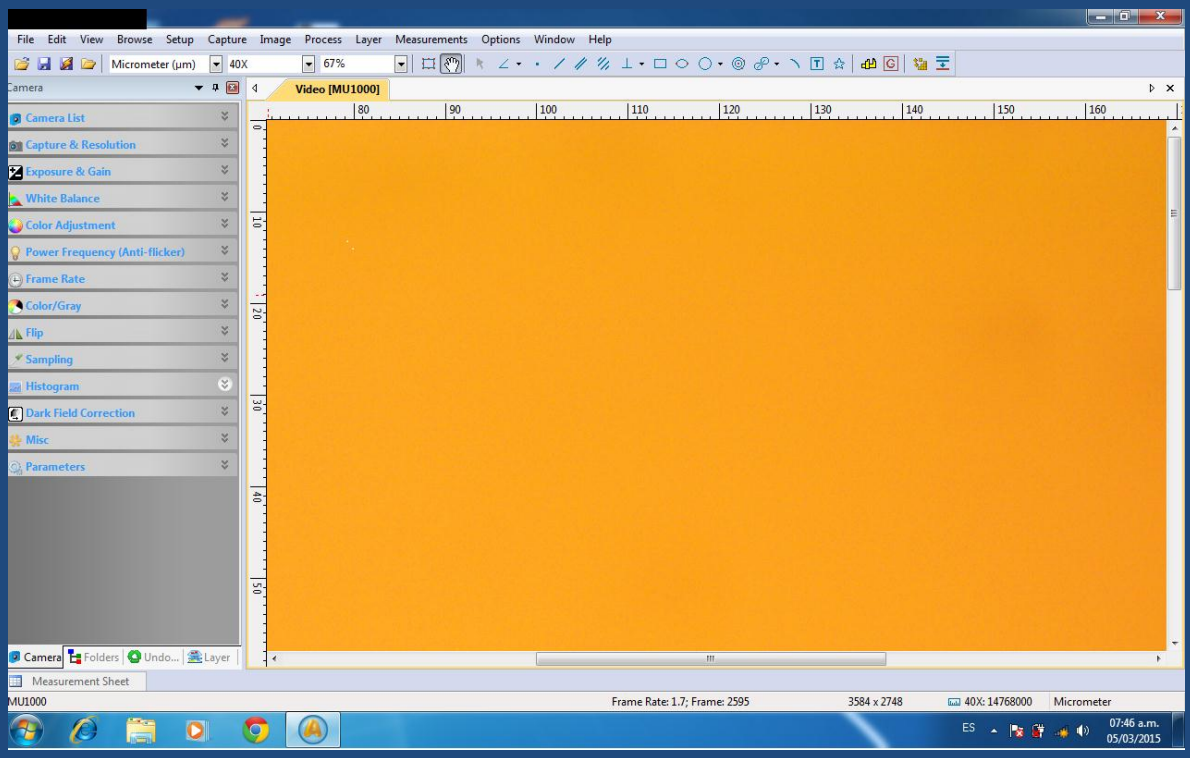
It is also important to say that you can sometimes achieve more resolution when you vary the contrast; this is clearly seen between illumination methods that for the moment are out of the scope of this article.

The present article has the purpose of analyzing all the potential that you can achieve if you have a camera, no matter how complex or simple it may be. You can take advantage of it, it has tons of functions that you can use to observe your samples.

I will analyze with you the advantages of my camera which is a 10 MP camera and that I am totally glad to have. Let me tell you that I started to use it three months ago and have learned a lot about the marvelous things that can be done with it.

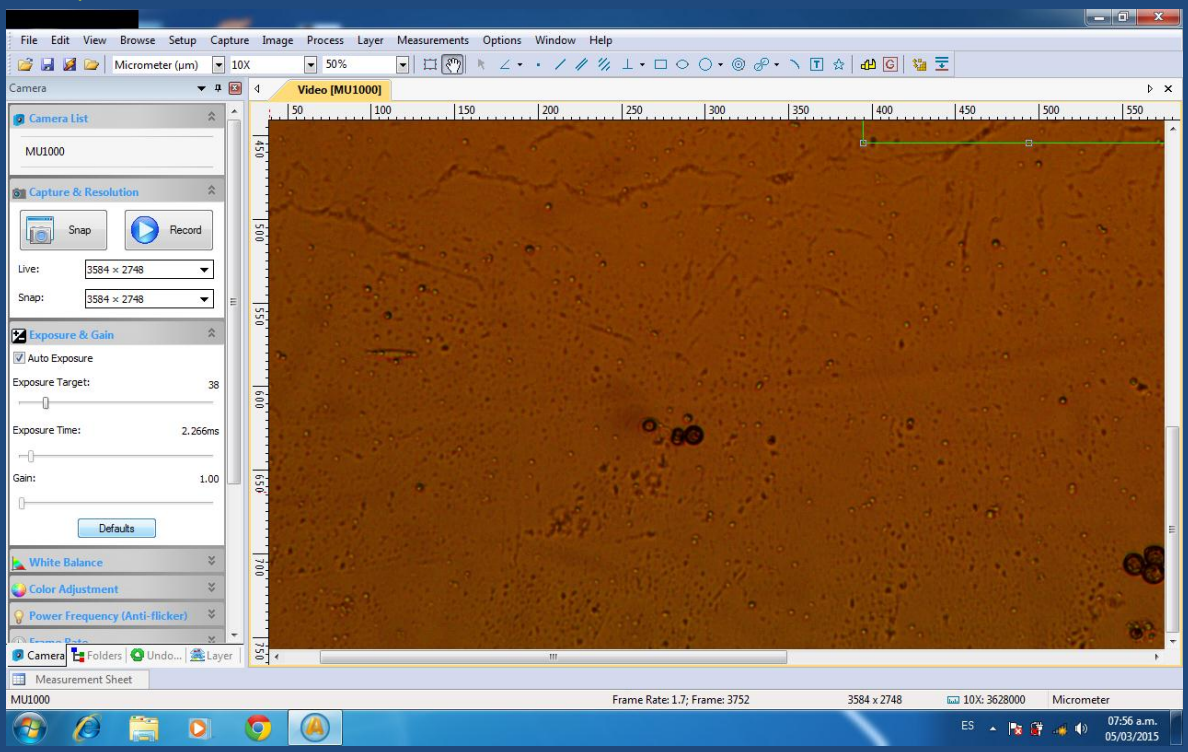
I am going to show you images of the screen of my software´s camera so that you can see how easy it is to obtain contrast in the samples if you count with one microscope and one camera.

- HERE ARE THE PICTURES USING THE BRIGHT FIELD CONDENSER

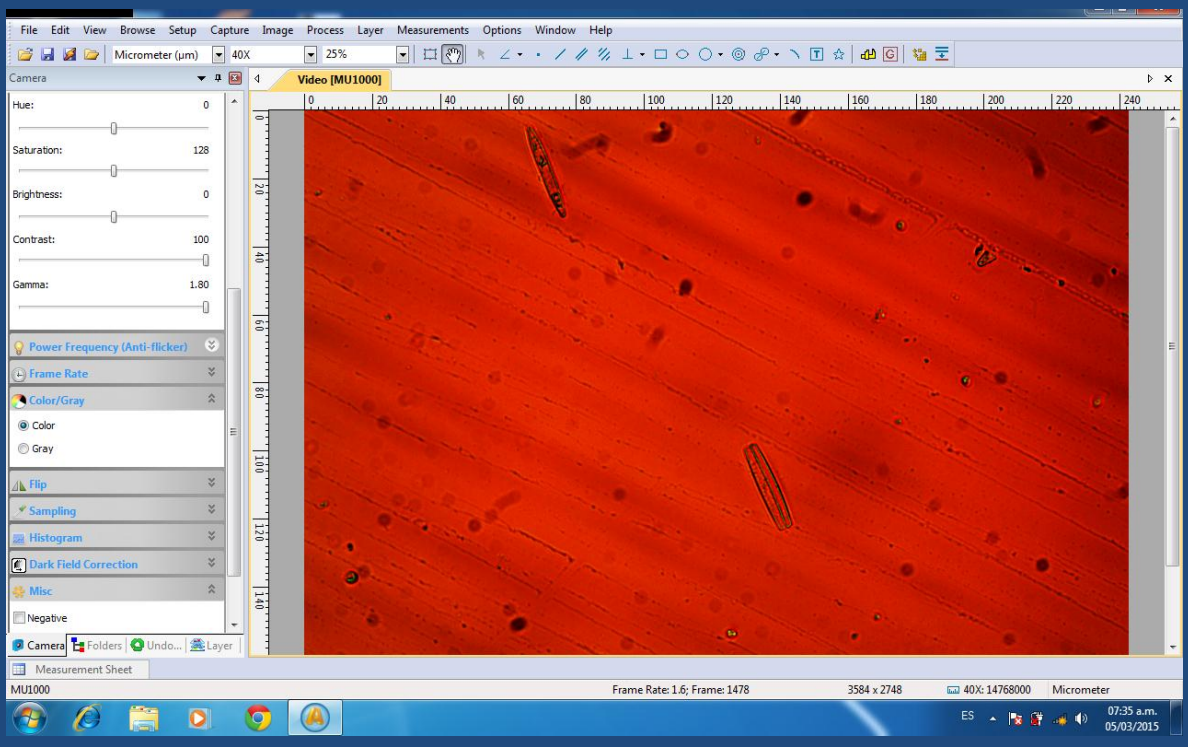


1. The functions in every camera are more or less the same; here it should be appreciated that the menu and the field that is shown when you turn on your microscope and open the software of the camera, it is in yellow and almost orange because of the warm light that produces an halogen bulb, like the one of my microscope and this by itself would be a very, very simple form of staining

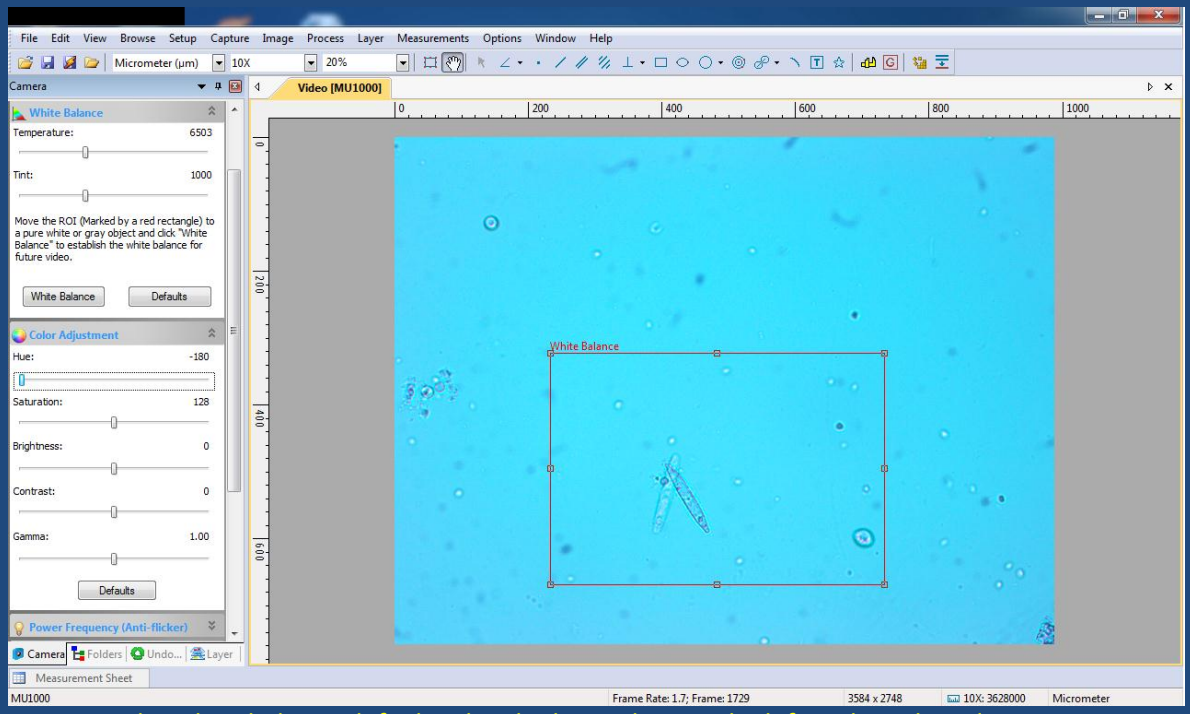
a sample.



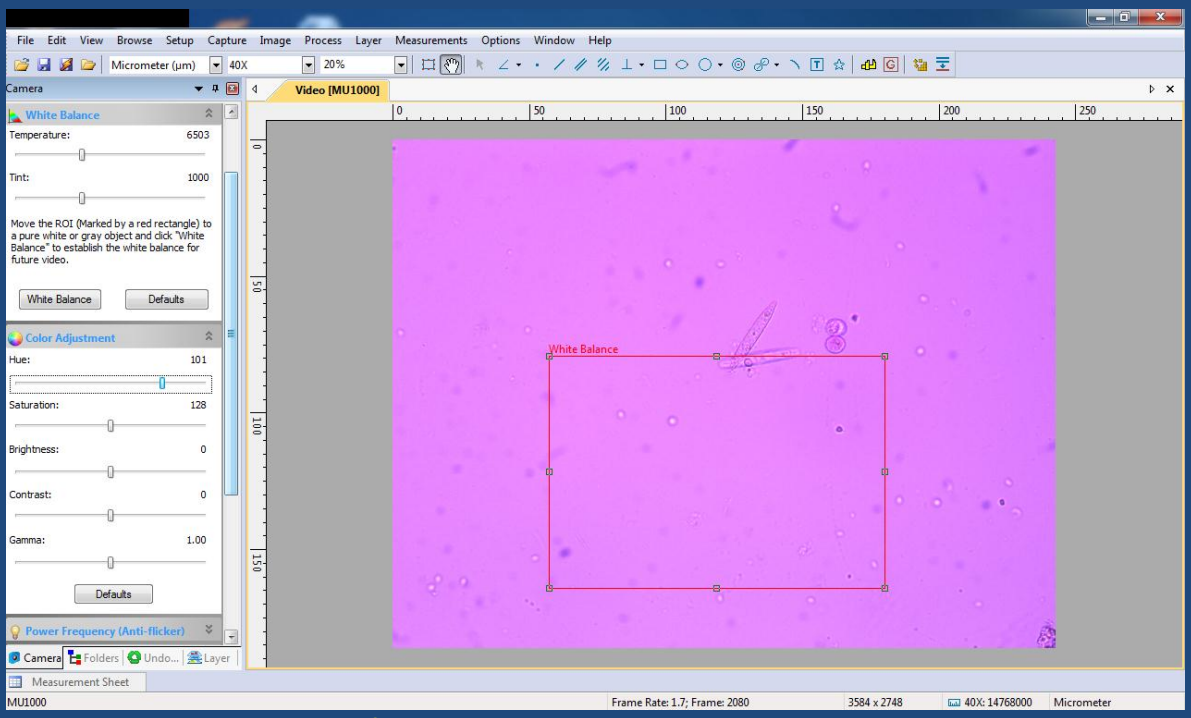
2.In the picture above we show the camera view, the resolution for the video and the photo; you can stain the sample by varying the exposure target to the left or to the right.



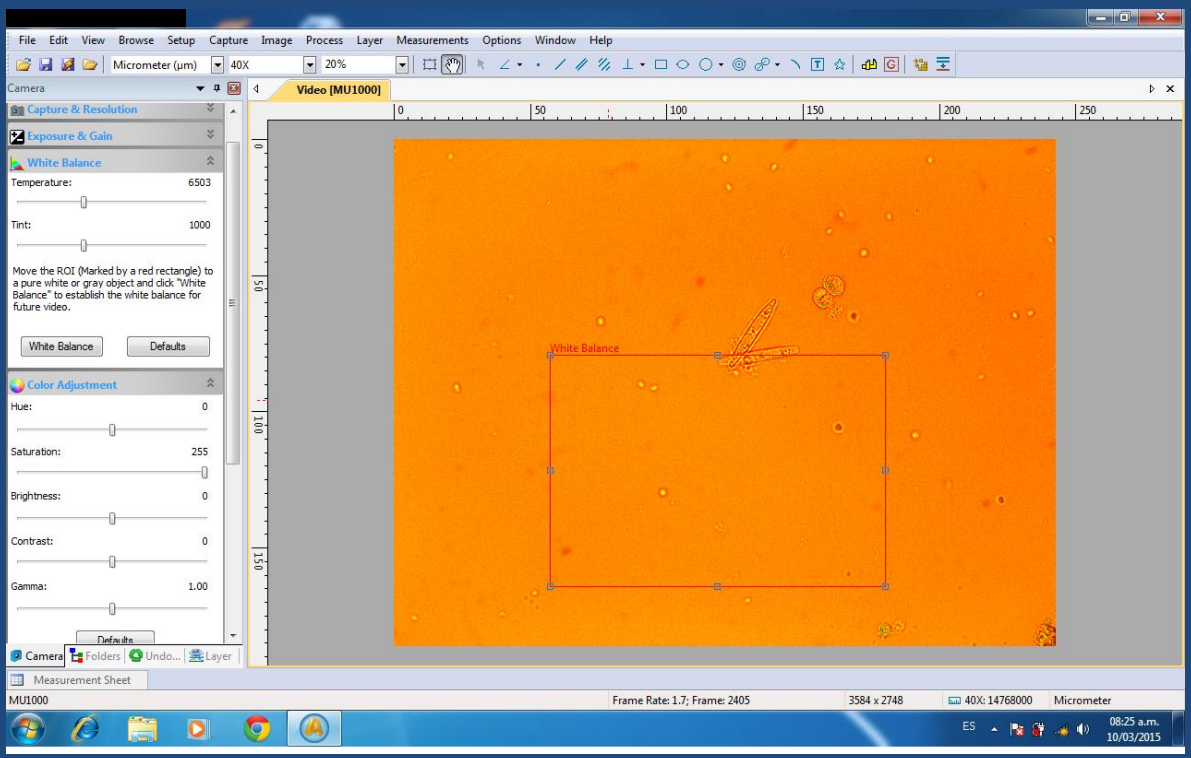
3. In this picture you can see more contrast just by moving the contrast and gamma values to the maximum using the color scale.



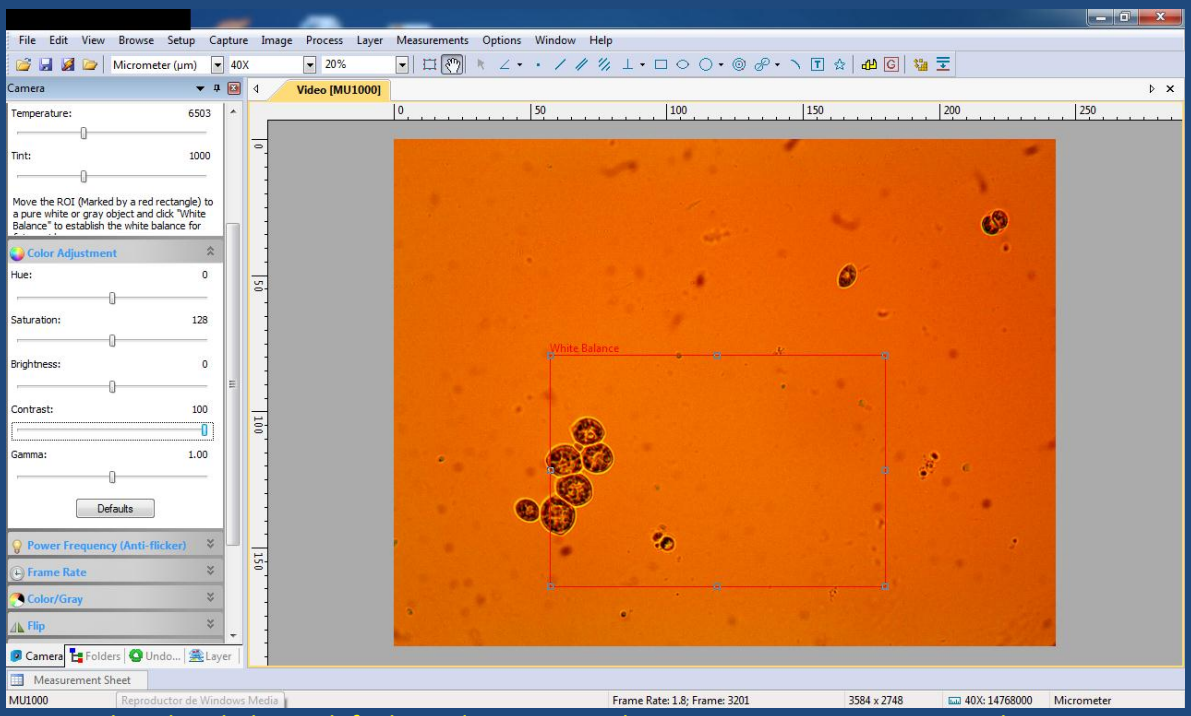
4. Here is the white balance defaults plus the hue values to the left in the color adjustment menu.



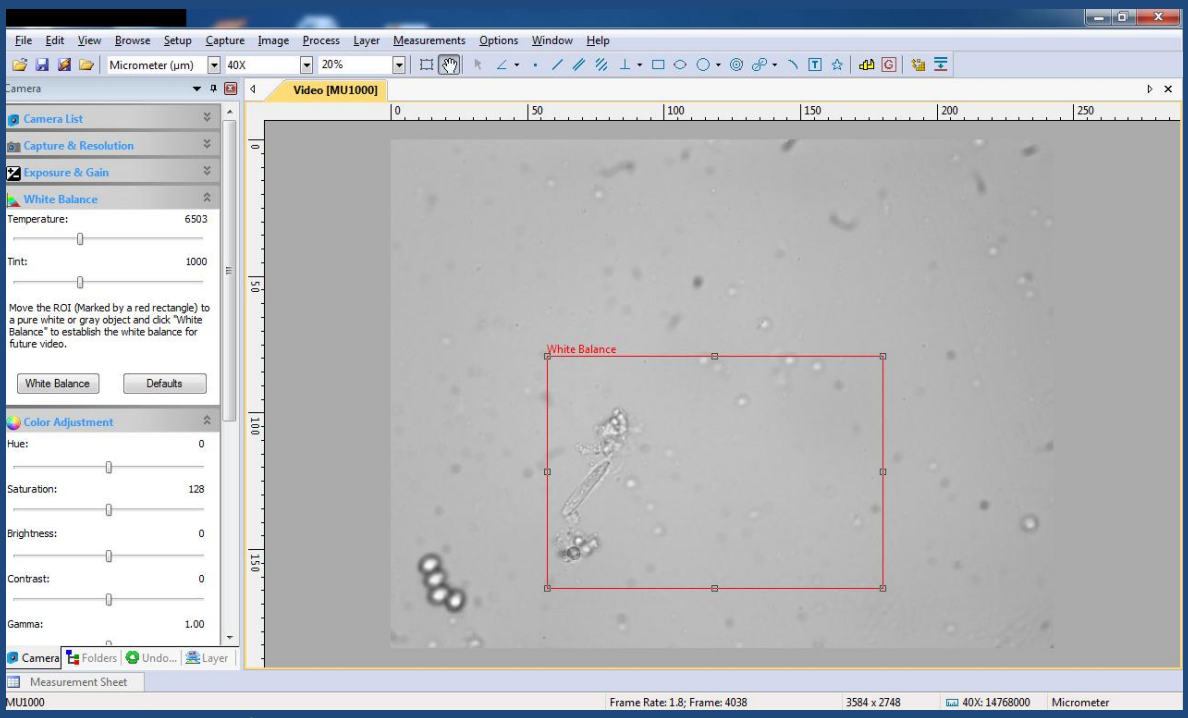
5.Here is the white balance defaults plus the hue values to the right in the color adjustment menu.



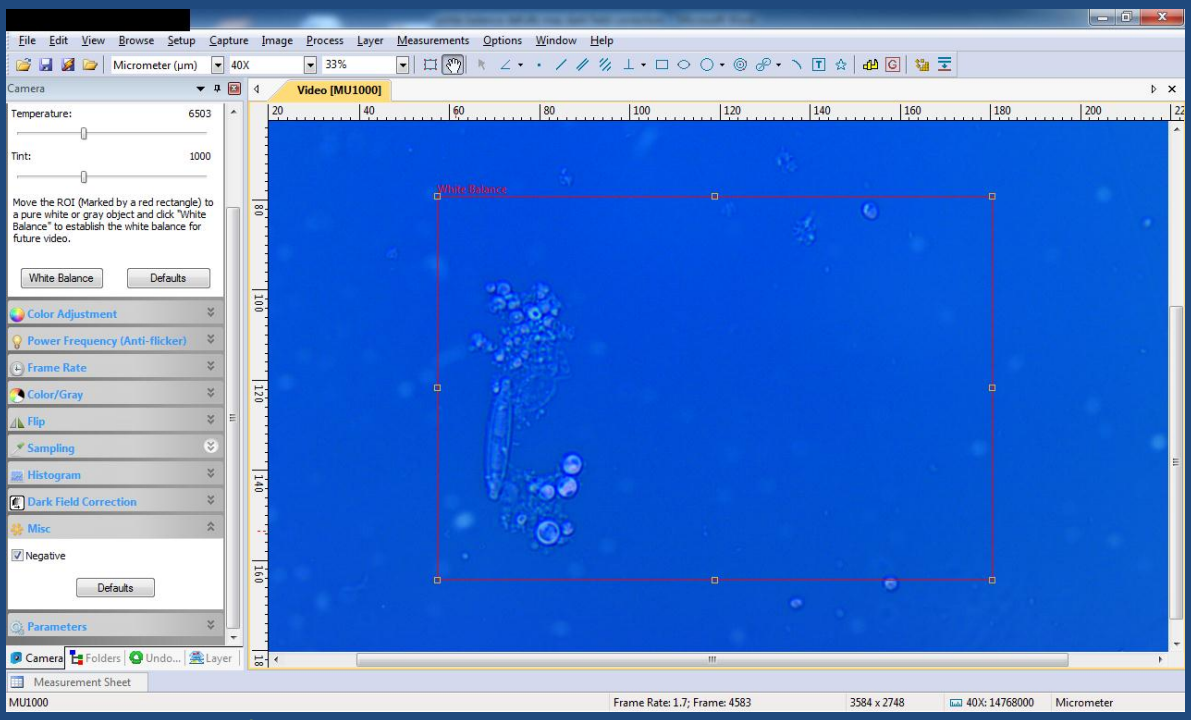
6.Here the white balance defaults and saturation to the maximum .



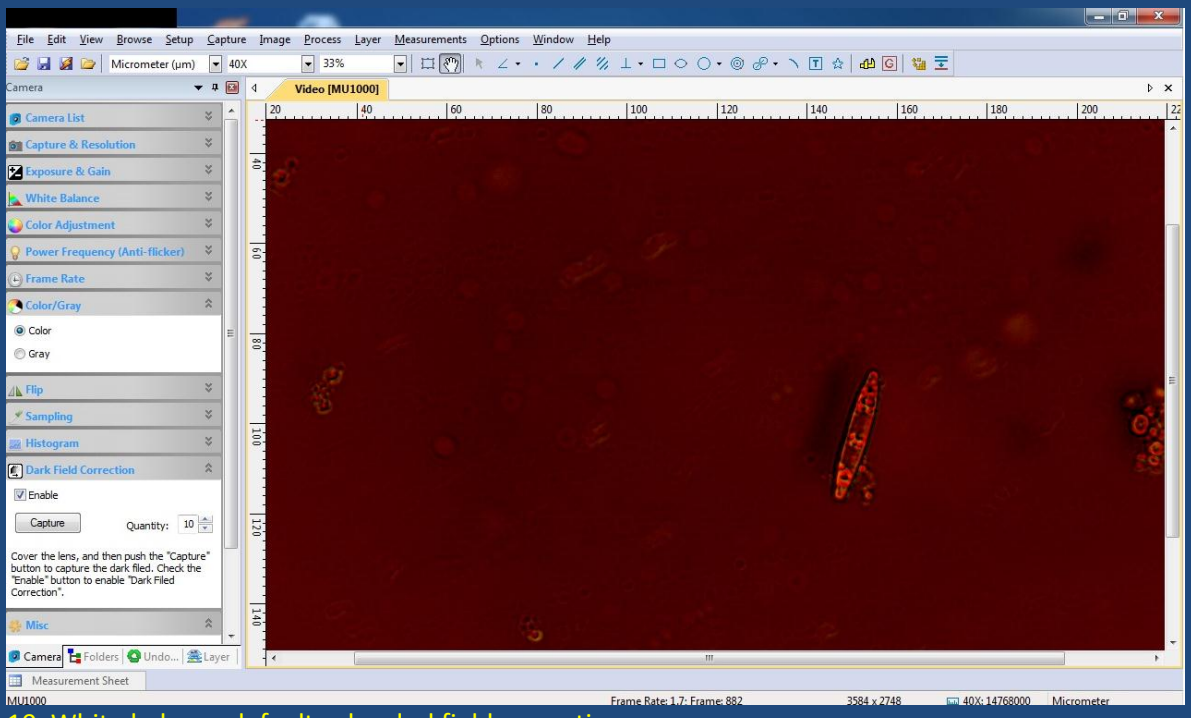
7. Here the white balance defaults and contrast to the maximum. Pay attention to the enhancement of the colors in the sample.



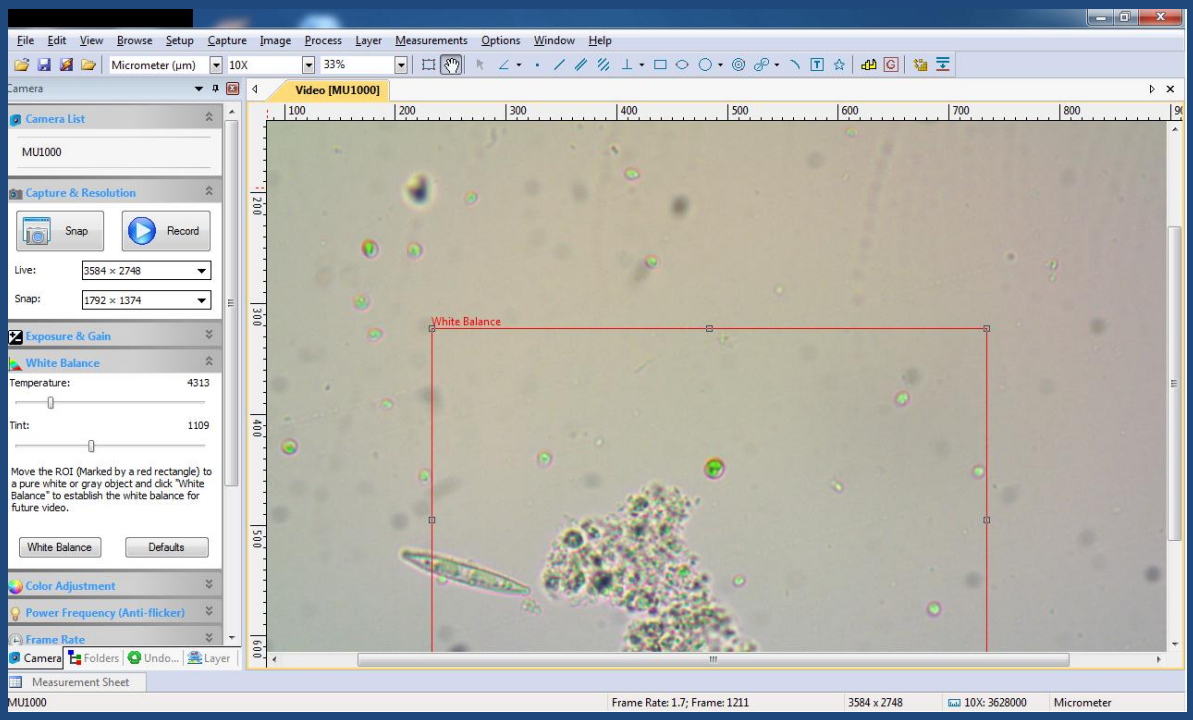
8. White balance defaults plus gray scale.



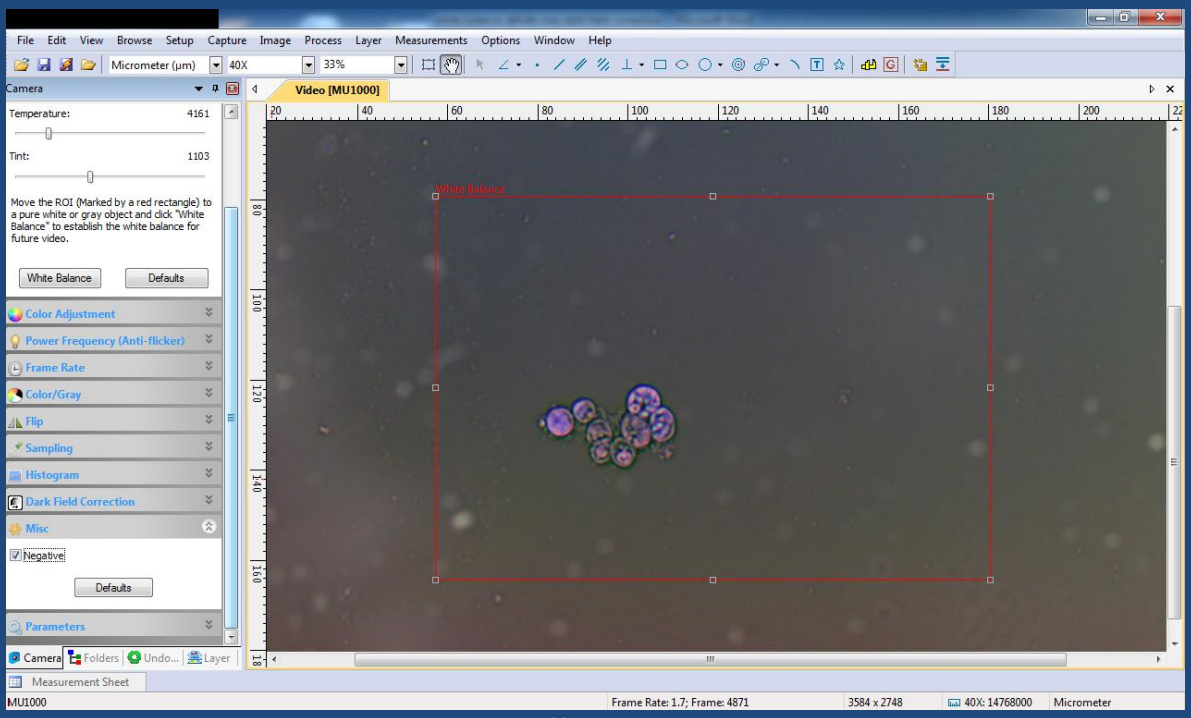
9. White balance defaults plus negative.



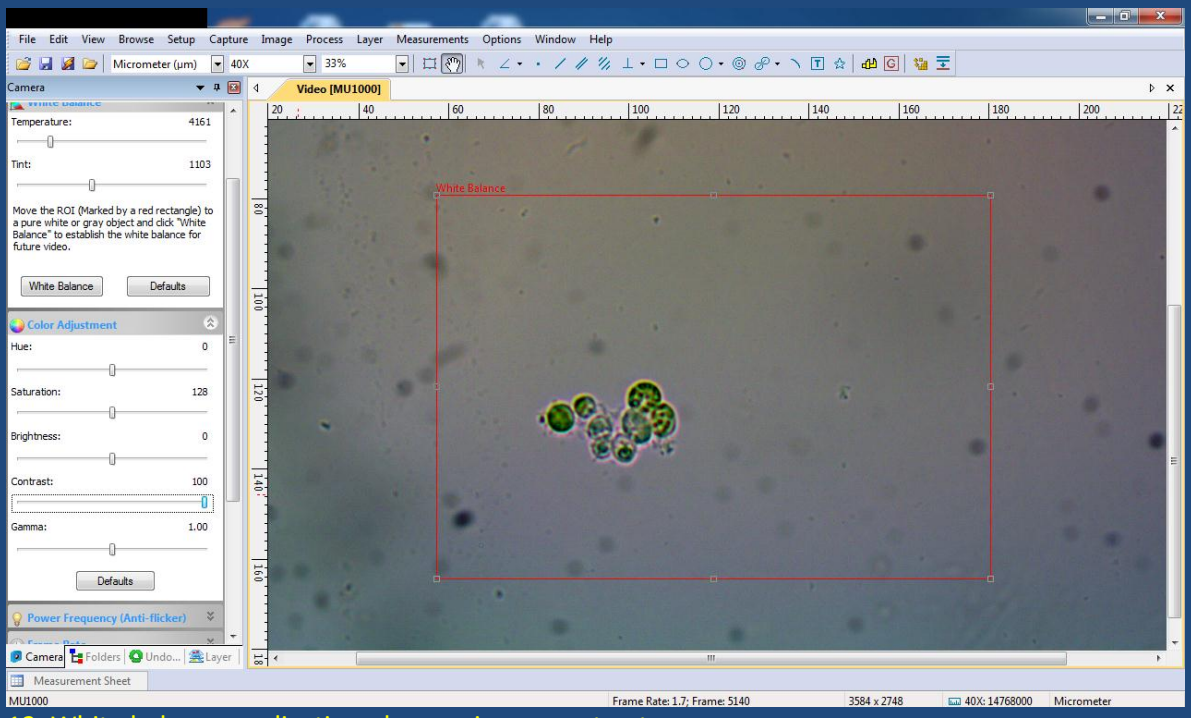
10. White balance defaults plus darkfield correction.



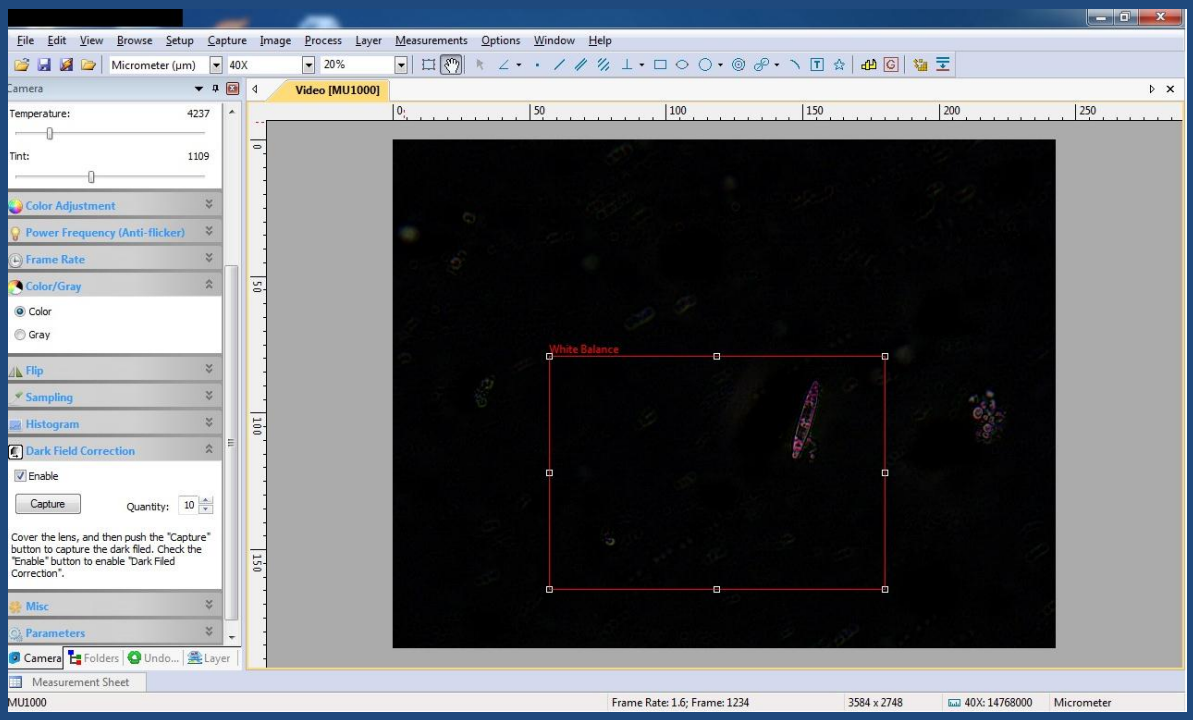
11.When you apply the white balance, the camera gives you an approximation to the colors you expect from the sample as it is appreciated in this picture the algae for example appears in green.



12. White balance application plus negative effect

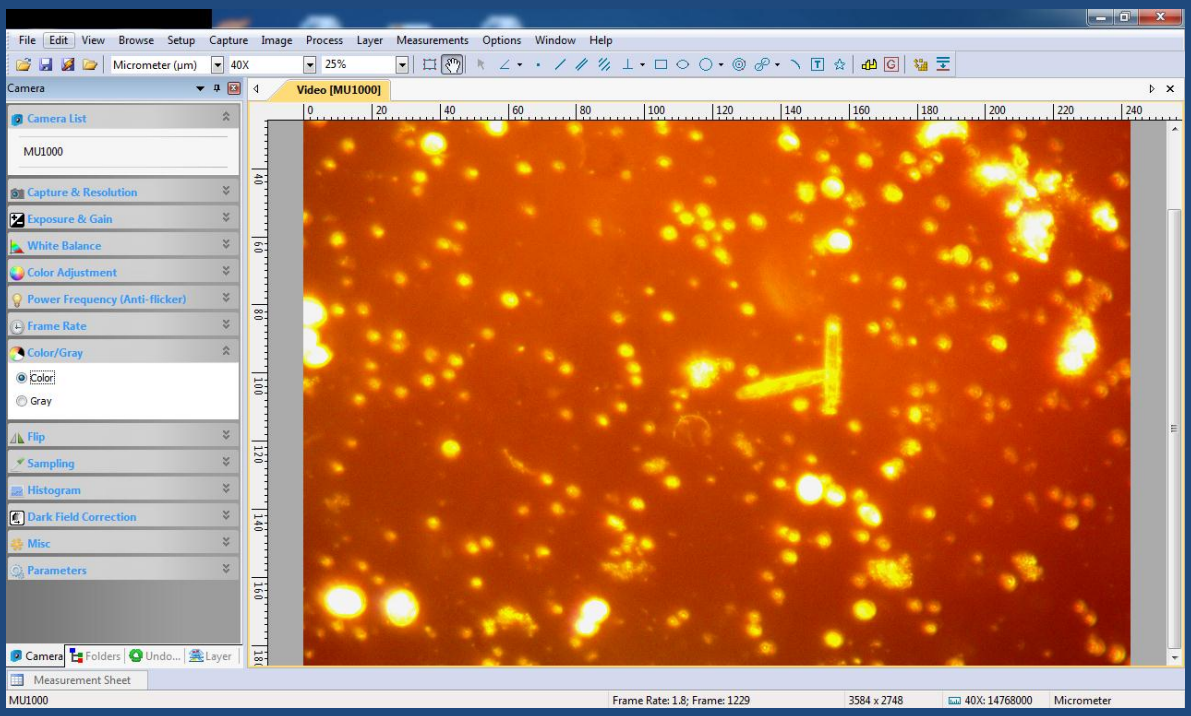


13. White balance application plus maximum contrast

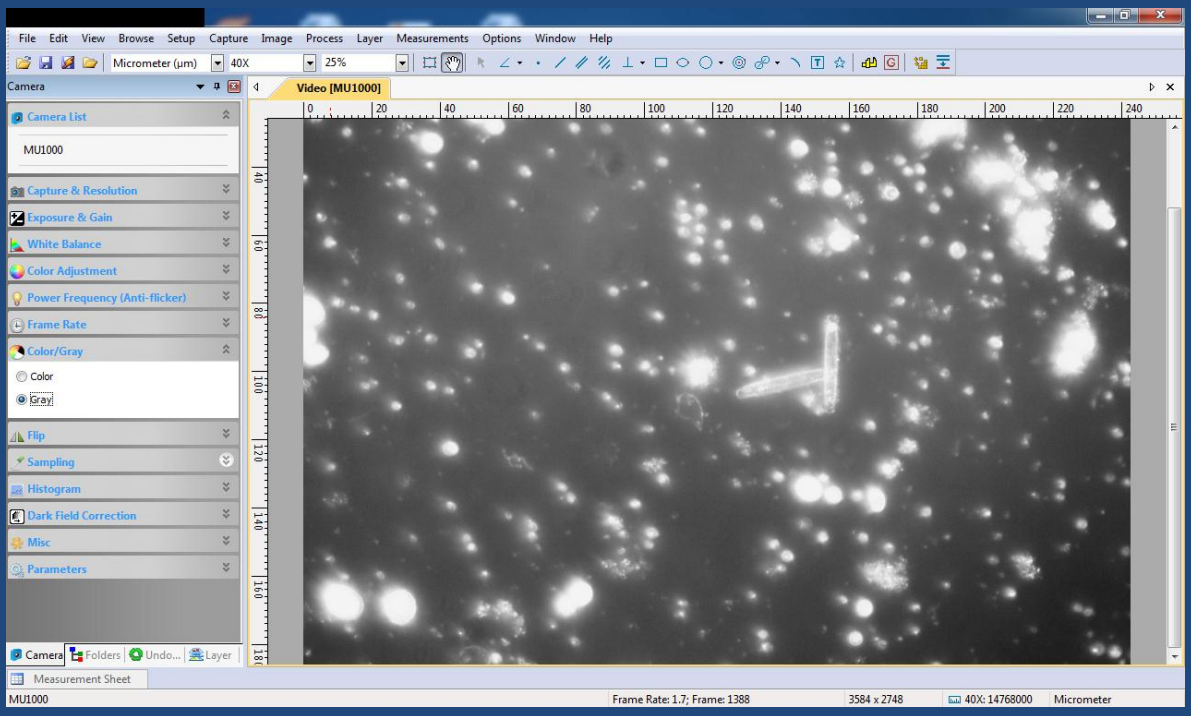


14. White balance application plus darkfield correction.

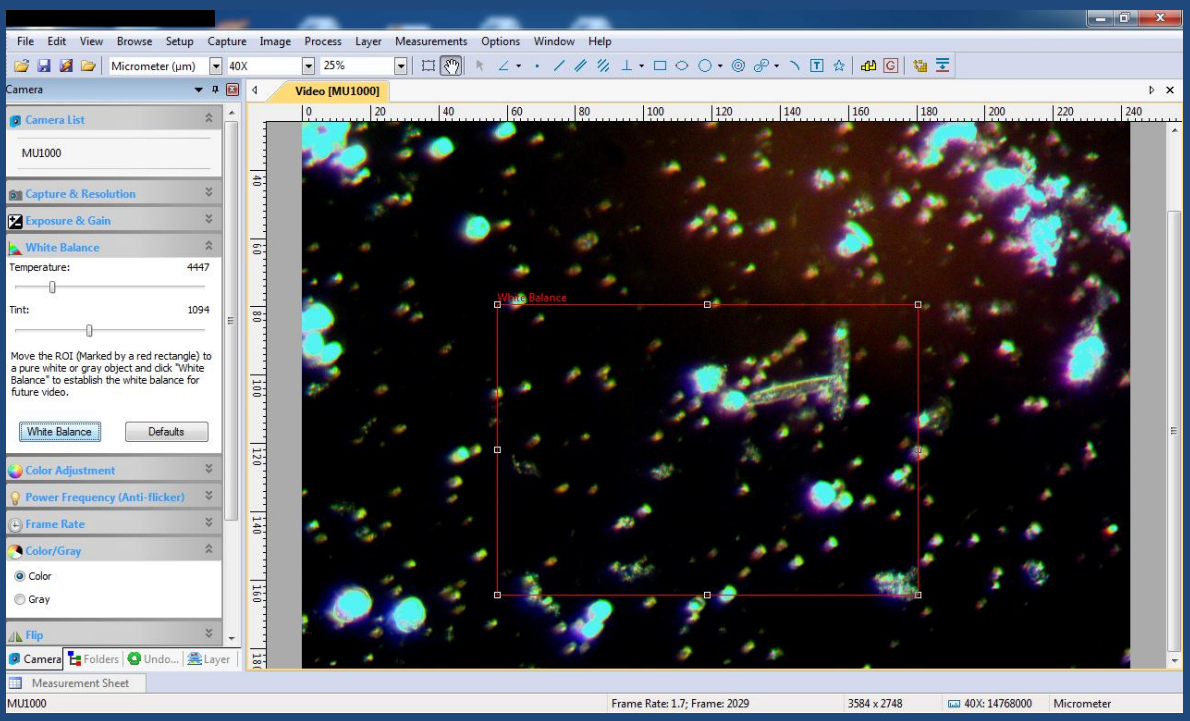
- NOW WE HAVE BELOW THE COMBINATION OF THE CAMERA EFFECTS WITH THE DARKFIELD CONDENSER



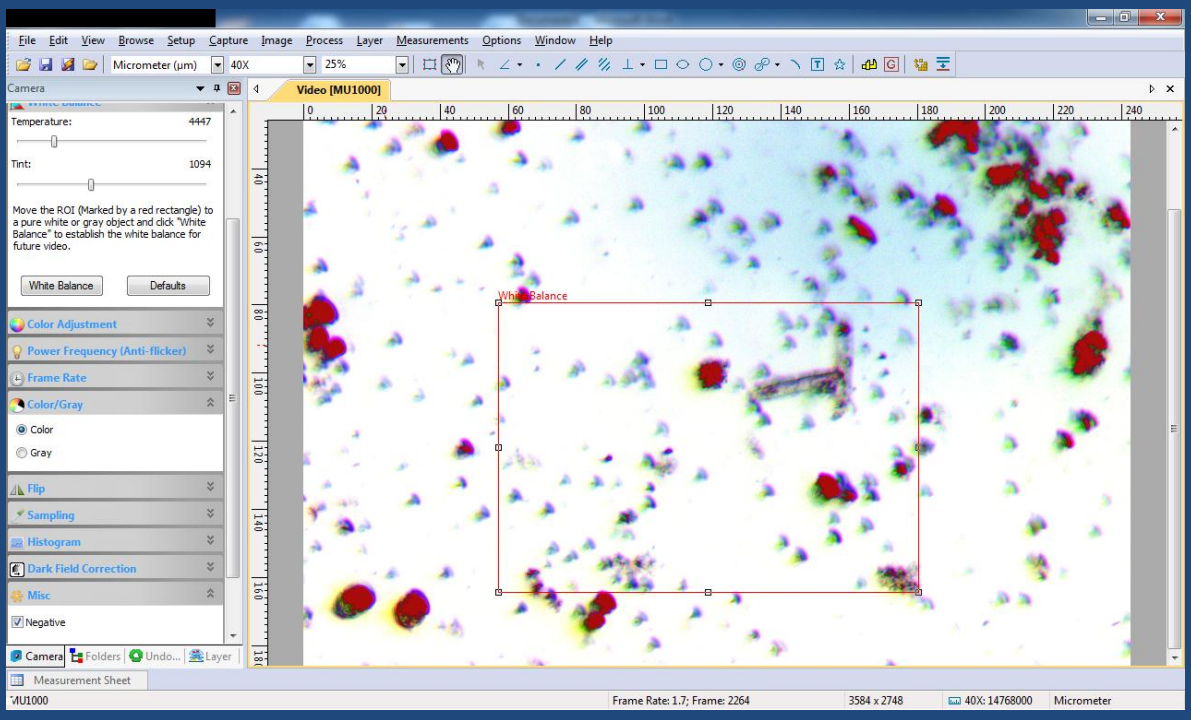
15. White balance defaults plus color scale



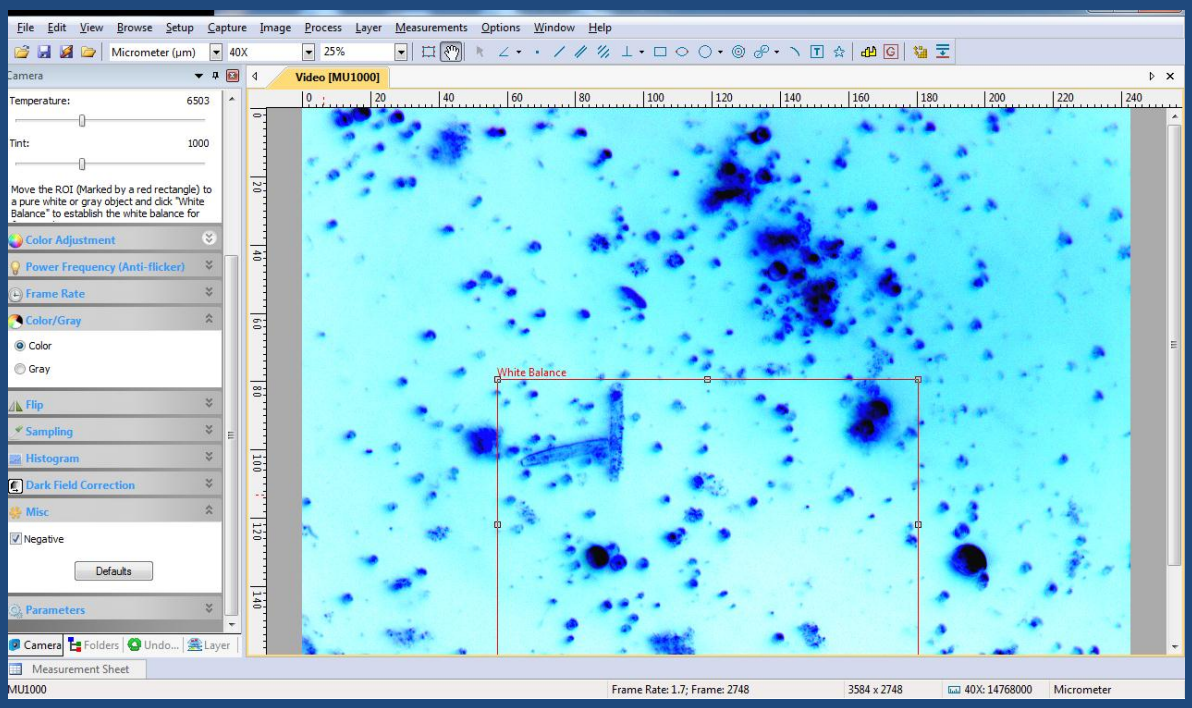
16.White balance defaults plus gray scale



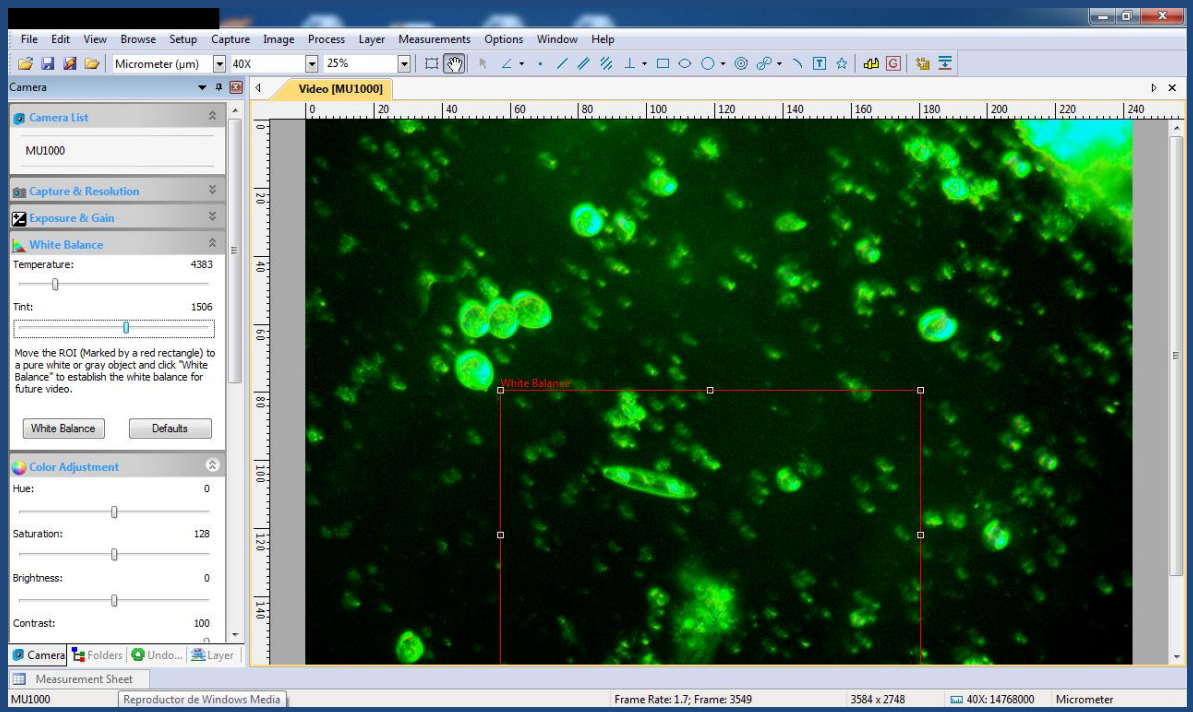
17. White balance application plus color scale



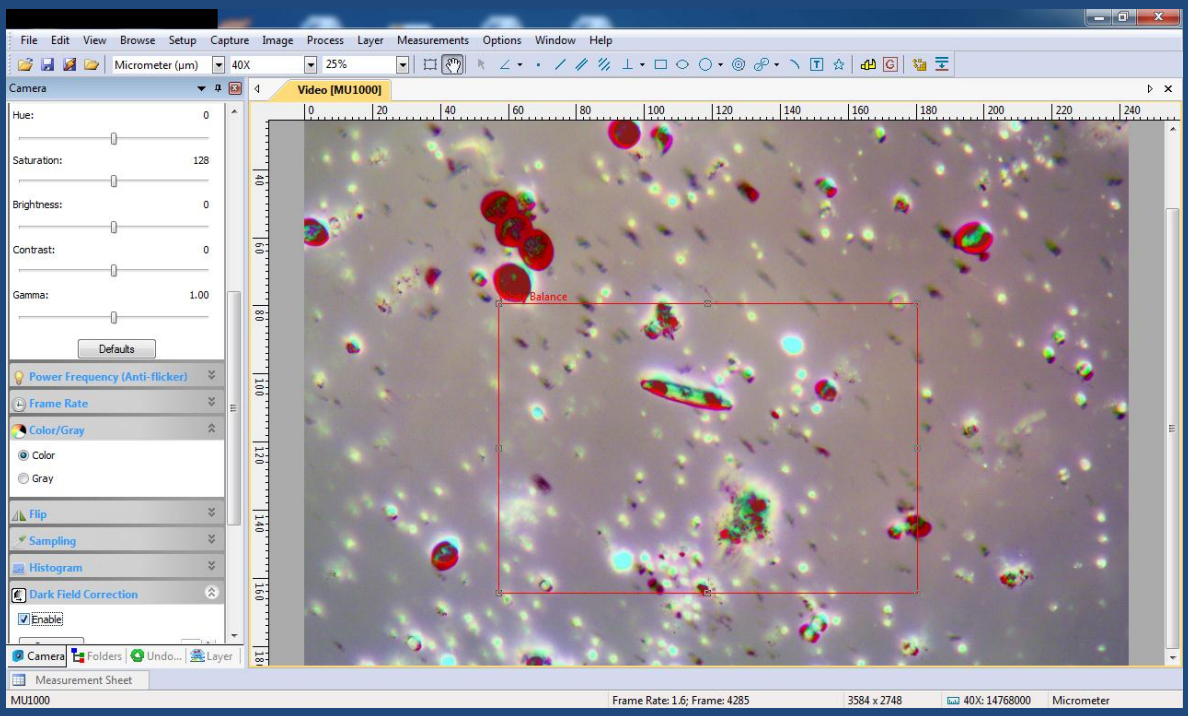
18. White balance application plus color scale plus negative effect



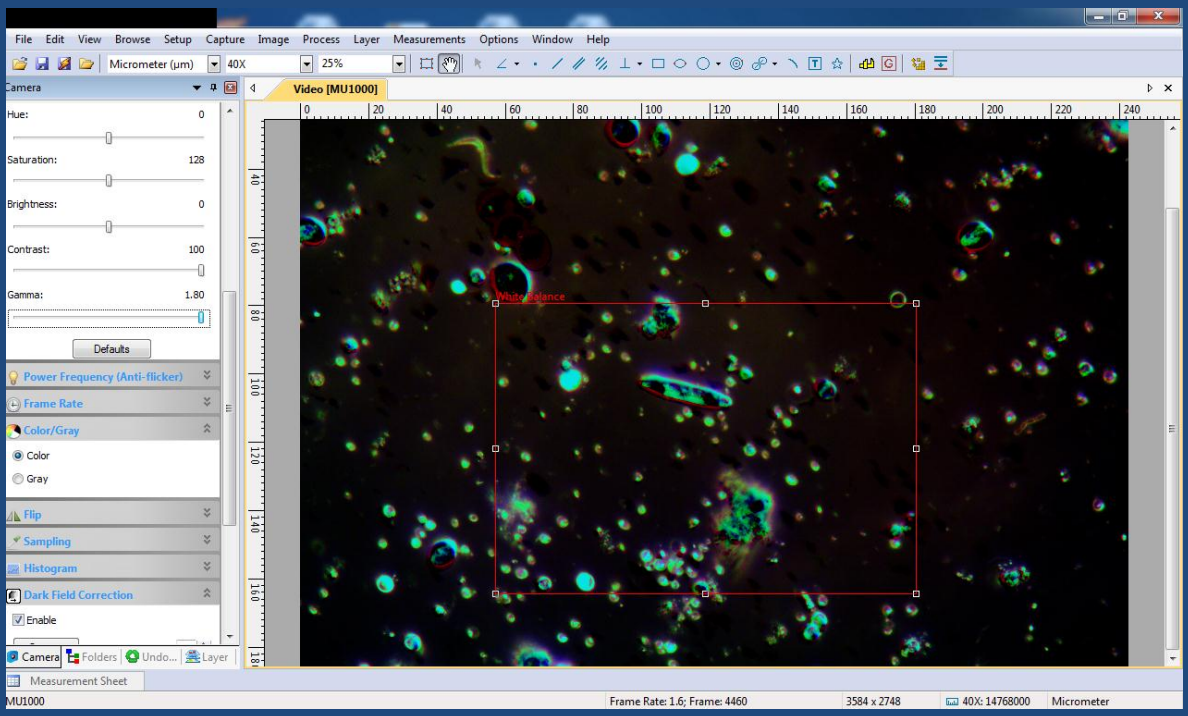
19.White balance defaults plus color scale plus negative



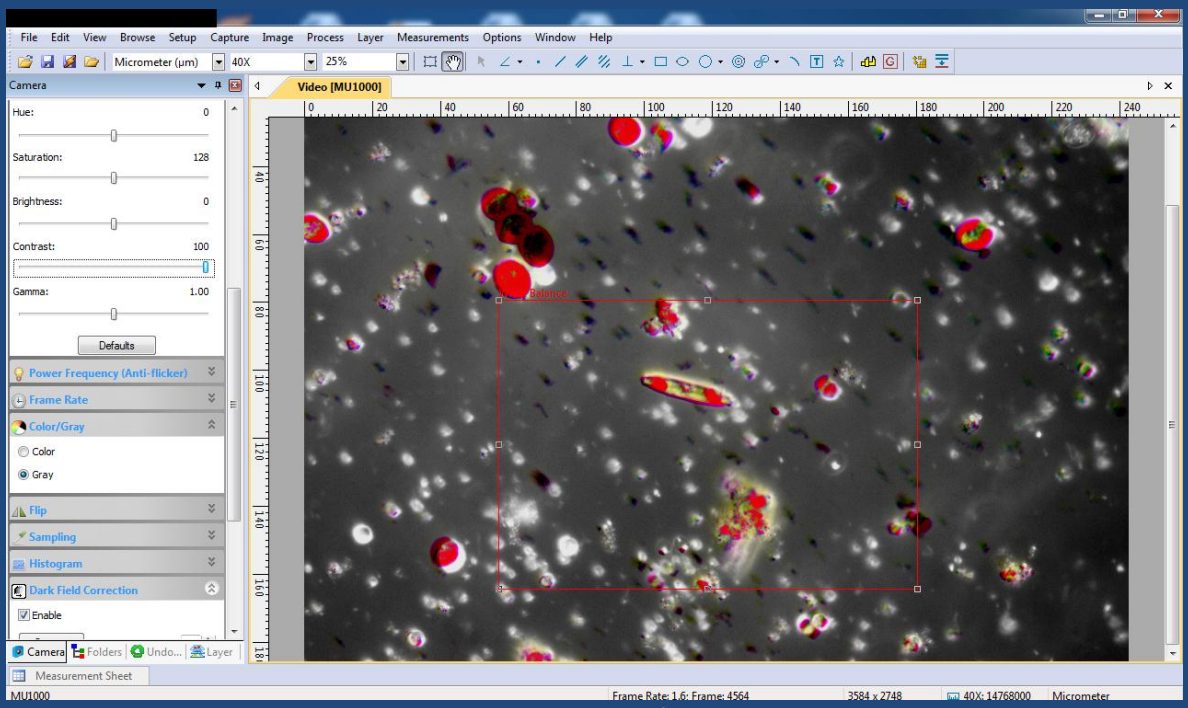
20. White balance application plus tint to the right



21. White balance application plus darkfield correction plus color scale



22. White balance application plus darkfield correction plus color scale contrast and gamma maximum values



23. White balance application plus gray scale plus darkfield correction.

CONCLUSION :

We live in a modern era, we have lots of advantages today, we cannot only record our observations with the help of our cameras, we can give them color, and obviously the colors and the possibilities will vary with every kind of sample. In this article I used diatoms and algae.

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(Above in anti-spam format. Copy string to email software, remove spaces and manually insert the capitalised characters.)

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