

## OPTICAL STAINING PART 3 Rheinberg illumination DIY-FILTERS

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### INTRODUCTION:

As presented in previous articles of this series, improving contrast in a microscope sample is to some extent easy and does not necessarily require stains or inks to be used. It can be achieved by using optical methods such as camera features, darkfield illumination condensers or, as in the case of the present article, a method that not only enhances the sample but it also adds color to the background and the object, I refer to RHEINBERG ILLUMINATION.

### Development:

Named after Julius Rheinberg who first described it in 1896, Rheinberg illumination is a kind of DARKFIELD with the same principle of preventing the entrance of light directly to the sample with a stop. Nevertheless with the difference that this time the stop is not opaque as in the case of DARKFIELD but it now has color and the stop is translucent. The sizes of the stops can vary, the only requirement is that it must be big enough to surpass the numerical aperture of the objective.

The simplest Rheinberg filter is made of a color stop, this will produce a colored background illuminated with the color of the stop; so a blue one will give you:

Then when you place the sample in the field, the light that passes around it will be diffracted by the object producing the shape of the object in the sample which will appear in yellow or white depending on the type of illumination you have in your microscope, i.e. halogen or led

the same principle as in darkfield.

Another form of doing Rheinberg is combining two techniques by using both colored stop and a colored ring "annulus"

so the sample will have a background illuminated by the color of the stop and the object in the sample illuminated by the annulus.

Exactly the same as darkfield illumination, it is outstanding isn't it?

Rheinberg filters originally were made of colored gelatin or glass filters, nevertheless for most of the time they are difficult to get. For that reason many authors of enthusiast microscopy have shown how to make Rheinberg filters on a DIY basis and there are several ways of producing them. Here I will show how I created mine.

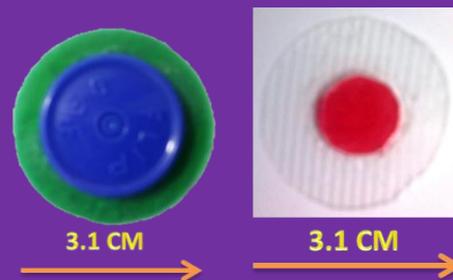
I employed two main techniques, first of all collecting any colored TRANSLUCENT plastic pieces that I could find; mainly I got the stops from injectable medication covers

and transparent colored covers that are used to bind a book or school homework,

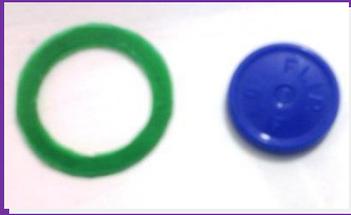
I also used plastic from a sample container cap for this stop and from a lipstick for dry lips for the one here

And the rings just from transparent colored covers for books.

All the rings used and the transparent holders of the color filters were of 3.1 cm which were made according to the size of the colored filters provided by the microscope manufacturer so that they fit perfectly in the filter tray of the brightfield condenser.



You can make the filters detachable



or permanently glued.

Rheinberg illumination is for most of the time limited to low and medium magnification powers 4x, 10x, so when you see through the ocular of the microscope no matter, the effect is always the wanted one for each combination. The problem comes when it is exposed to the camera, for achieving the expected colors you have to keep the camera in WHITE BALANCE DEFAULTS. If you apply white balance you will get a colored sample but with different tonalities. For getting the best result I invite you to check the article [DARKFIELD ILLUMINATION AND THE DIGITAL CAMERA BY HOWARD WEBB, MISSOURI, USA on MICSCAPE.](#)

As Rheinberg illumination follows the same principle as DARKFIELD then the same principles apply.

Other methods to obtain the best result is by decreasing the intensity using the blue filter, especially if it is an halogen bulb like mine. As I said in the article about darkfield published in June this year, if you don't have the blue filter which most of the manufacturers provide with the microscope at the moment of purchasing,



you can create one using some light blue plastic or light blue cellophane.



Another detail to be taken into account is that when exposed to the camera some colors, once you take the photo, appear dark, which could cause some disappointment. One thing that has worked for me in a very good way is to use the program MICROSOFT PICTURE MANAGER 2010, this program as I have described in other articles of this series works like "turning on" the light to the picture with the advantage of keeping the colors, only giving illumination, it seems as if you have used a flash at the moment of taking the photo.



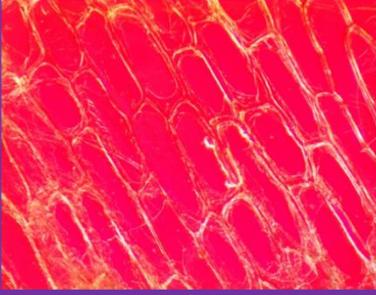
Now with the effect of MICROSOFT PICTURE MANAGER 2010, it is clearer.



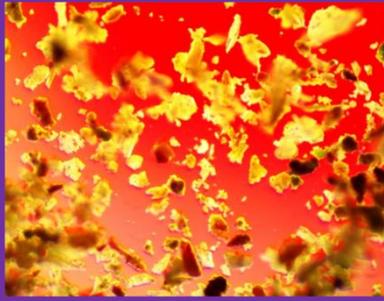
Results:

In order to see Rheinberg illumination effects I group them "by filter",

First I place the photos taken with just one colored stop on the center of a piece of transparent plastic.



**ONION CELLS 10X**

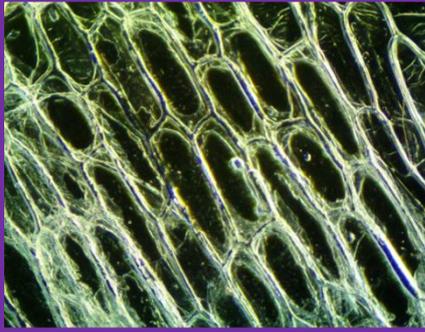


Peppermint powder 10x



**COFFEE POWDER 10X**

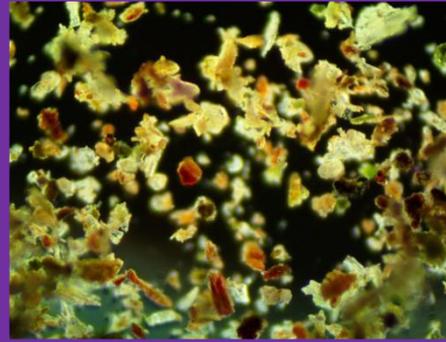




**ONION CELLS 10X**



**DAPHNIA 4X**



Peppermint powder 10x



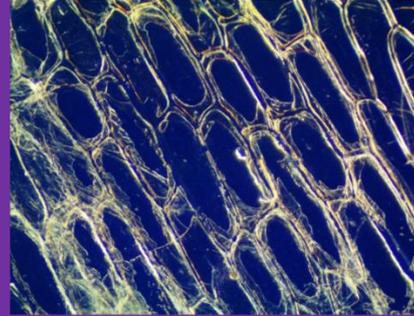
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**SUGAR 4X**



**DAPHNIA 4X**



**ONION CELLS 10X**



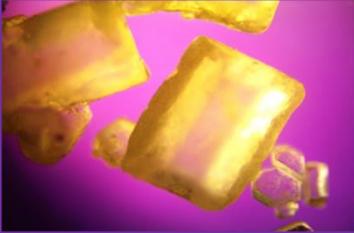
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**COFFEE POWDER 10X**



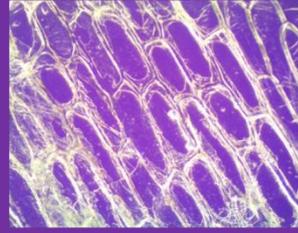
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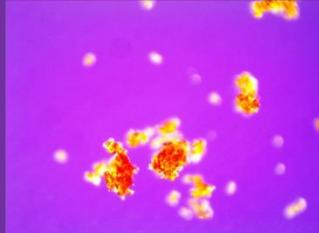
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**ONION CELLS 10X**



**COTTON FIBERS 10X**

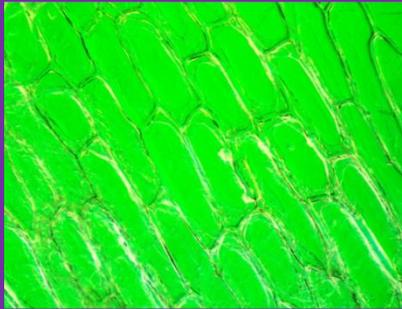


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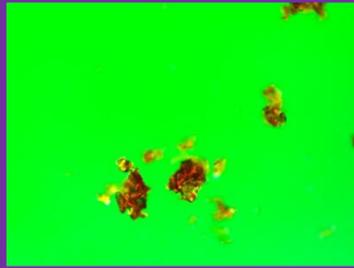


**DIATOM 10X**

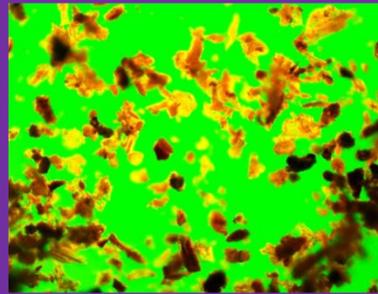




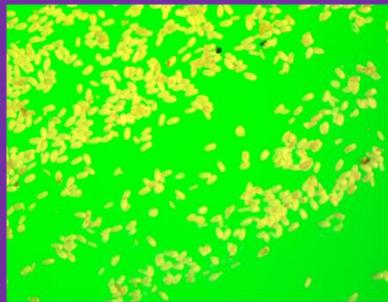
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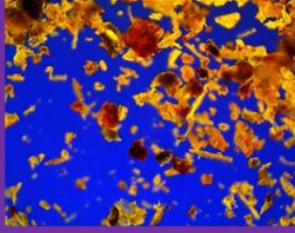


Peppermint powder 10x



**POLLEN OF SPATHIPHYLLUM WALLISII 10X**

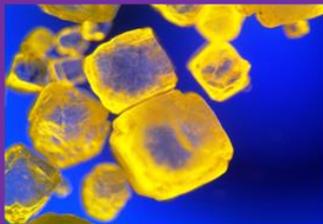
Here we have the combination of two different colors one in the center ; it is called a stop and one on the periphery is called the annulus ring.



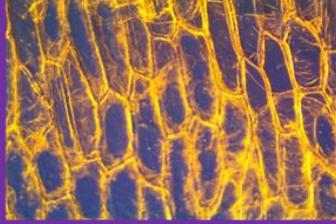
Peppermint powder 10x



DIATOM 10X



SALT CRYSTALS 4X



ONION CELLS 10X



MOSQUITO 4X



POLLEN OF SPATHIPHYLLUM WALLISII



DAPHNIA 4X

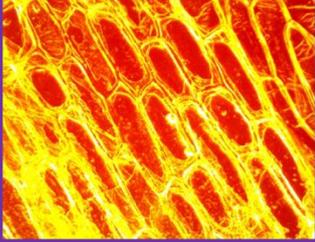


LILY POLLEN 10X

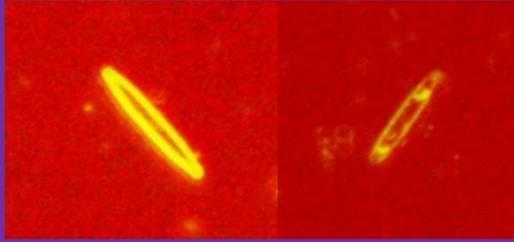


COTTON FIBERS 10X

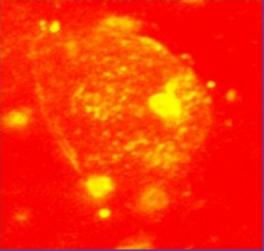




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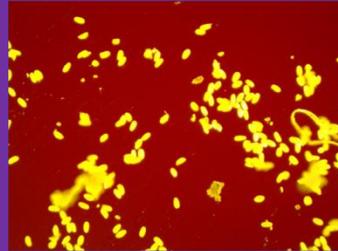
DIATOMS 10X



MOUTH EPITHELIAL  
CELLS 10X



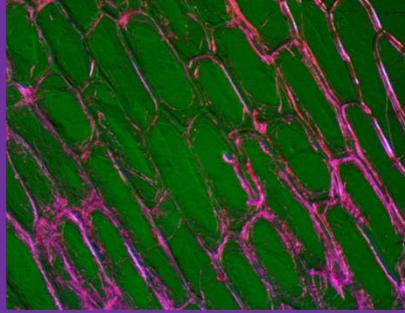
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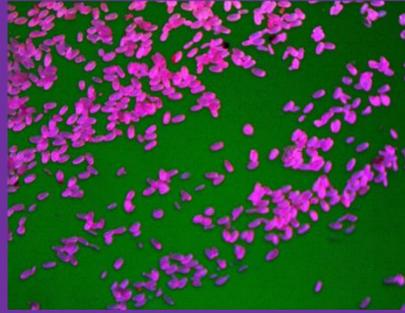
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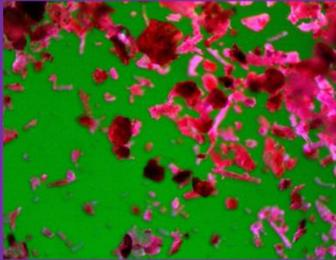
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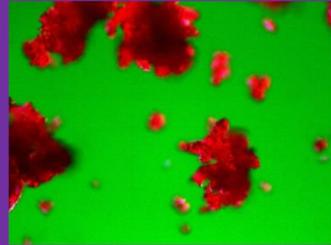
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**POLLEN OF SPATHIPHYLLUM WALLISII**



**Peppermint powder 10x**

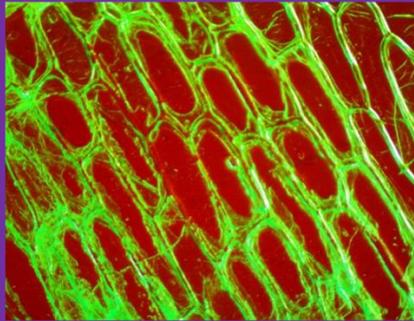


**COFFEE POWDER 10X**

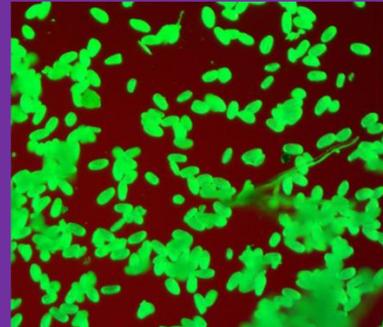




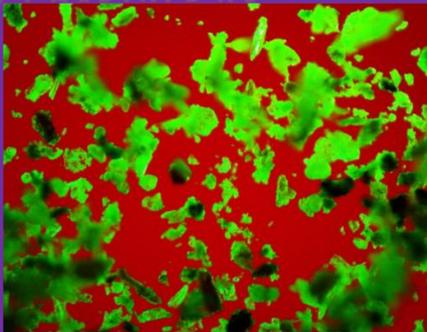
**SALT CRYSTALS 4X**



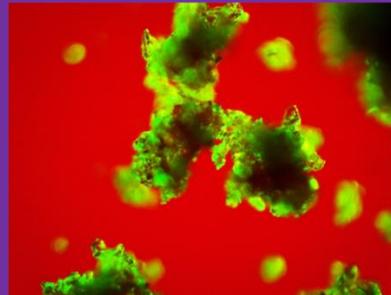
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POLLEN OF SPATHIPHYLLUM WALLISII



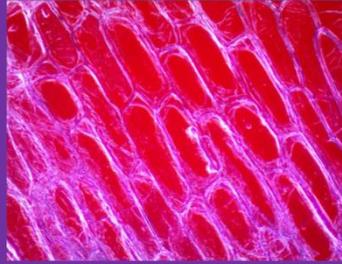
Peppermint powder  
10x



**COFFEE POWDER 10X**



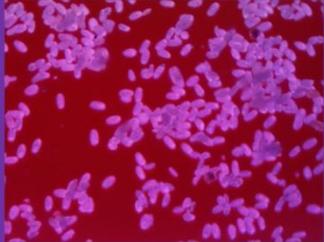
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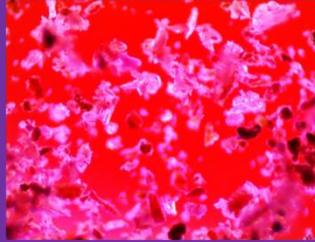
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**SALT CRYSTALS 4X**



**POLLEN OF SPATHIPHYLLUM WALLISII 10X**

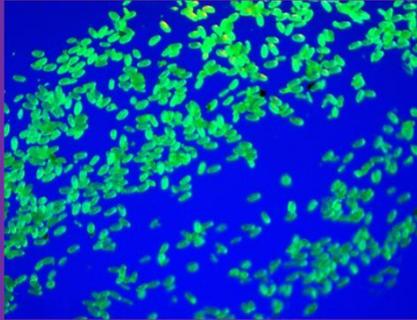


**Peppermint powder  
10x**



**COFFEE POWDER 10X**

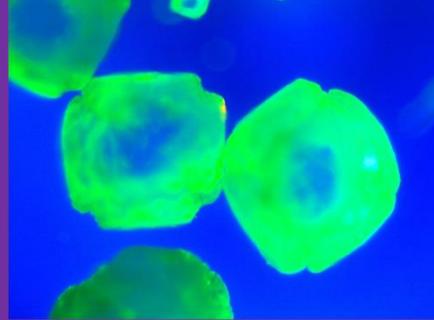




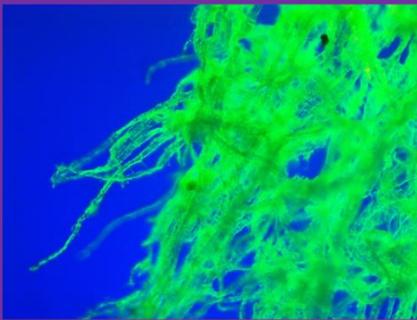
POLLEN OF SPATHIPHYLLUM WALLISII 10X



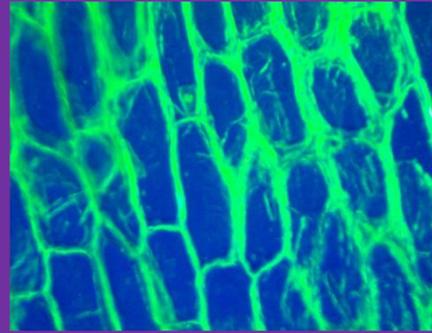
DAPHNIA 4X



SALT CRYSTALS 4X



BATHROOM TISSUE 10x



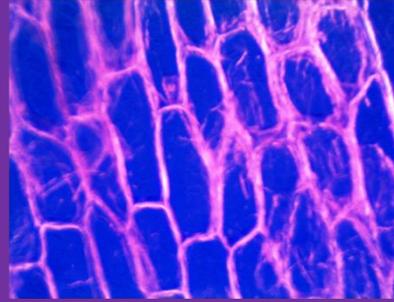
ONION CELLS 10X



peppermint powder 10x



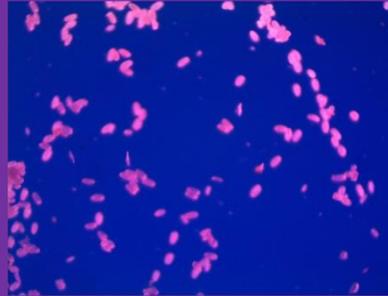
**DAPHNIA 4X**



**ONION CELLS 10X**



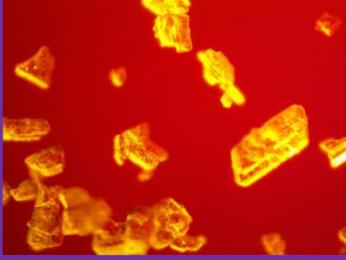
BATHROOM TISSUE 10x



POLLEN OF SPATHIPHYLLUM WALLISII 10X



**SALT CRYSTALS 4X**



Sodium bicarbonate  
crystals 10x

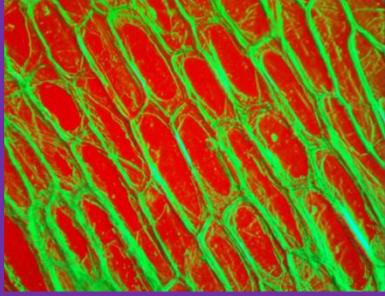


peppermint powder 10x

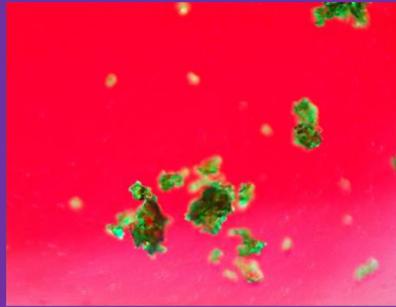


**COFFEE POWDER 10X**

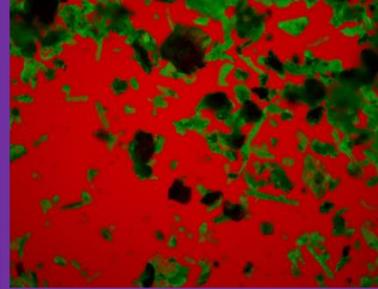




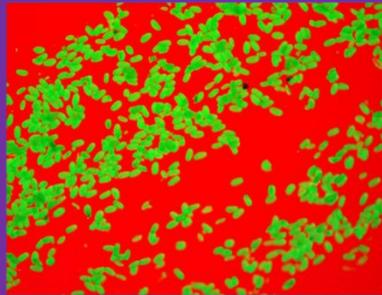
**ONION CELLS 10X**



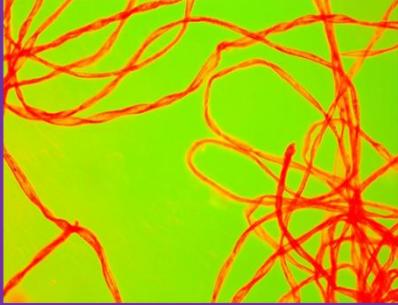
**COFFEE POWDER 10X**



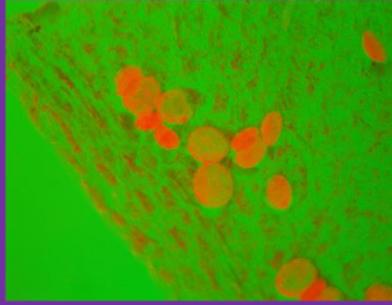
Peppermint powder 10x



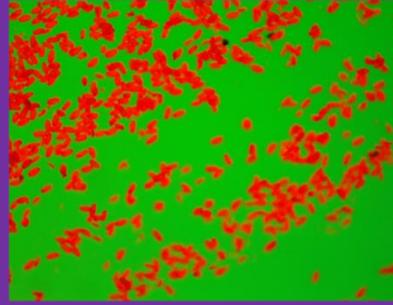
**POLLEN OF SPATHIPHYLLUM WALLISII 10X**



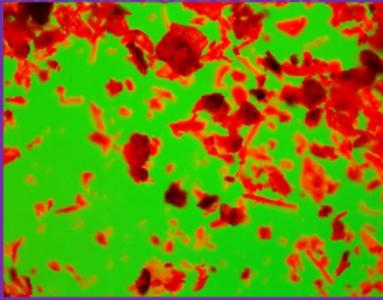
**COTTON FIBERS 10X**



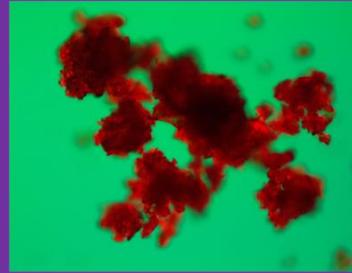
Pollen of a yellow lily upon a petal  
10x



**POLLEN OF SPATHIPHYLLUM WALLISII 10X**



peppermint powder 10x



**COFFEE POWDER 10X**

#### Conclusion:

Rheinberg illumination technique is a form of OPTICAL STAINING. It is as marvelous as darkfield and very easy to achieve since everywhere it is possible to get colored plastic. Combining colors to see what gives the best result is a matter of trial and error. There are no rules, so again only our imagination and curiosity are the limits. I invite you if you have not tried Rheinberg illumination before to make your own filters placed one by one on the filter tray of your microscope. Focus the condenser and get a very funny and rewarding experience with this kind of DARKFIELD.

#### References:

- [www.microscopy-uk.org.uk/mag/artsep00/chillum.html](http://www.microscopy-uk.org.uk/mag/artsep00/chillum.html)
- <http://micro.magnet.fsu.edu/primer/techniques/rheinberg.html>
- <http://www.quekett.org/resources/making-rheinberg-illumination-discs>
- <http://microscopy.berkeley.edu/courses/tlm/df/rheinberg.html>
- <http://www.microscopy-uk.org.uk/mag/artapr02/contrast.html>

