EPI-RHEINBERG

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INTRODUCTION:

Rheinberg illumination was named after Julius Rheinberg who discovered this property of color filters to add contrast to microscopy observations. It consists of placing between the light source and the sample, color filters typically made of Wratten gelatin or glass. It may be just one color adding to the sample the color of the filter but it can also be bi- (or multi-) colored: I mean that it has two (or more) parts, one central which is the part called a stop that is going to illuminate the background and a peripheral part called a ring that is going to illuminate the contents of the sample. This follows the exact principle as in DARKFIELD illumination but with color.

In August 2015 I had the privilege of presenting in MICSCAPE magazine an article analyzing Rheinberg illumination that was called OPTICAL STAINING PART 3 Rheinberg illumination DIY FILTERS in which I showed how easy it was to create Rheinberg illumination out of things around home by making by myself the filters. On that occasion I used mainly pieces of those plastic covers that are employed at school to bind books and that are transparent, obtaining very good results for transmitted light with transparent samples. But what about if the sample is thick and it is not possible to illuminate with transmitted light? Is it possible to color it optically? The answer is YES, IT IS POSSIBLE and with very good results too, see below.

For the fact mentioned previously that the sample in this case is thick and the does not allow to pass light through it at least not visible light, so it must be illuminated from above making this way reflected or epi- illumination.

Last April 2016 I achieved epi –illumination with a brightfield background and a darkfield one with my conventional microscope by creating on a DIY basis a LED illuminator which was held to the objectives with some elastic and some insulating plastic wires and powered by a pair of AA batteries.

All this mentioned above has inspired me to join these two topics and make EPI-RHEINBERG ILLUMINATION, taking for granted that Rheinberg means adding color to a sample and epi-illumination means giving light to the sample from above, I have obtained very good results.

DEVELOPMENT:

For creating epi-Rheinberg illumination I used the LED illuminator that I had designed for the articles mention above

One for the 4x objective and one for the 10x objective that are the ones that allow a user to easily place the illuminator because of the working distance below them.

And as filters I used small pieces cut with a paper punch from the same covers that I had employed for transmitted Rheinberg.

I also used small pieces of those covers that come with the needles of syringes.

I cut in the narrower part so that it may fit the LED of the illuminator and I glued the small pieces of color plastic in front of these so that the light of the LEDs were projected with color upon the sample in this way:

Placed on the microscope they looked like this:

I tried to use color LEDS but the results were not good enough to consider them because of the intensity of the light projected was very weak to illuminate the sample from above.

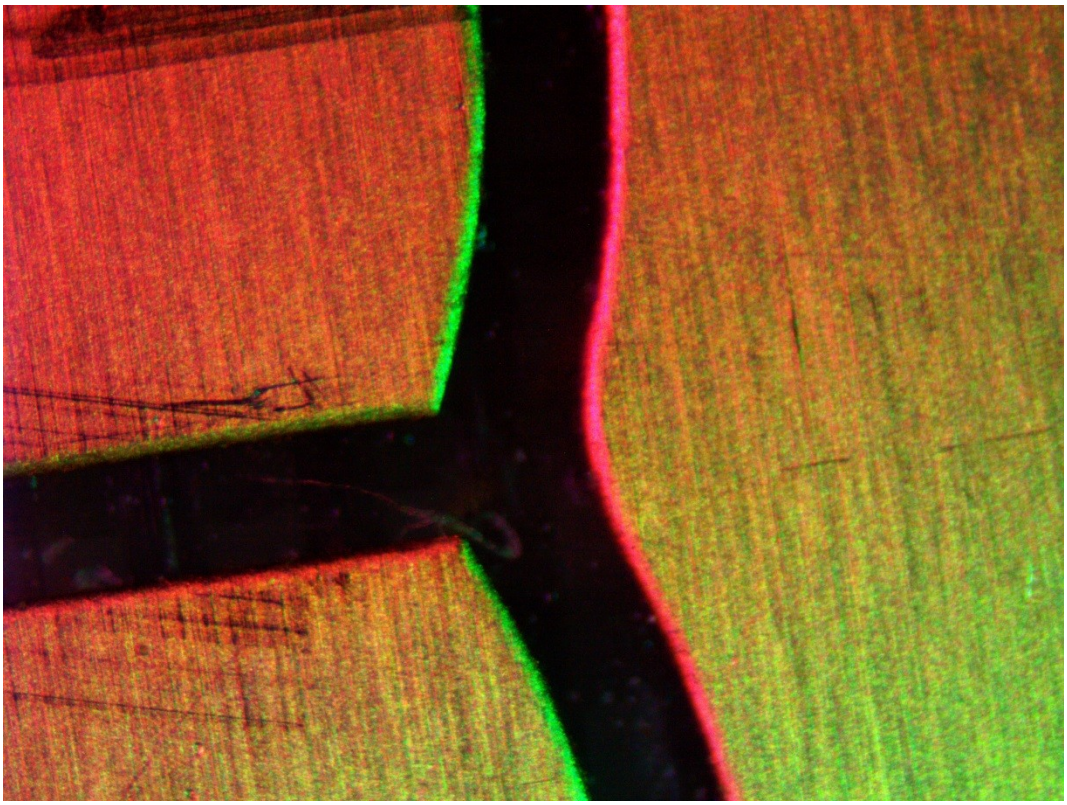
I also tried small bicolored circles in front of the LEDS but the problem was that when projecting the colors upon the samples they illuminated “big areas” of the samples so the objective “saw” just the part illuminated by one of the colors. Probably it is Rheinberg because it added color to the sample, nevertheless it is simple.

So the best form to illuminate a sample from above with color is using the bright white LEDs covered with the filters made as described above.

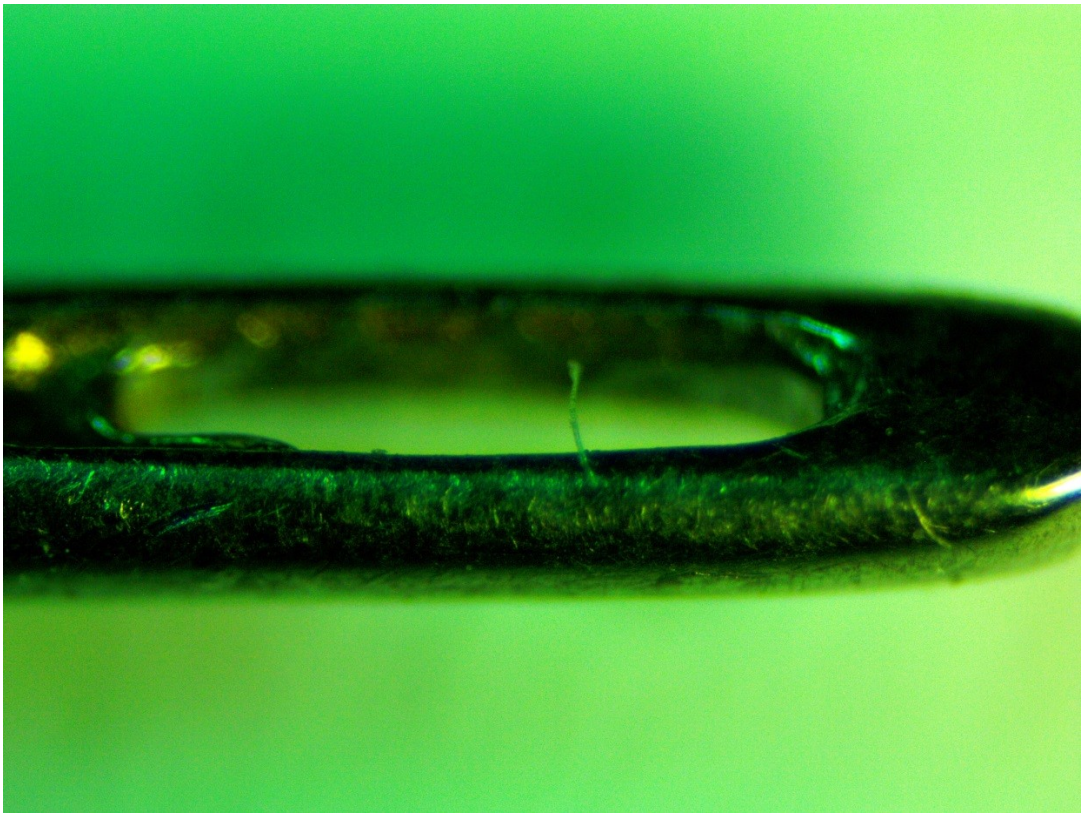
Two of the filters are taken and place one on each LED of the illuminator so that both colors are projected upon the sample, SEE RESULTS.

RESULTS:

First with thick samples:



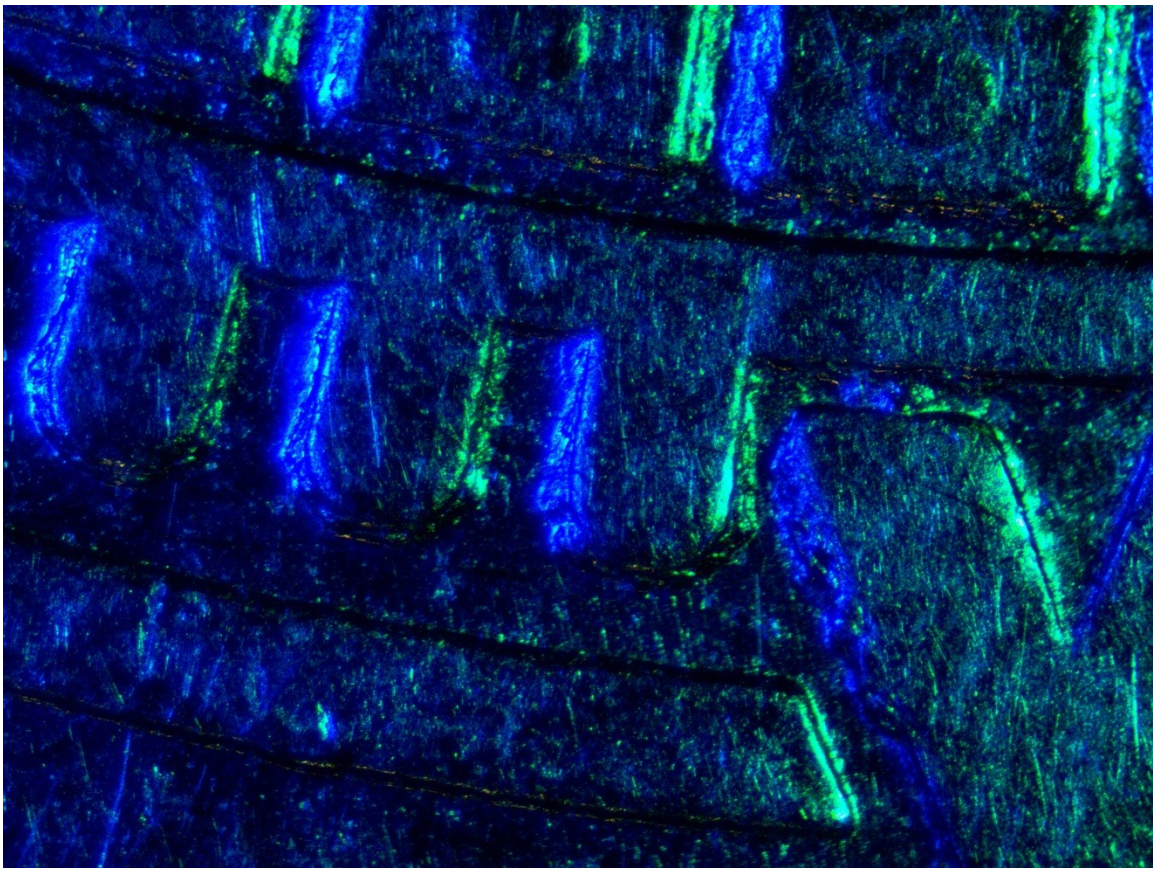
Part of a phone SIM card 4x illuminated with red and green filters.



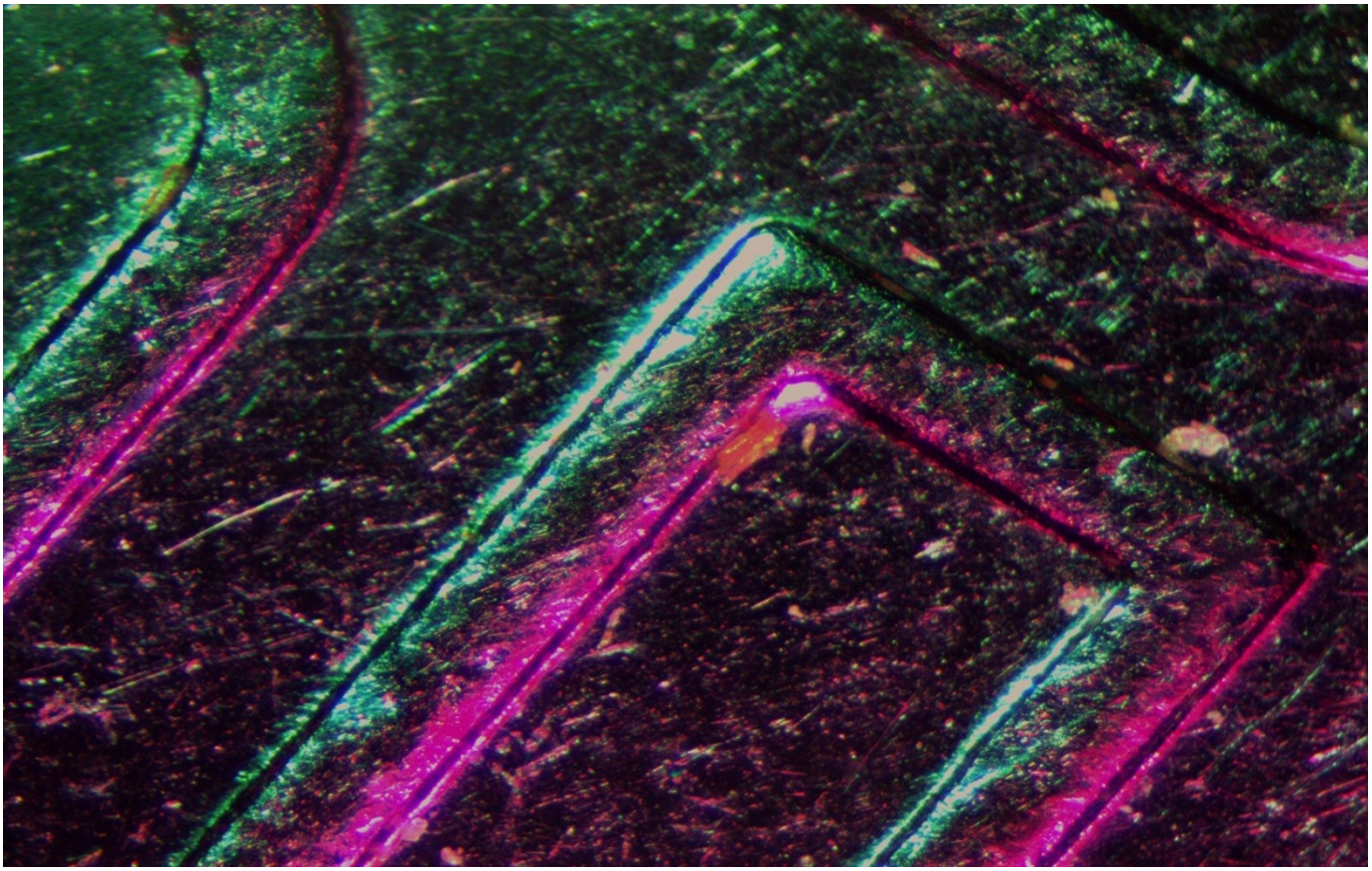
Needle eye upon a clear background illuminated with yellow and green filters.

Year of coining of a Mexican one peso coin 4x red and green filters.

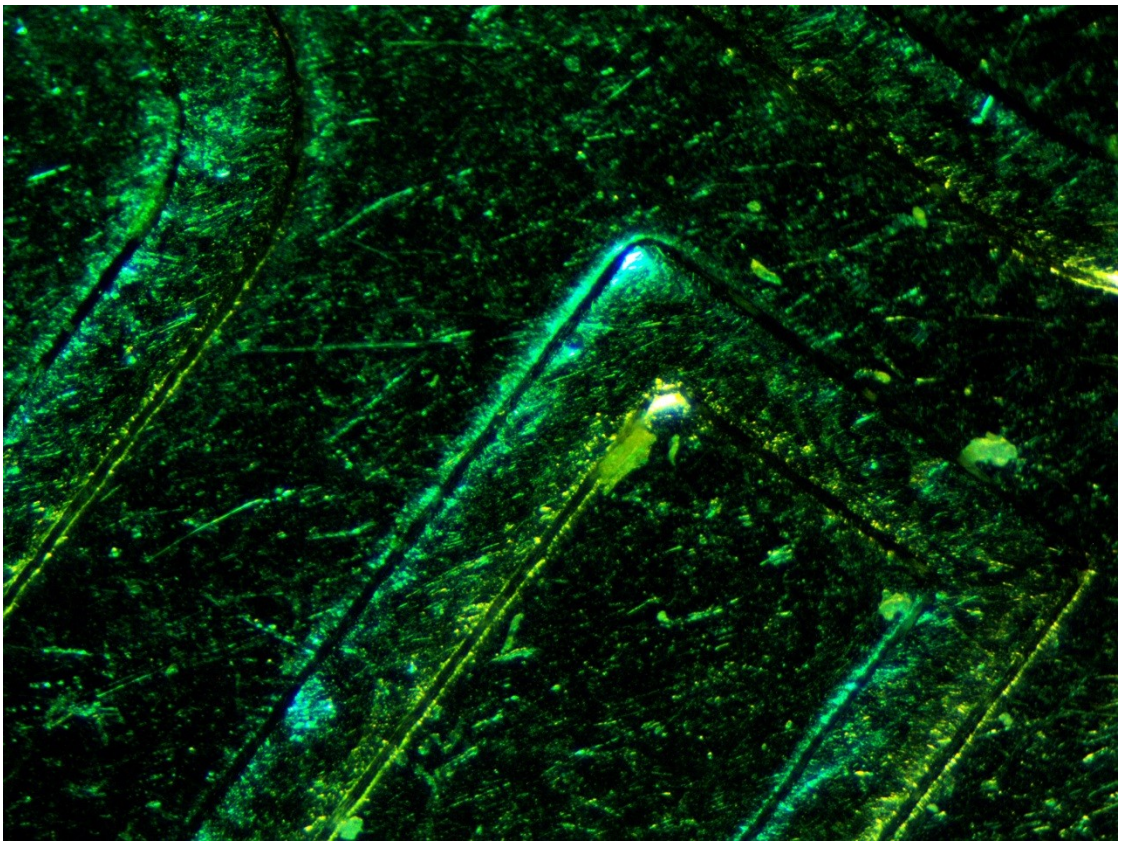
Year of coining of a Mexican one peso coin 4x purple and green filters.



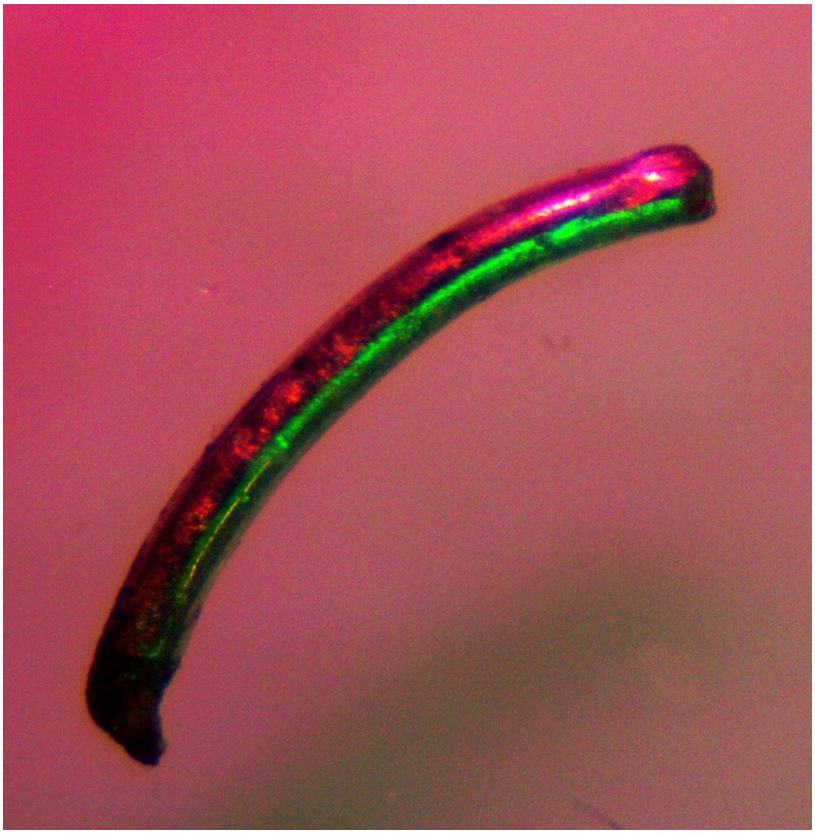
Part of a Mexican one peso coin, 4x blue and green filters.



Part of a Mexican 50 cent coin illuminated with red and green filters

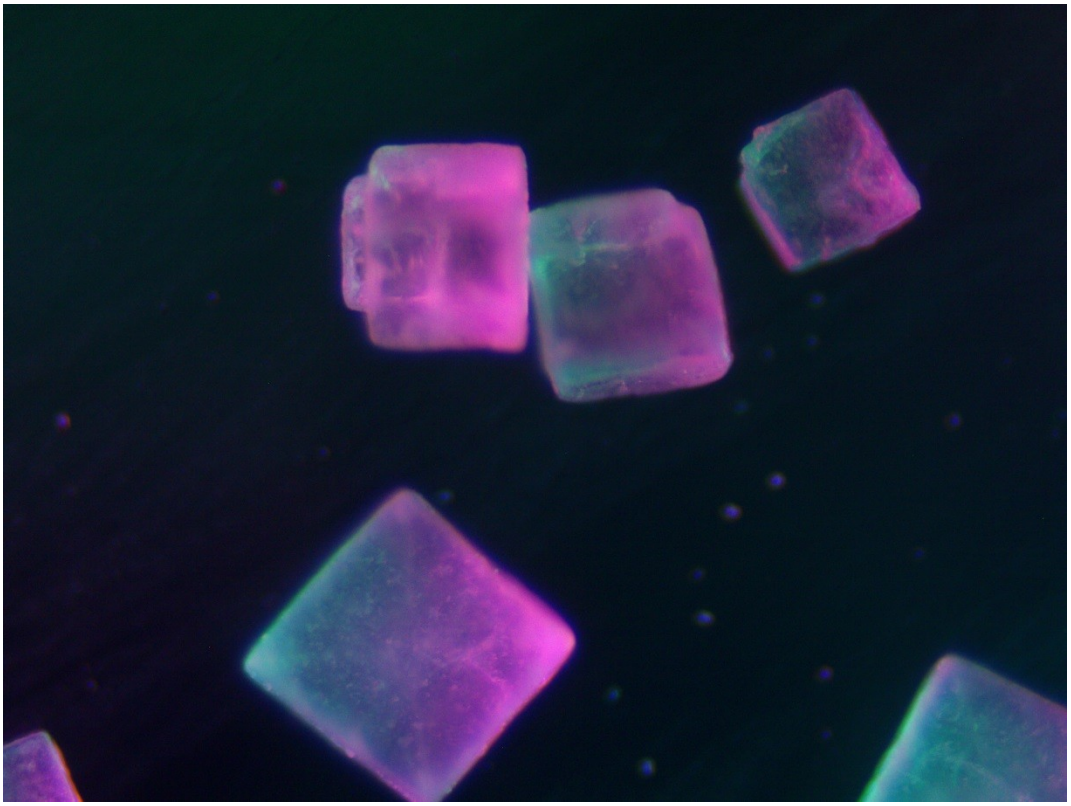


Part of a Mexican 50 cent coin illuminated with yellow and green filters.

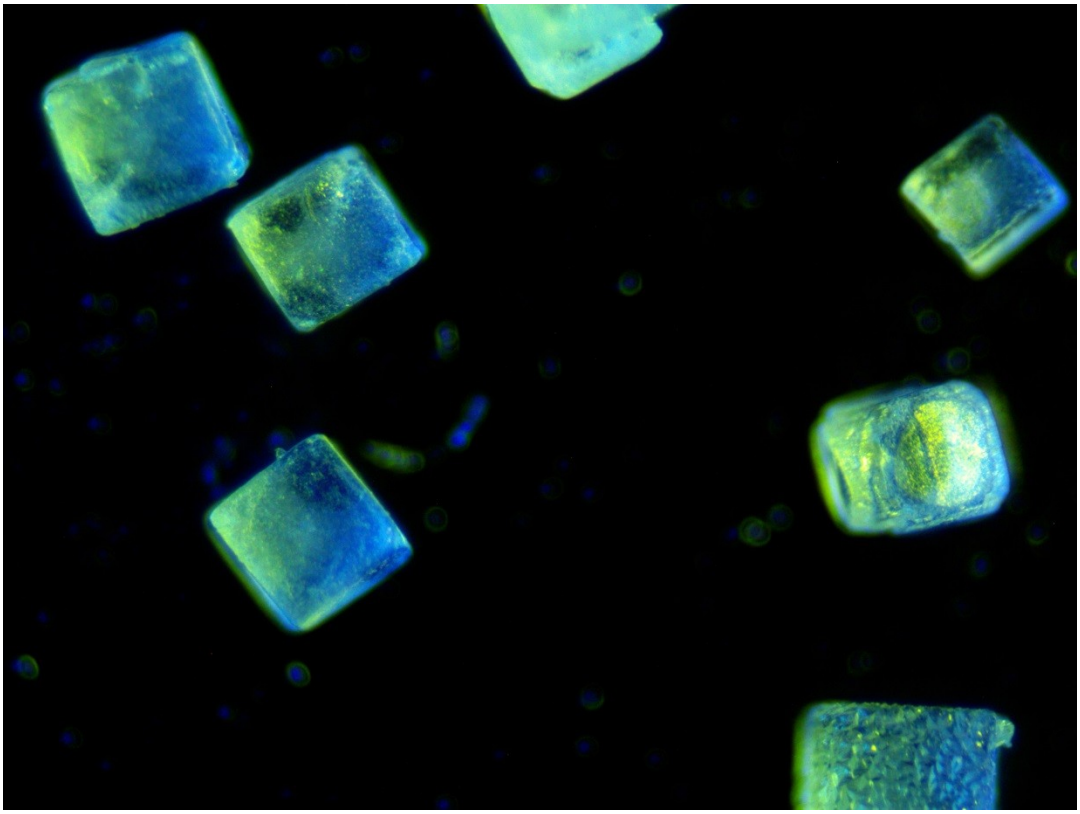


A bit of copper wire upon a clear background, 4x red and green filters.

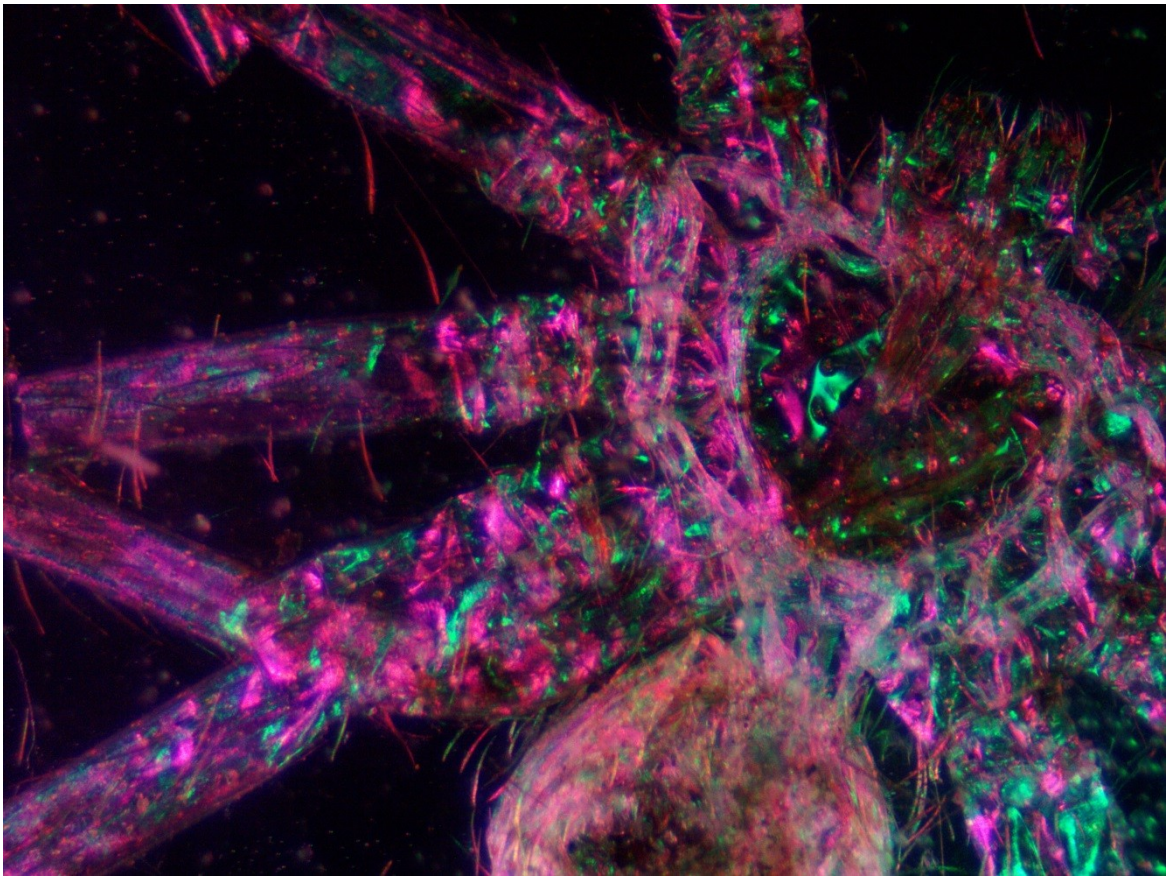
Now with transparent samples:



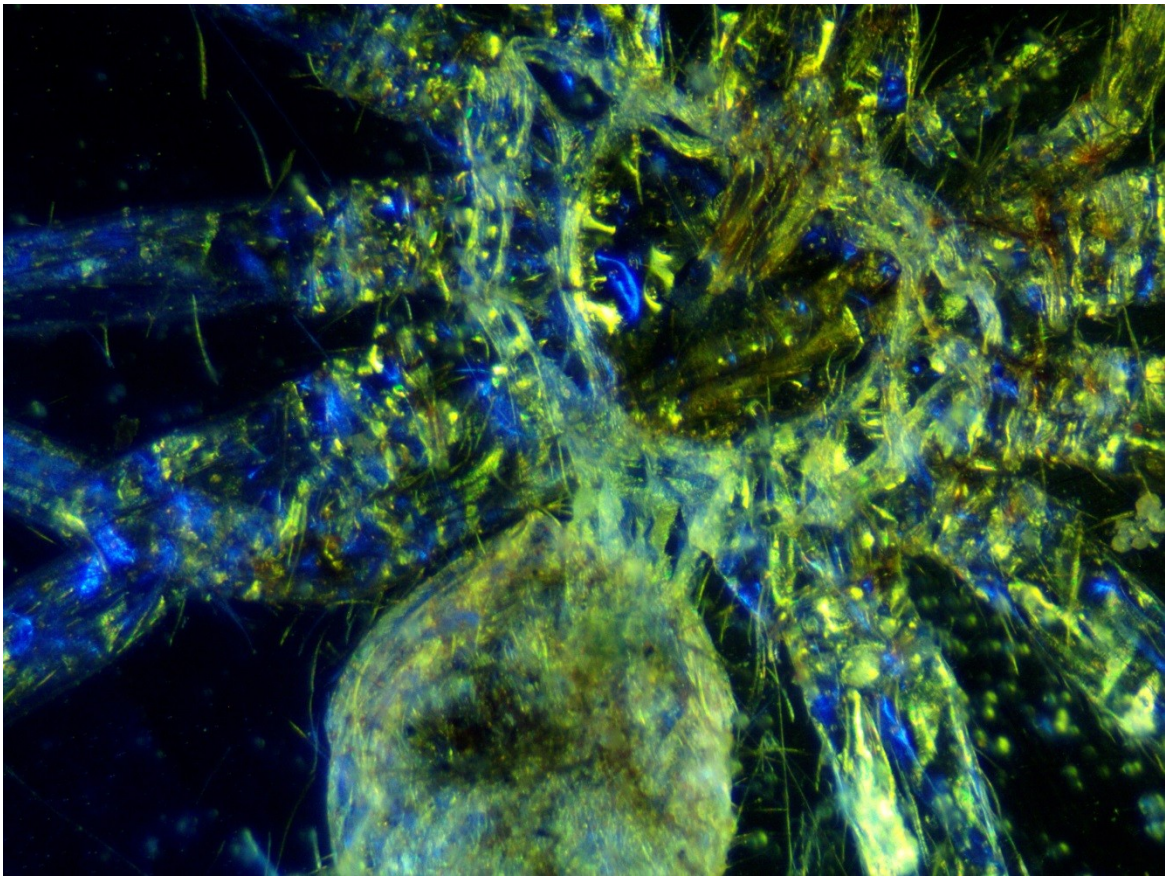
Salt crystals upon a black background 4x illuminated with red and green filters.



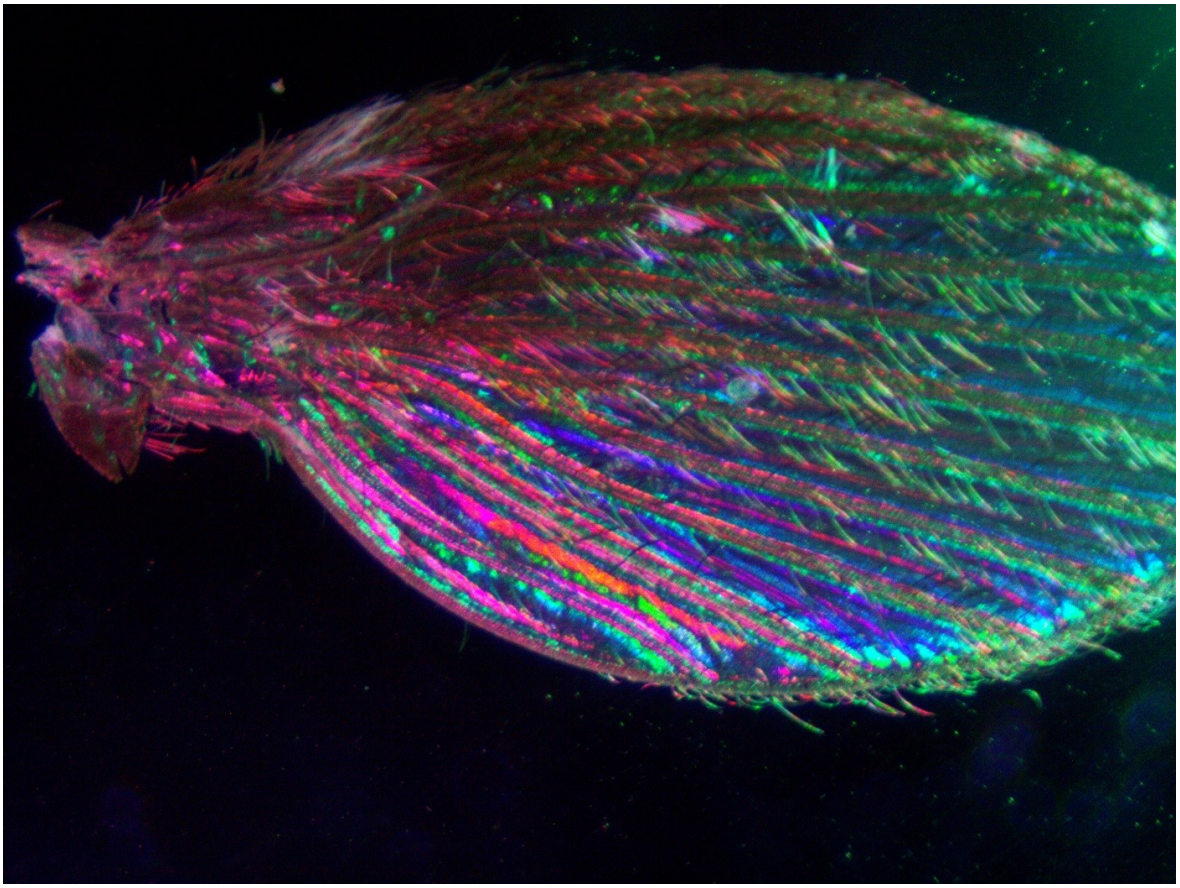
Salt crystals upon a black background 4x illuminated with yellow and blue filters.



Spider upon a black background 4x red and green filters.



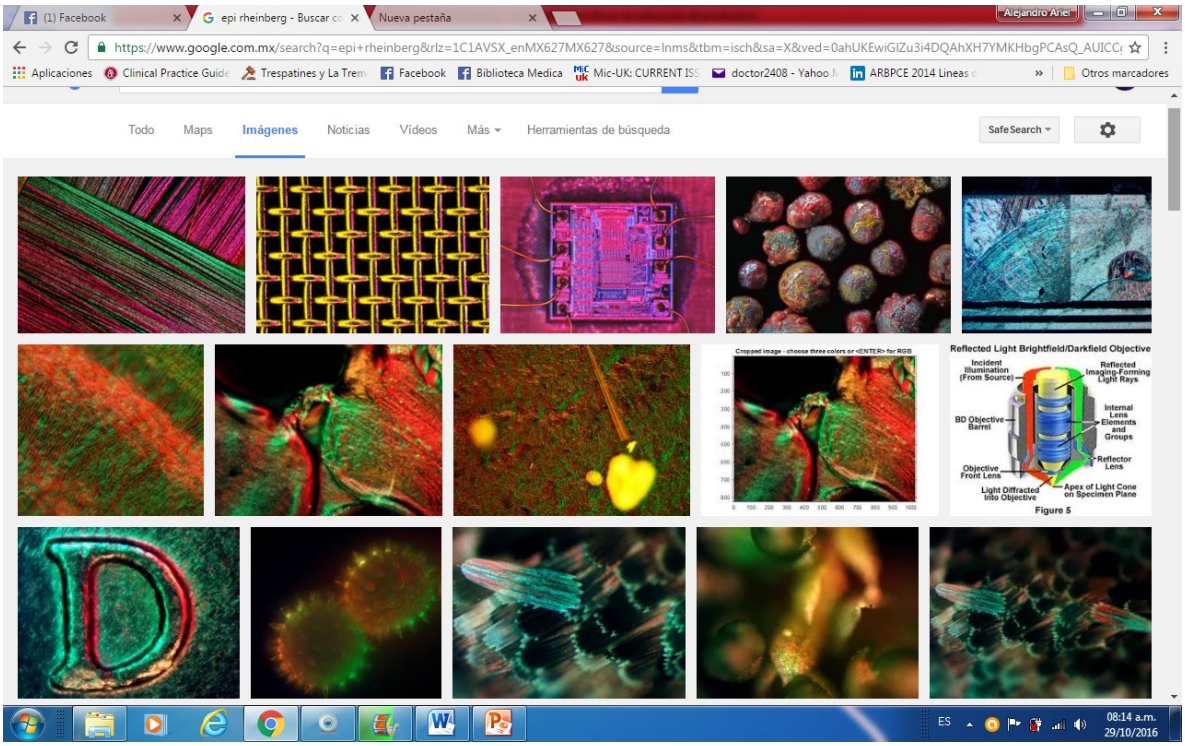
Spider upon a black background 4x yellow and blue filters.



Wing of *Psicoda* 4x red and green filters.

CONCLUSION:

The results showed in this article are comparable to those results that appear on the Internet that were produced with real epi-Rheinberg systems.



So the technique is going to be very useful to create beautiful images for both thick and transparent subjects illuminated from above.

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capitalised characters.)

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