

Darkfield Reflected Rheinberg Microscopy (A.K.A. “Disco Lights Illumination”)

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Introduction

Rheinberg illumination is a darkfield technique, which is attributed to British microscopist Julius Rheinberg (1896). This well-known technique is described in some detail on sites such as <https://micro.magnet.fsu.edu/primer/techniques/rheinberg.html>. It is often employed for transparent specimens such as salt crystals, as in Figure 1, and snowflakes, giving contrast and beautiful colors to these types of subjects. With standard darkfield microscopy, the central annulus of the illumination path through the condenser is blacked out with a central stop, with only light striking the specimen with a numerical aperture sufficiently large so as to not be collected by the objective being used. The specimen is then viewed not in brightfield (with illumination NA within the acceptance cone of the objective), but only via scattering and diffraction from the specimen. This creates a black background and provides excellent contrast to the subject of interest. This is often used for diatoms and the like, as shown in Figure 2.

The ideal position for a darkfield central stop is in the condenser, in the same location as annuli for phase contrast microscopy. Many condensers have turrets or sliders to allow brightfield, darkfield and phase contrast options. Some manufacturers have provided slots near this location to obtain oblique illumination or Rheinberg as well. The difference between standard darkfield and Rheinberg illumination is that there are colored filters which provide the light to the specimen. It can be a single color or split into different sectors. Often, instead of a blackened central stop, a dark colored filter is used as the central illumination. The effect of this is to create a background for your image which is not black, but can be colored as desired, giving a unique character to certain subject types. Zeiss produced a specialty condenser for Rheinberg illumination (Mikropolychromar), but many manufacturers, such as Leica, provide a slot to insert filters in a standard condenser. Nikon does not appear to be one of these manufacturers.

It is also possible to use darkfield in an episcopic (reflected light, or epi) system, as shown in Figure 3, a lacewing egg. It takes a little different system for reflected light, since the objective lens is also acting as the condenser lens. The solution is to have a separated outer annulus which provides the high NA illumination, and a standard objective to receive the scattered/diffracted light from the specimen.

These may be referred to as “Epi”, “BD” or “ED” objective types. They are characterized by unusually large barrels and generally larger threads, such as M26 or M27, as opposed to RMS (20.32 mm) or M25. This requires a special objective turret and specialized hardware in the episcopic illuminator.

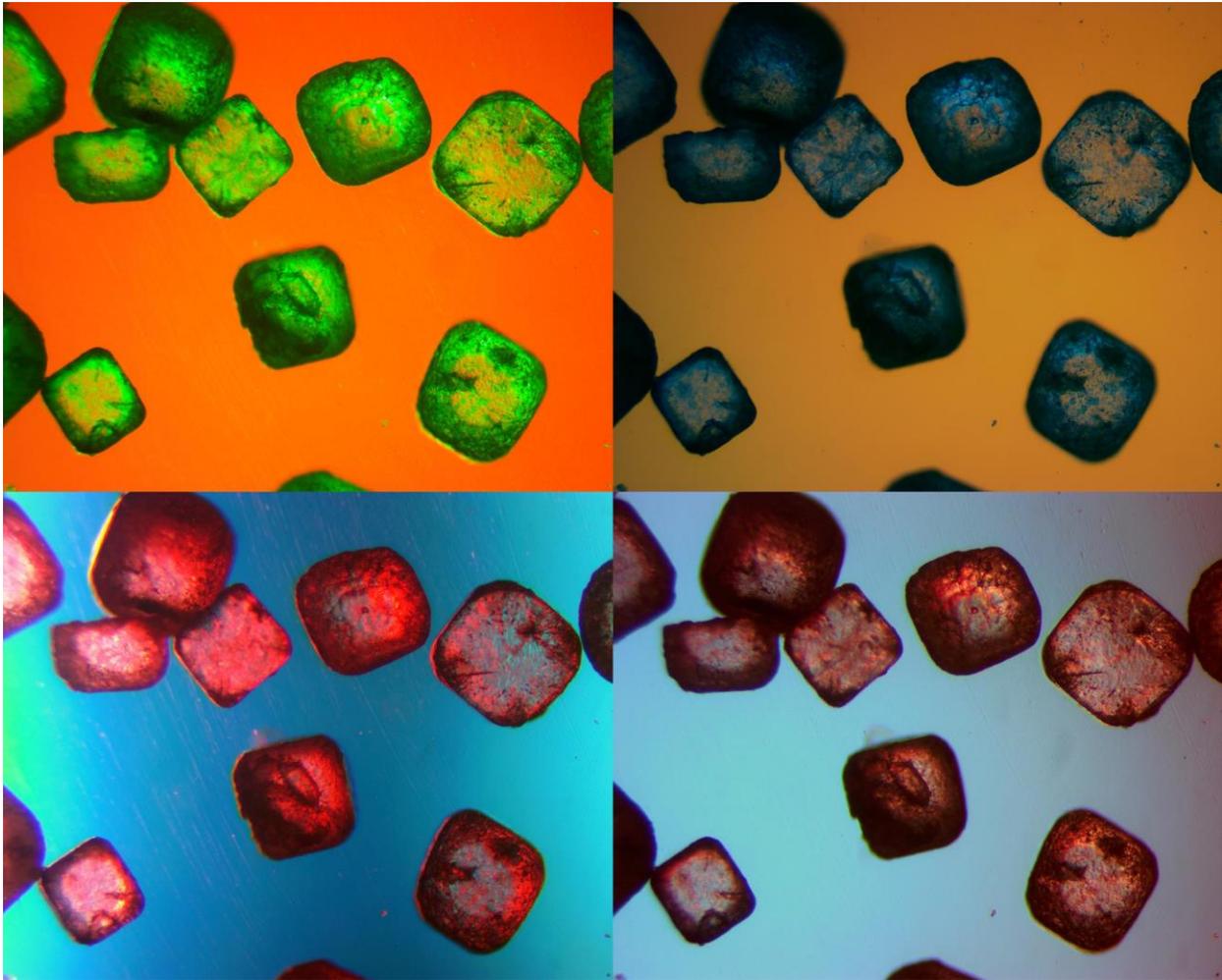


Figure 1 – Table salt (NaCl) crystals with different filter combinations in traditional transmitted Rheinberg illumination 4x.

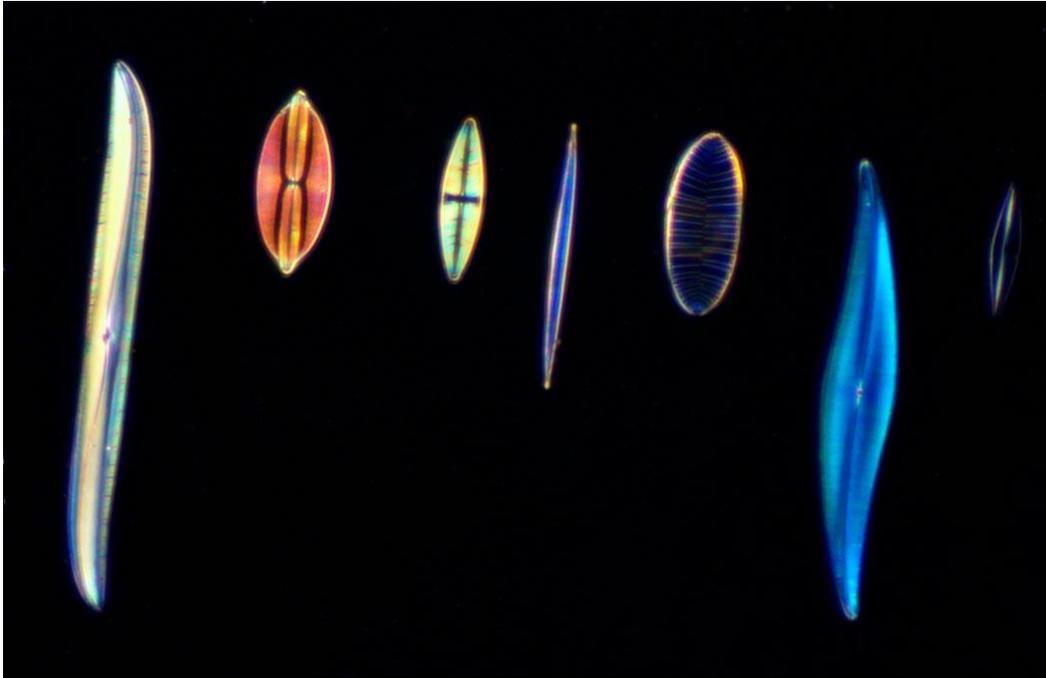


Figure 2 – Diatom test slide with transmitted light darkfield 10x.

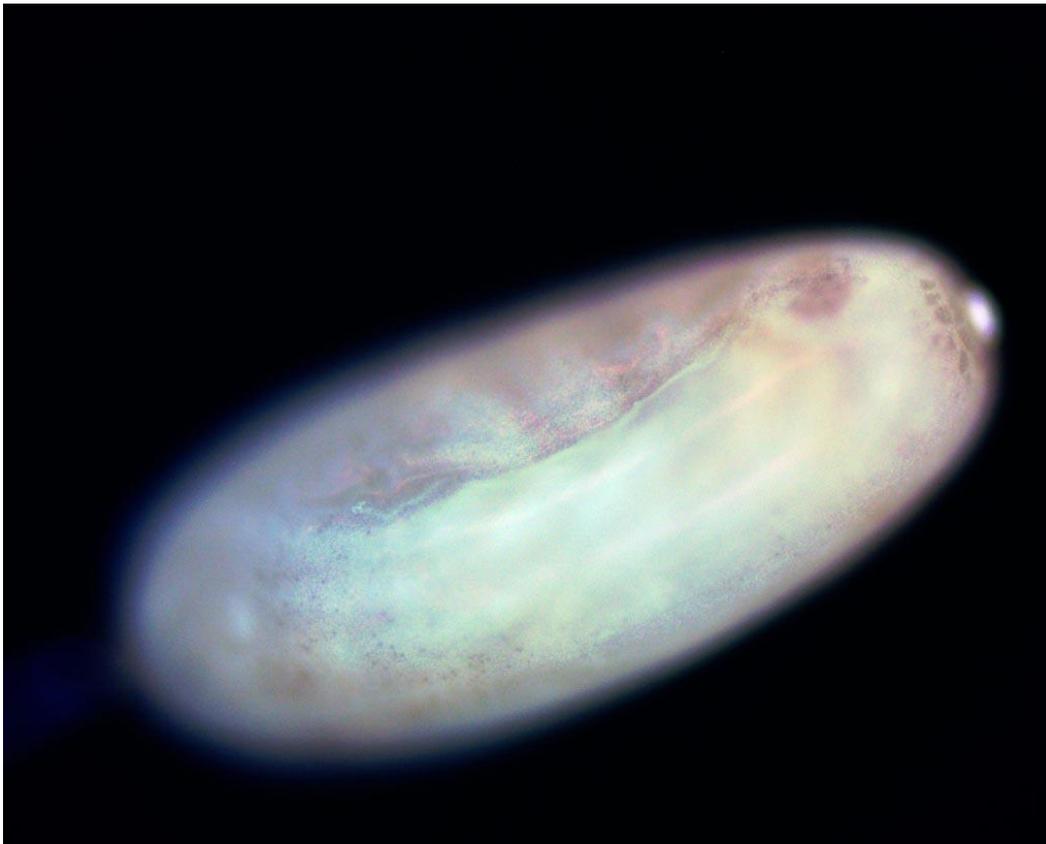


Figure 3 – Lacewing egg with epi darkfield 10x.

Darkfield Reflected Rheinberg

While trying to figure out how to best do Rheinberg on a Nikon microscope, without hacking up a condenser, it occurred to me that based on the preceding description of Rheinberg and reflected darkfield, that the two ideas can be combined. The moniker “darkfield reflected Rheinberg”, while accurate, doesn’t quite have the ring to it that “Disco Lights Illumination” (DLI) has. This name was inspired by the photo I took of the output of the BD Plan objective, with the BD illuminator switched to darkfield mode, as seen in Figure 4.

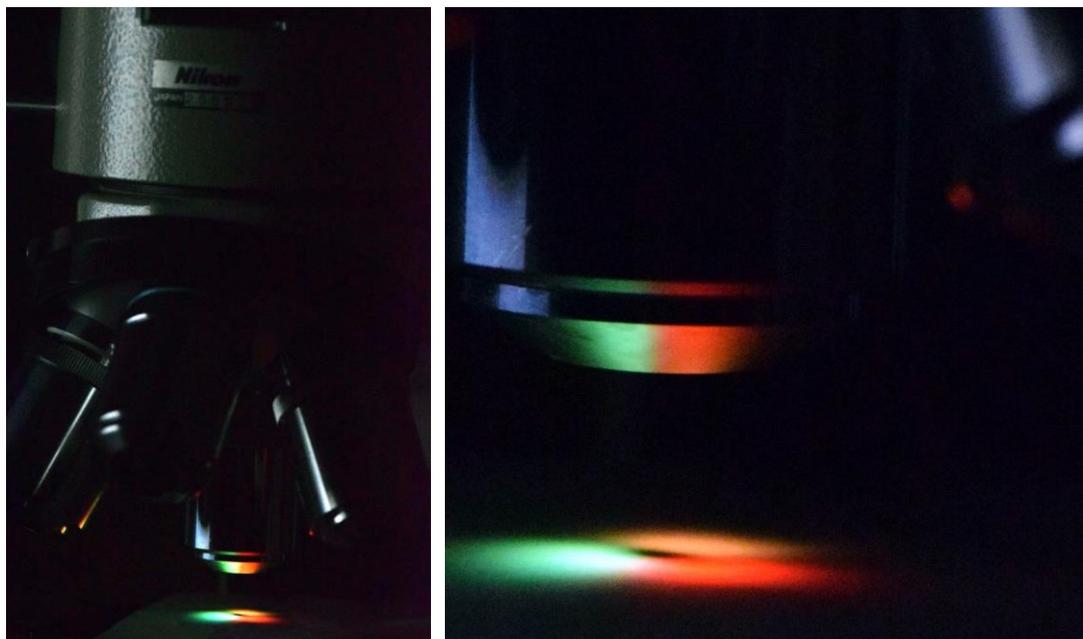


Figure 4 – Colored light exiting the BD Plan objective with colored filters over the three sectors.

It has since occurred to me that a fairly simple modification to a Hoffman modulation contrast condenser for an inverted Nikon Diaphot-TMD will allow user-created filters to be installed in an appropriate location for transmitted light Rheinberg (as shown in Figure 1).

Typically, Rheinberg filters are 2-3 mm thick and are slid into or under a condenser in transmission. With the BD setup, there is no such space, but with a very thin filter, it can be placed between the bottom of the episcopic illuminator and the top of the objective turret (nosepiece), on top of each of the three sectors. You must leave the central portion open, or else it will color everything headed up to the observation head. The photos in Figure 4 are taken with three hand-cut slivers of plastic shim stock, as seen in Figure 5. As you can see, great precision is not needed to achieve results.

Figure 5 is shown with the vertical illuminator removed, which is inconvenient – it is large, awkward and potentially hot, if you have been using it for any length of time (it has a 50 W halogen bulb). It does, however, provided easy access to place the filters just where you want them on top of the nosepiece sectors. The other method is to leave the illuminator on, but remove the nosepiece itself. While possible, it is tricky and a little frustrating to have the filters stay in place as you slide the nosepiece back into its circular dovetail slot. Careful application of small pieces of tape can help this issue.



Figure 5 – Rheinberg BD filters cut from plastic shim stock (green, red and yellow in this case).

Another method that is possible to use is to create a colored mask and print it on transparencies, then trimming it out as a single annulus. The colors may not be as rich, but it is possible to stack more than one filter ring to deepen the colors produced. Figure 6 shows a sample of the masks I have printed. These, especially for multiple layers, need to be carefully taped (not intruding into the apertures).

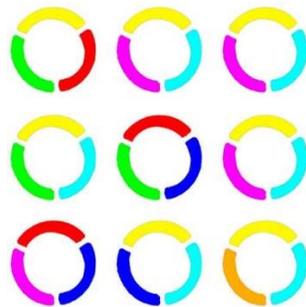


Figure 6 – A small sample of the 7 x 9 grid of filters that can be printed on a single 8.5 x 11-inch sheet.

The balance of colors is important to achieving the most striking images. There are really two issues here, intensity and “richness” of color, as described in the previous paragraph, which is really the saturation of the three colors. Several methods present themselves to improve the balance. First, if the saturation is poor, you can choose a deeper filter, if possible. You can also stack more than one filter layer together, either for all three sectors or for any offending sector individually. To achieve balance, while not generally recommended, it may be possible to misalign the lamp filament to achieve the desired balance (just don’t forget to fix your Köhler alignment when finished!)

There are a couple of other important points to keep in mind. As with normal BD darkfield, you must have the field stop all the way open, or no light comes out! Also, ensure that there are no colored filters in the illumination path. The green interference filter is obvious, but it is easy to forget to remove a blue filter that is normally there for standard color balancing. This reduces the efficiency of yellow filters significantly. In fact, one or two of the example images below were taken inadvertently this way.

Example Images

Next is a gallery of example images taken with various power BD Plan objectives with DLI.

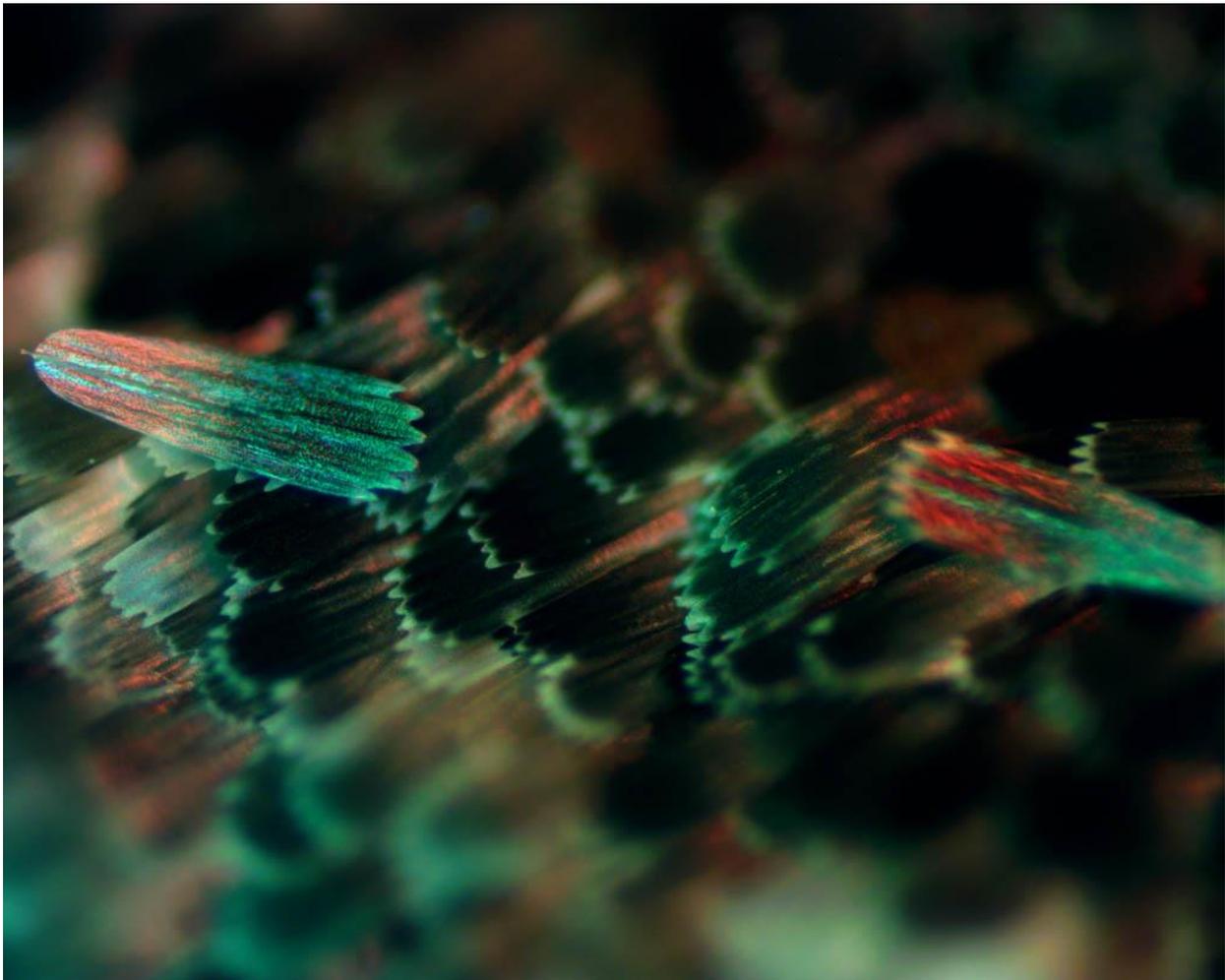


Figure 7 – Moth wing scales 10x.

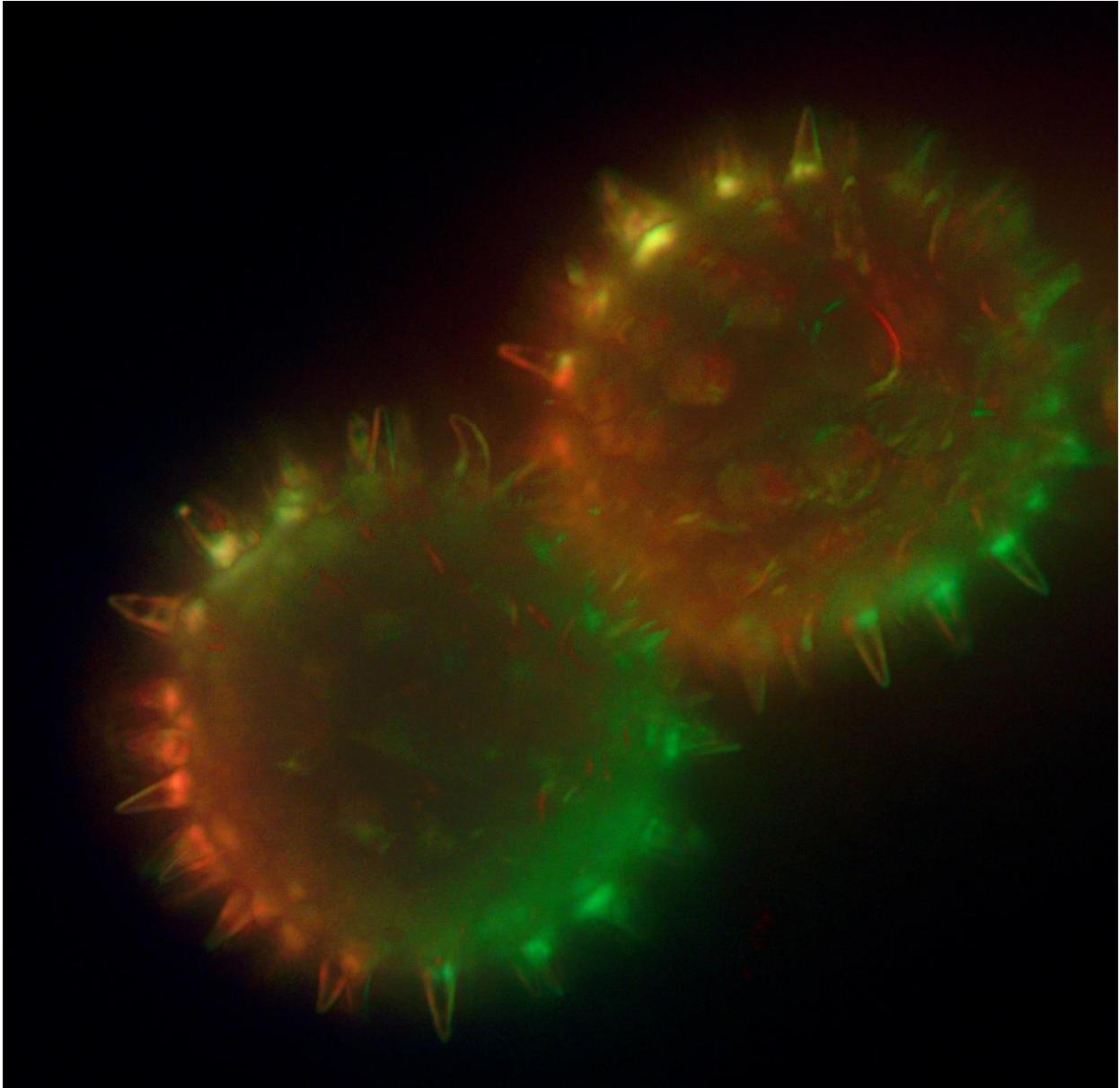


Figure 8 – Sunflower pollen (brightest pixel focus stack) 100x.

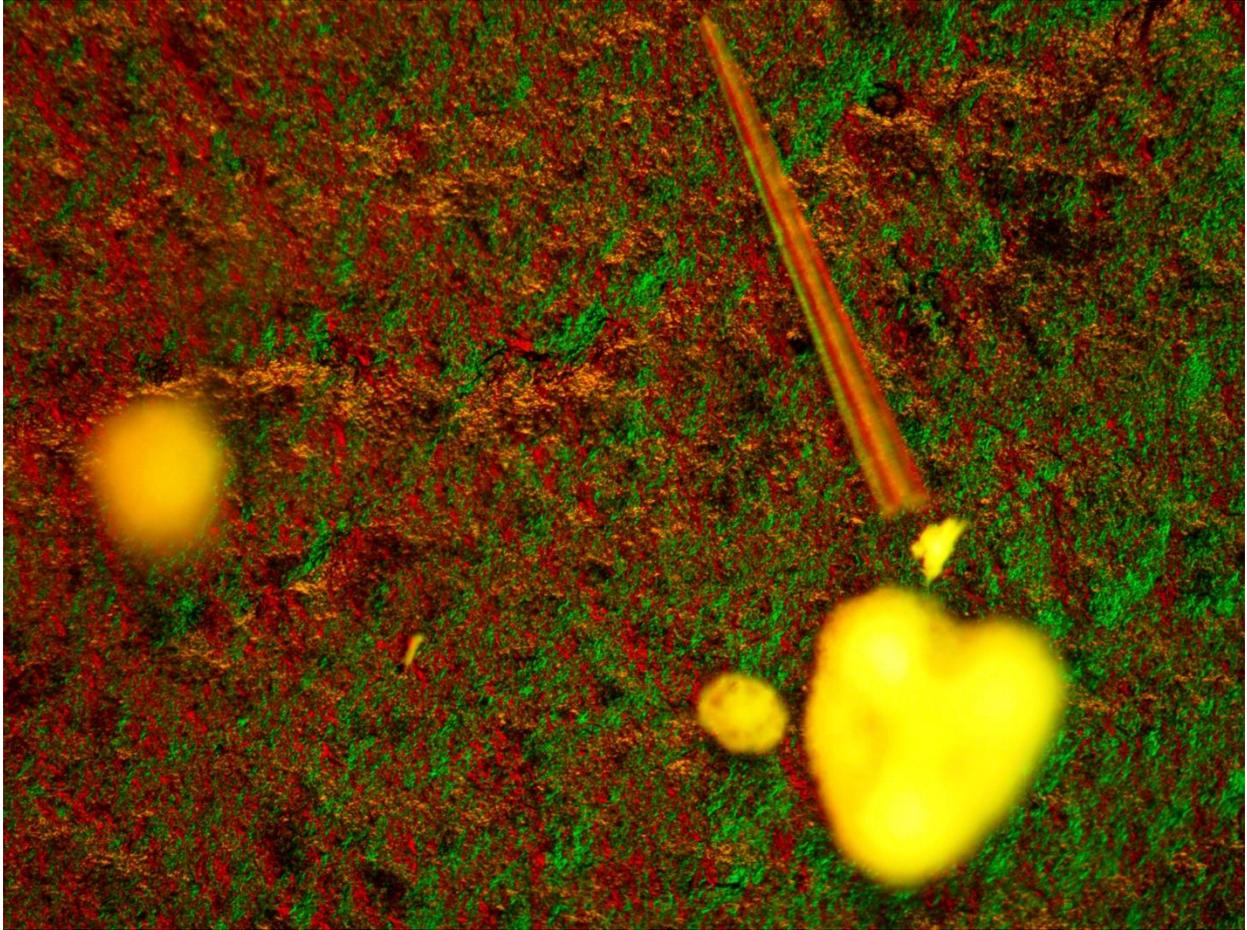


Figure 9 – Dry mounted radiolarian fossils on black paper – focused on the background 10x.

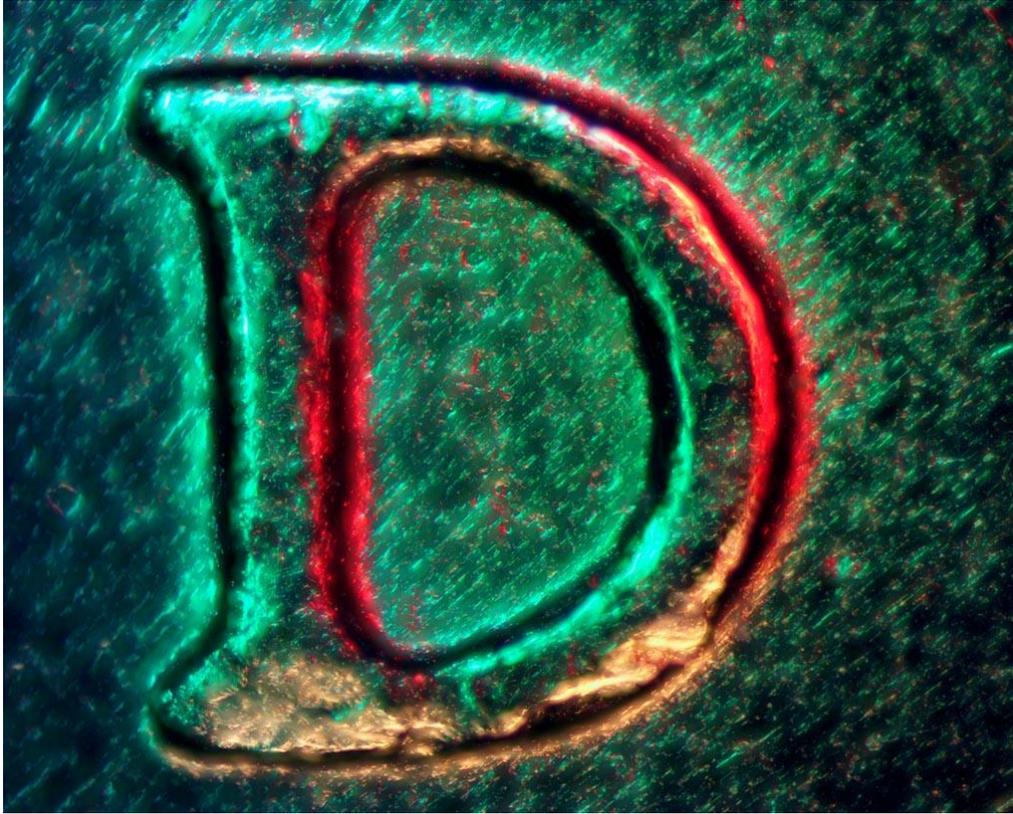


Figure 10– Denver mint mark on U.S. nickel coin (brightest pixel focus stack) 10x.

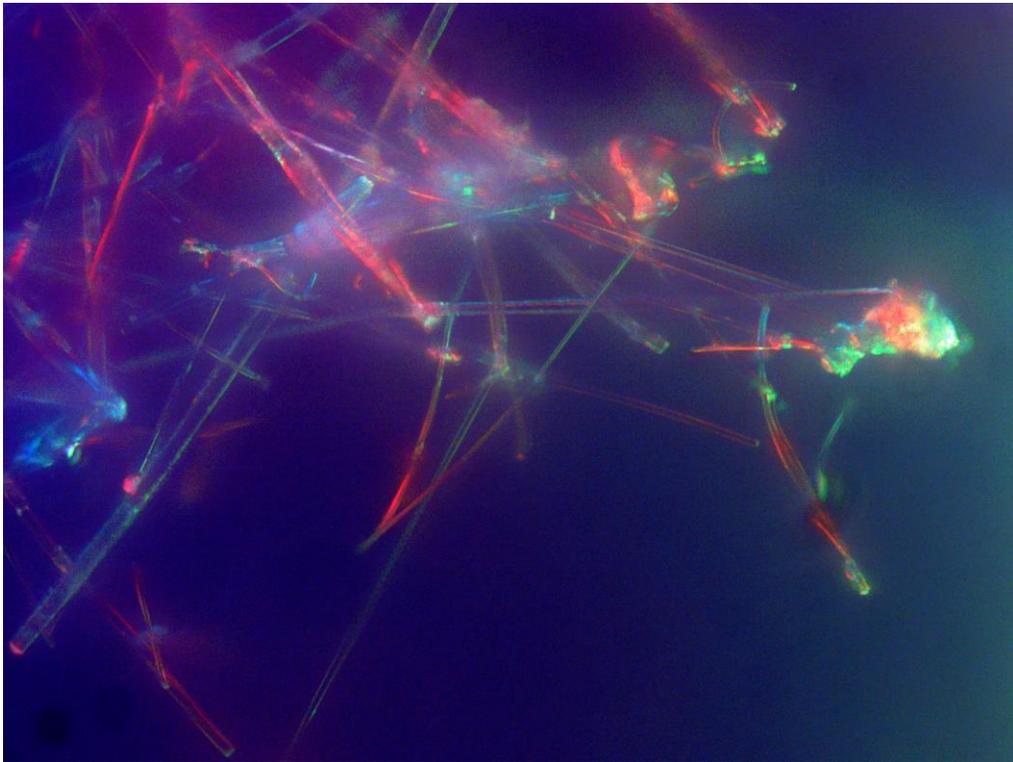


Figure 11 – Ceramic refractory fibers (brightest pixel focus stack) polarized 40x.

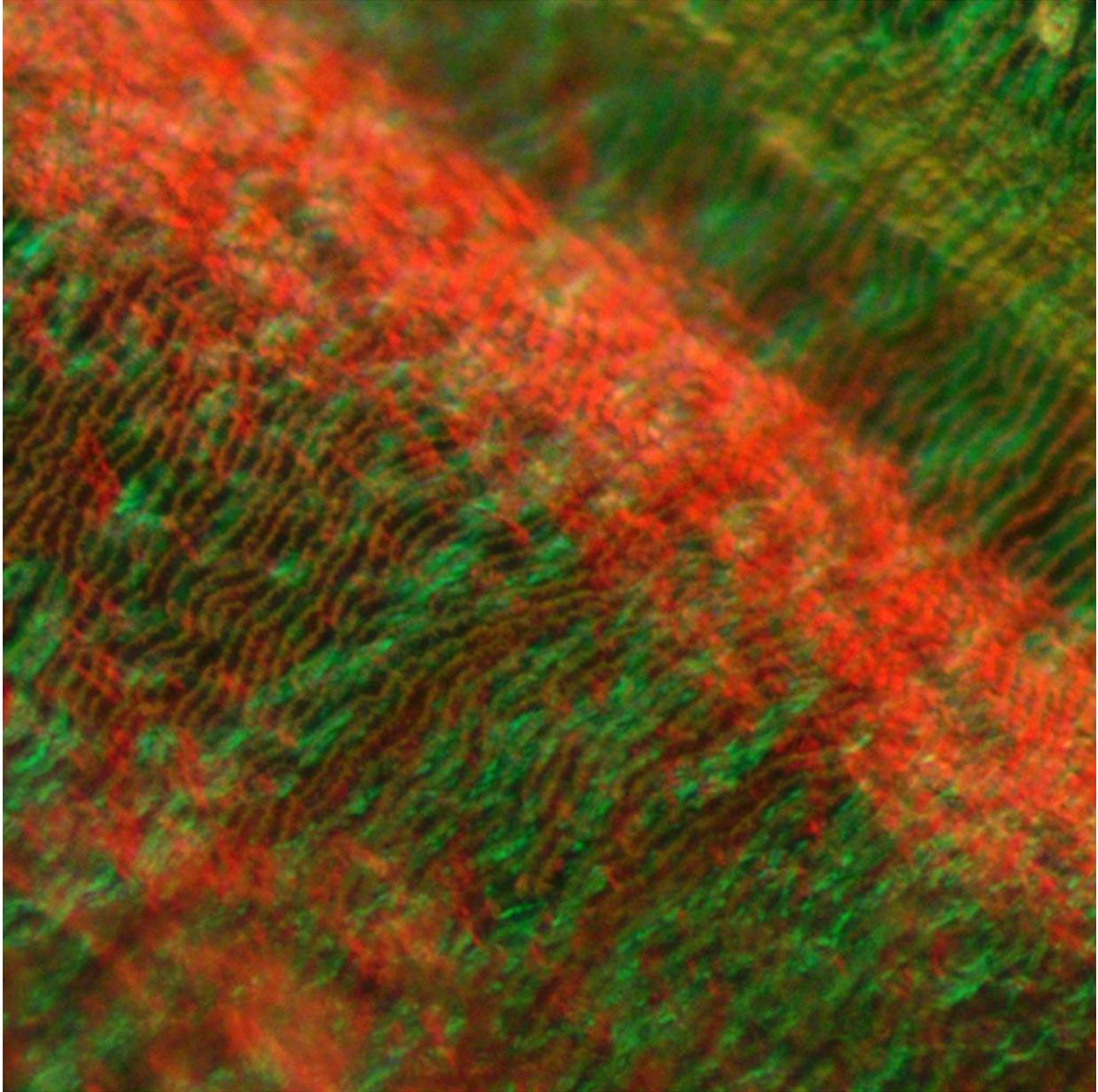


Figure 12 – Snake skin (belly) (single exposure, cropped) 10x.

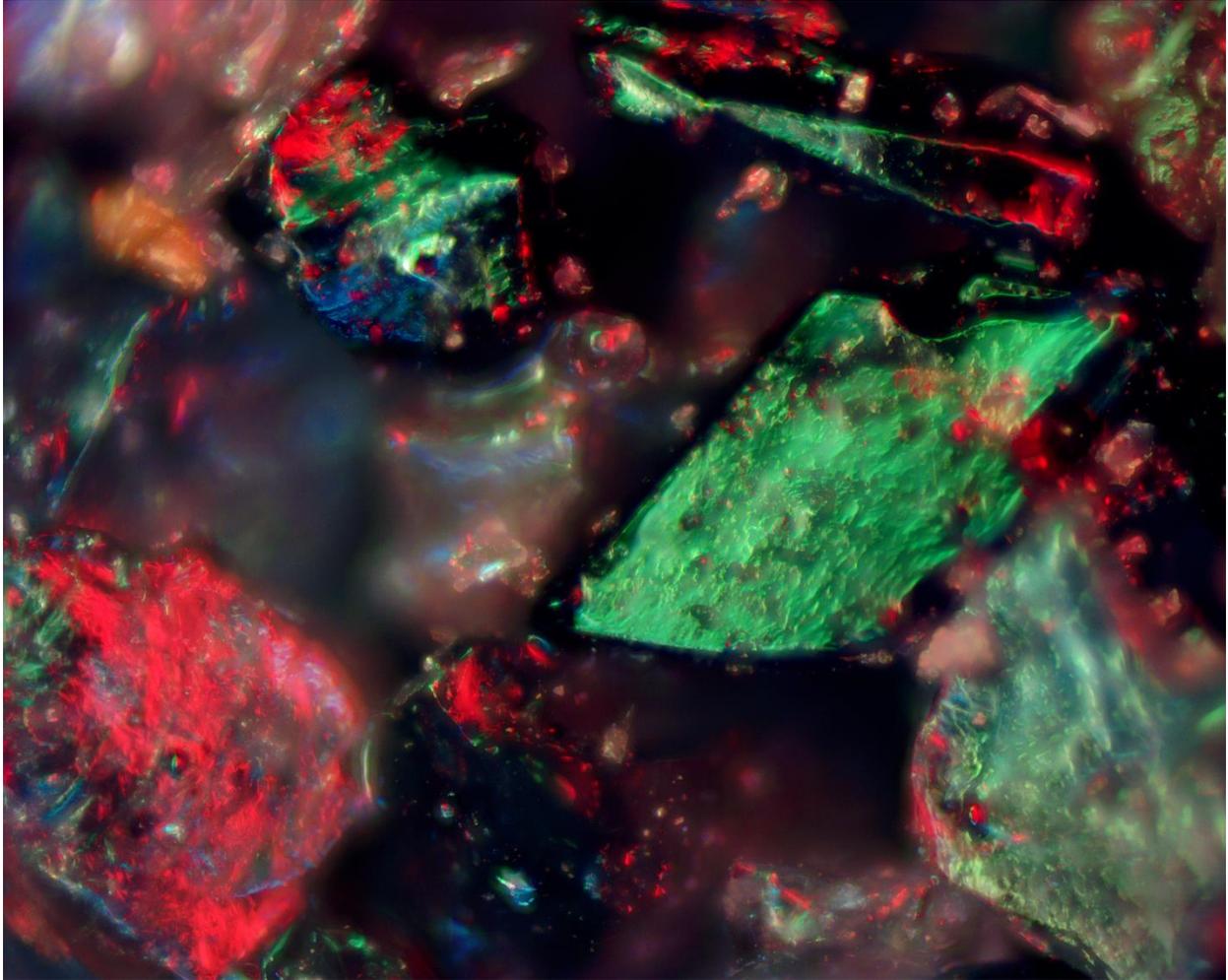


Figure 13 – Silicon carbide dressing stone (averaged focus stack) 10x.

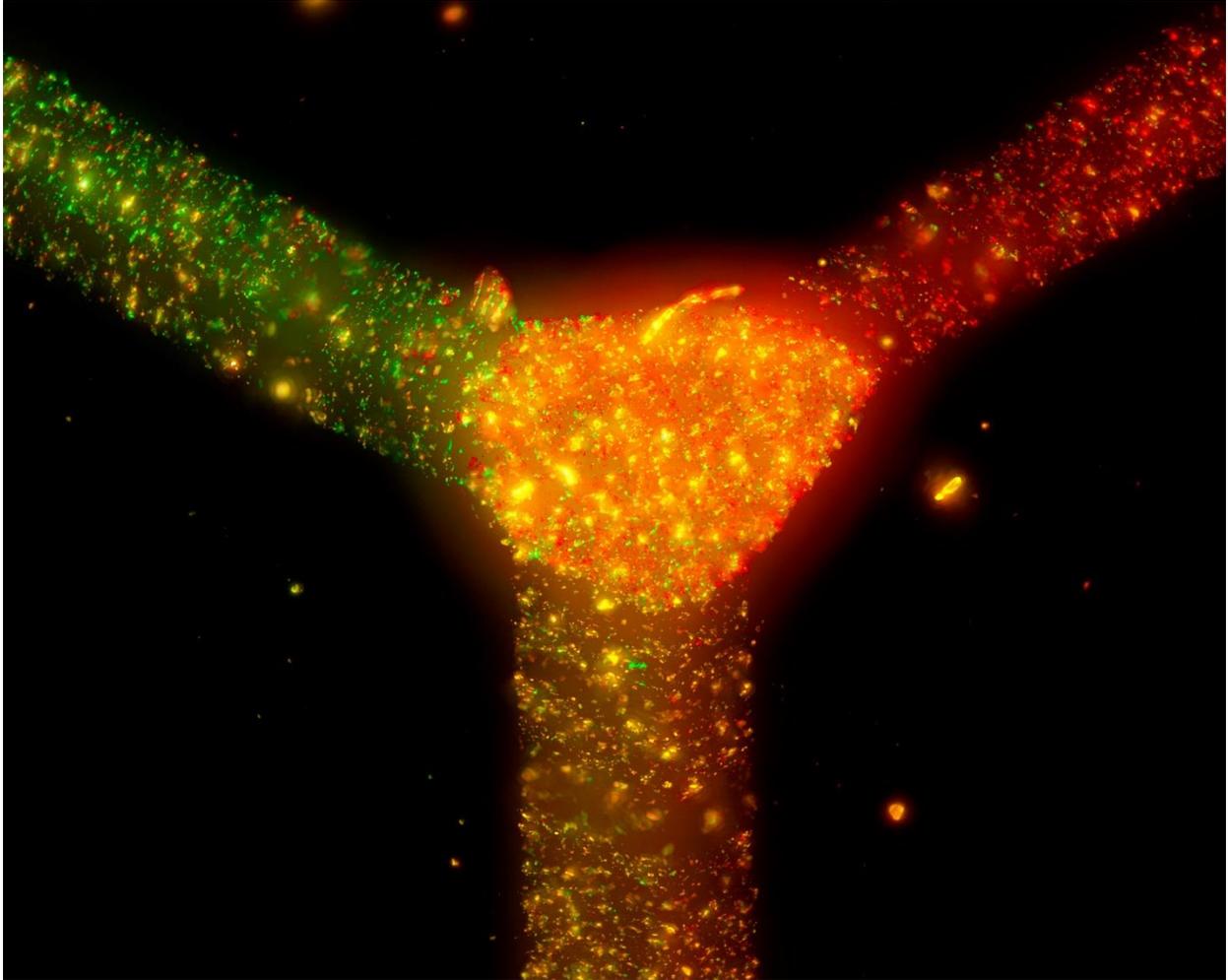


Figure 14 – Ground glass (black is polished glass) of the tip of a cornercube prism (brightest pixel focus stack) 10x.

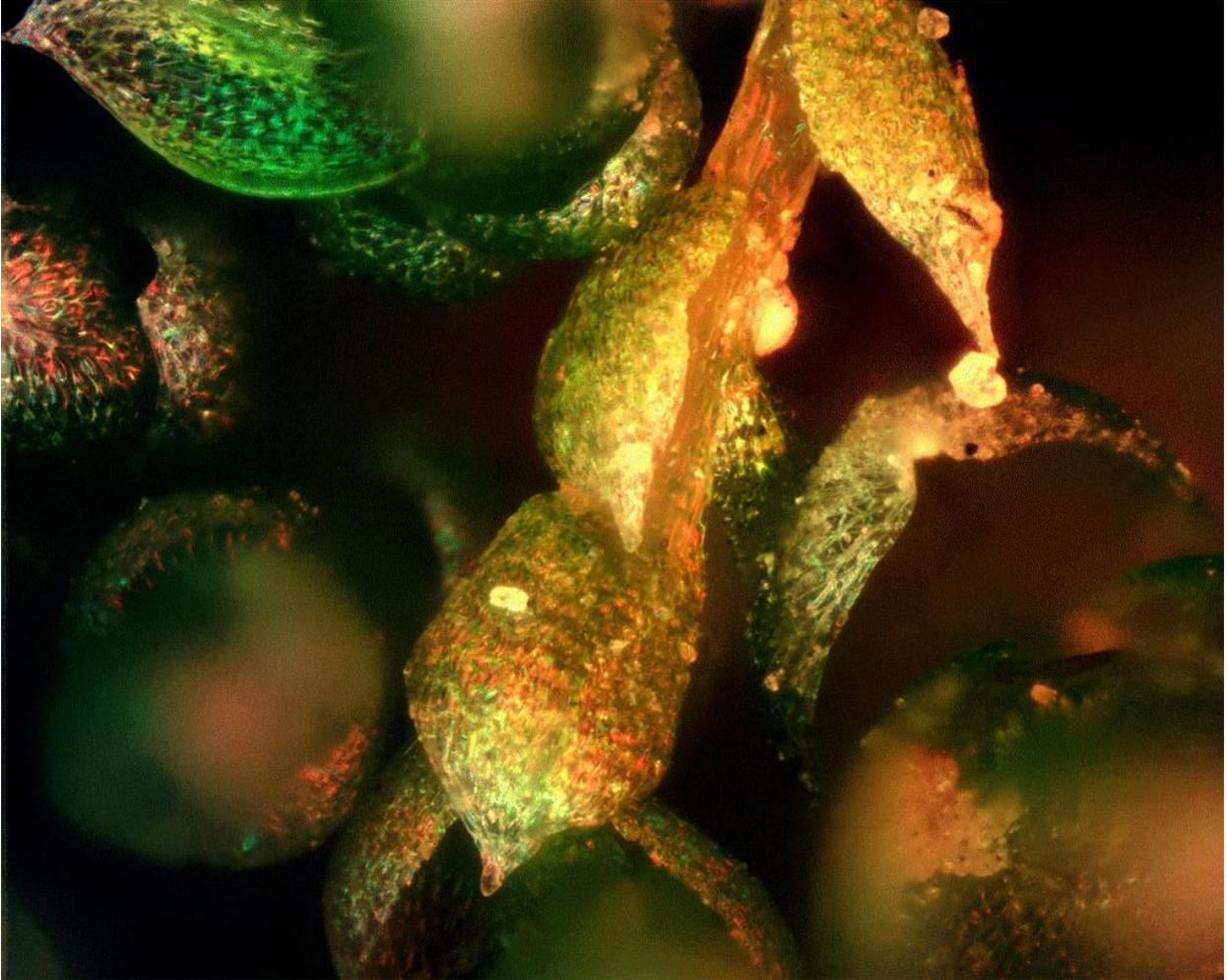


Figure 15 – Fresh moss (focus stack with Zerene Stacker) 10x.

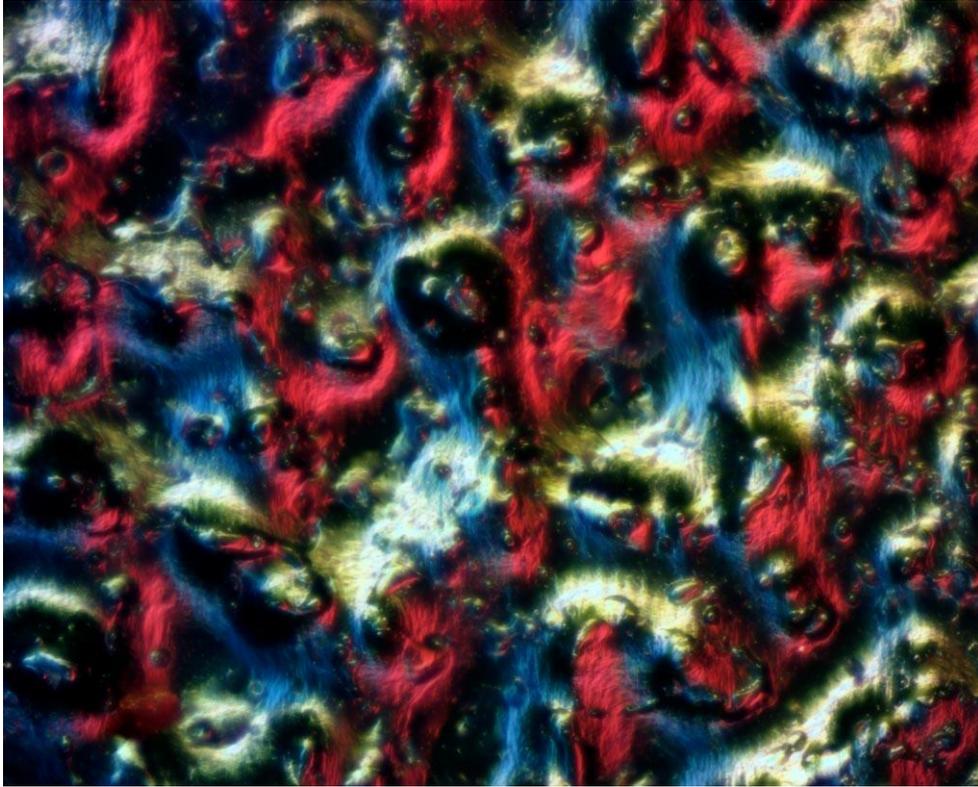


Figure 16 – Textured black plastic cap (averaged focus stack) 10x.

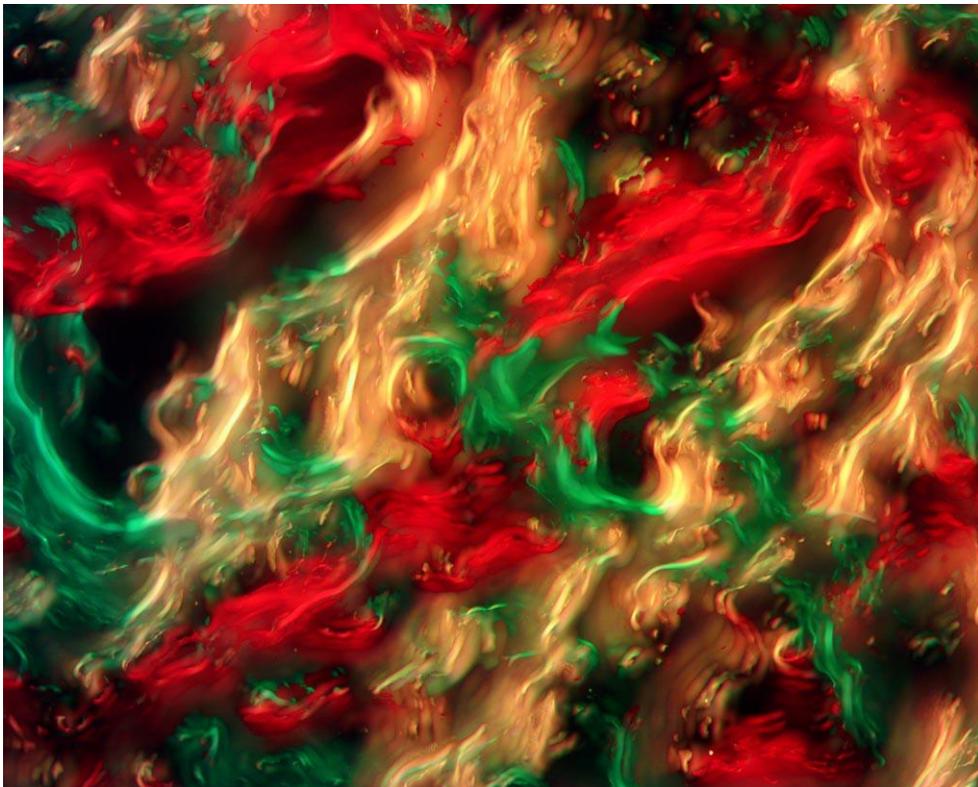


Figure 17 – Glass cut with diamond bit Dremel tool (brightest pixel focus stack) 40x.

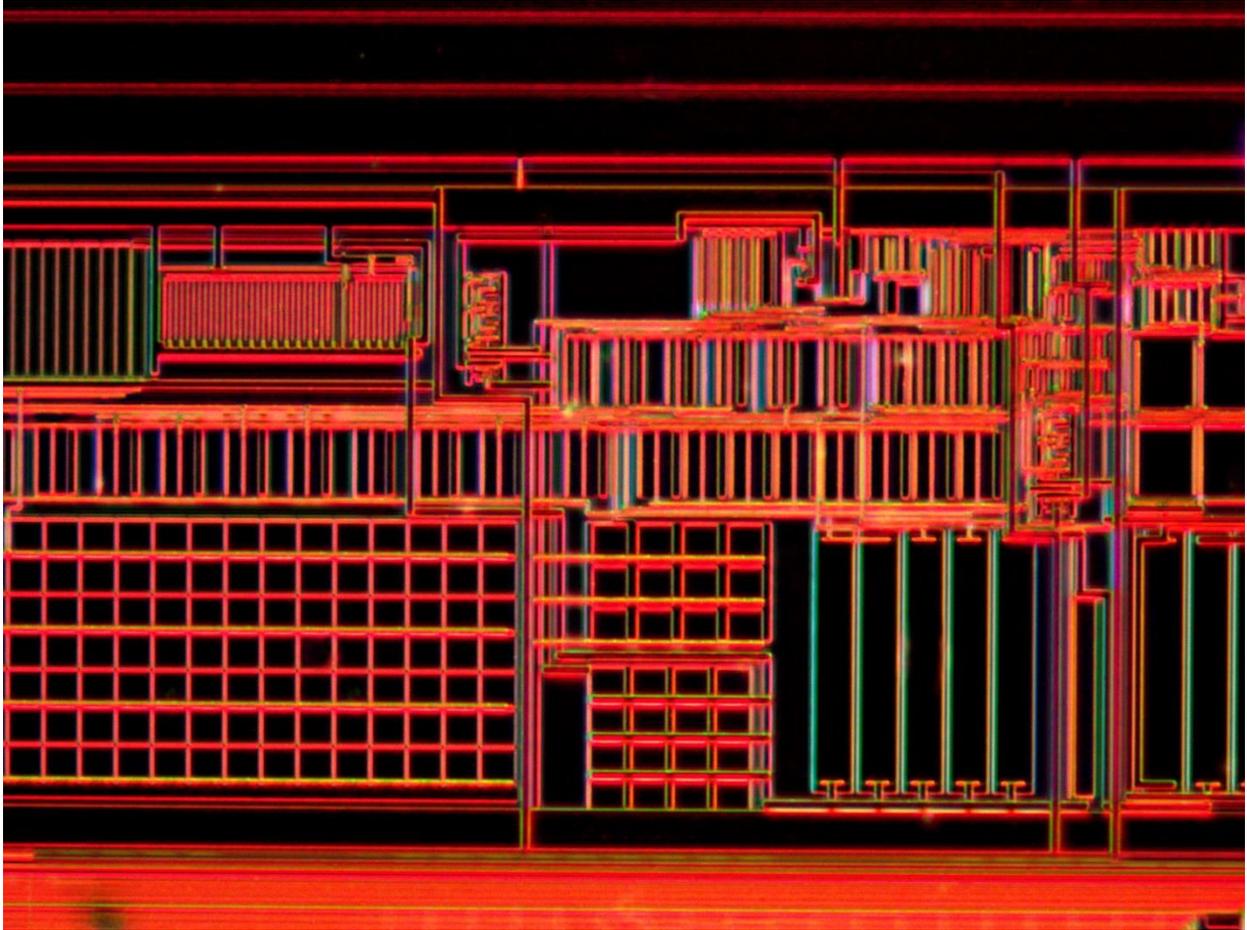


Figure 18 – CMOS camera sensor electronics (single exposure, cropped) 40x.

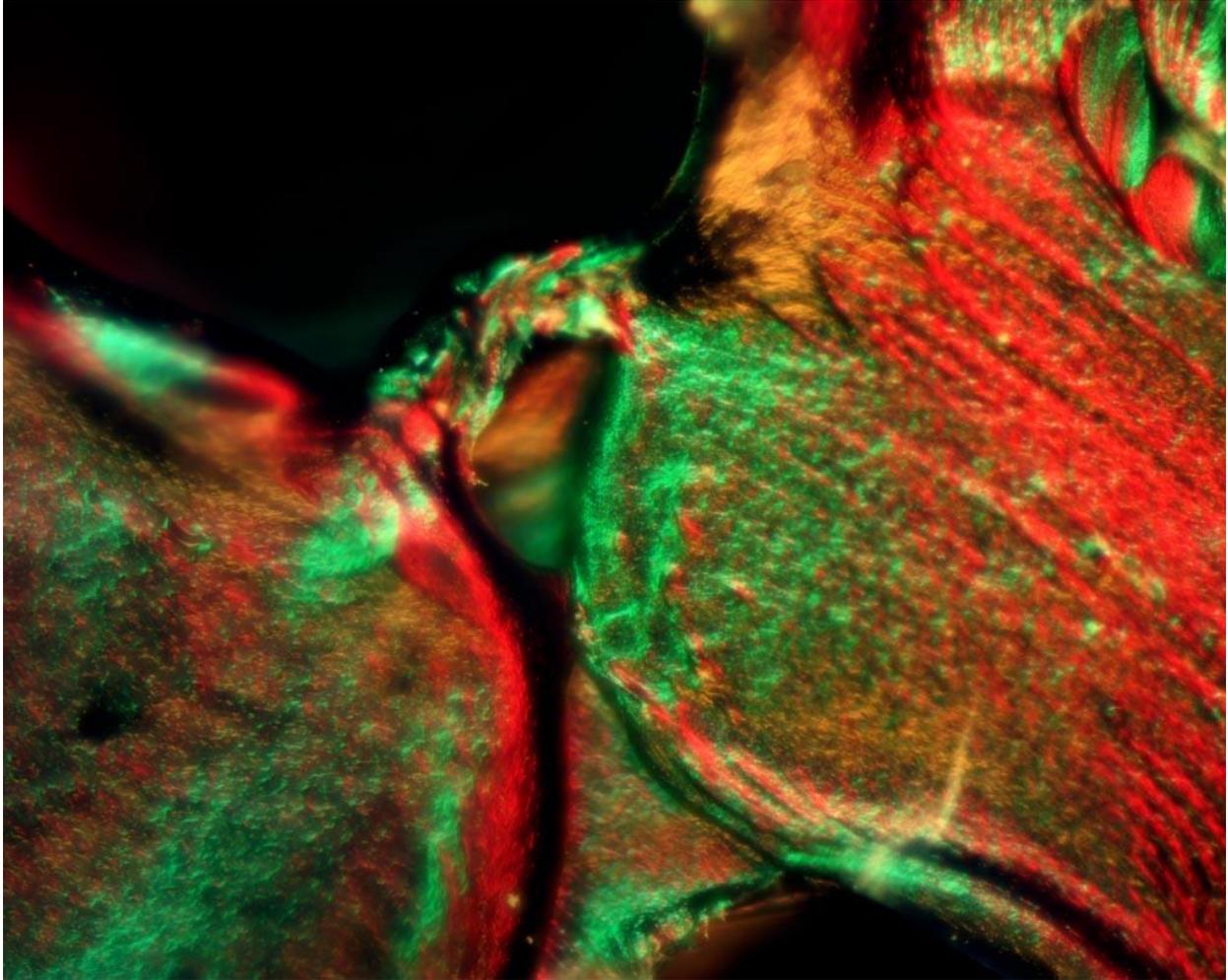


Figure 19 – 3D printed black ABS plastic hexagonal fill (averaged focus stack) 10x.

3-D Reconstruction

Keeping in mind that the geometry of illumination is known, it seems at least possible to use a single image such as Figure 19 to reconstruct the approximate topology of the specimen. As you might imagine, not all images lend themselves to this approach, so a robust, accurate, and linear algorithm may not be possible. However, a description of one such technique will be described.

We will start with the image in Figure 19, which has been stacked through focus. This in itself presents a bit of an issue in certain cases, if the depth is large. For example, the cornercube prism shown in Figure 14 has the ground bevels going down at a 45° angle, but is presented as “flat”. Clearly, this wouldn’t give a very accurate 3-D representation of the image. Figure 19 has much less depth, only a few microns, so we will begin there.

The major assumption (which really is not valid) is that the brightness of a given pixel is proportional to the local slope. In other words, as it is locally tilted more, more light makes it into the objective via

specular reflection. This is of course not how high-NA darkfield works at all, but we have to make some type of simplifying assumption, or this problem is a non-starter.

In this case, the programming language MATLAB is used. The first step is to load, display, and potentially crop the image, as shown in Figure 20.

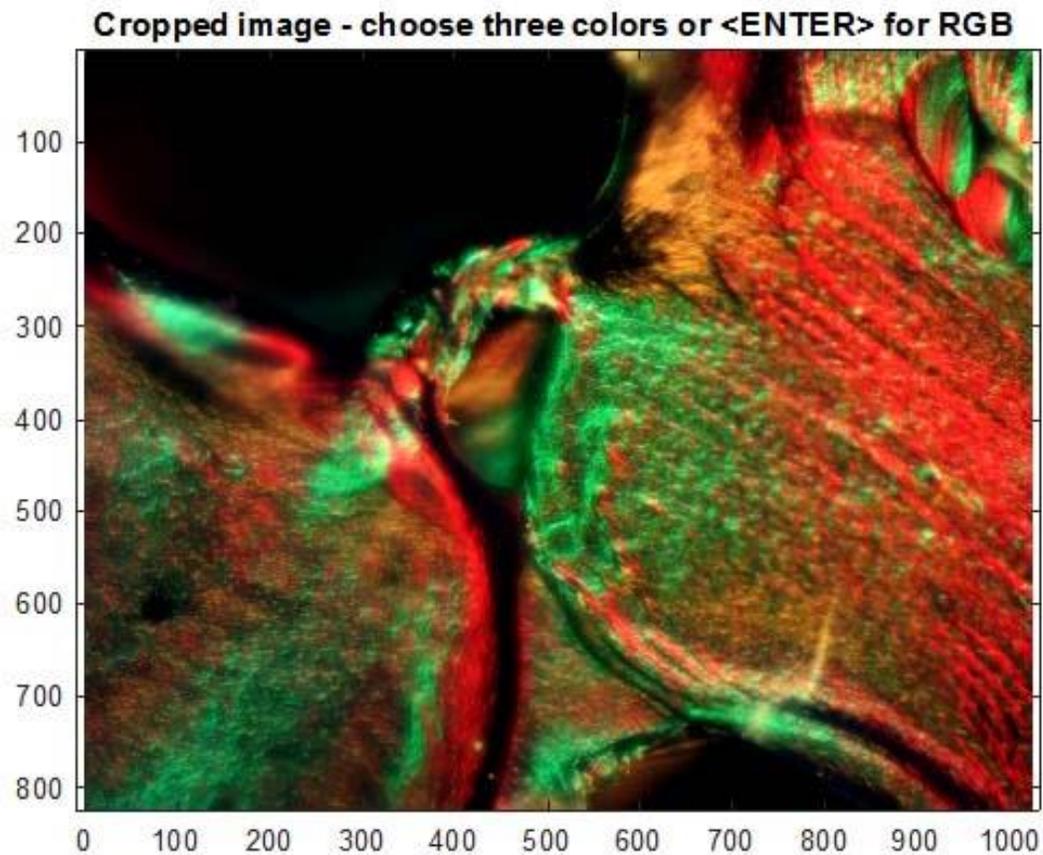


Figure 20 – Input image after cropping (none done in this case).

Next, three color channels are selected (either RGB by default, or by clicking three representative pixels). The image is displayed as the three RGB channels and then as the image decomposed into your selected colors. In this case, the red, green and yellow filters were used, so those colors were selected.

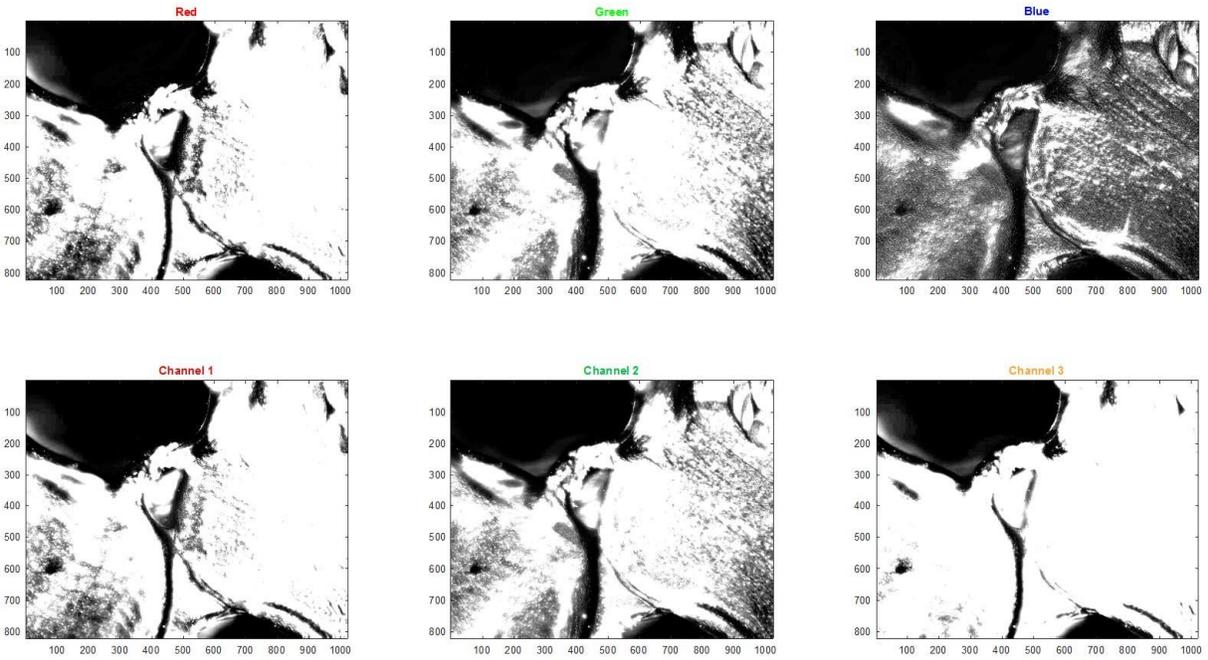


Figure 21 – Image decomposed into three color channels (RGB and custom colors). Note that yellow is the complement of blue, so the blue channel has very little brightness, but the yellow has much more.

Knowledge of the geometry (which direction each color is coming from), allows each channel to be decomposed into X (top row) and Y (bottom row) components, shown in Figure 22. Note that there is no X contribution from the yellow filter, which is approaching from the -Y direction, as expected.

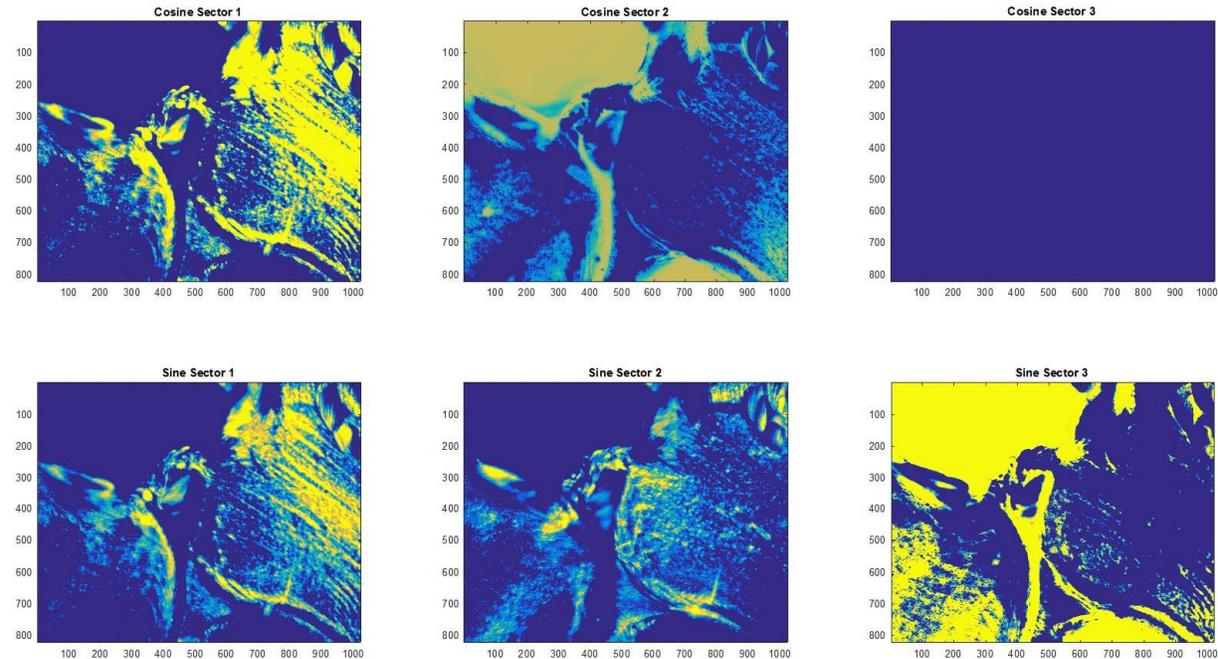


Figure 22 – X/Y decompositions for each custom color channel.

Simple summation gives the full X/Y contributions from all three channels, as seen in Figure 23.

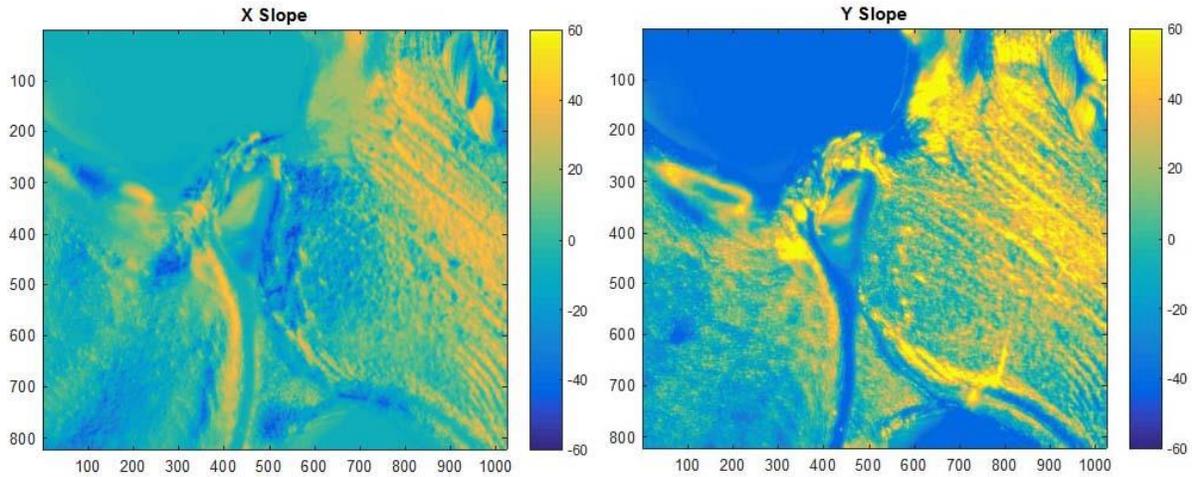


Figure 23 – X and Y “slope” maps derived from Figure 22. The Y component shows more “slope” because of a manual scaling selection error to compensate for perceived brightness.

The real assumption in the process is now applied, considering the decomposed color channels to represent slopes being proportional to brightness in the image. If we assume that these are slopes, then we can integrate them together to come up with a surface height map, as seen in Figure 24, and displayed in 3-D in Figure 25.

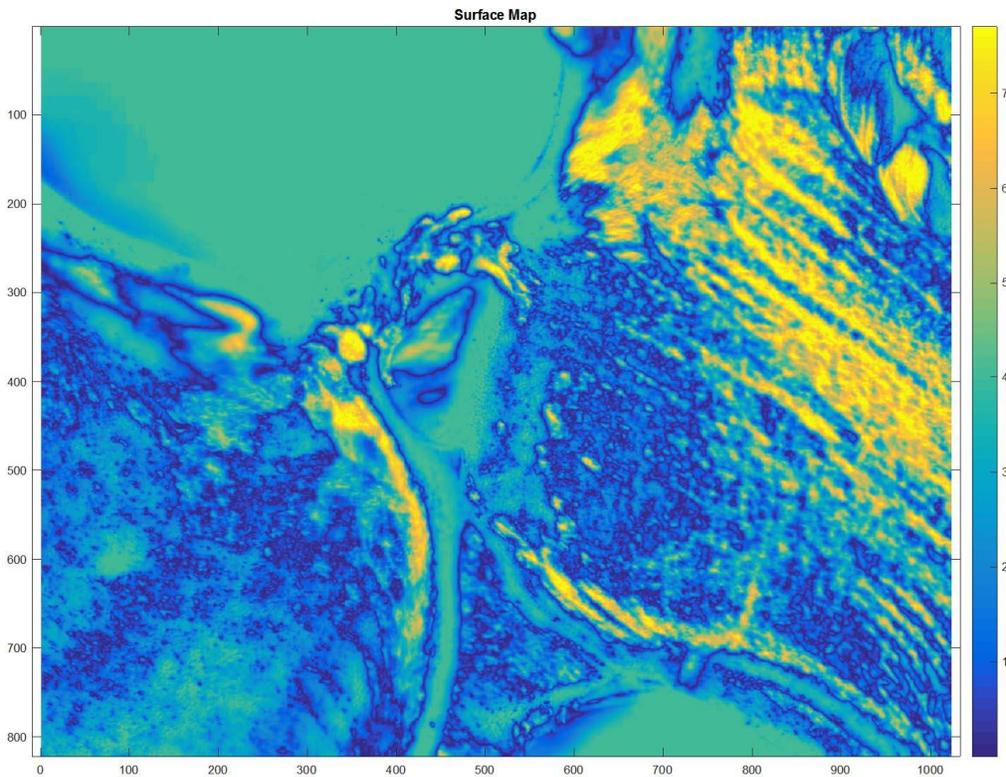


Figure 24– Output height map in arbitrary units.

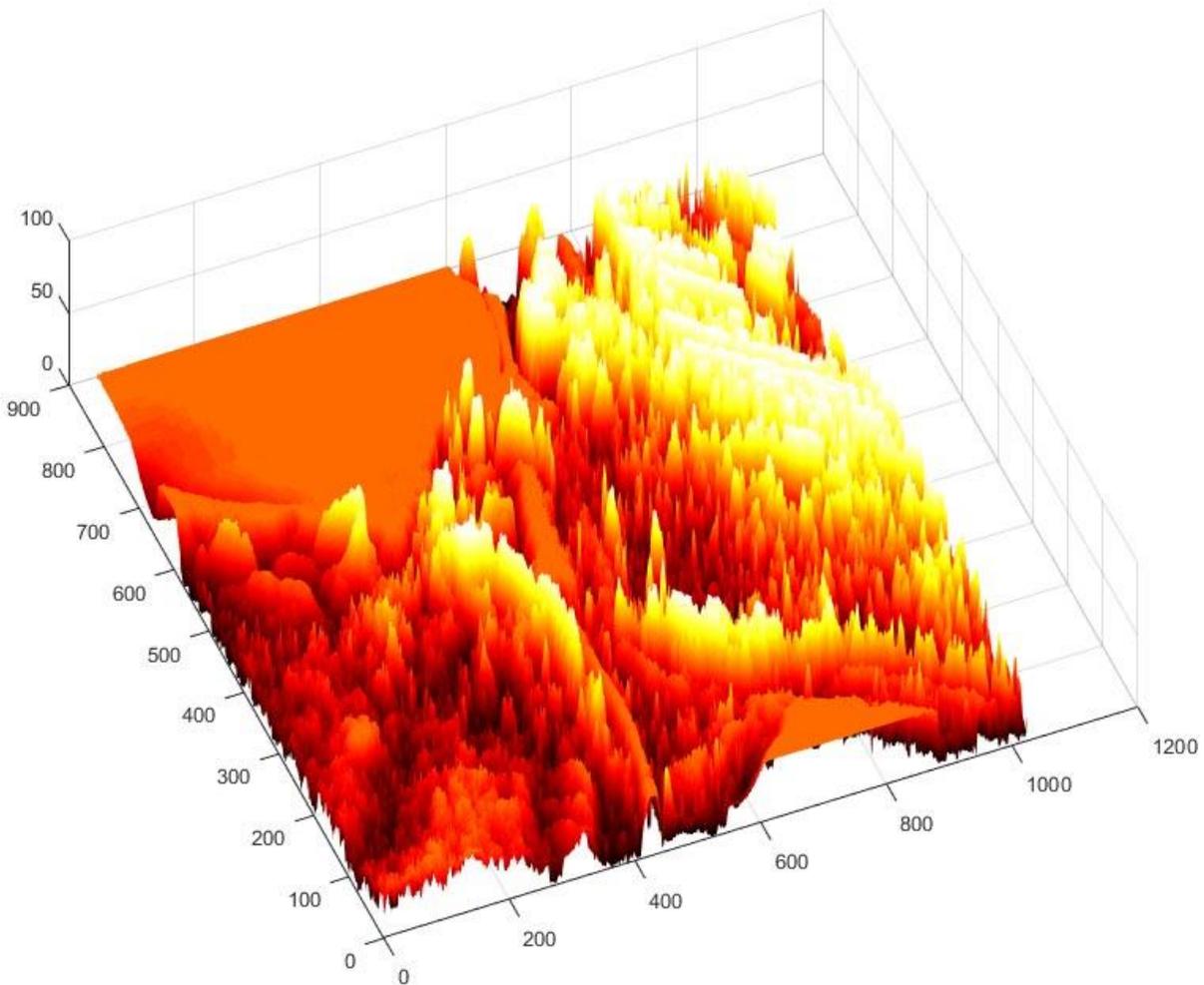


Figure 25 – Output map shown in a 3-D plot, which can have the perspective and scale changed.

Going back to the input of Figure 20, it appears as though there are ridges which are higher areas of the plastic material. The 3-D reconstruction also shows those as being ridges. The black areas (the holes in the honeycomb pattern) are shown as featureless flat areas. In the southwest corner, the green area appears to be higher than the surrounding trenches – which is shown in the reconstruction.

Some parts don't make as much sense, such as the central green area, which seemingly is represented much too low in the reconstruction.

It should be noted that we cannot say what the absolute height variation is, nor is the map particularly linear, but in this case, the map makes more sense than not. As mentioned, this algorithm is not particularly robust, failing to give sensible answers more often than not. One of these cases has a fairly obvious explanation. The clearly 3-D "D" mint mark in Figure 10 has local slopes that are so high that some areas are completely black, making the slope map discontinuous. As you may expect, a good representation of this feature is not reproduced, as shown in Figures 26 and 27.

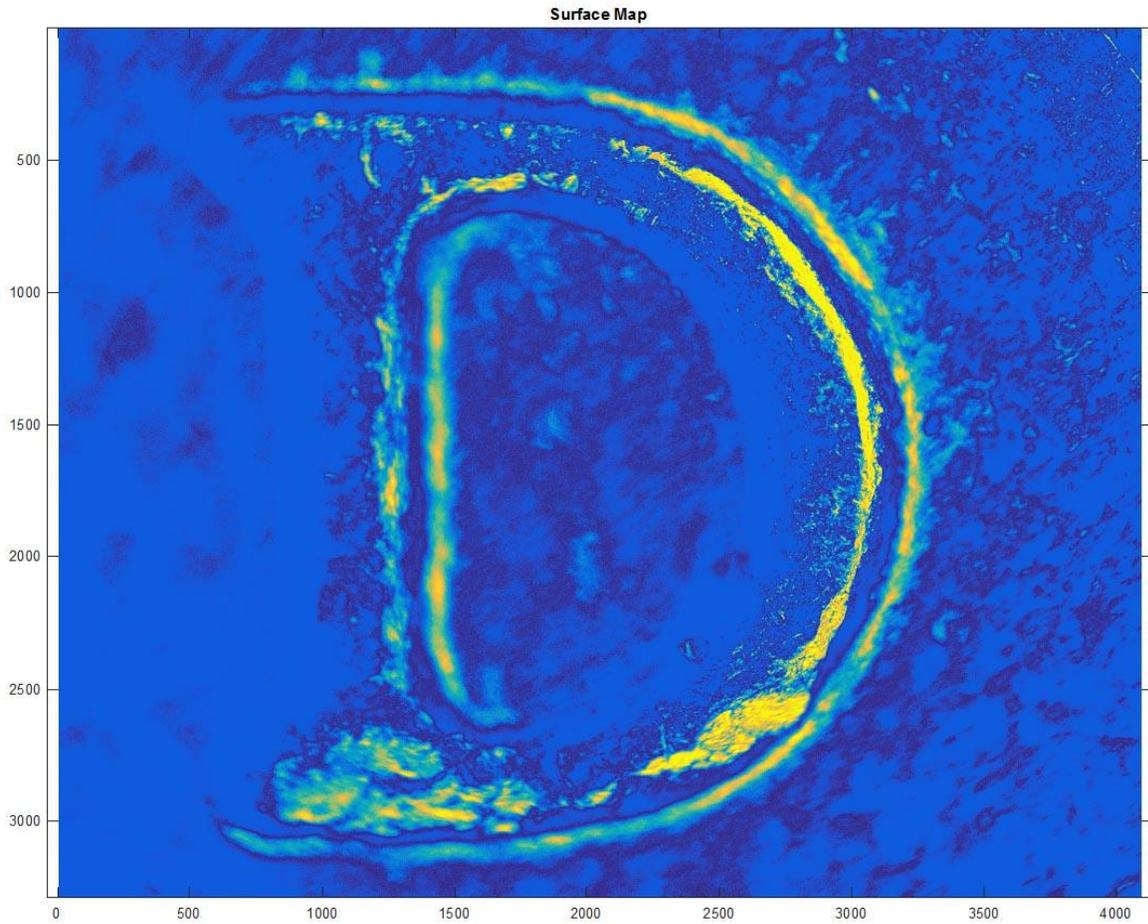


Figure 26 – Top view of “D” mint mark reconstruction.

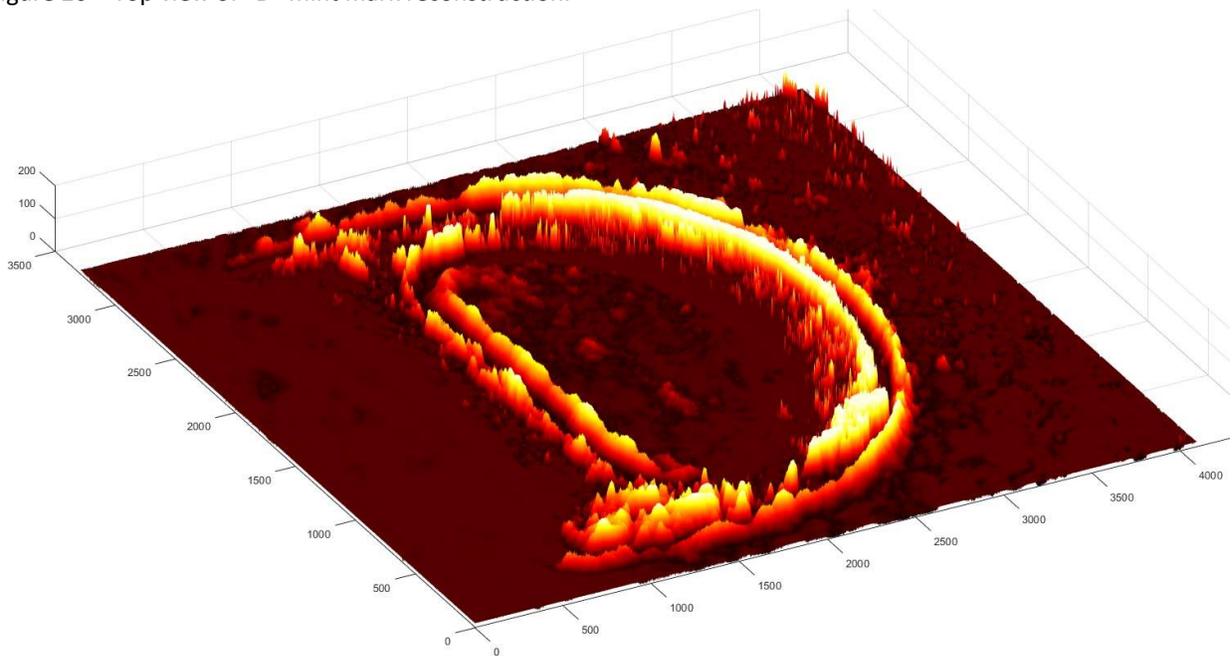


Figure 27 – Perspective view of “D” mint mark reconstruction.

Conclusions

Darkfield reflected Rheinberg (A.K.A. Disco Lights Illumination) can be a very useful technique to image the surface of opaque materials in reflection. It combines traditional darkfield Rheinberg illumination with the powerful "BD" system of episcopic darkfield. As with darkfield imaging in general, it works the best and easiest with low magnifications, but with unusually "hot" camera settings, can work even with 100x BD objectives, as seen in Figure 8, a particularly challenging subject.

As with all techniques, certain subjects lend themselves to spectacular results, and with others, very little additional information is obtained. Sometimes the results are simply beautiful, but with careful inspection, useful topological information can be gleaned. Occasionally these images can be processed to obtain an approximate 3-D representation of the specimen from a single image.

The technique itself is fairly simple, with filters that can be crudely cut from colored thin plastic or even printed on transparencies. All images presented here use the cut shim-stock method, shown in Figure 5, with the exception of Figure 16, which used laser printed transparencies such as Figure 6. With a rotating specimen stage, some interesting effects can be observed with the non-symmetric illumination.

While the image brightness, contrast and color depth may be significantly reduced, it may be possible to use external illumination, such as from a ring of LEDs around the specimen, with colored filters, or even simply colored LED illumination (variable RGB LEDs could have interesting effects) from around the edge.

Along similar lines, approximately comparable results could be obtained in the larger world with colored spotlights around a subject, that has a 3-dimensional profile, which is photographed from above.

While the BD system technique described here is thus far not well documented, this isn't the first application of epi-Rheinberg. *Photography with a Microscope* by Fred Rost and Ron Oldfield (2000) describes an integrated circuit image that they believe is the first published example of the technique. Additionally, award-winning micrographer Spike Walker has produced some stunning images with Zeiss microscopes using similar techniques. Upon reaching out to Spike, he responded with the following details about his setup for epi-Rheinberg:

For the past 30 years my main workhorses for photomicrography have been Zeiss Ultraphots (see my website www.the-ultraphot-shop.org.uk). For epi-Rheinberg I used one of the epicondensers supplied for these instruments ('IIC' or 'IIIC') together with an annular darkfield 'Reflector D', x4, x8 or x12 'Epiplan HD' bright/darkfield objectives, and suitably coloured disks. My first disks were simply divided into 2 equal halves in contrasting colours or were similarly quartered, and were placed in slots in the epicondenser from where they could be rotated for best effect. Later designs were more 'weird and wonderful' but by then I was doing most of what little polished material I was called upon to photograph with epi DIC and have seldom visited the technique since. The attached examples were shot on Velvia in the 35mm photohead of the Ultraphot.

Spike has occasionally worked with epi-Rheinberg for approximately 20 years on the Ultraphot. A gallery of his work is at <http://www.microscopy-uk.org.uk/mag/artoct10/sw-gallery5.html>.

Many thanks to GaryB from PhotoMacrography.net for the perfect addition of John Travolta to Figure 4!



"That's the way...uh-huh, uh-huh, I like it!"

Email author: mtuell AT email DOT arizona DOT edu

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

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