KRISTIANSEN ILLUMINATION

A NOVEL MICROSCOPY TECHNIQUE

A short, illustrated introduction on the application of a new fast, cheap, and easy pseudo DIC illumination technique for high contrast live cell and micro animal imaging using a simple compound microscope.

By Martin Kaae Kristiansen, M.Sc.

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INTRODUCTION

My name is Martin Kaae Kristiansen and I have a master's degree in biomedical science. During my education I did a larger project using different microscopes and a technique called expansion microscopy. After that I fell in love with microscopy and the microscopic world. The next natural step was of course to buy a microscope of my own and start investigating different samples from nature, like moss, pondwater, marine water, lichen and so on. I quickly realized that I had to share all the amazing organisms I found with other people. For that purpose, I started '*My Microscopic World*' on social media like *Instagram*, *Facebook* *and* *Twitter*. My Microscopic World quickly grew and allowed me to work with large companies like *Motic Europe*, *LabCam*, *UNILAD*, *off-White*, as well as local and international television. As my audience grew so did my skills and in 2020, I got a 3rd place in Nikons '*Small world in motion*' contest.

With my relatively cheap microscope (Motic BA310E) and an iPhone I was able to produce beautiful images and videos. However, I was limited by the few available microscopy techniques for amateurs, such as regular brightfield, darkfield, polarized light, and oblique illumination. But naturally these were not able to produce images as other more expensive microscopes such as a *differential interference contrast* (DIC) microscope.

One day when I was filming a video using darkfield microscopy I adjusted the white balance on my phone with a piece of lens paper underneath the sample, and I noticed something. The contrast changed in an unexpected way. Over the next few months, I experimented a lot with different settings, materials, and filters to find the optimal setup for the new illumination technique I had discovered, and called '*Kristiansen illumination'*.

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THE SETUP – FAST, EASY, AND CHEAP

The overall setup to achieve this type of illumination is incredibly simple, and all it requires, other than a regular compound microscope, is a cheap plastic darkfield or oblique filter (*image 1*) and a coverslip with a piece of translucent tape on it (*image 2+3*).

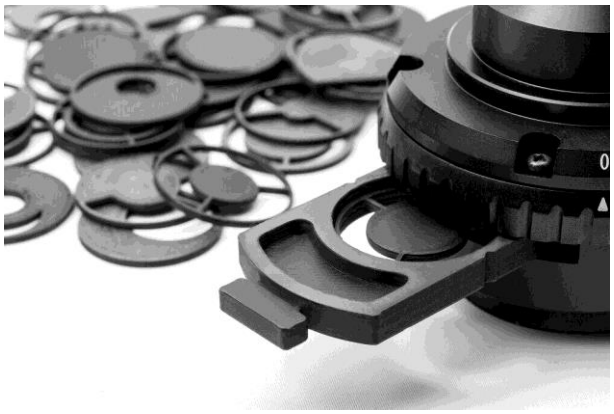

IMAGE 1: A 3D printed filter holder and different oblique and darkfield filters (Image from the seller *'bauras/ µstuff'* on eBay).

IMAGE 2: The translucent tape I use.

HOW TO DO IT

1. For the best results you will need a clean coverslip before you add the tape. Cheap coverslips are just fine but might need to be precleaned with some hot water and dish soap to get rid of dust and grease on the glass.

2. Make sure to use TRANLUCENT and NOT transparent tape. When applying the tape avoid getting small air bubbles stuck under the tape. This will yield the most uniform background with as few optical artifacts and disturbances as possible.

3. The coverslip with the tape goes on top of the condenser, tape side DOWN (image 3). Tape side up works as well, but in my experience tape side down gives a much better result.

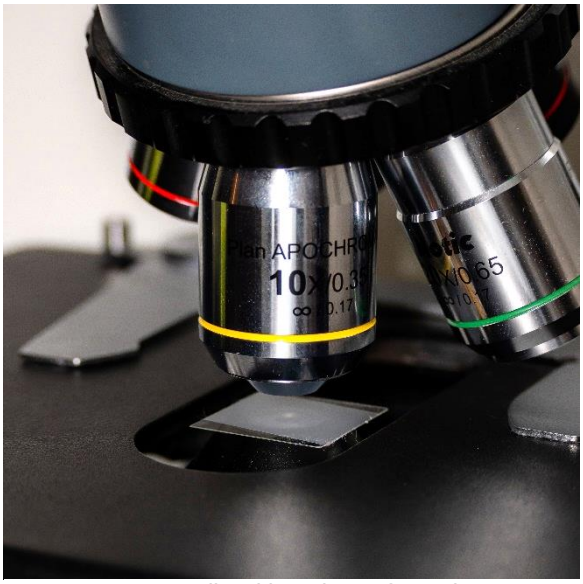

IMAGE 3: A coverslip with a piece of translucent tape on top of the condenser.

4. Apply a darkfield filter in the condensers filter holder. I use one of several cheap 3D printed filters bought on eBay[1] (image 4) but the one in image 5 is the one I use the most. Several kinds of darkfield filters and oblique filters will be able to achieve the contrast effect, but with different results. This will be discussed further down in the section '*Adjusting the illumination*'.

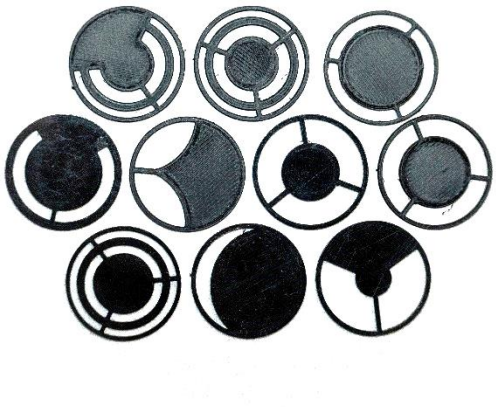

IMAGE 4: Different filters able to produce Kristiansen illumination when coupled with a translucent material.

IMAGE 5: The filter I use most of the time

5. As a starting point I set the condenser as high as possible and open up the condenser diaphragm and field diaphragm, if you have one, all the way. Now you are ready to get started. The adjustment of the illumination will be discussed further down in the section '*Adjusting the illumination*'.

HOW IT WORKS – A HYPOTHESIS

During my experiments with different materials, filters, and settings. Combined with my knowledge on how darkfield microscopy work, the following is my current hypothesis on how the contrast effect is achieved.

THE BASICS

The way a regular compound microscope produces an image, is by illuminating the specimen from below. The light then travels through a condenser which focuses the light onto a smaller area where your specimen is. The way the condenser does this is by bending the column of light from the light source and turning it into a cone of light instead. This yields an image of your specimen on a white background.

[1] https://www.ebay.com/usr/bauras

In darkfield microscopy we use a filter, like some of the filters in image 4, to block some of the light from the source before it gets to the condenser and gets turned into a cone. When the center of the light column gets blocked the result is a tube of light. And when the tube hits the lens in the condenser it gets converted into a hollow cone of light (image 6). This means that there is no longer any light to illuminate the background but only light coming from the sides at an angle illuminating the specimen. Which yields a “glowing” specimen on a black background. You can find a short video describing darkfield illumination here[2].

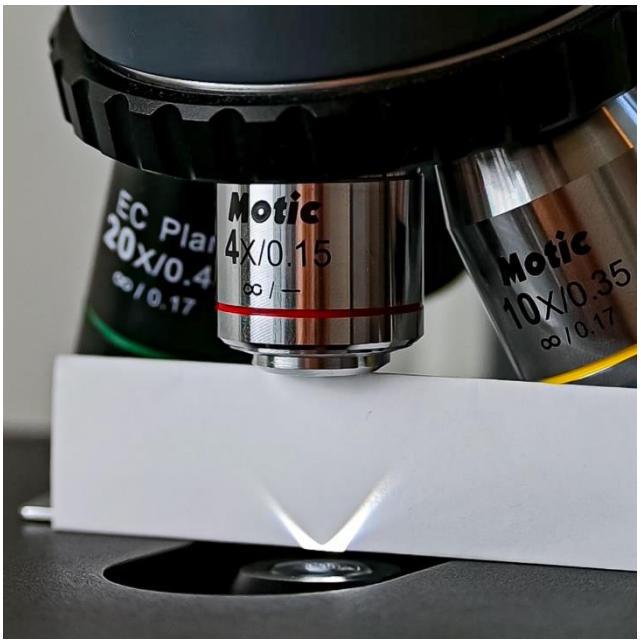

IMAGE 6: Visualization of the hollow cone of light in darkfield microscopy.

Now, if we add a piece of translucent material on top of the condenser, we can scatter the darkfield light coming at an angle to “re-illuminate” the background turning it grey. And because the light is still coming from an angle, due to the filter, the intensity will be greatest from certain angles (depending on the type of filter used). All of this together provides a circular oblique illumination on a greyish to blue background, thereby enhancing contrast while still utilizing the increase in color vibrance from darkfield microscopy.

But to enhance contrast even further we can make a few different adjustments.

ADJUSTING THE ILLUMINATION

Depending on the microscope there are a few different adjustments to make, besides light intensity:

1. Condenser height
2. Condenser diaphragm
3. Field diaphragm (not on all microscopes)
4. Filter type

Through my experiments I have found what works best for me, and the following is the work flow I usually follow to adjust the illumination. As mentioned in step 5 of the setup, as a starting point I set the condenser as high as possible and open up the condenser diaphragm and field diaphragm, if you have one, all the way.

[2] https://www.instagram.com/tv/CQZFYRNjVRI/?utm_medium=copy_link

CONDERNSER HEIGHT

When starting at the top position the background might have a few artifacts and irregularities. The condenser needs to be lowered until these disappear, and a uniform and smooth background has been achieved. However, be careful not to lower it too much as this will affect the light intensity very quickly. The ideal height would be as high as possible to retain as much light intensity as possible.

CONDENSER DIAPHRAGM

The condenser diaphragm can be close a bit to get rid of a bit of noise and to lower overexposed highlights. Closing the condenser diaphragm will also affect the light intensity quickly, the ideal adjustment is therefore as little as possible.

Adjusting the condenser diaphragm will also shift the color to a more yellowish tone (at least when this illumination is done with an LED light source). So, it is possible to compensate for a very blue/green shifted image by closing the diaphragm a bit.

FIELD DIAPHRAGM

This adjustment will make the background darker while retaining much of the light intensity coming from the specimen itself. While fully opened, the background will be a light grey color (depending on the filter type) and as the diaphragm is closed the background will shift towards a black darkfield background.

FILTER TYPE - FROM GREY TO BLUE BACKGROUND

To simplify the explanation of filter type I will explain it in respect to three regular darkfield filters. One with a small center stop, one with a medium center stop, and one with a large center stop. The smaller the center stop the greyer the background will be. By increasing the size of the center stop the color will shift to a bluer tone. Furthermore, depending on the objective magnification the center stop needs to be bigger as the objective magnification increases to obtain the illumination effect. I found that a medium size stop will give the perfect balance between grey and blue for both 4x, 10x and 20x objectives, and the rest can be adjusted with the other adjustments mentioned above.

Irregular filter shapes impact the illumination in a different way but should follow the same ground principles as described here.

GEAR AND SAMPLE REQUIREMENTS

GEAR

All my experiments and images have been done using a Motic BA310E with an LED light source. The objectives I have tested this illumination technique with are both Motics EC plan objectives, as well as Motic plan APO objectives. This revealed that the effect is only possible to achieve in the same range as a darkfield illumination. For my microscope that means I am able to use the effect with my 4x, 10x and 20x objectives. However, not my 20x plan APO objective but perfectly with my EC plan objective. I suspect this is due to the high NA of the plan APO objective compared to the condensers. My hypothesis is therefore that a condenser with a higher NA, or objectives with lower NA might work at higher magnifications as well.

Even though I have only tested the illumination on my Motic BA310E, I know others that have tried it with other bands as well with good results.

SAMPLE

The samples and specimens I found to work best with this illumination are large cells such as frontonia spp., stentor spp., vorticella spp., spirostumum spp. and more. As well as micro animals such as tardigrades, rotifers, small worms, and crustaceans (for examples see image section further down).

IMAGES

The following images are all produced using a Motic BA310E with an LED light source.