

How to turn your microscope into a phase contrast microscope

Gerard 't Hooft

Institute for Theoretical Physics
Utrecht University

Postbox 80.089
3508 TB Utrecht, the Netherlands

e-mail: g.thoof@uu.nl
internet: <http://www.staff.science.uu.nl/~hooft101/>

Abstract

Many microscopic samples are highly transparent so that they produce little contrast when viewed in an ordinary microscope. However, they often do produce small phase rotations, due to local variations of the index of refraction. Phase contrast is a technique to turn phase differences of light into intensity differences. It was invented by Frits Zernike, a revolutionary invention that earned him the Nobel Prize in 1953. He achieved that the light variations seen, accurately represent phase shifts. Here, I ask a simpler question: if I just want phase shifts to turn into intensity differences, what is the easiest way to accomplish this, given a very mundane microscope? In this short treatise I illustrate the method I found when I was a student in theoretical physics. My microscope indeed produced impressive images.

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The method proposed here, at first sight, seems to be not much more than a somewhat unusual way to illuminate an object from the back, while avoiding direct light to enter into the eye piece.

Imagine a small opaque (black) disk attached in the middle of the objective lens. Then, when illuminating an object to be studied in the microscope, we first arrange the condenser lens in such a way that it projects an image of the light source on the disk; consequently, the disk actually blocks the light from entering the microscope, and direct light will not be seen. When we put an object in the appropriate place, it will be illuminated from the back, and if it diffuses the light, then that light will enter the objective away from the disk. One then expects to see the object illuminated, while surrounded by a black background.

See Figure 1. At this point, one would comment that this seems to have little to do with phase contrast; we are dealing with classical optics here. Now, one *could* argue that any object with variations in its index of refraction, would scatter infalling light, so a relation with phase contrast can be suspected. But does this really have anything to do with the real thing? Let me here explain why it does indeed.

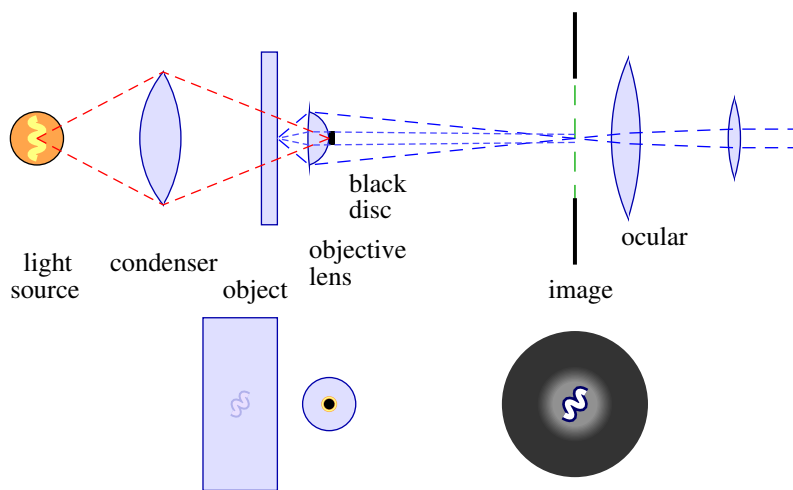


Figure 1: Schematic setup (not to scale)

Consider the case when the diameter of the disk is quite small compared to the diameter of the objective. actually, this was the configuration that Zernike wanted to avoid: focussing the light source on the disk would force us to use a very tiny light source, and this implies poor lighting. Indeed, this is a price to pay. In practice, we will have to search for the optimal diameter of the disk. I found that, choosing the disk to be about $1/10^{\text{th}}$ to $1/20^{\text{th}}$ of the diameter of the objective, will serve our purpose quite well.

Now let us use the language of wave mechanics to describe what happens. First, take the case that there is no object at all. If there is no disk, we will see an even distribution of light in the image. When putting the disk back, the image becomes dark.

In terms of wave mechanics, we now argue that the disk acts as a light source with the light in opposite phase as compared to light coming from the source directly. We regard the black image that is produced as being the consequence of completely destructive interference.

But the two interfering light sources are not identical. The original source comes through the entire objective. The interfering effect from the disk is passing through a 'lens' that replaces the disk, so that the diameter of this beam is much smaller.

Now, consider an object in the proper focus point of the microscope. The light that passes through the entire objective is focussed well in the image plane, so that produces a sharp image of the object. However, the destructively interfering light from the disk has a diameter that is so small that it cannot generate such a sharp image. Therefore, what we will see is two images interfering with one another: a sharp image of the object, and a diffused image, where all details are smeared. If the disk is $1/10^{\text{th}}$ of the diameter of the objective, and the microscope works close to the optical limit, then the details of the secondary image are smeared by a factor 10.

In short, we see the image interfering with a blob of light about 10 times the diameter of the sharp details that we are interested in. In practice, this is often what we want. The diameter of the blob of reference light is not as large as it could be in the more professional phase contrast devices, but it can serve its purpose quite well.

Note that, in the absence of any object with shifted phases, the image will always be dark. If there is an object, we will see light, but that does not reveal whether the phase shift is positive or negative. To make the sign visible as well, one needs more professional devices, for instance, a transparent disk that shifts the phase by some fixed angle. A black disk can be attached simply by putting a dot of black ink on the objective lens. For studying microscopic life, this simplified setup may often suffice.

Finally, how does this compare with the real thing? See the Wikipedia article on phase contrast. In modern phase contrast techniques, one uses one or more rings rather than disks or dots. The rings produce background blobs, just as my disk or dot does, but they have a larger surface, while less light is wasted. This is obviously an advantage, though the disks are much easier to produce. Furthermore, one can make the disks or rings to rotate the phase (by etching, for instance), rather than having them simply opaque. This yields qualitatively much better results.

So yes, the best way to produce phase contrast is to buy a good microscope equipped with this capability. But if you want to know what phase contrast is, and how it works in principle, you can make it yourself.