NOVEL METHODS FOR AXIAL INTERFERENCE CONTRAST MICROSCOPY

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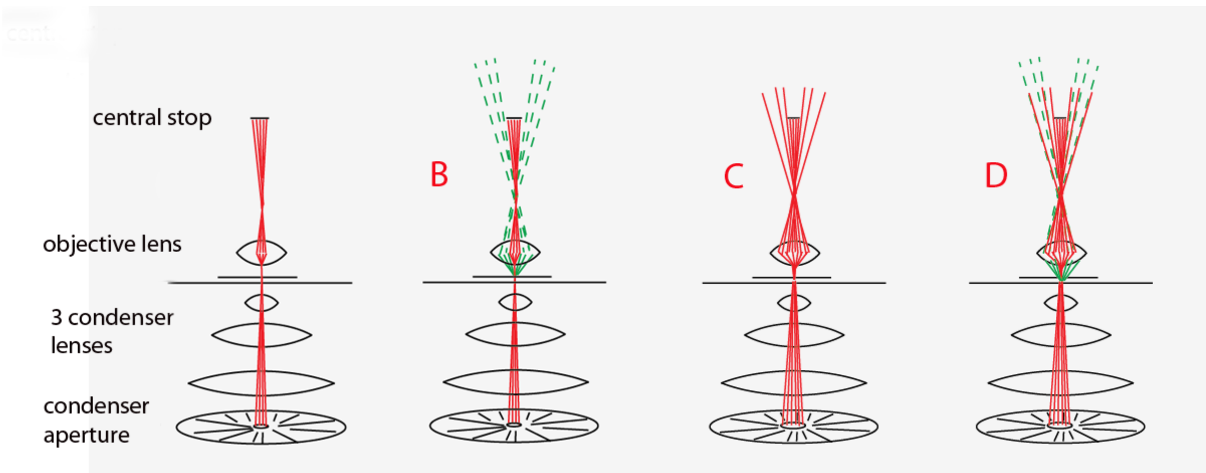
Below is a diagram that shows Axial Interference Contrast for light microscopes (AIC). This particular method is one of the simplest Innovations of Joerg Piper. It uses a small solid disc stop mounted above the objective within the back focal plane. The disc stop obscures the optical axis, leaving a shadow in the centre of the image. It also uses a narrow cone of illumination. The angle of the illuminating cone can be controlled by the condenser aperture and / or by using a mask within the condenser.



A Small axial aperture. All unscattered light is blocked
B Small axial aperture. Most scattered light is NOT blocked, resulting in axial darkfield luminance contrast
C Slightly wider axial aperture. Some unscattered light is not blocked, resulting in a partial brightfield image
D Axial interference contrast superimposes a partial luminance darkfield image on top of a partial brightfield image, resulting in light interference

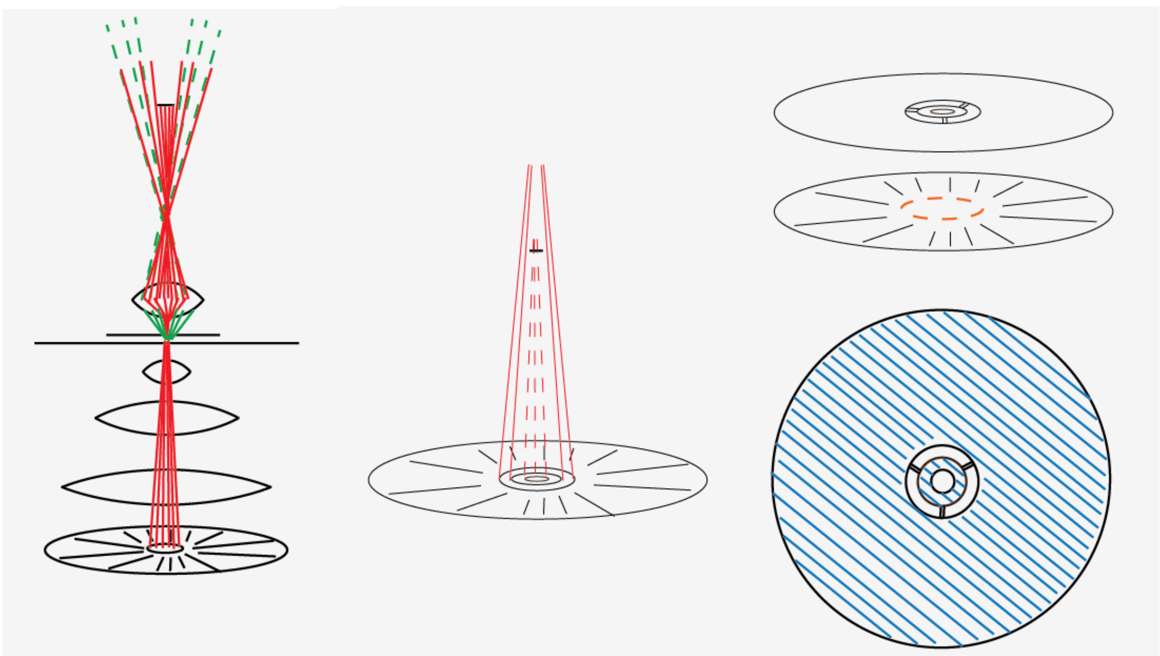
SIMPLE PIPER AXIAL STOP ILLUMINATION METHODS

Figures 1A and 1B show a narrow Axial illuminating beam. Photons that do not undergo scattering will all strike the solid disc obstruction. Other photons that are scattered when being refracted through this specimen undergo scattering in many directions. The diagram shows that some of the scattered light is not blocked by the solid stop (green dashed lines). This light can pass up the microscope to the eyetube. The resulting image is a darkfield image. Joerg Piper gave the name “luminance darkfield contrast” for this type of image.

In figures 1C and 1D, the diameter of the condenser aperture has been widened, resulting in a slightly wider cone of Illumination. In this case, some of the unscattered light can now pass into the eyetubes (solid blue lines). The resulting image is actually composed of two superimposed images. A darkfield luminance contrast image is superimposed on a brightfield image. The light waves in both these images undergo interference which results in visual contrast similar to phase contrast and DIC. Piper gave the name luminance interference contrast for these types of images. in my videos and articles I have used the word Axial Interference Contrast instead.

Piper noted a comparison between conventional darkfield images versus axial darkfield images. He found that the axial method was superior for showing internal detail. On the other hand, conventional darkfield microscopes use a thin illuminating cone with a very wide angle. This can be useful to highlight edge details, but often internal detail can be lost. The typical blooming and halo artifacts seen in phase contrast and conventional darkfield were absent or greatly reduced.

The next diagram shows a modification of the condenser, using a mask. The mask has a small central hole as well as an adjacent thin concentric ring. If light is allowed to pass up through the thin concentric ring, an axial interference contrast image can be formed. In this improved method, the angle of light in the brightfield image is now slightly wider. This can improve the amount of contrast in the image. Piper noted that it was necessary to adjust the diameter of the condenser aperture to balance the brightness of the darkfield image against the brightness of the brightfield image to optimise the amount of contrast in the image. If brightfield tends to dominate, then Interference contrast relief is greatly reduced.



Joerg Piper commented that there was some significant loss of wasted or blocked light within the condenser. In some cases it was recommended to use a brighter lamp source. Piper also recommended using colour contrast by using various different colour filters. This idea will be discussed again later. Joerg and Timm Piper have also developed some other more advanced innovations that are beyond the scope of this article.

Below is a diagram used by Piper from his article at www.luminance-contrast.com
Note the side view of the condenser mask showing the small central hole and the adjacent annular ring. In the diagram, the condenser aperture is closed down such that no light passes through the annular ring. This is the configuration for pure luminance darkfield. If the condenser aperture is opened further to allow light into the annular ring, a brightfield image is superimposed onto the darkfield image. These images undergo interference to allow luminance interference contrast (or Axial Interference Contrast).

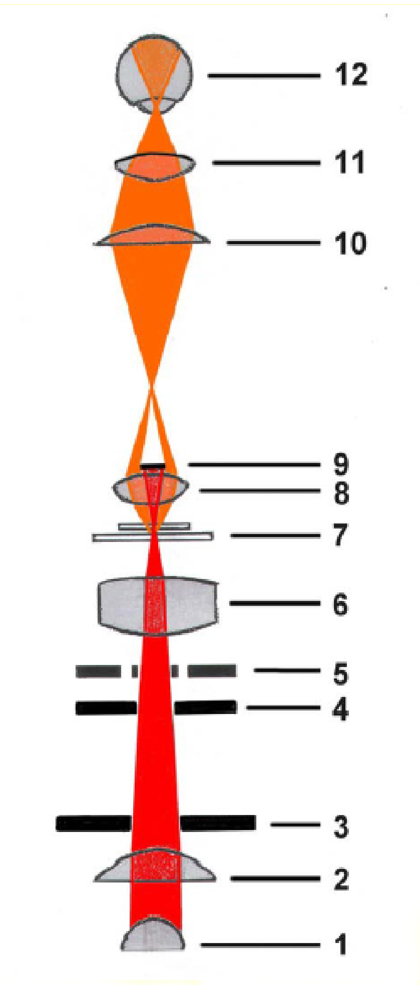
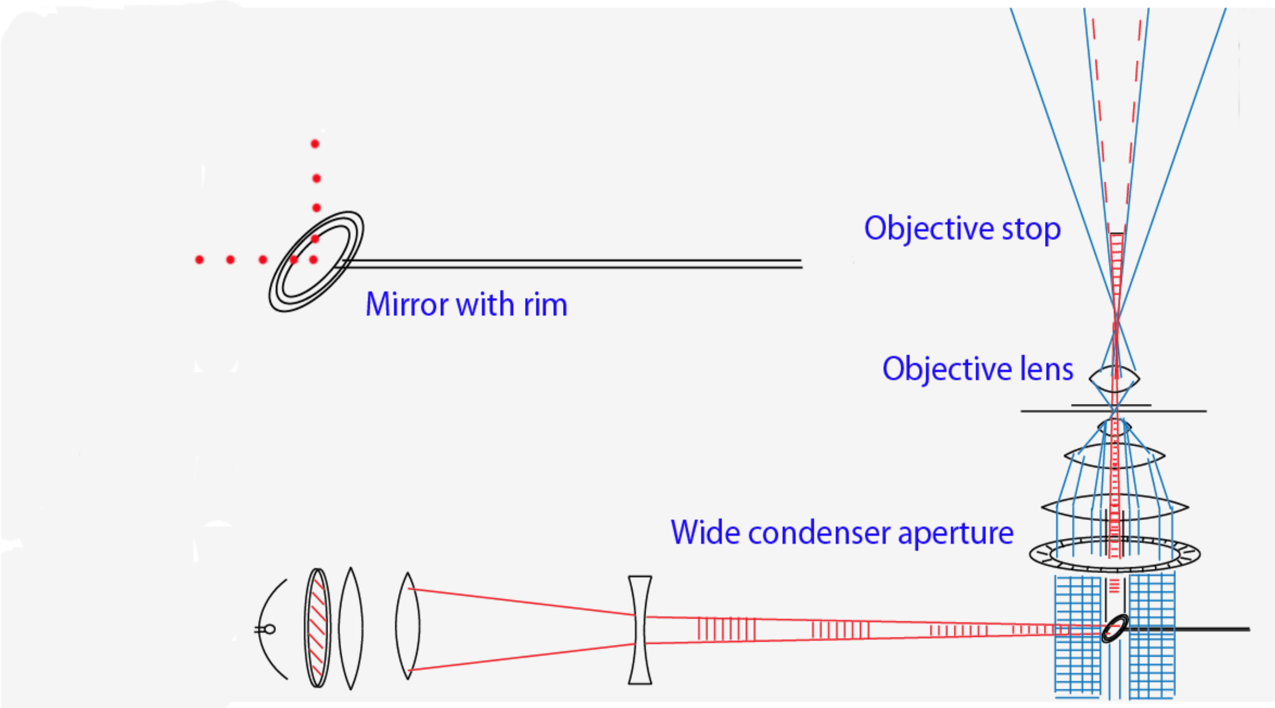


Fig. 3: Light path in luminance contrast / luminance dark field

1 = light source
2 = collector lens
3 = field iris diaphragm (light diaphragm, Köhler illumination)
4 = condenser aperture diaphragm (iris diaphragm)
5 = special light mask (facultative, see fig. 7b)
6 = condenser
7 = specimen (slide and cover slip)
8 = objective
9 = light stop (within the objective´s back focal plane)
10 = eyepiece (field lens)
11 = eyepiece (loupe lens)
12 = eye

MIRROR AXIAL INTERFERENCE CONTRAST CONCEPT

The diagram below shows a proposed novel method that can allow all of the light from the lamp to be used to form the image, with minimal wastage. I have called this the Mirror Axial Interference Contrast concept. The condenser aperture can be fully opened if desired.

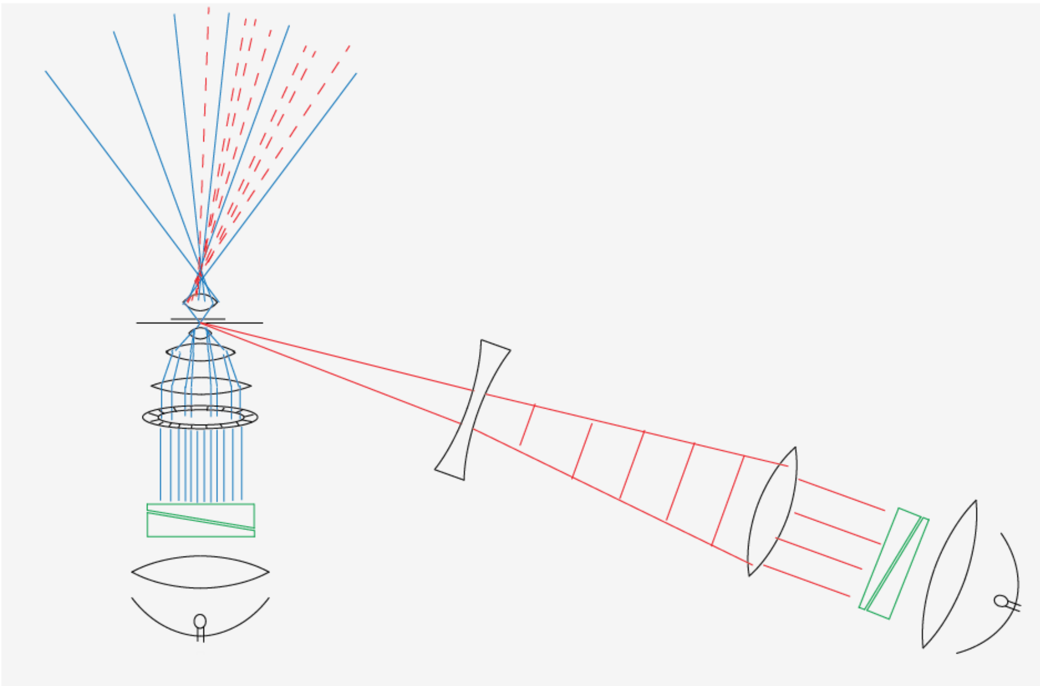


MIRROR AXIAL INTERFERENCE CONTRAST CONCEPT Copyright 2018 Alan Blood

This diagram shows an axial illuminating beam in a contrasting red colour. The axial darkfield illuminating beam is reflected off a 45 degree mirror mounted into the optical axis of the condenser. The mounting plate for the mirror will obstruct the blue transmitted beam, creating a shadow in the centre of the blue brightfield Illumination beam. The full brightness of the horizontal lamp can be used if desired. In some cases, it might be necessary to reduce the intensity of the transmitted beam. As discussed by Paul Martin, the use of wide open aperture can maximize the theoretical resolution within the brightfield image. One disadvantage of wide aperture is a very narrow depth of field in the brightfield component. The depth of field can be increased by closing down the condenser aperture, however the theoretical resolution would be decreased somewhat. In these innovations, the darkfield component has large depth of field, so it might not be necessary to close down the substage condenser aperture.

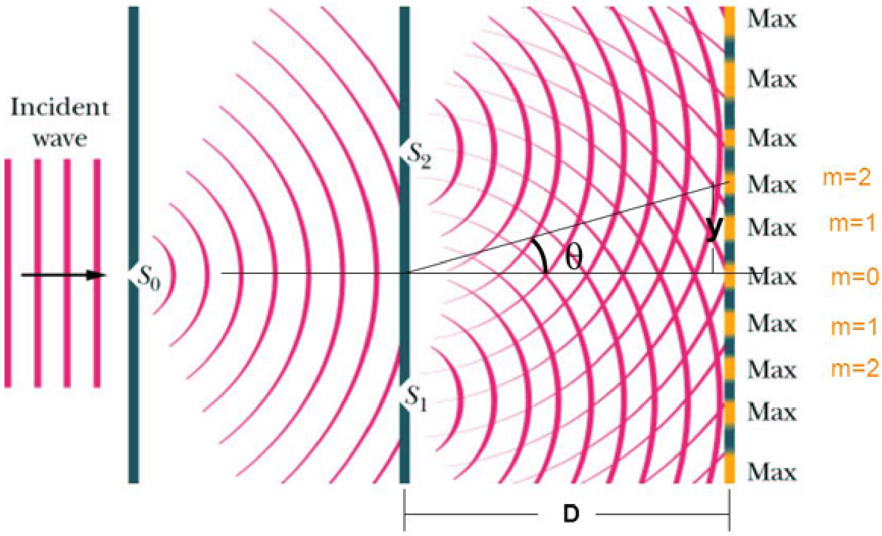
RIFE TWO-COLOR ILLUMINATION

The next diagram shows the configuration that Royal Raymond Rife may have used for colour contrast. It uses a narrow highly oblique monochromatic beam shining from one direction, which creates a red darkfield image. This is superimposed on a blue brightfield image. The diagram also shows how Rife used Risley prism monochromators instead of color filters (shown in green). In this example, the substage condenser aperture is wide open. Note that here, there is no objective stop used, so axial interference contrast would not occur. However some cases, visual contrast is obtained between red detail and blue background, similar to Rheinberg color contrast.



SOME PHYSICAL CONCEPTS IN INTERFERENCE CONTRAST

When light passes through two fine slits, or when light passes on either side of a small object in a microscope image, we can see diffraction effects. One simple diagram shows the Airy disk. This is the image of a very fine point seen through a lens, sometimes called the Point Spread Function. Here we can see the bright central maximum, and on either side we can see bright and dark rings caused by constructive interference and destructive interference. The brightest central zone is called the zeroeth order bright spot. The adjacent rings are the higher order rings, or diffraction circles, such as first order, second order etc. Higher order rings are sometimes known as “sidebands” or “side lobes” of light.



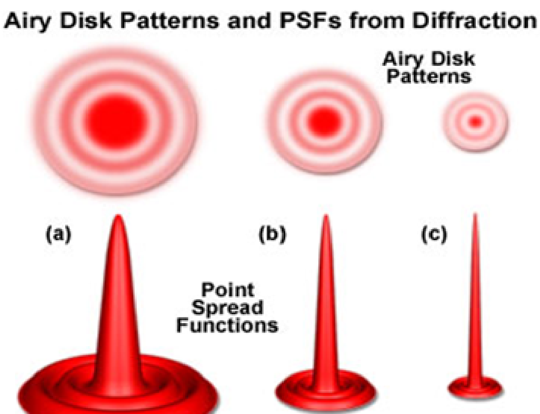
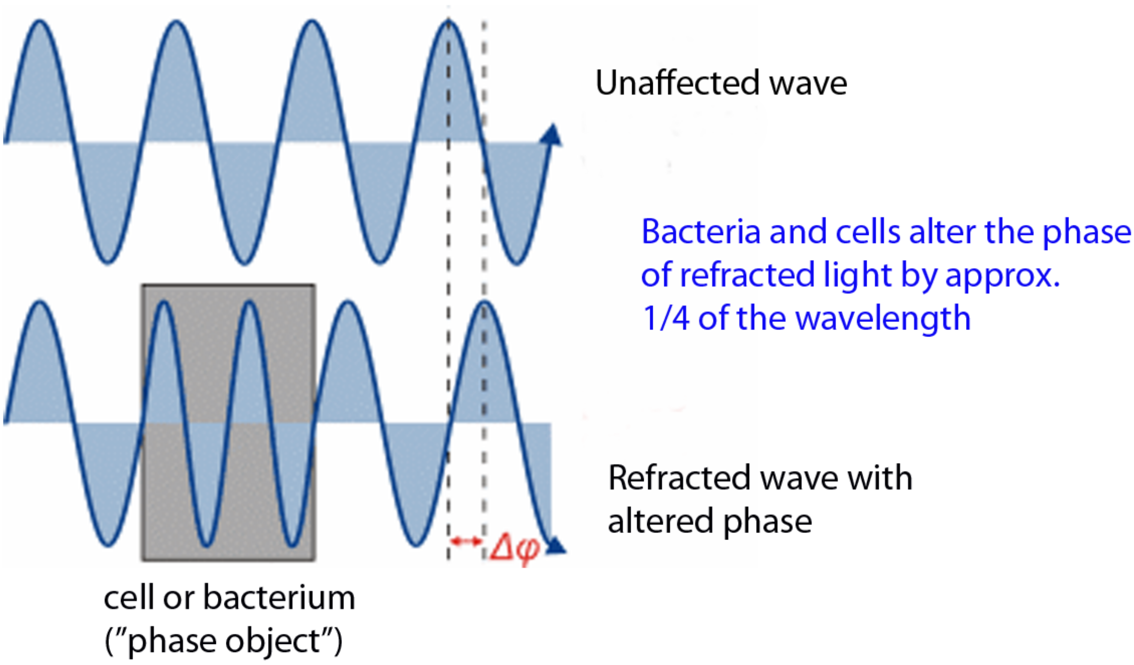


Figure 5

Biological materials like bacteria and cells tend to alter the phase of refracted light by approximately 1 / 4 of a wavelength, or 90 degrees. These can be called “phase objects”. Objects that are transparent are invisible in an ordinary microscope, but they can easily be observed using Phase Contrast microscopes or by using the Piper method.

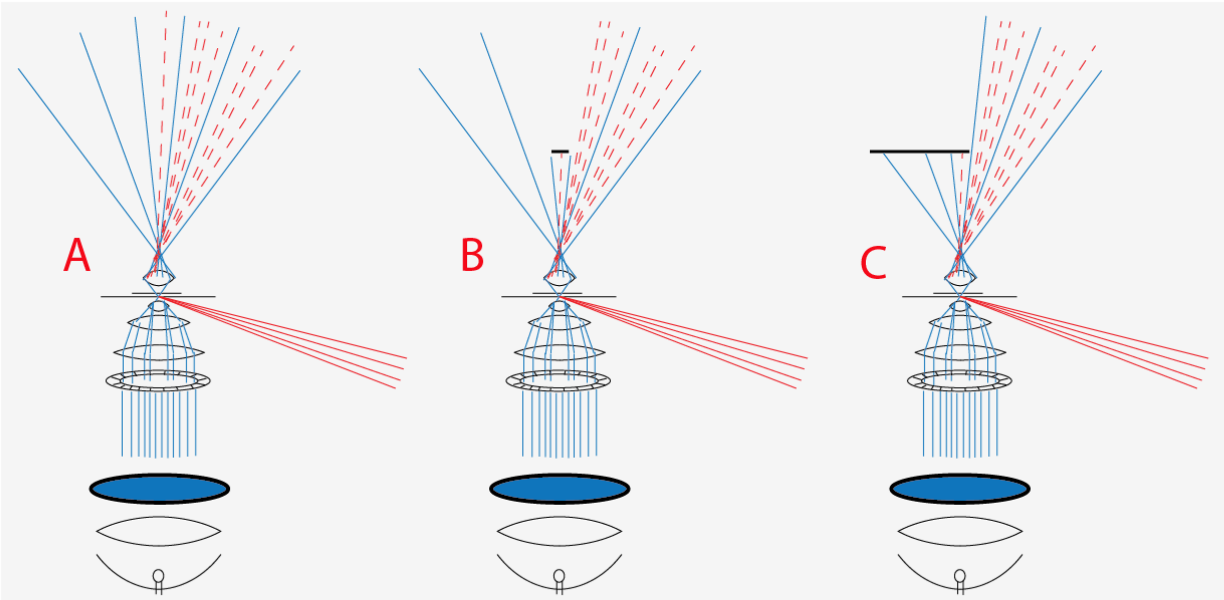


In methods of oblique microscopy, the source of light is unbalanced, for example by moving the condenser slightly to one side, or by masking part of one side. The higher order sidebands progressively increase in phase shift with respect to the phase of the zeroeth order. In balanced conditions, interference effects of higher sidebands tend cancel each other out, with no net effect on the zeroeth order light. However unbalanced lighting can result in suppression of the side bands on one side, allowing the remaining sidebands to interfere with zeroeth order light, giving a directional highlight and shadow effect.

In the Piper, method the zeroeth order is absent in the darkfield component, but the higher order sidebands can interfere with the zeroeth order within the brightfield component.

AXIAL CONTRAST IN RIFE ILLUMINATION

Now we discuss the Rife two-color illumination scheme again to show how Rife could achieve axial interference contrast.



TWO-COLOR ILLUMINATION USING
A LARGE ECCENTRIC OBJECTIVE STOP

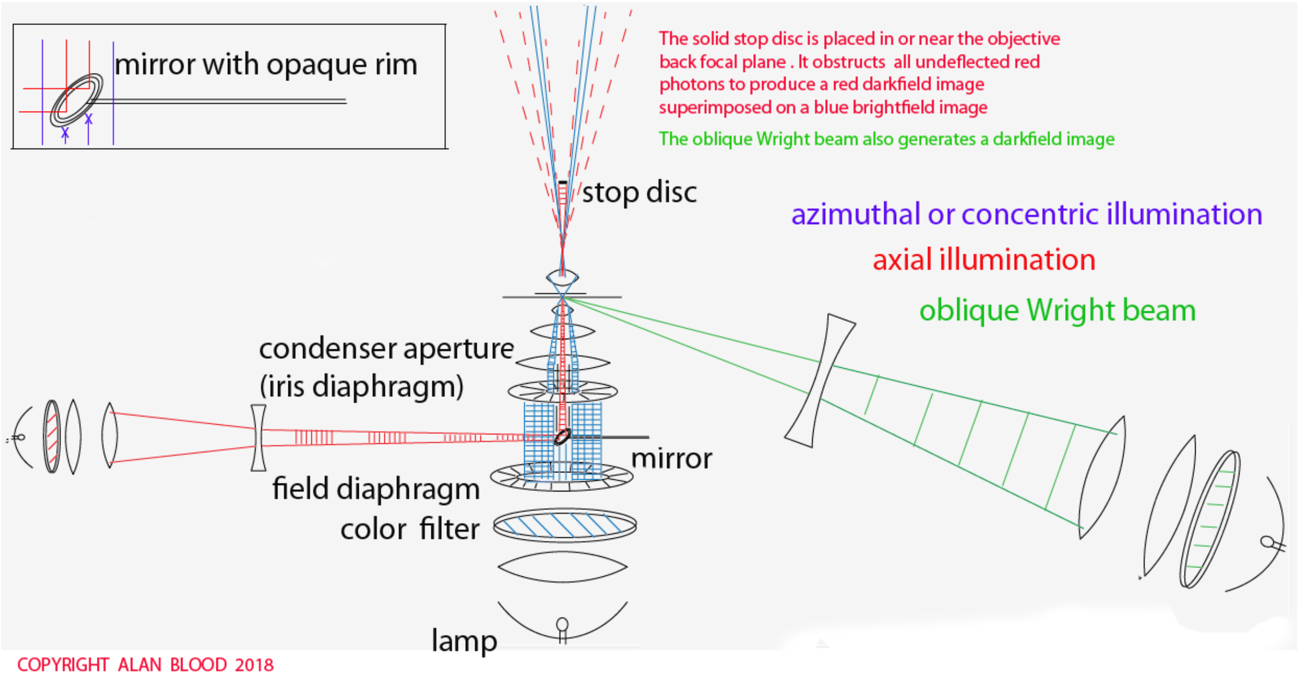
The first diagram on the left shows the simplest system using a wide condenser aperture to generate a blue brightfield image. A red highly oblique beam generates a superimposed red darkfield image. Scattered light of very finely resolved detail can enter the objective. This maximizes resolution in the plane perpendicular to the beam. Also, because the light in the image is unbalanced between left and right sidebands, oblique interference contrast can be observed that has a directional relief effect. Using two colors can often improve visual contrast.

In the middle diagram, a small solid disc stop is placed above the objective. In theory this generates a blue darkfield component, However in this example, contrast is not visible because the brightfield component is too intense. It would be necessary in wide aperture AIC applications to restrict brightfield intensity e.g. by using a neutral density filter with a central axial hole, or to use a mask to create a wide hollow peripheral light cone, or to use a mask with radial arms.

In the third diagram on the right, a larger solid stop has been used in an eccentric or offset position. It obstructs the optical axis as well as the whole left side of the image. It also creates a new directional oblique contrast effect. The large stop can be modified to an L-shape that obscures the optical axis plus three quadrants of the image. The unobscured quadrant can be digitally cropped to fill the monitor screen. If desired, pixel density can be increased within the camera, allowing ultra-high empty magnification images e.g. 6,000 X to be captured.

THREE-COLOR AXIAL INTERFERENCE CONTRAST

The diagram below shows a three-color contrast configuration that combines the Rife dual illumination system with the mirror Axial interference illumination system. In this example, the condenser aperture is partially closed down. It may be also possible to use wide aperture.



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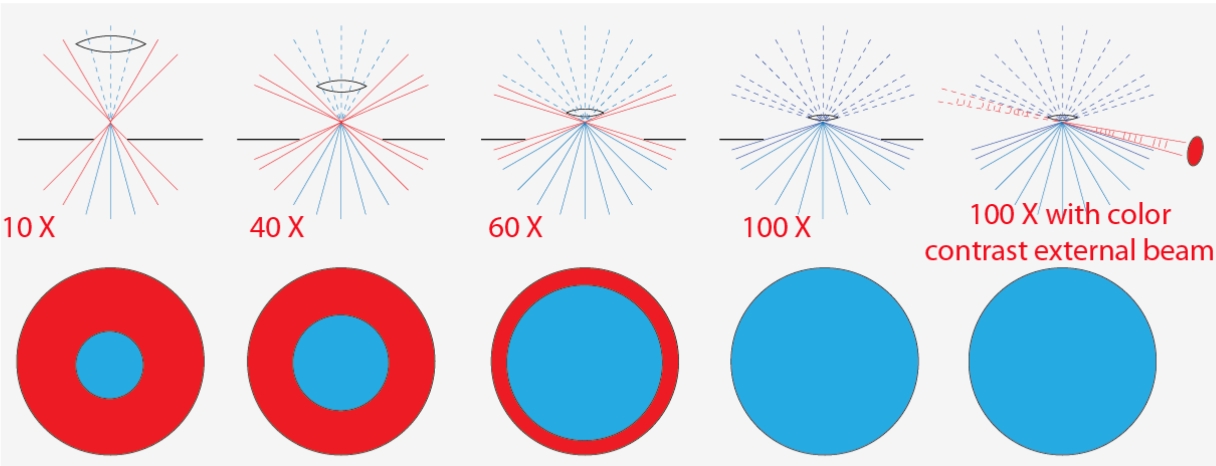
THREE-COLOR AXIAL INTERFERENCE CONTRAST CONCEPT

In these innovations, axial interference contrast images can be obtained that are brighter than images obtained using the Piper method. In some cases this can allow the images to be highly magnified without suffering loss of brightness.

ACCEPTANCE ANGLES FOR DARKFIELD COMPONENT

The diagram below shows the layout for simple Rheinberg color contrast filters for objectives with NA increasing from 10 X up to 60 X. For 100 X objectives, brightfield illumination fills the acceptance aperture of the objective. Therefore red darkfield superimposed images can only be achieved by using a narrow focused external Unidirectional Highly Oblique Monochromatic Beam (UHOMB) with long focal length. Alternatively, Paul Martin has used a concentric array of external LED sources (not shown), but no means of beam focusing was available, which limited Martin's darkfield intensity.

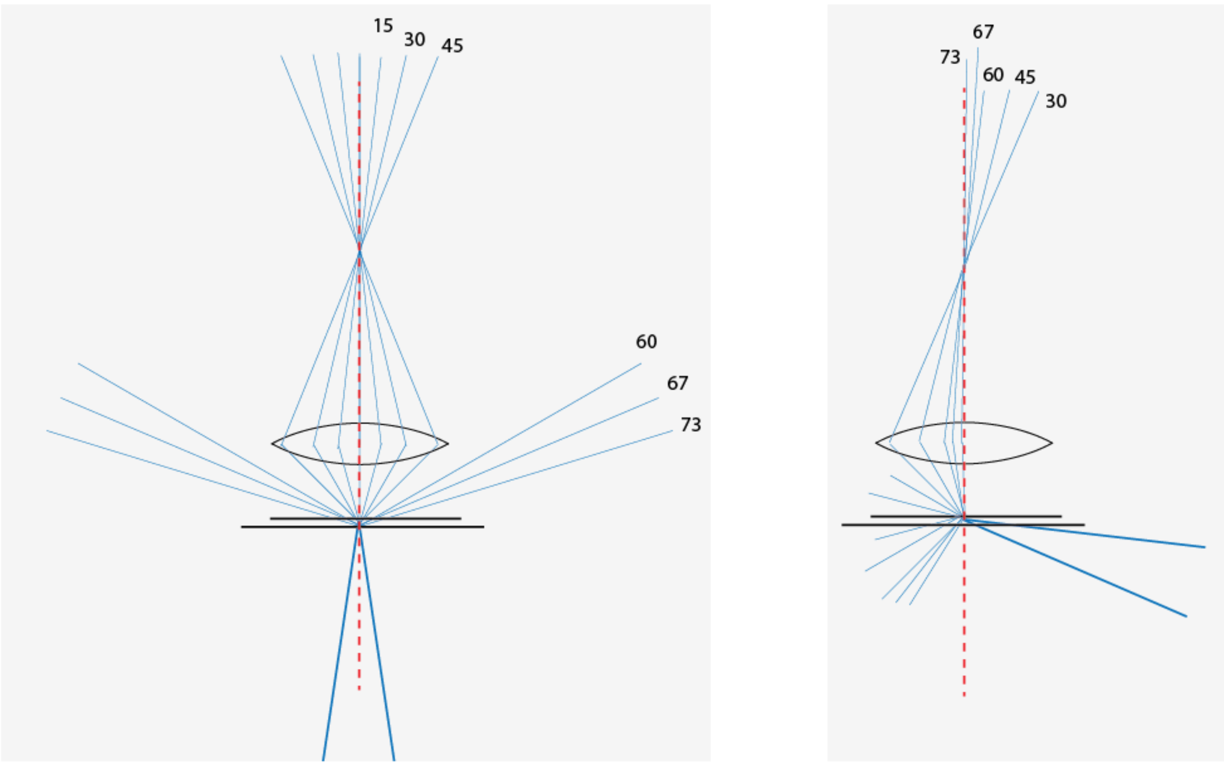


Scattered red photons can enter the objective

COLOR CONTRAST AND DIFFRACTION CIRCLES

ENHANCED DARKFIELD RESOLUTION USING UHOMB

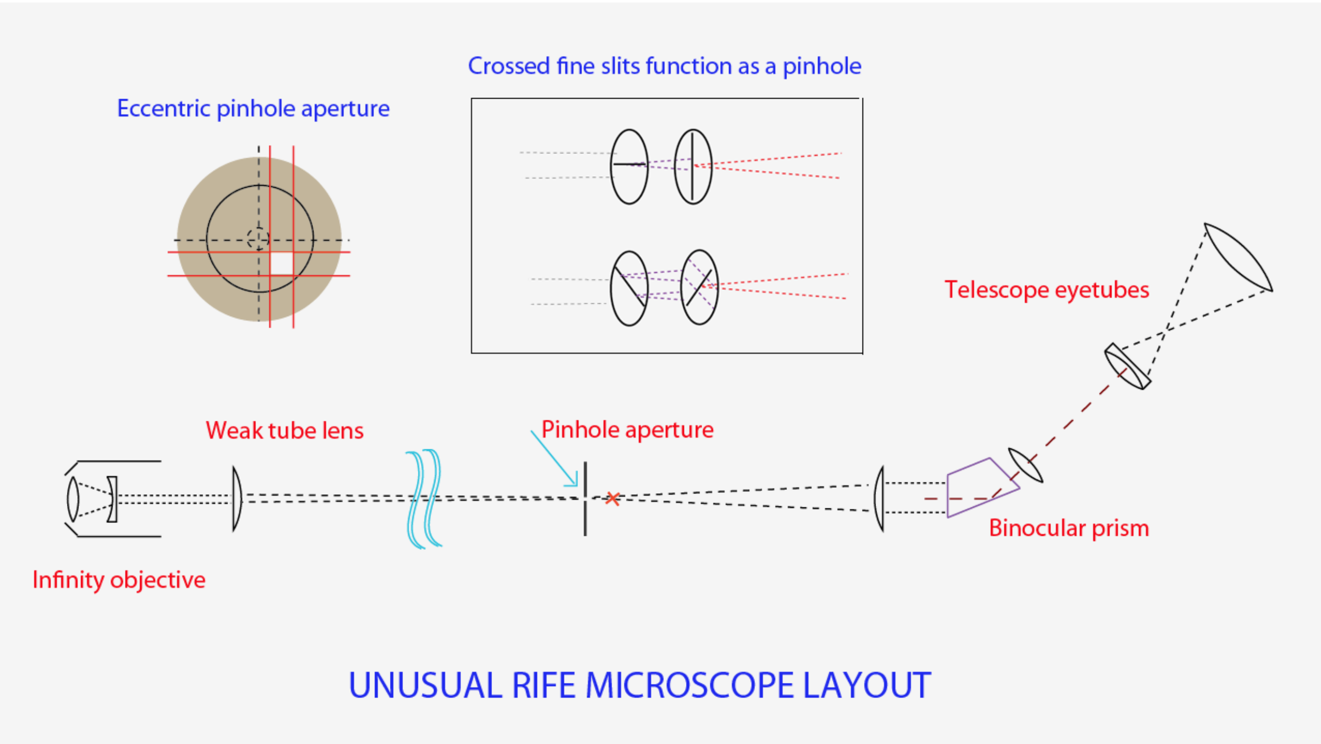
The next diagram shows how the widest diffraction circles can enter the objective when using a highly oblique monochromatic illuminating beam (right side) compared to substage illumination (left side). In theory this would give some improvement to resolution of the red darkfield component in the plane normal to the oblique beam axis. Note also that the highest order scattered red light becomes concentrated in a single quadrant of the image. It is proposed to align the L-shaped AIC stop to allow this quadrant to be unobscured.



HIGHLY OBLIQUE ILLUMINATION ALLOWS IMPROVED RESOLUTION BECAUSE THE HIGHEST ORDER SIDEBANDS CAN ENTER THE OBJECTIVE APERTURE

RIFE MICROSCOPE PINHOLE FUNCTIONING AS AN AIC STOP

In a previous video and article, I presented an unusual design for Rife bench microscopes that uses a pinhole created by two intersecting fine slits. If the pinhole is positioned eccentrically offset from from the optical axis, the resulting image is similar in concept to the example presented above using the large L-shaped objective stop.

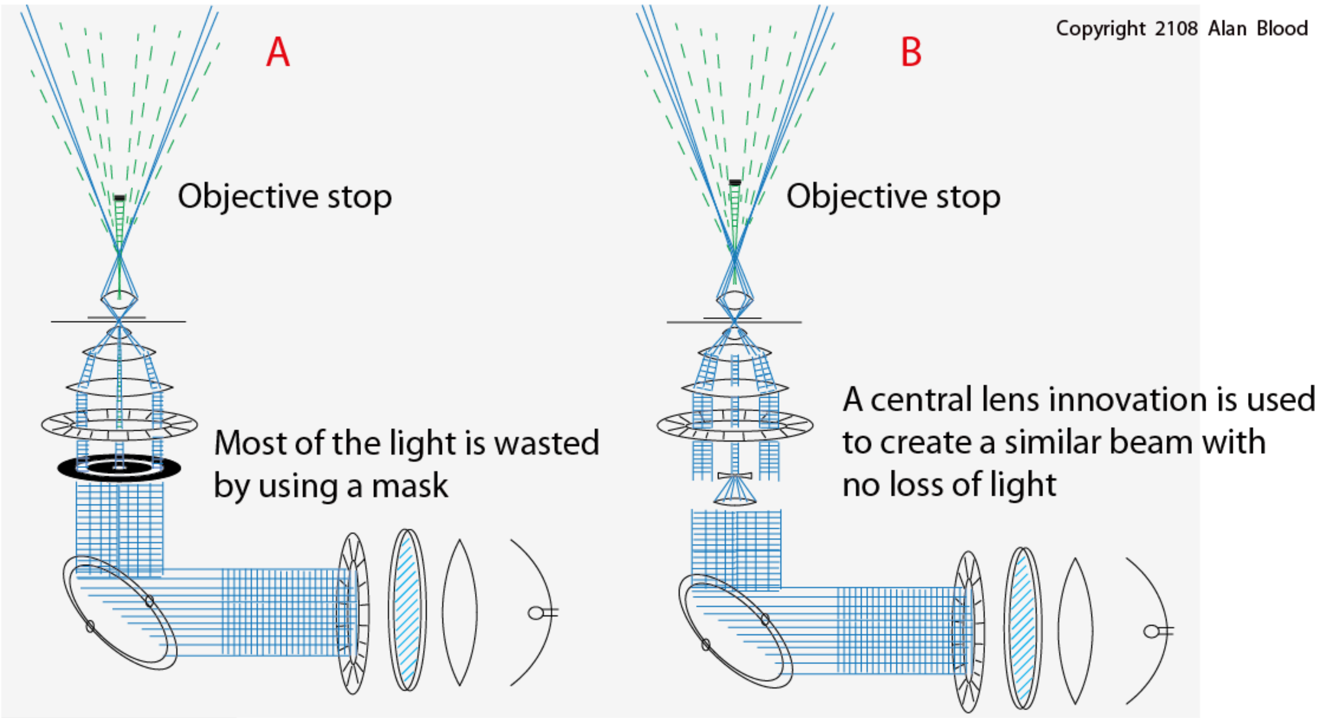


The original Rife microscopes may have used a method of projecting an expanding beam emerging from the pinhole before it reaches the ocular to achieve ultra-high magnifications. Thus the pinhole expansion effect would create a third stage of magnification.

A simpler method to increase the magnification using AIC in conventional microscopes might be to simply increase the pixel density within a digital camera. Typically digital microscope cameras might use a pixel diameter of 100 nm. (The convention is pixel sampling at double the nominal resolution of 200 nm). The pixel density could in theory be increased to 50 nm. Thus a typical image magnification of e.g.1500 X could be increased up to 6000 X if desired. In many cases the optical resolution cannot be improved simply by increasing magnification. However in some cases e.g. where fine line detail can be observed in a sparse or empty background, increased magnification can be useful. In images that include a large central shadow, the monitor display can show (for example) only the top left hand corner of the large image.

AIC CENTRAL LENS INNOVATION

Another suggested AIC innovation is to use a central lens assembly beneath the condenser to concentrate a relatively wide central cone of light into a narrow axial beam (figure B). This innovation greatly increases the axial darkfiled illumination intensity. It would thus improve the balance between brightfield and darkfield intensity in wide aperture applications, and would allow brighter images because there would be less need to restrict brightfield intensity. The method also may allow mutual coherence to be retained between the two beams because they derive from a single source. For comparison, a typical configuration for AIC using a mask is shown in figure A. It may be possible to substitute the blue color filter with a simple Rheinberg color filter with an appropriate inner diamater to generate contrasting colors in the central versus peripheral beams (not shown).



AXIAL INTERFERENCE CONTRAST WITH CENTRAL LENS INNOVATION