Rheinberg Stop Contrast Illumination

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Introduction

RHEINBERG DIFFERENTIAL CONTRAST ILLUMINATION is a development of Darkfield Illumination where the usual opaque patch stop is replaced with a transparent, coloured stop and the use of a transparent, coloured annular stop in the path of the usual white light. The result from this combination of coloured central and annular stops are specimens appearing in any colour on a background of any other colour. This paper describes a variation of Rheinberg Illumination that further raises the level of information in transluscent and transparent specimens, and with those specimens having sufficient depth, produces a pseudo-stereoscopic image.

One problem with Rheinberg Differential Colour Illumination, particularly with transparent specimens, is that whilst it assists the imaging of specimen edge boundaries, internal structures and/or surface detail is frequently lacking. A partial solution to this problem is to use darkfield central stops with Rheinberg filters, especially those filters comprised of several different colours in the form of an annulus. The result of having a coloured image on a dark field is to raise the contrast between the weakly coloured structures of the specimen and the background.

With many types of specimens all that is required to enhance the image of the specimen is to reduce the intensity of the background colour. This is easily achieved by placing a stop of polarizing filter material over the transparent coloured central stop. With a polarizer rotated between the light source and the filter, the intensity of the background colour is easily changed from maximum intensity through darkfield without affecting the annular colours.

Rheinberg Stop Contrast Illumination

From my experiments with Rheinberg filters, which involved varying the diameters of both the coloured central stops and darkfield patch stops, I produced results similar to 'Three Coloured Darkground', described by P. S. Hewlett (1983). Whilst performing these experiments I observed that when a darkfield patch stop of a sufficiently small size was used, specimens of sufficient depth appeared as a pseudo-stereoscopic image on a brownish background. Increasing the diameter of the coloured central stop combined with the small darkfield patch stop, resulted in the specimen appearing in the colour of the annulus on a background colour of reduced intensity. Also, and quite noticeable, was an increase in the quality and quantity of topographical information over that achieved with conventional Rheinberg Illumination.

A further refinement to the Rheinberg Stop Contrast filters hereafter referred to as RSC, is a small reduction in the outside diameter of the annular to provide a narrow clear ring around the coloured annulus. With the condenser diaphragm fully open this ring of white light further enhances the

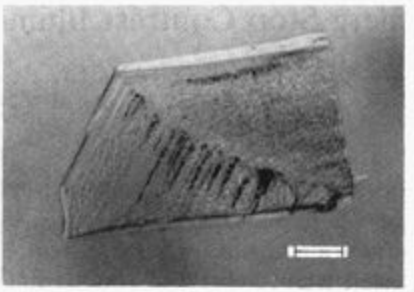

FIG. 1. Acetaminophyn crystal, brightfield. Bar = 50 μm.

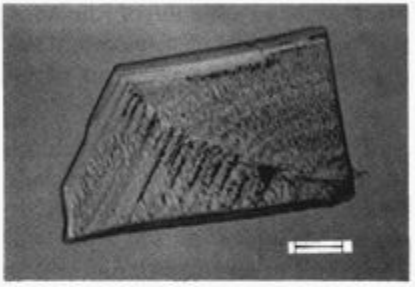

FIG. 2. Acetaminophyn crystal. Bar = 50 μm. Rheinberg illumination, yellow stop/blue annular.

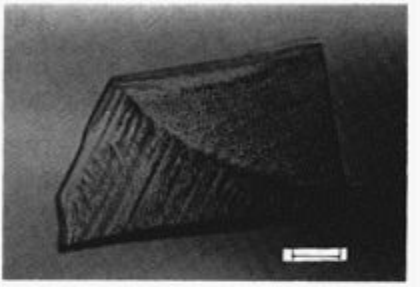

FIG. 3. Acetaminophyn crystal. Bar = 50 μm. Rheinberg stop contrast, yellow stop/blue annular.

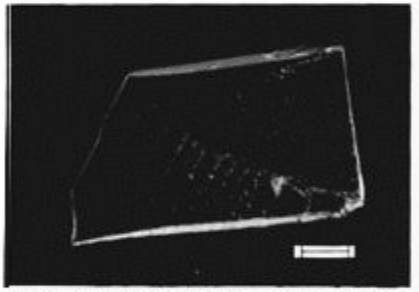

FIG. 4. Acetaminophyn crystal. Bar = 50 μm. Rheinberg illumination, blue stop/yellow annular.

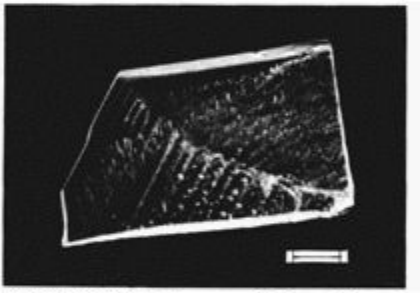

FIG. 5. Acetaminophyn crystal. Bar = 50 μm. Rheinberg stop contrast, blue stop/yellow annular.

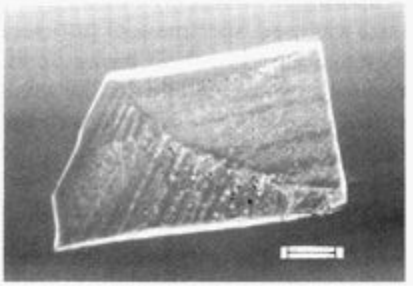

FIG. 6. Acetaminophyn crystal. Bar = 50 μm. Rheinberg illumination, red stop/yellow annular.

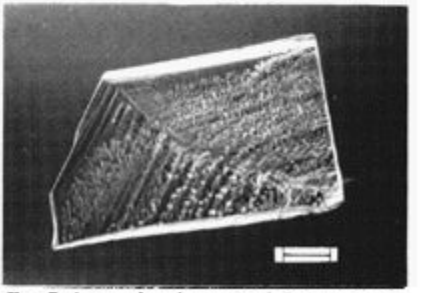

FIG. 7. Acetaminophyn crystal. Bar = 50 μm. Rheinberg stop contrast, red stop/yellow annular.

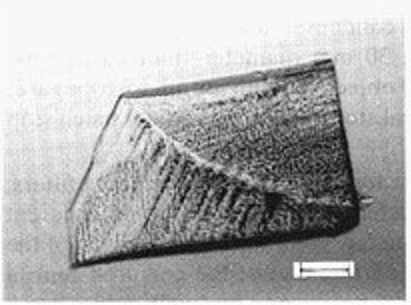

FIG. 8. Acetaminophyn crystal. Bar = 50 μm. Rheinberg stop contrast, yellow stop/green annular.

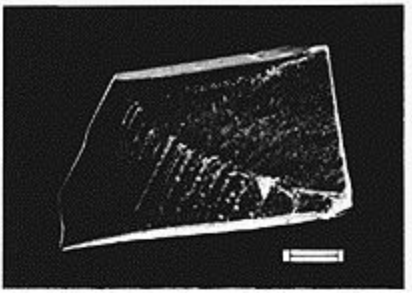

FIG. 9. Acetaminophyn crystal. Bar = 50 μm. Rheinberg stop contrast, green stop/yellow annular.

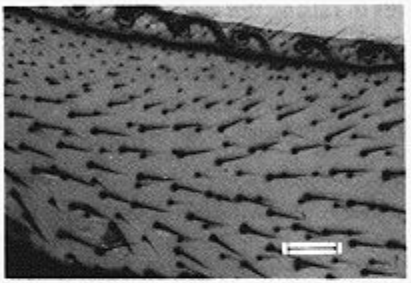

FIG. 10. Hoverfly wing. Bar = 50 μm. Rheinberg stop contrast, yellow stop/green annular.

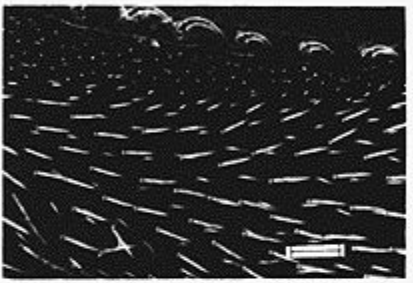

FIG. 11. Hoverfly wing. Bar = 50 μm. Rheinberg stop contrast, green stop/yellow annular.

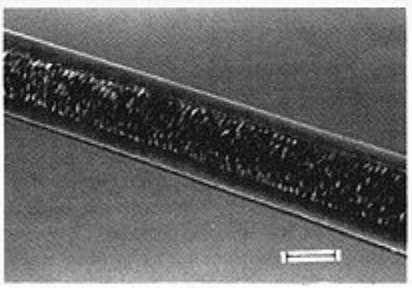

FIG. 12. Mink hair. Bar = 50 μm. Rheinberg stop contrast, red stop/yellow annular.

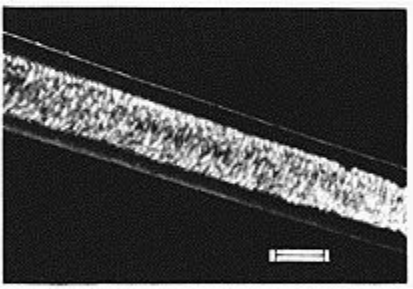

FIG. 13. Mink hair. Bar = 50 μm. Rheinberg stop contrast, green stop/yellow annular.

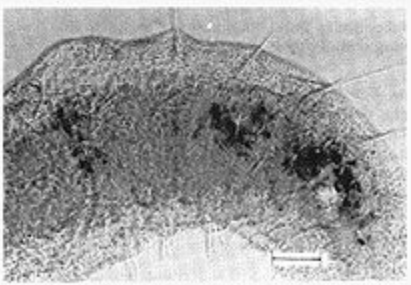

FIG. 14. Annelid worm — Naididae. Bar = 50 μm. Rheinberg stop contrast, red stop/yellow annular.

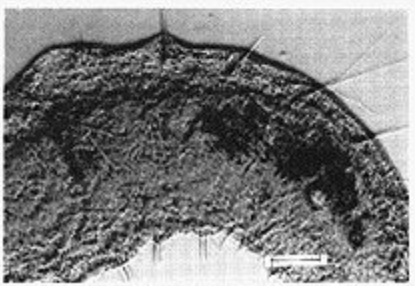

FIG. 15. Annelid worm — Naididae. Bar = 50 μm. Rheinberg stop contrast, yellow stop/green annular.

specimen. Varying the aperture of the condenser diaphragm controls the intensity of the annular colour. With a 30 mm diameter filter carrier the measurements for an RSC filter for a × 10 objective (with my microscope) are: coloured annular 22 mm⌀, coloured central stop 10 mm⌀, darkfield patch stop 6 mm⌀.

Fabrication of RSC filters is the same as for conventional Rheinberg filters; trials will, however, have to be conducted with patch stops of different diameters to determine the optimum diameter. Generally the diameter of the patch stop will be approximately three-quarters that of the coloured central stop; too large, and a darkfield effect will be produced, too small, contrast will be reduced.

As an imaging technique, Rheinberg Stop Contrast filters have proven to be useful with low to medium power objectives in raising the level of information in transluscent and transparent specimens. For the photomicroscopist, they provide a means of producing colourful, high information pseudo-stereoscopic photomicrographs. In black and white photomicrography the optical shadowing effect of the filters greatly assists the expansion of the grey scale.

REFERENCES

HEWLETT, P. S. (1983). Three-colour dark-ground images of transparent colourless objects. *Microscopy*, **34**, 522-528.

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POSTAL MICROSCOPICAL SOCIETY

TENTH NATIONAL MEETING AND EXHIBITION

Saturday 5 May 1990

THE 10th National Meeting and Exhibition of the Postal Microscopical Society will take place at St Mary's Community Centre, Bramall Lane, Sheffield, on Saturday 5 May 1990. This is a popular venue in the centre of the city, easily accessible by road or rail (there is ample parking space). The successful formula of Conversazione, Exhibition and presented papers on topics of microscopical interest will be followed. Luncheon and other refreshments will also be provided. A warm invitation is extended to all microscopists to attend — further details from Frank Rowntree at the address below:

Main Papers:

Blue Green Algae — A Threat to Life and Limb by Professor A. J. Brook.
Photomicrography — A New Art Form? by M. I. (Spike) Walker.
The Microscopist in Africa — Some Reflections by David Williamson.
The RMS 150th Anniversary — A Retrospective by Dr P. Evenett.

Demonstrations:

Video Microscopy — Getting Started by A. V. Dodge.
Use of Video Camera and Microscope by M. E. Chaplin.
Foraminifera Sand from Connemara by Roy Wimby.
Measurement of Diatoms by SEN by J. B. Saunderson.
Some Aspects of Illumination by B. Ellam.
The Home-Made Microscope by Mike Smith.

Poster Demonstration:

Microscopy in Renal Transplantation Research by J. B. Saunderson.

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