Rheinberg Illumination

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Microscopists have continually developed new techniques for enhancing specimen contrast since Zacharius first built the first compound light microscope around 1595. His simple two lens microscope became the basis for the evolution of light microscopes until 1828. That date is when the first polarizing light microscope was invented by Nichols. Since then, it has served as the building block for most research microscopes that are fitted for accessories made to enhance contrast or measure specific optical properties.

Julius Rheinberg devised a simple contrast enhancement method in 1896. He published his findings in the journal of the Royal Microscopical Society for the benefit of others. Today, Rheinberg illumination (RHI) has given way to phase contrast, differential interference contrast, confocal, laser microscopy, scanning probe and atomic force microscopy. The latter four are very recent technological advances. They could not be used for applied research, until our knowledge of computers technology elevated to a point that where we could handle the extensive algorithms necessary to exploit them. Just like Rheinberg, light wavelength, amplitude or other physical property is retarded (slowed down), to produce a dramatic difference in contrast that can not be perceived otherwise.

When contrast enhancement is used, light rays do not directly enter and pass through specimens. Instead, they are reflected, refracted or diffracted back to the objective when it hits the specimen. The amount of retardation corresponds to a given wavelength (nm) that is dependant on the color of filters chosen to produce a "stained" particle color. The amount of contrast produced depends on the nm difference of the colored filters selected. Selection of filters can sometimes be hit or miss, but in general, it is best to choose colors that are far apart from each other in the spectrum of light. Two good choices are a red and green filter (figure 1) and a blue and red filter (figure 2).

The placement and arrangement of filters can be varied. The most common configurations are in the microscope filter holder, or on top of the condenser objective lens. Figure 1 shows the first placement configuration with a green central and a red annular stop. Low contrast crystals would appear nearly invisible in plane polarized transmitted light (figure 3). Rheinberg illumination using a blue and red stop increases the contrast of the specimen as seen in figure 4. With Rheinberg, the color of the background will be the color of the central filter, and the particle will be imparted with the color of the annular filter. Edges of the particles will be transitional and diffuse, from green to red. This is because light is refracted at varying wavelengths corresponding to the wavelength difference of the filters, at steep angles to the crystal facets. In theory, it is possible to determine refractive index using this technique, albeit quite difficult, making it useless in practicality.

Contrast can also be varied by changing the size of the annular and central stops, opening-closing the substage condenser diaphragm, or to have two annular stops (figure 2). Another configuration would have the annular stop on the bottom condenser, and the central stop on the objective lens. This configuration can be even more complex, making quadrants of one of the annular stops two different colors.

There are a wide variety of gelatin filters that can be purchased from scientific suppliers, or from a photo shop. Special filter sets and condensers have been made in the past by microscope manufactures (Zeiss, Leica). Most of these, however, are difficult to find anymore, and are considered to be rare collectibles. Still, that does not take away from the useful objective of using Rheinberg Illumination.....to acquire esthetically pleasing high contrast images of particles. Buy your gelatin filters and get started now for sending special Christmas card to friends, family and colleagues. ■

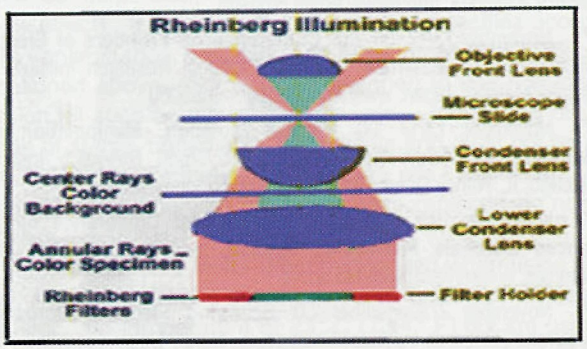


Figure 1: Rheinberg Illunincation Configuration

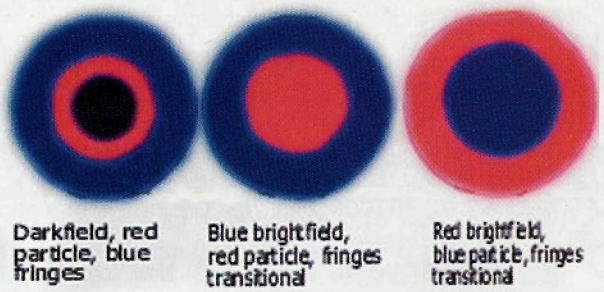


Figure 2: Three Rheinberg filter configuraons

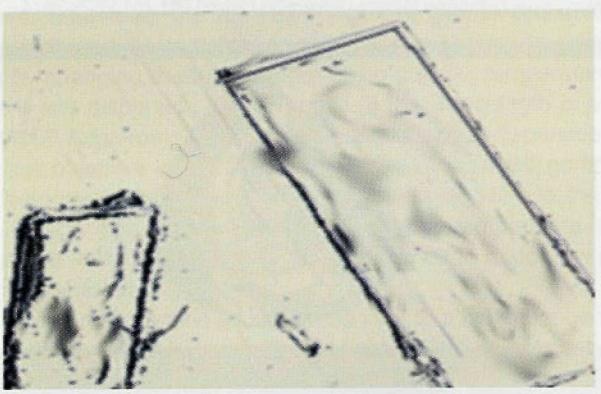


Figure 3: Crystals, plane polarized light, 100X

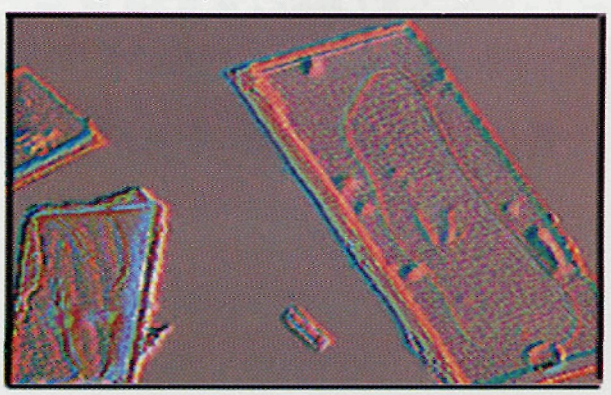
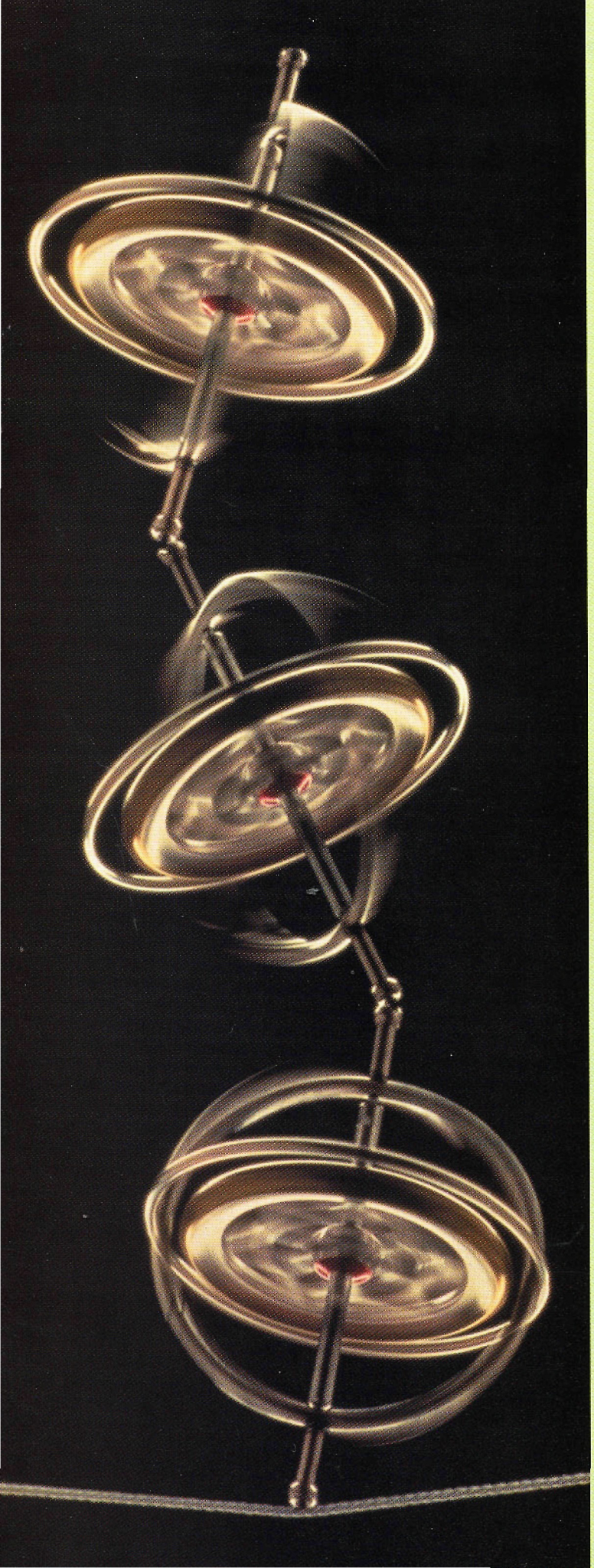


Figure 4: Crystals, Rheinberg, 100X



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