Multicolor Contrast Effects by Monochromatic Astronomic Filters – Utilization in Light Microscopy and Photomicrography

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Introduction

Several color filters have been developed for astronomic use in order to improve image quality in visual observations and astronomic photography. Various positive effects, which can be achieved in astronomic practice, have already been described by several authors, manufacturers and distributors [2, 4, 5, 9, 10, 12]. Monochromatic narrow-band filters are widely used by astronomers. These filters are constructed as interference filters; they select small ranges within the spectrum of the visible light corresponding with characteristic radiation emitted by celestial nebula or the sun and its protuberances. This way, scattered terrestrial radiation caused by "light pollution" and non visible infrared or ultraviolet spectral components are blocked. Fundamental improvements of image quality will result from these filters when refractors or reflector telescopes are fitted with them.

Table 1: Technical data of monochromatic astronomic filters

Filter	Element	Wavelength	Half-Intensity Width	Color
K	calcium	395 nm	6 nm	blue-violet
H beta	hydrogen	486 nm	8,5 nm	blue
O III	oxygen	500 nm	8,5 nm	blue-green
Solar Continuum	-	540 nm	8 nm	green
H alpha	hydrogen	656 nm	35 and 7 nm	red
S II	sulfur	670 nm	8 nm	red

Additionally, in light microscopy and photomicrography, astronomic filters are capable of improving the resulting image quality. Initial experiences of this have already been published in this magazine [11]. In the meantime, several astronomic monochromatic filters were intensively tested with regard to their utilization in microscopic applications. The results of these tests are presented and discussed now.

Materials and Methods

Six types of monochromatic filters often used in astronomy were tested, manufactured by the Baader Planetarium Company, Germany. **Table 1** gives an overview of the basal characteristics of these filters [data from 3, 7, 8, 12]. The Solar Continuum filter is constructed for sun observations in monochromatic green light [3]. The K-line filter is normally used for solar observations in blue-violet [3, 11]. The H alpha filter works as a monochromatic red filter suitable for observations of sun protuberances and some particular emission nebula [3, 7, 8]. The other filters (H beta, O III, S II) are also offered for observations of various celestric nebulas in red or blue spectra [3, 7, 8].

These filters were used for selected monochromatic illumination of microscopic specimens in red, green, blue or violet. In the optical manufacturing process, achromatic and apochromatic

Table 2: Contrast characterization of monochromatic red, green and blue filters with regard to the primary and secondary colors of the specimen

	Colors of the specimen								
filter	red	orange	yellow	green	blue	violet	black	grey	brown
red (656, 670 nm)	-	-	-	+	+	-	+	+	+
green (540 nm)	+	+	-	(-)	+	+	+	+	+
blue (486 nm)	+	+	+	+	(-)	(+)	+	+	+
green (500 nm)	+	+	+	+	+	+	+	+	+

\+ = high contrast (-) = low contrast
(+) = medium contrast - = invisible or nearly invisible

microscope objectives are usually designed based on the following reference wavelengths [6]: 656 nm (red), 546 nm (green), 486 nm (blue), 405 nm (violet). In achromatic lenses, the refraction is corrected for red and blue light, in apochromatic lenses for red, green and blue; moreover, the aberration of violet is reduced in apochromatic systems. In this respect, the H alpha, H beta and Solar Continuum filters may be of special interest with regard to microscopic applications as their transmissions were identical or nearly congruent with the respective corresponding reference wavelengths microscope lenses are based on.

All filters were inserted into the illuminating light path. Observations were made by a standard laboratory microscope in transmitted light (bright and dark field, phase and interference contrast, polarized light). Also in concentric epi-illumination (bright and dark field) using a special microscope for wafer inspection. The microscopes were equipped with plano apochromatic objectives, achromatic lenses and mirror objectives, including some systems specially adapted for long focal distances and dark field inspection based on epi-illumination. Digital images were taken by a 7.1 Mp camera (Olympus Camedia C 7070) with bulb and flash light. For computer-based reconstruction of multicolor images, where individual images taken with different color filters are combined, several image stacking software packages were utilized (Helicon Focus, Picolay, Combine Z 5).

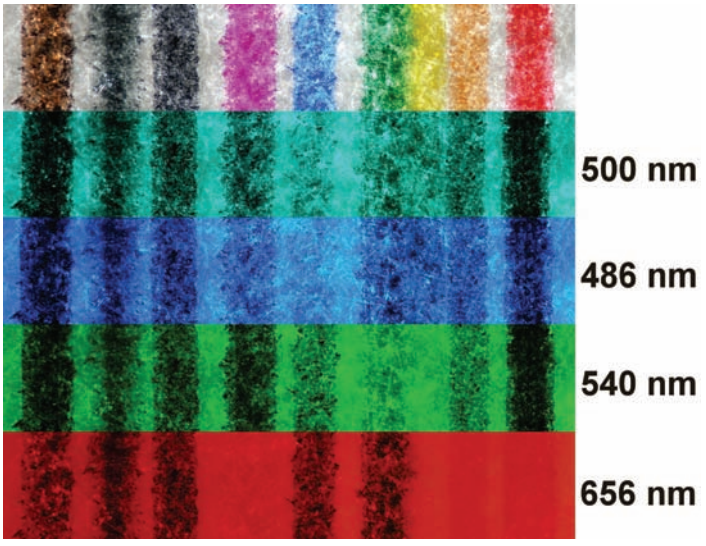

Fig. 01: *Multicolor stripes, taken in white and monochromatic light (blue, green, red), bright field, objective 2,5x, ocular 6,3x, horizontal field width (HFW): 6 mm*

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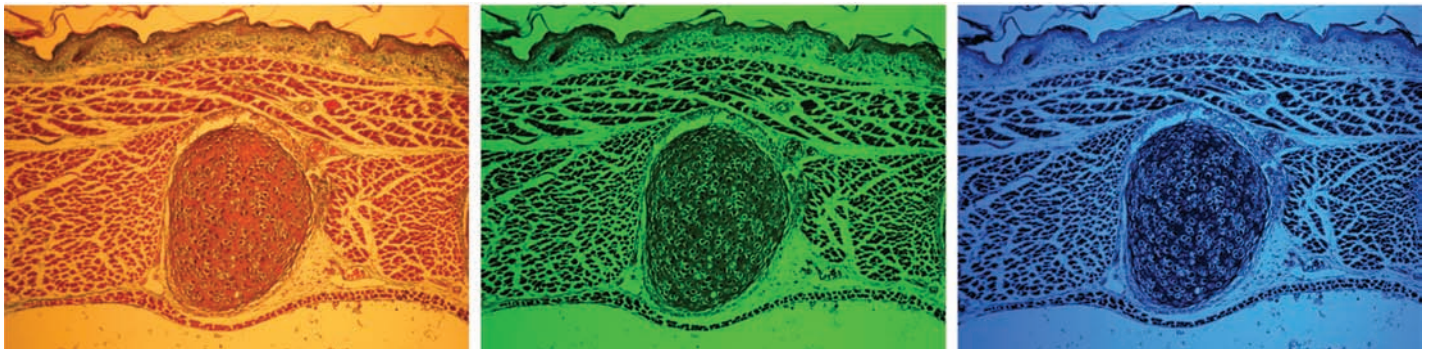

Fig. 02: *Mouse embryo, epidermis, muscle and cartilage cells, bright field, bulb light, monochromatic green light (540 nm) and blue light (486 nm), long distance objective 20x, ocular 10x, HFW: 0,5 mm*

Results

General aspects

The monochromatic astronomic filters can be divided into four different types: red filters (H alpha , S II), green filters (solar continuum, O III), blue filters (H beta) and violet filters (K-line filter). With regard to the native colors of specimens, the contrast characterizations of these filters are different from each other. **Fig. 1** shows a pattern of multicolor test stripes in bright field, illuminated by white light and monochromatic red, green and blue light. The monochrome images were taken with H alpha (red, 656 nm), Solar continuum (green, 540 nm), O III (green, 500 nm) and H beta (blue, 486 nm) filters. The corresponding contrast characterizations and typical color detections are presented in **table 2.**

When specimens are illuminated in monochromatic light, any degradation of image quality caused by chromatic aberration is eliminated. **Fig. 2** presents a histological routine section stained in red, photographed with a basal corrected achromatic objective for long working distances, taken in normal bulb light, monochromatic green and blue light. It is evident that the monochromatic techniques lead to visible improvements of sharpness, resolution, and contrast.

Nevertheless, the focal plane can differ when a specimen is successively illuminated in red, green and blue light so for maximum sharpness the image has to be refocused. The lower the achromasia of the complete optical system and its interacting components (objective, ocular, tube-lenses, and lenses within the camera or camera adapters) the larger the resulting differences in focus may be. According to our own measurements, the maximum shift of the working distance necessary for a constant sharpness can be up to 8 µm when monochromatic illumination is changed from green (540 nm) to blue (486 nm). Monochromatic filters should preferably be combined with an ultraviolet- and infrared cut filter for eye protection.

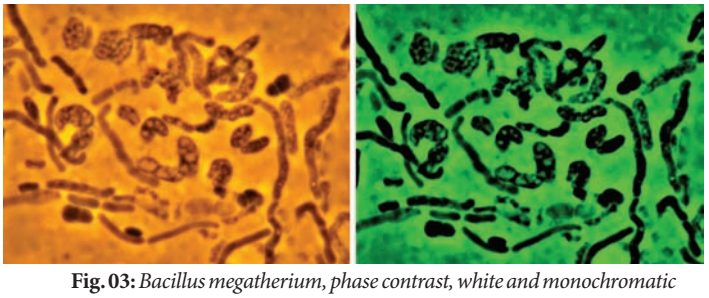

Fig. 03: *Bacillus megatherium, phase contrast, white and monochromatic green light (540 nm), objective oil 100x, ocular 12,5x, HFW: 20 µm*

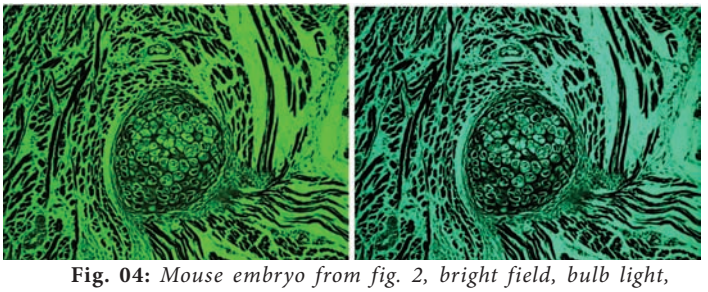

Fig. 04: *Mouse embryo from fig. 2, bright field, bulb light, monochromatic green light, 540 nm (left), 500 nm (right), objective 10x, ocular 10x, HFW: 1 mm*

When specimens are illuminated both in epi-illumination and in transmitted light, new color contrast effects are achievable when the transmitted and incident light are filtered in different colors. All effects described as follows are strongly dependent on monochromatism. When simple color filters are used that are not monochromatic, relevant enhancements of image quality *will not result* because such filters let pass a high range of different wavelengths. On the contrary, simple red and blue filters can lead to severe decreases of contrast and contour sharpness in life microscopy and photomicrography so that the global image quality will be worse. In photomicrography, monochromatic filters can be used for bulb light as well as for flash light. When mirror objectives are used, all contrast effects achievable by these filters are visible in the same manner as in common glass lenses.

Monochromatic green filters

Monochromatic green filters lead to fundamental enhancements of sharpness, resolution, and contrast in most situations of microscopic routine, in visual observations as well as in photographic images. Fine structures of specimens can be distinguished over the full range of their potential native colors. Thus, the quality of a monochrome green image is not influenced by the original color of the specimen or the particular technique used for staining. Similar improvements are also achievable in an unstained specimen being examined in dark field, phase, or interference contrast. In this respect, monochromatic green filters can be regarded as a universally applicable tool, especially suitable for black and white imaging. Pure yellow is not visible in monochromatic green light, when a Solar continuum filter (540 nm) is used; it is strongly contrasted by the O III filter (500 nm). In this respect, the O III filter may be regarded as a superior green filter, combining the contrast effects of the solar continuum filter and the H beta filter (see table 1).

When a digital camera is used for photomicrography, green filters contribute to a maximized sharpness and resolving power in digital images, as the majority of pixels (50 %) are sensitive for green light, whereas just 25 % of the pixels are capable of detecting red and blue. The superior quality of monochrome digital images

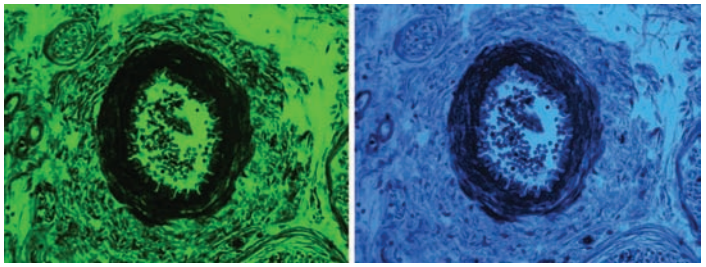

Fig. 05: *Mouse embryo, wall of an artery, bright field, monochromatic green light (540 nm) and blue light (486 nm), long distance objective 20x, ocular 10x, HFW: 0,5 mm*

taken in monochromatic green light is demonstrated in **fig. 3**. **Fig. 4** shows the different characters of the 540 nm and 500 nm green filters in a histological routine section stained in red.

Monochromatic blue filters

The utilization of monochromatic blue filters is dependent on the specimen´s color. When structures are colored in blue, they may be poorly visible or invisible in monochromatic blue light. On the other hand, specimens that are not blue may appear with superior quality when illuminated in blue light vs. green light. In monochromatic blue light, pure yellow is especially visible in high contrast. Fine detail on the resolving limit of the respective optical systems can be realized and the variability of tonal values visible in life observation and microscopic photographs may appear in a superior clarity. Lateral resolution is maximized as the wavelength of visible light transmitting these filters is minimized. These effects are visible in stained and unstained specimens when examined in routine applications. **Fig. 5** shows the vessel wall of an embryonic artery taken in monochromatic green and blue light for comparison. With regard to the concentric layers of the vascular muscle cells, monochromatic blue light leads to a more subtly differentiated image.

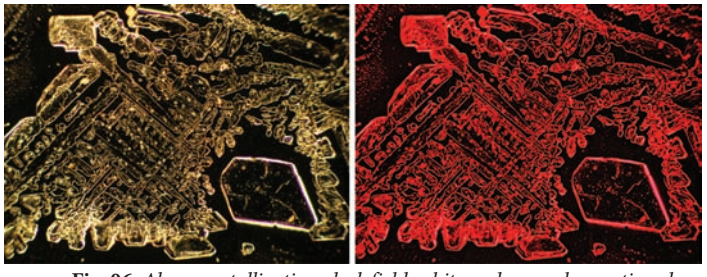

Fig. 06: *Alum crystallization, dark field, white and monochromatic red light (656 nm), long distance objective 20x, ocular 10x, HFW: 0,5 mm*

Monochromatic red filters

Monochromatic red filters can be used for selective contrast enhancements in specimens colored or stained in blue or green. Otherwise, structures in red, orange, yellow or violet are not visible in monochromatic red light. In digital cameras, it may be difficult to obtain images exactly in focus as all structures in monochromatic red are poorly detected by the camera display. Therefore, a series of images should be taken in different focal planes so that sharp images can be selected afterwards. In most cases, the camera automatic metering mode does not work correctly and the exposure has to be corrected manually. On the other hand, monochromatic red filters can be useful in optimizing visual observations in high contrast modes especially in unstained specimens appearing at maximized brightness. Thus, in dark field examinations of colorless specimens, the recognizability of small detail can be significantly improved by monochromatic red light. Moreover, blooming and swamping caused by scattered light can be reduced with the help of such filters. According to our own observations, transparent colorless structures situated in a black and dark background are visible in best contrast and clarity when illuminated by monochromatic red light; dazzling seems to be lower than in green, blue, or white light. In summary, monochromatic red filters can in the first place be regarded as helpful tools for visual observations in dark field or similar techniques associated with an extraordinary high range in brightness and contrast.

According to our own experience, S II and H alpha filters (two versions with 35 nm and 7 nm half-power-width) are nearly equal in their practical use. The H alpha filter with 7 nm half-intensity-width absorbs more light intensity than the variant with 35 nm half-power-width and the S II filter being near the infrared-level produces a darker red color than the H alpha filters. In our opinion, these marginal differences are not significantly relevant in practice. **Fig. 6** demonstrates the effects of monochromatic red light (H alpha, 35 nm half-intensity-width) in dark field observations of a colorless specimen in comparison with white light.

Monochromatic violet filters

The violet K-line filter is the only one that cannot be successfully used in conventional light microscopy. When this filter is inserted into the illuminating light path, images are indistinct and in very low contrast, even in catoptric mirror objectives free from glass lenses and chromatic aberration. It should be taken into account that glass lenses within common eyepieces and objectives are not corrected for the short wave length of this filter.

Special aspects

When a specimen is sequentially photographed in monochromatic red, green and blue light, three single images can be created based on these three reference colors. Each image will be characterized by a maximized sharpness and minimal chromatic aberration. Dependent on the color characteristics of the respective specimen, one special color version might show more detail and

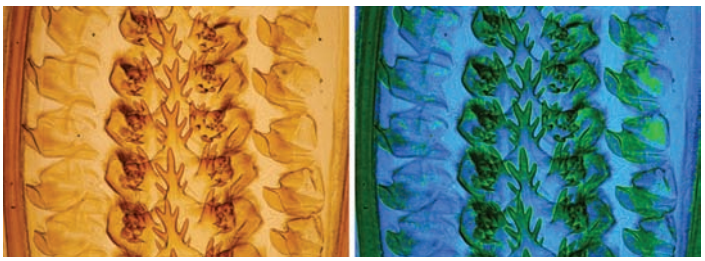

Fig. 07: *Stomach of a cricket, bright field, white light image and bicolor sandwich (monochromatic blue and green light), objective 10x, ocular 10x, HFW: 1 mm*

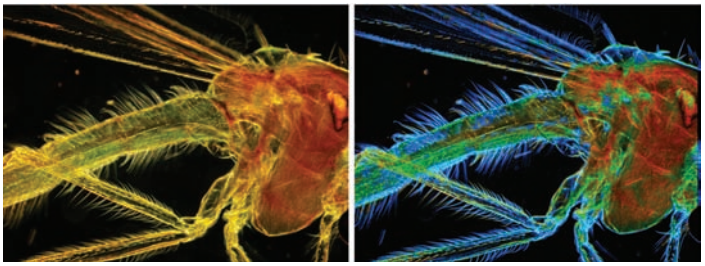

Fig. 08: *Gnat, dark field, white light image and tricolor sandwich (white light, monochromatic blue and green light), objective 4x, ocular 6,3x, HFW: 2,5 mm*

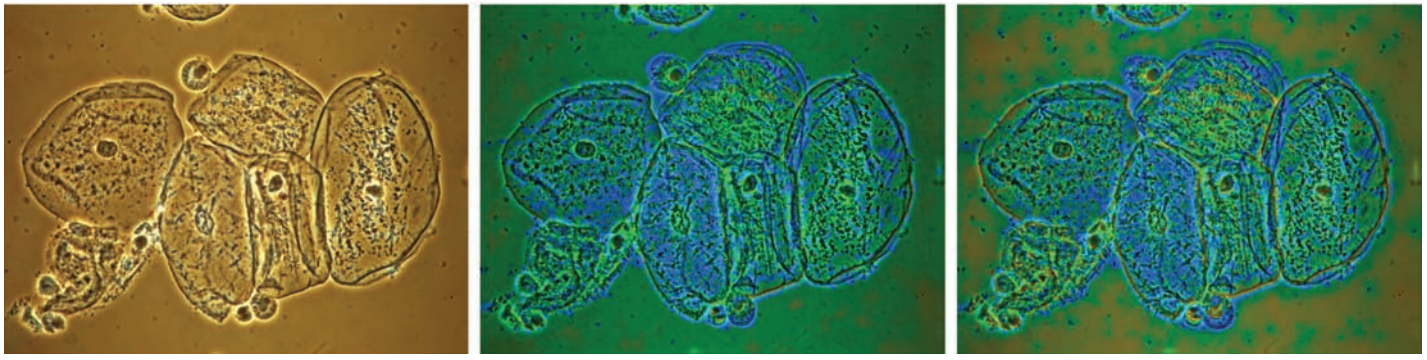

Fig. 09: *Native epithelial cells, phase contrast, white light image, bicolor sandwich (monochromatic blue and green light), tetra color sandwich (white light, monochromatic red, green and blue light), objective 40x, ocular 10x, HFW: 250 µm*

Fig. 10: *Hydro soluble pigment, thin-layer crystallization, phase contrast, white light image, bicolor sandwich (monochromatic red and blue light), tricolor sandwich (monochromatic red, green and blue light), objective 40x, ocular 10x, HFW: 250 µm*

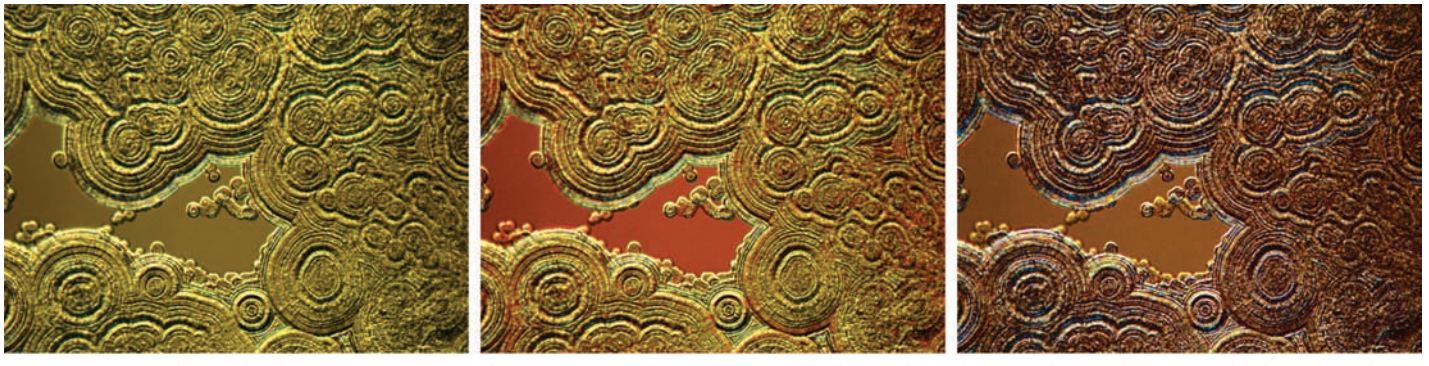

Fig. 11: *Polymer, thin layer preparation, interference contrast, white light image, bicolor sandwich (white and monochromatic red light), tetra color sandwich (white light, monochromatic red, green and blue light), objective 40x, ocular 10x, HFW: 250 µm*

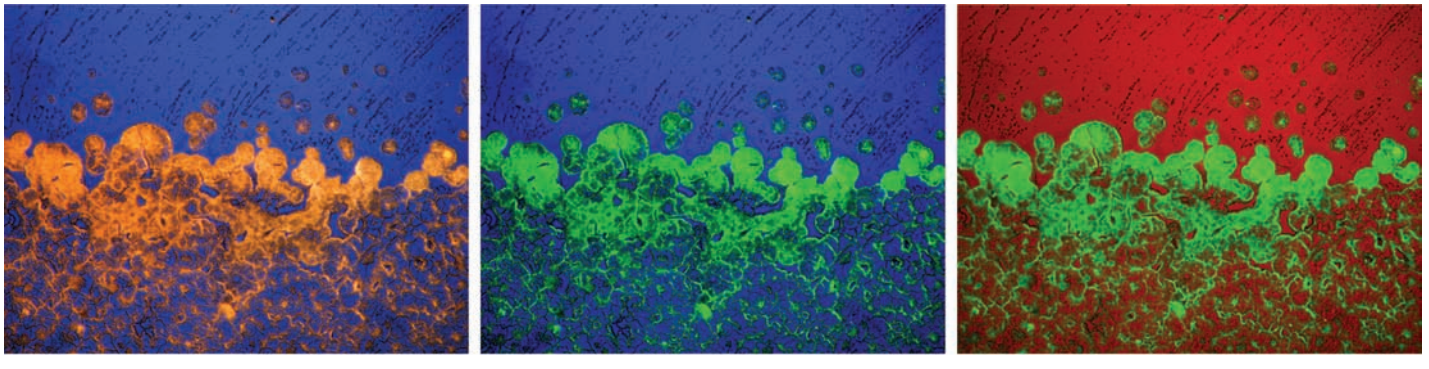

Fig. 12: *Alum crystallization, simultaneous illumination in bright and dark field, bright field in transmitted monochromatic blue or red light, dark field in epi-illumination with orange or green light, bicolor double contrast, epi-darkfield objective 10x, ocular 10x, HFW: 1 mm*

better contrast that the others.

A higher variability of contrast effects may occur when these three monochrome single images are superimposed by software-based postprocessing or when two images taken in different colors are matched as a pair. Thus, several bi- or multicolor sandwiches can be created. The clarity of fine structures within the specimen and the contrast of the specimen itself can be substantially improved by this technique; some effects achievable are similar to solarisation. Moreover, an image taken in white light showing the specimen in its natural color can be superimposed with one, two, or three monochrome images. Also by this averaging technique, the visual information in the resulting composite image might

well be enhanced when compared with corresponding single images taken in white or monochromatic light. The high variability of contrast effects in multicolor sandwiches is demonstrated in the **figures 7 – 11** showing doubles or triples of conventional white light images and multicolor sandwiches. The monochrome images were taken with the H alpha (35 nm half-width), H beta and Solar continuum filters.

Some transparent or semi-transparent specimens can be simultaneously examined in transmitted light and epi-illumination. In this case, contour sharpness can be enhanced when the transmitted light is monochromatic. Dependent on the specimen´s individual color, red, green, or blue light might be preferred for background illumination. When the incident light is not filtered, the specimen will appear in its native color surrounded by a monochrome background. When epi-illumination is combined with monochromatic dark field illumination in transmitted light, some detail within the specimen can be shown in a distinctive color contrast. Moreover, the incident light can also be filtered in a defined color different from the background. In this way, bicolor contrast effects can be achieved that are comparable with the visual aspects of ancient "Rheinberg" illumination images[1]. In contrast to the common "Rheinberg" technique, the brightness of the background and the specimen can be selectively regulated and adjusted as the intensities of incident and transmitted light are modified separately from each other. Some examples for bicolor contrast images based on simultaneous applications of transmitted and incident light are shown in the **figures 12 and 13**, taken with the same filters as used for the figures 7 – 11.

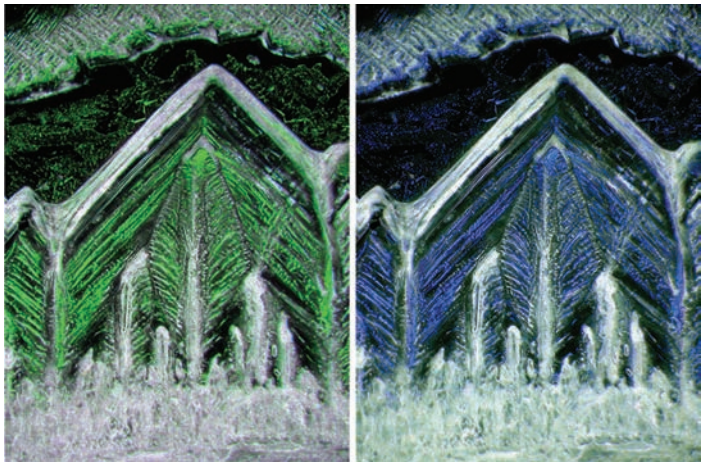

Fig. 13: *Alum crystallization, simultaneous dark field illumination in transmitted monochromatic green or blue light and incident white light, epi-darkfield objective 10x, ocular 10x, HFW: 1 mm*

Conclusions for practice

Monochromatic astronomic filters designed as red, green, or blue filters are useful in light microscopy. They can lead to fundamental improvements of sharpness, resolution, and contrast. When a specimen is successively filtered in red, green and blue light it will be dependent on the specimen´s native color which monochromatic filter will lead to the best result. Monochromatic violet filters do not seem to be useful for conventional applications in light microscopy. Nevertheless, such filters might be of interest in ultraviolet (UV) microscopy when the optical equipment is completely based on mirror systems or special quartz lenses. In this case, violet filters could be suitable for visual observations in monochromatic light near the range of UV radiation.

Monochromatic green filters are the most universally applicable tools for all fields of microscopic practice; they can contribute to optimized visual information over the full range of natural colors. Monochromatic blue filters are suitable for enhancements of resolution and sharpness in specimens or structures that are not colored blue. Both types of filters are useful for visual observations as well as for photomicrography. Physically, monochromatic blue filters promise the highest resolutions in microscopic images possible within the utilizable range of the visible light spectra as the wavelengths of the illuminating light beams passing these filters are shorter than in the other filters.

In some cases, monochromatic red filters can improve the visual aspect of colorless specimens when examined in dark field illumination. In special situations, these filters can also be used for selective contrast enhancements of fine structures colored blue. In photomicrography, focusing and exposure metering are associated with increased difficulties. Nevertheless, these filters can be used very well for background illumination in transmitted light when the specimen is simultaneously illuminated in epi-illumination by white or otherwise colored incident light. The visual information and the aesthetic aspect of photomicrographic images can be enhanced by bi- or multicolor sandwiches based on serial images taken in different monochrome colors (red, green, blue).

Thus, I would recommend these filters for all microscopists who are interested in creative photomicrography and new and improved contrast effects. ■

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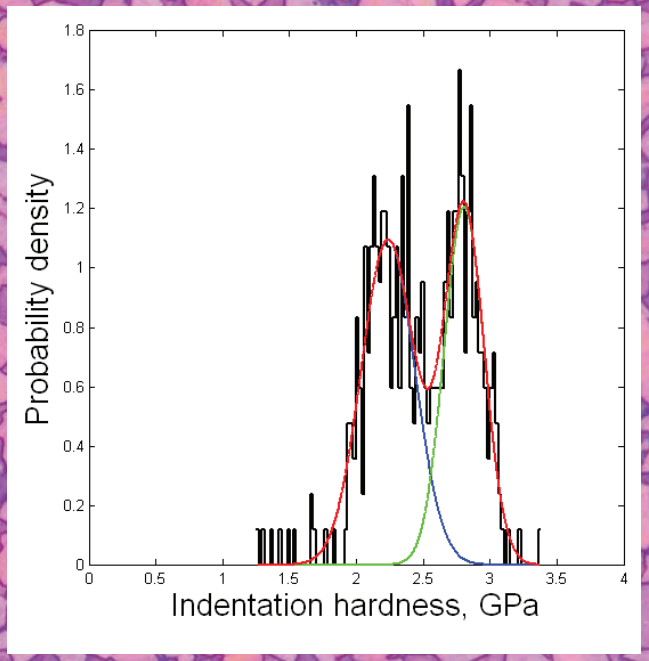

Microstructure of Naval Brass, CDA 464, Cu – 39.7% Zn – 0.8% Sn, in the hot rolled and annealed condition. The twinned alpha - Cu phase is visible through the use of polarized light and sensitive tint, ~500x. The graph shows the distribution of hardness measurements in a 20 x 20 array of nano-indents made at 2 mN over the surface of a polished specimen of Naval Brass revealing the hardness distribution for the alpha phase (left peak) and the beta phase (right peak). A Nanoindentation Tester (CSM Instruments Inc.) was used to perform the nanoindentation tests.

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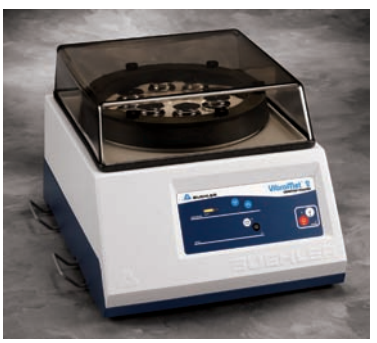

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