RGB-Splitting and Multi-Shot Techniques in Digital Photomicrography–Utilization of Astronomic RGB-Filters in True Color Imaging

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Introduction

A great many filters in common use in astronomy are used in order to improve the resulting optical quality in observations and photographs [1-4]. The majority of these filters are also capable of improving image quality in microscopy and photomicrography. Several instances of this have already been reported in this magazine [5-6]. Some new types of RGB-CCD astro filters have recently been developed for multi-shot techniques [2]. These filters can also be regarded as interesting tools for photomicrography that can maximize the quality of true color imaging based on RGB-splitting and multi-shot techniques. The respective methods and their technical benefit are presented and discussed below.

RGB-splitting and multi-shot techniques are routine tools in some fields of professional studio photography and astronomical photography. They can lead to visible improvements in sharpness, resolution and color balance. To obtain maximum image quality, special multi-shot cameras can be used. These are fitted with a multi-shot sensor that does not require any color filters. Therefore, each pixel is sensitive for all colors and pixel interpolations are not necessary.

In contrast to this, all pixels in common CCD chips or CMOS sensors are coated with color filters for red, green, or blue. 50% of the pixels are sensitive for green light, 25% for red or blue light (the so-called BAYER model). Structures that are not colored in red, green or blue are not detected by the respective pixels but reconstructed by interpolation. Several artifacts and loss of resolution and sharpness can result. When parts of the non-visible light spectra (ultraviolet and infrared) are not completely blocked within a digital camera, some more degradation of image quality may occur, which leads to lower contour sharpness or color fringes, caused by interferences with visible light components. Therefore, RGB-splitting and multi-shot techniques are suitable for improvements of image quality, even in photomicrographs taken with common tricolor chips.

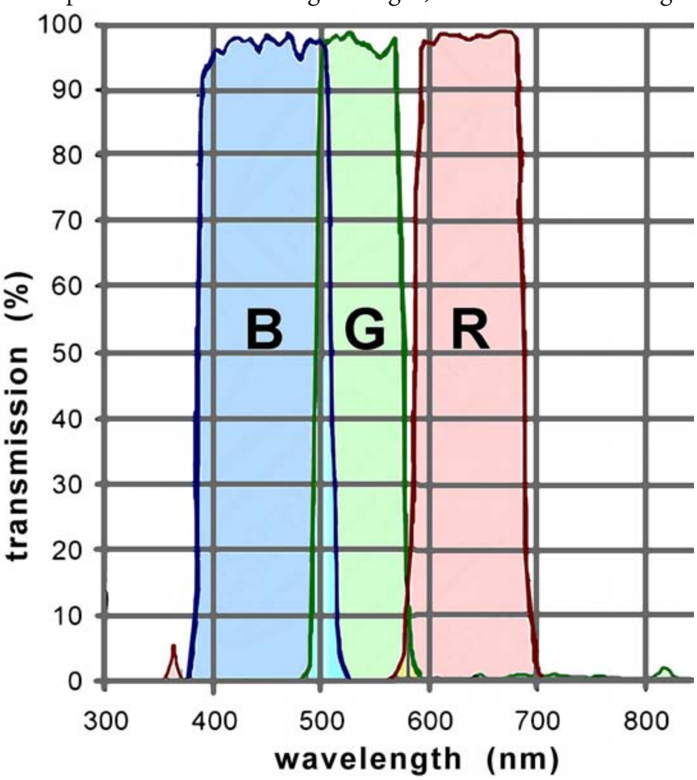

Fig. 1: *Transmission of the Baader RGB-CCD-filters (Baader, 2008)*

Principles of RGB-splitting and multi-shot techniques

At first, three single images have to be taken of a specimen, filtered in red, green and blue (**fig. 2 a-c, 4 a-c, 5 a-c**). The appropriate filters are inserted into the illuminating light path, as is the case in other filter techniques. Non-visible light spectra are blocked by the respective color filters. The character of the resulting final image can be different, depending on the optical design of the particular set of color filters.

In a second step, the three monochrome images taken in red, green, and blue have to be superimposed, so that a true color image can be reconstructed (**fig. 2e, 3b, 4e, 5e**). By use of image processing software, basal quality determining parameters can be separately adjusted for each color channel (e.g. gradation, histogram, brightness, contrast, color saturation). Because of this, separation, balance and purity of color can be optimized in a superior manner, and deviations in color tinge are minimized. Moreover, all existing advantages of monochromatic light illumination can be transferred into the reconstructed true color images, especially when monochromatic narrow band filters are used. The smaller the range of transmission, the higher the degree of coherence and visible enhancement of lateral resolution and contour sharpness will be.

Table 1: Technical data of RGB-CCD-filters

Filter	Transmission	Reference Wavelength	Half-Intensity Width	Color
RGB B-CCD	380-520 nm	450 nm	140 nm	blue
RGB G-CCD	500-580 nm	540 nm	80 nm	green
RGB R-CCD	580-700 nm	640 nm	120 nm	red

Table 2: Technical data of monochromatic narrow band filters

Filter	Element	Wavelength	Half-Intensity Width	Color
H beta	hydrogen	486 nm	8,5 nm	blue
Solar Continuum	-	540 nm	8 nm	green
H alpha 7 nm	hydrogen	656 nm	7 nm	red

Material and Methods

RGB-splitting was carried out with different types of color filters, manufactured by the Baader Planetarium Company, Germany. First, a new set of three RGB-CCD filters were tested recently that were developed for multi-shot techniques in astronomy [2]. The transmission spectra of these filters are matched with the

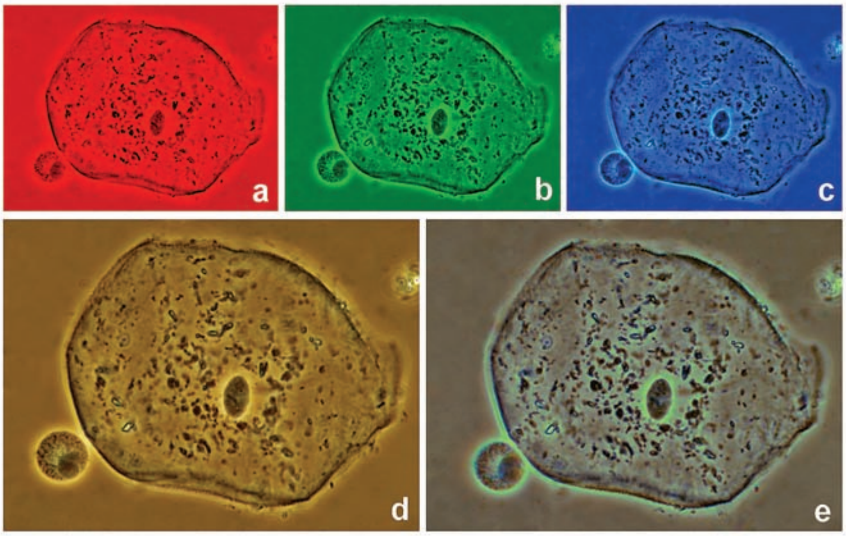

Fig. 2: *Human epithelial cell from the oral mucosa, live preparation, phase contrast, objective 40×, eyepiece 10×, bulb light, monochrome images taken with RGB-CCD-filters (a-c), single shot image taken without any filter (d), RGB-reconstruction from a-c (e)*

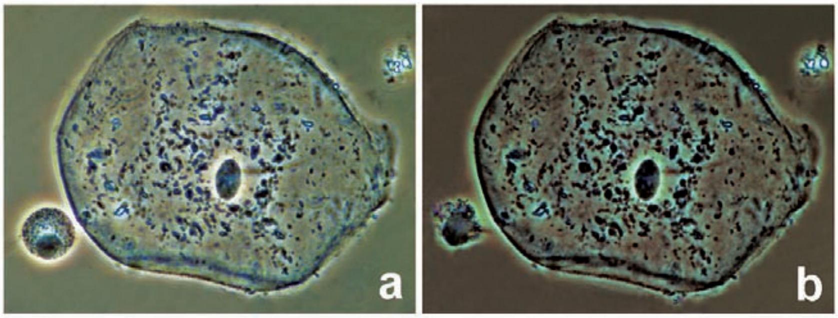

Fig. 3: *Specimen, equipment and procedure from fig. 2, flash light illumination, single shot image taken without any filter (a), RGB-reconstruction based on RGB-CCD-filters (b)*

red, green and blue color filters the pixels in common CCD or CMOS sensors are coated with; their half-intensity-width ranges from 80 – 140 nm. The corresponding technical data and characteristic transmissions of these CCD filters are presented **in table 1 and fig. 1**. Additionally, three monochromatic astro-filters were used for multi-shot techniques [1]: H alpha filter (red, 656 nm), solar continuum filter (green, 540 nm) and H beta filter (blue, 486 nm). The half-intensity width of these narrow band filters is lower than 10 nm (**table 2**).

All RGB-triplets (single images, filtered in red, green, and blue) were superimposed with the help of the freeware DRI-Tool (synonym: Image Stacker). This software has primarily been designed for equalizing brightness and contrast within a series of several single images differing in their exposure. When three images in red, green, and blue are superimposed, the color tone of the resulting image will be well balanced in most cases. The purity and authenticity of colors can be improved further when histogram and gradation parameters are manually and selectively adjusted for each color channel.

All tests were carried out with bulb and flash light, using a common laboratory microscope in bright field and phase contrast. Digital images were taken with a 7.1 MP digital camera (Olympus Camedia C-7070), equipped with a Leitz / Leica vario ocular 5× – 12,5×.

Results

RGB-CCD filters are suitable for bulb light as well as for flash light, because the brightness of illumination is just moderately reduced (by about –1 or –2 EV values).

The light absorbing effects of monochromatic narrow band filters are much stronger— reductions of the remaining brightness by –5 or –6 EV values are normal —so that in most cases these types can only be used for bulb light illumination. On the other hand, narrow band filters can lead to superior enhancements of sharpness and resolution when compared with RGB-CCD filters, as their range of transmission is much smaller and their coherency much higher.

Any degradation of the image quality caused by potential chromatic aberration associated with all components of the optical system (microscope objectives, eyepieces, lenses within the microscope tube and the digital camera) can be massively reduced or eliminated.

Further improvements of the global sharpness will occur when each monochrome single image is focused separately and individually. In digital photomicrography, the focal plane can significantly differ with regard to the dominant color or wavelength. According to our own measurements, the maximum shift of the focal plane can be up to 8 µm, when the color is changed from red or green to blue. Thus, in a single exposure, images on a single focal plane can be regarded as a compromise, when flat specimens stained in red and blue are photographed in the usual manner. When red structures are optimally focused, no blue structures within the same plane will be in an ideal focus, and when the blue zones are in an optimum focus, the corresponding red structures will not be focused perfectly. These considerations are important for practice if a multicolor specimen is photographed in high magnification and high-end images are desired.

The examples shown in **fig. 2-5** demonstrate the visible enhancements of image quality achievable by multi-shot techniques based on RGB splitting. Fundamental improvements in color balance are visible in bulb light as well as in flash light, in bright field as well as in phase contrast. Any relevant deviations related with the hue of color can be eliminated, both the yellowish tint of bulb light as well as the blue tint of flash light. In most situations, the character of colors can be transformed into ideal white light. The color adjustments achievable by these techniques are much more accurate and precise than those resulting from manipulations of the color or white balance based on camera presets or the usual image processing software.

Three monochrome images can be superimposed within a few seconds if the suitable software (DRI Tool / Image Stacker) is installed on an up-to-date computer. If necessary, gradation and histogram parameters can be easily adjusted for each color

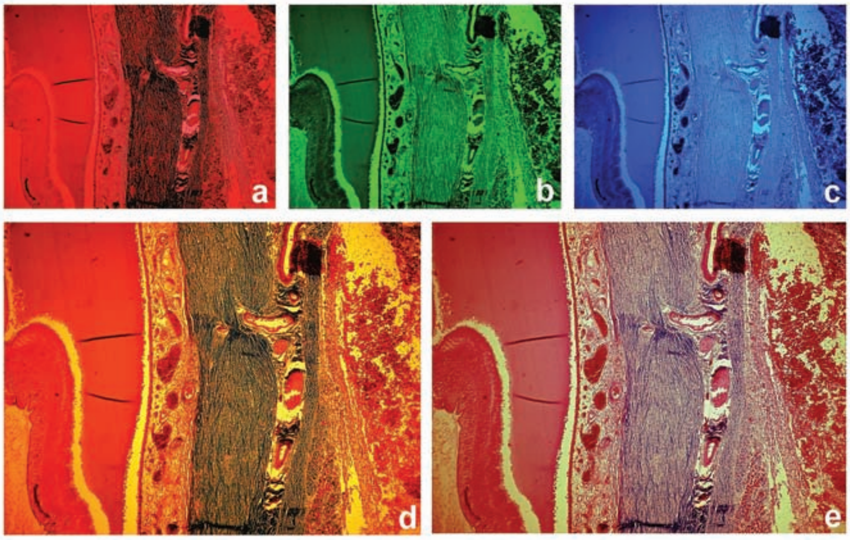

Fig. 4: *Stained section of the human eye, bright field, objective 4×, eyepiece 6×, bulb light, monochrome images taken with RGB-CCD-filters (a-c), single shot image taken without any filter (d), RGB-reconstruction from a-c (e)*

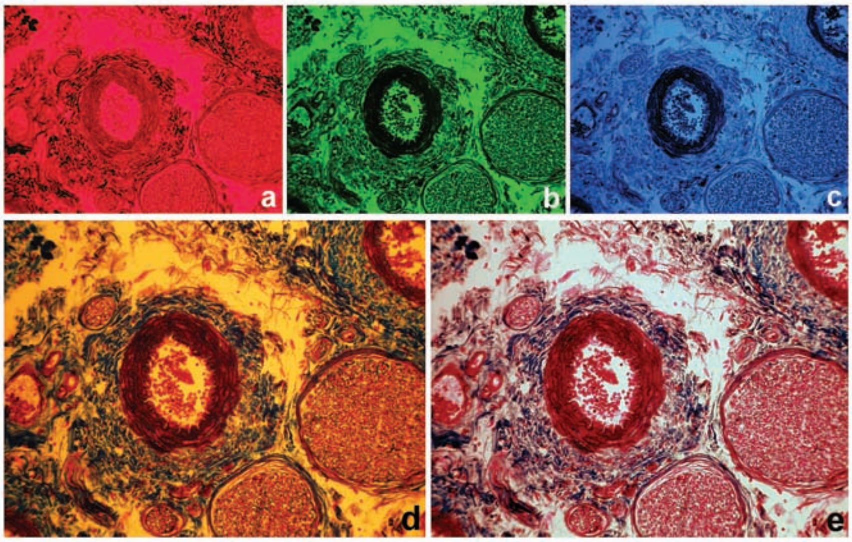

Fig. 5: *Stained section of a mouse embryo, bright field, special objective for long working distance L 20×, eyepiece 10×, bulb light, monochrome images taken with monochromatic narrow band filters from table 2 (a-c), single shot image taken without any filter (d), RGB-reconstruction from a-c (e)*

channel in a few steps, so that, overall, the final reconstruction of a true color image will be finished within a few minutes.

Discussion and Conclusions for Practice

In digital photomicrography, RGB-splitting and multi-shot techniques will lead to the best results if a static specimen has to be photographed in optimum quality. The purity, differentiation, and authenticity of colors will be maximized, and any loss of quality caused by potential chromatic aberration will be avoided. In multicolor specimen, all structures will be ideally focused, as the optimum focal plane will no longer be dependent on the regional color or dominant wavelength. Bright field images will occur in ideal white, phase contrast in ideal grey tones. These positive effects can all be achieved in bulb and flash light illumination.

When low or moderately corrected optical systems are used, e.g. special lenses for long working distances that are not designed as apochromates because of their optical compromises, the resulting image quality can be nearly apochromatic when true color images are reconstructed from three monochrome RGB images, and in highly corrected apochromatic lenses, the resulting final quality can be enhanced further in photomicrography.

As RGB-CCD and narrow band filters differ in their transmission it must be determined by specific circumstances (optical characteristics of the specimen, light source, illumination mode) whether the new RGB-CCD filters or traditional narrow band filters will lead to better results.

The extraordinary high grade of color purity and optimization in resolution and sharpness are not achievable by current techniques (changes of the white balance and other camera presets, software-based postprocessing of single shot images, use of daylight conversion filters or other light modulating filters). Nevertheless, these alternative means remain useful in motile specimens which can only be photographed by single shots. It may be regarded as a noteworthy fact that all techniques described in this article lead to relative improvements in standard equipment utilizing commercial digital cameras.

Fundamental further improvements might be expected if a multi-shot camera designed for astronomy were used for photomicrography. In this way, any pixel interpolation could be avoided so that the lateral resolution and contour sharpness might be enhanced further. Moreover, visible signal noise could be suppressed as multi-shot astro cameras are usually equipped with a sensor cooler. For this reason, especially in fluorescence microscopy and other low-brightness techniques associated with long-time exposures, significant enhancements of the final image quality could be expected in photomicrography. ■

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