Preparing Monochromatic Images for Publication: Theoretical Considerations and Practical Implications

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Introduction

In light microscopy, monochromatic images are produced using monochromatic color filters inserted into the illuminating light path. When compared with true-color images, the image quality can be enhanced by this sort of monochromatic light filtering in special circumstances. In particular, contrast, sharpness, and lateral resolution can be maximized and potential chromatic aberration can be avoided. Of course, the specific properties of the specimen and the respective optical equipment will determine whether monochromatic light filtering can improve imaging results compared with unfiltered white-light illumination.

Moreover, monochromatic light is intimately involved in fluorescence microscopy. Fluorescent specimens are illuminated with monochromatic excitatory light (for example, ultraviolet, blue, or green) and the emitted fluorescent light is normally monochromatic as well. Of course, the wavelength of the emitted light is longer than that of the excitatory light. Fluorescent dyes are characterized by specific absorption spectra, emission peaks, and bandwidths. One fluorescent molecule, DAPI (4',6-diamidino-2-phenylindole), absorbs ultraviolet light (absorption maximum: 358 nm), and it emits blue fluorescence light (emission peak: 461 nm). The corresponding transmission and emission spectra are shown in Figure 1 (modified from [1] and [2]).

When digital color photomicrographs are taken with monochromatic or polychromatic illumination, the resulting images are normally based on the red-green-blue (RGB) gamut or color space; they can usually be observed on a screen (display, monitor) in appropriate clarity, that is, adequate luminance, contrast, and tonal values. But severe problems can arise when color prints have to be made from monochromatic images regardless of whether they are carried out as photo prints (based on the RGB gamut) or as cyan-magenta-yellow-black (CMYK)-based hardcopies, inkjet, laser, or offset prints. In particular, extraordinary difficulties can be apparent when high-quality monochromatic RGB images have to be converted into the CMYK color space in order to be processed in a print workflow. In this case, it sometimes seems nearly impossible to achieve satisfying results; all types of prints made from the respective monochromatic image can appear very poor, with low brightness, contrast, and clarity. So much so that a lot of detail that can be well-perceived on a color screen or monitor will be invisible in the printed image—even when brightness and contrast are maximally enhanced by image processing software before the image is sent to print.

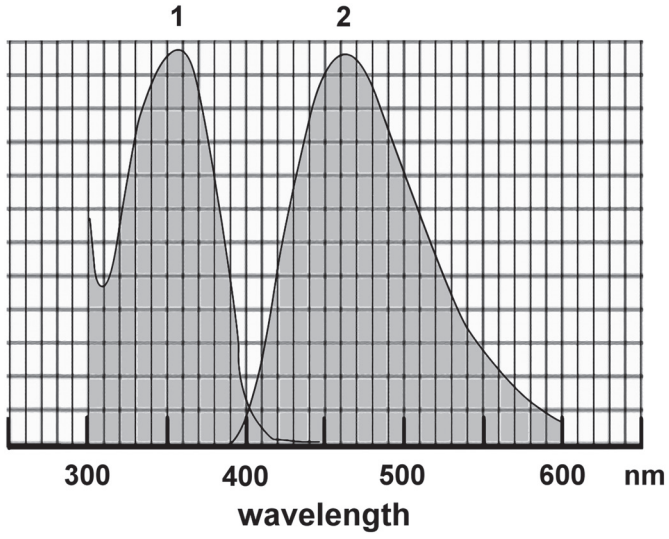

Figure 1: Excitation (1) and emission (2) spectra of DAPI (4',6-diamidino-2-phenylindole), absorption maximum: 358 nm, emission peak: 461 nm (modified from [1] and [2]).

In view of these technical problems, certain theoretical aspects are compiled in this article, which can be regarded as "causers" of the respective difficulties. Then some practical solutions are presented, which seem to be well suited to improving quality in monochromatic image prints.

Fundamental Reasons for Poor Quality in Monochromatic Image Prints

Colorspace limitations. When monochromatic color prints appear poor in quality, limitations of the respective color space should be taken into account as a possible causal factor. Both color spaces, RGB and CMYK, are not congruent with the color perception of the human eye, and the CMYK color space deviates more than the RGB gamut [3]. In the eye, three different retinal cones act as color receptors [4]: The S-cones detect short wavelength blue light (maximum sensitivity: about 445 nm), the M-cones medium wavelength green light (maximum sensitivity: about 540 nm), and the L-cones long wavelength red light (maximum sensitivity: about 565 nm). The half-intensity width of the visible light spectrum is about 110 nm for the M- and L-cones and 55 nm for the S-cones. Approximately 5–10 percent of all retinal cones are blue-detecting S-cones, 30–35 percent are green-detecting M-cones, and 55–60 percent are red-detecting L-cones (including long-wave green and yellow wavelengths). Thus, the sensitivity of the human eye is different for various colors or wavelengths as shown in Figure 2 (modified from [5]). The following fundamental facts can be derived from these findings: (a) All colors that can be perceived by the retina result from the perception of three primary colors: red, green, blue. (b) The color sensitivity of the human eye is maximized for green and green-yellow. (c) The perception of all existing colors that can be visualized by the retina is based on an additive color mix.

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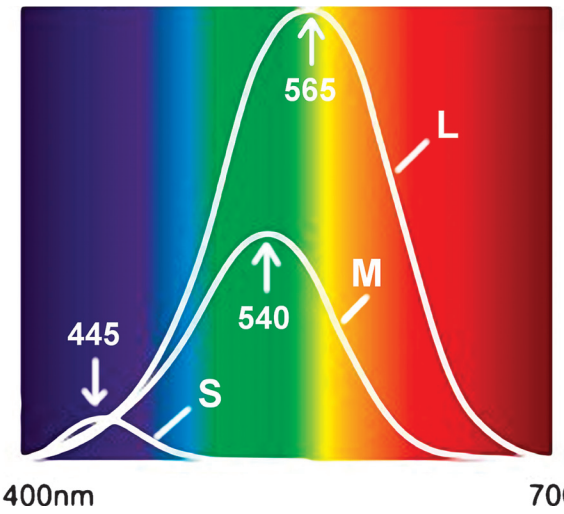

Figure 2: Spectral sensitivity of the human eye, presented for S-, M-, and L-cones, further explanations in the text (modified from [5]).

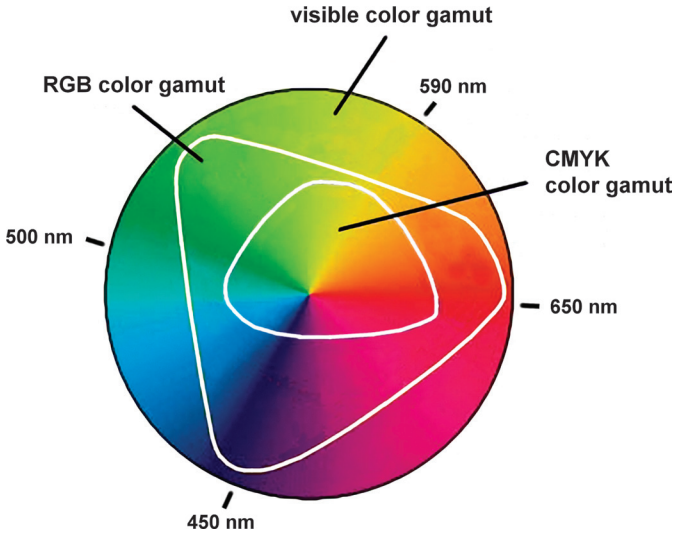

Figure 3: Visible, RGB, and CMYK color gamut (modified from [6]).

Digital photographs (and photomicrographs) are normally taken in the RGB color space. The RGB gamut is partially adapted to the physiological color perception of the retina, because all colors are generated by additive color mixing of the same three primary colors. Moreover, in standard color sensors, the proportion of green, red, and blue detecting pixels is approximately adapted to the color sensitivity of the eye, 50 percent of pixels detecting green and 25 percent detecting red and blue. But, the RGB gamut can just display only 70 percent of the colors that can be perceived by the human eye [6].

When RGB images are visualized on color screens (monitors, displays, LCD, TFT, and CRT/vacuum tube), they are presented in the RGB color space, because also this hardware works with the same three primary colors. And color photo prints created by exposure of light-sensitive photographic papers also are based on the RGB color space. Moreover, in all monitors and color photo printers the nuances of colors are created by additive color mixing.

When RGB images are to be printed in color by any type of printer (inkjet, laser, offset, and other professional systems), however, they first have to be converted into the CMYK color space. This color gamut is much smaller than the RGB color space, reproducing just circa 20 percent of the colors visible to humans [6]. Figure 3 illustrates the different limitations of the RGB and CMYK color spaces when compared with the human visible color gamut in an instructive manner (figure modified from [6]). As demonstrated in Figure 4 (modified from [6]), the RGB color gamut on which a true-color monitor is based can be compared approximately with the color gamut of a photographic (analogue) color film. Whereas, the CMYK color gamut is significantly smaller within the whole range of print qualities (newsprints up to high-end art papers). Moreover, Figure 4 shows that the RGB gamut shown in a true-color monitor reaches to the shortest wavelengths within the visible light spectrum, even when compared with an analog photo film. Thus, monochromatic RGB images colored in short wavelengths (blue or violet) can be observed in best quality when visualized on a high-quality true-color monitor. But they cannot be printed in color in satisfying

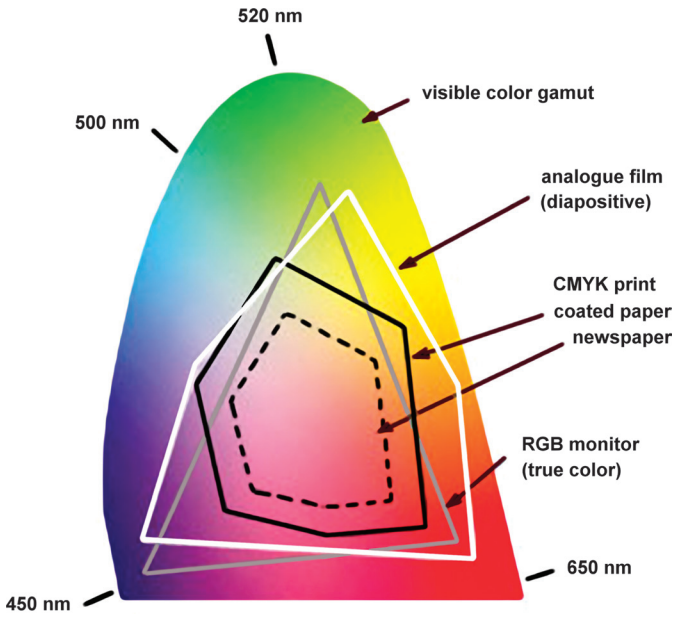

Figure 4: Color presentation in analogue films (diapositives), true-color monitors (RGB-based) and color prints (CMYK-based), used for high-end art papers and newspapers (modified from [6]).

quality when their monochromatic color is outside the CMYK gamut.

Finally, for each color space (RGB and CMYK) particular color tones exist in reality that cannot be authentically reproduced by the respective gamut (so-called out-of-gamut colors). Therefore, serious problems will regularly occur, especially with monochromatic light when its wavelength is outside the respective color space used for imaging or printing.

Limitations in luminance and dynamic range. Further limitations result from differences in the maximum luminance and dynamic range achievable in photographs, prints, and publications. The luminance (parameter of brightness) is measured in lux or candelas per square meter (1 lux = 1 cd / m^2). Dynamic range is defined as the ratio between the maximum

and minimum values of luminance within a specimen or image. The normal dynamic range in natural, everyday daylight scenes is circa 100,000:1, that is, the brightest visible structures are 100,000-fold brighter than the darkest environmental components. Dynamic ranges higher than 10,000:1 are defined as high dynamic ranges (HDR). Thus, the existing high differences in natural brightness cannot be transferred to any photograph or print.

When an analog camera is used, dynamic ranges up to 10,000:1 can be recorded on the film used (color slide). When the dynamic range of an image is 10,000:1 or lower, it is defined as a low dynamic range image (LDR). In digital cameras, the maximum dynamic range detectable by their sensor is lower than in analog cameras (about 1000:1). In common screens (laptop, computer, TV), visible dynamic ranges are reduced further (typically 100:1 to 800:1). With regard to different types of monitors, in up-to date thin-film-transistor liquid-crystal display (TFT LCD) screens, the maximum luminance is significantly higher (500–800 cd / m^2) than in CRT monitors (circa 100–150 cd / m^2) so that much greater differences in brightness and tonal values can be perceived with a TFT than using a CRT monitor. In all types of paper-based prints (photo prints, inkjet, laser, offset, and other high-end hardcopies) the visible dynamic ranges are reduced still more (about 32:1 to 64:1). Thus, the sensitivity in visualization of differences in brightness is radically diminished when digital images are transformed from a screen view to a print or hardcopy image. Some more details about this topic are compiled in a previous contribution [7].

Because of these facts, various problems can arise when monochromatic images must be converted into paper-based prints, especially when fine differences in brightness and contrast are to be discriminated. The dynamic range of a color print is so low compared to that of either the digital camera or the LCD monitor that higher dynamic range in the latter devices will not improve a color print.

Materials and Methods

Preparations of human chromosomes (cell cultures of lymphocytes, mitoses blocked in metaphase by colchicine) dyed with DAPI (4',6-diamidino-2-phenylindole) were examined in fluorescence illumination (ultraviolet excitatory light) using a plane achromatic oil immersion lens 100/1.30. The blue emitting fluorescent specimen was selected because monochromatic blue can lead to greater problems in authentic color printing than monochrome green or red. Thus, the out-of-gamut problem is often more apparent with shorter wavelengths.

In this illumination technique, the chromosomes appeared in monochromatic blue fluorescence light (peak wavelength: 461 nm). Digital color photomicrographs were taken in standard technique using a 7.1 megapixel (MP) camera equipped with a 1/1.8" RGB color chip adapted to a Leitz/Leica Vario-Photo-Ocular 6.3–12.5×. A representative photomicrograph showing an aggregation of chromosomes in adequate separation was used for different experiments carried out in order to evaluate and improve print quality. Color prints (CMYK-based) were made with a standard HP inkjet printer using high-quality glossy paper, and photo prints (RGB-based) were made by a local photo print service.

Improving the Quality

To improve the quality in prints employing monochromatic colors, hardware-based and software-based solutions were evaluated. After explanations of these solutions in the following sections, examples will be given in the results section.

Hardware-based solutions. When monochromatic photomicrographs are to be printed in the original version without being rendered in any postprocessing procedure, the photograph in question can be presented on a high-resolution TFT monitor, and digital color reproductions can be made from this screen by use of a normal digital camera with standard presets (automatic white balance, automatic exposure, activated autofocus). According to the author's experience, a high-quality TFT screen is more suited for this task than a CRT monitor. For practical evaluations, we used a laptop Dell XPS M 1730. This gamer laptop is fitted with a high-quality TFT screen characterized by the following technical data: WUXGA (full-HD), resolution of 1920×1200 (2.3 megapixels), refresh rate: 60 hertz, pixel size = 0.191 mm, diagonal size = 17 inches, luminance = 250 cd / m^2, and dynamic range = about 800:1. Monochromatic color images presented on this screen in true color (32-bit) were photographed with 6 MP and 14.5 MP digital cameras. According to the refresh rate of the screen (60 hertz), the exposure time (shutter speed) was 1/60 or 1/30 second.

Thus, the screen showing an original monochromatic image can be photographed like a normal multicolor scene. Monochrome structures within the screen appear in a particular color and are photographed like other colorized objects without remaining monochromatic. So, the range of printable tonal values is higher than in the original monochromatic image, and the image can be printed as a color image with enhanced clarity.

Software-based solutions. When a reproduction of the color in question is not needed in digital monochromatic images, the respective original photomicrograph can be converted into black and white by computer-based postprocessing. It is essential for good results that the color channels (red, green, blue) are able to be balanced separately from each other when the original color image is modified to black and white. Figure 5 shows the control panel of a well-suited Photoshop plugin (convert, RGB to gray). In the standard preset used for multicolor images, the monochromatic blue image is black so that the blue fluorescent chromosomes cannot be detected (Figure 5a). Only by a strong selective enhancement of the blue dominance (blue channel) can the chromosomes be well contrasted (Figure 5b). By this "trick," 256 tonal values can be maximally used for reproductions of fine nuances in originally blue structures when the color image is taken at 8-bit shade depth. The black-and-white image thus-created can be printed in satisfactory quality by any technical procedure.

The converted black-and-white image described, showing the existing fine tonal values in appropriate clarity, can be also used for improvements of color prints where monochromatic images are to be presented in color. To achieve this, the black-and-white conversion can be superimposed on the original blue monochromatic image. In the composite sandwich image thus-created, monochrome structures appear in many more tonal nuances and better contrast. In some cases, the clarity of printed details can be accentuated still more when the color

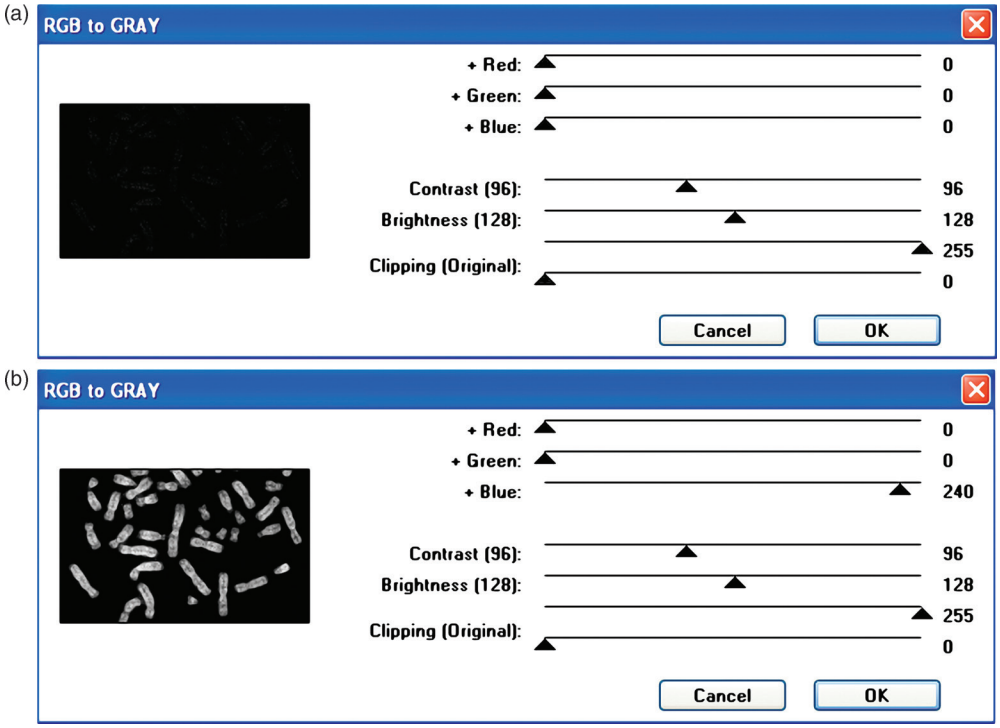

Figure 5: Control panel of the RGB-to-Gray plugin. Standard preset, color channels equalized (a), selective amplification of blue (b), further explanations in the text.

balance is shifted a little bit to red and green so that a few red and green pixels are combined with the preexisting blue pixels. Of course, such color shift has to be carried out carefully so that the fundamental color is not changed and remains dominant. The freeware DRI-Tool or Image Stacker are useful for such superpositions. When the doublet of color and black-and-white images is selected, both images are equalized with regard to their brightness and contrast and then combined with each other in an automatic run so that they contribute to the composite image in the same percentage (50:50). Alternatively, the images can be superimposed with other software for DRI (dynamic range increase) or HDR rendering. The software Photomatix Pro could be successfully used for this task. In contrast to the freeware mentioned above, the final image resulting from superposition can be adjusted in the Photomatix software with regard to several parameters so that existing fine tonal nuances and details can be accentuated by contrast amplification without producing any "overdriving" or blooming. Moreover, high-grade contrast equalizations can be carried out so that the brightness of specimens and background can converge in order to avoid large high differences in brightness and contrast. In Photomatix Pro, the so-called details enhancer is well suited for this task in many cases. The final quality of prints and hardcopies can be enhanced further by these particular rendering techniques. Further information about the software mentioned above is given in a previous publication [7].

Results

A high-quality digital photomicrograph of human chromosomes dyed with DAPI and appearing in monochromatic blue fluorescence light (emission peak: 461 nm) was used for our technical tests. When presented on true-color monitors (TFT LCD and CRT), all chromosomes were visible in excellent quality: background was black and the chromosomes were well contrasted showing a strong blue luminance, a high complexity of tonal values, and fine details. The same digital photomicrograph looked inferior when an inkjet print (based on CMYK) was made with a HP standard color printer and high-quality glossy paper (Figure 6a). The contrast was very low, the background somewhat gray, and the blue chromosomes appeared very flat: the brilliance and fine details were lost. This poor result could not be influenced by maximizing contrast, gradation, or brightness.

Color photo prints based on RGB lead to better results than color prints made with a CMYK printer. From the same photomicrograph above, a color photo print (based on RGB) was made by a professional photo service (color scan in Figure 6b). In this photo print, the background was a little bit darker and the chromosomes showed a higher level of luminance and contrast than in Figure 6a, but the existing high variability in tonal values and fine details, which could be observed on a true-color monitor, were not properly reproduced. In Figure 6b the brightest blue appears to be saturated because the dynamic range is so limited in color prints. All in all, it was not possible to produce satisfying prints of this photomicrograph when the original digital image was directly used for print, regardless of whether papers were printed based on the CMYK or RGB color space.

Next, digital color photographs were taken from the high-resolution TFT screen of the Dell laptop. The photomicrograph was presented on this monitor at full screen size so that the monitor's maximum resolution (2.3 MP) could be used. As described above, digital photographs were taken from this screen with two different cameras (Figure 7). In principle, both cameras led to useful results, but the image taken with the 14.5 MP camera (Figure 7a) showed more tonal values and fine details than that taken with the 6 MP camera (Figure 7b). Although the TFT LCD screen of the Dell laptop was high-resolution, the monitor's pixel were photographed and projected onto the pixels of the respective camera sensor so that moiré artifacts were sometimes apparent. In the 14.5 MP camera, each single monitor pixel was imaged by 6.3 camera pixels (14.5 : 2.3), whereas in the 6 MP camera just 2.6 camera pixels corresponded to a monitor pixel. For this reason, moiré artifacts were more likely to be apparent when the 6 MP camera was used. Of course, moiré effects can often be removed with the help of image processing software.

All color images taken from the high-quality color screen could be printed in much better quality when compared with

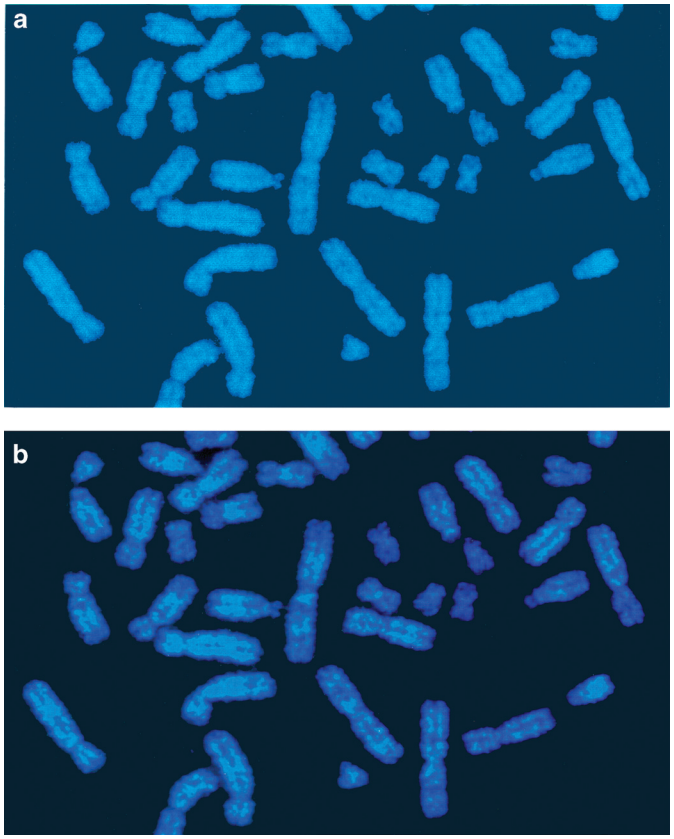

Figure 6: Human chromosomes, DAPI, UV-excitation, blue fluorescence, further explanations in the text. Color scans of an inkjet print (a) and a conventional photo print (b).

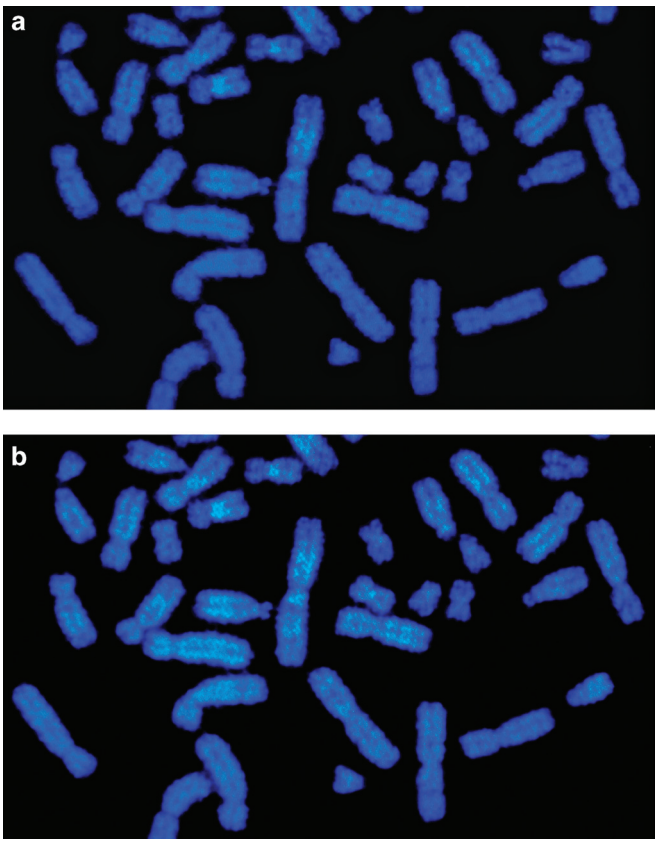

Figure 7: Digital photographs of a TFT monitor screen, Dell Laptop XPS M 1730, further explanations in the text. (a) 14.5 MP camera, (b) 6 MP camera.

prints from the corresponding original photomicrograph. In the color prints shown in Figure 7, the chromosomes appeared in significantly higher clarity even when made with a simple inkjet printer. The background was black, the blue color was well saturated, and more tonal values and details were visible than in the original prints made from the printed original photomicrograph shown in Figure 6.

Examples of postprocessing are presented in Figure 8. The high variability of tonal values can be reproduced in optimum contrast and clarity when the monochromatic blue image is converted into black and white as described above (Figure 8a). When authentic color prints are needed, the black-and-white image can be digitally superimposed on the original monochromatic color image in the way already described. Figure 8b gives an example of this technique carried out with the DRI Tool in an automatic running procedure. Figure 8c shows a well-balanced superposition made with the Photomatix Pro software (command: "combine highlights and shadows"), presenting the blue color in higher intensity.

Using the "Details Enhancer" fitted on the Photomatix Pro software, fine details existing at the perception limit of standard reproductions—even minimal irregularities at the surface of the specimen slide—can be accentuated and visualized in highest quality when both images—the black-and-white and the original color image—are rendered (Figure 9). Of course, the character of the resulting digital reconstruction deviates from that of the original fluorescence image, but the finest details can be printed in superior clarity.

Discussion

In this paper, several techniques are described that can lead to superior results when prints are to be made from monochromatic images. Significant improvements can be expected, especially in structures imaged in out-of-gamut colors. Taking digital color reproductions from a high-resolution TFT monitor might be the simplest way to achieve printable results, but the resolution of the camera sensor should be as high as possible so that potential moiré artifacts will be reduced or avoided. Moreover, the usable resolution of the reproduction is determined by the maximum resolution of the monitor screen. Alternatively, monochromatic color images can be converted into black and white and then superimposed on the corresponding original image by use of specialized software. In this way, the black-and-white image is colorized, and fine tonal values, which are solely visualized in the black-and-white image, are transferred into the color image. In our opinion, both variants (hard- and software-based) are useful for didactic purposes when the character and colorization of monochromatic color fluorescence images, for example, achievable with DAPI, are to be presented in publications.

All techniques carried out with monochromatic images based on a single color can also be used for optimization of prints, which have to be made from multiply-stained fluorescent specimens (living cell cultures, for instance, dyed with DAPI, Mito Tracker Red, and Alexa Fluor phalloidin) where the quality of standard color prints of the original images will not be satisfying.

The fact that high-end TFT LCD monitors promise the best results in visualizations of such problem images might

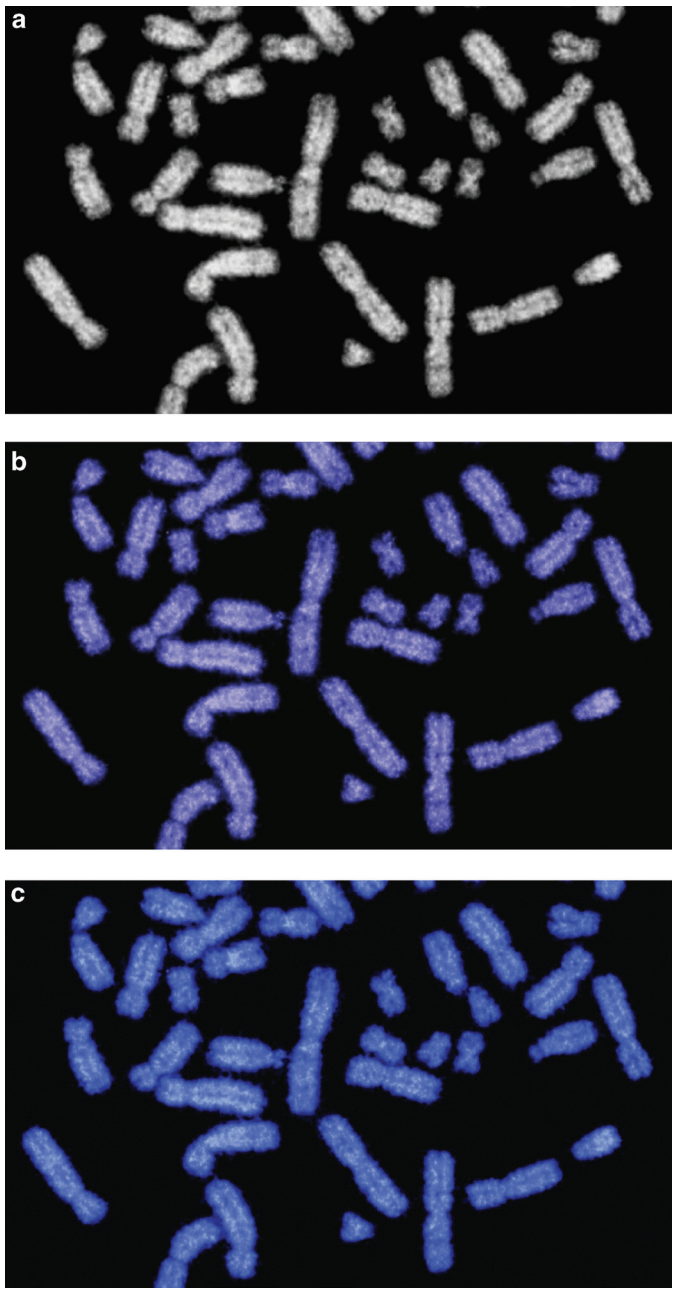

Figure 8: Examples for digital postprocessing, (a) black-and-white conversion; (b) superposition of genuine image and black-and-white conversion, sandwiches made with DRI-Tools; and (c) Photomatix Pro.

be regarded as a strong argument for online publications in addition to hardcopy-based print media. When color reproductions are not needed, color images that are out-of-gamut can also be presented without any loss of quality when properly converted into black and white.

Because professional printing techniques are based on CMYK printers, potential monochromatic "problem" images should at first be test-printed in color using a standard inkjet printer set to CMYK before they are submitted for publication in print media. In this way, difficulties associated with color prints should be detected in advance, and problem images that should be rendered by the methods described here could be preselected.

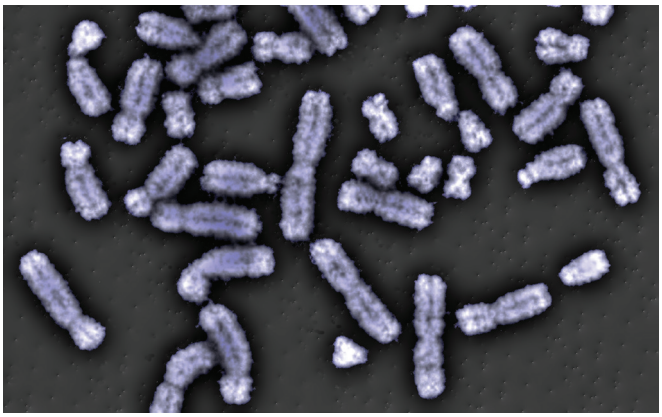

Figure 9: Superposition of genuine color and converted black-and-white images by use of the "Details Enhancer" software, Photomatix Pro.

Conclusions

This contribution deals with hard- and software-based techniques for optimization of color prints made from monochromatic images that cannot be properly printed by standard techniques. Fundamental reasons for technical difficulties are compiled, and specific recommendations of suitable solutions are presented.

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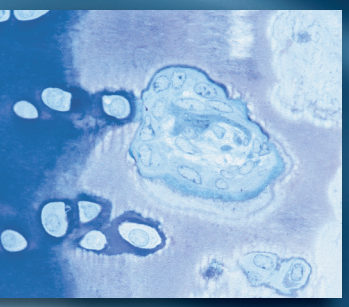

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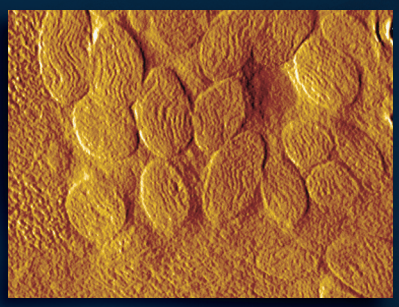

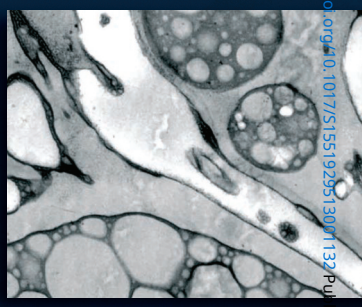

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