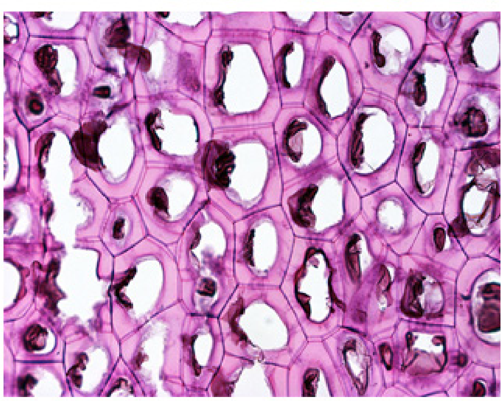
LUMENERA **WHITE PAPER SERIES**



# Importance of Color Reproduction in Scientific Images

## WHAT'S INSIDE

# INTRODUCTION

Through our eyes, seeing is not always believing. Under different lighting conditions, we tend to see the same objects as having the same color. For example, an apple will appear red whether it is lit by daylight or candlelight and a white sheet of paper will be perceived as being white regardless of the light source. This is something that we do subconsciously and is known as 'color constancy' or 'chromatic adaptation'. Our eyes receive light via two photoreceptors: cones and rods. The visual cortex processes this information and color constancy allows our brains to recognize objects regardless of lighting conditions.

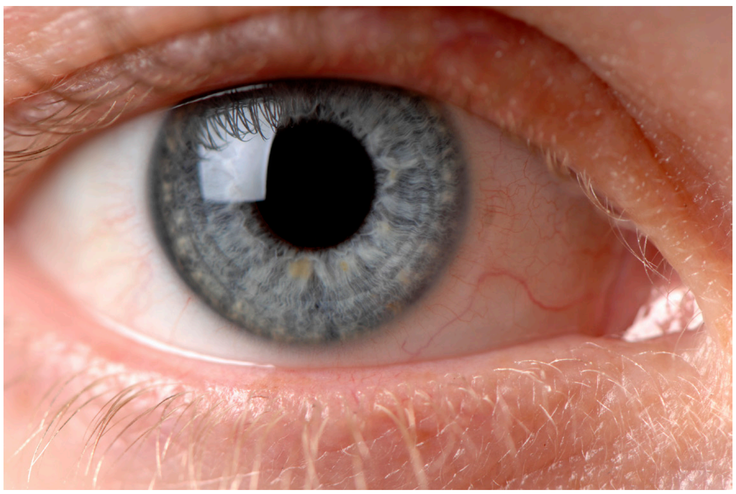
This is all very well when we are interacting with the ever-changing contrast of the world around us. However, when it comes to viewing microscopic specimens with a monitor or capturing microscopy images with a camera, the colors we perceive down the eyepieces will differ to those viewed on the screen or captured by the camera.

## The Importance of
# COLOR REPRODUCTION

Does it matter if the images we see down the microscope don't match those captured by the camera? Well, for samples involving color, such as histology slide stained with hematoxylin and eosin, it is very important. This is to ensure the image can be correctly analyzed and can also be compared with previous samples. With poor color reproduction there is a chance that important information may be missed because the contrast between colors isn't clear. Therefore, we need to adjust images taken on the microscope to account for the light source.

In image processing, this chromatic adaptation is referred to as 'white balance' or 'color balance'. Data is collected by image sensors (such as photomultiplier tubes or camera sensors) and processed for color reproduction on computer screens or camera displays. Obviously, electronic image sensors and processors don't match our cones and rods, and those sensors and processors can't always tell that an apple is red in candlelight. Therefore, it's important to take color correction into account when viewing and capturing images from microscopes.

# COLOR CORRECTION by Camera Sensors



> **THE HUMAN EYE** and the image sensors employed in microscopy differ in perception of light and color.

It's important to match, as closely as possible, the colors we view down the eyepieces to the colors displayed on a monitor or camera.

Two of the most common image sensors in microscope camera are the Charge Couple Devices (CCD) and Complementary Metal Oxide Semiconductors (CMOS).

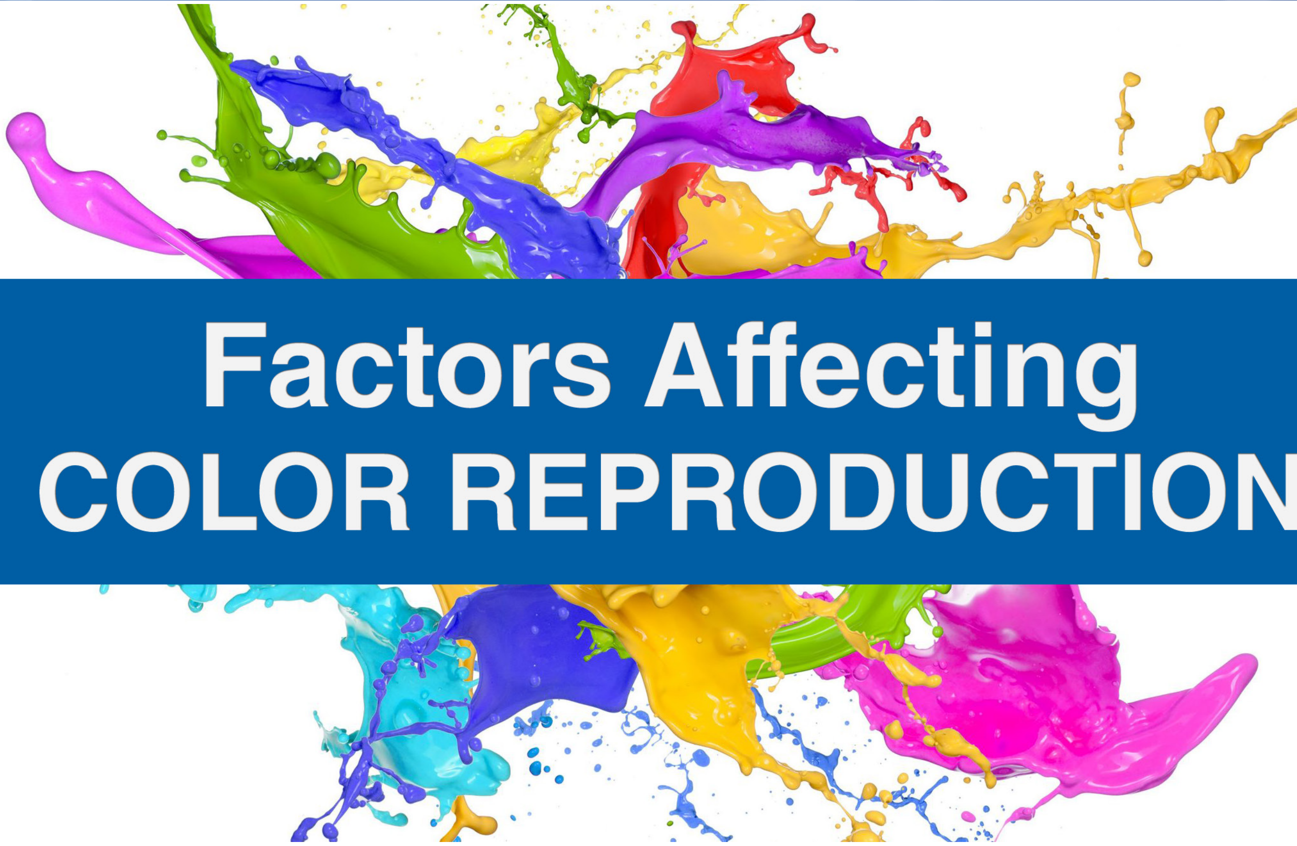
Cameras with either CMOS or CCD sensors are sensitive to infrared (IR) light, which can have the effect of reducing image contrast. Some cameras incorporate IR filters that can compensate for this sensitivity, or filters can be used in the microscope set-up.

Color reproduction will also vary between microscopes, room set-ups, and lighting conditions. Furthermore, colors will also vary depending on the samples, stains or fluorophores that are used. If possible, always use the same microscope system and ensure it is correctly aligned for Koehler Illumination.

## WHAT IS Koehler Illumination?

Koehler Illumination is a method that provides optimum contrast resolution by focusing and centering the light path and spreading it evenly over the field of view.

This process is used to achieve bright and even illumination across the sample, whilst ensuring that the illumination source is invisible to the resulting image. This helps to create the best possible image quality and is the illumination method of choice for the majority of modern microscopes.



# Factors Affecting COLOR REPRODUCTION

## Light Source

You've probably heard colors being referred to as 'warm' or 'cool' referring to 'color temperature'. Color temperature is measured in Kelvin (symbol 'K'). A bit counter-intuitively, higher temperature colors are called 'cool colors' (such as blue or white) and the lower temperature colors are known as 'warm colors' (such as red and yellow).

Microscope light sources vary with color temperature. For example, daylight is regarded as 5000 K and a tungsten halogen lamp has a temperature of around 3200 K. Filters can be used in microscope systems to raise or lower the temperature of the light source. Color temperature isn't everything, though. A variety of light sources may have the same color temperature, but have different spectral properties. Also, color temperature isn't a reliable prediction of how specimens will be viewed and processed by the microscope imaging system.

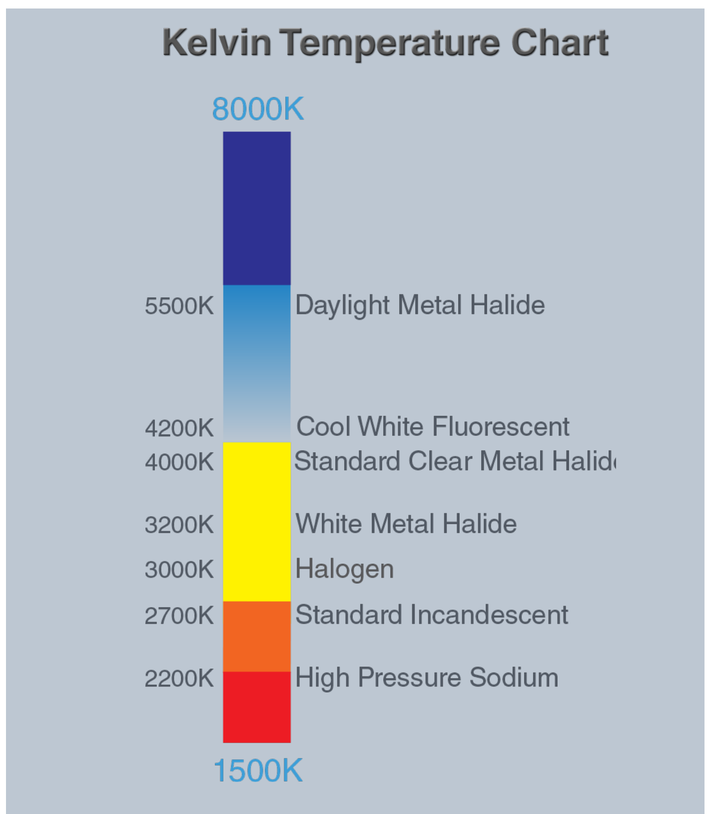



*FIGURE 1. Color temperature chart*

## Camera Type

CCD and CMOS cameras can be adjusted electronically for white balance. However, the light sensing elements of these sensors are monochromatic and color image is obtained by detecting the light that passes through red, green and blue (RGB) filters, which cover each individual pixel in the sensor array. Different cameras have different color correction systems. The software alone controls some systems and others require both software and hardware adjustments. Some settings cannot be altered by the user, meaning it is important to select a camera that has good color reproduction and color correction.

Lumenera designed one software adjustment system for use in their cameras and specific applications called 'Color Correction Matrices' (CCM), which uses a color reference matrix to compare each color component of the image.

## Improper White Balance

White balance is the process of removing unrealistic color cast in an image. Because light sources vary in color temperature, this will have an effect on the white balance of an image on screen or captured by a camera. Figure 2 shows images before and after white balance has been applied, you can see how the colors differ greatly before and after adjustment.

One way to ensure the correct white balance settings are applied on the camera is to use Lumenera's 'INFINITY Software White Balance' functions, which provide several methods for adjusting the color balance. White balance adjustments should be performed any time the lamp intensity is altered or filters are inserted in the optical path.

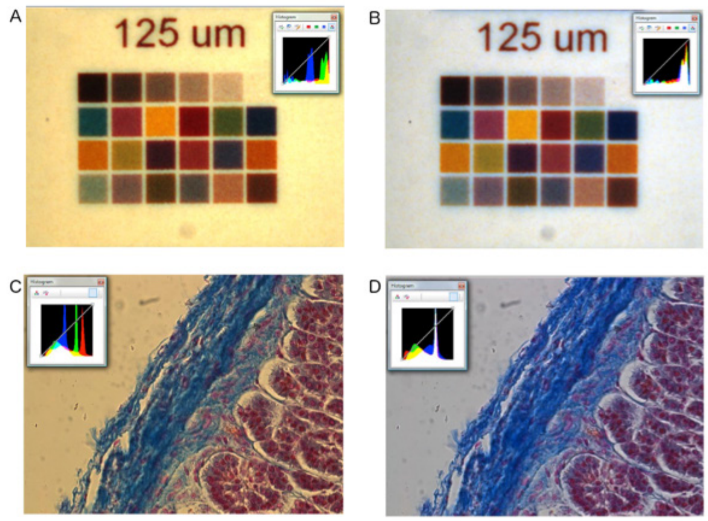



*FIGURE 2. Effect of white balance on color reproduction. A) Microscope Slide color chart; B) Microscope Slide color chart after white balance. C) Feline Adrenal gland stained with hematoxylin and eosin stain D) Feline Adrenal gland stained with hematoxylin and eosin stain after white balance. Images were taken usingOlympus BX51, Halogen lamp, with daylight filter and a INFINITY3-3URC.*

If you feel the automatic white balance adjustment has not correctly white balanced your sample, you can also manually adjust color channel gains while referring to the histograms for an empty field of view. Figure 3A shows a histogram with poor white balance – each of the channel outputs has distinct peaks. You can manually adjust each channel output so that the three peaks overlap as seen in Figure 3B. For a detailed guide for white balance adjustments using the INFINITY ANALYZE view this video tutorial.

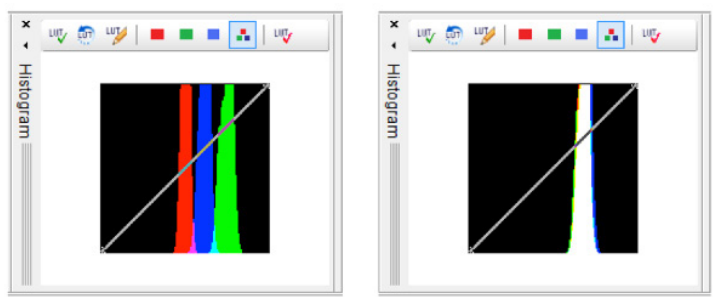
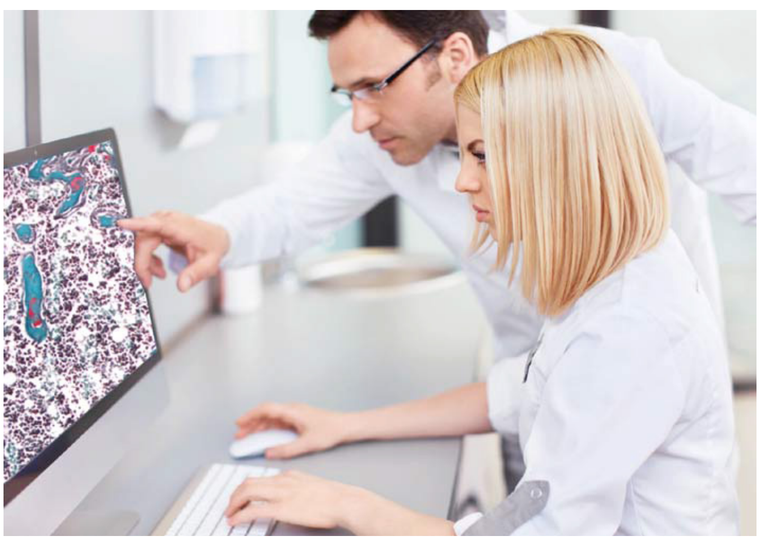



*FIGURE 3A & B Using histograms to aid in white balance adjustments. Left) Histogram showing poor white balance – each of the channels are visible separately. Right) Histogram following white balance – the channels are now overlapping.*

## Choice of Monitor

Color reproduction will vary between monitors. Monitors must be calibrated when first installed. Calibration should be performed at regular intervals over the lifetime of the display. Additionally, if correct color balance is important for imaging of your samples, then several companies offer medical display monitors. These specialized monitors offer a more accurate reproduction of color than standard monitors.



## Medical RGB

Even with specialized medical display monitors, there is still a concern over standardized color reproduction. There is currently no standardization of color management across platforms. In order to address this issue, The International Color Consortium (ICC) was set up in 1993. Within the ICC there is a Medical Imaging Working Group that promotes the correct use of color management across a variety of medical and research areas. The group is currently addressing the fact that there is no suitable color display calibration objective for medical imaging displays designed to show color images. The working group is also examining the production of a suitable calibration slide for use in histopathology to enable consistent color reproduction between different imaging systems.

# How to OPTIMIZE COLOR PERFORMANCE for Lumenera's USB cameras

**This section provides an overview** of the camera processes and settings that are used to obtain the best color possible from any Lumenera USB camera.

Once the camera, illumination source, and optics have been configured, the application software should be used to display the camera image and manipulate the camera settings. When you first start the camera control software, refer to the live video preview from the camera, and begin by adjusting the settings for Exposure & Gain. It is important to manipulate these two settings first, regardless of how the colors appear in the image on the monitor.

## STEP 1
## Exposure & Gain Adjustments

It is important for the image to be properly exposed before making adjustments to any other camera properties. Verify that the camera gain is set between a value of 1 & 3 to begin.

Next adjust the exposure to increase or decrease the intensity of the scene. This can be accomplished by moving the exposure slider control, typing a specific exposure value, or enabling the Continuous Auto Exposure control. If the Auto Exposure control is used, choose an average pixel intensity value in the range of 150 to 200, to begin.

Increasing the camera's exposure time will result in the frame rate performance being reduced. For example, if each frame requires 100ms (1/10th second) of exposure, then the camera will only be able to deliver 10 frames per second, and an exposure time of 200ms (1/5th second) will result in only 5 frames per second. Therefore it is generally preferable to keep the camera operating at shorter exposure times. If the illumination level cannot be increased, then the camera gain may need to be increased. The gain adjustment is an amplification of the signal from the sensor chip. Too much gain being applied will result in the image developing the appearance of electronic noise in the output.

# STEP 2
## White Balance


*FIGURE 4A. Image before white balance*


*FIGURE 4B. Image after white balance*

Once the camera settings are providing a live preview image with suitable brightness, the next step is to balance the camera's Red, Green, and Blue channel output for the light source. A correct white balance can only be obtained on a camera where the output is below the maximum intensity. For this step, place a white (or uniform grey) card in front of the lens. If the camera is used on a microscope with transmitted light, use the background light source for the white balance operation, by removing the sample from the field of view. Click on the White Balance function in the application software. The live preview image should now show a grey result. Replacing the sample in the field of view, or removing the white card from in front of the lens should result in the live preview image showing colors that look accurate – however, there may be further fine-tuning required to obtain the best results.

Be aware that many illumination sources will vary in color temperature as the intensity is adjusted. The ratio of Red, Green, and Blue wavelengths in the lamp will change as the power level increases or decreases, or if optical filters are added to the light path. A new white balance adjustment may be required each time the lamp intensity is altered. For this reason, it is recommended that the camera's exposure slider be used as the preferred adjustment when the amount of light reaching the camera varies.

Individual display monitor settings can vary greatly between manufacturers. It is important to understand that the appearance of the image produced by the camera is ultimately influenced by the adjustments programmed into the monitor itself. If the color performance is not meeting your expectations, ensure that the monitor adjustments are within a normal operating range.

## STEP 3 Further Adjustments

**Color Correction Matrix (CCM):** A camera's response varies with different illumination sources, due to the fact that artificial lighting cannot produce a full spectrum of light. This means that they do not produce an even amount of light across all possible color frequencies. The purpose of a color correction matrix is to allow the camera to reproduce the color of the scene as faithfully as possible by compensating for the missing color frequencies in the light source. Sunlight is the only full spectrum illumination source.

Each camera supports several CCMs, depending on the application software. The basic ones are: incandescent, fluorescent, halogen and daylight. Each CCM is tuned for the spectral response of the light source, to provide the optimal color performance that matches the specific sensor characteristics in each camera model. In certain situations, the illumination used may not be accurately represented by any of the default CCMs, so an option is available to define a custom color correction matrix. In this case, the custom CCM would be created to compensate for the unique spectral characteristics of the light source to ensure that the camera is reproducing the colors correctly.

As the appearance of the camera image is impacted by so many configuration settings (including the monitor), select a CCM that produces the color response that you desire, rather than relying solely on the name of the CCM that matches your lamp type.

*FIGURE 5A. Daylight CCM*


*FIGURE 5B. Incandescent CCM*


*FIGURE 5C. Fluorescent CCM*


*FIGURE 5D. Halogen CCM*

**Gamma:** Setting a suitable gamma correction value is based on the type and quality of the display monitor, in addition to the target scene and illumination. Gamma correction is implemented as a look-up table on-board the camera, where the intensity values are altered in real-time, based on the numerical value of this setting. A default value of 1.0 is suitable for most flat-screen monitors. This value can be adjusted based on the monitor performance, until it provides the best color and separation of the range of intensity values.

For example, if insufficient detail is discernible in the darker regions of a scene, this normally means that the gamma value should be increased. This will brighten up these regions and provide images with an improved color performance.


*FIGURE 6A. Gamma: 1.0*


*FIGURE 6B. Gamma: 1.25*


*FIGURE 6C. Gamma: 1.5*

**Saturation:** This parameter alters the manner in which output colors are presented. Saturation is a characteristic of the observation of color. Saturated colors are called strong or vivid. De-saturated colors are referred to as weak, or washed out. By default, the camera does not apply any saturation. Values greater than the default value make the images more saturated in color, i.e. the colors become more vibrant, while values less than the default setting make the images less saturated. Reducing the saturation setting to the minimum value removes all of the color information from the images, thus producing a monochrome result.

For example, increasing saturation will make red areas become redder, green areas greener, etc. There is a limit to this, beyond which incorrect hues are introduced. Normally, saturation will only need to be adjusted by in the range of +-30% to achieve optimal response from the camera. Values outside of this range will affect the image dramatically and result in poorer color performance.


*FIGURE 7A. Saturation: Minimum*


*FIGURE 7B. Saturation: Unity*


*FIGURE 7C. Saturation: +25%*

**Contrast, Hue, Brightness:** It is recommended that these three camera parameters be left at their default values at all times. Even subtle alterations to the Hue will produce an output from the camera with wildly varied colors. If you are having any difficulties in obtaining an accurate color response from your camera, verify that these settings are reset (Contrast:0, Hue:0, Brightness:0).

**Demosaicing Method:** Most color camera sensors use a monochrome sensor with a color filter mask of Red, Green, or Blue over each pixel to capture color information. The typical layout of this arrangement is known as a Bayer filter. The raw images returned from the camera consist only of intensity measurements taken at each pixel. To extract the color information out of the images, a demosacing algorithm is used to merge the values of neighboring pixels to determine the appropriate missing color values for each pixel location. The number of pixels and the demosaicing method used both determine the accuracy of the color interpretation for each algorithm.

Typically, the camera will use a faster, but lower quality, demosaicing method for the live video preview as the video refresh rate takes precedence over the finest details being resolved. The application software will use the highest quality demosaicing method when capturing an image from the camera.

In most cases, setting the appropriate CCM and demosaicing method along with a proper white/color balance will produce excellent results. There are occasions where it may be necessary to increase the gamma and saturation to improve the color performance of the images and to make the colors more vibrant.

# About The Company: LUMENERA CORPORATION

Lumenera Corporation, a division of Roper Technologies, headquartered in Ottawa, Canada, is a leading developer and manufacturer of high performance digital cameras and custom imaging solutions. Lumenera cameras are used worldwide in a diverse range of industrial, scientific and security applications.

As a global market leader Lumenera provides an extensive range of high quality digital cameras with unique combinations of speed, resolution and sensitivity to satisfy the demands of today's imaging applications. Lumenera also offers custom design services to OEM partners requiring specialized hardware and software features.

Core competencies include digital bus technologies such as USB 3.0, USB 2.0, Ethernet, HDMI and Gigabit Ethernet (GigE) as well as a complete command of digital imaging hardware and software built around CMOS and CCD based imagers. Our diversity provides our customers with the benefits of superior price-to-performance ratios and faster time-to-market.

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