Displays 33 (2012) 214–220

Contents lists available at SciVerse ScienceDirect

Displays

journal homepage: www.elsevier.com/locate/displa

Colour management system for displaying microscope images

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ARTICLE INFO

Article history:
Available online 14 March 2012

Keywords:
Digital microscope
Display
Colour management
Colour reproduction

ABSTRACT

A four stages colour management system is developed in order to truly display microscope images on a display and to objectively assess colour properties for cytology samples. By using 35 stained glasses, the colour outputs of two microscopes were simulated. Each stage of the processing was performed and the microscope images captured by two different microscope camera systems were reproduced to a single LCD panel. The management system evaluated was found to be effective in terms of colour image reproduction and could be applied to situations when objective assessment is required.

1. Introduction

The staining of cytology samples has been widely used for many biological and medical applications. In the field of medicine and dentistry, its main applications are in branches of histopathology and microbiology. Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease. However, microbiology refers to study of microorganisms such as bacteria, yeast and virus. When stained cytology specimens are examined, colour property is one of the important measures for appropriate identification of cell types, morphology and nature of the examined specimens [1–3]. One problem using this technique can be that colour variation is often generated during the staining process. In practice, staining results often differ among separate laboratories, as well as within the same laboratory at different times, which can make examination and interpretation difficult. Although this problem has been well recognized by observers, to the best of our knowledge there is no objective measurement method available for quantifying this colour variation due to the limitations of colour measuring equipment for these small and non-uniform samples.

Digital microscopes are relatively new instruments and often incorporate a digital camera to capture images of the specimens observed. They usually facilitate image capture through the optical microscope, thereby enabling the examiner to observe and examine the specimen slides on a colour display or monitor. A key advantage of this method is the ability to electronically store and communicate results. Through the application of this new technique, considerable savings in both time and effort for both technical and medical staff can be achieved by delivering consistent results with readily available images. Furthermore, the results obtained through this type of image processing have the potential to be far more objective than the relatively subjective human assessment that is carried out at the moment. Until recently there have not been compelling reasons for replacing conventional microscopes for medical assessment and diagnosis, although momentum is gaining in the fields of interactive and automated virtual microscopy to help in the assessment and diagnosis of medical conditions [4,5]. One reason for this is that although digital microscopes are capable of producing images that can lead to a diagnosis/interpretation of a tissue sample by analysing the cell type present, it cannot accurately and consistently reproduce colour content. This mismatch is particularly evident when comparing the view seen through a conventional microscope and the colours viewed of a digital image produced by a digital microscope. This difference is due to the RGB colour space used by digital cameras and displays, being different to the corresponding visual stimulus [6]. More specifically, there are two major issues with the colour signals generated by device (RGB) colour space. Firstly, they are all device dependent [7]. That is to say, different digital cameras or displays are known to produce quite different colour signals (RGB) for the same scene. The second problem is that they have no colorimetric basis. As a consequence, the device RGB colour signals cannot be directly linked or correlated to that perceived by the human visual system.

In order to reproduce colour microscope images accurately on a target display, device colour characterisations [8] have to be conducted to connect each device (RGB) colour space to the human eye response. In this study, a whole colour management system/protocol for displaying microscope images is developed and an evaluation of image reproduction from two microscope systems to one LCD display was undertaken.

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http://dx.doi.org/10.1016/j.displa.2012.03.001

2. Methodology and processing

Conventional methods that are widely used for colour management and colour image reproduction were used in this study for the colour correction of microscope images [8]. In order to translate a colour microscope image accurately to a display, four steps are proposed, as shown in Fig. 1. In the first step, an image is captured by the microscopes built in digital camera with an appropriate calibration setting. Each microscope image is set as the original/control image and recorded in camera (RGB) for each image pixel. In the second step, a forward camera characterisation is conducted to transform the original microscope images from camera (RGB) to CIE *XYZ* tristimulus values [8]. The third step involves a reproduction microscope image that is generated for the target display by transforming the CIE *XYZ* tristimulus values to the display RGB values by using the reverse display characterisation model [8]. In the final step, the new generated reproduced image is displayed on the target display.

To develop and evaluate the colour management system, microscope images from two microscope image systems (a) Nikon E800 microscope with a Nikon Coolpix 4500 digital camera and (b) Olympus BX45TF microscope linked to an Olympus Camedia C-3030 ZOOM digital camera were reproduced and displayed on a DELL LCD display. Both microscope cameras offered full manual control over aperture and shutter speed for exposure control and were targeted at users ranging from amateurs to advanced, and more experienced digital photographers. The 21″ DELL LCD display was rendered by a DELL T3400 PC with a Nvidia FX5700 graphics card. Each step for the proposed colour management system is described below.

2.1. Testing samples

When a colour management system is developed, numerous training colour samples should be used, ideally encompass the entire colour spectrum of the reproduction device, and be distributed uniformly throughout that colour range. However, a balance does have to be achieved as practical and processing considerations have to be taken into account. This is because, in theory, the more training colours you have the more accurate the colour reproduction model should be. However, reducing the number of training colours used improves and simplifies (and hence shortens) the reproduction process. Therefore, any decision on the number of training colours used in the colour management system process would be a compromise between model accuracy and the complexity of the characterisation process.

For this study, 35 colour stained glass samples with a uniform colour output were selected as the training colour samples to represent those seen by the human eye through the microscope and the colour specification of each colour slide was then measured under a standard viewing condition. Conventional colour measuring instruments, such as colorimeters and tele-spectroradiometers have limitations due to the practical geometric constraints inherent with the small aperture of a microscope's output. Thus, the viewing conditions of the microscopes had to be simulated to provide meaningful colour measurement data. In this study, a Veri-Vide Luminaire 120 daylight simulator was used to simulate microscope lighting. The 35 stained glass samples were placed in the middle of the lighting unit in a dark room, and colour values were measured using a Photo Research PR650 tele-spectroradiometer in terms of absolute CIE tristimulus values [9]. To reproduce conditions similar to those seen when using a microscope, the PR650 was positioned 70 cm above each sample using a copystand. Colour specifications from the 35 stained glass were then plotted – CIELAB a^*b^* chromatic diagram [9] (Fig. 2). From these results it can be seen that although the colours samples represent a broad spectrum of colour values, there were less samples in the red–blue area.

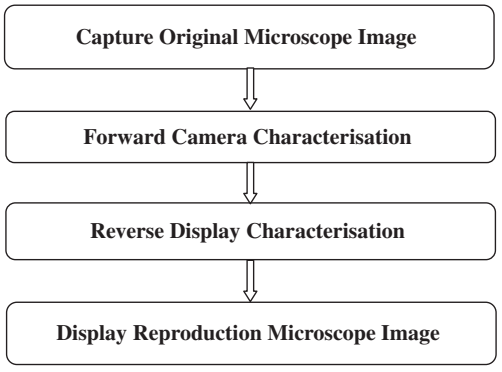

Fig. 1. Steps of colour management system.

2.2. Image acquisition

Microscope images are affected by both the optical component of the microscope and the built in digital camera. Therefore the settings on both are critical for accurate image acquisition so that the digital camera can deliver meaningful and repeatable data. The set-up of the microscope should follow the guidelines for "ideal" slide examination. For example, in this study, we followed the recommendations set out by NHSCSP – a 10× magnification lens with an NCB11 blue filter is used in conjunction with the microscope's 12V 100W LL Halogen Lamp illumination at a luminance setting of 5 [10]. The illumination level of the microscope was fixed throughout the whole characterisation process.

For the digital camera, a combination of lens aperture size and exposure time determines the amount of light reaching camera's imaging sensors. The signals generated by the sensors vary, and are dependant on the amount of light stimulating them. Therefore, the aperture size and exposure time were fixed during the whole period of image acquisition. Special attention was paid to the exposure setting to avoid any "colour clipping", i.e. the saturation of one or more of the three RGB channels. For both cameras, their white balance was pre-set according to the microscope lighting. The camera focus was set to automatic and zoom was fixed. After capturing a range of images, one exposure settings was adopted for

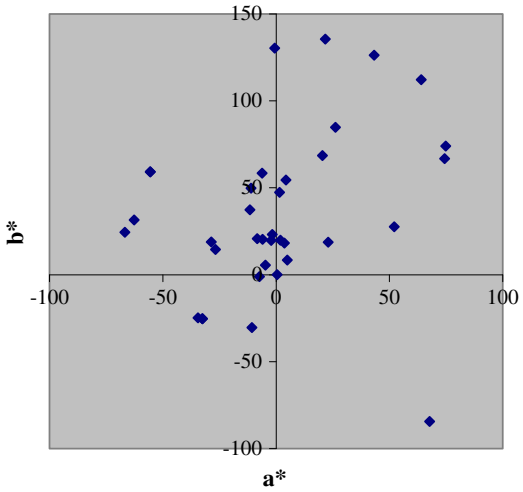

Fig. 2. Colour specification for 35 colour stained glass specimens.