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Colour management system for displaying microscope images

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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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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.

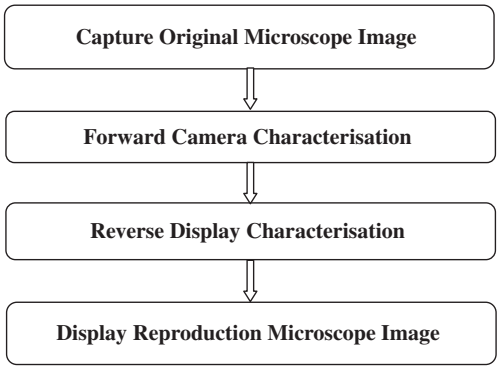

Fig. 1. Steps of colour management system.

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

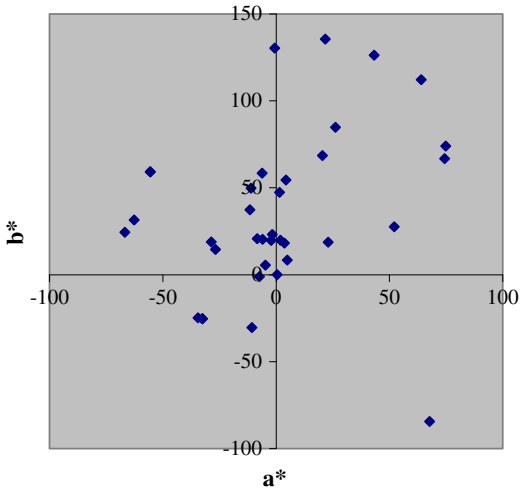

Fig. 2. Colour specification for 35 colour stained glass specimens.

both Nikon and Olympus microscope systems. The camera settings for both microscope systems are listed in Table 1.

The colour data from the 35 stained glass samples was captured by each microscope system using the respective settings. For each acquired image, the camera (RGB) for each pixel was determined and averaged to represent the overall RGB camera response. This is represented diagrammatically and Fig. 3 illustrates the colour outputs in three dimensional camera (RGB) for the two microscope camera systems. In Fig. 3, dr dg and db represents camera RGB for each channel where the white and black dots plotted represent the colour outputs from the Nikon and Olympus microscope camera systems respectively, for all 35 training colour samples. For each training colour, a black solid line was used to connect colour outputs from the Nikon and Olympus microscope camera respectively, and to indicate the colour shift between the two systems. It can be seen that when comparing the two microscope systems, their colour outputs were significantly different and that the colour shifts observed were not predictable i.e. no direct relationship was observed. These data demonstrate the device dependency of camera RGB and their inconsistency when compared to each other.

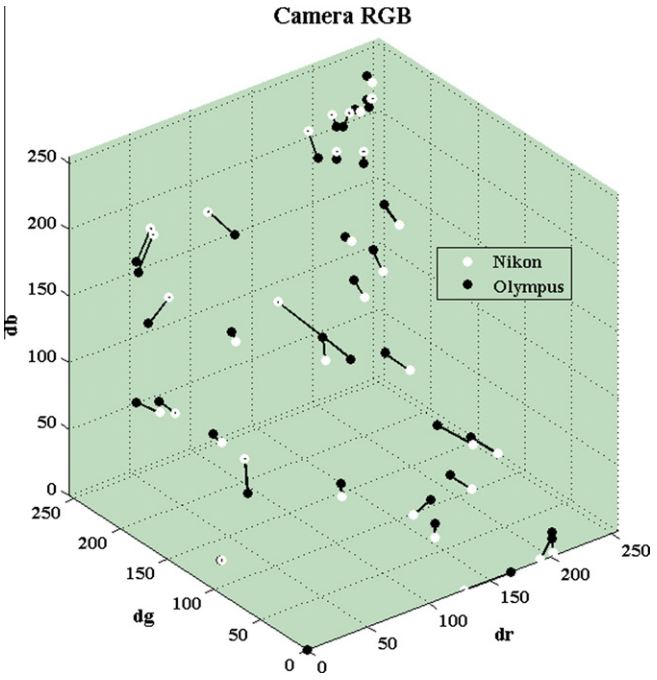

Fig. 3. Camera rgb for 35 colour slides in two microscope camera systems.

2.3. Camera colour characterisation

Camera colour characterisation enables the transformation of device dependent camera RGB to device independence CIE *XYZ* tristimulus values. More specifically, camera colour characterisation represents a mathematical model that can calculate the relationship of colour response between a specific digital device/camera and the human eye. As discussed, one of the most straightforward methods to enable colour characterisation of a microscope camera system is to use a number of training colour samples. These colours images can then be captured by a digital microscope and measured by a spectrophotometer to obtain their camera (RGB) values and the corresponding CIE *XYZ* values. Then standardized modelling techniques can be employed including three-dimensional lookup tables with interpolation [11], least-squares polynomial regression [12] or neural networks [13], to derive a transformation between the camera (RGB) and CIE *XYZ* values.

However, methods based on 3D lookup tables or neural networks require a large number of reference samples to achieve a reasonable performance, and thus are considered impractical for digital microscope applications. In contrast, polynomial regression based on the least-squares method is widely employed for camera characterisation due to its accurate results and ease of implementation [14]. Thus, the latter was adopted to undertake the microscope camera characterisation using the 35 colour stained glasses.

In this project both second order and third order polynomial regression and a direct 3×3 matrix were used to address the relationship between the two digital microscope camera systems. A third order polynomial regression that provided best performance for both camera systems was finally adopted and their camera characterisation models developed. Calculation for 3rd order polynomial regression is listed in Eq. (1). By using the training colours, Matrix M is achieved and used to predict CIE *XYZ* tristimulus values from any camera RGB values for microscope image.

$$M = (R^T R)^{-1} R^T X \tag{1}$$

where $R' = [r\ g\ b\ r^2\ g^2\ b^2\ rg\ rb\ gb\ r^3\ g^3\ b^3\ r^2g\ rg^2\ r^2b\ rb^2\ g^2b\ gb^2\ rgb\ 1']$

where R and X represents RGB value and CIE *XYZ* values, the symbol T or -1 denotes either transpose or inverse.

Camera (RGB) and the corresponding CIE *XYZ* tristimulus values for each microscope camera system were then plotted to reveal the relationship between in each colour channel Fig. 4a–f. Fig. 4a–f represents the red, green and blue channels respectively and detail the descriptor for each of the Nikon and Olympus microscope camera systems. Both training data (represented by dots) and fitted 3rd order polynomial regression model (represents by solid line) are plotted in each sub figure. It can be seen from Fig. 4, each model indicates a good relationship for the training data and in general, performance for the blue channel is better than that for either red channel or green channel.

Therefore, by using a forward camera colour characterisation model, each pixel of captured camera (RGB) from the original microscope image can be transformed to CIE *XYZ* tristimulus values.

2.4. Display colour characterisation

Display colour characterisation enables a direct correlation to be made between display input (RGB) and output CIE *XYZ* tristimulus values. The display techniques can be described in two stages [15]: firstly, non-linear display optoelectronic transfer function (OETF) relating the (RGB) digital signal is used to drive a display channel to the relative luminance produced by that channel; sec-

Table 1
Camera settings for the capturing microscope images.

Microscope camera systems	White balance	Focus	Zoom	Exposure	
				Aperture	Speed
Nikon camera	Measured	Auto	Fixed	6.2	1/1000
Olympus camera	Measured	Auto	Fixed	5.0	1/800

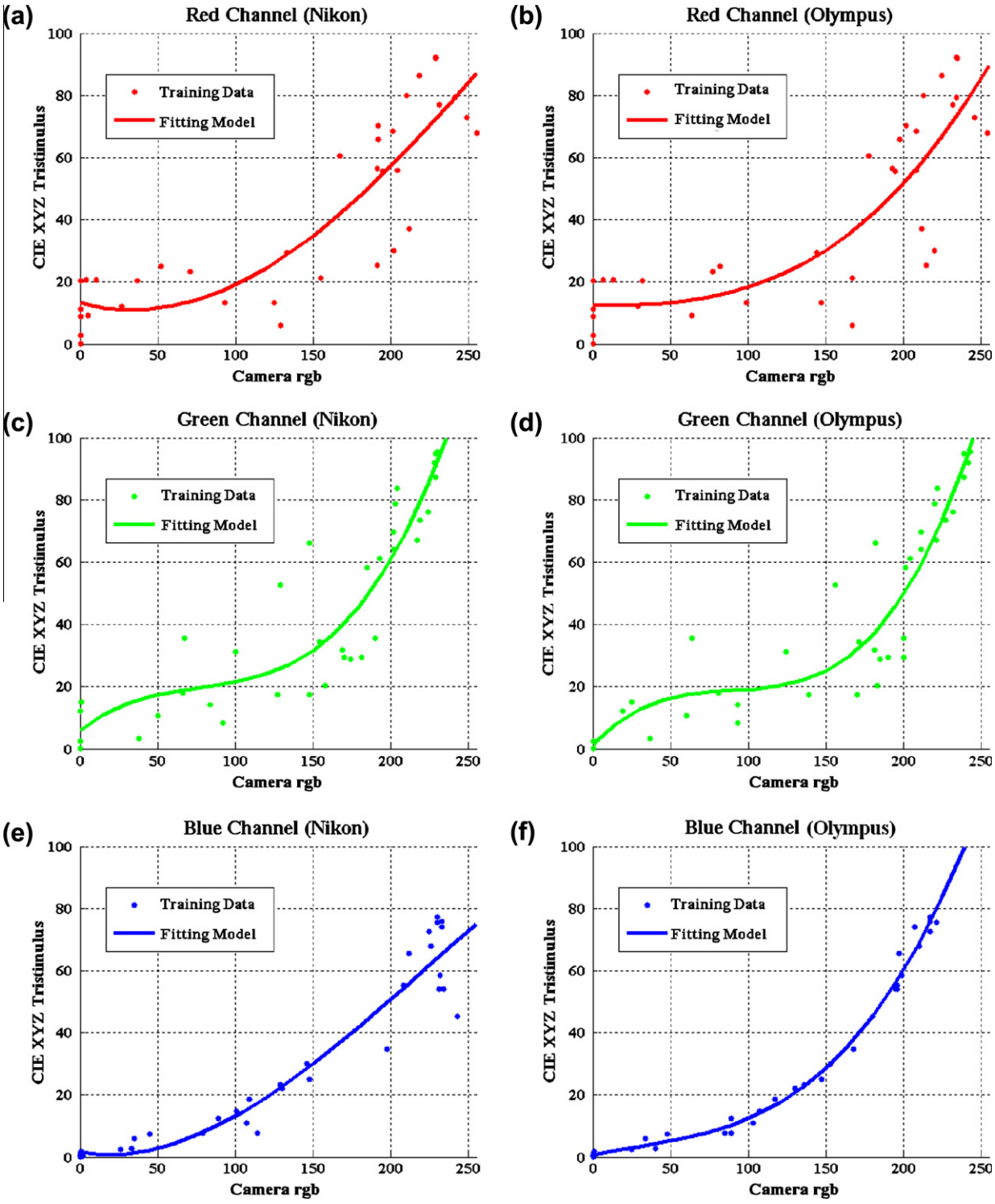

Fig. 4. Relationship between camera (RGB) and CIE *XYZ* tristimulus values for microscope camera system.

ondly, a 3×3 linear transformation to convert the relative (RGB) luminance to device-independent CIE *XYZ* tristimulus values. An accurate colour characterisation model can then be achieved by measuring CIE tristimulus values for numbers of colour output (grey scale and display peak outputs) and then model them by using mathematically models, such as GOG model [16] or PLCC model [17].

In this case the DELL LCD panel with a D65 white point was used to display the microscope images. In order to develop the correct colour characterisation model for the panel, display peak colour outputs for each colour channel and 18 grey scale samples were prepared. Each colour sample was displayed on the centre of screen and measured using a PhotoResearch PR650 tele-spectroradiometer in term of its CIE *XYZ* tristimulus values (cd/m^2). Based on the grey scale samples, the non-linear OETF function was then used to transform the display (RGB) to RGB relative luminance, by using PLCC models, which have been shown to give accurate predictions for LCD displays [18]. A linear transform was then developed using

measured peak colour outputs to transform relative luminance to CIE *XYZ* tristimulus values. Fig. 5a and b represents plots of these data and illustrate display non-linear OETF function (between display (RGB) and RGB relative luminance) and linear function (between RGB relative luminance and CIE *XYZ* tristimulus values) for the DELL LCD panel in each colour channel.

In order to accurately reproduce microscope images, the reversed display colour characterisation used to transform the CIE *XYZ* tristimulus value to display (RGB) values was adopted in the colour management system. Based on this process, the achieved *XYZ* tristimulus values for each original microscope image in the previous step were transformed to display (RGB) and named as the reproduction microscope images.

2.5. Microscope image display

The reproduced microscope images were displayed on the target display using the pre-determined settings when display characterisation was performed.

3. System evaluation

A colour management system for reproducing microscope images on an LCD display was then developed. Special attention was paid to true colour transformation and the performance of colour reproduction for the documented systems. Thirty five microscope images that captured data from colour stained glass were used as original images. By using the proposed colour management system, each original colour image was transformed to a reproduction image. Both original colour images and reproduction colour images were displayed on the target DELL LCD panel and output data measured using the PhotoResearch PR650 TSR, as CIE *XYZ* tristimulus values. Then, colour shift from the simulated colour output of the microscope to colour stimuli for the either original microscope image or reproduction microscope image on the target display was represented by the predicted colour difference unit using the CIE DE2000 colour difference formula [19]. It should be remembered that a colour-difference formula is designed to give a quantitative representation of perceived colour difference between two colours – CIE DE2000 colour difference formula is the current recommendation by CIE TC 1-47 for industry colour difference assessment, and has been shown to be the most uniform state-of-art colour difference equation. Furthermore, it is directly correlated to human visual perception in terms of colour difference [20]. It is acknowledged that a smaller difference between colours in simulated microscope images and on electronic displays represents better colour image reproduction. The performance of the colour management system was evaluated by assessing the improvement of colour reproduction from the original microscope image to the reproduction image for the target display. The mean, maximum and standard deviation of colour differences between simulated microscope images and the two sets of display images for the 35 test colour samples were calculated and are represented in Table 2. Fig. 6a and b were also plotted to indicate the performance of colour reproduction of all 35 stained glass specimens for both original and reproduction microscope images for Nikon and Olympus camera system, respectively.

From Table 2 and Fig. 6, it can be seen that comparing colours from simulated microscope images, for both the Nikon and Olympus systems, reveal that the reproduction images have an average colour difference of approximately 5 and 4 ΔE_{00}, whereas the original images have approximate average colour differences of 15 and 12 ΔE_{00}, respectively. This demonstrates that significant improvements in term of colour accuracy can be achieved by using the proposed colour management system for microscope images.

It is interesting to note that all the colour management formulae developed were based on comparing the colour differences of uniform colour patches, where the visual responses to colour difference was most sensitive. Studies on colour difference for complex images [21] have shown that the there is more tolerance for detecting colour difference in these situations, and suggested that when comparing complex images, the perceptibility threshold was about 2 ΔE_{00} units, and the acceptability threshold was around 4 ΔE_{00} units. The performance of a proposed method that produces a result of 4–5 ΔE_{00} units means the observer will probably detect only a subtle colour difference between the view through the microscope and its reproduction on a display. However such a difference may be considered just above or close to an acceptable range. Therefore, the methodology developed would be considered effective for this application. The fact that the results from the two microscope systems were very similar, implies that the methodology developed may be independent for the digital microscope systems used, and comparable results can be expected from digital microscopes with similar specifications.

4. Discussion

Although colour and digital colour images are a relatively small component within telepathology they are none the less important [22]. With advances in virtual/digital microscopy being made at

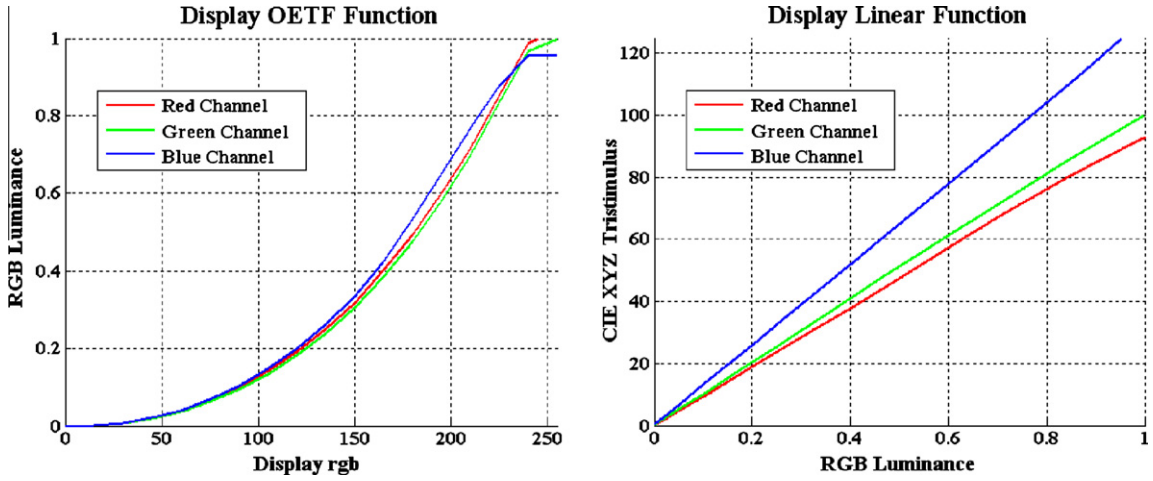

Fig. 5. Two stages colour characterisation for DELL LCD panel.

Table 2
Colour difference between microscope and target display in term of ΔE_{00}.

Nikon system	Mean	Max	STDEV	Olympus system	Mean	Max	STDEV
Original	14.9	66.5	10.9	Original	12.2	63.2	9.6
Reproduction	4.8	15.2	3.6	Reproduction	4.3	11.3	3.0

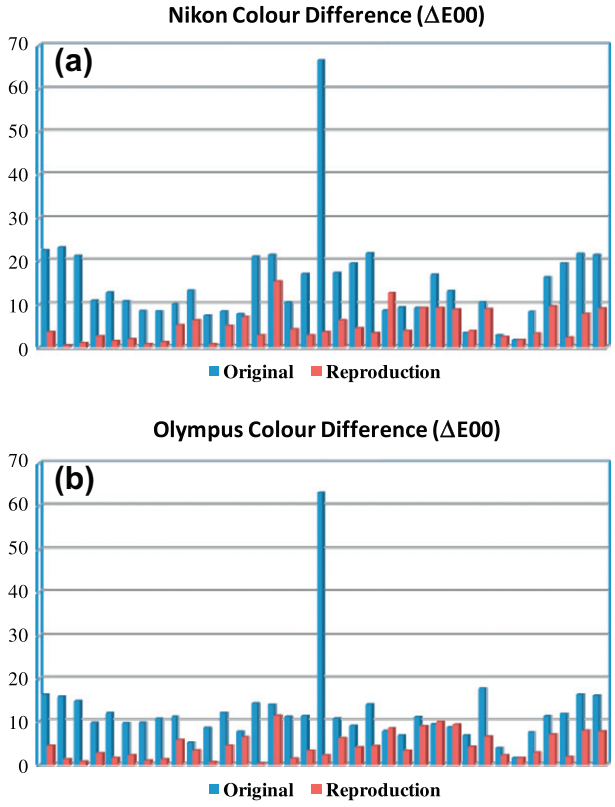

Fig. 6. Performance of colour reproduction for two microscope camera system.

what appears to be an exponential rate, thought should be given to addressing issues around colour and its management within this developing field [23]. Digital images used in interactive and virtual microscopy have many advantages over conventional methods (– subjective assessment with the human eye from glass slides). These advantages include rapid scanning over larger fields of view, construction of whole slide imaging, electronic transfer of images between different clinicians, storage of information and ease of access, to name but a few. Furthermore, methods can be employed to reduce the subjectivity that is often seen when interpreting histological slides by eye. It is generally acknowledged that a significant part of the overall process in trying to reach a diagnosis is operator assessment of the final image. However, when it comes to assessing either histological slides or digital images there are elements of inter-operator variation and these include limited field of vision and variations is colour interpretation [4]. These variations are further compounded by differences in the quality and consistency of digital images (colour or otherwise) on displays/monitors, and their ability to be manipulated. Variations in colour can be the result of colour shifts during the digital processing of such images. These can include changes that occur with different lights, levels of illumination, filtration, projection, displays/screens/monitors as well as changes that occur throughout the electronic translation of digital data through processors and computer chips. These internal or operator independent variations can be considered different from external changes that can occur as a result of active manipulation of images. Again, these can have a significant effect on the outcome of such processes where deliberate manipulation of image colour (brightness, hue, contrast, etc.) can at best lead to differences of opinion and at worst lead to inaccurate diagnoses [22–24]). Although important it is acknowledged that these specific points lie outside the scope of this article.

In this study, both the microscope camera and display adopted in this study could be considered reputable and high quality products, and suggests the colour management system is not only required for high end products but are in fact appropriate for general applications.

The primary mathematical processing for the colour management system is microscope camera characterisation and display characterisation. This study suggests that the characterisation models only need designing once and can be used throughout the life span of the equipment as long as each device works properly. Furthermore, each device characterisation can be used independently in order to meet the requirements of different apparatus. For instance, when a slide is examined in a different location, display characterisation needs only to be conducted for each target display in the different location, whereas the camera characterisation need only be conducted once the microscope image is captured using a particular microscope system. Thus, if a series of observers use different microscope systems and would like to compare their findings on one particular display, camera characterisations only needs to be performed for the microscope camera system used, whilst the display characterisation only needs to be conducted for one target display.

This process can be time consuming so to improve the processing efficiency, a full range of RGB Look-UP-Tables (LUTs) could be produced to connect original camera (RGB) to the reproduction display (RGB) for 24 bit (RGB) colours. Then when a microscope image displays on the target display, the RGB LUT is used to map the camera (RGB) to display (RGB) for that particular image. By using RGB LUT, whole processing takes less than 0.1 s for most of computers although a relative large size of LUT file needs to be saved on the hard drive.

The model performance of approximately 4–5 ΔE_{00} for the two microscope systems indicates an acceptable colour difference for a complex image. However, the performance of the model could be improved by utilizing more training samples for camera characterisation and a larger grey scale for display characterisation.

By using this colour management system for digital microscope, each specimen is transformed digitally and it's true colour information is accurately and consistently translated from the microscope to the display. It also makes an objective assessment of the colours from different sources possible. For instance, colour variation between stained specimens can be evaluated objectively by calculating their colour difference directly. The technique can be further developed to incorporate with colour image processing algorithm to meet specific applications. For instance, colour for a specific area of specimen can be automatically detected and compared by using technique of pattern recognition.

To date it would appear that there are no general accepted set standards in relation to digital microscopy – hardware, software, digital monitors and storage [5]. Furthermore, although the interpretation of colour is only one of several steps within the process of telepathology, setting image standards including optimization

and standardization of colour would go some way to increase the accuracy and consistency of virtual microscopy [2,23]. This article demonstrates a colour management system that can truly reproduce colour microscope images on a target display and thus, may go some way to help achieve the standards that are required to improve digital/virtual tissue based diagnosis.

5. Conclusions

In this study, a colour management system was developed for consistently capturing colour microscope images and accurately reproducing them on a target display. Two digital microscope systems (Nikon and Olympus) and one target display (DELL LCD Panel) were studied using stained glass samples as training colours. Each sample was measured using a PR650 tele-spectroradiometer under simulated viewing conditions similar to those in which an optical microscope is used. The performances of colour reproduction using the proposed colour management system was evaluated and proved to be very effective. Using this proposed method, microscope colours can be objectively quantified, which could provide a platform for consistently quantifying the colour variation among microscope slides and thus aid specimen evaluation and diagnosis between different observers. The methodology developed could also be easily applied to all cytology applications using microscopes.

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