Yagi Digital Microscope Calibration

Method summary, assessment and suggestions for improvement

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Introduction

In the area of pathology, a type of digital microscope for whole slide imaging (WSI) has been developed by a number of manufacturers. These systems, often referred to as scanners, capture a high resolution colour image of around 4000 pixels per mm with a typical maximum imaging area of 25 x 50 mm. Many different image formats are used and most of the image viewers are proprietary. There is significant concern expressed by pathologists that the colour produced by these systems is significantly different from each other and there is a strong desire, including from some of the manufacturers, to define a colour framework for WSI. Probably the best candidate for such a colour framework is that defined by the International Color Consortium which would provide an open solution compatible with many existing tools and applications.

Dr Yakuko Yagi of Massachusetts General Hospital (MGH) and Harvard University has pioneered a method of colour correction for digital microscopes. This method has been applied successfully to images produced by WSI systems but in principle could be applied to digital microscopes in general. To some extent the method assumes that a calibration adjustment of some kind (at least white balance and tone adjustment) has been done when the slides are scanned.

This document provides a summary of the Yagi Digital Microscope Calibration method and colour framework. The colour framework is described, possible areas of improvement identified and some suggestions made to show how this method can be adapted to support ICC colour management. In concert with the development of a standard for colour displays for medical imaging[1], an ICC framework would provide a significantly higher level of colour accuracy required for critical colour assessment.

We should note that there is currently no agreement between pathologists as to how to apply coloured stains to slides for viewing by a digital microscope. Slides are often stained to accommodate the pathologist's colour preference. This means that the colours of the original slides have significant variation even when the same staining protocol has been used. While standardisation of slide staining is an area of active discussion, this paper deals only with the process of scanning and subsequent display of these slides.

The aim of this part of the process is to faithfully reproduce the colours that appear on the slide on a display. Some means to confirm that this end-to-end calibration has been correctly performed is essential.

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[1] One idea for such a standard is that of mRGB proposed by Michael Flynn of AAPM which uses the DICOM GSDF as the definition for the neutral scale. This would not be a single colour space but would define a set of colour spaces for different viewing environments.

Status quo

Today ICC colour management is seldom used in WSI even though it is required by DICOM. This has led to a situation where scanner manufacturers all have a different view of how to calibrate their system; a few provide ICC Profiles for the images produced by their systems but many do not.

Furthermore, pathologists often view images on un-calibrated displays and in uncontrolled viewing environments and would like to have a solution that produces a similar colour result for all WSI systems.

The Yagi calibration method provides a means to ensure that the displayed colour is approximately correct for all of today's WSI systems and in addition provides a simple method to determine whether a given display is suitable for colour assessment.

System overview

The Yagi calibration system has three key elements: a calibration slide, a method to check the display to ensure that it is suitable for colour assessment and a method for calibration (or normalisation) of WSI images.

The display assessment method assumes that the display to be used is nominally sRGB and provides an inexpensive means to check the display. The method has been designed to make it easy for pathologists to use and is to some extent independent of viewing conditions.

The calibration method assumes that the WSI scanner has a reasonable calibration aim defined by the manufacturer. The method makes use of a 9-patch slide created using Rosco filters for which sRGB colour values for each patch are calculated and used as the colour aim. The slide is scanned using the system to be calibrated and RGB colour values from the scanned image are used along with the sRGB colour aims to estimate a 3-3 colour transform. This transform is then used by viewing software to correct images as they are displayed[2].

Calibration slide

Both the display assessment and calibration / normalisation methods use a calibration slide, an example of which is shown in the figure below.

The numbers and names identify the Rosco filter[3] used for each patch. This set of patches represents just one possible set and other variations, for example including neutral patches, have been produced and tested by Dr Yagi's research team. One objective when selecting the set of patches is to choose a set that is small enough for pathologists to check easily but which includes sufficient colours to provide sufficiently accurate colour check.

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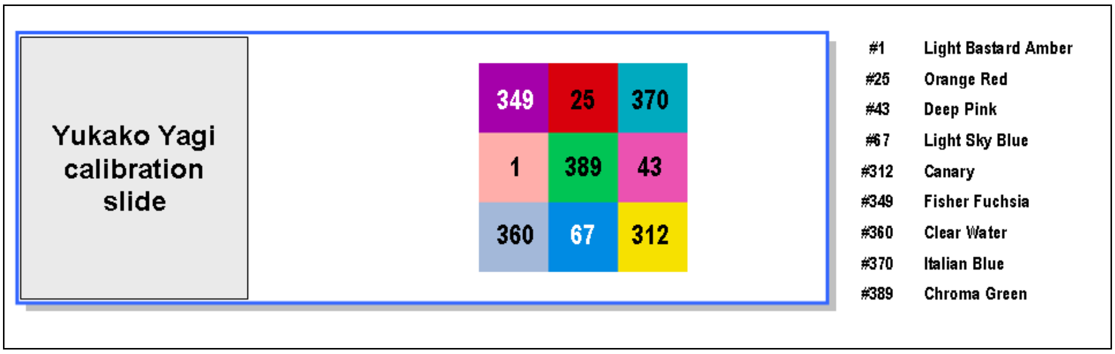
[2] Under Dr Yagi's direction a viewer has been developed which is capable of reading a number of popular WSI image formats and applies a colour correction transform when displaying images.

[3] Details of Rosco filters including spectral transmittance are available from the Rosco web site: http://www.rosco.com/.

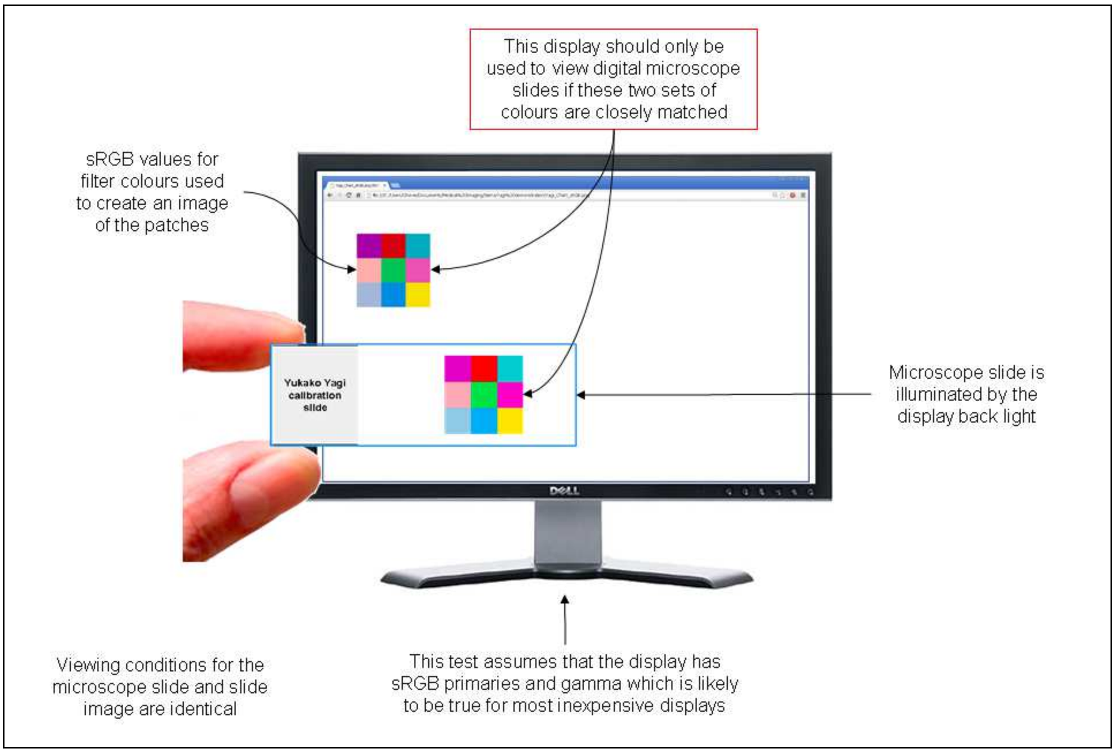
Spectral transmittance of these patches can be measured and sRGB reference values for each coloured patch can be estimated by making some assumptions about the illuminant and viewing environment[4].



Display assessment

The display assessment method provides visual confirmation that the display to be used to view images is approximately sRGB.

A web page that includes an sRGB image of the set of calibration patches is used. The image includes a set of patches of approximately the same size and layout as the patches on the calibration slide; the remainder of the page has a white (R=G=B=1) background. The calibration slide is held close to the display over the white background as shown in the figure below and a visual comparison of the two sets of colours is made by the pathologist.



While there are some sources of error in this colour evaluation, the method does provide some level of compensation for different viewing conditions and

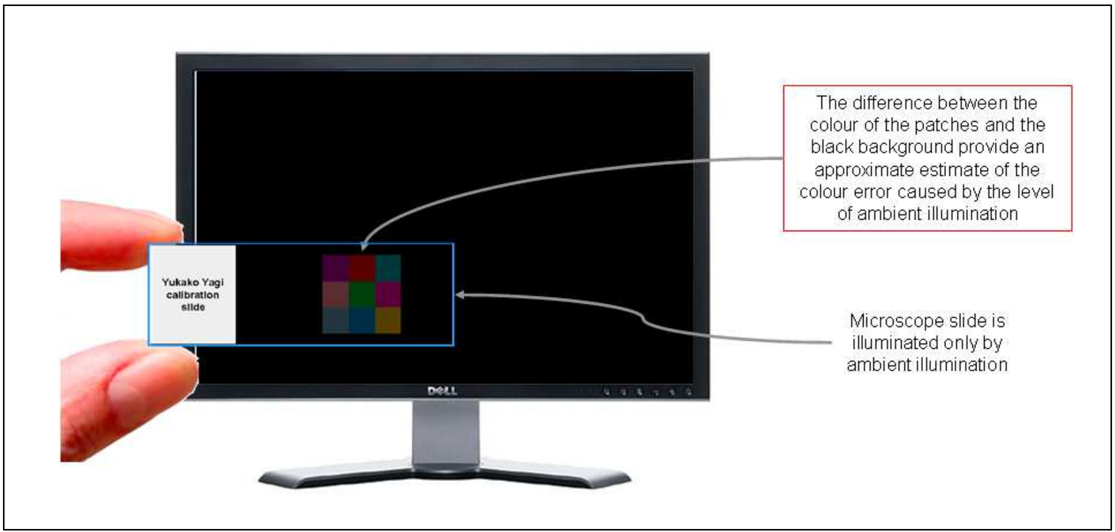
4 Different assumptions result in different RGB values.

provides a reasonably accurate visual check. In absence of a widely adopted ICC framework this system provides a simple check for non-critical colour evaluation that would be difficult to improve although there may be other choices of patches used for some kinds of work. Note that this solution only provide a check that the display and intended viewing environment is suitable or not and does not provide any solution for displays that are insufficiently accurate.

When considering sources of error in this system, the first case to consider is where the viewing conditions are as specified in ISO/IEC 61966-2-1 (the sRGB standard). In practice the use of the sRGB reference viewing environment is unlikely as the required ambient lighting is very low (64 lux) and many displays used do not fully support the sRGB colour gamut. There are two main sources of error: the illuminant used and the ambient reflection from the colour patches on the slide. Both of these errors are likely to be small relative to the accuracy required by the pathologists when performing non-critical colour evaluation.

When calculating the sRGB reference values from the filter transmittance D65 is used, whereas the illuminant used when viewing the calibration slide is the display's back light. Since the display is an sRGB display, the white point has a correlated colour temperature of D65 which means that the reference white point for the slide image and the reference white point for the slide have the same colorimetry. However the spectral content of the display is likely to be significantly different from D65 and this introduces a small difference between the colour of the image patches and the transmitted colour of the calibration slide.

The second source of error occurs where there is some level of ambient illumination. In this case there is a reflection component of all colours which combines with the emission component of the slide image and with the transmission component of the backlit slide. This reflection component is almost certainly different for the displayed image and calibration slide and this difference increases as the ambient illumination increases. An approximate visual estimate of the size of this error can be made by displaying a black background and comparing this with the coloured areas on the slide.



In cases where the display deviates from sRGB primaries, white point and tone reproduction additional errors are introduced. Significant additional errors may be introduced when the viewing environment is not that assumed by sRGB, in particular the level of ambient illumination is likely to introduce significant colour differences.

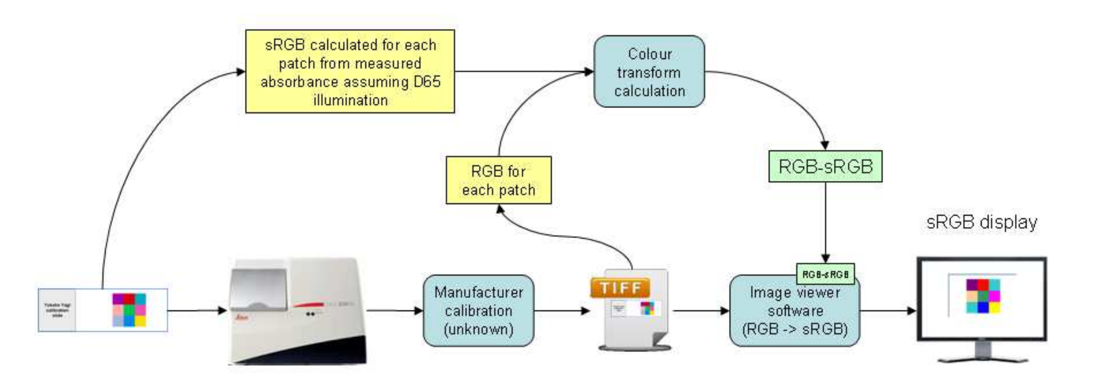
Creating an ICC Profile for the display using standard display profiling software provides a means to reduce some of these errors although it should be noted that many display profiling packages do not include the effects of ambient illumination when creating profiles.

Calibration correction of WSI images

The colour images produced by WSI systems are not standardised and different calibration aims are used by different manufacturers. Some have already adopted a standard method using ICC Profiles but until such a system is widely adopted the Yagi method provides a reasonably effective means to normalise images from different systems. Furthermore when an ICC framework is adopted, this method (with some minor changes) can be used to provide legacy support for systems that have not adopted ICC colour management.

An overview of the calibration method and integration with a viewer is shown in the figure below.

The calibration slide is scanned by the digital microscope to create an image of the slide. Although this is shown as a TIFF image it is more usual for some other format (often based on a variant of TIFF) to be used. RGB values for coloured patches of the image are found by examining each region and averaging values where there is some variation. The sRGB reference values for the slide are used along with these image colour values to calculate an image RGB to sRGB colour transform (RGB-sRGB). This colour transform is then used by the viewer software when viewing the image.



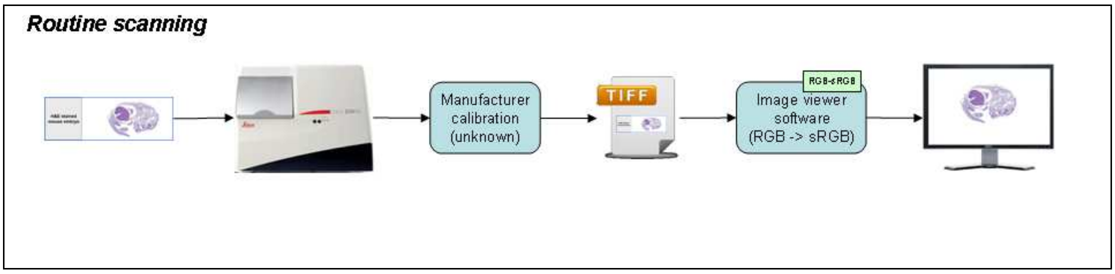
Scanner calibration

The end-to-end result can be checked using the display assessment method described above using the scanned image rather than the sRGB reference.

Subsequent images of microscope slides scanned using the calibrated WSI system can be scanned and displayed using this RGB-sRGB transform (see below). A transform can be constructed in this way for each WSI system.

While this is a good practical solution for current systems there are a number of limitations that may limit its use for critical colour evaluation.

The solution ignores ICC Profiles in cases where they are provided. If the manufacturer has provided an ICC profile of good quality this is likely to provide a better staring point for calibration. At the time of writing, however, we are not aware of any work to evaluate the quality of these ICC profiles.



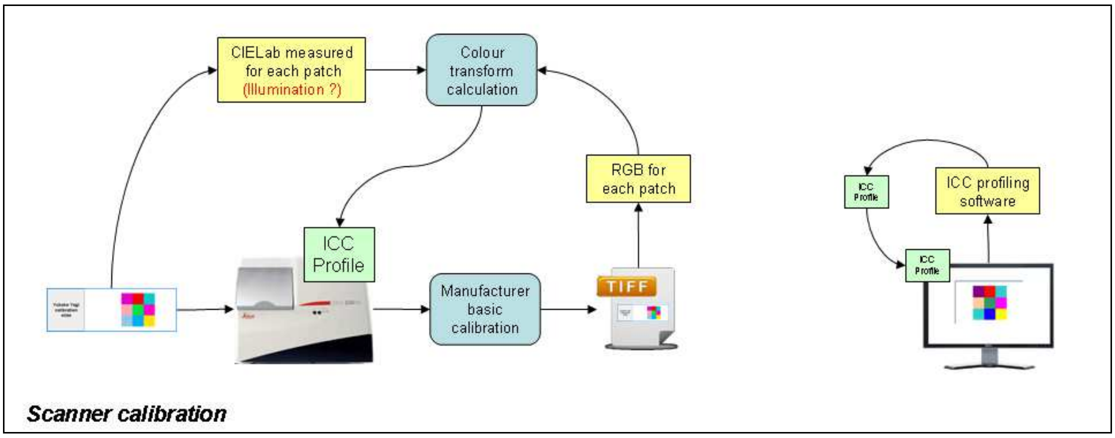
The solution is limited to viewers that can support the RGB-sRGB colour transform and applies a colour conversion to the colour conversion performed by the system (manufacturer calibration). Applying multiple colour conversions in this way almost always introduces errors and in some cases these could be significant as the manufacturer's calibration aim is unknown. Using the ICC framework, colour conversion is deferred until the image is displayed and a single colour transform is calculated by combining the ICC profile for the scanner with that for the display. In principle this provides a means to reduce colour errors.

Colour calibration is approximate and may be limited when critical evaluation is needed and, as previously observed, large changes in viewing environment may introduce significant colour errors.

Providing support for ICC profiles

The current calibration system could be adapted relatively easily to use ICC profiles instead of a proprietary transform. At the simplest level, the RGB-sRGB transform could be encoded and communicated in an ICC DeviceLink profile. This would allow other viewers to be developed by third parties that could use the calibration method.

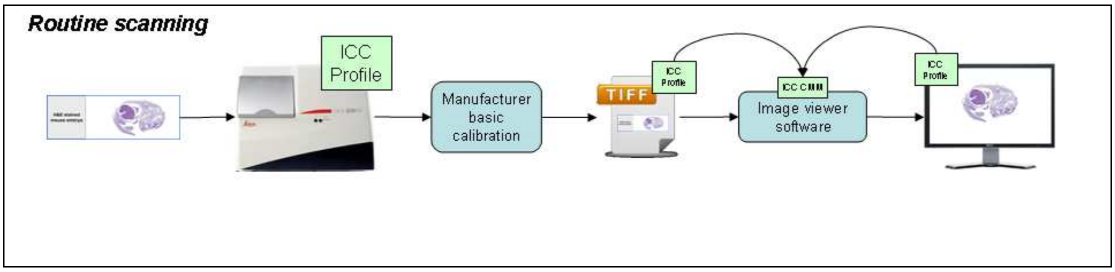
A better and more complete solution is shown in the figure below.



As can be seen from this figure, the steps are very similar but instead of calculating an RGB-sRGB transform, an ICC profile describing the colours in the image produced by the scanner is created. This ICC profile includes an RGB-to-CIE_XYZ or RGB-to-CIE_Lab transform.

Display profiling is performed separately and an ICC profile produced for the display using display calibration software which is widely available. If display profiling software is not available and an sRGB display is being used for viewing, a standard sRGB ICC profile can be used[5]. While this is unlikely to describe the display colour as accurately as a custom made profile this should provide equivalent colour accuracy to that achieved by the current calibration method.

These two profiles (scanner and display) are combined by the viewer when images are displayed as shown in the figure below.



There are a number of benefits to this system which can only be fully realised by its adoption by all of its stakeholders.

The scanner calibration and display calibration can be separated which makes it easy to support multiple scanners and multiple displays. Images from different scanners can be displayed on different displays with matching colours.

This solution is not limited to sRGB displays and can make use of the significantly larger colour gamut available on some displays. This may be important for critical colour viewing as some of the colours that appear on digital microscope slides are out of the sRGB colour gamut[6].

A single colour conversion is used to minimise the number of conversion steps each of which introduces quantisation and interpolation errors.

Calibration chart for critical colour

The small set of colours in the calibration chart may not be sufficient to provide a good calibration for a digital microscope and may need to be extended to include additional colours, for example neutrals and tints.

Ideally such a calibration chart would have a set of colours whose spectra are similar to the set of colours to be scanned.

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[5] One example of such an ICC profile is at http://www.color.org/srgbprofiles.xalter#v2.

[6] One example of a colour that is out of sRGB colour gamut is Eosin which is one component of H&E staining which is the most commonly used staining protocol (more than 80% in many labs).

Incorporating preference

An additional step could allow pathologists to adjust colour according to their preference and this preference could be remembered for each pathologist and communicated in the form of an ICC ColorSpace profile. In this way a viewer could be developed that combines the profile from each image with the pathologist preference profile and the display profile to create a solution that can show images in a consistent way.

Conclusions

The calibration system developed by Dr Yagi is easy to use and provides a basic method of system calibration and display assessment. This method does have a number of limitations that may make it unsuitable for critical colour evaluation. A framework based on ICC colour management should be defined and its adoption encouraged.

It may be useful to define two quality levels for colour evaluation: one for critical colour assessment and one for 'every day' assessment. The current calibration system could be adapted to make use of ICC profiles and would provide a good, inexpensive solution for 'every day' assessment of colour images.

The use of the 9-patch reference slide (or something very similar) to check the end-to-end performance of the system should be tested and guidelines produced for its use in checking the overall system performance.