

Estimation of the calibration error associated with the use of a film calibration target

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FFEI Limited

Digital microscope film calibration target

HutchColor HCT
Ektachrome™
35 mm



It has been observed in previous discussions in the ICC Medical Imaging Working Group that calibrating a digital microscope using a film target will introduce errors. These errors arise because the spectral characteristics of the dyes used in photographic film are significantly different from the stains used in pathology.

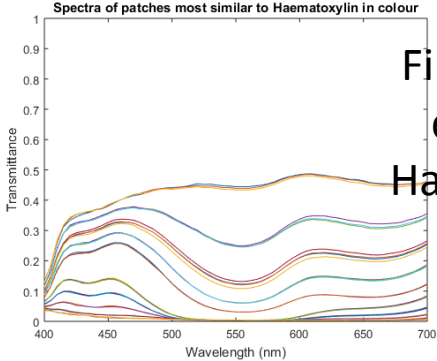
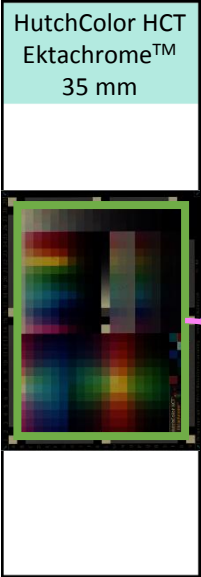
The aim of this project is to estimate the likely size of the error introduced compared to calibration using a calibration method that takes into account the spectral characteristics of the stains.

The magnitude of the errors introduced by a mismatch between the spectral characteristics of the calibration patches used to calibrate the system is difficult to estimate precisely as they depend on a number of factors including the light source and sensor sensitivities.

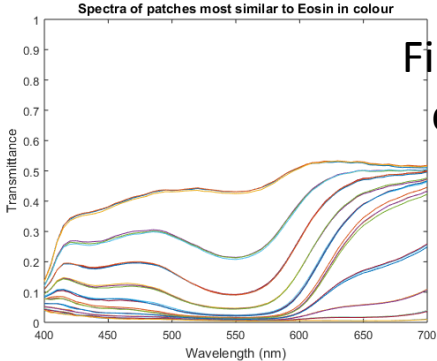
This presentation uses a representative source and typical sensor sensitivities to estimate the likely size of errors – in many cases the errors will be substantially larger than those estimated here.

Measurements of film and stained tissue

Measurements of calibration slide



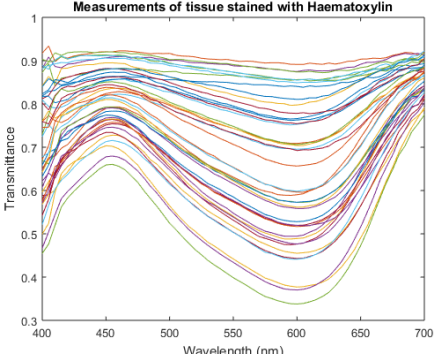
Film patches closest to Haematoxylin



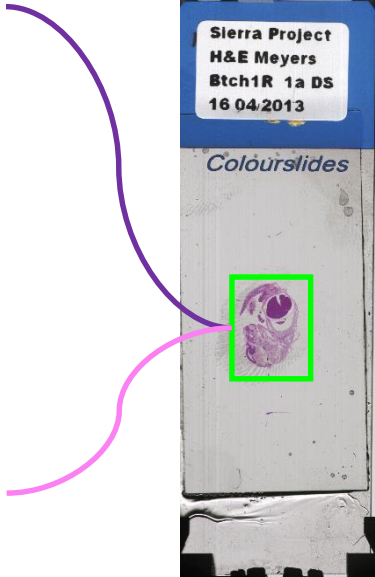
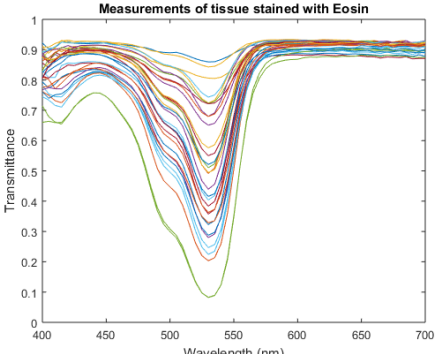
Film patches closest to Eosin

Measurements of H&E stained tissue

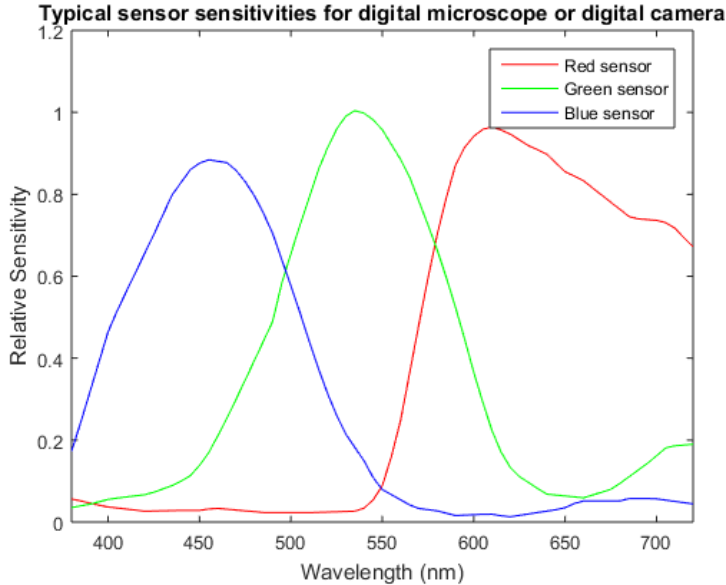
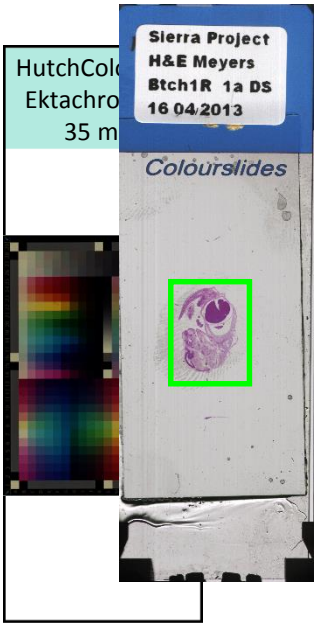
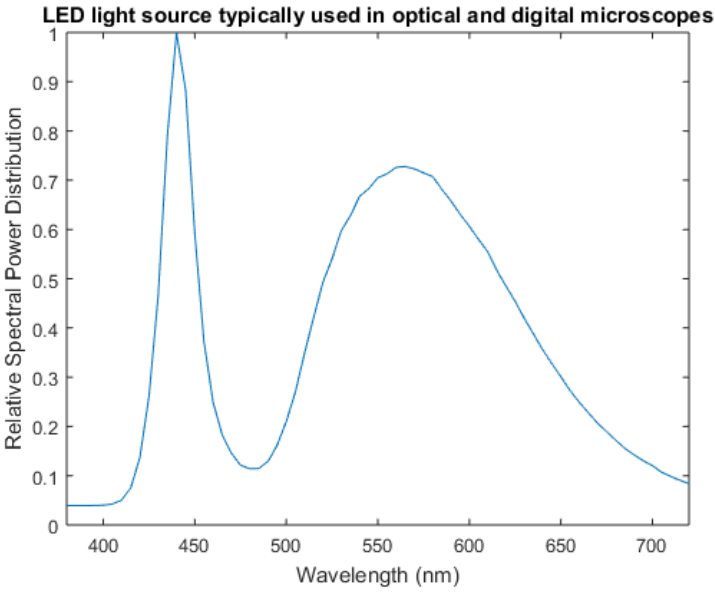
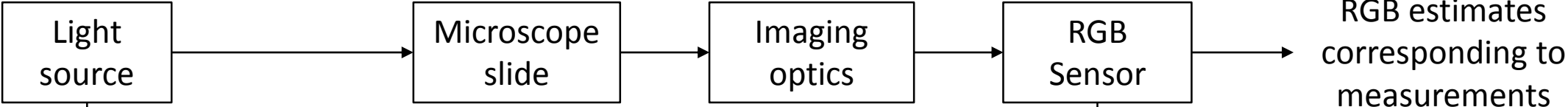
Measurement of tissue stained with Haematoxylin



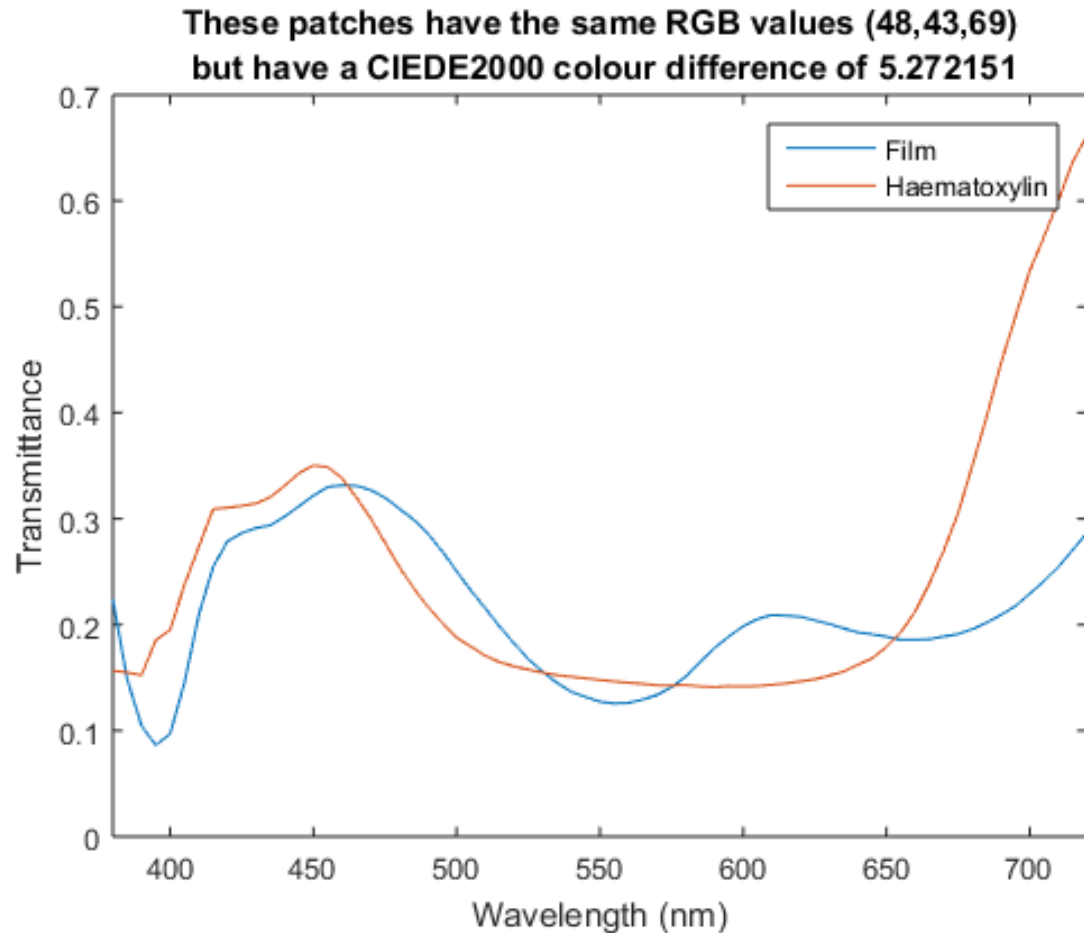
Measurement of tissue stained with Eosin



RGB scanner model



Haematoxylin

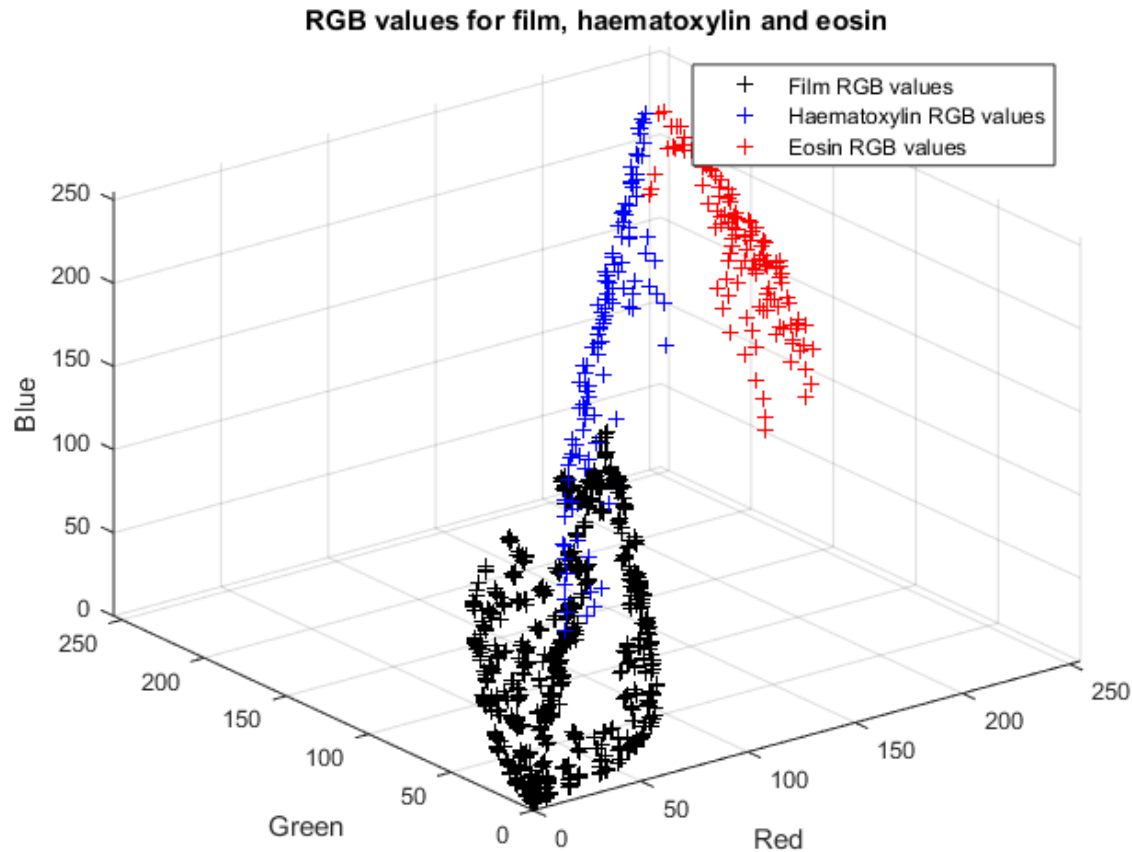


This figure shows the spectral transmittance for a measurement from the film target (blue) and a measurement of Haematoxylin-stained tissue (red)

These patches have the same RGB values (48,43,69) but differ in colour by more than 5 CIEDE2000

This error is a systematic error and cannot be corrected by the calibration software and is larger than the total colour errors we aim to achieve for a calibrated digital microscope system

Different RGB gamuts



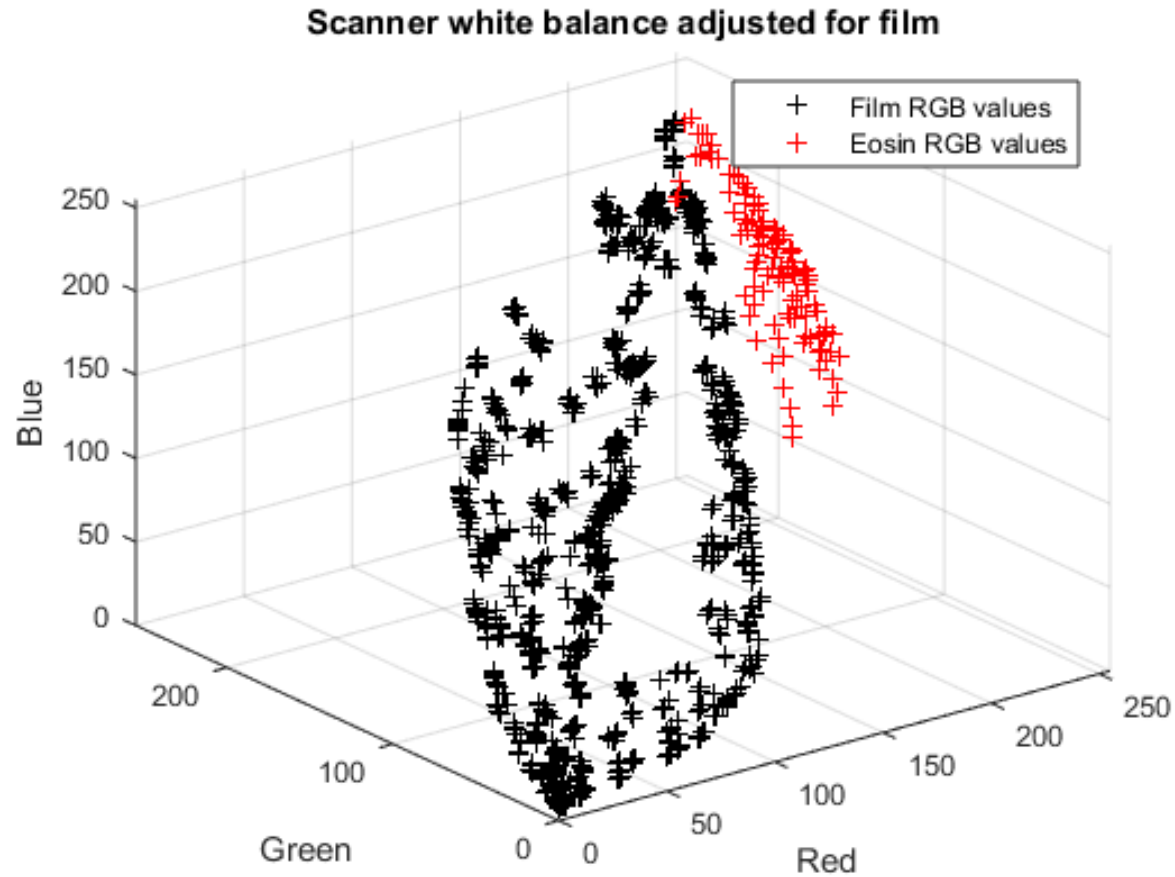
This figure shows measurements from film (black) Haematoxylin-stained tissue (blue) and Eosin-stained tissue (red)

Film is more dense than tissue and this means that most of the RGB values that arise when scanning stained tissue are not seen by the scanner during calibration

This means that the colour corresponding to RGB values for Haematoxylin and Eosin cannot be estimated with any degree of precision

It is not easily possible to estimate the size of these errors precisely but typically errors in estimating colours outside the gamut of the training set (the calibration target) **are likely to be greater than 10 CIEDE2000**

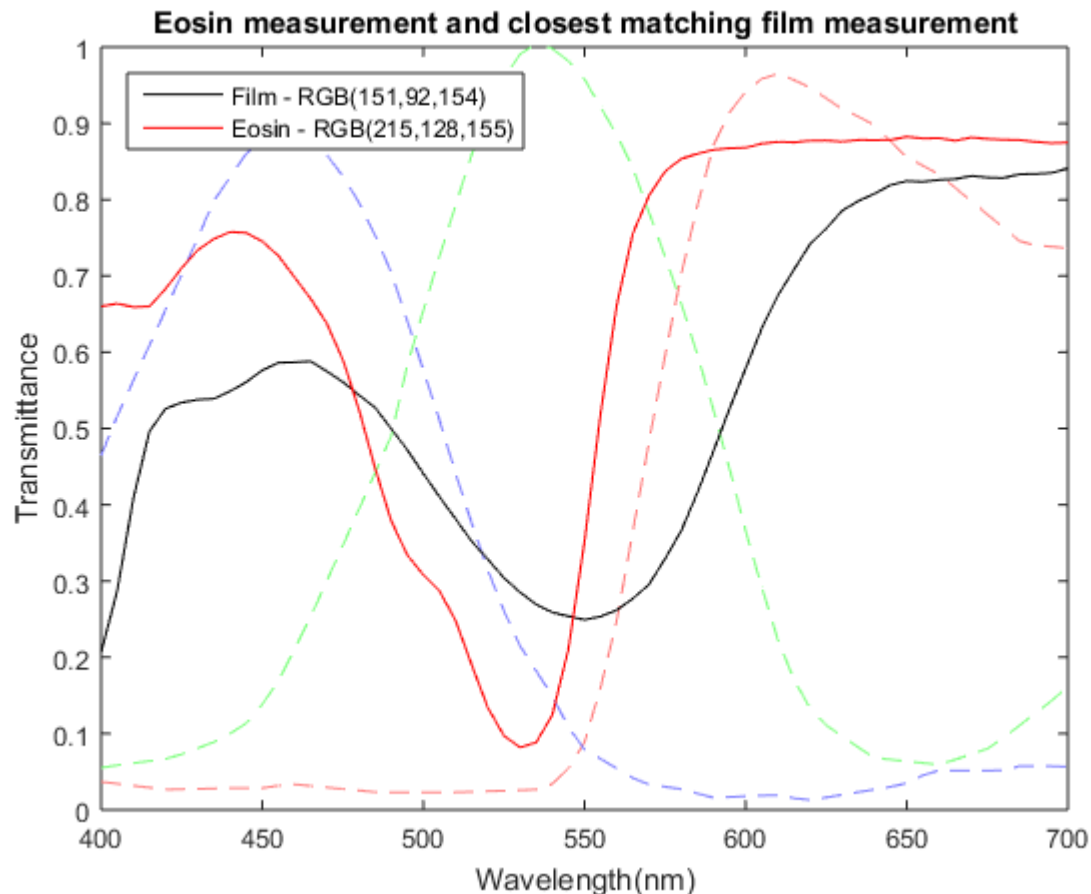
Eosin



Even if the effect of the film base is removed, for example by white balancing, Eosin RGB values are significantly outside the film gamut

Using a different white balance for calibration and scanning of tissue samples introduces additional errors

Eosin vs film



By comparing the shape of the spectrum for an Eosin patch and the closest matching film patch with the sensor sensitivities it can be seen that they are very different shapes

This difference in shape when compared with the shape of the sensor sensitivities explains the reason for the very different colour gamuts

Trying to estimate the colour of eosin from the RGB values of the scan of the calibration target will include a large uncertainty