Project Sierra

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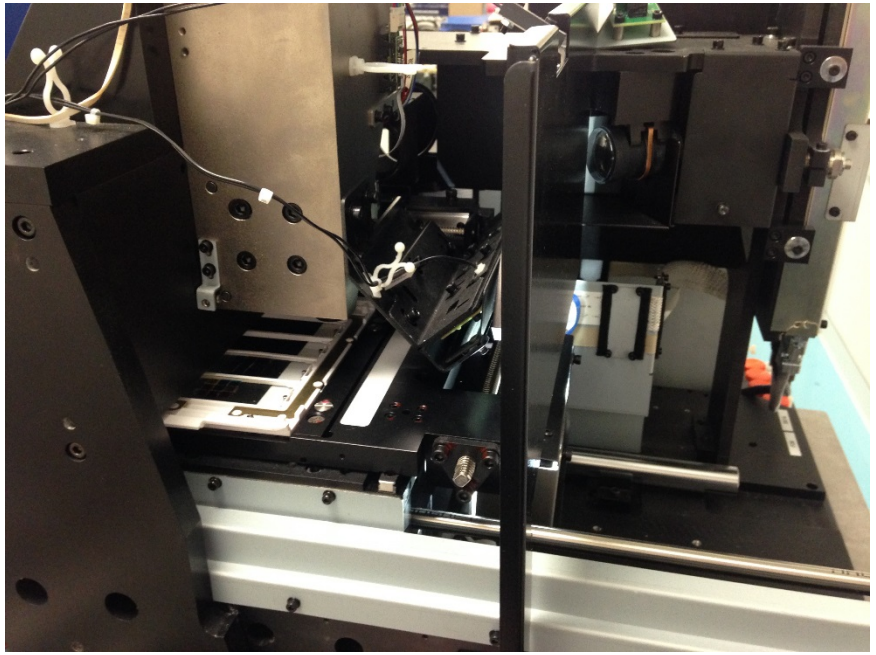
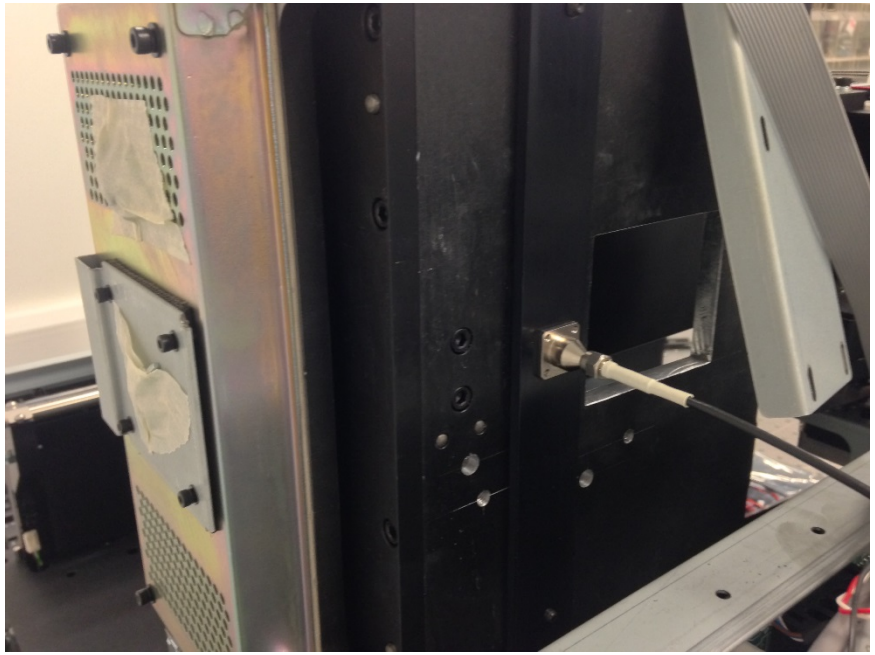
FFEI Limited

Project Sierra overview

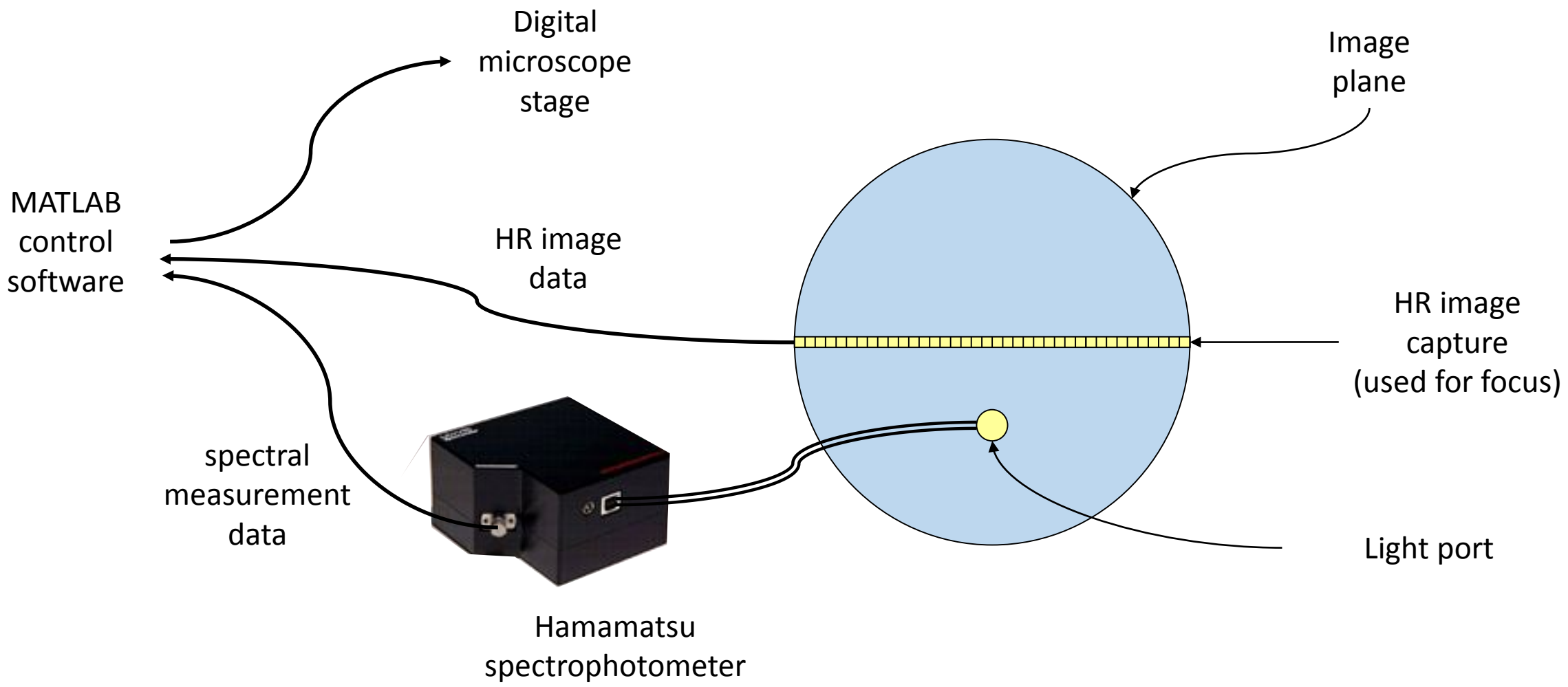
- Partners
  - FFEI Limited
  - Leeds Teaching Hospitals NHS Trust and University of Leeds
  - Funded in part by UK Technology Strategy Board
- Objectives
  - develop materials and methods to assess imaging characteristics of digital microscopes
  - includes colour, dynamic range and resolution
- Status
  - prototype colour calibration assessment slides are available
  - a method to determine light exposure of a slide has been proposed
  - a method to assess staining systems has been proposed
- Patent applications
  - FFEI has filed a number of patent applications relating to technology developed as part of the Sierra project – details will be provided on request

Measurement system based on digital microscope

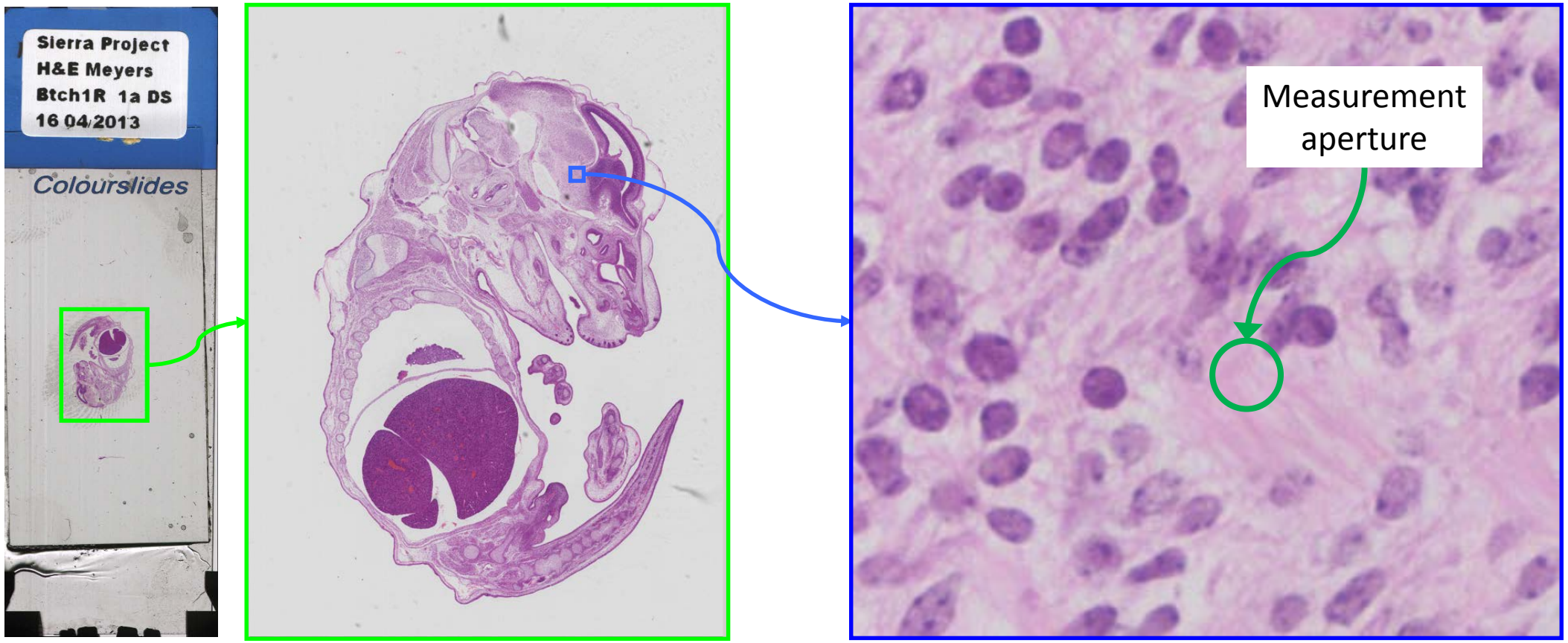




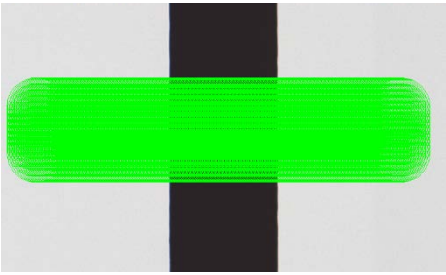
Digital microscope measurement system schematic

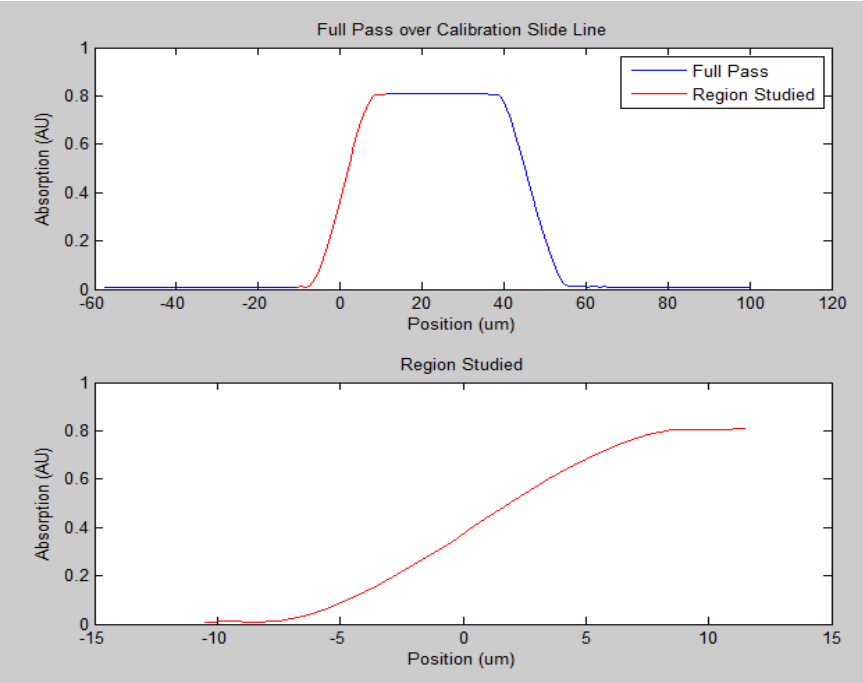


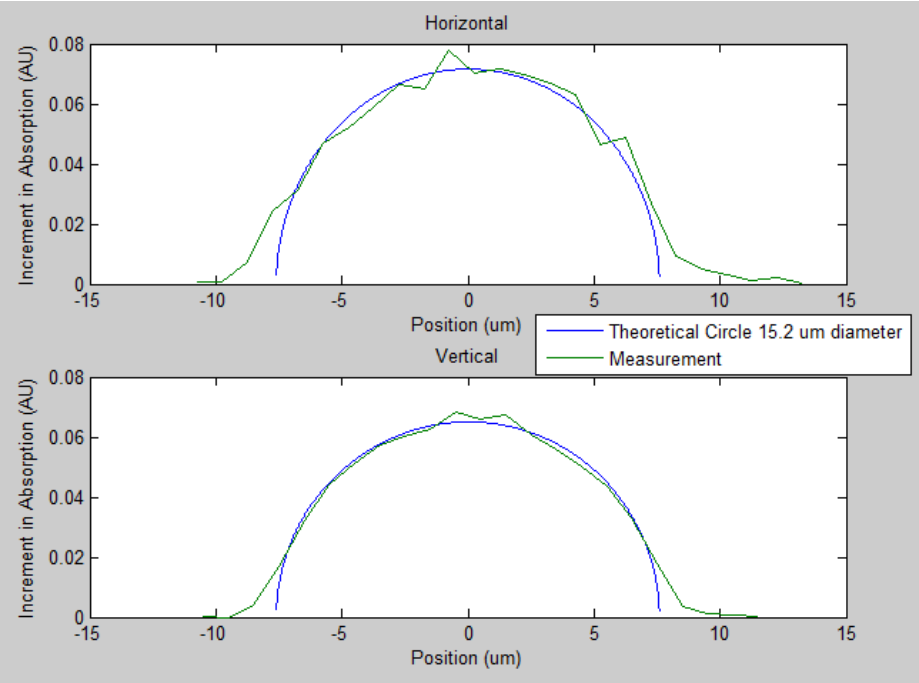
Viewer showing measurement position and aperture size for a typical slide measurement



Estimation of measurement aperture size

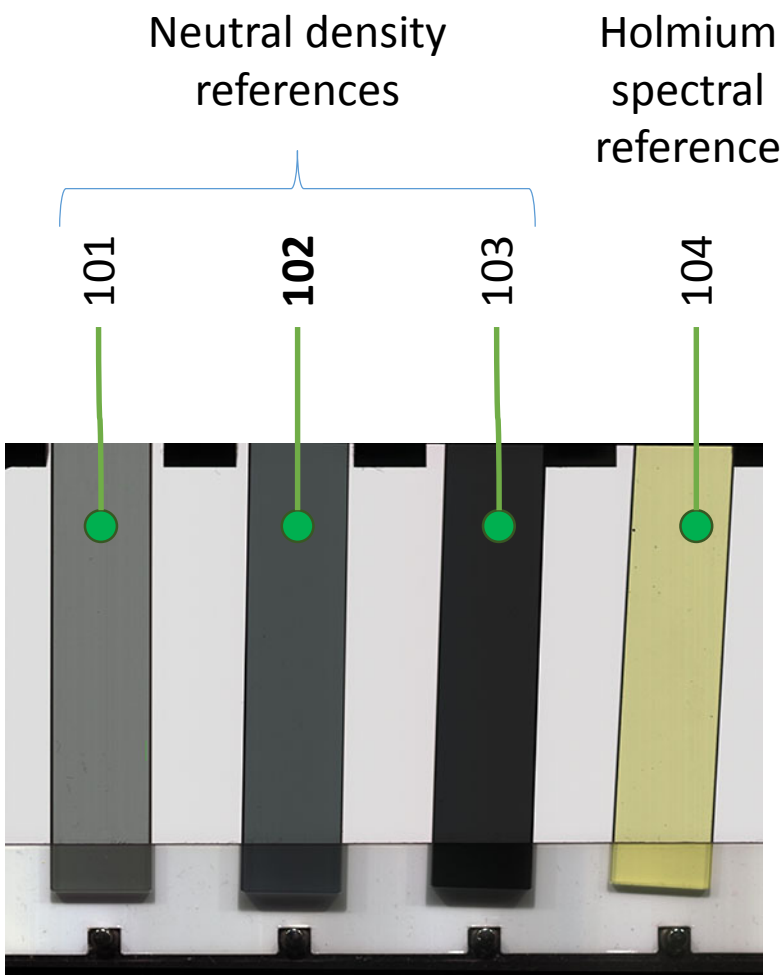


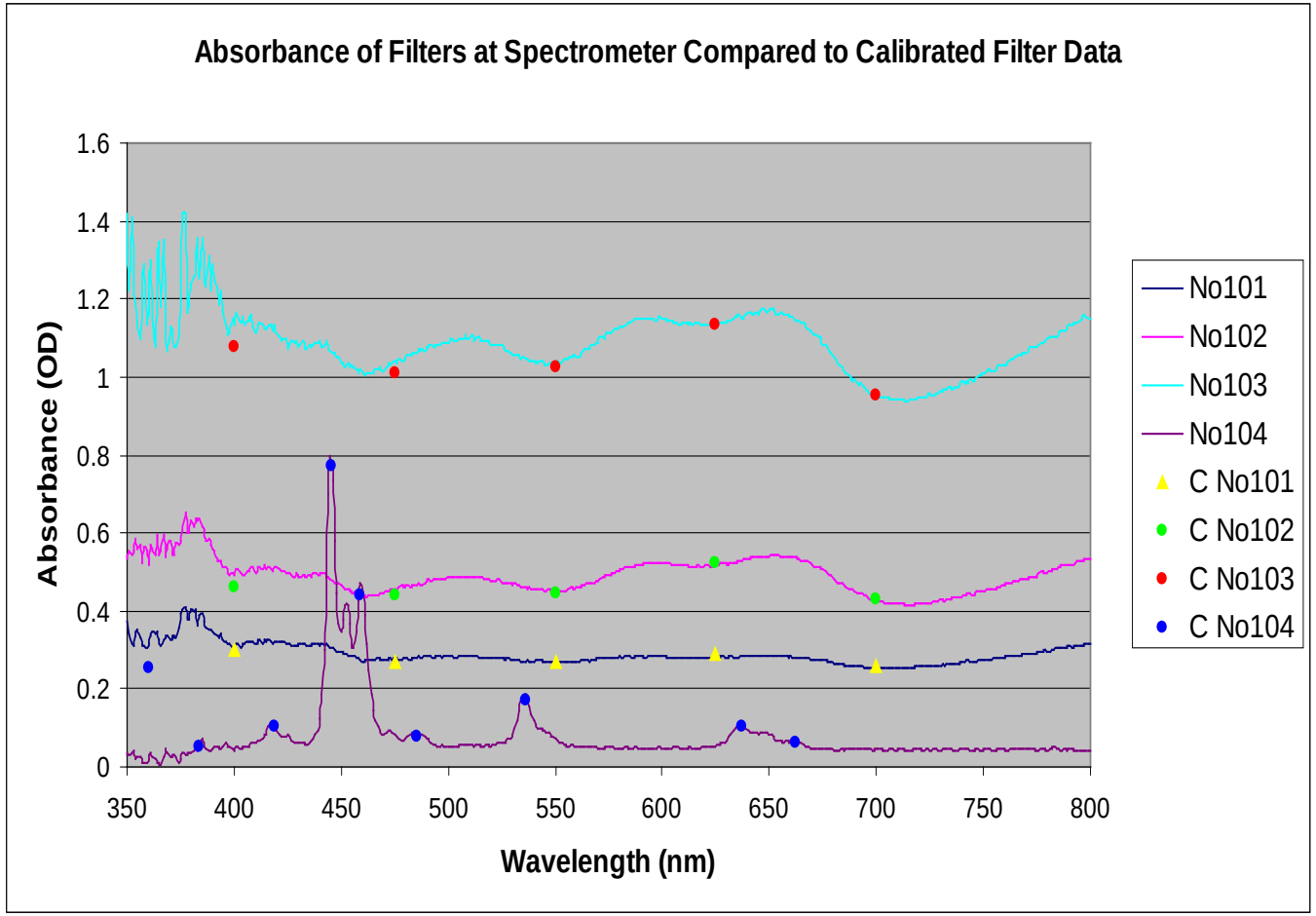




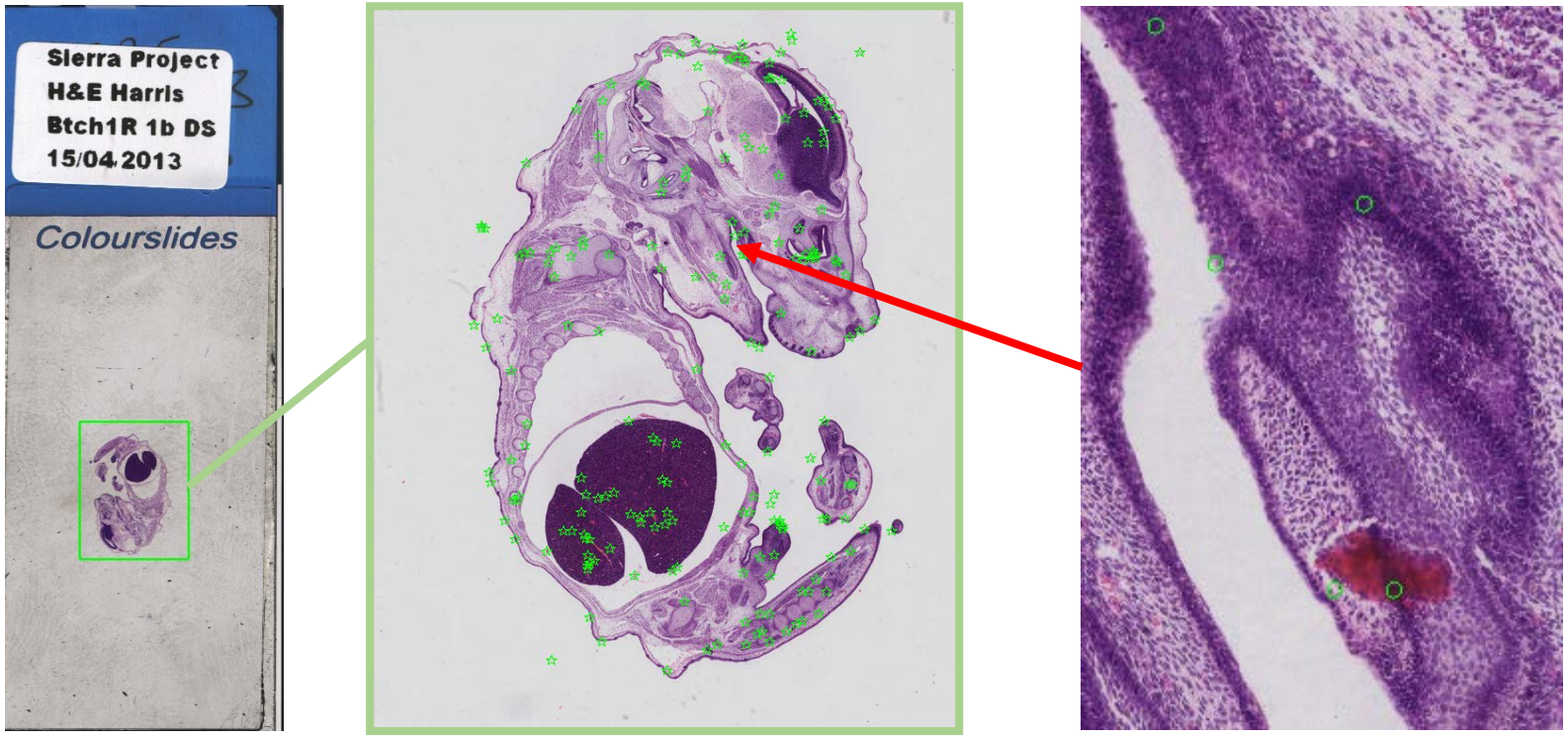
Measurement region is around 15 μm

Certified reference materials

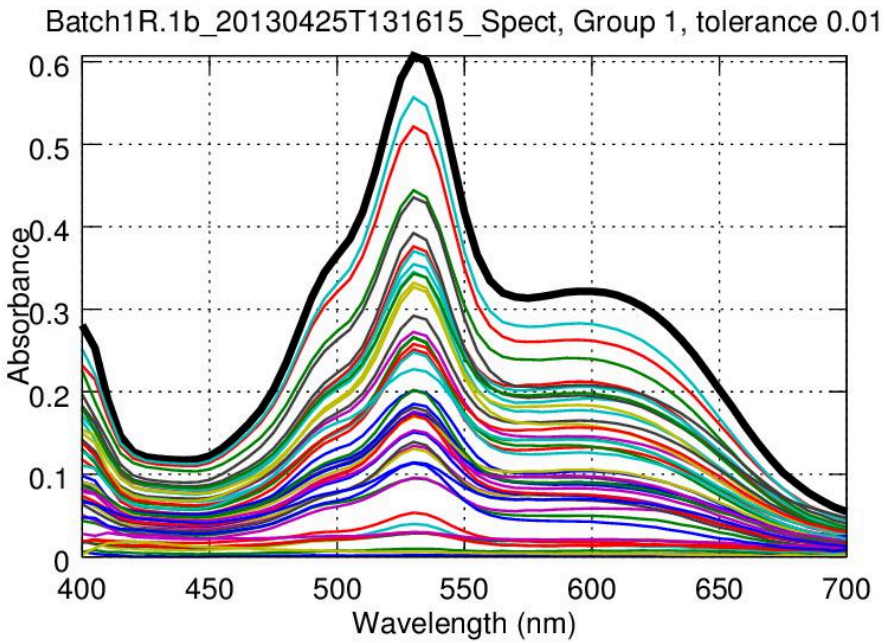


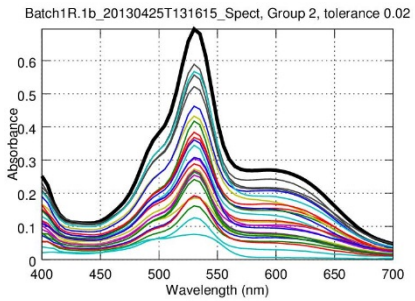


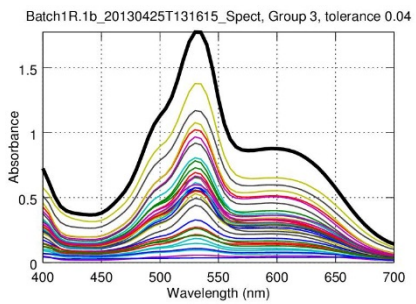
Example of slide measurement (Harris H&E stain)

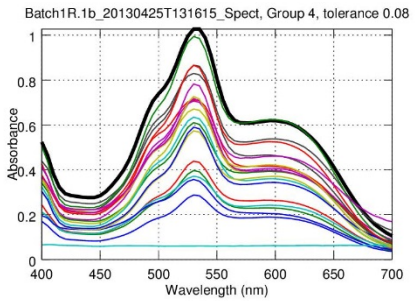


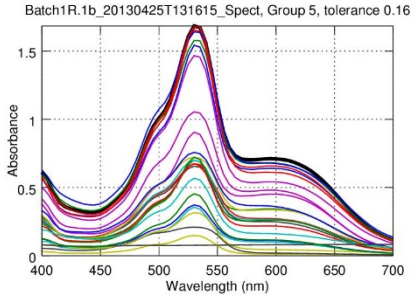
Grouping of colours found in Harris H&E stained sample

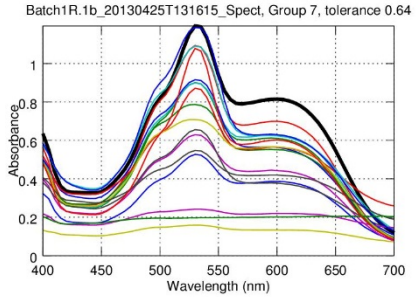


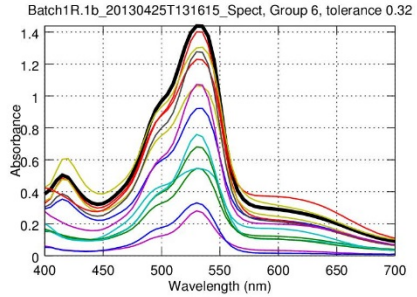


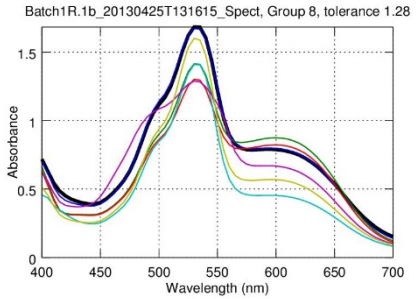


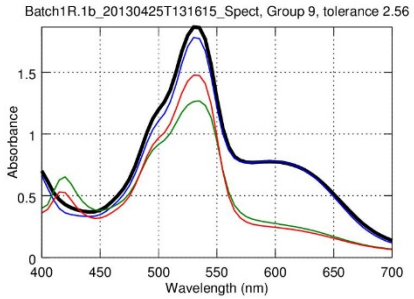


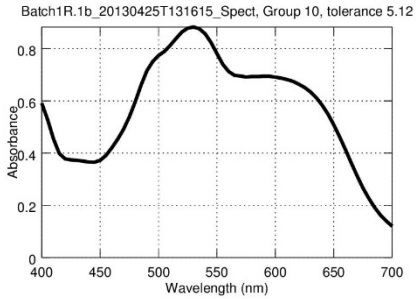








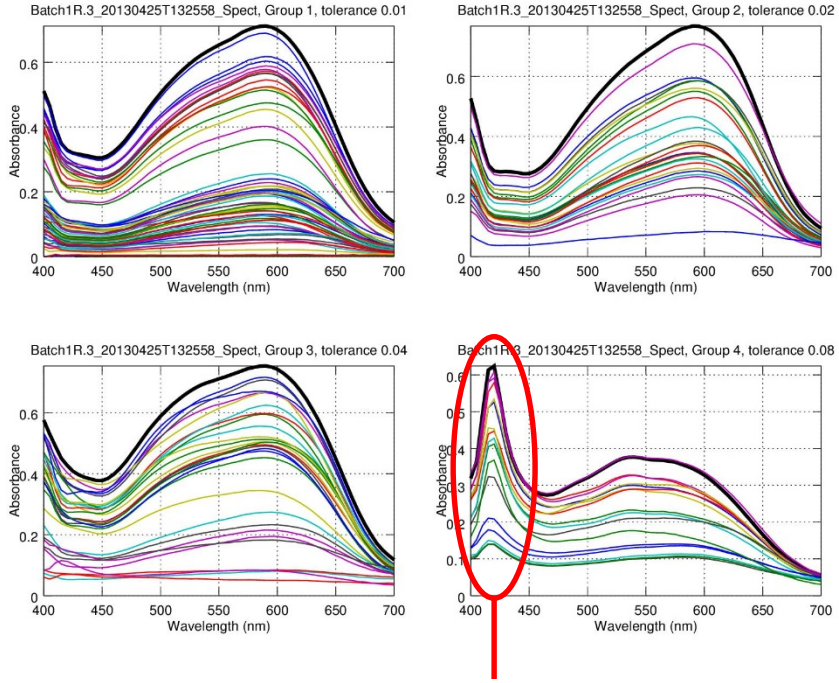




Each coloured line in these graphs represents a spectral measurement of a colour present on the slide. The figure above shows the colour group with most colours – the other nine show the remaining groups.

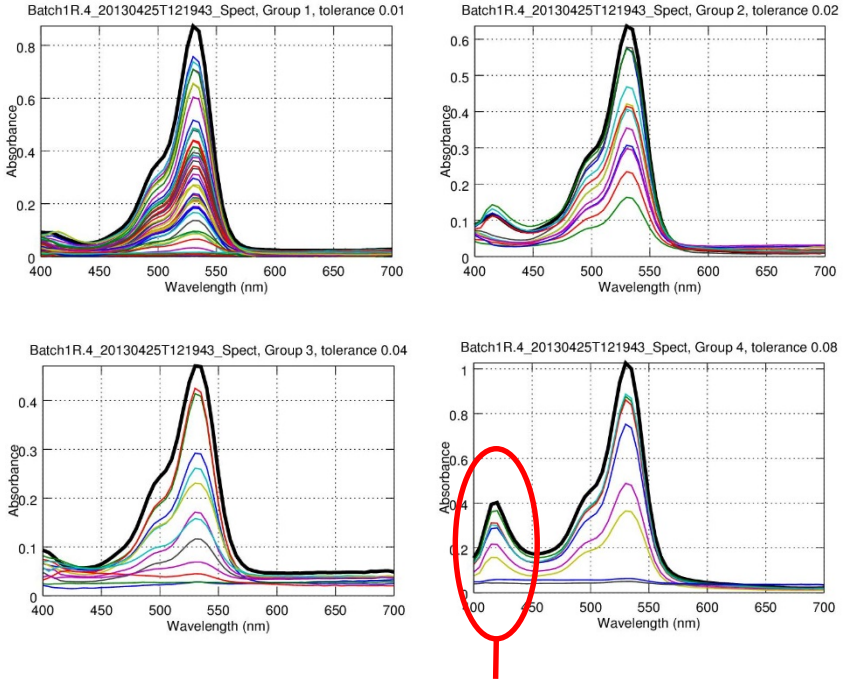
Grouping of colours found in single stain slides

Colour grouping for sample stained with Haematoxylin only



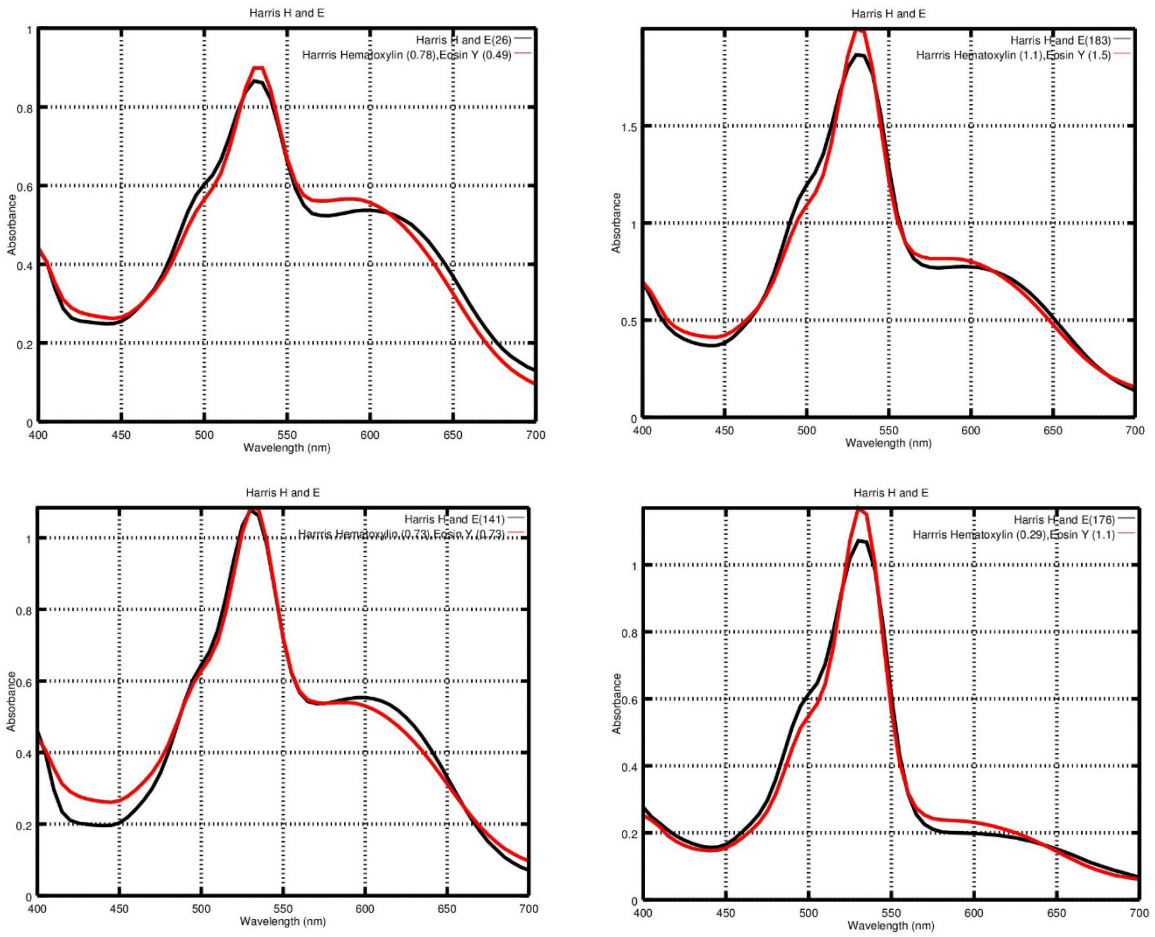
Haemoglobin

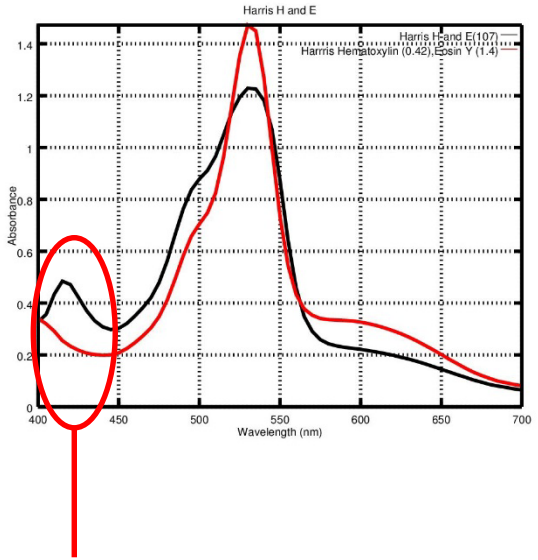
Colour grouping for sample stained with Eosin only



Haemoglobin

Matching Harris H&E colours using single stains

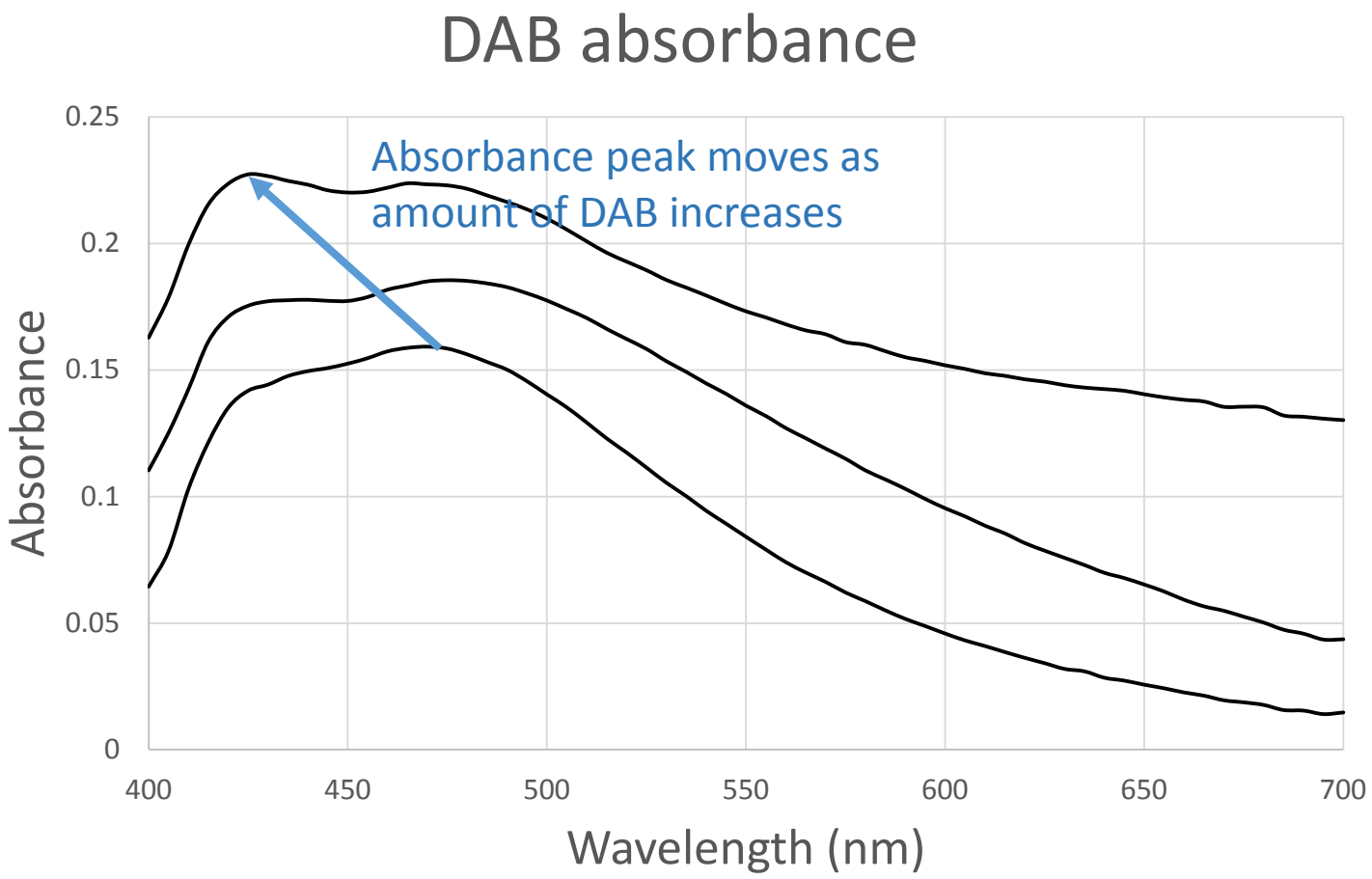




Error in this case is due to presence of Haemoglobin in the sample

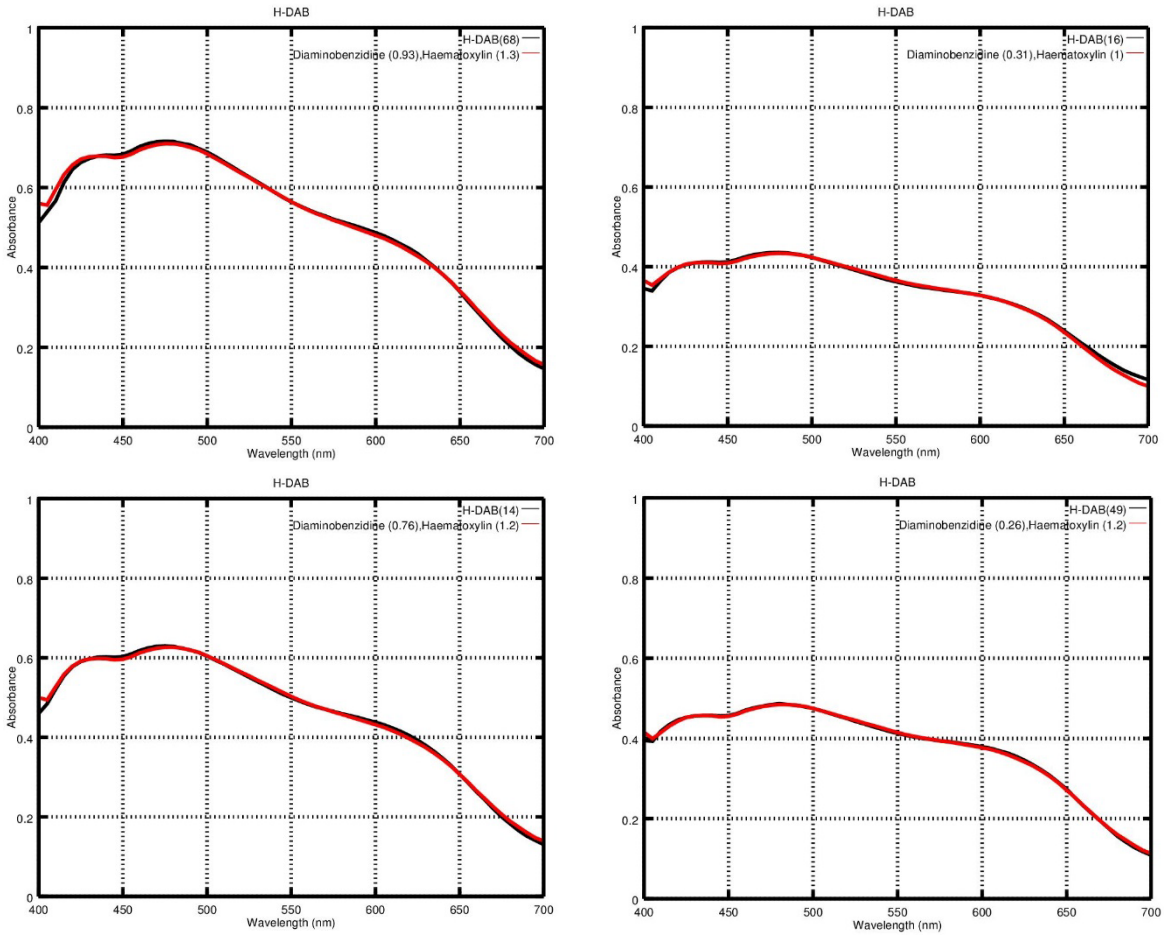
Demo

Diaminobenzidine (DAB) absorbance



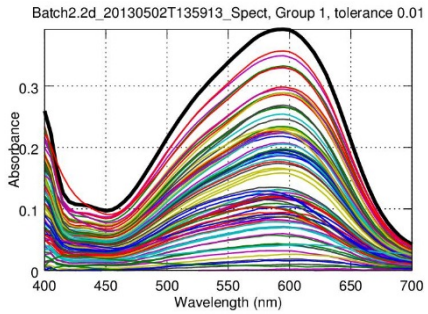
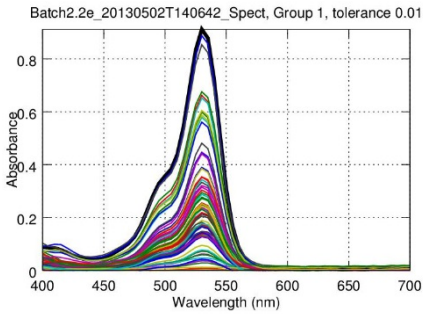
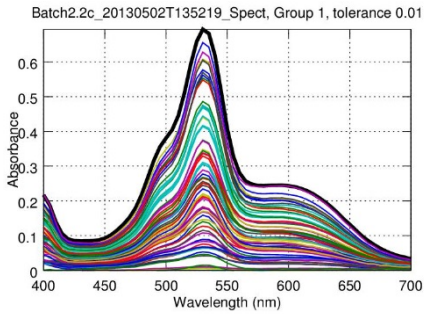
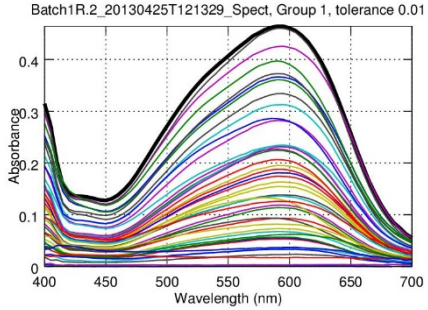
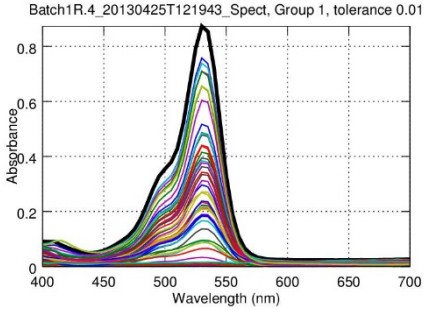
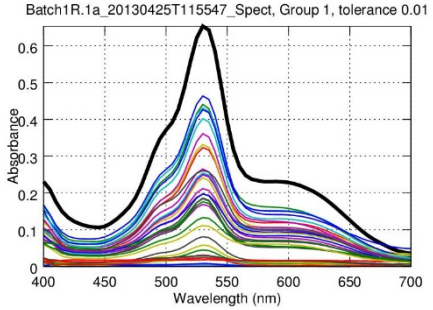
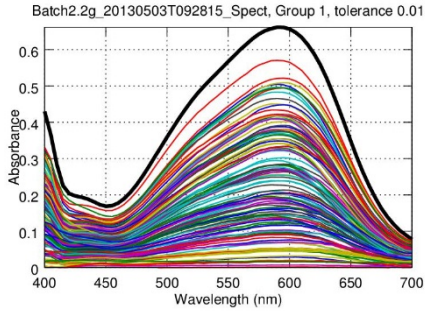
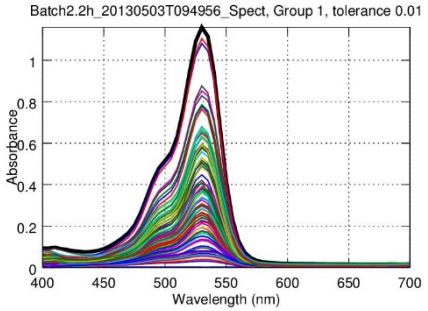
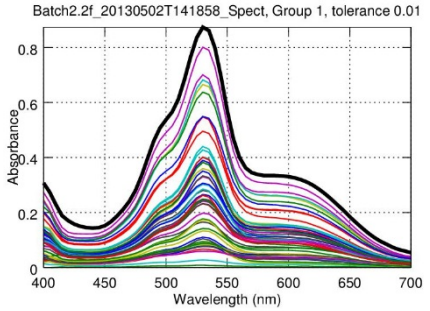
DAB staining does not follow Beer-Lambert

H-DAB analysis



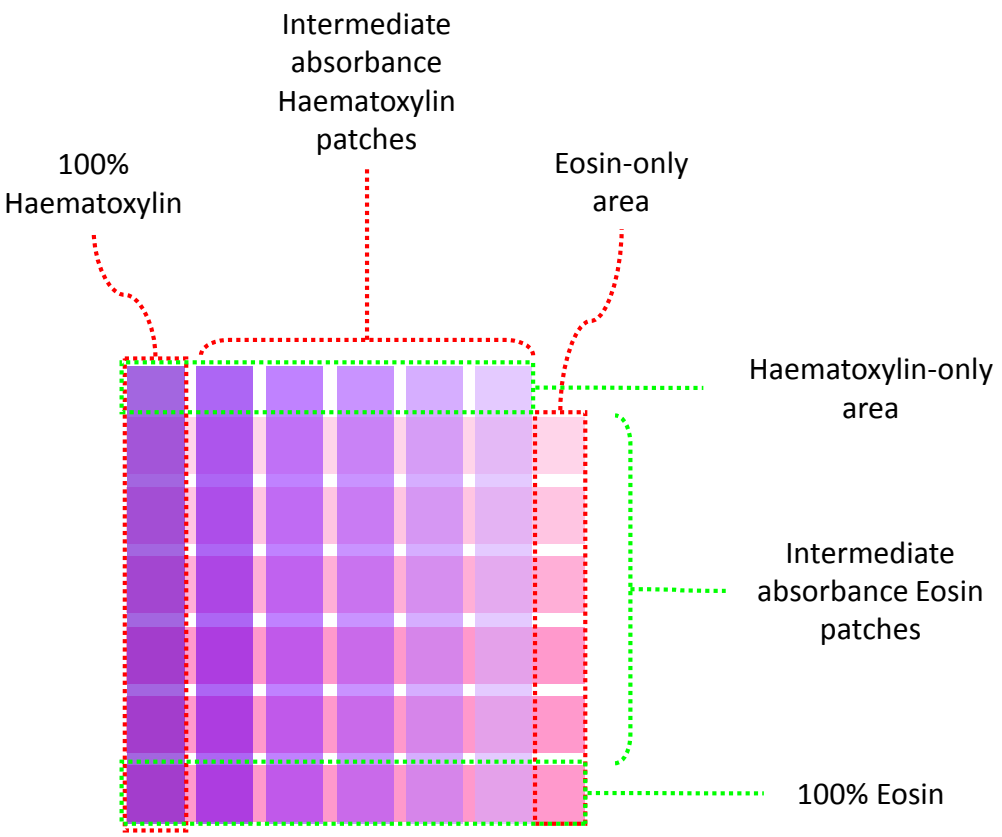
DAB with Haematoxylin counterstain can be simulated by simple linear addition

Effect of stain thickness

	Haematoxylin	Eosin	H&E
2μm tissue section			
5μm tissue section			
12μm tissue section			

H&E stains combinations

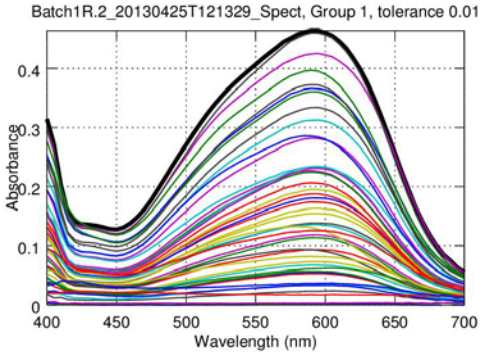
Requires N sheets stained with different amounts of Haematoxylin and N sheets stained with different amounts of Eosin to produce N patches with different levels of each single stain and NxN different stain combinations

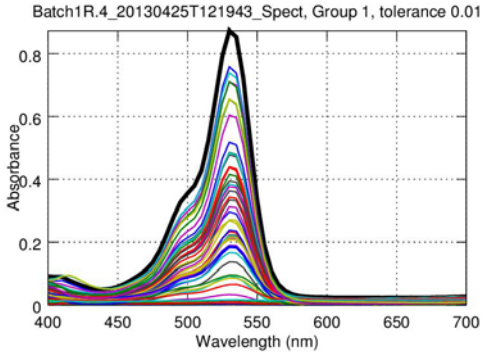


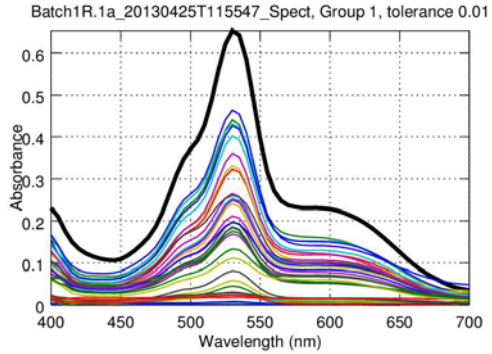
Comparison between slide colours and stained tissue samples

Haematoxylin | Eosin | H&E stained

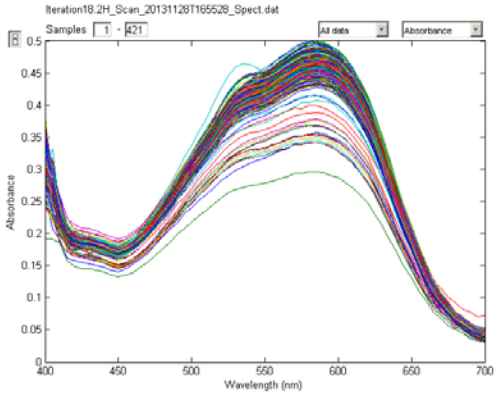
Stained tissue measurements

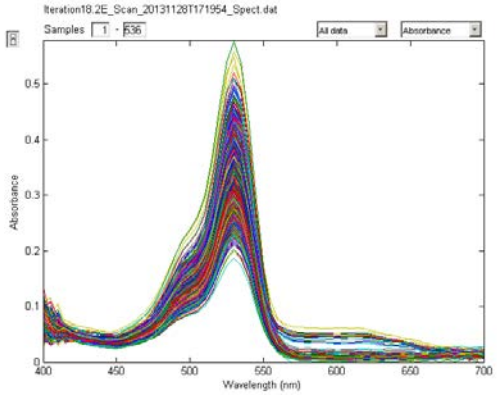


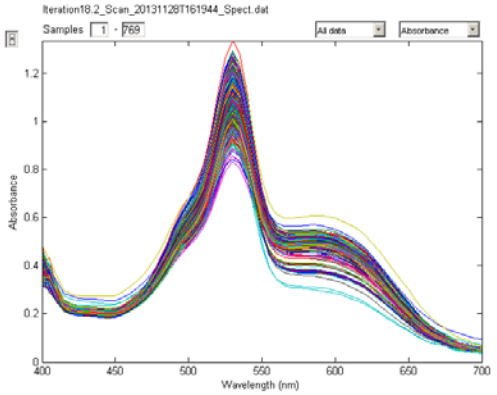




Stained biopolymer measurements

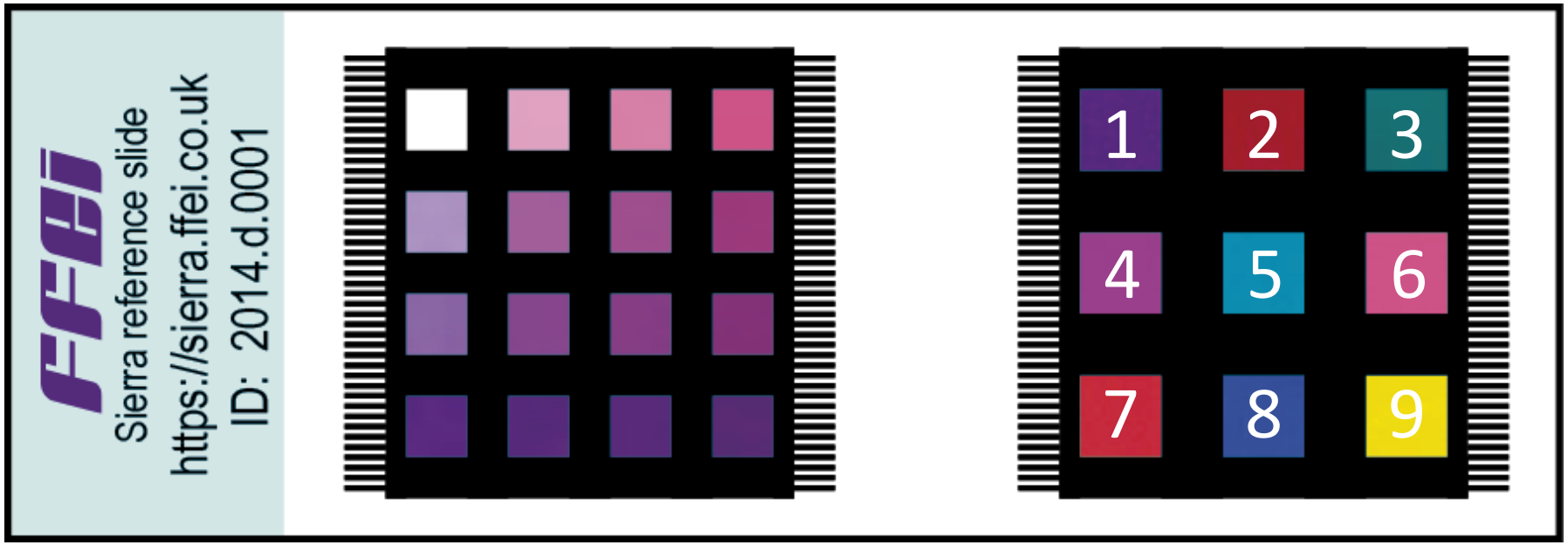






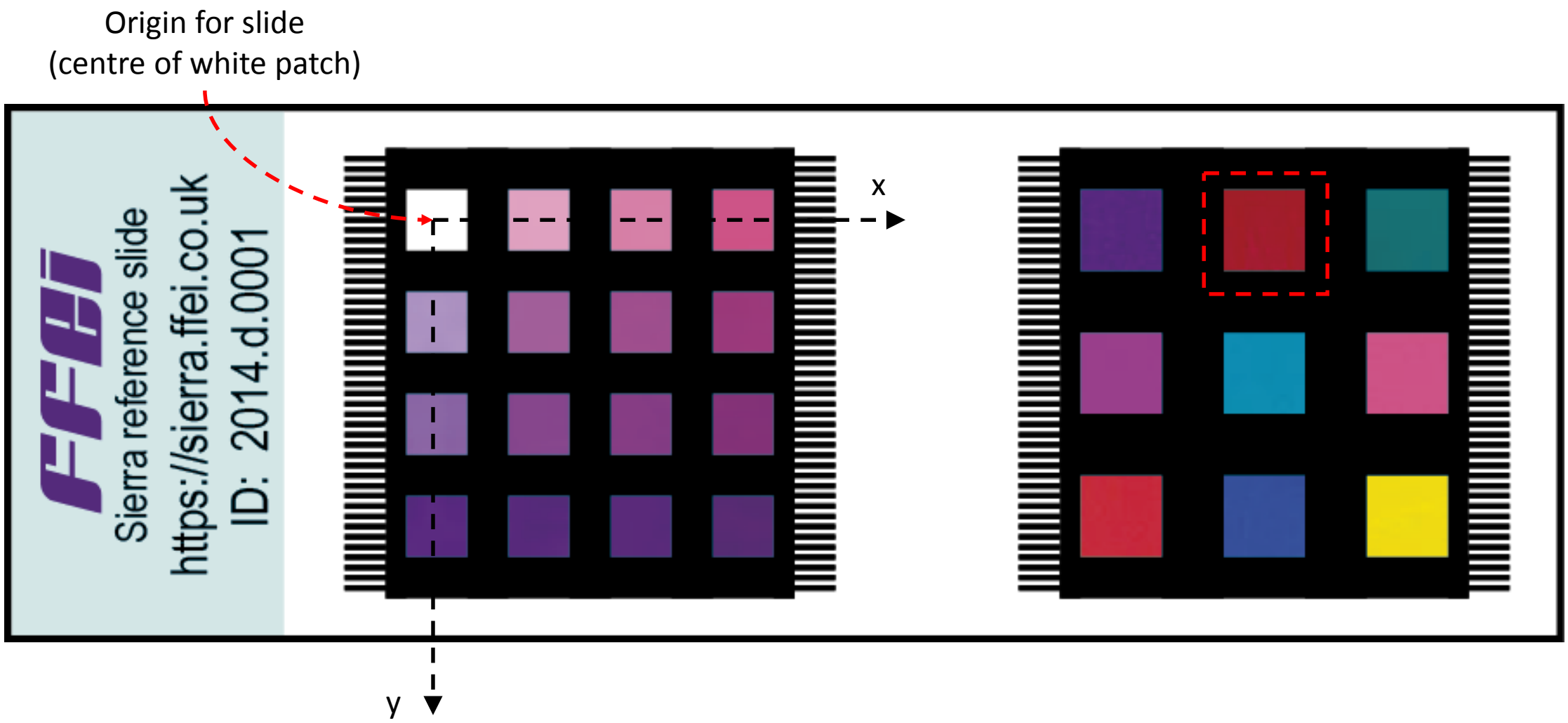
Sierra calibration assessment slide from FFEI

H&E stain area

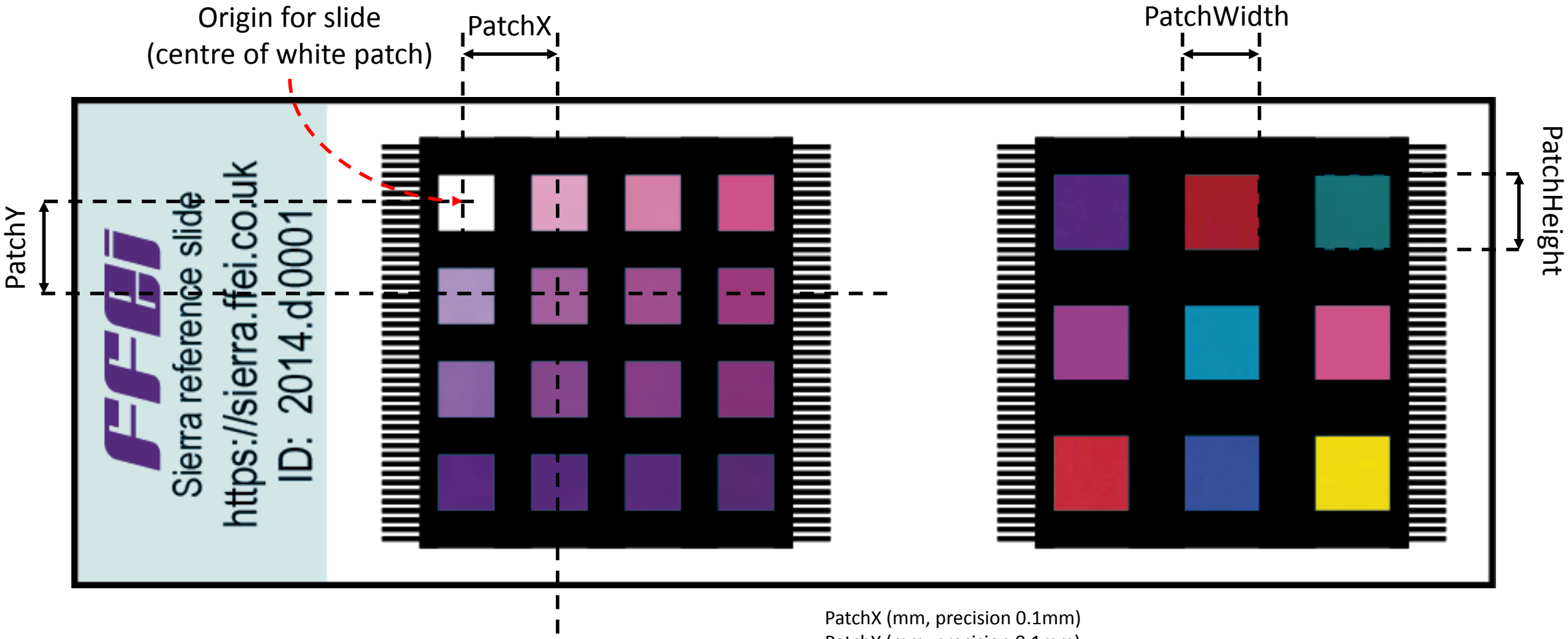


1. Haematoxylin
2. Neutral Red
3. Light Green FS
4. PAS
5. Methyl Green
6. Eosin
7. Ponceau Fuchsin
8. Aniline Blue
9. Tartrazine

FFEI Measurements: slide coordinate system

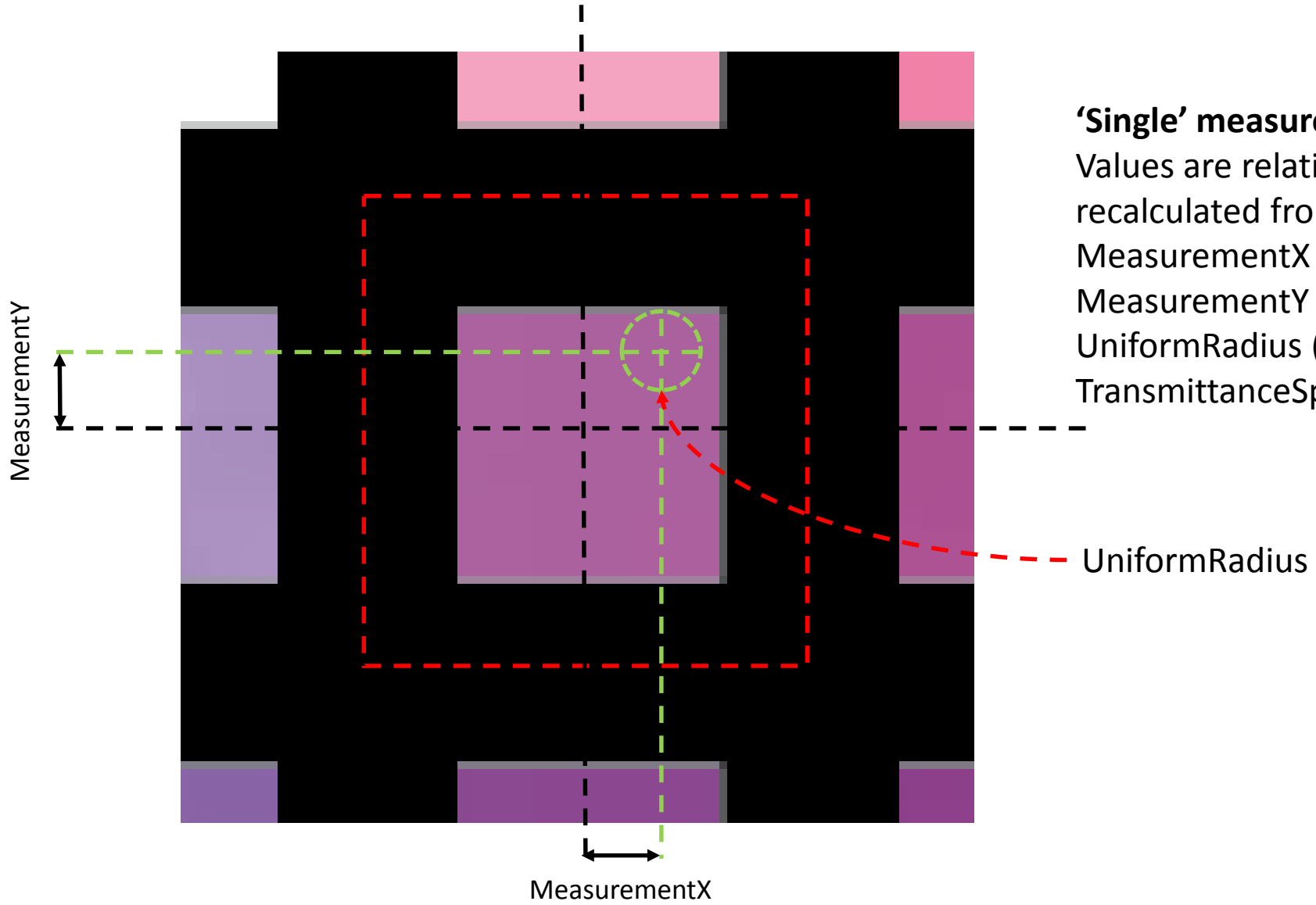


FFEI Measurements: patch position



PatchX (mm, precision 0.1mm)
PatchY (mm, precision 0.1mm)
PatchWidth (mm, precision 0.1mm)
PatchHeight (mm, precision 0.1mm)
TransmittanceSpectrum (samples at 380:10:800 nm)

FFEI Measurements: patch coordinate system



‘Single’ measurement metadata

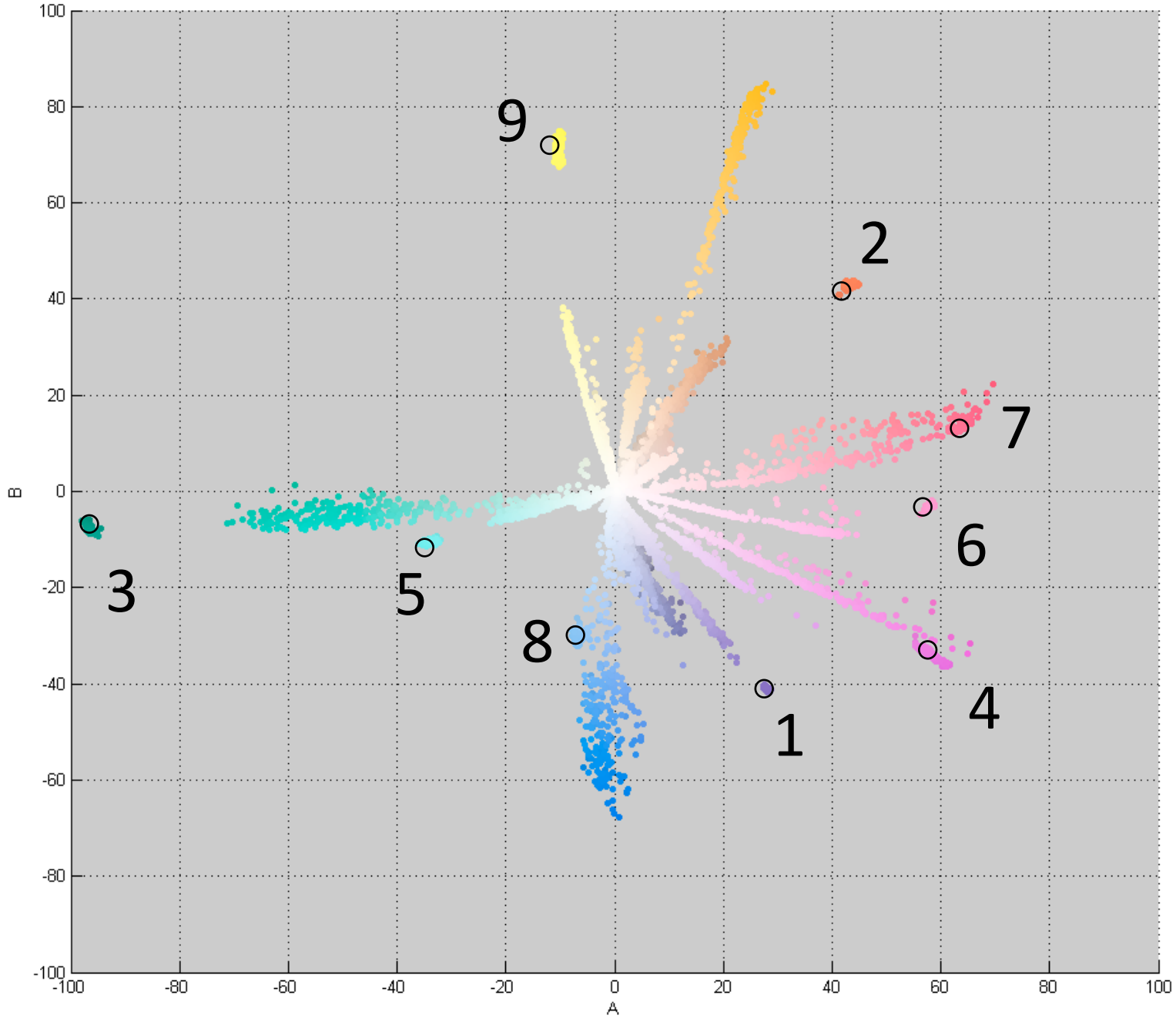
Values are relative to patch centre which should be recalculated from image

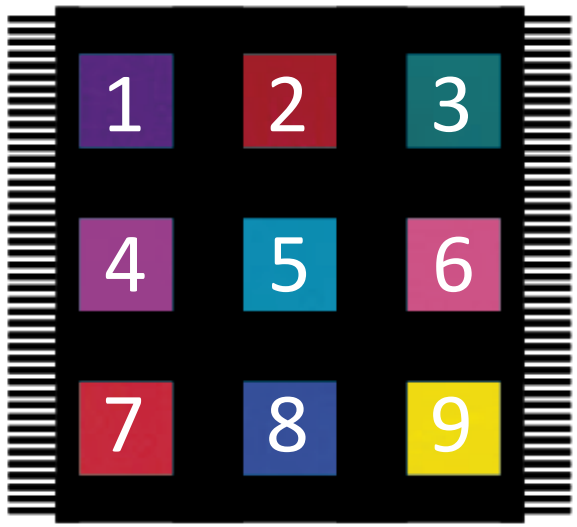
MeasurementX (mm, precision 0.0001 mm)

MeasurementY (mm, precision 0.0001 mm)

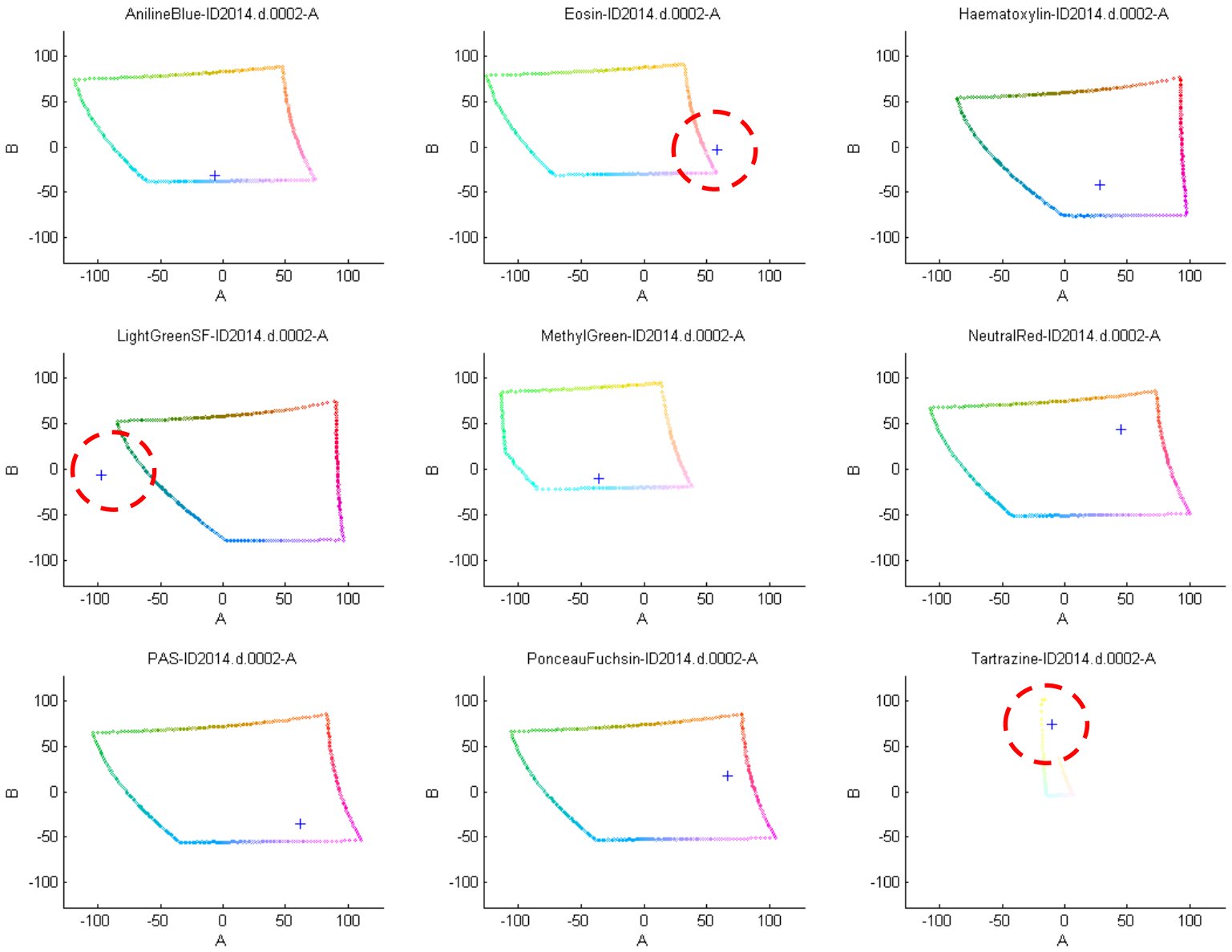
UniformRadius (mm, precision 0.0001 mm)

TransmittanceSpectrum (samples at 380:10:800 nm)





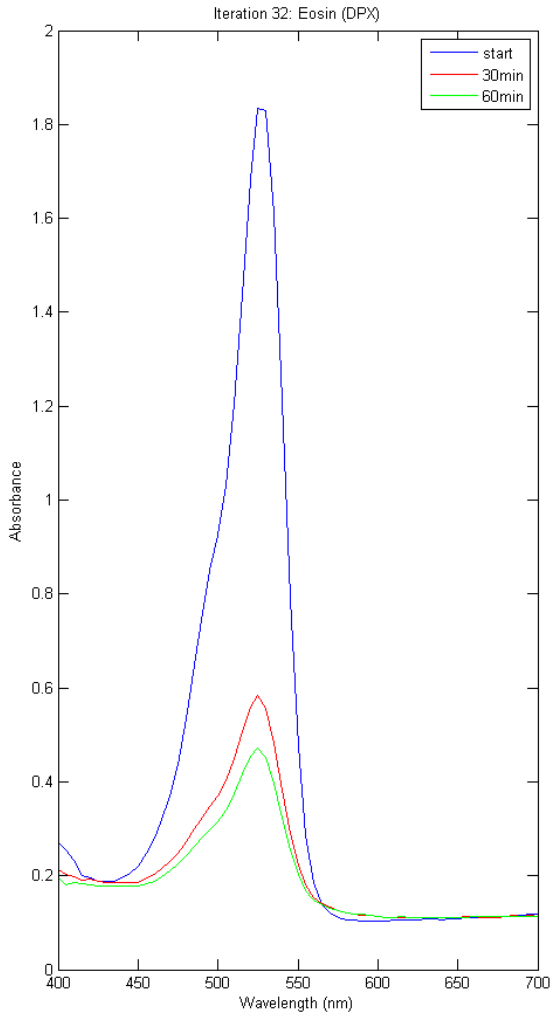
Plot shows the colours found on a selection of stained tissue slides in relation to the colours of the nine patches of the reference slide

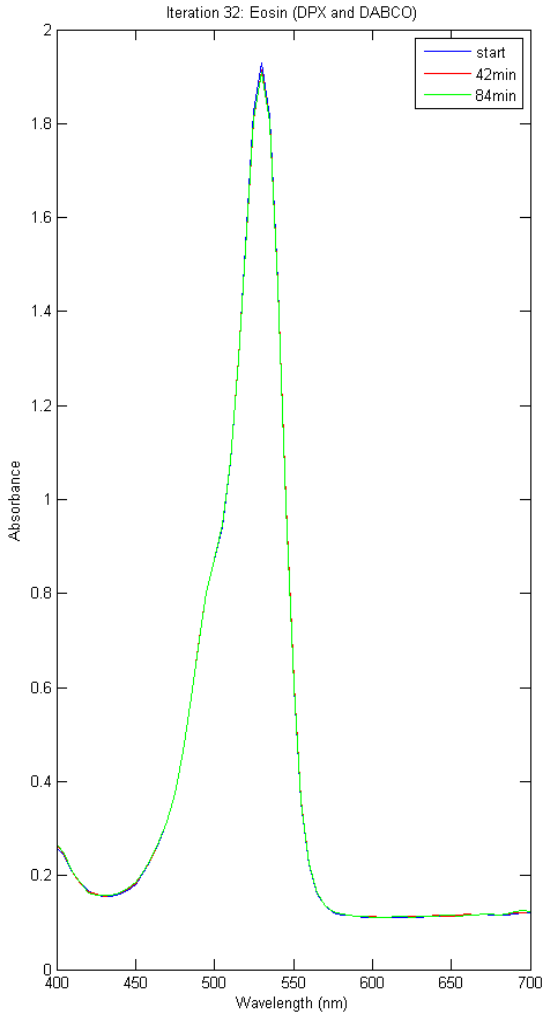


Comparison with typical display colour gamut AdobeRGB+

Eosin stabilisation

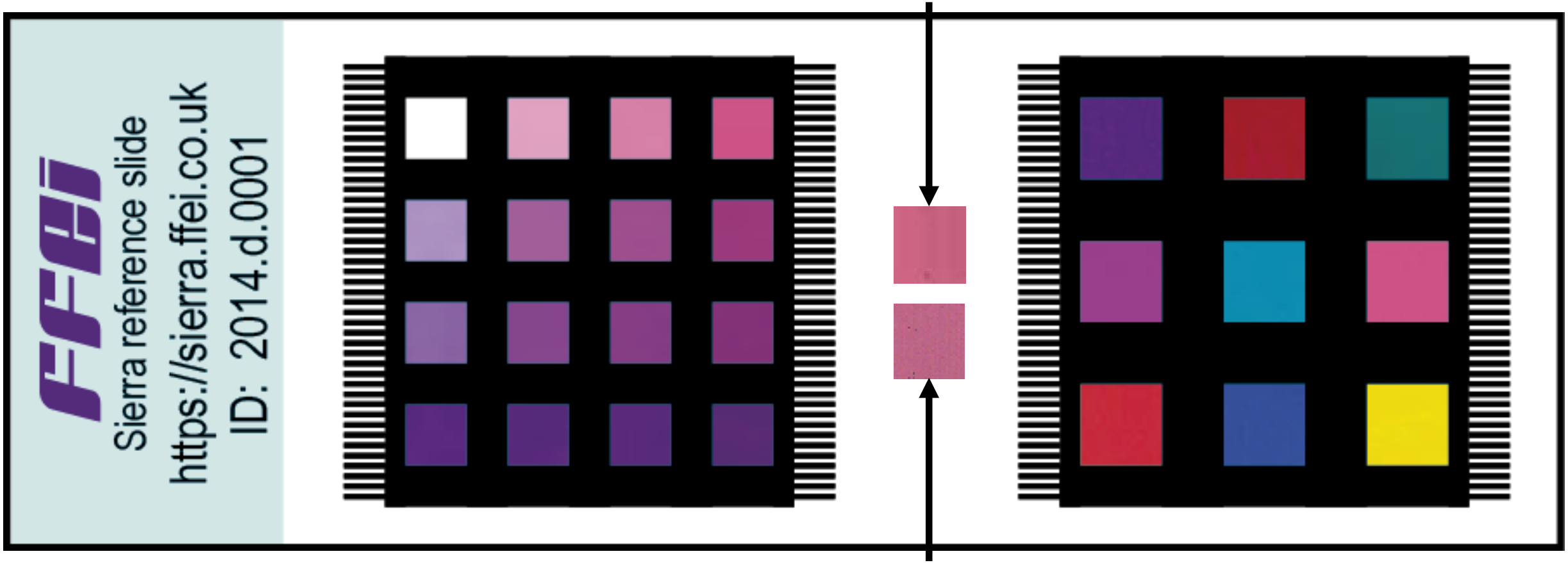
Untreated Eosin
absorbance has dropped to 1/3 of its initial value after 30 minutes exposure to high intensity light source



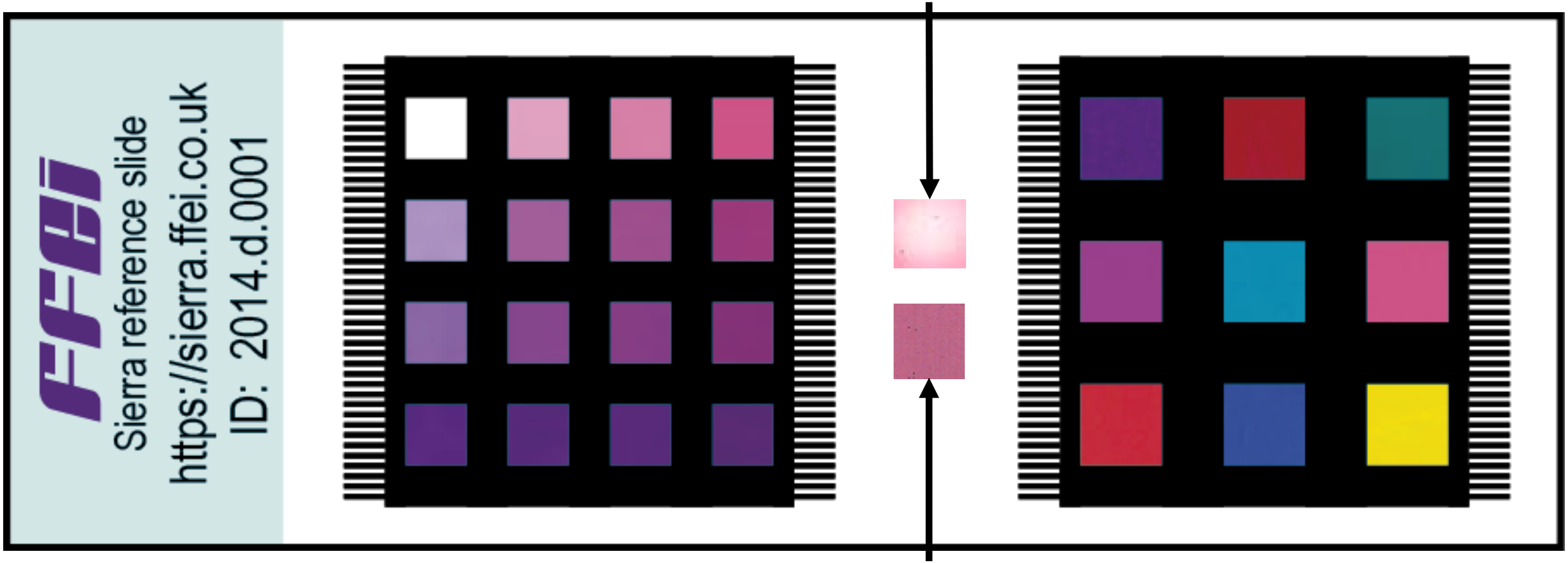


Eosin treated with DABCO
no change in absorbance after continuous exposure to the same high intensity light source for 84 minutes

Untreated Eosin stained sample



Eosin stained sample treated with DABCO or similar



Untreated Eosin stained sample

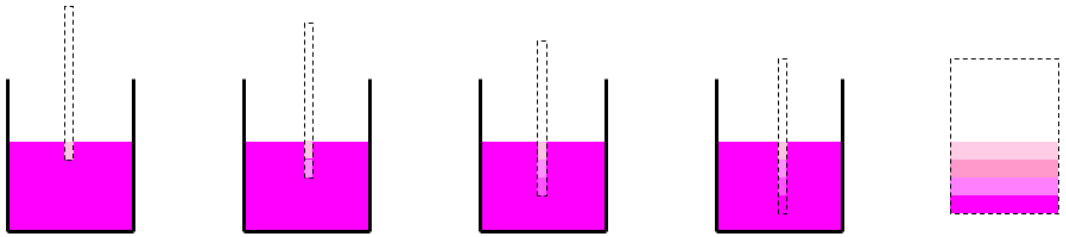
Eosin stained sample treated with DABCO or similar

Planned improvements

- Patch uniformity
  - modify staining process (a number of improvements have been identified)
  - improve handling in manufacturing
- Patch size reduction
  - staining jig used to stain multiple levels
- Additional colours
  - add multiple intensity levels
  - add neutral patches

Patch size reduction

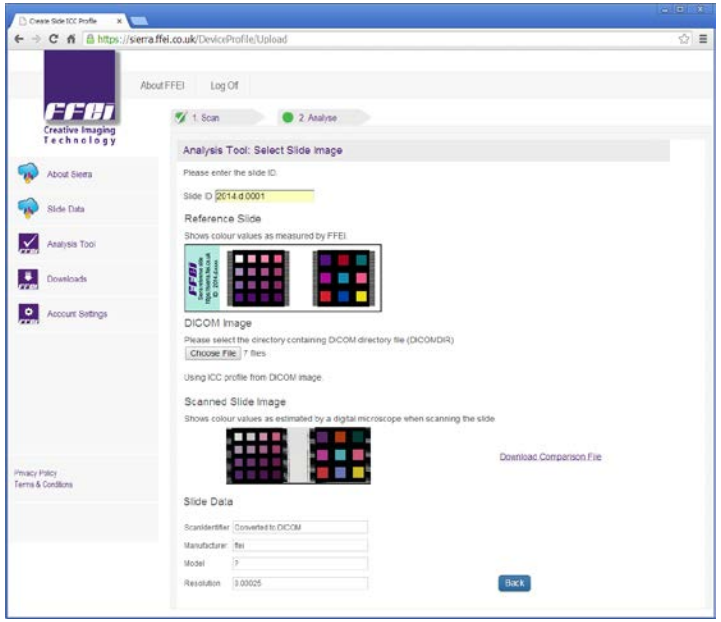
staining jig can be used to stain bands of multiple stain intensities on single sheet



FFEI tools

- As part of the Sierra project we have developed a number of core technologies some of which we are providing for use in assessing the calibration reference slide
- Analysis tool
  - HTML5 web-based DICOM image analysis
  - shows colour values for Sierra reference slide image
- Sierra whole slide image viewer
  - basic viewing capability
  - high performance colour management
- Sierra OpenSlide to DICOM converter
  - converts image formats supported by the OpenSlide library to DICOM
  - adds an ICC Profile for the calibrated scanner

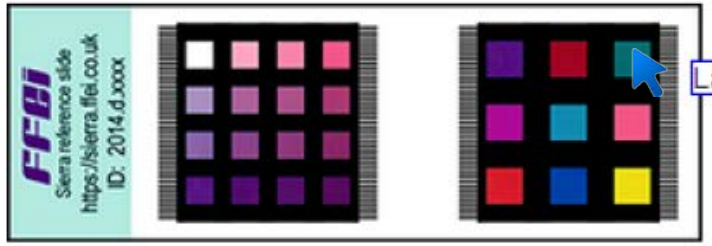
Analysis Tool



HTML5-based tool – works best in Chrome currently
Requires a DICOM image of slide on local PC

Reference Slide

Shows colour values as measured by FFEI.



Lab_Ave(53.01, -88.75, -7.85), Lab_Point(52.12, -87.63, -8.05)

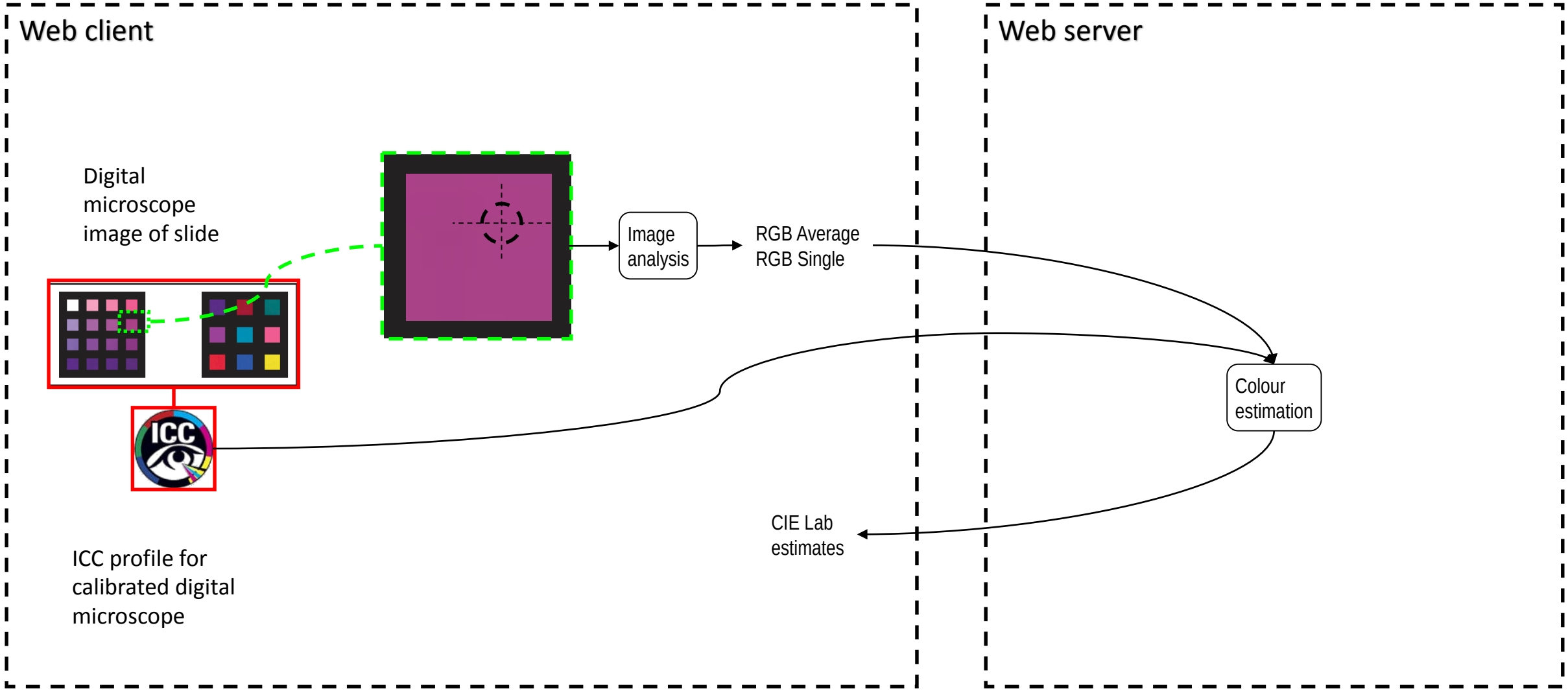
Scanned Slide Image

Shows colour values as estimated by a digital microscope when scanning the slide.

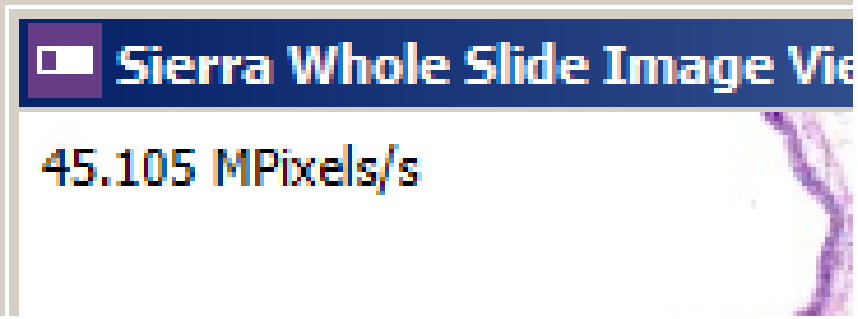
Lab_Ave(50.09, -84.64, -11.87), Lab_Point(49.28, -85.68, -10.93)

Download Comparison File

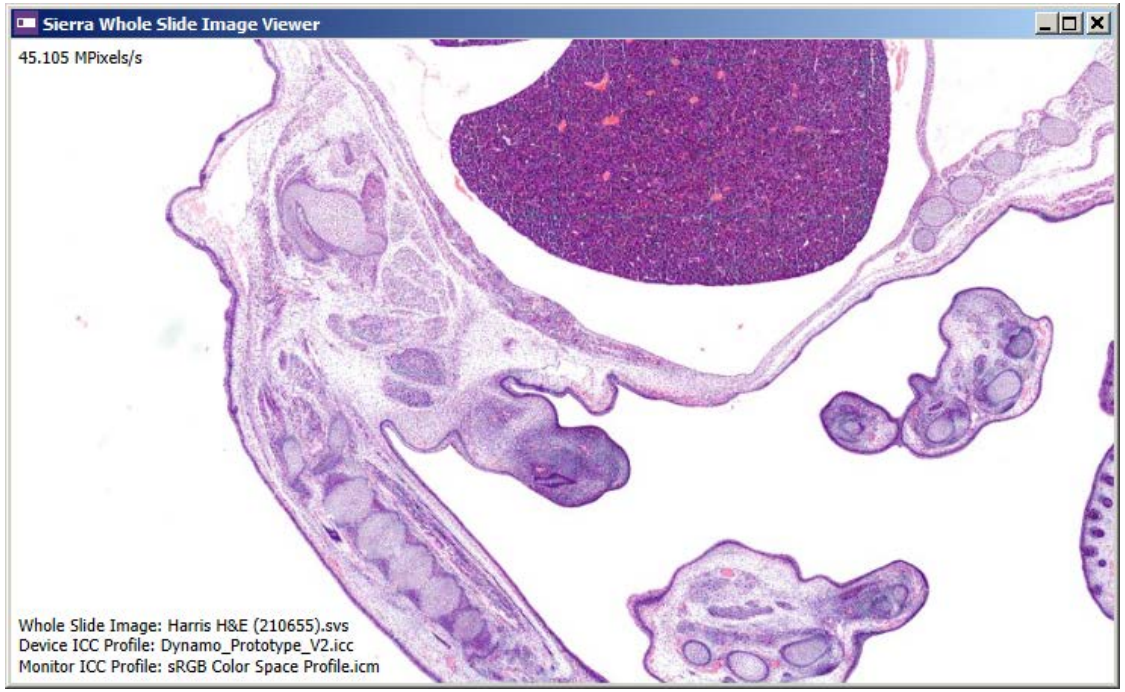
Analysis Tool



Whole Slide Image Viewer

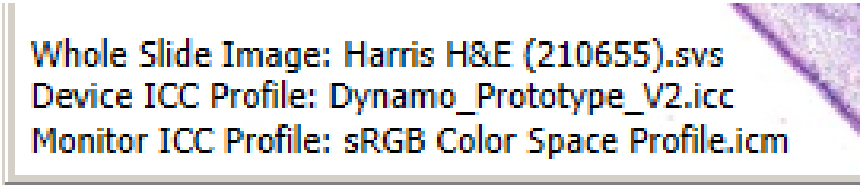


Shows number of colour conversions in pixels per second



Can be downloaded from Sierra web site Supports basic pan and zoom functions

Shows Image and profile name and the display profile being used for colour conversion

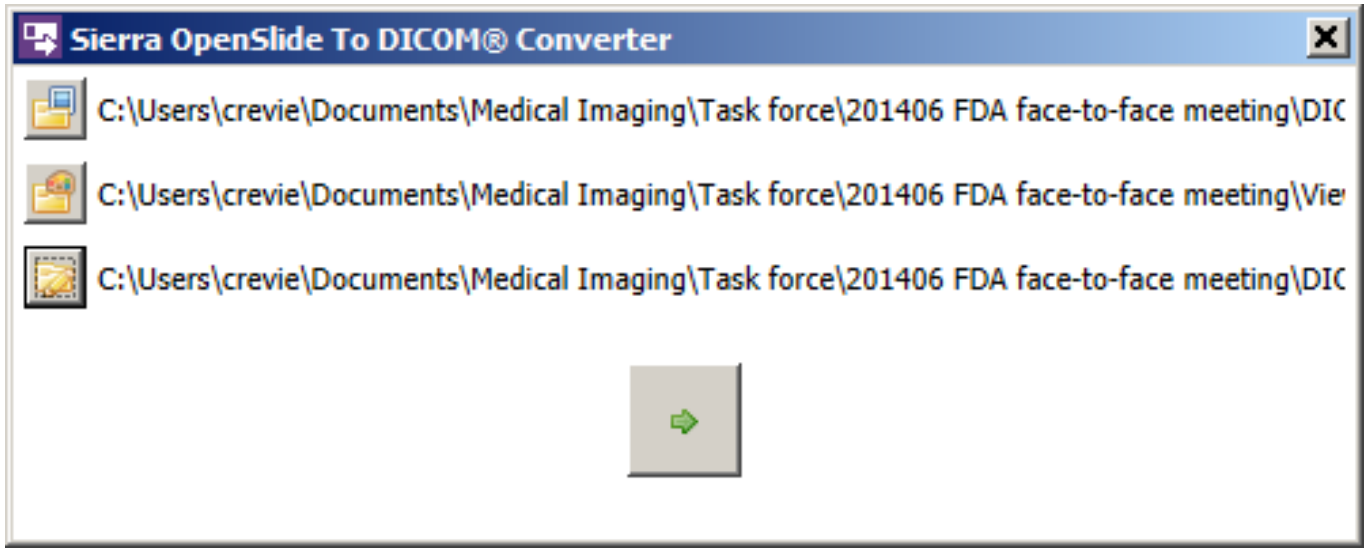


Sierra OpenSlide to DICOM Converter

File to be converted

ICC Profile for calibrated scanner

Folder to be used for DICOM image



Command line utility and GUI versions available for download from Sierra web site

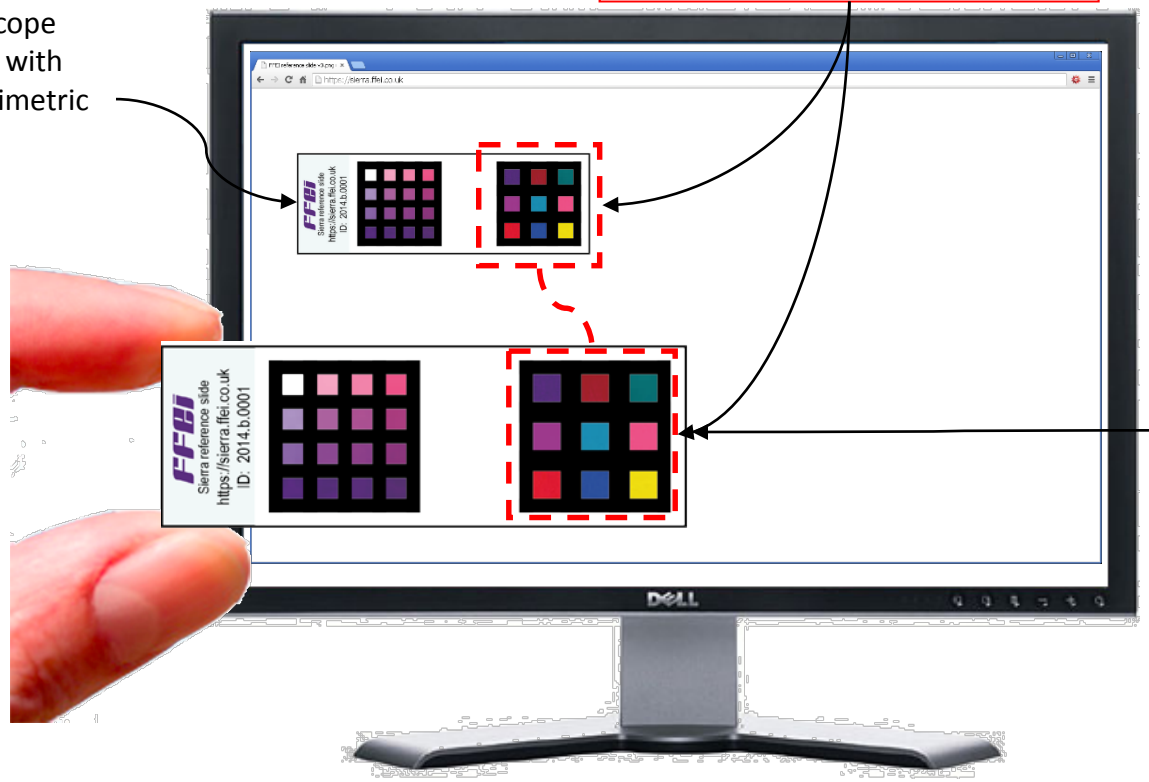
Some notes about the tools

- Tools are provided ‘as is’ and should not be considered product quality as they demonstrate a limited set of functionality
- Please feed back suggestions for improvement and report problems to sierra.support@ffei.co.uk
- Although we are using HTML5 as the basis for our work we have only tested these products using Google Chrome – we are aware of performance degradation and some user interface problems when using other browsers which we expect to address in due course
  - to select from Slide ID menu - click on the RHS of the control to display the menu, a double click is required in Firefox
  - on the Slide Data page to get the table to update with the latest selected Slide ID, click outside the menu or hit the tab key

Visual assessment

System should only be used to view digital microscope slides if these two sets of colours are closely matched

Digital microscope image of slide with Relative Colorimetric rendering



Microscope slide is illuminated by the display back light

Viewing conditions for the microscope slide and slide image are identical

Based on a method developed and promoted by Yukako Yagi and Pinky Batista

Future: fully automated system calibration check

An image of the slide is scanned periodically on a calibrated digital microscope and held for future use in calibrating the system – the slide ID is held as part of the image data

At the start of each review session the pathologist performs a system calibration

Colour from each patch of image is displayed using a calibrated WSI viewer

Measurements are made using standard display measurement instrument

These measurements are compared with the reference measurements for the slide

The calibration status for the system is displayed and automatically recorded in the images reviewed during the session

Questions and discussion