**Calibration Slide for Histopathology task force**
**Teleconference**
19 June 2014 • 13:00 (EST)

The meeting was called to order at 13:00 pm (EST) by Craig Revie, chair of MIWG, with the following attendees:

Rich Amador, Canon U.S.A., Inc.
Chris Bai, BenQ Corporation
Pinky Bautista, MGH PICT center
Vipul Baxi, Omnyx Integrated Digital Pathology
Wei-Chung Cheng, Federal Drug Administration
David Clunie, Bioclinica & PixelMed
Brian Cote, Eizo Corporation
Scott Forster, Roche Ventana
Phil Green, Gjøvik University College, Norway
Bas Hulsken, Philips Healthcare Incubator
Po-Chieh Hung , Konica Minolta
Francisco Imai, Canon U.S.A., Inc.
Bryan Kennedy, KARL STORZ Imaging
Stephen Lansel, Olympus
Changjun Li, Liaoning University of Science and Technology
Takashi Matsui, Eizo Corporation
Efrain Morales, KARL STORZ Imaging
Allen Olson, Leica Biosystems
Craig Revie, Fujifilm Corporation
Christye Sisson, Rochester Institute of Technology
John Sweeney, BenQ Corporation
Dave Wyble, Avian Rochester, LLC
Kaida Xiao, Technical Consultant
Albert Xthona, Barco NV
Masahiro Yamaguchi Tokyo Institute of Technology
David Brettle, James's University Hospital Leeds

After self-introductions and a check of the sound quality Mr. Revie reviewed the agenda for the meeting as follows:

1. FFEI calibration slide
2. Framework for multispectral imaging in pathology
3. Point of Use QA in Digital Pathology Slides
4. Color calibration methods in Phillips scanners

**1. FFEI calibration slide**

1.1 Overview. Mr Revie gave an overview of the Sierra calibration slide project [see attached]. The measurement system was based on a digital microscope adapted as a spectrophotometer, using a Hamamatsu spectrometer. He described the slide measurement procedure, which captured transmittance over the range 380-800nm.

The FFEI development platform currently obtains errors in the region on 5-10 CIELAB $\Delta E^*_{ab}$, but with further work this is expected to be reduced.

Mr Revie stated that the measurement used CIE Illuminant E (i.e. equal spectral power at all visible wavelengths) in computing colorimetric quantities, while the resulting profile converted the scanned data to D50 colorimetry. Discussion on the illuminant was deferred to a future meeting.

Mr Revie showed the spectral absorbance of biopolymer stains measured using this setup, and compared them with stained tissue samples. Stains were measured at different thicknesses, and the results were dependent on the thickness.

He indicated that the stained biopolymer used in the Sierra calibration slide carried a greater stain intensity. There was some non-uniformity in the measurements. He outlined planned improvements to the calibration slide, including patch size and uniformity and the number of colors in the slide. Currently patches are 3 mm and 4 mm square and smaller patch sizes will be required on future slides.

Wei-Chung Cheng of the FDA indicated that in their study conducted in conjunction with the Biological Stains Commission (BSC) they discovered that Haematoxylin was the most variable stain, and there was also regional variation. FFEI intend to study this further with help from Wei-Chung. Five different labs have been assessed to date. The results are not yet published but will be shared with the group. Haematoxylin had the highest variation. Staining protocols were varied and documented in the study.

Slide colors were compared with display color gamut, and several, most notably Eosin and Light Green SF stains were outside the extended-gamut Adobe RGB (1998).

Mr Revie showed pages from the project web site (https://sierra.ffei.co.uk) [see attached]. Captured slide images can be downloaded from the Sierra web site and it is also possible to share evaluation data through the web site. He asked the meeting for feedback on anything that should be included on the project web page.

Mr Revie also showed tools available from FFEI for use with the calibration slide. These included tools for image analysis, slide viewing, and a DICOM converter which embeds an ICC profile.

1.2 Calibration slide evaluation. He described the schedule for round robin tests [see attached], and the evaluation procedure and timescale. Most whole-slide imaging vendors have already disclosed calibration

methods, and the proposed calibration slide is intended to provide a vendor-independent method of evaluating the calibration. Mr Revie agreed to provide results on changes in the stains over time.

2.3 Mr Revie stated that he would like other vendors to help in developing an extended calibration slide and taking that slide into production such a cooperation is expected to be done under an NDA with the aim that the resulting slide will be available for use by everyone.

**2. Framework for multispectral imaging in pathology**

Professor Masahiro Yamaguchi presented an outline of applications for multispectral imaging in pathology [see attached]. The applications described were brightfield and fluorescence microscopy. He summarised the use of color 'unmixing' to estimate the amount of dye present or to detect fluorescent markers commonly used in FISH imaging.

Professor Yamaguchi summarised requirements for DICOM and showed solutions using ICC v4 Device Link profiles and ICC v5 Material Connection Space profiles.

The work of CIE TC8-07 on multispectral file formats was outlined. Dr Hulsken suggested that there was a need to discuss in DICOM WG6, where there could be a 6-12 months timeframe for adoption. DICOM want consistency with the ICC specification, not something new.

**3. Point of Use QA in Digital Pathology Slides**

Dr David Brettle presented an analysis of point-of-use quality assurance for digital pathology slides [see attached]. He showed examples of problems with misinterpretation of poor images. A proposed solution was a reference object that can be used to assess the degradation of the image. An image integrity index can be computed.

Dr Brettle's basic idea is to use the biopolymer developed as part of the Sierra project and place that on an unstained slide. The biopolymer patch then passes through the staining process and accepts stain in a predictable way. This stained patch then serves as a point of reference for images of the slide.

This approach is expected to lead to improved diagnostic accuracy, especially for less experienced users. Dr Brettle showed prototypes of absolute and relative color references for pathology slides, and described benefits of the approach including calibration slide assessment and whole-life QA. The reference patch would go on every slide, and use the same biopolymer as the FFEI calibration slide. A stain-specific biopolymer is also being considered.

Dr Brettle also proposed including a small physical marker close to the sample being imaged.

**4. Color calibration methods in Phillips scanners**

Dr Bas Hulsken presented a description of calibration methods used in Phillips scanners [see attached]. He showed results from the calibration process, in which he had found a linear correction matrix worked best. The target used was based on the IT8.7/1 target and the procedure is colorimetric rather than spectral.

Dr Hulsken also described the use of plasmonic beads, which could be used to create an interesting calibration phantom. Beads with different spectral characteristics can then be combined to create a large variety of possible spectra. The phantom is at development stage, and Phillips are experimenting with the manufacturing process.

Phillips are also experimenting with these plasmonics to generate well-defined spectra, which could have advantages over real dyes for stability. A focused ion beam is used, and color arises from the crystal lattice structure coupling phonons. The process could be made to mimic dye spectra, and it retains homogenous color at high magnification. Dr Hulsken stated that this was Phillips' contribution to scanner calibration development, and emphasised that his company want to work with other vendors to develop a solution for the community.

Mr Revie closed the meeting at 16:50pm.

A partial recording of the meeting is available at http://www.npes.org/Portals/0/standards/2014-06-19%2018.11%20New%20Meeting.wmv

**Action items from the meeting:**

| | |
|---|---|
| **MIWG-14-33** | Contact Craig Revie to schedule participation in assessment slide evaluation round-robin (interested vendors) |
| **MIWG-14-34** | Provide results on permanence of calibration slides to group (Craig Revie) |
| **MIWG-14-35** | Present summary of work on the calibration slide for FDA meeting on 20 June (Craig Revie) |

# Project Sierra

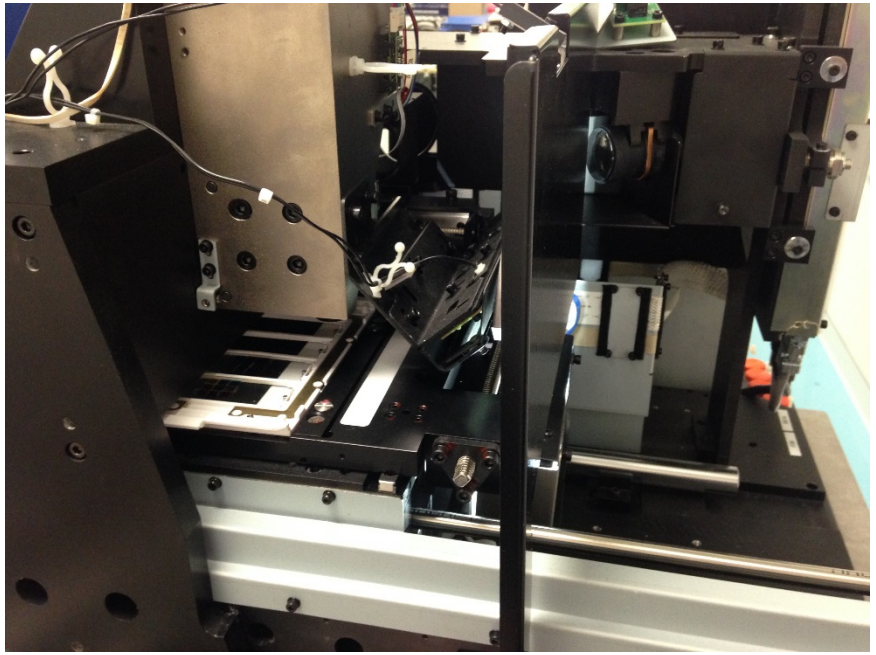
W Craig Revie

FFEI Limited

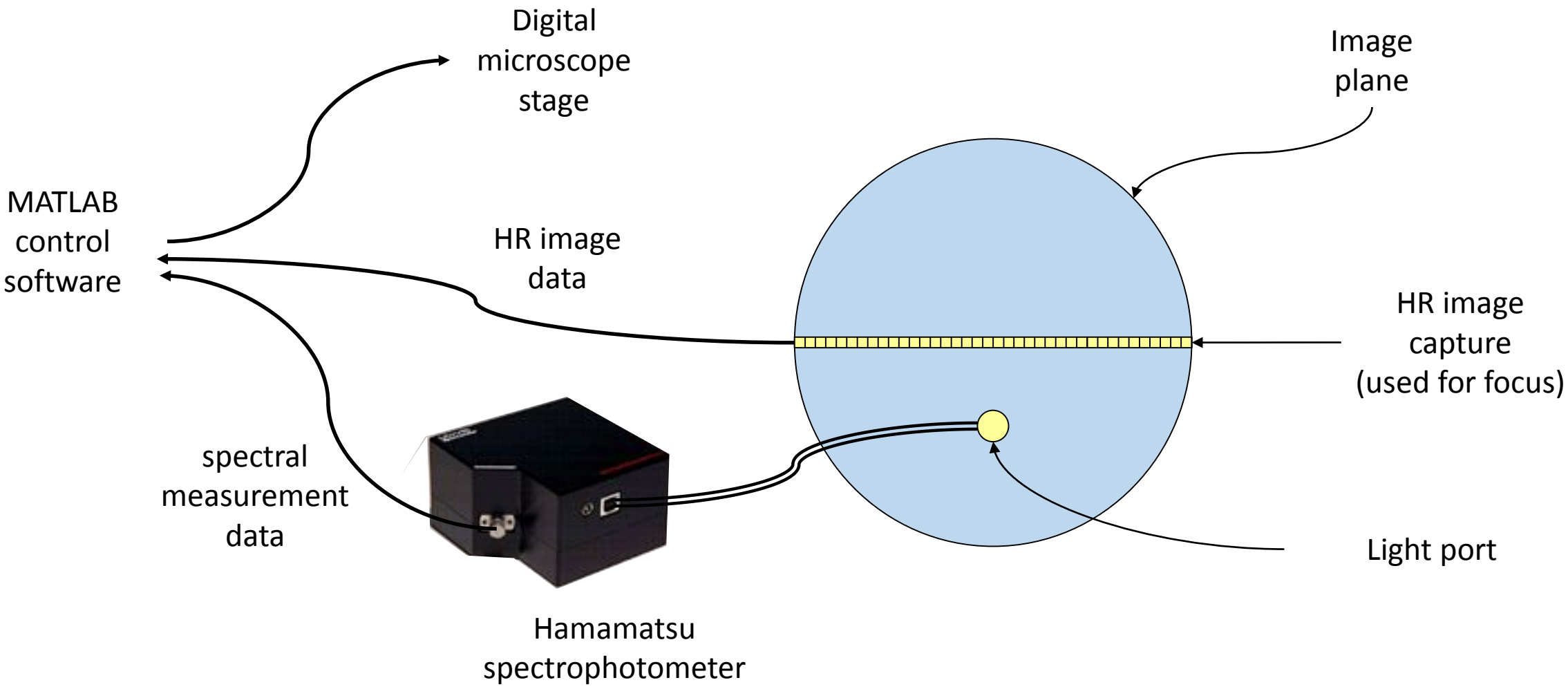
# Project Sierra overview

- 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

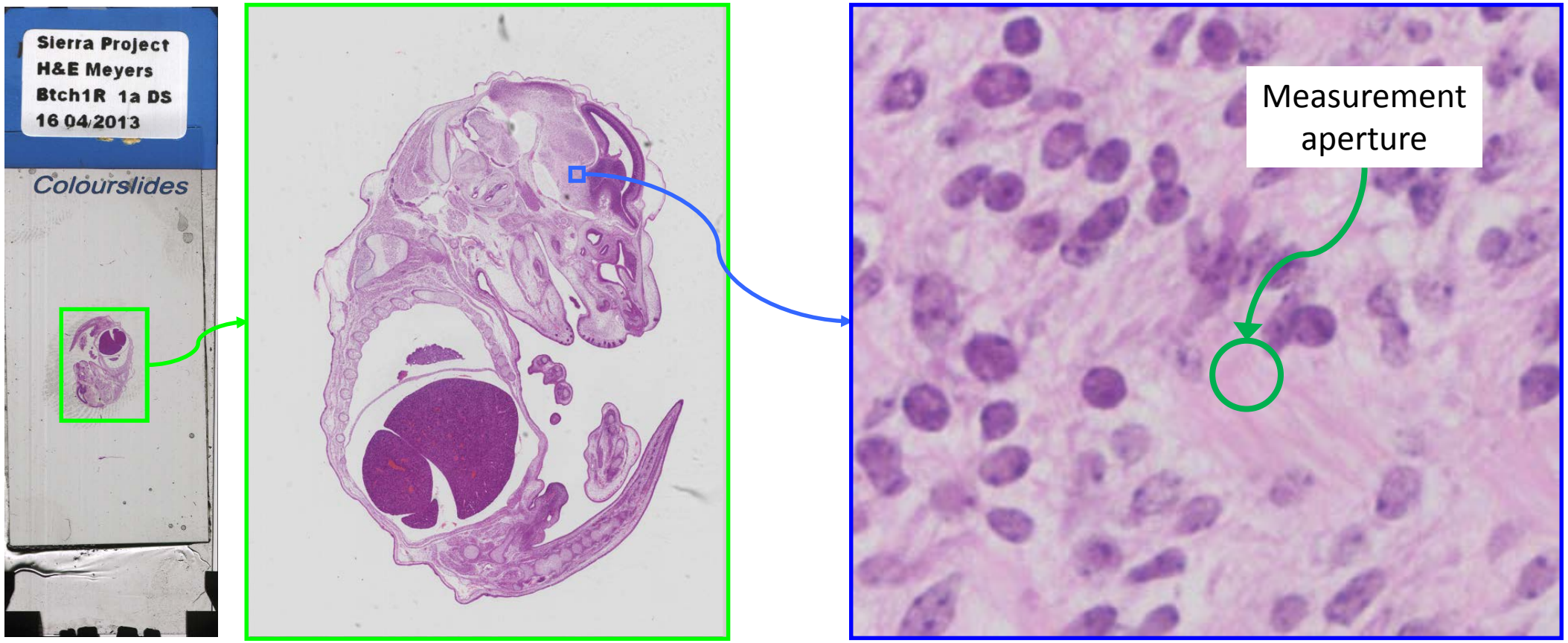
# Measurement system based on digital microscope



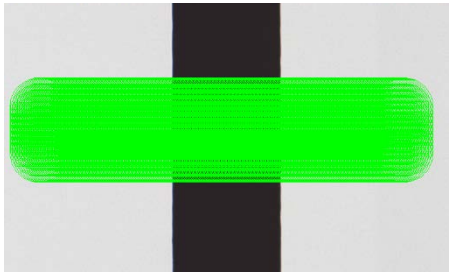
# Digital microscope measurement system schematic

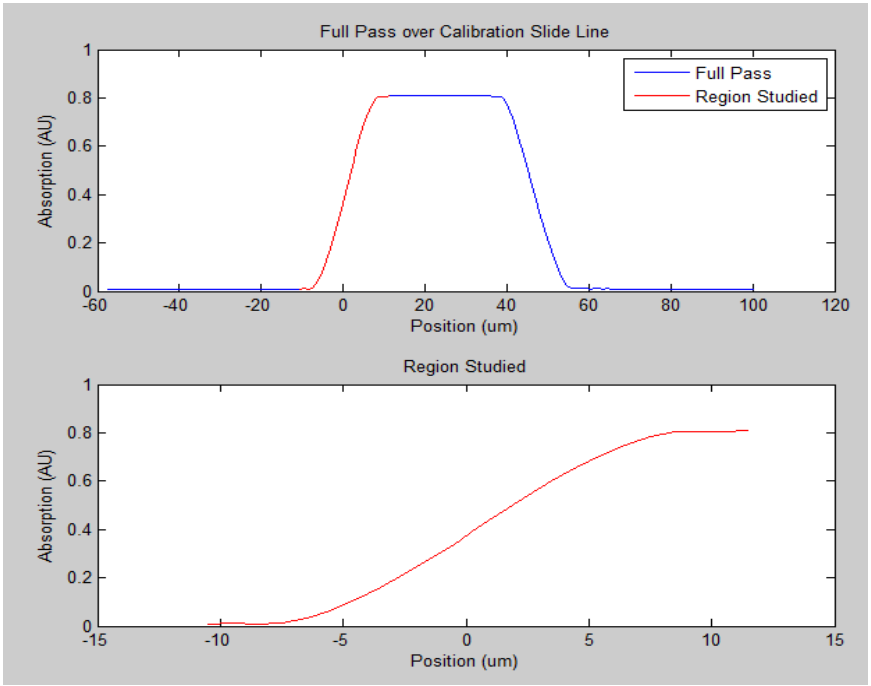


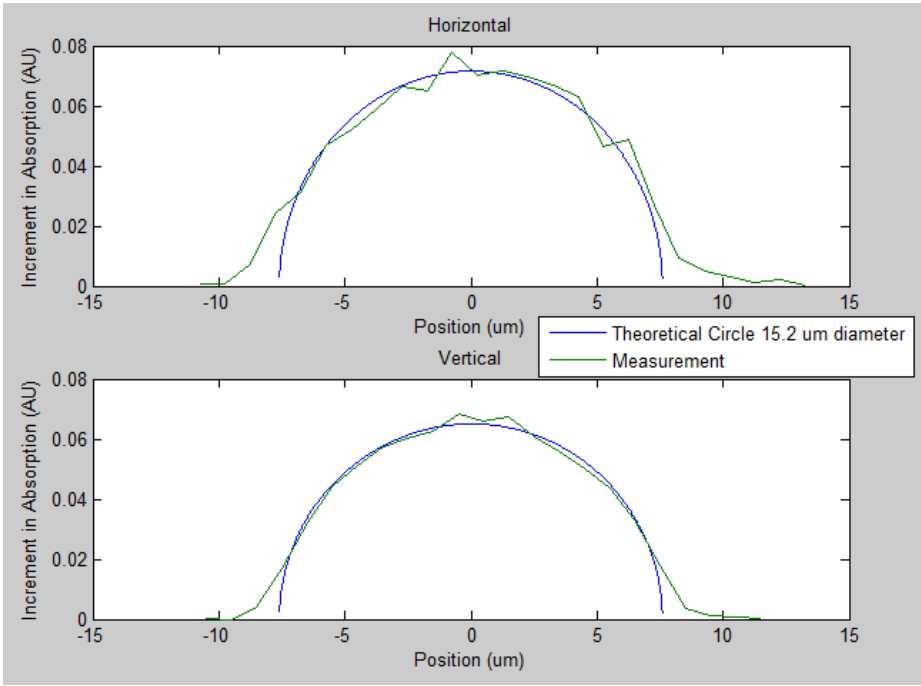
# Viewer showing measurement position and aperture size for a typical slide measurement



# Estimation of measurement aperture size

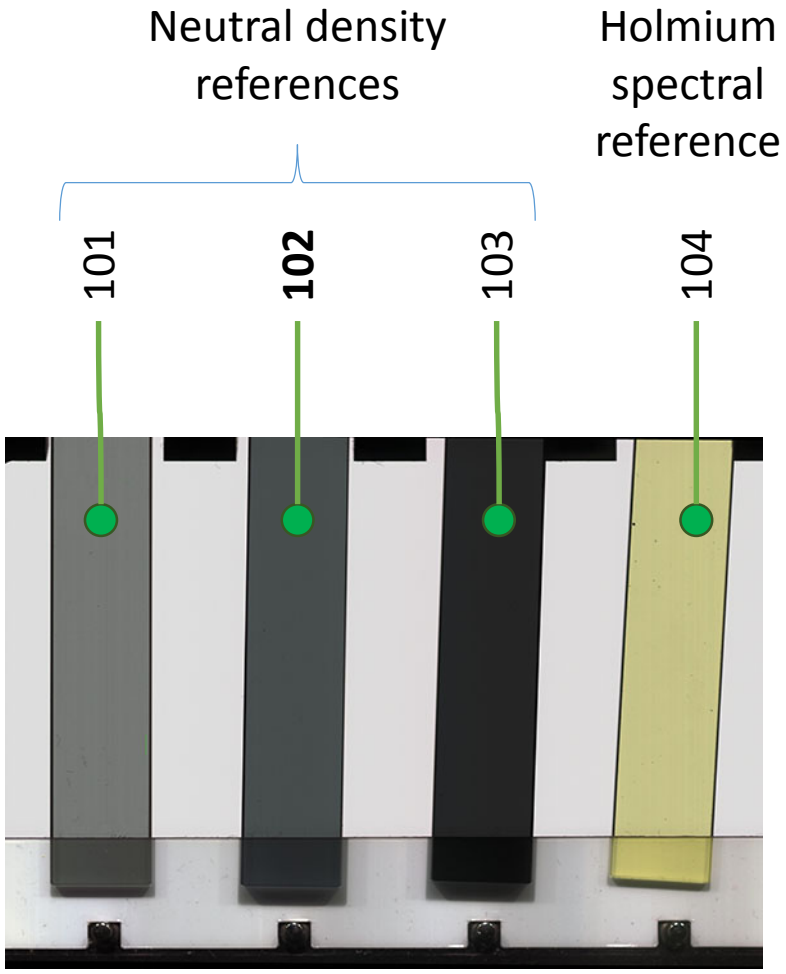


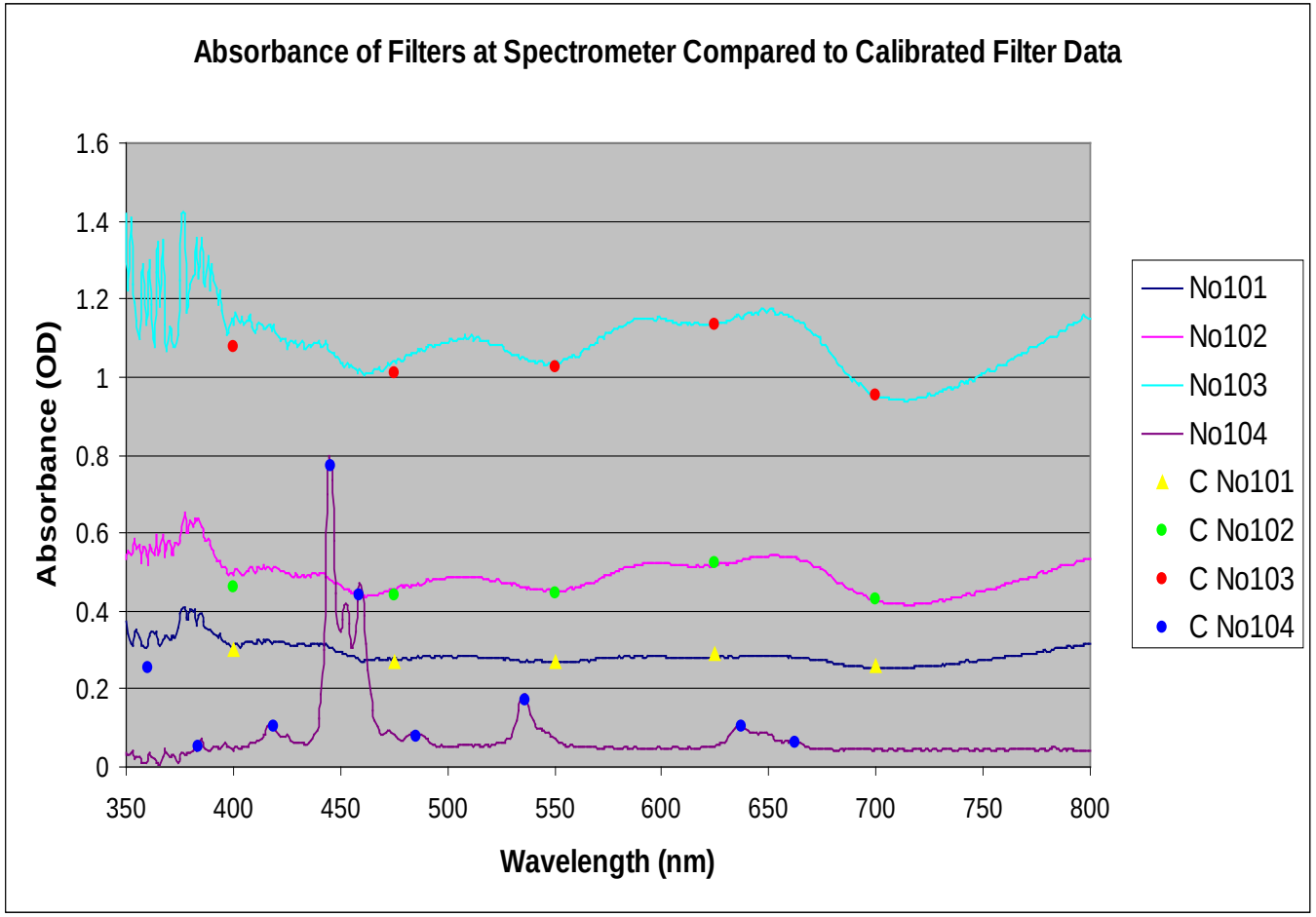


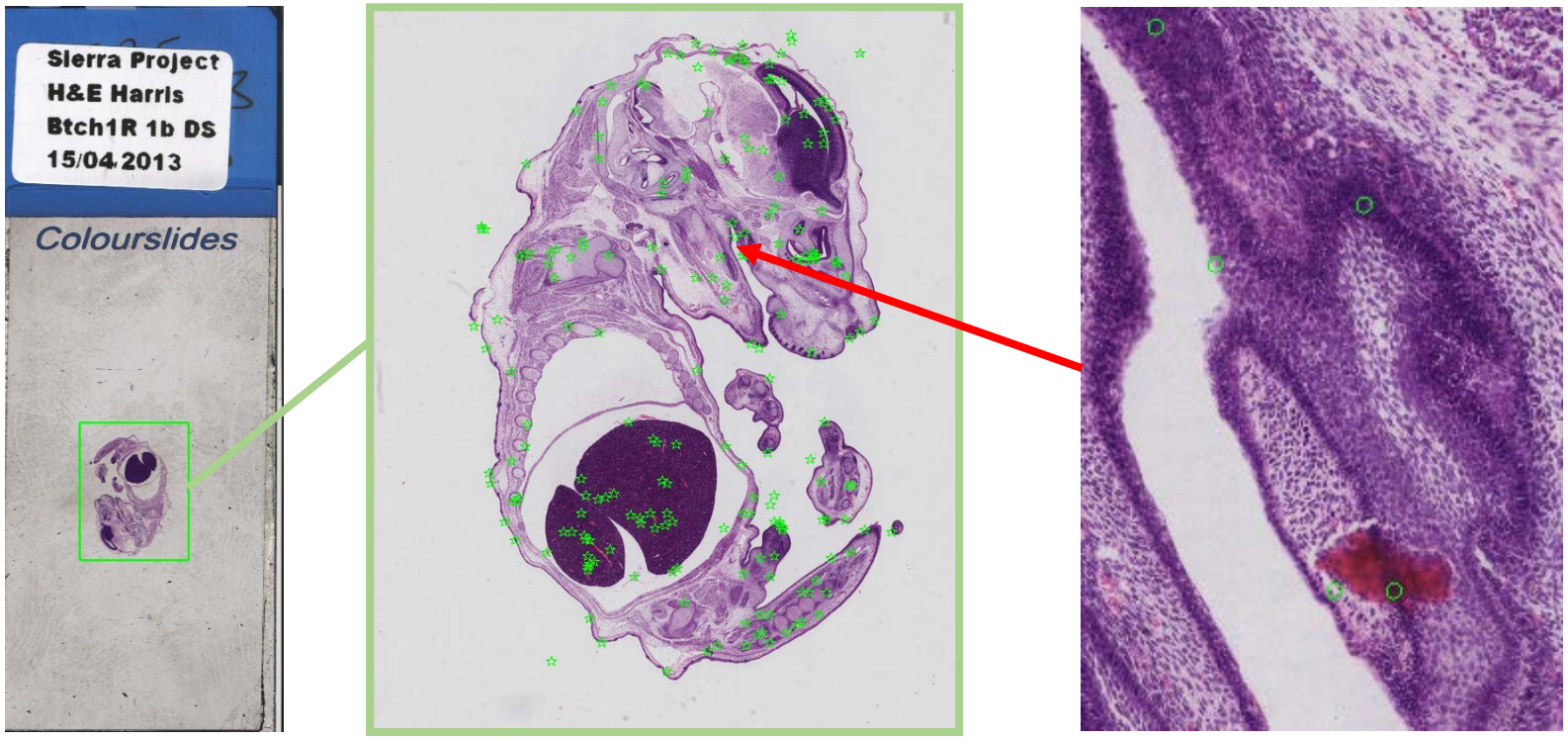

Measurement region is around 15 µm

# Certified reference materials

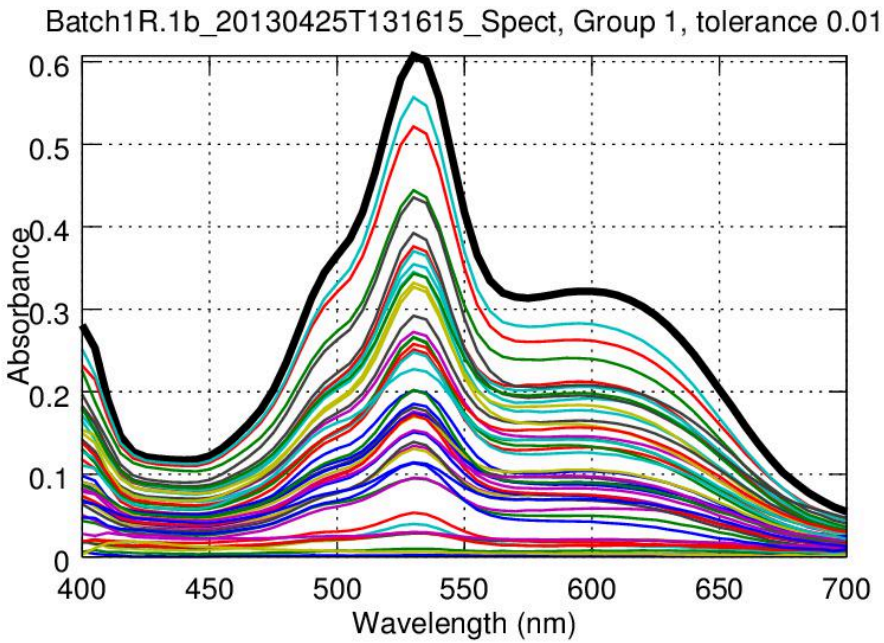


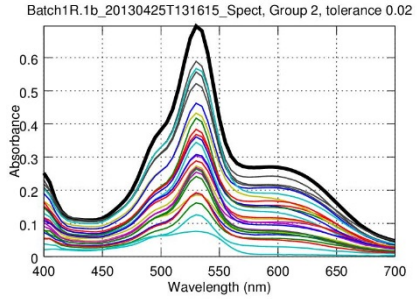



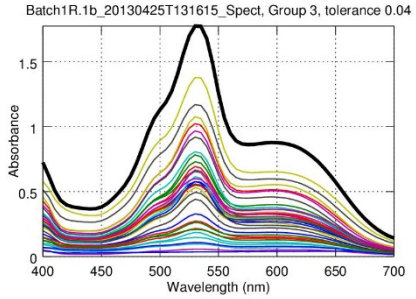
# Example of slide measurement (Harris H&E stain)

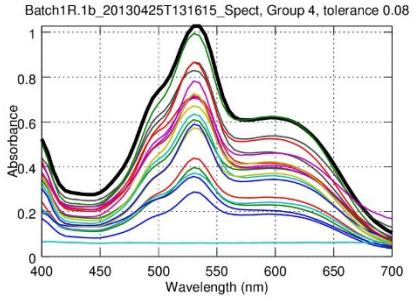


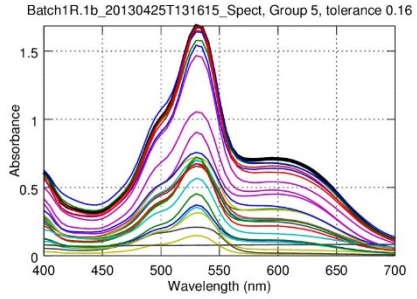
# Grouping of colours found in Harris H&E stained sample

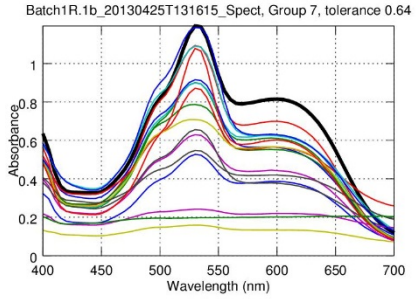


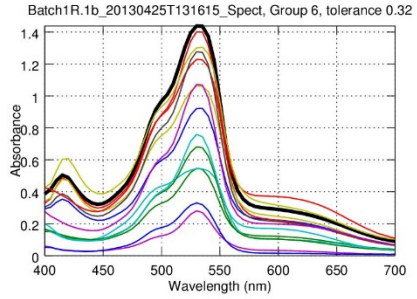



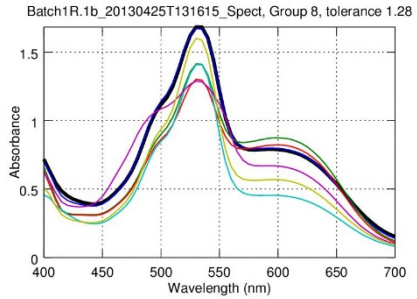



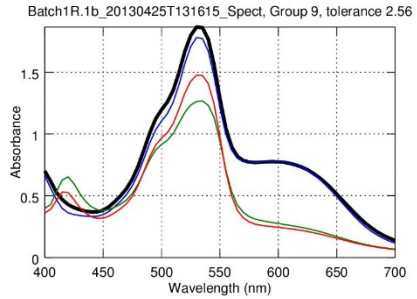



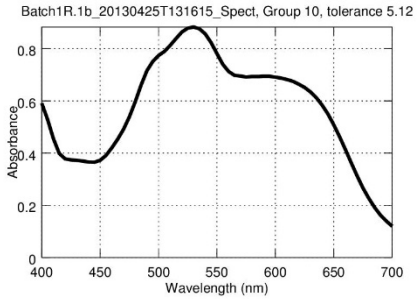



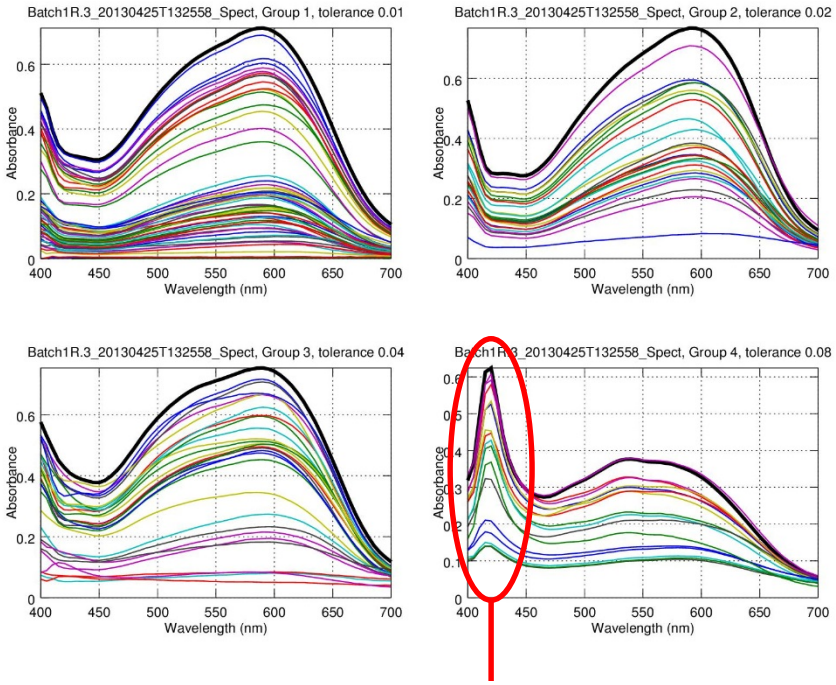




Each coloured line in these graphs represents a spectral measurement of a colour present on the slide. The figure above shows the colour group with most colours – the other nine show the remaining groups.

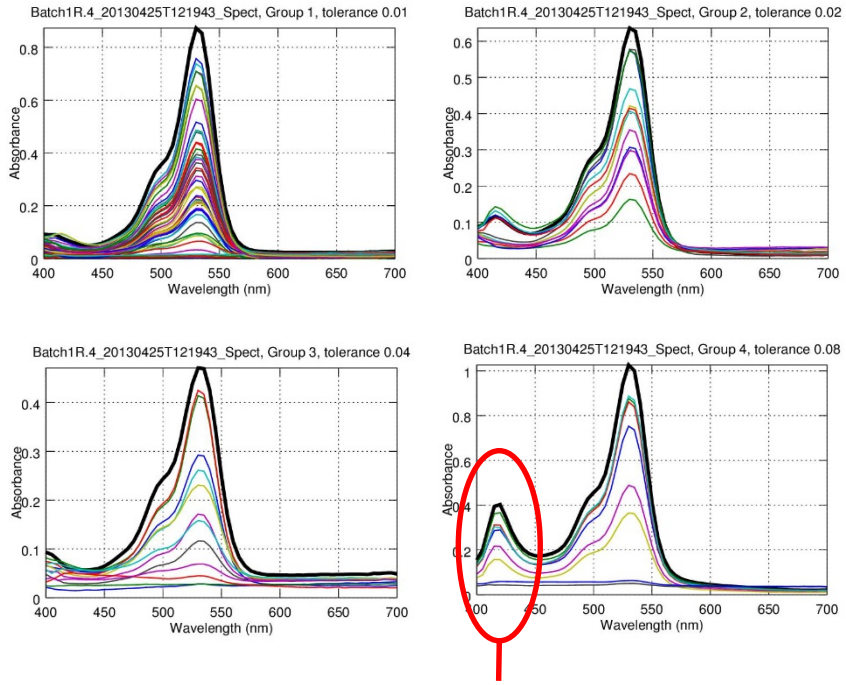
# Grouping of colours found in single stain slides

Colour grouping for sample stained with Haematoxylin only



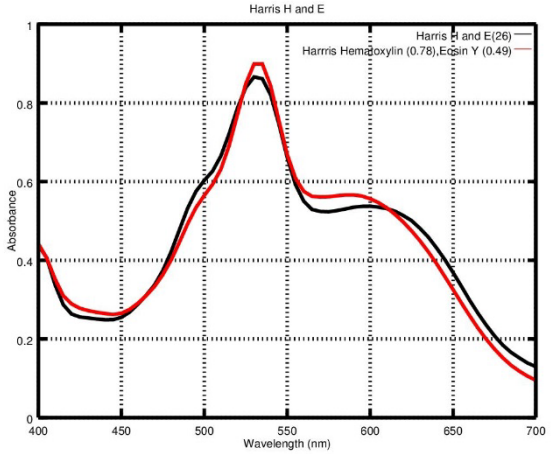

Haemoglobin

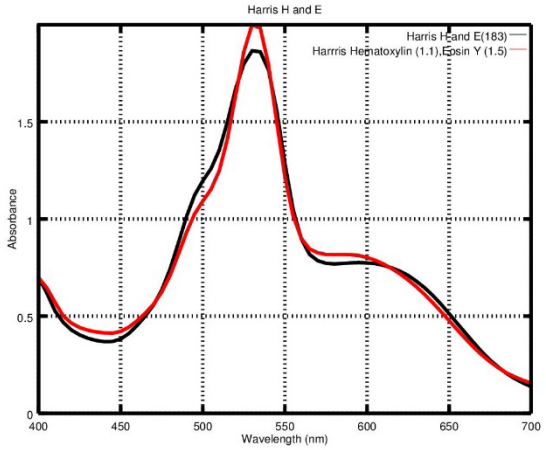
Colour grouping for sample stained with Eosin only

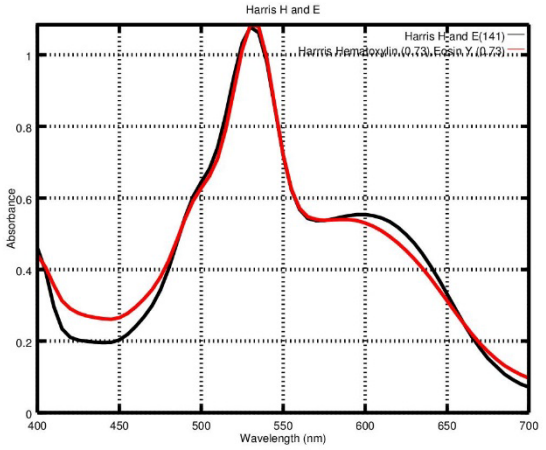


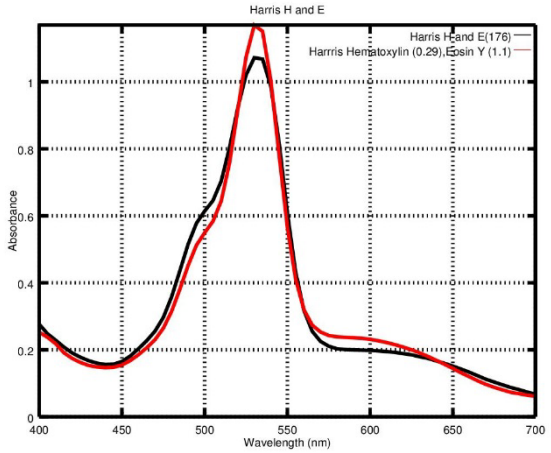

Haemoglobin

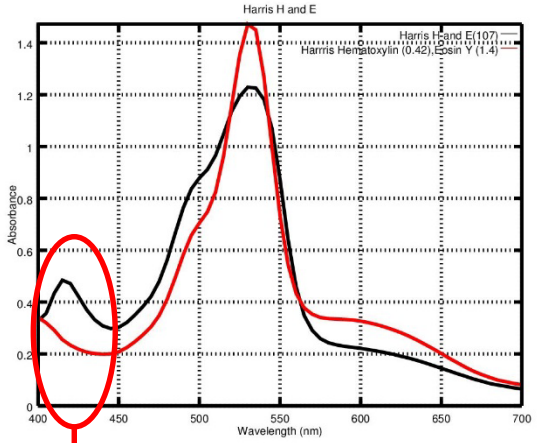
# Matching Harris H&E colours using single stains



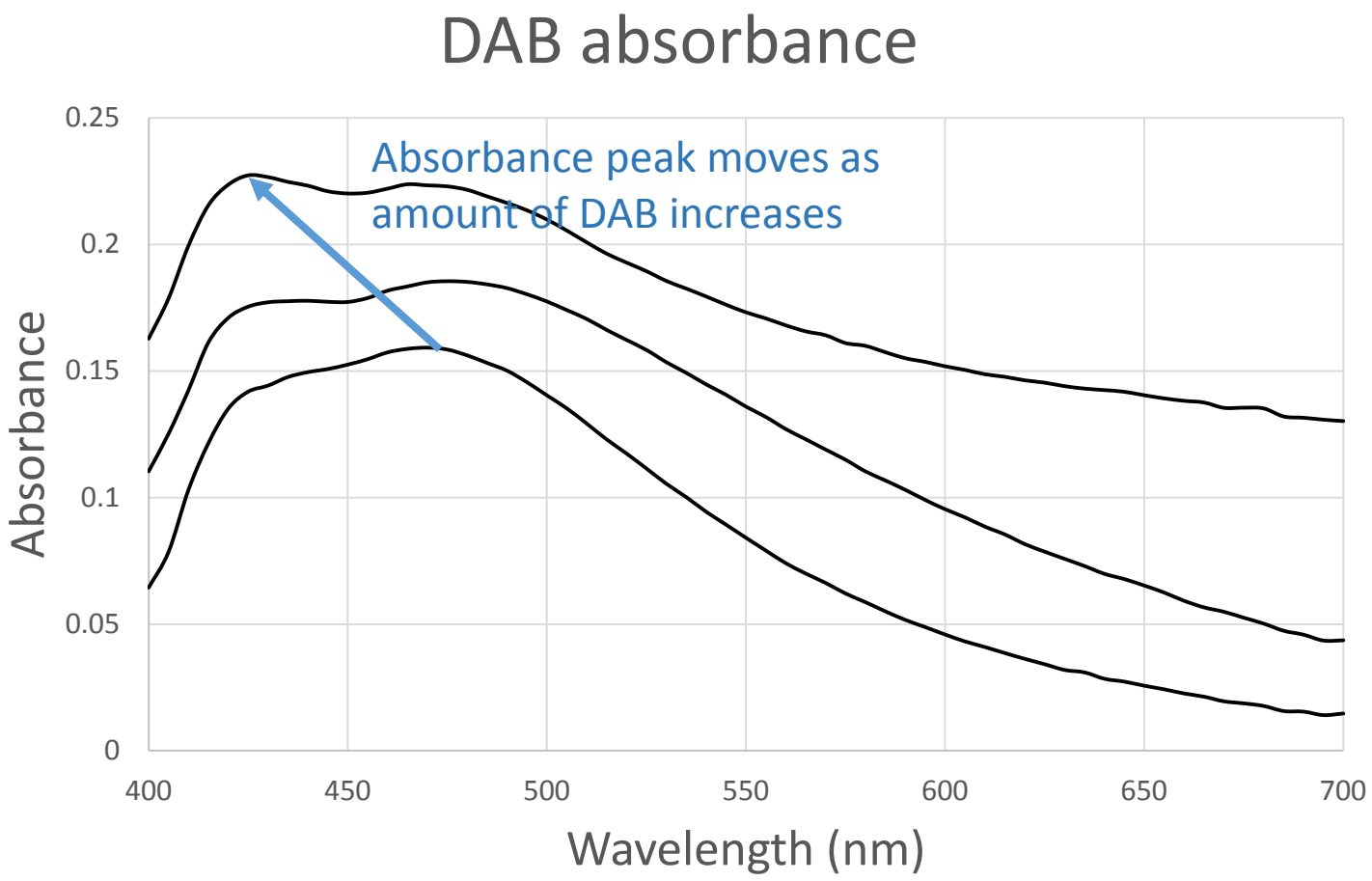




Error in this case is due to presence of Haemoglobin in the sample

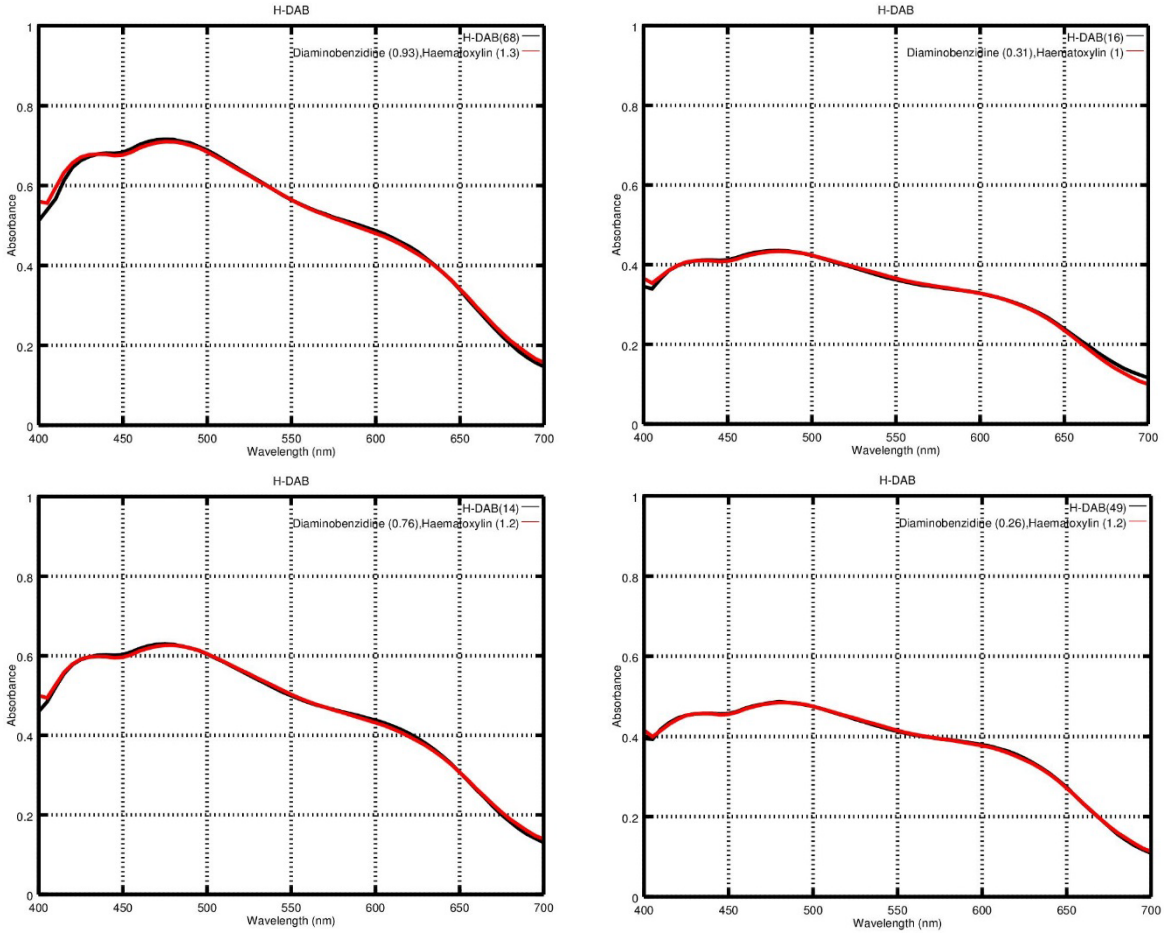
Demo

# Diaminobenzidine (DAB) absorbance



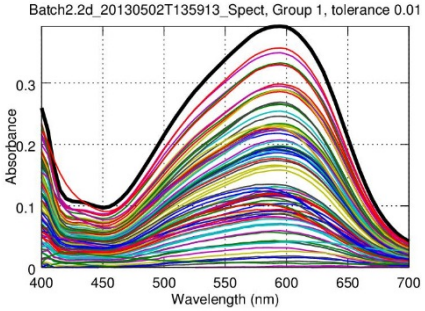
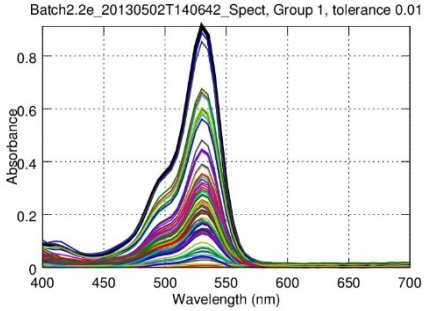
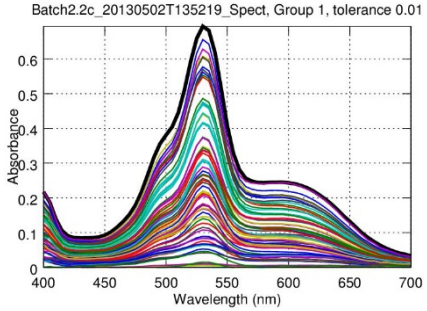
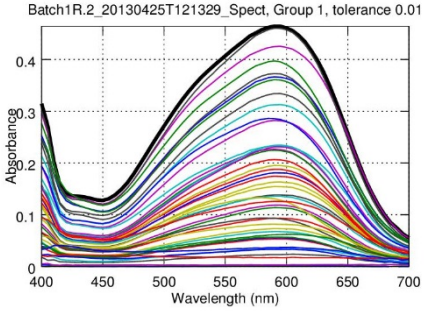
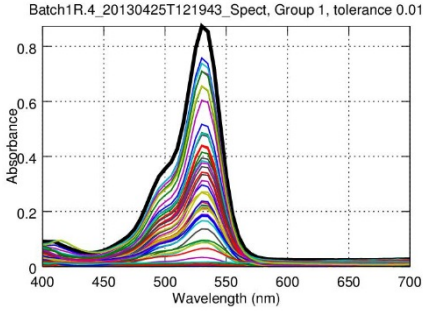
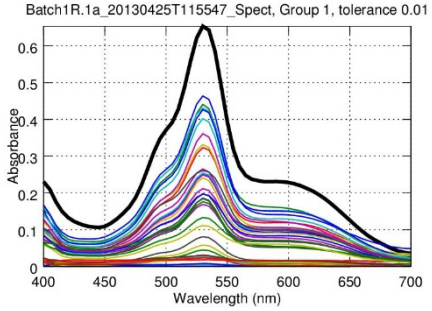
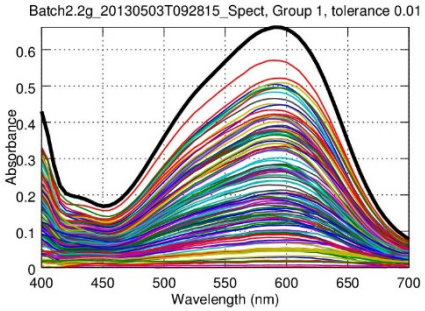
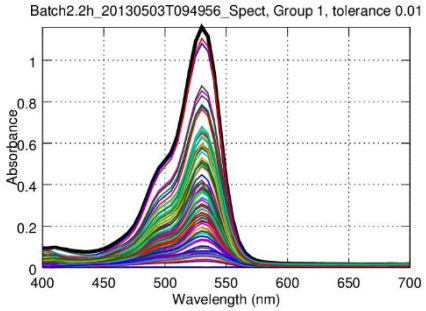
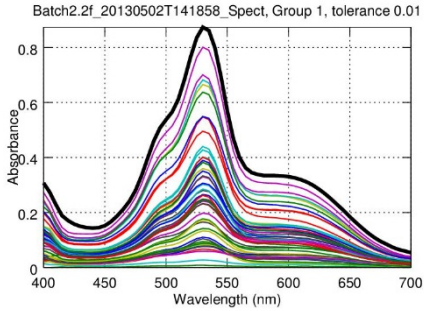

DAB staining does not follow Beer-Lambert

# H-DAB analysis



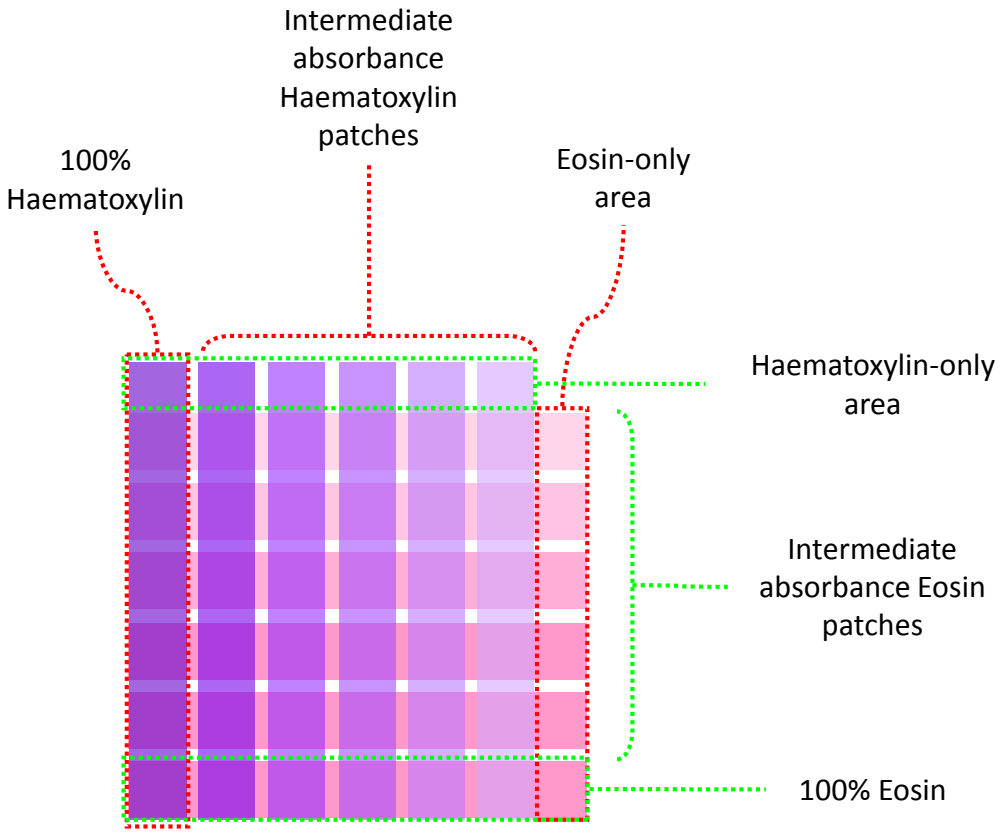

DAB with Haematoxylin counterstain can be simulated by simple linear addition

# Effect of stain thickness

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

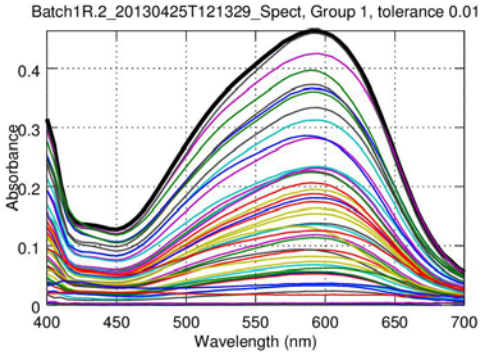
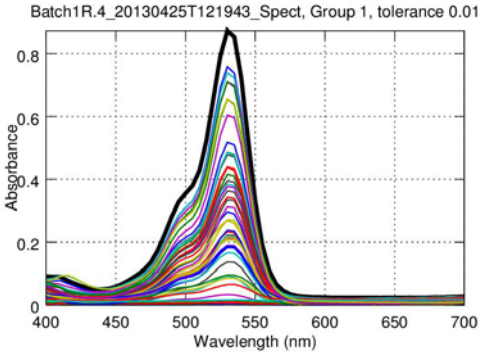
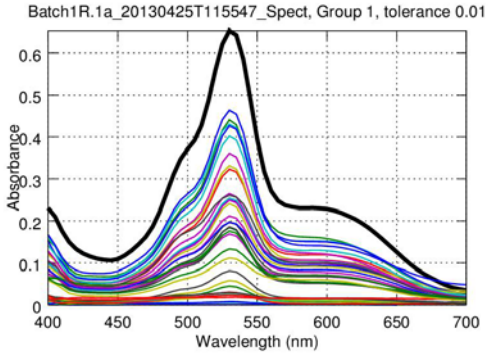
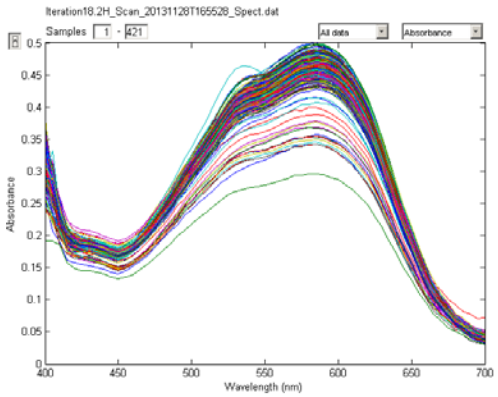
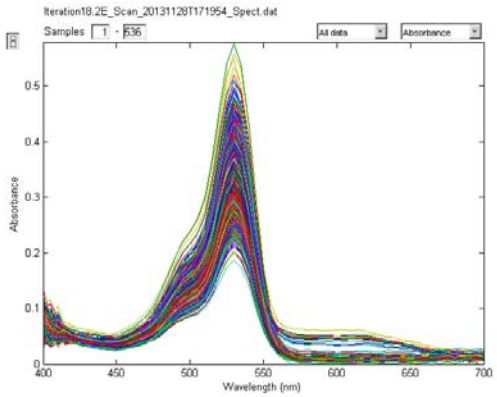
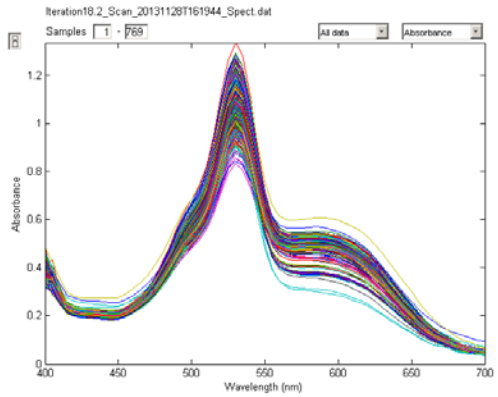
# H&E stains combinations

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

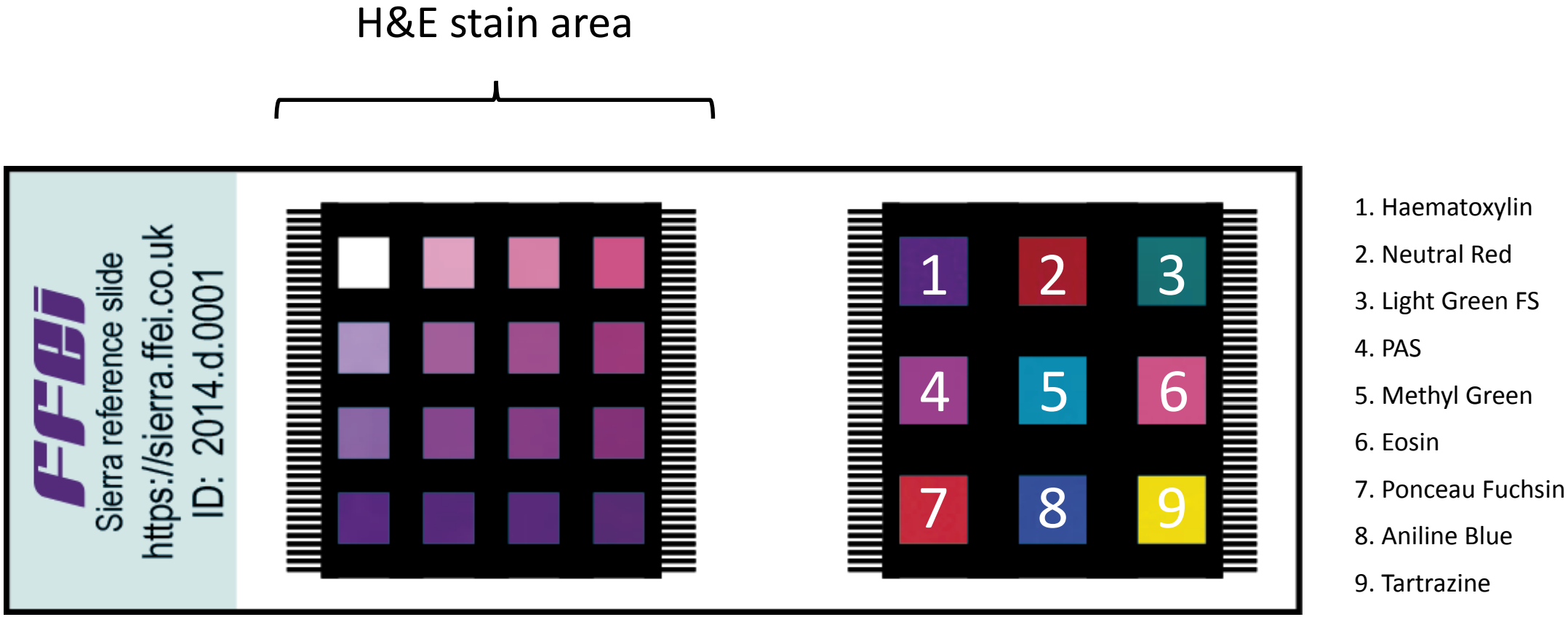


# Comparison between slide colours and stained tissue samples

Creative Imaging Technology

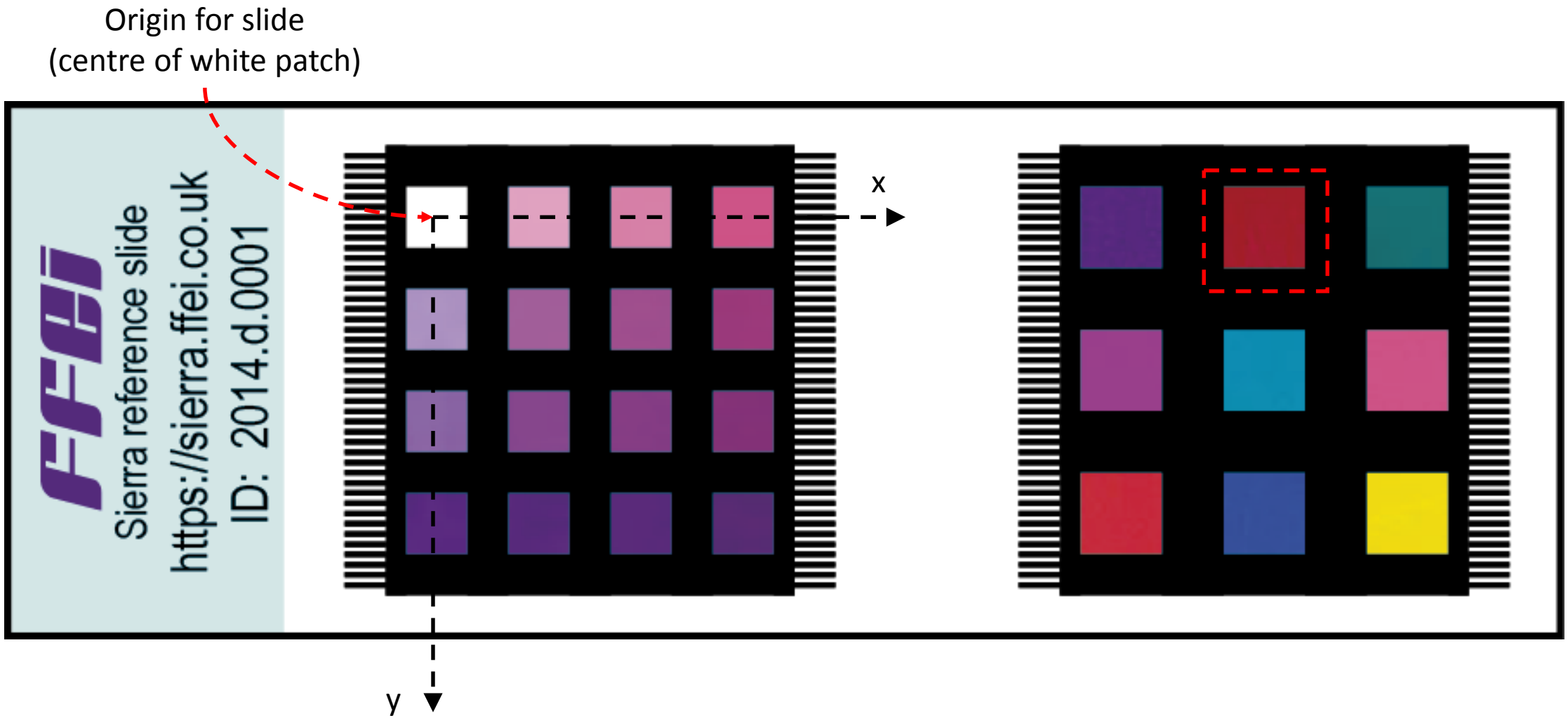
| | Haematoxylin | Eosin | H&E stained |
|---|---|---|---|
| Stained tissue measurements |  |  |  |
| Stained biopolymer measurements |  |  |  |

# Sierra calibration assessment slide from FFEI

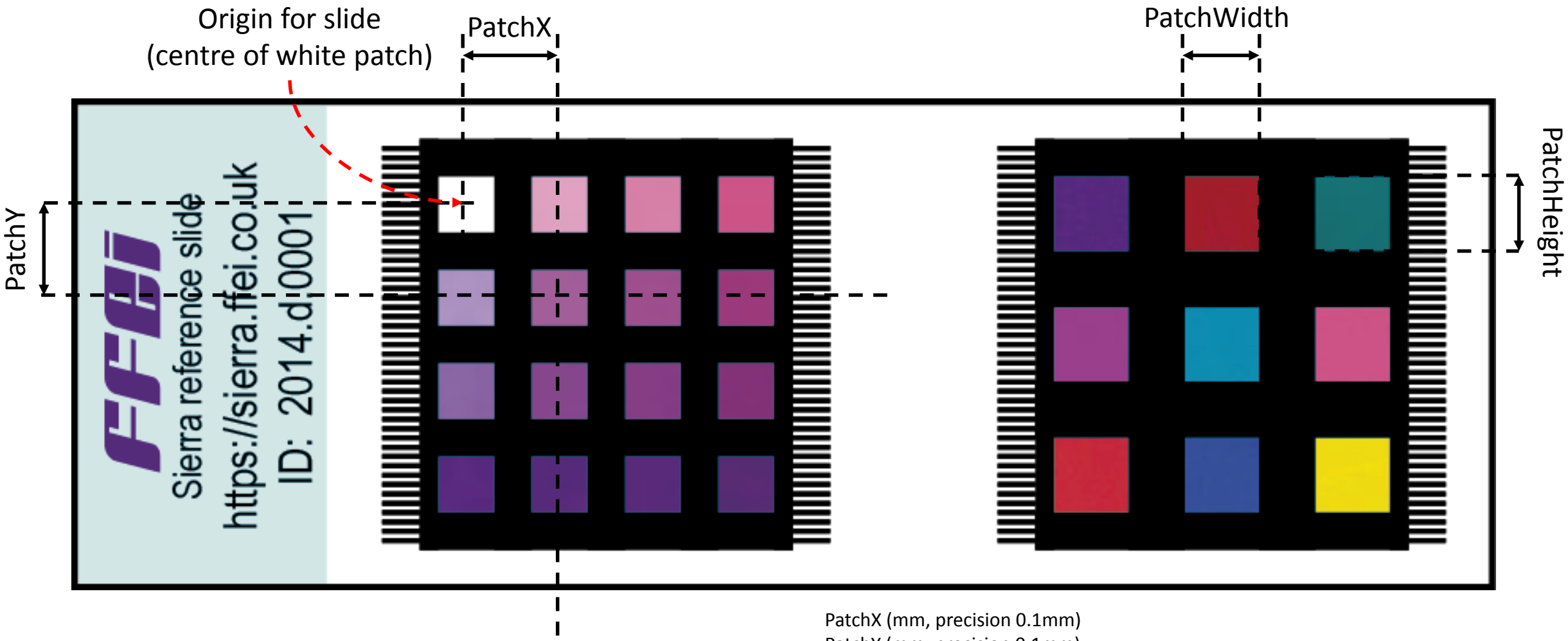



1. Haematoxylin
2. Neutral Red
3. Light Green FS
4. PAS
5. Methyl Green
6. Eosin
7. Ponceau Fuchsin
8. Aniline Blue
9. Tartrazine

# FFEI Measurements: slide coordinate system

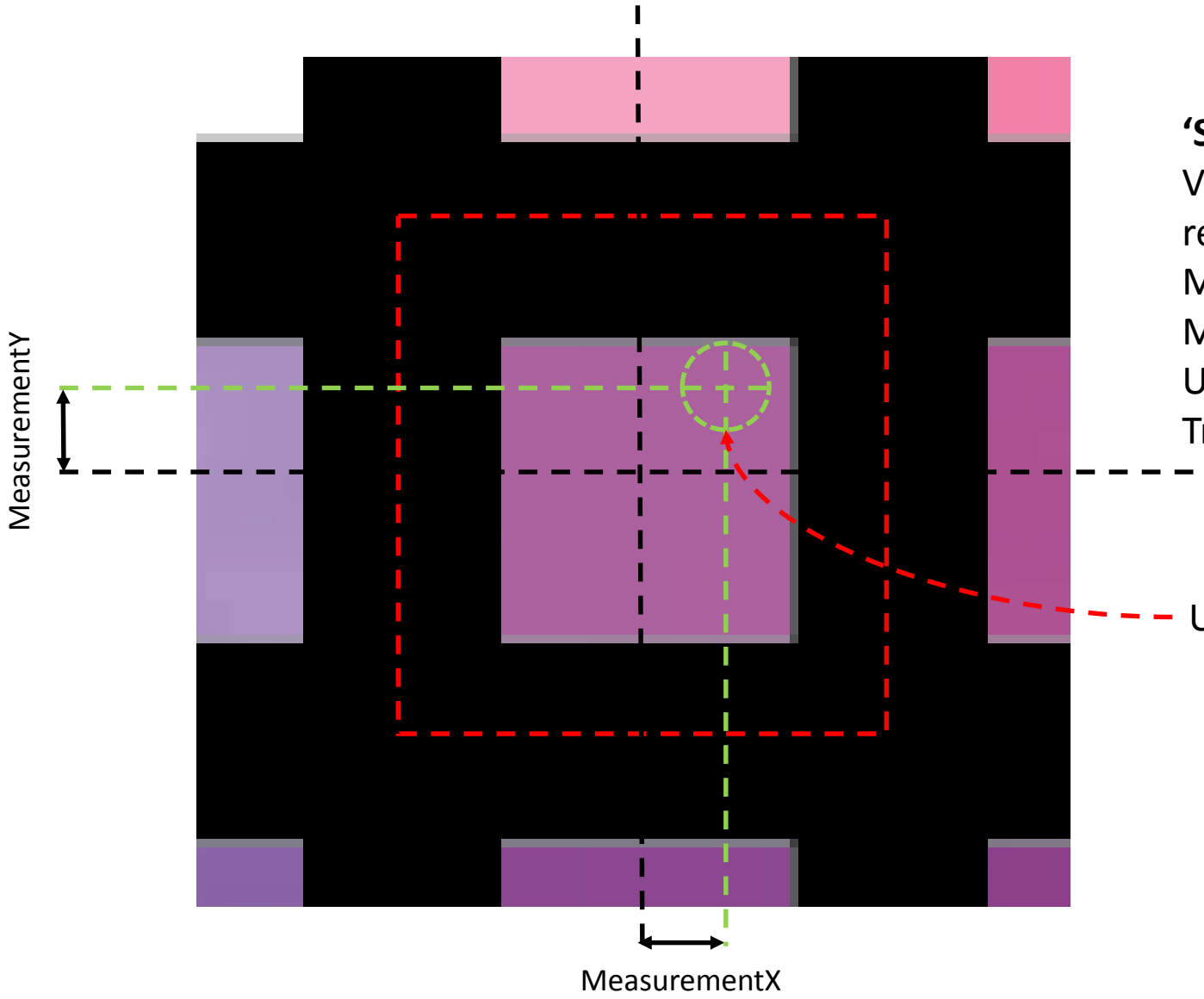


# FFEI Measurements: patch position




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**

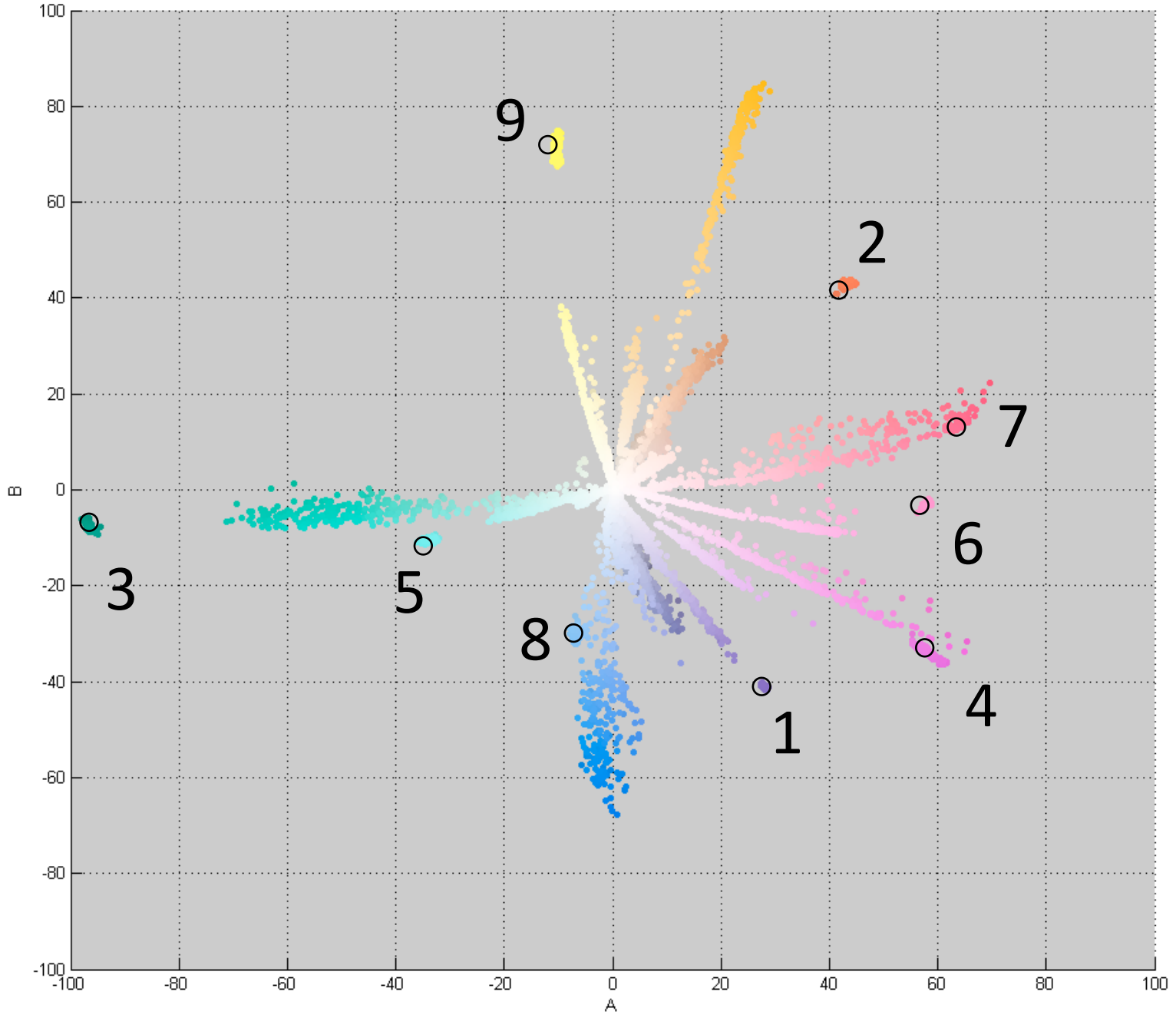
Values are relative to patch centre which should be recalculated from image

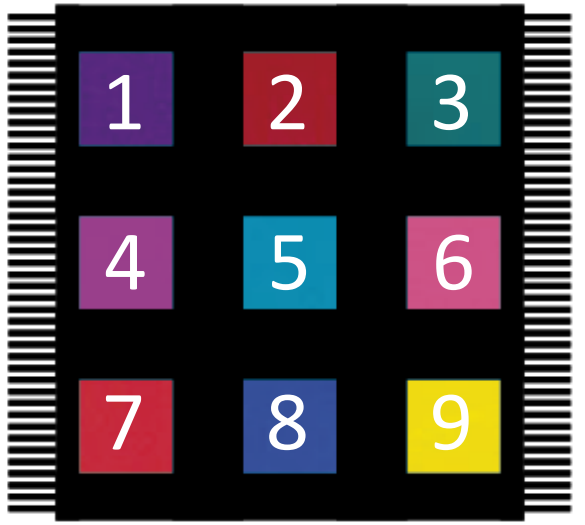
MeasurementX (mm, precision 0.0001 mm)

MeasurementY (mm, precision 0.0001 mm)

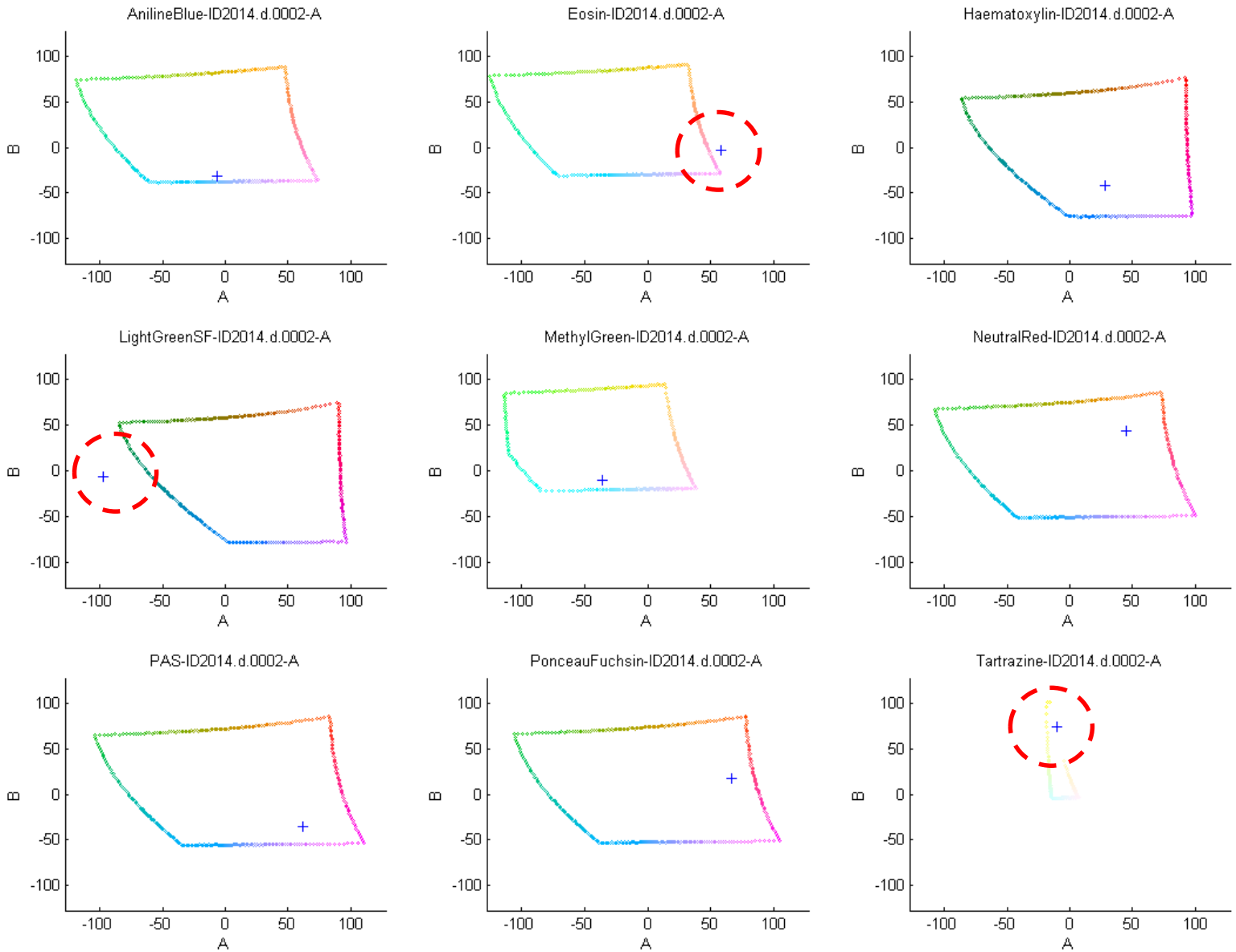
UniformRadius (mm, precision 0.0001 mm)

TransmittanceSpectrum (samples at 380:10:800 nm)



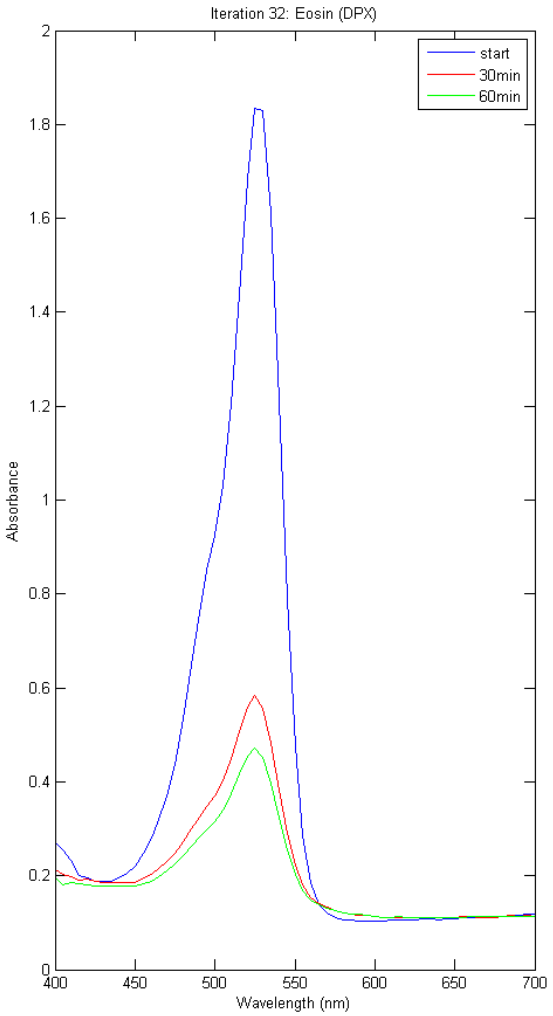

Plot shows the colours found on a selection of stained tissue slides in relation to the colours of the nine patches of the reference slide

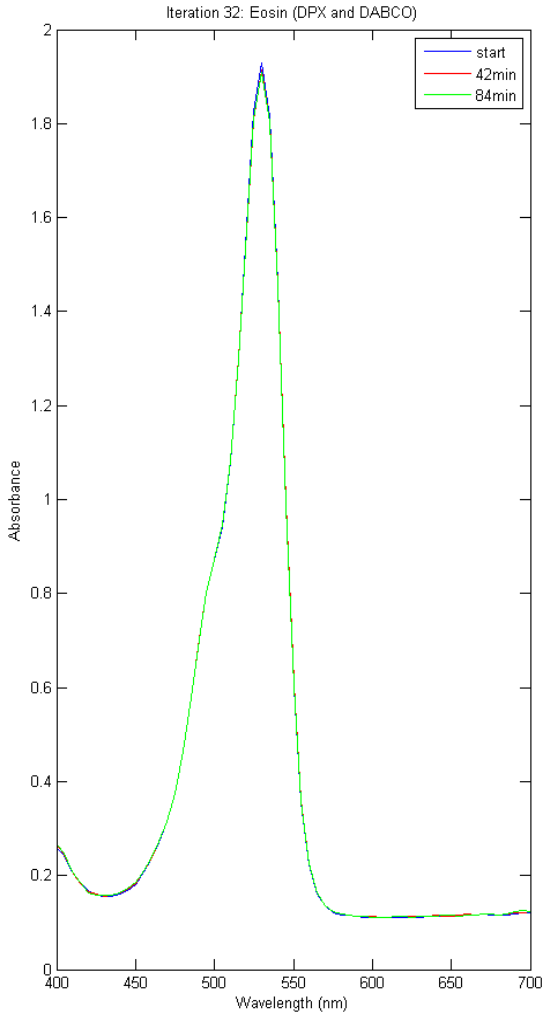



# Comparison with typical display colour gamut AdobeRGB+

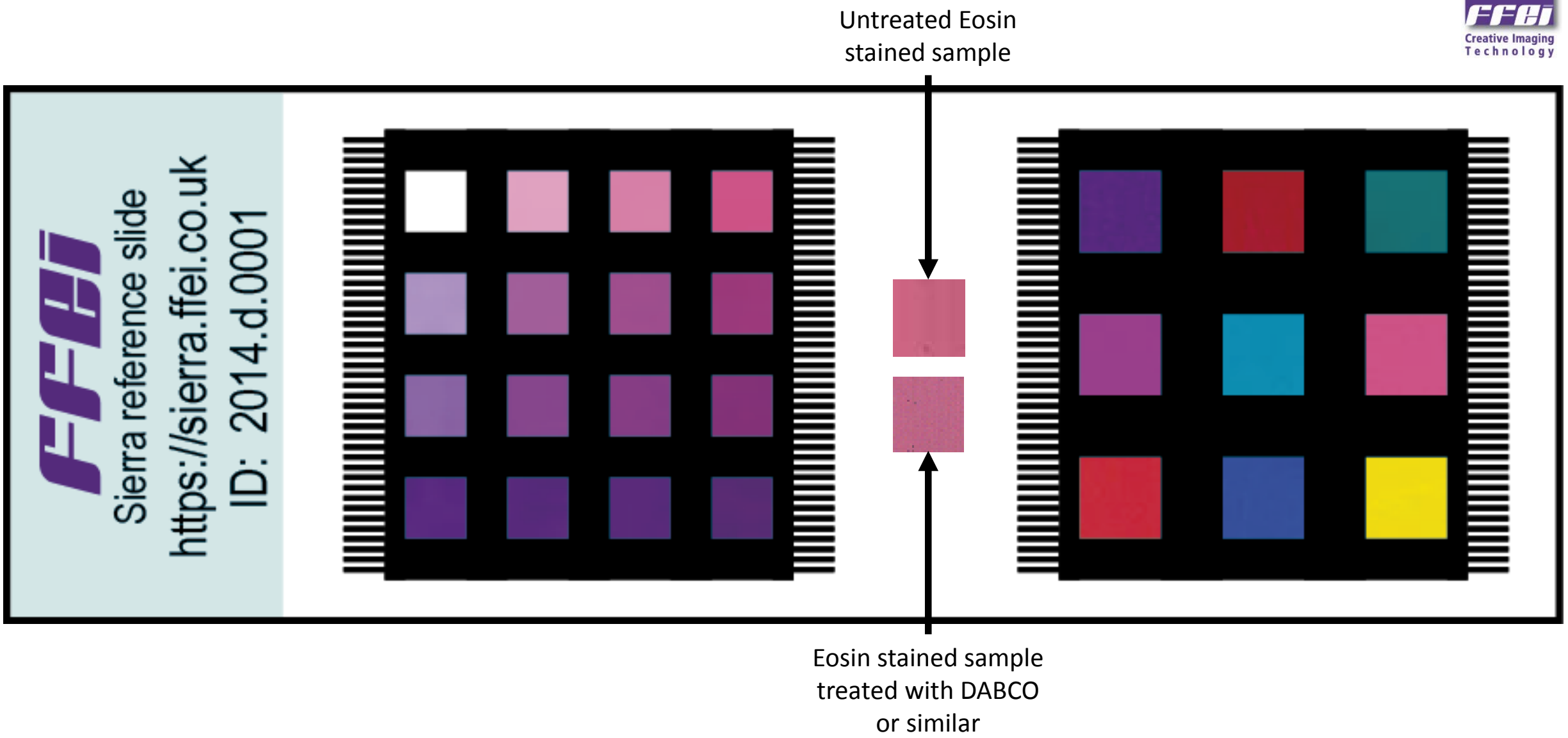
# Eosin stabilisation

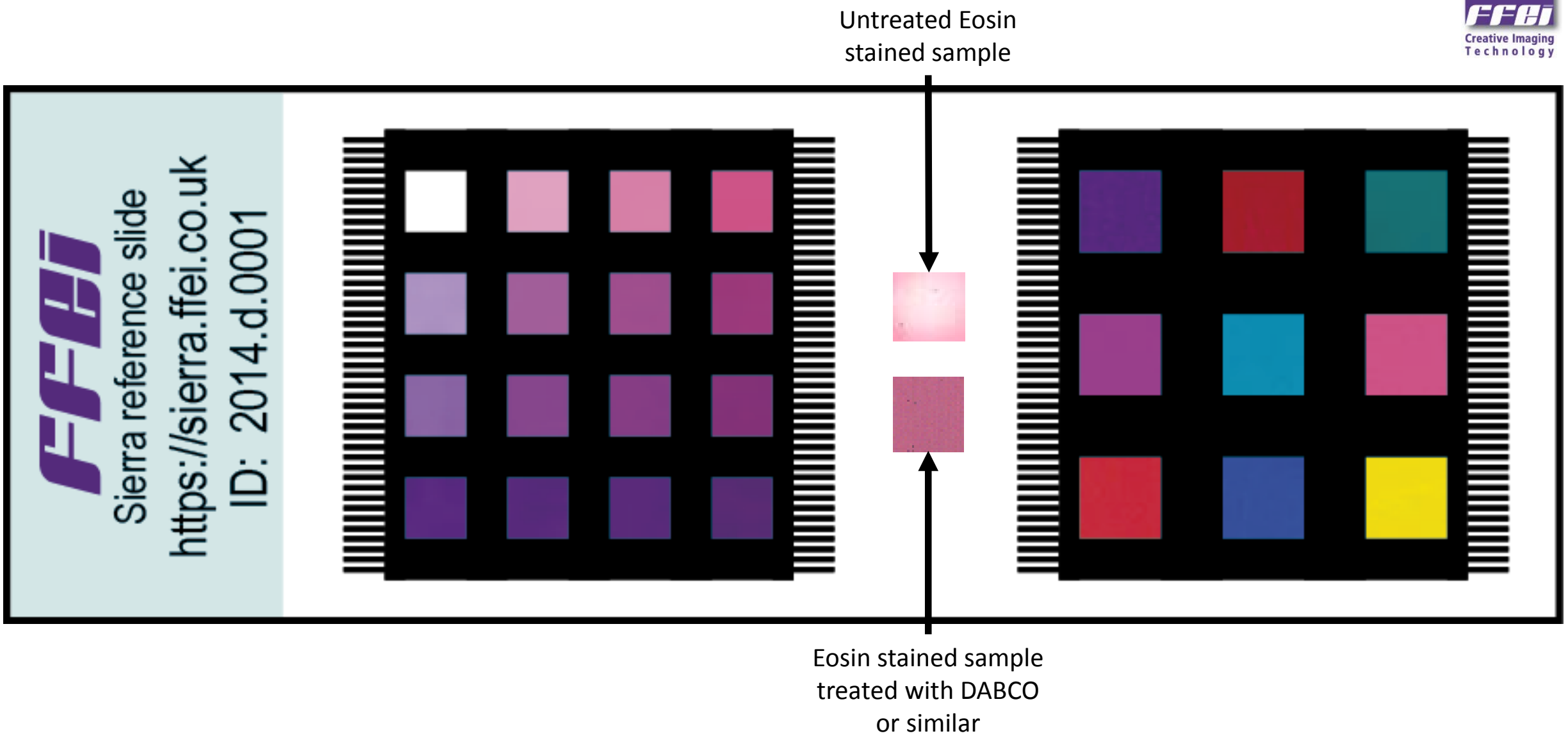
**Untreated Eosin**
absorbance has dropped to 1/3 of its initial value after 30 minutes exposure to high intensity light source



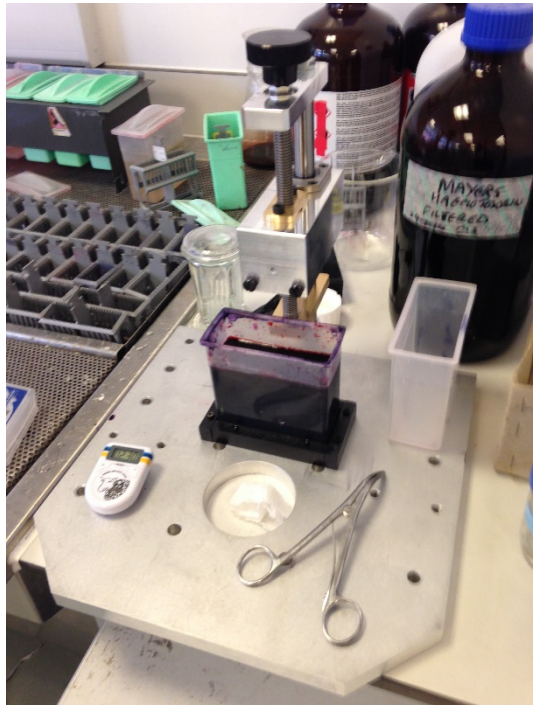

**Eosin treated with DABCO**
no change in absorbance after continuous exposure to the same high intensity light source for 84 minutes


Untreated Eosin
stained sample
FFEI
Sierra reference slide
https://sierra.ffei.co.uk
ID: 2014.d.0001
Eosin stained sample
treated with DABCO
or similar


Untreated Eosin
stained sample
FFEI
Sierra reference slide
https://sierra.ffei.co.uk
ID: 2014.d.0001
Eosin stained sample
treated with DABCO
or similar

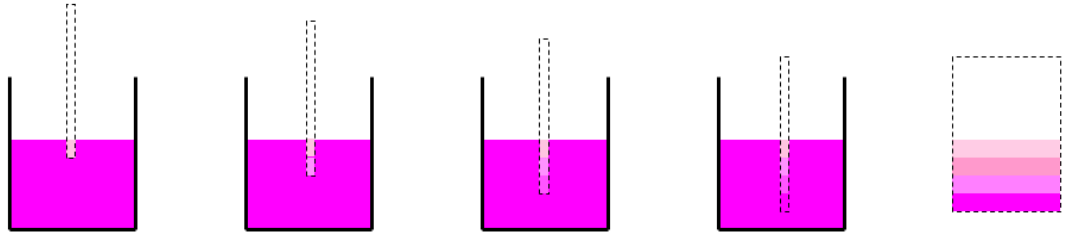
# Planned improvements

- 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

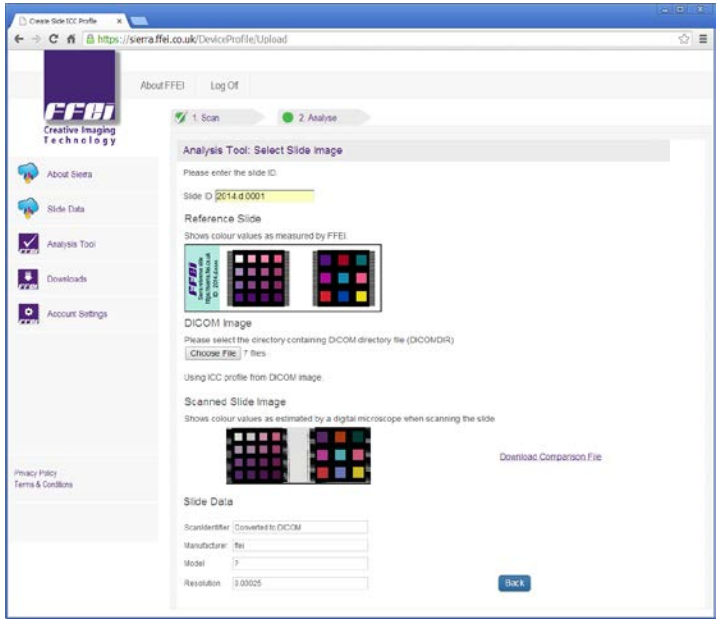
staining jig can be used to stain bands of multiple stain intensities on single sheet



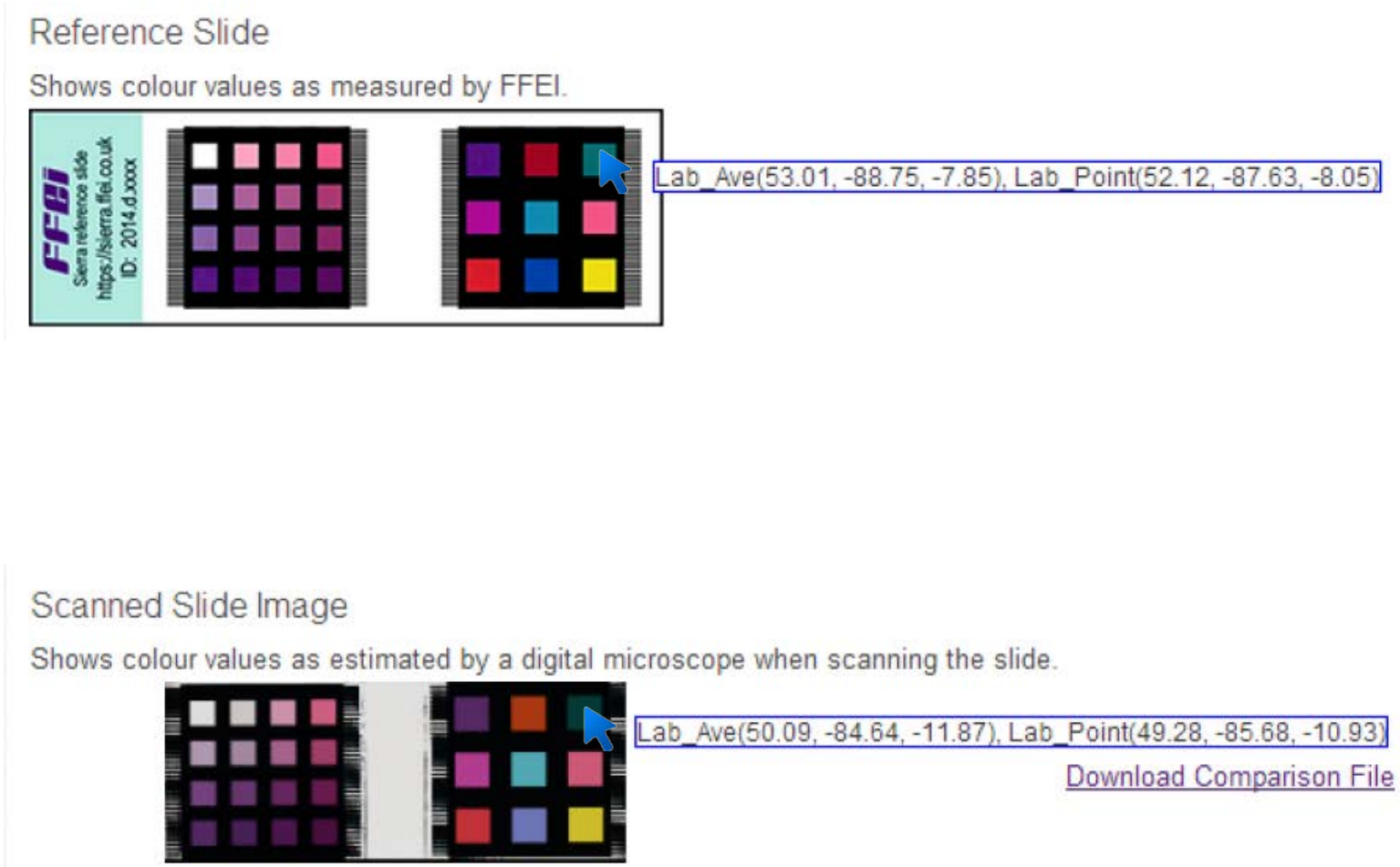
# FFEI tools

- 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

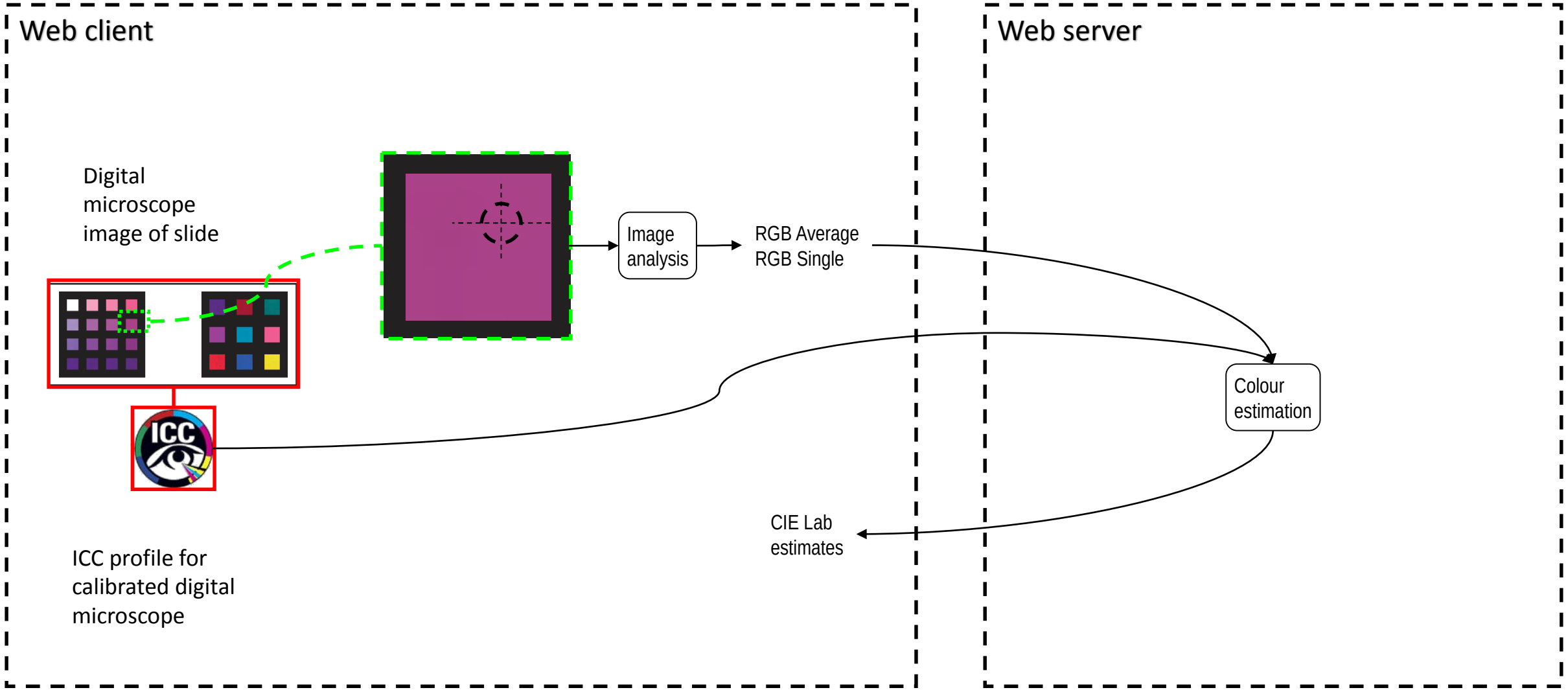
# Analysis Tool



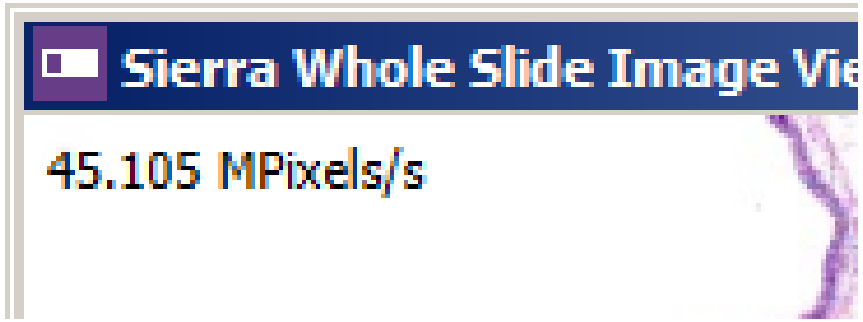

HTML5-based tool – works best in Chrome currently
Requires a DICOM image of slide on local PC



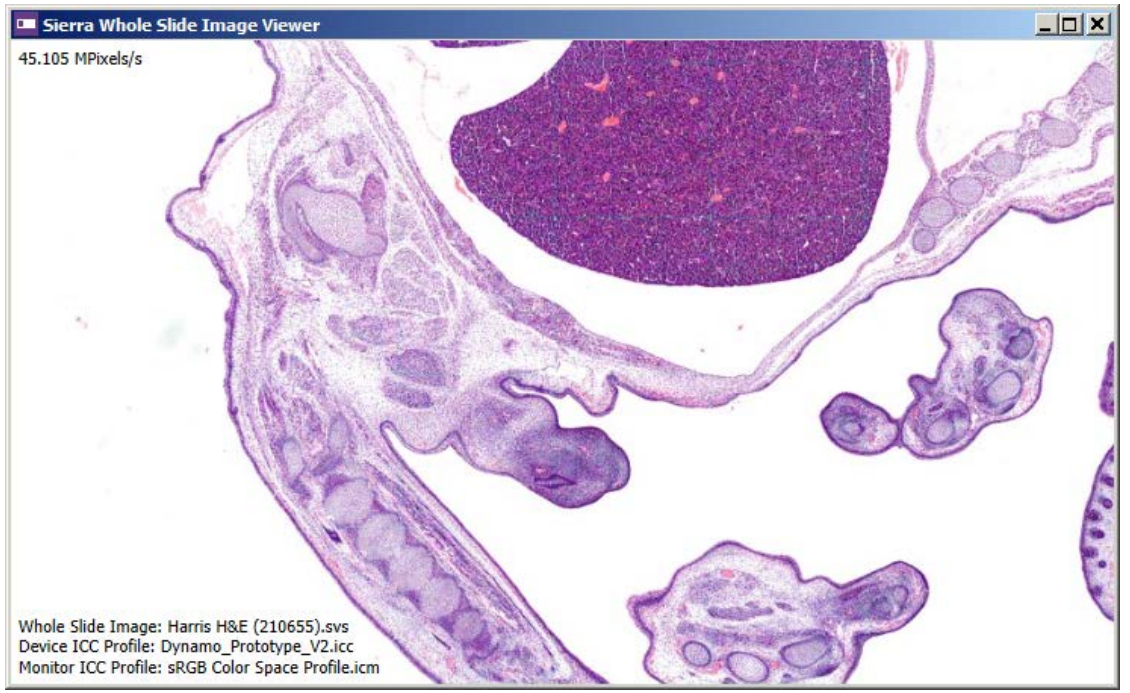
# Analysis Tool



# Whole Slide Image Viewer

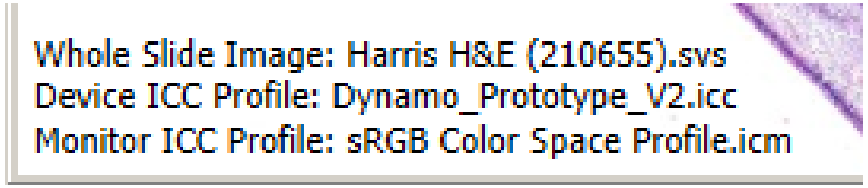



*Shows number of colour conversions in pixels per second*




*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*

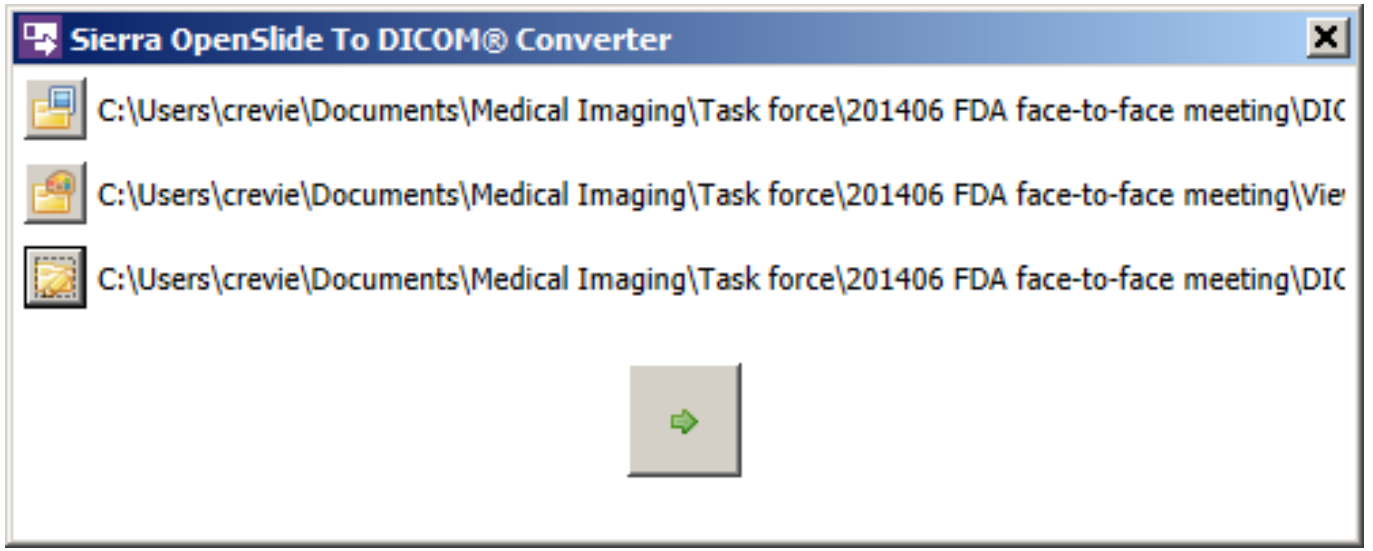


# Sierra OpenSlide to DICOM Converter

*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*

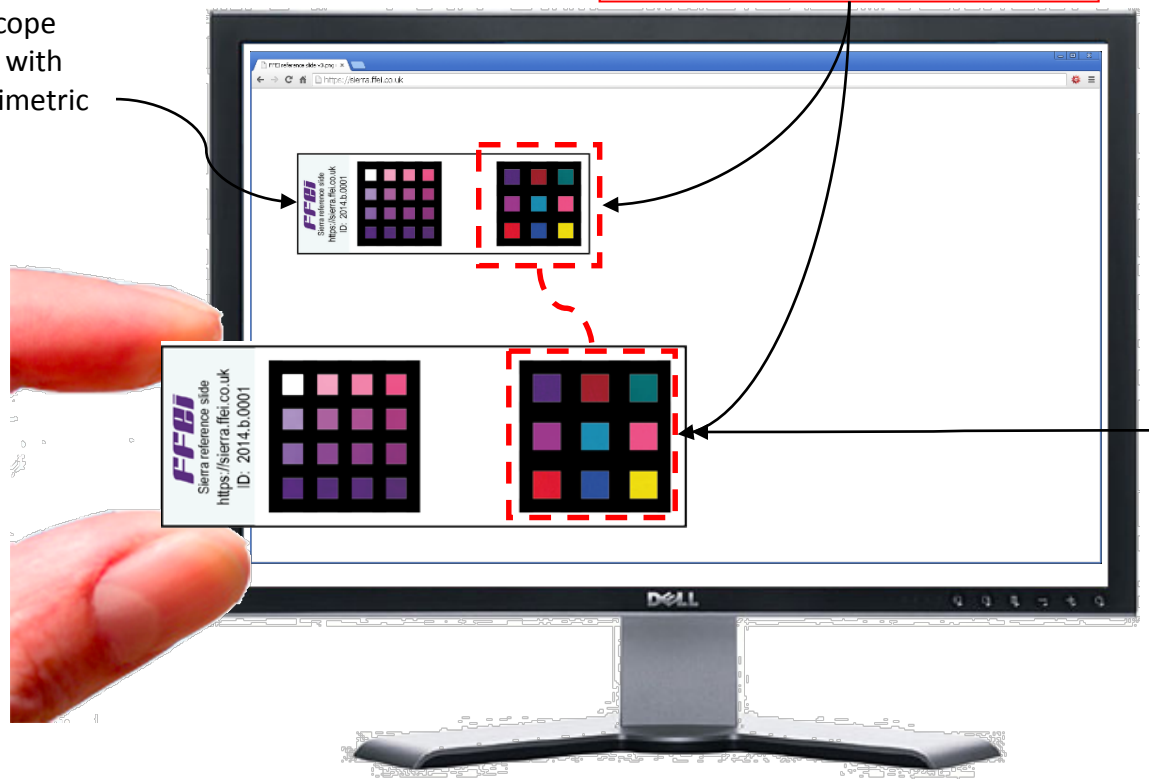
# Some notes about the tools

- 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

FFEI Sierra MicroColour

https://sierra.ffei.co.uk

About FFEI

Creative Imaging Technology

# FFEI Sierra MicroColour

Web based, colour profiling for digital microscopes.

Register as Beta slide partner

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FFEI

Remember me on this computer

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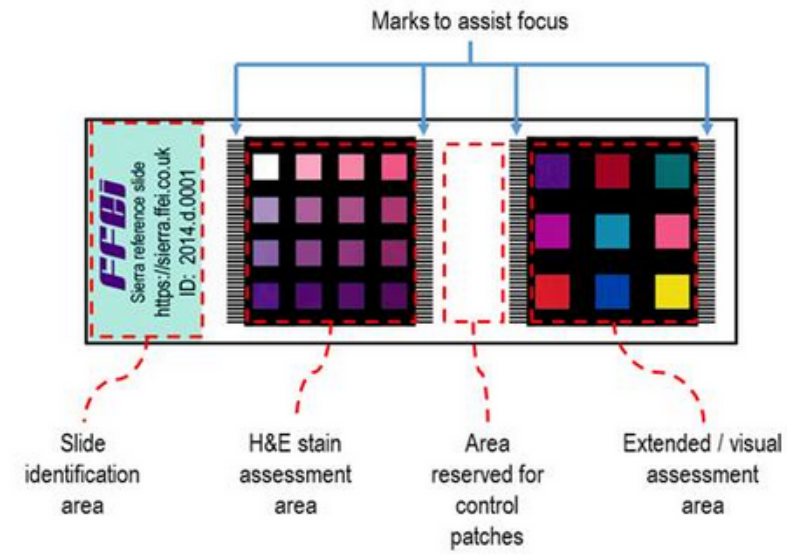
# About Sierra

Project Sierra is an FFEI research project carried out in conjunction with Leeds Teaching Hospitals NHS Trust and the University of Leeds. This project was funded in part by a grant from the UK Technology Strategy Board and includes a number of elements relating to digital microscope performance including the development of test objects and methods for the assessment of colour, dynamic range and resolution.

As part of this project we have created the Sierra Reference Slide which is designed to be used to assess the colour reproduction of a digital microscope system. This slide uses a set of stains commonly used to stain biological samples which have been applied to a biopolymer that has been selected based on its ability to maintain the colour characteristics of the stained biological samples.




A number of prototype slides have been created and these are being made available to members of the International Color Consortium Medical Imaging Working Group for evaluation. We expect the evaluation to include a number of aspects including (a) measurement of patches using a spectrophotometer, (b) scanning of slide using a variety of digital microscopes and (c) comparison of 'calibrated images' with measurements. The Sierra web site has been set up to enable exchange of data when assessing the Sierra Reference Slides and is restricted to those involved in the assessment of the slides. For further details please contact the ICC Medical Imaging Working Group.

For further details of the Sierra project, including details of the Sierra Reference Slide assessment program please contact sierra.support@ffei.co.uk.

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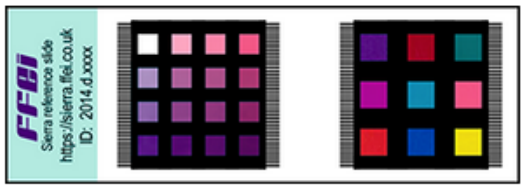
## Slide Data

This page provides a way for Sierra participants to share information relating to Sierra digital microscope assessment slides. Entering the Slide ID for the Sierra slide shows information posted by other Sierra participants and provides a means to allow participants to add information. Details of Sierra slides




Slide ID 2014.d.0001

Show 10 entries

| Slide ID | User | Measurement File | Slide image URL | ICC Profile | Comment |
|---|---|---|---|---|---|
| 2014.d.0001 | FFEI | ID_2014.d.0001_Measurements.csv | | Dynamo_Prototype_V2.icc | |

Showing 1 to 1 of 1 entries (filtered from 4 total entries)

Each Sierra user may upload the following files. The upload process may be repeated multiple times but only the latest version will be retained and visible to other Sierra participants.

Measurement File Choose File No file chosen Sierra file format

Slide Image URL

ICC Profile Choose File No file chosen

Comment File Choose File No file chosen

Submit

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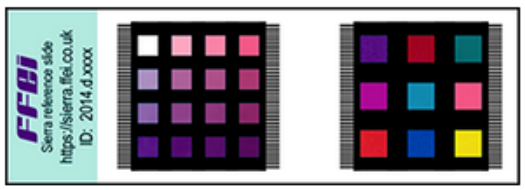
1. Scan | 2. Analyse

## Analysis Tool

The Sierra digital microscope image analysis tool provides a way for digital microscope users to assess how well their digital microscope captures colour. This on-line analysis tool is designed to be very easy to use, requiring only a Sierra calibration assessment slide and a standard web browser that supports HTML 5.

This software assumes that a digital microscope has been calibrated and that an ICC Profile that describes the device's colour is available – ideally as part of the scanned image. A Sierra digital microscope slide (similar to that shown below) is scanned using a calibrated digital microscope and a whole slide image created. User guide and discussion of measurement comparison issues.

Note that this version of the software requires the data to be stored in a single image segment of a DICOM image. Although there is relatively poor support for the DICOM whole slide imaging format this was selected as we anticipate that this may become the standard export format for most systems. FFEI has also provided an image format conversion tool based on the OpenSlide library which will convert a number of proprietary formats to DICOM.




To begin you will need to scan your calibration slide using the scan condition you wish to calibrate and create a DICOM image.

Having scanned the slide, the user selects the slide image file using a standard web browser. HTML 5 compatible web client software then analyses the image and determines the RGB values of each patch. These RGB values are then used along with the ICC Profile that describes the calibrated scanner to produce Lab colour estimates for each patch.

Next

Create Slide ICC Profile

https://sierra.ffei.co.uk/DeviceProfile/Upload

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1. Scan | 2. Analyse

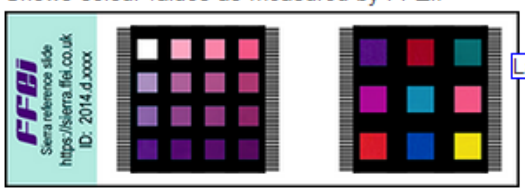
## Analysis Tool: Select Slide Image

Please enter the slide ID.

Slide ID 2014.d.0001

### 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)

### DICOM Image

Please select the directory containing DICOM directory file (DICOMDIR)

Choose File No file chosen

### Scanned Slide Image

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

### Slide Data

| | |
|---|---|
| ScanIdentifier | |
| Manufacturer | |
| Model | |
| Resolution | 0 |

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## Analysis Tool: Select Slide Image

Please enter the slide ID.

Slide ID 2014.d.0001

### Reference Slide

Shows colour values as measured by FFEI.

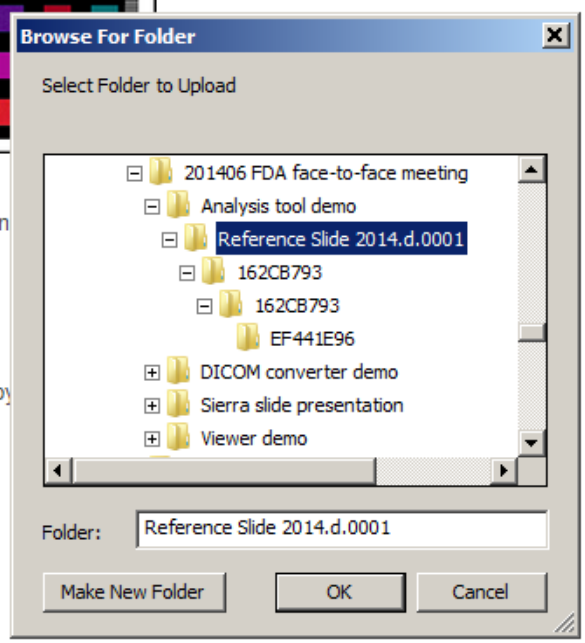

### DICOM Image

Please select the directory containin

Choose File No file chosen

### Scanned Slide Image

Shows colour values as estimated by




### Slide Data

| | |
|---|---|
| ScanIdentifier | |
| Manufacturer | |
| Model | |
| Resolution | 0 |

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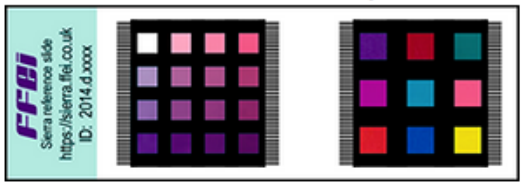
## Analysis Tool: Select Slide Image

Please enter the slide ID.

Slide ID 2014.d.0001

### Reference Slide

Shows colour values as measured by FFEI.




### DICOM Image

Please select the directory containing DICOM directory file (DICOMDIR)

Choose File 7 files

### Scanned Slide Image

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

### Slide Data

| | |
|---|---|
| ScanIdentifier | Converted to DICOM |
| Manufacturer | ffei |
| Model | ? |
| Resolution | 0.00025 |

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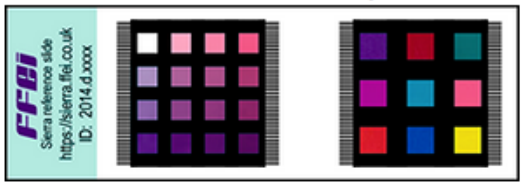
## Analysis Tool: Select Slide Image

Please enter the slide ID.

Slide ID 2014.d.0001

### Reference Slide

Shows colour values as measured by FFEI.




### DICOM Image

Please select the directory containing DICOM directory file (DICOMDIR)

Choose File 7 files

Using ICC profile from DICOM image.

### 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

### Slide Data

| | |
|---|---|
| ScanIdentifier | Converted to DICOM |
| Manufacturer | ffei |
| Model | ? |
| Resolution | 0.00025 |

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# Downloads

## Windows Binaries

FFEI Ltd. has provided these tools which may help in the process of evaluating digital microscope performance.

**Sierra Whole Slide Image Viewer:** can be used to view any of the vendor whole slide image formats supported by the open source OpenSlide library modified to include the FFEI Dynamo and DICOM® media file format for VL Whole Slide Microscopy IOD images.

**Sierra OpenSlide to DICOM Converter:** a graphical user interface and a command line utility, both provide the capability to convert any of the whole slide image formats supported by the OpenSlide library to DICOM media file format for VL Whole Slide Microscopy IOD images.

| | | | |
|---|---|---|---|
| Sierra Whole Slide Image Viewer | 32-bit | 64-bit | User Guide |
| Sierra OpenSlide to DICOM Converter | 32-bit | 64-bit | User Guide |

## Source

| | |
|---|---|
| OpenSlide Library (Modified by FFEI Ltd.) | zip |

User Information

https://sierra.ffei.co.uk/User

About FFEI | Log Off

Creative Imaging Technology

- About Sierra
- Slide Data
- Analysis Tool
- Downloads
- Account Settings

Privacy Policy
Terms & Conditions

## Details

Name FFEI — Change Password...

Email craig.revie@ffei.co.uk — Change Email...

# Sierra
# Calibration Assessment Slides

W Craig Revie

FFEI Limited

# Sierra calibration assessment slides

- As described in previous Medical Imaging Working Group presentations, FFEI has developed a prototype calibration assessment slide
  - now available for assessment by a group of experts
  - need a plan to make these slides available on a commercial basis
- In this presentation:
  - Sierra project overview
  - Sierra web site
  - review of round-robin proposal
- Related topics
  - exposure control / slide lifetime assessment
  - staining assessment

# Sierra project overview

# Sierra web site

# Questions from email

- What will you do with the slide as part of the round-robin
  - when can you do it?
- Can you create DICOM WSI image files for analysis?
- What is the digital microscope system colour rendering aim?
  - reference light source
  - numerical aperture
  - rendering intent
- Assessment of colour performance
- Will your company join the work to take the slide into production?

# Round robin: rules of engagement

- Confidentiality
  - the aim of the group is to publish the results of the round-robin assessment however during the period of the round-robin all of the data files and discussion shared with the group shall not be shared outside of the group
- Web site access and use
  - the Sierra web site https://sierra.ffei.co.uk will be managed by FFEI
  - access shall be granted to suitably qualified participants
  - FFEI will maintain a roster of members which will be made available to group members
- Objectives
  - make and compare measurements of slides
  - scan slides on a range of digital microscopes and provide feedback
  - provide feedback on framework for digital microscope evaluation
- Timescale
  - time allocated to group members for assessment is strictly limited and group members should complete their assessment in a timely manner

# Round-robin assessment time slots

| Slide ID | Jul 2014 | Aug 2014 | Sep 2014 | Oct 2014 | Nov 2014 |
|---|---|---|---|---|---|
| 2014.d.0001 | FDA | TIT | | Konica Minolta | Slides re-measured by FFEI |
| 2014.d.0002 | Leica | GE Omnyx | | | |
| 2014.d.0003 | Ventana | | | | |
| 2014.d.0004 | Philips | MGH | | | |

# FFEI has demonstrated feasibility – we would now like broader collaboration in Sierra

- Our long term vision is to develop a pair of slides
  - a slide that can be used to calibrate a digital microscope and
  - a slide that can be used to assess the calibration state of a digital microscope
- FFEI has funded the development to date and we have drawn on expertise within our organisation but we would like to identify a small group of partners to take this forward to production and deployment
- For this project to be successful we need funding, expertise and technical resources and would like help from members of the ICC Medical Imaging Working Group
- If you would like to join our work please make a detailed proposal as to how you will contribute – FFEI will select a small group with the necessary skills and resources necessary to set up reliable production of the slide on a commercial basis
- Work within the group will be subject to mutual NDA with the eventual goal of making the slides available to all

# Questions and discussion

# Framework for Multispectral Imaging Application to digital pathology

Masahiro Yamaguchi, Tokyo Institute of Technology

Bas Hulsken, Phillips

Max Derhak, Onyx Graphics Inc.

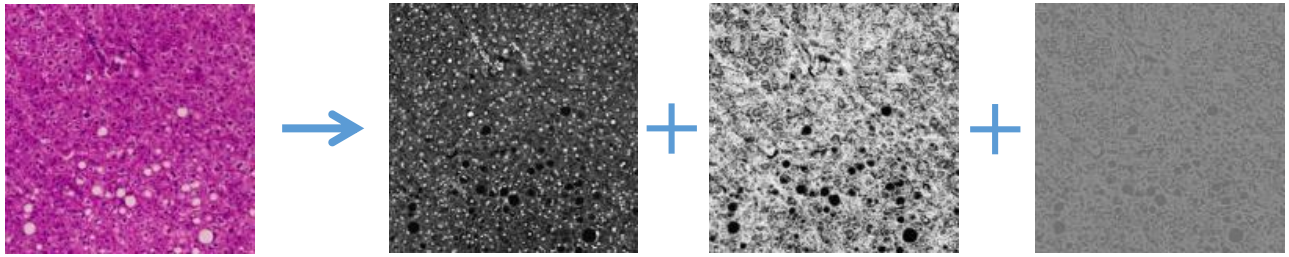
# Multispectral imaging in pathology

- Brightfield
  - Object detection, segmentation
  - Color unmixing – Stain amount image
  - Digital adjustment of staining strength
  - Digital staining

- Fluorescence
  - Simultaneous imaging of multiple markers
  - Cross-talk, auto-fluorescence removal
  - Combined brightfield and fluorescent images

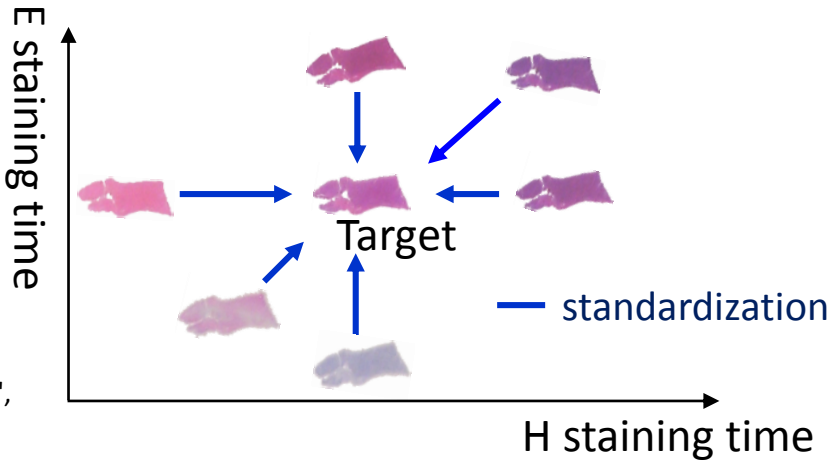
# Estimating dye amount image

- Color unmixing
  - Estimation of dye amount image




Residual is not actual data

- Adjustment of dye amount
- Standardization of staining condition



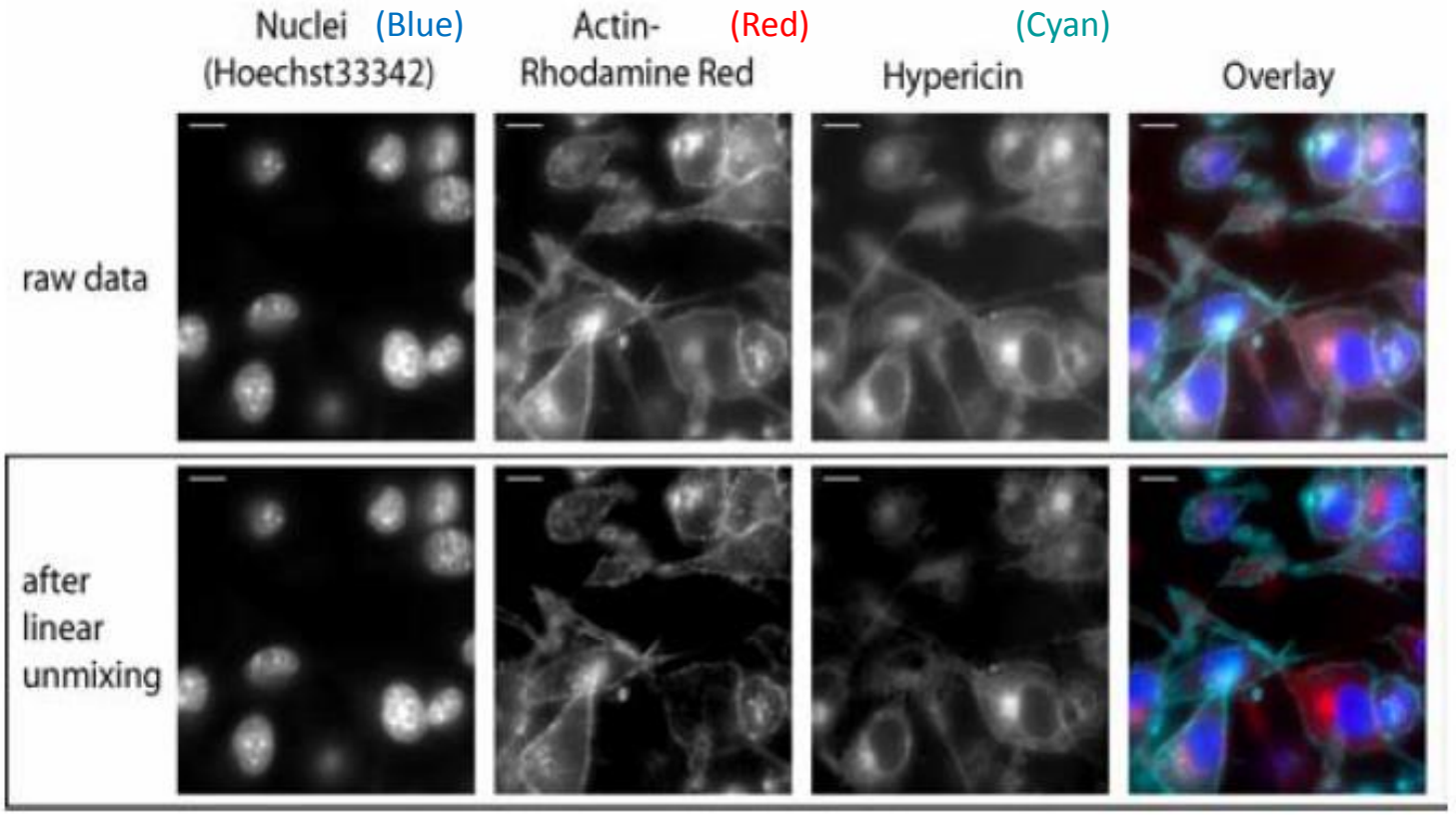

K. Fujii, M. Yamaguchi, N. Ohyama, K. Mukai, "Development of support systems for pathology using spectral transmittance: the quantification method of stain conditions", Proc. SPIE 4684, 1516 (2002)

T. Abe, Y. Murakami, M. Yamaguchi, N. Ohyama, Y. Yagi “Color correction of pathological images based on dye amount quantification,” Opt. Rev., 12, (4), 293-300 (2005).

# Fluorescent imaging for molecular pathology

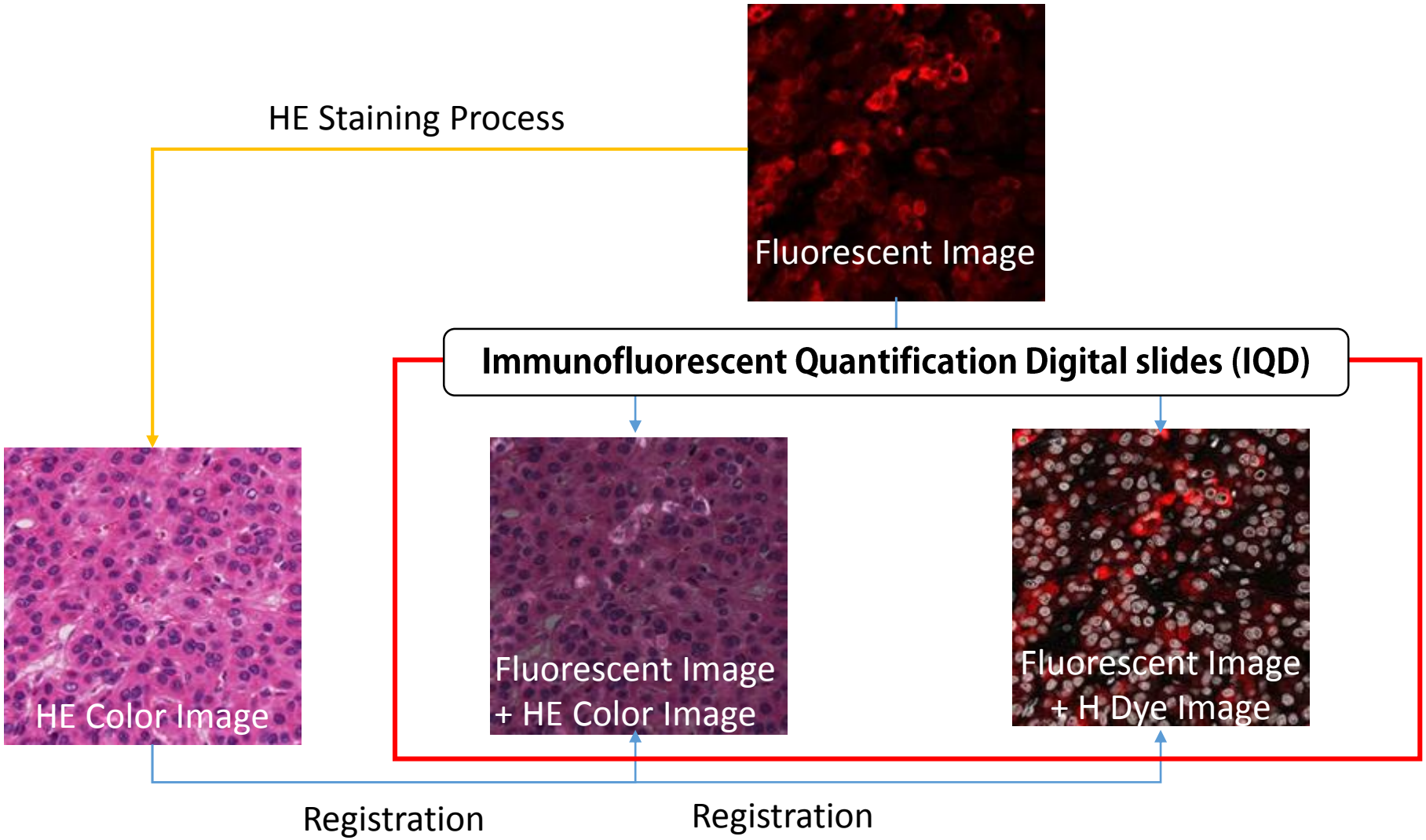
- Necessary to test various biomarkers
  → Multispectral imaging with using multiple fluorescent dyes
- Crosstalk problem
  - Crosstalk between different fluorescent dyes
  - Auto-fluorescence
  → Color unmixing
- Quantification / semi-quantification of marker expression,
  - Identification of tumor, tissue, nuclei, or membrane needed
  → Combination of fluorescent and H- or HE-stain images

# Color unmixing for unwanted fluorescence removal




Modern Research and Educational Topics in Microscopy.
A. Méndez-Vilas and J. Díaz (Eds.)

# Combination of Fluorescent and HE-stain




A. Hashiguchi et al 'Using immunofluorescent digital slide technology to quantify protein expression in archival paraffin-embedded tissue sections' *Pathol Int* 2010; 60: 720–5.



*Department of Pathology,*
*School of Medicine, Keio University*

# Requirements from DICOM WG26

1. Ability to define how to display multi-spectral images as true color visible light images.

   Color reproduction: ICC framework can make it!

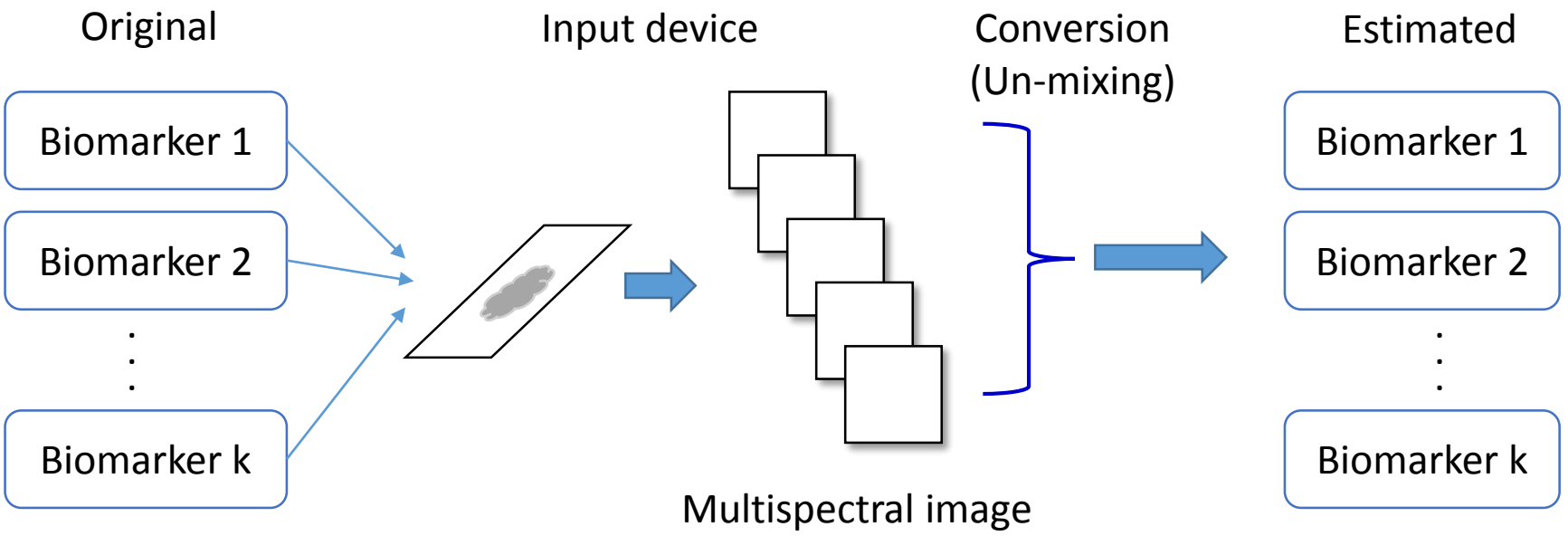
2. Ability to define how to un-mix multispectral input channels for the purpose of deriving quantitative representations of individual biomarker intensities.

   **Not the issue of color reproduction: PCS is useless…**
   **Can we adopt ICC framework?**

3. Ability to define how to display (un-mixed) multi-spectral images as pseudo color images.

   Color reproduction: ICC framework can make it!

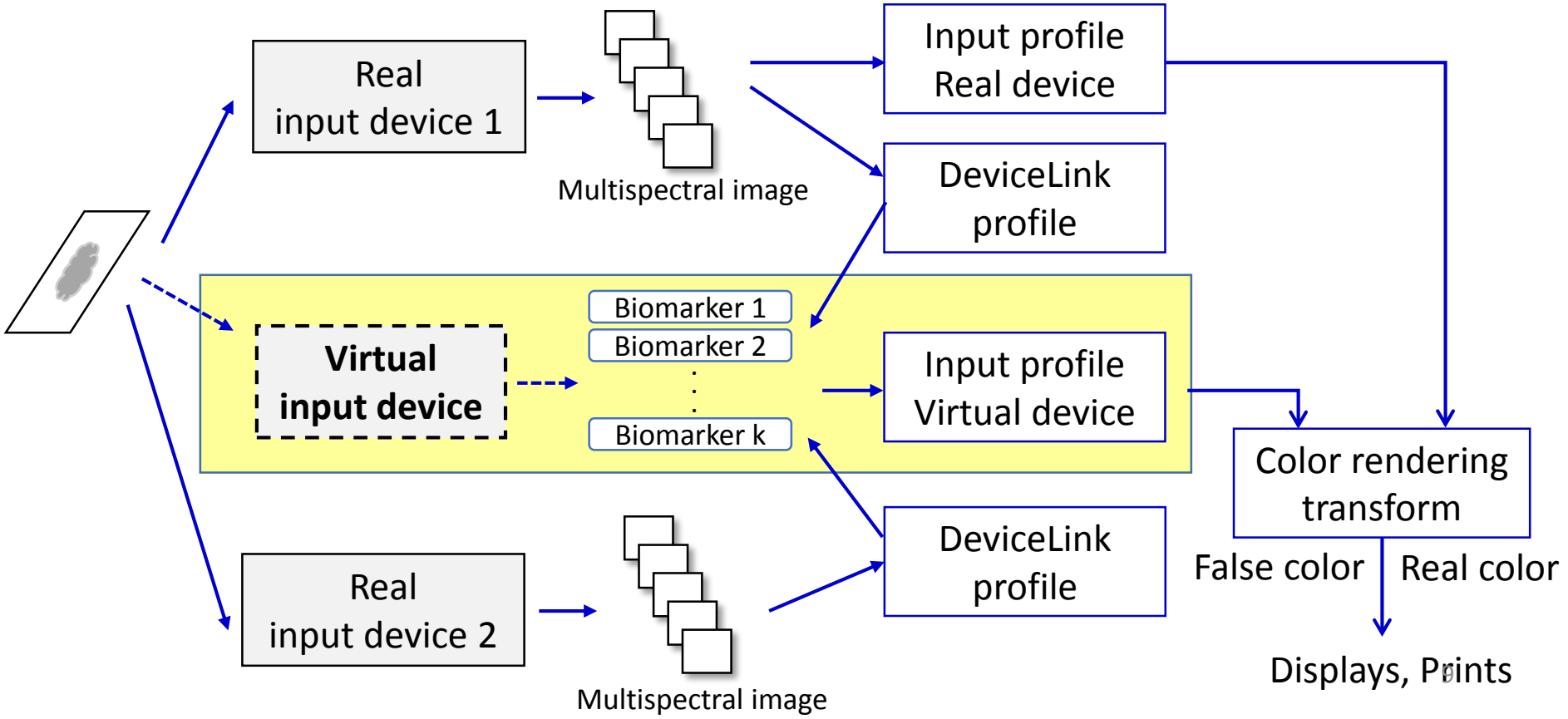
# General model for multispectral un-mixing




PSA, Ki-67, CK-19
HER2, ER, PgR, …

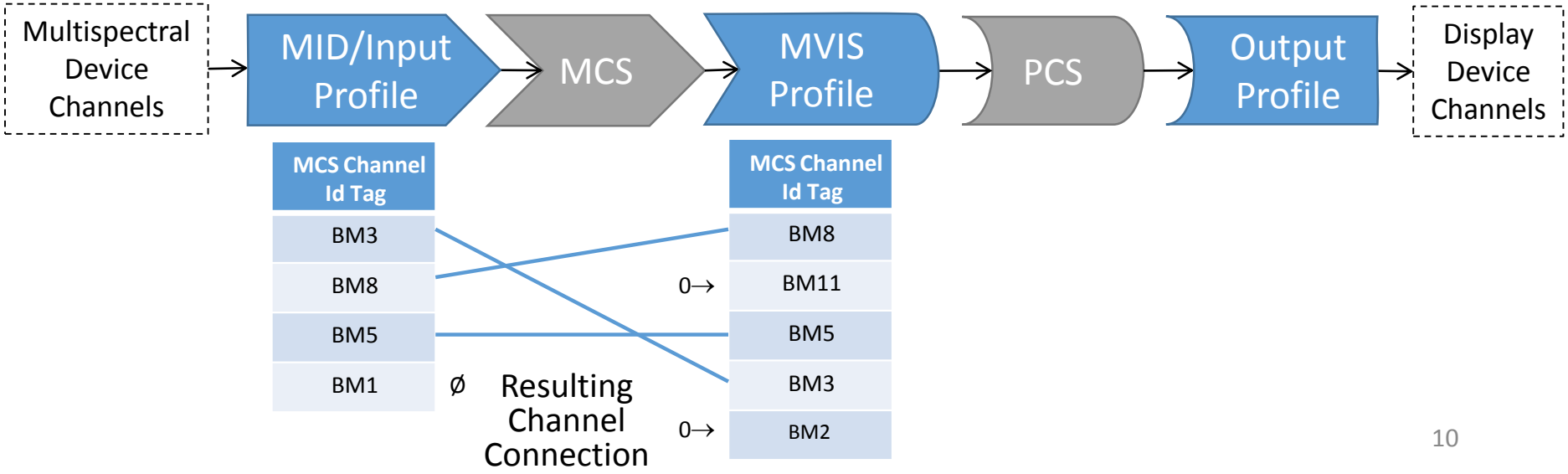
# Solution to color unmixing by ICC v4

- Consider a virtual input device
  that can directly capture un-mixed biomarker images
- Use DeviceLink profile



# Solution to color unmixing by ICC Labs

- RefICCLabs - - - ICC v5
  - Spectral profile
  - Calc element
  - …
- Proposal of “Material Connection Space” Profiles
  - MCS connection allowed between source biomarker Material Identification (MID) and destination biomarker Material Visualization (MVIS) profiles



# For reference:
# Requirements in the Technical Report draft “Multispectral format” of CIE TC8-07

http://www.multispectral.org

- Basic
  - Platform independent
  - Lossless compression (ZIP, LZW, etc.)
  - Lossy compression (JPEG, Wavelets, etc.), independent for each channel
  - geolocation information (e.g., latitude and longitude of the corner pixels)
  - High Accuracy: many channels, many pixels, many bits
  - Ability to store raw data such as naked camera outputs, DC bias values, camera gain
  - Unevenly sampled spectral signal
  - Royalty free
  - File size limitation
  - Archiving Application (details)
- Highly desirable
  - Ability to store uncompressed data
  - Geometric registration information including pin cushion model along with a grid of registration control points
  - Possibility to store company specific information (native tags)
  - Storage compatible with Lab, RGB possible (e. g., L, a, b, ch4, ch5, ch6, ... )
  - Reconstruction matrix stored as
  - Simple usage, easy spectral reconstruction (e.g. just multiply the channels by a matrix in order to yield the spectra)
  - All information in one file
  - Units of the data (e.g. reflectance (per cent for fraction)  or radiance (watts/sq-meter, etc.))
  - Planar packing available
  - Industrial Application (simple reconstruction)

# Next step

- Consider adoption of ICC v4 keeping in mind the upper compatibility in v5.
- Documentation for implementation to DICOM.
- Investigate the advantage and feasibility of ICC v5 application.

The Leeds Teaching Hospitals NHS
NHS Trust

# Point of Use QA: Digital Pathology Slides

David Brettle, Darren Treanor (LeedsTH NHS Trust)
Craig Revie (FFEI), Mike Shires (Leeds University)

Leeds Cancer Centre

# Outline

- Building on existing work in X-ray imaging, will illustrate the need of point of use QA as an adjunct to component QA.
- To present early stage design considerations specifically for digital pathology.
- Propose possible solution to quantify  image integrity.

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# What's the problem?

- Analogue:

  Acquisition and display are linked proving image context or ‘frame of reference’.

- Digital:

  Acquisition and display are unlinked removing context;  There is no ‘digital frame of reference’.  This is compounded by the potential for unlimited post acquisition processing.

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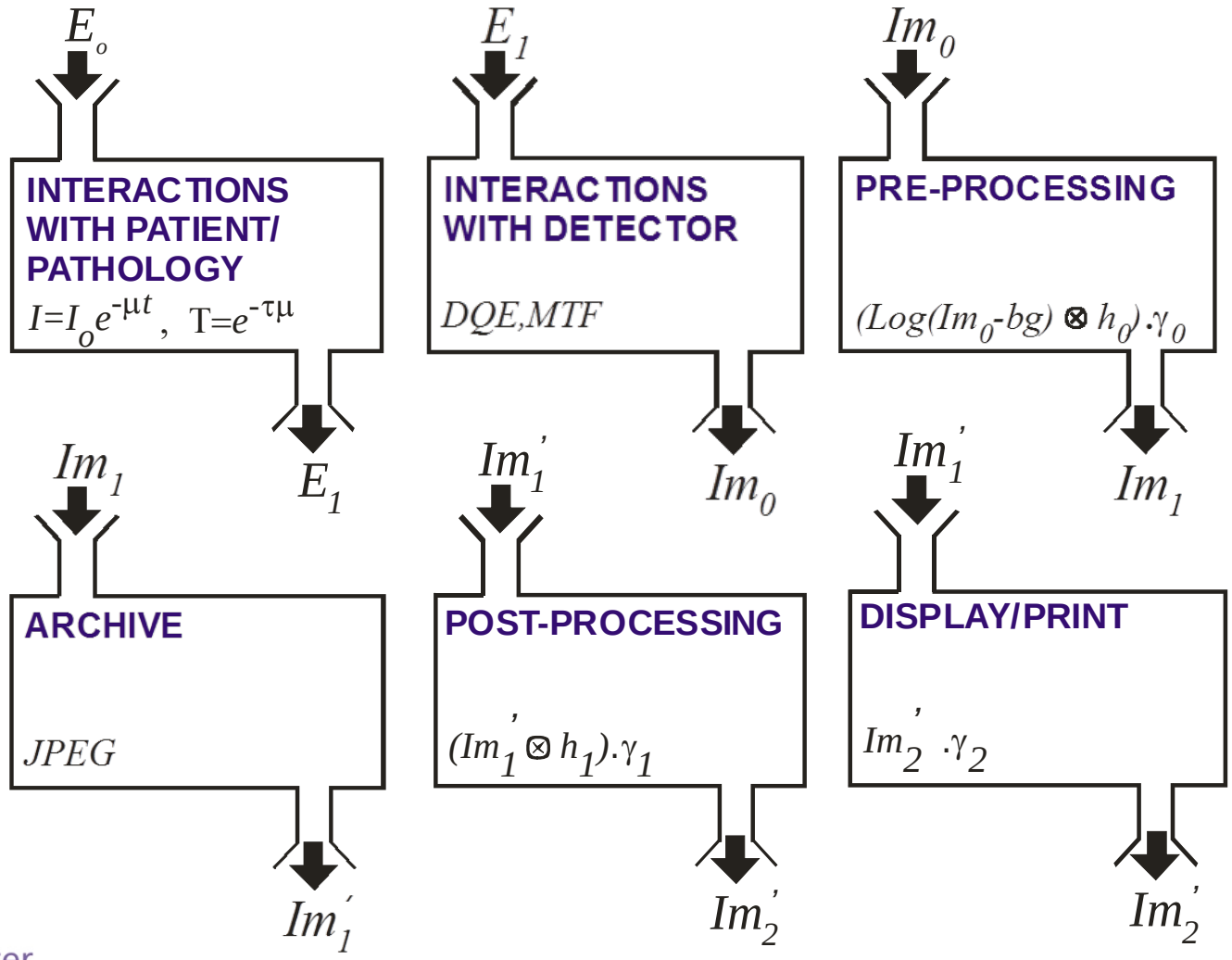
# The Digital Image Chain

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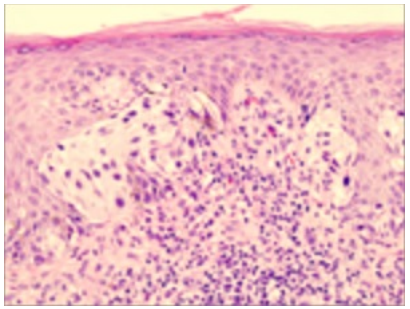
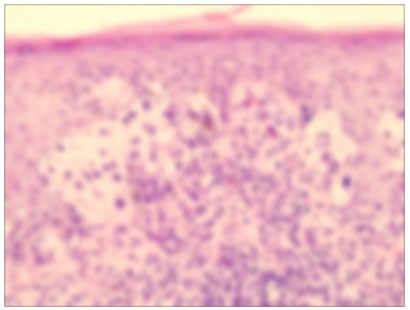
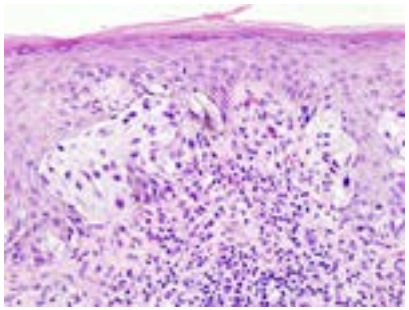
# The Digital Image Chain



Transmission

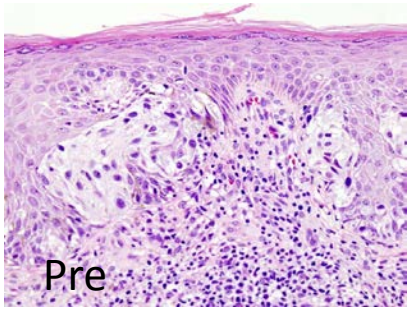


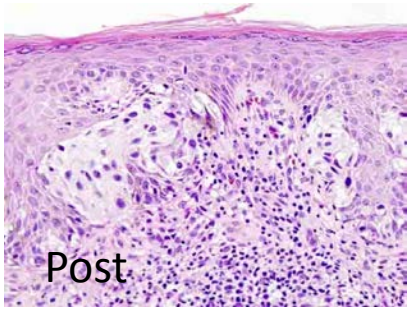
Detection

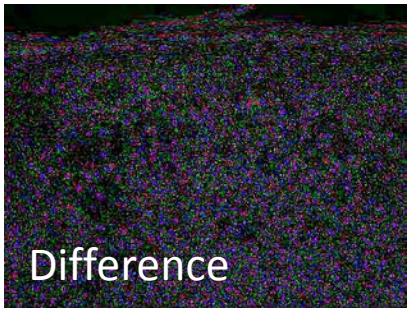


Pre-processing

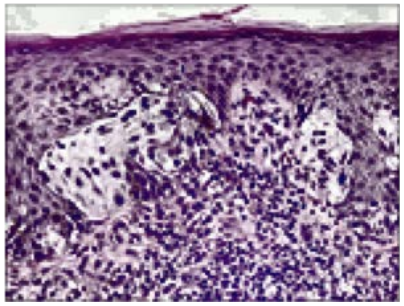
Compression

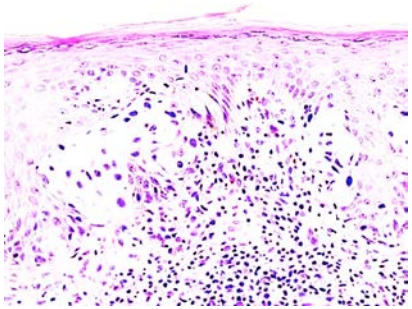


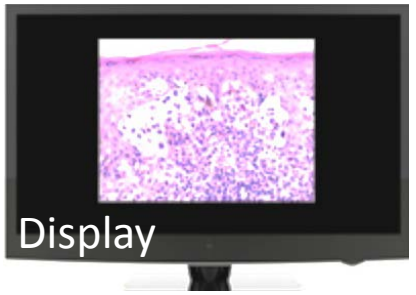




Post-processing





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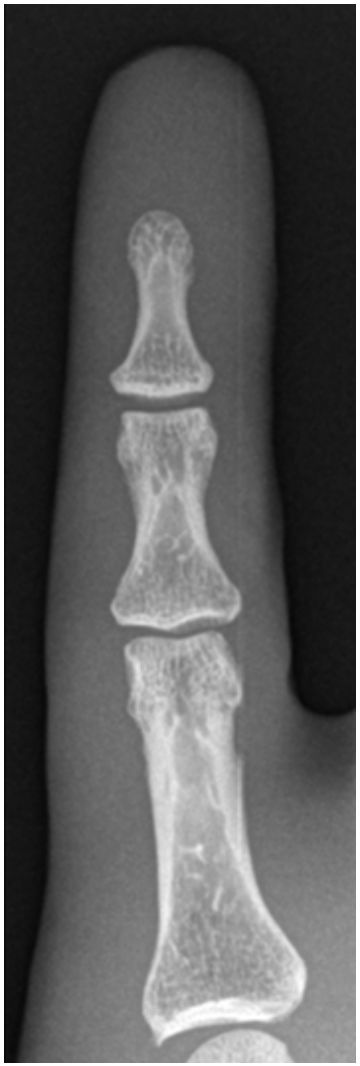
# Pre-processing gone wrong



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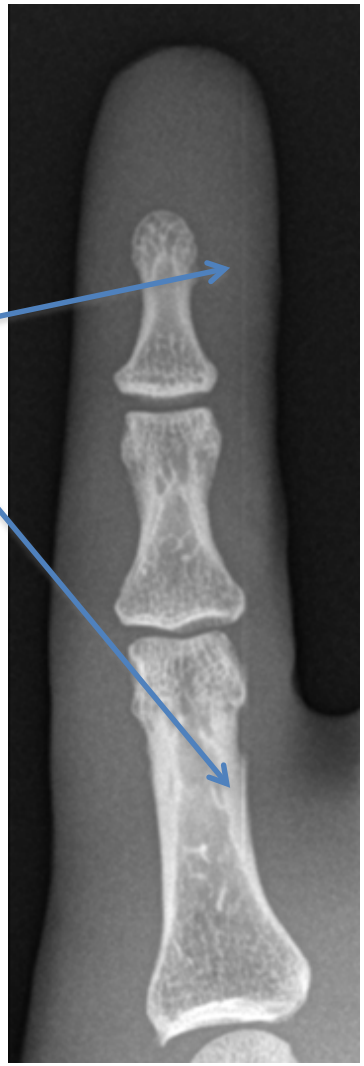
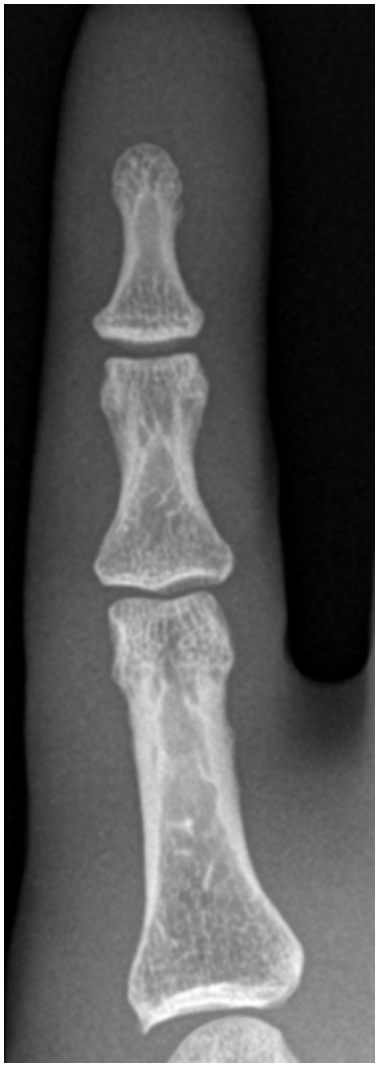
# Pre-processing gone wrong

Stitching artefact





Corrected Algorithm

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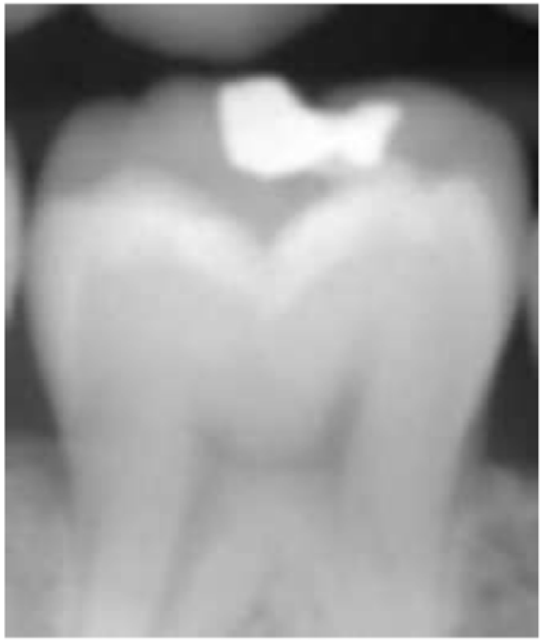
# Post Processing gone wrong: Recurrent Caries?



Original

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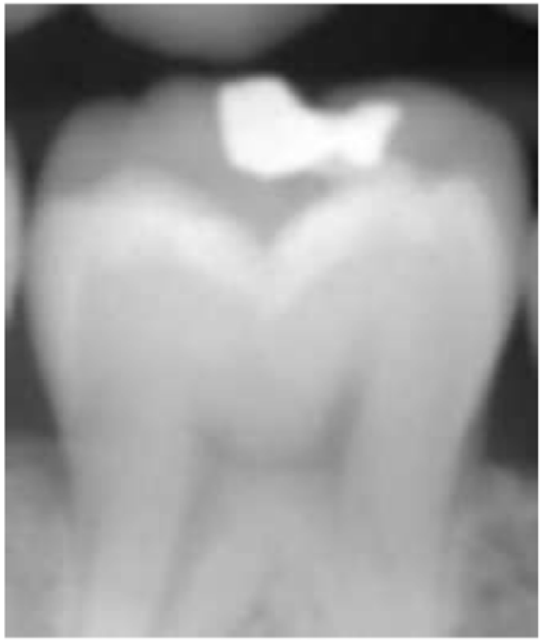
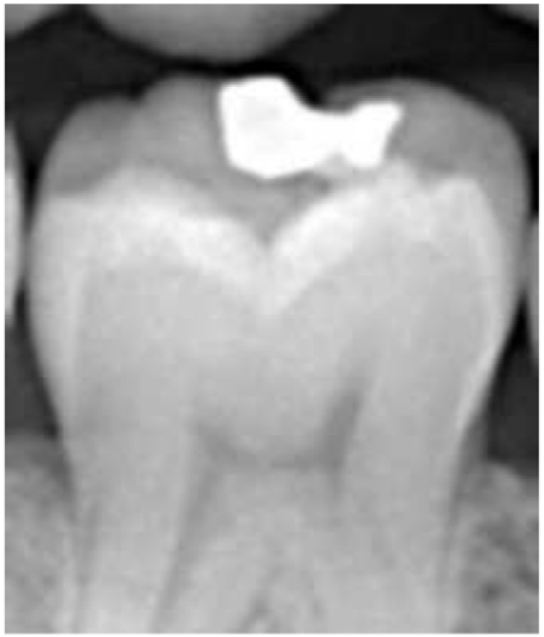
# Post Processing gone wrong: Recurrent Caries?



Original



Processed,
but mimicking pathology

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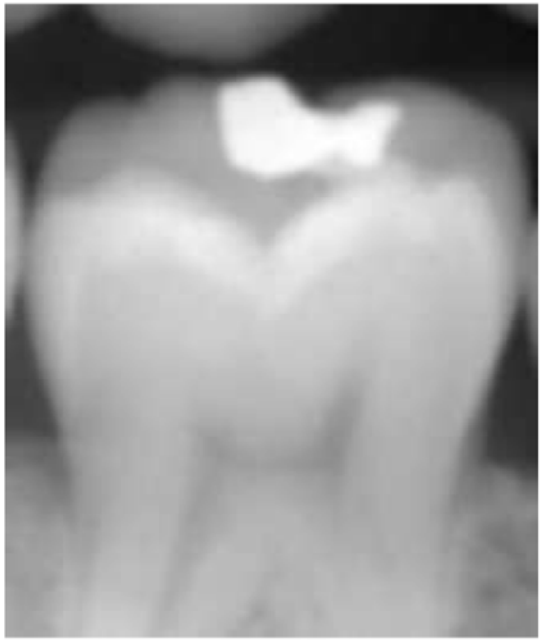
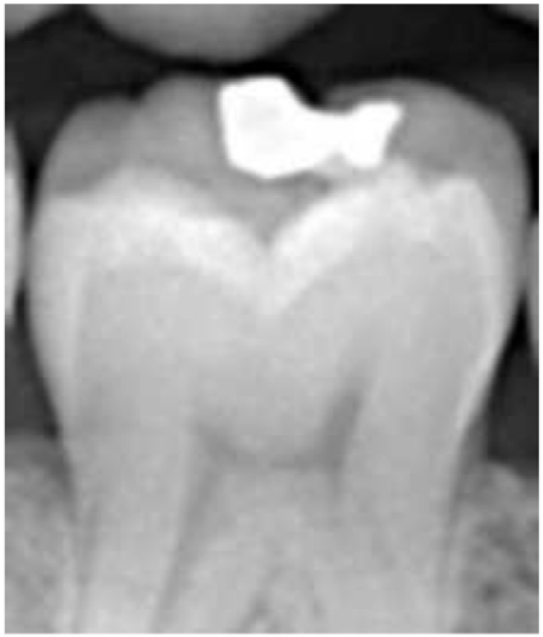
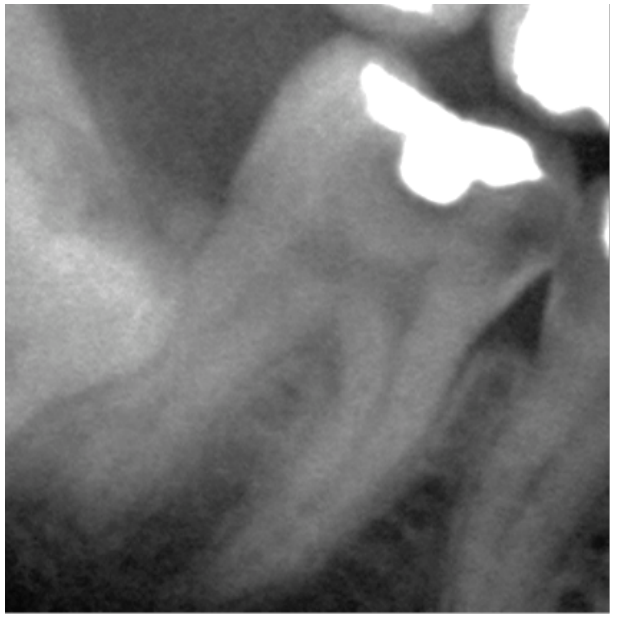
# Post Processing gone wrong: Recurrent Caries?



Original



Processed,
but mimicking pathology



Real Pathology

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Brettle D. S and Carmichael F.
*The impact of digital image processing artefacts mimicking pathological features associated with restorations.*
British Dental Journal (2011). 211(4), 167–170.

# The ‘So what?’ factor - Local context

- Local experience can compensate for factors affecting the image chain. *“So what!..That doesn't bother me, we see that on all the images”*.

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# The ‘So what?’ factor - Local context

- Local experience can compensate for factors affecting the image chain. *“So what!..That doesn't bother me, we see that on all the images”*.

BUT

- If the image becomes orphaned from the host institution this local knowledge and ‘compensation’ is missing.
- A system can be within specification but still apply deliberate processes that may compromise an image.

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# Concept Solution

An embedded environment in the image that is responsive to all the processes applied to the image, from acquisition to display, and allows assessment of integrity at any time or point in the image life.

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# General Design Criteria

- Responsive to image generation factors.
- Non-intrusive in the image.
- Responsive to essential image components: frequency, contrast, color and image processing.
- Allows quantification of the degradation of the image and subjective assessment.
- Accessible at all stages in the image life-cycle.
- Allows image ‘correction’.

# Proposed solution (X-ray)

- Digital Frame of Reference ‘DFOR’
- Already proposed as a software reference
- Now a physical radiographic ‘side marker’ with responsive features.


Physical Marker

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# Proposed solution

- Digital Frame of Reference ‘DFOR’
- Already proposed as a software reference
- Now a physical radiographic ‘side marker’ with responsive features.

Physical Marker

X-ray

Leeds Cancer Centre

# Proposed solution

- Digital Frame of Reference ‘DFOR’
- Already proposed as a software reference
- Now a physical radiographic ‘side marker’ with responsive features.

Physical Marker

X-ray

Processed USM

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# Also ..'Image Integrity Index' (III)

- $g_{x,y} = f_{x,y} \otimes h_{x,y}$
- $G_{u,v} = F_{u,v}H_{u,v}$ (In Fourier space)
- $H_{u,v} = G_{u,v} / F_{u,v}$

- To find H need to know G and F. Normally this is not possible but with the 'Digital Frame of Reference' (DFOR) this is.
- Therefore summing H gives a net indicator of deviation from the original.
- Can be indexed to a pure digital reference or acquisition reference.

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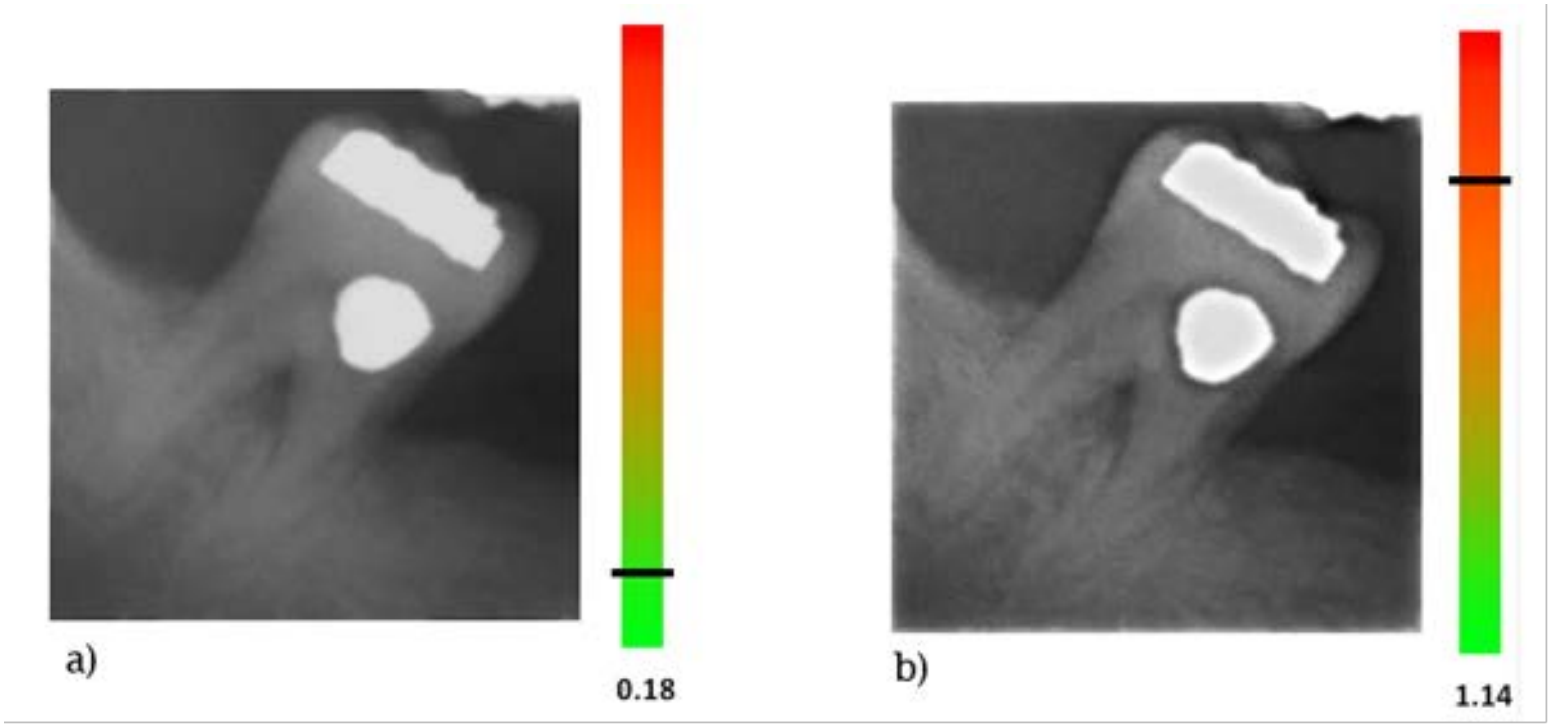
# Image Integrity Index

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# Digital Pathology

- There are parallels between digital X-ray imaging and digital pathology.
- Local acquisition and image generation dependencies.
- Orphan images.
- Potential clinical impact of not knowing what has happened to an image.

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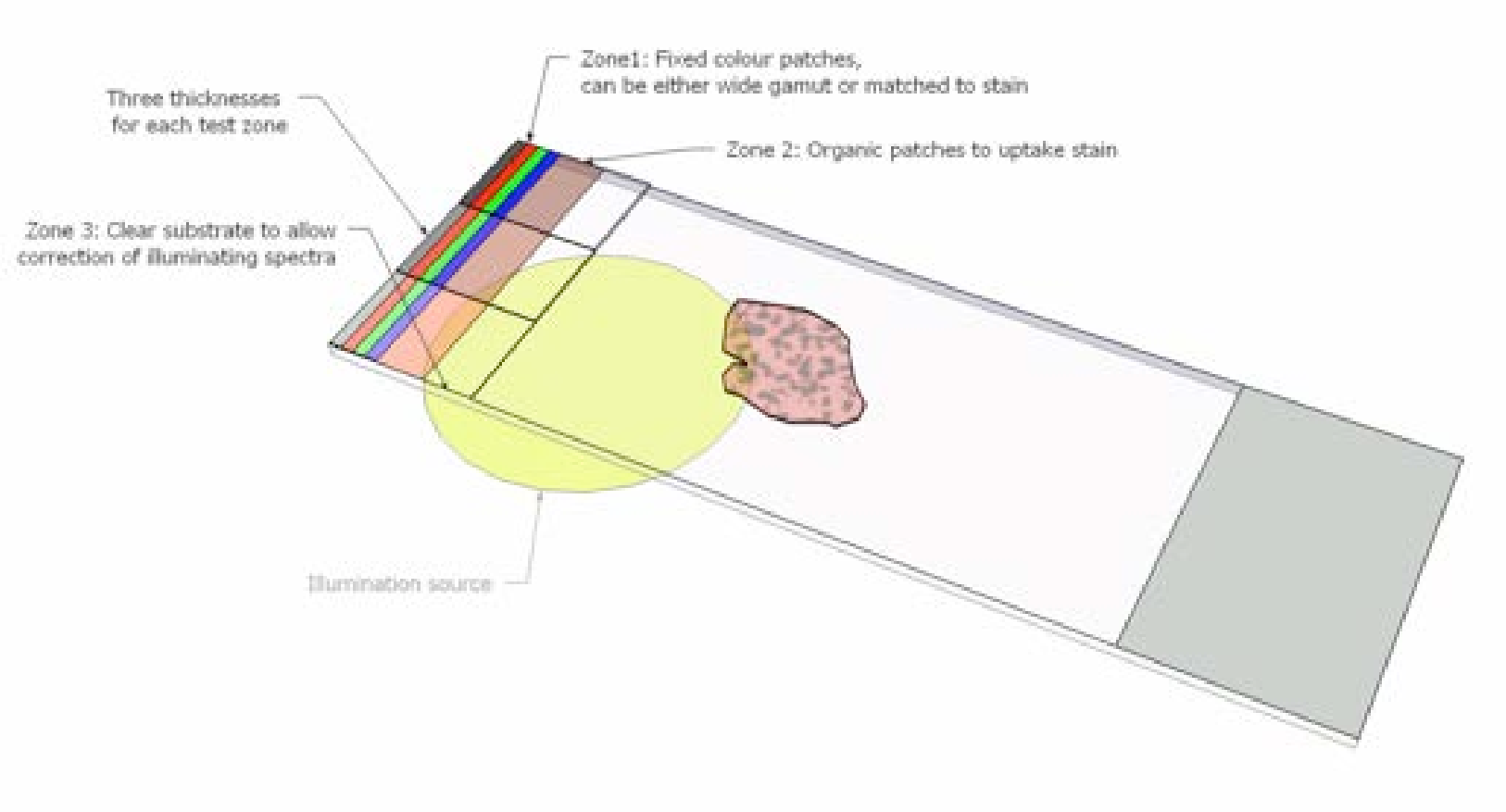
# Original Concept

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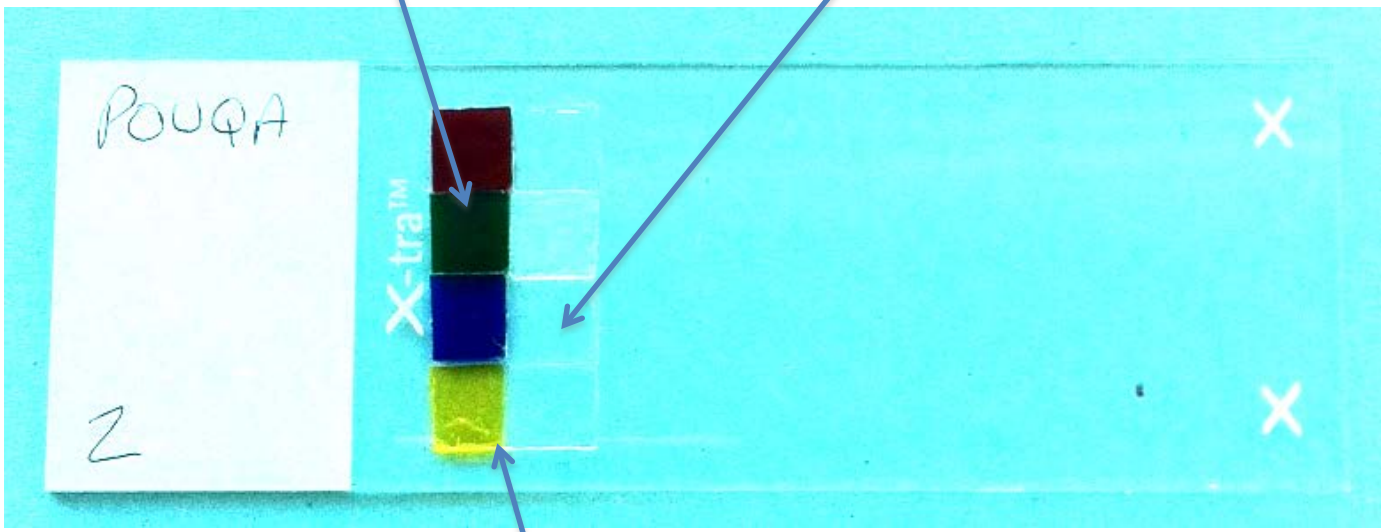
# Considerations for DP POUQA

- **Staining**: Need substrate that takes up stain in a reproducible way which may need to be tailored to each stain type.
- **Scanning**: System factors can alter colour response, can even be a conscious system design criteria therefore colour reference is needed.
- **Digitisation**: The data sets for DP are huge, compression is a key factor, whereas in X-ray loss of high frequency may not be significant in DP may be profound impact.
- **Fading**: Scanning of a slide may not take place immediately, particularly with a transitional stage from analogue to digital. The substrate therefore needs to fade at the same rate at the tissue.
- **Stain variability**: Different stains interact with the tissue in different ways, therefore all staining mechanisms need to be considered.

# Prototype

Absolute color reference:
4 thickneseses

Relative colour reference:
4 substrate thickneseses




Derived system information:
ESF, MTF, SNR etc

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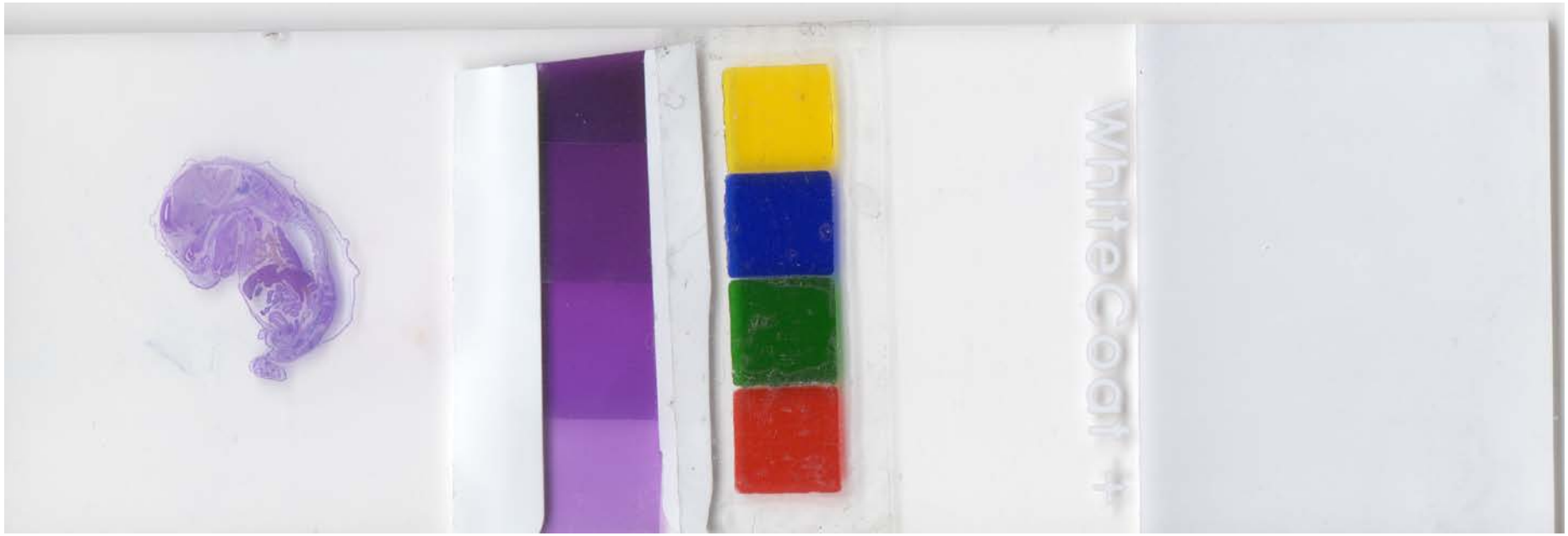
# Proof of concept

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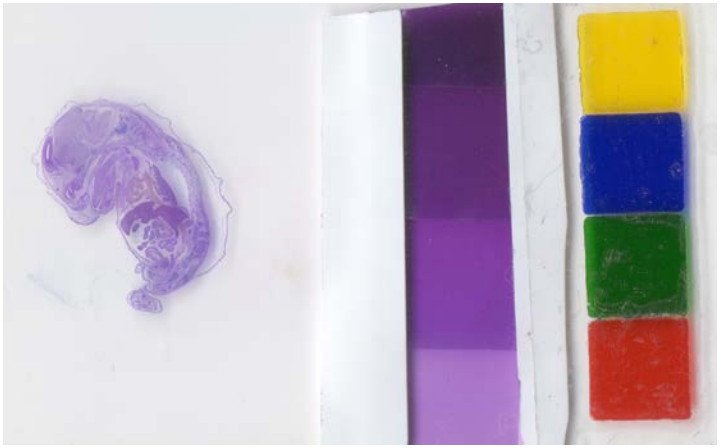
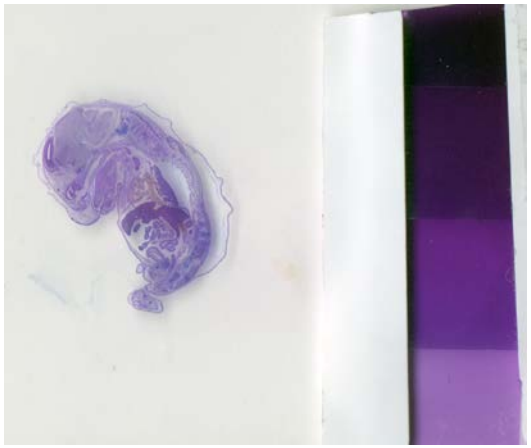
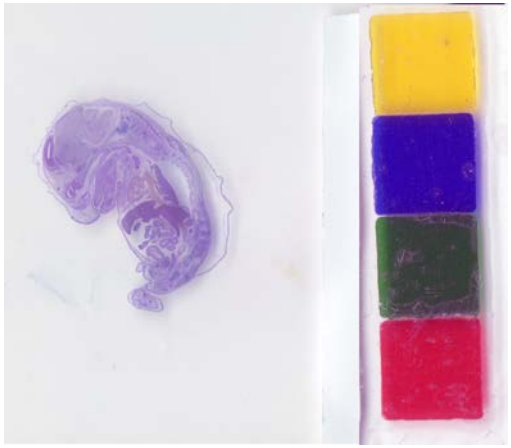
# Ability to ‘optimize’ every slide



Original



Relative color re-mapping



Absolute colour re-mapping

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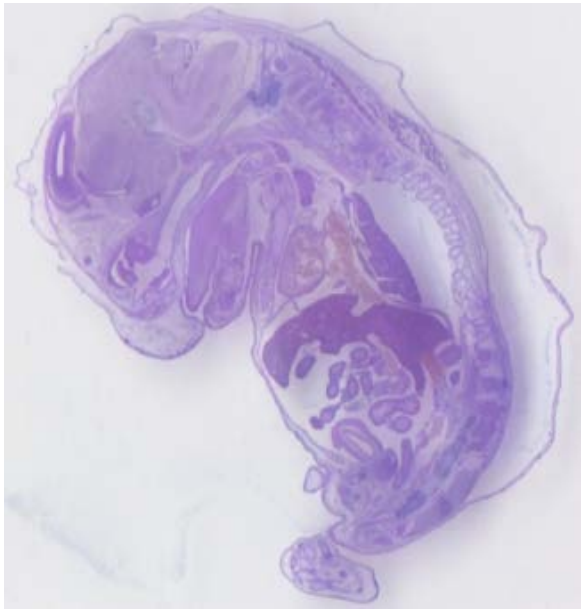
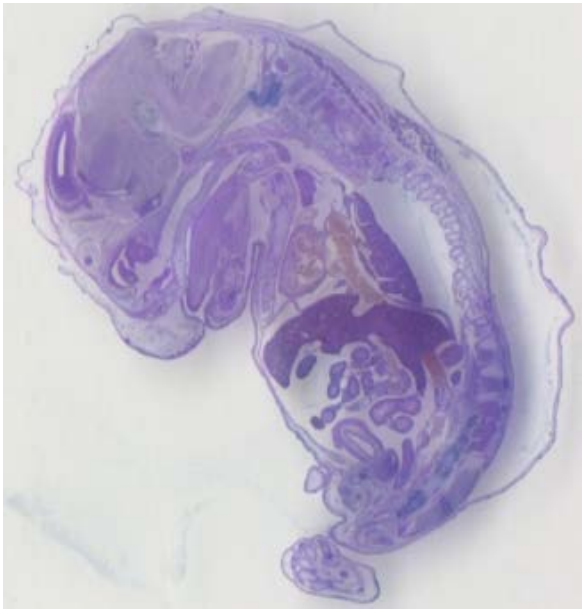
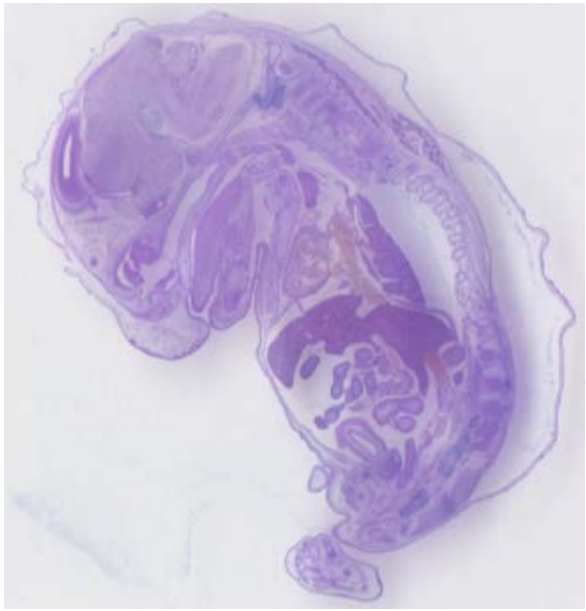
# Zoomed in.



Original



Relative color re-mapping



Absolute colour re-mapping

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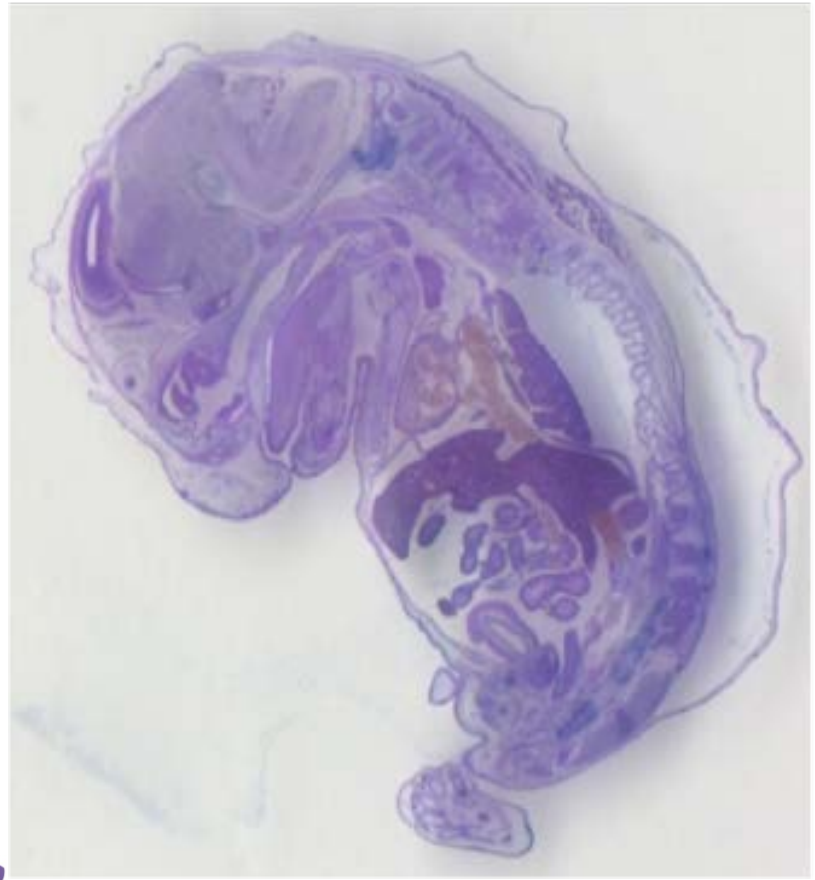
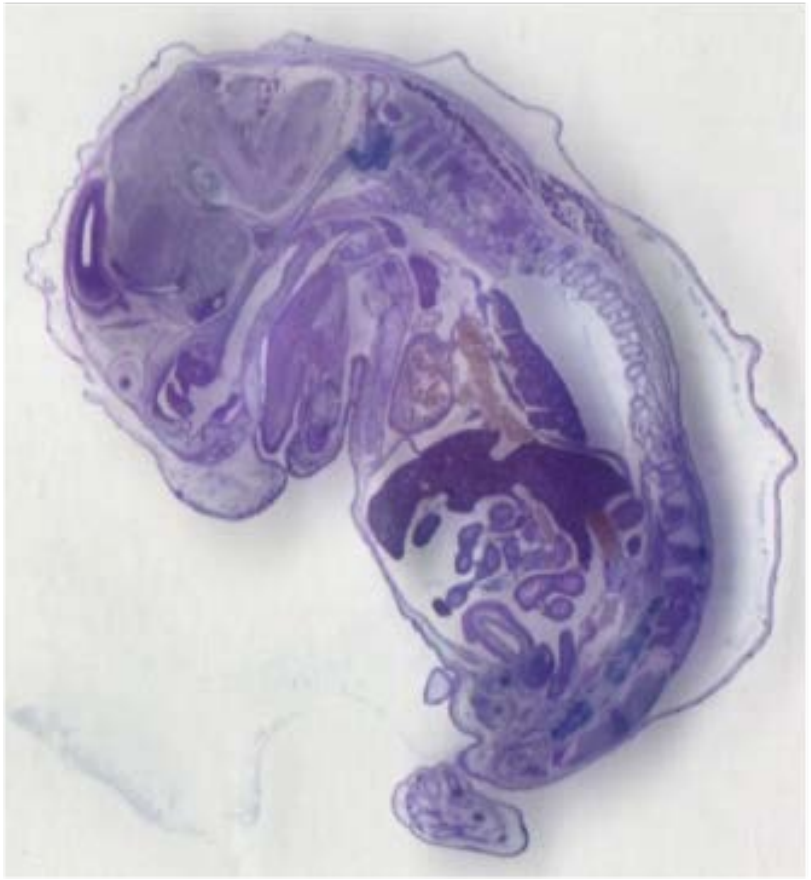
# …with processing





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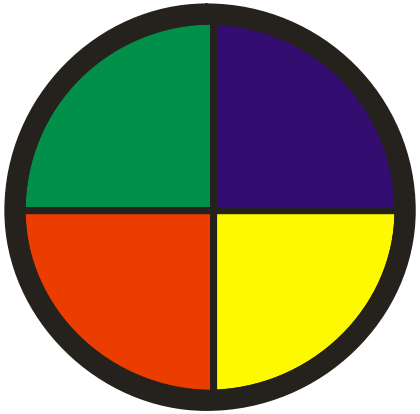
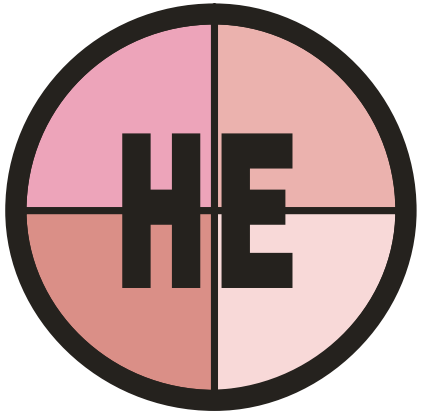
# Possible solution?



Absolute Colour Reference Patch


Substrate Patch




Option: Relative Colour Reference patch

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# Options

- Discrete markings on the zones will facilitate focussing. Could also be used as a LSF for MTF especially if 45º lines used giving net resolution.
- The zones could be separate stickers allowing optional/easier positioning.
- Different substrate material depending on the stain/cell structure of the sample allowing reference to the specific diagnostic task.
- And/or absolute reference colours for the stain used
- Simple ‘Stain used’ indicator possible.

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# Benefits

- Can be used to check scanner calibration.
- Allows relative stain calibration.
- Allows absolute colour calibration (if required).
- Subjective assessment of slide and image quality
- Quantitative indication of staining variability
- Secondary measurements MTF, DQE etc
- Display normalisation
- Image Integrity Index.
- Whole image life cycle QA

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# To Do

- Improve ‘on slide’ staining.
- Investigate other substrate/stain behaviours
- Develop Image Integrity Index and primary quality metrics

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# Conclusions

- In digital imaging there are many factors that can affect image integrity.
- Component QA is important but…
- Without being able to quantify and communicate all the image degradation factors negative clinical impact is possible.
- A POUQA solution is proposed utilising a test environment embedded in every ‘mission critical’ image; e.g. X-ray, Pathology, Forensics  to allow subjective and quantitative assessment of image integrity at every stage.

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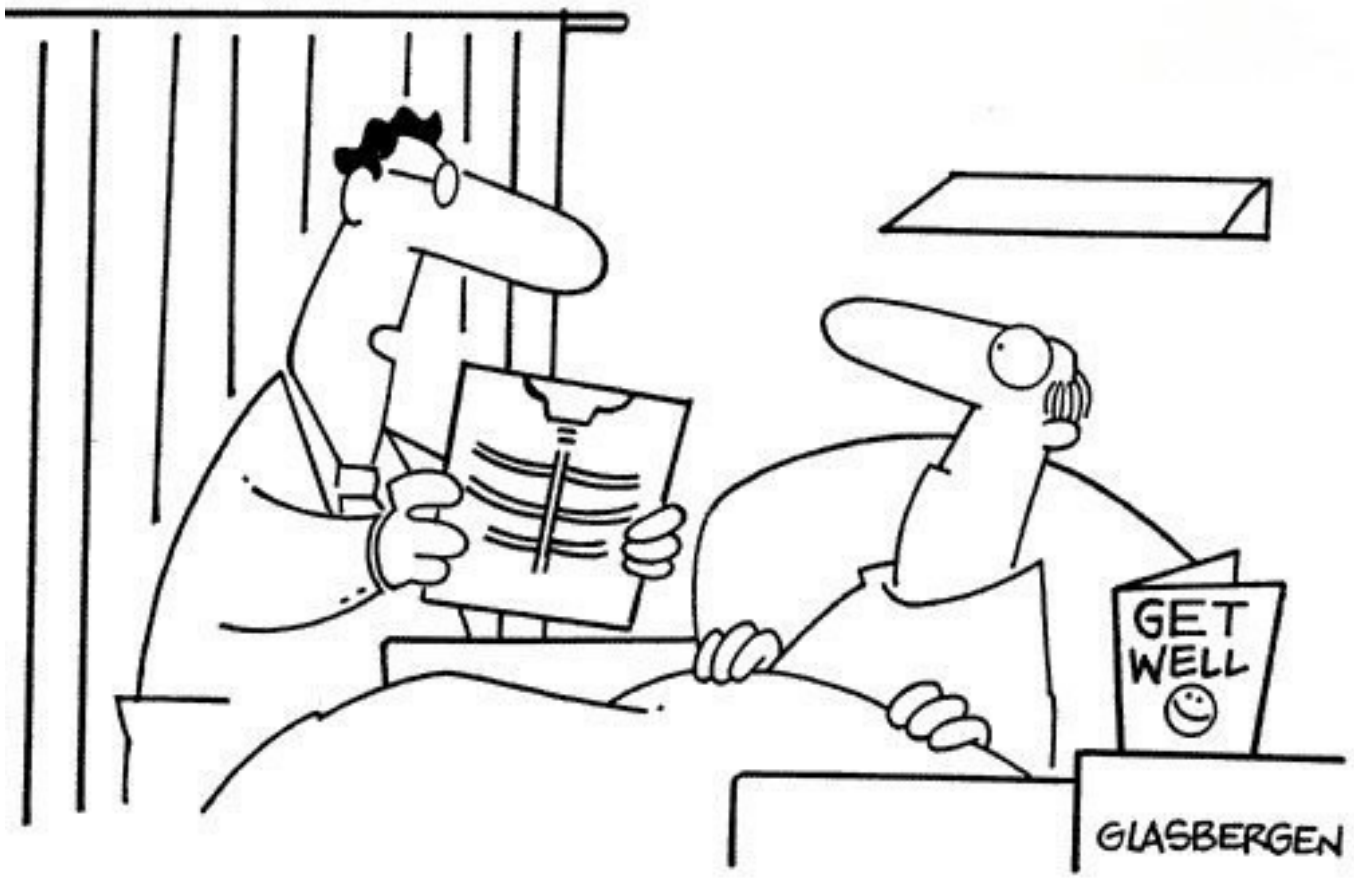
# The potential is endless!




**"Your x-ray showed a broken rib, but we fixed it with Photoshop."**

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**Contact: davidbrettle@nhs.net**

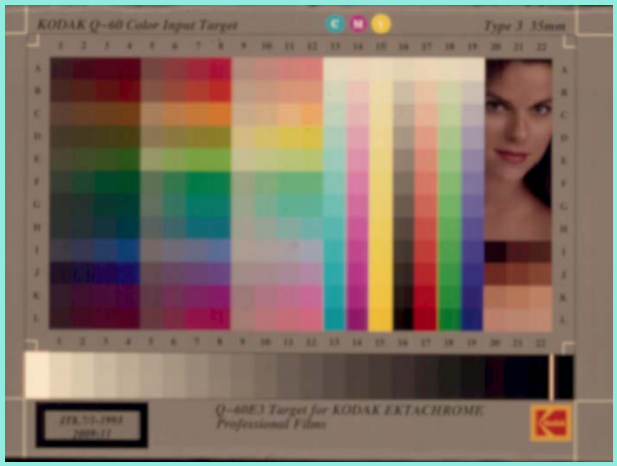
# (Color) Calibration update

**Bas Hulsken**
Philips Digital Pathology Solutions
June 19, 2014

innovation + you

PHILIPS

# Color calibration in the current Philips scanner




Standarized Color Target

Scanner

Residual Error (ΔE)

Color Profiler

Correction Matrix

Reference



PHILIPS

# Results

- Calibration makes scanner colors more consistent
  - Before: mean scanner: 1.8+/-1.0ΔE, worst scanner 4.7ΔE
  - After: mean scanner: 1.2+/-0.7ΔE, worst scanner 3.5ΔE
- Calibration brings scanner colors closer to the "ground truth"
  - Before: mean scanner: 10.1+/-0.4ΔE
  - After: mean scanner: 3.5+/-0.3ΔE
- Color targets (phantoms) can be made in a reproducible fashion
  - Mean error between color phantoms: 0.6+/-0.3ΔE
  - Resolution and contrast and noise influence color perception (and overall image quality perception) even if they don't quantitatively influence color.
- Best "calibration mode" found:
  - Linear correction Matrix works best! (better than non-linear LUT)
  - Because: over-fitting and local-optimization (film ≠ stained tissue)

# Exploratory “Phantom” work



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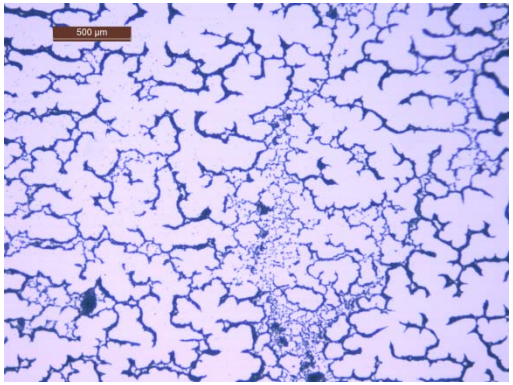
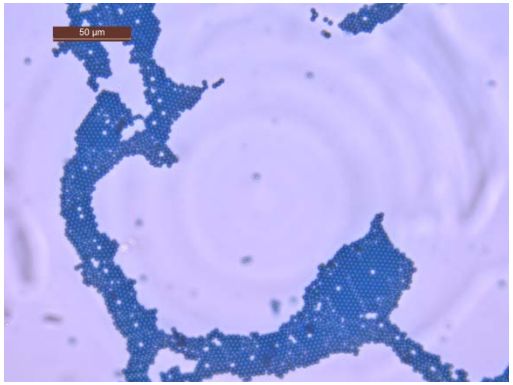
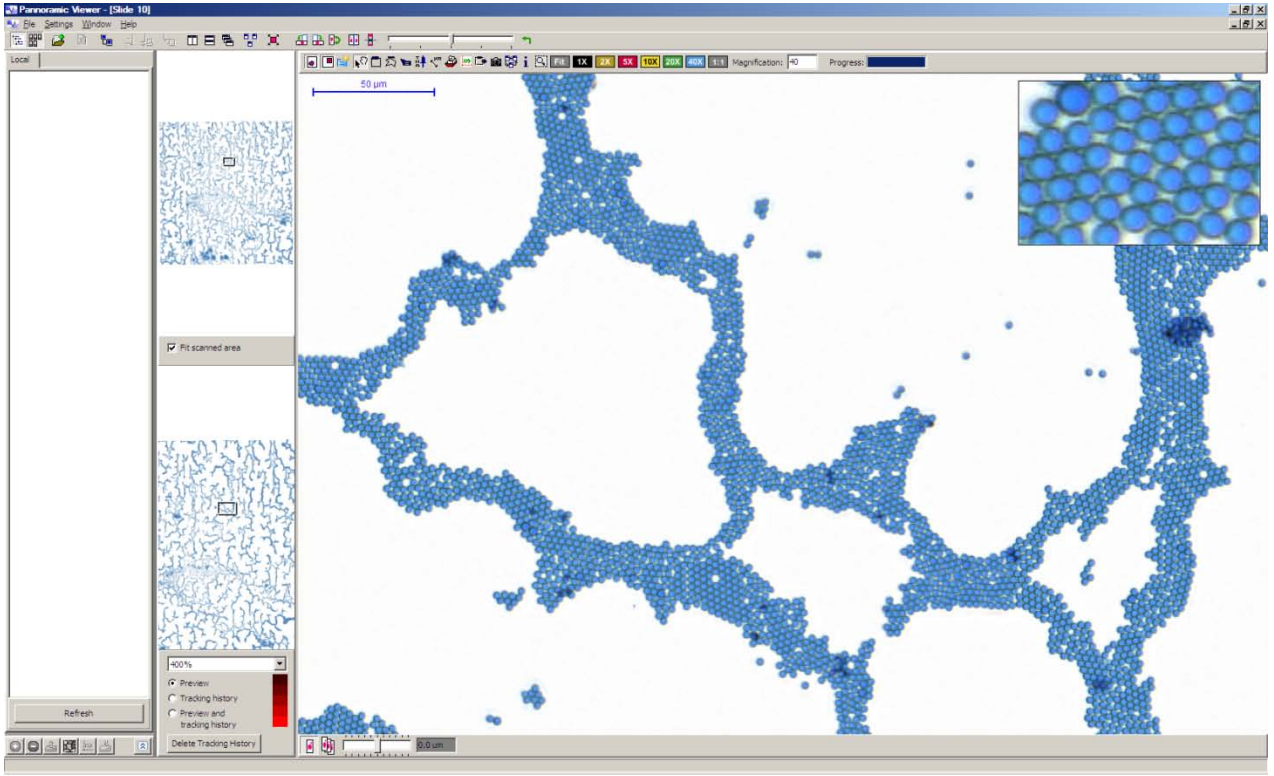
# Stained beads

- Tissue-like patterns, monolayers of beads, tunable bead size (1um to 10um)
- Individual beads can be resolved



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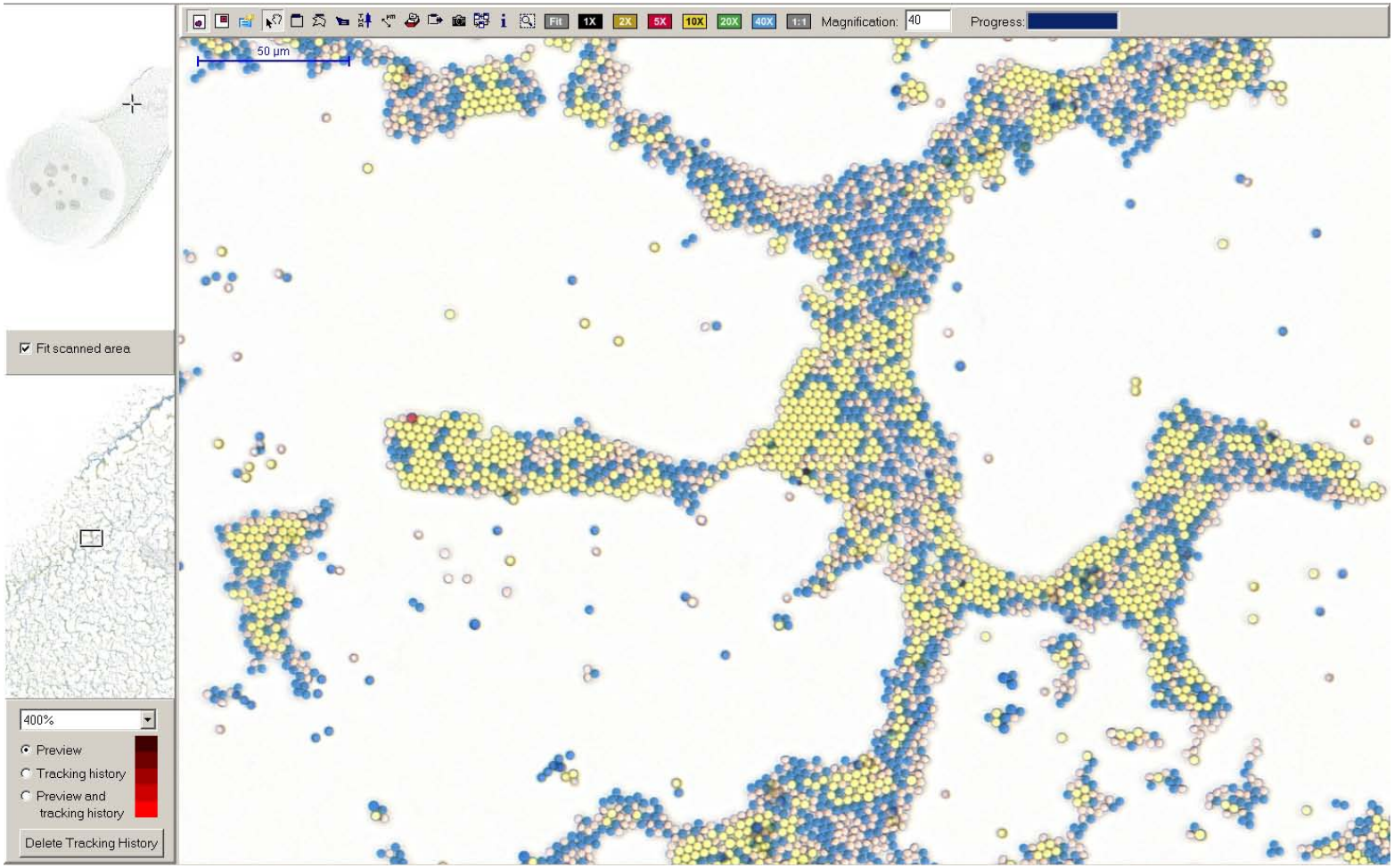
# Mix multiple colors



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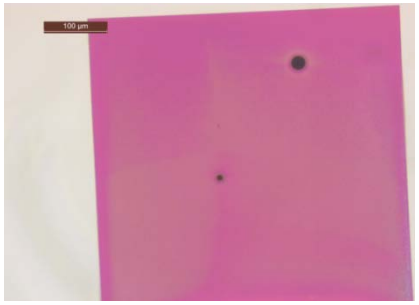
# Plasmonics, a possible way towards stable, and well defined color phantoms

- Well defined colors
- Transmission &
- Reflection

4x

10x



20x

40x



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