Calibration Slide for Histopathology task force
Teleconference
21 January 2014 • 15:00 (UK) / 10:00 (EST)

The meeting was called to order at 10:00 am (EST) by Craig Revie, chair of MIWG, with the following attendees:

Pinky Bautista	MGH PICT center
Wei-Chung Cheng	FDA
Scott Forster	Ventana
Phil Green	ICC
Bas Hulsken	Philips Healthcare Incubator
George Hutchinson	FFEI
William Li	Eastman Kodak
Tom Lianza	X-Rite
Allen Olson	Leica Biosystems
Debbie Orf	NPES
Craig Revie	FFEI
Darren Treanor	Leeds University
Viktor Varga	3DHISTECH
Chih-Lei Wu	FDA
Dave Wyble	Avian Rochester, LLC
Yukako Yagi	MGH/Harvard Medical School

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

1. Response to call for assessment methods
   - Calibration assessment slide using filters
   - Pros and Cons relating to use of film targets for scanner calibration
   - Slide measurement apparatus and procedure
   - Two-slide solution for calibration
   - FFEI calibration slide using pathology stains
2. Digital microscope test materials and test methods
3. Any other business
4. Next meeting

Mr Revie reviewed the goals of this project [see attached] and suggested that this meeting should decide on candidate test materials.

1.1 Calibration assessment slide using filters

Professor Yukako Yagi presented an outline of the filter-based method she had developed [see attached], with a focus on the purposes, benefits and assessment criteria.

Users of whole-slide image capture systems do not necessarily learn microscopy, so the images are not perfect. Ideally a system would be able to calibrate a capture to a perfect colour reference, but at present we do not know what that is. Yagi-sensei had observed that observers prefer the slide white point to correspond to the display white in the case of digital captures, indicating that a media-relative colour reproduction goal would be appropriate.

In her system two different calibration slides are used, which makes it possible to determine the cause of colour problems. It was important for vendors to understand colour variation in the system and between devices such as scanners, and this would help with standardization.

The benefits of the approach were that the calibration slides were easy to reproduce, low cost and easy for users to understand their use. Currently there are 9 colours filters on a calibration slide, and addition of greyscale filters is being considered. The spectral transmittance of the slides is measured before they are shipped.

Assessment criteria that could be tested by the calibration included the reproducibility of the scanner and display and the effect of viewing and processing software. Her algorithm corrects the slide colours by calibrating the whole-slide capture system 'in the factory' and then a manual recalibration of the monitor is performed in the laboratory if such a correction is needed.

Dr Allen Olson noted that differences in microscope illumination source and other factors might result in the calibration not predicting a match of a pathology slide, particularly as different scanner spectral response would give different responses and thus introduce metamerism. Professor Yagi reported that the system had been tested on different systems and appeared to work satisfactorily.

It was agreed that Professor Yagi would make the slides and procedure available so that companies participating in the call could assess the calibration system.

1.2 Pros and Cons relating to use of film targets for scanner calibration

Mr Tom Lianza presented some observations based on his experience in measurement and calibration in graphic arts [see attached]. The IT8.7/1 calibration slide standard defined measurement geometry and target colour values, and was designed to calibrate the scanner and film combination, since it was well known that the dyes in different film products had different spectral characteristics. Diffuse scattering is a problem in transmission densitometry, and in practice measurement includes scatter characteristics which may differ from the microscopy capture. He suggested a single calibration target design should be the goal. His recent experience working with the motion picture industry on their move to digitization of film had found that once the calibration was done, all the scanners were within specification. The goal of this group should be to define a specification and tolerances for standardization purposes.

For the mouse embryo image in Yagi-sensei's procedure, one could look at the histogram in *x,y* or *u',v'* chromaticity. Devices will give different scattering results depending on the dye properties. When

comparing the output of a test slide and a reference, one should see a bimodal distribution, and one can evaluate the mean values between two scanners to see if they match. It was agreed that it was necessary to control for illumination in this procedure.

Dr Bas Hulsken noted that some vendors have already used the IT8.7/1 slide for microscope calibration, and agreed to share results and physical slides in the following week.

1.3 Slide measurement apparatus and procedure

Dr David Wyble presented some experience on the measurement apparatus for slide calibration [see attached] based on his work in this field with Omnyx. He had measured spectral transmittance of colour patches, with a setup based on the recommendations of ASTM E1348-11. His arrangement mounted a ColorChecker-like slide design onto glass using adhesive and a transparent cover sheet. The measurement zone was 1mm, and the aperture underfills the port of the spherical diffuser. A Konica-Minolta CS2000 was used to record the spectral measurement, and in addition a camera was used to record the actual measurement area for verification purposes.

Mr Revie noted that this design is different from the microscope geometry, where the light beam is parallel and the depth of field is also different. Dr Wyble agreed and stated that he had converged on this geometry in order to adhere to a published standard. Mr Revie reported that his company, FFEI, have developed a measurement system based on a modified scanner, whose geometry replicates the geometry of the slide scanner. It measures a 5 micron area, averaging the capture on the same region as seen by the scanner. The whole slide is illuminated using the actual microscope illumination. He may be able to share more details of this system with the group in the future.

It was agreed to hold a round robin to compare measurement results from different vendors for a single slide.

1.4 Two-slide solution for calibration

Dr Allan Olsen discussed some ideas around a two-slide solution for calibration. He suggested the problem is best split into calibration and assessment, with calibration performed by vendors of a device or system. A modular approach to calibration was preferred, although ultimately an end-to-end calibration could also be carried out once the different system components were calibrated. The working group could make recommendations on suitable calibration methods.

For validation, the things to include were testing against requirements, and the correct operation of the system. Different types of test materials may be needed for calibration and validation. The latter is an infrequent test, while the operational test is simpler and could use materials scanned following primary calibration in order to provide transfer standards for end use.

A test slide must be tested using histology stains or materials with equivalent spectral properties. His company used a spectral approach to calibration and characterization.

1.5 FFEI calibration slide using pathology stains

Mr George Hutchinson presented a proposal for a slide to test a calibrated system [see attached]. This had been developed in conjunction with the University of Leeds. They had developed a biopolymer which absorbs stain in a similar way to biological tissue, and built a measurement system using a modified scanner. They have access to a database of measurements to act as a reference.

They investigated the interaction of stain colours and found the results were essentially a linear addition in density. Their design overlaid strips of different densities to generate 48 combinations of two stains. The proposed slide design has space for a barcode or other identification, and additional stain combinations. Tests showed good results for a wide range of two-stain protocols, and they are working on three-stain ones. Their goal is a single calibration slide.

The biopolymer thickness can be controlled to match actual tissue samples, with a 10-30 micron range.

The slide could be considered as a calibration standard candidate. FFEI's goal was to make the slide commercially available to end users at a reasonable cost. They have filed IP on the process but would welcome feedback in order to decide how to direct the commercial development of the product. FFEI would consider licensing or sub-contracting the manufacturing and/or marketing of the end product. They could also consider collaborating with others, such as Professor Yagi to develop a 9-patch slide with different stain protocols.

Dr Wyble suggested that it was important to avoid over-stating the need to match actual stain spectra, as optical properties such as scattering could be more important than the spectra. He agreed that the FFEI proposal potentially meets the requirements of matching the stain spectra and the capture geometry.

Dr Olsen suggested that it was necessary to know the purpose and the precision and accuracy requirements of the calibration process, as it was possible that an existing film-based target might be sufficient. The issue to test is whether there are stain combinations that are seen as a single colour by the human visual system, but as different by a device.

Mr Revie agreed, noting that the approach allows two of three different stain protocols to be included and the different protocols could be assigned different weights for different applications. FFEI have studied differences in haematoxylin, and in this case found the differences to be very small and not requiring different calibration targets.

They had also studied slide stability. In non-bright storage conditions some fading had been found, especially in the fluorescent dyes. In general the slides were stable for 6-12 months if the storage conditions were good, so anticipate that slides would have an expiry date. They are also working on modelling the decay over time to extend the expiry.

Dr Olsen stated that it was important not to specify a calibration procedure that locks users in to a specific product and vendor. Mr Revie responded that they were looking at a consortium approach where an industry body or association funds the development. He asked the group to consider this, and suggested a follow-up discussion by email.

2. Digital microscope test materials and test methods
The document has been updated and Mr Revie undertook to circulate the latest version to the group.

3. Any other business
There was no other business

4. Next meeting
The next meeting was scheduled for February 20 at 15:00 GMT (10:00 EST). Two hours will be scheduled for this call, and other MIWG members invited to participate.

The next full meeting of MIWG is 3 March in Tokyo. The timing will be inconvenient for the US East Coast, but will allow for participation by Asian, European and West Coast vendors.

Actions:

MIWG-14-05 Share slides and procedure for testing by other members of the group (Yukako Yagi)

MIWG-14-06 Organise circulation of a single slide to vendors with measurement capability for a round robin test (Craig Revie)

MIWG-14-07 Circulate proposal for biopolymer-based calibration slide for consideration by the group for consortium funding or other development (Craig Revie)

International Color Consortium

Calibration slide for histopathology

Teleconference

January 21st 2014

Calibration slide for histopathology

January 21st 2014 (2-hours teleconference)

- **Response to call for assessment methods (see next slide)**
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- **Digital microscope test materials and test methods (Craig Revie)**
- **Any other business**
- **Next meeting 20th February 2014 (task force update)**

- **Deferred**
  - Scott Forster plans to make a presentation in a future meeting
  - Stephen Hewitt has been working on some ideas for a calibration assessment slide but has declined to make a presentation at this stage

Call for digital microscope colour assessment test materials

- **We need a test to demonstrate digital microscope colour accuracy**
  - objective test that needs no knowledge of how the digital microscope operates
  - no knowledge of the calibration scheme should be required to perform the test
  - aim is to demonstrate that colours in the images produced by the digital microscope accurately reflect the colour in the material being scanned
- **Simple test**
  - reference microscope slide is scanned to create an image of the slide
  - colours in this image are then analysed and compared with the colour values as measured on the reference slide
- **This call is to identify suitable test materials**
  - ideally we will agree a single method to assess these devices
  - perhaps create a short list of options and identify the benefits and shortcomings of each method
  - conduct round-robin testing using the materials provided

Calibration slide for histopathology

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Calibration assessment slide using filters

Yukako Yagi, PhD
yyagi@partners.org
Director of the MGH Pathology Imaging & Communication Technology Center
Assistant Professor of Pathology, Harvard Medical School
Affiliate Faculty, Wellman Center for Photomedicine, MGH

MASSACHUSETTS GENERAL HOSPITAL
PATHOLOGY

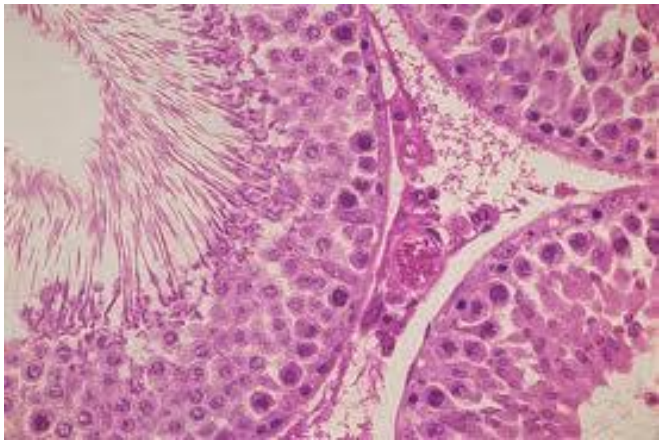
HARVARD MEDICAL SCHOOL

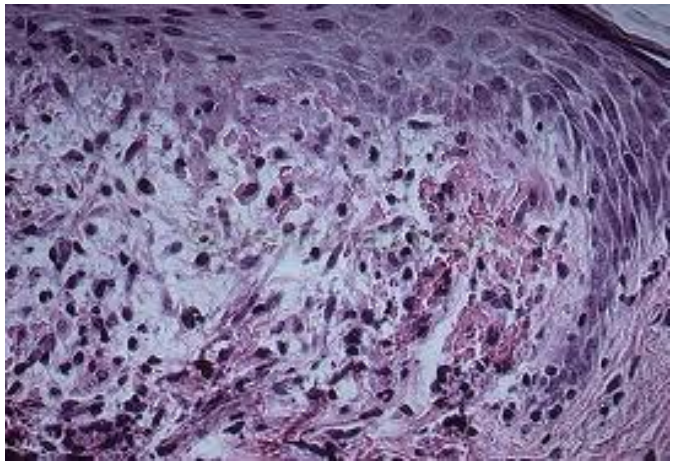
Today's Topics

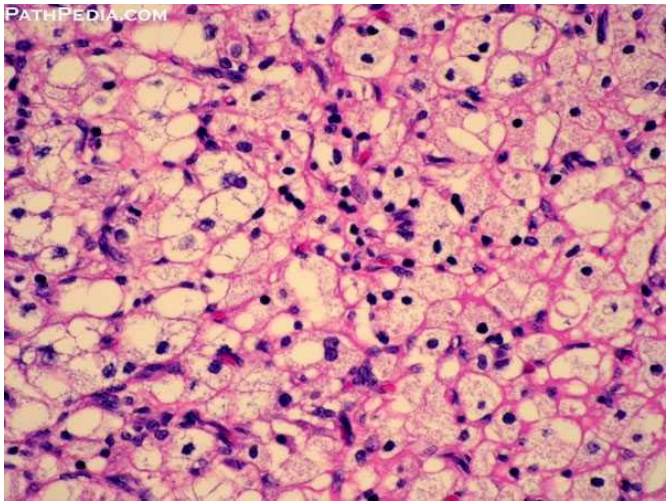
- Purposes
- Benefits
- Assessment Criteria

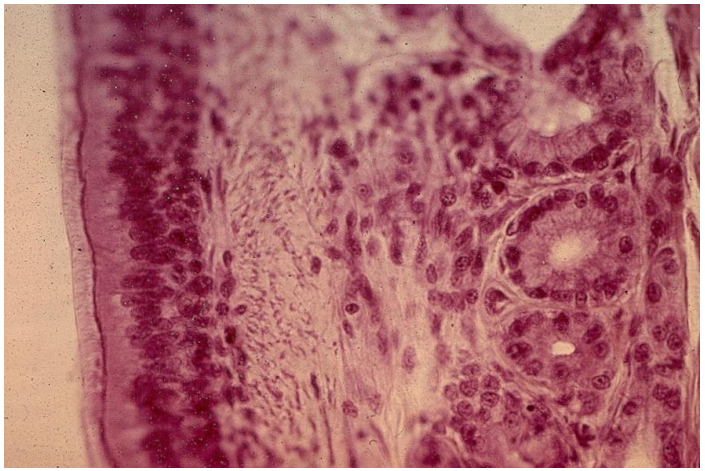
Background

Typical H&E Color under the pathologist's microscope



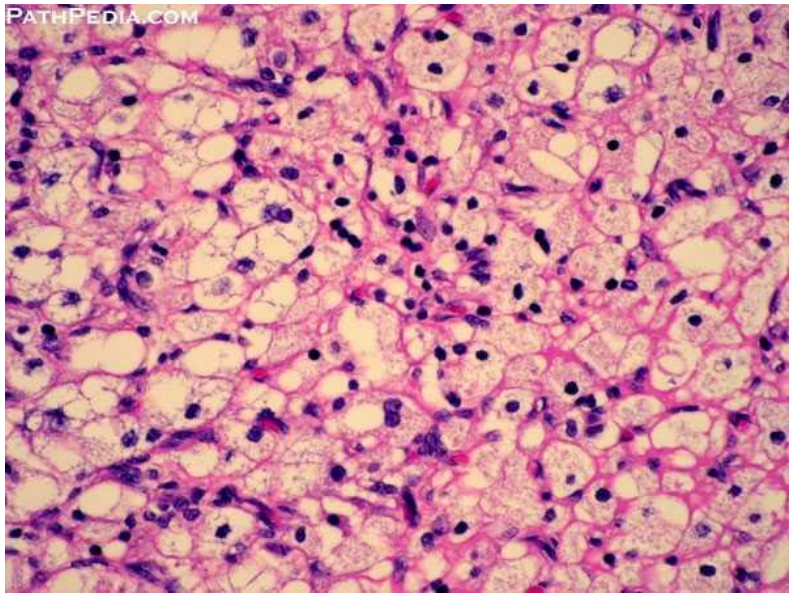




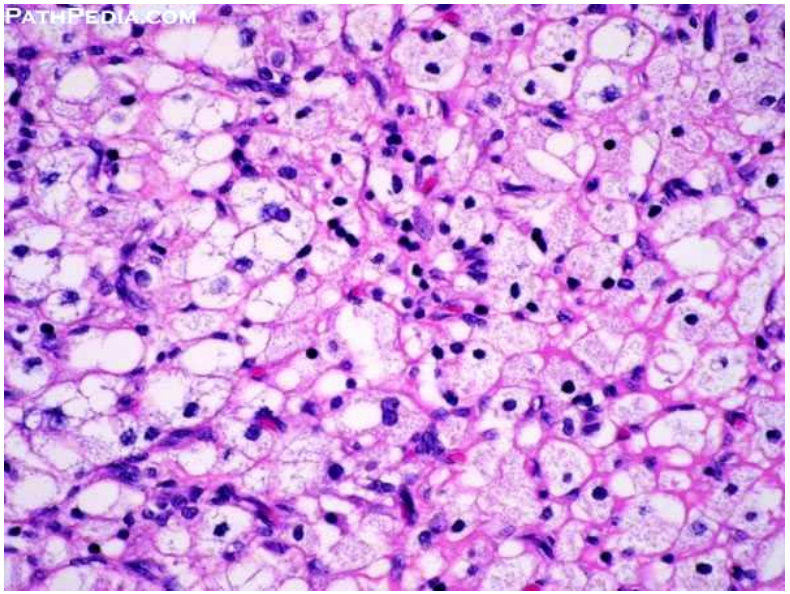


Background

Microscope



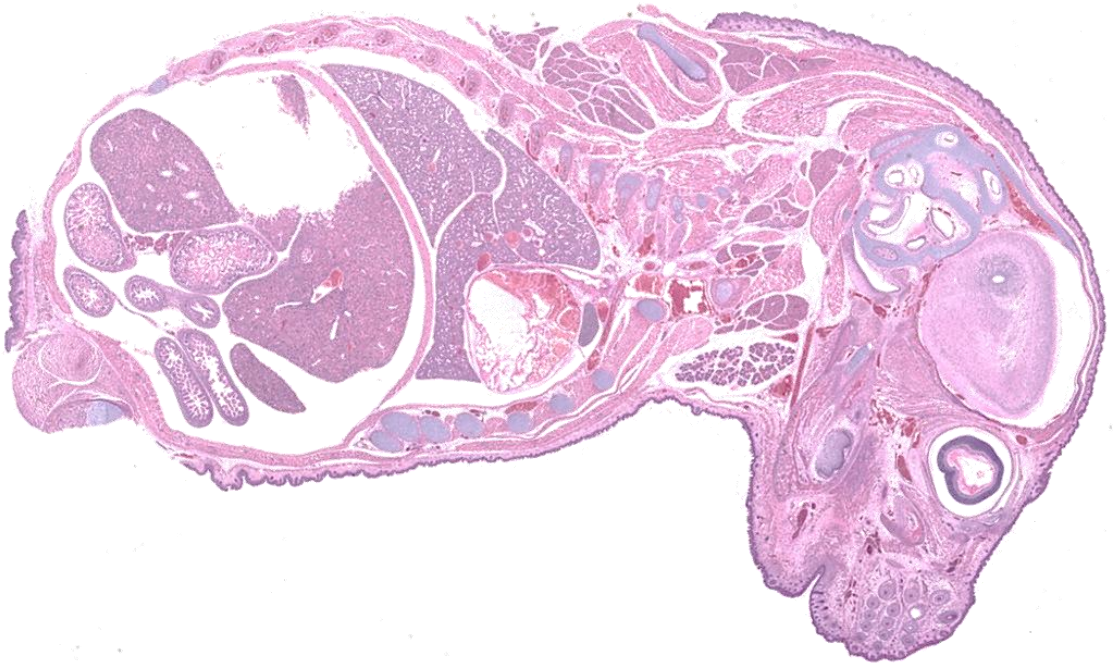
Digital Camera



Pathologists prefer white background

Background

WSI



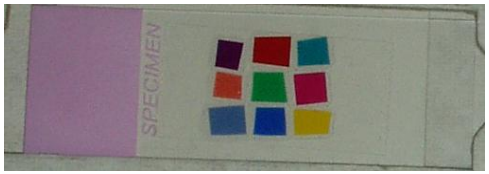
perfect color

↓

Reference

Pathologists want to see the "perfect color"

Purposes



As Scanner Users

- To find the causes of color issues
- To demonstrate the color variations

Pathologists

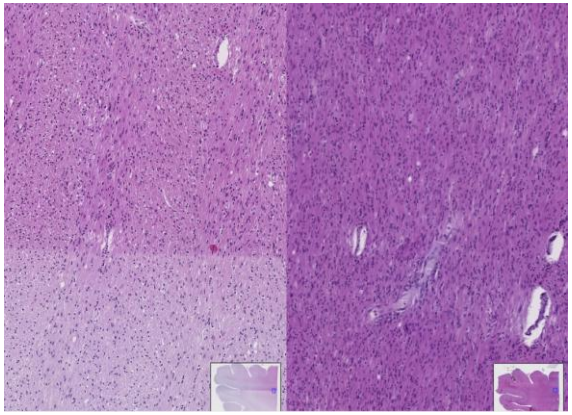
- Have pathologists to know the cause of color issues issues is not only “Scanner”
- What pathologists can responsible

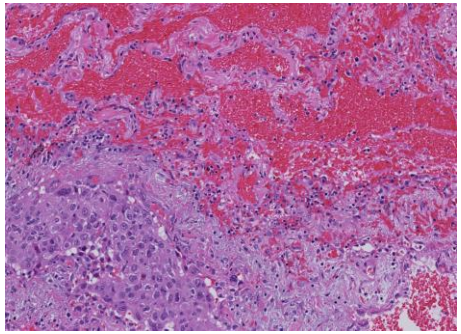
Vendors (mainly scanners and monitors)

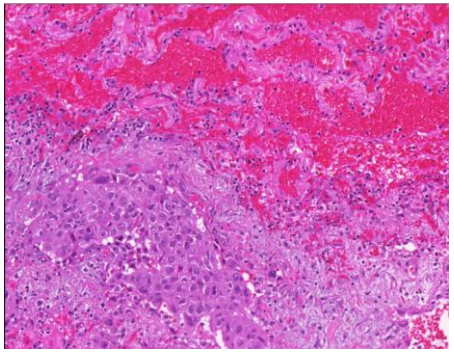
- Have vendors to understand color variations within a system, a product, and between other scanners.
- To support vendors to standardize the color

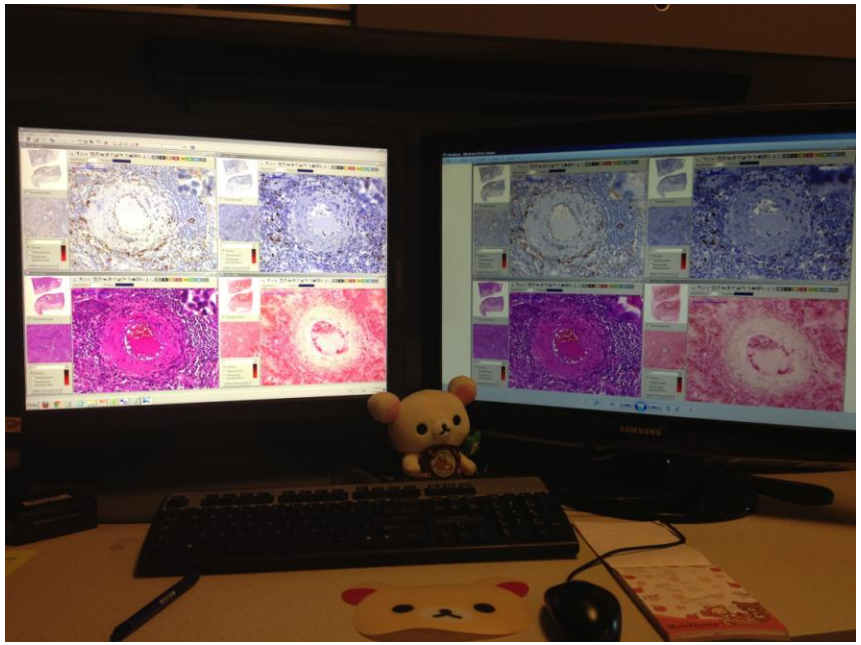
Towards Standardization

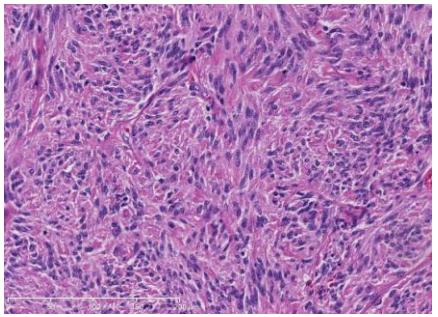
Color variations

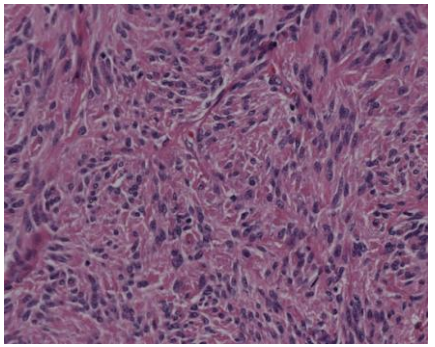








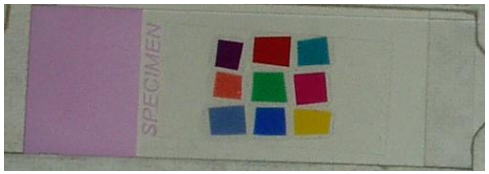




Today's Topics

- Purposes
- **Benefits**
- Assessment Criteria

Benefits



- Easy to reproduce
- Low cost

Pathologists

- Easy to understand the concept
- Easy to understand the importance of standardization
- Feel comfortable with “mouse embryo” H&E slide more than just the color chart

Vendors (mainly scanners and monitors)

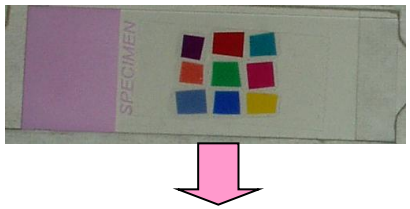
- Easier for maintenance
- Easier to communicate remote users

Towards Standardization

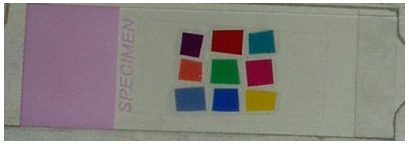
Concept

We measure the spectral information of each patch before shipping

Scanning

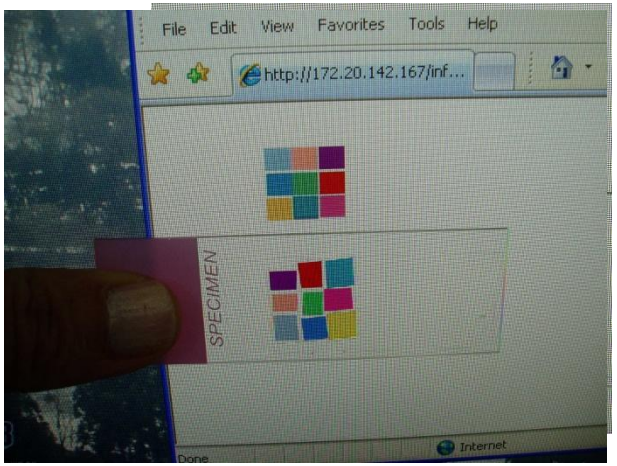


VS



(Review) Display

The Imaging web site has the colors of the Calibration slide.



Compare the displayed colors of the calibration slide to their actual colors to understand the difference

The Imaging web site has Calibration slide.

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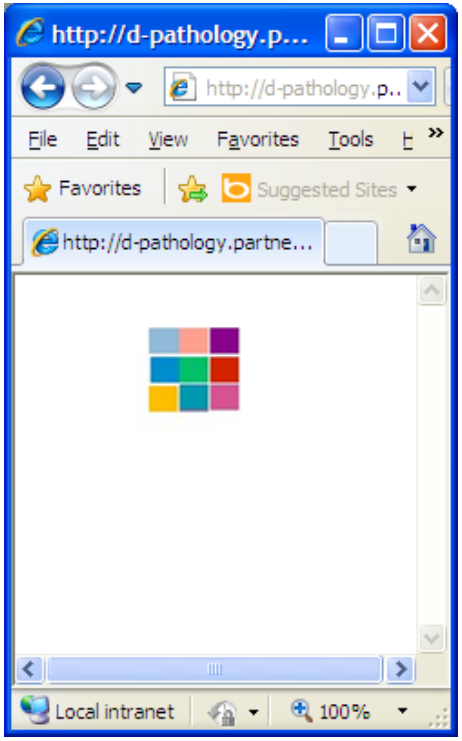
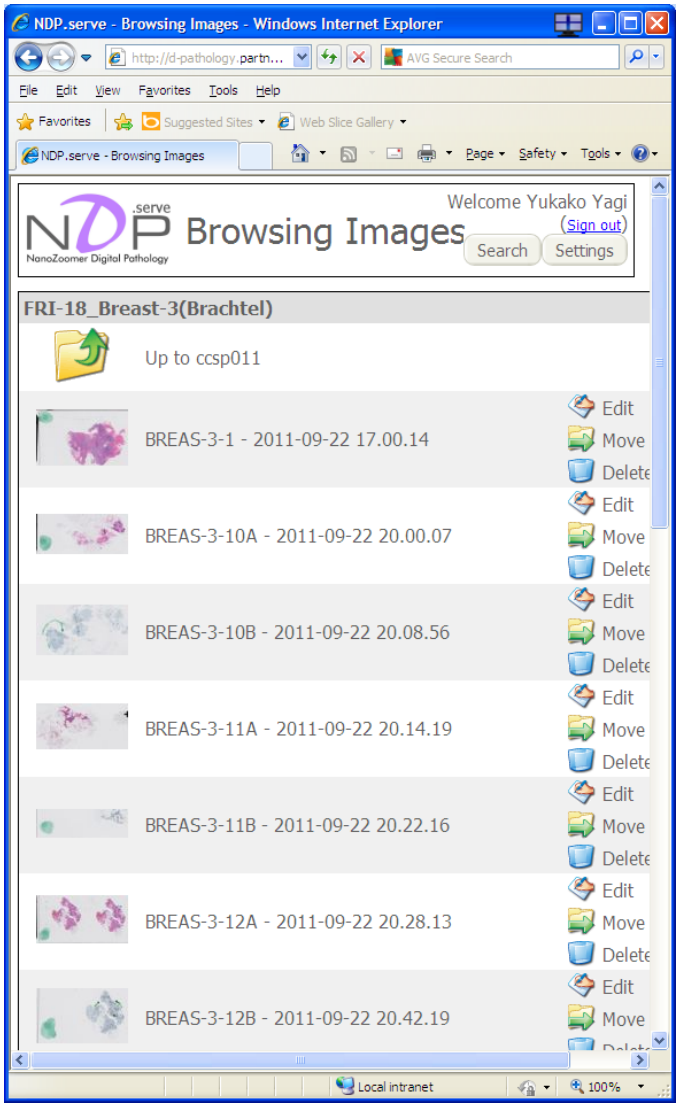
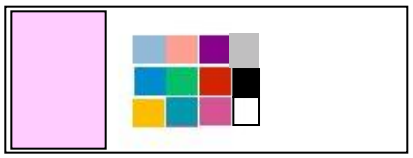
Concept

Before looking at WSIs, the pathologists check the color chart like some Radiology Image Viewing system





Additional filters?

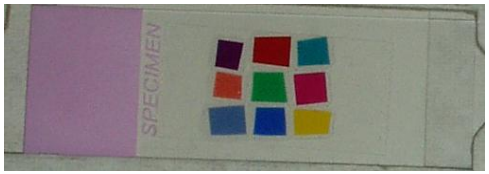


- ND: 50%
- Black
- White

Today's Topics

- Purposes
- Benefits
- **Assessment Criteria**

Assessment Criteria



Scanner

- **Reproducibility: Over time (often change by the program update)**
- **Between scanners:**

Monitor

- **Reproducibility: Over time (often change by the program update)**
- **Between monitors**

Software (such as viewer or image analysis..)

- **To make sure the viewer does not change the color without intention.**

Towards Standardization

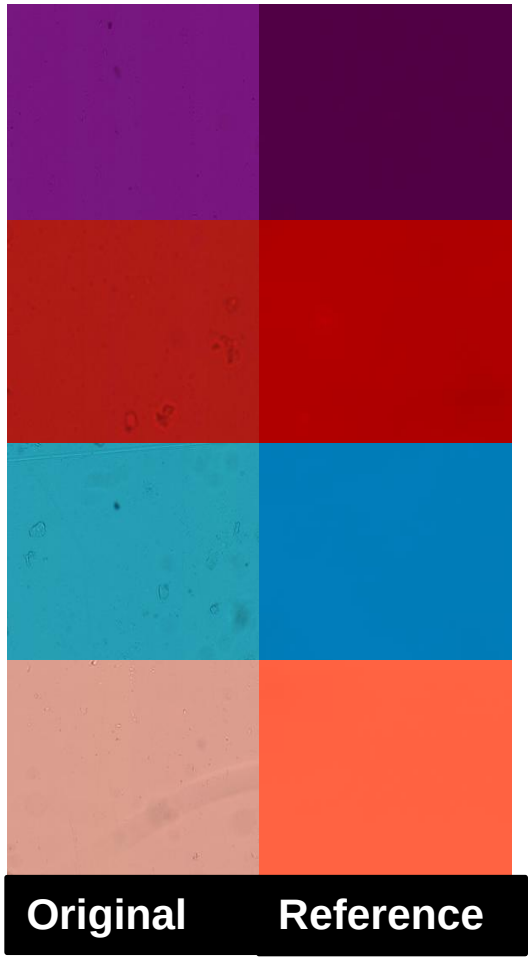
Color transformation matrix per scanner

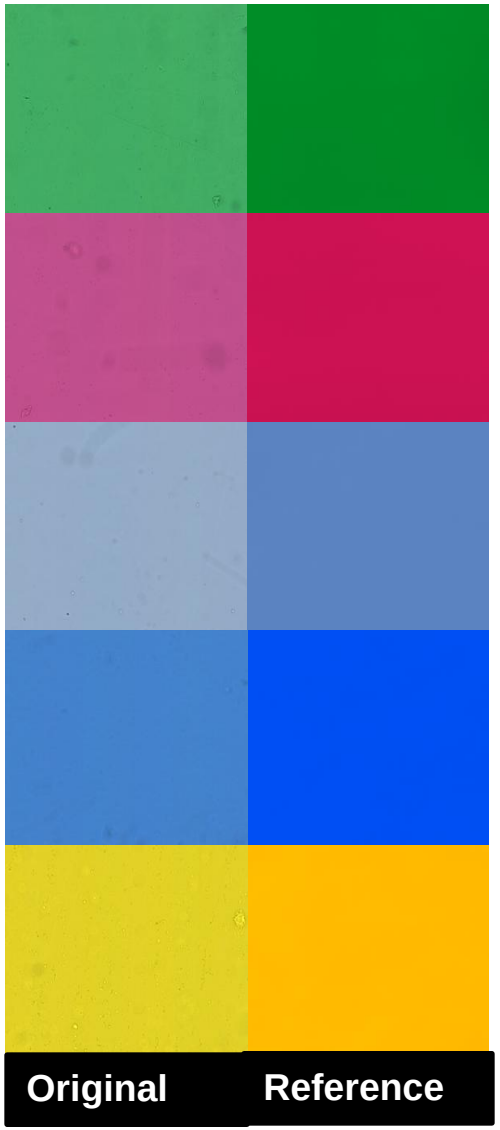
Color patches

- Colors are not accurate enough
- Standardize using the original and reference color patches

Original - Produced by a whole slide scanner

Reference - Produced by using spectral information of the patches





Polynomial transformation

$$\begin{pmatrix} R \\ G \\ B \end{pmatrix} = \begin{pmatrix} a_{1,R} \ldots . a_{m,R} \\ a_{1,G} \ldots . a_{m,G} \\ a_{1,B} \ldots . a_{m,R} \end{pmatrix} \left[\theta_m \begin{pmatrix} R \\ G \\ B \end{pmatrix} \right]$$

Color of the patches as produced by a particular scanner

Reference color of the color patches

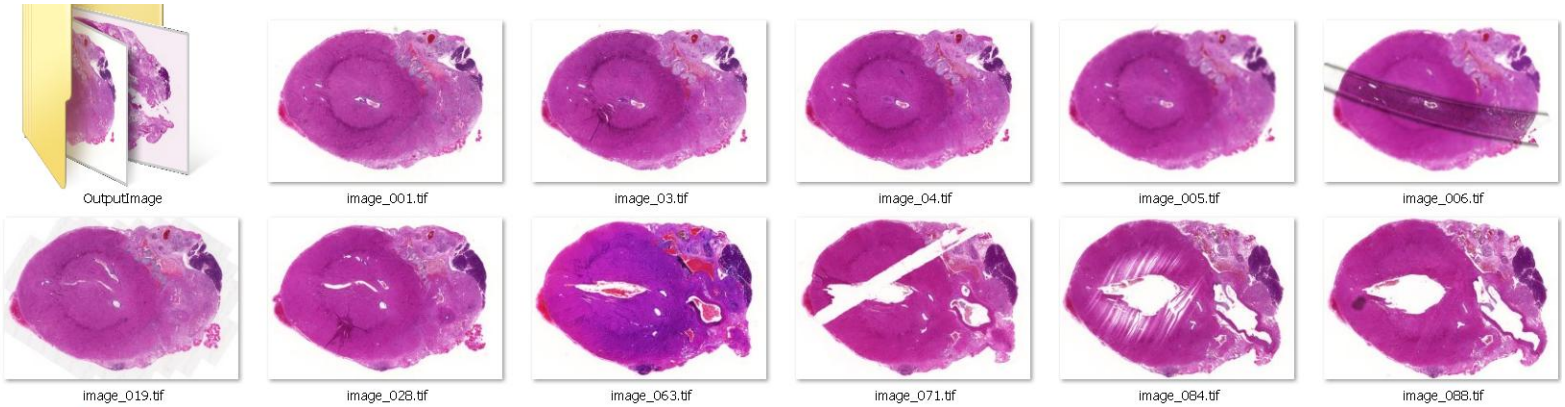
Color transformation matrix will be stored for used in color standardization

Each scanner will have its own Color transformation matrix

Pathologists have been changing..

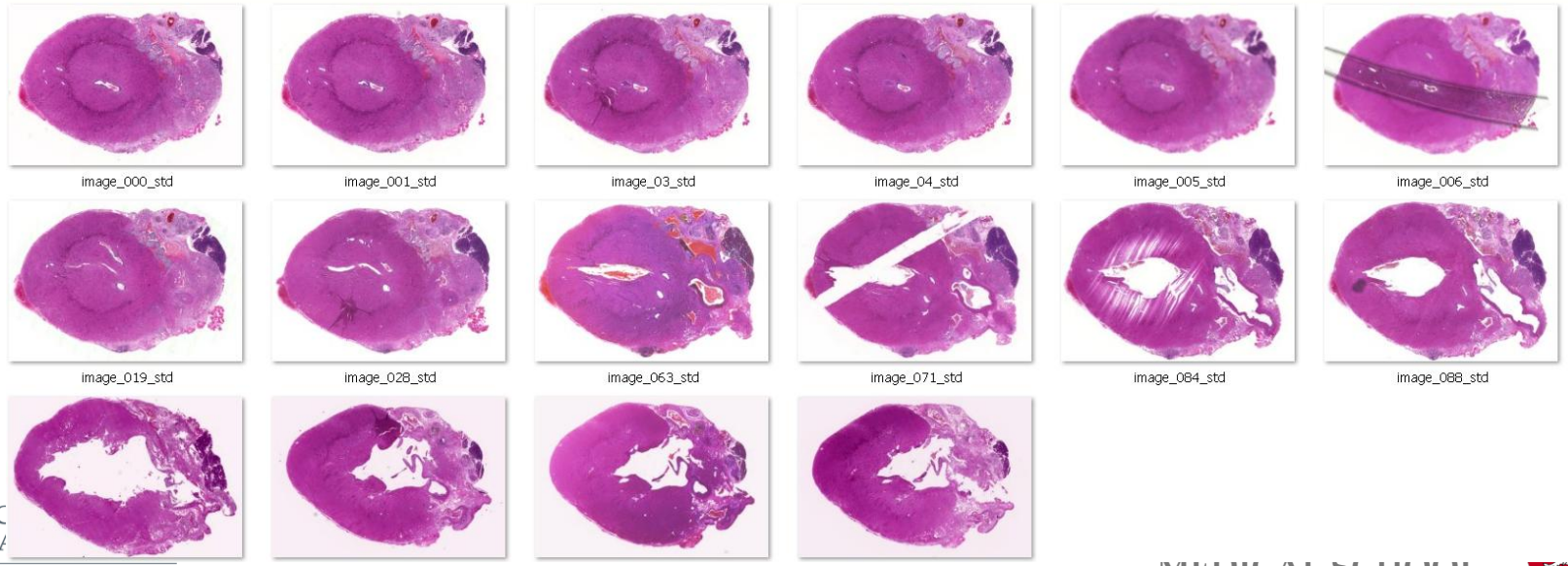
Pathologists are asking us to standardize the color of digital images

Examples: Color issues in WSI 3D (Staining)



Original

Standardized



Thank You!

SequelCsi

Pros and Cons relating to use of film targets for scanner calibration

Tom Lianza

SequelCsi

Original Standard

- *IT8.7/1 - 1993 (R2003) - Graphic technology - Color transmission target for input scanner calibration: This standard defines an input test target that will allow any color input scanner to be calibrated with any film dye set used to create the target. It is intended to address the color transparency products that are generally used for input to the preparatory process for printing and publishing. This standard defines the layout and colorimetric values of a target that can be manufactured on any positive color transparency film and that is intended for use in the calibration of a photographic film/scanner combination.*

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Important note:

- The target is designed to calibrate different scanners to measure a specific film type.
- This method of calibration does not anticipate accurate results with different color media.
- Differences in scanner spectral sensitivity functions will be minimized (probably) but accuracy with other media is not guaranteed
- The film target is sensitive to optical geometry as well. Diffuse scattering is a real issue in transmission densitometry.

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Recommended studies

- Determine the desired colors on the target
- Compare these colors spectrally to the measured colors of the various stain samples
- Run an arms length Round-Robin experiment with a single target shared by multiple vendors to compare calibration target rendering capability

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Pros and cons

- Film targets are routinely made (Pro)
- Film targets can be calibrated (Pro)
- Film targets have limited spectral content (con)
  - Generally only three dye components.
- Film targets may have different scattering properties than (con)
- The optical geometry used to calibrate may not simulate the optical geometry of the scanner (potential con)

Measurement Apparatus for Film Slide Calibration

David R Wyble

ICC Medical Imaging Working Group
Calibration Slide for Histopathology Teleconference
January 21, 2014

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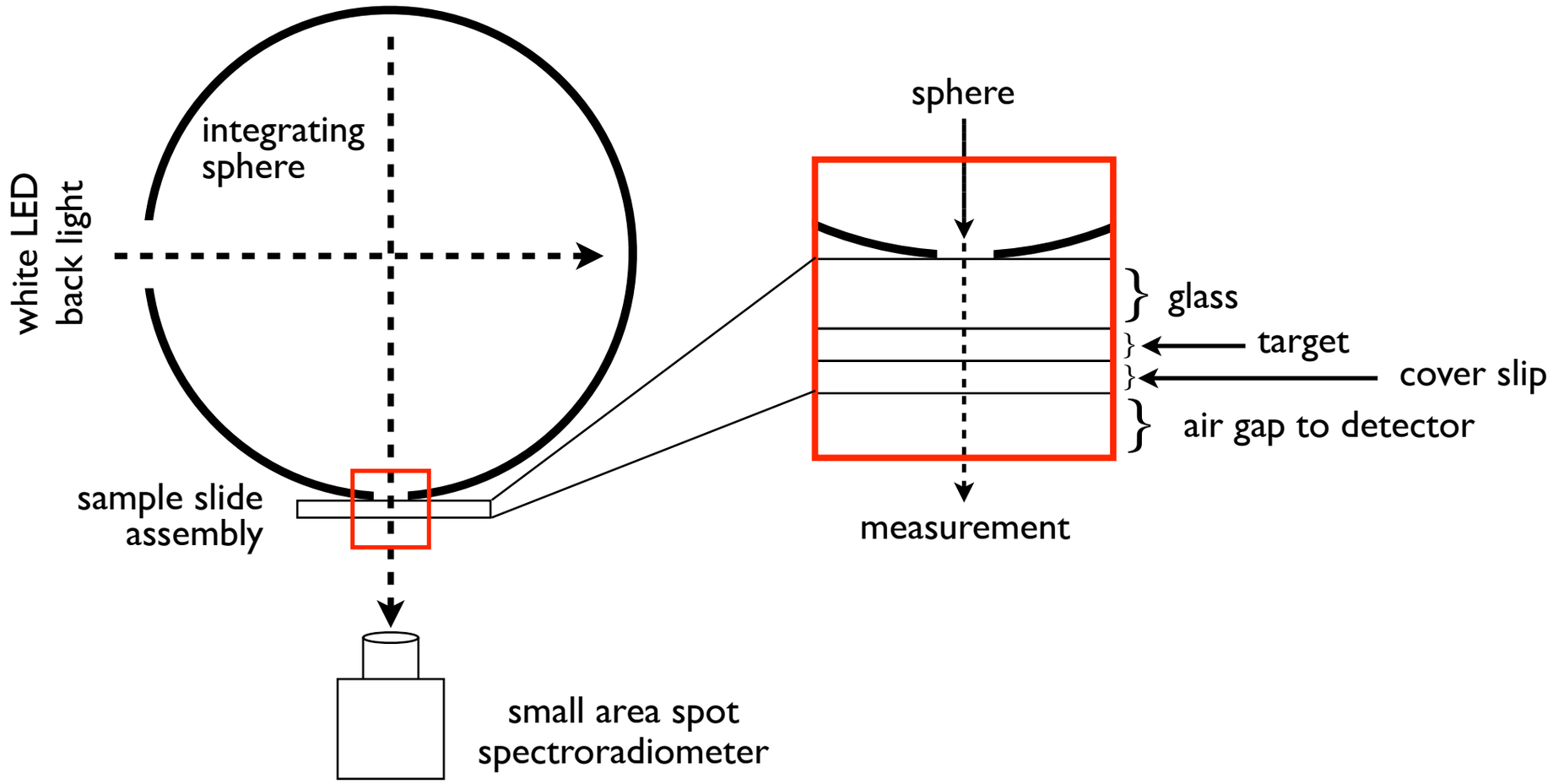
Geometry Overview "d:0°"



ASTM E1348-11: "*Standard Test Method for Transmittance and Color by Spectrophotometry Using Hemispherical Geometry*"

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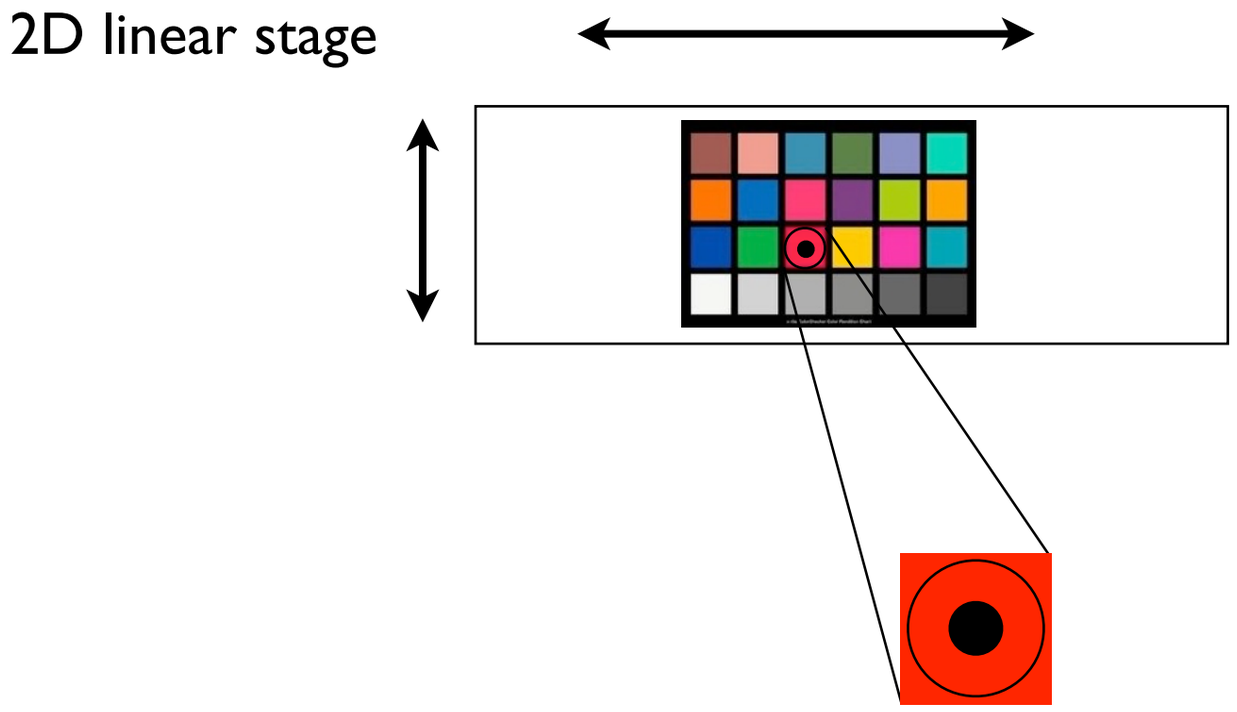
Sample Area Dimensions



red = sample square (4.25mm)
circle = sphere port (3mm)
solid dot = measurement zone (1mm)

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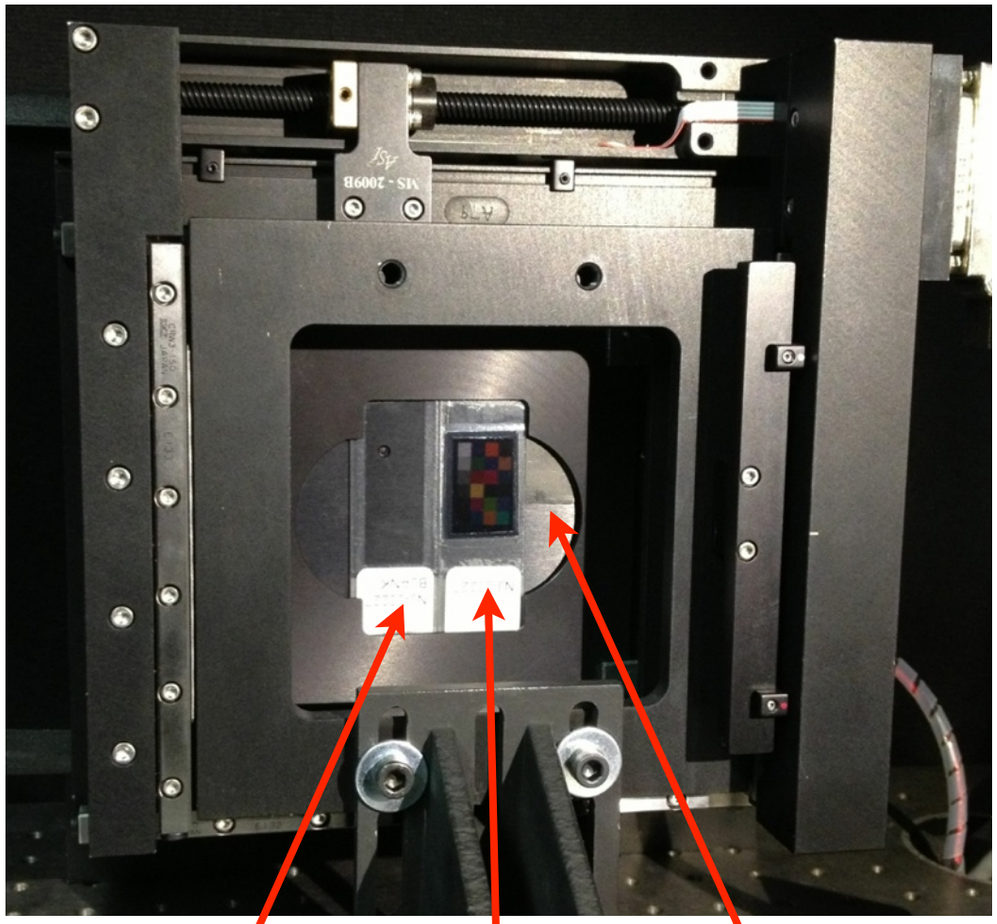
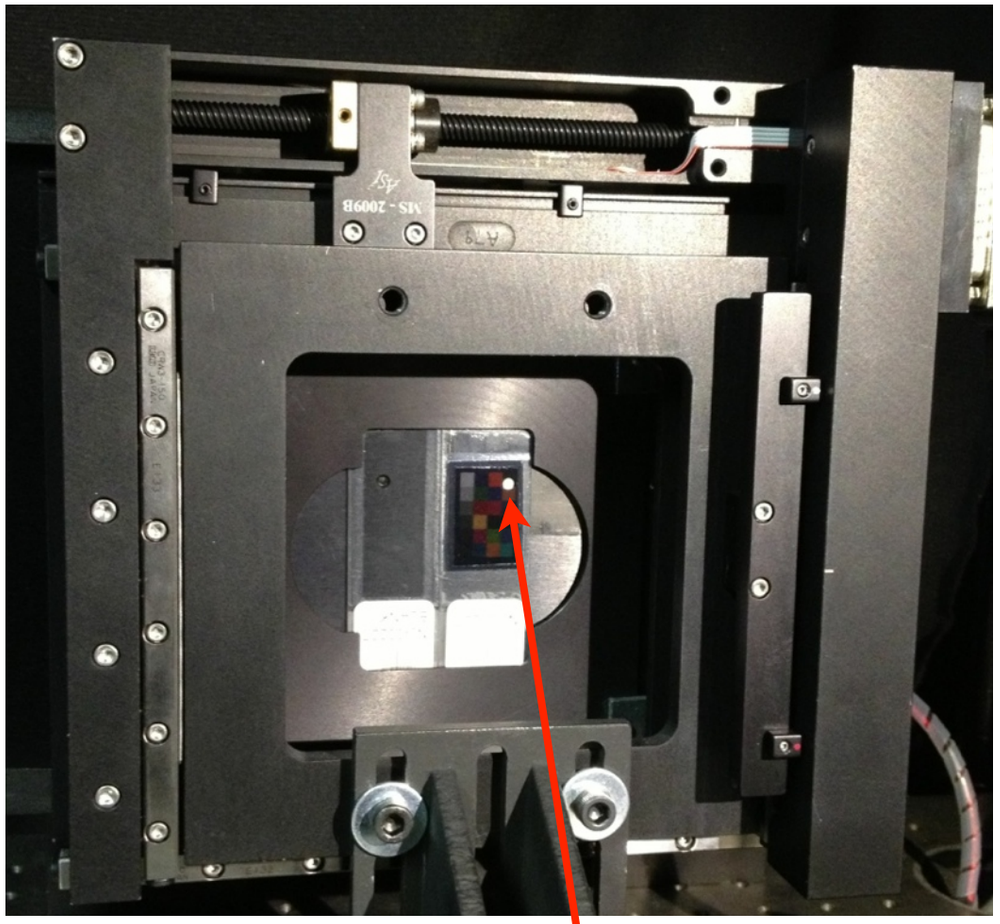


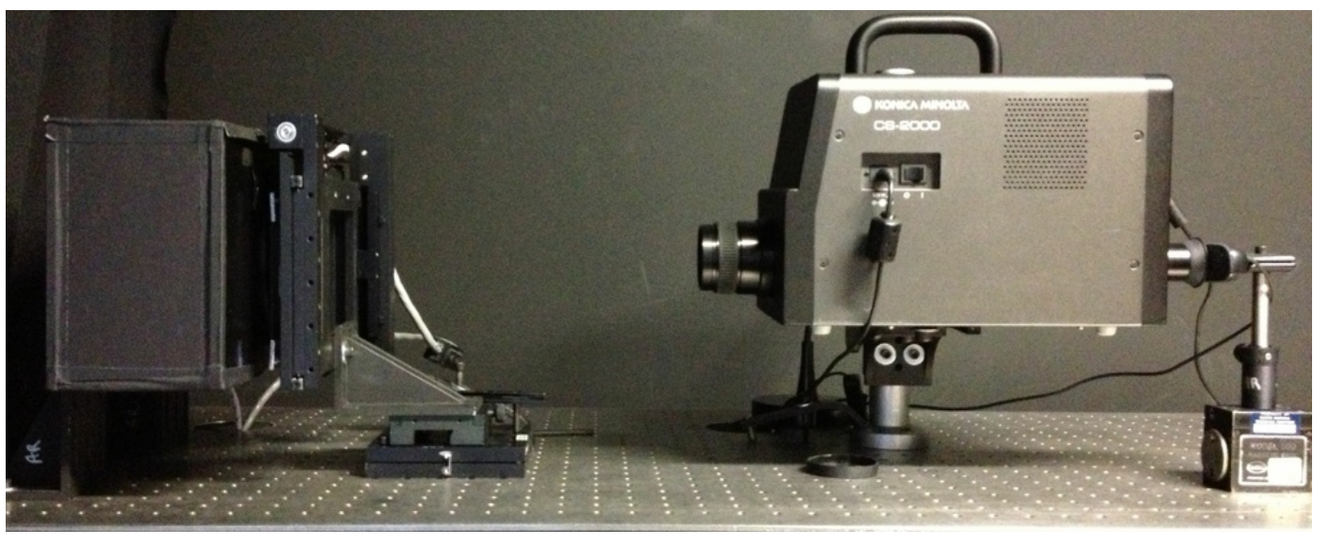
blank slide

color slide

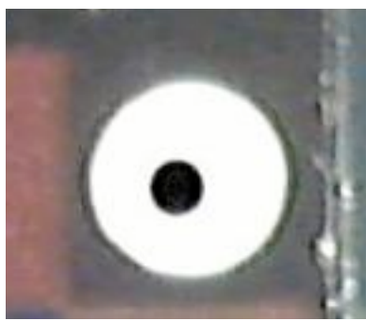
“open port” area



sphere port (LED on)



sphere xy stage radiometer camera



camera records every measurement location

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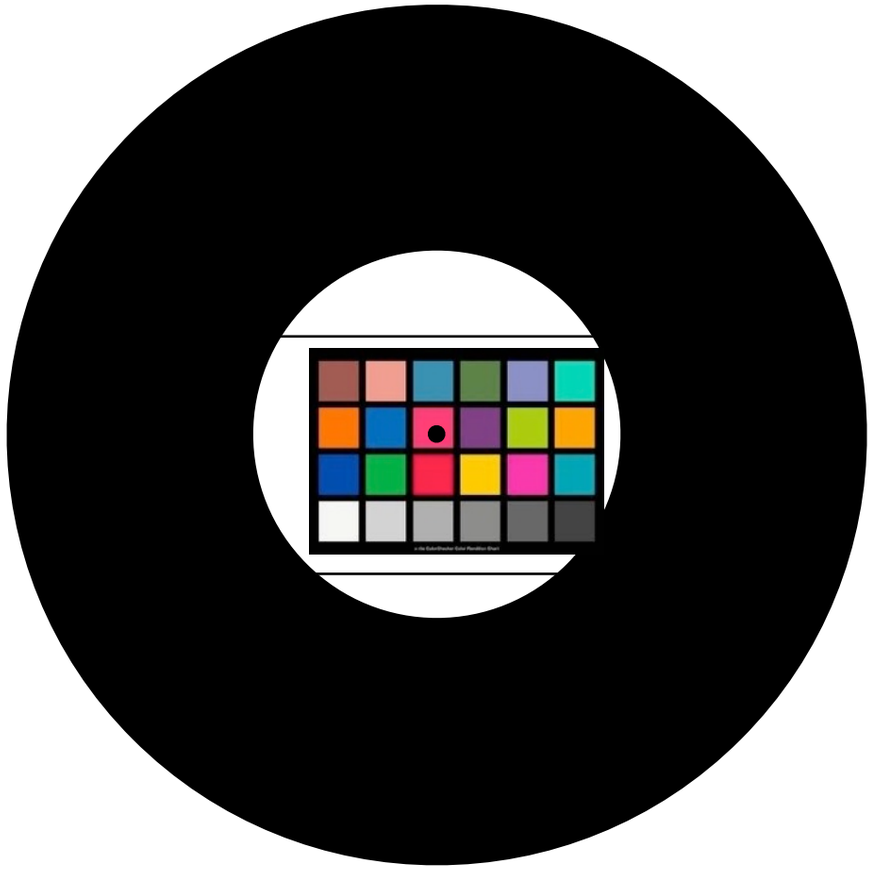
Considerations and Questions

large aperture sphere



(Near) whole slide illumination?

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FFEI proposal for calibration assessment slide

George Hutchinson
FFEI Limited

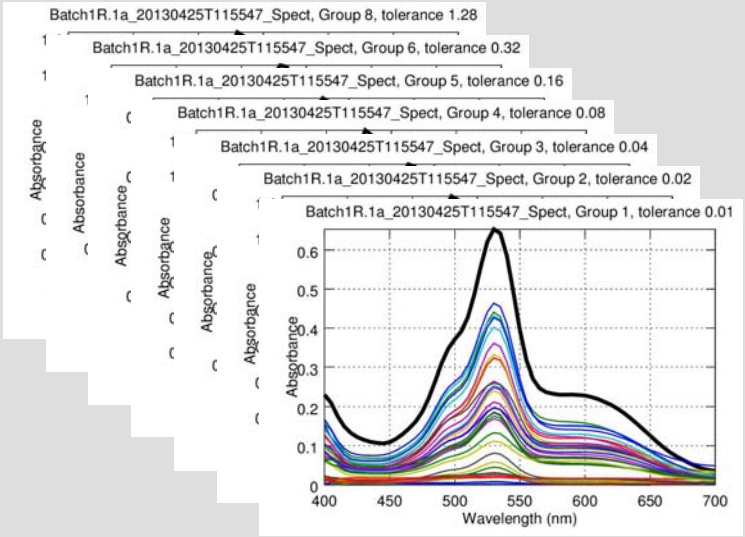
Summary

- The method described in these slides is for calibration assessment and applies to any calibration method
  - can be used for pre-market acceptance or post-market constancy testing
- The method is not intended to be used for calibration but may be used to check the performance of any calibration system
  - black-box assessment method
- Can be used for:
  - Product qualification
  - Installation Qualification
  - Operational Qualification
  - Equipment Performance Verification

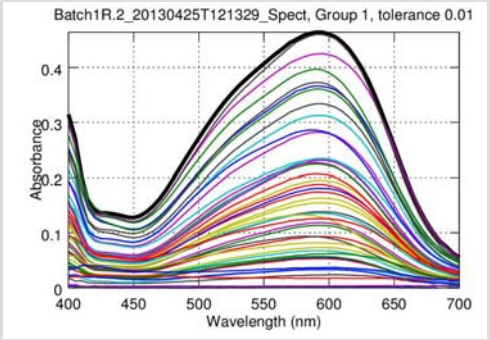
Project Sierra (FFEI and Leeds University)

- Create a slide that includes a set of coloured patches that have the same spectra as those found in tissue samples
  - using tissue samples directly is undesirable as there is significant 'structure' which leads to uncontrolled variation in colour over small areas
- Identify a non-tissue substrate that can be stained using the same set of stains as are used to stain tissue samples
  - should accept all of the stains commonly used for pathology
  - should have similar range of colour absorbance to that of stained tissue
  - staining level can be controlled
- Understand how stains operate
  - is there any 'interaction' between stains, e.g. chemical reaction / modification?
  - can the stain colours be modelled using Beer-Lambert assumptions (for example is there significant scattering as documented for DAB stain)?

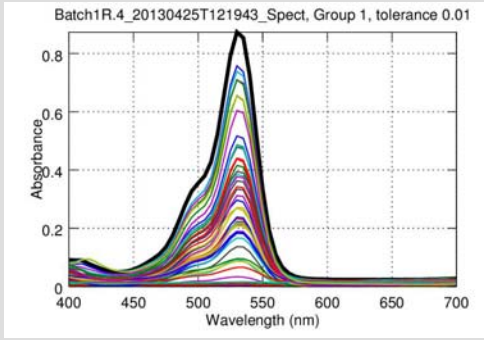
FFEI stains database



Spectral measurements of standardised tissue sample stained following typical H&E staining protocol



Spectral measurements of standardised tissue sample stained with Haematoxylin only



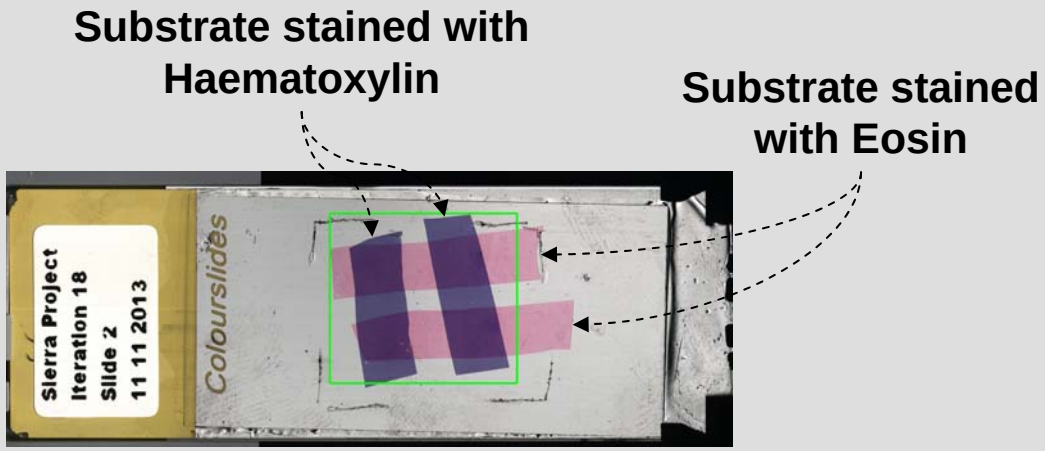
Spectral measurements of standardised tissue sample stained with Eosin only

Slides with tissue samples stained using 22 commonly used staining protocols and slides with tissue samples stained using 47 individual stains were analysed

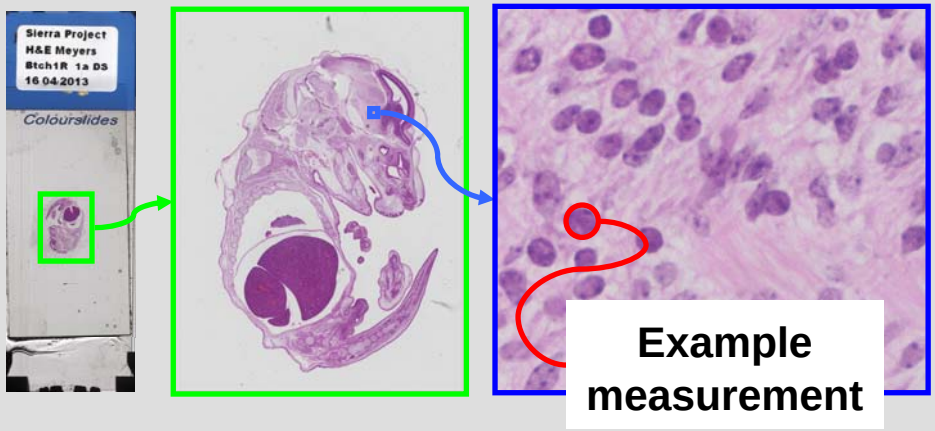
up to 400 spectral measurements of regions of distinct colours made for each slide

Comparing how stains work in substrate and in tissue

Stained substrate

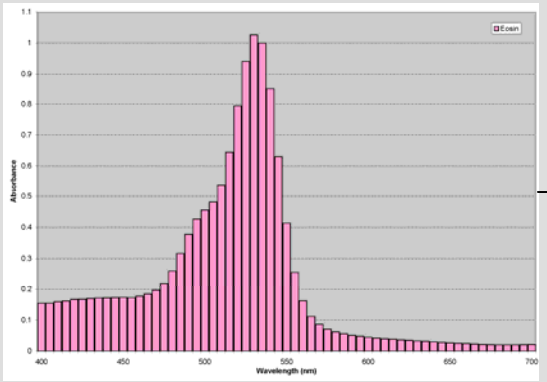


Stained tissue



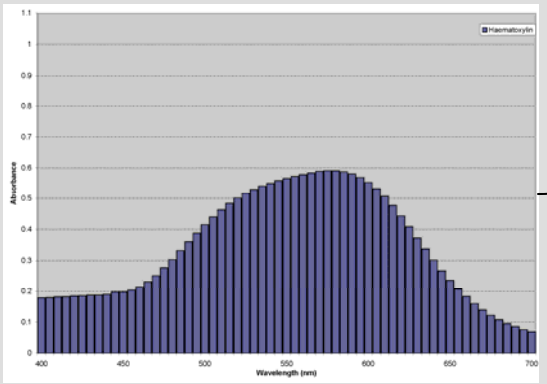
Behaviour of stains
(example – other stains operate similarly)

Eosin



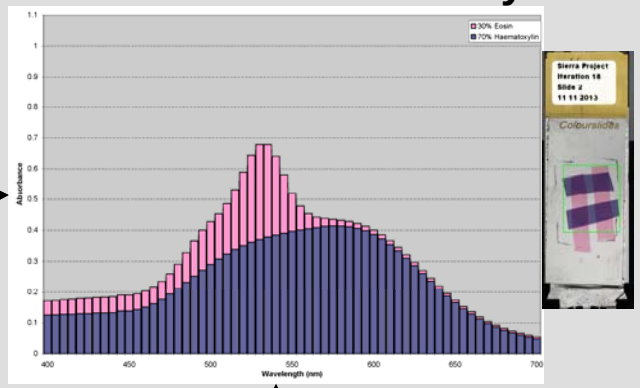
30%

70%



Haematoxylin

30% Eosin + 70% Haematoxylin



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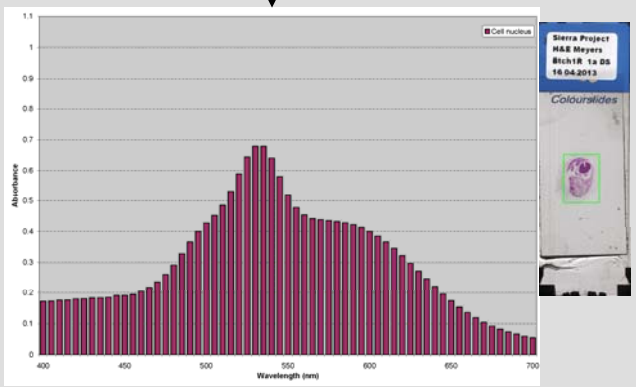
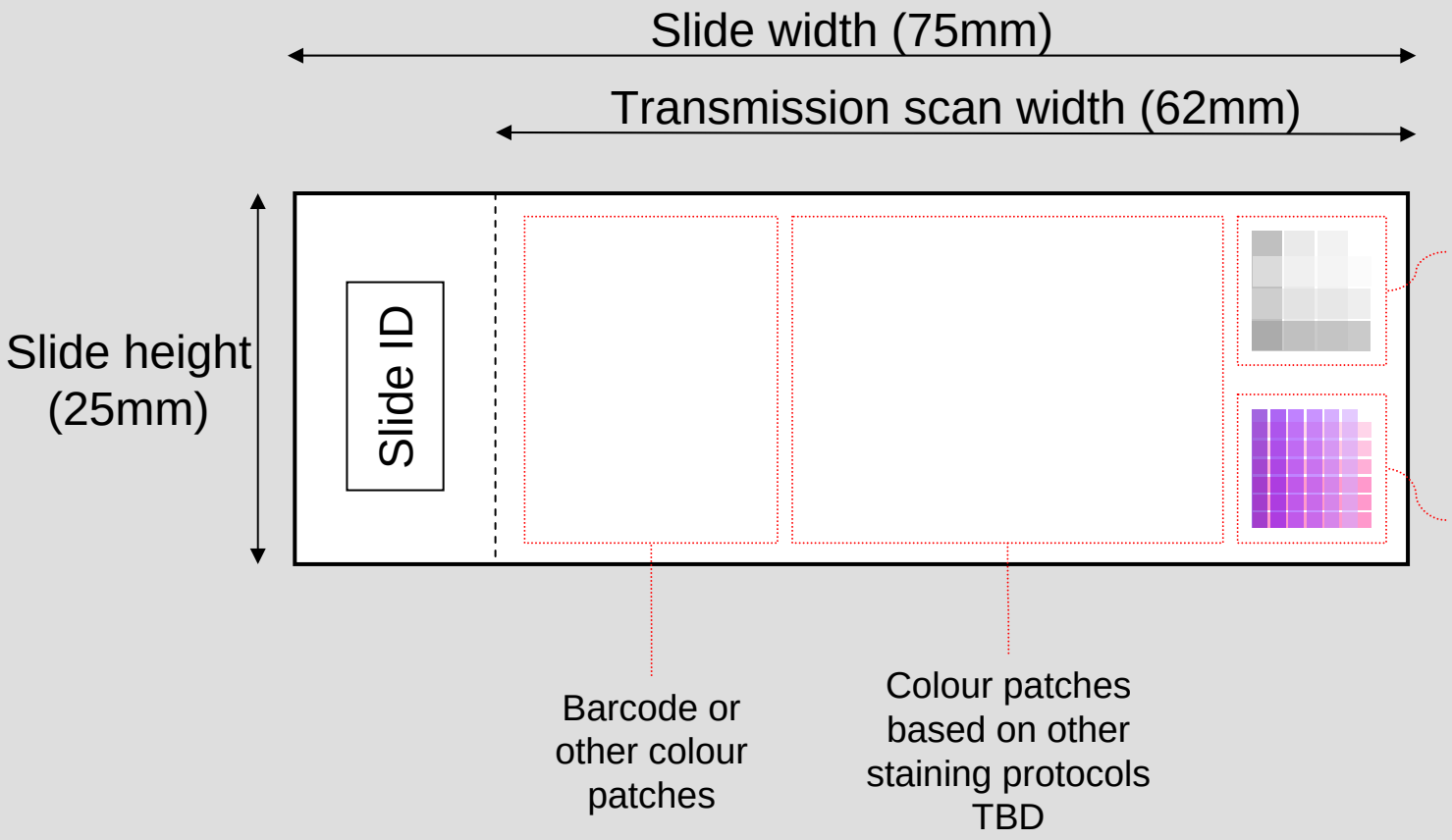


Example colour spectrum is simple linear addition of 30% Eosin and 70% Haematoxylin

Overview of proposed slide



Neutrals area
6 strips combine to produce 15 different neutral density areas

H&E stains area
6 Haematoxylin and 6 Eosin strips combine to produce an array of 48 different H&E stain combinations

Key points

- Actual pathology stains can be used avoiding metamerism
- We have identified a non-tissue substrate that accepts stains in the same way as tissue
- 2-D arrays of coloured patches can be produced for each staining protocol and multiple staining protocols can be included on a single slide
- Colour is relatively uniform across each patch
- Good correspondence between spectra that arise in stained tissue and spectra of coloured patches on slides

- Datums for each group of patches provide positional references and detail to focus on
- Spectral measurement data can be incorporated on slide or in an internet file referenced by ID

Scope

- Our analysis includes the following staining protocols:
  - Haematoxylin and Eosin (H&E), Diaminobenzidine (DAB) with Haematoxylin counter stain, Papanicolaou (PAP), Perls' Prussian blue, Periodic acid-Schiff (PAS), Reticulin, Millers elastic Van Gieson, Shikata, Giemsa stain, Ziehl Neelsen technique, Grocott, Alcian blue PAS, Jones methenamine silver, Gram, Congo red stain for amyloid, Masson trichrome
- Our method works well for the 2-stain protocols, a subset of colours must be identified for 3-stain protocols

- This approach could be used to produce a single slide that could be used for Performance Qualification, Installation Qualification, Operational Qualification and Equipment Performance Verification

Current status

- We have developed a 'proof of concept'
- FFEI is a technology development company and we could develop this product commercially if there was sufficient interest from the group