Calibration Slide for Histopathology task force
Teleconference
20 February 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:

Craig Revie, FFEI
Hong Wei, Datacolor
Mark McNulty, Datacolor
Phil Green, ICC
Pinky Bautista, MGH PICT center
Wei-Chung Cheng, Food and Drug Administration
Glenn Davis, Ventana Medical Systems
Po-Chieh Hung, Konica Minolta
James Chang, Sharp Labs
Michael Flynn, Henry Ford Health System
Mike Brill, Datacolor
William Li, Kodak
Andy Masia, X-rite
Masahiro Yamaguchi, Tokyo Institute of Technology
Elizabeth Krupinski, University of Arizona
Sharon Johnson, The Shareden Group, Inc.

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

1. Introduction and summary of current status
   - Next meetings
   - ICC web site support for MIWG
2. Virtual Display Color Processor
3. Datacolor ChromaCal calibration slide
4. FFEI assessment slide evaluation proposal
5. Other business

1. Introduction and summary of current status

1.1 Next meetings

Mr Revie showed details of future MIWG meetings [see attached]. The next full meeting is March 3 in Tokyo (see web site for full details). A telecom on Medical Photography is scheduled for March 20 at 15:00 GMT (10:00 EST).

Subsequently there is a telecon on 17 April (Mobile Displays).

Face-to-face meetings will be held on June 19-20 at FDA (co-located with CIE D1 and ISCC Special Topics meetings), and on October 30-November 1 in Boston (co-located with CIC and ADP).

1.2 ICC web site support for MIWG

Mr Revie reminded the call of the MIWG web site at http://www.color.org/groups/medical/medical_imaging_wg.xalter. The site includes details of future meetings, records of previous meetings, listing of activities and participants, and resources relevant to work of the group. A wiki has also been established (details from Phil Green) and MIWG members are encouraged to provide suggestions on how this can be used.

2. Virtual Display Color Processor

Mr Wei-Chung Cheng of the FDA presented an outline of an evaluation method for whole-slide imaging systems [see attached]. He showed the methodology and design philosophy, which included evaluation of the capture and display components of the workflow, and described the framework for obtaining colour measurements of slides using a spectroradiometer. The test slide is based on a 140-patch ColorChecker imaged into Fuji Velvia, which is measured.

He also showed the Virtual Display Color Processor, which is a hardware interface board that captures the RGB frame data that is sent to the display. He confirmed that the evaluation of the display is against the intended colour space or profile, rather than measurements of the display.

Mr Cheng acknowledged that the spectrum of the test slide is different from stains or tissue. The intention is not to improve calibration or characterization, but to define a common protocol for evaluating the performance of WSI systems.

3. Datacolor ChromaCal calibration slide

Mr Mark McNulty of DataColor presented a description of the Chromacal calibration system [see attached]. The goal of the system is to improve accuracy and consistency. The target of the current system is limited to transmitted light brightfield microscopy.

Chromacal includes calibration software for the digital microscope, a calibration slide, and display calibration equipment. Each item is available separately.

A range of magnifications are supported by the slide design, and it has the advantage that the whole colour patch array can be viewed at once in the microscope. Mr McNulty reported that the system provided an accuracy of around 4 CIELAB ΔE^*_{ab}, and made it possible to achieve inter-microscope consistency of around 2.8 CIELAB ΔE^*_{ab}. He emphasised that Chromacal does not modify the source image but appends the calibration transform to the file. An audit trail for the adjustments made is available in the system.

The calibration slide uses narrow-band interference filters, so is not designated for a single stain protocol.

4. FFEI assessment slide evaluation proposal
Mr Revie presented an update of the FFEI calibration slide proposal [see attached]. The slide design uses a biopolymer staining technology developed by FFEI and University of Leeds, UK. Slides have a block of 15 different combinations of H&E staining protocol and a reference white, together with a block of 9 base stains that are used in many staining protocols.

The workflow is separated into calibration of the capture system (using measurements of the calibration slide as reference), the display RGB data (using the VDCP method) and the physical display (using measurements of the display). An ICC profile is generated from the capture of the reference slide and used to transform the test slide data to the ICC PCS. Mr Revie also described a visual assessment of the display calibration, using the method developed by Yagi and Bautista, illuminating the slide with the display backlight.

FFEI would like vendors to evaluate this system, and have provided a web site for upload of evaluation data (https://sierra.ffei.co.uk). Mr Revie proposed a round-robin assessment. The group of participants will review and agree how to publish the results of the round-robin test. A number of vendors and organisations have already agreed to participate.

Mr Masia recommended that multiple measurements and captures should be performed to reduce the effects of variability on the capture and display systems.

Mr Revie confirmed that FFEI have attempted to maximise the life of the reference slide by adding a stabilising agent to prevent photobleaching and by carefully selecting the choice of stains.

5. Any other business
There was no other business and the meeting closed at 11.07 EST.

A full recording of the meeting is available at http://www.npes.org/Portals/0/standards/2014-02-20%2010.05%20Calibration%20Slide%20for%20Histopathology%20meeting.wmv

Action items from the meeting:

MIWG web site and wiki

MIWG-14-12 Provide suggestions for content to Phil Green (all)

FFEI assessment slide

MIWG-14-13 Participate in round-robin test (interested members)

Actions from previous meeting:

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

February 20th 2014

Calibration slide for histopathology

February 20th 2014 (2-hours teleconference)

- **Introduction and summary of current status (Craig Revie)**
  - Next meetings
  - ICC web site support for MIWG
- **Virtual Display Color Processor (Wei-Chung Cheng)**
- **Datacolor ChromaCal calibration slide (Mark McNulty / Mike Brill)**
- **FFEI assessment slide evaluation proposal (Craig Revie / George Hutchinson)**
- **Other business**

Next meetings

- **Tokyo MIWG meeting (3rd March 13:00-17:00)**
  - followed by a networking reception
  - local contacts are Masahiro Yamaguchi and Takashi Matsui
  - teleconference option available – please register with Debbie
- **Next teleconferences – save the date!**
  - 20th March : teleconference (Medical photography)
  - 17th April : teleconference (Mobile)
- **Washington DC (19th and 20th June)**
  - FDA White Oak Conference Center
  - format will be working group meetings rather than presentations
- **Boston (30th October – 1st November)**
  - ICC meetings will be followed by ICC DevCon, IS&T Color and Imaging Conference (CIC22) and the 2nd International Congress of the International Academy of Digital Pathology (IADP)

ICC MIWG Web Site: new resources

- **ICC web pages for Medical Imaging**
- **DPA Whole Slide Imaging Repository**
  - web page lists sources for whole slide and static image examples
- **Virtual Display Color Processor** code
- **SPIE Medical Imaging Paper by Tom Kimpe et al.**
  - Does the choice of display system influence perception and visibility of clinically relevant features in digital pathology images?

- **There is also Wiki support and we could use that for some of our work in future – contact Phil Green for details**

Assessing Whole-Slide Imaging Systems

Wei-Chung Cheng
Division of Imaging and Applied Mathematics
OSEL, CDRH, US Food and Drug Administration

Whole Slide Imaging
ICC Medical Imaging Working Group
2-20-2014

Introduction

- Not proposing a calibration slide, but an evaluation method
- Purpose: Evaluating *color reproducibility* for regulatory purposes
  - "Colorimetrical color difference between digital image and slide truth"
    - Not perceptual difference; not based on optical microscopy
    - For evaluation only, not for calibration/correction
- Methodology (SPIE MI 2013) consists of
  - A. Producing the color phantom
  - B. Establishing the truth
  - C. Retrieving the digital display data (SID 2012)
  - D. Calculating the color difference
- Design Philosophy
  - Least burdensome – simple enough to be doable or available for anyone
  - Quantitative (comparing systems and components – intra-device, inter-device, inter-vendor)
  - Objective (universal and repeatable)
  - Flexible -- mix-and-match stages (e.g., C+D in-house, A+B outsource)

Framework (SPIE MI 2013)

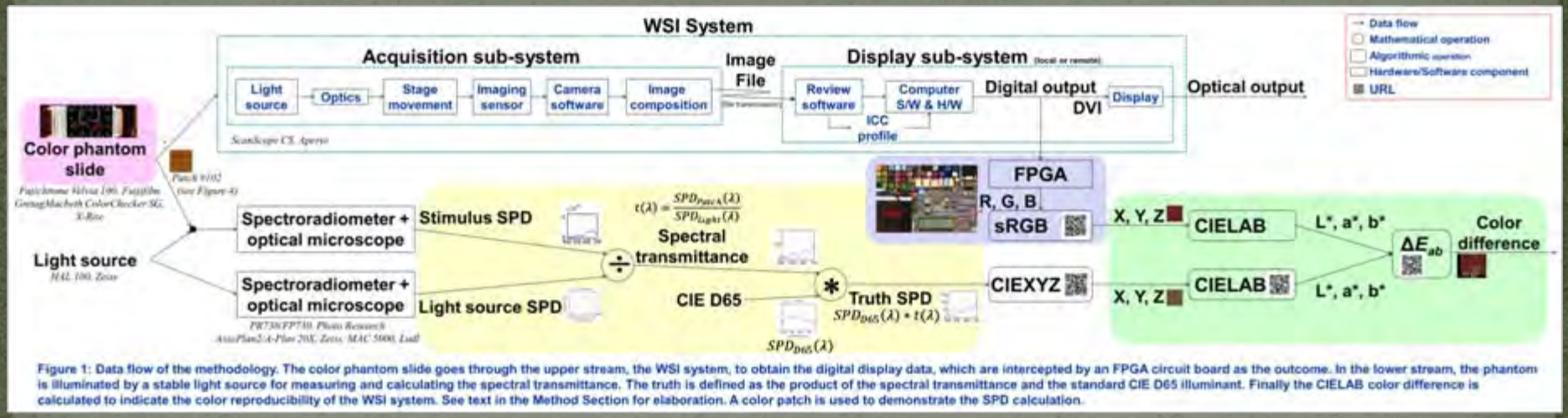


Figure 1: Data flow of the methodology. The color phantom slide goes through the upper stream, the WSI system, to obtain the digital display data, which are intercepted by an FPGA circuit board as the outcome. In the lower stream, the phantom is illuminated by a stable light source for measuring and calculating the spectral transmittance. The truth is defined as the product of the spectral transmittance and the standard CIE D65 illuminant. Finally the CIELAB color difference is calculated to indicate the color reproducibility of the WSI system. See text in the Method Section for elaboration. A color patch is used to demonstrate the SPD calculation.

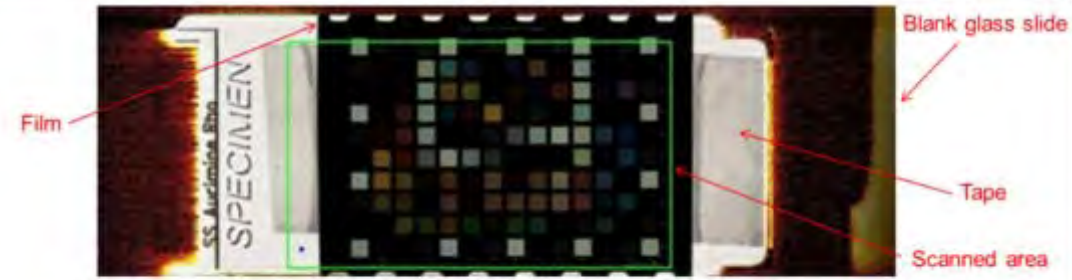
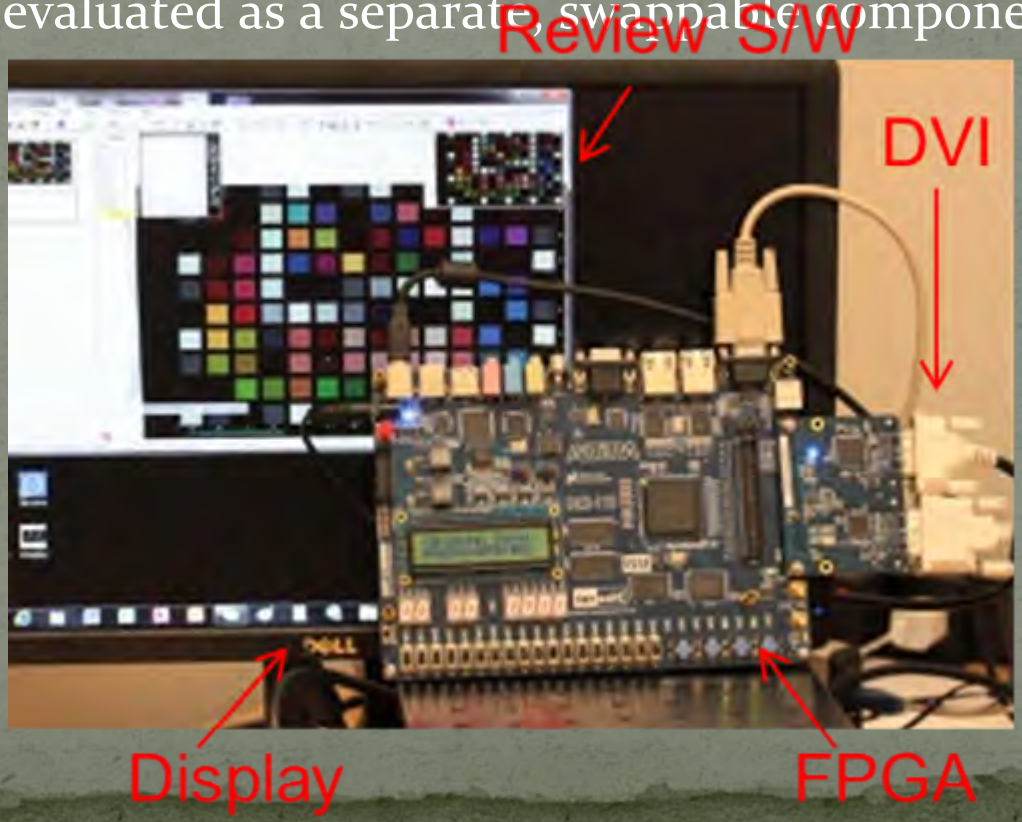


Figure 2: Snapshot of the color phantom taken by the WSI scanner.

VDCP (SID 2012)

- Virtual Display Color Processor
- A circuit for retrieving RGB values from the DVI or HDMI cable
- Robust digital reading without time-consuming optical measurement
- Account for effects of review software/hardware and color management
- Display can be evaluated as a separate, swappable component



References

- Cheng, Wei-Chung, et al. "Assessing color reproducibility of whole-slide imaging scanners." *SPIE Medical Imaging*. International Society for Optics and Photonics, 2013.

- Cheng, Wei-Chung, and Aldo Badano. "70.2: Virtual Display: A Platform for Evaluating Display Color Calibration Kits." *SID Symposium Digest of Technical Papers*. Vol. 42. No. 1. Blackwell Publishing Ltd, 2011.

- Papers and code available at http://code.google.com/p/virtualdisplay/

datacolor

CHROMACAL™ Color Calibration System for Optical Microscopy

Presentation to:
ICC Medical Imaging Working Group

February 20, 2014

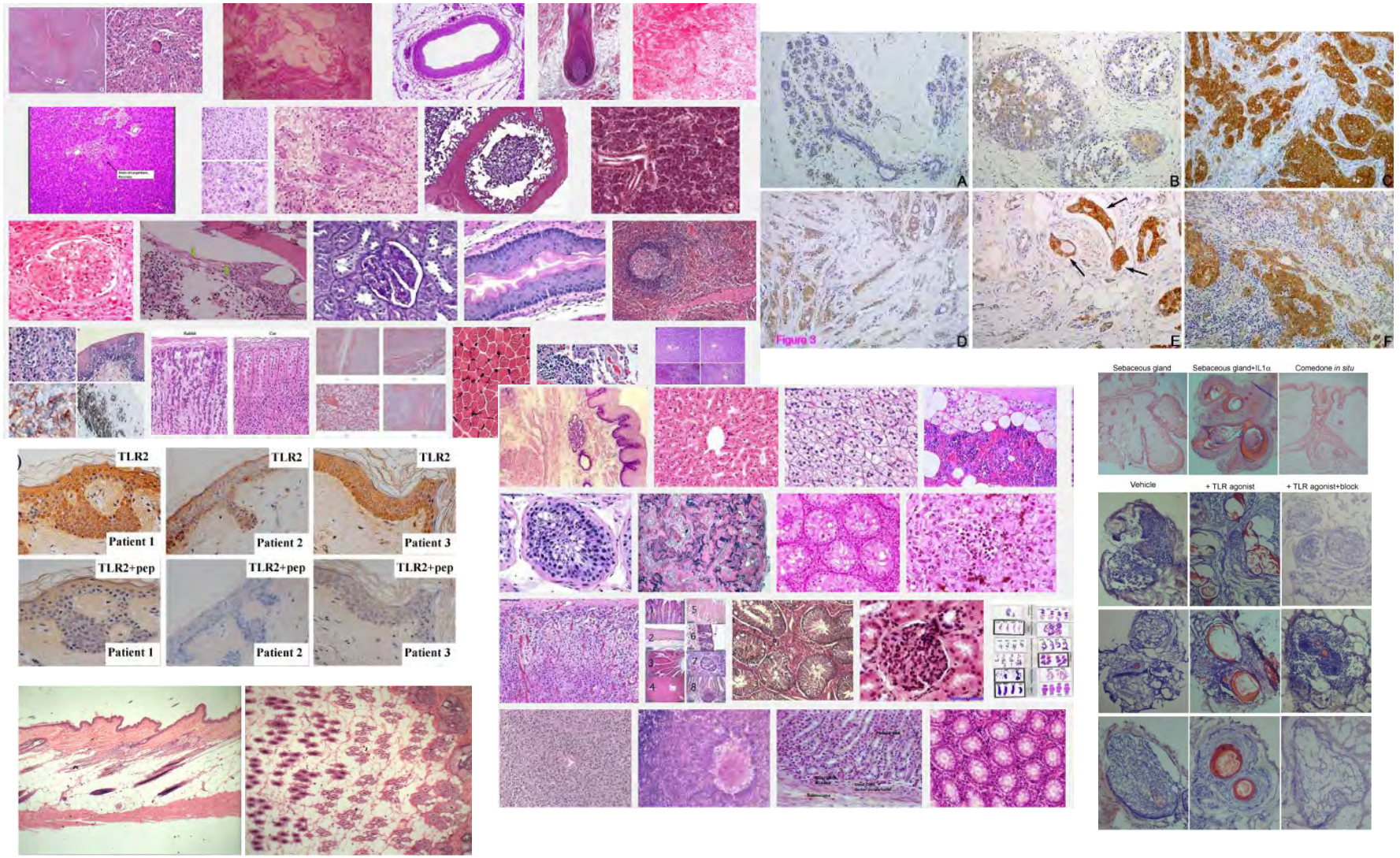
From Datacolor

- Mark S. McNulty – General Manager
- Dr. Michael H. Brill – Director of Research
- Dr. Hong Wei – Sr. Optical Engineer

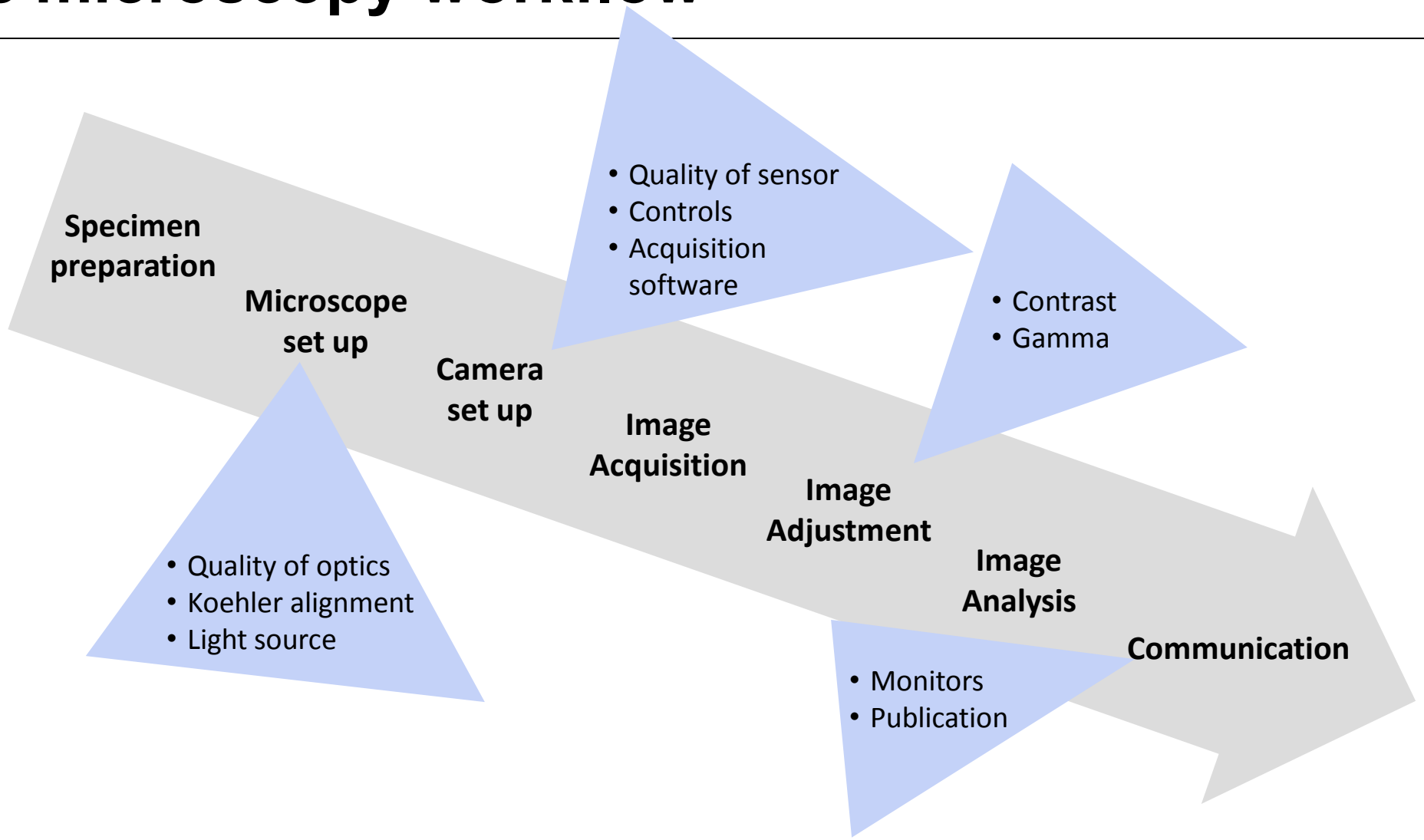
Topics

- Why calibrate color in digital brightfield microscopy?
- Imaging workflow and color variability
- Color calibration system for brightfield microscopy

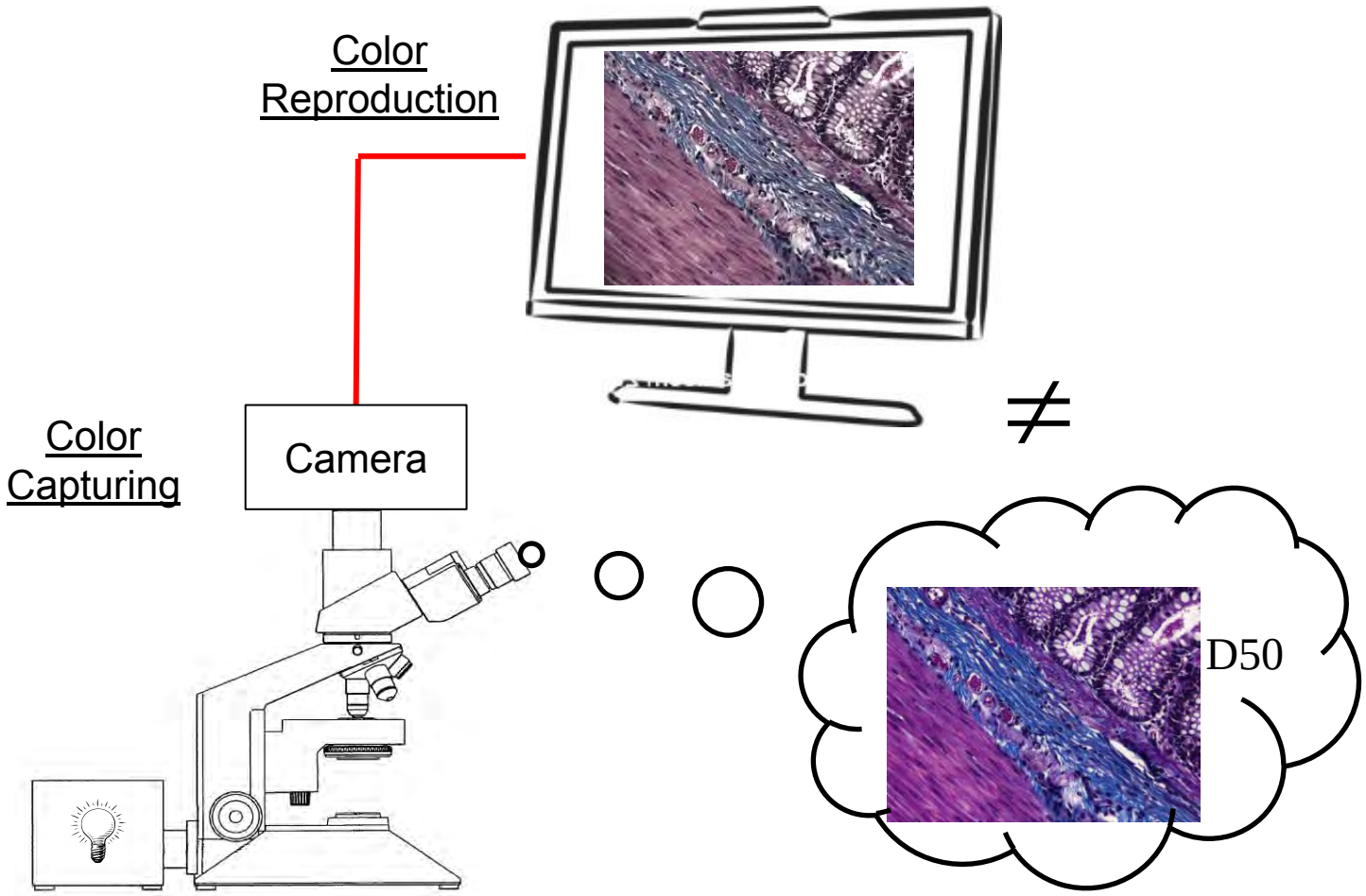
Why color calibration for microscopy?



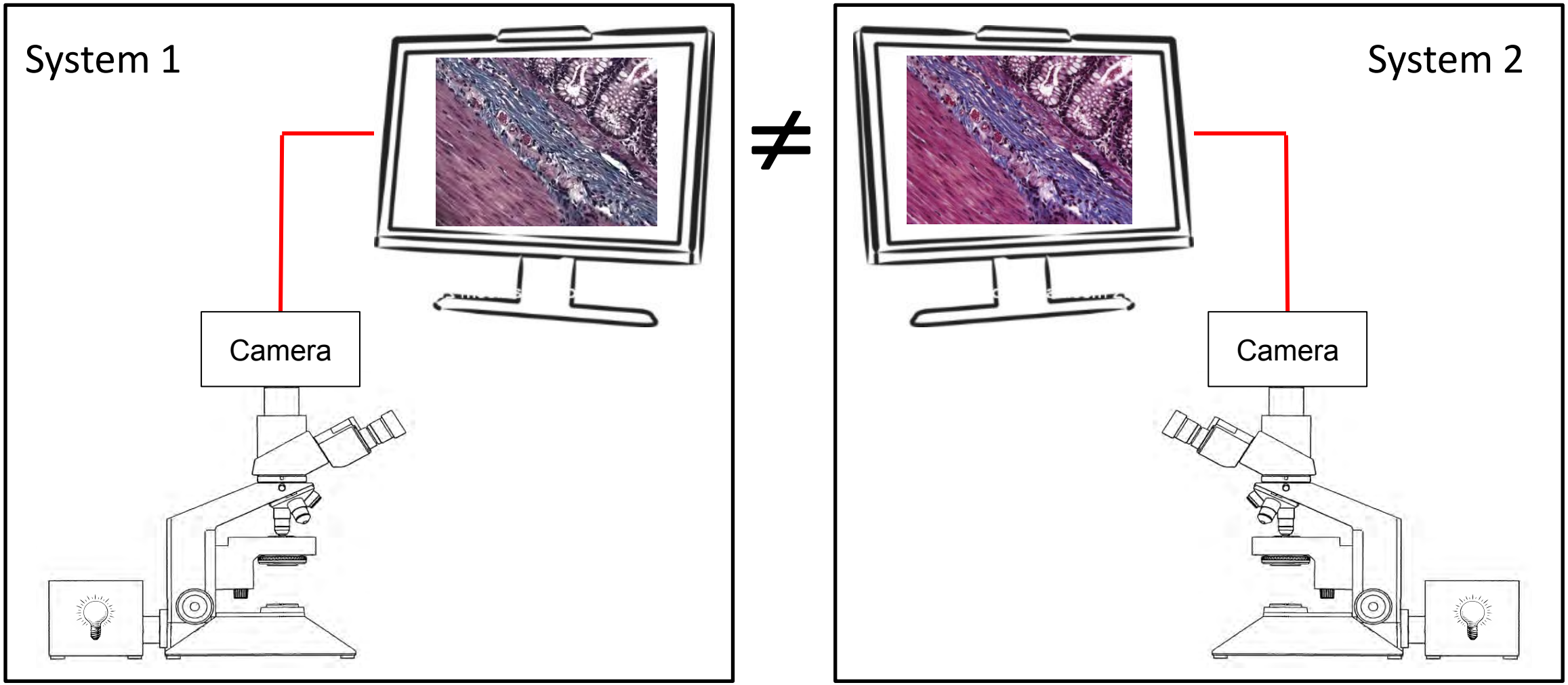
Basic microscopy workflow



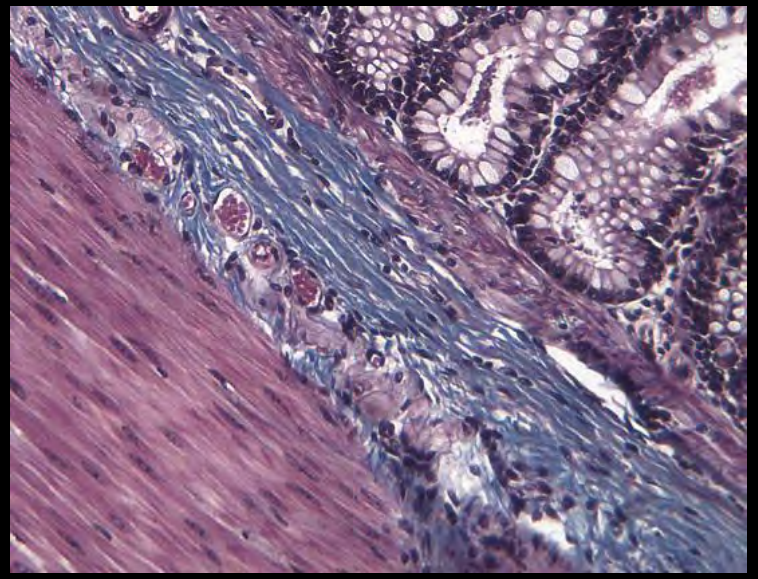
Color management challenge



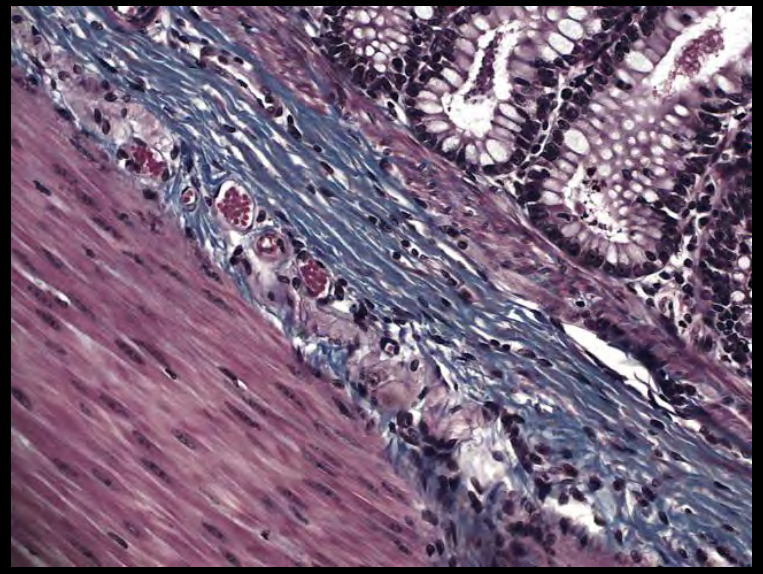
Color management challenge



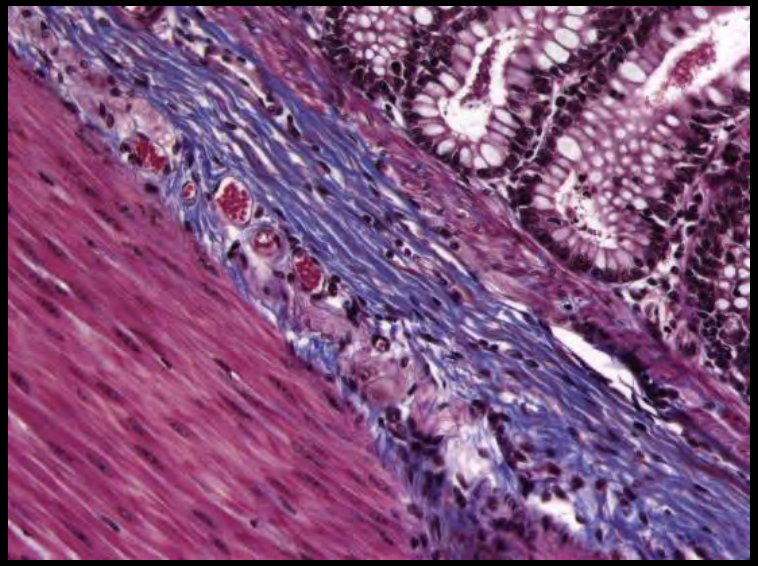
Motic Moticam 3MP
on Motic AE31



Motic Moticam 3MP
on Zeiss Axio Lab.A1



QImaging QIClick on
Zeiss Axio Lab.A1

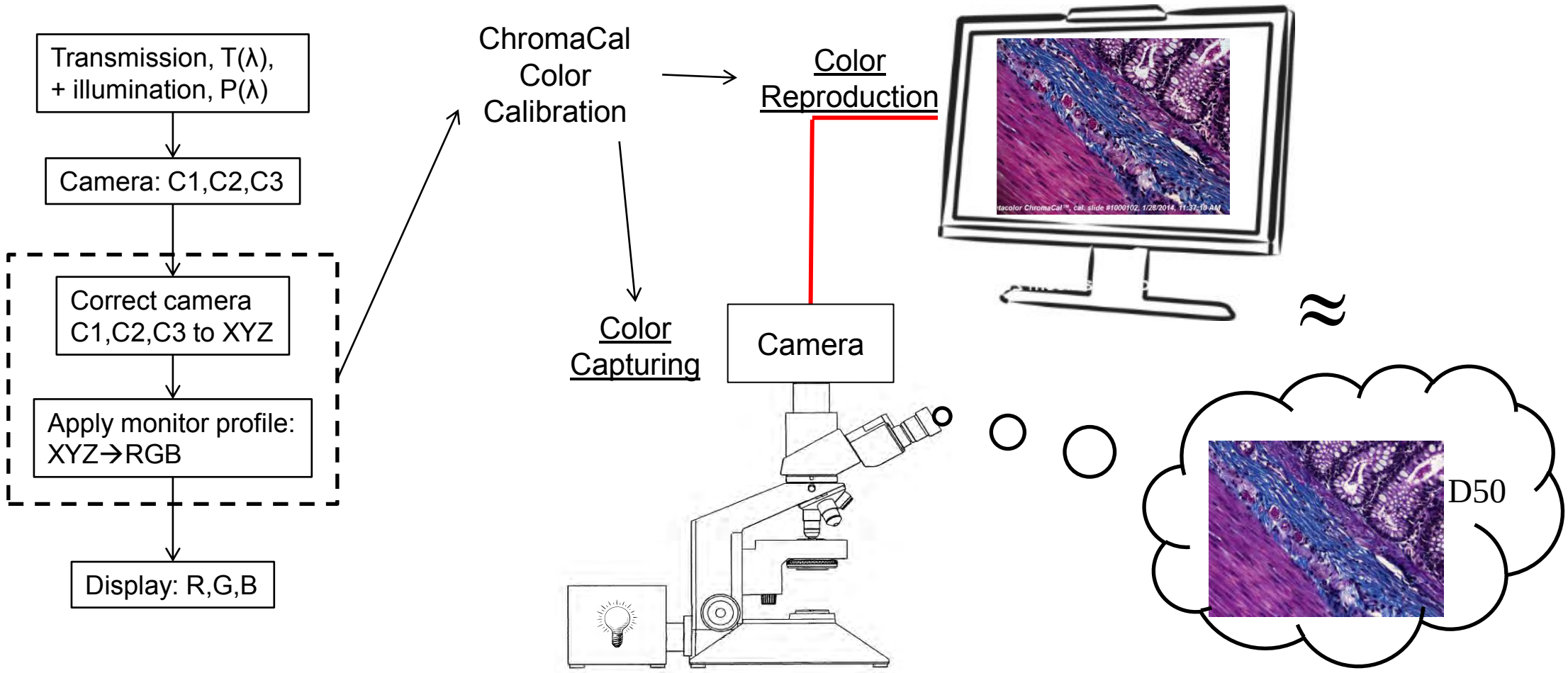


Uncalibrated Images

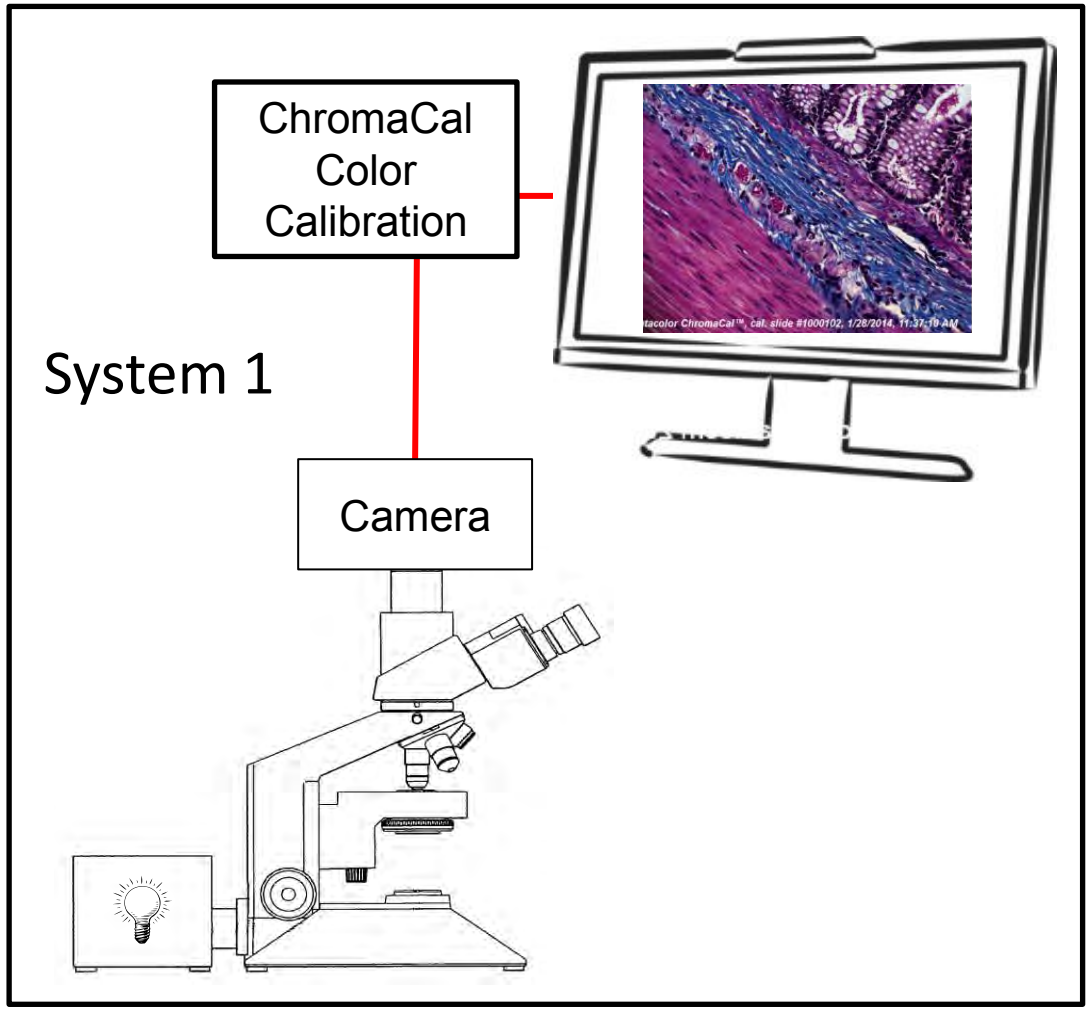
What is ChromaCal?

- **Easy to use color calibration system for images from transmitted, brightfield microscopes**

Color management solution with ChromaCal



Color management solution with ChromaCal



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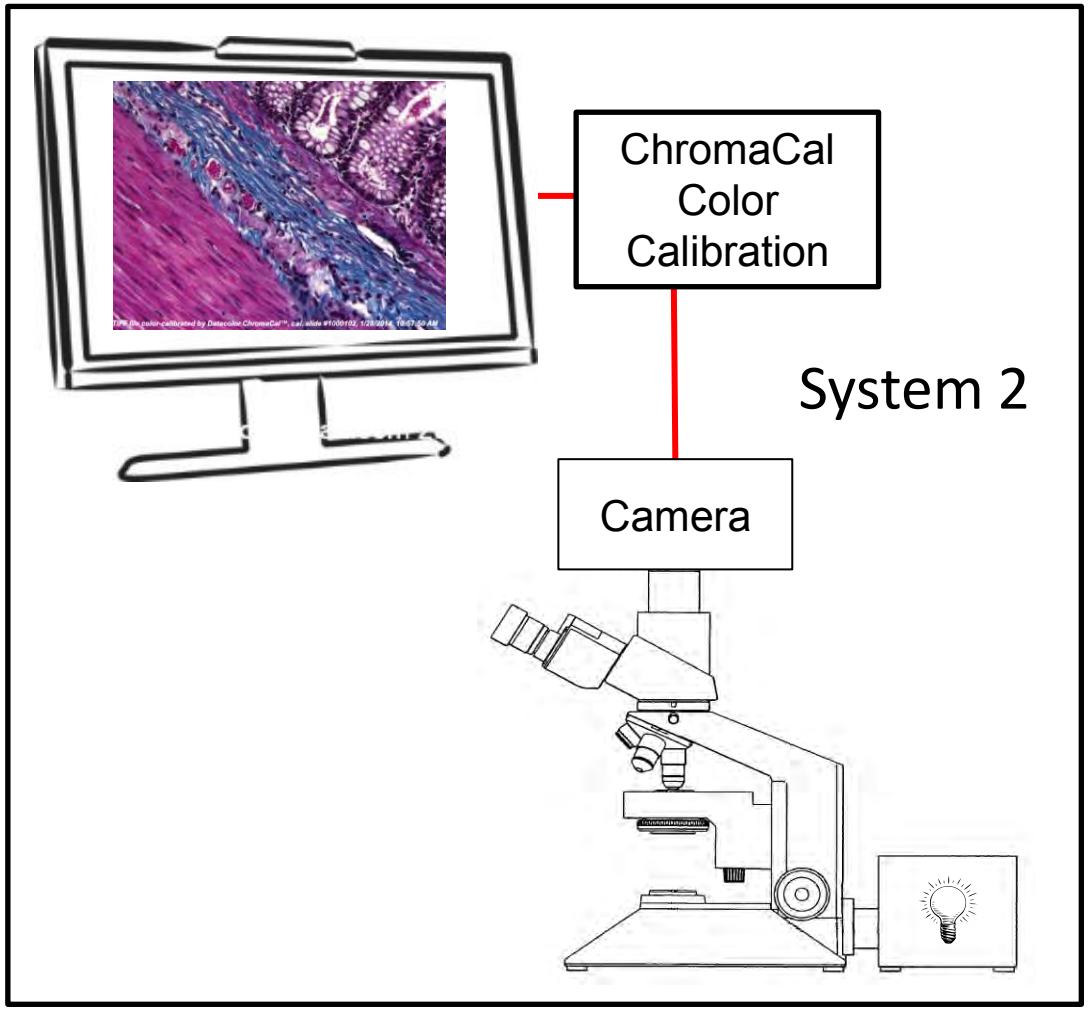


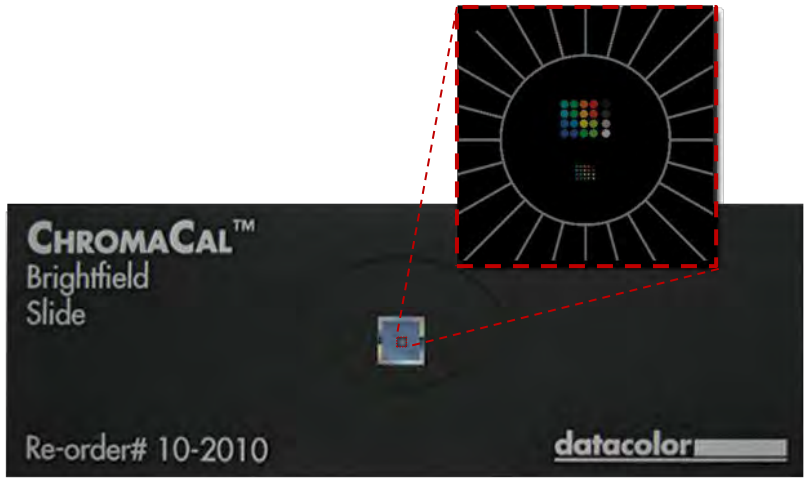
CHROMACAL Product Family

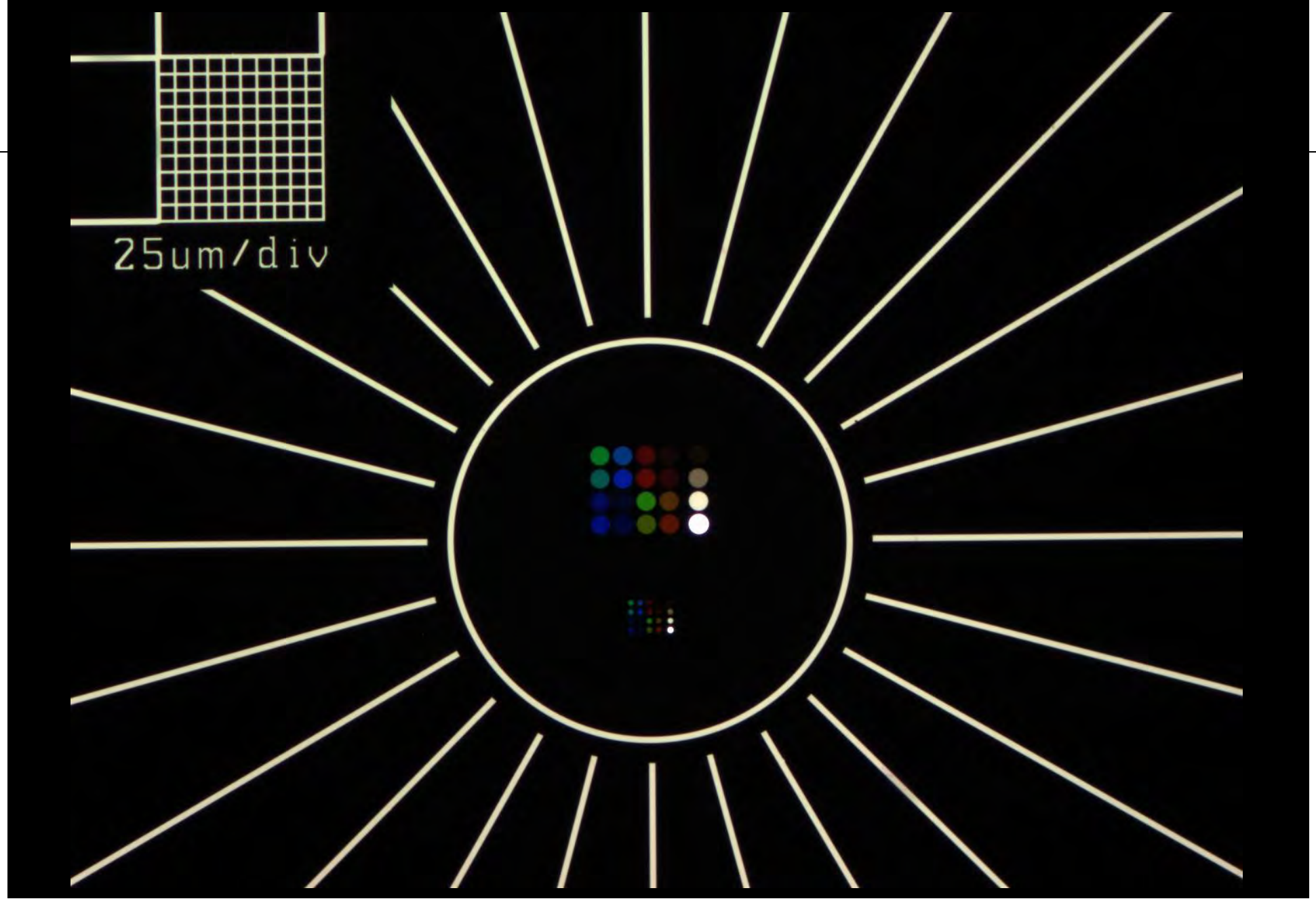
1. **Calibration system***
2. **Calibration slide**
3. **Monitor calibration set**

* Not for clinical diagnostic use in the U.S.

The ChromaCal Workflow

- **Step 1**
  - Capture your specimen images and an image of the calibration slide

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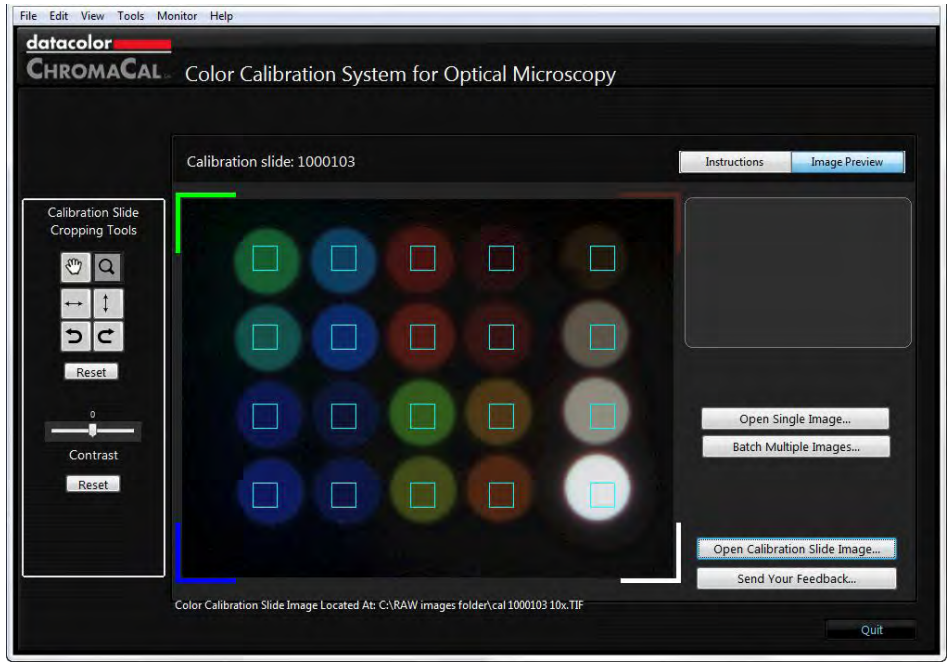
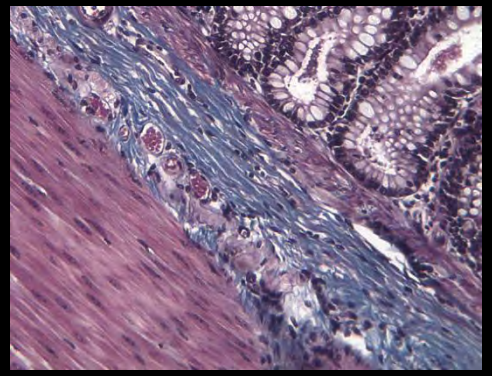
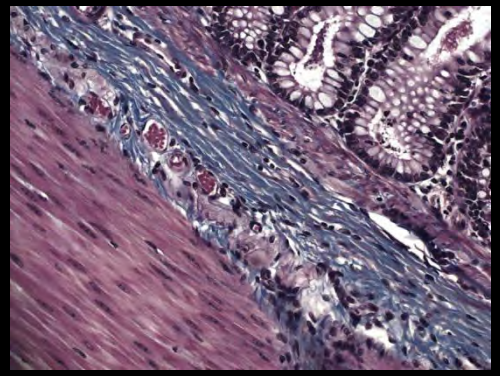
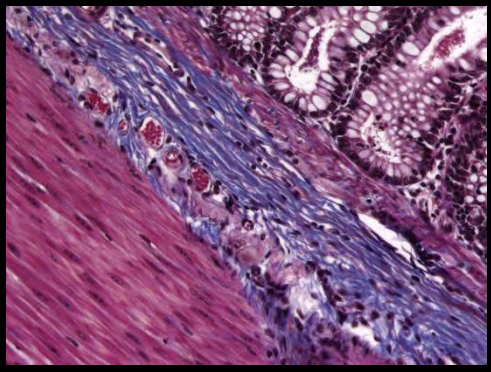
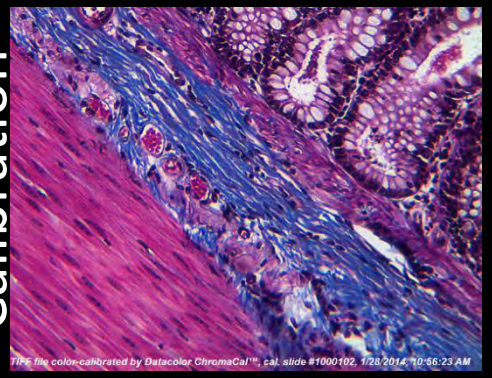
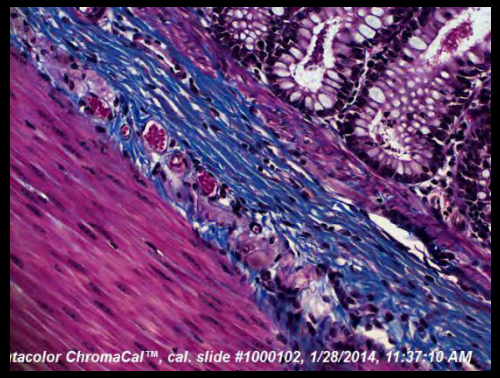
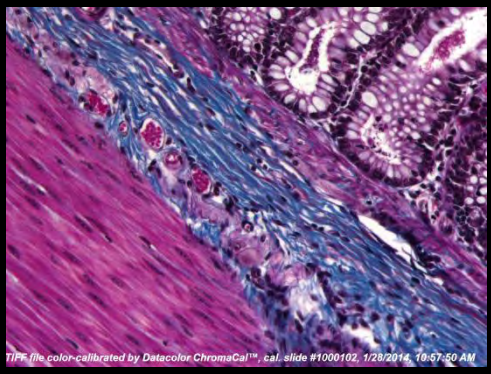
The ChromaCal Workflow

- **Step 2**
  - Calibrate your images with one touch, in single or batch mode

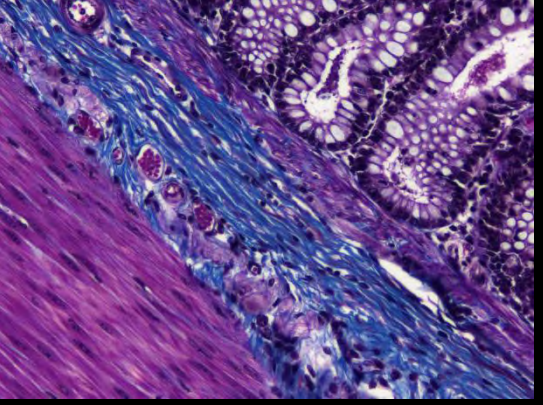


The ChromaCal Workflow

- **Step 3**
  - Communicate your calibrated images on calibrated monitors
  - Monitor calibration is done once, and then updated periodically

	Motic Moticam 3MP on Motic AE31	Motic Moticam 3MP on Zeiss Axio Lab.A1	QImaging QIClick on Zeiss Axio Lab.A1
Before calibration			
After ChromaCal Calibration			

Colorimetric Accuracy



True color from multispectral camera on Zeiss microscope

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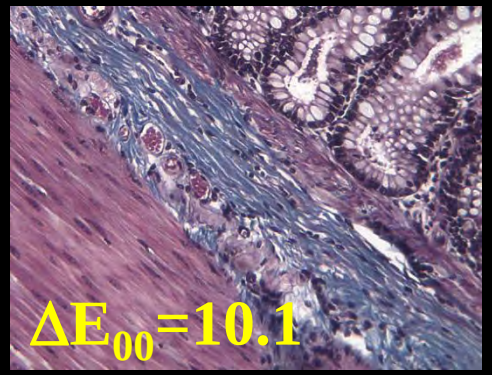
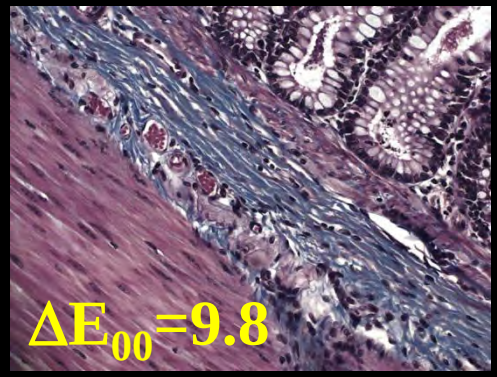
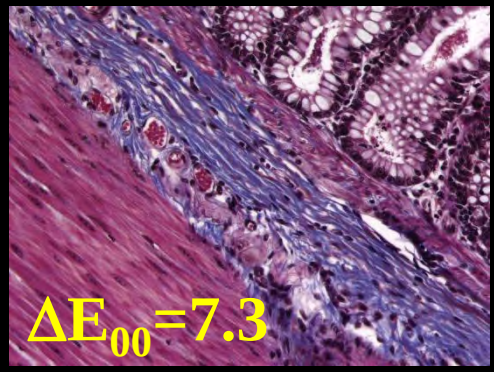
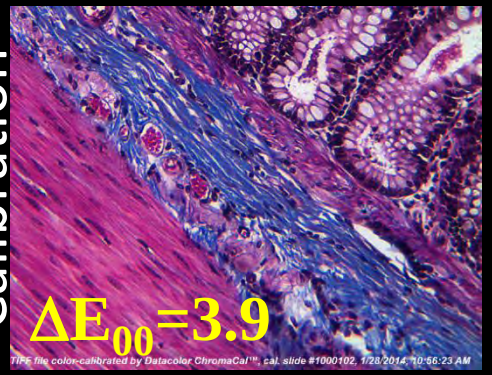
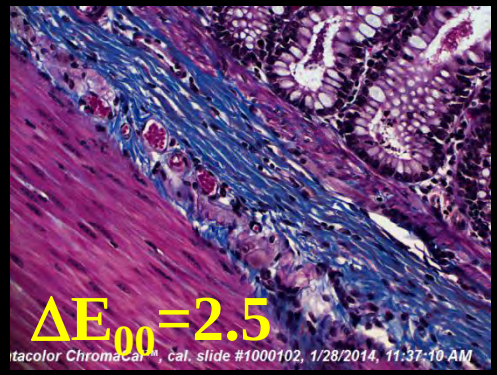
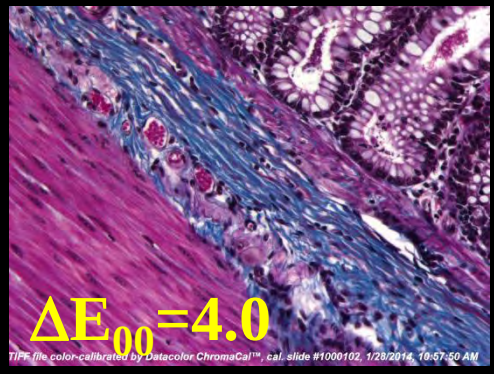
Before calibration			
After CHROMACAL Calibration			

Color Consistency

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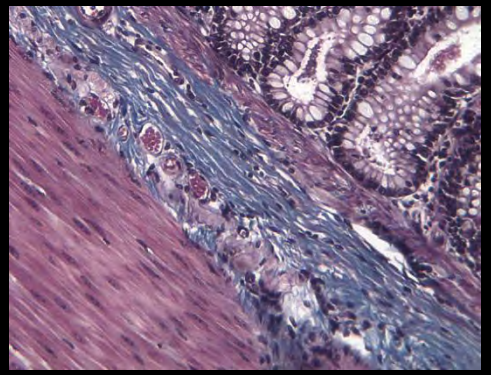
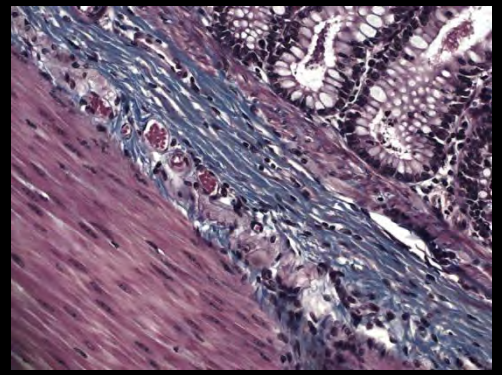
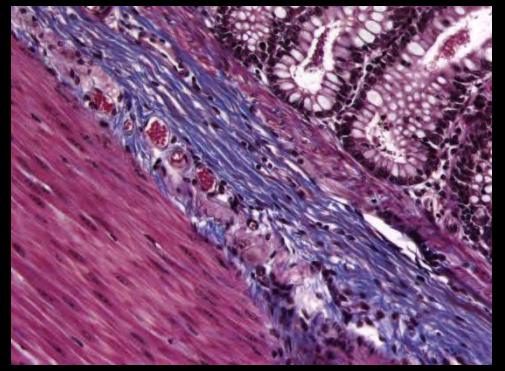
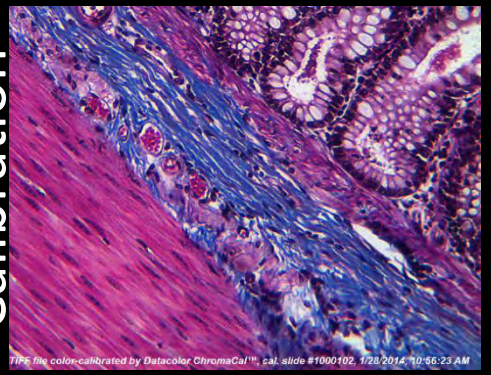
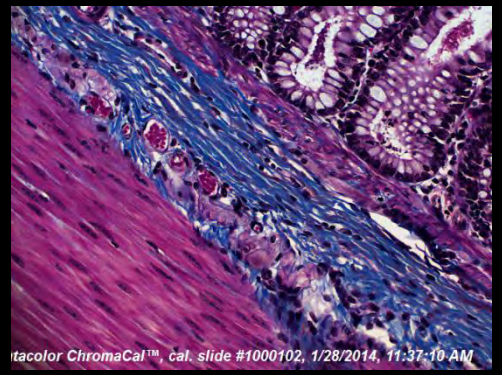
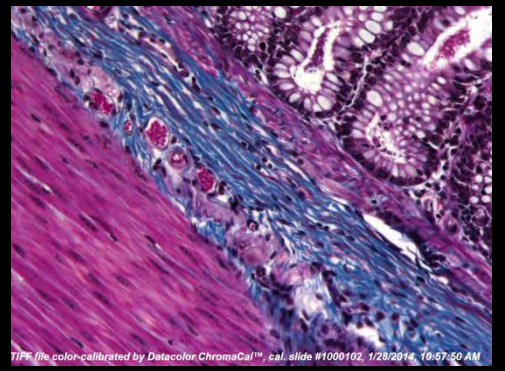
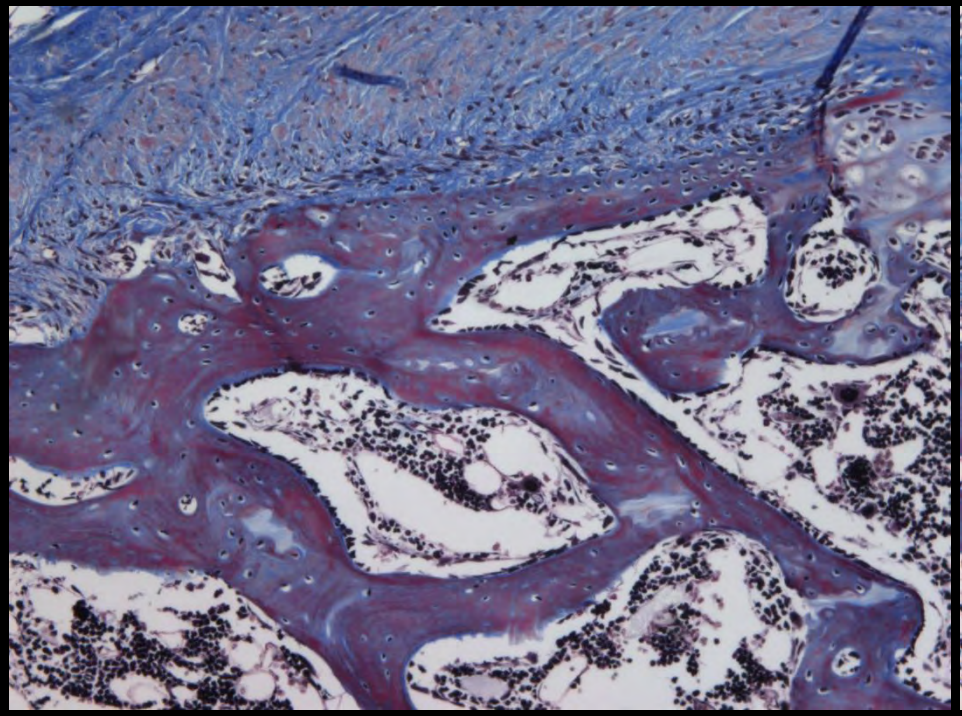
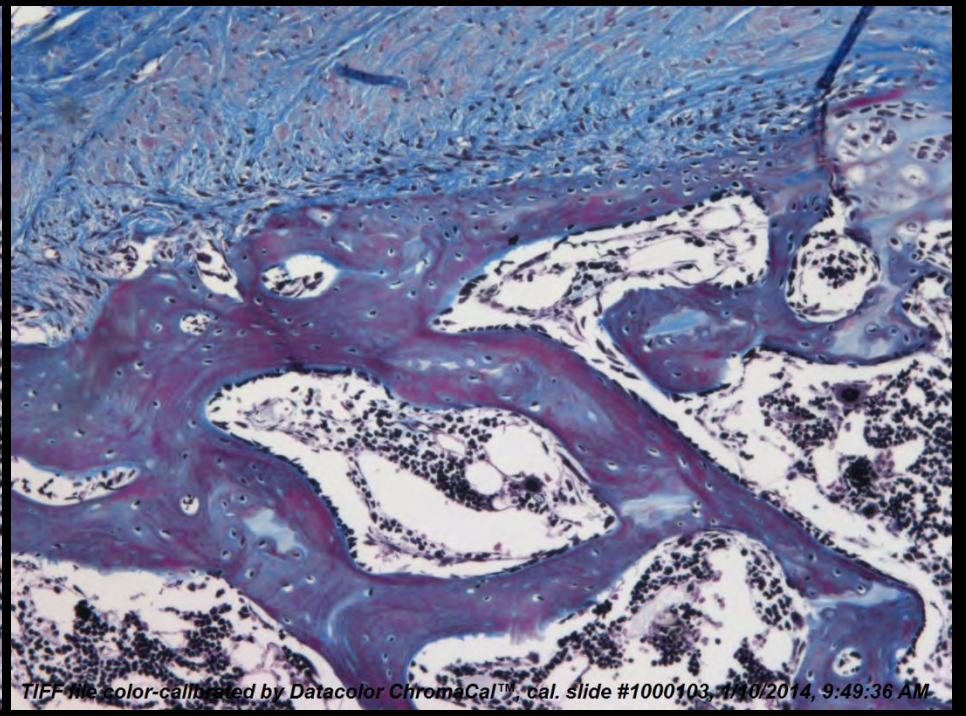
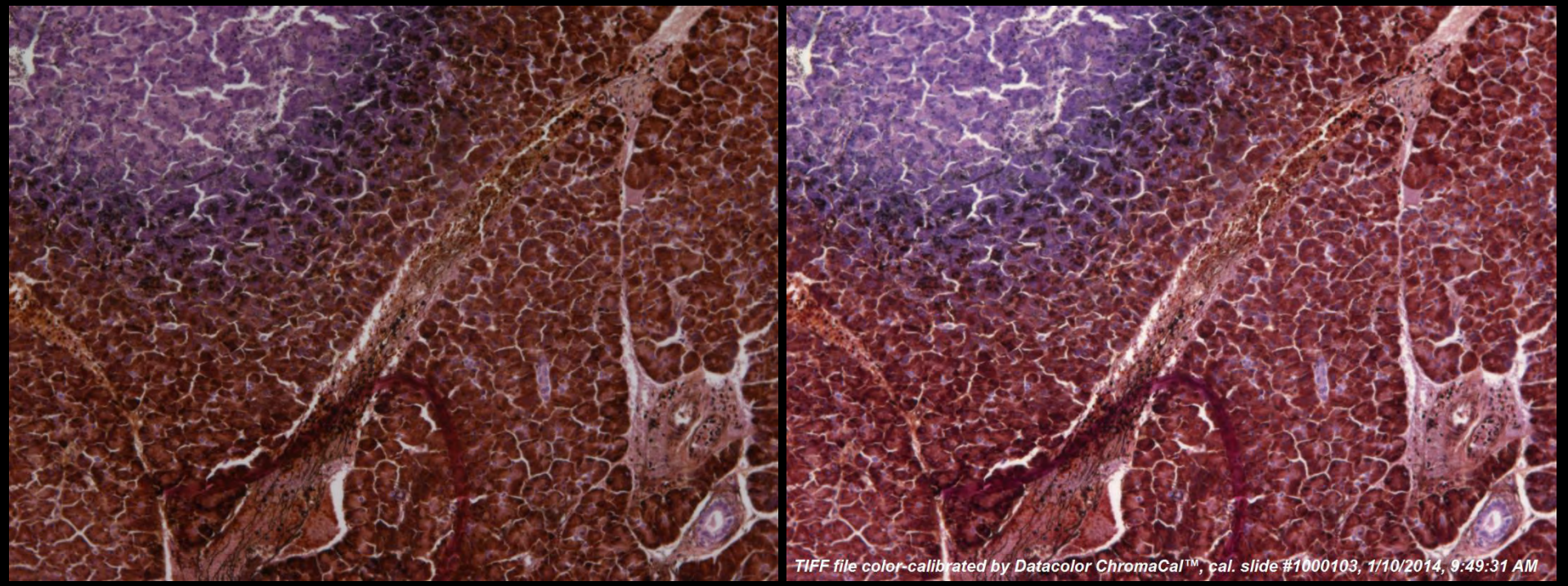
Before calibration				ΔE_{00}=4.9
After CHROMACAL Calibration				ΔE_{00}=2.8





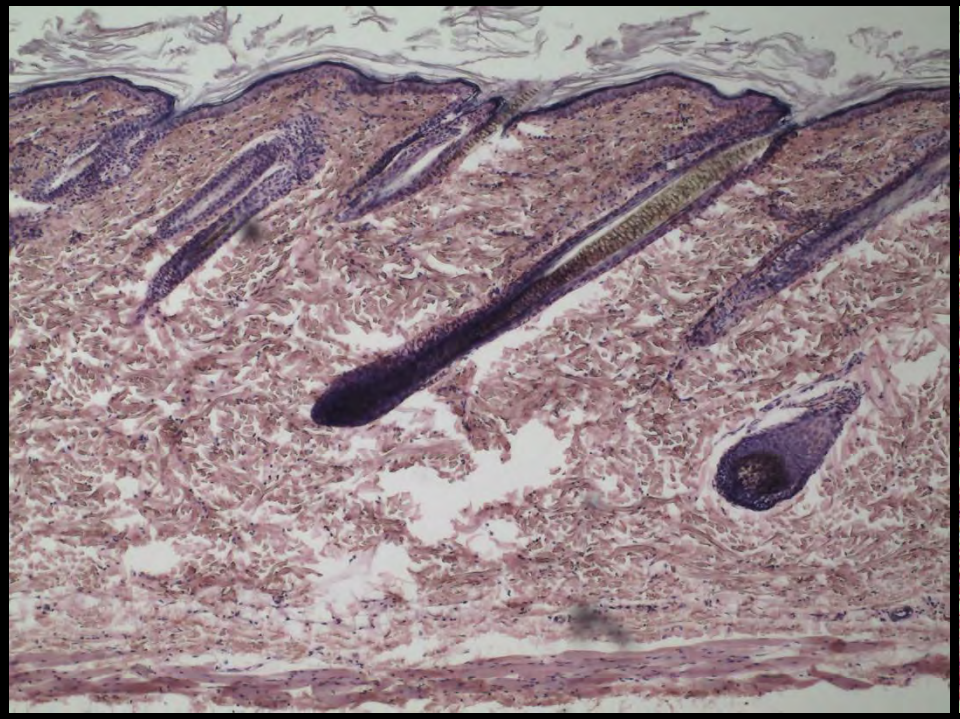
Before

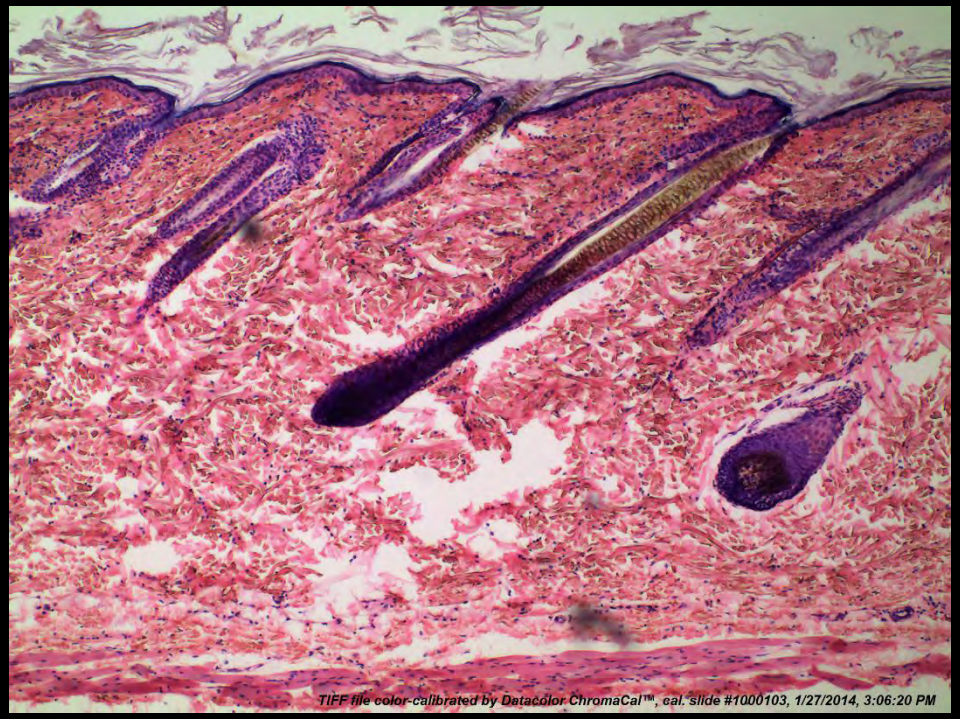
After



Before

After





Before

After

View Calibrated Images

Use this feature to browse through and preview calibrated images (TIFF files created by ChromaCal with calibration applied). After you select a Source folder, all calibrated TIFF files in this folder will be added to the list. Scroll through the list and select an image to view. You can also use the Magnifier and Hand to zoom/scroll/examine the contents of the calibrated image file more closely.

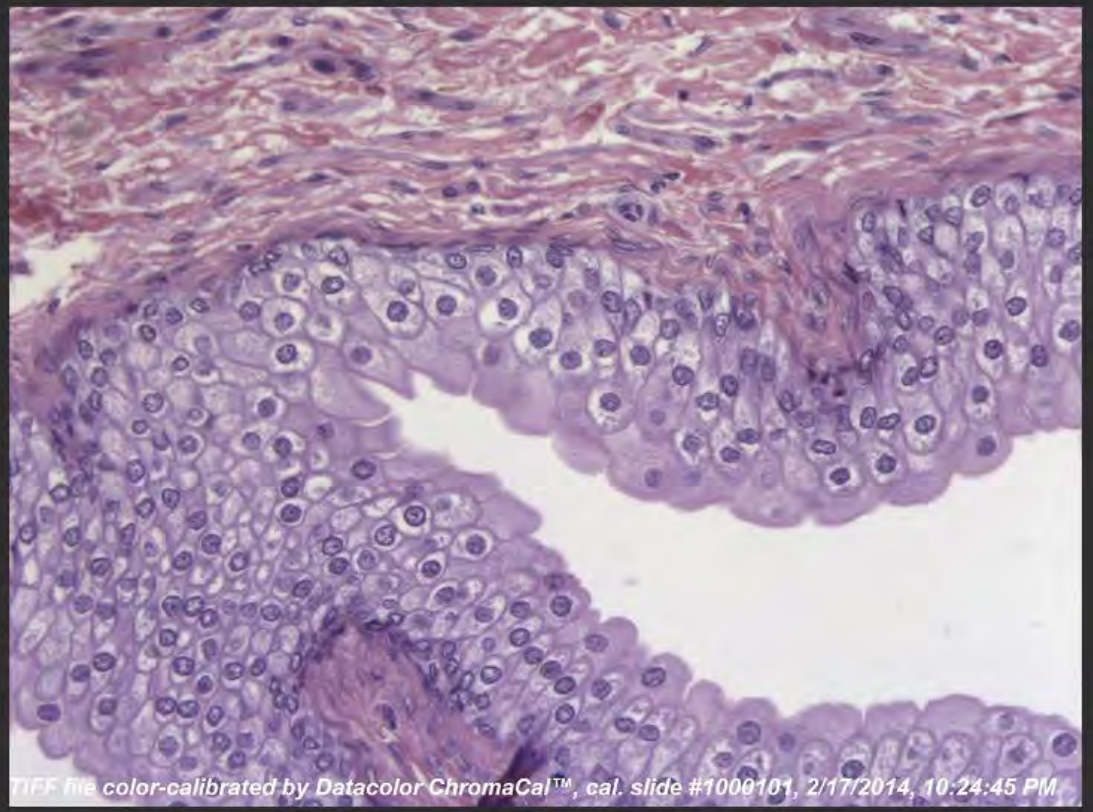
Select Calibrated Images Folder:

C:\Users\mark.mcnulty\ChromaCal testing\calibrated batch images\

Name

HA 5-1 ChromaCal-calibrated.TIF
HE_10x ChromaCal-calibrated.TIF
HE_50x - Copy (1) ChromaCal-calibrat...
HE_50x ChromaCal-calibrated.TIF

Reset



There are 4 calibrated images in this folder.

File: HA 5-1 ChromaCal-calibrated.TIF

Tiff file color calibrated by Datacolor ChromaCal Image Calibration, Contrast 0 on 2/17/2014, 10:24:45 PM with slide serial number: 1000101, ChromaCal Image Calibration Version 1.0.

Finished

datacolor

CHROMACAL™

Empowering Science with Color Integrity

FFEI proposal for calibration assessment slide status update

Craig Revie on behalf of George Hutchinson
FFEI Limited

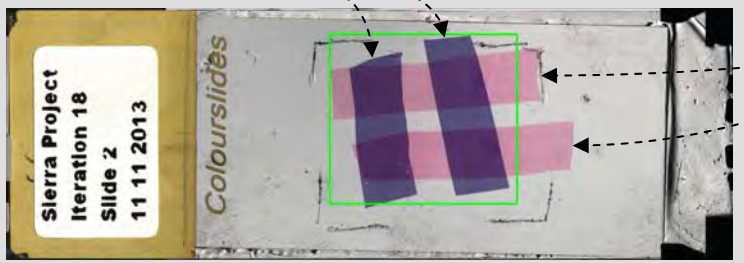
From previous telecon

Stained biopolymer compared with stained tissue

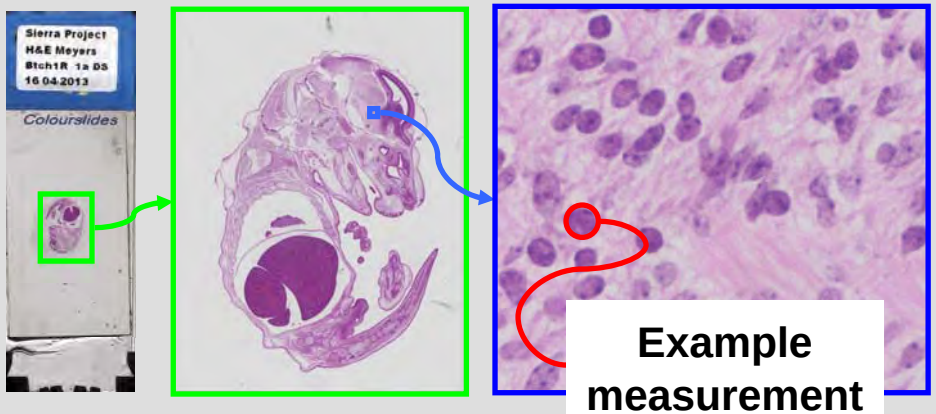
Stained biopolymer

Biopolymer stained with Haematoxylin

Biopolymer stained with Eosin



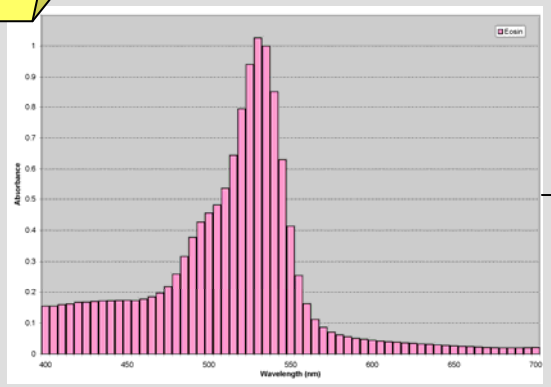
Stained tissue



From previous telecon

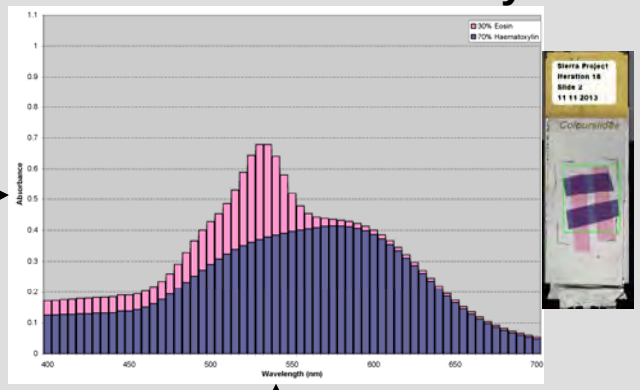
Behaviour of stains (example shows H&E)

Eosin



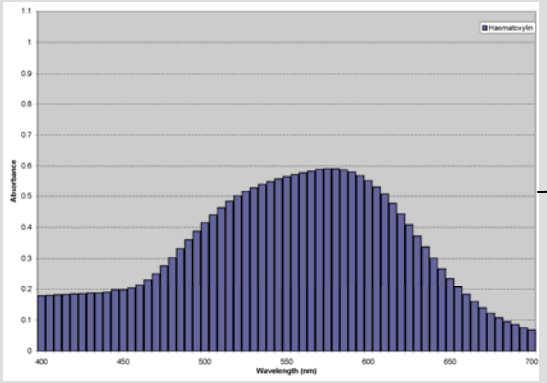
30%

30% Eosin + 70% Haematoxylin

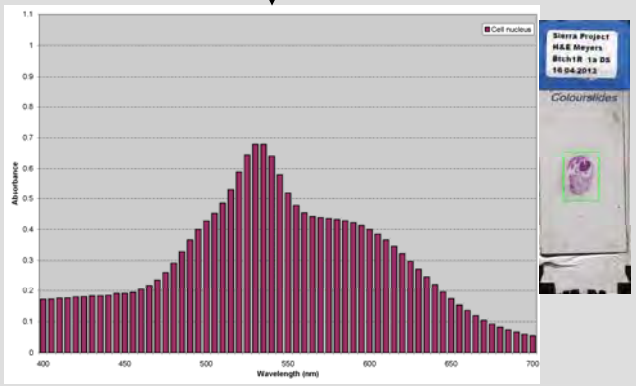


=

70%

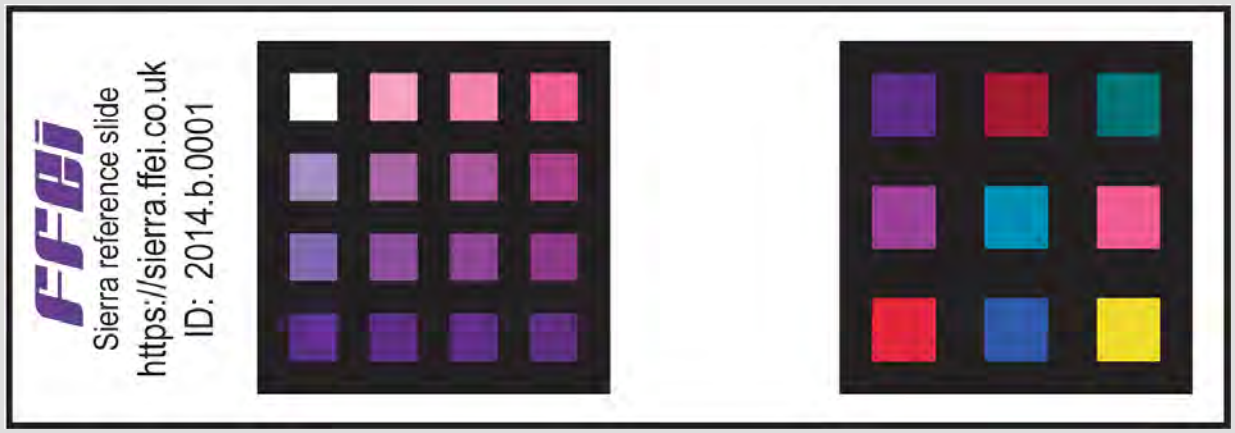


Haematoxylin



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

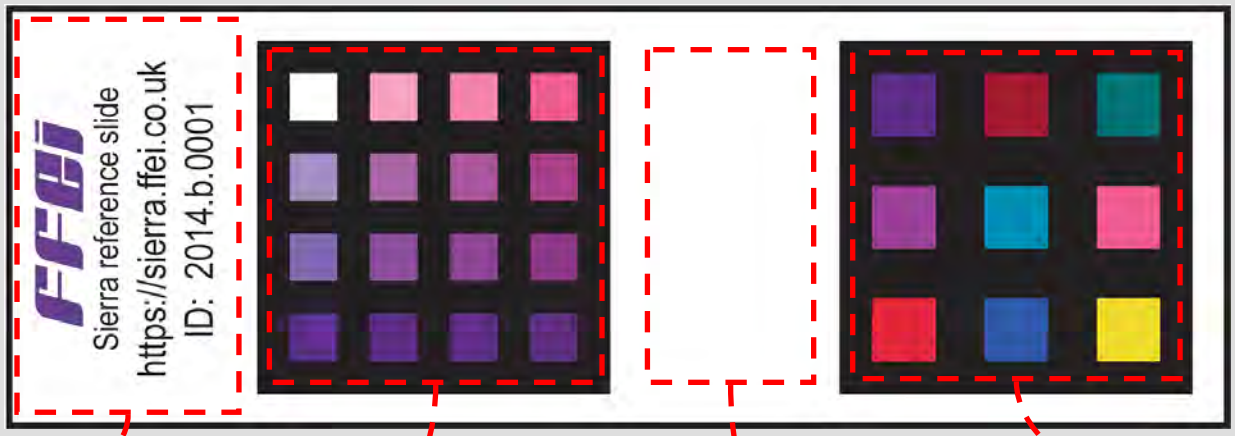
Calibration assessment slide



Slide uses FFEI's biopolymer staining technology to create a set of typical pathology colours

Unlike real pathology samples, coloured patches are uniform and are relatively easy to measure

Calibration assessment slide



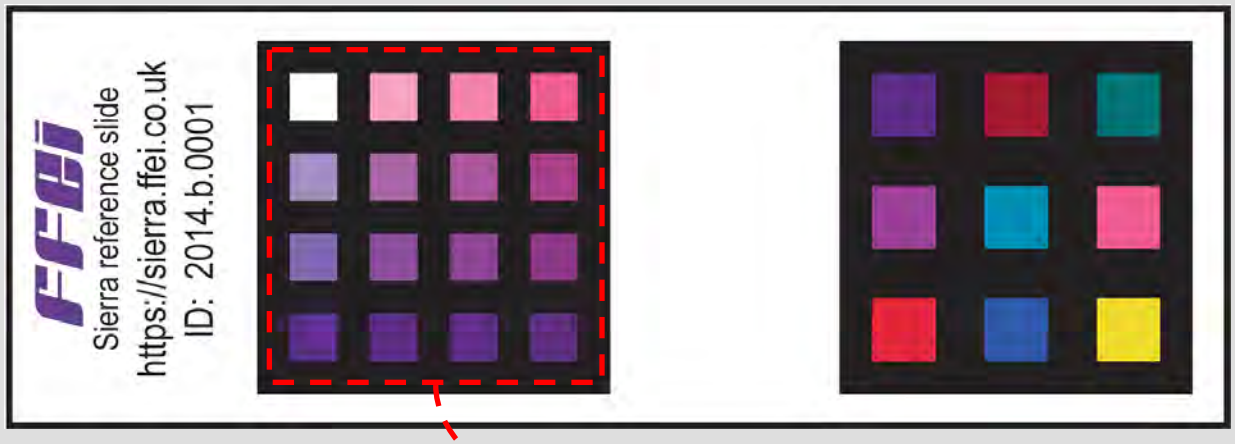
Slide identification area

H&E stain assessment area

Control patches area

Extended / visual assessment area

Calibration assessment slide

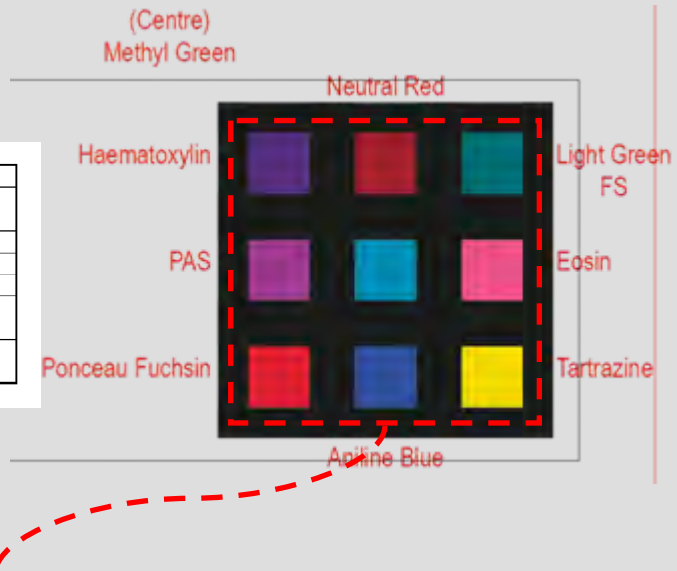


H&E stain assessment area

- 15 colours from the gamut of colours that appear on H&E stained slides and a 'reference white'

Calibration assessment slide

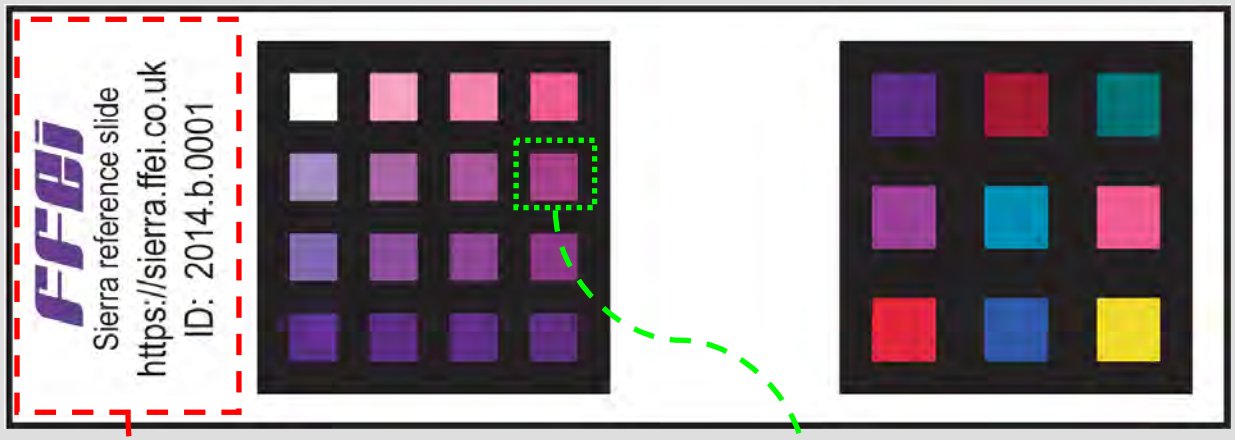
Stain	Staining protocol
Haematoxylin	Mayer's H&E, Harris H&E, H-DAB, PAP, Congo Red for Amyloid, Masson's Trichrome
Eosin	Mayer's H&E, Harris H&E, PAP
PAS	Periodic acid-Schiff, Alcian Blue PAS,
Ponceau Fuchsin	Ziehl Neelsen, Millers elastic Van Gieson, Masson Trichrome
Neutral Red	Gram Neutral Red
Aniline Blue	MSB
Methyl Green	Methyl Green
Light Green SF Yellowish	Papanicolaou, Jones methenamine silver, Masson trichrome
Tartrazine	Shikata

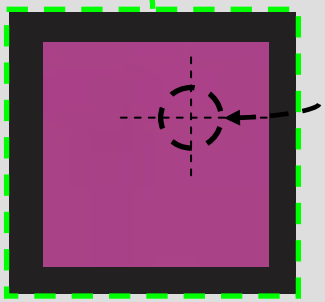


Extended / visual assessment area

- 9 stains that form the basis of a number of commonly used staining protocols
- set of colours selected to cover the gamut of colours found in stained pathology samples

Calibration assessment slide





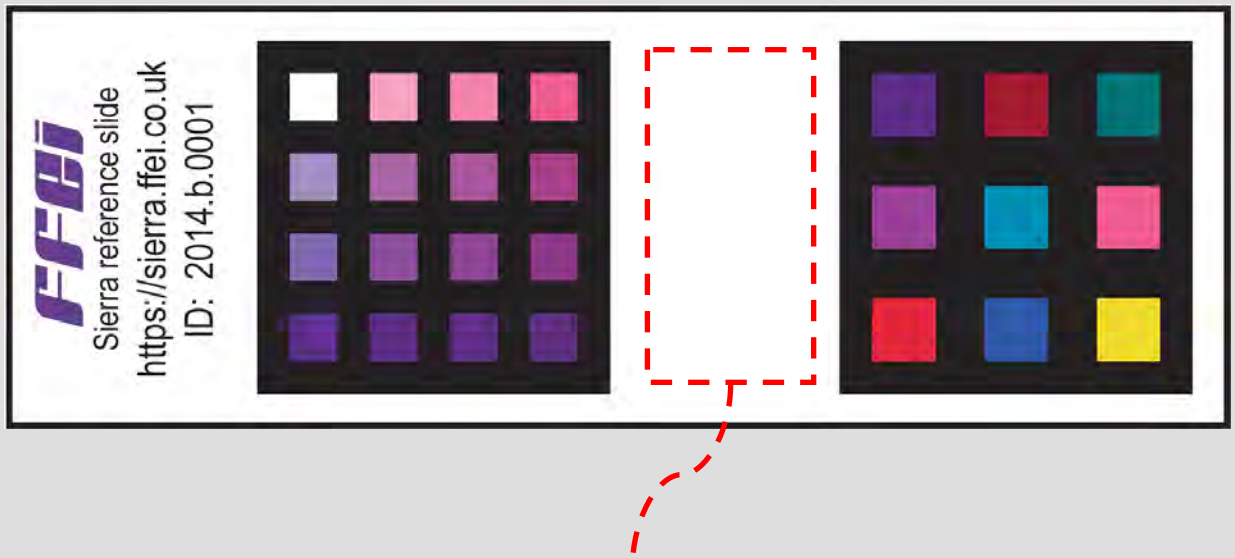
Individual measurement point

Slide identification area

- measurements for each slide and other data can be shared on a web site developed by FFEI (https://sierra.ffei.co.uk)

Average measurement value for each patch and colour of identified measurement point will be available

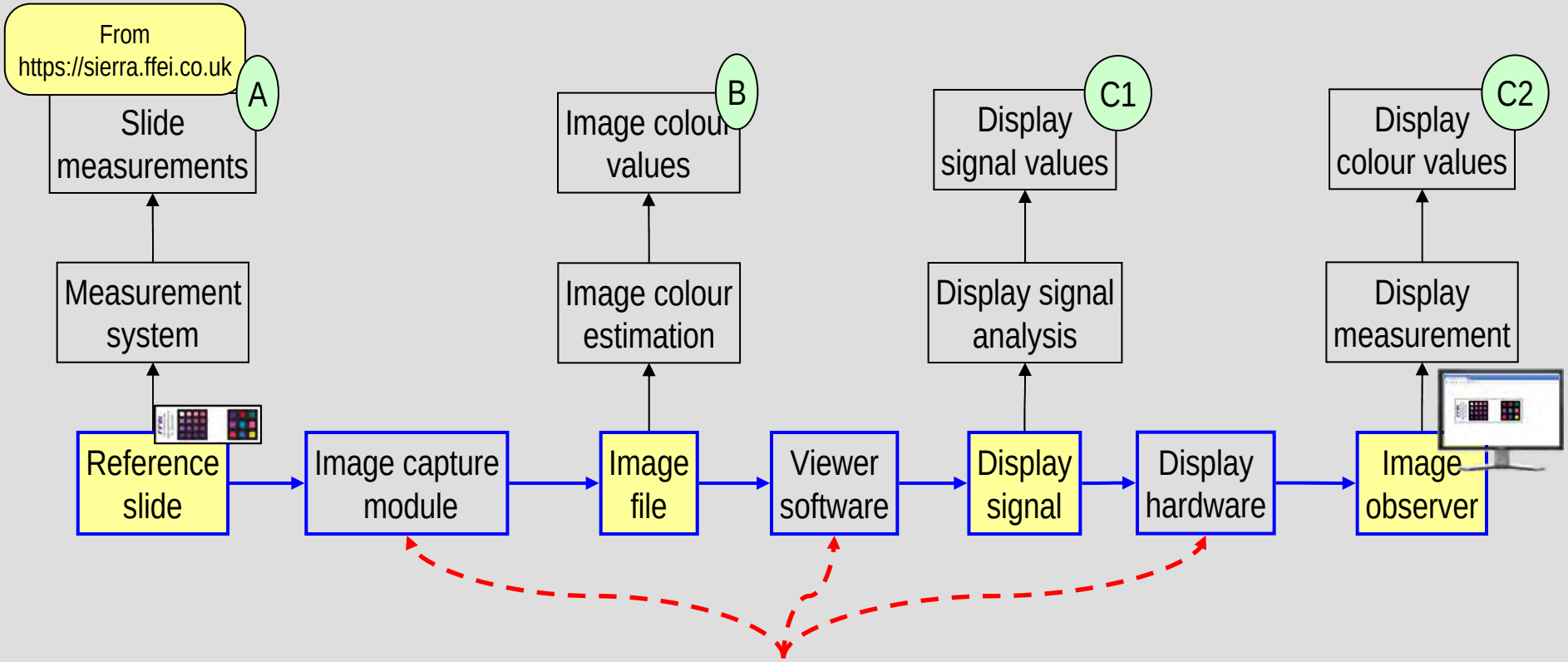
Calibration assessment slide



Control patches area

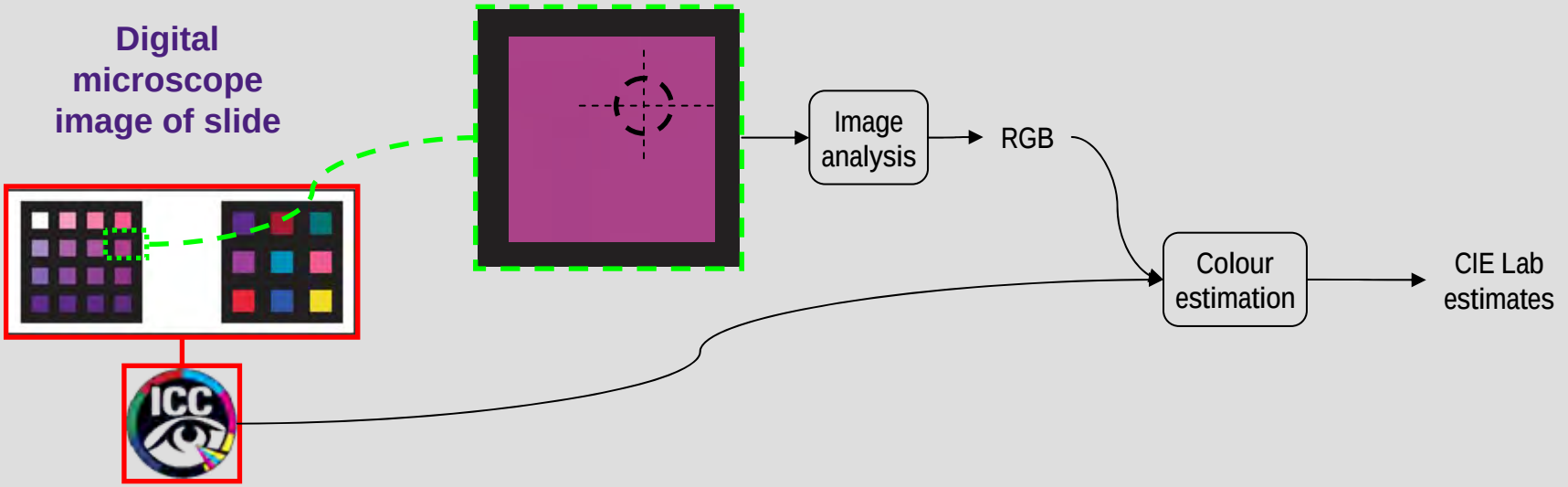
- will include additional patches to be used to indicate when the slide must be replaced (TBD)
- possibly add a set of neutral patches

How we expect the slide will be used



System components are calibrated using manufacturer's calibration method
The aim of the calibration assessment slide is to demonstrate / check that the system is able to handle colour with sufficient accuracy

Assessment step B: image colour values



ICC profile for calibrated digital microscope

Image processing software identifies average image RGB value for entire patch and image RGB value for the identified measurement point

These values are used in conjunction with the ICC Profile to determine the colour as seen by the digital microscope (CIE Lab estimates)

These values can then be compared with the slide measurements to determine the accuracy of the digital microscope capture system

Assessment step C1: Virtual Display

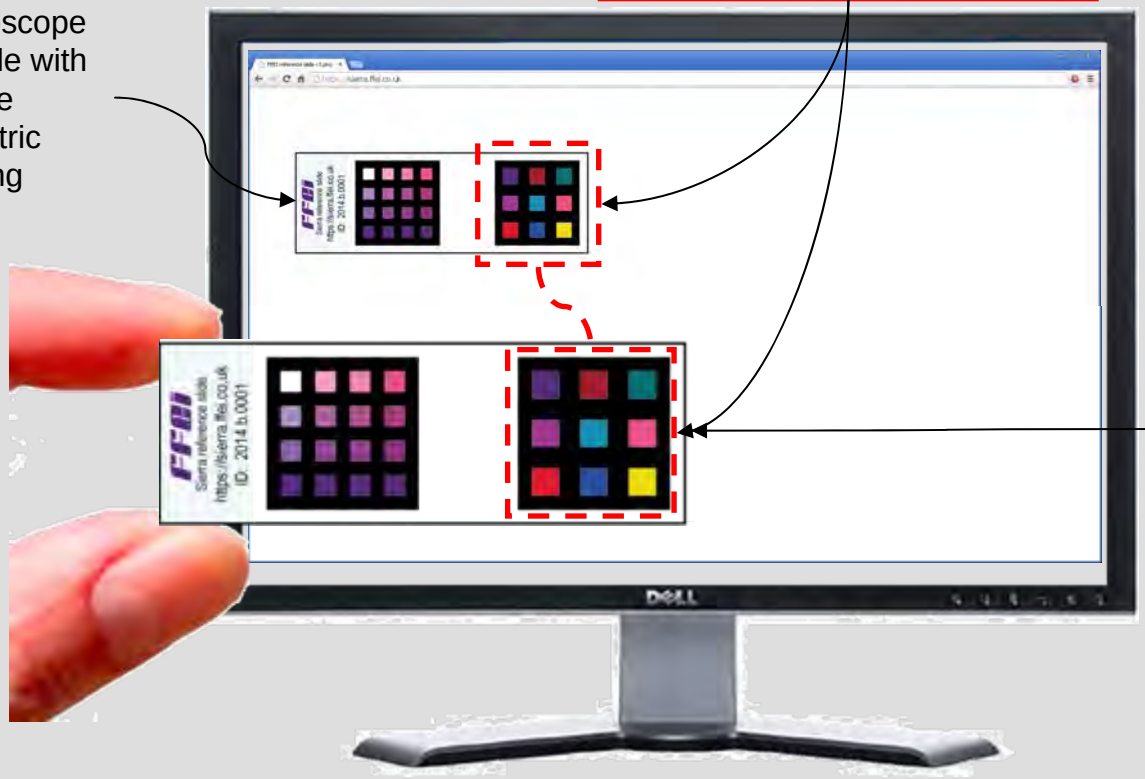
- Method proposed by Wei Chung Cheng (or similar) can be used to calculate average image RGB value for entire patch and image RGB value for the identified measurement point
- This data can be used in conjunction with the display ICC profile to calculate the colour values being presented for display

- See http://www.color.org/groups/medical/VDCP.xalter for details of the Virtual Display Color Processor proposed by Wei Chung

Assessment step C2: 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

Round-robin slide assessment proposal

- **Objectives**
  - check that all of the vendors are able to scan the slide and produce an image / ICC Profile
  - check that colour errors can be estimated reliably for this image by the participants
  - identify additional patches needed
- **Procedure**
  - FFEI manufacture, measure and scan slide
  - each company measures (where possible) and scans slide
  - slide returned to FFEI for measurement by the 'initial' measurement system to ensure that patch colours have not changed
  - measurements and scans to be shared between participants only
    - we could use https://sierra.ffei.co.uk for this purpose
- **If necessary second revision of slide created**
  - set of patches modified to include other or additional stains
  - geometry modification
  - . . .
- **Result of round robin assessment published**
  - will show the current calibration capability
  - can be used to define requirements for calibration assessment

Participants

- FFEI Limited (will manage the round-robin process)
- FDA (Aldo Badano, Wei-Chung Cheng)
- MGH (Yukako Yagi, Pinky Batista)
- Leica Biosystems (Allen Olson)
- Ventana (Scott Forster – to be confirmed)
- . . . others . . .

Call for participation

FFEI is currently exploring a number of options to fund the development and commercialisation of this slide

Contact George Hutchinson at FFEI

George.Hutchinson@ffei.co.uk