Evaluation of Targets for Color Calibrating Digital Color Images from an Optical Bright-Field Transmission Microscope

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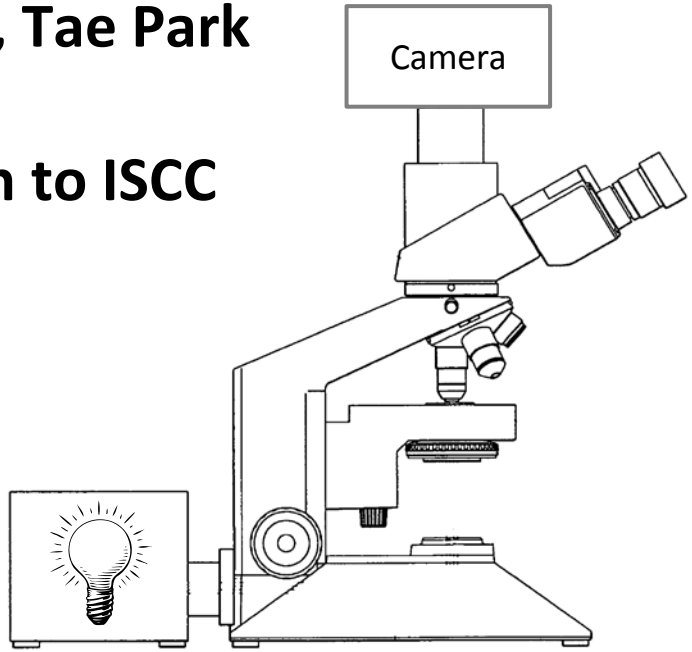
DATACOLOR
06/18/2014 Presentation to ISCC



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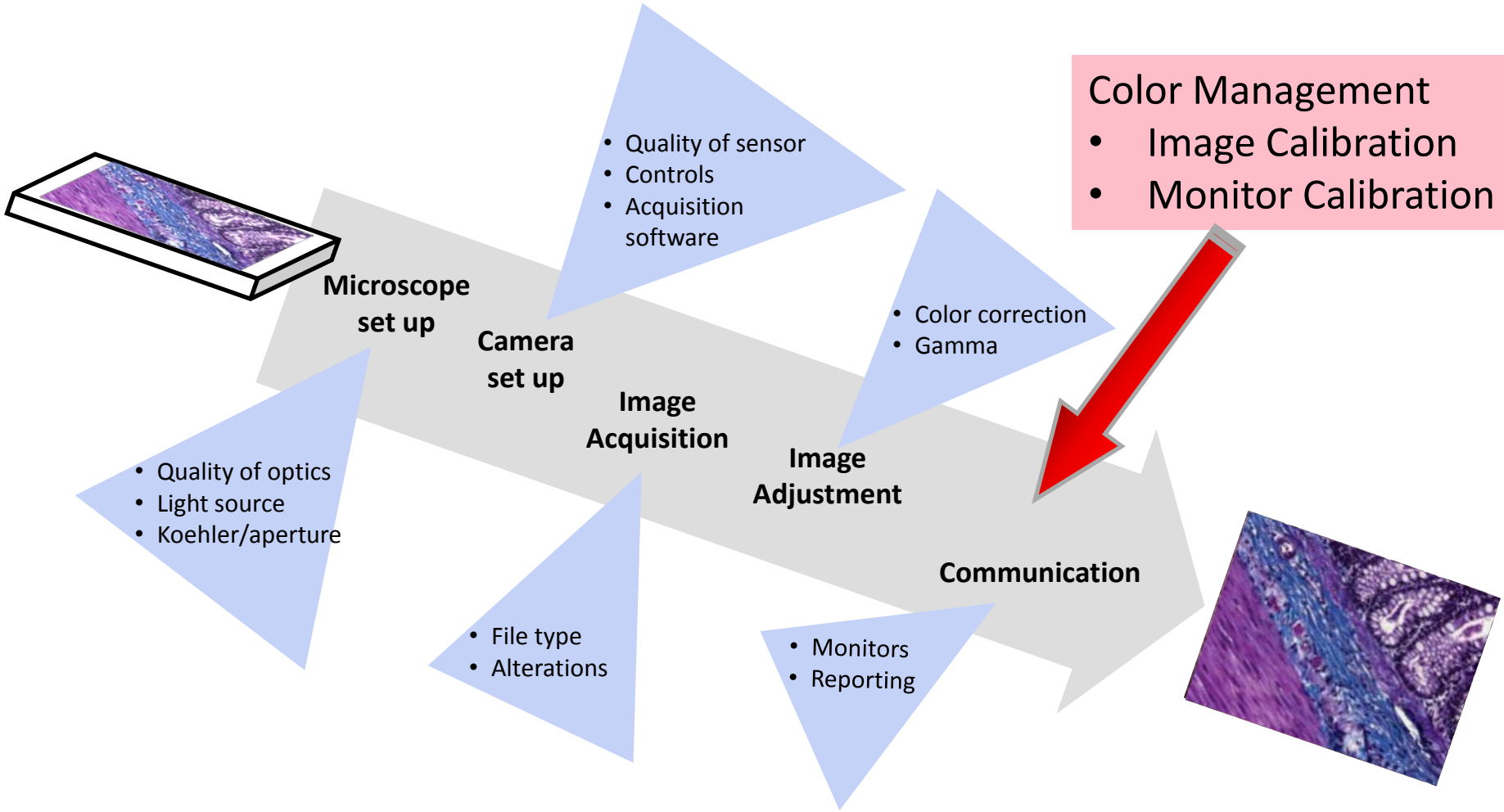
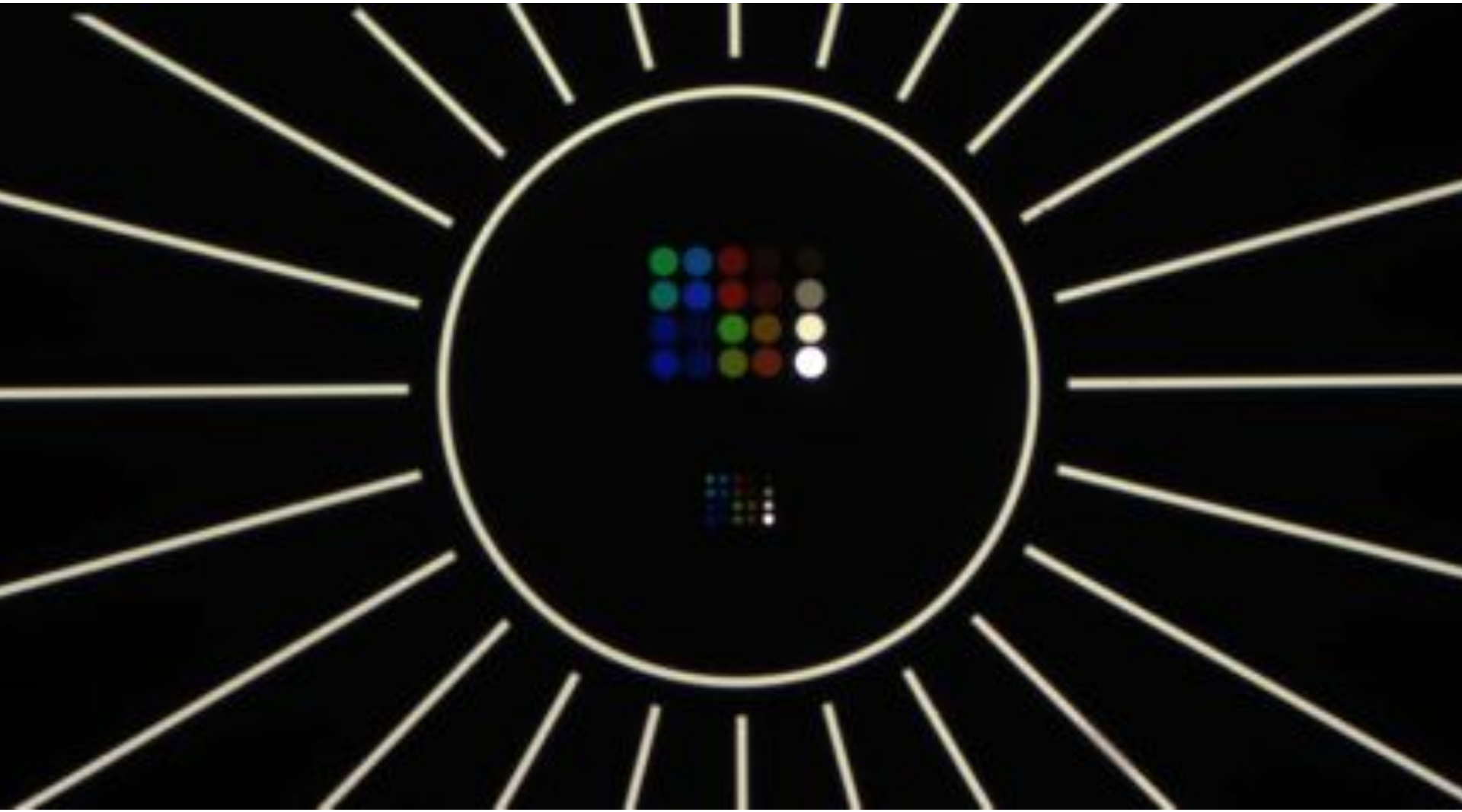
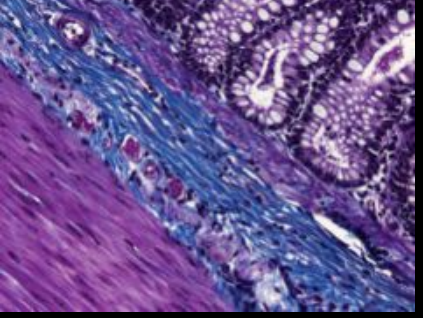
Sources of Color Variability



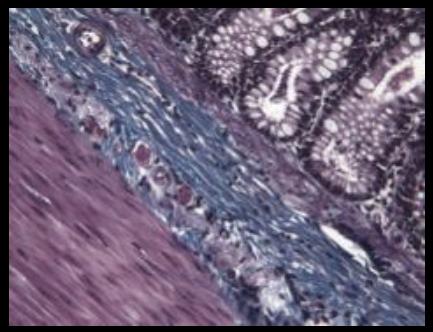
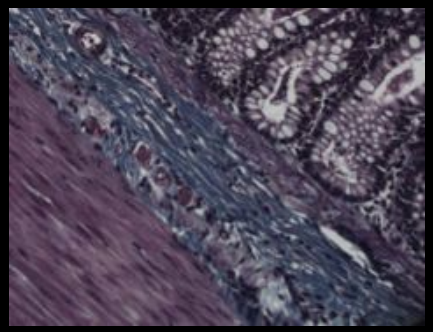
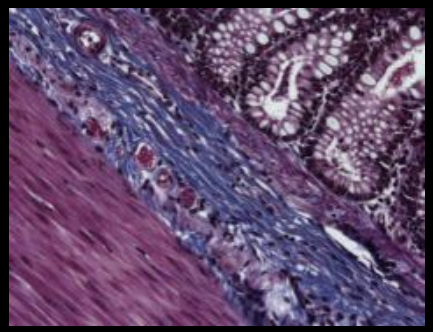
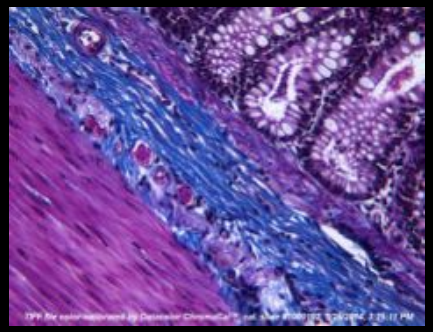
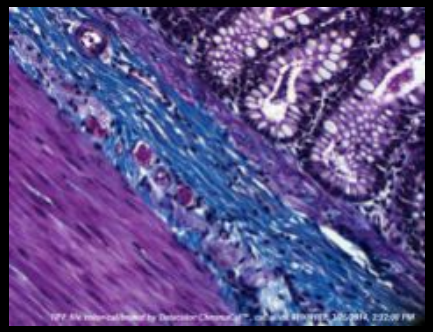
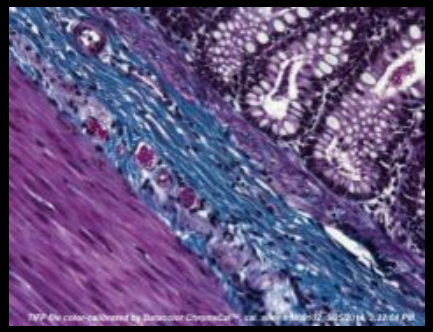
ChromaCal Calibration Slide



ChromaCal Example of Use



Ground truth color from multispectral camera on microscope 2

	Camera 1 on Microscope 1	Camera 1 on Microscope 2	Camera 2 on Microscope 2
Before Calibration			
After CHROMACAL Calibration			

Scope

- Develop methodology to assess ChromaCal technology
  - ✓ Create reference slide for testing.
  - ✓ Evaluate ChromaCal using the reference slide following ICC MIWG guidelines.
  - ✓ Provide the microscopy community with potential tools to test their own technologies.

Challenges

- No commercially available test color chart (slide) that is designed specifically for digital microscope images.
- Insufficient published data on the stain colors.
- No standard color management solution for digital microscope images.

Reference Slide & Development Algorithm

Reference Slide Development – 4 steps

1) Collect transmission spectra of biological samples with certain stain(s)* by multispectral imaging.

2) Compute the color coordinates of the sample with the stain(s) using a preferred illuminant, such as CIE D50.

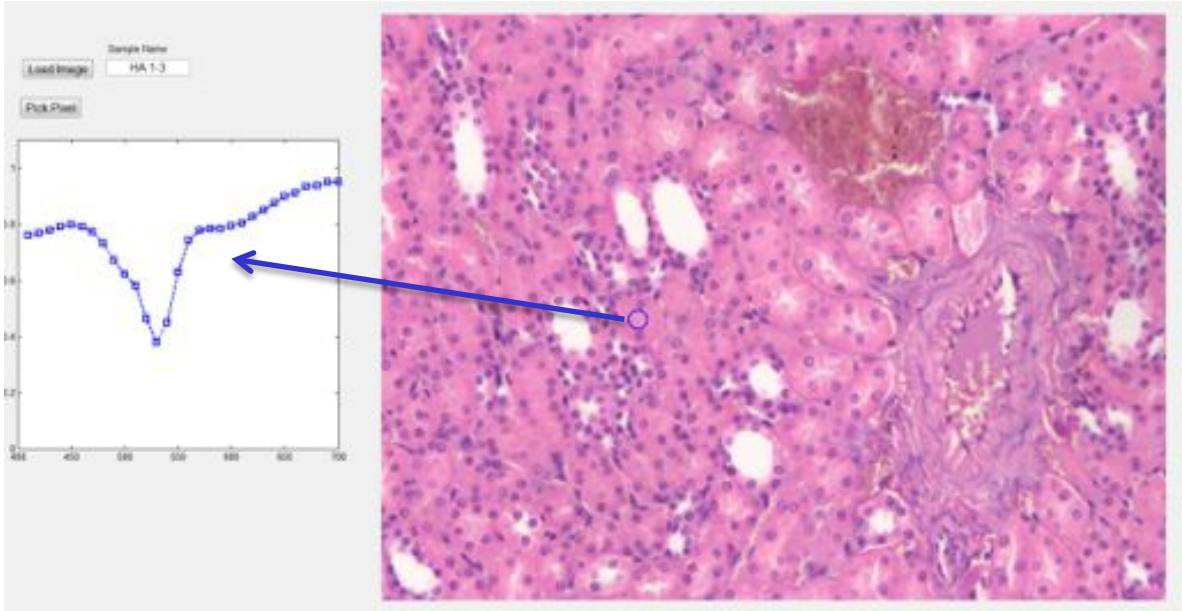
3) Group all the found colors together in the color space and delimit from them the color gamut of the stain(s).

4) Identify test color filters that match or close to the stain colors.

* Currently, the activity has been limited to Hematoxylin & Eosin stains only. But the same concept can be applied to other stains.

Step 1: Transmission Spectra Collection with Multi-spectral Camera

- **Band**: 31 bands, 400-700nm, 7nm bandwidth
- **Dynamic Range**: 12 bits
- **Pixel format**: 1392 x 1040 (1,447,680 transmission spectra)
- **Color Accuracy**: ~1 ΔE to a NIST certified spectrometer
- **Samples**: 22 H&E slides
- **Total of transmission spectra collected**: **31,848,960**



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Step 2&3:
Color Coordinates Calculation & H&E Color Gamut

Transmission Spectrum

↓

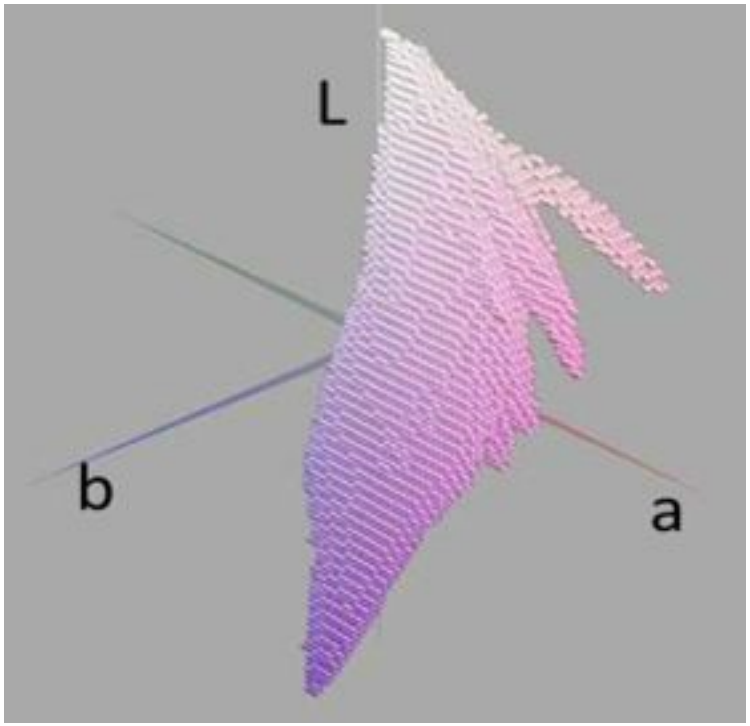
CIE XYZ @D50

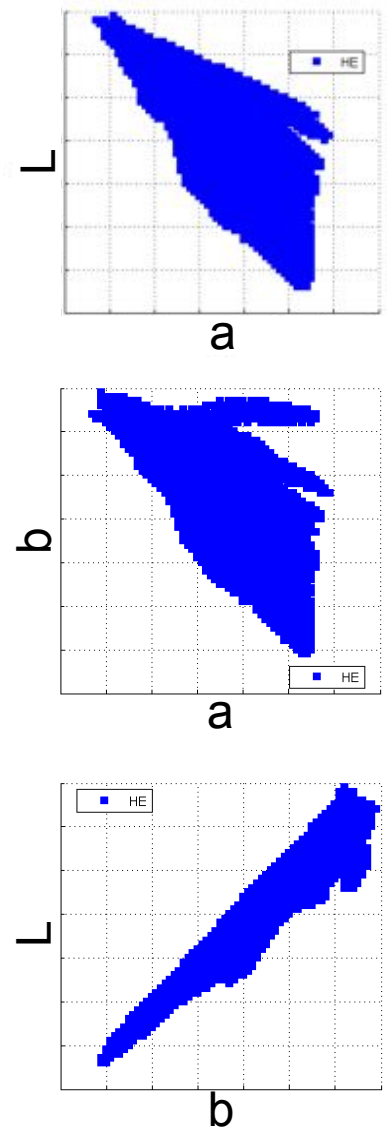
↓

CIE Lab

×31,848,960

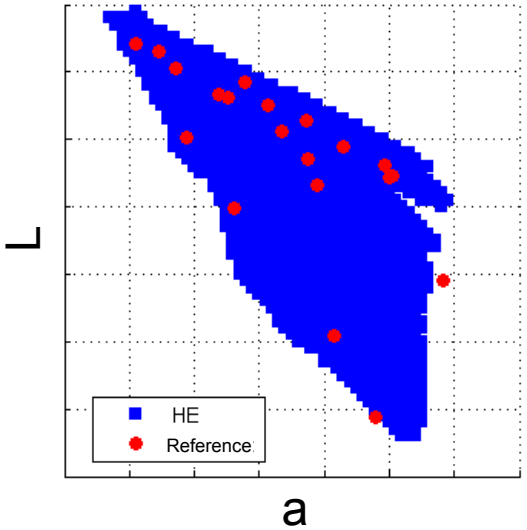
H&E Color Gamut

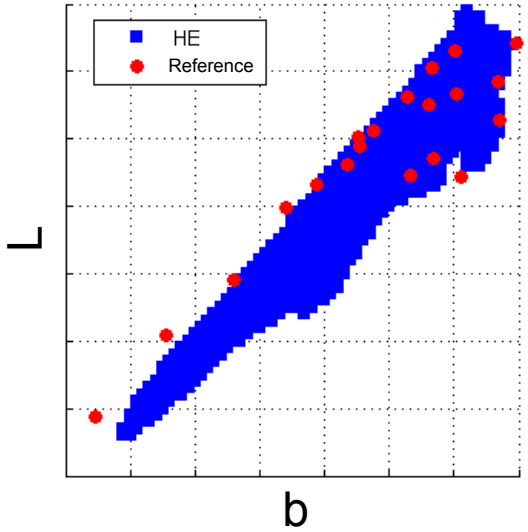


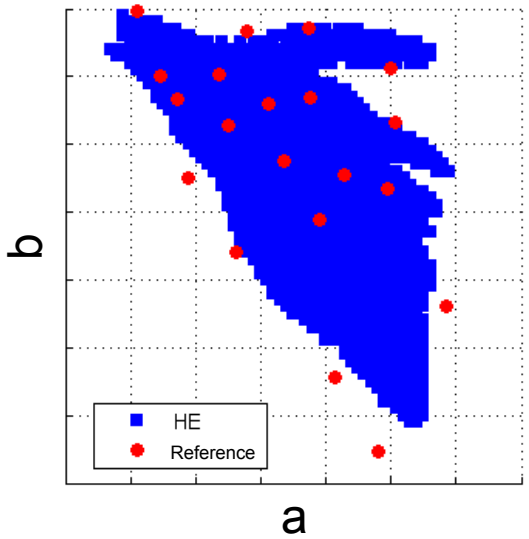


Step 4: Identify Reference Color Filters

H&E Stain Reference Slide







Features against ICC Requirements

ICC Proposed Requirements*	Features of H&E Reference Slide
The colors should be representative of the colors of stains;	All the 20 colors are within or very close to the H&E stain color gamut;
The colors should be measurable. The measurement should be repeatable;	Large patch size (~3.8mm), allowing easy measurement with typical measurement setup;
The color should be stable over time;	Stable solid color filter sandwiched between glass slide and cover glass with optical cement;
The slide should be clearly identified;	Large space for barcode;
Easy scanning of the slide on digital microscope systems.	Glass slide, same 3”x1” dimension

** Adapted from ICC MIWG (Medical Imaging Working Group) white paper at work*
http://www.color.org/groups/medical/Digital_microscope_test_materials_and_test_methods-v3.pdf

ChromaCal Performance Testing

Color Performance Test Work Flow

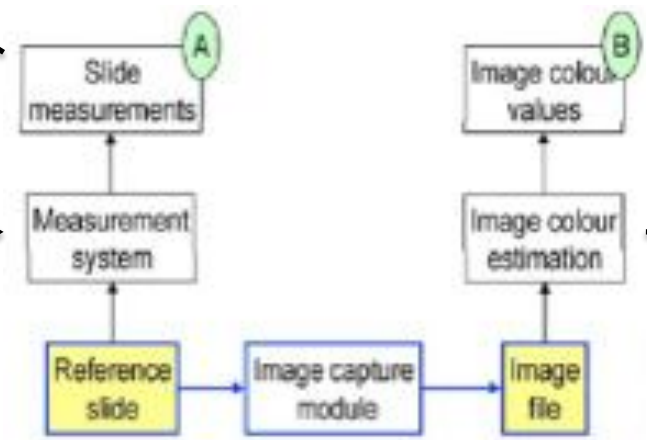
True Labs of 20 test colors, D50

Raw_ΔE, 2000

Rendered Labs of 20 test colors

Multispectral Imaging

CIE XYZs (sRGB to XYZ, D50)



http://www.color.org/groups/medical/Digital_microscope_test_materials_and_test_methods-v3.pdf

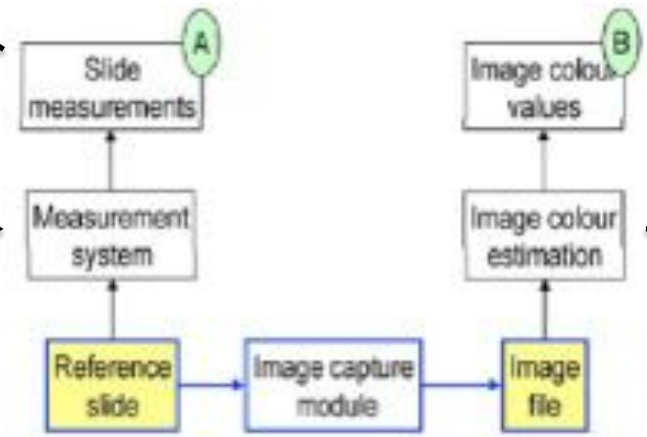
Color Performance Test Work Flow

True Labs of 20 test colors, D50

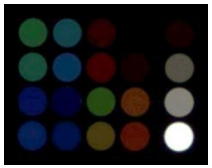
Cal_ΔE, 2000

CIE Labs of 20 test colors

Multispectral Imaging



CIE XYZs after **ChromaCal** Calibration (D50)



http://www.color.org/groups/medical/Digital_microscope_test_materials_and_test_methods-v3.pdf

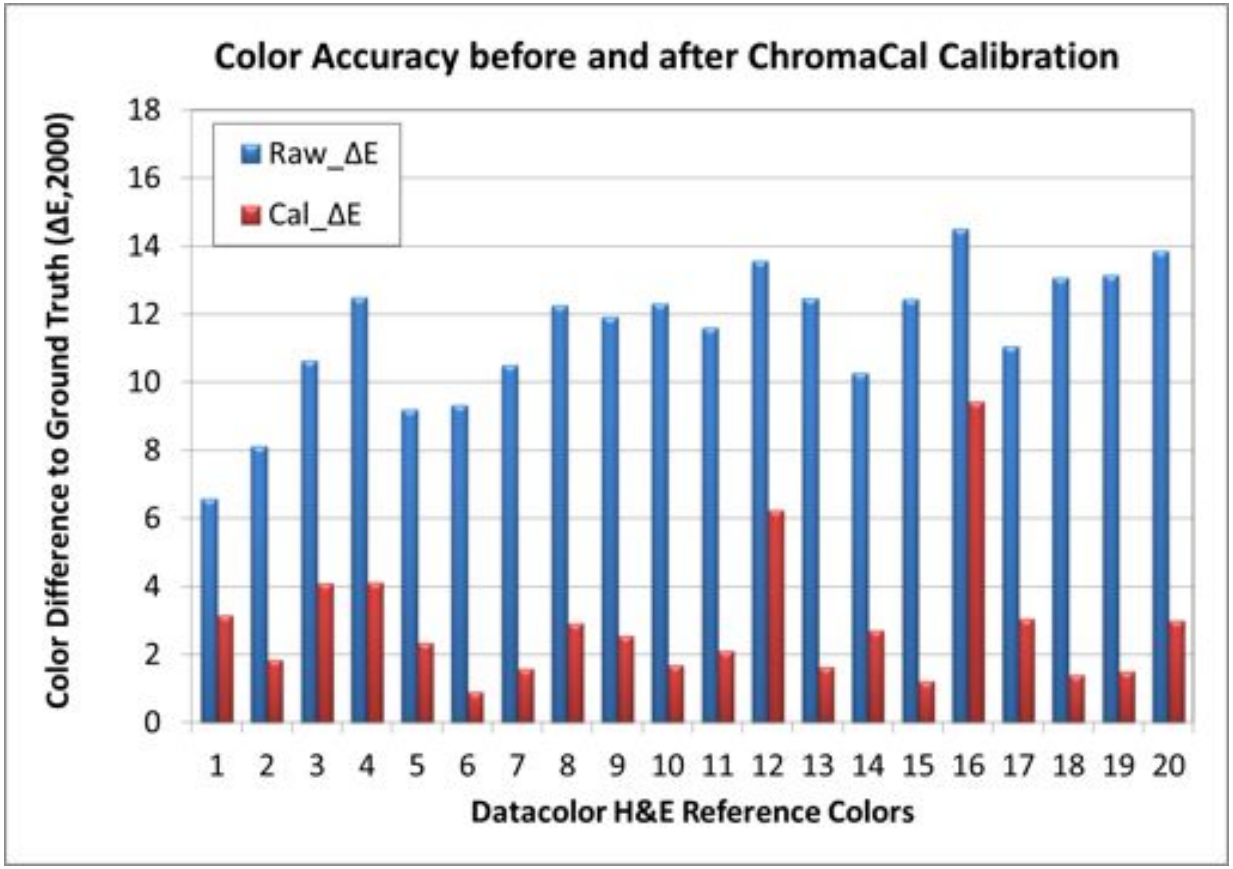
ChromaCal
Color Accuracy – with Tungsten light @ 3000K

Microscope:
Zeiss Axio Scope A1

Objective:
Zeiss Achroplan
20x/0.45

Digital Camera:
Lumenera Infinity 2-1R
1.4M pixels CCD

Light Source:
Tungsten Halogen
@3000K



	Raw_ΔE	Cal_ΔE	Improved Accuracy
Mean	11.46	2.87	**75%**
Max	14.49	9.44	**35%**

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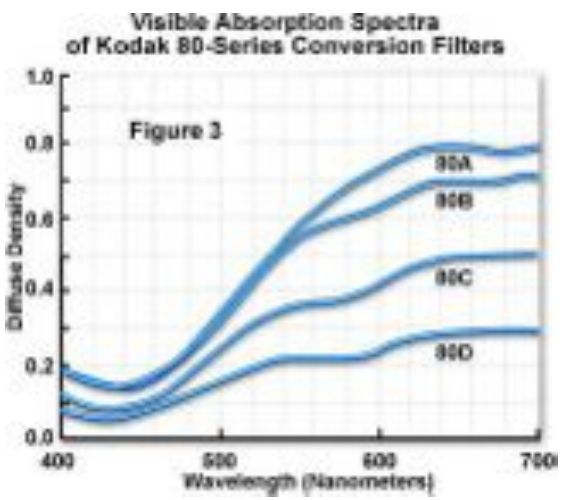
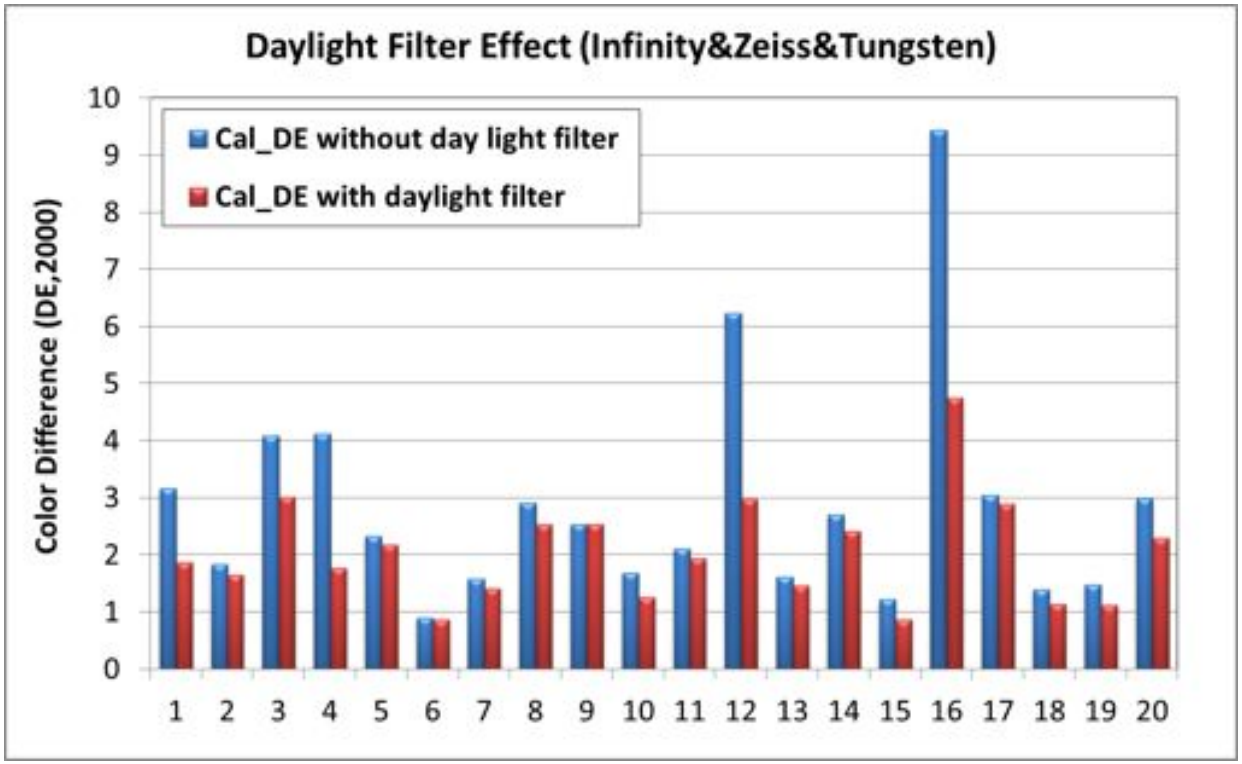
ChromaCal
Color Accuracy – Tungsten with Daylight Filter

Added a daylight filter (Kodak 80A) to increase the CCT of the light source from 3200K to 4830K



Adapted from http://www.olympusmicro.com/primer/photomicrography/colortemperature.html



	Without Daylight Filter	With Daylight Filter
Mean ΔE	2.87	2.05
Max ΔE	9.44	4.75

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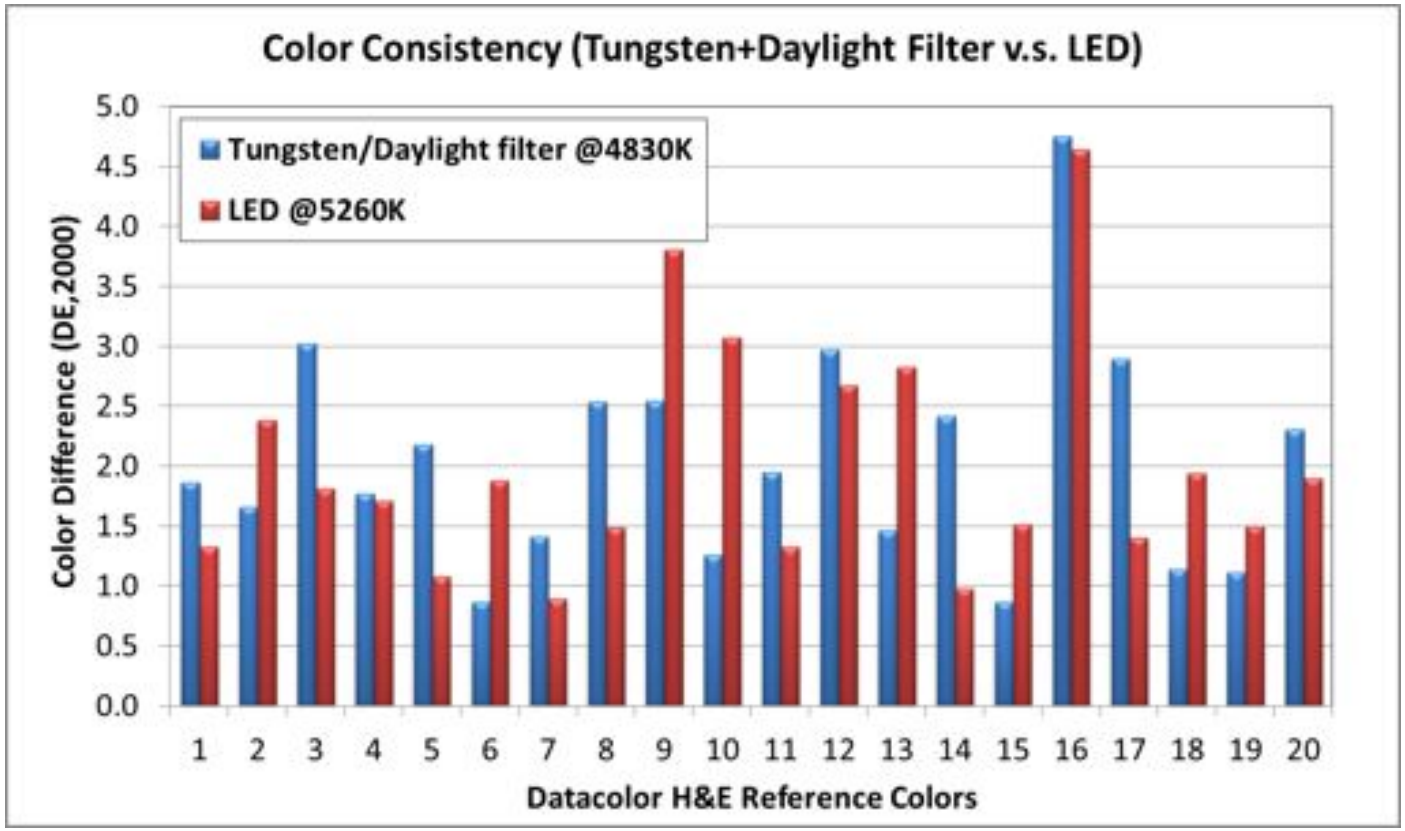
ChromaCal
Color Accuracy – Tungsten vs. LED



	Tungsten w/daylight filter	LED
Mean ΔE	2.05	2.01
Max ΔE	4.75	4.64

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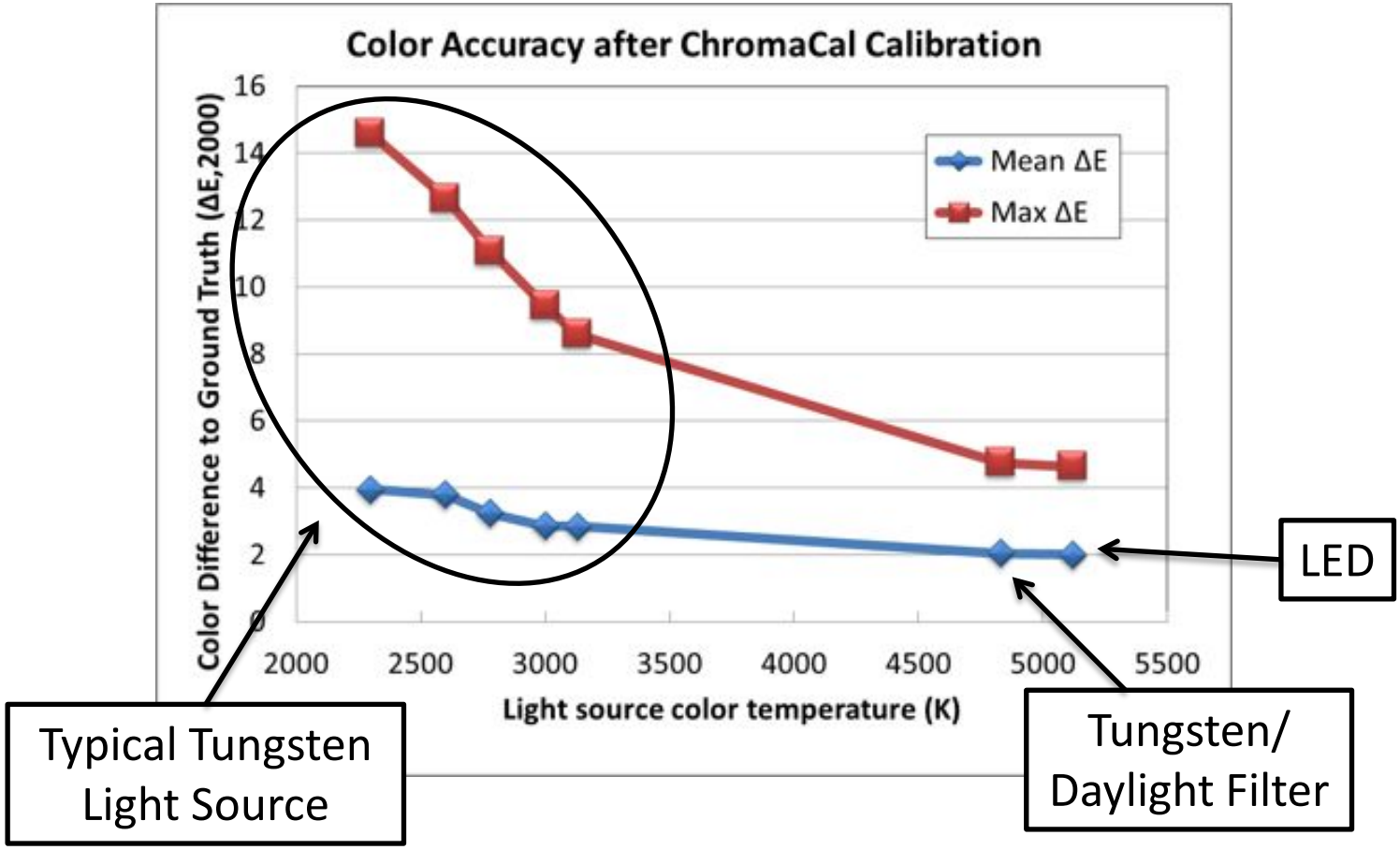
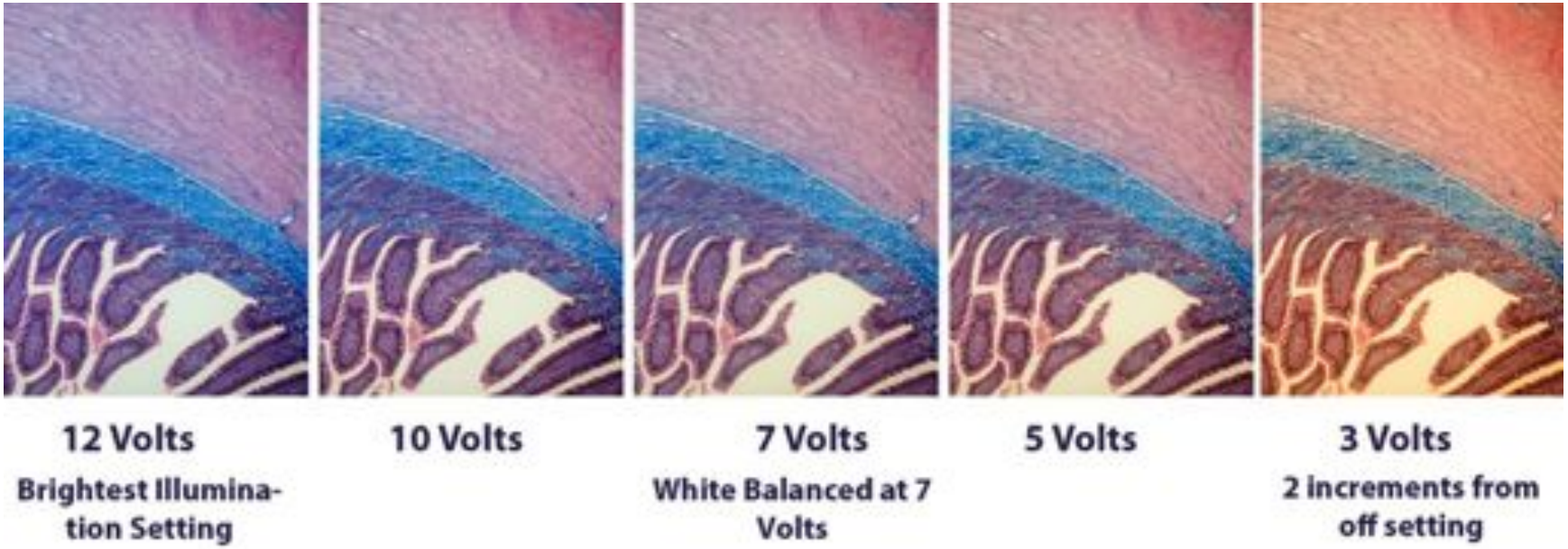
ChromaCal
Color Accuracy is sensitive to light source CCT



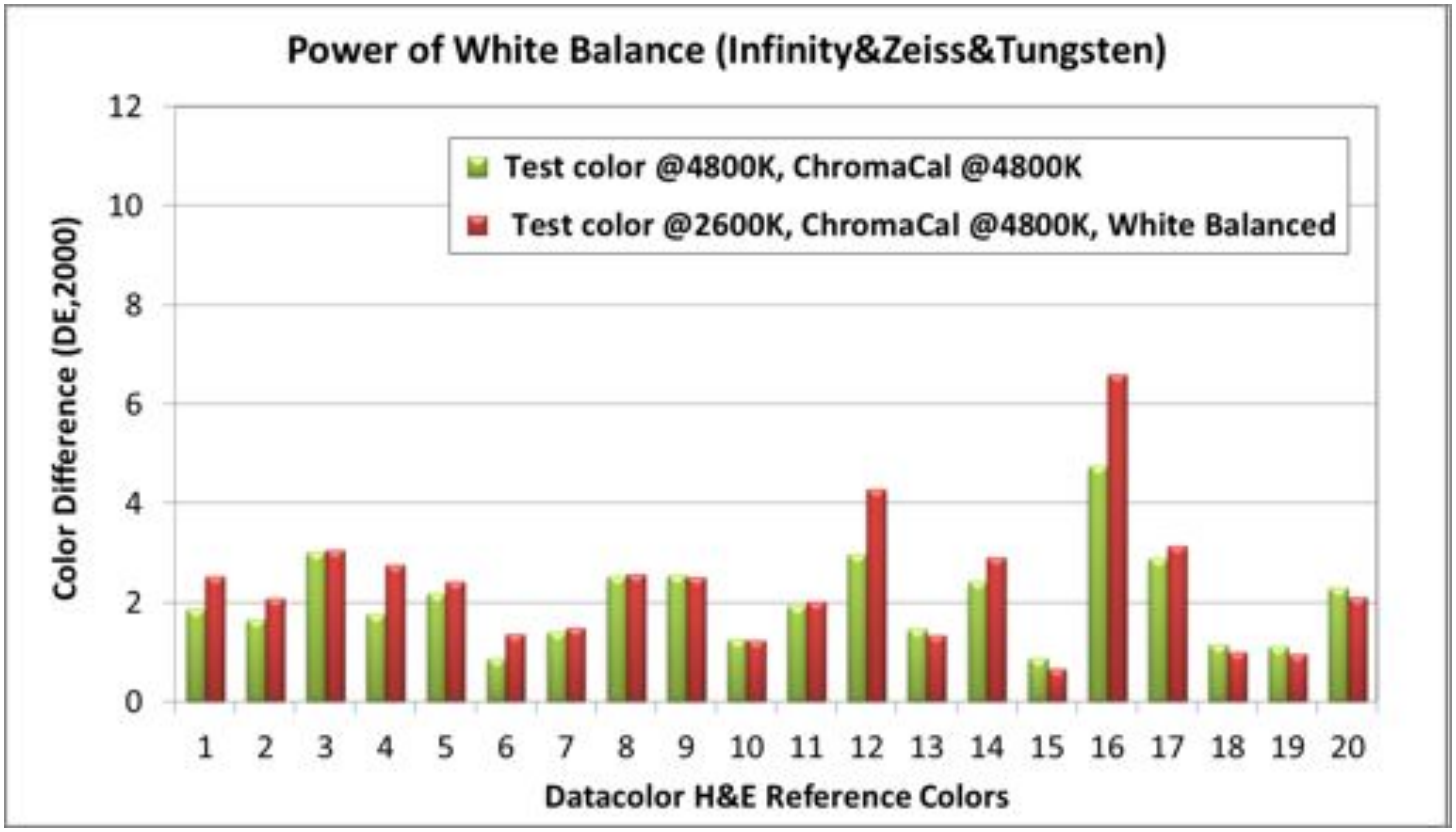
Light Source Color Temperature

Image courtesy of Jerry Sedgewick, Imaging & Analysis, LLC



Note: These slides have identical magnification. Generally change of magnification is what necessitates change of intensity and CCT

ChromaCal
Power of Auto White Balance



Cal_DE	Test color @4800K ChromaCal @4800K	Test color @2600K ChromaCal @4800K	Test color @2600K ChromaCal @4800K, AWB
Mean	2.05	6.77	2.35
Max	4.75	10.23	6.60

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Summary

- ✓ Developed a H&E stains reference slide containing 20 large color patches.
- ✓ Meets the requirements from ICC MIWG.
- ✓ Evaluated the color Performance of the ChromaCal™ Image Calibration using this reference slide.

Future Work

- ➢ Test more samples with H&E stains to improve the complicity of the H&E color gamut
- ➢ Conduct the similar work on other stains.