Whole Slide Imaging Sierra round robin initial results and proposed next steps

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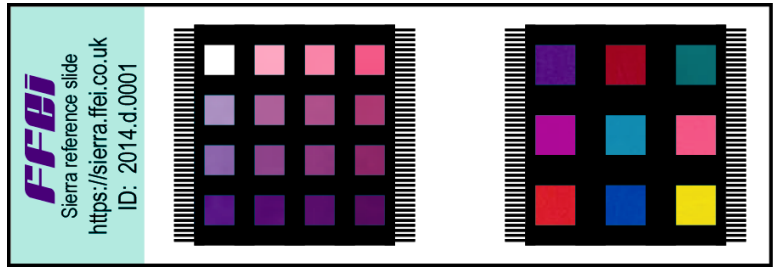
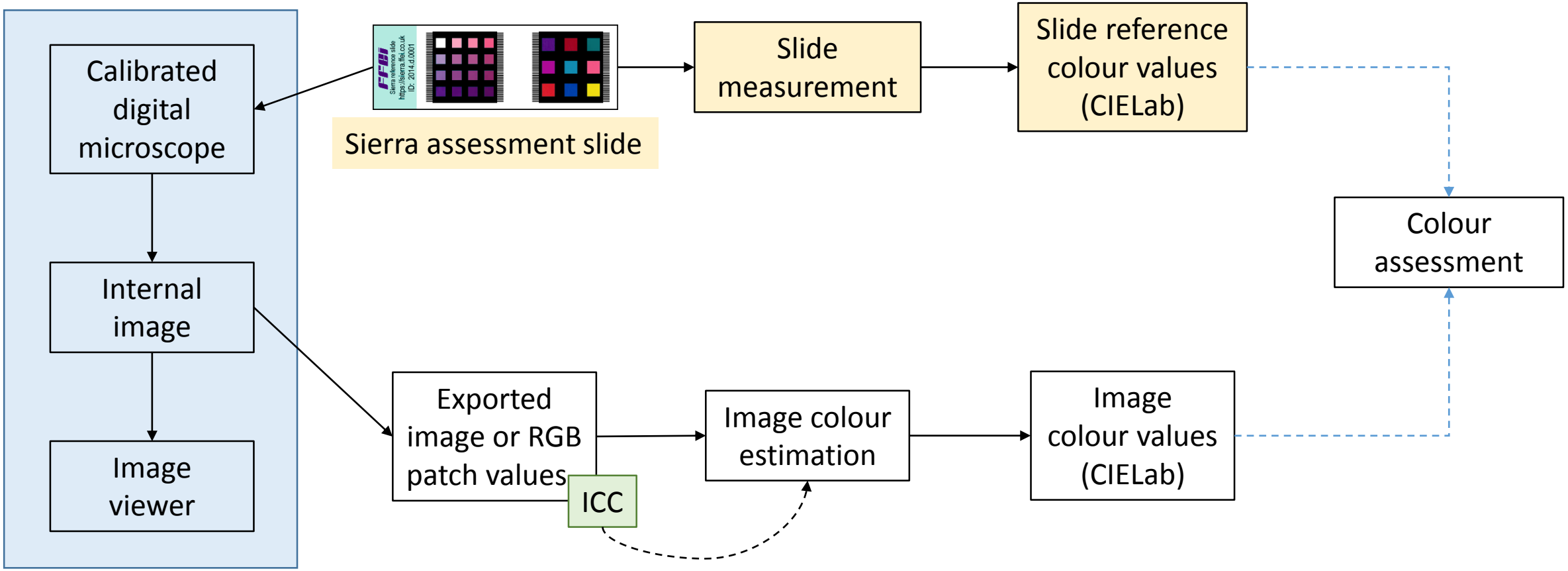
Wednesday 4th March 2015



Assessment method



Calculation of slide reference CIEXYZ values

$$X = \sum_{\lambda} S_\lambda \times T_\lambda \times \bar{x}_\lambda \qquad X_n = \sum_{\lambda} S_\lambda \times \bar{x}_\lambda$$

$$Y = \sum_{\lambda} S_\lambda \times T_\lambda \times \bar{y}_\lambda \qquad Y_n = \sum_{\lambda} S_\lambda \times \bar{y}_\lambda$$

$$Z = \sum_{\lambda} S_\lambda \times T_\lambda \times \bar{z}_\lambda \qquad Z_n = \sum_{\lambda} S_\lambda \times \bar{z}_\lambda$$

T_λ is the ***relative spectral transmittance*** of the patch (relative to white patch)
X_n, Y_n, Z_n are the tristimulus values for the illuminant
S_λ is the spectral power distribution of the ***reference illuminant (D50)***
$\bar{x}_\lambda, \bar{y}_\lambda, \bar{z}_\lambda$ are the CIE 1931 colour matching functions

Data for D50 and for $\bar{x}_\lambda, \bar{y}_\lambda, \bar{z}_\lambda$ is available from http://files.cie.co.at/204.xls

Calculation of slide reference CIELab values

$$L_x = f\left(\frac{X}{X_n}\right) * 116 - 16$$

$$L_y = f\left(\frac{Y}{Y_n}\right) * 116 - 16$$

$$L_z = f\left(\frac{Z}{Z_n}\right) * 116 - 16$$

$$f\left(\frac{X}{X_n}\right) = \left(\frac{X}{X_n}\right)^{\frac{1}{3}} \quad \text{if } \left(\frac{X}{X_n}\right) > \left(\frac{6}{29}\right)^3$$

$$f\left(\frac{X}{X_n}\right) = \left(\frac{841}{108}\right)\left(\frac{X}{X_n}\right) + \frac{4}{29} \quad \text{if } \left(\frac{X}{X_n}\right) \leq \left(\frac{6}{29}\right)^3$$

With similar equations for Y and Z.

Source ICC Profile Specification Annex A
http://www.color.org/specification/ICC1v43_2010-12.pdf

Relative spectral transmittance of the patch (T_λ)

Spectral transmittance (relative to clear patch) from 380:800 nm (T_λ)

Measurements file for each slide was created by FFEI and is available from https://sierra.ffei.co.uk/

ISO28178																	
ORIGINAT	FFEI Limited																
DESCRIPT(	Sierra calibration assessment slide																
SERIAL	2014.d.0002																
CREATED	06/06/2014																
FORMAT_'	1																
INSTRUME	FFEI / Hamamatsu test rig																
NUMBER_	53																
BEGIN_DATA_FORMAT																	
SAMPLE_I	PatchName	Measuren	PatchX	PatchY	PatchWid	PatchHeig	Measuren	Measuren	UniformR	SPECTRAL_380	SPECTRAL_390	SPECTRAL_400	SPECTRAL_410	SPECTRAL_420	SPECTRAL_430	SPECTRAL_440	SPECTRAL_450
END_DATA_FORMAT																	
NUMBER_	50																
BEGIN_DATA																	
A1	H0E0	Mean	0	0	3	3	0	0	0	1	1	1	1	1	1	1	1
A2	H0E25	Mean	5	0	3	3	0	0	0	0.898890193	0.885033823	0.894140688	0.901182362	0.904598719	0.905642286	0.902823798	0.903809112
A3	H0E50	Mean	10	0	3	3	0	0	0	0.869149593	0.857141506	0.856683901	0.86377907	0.86536508	0.865037301	0.855936817	0.826616779
A4	H0E100	Mean	15	0	3	3	0	0	0	0.842899411	0.81303741	0.803548899	0.810905191	0.817135858	0.811522362	0.784411469	0.725915665
A5	H25E0	Mean	0	5	3	3	0	0	0	0.839513194	0.830188825	0.827022335	0.825572831	0.815986662	0.815908136	0.813458709	0.81388336
A6	H25E25	Mean	5	5	3	3	0	0	0	0.757256251	0.747400329	0.739387115	0.737767397	0.732518013	0.735597478	0.731522425	0.729656824
A7	H25E50	Mean	10	5	3	3	0	0	0	0.741723679	0.722278172	0.714161774	0.707186605	0.707332265	0.700006611	0.691916828	0.67166944
A8	H25E100	Mean	15	5	3	3	0	0	0	0.71395145	0.687782277	0.677039949	0.677111531	0.674746676	0.669358169	0.649050646	0.595294184
A9	H50E0	Mean	0	10	3	3	0	0	0	0.71148624	0.706612241	0.689705307	0.660026203	0.636598656	0.6259645	0.619007147	0.62187989
A10	H50E25	Mean	5	10	3	3	0	0	0	0.644645257	0.628303039	0.615297021	0.593576311	0.570755635	0.558255758	0.553926747	0.550709453
A11	H50E50	Mean	10	10	3	3	0	0	0	0.633889875	0.61104625	0.596975535	0.57314085	0.552408002	0.536523188	0.525511399	0.508116682
A12	H50E100	Mean	15	10	3	3	0	0	0	0.609181424	0.588971754	0.563112012	0.543311221	0.521187869	0.506568571	0.485436455	0.447533144
A13	H100E0	Mean	0	15	3	3	0	0	0	0.615513872	0.591437474	0.566943285	0.538905358	0.508863936	0.489924805	0.481103081	0.478556718
A14	H100E25	Mean	5	15	3	3	0	0	0	0.596657812	0.566484129	0.543282355	0.509588867	0.48239242	0.463519798	0.455023255	0.449676711
A15	H100E50	Mean	10	15	3	3	0	0	0	0.578287631	0.545536544	0.521231659	0.496317804	0.463264578	0.442907237	0.431389391	0.41292865

Patch measurements have been made by a number of participants which show good agreement with published values

Use of D50 as a reference illuminant

- D50 is **probably not the best reference** but was used for ease of comparison
  - All other options require some form of chromatic adaptation (see http://www.brucelindbloom.com/index.html?Eqn_ChromAdapt.html)
- Other candidates
  - The measured SPD of a ‘typical’ optical microscope, perhaps an average of some kind
  - The SPD of the whole slide imaging system (this is never viewed)
  - CIE Illuminant E (a synthetic flat spectrum)

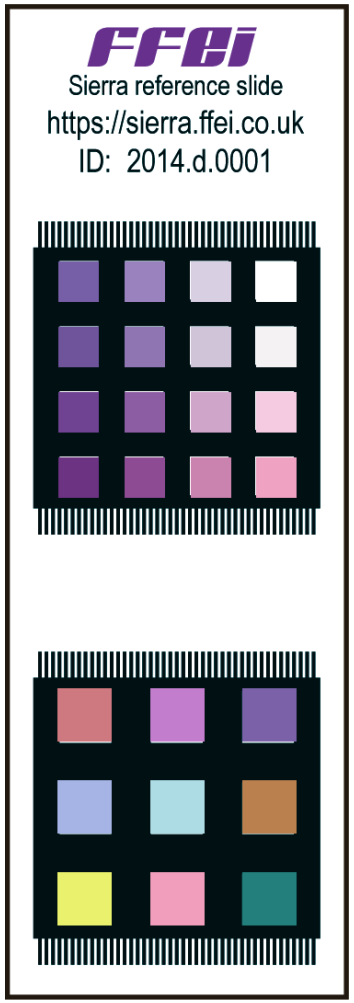
- Data for D50 and for $\bar{x}_\lambda, \bar{y}_\lambda, \bar{z}_\lambda$ is available from http://files.cie.co.at/204.xls

Reference slide CIELab values

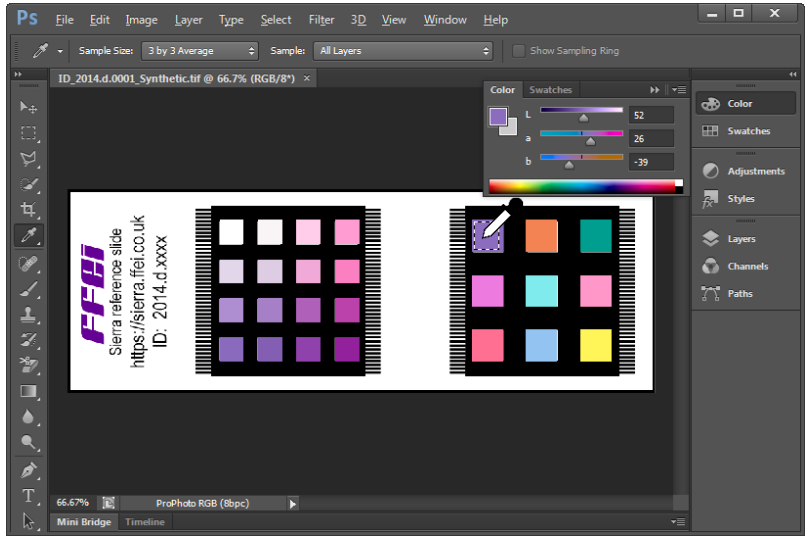
Synthetic reference image in ProPhoto RGB for slide ID_2014.d.0001

When displayed on a calibrated wide gamut display this image matches the reference slide illuminated by D50

Note that several of these patches are outside of the colour gamut of some displays (see following slide)

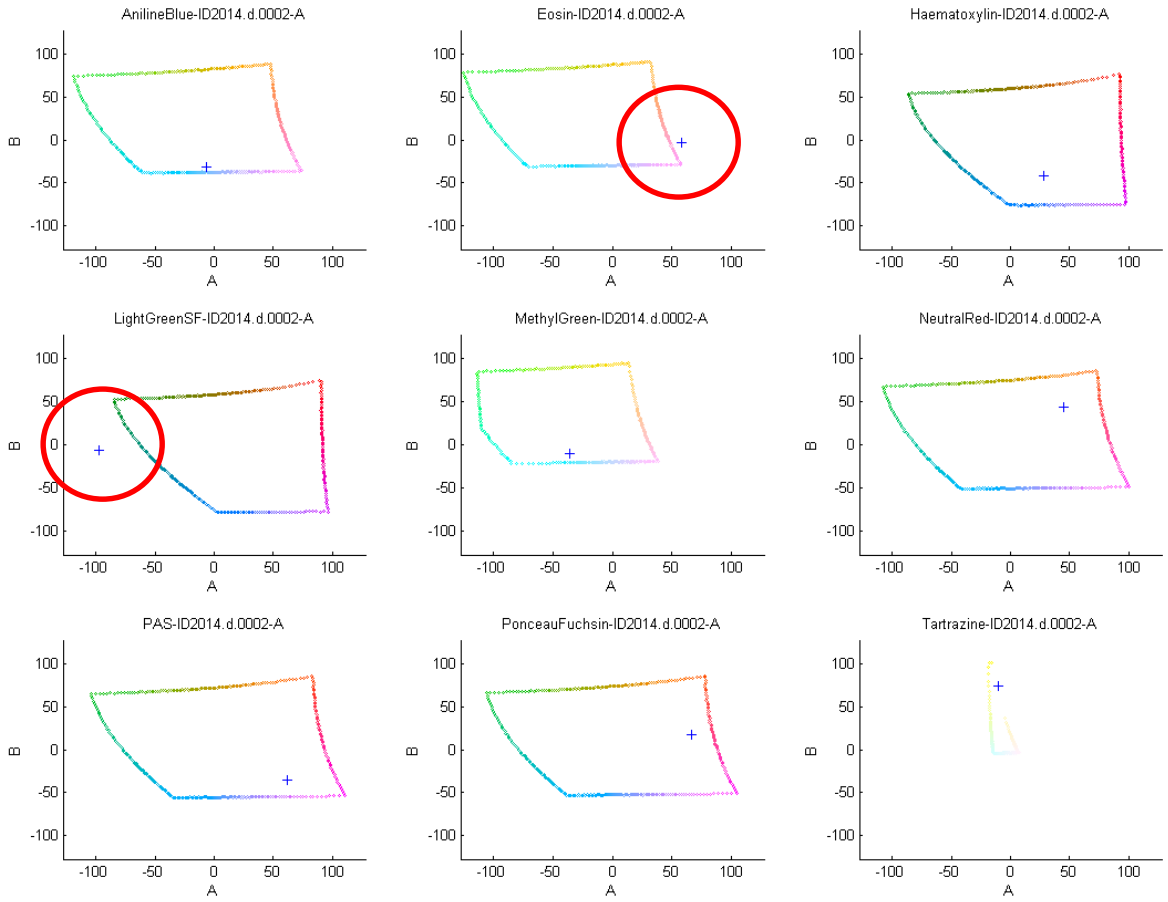


Patch Name	Lab_L	Lab_a	Lab_b
H0E0	99.9988	0.0033	-0.0044
H0E25	96.6426	2.2968	-0.3818
H0E50	88.8474	27.9223	-4.6433
H0E100	80.1688	53.3615	-4.1687
H25E0	87.1097	6.9449	-8.7922
H25E25	84.2053	9.0656	-8.5593
H25E50	77.5895	31.5652	-12.2809
H25E100	69.6112	53.5979	-11.3816
H50E0	64.0146	21.7882	-29.9941
H50E25	59.5005	24.7506	-30.794
H50E50	53.2755	42.6467	-32.911
H50E100	49.2011	57.2553	-31.1975
H100E0	50.9944	25.9697	-39.4892
H100E25	47.0626	28.5534	-40.0369
H100E50	42.4234	44.7031	-42.1123
H100E100	37.7625	55.9028	-40.2539
Haematoxylin	51.9317	25.364	-38.4756
NeutralRed	67.48	41.1816	45.0063
LightGreenSF	52.984	-91.0031	-10.5381
PAS	67.5002	54.2691	-31.4764
MethylGreen	86.6407	-32.4549	-10.5291
Eosin	79.564	55.571	-1.1358
PonceauFuchsin	68.1692	61.1493	13.2545
AnilineBlue	76.7327	-7.7321	-27.6187
Tartrazine	95.6619	-9.1693	73.1616



Photoshop can be used to inspect patch colour values using Eyedropper tool

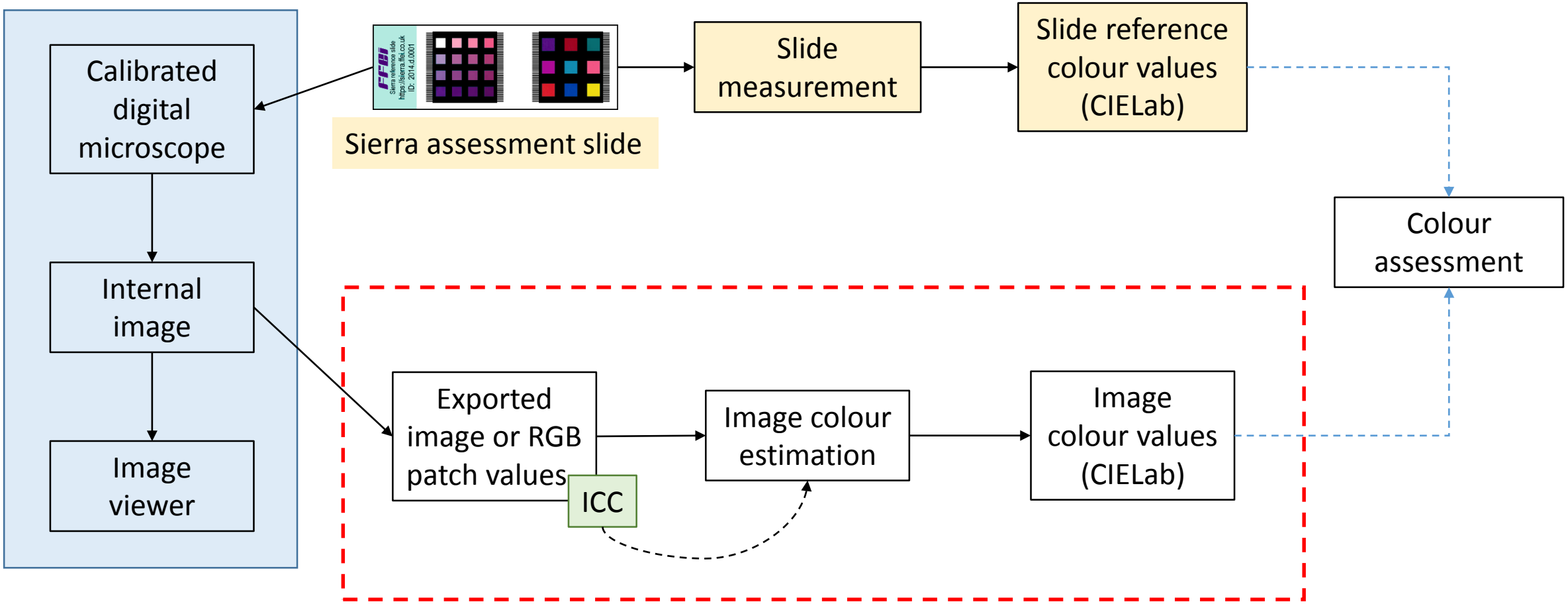
Reference slide colours vs Adobe RGB



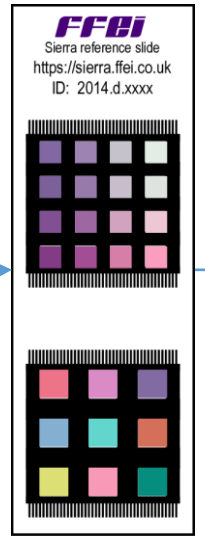
Note that some colours are out of gamut for Adobe RGB which has a significantly larger colour gamut than sRGB

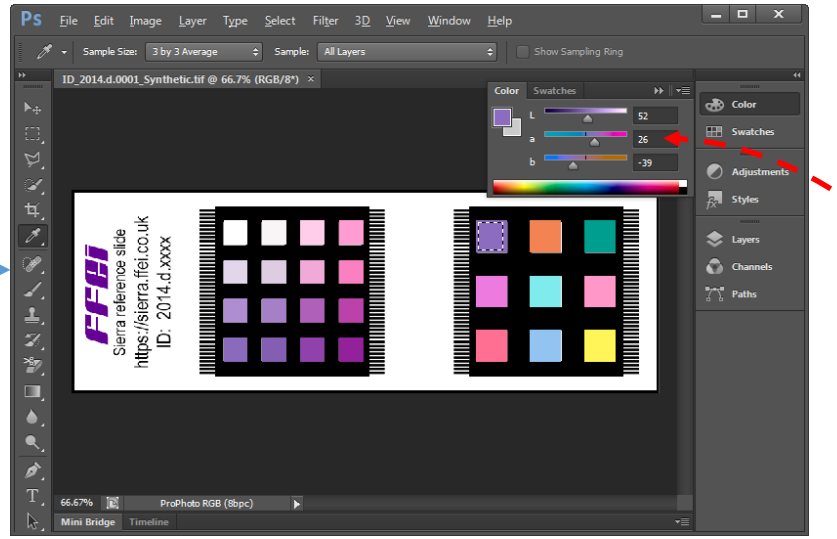
This may result in some clipping – as far as I am aware no one has conducted a study to determine whether this could affect diagnostic outcome but care should be taken when preparing colours for an sRGB workflow

Assessment method



Calculation of Lab values from RGB





RGB values
+ ICC Profile

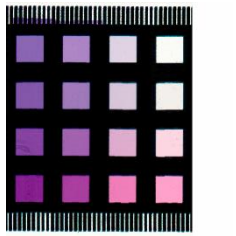
MATLAB method
Uses CIELABD50.icc – a simple Lab ICC Profile (does not work with the latest version of Photoshop)

Image Lab values

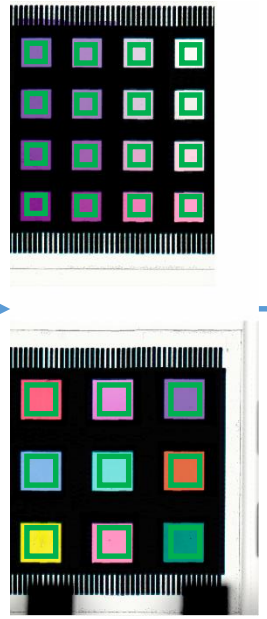
Sanity check

Calculation of Lab values from scanned image

ICC Profile may be embedded in image



Typical scanned image (TIFF)



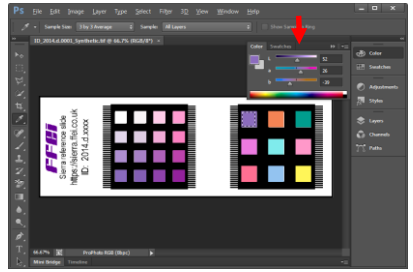
Patches identified

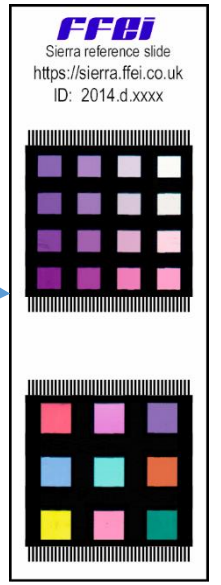
Average RGB values for central 70% calculated

RGB to Lab conversion

Image Lab values

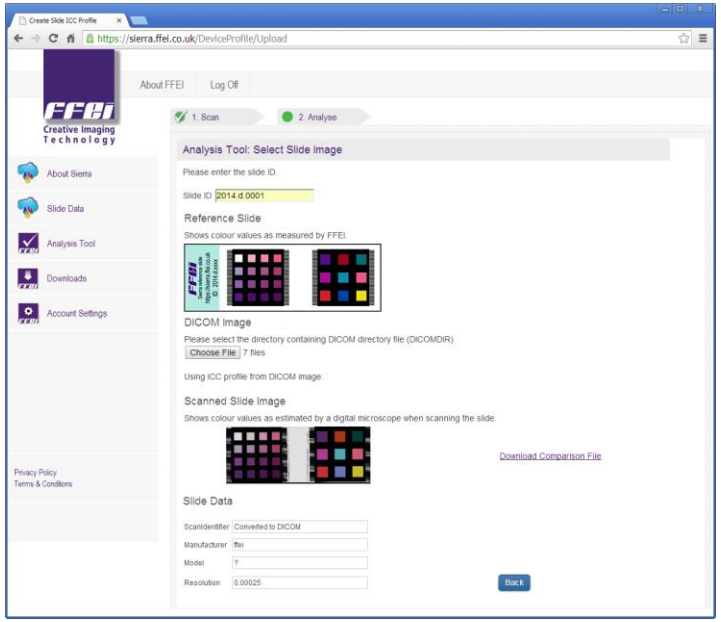
Sanity check





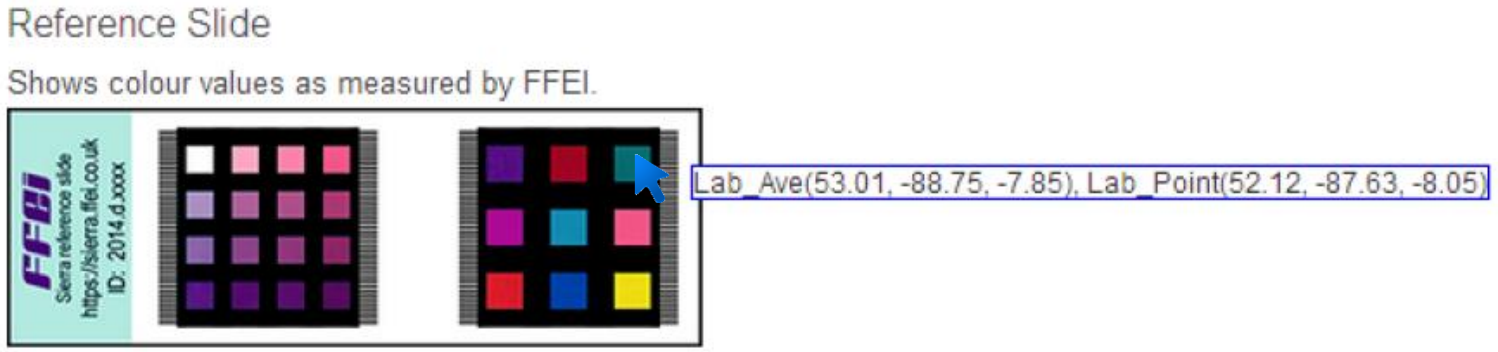
Synthetic image with embedded ICC Profile

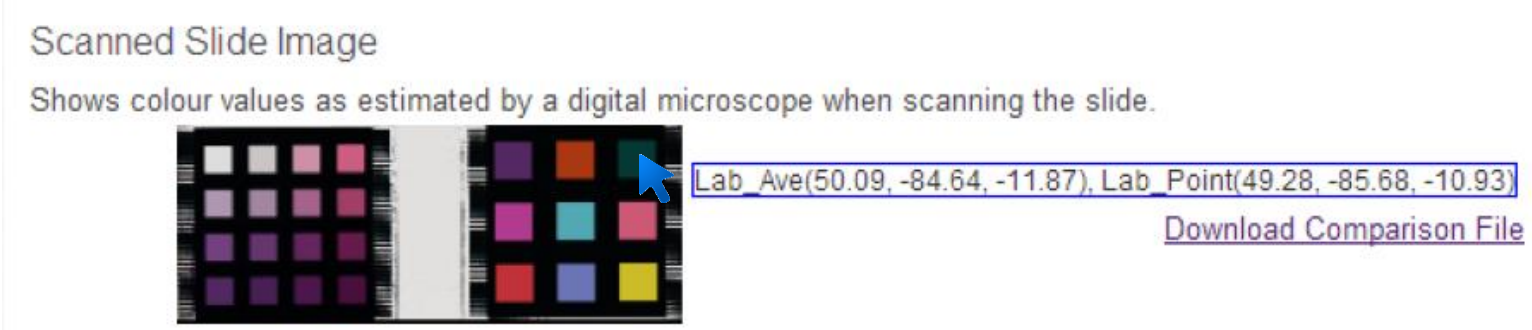
Calculation of Lab values (Sierra Analysis Tool)



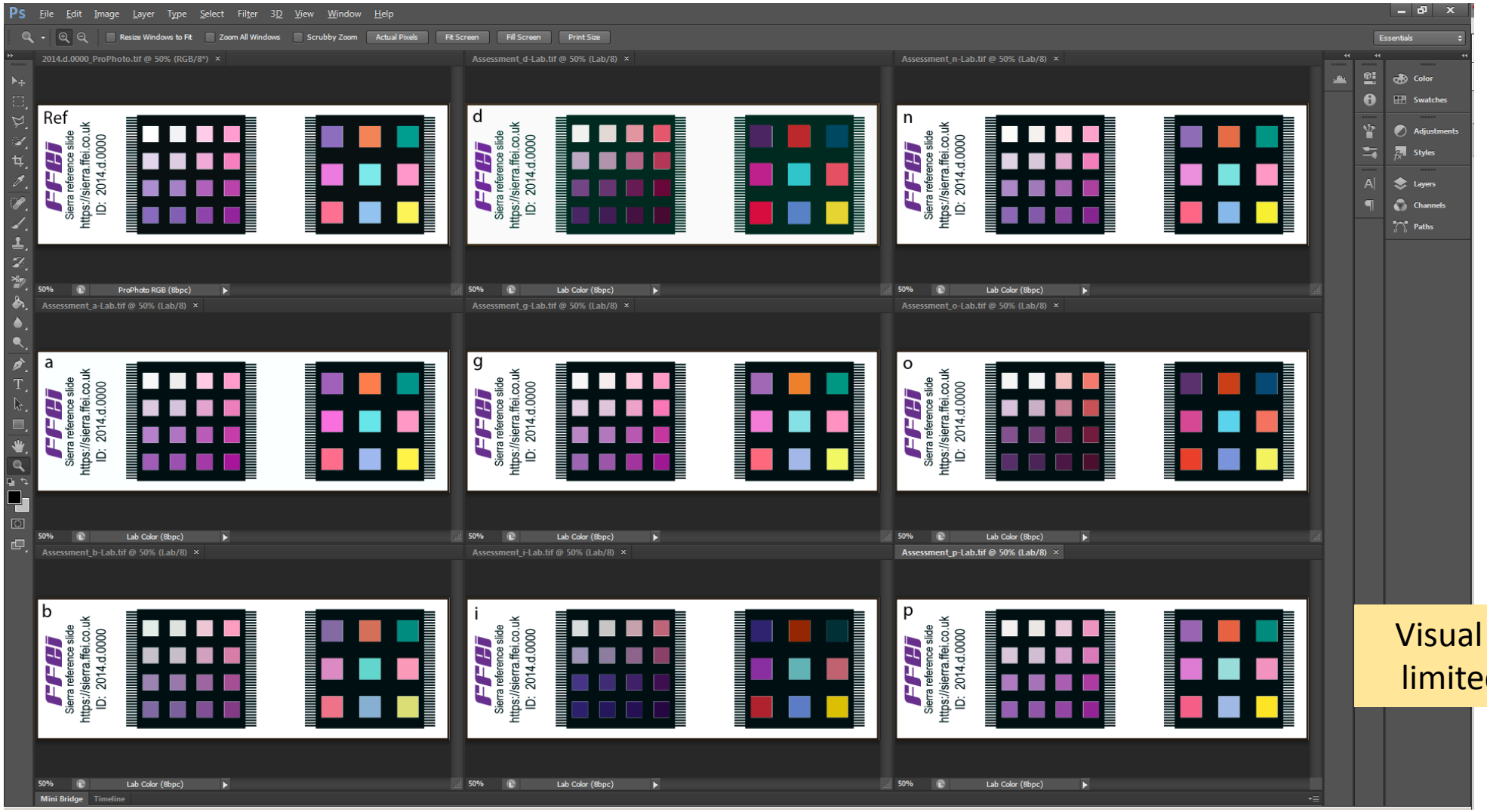
HTML5-based tool – works best in Chrome currently

Requires a DICOM image of slide on local PC





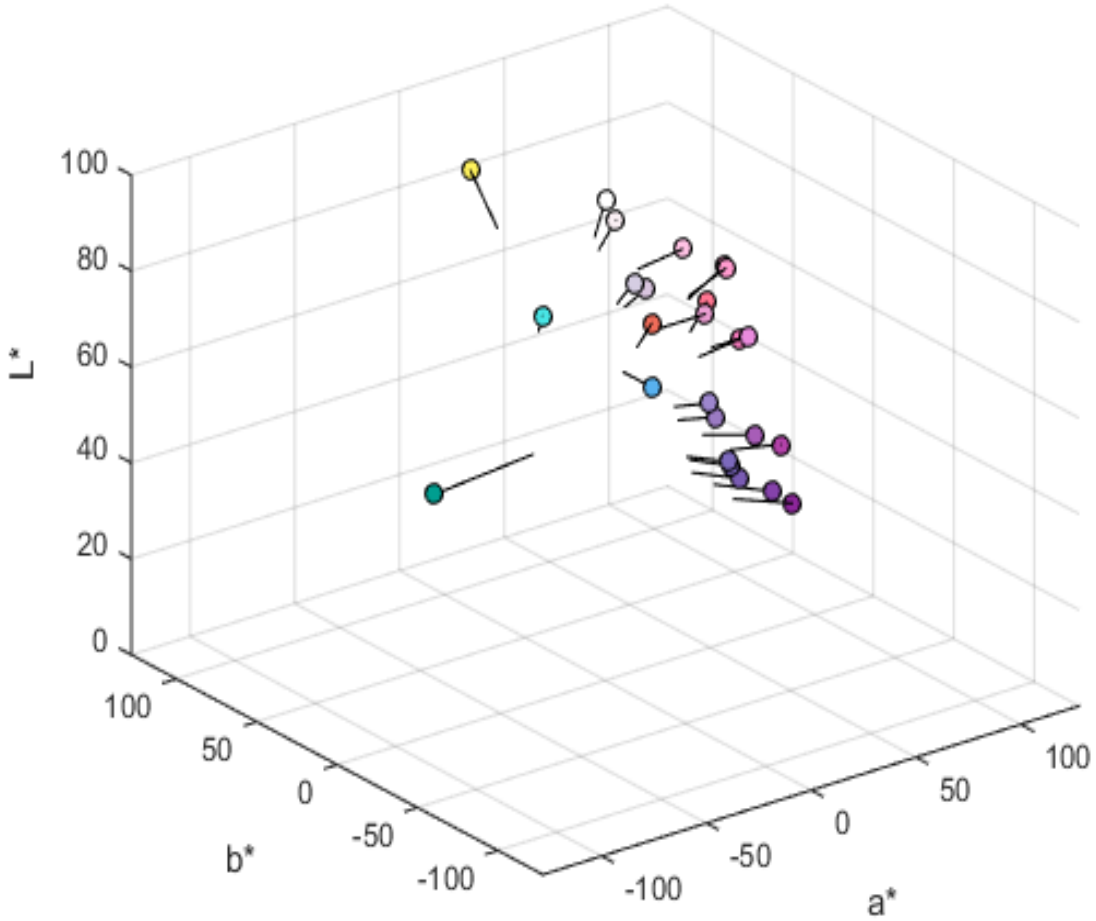
Analysis: synthetic images

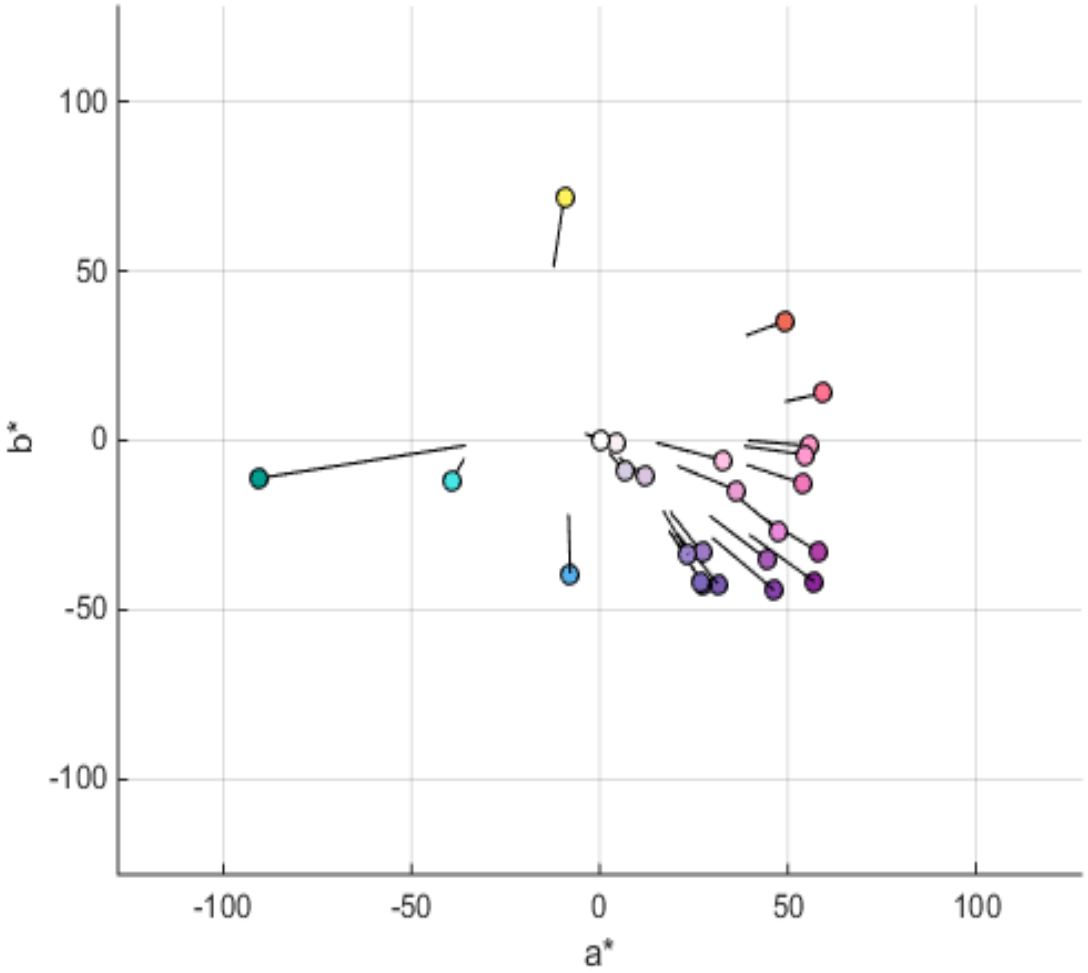


Visual comparison limited by display

Analysis: plot of Lab values

Lines show difference between Reference Lab (marked with circle) and Image Lab values





Reference white

- In most cases the reference white assumed by the viewer is lighter than the white of the clear patch
- In order to compare CIELab values of the image against the reference CIELab values, a set of image CIELab values has been produced with the clear patch used as the white reference
- This is done in the following way:
  - CIELab values for each patch are calculated from the image / ICC Profile (MATLAB)
  - These values are converted to CIEXYZ using D50 as the white point
  - The CIEXYZ value of the clear patch is identified
  - The XYZ values are converted back to CIELab using the clear patch as the reference white

Results

- Sierra images
  - An Lab image created with Lab Reference Values for ‘average slide’
  - Synthetic image of the slide with average RGB values for each patch
  - ICC Profile assigned to image which was then converted to Lab using Photoshop
  - Given a suitable display these images show colour differences
- Sierra data files
  - Shows reference vs Lab values using selected white point and
  - Lab values relative to clear patch
- Sierra MATLAB plots
  - Plots showing difference between reference Lab values (circles) and image Lab values
- Sierra Colour Plots
  - PDF file showing several views of the MATLAB plots

FDA Guidance Document Review

Discussion: possible next steps

- Second round-robin evaluation
  - Primarily between the manufacturers of WSI systems
  - Objective to determine ‘reasonable tolerances’ for the image colours (at least for H&E stains)
  - Reference measurement variation should be reduced
- Specify reference illuminant
  - In my assessment I used D50 as the illuminant as this simplifies the assessment
  - In practice the light source of microscopes is never D50 and usually has a significantly different spectrum
- Image white point
  - The image white point is unlikely to be that of the white patch as there is usually a need to allow some ‘headroom’
  - The choice of white point is, however, important and some guidelines may be appropriate for this
- Update Digital microscope test materials and test methods
  - Use Sierra slide as an example of a suitable colour reference
  - Add details of evaluation method
  - Add recommendation for ‘reference illuminant’ and possibly ‘reference white’ for scanner
- Slide manufacture
  - FFEI has not yet identified a suitable manufacturing partner but is exploring a few options