Understanding Fluorescence – Measuring, Simulation, and Correction

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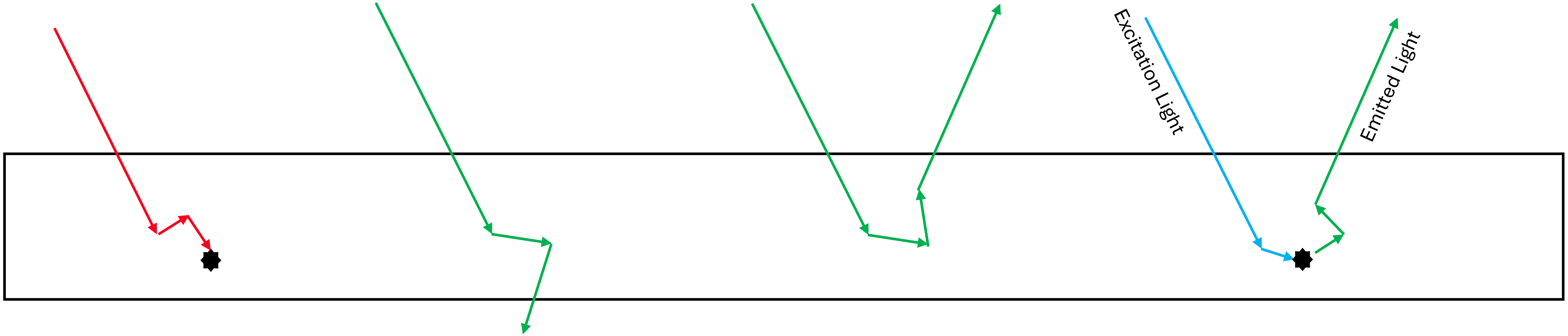
***Viewers Advisory**: Some slides may contain periodic expressions of "math". Figures, tables and discussion are provided to add greater understanding.*

Measurement

Reflectance versus Fluorescence

Pathways of Light – Reflectance vs Fluorescence



Absorption

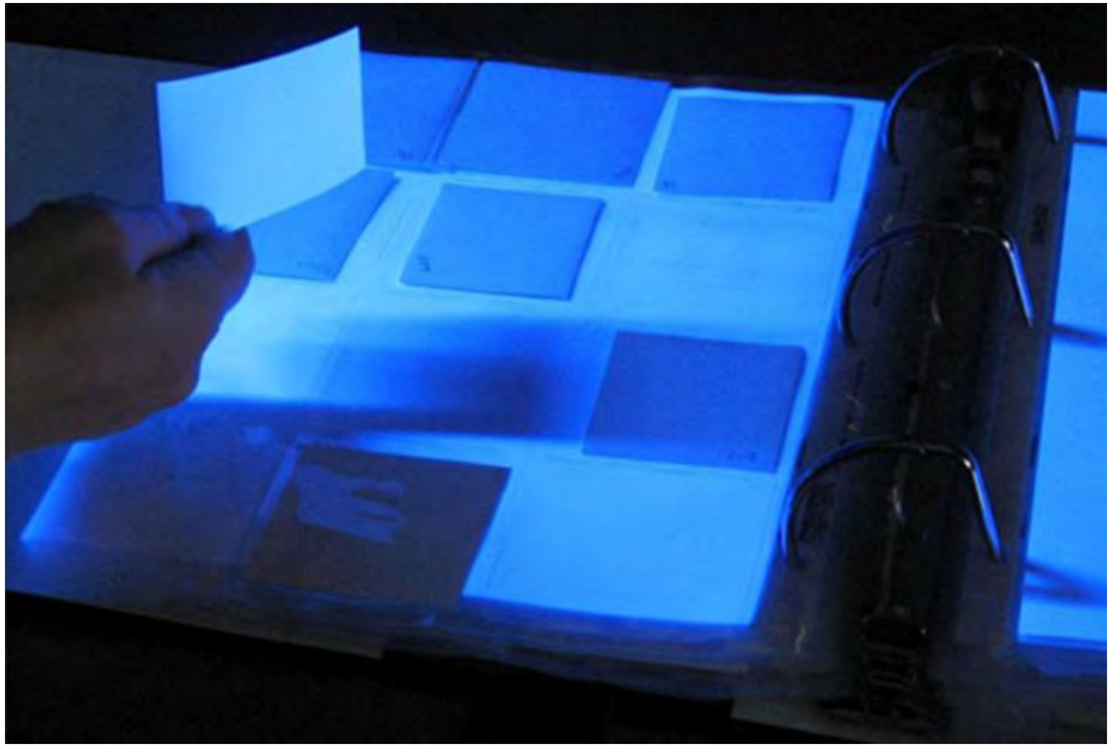
Transmittance

Reflectance

Fluorescence

Examples of Wavelength Changes Due to Fluorescence

(Picture from https://www.keech.org.uk/about/news-media/273-fluorescent-fun-for-keech-mum)



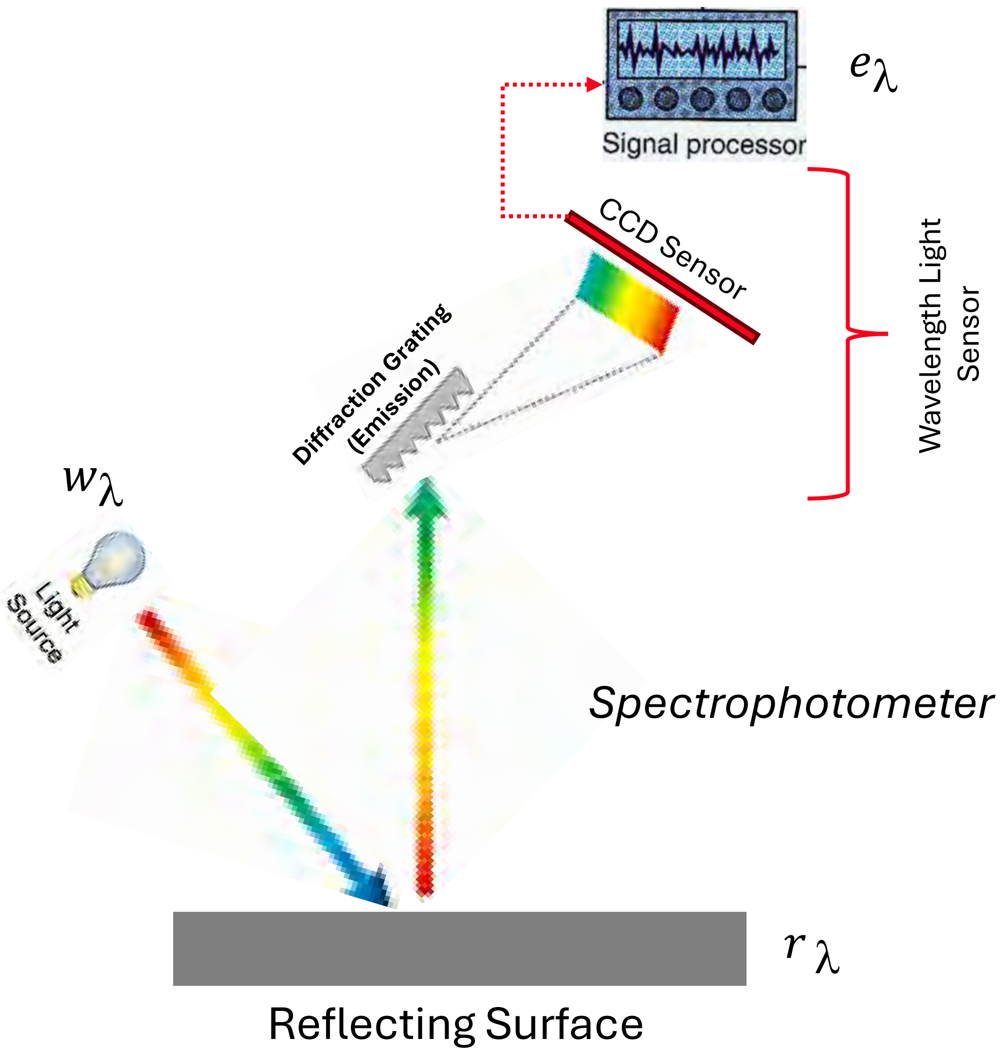
(Picture from http://news.yale.edu/2015/02/19/yale-launch-lens-media-lab-photograph-research-and-conservation)

Measuring reflectance

- Light reflects off a surface and is separated into component spectra that are measured for each wavelength
- Reflectance factor is the amount of light measured for each wavelength divided by the amount of source light at each wavelength

$$r_\lambda = \frac{e_\lambda}{w_\lambda}, \quad \mathbf{r} = \begin{bmatrix} r_1 \\ \vdots \\ r_m \end{bmatrix} \text{ for } \lambda\text{=1 to } m$$

Note: Reflectance is measured as a **vector**



Measuring fluorescence

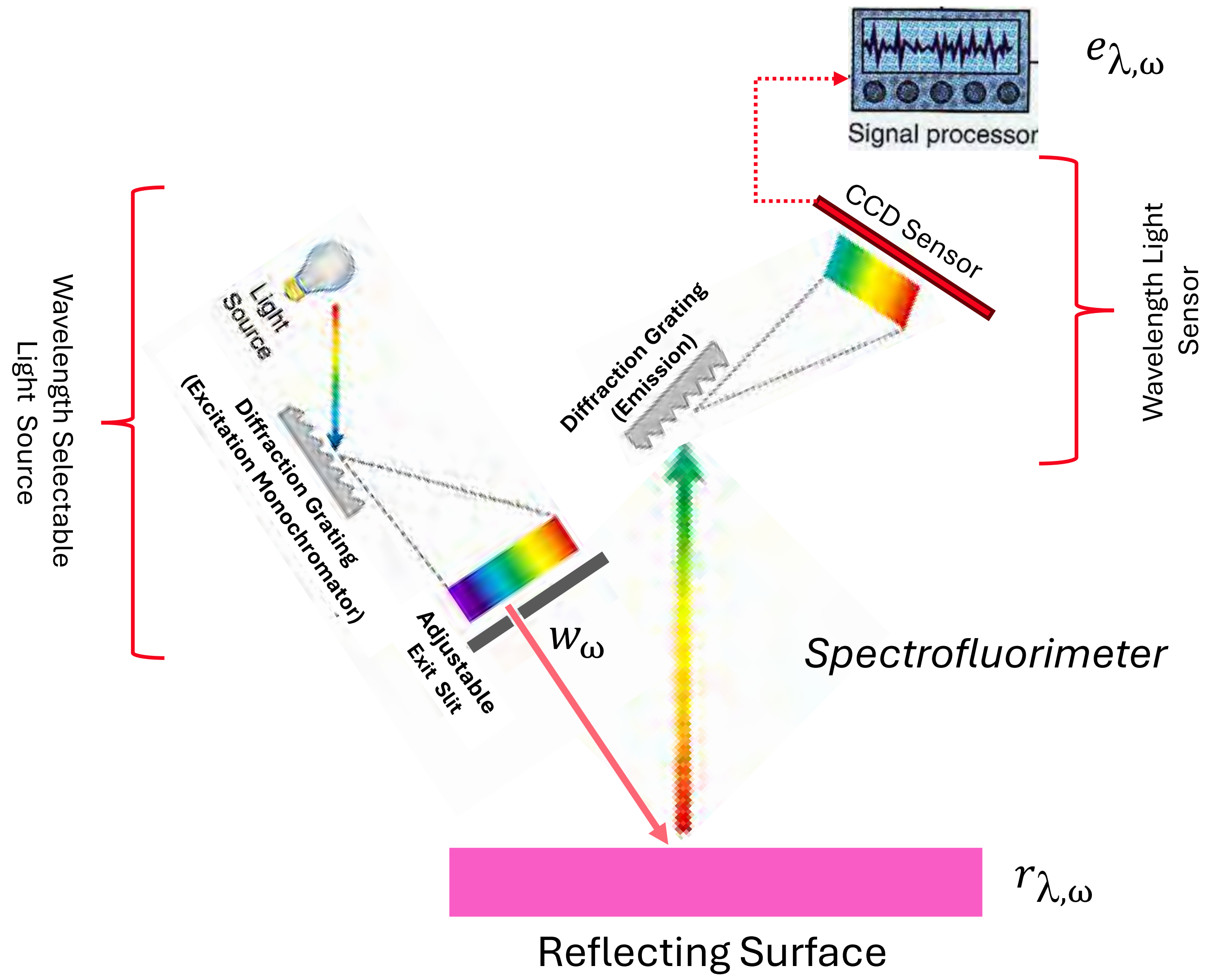
- Light is separated into competent spectra and narrow bands are reflected off surface which is separated into component spectra that are measured for each emitted wavelength
- Reflectance factor is determined for each ω th source band of light

$$r_{\lambda,\omega} = \frac{e_{\lambda,\omega}}{w_{\omega}}\ , \quad \mathbf{r}_{\omega} = \begin{bmatrix} r_{1,\omega} \\ \vdots \\ r_{m,\omega} \end{bmatrix} \text{ for } \lambda\text{=1 to } m$$

- Fluorescence represents the combination of the reflectance factors

$$\mathbf{F} = [\mathbf{r}_1 \quad \cdots \quad \mathbf{r}_n] \text{ for } \omega\text{=1 to n}$$

Note: Fluorescence is measured as a **matrix** (Commonly referred to as a Donaldson matrix)



Example Fluorescent Measurement

Emission wavelengths (380nm – 780nm)

$$\mathbf{F} = \begin{bmatrix}
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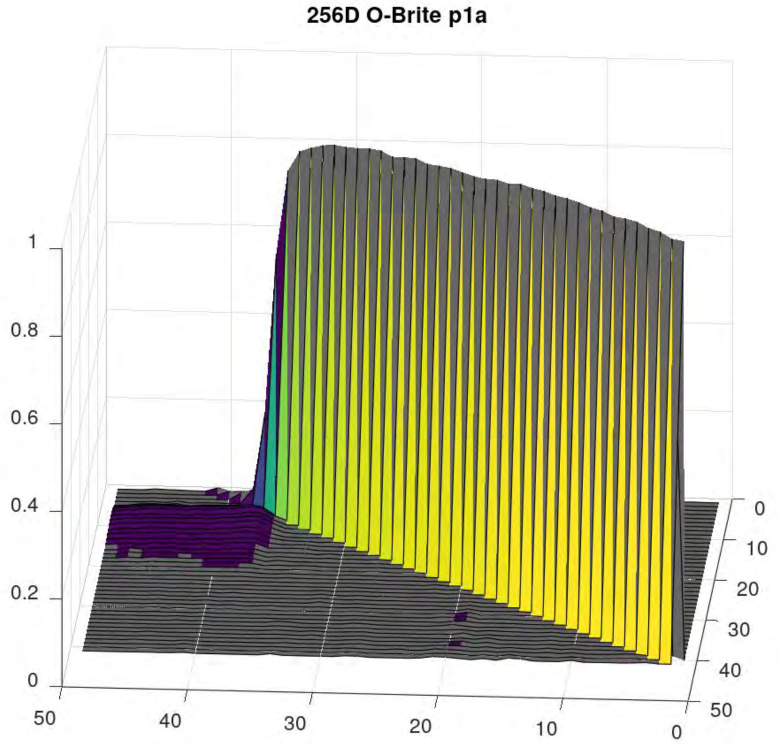
\end{bmatrix}$$

Excitation wavelengths (300nm – 780nm)



Spectroflourescence measurements curtesy of Brian Gamm

Emitted light considerations

- The predicted light reflected (emitted) off an object is found by multiplying the excitation light by the reflectance or fluorescence

$$e_{r,\lambda} = r_\lambda w_\lambda$$

$$\mathbf{e}_r = diag(\mathbf{w})\mathbf{r} = \mathbf{w} \cdot \mathbf{r} = \mathbf{r} \cdot \mathbf{w}$$

$$\mathbf{e}_f = \mathbf{F}\mathbf{w}$$

Where:

$$diag(\boldsymbol{w}) = \begin{bmatrix} w_1 & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & w_\mathrm{n} \end{bmatrix}$$

- Colorimetry is determined by multiplying the emitted light by normalized color matching functions

$$\mathbf{C} = \frac{1}{k}\begin{bmatrix} \bar{\mathbf{x}}^T \\ \bar{\mathbf{y}}^T \\ \bar{\mathbf{z}}^T \end{bmatrix}, \quad \mathbf{c}_r = \begin{bmatrix} X_r \\ Y_r \\ Z_r \end{bmatrix} = \mathbf{C}\mathbf{e}_r, \quad \mathbf{c}_f = \begin{bmatrix} X_f \\ Y_f \\ Z_f \end{bmatrix} = \mathbf{C}\mathbf{e}_f$$

Note:

$$diag(\boldsymbol{w})^{-1} = \begin{bmatrix} \frac{1}{w_1} & \cdots & 0 \\ \vdots & \ddots & \vdots \\ 0 & \cdots & \frac{1}{w_\mathrm{n}} \end{bmatrix}$$

Measuring reflectance when fluorescence occurs

- Reflectance factor is found by multiplying the reflected light by the inverse of the measurement light source

$$\mathbf{r}_r = diag(\mathbf{w}_{meas})^{-1}\mathbf{e}_r \quad and\ substituting\ for\ \mathbf{e}_r$$

$$\mathbf{r}_r = diag(\mathbf{w}_{meas})^{-1} diag(\mathbf{w}_{meas})\mathbf{r} = \mathbf{I}\cdot\mathbf{r} = \boldsymbol{r}$$

  - Reflectance factor is **independent** of the light source used to measure when **no fluorescence occurs**
- Measuring reflectance factor (with a spectrophotometer) when fluorescence is present is equivalent to

$$\mathbf{r}_f = diag(\mathbf{w}_{meas})^{-1}\mathbf{e}_f \quad and\ substituting\ for\ \mathbf{e}_f$$

$$\mathbf{r}_f = diag(\mathbf{w}_{meas})^{-1}\mathbf{F}\,\mathbf{w}_{meas}$$

  - Reflectance factor is **dependent** on the light source used for measuring when **fluorescence occurs**

Problem with using spectral reflectance measurement of fluorescent object

- The predicted emission from an object that simply reflects is the term-by-term product of the light source and the reflectance

$$\mathbf{e}_{r,alt} = diag(\mathbf{w}_{alt})\mathbf{r} \qquad (1)$$

- The correct predicted emission when fluorescence occurs is

$$\mathbf{e}_{f,alt} = \mathbf{F}\ \mathbf{w}_{alt} \qquad (2)$$

- From previous slide, the measured spectral reflectance when fluorescence occurs is expressed as

$$\mathbf{r}_f = diag(\mathbf{w}_{meas})^{-1}\mathbf{F}\ \mathbf{w}_{meas} \qquad (3)$$

- Combining (1) and (3) reveals that the only light source ($\mathbf{w}_{alt}$) that can be predicted correctly with fluorescence is the measurement light source

$$\mathbf{e}_{f,alt} = diag(\mathbf{w}_{alt})diag(\mathbf{w}_{meas})^{-1}\mathbf{F}\ \mathbf{w}_{meas} \qquad (4)$$

Simulation Using Substrate Correction

Getting Fluorescence from Reflectance

Using Substrate Correction to derive fluorescent measurements (Part 1)

- Substrate correction uses “media relative adjustment” to get colorimetric aims for alternate media

$$\begin{bmatrix} X_{alt} \\ Y_{alt} \\ Z_{alt} \end{bmatrix} = \begin{bmatrix} {X_{w\_alt}}/{X_{w\_src}} & 0 & 0 \\ 0 & {Y_{w\_alt}}/{Y_{w\_src}} & 0 \\ 0 & 0 & {Z_{w\_alt}}/{Z_{w\_src}} \end{bmatrix} \begin{bmatrix} X_{src} \\ Y_{src} \\ Z_{src} \end{bmatrix}$$

$$\mathbf{c}_{alt} = diag\left(\mathbf{c}_{w\_alt}\right) diag\left(\mathbf{c}_{w\_src}\right)^{-1} \mathbf{c}_{src}$$

Using Substrate Correction to derive fluorescent measurements (Part 2)

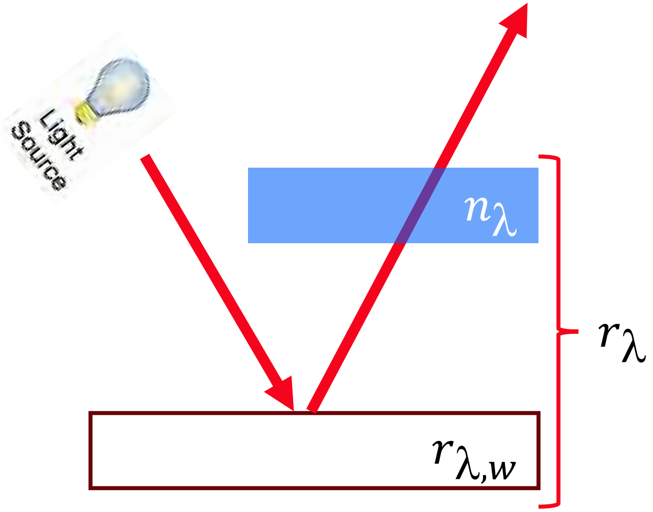
- Similarly - substrate correction can also be used to derive reflectance factor aims for an alternate media

$$\mathbf{r}_{alt} = diag\left(\mathbf{r}_{w\_alt}\right) diag\left(\mathbf{r}_{w\_src}\right)^{-1} \mathbf{r}_{\boldsymbol{src}}$$

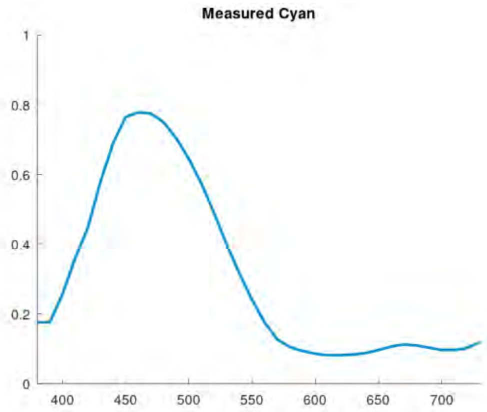
$$r_{\lambda,alt} = r_{\lambda,w\_alt} \frac{r_{\lambda,src}}{r_{\lambda,w\_src}}$$

- The fraction represents a normalization that separates colorant reflectance from media reflectance and represents the physical path of light reflecting off the media and then being transmitted through the colorant

$$n_\lambda = {}^{r_\lambda}/_{r_{\lambda,w}} \quad \therefore \quad r_\lambda = r_{\lambda,w}\, n_\lambda$$



Example Spectral Substrate Correction


Measured Cyan

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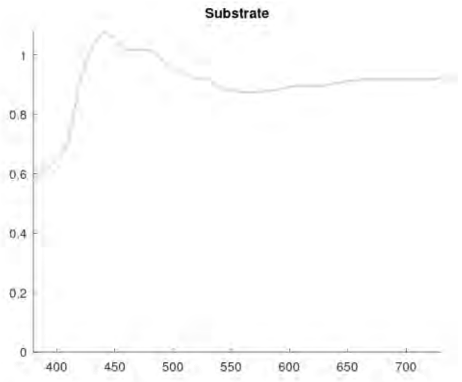
Substrate

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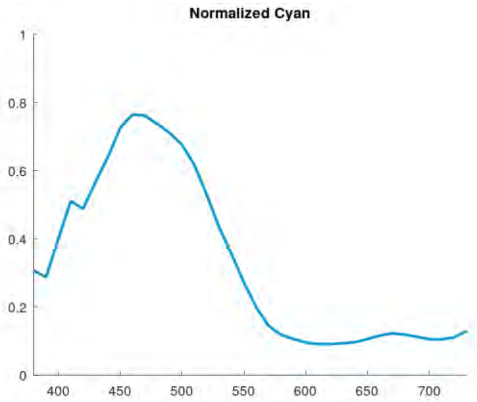
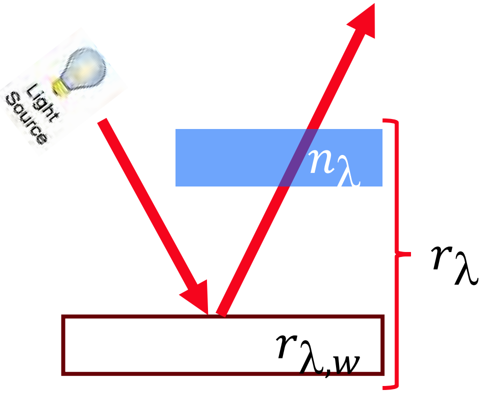
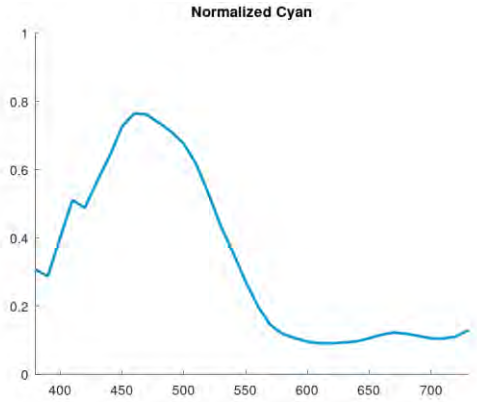

Normalized Cyan




Normalized Cyan

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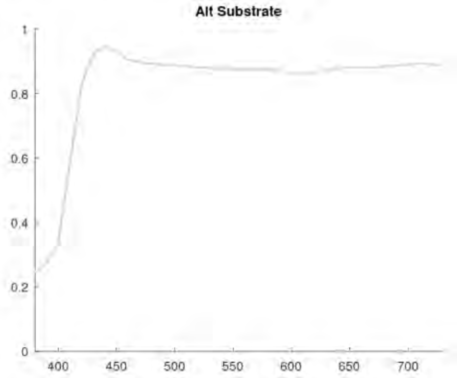
Alt Substrate

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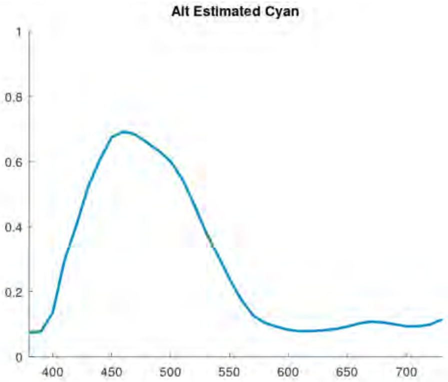

Alt Estimated Cyan

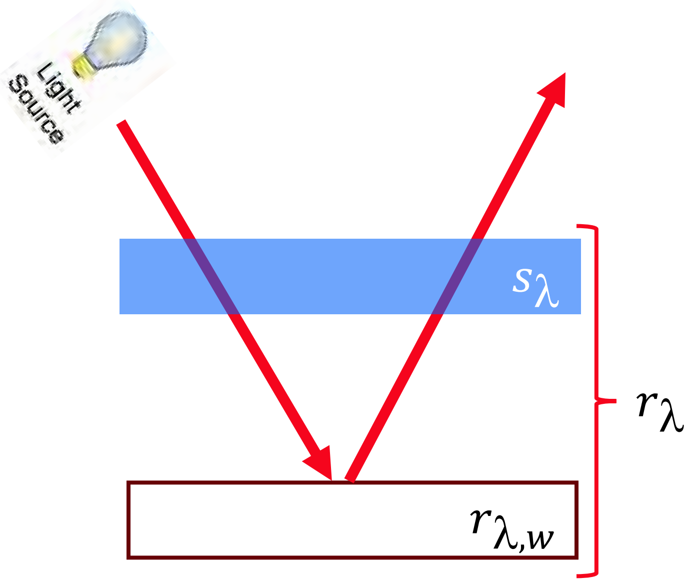
Using Substrate Correction to derive fluorescent measurements (Part 3)

- The wavelength spectral substrate equation can be rewritten to express the path of light going through the colorant and then reflecting off the media and finally going back through the colorant

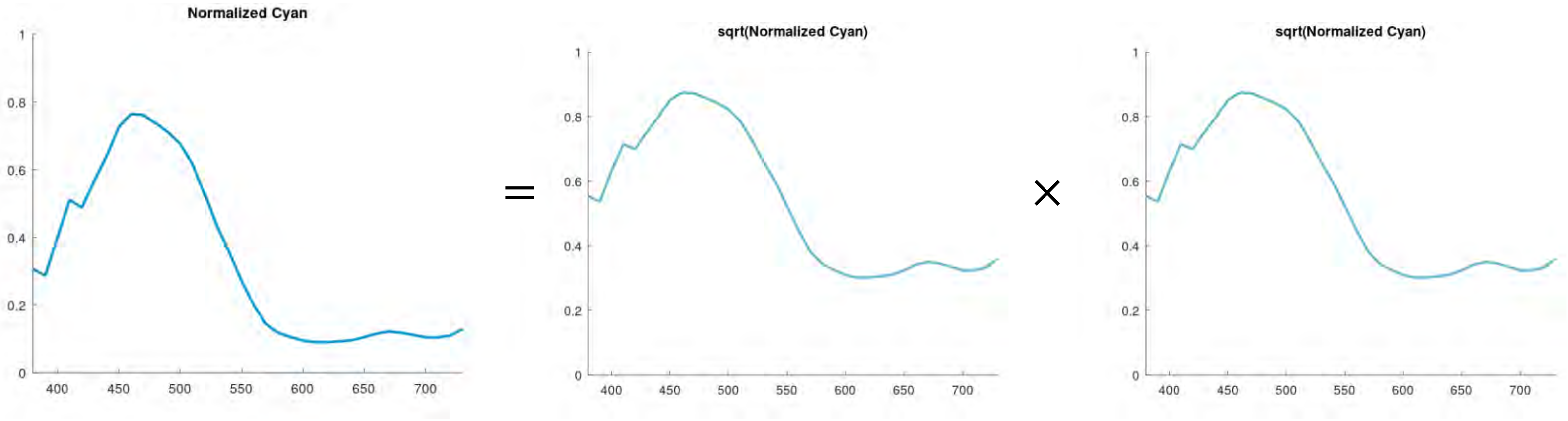
$$s_\lambda = \sqrt{n_\lambda} = \sqrt{\frac{r_\lambda}{r_{\lambda,w}}}$$

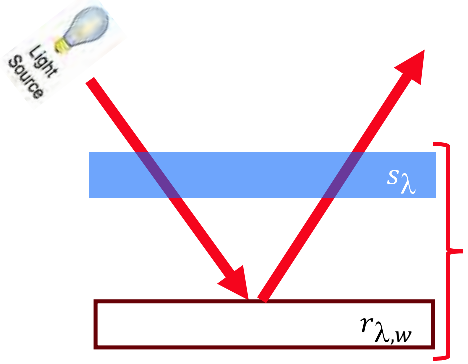
$$r_\lambda = s_\lambda r_{\lambda,w} s_\lambda$$

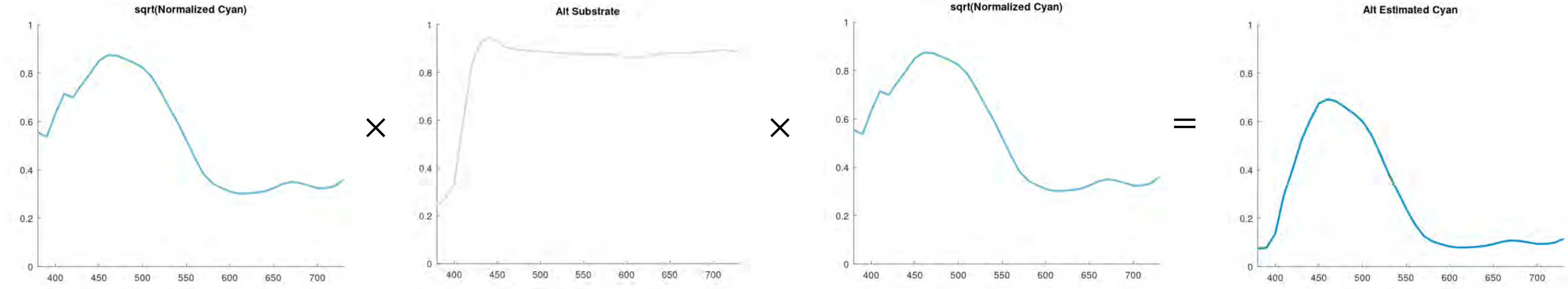
$$\mathbf{r} = \mathbf{s} \cdot \mathbf{r} \cdot \mathbf{s} = diag(\mathbf{s}) diag(\mathbf{r}_w) \mathbf{s}$$



Light going through and back





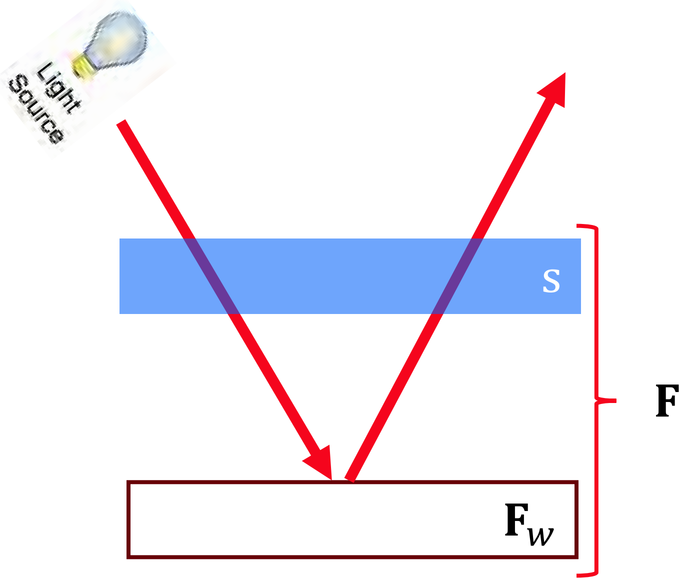


Using Substrate Correction to derive fluorescent measurements (Part 4)

- A formula for substrate correction with a media that fluoresces can be expressed by replacing the s_λ wavelength terms with vectors in diagonal matrices before and after a Donaldson matrix for the fluorescence of the media

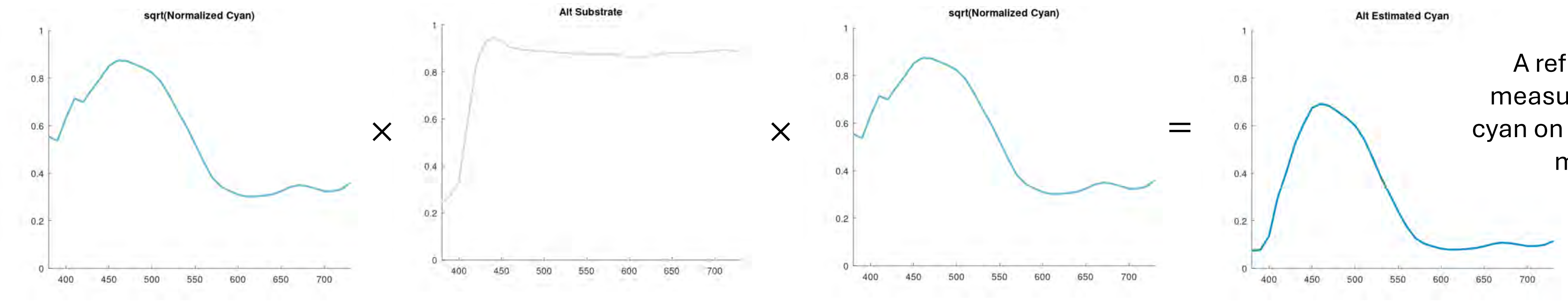
$$\mathbf{F} = diag(\mathbf{s}_{emmision})\mathbf{F}_w diag(\mathbf{s}_{excitation})$$

- This equation allows for spectrofluorescent measurements on media that fluoresces to be derived from reflectance factor measurements on media that doesn't fluoresce

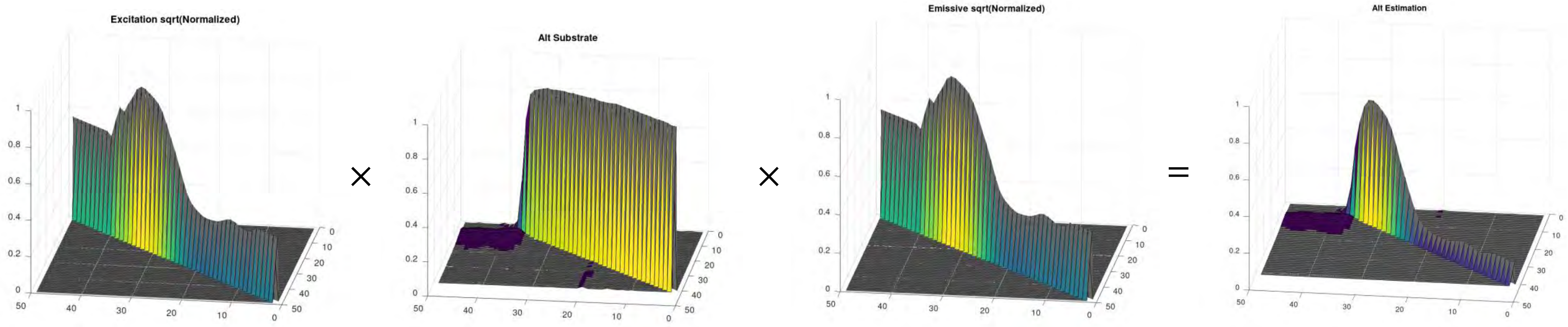


- Note: the $diag(\mathbf{s}_x)$ matrices before and after the media term are likely different dimensions to agree with the dimensions of $\mathbf{F}_w$ (because $\mathbf{F}_w$ is not square).

Example fluorescent substrate correction



A reflectance measurement for cyan on an alternate media

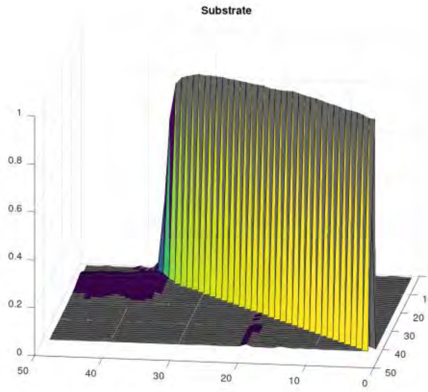


A fluorescence measurement for cyan on a fluorescent media

Generating spectrofluorescence measurements of an IT8 Chart

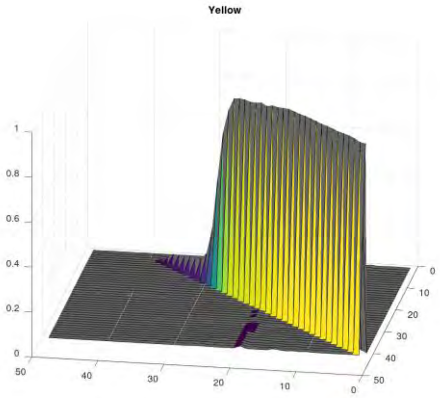
- Plausible Donaldson fluorescence matrices were determined using fluorescent substrate correction (FSC) with a spectrofluorescence measurement of a test substrate and offset press reflectance measurements of patches in an IT8 chart
- A hypothetical spectrophotometer can be used with these measurements to assess using different measurement light sources and viewing light sources
  - Mismatches between colorimetry from spectrofluorescence and spectral reflectance measurements can then be quantified

Generated Donaldson Matrices for Primary Colors

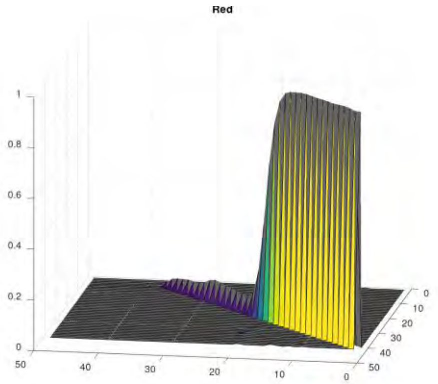


Substrate

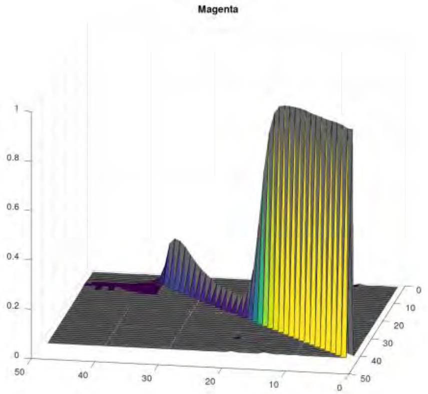
Yellow

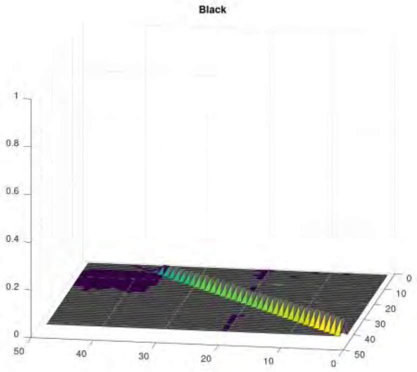


Red

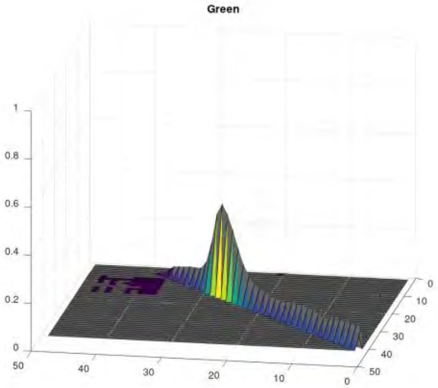


Magenta

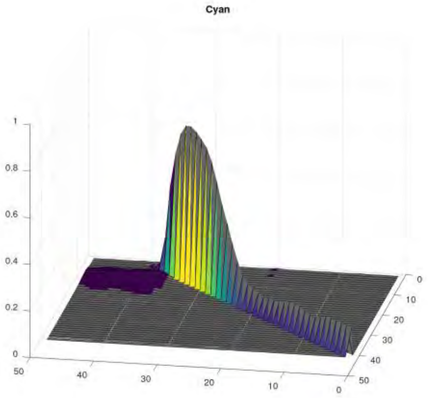




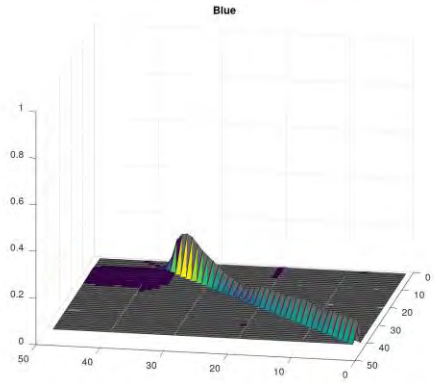
Black



Green

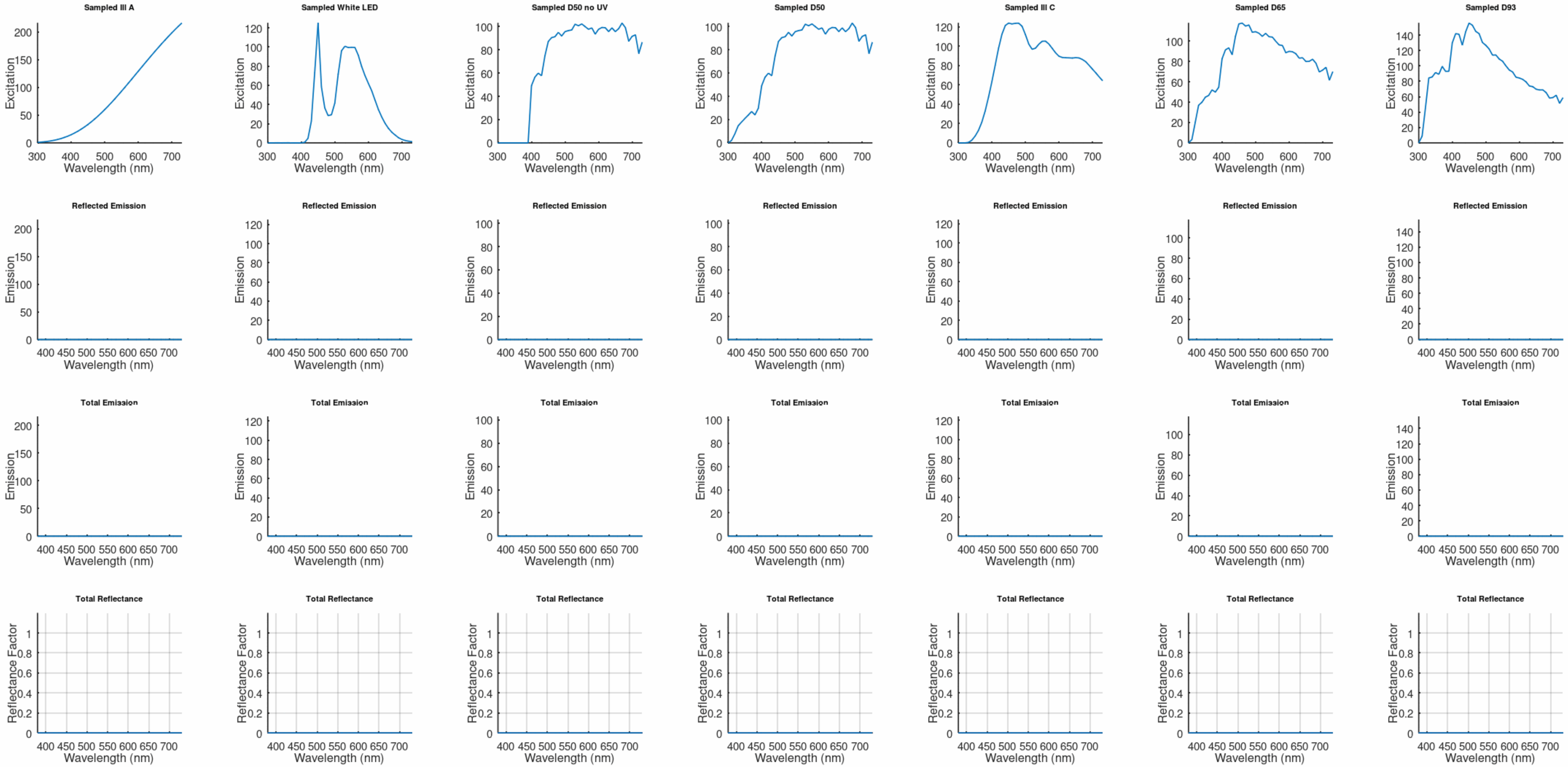


Cyan

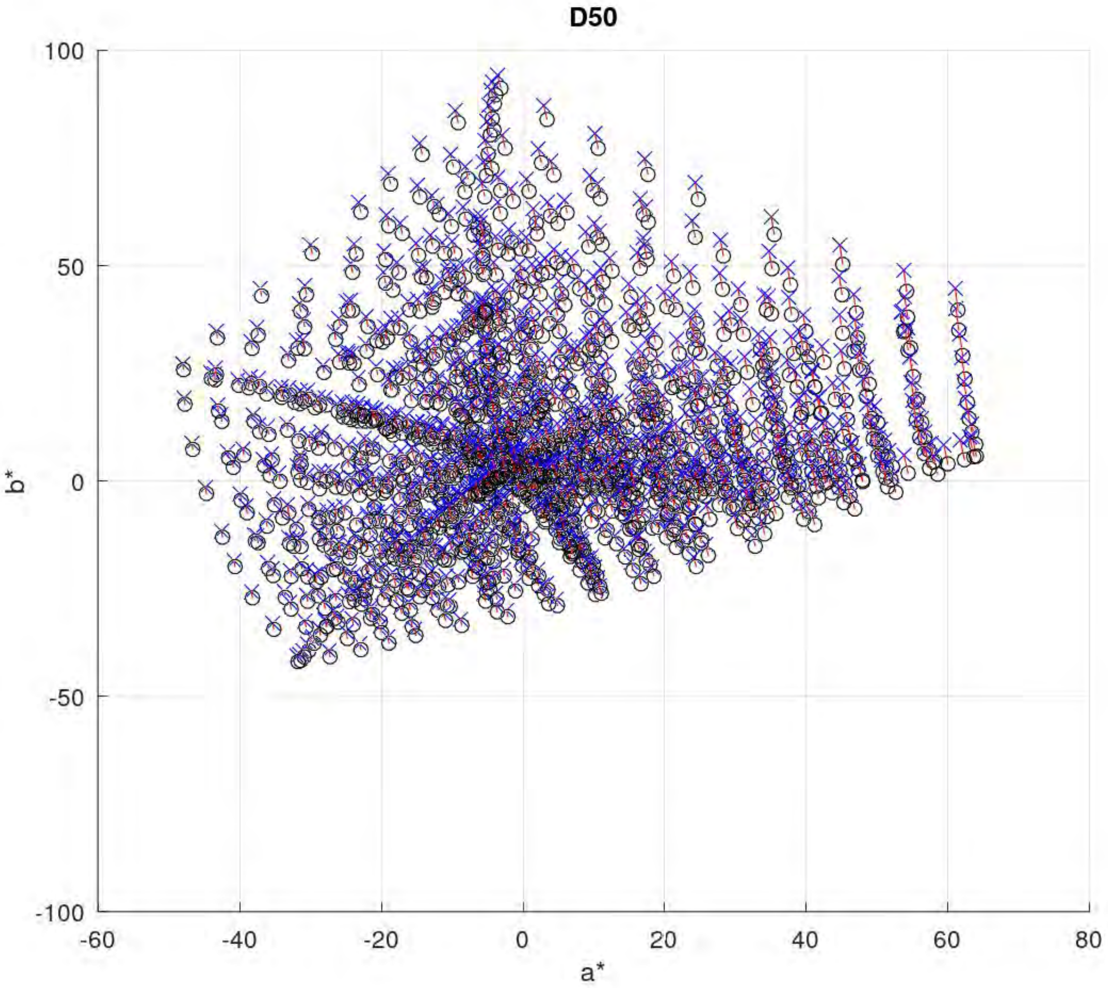


Blue

Measuring 80% cyan with different light sources



Expected IT8 observational errors



Key

O - $\mathbf{CFw}_{D50}$

X - $\mathbf{C}diag(\mathbf{w}_{D50})diag(\mathbf{w}_{illA})^{-1}\mathbf{Fw}_{illA}$

Actual (fluorescence) Colormetry

The O's represent observed colorimetry of IT8 patches under D50.

Measured (reflectance) Colorimetry

The X's represent measuring spectral reflectances of IT8 patches with illuminant A in device and then deriving D50 colorimetry from reflectances.

IT8 fluorescence colorimetry versus reflectance colorimetry

ΔE_{ab} color differences between $\mathbf{CFw}_{view}$ and $\mathbf{C}diag(\mathbf{w}_{view})diag(\mathbf{w}_{meas})^{-1}\mathbf{Fw}_{meas}$

Viewing Condition Light

Measurement Light

	D93	D65	D50	D50 no UV	C	A	White LED
D93	0.00	2.62	4.99	5.01	2.48	9.34	5.25
D65	2.64	0.00	2.38	2.40	0.77	6.83	4.61
D50	5.05	2.40	0.00	0.04	2.73	4.63	5.57
D50 no UV	5.23	2.49	0.04	0.00	2.82	4.70	5.51
C	2.55	0.74	2.78	2.80	0.00	7.15	4.14
A	9.92	7.17	4.79	4.77	7.34	0.00	9.25
White LED	3.43	3.47	5.16	5.16	3.12	9.35	0.05

Average color difference

Viewing Condition Light

Measurement Light

	D93	D65	D50	D50 no UV	C	A	White LED
D93	0.00	7.53	14.94	14.95	5.68	28.36	11.93
D65	7.33	0.00	7.19	7.20	3.33	20.29	14.83
D50	14.17	7.01	0.00	0.06	9.74	12.99	18.54
D50 no UV	13.67	6.77	0.06	0.00	9.39	12.56	18.55
C	5.70	3.21	9.54	9.55	0.00	21.48	14.59
A	25.76	19.03	12.56	12.54	21.03	0.00	29.41
White LED	10.19	13.76	18.00	17.99	13.48	28.81	0.08

Maximum color difference

Measurement Conditions

M0=A, M1=D50

TAGA NextGen

Correction

Getting better reflectance measurements

Correcting reflectance measurements to better predict observed results

- It has been shown that using reflectance measurements to determine colorimetry for non-measured viewing lighting conditions may not predict observations when fluorescence occurs
    - Thus, when $view \neq meas$:
      $\mathbf{CFw}_{view} \neq \mathbf{C}diag(\mathbf{w}_{view})\mathbf{r}_{F,meas} = \mathbf{C}diag(\mathbf{w}_{view})diag(\mathbf{w}_{meas})^{-1}\mathbf{Fw}_{meas}$

- Question: Can a correction to reflectance measurements be found that better predicts observations?
    - Thus, can a correction matrix $\mathbf{M}$ be found that results in the following?

$$\mathbf{CFw}_{view} \approx \mathbf{C}diag(\mathbf{w}_{view})\mathbf{Mr}_{F,meas}$$

Determining a correction matrix

$$\mathbf{C}\mathbf{F}_i\mathbf{w}_{view} = \mathbf{C}diag(\mathbf{w}_{view})\mathbf{M}\mathbf{r}_{F_i,meas}$$

solving for $\mathbf{M}$

$$\mathbf{C}\mathbf{I}\mathbf{F}_i\mathbf{w}_{view} = \mathbf{C}diag(\mathbf{w}_{view})diag(\mathbf{w}_{view})^{-1}\mathbf{F}_i\mathbf{w}_{view} = \mathbf{C}diag(\mathbf{w}_{view})\mathbf{M}\mathbf{r}_{F_i,meas}$$
$$\mathbf{C}diag(\mathbf{w}_{view})\mathbf{r}_{F_i,view} = \mathbf{C}diag(\mathbf{w}_{view})\mathbf{M}\mathbf{r}_{F_i,meas}$$
$$\mathbf{r}_{F_i,view} = \mathbf{M}\mathbf{r}_{F_i,meas}$$

Let

$$\mathbf{R}_{view} = [\mathbf{r}_{F_1,view} \quad \cdots \quad \mathbf{r}_{F_n,view}],\ \mathbf{R}_{meas} = [\mathbf{r}_{F_1,meas} \quad \cdots \quad \mathbf{r}_{F_n,meas}]$$

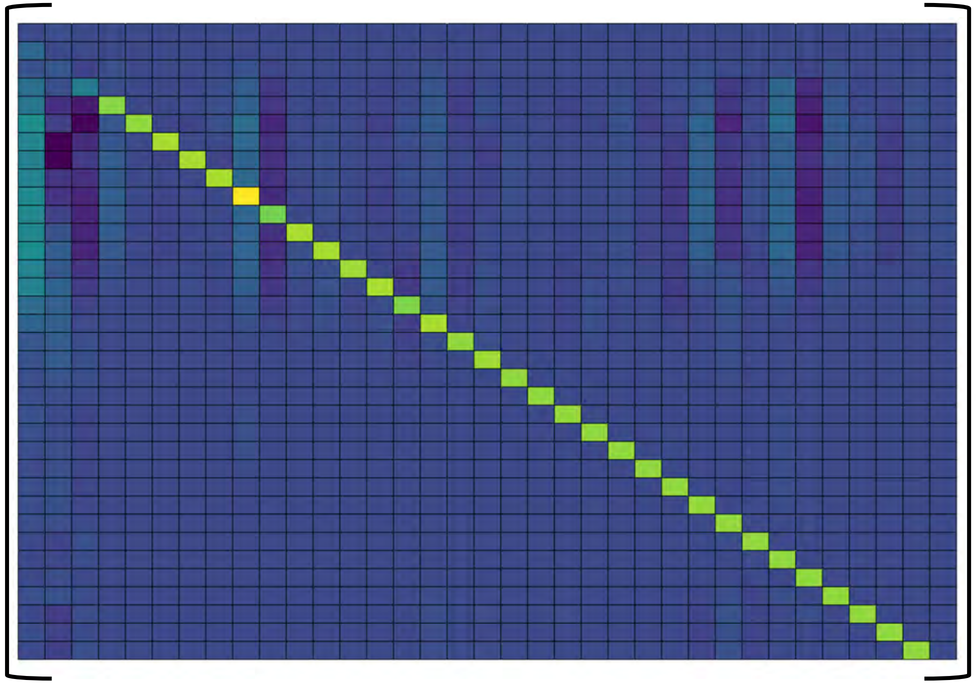
$$\mathbf{R}_{view} = \mathbf{M}\mathbf{R}_{meas}$$

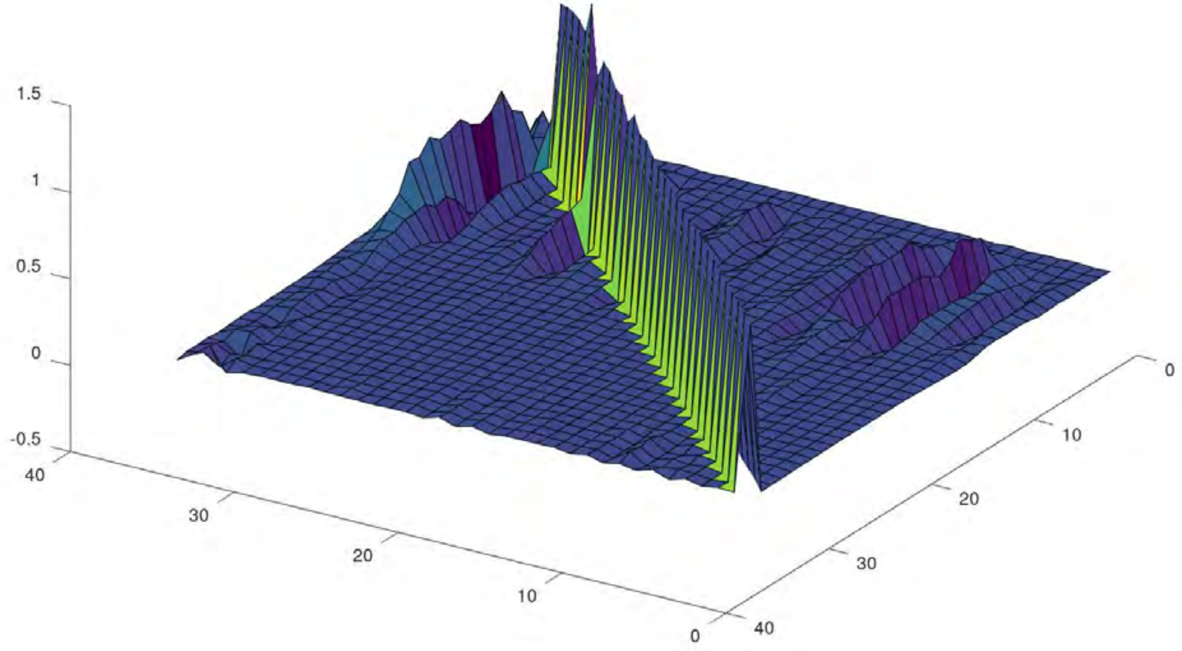
$$\mathbf{M} = \mathbf{R}_{view}pinv(\mathbf{R}_{meas})$$

Note: This requires that reflectances measured under both *meas* and *view* light sources are known!

Example correction for A *meas* to D93 *view*

$$\mathbf{M}_{AtoD93} =$$





IT8 fluorescence colorimetry versus corrected reflectance colorimetry

ΔE_{ab} color difference between $\mathbf{CFw}_{view}$ and $\mathbf{C}diag(\mathbf{w}_{view})\mathbf{M}diag(\mathbf{w}_{meas})^{-1}\mathbf{Fw}_{meas}$

Viewing Condition Light

Measurement Light

	D93	D65	D50	D50 no UV	C	A	White LED
D93	0.00	0.02	0.04	0.07	0.05	0.06	0.08
D65	0.03	0.00	0.02	0.05	0.03	0.04	0.07
D50	0.05	0.02	0.00	0.03	0.02	0.02	0.06
D50 no UV	0.08	0.05	0.03	0.00	0.02	0.02	0.02
C	0.05	0.03	0.01	0.02	0.00	0.02	0.03
A	0.07	0.04	0.02	0.02	0.01	0.00	0.06
White LED	0.17	0.13	0.09	0.04	0.07	0.07	0.00

Average color difference

Viewing Condition Light

Measurement Light

	D93	D65	D50	D50 no UV	C	A	White LED
D93	0.00	0.19	0.34	0.57	0.46	0.52	0.68
D65	0.24	0.00	0.19	0.47	0.33	0.40	0.62
D50	0.59	0.26	0.00	0.38	0.18	0.24	0.63
D50 no UV	0.82	0.53	0.30	0.00	0.19	0.13	0.26
C	0.48	0.27	0.09	0.14	0.00	0.22	0.26
A	0.71	0.41	0.16	0.20	0.07	0.00	0.62
White LED	1.32	1.06	0.79	0.29	0.53	0.59	0.00

Maximum color difference

Measurement Conditions

M0=A, M1=D50

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Conclusion

That’s all folks!

Summary

- Fluorescence involves light being emitted from surface at different wavelengths than are absorbed
- Spectral reflectance measurements are dependent on the light source in the measurement device when fluorescence occurs
  - Applying arbitrary illuminances to measured spectral reflectances (that have fluorescence) will likely result in errors in predicting observed color
- Substrate correction approach can be applied to both spectral reflectance as well as media that fluoresces to get estimates of spectral fluorescence
- It might be possible to adjust measured spectral reflectances (with fluorescence) to better apply to alternate viewing light sources
  - However, the demonstrated method requires knowing the reflectance measured under the alternate viewing light source

Thank You!

For your kind attention

Questions?