Calibration of Leica Scanscope AT2

Allen H. Olson, PhD
Aperio ePathology, Leica Biosystems

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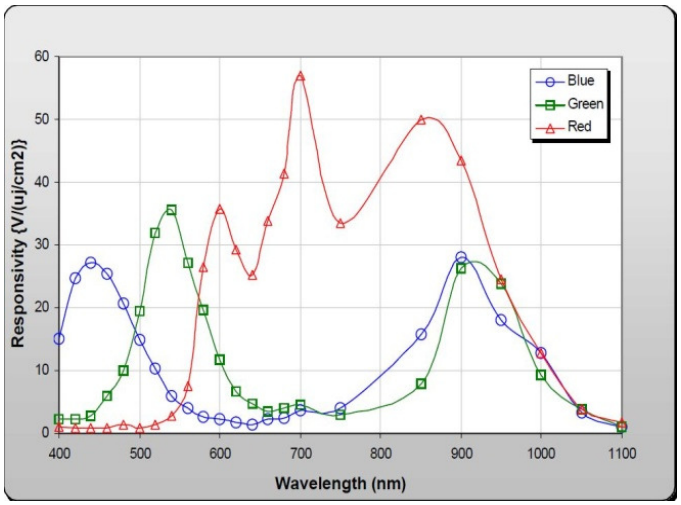
Overview

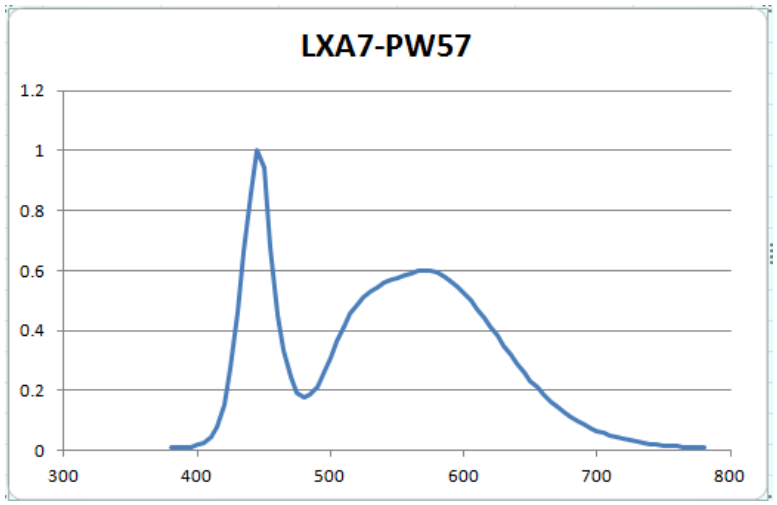
- Spectral Models for Scanner and Microscope
- Histological Stain Spectra (examples from literature)
- Construction of Color Transform
- Viewing of Digital Slides (ICC Profile using 3D LUT)
- Validation of Spectral Models (IT8.7 Film Target)
- Measuring Scanner Spectral Response
- Slide-Specific Color Profiling

Spectral Model for Scanner



Dalsa Piranha PC-30 Camera

$< \bar{r}(f), \bar{g}(f), \bar{b}(f) >$



LED Light Source

$L_S(f)$

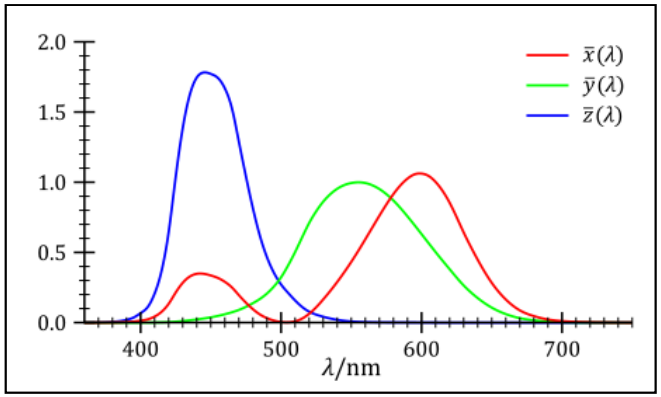
$$< R, G, B > = \frac{1}{< W_r,\ W_g,\ W_b >} \int < \bar{r}(f), \bar{g}(f), \bar{b}(f) > L_s(f)\, T(f)\, df$$

$< R, G, B >$
Scanner Output

$< W >$
White Balance
$[T = 1.0]$

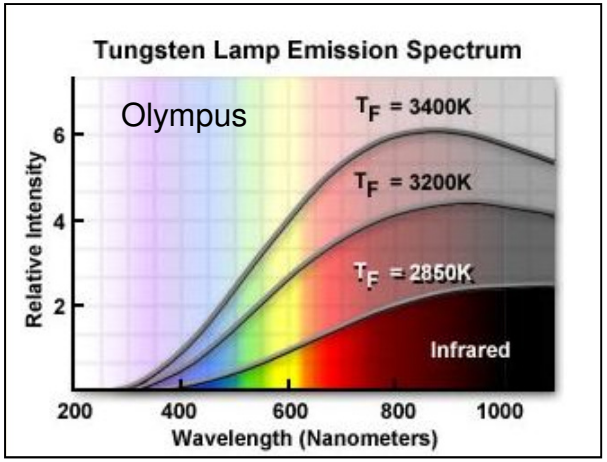
$T(f)$
Stain Transmission Spectra

Spectral Model for Microscope



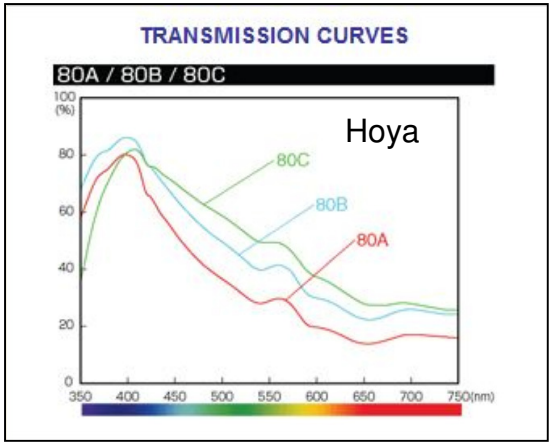
CIE Standard Observer

$< \bar{x}(f), \bar{y}(f), \bar{z}(f) >$



Tungsten Lamp

$L_m(f)$



Daylight Filter

$F(f)$

$$< X, Y, Z > = \frac{1}{W_y} \int < \bar{x}(f), \bar{y}(f), \bar{z}(f) > L_m(f)\, F(f)\, T(f)\, df$$

$< X, Y, Z >$

CIE Tristimulus Values

W_y

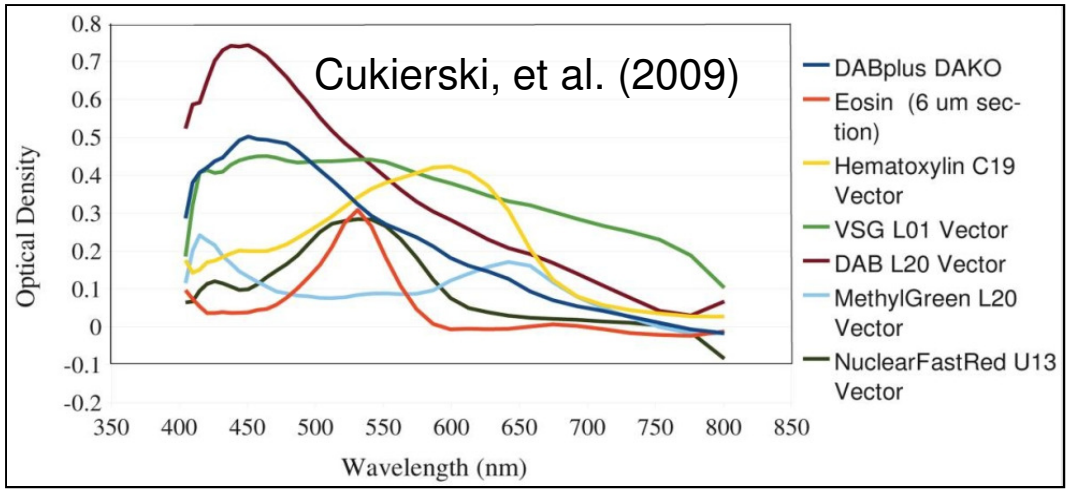
Normalization

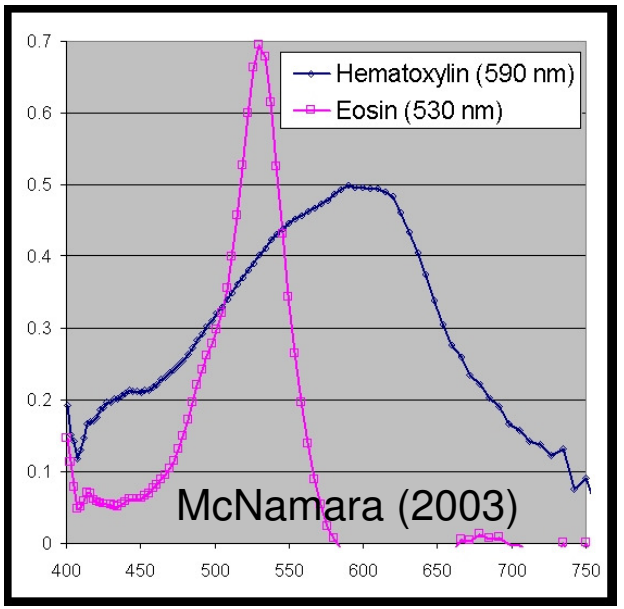
$(Y_{max} = 1)$

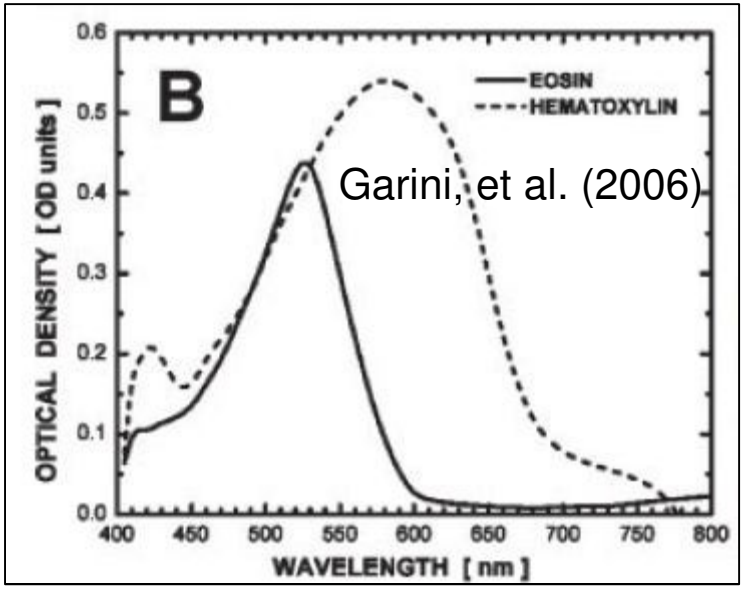
$T(f)$

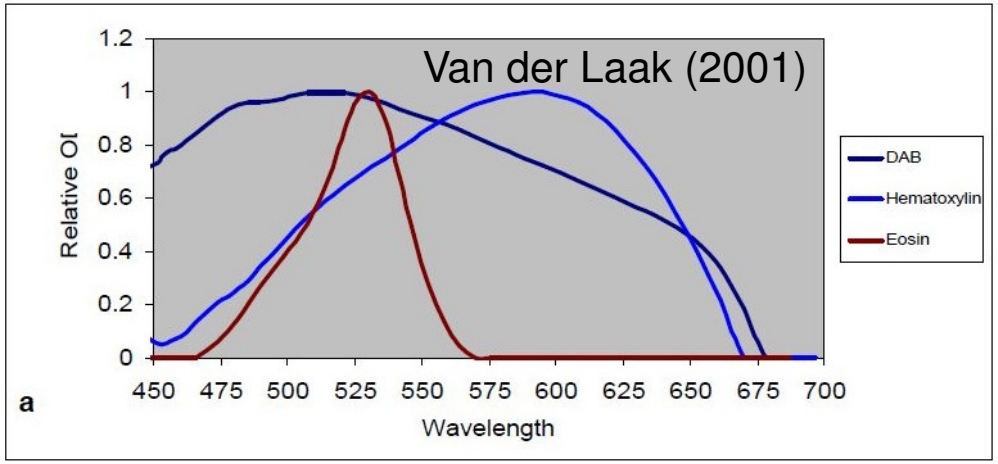
Stain Transmission Spectra

Histological Stain Spectra $T(f)$





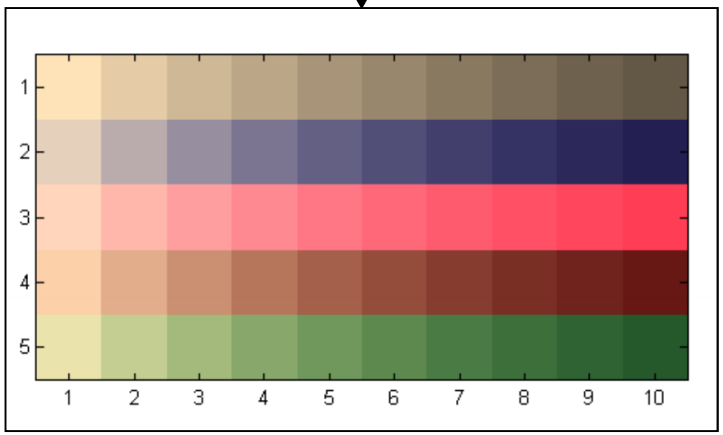




Construction of Color Transform

Stain Spectra $T(f)$
Neutral Gray
Hematoxylin Eosin
DAB
Methyl Green

Target Spectra
$[T_k(f)]^{\gamma}$ x $[T_l(f)]^{\alpha}$



Scanner Model

$< R_i , G_i , B_i >$

Microscope Model

$< X_i , Y_i , Z_i >$

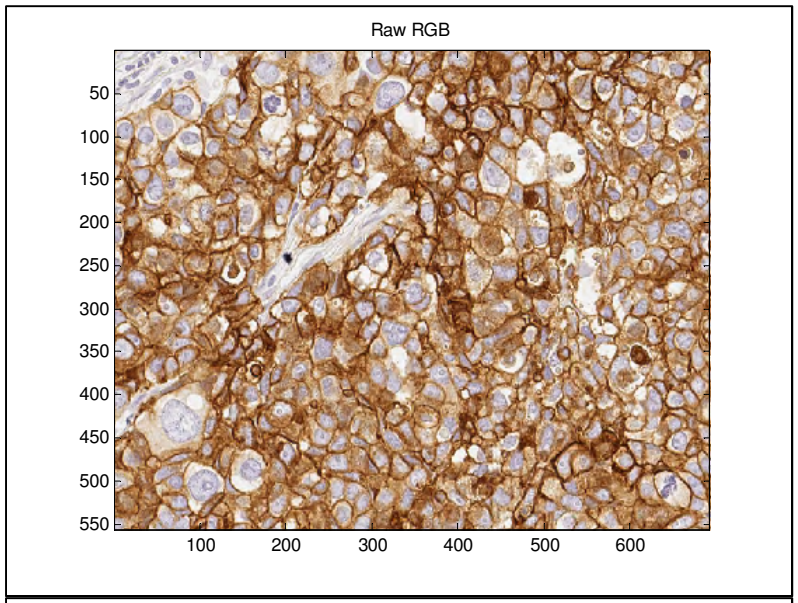
Interpolator

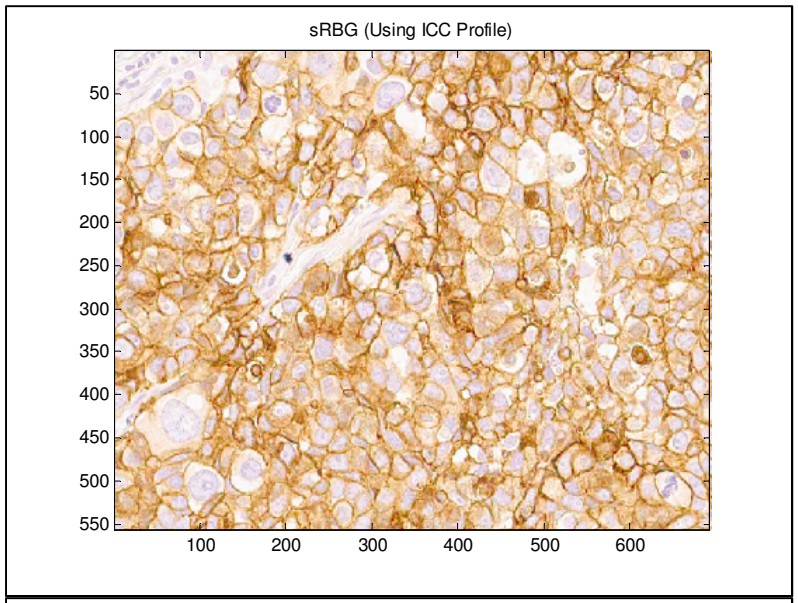
3D LUT
$< X, Y, Z > = LUT(< R, G , B >)$

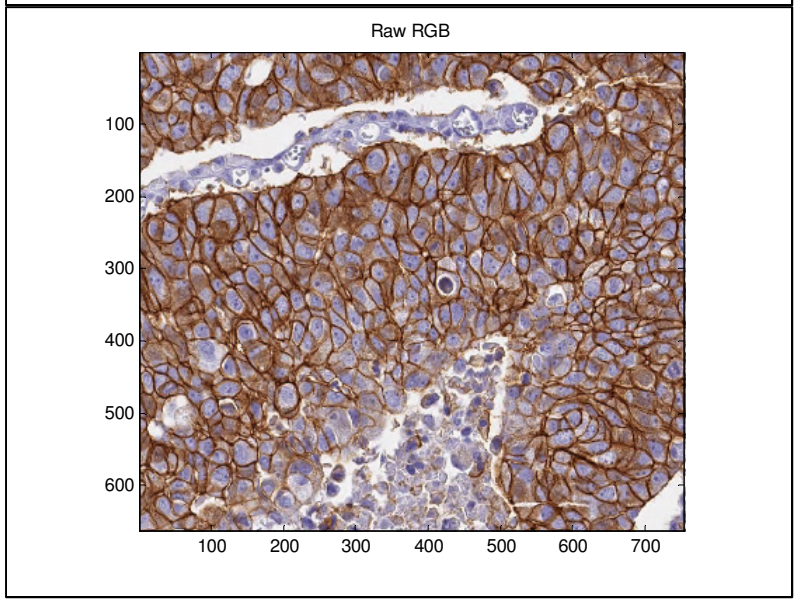
Viewing of Digital Slides

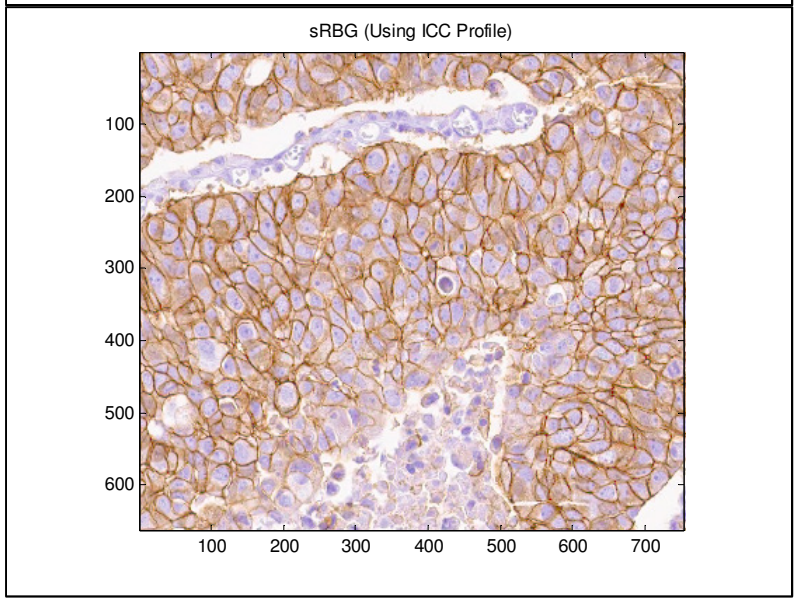
- Color Management – ICC Profile Workflow
- Digital Slide ICC Profile
  - Use “create_CLUT_profile” application (ICC website)
    - Chromatic Adaptation to D50 connection space
    - Microscope White Point = (0.9984, 1.0000, 0.5423)
- Monitor ICC Profile
  - sRGB mode for monitor
  - Use generic sRGB profile
- Viewing Software
  - Aperio ImageScope – LCMS library
- Microscopic Viewing
  - Nikon Eclipse E400 with Hoya 80A filter

Viewing of Digital Slides

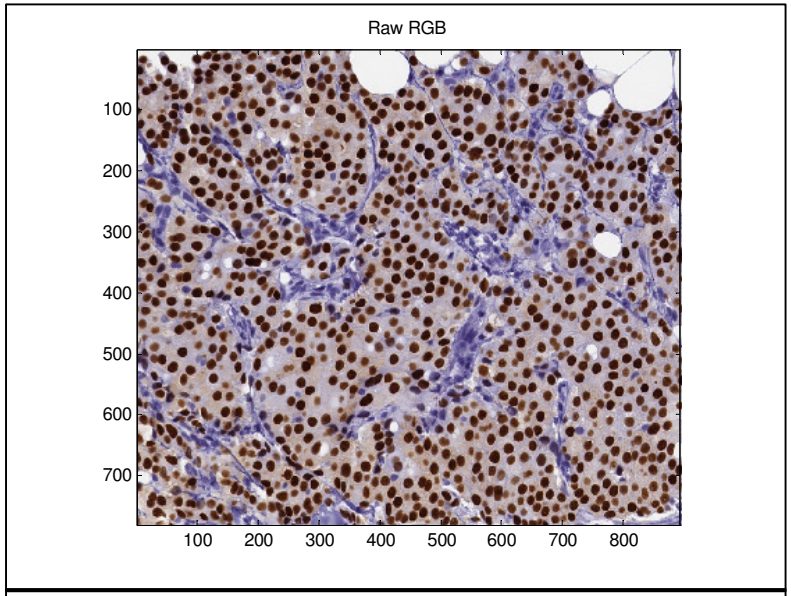


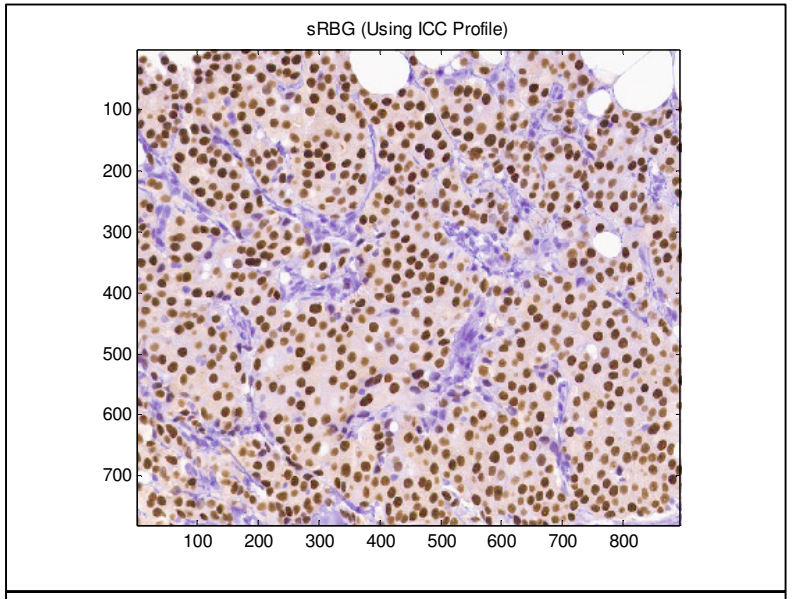


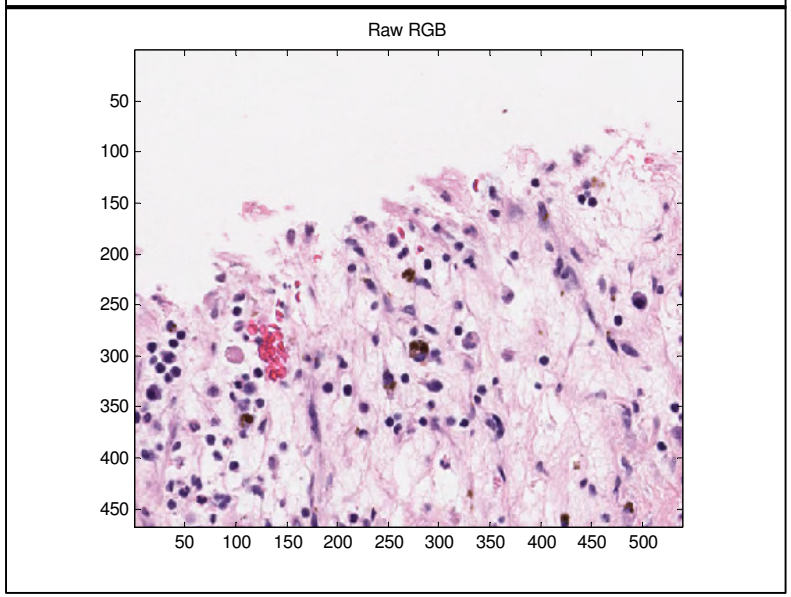


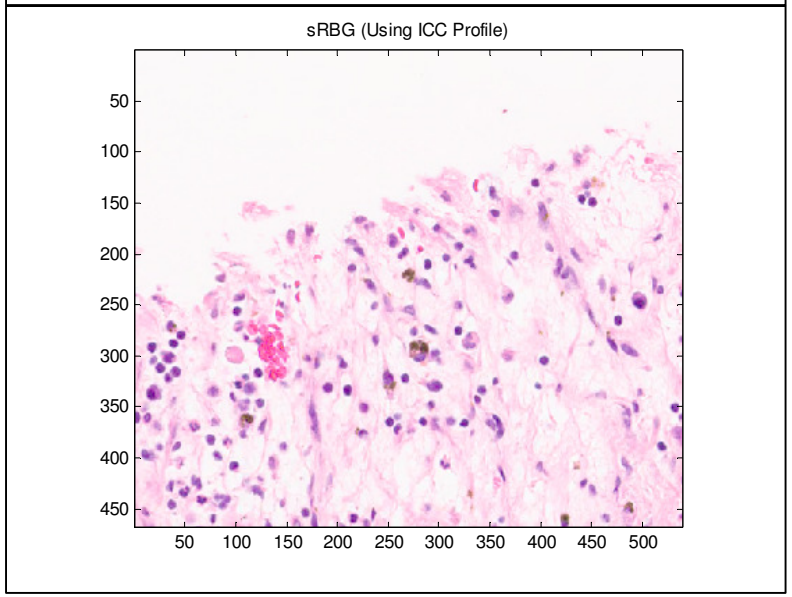


Viewing of Digital Slides

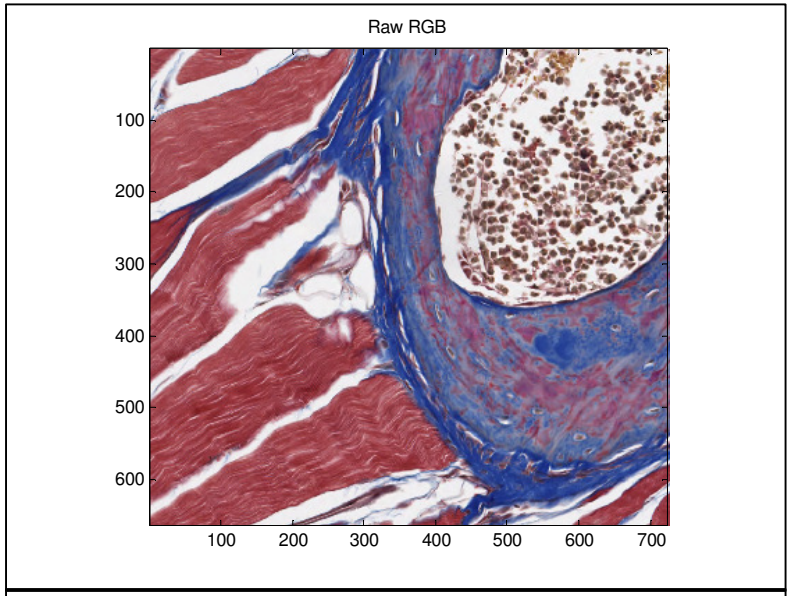


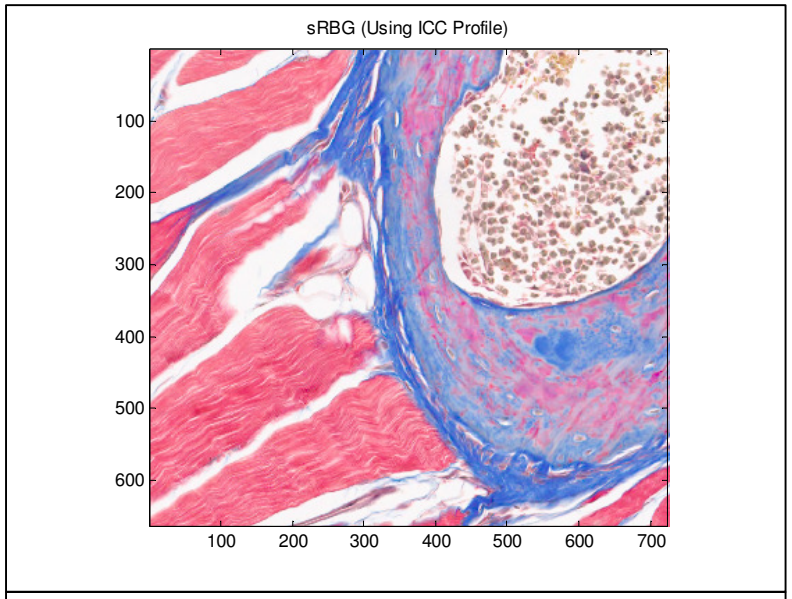


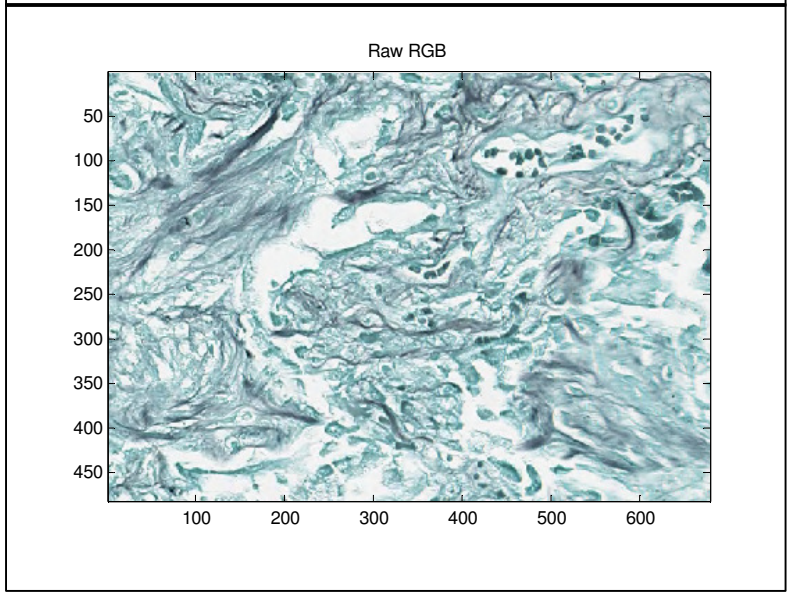


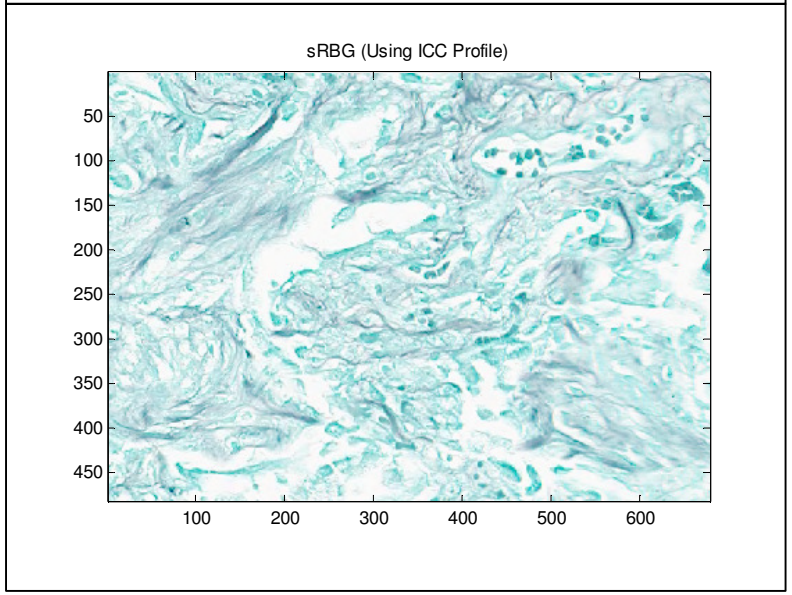


Viewing of Digital Slides

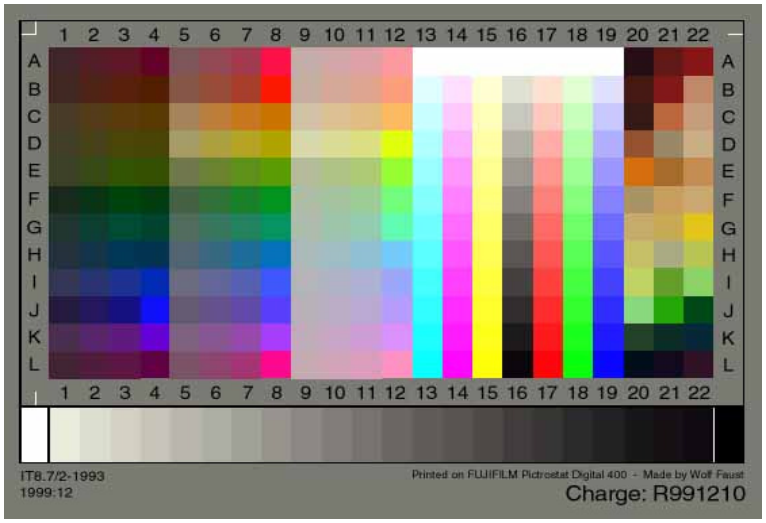








Validation of Spectral Models

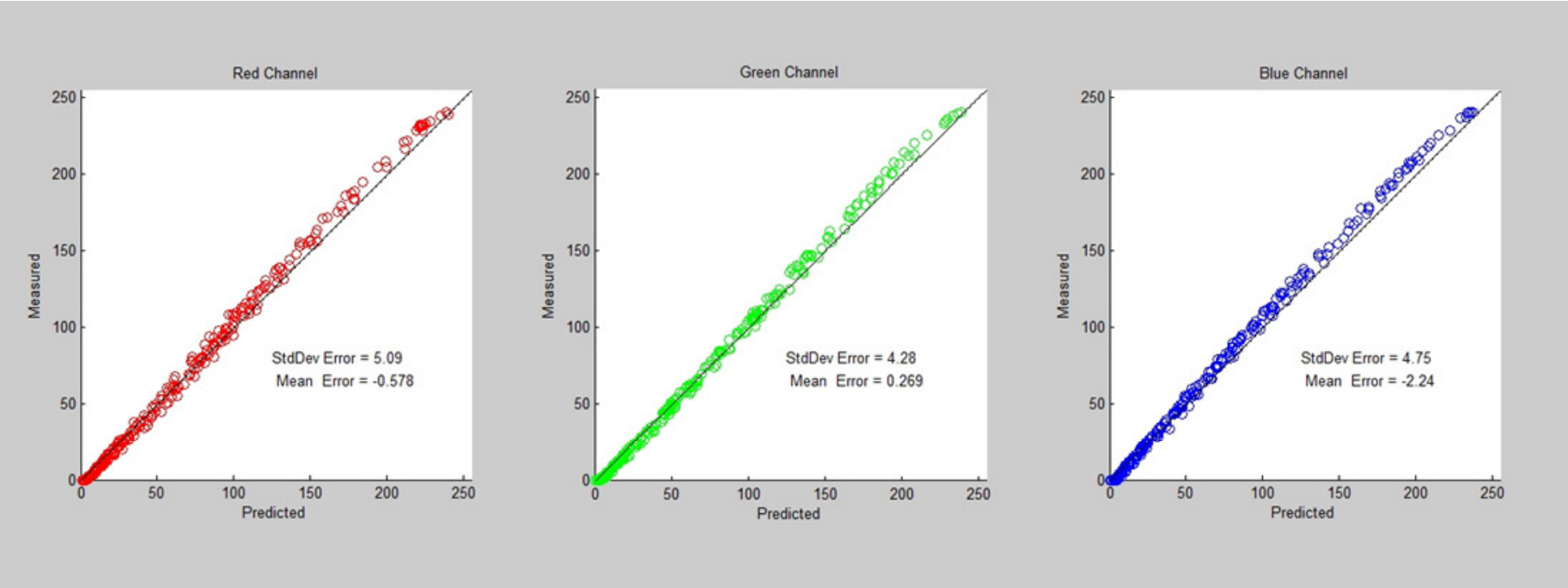


- IT8.7 Ektachrome Film Target
  (Wolf Faust)

- Calibration File
  XYZ (D50)
  Spectral Transmittance
  380-780nm (10nm)

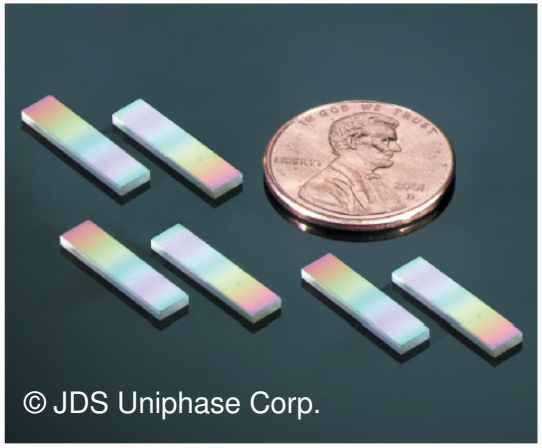
- Scanner Model Validation
  - Scan/Measure Target RGB values
  - Calculate Model-Predicted RGB values
  - Compare Measured vs Predicted values
- Microscope Model Validation
  - Change Lamp to D50 (no filter)
  - Calculate Model XYZ values
  - Compare with calibration XYZ values

Validation of Spectral Models

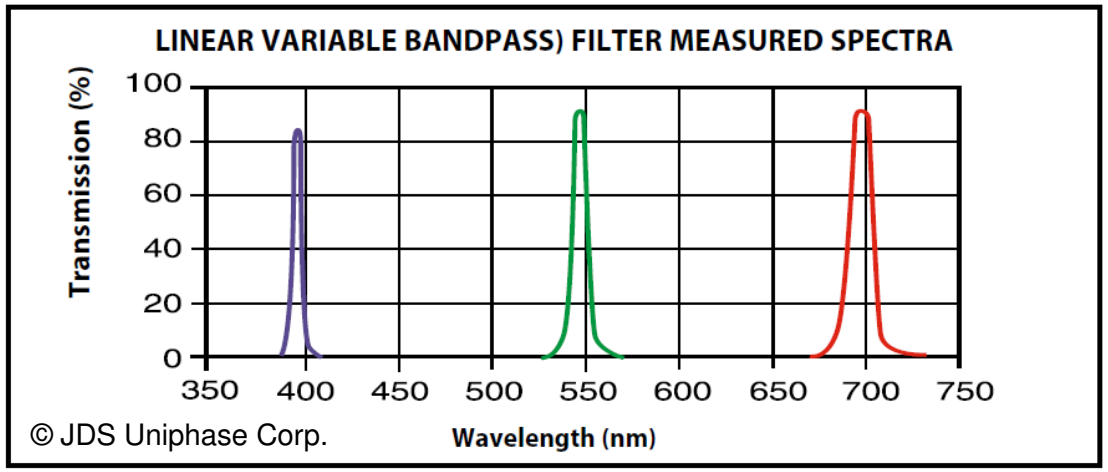


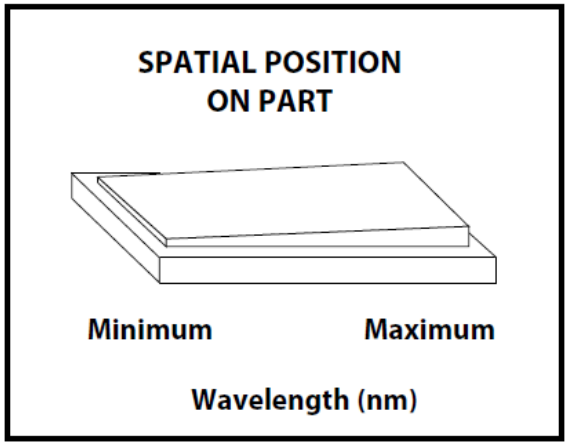
- Scanner Model
  - Standard Error 4-5 counts (shown above)
  - All Model data based upon manufacturer data sheets
- Microscope Model
  - D50 values agree to 10^{-4} (precision of spectral data)
  - Obviously manufacturer calculated these too

Measuring Scanner Spectral Response



Linear Variable Filter





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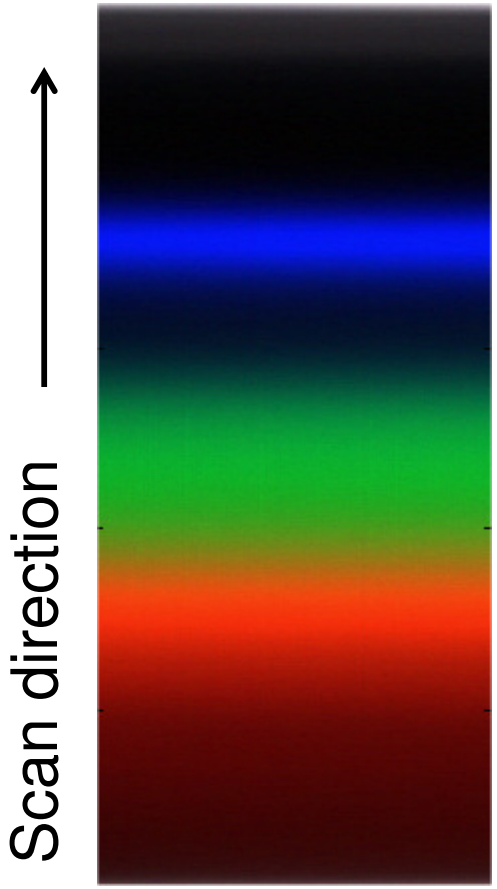
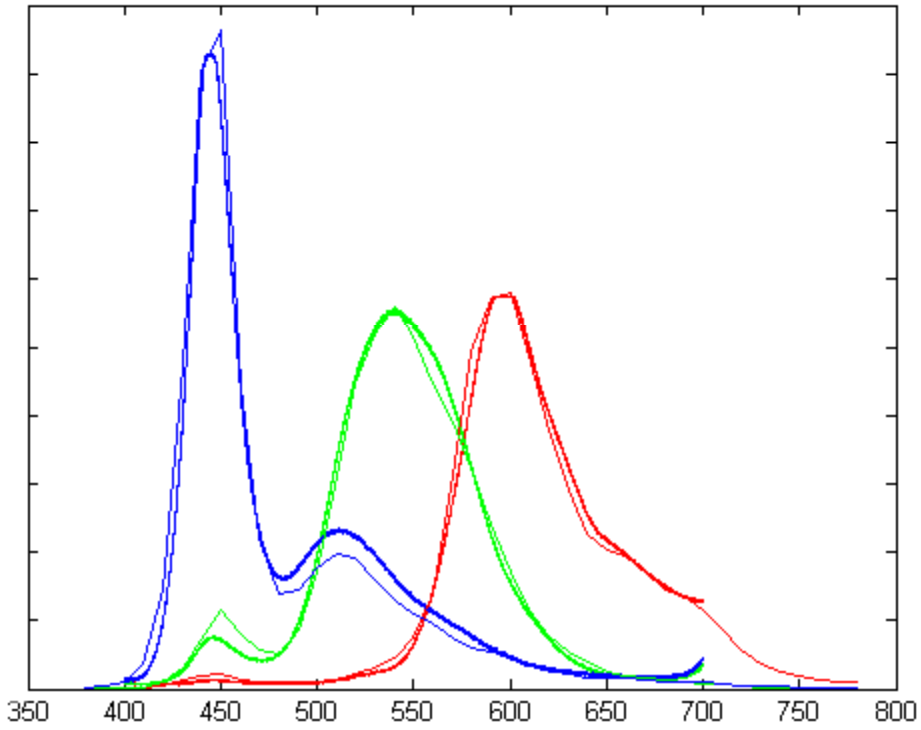
1. Scan along length of filter
2. Spectral response $R(f)$, $G(f)$, $B(f)$
3. Compare to model camera/light response

Measuring Scanner Spectral Response

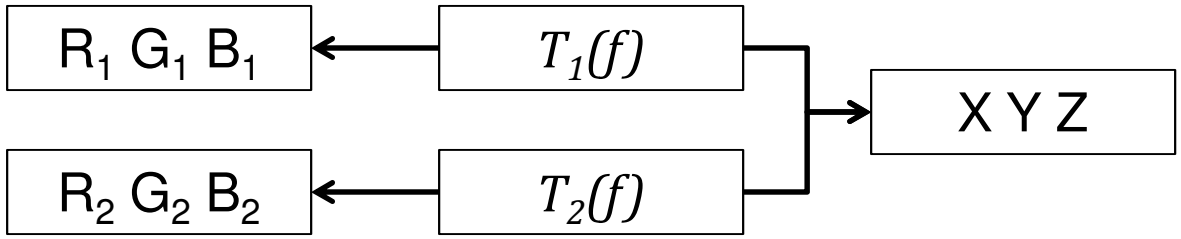




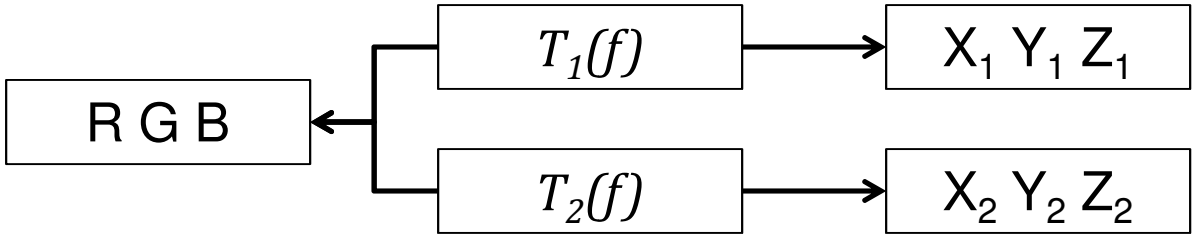
❖ Semrock Quad Band Filter: FF01-440/521/607/700 used for referencing the scan axis to nm.

Slide-Specific Color Profiling

Observer Metamerism



Device Metamerism



- This problem can be avoided altogether for histology.
- Histology slides mostly have two or three stains, designed to not be observer metameric – likely not device metameric either.
- A color transform can be calculated for each slide, based upon the specific stains and their spectral properties.

Conclusions / Questions

- Spectral models for scanner and microscope were combined to generate a color transform based upon manufacturer data specs and stain transmittance spectra from published literature.
- Significantly, the color transform was calculated without actually scanning a target slide.
- The models were then validated using an IT8.7 film target, having known spectral transmittance.
- Calibration of the scanner's transfer function was also performed using a Linear Variable Filter (LVF) and compared favorably to the generic model.
- This approach suggests the possibility of generating slide-specific profiles for each digital slide, based upon pre-calibrated spectral properties of the actual stains.