Determining the color truth of histological tissue slides with a hyperspectral imaging system

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**Abstract:** A hyperspectral imaging microscopy system was developed for obtaining the spectral transmittance of histological tissue slides on the pixel level.

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1. Introduction
2. Body

3.1 Hardware

The multispectral imaging system was implemented by retrofitting a conventional light microscope. It comprised four components –– microscope, tunable light source, camera sensor, and control software. The hub of the multispectral image system was an upright light microscope (AxioPhot 2, Carl Zeiss Microscopy, NY, USA) in bright-field mode with a 10X objective (Carl Zeiss A-Plan 10X/0.25 Ph1). A motorized XY-stage (MAC 6000, Ludl Electronic Products Ltd., Hawthorne, NY, USA) translationally moved the glass slide under the objective to select the desired region of interest (ROI). The glass slide was illuminated by, in lieu of a conventional tungsten halogen lamp, a tunable light source (OL490 Agile Light Source, Gooch and Housego, TX, USA). On the detector side, a scientific monochrome CCD camera (Grasshopper3 9.1 MP Mono USB3 Vision, Point Grey Research Inc., BC, Canada) calibrated to a linear response was used to measure the luminance of each pixel in the field of view. After mounting the light source and camera, Kohler illumination was attained by refocusing the condenser (Zeiss achromatic-aplanatic condenser system, aperture 0.9) accordingly. The image was focused manually with 550-nm (green) light.

Inside the tunable light source, the broadband white light from a xenon lamp was dispersed into various wavelengths by prisms. In this study, the 150 micron aperture was selected to generate the narrowest bandwidth. A fast-switching MEMS-based Digital Light Processor (Texas Instruments Incorporated, TX, USA) with 1,024 columns was software-controlled to reflect a set of selected wavelengths. The mapping between the 1,024 columns and the wavelength was nonlinear and needed to be determined at factory as a calibration file. The factory software looked up the calibration file and actuated the corresponding columns based on the user's choice of wavelength. The wavelengths reflected by the actuated columns were then combined and delivered through a liquid light guide. The liquid light guide was coupled with the light microscope with a collimating adapter (LLG5A4-A, Thorlabs, Newton, NJ, USA).

The pixel count of the camera was 3,376×2,704 at 9 fps. The size of the CCD sensor (ICX814, Sony Electronics Inc., Park Ridge, NJ, USA) was the 1-inch format that covers a major portion of the field of view of the microscope. The resolution of the microscope system when using the 10X objective was 370 nm per pixel. The tunable light source, motorized stage, and camera were all controlled by programs written in Matlab 2015b (Mathworks, MA, USA) running in the Microsoft Windows 7 Professional 64-bit environment.

3.2 Software

The goal of the multispectral imaging system was to measure the spectral transmittance, T, of the glass slide for each pixel. The glass slide was illuminated by the light source with spectral power distribution L. The spectral sensitivity of the camera, including both the sensor and optics, is C, which also depended on the camera parameters such as the exposure, gain, and brightness settings. The camera detects the luminance of the target Y and reports in digital count D. The detected luminance Y is proportional to C, L, and T over the visible light wavelength λ:

|  |  |
| --- | --- |
|  | (1) |

By fixing the settings of the camera and the spectrum of the light source, C and L can be considered constant so the detected luminance at a specific wavelength is proportional to the transmittance of the tissue sample on the glass slide only:

|  |  |
| --- | --- |
|  | (2) |

If the camera has a linear response between the detected luminance Y and the output digital count D, then the relationship between the transmittance and the digital count can be expressed as a linear function:

|  |  |
| --- | --- |
|  | (3) |

The two coefficients and can be found by measuring two different glass slides with known transmittance. In this study, a transparent target ( =1) and an opaque target ( =0) were used. The corresponding camera digital counts are, respectively:

|  |  |
| --- | --- |
|  | (4) |
|  | (5) |

The transmittance can then be calculated via interpolation:

|  |  |
| --- | --- |
|  | (6) |

Recall that the spectral power distribution of the tissue sample, , is the product of the spectral power distributions of the light source and the transmittance. Although the resulting spectral transmittance is the ground truth of the glass slide, it cannot serve as the color truth for comparison until a light source is applied. In this study, the CIE D65 illuminant was used.

(7)

The CIEXYZ tristimulus XYZ was calculated by

(8)

(9)

, (10)

where , , and are the CIE 1931 color matching functions.

Then the tristimulus of the CIE D65 illuminant was calculated similarly and used as the reference white, XnYnZn, to calculate the CIELAB L\*a\*b\* values.

(11)

(12)

(13)

(14)

(15)

The CIELAB L\*a\*b\* values would be used as the truth to evaluate the WSI scanners. For visualization and presentation purposes, the CIELAB data were also converted into the sRGB color space to reconstruct the truth image12. Notice that the sRGB color space was not used to process the truth data because some eosin-stained shades with high lightness values are out of the sRGB color gamut. Nevertheless, many commercial WSI scanners use sRGB to store and display images. In this study the CIE D65 illuminant was used to establish the truth. Alternative illuminants can be used for specific viewing environments or applications.

The conceptual procedure of multispectral imaging is described in the following pseudo-code.

Adjust the camera settings

For each wavelength λ=380:10:780

Adjust the tunable light source for λ

Image the opaque target to obtain Dmin for T=0

Image the transparent target to obtain Dmax for T=1

Measure the target slide to obtain D for each pixel

Linearly interpolate (D,T) from (Dmin,0) and (Dmax,1)

End

Reconstruct spectral transmittance for each pixel

3.1 Workflow

A series of 41 images were captured at wavelengths from 380 to 780 nm at the interval of 10 nm. The spectra of the light source are shown in Fig 2. The set of 41 images were compared with both flat-field bright and dark images to calculate the spectral transmittance. The flat-field bright image was acquired by imaging a blank area on a glass slide with coverslip mounted. The camera exposure settings were adjusted such that the bright pixel values did not saturate. These pixel values, Dmax, which may vary among pixels due to spatial non-uniformity, indicate the 100% transmittance, which already included the effects of the glass slide and the coverslip. The flat-field dark image was acquired by imaging a black plate that does not transmit light. These pixel values, Dmin, indicate the 0% transmittance. For each wavelength, each pixel reading was linearly interpolated between the bright and dark pixel values to obtain the per-wavelength transmittance (Equ 6). After collecting the per-wavelength transmittance data from 41 images, the complete spectral transmittance of each pixel was reconstructed (Fig 1, Spectral Transmittance). The spectral transmittance was multiplied by the spectrum of the CIE D65 illuminant (Equ 7) to obtain the spectral power distribution (Fig 1, SPD). The spectral power distributions of the tissue and the reference white were converted to CIEXYZ (Equ 8-10), which generated CIELAB (Equ 11-15). The procedure was implemented in Matlab conceptually but not verbatim because data vectorization was required to minimize the computation time for large images.

1. Accuracy Evaluation
   1. Paul’s uncertainty data
   2. Firdous’ data
2. Applications and Examples

4.1 Evaluate WSI scanners

4.2 Create ICC profiles

4.3 Evaluate staining variation

1. Funding, acknowledgments, and disclosures

5.1 Funding

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5.3 Disclosures

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