Color aspects and Color Standardization in Digital Microscopy

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Today’s Topics

- Towards Standardization
- Color Aspects
- Types of Color Issues in WSI
- Color Standardization
Towards Standardization
Standardization of the image quality and the color displayed are important aspects of digital pathology implementation. While the most common reason for the variations of color and image quality is the variance in the protocols and practices in the histology lab, the image displayed can also be affected by variation in capture parameters, image processing and display factors in the digital systems themselves. It is difficult to identify which exactly causes the problem.
Steps: Towards Color and Image Quality Standardization

1. To Notice
   - To realize the image quality and color issues are often present in the images we use

2. To Identify
   - To identify the causes of issues in WSI

3. To Solve
   - To develop the methodologies to improve the color and image quality of WS images

4. To Promote
   - To introduce the methods solutions to the public

Today, we focus on “color” in Whole Slide Imaging (WSI)
Color Aspects
Color Aspects in Digital Pathology

- Thickness of Specimen
- Staining
- Scanner or Scanning process
- Viewer Software
- Display
Color Aspects in Digital Pathology

- Thickness of Specimen
- Staining
- Scanner or Scanning process
- Viewer Software
- Display
Thickness of Specimen & Staining

Thicker sections are stained more by the automated staining machine.
Thickness of Specimen & Staining

More details can be seen on slides of thinner sections
Thicknes of Specimen & Staining

The appearance of stained slide varies between laboratories or institutions.

Examples of H&E stained variations caused by variations in staining protocols.
Color issues in WSI 3D (Staining)

Original

Standardized
After color normalization
Thickness of Specimen & Staining issues in serial sections of WSI
Thickness of Specimen & Staining issues in serial sections of WSI

Color appearance

slides from same block
Color Aspects in Digital Pathology

- Thickness of Specimen
- Staining
- Scanner or Scanning process
- Viewer Software
- Display
Scanner or Scanning Process

Same slide, different scanners
Scanner or Scanning Process
Same slide, different scanners
Scanner or Scanning Process

Scanner A

Scanner B

IHC

IHC Double Stains
Scanner or Scanning Process

Scanner A

Scanner B

IHC

H&E
Color Aspects in Digital Pathology

- Thickness of Specimen
- Staining
- Scanner or Scanning process
- Viewer Software
- Display
Viewer Software

Same scanner, same slide, two different viewers
Color Aspects in Digital Pathology

- Thickness of Specimen
- Staining
- Scanner or Scanning process
- Viewer Software
- Display
Same images in same PC were viewed by 2 different displays
Display

Same image in same PC was viewed by 3 different displays
Example Experiment: Color of Display
In color-related fields, a color chart is a physical arrangement of standardized color samples, used for color comparisons and measurements such as in checking the color reproduction of an imaging system. Color charts are used to calibrate and to profile graphic devices, such as digital cameras and scanners. Therefore standardized IT8 targets are made by several companies.
Display Experiment with Macbeth Color Chart at the Department of Pathology in MGH

The standard displays of our Department are of 2 different models. We randomly selected 23 standard displays from one of the two models for this experiment. All driver software and display settings were exactly the same for all the 23 displays.

We measured the each color on each display by Display Analyzer. If the data is too offset, we calibrated using Monitor Calibration tool.
Red Value 23 Displays

Red Value

Chart Color #
Green Value 23 Displays
Blue Value 23 Displays

Blue Value

Chart Color #
Example of Color differences: before calibration and after calibration
Results: Experiment with Macbeth Color Chart at Dept of Pathology, MGH

Pathologists were looking at same image without noticing the differences in color. After the calibration, the color differences were clearer.

Probably, it is not good to use the WSI ?? User should be able to notice the color shift of his own display
Until we showed the result, no one noticed how bad our displays were
Why is it problem?
Is it problem?

Scanning → Staining → Display

HARVARD MEDICAL SCHOOL

MASSACHUSETTS GENERAL HOSPITAL
Pathology
Is it problem?

Scanning

Staining

Display

Pathologist looks at an actual slide under the microscope

Yes
Is it problem?  Yes

When a pathologist looks at the image on the monitor without a glass slide, it is difficult to know if the color of the image is accurate or not.

It may cause diagnostic error; or pathologists may be uncomfortable to make a diagnosis.
Is it problem?
Is it problem? Yes

8.8% 7.9%
Is it problem? Yes

- When we use it for Computer Aided Diagnostic System or image analysis

8.8%  7.9%
Color Standardization in WSI

• To prevent diagnostic errors

• To use WSI for Computer Aided Diagnostic System
The reason of Color Standardization for us

- Between scanners
- To make sure the color (WSI) is safe to use before showing pathologists or using for analysis
Color Standardization in WSI

From Staining to Display

Staining → Scanning → Viewer software → Display
Color Standardization in WSI: From Staining to Display

Today’s topics

Staining

Multispectral Imaging application

Scanning

Display
How can we identify the cause of the difference in color and standardize?
To identify the causes of issues in WSI

We have developed a slide set at MGH

Calibration Slides for Scanner

Image Quality & color

Color

Calibration Slides for Pathologist (Display)

Color
Color Calibration Slide

(Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future, Ronald Weinstein et al. In Human Pathology, 2009)

9 color filters were selected for Histology Stained Slides, which especially works best with H&E stained slides. The filter selection was based on spectral information of each color. Previously, a research study was conducted.

Original Slide for Microscope
15-day old or older mouse embryo paraffin block is sectioned by automated sectioning machine with 3um/section. (100 slides at a time)
Image Quality Slide

- H&E stain is performed with an automated staining machine at the same time.
- All Slides are scanned with one of the scanner in the lab and scanned images are posted on the web site.
Display for the Viewer

Go to Calibration slide web of PICT Center, MGH

Compare the color of calibration slide vs calibration slide on the display. If it is too far, contact HELP DESK

This Slide is hand made in the lab. The cost is very close to 0. It can be given to all pathologists
Scanner

Scanning

Review Display

The Imaging web site has the colors of the Calibration slide.

Compare the displayed colors of the calibration slide to their actual colors to understand the difference.

The Imaging web site has Calibration slide.
Almost all colors are wrong
Results: Scanner 2

Better than Scanner 1. Especially Pink and Blue are wrong.
Results: Scanner 3

Better than Scanner 1. Especially Green and Yellow are wrong.
Results 20x vs 40x of the scanner 1

Original

20x

R=98  G=124  B=152
R=187  G=107  B=84
R=65  G=16  B=46
R=31  G=80  B=158
R=204  G=1585  B=45

40x

R=112  G=142  B=178
R=219  G=126  B=92
R=85  G=17  B=50
R=44  G=89  B=187
R=233  G=182  B=39
R=19  G=98  B=137
Results

We have tested 5 different scanners with the calibration slides. No scanner produced exactly same color with the original even after the adjustment of the error of each Display.
Image Quality Evaluation & Color Standardization
Color Standardization
Color patches

- Colors are not accurate enough

- Standardize using the original and reference color patches

Original - Produced by a whole slide scanner

Reference - Produced by using spectral information of the patches
Each scanner will have its own **Color transformation matrix**

$$\begin{pmatrix} R \\ G \\ B \end{pmatrix} = \begin{pmatrix} a_{1,R} & \ldots & a_{m,R} \\ a_{1,G} & \ldots & a_{m,G} \\ a_{1,B} & \ldots & a_{m,R} \end{pmatrix} \begin{pmatrix} R \\ G \\ B \end{pmatrix}$$

Reference color of the color patches

Color transformation matrix will be stored for used in color standardization

Color of the patches as produced by a particular scanner
Whole slide scanners and Color Imaging

Whole slide scanner 1 (WSI 1)

Whole slide scanner 2 (WSI 2)

Use the mouse embryo slide to confirm the color transformation matrix
Results in Liver
Thumbnail images of the original whole slide images

Scanner A  
Liver

Scanner B

There is color variation....
Thumbnail images of the standardized whole slide images

应用色校正可以最小化颜色差异……
Liver

Without color correction...
Liver

Result of color correction...
There is separation in the distribution->
Corrected

Overlap in the color distribution ->
There is separation in the distribution->
Corrected

Overlap in the color distribution ->

Cytoplasm (Corrected)

Blue channel

Red channel
Results in Lymphoma
Thumbnail images of the original whole slide images

Scanner A

Scanner B

Lymphoma

There is color variation....
Thumbnail images of the standardized whole slide images

Scanner A

Scanner B

Lymphoma

Application of color correction minimizes the color differences.....
There is color variation....

(no correction)
Application of color correction minimizes the color differences....
There is separation in the distribution->
Overlap in the color distribution - >
There is separation in the distribution -> dissimilar color
Overlap in the color distribution - >

Cytoplasm(Corrected)

A

B

Blue channel

Red channel
Image Quality Evaluation
# Image Quality Evaluation Algorithm

<table>
<thead>
<tr>
<th>Image Quality</th>
<th>Multiple regression analysis</th>
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<tbody>
<tr>
<td>Definitive evaluation index $q$, is calculated by $q = \alpha + \beta s + \gamma n$</td>
<td></td>
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<tr>
<td>$\alpha, \beta, \gamma$ are derived from training data.</td>
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Image Quality Evaluation Method for Whole Slide Scanning

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Introduction

What is whole slide imaging (WSI)?
- WSI refers to the conversion of a conventional glass tissue slide into a digital image.
- So, users can access the image remotely on a computer monitor as if they are using a microscope.
- WSI has many advantages.
  - High-quality images are essential to perform an accurate image analysis and to provide a correct diagnosis.
  - Also, an improved image quality can help to improve the efficiency of a WSI system.

The purpose of the study
1. To develop an image quality evaluation algorithm for whole slide scanning.
2. To determine the appropriate image quality parameter values.
3. To investigate how to implement the algorithm in whole slide scanners.

Evaluation Algorithm

Evaluation method is based on sharpness (focus) and noise.

1. Sharpness evaluation
   - The edges in the image are detected using the Canny algorithm.
   - Pixel values of the detected edges are determined and the average value is used as the sharpness index.

2. Noise evaluation
   - A similarity masking technique is used to detect the edges and noises in the image.
   - The center pixel (3x3-sized window) is replaced with the minimum difference between its surrounding pixels in order to leave only the noises.
   - The mean-square of replaced pixel values is used as the noise index.

Multiple regression analysis

Definitive evaluation index, \( I \), is calculated by

\[ I = \alpha \times \text{Sharpness} + \beta \times \text{Noise} + \gamma \]

We can choose the arbitrary index for multiple regression depending on the requirement of user's application. If we use the subjective evaluation values, the image quality for diagnostic application is calculated. Otherwise, the evaluation index will be used to show the image quality required for image analysis.

Experiments

1. Evaluation of the algorithm

50 images were captured from the various types of slides scanned by NanoZoomer 2 HT (Hamamatsu, Japan) and trimmed into 400x400 pixels. We conducted a survey to give the subjective score of pathologists, technicians, and image specialists. The images were rated on a scale of one to five, i.e., 1 was the best quality and 5 was the worst. The average score of each image was used for multiple regression analysis, in which we investigated the correlation between the subjective scores and the objective scores. From the regression analysis results, we determined the appropriate image quality parameter values, i.e., threshold value between good and bad quality image.

2. Application to WSI

We applied the proposed image quality evaluation method to the WSI of an H&E-stained mouse embryo. Its size was 56,000x24,000 pixels.

The entire image was divided into 400x400-pixel blocks, and the evaluation algorithm was applied to all blocks. The equation derived by multiple regression analysis was used to estimate the image quality of each block. The block whose evaluation index was greater than the threshold value (based on the results of the subjective experiment) was considered with the original image. Otherwise, each block was discarded depending on the evaluation index. The blocks, which had more white pixels than 70% of the blocks, were regarded as background and also visualized with the original color.

Discussion

We performed a simulation on the application of image quality evaluation in whole slide scanning. In the simulation, a slide was first scanned in automatic scanning mode. Then, the image quality of the scanned image (WSI) was evaluated using our proposed method. The slide was re-scanned in an automatic focusing mode. Then, the image quality of the re-scanned image was evaluated again. The results showed that the image quality improved when the slide was re-scanned.

Conclusion

The image quality evaluation algorithm is extremely important for WSI. Reality of our experiment shows that by incorporating the proposed image quality evaluation method, the quality of whole slide imaging is improved. Also, we are planning to integrate the scanning procedure of digital slides. The effectiveness of the evaluation method will be tested in our future work.
Discussions

- The two types of calibration slides helped users to improve the color accuracy of the images they are looking at.
- Two algorithms for color and quality are working well for 5 scanners.
- We have developed additional calibration slides to improve the reliability of WSI system.
- Many pathologists have started to realize that accurate color and image quality are important in WSI.
Summary: Standardization

Scanning

Display

Online Management System is available

Color

Staining

Standardization

Algorithm

Digital Staining Standardization is available

Image Quality Evaluation Algorithm
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