Color aspects and Color Standardization in Digital Microscopy

Yukako Yagi, PhD
yyagi@partners.org
Director of the MGH Pathology Imaging & Communication Technology Center
Assistant Professor of Pathology, Harvard Medical School
Affiliate Faculty, Wellman Center for Photomedicine, MGH

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Today's Topics

- **Towards Standardization**
- **Color Aspects**
- **Types of Color Issues in WSI**
- **Color Standardization**

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Towards Standardization

Standardization in Digital Microscopy

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

Standardization

Today, we focus on "color" in Whole Slide Imaging (WSI)

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Color Aspects

Color Aspects in Digital Pathology

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

Color Aspects in Digital Pathology

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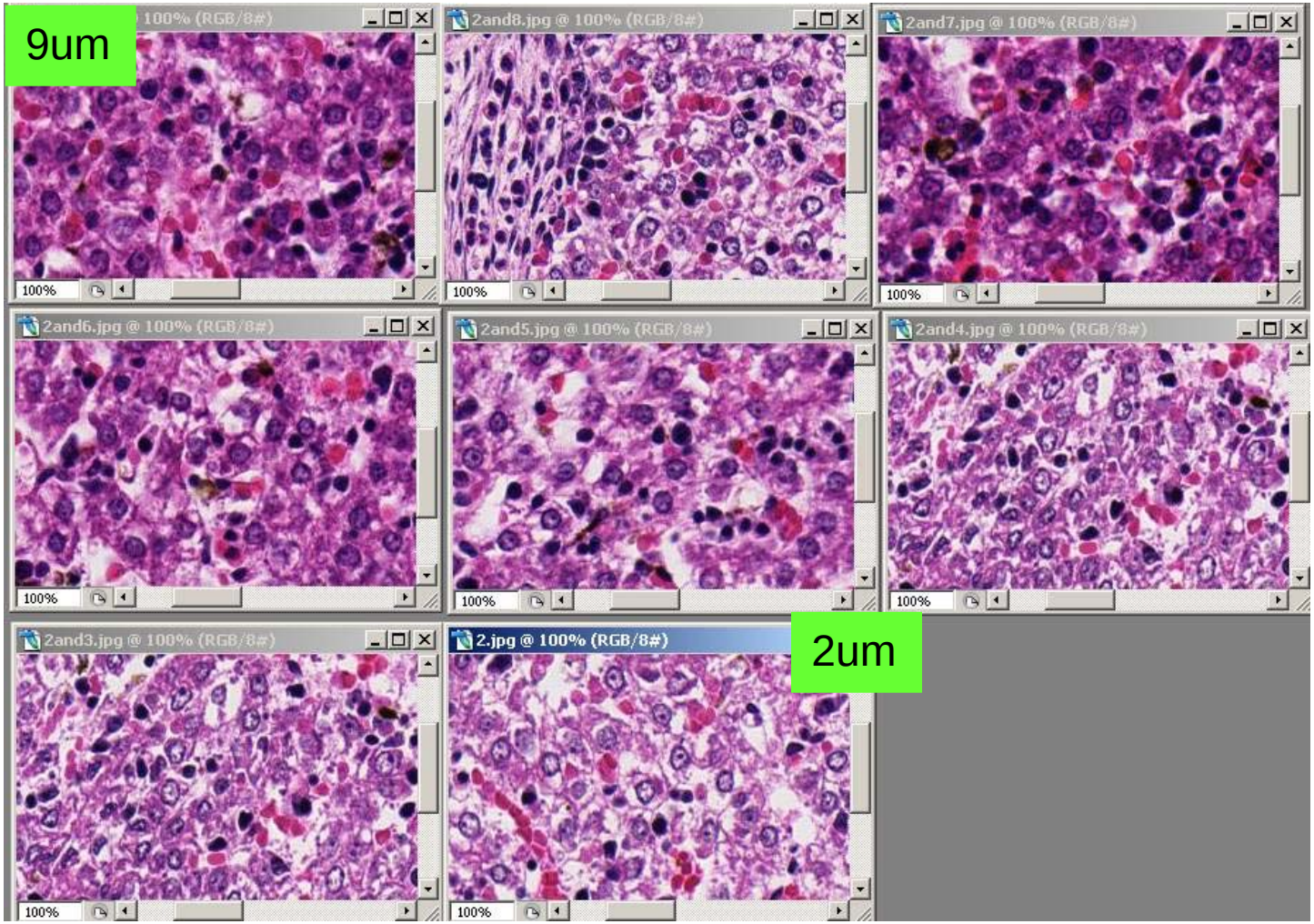
Thickness of Specimen & Staining

Thicker sections are stained more by the automated staining machine

Thickness of Specimen & Staining



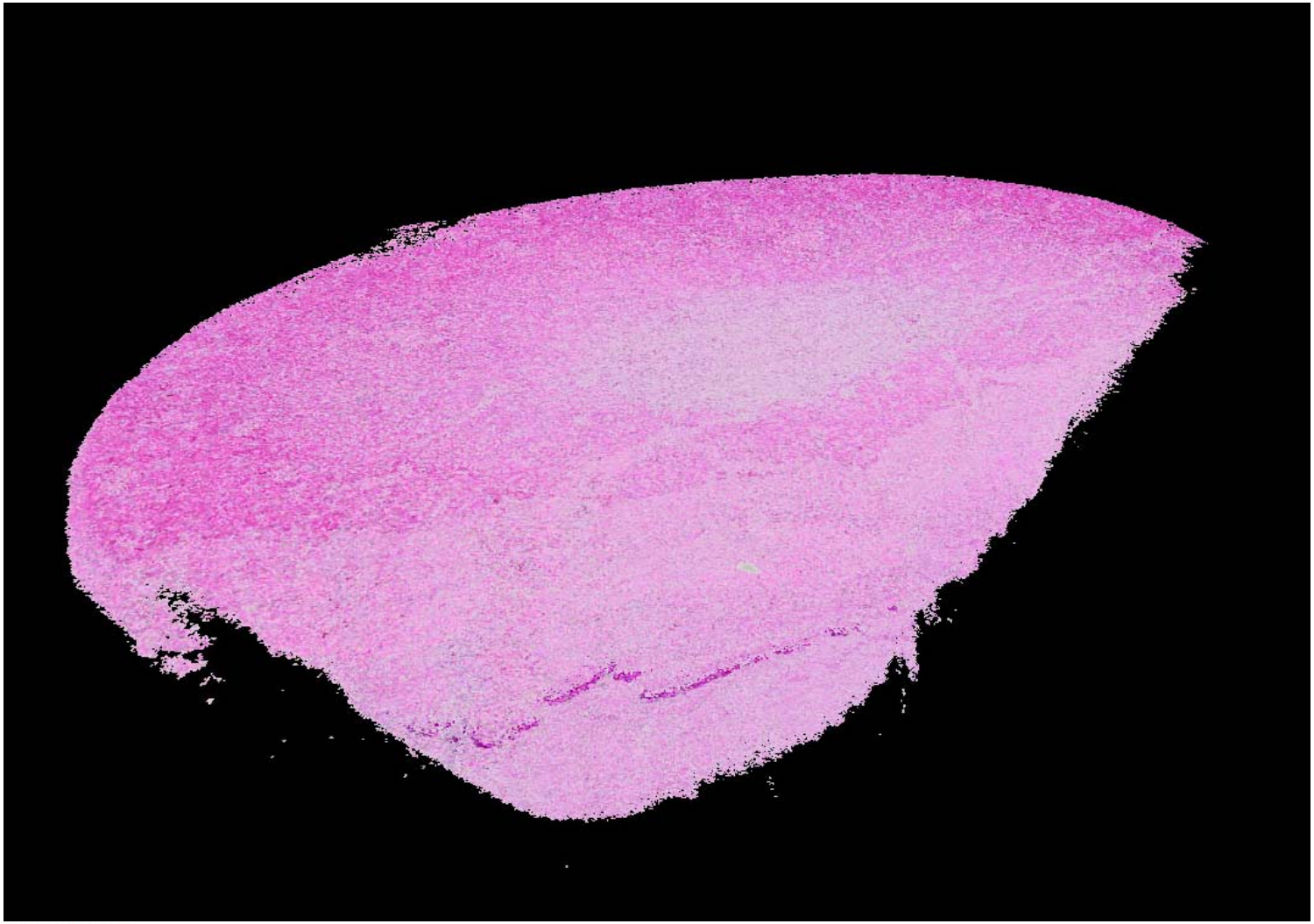
More details can be seen on slides of thinner sections

Thickness of Specimen & Staining

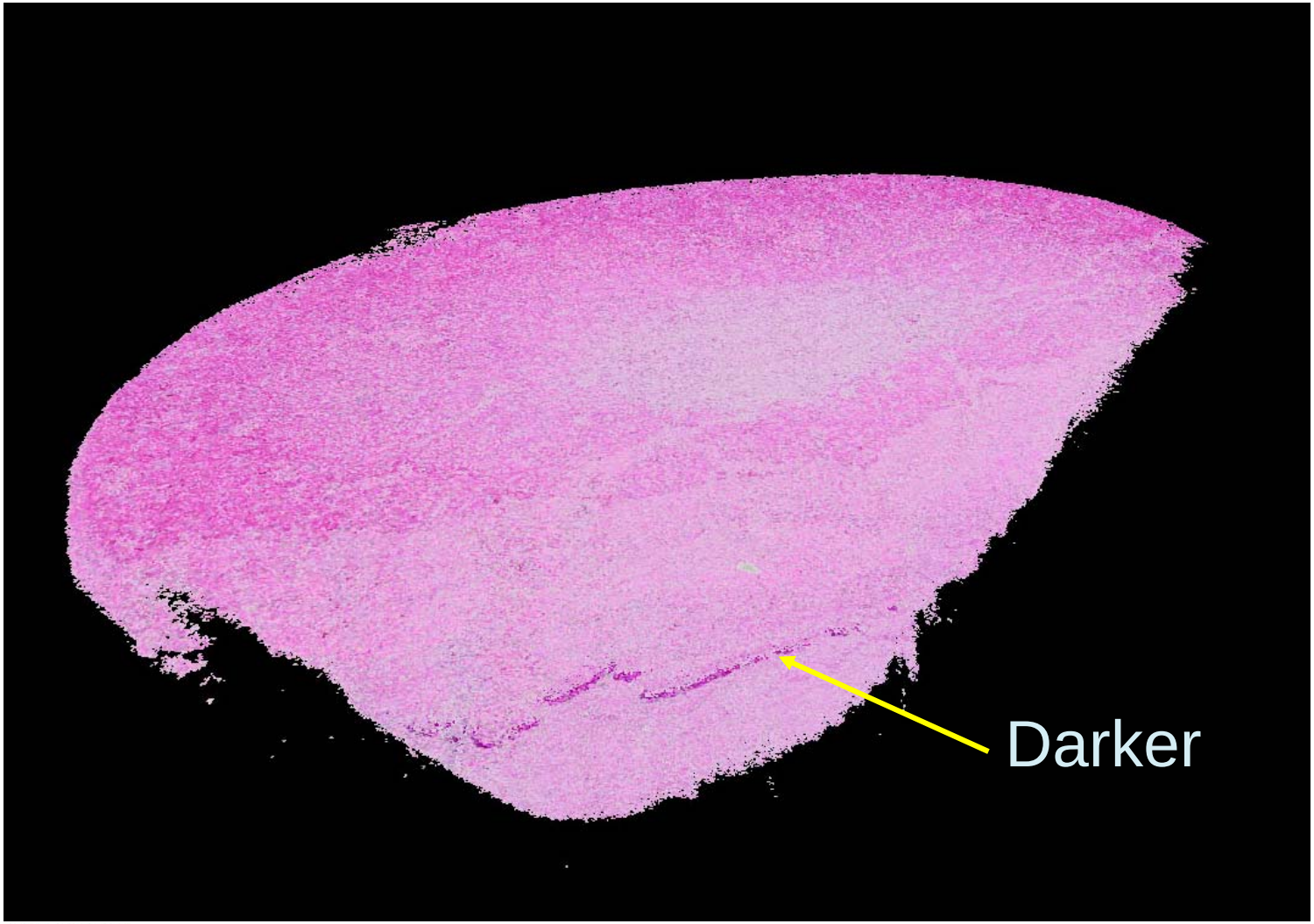
The appearance of stained slide varies between laboratories or institutions

Examples of H&E stained variations caused by variations in staining protocols

Staining Issues in serial sections of WSI based 3D Imaging



Effect of Staining Issues in serial sections of WSI based 3D Imaging



Staining Issues in serial sections of WSI based 3D Imaging

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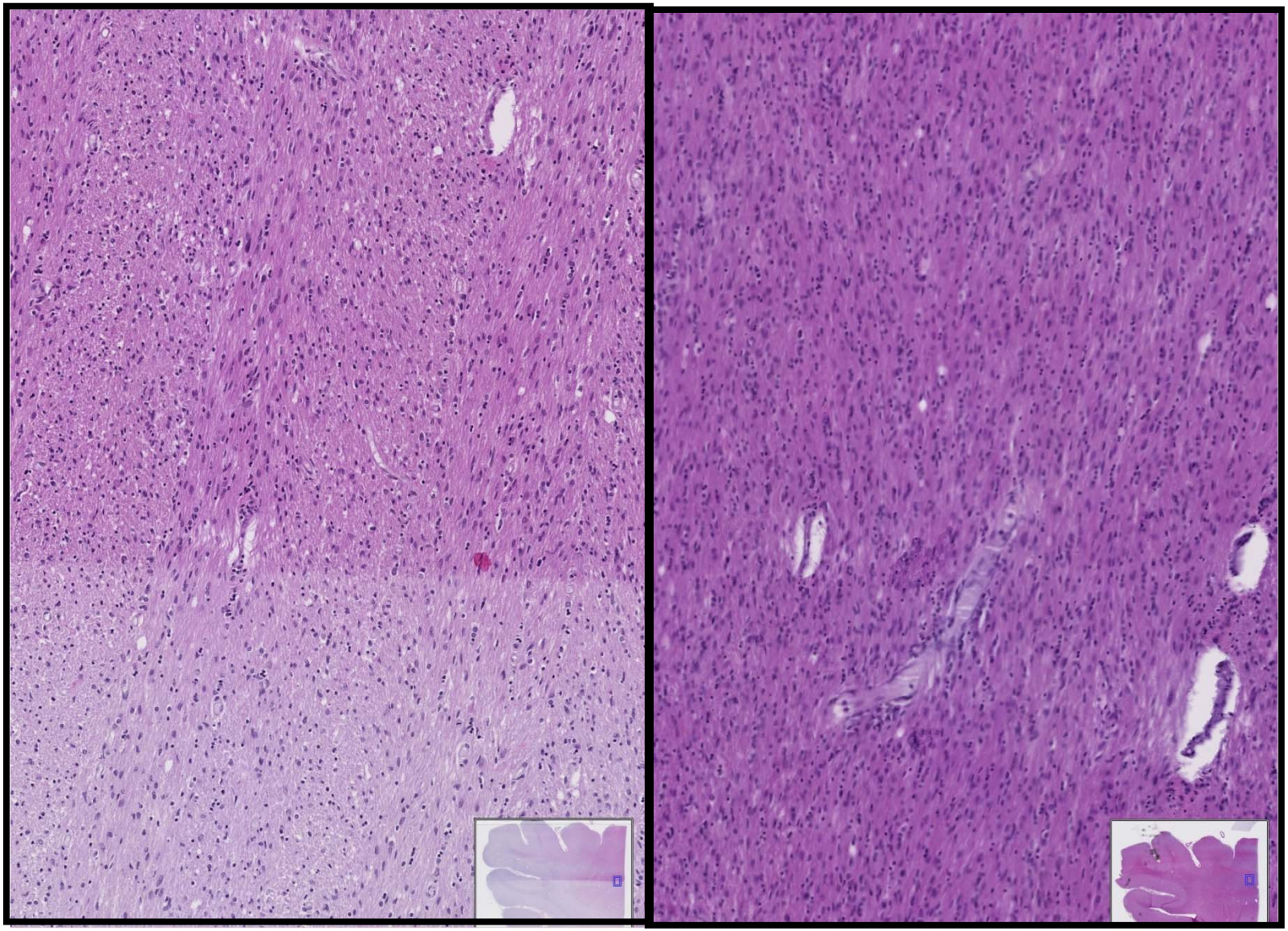
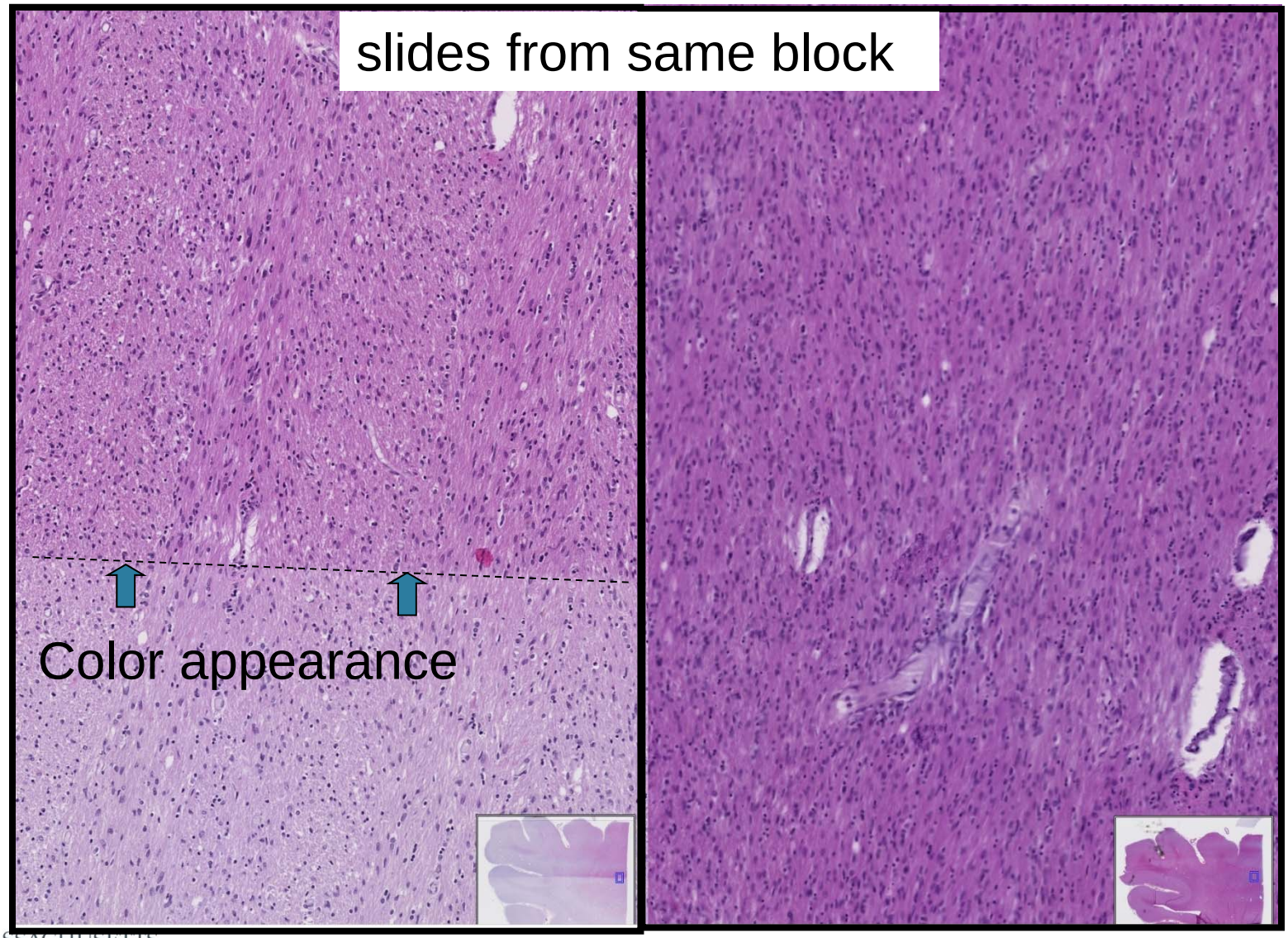
Thickness of Specimen & Staining issues in serial sections of WSI



Thickness of Specimen & Staining issues in serial sections of WSI

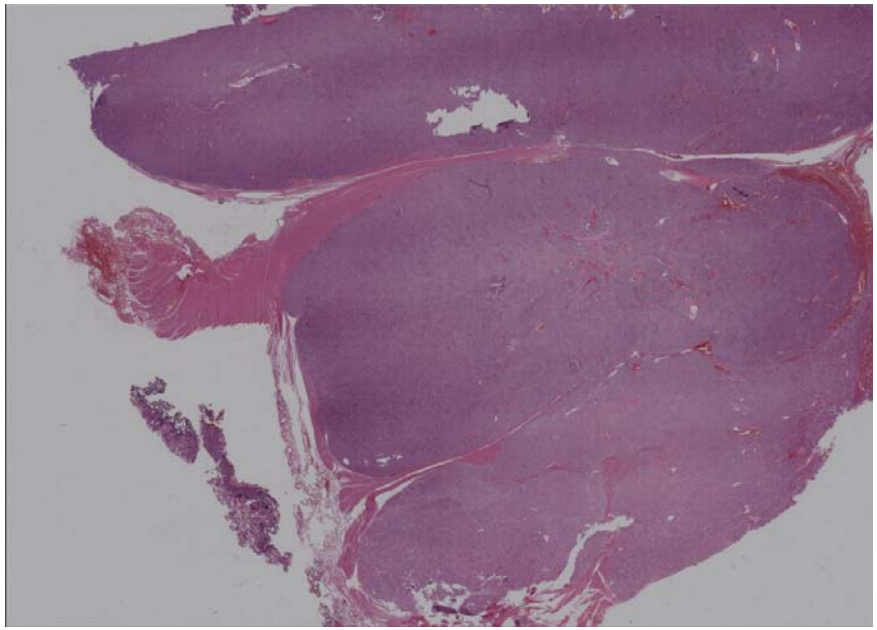


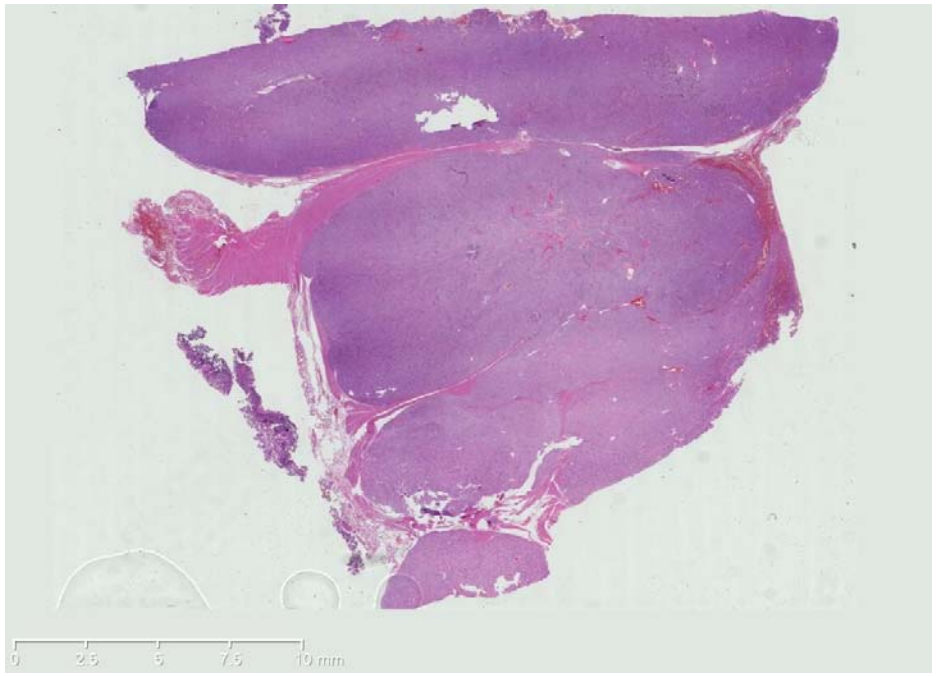
Color Aspects in Digital Pathology

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

Scanner or Scanning Process

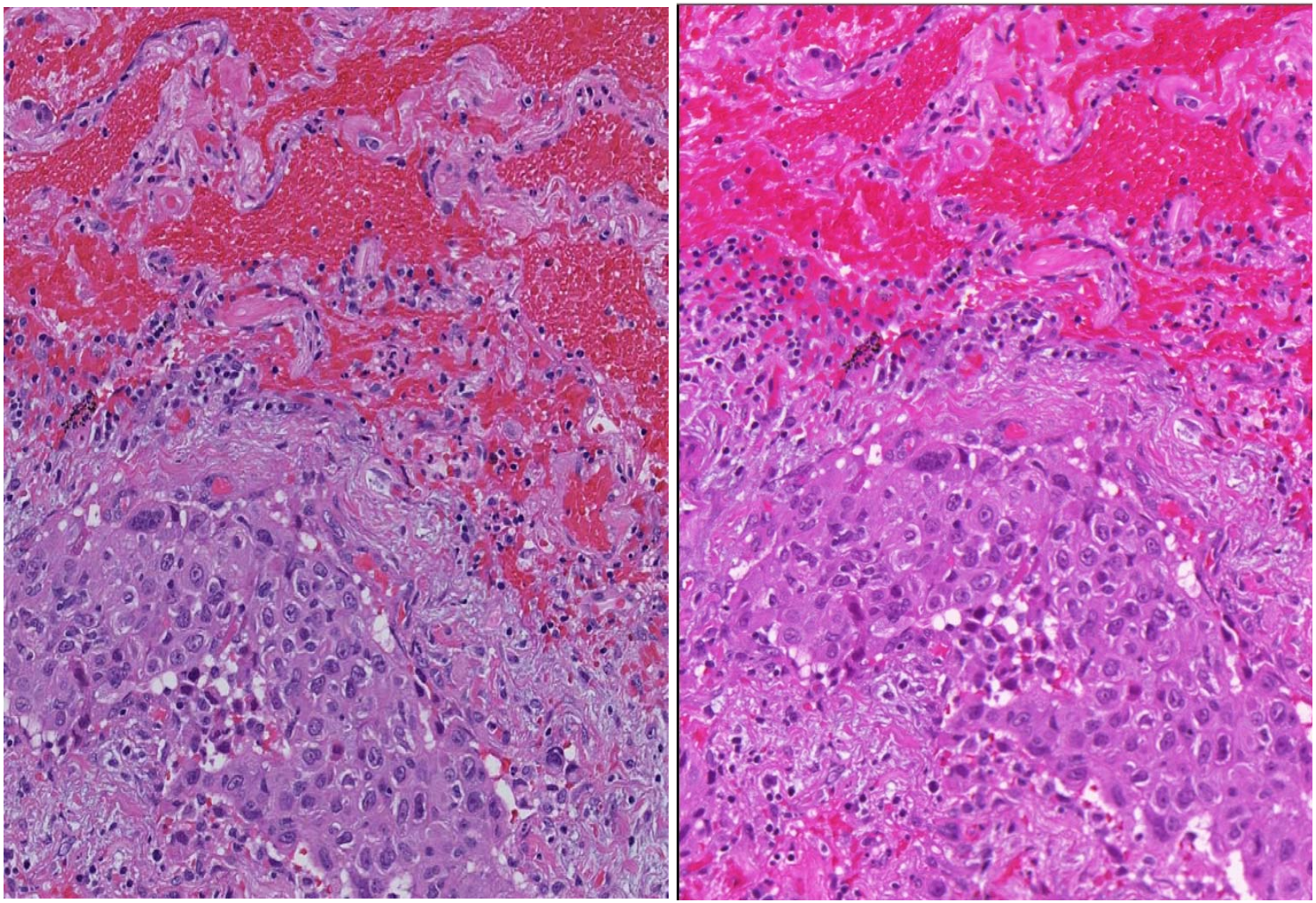
Same slide, different scanners



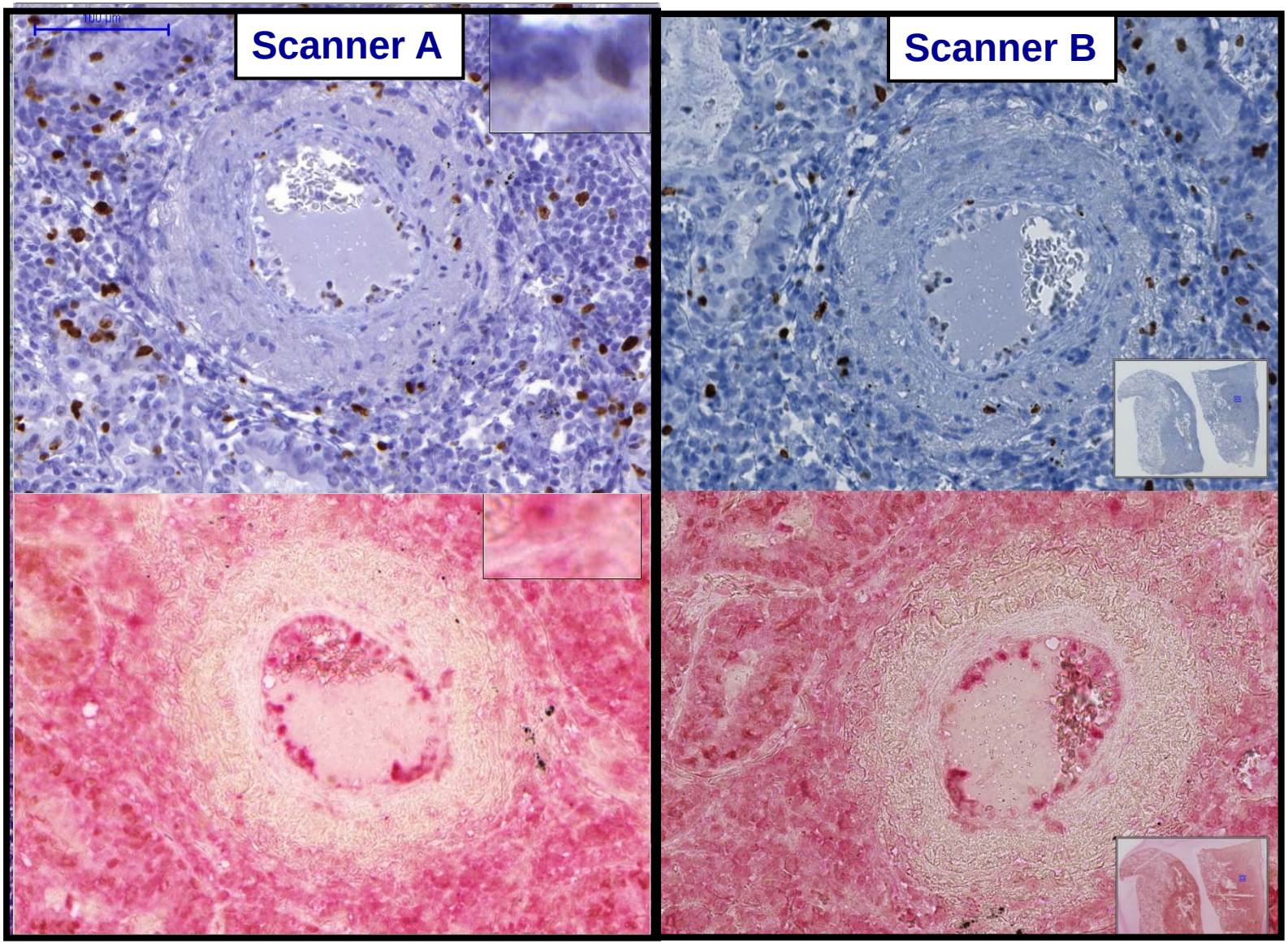


Scanner or Scanning Process

Same slide, different scanners



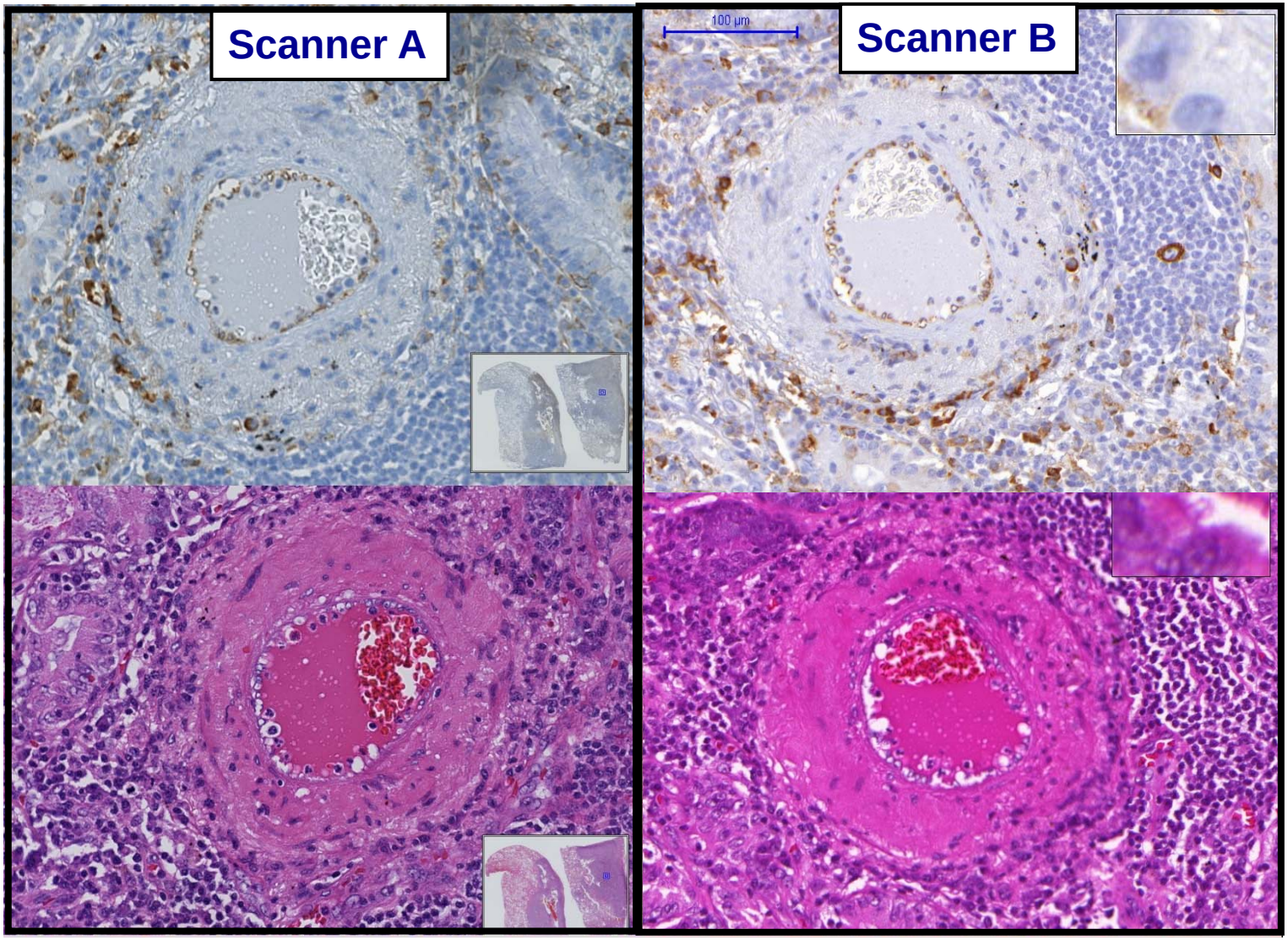
Scanner or Scanning Process



IHC

IHC Double Stains

Scanner or Scanning Process

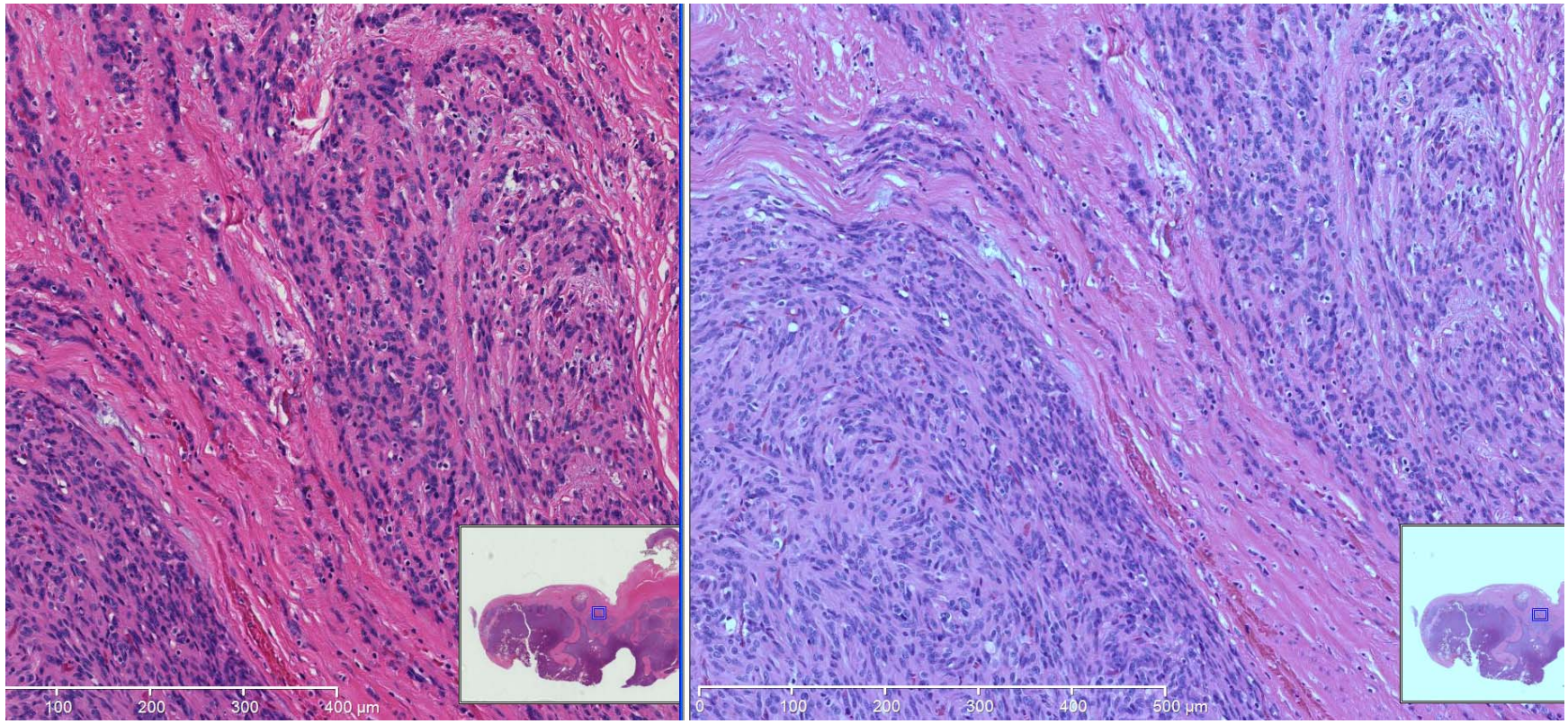


Color Aspects in Digital Pathology

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

Viewer Software

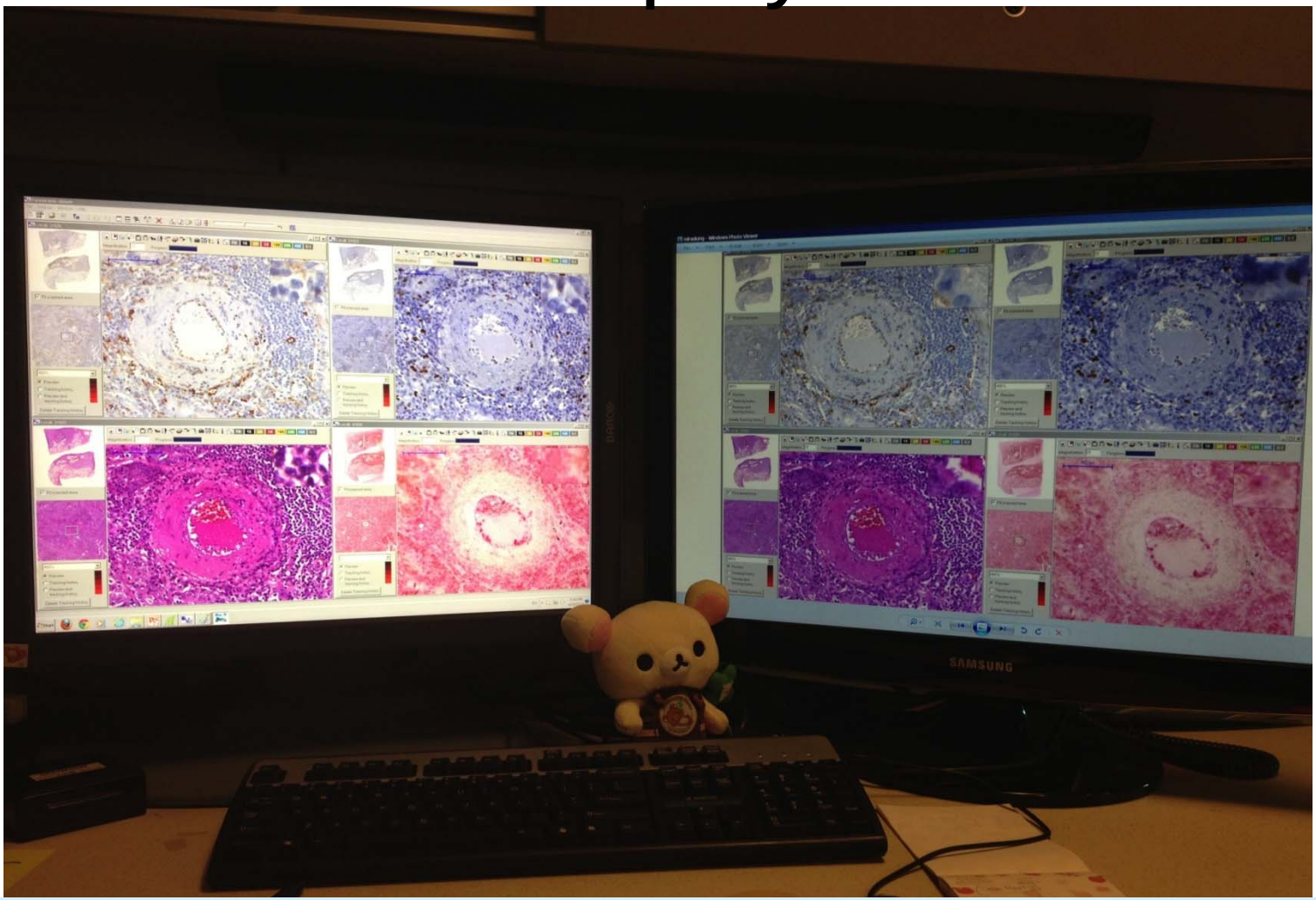
Same scanner, same slide, two different viewers



Color Aspects in Digital Pathology

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

Display



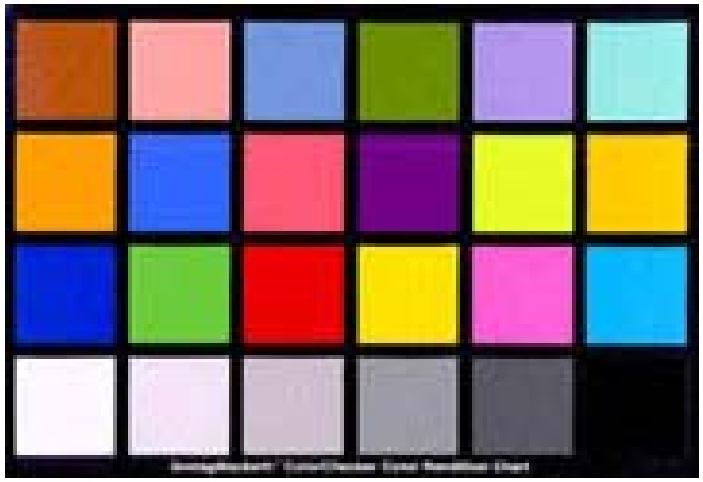
Same images in same PC were viewed by 2 different displays

Display

Same image in same PC was viewed by 3 different displays

Example Experiment: Color of Display

Macbeth Color Chart



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

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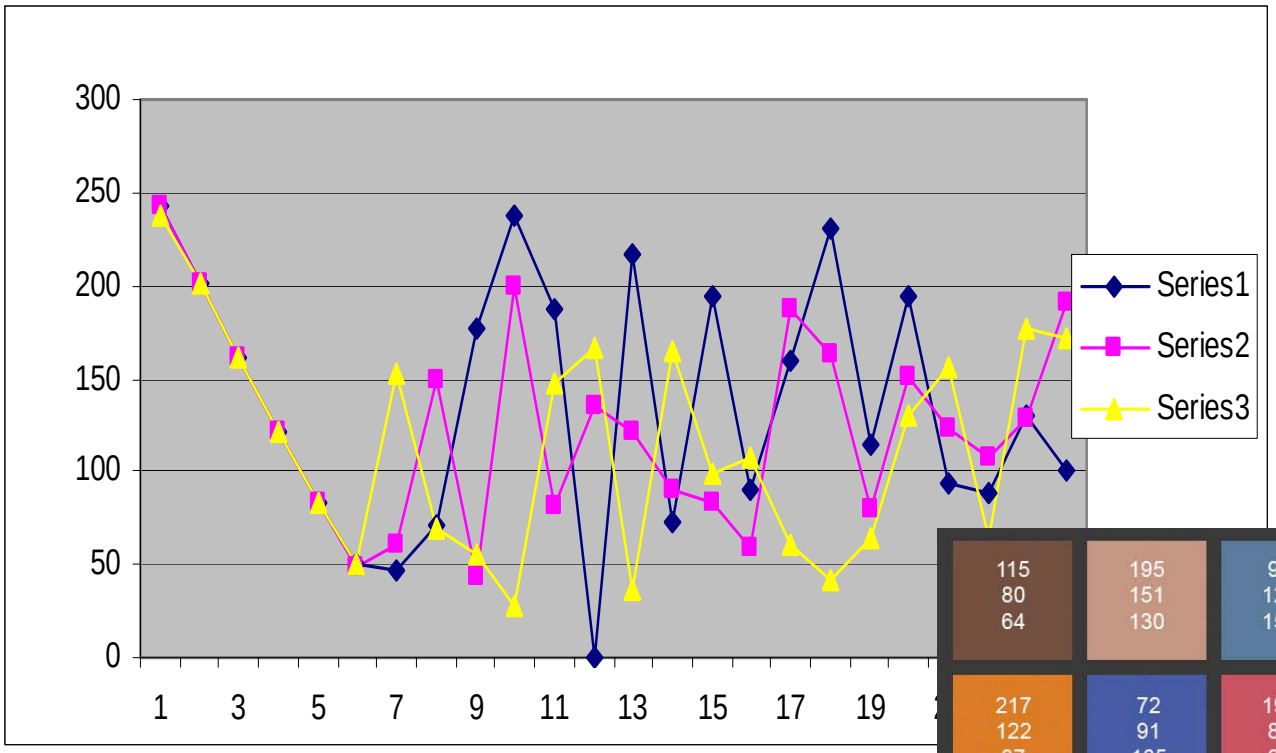
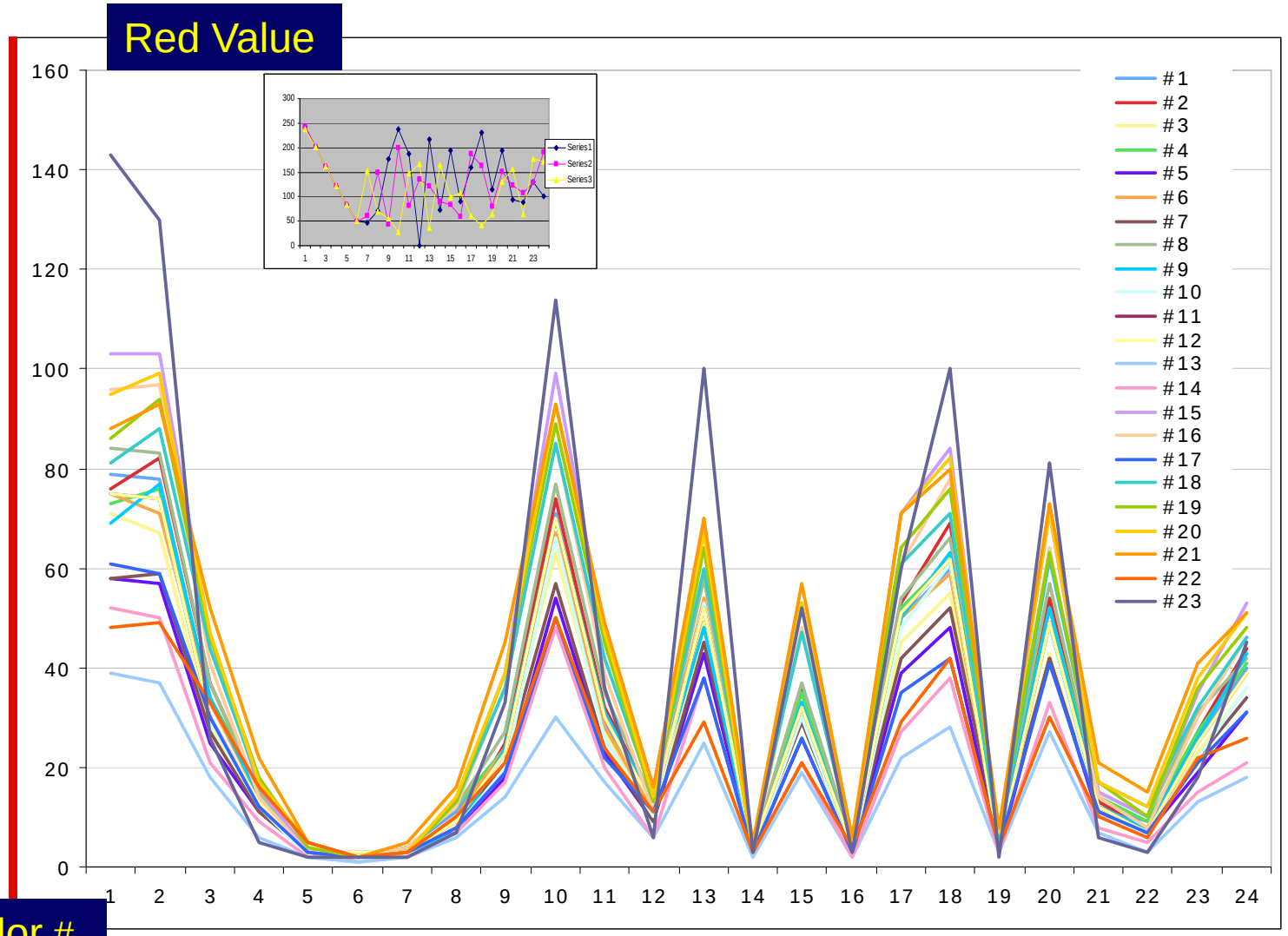
Macbeth Color Chart RGB Value

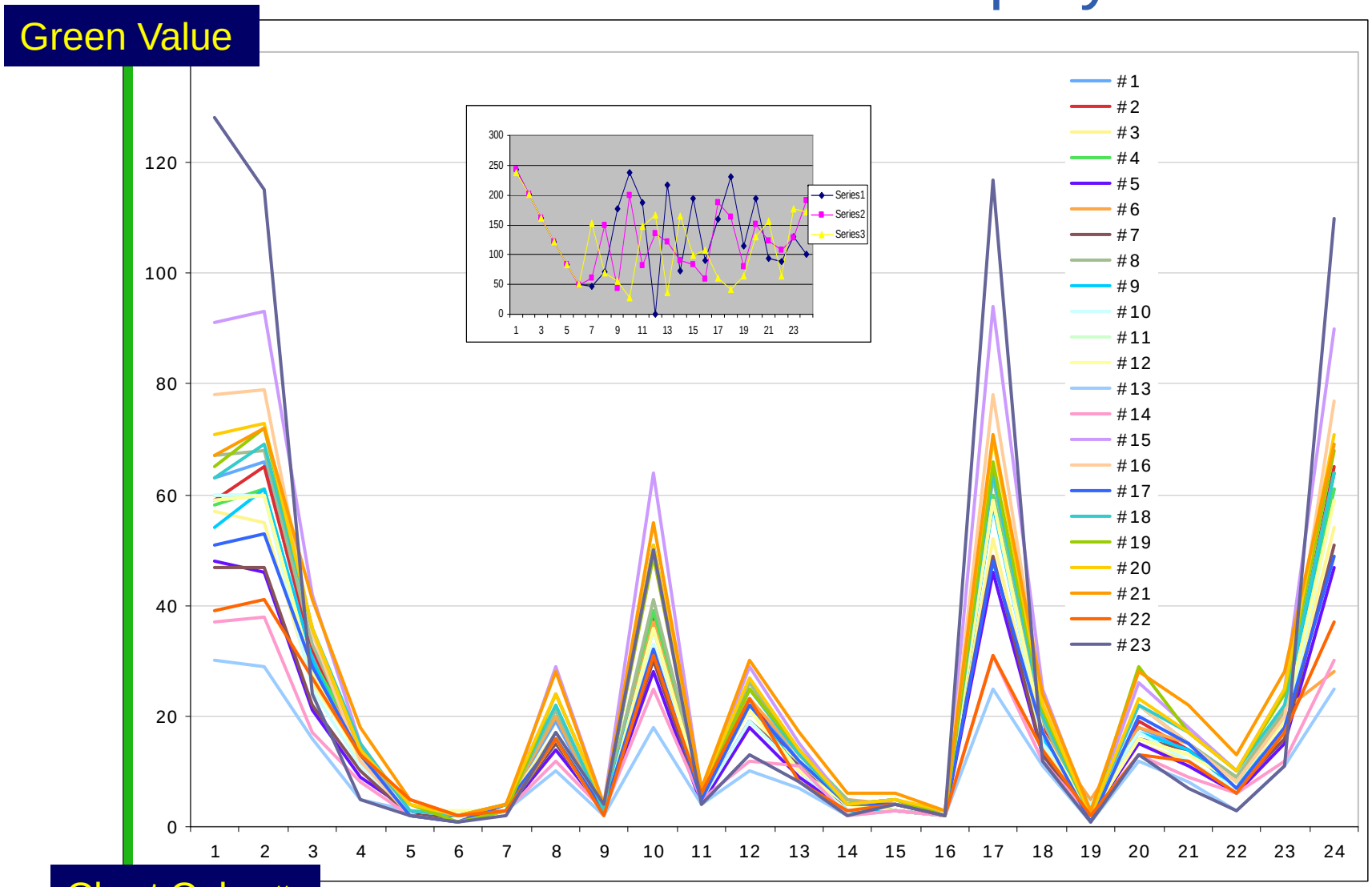


115 80 64	195 151 130	94 123 156	88 108 65	130 129 177	100 190 171
217 122 37	72 91 165	194 84 98	91 59 107	160 188 60	230 163 42
46 60 153	71 150 69	177 44 56	238 200 27	187 82 148	0 135 166
243 242 237	201 201 201	161 161 161	122 122 121	83 83 83	50 49 50

Red Value 23 Displays



Green Value 23 Displays



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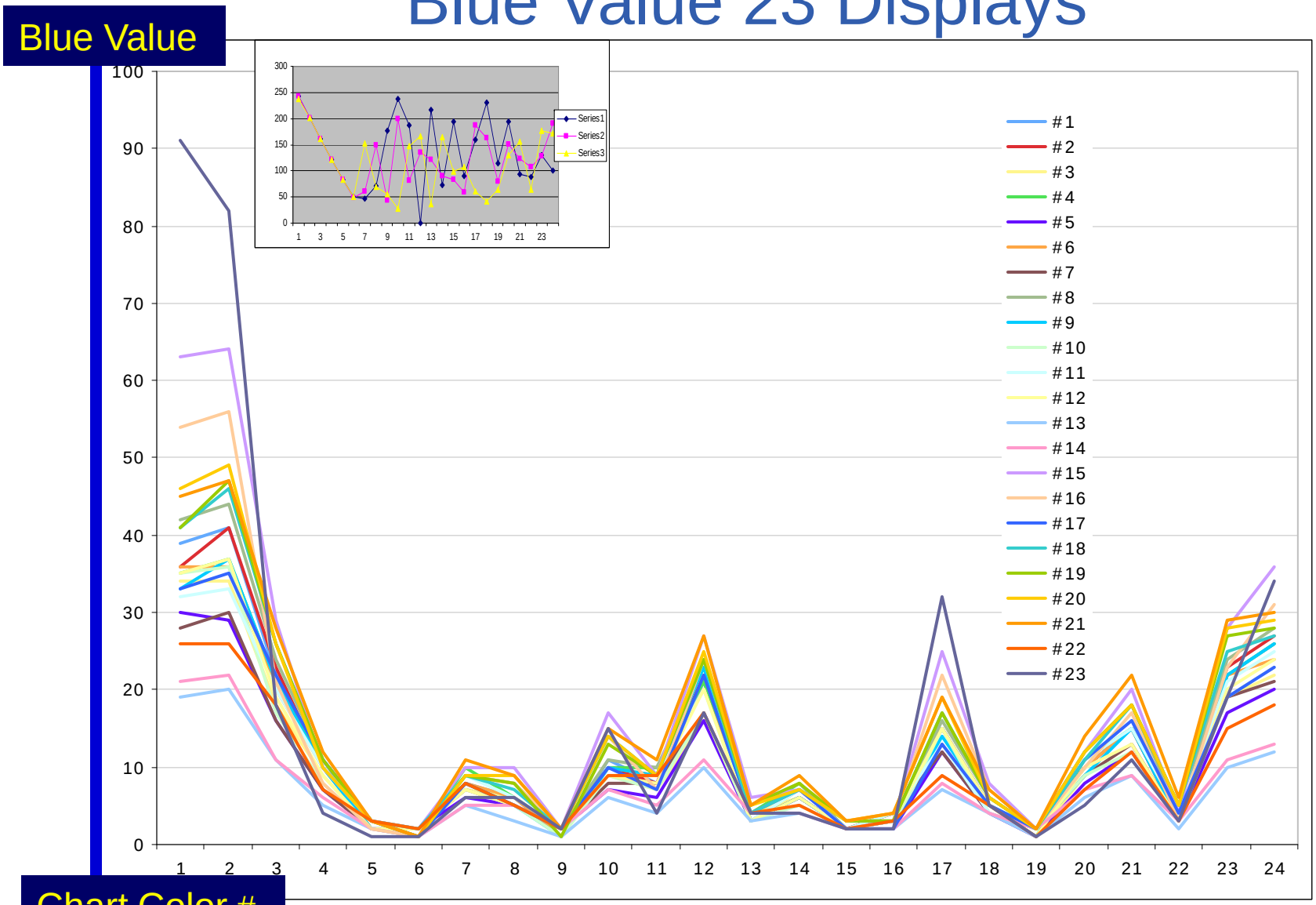
Blue Value 23 Displays



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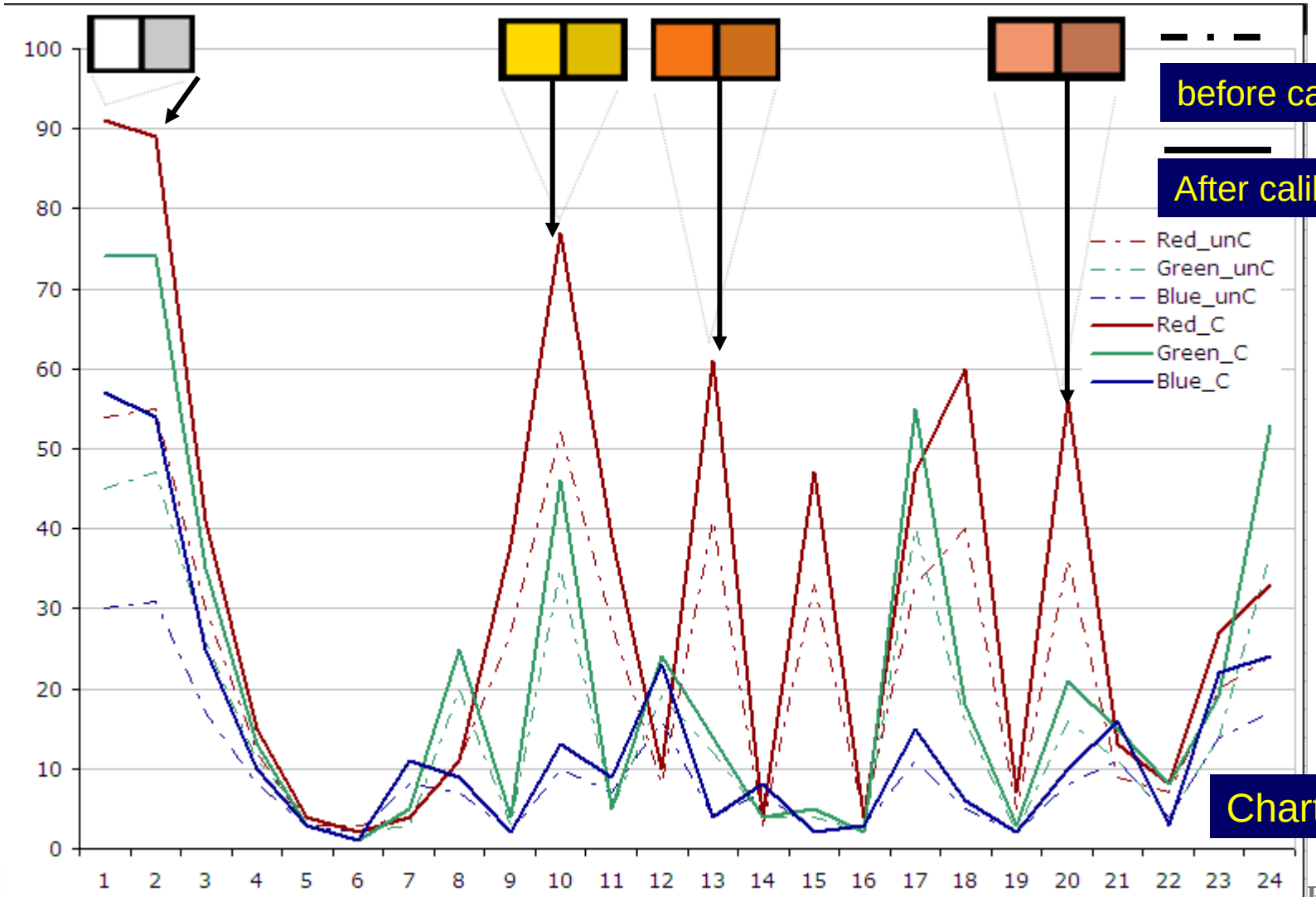
Example of Color differences: before calibration and after calibration



before calibration

After calibration

Chart Color #

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

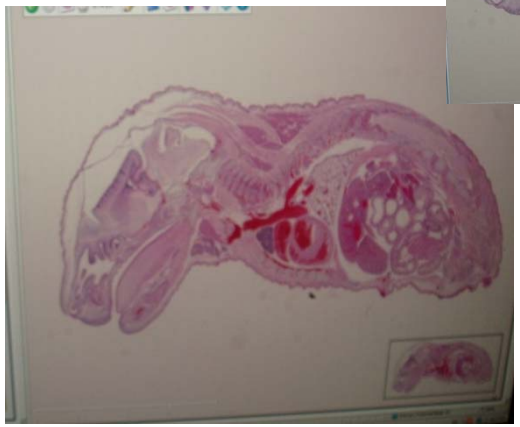
Why is it problem?

Is it problem?

Staining

Display

Scanning



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Is it problem?

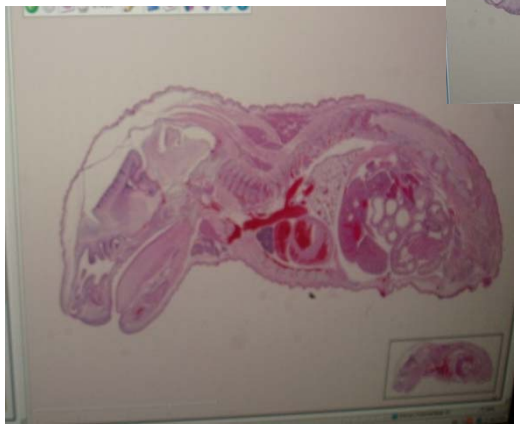
Yes

Staining

Display

Pathologist looks at an actual slide under the microscope

Scanning



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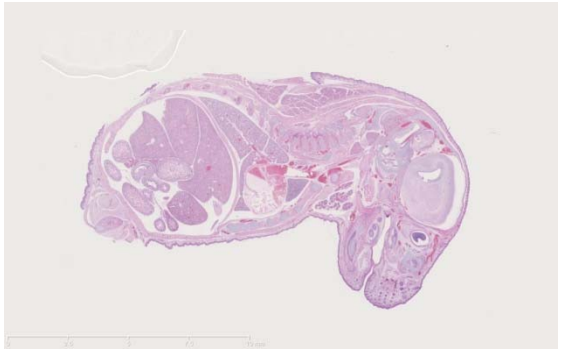
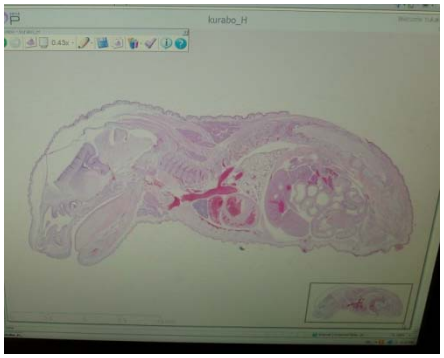
Is it problem?



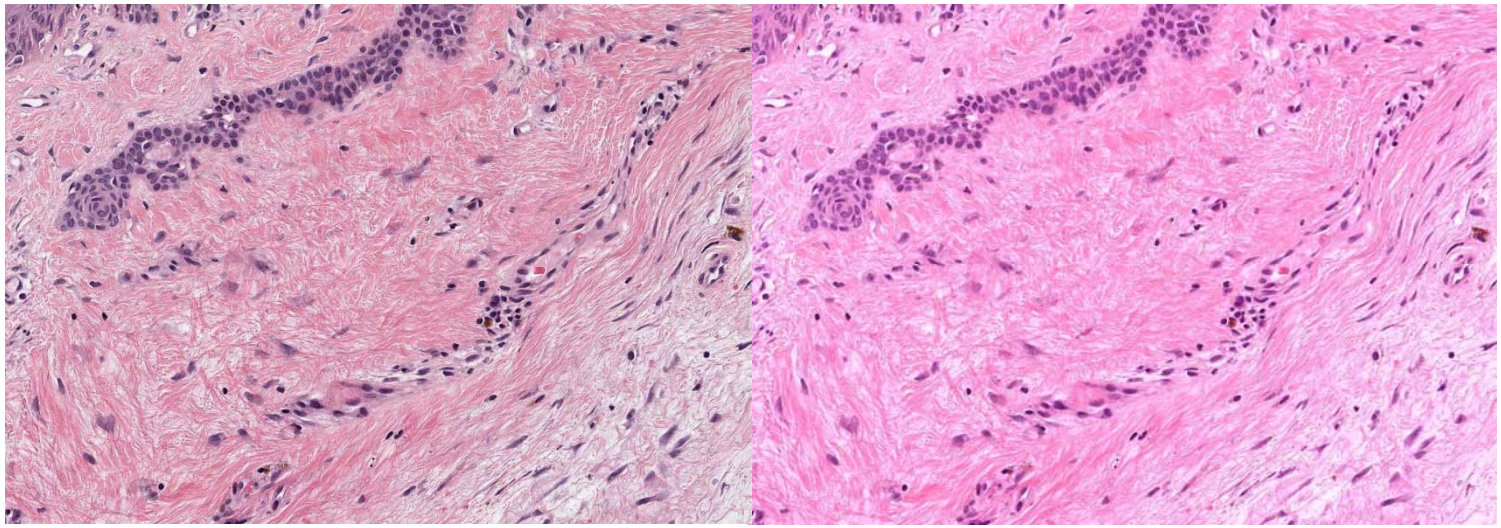


Yes

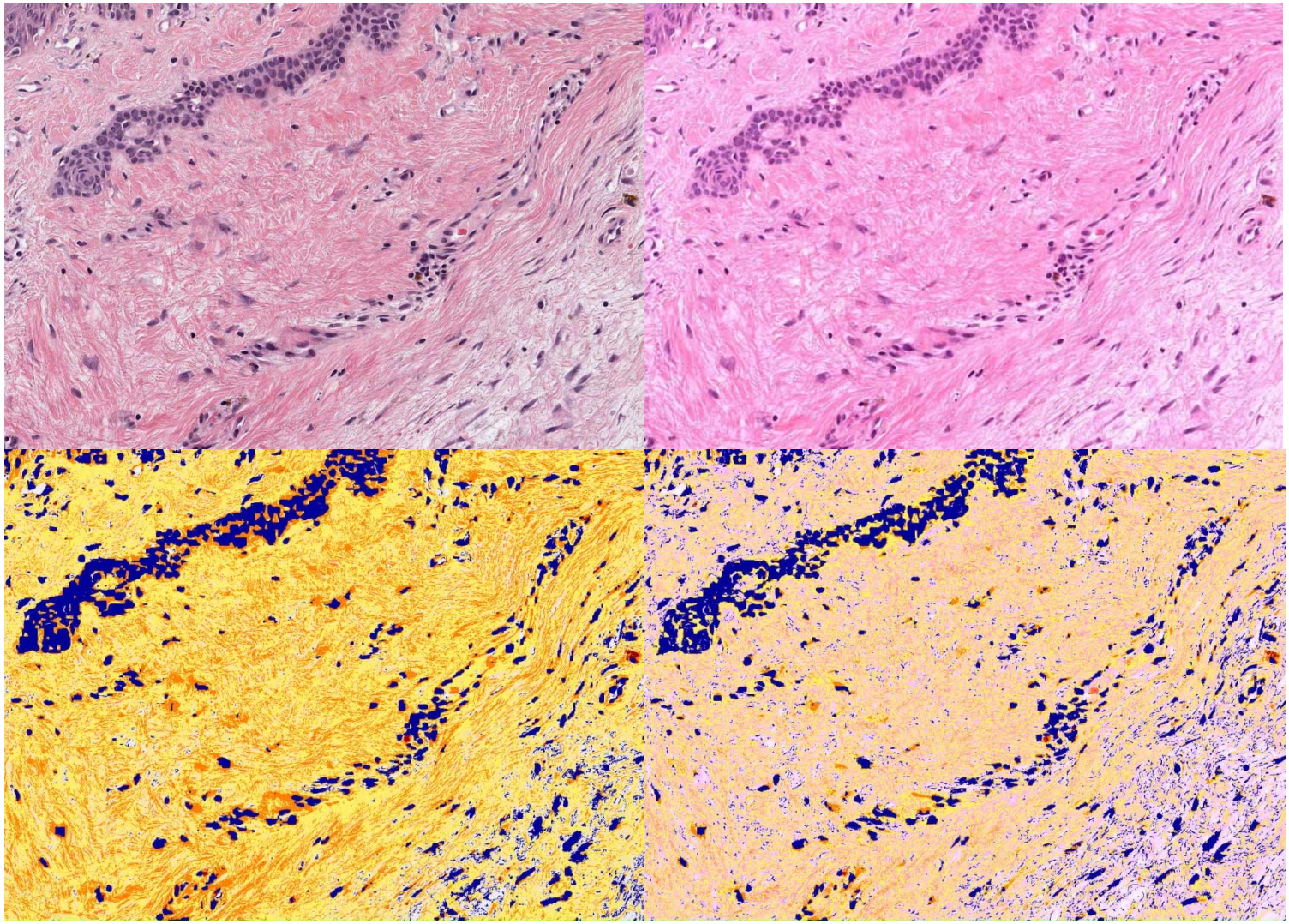
When a pathologist looks at the image on the monitor without a glass slide, it is difficult to know if the color of the image is accurate or not.

It may cause diagnostic error; or pathologists may be uncomfortable to make a diagnosis.

Is it problem?



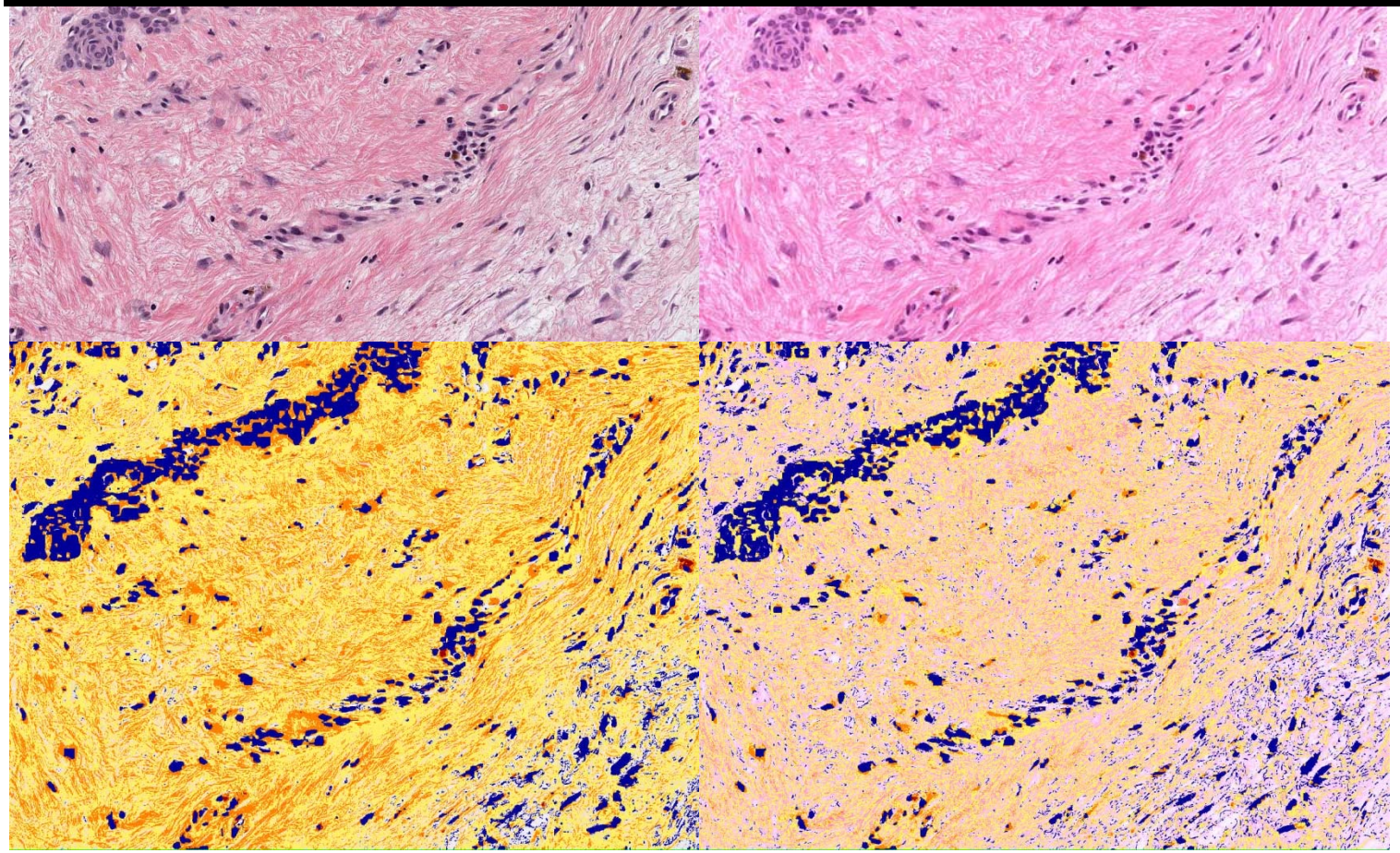
Is it problem? Yes



8.8% 7.9%

Is it problem? Yes

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



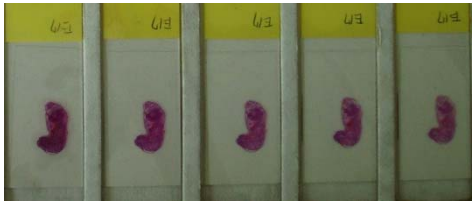
8.8% 7.9%

Color Standardization in WSI

- To prevent diagnostic errors
- To use WSI for Computer Aided Diagnostic System

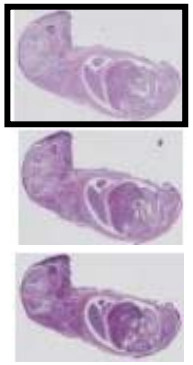
Color Standardization in WSI

From Staining to Display



Staining

Scanning

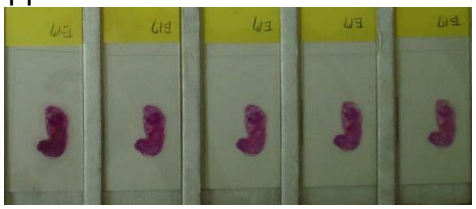


Display

Color Standardization in WSI: From Staining to Display

Staining

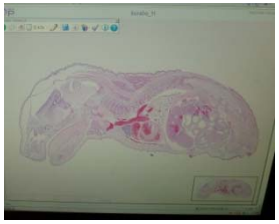
Multispectral Imaging application



Today's topics

Scanning

Display



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How can we identify the cause of the difference in color and standardize?

To identify the causes of issues in WSI

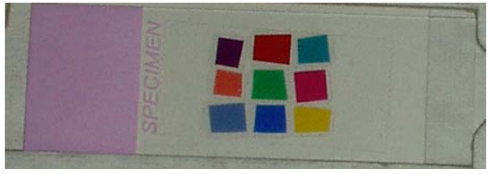
We have developed a slide set at MGH

Calibration Slides for Scanner

Image Quality & color

Color

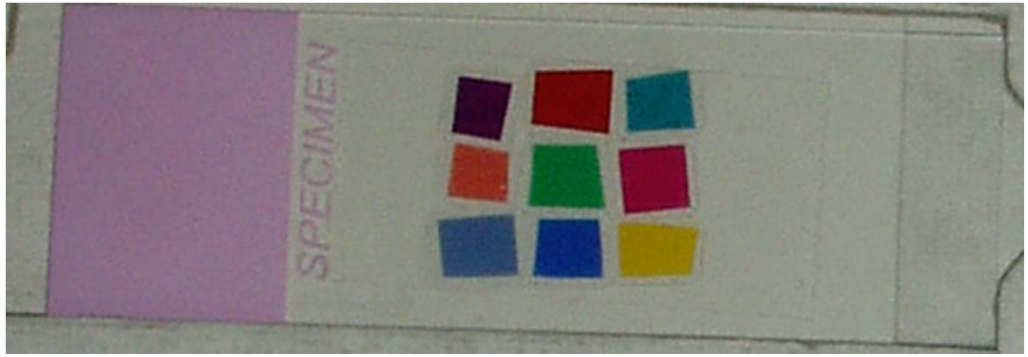
Calibration Slides for Pathologist (Display)

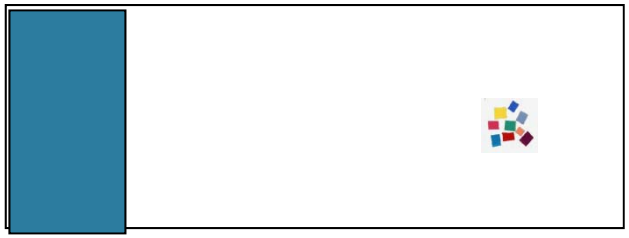


Color

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

Image Quality Slide

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

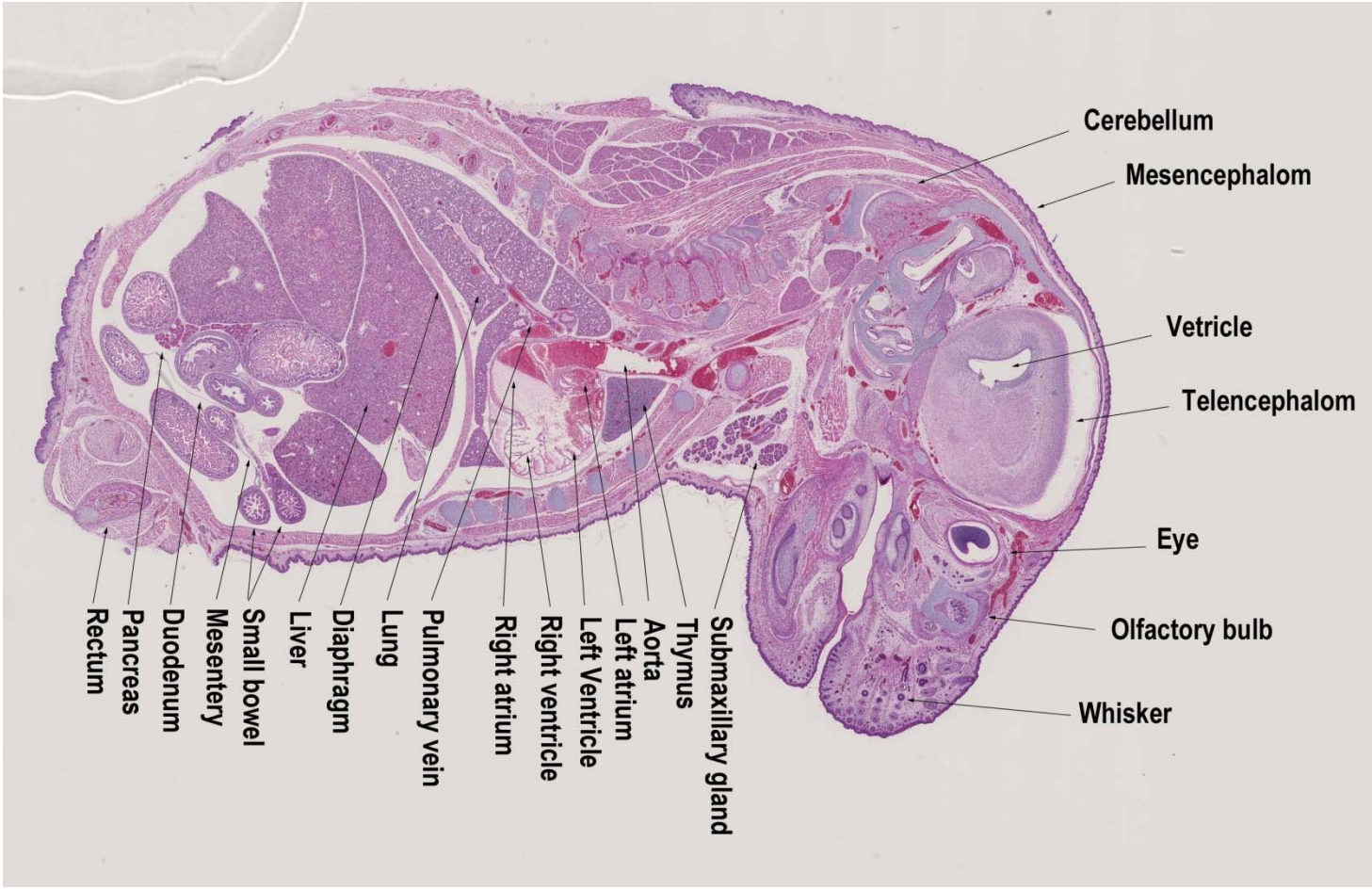
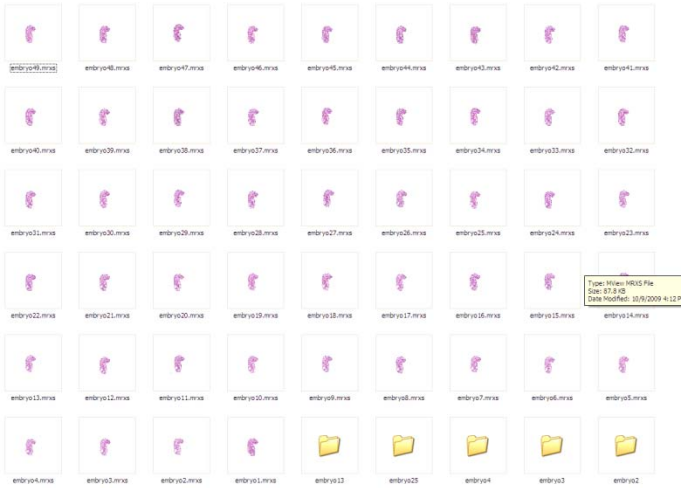


Image Quality Slide

- 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.



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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

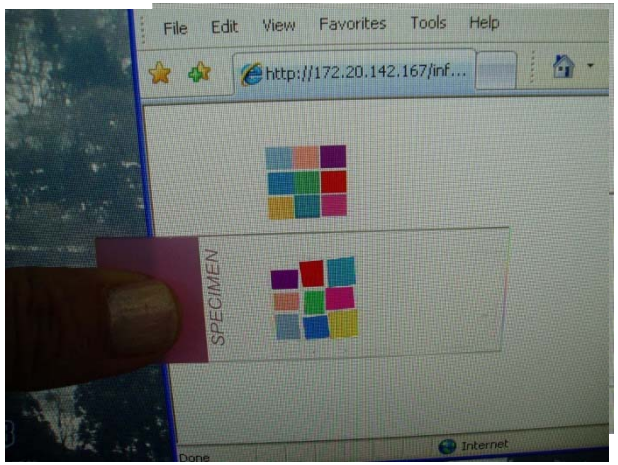
Scanner

Scanning

VS

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.

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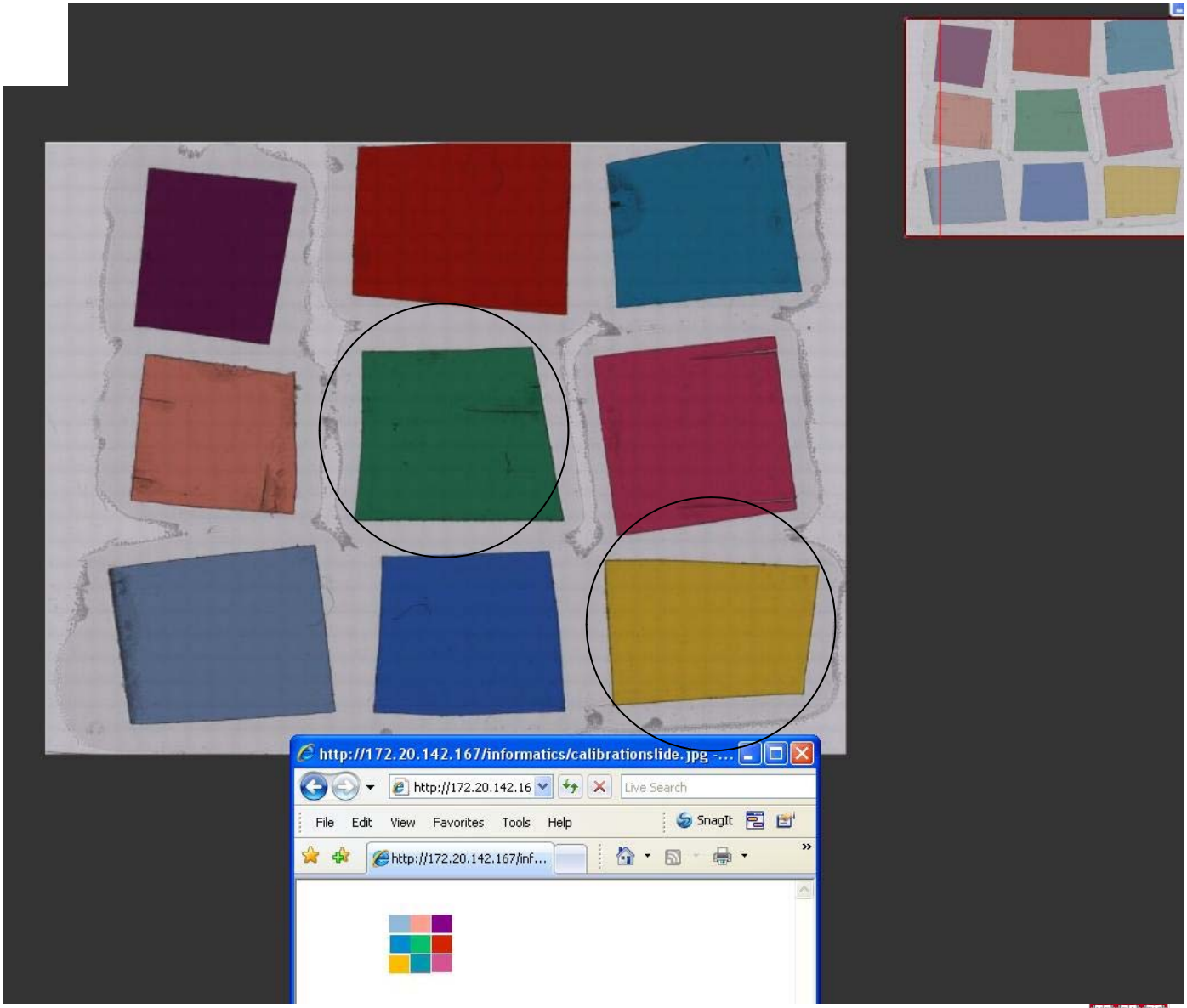
Results: Scanner 1

Almost all colors are wrong

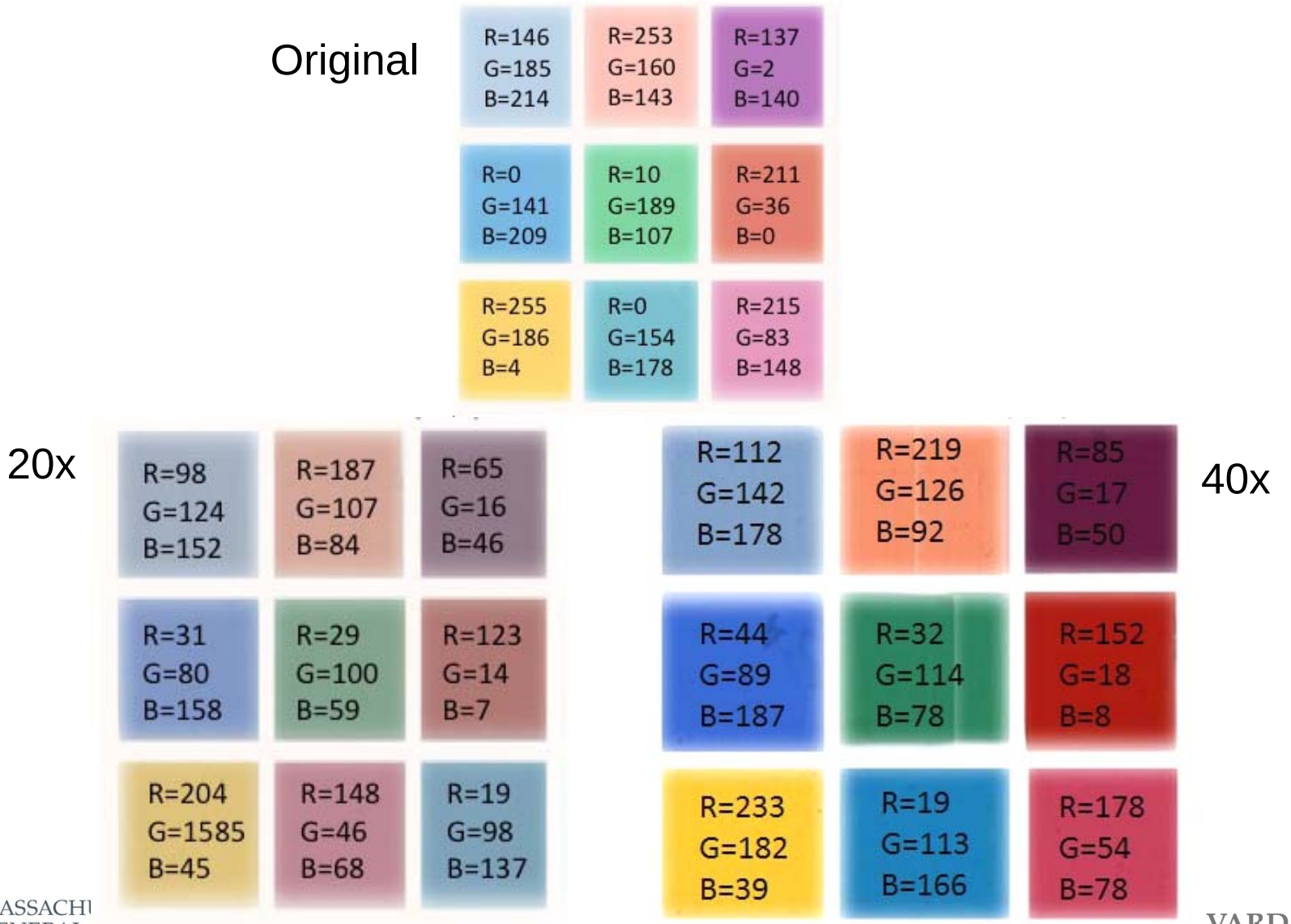
Results: Scanner 2

Better than Scanner 1. Especially Pink and Blue are wrong

Results: Scanner 3



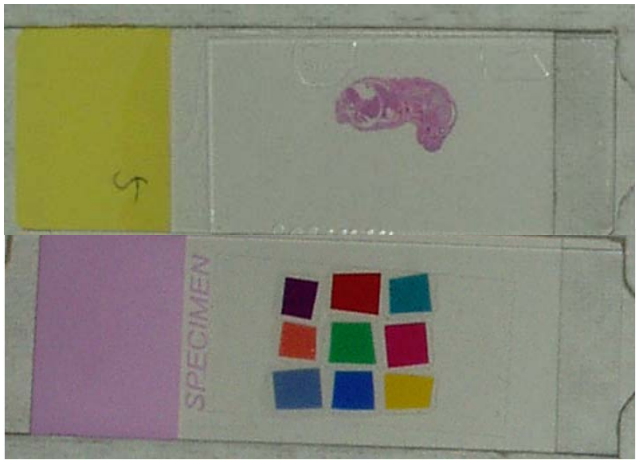
Results 20x vs 40x of the scanner 1



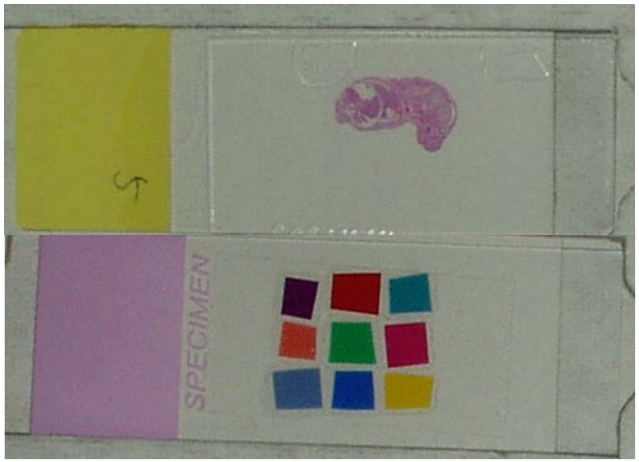
Results

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



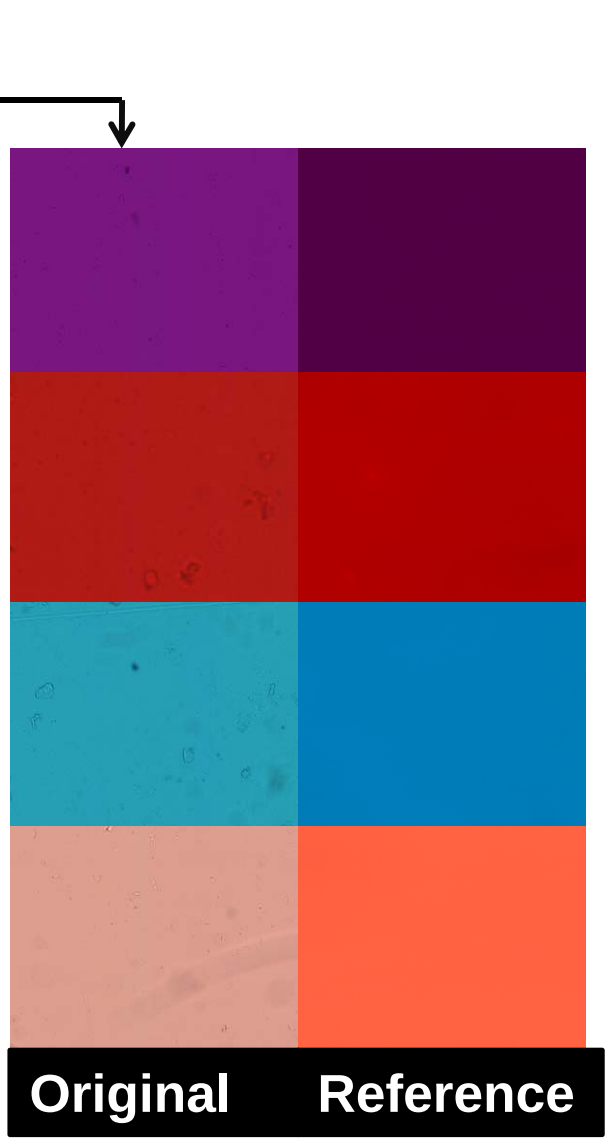
Color patches

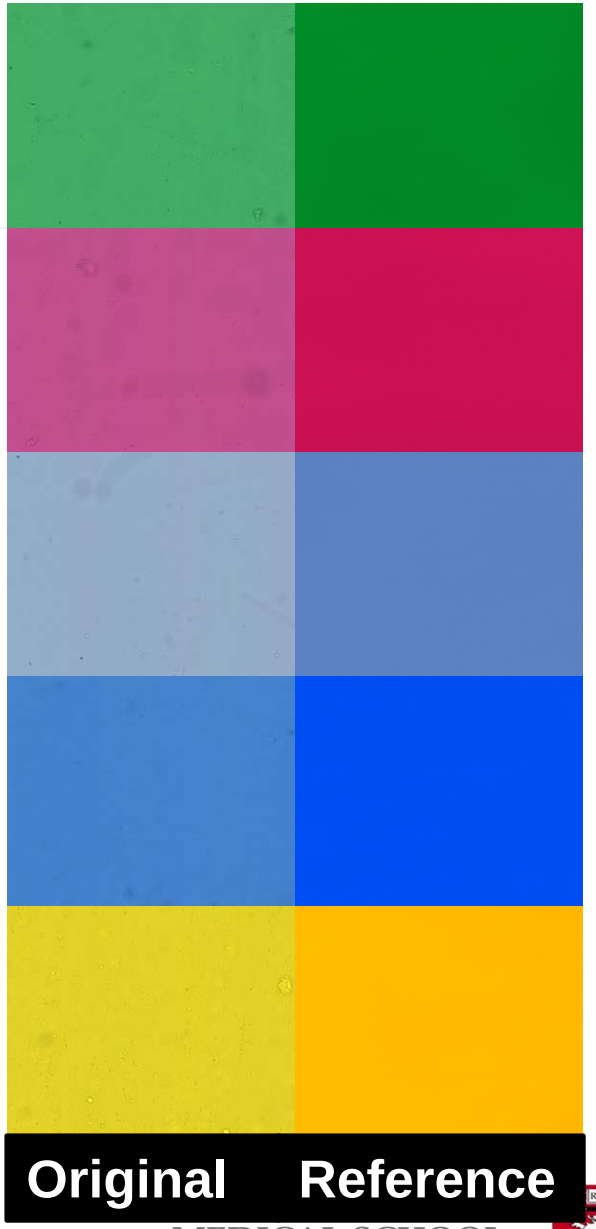
- **Colors are not accurate enough**

- Standardize using the original and reference color patches

Original - Produced by a whole slide scanner

Reference - Produced by using spectral information of the patches





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Polynomial transformation

$$\begin{pmatrix} R \\ G \\ B \end{pmatrix} = \begin{pmatrix} a_{1,R} \ldots . a_{m,R} \\ a_{1,G} \ldots . a_{m,G} \\ a_{1,B} \ldots . a_{m,R} \end{pmatrix} \left[\theta_m \begin{pmatrix} R \\ G \\ B \end{pmatrix} \right]$$

Color of the patches as produced by a particular scanner

Reference color of the color patches

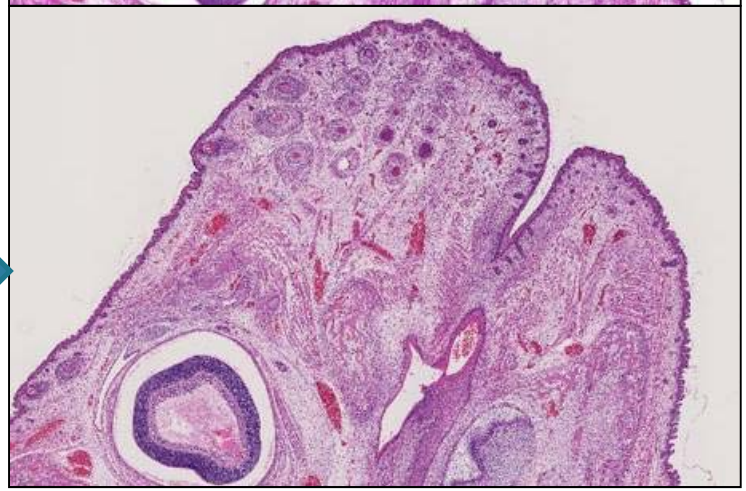
Color transformation matrix will be stored for used in color standardization

Each scanner will have its own Color transformation matrix

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

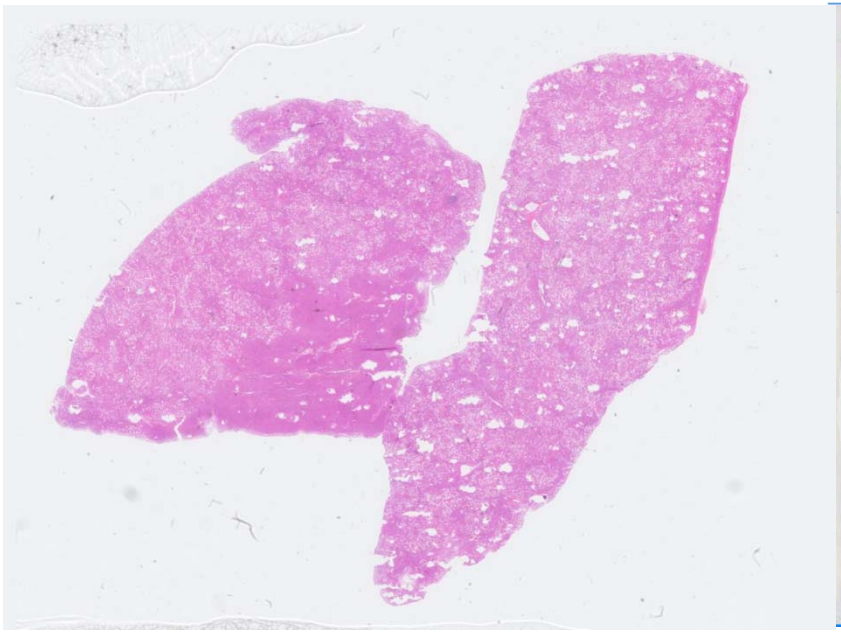
Scanner A

Scanner B

There is color variation….

Thumbnail images of the standardized whole slide images

Scanner A

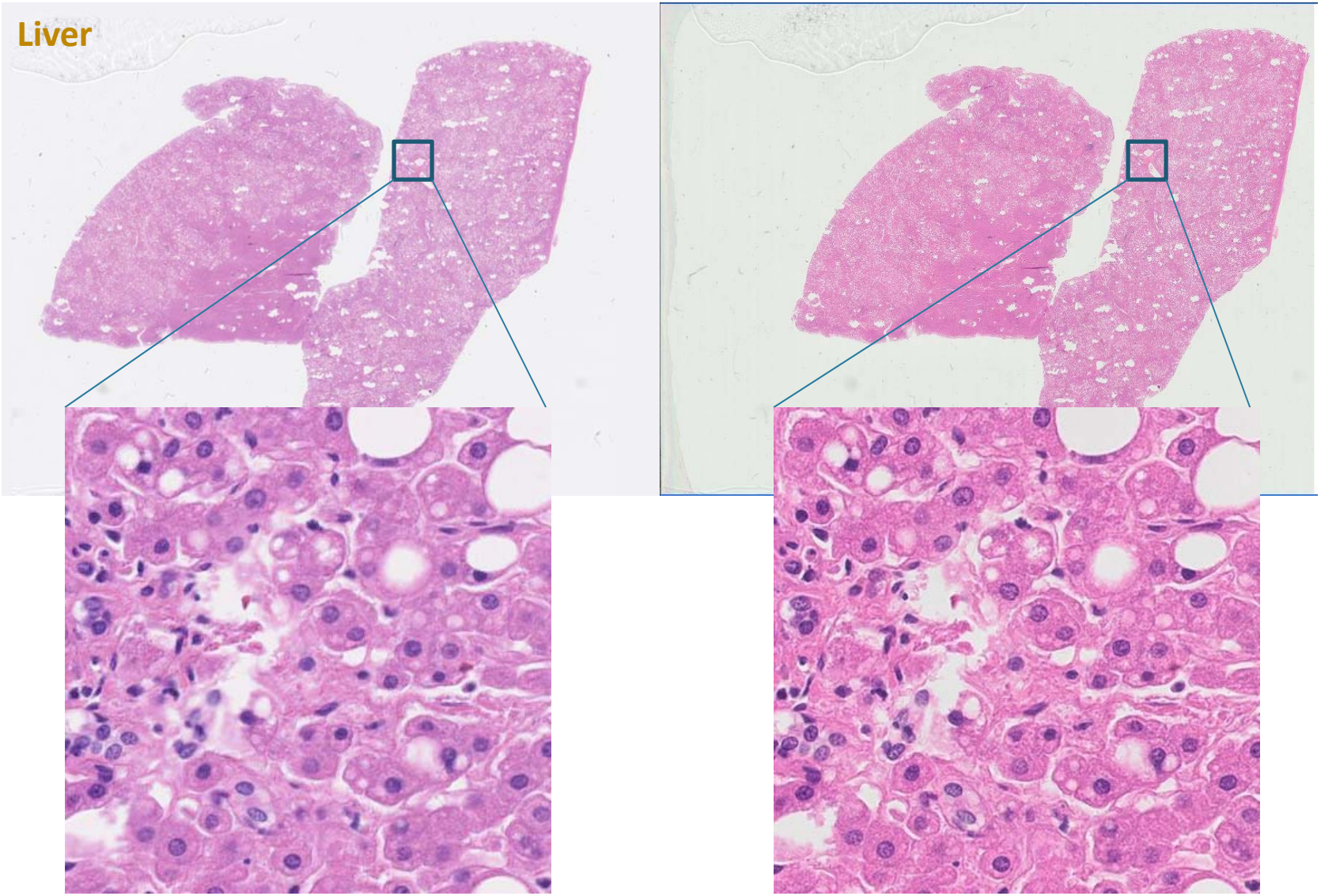


Scanner B

Application of color correction minimizes the color differences…..

Scanner A

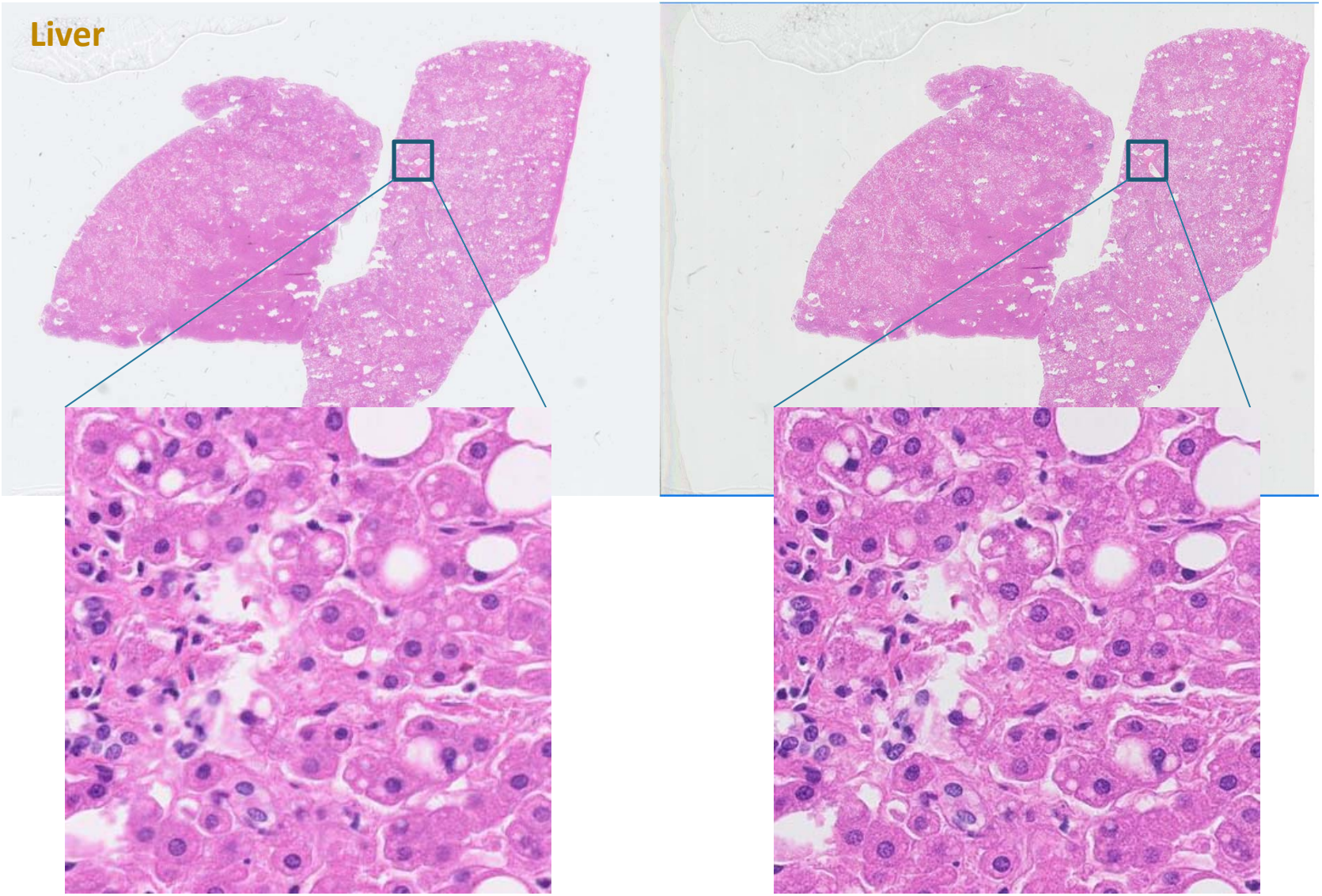
Scanner B



Without color correction...

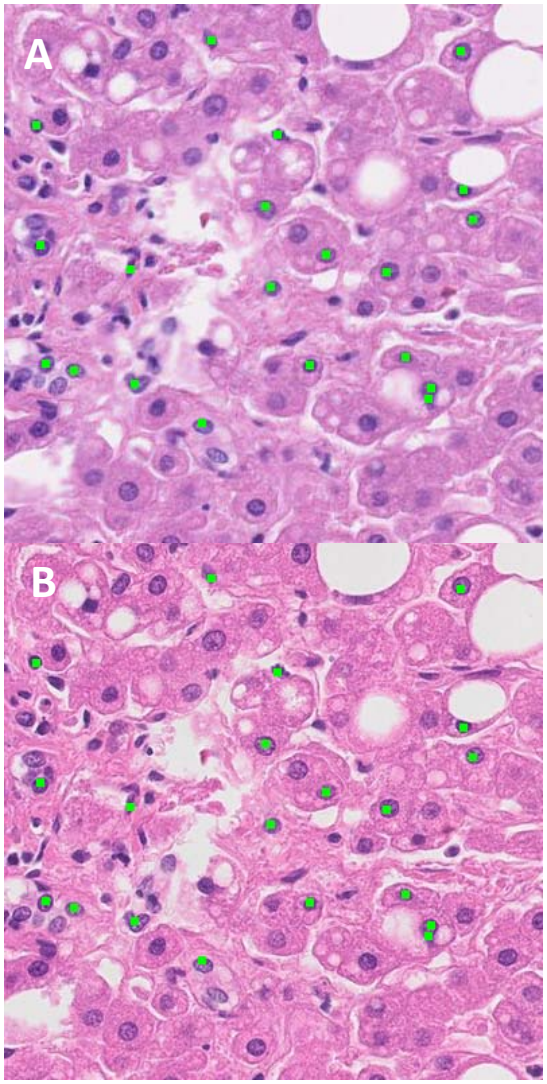
Scanner A

Scanner B

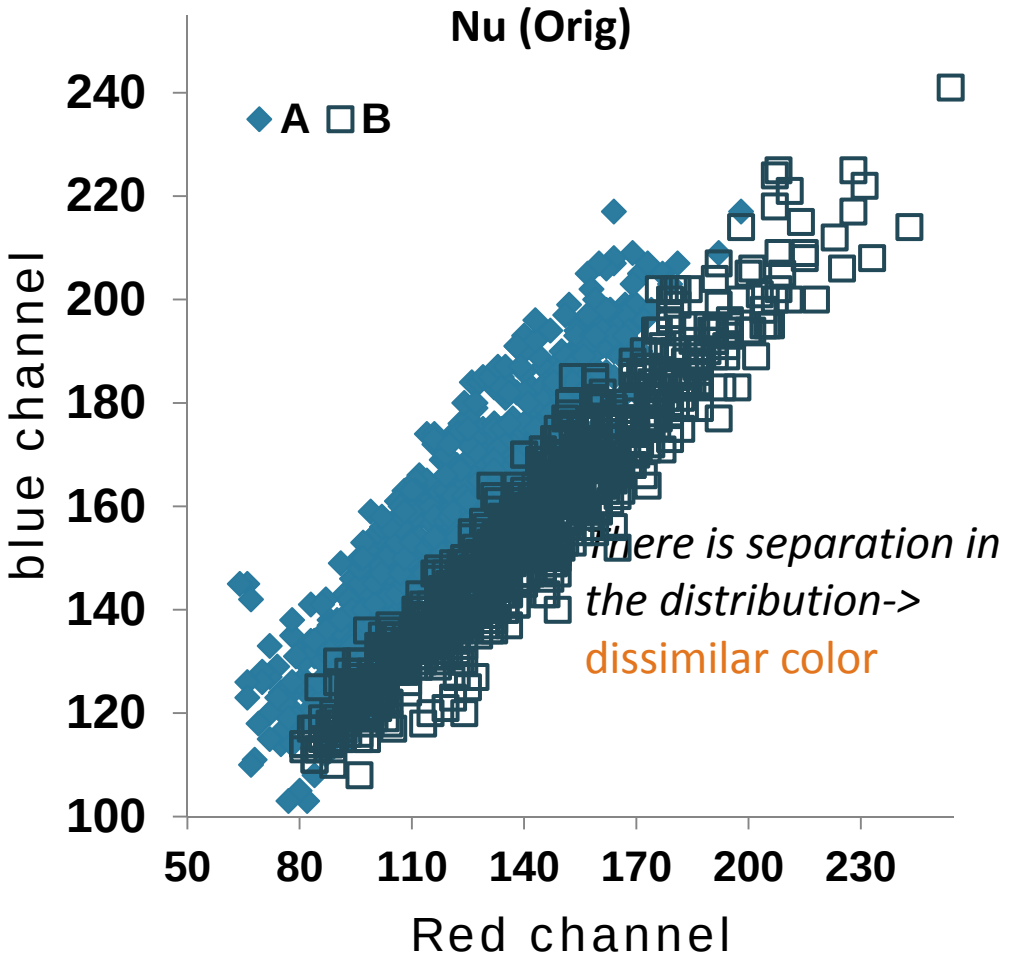


Result of color correction...

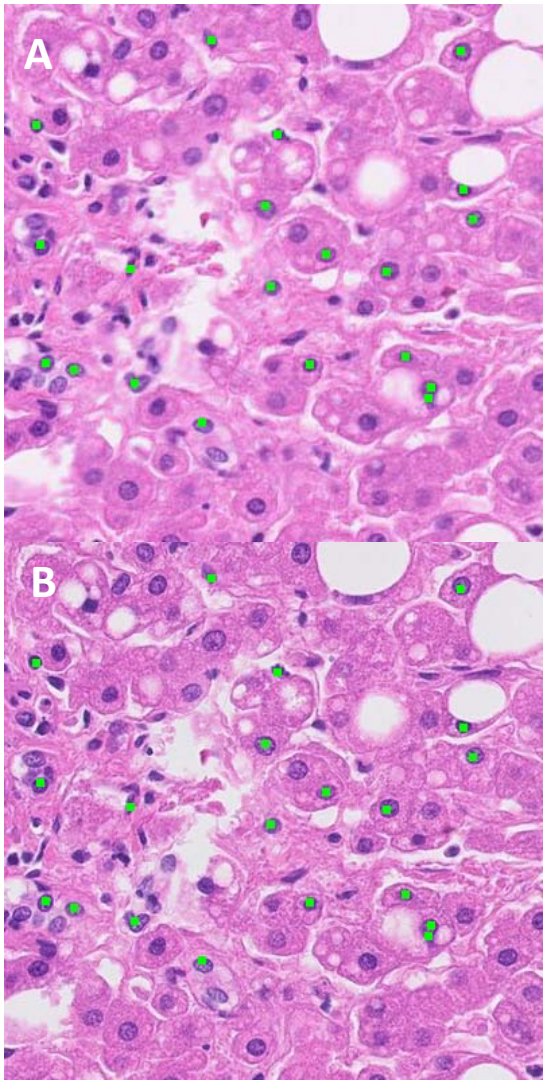
Original



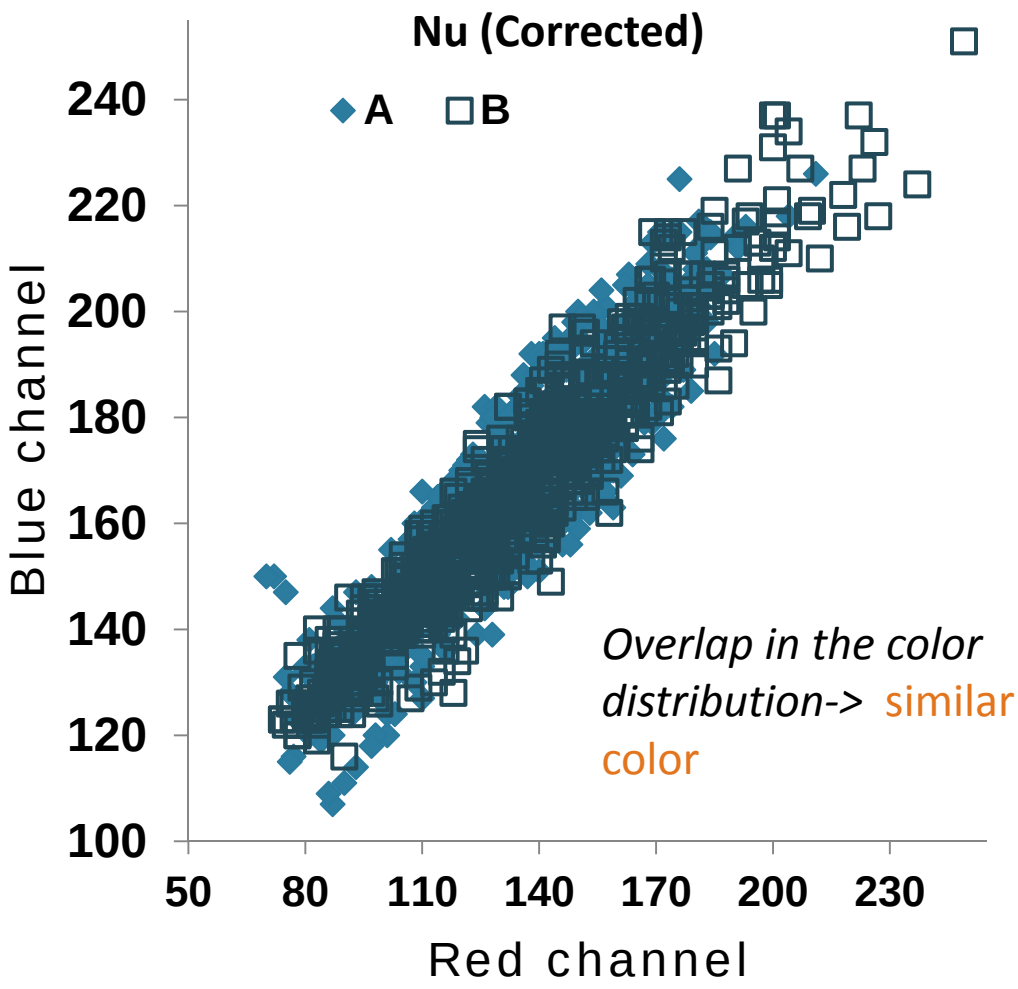
RGB color distribution



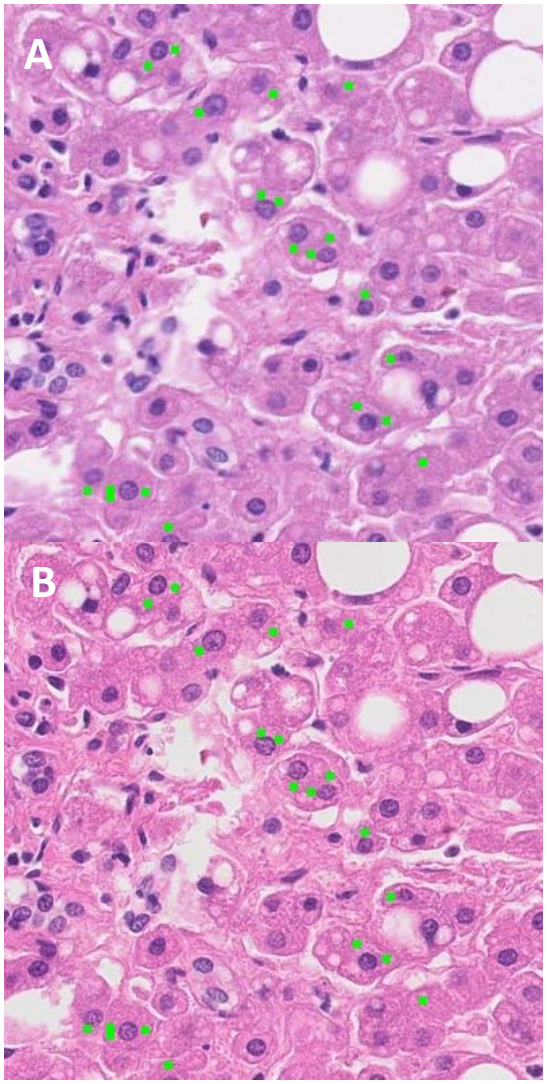
Corrected



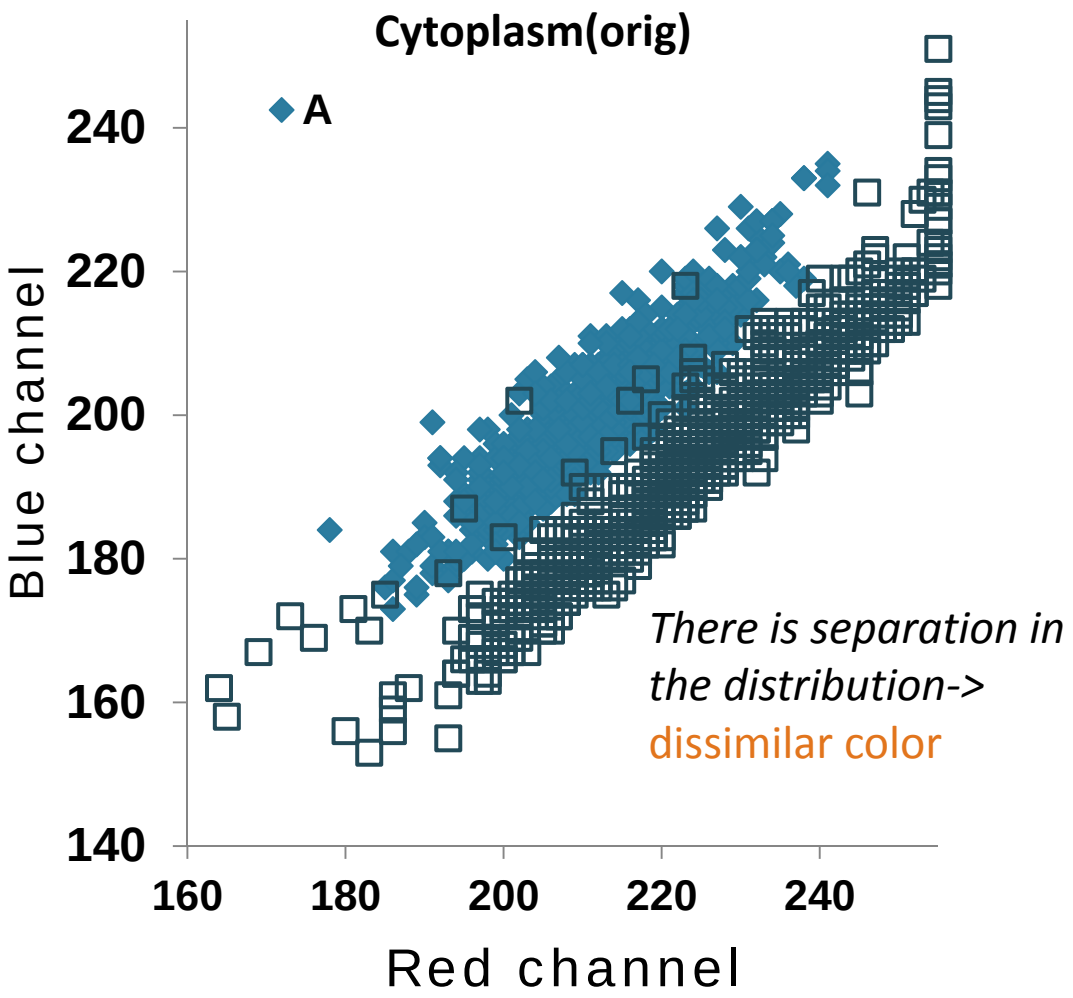
RGB color distribution



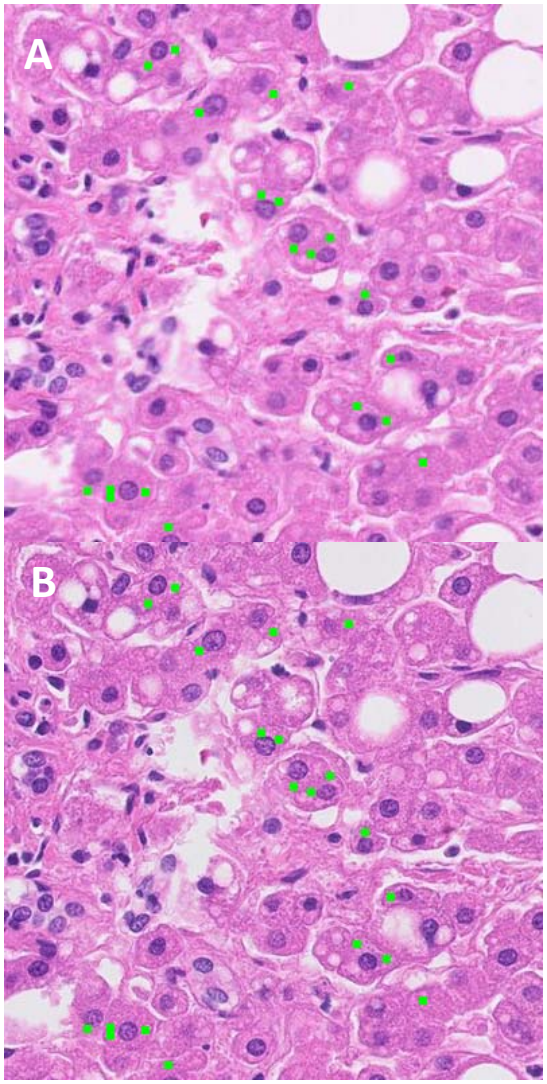
Original



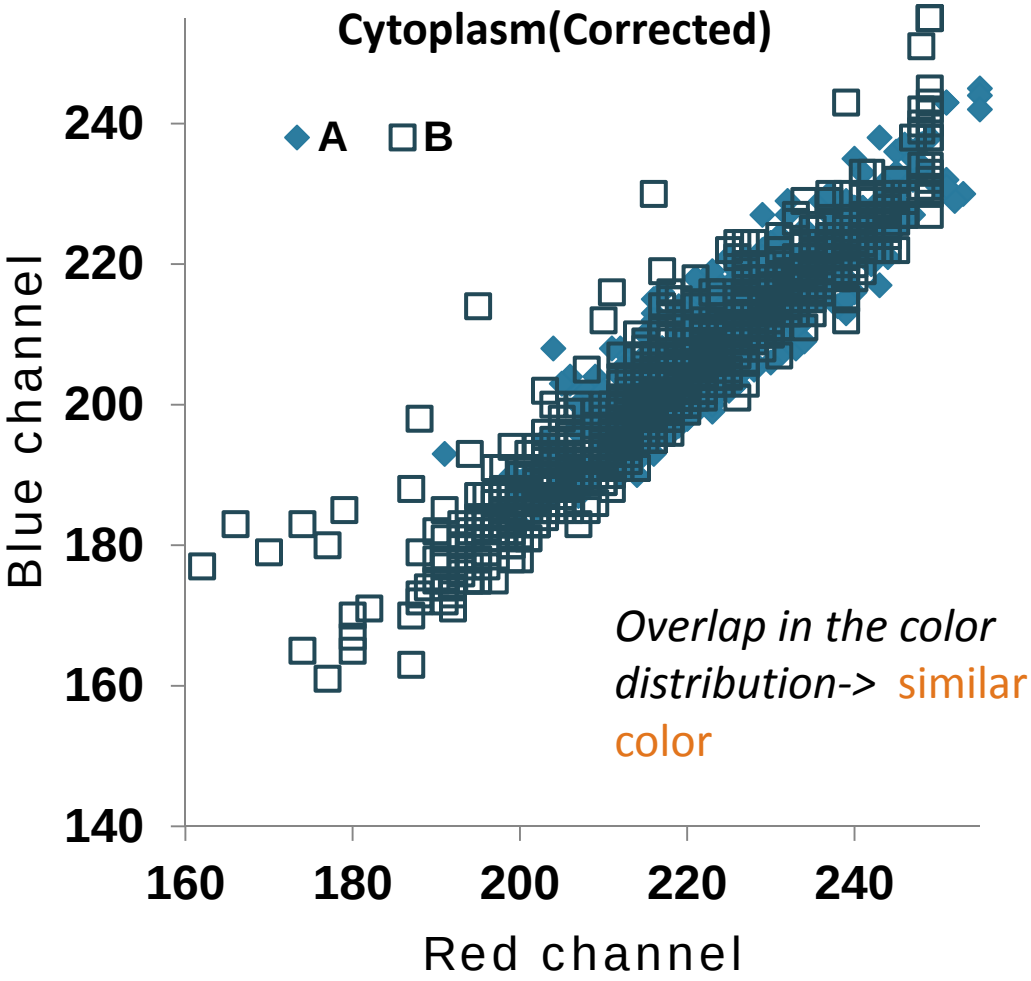
RGB color distribution



Corrected



RGB color distribution



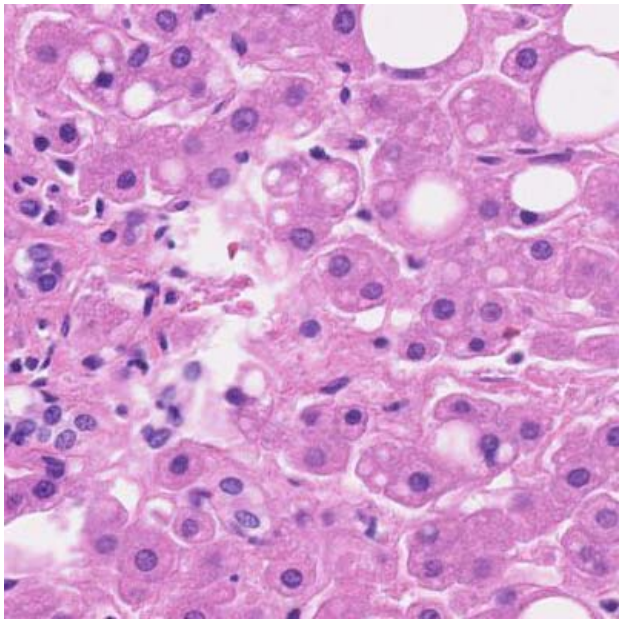
MASSACHUSETTS GENERAL HOSPITAL
PATHOLOGY

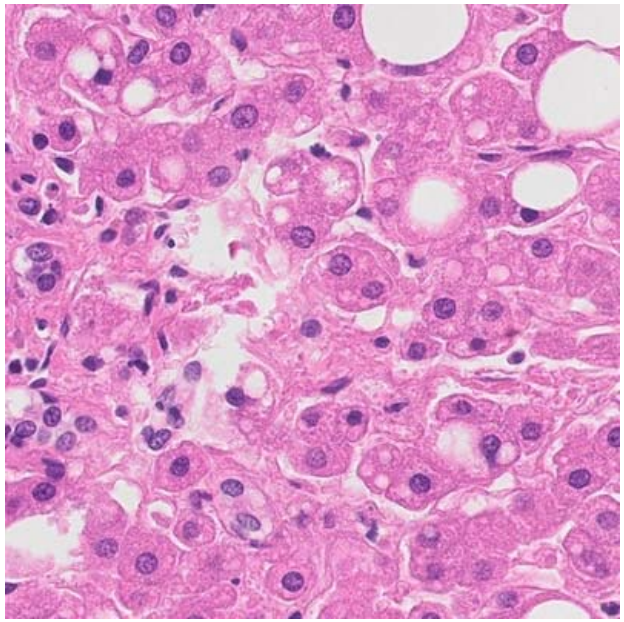
HARVARD MEDICAL SCHOOL

Scanner A

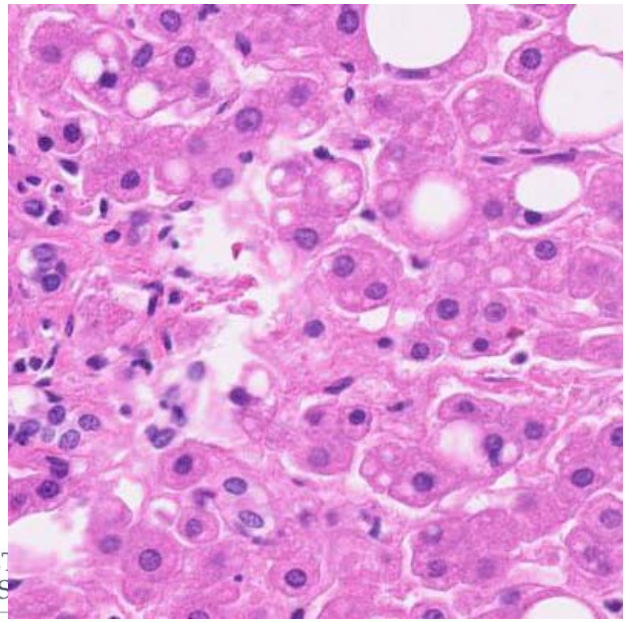
Scanner B

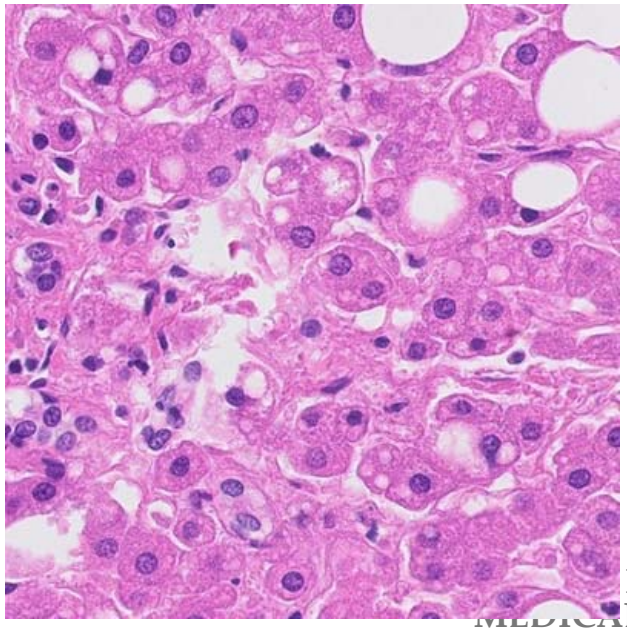
Original





Corrected

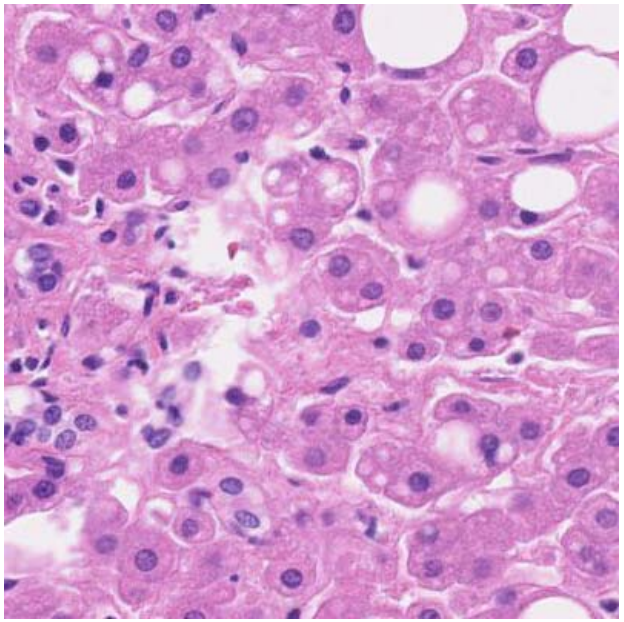


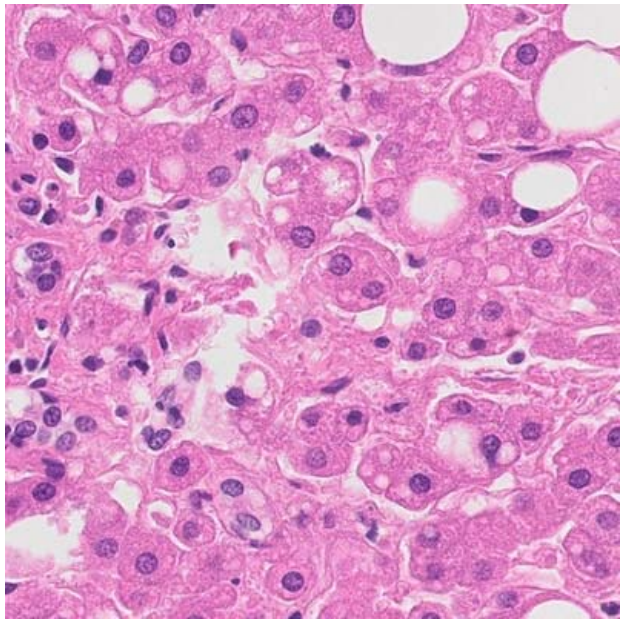


Scanner A

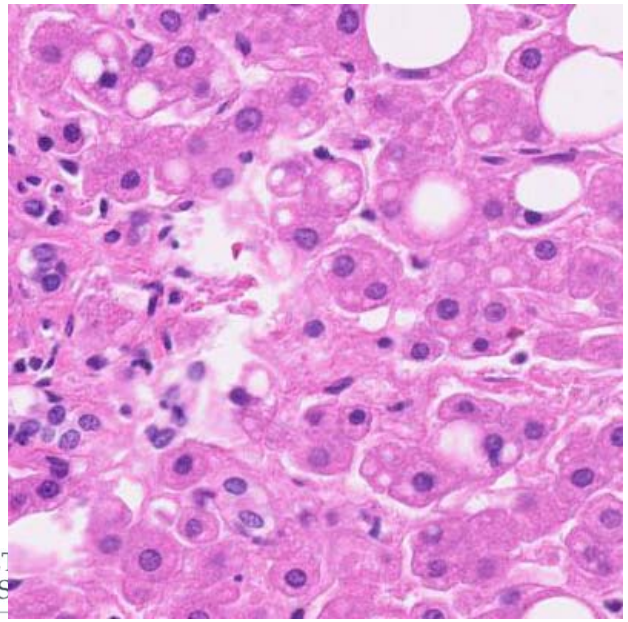
Scanner B

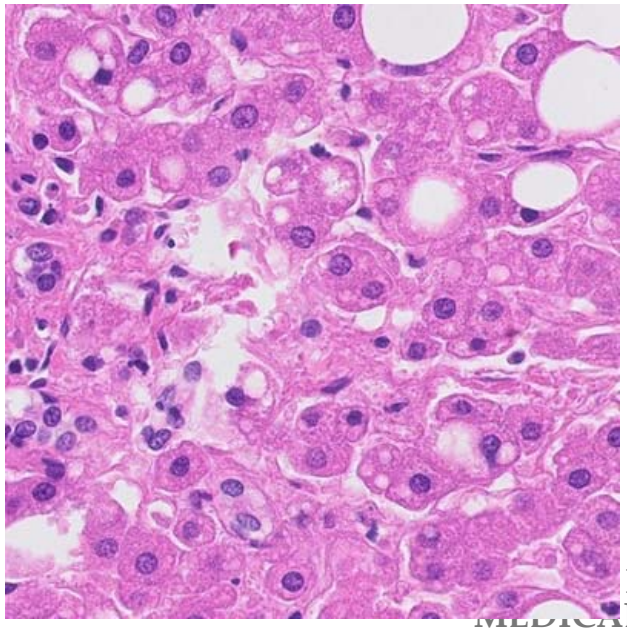
Original





Corrected

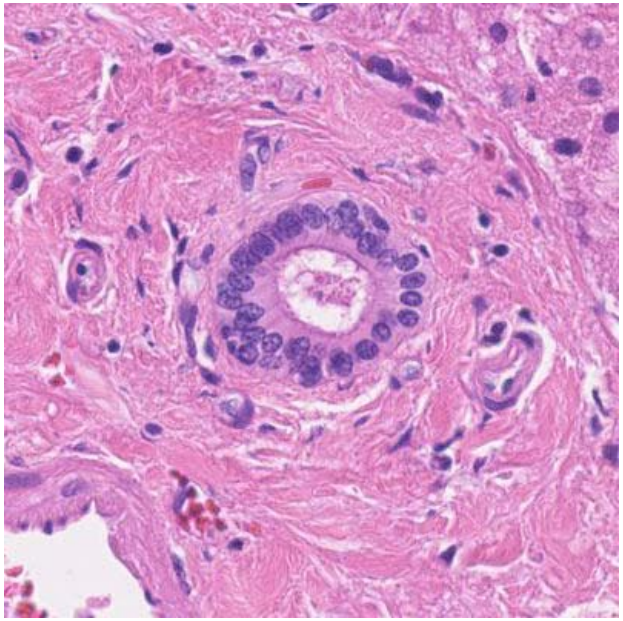


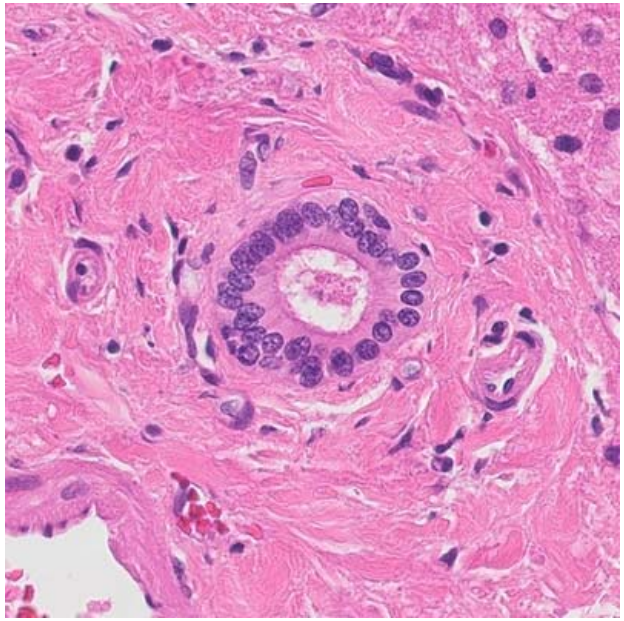


Scanner A

Scanner B

Original





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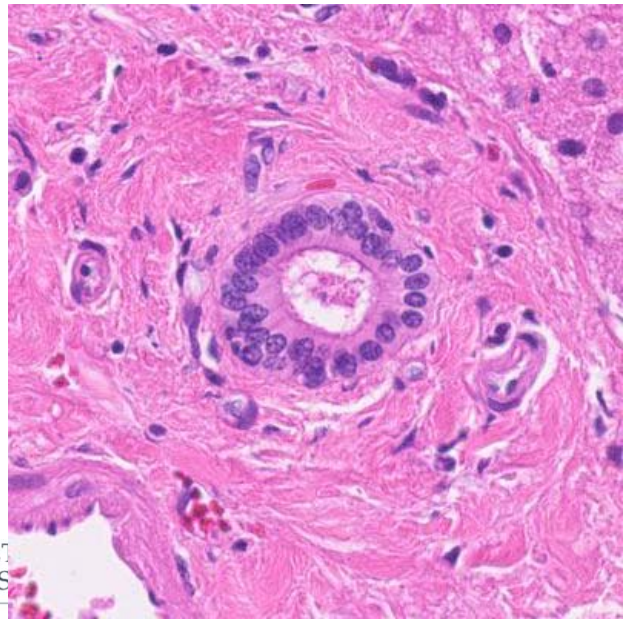
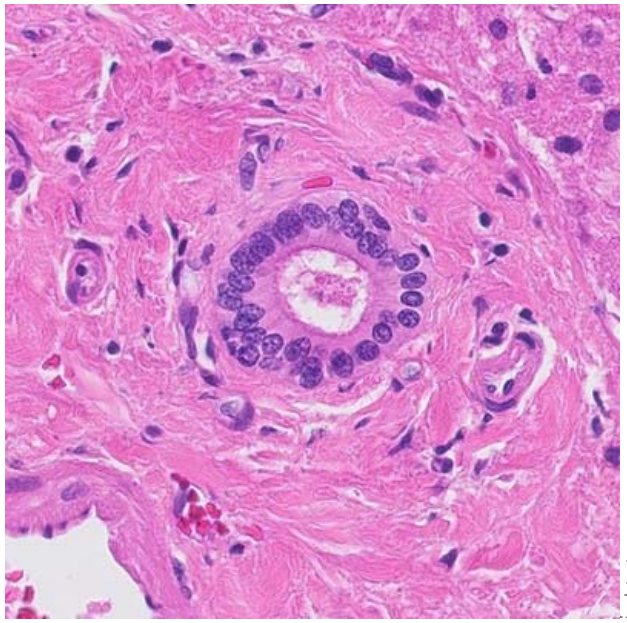
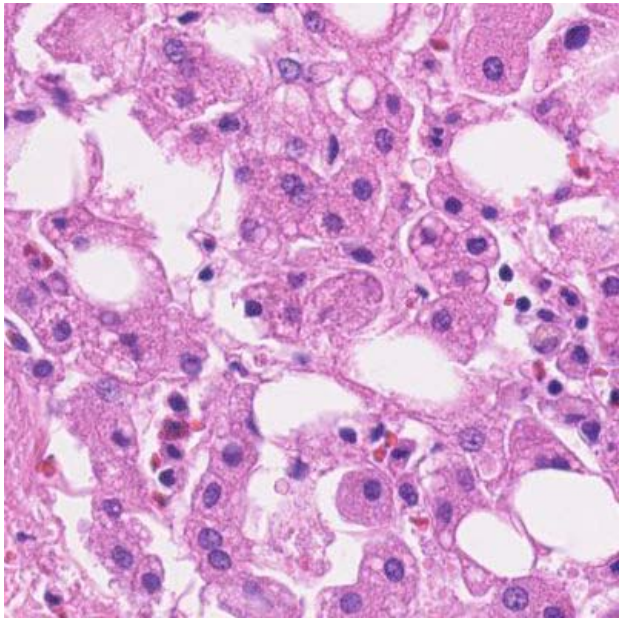


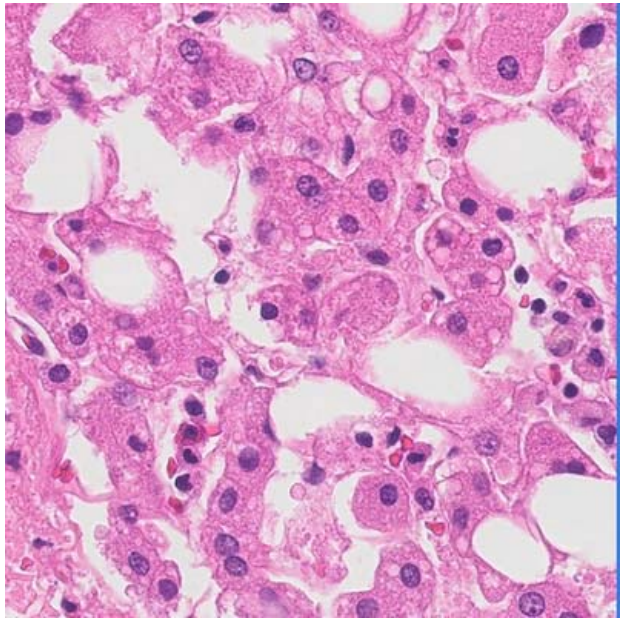


Scanner A

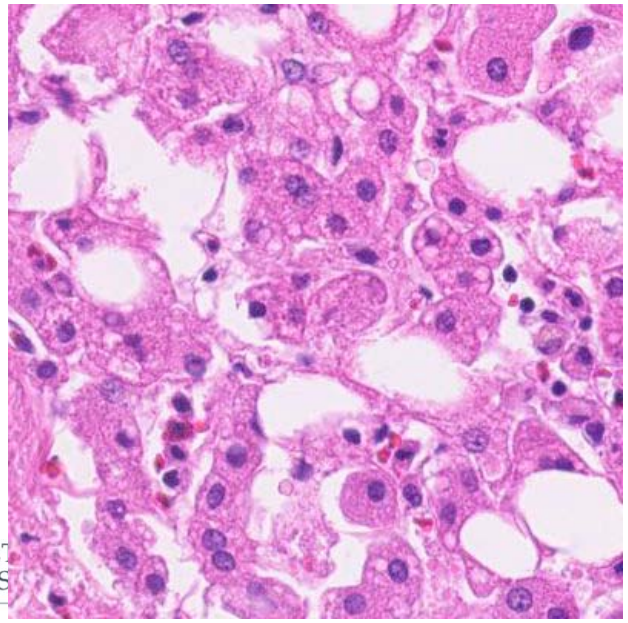
Scanner B

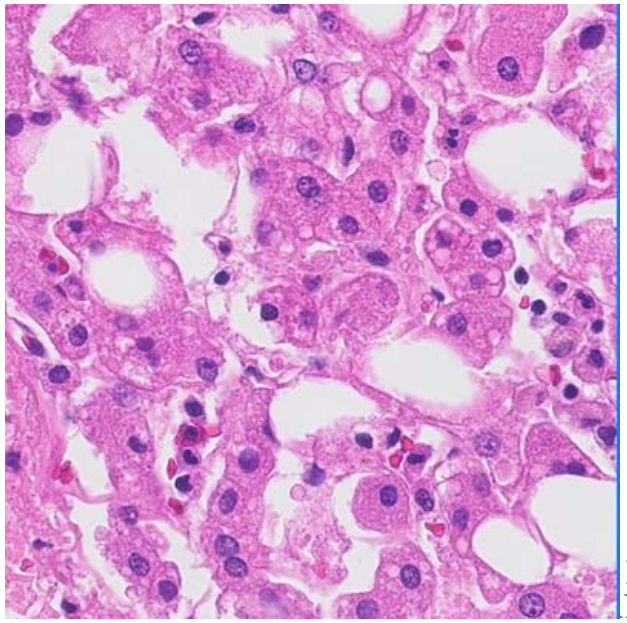
Original





Corrected





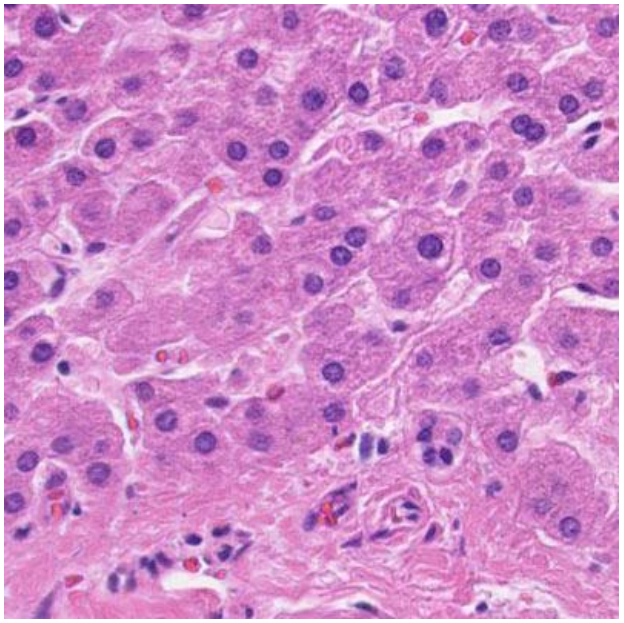
MASSACHUSETTS GENERAL HOSPITAL
PATHOLOGY

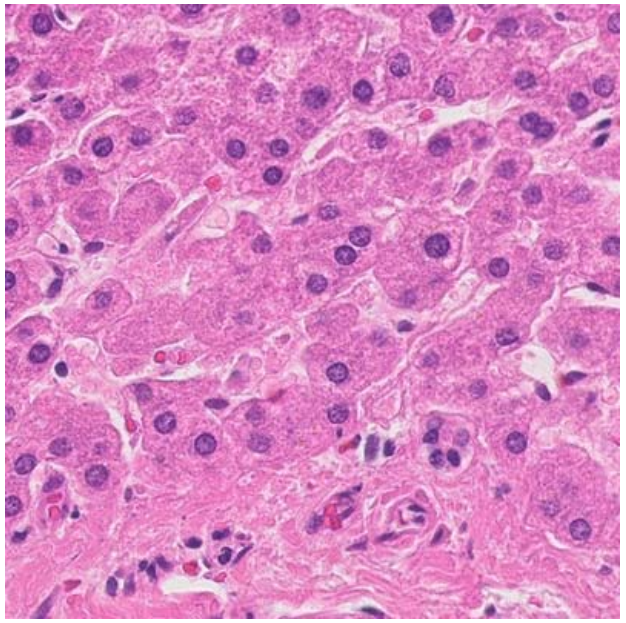
HARVARD MEDICAL SCHOOL

Scanner A

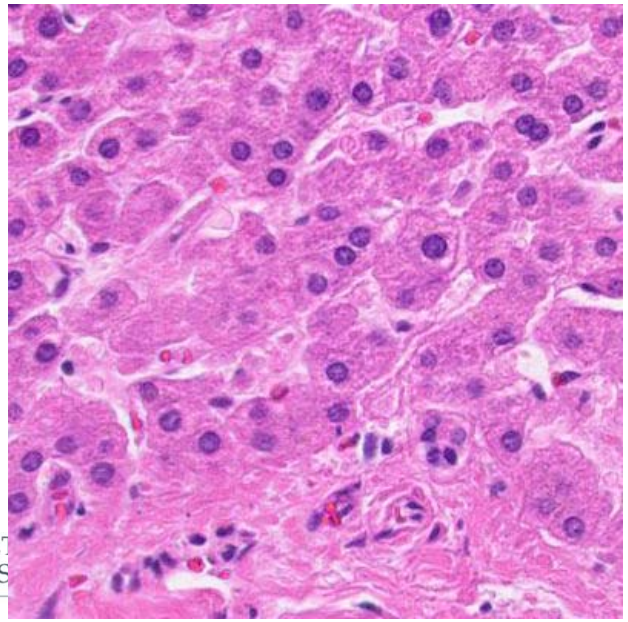
Scanner B

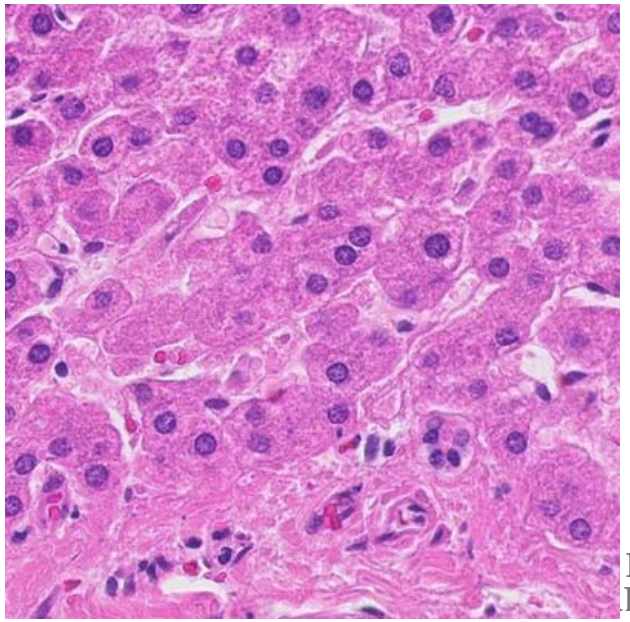
Original





Corrected





MASSACHUSET
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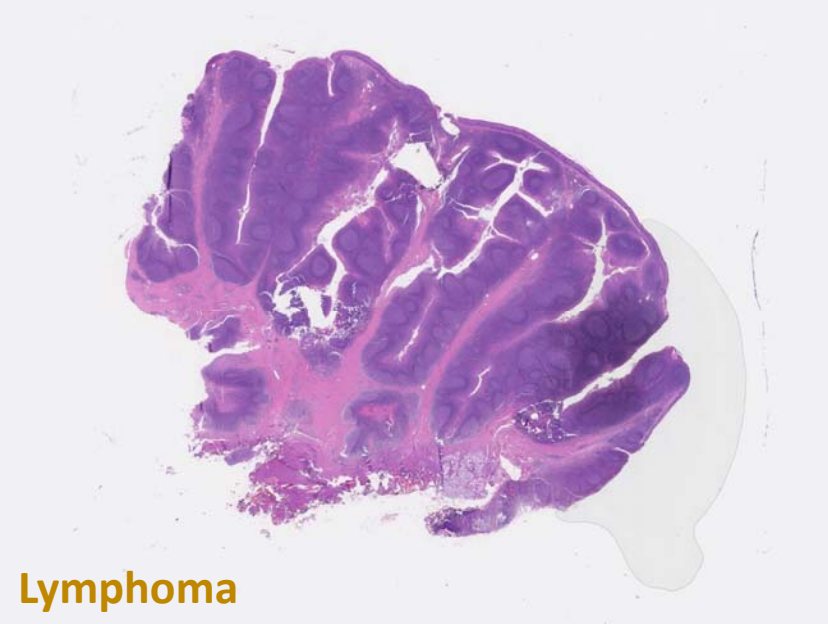
PATHOLOGY

HARVARD
L SCHOOL

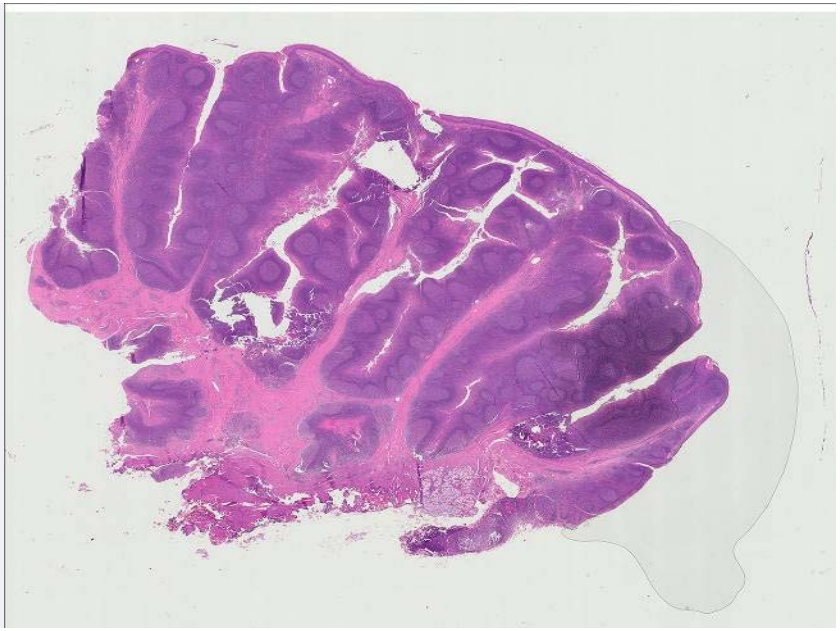
Results in Lymphoma

Thumbnail images of the original whole slide images

Scanner A



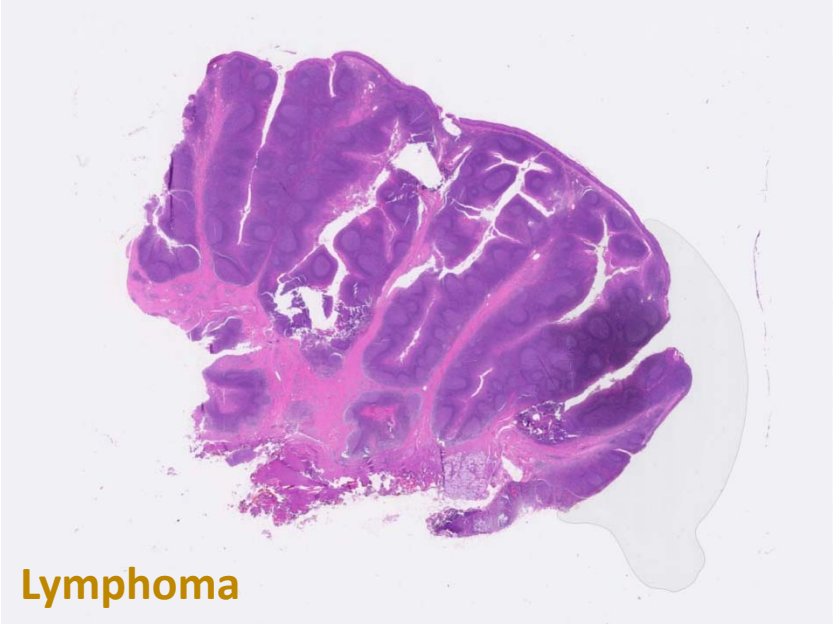
Scanner B



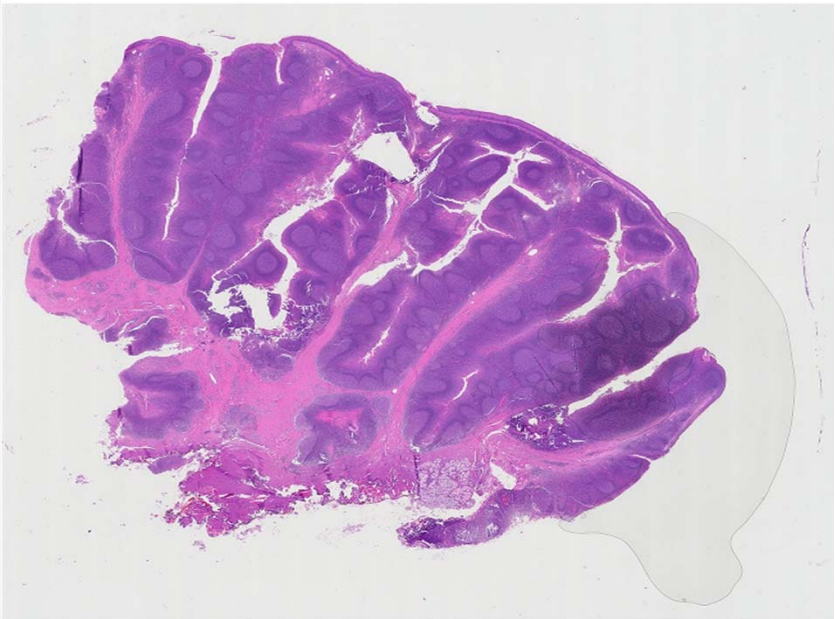
There is color variation….

Thumbnail images of the standardized whole slide images

Scanner A



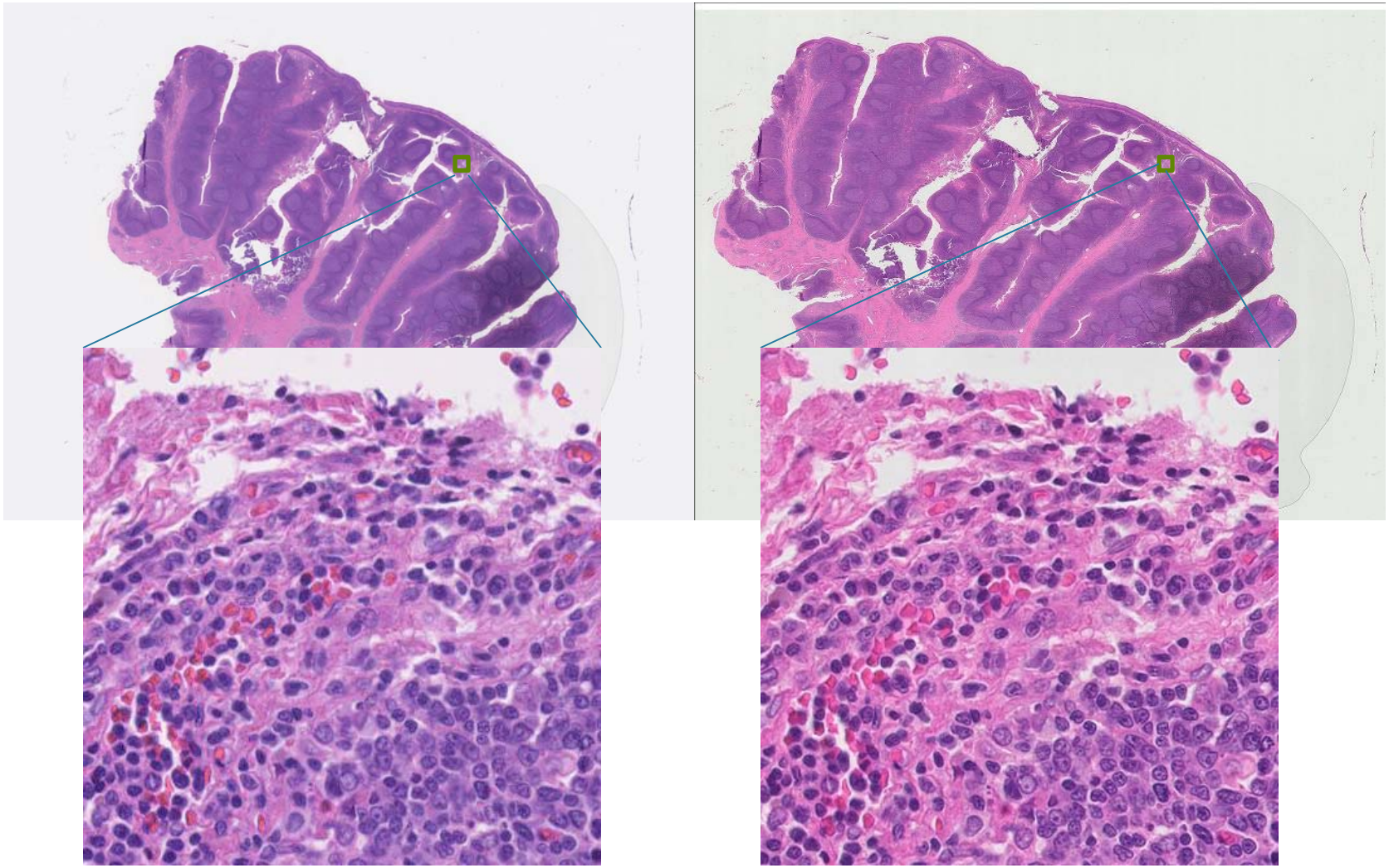
Scanner B



Application of color correction minimizes the color differences…..

Scanner A

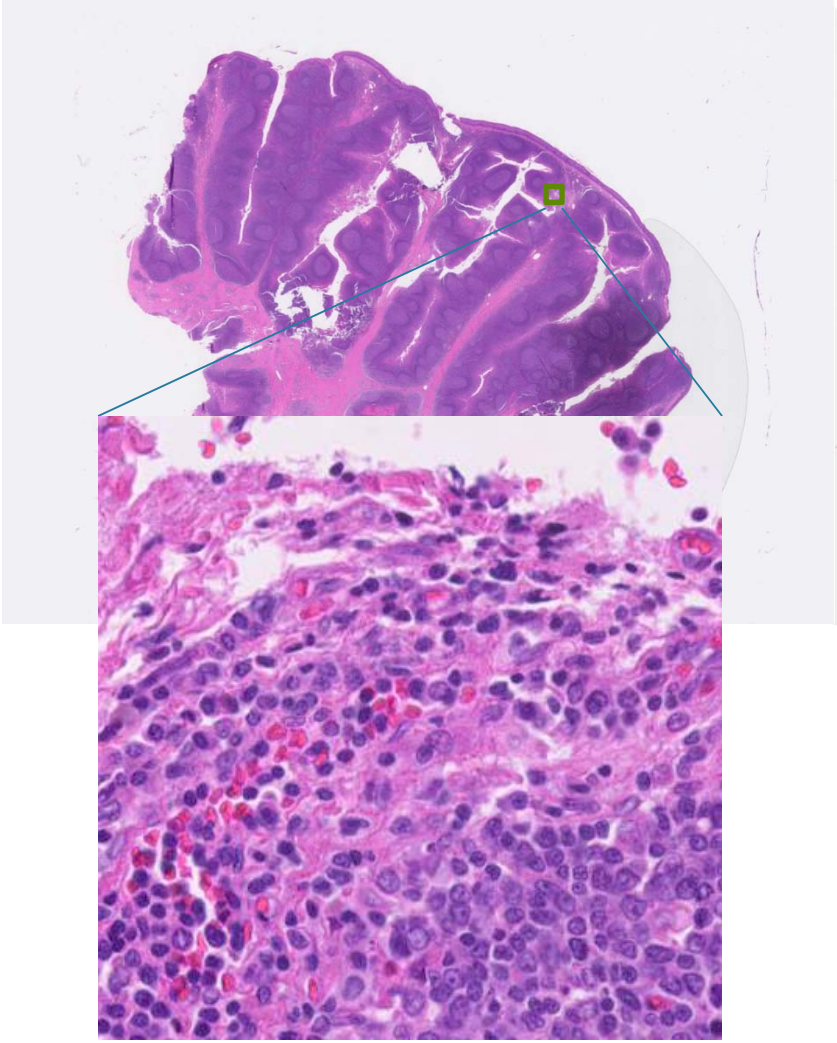
Scanner B



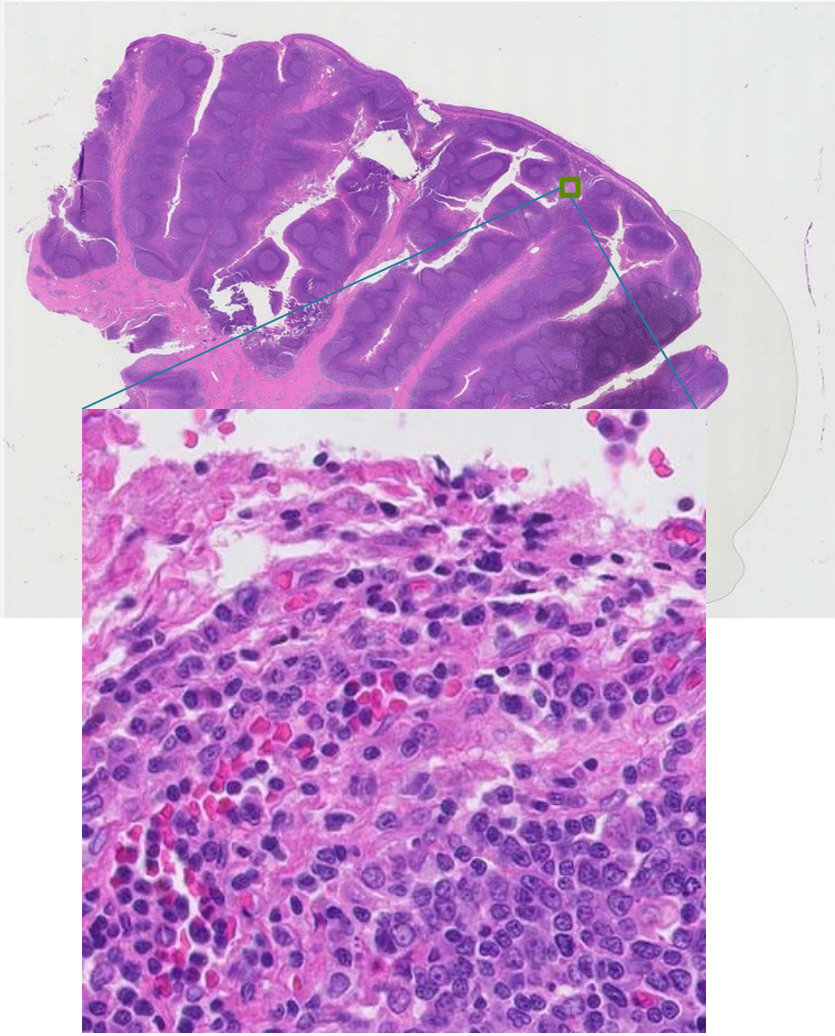
There is color variation....

(no correction)

Scanner A

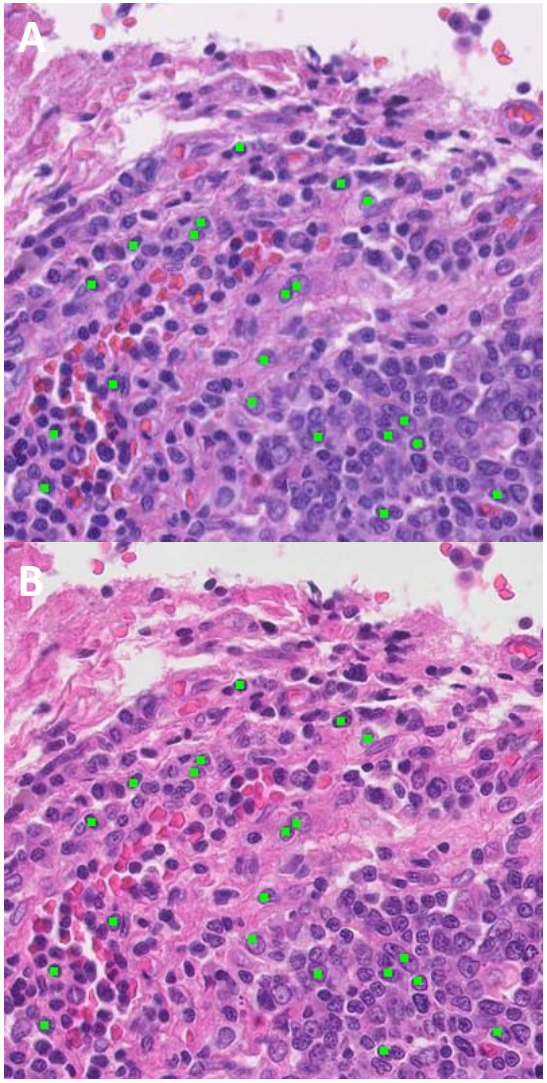


Scanner B

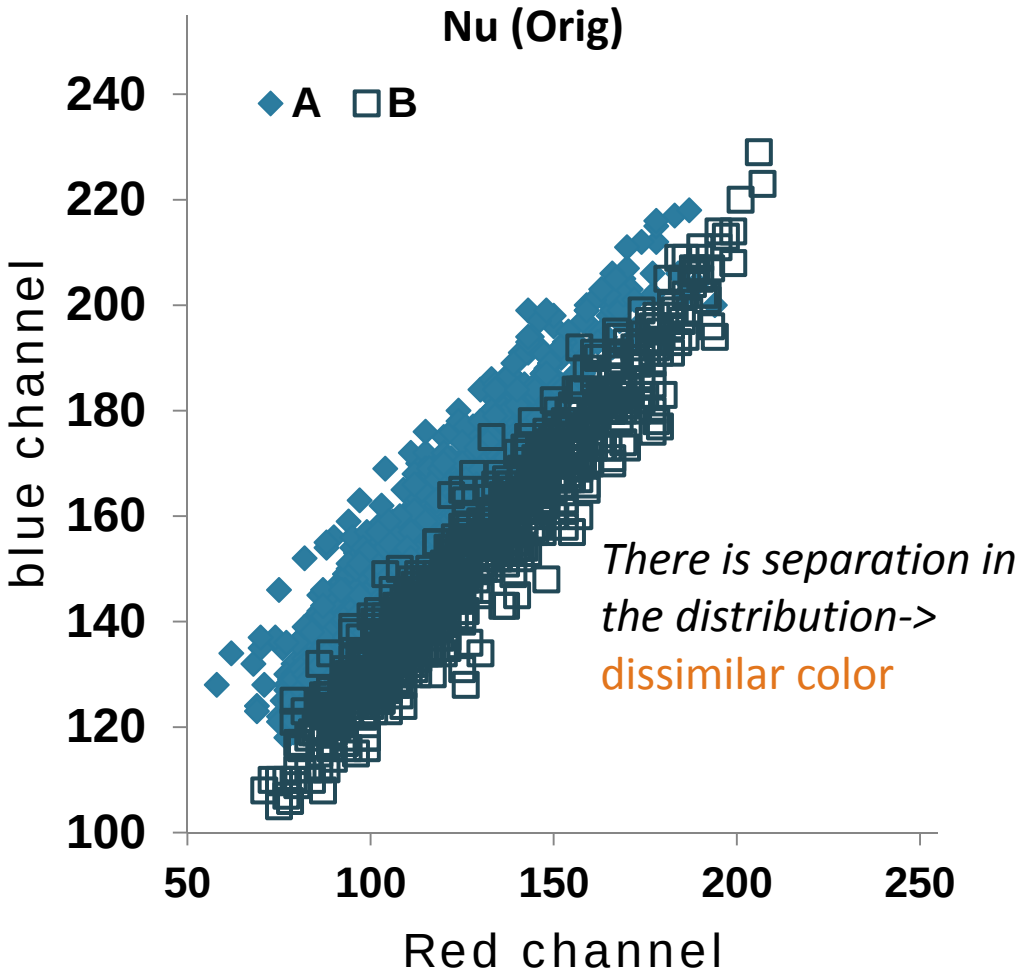


Application of color correction minimizes the color differences.....

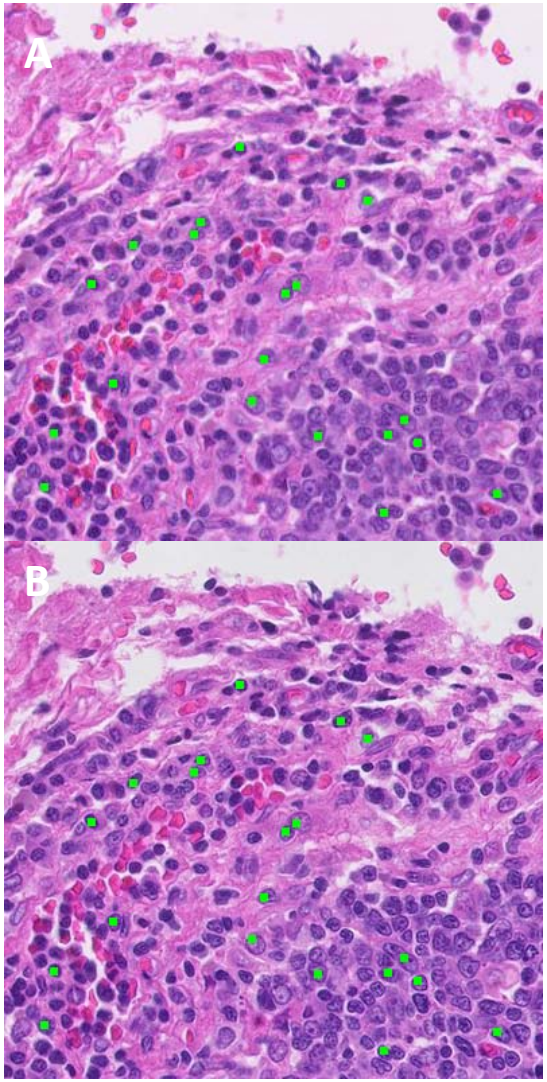
Original



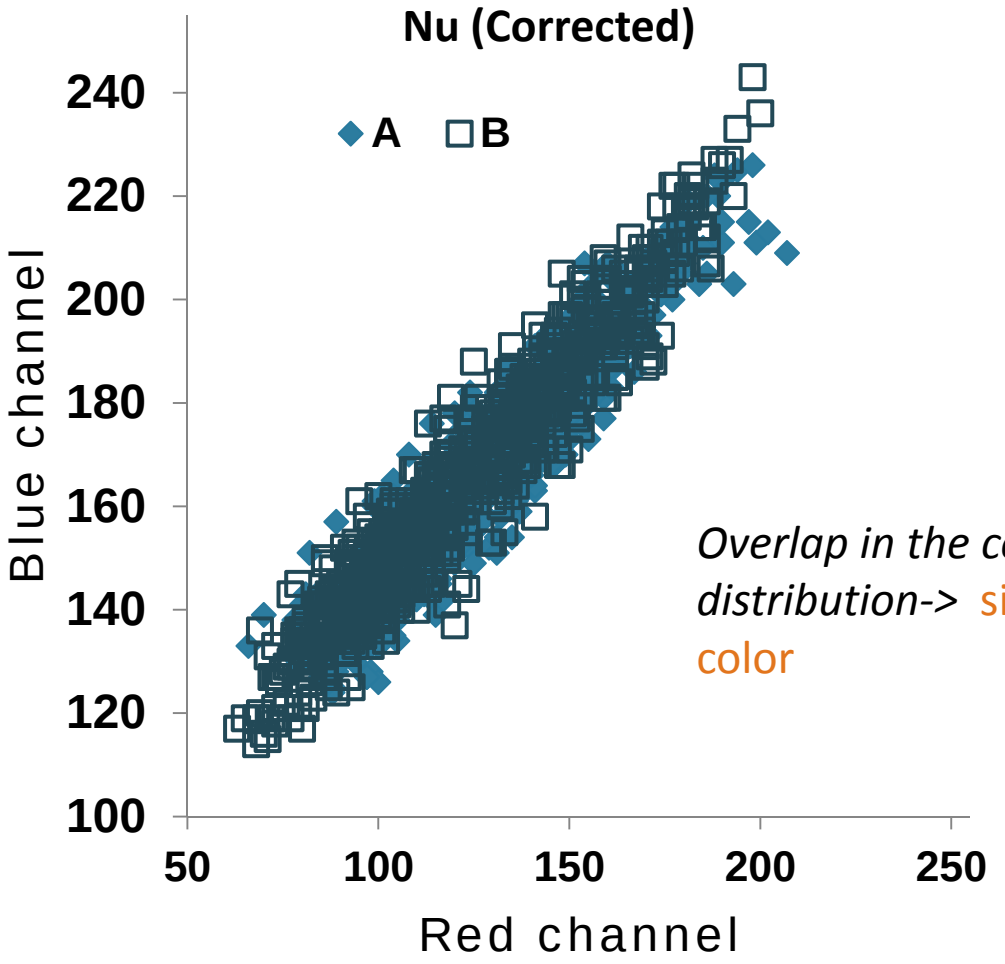
RGB color distribution



Corrected



RGB color distribution



Overlap in the color distribution-> similar color

MASSACHUSETTS GENERAL HOSPITAL
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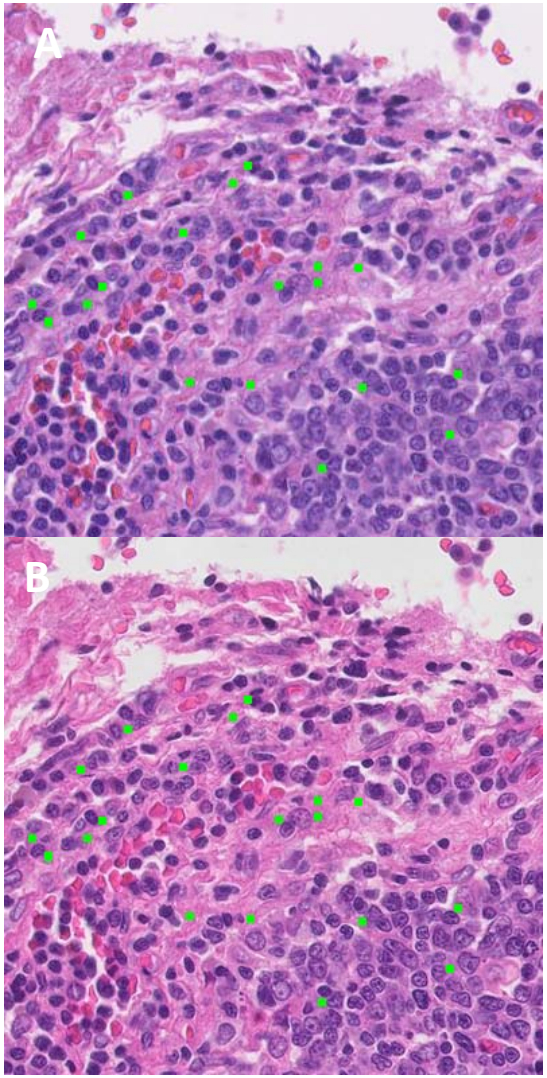
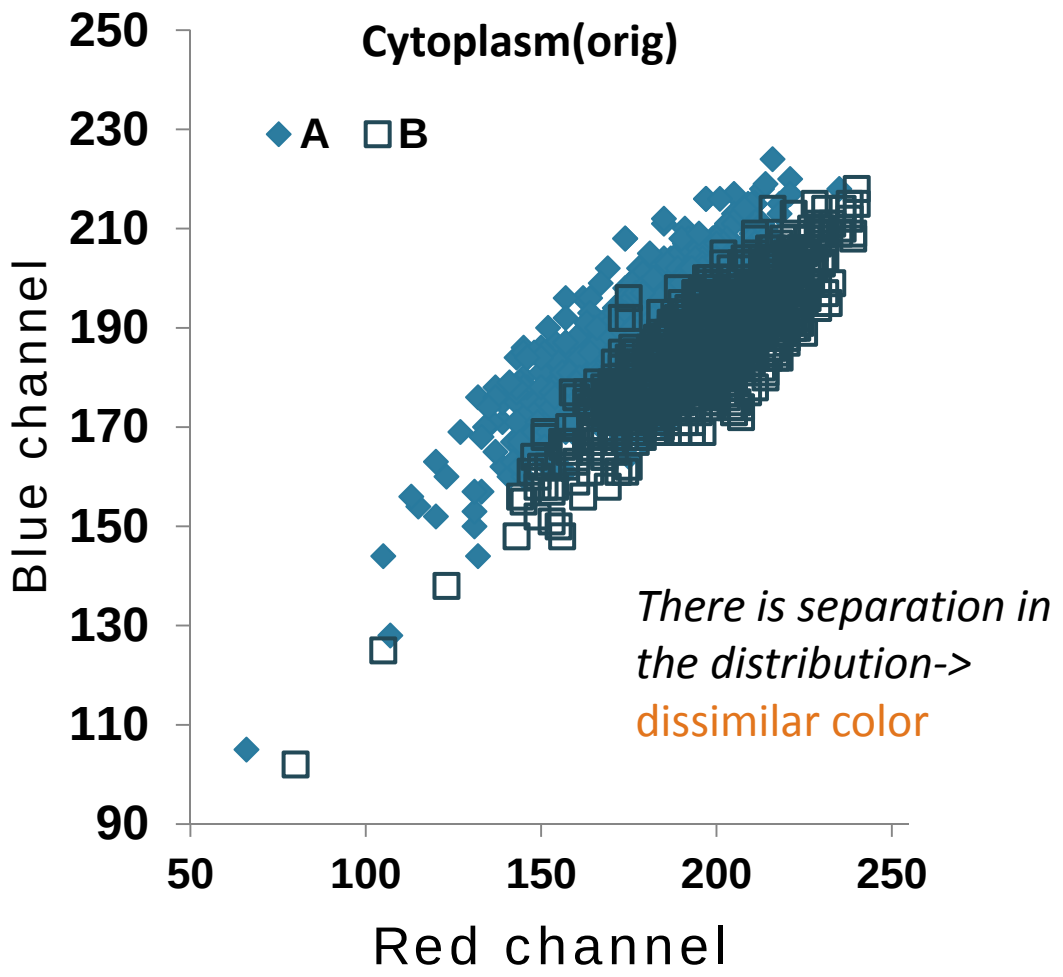
HARVARD MEDICAL SCHOOL

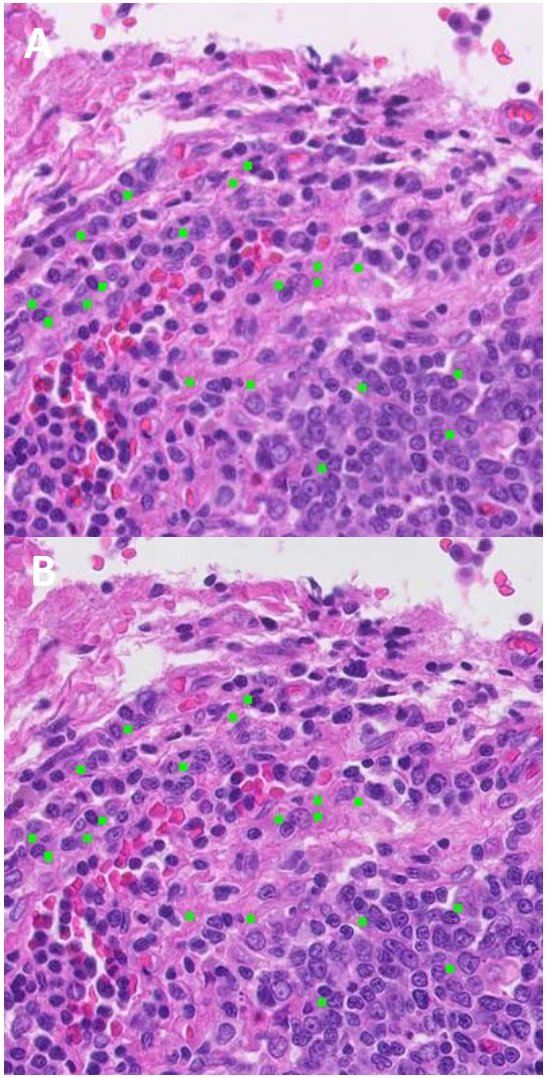
Original



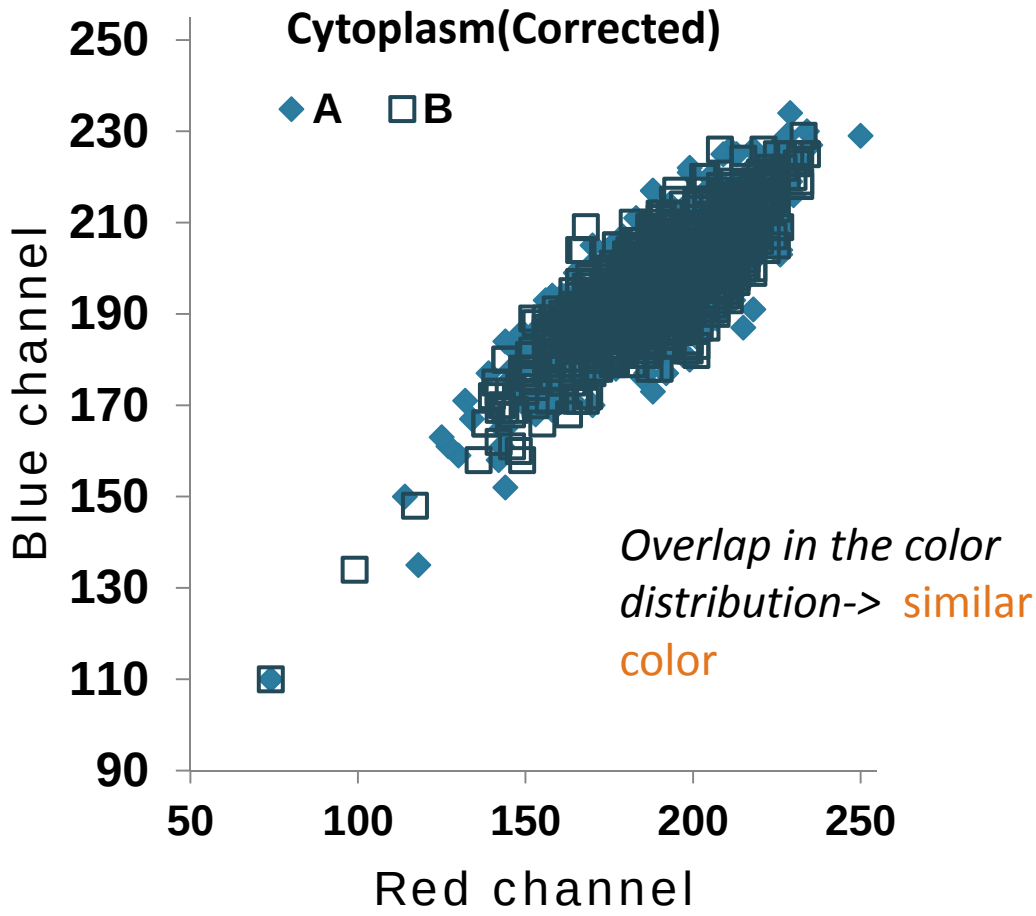
RGB color distribution



Corrected



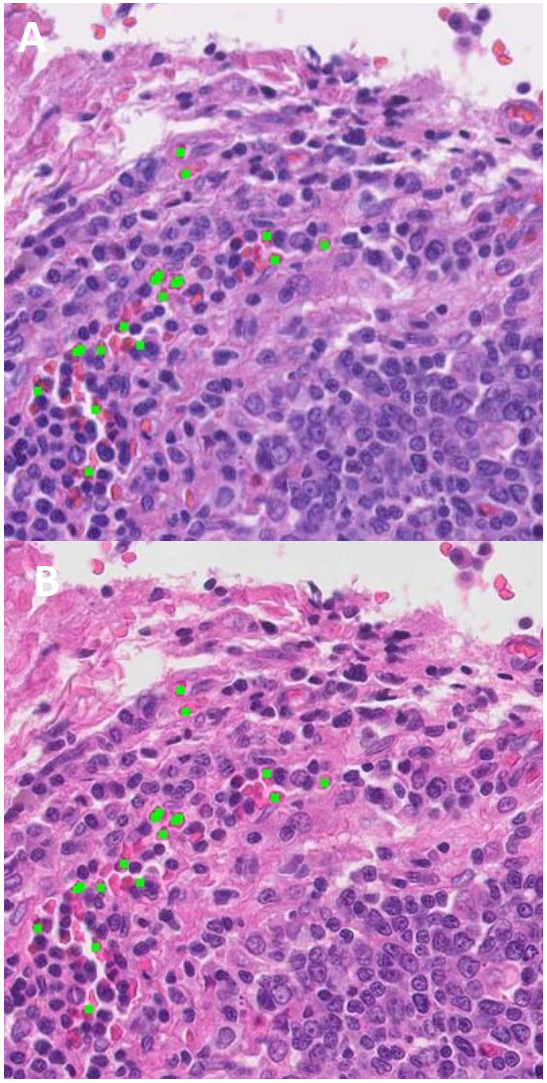
RGB color distribution



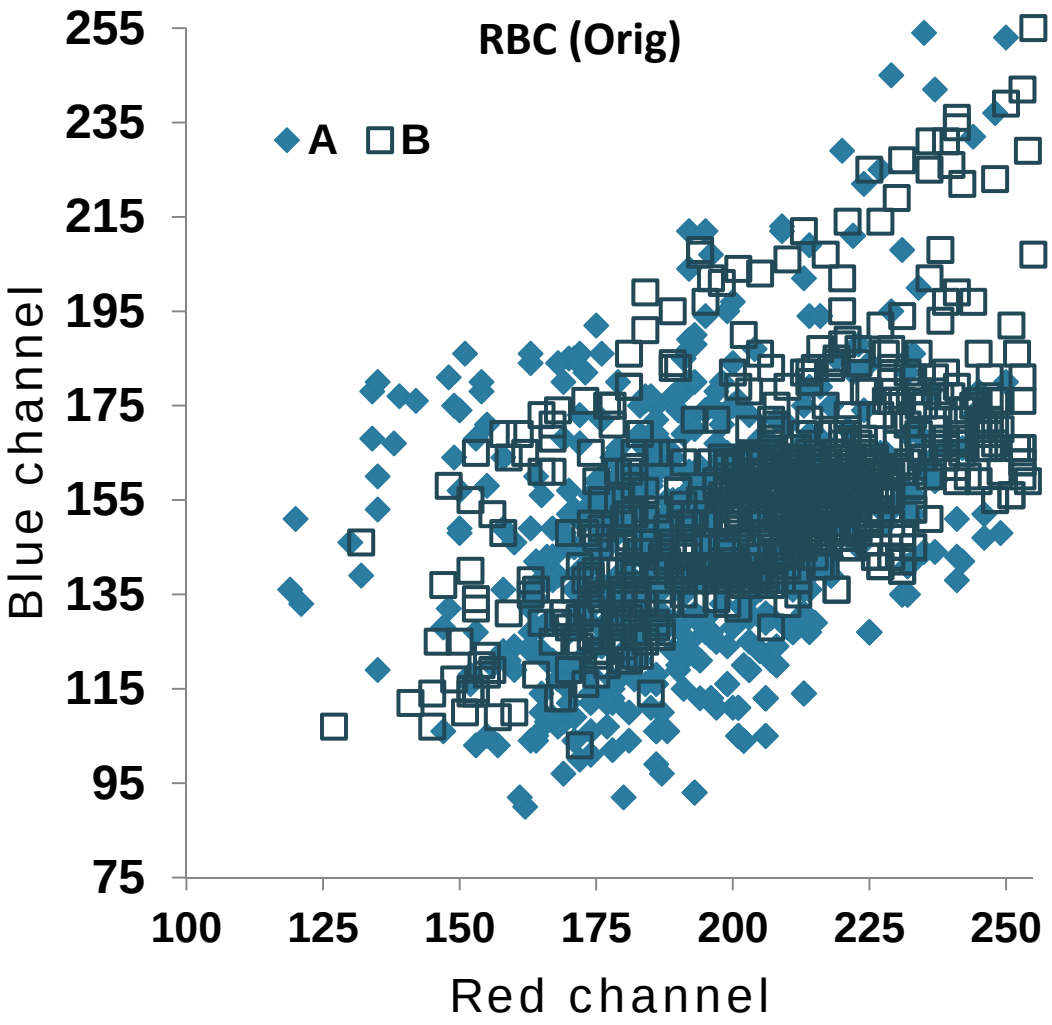
MASSACHUSETTS GENERAL HOSPITAL
PATHOLOGY

HARVARD MEDICAL SCHOOL

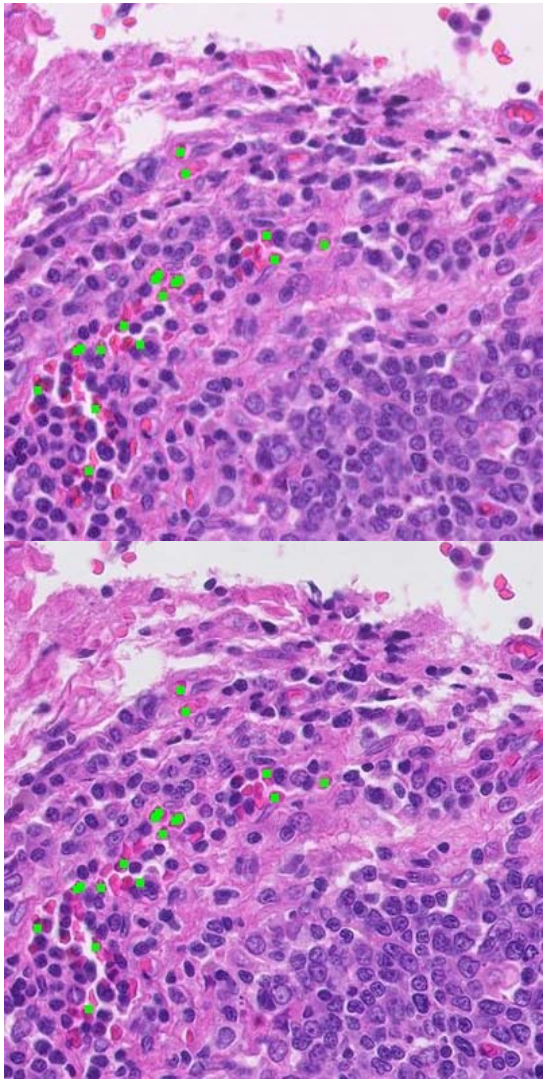
Original



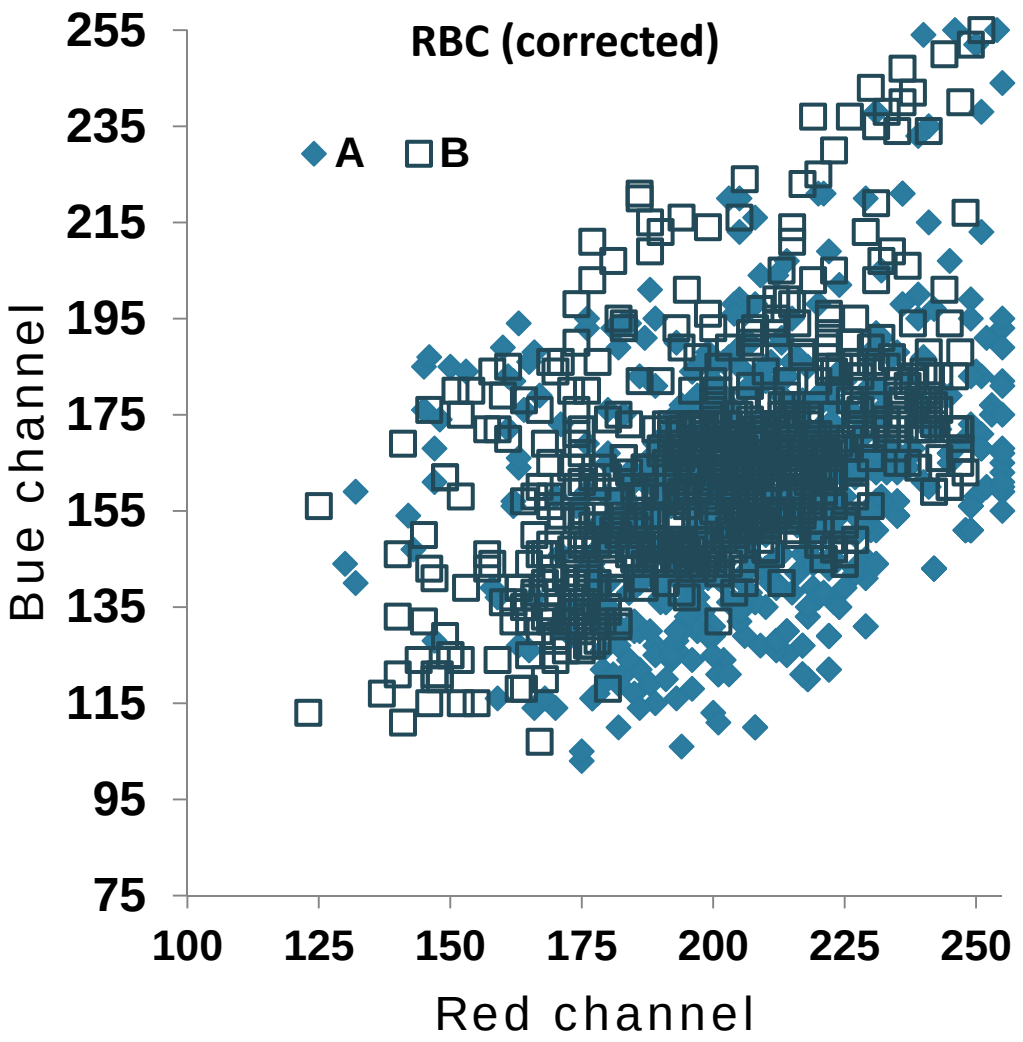
RGB color distribution



Corrected



RGB color distribution

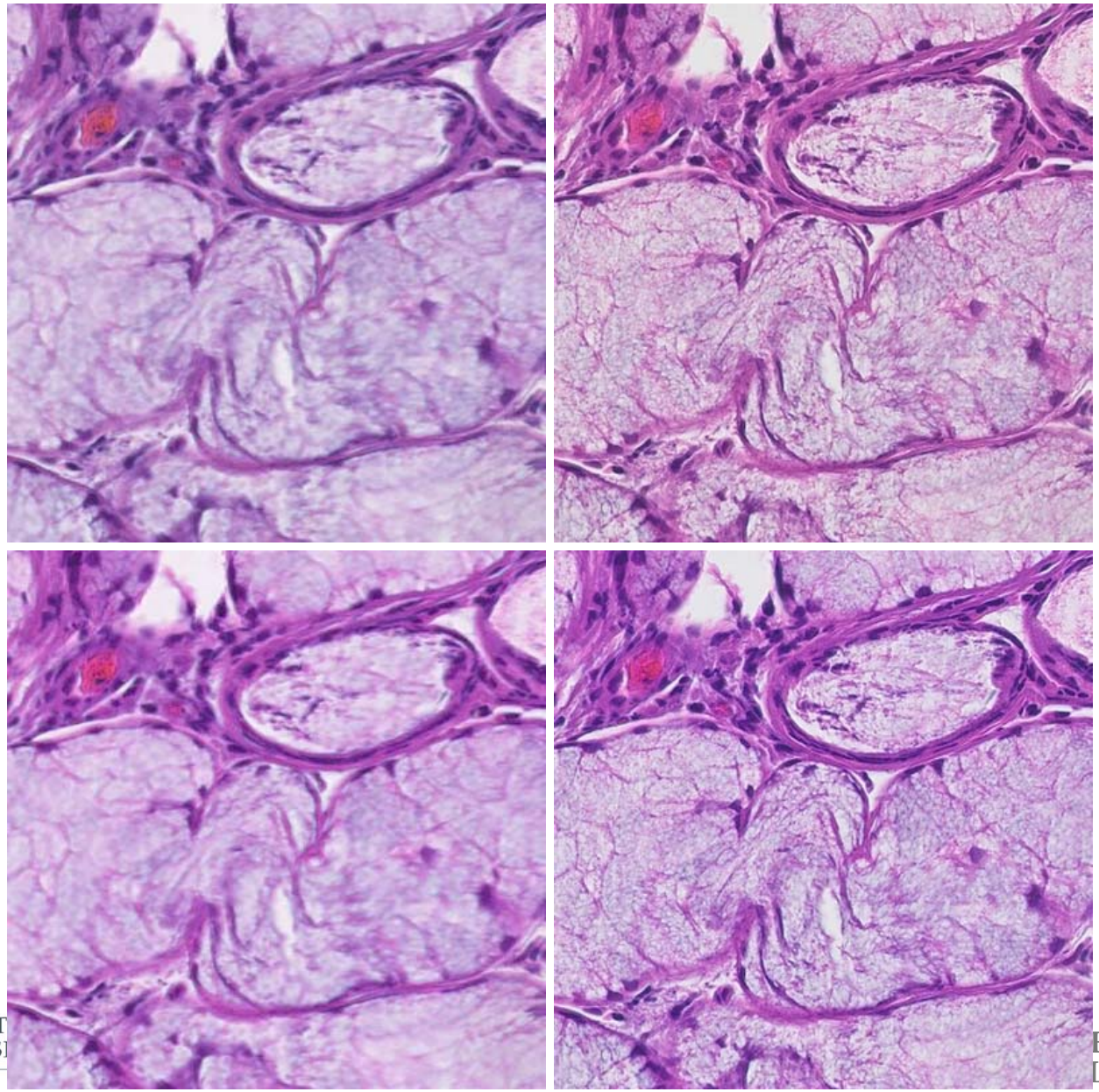


Scanner A

Scanner B

Original

Corrected



MASSACHUSET
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PATHOLOGY

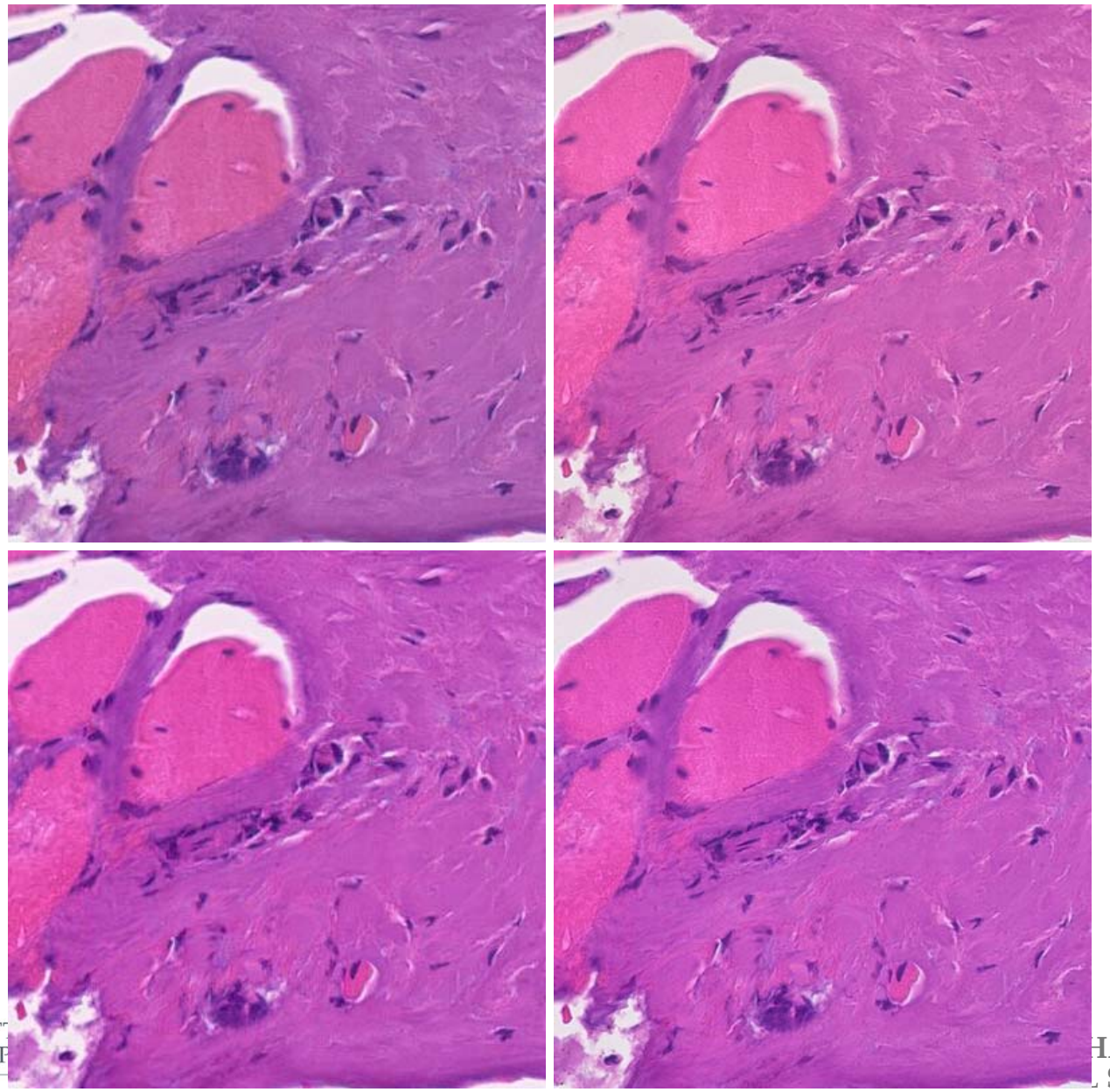
HARVARD
L SCHOOL

Scanner A

Scanner B

Original

Corrected



MASSACHUSETT
GENERAL HOSP
PATHOLOGY

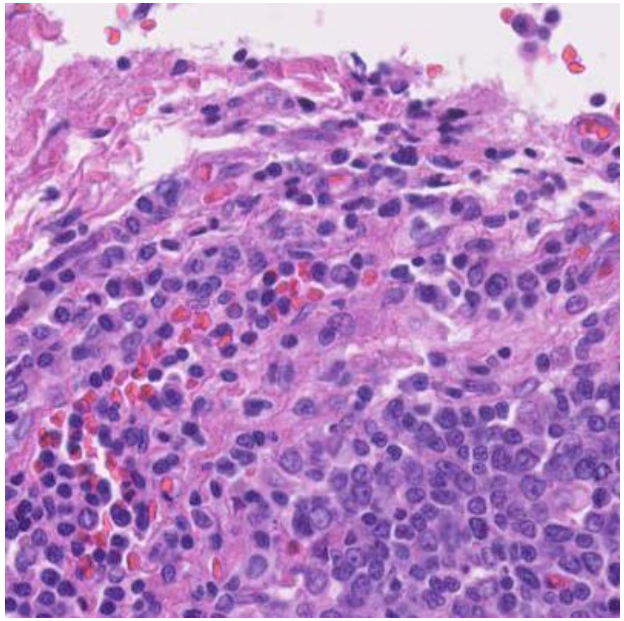
HARVARD
SCHOOL

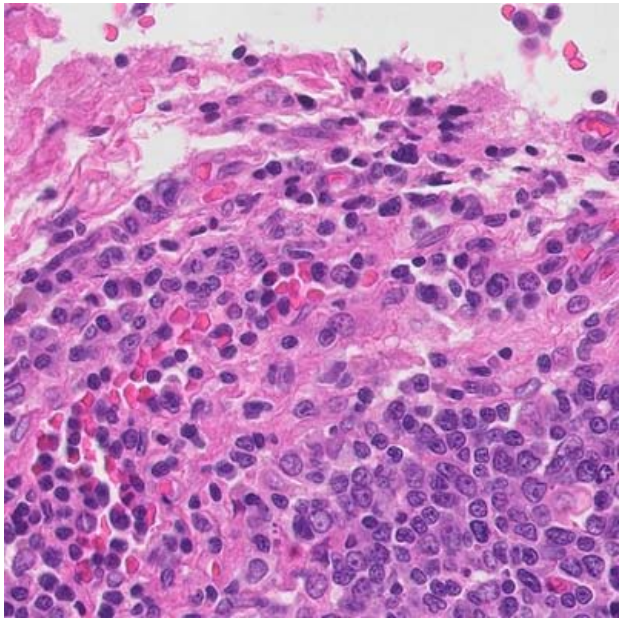
Scanner A

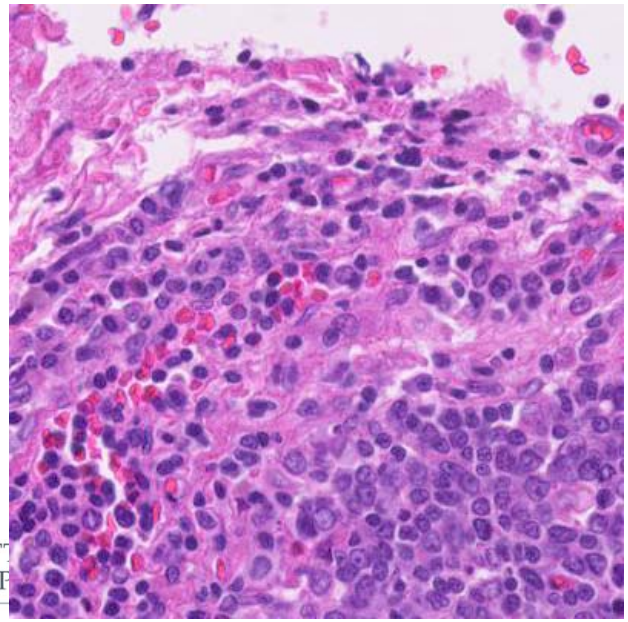
Scanner B

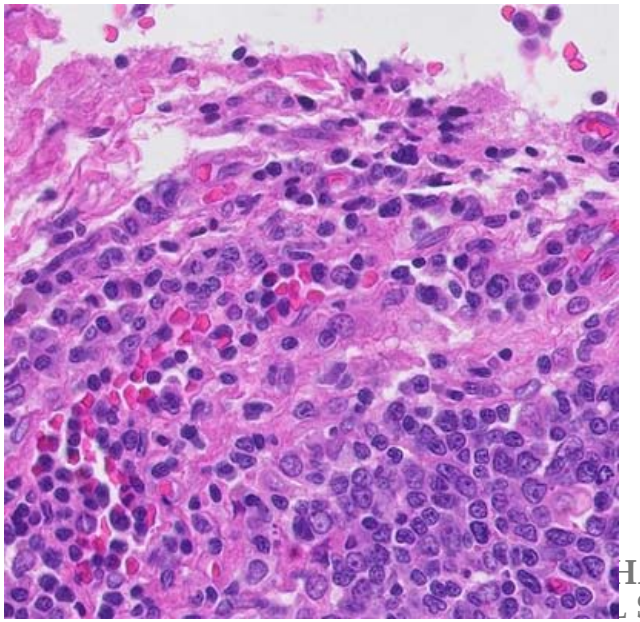
Original

Corrected









MASSACHUSETT
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PATHOLOGY

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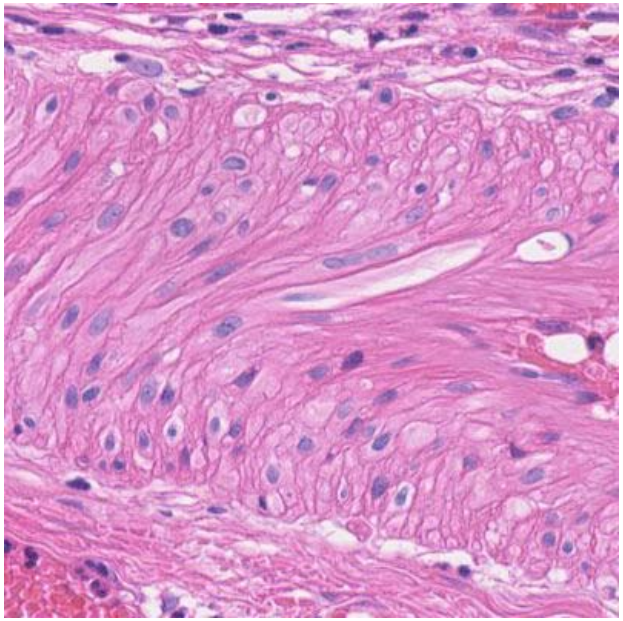
SCHOOL

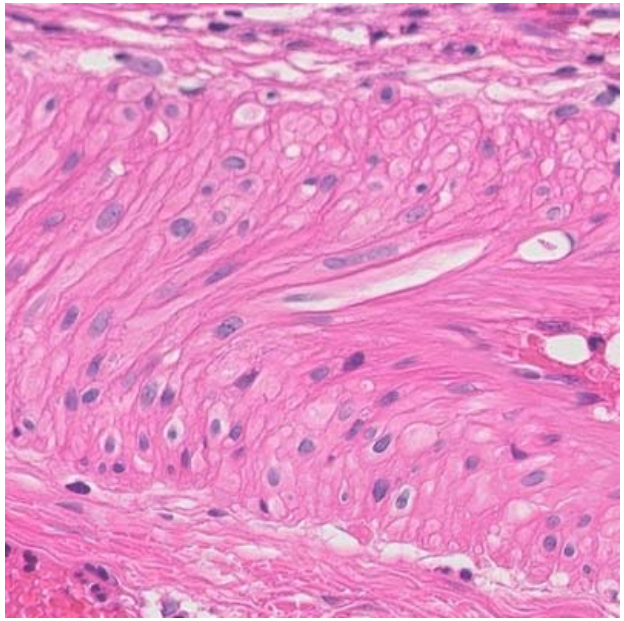
Results in Intestine

Scanner A

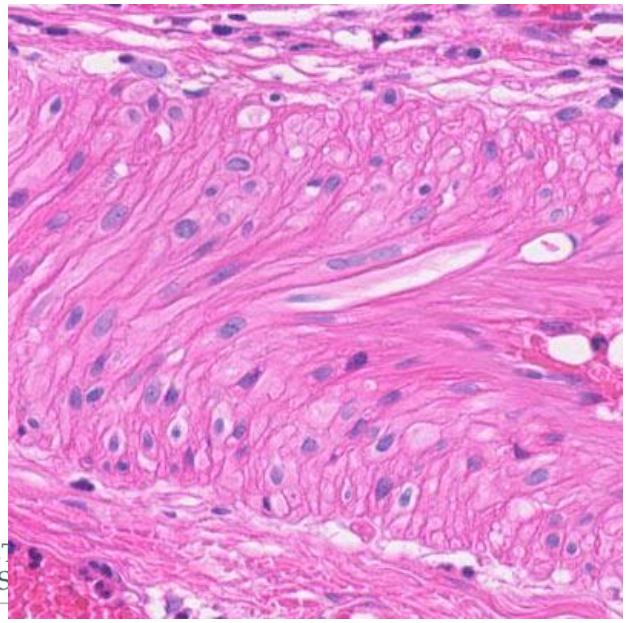
Scanner B

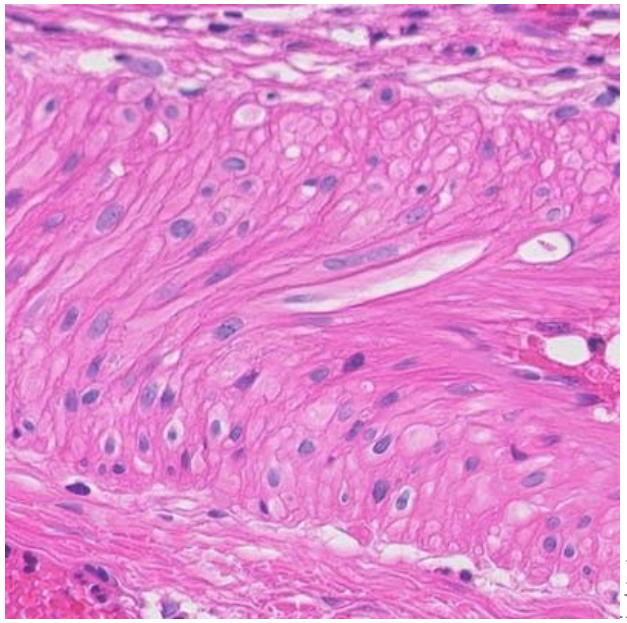
Original





Corrected





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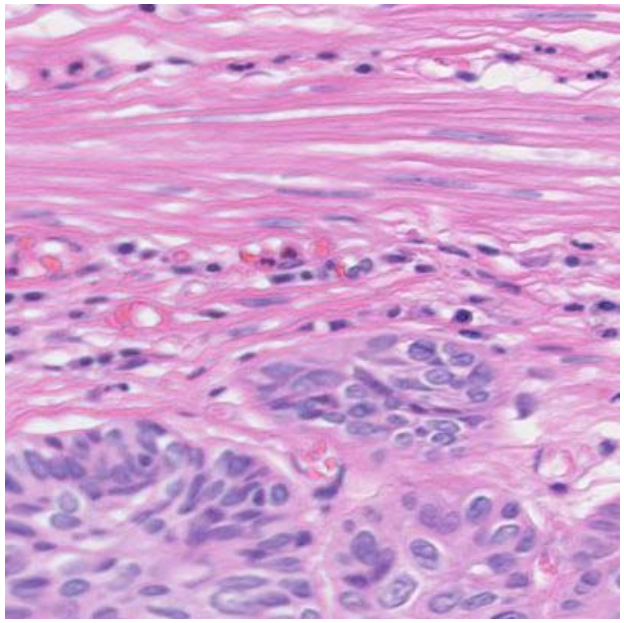
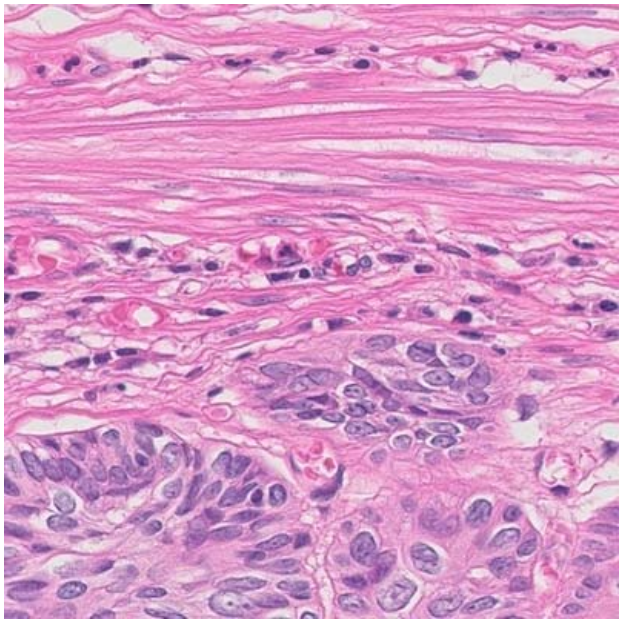
Scanner A

Scanner B

Original





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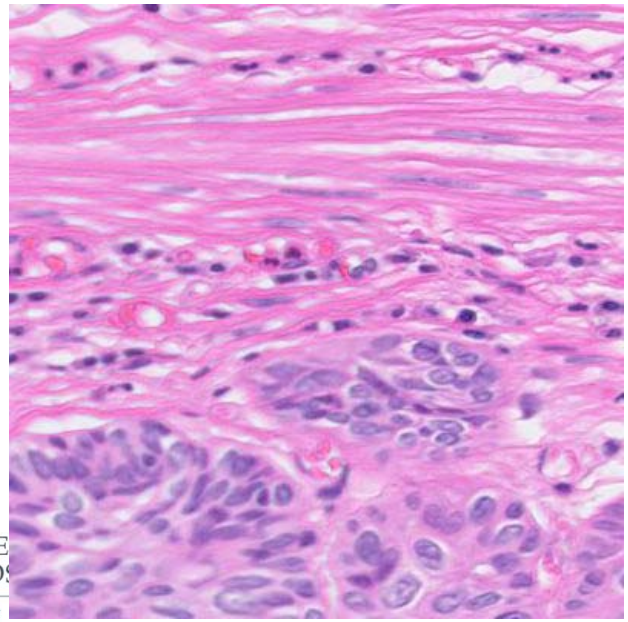

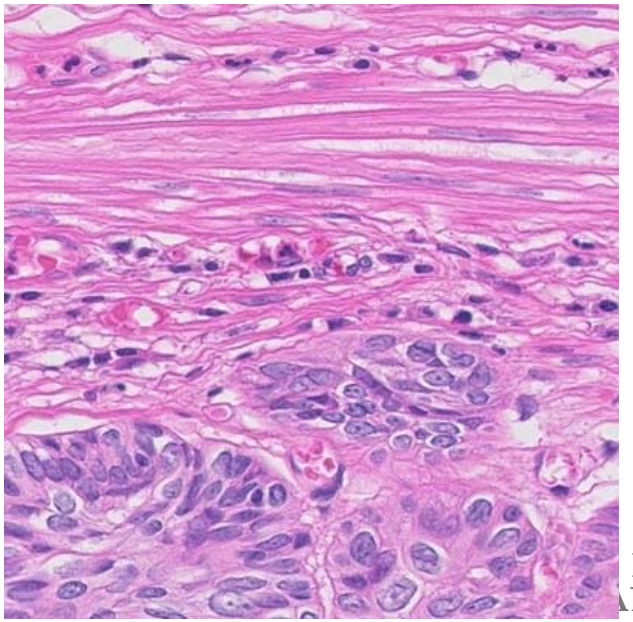

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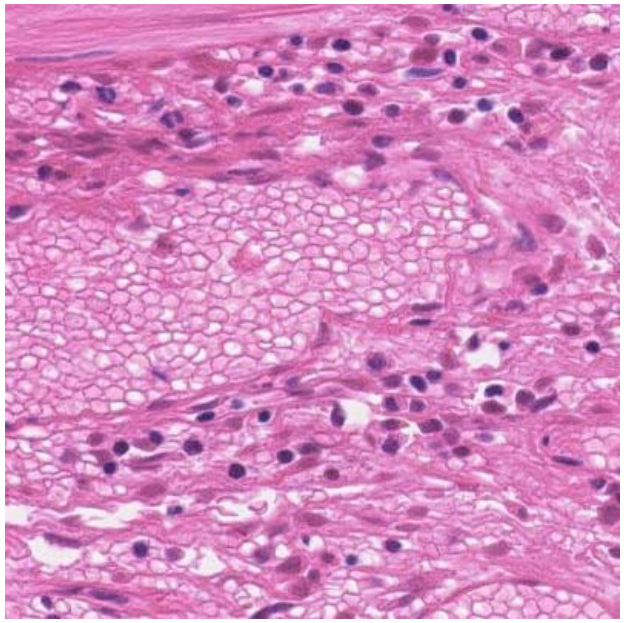
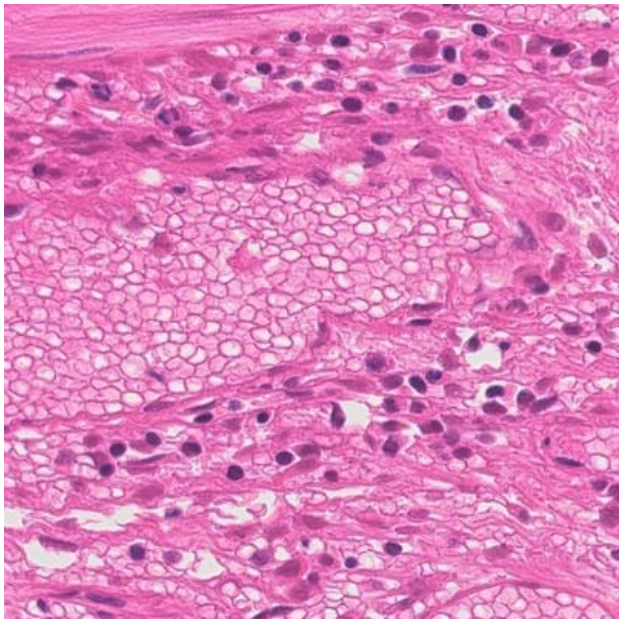
Scanner A

Scanner B

Original





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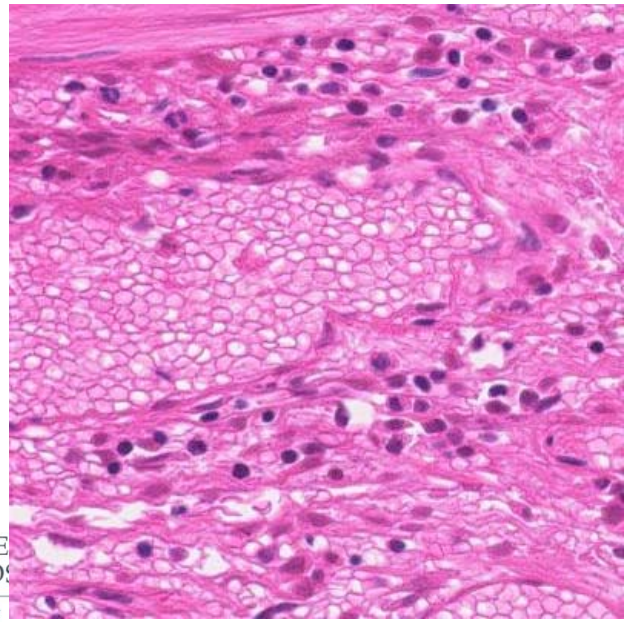

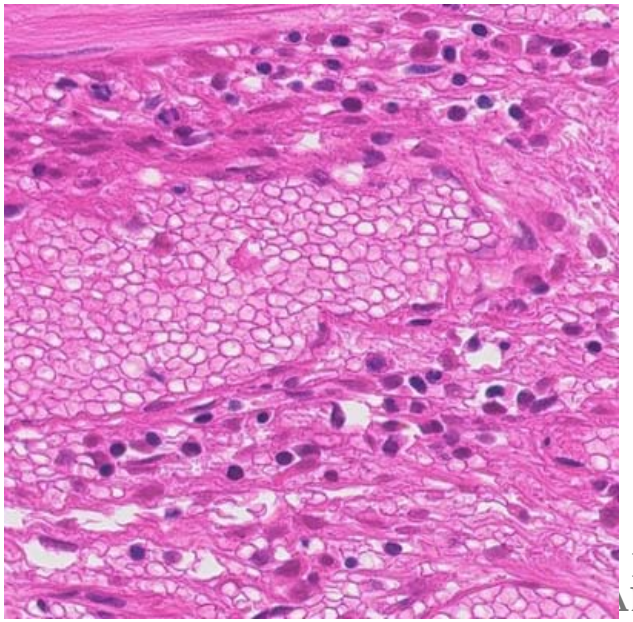

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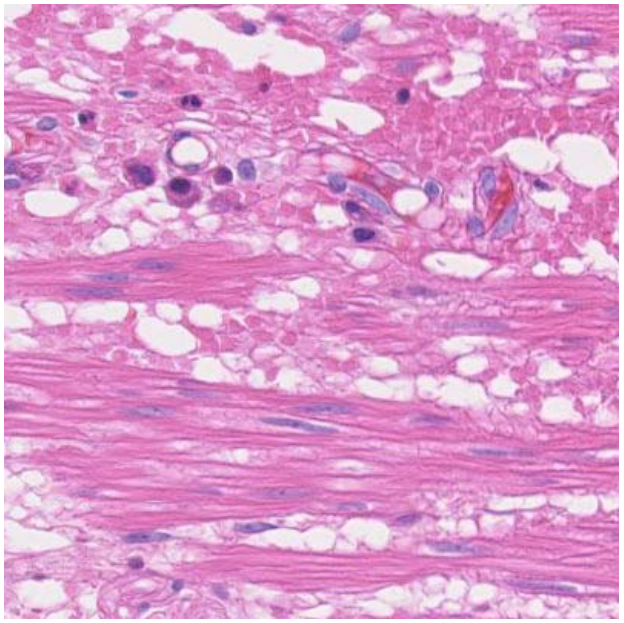
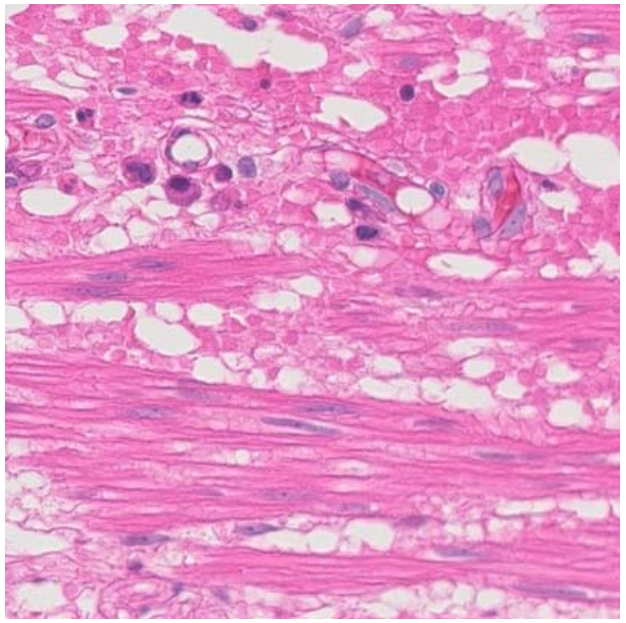
Scanner A

Scanner B

Original





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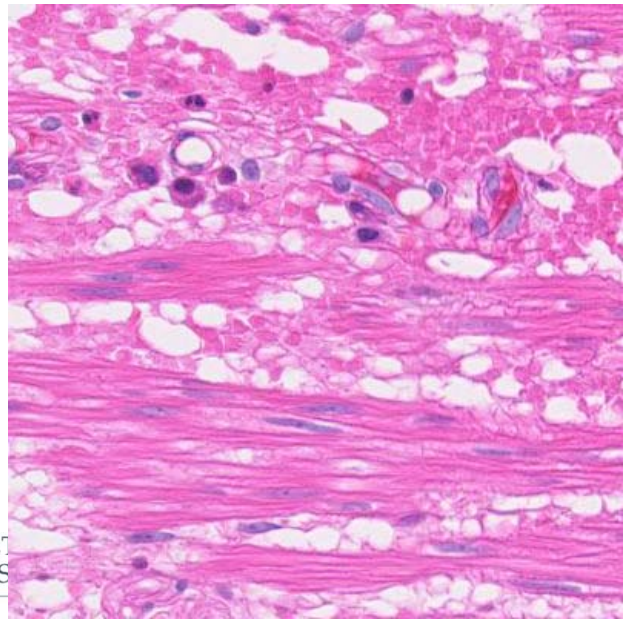

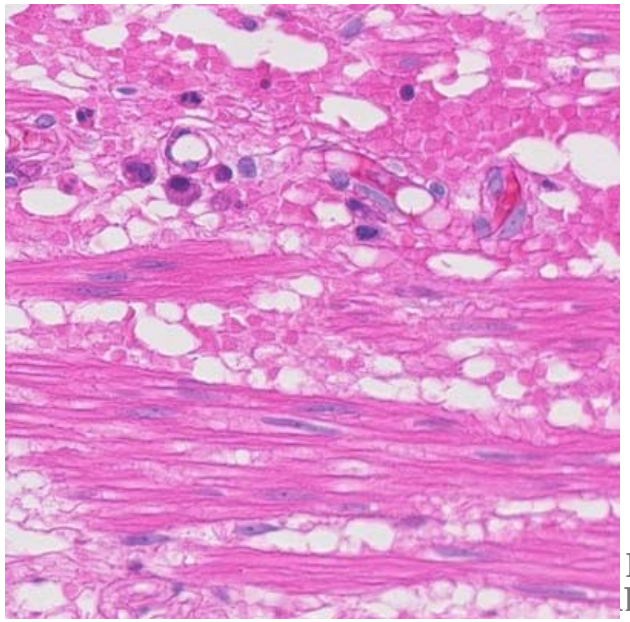

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Image Quality Evaluation

Image Quality Evaluation Algorithm

Image Quality Multiple regression analysis

Definitive evaluation index q is calculated by $q = \alpha + \beta s + \gamma n$

α, β, γ are derived from training data.

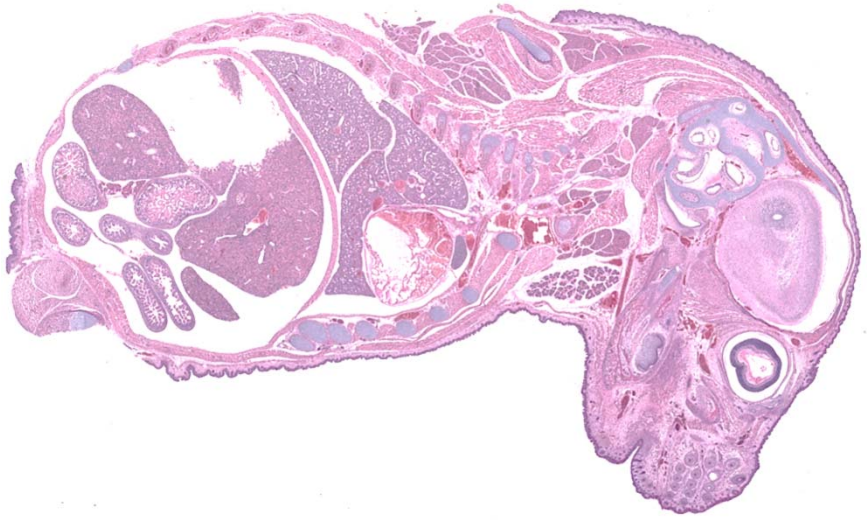

Image Quality Evaluation Method for Whole Slide Scanning

MASSACHUSETTS GENERAL HOSPITAL PATHOLOGY

Noriaki Hashimoto[1], Pinky A. Bautista[2,3], Masahiro Yamaguchi[1], Nagaaki Ohyama[1], Yukako Yagi[2,3]

[1]Tokyo Institute of Technology, [2]Massachusetts General Hospital, [3]Harvard Medical School

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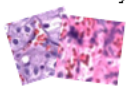
Introduction

What is whole slide imaging(WSI)?

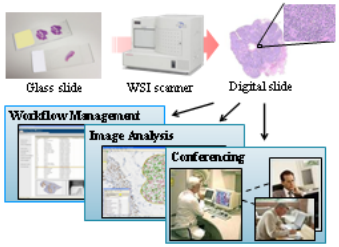
- WSI means to transform a conventional glass tissue slide into a digital image so users can access the image remotely on a computer monitor as if they are using a microscope

Issues with WSI

- WSI has to provide consistent high-quality images
- The images need good color representation
- Image data needs a standard format for storage



A high image quality is fundamental 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 scanner.



The purpose of this 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

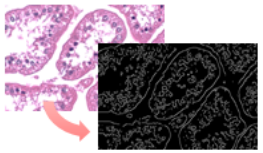
Evaluation Algorithm

Evaluation method is based on sharpness (focus) and noise

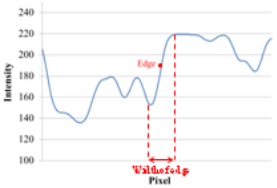
1. Sharpness evaluation

a. The edges in the image are detected using the Canny algorithm.

b. Pixel-widths of the detected edges are determined and the average value is used as the sharpness index,5.



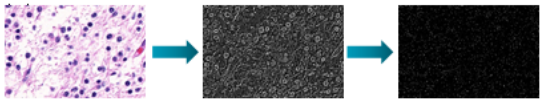
Edge detection



2. Noise evaluation

a. A unsharp masking technique is used to detect the edges and noises in the image.

b. The center pixel (3x3-pixel 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



Unsharp masking (Edge and noise detection)

Calculate the minimum difference within 3x3 pixels (Edge reduction)

Multiple regression analysis

Definitive evaluation index q, is calculated by

$$q = \alpha + \beta s + \gamma n$$

α, β, γ are derived from training data.

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, using the objective evaluation values allows the result 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 2.0HT (HAMAMATSU), and trimmed into 400x400 pixels.

We conducted a survey to get the subjective scores of pathologists, technicians, and image specialists. The images were rated on a scale of one to five, i.e., 5 was the best quality and 1 was the worst. The average scores of each image were used for multiple regression analysis, in which we investigated the correlation between the computed results using our algorithm and the subjective scores. From the regression analysis results, we determined the appropriate image quality parameter value, i.e., threshold value between good and bad quality image.

The screenshots of the survey

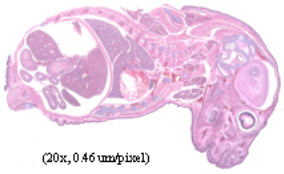
2. Application to WSI

We applied the proposed image quality evaluation method to the WSI of an H&E stained mouse embryo. Its image size was 36,000x24,000 pixels.

The entire image was divided to 400x400-pixel blocks, and the evaluation algorithm was applied to all blocks. The equation derived by multiple regression analysis was used to evaluate the image quality of each block.

In this experiment, the block, whose evaluation index was greater than the threshold value based on the results of the subjective experiment, was visualized with the original color. Otherwise, each block was shaded depending on the evaluation index. The blocks, which had more white pixels than 75% of the block, were regarded as background and also visualized with the original color.

Original image



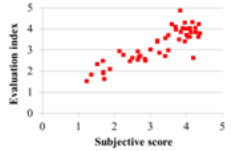
(20x, 0.46 um/pixel)

Results

1. Evaluation of the algorithm

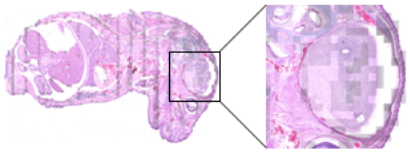
In the result of multiple regression analysis, the correlation coefficient was 0.869.

This confirms the evaluation indices are highly correlated to the subjective scores. In this case, we defined 3.5 as the threshold for the good-quality image.



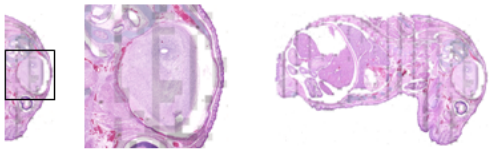
2. Application to WSI

We scanned the mouse embryo slide in automatic focusing mode and applied the proposed image quality evaluation method.



The image quality evaluation result of the automatically scanned slide (darker is poor/slow lower quality)

Some regions of the evaluating result indicated the low image quality. We specified a focus point on such region manually and the mouse embryo slide was re-scanned. Then, the image quality evaluation method was applied to the re-scanned image.



The image quality evaluation result of the re-scanned slide (darker is poor/slow lower quality)

Stitched image where the re-scanned region was integrated into the original image

The image qualities of the regions, which had low-quality indices in the original image, were improved. By integrating improved regions of the re-scanned image into the original image, the image quality of the entire image improves.

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 focusing mode. Then, the image quality of the scanned image (WSI) was evaluated using our proposed method. The slide was re-scanned wherein a focus point was specified on regions in the whole slide image which exhibited low image quality values. These regions were replaced with their counterparts from the re-scanned image. In the actual implementation however, the scanner's protocol could be configured such that only the regions with low image quality values will be re-scanned. The results presented above show the effectiveness of the present image quality evaluation method in identifying the quality of the image.

The current scanner that we used in our experiment implements line scanning. So that, the shaded strips present in the whole slide images correspond to edges of the line. It is part of our future work to investigate the performance of the present image quality evaluation on other scanners with different scanning method.

Conclusion

The image quality evaluation algorithm is extremely important for WSI. Results of our experiments show that by incorporating the proposed image quality evaluation method, the quality of whole slide images is improved. The image quality evaluation method that we presented could be integrated to the scanning procedure of digital slides. The effectiveness of the evaluation indices used in our experiments were confirmed through linear regression analysis.

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

Color Standardization Algorithm

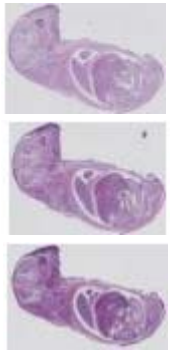
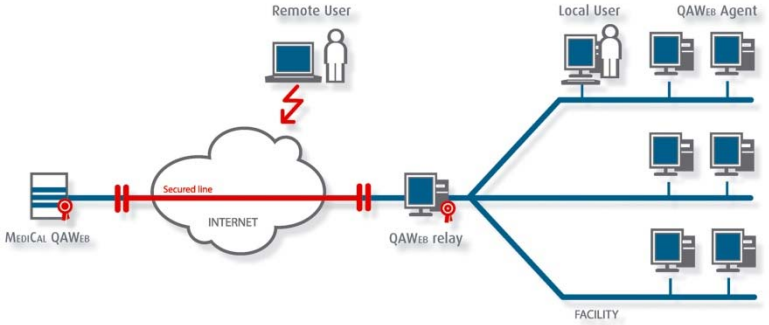


Image Quality Evaluation Algorithm

Display

Online Management System is available



Staining

Digital Staining Standardization is available

Acknowledgements

- This research was partially supported Olympus, Canon, 3DHISTECH, Kurabo
- PICT Lab, Pathology Informatics, Department of Pathology at MGH

Thank You!