Color Within The Context Of Whole-Slide Imaging

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Disclosure

- “Employee” Of The US Federal Government
- Clinical and Laboratory Standards Institute
  - Member, Consensus Committee On Immunology and Ligand Assays
  - Former Co-Chairman, Subcommittee On Quality Assurance For Immunohistochemical Procedures
- Center For Device & Radiological Health, Food & Drug Administration
  - Consultant, Hematology & Pathology Devices Panel
  - Collaborator, Critical Path Initiative On WSI
- Society Leadership Roles
  - Histochemical Society, Councilor
  - Association for Pathology Informatics, Program Chair-Elect

Veritas Vos Liberabit

The Truth Shall Set You Free

Quid Est Veritas?

What Is Truth?

Truth Function

- One Of The Deepest Debates Of Philosophy
- Scientific Approach – Demonstratable Based On The “Laws of Nature”
- “Fundamental Truths” Based On Thought & Belief

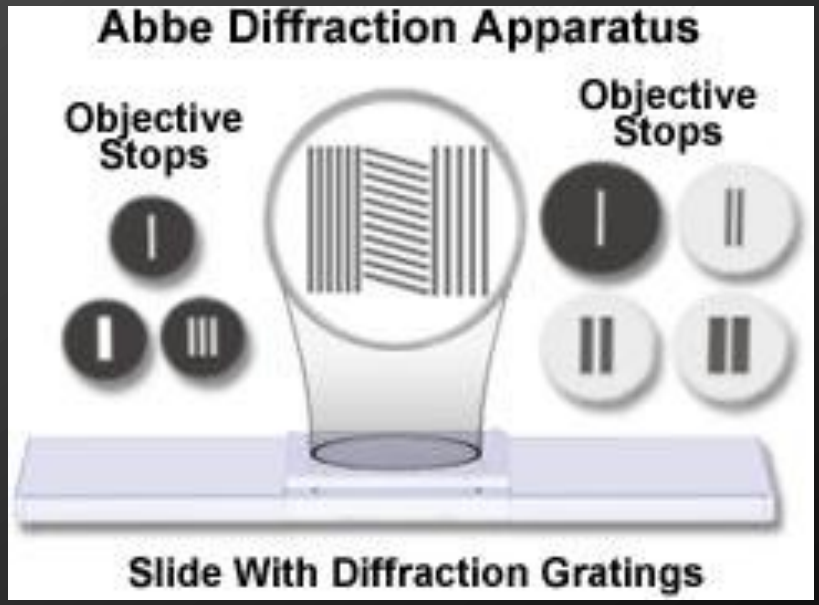
Color In The Context Of Physics

- Wavelength
- Photon Number
- Photon Density

- Changes In Color With Transmission Across Space and Media Are Defined

Defining Color As An Absolute

- Wavelength per unit volume
- Can be objectively evaluated within the context of *total test* by means of a Abbe Diffraction Grating



http://micro.magnet.fsu.edu/optics/timeline/people/abbe.html

Color Within A System Of Relativism

Je pense donc je suis

Rene Descarte

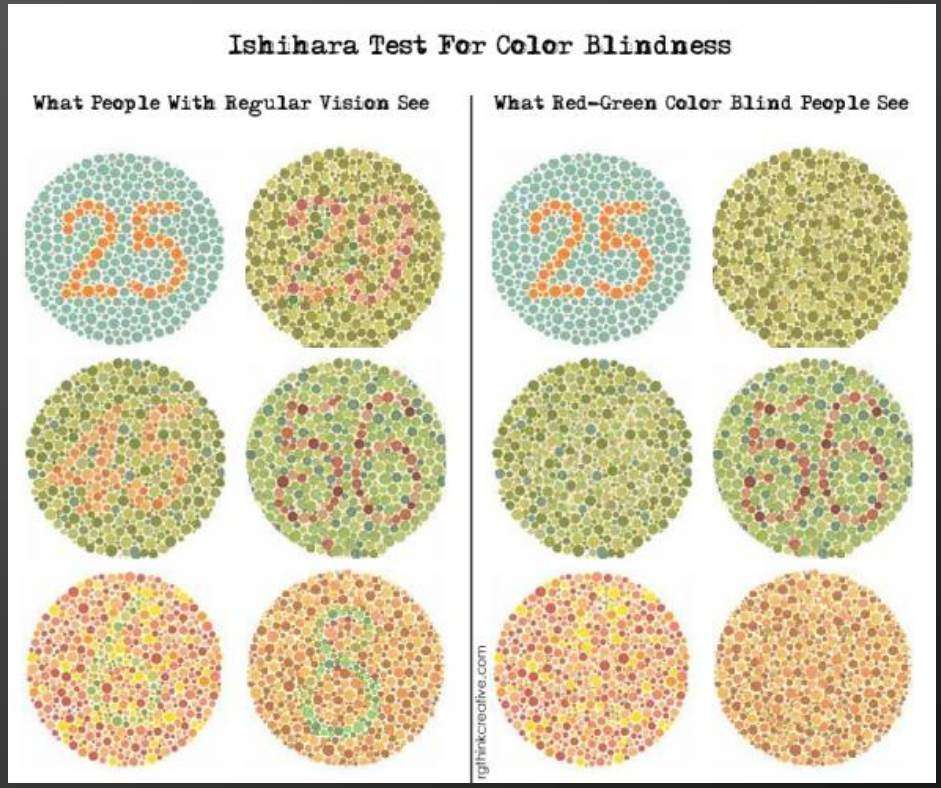
I Think Therefore I Am

I See, Therefore There Is Color

SMH

Color In The Context Of Perception

- Relative
  - Color Is Defined By Comparison
  - Colorblindness
- Impacted By:
  - Physiology
  - Age
  - Experience
  - Viewing Environment/ Illumination



Ends, Means, Goals

- Philosophy Of Science Model Proposed By Peter Achinstein
- Balances Scientific Knowledge With Empiricism & Anarchy/Nihilism
- Constitutes A “Fit-For-Purpose” Model Where A Gold Standard Is Neither Feasible Or Required, But Encompasses “Intended Use”

Ends, Means Goals
In The Context Of Color In Pathology

- Human Visual Perception/ Discrimination
  - Fund of Knowledge
  - Contrasting Colors
  - Accommodates Limitations Of Observer
    Color Blindness Aging
- Multiple Externals Impact Color
  - Preanalytic Factors
  - Analytic Factors
  - Instrumentation (Microscope) Factors

- Wide But Definded Boundaries Of Acceptable Color

As The Detection System Changes, So Does The Way Use Images

Goal Of WSI In Medical Imaging

- What Is The Rational For Adoption WSI for The Evaluation Of Pathology Slides?
  - What Benefit Will It Provide?

- Clearly The Goal Is Improved Patient Care
  - What Are The Intermediates To Improving Care?
- Pathology Is Based On A Shared Fund Of Knowledge
- Therefore The Capacity To “Share” Slides Is Required

Success of WSI In Clinical Practice

- Color Must Be Reproducible Between All Systems
  - In The Past The Microscope Was The Medium For The Pathologist To View The Slide (The Truth Object)
  - The Pathologist Could Control The Microscope
- In The Instance Of WSI, The Image Becomes The Medium Of Viewing
  - The Pathologist Can Modify The Image With Software
  - The Image Must Have Veracity To The Slide

Redefining The Question Of Color In WSI

- What Is Required To Ensure That A Slide (The Primary Truth Object) Is Faithfully Reproduced In Electronically To Become A Surrogate Truth Object?
- Addresses
  - Human Reading Of Slides
  - Sharing Of Slides
  - Computer Aided Diagnosis
- Consequence
  - May Require The Instruments To Define Limits Of What They Can Faithfully Reproduce

Practical Considerations In WSI

- Automation Drives Standardization
- Instrumentation Should Have A Required Specification Of An Acceptable Specimen For Satisfactory Performance
- Physical Attributes Of The Slide
- Stain Characteristics Of The Slide

It Is More Than An Issue Of “Color”

- The Generation Of An Image Based On What The Detectors Capture Is Trivial
- Display Of The Image Is Obtainable
- The Optics & Illumination Of What Is Presented To The Detector Is Critical
  - This Appears To Be The Substantial Challenge
  - Illumination To Generate The Full Dynamic Range & Contrast Features

Optics Of Image Generation

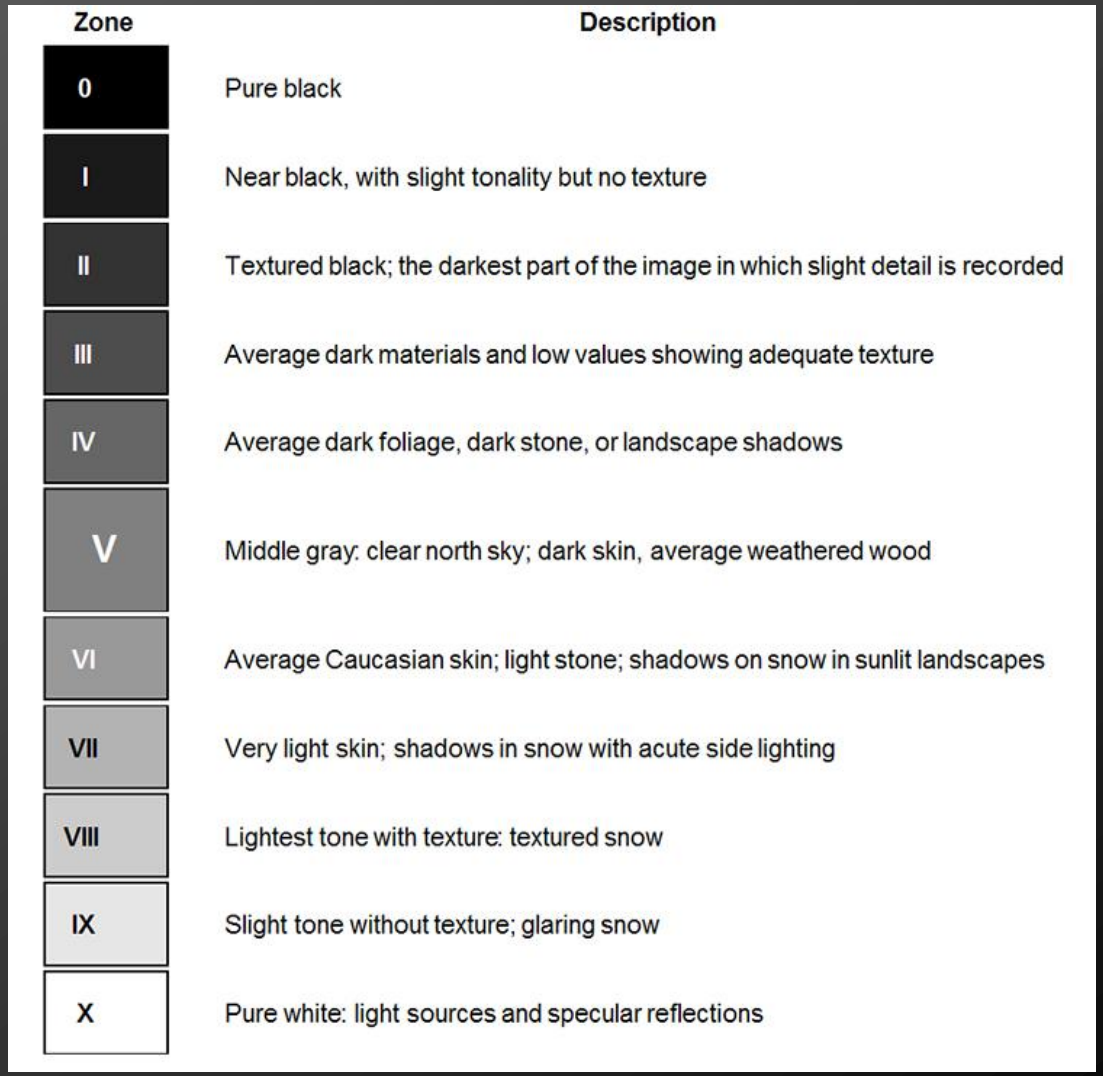
- Objective
  - Aberrations
  - Numerical Aperture
- Condenser
  - Design
  - Numerical Aperture
- Light Source
  - Color Temperature
  - Stability

- Image Object
  - Absorption-Based Color
    - Stains
      - Beer's Law
  - Defraction Based Color
    - Scatter Based Contrast
    - Cellular Organelles
    - Silver Impregnations
  - Object Of Finite Thickness
  - Difficult To Reconcile

Goal: Generate An Image That Replicates What A Pathologist Sees With A Standard Microscope

Dynamic Range

- Multiple Methods To Describe
- Commonly Bastardized To 8-Bit Color
- Can Be Applied To Color
- Critical Issue Is The Loss Of Information At Both Ends Of Scale



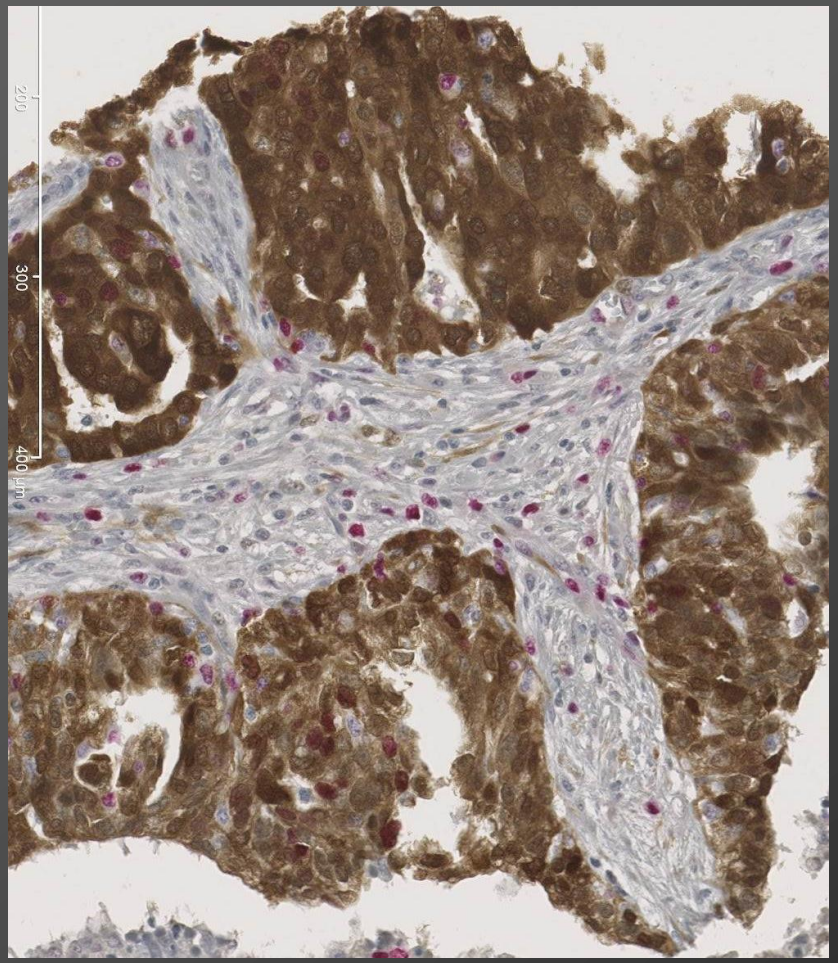
Zone	Description
0	Pure black
I	Near black, with slight tonality but no texture
II	Textured black; the darkest part of the image in which slight detail is recorded
III	Average dark materials and low values showing adequate texture
IV	Average dark foliage, dark stone, or landscape shadows
V	Middle gray: clear north sky; dark skin, average weathered wood
VI	Average Caucasian skin; light stone; shadows on snow in sunlit landscapes
VII	Very light skin; shadows in snow with acute side lighting
VIII	Lightest tone with texture: textured snow
IX	Slight tone without texture; glaring snow
X	Pure white: light sources and specular reflections

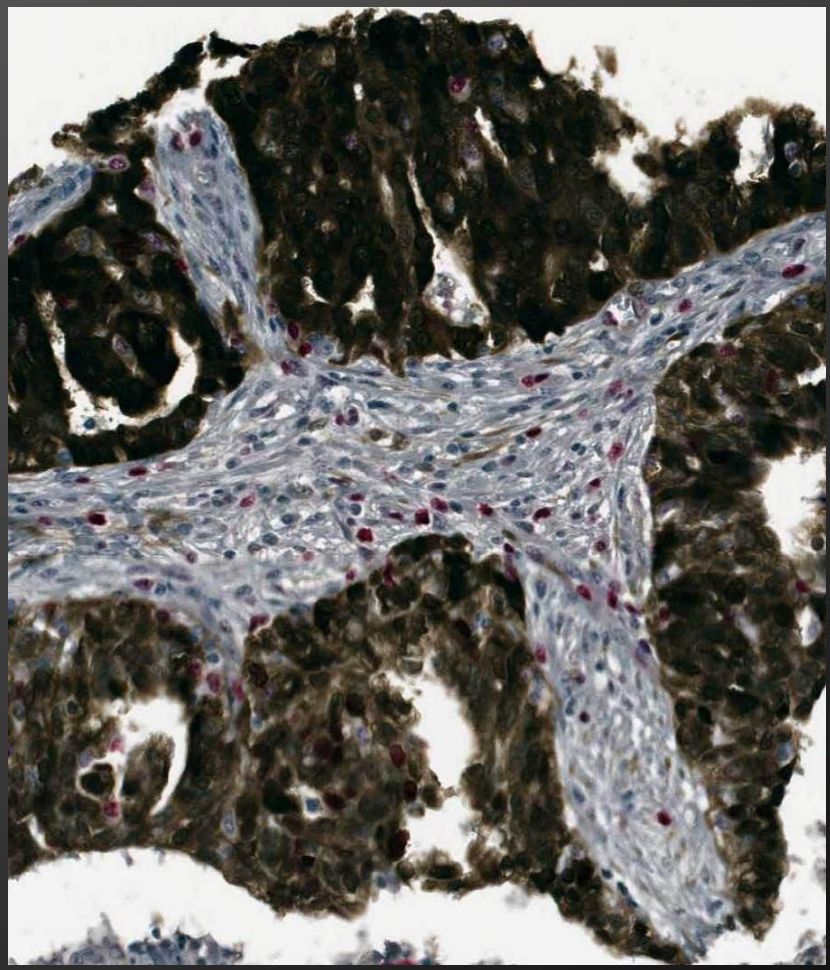
The Challenge Of Whole Slide Imaging

- Making The WSI Is Easy
- Making The WSI *Robust* Is A Work In Progress:
  - Contrast
    - Illumination & Optics
  - Color
    - Reference Standards
  - Spatial Reproduction / Scale
    - Reference Standards

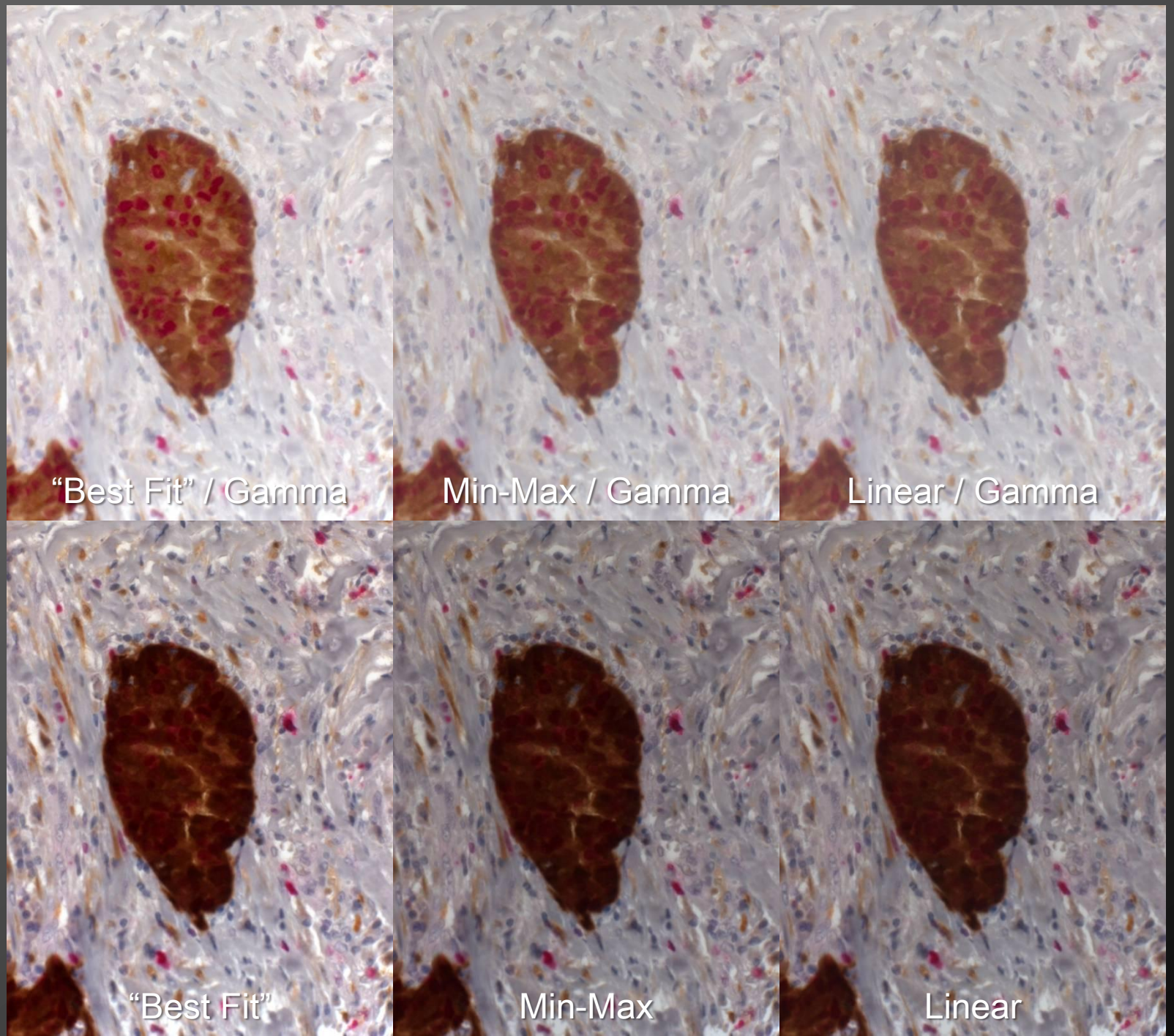
Which Is Truth?

Difference Between Images Is A Result Of Condenser Design

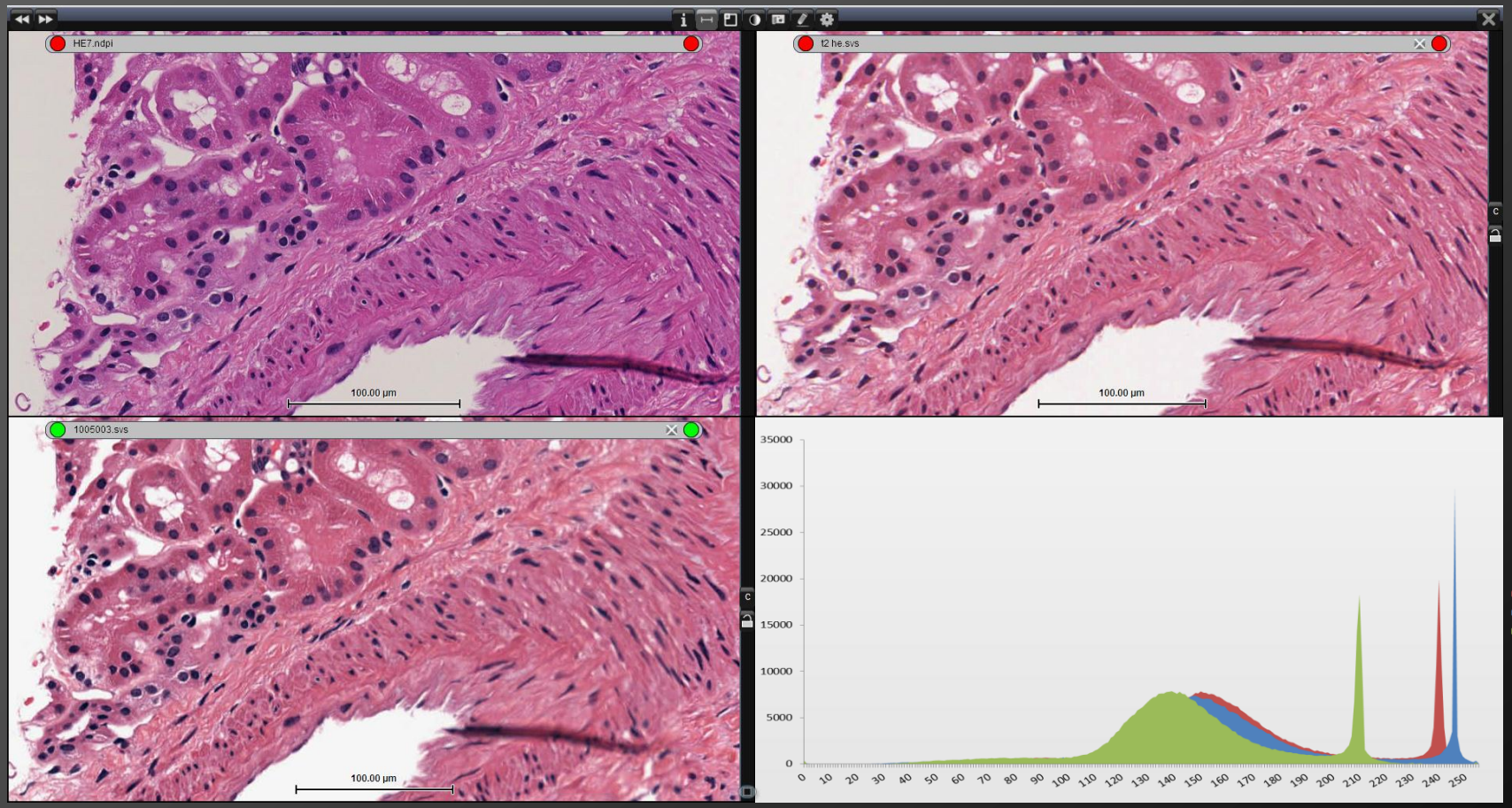




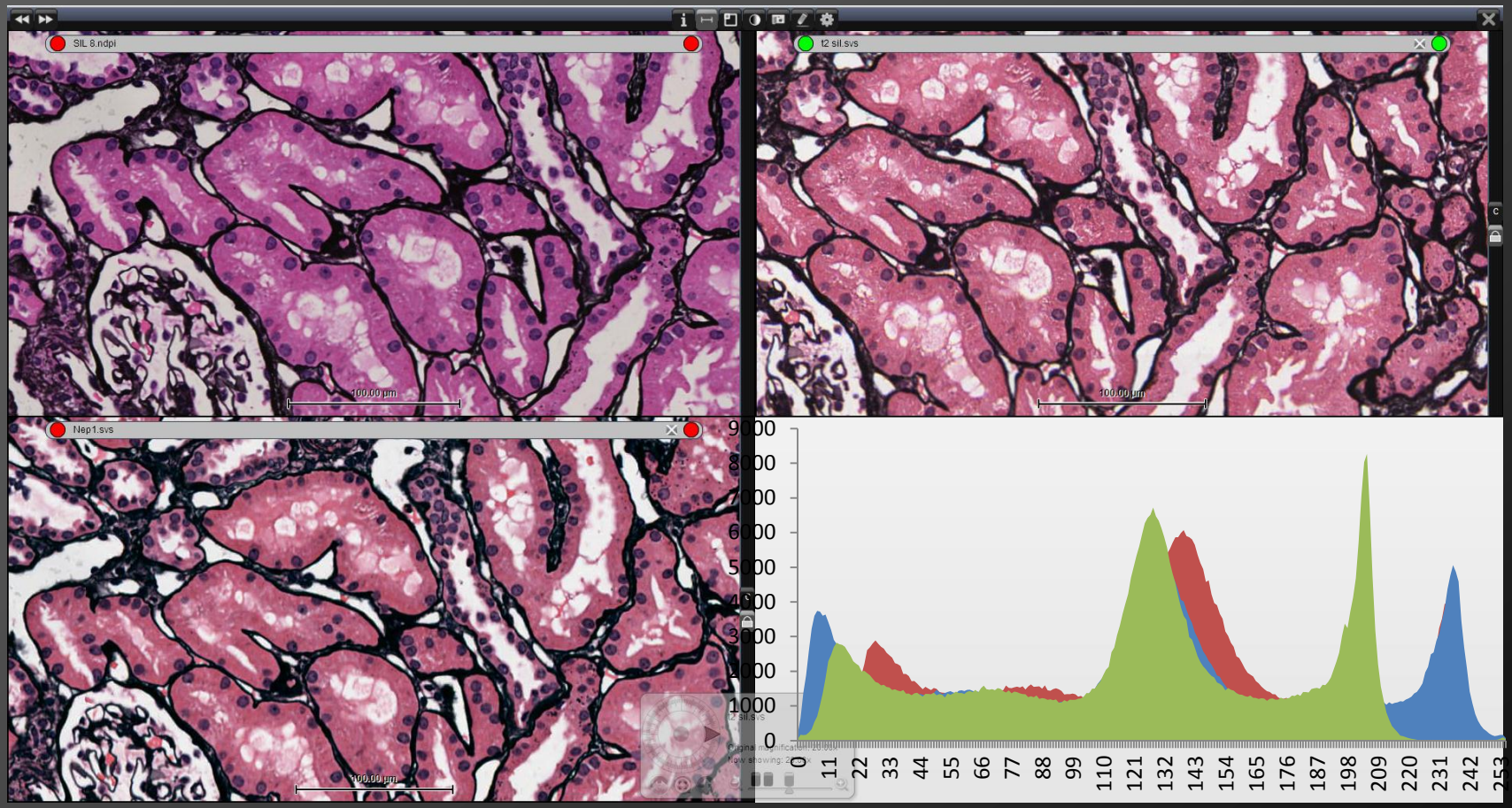
Pass **Intended Use** **Fail**


“Best Fit” / Gamma
Min-Max / Gamma
Linear / Gamma
“Best Fit”
Min-Max
Linear

Differences In Color



A Silver Stain Example



Truth In Color

- Defining True Color Is Challenging
- Reproduction Of Color Between Instruments Should Not Be Difficult
- A Color Standard Allows Interchange Of Images As The “Truth Object” In Place Of Slides

- Defining Boundaries Of Acceptable Slides For Imaging Is Encouraged

Accurate Reproduction Of “Color”

- Reproduction Of Color
- Reproduction Of Contrast
- Reproduction Of Dynamic Range

- Instrument
  - Illumination, Optics & Detector
- Software
- Displays

Final Thoughts

- A “Fit-For-Purpose” Definition Of Color Is Needed
- Whole Slide Imaging Is About The Exchange Of Images By Definition
- An Obtainable, Reproducible Standard Of “Color” Is An Appropriate Goal
- The Spectrum Of Colors & Performance Features Should Replicate The Entire Spectrum Of Colors & Features That Can Be Encountered In Microscopy