Whole slide imaging: what the future holds now that the FDA has approved a system for routine diagnostic pathology case reporting

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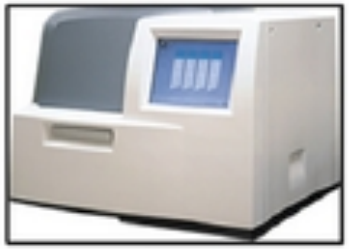
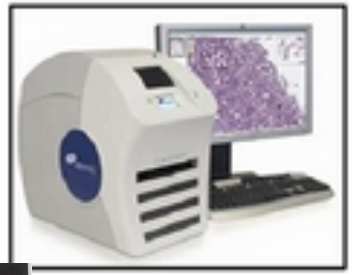
Dr. Boyce has no conflicts

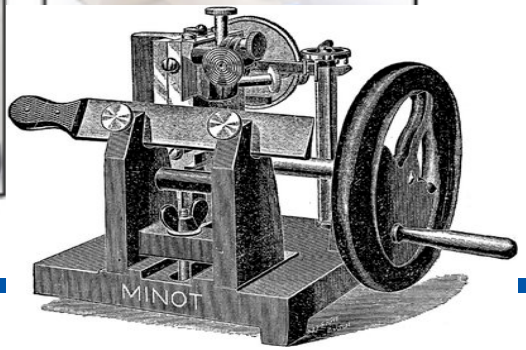
Pathologists have been using imaging devices to view and analyze glass slides for more than 150 years

Types of imaging devices

Microscope with digital camera	Whole slide scanners	Robotic microscopes	Image analysis systems







What is Whole Slide Imaging?

Glass Slide

Scanner

Storage

A robotically controlled microscope scans slides and converts captured images into digital files using computer software and hardware designed to impersonate a traditional microscope for subsequent viewing of glass slides

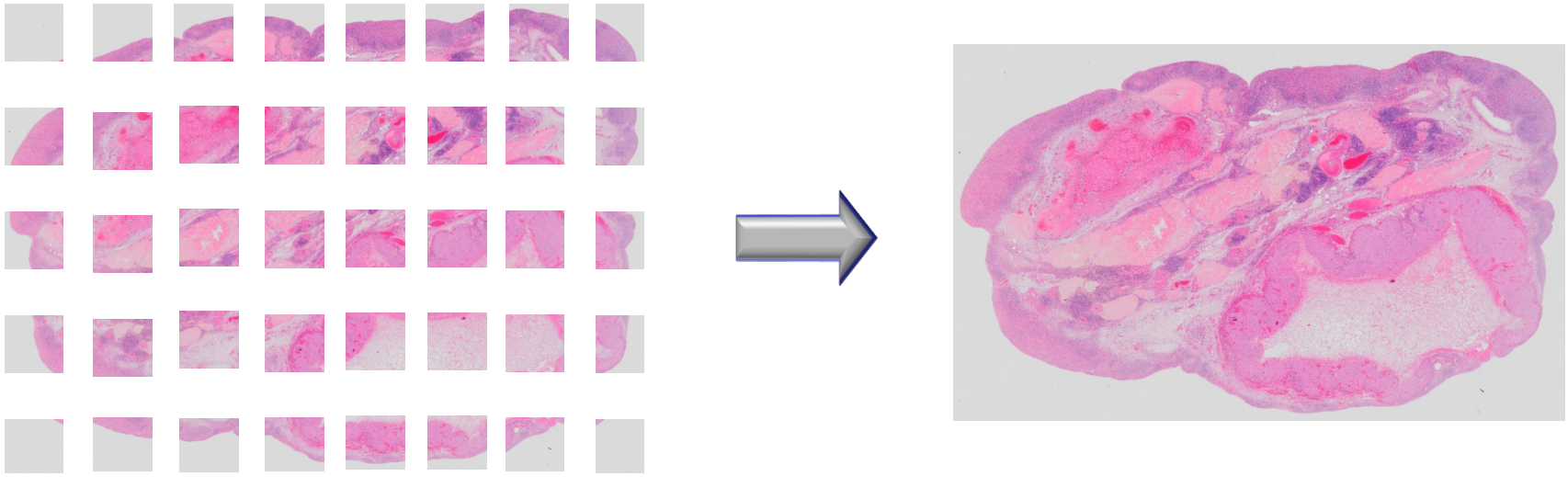
• These digital images should be equivalent to the images observed when original glass slides are viewed using conventional microscopes.

Whole Slide Imaging

A virtual slide is a **digitally captured glass slide** comprised of multiple high quality portions of the images stitched together electronically.

A computer and monitor are used to **view, navigate, change magnification, and focus** throughout the virtual slide with speed and ease.

A Virtual Slide file size is typically **hundreds of Megabytes to a few Gigabytes** depending on the area scanned and the objective used.



Virtual Microscopy Applications

- **Hospitals**
  - **Slide image storage**
  - **Retrieval of slides for on-site or remote review**
  - **Interpretation of H&E-stained regular and frozen sections and of special stains, including IHC**
  - **Teaching residents and other students/staff**
  - **Research studies**

Slide Storage and Handling at URMC 2018

Slide Drying before Storage at SMH 2018

Medium-term Slide Storage at SMH 2018

WSI can eliminate short-long-term glass slide storage, but requires very large storage capacity, similar to radiology

Estimate of glass slide storage at Strong Memorial Hospital, Rochester, NY in 2028

In 2004, we accessioned >39,000 cases,
These generated >113,000 blocks and **>170,000 slides**.

In 2014, we accessioned >55,000 cases
These generated >171,000 blocks and **>240,000 slides**
This is a 35% increase over 10 years, totaling **>2 million slides**.
With a 35% increase to 2024, we estimated storing **>3 m slides**

After some hospital reorganization in 2016,
we accessioned >90,800 cases from 3 hospitals
These generated >310,600 blocks and **>549,900 slides**
With a 35% increase to 2028, we estimated storing **>6.6 m slides**

NYS requires retention of slides for 20 years
We currently store slides from the past 20 years in >1000 cabinets that occupy ~380 sq. ft. within the hospital.

Advantages of digital slide storage

1. Virtual slides stored off site on servers in the “cloud”
2. The staining will not fade with time
3. Mounting medium will not dry out
4. Slides will not be taken or broken and not returned
5. Slides can be viewed on a computer anywhere by personnel with access privileges for diagnosis and teaching
6. Fewer requests for recuts

Limitations of digital slide storage: Costs

Whole slide imagers: $100-300K/instrument
Maintenance contracts: $15-20K per instrument
Set up and continuing IT support:

Isaacs et al. (2010) estimated that they made an initial capital investment of $2 million to purchase all hardware and software needed to establish a fully integrated system and projected a yearly indirect cost of approximately $650,000 for support personnel.

Developing a working interface between the WSI system and the LIS estimated to be approximately $70,000.

Limitations of digital slide storage: Costs

File size can be reduced to ~300-500MB from 1-5GB, but with ~500 slides/day would need >175 GB/day and up to 5 terabytes/month.

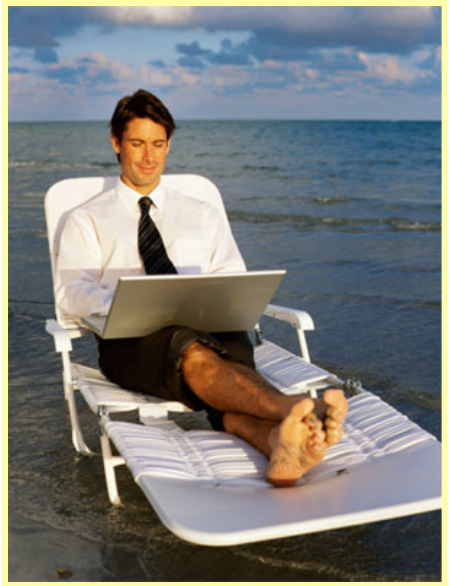
Webster and Dunstan (2014) reported the annual cost for storage with back-up and security for 2,000 images with an average file size of 500 MB (these would fill a terabyte-sized file) ranged from $3,000 to $10,000, i.e., $1.5−$5/digital slide/year

Our costs for storing images of this size for the >2 million slides we have generated during the past 10 years would be $3−10 million/year for the 1000 terabytes of storage required!

These costs could be limited by storing only selected slides: Which ones?

Virtual Microscopy Applications: Clinical Practice

- **Rendering primary diagnosis on routine cases**
  - **Local or remote diagnosis; remote local or expert consultation**
  - **Frozen section diagnosis, with consultation from expert colleagues**
- **Tumor boards**
  - **Eliminates need to have a microscope in each room or to take pictures for PPT presentations**



Limitations of use in regular clinical cases: Pathologist's unfamiliarity with the technology

- Most are unfamiliar with the technology and have not used it.
- Residents loading slides for remote review by pathologists out of hours would need to maintain these skills

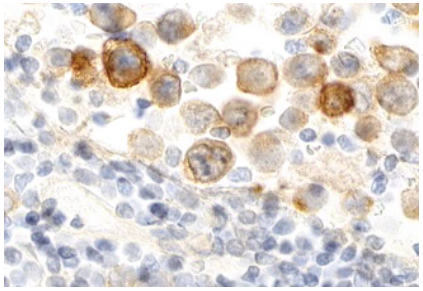
- Initially will take a significant effort to learn how to use it.

- Will seem much slower than placing slides on a stage and dictating a diagnosis.

- May be resistance to give an opinion remotely on challenging cases

  - **Was approved in Canada and parts of Europe 3-4 years ago, but so far very limited uptake.**

Virtual Microscopy Applications



- **Immunohistochemistry (IHC)**
- **- Multiple diagnostic and research applications especially when linked to an image analysis system**
- **- Automated quantification of percentage of Ki67 positive cells or intensity of staining of Her2 in breast cancer cases possible**
  - **Currently this is done by eye and estimated**
  - **There is no formal training for residents to quantify anything in histopathology, yet these are estimated by pathologists**

HER2 scoring today

Microscope-based

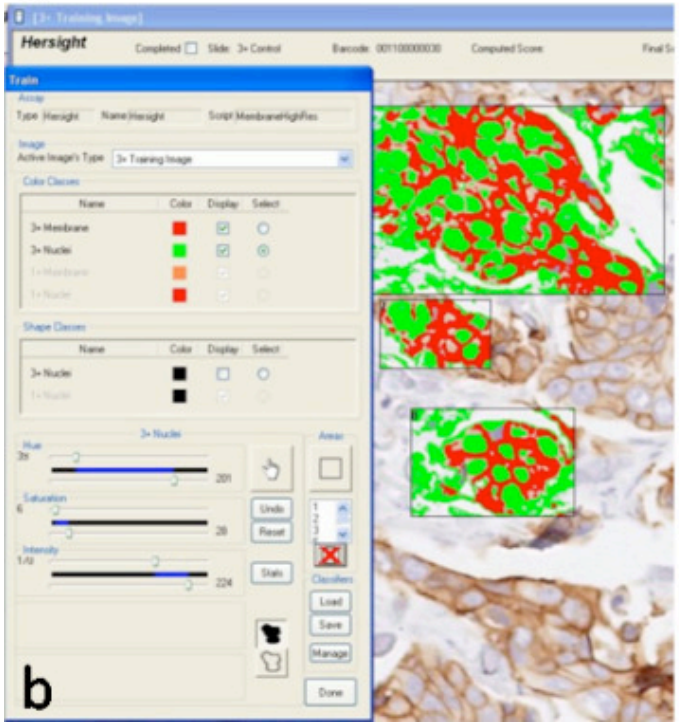
- Immunohistochemistry using glass slides
- Subjective
- Prone to inter- and intra-observer variation

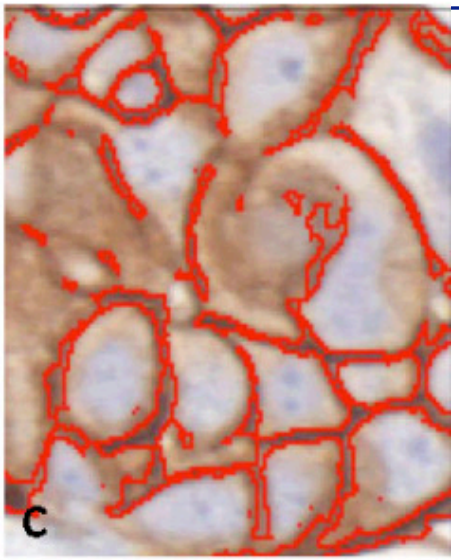
Limitations of WSI for quantification of IHC staining

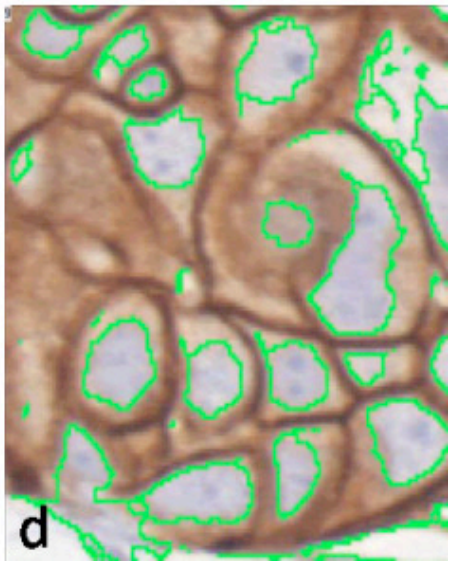
Although the FDA has approved the use of WSI for quantification of Her2 staining, few labs are using it, in part because few hospital labs have a WSI system or the one approved for this purpose.

Grading and quantifying negative, 1+ and 3+ staining is straightforward, but accurately grading and quantifying 2+ staining is problematic for glass and digital slide reading

Her2 can be scored today using FDA-approved image analysis systems (e.g. ACIS; ARIOL)







a) Ariol automated image analysis system

b) Training window displaying the 3+ membrane and nuclear colors with fill mask.

c) Outline of membrane as detected by the color classifier for the 3+ membrane color class.

d) The border mask of nuclei as detected by the color classifier for the 3+ nuclei color class.

(BMC Cancer 2009, 9:165)

There is large inter-observer variability in HER2 scoring using conventional microscopy

- Positive rate of HER-2 using results of immunohistochemical analysis was reported to vary from 2% to 89%

  (Am J Clin Pathol 2002;118:693-698)

- **However, HER2 gene expression is amplified in only 10–35% of invasive breast carcinomas**

Evaluation of inter-observer reproducibility of HER2 scoring among 5 pathologists

- 46 cases of invasive breast carcinoma studied.
- Complete agreement in 22 (48%) of 46 cases.
  - Generalized kappa values indicated substantial agreement (0.80).
- Distinguishing weakly (2+) from strongly (3+) positive results showed agreement in only 13 (59%) of 22 positive cases (kappa = 0.38).

(Am J Clin Pathol 2002;118:693-698)

Findings of a study comparing conventional glass slide and digital reading of HER2 staining in breast cancers by 3 pathologists at 2 sites

- **25 - 34% discordance rate for HER2 IHC scoring among 3 experienced breast pathologists at each of 2 sites reading the same slides on a microscope *or* digitized images on a computer screen.**
- **Tendency towards more variation with digital reading**
- **One pathologist's scoring varied significantly from that of the other 2 pathologists at each site.**
- **4-8% of patients could have been given treatment erroneously, based on digital scoring**
- **More study is needed to determine if inter- and intra-observer variation in scoring can be decreased as pathologists become more aware of their scoring history and more familiar with virtual pathology microscopy**

Optimal tissue handling requirements

- Time from tissue acquisition to fixation should be ≤ 1 hour; samples for HER2 testing are fixed in neutral buffered formalin (NBF) for 6–48 hours.
- Time tissue is removed from patient, cold ischemia time, and time the sample was placed in NBF must be recorded.
- Initial test validation requires 25–100 samples tested by an alternative validated method in the same laboratory or by a validated method in another laboratory.

Optimal IHC testing requirements

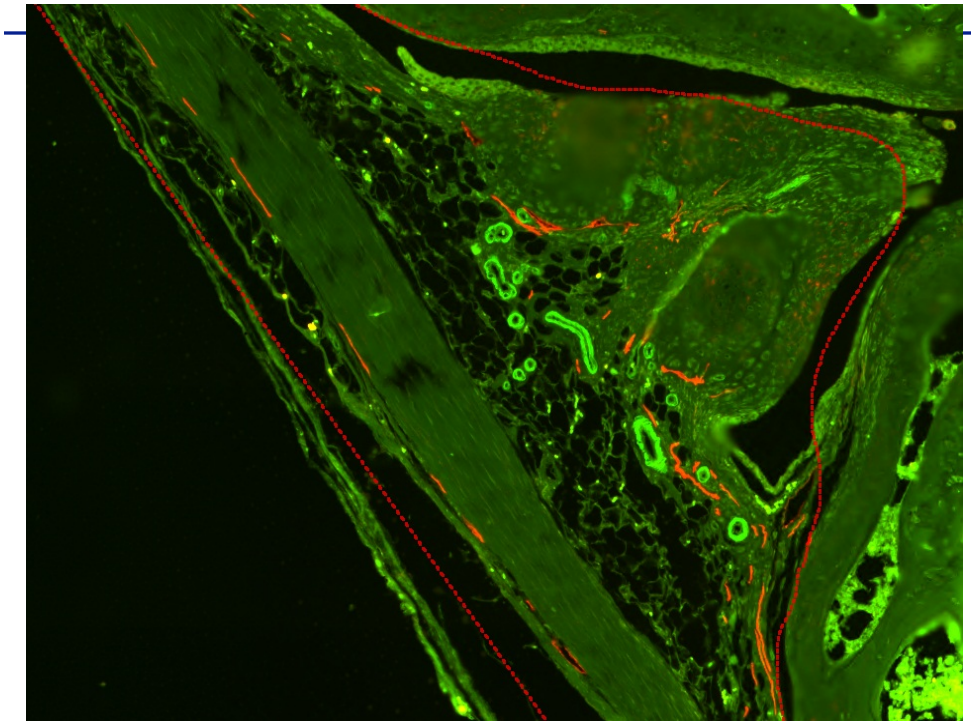
- Fixation for < 6 hours or > 48 hours is not recommended.
- Sample has strong membrane staining of normal breast ducts (internal control).
- Positive HER2 result requires homogeneous, dark circumferential (chicken wire) pattern in >30% of invasive tumor.
- Interpreters must have methods to maintain consistency and competency.

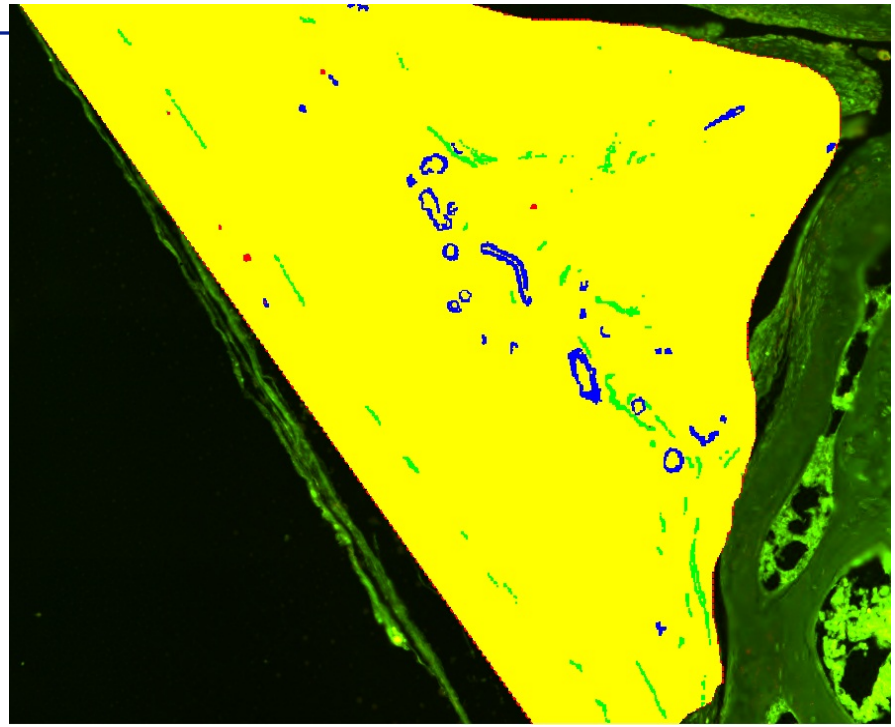
Virtual Microscopy Applications

Immunofluorescence

- Multiple diagnostic and research applications, including skin and kidney biopsy interpretation
- Major advantage is that slides are scanned once
- The high quality images are then stored for multiple subsequent assessments without the certainty of the signal diminishing and ultimately disappearing
- Quantification of features of images can be done without quenching of the signal intensity

Lymphatic vessels analysis in soft tissue of knee joint of a mouse with osteoarthritis



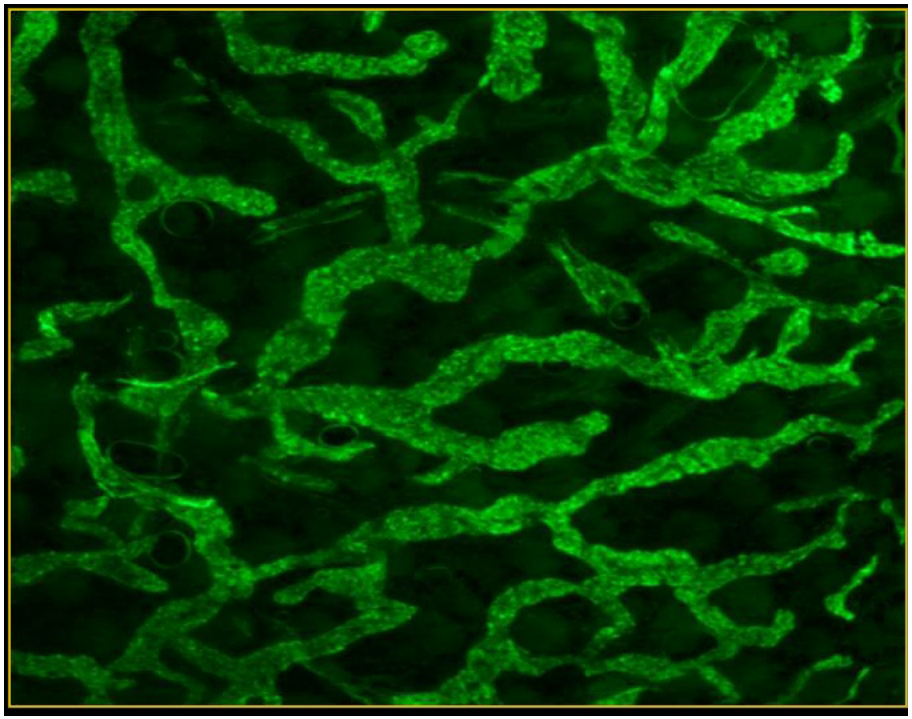


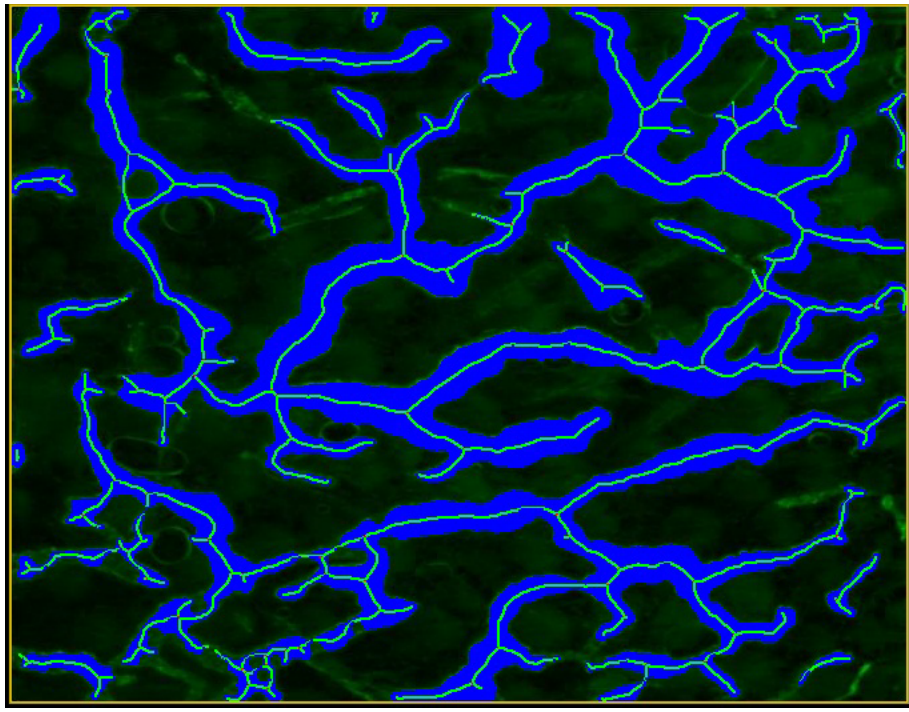
Anti-podoplainin(red) and anti-SMA (green) immunofluorescent staining

Result	Value
red area	4598.62379066178
green area	5305.90508353539
yellow area	103.630958662801
red and green area	9904.52887419717
red,gree, yellow area	10008.15983286
bg area	600696.851888924
total area	610705.011721784
red area ratio	7.53002464757363E-03
green area ratio	8.68816364970749E-03
yellow area ratio	1.69690696283349E-04

Podoplanin is expressed by lymphatic endothelial cells.
SMA is expressed by blood vessel smooth muscle cells

Automated lymphatic vessel analysis in mouse ear





Immunostaining of lyve-1, a lymphatic endothelial cell marker;

Conversion of image to blue color using Visiopharm software

Result	Value
No Branchpoints	103
Area Vessels	3952755723.97661
Total Length	245726729.824561
Mean Diameter	16.0859818823891

Virtual Microscopy Applications

- **Education:**
  - **Medical Student, Resident, Histotechnologist, Pathologist's Assistant**
  - **Standardization of teaching samples**
  - **24-hour access to samples**
  - **Virtual Teaching Lab**
  - **Residency training and assessment of skills**

Virtual Microscopy for Teaching

- **Many Medical Schools now use digital slides to teach medical students:**
  - **Students and pathologists generally like it**
  - **All students now look at the same slide and quality does not change with time.**
  - **No need to cut up to 240 slides to replace old slides**
  - **Easy to switch out slides to change focus of labs**
  - **Standardization of teaching samples**
  - **24-hour access to samples**
  - **Virtual Teaching Lab**
  - **Residency training and assessment of skills**
    - **CAP checklist slides are now digital images**

Virtual Microscopy for Teaching

- **Residency training and assessment of skills**
  - Used to assess the diagnostic skills of pathology residents in the RISE exams and in the Board examinations of the College of American Pathologists.
  - Also are offered as an alternative to glass slides by the American Society of Clinical Pathology (ASCP) for their CheckPath continuing medical education/ competency assessment instrument for practicing pathologists and residents.
  - Pathologists submitting cases to ASCP for the CheckPath program would need to submit only one slide, not scores of them

CAP Validation Recommendations

- Validation of the entire WSI system, involving pathologists trained to use the system, should be performed in a manner that emulates the laboratory's actual clinical environment.

- Validation study should include at least 60 routine cases per application, comparing intra-observer diagnostic concordance between digitized and glass slides viewed at least 2 weeks apart.
- Slides should be of high quality
    - (does this include bone sections?).

CAP Definition for Validation

- Validation, in the context of new technology or instrumentation, refers to a process that aims to demonstrate that the new method **performs as expected for its intended use and environment prior to its application for patient care**.

- Therefore, validation is recommended to determine that a pathologist can use a WSI system to render an **accurate diagnosis with the same or better level of ease as with a traditional microscope and without interfering artifacts or technological risks to patient safety**.

CAP Precautions for WSI

- Image quality
- Missed tissue on WSI
- Lack of experience of pathologist with WSI
- Specific microscopic details sometimes difficult to identify because of poor image resolution at high magnification or they go undetected (H. Pylori and mitoses/HPF)
- Virtual slide may take longer to review than the same glass slide using regular microscopy

Validation of whole slide imaging for primary diagnosis in surgical pathology

- Slides from 1,214 consecutive cases interpreted 1 year previously by 2 sub-specialty pathologists were retrieved from files at Cleveland Clinic.

- 607 alternate cases were scanned at original mag. of ×20.
- Each pathologist reviewed his or her cases using either a microscope or WSI.

- Independent pathologists identified and classified discrepancies.

Bauer TW, Arch Pathol Lab Med. 2013,137(4):518-24

Validation of whole slide imaging for primary diagnosis in surgical pathology

- Slides from 1,214 consecutive cases interpreted 1 year previously by 2 pathologists (1 orthopedic and GI; 1 general) were retrieved from files at Cleveland Clinic.
- 607 alternate cases, including 1,025 parts were scanned at original magnification of ×20.
- Each pathologist reviewed his or her cases using either a microscope or WSI.
- Both pathologists had reviewed several hundred WSI slides beforehand
- Independent pathologists identified and classified discrepancies. (Bauer TW, Arch Pathol Lab Med. 2013,137(4):518-24)

Validation of whole slide imaging for primary diagnosis in surgical pathology

- The major and minor discrepancy rates:
  - For WSI reviews were 1.65% and 2.31%, respectively.
  - For glass slide reviews were 0.99% and 4.93%, respectively.
- The authors conclude that WSI is not inferior to glass slide reading and that theirs is perhaps the most rigorous study published to date supporting the safe and efficacious use of WSI for primary diagnosis.

(Bauer TW, Arch Pathol Lab Med. 2013,137(4):518-24)

Validation of digital pathology imaging for primary histopathological diagnosis.

Largest WSI validation study before FDA approval

- 17 pathologists re-reported 3017 cases using WSI.
    - Of these, 1009 were re-reported by the same pathologist, and 2008 by a different pathologist.
- 97 cases (3.2%) required rescanning before a report could be issued

- Re-examination of 10,138 scanned slides (2.22 terabytes) produced 72 variances between glass slide and WSI reports, including 21 clinically significant variances.

Snead D, et al., (2016) Histopathology. 68, 1063-72.

Validation of digital pathology imaging for primary histopathological diagnosis.

- **'Ground truth' lay with glass slides in 12 cases and with WSI in 9 cases.**

- **In 3 cases, WSI accounted for the variance, including a gastric biopsy, where H. pylori only became visible on slides scanned at x 60, and bronchial and penile specimens, where dysplasia was reported by WSI, but was not present on glass slides.**
- **The results show that WSI is non-inferior to glass slide review.**

- This is one of the largest studies showing that WSI is equivalent to glass slides for the diagnosis of histopathology specimens.

Snead D, et al., (2016) Histopathology. 68, 1063-72.

Whole Slide Imaging Versus Microscopy for Primary Diagnosis in Surgical Pathology: A Multicenter Blinded Randomized Non-inferiority Study of 1992 Cases (Pivotal Study).

Using the FDA-approved Philips IntelliSite Pathology Solution

- **A blinded randomized non-inferiority study across the entire range of surgical pathology cases (biopsies and resections, including H&E, IHC, and special stains) from 4 institutions using the original sign-out diagnosis as the reference standard.**

- **16 pathologists interpreted 1992 cases, resulting in 15,925 reads by microscopy or WSI, followed by a wash-out period of ≥4 weeks, after which cases were read by the same observers using the other modality.**

(S. Mukhopadhyay, Am J Surg Pathol. 42, 1, Jan. 2018)

Whole Slide Imaging Versus Microscopy for Primary Diagnosis in Surgical Pathology: A Multicenter Blinded Randomized Non-inferiority Study of 1992 Cases (Pivotal Study).

Using the FDA-approved Philips IntelliSite Pathology Solution

Technical issues:

- In the first scan of 3390 slides, the Philips IntelliSite Pathology Solution was able to automatically detect an issue, such as no tissue or label detection, for 77 slides (2.3%).
- The images from 70 slides (2.1%) did not pass the image quality check by the scanning operator for slide-related issues such as prior ink markings, broken slides or debris on the slide.
- For 55 images (1.6%) the scanning technician identified an out of focus image (54 images, 1.6%) or missing tissue (1 image, 0.03%).

Measures aimed at accurately assessing intra-observer variability and mitigating the risk of bias, including selection bias and recall bias

- Selection of consecutive cases.
- Inclusion of a validation pathologist to validate cases selected by the enrollment pathologist.
- Randomization of reading order, division of cases evenly into batches, of cases between reads.
- Alternation of reading modalities by batch (ie, a batch of microscopy cases was followed by a batch of WSI cases on a different day).
- Blinding of reading pathologists to the reference standard diagnosis.
- Adjudication of concordance by pathologists different from reading pathologists

Whole Slide Imaging Versus Microscopy for Primary Diagnosis in Surgical Pathology: A Multicenter Blinded Randomized Non-inferiority Study of 1992 Cases (Pivotal Study).

Using the FDA-approved Philips IntelliSite Pathology Solution

- **The major discordance rate with the reference standard diagnosis was 4.9% for WSI and 4.6% for microscopy. The difference between major discordance rates for microscopy and WSI was 0.4%.**
- **The difference in major discordance rates for WSI and microscopy was highest in endocrine (1.8%), neoplastic kidney (1.5%), urinary bladder (1.3%), and gynecologic pathology (1.2%) where discrepancy rates between each modality and the reference standard was 5-6%.**
- **The authors conclude that WSI is non-inferior to microscopy for primary diagnosis in surgical pathology.**

Now that WSI has been approved for general use – what can we expect?

1. Improved efficiency and accuracy of pathology diagnosis – invest $$$, but save $$$
2. Better reproducibility of H&E and other ‘special‘ stains.
3. Advanced personalized diagnostics - already happening – one by one.
4. Double and multiplex IHC interpretation
5. Quantitative IHC – accurate, reproducible, measurements of analyte/cell or /area of tissue
6. Morphometry – new criteria for pathology primary diagnosis

Now that WSI has been approved for general use – what can we expect?

- Although up to 300 slides can be loaded and scanned automatically by some WSI, digital slides need to be examined individually by technical staff for defects, such as bubbles, folds, and other artifacts.
- One study reported that 18% of scanned slides were considered unsuitable for interpretation after scanning. (Al-Janabi et al. 2012)
- Others reported that the unacceptable rate can be lowered to between <1% to 5% by careful review and rescanning of unacceptable digital slides by trained technologists (Pantanowitz et al. 2012).
- Departmental IT staff also needs to be involved regularly to deal with logistic and network issues and ensure that interfaces with the laboratory and other hospital information systems are functioning optimally as part of a fully integrated digital pathology service (Griffin & Treanor 2017)

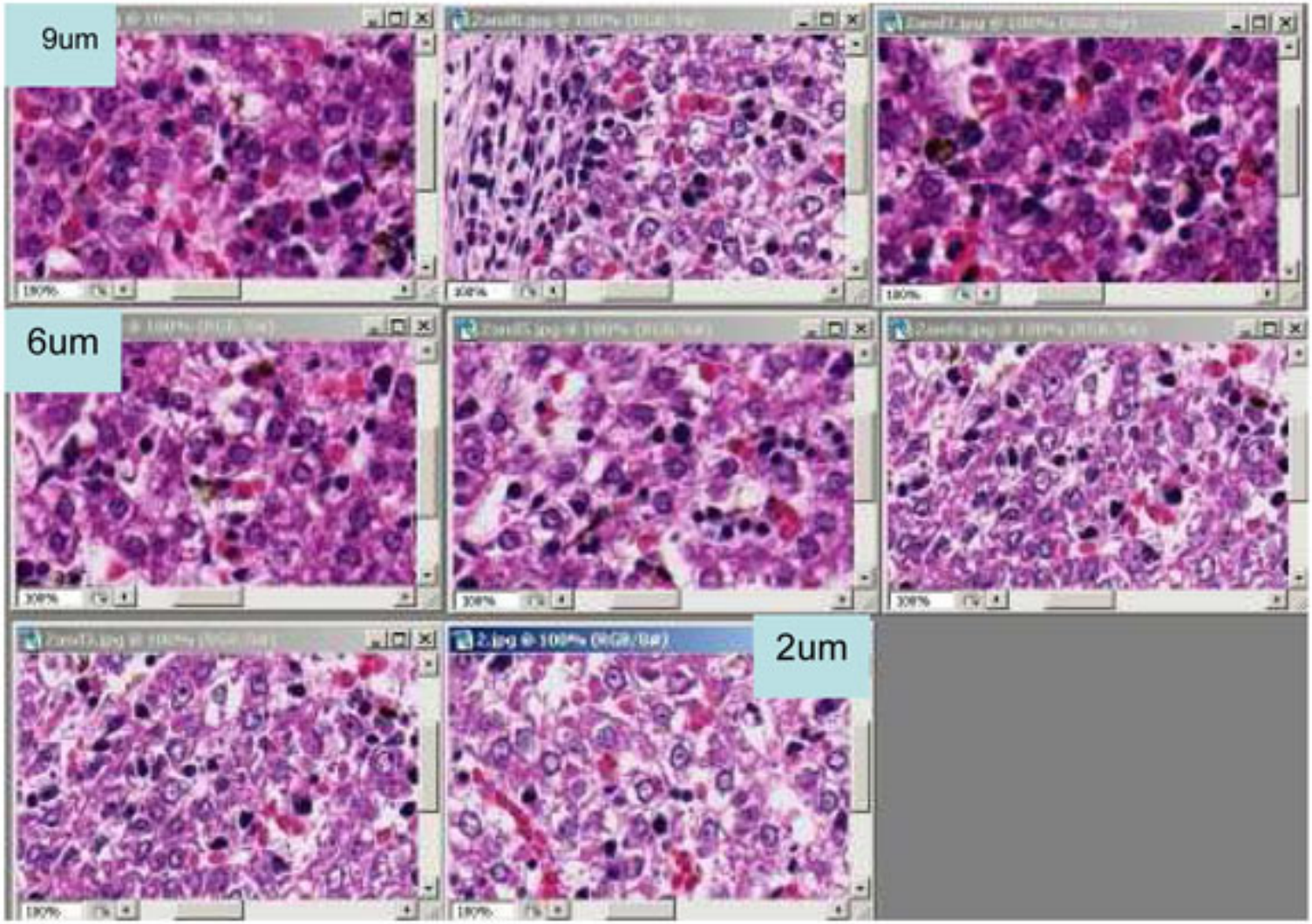
Are there quality limitations in the use of virtual slides?

Section thickness influences the image observed in virtual slides

Differences in stain dyes absorbance by tissue thickness

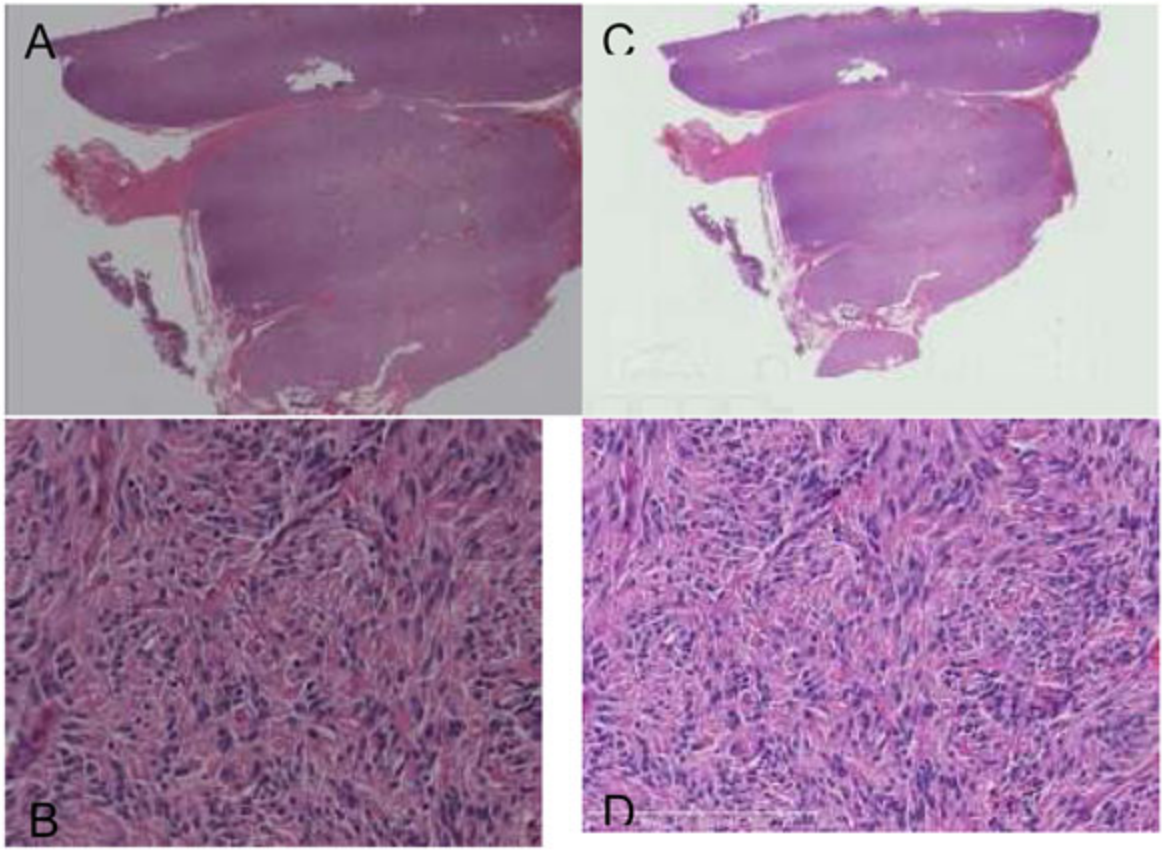
Yagi et al. Diagnostic Pathology 2011, 6 (Suppl 1):S15

Thicker sections show darker and unclear details of tissue.



Yagi, et al. Diagnostic Pathology 2011, 6 (Suppl 1):S15

Color differences in sample images may be seen between scanners.



Yagi et al. Diagnostic Pathology 2011, 6 (Suppl 1):S15

Summary

- Whole slide imaging has multiple uses for clinical diagnosis, teaching, and research.

- Slide storage and retrieval can be achieved, but cost will be high.

- Whole slide imaging and image analysis hold promise for numerous settings in pathology

- Are pathologists and histotechnologists ready for it?