Digital Pathology for Routine Histopathology Diagnosis

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Outline of Talk

PART A

- Introduction to Digital Pathology for histopathology diagnosis
- Imaging technology with Whole slide imaging (WSI)
- Data Storage

PART B

- Integration of databases
- FDA approval
- Validation of a newly acquired WSI system
- Technical problems with WSIs
- Issues in WSI adoptation
- Advantages of Digital Pathology

Introduction

- Digital pathology is rapidly evolving, driven by developing technology, decreasing costs, and regulatory
- Complete digital pathology and whole slide imaging (WSI) for routine histopathology diagnosis is currently in use in some laboratories worldwide (*V. N. Newitt, CAP Today Sept 2019*)
- Digital pathology is a medium with adequate image resolution for primary histopathology diagnosis. Successful digitization relies on existing sample tracking and integration of the information technology infrastructure. Rapid and reliable scanning was key to the transition to a fully digital workflow.

Introduction

- The basis of digital pathology lies in obtaining a digital replica of the histologic slide, called a whole slide image (WSI)
- Digital pathology resulted in (a) efficiency gains in the preanalytical and analytical phases, (b) created the basis for computational pathology, i.e. the use of computer-assisted tools to aid diagnosis
- Ultimately, digital pathology aims to promote diagnostic precision by providing digital tools for accurate histologic assessment, to facilitate collaborations, remote consultations, education, and conferences (within pathology or with oncology).

Imaging Technology

- Scanners capture images of tissue sections tile by tile or in a line-scanning fashion. The multiple images (tiles or lines, respectively) are captured and digitally assembled (‘‘stitched’’) to generate a digital image of the entire slide
- Scanning can occur at multiple magnifications. Scanning at 20 and 40 magnification is usually acceptable for standard viewing and interpretation, including routine image analysis of hematoxylin-eosin (H&E) and IHC slides
- Different scanner models vary not only in their scanning modality, but also in their slide-loading capacity and scan time. High-throughput scanners have loading units that can hold up to 400 slides. Scanning times per slide (standard size 75x25 mm) range from 30 seconds (20x) to 2 minutes (40x). Scanning at higher magnification (i.e. 20 versus 40), digitization of larger tissue sections, can further increase the scan time (as well as file size).

A Typical WSI Scanner

Slide Loading

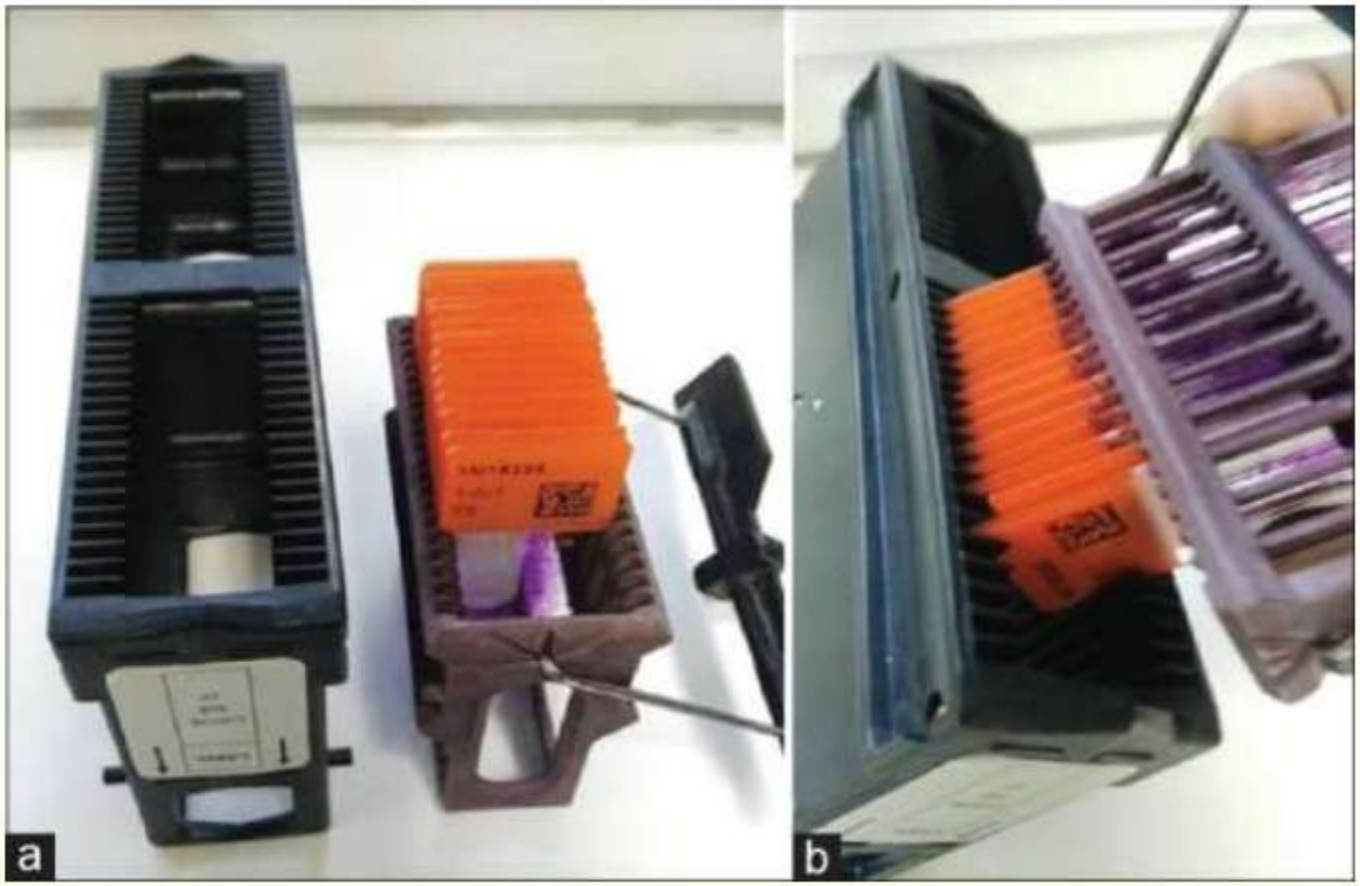


Figure 3

(a) Compatible scanner and staining slide racks (b) allow slides to be easily loaded for scanning with minimal laborious human intervention

F Fraggetta. J Pathol Inform. 2017; 8: 51

Imaging Technology

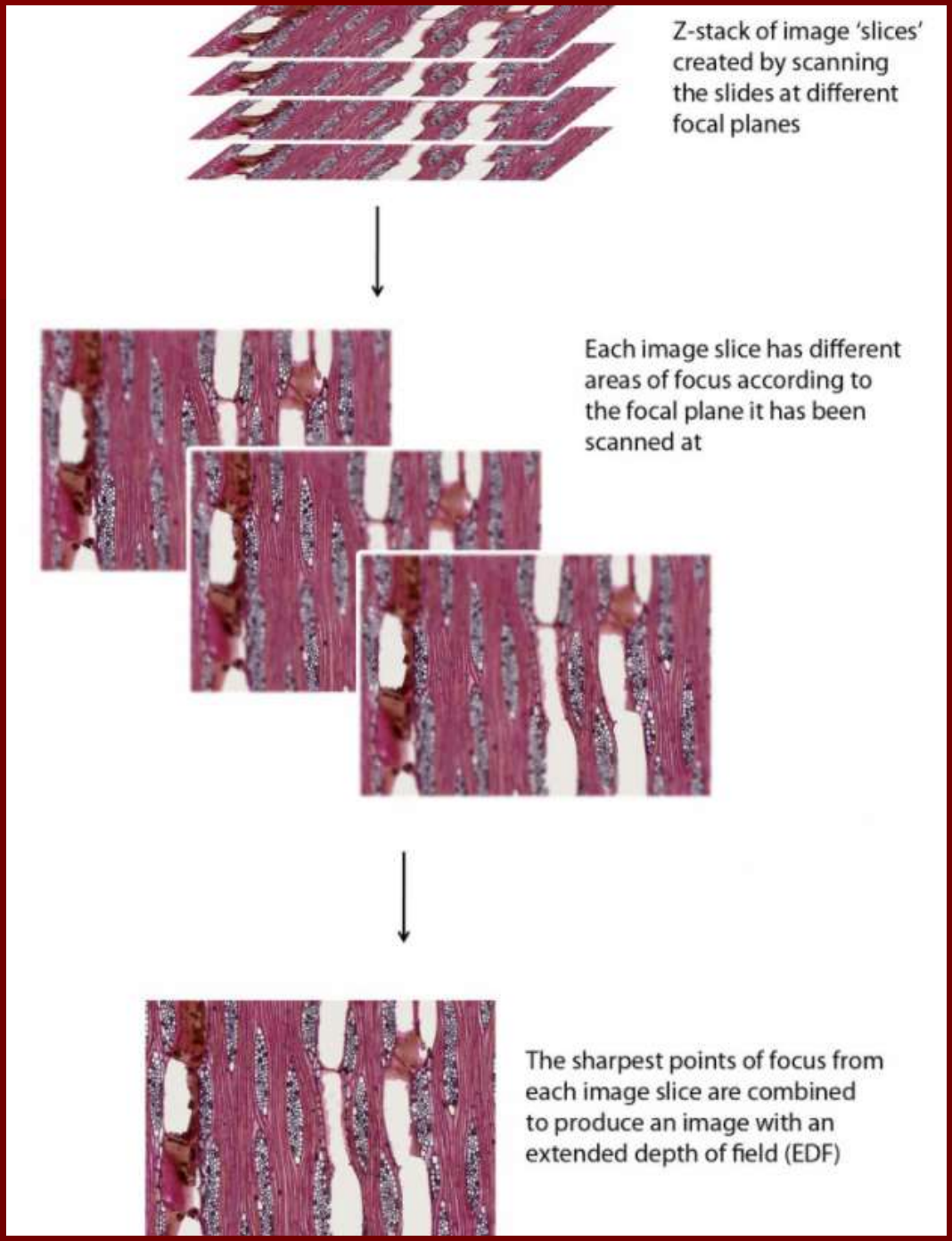
- Most modern scanners are self-calibrating, and incorporate a continuous autofocus mechanism
- WSIs with technical issues are stilled scanned and WSIs go to a dedicated system folder (called ‘‘action required’’). These slides require the scanning histotechnician to open the action-required folder and manually remedy the problems. This permits unsupervised overnight scanning.

Z-Stacking

- WSI is a useful tool for cytopathology. However, it is currently being used more for educational purposes than for routine diagnostic work. This is because scanning cytology slides is difficult if they contain thick smears or if specimens have three-dimensional (3D) cell groups (eg, Pap smears)
- To overcome this “focusing” challenge, some WSI scanners provide z-axis scanning. Z-stacking involves scanning a glass slide at different focal planes along the vertical z-axis and stacking the images on top of each other to produce a composite multiplane image
- Z-stack scanning, however, takes longer and produces larger digital files

Z-Stacking

Morphle Lab
https://www.morphlelabs.com/

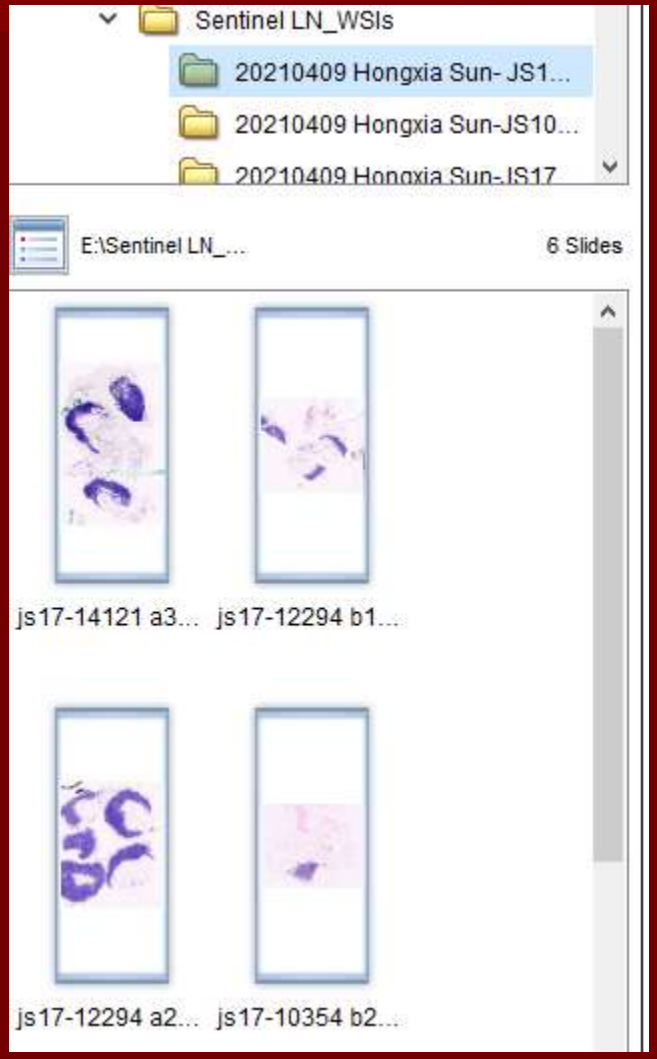


Virtual Slide Tray

- Immediately after scanning, the digital images are available in the image management system (IMS), which has an array of digital tools for image evaluation and creates virtual slide trays for each case.
- These virtual trays (thumbnail images of slides), using the information supplied by the LIS, emulate the physical trays that a pathologist would use in conventional light microscopy

Virtual Slide Tray

Sample from DL Breast Cancer Screen Project, Motic Digital Slide Assistant



Imaging Viewing

- Many WSI systems include image viewing software that can be installed locally on user computers
- Other vendors offer this ability as part of a larger software suite residing on network servers, enabling users to view whole slide images in Web browsers
- Most image viewers offer commonly used features including zooming, adding annotations, performing measurements, taking snapshots, exporting images, and making image adjustments such as brightness, contrast, sharpening, or color intensity
- A number of vendors offer image analysis algorithms (eg, for scoring estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2, etc.).

IMAGE PROCESSING

IMAGE PROCESSING

Once the glass slide information is transferred into a digital format, various handling procedures can be processed:

- Zoom-in and out
- Free annotating
- Measurements
- Application of grids, masks
- Side-by-side image comparison
- Image processing, adjustment and enhancement

A multiplication of data users is enabled by:

- Cloud and APP support
- Data export for 3rd party compatibility
- Network browsing
- Motic's Digital Slide Management software for internet slide library, also for iPad

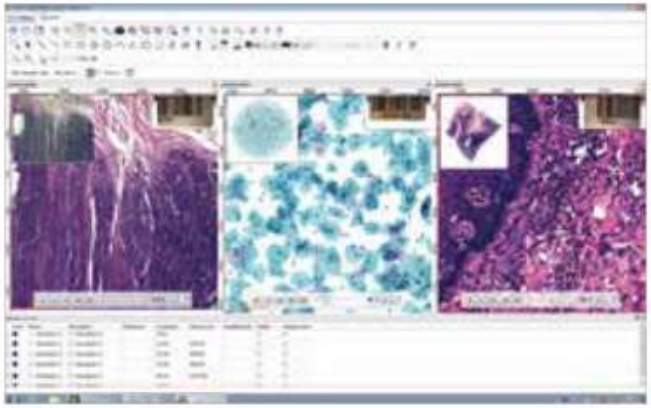


Image Management System (IMS)

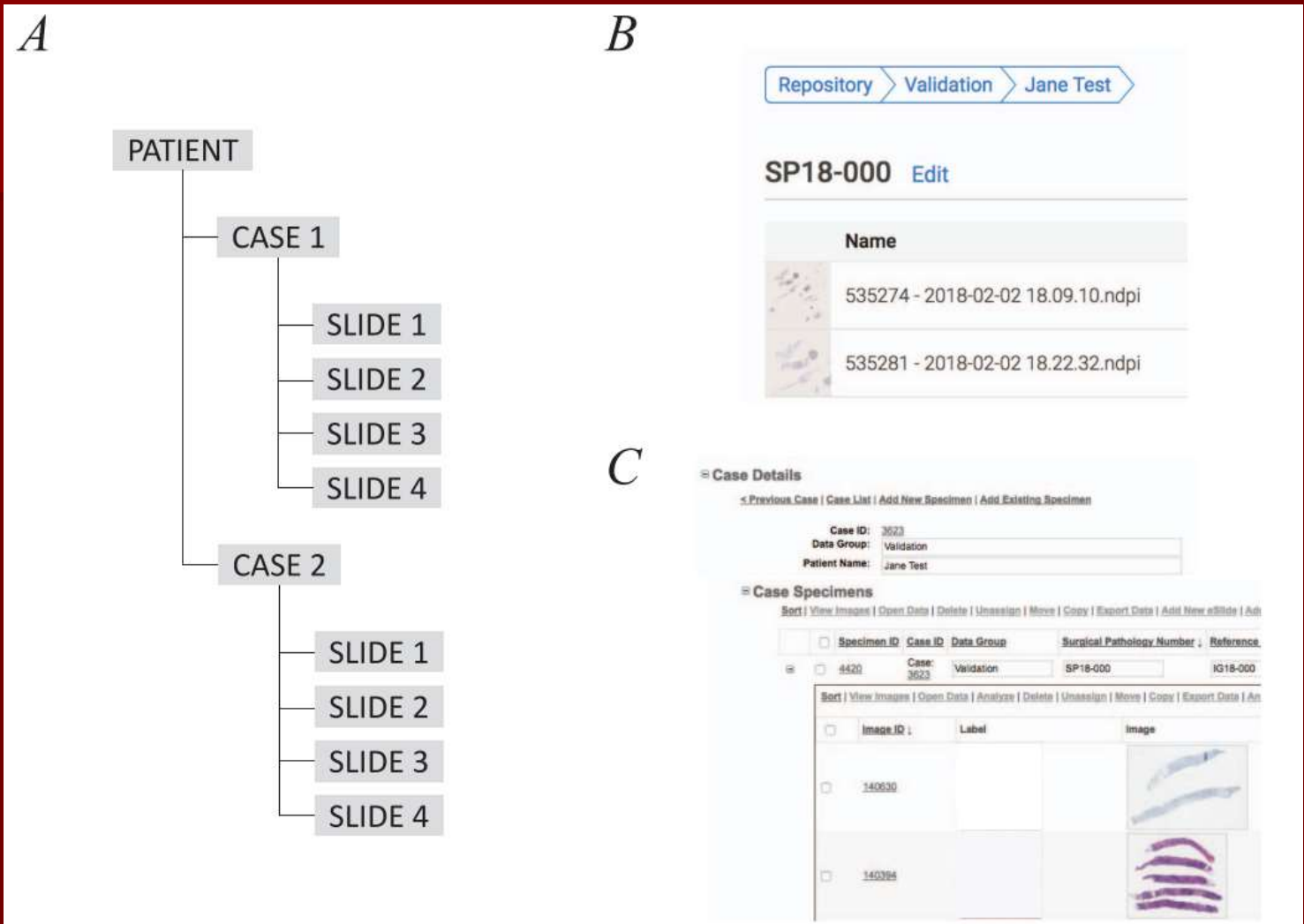


Figure 3. *A sample hierarchy for the organization of whole slide images in a clinical workflow (A). The top level is indexed by patient identifier and contains all cases and specimens for that patient. Individual whole slide images reside underneath the case level. Examples of this organization are shown for patient "Jane Test" with specimen identifier SP18-000 in the (B) PathcoreFlow (Pathcore, Toronto, Ontario, Canada) and (C) Aperio eSlideManager (Leica Biosystems, Wetzlar, Germany) image management systems.*

WSI Sample Hierarchy (Zarella et al, Arch Pathol Lab Med—Vol 143, Feb 2019, pp222-234)

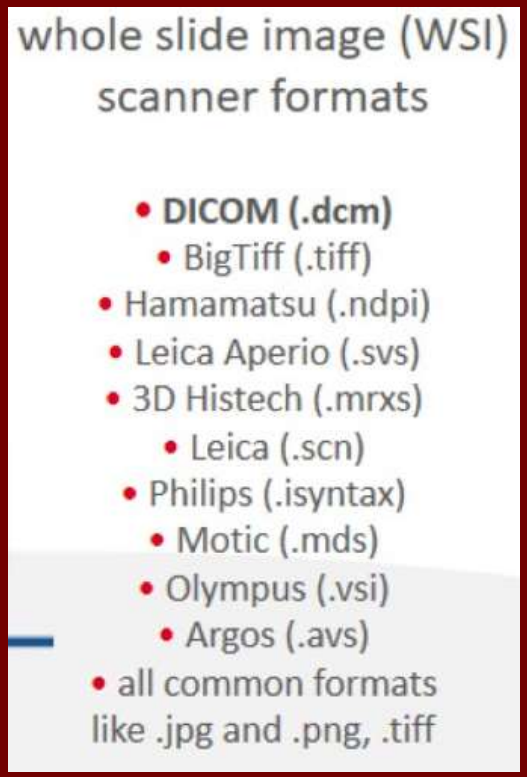
Image Files

- The resolution is defined as the minimum distance at which 2 distinct objects can be identified as separate events.
- A typical whole slide image scanned at x40 magnification has a resolution of about 0.25 um per pixel and 24-bit color depth
- Despite methods of reducing file size, a single WSI in practice often exceeds 1 GB in size. Files of this size can be prohibitive to download. Whole slide images are stored at multiple resolutions to accommodate a streamlined method for loading images.
- The image can be loaded at a low resolution. Conversely, when users examine tissue at high magnification, only a small field of view is visible on the monitor at any given time, and so the image does not need to be loaded in its entirety.

Image Files

- For example, a sample WSI acquired at x40 is accompanied by the same image down-sampled at x20, x10, and x5, as well as a thumbnail image that represents the entire tissue fit within a ~1-megapixel frame
- This multi-resolution representation is commonly referred to as an image pyramid and enables more efficient data throughput by precomputing lower-resolution versions of the WSI
- Adequate network bandwidth (typically 1Gb/sec) is needed for image viewing. Otherwise, unacceptable lag and pixelation will result in viewing instability.

Image File Formats

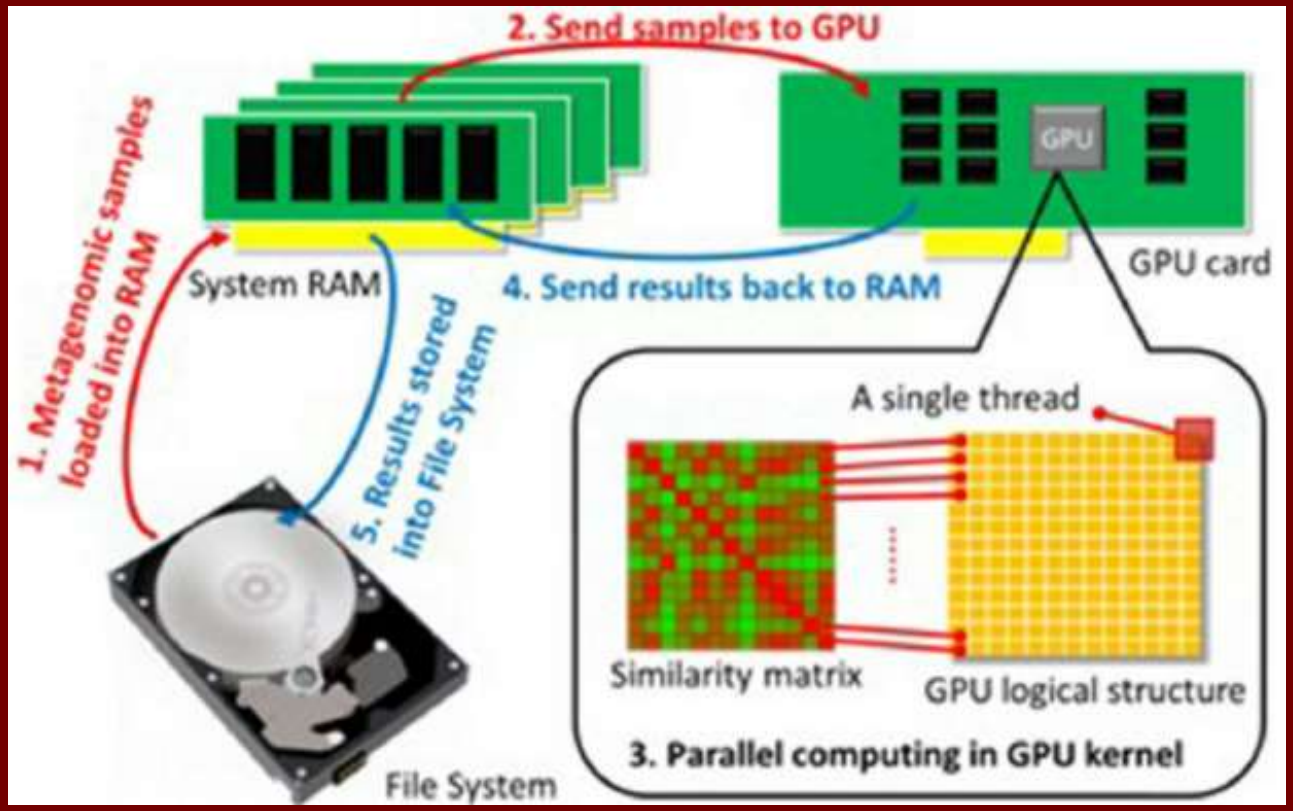


Hardware for Display

- Adequate workstation with graphics processing unit (GPU) for graphics-intensive viewing software (image files in the form of matrix require parallel processing by GPU cores). Typical example: Intel Xeon CPU E5-1620 v3 at 3.50-GHz processor, 16 GB RAM, and an NVIDIA Quadro K4200 GPU card
- Adequate high-resolution display monitors are needed to examine the WSI. Typical example: 24-in Philips 246V5L LED monitor with a resolution of 1920 x 1080 pixels

Graphics processing unit (GPU)

GPUs have hundreds to thousands of cores that facilitate matrix computation (essential for image display and manipulation)



Yang et al. BMC Systems Biology 2014, 8(Suppl 4):S7
http://www.biomedcentral.com/1752-0509/8/S4/S7

WSI Storage Technology

- A robust, scalable data storage platform is needed. Bandwidth needs to be adequate for images to be uploaded from scanners to servers
- Organizations must decide whether cloud-based or (local) server-based network access better suits the needs of the users and conforms to the information system protocols of the organization
- For cloud-based solutions especially, a main consideration can be the cost that accompanies the bandwidth and data throughput necessary to achieve desired performance
- Hybrid solutions that involve local and cloud-based storage and access, or hub-and-spoke models for multisite organizations, can also be effective strategies


Delicate Arch, Moab National Park, UT
May 2021

Integration of Databases

- A crucial step toward caseload digitization is the integration between the IMS and the existing laboratory information system (LIS)
- The digitized WSI is identified by its bar code (2D or Quick Response, QR) and matched with the corresponding patient and sample details fed to the IMS by the LIS

Integration of Databases

- The sample details include specimen site, staining techniques, number of glass slides produced per case, and the assigned pathologist
- The communication between the LIS and the IMS is bidirectional and done by means of Health Level 7 messaging protocol. It is essential that both the LIS and the image viewing software vendors be involved in the integration process, and that their software be open and flexible to permit the necessary data interchange.

A Typical Database Integration (IMS and LIS)

Retamero et al. Arch Pathol Lab Med—Vol 144, Feb 2020, pp221-228

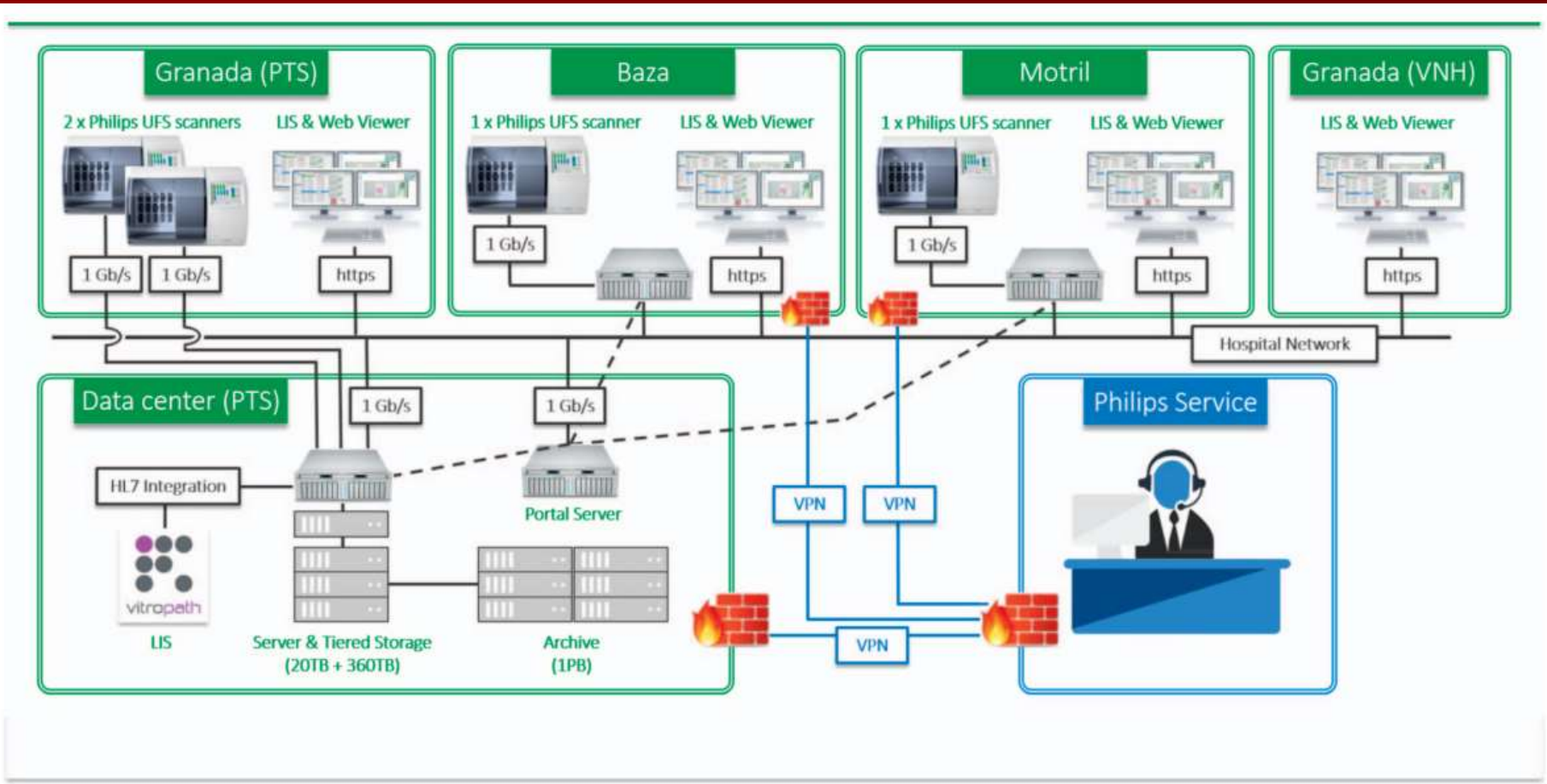


Figure 2. *Schematic representation of the system architecture at Granada University Hospitals. Thanks to the portal architecture, all digitized images are available throughout the entire network. Abbreviations: VNH, Virgen de las Nieves Hospital; PTS, Campus de la Salud Hospital; LIS, laboratory information system; UFS, ultrafast scanner.*

A Typical Pathologist Workstation

Retamero et al. Arch Pathol Lab Med—Vol 144, Feb 2020, pp221-228

EMR LIS IMS

The microscope is no longer required for routine histopathology diagnosis

FDA Approval of WSI System

M.D. Zarella et al.Arch Pathol Lab Med. 2019;143:222–234

- An important breakthrough in regulation occured when the FDA allowed the first system, the Philips IntelliSite Pathology Solution, to market its WSI device for primary diagnostic use of surgical pathology slides in the United States This was accomplished by close collaboration between the Digital Pathology Association and the FDA
- Previously, the FDA had designated WSI systems as class III medical devices, i.e. considered as ‘‘highest risk’’, and are are therefore the most highly regulated medical devices
- Now, FDA has declared WSI systems as class II (moderate-risk devices that already have a predicate device on the market). The FDA published in their classification order that this device, and equivalent devices of this generic type, should be classified as class II devices (a more clear-cut pathway)

FDA Approval of WSI System

Esther Abels1 et al, J Pathol Inform 2017;8:23

- For regulatory purposes, a WSI system is defined as consisting of two integrated subsystems, the (a) whole slide scanner that converts the content of a glass slide into a digital image file, and (b) a workstation environment, including the display, for viewing digital images
- Whole slide imaging platforms are best validated as integrated (closed, or locked-down) systems, and it is not necessary to validate individual components such as a computer monitor

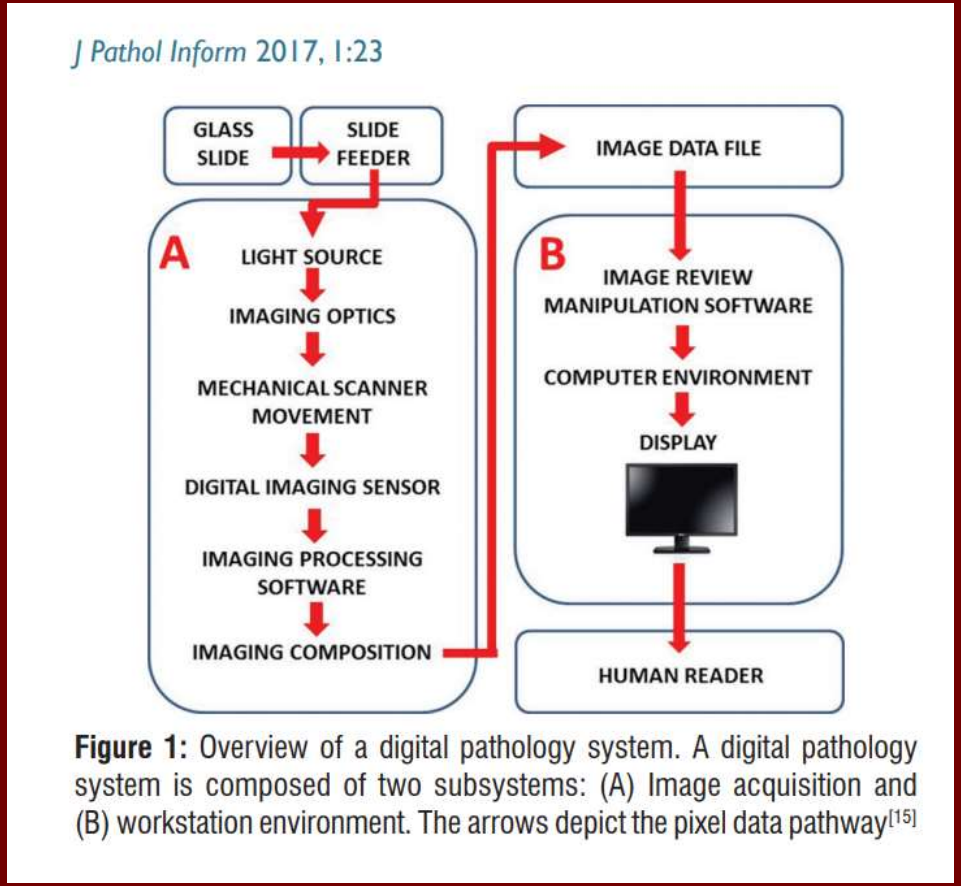


Figure 1: Overview of a digital pathology system. A digital pathology system is composed of two subsystems: (A) Image acquisition and (B) workstation environment. The arrows depict the pixel data pathway[15]

FDA Approval of WSI System

Esther Abels1 et al, J Pathol Inform 2017;8:23

- Following the first FDA approval of Philips IntelliSite Pathology Solution in 2017, subsequent manufacturers can apply for a 510(k) clearance for FDA approval. This first authorized WSI predicate device will pave the path to show substantial equivalence for other devices

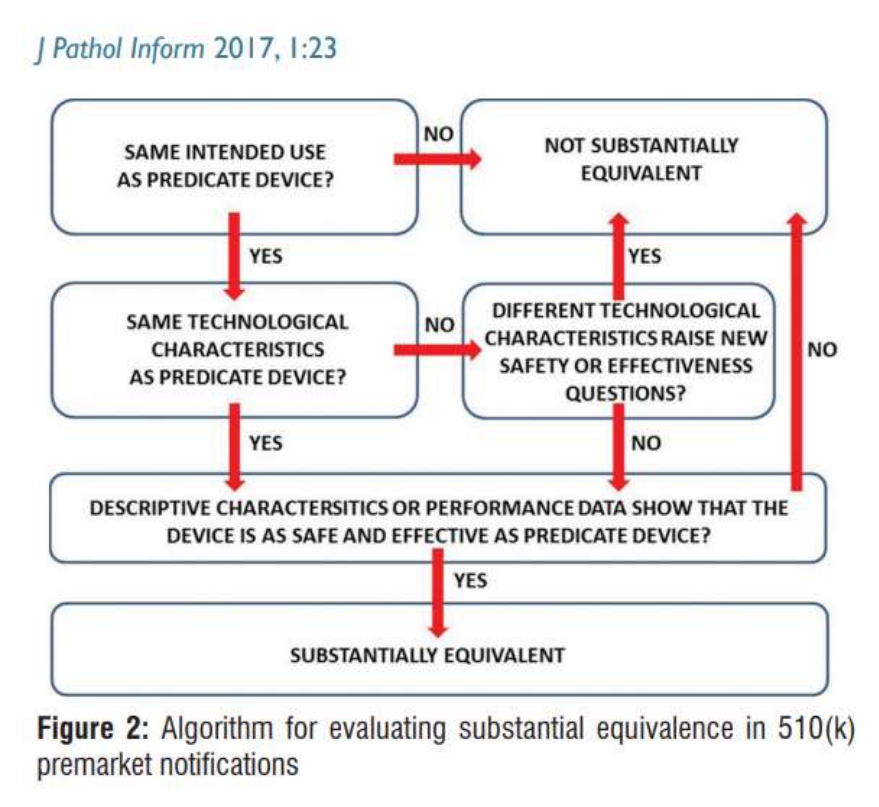


Figure 2: Algorithm for evaluating substantial equivalence in 510(k) premarket notifications

FDA Approval of WSI System

Esther Abels1 et al, J Pathol Inform 2017;8:23

- The Phillips' large multicenter, retrospective clinical study demonstrated that diagnosing 2000 surgical pathology cases (15,925 readings) with the Philips IntelliSite Pathology Solution was noninferior to optical microscopy. The clinical trial yielded a digital to optical interpretation rate difference of 0.4% with a 95% confidence interval
- The second WSI system was approved by FDA in 2019:

Leica Biosystems' Aperio AT2 DX System AT2 DX System

Validation Process (CAP, 2013)

Evans, Andrew J et al. Archives of Pathology & Laboratory Medicine, July 1, 2017

- A WSI system should focus on its intended clinical use(s) (eg, primary diagnosis, frozen sections, or immunohistochemistry review)
- Whole slide imaging platforms are best validated as integrated systems, and it is not necessary to validate individual components such as a computer monitor
- The guidelines recommend that at least 60 representative cases (irrespective of the number of slides per case) be examined in a validation study for any new clinical use, with at least a 2-week "washout" period. A washout period is the time between review of a glass slide with a traditional microscope and review of the same case using a digital slide (or vice versa).
- Intra-observer diagnostic concordance between glass slide and WSI diagnosis on the same slide is the most important "performance characteristic" to assess.

Validation Process (CAP, 2013)

- The average intra-observer variability rate between the optical and digital diagnoses observed during most validation process was below 1% *[Retamero et al. Arch Pathol Lab Med—Vol 144, Feb 2020, pp221-228]*
- Completing an internal validation process should satisfy all users that WSI can be used to make accurate and complete diagnoses.
- It also provides an opportunity to identify histology-related issues that may require further attention to ensure optimal image quality, as well as allowing pathologists to identify potential limitations with WSI that may require ancillary procedures for digital sign-out

Technical Issues in WSI Scanning

- Although the rates of scanning errors have been low, the adoption of digital pathology at large scale has required some effort by histotechnicians to minimize these errors
- The histologic preparations need to be clean of artifacts, have carefully placed coverslips that are well aligned and free of excess mounting media, which could interfere with the mechanical elements of the scanning system
- In addition, the labeling should be fully readable and free of any pigment splatters that could hinder label identification. This issue has been addressed with the introduction of glass printers, which imprint the slide ID and bar codes on the glass slide itself and are thus less prone to artifacts.
- The implementation of these workflow changes has brought the technical error rate from approximately 1.5% down to 0.1% at current.

Technical Issues in WSI Scanning

- Special WSI scanners are needed for

  -Frozen section

  -Whole mount

  -Cytology (needs z-stacking as discussed)

- -Immunofluorescence

  -"Wet heme" slides such as blood smear, bone marrow aspirate

  (with oil-immersion)

- Most scanners generate proprietary image file formats (eg, .SVS, .RTS, .NDP) that require specific viewing software to be viewed. While some files may be converted to open formats (eg, .JPEG, .TIFF), proprietary digital files generated by one scanner may not be viewable with viewers from other vendors.

Issues in WSI Adoption

- Pathologists' discomfort with using WSI has an emotional component to consider. Primary among these is fear of making a diagnostic error when using WSI instead of more familiar conventional microscopy
- Anger may exist over being mandated by others (eg, hospital administration, clinicians, department chairs) into using WSI
- Pathologists may have little or no training or experience in using WSI systems. Fundamental mechanical and ergonomic differences from conventional microscopy exist when using WSI for slide review. Examples include the need for an input device such as mouse or touch pad to navigate slides and the fact that the field of vision on a computer screen may not correlate exactly with the field of vision on a microscope.

Issues in WSI Adoption

- Owing to the low discrepancy rates observed in various validation studies, the majority of pathologists may be confident and safe reporting cases using WSI, which reflects its appropriateness as a diagnostic medium
- However, some users may welcome the aid of training sets in the transition to digital pathology, to ensure pathologists gain the sufficient experience and confidence in their newly acquired digital skills. This may be particularly relevant for certain aspects, such as training to identify neuroendocrine chromatin, or nuclear features of papillary thyroid carcinoma
- WSI cases may take more time to review than routine glass slide cases, especially when a pathologist is relatively new to reviewing cases digitally. A number of studies have shown that more time per slide or per case for WSI compared to glass is necessary, sometimes up to 20% increase per case.

Training Pathologists for WSI Adoption

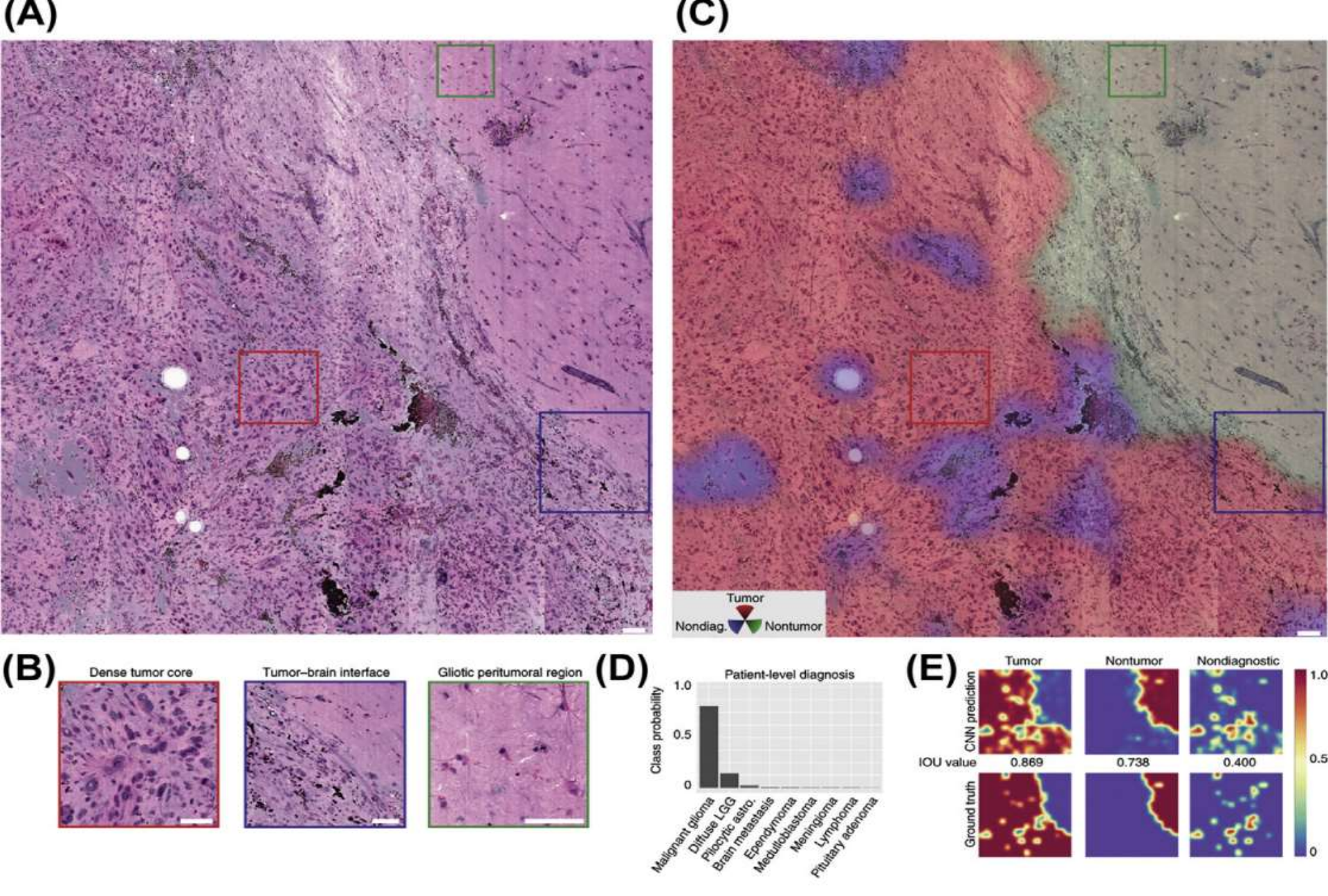
- Training with respect to incorporating WSI into clinical practice has 2 components: (a) training pathologists on how to access scanned slides and use the WSI software to review them, (b) training the pathologist how to execute the entire workflow for intended uses of WSI in the department.
- WSI workflow: how and when WSI cases become available for a pathologist to review, how to find one's own WSI cases in the system, processes for deferring to glass slide review, and how reports will be created and distributed
- During the transition period from glass slide to WSI-based diagnostics, one should expect to encounter challenges associated with a hybrid glass slide-WSI workflow.

Image analysis

Aeffner F, Zarella et al . J Pathol Inform 2019;10:9

- There are numerous software packages, which are used by pathologists and researchers to enable interpreting the image data and guide the analytic process.
- The GUI would include a number of user-input parameters such as:

1. Color definition – Ability to set the color for each of the stains. For example, blue for hematoxylin, brown for Ki-67, and red for CD3
2. Nuclear detection – with parameters such as nuclear size, color threshold, roundness, eccentricity, and other parameters to identify individual nuclei while excluding nonnuclear structures
3. Marker detection for each biomarker – with biomarker color (e.g., brown for Ki-67), cellular compartment (nuclear, cytoplasm, and membrane: e.g., nuclear for Ki-67)
4. Expression categories – with values to separate detected cells into negative, low, medium, or high (0, 1+, 2+, or 3+) based on the intensity thresholds.



Nature Medicine, 2020;26(1):52e58

Image analysis

Aeffner F, Zarella et al . J Pathol Inform 2019;10:9

- There are basically three types of algorithm available currently in the diagnostic space:

(1) FDA-cleared – FDA has determined that the commercially available product is substantially equivalent to another legally marketed algorithm for the same purpose as safe and effective. A premarket notification is referred as to 510(k);[135]

(2) non-FDA approved commercially available – these products can be FDA registered for investigational use only

(3) laboratory developed test – the algorithm is designed and used within a single laboratory according to the laboratory's own procedure. When laboratories choose to use quantitative image analysis for clinical purpose, the quantitative image analysis system and algorithm must be validated for diagnostic interpretation. Validation is designed to gather and document evidence that a system or test will consistently produce a result that meets predetermined acceptance criteria.

Image analysis

Aeffner F, Zarella et al . J Pathol Inform 2019;10:9

- FDA-cleared quantitative image analysis systems and algorithms have less validation burden than non-FDA-cleared products for the laboratory

Discussion on Advantages of Digital Pathology

Retamero et al. Arch Pathol Lab Med—Vol 144, Feb 2020, pp221-228

- The creation of a full digital multisite network, where all slides are available to any user from any location, has brought about several advantages
- The most important one is the ability to assign caseloads according to specialty interest among our pathologists, and not by geographical site. Also, pathologists based at the peripheral hospitals can request immediate consultations from the specialists located at the central campuses
- On-call pathologists covering another site can report their routine cases from within the hospital network, and transport of physical glass slides is no longer required
- Histologic images can be easily identified, tagged, and displayed during multidisciplinary team meetings.

Discussion on Advantages of Digital Pathology

- Glass slides must be scanned before WSIs are available for examination. However, the loss of time in scanning can be compensated by saving time in transporting and distributing glass slides to individual sites
- The existence of a digital archive enables the immediate availability of previous slides, which is helpful, for instance, when comparing the findings in resection specimens with those of the initial incisional biopsy
- The retrieval of the digital slides from the archive takes a few minutes instead of the hours it took previously to request, find, and retrieve the slides in a traditional analog archive
- The adoption of digital pathology has resulted in improved workflow efficiency. Pathologists signed out on average 21% more cases after adopting digital pathology in one study.

Discussion on Advantages of Digital Pathology

M.D. Zarella et al.Arch Pathol Lab Med. 2019;143:222–234

- Furthermore, WSI enables institutions to retain digital copies of cases sent for consultation, which ensures that the images are available while the glass slides are transported back and forth
- Similarly, a permanent record of the slide can be kept when the tissue from a slide is needed for molecular testing
- Slides can also be archived for medicolegal and forensic purposes
- Remote intraoperative consultation was one of the first use cases for digital pathology and arguably one of the most widely cited reasons for the adoption of digital pathology
- Using WSI in place of static images, multi-headed microscopes, and projection microscopes for presentation in multidisciplinary tumor boards can improve the experience for both the presenter and the audience
- Whole slide imaging does not require a multi-headed microscope or a microscope connected to a projector in the presentation venue, which may not always be present in a conference room.

Discussion on Advantages of Digital Pathology

M.D. Zarella et al.Arch Pathol Lab Med. 2019;143:222–234

- For pathology residents and medical students: collections of carefully curated and organized WSI cases, some of which are freely available in public repositories, can provide the core material for trainees to build knowledge
- For practicing pathologists: virtual slides are now ubiquitously used in pathology meetings to provide interactive learning and simulate real life practice
- In order to improve proficiency and quality, pathologists participate in competency assessment programs such as the College of American Pathologists proficiency survey, which has incorporated a WSI component into many of its programs

Increased Productivity with Digital Patholgy

Retamero et al. Arch Pathol Lab Med—Vol 144, Feb 2020, pp221-228

Table 3. Yearly Caseload Variation at Granada University Hospitals (2015–2018, Numbers Rounded)[a]

Year	No. of Pathologists	Histology Samples	Caseload Change From Previous Year, %	Histology Cases per Pathologist	Histology Cases per Pathologist % Change (Compared With 2015)	Total RVU	RVU per Pathologist	RVU per Pathologist % Change (Compared With 2015)
2015	24	53 500		2229		1 375 544	57 314	
2016	22	56 500	6	2568	15	1 450 225	65 919	15
2017	23	61 500	9	2674	20	1 581 231	68 749	20
2018	23	64 500	5	2804	26	1 687 039	73 350	28

Abbreviation: RVU, relative value unit.

[a] Only histology cases are depicted. The caseload has increased yearly between 6% and 9%. That, together with variations in the number of pathologists, has resulted in an increase in the percentage of cases per pathologist of between 15% and 26% each year compared with 2015, the year prior to full digitization. The RVUs show similar increases.

A cost-benefit model proposes that improvements in productivity of at least 10% to 15% are required to amortize the investment after 1 to 2 years

Discussion on Advantages of Digital Pathology

M.D. Zarella et al.Arch Pathol Lab Med. 2019;143:222–234

- Digital pathology creates the basis for computational pathology, i.e. the use of computer-assisted tools including deep learning to aid diagnosis (tumor region identification, detection of metastatic foci, tumor classification, and prediction of gene mutations, etc.). These tools also determine prognosis of patients
- Deep learning algorithms likely will push digital pathology needs. Pathologists are becoming aware that algorithms plus pathologists are more accurate than operating alone. Algorithms can help pathologists reach results that are more accurate and precise. They will also help oncologists to make better informed decisions on treatment.

Lewis Ginter Botanical Garden, Richmond, VA
Oct 2021