

REVIEW

Digital pathology: *semper ad meliora*

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This review is an evidence-based summary of digital pathology: past, present and future. It discusses digital surgical pathology and the cytopathology digitisation challenge as well as the performance of digital histopathology and cytopathology as a diagnostic tool, particularly in contrast to user perceptions. Time and cost efficiency of digital pathology, learning curves, education and quality assurance, with the importance of validation of systems, is emphasised. The review concludes with a discussion of digital pathology as a source of 'big data' and where this might lead pathologists in the digital pathology future.

Key words: WSI; whole slide images; digital microscopy; digital pathology; cytopathology; histopathology.

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INTRODUCTION

Pushing forward with digital pathology research and translation of findings into clinical practice, as well as education and quality assurance, is essential. Each step in our learning curve, each collaboration and publication, results in cumulative improvements in knowledge with the goal of evidence-based change in the workplace. Glassy asks '*quo vadis?*';¹ the literature suggests this way.

DIGITAL SURGICAL PATHOLOGY

Certainly, digital surgical pathology is now accepted as equivalent to traditional microscopy for diagnostic accuracy.^{2–11} It has been suggested that digital pathology also lends itself more easily to measurements of tumour depth, as well as margin clearance from tumour.¹² The numerous advantages of digitising pathology have already been covered extensively.^{13–18} These include multiple-site access to cases, permanent archiving of well-stained slides and potential cost savings on slide storage real estate. Importantly, whole slide imaging (WSI) may represent a rich source of data for computer-assisted detection and diagnosis, as well as for predictive and prognostic tools.

The amount of manipulation and consideration required for the digital specimen exists on a spectrum. Large histopathology specimens are presented in monolayers which lend conveniently to digitisation. Small biopsies (particularly those specimens containing *Helicobacter pylori*^{2,8} or eosinophils⁸) can present difficulties. However, solutions have been given by groups such as Snead *et al.* and Kalinski *et al.*^{2,8}

For small histopathology specimens sometimes z-axis capability² or specific scanning parameters⁸ are required. Universal consensus on ideal distance between z-axis focal planes and the number of focal planes is yet to be reached. Snead *et al.* in their study of 3017 small biopsy specimens (10,138 whole slide images) found that scanning gastric biopsies at 60× was necessary to accurately detect *Helicobacter pylori*.⁸ They also suggested scanning at 60× for all renal biopsies. However, even at 60×, there was noted interpretation difficulty for membranous nephropathies.⁸

Based on their study of 144 gastric biopsies, Kalinski *et al.* suggest z-stacking for all gastric WSI to accurately detect *Helicobacter pylori* numbers, with nine focal planes suggested as ideal.² All other parameters in the biopsy were adequately diagnosed with one focal plane. An image of WSI demonstrating *Helicobacter pylori*, with a link to the slide, is provided in Fig. 1.

DIGITISING CYTOPATHOLOGY

Digitising cytopathology specimens of course represents a significant challenge. Problems we all worry about are: cytopathology specimens especially fine needle aspiration biopsies (FNAs) containing 3-dimensional cell groups, cells being dispersed across multiple different focal planes in the z-axis and the diversity of cytopathology specimens. This is part of the cytopathology digitisation problem and each of these unique cytopathology sample types needs to be approached differently during image acquisition.^{19–21}

It does seem possible to negotiate the hurdles, however. Familiarisation with the technology and adaptation of the work flow may be the answer.²¹ It is possible that we will need a different approach for specimen collection, slide preparation and whole slide image editing.²²

Z-stacking is possible for cytopathology specimens but the more z-stacks the greater the file size and storage requirement. Navigating cytopathology WSI can be slow and this is exacerbated when there are cells widely dispersed across multiple focal planes. When material on the slide is scant, manoeuvring and navigating the resulting WSI is difficult, increasing the time to screen a slide and increasing the risk of missing diagnostic material.

To leverage the emerging technology by 'marking up' the slide to remove dead-space or other unsuitable areas prior to scanning may be a solution. Editing the resulting WSI with cropping and extracting areas works well for educational WSI but may also be suitable as part of specimen preparation during the diagnostic workflow. Dee and co-workers found that digitising less than the entire glass specimen did not compromise accuracy.¹³

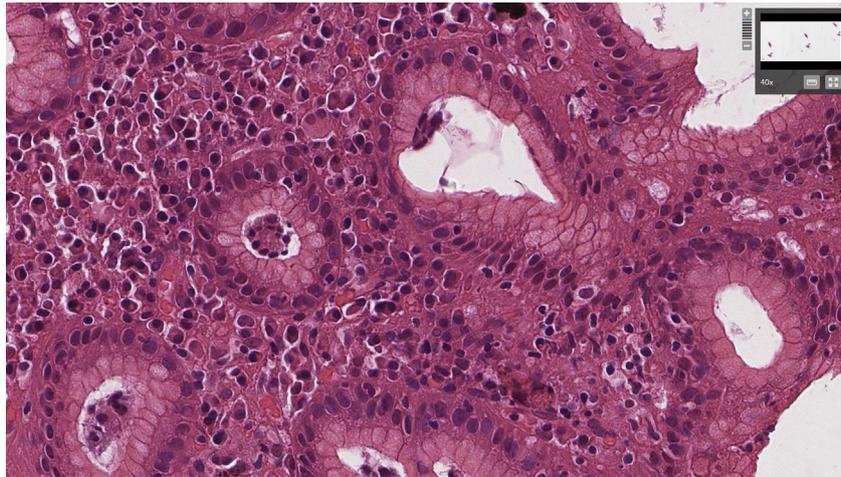


Fig. 1 Example of whole slide imaging H&E stained gastric biopsy showing *Helicobacter*-associated gastritis: http://bit.ly/BESTeduau_Gastritis.

Additionally, a range of alternatives to z-stacking have also been offered for cytopathology WSI. Capitano *et al.* refer to these techniques as deep focusing, which have the advantage of reasonable file size.²³ Some slide scanners are equipped with extended focusing algorithms (EF), producing extended focus images (EFI) as alternatives to z-stacked or multi-plane WSI. These functions extract focused areas from each focal plane, then assemble them together into a single image. Mori *et al.* also refer to this technological attempt to assimilate multiple focal planes into a single plane as ‘focus fusion’.²⁴ Lee and colleagues compared EFI technology to z-axis WSI. EFI file size was small (200 MB) compared to WSI (1.5 GB) with seven focal planes.²⁵ There was also increased speed during the EFI evaluation and screening process, compared to z-axis WSI. The disadvantages are that the scan time for EFI is not reduced because the multiple planes in the z-axis still need to be acquired; additionally, once combined into the EFI, the separate z-axis planes are permanently discarded. There can be problems with background and resolution: the algorithm is also applied to the extracellular background debris throughout the thickness of the slide. This may result in a grainier appearance with lack of crisp detail for intercellular borders and nuclear detail.^{25,26}

Lahrman *et al.* describe semantic focusing as an improvement over multilayer scanning. A cell-master-focus layer based on a full 3D map of the specimen is created, where debris is ignored. The automation is based on cell-focus algorithms that have high focus quality (sensitivity and specificity of 94.2% and 95.9%, respectively) and reasonable scan times. Algorithms can also be implemented to deal with cell clusters.²⁷

DIGITAL CYTOPATHOLOGY: IS IT INFERIOR OR SUPERIOR?

This depends. Overall, studies show that diagnostic accuracy using digital cytopathology is not inferior,^{13,28–31} with suggestions from one study that it could be superior,³¹ to using a glass slide and microscope. However, results are dependent on the number of planes acquired in the z-axis on WSI, the distance between planes and whether the z-stacks are attached as focal annotations or not.^{13,28–31}

Evered and Dudding scanned 20 Sure Path cervical cytology slides with 5 z-axis planes and then again with 21

focal planes. Overall there was no difference in accuracy using WSI versus traditional microscopy for diagnosis. However, when the groups were analysed separately, the accuracy rates were inferior for WSI with only 5 focal planes and superior (although not reaching significance) with 21 focal planes compared to the equivalent glass slides.³¹

Dee and colleagues circulated 5 glass slides, equivalent WSI with x- and y-axes and equivalent WSI with focal z-stacks, to 51 cytotechnologists and 28 cytotechnologist-trainees. There was no statistical difference in diagnostic accuracy rates for these liquid-based cervical cytology cases.¹³

Further, Hanna *et al.* in their study, scanned 30 cytopathology cases from a range of specimen types (gynaecological, FNA and fluid). The cases were scanned firstly with only the x- and y-axes and then rescanned with ‘video-type’ focal z-stacked annotations at regions of interest (ROIs) where a cytologist had manually zoomed in the z-axis whilst screening on a traditional microscope. Although the WSI were statistically perceived to be inferior to the glass slides, the actual diagnostic accuracy rates of the WSI were not inferior.³⁰

OVER-CALL AND UNDER-CALL OF DIAGNOSTIC FEATURES AND THE IMPORTANCE OF SYSTEM VALIDATION

Studies have found a range of features that are demonstrated either more clearly or alternatively less clearly on WSI compared to their glass counterparts. For example, structures like *Candida* hyphae are more easily picked up on WSI.⁸ Flotte and Bell also correctly point out that colour is sometimes not accurately translated from optical microscope to WSI.³² Snead *et al.* found that red stain intensity was an issue on haematoxylin and eosin (H&E) WSI when examining eosinophils, mycobacteria in Ziehl–Neelsen stains and fungi in DiPAS stains.⁸ Potential difficulties assessing textural or tinctorial qualities on WSI such as for amyloid or mucin has also been raised by Williams *et al.*³³ This underscores the need for monitor calibration as well as ongoing validation and quality assessment of these systems. Digital display devices need to be regulated for factors such as physical size, pixel dimension, pixel defects, luminance, contrast, resolution and noise, chromaticity at centre, colour gamut and accuracy, as

well as assessing ideal background lighting.¹⁴ Additionally the ideal number of displays for effective and efficient diagnosis needs to be determined.

Snead *et al.* in their study of 3017 biopsies also found nuclei to appear darker on WSI, thus leading to over-calls of dysplasia.⁸ Ordi *et al.* noted that, on the one hand, high grade squamous intraepithelial lesions (HSIL) on uterine cervical biopsies were more likely to be under-called as low grade, but that reactive changes were over-called as low grade intraepithelial lesion (LSIL) in one case.¹² Houghton *et al.* in their study of 100 surgical pathology cases found that both metaplasia and dysplasia were under-called in several cases.⁵ Gui *et al.* in their study of 42 diagnostically challenging biopsies containing degrees of upper gastrointestinal dysplasia, found that intraobserver variability was wider for WSI compared to glass.³⁴ Additionally they found that there was a trend toward downgrading dysplasia with WSI. In their study of 96 skin specimens, Nielsen *et al.* found that actinic keratoses caused issues with diagnostic discrepancies in general.³ Fig. 2 shows WSI demonstrating actinic keratosis, with a link to the slide.

More recently, Williams *et al.* performed a systematic review of glass to digital diagnostic discordances from validation studies (8069 pairs of digital to glass comparisons).³³ Dysplasia diagnosis resulted in 108 of the total 335 discordances. This review reported a tendency to under-call dysplasia as benign or reactive, and where dysplasia was being graded there was a tendency to undergrade the lesion.

On cytopathology WSI, Hanna *et al.* found that atypia was sometimes under-called.³⁰ Dee *et al.* presented similar findings: that atypia was under-called on some of their WSI gynaecological cytology cases.¹³

It has been suggested that these sorts of discrepancies may just be in line with expected intraobserver variability.¹² Certainly, the decreasing discrepancy seen with dysplasia diagnosis on WSI compared to the glass slide over time, is reassuring.¹² However, these features that cause problems on WSI certainly need continued surveillance. Solutions to some WSI diagnostic issues have been offered: Snead and colleagues have suggested that small biopsy cases potentially containing bacteria, or granulocytic inflammatory cells should be scanned at 60 \times .⁸ It is also possible that over- or under-call may be a function of the scanning and viewing

modalities used and this underscores the need for constant validation of the individual system, regardless of whether it be for diagnosis, education or quality assurance (QA). Optimisation of the image is essential through collection of data on the best z-axis scanning parameters^{29,31} or optimal scanning magnification for specific diagnostic features.^{2,8} Evaluation and validation of scanners (as well as the displays as discussed above) is also important.³⁵

To address such validation issues the College of American Pathologists has produced an evidence-based summary of recommendations for laboratories to validate the accuracy and reliability of using WSI systems. The recommendations are related to work by Pantanowitz *et al.*³⁶ and points to emphasise when validating these systems include the need for a washout period of at least 2 weeks between viewing digital versus equivalent glass slides. This is because pathologists may recall images for considerable lengths of time. However, it is emphasised that long washout periods also need to be avoided because a pathologist's experience as well as diagnostic criteria could change over time. The validation study should also establish diagnostic concordance between digital and glass slides for the same observer (i.e., intraobserver variability) and all the material present on a glass slide needs to be included in the digital image. Additionally, a pathologist(s) also should be adequately trained to use the WSI system that is involved in the validation process.

In 2018, The Royal College of Pathologists (UK) also published 'Best practice recommendations for implementing digital pathology'.³⁷ The difficulties in diagnosis on WSI with particular types of specimens was highlighted: epithelial cell dysplasia, small objects such as micro-organisms, or large expanses of tissue needing assessment for rare events such as micrometastases.³³ These guidelines provide very practical solutions, referring to 'risk reduction' with such cases. So, it is likely that there will persist a small nucleus of histology and cytopathology cases that will require examination of glass slides, glass slide-WSI combination or some other measure, for some time into the future. There may also be the need for glass slide deferral when the WSI quality is poor, or when there is scant material in the specimen,³⁸ or in the rare event of all laboratory scanners malfunctioning.⁶ Further issues to consider during implementation of WSI into clinical practice are also well covered by Evans and colleagues.³⁹

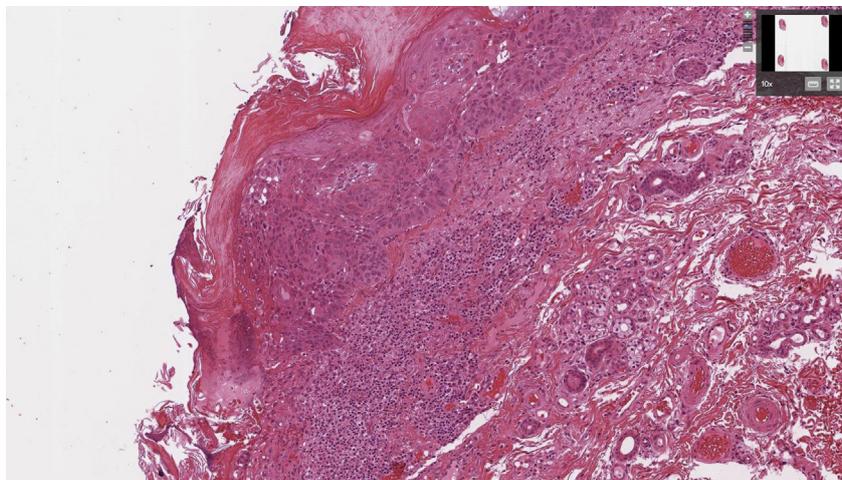


Fig. 2 Example of whole slide imaging H&E stained skin biopsy showing actinic keratosis: http://bit.ly/BESTeduau_Keratosis.

Table 1 Time efficiency for surgical pathology WSI diagnosis

Group	Year	Average time for WSI (mins) per slide/case		Average time for glass (mins) per slide/case	z-axis	Comments	Washout
Velez <i>et al.</i> ⁴¹	2008	In-house viewer: 34	Vendor viewer: 38	23	No	45 dermatopathology cases 3 pathologists 2–7 slides per case	NS
Gui <i>et al.</i> ³⁴	2012	2.1		1.4	No	42 H&E stained slides Gastrointestinal biopsies Varying degrees of dysplasia 4 pathologists	>3–4 weeks
Jen <i>et al.</i> ⁴⁰	2013	15.3		10.8	No	25 renal biopsies: H&E and PAS 6 pathologists	>2 weeks

H&E, haematoxylin and eosin; NS, not specified; PAS, periodic acid–Schiff; WSI, whole slide imaging.

As digital microscopy is increasingly being incorporated into diagnostic practice, validation of systems outside the laboratory itself also need to be considered. Validation of viewing systems as well as security systems for remote sign-out locations may be necessary. Further potential hurdles are implementation of training for new digital systems as well as decisions regarding which platforms to use.

IS IT A MATTER OF TIME AND IS IT COST EFFICIENT?

Most studies demonstrate that it takes longer to examine, screen and/or arrive at a diagnosis with a digital slide compared to a traditional glass slide. It takes even longer with z-axis viewing.

Gui *et al.* found that the amount of time to review gastric biopsy WSI to assess dysplasia compared to a glass slide was 50%–400% more and Jen *et al.* found five out of six pathologists spent longer examining renal allograft biopsies when using WSI (Table 1).^{34,40} Findings of Velez and colleagues were similar with dermatopathology WSI.⁴¹

A number of studies have examined time efficiency for cytopathology WSI screening and diagnosis. Dee *et al.* found that arriving at a diagnosis on WSI took almost twice as long compared to using glass slides (Table 2), even though only one quarter of the surface of the liquid-based preparation was captured.¹³

Stewart *et al.* described three proficiency tests, each consisting of 10 Thin Prep slides with no z-axis planes. Participants examined cases as per usual laboratory screening and diagnostic procedure. Participants could not screen WSI in the expected time according to Centres for Medicare and Medicaid (CMS) guidelines, and final time to finish the ten WSI cases was markedly more than the time expected to reach a diagnosis on equivalent glass slides.⁴²

Evered and Dudding recruited 24 experienced cytologists to examine 20 Sure Path cervical cytology slides in a cross-over design trial. Times were approximately 18 minutes for WSI compared with 8 minutes or less for glass slides.³¹

House *et al.* found that it took 1.5 times longer to diagnose gynaecological and non-gynaecological cytology cases on WSI compared to glass slides. For senior residents in the trial, it took nearly 2.5 minutes longer to make a diagnosis on the WSI.⁴³

In the study by Hanna *et al.*, no matter what cytology specimen type the group was analysing, the z-axis images

took 5.5 times longer on average to diagnose or screen compared to examining glass slides and WSI with x- and y-axes only took 1.8 times longer than the glass slides.³⁰

Increased reporting time is decreased laboratory income, and in addition, whole slide imaging does not reduce the laboratory time and cost of generating the original glass slides prior to scanning. In contrast to most histopathology specimens, cytology is a low volume test, so in this situation glass slides need to be produced pre-scanning and also need to be retained, because there is no tissue block.

Interestingly, a recent study where a pathologist reported 400 consecutive cases (histopathology, non-gynaecological cytology and FNA cytology) firstly as WSI and then by traditional glass slides 6 months later, showed that when the entire diagnostic workflow was analysed the sign-out time for digital cases overall was less than that for the conventional caseload (with a light microscope).⁴⁴ This may be due to a number of factors such as better ergonomics, larger viewing fields, faster case assembly, less physical slide handling, faster case retrieval for both current or archived cases or faster second opinions. Reporting was carried out over 20 reporting sessions each containing 20 cases; 16 of the 20 sessions resulted in a faster sign-out for digital compared to glass. A sessional log was recorded in order to identify potential factors that were resulting in better or worse diagnostic time efficiency for WSI. Those cases that required more use of 20× magnification or greater, resulted in a slower digital time compared to the traditional microscope. The range of cases that were associated with examination at higher power were low bacterial loads, for example in cases with *Helicobacter pylori* infection, as well as low tumour loads in tissue, or alternatively where there was need to examine nuclear detail.⁴⁴ Larger display monitors have been found to improve user experience for work requiring magnification greater than 20×,³⁸ so this may be a partial solution for these WSI diagnostic problems. Additionally, entire specimens often fit on the viewing screen with magnifications up to 4×, allowing more rapid assessment and diagnosis of many entities, particularly for small biopsies.⁴⁴

Vodovnik further explains that turnaround consists of diagnostic and non-diagnostic time. This author estimated that the shorter digital diagnostic time shown in their study amounted to an additional 250 cases per year per pathologist. They found the non-diagnostic time harder to measure but surmised that the consolidation of multiple tasks in digital

Table 2 Time efficiency for cytopathology WSI diagnosis

Group	Year	Average time for WSI (mins) per slide/case			Average time for glass (mins) per slide/case		z-axis	Comments	Washout
Dee <i>et al.</i> ¹³	2007	8 (cytotechnologists) 11 (cytotechnologist students)			5 (cytotechnologists) 6 (cytotechnologist students)		Yes	51 cytotechnologists and 28 cytotechnologist students Gynae-cytology: No difference in average time per slide with or without z-axis	WSI first then glass; dependent on when rotated glass slides reached the lab (up to 3 months)
Stewart <i>et al.</i> ⁴²	2007	18.9, 15.0, 7.5 (primary screeners) 7.6, 7.4, 4.4 (secondary screeners – pathologists)			(Reference interpretation) CMS guidelines		No	3 tests (10 gynae ThinPrep each) 3 cytotechnologists followed by 2 pathologists Comparison with reference interpretation	NA
Evered and Dudding ³¹	2011	18			8 or less		Yes 5 z-stacks then 21 z-stacks	20 SurePath slides 24 cytologists	NA, a cross-over design used
House <i>et al.</i> ⁴³	2013	3.0 (cytotechnologist) 4.2 (cytopathologists without DP experience) 3.1 (cytopathologist with DP experience) 5.6 (senior pathology residents)			1.7 (cytotechnologist) 2.9 (cytopathologists without DP experience) 2.0 (cytopathologist with DP experience) 3.1 (senior pathology residents)		No	22 cytopathology cases (gynae and non-gynae)	3 days; WSI first followed by glass slides
Hanna <i>et al.</i> ³⁰	2017	Specimen	Panoptiq	Aperio	Specimen	Glass	Yes	Panoptiq system: HP z-stacks on ROIs 30 cases 1 cytologist and 3 cytopathologists	3 weeks
		Total	11.0	3.5	Total	2.0			
		Gynae	24.5	7.3	Gynae	3.8			
		Non-gynae	4.2	1.6	Non-gynae	1.1			

CMS, Centres for Medicare and Medicaid guidelines; DP, digital pathology; Gynae, gynaecological; HP, high power; mins, minutes; NA, not applicable; ROI, regions of interest; WSI, whole slide imaging.

reporting systems could also be a contributory factor in shortening of the non-diagnostic time as well.⁴⁴

Potential implementation costs and incompatibilities that accompany obsolescence of technology are major concerns for institutions from a financial perspective. Interoperability or a vendor-neutral image standard would be a useful springboard for progression but represents a current road-block. Additionally, integration of new software and hardware with current laboratory infrastructure including the laboratory information systems (LIS) is important.⁴⁵ The real estate cost-savings for glass storage needs, mentioned previously, need to be balanced against a robust data storage strategy and costings.

To integrate WSI fully into the diagnostic workflow, potential costs of updated infrastructure include: hardware (scanning, storing, displays); software (viewing/annotating and incorporating WSI into reports); costs associated with process validation and quality assurance programs, as well as updating regulatory, medicolegal and billing items.

Vodovnik has reported cost benefits based on increased laboratory and reporting efficiency as well as productivity with digitisation, in a setting where there was marginal IT investment required.³⁸ In contrast, Pantanowitz describes significant investment of time and money.¹⁴

Thorstenson and colleagues describe their experience with implementation of WSI into the diagnostic workflow. Scanners run continuously with a concentration of workload overnight, and maintenance of operating systems is by industry supplier. Extra quality assurance steps are added into the laboratory workflow, such as decreasing cut up size of sections to ensure no over-hang of tissue on the slide that would interfere with scanning. Laboratory staff also check that all material on every glass slide has been included on the corresponding WSI. They describe a storage capacity for WSI of 130 TB (terabytes) accounting for 0.4 GB per slide, and digital slides are not stored indefinitely in this set up.⁶

LEARNING CURVES

It is important to note that when first starting to assess slides digitally, a learning curve period does exist, during which time there might be discrepancies in diagnosis, compared to using glass slides, as well as time inefficiency in making a diagnosis.^{3,42,46} For example, Ordi *et al.* found that discrepant diagnosis decreased over time when comparing gynaecological biopsy diagnosis on glass compared to WSI.¹²

So both speed and accuracy increase with use and familiarity with the technology, and this occurs in both the diagnostic as well as the educational arena. Van Es *et al.* in a randomised cross-over design compared WSI and associated digital online learning technology to traditional methods for learning cytopathology. The trial took place over three learning phases (gynaecological cytology, FNA cytology and exfoliative cytology), with a timed online examination using WSI at the end of each phase. This group found that the assessment scores for both intervention and control groups progressively increased with each phase, supporting the notion of a learning curve with this technology.^{15,47} Additionally, surveys also reveal that satisfaction from using digital pathology shows steady improvement with time.^{48,49} So, it is arguable that some of these reasons for diagnostic inaccuracy and time inefficiency on WSI are correctable by

increasing exposure of doctors and doctors-in-training to digital technology during their training and education.

ACCURACY IS IN THE EYE OF THE BEHOLDER

Flotte and Bell, suggest that 'in the opinion of many pathologists the images are still not as good as those viewed through an optical microscope'.³² This is true: historically research shows that users still perceive digital microscopy, especially digital cytopathology, as less accurate for diagnosis and more cumbersome and time-consuming to view.^{13,30,50} In their systematic review, Goacher *et al.* found that in the few studies that reported it, diagnostic confidence was less with WSI compared to a glass slide and a microscope.¹⁰

However, these perceptions usually do not correlate with the quantitative comparisons of glass versus digital accuracy with respect to diagnosis as well as education.^{13,15,30,47} Glassy coined the term 'mythinformation' when discussing the pathology profession's perception, in general, of digital pathology.¹ However, he rightly points out, that almost every study shows that digital microscopy is not inferior to a glass slide and a microscope. In a diagnostic setting, Evered and Dudding's results may even suggest that digital gynaecological cytology with z-stacking is superior to using glass slides.³¹

Digital microscopy is strongly accepted for education but there is variable hesitance to use WSI for assessment purposes⁴⁶ especially in cytopathology¹³ because of perception that performance might be slower in this fixed time setting.^{13,31,42} Pathology trainees also comment that examining WSI can be 'frustratingly stop-start'.¹⁵ Ross *et al.* mention that their participants in pathology quality assurance programs expressed reluctance early on to accept digital technology particularly in cytopathology. However, their results suggest that diagnostic accuracy with WSI in these cytopathology quality assurance programs was acceptable.¹⁸

DIGITAL PATHOLOGY FOR EDUCATION

In an educational setting Van Es *et al.* found that cytopathology WSI and associated adaptive online tutorials worked just as well for anatomical pathology trainees, compared to textbooks and glass slide teaching sets, in order to prepare them for subsequent assessments.¹⁵ For their senior medical student cohort, WSI and associated online adaptive tutorials were superior to traditional educational resources when learning to make a diagnosis on FNA cases.

Digital pathology provides equity and standardisation of learning resources for medical students as well as doctors who are training to be pathologists. Doctors in training now consider WSI repositories as essential and invaluable for learning pathology.¹⁵ WSI 'make it an even playing field for trainees',¹⁵ particularly for those in remote locations where they may not have exposure to the same range of teaching sets or diagnostic cases compared to those trainees working in larger centralised laboratories. Surveys have shown that doctors in training find it easier to learn histopathology and cytopathology from the interactivity of WSI and associated technology.¹⁵ Learning platforms are now available that can incorporate WSI providing individualised visual and written feedback and giving immediate reinforcement of correct responses and remediation of misconceptions in real-time.⁵¹

Such platforms can analyse learner accuracy and misconceptions in real time. This can enable the teacher to monitor the level of complexity of the lesson for the user/learner cohort and whether the adaptive feedback provided is functioning effectively to remediate misconceptions. Learning platforms are also available that allow diagnostic features to be annotated on WSI and these annotated layers to be shared (see Fig. 3).

Digital pathology is also useful for assessment purposes not only for medical students but also for specialist pathology trainees. Candidates for part I and part II anatomical pathology examinations for the Royal College of Pathologists of Australasia (RCPA) may originate from all states in Australia, as well as New Zealand, Malaysia and Singapore. So, ensuring relative standardisation of the material on the glass slides used for these examinations represents a large burden in both time and expense for the RCPA and distributing identical diagnostic material to all these examination sites is logistically challenging. Digital pathology is an ideal medium for assessing cytopathology, haematology and small biopsy histopathology, where material is of low volume, and thus particularly difficult to replicate and standardise. Digitisation also allows assessment material to be easily archived as well as facilitating inclusion of rare entities that otherwise could not be included in assessments when using glass slide specimens. WSI can also be easily incorporated into online assessments in pathology authored through programs such as Questionmark Perception (Questionmark Computing, UK) for both the medical student and specialist trainee setting.^{15,47}

The areas which can cause difficulty in digital pathology have now been well documented such as assessment of dysplasia, microorganisms,³⁷ and structures relying on red-stain intensity such as eosinophils.⁸ This will allow caution with such cases in the assessment setting. Cytology WSI, to a degree, is regarded with caution for assessment. This is due to features such as 3-dimensional cell groups and distribution of potentially diagnostic cells across multiple focal planes,¹⁵ as well as ergonomic difficulties perceived when examining

cytopathology WSI (especially if there are focal z-axis annotations), in a fixed time setting. However, cytopathology WSI which have z-axis across the entire slide may be met with greater acceptance for these purposes.²²

There are some complexities associated with the inclusion of digital microscopy into the assessment process, and it is useful to be guided by the experience of others. Van den Tweel and Bosman give a detailed report on their 2-year experience using WSI for European pathology training (including histology training) and postgraduate assessments.⁵²

DIGITAL PATHOLOGY FOR QUALITY ASSURANCE

Quality assurance programs (QAP) are important to support scientific and medical communities. Little has been published on digital pathology for quality assurance purposes, particularly in the difficult discipline of cytopathology,^{18,53,54} despite the fact that glass slide based programs are fraught with impracticalities.^{18,53} Digitisation of quality assurance programs has guaranteed that the same diagnostic material is present on the slide that is accessed by all participants no matter how remotely these participants are distributed. Multiple advantages are cited such as standardised case material, opportunity for more powerful statistical analysis, efficiency of case archiving and retrieval, reduced postage and customs logistics, as well as retention of cases by participants for future educational activities.¹⁸ Additionally, digitisation of tissue biopsy slides allows preservation of tissue for clinical practice, for example, future molecular studies.

DIGITAL PATHOLOGY AS A SOURCE OF 'BIG DATA'

Computer-assisted diagnosis (CAD) promises to be a useful support and quality assurance tool for anatomical pathologists to assist with issues such as assessment of nuclear pleomorphism, identification of mitoses or decisions on

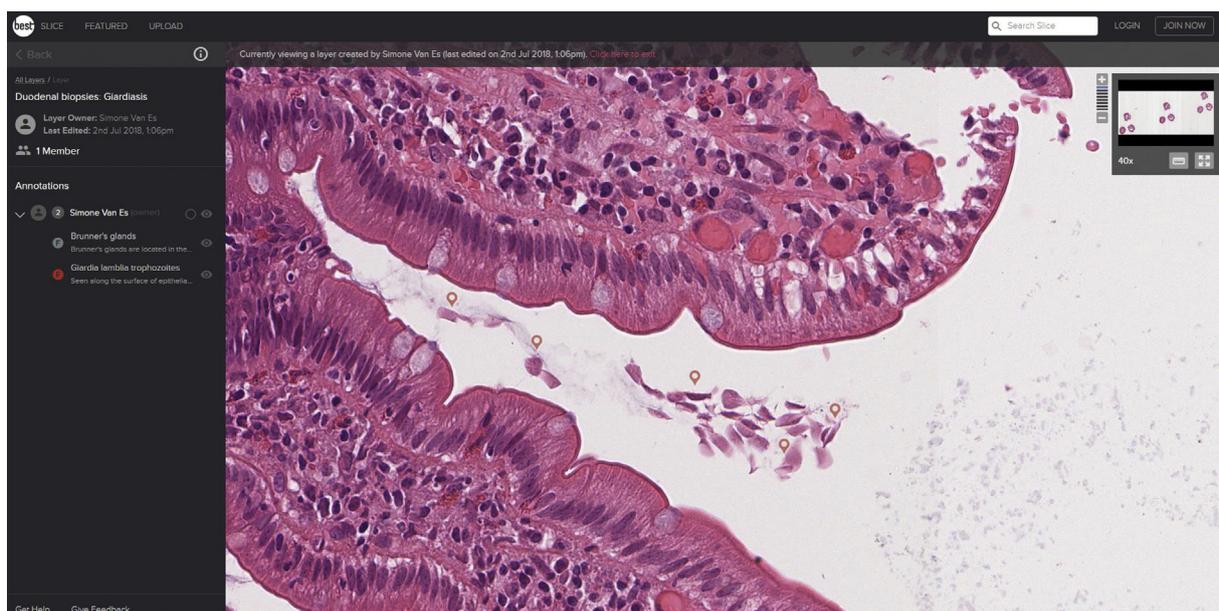


Fig. 3 Example of annotated layer on H&E-stained duodenal biopsy whole slide imaging: http://bit.ly/BESTeduau_Giardia.

whether tumours are benign or malignant.⁵⁵ Initial attempts to automate cervical Pap test screening began in the 1950s. However, the most productive and focused workflow benefits only occurred after the introduction of liquid-based cytology in the mid-1990s. Such systems have continued to be refined⁵⁶ and CAD ranges from the characterisation of nuclei in benign and malignant tissue on H&E WSI to algorithms designed to identify stromal-specific features that characterise malignant tumours.⁵⁷

To deal with the challenge of exponential rise in data associated with WSI, sub-region searches are being developed to automatically match image patches of a particular pattern or architecture against a given queried region of interest (ROI) on WSI being examined.⁵⁸ Algorithms developed by Qi *et al.* for example, mimic the two-step process used by a diagnostic histopathologist through a low power overview search for abnormal (coarse searching in their proposed algorithm), followed by a high power more refined analysis of the disease process (fine searching in their proposed algorithm).⁵⁸

Madabhushi and Lee describe the potential to mine 'sub-visual' data within WSI that is beyond the ability of the human eye to detect.¹⁶ There may be such opportunity of better quantitative modelling of the abnormal on a data-dense pathology slide than can ever be provided by a highly trained pathologist. There is also a huge potential for combining data from WSI with the patient's radiographic images, proteomics and genomics for better prognostic and predictive output, contributing to the concept of precision medicine where treatment is directed towards the signature characteristics of that individual's tumour.¹⁶

Our true pathological future, however, may embrace 'deep machine learning'. In this situation, vast amounts of data are provided to the digital neural network or 'deep neural networks' (DNN) to produce and then implement decision algorithms. Historically, pathologists are trained to arrive at a morphological diagnosis on a histology slide by manually progressing, stepwise, through a set of specific learnt decision trees. In clinical practice, to improve this morphology-based decision accuracy, which is complicated by intra- and inter-observer variability, adjunct investigations have been introduced such as molecular-based testing. However, it is possible that when provided with well annotated training sets, potentially with clinical and genomic data, the outcome for these DNN is automation in diagnosis and prognostication at a level more sophisticated than that obtainable by an unaided pathologist.^{16,59} If neural networks are given accurate and extensive enough training data, deep learning algorithms can outperform a human expert, for example, in strategic board games, or can perform with similar accuracy in diagnosing skin lesions compared to expert dermatologists.⁵⁹ Olsen and colleagues recently have described impressive accuracy rates for nodular BCC (basal cell carcinoma), dermal naevi and seborrheic keratoses with artificial intelligence using deep learning algorithms.⁶⁰

Challenges for our artificial intelligence (AI)-based future in pathology include the huge amounts of data included in WSI scanned with x- and y-axes and this will be exacerbated as WSI with z-stacking starts to become more routine. The training data itself needs to include not only all potential variants and possibilities of a certain type of diagnostic entity but also the potential range of colour and shades and staining of the tissue that may occur within and between

laboratories,^{16,55} and possibly between scanners. Potentially this might include faded stains as well. This essential data needs to encompass the range of visual data that an experienced pathologist would encounter over many years of practice. The selection of such data seems to be fundamental to the success of deep neural networks. Obviously, pathologists are best placed to make these data selections.

CONCLUSION: THOUGHTS FOR OUR DIGITAL PATHOLOGY FUTURE

Medical schools are adopting digital microscopy and online learning as a way of teaching histopathology, cytopathology and the pathological basis of diseases. This is due to a combination of costs, need for equitable resources across widely distanced and even offshore campuses as well as educational value that can be harnessed from this rapidly evolving technology.

That means that all our trainees as well as our more recently qualified specialists have pursued their basic medical training with digital microscopy not glass slides and microscopes. Digital microscopy is what will give them more speed and accuracy because of their familiarity with this technology.

Laboratories in The Netherlands,^{61,62} Sweden,⁶ Spain,⁶² Canada,⁶³ Singapore^{19,62,64} and the UK⁶⁵ use WSI for primary diagnosis, with recent approvals by the United States Food and Drug Administration (FDA)⁶⁶ and expansion of performance for WSI primary diagnosis in Japan.¹¹ Future-proofing product compatibility and product evolution as well as working on image standardisation are important issues.

Harnessing the data from the most visual discipline in pathology through artificial intelligence is obviously the way forward. However, we pathologists still need to utilise our natural intelligence to interpret the input as well as final output. It is likely that pathologists' expertise will be needed for a long time yet, although their current role within the workflow may slowly morph. As Glassy comments, our approach should be optimistic.¹ Regarding digital pathology, he recently enquired '*quo vadis*'—where are we going?¹ Well, evidence strongly suggests *semper ad meliora*—always towards better things.

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