Digital Pathology - Whole Slide Images and Virtual Microscopy Adaptive Tutorials

Educational tools in cytopathology for anatomical pathology trainees and senior medical students

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A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy

UNSW Medicine
School of Medical Sciences
2015
To my children,

You continue to teach me something new every day.
Docendo discimus…

.... We learn by teaching

(Seneca the Younger, Epistulae Morales ad Lucilium)
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Abstract

Background:

Diagnostic cytopathology is an essential part of clinical decision-making. It forms the basis for diagnosis of many cancers and infections. However, there is lack of standardised high quality cytology teaching materials to assist learning by specialist pathology trainees, as well as medical students. In addition, there is inequity of learning opportunities for trainees and students between departments and campuses. This project addressed the above issues by creating and evaluating digital educational material in cytopathology.

Methods:

1. Glass cytopathology slides were digitised as whole slide images (WSI). These were utilised for the creation of 25 virtual microscopy adaptive tutorials (VMATs). The acceptability of these resources for learning by pathology trainees and specialist pathologists was determined by deploying 3 VMATs in a pilot study, hosted by the Royal College of Pathologists of Australasia (RCPA).

2. To formally evaluate the efficacy, perceived efficiency and acceptability of WSI and VMATs for learning cytopathology, two randomised cross-over trials were conducted:
   
   a. Comparing WSI and VMATs with traditional glass slides and textbooks for pathology trainees
b. Comparing WSI and VMATs with online textbooks and atlases for senior medical students

Results:

Efficacy of learning with WSI and VMATs was at least equivalent to existing methods. Efficacy, efficiency and equity of learning provided by WSI and VMATs were prominent themes in evaluation surveys completed by pathology trainees. For medical students, exposure to VMATs resulted in significantly improved diagnostic accuracy for fine needle aspirates. Medical students' perceptions of VMATs were positive, particularly regarding immediate feedback, interactivity and equity.

Conclusion:

These digital pathology educational resources were found to be effective, efficient and acceptable e-learning tools for learning cytopathology, with the potential to provide widespread equitable access to high quality, consistent teaching material for a wide range of users.

Keywords: virtual microscopy, virtual pathology, digital pathology, whole slide imaging, WSI, virtual slides, vslides, cytopathology, adaptive tutorials, medical education, postgraduate pathology education, virtual microscopy adaptive tutorials, VMATs.
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Abbreviations, symbols and definitions

WSI – whole slide images

Vslides – virtual slides

VMATs – virtual microscopy adaptive tutorials

RCPA – Royal College of Pathologists of Australasia (RCPA is the specialist body which oversees the specialist training of doctors in all disciplines of Pathology for Australasia)

FRCPA – Fellow of the Royal College of Pathologists of Australasia

UNSW – The University of New South Wales

UNSW Medicine – The University of New South Wales Medicine Program (6 year undergraduate Medical Program)

AeLP – Adaptive eLearning Platform: this is an intelligent tutoring system developed by Smart Sparrow™.

3-D - three dimensional

GB – gigabytes (10^9 bytes)

TB – terabytes (10^{12} bytes)

PB – petabytes (10^{15} bytes)

FNA – fine needle aspiration

Gyne – gynaecological
H&E – haematoxylin and eosin

Thin Prep – Liquid-based gynaecological/cervical cytological preparation (Cytyc Corporation, Marlborough, MA)

eLearning – learning conducted using electronic media, typically via the internet

URL – stands for ‘Uniform Resource Locator’ which is a type of Uniform Resource Identifier (URI). URIs and URLs are generic terms for names and addresses of objects on the World Wide Web

HTML5 – is a type of language used for presenting content on the World Wide Web. HTML5 is the fifth revision of the HTML standard from the World Wide Web Consortium (W3C).

Hrs – hours

HSIL - high grade squamous intraepithelial lesion

SCC - squamous cell carcinoma

LSIL - low grade squamous intraepithelial lesion

HPV - human papilloma virus

HSV - herpes simplex virus

TB – tuberculosis

NHL - Non Hodgkin Lymphoma
Acknowledgements

People and Institutions:

I would like to acknowledge the following people who have assisted with various aspects of my research:

Image acquisition: Dr Maria Sarris (Histology and Microscopy Unit, UNSW), Ms Fifin Intan (RCPAQAP), Ms Jambhika Godara (Olympus Australia); Technical Support: Mr Jake Surman (UNSW), Mr Peter Zarzour (UNSW), Mr Zack Belinson and Mr Gary Goldie at Smart Sparrow; the BEST Network; Statistics assistance: Dr Michael Bennett (Prince of Wales Hospital, Sydney), Dr Roy Wilson (School of Mathematics & Statistics, UNSW), A/Prof Boaz Shulruf (Medical Education, UNSW); RCPA Participant Recruitment: Ms Vanessa White (Royal College of Pathologists of Australasia); Glass cytopathology slides: Ms Joanne La Malfa (Anatomical Pathology Department, Prince of Wales Hospital, Sydney).

I would like to thank the pathology registrars/trainees and medical students who agreed to participate in these trials and the pathologists and trainees who gave feedback on the Pilots. I would also like to thank the Royal College of Pathologists of Australasia, including the senior project officer, Vanessa White, as well as the training coordinators and supervisors throughout Australia, New Zealand and Malaysia who gave enthusiastic support to my project.
Finally, I would like to thank my primary supervisor Associate Professor Gary M. Velan and my co-supervisors Professor Rakesh K. Kumar, Dr Wendy M. Pryor, and Associate Professor Elizabeth L. Salisbury. The support and assistance with my progression through this process was appreciated.

**Funding**

This project was supported in part by the Royal College of Pathologists of Australasia (RCPA) which has received Australian Government funding under the Specialist Training Program (#247).

**Ethics:** Prior to the commencement of the studies in this project, approval had been obtained (UNSW HREC 11311, UNSW HC 14354, the Board of Censors Royal College of Pathologists of Australasia (RCPA) and the RCPA Training Overseeing Committee).
Publications

Publications deriving directly from this PhD research project (Some of the work presented in this thesis has been included in these articles):

Journal articles:

Van Es SL, Kumar RK, Pryor WM, Salisbury EL, Velan GM. (2015). Cytopathology whole slide images and adaptive tutorials for postgraduate Pathology trainees: a randomized crossover trial. Human Pathology. 46(9), 1297-1305. doi: 10.1016/j.humpath.2015.05.009


Undergoing peer review:

Van Es SL, Kumar RK, Pryor WM, Salisbury EL, Velan GM. Cytopathology whole slide images and adaptive tutorials for senior medical students: a randomized crossover trial.

Publications associated with this research topic and published or presented during the time of this project:


Conference presentations


http://journals.lww.com/pathologyrcpa/toc/2012/00001


**Conference posters**


[https://teaching.unsw.edu.au/engaging-students-blended-learning-landscape](https://teaching.unsw.edu.au/engaging-students-blended-learning-landscape)
1 Introduction

1.1 Thesis Outline

Advances in technology associated with virtual microscopy offer opportunities to enhance teaching and assessment of pathology at specialist-trainee level, as well as expanding on its established value for education in pathology for medical students (Velan et al., 2009). The key driver to employ this technology at the pathology trainee level is the need for equitable access to scarce high quality instructive clinical material across diverse training environments, and to achieve better standardisation of assessment. At the medical student level, enhanced use of virtual microscopy offers the potential means to deliver efficient, engaging and effective teaching.

This thesis focuses particularly on the efficacy and user experience provided by whole slide images (WSI) and virtual microscopy adaptive tutorials (VMATs) in cytopathology. Cytopathology is an important aspect of pathology currently neglected in medical curricula, and an area where clinical material of consistent quality and adequate range is difficult to provide for pathology specialist trainees.

The following review of the literature describes the current use, value and limitations of digital pathology, particularly in medical student and pathology specialist training and education, and identifies priority areas for development. The process of developing materials for the studies described in this thesis highlighted technical challenges and has resulted in recommendations for others who are considering making use of this technology.
Evaluation of educational interventions using these technologies with specialist trainees and medical students has demonstrated a high level of user acceptability as well as learning benefits at least equal in effectiveness to conventional methods with the significant advantage of much greater accessibility. The thesis concludes with a discussion of the potential for further development as well as potential applications of digital microscopy technology for pathology education and practice.

1.2 Terminology

At the time that this research project was initiated ‘virtual slide’ (‘vslide’) was the terminology of choice for the 2-dimensional (2-D) or 3-dimensional (3-D) image that had been scanned or ‘acquired’ from a glass slide. This term has now become outdated and has been replaced by the term, ‘whole slide images’ (WSI). Throughout this thesis, the terms WSI and vslides are used interchangeably. ‘Digital’ and ‘virtual’ are interchangeable terms that are also used throughout this text.

Virtual microscopy adaptive tutorials (VMATs) are referred to extensively through this text. These are adaptive online tutorials which incorporate WSI. VMATs are a type of intelligent tutoring system and they are novel in that they provide remedial feedback tailored to incorrect responses to key questions. This enables a teacher to create adaptive, personalised and interactive learning experiences for the user or learner. Both WSI and VMATs will be described in detail later in the text.

1.3 What are ‘virtual’ microscopy and WSI?

A real image on a glass slide must first be ‘captured’ to become a WSI. This process is facilitated by a device such as a scanner or camera that converts the original image, through a sensor that generates electronic signals, into a two-dimensional array
of dots or small squares called pixels. Each pixel has a specific colour representative of the colour that was present on the original image at that point. In order to visualise and differentiate cells from their surrounding structures image contrast is utilised. This is a function of the lenses and the image sensors. If the spaces between pixels in the sensor are small, the number of pixels and resolution of the lens is high, as well as the resolution of the lens used being very good, then the resulting optical image is more finely resolved without sampling distortion. The more pixels there are in the array the better the resolution of the image. Greater resolution obviously means higher memory requirements to store the visual data and the need for higher bandwidth capacity for a server to deliver those images to remote sites over networks and the internet.

Most computers will store WSI in a compressed format, resulting in a reduced image size. Data compression is important, particularly with 3-D WSI, which can contain many gigabytes (GB) of data. Data compression can be either ‘lossless’ or ‘lossy’. Lossless compression, as the name suggests, results in no data loss and the image remains identical; the most common type of lossless compression is LZW, an acronym that represents the 3 mathematicians who developed it – Lempel, Ziv and Welch (Aperio, 2005). However file size can only be reduced by a factor of two or three times with this technique.

Compression algorithms resulting in so called ‘lossy compression’ usually result in some loss of detail influencing the image quality (‘compression artefacts’). However, they can reduce the file size by 10 to 50 times depending on the technique used and the desired quality of the resulting image. Two common types of lossy compression are JPEG and JPEG2000. Lossy compression is most appropriate for diagnostic and educational digital microscopy because of the associated large amounts
of data. Compression ratios, ideal pixel resolution, monitor resolution and contrast ratios have been determined for diagnostic accuracy when using WSI (Kalinski et al., 2009).

The data for a whole slide image is stored in files which are vendor-specific. Common file formats include .SVS, .JPG (JFIF/JPEG), .TIF (TIFF), .JP2 (JP2/JP2000) or .GIF (Aperio, 2005). The terminology can become confusing, as the most common file format for image data is called JFIF (JPEG File Interchange Format). However, JFIF stores images that have been compressed with JPEG compression so for this reason are often simply called ‘JPEG files’ (Aperio, 2005). SVS files are actually TIFF file and have many advantages for image-viewing such as containing different resolution levels, using a tiled image organisation (which will be described below) and having the capability to use either lossless compression with LZW or lossy compression with either JPEG or JPEG2000 (the latter is preferred for better image quality).

Digital slides are captured with either x20 or at x40 objective lens. The higher the magnification of the objective lens the higher the resolution of the acquired image. The process of WSI acquisition can be quite lengthy particularly at high magnifications, (e.g., 40 X), which is necessary for cytology slides, with scanning of one slide taking up to 30 to 40 minutes, or longer if multiple focal planes are incorporated. An array microscope-based ultra-rapid WSI processor has been described (Weinstein et al., 2004) to accelerate this process. It is a lens array ensemble, consisting of stacks of numerous uniquely shaped lenses constituting many ‘miniaturised microscopes’ which overlie a glass slide. In one system there can be as many as 80 of these ‘mini’ microscopes. This array microscope produces a seamless two-dimensional
image, processing WSI in under one minute with outputs of 1000 WSI per day. The machine is accompanied by an automatic slide loader which can process up to 40 slides per hour without operator intervention. Validation studies by senior pathologists show good diagnostic correlation using WSI scanned using this technique compared to equivalent glass slides (Weinstein, 2005).

The quality of WSI can also vary from scanner to scanner (Walkowski and Szymas, 2011). Walkowski and colleagues (2011) demonstrated, through both automated objective assessment and subjective pathologist opinion, that there was a marked difference in quality factor of WSI acquired with two different scanners.

Current bandwidth requirements for WSI transfer via intranet or internet can be achieved in most university campuses and hospital departments. Bandwidth requirements for viewing of WSI are not high because the entire image file does not need to be transferred, as will be discussed below. The image is stored on a remote server, and only segments of the entire image selected are transferred as required. Nevertheless, network and computational bottlenecks can be improved to a degree by automated pre-setting of ‘regions of interest’ (ROIs), and creating ‘probabilistic maps’ (Romo et al., 2011). Models have been proposed, albeit with recognised limitations, to automatically calculate diagnostic areas or ROIs on WSI, by creating algorithms based on expert pathologists’ selections of diagnostic locations on the slide (Romo et al., 2011).

1.4 How are WSI viewed?

WSI are viewed on a computer screen or monitor, simulating the visual experience of viewing glass slides with a traditional microscope. Sharpness and colour
contrast contribute to the image quality and these vary depending on the hardware, software and screen monitor. The user interface needs to be intuitive (Pantanowitz et al., 2011b). There needs to be smooth scrolling, short access times and several options for magnification. Most slide scanners on the market use their own proprietary file formats, as previously described, often needing specific proprietary viewing software (Isaacs et al., 2011; Pantanowitz et al., 2011b). However, browser-based-viewing software does exist, enabling images to be accessed with a standard web browser, independent of their device’s operating system (Marchevsky et al., 2003; Helin et al., 2005).

The better the resolution of the digitised image, the greater the file size, storage requirements and bandwidth needed to serve these images over the web. However these files can be highly compressed to sizes of approximately 1 GB. Grouped aggregates of pixels are stored and loaded for viewing either as horizontal strips/lines or alternatively as squares/rectangles called tiles, depending on the file format. A tile is a fixed size digital element (commonly 240 x 240 pixels) that can be quickly rendered in a viewer. Tiles are combined (known as ‘stitching’) into yet larger arrays to produce a final image file that recapitulates the glass slide. Adjacent images and tiles may have differences in brightness and contrast due to varying locations of the camera when the images were acquired. This is known as shading; WSI viewers overcome these issues through techniques referred to as blending and histogram equalization (Sucaet and Waelput, 2014).

JPEG files have a stripped or lined organisation of their image pixels, so that when a small sub portion of the whole image is selected anywhere on the slide, all the strips above and to the side of it, including the strip in which the sub portion is
contained, must first be loaded from left to right, before this sub portion of the image can be viewed or processed. The result is that parts of the images load very slowly. TIFF files have the choice of either stripped or tiled organisation. The tiled format requires that the image pixels are stored in squares or rectangles. This is more practical for random access when coming to view a slide as only the tiles directly surrounding the desired portion of the slide need to be loaded to view that area, resulting in faster viewing (Aperio, 2005; Sucaet and Waelput, 2014).

The viewing software reduces the amount of data required by selecting only those tiles that pinpoint the users selected location on the WSI. Efficient zooming and panning on WSI needs a tiled organisation of the images, as well as multiple images from the same region on the glass slide taken at different resolutions. Zooming on a traditional microscope refers to increasing the magnification on the glass slide by changing the lens objective. Simulated zooming in WSI relies on identically-sized tiled images of different resolutions being stored and accessible from a pyramidal file arrangement. The base of the pyramid contains an image with the greatest resolution, from which the simulated ‘high power’ image is taken; this comprises most of the size of the tiled WSI file. Zooming on WSI requires changing the display resolution of the image, as with a real microscope. A thumbnail of the image can also be included and this image would have the absolute lowest resolution of all the images from that grid, appearing at the top of this pyramidal arrangement. In order to pan around a WSI, in the same way as one would by moving the stage on a traditional microscope, the viewing window of the computer displays constantly changing windows. This panning is easily achieved by using a tiled file organisation, as the amount of data needing to be loaded is only a fraction of the total image size.
Viewing of WSI is considered an issue by many practicing pathologists. Currently, most pathologists are not willing to incorporate WSI into their routine diagnostic practice (Isaacs et al., 2011). This is partially because they may be required to move back and forth between different software packages for diagnosis, report writing and other parts of the e-health record. Management systems that use a shared interface between laboratory and imaging systems have been developed and successfully employed to circumnavigate these issues (Isaacs et al., 2011).

Additionally, in most studies reaching a diagnosis by viewing WSI on a monitor has been shown to take longer than using glass slides with traditional microscopes (Steinberg and Ali, 2001; Dee et al., 2007; Furness, 2007; Stewart et al., 2007; Evered and Dudding, 2011). However, a number of studies have shown that diagnostic efficiency improves significantly with increasing familiarity with the technology (Weinstein et al., 2001; Stewart et al., 2007; Jara-Lazaro et al., 2010; Nielsen et al., 2010; Szymas and Lundin, 2010). Furthermore, viewing WSI using a high resolution array of a large number of computer screens, called a Powerwall, has been found to be as efficient as a traditional microscope, with the potential to outperform conventional microscopes in histopathological diagnosis (Treanor et al., 2009a).

1.5 History and uses of virtual microscopy

There are extensive descriptions in the literature over the last few decades of the use of digital imaging technology in pathology. ‘Digital’ or ‘virtual’ slides (vslides) or ‘whole slide images’ (WSI) with x and y axis capability are widely used (Isaacs et al., 2011; Khalbuss et al., 2011): these are scanned images of tissue sections or cytology smears, typically stored in a multi-resolution pyramidal format, as described
above. Scanning devices for creating digital images are well described and illustrated by Khalbuss and colleagues (Khalbuss et al., 2011).

Digital Pathology technologies include telepathology (Weinstein, 1986; Weinstein et al., 1987; Hitchcock, 2011), transmitted still images (van den Tweel and Bosman, 2011), WSI with z-axis capability (so-called z-stacking) (Dee et al., 2007; Kalinski et al., 2008; Zwonitzer et al., 2010; Evered and Dudding, 2011), WSI with extended focus imaging (EFI) (Lee et al., 2011) or so-called ‘focus fusion’ (Mori et al., 2011), video image capture (Melin-Aldana et al., 2008) and virtual pathology tracking software for training and assessment purposes (Krupinski et al., 2006; Treanor et al., 2009b; Mello-Thoms et al., 2012; Krupinski et al., 2013). Digital pathology has also been successful in developing tumour biorepositories (Isabelle et al., 2006; Teodorovic et al., 2006) and in supporting Pap smear screening practices (Pantanowitz et al., 2009).

The term ‘telepathology’ started appearing in the scientific literature in the mid to late 1980’s. Weinstein and colleagues made first reference to telepathology (Weinstein, 1986; Weinstein et al., 1987). This term described the sharing of a glass slide in real time over long distances employing high–resolution video cameras and monitors via a telecommunication linkage. The image continuously updates on a screen at a remote site using an internet connection. Telepathology compares well to traditional glass slides ("American Society of Cytopathology Abstracts: 54th Annual Scientific Meeting," 2006; Kerr et al., 2008; Alsharif et al., 2010; Heimann et al., 2012). This method is particularly advantageous for consultations and second opinions. Real-time telepathology can also be used to interpret frozen sections remotely.
There are options for the microscope to be either controlled onsite or alternatively remote-controlled. There are obviously no issues with z-axis (vertical focus) capability. However, remotely manipulating the microscope and slide, including changing magnifications, can be slow and labour intensive. High resolution real-time image transfer also requires large bandwidths. Therefore, real-time imaging with telepathology is less useful for applications requiring high magnification searching whilst screening, such as is required for screening cytopathology slides. It is obviously less helpful for self-directed learning techniques and education. An additional disadvantage with this method is that it potentially introduces diagnostic bias because the whole slide is not necessarily available for review. Additionally this method does not result in the storage of a permanent archived image for both medical records and education.

Descriptions of the term ‘virtual pathology’ started appearing in the medical literature around the turn of the millennium. ‘Digital laboratories’ were predicted for the future based on WSI having the greater potential compared to traditional microscopy for the correlation of clinical, imaging and immunohistochemical data that can accompany the virtual image (Barbareschi et al., 2000). One of the first descriptions of a client/server architecture providing a realistic simulation of a high power microscope was by Ferreira and colleagues in 1997 (Ferreira et al., 1997). They described client viewing software that runs on the user’s PC whilst the database software for storing, retrieving and processing the microscope image data runs on a high performance server located remotely.

Virtual microscopy can be employed for many educational purposes. WSI technology can be used to study hotspots or ‘regions of interest’ (ROIs) (Mello-Thoms,
2011) on a WSI, such as the areas on a WSI that attract longer examination times because they contain diagnostic information compared to those areas studied quickly because they contain only auxiliary information. A user’s slide exploration strategy of WSI can also be recorded as animated movies and analysed (Mello-Thoms et al., 2012). Software has also been developed to produce visualisations of the diagnostic track on WSI of an expert pathologist compared to students (Treanor et al., 2009b). Another developing area of research in virtual pathology is eye tracking (Krupinski et al., 2006; Krupinski et al., 2013). This involves use of headsets equipped with technology that monitors and records eye movements of subjects while examining WSI. The amount of time spent examining each area on the slide can also be recorded. The visual pathways utilised to arrive at a diagnosis can be compared between cohorts, for example between expert pathologists and less experienced pathology trainees or even medical students. Studies have shown that accuracy, total time examining a slide and complexity of visual pathways are all related to the experience of the user (Krupinski et al., 2006; Krupinski et al., 2008; Krupinski and Jiang, 2008; Krupinski and Berbaum, 2009; Krupinski et al., 2013). However, the eye-tracking technology use for such studies is very expensive, and is therefore not suitable for evaluation of large cohorts of participants, who may be geographically dispersed.

Finally, Zito and colleagues describe digitised cytopathology slides in JPEG format which are processed by virtual reality software to create a Quick time virtual reality movies (QTVR), developed by Apple Computer Inc. (Cupertino, CA) for Macintosh and Windows platforms (Zito et al., 2004). These virtual reality movies simulate conventional microscopy by allowing the viewer to navigate within the movie, to magnify or zoom out where desired, and to connect areas of interest to each other by
‘hotspots’. The system described was a simple and inexpensive way to create reservoirs of educational material. However, it was non-automated, thus only useful for slides containing small amounts of material.

1.6 Z-axis viewing

Scanning over multiple focal planes in WSI to match a traditional glass slide is not currently a viable option for routine educational and diagnostic practice due to limitations in processing power, memory, networking and storage space. Each additional focal plane that is scanned in the vertical dimension on WSI increases both the scanning time and the file size per slide. Recently, Evered and Dudding used both 5 focal planes at 1.0 µm intervals and 21 focal planes per slide at 1.5 µm intervals (Evered and Dudding, 2011). The time to scan each slide was considerable, (i.e. several hours), resulting in file sizes of WSI in excess of 7 GB. With current Internet bandwidth, such large file sizes result in network congestion when multiple users are trying to access and use the WSI via a server.

There has been much consternation over the lack of vertical focus on WSI compared to glass slides. The greatest advantage of the traditional microscope, when diagnosing or learning from a cytopathology slide, is a continuous range through an infinite number of focal planes along the vertical axis. Nevertheless, there do not seem to be consistent differences in diagnostic accuracy comparing 2-D WSI, 3-D WSI and glass slides based on several studies, described as follows. Dee and colleagues (2007) found that similar diagnostic accuracy could be achieved when using 2-D WSI and matched glass slides for cytopathological analysis. The same study showed that the diagnostic accuracy using 3-D WSI with z-axis capability was high, but slightly lower than glass slides (94% versus 96%). However this difference was not statistically
significant (Dee et al., 2007). Even though 2-D cytopathology WSI do not allow focus into collections of cells in the vertical planes, it may be that there are enough diagnostic cells in the single focal plane available for an accurate diagnosis. This group also found no differences in the time required to analyse individual 2-D WSI compared with 3-D WSI. However, Dee and colleagues (2007) and other authors (Evered and Dudding, 2011) found that screening WSI takes longer than screening a glass slide on a microscope.

Interestingly, Evered and Dudding (2011) found that increasing focal depth (21 vertical planes) resulted in superior diagnostic accuracy compared to glass slides and also to WSI with reduced focal depth (five focal planes). However overall, when these two sets of results were combined, there was no statistically significant difference in diagnostic accuracy between 3-D WSI and glass slides. A major disadvantage of WSI with z-axis capability is that scanning time is much longer compared to scanning only one focal plane for a 2-D WSI. 3-D WSI are also typically multiple GB in size, and are therefore cumbersome to view via the Internet. File sizes are proportional to the number of focal planes scanned. There is also no standardisation of depth between focal planes for different scanners (Lee et al., 2011).

Small biopsy histopathology pathology, in theory, also has similar 3-D viewing requirements to cytopathology specimens. However similar concordance rates for diagnostic accuracy are seen for 2-D small biopsy WSI (gastric biopsies) compared to traditional microscopy (Molnar et al., 2003).

An alternative to 3-D WSI are extended focus images (EFI). Some slide scanners are equipped with extended focusing algorithms (EF). These functions extract
focused areas from each focal plane, then assemble them together into a single image. The use of EFI in virtual cytology has thus far received minimal attention. Mori et al refer to this technological attempt to assimilate multiple vertical planes into a single plane as ‘focus fusion’ (Mori et al., 2011).

Lee and colleagues (2011) compared EFI technology to z axis scanned slides using three different brands of scanner, each having different numbers of focal planes as well as different size gaps between planes. File size for the EFI was small (200 MB) compared to the z-axis WSI files (1.5 GB) for seven focal planes. In addition, there was only one focal plane for EFI so there was increased speed during the evaluation process, with no need to move up and down through the vertical plane, compared to z-axis WSI.

The main disadvantages of EFI are that all vertical axes must still be acquired, which increased scanning time. Once Combined into EFI the information from the separate vertical planes is discarded permanently. Also there can be problems with background and resolution. Cells that were blurry in the multilayer stack are brought into focus with EFI. However the EFI algorithm is also applied to the extracellular background debris throughout the thickness of the slide resulting in a grainier appearance of the EFI and a lack of crispness and detail. This can translate into difficulty discerning intercellular borders and nuclear detail especially chromatin. These issues with visual quality are present no matter how many extra numbers of focal planes are included in the EFI algorithm (Qayyum et al., 2009; Lee et al., 2011).
1.7 Advantages of virtual microscopy

The constant growth in the body of knowledge in medicine requires pathologists, pathology trainees and medical students to engage in continuing education. Providing these groups with equitable access to an efficient and effective form of education (especially in remote and rural settings) is important but challenging. Virtual microscopy may provide a solution.

From an educational and diagnostic perspective, the advantages of virtual microscopy are numerous, including: (a) rapid access via a web browser to an image database; (b) relative permanence compared to glass slides, which are prone to fading and breakage; (c) ability to provide ideal teaching cases to large and/or dispersed audiences simultaneously; (d) multi-site consultation and/or education, including using annotation (Scoville and Buskirk, 2007; Stewart et al., 2008; Weaker and Herbert, 2009; Triola and Holloway, 2011; Elms et al., 2014; Elms et al., 2015), hot-linking (Fujita and Crowley, 2003) or incorporation into online tutorials (Velan et al., 2009); (e) straightforward incorporation into formative and/or summative online assessments (Velan et al., 2008), (f) Efficiency of slide archiving and retrieval both for the diagnostic and educational setting. In addition, there is the potential to view multiple slides either on the same screen or on adjacent screens for comparison.

Production of reservoirs of histopathology WSI also eliminates the need to produce multiple recuts of one slide, thereby minimising tissue waste from the paraffin block and saving laboratory resources and the time of technical staff. Production of histopathology and cytopathology WSI standardises material used for multidisciplinary meetings, tumour board boards, and clinical trials where slides require consensus review. Creating digital slide reservoirs for cytopathology and small biopsy pathology
provides standardised material for examination and proficiency testing nationally and internationally.

Advantages of digital pathology include replication of WSI, with minimal cost, storage, cataloguing and management. Unlike glass slides, the images do not deteriorate or fade. WSI allow networking for consultation or education, and they are amenable to annotation for consultation, meetings or education and training. WSI are also easily amenable to image analysis, internal audit and computer-aided diagnosis.

From a diagnostic viewpoint, advantages of digital slide acquisition and archiving are manifold. Previous slides from the same patient are easy to access and there is no potential need to re-stain or do without, if the old slide is faded and no further tissue remains in the paraffin block, as seen in Figure 1.
Figure 1a and 1b: Original H&E stained slide of a heart valve affected by subacute endocarditis compared to the recut stained slide. 1a) The slide in 1a is faded and diagnostic features can no longer be discerned. 1b) an H&E stained recut of the same heart valve specimen that is adequately stained. However as a result of cutting further into the paraffin block there is less diagnostic material in the block. This problem could be avoided if all glass slides were scanned and digitally archived at the time of diagnosis.

Pantanowicz and colleagues (2011a) observed that the ‘real power of a digital image resides in the computer applications that can be leveraged to analyse the information they hold’. Computer-assisted image analysis using digital images is not
possible with the light microscope. Thus, this is another aspect of digital pathology that promises to improve our accuracy, reliability, specificity and productivity. Automated screening systems used to quantitate immunoreactivity for diagnosis are becoming important diagnostically. It is possible to automate such screening using WSI (e.g., oestrogen and progesterone receptor immunoreactivity in breast cancer). Imaging algorithms have been described which utilise colorimetric as well as intensity determinations to analyse pixels on the WSI and corresponding immunohistochemical slides (Sharangpani et al., 2007).

Scaffolding existing pathology services in under-resourced countries, with the aim of improving patient care and survival, is another advantage of telepathology and virtual pathology for both education and diagnosis (Hitchcock, 2011; Hetzmann et al., 2014; Stauch et al., 2014). Virtual pathology repositories can be created by well-resourced countries to be made accessible to under-resourced countries to improve training and diagnostic skills.

Real-time telepathology can also facilitate rapid consultations and improve service and education for under-resourced locations, in the same way. The only potential issues are lack of appropriate bandwidth. Hitchcock describes mailing DVDs of WSI to centres in under resourced countries so that WSI can be viewed on local computers (Hitchcock, 2011). This approach can assist local pathologists and technicians in developing nations to develop their skills even if they do not have the infrastructure to support virtual microscopy.

WSI can also be included in electronic pathology publications (Kayser et al., 2011). Authors can submit the article at the same time as their accompanying glass
slides for digitisation and documentation. The images then are stored in a separate image data bank which is linked to the article. Alternatively the link to the WSI on another server can simply be included within the manuscript. Most journals are now available electronically. By providing WSI as supplements to research articles, the results can be verified by any reader of the article (Lundin et al., 2004). Those reading case studies or articles on disease entities can potentially learn more from the article by being able to view the attached or linked WSI. Lundin and colleagues (2004) point out that ‘virtual microscopy responds to the increasing demands on openness and transparency in scientific reporting’.

There are also some disadvantages of digital pathology, notably: (a) lack of familiarity compared to traditional microscopy; (b) limited resolution; (c) potential effects of image manipulation (Pinco et al., 2009; Jara-Lazaro et al., 2010); (d) issues with storage and delivery of large files, especially z-stacked images; (e) lack of standardisation with respect to image format, operating systems and viewing software (Tuominen and Isola, 2009);(f) initial cost outlay.

Many regard the disadvantages associated with WSI a barrier for use in education and diagnosis. Pathologists are very comfortable and familiar with traditional microscopy equipment with its full control of focal depth. WSI are limited in resolution by the technical features of their acquisition. Virtual images of course can also be manipulated as already described (rotation, brightness, red-green-blue colour, luminosity) which might have an impact on diagnostic accuracy and reliability of the image (Pinco et al., 2009; Jara-Lazaro et al., 2010), even as an educational tool.
More disadvantages include the size of WSI files both individually and as a collection. One whole slide image scanned with x and y axes may have a file size of between 1.5 GB up to about 8 GB depending on the size of the tissue on the slide and the detail therein. The file size for WSI is dependent on the magnification used (i.e., 40x scan has more data than a 20x scan), the size of the region scanned and the number of z-stacked slices at each locus. The number of focal planes needed to be scanned in the vertical axis of a whole slide image in order to achieve maximum diagnostic accuracy is also unknown to date. On the other hand, current bandwidth available for viewing of 2-D WSI via an intranet or the Internet is adequate in most departments and on most home computers. This is because the entire image file does not need to be transferred, only the portions of the image that are being examined at any point in time.

1.8 Virtual microscopy in the diagnostic setting

It is anticipated in the medium-term, that digital pathology will become increasingly commonplace in the pathology work environment. Rapid advances in technology are continuing to improve the speed at which data can be acquired and viewed. Access to virtual imaging technology is increasing as costs of acquiring and maintaining these systems are decreasing. Advantages of incorporating virtual pathology into the diagnostic setting, as previously described, include providing a diagnostic service to remote locations where employing a full time pathologist may not be cost effective (Weinstein et al., 2009; Alsharif et al., 2010; Pantanowitz et al., 2011b) or alternatively, allowing for a rapid second opinion where a single pathologist is working in the remote location (Alsharif et al., 2010; Pantanowitz et al., 2011b; Wilbur, 2011). Virtual microscopy also opens up the potential for virtual group practices to circumnavigate shortages of specialist pathologists.
Numerous studies have examined the value of virtual histopathology and cytopathology for diagnosis (Dee et al., 2007; Furness, 2007; Koch et al., 2009; Nielsen et al., 2010; Chargari et al., 2011). Digital and traditional techniques are comparable for accuracy (Harris et al., 2001; Heidger et al., 2002; Dee et al., 2007; Mill et al., 2007; Nielsen et al., 2010; Evered and Dudding, 2011; Triola and Holloway, 2011), however there are occasional reports of significant diagnostic features being missed on WSI compared with the original glass slide. For example, Kalinski and colleagues found that the identification of *Helicobacter pylori* in small gastric biopsies using 2-D WSI led in several cases to incorrect diagnoses compared with conventional microscopy (Kalinski et al., 2008). This could have resulted in failure to treat patients appropriately, with the potential for ensuing harm. The use of 3-D WSI (with 9 focal planes), however, in the same study resulted in similar diagnostic rates for this feature compared to conventional microscopy (Kalinski et al., 2008).

Purported reasons for inaccurate diagnosis using WSI are varied and include lack of colour fidelity (Ho et al., 2006; Jara-Lazaro et al., 2010); lack of adequate focal depth (Kalinski et al., 2008); lack of familiarity with the technology (Nielsen et al., 2010); system speed (Steinberg and Ali, 2001; Dee et al., 2007; Evered and Dudding, 2011); out-of-focus areas (Steinberg and Ali, 2001; Dee et al., 2007; Evered and Dudding, 2011); lack of resolution at high magnification (Evered and Dudding, 2011); problems with user interface (Isaacs et al., 2011); inability to integrate clinical data into the diagnostic interface (Isaacs et al., 2011); and lack of confidence with the interface and software (Jara-Lazaro et al., 2010).

Formulating a diagnosis on WSI can take roughly twice as long for cytopathology (Dee et al., 2007; Stewart et al., 2007; Evered and Dudding, 2011) or
even longer for small biopsy specimens (Furness, 2007) compared to diagnosing equivalent glass slides. However, it is important to note that when first starting to assess slides digitally, a learning curve period does exist, during which there might be discrepancies in diagnosis compared with glass slides (Stewart et al., 2007; Nielsen et al., 2010) as well as time inefficiency in making a diagnosis. Surveys have also shown that user satisfaction with digital pathology shows steady improvement with time (Szymas et al., 2010; Szymas and Lundin, 2011). It is arguable that some of these reasons for diagnostic inaccuracy on WSI are correctable by increasing exposure of doctors and doctors in training to digital technology during their training and education.

Many groups have alluded to the need for rotations in digital pathology and digital cytopathology as a necessary component of pathology specialist training (Fonyad et al., 2010). Virtual rotations in pathology informatics for pathology residents have been advocated in the USA due to the increased usage of WSI in pathology and cytology education and to a lesser extent in diagnosis (Kang et al., 2009). Isaacs and colleagues (2011) found that there was significant variation in the experience of both trainees and staff in their department regarding digital images, viewing software and the willingness to incorporate WSI into their routine diagnostic practice. Instituting virtual microscopy and associated technology early on in pathology education and training might help to alleviate this resistance to using technology for diagnosis.

Educational benefits of WSI have also been described: integrating WSI into diagnostic workflow enables efficient production of histopathology and cytopathology digital slide sets (Isaacs et al., 2011). This facilitates viewing of selected WSI remotely from any location in order to support independent study, practical laboratory classes,
conference sessions, reservoirs for examination purposes, quality assurance, as well as many other educational activities.

1.9 Virtual microscopy in the educational setting

Numerous studies have examined the use of virtual histopathology for teaching (Blake et al., 2003; Kumar et al., 2004; Kumar et al., 2006; Dee and Meyerholz, 2007; Mill et al., 2007; Dee, 2009; Weaker and Herbert, 2009; Fonyad et al., 2010; Triola and Holloway, 2011). Virtual microscopy is accepted as a reliable teaching tool through a wide range of disciplines in the health professions (Neel et al., 2007; Sims et al., 2007; Szymas and Lundin, 2010; Szymas et al., 2010; Monaco et al., 2011; Szymas and Lundin, 2011). The essentials for setting up WSI libraries have been well described (Coleman, 2009).

The educational value and reliability of virtual microscopy compared to traditional microscopy has been extensively assessed (Blake et al., 2003; Dee et al., 2007; Dee and Meyerholz, 2007; Dee, 2009; Triola and Holloway, 2011) with virtual methods scored as equal or even superior, in one or both respects (Harris et al., 2001; Heidger et al., 2002; Dee et al., 2007; Triola and Holloway, 2011). A number of studies indicate that students prefer learning microscopic pathology with virtual technology (Scoville and Buskirk, 2007; Monaco et al., 2011) particularly regarding time efficiency, accessibility and overall educational value (Harris et al., 2001; Heidger et al., 2002).

WSI can be incorporated into digital educational programs to teach pathology (Velan et al., 2009). Additionally use of online assessments or quizzes utilising
programs such as Questionmark Perception™ can incorporate digital images or links to WSI (Velan et al., 2002b; Glatz et al., 2006; Velan et al., 2008).

Virtual pathology is becoming standard practice in tertiary education and is also beginning to play a role in postgraduate pathology training and proficiency testing (Marchevsky et al., 2003; Stewart et al., 2007; Bruch et al., 2009; van den Tweel and Bosman, 2011). For example, when virtual microscopy was compared to traditional microscopy in a cohort of veterinary science students, there were significantly higher ratings for virtual microscopy as a learning tool. The students commented that it offered clearer images, greater opportunities for collaborative learning, time efficiency and flexibility (Mill et al., 2007). Other veterinary science groups have also described their success with virtual microscopy in pathology education (Sims et al., 2007). However, one study found that students preferred traditional microscopy in graded practical examinations (Neel et al., 2007).

There are similar findings in cohorts of cytology-scientists who were positive regarding the role of WSI in education (Dee et al., 2007). However, as described in previous studies (Neel et al., 2007), there was a preference for proficiency testing to resemble the real-life diagnostic situation. In that regard, even 3-D cytopathology WSI were not quite as acceptable to the participants as glass cytopathology slides (Dee et al., 2007).

Thus overall, most studies show strong acceptance of digital pathology as a learning tool, with some reluctance with its deployment for assessment purposes (Koch et al., 2009), especially in cytopathology (Dee et al., 2007). This is primarily due to the
perceived reductions in efficiency when using virtual cytology (Dee et al., 2007; Stewart et al., 2007; Evered and Dudding, 2011).

1.10 Virtual cytology

Cytopathology is an area of pathology where it is difficult to distribute multiple identical high quality glass slides for training purposes, continuing education and quality assurance. There is typically only one copy of each glass slide specimen, which is at risk of fading or breaking once archived. Digital pathology may represent a solution to these problems.

Microscopic examination of a glass slide is not limited by focal depth, whereas focusing through different planes cannot be achieved with a wholly or partially two-dimensional WSI. Cytopathology is z-axis dependent because cell aggregates and single cells are suspended at multiple planes in an irregular thick 3-dimensional matrix layer between coverslip and glass slide. Cytology slides may be much thicker than histopathology slides (which are usually approximately 4um thick) and the cells aggregates and single cells may be in any focal plane throughout this depth.

Cervical Pap smears and FNAs (fine needle aspiration)/FNABs (fine needle aspiration biopsy) often have clumps of material (secretory, debris, solid blood clot, cellular) or collections of cells, all of which require vertical focus to adequately visualise the features and abnormalities. Small tissue fragments can also sometimes be aspirated (micro biopsies) during FNAs giving an obvious vertical dimension to these collections of cells. The need for depth of focus is diminished somewhat with use of liquid-based cytology (Marchevsky et al., 2003; Stewart et al., 2007), as aggregates and clumps of large numbers of cells are reduced. These fluid-based cytology preparations
present cells in a monolayer on the slide, which reduces the number of planes to focus through for visualisation.

Slide thickness from one glass cytology slide to the next may also not be uniform, so this adds to the difficulty of knowing how many focal planes to scan for WSI – each additional plane adding to the scanning time and the file size and thus storage space. Proprietary scanners offer different numbers of focal planes as well as distances between planes, although some scanners allow for manual adjustment of this parameter. Some scanners enable up to 20 z-stacks but use of this capacity results in large file sizes that create difficulty with network image transfers. Z-stacking requires storage of files that are multiples of the already large single plane WSI (Evered and Dudding, 2011) resulting in slower computer response times.

Studies comparing the diagnostic accuracy of virtual cytopathology specifically, to traditional cytopathology, reveal the methods to be similar (Dee et al., 2007; Stewart et al., 2007) or reveal small differences between the two methods (Marchevsky et al., 2003; Gagnon et al., 2004; Wilbur et al., 2009). Users, do however, feel more comfortable if vertical focus is available on cytopathology WSI (Steinberg and Ali, 2001; Dee et al., 2007; Mori et al., 2008; Evered and Dudding, 2011). In addition some feel that an added disadvantage of cytopathology WSI is the difficulty in digitally marking the WSI in the same way as one can dot a glass slide with a pen to mark an organism or abnormal cell interest (Steinberg and Ali, 2001).

Consequently, modified approaches are potentially required for scanning and viewing virtual cytopathology. Due to the mono-planar flat nature of most but not all histopathology slides (apart from small biopsy specimens) single plane digital scans are
usually suitable and recapitulate the original image well. Current scanning technology often does not satisfactorily deal with cytopathology smears. Therefore more advanced manipulations are required for cytopathology specimens to obtain a more exact digital copy of the original specimen. These manipulations include image acquisition of multiple images in the z-axis or alternatively EFI (Lee et al., 2011).

1.11 Anatomical Pathology training in Australasia

Definition of an Anatomical pathologist:

Anatomical pathologists (APs) in Australia are medical doctors who have completed five to six years of supervised postgraduate training and passed three sets of examinations to qualify as medical specialists in pathology (Fellows of the Royal College of Pathologists of Australasia, (FRCPA). Examinations for Fellowship are difficult and complex (RCPA, 2015) and include diagnosis of cytopathology specimens. Pass rates vary but have been low in previous years (HWA, 2005).

Anatomical pathologists provide diagnostic reports on tissue samples, including in the areas of histopathology, cytopathology and autopsy pathology, utilising microscopy to evaluate fixed, stained tissue sections (histopathology) or collections of individual cells (cytopathology).
1.12 Undergraduate and postgraduate medical training in the remote or rural setting

In the supply of health services, the definition of regional, non-metropolitan, rural and remote is a difficult one, but usually refers to an area of ‘geographic disadvantage’ in the context of the item being studied. The Australian Government’s Department of Health uses the ARIA definition of rural and remote areas: ‘...identified with lack of accessibility to services regarded as normal in metropolitan areas’ (DoHA, 2001).

However, this definition refers to access to services and social interaction. Others have defined ‘remote medical context’ as including ‘locations that are geographically, professionally and personally isolating with limited sophistication of medical and logistics support, limited access to peers,…’ (Smith et al., 2008).

For the purposes of this thesis: ‘rural’ will be defined as outside a major metropolitan area, and ‘remote’ location is one where there is a discrepancy in access and equality of services. For cytopathology training, ‘remote’ could reasonably include any laboratory where there is minimal or no access to a range of cytological diagnostic cases, cytological teaching sets and pathologists who have both the skills and time to teach cytopathology. It would also include those locations where workloads are so high and pathology manpower so low that time for teaching registrars is minimal.

Based on these definitions, many laboratories in metropolitan locations could be included under this umbrella, including Anatomical Pathology laboratories in large paediatric hospitals, where fine needle aspiration biopsies are very rarely, if ever, performed and gynaecological cytology, a service that is not required.
Laboratories around Australia (RCPA, 2006) - and worldwide (Ford, 2010) - have undergone a critical workforce crisis in trained cytopathologists. Introduction of VMATs for teaching pathology trainees about cytopathology could be useful in this setting, given the demonstrated success of this approach in undergraduate histopathology (Velan et al., 2009).

1.13 Medical student education in pathology and cytology

Beginning with imprint smears in the 1830s and progressing to needle aspiration in the 1920s, diagnostic cytopathology has increasingly become an essential part of clinical decision-making, with application to samples ranging from body fluids to solid tumour masses (Hajdu and Hormoz, 2008; Diamantis et al., 2013). Cytological examination is the basis for initial diagnosis of most tumors and many infections. It is also widely relied upon as an effective and relatively non-invasive screening tool for many diseases.

It could be argued that quality instruction for medical students in this area could enhance patient care. Remarkably however, this is an area that has received little or no formal attention in most medical school curricula (Ford and Pambrun, 2015). Even though most medical students will not become pathologists, they will request or perform cytopathological investigations and will rely on cytopathological diagnoses that have been reported by pathologists. Hence, medical students should understand the language of cytopathology and should have a basic sense of how cytopathological diagnoses are rendered.

Meanwhile, structural reform has resulted in fierce competition for space in medical curricula, potentially resulting in decreasing exposure of medical students to
microscopic pathology and its role in diagnosis. In the past decade, a world-wide pathology workforce crisis led to a decrease in exposure of medical students to pathologists, as well as teaching in pathology (Ford, 2010; Hung et al., 2011). Pathology in undergraduate curricula is also increasingly being taught by non-pathologists (Smith et al., 2010), with ‘little attention given to appropriate medical student education in the area of laboratory medicine’ (Smith et al., 2010).

Consequently, the evaluation of effective and efficient digital educational material for undergraduate teaching in cytology is important in an era where there is a decreasing availability of traditional cytology teaching materials (glass slides), as well as a shortage of pathologists to teach cytopathology (Ford, 2010; Hung et al., 2011). Indeed, evidence is emerging that focused exposure of medical students to quality pathology teaching (including electronic educational material) has a positive impact on their understanding of pathology in clinical practice in addition to influencing their choice of pathology as a career pathway (Van Es et al., 2015).

Virtual microscopy using WSI has proved to be an efficient method for teaching both histology and histopathology (Blake et al., 2003; Kumar et al., 2004; Kumar et al., 2006; Dee and Meyerholz, 2007; Dee, 2009; Weaker and Herbert, 2009; Fonyad et al., 2010; Triola and Holloway, 2011). Whole slide imaging is also useful for diagnostic histopathology and cytopathology (Dee et al., 2007; Koch et al., 2009; Nielsen et al., 2010). Although there are potential limitations for cytopathology, because WSI have difficulty displaying the z-axis, comparative studies have found no evidence of major inaccuracies (Marchevsky et al., 2003; Gagnon et al., 2004; Dee et al., 2007; Stewart et al., 2007; Evered and Dudding, 2011).
There is thus an opportunity to provide medical students with a meaningful introduction to cytopathology using WSI, but to date, development of such educational resources has not been reported.

Importantly, not only does virtual microscopy provide students with convenient access to consistent, high quality educational materials, but in addition WSI can be combined with so-called ‘intelligent tutoring systems’ such as virtual microscopy adaptive tutorials (VMATs), with distinct practical and educational benefits in using WSI and VMATs to teach pathology (Velan et al., 2009).

1.14 Interactive learning

Digital technology could allow medical students and postgraduate specialists-in-training in fields including pathology, obstetrics, surgery, medicine, and dermatology to interact in an online environment via interactive computer programs. Increasingly these interactive programs are playing important roles in medical training (Seymour et al., 2002; Banks et al., 2007; Pospischil et al., 2007; Kanthan, 2009; Schlickum et al., 2009; Ho et al., 2014; Rimoin et al., 2015). There are many advantages of educational simulation. To date there have been minimal reports in the literature of interactive programs which are efficient, cost-effective and successful in teaching cytopathology and histopathology.

When considering successful implementation of eLearning activities, interactivity, feedback and flexibility are essential for engagement and learning impact (Marcus et al., 2011; Tochel et al., 2011). Flexibility is particularly important in a postgraduate or continuing education setting. Self-paced guided learning has also been
shown to improve acquisition of knowledge (Prusty and Russell, 2011; Polly et al., 2014).

1.15 Online learning modules in pathology

It has been established that virtual microscopy is useful for learning by medical students, but provision of WSI alone for teaching may not be sufficient to convey salient diagnostic features (Weaker and Herbert, 2009). Velan and colleagues (2009) found that simply using browser-based virtual microscopy was not sufficient for students who have conceptual difficulties, because teachers cannot provide one-on-one support for large cohorts of students. Thus some students are not optimally engaged in learning with WSI.

Additional support for students via an interactive eLearning environment is now readily achievable and may be particularly valuable for image-based disciplines such as microscopic pathology (Pospischil et al., 2007). Recent research suggests that visual action programs enhance areas in the brain associated with spatial attention and sensorimotor function, improving field of view, spatial resolution of vision, contrast sensitivity and oculomotor performance (Gong et al., 2015). Additionally interactive virtual teaching modules have been shown to improve general pattern recognition (Bejjanki et al., 2014) and pathology examination performance (Kanthan, 2009) and are an effective and acceptable way for medical students to learn pathology (Velan et al., 2002a; Velan et al., 2002b; Kumar et al., 2004; Kumar et al., 2006; Velan et al., 2008; Velan et al., 2009; Kumar et al., 2011; Ho et al., 2014).
1.16 Virtual microscopy adaptive tutorials (VMATs)

VMATs are a type of online tutorial, which requires users to interact with WSI. They are novel in that they are not only interactive (Velan et al., 2009), but they also emphasise learning by exploration of WSI (Ben-Naim et al., 2008) with individualised remediation of errors and misconceptions. Each VMAT is self-paced in nature with the aim of increasing the learners’ motivation through feedback and visual reward.

VMATs are developed using the Adaptive e-Learning Platform (AeLP), an intelligent tutoring system developed by Smart Sparrow™. The AeLP enables remediation of misconceptions and adaptive sequencing of questions and activities for users/learners (Ben-Naim et al., 2008). Immediate visual and written feedback can be provided to the individual user/learner based on their interactions in the online environment. Furthermore, analytics provided in real-time by the software platform provides teachers with evidence of the effectiveness of the adaptive feedback used in the tutorial.

The VMAT author can track analytical data available in real-time from the AeLP’s web interface, which reveals the spectrum of errors caused by participants’ underlying misconceptions and consequently adapt the content and remedial feedback states of the tutorial. This provides scope to constantly monitor, improve and streamline the feedback provided in each VMAT, mimicking real-life practice more than other currently available online formats. The successful use of VMATs to effectively engage undergraduate students in learning histopathology from WSI has been reported (Velan et al., 2009). However, the use of VMATs for learning cytopathology has not been described before embarking on this project.
There are a small number of other online cytopathology tutorials available from institutions worldwide. Examples include, ‘Cytopathology Tutorial’, an online cytopathology teaching resource of the Johns Hopkins University, [http://pathology2.jhu.edu/cyto_tutorial/Index.cfm](http://pathology2.jhu.edu/cyto_tutorial/Index.cfm); and ‘Cytology Stuff’, [http://www.cytologystuff.com/](http://www.cytologystuff.com/), an online educational resource of Hologic Inc. (Bedford, USA). However, it is arguable that without detailed analytics and subsequent adaptation of feedback, the desired dialogue between teachers and students in the online environment is diminished. Laurillard (2002) describes intrinsic feedback as essential for learning, where the feedback is adapted and tailored to the individual learner, ‘which helps to build their understanding of the internal relations between theory and practice’. This type of individualised adaptation of feedback helps the learner to ‘know how close they are to a good performance, and what more they need to do’ (Laurillard, 2002)

1.17 Virtual microscopy for assessment purposes – feasibility on a large scale

Digital pathology offers advantages for training material and for examination purposes particularly for specialist colleges. This is especially the case for cytopathology and small biopsy pathology where standardisation of material is essential for examination purposes.

Candidates for part I and part II anatomical pathology examinations for the Royal College of Pathologists of Australasia originate from all states in Australia, as well as New Zealand, Malaysia and Singapore. Distributing identical diagnostic material to all these examination sites is logistically challenging. Ensuring relative standardisation of the material on the glass slides that need to be used for these examinations represents a large burden in both time and expense for RCPA.
Furthermore, distributed glass slides will never be identical, particularly for cytopathology cases. Only WSI can ensure precise standardisation of assessment material.

However there are 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 two-year experience using WSI for European pathology training (including histology training) and postgraduate assessments (van den Tweel and Bosman, 2011). The authors describe of the hosting server architecture requirements and the software management requirements. The European pathology assessment and learning system partnered with iPath Diagnostics Ltd, a company specialising in the utilisation of WSI in pathology. Experience was reported with five progress tests for hundreds of participants undergoing specialist pathology training.

There were some initial issues with accessibility but this improved with time. This was influenced both by the hosting server capacity and participants’ internet bandwidth. The most obvious advantage they describe of employing WSI for their assessments instead of the traditional glass slides is that it allowed the deployment of the same examination and the same material simultaneously throughout Europe.

For the assessment of learning in pathology by medical students, there has been success with online assessments in pathology for undergraduate medical students using still images and histopathology WSI (Velan et al., 2002b; Velan et al., 2008). Velan and colleagues (2002b) have also shown that online formative assessments in pathology authored through Questionmark Perception™ (Questionmark Computing
1.18 Rationale for virtual cytopathology

Despite apprehension surrounding the use of virtual cytopathology as an educational and/or diagnostic tool, there are many potential advantages of exploring the reliability and acceptability of virtual cytopathology in this project, as a tool for learning and assessment:

1. EDUCATION:

i. Improve access to valuable learning tools - subtle individual cell abnormalities and cell patterns need to be learnt and recognised from cytopathology slides, which is difficult to achieve by independent study. In that context, interactive e-Learning tools such as VMATs provide individualised feedback and guidance, which might enhance learning of cytopathology by both postgraduate pathology specialist trainees as well as medical students.

ii. Gynaecological cytopathology: cervical Pap smear cytology, in particular, is difficult to learn. Access to gynaecological cytopathology WSI (particularly cervical Pap smears) and VMATs may represent a way to improve the learning of gynaecological cytology for all pathology trainees (as well as medical students) not just for those in specialised laboratories.

iii. Standardisation of material for teaching: there is currently lack of standardised access to diagnostic cytopathology teaching material in laboratories and medical schools. This is particularly the case for gynaecological cytology specimens, where a large proportion is handled in
private laboratories rather than public hospitals. This reduces the exposure of pathology trainees and students to these specimens.

iv. Permanent archiving of educational cases

v. Rural and remotely located pathology training: There is an increasing number of pathology trainees and medical students in remote and rural locations. However, many of these laboratories refer their cytology specimens to central laboratories for diagnosis as they do not have the expertise or manpower to handle these specimens, leading to inequality of specimens to learn from for pathology trainees and medical students in those laboratories or campuses.

vi. Networking and collaborative study: access to large sets of instructive cytopathology WSI and VMATs online may promote networking and the chance to study collaboratively.

vii. Targeting to multiple disciplines: Although this project will examine the educational effectiveness, efficiency and perceived value of virtual cytopathology, the results may translate to virtual histopathology (especially small biopsy pathology) and haematology as well.

2. EFFICIENCY

Saving of cost and time – once the initial cost of slide scanning equipment, server and software has been met, there are potential savings when high quality WSI and VMATs can be easily and rapidly accessed from large educational reservoirs for learning and for assessment purposes.

3. MAINTENANCE OF QUALITY
Increase in medical student and trainee numbers - from 2005 to 2012 the projected increase of medical student number was 81% and the recommendations at that time were ‘most urgently, postgraduate medical training will require substantial injection of resources to expand opportunities for clinical training, without compromising quality’ (Joyce et al., 2007).

Latest figures show that there were 16 837 medical students in Australia in 2014 (MDANZ, 2014). Currently there are 1 689 pathologists in active practice in Australia (RCPA, 2013a), however only 41.5 % of these are anatomical pathologists (AMWAC, 2003), trained to interpret and teach histopathology and cytopathology. Pathologists’ diagnostic workloads are teaching-prohibitive with one pathologists available for diagnostic work per head of 13 215 population in Australia (RCPA, 2013a). Pathologist workforce is aging with 20% of practicing pathologists across Australia and New Zealand over 60 years of age (RCPA, 2013a). Despite this imbalance of pathologists available for teaching compared to the numbers of those needing instruction in pathology, introduction of WSI and VMATs, may give pathology trainees and medical students equitable access to standardised and high quality pathology educational resources.

### 1.19 Aims and hypothesis

The preceding literature review has indicated that while digital pathology has great potential to enhance education in pathology, its effectiveness and acceptability for learning cytopathology has not been assessed in the setting of Australian pathology specialist training or medical education.
Experience with WSI and virtual microscopy adaptive tutorials (VMATs) for education and proficiency in cytopathology has never been assessed in the Australasian pathology setting, either for pathology trainees or medical students. No published evidence of efficacy, efficiency and acceptability of cytology WSI currently exists for these cohorts. In addition, VMATs, a new interactive form of online learning allowing individualised remediation of errors, has never been tested in any setting for learning cytopathology. Access to high resolution histopathology and cytopathology WSI and access to VMATs may be a potential means to enable engaging and interactive learning experiences in Pathology in a convenient, effective and time efficient manner in these settings. The numbers of medical students and pathology specialist trainee numbers in Australia are increasing. In contrast the specialist pathology workforce must cope with high workloads as well as teaching students and trainees. Thus, research into more equitable, effective and efficient means of learning pathology for specialist trainees and medical students, especially in the complex area of cytopathology, is timely.

Consequently, the main aims of this project are as follows:

1. To evaluate the efficacy, efficiency and acceptability of both WSI and VMATs compared to traditional glass slides and textbooks for learning cytopathology by postgraduate Anatomical Pathology trainees;

2. To evaluate the efficacy, efficiency and acceptability of both WSI and VMATs for learning cytopathology compared to existing methods of learning cytopathology (textbooks and atlases) for an Australian medical student cohort

3. Through qualitative evaluation, to develop understanding of how pathology trainees, as well as senior medical students and even pathology specialists
perceive the role of virtual microscopy (in particular the use of WSI and VMATs) in learning cytopathology.

In conjunction with these aims, the following hypotheses will be tested:

1. WSI and VMATs are at least equally as effective, efficient and acceptable as existing methods of learning cytopathology for Australasian anatomical pathology trainees
2. WSI and VMATs are at least equally as effective, efficient and acceptable methods as existing methods of learning cytopathology for senior medical students in Australia

To evaluate the above hypotheses, the following studies were performed and are described in the following sections of this thesis:

1. Pilot study: VMATs were associated with six WSI in order to evaluate usability and acceptance of cytopathology WSI and associated VMATs for trainees and specialists (Fellows) in Pathology.
2. Trial for specialist trainees in pathology: a randomised crossover trial was employed to compare efficacy, efficiency and acceptability of WSI and VMATs with traditional glass slides and textbooks for learning of cytopathology by postgraduate Anatomical Pathology trainees, who were training with the Royal College of Pathologists of Australasia.
3. Trial for senior medical students: a similar randomised crossover trial was employed to compare efficacy, efficiency and acceptability of WSI and VMATs with existing methods for learning (online textbooks and atlases) for medical students.
2 Methods

2.1 Scanning and deployment of whole slide images

Whole slide images

WSI were acquired at ×40 magnification using an Aperio Scanscope XT (Leica Biosystems Inc., Vista, CA, USA). A total of 154 virtual cytopathology slides were scanned for this project. Additional focal points were manually inserted to maximise the in-focus areas on the final WSI. Hundreds of individual adjacent high power images from each glass microscope slide were created in JPEG format and fused, “stitched” or “tiled” together, compressed into multi-resolution file format (.svs) to create one almost seamless image. Slides were scanned in two dimensions (x and y axes) and zoom was possible through the multiple magnifications that had been captured.

Files were cropped to reduce final size to less than 2 GB. The WSI were stored in a biomedical image database known as Slice (https://www.best.edu.au/slice/featured) and delivered using a custom-built whole slide viewer. Slice incorporates a server and an innovative whole slide image viewer developed by the BEST Network (http://www.best.edu.au). Slice is supported by Internet Explorer 9 or later, also Safari v.5.0 and later, Chrome latest versions and Firefox v.17 and later.

Accompanying VMATs were developed to augment the majority of WSI, while a subset of WSI was used for online assessments. Examples of a cytopathology WSI and the Slice interface can be viewed in Figure 2 or via the following links: https://www.best.edu.au/s/ruj9leul/vadul81r and
The slides were automatically pre-scanned using a low power objective (x2). Some slides were divided into two parts due to original large file sizes. Image file size ranged from 800 MB to just over 2 GB. Those slides which had a file size greater than 2 GB were reassessed and any non-diagnostic areas including those areas on the peripheral aspects of the slide were cropped manually in order to improve interaction with the interface. Time to scan was variable depending on the size of the material on the glass slide, but was typically 30 to 40 minutes per slide.
Figure 2: Cytopathology WSI (Papanicolaou stain) of a lymph node specimen. When viewing the slides on Slice, left clicking the mouse will allow the viewer to pan around the slide and using the mouse wheel allows the viewer to move the slide through multiple magnifications. The control box in the upper right hand corner of the webpage also allows the user to adjust the slide through multiple magnifications.

When WSI are viewed remotely, a user-selected tile is downloaded via a web browser. The selected tile is much smaller in file size compared to the whole slide, as described in the previous chapter, facilitating faster download speeds with typical broadband internet connections. The WSI viewer runs on the server and is viewed by “thin-client” arrangement using internet access.

WSI stored in the Slice database were used for creation of VMATs. In order to keep the WSI for the project ‘private’ or ‘unseen’, they were not ‘tagged’ and had no associated metadata on the server, thus preventing them from being discovered by a
search engine. This assured that participants in the trial could not get access to the digital pathology educational material unless and until they were provided with URLs by the investigators.

WSI were viewable on tablets, laptops and PCs. VMATs were viewable on laptops and PCs. The authoring environment utilises Adobe Flex/Flash/AS3 technology. A current limitation of this technology is that currently, drag-and-drop as well as drop-down list interactions include a Flash component (.swf file). As a consequence, the Adobe Flash Player™ plug-in must be installed and enabled on the browser used to access VMATs. This means that such interactions are not supported by Apple’s mobile devices (iPads and iPhones), for which Adobe Flash player is not enabled. The screen sizes, resolutions and colour qualities of participants’ viewing devices were not recorded for the purposes of this project.

2.2 Development of VMATs

VMATs were created using the Adaptive e-Learning Platform (AeLP), an intelligent tutoring system developed by Smart Sparrow Ltd. (https://www.smartsparrow.com/). The AeLP functions as an SaaS (Software-as-a Service) cloud-based platform, accessed via the web, with support for all major operating systems, browsers and devices.

VMATs are created by educators using the Annotate™ tool available via the AeLP. Each VMAT incorporated one or more WSI. The whole slide image appears within a window on a screen wherein users can zoom, and interact with the WSI, for example by dragging and dropping markers on regions of interest, or by identifying
features by selecting from drop-down lists. Wizards support the development of drag-and-drop, as well as drop-down list interactions with WSI.

Within each interaction in VMATs, authors can flag incorrect responses that indicate misconceptions - such responses are termed ‘trap states’. Remedial feedback can be tailored to each ‘trap state, thereby facilitating an adaptive and individualised learning environment.

Published VMATs are accessible via URLs, which can be distributed to participants by email, or via a Learning Management System (LMS), such as Moodle™ (https://moodle.org/).

Technical requirements to use the VMATs were a modern web browser, e.g. Chrome 30, Firefox 24, Internet Explorer 9 or Safari 5.1 or later, with Adobe Flash Player 11.1 or later installed.

VMAT interface:

The VMAT user interface is intuitive. However, information on the first screen of each lesson provides instructions on how to navigate the adaptive tutorial and to quickly orientate the user. Continual functions include forward and back options and a “restart” button to return to main menu button on each screen (Figures 3, 4 and Figures 5, 6).
Figure 3: VMAT Interface. An example of a drag-and-drop question.

Abbreviations: VMAT, virtual microscopy adaptive tutorial
Figure 4: VMAT Feedback. Individualised visual and written feedback provides immediate reinforcement of correct responses and remediation of misconceptions.

Abbreviations: VMAT, virtual microscopy adaptive tutorial
Figure 5: Further example of an interactive question within a VMAT. Abbreviations: VMAT, virtual microscopy adaptive tutorial

Figure 6: Example of a VMAT question with embedded educational video. Abbreviations: VMAT, virtual microscopy adaptive tutorial
Examples of VMATs created for this project can be accessed via the following links:

VMAT for Pilot Cytopathology Case 1:
https://aelp.smartsparrow.com/learn/open/5e8865181678420688b52345849916c3

VMAT for Pilot Cytopathology Case 2:
https://aelp.smartsparrow.com/learn/open/51b61dd4c4f64527bb6c32ad77dd8143

VMAT for Pilot Cytopathology Case 3:
https://aelp.smartsparrow.com/learn/open/d7e9ba6c954b41bfb48908df869f5e2d

Examples of trial VMATs are as follows:

https://aelp.smartsparrow.com/bronte/viewer/open/9r43hp68
https://aelp.smartsparrow.com/bronte/viewer/open/s581vrzn

Adobe Captivate version 6 was used to create short instructional videos with file sizes between 2 to 8 MB to augment some VMATs (Figure 6). The files created by Adobe Captivate are saved by default as .mp4 video files, and can then either be linked from the VMAT (e.g. on YouTube – https://www.youtube.com) or embedded via Annotate as .flv (Flash video) files.

VMATs were used to introduce cytopathology terminology as well as common inflammatory/reactive changes and associated micro-organisms. In WSI demonstrating
neoplasia, VMATs emphasised diagnostic features, as well as how to differentiate primary from secondary tumours including appropriate use of ancillary tests.

In total, 25 VMATs were created. Only one case was explored per tutorial. Number of WSI included in each tutorial ranged from 1 to 3. The first 3 VMATs were exemplars used in the pilot study to assess acceptability of this learning technique. The cases each represented a common entity from each branch of cytopathology: gynaecological cytopathology; fine needle cytopathology; and fluid/exfoliative cytopathology.

The remaining 22 VMATs were subsequently created for the randomised trials. Again the subject matter of each of the 22 VMATs covered common entities of the traditional topics in the three branches of cytopathology as mentioned above (gynaecological cytopathology, fine needle cytopathology and fluid/exfoliative cytopathology).

Feedback was obtained on three online pilot cytopathology VMATs from pathology trainees and specialist pathologists. This feedback was utilised when developing a further 22 VMATs for the subsequent trials for pathology trainees and medical students. Storyboards for each VMAT were initially drafted in Word documents, including screen captures showing location of diagnostic features on WSI. Questions, correct answers and feedback were subsequently entered into the Annotate authoring interface via cut and paste functions.

**Interactive features of VMATs**

Engagement and interactivity are promoted within the adaptive tutorials by utilising a variety of question formats (e.g. multiple-choice, drop-down lists, drag and drop type questions, fill-in-the blank), through which users can proceed at their own pace. An example of a drag and drop question and associated feedback are seen in
Figures 3, 4, 5 and 6. Immediate feedback is provided following the user’s/learner’s submission of responses. Feedback may contain useful educational information about the area on the slide, sometimes with the appropriate area being highlighted with different shading or arrows on the feedback screen. The screens generally do not permit progress until the question has been attempted. The author can limit the number of attempts for a question. Users can return to the question screen after feedback is provided on an incorrect answer, unless the maximum number of attempts has been exceeded.

Detailed analytics are accessible to the VMAT author. Analytics include lesson usage, completion rates, question scores and the proportion of adaptive feedback in use. This information enables the author 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. The average score for each question is provided, which may also indicate whether the level of difficulty is appropriate for learners. For drag and drop questions, heat maps (Figure 7) are available, thereby facilitating a quick visual analysis of learners’ areas of weakness and strength, as well as common misconceptions.
2.3 Pilot study

A pilot study was utilised to gauge usability and acceptance of virtual cytopathology slides and associated VMATs by trainees and specialists in Pathology. Three cytopathology pilot VMATs, associated with relevant WSI, were developed using the adaptive e-learning platform (AeLP).

Ethics approval for the pilot study was obtained from the Board of Censors and Training Network Overseeing Committee, Royal College of Pathologists of Australasia (RCPA), as well as UNSW Australia (HREC 11311). Open access to pilot VMATs was provided to all RCPA fellows and trainees over a period of nine months in 2013 for use, feedback and comment.

The availability of pilot WSI and VMATs was advertised through the monthly RCPA newsletter, *Pathology Today*. The educational material was password-accessible to RCPA members (Pathology trainees and specialist
Pathologists) via the RCPA education web portal (https://www.rcpa.edu.au/Education). An evaluation questionnaire was also incorporated at the end of each adaptive tutorial. The questionnaire comprised 5-point Likert scale items, (strongly agree, agree, neutral, disagree, strongly disagree) and enabled users to evaluate perceived impact of VMATs on learning and engagement. The items were as follows:

1. The interactive questions using whole slide images were helpful;
2. The interactive questions using whole slide images improved my understanding;
3. I clearly understood what was required of me in performing the tasks;
4. I would like to see more interactive questions using whole slide images in other practical classes;
5. I learned more from interactive questions using whole slide images than from exploring virtual slides independently;
6. I learned more from interactive questions using whole slide images than from exploring glass slides independently.

A comment box was provided for written responses. All VMATs and questionnaires were completed anonymously.

The analytics tab in each VMAT was accessed to record lesson usage, completion rates, and the proportion of adaptive feedback in use. This analysis indicated whether the adaptive feedback in each VMAT was adequate to effectively remediate misconceptions. Figure 8 shows an example of the overview tab from the analytics page provided by the AeLP.
Graphical analysis of the number of responses to each survey question was also provided by the AeLP for each VMAT. It should be noted that the analytics are updated in real-time in the AeLP, whilst the VMAT remains active or ‘live’. The responses to survey items were accessed at a later time point than the overview analytics. The responses for each Likert scale item were combined from all three Pilot surveys, and subsequently analysed in a spreadsheet.

2.4 Trial with anatomical pathology trainees (trial 1)

**Trial design and analysis**

This trial focused on the main themes of specialist cytopathology training, including gynaecology, fine needle aspiration (FNA) and exfoliative/effusion fluid cytopathology. Participants were volunteer Anatomical Pathology trainees from all states in Australia, as well as from New Zealand and Malaysia. They were recruited by advertisement and broadcast email through the Royal College of Pathologists of
Australasia (RCPA). Informed consent was obtained from all participants, who had the option of withdrawing at any time. No inducement was offered to the students to participate in the trial. The trial was divided into three phases as seen in Table 1, with a cross-over between phases and an online assessment at the end of each phase. The cross-over design was employed, to minimise potential bias caused by differences between groups with respect to prior knowledge, experience with cytology, or pre-trial familiarity with WSI. Prior to the commencement of this project, human research ethics approval had been obtained (UNSW HREC 11311 and the Board of Censors Royal College of Pathologists of Australasia).

A preliminary power analysis had indicated that to demonstrate a 30% difference between groups with >90% probability, 9-10 participants per group were required for the study. Participants were randomised into two groups such that there were equal numbers of junior (years 1 and 2) and senior (years 3, 4 and 5) trainees. Participants were then grouped geographically, each group having an approximate equal number of participants from major metropolitan teaching hospitals and rural or regionally located departments. Geographic grouping was intended to reduce or eliminate sharing of the different educational material by participants from different groups (intervention and control). In addition, this helped to avoid bias caused by potential inequities in teaching resources (existing/traditional methods of learning) based on metropolitan or rural location.

At the commencement of each 2-week phase of the trial, all members of a group either received links to WSI and VMATs, or a list of diagnoses/disorders to be studied in the traditional way using departmental teaching slide sets and textbooks. Participants
were asked to record the amount of time they spent studying cytopathology during each phase.

Table 1: Timeline and format of virtual cytopathology trial for RCPA trainees (trial 1)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Timeline (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynecological cytology (Phase 1)</td>
<td>VMATs and WSI</td>
<td>VMATs and WSI</td>
<td>0-14</td>
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<tr>
<td></td>
<td>ONLINE ASSESSMENT– Gynecological cytology</td>
<td></td>
<td>15-16</td>
</tr>
<tr>
<td>Fine needle aspirate cytology (Phase 2)</td>
<td>VMATs and WSI</td>
<td>Traditional</td>
<td>17-31</td>
</tr>
<tr>
<td></td>
<td>ONLINE ASSESSMENT- Fine needle aspirate cytology</td>
<td></td>
<td>32-33</td>
</tr>
<tr>
<td>Fluid/Exfoliative cytology (Phase 3)</td>
<td>Traditional</td>
<td>VMATs and WSI</td>
<td>34-48</td>
</tr>
<tr>
<td></td>
<td>ONLINE ASSESSMENT– Fluid cytology</td>
<td></td>
<td>49-50</td>
</tr>
</tbody>
</table>

Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials
<table>
<thead>
<tr>
<th>Topic</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gynecological cytology</strong></td>
<td><strong>VMATs and WSI</strong></td>
<td><strong>VMATs and WSI</strong></td>
</tr>
<tr>
<td>(Phase 1)</td>
<td>Case 1: HSIL (1xVMAT; 1xWSI)</td>
<td>Case 1: HSIL (1xVMAT; 1xWSI)</td>
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<tr>
<td></td>
<td>Case 2: HSIL/SCC (1xVMAT; 2xWSI)</td>
<td>Case 2: HSIL/SCC (1xVMAT; 2xWSI)</td>
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<td></td>
<td>Case 3: LSIL/HPV (1xVMAT; 2xWSI)</td>
<td>Case 3: LSIL/HPV (1xVMAT; 2xWSI)</td>
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<td></td>
<td>Case 4: Normal pap smear (1xVMAT; 2xWSI)</td>
<td>Case 4: Normal pap smear (1xVMAT; 2xWSI)</td>
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<td></td>
<td>Case 5: HSV (1xVMAT; 1xWSI)</td>
<td>Case 5: HSV (1xVMAT; 1xWSI)</td>
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<td></td>
<td>Case 6: Endometrial carcinoma (1xVMAT; 2xWSI)</td>
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<td>Case 7: Candida albicans (1xVMAT; 2xWSI)</td>
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<td></td>
<td>Case 8: Adenocarcinoma in situ cervix (1xVMAT; 2xWSI)</td>
<td>Case 8: Adenocarcinoma in situ cervix (1xVMAT; 2xWSI)</td>
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<td><strong>Fine needle aspirate cytology</strong> (Phase 2)</td>
<td><strong>VMATs and WSI</strong></td>
<td><strong>Traditional (Books and glass slides): list of diagnoses to study</strong></td>
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<tr>
<td></td>
<td>Case 1: TB (1xVMAT; 2xWSI)</td>
<td>TB</td>
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<tr>
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<td>Case 2: Papillary carcinoma of the thyroid (1xVMAT; 2xWSI)</td>
<td>Papillary carcinoma of the thyroid</td>
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<td></td>
<td>Case 3: Melanoma (1xVMAT; 2xWSI)</td>
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<td></td>
<td>Case 4: NHL (1xVMAT; 2xWSI)</td>
<td>NHL</td>
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<tr>
<td></td>
<td>Case 5: Adenocarcinoma Lung (1xVMAT; 2xWSI)</td>
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<tr>
<td></td>
<td>Case 6: Fibroadenoma (breast) (1xVMAT; 2xWSI)</td>
<td>Fibroadenoma (breast)</td>
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<tr>
<td></td>
<td>Case 7: Breast carcinoma (1xVMAT; 3xWSI)</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Fluid/Exfoliative cytology (Phase 3)</td>
<td>Traditional (Books and glass slides): list of diagnoses to study</td>
<td>VMATs and WSI</td>
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<td>------------------------------------</td>
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<td>Reactive pleural fluid</td>
<td>Case 1: Reactive pleural fluid (1xVMAT; 2xWSI)</td>
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<td>Mesothelioma</td>
<td>Case 2: Mesothelioma (1xVMAT; 2xWSI)</td>
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<tr>
<td>Gastric carcinoma</td>
<td>Case 3: Gastric carcinoma (1xVMAT; 2xWSI)</td>
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<tr>
<td>Urine</td>
<td>Case 4: Urine (1xVMAT; 1xWSI)</td>
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<tr>
<td>Urothelial carcinoma</td>
<td>Case 5: Urothelial carcinoma (1xVMAT; 1xWSI)</td>
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<td>Benign bronchial features</td>
<td>Case 6: Benign bronchial features (1xVMAT; 1xWSI)</td>
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<tr>
<td>Ovarian serous carcinoma</td>
<td>Case 7: Ovarian serous carcinoma (1xVMAT; 3xWSI)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials; HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; LSIL, low grade squamous intraepithelial lesion; HPV, human papilloma virus; HSV, herpes simplex virus; TB, tuberculosis; NHL, Non Hodgkin Lymphoma

As seen in Table 1 and Table 1a, Group 1 and Group 2 were both given the same educational material in the first phase, followed by an online assessment, to ascertain the level of cytopathology knowledge in each group and also to ascertain level of familiarity with virtual technology.

The diagnostic categories of the 22 VMAT-supported cases are listed in Table 2. The tutorials pointed out normal cells and patterns, inflammation/reactive changes and organisms. In neoplastic cases there was an effort to flag anything that may help diagnostically to differentiate primary from secondary tumours including ancillary tests.
Table 2: Diagnostic categories of the 22 Cases (VMATs and WSI) studied during the trials

<table>
<thead>
<tr>
<th>Gynaecological Cytopathology (Cervical smear/thin prep category)</th>
<th>Specific Diagnosis</th>
</tr>
</thead>
</table>
| Negative                                                      | 1. with endocervical cells, mature squamous cells, squamous metaplastic cells.  
                                                                  2. with Herpes Simplex virus  
                                                                  3. with Candida albicans |
| Atypical                                                      | 4. LGSIL – CINI/HPV  
                                                                  5. HSIL – CIN III  
                                                                  6. Squamous cell carcinoma  
                                                                  7. Endometrial adenocarcinoma  
                                                                  8. Adenocarcinoma of endocervix |

<table>
<thead>
<tr>
<th>Fine Needle Aspirate Cytopathology (Organ)</th>
<th>Specific Diagnosis</th>
</tr>
</thead>
</table>
| Lung                                       | 1. Granulomatous inflammation  
                                                                  2. Adenocarcinoma |
| Thyroid                                    | 3. Papillary carcinoma |
| Lymph node                                 | 4. Metastatic melanoma  
                                                                  5. Non Hodgkin Lymphoma |
| Breast                                     | 6. Fibroadenoma  
                                                                  7. Ductal carcinoma |

<table>
<thead>
<tr>
<th>Fluid/Exfoliative Cytopathology (Specimen type)</th>
<th>Specific Diagnosis</th>
</tr>
</thead>
</table>
| Pleural Fluid                                  | 1. Reactive mesothelial cells  
                                                                  2. Mesothelioma |
| Peritoneal Fluid                               | 3. Metastatic gastric adenocarcinoma  
                                                                  4. Ovarian serous carcinoma |
| Urine                                          | 5. Normal  
                                                                  6. Urothelial carcinoma |
| Respiratory Tract/ Bronchial brushing          | 7. Benign/reactive |
Distribution of WSI and VMATs amongst the trial phases are as follows. Twenty two VMATs were developed for this trial. Eight for phase 1 (gynaecological cytopathology) and 7 for phases 2 and 3 (FNA and exfoliative/effusion fluid cytopathology respectively). For phase 1, there were 14 associated WSI, 15 WSI for phase 2 and 12 WSI for phase 3. Slide numbers that were available for each case varied by their nature (even though case numbers themselves were the same for phase 2 and 3). The explanation for this is that the nature of the specimen dictates the number of slides that are usually produced for a certain case: Gynaecology cytology often produces 2 slides (one cervical smear and one Thin prep), FNA cases typically produce a fixed smear, an air dried smear and possibly a slide from a cell block whereas, a fluid such as a urine specimen would usually only result in one fixed specimen slide per case. 18 WSI were kept aside to be incorporated into the Questionmark Perception™ (Questionmark Computing Ltd, London, UK) quizzes for the 3 separate online assessments at the end of each phase.

Performance at the end of each phase of the trial was measured by a timed (60 minute) online virtual cytopathology assessment containing links to WSI (which did not always contain the screener’s diagnostic marks) using Questionmark Perception™ (Questionmark Computing Ltd, London, UK). Each assessment covered the theme that had been studied during that phase of the trial by both groups. The assessment following each module was subject matter specific, with the purpose of isolating any potential carry over effect in this cross-over trial design. Each assessment could only be attempted once and participants agreed to adhere to an honour code, which banned use of any outside aids or assistance (e.g. consultation with colleagues, access to text books...
or internet sources). If participants exceeded the time limit, answers were automatically submitted.

On average, each assessment comprised 7 WSI with 1-3 associated multiple choice questions. Following submission of answers, participants received immediate automated feedback. WSI, rather than glass slides, were utilised in the assessments because participants were distributed widely across Australia, New Zealand and Malaysia. Given the nature of cytology specimens, it is not possible to distribute identical glass cytopathology slides to all participants. Using whole slide images guaranteed that all participants received identical cytological material thereby ensuring that variations in assessment scores could not be attributed to any differences in the assessment materials.

Diagnostic categories in the first online assessment included negative smears (including changes associated with microorganisms) and atypical smears (low grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), squamous cell carcinoma, endocervical adenocarcinoma-in-situ/adenocarcinoma). The second online assessment on fine needle aspiration cytology included specimens from the thyroid (papillary carcinoma), lymph nodes (metastatic melanoma, non-Hodgkin lymphoma), breast (ductal adenocarcinoma), and lung (adenocarcinoma, squamous cell carcinoma, small cell carcinoma). The final online assessment on fluid/exfoliative cytology included pleural fluid specimens (reactive, metastatic adenocarcinoma), sputum specimens (normal, small cell carcinoma), bronchial brush specimens (benign) and a urine specimen (urothelial carcinoma).
At the conclusion of the trial, participants were also asked to complete anonymous online questionnaires regarding their perceptions of WSI and VMATs as educational tools for cytopathology (using a scale of 1=low, 10=high) and other aspects of their experience (using Likert 5 point scales). The questionnaire also invited open-ended responses about the best features of the WSI and VMATs, suggested improvements and how these methods of learning cytopathology compared to traditional methods.

Assessment scores and hours of study were compared using an unpaired Student's t-test. Data shown are mean ± standard error of the mean (SEM) unless otherwise stated. The questions were categorised for purposes of analysis into one of two categories: 1. Select a favoured diagnosis from a list of alternatives (“Diagnosis”); and 2. Identify cellular features (“Identification”), and the analysis of assessment results was stratified accordingly.

For Likert scale and rating items in the questionnaire, median ratings were compared using Mann-Whitney tests. Responses to open-ended questionnaire items were exported into a spreadsheet to facilitate thematic analysis. The PhD student and her supervisor (SLVE, GMV) independently identified common themes. Responses were coded and emergent themes were subsequently identified.

2.5 Trial with senior medical students (trial 2)

The same 22 VMATs and accompanying WSI previously described (Table 1 and Table 1a), for the trial with the anatomical pathology trainees (trial 1), were utilised for this trial with a cohort of senior medical students.
**Trial design and analysis**

The trial was approved by the UNSW Human Research Ethics Committee (HC 14354). As with trial 1, this trial also focused on the same main themes of cytopathology, including gynecology, fine needle aspiration (FNA) and exfoliative/effusion fluid cytopathology. The diagnostic categories of the 22 VMAT-supported cases have been previously listed in this chapter.

Participants were volunteer year 5 and year 6 medical students from the 6-year undergraduate Medicine program at the University of New South Wales (UNSW) Australia, recruited by broadcast email. Informed consent was obtained from all participants, who had the option of withdrawing at any time. No inducement was offered to the students to participate in the trial. The trial was divided into three phases as seen in Table 3, with a cross-over between phases and an online assessment at the end of each phase. Again, the cross-over design was employed to account for differences between groups with respect to knowledge, possible experience with cytology, and pre-trial familiarity with WSI.

Diagnostic categories were the same as in the Anatomical Pathology trainee trial (trial 1). In the first online assessment on gynecological cytology categories included negative smears (including changes associated with microorganisms) and atypical smears (low grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), squamous cell carcinoma, endocervical adenocarcinoma-*in-situ*/adenocarcinoma). The second online assessment on fine needle aspiration cytology included specimens from the thyroid (papillary carcinoma), lymph nodes (metastatic melanoma, non-Hodgkin lymphoma), breast (ductal
adenocarcinoma), and lung (adenocarcinoma, squamous cell carcinoma, small cell carcinoma). The final online assessment on fluid/exfoliative cytology included pleural fluid specimens (reactive, metastatic adenocarcinoma), sputum specimens (normal, small cell carcinoma), bronchial brush specimens (benign) and a urine specimen (urothelial carcinoma).

Participants were randomised into two groups such that there were equal numbers of year 5 and year 6 students in each group. At the commencement of each one-week phase of the trial, each group either received links to WSI and VMATs, or to a list of diagnoses/disorders to be studied according to existing methods, including links to a comprehensive cytopathology e-textbook and online atlas via the UNSW library and to a well-known cytopathology educational website. Participants were asked to record the amount of time they spent studying cytopathology during each phase. In online questionnaires, participants were also asked to rate the overall value of WSI and VMATs as educational tools (on a scale from 1 to 10) and to evaluate other aspects of their experience (using 5 point Likert scale items). The questionnaire also invited open-ended responses. These questionnaires were provided to all participants at the end of the phase 1 (Gynaecological cytopathology phase), to group 1 (intervention group) at the end of the second phase (FNA phase) and to group 2 (intervention group) at the end of the third phase of the trial (fluid/exfoliative cytopathology phase). The students in this trial were surveyed more frequently compared to the pathology trainees in trial 1, because the students were completely cytopathology-naïve, therefore monitoring of their perceptions as they progressed through the trial was particularly important.

Knowledge of relevant aspects of cytopathology was assessed at the end of each phase by using a time-limited (60-minutes) online virtual cytopathology quiz
authored in using Questionmark Perception™ (Questionmark Computing Ltd, London, UK). Each assessment covered the theme that had been studied during that phase of the trial. Each assessment could only be attempted once, and participants agreed to adhere to an honor code, forbidding use of any outside aids or assistance (e.g. consultation with colleagues, access to textbooks or internet sources). If participants exceeded the time limit, answers were automatically submitted. On average, each assessment comprised 7 WSI with 1-3 associated objective items. Following submission of answers, participants received immediate automated feedback. The assessments, WSI and VMATs had previously been validated in the trial involving the postgraduate pathology specialist trainees.

All questions within each assessment contained a link to a whole slide image (which did not always contain the screener’s diagnostic marks). The assessment questions were identical to the previous trial. The questions were categorised for purposes of analysis into one of two categories: 1. Select a favoured diagnosis from a list of alternatives (“Diagnosis”); and 2. Identify cellular features (“Identification”), and the analysis of assessment results was stratified accordingly.
Table 3: Timeline and format of virtual cytopathology trial for UNSW Medicine students (year 5 and 6) (trial 2)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Timeline (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynecological cytology (Phase 1)</td>
<td>VMATs and WSI</td>
<td>VMATs and WSI</td>
<td>0-7</td>
</tr>
<tr>
<td></td>
<td>ONLINE ASSESSMENT - Gynecological cytology</td>
<td></td>
<td>8-11</td>
</tr>
<tr>
<td>Fine needle aspirate cytology (Phase 2)</td>
<td>VMATs and WSI</td>
<td>Traditional</td>
<td>12-18</td>
</tr>
<tr>
<td></td>
<td>ONLINE ASSESSMENT - Fine needle aspirate cytology</td>
<td></td>
<td>19-23</td>
</tr>
<tr>
<td>Fluid/Exfoliative cytology (Phase 3)</td>
<td>Traditional</td>
<td>VMATs and WSI</td>
<td>24-30</td>
</tr>
<tr>
<td></td>
<td>ONLINE ASSESSMENT - Fluid cytology</td>
<td></td>
<td>31-34</td>
</tr>
</tbody>
</table>

Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials

Data shown are mean ± standard error of the mean (SEM) unless otherwise stated. Student’s t-test was used to compare the prior academic performance of the students in each group, based on mean weighted average mark (WAM). Total scores in each assessment, scores on items in the “Diagnosis” and “Identification” categories within each assessment, as well as self-reported hours of study in each phase of the trial.
were compared in the same way. Median ratings for questionnaire items were compared using Mann-Whitney tests.

Responses to open-ended questionnaire items were exported into a spreadsheet to facilitate thematic analysis. The PhD student and her supervisor (SLVE, GMV) independently identified common themes. Responses were coded and emergent themes were subsequently identified.

2.6 Statistical software

GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) was used for the quantitative statistical analysis and graphical presentation of data from both trials. Analytics for the pilot were provided by the AeLP (Smart Sparrow Pty Ltd, Surry Hills NSW Australia) and graphs produced with Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington USA)

2.7 Notes about Methods

2.7.1 Preliminary techniques with image acquisition and scanning software.

Importance of slide preparation

Histopathological and cytopathological material in pathology is processed onto a glass slide in order to be assessed with a microscope. As see in Figures 9 to 12, the stained tissue on these glass slides are prone to deterioration with time e.g. breaking, fading and collecting dust and debris, as well as detachment of cover slips. As the glass slides were scanned for this project, it became apparent that the quality and standard of the original glass slide affected the final result of the acquired whole slide image.
Figure 9a and 9b: Small intestinal biopsies, as an example, comparing quality of archived and recent specimens. 9a) Note the H&E stain is faded and the slide covered in dust resulting in a glass slide that is non diagnostic and suboptimal for scanning. The smaller the biopsy specimen the more rapid is the loss of staining; 9b) a similar recent slide for comparison. Glass slides should be scanned soon after preparation, when staining is optimal.
Figure 10: Papanicolaou-stained smear of a pleural fluid specimen. With time the cover slip has started to lift and the stain has faded. As cytology slides are often unique (i.e. there is no paraffin block from which to re-cut sections) this diagnostic medical record and educational pathology material, will be lost if not scanned soon after the original staining and cover-slippping.

Figure 11: Papanicolaou-stained cervical smear with fading. The cytoplasm of mature squamous cells and the neutrophils surrounding them should stain a sky-blue colour and their nuclei a dark blue, as seen on the right of the image. On the left, the remaining cells have faded to a ghost orange-pink. It is difficult to discern any important diagnostic features in these poorly stained cells. Scanning these slides immediately following preparation would have allowed a permanent digital archive.
Figure 12: A well-stained cervical Papanicolaou smear, for comparison to Figure 11. Cytoplasm of mature squamous cells, metaplastic cells, neutrophils and their nuclei are all staining appropriately in this image of a recently prepared cytology slide.

Faults on glass slides increase with time. This issue presented difficulty with image acquisition for this project, as many of the glass slides used for scanning were from teaching sets or older cases that were archived for some time. Although the original number of cases to include in this project was very large, the selection of quality glass slides for scanning was time-intensive and necessitated selecting only those slides that had a minimal deterioration. Consequently, to achieve high quality WSI, several factors needed to be taken into account. The quality of the original histological section or cytological slide needed to be excellent with no faults in the tissue, no fading of the stained material on the slide, no dust on the slide and no air bubbles in the mounting medium between the slide and the coverslip.
Annotations

As cytology can be z-axis dependent, depending on the specimen type, the scanning process of such specimens represented a challenge with manual insertion of focal points and rescanning required to improve focus on diagnostic areas. Typically when a glass cytology slide is examined for diagnosis, it is screened by a cytology scientist who places pen or paint marks around diagnostic cells or collections of cells. A paint, pen or felt pen marker can be used, as seen in Figure 13.

![Cytological smear on a glass slide with localising pen marks. Sputum specimen with cells consistent with small cell carcinoma. Note the thick dark pen marks on the glass coverslip, localizing diagnostic collections of cells.](image)

The slide is then examined by a second screener or pathologist before the case is signed out and verified. When scanning glass slides, the thickness and lack of transparency of the marking material on the coverslip often distracted the scanning sensor causing the focal plane of the image to be at the level of the coverslip. This resulted in pen marks being most in focus, rather than the specimen material. A
transparent lighter-coloured marker on the glass coverslip was found to provide least
distraction for the scanning sensor, allowing these diagnostic marks, which are helpful
for educational purposes to remain in place.

An alternative to leaving screener’s pen marks in situ on the glass slides prior
to scanning would be to scan the slide prior to screening and use an annotation tool to
mark diagnostic areas during screening of a slide in digital format. Annotations in Slice
(Figure 14) may be appropriate as a digital screening tool in pathology.

![Figure 14: WSI using an annotation to mark a cell collection of interest](image)

Others have used Google Maps Javascript Application Programming Interface
(API) for this purpose, based on its speed and ability to handle large amounts of data
(Triola and Holloway, 2011). A similar technique has been described in a separate
project using Slice (Elms et al., 2014; Elms et al., 2015).
Z-stacking

Z-stacking was attempted with the Aperio Scanscope XT (Aperio Technologies Inc., Vista, CA, USA). The slide was scanned in x and y axes, then whilst the glass slide was still on the stage, the diagnostic areas were selected for z-stacking and scanned as annotations on the slide. These appeared as a small green square on the WSI at low power which could be zoomed into along the vertical plane (Figure 15).

Figure 15: WSI of an FNA of the thyroid (x2.5 magnification) with z-stacking annotations. There are two z-stacked foci present on the WSI, represented by the green squares. These foci were selected manually. Note the magnification zoom bar and the focus bar to allow z-axis movement in the left upper corner of the window.

Only a small area of each slide could be z-stacked. If the relevant collection of cells was quite large, only a portion of the aggregate could be z-stacked. The end product was a collection of .svs files (one for the x-y axis slide and one for the z-stack
annotations). An .xml file directed the viewer to the correct location of the z-stacks on the slide. The WSI with z-stacked annotations were labor and time-intensive to create.

Additionally, browser based 3-D WSI viewing software was not available to view z-stacked WSI online for this study. Delivering WSI to participants via DVD was explored as an option, but the logistics of delivering these in a timely fashion to trial participants was not feasible. The few z-stacked WSI created were also not able to be incorporated into the VMATs.

An alternative to z-stacking is extended focus imaging (EFI), where in-focus areas from multiple focus planes are collapsed into a single plane WSI using complex algorithms. While the final file for the EFI uses the memory and transfer requirements of a single plane scan, scanning time is still as long as creating a multi-layered z-stack. EFI technology was utilised for a sample fluid-based cytology WSI - Olympus VS-120 (Olympus Corporation, Tokyo Japan) - and compared to a similar area on the z-stacked portion of the WSI. Subtle differences in these areas of the slide could be seen (Figure 16), similar to the those described by other groups (Lee et al., 2011). In particular, EFI scans are less crisp compared to 2-D and z-stacked WSI. This technology was therefore not pursued further in this project.
16a) x 40

16b) x40

Figure 16a and 16b: Pleural fluid comparing EFI technology versus multi z-stacking. 16a) Pleural fluid EFI; 16b) the same sample scanned with z-axis annotation

2.7.2 Justification for trial design

A crossover design was selected for both trials in this project, in order to control for prior potential exposure both to cytopathology and to digital technology. A crossover design was an attempt to deal with any different attributes regarding technology or education in the different groups that are otherwise impossible to control for. A crossover design is also more statistically effective because it requires fewer participants for the same statistical power (Senn, 2002).

There are a large number of studies which describe the use of WSI technology alone. The vast majority are descriptive in nature, some with cross-sectional analysis of frequency of use, effectiveness or perception (Harris et al., 2001; Heidger et al., 2002; Blake et al., 2003; Kumar et al., 2004; Kumar et al., 2006; Mill et al., 2007; Sims et al., 2007; Dee, 2009; Weaker and Herbert, 2009; Fonyad et al., 2010; Szymas et al., 2010; Monaco et al., 2011; Szymas and Lundin, 2011; Triola and Holloway, 2011; van den Tweel and Bosman, 2011). A number of these studies are observational or cross-
sectional in nature and quantitate the accuracy of WSI against the gold standard diagnosis that had been previously provided by glass slide analysis, as described below.

There are few studies, examining the accuracy of WSI, that use a traditional experimental research design (Furness, 2007; Koch et al., 2009; Evered and Dudding, 2011). Evered and Dudding (2011) used a randomised controlled trial with a crossover in order to attempt to control for bias where they looked at 3-D WSI accuracy compared to glass slides. Most groups employ a sequential study method, where a group are asked to diagnose a set of WSI and these diagnoses are either directly compared to a previously provided gold standard diagnosis (from the glass slide) (Stewart et al., 2007; Bruch et al., 2009), or after a variable washout period the participants are then asked to provide diagnosis on the same slides in glass format (Marchevsky et al., 2003; Dee et al., 2007; Kalinski et al., 2008; Nielsen et al., 2010; Chargari et al., 2011). In the sequential-type studies all subjects serve as their own controls and the major advantage is reducing the sampling size that is needed. The statistical tests would assume randomisation. Disadvantages of this technique include knowing the ideal washout period (which varied from 3 weeks to 1 year in the studies reviewed) (Marchevsky et al., 2003; Dee et al., 2007) and the risk of carry over.

2.7.3 Qualitative analysis

A qualitative approach identifies participants’ experience through an analysis of reflection. It can provide deeper and more lateral information on the educational techniques being tested and add value to the final discussion and translation of the findings. One of the aims of the research in this project was to develop an understanding of how pathology trainees and pathology specialists, as well as senior medical students, feel about the use of WSI and VMATs for learning pathology,
particularly cytopathology. A qualitative approach was employed to appropriately address this question. A qualitative approach could provide additional information on user perception via the collection, ordering, description and interpretation of textual data. There are a diversity of approaches for analysis of qualitative data, however a very useful approach has been described by Kitto and colleagues (Kitto et al., 2008).

Important issues when using qualitative analysis are:

- justification of why this research approach was employed to answer a specific research question;
- procedural rigour;
- representativeness interpretation (such as, have negative cases been included and discussed);
- transferability (e.g., relevance of the findings to current research and knowledge and is the study cohort a good representation of the entire group).

Following these conceptual guidelines allow generalisations from the textual data (Kitto et al., 2008).

In order to describe and gauge user perception of cytopathology WSI and VMATS as an educational tool, participants were given questions with both scaled response formats and open comment formats. In both trials, the participants were invited to answer a number of open-ended questions about using cytopathology WSI and VMATs to learn cytopathology. These questions are in the appendix of this document. Individual responses could be correlated with performance in the online assessments.
In order to enhance interpretive rigour, two researchers were involved in the qualitative analytical process to increase the validity and reliability of the findings. However, the level of consensus amongst the researchers in this qualitative analysis was not necessarily paramount (Kitto et al., 2008) compared to the discussion of and reflection process of the researchers. Discussion of the analysis by the researchers provided opportunity for refining and further developing coding. All written responses to each question were provided in a spreadsheet. This was transferred to a word document. Themes were developed by the first researcher (SLVE) whilst reading all responses and subsequent codes created. These codes were inserted into the written responses. The code frequency was counted. Although the specific numbers were not necessarily important, the frequency allowed assessment of emerging and stronger themes.

Both the spreadsheet with the written responses and questions as well as the coded responses were given to a second researcher (GMV), to gauge agreement with the emerging and strong themes that had arisen from the textual data. Actual numerical frequency of the themes is provided in tables in the results chapter although these numbers in themselves are not paramount in qualitative analysis.
3 Results

3.1 Pilot study

Pilot Cases 1, 2 and 3

The pilot study of 3 VMATs and associated WSI was used to gauge usability and acceptance of this technology by pathology trainees and specialists for learning cytopathology.

Usage figures were derived from the AeLP, together with information about the average time users spent on each VMAT, and whether individual users completed the lesson. The majority of users spent a small amount of time (median 5 minutes) engaging with each adaptive tutorial. 31% of 119 users (n=37) completed pilot case 1, including the questionnaire. 41% of 32 users (n=13) completed pilot case 2, whereas 40% of 30 users (n=12) completed pilot case 3. Overall 62 out of 181 users were deemed to have completed a VMAT contingent on submission of the survey at the end of the tutorial. As the responses were anonymous, there was no indication whether the respondents were specialist pathologist or pathology trainees. Correspondingly, there was no indication as to whether any participants completed all three cases.

Evaluation Questionnaires

Anonymous responses to an online questionnaire available to participants upon completion of each VMAT case were combined and results are displayed in Figure 17. There was strong overall agreement from users that VMATs helped understanding and development of skills. There was also broad agreement that users learned more from VMATs than traditional glass slides or WSI alone.
There was a consistently strong positive perception of the VMATs with more than 80% of users responding positively to the majority of questionnaire items. 92% (59 out of 64) agreed that VMATs were helpful in developing diagnostic skills in cytopathology; 88% (56 out of 64) agreed that VMATs improved their understanding of cytopathology; 95% (61 out of 64) agreed that the instructions in the VMATs were clear; 91% (58 out of 64) agreed that they would like to use more VMATs; 88% (56 out of 64) agreed that they learned more from VMATs compared to WSI alone; 65% (35 out of 54) agreed that they learned more from VMATs than they would have done from a glass slide alone.
Figure 17: Likert-scaled responses in feedback questionnaires for the pilot study, quantified in numbers (above) and percentages (below). Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials.
User perceptions of VMATs

Open-ended feedback included positive comments about the flexibility and user experience provided by VMATs, e.g. ‘well-constructed and informative’ and ‘it is a great tool’.

Users’ comments included statements that the VMATs were beneficial for learning, e.g. ‘good basic material for those starting out in cytopathology’, ‘a well-designed resource’, and ‘pointed out the features of the virtual slide with useful explanations’. Some commented that the VMATs promoted efficiency, e.g. ‘I think being guided through with the interactive questions is particularly helpful if studying when tired’.

Engagement with the VMATs was reflected in comments such as ‘fun to do’, and ‘particularly enjoyed the interactive star/arrow style questions – great as it points out the features on the slides’.

Users offered helpful suggestions to improve the layout and functionality of future VMATs, e.g. ‘when the feedback window opens it is in the centre of the screen…perhaps it would be better if the feedback window could open slightly to the right to allow the user to view the cytological features while reading the feedback’.

3.2 Trial with anatomical pathology trainees (trial 1)

In this trial anatomical pathology trainees were randomised into two groups to receive either digital educational materials in cytopathology (WSI and VMATs) or, alternatively, to study with traditional cytopathology resources (glass microscope slides from pathology department teaching sets and textbooks).
43 Anatomical Pathology trainees volunteered for this trial. Of these, 21 were randomised to Group 1 (11 from year 1 or 2, 10 from year 3, 4 or 5) and 22 to Group 2 (10 from year 1 or 2, 12 from year 3, 4 or 5). There was no significant difference between the two groups in years of training (P= 0.4). These 2 groups were then provided different educational resources at different stages of the trial. At the end of each phase both groups were invited to complete an online assessment relating to the theme for that phase. In addition, each question in the assessments fell into one of two categories: 1. Select a favoured diagnosis from a list of alternatives (‘Diagnosis’); or 2. Identify cellular features (‘Identification’), and the assessments were analysed as such.

In phase 1 of the trial, both groups were provided with the WSI and VMATs relating to gynecological cytopathology. There was no difference between the two groups in the mean scores of those who completed the first online assessment, (all questions for this assessment were in the ‘Diagnosis’ category (Group 1: 55.6 ± 5.6, n=19; Group 2: 51.1 ± 6.0%, n=19; P=0.5) (Figure 18a).

In phase 2 of the trial, which focused on FNA cytology, only Group1 received WSI and VMATs. There were no significant differences in overall scores between groups, (Group 1: 91.8 ± 2.3%, n=12; Group 2: 82.6 ± 4.3%, n=15; P=0.09) (Figure 18b). There were also no significant differences between groups in mean scores for items in the ‘Diagnosis’ category (Group 1: 90.3 ± 4.3%, n=12; Group 2: 77.73 ± 5.6%, n=15; P= 0.10) or the ‘Identification’ category (Group 1: 93.3 ± 2.8%, n=12; Group 2: 88.0 ± 4.7%, n=15; P= 0.37) (Figure 18b).

In phase 3 of the trial, emphasising fluid cytopathology, only Group 2 received digital resources. Again there was a no significant difference overall between the mean
assessment scores of the groups, (Group 1: 77.3 ± 3.2%, n=12; Group 2: 72.5 ± 2.8%, n=17; P=0.28). There were also no significant differences in mean scores for items in the ‘Diagnosis’ category (Group 1: 66.8 ± 3.6%, n=12; Group 2: 62.7 ± 3.4%, n=17; P=0.41) or the ‘Identification’ category (Group 1: 87.8 ± 3.4%, n=12; Group 2: 82.5 ± 3.0%, n=17; P=0.27) (Figure 18c).
Figure 18: Group 1 and Group 2 trainee assessment scores ‘Overall’, for ‘Diagnosis’ questions and for ‘Identification’ questions respectively, after studying for: (a) Gynaecological cytology (b) FNA cytology and (c) Fluid/effusion cytology; (Please note: Gynaecological cytology assessment consisted of ‘diagnostic’ questions only; For FNA assessment, Group 1 =
Abbreviations: Gyne, Gynaecological; FNA, fine needle aspiration; WSI, whole slide image; VMAT, virtual microscopy adaptive tutorial.

Further, there was no significant difference between groups in their self-reported time spent studying the material in any phase of the trial (Phase 1, Group 1: 2.7 ± 0.3 hrs, n=18; Group 2: 4.2 ± 0.8 hrs, n=17; P=0.11; Phase 2, Group 1: 3.7 ± 1.2 hrs, n=16; Group 2: 4.3 ± 0.7 hrs, n=16; P=0.68; Phase 3, Group 1: 4.5 ± 1.1, n=12; Group 2: 5.6 ± 1.2 hrs, n=17; P=0.53). Comparing WSI and VMATs for learning in questionnaire responses, there was a non-significant trend towards a preference for the VMATs (median rating 8 out of 10, n=26) over WSI (7 out of 10, n=23) (P=0.06). Quantitative responses to questions on efficiency and usability of WSI and VMATs are presented in Figure 19.

Figure 19: Perceived benefit by trainees of WSI and VMATs as a cytopathology learning tool, where 5=strongly agree and 1=strongly disagree. Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials
Open-ended questionnaire responses

Qualitative analysis of open-ended questionnaire responses resulted in the emergence of a number of themes. A total of 25 participants commented on the cytology VMATs, and the dominant positive themes that emerged were: the value of VMATs as a high-impact learning tool; followed by convenience; equity of training opportunities; and time efficiency (Table 4).

Table 4: Selection of stronger emerging themes from trainees for VMATs*

<table>
<thead>
<tr>
<th>Themes</th>
<th>Response rate: N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valuable/High impact learning</td>
<td>45 (63)</td>
</tr>
<tr>
<td>Equity of training</td>
<td>8 (11)</td>
</tr>
<tr>
<td>Convenient/Easy access (at home, in-transit)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Time efficient</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Needs to be tailored to level of knowledge/training</td>
<td>5 (7)</td>
</tr>
</tbody>
</table>

*Many responses containing multiple themes

VMATs were considered by trainees as likely to be ‘one of the main sources of teaching for the (pathology) trainee in the near future’. They made a ‘huge difference to those of us who are junior’ and made it ‘feel like you are getting a personalised experience compared to traditional teaching’. Trainees also commented that ‘the interactive nature made it easier to understand and retain the information’ and that learning with VMATs was ‘much more appealing than the WSI alone and reading from
a textbook’. The theme of equity emerged with comments such as ‘even if you didn’t have access to teaching material or teachers you can learn via the VMATs’. Whilst trainees appreciated the VMATs as highly valuable learning tools, one participant commented on the difficulty of screening WSI in the window provided on VMAT screens.

For WSI, dominant positive themes were: convenience, followed by equity of training and valuable/high-impact learning (Table 5).

Table 5: Selection of stronger emerging themes from trainees for WSI*

<table>
<thead>
<tr>
<th>Themes</th>
<th>Response rate: N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Convenient (at home, in-transit on iPad)</td>
<td>23 (19)</td>
</tr>
<tr>
<td>Equity of training</td>
<td>19 (16)</td>
</tr>
<tr>
<td>Resolutions inferior to traditional microscopy</td>
<td>19 (15)</td>
</tr>
<tr>
<td>Valuable/High impact learning</td>
<td>16 (13)</td>
</tr>
<tr>
<td>Screening the slide tedious and slow</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Lack of 3-D focus</td>
<td>9 (7)</td>
</tr>
<tr>
<td>Archiving advantage – no fading, reservoir</td>
<td>7 (6)</td>
</tr>
</tbody>
</table>

*Many responses contained multiple themes

WSI were seen by trainees to be very useful for ‘learning on the go’, with touch screen devices providing pleasing resolution and easy panning as well as pinch/zoom changing of magnification. Comments included that equity was ensured by ‘access to great cases’ in every laboratory and ‘easy access to study from home’. A
representative comment on the theme of equity was ‘I guess the availability of resources in each department is variable. The virtual slides can make it an even playing field for trainees,’ and on the theme of valuable learning was ‘a comprehensive repository of cytology virtual slides would be invaluable’.

An emerging negative theme was that analysing a virtual cytopathology slide could be cumbersome and slow in comparison to a traditional glass slide, with comments such as ‘scrolling around on the (virtual) slide is much more time-consuming compared to a microscope’ and ‘I found screening (the WSI) in the exam really time-consuming’. Another negative theme concerned the perception that the resolution of WSI was inferior to glass slides. The lack of 3-D focus, although mentioned, was not a strong theme.

Negative comments regarding poor resolution and lack of 3D focus did not correlate with poor scores in the online assessment that was completed just prior to the survey (participants were provided automated feedback on their performance immediately prior to completing the survey). Negative comments came from participants with a range of scores from 98% to 38%. Participants’ comments are summarised in tables 6 and 7.
Table 6: Representative participant comments from trainees on VMATs

<table>
<thead>
<tr>
<th>Theme</th>
<th>VMAT comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>High learning Impact</td>
<td>- Made a huge difference, especially to those of us who are junior.</td>
</tr>
<tr>
<td></td>
<td>- Sense of achievement after completing a VMAT.</td>
</tr>
<tr>
<td></td>
<td>- Feel like you are getting a personalised experience compared to traditional teaching</td>
</tr>
<tr>
<td></td>
<td>- Interactive nature made it easier to understand and retain information.</td>
</tr>
<tr>
<td></td>
<td>- Much more appealing than the virtual slides alone and reading from a textbook.</td>
</tr>
<tr>
<td>Efficiency</td>
<td>- Very clear and efficient</td>
</tr>
<tr>
<td></td>
<td>- Ideal initial learning method.</td>
</tr>
<tr>
<td>Equity of Training</td>
<td>- Even if you didn't have access to teaching material or teachers you can learn via the VMATs</td>
</tr>
<tr>
<td></td>
<td>- Traditional methods are very dependent on center</td>
</tr>
</tbody>
</table>

Abbreviations: VMATs, virtual microscopy adaptive tutorials
## Table 7: Representative participant comments from trainees on WSI

<table>
<thead>
<tr>
<th>Theme</th>
<th>WSI comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-D</td>
<td>Ability to focus up and down through thick clusters of cells is invaluable - would be nice to have on a WSI.</td>
</tr>
<tr>
<td>Slide screening</td>
<td>Much harder to screen a slide on computer than on microscope.</td>
</tr>
</tbody>
</table>
| Resolution                   | Best quality as if we were seeing it on the glass slides  
                                | The resolution (of all WSI, not just these cytopathology WSI) never compares to using a real microscope.                                           |
| High Impact Learning         | A comprehensive repository of cytology WSI would be invaluable                                                                               |
| Efficiency                   | Best features- less impact on pathologist's time                                                                                               |
| Convenience                  | Best features include being able to use them at any location - can use them at home even if you don't have a microscope                        |
| Health, safety and ethics    | Avoids problems of glass slides (e.g. confidentiality issues with taking them off site, damaging/losing slides)                                |
| Interface                    | Easy to learn how to use the software                                                                                                           |
| Equity                       | WSI can make it an even playing field for trainees                                                                                              |

Abbreviations: WSI, whole slide images
3.3 Trial with senior medical students (trial 2)

In this trial, senior medical students were randomised to either receive the digital educational materials in cytopathology (WSI and VMATs) or, alternatively, to study with existing methods for learning cytopathology (etextbooks and atlases via UNSW library and the internet).

46 senior medical students volunteered to participate in this trial and 23 were randomised to each of Groups 1 and 2. However, possibly because cytopathology was non-assessable supplementary material, several students withdrew early. As a result, the number of participants who completed one or more assessments was reduced to 17 students in Group 1 and 18 students in Group 2.

There was no significant difference between the two groups in terms of seniority, with 10 students from year 5 and 7 from year 6 students in Group 1, compared with 11 from year 5 and 7 from year 6 in Group 2. Prior academic performance, as indicated by mean weighted average mark, was indistinguishable between groups (P=0.96).

There were also no significant differences between groups with respect to mean time spent studying the material in each phase of the trial (Phase 1, Group 1: 2.6 ± 0.3 hrs, n=17; Group 2: 2.8 ± 0.3 hrs, n=18; P=0.75; Phase 2: Group 1: 1.8 ± 0.2 hrs, n=15; Group 2: 2.4 ± 0.4 hrs, n=18; P=0.18; Phase 3, Group 1: 1.6 ± 0.2 hrs, n=11; Group 2: 2.1 ± 0.4 hrs, n=16; P=0.31).

As in the previous trial, the 2 groups in this medical student cohort were provided with different educational resources at different stages (phases) of the trial, followed by an online assessment at the end of each phase, which related to the theme
studied in that phase. In addition, each assessment question fell into one of two categories: 1. Select a favoured diagnosis from a list of alternatives (‘Diagnosis’); or 2. Identify cellular features (‘Identification’), and the assessments were analysed as such.

In phase 1 of the trial, both groups were provided with WSI and VMATs relating to gynecological cytopathology. There was no significant difference between groups in mean assessment scores for that topic (all questions for this assessment were in the ‘Diagnosis’ category (Group 1 = 35.4 ± 5.9%, n=17; Group 2 = 27.1 ± 3.6%, n=18; P=0.24) (Figure 20a).

In phase 2 of the trial, which focused on FNA cytology, only Group 1 received the WSI/VMAT resources, whilst Group 2 studied with existing resources (cytopathology atlas and e-textbook). The mean assessment score of the intervention group was higher, with a trend towards a significant difference (Group 1: 71.7 ± 4.8%, n=15; Group 2: 58.2 ± 4.8%, n=18; P=0.06). Further analysis revealed there was a significant difference in favor of Group 1 for items in the ‘Diagnosis’ category (Group 1: 74.3 ± 5.8%, n=15; Group 2: 55.6 ± 5.4%, n=18; P=0.025), but not in the ‘Identification’ category (Group 1: 66.7 ± 6.3%, n=15; Group 2: 62.5 ± 5.8%, n=18; P=0.63) (Figure 20b).

In phase 3 of the trial, emphasising fluid cytopathology, only Group 2 received the WSI/VMAT resources, whilst Group 1 studied with existing methods. There was no significant difference between groups in total assessment scores, (Group 1: 53.6 ± 5.7%, n=11; Group 2: 58.0 ± 4.5%, n=16; P=0.55). There were also no significant differences in mean scores for items in the ‘Diagnosis’ category (Group 1: 39.8 ± 5.2%,
6.9%, n=11; Group 2: 45.3 ± 6.0%, n=16; P=0.56) or the ‘Identification’ category (Group 1: 67.1 ± 5.9%, n=11; Group 2: 70.30 ± 3.9%, n=16; P=0.64) (Figure 20c).
Figure 20: Group 1 and Group 2 student assessment scores ‘Overall’, for ‘Diagnosis’ questions and for ‘Identification’ questions respectively, after studying for: (a) Gynaecological cytology (b) FNA cytology and (c) Fluid/effusion cytology. (Please note: Gynaecological cytology assessment consisted of ‘diagnostic’ questions only; For FNA assessment, Group 1 = intervention group; For Fluid/effusion assessment, Group 2 = intervention group)
Abbreviations: Gyne, Gynaecological; FNA, fine needle aspiration; WSI, whole slide image; VMAT, virtual microscopy adaptive tutorial; * = statistically significant result (P=0.02)

While both WSI and VMATs were positively received, there was a significant preference for the VMATs (median rating 8 out of 10, n=53) over WSI (7 out of 10, n=58) (P=0.0002). In Likert scale items (Figure 21), VMATs were also perceived as more useful in developing diagnostic skills in cytopathology (P<0.01), more time efficient (P=0.01), and providing more equitable learning opportunities (P<0.01) than WSI alone. There was a strong preference for both VMATs and WSI over existing methods of learning cytopathology, with a further significant preference for VMATs over WSI alone (P=0.03).

Figure 21: Perceived benefit by students of WSI and VMATs as a cytopathology learning tool expressed as median and range, where 5=strongly agree and 1=strongly disagree.

Abbreviations: WSI, whole slide images; VMATs, virtual microscopy adaptive tutorials
Open-ended questionnaire responses

Qualitative analysis of open-ended questionnaire responses resulted in the emergence of a number of themes. There were 84 comments on the VMATs (Table 8) and 101 comments on WSI during the three phases of the trial (Table 9). The dominant positive themes regarding VMATs were their value as a high-impact learning tool, benefits regarding equity of learning opportunities for students at rural campuses, immediate feedback and interactivity. One negative theme that emerged was the need for VMATs to be tailored to the level of understanding of the learner.

With respect to WSI, positive themes related to convenience, ease of use, equity of learning opportunities and adequate visualisation. Negative themes included the lack of interactivity and annotations, without which WSI were perceived as insufficient to learn cytopathology. None of the participants commented on the lack of z-axis capability. Representative quotes for each theme can be seen in Tables 10 and 11.
Table 8: Selection of stronger emerging themes from students for VMATs*

<table>
<thead>
<tr>
<th>Themes</th>
<th>Response rate: N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valuable or High impact learning</td>
<td>41 (28)</td>
</tr>
<tr>
<td>Needs to be tailored to level of knowledge/training</td>
<td>25 (17)</td>
</tr>
<tr>
<td>Immediate feedback</td>
<td>18 (12)</td>
</tr>
<tr>
<td>Interaction</td>
<td>18 (12)</td>
</tr>
<tr>
<td>Equity of training</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Convenient/Easy access (at home, in-transit)</td>
<td>11 (7.4)</td>
</tr>
<tr>
<td>Time efficient</td>
<td>10 (6.7)</td>
</tr>
</tbody>
</table>

*Many responses contained multiple themes

Abbreviations: VMATs, virtual microscopy adaptive tutorials
Table 9: Selection of stronger emerging themes from students for WSI*

<table>
<thead>
<tr>
<th>Themes</th>
<th>Response rate: N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not enough to learn from in isolation – Need VMATs or other</td>
<td>22 (13)</td>
</tr>
<tr>
<td>Convenient (at home, in-transit on IPAD)</td>
<td>22 (13)</td>
</tr>
<tr>
<td>Intuitive interface /easy to use</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Slides need annotations or ‘on-off’ labels</td>
<td>15 (9)</td>
</tr>
<tr>
<td>Preference for screener’s marks</td>
<td>15 (9)</td>
</tr>
<tr>
<td>Better/Easier than glass slides alone</td>
<td>14 (8.3)</td>
</tr>
<tr>
<td>Valuable or High impact learning</td>
<td>13 (7.7)</td>
</tr>
<tr>
<td>Equity of training</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Resolution is good</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Incorporate function to practice locator skills on slide and approach to a slide</td>
<td>9 (5)</td>
</tr>
</tbody>
</table>

*Many responses contained multiple themes

Abbreviations: WSI, whole slide images
Table 10: Representative participant comments from students on VMATs*

<table>
<thead>
<tr>
<th>Themes</th>
<th>VMAT comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valuable or High impact learning</td>
<td>- improved understanding</td>
</tr>
</tbody>
</table>
| Equity of training | - ensures consistency amongst students and different locations - very helpful in that regard.  
- makes learning across the different campuses more equitable  
- as a student of a rural campus, I find adaptive tutorials a very good learning resource  
- I think the VMATs are incredibly useful for learning cytopathology (particularly in rural campuses where we are unable to participate in Sydney classes) |
| Convenient/Easy access (at home, in-transit) | - accessible anytime, anywhere (e.g. uni, home).  
- learning is not confined to just the single tutorial at uni.  
- It allows students to return to the teaching material outside of class times, to further consolidate their understanding. |

*Many responses contained multiple themes*

**Abbreviations:** VMATs, virtual microscopy adaptive tutorials
<table>
<thead>
<tr>
<th>Themes</th>
<th>WSI comment</th>
</tr>
</thead>
</table>
| Not enough to learn from in isolation –    | - if the WSI were interactive more like a virtual tutorial I feel they would be more useful. I found the majority of slides very confusing  
- Need VMATs or other                        | - without any corresponding explanation or adaptive tutorial they (WSI) are somewhat less useful.  
- Cytopathology WSI were useful as an adjunct to the VMATs. If considering the WSI on their own however, I would have found myself confused  
- These slides are of good quality but it is difficult to gain much from just studying the slides themselves. I found incorporation of questions e.g. via the VMATs were much more effective |
| Convenient (at home, in-transit on iPad)   | - I really like virtual microscopy for its convenience  
- learning is not limited to the availability of a classroom with microscopes and glass slides  
- It allows students to return to the teaching material outside of class times, to further consolidate their understanding |
| Intuitive interface /easy to use            | - easy platform to use for zooming in |
and out
- easier to view than actual (glass) slides

<table>
<thead>
<tr>
<th>Slides need annotations or ‘on-off’ labels</th>
<th>- It would be good if the slides could be labelled and annotated (but the annotation can be ‘switched off’ or ‘hidden’)</th>
</tr>
</thead>
</table>

| Resolution is good                      | - clear images, easy to maneuver and find cells.  
- The WSI are really helpful and clear  
- great images  
- clarity of images |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|

<table>
<thead>
<tr>
<th>Equity of learning</th>
<th>- WSI are certainly more useful than glass slides (particularly in rural campuses) and make sure that all students are able to look at the same slide</th>
</tr>
</thead>
</table>

*Many responses contained multiple themes

Abbreviations: WSI, whole slide images

### 3.4 Summary of educational contents of VMATs and intended learning outcomes

As previously stated, 25 VMATs were created in total. Only one case was explored per tutorial. The first 3 VMATs were ‘taster’ VMATs to assess acceptability of this learning technique. Each one represented each branch of cytopathology:

Gynaecological cytopathology, fine needle cytopathology and fluid/exfoliative cytopathology.
The remaining 22 VMATs were subsequently created for the randomised trials. Again the subject matter of each of the 22 VMATs covered most of the traditional topics in the three branches of cytopathology as mentioned above (Gynaecological cytopathology, fine needle cytopathology and fluid/exfoliative cytopathology) The intended learning outcome for each tutorial are outlined in Table 12.

Table 12: Summary of delivered educational content of each VMAT

<table>
<thead>
<tr>
<th>CASES</th>
<th>Title of VMAT</th>
<th>VMAT TOPIC</th>
<th>WSI incorporate d into VMAT</th>
<th>Learning to be achieved</th>
<th>Additional learning tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>PILOT CASES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilot Cytopathol ogy Case 1 – Gynaecological cytology</td>
<td>Cytology Pilot Case 1 – Cervical Cytology</td>
<td>HSV HPV LSIL</td>
<td>1.Cervical Thin Prep</td>
<td>1.Microbiology and cytopathology of Pap smears</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Cervical cytology reporting categories</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.Reporting categories for breast FNA specimens</td>
</tr>
<tr>
<td></td>
<td>Pilot Cytopathology Case 3 – Fluid/exfoliative cytology</td>
<td>Cytology Pilot Case 3 – Urine cytology</td>
<td>High grade urothelial neoplasm</td>
<td>1.Pap-stained urine sample</td>
<td>1.Cytological features of urine specimen – malignant features and normal background features</td>
</tr>
<tr>
<td>TRIAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| CASES | GYNAECOLOGICAL
| Cytopathology
| Case 1 | Gynaecologic
| Cytopathology
| Case 2 | Gynaecologic
| Cytopathology
| Case 3 | Gynaecologic
| Cytopathology
| Case 4 | Gynaecologic
| Cytopathology
| Case 5 |
|-------|--------------|---------------|--------------|---------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|--------------|---------------|--------------|--------------|---------------|--------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|-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| Gynaecological Cytology Case 7 | Gynaecologic Cytology Case 7: Candida | Cervix – cytological features of Candida albicans | 1.Cervical Thin Prep | 1.Microbiology and cytology of Pap smears |
| Gynaecological Cytology Case 8 | Gynaecologic Cytology Case 8: Adenocarcinoma/Adenocarcinoma-in-situ cervix | Cytological features of high grade glandular lesion cervix | 1.Cervical Pap smear | 1.Cytological features of a high grade cervical glandular lesion cervix |
| | **FNA Cytology Case 2** | FNA Cytology Case 2: Papillary carcinoma of the thyroid | Papillary carcinoma of the thyroid | 1.Pap-stained FNA smear thyroid lesion | 1.Cytological features of papillary carcinoma 2.Reporting categories for thyroid FNA |
| | **FNA Cytology Case 4** | FNA Cytology Case 4: NHL (Lymph Node) | NHL | 1.Pap-stained FNA smear | 1.Endobronchial ultrasound-guided FNA 2.Cytological |
|---------------------|---------------------------------------------|---------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| FNA Cytology Case 6 | FNA Cytology Case 6: Fibroadenoma (breast)   | Fibroadenoma (breast) | 1. Pap-stained smear FNA breast 1. Cytological features of fibroadenoma breast 2. Cytological features favouring a benign versus malignant breast lesion | 1. Cytological features of fibroadenoma breast 2. Cytological features favouring a benign versus malignant breast lesion |
| Fluid Cytology Case 1 | Fluid Cytology Case 1: Reactive (Pleural) | Reactive pleural fluid | 1. Pap-stained smear of pleural fluid | 1. Cytological features favouring benign versus malignant  
2. Cytological features of mesothelial cells  
3. Cytological features of inflammation |
|---------------------|------------------------------------------|------------------------|--------------------------------------|---------------------------------------------------------|
| Fluid Cytology Case 2 | Fluid Cytology Case 2: Mesothelioma (Pleural Fluid) | Mesothelioma | 1. Pap-stained smear of pleural fluid | 1. Cytological features of mesothelioma  
2. Immunohistchemistry and electron microscopy of mesothelioma and adenocarcinoma |
| Fluid Cytology Case 3 | Fluid Cytology Case 3: Gastric carcinoma (Peritoneal Fluid) | Gastric carcinoma | 1. Pap-stained smear peritoneal fluid | 1. Cytological features of adenocarcinoma  
2. Immunohistchemistry and electron microscopy of and adenocarcinoma versus mesothelioma |
| Fluid Cytology Case 4 | Fluid Cytology Case 4: Normal Urine | Urine | 1. Pap-stained specimen | 1. Cytological features of normal urine specimen  
2. Cytology reporting for urine specimen |
| Fluid Cytology Case 5 | Fluid Cytology Case 5: Urothelial carcinoma | Urothelial carcinoma | 1. Pap-stained specimen | 1. Cytological features of high grade urothelial lesion - urine specimen  
2. Cytology reporting for |
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<th>Fluid Cytology Case 7: Ovarian Carcinoma (Peritoneal Fluid)</th>
<th>Benign bronchial features</th>
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Abbreviations: HSIL, high grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; LSIL, low grade squamous intraepithelial lesion; HPV, human papilloma virus; TB, tuberculosis;
4 Discussion

4.1 General

Histopathology and cytopathology are image-based disciplines that are well-suited to use of digital technology for education. The studies performed in this project have shown that virtual microscopy and virtual microscopy adaptive tutorials (VMATs) are at least as effective as existing methods for learning cytopathology amongst pathology specialist trainees. The same digital educational material, when subsequently trialed with a cohort that was completely cytopathology-naïve (medical students), was even found to be superior to existing methods, for learning how to accurately diagnose cytopathology specimens. In addition, VMATs were perceived more positively by medical students, trainees and specialists for cytopathology education compared with existing methods.

The measurable and perceived benefits for learning cytopathology with WSI and VMATs in these cohorts are in keeping with previous studies, which have demonstrated the successful use of VMATs to effectively engage students in learning histopathology with WSI (Velan et al., 2009). Pathology trainees found the WSI and VMATs a very convenient way of learning, providing equitable access to standardised high quality teaching material. Medical students also found WSI and VMATs to be valuable learning tools, with immediate feedback and the interactivity provided by these tools being highly valued.

When considering the efficacy of educational tools in general, feedback and flexibility are considered essential for engagement and learning impact (Marcus et al., 2011; Tochel et al., 2011). Self-paced, guided interactions have also been shown to
improve the acquisition of knowledge (Prusty and Russell, 2011; Polly et al., 2014). The reward of immediate visual feedback has also been shown to enhance the learning experience, (Seymour et al., 2002; Kanthan, 2009; Schlickum et al., 2009; Bejjanki et al., 2014).

Left to their own devices to analyse and learn from a cytopathology WSI, many participants in the current project found it ‘frustratingly stop-start’. Participants commented that having diagnostic cells or patterns on cytology WSI highlighted in different colours by adaptive feedback in VMATs (cf. Figures 4 and 6 in the Methods Chapter), really helped reinforce learning. Some participants in the trials also found that immediate feedback, in the VMATs and also in the online assessments was very helpful: ‘the instant feedback at the end of the test was great along with the explanation of the relevant features’.

Additionally, participant feedback from the current project indicated that VMATs are particularly useful as a guide for more junior pathology trainees who have had minimal previous exposure to cytopathology. However, it was also clear that VMATs need to be tailored to the prior experience of the intended learners. Students, pathology trainees and specialists alike found VMATs easy to use, even though those groups did not have prior experience with these learning tools.

In contrast to previous studies (Steinberg and Ali, 2001; Koch et al., 2009), participants in the pilot and in both trials reported minimal issues regarding technical difficulty, speed of viewing and clarity of the WSI and VMAT images. Feedback from the pilot study indicated that pathologists and pathology trainees were open to the introduction of WSI and VMATs to assist with learning of cytopathology.
Importantly though, there does appear to be a learning curve with this technology, which may impact on initial examination performance. At the end of phase 1 in both trials, scores for the Gynaecological cytopathology assessment were low for both groups but improved with increasing familiarity, in the subsequent phases for both groups 1 and 2 (cf. Figures 18 and 20 in the Results chapter). This finding is in keeping with previous studies (Stewart et al., 2007; Nielsen et al., 2010).

4.2 Pilot - cytopathology WSI and VMATs

Feedback obtained from Likert scale items and written responses in the pilot study indicated that users perceived VMATs as useful to improve understanding of cytopathology, particularly when compared to WSI alone, or even glass slides. The majority of participants were also interested in using more VMATs. In response to user feedback obtained during the pilot study, VMATs were improved by clarifying instructions for tutorials used in subsequent trials. Comments from users on the content of the tutorials also allowed improvement and correction of errors.

The main benefits of WSI and VMATs, perceived by participants in the pilot study, were that these methods were efficient, effective and fun. The VMATs are novel in that they are specifically designed to ‘adapt’ to the user’s decision making process through remediation of misconceptions. Furthermore analytics provided by the Adaptive eLearning Platform (AeLP) provides teachers with evidence of the efficacy of the adaptive feedback used in the tutorial.

A modest proportion of users completed any of the three VMATs in the pilot study. These are novel learning tools for learning histopathology and cytopathology, and interest in them from pathologists and trainees might be expected to be high.
However, VMATs are quite intensive and there was no incentive offered for completion for these anonymous participants. In addition, a considerable proportion of the anonymous participants may have been qualified pathology specialists; Because the participation was completely anonymous there was no way to discern specialist participants from trainees. Specialists would utilise the VMATs, not to learn the basic subject matter for exams, as would a trainee, but rather to be aware of the educational technology that will potentially be used by trainees under their supervision. Moreover, VMATs were designed for learning by trainees, rather than a teach fully qualified specialists. However, specialists were included in the pilot study to introduce them to VMATs, with the purpose of engendering their support for use of VMATs by trainees. Therefore, in this time-poor cohort (which included specialists), it is unsurprising that there was a substantial drop out rate.

Further, the aim of the pilot was to gain feedback in order to improve the VMATs being developed for the randomised trials. Low completion rates, which might have been related to time required to complete the VMATs, as well as constructive feedback were taken on board in order to streamline VMAT development and usability. Pilot feedback suggested that the layout and functionality of the VMATs could be improved and this was a substantial focus when developing the 22 VMATs for the trials. Completion rates for VMATs were much higher for the randomised trials where the cohorts were pathology trainees and medical students respectively.

From an educational perspective, the advantages of introducing virtual microscopy for pathology education via a large cloud-based image repository, such as that described in this project, were seen to be numerous. Based on feedback in the pilot and observations of others these include: (a) rapid access; (b) relative permanence
compared to glass slides, which are prone to fading and breakage; (c) ability to provide ideal teaching cases to large and/or dispersed audiences simultaneously; (d) multi-site consultation and/or education, including using annotation (Stewart et al., 2008; Triola and Holloway, 2011) or incorporation into online tutorials (Velan et al., 2009); (e) straightforward incorporation into formative and/or summative online assessments (Velan et al., 2008).

From an educational perspective, access to large sets of instructive virtual cytopathology and histopathology VMATs has the potential to improve equity of learning opportunities and standardisation of teaching material, as well as promoting networking and collaborative study. This is particularly true for pathologists and trainees in rural or remote locations. This highlights the importance of the continued development of VMATs. Subsequent evaluation by randomised trials comparing this educational method to traditional techniques for learning cytopathology in a number of different cohorts would be desirable.

4.3 Cytopathology WSI and VMATs for anatomical pathology specialists in training

There are a number of previously reported evaluations of virtual cytopathology for diagnostic accuracy and efficiency (Steinberg and Ali, 2001; Marchevsky et al., 2003; Gagnon et al., 2004; Stewart et al., 2007; Stewart et al., 2008; Evered and Dudding, 2011; Lee et al., 2011). However, the current project is novel, in that it has additionally evaluated an online tutoring system (VMATs) in association with cytopathology WSI.
In this trial, it was found that the reliability and diagnostic accuracy of cytopathology WSI and VMATs as learning tools for pathology trainees was similar to that of existing methods (glass teaching slides and textbooks). These results are supported by others who found digital methods to be equivalent to traditional methods for diagnostic accuracy in pathology (Harris et al., 2001; Steinberg and Ali, 2001; Heidger et al., 2002; Marchevsky et al., 2003; Gagnon et al., 2004; Kumar et al., 2004; Kumar et al., 2006; Dee et al., 2007; Mill et al., 2007; Evered and Dudding, 2011; Triola and Holloway, 2011). Moreover, the learning process with WSI and VMATs in the current study was perceived more positively by trainees compared to learning with glass slides and textbooks. This indicates a potential role for integration of WSI and VMAT technologies into specialist training.

The overwhelming advantage of WSI was perceived to be the convenience and flexibility of access from anywhere at any time. WSI were seen to be very useful for ‘learning on the go’ with touch screen devices providing pleasing resolution and easy panning and focusing. A limitation that should be noted, however, is that VMATs will not currently function on iPads or iPhones because Apple does not permit the Flash Player to be installed on its mobile browsers. This limitation would be overcome by elimination of the Flash component of VMATs. There are plans for the AeLP to undergo conversion to an HTML5-based platform, which would enable use of this technology with any recent web browser.

WSI were also thought to provide equity in learning and training for pathology trainees with ‘access to great cases’ in every training location as well as allowing ‘easy access to study from home’. This was a perceived learning benefit in remote and rural pathology departments, in which the number of trainees is increasing. This would also
benefit smaller Australian pathology laboratories which refer their cytology specimens elsewhere for diagnosis.

Consistent with data obtained previously (Steinberg and Ali, 2001; Dee et al., 2007; Evered and Dudding, 2011), participants in the current trial expressed frustration and uncertainty about the lack of 3-D focus. However, also concordant with previous reports, the current trial demonstrated that proficiency in cytopathological diagnosis using only two-dimensional virtual technology is equivalent to traditional techniques (Dee et al., 2007).

Several potential limitations of this trial are acknowledged. Of note, this study had a small sample size, reducing the likelihood of discovering significant differences between groups. It is also possible that the online assessments may have had insufficient items to reliably demonstrate differences between groups. However, it might have been unreasonable to expect busy pathology trainees to undertake longer assessments than were offered in this trial. There was an attempt to deal with some other potential confounders, such as ensuring a balance of seniority in training within each group. Additionally, following randomisation, participants were organised geographically to minimise potential sharing of educational material between groups, which could otherwise have affected the validity of the results. To ensure that there were no pre-existing differences between groups, familiarity with digital slide technology was assessed via the gynaecological cytopathology ‘pre-test’ at the end of the first phase of the trial, when all participants in both groups 1 and 2 received the same digital teaching material.

Because both groups were exposed to WSI and VMATs in the first phase, we cannot completely exclude the possibility that carryover effects might have minimised
differences between groups in subsequent phases of the trial, although the themes of the educational material in each phase of the trial were quite distinct. A possible measure that could be employed to minimise carry-over is to introduce a “washout period” between phases of the trial, as described by Senn (2002). However, such time gaps in the trial would have increased the risk of participant drop out in this time-poor cohort.

Another confounder that is almost impossible to eliminate is selection bias. All participants volunteered for the trial, which may reflect an interest in online learning technology. This might have contributed to the overwhelming positive feedback on the WSI and VMATs. It could also be argued that assessments in each phase of the trial should have employed glass slides, because they are used in current diagnostic practice. However, using WSI in the assessments guaranteed that all participants received identical cytological material, thereby ensuring that any difference in assessment scores could not have been caused by variations in the material examined. Lastly, this trial was designed to be a short-term study. Further studies to look at the long-term effect of this learning methodology, after a significant wash-out period, might be useful.

Regarding the qualitative data, it should be noted that the results from this trial have transferability and generalisability, as the trial cohort represents the current Australasian group of anatomical pathology trainees well in terms of geography and level of training.

The positive findings associated with the use of VMATs in this study may have broader implications for specialist practice, including continuing professional development, maintenance of competence and quality assurance. Adequate training in
cytological diagnosis will remain essential for anatomical pathology trainees and specialists alike.

Thus access to large sets of instructive cytopathology WSI and associated VMATs have the potential to provide equitable access to high quality teaching material for pathology trainees, anywhere and at any time. Additionally, these technologies could be utilised to promote networking and collaborative study for all trainees, which is of particular importance for those in rural or remote locations.

4.4 Cytopathology WSI and VMATs for medical students

Cytopathology is a field of pathology to which medical students have minimal exposure. This is despite the importance of cytopathology in ‘real-life’ clinical decision-making. Importantly, demonstration in this study of the effectiveness of WSI and VMATs for learning subject matter that is both difficult and completely new to the participants (cytopathology) supports the notion that VMATs are an effective educational tool.

One previous study described students’ perceptions and learning outcomes following exposure to VMATs authored through AeLP (Velan et al., 2009). However this is the first randomised trial that has evaluated efficacy of VMATs in learning pathology. There is also no data on the efficacy of virtual microscopy, in general, to help medical students to learn cytopathology. Furthermore, this study is the first to demonstrate that by utilising WSI and interactive learning tools such as VMATs, it is possible to provide medical students with a meaningful introduction to cytopathology and to improve their diagnostic skills. The study outcomes indicate it is possible to
effectively engage students, even when the material being studied is difficult and supplementary to their curriculum.

For this cohort of Australian senior medical students, VMATs proved to be at least as effective as existing digital methods for learning cytopathology. Importantly VMATs were superior in some respects, e.g. ability to make an accurate diagnosis on a whole slide image. This superiority was clearly evident in the second phase of our trial, but was no longer apparent in the third phase, possibly because of carry-over effects reducing differences between the control and intervention groups.

Of course, most medical students will not become pathologists. However, providing medical students with opportunities to understand the diagnostic process employed by anatomical pathologists in clinical practice, has the potential to improve clinico-pathological correlation skills which are desirable for any doctor. The exposure to digital microscopy and effective educational tools in pathology may also positively influence a student’s choice of pathology as a career pathway (Van Es et al., 2015). Digital educational tools in pathology that have been demonstrated to be effective in these trials may also help provide training in digital pathology which is becoming a requisite for specialist trainees in this discipline (Rao and Gilbertson, 2014; Riben, 2014).

An interesting aspect of this trial was that medical students were successfully introduced to the diagnostic process employed by anatomical pathologists with respect to cytopathology samples. Learning about diagnostic cytopathology may help improve attention to key diagnostic features and enhance cognitive integration of multiple clues (Mello-Thoms et al., 2012; Krupinski et al., 2013). It is thought that improvement in
attention to key diagnostic areas on a slide and the subsequent visual processing is important in the development of consistent, accurate and efficient diagnostic performance (Krupinski et al., 2013), two key areas that are assisted by cytopathology VMATs.

Thus, introducing medical students in Australia to this sub-discipline may be valuable not only because of its ‘real world’ relevance but also because it might enhance their overall diagnostic and decision-making skills in practice. By engaging students through interaction as well as highlighting essential diagnostic visual clues on WSI, VMATs cement a diagnostic pathway.

Medical students’ perceptions of VMATs were very positive. Key points to emerge from the analysis of questionnaires were that students regarded the best features of VMATs as their immediate feedback and interactivity. There was also strong agreement that VMATs helped improve equity of learning between multiple clinical campuses, including rural campuses. While both WSI and VMATs were preferred to existing methods for learning, there was a significant preference for VMATs over WSI alone.

These VMATS were originally authored for junior anatomical pathology trainees. Therefore it was unsurprising that some medical students felt they needed more introduction to the basics of cytopathology and instruction on how to approach a slide, a skill set that pathology trainees already possess. When authoring VMATs for cohorts with different knowledge backgrounds in the subject, the material needs to be tailored appropriately. Nevertheless, the majority of medical students still considered VMATs both enjoyable and straightforward to learn from. In contrast to previous
studies, (Steinberg and Ali, 2001; Dee et al., 2007; Evered and Dudding, 2011), as well as the trial with specialist pathology trainees in this project, the lack of 3-D focus was not mentioned as an issue by medical students. However, this is not surprising given that this cohort have had no prior exposure to cytopathology.

Several limitations of this trial are acknowledged. Because of the optional nature of the material, the number of students who completed the assessments was modest, so this trial may have been underpowered with respect to detecting significant differences between groups. Other limitations include the fact that students were all from a single medical school, which might affect the generalisability. As with the specialist trainee trial, there is also the potential impact of selection bias. All participants were volunteers, which may reflect an interest in and positive perception of online learning technology such as WSI and VMATs.

Learning by medical students of microscopic pathology increasingly relies upon digital learning resources, and students have high expectations of the quality of those resources. In this context, the findings of this study could open up opportunities to expand the use of VMATs in teaching of microscopic pathology.

4.5 Validation, regulatory practices and legal issues for educational and diagnostic virtual microscopy

The work carried out in this thesis on virtual microscopy for educational purposes in a number of cohorts is important because its introduction into teaching for undergraduate and postgraduate education requires validation of its efficacy, efficiency and acceptability. Additionally though, regulatory standards are required. This is to
ensure maintenance of potentially sensitive teaching material and confidentiality for the patients from which WSI were derived. This includes separation of any identifying material, including history or other data, from individual WSI. Hospital and university ethics committees need to be consulted for advice when storing this material in cloud repositories.

As educational material in cytopathology derives directly from diagnostic material, brief consideration of diagnostic guidelines in digital pathology is warranted in this discussion. From the diagnostic viewpoint, further strict regulatory and validation requirements still need to be developed regarding WSI. Additionally, measures must be in place to safeguard against intentional or unintentional corruption or manipulation of the specimens and data (Pinco et al., 2009). Primary diagnosis and secondary opinion directly impact on patient care and safety, consequently clear regulations need to be in place to maintain quality, ensure patient safety and to identify incontestable lines of responsibility (Leung and Kaplan, 2009). A particular issue needing consideration for diagnostic WSI is the responsibility for incorrect diagnosis where important diagnostic cells were not included in the scanned slide’s focal planes.

Well researched legal and policy guidelines must be in place for the continuous maintenance of patient identification, confidentiality and image data security (Ranson, 2007; RCPA, 2013b). Instruction and reflection on these guidelines and issues is something that potentially needs to be incorporated into pathology specialist training, as pathology is increasingly becoming digital in education, in diagnostic settings and quality assurance.
4.6 Online assessment using WSI

Equity of learning opportunities provided by the use of WSI was a strong theme to emerge in the feedback from these studies, particularly in the pathology trainee cohort. It is important for examination purposes, particularly for qualifying examinations for medical specialists in pathology, to provide all participants with identical examination material. This is not currently possible where glass slides are used for this purpose. Only a small number of glass slides are produced from each cytopathology specimen, regardless of whether it is a cervical Papanicolaou smear, fine needle aspirate or a fluid-based specimen. The usual number of slides range from one to four. In addition, the collection and range of material on each glass slide is non-identical and it may be that only one of the slides in the case contains readily identifiable diagnostic material.

This situation is in contrast to histopathology. Large specimens, where there are multiple paraffin blocks containing diagnostic material, ensure a ready supply of almost identical assessment material. For small biopsy histopathology, though, there are only millimetres of tissue in the paraffin block, so the situation is similar to that for a cytopathology sample.

To ensure standardisation of assessment material in pathology, WSI can be incorporated into online assessments in histopathology and cytopathology. The online assessments in the two current trials were successfully deployed to Medicine students at metropolitan and rural campuses. In the trial for pathology trainees, the assessments were successfully deployed even more widely to participants in all states of Australia, New Zealand and Malaysia.
As others have found (van den Tweel and Bosman, 2011), WSI within an online assessment better assesses the skill of the pathology trainee in identifying the diagnostic areas compared to a still image. Moreover, this approach has the added advantage of providing the question adjacent to the WSI being examined.

Immediate feedback, even in an online assessment, is an essential part of the learning process (Velan et al., 2009; Marcus et al., 2011; Tochel et al., 2011) and this was supported by the responses of students and trainees during the trials in this project. Similar findings in online pathology assessment on a more extensive scale are described by van den Tweel and Bosman over a number of years (van den Tweel and Bosman, 2011). Their online proficiency testing used WSI for pathology candidates widely dispersed over geographical areas in Europe. Their assessments also used objective items, so that they could be marked in an automated fashion, allowing the outcomes to be readily analysed (van den Tweel and Bosman, 2011). However, the authors had incorporated the images into the same browser window as the assessment. The authors reasoned that this option made it easy to navigate the slide whilst answering the question set for that slide. This approach was utilised in the VMATs in the current trials. Feedback from participants indicated that WSI windows, within the same browser window as associated questions in the VMATs, were quite hard to manoeuvre. In contrast, online assessments for the trials in the current project, presented WSI in a different browser window to the question.

Issues to consider when introducing digital pathology for assessment purposes include: the field of view of WSI is smaller than that of a real glass slide and pathologists or pathology trainees may not be familiar with the interface or software in the same way that they are familiar with a microscope and glass slide. Thus the amount
of time required to screen a whole slide may be increased for them (Dee et al., 2007; Stewart et al., 2007; Evered and Dudding, 2011). Many of the trial participants in the current project felt that analysing cytology WSI was more cumbersome, labour-intensive and time-consuming when compared to a glass slide on a traditional microscope. This feedback is in keeping with previous studies, both in virtual cytology (Steinberg and Ali, 2001; Dee et al., 2007; Stewart et al., 2007; Evered and Dudding, 2011) and small biopsy histology (Furness, 2007).

Consequently although virtual microscopy holds promise for assessment purposes, it is important to ensure that participants are already familiar with the technology throughout their training, because, as already discussed, this technology has a definite learning curve.

4.7 Annotating cytopathology WSI to maximise learning

Being able to highlight or flag diagnostic areas on WSI is important to maximise learning from the pathology slide. This can be done by incorporating the WSI into a VMAT. However annotating WSI, in the same way that glass pathology slides are marked by a pathologist with a pen in order to flag diagnostic cells, holds merit. There were comments from the medical student cohort in this project that WSI would be more useful if there was an easy way to annotate or label an area in order to bring it to the attention of others. This sentiment is in keeping with that from other studies (Steinberg and Ali, 2001). Some of the students in the medical student trial reasoned that, ideally, there should be a function such as ‘a layer that you can turn on and off’ to ‘label the features on the slides’. Some went further to add that if ‘there were any labels on the WSI linked, I could have looked over features I was not particularly clear about from the tutorial’. Another group has described similar comments from their study
participants (Steinberg and Ali, 2001), where they expressed frustration regarding the inability to digitally mark the WSI in the same way as dotting or circling on glass slides in order to highlight an area of interest and thus be able to return to it when needed.

Pathology trainees also prefer screening marks denoting diagnostic areas to be left on the glass slides during image acquisition. However this created difficulties during the scanning process as described in the Methods chapter. Consequently, annotating the WSI - a recently developed function in Slice, (Elms et al., 2015) - is a potential solution to this problem and represents an area of study for the future. This capability has been developed for the Slice database of WSI (https://www.best.edu.au/s/featured).

4.8 Digitising slides for education as part of diagnostic workflow

The findings in this study support the hypotheses proposed in the introductory chapter of this thesis, i.e. that WSI and VMATs are at least as equally effective, efficient and acceptable for learning cytopathology, compared with existing methods, for both pathology trainees and senior medical students. A strong theme emerging from participant feedback was that WSI and VMATs ensured equity of learning in pathology with ‘access to great cases’ and trial participant recommendations included that ‘a comprehensive repository of cytology virtual slides would be invaluable’.

In order for the quality and quantity of WSI to be acceptable and useful for widespread education in histopathology and cytopathology, no matter what level of training they are used for, a standardised method for routine acquisition of high quality images is needed. Histopathological and cytopathological material in pathology is processed onto a glass slide in order to be assessed with a microscope. As found in this
project and others (Jara-Lazaro et al., 2010), faultless glass slide preparation is an essential pre-imaging step. As described already, stained tissue on these glass slides is also prone to deterioration, e.g. breaking, fading and coverslips detaching. Whole slide images represent an excellent way to accurately archive this tissue, which would then serve as both as a medical diagnostic record and a readily available teaching reservoir for pathology and medical education in general.

Issues that were encountered with acquiring high quality WSI for this project have already been summarised. Potential solutions and recommendations for digitising the diagnostic workflow for education purposes are discussed here. In order to achieve standardised high quality WSI, several factors need to be taken into account. The quality of the original histological section or cytological slide needs to be very good with no faults in the tissue, no dust on the slide and no air bubbles in the mounting medium between the slide and the coverslip. Faults on the glass slide increase with time, necessitating scanning at the time of creation of the glass slide, ideally, for maximum quality of the digitised product. All the tissue from the original glass slide needs to be accessible on WSI, if they are also to be used as a diagnostic archive. Obviously sharpness, contrast, colours, hardware, software and screen monitor quality all contribute to the image quality and need to be standardised, as previously discussed.

Scanning a whole slide with acceptable resolution even in just two dimensions does create a large file, especially if the specimen on the slide is large. For histology this is relevant for large resection specimens from cut up. For cytology this means that smears covering most of the glass slide will potentially have large file sizes. Each non-cropped WSI can have a size of 1.5 GB to 4 GB or more.
High resolution scanning at 40x, which is needed for cytology specimens, can also take up to 30 to 40 minutes, although automated high-speed solutions have been described for this process (Weinstein et al., 2004). Radiological medical imaging technology is mature, but the techniques for organisation and presentation of digital pathology slides are more complex, and are lagging behind (Sinn, 2013). For adoption of digital technology into daily diagnostic laboratory workflow, even in part, integration with the laboratory information system would be required. There would be a need for continuous automated scanning, high bandwidth connectivity, large storage capacity and intuitive user interfaces. Current limitations include lack of standardised software, bandwidth limitations of networks and limited infrastructure support in the majority of institutions.

Many hurdles still need to be overcome if ‘slide-less’ laboratories are to become a reality. This would enable digital archiving of all glass slides in the diagnostic laboratory, from which all pathology teaching material derives. Potential barriers to be addressed include standardisation protocols, validation issues, and potentially medico-legal and practitioner registration issues. As an estimate, presuming an average load of 200 cases per day for a laboratory, an approximation of 10 slides per case, and average file size of 2 GB per slide (presuming x and y axis scanning only), a server storage capacity of 4000 GB per day would be needed; translating into a 100 TB capacity needed for a 12 month period and 1000 TB or 1 PB needed for an archive period of 10 years. An additional requirement for all routine slide work to become digitised in the laboratory is a rapid slide scanner. With the above conservative estimate of 2000 slides per day in an eight hour working day scanning time would only allow for two minutes per slide. This presumes that scanning only takes place during the working
day rather than around the clock. These rapid scanning times are not currently feasible in most laboratory situations, but are important challenges for the future.

There are studies that have analysed the cost of the diagnostic set up for digital pathology (Isaacs et al., 2011), including hardware, software, potential cost of any diagnostic inaccuracies and the problem of redundancy. The latter arises because the original tissue still has to be produced as glass slides initially, but then additional staff need to be employed to create digital copies of the exact same slides. Realistically, incorporating digital pathology into diagnostic workflow would require two extra personnel dedicated to this process: a digital imaging technician and an IT support person. In their study, Isaacs and colleagues (2011) looked at value added to their practice through the use of digital pathology according to 3 criteria: cost-saving, time-saving and improved patient care.

Such outcomes are achievable, but require careful initial planning. One hospital laboratory in Sweden has become totally slide-less, digitising all of their glass slides (Thorstenson et al., 2014). In that context, essential elements for success were full integration with a digital pathology system and laboratory information system, reliable scanning and continuous use of a slide scanner with good image quality and limited use of lab personnel (Pantanowitz et al., 2011b).

**4.9 Virtual microscopy for quality assurance and continuing education**

The findings in this project have potentially broader implications for quality assurance and continuing education in pathology. Continuing education of pathologists is extremely important to keep diagnostic error rates in check in a field of medicine where they are thought to range from anywhere between 1% and 43% (Raab et al.,
Such continuing education is a compulsory part of practice for many pathology institutions worldwide including the Royal College of Pathologists of Australasia (James, 2005) and the College of American Pathologists. Quality assurance plays a major role in continuing education in histopathology and cytopathology.

WSI are amenable to quality assurance outcomes because they can be easily annotated (Stewart et al., 2008; Elms et al., 2014), as previously discussed. In addition, the findings of the current project, as well as those of previous studies (Velan et al., 2008; Velan et al., 2009), have shown that web-based programs that provide immediate feedback, whether it is in an online tutorial or as part of an online assessment, are readily acceptable learning tools. In that regard, online assessments that can incorporate WSI, as well as provide automated marking and immediate remedial feedback, hold promise for the future.

4.10 Medical student and medical professional education (recommendations for the future)

The work presented in this project has established that cytopathology WSI and VMATs are at least as effective as existing ways of learning cytopathology for pathology specialist trainees as well as medical students. In fact, these digital techniques are even more effective than existing methods, for medical students learning how to make a cytopathology diagnosis. In addition, for both these cohorts studied, virtual microscopy was perceived to be a more acceptable and efficient way to learn pathology, for a number of reasons, compared to existing methods.

This verification is all the more important because, as educational technology is rapidly advancing, medical students, pathology trainees and specialists will have increasingly higher expectations about how they should be able to engage with
pathology educational resources. WSI are becoming part of the specialty examination process in Anatomical Pathology in Australasia, and are incorporated into certifying and proficiency examinations in the United States (Weinstein, 2005) and Europe (van den Tweel and Bosman, 2011). Worldwide, the diagnostic workflow is increasingly incorporating digital technology (Pantanowitz et al., 2011b; Al-Janabi et al., 2012).

However, it will take time to adjust to using this technology for cytopathology and histopathology, with learning curves demonstrated in this project and also by others (Weinstein et al., 2001; Nielsen et al., 2010). These data suggest that the more the profession uses this technology, the more efficient and accurate its use will become. In response to an increasingly urgent need to address this learning curve in digital pathology, for assessment purposes and daily practice, pathology resident rotations in digital pathology technology are being introduced in the United States (Henricks et al., 2003; Kang et al., 2009; Rao and Gilbertson, 2014) and medical informatics has even recently become a board certified specialty in that region (Quinn et al., 2014; Rao and Gilbertson, 2014; Riben, 2014).

The digital educational and assessment technology tested and described in this thesis been shown to be effective, efficient and acceptable at different stages of medical training. As a consequence of the development of this educational material VMATs have now become part of the anatomical pathology trainee educational resources provided by RCPA. Based on positive findings from this study, it is anticipated that learning with VMATs by anatomical pathology trainees will continue to expand. Based on the literature described above and the findings in the current studies, it is recommended that such technology be included in standard anatomical pathology training in cytopathology and histopathology. In addition, incorporation of assessment
technology as described in this thesis, would strengthen equity and standardisation of current slide examination procedures in anatomical pathology. Moreover, the use of this type of educational technology could be considered, not just for pathology trainees and medical students, but also for dermatology trainees or even cytology scientist trainees. Both these groups are involved with dermatopathology and cytopathology samples respectively, in their daily practice and during their professional assessments. These tools could also be used for specialists in the medical professions described above (pathologists, dermatologists and cytology scientists), for quality assurance purposes and continuing educational purposes. Furthermore, the educational content of the VMATs could easily be adapted to the disciplines of dermatopathology or general surgical pathology.

From a practical perspective, the AeLP itself is straightforward to use to develop VMATs. However, the preparation of educational content to accompany each whole slide image within the VMAT requires diagnostic expertise and understanding of potential misconceptions. Storyboarding of the proposed content and development of adaptive feedback associated with trap states (incorrect responses that indicate misconceptions) is also needed. VMATs can be built by non-specialists trained in using the authoring software, provided that there is adequate storyboarding of the proposed content and accompanying feedback. The average development time for each VMAT is between 15 to 30 hours, depending on the complexity of both the educational content and the layering of interaction within the VMATs. Cost and time commitment is also dependent on the availability of pre-scanned WSI, availability of local image acquisition services, and a licence agreement for authoring and deploying VMATs using the AeLP.
4.11 Novelty of this material and research

Both the trials and the VMATs utilised in this project are novel in a number of ways. Several groups have evaluated cytopathology WSI for accuracy and efficiency (Steinberg and Ali, 2001; Gagnon et al., 2004; Dee et al., 2007; Stewart et al., 2007; Stewart et al., 2008; Evered and Dudding, 2011). The cohorts in those studies comprised qualified cytopathologists, cytotechnologists or cytotechnology students. However, none of those studies involved either pathology specialists-in-training or medical students, and none used participants from Australia. Furthermore, only one prior study examined the effectiveness of WSI as an educational tool, (Stewart et al., 2008), which focused on annotations on WSI. However that study was descriptive rather than a randomised controlled trial of the efficacy of the intervention.

No studies of the efficacy of WSI for learning have been performed in the Australian setting and none have evaluated the efficacy of WSI for learning in medical students. Cytopathology VMATs have not been authored prior to this project.

4.12 Further research

Based on the work presented in this thesis and having established the efficacy, efficiency and acceptability of these digital pathology learning tools, there are a number of ways that the current work could be further extended. Areas that merit further investigation include:

i) **Long term retention of knowledge from cytopathology WSI and VMATs** – a question that remains unanswered from this study, is whether students who learn interactively retain the material better, even if there were no immediate significant differences in assessment compared to existing learning techniques for cytopathology. Similar studies to those described in
this thesis assessing medical students and anatomical pathology trainees after exposure to similar interactive educational material with long term follow-up would be useful.

ii) **Efficacy and efficiency of histopathology VMATs compared to traditional learning with glass slides and microscopes**

iii) **WSI with EFI and z-axis capability, compared with 2D cytopathology.**

There are no studies to date which have analysed average times for pathologists to screen and diagnose slides which have multilayered stacking versus WSI with extended focus. There are also no studies that have compared diagnostic accuracy of extended focus imaging compared to conventional glass slides. This would be particularly useful information for the diagnostic cytopathology setting, given the advantages and disadvantages of both these techniques.

iv) **Digital small biopsy pathology (including dermatopathology, which are typically small specimens) versus traditional methods including assessment of diagnostic accuracy rates and efficiency.** Small biopsy pathology falls into the spectrum between cytopathology and large surgical specimens. As these specimens are small, they do rely on z-axis capability to a degree. There has been a small amount of work done in this area of pathology outside of Australia and WSI were found equally reliable for diagnosis compared to glass slides (Helin et al., 2005; Koch et al., 2009; Mooney et al., 2011; Houghton et al., 2014) for most but not all diagnostic features (Molnar et al., 2003; Kalinski et al., 2008).
v) Digital cytopathology compared with traditional methods for the cytology scientist profession in the Australasian setting.

vi) Effectiveness of annotating both histopathology and cytopathology WSI for collaborative study in pathology trainees.

vii) Annotating WSI versus marking equivalent glass slides during cytology screening sessions.

viii) Exploring delivery and accuracy of digital pathology with Powerwall technology. Powerwalls are high resolution arrays of a moderate number of computer screens for viewing WSI more efficiently. These have been shown to aid the efficiency of diagnosis with the potential to outperform conventional microscopes in accuracy of histopathological diagnosis (Treanor et al., 2009a).

4.13 Conclusions

The results from the pilot study and trials in this project support the hypotheses that WSI and VMATs are at least equally as effective, efficient and acceptable for learning cytopathology as existing methods for pathology trainees as well as medical students. The data from this project has demonstrated measurable and perceived benefits of WSI and VMATs for pathology trainees, as well as medical students. There were also perceived benefits for pathologists revealed by the pilot study. This is a step towards validation of digital pathology for assessment and education and possibly even diagnostic workflow, in Australasia.

Cytopathology is a difficult area of pathology to master. Learning resources in cytopathology are not currently standardised or equitably available. The initial question at the beginning of this project was: do WSI improve learning in cytopathology for the
Australasian pathology trainee cohort? Further, is there additional technology that can enhance cytopathology learning with WSI? The digital learning tools created and described for this thesis in that cohort were indeed shown to be useful for learning cytopathology.

However, it was uncertain whether prior cytopathology exposure in this cohort influenced this positive outcome. To attempt to answer that question, the same digital cytopathology learning resources were trialed in medical students, a cohort that was completely cytopathology naïve. These same learning resources were shown to be successful in the latter group as well, even being superior in some respects to existing resources for learning cytopathology.

These sets of positive findings may have broad implications. The learning and assessment tools tested and described in this thesis have been shown to be effective, efficient and acceptable pathology learning tools at different levels of medical training. Their use could be considered for teaching and assessment for pathology trainees and medical students, not just in cytopathology, but also for similar subjects in pathology such as dermatopathology also part of anatomical pathology training and a difficult area like cytopathology), and for other specialty groups, as well, such as dermatology trainees. These educational tools could also be considered for related professions such as cytology scientists and their trainees. Furthermore, the use could be expanded to specialists in these professions, for ongoing quality assurance and continuing education. Importantly, these digital pathology educational resources are readily accessed and shared, ensuring standardisation and equity of learning opportunities for the medical profession.
Appendix

Open-ended survey questions for senior medical students

1. Please comment on the best features of cytopathology virtual slides
2. Please suggest improvements that you would like to see in cytopathology virtual slides
3. Please comment on the use of virtual cytology slides for undergraduate cytology education, particularly in comparison to the use of traditional training methods in cytology
4. Please comment on the best features of cytopathology VMATs
5. Please suggest improvements that you would like to see in cytopathology VMATs
6. Please comment on the use of cytopathology VMATs for undergraduate cytopathology education, particularly in comparison to the use of traditional training methods in cytology

Open-ended survey questions for pathology specialist trainees

1. Please comment on the best features of cytopathology virtual slides
2. Please suggest improvements that you would like to see in cytopathology virtual slides
3. Please comment on the use of virtual cytology slides for postgraduate cytopathology education, particularly in comparison to the use of traditional training methods in cytology
4. Please comment on the best features of VMATs

5. Please suggest improvements that you would like to see in cytopathology VMATs

6. Please comment on the use of cytopathology VMATs for postgraduate cytology education, particularly in comparison to the use of traditional training methods in cytology

Questions with scaled responses for pathology trainees regarding cytopathology WSI and VMATs where strongly agree=5, agree=4, neutral=3, disagree=2, strongly disagree=1

1. Cytopathology virtual slides were helpful in developing my diagnostic skills in cytopathology.

2. I preferred cytopathology virtual slides to traditional methods of learning cytology.

3. Use of cytopathology virtual slides was more time-efficient than traditional methods of learning cytology.

4. Learning to use the viewing software for the cytopathology virtual slides was straightforward.

5. Availability of cytopathology virtual slides would improve the equality of training in cytopathology between departments.

6. Access to a repository of cytopathology virtual slides would enhance training in rural and remote hospitals.

7. Use of cytopathology VMATs was helpful in developing my diagnostic skills in cytopathology.
8. I preferred VMATs to traditional methods of learning cytopathology.

9. Use of VMATs was more time-efficient than traditional methods of learning cytopathology.

10. Learning to use the interface for VMATs was straightforward.

11. Availability of VMATs is likely to improve the equality of training in cytopathology between departments.

12. Access to a repository of cytopathology VMATs would enhance training in rural and remote hospitals.

Questions with scaled responses for medical students regarding cytopathology WSI and VMATs where strongly agree=5, agree=4, neutral=3, disagree=2, strongly disagree=1

1. Cytopathology virtual slides were helpful in developing my diagnostic skills in cytopathology.

2. I preferred cytopathology virtual slides to traditional methods of learning cytology.

3. Use of cytopathology virtual slides was more time-efficient than traditional methods of learning cytology.

4. Learning to use the viewing software for the cytopathology virtual slides was straightforward.

5. Availability of cytopathology virtual slides would improve the equality of training in cytopathology between campuses.

6. Access to a repository of cytopathology virtual slides would enhance training in rural and remote hospitals.
7. Use of cytopathology VMATs was helpful in developing my diagnostic skills in cytopathology.

8. I preferred VMATs to traditional methods of learning cytopathology.

9. Use of VMATs was more time-efficient than traditional methods of learning cytopathology.

10. Learning to use the interface for VMATs was straightforward.

11. Availability of VMATs is likely to improve the equality of training in cytopathology between campuses

12. Access to a repository of cytopathology VMATs would enhance training in rural and remote hospitals.

Questions with scaled responses for Pilot study participants regarding cytopathology WSI and VMATs where strongly agree=5, agree=4, neutral=3, disagree=2, strongly disagree=1

1. The interactive questions using virtual slides were helpful in developing my diagnostic skills in cytopathology.

2. The interactive questions using virtual slides improved my understanding.

3. I clearly understood what was required of me in performing the tasks.

4. I would like to see more interactive questions using virtual slides.

5. I learned more from interactive questions using virtual slides than from exploring virtual slides independently.
6. I learnt more using the adaptive tutorials compared to studying traditional glass slides on my own.

**Questions with scaled responses for Pathology trainees regarding cytopathology WSI and VMATs (1=lowest, 10=highest)**

1. Rate the overall value of cytopathology virtual slides as a learning resource (1=lowest, 10=high)
2. Rate the overall value of VMATs as a learning resource (1=lowest, 10=high)

**Questions with scaled responses for Medical Students regarding cytopathology WSI and VMATs (1=lowest, 10=highest)**

1. Rate the overall value of cytopathology virtual slides as a learning resource (1=lowest, 10=high)
2. Rate the overall value of VMATs as a learning resource (1=lowest, 10=high)
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