Development and Evaluation of The Virtual Pathology Slide

A New Tool For Understanding Inter-Observer Variability in Diagnostic Microscopy

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Development and Evaluation of The Virtual Pathology Slide

A New Tool For Understanding Inter-Observer Variability in Diagnostic Microscopy

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June 30th 2004

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

The VPS (Virtual Pathology Slide) is a microscope emulator enabling the examination of pathology slides via the Internet or CD-Rom. A novel feature of the VPS is the ability to record the migratory traces (image viewed and magnification) of pathologists examinations on a remote relational database located in Dublin City University.

In order to evaluate the VPS, Ten breast needle core biopsies were randomly selected and presented to 17 pathologists or trainee pathologists with at least 2 years experience in pathology practice. Participants were required to examine each case online and provide a diagnostic classification using online feedback forms, based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology. The recorded data permitted examination of inter-observer variability and user satisfaction.

The study demonstrated that Pathologists can make a correct diagnosis using the VPS. Consensus glass diagnosis agreed with consensus VPS diagnosis in 9 out of 10 cases. Consensus diagnosis for Slide 8 differed from glass slide diagnosis by one classification grade. Several Participants using the VPS achieved strong individual performance, with 10 of the 17 participants displaying "good" to "*excellent*" (>0.6) agreement with VPS consensus, based on a weighted Kappa rating.

Modification of diagnostic classification based on a review of text diagnosis resulted in VPS consensus diagnosis for Slide 8 concurring with glass slide diagnosis and demonstrated a lack of familiarity and understanding amongst participants in the application of the applied diagnostic guidelines, particularly in the diagnosis of Intraductal Pappilloma. Modification of diagnostic classification based on text diagnosis increased average overall slide consensus from 66.5% to 69.4% but decreased individual Kappa performance by 0.76 to 0.72.

Participants diagnostic performance was found to be unrelated to their confidence in making a diagnostic decision using the VPS. Perception of image quality was

demonstrated to be clearly dependent on participants screen resolution and colour depth, but was shown not to influence diagnostic performance.

Perception of download speed was found to be unrelated to individual diagnostic performance. However, it was demonstrated that there is an increase in the number of fields of view examined by participants as their perception of download speed improves.

The number of fields of view examined per slide was found to be representative of the histological difficulty in interpreting a case. In general, as slide consensus decreases, the number of fields view examined for that slide increases.

The number of fields of view examined at a particular magnification was found to be unique for each slide and dependent on the histological complexity of each slide.

To elucidate reasons for diagnostic inconsistency, a software application called 'Bitmapper' was developed. This generates a graphical representation of a diagnostic trace using data stored on the VPS database. This takes the form of 128x128 pixel bitmap image, where each pixel is representative of an individual field of view on a VPS slide at the highest magnification available. The colour value of each pixel is determined by whether the field of view it represents has been viewed, and if so, at what magnification.

This diagnostic trace was used to locate hotspot regions of potential diagnostic importance within a slide. For each of the slides a pathologist, specialist in breast disorders, examined images from these hotspots and successfully deduced a reason for diagnostic inconsistencies. This demonstrated that Bitmapper is an extremely useful tool for determining reasons for observer variation.

The development of the VPS and ancillary software tools was successful in that pathologists were willing to use the system. Pathologists could make a correct diagnostic decision using the system. The degree of observer variation could be quantified and using Bitmapper, reasons for observer variation could be determined.

This technology has applications in determining the cause of observer variability and will prove a useful tool in external quality assurance studies (EQA) in pathology.

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The completion of this thesis has been an 'experience' and in the words of C.S. Lewis, *"Experience: that most brutal of teachers. But you learn. My God do you learn".*

Some would argue that studying for a PhD is a lonely, isolated road, the successful completion of which is down to the individual. I have found this to be untrue. I have completed that journey and will not forget the help, support, guidance and forbearance of the people I have met along the way. Thank you all.

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To Burnboy, Playboy and Spcinter boy, "*My friends are my estate.*" *Emily Dickinson.* Denis you don't live with me, you never have, so why is your food in my fridge?

Looking at a tissue sample through a microscope, you see a person up close in a manner that they have never even viewed themselves; yet, despite this intimacy you could not be more distant from them. This project revolves around 10 needle core biopsy samples taken from 9 women suspected of having developed a breast tumour. It does not describe the fear, anguish, and pain that they and their families endured. It does not describe the severity and length of their treatment, the quality of their lives or their prognostic outcome. When you read this thesis, think and pray for them.

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Chapter 1:- Overview of Telepathology and Telemedicine.

1.0 Overview Of Telemedicine

Telemedicine is defined as diagnosis at a distance.[1] In a broader sense it has evolved to describe any medical activity that involves the use of telecommunication and the element of distance.[2] Additional terminology to describe such activity has flourished in recent years such as telehealth, online health, e-health and cybermedicine. [2-4] The above descriptors define both the flow of information between medical personnel, patients and their physicians and amongst patients themselves. The term 'Telemedicine' has however come to describe and encompasses the use of telecommunications by medical professionals to make clinical consultations and decisions.[2-4]

Utilisation of telecommunications infrastructure to transfer medical data originated at the birth of the invention of the telephone. The very first telephone conversation in 1876, may be interpreted as the first telemedicine call when an ill Dr Alexander Graham Bell uttered 'Come here, Watson, I need you.[15] The first electrical stethoscope was used in 1906 when Einthoven described the transmission of electrocardiograms.[16] The first transfer of a still image by telephone line was performed in 1907, between Paris and Bordeaux, via Lion, however the images formed were too primitive for telemedical purposes[6]. The image dependent sub-disciplines of medicine had to wait much longer for the development of technology suitable for transmitting images at low cost and at sufficient speed and quality.

The first routine telemedicine service involved the use of ship to shore radio by mariners requesting medical advice. The diffusion of radio communications in the 1920's was also observed as a new medium for the practice of telemedicine. The front cover of the magazine 'Radio News' in 1924 depicted a mother standing with her son in front of a modified radio telling her son to 'Put your tongue out for the doctor Johnny' while the doctor sitting in front of another modified radio, in a remote location, views an image of the tongue.[15]

In the 1950s NASA's scientists were concerned with the physiological effects of zero gravity on their astronauts. They developed sophisticated telemetry and telecommunication systems to constantly monitor astronauts' physiological functions, such as blood pressure, respiration rate, ECG and temperature. NASA also pioneered

the development of a medical support system encompassing diagnosis and treatment of in-flight medical emergencies, in addition to development of complete tele-medical delivery systems.[17-18]

Some early experiments were carried out in teleradiology in 1959, which involved transmitting images over 9km of coaxial cable.[7] One of the earliest demonstrations of a telemedicine service involving the electronic transmission of images occurred in the 1960s when Dr. Kenneth Bird at Massachusetts General Hospital (MGH) in Boston, MA, USA created a television linkage between the hospital and Logan Airport, four miles away. Dr Bird set up a diagnostic "shop" in the medical station of Logan airport. Doctors passing through were invited to bring X-rays and patient data to a telemedicine room on the passenger concourse. The X-rays were illuminated by a light box, scanned by a television camera and the images transmitted to a monitor in the MGH's radiology department. The case was then discussed with MGH radiologists via an ordinary telephone line. [1,7, 19,20]

Terrestrial utilisation of technology developed by NASA took place in the 1970's when satellite communication between ground stations was used to provide a health care service to members of the Papago native American tribe living in remote areas of the Papago Indian Reservation in Arizona. This program known as the Space Technology Applied to Rural Papago Advanced Health Care (STARPAHC) and lasted for 20 years. [20-21]

Throughout the 1970's the practice of telemedicine using satellites showed much promise. The most notable project involved a Canadian satellite, The Communications Technology Satellite (CTS), later renamed Hermes. This was designed to serve the communications needs of remote areas of Canada.[23]

Three telemedicine experiments were conducted using Hermes. The first involved the Ontario Ministry of Health testing the feasibility of monitoring vital signs such as heart rate, respiration, temperature, ECG, while a patient was evacuated from a remote community in northern Ontario. The second experiment consisted of a 5-month trial using Hermes for teleconsultation and education. It linked the University of Western

Ontario with Moose Factory General Hospital and the Kashechewan Nursing Station on James Bay. The third experiment involved an on-going medical education programme supported by Hermes between a hospital in St. John's, Newfoundland, a hospital in Stephenville, and a hospital in Labrador City and Goose Bay.[23]

The recent growth in telemedicine has spawned from the adoption of modern telecommunication technology by almost every specialist area of medicine.[5] We are now witness to the routine practice of consultations in the fields of telepsychiatry, teledermatology, telecardiology, telepathology, teleophthalmology and teleradiology, to name but a few. Such technologies have been successfully adapted for the provision of medical services in prisons, ships, airports, islands, developing countries and at disaster sites.[1,5-6]

Medicine has always been quick to utilise and has frequently been the driving force behind advances in technology. The development and adoption of telemedicine, has been in response to a need, to provide medical services to communities where they do not exist, to improve and reduce the cost of services where they do exist and to reduce and improve the efficiency of the physician's ever increasing workload.

One of the greatest threats to the success of telemedicine is poor planning and implementation of a telemedicine strategy or system. The end-user has to be willing to use the system. The system has to increase efficiency, reduce workload, be perceived as a useful tool and not an incumbent chore.

The successful implementation of telemedicine requires comprehensive assessment of the clinical and economic advantages and disadvantages, including installation and running costs, the changes required in work practices, training requirements and any ethical or legal considerations. To date the universal acceptance and implementation of telemedicine has been impeded by insufficient analysis of any of the above.[7-11]

The initial financial investment required to implement a telemedicine system has been a significant barrier to its widespread acceptance. It is difficult to measure the cost saving achieved from having a system, to guarantee reimbursement and justify investment.

The telemedicine market is very competitive with companies aggressively vying for market share. While the manufacturing cost of telemedicine equipment is still prohibitively expensive, it has dropped in recent years and looks set to continue to do so permitting many to consider telemedicine as an economically viable option for the first time.[8-12]

In terms of diagnostic accuracy, it is difficult to compare the performance of a telemedicine system to that of conventional practice.[18] There still exists a lack of standardisation and procedures and it is difficult to differentiate the skill of the physician from the performance of the system. Numerous studies have, however, been performed in an attempt to benchmark telemedicine systems to that of conventional practice.[25-18] This has resulted in a growing confidence amongst the medical fraternity in the use of telemedicine. In some branches of medicine such as radiology the use of such systems has become a standard part of training. This will contribute significantly to the change in attitude and growth in use of telemedicine.[7]

There has been considerable debate as to the ethical and medico legal implications in the use of telemedicine. The international and cross-jurisdictional nature of telemedicine presents a complexity that is not encountered in any other area of medicine. While numerous assumptions as to the legal implications of practising or mal-practising of telemedicine exist, the modern nature of telemedicine means that these assumptions remain untested, making many physicians fearful of its use.[13-14]

Very often the technology infrastructure, especially a satisfactory telecommunication network does not exist. This has paralysed the roll-out of telemedicine, especially in remote and developing regions where its need is greatest. The advent of wireless and other inexpensive technologies will make telemedicine increasingly available in areas where its benefits can be felt most.

1.1 Overview of Telepathology

Telepathology is a subset of telemedicine. It is the practice of diagnostic pathology by a remote pathologist utilizing images of tissue specimens transmitted over a telecommunications network[1,5]. It is a hybrid of telemedicine and pathology involving

the exchange of pathology images and data through telecommunication for the purposes of diagnosis, consultation, research education or quality assurance. Traditionally telepathology is classified based on the software and hardware used to acquire, process, transmit and review images.[1-5,6]

Use of telepathology technology specifically in the field of pathology first occurred in 1973, when pathology images of peripheral bone marrow smears were transmitted via satellite from a ship moored in Brazil to Washinghton for teleconsultation. However, it wasn't until 1986 that the term telepathology was first cited in *Human Pathology,* where Weinstein[1] proposed, in an editorial, the integration of robotic microscopes, imaging and broadband telecommunication connections in wide-area pathology networks[1]. The first use of the internet for telepathology was reported in 1989[23,24]. In the same year the first telepathology service was initialised when investigators in Norway set up a network of telepathology workstations in rural hospitals to provide diagnosis on intra-operative frozen section slides. *Human Pathology* continued interest in the development of telepathology, led to an entire issue devoted to telepathology in 1996.[23] However, early promoters of telepathology, may have been over-optimistic in predicting that telepathology would replace the microscope. [5,7 15-25]

The term Telepathology transcends a number of methodologies for delivering pathology diagnosis at a distance. These may be classified based on the technology used and are described below.

1.1.1 Static Telepathology

Static telepathology refers to the capture and digitalisation of macroscopic and/or microscopic images, selected by a pathologist or pathologist's assistant, which are transmitted by electronic means to a telepathologist. Typically, a static-telepathology imaging system is composed of a microscope, camera and an internet or direct network connection. The images captured are usually high quality and, if uncompressed, require large storage space and bandwidth. With static telepathology, the images are limited in number and represent only a fraction of the entire histological specimen. Image transmission maybe performed via electronic mail (e-mail), file transfer protocol (FTP), Hypertext transfer protocol (HTTP), a proprietary protocol or CD-ROM/DVD. With e-

mail, image files are sent as attached files in a message containing the basic information about the case. Alternatively, FTP is used to upload files directly onto a server from which a reviewing telepathologist can download them.

A more popular method is to include both the images and the information about the case in a web page providing simultaneous access to several pathologists in different parts of the world. Static telepathology is also referred to as store and forward telepathology[5-8]. Recent improvements in internet-browser technology have facilitated the development of interactive store-and-forward Web pages. These feature the ability to show the spatial relationship between individual images in low-power and high-power views. This technology is commonly visualized using a small image gallery constructed from one or two microscopic fields out of a possibility of thousands, displaying images of the same fields at higher magnifications [6,5].

Field selection and interpretation are thought to be the primary reasons, specific to store-and-forward telepathology, that account for its discordance with diagnosis in a conventional pathology setting.[13] Studies involving multiple pathologists provide the most robust and accurate method of assessing a telepathology technique. However it is difficult to distinguish the performance of the technology from the skill of the pathologist and the degree of difficulty of the cases being presented.[13] While this method has been shown to be popular, accurate and inexpensive, studies have highlighted the potential for sampling bias in the pre-selection of areas of interest and consequent potential to misinterpret a case[23-27].

1.1.2 Dynamic Telepathology

Dynamic systems allow a telepathologist to view images transmitted in real time from a remote robotic microscope that permits complete control of the field of view and magnification by the examining pathologist.[3] In its simplest form it consists of a microscope operator who moves the stage under telephone instructions from the telepathologist. This concept is referred to as a 'virtual double headed microscope'.[28] Typically, in its more developed format, dynamic telepathology involves the use of a telerobotic microscope, remotely operated by the telepathologist who makes the diagnosis. This robotic system may include one or more of the following elements:

movement of the microscopic stage, rotating nose-wheel for changing objectives, automated focusing and light adjustment. Dynamic telepathology requires a stable broad-bandwidth connection between sender and recipient and the images captured are usually of lower resolution that those acquired in static telepathology, but are continuous and allow the receiving pathologist to examine the entire slide. Some dynamic systems feature teleconferencing allowing simultaneous voice communication between the referring and the consulting pathologist. Dynamic telepathology systems are generally more expensive than the static systems, more complex to set-up and maintain, but are ideal for running a high-volume service that deals primarily with routine cases, frozen-section or for use in remote understaffed locations [5,8 25,29].

1.1.3 The Virtual Slide-A Static-Dynamic Hybrid

In recent years, advances in browser technology has led to the development of hybrid systems that contain elements of both static and dynamic telepathology. These have commonly become known as virtual slides. Virtual slide systems utilize an automated scanner that sequentially records images of the entire slide, which can then be forwarded to another location or examined remotely using customised viewing software for diagnosis. The dedicated software acts as a microscope emulator that displays digitized representations of tissue slides, allowing inspection of numerous fields of view, over a wide range of magnifications. It has numerous advantages over dynamic telepathology. The slide is digitally archived, a pathologist or technician is not required to be present at the host station as is sometimes the case in real time teleconsultation and the slides may be viewed at any time. [29-37]

The everyday use of virtual slides has been inhibited by the time taken to scan a virtual slide. To date this has been due to limitations in optics, cameras, stages, software for acquisition and display, image compression, the bandwidth, acceptance by end user and cost. Depending on the scanning system, scan magnification and amount of tissue on a slide, scan time can take between a few minutes to a few hours.

Given that almost every other aspect of a pathology lab workflow is automated, there is an increasing need for the development of high-throughput virtual slides scanners. It is likely that the future will realise virtual slide archives as an integrated component of a

HIS (Hospital Information System) in a similar manner in which hospital PACS (picture archiving and communication systems) have revolutionised X-Ray departments.

The advent of ultra-fast slide scanners such as Aperio[33], DMetrix[35], Trestle[51] and 3Dhistech[38] is encouraging, demonstrating a definite trend towards the development of a fast, efficient, automated system capable of scanning high-quality images.

Apart form the growing potential of using Virtual Slides for teleconsulation there are other applications of this technology such as digital image archiving, EQA (External Quality Assurance Studies), education and research.

The increasing requirement of high throughput image analysis, especially in the area of tissue micro arrays promises a bright future for virtual slide in both academic research institutions and industrial BioPharma. Currently however, the greatest number of research papers on virtual slides describing its use as a educational tool.[29,33-35]

Recent publications include a variety of web technologies for delivering virtual slide educational tools such as Quicktime Virtual Reality (QTVR) technology (Apple Computers Inc.) by Zito *et al* (2004).[32] to provide pathology teaching cases at the National Cancer Institute, Italy. Blake *et al* (2003) used a MrSID viewer (LizardTech software) to deliver Virtual Slides as part of a histology course at the Medical University of South Carolina.[39]

Dee *et al* (2003) used a FlashPix (EastMan Kodax) image file format to provide virtual microscope slides in the annual pathobiology of cancer workshop laboratory in the University of Iowa. FlashPix embedded in a Macromedia Flash viewer was created by the Institut für Pathologie, Basel Switerzerland to develop a virtual slide teaching tool 'vMic', while Crowley *et al* (2003)[39] used FlashPix embedded in java viewer to create a 'virtual slide tutor'.[38]

A image file format called Zoomify with functionality similar to FlashPix has been used by manufactures of virtual slide scanning hardware such as Aperio Technologies

and MicroBrightfield to publish virtual slides.[40] An unique feature of these technologies is the ability to annotate slides. This is of particular use in education.

The diversity in technologies used to present virtual slides promises an exciting future for this technology. Of interest is the expanding functionality of virtual slide viewers to not just present images, but to allow users to annotate images and perform rudimentary images analysis.

1.2 Technical Considerations of Telepathology

When determining the technical requirements of a telepathology system it is useful to consider the necessary hardware and software in terms of image acquisition, image storage and display, and image transmission. Under these headings it is possible to define the components required and their functional characteristics.

1.2.1 Image Acquisition

A typical image acquisition system in telepathology is comprised of a digital or analog camera mounted on a light microscope. The microscope may have differing degrees of automation. It may have a robotic stage, robotic objective nose wheel, autofocusing, auto-illumination and filters. All major microscope vendors such as Olympus, Nikon, Leica, and Zeiss offer fully motorised/robotic imaging microscopes. The automated features available on such motorised imaging systems include:

- motorised stage in the x,y direction.
- motorised focus wheel, usually controled by auto-focus software.
- motorised nose wheel for changing objectives.
- motorised filter wheels for fluorescence work.
- adjustable light intensity.
- remote control of a microscope and image viewing over a network.
- mounted 3-chip CCD camera for live feed or digital camera for store and forward.
- proprietary software for controlling and viewing images.

Despite being available for a number of years, the fully automated solution remains prohibitively expensive with prices in excess of €100,000 (2004).[41] Many users feel frustrated with such systems by having to pay for superfluous features or being restricted to the use of proprietary stage and imaging software.

The alternative solution is to purchase modular motorised features such as motorised stages and focus controllers and integrating them into conventional microscopes. Users have much greater freedom and choice in selecting third party imaging and microscope controlling software or indeed in developing their own stage and image management software.

1.2.1.1 Robotic Microscopes

There are a number of manufactures offering motorised robotic stages that one can integrate with a conventional microscope. The most popular vendors in Europe are Prior, Marzhauser, and Ludl.[42-45] This is usually a less costly affair than buying a purpose built robotic microscope.

Motorised stages are driven by stepper motors. Typically the stepper motors used can be programmed to operate in microsteps of less than 1/256 increment of a total revolution.[45-46] The stages are based on a platform design where metal plates are moved using precision screws with mechanical interfaces comprised of crossed-roller or ball-bearing guides. Stages are available with different travel ranges and with different dimension and capacity slide holders.

When using a motorised stage the motor will naturally heat up. The thermal displacement for a 5°C temperature rise can extend the length of a typical 200mm screw by as much as 12μm. [45-46]

There are three factors to consider when comparing quality and performance between motorised stages.

Stage Resolution:- refers to the minimum stage movement and is determined by the pitch of the screw and the step angle of the stepper motor driving the screw. Resolution can vary from 0.025μm to 25μm.[45-46]

Repeatability or Precision:- is a measure of the capability to reposition the stage to the same coordinates time after time. Stages incorporating zero backlash nuts can contribute to providing a precision of 5μm or less. [45-46]

Absolute Accuracy:- is a measure of the capability to position the stage to exact coordinates as determined by a standard (eg a calibrated reticule). There are two

components to this specification; the runout error which is the total deviation accumulated over the length of the screw and the rolling error which is the deviation from nominal at any position.[45-46]

Traditionally, stepper motors for robotic stages were controlled by a PC via a RS232 Serial/Com port connection to a controller box. Serial port connections are a slow means of communication between a PC and a peripheral device. Many manufactures now offer USB connection to their motor controllers. One manufacturer who offers a internal PCI controller card for direct connection between the PC BUS and the stage claims data communication speeds of up to 100 times faster than the traditional RS232 to controller box configuration.[43]

1.2.1.2 Microscope Optics

Despite playing a crucial role in the quality of a captured image, the requirement for a good quality objective lens is routinely overlooked by telepathology vendors and customers alike. This is largely due to complexity in understanding the functionality of a microscope and lens. When purchasing a microscope some terms need to be understood in order to make the best choice.

In the context of using an optical microscope, 'Image Resolution' may be defined as the minimum detectable distance (by the human eye) between two closely spaced specimen points. Three factors that affect image resolution include the separation distance between the specimen and the lens, the illumination wavelength, medium refractive index and angular aperture of the objective lens.

Of the above four, factors the angular aperture of the objective lens is the most important factor in determining resolution. This dictates the size and shape of the 'illumination cone' as it enters the objective lens. For convenience, the angular aperture is multiplied by the refractive index and the product expressed as the Numerical Aperture (NA). Oil immersion has a higher refractive index than air resulting in a increase in resolution of up to 1.4. [47]

Values of NA range from 0.1 for very-low magnification objectives (<4x) up to 1.4 for high quality, high magnification, oil, immersion lenses. The NA is the most important selection criteria in conjunction with magnification when purchasing a microscope

objective. High NA objectives are often better corrected for aberration, They capture more light and produce a brighter, more corrected image with a greater the amount of resolved detail. In general, the higher the NA, the more expensive the objective. [47]

Apart from NA and magnification, other details written on the side of most lenses include the Greek infinity symbol indicating the tube length (250 millimetres) for which the objective is designed to render optimum images, the thickness of the cover slip (usually 0.26 millimetres) for which the lens is optimised in order to correct for spherical aberration and whether the lens is designed for oil-immersion. The aperture ratio may also be printed on the barrel an objective, this should be as low as possible for a high NA. [47]

Nikon offer four classifications of objectives: achromats, plan achromats, plan fluorites, and plan apochromats, the final two classifications provide the highest quality image and are the most expensive due to complexity of manufacture and expense of materials.

Lenses are manufactured to correct for spherical and chromatic aberration and field curvature. Chromatic aberration or colour dispersion is the phenomena of white light splitting into its constituent colours along the optical axis of a lens while spherical aberration is the deviation of light from the focal point along the optical axis of a lens.

Plan or flat field objectives are designed to correct for field curvature. These lenses are suitable for general microscopy such as the scanning of tissue cultures, performance of cell counts and photomicrography. [47]

It is important to understand the characteristics of each lens classification in order to obtain the best quality image. For example correction for spherical and chromatic aberration in achromats lenses occurs in the yellow/green wavelength. These are the colours most sensitive to the human eye. Use of a green filter in combination with an achromat lens will produce a high quality monochrome image. Table 1.1 below is a reproduction of the objective lens offering by Nikon and shows how each lens technology attempts to compensate for inherent errors.

Table 1.1 Illustration of Common Objective lenses and their corrective properties[47]

Objective Type	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 Colour	2 Colour	No
Plan Achromat	1 Colour	2 Colour	Yes
Fluorite	2-3 Colour	2-3 Colour	No
Plan Fluorite	3-4 Colour	2-4 Colour	Yes
Plan Apochromat	3-4 Colour	4-5 Colour	Yes

1.2.1.3 Image Capturing Devices

CCD (solid-state charge-couple device sensor) cameras contain a rectangular chip, the surface of which is composed of an array of small, light-sensitive photocells that produce a charge, the strength of which is proportional to the amount of light they receive. CCDs are usually arranged as either a linear or a rectangular array of cells. The performance of a CCD camera is measured by its output, which is a function of the number of photosites on the CCD surface. Initially mono or single chip cameras were produced, these outputted a black and white (greyscale) image. Single chip cameras were later produced that had a colour capability. This was achieved by coating alternative rows of cells in red, green, and blue filters. However this produced a poor quality colour image as only one in three photocells sensed one of the three colours. This problem was eliminated by the development of three chip cameras. This configuration consists of three identical CCDs each handling one of the primary colours and a prism incorporating dichroic filters improve colour and sensitivity [7, 48-49].

CCD Resolution (also known as the pixel resolution or the spatial sampling frequency) is a function of the number of photodiodes and their size relative to the magnification of the image projected onto the chip's surface by the microscope lens. The higher the magnification and the smaller the pixel, the higher the resolution. Very often spatial sampling frequency, rather than the optical resolution, is the limiting factor in overall system resolution. Nikon provide a useful dynamic webpage for optimising the microscope optical resolution to a specific camera, in order to determine the necessary pixel density that adequately captures all of the image data from the microscope lens.[50]

Given that each CCD only senses one colour, the output signal is at full system resolution. However these cameras are larger, heavier and considerably more expensive.

The field of view captured by a CCD camera is rectangular and smaller in area than the circular field of view acquired through the microscope oculars. This is due to the shape and size of the CCD chip. CCD cameras are manufactured with 1/3, 1/2 and 2/3 inch chips. Alternatively the area captured by camera may be increased using optical adaptors between the camera and the microscope [7,48-49].

CCD cameras may be either analog or digital. Traditionally, video camera technology has been analog. An analog signal is suitable for direct connection to a TV monitor, or video recorder. However the analog signal outputted by such cameras cannot be interpreted directly by a computer. The signal has to be digitised. This is accomplished using a video capture board called a framegrabber. A framegrabber receives analog signal and converts each individual frame into a series of bitmapped images which can be displayed and manipulated using dedicated imaging software on a PC. It accomplishes this by taking one horizontal line at a time and dividing each into uniform sections, and calculating the red, green, and blue values of the signal for each section. This arises out of the 4:3 aspect ratio of television pictures, for which most cameras are designed for [3,48-49].

Analog cameras typically output four different types of video signal [49]:

- RGB signal: This is a 3-channel signal where each channel carries a signal for each color, red, green, blue, and sync. The sync is usually conveyed on the green channel and describes the frequency of the analog wave. This signal produces the highest quality image and is the most commonly used in telepathology where analog cameras are used.
- Component YCrCb: This is a 2-channel signal format. The Y signal carries luminance information. Cr describes the Red value and Cb describes the Blue values. The green value is deduced by subtracting the red and blue values from the total signal.
- S-Video Y/C: 2-channel format, Y signal carries luminance while the C signal carries chrominance values. This format is commonly used in security cameras.

- Composite signal. This is a compound signal. The chrominance, luminance and the synchronizing signal are modulated into one single signal. This is the most commonly used signal for video and TV. However, the signal resolution is generally regarded as being of insufficient quality for machine vision applications and telepathology.[6]

Depending of the camera type, it may be connected to a video card or framegrabber card housed in a dedicated imaging workstation via firewire, USB, or RGB-coaxial cables. The stage is usually connected to the imaging workstation via RS232 (serial), parallel ports or USB. Using dedicated software the workstation controls the microscope and image acquisition. In static telepathology, the imaging workstation is configured as a stand-alone system for acquiring and manipulating images. In dynamic telepathology, the workstation is networked allowing it to function as a slave or server to a distant workstation that can control in real time all automated aspects of the system remotely. Use of PCI (Peripheral Component Interconnect) cards, such as framegrabbers, is becoming increasingly redundant in modern computers [48].

Storing a typical frame grabber image of 768x574, even in temporary memory, requires 1.3MB of memory. This is because each pixel within an image requires three bytes to store the red, green and blue value. To alleviate the memory overload, this places on a computer's RAM, framegrabbers have inbuilt frame buffer memory which provides sufficient frame buffering to store the video image displayed on the screen. Typically framgrabbers can process up to 25 frames per second, producing near real time digital video feed directly to the imaging software for subsequent acquisition and manipulation.[61]

The design and development of camera technology has always been commercially driven. The greatest market share for cameras has been in TV, home video, and security surveillance. Traditionally the majority of these cameras were expensive and designed to produce analog composite output (NTSC or PAL) to view on a television set. The result is that cameras not specifically designed for microscopy have had to be used in the practice of telepathology. Obviously this configuration has had a detrimental effect on image quality [7].

Recent development has seen the availability of inexpensive digital cameras. These use the same CCD technology as analog cameras. However, an analog to digital converter is also housed in the camera. Therefore, these devices have a digital output, and are usually connected to the computers motherboard through a direct connection to the *small computer system interface* (SCSI) bus or other high-speed connection such as the *universal serial bus*. (USB). A popular method of downloading images from a digital camera to computer is the use of Firewire (also known as IEE1380 iLink, or DV). Data transfer via Firewire is faster than USB and has a smaller neater connection interface than a SCSI. Digital cameras reach a higher spatial and colour resolution (up to 5000x5000 pixels) than analog. However, current image acquisition speeds (>20 frames per second for high end digital cameras) makes it suitable mainly for store-and-forward telepathology [48-49].

To mount a camera on a microscope an adapter usually called a C-mount is required. A number of adaptors are available and one must be selected that is compatible with both the microscope and the camera. For some low cost cameras, adaptor rings designed to fit the eyepiece have been developed.

1.2.1.4 Ultrafast Slide Scanners

The last four years has seen an emergence of a new type of technology capable of rapidly scanning a glass slide and generating a virtual slide. The advent of ultrafast high-speed slide scanners represents a revolutionary breakaway from the conventional method of mounting a camera and motorised stage on a microscope. Mechanically and optically, these devices are custom-built for high throughput, incorporating slide loaders and barcode readers for handling in excess of 50 slides at a time. The exact operational characteristic behind each device varies, but consist of technology such as high speed robotics, linear CCD array sensors and even arrays of multiple objective lenses (the array microscope).[35] Some of these devices are driven by software that is further designed to reduce scanning time by scanning only areas that contain tissue.

Some manufacturers are attempting to offer 'one touch scanning' whereby at the touch of a button multiple slides are scanned and automatically made available online.[33] The elimination of human intervention in the scanning process and the rapid online publication of a virtual slide make it an attractive competitor to real-time dynamic

telepathology for online tele-consultation. Virtual slides created in this manner are available almost in real time. They may be examined at any time but, unlike dynamic telepathology, the referring pathologist does not need to be present, the viewing pathologist does not need any complicated or expensive software and the slides can be archived in a database for future reference and may be retrieved with greater efficiency than glass sides. Current vendors of high-speed scanners include Interscope, Aperio, 3DHistech and DMetrix.[29-33,51]

The use of ultrafast scanners has increased the potential of virtual slides to make a real impact in education, consultation, quality assurance and research.

1.2.2 Image Storage and Display

1.2.2.1 Digital Image Attributes-Pixels, Pixel Depth, file Size and Resolution

In order to understand and compare the functionality of image capturing devices, an understanding of the attributes of a digital image such as pixels, bit depth, resolution and image file size is required. Pixel is an abbreviation for 'Picture Element'. A pixel is the smallest element in a digital image. Digital monitors display images dividing the screen into thousands (or millions) of pixels, arranged in rows and columns.

Display resolution in the context of a digital image is a measure of pixel density. For a given image the smaller the pixel size, the greater the number of pixels can be used to describe that image and the higher the resolution. As resolution increases, the 'sharpness', 'clarity' or detail of an image also increases.

The quality of a display system depends on its resolution, how many pixels it can display, and how many bits are used to represent each pixel. For monitors, the screen resolution refers to the number of pixels on the entire screen. A VGA monitor (video graphics array standard for monitors, introduced by IBM in 1987) displays only 640 by 480, or about 300,000 pixels. In contrast, SVGA (Super video graphics array standard for monitors introduced by a number of manufacturers) monitor displays 800 by 600, or 480,000 pixels.

For printers, the resolution is described in number of dots per inch (dpi). For example, a 300 dpi printer is one that is capable of printing 300 distinct dots in a line 1 inch long. Printers, monitors, scanners, and other I/O devices are often classified as high resolution, medium resolution, or low resolution. The resolution ranges for each of these grades continually shifts as the technology improves.

Spatial resolution is expressed as microns per pixel. It is a function of the properties of the objective used to acquire the image and the physical dimensions of the CCD-chip. It is a valuable descriptor of the physical area that an image represents and is an unambiguous means of indicating the level of detail and magnification of an image. Some hardware manufactures, such as Aperio, use this as a method of communicating their pixel density. Aperio define their image resolution as 0.47 μm/pixel with 20x objective and 0.23 μm/pixel with 40x objective.

Bit depth represents the number of colours or shades of grey can be used to define each pixel. For example, in 8-bit color mode, a colour monitor uses 8 bits for each pixel, making it possible to display $2^8 = 256$ different colors or shades of gray. True Color systems uses 24 bits per pixel, allowing them to display more than 25 million different colors.

Image file size is a function of resolution and bit depth. The greater the number of pixels in an image and the higher the bit depth (to describe each pixel) the larger the image file size. This relationship may be expressed as follows:

Memory in Bytes = (X-Resolution * Y-Resolution * Bits-Per-Pixel).[48-49]

Storage resolution is expressed as the pixel width and height of an image by the colour depth. This provides an indication of the quality of the image, the physical size of the image and the memory requirements for storing an uncompressed version of the image. An example of an expression of storage resolution is 768x574 x 24 bit colour.

1.2.2.2 Image Quality in Telepathology

Initial studies in the use of telepathology were concerned with whether image quality was of a sufficient standard to render a correct diagnosis. Image quality and the ability to make diagnostic decisions from compressed digital images is still a contentious issue [5,7-8]. In order for telepathology to be of clinical use, studies have attempted to access the diagnostic accuracy of store-and-forward telepathology, and have shown accuracy in the range of 77% to 100% [27]. The diverse nature of this technology makes it difficult to draw comparisons between studies, or to form a consensus on a method of best practice. There is no universally-accepted standardization in hardware, software, image resolution, colour-depth, or image compression and storage.[3,13,55-57]. To contend with such nonstandardization, guidelines have been formulated for the capture and manipulation of diagnostic images and for the practice of telepathology[7,56-57]. Development in the production of low-cost high-resolution monitors has addressed part of this problem. The most crucial part of this problem is the technology used, both hardware and software to capture an image, convert it to a digital form, and in non-dynamic telepathology, the method used to store it.

To display colour at different intensities, colour monitors combine the primary colours red green and blue to different degrees, with the potential to generate up to 24 million colours. This is known as true colour or 24-bit colour. Images composed from a 256-greyscale palette are known as greyscale images of 8-bit image. 8-bit images require less than three times the storage capacity of 24-bit colour images and are considered to be of sufficient quality for teleradiology [7]. Initially, Doolittle *et al* (1997) published work suggesting that 8-bit colour may be of sufficient quality for histological image assessment. However, 24-bit colour images have become the preferred standard. Some scanners and graphics cards are capable of displaying 32-bit colour. However, this colour depth is of more use for images intended for machine vision solutions and image analysis [6-7].

Early publications in the field of telepathology were concerned with the effect of compression on image quality and ultimately the point at which the rate of image compression compromised the ability of the viewing pathologist to make a correct diagnosis [1,6-7]. It is understood that where image analysis is being performed, such as in the area of automated diagnosis, it is desirable that image integrity is maintained through the use of uncompressed lossless formats.[6] For medico-legal reasons, there are advocates for the preservation of image data using uncompressed or lossless formats. However, in the practice of telepathology, a number of image compression algorithms (lossy format) have been employed to reduce the size of images to a more practical size for transmission over a network [48]. The most common types of image formats used in telepathology are discussed here[55].

1.2.2.3 Image File Formats

There are several image file formats available. Each has unique characteristics. Some compress images resulting in data loss and a reduced image file size (lossy), while others are uncompressed formats that retain all the bit data describing an image (lossless). Some image file formats are open standard such as *.jpg (Joint Photographics Experts Group) or *.bmp (Bitmap) and can be opened by any imaging application while others are proprietory, such as *.psp (Paint Shop Pro) or *.eps (Encapsulated Post Script). The most popularly used file formats for telepathology are described below.

Tagged Image File Format (TIFF)

This is a complex, customisable file format, varying from greyscale images to 24-bit colour bitmaps, which may use the lossless LZW compression algorithm used by the GIF format. Not all imaging software supports all the possible TIFF formats. TIFF images are the preferred format in telepathology for image analysis due to the image data being non-compressed[60].

The Joint Photographics Experts Group (JPEG)

This image file format was originally established for the purpose of storing high-resolution photographic images. It is the dominant image file format for still-image compression and is well supported by web browsers and imaging software. The JPEG format uses a raster file compression algorithm based on a Discrete Cosine Transformation that permits configurable compression ratios between 1-100 with 100% compression resulting in the highest degree of image compression by the JPEG transformation. The JPEG transformation loses low frequency hues resulting in high contrast areas such as the edge of tissue becoming distorted if compression is too high. JPEG is accredited as an industry standard by the International Standards Organisation (ISO) [58].

Wavelet scalar quantification was developed for the FBI (United States Federal Bureau of Investigation) for archiving and transmitting fingerprint data. It is a lossy compression algorithm considered to have a better compression ratio and preservation

of image detail. There are a number of vendors offering wavelet compression software such as Dr Solomon, but no industry standard or compatibility between different companies. Without a recognised standard, images saved using one type of wavelet compression will only be able to be viewed by those with similar software. A number of companies such have Adobe (manufacturers of the industry-standard graphics software, Photoshop™) have stated that they will not support wavelet compression until a standard has been agreed.[58-59]

JPEG 2000 is a new image coding system that uses compression techniques based on wavelet technology. Its improved compression algorithm will probably replace the older JPEG format. Although it is not inherently supported in Windows™ applications, plugins can be downloaded to enable browser support and a number of telepathology vendors offer free JPEG 2000 viewers [29-32,36].

Flashpix (FPX)

Flashpix™ was developed by Eastman Kodak Company and offers a number of unique features, including:

- The ability to store a number of resolutions of the same image in a single file (multilayering of image resolutions).
- Use of Microsoft's OLE structured storage format. This will enable developers to extend the format.
- Linking support. This permits different applications to link to the same image in different ways such as linking to applications that embed digital watermarks.

Flashpix has been used successfully in a number of telepathology applications.[32-35] However, it is not yet supported by most imaging software applications.

1.2.2.4 Data and Image Storage Devices

The hard drive offers the fastest access and greatest convenience for storing images. Size and speed of the hard drive is important in determining how long it takes to save or retrieve images. The type of connection between the peripheral device (e.g. hard drive) and the motherboard is also important in determining the time it takes to save or retrieve images. Currently, the Ultra-ATA IDE (Attachment Integrated Drive Electronics) is the standard interface offering 33 megabytes per second (MBps) data transfer. Alternative faster architecture exists such as the SCSI (Small Computer System Interface) offering 40 MBps or the increasingly popular Universal Serial BUS (USB). Depending on the USB device specification, the data transfer rate of a USB can be as fast as 480Mbps [7,55,56].

The feasibility of storing images locally on a hard drive has increased in recent years as the cost of memory decreases and the standard size of hard drives offered by manufactures increases. A number of ultrafast virtual slide vendors such as Aperio and DMetrix offer terabyte servers as part of their integrated solution.[33,35] However storage of an image archive on a removable media is still encouraged as the best form of data backup and is a useful method of data transfer where bandwidth is low. The most popular form of removable media used in telepathology is CD-R which can store up to 700MB. As DVD readers and writers are becoming more readily available, they are more likely to replace the CDR. DVD can store between 2.6 GB-10GB.

1.2.3 Image Transmission

1.2.3.1 The Internet

It could be argued that the origins of the Internet date as far back as the laying of the Atlantic cable in 1858 and 1866, a communication network that remained in service for almost one hundred years. The concept of the Internet, as it is known today, may however be traced back to the late 1960s. However, it was not until the development of user-friendly browsers, hypertext, ancillary clients, server side languages and the proliferation of Internet Service Providers (ISP's, commercial companies that provide access to the Internet) in the early nineties, that the growth in Internet usage as we know it today, finally occurred. It is probably the most important scientific instrument of the late twentieth century given the increasing pace of scientific research, resulting from data and technology exchange via the Internet. [61-63.]

Issues that restricted the proliferation of the Internet, have also to be overcome by telepathology if it is to gain widespread acceptance. This includes bandwidth, image compression, availability and cost. The Internet has become an intrinsic means of delivering telepathology services. Almost all telepathology systems rely on the Internet network infrastructure for communication. This includes e-mail consultation services, telepathology webpages and the use of TCP/IP connection to control robotic microscopes. Consequently knowledge of telecommunication protocols and network technology is necessary in order to appreciate this crucial limiting factor.[64]

1.2.3.2 Network and Data Security

Use of the Internet for telepathology purposes has raised concerns in terms of the security and privacy of data transmitted. Major IT security firms such as Symantec and Mcafee have identified three considerations when dealing with security of electronically stored data.[65]

> *Confidentiality:* information should be available only to those are authorized.
> *Integrity:* information should be modified only by authorised individuals.
> *Availability:* information should be accessible to authorised individuals when they need it[65]

In the US, these three principles form the cornerstone of HIPAA compliance, a necessity for any healthcare informatics system storing patient data. HIPAA is an acronym for the Health Insurance Portability and Accountability Act of 1996. The HIPAA was passed to a uphold patients right to privacy, maintain their health insurance despite serious health conditions and prevent 3rd parties (insurance companies) from ascertaining confidential medical information about a patient to use against them. Compliance of HIPAA regulations ensures best practice in maintaining cconfidentiality, integrity and availability of patient data.[66]

HTTP is the protocol for moving files such as webpages across the Internet between a webserver and a web browser.[67] It has been identified, in particular by e-commerce, as a convenient but insecure and vulnerable method to transfer data. To counter this, additional protocols for secure Internet connection have been developed, such as SSL (Secure Sockets Layer) and TLS (Transport Layer Scurity).[67] These are an additional set of agreements or protocol that lie between the TCP/IP network protocol and HTTP(hypertext transfer protocol).[67]

HTTPS is increasingly becoming the method of choice in telepathology for securely conducting teleconsultation. For example, the Armed Forces Instutite of Pathology, the UIPCC Telepathology Consultation Centre (Institute of Pathology, Charité, Gremany) and iPATH (University of Basel, Switerland) all use HTTPS as part of their consultation process. Other telepathology users have also implemented HTTPS in their system Della Mea *et al*(2000). In addition, telepathology vendors such as Dmetrix, who supply servers specifically configured for serving teleathology images and ancillary data are now using a SSL as standard.

The SSL and TSL are a protocol layer placed between the TCP/IP network protocol and HTTP (hypertext transfer protocol) application protocol providing secure communication between a client web browser and a server. This is accomplished by allowing mutual authentication, the use of digital signatures for integrity, and encryption. It is the standard used for Internet banking and online credit card payments.

It is possible to determine if a client webrowser is connected using SSL or TSL to a webpage hosted on a 'secure' server by ensuring that the HTTP tag of the URL changes to HTTPS (hypertext transfer protocol) in the browser address bar. It should also be

possible to view a a third part certificate indicating that SSL is implemented correctly on the server by right-mouse clicking on the properties menu of the webpage.

1.3 The Economics of Telepathology

1.3.1 Telepathology Market

According to the Federal Trade Commission in the USA, the Internet is already dramatically changing the way consumers access health information, receive diagnostics and purchase pharmaceuticals.[68] A telemedicine report by market research company 'Business Communications' projects that over fifty million Americans will seek health information online by 2005.[69] It will create opportunities for healthcare providers and niche players producing and supplying within the information and wireless technology industry. The cost-benefits of telemedicine will speed the transmission of the technology to wider markets and shift the delivery of healthcare away from traditional models of service.[69]

According to market analysts Frost & Sullivan, the US telemedicine systems and services market will reach $302.6 million by 2007—up from $131.2 million in 2001. Technology is reducing costs, improving care, and creating new types of services, many made possible by sophisticated Internet Protocol (IP) networks.[69,72]

The Internet is emerging as a key tool in telemedicine service delivery, primarily of the store-and-forward variety. About 10% (31) of a total sample of 296 program managers indicated that they use the Internet for a number of purposes, including medical image transfer (74.2%), patient-care consulting (51.6%), patient records (41.9%), and video transfer (38.7%). The latter statistic is a key indicator of advances in high-speed networking and desktop video and how they are being integrated with healthcare networks.[69,72]

Telemedicine implementers were seeing the service as a way to increase operational efficiency through better use of staff resources (18.3%), provide rapid information transfer (13.3%), achieve better physician collaboration (12.2%), compete in managed-care climates (11.3%), and provide medical education (7.8%).[69,72]

As an industry telemedicine can be subclassified according to the medical speciality in which it is deployed. Telemedicine-mediated cardiology is provided at 46% of sites, followed by dermatology (44%), psychiatry (43.5%), energy medicine (39.8%), home healthcare (23.1%), pathology (21.3%), and oncology (20.4%).

According to EUROPATH; (European Pathology Assisted by Telematics for Health), annual pathology activity within the EU, totals some 180 million microscopic preparations, examined by highly-dedicated specialists in 2500 laboratories at a cost of 1800 million euro.[72]

Telepathology, once viewed as a passing fad, is now regarded as an integral part of the promising future of telemedicine. Further inroads into the telecoms arena, in conjunction with a greater acceptance from pathologists, are expected to promote strong growth in the embryonic European telepathology market.[68-70]

Analysts suggest that technological progress in the field is expected to drive the market for telepathology. In particular, information and communication technology (ICT) developments, virtual microscopy, and integration with Hospital Information Systems (HIS), are likely to propel demand. [68-70]

The integration with HIS is in keeping with the strategy of widening the benefits provided by telepathology, thereby promoting market expansion. Axel Baccari, the healthcare research analyst with Frost & Sullivan, (www.healthcare.frost.com), notes:

> *"Telepathology needs to be used as a tool, rather than being considered by itself. Most importantly, we need to push development of the uses and users of telepathology. Telepathology is just starting to do that, therefore it becomes crucial for vendors to follow this evolution,"*[70]

Revenues generated in Europe by the sale of telepathology systems came to $6.1 million in 2001 and are forecast to reach $8.8 million by 2006.[78] With telepathology expected to gain increasing acceptance within the next three years, the timing of a new technology will influence its level of success. Frost & Sullivan have concluded that:

> *"This issue is of particular importance since we are in a market which is on the one hand technology-driven, and on the other, based on a target market with a conservative approach. The challenge today is not so much in overcoming technical barriers, but rather why, where, and how to implement which technology,"*

A related challenge will be to identify a marketing strategy capable of convincing end-users that telepathology is not a substitute for conventional diagnostic procedures, but a real advancement in the world of pathology. [70]

As competition intensifies in the European telepathology market, both microscope as well as software companies stand to gain. Microscope vendors will benefit from continued customer loyalty to their well-established brand names. However, software companies and the new breed of vendors offering innovative high-speed scanners will be in an advantageous position, since store and forward telepathology is likely to experience stronger growth than robotic telepathology.[70]

1.3.2 Benefits Versus Costs of Telepathology Practice

Telepathology can cut health care costs and reduce demands upon hospitals. Considering that a significant proportion of a hospital budget is expended on meals and ancillary services, any method that can reduce a patient's hospital stay should be explored. Rapid diagnosis of patients using telepathology systems reduces a patient's hospital stay and consequently hospital waiting lists. For example, a considerable number of studies examining the applicability of telepathology in the diagnosis of frozen sections have been performed [73,26,52-54].

Telepathology can reduce the transport costs and risk incurred by having to transport pathology specimens over long distances, sometime over rough terrain from referring to consultant pathologists. In remote regions, telepathology can save patients time and money by eliminating the need to travel to distant doctors and hospitals for biopsies. In addition, telepathology reduces the stress and anxiety on patients and their families that can be incurred by travel[9-12,74-75].

Telepathology can lead to improved consultations and second opinions. It enables pathologists to consult quickly with a specialist some distance away[74]. Analysts predict that, in the future, more patients will be diagnosed using telepathology, especially patients in smaller remote hospitals that do not have the facilities of larger ones[74-75].

Theoretically, telepathology is a universal service that provides access to centres of excellence for various specialities from anywhere in the world. It has the potential to share among a much greater number of patients, the limited resources of specialists and expensive equipment.

For health care services, telepathology offers not only the possibility of reducing the expense of pathology services, but also should be seen as a source of revenue. It is a sophisticated industry composed of hardware manufacturers, service providers, telecom networks and pathology labs, all of who have the potential to generate revenue from the sale and provision of their products and services. There are commercial opportunities for those who view telepathology as a source of revenue. This has been realised by a number of companies and institutions, such as the US AFIP (Armed Forces Institute of Pathology) [76].

Telepathology offers obvious cost savings in training and education. Pathologists working in rural areas of developing countries, access to pathology databases on the Internet, for example, can inexpensively keep up to date with what is happening in their field, and use bulletin boards to pose and address questions to other pathologists from all over the world. Telepathology websites are forming an important source of case study material from every part of the world. Many have sections dealing with rare or difficult cases and provide forums for online quizzes.[77-79]

Della Mea *et al* (2000)[75] performed an evaluation of the economics of a telepathology frozen-section service in comparison to an ambulance service. Due to the initial investment required for a telepathology system, they found that conventional services were more cost effective for low caseloads while the telepathology service was more economic for high caseloads They suggested that the unknown economic consequences of adopting telepathology is an obstacle to its diffusion[75].

An initial investment in telepathology equipment provides the greatest resistance to purchase for any pathologists wishing to integrate telepathology into their practice.[77,80] Each pathology practice is different and therefore requires a sensible appraisal as to the economic benefit of implementing a particular telepathology system. From a cost/

benefit viewpoint, pathologists need to know that they will be reimbursed by a reduction in their workload, an increase in their efficiency and greater faster access to specialist opinion. A telepathology system can cost between €50,000-€150,000 depending on the type and degree of automation and ancillary software and support. Even the simplest telepathology systems require greater manual intervention and longer examination time than when using a conventional glass slide examination. However, the last 18 months has seen a paradigm shift in the performance, capability and cost of systems available. The emergence of ultra-fast high speed scanners represents a new generation of technology that looks set to come close to replicating the digital revolution initialised in radiography two decades ago. Some of these systems such as the Scanscope™ by Aperio and DMetrix™ appear capable of scanning up to a slide a minute with multiple slide loaders for high throughput. Conversely a low cost solution to dynamic telepathology that also deviates in design and operational principle from the conventional microscope, is the Coolscope™ by Nikon. This system retails for €26,500 (April 2004) and permits full remote examination of a slide. Trends in the development of telepathology systems offered to the pathologists at a lower cost, look set to soon dissipate concerns over the economic benefits of telepathology.

1.4 Medico-Legal Implications of Telepathology

1.4.1 Legal and Ethical Considerations in The Practice of Telepathology

Everyday clinical decisions are made by doctors using fax and telephone without medico-legal implications and fear of litigation, yet this has been identified as a major concern by pathologists and forms part of the reluctance for the widespread diffusion of telepathology.[14-15] The global, cross-jurisdictional nature of telepathology introduces considerable complexity for the medico-legal fraternity. Several legal and regulatory issues have been identified such as quality, patient confidentiality, licensing, evaluation, practice standards, reimbursement, patient acceptance, expense, malpractice, hardware/software standards and compatibility [14-15].

Bamford *et al* (2003)[77] encountered severe reluctance on the part of a number of pathologists while attempting to set up a telepathology network on the UK. To counter this fear and encourage participation, Bamford's research team published a letter on their website outlining the UK Medical Defence Union viewpoint. The letter indicated that "the law makes no distinction between medicine and telemedicine; in either case the practitioners owe a duty of care to the patient". The letter advised that pathologists should "not offer opinions where they are not properly trained or qualified and should admit uncertainty if it exists".[77] Working in an even darker climate of litigation, pathologists in the US share the malpractice concern. Many are of an opinion, that should a diagnosis be rendered by means of telepathology, a statute of limitations should be included and the images should be stored and retained in the digital data banks of both the sender and the recipient.

1.4.2 Organisational and Personal Liabilities in the Practice of Telepathology

Confidentiality, where information that could identify a patient, is transmitted over an open system like the World Wide Web, is also of great concern to pathologists. However, this concern lies mostly with pathologists who are unfamiliar with telepathology and unsure of its underlying technology. Bamford *et al* (2003) [77] warned participations using their system not to transmit data that could breach confidentiality. Hosch (2001)[80] identified a number of individuals involved in the practice of telepathology who are legally vulnerable. These include the surgeon who performs the biopsy, the technician who prepares the slide, the general pathologist or pathology

technician who refers the case, the consulting pathologists and the informatics specialist responsible or the system. They suggested that the current pathology liability principles are valid for telepathology whereby the head of the health care facility and the head of the pathology department have an organisational legal responsibility to ensure correct medical processes. However, within the general liability situation, it is the clinician treating the patient who bears the ultimate responsibility for the well-being of their patient. Many other telepathology practitioners advise that where telepathology is to be used to render a diagnosis, written consent should be obtained from the patient and the tele-expert should check for the existence of such an agreement with the clinician. The creation of such a direct legal link between the telepathologist and the patient diminishes the risk of liability for all concerned [80].

In Germany, Norway, and Japan the legal position of the telepathologist is considered to be the same as a pathologist making a diagnosis by conventional means. That is, the telepathologist is liable when a false diagnosis is made, regardless of whether the correct sampling of images occurred. In the UK, the legal responsibility lies with the referring pathologist.[56] The United States Armed Forces Institute of Pathology (AFIP) attempt to share legal responsibility by stipulating that the pathology consultation report includes a statement "This report is not valid until countersigned by the originating pathologist".[76] Persistent confusion and uncertainty in this area and the absence of legal precedence is a major contributing factor to acceptance and use of telepathology.

1.4.3 Licences Regulation and Jurisdiction in the Practice of Telepathology

Another concern highlighted in the US relates to problems with licensing agreement in the practice of telepathology. Most state licences permit doctors to practice only within the state where they have their licence [80]. There are some cross-state licences, such as those within the Department of Veteran Affairs and the Indian health service. Medical licences in the US are used as a form of quality assurance, ensuring that patients receive the best possible treatment by competent health care professionals [80]. The practice of telepathology may conflict with this, in that a patient may be treated locally, by under-qualified staff who are being advised by an online expert. Medical licences also exist in order to regulate the commercial activities of those that practice in health

care.[55] This has the advantage of protecting a pathologist's market from out of state competition. The trans-state practice of telepathology conflicts with such regulation. In 2003, a federal bill *'Medicare Telehealth Validation Act of 2003'* was brought before the legislature.[81] The bill predominantly covers the funding requirements to expand access to telehealth services throughout the US, including the provision of telehealth services and networks across state boundaries and establishes the duties and responsibilities of 'The Joint Working Group on Telemedicine'. Currently the bill is before the Houses of Congress 'Subcommittee on Health'. Currently legal precedents with regard to remote liability are not established. Confusion as to whether a telepathologist has to be licensed in their own state, the state the patient is being treated in or in both has prevented the widespread use of telepathology.[80-81] Clearly the same level of confusion applies to the practice of international telepathology. If a malpractice suit does occur, where it is litigated can have serious implications in terms of the laws that will actually govern the case and the financial cost.[80-81]

There is uncertainty as to whether malpractice insurance policies provide coverage for telemedicine. Hosch (2001)[80] advises that to avoid financial risk, telepathologists should inform their insurance companies that they are practising telepathology and request by written statement or insurance contract, confirmation of their coverage. In addition, they advise to limit liability by way of a contract between participating parties. In most circumstances the referring pathologist asks the opinion of a consultant they are personally familiar with. This is important in increasing the confidence performing a diagnosis. In Norway, two consulting pathologists usually perform the telediagnosis thereby reducing the possibility of error [80].

1.4.4 Duty of Case Documentation

Duty of case documentation is also an important consideration. A general rule is that the patient does not have to prove a treatment mistake, however, the doctor must be able to prove correct treatment processes. Hence, proper documentation and glass slide archiving is normal practice in conventional pathology. Case documentation and glass slides are normally stored for between 10 and 30 years, if not indefinitely. In normal glass slide consultation, the consultant may return the slide. If the consultant pathologist wishes to query the case at a later date they may only have their report to

review. With telepathology the opportunity exists to record fields of view, cursor movements, annotated comments, and magnifications utilised. Such comprehensive recordings have the advantage of permitting a quality review of a consultant's performance. [14-15,80]

1.4.5 Electronic Records and The Patient's Right To Privacy

In 1996 the US Congress introduced the Health Insurance Portability and Accountability Act (HIPAA, Public Law 104-191).[81] This act was introduced to serve two functions; a reduction in the administrative overhead of health care entities and the protection of individually, identifiable health information with particular reference to computer databases. The US Department of Health and Human Services refers to this as the "Standard for Privacy of Individually Identifiable Health Information" and has been applied broadly to all aspects of telehealth where personal information is stored or transmitted electronically, whether as financial/administrative transactions or the practice of telemedicine. [81]

Within the EU, Article 42 of the Charter of Fundamental Rights of the European Union provides a right of access to documents of the European Parliament, Council and Commission. It is upon this precedent, that freedom of information laws exist, which provide EU citizens within their respective countries, access to official information held by government and other public authorities. This includes medical records and other personal information held by hospitals.[82]

In Ireland, the Freedom of Information Act (1997) and the Freedom of Information Amendment Act (2003), establishes three statutory rights for members of the public where information relating to them is held by a public body. The rights of the individual as asserted by the act are summarized below.[83]

- a legal right for each person to access information held by public bodies
- a legal right for each person to have official information relating to him/ herself amended where it is incomplete, incorrect or misleading

- a legal right for each person to obtain reasons for decisions affecting him/herself

Under section 15 and 25 of the Act all public bodies are required to provide a publicly available booklet that must include an explanation of the records held by the and the procedures and facilities in place for requesting information.[84]

In addition to the Freedom of Information Act, the Data Protection Act, 1988 & 2003 exists for the protection of the individual's fundamental right to privacy, and their right to exercise control over how their personal information is used or disclosed. The Data Protection Act, 1988 &2003 enforces within Irish law the following key EU Directives:[84]

- EU Directive 95/46/EC - the Data Protection Directive
- EU Directive 97/66/EC - the Telecommunications Directive
- EU Directive 2002/58/EC - the e-Communications Directive

Under Section 25 of the Irish Data Protection Act, 1988, individuals such as doctors or medical personnel are required to register with the Office of the Data Protection Commissioner if they record details on a computer database relating to the physical or mental health of identifiable individuals.[84]

One case brought before the Irish Data Protection Commissioner that has relevance to the practice of telepathology relates to an incident in which the clinical notes of patients under the care of a medical consultant were disclosed for external review to a UK based risk management group with out the consultant knowledge or consent. The Data Protection Commissioner expressed the following viewpoint which has implications within Ireland as to who retains ownership and responsibility/liability for data. [84]

> *"In this case and indeed for patients in acute public hospitals it has to be recognised that the health board or the hospital is the data controller and not the consultant. However where a consultant has private patients then he/she becomes the controller if he/she is treating them in a private hospital or in his/her private rooms."* [98]

In the UK, the EU directives listed above are implemented under the Data Protection Act, 1988. This is in addition to other statutes including the Computer Misuse Act 1990

and '*The Common Law Duty of Confidentiality*'. In addition to this the UK General Medical Council and NHS have published guidance on the legal and ethical duties of medical professionals to protect the privacy of patient information in the documents entitled '*The Protection and Use of Patient 'Information'* (HSG(96)18) and '*Using Electronic Patient Records in Hospitals; Legal Requirement and Good Practice'* (HSC1998/153).

One of the basic principles of health care is the patient's right to informed consent. Where adequate information has been provided to the patient and the patient comprehends what is proposed when consent is obtained. This is both a legal and ethical issue and is intrinsically linked to the issue of confidentiality and the transmission/disclosure of electronic medical records or images to third parties.[14-15]

Who bears ultimate responsibility and liability for a patient treated using telemedicine?[80,85] Who decides what the standards are for the transmission, compression and resolution of images and data in the practice of telemedicine? [80,99] Thompsons Solicitors the largest UK law firm involved in clinical negligence cases provide the following advice to would be clients. [86]

> *There are a number of elements to establishing that medical treatment was negligent. The patient has to prove (on "the balance of probabilities") that the clinical practitioner has been negligent.*
>
> *This means showing that the standard of care fell below what could "reasonably have been expected'. Medical opinion often differs over treatment for a particular ailment and it is a valid defence if it can be shown that the treatment was in accordance with the views of "a responsible body of medical opinion".*
>
> *Even if you can show that the standard of care was negligent, you still have to prove that the negligence actually caused the injury.*[86]

It is crucial to note from the advice above, that within existing medical law, the precedent exists whereby a doctor cannot be found negligent when acting in accordance with a practice or procedure laid down by a body of clinicians expert in that medical area ("*a responsible body of medical opinion*") even if a body of opinion exists which takes the opposite view.[85-86]

Stanberry *et al* (2001)[99] reported on a case in the UK concerning product reliability and safety. The plaintiff was born with a skin-covered protrusion on their spinal cord, which had been missed by an ultrasound scan. It emerged during the court proceedings that two months previously the consultant radiologist had complained in writing to the chief executive of the trust that the quality of images obtained from the scanner were becoming increasingly variable to the point where '*we now have considerable problems interpreting the scan.*' In this situation it was demonstrated that the hospital was liable for the provision of functioning equipment. This has implications of the exposed liability of telepathology providers where images may degrade or become lost or corrupted with or without the knowledge of the pathologists making the decision. [85]

In a separate publication, Stanberry *et al* (2001) also identified a case in the UK (*R. vs. Department of Health, ex parte Source Informatics Ltd)* where Source Informatics Ltd attempted to challenge the UK Department of Health guidelines on '*The Protection and Use of Patient Information*' .[101] Source Informatics Ltd were hoping to collect data describing the prescribing habits of general practitioners by paying pharmacists a fee for the information with general practitioners consent. The data would not contain any information that would allow a patient to be identified. The court ruled that the 'dispensing information without the *consent* of patients would be an unlawful breach of confidence'. This ruling was made with respect to the use and ownership of anonymous patient data for commercial purposes. However, Stanberry *et al* (2001) point out that while it throws into question the legality or a whole range of routine uses of non-personalized patient data in clinical audit, statistical, research and education, if the use and disclosure of data is not for commercial reasons and in the public interest or if the patients give express or implied consent to the use of their records for research, the court believed that the law would not have been broken. Stanley *et al* (2001) suggest that where doubt exists an explicit expression of informed consent from the patient should be sought [87].

In terms of violating patient privacy, practitioners of telemedicine are vulnerable to litigation if:[80,85]

- they do not guarantee the right of privacy of a patient's electronic records by having acceptable protocols in place for the transmission, storage and disclosure of patient data.

- they do not fully inform patients as to the intended use of their data, or of other practitioners that are involved in the telemedicine consultation.
- They do not obtain informed consent from their patients for the transmission/disclosure of personally-identifiable information.
- They do not obtain informed consent from their patients to perform the teleconsultation.

Knowledge of the law was is now an intrinsic part of clinical training. It is an important part of every clinical and diagnostic decision that is made. As with all practitioners of medicine, pathologists engaged in the practice of telepathology need to be aware of the directives, statutes, articles, guidelines and legal precedence discussed above and as with all their other clinical duties, perform telepathology with similar legal diligence.

1.5 Standardisation In The Practice Of Telepathology

1.5.1 Digital Imaging and Communications in Medicine (DICOM)

Standardisation in telepathology from both technical and medical perspectives is a crucial perquisite for its acceptance as an integrated part of the whole health care delivery system.[7,88-90] From a technical perspective, the area where standards and protocols need to be agreed upon is in the standardisation of data formats. There is a number of data types associated with pathology cases including administrative data, such as payment and insurance details, patient data such as name, address, and age and medical data, such as laboratory data, and therapies.[89]

The birth and revolution in the use of digital medical images in standard medical care must be attributed to the field of radiology. The increasing use of digital images in radiology in the 1970s and 1980s led the American College of Radiology (ACR) and the National Electrical Manufactures Association (NEMA) in 1983 to forming the 'ACR-NEMA' committee in order to create a standard method for the transmission of medical images and associated information. In 1993, this committee formally became known as DICOM (Digital Imaging and Communications in Medicine). DICOM's objective is to achieve compatibility and uniformity and improve workflow efficiency between imaging and other informatics systems in healthcare services worldwide.[91-92]

Prior to the establishment of DICOM, most devices stored images in a proprietary format and transferred image data proprietary formats over a network or on removable media in a non-compatible manner. The DICOM standard specifies a network protocol utilising TCP/IP. Under DICOM standards, medical data is object-oriented so that it can be uniquely identified as it is acted upon across a network. This includes not only image data but also data pertaining to patients, studies, reports, and other data groupings [105-106].

DICOM has become a cooperative standard in that all major diagnostic medical imaging vendors have incorporated the standard into their products and actively participate in the enhancement of that standard via public demonstrations, the Internet and private test sessions. It is envisaged that DICOM will be utilised by every medical discipline that utilises images including cardiology, dentistry, endoscopy,

mammography, ophthalmology, orthopaedics, pathology, paediatrics, radiation therapy, radiology and surgery. DICOM is also used in veterinary medical imaging [91-92].

The acceptance of DICOM and its cross platform compatibility across a multi vendor environment has facilitated the development of picture archiving and communication systems (PACS) and interfacing with medical information systems.

Currently, there is no telepathology standard that permits end-to-end interoperability and communication between telepathology systems and other healthcare information systems. However it is proposed that DICOM standards are to be set by the Image Exchange Committee of the College of American Pathologists.

1.5.2 iPath - an open source server communication forum

iPath was originally developed by the Department of Pathology at the University of Basel in 1999 as a telepathology solution.[93] The open-source, low cost nature of the technology has, however, found its way into many other telehealth applications. iPath is comprised of a database driven webserver and provides a medical discussion forum, where cases presented by clinicians within dedicated user groups can be discussed and commented. iPath is successfully used for a number of telemedicine applications such as frozen-section diagnosis for a rural hospital in the Swiss mountains, primary consultations for the National Referral Hospital in the Solomon Islands and secondary consultations among German bone tumour specialists. Pathologists in both developing and developed countries such as Bangladesh, Germany, India, Iran, Poland, South Africa, Switzerland, Thailand, USA and Vietnam have commenced using iPath to discuss difficult cases. It is envisaged by iPath developers, that a telemedicine network using iPath, will be deployed in Cambodia, Laos, Nepal and Ethiopia. The open source nature of this technology has been its greatest accelerant. The use of HTTP/HTTPS to network computers to iPath has avoided issues with firewalls which have stunted the availability and acceptance of telepathology networks until now.[93]

1.5.3 United States Armed Forces Institute of Pathology

Within the US Military Health Care System (MHCS), there are several individual pathologists around the world who are isolated from regional medical centres. It is the ideal setting for the provision of telepathology services. Much pioneering work in the

application of telepathology has been performed by the Institute, which successfully runs a telepathology consultation service via e-mail and the Internet[94].

In validating the suitability of a web-based telepathology system for frozen section consultation within the US Army's Medical Corps, Kaplan *et al* (2002)[54] performed the examined intra-observer agreement and concordance with glass-slide diagnosis for 120 frozen section cases diagnosed at a distance by three pathologists. 75% of the cases used were of unknown disease process (for primary diagnosis) while the remaining 25% were for staging to determine the extent of resection. The diagnostic concordance between telepathology and glass slide diagnosis was 100%. Examination time was on average 2 minutes 50 seconds. Kaplan has subsequently been responsible for installing 21 Trestle MedMicro real time telepathology workstations in US military locations around the world. (Kaplan, personal communication).

1.5.4 International Union Against Cancer Telepathology Consultation Center (UICC-TPCC)

The UICC-TPCC enables pathologists to exchange digital images and diagnostic opinions, via the World Wide Web. This service is coordinated from the Charité University Hospital Institute of Pathology in Berlin.

Using the system, a requesting pathologist submits a new case, which is reviewed by a UICC-TPCC pathologist who assigns it to an appropriate expert. One of the internationally-recognized pathologists who have volunteered to serve as UICC tumour experts reads the images and makes diagnostic suggestions that are sent to the referring pathologist. Case turn around is guaranteed within three working days.

The program is focused on providing services to developing countries, offering local pathologists access to high-quality histopathological diagnoses. The service is also offered to smaller institutes of pathology, particularly those in Eastern Europe, Asia, and Africa. UICC-TPCC has a roster of more than 50 experienced cancer experts. The consultation centre approach could potentially be expanded to other fields in the future, including teleradiology and tele-ultrasound.

Mireskandari *et al* (2004) recently published a study comparing the use of iPath and UICC-TPCC where cases were sent to both centres from Germany and Iran and demonstrated that such specialist centres were a feasible solution to a pathologist in a remote location to receive accurate and timely second opinion diagnosis.

1.6 Perception and Acceptance of Telepathology by Pathologists

1.6.1 Human factor-attitudes towards the acceptance of telepathology

Mairinger *et al* (1998)[95] surveyed the attitudes of 256 pathologists towards telepathology and found that the majority believed telepathology saved time and money, and was useful in any of the proposed applications. However its use was met with 'unfocused scepticism' due to a knowledge gap, and misunderstandings about data security and image quality. All these problems have been largely resolved over the last few years, however Mairinger *et al* (1998)[95] concluded that misunderstandings still exist, that telepathology is an international affair and that the legalities as to liability need to be clarified in order to diffuse fears about its use.

Mairinger *et al* (1998) [95] concluded that the flow of information from telepathology experts to potential users is insufficient and until improvements occur, the 'second birth' of telepathology will fail to materialise. In a subsequent publication, Mairinger *et al* (2000)[8] identified five requirements for the spread of telepathology.

> *"(1)communication and influence; (2) economic costs and benefits; (3) knowledge barriers and learning; (4) feasibility of techniques offered for the demands of the users; (5) clarification of the legal status and other factors concerning international collaboration."*[95]

They also suggested that the real obstacles to the acceptance of telepathology are the prejudices and habits of pathologists, some of whom see telepathology as an unwelcome gateway to international competition between pathology service providers, and that telediagnosis is not as easy as it may seem.

The teleconferencing system developed by Bamford *et al* (2000)[77-78], referred to as "the virtual double headed microscope" system utilised existing microscope and computers in a pathology laboratory and required the purchase of a relatively inexpensive digital camera and video card. The software utilised was free (Microsoft Netmeeting) or inexpensive (Webcam32). The authors advertised the service in histopathology, offering free installation, training and technical advice. The response they received was

promising. Installation was completed in 35 laboratories with 66 registered users. However, since installation, only 29% of registers have used the system at least once while a questionnaire was sent to all registered users of which only 23% were returned. The authors found that acceptability and use of the system was compounded by both human and technical factors within the NHS system. Despite training sessions being provided, pathologists were reluctant to learn how to use the system, while hospital IT staff required for administrative access to the hospital network did not perceive the project as part of their work, and were reluctant to assist. Image quality was cited by some as a reason for not using the system. However, this was not seen as a issue by those who actively used the system. Fears regarding medico-legal, patient confidentiality and litigation led to some pathologists declining to participate. From the questionnaires, the authors ascertained that unless telepathology can be shown to substantially reduce the workload of pathologists, its acceptability in routine work will remain in doubt. The authors concluded that in a densely populated country such as the UK, with a well-developed postal system, pathologists do not believe telepathology has any advantage over conventional means of consultation. The authors suggested that for the moment, efforts in the UK in developing telepathology is best directed at education and standardisation rather than expert consultation. [77-78]

1.7 Applications of Telepathology.

The diversity in telepathology systems reflects growing technological expertise in this area and the increasing importance of telepathology in education, training, quality assurance, and teleconsultation.[18,20,25,29] Numerous pathology archives abound on the Internet providing links to both educational and commercial telepathology websites. These offer access to either static or dynamic image delivery systems.[29-33,35-36,51]

1.7.1 Teleconsultation.

Pathology is the medical sub-discipline concerned with the causes and nature of disease, and with changes wrought by disease. In pathology, the volume of information, both diagnostic and prognostic, that is available from the examination of biopsy material, requires an extensive knowledge of diseases and clinical implications. As a consequence, consultations are an important practice in pathology, as pathologists frequently require opinions from those who specialize in various diseases. Usually this requires that pathology material is sent to the consultative site. This process is time consuming, a latitude that the patient may not have, and costs money. The application of telepathology is principally dependent on cost and the availability of appropriate resources and expertise[1-5,6,].

1.7.2 Interdisciplinary Telepathology

The practice of interdisciplinary telepathology comprises a pathologist and another specialist such as a radiologist or endoscopist. It is used to ensure correct tissue sampling, provide additional information to the pathologist and to facilitate collaborative or therapeutic decision-making. Studies involving interdisciplinary telepathology are rare and usually specific to a local institution. However, given the current trend in the development of medical informatics and integrated health care systems, the future of interdisciplinary telepathology is promising.[96]

1.7.3 Telepathology in Education

The most successful and widely used application of telepathology is in the field of pathology education. Internet sites abound dedicated either to cooperative workshops or to publishing image and clinical reference databases for educational purposes.[34,38,53,96]

The use of telepathology in education has distinguished itself under three themes: the development of the virtual slide classroom, as part of a pilot study in Iowa University virtual slides have replaced the microscope.[38] the development of internet forums such as iPath for ongoing education and support,[34] and as a research tool for investigating the cognitive processes of learning in education, Crowley *et al* (2002) used a telepathology system to distinguish the learning requirements of 'novice', 'intermediate' and 'expert pathologists' and went on to develop a system with protocols that would provide the appropriate level of assistance to the 'student' depending on their level of experience.[37,97,98]

The greatest paradigm shift in the use of telepathology comes under the first heading. Following on from the pilot study carried out in Iowa University, a number of universities in the US are replacing the microscope laboratories with computer laboratories where histology is increasingly being taught using virtual slides. These include the University of Pittsburgh, University of South Carolina, and University Columbia[154,155,156] The economic benefits are driving this change, as is user satisfaction. Students can use computer laboratories for a variety of other functions. This is not so with a microscope laboratory. Indeed a PC is considerably less expensive than a teaching or multi-headed microscope. For any teaching pathologists it is difficult to build up and maintain a collection of high quality teaching glass slides. During the course of teaching they can become lost or broken. Using Virtual Slides they may be widely distributable and more readily available to students.

The virtual slide will not completely replace the conventional microscope. Students must still master the set-up maintenance and use of a microscope. However, where histology is being taught, not only to pathologists, but medical students and life scientists there are considerable advantages in using virtual slides.

1.8 Telepathology Applications In Quality Assurance

1.8.1 Factors That Contribute to Diagnostic Accuracy In Pathology

Histopathological diagnosis forms the cornerstone of modern oncology. In the diagnosis and management of any tissue abnormality, its biologic significance as well as its diagnostic reproducibility is crucial in optimising patient treatment[102]. Errors in histopathological diagnosis can critically affect patient outcome and have, on occasion, become the subject of media concern.[99-104] There are a multitude of factors which contribute to diagnostic error, some of which are outlined below.

- *Specimen Type*

With some specimen types, such as bone and haematological cases where cells pertinent to a correct diagnosis are rare, it is more difficult to make a correct diagnosis than with other tissues. In addition, the higher the prevalence of a given disease, the greater the probability that a diagnosis will be "correct". This predictive value can be improved by selecting a population in which the disease has a higher prevalence, for example, breast cancer screening of "at risk" groups rather than the screening of the whole population.[80,99]

- *Specimen quality*

Diagnostic accuracy in microscopic examination is dependent on the quality of the tissue specimen. It is important that a biopsy is of sufficient quality and quantity and is removed from the correct area so as to be thoroughly representative of the tissue being examined. An inadequate biopsy may result in an incomplete or inaccurate diagnosis.[80]

- *Sample handling*

Macroscopic or gross examination is an important part of pathology. Macroscopic examination determines from where tissue will be sampled for microscopic examination. A diagnosis may be formulated from macroscopic examination to be confirmed upon microscopic examination. It is crucial that tissue is sampled from the correct area. It is important that the procedure for slide preparation is

automated, consistent and of a high quality. If slide preparation is not optimal misinterpretation may occur.[120,80]

- *Case information*

The provision of appropriate and relevant clinical information is essential if a pathologist is to make a correct diagnosis.[120,80]

- *Training & Experience*

If a pathologist is not a specialist in diseases associated with a particular specimen type, they are less likely to provide the correct diagnosis. Inexperience coupled with time critical decisions, such as with frozen section examinations and knowledge of the strong therapeutic consequences a diagnostic decision will have, has been shown to contribute to diagnostic error.[120,80]

1.8.2 Effect of The Use of Telepathology on Diagnostic Accuracy

There has been a considerable number of studies that consider the effect of telepathology on diagnostic accuracy. Shimosato (1992)[104] and Callas (1997)[105] showed that the skill and accuracy of the pathologist improved with experience in using a telepathology system while Weinstein (1997)[4], demonstrated that diagnostic accuracy using telepathology was related to a pathologists prior experience with video monitors such as computers and even video games. Hosch (2001)[80] suggested that a number of features of telepathology can improve the level of diagnostic accuracy such as the ability to compare multiple reference images at once. They considered that the use of telepathology removes 'professional isolation' as an error factor in that telepathology facilitates by providing rapid access to colleagues. In conclusion, they suggested that a pathologist reluctant to make a diagnosis using telepathology, always retains the right to defer diagnosis and refer to the glass slide.

1.8.3 Quality Assurance in Microscopic Diagnosis

Quality assurance has rapidly become a fundamental part of the practice of histology. Within each speciality and sub-speciality, there is a myriad of professional bodies that on a departmental, regional, national, EU and international basis, frequently perform quality assurance audits. Quality assurance (QA) is a planned and systematic review

process to determine that acceptable standards are being maintained and enhanced. Quality assurance can take the form of internal (IQC) and external (EQA) testing and validation.[107-108]

In most situations, both co-exist and complement each other. In the histopathology and cytopathology setting, IQC consists of procedures that pathology laboratories use to monitor within. The ISO definition, EQA (also known as 'proficiency testing') refers to a system of objectively checking laboratory results by means of an external agency, including comparison of a laboratory's result at intervals with those of other laboratories.[107-108]. IQC and EQA schemes are effective means for the assessment of pathology laboratory performance. EQA involves sending participating pathology laboratories slides on a regular basis. The participating pathologists submit a report on each slide. The reports are collated and analysed and used to measure the consistency of reporting in a number of key areas, such as diagnosing the major categories of disease and prognostic features of carcinomas. Such audits permit the measurement of agreement or disagreement between users and can be used as indicators of the proficiency between users and the robustness of grading schemes or diagnostic algorithms [107-108].

The professional bodies that run quality assurance studies are eager to perform such studies as frequently as is practical However time, effort, and cost are major limiting factor. There is considerable expenditure associated with the logistics of performing conventional quality assurance studies, predominantly associated with the inefficient use of human resources. A registry of participants must be maintained, slides must be mailed and completed forms must be manually collated, interpreted and undergo statistical analysis so that global and individual results are published. It is also inefficient for similar specialist groups to perform similar QA studies. The lack of centralisation breeds inconsistency and non-standardisation. The level of manual intervention is also a cause of concern in terms of introducing error. [107-108]

To illustrate such schemes, their inherent limitations and the suitability of telepathology to overcome such limitations, the following two examples are presented. Under the National External Quality Assessment (NEQAS) in the UK, a number of schemes for various medical disciplines are conducted, including one for breast screening. The

scheme involves distributing 12 pathology glass slides to over 400 pathologists. The participants submit a diagnosis using a standardised report form, which is compared with the diagnosis preferred by a panel of pathologists expert in breast disorders. The logistics of the scheme are such that each assessment takes five months and the scheme can only be conducted twice a year.[107]

The second example is of the Committee on Quality Assurance Training and Education (QUATE) of the European Federation of Cytology Societies (EFCS). This is organised by the European Union (EU) and the European Federation of Cytology Societies (EFCS). In order to become QUATE certified, participants must attend EFCS tutorials and meetings where proficiency tests are conducted. Such tests consist of scoring fifty multiple questions within an hour, passing an oral examination, screening and reporting on 10 cervical smears within 2 hours and the ability to make a diagnosis from fixed fields on twenty slides within half an hour. The certification lasts for three years before being renewed.[108]

Lee *et al* (2003)[109] examined the reproducibility and accuracy of telecytology using digital still images for the purpose of QA. They interpreted interobserver variation using Kappa statistics and an intra class correlation coefficient. Using three pathologists and three cytotechnologists who examined 50 slides, they determined that 40x was too low a magnification to determine a diagnosis and that 100x was necessary. Lee *et al* (2003) proposed that the aim of QA studies is to test the participant's ability to make a correct decision on a specific abnormal finding rather than to test their ability to screen an entire slide.[109]

1.8.4 Quality Assurance and Telepathology

The growing practice of performing QA in pathology represents a giant leap in terms of improving the quality, accuracy and consistency of diagnosis.[105-110] However, in today's informatics age, it is an inefficient system requiring a high degree of streamlining, automation and centralisation. Recent technological developments have spawned a new age where even the most mundane housekeeping task, grocery shopping, can be accomplished online, it is time that pathology utilises such advances.

The online performance of QA studies in microscopic diagnosis using telepathology offers significant benefits including:

- Apart from automation, centralisation and efficient use of resources, the widespread use of telepathology provides a capability for real-time analysis and feedback for participants and an online repository of results for third party scrutiny or historical/trend analysis.

- The use of telepathology eliminates the possibility of human error associated with the 'manual handling of data', glass slides would no longer get lost or broken in transit and turnaround time from the start of a study to the publication of results would be much quicker.

- Telepathology-driven QA studies could be performed using biopsy samples where tissue is limited such as with needle core biopsies and all participants would review the same digital slide, rather than, for example, different slides from the same paraffin block.

There are, however even greater benefits to be reaped by harnessing the resources of telepathology. A QA study performed using conventional means is capable of quantifying the degree of diagnostic agreement, reproducibility or consistency, of diagnosis. This is usually expressed as the percent agreement or concordance of diagnostic categorisations. Frequently, the Kappa statistic is also used to determine if the observed percent concordance is greater than would be expected to occur by chance.

The above methods are useful for identifying the presence and the extent of diagnostic variability, and, by convention,are the methods used to report inter-observer variability. However, they are of limited value given that they indicate the degree of diagnostic error but do not explicitly identify the cause.

1.8.5 Defining the Need For The VPS

To identify why a diagnostic error is made during microscopic examination of a slide, discrete variables describing the area of tissue viewed, the magnification it is viewed at and the time spent viewing the selected area of tissue must be measured and recorded in order to formulate a trace of the diagnostic process when examining a slide. Analysis of this data provides an opportunity to establish trends amongst pathologists in their examination of a particular slide and the ability to profile personal traits of individual pathologists in their performance of slide examinations.

It is not possible to record such variables by conventional means. Indeed, currently there is no commercial or academically available telepatholgy system available, which, can log and record variables describing a pathologist's examination. Some pathologists such as Crowley *et al* (2003)[37] proposed a methodology of videotaping pathologists examination. The examining pathologist used 'think aloud' protocols to describe why and where they were looking. It did not record their diagnostic trace as a discrete dataset. [37]

This present work focused on the development of a method for measuring, quantifying and identifying reasons for diagnostic error in microscopic diagnosis. This will be accomplished This will be accomplished through the implementation of the following objectives.

- To create a virtual microscope, known as the Virtual Pathology Slide (VPS) that simulates the experience of operating a conventional microscope, by presenting users with an image of a pathology slide and allowing the user to examine that slide by changing magnification and scrolling laterally.

- To incorporate novel features such as the ability to record a diagnostic trace of a pathologists examination of a virtual slide, in conjunction with the diagnostic decisions made by pathologists for their examinations, where the diagnostic trace is a discrete dataset (spatial location, magnification and time) that describes their examination of a slide.

- To develop novel data analysis tools that utilise the recorded examination datasets of pathologists, to elucidate the reasons behind inter-observer and intra observational variation, errors in diagnosis and provide graphical representations of diagnostic traces.

- To evaluate the system by presenting a number of pathology cases via the VPS, to pathologists and determining their diagnostic performance and overall perception of the system.

- To use the recorded datasets to analyse and determine reasons for diagnostic inconsistency.

It is hoped that the system will provide a novel insight into how and why diagnostic errors are made in a manner that previously could not be measured and that the system developed will be of particular use to pathologists engaged in behavioural studies, quality assurance studies and pathology education.

1.9 Conclusion

Until recently, the development of a tool for routine diagnosis and teleconsultation was the driving goal for the evolution of telepathology systems. Initial expense, lack of broadband Internet connections, potential liabilities, and a lack of knowledge transfer from expert to potential user have all contributed to preventing the incorporation of telepathology into everyday practice. However, the success of frozen section telepathology services offered in Norway and by the AFIP demonstrate that, in certain circumstances, real time remote diagnosis by means of telepathology can offer rapid quality diagnosis, improved patient care, and still be cost effective. The emerging role of telepathology in the area of education and quality assurance is not encumbered by the same difficulties. It has been demonstrated that the application of telepathology in such roles has the advantage of lower cost, less logistical effort, and a positive response to its use by the end user. Coupled with the growing presence of ultra-fast slide scanners, this should ensure an increasing role for telepathology in this area. [29-30,32-46,51]

Over the past forty years, vast resources have been expended by governments and intuitions on cancer research, however the cure remains elusive. In that time it has been shown that the accurate and timely diagnosis of disease can provide immediate benefit and relief for many patients. Telepathology has the potential to play a major role in this. The advantages of this technology are becoming increasingly apparent to pathologists. Currently, with the increasing desire for second opinions, the expansion of quality control programs and the economic need to deliver an efficient health care service, many countries use this technology.

A search of Pubmed (April 2004)[153] reveals more than 500 publications containing the word telepathology. However the rapidly emerging technology has some limitations. This includes expense, lack of standardisation in hardware, software, communication protocols, and indeed telepathology practice. The limited availability of broadband is also a major prohibitary factor. Despite such drawbacks, the potential advantages of telepatholgy will drive the technology forward. Provided that telepathology can be successfully integrated into the pathologist's workflow and the development of a telepathology culture within the pathology community, telepathology will become part of the pathologist's daily practice in the very near future.

Chapter 2:- Development of the Virtual Pathology Slide (VPS).

2.1. Introduction

Digitising a pathology glass slide at a spatial resolution sufficient to make a diagnosis is challenging. The intrinsic difficulties of this process are often overlooked by the end user who need not be aware of the significant technological issues which have had to be solved in order to derive a virtual slide. Initial attempts were made to create virtual slides including the use of line scanners or flat bed scanners.[6-7] These, however failed to achieve the image quality necessary to make a diagnosis, even where high resolution CCD sensors are combined with that of low magnification wide field of view objectives. To overcome the deficit in spatial resolution, imaging techniques have also been used whereby pixel values are interpolated in order to achieve an artificial magnification. However, interpolation of pixel values has been a contentious issue amongst pathologists and has not found acceptance. [6-7]

Bacus Laboratories were the first commercial entity to obtain a patent for the generation of virtual slides.[29] Bacus use a microscope with a motorised stage and mounted CCD camera to create a virtual slide. Initially they perform a low power scan using a 1.25x objective to obtain a thumbnail image of the slide from which the area to be scanned at a high power magnification is accomplished by changing the objective lens to 20x and 40x. The high-powered microscope objective then acquires individual fields of view at a high magnification, which may then be tiled together in order to present a high magnification digitised image of the slide. In order to present representations of the slide at a reduced magnification, an image pyramid system can be used whereby the dimensions of high magnification images are resized while their spatial resolution is maintained. The application of an image pyramid algorithm to resize images while maintaining spatial resolution is a classic image processing solution. The images may then be viewed using a propriety Web Slide Viewer. [29]

The generation of virtual slides by other vendors and academic groups invokes a variation of the above protocol. All revolve around the principle of acquiring and tiling fields of view at high magnification and using an image pyramid algorithm to generate images of lesser magnification.[30-33,35,36,51]

However, automated scanning at high magnification (40x or above) has presented its own difficulties due to the variable nature of slide preparations and, in particular, the

variable thickness of tissue sections. At high magnification (40x or above) auto-focusing of the lens may be required for every field of view depending on the presence of a variable focal plane and the height of numerical aperture of the lens. However, this can significantly extend the length of time required to scan a slide. By decreasing the numerical aperture of the lens used, the depth of field increases, this allows for increased variability of tissue thickness before focusing is required. However, it also causes a reduction in spatial resolution and image quality. Typically, software for autofocusing functions by analysing the signal to noise ratio of a series of images acquired at different focal planes. The image is deemed to be in focus when signal noise is greatest, when tissue is in focus the grey value is highest. However, autofocusing algorithms must be robust enough during slide scanning to detect the signal to noise threshold above which focusing is required, and in the absence of tissue, (for example at the edge of a tissue section) not to lose focus entirely in a search for non existent tissue.

An additional challenge in the creation of virtual slides involves the tiling or stitching together of images to form a seamless montage. If the repeatability and accuracy of a microscope stage is not at a sub-micron level, or if the camera is misaligned with the x,y axis of the stage, a combination of overlap or under lap will occur resulting in apparent misalignment between individual images within the montage. The degree of misalignment that is present is exacerbated the higher the magnification. Use of a high quality stage in addition to proper alignment of the camera are obvious solutions to this problem. The seamlessness of the alignment can also be improved using image processing algorithms that use a form of pattern recognition to align images.

To overcome the bottleneck of available storage capacity and broadband, virtual slides are typically saved using image file formats such as Zoomify™,[153] Flashpix™,[32-35] or JPEG2000™.[58] It is the web slide browser software that provides the functionality to the end user to allow the examination of tissue similar to that of using a conventional microscope. Currently available web slide browsers are typically built using Microsoft C++, or Java from Sun Microsystems.[154] Other virtual slides viewers have been developed that simply display the image and provide 'microscope functionality' in a conventional web browser such as Internet Explorer and use web technologies such as Macromedia Flash.[155] The level of functionality of virtual slide viewers has grown

beyond that of attempting to replicate the experience of using a conventional microscope. For example, many virtual slide viewers permit images to be annotated and artefacts marked within an image or an increasing ability to perform some types of image analysis such as area and distance measurement. This represents an important coming of age for telepathology, the realisation that perhaps the greatest advantage in using virtual slides lies not with the image itself but with data associated with the image.

This chapter describes the methodology utilised in the development and generation of a VPS. The chapter documents the initial scanning of the tissue section and publication of acquired images on the web. The chapter also documents the manner in which the functionality of the VPS browser application delivers a simulated microscope experience. In an important new departure from that of alternative virtual slides, the development, architecture and functionality of the user-tracking feature of the Virtual pathology is also described.

2.2. Equipment

2.2.1 Hardware and Software Utilised

An imaging workstation was developed in-house to fulfil the needs of the project. An Olympus BX-40 microscope (Olympus, Melville, NY) incorporating a 40x Plan Apochromat lens with a 0.95 numerical aperture was used. The microscope was fitted with a computer controlled motorized stage (Prior Scientific Inc., Rockland MA), accurate to 0.1μm in the Cartesian X and Y planes and to 0.02μm in the Z (focus) plane, with integrated focus processor via an RS232 connection. The focus processor required a direct camera signal via a conventional video cable (S-Video signal). The video camera utilised was a JVC KY55B 3-CCD (3 chip charged couple device) with an RGB (Red Green & Blue) digital signal outputted to an Imaging Technologies IC-RGB frame grabber board. (Coreco Imaging, Incorporated, Bedford, MA). The framegrabber was housed in a personal computer with 64MB RAM and 50GB local hard drive capacity. The computer operating system utilised was Windows NT4 (service pack 6) (Microsoft Corp, Redmond, WA). Figure 2.1. depicts the VPS imaging workstation used to scan pathology slides.

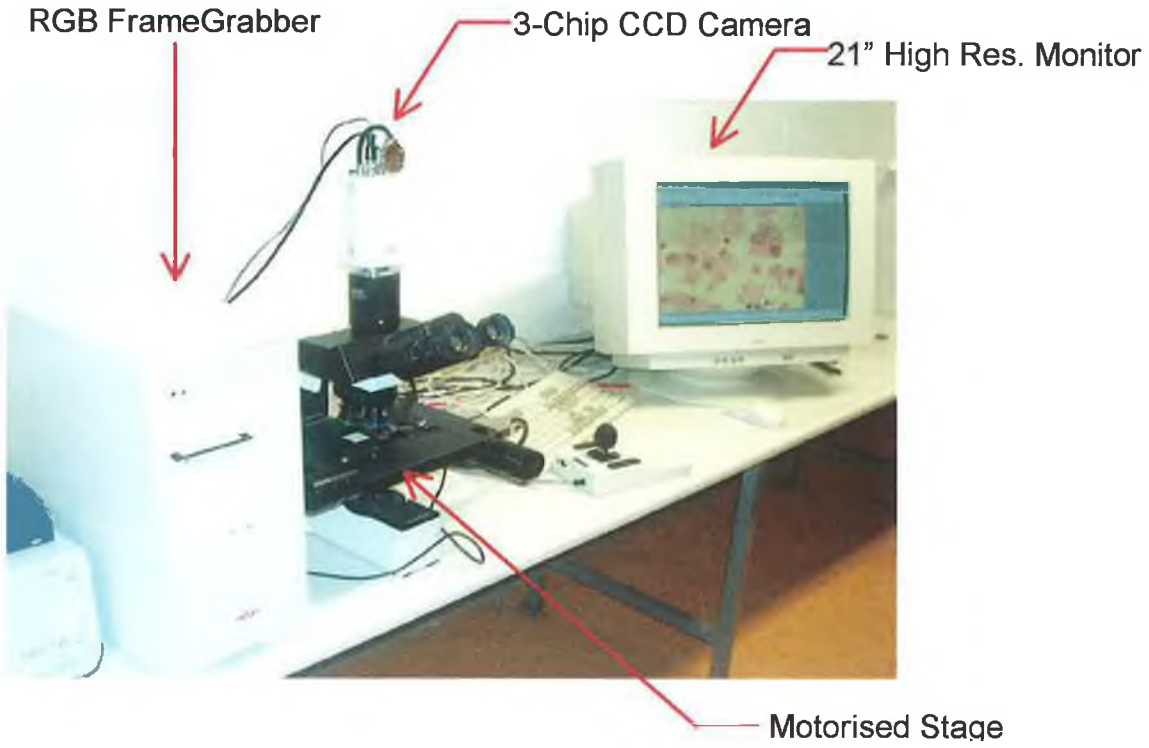


Figure 2.1 Depiction of Imaging Workstation Used To Scan Pathology Slides For The VPS.

2.3.Construction Of A Virtual Pathology Slide

2.3.1 Slide Digitisation

The first stage of the construction of a Virtual Pathology Slide is the digitisation of the tissue of interest at the highest required magnification (40x objective). All imaging applications were developed within Optimas 6.5, image acquisition and analysis software (Media Cybernetics, Carlsbad, California, USA).[111] Optimas is capable of interfacing and acquiring images from a framegrabber which can subsequently be manipulated using Optimas's programmable platform, which facilitates the creation of image analysis algorithms using ALI (Analytical Language for Images). The software also allows programmable control of RS232 interfaced devices, such as the robotic stage. An algorithm was constructed in ALI to perform a programmed raster scan of 15.53mm x 11.61mm ($180mm^2$) of tissue, and build subsequent layers of lesser magnification.

2.3.2 Raster Scan - Generation Of Image-Layer 1

The raster scan involves acquiring 128 x 128 images in both the X and Y Cartesian directions, one row at a time. The scanning algorithm is manually seeded with the Cartesian X, Y coordinates of the tissue midpoint. Using the midpoint coordinates, the scanning algorithm determined the boundaries of the scan.

At high power magnification, variations in tissue thickness require constant adjustment of fine focus while scanning. This is achieved using an autofocus utility integrated in the system. However, due to mechanical slippage, the system had potential to lose focus over time. To compensate for this shortcoming, a custom focus determination was utilised.

Before scanning commences, the stage is manually moved to a reference point within the tissue section, representative of the topography of the entire section. An autofocus is performed at this point and a value representing the focal depth recorded. This focal value is subsequently used as a reference point during the raster scan. At the beginning of each row, the stage moves to this reference point, refocuses and recalculates a new focal depth. The offset between new and previous focal depth is a measure of the

slippage that has occurred. This offset is then utilised to correct any focal depth aberration while scanning the subsequent row.

During the raster scan, for each field of view, the standard deviation of grey values is determined using a ALI function called within the scanning algorithm. If the value is above a given threshold, tissue is deemed to be present and image capture is required. A fine auto-focus is performed and the image saved. This procedure reduces processor workload and memory storage requirements for a slide, as only images that contain tissue are saved.

Two three-digit numbers ranging from 101 to 228 are generated, describing the grid reference of each image on the Cartesian X, Y plane. These numbers are concatenated into a seven-character string separated by an arbitrary assigned delimiter (k) to form the file name for each saved image. For example

X=1 Y=2 (X, Y Cartesian coordinate of an image)
101K102.jpg (Image file name)

Each acquired image represents 0.011 mm^2 at a resolution of 768 pixels by 574 pixels and saved as both bitmap (*.bmp) and JPEG (Joint Photographic Expert Group). Layers of lesser magnification were built using the uncompressed lossless Bitmap version. The Bitmap version was then deleted to conserve space. The JPEG images were compressed by 10%, resulting in image file sizes in the range of 100-150kbs.

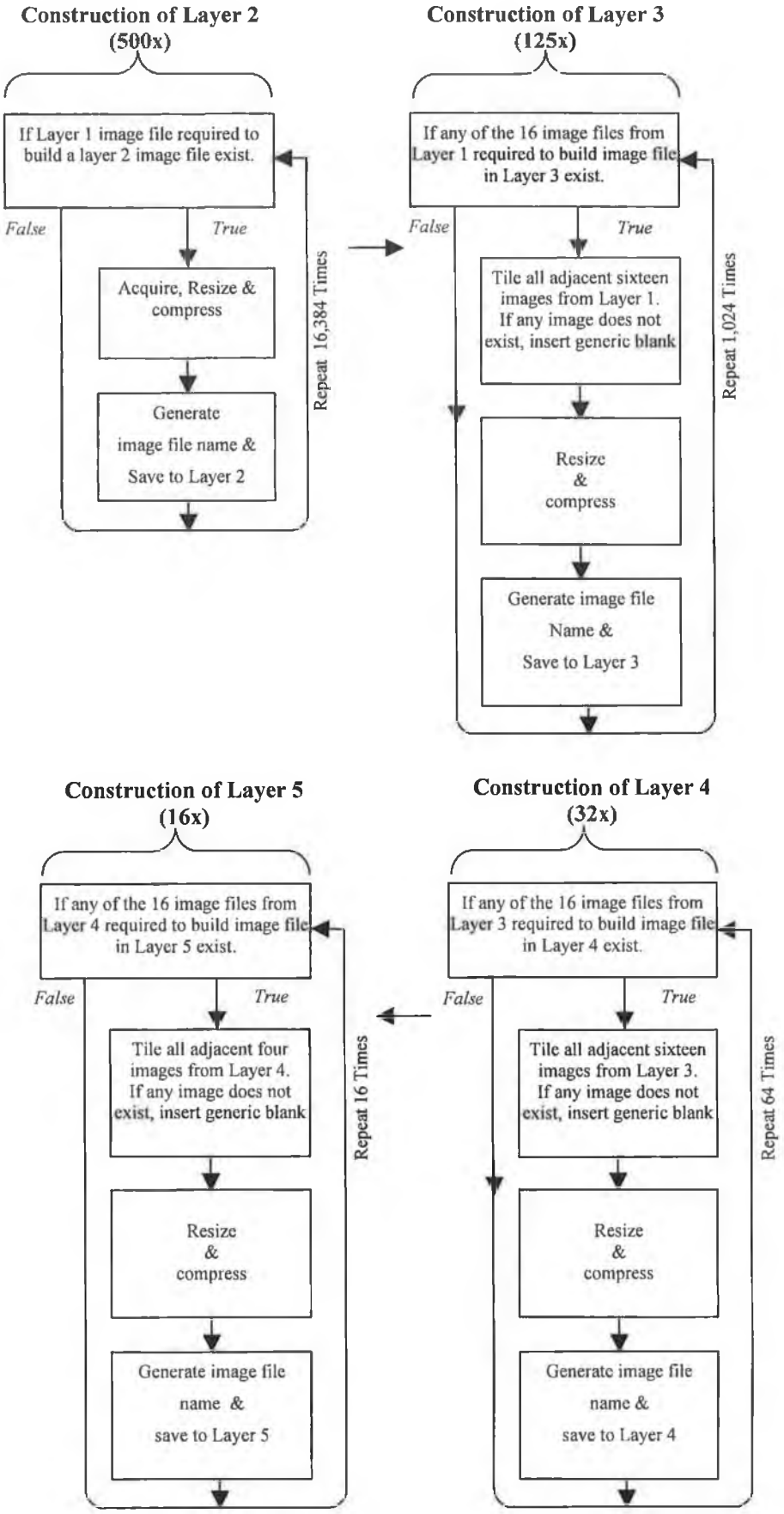


Figure 2.2 -Flow Diagram Describing The Algorithm Used To Construct Image Layers 2 to 5 From Image Layer 1.

2.3.3 Scaling of Image Layers 2-5

To fulfill the range of required magnifications for display, four image layers of lower magnification but equal resolution to Image Layer 1 are generated using a scalable imaging algorithm as described in Figure 2.2. The image layers were constructed as follows.

- Image Layer 2 was constructed by resizing all the images in Image Layer 1 to 1/16 of their original size (192 pixels *by* 143 pixels).

- Image Layer 3 was constructed by tiling 16 spatially related images (4 horizontal x 4 vertical) from Image Layer 2, resizing the tiled composite to 1/16 of its size and saving as one image (192 pixels *by* 143 pixels). This process was performed 1024 times to create a complete Image Layer.

- Image Layer 4 was constructed in the same manner as Image Layer 3, however images from Image Layer 3 were used as the source images for all operations. The process resulted in the production of 64 images (192 pixels *by 143* pixels).

- Image Layer 5 was constructed by tiling 4 spatially related images (2 horizontal x 2 vertical) from Image Layer 4, resizing them to 1/4 of their size (192 pixels *by 143* pixels) and saving as one image. This resulted in the production of 16 images with an apparent magnification of 16x.

When tiling and creating a composite image during the generation of Image Layers 3 to 5, one or more of the 16 spatially related images may not exist, as only images that contain tissue were originally saved during the raster scan. To complete the composite, a blank image generated at the beginning of the raster scan was inserted.

Image filenames for each Image Layer are generated in a similar fashion to Image Layer 1. Each Image Layer is saved to an individual folder. Images are saved as JPEG image files with a 10% compression ratio. This results in image file sizes of approximately 8kb. The total number of images generated and resultant file size for a complete digitilised VPS slide is dependent on the amount of tissue present. Typically

for a needlecore biopsy 6000 images are saved. This requires approximately 600Mb of storage.

Table 2.1 illustrates the maximum possible number of images generated, image file name range and resultant magnification for each image layer.

Table 2.1 Illustration Of The Apparent Magnification, Image File Name Range and Maximum Potential Number Of Images For Each Image Layer Within a VPS.

IMAGE LAYER	MAGNIFICATION	FILE NAME RANGE	MAXIMUM NO. OF IMAGES
1	2000x	100K100.jpeg→228k228.jpeg	16,384
2	500x	100K100.jpeg→228k228.jpeg	16,384
3	125x	100K100.jpeg→132k132.jpeg	1,024
4	32x	100K100.jpeg→108k108.jpeg	64
5	16x	100K100.jpeg→104k104.jpeg	16

2.3.4 File Transfer to Server

The web server utilised for the study was a Sun Microsystems Enterprise 450 (Sun Microsystems, Palo Alto, CA). It supports up to four 400-MHz/4-MB or 480-MHz/4-MB UltraSPARC-IITM processors, a 1.6-GB/sec UPA interconnect, and a 1GB/sec PCI I/O subsystem. The server can be deployed with up to 4-GB of main memory, up to 364-GB of fast hot-swap UltraSCSI internal storage, and over 6-TB of external storage capacity. Figure 2.3 depicts an image of the Sun Microsystems Enterprise 450 server used to host VPS images.

Figure 2.3 Depiction Of The Sun Microsystems Enterprise 450 Server Used To Host VPS Images.

The server is connected to Ireland's academic and research network, which is known as HEAnet.[112-113] HEAnet provides connections to networks in Europe by means of its 10Mbps link with the TEN-155 (Trans-European Network at 155 Megabits per second) backbone, and a 2 Mbps circuit links HEAnet to JANET, the UK education and research network. HEAnet is connected to UUnet via DANTE in New York, USA, with transit to all parts of the Internet, including a backup connection to the UK and Europe.

At New York, HEAnet connects to other Internet 2 networks, including the Abilene project.[113]

A file transfer protocol application called FTP Explorer is used to upload images to the server. This is available on the Internet at www.ftpx.com.[114]

Image Layers are archived in an identical fashion on the server as on the local hard drive, where each magnification layer maintains its own separate folder. Therefore, for each slide, there are 5 folders, one for each magnification. The folders are named "BUILDn" where 'n' is a numerical value indicating the magnification layer it contains. For example 'BUILD5' contains 16x images, 'BUILD4' contains 32x images, 'BUILD3' contains 125x images 'BUILD2' contains 500x images and 'BUILD1' contains 2000x images.

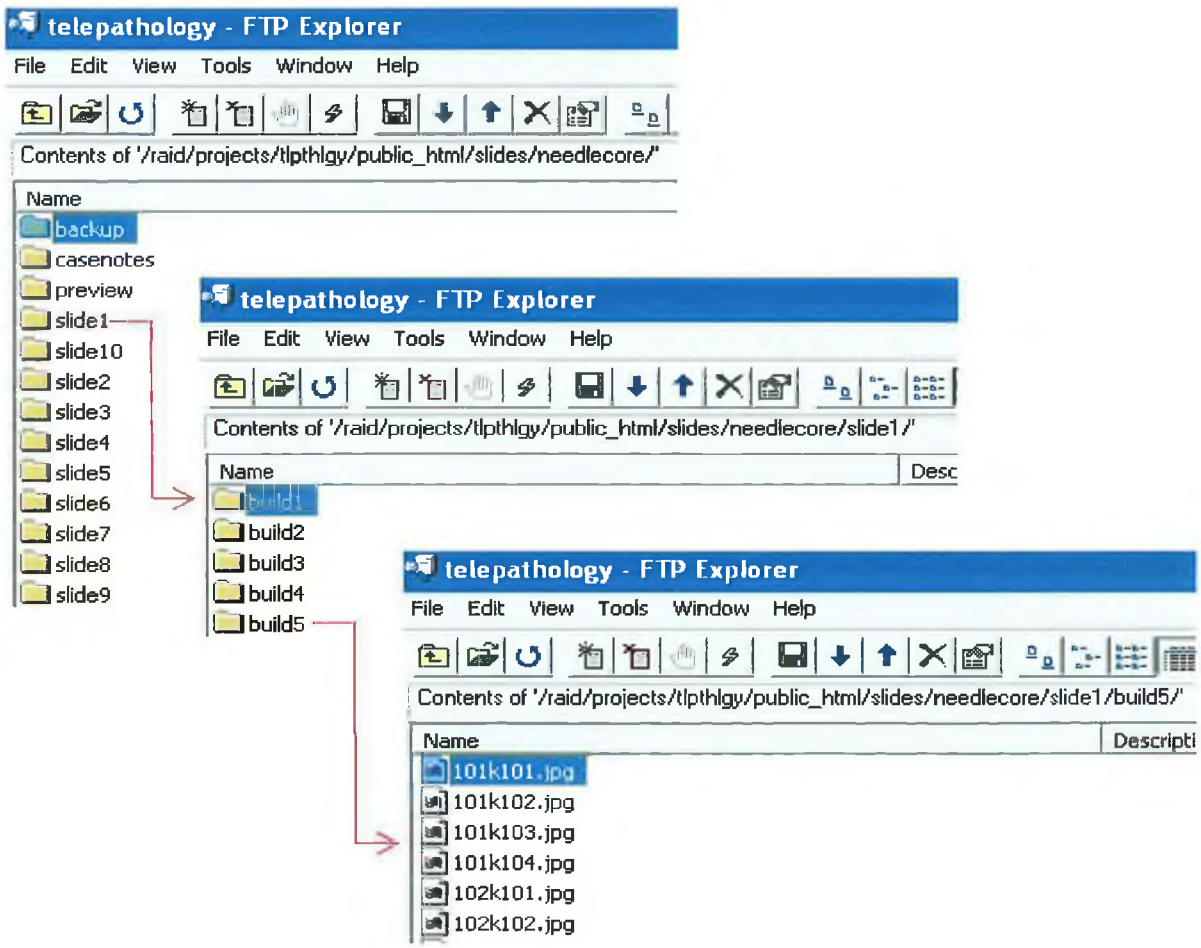


Figure 2.4 Depiction of Folder Tree Structure of Slides Stored on The VPS Server.

2.4. VPS Presentation

2.4.1 VPS Application

The VPS user interface is a web application that can be displayed by any conventional browser connected to the Internet. Figure 2.5 illustrates the components of the VPS interface while Figure 2.6 depicts the VPS ability to zoom in on an area of interest (Figure 2.6(a-d)), scroll laterally (Figure 2.6(d)), and then zoom in to the highest magnification (Figure 2.6(f)). The VPS user interface is composed of sixteen images tiled seamlessly within a table of 4x4 cells. The range of apparent magnifications experienced is dependent on the user's system configuration. However, a magnification of 2000x is experienced, when using a 19-inch (48cm) monitor with a screen resolution of 1024x768. The full range of magnifications experienced at the above resolution is 2000x, 500x, 125x, 32x and 16x.

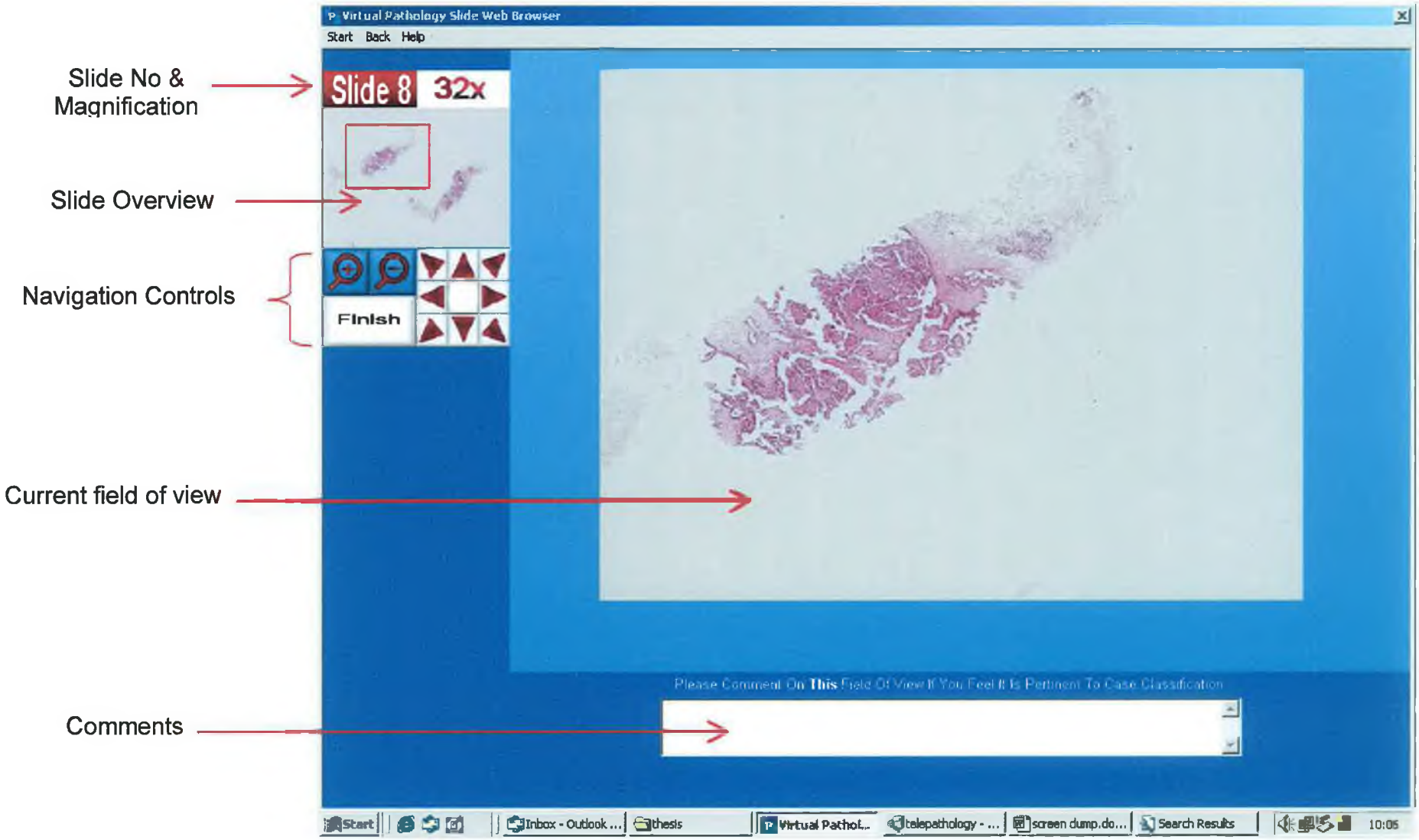


Figure 2.5 Screen Dump Of The VPS Microscope Emulator Interface With Standard Components Labelled.

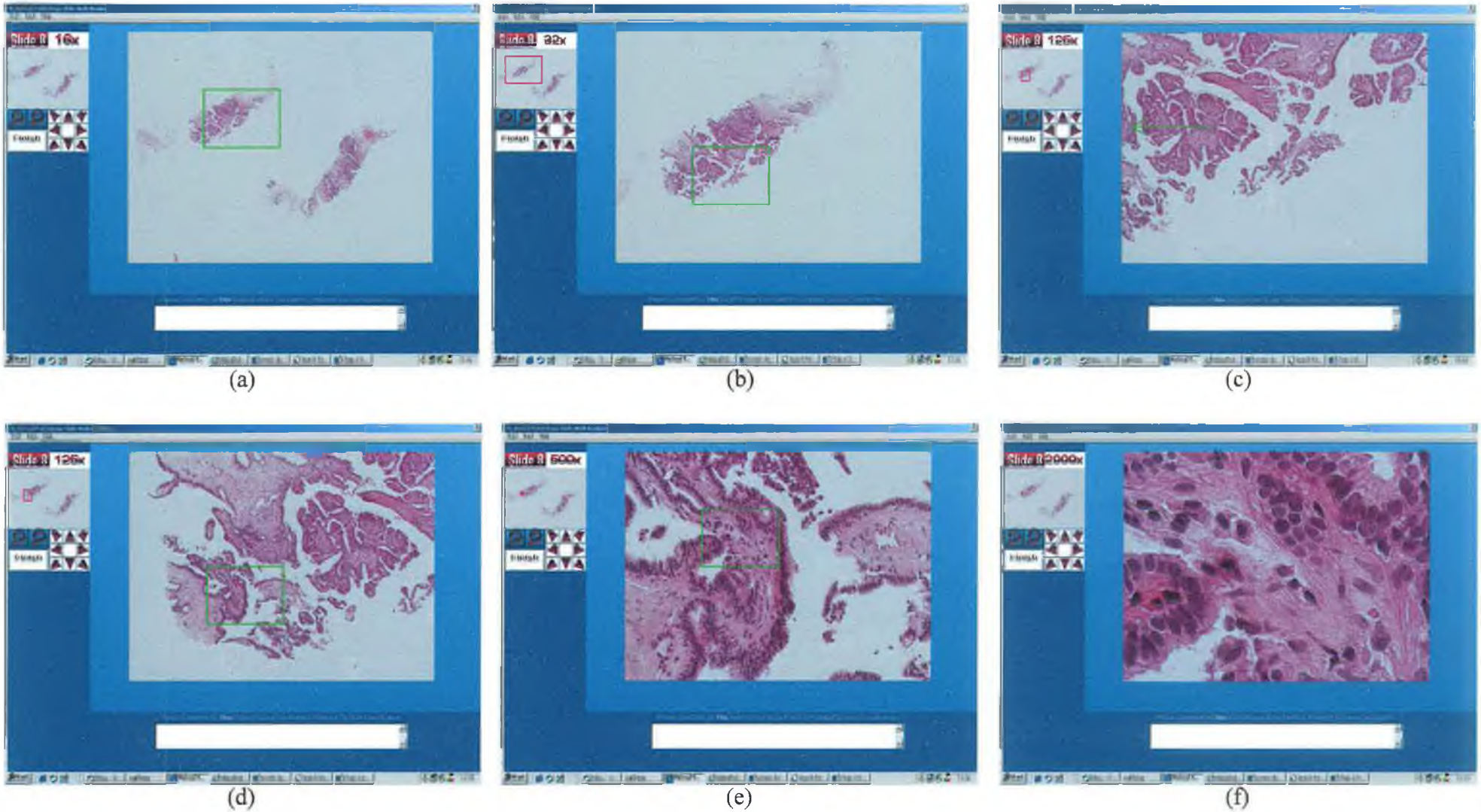


Figure 2.6 Migrating Through a VPS. (a) Initial view of VPS at 16x (b) Zoom to 32x on area of interest. (c) Zoom to 125x on area of interest. (d) Move laterally around area of interest.(e)Zoom to 500x on area of interest. (f) Zoom to 2000x on area of interest.

2.5 VPS Functionality and The Client/Server Relationship

2.5.1 Client Action

Sharing information on the Internet is a simple process of a client using a Web browser to request a file from a Web server. The Web server sends a response that contains the file. The Web browser interprets the HTML instructions within the file to format and display the data in that file. The VPS interface is written in HTML (Hypertext Markup Language). HTML is a universal browser language for creating Webpages.

A modification to the above model involves the generation of HTML dynamically, either on the side of the Web client (using client-side scripting, DHTML, XML/XSL or other client applications) or on the side of the Web server (using PHP, CFM, ASP, ASP.NET, COM+, ISAPI, or other server interface).

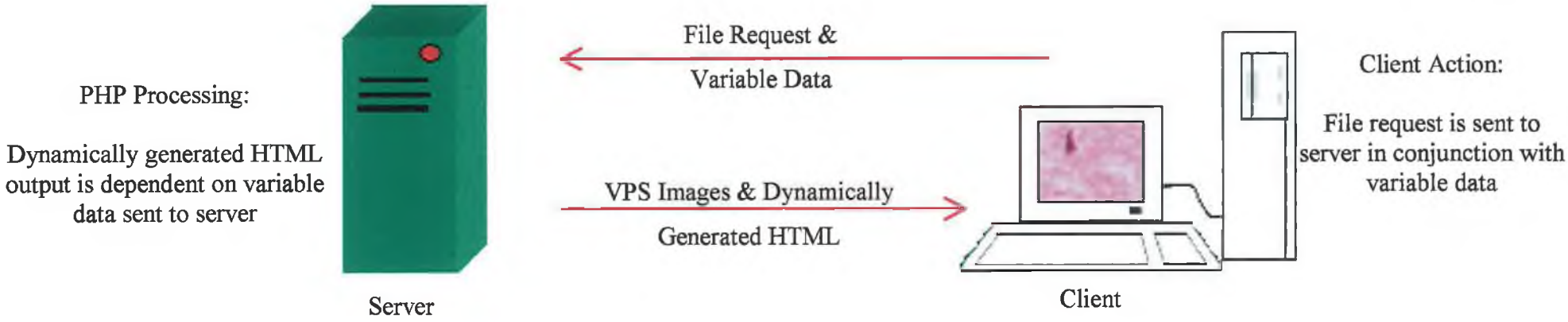
The VPS application uses server side scripting in PHP, to generate HTML dynamically. The ability to zoom in, zoom out, and scroll laterally within a VPS slide is dependent on:

(i) A client action. When the user interacts with the client, an Internet browser the user employs to view the VPS, variable input data is sent from the client to the server.

(ii) The server response. The server processes the information and dynamically creates VPS content to send to the client.

(iii) Client-Server communication, the ability of the server to receive, process and respond to variable input data from the client.

The Client/Server Relationship is illustrated in Figure 2.7.



- Variable data describing a user's interaction with a web-page may be sent from the client to a web server, in addition to a file request, when a user clicks a hyperlink, submits a form or enters a URL.
- Using the variable data submitted by the client, the server processes the PHP script contained within the requested file resulting in the generation of HTML dynamically, which is sent to the client.

Figure 2.7 Schematic Representation of Client Server Interaction

2.5.2 Server Response

The server is utilised as a store for information, requested by the client. Under certain conditions, a server can be utilised to perform some processing at the request of the client. This feature is generally facilitated using server side scripting. Server side script is incorporated in certain HTML based pages requested by the client. To identify pages containing script, such pages are appended with a special extension (an extension other than "*.htm"). For Microsoft Active Server Pages, the extension is "*.asp", for PHP, the extension is "*.php".

PHP, originally known as PHP/FI (Personal Home Page/Forms Interpreter) was created by Rasmus Lerdorf in 1995.[115] It has subsequently undergone continuous development by the open source community, into a powerful and popular web programming language. It is estimated that PHP is installed on over 20% of the domains on the Internet[134]. PHP is an open source, server side, cross-platform, scripting language[134]. With syntax similar to the C language it has evolved from other server-side programming tools such as PERL. When the server receives a request from a client for a page with "*.php" extension, the server will open the file and parse the contents until it identifies a PHP tag within the text. The server then processes the commands within the start and finish PHP tags and replaces this content with static HTML, based on the outcome of the processing.[115]

2.5.3 Client-Server Communication

For any dynamic processing to be conducted on the server, the client needs to be able to send the server variable data, which can subsequently be utilised in calculations to generate variable HTML output. The client and the server communicate using a URL. (Uniform Resource Locator). (i.e. www.telepathology.dcu.ie/index.htm)

The URL describes the location of the server on the Internet. Using the http GET method, variables can pass between a server and a client directly within a URL, as in the following example.

www.telepathology.dcu.ie /slides/slide2/main.php3? DIRECTION=2&LAYER=3&IMAGE=101K104

These variables can be utilised within the PHP script of "main.php3". In the above example, the variables DIRECTION, LAYER and IMAGE are passed to the page, "main.php3". Every time the user clicks on an image or a navigational control button (Figure 2.5), a hyperlink is activated calling the server page, "main.php3". However, the value of the variables returned to the server changes, depending on the magnification the user is currently viewing, the images displayed on the VPS and the image or navigational control button the user clicked. Figure 2.7 and Figure 2.8 illustrates the client-server communication and response for the VPS.

The value of the DIRECTION variable indicates the direction the user wished to migrate to (Zoom in, Zoom out, Lateral motion). On reading the value of the DIRECTION variable, the server parses the PHP script and processes only the part of the PHP script that calculates the correct set of output images for the desired move.

The value of the LAYER variable indicates the current magnification the user is viewing. It is used within the PHP script to decide which Image Layer folder to retrieve images from, when generating a set of output images. Table 2.1 illustrates the possible range of values for DIRECTION and LAYER.

If the user wishes to zoom, by clicking on an image in the VPS, the value of IMAGE identifies the image the user clicked on. If the user wishes to zoom or scroll by clicking on a navigational control button (Figure 2.5)), IMAGE identifies the top left image of the sixteen images currently displayed on the VPS (Figure 2.5, "Image1"). If the user wishes to zoomout, IMAGE identifies the image that is diagonally down and to the right of the top left image for Image Layers 2 to 5 (Figure 2.9, "Image6"). If the user is viewing Image Layer 1 (2000x), it references the single image on display.

The client may sometimes request images that do not exist on the server, for example when viewing images around the edges of a tissue section. When this occurs, a generic blank image is inserted into the cells of the HTML table resulting in a seamless field of view.

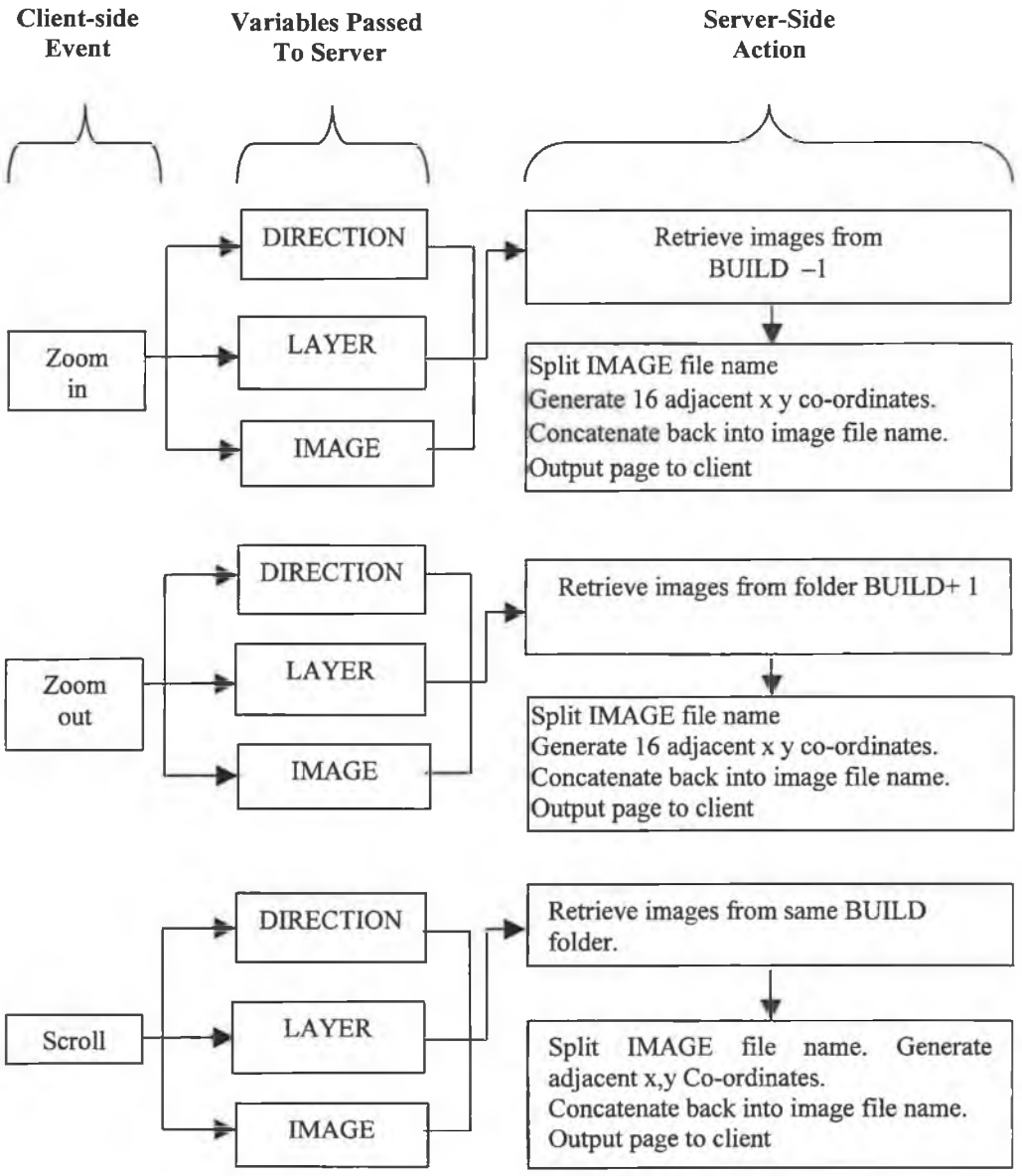


Figure 2.8 - List Of Server-Side Actions Based On Client Side Events.

2.5.4 Spatial Relationship Between Images of the Same Magnification

The naming structure of an image file describes the geographical x and y location on the slide that the image file represents. For example, when the image file name 105K101.jpg is parsed, the x and y coordinates are returned where x=105 and y=101. The file name of the image directly beside image 105K101.jpg may be determined by incrementing or decrementing the x or y value appropriately. For example, the image file name of the image that is tiled directly to the left of 105K101.jpg is called 104K101.jpg. The image that is tiled directly below image 105K101.jpg is called 105K102.jpg. Figure 2.9 illustrates the image file naming structure of 16 tiled images that comprise a field of view of the VPS where the top right image file name is 105K101.jpg

2.5.5 VPS Zoom

The first field of view the user experiences is the tissue at 16x magnification. This view is composed of 16 tiled images (4 horizontal, 4 vertical) from Image Layer 5. As the user clicks on one of the 16 images, an associated hyperlink is activated. This calls "main.php", with the variable IMAGE in the hyperlink identifying the calling image to the server.

If the server reads the variable DIRECTION=1, this results in the activation of the PHP script that constructs a field of view of higher magnification to the current field of view.

The server reads the variable LAYER (for example, LAYER=5) and decreases its value by 1 (LAYER=4). This results in the output set of images being retrieved from Image Layer 4, which contains images at 32x magnification. Using the delimiter (k) as a recognition character, PHP splits the IMAGE variable into its X and Y coordinates (e.g. IMAGE=105K101 returns X=105 Y=101). The X and Y coordinates (Xn and Yn) of the new top left image (Figure 2.9, "Image1") is calculated. This is based on the spatial relationship between images on different Image Layers. For example, if DIRECTION=0, the user wishes to zoom.

Variables passed to server

DIRECTION=0
LAYER=3 (Assumption for the purposes of this example).
IMAGE=105K101.jpg (Assumption for the purposes of this example).

Parse IMAGE to obtain Coordinates of new top left image 'Image1'.

(i)	X=105	Y=101
(ii)	Xn=4*(X-100)+97	Yn=4*(Y-100)+97
(iii)	Xn=107	Y=101

The X and Y coordinates of the fifteen adjacent images are generated by manipulating the value of Xn and Yn appropriately (Figure 2.9), resulting in the creation of a two-dimensional reference grid. The sixteen new pairs of X and Y coordinates are concatenated with the delimiter (k) and reconstructed into a valid image file name (e.g. Xn=105 Yn=101 ∴105K101.jpg). A condition is included to ensure all new image file names are valid for that particular layer. This prevents incorrect X and Y coordinates being generated and ensures that the 16 images called by the client, are within the bounds of the image set.

This method is used to zoom in until the penultimate layer (Image Layer 2) is reached. In the penultimate view (500x), if the user clicks on one of the 16 tiled images, the selected image is retrieved from Image Layer 1 (2000x) and presented to the user.

Image 1 x=xn y=yn x=105 y=101 File=105K101.jpg	**Image 2** x=xn+1 y= yn xn=106 yn=101 File=106K101.jpg	**Image 3** x=xn+2 y=yn xn=107 yn=101 File=107K101.jpg	**Image 4** x=xn+3 y=yn xn=108 yn=101 File=108K101.jpg
Image 5 x=xn y=yn+1 xn=105 yn=102 File=105K102.jpg	**Image 6** x=xn+1 y=yn+1 xn=106 yn=102 File=106K102.jpg	**Image 7** x=xn+2 y=yn+1 xn=107 yn=102 File=107K102.jpg	**Image 8** x=xn+3 y=yn+1 xn=108 yn=102 File=108K102.jpg
Image 9 x=xn y=yn+2 xn=105 yn=103 File=105K103.jpg	**Image 10** xn+1 yn+2 xn=106 yn=103 File=106K103.jpg	**Image 11** x=xn+2 y=yn+2 xn=107 yn=103 File=107K103.jpg	**Image 12** x=xn+3 y=yn+2 xn=108 yn=103 File=108K103.jpg
Image 13 X=xn yn+3 xn=105 yn=104 File=105K104.jpg	**Image 14** x=xn+1 y=yn+3 xn=106 yn=104 File=106K104.jpg	**Image 15** x=xn+2 y=yn+3 xn=107 yn=104 File=107K104.jpg	**Image 16** x=xn+3 y=yn+3 xn=108 yn=104 File=108K104.jpg

Figure 2.9 Illustration Of How The X and Y Coordinates Of Sixteen Adjacent Images Displayed In The VPS Interface Are Generated By Manipulating The Value Of Xn and Yn For Magnifications, 16x, 32x, 152x, 500x.

2.5.6 VPS Zooming Out

When zooming out using the navigation button on the control panel (Figure 2.8), it is perceptually important to centre the previous view within the zoomed out view. This is accomplished by assigning the previous location of Figure 2.9 -"Image 6", to the previous view in the zoom out view.

The server reads the variable, DIRECTION=2 (Table 2) resulting in the "zoomout" section of the PHP script being processed. The value of LAYER is increased by 1, so that image files will be retrieved from a folder containing lower magnification images.

The server reads the variable, IMAGE. As with zoom, the server splits IMAGE into its X, Y Cartesian coordinates. A condition exists to ensure that both X and Y are even numbers. If either number is not, a value of 1 is added. The Cartesian X, Y coordinates (Xn and Yn respectively) of the zoomed out top left image, is calculated using an algorithm that describes the spatial relationship between the most central image (Figure 2.9, "Image 6") on the lower-magnification Image Layer and the top left zoomed out image. For example, if DIRECTION=1, the user wishes to zoom out.

Variables passed to server

DIRECTION=1
LAYER=2 (Assumption for the purposes of this example).
IMAGE=105K101.jpg (Assumption for the purposes of this example).

Parse IMAGE to obtain Coordinates of new top left image 'Image1'.

(i)	X=117	Y=101
(ii)	Xn= X=(X-100)/2+97	Yn=(X-100)/2+97
(iii)	Xn=105	Y=101

The Image File name of the new top left image 'Image1' will be 117k101.jpg. In an identical fashion to zoom in, manipulating the value of Xn and Yn generates the coordinates of the fifteen adjacent zoom-out images. Conditions within the PHP script

ensure the newly created X, Y co-ordinates are valid for that Image Layer. The X and Y co-ordinates of the sixteen images are then concatenated with the delimiter and constructed into a valid image file name and attached to a dynamically created hyperlink. This results in a set of sixteen output images being sent to the client.

2.5.7 VPS Scrolling

When scrolling within an Image Layer (using the navigational buttons on the control panel (Figure 2.5), the following events occur. DIRECTION will have a value between 3 and 10 depending on which navigational button the user clicked (Table 2.1).

The server retrieves images from the same Image Layer folder (the value of LAYER remains constant). The server splits the value of IMAGE, representing the top left image file name (Figure 2.9, "Image 1"), into its X and Y co-ordinates. Based on the input value of the DIRECTION variable, an algorithm is used to generate the new X, Y coordinates (Xn and Yn respectively) for the top-left image of the new field of view. For example, if DIRECTION=6, the user wishes to scroll right.

Variables passed to server

DIRECTION=6
LAYER=3 (Assumption for the purposes of this example).
IMAGE=105K101.jpg (Assumption for the purposes of this example).

Parse IMAGE to obtain Coordinates of new top left image 'Image1'.

(i) X=105 Y=101
(ii) Xn=x+2 Yn=Y
(iii) Xn=107 Y=101

The Image File name of the new top left image 'Image1' will be 107k101.jpg. In an identical fashion to Zoom, the X, Y coordinates of the fifteen adjacent images are

created and concatenated back with the delimiter (k) and file format (.jpg) into valid file names. The new images will be retrieved from folder BUILD3.

If scrolling at 2000x (Image Layer 1), the server produces an output page containing a single image that is adjacent in the direction of scrolling, to the image in the input page. For example, if DIRECTION=6, the user wishes to scroll right.

Variables passed to server

DIRECTION=6
LAYER=1 (Assumption for the purposes of this example).
IMAGE=105K101.jpg (Assumption for the purposes of this example).

Parse IMAGE to obtain Coordinates of new top left image 'Image1'.

(i) X=105 Y=101
(ii) Xn=x+1 Yn=Y
(iii) Xn=106 Y=101

Image file name 106k101.jpg from folder 'BUILD1' is displayed.

2.6 User Tracking

2.6.1 Fundamental Concepts of Databases

A database is a collection of data that is organised so that its contents can easily be accessed, managed, and updated. Databases contain aggregations of data records or files, such as sales transactions, product catalogues and inventories. Databases are prevalent in large mainframe systems, but are also present in smaller distributed networks via database servers and on personal computers.[118-120]

The structure, constraints and description of data stored in a database is controlled by a Database Management System (DBMS). The DBMS is a software application in control of access, security, storage retrieval, backup and other service support functions for the database system. This allows it to manage the large, structured sets of persistent data, which make up the database, and provide access to the data for multiple, concurrent users whilst maintaining the integrity of the data.[118-120]

The DBMS provides security facilities in a variety of forms, both to prevent unauthorized access and to prevent authorized users from accessing data at the same time as each other or overwriting information, which they should not. Usernames and passwords are used to identify operators, programs and individual machines and assigns access privileges to them. These privileges can include the ability to read, write and update data in the database.

Database design is the process of taking the requirements for the database such as what information is to be stored, who can access it, how many people can access it simultaneously and designing a system which can accommodate this.

To design a database, a schema is developed which defines what data is stored, how it relates to other data, and who can access, add and modify data. Depending on the type of database, the schema is broken into several sub-schemas, which define individual tables and relationships between the tables and the data contained within.[118-120]

2.6.1.1 Classification of databases

Data in a database can be structured in several different ways using different models, depending upon the type of information being stored and its use. The following outlines a number of models that have been used. [118-120]

- Flat File Database*:* The simplest database structure known as a flat file database consists of single files. Flat files are data files that contain records with no structured relationships. Although momentarily popular in the 1970 due to their simplicity, flat file databases are considered to be an obsolete, inefficient storage manner for interpreting, querying editing and updating data. Many database management systems offer the option to export data to a comma or tab delimited file, for example in order to export data to Microsoft Excel™. This type of file contains no inherent information about the data and interpretation requires additional knowledge. For this reason, this type of file can be referred to as a flat file. [118-120]

- Hierarchical Database: Organizational Model for data. This is the oldest organizational database model. It was used during the mainframe era of the 1950 and 1960's. The hierarchical model uses multipl,e single-tree, parent-child relationships but is not able to cope with linking between branches or over multiple layers. As a consequence it is inefficient, while some legacy systems may exist, it is not in widespread use today.[118-120]

- Relational Databases: This is the most widespread model on which most commercial systems are based. The concept of relational databases was first outlined in a paper published by Codd (IBM) in 1969.[120] The model is based on set theory and predicate logic and provides a high level of abstraction. [118-120]

  A relational database is a set of tables containing data fitted into predefined categories. Each table (sometimes called a *relation*) contains one or more data categories in columns. Each row contains a unique instance of data (called a primary key) for the categories defined by the columns. [118-120]

  When creating a relational database, the *domain* of possible values in a data column and further *constraints* that may apply to that data value may be defined. For example, a domain of possible customers could allow up to ten possible customer names but be constrained in one table to allowing only three of these customer

names to be specifiable. The definition of a relational database results in a table of metadata or formal descriptions of the tables, columns, domains, and constraints.

Relational databases are relatively easy to create and access and have the important advantage of being easy to extend. After the original database creation, a new data category may be added without requiring that all existing applications be modified. [118-120]

- Object Oriented Databases: Based on the object-oriented philosophy that developed during the late 1970. The first commercially available object oriented DBMS became available in the mid-1980 (Objectivity). Historically, computer programs were written as logical procedures that take input, process, and produces output data. The programming objective was how to write logic, not how to define data. The focus of object-oriented programming is on the objects to be manipulated instead of the logic required to manipulate them. In other words, object-oriented programming (OOP) is organized around "objects" rather than "actions". In object-oriented databases objects are a collection of data items and the operations, which can be executed on them. Users create their own objects and specify how these relate to each other. [118-120]

  Unlike relational databases, object-oriented databases do not have a high level language such as SQL (Structured Query Language). This has the advantage of providing the database programmer with a low level control of the system. [138]

  In object-oriented databases, data is stored as a persistent object, and not as a row in a table making it more efficient in terms of storage space requirements.[138]

- Object Relational Databases: This is a relatively recent development that adds new functionality to relational systems, by allowing data to be manipulated in the form of objects, in additional to providing the traditional relational interface. It is a hybrid of the relational and the object oriented models.[118-120]

  The development of this model occurred because the relational model was not designed for, and able to cope effectively with, the new types of data it is expected to store such as audio, video and image files as well as user defined types. Also, the

increasing use of object-oriented programming languages led to the realization that there is an increasing degree of impedance mismatch in DBMS (database management system) software. Impedance mismatch refers to the difference in the way programmers structure data and the way the database structures it, and the resultant large volume of code required to convert data into a format which can be inserted or retrieved. [118-120]

- Distributed Databases: Model for databases which are spread out over several systems, but which appears to users to be a single system, used where the quantity of information in a database becomes too large for a single system can to cope with all the information that needs to be stored, sorted and queried.

Distributed databases have common characteristics, they are stored on two or more computers, called nodes, and these nodes are connected over a network. They may be homogeneous (use the same DBMS) or heterogeneous (use different DBMS or may integrate legacy systems). Useful for critical systems where one node may mirror another so that if one node crashes, the other can continue to provide access to the database. [118-120]

- Multimedia Databases: Model for storing several different types of file i.e. text, audio, video and images in a single database. Prior to broadband and low-cost storage space this model was not considered feasible. It is now an intricate part of web services. Metadata is assigned to each file that provides information about the file the information it contains and its location. [118-120]

- Network Databases: A Network Database, does not refer to the fact that the database is stored on a network, but to the way data is linked and organised to other data in a network of linked records. It is an early form of database quickly superseded by relational databases and is no longer used. The Network database model was first introduced in 1971 by CODASYL Data Base Task Group was also referred to as the DBTG Model. It is considered a contemporary of the relational model, both in terms of its age and its basis in research done in the 1960. Network

databases structure data in the form of a network of records and sets which are related to each other, forming a network of links. To do this it uses records, record types and set types. Network database are only used in legacy systems. [118-120]

2.6.1.2 SQL-Structured Query Language

SQL is an ANSI standard computer language for accessing and manipulating databases. SQL is utilised by the DBMS to access, define, and manipulate data in Oracle, DB2, Sybase, Informix, Microsoft SQL Server, Microsoft Access, MYSQL and almost every other database systems.[121]

Codd, an IBM researcher first published the original concept behind relational databases in 1969. IBM proceeded to develop the first prototype of a relational database in 1974 known as System R. The Query Language was named SEQUEL (Structured English Query Language). It was quickly renamed SQL due to trademark difficulties. SQL was developed as a fully functional DBMS to manage IBM commercial products based on System R. The first SQL/DS was released in 1981.[121]

However, despite IBM performing almost all of the research, the first RDBMS was released two years previously in 1979 by Relational Software (later to be known as Oracle).

SQL became the official standard through the American National Standards Institute (ANSI) certification in 1986 (X3.135-1986) the International Standards Organization (ISO 9075-1987). The standards have continually been updated (SQL-1999) The existence of SQL standards have been crucial in ensuring that SQL is widely supported by almost every DBMS for communication. [118-121]

2.6.1.3 Oracle 8i Database Management System.

The DBMS utilised for the development of the VPS is ORACLE™ 8*i* Database Enterprise Edition. This is installed on a separate disc local to the VPS Web server. ORACLE™ *8i* has a nested architecture described in Figure 2.10. It consists of a kernel, data dictionary, and SQL layer. The kernel exploits the facilities of the host operating system, in this case UNIX, in executing internal code for handling and optimising communication with the database such as data input and retrieval. [122]

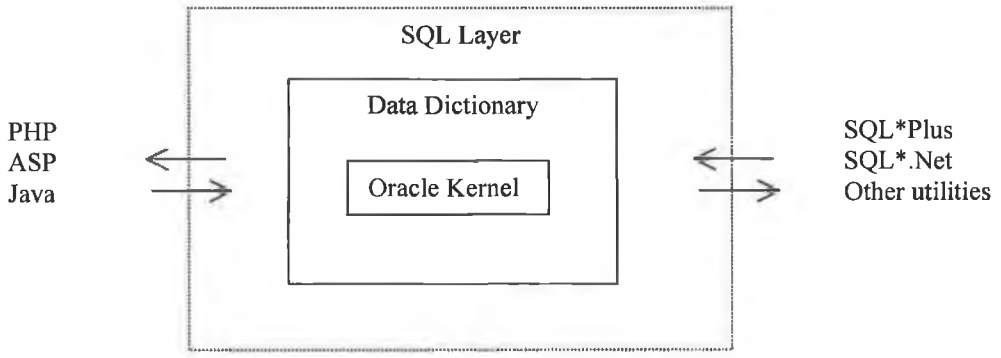


Figure 2.10 Schematic Illustrating Nested Architecture of ORACLE™ *8i* Web Database. [122]

The Data dictionary is meta data (data describing data). The Data Dictionary manages and documents ORACLE™ objects such as the schema for tables, usernames, passwords, and permissions. The SQL layer provides a communication interface for passing instructions and requests between the kernel and external layers supported by ORACLE™ *8i* such as PHP, Java or ASP. There are a number of methodologies for external client layers to connect via SQL to ORACLE™ *8i,* such as ODBC (Open DataBase Connectivity, a Microsoft Windows standard for accessing different database systems). PHP has in-built functions for interacting with ORACLE™ called OCI functions (ORACLE™ Call Interface). Using these functions, PHP scripts perform the following sequence of events interacting with the database:

- Connect with ORACLE™, pass an SQL instruction
- Return the result.
- Perform any outstanding database transaction
- Close the connection.

2.6.2 Data Recorded by VPS Database:

There are three specific data types recorded by the VPS.

- *System configuration data*-This consists of data submitted by a client browser to the VPS server as they interact with the VPS. This includes parameters such as client browser version, OS screen resolution, screen colour depth and IP address.
- *Client tracking data*-This data describes client "movements" as a pathologist examines a series of pathology images using the VPS. This data set is sufficiently comprehensive to replay the online examination of a VPS pathology slide by a client, in real time.
- *User submitted data*- This is data submitted to the VPS server by pathologists, using simple HTML forms.

2.6.3 Additional Variables Passed From The Client To The Server.

Bandwidth is a measurement of the maximum amount of data that can be transferred across a network over time. The higher the bandwidth, the faster pathology images download and the better the user experience of the telepathology system. The cost of broadband and its limited availability have traditionally been a barrier to the acceptance of telepathology (Bamford). Broadband refers to an increased ability to rapidly transfer large volumes of data across the network. It requires a high speed connection such as xDSL or ISDN[77] between the client and the ISP (Internet Service Provider). Broadband is a practical necessity for implementing a telepathology service. It is becoming increasing available in developed countries at affordable costs making telepathology a viable option. However, in remote regions and poorer countries where the benefits of telepathology were first envisaged and could truly be realised, broadband availability remains elusive, both in terms of cost and technical availability.

The VPS was developed with an ability to monitor a user's bandwidth when downloading images from the VPS server. Using client-server communication, the file size of each image sent to the client, in conjunction with the time taken to download the image, is recorded. This data is used to calculate the rate of data transfer per second between the server and the client. This is commonly referred to as "bandwidth" and is a

descriptor of the performance of the network connection between the client and the server. When performing the calculation, data is discounted where the client has viewed an image more than once as the image may reside on the client's cache. The time spent viewing a set of images is also passed from the server to the client in order to determine the amount of time a user spent examining a field of view, as distinct to the amount of time a user spent downloading a field of view.

2.6.4 *Configuration of VPS Data Archive:*

The data described above is written to an Oracle™ database, on the VPS server, using PHP. The architecture of the VPS data archive is structured in a format that mirrors the sequential steps pathologists undertake when registering with the VPS website, logging on, examining a slide and submitting a report. This sequence of events is illustrated in Figure 2.11

The database is composed of seven tables. A detailed description of the database structure is tabulated in Table 2.2.1-2.2.6.

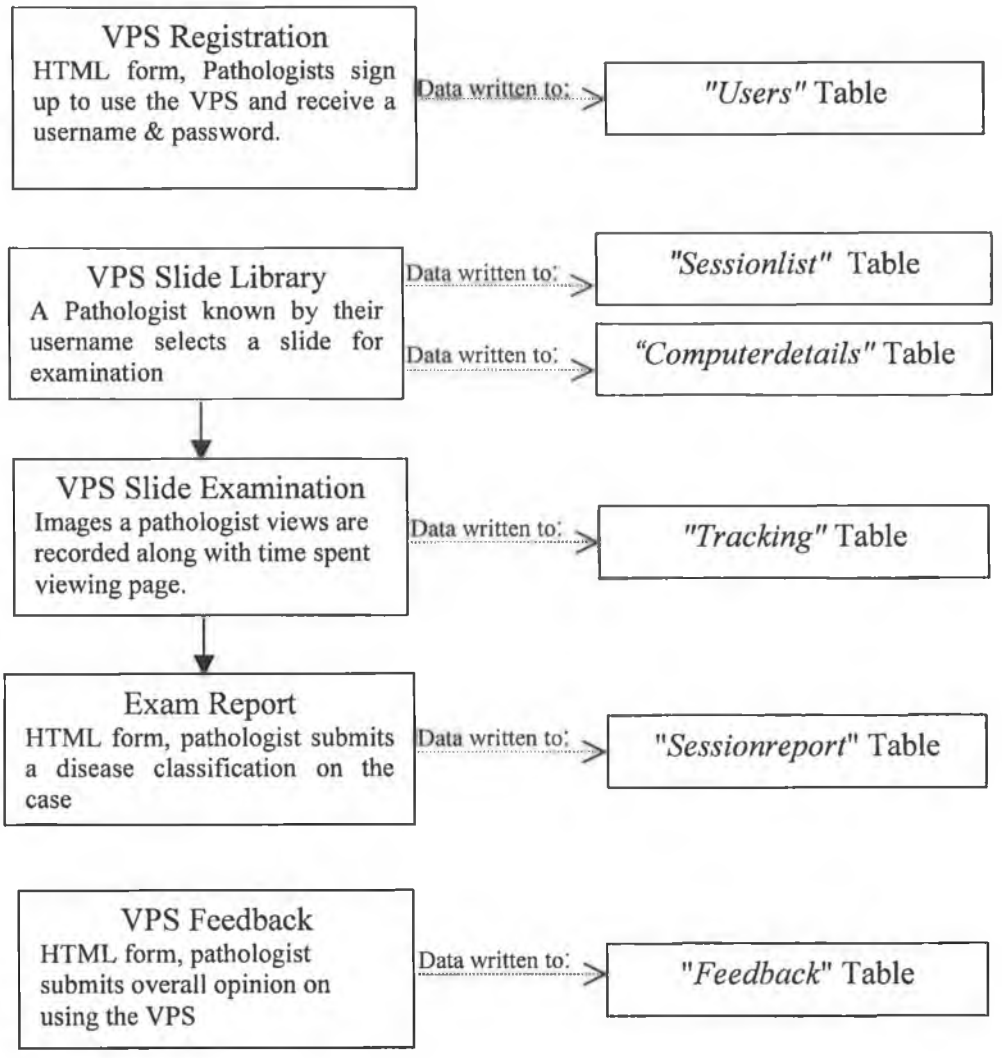


Figure 2.11 Schematic Indicating Origin Of Data Stored in VPS Database

Table 2.2.1 Structural Content of VPS Oracle Database For Table Name USERS

Data is submitted from a user registration HTML form. Each submission contains the following fields.

Column Number	Column Name	Data Type	Length	Description
0	IDNUM	CHAR	5	Unique number assigned to each user.
1	LOGDATE	CHAR	20	Date and Time of Registration.
2	EMAIL	CHAR	40	E-mail Address.
3	EXPERIENCE	CHAR	20	Number of years of experience in pathology.
4	USERNAME	CHAR	16	Username.
5	PASSWORD	CHAR	16	Password.
6	IPNO	CHAR	20	Internet Protocol Address describing the users domain name server(DNS).
7	PROFILE	VARCHAR	1	Number assigned to user by DB Administrator that assign permission to user to access study.
8	FIRSTNAME	CHAR	30	First name.
9	LASTNAME	CHAR	30	Last name.
10	ADDRESS	CHAR	200	Address.
11	PHONE	CHAR	30	Telephone Number.
12	FAX	CHAR	30	Fax Number.

Table 2.2.2 Structural Content of VPS Oracle Database For Table Name SESSIONLIST

Upon logging in using their username and password, a pathologist selects a slide for examination. This results in the following data specific to the slide examination being written to the database.

Column Number	Column Name	Data Type	Length	Description
0	IDNUM	CHAR	5	User ID Number.
1	SLIDENO	CHAR	5	Slide number user has selected for examination.
2	LOGTIME	CHAR	10	Time.
3	LOGDATE	CHAR	15	Date.
4	SESSNO	CHAR	5	Number unique to a particular slide examination.
5	IPNUM	CHAR	15	Internet Protocol Address describing the users domain name server(DNS).
6	SCREENW	CHAR	4	User's Screen Width.
7	SCREENH	CHAR	4	User's Screen Height.
8	IMAGSRC	CHAR	5	Indicates whether User is downloading images for Internet or from CD (value is 'WEB or 'CD'')
9	STUDY	CHAR	5	Value to indicate slide set users is examining. Value is '0' for breast needle core study.

Table 2.2.3 Structural Content of VPS Oracle Database For Table Name TRACKING

Upon selecting a slide for examination, a "*Tracking" table* is created and named after the session number. The table contains a log of each move within a slide examination and records the following parameters.

Column Number	Column Name	Data Type	Length	Description
0	SESSNO	NUMBER	22	Number unique to a particular slide examination.
1	MAGLEVEL	NUMBER	22	Magnification User is viewing
2	SLIDEX	CHAR	5	X –coordinate used in conjunction with SLIDEY to describe field of view user is viewing
3	SLIDEY	CHAR	5	Y–coordinate used in conjunction with SLIDEX to describe field of view user is viewing
4	BUTTON	CHAR	2	Record of button User clicked e.g. zoomin and zoomout,
5	DOWNLOAD	NUMBER	22	Amount of data downloaded (Bytes)
6	TIMETAKEN	NUMBER	22	Time taken to download a field of view
7	TIME	CHAR	10	VPS Server Time
8	TIMEVIEW	NUMBER	22	Time spent viewing a field of view
9	SLPRESS	CHAR	5	Record of lateral scroll of button User clicked. Up, Down Left, Right etc.
10	UCOMMENT	CHAR	500	Record of User's comments for a particular field of view.
11	IPNO	CHAR	20	Internet Protocol Address describing the Users domain name server (DNS).

Table 2.2.4 Structural Content of VPS Oracle Database For Table Name SESSIONREPORT

Upon completing a slide examination, the User submits a HTML form the contents of which are recorded in the table below.

Column Number	Column Name	Data Type	Length	Description
0	IDNUM	CHAR	5	User ID Number.
1	SESSNO	CHAR	5	Number unique to a particular slide examination.
2	DSPEED	CHAR	20	Discrete rating of image quality by user for a particular slide
3	IMAGEQ	CHAR	20	Discrete rating of image quality by user for a particular slide
4	UCOMMENT	CHAR	500	User's comment on image quality for a particular slide
5	CLASSIFICATION	CHAR	20	Diagnostic grade submitted by User for a particular slide examination
6	SUBCLASS	CHAR	20	Record of sub classification of a malignant classification into invasive or in-situ
7	DIAGNOSIS	CHAR	1500	Diagnostic comments submitted by User for a particular slide examination
8	IPNO	CHAR	20	Internet Protocol Address describing the User's domain name server (DNS).
9	REEXAMINATION	VARCHAR	3	Value indicating whether the user has submitted a report for a particular slide more than once.

Table 2.2.5 Structural Content of VPS Oracle Database For Table Name COMPUTERDETAILS

Data Submitted when a User select a slide for examination for the first time.

Column Number	Column Name	Data Type	Length	Description
0	USERID	CHAR	5	Unique number assigned to each user.
1	IPNO	CHAR	20	Internet Protocol Address describing the users domain name server (DNS).
2	SCREENH	CHAR	5	Users screen resolution width.
3	SCREENW	CHAR	5	Users screen resolution height.
4	OSBROWSER	CHAR	200	Users Operating System & browser version.
5	COLORSIZE	CHAR	5	Users Screen Colour Depth

Table 2.2.6 Structural Content of VPS Oracle Database For Table Name FEEDBACK

Contains data submitted from an online questionnaire that was circulated to Users after they had used the VPS.

Column Number	Column Name	Data Type	Length	Description
0	IDNUM	CHAR	5	User, ID Number.
1	COMPETENCE	CHAR	30	Users self-evaluation of computer competency. Contains the following discrete values. 'Poor' 'Adequate' 'Competent' 'Advanced'.
2	FREQUENCY	CHAR	30	User,'s self-evaluation of how frequently they use a telepathology system. Contains the following discrete values. 'Never', 'Infrequently', 'Monthly', 'Daily'.
3	IMAGEQ	CHAR	30	User's overall evaluation of the quality of VPS digital images: Contains the following discrete values. 'Poor', 'Adequate', 'Good', 'Excellent'
4	PREPQUAL	CHAR	30	User's overall evaluation of the quality of VPS slide preparation contains the following discrete values. 'Poor', 'Adequate', 'Good', 'Excellent'.
5	DSPEED	CHAR	30	User's overall evaluation of the download speed of VPS. Contains the following discrete values. 'Very Slow', 'Adequate', 'Good', 'Very Fast'.
6	EASEUSE	CHAR	30	Please indicate the ease of use of VPS. Contains the following discrete values. 'Very difficult', 'Difficult', 'Easy', 'Very Easy,.
7	CONFIDENCE	CHAR	30	User's self-evaluation of their confidence in making a diagnostic classification using the VPS. Contains the following discrete values. 'Not Confident', 'Reasonably Confident', 'Confident', 'Very Confident'.
8	WHYNOT	CHAR	1000	User's response to question 'If you were not confident in using the VPS to make a diagnostic classification please indicate why?'
9	IMPROVE	CHAR	1000	User's response to question 'What aspects of the VPS would you like to see improved?'.
10	UCOMMENT	CHAR	1000	User's response to question 'Additional comments?'

2.6.5 Examination Time:

When a user selects a slide for examination a server timestamp is recorded in the 'sessionlist table' of the VPS oracle database. When a user submits a summary report form following a slide for examination a server timestamp is recorded in the 'sessionreport' table of the VPS oracle database. The duration of a slide examination can be determined by subtracting the 'sessionreport' timestamp interval from the 'summaryreport' timestamp.

The above methodology, does not however, provide a true reflection of the amount of time spent by a user examining tissue, in that it includes the data transmission time from when the client sends a request to the server, to when all the images are downloaded. If for example, a user had a slow internet connection, they may only view a few fields of view however, their examination time may be equivalent to that of a user with a fast internet connection who had examined several fields of view.

Another scenario may entail a user examining a slide, halting their examination to answer the phone or for some other distraction, and then recommencing their examination.

In order to determine that actual amount of time spent viewing tissue during an examination an alternative method was also implemented. Each time a new filed of view is displayed to a user on their monitor a timer is started. When the user clicks a button on the VPS to select another field of view, the timer stops, the data is sent to the server and logged in the 'tracking' table of the VPS oracle database in addition to a server timestamp.

The JavaScript timer function that starts and stops the timer is 'called' by a "Body OnLoad" event handler and an "OnClick" event handler respectively. The "Body OnLoad" event handler calls the timer function only when the entire page has been rendered on the clients monitor. The "OnClick" event handler calls the timer function when the user clicks a button or hyperlinked image. Consequently the measured time only represents time spent viewing tissue and does not include data transmission time. The sum of each time in the 'tracking' table for a particular slide examination (session number) is equal to the actual time spent viewing tissue for that slide examination.

Using the above method, it was intended to perform the following analysis.

- Comparative analysis of examination time versus diagnostic performance to determine whether individuals with high diagnostic agreement spent longer examining tissue than individuals with low diagnostic agreement.

- To determine whether examinations times for a slide could be related to the diagnostic difficulty (level of consensus agreement amongst participants) of that slide.

During the course of the study documented in this thesis, it was noticed by the author that the time recorded for examining tissue appeared erroneous for some moves as the value recorded exceeded the time interval between the server timestamps recorded for the subsequent move. Further testing revealed that on some occasions the OnLoad event handler did not call the timer function correctly, on other occasions an excessively large value was passed to the database for recording. An example of such observed inconsistency for session number 690 as represented in the database is shown below.

Move	Server Timestamp(hrs:min:s)	Time examining Tissue(s)
2	20:13:36	1039202288
3	20:13:43	3
4	20:13:49	2

The error in measuring time spent examining tissue was not detected during the testing phase of this project. It is felt by the author that the reason why such a crucial 'bug' went unnoticed is largely due to testing having occurred using a broadband connection. This belief is supported by the results of a subsequent testing phase where suspicious values could not be reproduced using a high-speed internet connection, however, using a 56kb/s modem the unreliability of the timing system became more apparent.

Given the obvious corruption of some portions of the timing data it was decided by the author to omit analysis of the timing data. The portion of the application that records the time spent by a user examining tissue has been rewritten in subsequent versions of the VPS and thoroughly tested to ensure correct time measurement is recorded.

2.6 User Anonymity:

Upon registration, every user is assigned a unique user identity number (IDNUM). When examining a VPS slide, it is this number that is associated with the data that is recorded in the VPS database. This preserves their anonymity and ensures any data submitted to the server can be stored in the strictest confidence.

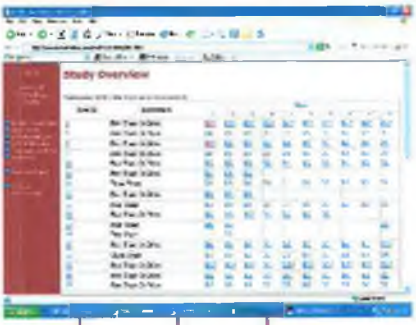
2.7 Data Retrieval:

In order to monitor user's interaction with the VPS and perform statistical analysis of recorded data, a password protected PHP driven website was developed. The PHP script dynamically writes SQL queries that retrieve requested information from the database.

Figure 2.12 illustrates the folder tree-like structure of the website, while Figures 2.13-2.16 illustrate screenshots of the Study Overview Page, and the three pages directly available for the Study Overview Page including a Users Profile Page, a Slide Profile Page and a Slide Examination Profile Page.

In addition to retrieving data from the database a number of database administrator WebPages were created to edit table contents, for example, to delete slide examination data during VPS testing and development.

Hyperlinked Overview of Details of All The Users, Slides, and Slide Examinations.



Details About A Particular Slide

Details About A Particular User

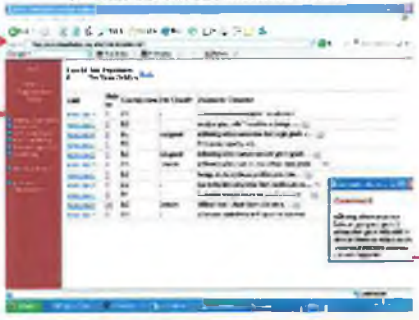
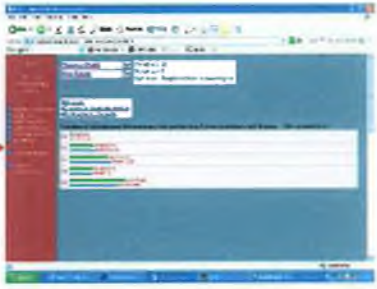


Figure 2.12 Sitemap of Data Retrieval Web Pages

Comparison of Diagnostic Classification Between All Users.

Comparison of Diagnostic Classification Between Users With Greater Than 5 Years Experience.



Diagnostic Classification Between Users With Greater Than 5 Years Experience and All Users.

Details About A Particular Slide Examinatio Including:

- Users Computer Specification
- Moves Details (How Man Zoom, Zoom Out, Latera scrolls)
- Time Details (Duration o examination and between moves
- Area Viewed Details (Are

Click On Any Slide Number To Obtain Data Recorded For All Examinations of a Particular slide.

Click On Any 'User Id' To Obtain Data Recorded About A Particular User For All Slide Examinations.

Click On Any Diagnostic Grade To Obtain Data Recorded For a Particular Slide Examination.

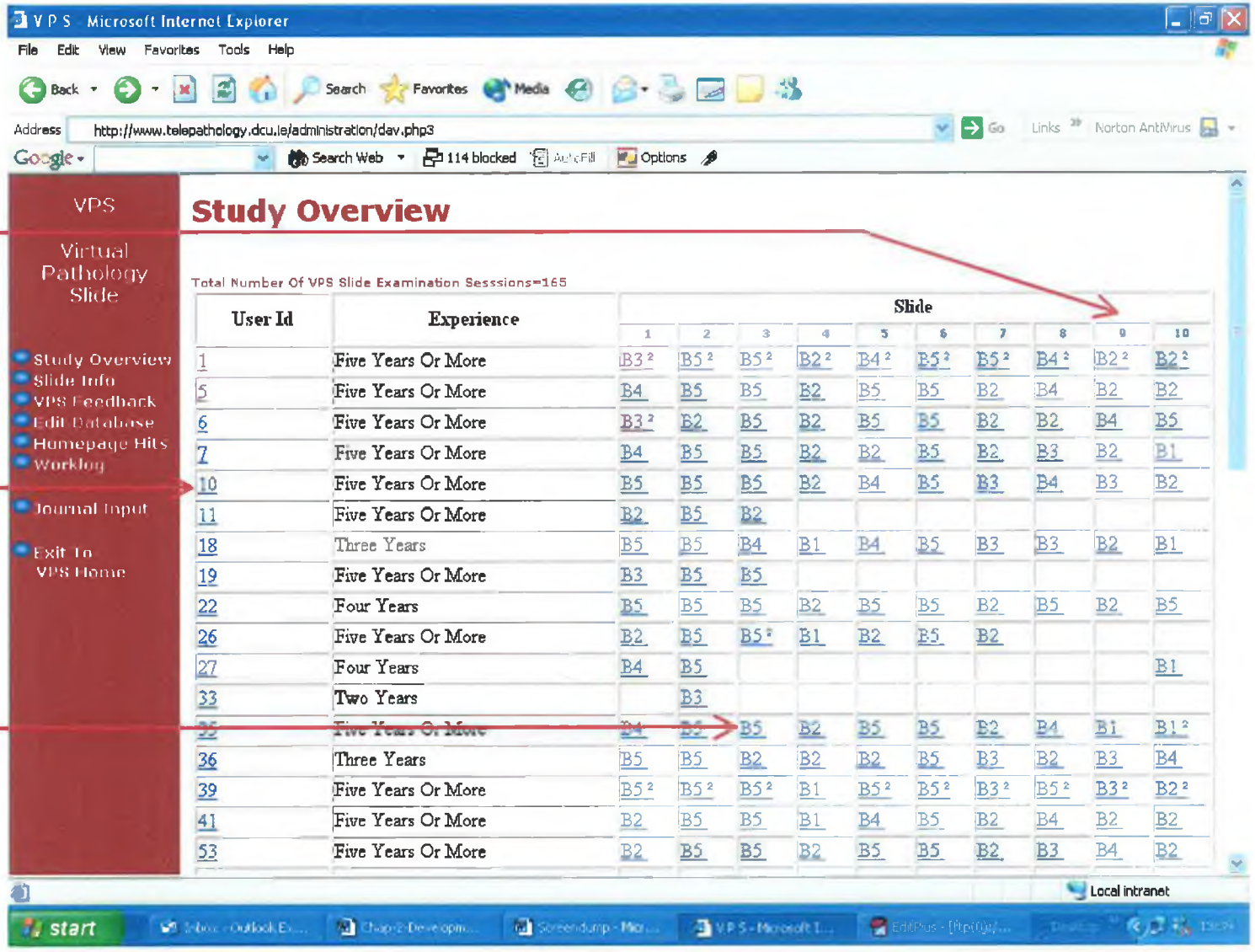


Figure 2.13 Homepage of Data Retrieval Web Site

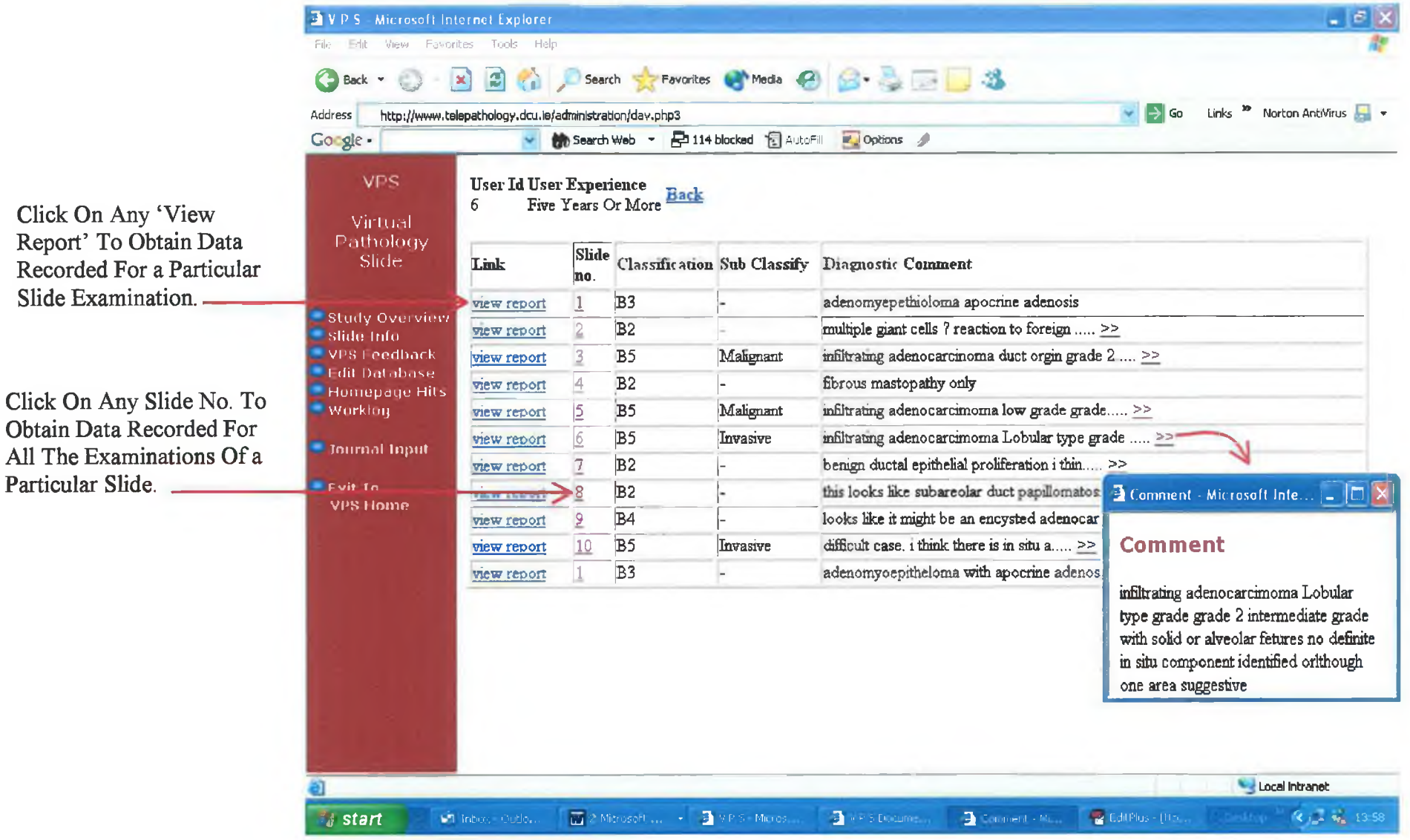


Figure 2.14 Record of Examinations Submitted by a Particular User-Linked Directly From Homepage of Data Retrieval Web Site

Click To View Comparative Analysis Graphs Between Different Users For a Particular Slide

Click On Any 'View Report' To Obtain Data Recorded For a Particular Slide Examination.

Click On Any 'User Id' To Obtain Data Recorded About A Particular User For All Slide Examinations.

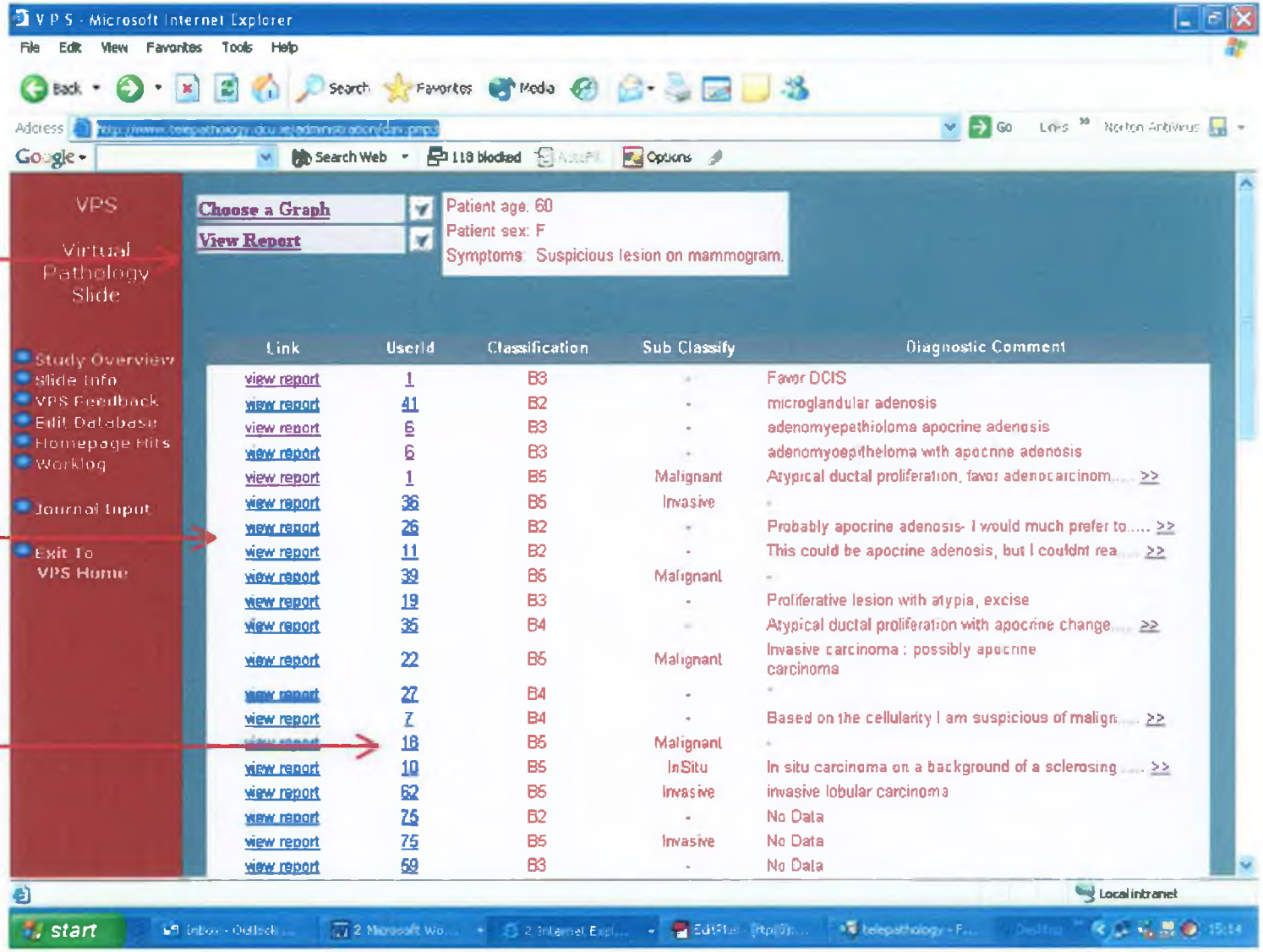


Figure 2.15 Record of All Examinations Submitted For a Particular Slide-Linked Directly From Homepage of Data Retrieval Web Site.

Click To View Specific Data Pertaining To A Particular Slide Examination

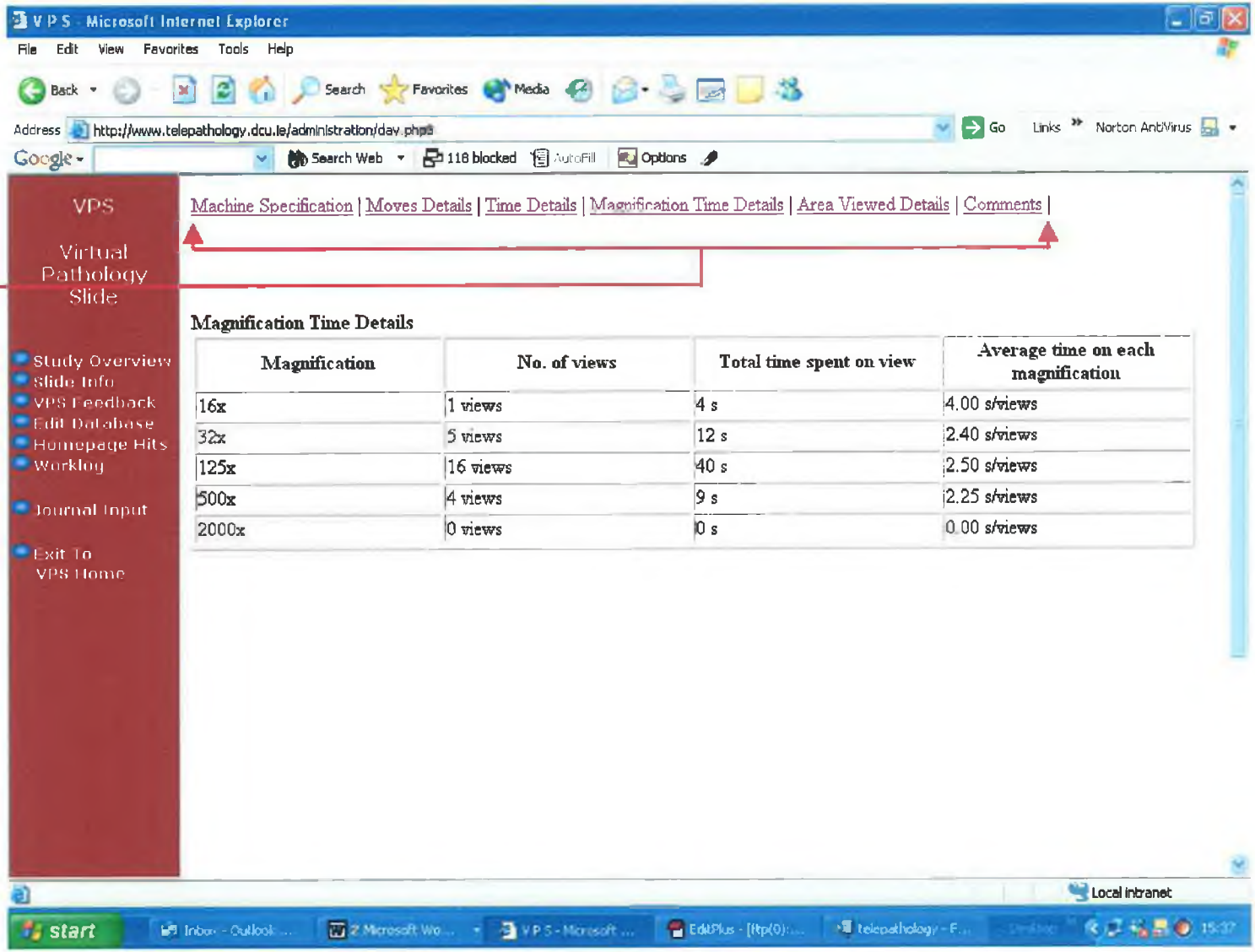


Figure 2.16 Record of a Particular Slide Examination-Linked Directly From Homepage of Data Retrieval Web Site

2.8 VPS Deployment

2.8.1 VPS Customised Browser

The VPS customised browser was developed to control access for users during VPS dedicated studies, optimise the integrity of recorded data and to provide a uniform experience for users who would otherwise experience subtle differences due to the wide number of web browser versions currently in existence.

Constructed using Visual C++ application wizard, the VPS Customised Browser is a Microsoft Foundation Class (MFC) application that utilises Internet Explorer file libraries in order to behave as a customised browser. The VPS Customised Browser opens up a prescribed webpage on the VPS server. The VPS browser application is optimised for PC users with Internet Explorer 5. [140-141] The VPS browser application may be downloaded from www.telepathology.dcu.ie/vpsbrowser.exe

2.8.2 VPS CD-ROM

Broadband Internet connectivity is currently still unavailable to many pathologists. This is currently a major limiting factor for acceptability of the web-driven telepathology due to the time taken to download large number of images over the Internet. In order to improve user's experience, the VPS interface has been adapted to download images locally from a VPS-CD, if one is present in the CD-ROM drive. Otherwise images are downloaded from the VPS server.

2.9 Conclusion

The advent of virtual slides represents a significant breakthrough in the manner in which pathologists share information. Prior to such developments, static images or photomicrographs of a single field of view at a single magnification was the sole method to widely distribute information contained on a glass slide. The virtual slide has functionality similar to that of a conventional microscope, in terms of being able to zoom and move laterally to regions of interest. However, it also maintains distinct advantages over that of using conventional microscopes. In addition to the ubiquitous nature of virtual slides, they also provide the ability to view macro overviews of tissue sections not possible using the traditional microscope. This has been likened to being able to 'back-off and view the forest in addition to the trees'. [142] However, perhaps the greatest advantage in using virtual slides lies not with the image itself but data associated with the image.

Telepathology systems have been developed that provide varying degrees of information when an image is viewed such as the stain, organ, orientation, some patient data pertinent to diagnosis and the diagnosis itself. However, the capability to exploit the possibilities of appending information to an image remains largely unexploited in conventional telepathology, in comparison to the realm of bioinformatics and tissue micro array analysis where high throughput molecular and immunohistochemical screening has demanded an efficient data management strategy. The three most established virtual side vendors in the US all offer software for tissue scoring of TMAs by multiple users and image annotations with more mainstream bioinformatics companies offering data and image management software for different types of image analysis.

The development of such technologies has become increasingly affordable with decreasing storage costs and the availability of open source enterprise level platforms such as MySQL database management system. Furthermore, the application of XML (extensible mark-up language) to describe histology and tissue related data has added a huge impetus to the development of such discovery tools, not only by permitting various applications to exchange data but also by allowing computer and life scientists alike, to finally communicate in a manner comprehended by both parties.

It has been argued that the widespread diffusion of telepathology in pathology practice has been stunted by limitations of the technology itself. However, it is the advances in information technology, which are now driving changes in pathology in terms of high throughput screening, increased automation, and improved accuracy and quality of diagnosis.

The VPS exploits recent advances in database driven web technology in its ability to present users with an image of a pathology slide and allow the user to examine a slide by changing magnification and scrolling laterally. The VPS design also achieved the ability to record a user's diagnostic trace on a database. The VPS system is reliable, robust and scalable in terms of the number of slides it can present and the number of slide examinations it can record. The development of the VPS represents the beginning of a new chapter in pathology informatics, quality assurance and teaching.

Chapter 3:- The Virtual Pathology Slide (VPS) Evaluation Study.

3.1 Introduction

User perception and acceptance of telepathology has been the mitigating factor in determining the success of telepathology. Its success has also been measured using numerous descriptors such as cost benefit analysis, whether the user is reimbursed by the system due to reduced workload, increased throughput or increased efficiency. However, the diagnostic accuracy of a telepathology system, in comparison to using a conventional microscope, has consistently concerned pathologists and is generally the yardstick by which the performance of a system is described.

The simplest form of telepathology, static telepathology, has become a common tool for second opinion diagnosis, despite the inherent presence of sampling bias. The services offered by AIFP iPath and UICC are a tribute to its success and its acceptance amongst pathologists as a means of providing expert second opinion diagnosis. However, the gradual acceptance of this technology has been shadowed by a continual debate amongst pathologists as to what constitutes an acceptable level of diagnostic accuracy. Over the years, comparison studies between glass slides and still images have yielded a wide ranging consensus between 52% and 96%.[153] However recent studies have produced very high consensus suggesting improving technology standards or increasing expertise and experience by pathologists in selecting images for referral and in making a diagnosis using a digital image.

Using a static images system, Della Mea *et al* (2000)[73] conducted a study involving 184 individual cases (frozen sections (60), gastrointestinal pathology (64), and urinary cytology (60)) and demonstrated a diagnostic accuracy of between 90-100%. Demichelis *et al*(2001)[119] achieved comparable results by achieving 98.6% concordance. They used a static image based system that permitted full remote control by the consultant pathologist. Molnar *et al* (2003)[135] performed a study using virtual slides involving 103 gastrointestinal biopsy specimens achieving 94.1% consensus. Conversely Nordrum *et al* (2004)[26] achieved a relatively poor consensus having obtained 29 discordant diagnosis out of 90 in a study on static images. However, they remained strongly supportive of the practice of using still images in obtaining second opinion diagnosis and referred to the 'observer variability in diagnostic histopathology

in general' to partly explain their discordance. Of interest is a recent study involving static images of immunofluorescence images conducted by Lanschuetzer *et al* (2004).[153] Consensus was reached in 14 out of 17 cases (82%). The use of immunofluorescent images is an unusual departure for a telepathology study given the technical difficulties in acquiring a fluorescent image of diagnostic quality and perhaps represents a coming of age of technology.

Despite increasing availability of broadband, uptake in the use of robotic dynamic telepathology has remained poor. Equipment costs are prohibitive and examination times are longer than using a conventional microscope. Sampling bias is not an issue with dynamic telepathology, therefore, one would expect to observe a radical rise in diagnostic accuracy when using dynamic telepathology, in comparison to that of using static telepathology. This is not the case. The level and range of values reported is very similar. A robotic dynamic telepathology study involving forty-seven fine needle aspiration smears, reported by Singh *et al* (2002)[53] showed a diagnostic accuracy of 80.9%. Singh *et al* also emphasised the 'operational and technical problems that made remote diagnosis tedious and lengthy'. On the other hand, Kaplan *et al* (2002)[54] performed a dynamic telepathology study involving 120 frozen cases and achieved 100% diagnosis consensus.

To date, there are few reports on the diagnostic accuracy of virtual slides. This is because virtual slides are relatively new. While the role of virtual slides in quality assurance has been suggested by some, recent reports on the use of virtual slides are concerned predominantly with its application as an educational tool rather than for primary or secondary diagnosis, where concerns as to the applicability of the technology lies not with diagnostic accuracy but with the ability of the virtual slide to illustrate the nuances of anatomical histopathology.[38,39,40]

While the diagnostic accuracy of telepathology systems is high and considered by most authors as sufficiently acceptable to replace conventional means, there is considerable inconsistency in results between studies. Given the subjective nature of microscopic diagnosis, this is to be expected. However, this inconsistency highlights the multivariate nature of implementing a telepathology service and demonstrates that no

two telepathology services are identical, even if the same equipment is used. The attitudes, experiences, and specialisms of pathologists amongst telepathology 'groups' varies as does the IT infrastructure. Ultimately, before a telepathology service is commissioned, there is a requirement for it to be evaluated in order to instil confidence and credibility amongst its users.

From the programmer's perspective, evaluation is an important part of the software development cycle. It provides an opportunity to determine whether the software produced actually realises the end-user's expectations and affords end-users the chance to identify flaws and contribute recommendations to the development team.

In order to determine the diagnostic accuracy, usefulness and acceptance of the VPS, an evaluation study was conducted in which a group of pathologists were asked to examine and categorise 10 breast needle core biopsy slides. The primarily object of the evaluation study was to determine:

- Can users make a correct diagnostic decision using the VPS?
- Are users confident making a diagnostic decision using the VPS?
- Do users feel that the image quality presented by the VPS is of sufficient quality?
- Are users expectations met with respect to the functionality/navigability of the system?

The study also provided an opportunity to assess unique features of the VPS such the VPS user tracking system. Diagnostic trace data from the study was used to quantify and analyse reasons for the occurrence of inter-observer and intra-observer variation amongst pathologists.[125-128]

The study involving the VPS specifically used breast tissue, a suitable choice for the measurement of inter-observer variability. Inconsistency by pathologists in the diagnostic classification of proliferative breast lesions into specific diagnostic categories is well recognised. Lack of agreement can occur between pathologists (inter-observer variability) and for the same pathologist at different times (intra-observer

variability). There is no accepted standard classification for such lesions. As such, "false negative" and "false positive" diagnoses cannot be defined. As a consequence, sensitivity (true-positive rate) and specificity (true-negative rate) measures have not been developed to describe the accuracy of histological diagnosis. Instead, diagnostic agreement, and diagnostic reproducibility or consistency has been described in terms of the percent agreement or concordance of diagnostic categorisations. This method of reproducibility does not require comparison with a standard. However; some studies have reported the percent of diagnoses that agree with the majority diagnosis, or with the diagnosis made by an "expert panel" This is usually referred to as consensus agreement.[99]

The Kappa statistic can be used to determine if the observed percent concordance is greater than would be expected to occur by chance. The value of Kappa ranges from 0 to 1; a Kappa greater than 0.75 denotes excellent reproducibility, Kappa between 0.4 and 0.75 denotes good reproducibility, and Kappa less than 0.4 denotes marginal reproducibility.[129-133]

With respect to histopathology quality assurance studies, Kappa statistics are popular for two reasons. Firstly, Kappa values may be used as a test to indicate that there is no more agreement between two observers than would ordinarily occur by chance. Secondly, Kappa values may be used to quantify the 'level of agreement' between two observers. [129-133]

There is some disquiet in the use of Kappa statistics as a chance corrected method to quantify the 'level of agreement'. Ubersax (1987)[132] reported that low Kappa ratings may be achieved even though individual ratings are accurate, Kappa is considered an omnibus index of agreement in that it does not distinguish between different sources of agreement. However, Kappa statistics are easily calculated, widely understood and software is readily available. Kappa statistics remain the statistic of choice as a measure of inter-observer variation.

3.1 Breast Cancer – "The Nature of The Beast"

The female breasts are modified sweat glands composed of lobes and lobules interspersed with adipose tissue and connective tissue. Ducts drain from each lobule. These converge to form a lactiferous duct that drains from each lobe. The lactiferous ducts merge just beneath the nipple to form a lactiferous sinus.

The functional secretion unit in lactation is the terminal duct lobular unit. Each duct has a lining of epithelium surrounded by a thin myoepithelial cell layer responsive to oxytocin, the hormone that stimulates lactation.

Neoplasms or tumours may arise in the ductular epithelium, lobules, or the stroma. However, the majority of cancers arise in the ducts.

Breast cancer is rarely diagnosed in women younger than age 25. The incidence rises steadily to reach a peak around the age of menopause. The rate of increase is lessened after menopause, however older women are still at increasing risk over time. Male breast cancer is rare, accounting for less than one percent of all diagnosed breast cancers and usually affect men in their sixties or older.

In Europe and North America about 1 in 8 women will develop breast cancer. However, breast cancer is much less common in Asia. While the incidence rate for breast cancer is increasing (in particular the detection of localised cancers) the mortality rate is not, this indicates that early detection is improving patient prognosis.

While a specific cause for breast cancer has not been identified, there are risk factors that increase the likelihood for breast cancer development. These risks include: Maternal relative with breast cancer, previous cancer history, obesity and fat intake, nulliparity or later age at first pregnancy, are all known to increase the likelihood of breast cancer.

The expression of BRCA1 and BRCA2 genes may explain some familial cases, and contributes to the etiology for about 1% of breast cancers.

Fibrocystic changes such as atypical epithelial hyperplasia are usually benign breast "lumps" the presence of such atypical changes increases the risk of malignancy.

Arguably the best method for detection of breast abnormalities is self-examination or routine physical examination performed by a health care worker. However, a breast cancer may have been present for 5 to 10 years before reaching a size (approximately 1 cm) that is detectable by palpation.

The most sensitive and specific method to detect breast cancer is mammography. This screening and diagnostic technique can detect masses that are not palpable, because carcinomas generally have a density greater than the surrounding breast tissue.

The major purpose of a screening mammogram is to separate normal from abnormal findings and to identify patients who need further evaluation. If the patient has an abnormal screening mammogram or signs and symptoms of a breast abnormality, then a diagnostic mammogram is performed.

Following detection of an abnormality by palpation and/or by mammography, a tissue sample can be obtained.

For small lumps that are not clearly cancers, a procedure called "fine needle aspiration" (FNA) or a wide bore needle core biopsy is performed. The cells taken from a needle core biopsy are placed on glass slides, stained to highlight the cells, and then examined by a pathologist. The pathologist tries to determine if malignant cells are present.

Usually this initially involves examining the slide at low power magnification in a manner similar to a raster scan pattern. Based on the types and patterns of cells on the slide, the structures of the breast (e.g. ducts, lobules) that are present may be identified. It is frequently possible to determine the region of the breast the tissue specimen was removed from.

Depending on the degree and extent of atypia, hotspots regions where disease may be present can be identified. These hotspots are examined at higher magnification from which a diagnostic decision can be made. The type of carcinoma present will be determined by the area and structure of the breast that is affected and the degree of differentiation. Carcinomas can be invasive (extending into the surrounding stroma) or non-invasive (confined to the ducts or lobules).

Breast biopsy is performed to remove a lesion and make a definitive diagnosis, if a malignancy has not been demonstrated by needle core biopsy but is still suspected (due to, for example, insufficient sampling), or if a lump is likely to be malignant. Such a biopsy can be done under local or general anesthesia. The biopsy can also be directed radiographically (sterotactically) by placing a needle and/or coloured dye into the area that is abnormal. The biopsy can be examined by frozen section by the pathologist for a quick, preliminary diagnosis. Usually however, the biopsy is processed routinely, and a diagnosis is made. If a malignancy is found, the biopsy can be further studied via immunohistochemical staining to determine receptor status.

The grade of a cancer refers to how quickly, or aggressively, it is growing. The staging of cancers is an evaluation of the extent of tumour spread. Grade and stage are the most important factors for predicting patient prognosis and optimising treatment. Due to the

wide variety of histological cell types, a uniform system of grading for breast cancers is not possible. The varied cell types, in addition to the invasiveness of the cancer, help predict the biological behaviour of the cancer.

Classification of a breast cancer may also involve determining the hormone receptor status of breast cancer cells. This is useful for deciding on treatment and prognosis. In general, cancers in which the cells express estrogen receptor (ER) in their nuclei will have a better prognosis. They are usually differentiated and are likely to respond to the drug tamoxifen. The receptor status of other hormones such as progesterone may also be used to classify a breast cancer.

Flow cytometry may be used to classify a breast carcinoma. It is a technique that can measure the amount of DNA in the nuclei of a breast carcinoma cell. Normal and benign cells typically have a single homogenous population of cells with a "euploid" DNA content. However, malignant cells are less differentiated and have abnormal "aneuploidy" expression of DNA content. Prognosis can be related to the degree of cellular aneuploidy.

Carcinomas have a propensity to spread via lymphatics. Breast cancers, when they metastasize, often spread initially to the axillary lymph nodes where most lymphatics from the breast drain. Spread of carcinoma to the dermal lymphatics produces a so-called "inflammatory carcinoma" which is a descriptive term, not a histological type. Other organs can be sites of metastases, and such sites as lung, bone, and liver are more common.

Prognosis is considered to be dependent on patient age, stage, grade, tumour type and hormone receptor status. Treatment of breast cancer takes a variety of forms, depending

upon the grade and stage of the cancer as well as the overall health of the patient and the wishes of the patient. Therapy needs to be appropriate for each individual.

A localized carcinoma can be removed completely with local excision (lumpectomy also termed breast conserving surgery or BCS) with margins free of tumour. At the same time sampling of axillary lymph nodes occurs to determine if lymph node metastases are present from which it may be necessary to perform a total mastectomy with removal of the breast.

Depending upon cancer type and hormone receptor status surgical intervention may be combined with radiation therapy and or chemotherapy, Radiation, coupled with BCS, may help to reduce the incidence of a second cancer in the breast when intra-ductal carcinoma is diagnosed. More extensive cancers may be treated with a modified radical mastectomy with removal of the entire breast and axillary lymph nodes. Breast carcinomas that have a higher stage may be amenable to aggressive chemotherapy, coupled with total body radiation and bone marrow transplantation[94].

3.2 Slide Selection

Ten needle core biopsies were obtained from the Department of Pathology, Mater Misericordiae Hospital, Dublin, Ireland. The slides were randomly selected by an experienced pathologist with a special interest in breast pathology. The slides represented a wide range of diagnostic classifications. A profile of the 10 slides utilised in the study are summarised in Table 3.1.

3.3 Study Participants

Fifty-four pathologists with at least 2 years experience in pathology practice registered for the study. Participants were recruited through personal contact and with the

assistance of Professor Peter Dervan, Mater Misercordiae Hospital, Dublin. Of the 54 pathologists recruited, 17 examined all 10 slides and 8 initiated the study but did not complete it. Of the 17 participants who completed the study, 8 were members of the European Working Group of Breast Screening Pathology. Of the 17 participants who examined all 10 slides, 13 subsequently completed a questionnaire on user perception of the VPS. A further 8 participants initiated the study but did not complete it, 3 of when completed the questionnaire. A full break down of study participants in provided in Table 3.2.

Table 3.1 Summary Profile Of The Ten Glass Slides Used in The VPS Validation Study

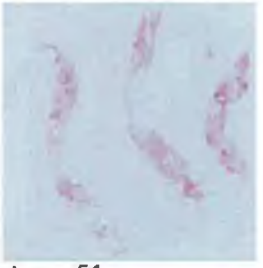
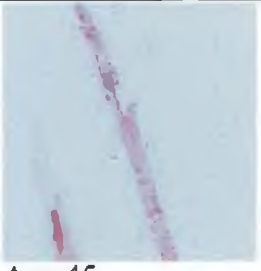
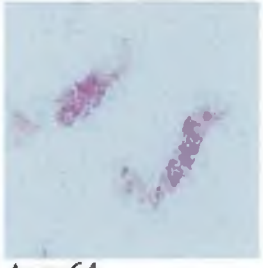
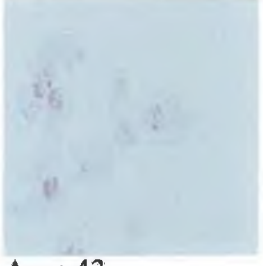
Slide 1	Slide 2	Slide 3	Slide 4	Slide 5
				
Age: 60 **Notes:** Suspicious lesion on mammogram. **Glass Slide Diag:**B5 Invasive Ductal Carcinoma With Apocrine Change	**Age:**56 **Notes**: Lump right breast **Glass Slide Diag:** B5 Invasive Ductal Carcinoma	**Age:** 57 **Notes**: Lump left breast **Glass Slide Diag:** B5 Invasive Ductal Carcinoma	**Age:** 51 **Notes**: Suspicious mammogram. **Glass Slide Diag:** B2	**Age**::67 **Notes**: Lumpectomy 5 years a new lump in same region, Recurrence? **Glass Slide Diag:** B5 Invasive Ductal Carcinoma
Slide 6	**Slide 7**	**Slide 8**	**Slide 9**	**Slide 10**
				
Age:67 **Notes**: Lump right breast. **Glass Slide Diag:** B5 Inv Ductal Carcinoma	**Age:**45 **Notes**: Ill-defined lump right breast, previous cytology suspicious for malignancy **Glass Slide Diag:** B2 Benign Usual Epithelial Hyperplasia	**Age:**64 **Notes**: Ultrasound shows cyst with solid component in wall **Glass Slide Diag:** B3 Intraduct Papilloma	**Age:**64 **Notes**: Ultrasound shows cyst with solid component in wall **Glass Slide Diag:** B2	**Age:** 43 **Notes**: Micro-calcification on mammogram **Glass Slide Diag:** B2 Fibrocystic Change

Table 3.2 Summary Profile of 17 Participants Examined All Ten Slides

No.	User ID	Geographical Location	Experience	Examined all Slides?	Completed Questionnaire?
1	1	USA	>5 Years	√	√
2	5	Ireland	>5 Years	√	√
3	6	Ireland	>5 Years	X	√
4	7	Ireland	>5 Years	√	√
5	8	Ireland	2 Years	X	√
6	10	Ireland	>5 Years	√	√
7	11	Ireland	>5 Years	X	√
8	18	Ireland	3 Years	√	√
9	19	Ireland	>5 Years	X	√
10	22	Ireland	4 Years	√	√
11	35	Ireland	>5 Years	√	√
12	36	Ireland	3 Years	√	√
13	39	Ireland	>5 Years	√	√
14	41	Ireland	>5 Years	√	√
15	55	Italy	>5 Years	√	√
16	62	Finland	>5 Years	√	X
17	65	Portugal	>5 Years	√	X
18	68	Denmark	>5 Years	√	X
19	75	Hungary	>5 Years	√	X
20	87	UK	>5 Years	√	√

3.4 Examination Procedure

Upon launching the VPS browser, participants were prompted to log in using the username and password they received at registration. This made them identifiable to the system. On successful log-in, the VPS needle core examination guidelines were displayed[124]. Figure 3.1 illustrates the process by which participants logged in, examined the ten slides and subsequently completed a user perception questionnaire.

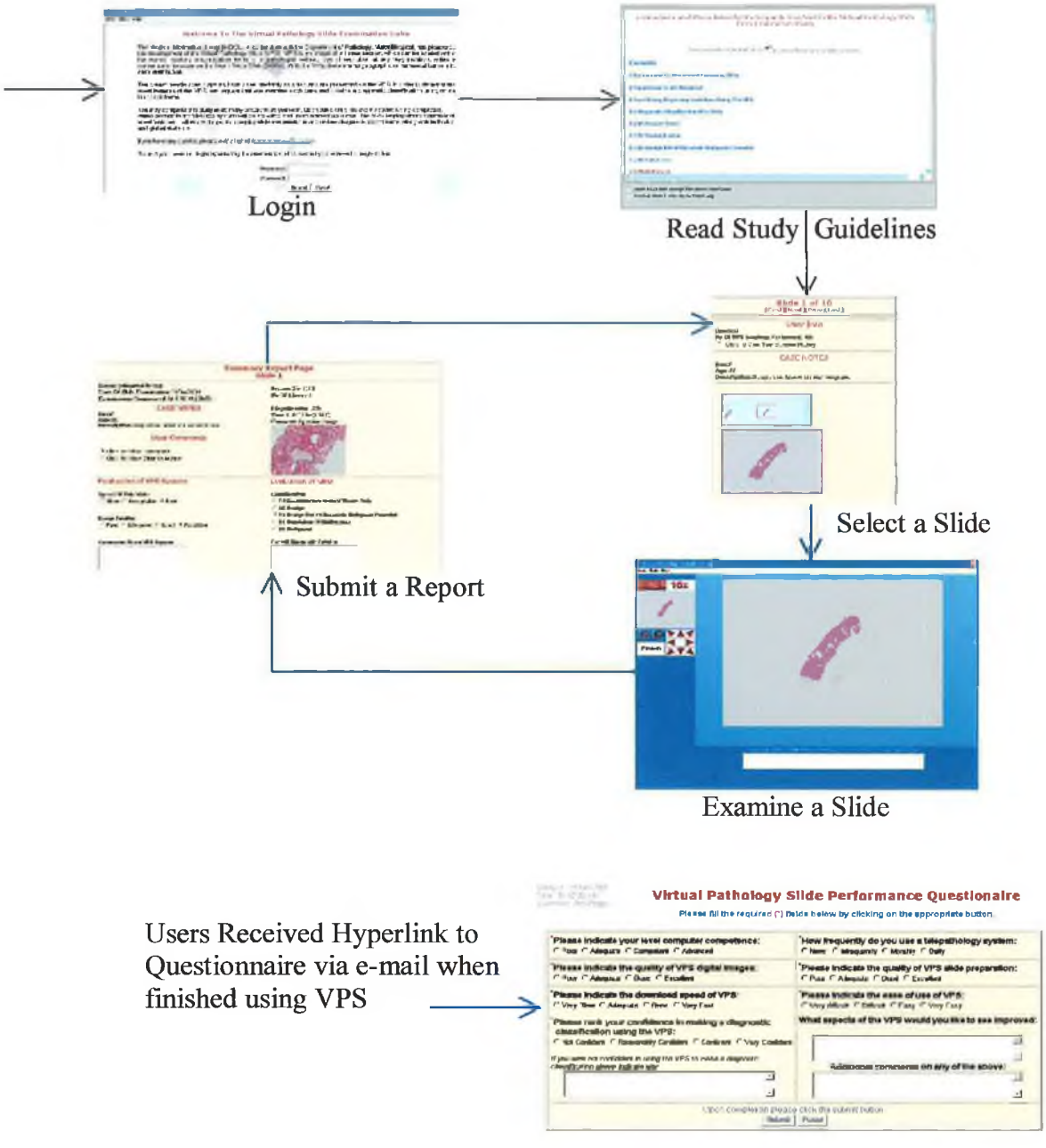


Figure 3.1 Screen Shots of Slide Gallery, VPS Interface, Summary Report Form and User Questionnaire. Figure Depicts The Navigation Process Users Underwent When Examining All 10 Slides.

After stating that they had read the guidelines, participants were permitted to browse the slides available for examination and select one from a slide gallery. The slide gallery displayed a thumbnail image of each slide and indicated the patient's age and sex, and a brief case description. A screen dump of the slide gallery is depicted in Figure 3.2.

Figure 3.2 Slide Gallery Used To Allow Study Participants Browse and Select A Slide For Examination. Participants Were Instructed to Select A Slide By Clicking On A Thumbnail.

Upon selecting a slide for examination, participants were presented with the VPS user interface (Figure 2.4). While examining a slide, participants could, if desired, annotate the fields of view using the text area provided. Upon completing a slide examination, participants submitted an online report that provided a diagnostic classification for the

case, using an adaptation of the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology[96]. The study guidelines as presented to Users are included in Appendix 1. Users were requested to classify the slides as one of the following:

B1: Unsatisfactory/normal tissue only.

B2: Benign.

B3: Benign but of uncertain malignant potential.

B4: Suspicious of malignancy.

B5: Malignant.

For slides categorized as B5, participants were required to sub classify their decision as malignant, in-situ, or invasive. Upon making a classification, participants were returned to the slide gallery from which another slide could be selected for examination. A screen dump of the report page submitted by participants is depicted in Figure 3.3.

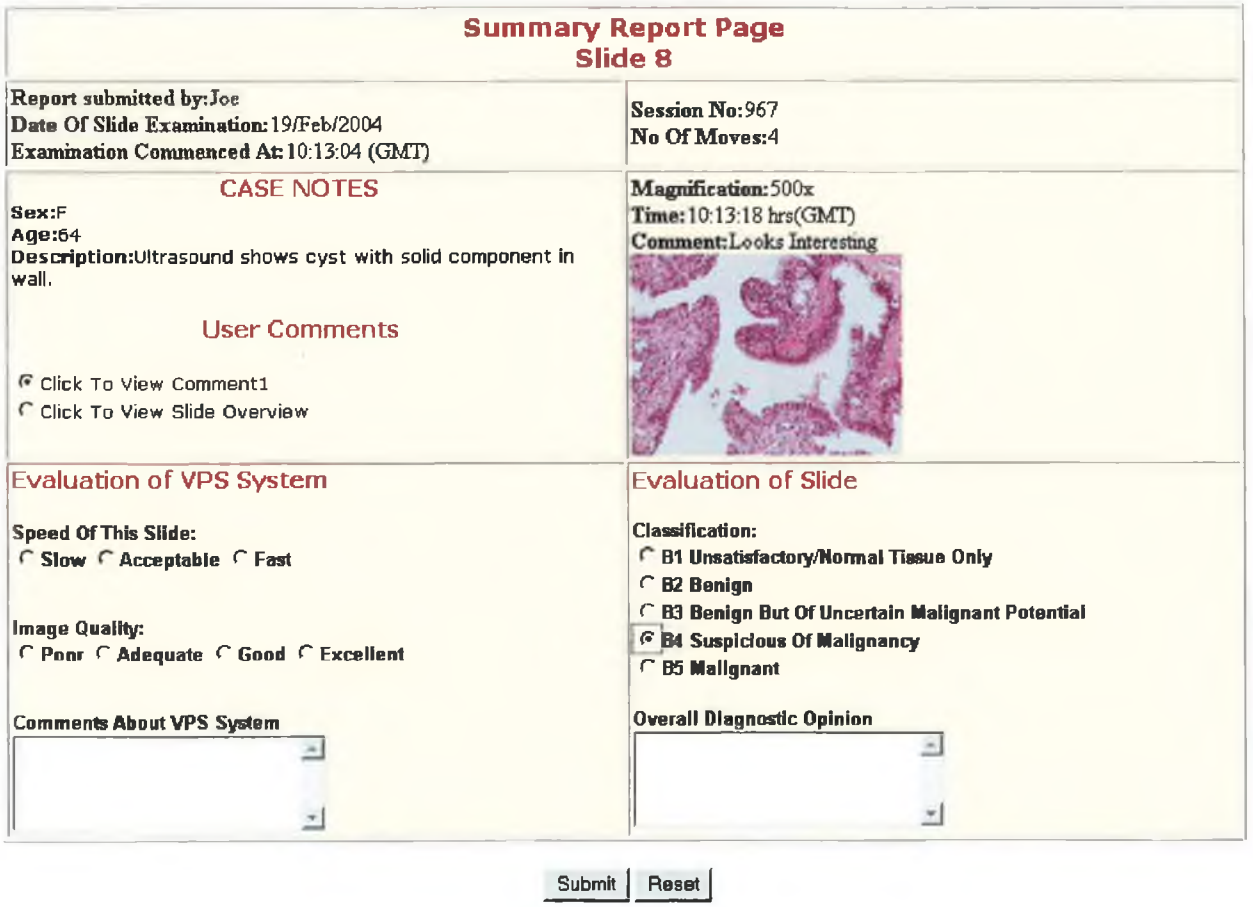


Figure 3.3 Example of Summary Report Page Used by Participants to Submit A Diagnostic Classification on A Slide

Utilization of this data submitted using the Summary Report Form allowed the following to be determined:

- Percentage concordance for a user, calculated as the number of slides (expressed as a percentage) for which the user's diagnosis is in agreement with the consensus VPS diagnosis.
- Percentage concordance of a slide, calculated as the percentage of users who concur as to the correct diagnosis of a slide.
- Cohen's Kappa[126-140], a measure of agreement between observers taking into account agreement that could occur by chance. Kappa values range from 0 to 1 with a score greater than 0.7 indicating "substantial agreement."

3.5 Calculation of Individual Kappa Score

With respect to the VPS validation study, Kappa provides a measure of the degree to which two observers concur in their respective sorting of 10 slides into 5 mutually exclusive diagnostic categories. In this context, one of the observers, consists of the VPS consensus diagnosis for the set of 10 slides.

The original and simplest version of kappa is the un-weighted kappa coefficient (Cohen 1960) [129]

$$\text{Kappa} = \frac{\text{Observed Agreement - Chance Agreement}}{\text{Total Observed-Chance Agreement}} \quad \textbf{Eqn 3.1}^{134}$$

- ***Kappa*** is 1 when there is perfect agreement between the classification systems.
- ***Kappa*** is 0 when there is no agreement better than would occur by chance.
- ***Kappa*** is negative when agreement is worse than would occur by chance.

The level of interobserver agreement between two observers may be interpreted from their Kappa value as follows.

Table 3.3 Relationship between Kappa values and level of agreement.[133]

Value of kappa	Strength of Agreement
<0.20	Poor
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.8	Good
0.81-1.00	Excellent

To calculate Kappa, a contingency table is constructed for each participants against the consensus diagnosis (Table 3.4). This is a two-dimensional table that compares the number of observations of a participant for each diagnostic category, to that of the consensus agreement. It illustrates the number of disagreements between the participant and the consensus and the degree to which they disagree.[130-134]

Table 3.4 Example of Contingency Table Between Consensus Agreement For Each Slide and Participant 5.

	Consensus					
User 5	**B1**	**B2**	**B3**	**B4**	**B5**	
B1	**0**	0	0	0	0	**0**
B2	0	**4**	0	0	0	**4**
B3	0	0	**0**	0	0	**0**
B4	0	0	0	**1**	**1**	**2**
B5	0	0	0	0	**4**	**4**
	0	**4**	**0**	**1**	**5**	**10**

Using the contingency table illustrated in Table 3.4, the probability of chance agreement between Participant 5 and consensus for each diagnostic category is calculated, from which the overall probability of chance agreement is determined (38%).

Given that Participant 5 achieved 90% agreement with consensus, and that there was a 38% probability of this occurring by chance. An unweighted Kappa for Participant 5 may then be calculated using Eqn 3.1.

Table 3.5 Determination of Kappa for Participant 5.

Probability of Chance Agreement for B1	(0x0)	0%
Probability of Chance Agreement for B2	(4x4)	16%
Probability of Chance Agreement for B3	(0x0)	0%
Probability of Chance Agreement for B4	(1x2)	2%
Probability of Chance Agreement for B5	(5x4)	20%
Overall Probability of Chance Agreement		38%
Overall Agreement	(0+4+0+1+4)	90%
Unweighted Kappa =(90-38)/(100-38)		0.83

However where there is greater than two scoring categories, differences in scoring are usually weighted according to the difference between the two observers. The weighting may be set by different methods. Typically the weight is multiplied by the degrees of distance between the two observers. [101]

If there are k categories, then the maximum degree of disagreement is k-1. In the above example there are 5 diagnostic categories, therefore the maximum degree of disagreement is 4. (5-1).

A weight for each degree of disagreement may be calculated using the following formula.

$$Weight = 1 - \left(\frac{i}{k-1}\right) \quad \begin{matrix} Where\ i=5,4,3,2 \\ k=5 \end{matrix} \qquad \textbf{Equation 3.2}^{134}$$

Where there is full agreement, weight is equal to 1. Weight tends to zero as the degree of disagreement increases.

The table below shows that out of the 10 slides, User 5 agreed with consensus diagnosis in 9 out of the 10 slides (Degrees of Disagreement =0). Where the users agreed with consensus, a weight of 1 was applied when calculating the probability of this occurring by chance.

In the one scenario where User 5 diagnostic classification disagreed with consensus by one degree, a weight of 0.75 was applied when calculating the probability of this occurring by chance.

Table 3.6 Tabulated Data For Calculation of Weighted Kappa For User 5

Degrees of Disagreement	User 5	Expected	Weight	Weight x User 5	Weight x Expected
0	9	10	1	9	10
1	1	0	0.75	0.75	0
2	0	0	0.67	0	0
3	0	0	0.5	0	0
4	0	0	0	0	0
Total	10	10		9.75	10

The Overall Probability of Chance Agreement (9.75/10) =0.975

Overall Probability of Agreement (10/10)=1

Kappa =0.975.

3.6 User Performance Using the VPS

One of the most challenging aspects of evaluating a telepathology system is separating the performance of a pathologist examining a slide using the telepathology system, from their performance when examining a glass slide. Table 3.7 shows strong diagnostic agreement between original glass-slide diagnosis and the most-common diagnosis offered by users of the VPS, with agreement being reached in 9 out of the 10 slides. Disagreement by 1 diagnostic degree occurred with slide 8 (glass slide diagnosis was B3; most- common VPS diagnosis was B4). The diagnostic classification of slide 8 had the lowest level of agreement between participants at 38.5%. The second most popular choice for slide 8 was split between B3 and B2, 6 participants (35.3% of users) classified it as B4 while 4 participants (23.5% of users) classified it as B3 and 4 participants (23.5% of users) classified it as B2). Participants with the 4 highest Kappa scores (23.5% of users) classified slide 8 as B4.

Table 3.7 Comparison of glass slide needle core surgical biopsy diagnosis and most-common Virtual Pathology Slide (VPS) diagnosis, in order of level of agreement (concordance) for each slide.

	Virtual Pathology Slide									
	S6	**S2**	**S3**	**S4**	**S7**	**S9**	**S10**	**S1**	**S5**	**S8**
Consensus Diagnosis (Glass)	B5	B5	B5	B2	B2	B2	B2	B5	B5	B3
Consensus Diagnosis (VPS)	B5	B5	B5	B2	B2	B2	B2	B5	B5	B4
% Concordance	100	94.1	82.4	76.5	64.7	58.8	52.9	52.9	47.1	35.3

A more-detailed analysis of the diagnostic classifications made by participants is described in Table 3.8. The average percentage concordance between participants on all cases was 66.5%. Of the 17 participants, 14 attained a percentage concordance of between 90% and 60%. This indicates very strong individual performance using the VPS.

Table 3.8 For each participant: years of experience in pathology practice, diagnostic classification of slides and level of agreement with each other (% concordance and Kappa index).

[1]ID	[2]EXP	Virtual Pathology Slide										[3]Concordance,%	[4]Kappa
		S6	S2	S3	S4	S7	S9	S10	S1	S5	S8		
5	5	B5	B5	B5	B2	B2	B2	B2	B4	B5	B4	90	0.98
62	5	B5	B5	B5	B2	B2	B3	B2	B5	B4	B4	80	0.94
35	5	B5	B5	B5	B2	B2	B1	B1	B4	B5	B4	70	0.94
10	5	B5	B5	B5	B2	B3	B3	B2	B5	B4	B4	70	0.91
39	5	B5	B5	B5	B1	B3	B3	B2	B5	B5	B5	60	0.90
55	5	B5	B5	B5	B2	B2	B2	B2	B5	B3	B3	80	0.87
87	5	B5	B5	B5	B1	B2	B2	B2	B3	B5	B3	70	0.86
18	3	B5	B5	B4	B1	B3	B2	B1	B5	B4	B3	40	0.86
68	5	B5	B5	B5	B2	B3	B2	B2	B5	B4	B2	70	0.85
65	5	B5	B5	B5	B2	B2	B3	B1	B3	B4	B4	60	0.80
22	5	B5	B5	B5	B2	B2	B2	B5	B5	B5	B5	80	0.75
41	5	B5	B5	B5	B1	B2	B2	B2	B2	B4	B4	70	0.75
7	5	B5	B5	B5	B2	B2	B2	B1	B4	B2	B3	60	0.73
1	5	B5	B5	B4	B2	B4	B2	B2	B5	B5	B1	70	0.67
75	5	B5	B5	B5	B2	B2	B2	B5	B4	B5	B2	70	0.65
36	3	B5	B5	B2	B2	B3	B3	B4	B5	B2	B2	40	0.26
6	5	B5	B2	B5	B2	B2	B4	B5	B3	B5	B2	50	0.23
Average												66.5	0.76

1-ID = identification number of participant.
2-EXP = years of experience in pathology practice.
3-Concordance = number of slides (expressed as a percentage) for which the user's diagnosis is in agreement with the consensus Virtual Pathology Slide diagnosis.
4-Kappa = Cohen's Kappa, a measure of agreement between observers, taking into account agreement that could occur by chance. Kappa greater than 0.7 indicates "substantial agreement."

with 10 of the 17 participants displaying excellent agreement with VPS consensus based on Kappa rating. User 5 achieved 90% concordance with consensus VPS diagnosis achieving 0.98 Kappa.

The average Kappa value achieved by participants was 0.76. Participants 36 and 6 achieved a Kappa of 0.26 and 0.23 respectively indicating "fair agreement" with other participants while the remaining 15 participants achieved a Kappa of between 0.97 and 0.65 indicating good (where Kappa ranges from 0.6-0.8) to excellent agreement (where kappa ranges from 0.8-1.0) [97-101]

The average percentage concordance for slides was 66.5% with a minimum concordance of 35.3% for Slide 8 and a maximum concordance of 100% for slide 6. The percentage concordance for slide 5 was 47%. For all remaining slides there was greater than 50% agreement between participants.

The calculation of Kappa was weighted to reflect the degree of variation of a participant's diagnostic decision from the most popular choice. For example, Participant 18 achieved a high Kappa of 0.86 despite being in agreement with other Participants for 4 out of the 10 slides. This is because for each of the other 6 slides, Participant 18 was inconsistent with the consensus categorisation by one degree. Participant 36 achieved the same percentage concordance as participant 18 but only achieved a Kappa of 0.26. This is because the diagnostic categories selected by participant 36 deviated to a greater degree from the popular choice than those selected by participant 18[97-101]. Figure 3.4 illustrates this relationship. Participants 36 and 6 are clearly identifiable as outliers having achieved poor consensus agreement and kappa compared to the rest of the group.

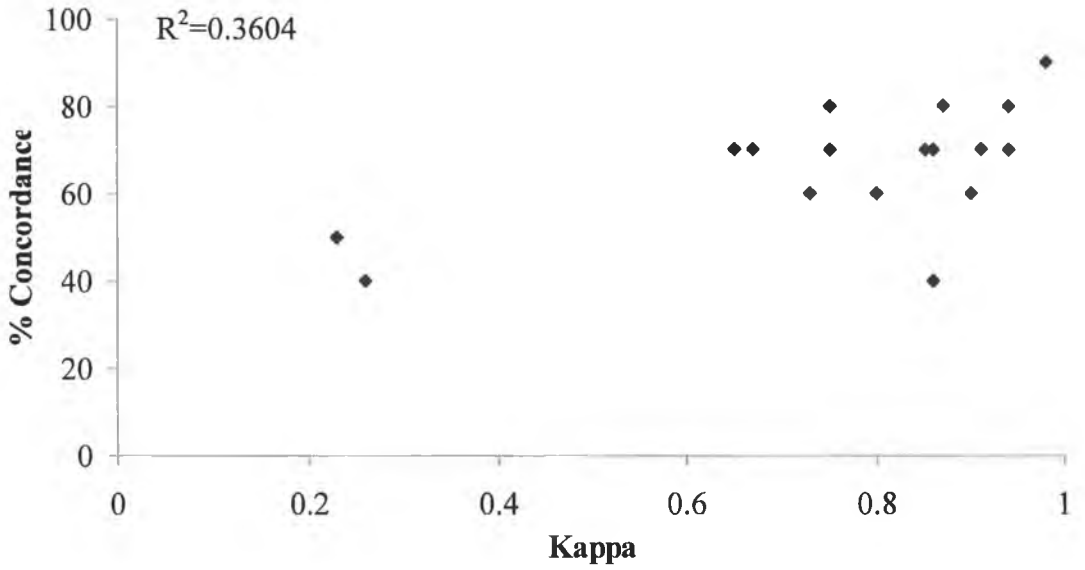


Figure 3.4 Plot of % Concordance VS kappa for each participant: years of experience in pathology practice, diagnostic classification of slides and level of agreement with each other (% concordance and Kappa index).

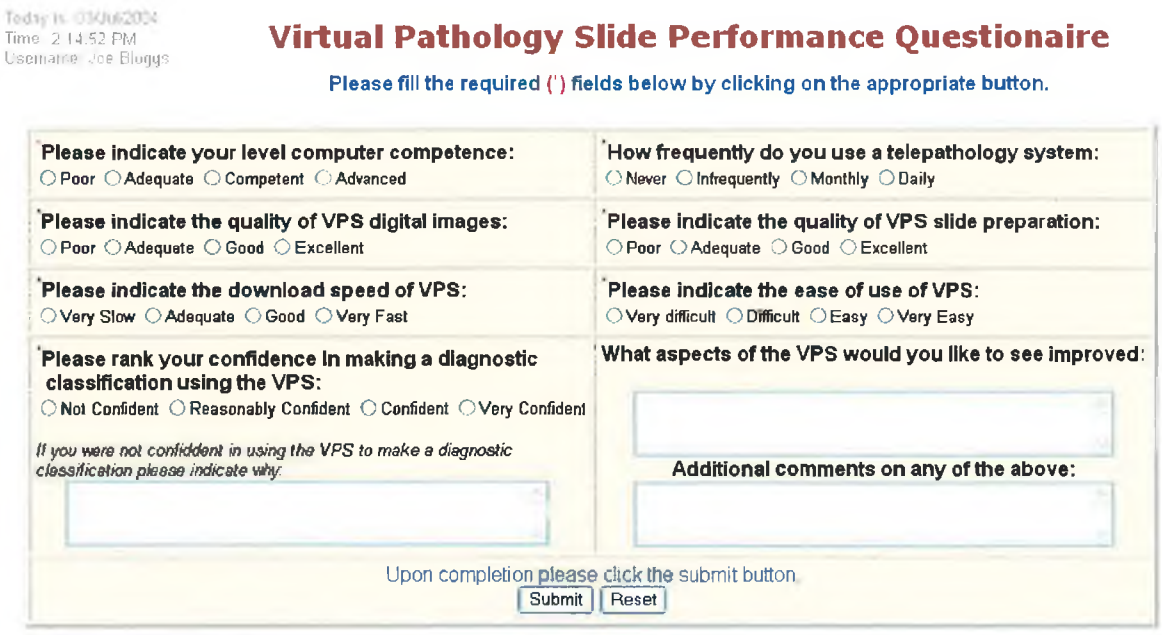
The average percentage concordance for the entire set of slides was 66.5%. Full agreement between participants was achieved for Slide 6. This demonstrates that full agreement can be achieved using the VPS on unambiguous cases.

Slide 8 had the lowest level of concordance at 35.3%. This reduced the average percentage concordance for the set of slides by 3.46%. Table 3.8 shows there is a broad distribution of diagnostic categorization for slide 8 by participants.

Although agreement for Slide 7 was quite high (64.7%) discrepancy in slide classification for this slide had the greatest clinical significance. This is because inconsistency in classification for Slide 7 was split between B2 and B3. A B2 classification would result in no further treatment for the patient whereas a B3 classification would almost certainly result in surgical resection. The consensus diagnosis for Slide 7 was B2. Participants who submitted a B3 classification may represent an over cautious approach.

3.7 User Perception of the VPS

All Participants who used the VPS but did not necessary complete examination of all 10 slides were subsequently requested to complete an online questionnaire describing their experience using the VPS. 13 of the 16 Participants who completed the questionnaire examined all 10 slides. The other 3 who did not complete examination of all 10 slides were not included in the calculation of consensus and kappa statistics. Participants were asked to assess their own computer competency and indicate the frequency with which they use a telepathology system. Participants were also asked to give a subjective evaluation of their diagnostic confidence in using the VPS, reasons for uncertainty, an evaluation of image quality, slide preparation, ease of use and perceived download speed. Figure 3.5 depicts a screen shot of the questionnaire participants were requested to complete.


Today is: 03/06/2004
Time: 2:14:52 PM
Username: Joe Bloggs

Virtual Pathology Slide Performance Questionaire

Please fill the required (*) fields below by clicking on the appropriate button.

*Please indicate your level computer competence:
○ Poor ○ Adequate ○ Competent ○ Advanced

*How frequently do you use a telepathology system:
○ Never ○ Infrequently ○ Monthly ○ Daily

*Please indicate the quality of VPS digital images:
○ Poor ○ Adequate ○ Good ○ Excellent

*Please indicate the quality of VPS slide preparation:
○ Poor ○ Adequate ○ Good ○ Excellent

*Please indicate the download speed of VPS:
○ Very Slow ○ Adequate ○ Good ○ Very Fast

*Please indicate the ease of use of VPS:
○ Very difficult ○ Difficult ○ Easy ○ Very Easy

*Please rank your confidence in making a diagnostic classification using the VPS:
○ Not Confident ○ Reasonably Confident ○ Confident ○ Very Confident

If you were not confiddent in using the VPS to make a diagnostic classification please indicate why:

*What aspects of the VPS would you like to see improved:

Additional comments on any of the above:

Upon completion please click the submit button.
Submit Reset

Figure 3.5 Screen Dump of Questionnaire Completed By Participants

Figure 3.6 illustrates participants self-assessment of their own computer competency. There is a natural human tendency to embellish ones own ability. The age profile of European pathologists would also suggest a poor computer competency. Figure 3.6 depicts participants perception of their abilities, rather than a measure of their actual abilities. However, Figure 3.6 shows that of the participants who completed the questionnaire 18.75% of participants described themselves as "advanced", 18.75% described themselves as "competent" and 62.5% described themselves as "adequately competent" with computers.

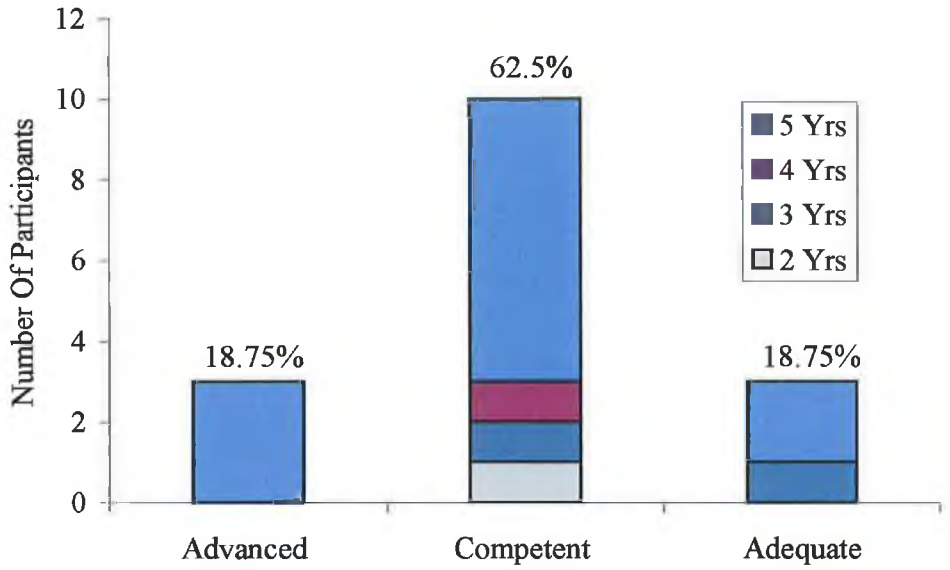


Figure 3.6 Participants self-assessment of competence in IT. Yrs = years of pathology experience. Percentages = percentage of 16 participants for that rating.

Of the participants who completed the questionnaire, 44% indicated they had never used a telepathology system prior to the study. This is indicative of limited success in the diffusion of telepathology throughout Europe. While telepathology may not replace the conventional microscope, many might accept that as systems become more affordable, and broadband becomes more readily available, it will have an increasing role to play in the practice of histopathology.

Figure 3.7 shows that of the participants who completed the questionnaire, 6.25% of Participants use a telepathology system *'daily'*, 18.75% use a telepathology system *'monthly'*, 31.25% use a telepathology system '*infrequently*' while 43.75% have '*never*' used a system. It is probable that the successful adoption of this technology

hinges on its use being incorporated into the training of pathologists. It is therefore disappointing to note from Figure 3.7 that all participants with less than 5 years experience indicated they had never used a telepathology system, except one participant with 3 years experience who uses telepathology monthly.

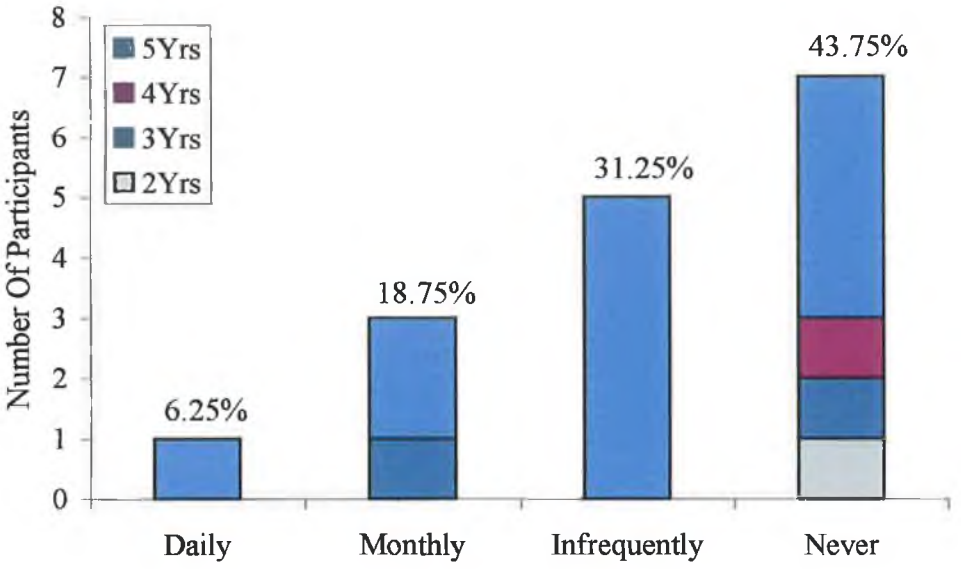


Figure 3.7 Declared Frequency Participants Use a Telepathology System. Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

Images for the VPS were captured using a high quality 40x lens with a high numerical aperture (0.95nm). Participants did not complain about images being out of focus. However, image compression was used and concerns amongst pathologists about the effects of image compression on image quality will remain as long as lossy image file formats are used in telepathology.

While it is possible to remotely determine a participant's screen resolution and colour depth, it is not possible to determine the brightness and contrast setting of their monitor, whether they are viewing the slides on a laptop or flat screen, or whether their screen is exposed to glare while they are viewing the slides.

Figure 3.8 illustrates that 87.5% of participants expressed satisfaction with the image quality with 43.75% indicating the quality as "adequate," 25% as "good," and 18.75% of participants indicating the image quality as "excellent."

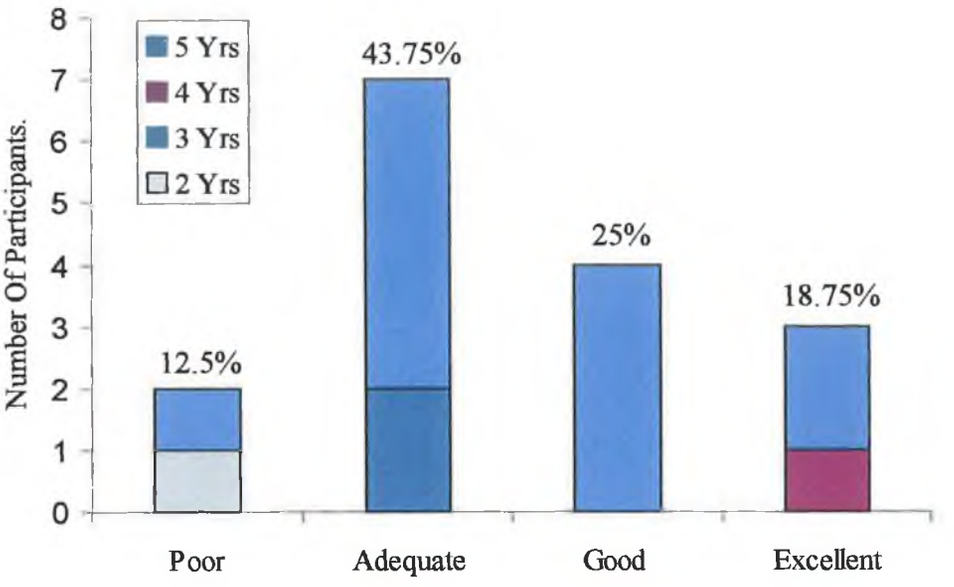


Figure 3.8 Participants Perception of VPS Image Quality. Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

It is difficult to discern whether comments regarding image quality actually referred to the quality of the image or the quality of the slide. The glass slides chosen for this study were not specifically prepared for telepathology. Consequently the quality of their preparation is representative of the scenario encountered by histopathologists every day. It was noted that Slide 2 in particular was badly prepared. Despite this, it achieved the second highest consensus agreement.

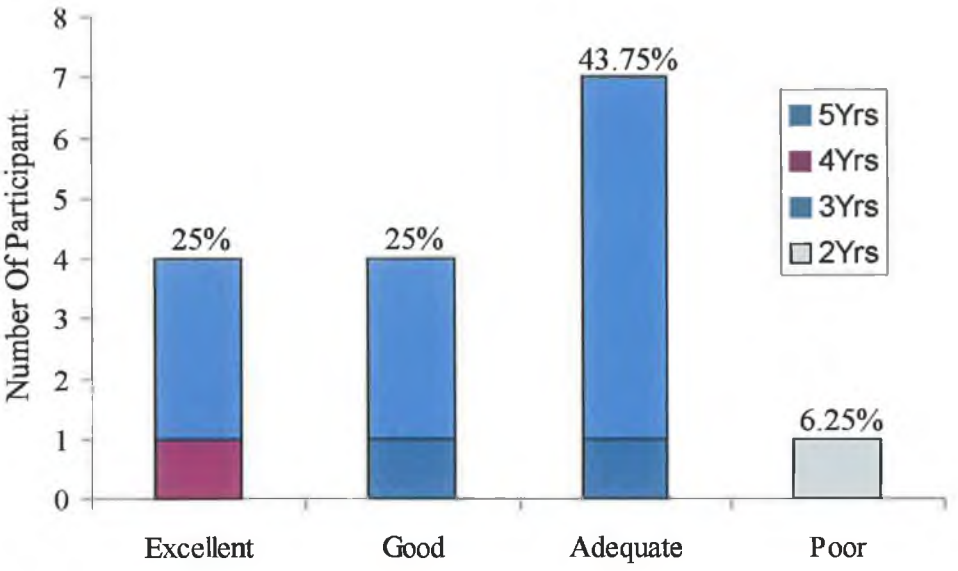


Figure 3.9 Participants Perception Of The Quality of Slide Preparation. Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

Figure 3.9 illustrates that 50% of participants indicated that the quality of slide preparation was *'Good'* or *'Excellent'*, 43.75% described the quality as '*Adequate*' while one individual who had 2 years experience and did not examine all 10 slides rated the quality of slide preparation as poor.

Participants were requested to rate their degree of confidence in making a diagnostic decision using the VPS. In order for a telepathology system to be successful, it is crucial that pathologists using the system have confidence that they can make a diagnostic decision. It is difficult to determine whether a pathologist's lack of confidence in using a system is due to the system itself or the individual pathologist's experience, skill and attitude towards the adoption and use of the technology.

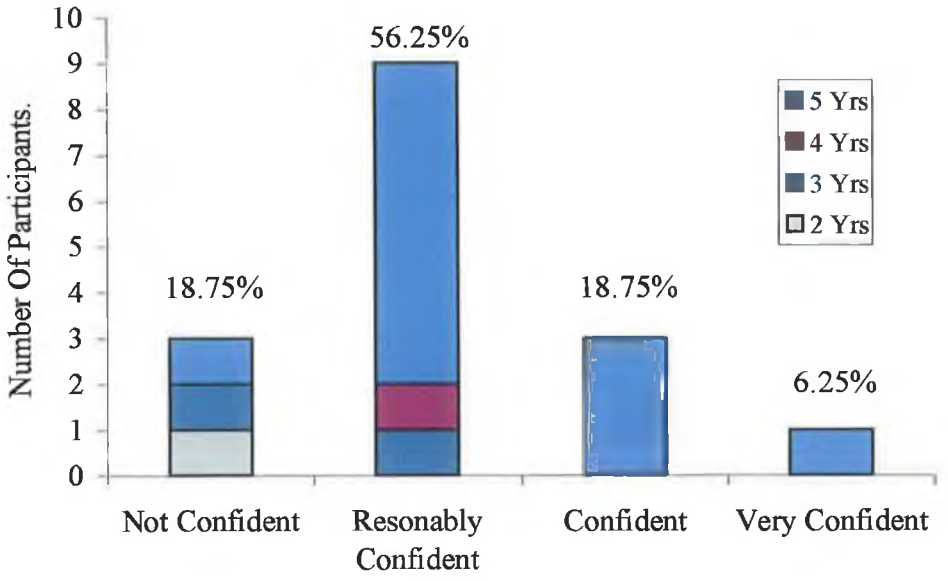


Figure 3.10 Degree of Confidence In Using the Virtual Pathology Slide (VPS) to Make a Diagnostic Decision. Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

Figure 3.10 illustrates that 80.25% of participants expressed confidence in using the VPS with 56.25% indicating they were "reasonably confident," while 18.75% were "confident," and 6.25% were "very confident" in making a diagnosis.

It is of interest that two of the three participants who expressed a lack of confidence in using the VPS to make a diagnostic decision had less than 5 years experience.

Participants were asked to rate their perception of the download speed of the VPS. Broadband has been identified as a limiting factor towards the acceptance of telepathology. However, the increasing availability and decreasing cost will hopefully remove broadband as a barrier to the widespread use of telepathology. How, participants rated download speed in this question is more a measure of participants expectations than an actual measure of their download speed.

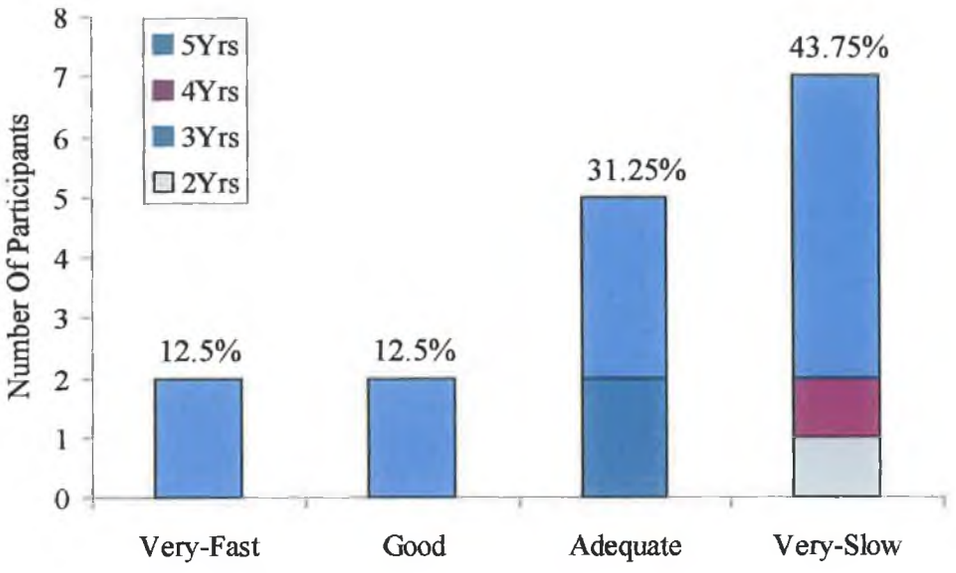


Figure 3.11 Participants Perception Of Download Speed. Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

Figure 3.11 illustrates that 43.75% of Participants who completed the questionnaire perceived the download speed of images used on the VPS as '*Very-Slow*'. 31.25% found the download speed to be *'Adequate'* while 25% of Participants found the download speed to be '*Good*' or '*Very-Fast*'. It is of interest to note that 25% of Participants who perceived the download speed to be slow had less than 5 years experience in pathology. One could postulate that younger pathologists have greater experience of the Internet and broadband than their older colleagues and may therefore have higher expectations of download speeds.

Figure 3.12 illustrates that 68.75% of participants rated the VPS "easy" (62.5%) to use or "very easy" to use (6.25%). This is of interest given the high percentage of participants who have never used a telepathology system prior to using the VPS. Of the two participants who found the VPS *'Very-Difficult'* one had less than two years experience.

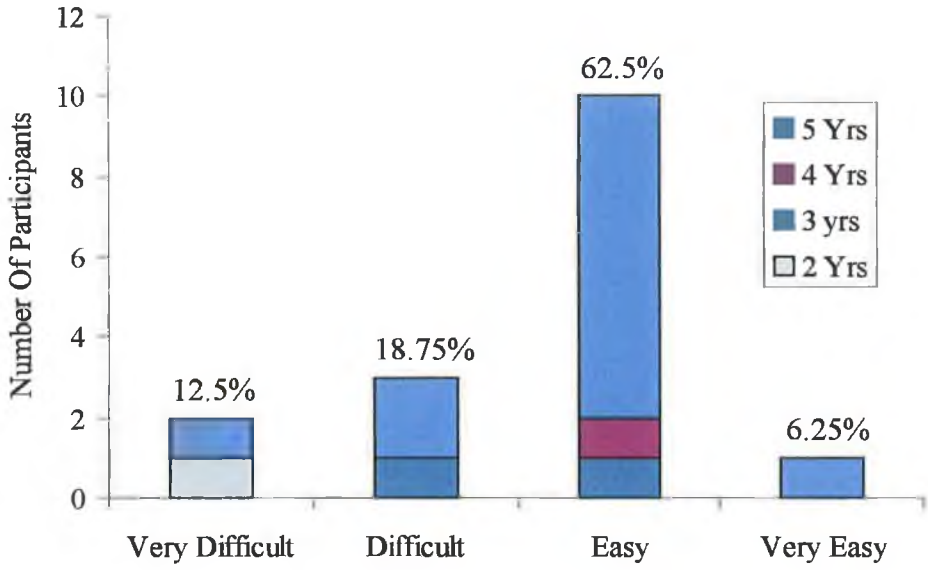


Figure 3.12 Ease of Use of The Virtual Pathology Slide (VPS). Yrs = Years of Pathology Experience. Percentages = Percentage of 16 Participants For That Rating.

Table 3.9-Summary of Users Perception of Image Quality and Diagnostic Confidence vs Performance Based on Percentage Concordance and Kappa, Ranked by Kappa.

User Id Number	Image Quality	Confidence Making a Diagnostic Decision Using The VPS	% Concordance	Kappa
5	Excellent	Very-Confident	90%	0.98
35	Adequate	Reasonably-Confident	70%	0.94
10	Adequate	Reasonably-Confident	70%	0.91
39	Good	Confident	60%	0.9
22	Good	Reasonably-Confident	80%	0.87
55	Poor	Reasonably-Confident	80%	0.87
18	Adequate	Not-Confident	40%	0.86
87	Adequate	Reasonably-Confident	70%	0.86
41	Excellent	Reasonably-Confident	70%	0.75
7	Adequate	Reasonably-Confident	60%	0.73
1	Good	Confident	70%	0.67
36	Adequate	Reasonably-Confident	40%	0.26
6	Excellent	Confident	50%	0.23
8	Poor	Not-Confident	-	-
11	Poor	Not-Confident	-	-
19	Adequate	Reasonably-Confident	-	

Table 3.9 illustrates that Participants confidence is not necessary related to their diagnostic performance. For example Participant 18 indicated that they were '*Not-Confident'* however they achieved a Kappa score of 0.86. Conversely, Participants 36 and 6 indicated they were '*Reasonably-Confident*' and '*Confident*' respectively. Table 3.9 also illustrates that while some participants such as Participant 55 indicated image quality as '*Poor*' this did not affect their performance having achieved a Kappa of 0.87. Table 3.10 details comments submitted by participants who were not confident using the VPS. Table 3.11 details additional comments submitted by participants on any aspects of the VPS that they would like to see improved. There were three recurring issues evident from the VPS users comments: download speed, image quality and navigation.

During the course of the study, the issue of download speed was considered to be a limiting factor towards uptake and completion of the study. To remedy this, a CD-Rom

version of the VPS was developed. This functioned by download images from the CD while sending tracking data via the Internet to the VPS server in DCU. This improved the speed of the VPS as large image files were no longer being downloaded across the Internet. The interface and functionality of the VPS remained otherwise unchanged.

Apart from Participant 55, all other participants who indicated dissatisfaction with download speed downloaded images via the Internet rather than from CD-ROM. Participants who utilised the CD-Rom version of the VPS did not indicate that download speed adversely affected their experience.

Since this study was performed, a new version of the VPS browser has been developed that incorporates threading processes. This permits the browser to carry out multiple processes simultaneously and independently of each other, rather than wait for one process to finish before commencing another. In the VPS, this has the effect whereby tracking data is sent to the VPS database server in a independent thread so that users do not have to wait for a server response and are presented with images the instant they are available. The effect of this process efficiency is reduced download times for the user.

Participants 55, 11, 7, 8 and 10 attributed poor image quality to the VPS. It is noteworthy that, apart from Participant 7, all other participants concerned with image quality viewed the VPS with a screen resolution of 800x600 pixels while participant 7 viewed the VPS with a screen resolution of 480x680 pixels for the first two slides and 800x600pixels for the remaining 8 slides.

Some participants indicated that they did not find navigating through a slide examination intuitive. For all participants, it was their first time using the VPS and for almost half, it was their first time using a telepathology system. As with all software, there is a leaning curve to be overcome by the user before they are comfortable at using a system. Participants 11, 87, 55, and 7 all suggested additional intermediate and low power magnifications with improved resolution. A new version of the VPS has been developed which reflects this request. It incorporates nine magnification levels. (4x, 8x,16x, 32x, 64x, 125x, 250x, 500x and 1000x) This was achieved by modifying the manner in which magnification levels are built. Difficulty was also expressed with

respect to the centring of regions a participant zooms in on. This has also been addressed with the development of a new VPS where users select using a 'zoom box' the boundaries of the region they wish to zoom to.

It is important to note that apart from participants 7,8,11 and 18, other participants who expressed dissatisfaction at download speed, image quality or slide navigation, still indicated that they found the VPS easy to use or were confident in using the VPS to make a diagnostic decision.

Responses by participants to the VPS questionnaire are available for viewing online at http://www.telepathology.dcu.ie/administration/adm4.php3?id=true.

Table 3.10 Summary of Data Recorded by VPS User Perception Questionnaire where Users submitted a comment indicating they ' were not confident in using the VPS to make a diagnostic classification please indicate why Ranked by '*Confidence in making a diagnostic classification*'.

User ID	IT Competency	Experience in Pathology	The ease of use of VPS	Confidence in making a diagnostic classification	Screen Resolution	Image Quality	If you were not confident in using the VPS to make a diagnostic classification please indicate why?
8	Competent		Very-Difficult	Not-Confident	800x600	Poor	*Too long to download images*
11	Competent	>5	Very-Difficult	Not-Confident	800x600	Poor	*Image quality was poorDownload speed was extremely slow and made the viewing experience disjointed and basically unworkableNavigation within the slide was disjointed and it was difficult to maintain perspective whilst moving from field to field. The range of magnifications was too limited, especially in the intermediate magnification range*
18	Adequate	3	Difficult	Not-Confident	800x600	Adequate	*Takes long to move around on slide/change mag. etc. Although the system worked fast it is still very slow compared to normal light microscopy. Also, cannot focus or change light which I sometimes find useful (e.g. to look at possible mitotic figures it can be useful to focus up/down.*
19	Competent	>5	Difficult	Reasonably-Confident	1024x768	Adequate	*Speed of navigation made me impatient*
87	Advanced	>5	Easy	Reasonably-Confident	768x1024	Adequate	*The system would benefit from higher resolution low power images as much of the diagnosis by experienced pathologists is based on the low power identification of diagnostic areas*
6	Competent	>5	Easy	Confident	1024x768	Excellent	*problems with assessing significance of small subtle lesions without having the whole slide to look at*
5	Competent	>5	Very-Easy	Very-Confident	1024x768	Excellent	*artificial as I picked the slides*
1	Advanced	>5	Easy	Confident	1280x1024	Good	*Quality of slides; not limited by quality of system. Also, breast pathology is controversial issue and several mimickers of b9*

Table 3.11 Summary of Additional Comments Submitted By Participants Who Completed The Questionnaire

IDNUM	What Aspects Of The VPS Would You Like To See Improved
1	*Zoom controls while maintaining resolution at higher powers.*
5	*navigation*
6	*unable the click on the precise area of slide with the cursor which often seemed to be off centre* *had difficulties moving slide laterally and up and down ito get the precise area i wanted*
7	*Image quality especially in lower power images. Downloading images was slow especially for the higher powers. If the intermediate power images were improved it might reduce the number of high power images that have to be downloaded.*
8	*problem was on my end, slow connection*
10	*I think the overall quality of the images could be improved although the majority were reasonably good. The downloading speed would be a limiting factor in the routine use of this.*
11	*Image qualityNavigation mechanismRange of magnificationDownload speed (although I fully accept that this is probable limited by the user\s computer and internet connection)*
18	*Speed!!!!Ability to move slide in a continuous manner as with ordinary microscopy. It was the first time I have used telepath.. Maybe with more practice I would be more confident.*
19	*Speed and ease of navigation* *Apologies for not completing but was time-consuming- worthwhile venture - keep at it*
22	*It would be better if the field that is magnified each time matched the most cellular area on the slide, so that the area of interest could remain centre-stage. I dont know if it would be possible to increase the screen size so that more of the slide could be viewed for any given magnification - I do realise that this would mean longer download times!!*
35	*Download speed too slow. Resolution will require inprovement.*
36	*Moving around the field. In my opinion the scrolling system is better.Also in changing magnification, I perceive the field are already demarcated, which subsequently increase the number of movement. Enlarging the field and scrolling may be better.Enhance the image with betterm colour resolution*
39	*speedy download of higher magnification images*
41	*no comment*
55	*improve quality of imagesimprove quality of histological slides(to acquire images, slides should be of EXCELLENT quality), add intermediate magnifications because sometimes they are too low or to high and it is impossible to see both the entire lesion end histological details.the download speed is very slow in same cases the quality of slides is poor*
87	*Better low power resolutionBetter navigation using the mapespecially where it is necessary to change areas quickly.*

3.8 Conclusion

Participant 36 and participant 6 attained the lowest Kappa scores of 0.26 and 0.23 respectively. Confidence in using the VPS was described as "reasonably confident" by participant 36, who had 3 years experience in pathology. Further analysis of the images viewed is necessary to elucidate reasons for the diagnostic decisions made by participant 36; however, inexperience with breast pathology coupled with insufficient examination of the slides may have contributed to poor performance.

The clinical importance of submitting a particular diagnostic classification is greater for some classifications than for others. Submitting a B1 classification indicating the biopsy contained normal tissue, usually arouses suspicion that the suspected lesion was missed and results in further biopsies being taken from the patient. To avoid unnecessary re-sampling, it is unusual for pathologists to submit a B1 classification except in unambiguous circumstances. Submitting a B2 classification indicating a benign biopsy will result in the patient not receiving further treatment. It is important that a pathologist is not suspicious about a case when rendering this diagnosis otherwise a patient will be given not receive further treatment .

If a pathologist is suspicious of the presence of a carcinoma they will not rely solely on their diagnosis of a H&E stain. Usually a number of immunohistochemisty tests are performed on the biopsy before a pathologist would finally provide a classification for a case.

In terms of clinical outcome, it is possible to group the five diagnostic classifications into two groups, A B1 and B2 grouping would result in a patient not receiving further treatment while B3,B4 and B5 would all result in the patient receiving subsequent treatment, usually surgery. Discordance is therefore of greater importance between a B2 and a B3 diagnosis than between a B3 and a B4 or even a B3 and B5 diagnosis. This could be reflected in the calculation of weighted kappa by putting a greater weight on a discrepancy between the two groups.

Slide 7 had the 5th highest level of agreement where a percentage concordance of 64.7% was attained. It is arguable, however, that the clinical significance of discordance in agreement for Slide 7 is greater than any other slide given that the discrepancy in agreement lies predominantly between a B3 and a B2 category.

Use of telepathology was described as "infrequently" by participant 6 who was "confident" in making a diagnostic classification using the VPS and described the use of the VPS as "easy." However, participant 6 attributed some diagnostic uncertainty to "problems with assessing significance of small subtle lesions without having the whole slide to look at."

Participants with 3 years or less experience expressed least satisfaction with the VPS in terms of ease of use, image quality, and diagnostic confidence. All 3 participants, who indicated they were "not confident," attributed difficulty in using the VPS to poor download speed, with comments such as "Poor download speed was extremely slow and made the viewing experience disjointed and basically unworkable." "too long to download images" and "problem was on my end, slow connection."

High-speed broadband Internet connectivity is still unavailable to many pathologists. This is a major limiting factor for acceptability of Web-driven telepathology due to the time taken to download large image files over the Internet[2-3,5]. To overcome this, a CD-ROM VPS system was developed and distributed to selected participants. This facilitates rapid retrieval of images from a CD while data pertaining to the examination is transmitted and stored on the VPS web server.

Participants were asked to comment on improvements to the VPS that they would like implemented. A number of participants suggested they would like additional magnification ranges. For example:

> "*Navigation within the slide was disjointed and it was difficult to maintain perspective whilst moving from field to field. The range of magnifications was too limited, especially in the intermediate magnification range.*"

It is of interest to note that members of the ECWGBSP who took part in evaluting the VPS published a study in 2000[134] that attempted to measure and identify causes of inconsistency in diagnosing and classifying intraductal proliferations of the breast. The study comprised of 32 sections distributed to the study members. A follow on study occurred three years later where one or two digitised images were taken at medium or high magnification of one area from each of the sections and printed on high quality paper. The areas were selected by the pathologist at the co-ordinating centre and were deemed to be the most representative of the lesions in the sections. Kappa statistics for diagnosing hyperplasia of usual type, atypical ductal hyperplasia and ductal carcinoma in situ were 0.54, 0.35 and 0.78 for sections and 0.47, 0.29 and 0.78 for images, respectively. The poor Kappa performance is indicative of the difficult of the cases used in the study and demonstrated that most of the inconsistency was due to differences in morphological interpretation.

In terms of ability to mimic a conventional microscope, accessibility via the Internet, and simplicity of operation, the VPS system is a realistic alternative to conventional means of performing quality assurance studies or delivering histopathology education.

There are a growing number of interactive pathology sites available via the Internet. The diversity in their principle of operation, their application in telepathology, and their degree of sophistication promises an encouraging future in telepathology. The contribution of the VPS to the field of telepathology is notable in that it records the diagnostic pathway of a pathologist's slide examination.

The study described in this chapter was of benefit in evaluating the VPS as a useful telepathology tool and in eliciting the opinions of pathologists as to its advantages and deficits. It is evident that a correct diagnostic decision can be made using the VPS. However, as with any histopathological study, observer variation did occur. The remainder of this thesis evaluates the ability and usefulness of the VPS tracking system in providing an insight as to the cause of observer variation.

Chapter 4:- Analysis of Inconsistencies Between The Diagnostic Classification and Histological Description of 10 Breast Needle Core Biopsy Slides Using the VPS.

4.1 Introduction

Histopathological diagnosis forms the cornerstone of modern oncology. In the diagnosis and management of any tissue abnormality, its biologic significance as well as diagnostic reproducibility is crucial in optimising patient treatment.[99] Errors in histopathological diagnosis can critically affect patient outcome and has, on occasion, become the subject of media concern.[100-103] The existence of interoberver variability has important implications for patient care, diagnostic error and medical litigation. Interobserver variability is an increasingly important component of morphological evaluation. However, it has not been measured adequately or reasons for its occurrence has not been identified accurately.[136-139]

Reports of observer variability in histopathology have been with us for some time. In 1968, Cocker *et al* noted "serious inconsistencies in diagnosis between the various pathologists and the diagnosis made by individual pathologists studying the same specimen at various times".[140] Similar audits have supported these findings.[141-144]

Ramsay (1999)[99] surveyed six audits of errors in Histopathology. Three of the studies reported a diagnostic error rate of between 1.1 and 1.4%, while the fourth reported a much lower 0.26%. The authors identified a lack of consensus in what constitutes a statistically significant error and suggested this as the reason why no diagnostic errors were reported in two of the studies surveyed.

A study published in 1989 conducted by Oneson *et al* reported an increase in error when examining frozen sections.[145] Of 1000 frozen sections, 6.1% of diagnoses were deferred at the time of surgery and 3.5% of diagnoses were revised after routine paraffin-section examination.[140] A subsequent multi-centre study conducted by Howamitz *et al* (1990), consisting of 1952 frozen sections in 34 hospitals, also showed a 3.5% discrepancy rate between frozen and paraffin section diagnoses and a deferred diagnosis rate of 3.9%.[146] Ramsay (1999)[99] divided the types of errors pathologists were making into two categories; oversight errors, and misinterpretation errors. Oversight errors refer to where the pathologist has missed significant pathology. Misinterpretation errors refer to where pathological changes had been incorrectly

interpreted. Of the mistakes detected in this study, 57% were oversight errors and 43% were due to misinterpretation.

In a similar study, Zarbo *et al*(1991)[147], conducted an analysis of the diagnosis of 67 discordant frozen section diagnoses. The study revealed 29 cases in which non-representative tissue was examined and 29 examples of pathological misinterpretation.

In a five year audit at Southampton General Hospital (Ramsay 1999), 45 errors were detected, 65% due to misinterpretation, 31% due to pathological oversight and 4% due to the failure to answer a specific clinical query.[146] Pathologists who re-examined these cases recognised their error of oversight or misinterpretation, suggesting such errors are rarely attributable to an inability by the pathologists to make a correct decision.

Due to the rising cost of litigation, the statistics surrounding malpractice claims as a consequence of errors in histopathological diagnosis are of increasing interest.[148] Malpractice claims in North America involving 53 histopathological diagnoses indicated four major histopathological areas where lawsuits frequently occur.[149] These include failure to diagnose malignant lymphoma, malignant melanoma, and diagnostic problems with prostate needle biopsies. The final category; diagnostic errors by "expert consultants", referred to diagnostic disagreement in cases where two or more pathologists had reviewed a case. 87% of claims were attributed to oversight errors, while misinterpretation errors were attributed to the prostate biopsies where false positive diagnosis of carcinoma is known to be high.[149]

Ramsay (1999)[99] noted that, in many areas of pathology, the 'correct' diagnosis is not well defined, due to inadequate diagnostic criteria, inappropriate classification methods and limitations of morphological diagnosis. This is not always understood by other areas of medicine.[118] It is evident from literature that there is a lack of consensus when scoring or grading pathology specimens.[15-154] Morris (1994)[154] stated that the assignment of scores or grades requires an arbitrary division along an underlying biological continuum that requires the pathologist to assign an ordinal numerical score or grade rather than a nominal text diagnosis and that the use of ordinal diagnosis is bound to increase observer variation. He also stated that pathologists are usually trained

in nominal classification, and possibly possess no expertise on the area of ordinal classification.

The above issue has particular pertinence to the VPS study where an ordinal classification regime was imposed on participants completing the study. It is evident from the nominal text diagnosis submitted by some participants that the use of such a classification regime to score slides contributed substantially to inter-observer variation.

This chapter summarises findings of a comparative analysis between the classifications submitted by each participant for each slide and their text diagnosis. The implications of inconsistencies for individual Kappa and consensus statistics and the overall consensus for each slide are also highlighted.

4.2 VPS Reporting Guidelines

When participants logged into the VPS, they were presented with an online set of classification guidelines adapted from the Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer Screening.[124] In order for a participant to proceed to the slide gallery (Figure 3.2) and select a slide from examination, participants had to click a radio button indicating *'I Have Read and Accept The Above Guidelines'* Figure 4.1 is a screenshot of the interface participants were presented with. During the course of a slide examination, it was possible for participants to refer to the guidelines at any time by selecting the '*Help*' option from the VPS browser menu bar and return to their examination when finished. It was also possible to download and print out a Microsoft Word™ (see Appendix 1) version of the guidelines from the interface shown in Figure 4.1.

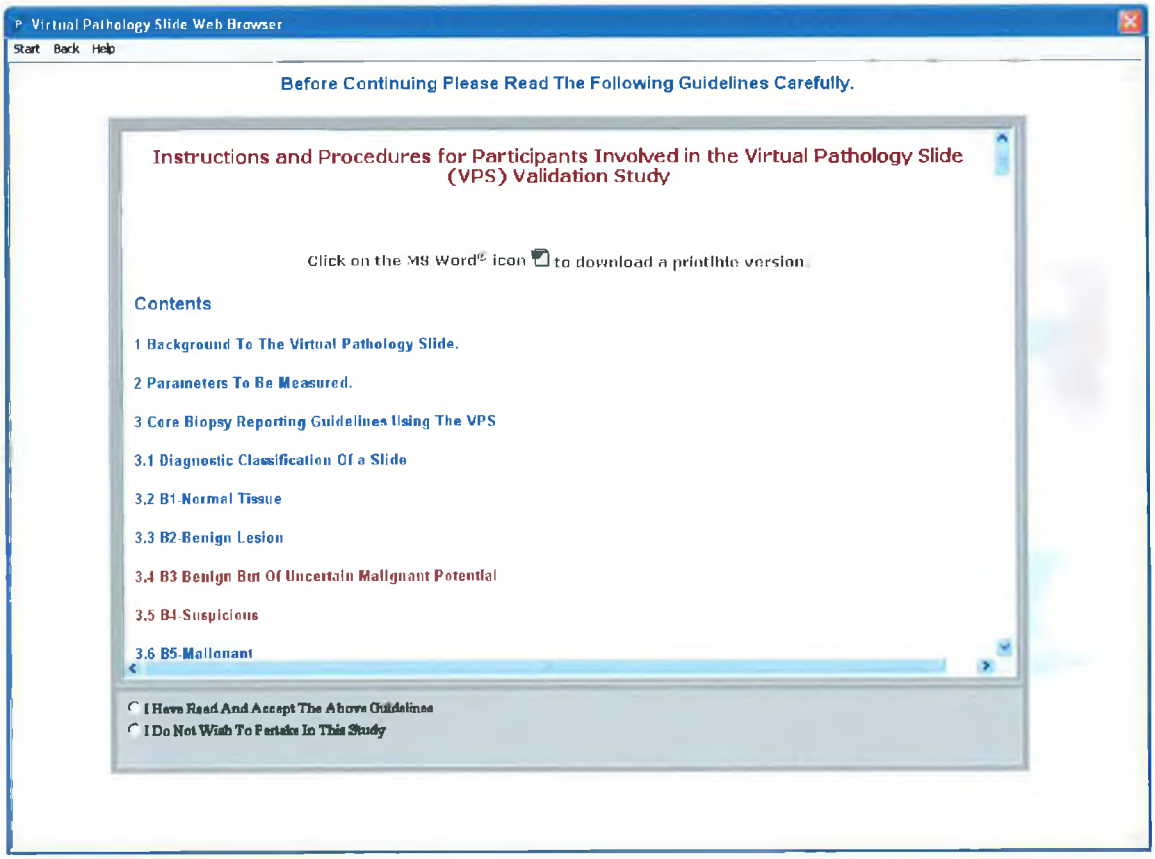


Figure 4.1 Screenshot of The VPS Reporting Guidelines As Presented to Study Participants

4.3 Submission of Diagnostic Comments

While it was obligatory to submit a diagnostic classification, it was not obligatory to submit diagnostic comments. However, some participants did avail of this functionality. All comments submitted by participants for each of the 10 slides can be reviewed in Appendix 2.

Professor Peter Dervan reviewed the comments submitted by participants and compared them to the diagnostic classification they submitted. Professor Dervan is head of Pathology, University College Dublin and consultant Pathologist at the Mater Miscoradae Hospital, Dublin. He is internationally recognised as a specialist in Breast Pathology and has chaired the Irish 'National Breast Screening Steering Group' and the 'National Quality Assurance Committee, Breast Cancer Screening'. Professor Dervan is also a member of the EU and UK Working Group on Breast Screening Pathology. Professor Dervan has authored a number of books on the subject of breast cancer aimed at both patients and clinicians. On the basis of the above credentials, Professor Dervan was considered the most suitably qualified pathologist in Ireland to render an interpretation on comments submitted by participants and compare them to the diagnostic classification they submitted.

It was evident to Professor Dervan that, in some cases, a participant submitted an incorrect classification based on their diagnostic comment. This indicated that:

- Some participants where not sufficiently knowledgeable of the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer Screening that were used in this study.
- The Core Biopsy Reporting Guidelines were not robust enough to account for the ambiguous histology of some lesions such as intraductal papilloma.

Table 4.1 to 4.4. illustrates the comments that were submitted for slides where Professor Dervan identified discrepancies between diagnostic comments and diagnostic classification submitted.

4.3.1 Review and Reclassification of Slide 1 Diagnoses Based on Diagnostic Comments

Apocrine cells are identifiable from the characteristic pink colour of cytoplasm under haematoxylin and eosin stains coupled with a prominent central nucleolus. While it is considered a common condition it is highlighted under *'Diagnostic pitfalls in interpretation'* within the reporting guidelines where it is noted that:-

> *'One particularly difficult lesion is atypical apocrine change in sclerosing adenosis, especially if this is associated, as it often is, with a complex sclerosing lesion.'*

Due to the presence of apocrine change, Slide 1 is a difficult case. It is important to note that where there is suspicion of ductal carcinoma in conjunction with the presence of Apocrine, additional evidence of carcinoma than is normally required is necessary before a B5 classification can be submitted.

> *"Pure apocrine DCIS is relatively rare, and when an apocrine proliferation is seen within ducts in a core biopsy, additional features of malignancy such as significant atypia, intraluminal necrosis and the presence of mitoses as well as multiple duct involvement should be sought for confirmatory evidence."*

Both participant 1 and 35 who had their classification amended based on their diagnostic comments noted *'Atypical ductal proliferation'*. In this instance the reporting guidelines advise that

> *"Mild or moderate degrees of apocrine proliferation with atypical features in a duct space should be assessed with caution, and it may be prudent not to record a definite diagnosis but to classify such a process as B3 (uncertain malignant potential".*

For participant 35 who submitted a B4 classification, the presence of apocrine cells may have masked the severity and degree of atypia indicating invasive ductal

carcinoma. Their comment required a statement indicating suspicion for malignancy based on the extent and severity of atypia in order to classify as B4. Hence Professor Dervan reclassified their diagnosis as B3.

Participant 1 indicated suspicion of carcinoma *'favour adenocarcinoma'*. However, they did not unequivocally indicate carcinoma as required by the guidelines. Hence Professor Dervan reclassified their diagnosis from B5 to B4.

Despite reclassification of the diagnostic categories submitted by Participant 1 and 35, their reclassified diagnosis was inconsistent with the consensus diagnosis of Slide 1.

Table 4.1 Diagnostic comment and classification submitted by participants for Slide 1. Where appropriate, a corrected classification based on participants diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 1- Glass slide diagnosis B5 Invasive Ductal Carcinoma With Apocrine Change			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
41	B2		microglandular adenosis
6	B3		adenomyepethioloma apocrine adenosis
87	B3		Atypical apocrine hyperplasia ? in papilloma
5	B4		probably invasive carcinoma with apocrine change
7	B4		Based on the cellularity I am suspicious of malignancy but I was not able to see adequate cellular detail to make a confident diagnosis (see comment on VPS system)
35	B4	B3	Atypical ductal proliferation with apocrine change. Complete excision required
10	B5		In situ carcinoma on a background of a sclerosing lesion
62	B5		invasive lobular carcinoma
68	B5		Invasive ductal carcinoma
1	B5	B4	Atypical ductal proliferation, favor adenocarcinoma.
22	B5		Invasive carcinoma : possibly apocrine carcinoma

4.3.2 Review and Reclassification of Slide 8 Diagnoses Based on Diagnostic Comments.

Participants 6, 36 and 68 classified Slide 8 as B2 despite correctly identifying a papillary lesion in their diagnostic comments. The reporting guidelines indicate that:

"the majority of papillary lesions will be designated B3"

Consequently, Professor Dervan reclassified their diagnosis from B2 to B3.

As Figure 4.2 illustrates, there is a requirement in the text diagnosis to indicate that the degree and extent of atypical epithelial proliferation is sufficient in order to classify a papillary lesion as B4 suspicious of carcinoma. Given that a papillary lesion is usually classified as B3 and that Participants 1, 5, 35 and 41 classified it as B4 without clear justification in their diagnostic comment, Professor Dervan reclassified their diagnoses from B4 to B3.

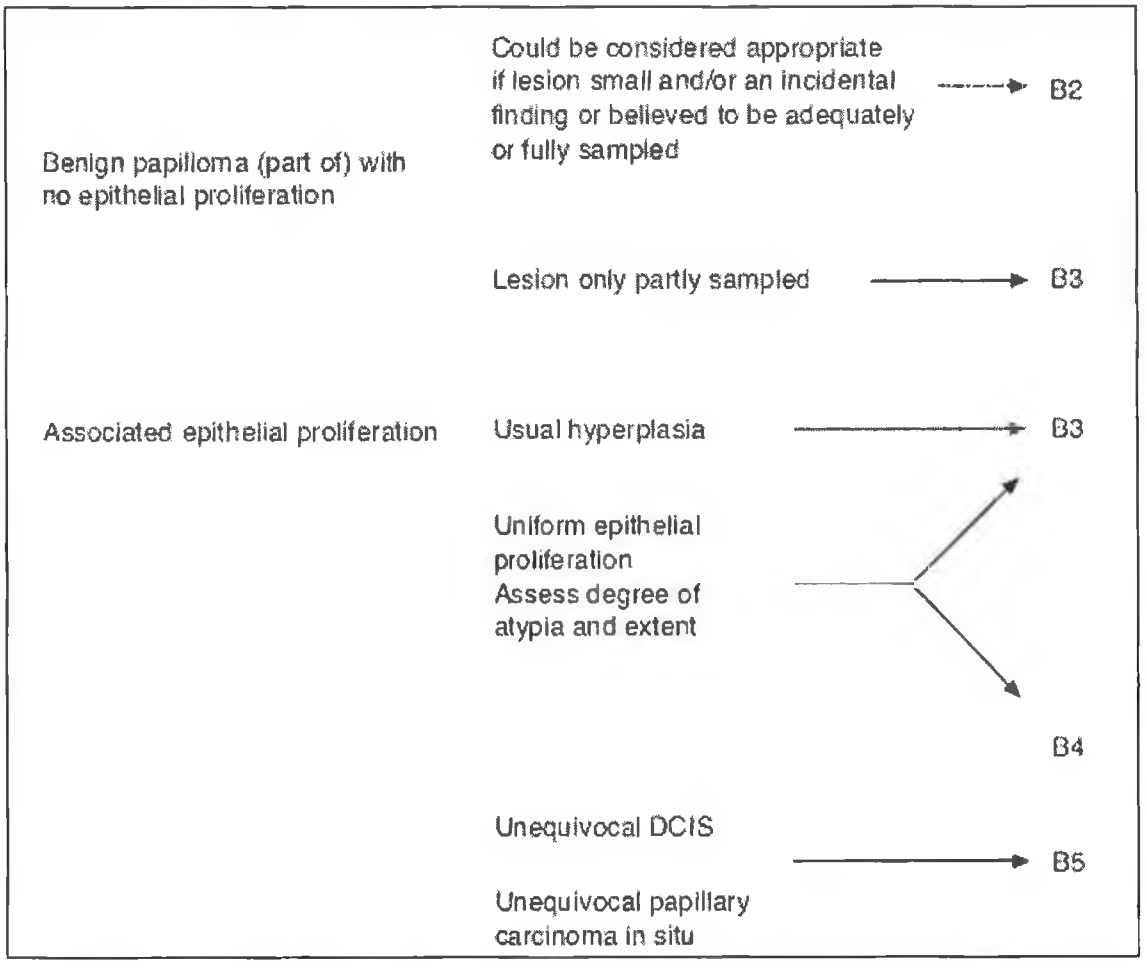


Figure 4.2 Guidelines for Diagnosing Papillary Lesions (reproduced from the Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer Screening).

Figure 4.2 illustrates the difficult and ambiguous nature of diagnosing papillary lesions. The fork tailed arrows between B3 and B4 classification emphasise that there is no discrete division between B3 and B4 and that a diagnostic decision made by a pathologist between a B3 and B4 categorisation is a purely subjective one.

Participant 62, who classified the slide as B4 stated in their diagnosis "*Papillary carcinoma*". This is an unequivocal statement indicating carcinoma,hence Professor Dervan reclassified their diagnosis from B4 to B5 as required by the reporting Guidelines.

Table 4.2 Diagnostic comment and classification submitted by participants for Slide 8. Where appropriate a corrected classification based on participants diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 8 B3 Glass slide diagnosis intraductal papilloma.			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
6	B2	B3	this looks like subareolar duct papillomatosis. what part of the breast is the biopsy from?
36	B2	B3	Ductal papillomatosis
68	B2	B3	Intraductal papilloma
7	B3		Papillary lesion - looks like a papilloma but needs biopsy
55	B3		papillary lesion
87	B3		Papilloma
1	B4	B3	Intraductal papilloma
5	B4	B3	intraduct papilloma with atypia. at least b3 probably b4. needs excision
10	B4		suspicious for intracystic papillary carcinoma
35	B4	B3	Intraductal papillary with atypia
41	B4	B3	papillary lesion, resection needed
62	B4	B5	Papillary carcinoma
22	B5		Intracystic papillary carcinoma. Advise excision.
39	B5		Intracystic papillary carcinoma
39	B5		Papillary carcinoma

4.3.3 Review and Reclassification of Slide 5 Diagnoses Based on Diagnostic Comments

The consensus diagnosis for Slide 5 was B5 invasive ductal carcinoma. Participant 36 classified Slide 5 as B2 and submitted the following diagnostic comment:

> *'Ductal papiloma in addition there is fibrocystic changes'.*

Fibrocystic changes is a common benign feature of a breast biopsy. It would normally elicit a B2 classification. However, papilloma lesions are classified by the reporting guidelines as an uncommon lesion. There exists considerable ambiguity and subjectivity as to its correct classification as Figure 4.2 illustrates. The reporting guidelines state:

> *'It is anticipated that the majority of papillary lesions will be designated B3'.*

Hence, Professor Dervan reclassified Participant 36 diagnosis from B2 to B3.

Table 4.3 Diagnostic comment and classification submitted by participants for Slide 5. Where appropriate a corrected classification based on participants diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 5-Glass Slide Diagnosis B5 Invasive Ductal Carcinoma			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
7	B2		Area of sclerosis suggestive of radial scar.
36	B2	B3	Ductal papiloma in addition there is fibrocystic changes
1	B5		Favor DCIS with fibrocystic changes and sclerosis.
41	B4		suspicious of radial scar, rule out tubular carcinoma
68	B4		Radial scar and ADH.
5	B5		mixed tubular ca and nos
35	B5		Invasive ductal carcinoma
39	B5		?recurrence
6	B5		infiltrating adenocarcimoma low grade grade 1 with foci of dcis low grade
22	B5		Invasive ductal carcinoma NOS with associated low grade DCIS

4.3.4 Review and Reclassification of Slide 7 Diagnoses Based on Diagnostic Comments

In slide 7, participant 36 submitted a B3 classification indicating "*Possibly sclerosing adenosis*". This is a benign condition and according to the reporting guidelines it should be classified as B2. However, sclerosing lesions are highlighted within the reporting guidelines under '*Problems and Pitfalls in Diagnosis*' where it is stated.

> *" There is a risk of over diagnosis of invasive carcinoma when confronted by sclerosing adenosis in a core biopsy".*

It is possible participant 36 was being over cautious when they classified Slide 7 as B3. Based on their diagnostic comment and the reporting guidelines Professor Dervan reclassified their diagnosis from B3 to B2 resulting in participant 36 agreeing with the consensus diagnosis for Slide 7 of B2- Benign Usual Epitheial Hyperplasia.

Participant 39 stated "*Atypical ductal hyperplasia/low grade DCIS*". Atypical ductal hyperplasia normally receives a B3 or B4 classification depending on the extent of atypia. However, participants 39 indicated low grade DCIS. According to the reporting guidelines this is categorised as B5. Hence Professor Dervan reclassified their diagnosis as B5. This reclassification increased the degree of disagreement between Participant 39 and the consensus diagnosis of Slide 7.

Table 4.4 Diagnostic comment and classification submitted by participants for Slide 7. Where appropriate a corrected classification based on participants diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 7 Glass slide diagnosis B2- Benign Usual Epitheial Hyperplasia			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
5	B2		sclerosing adenosis
6	B2		benign ductal epithelial proliferation i think it looks like blunt duct adenosis. foci of hemorrhage present consistent with previous fna
7	B2		fibrocystic disease with sclerosing adenosis
22	B2		Florid epithelial hyperplasia, of usual type.
41	B2		fibrocystic changes with epithelial hyperplasia of the usual type
55	B2		Hiperplasia usual type
10	B3		Best regarded as ADH
36	B3	B2	Possibly sclerosing adenosis
39	B3	B5	Atypical ductal hyperplasia/low grade DCIS
1	B5		DCIS

4.4 Impact of Reclassification on Slide Consensus and on Individual Performance

It is noteworthy that the slides with the three lowest consensus were all reclassified. Slides 1,5,and 8 who achieved the lowest consensus were all reclassified is illustrated in Table 4.5. The average percentage concordance for the ten slides increased from 66.5% to 67.1%.

A major consequence of the reclassification of diagnosis submitted is that the VPS consensus diagnosis for Slide 8 changes from B3 to B4. Individual percentage concordance and Kappa values were recalculated as they are based on the consensus classification for each of the ten slides (Table 4.6).

Table 4.5 Comparison of glass slide needle core surgical biopsy diagnosis, consensus Virtual Pathology Slide (VPS) diagnosis based on original classification submitted by Participants and modified classifications based on Participants diagnostic comments, in order of level of agreement (concordance) for each slide.

	Virtual Pathology Slide									
	S6	**S2**	**S3**	**S4**	**S7**	**S9**	**S10**	**S1**	**S5**	**S8**
Consensus Diagnosis (Glass)	B5	B5	B5	B2	B2	B2	B2	B5	B5	B3
Original Consensus Diagnosis (VPS)	B5	B5	B5	B2	B2	B2	B2	B5	B5	B4
Modified Consensus Diagnosis (VPS)	B5	B5	B5	B2	B2	B2	B2	B5	B5	B3
Original % Concordance	100	94.1	82.4	76.5	64.7	58.8	52.9	52.9	47.1	35.3
Modified % Concordance	100	94.1	82.4	76.5	70.5	58.8	52.9	47.1	47.1	64.5

It is noticable that where Professor Dervan suggested amendments to diagnostic classifications should be made, an amendment to Participant 36 classification was suggested for three of the four slides. Obviously it is extremely important to have a good knowledge of the diagnostic guidelines that one is using. This would have increased Participant 36 concordance with slide consensus from 40% to 70% and increased their Kappa from 0.26 to 0.46. Participant 36 Kappa rating would only have improved from "Poor" to "Moderate" as they were still several degrees away from consensus agreement on a number of other slides, such as Slide 3, where they submitted a B2 grade when consensus was B5.

Table 4.6 Comparison of original percentage concordance against recalculated percentage concordance and original kappa against recalculated kappa for each individual. Recalculated percentage concordance and kappa based on reclassification of diagnosis submitted from the diagnostic comment for each individual. Shaded cells indicate where there has been a change in Kappa performance by 0.05 or greater, or a change in percentage consensus by 10% or greater.

User ID	Original Consensus,%	New Consensus	Kappa	New Kappa
5	90	90	0.98	0.98
68	70	70	0.85	0.95
55	80	80	0.87	0.90
87	70	70	0.86	0.88
18	40	40	0.86	0.88
10	70	70	0.91	0.88
35	70	60	0.94	0.87
62	80	70	0.94	0.84
1	70	70	0.67	0.83
65	60	60	0.8	0.80
41	70	70	0.75	0.75
7	60	60	0.73	0.75
75	70	70	0.65	0.73
22	80	80	0.75	0.69
39	60	60	0.9	0.66
36	40	60	0.26	0.49
6	50	60	0.23	0.29
Average	66.5	67.1	0.76	0.72

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Table 4.6 Comparison of original percentage concordance against recalculated percentage concordance and original kappa against recalculated kappa for each individual. Recalculated percentage concordance and kappa based on reclassification of diagnosis submitted from the diagnostic comment for each individual. Shaded cells indicate where there has been a change in Kappa performance by 0.05 or greater, or a change in percentage consensus by 10% or greater.

User ID	Original Consensus,%	New Consensus	Kappa	New Kappa
5	90	90	0.98	0.98
68	70	70	0.85	0.95
55	80	80	0.87	0.90
87	70	70	0.86	0.88
18	40	40	0.86	0.88
10	70	70	0.91	0.88
35	70	60	0.94	0.87
62	80	70	0.94	0.84
1	70	70	0.67	0.83
65	60	60	0.8	0.80
41	70	70	0.75	0.75
7	60	60	0.73	0.75
75	70	70	0.65	0.73
22	80	80	0.75	0.69
39	60	60	0.9	0.66
36	40	60	0.26	0.49
6	50	60	0.23	0.29
Average	66.5	67.1	0.76	0.72

Incorrect classification based on the diagnostic comment submitted was most noticeable on Slide 8 (lowest slide consensus). The glass slide diagnosis for Slide 8 was intraductal papilloma. It is categorised based on its degree of progression. It is recognised as a lesion that can present difficulty in categorising correctly. Professor Dervan was of the opinion that this difficulty gave rise to the disparity between participants who submitted a classification. Using the classifications amended by Professor Dervan to re-calculate consensus, slide 8 would have achieved an increase in slide agreement from 35.3% to 64.5%.

Professor Dervan amended Participant 36's classification based on their diagnostic comment for Slide 5 (second lowest slide consensus) changing a B2 to a B3 where the participant had identified *"Ductal papiloma in addition there is fibrocystic changes"*. A ductal papilloma is a benign wart-like growth that can be precancerous. The consensus calssification for Slide 5 was B5 consequently, Professor Dervan amendments of Participant 36 classification did not affect the overall percentage agreement for slide 5.

Slide 1 achieved the third-lowest slide concensus. Professor Dervan amended 3 classifications submitted by participants based on their diagnostic comment. The consensus classification for Slide 1 was B5. Using the classifications amended by Professor Dervan to re-calculate consensus, Slide 1 would have achieved a decrease in slide agreement from 52.9% to 47.1%. Slide 1 was regarded by Professor Dervan as a very difficult case. The extensive presence of apocrine in Slide 1 makes it difficult to determine the true extent of ductal proliferation giving rise to the inconsistency between diagnostic comment and diagnostic classification.

Slide 7 achieved relatively good consensus agreement (64.7%). Professor Dervan amended Participant 36's classification based on their diagnostic comment, changing a B3 to a B2 where the participant had identified *"Possibly sclerosing adenosis"*. Sclerosing adenosis is a benign growth within the breast lobules and normally it does not require surgical intervention. Using Professor Dervan's amendment to Participant 36's classification the consensus agreement for Slide 7 would have increased to 70.5%.

Reclassification of Participant 36's diagnosis from B2 to B3 would not have affected the overall consensus agreement for Slide 5 (B5 Invasive Ductal Carcinoma).

4.5 Conclusion

It would have been beneficial in this study if all Participants had been obliged to submit a diagnostic comment in conjunction with a diagnostic classification for each examination. It would have given a valuable insight into participants knowledge and application of the guidelines and helped to explain discordance in diagnosis by giving clues as to how participants had interpreted the histology of the slides.

It would have been of interest to have recorded the participants who scrolled through the Online Reporting Guidlines or referred to them during a slide examination. However, it was assumed prior to the study that individuals, especially members of the European Working Group on Breast Screening in Pathology, would be familiar with diagnostic reporting guidelines.

Performance in terms of individual Kappa value decreased when pathologists text diagnosis was evaluated and classifications adjusted accordingly. However, the average overall slide consensus increased.

The participants examined the slides remotely in a unsupervised manner when examining the slides. It is therefore impossible to know if a participant was distracted when examining the slides. Participants were aware that they were being examined. It is therefore likely that they behaved in a more cautious manner than they would when examining slides during their daily routine.

The performance of a pathologist is dependent on their individual approach to slide examination. Some pathologists will have a greater amount of skill, knowledge and expertise in breast lesions than others. Some pathologists will be involved in the examination of breast histology more frequently than others, have greater access to continuing medical education and peer review or support.

It is not just necessary to have a thorough knowledge of specific lesions and disease progression. It is also necessary to have an in depth working knowledge of reporting guidelines. For example, in Chapter 3, Slide 8 was shown to have very low consensus amongst participants with respect to diagnostic classification. With a wide range of B2, B3, B4 and B5 classifications submitted. However, despite Slide 8 appearing as a difficult case, a review of the diagnostic comments of some participants indicated that

all had successfully identified an intraductal papillary lesion. Participants had failed to use the guidelines in assessing the degree of atypia and extent of uniform epithelial proliferation associated and submitting the correct classification accordingly. This is despite the Breast Needle Core guidelines making specific reference to the classification of papillary lesions

> *'It is anticipated that the majority of papillary lesions will be designated B3'.*

Histopathological diagnosis has traditionally been regarded as the 'gold standard' diagnosis against which all others are measured.[99] The value of this 'gold standard' is, however, questionable given the prevalence of inter and intra-observer variability. This is illustrated by the level of diagnostic discordance measured from the increasing number of studies being performed. Variability in the level of diagnostic agreement amongst pathologists and the lack of data to perform a statistically based predictive power on the likelihood of a particular diagnosis being correct, underlay a fundamental weakness in the ability of a histopathological examination to offer a 'gold standard' diagnosis.

Error may be considered an intrinsic property of microscopic diagnosis given that the majority of histopathological information that contributes to diagnosis is subjective. Error reduction requires constant vigilance by histopathologists themselves. Quality assurance and audit is becoming an increasingly integral part of clinical governance.[150-152] Such processes can reveal errors of omission such as typographical errors or of interpretation i.e. misdiagnosis. However, such studies as they currently exist, are limited in their ability to distinguish uncertainty from incompetence. Furthermore, it must be realised that disagreement between consensus agreement and expert opinion can be legitimately disputed.[150-152]

A reduction in the number of diagnostic categories will result in an increase in inter-observer agreement as measured by Kappa statistics, but a decrease in the amount of information transmitted. Given that the primary role of the pathologist is the transmission of information to the clinician, so that the most suitable treatment will be given to the patient, it could be argued that more diagnostic categories, rather than less

should be used[155-156]. However, an article in the Lancet by Foucar (1996)[157] entitled "Carcinoma-in-situ of the Breast: Have Pathologists Run Amok" argues that the monopoly which pathologists have on the naming, classification and categorization of disease, can have harmful physical and psychological consequences for patients, without any benefits.[118] The author suggests "their diagnosis of 'carcinoma-in-situ' has transmitted more fear than knowledge into the clinical arena". Foucar concludes that "patient's diagnosis should not be an anachronism sustained by anecdotes, conjecture and tradition" and that "terminology consumers" should start proposing disease classifications they would find most helpful.

Perhaps of greater use to the clinician is the provision of a percentage confidence rating by a pathologist describing their degree of certainty in the diagnosis they prefer on each individual case examined. This may have some benefit to the patient by providing the clinician with a rating of how confident the pathologists is that they are offering a "gold standard " diagnosis.

The incidence of errors in diagnostic histopathology reporting has been published in a number of studies. However, comparisons between studies is difficult due to inconsistency in what is regarded as a significant error. For example a misdiagnosis may only be accepted as a significant error where it leads to incorrect treatment of a patient. However, studies reveal errors in up to 4% of cases, with an overall rate of between 0.26% and 1.4% for cases in which the error is regarded as significant, as it risks patient welfare.[99]

Despite an analysis of the diagnostic comments submitted by participants, there still exists a substantial degree of unexplained inter observer variability. Chapter 5 attempts to utilise VPS tracking data to profile Participants diagnostic behaviour and determine whether Participants diagnostic performace is related to their perception of the performance of the VPS.

Chapter 5:- Use of VPS Tracking Data to Evaluate Participants Diagnostic Performance.

5.1 Introduction

Unlike other virtual slide systems, the Virtual Pathology Slide is unique in its ability to record several key parameters that describe user's interaction with the system. This chapter analyses these parameters, some of which provide valuable insight into participant's evaluation and experience of using the VPS system and determines whether these parameters provide insight into individual diagnostic performance and overall slide consensus.

One of the parameters investigated in this chapter is participant's perception of image quality for each slide examination. Factors that influenced participants perception of image quality are identified such as individual monitor settings. These are related to individual diagnostic performance. The relationship between overall slide consensus and perception of image quality is also investigated .

Image quality and the minimum standards required to make a correct diagnosis has remained a contentious issue in the field of telepathology. This is partly due to the lack of standardisation in the manner in which image quality is described. Doolittle *et al* (1997)[57] published a study indicating that as little as 8-bit colour depth is required for diagnosis from digital images. Given that an appreciation of colour, texture and staining intensity is a crucial part of histological assessment, 8-bit colour depth would seem insufficient. In a review of telepathology standards, Leong *et al* (1999)[6] makes reference to Doolittle's paper

> "*Work by Doolittle et al suggested that 8-bit colour may be sufficient for histological image assessment however the methodology behind that study has been called into question and 24-bit colour appears to be a de facto standard.*"

16 bit mode is referred to as "High Colour". A 16-bit display is only capable of producing 65,536 colours while 24-bit depth is also called "True Colour" because it can produce all the colours discernible to the human eye. There is a discernable difference between a histology image displayed at 16-bit and 24 bit colour depth.

Concerns over the issue of colour depth and screen resolution and their possible effect on diagnostic performance are likely to dissipate in the near future given that a minimum screen resolution of 1024 x 768 with a 32-bit colour depth are now being offered as standard by hardware manufactures.

Participant's perception of download speed for each slide examination is investigated in this chapter. An analysis is carried out to determine whether individual diagnostic performance or the number of fields of view examined is a function of participants perception of download. An analysis is carried out to determine whether there is a relationship between the overall perception of download speed for each slide and the number of fields of view examined.

A comparison is made between perception of download speed amongst participants who downloaded VPS images across the Internet and participants who downloaded VPS images from a CD-Rom.

Finally, an analysis is carried out to determine whether individual diagnostic performance is dependent on the number of fields of view examined or whether overall slide consensus is dependent on the number of fields of view. The number of fields of view examined at each magnification is also analysed to determine if examining a slide at a particular magnification contributes to slide consensus.

5.2 Evaluation of Image Quality Using The VPS Summary Report Form

For each slide examination performed by participants, a Summary Report Form was completed in which users submitted their classification (Figure 5.1). A section of the form required participants to assess the download speed and the quality of the images for that slide examination. Figure 5.1 is abstracted from the Summary Report Form and illustrates the portion of the form in which participants evaluated the VPS.

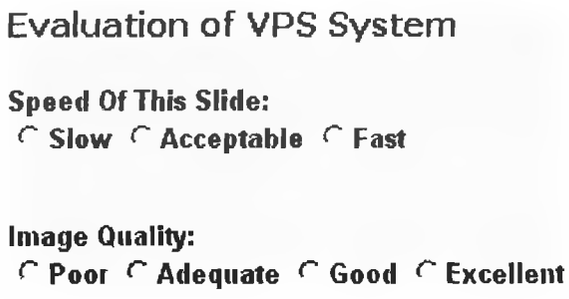


Figure 5.1 Section of the VPS Summary Report Form where participants were asked to review download speed and image quality.

Colour depth refers to the number of bits assigned to each pixel in an image. The higher the colour depth, the greater the number of colours that can be used to define the pixel values in an image and the higher the image file size will be. With respect to computer monitors, colour depth refers to the number of colours that any pixel can display. Both parameters may be adjusted at the user's discretion on computers with a Windows operating system from the 'Control Panel'. The screen resolution and colour depth capacity depend on the monitor and video card being used. Table 5.1 shows the number of colours a monitor can display depending on the colour depth setting.[158]

Table 5.1 Relationship Between Colour Depth and The Number of Colours a Monitor Can Display.[158]

Colour Bit Depth		Number of Colours a Monitor Can Display
8-bit	2^8	256
16-bit	2^{16}	65,536
24-bit	2^{24}	16.4 million
32-bit	2^{32}	16.4 million

A 32 bit colour depth displays the same number of colours as a 24 bit colour depth. This is because in a 32 bit colour image 24 bits are used for colour information and the

other 8 bits are used as a separate layer for representing levels of translucency in an object or image. These effects are normally used in digital video, animation and video games. [158]

Using the VPS tracking system, both the screen resolution and colour bit depth were recorded in an effort to survey the computer monitor settings of participants and determine whether this affected participants individual diagnostic performance or perception of image quality.

Table 5.2 Illustration of participant's perception of image quality for each slide in conjunction with screen resolution colour depth and individual diagnostic performance in terms of percentage consensus and Kappa. Table ranked by screen resolution.

User	Resolution	Colour Depth	Slide 1	Slide 2	Slide 3	Slide 4	Slide 5	Slide 6	Slide 7	Slide 8	Slide 9	Slide 10	Consensus %	Kappa
87	1024x768	16	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	70	0.88
5	1024x768	24	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	90	0.98
6	1024x768	32	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Poor	Poor	60	0.29
41	1024x768	32	Excellent	Excellent	Adequate	Good	Good	Good	Good	Good	Excellent	Excellent	70	0.75
62	1024x768	32	Good	Adequate	Adequate	Adequate	Adequate	Adequate	Good	Good	Good	Good	70	0.84
65	1024x768	32	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Good	Excellent	Excellent	60	0.8
1	1280x768	32	Good	Good	Adequate	Good	Good	Good	Excellent	Good	Good	Adequate	70	0.83
22	1280x768	32	Excellent	Excellent	Excellent	Good	Good	Good	Good	Adequate	Adequate	Poor	80	0.69
68	1280x768	32	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	70	0.95
7	800x600	8	*Poor	*Poor	Adequate	Adequate	Poor	Poor	Poor	Adequate	Poor	Poor	60	0.75
10	800x600	16	Adequate	Adequate	Poor	Adequate	Adequate	Adequate	Adequate	Good	Poor	Adequate	70	0.88
18	800x600	16	Adequate	Adequate	Adequate	Adequate	Adequate	Good	Adequate	Good	Poor	Adequate	40	0.88
35	800x600	16	Poor	Adequate	Adequate	Adequate	Adequate	Good	Poor	Adequate	Adequate	Poor	60	0.87
36	800x600	16	Adequate	Poor	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	Poor	Adequate	60	0.49
55	800x600	16	Poor	Adequate	Adequate	Adequate	Poor	Adequate	Poor	Poor	Poor	Poor	80	0.9
75	800x600	24	Adequate	Adequate	Good	Good	Adequate	Good	Adequate	Good	Good	Good	70	0.73
39	800x600	32	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	60	0.66

Table 5.2 illustrates that participants who recorded image quality for a particular slide as *"Poor"* predominantly had a low screen resolution of 800 x 600pixels and a colour depth of only 16 bit or less. Slide 9 and 10 were the only slides to receive a rating of *"Poor"* by participants with a screen resolution higher than 800 x 600pixels. The biopsy cores represented in slides 9 and 10 both contain a limited amount of tissue. Slide 9 achieved the highest rating for *"Poor"* (by 6 Participants). During the course of the study, the image quality of Slide 9 was reviewed, was deemed to be substandard and was rescanned.

Slide 1 and Slide 2 achieved the highest rating for image quality despite being the first two slides examined by individuals. This was surprising as Slide 2 was identified as a poorly prepared slide. This indicates that participants were distinguishing between poor image quality and poor slide preparation in their assessment of Slide 2.

For Slide 1 and 2 participant 7 had a screen resolution of 640 x 480. They subsequently increased this to 800 x 600 for the remainder of the slides. However, their bit depth setting remained at 8-bits. Consequently the tissue images reviewed by participant 7 for all 10 slides displayed only 256 colours. Under such circumstances, participant 7 performed remarkably well.

Figure 5.2 demonstrates that participants perception of image quality is predominantly dependent on the display setting of their own monitor. participants with a screen resolution of 800 x 600 pixels and a colour depth of 24 or 32 bit described image quality as "*Excellent*" " *Good*" or "*Adequate*". Participants with a screen resolution of 1024 x 768 or 1280 x 768 had a colour depth of 24 or 32 bit and described image quality as "*Excellent*" " *Good*" or "*Adequate*".

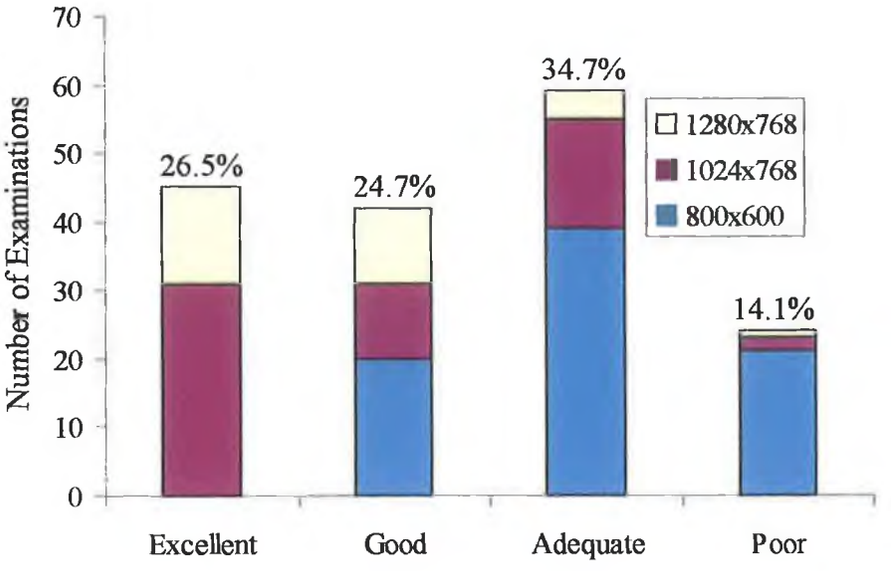


Figure 5.2 Stacked Bar Chart Illustrating The Distribution of Image Perception By Participants For Each Screen Resolution.

It is noteworthy that Participants 5 and 68 who had attained the highest and second highest individual Kappa recorded *'Excellent'* image quality for all 10 slides. Both had optimised screen settings. Participant 55 had a poor perception of image quality having rated 5 of the 10 slide as "*Poor*" and the remainder as "*Adequate*". This is probably due to their screen settings (800 x 600 screen resolution and a 16 bit colour depth). However, Participant 55 achieved the third highest individual Kappa.

Taking individual Kappa as a measure of diagnostic performance, the average Kappa value of participants with a screen resolution of 800 x 600 pixels was equal to the average Kappa value of participants with a screen resolution higher than 800 x 600 pixels. (Kappa=0.71). This supports the hypothesis that diagnostic performance is not affected by screen resolution or colour depth.

Table 5.3 Illustration of participant's perception of download speed for each slide in conjunction with the number of fields of view examined by each participant and the individual diagnostic performance in terms of percentage consensus and Kappa. Table ranked by number of fields of view examined.

User	IMG SRC	Slide 1	Slide 2	Slide 3	Slide 4	Slide 5	Slide 6	Slide 7	Slide 8	Slide 9	Slide 10	Fields of View	% Consensus	Kapp
75	CD	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Very Slow	Very Slow	Acceptable	116	70	0.73
7	WEB	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	121	60	0.75
36	WEB	Very Slow	Acceptable	Acceptable	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Acceptable	Very Slow	122	60	0.87
87	CD&WEB	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	129	70	0.88
10	WEB	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Very Slow	157	70	0.88
35	WEB	Acceptable	Acceptable	Acceptable	Very Slow	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	201	60	0.49
22	WEB	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	204	80	0.69
41	WEB	Fast	Fast	Very Slow	Very Slow	Fast	Fast	Fast	Fast	Fast	Fast	216	70	0.75
68	CD	Acceptable	Fast	Fast	Very Slow	Acceptable	Acceptable	Acceptable	Acceptable	Fast	Acceptable	232	70	0.95
65	CD	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	234	60	0.8
39	WEB	Very Slow	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	237	60	0.66
1	WEB	Acceptable	Fast	Acceptable	Acceptable	Acceptable	Fast	Fast	Very Slow	Fast	Acceptable	251	70	0.83
18	WEB	Very Slow	Acceptable	Acceptable	Very Slow	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Very Slow	252	40	0.88
55	CD	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Very Slow	Acceptable	Acceptable	Very Slow	289	80	0.9
5	WEB	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	306	90	0.98
62	CD	Acceptable	Acceptable	Very Slow	Acceptable	Acceptable	Very Slow	Acceptable	Very Slow	Acceptable	Acceptable	326	70	0.84
6	WEB	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	Fast	417	60	0.29

5.3 Evaluation of Download Speed Using The Summary Report Form

Table 5.3 illustrates Participants perception of download speed for each slide examination as a function for the number of fields of view examined and as a function of individual diagnostic performance.

Table 5.3 indicates that there does not appear to be a relationship between participants perception of download speed and individual diagnostic performance.

There is a visible trend indicating a positive perception of download speed as the number of fields of view examined by a participant increases. For example, participant 7 who rated the download speed for all ten Slides as '*Very Slow*' examined the second least number of fields of view (121). Participant 36 rated the download speed of seven out of ten Slides as '*Very Slow*' and the remainder as '*Acceptable*' reviewed the third least number of fields of view (122). In contrast Participant 6 and 5 who reviewed the largest and third largest number of fields of view rated the download speed of all 10 Slide as '*Fast*'.

Excluding Participant 87 who reviewed VPS slides using both CD and web download, the average number of fields of view examined by participants who reviewed images from CD was 239 The average number of fields of view examined by participants who reviewed images from CD was 226. Therefore there is no perceivable difference in the number of fields of view examined between participants who downloaded images from the web and Participants who download from CD.

There is no discernable difference in perception of download speed between participants who reviewed images across the Internet and participants who viewed images from CD. It is possible that Participants who used a CD-Rom had higher expectations of download speed than those who viewed images across the Internet.

5.4 Analysis Of The Number Of Fields Of View Examined Using The VPS.

The average number of fields of view examined by each participant was 22 per slide. Participant number 5, who achieved the highest Kappa, examined 306 views, while participant number 6, who had the lowest Kappa, examined 417 fields of view.

The highest number of number of fields of view examined for a particular slide was 118 by participant number 6 while examining slide 10. This slide had a percentage concordance between participants of 52.9%. The lowest number of views examined while examining a slide was 3; this was by participant 10 who achieved a Kappa score of 0.88 and agreed with the group consensus for slide 2. Diagnosis for slide 2 had a percentage concordance amongst participants of 94%.

Table 5.4 indicates that there is a tangible relationship between the number of fields of view examined on a slide and the percentage agreement for that slide. This relationship is graphically illustrated in Figure 5.3. As slide consensus decreases, the number of fields view examined for that slide increases. The linearity of this relationship improves if Slide 8 and Slide 9 are ignored. Slide 9 was rescanned during the course of the study due to substandard image quality on the initial scan. Slide 8 and Slide 9 were bilateral biopsies from the same patient. Despite being a benign tissue sample, Slide 9 was presented with the same case notes as Slide 8 "*Ultrasound shows cyst with component in cell wall*". This would have caused confusion and suspicion on the part of examining pathologists and resulted in a excessive number of fields of view being examined. The ambiguities of Slide 8 were discussed in Chapter 4 with respect to the number of participants who provided a correct text diagnosis which was inconsistent with the diagnostic classification they submitted. Slide 8 contained an intraductal pappilloma, which is the subject of much inconsistency in diagnosis. It is arguable as to the true consensus diagnosis for Slide 8. Figure 5.4 demonstrates that if data for slide 8 and slide 9 are removed the regression coefficient improves significantly from 0.35 to 0.61

Table 5.4 Record of the number of individual fields of view examined by Participants as they reviewed each slide Slides are ranked in order of percentage consensus, participants are ranked in order on number of fields of view reviewed.

User	Slide 6	Slide 2	Slide 3	Slide 4	Slide 7	Slide 8	Slide 9	Slide 10	Slide 1	Slide 5	Fields of View	Std Dev-Fields of View	% consensus	Kappa
75	10	7	11	7	9	12	3	18	19	20	116	5.7	70	0.73
7	11	5	10	15	11	11	6	16	17	19	121	4.6	60	0.75
35	8	6	13	19	12	11	7	20	11	15	122	4.7	60	0.87
87	4	17	9	13	12	10	11	22	11	20	129	5.4	70	0.88
10	11	3	18	11	23	14	26	18	20	13	157	6.7	70	0.88
36	11	5	18	35	23	11	34	33	21	10	201	11.0	60	0.49
22	20	10	15	34	40	14	21	8	23	19	204	10.1	80	0.69
41	14	21	53	13	13	11	6	19	21	45	216	15.3	70	0.75
68	8	10	17	32	24	16	4	27	36	58	232	16.1	70	0.95
65	17	7	22	19	67	9	23	17	28	25	234	16.7	60	0.8
39	24	32	28	28	33	28	8	31	9	16	237	9.3	60	0.66
1	12	12	29	19	42	17	13	28	45	34	251	12.4	70	0.83
18	10	16	42	27	26	20	24	30	27	30	252	8.7	40	0.88
55	9	17	43	36	32	15	22	39	30	46	289	12.6	80	0.9
5	5	5	58	27	33	47	29	37	37	28	306	16.5	90	0.98
62	30	18	47	33	38	31	17	27	55	30	326	11.7	70	0.84
6	39	17	30	42	43	22	32	118	27	47	417	28.5	60	0.29
Total	243	208	463	410	481	299	286	508	437	475	3810			
Mean	14.3	12.2	27.2	24.1	28.3	17.6	16.8	29.9	25.7	27.9				
Std Dev	9.2	7.6	15.9	10.4	15.2	9.8	10.3	24.1	12.3	13.9				
% Consensus	100	94.1	82.4	76.5	70.5	64.5	58.8	52.9	47.1	47.1				

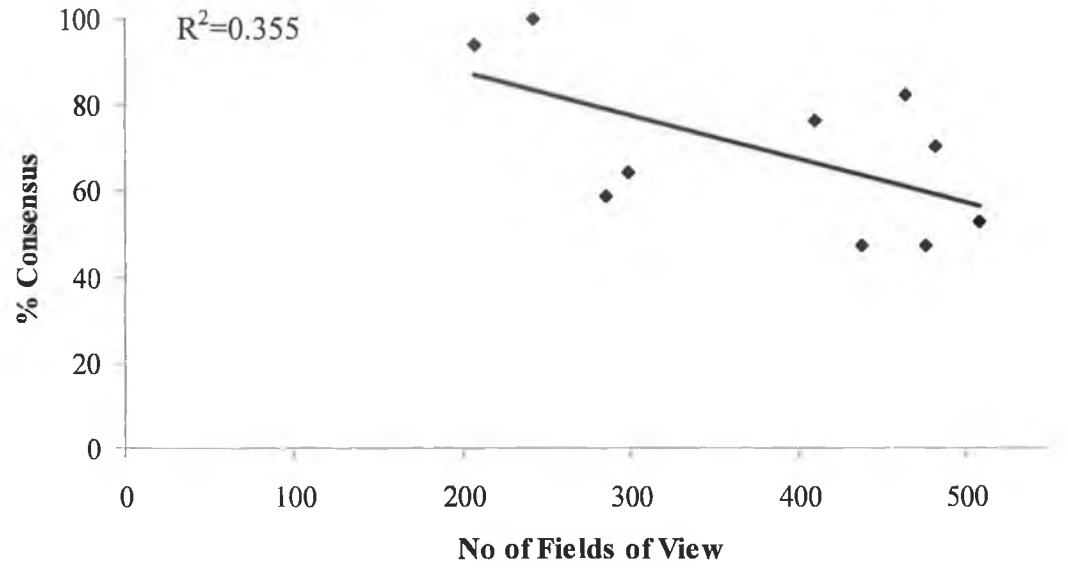


Figure 5.3 Scatter Plot Illustrating The Relationship Between Slide Consensus and Number of Fields of View Examined For Each Slide.

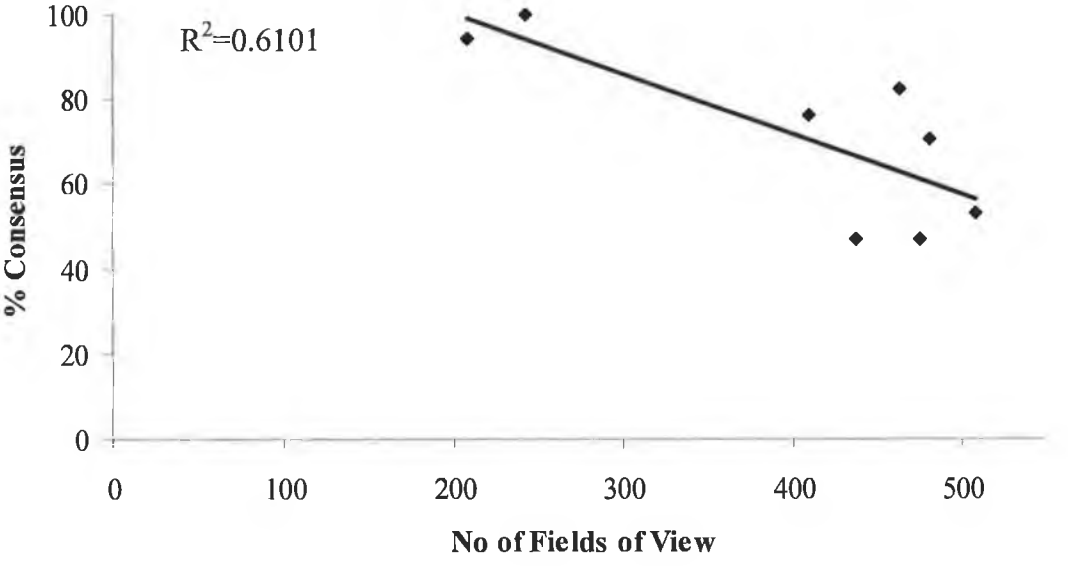


Figure 5.4 Scatter Plot Illustrating The relationship Between Slide Consensus And Number of Fields of View Examined For Each Slide, Where Contributory Data From Slide 8 and 9 Have Been Removed.

The case notes of Slide 10 indicated "*Micro-calcification on mammogram*" Confirmation of micro-calcification requires examination at high magnification. Professor Dervan examined Slide 10 and found no evidence of calcification. This is not uncommon as calcium crystals tend to fall out of samples during slide preparation. In addition, Professor Dervan indicated Slide 10 required examination at high

magnification in order to examine nuclear detail and exclude the possibility of carcinoma. Slide 10 received the second highest "*Poor*" rating for image quality after Slide 9. In addition, Participant 6 viewed 118 individual fields of view in Slide 10. This skewed the average number of fields of view examined in Slide 10. It is these factors, which have led to Slide 10 having a higher number of fields of view examined than any other slide.

Figure 5.5 Scatter Plot Illustrating The relationship Between Individual Kappa and Number of Fields of View Examined By Each Participant, Where contributory data From Participants 6 and 36 Have Been Removed.

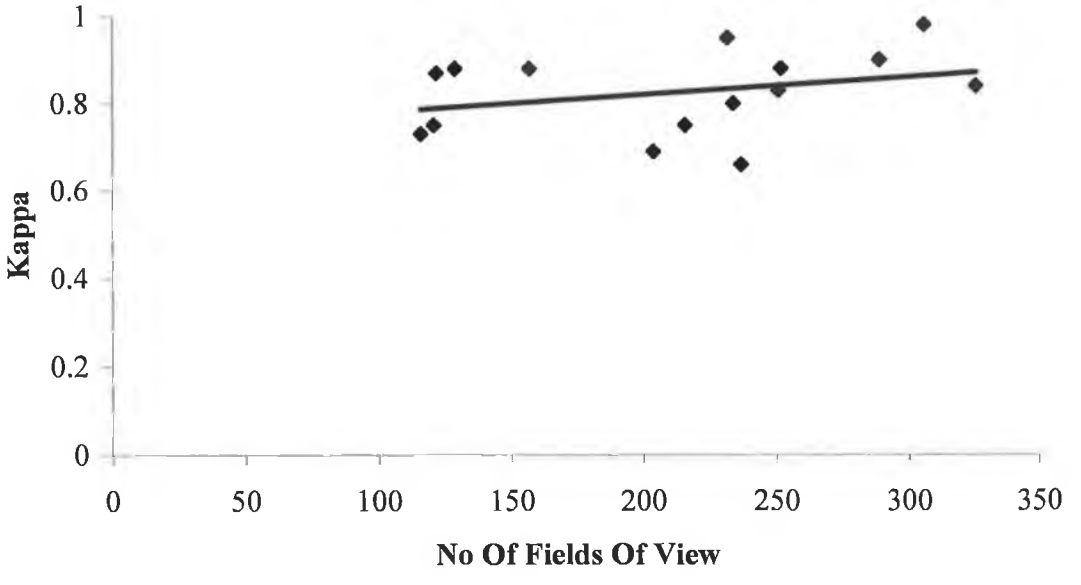


Figure 5.5 illustrates the relationship between diagnostic performance and the number of fields of view examined. Participants 6 and 36 who obtained the lowest and second lowest Kappa score respectively were identified as outliers and their data omitted. Despite the removal of contributory data from Participants 6 and 36 there appears to be only a tenuous link between the number of fields of view examined by a participant and their diagnostic performance.

Table 5.5. Record of The Number Of Fields of View Examined at a Specified Magnification For Each Slide.

Magnification	Slide 6	Slide 2	Slide 3	Slide 4	Slide 7	Slide 8	Slide 9	Slide 10	Slide 1	Slide 5	Total
2000x	15	9	37	6	22	13	10	24	22	29	187
500x	63	44	158	42	108	71	56	155	125	168	990
125x	79	101	166	159	227	89	89	158	175	176	1419
32x	54	33	75	157	78	80	84	117	82	73	833
16x	32	21	27	46	46	46	47	54	33	29	381
Total	243	208	463	410	481	299	286	508	437	475	3810
% Consensus	100	94.1	82.4	76.5	70.5	64.5	58.8	52.9	47.1	47.1	

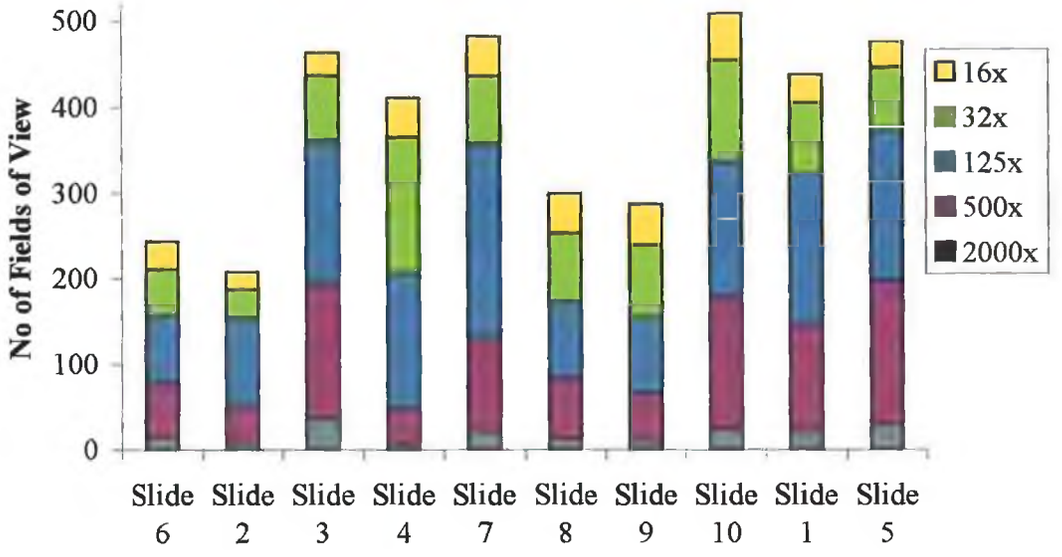


Figure 5.6. Stacked Bar Chart Illustrating The Proportion Of Fields of View Examined at a Specified Magnification For Each Slide

Table 5.5 and Figure 5.6 illustrate that there does not appear to be a relationship between consensus agreement for each slide and the number of fields examined at a specified magnification. There does not appear to be a clustering nship between number of fields of view examined at a specified magnification and the diagnostic classification of a slide. Slides 4, 7, 9, and 10 were classified as B2, while Slides 1, 5, 6, 2, and 3 were classified as B5.

The proportion of fields of view examined appears to be unique for each slide. This is probably because the histology of each slide is different. Some slides require greater examination at high magnification than others. For example, for Slide 3, a greater proportion of moves were examined at 500x and 2000x than Slide 4 where the majority of the tissue was examined at 32x and 125x. There does appear to be a relationship between the difficulty of a slide and the total number of fields of view examining it. Slides 3, 7, 10, 1 and 5 had the highest number of fields of view examined. These slides were known to contain histological features that were ambiguous or difficult to interpret. These are discussed in greater detail in Chapter 7. Slide 4 also had a high number of fields of view examined. This is probably because 3 needle core biopsies were presented in Slide 4. This was considerably more tissue than the other slide where only one or two cores were presented.

5.5 Conclusion

Perception of image quality was clearly dependent on participants screen resolution and colour depth. However it did not appear to substantially affect individual diagnostic performance. Perception of image quality was better for some slides than for others. Slide 9 and 10 achieved the greatest number of "*Poor*" ratings while Slides 1 and 2 achieved the greatest number of '*Excellent*' ratings. On the basis of this work a minimum screen resolution of 1024x768 with a 24-bit colour depth is now standard when using the VPS. Consequently, as pathologists upgrade their monitors and video cards, perception of image quality should become a diminishing issue in subsequent VPS studies. However, there are a number of variable affecting image quality that the VPS tracking system cannot measure such as the brightness and contrast setting of the monitor, or whether it is a flat screen. In addition, as conventional cathode monitors age, they undergo a process known as '*burn out*' where the quality of the image displayed depreciates with time.

Perception of download speed does not appear to be related to individual diagnostic performance However, there does appear to be a increase in the number of fields of view examined by a participant as their perception of download speed becomes more positive.

There was no noticeable change in perception of download speed between participants who viewed images from a CD compared to participants who viewed images from the Internet. It is believed that this is due to an increase in expectations by participants who used CD.

There is a tangible relationship between the number of fields of view examined on a slide and the degree of agreement for that slide. As slide consensus decreases, the number of fields view examined for that slide increases. However, there is no substantial relationship between the number of fields of view examined by a participant and their individual diagnostic performance.

There is no relationship between consensus agreement for each slide and the number of fields examined at a specified magnification. In addition there does not appear to be a relationship between diagnostic classification and the number of fields of view

examined. The number of fields examined for a case appears to be representative of the histological difficulty in interpreting a case. The number of fields of view examined at a particular magnification is unique for each slide and dependent of the histological properties of each slide.

While the data presented in this chapter is useful in providing a global overview as to the participants perception of the VPS and how this influenced their performance and in terms of analysing how magnification and number of fields of view examined affected slide consensus and individual diagnostic performance, it did not provide specific reasons as to the cause of observer variability and diagnosis. In order to investigate reasons for observer variability, a software tool was developed which utilises VPS tracking data to create a graphic visualisation of an individual slide examination and provide clues as to why certain diagnostic decisions were made. The development and use of this tool is discussed in the following chapters.

Chapter 6:- Development of a Graphical Representation of a VPS Diagnostic Migratory Trace.

6.1 Introduction

6.1.1 The Cognitive Process Diagnostic Pathology.

Diagnostic pathology is the practice of disease classification using tissue samples. The cognitive process of clinical diagnostic pathology is a multiple-agent paradigm involving attention process, limited channel capacity, pattern recognition and heuristics. Traditionally, exploration of the cognitive process has attempted to discern differences between novice and expert.[37]

Pattern recognition is the ability to recognise, identify and categorise information. It is a skill required for the performance of a number of clinical sub-disciplines such as radiology, dermatology and microscope diagnosis. In order to track and deduce the cognitive process in visual diagnostic tasks, a number of studies have utilised methods such as recording "think-aloud" protocols and eye-tracking and applied theoretical models to describe their results.[159-164]

The majority of work in this area has been carried out in radiology. Similar to diagnostic microscopy it is a visual diagnostic task dependent on pattern recognition and subject to observer variation. Kundel *et al* has published several studies that attempted to determine reasons for inter-observer variation in radiography. They used a eye-head tracker to measure the timing of decisions, where visual attention was directed, and how much time was spent fixating on a region of interest for each decision. This allowed Kundel to monitor eye position during initial and final decision phase, and decision times throughout. This allowed him to determine whether suspicious lesions were recognized and interpreted correctly.[157-159]

Comprehension of the interaction of competing cognitive process involved in microscopic pathology diagnosis is crucial in identifying sources of diagnostic error, improving educational schemata and benefiting from quality assurance programmes.

In order to identify and map discrete skills required in the development of expertise in microscopic pathology diagnosis, Crowley *et al* (2003)[39] performed a study involving 28 novices, intermediates, and experts who examined a standardized set of 8 breast pathology cases. The participants provided verbal protocols, which were recorded by a

video camera. The video camera was mounted on the microscope recorded the microscopic examination.

Crowley *et al* found differences among the novices, intermediates, and expert groups in terms of accuracy, certainty, and difficulty ratings. They also found differences in frequency and types of errors among groups.

The limitation of inter-observer studies involving eye tracking and think-aloud, video taped processes is that pathologists are acutely conscious that their behaviour and examination technique is being examined. This awareness will cause them to behave differently in the manner in which they examine a slide. In this regard the VPS may be considered a "*non invasive*" method of recording an examination technique.

A software application called Bitmapper was developed to aid the visualisation of a examination performed using the VPS. The application generates a graphical representation of a diagnostic trace, using data stored on the VPS Oracle database. Using image analysis techniques to interrogate the generated bitmap images, 'hotspot' areas from the original slide may be located, from which a diagnosis was obtained. The application proved useful in elucidating the decision-making behaviour of pathologists, and determining reasons for observer variation. This chapter describes the development of the system.

6.2 The Development of Bitmapper.

As outlined in Chapter 3, ten breast needle core biopsies were randomly selected and presented to 17 pathologists or trainee pathologists with at least 2 years experience in pathology practice. The recorded data permitted examination of interobserver variability and user satisfaction.

For each individual slide examination, the data recorded is referenced by a unique examination number. The User Tracking Data recorded for a particular slide examination is of sufficient detail so as to enable the slide examination to be replayed. Table 6.1 provides an example of the format in which data for a slide examination is recorded in the VPS tracking table.

In order to graphically represent the areas of tissue viewed during a slide examination and the magnification at which these areas were viewed, a Microsoft Foundation Class (MFC) [90-91] application, designated 'Bitmapper' was developed. Bitmapper retrieves spatial data from the database describing a participant's slide examination and uses this data to generate a greyscale bitmap image.

Table 6.1 Example of Data For a Slide Examination Recorded in The VPS Tracking Table

SESSNO	MAG-LEVEL	SLIDEX	SLIDEY	BUTTON	DOWNLOAD	TIMETAKEN	TIME	TIMEVIEW	SLPRESS	UCOMMENT	IPNO
654	5	101	101	1	26115	no data	20:22:39	no data	0	no data	62.253.64.8
654	4	102	102	1	34234	no data	20:22:59	13	6	no data	62.253.64.8
654	3	113	109	1	82282	no data	20:23:21	19	7	no data	62.253.64.8
654	2	153	141	1	98875	no data	20:23:46	22	10	no data	62.253.64.8
654	1	154	143	1	48017	no data	20:24:12	23	10	no data	62.253.64.8
654	2	153	142	2	93461	no data	20:24:24	6	0	no data	62.253.64.8
654	3	112	110	2	73698	no data	20:24:27	1	0	no data	62.253.64.8
654	3	112	108	4	83073	no data	20:24:36	6	0	no data	62.253.64.8
654	4	102	101	2	32933	no data	20:24:47	8	0	no data	62.253.64.8
654	5	101	101	2	26115	no data	20:24:55	4	0	no data	62.253.64.8
654	4	102	101	1	32933	no data	20:25:25	28	2	no data	62.253.64.8
654	3	109	101	1	61819	no data	20:25:39	11	2	no data	62.253.64.8

6.3 Fundamentals of Bitmaps

The origin of the Bitmap format may be traced back to the earliest days of computing with the Manchester University Mark I computer, developed shortly after the Second World War. This used a storage tube as its working memory. Phosphor dots were used to store single bits of data, which could be read by the user and interpreted as binary numbers. This type of bitmap became known as a device dependent bitmap (DDB), where during the early development of computers the output device was usually a monitor or printer.

Bitmap images, also called raster or paint images, are made of individual dots called pixels (picture elements) that are arranged and coloured differently to form a pattern. The pixel data is stored in a *raster* or grid of x and y coordinates with colour data that directly maps onto a display space. Examples of other raster type image files include TIFF, GIF, and JPEG files. An alternative to storing image file data is the use of vector or geometric graphics. Image data in this format is stored through a sequence of commands or mathematical statements that place lines and shapes in a given two-dimensional or three-dimensional space. However, to display a vector graphic, it must at some point be converted to a bitmap.

A bitmap file consists of a collection of structures contained under two elements, a header, a logical palette and bitmap data. A header contains information about the bitmap data found elsewhere in the file. The format of the header and the information stored can vary considerably depending on the application that makes the bitmap. Typically, a bitmap header is composed of fixed fields. None of these fields is absolutely necessary. Common data types that are included in the header include:

Palette, Bitmap Index, File Identifier, File Version, Number of Lines per Image, Number of Pixels per Line, Number of Bits per Pixel, Number of Color Planes, Compression Type, X Origin of Image, Y Origin of Image, Text Description, and Unused Space.

Data that cannot be stored in the file header may be stored in a Footer at the end of the file.

A logical palette is a colour palette that is created and associated with a given device context. Logical palettes defines the colours used and permits their use.

The bitmap data element of the bitmap file makes up the bulk of a bitmap format file. When bitmap data is written to a file, however, only one of two methods of organization is normally used: scan-line data or planar data.

The scan line data is the most common method for storing image data organized into rows. The scan line method organises pixel values into rows or scan lines so that a description of the image is made up of one or more scan lines. The pixel data in the file describing that image will be a series of sets of values, each set corresponding to a row of the image. Multiple rows are represented by multiple sets written from start to end in the file. Pixel data representing the image can be stored in the file in three ways: as contiguous data of one row after another, as strips of, for example, 3 or 4 rows or as tiles describing' blocks' of the image.

The planar data method of pixel value organization involves the separation of image data into two or more planes. For example, a true colour 24-bit image will be described by three planes for each of the primary colours. Files in which the bitmap data is organized in this way are called planar files. The method is used for output devices where one colour is handled at a time.

A bitmap size is related to the type of image it contains. Bitmap images can be either monochrome or colour. In an image, each pixel corresponds to one or more bits in a bitmap. Monochrome images have a ratio of 1 bit per pixel (bpp). The number of colours that can be displayed by a colour bitmap is equal to two raised to the number of bits per pixel. Thus, a 256-color bitmap requires 8 bpp (2^8 = 256).

In a 24-bit image, each image pixel corresponds to a 3-byte long pixel value in the file. Thus, an image 21 pixels wide requires 21 * 3 = 63 bytes of storage per row. On some machines and in some formats, however, rows of image data must be certain even-byte multiples in length. An example is the common rule requiring bitmap row data to end on long-word boundaries, where a long word is four bytes long. If the format requires

that the row starts be long-word aligned, 64 bytes would be required to hold the pixel values for each row.

Occasionally, 24-bit image data is stored as a series of 4-byte long pixel values, and each image row would then require 21 * 4 = 84 bytes. Storing 24-bit image data as 4-byte values has the advantage of always being long-word aligned.

6.4 Generation of a Bitmap using Bitmapper.exe

A test case 'Slide 1' was selected from the study from which user tracking data associated with the case was used to aid in the development of Bitmapper.exe. Slide 1 is a needle core biopsy obtained from a 60-year-old female, who presented with a suspicious lesion on a mammogram. Glass slide examination determined B5 classification of invasive ductal carcinoma with apocrine change.

The application generates a graphical representation of the migratory trace as follows:-

- Bitmapper provides a dialog interface that permits a user to enter a slide examination session number.

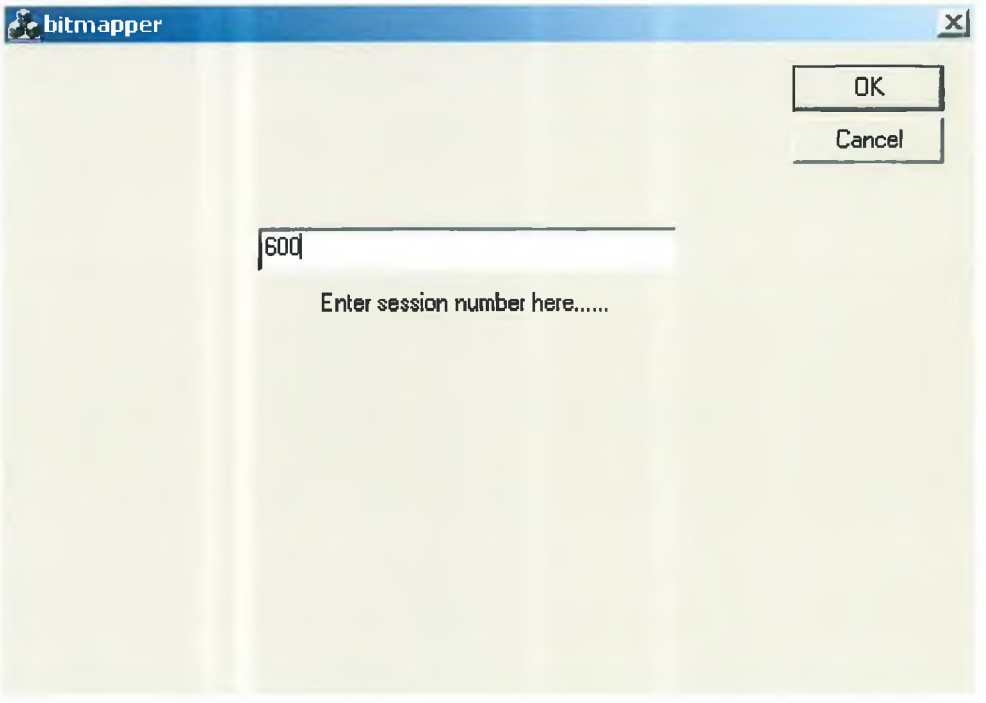


Figure 6.1. Illustration of '*Bitmappers*' user interface. The user inputs an examination number into the text field and a bitmap image depicting the examination trace is created.

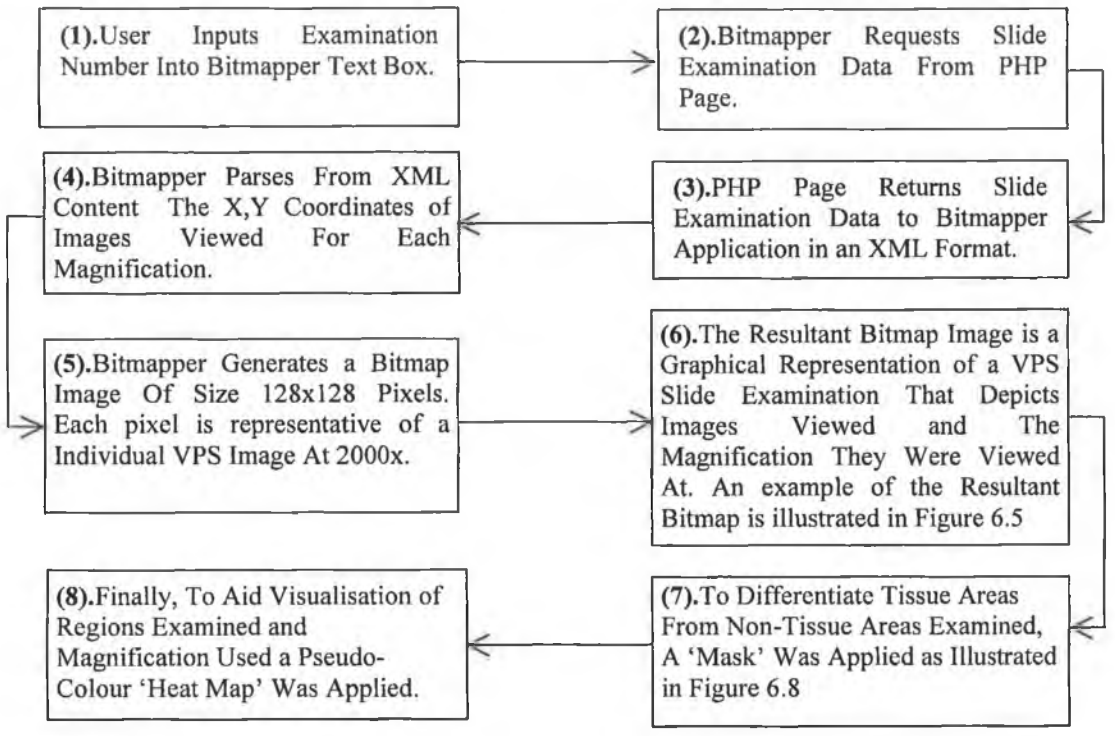


Figure 6.2 Flow Chart Illustrating The Process Involved in Generating Graphical Visualisation of VPS Examinations Using '*Bitmapper*'

- Upon receipt of this query Bitmapper formulates a URL (Uniform Resource Locater) with variables appended describing the session number.

- Utilising a CHTTP (Client Hyper Text Transfer Protocol) tunnel, Bitmapper connects to the internet and requests a page from the telepathology web server described by the URL.

- The page requested by Bitmapper is a server side PHP (Hypertext Preprocessor) script. Upon request, the page generates a SQL (Structured Query Language) command. This command is passed to the oracle server via a OCI (Oracle Call Interface). The returned values are parsed and a dynamically generated XML (Extensible Markup Language) file describing the migratory trace for that session number is created. The contents of this XML document are returned to Bitmapper via the CHTTP tunnel. Figure 6.3 illustrates the structure and content of the XML file. The PHP script that generated this script may be reviewed at the following URL.

http://www.telepathology.dcu.ie/Sqltest/seansql/imaging/mfcconnect.php3?exa

m

```
<?xml version="1.0" ?>
- <retriveddata>
  + <moveid0>
  + <moveid1>
  + <moveid2>
  + <moveid3>
  + <moveid4>
  + <moveid5>
  + <moveid6>
  + <moveid7>
  - <moveid8>
      <SESSNO>609</SESSNO>
      <MAGLEVEL>3</MAGLEVEL>
      <SLIDEX>113</SLIDEX>
      <SLIDEY>121</SLIDEY>
      <BUTTONS>10</BUTTONS>
      <DOWNLOAD>141669</DOWNLOAD>
      <TIMETAKEN />
      <TIME>12:32:35</TIME>
      <TIMEVIEW>3</TIMEVIEW>
      <SLPRESS>0</SLPRESS>
      <UCOMMENT />
      <IPNO>150.217.85.26</IPNO>
    </moveid8>
  + <moveid9>
  + <moveid10>
  + <moveid11>
  + <moveid12>
  + <moveid13>
  + <moveid14>
  + <moveid15>
  + <moveid16>
  </retriveddata>
```

Figure 6.3 - Structure and content of XML file with details of move 8 expanded to illustrate how an XML file describes the data it contains.

- Bitmapper parses the returned XML document for x, y and magnification coordinates.
- Bitmapper generates a palette matrix of 128x128 describing the grey scale colour value of 128x128 individual pixels. Each pixel represents one of the 16,384 individual fields of view that makes up 2000x magnification of a VPS slide. Bitmapper cycles through the array of x, y and magnification coordinates

- 3 times (for magnification 125x, 500x, and 2000x) and updates the palette matrix using the following logic:

- Data for 16x magnification is ignored because it consists of one field of view that is examined by all participants. Including data for 16x in the generation of a Bitmap would not contribute to identifying hotspot regions.

- If a pixel x,y coordinate is in a region that is unexamined during the course of a slide examination it is assigned a value of 0 (Black).

- If a field of view has been examined at 32x, 125x, 500x, or 2000x then the corresponding number of pixels representing that field of view are assigned a greyscale value as described in Table 6.2

Table 6.2 Illustration of the number of potential images each magnification is composed of and the corresponding number of individual fields of view. The number of pixels representative of fields of view for each magnification is also shown, in addition to the grey value assigned to a pixel by Bitmapper if it has been viewed at a given magnification.

Magnification	No of Images	No. of Individual Fields of View	No of Pixels Representative of a Field of View	Assigned Greyscale Value
16x	16	1	16,384	0(black)
32x	64	4	4,096	100
125x	1,024	64	256	150
500x	16,384	1,024	16	200
2000x	16,384	16,384	1	255(White)

- A subsequent 128x128 bitmap is generated with varying regions of greyness describing the area of a slide examined during a particular examination, at differing levels of magnification.

Figure 6.4 illustrates the values assigned to each magnification while Figure 6.5 shows a typical example of a 128x128 bitmap generated by Bitmapper for a slide examination The Class structure within the Bitmapper application and the functions responsible for the generation of Bitmaps are described in Figure 6.6.

16x	32x	125x	500x	2000x	*(Magnification)*
0	100	150	200	255	*(Pixel Grey Value)*

Figure 6.4 Grey Scale Spectrum used to generate palette for 128x128 pixel

Figure 6.5 Example of a 128x128 bitmap generated by Bitmapper for slide examination number 82 on Slide 1. The varying regions of greyness describe the area of a slide examined, at differing levels of magnification.

QueryVpsDb
Contains a function QueryTrackingSession that executes the following.

- Formulates a URL (Uniform Resource Locater) with variables appended describing the session number.
- Establishes an internet connection via CHTTP (Client Hyper Text Transfer Protocol) tunnel.
- Requests a PHP page from the telepathology web server described by the URL. The page generates a SQL (Structured Query Language) command that is passed to the oracle server via a OCI (Oracle Call Interface). The values are returned via the CHTTP tunnel in a dynamically generated XML (Extensible Markup Language) format and describe the migratory trace for that session number.
- Returns the contents of the XML page to function OnkillFocusEdit1 in the CbitmapperDlg class.

XmlParser
Contains a function XmlParser that executes the following:

- Find out how many moves were made for the slide examination
- Parse the x,y and magnification coordinates and store in storage class MoveData.

MoveData
Storage class, x,y and magnification coordinates for each.

MakeBitmap
Contains a function XmlBitmap that executes the following:

- Creates file name based on slide examination number.
- Loops through the moves five times for each magnification. Depending on the magnification a grey value is assigned to a pixel representing the xy co-ordinates of the move, thereby creating a matrix of pixel grey values.

Calls function WriteGreyScaleDataToBitmapFile in class CBitmapperHandler.

CBitmapperHandler
Contains a function WriteGreyScaleDataToBitmapFile that executes the following.

- Creates a palette where each pixel is an index in the palette with an associated greyvalue.
- Calls the function Write8BitBmpFile.
- Write8BitBmpFile fills and writes the bmp file header, info header, bmp palette data and finally saves and closes the file.

CbitmapperDlg.
Contains a function called OnkillFocusEdit1. This is called when the user inputs a slide examination number and is responsible for three actions.

- Calls a function in the QueryVpsDb that passes through a value for the slide examination number, queries the database and returns a result.
- Calls a function in XmlParser to parse the x,y and magnification of each move.
- Calls a function in CBitmapperApp to create a Bitmap.

Figure 6.6:-Class Structure Bitmapper And The Functions Responsible For The Generation Of Bitmaps.

In determining hotspot regions of tissue that were significant in coming to a diagnostic decision, a number of image analysis protocols were developed using Optimas 6.51 (Media Cybernetics, Carlsbad, California, USA).

6.4.1 Masking pixels representing non-tissue areas.

The resultant composite bitmap displays fields of view that were selected for examination by users whether tissue exists in that field of view or not. The following technique was developed to render the composite bitmap so that only migratory traces where tissue exists are represented.

A 24-bit RGB 128x128 sized overview bitmap image of the tissue biopsy was created. In order to differentiate tissue from non-tissue areas a threshold was manually applied where pixels whose values were within the threshold range represented areas that contained tissue and were differentiated as foreground. Pixels whose value fell outside the range represented areas that did not contain tissue and were differentiated into background.

A binary operation based on the manually selected threshold range was performed on the image. This segments the greyscale image into black or white by defining white as foreground and black as the background. A fill filter was applied to the binarised image to fills 'holes' in foreground regions. Holes are considered to be zero valued pixels (black) that are not four-connected to the background.

The resultant binarised greyscale image may be applied as a mask on migratory trace images. This is accomplished by performing an Optimas bit-wise AND arithmetic operation that combines the two images where the darkest pixel (pixel with the highest value) takes precedence. The resultant image represents migratory traces of fields of view that were examined where only tissue exists.

6.4.2 Generating an averaged composite migratory trace greyscale bitmapped image.

An algorithm was developed using Optimas bit-wise AVERAGE arithmetic operation to generate a greyscale bitmap image that represents the average migratory trace. The algorithm functions by calculating the sum of the values for each pixel in n number of images divided by n (where n is the number of migratory trace images from an average migratory trace image is being generated). This returns the average pixel value. The resultant image represents migratory traces of fields of view that were examined where only tissue exists. This technique permitted the generation of the following averaged composite migratory trace greyscale bitmapped images based on diagnostic categorisation.

- Average of B2 examinations (5 exams).
- Average of B3 examinations (8 exams).
- Average of B4 examinations (4 exams).
- Average of B5 examinations (10 exams).
- Average of all examinations (27 exams).

For slide 1, no slide examinations were recorded where a diagnostic decision of B1-Normal tissue was recorded.

Figure 6.7 128x128 greyscale bitmap representing the averaged composite migratory trace for all examinations of slide 1.

Figure 6.8 Intermittent images generated during the process of applying a binary mask to 'hide' pixels representing non-tissue areas from a graphical representation of the averaged composite migratory trace for all examinations of Slide 1. (a) 24 bit colour 128x128 pixel image of Slide 1. (b) Generation of binary mask representing areas where tissue exists. (c) Generation of averaged composite migratory trace for all examinations of Slide 1. (d) Application of mask to averaged composite migratory trace for all examinations of Slide 1.

6.4.3 Use of pseudo-colour to aid visualisation of diagnostically important 'hotspots'.

To aide recognition of 'hot spot' regions that were viewed with frequency by VPS users, a pseudo-colour ramp was applied. The pseudo colour ramp applied was a predefined macro available in Optimas. Pseudo colour changes the appearanee of a monochrome image by reassigning luminance values (red, green and blue) that correspond to features of the image that are to be enhanced. It is only the appearance of the image that is changed. This results in a colour map defined by a standard prism spectrum with blue representing black (where pixel value = 0) and red representing white (where pixel value =255).

The predefined macro available in Optimas permits a number of ranges of greyscale pixel values to be defined. e.g. from 0-149, 150-199 and 255-200. The luminance values of each pixel in the monochrome image will be adjusted depending on the range it falls into giving rise to a colour image.

Migratory trace images that have been treated with pseudo-colour are illustrated in Figure 6.9. An averaged composite for each of the diagnostic categories is also depicted. An overlaid red square denotes identified 'hotspots' regions that have been viewed by multiple participants for each classification.

6.4.4 Grade-by-grade analysis of Slide 1 using bitmapper

The grade-by-grade analysis of Slide 1 conducted during the development of the 'Bitmapper' consisted of 21 individual examination where four of the participants (Participants 1,55,75,39) had examined Slide 1 twice. The generation of Bitmaps as depictured in Figure 6.11 graphically suggests the following.

- There are distinctly visible "hotspots" within Slide 1 examinations.
- That these hotspots appear to be associated with final diagnosis. For example, users who categorized the slide as B2 predominantly examined an upper region of the slide, while users who concurred with conventional glass slide diagnosis, predominantly examined a lower region of the slide.
- That the magnification with which Slide 1 was examined appears to have an impact on slide diagnosis. For example, users who categorized the slide as B2 or B3 did not examine the slide using a high magnification while users who categorized the slide as B4 and B5 did.

Based on the above assumptions it is possible to conclude from the bitmaps generated for Slide 1 that there is a difference in the diagnostic behavior between pathologists who rendered different disease classifications, in terms of both the area viewed and the magnifications used.

The validity of the above arguments, however, can only be proven by requesting a pathologist who specialises in lesions of the breast to review the hotspots for each diagnostic classification that are identifiable from Bitmapper generated images. This is so that it can be determined whether each hotspot contained histological features that led to the diagnosis that was offered for each respective hotspot and whether the magnification used by participants had a role to play in the diagnosis they submitted.

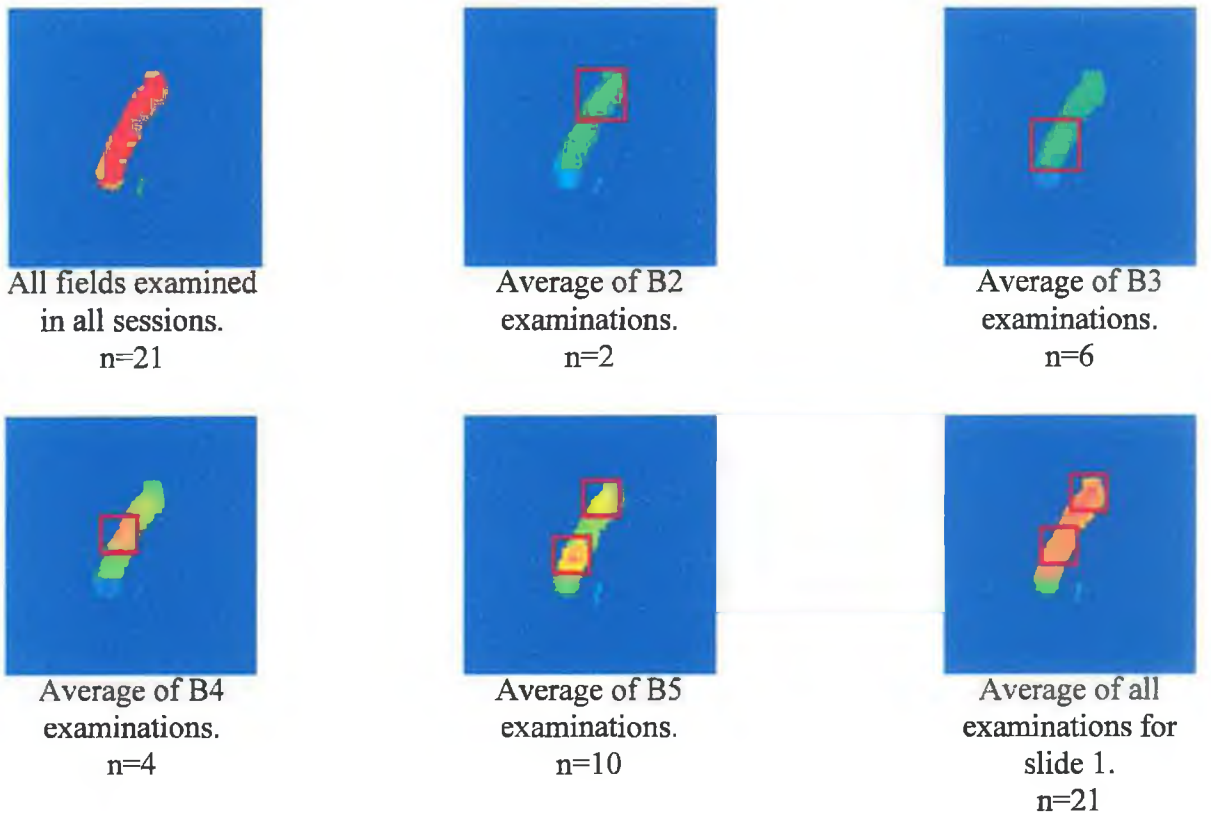


Figure 6.9 Pseudo-coloured, migratory trace composite of greyscale bitmapped images generated for Slide 1.

6.5 Conclusion:

The development of Bitmapper as an analysis tool provides an exciting insight into inconsistencies in histological diagnosis. It permits the profiling of individual slide examinations in terms of magnifications used and regions examined. For the first time it provides pathologists with the ability to obtain definitive reasons for observer variation based on improper fields selection.

Bitmapper was initially developed as a desktop application for analysis of migratory data from this study, however, there exists considerable scope for further development. The application is currently being developed to run on the VPS server as a Java servlet. For subsequent studies, it is proposed the servlet will run either as a scheduled task or through a user request from a HTML page. It is intended that the requirement for the use of third party software (Optimas) be removed, so that the generation of a tissue mask, removal of non-tissue areas, averaging and the treatment with pseudo-color occur all within the new application.

As stated in Chapter 5, the image quality of Slide 9 was substandard. The Slide was consequently rescanned. The image mask of the current scan that depicts where tissue exists therefore does not correspond to early examination traces. The generation of diagnostic traces of early examinations of Slide 9 would therefore be invalid. Therefore no attempt was made to analyze Slide 9 examinations using bitmapper.

In the next chapter, hotspots regions for each diagnostic grade are identified for each of the slides, apart from slide 9. The morphological features of these regions are examined by a breast specialist and an attempt is made to explain reasons for inconsistency in diagnoses submitted. This will permit the usefulness of Bitmapper as a forensic tool in determining diagnostic inconsistency to be evaluated by a pathologist specialist in breast lesions.

Chapter 7:- Analysis Of The Behavioural Process Of Microscopic Diagnosis Using Bitmapper

7.1 Introduction

The process of histopathological diagnosis is founded on adequate field selection. If the information for making a correct microscopic diagnosis is omitted, the pathologist will fail to make the correct decision. However, from the moment a tissue sample is selected for removal by a surgeon, the amount of tissue that will be examined at a microscopic level diminishes until the slide is finally prepared. This reduction in diagnostic material available for examination is illustrated in Figure 7.1.

Figure 7.1 graphically documents the entire process of microscopic diagnosis from sample retrieval through to selection, preparation, examination, and interpretation. A tissue sample is selected for removal by a surgeon, If a successful excision is performed, this will include the entire tumour in addition to a shaving of normal tissue around the margins. The pathologist may cut a sample of the tumour away for macro examination. Suspicious sample parts are selected for glass slide examination. The pathologist will perform a raster scan of the glass slide at a low magnification (usually in the order of 2.5x to 4x) so as to select suspect diagnostic fields of view. Each of these suspicious regions will receive a micro examination at a higher magnification (10x to 20x), from which diagnostically important fields of view will receive a thorough examination at 40x. [80] The removal, selection and preparation of tissue for examination are obviously critical to making a successful diagnosis. However, it is the process of micro examination and interpretation that this thesis attempts to quantify and analyse.

This chapter explores the utilisation of diagnostic traces generated by Bitmapper to determine if diagnostically important hotspots can be identified on a slide and whether examination of these areas is crucial to delivering a consensus diagnosis.

Bitmapper is an application that constructs a bitmap image. The image depicts the diagnostic trace of a VPS slide examination by graphically representing the tissue area examined and the magnification at which the area is viewed at. The development and functionality of Bitmapper is described in Chapter 7. The utilisation of imaging

techniques to develop an averaged composite diagnostic trace from multiple bitmaps is also described.

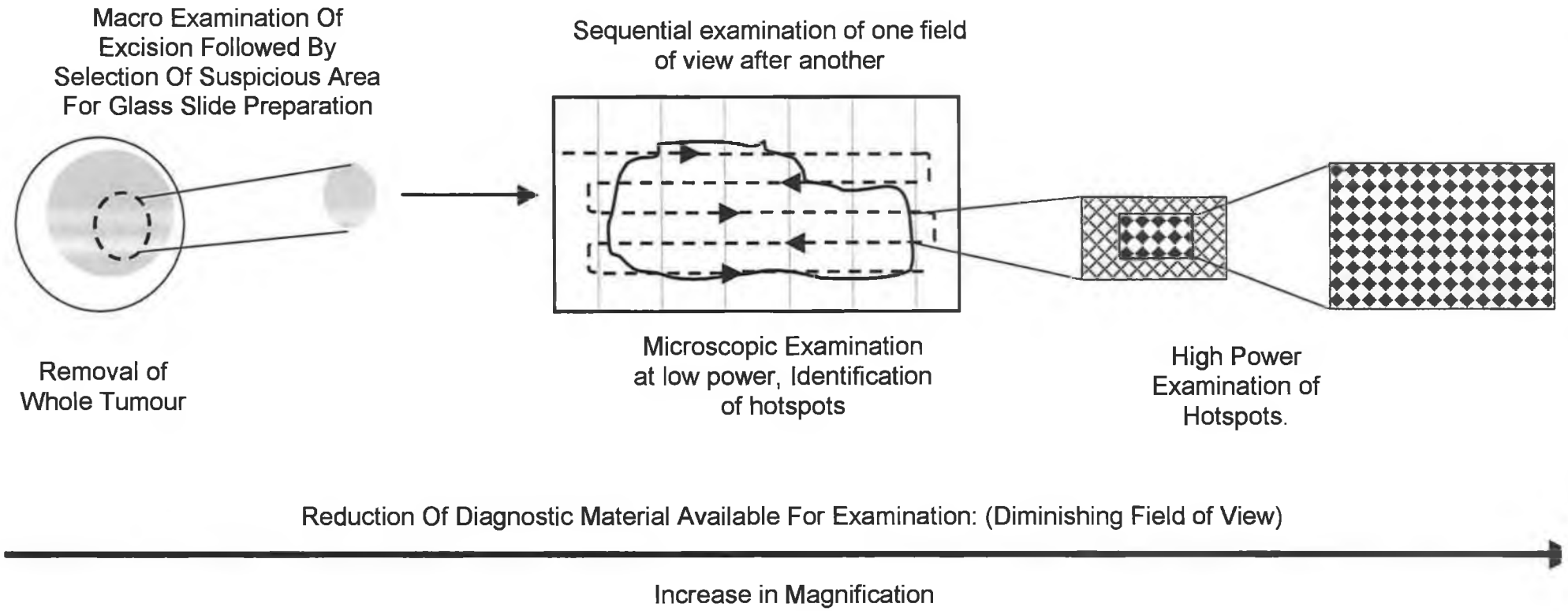


Figure 7.1 Illustration of a typical sequence of events leading to microscopic diagnosis from sample retrieval through to selection, preparation, examination, and interpretation. Illustration demonstrates the reduction in diagnostic material available for examination as the process proceeds.(Adapted from Hosch et al 2001)[80].

In order to evaluate the potential of Bitmapper and to provide insight into the examination behaviour of pathologists this chapter will attempt to determine the following.

- For a particular slide, determine if one or more diagnostically important hotspots for each diagnostic grade can be readily identified. Where a hotspot region is not readily observed, determine reasons why, such as, for example, an insufficient number of examinations for that grade.

- For a particular slide, determine if high or low magnification power predominantly contributed to the diagnostic grade submitted e.g. B2 examination was predominantly examined using low power whereas high power magnification was predominantly used by participants who submitted a B5 grade.

- If a hotspot for a diagnostic grade that disagreed with the consensus is in close proximity to a hotspot where diagnostic grade is correct, can it be inferred that participants who submitted the incorrect grade predominantly did so due to misinterpretation- *looking in the correct region but making an incorrect diagnosis.*

- If a hotspot for a diagnostic grade that disagreed with the consensus is geographically distant to a hotspot where diagnostic grade is correct, can it be inferred that participants who submitted the incorrect grade predominantly did so due to observational error- *looking in the wrong place*.

- Determine if participants who submitted the incorrect diagnostic grade did so due to observational error or mis-interpretational error or use of low magnification.

- Determine if participants who submitted the correct diagnostic grade predominantly used high power magnification and examined tissue in the correct region of interest.

- It is evident that observational error or mis-interpretational error or use of low magnification is the predominant cause of diagnostic error for a slide with poor diagnostic agreement.

- Review the diagnostic traces of a participant for each slide. Determine if the diagnostic patterns of a participant are similar for each slide. Determine if their errors are predominantly due to observational error or mis-interpretational error or use of low magnification.

In summary, this chapter evaluates the utilisation of diagnostic traces generated by Bitmapper to determine if diagnostically important hotspots can be identified on a slide and whether examination of such areas are crucial to delivering a consensus diagnosis. This chapter also explores whether the use of such diagnostic traces can elicit and differentiate behavioural traits of individual pathologists with both excellent and poor consensus and determine reasons for the submission of a discordant diagnosis. This will be accomplished by selecting images from hotspots identified by Bitmapper diagnostic traces and presenting them to a pathologist for their interpretation.

7.2 Evaluation of Bitmapper as a Useful Tool in Identifying Potential Diagnostic Hotspots.

To facilitate the evaluation of Bitmapper generated images and the screening of diagnostic traces of a specific participant or a particular slide, a PHP script was developed which permits users to select to display the following:

- Bitmaps describing the diagnostic trace of each examination for a particular slide, sorted by diagnostic classification. This dynamically generated page also includes a bitmap of the averaged diagnostic trace for all examinations of that slide and the averaged diagnostic trace for each diagnostic grade submitted for that slide

- Bitmaps describing the diagnostic trace of each slide examination for a particular user. For each slide examined by a user, this dynamically generated page also includes a bitmap of the averaged diagnostic trace for all examinations for that Slide and the averaged diagnostic trace for the diagnostic grade, which they submitted.

This PHP script is available for viewing at:
http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php

For each slide, fields of view were selected and presented to Professor Peter A Dervan for his interpretation. The exact protocol for selecting fields of view for examination by Professor Dervan is as follows.

- Potential diagnostic regions were identified from Bitmapper examination traces for each diagnostic category submitted for each slide.

- Fields of view were manually selected from the identified hotspots at 32x, 125x, 500x and 2000x.

- Images from each for each diagnostic category of each slide were presented to Professor Dervan who was asked to provide a diagnostic classification and

commentary of the histology of the images. During this process Professor Dervan was not aware of the slide he was examining or the consensus diagnosis of that slide.

- For image sets associated with discordant diagnosis, Professor Dervan was informed of the diagnosis submitted and asked to provide reasons for the discordant diagnosis submitted based on the histology of the images.

It is important to note that for some slides such as slide 7 the diagnostically important hotspot for each of the categories was located in the same region.

Where participants had performed extensive examination of a slide such as Slide 3 it was difficult to locate exact fields of view that could be of diagnostic importance. In this circumstance fields of view were manually selected at 32x, 125x, 500x and 2000x from lower, middle and upper regions of the Slide.

7.2.1 Examination Profiles of Slide 1.

Consensus diagnosis for Slide 1 was B5. There is significant variation in the diagnostic grades submitted for Slide 1, indicating a degree of difficulty with this case. This makes it particularly suitable for studying differential examination profiles in comparison to a case with low inter-observer variation.

Professor Dervan was presented with static images containing hotspots regions characterised from the averaged bitmap of B2, B3, B4, and B5 regions (Figure 7.2).

Professor Dervan's initial impression was that Slide 1 appeared to look like carcinoma '*however there is a considerable amount of apocrine change*'. This is a secretion from the exocrine glands in which part of the secreting cell is released with the secretion. This is what gives the tissue in slide 1 its rich pink appearance. After further examination, Professor Dervan felt that evidence of solid carcinoma and apocrine was present in all fields of view. The presence of so much apocrine increases 'the threshold for malignancy'. Consequently additional proof that Slide 1 is a carcinoma is required before a decision can be made. Professor Dervan felt that this was why there was such a spread of diagnostic categories submitted for Slide 1 and why Slide 1 achieved a poor consensus diagnosis.

There are distinctly visible hotspot regions for each diagnostic grade submitted. However, by reviewing the individual slide examination profiles it is evident that it is not necessary to review a hotspot region identified for a particular grade in order to submit that grade.

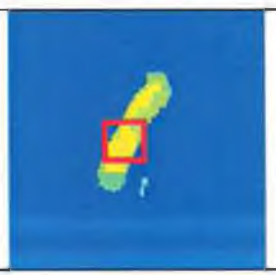
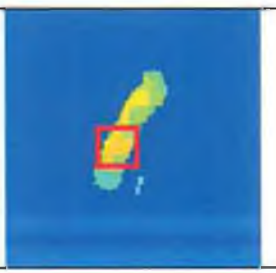
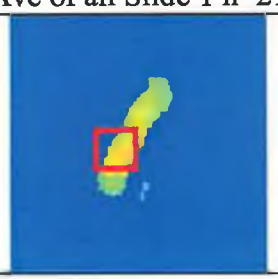
Participant 41 was the only Participant to submit a B2 diagnosis. This would have resulted in the patient not receiving the treatment they required. Professor Dervan was shown the fields of view examined by Participant 41 who submitted a B2 category and was of the opinion that the area viewed by Participant 41 was definitely not a benign proliferation and that they should have been suspicious of carcinoma.

Participant 41 submitted the diagnostic comment "*microglandular adenosis*". This is also known as microglandular hyperplasia. It is a rare benign irregular cluster of small tubules which are present in adipose or fibrous tissues, resembling tubular carcinoma but lacking stromal fibroblastic proliferation. Professor Dervan was strongly of the opinion that neither the fields of view nor indeed any part of Slide 1 contain evidence

of microglandular adenosis, that the term was 'carelessly' used by Participant 41 without any understanding of its meaning. Furthermore, microglandular adenosis retains the potential to evolve into carcinoma. According to the reporting guidelines if Participant 41 had truly believed it was microglandular adenosis they should have classified it as B3. Figure 7.3 depicts the region of Slide 1 that Participant 41 examined at high magnification.

Images used to compile Figure 7.2 and individual bitmaps for Slide 1 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=1

			
	Ave of all Slide 1 n=21	Ave of B2 For Slide 1 n=2	Ave of B3 For Slide 1 n=6
			
Ave of B4 For Slide 1 n=4	Ave of B5 For Slide 1 n=9	Session No=268 User Id=39 Classification=B5	Session No=29 User Id=41 Classification=B2

Percentage Consensus for Slide 1: 52.9%.
Consensus Diagnosis: B5.

Figure 7.2 Bitmaps depicting the average diagnostic trace for all examinations of Slide 1 and the averaged diagnostic trace for each diagnostic classification. Where possible, observable hotspots are identified.

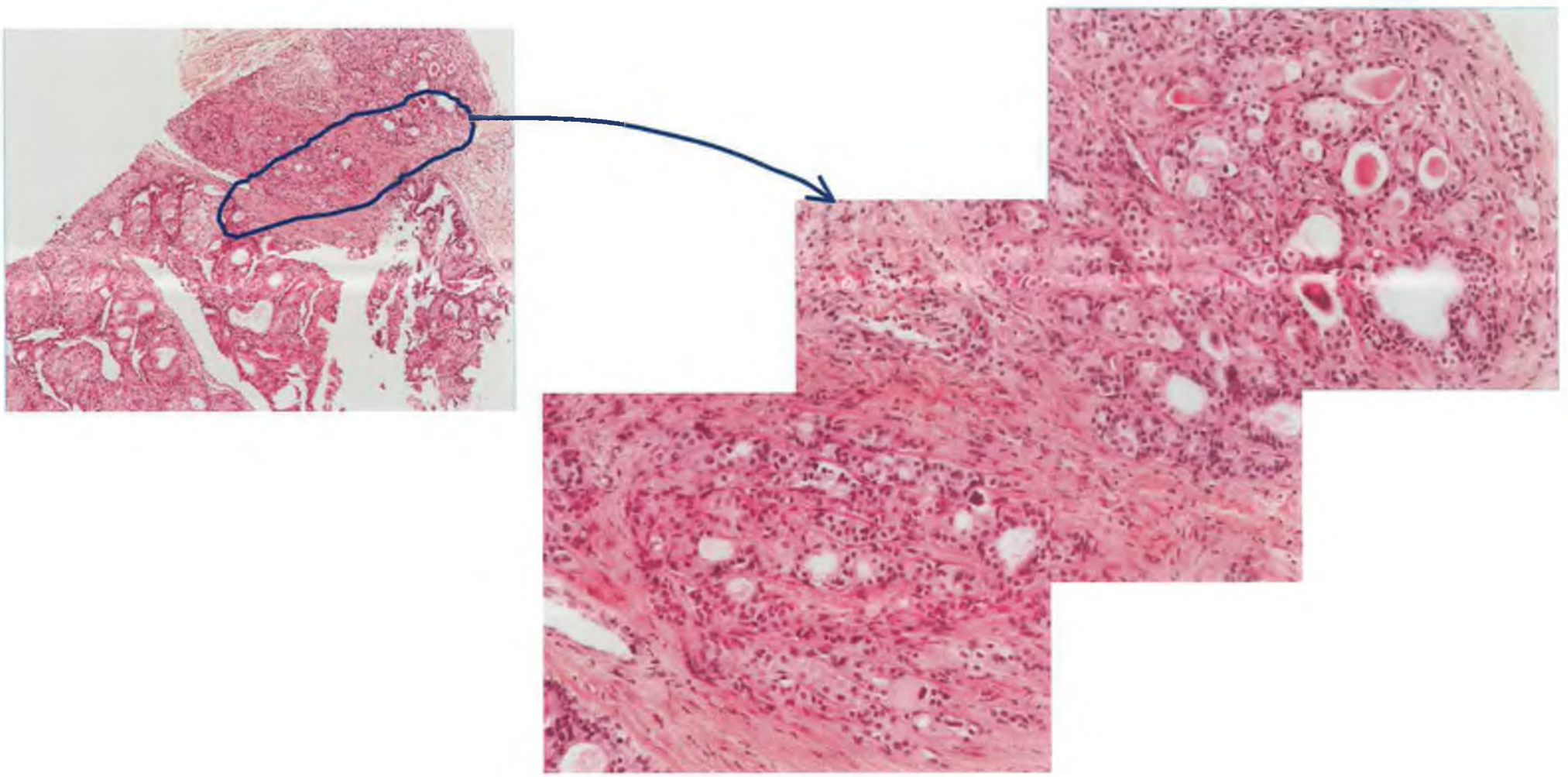


Figure 7.3 Sample area of region selected by Participant 41 for examination. Participant 41 misinterpreted the presence of *"microglandular adenosis"* from this region. According to Professor Dervan, the histology of the region examined by participant 41 in no way resembles *"microglandular adenosis"*.

7.2.2 Examination Profiles of Slide 2

Consensus diagnosis for Slide 2 was B5. Grade by grade analysis shows that of the nineteen examinations, only one participant, Participant 6, deviated from Slide consensus by submitting grade B2. This would have resulted in the patient not receiving the treatment they required.

For grade B5 it is difficult to discern 'hotspot' areas due to the dispersed diagnostic examination pattern amongst the participants.

Figure 7.4 illustrates that Participant 6 examined areas of the slide that other participants examined at the same magnification. It is possible to infer from the diagnostic trace that the discordant grade submitted by Participant 6 resulted from misinterpretation.

Without knowing the consensus classification for Slide 2, Professor Dervan was presented with the fields of view examined by Participant 6 (Figure 7.5) and was able to make the correct diagnostic classification indicating B5 infiltrating carcinoma.

From a technical perspective Slide 2 was poorly prepared and stained. In conjunction with the diagnostic classification B2, Participant 6 submitted the diagnostic comment

> *"multiple giant cells ? reaction to foreign material ?amyloid would like to see congo red stain would lijke to polarise as well"*

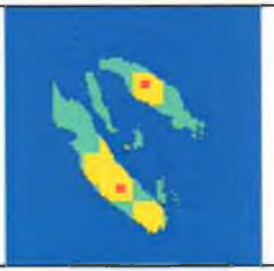
A foreign body giant cell is a very large cell formed by the fusion of multiple macrophages, typically epitheloid cells. The stimulus for fusion is a foreign particle that is too large for phagocytosis by one macrophage or has mechanisms to negate phagocytosis and killing mechanisms. The giant cell exhibits multiple nuclei, often arranged in the periphery of the cell, and can measure up to 60 micrometres in diameter. Foreign body giant cells are found at the core of granulomas produced by granulomatous inflammation.

Participant 6 also referred to amyloid presence which is a build-up of a fibrous protein. They also suggested use of Congo Stain. This is a stain used for amyloid detection in pathologic tissue. It gives red staining of amyloid and induces green birefringence under polarised light.

Professor Dervan reviewed the exact fields of view examined by Participant 6 and felt he understood why Participant 6 submitted a B2 category. Professor Dervan was of the opinion that the presence of giant multinucleated cells, could easily be misinterpreted as inflammatory multinucleated histocytes, However, this mistake should not have been made. Figure 7.5 illustrates fields of view examined by Participant 6 and the giant multinucleated cells they misinterpreted as B2 benign.

Images used to compile Figure 7.4 and individual bitmaps are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=2

			
	Ave of all Slide 2 n=19	Session No=34 User Id=6 Classification=B2	Session No=20 User Id=41 Classification=B5-Invasive
			
Session No=542 User Id=68 Classification=B5-Invasive	Session No=83 User Id=18 Classification=B5-Invasive	Session No=609 User Id=55 Classification=B5-Invasive	Session No=269 User Id=39 Classification=B5-Invasive

Percentage Consensus for Slide 2: 94.1%.
Consensus Diagnosis: B5.

Figure 7.4 Bitmaps depicting a examples of diagnostic traces of examinations for Slide 2 and the averaged diagnostic trace for each diagnostic classification.

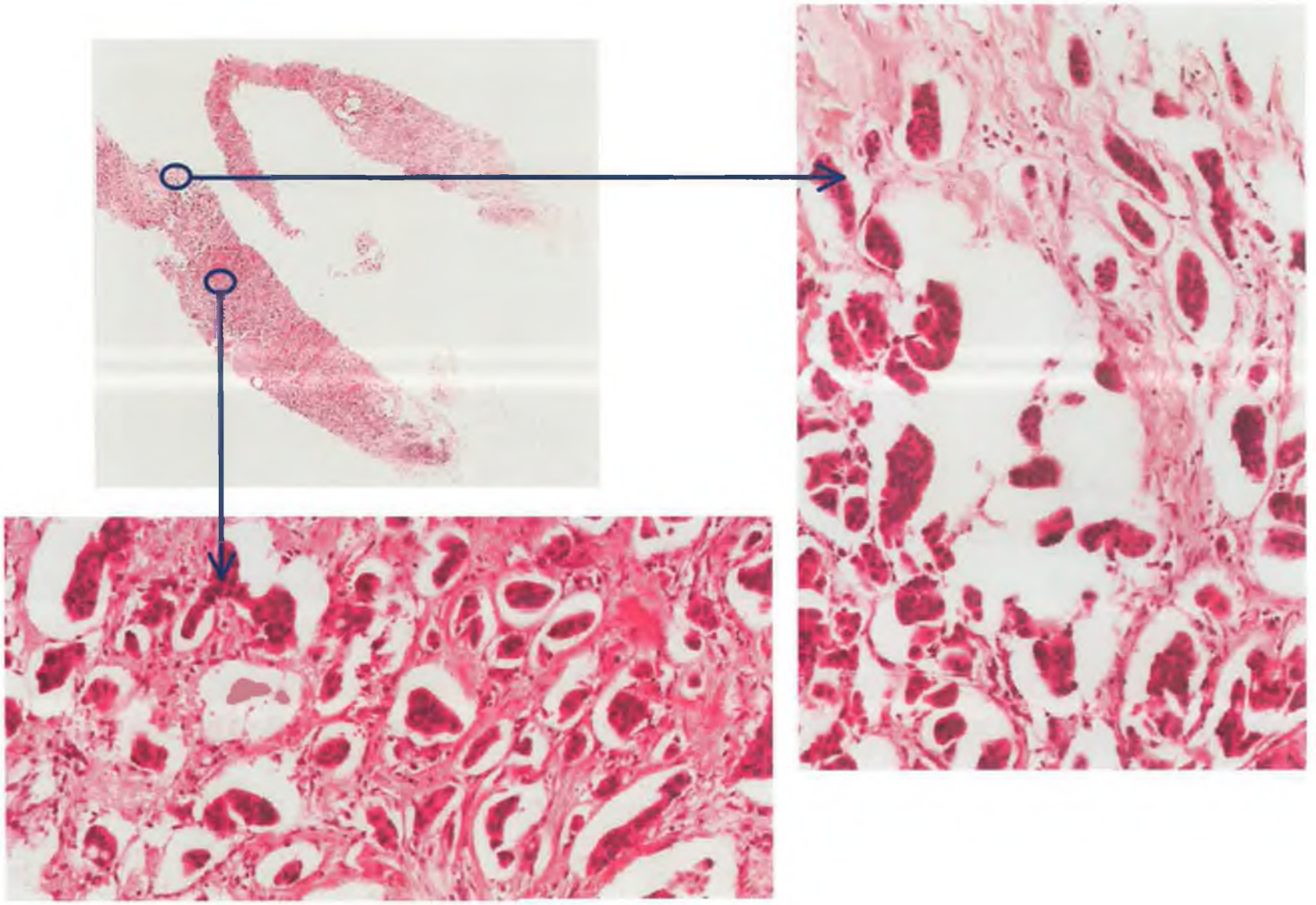


Figure 7.5 Fields of view Selected by Participant 6 for examination at 500x Participant 6 misinterpreted the presence of giant multinucleated cells as benign inflammation.

7.2.3 Examination Profiles of Slide 3

All Participants who examined Slide 3 used a medium to high power magnification. Consensus diagnosis was B5. Grade by grade analysis shows that of the nineteen examinations, sixteen graded B5, two graded B4 and one graded B2-Benign. (Participants 1, 55 and 39 examined Slide 3 twice).

The diagnostic patterns for grade B5 are well distributed. No hotspot was easily discernable. Participants tended to examine the upper, middle and lower section of the needle core.

Figure 7.6 shows the diagnostic pattern of participant 36 who submitted a B2 classification. Participant 36 examined regions at high magnification that are in close proximity to regions examined by other participants who classified Slide 3 as B5. Figure 7.6 shows diagnostic traces of participants who solely examined either the lower, middle or upper region of the Slide and submitted a B5 classification. It is therefore evident from the diagnostic traces that it was possible to render a B5 classification regardless of the region examined.

Fields of view at 125x and 500x from each of the lower, middle and upper regions were presented to Professor Dervan.

Still images from the lower region were initially examined from which it was immediately discernable that Slide 3 represented tubular carcinoma. The characteristic morphology of tubular carcinoma is illustrated in Figure 7.7.

Tubular carcinoma is described by neoplastic cells which form a single cuboidal layer in small round to teardrop or oval shaped ductules, widely spaced in a fibrous stroma. The prognosis tends to be better than for an intraductal carcinoma hence tubular carcinoma receives a category of B3 or higher. However, tubular carcinoma is known to be extremely difficult to interpret and is difficult to separate from sclerosing adenosis or radial scar. This explains why no readily discernable hotspot was identifiable from Slide 3. Participants were searching for additional evidence to allow them to categorise the severity of the carcinoma correctly.

The tissue in Slide 3 was considered by Professor Dervan to be extremely difficult to interpret. The slide was composed of 'innocuous glands'- some were normal and some were suspicious thereby distorting the normal architecture of the breast.

When examining the upper region of the slide, Professor Dervan identified the presence of solid components that indicated an invasive carcinoma. This has a much more serious implication for patient prognosis and provided Professor Dervan with the evidence required to render a B5 diagnosis.

Participant 36 submitted a B2 diagnostic category with the comment for Slide 3.

'*Sclerosing adenosis*'

This is a benign condition where extra tissue grows within the breast lobules. Professor Dervan was shown the exact fields of view examined by Participant 36 (Figure 7.7) and felt that Participant 36 did not '*appreciate the invasiveness of the tubules*'. As the diagnostic trace of Participant 36 in Figure 7.7 shows, Participant 36 only examined the mid region of Slide 3 at 125x and 500x. It is evident that Participant 36 misinterpreted tubular carcinoma for sclerosing adenosis. This is not an uncommon mistake. It is probable that had Participant 36 examined the upper region of the slide where evidence of invasive carcinoma is greatest, they would have made a more accurate diagnosis.

Three participants (7, 35, 39) submitted a diagnostic comment '*tubular carcinoma*'. All three Participants classified the slide as B5, perhaps indicating they appreciated the severity of the case. All other participants who classified the slide as B5 and submitted a diagnostic comment indicated they had identified the presence of infiltrating ductal carcinoma. Figure 7.7 illustrates from the diagnostic traces of Participants 35 and 39 that they had examined the upper region of the slide where there is extensive evidence of invasive carcinoma at 500x and 2000x magnification. Despite this, they only indicated the less serious presence of tubular carcinoma.

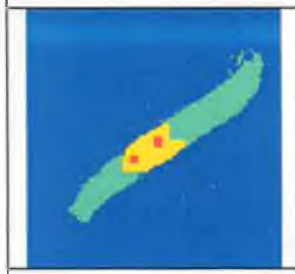
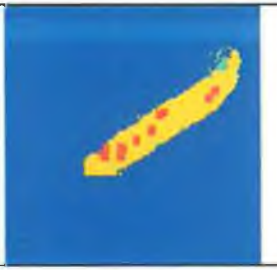
It is evident from Participant 7's diagnostic trace illustrated in Figure 7.7 that they only reviewed the lower region of Slide 3 at 500x and 2000x. Based on Professor Dervans examination of this region it is insufficient field selection by Participant 7 in the mid to upper region of the slide that prevented them from appreciating the presence of infiltrating carcinoma.

During the interview, Professor Dervan again voiced his opinion that the slide was difficult to interpret due to the level distortion of tissue architecture. The difficulty with this slide lay in deciding whether to categorise it as B3 or higher. This difficulty in interpreting the slide is reinforced by the comments of Participant 10 who submitted a correct B5 diagnostic category but indicated

> '*Not confident as unable to visualise cells adequately. My initial impression was that this was sclerosing adenosis*'.

Images used to compile Figure 7.6 and individual bitmaps are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=3

			
	Ave of all Slide 3 n=19	Ave of B4 For Slide 3 n=2	Ave of B5 For Slide 3 n=16
			
Session No=114 User Id=36 Classification=B2	Session No=304 User Id=7 Classification=B5-Invasive	Session No=278 User Id=35 Classification=B5-Invasive	Session No=685 User Id=39 Classification=B5-Invasive

Percentage Consensus for Slide 3: 82.4%
Consensus Diagnosis: B5.

Figure 7.6 Bitmaps depicting a examples of diagnostic traces of examinations for Slide 2 and the averaged diagnostic trace for each diagnostic classification.

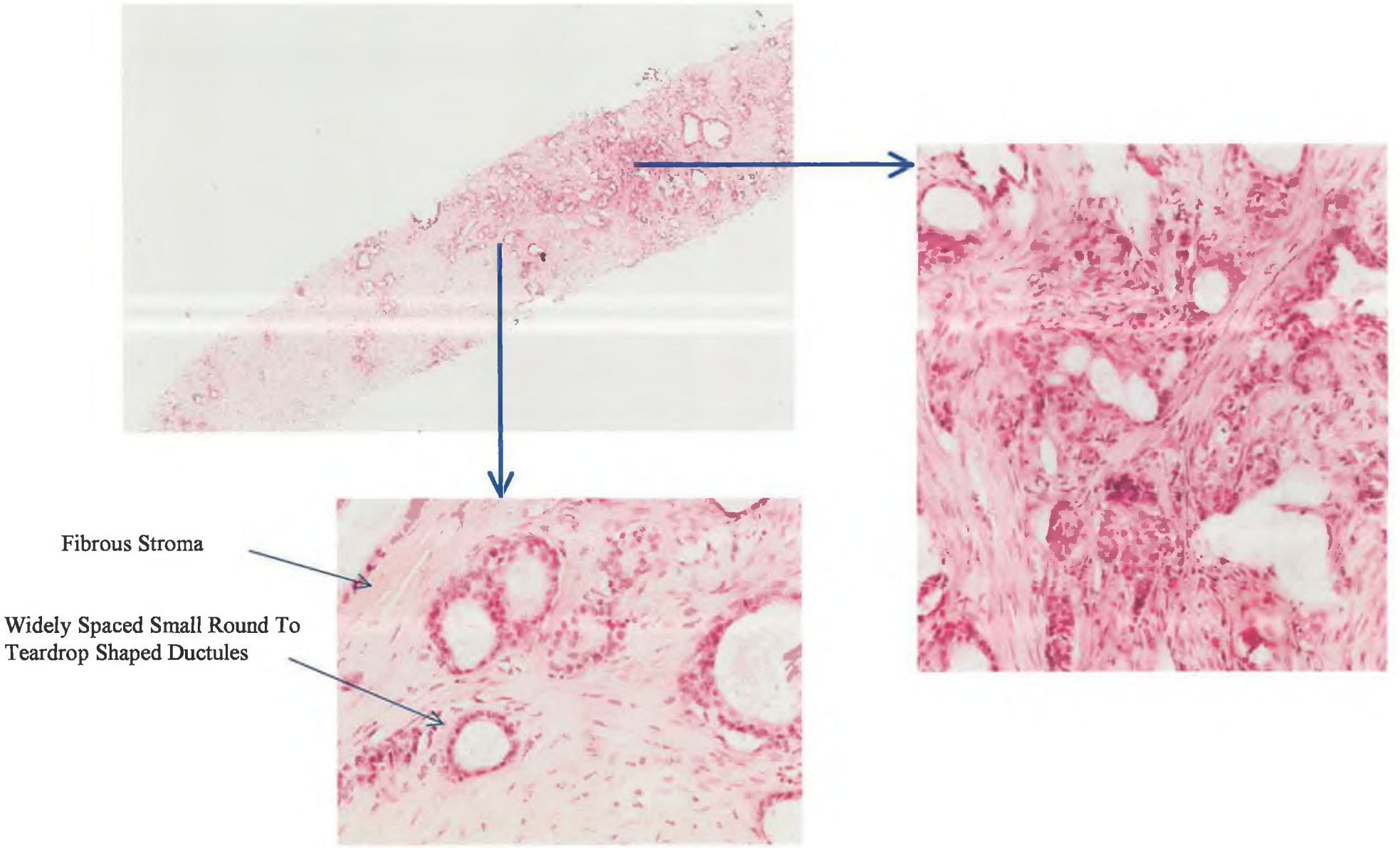


Figure 7.7 Fields of view selected by Participant 36 for examination at 500x Participant 36 failed to identify tubular carcinoma or the extensive presence of invasive cancer cells.

7.2.4 Examination Profiles of Slide 4

Three needle cores were presented in Slide 4. Of a total of nineteen examinations performed, four classified the slide as B1-Normal and the remaining fifteen examination classified it as B2-Benign.

For the B1 graded slide examinations a hotspot region is identifiable in the upper region of the core on the right. This area was examined at 500x by three Participants and at 2000x by two of the three Participants. For B2 graded slide examinations this area was also examined at 500x by seven Participants and at 2000x by five of the seven Participants.

For B2 graded slide examinations, a hotspot region is identifiable in the upper region of the core on the left. Eight participants examined this upper left region of the slide at 500x and four of the eight examined the area at 2000x. Only one participant (User 87) who graded B1 examined this area. Despite using 2000x to examine this region User 87 provided a discordant diagnosis.

All hotspot regions identifiable using Bitmapper individual diagnostic traces correspond to regions in the tissue that contain ducts. An example of ducts identified in this manner is illustrated in Figure 7.9.

According to Professor Dervan, high magnification was not necessary to render a diagnosis with this slide. Professor Dervan was presented with a 125x image depicting the hotspot region examined by participants who submitted a B1 category. Microcystic change was identifiable from the image. Professor Dervan categorised the images as B2 fibrocystic change. Fibrocystic changes are commonly present in otherwise normal breast and usually do not require treatment.

When asked why 4 participants had submitted a B1 category, Professor Dervan explained that there is a just cause as to why a biopsy takes place (palpable lump, suspicious ultrasound or mammogram) submitting a B1 category indicates that nothing was found on the slide to justify the sampling of a biopsy. This may indicate sampling error and result in another biopsy being taken. Submission of a B2 category justifies the

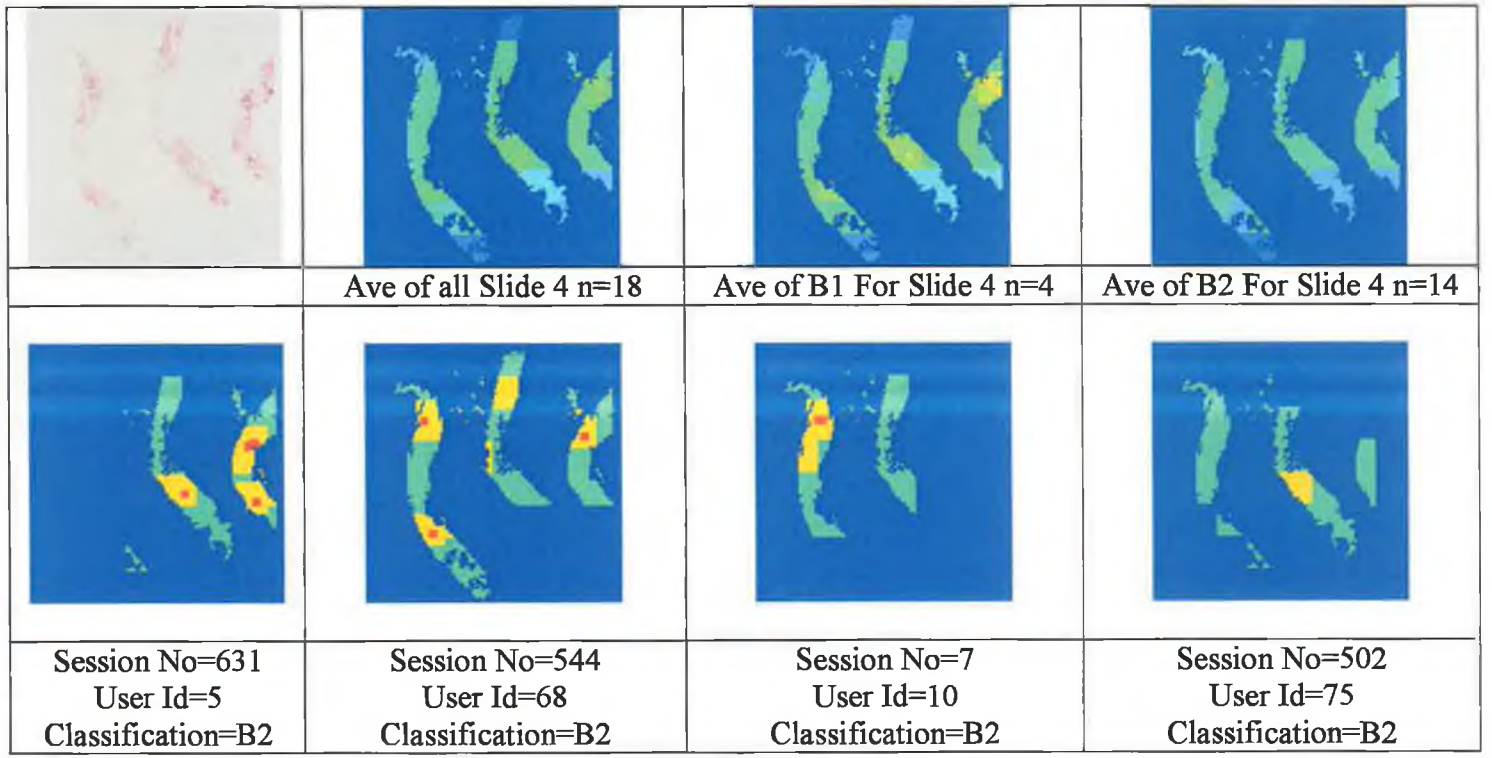
taking of a biopsy without further surgical intervention or treatment of the patient required.

Participant 75 submitted a B2 category for Slide 4 with an appended diagnostic comment 'Hamarthoma'. This is a circumscribed benign nodule composed of variable adipose tissue, fibrous stroma, and glandular tissue, often referred to as a 'breast within a breast'. Hamartoma of breast has distinct mammographic features with circumscription and fat and soft-tissue density surrounded by a thin radiopaque capsule.

Professor Dervan indicated that the histology of breast hamartoma is not considered to be well defined and that the condition is diagnosed by x-ray or ultrasound only. This is accomplished by means of identifying the peripheral radiolucent zone (radiopaque capsule). Pathologists would not use the term hamartoma unless they aware were of this distinctive mammographic finding. There was no mention of the term hamartoma in the case notes, it was therefore improper for Participant 75 to submit such a comment.

Images used to compile Figure 7.8 and individual bitmaps for Slide 4 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=4



Percentage Consensus for Slide 4: 77.5%.
Consensus Diagnosis: B5.

Figure 7.8 Bitmaps depicting the average diagnostic trace for all examinations of Slide 4 and the averaged diagnostic trace for each diagnostic classification. It is noticeable that the hotspots participants evaluated directly correspond to ducts in the tissue.

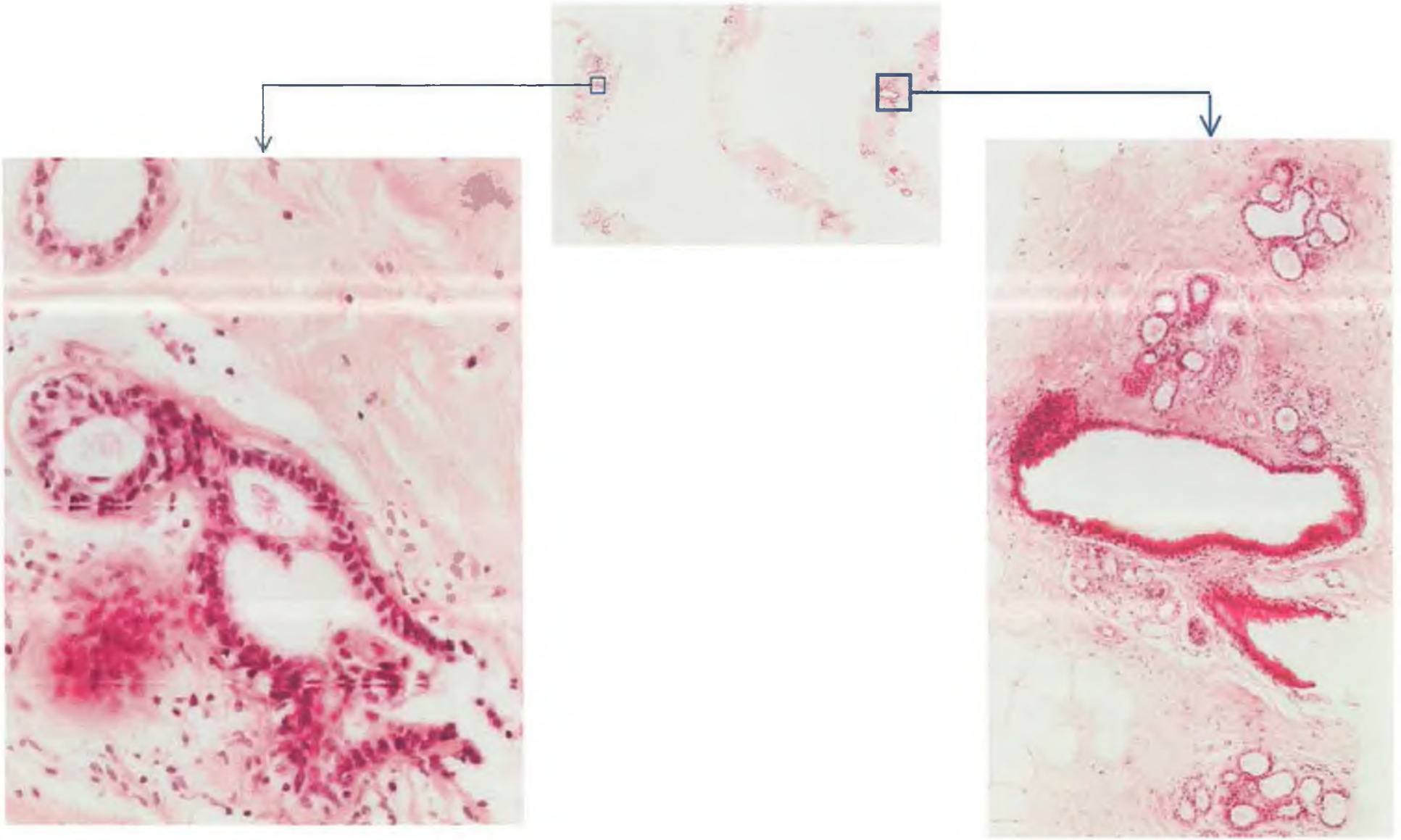


Figure 7.9 Fields of view showing normal ducts with evidence of fibrocystic change. These were selected from the diagnostic traces of a number of participants.

5 Examination Profiles of Slide 5

Slide 5 provides a very interesting profile in that 4 different diagnostic grades were submitted for this slide ranging from B2-Benign to B5-Invasive. This variability reflects the degree of difficulty in determining a correct diagnosis for this slide. Slide 5 achieved the second lowest slide consensus at 47.1%.

All participants examined areas of tissue at 2000x magnification apart from user 75 who submitted a consensus diagnosis of B5 and examined at the highest magnification power of 125x, For all diagnostic grades submitted for Slide 5, the region of diagnostic importance is in close proximity to each other and clearly definable. Participants 39 and 55 performed the most through examination at 500x and 2000x, despite both submitting a discordant diagnosis of B3.

Despite examining a clearly identifiable 'hotspot' at 500x and 2000x magnification, a diagnostic grade discordant with the VPS Slide and the glass consensus diagnosis of B4 was submitted for twelve examinations. It is reasonable to infer from the diagnostic traces that this was due to misinterpretational error. A description of the histology of this hotspot is illustrated in Figure 7.11.

Using 125x and 500x static images, Professor Dervan initially examined the hotspot region identified for participants who categorised the slide as B3, B4 or B5. This is the region is illustrated in Figure 7.11. Professor Dervan asserted from what he saw that the pink area which occupies most of the lower region of the hotspot was probably benign apocrine metaplasia. This describes an alteration of acinar epithelium of breast tissue to resemble apocrine sweat glands; seen commonly in fibrocystic disease of the breasts. Directly above this region Professor Dervan identified gland distortion and possible sclerosing adenosis. From these images Professor Dervan concluded that the Slide probably was a B2 category but that he was suspicious and would like to see additional areas of the slides.

Professor Dervan examined the hotspot region identified from the examination traces of the participants who graded B2. A duct filled with cells in a cribform pattern (lacy

pattern of cellular arrangement) was identified indicating hyperplasia or low grade DCIS.

Using the 125x image, an invasive radial scar was identified. A radial scar is a star-shaped abnormality in breast tissue. They are thought to be benign changes caused by normal ageing, However, some radial scars contain small carcinomas or show pre-cancerous changes. Professor Dervan indicated this slide was at least a B3 possibly a B5. After some time, Professor Dervan concluded from the still images that Slide 5 was a B4 category.

Professor Dervan was of the opinion that Participants 7 and 36, both of whom had submitted a B2 category had misinterpreted the radial scar as benign. This was evident from the comment made by Participant 7…

'*area of sclerosis suggestive of radial scar*'

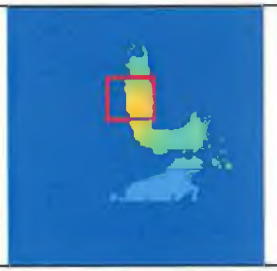
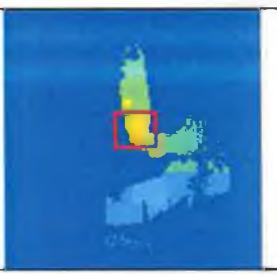
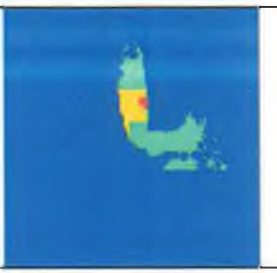
and Participant 36…

'*ductal papiloma in addition there is fibrocystic changes*'

Ductal papilloma is a benign wart-like growth. It can be precaneerous. According to Professor Dervan, Participant 36 should have classified their findings as B3.

Images used to compile Figure 7.10 and individual bitmaps for Slide 5 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=5

			
	Ave of all Slide 5 n=20	Ave of B2 For Slide 5 n=2	Ave of B3 For Slide 5 n=2
			
Ave of B4 For Slide 5 n=9	Ave of B5 For Slide 5 n=7	Session No=306 User Id=7 Classification=B2	Session No=117 User Id=36 Classification=B2

Percentage Consensus for Slide 5: 47.1%.
Consensus Diagnosis: B5.

Figure 7.10 Bitmaps depicting the average diagnostic trace for all examinations of Slide 5 and the averaged diagnostic trace for each diagnostic classification. Where possible observable hotspots are identified.

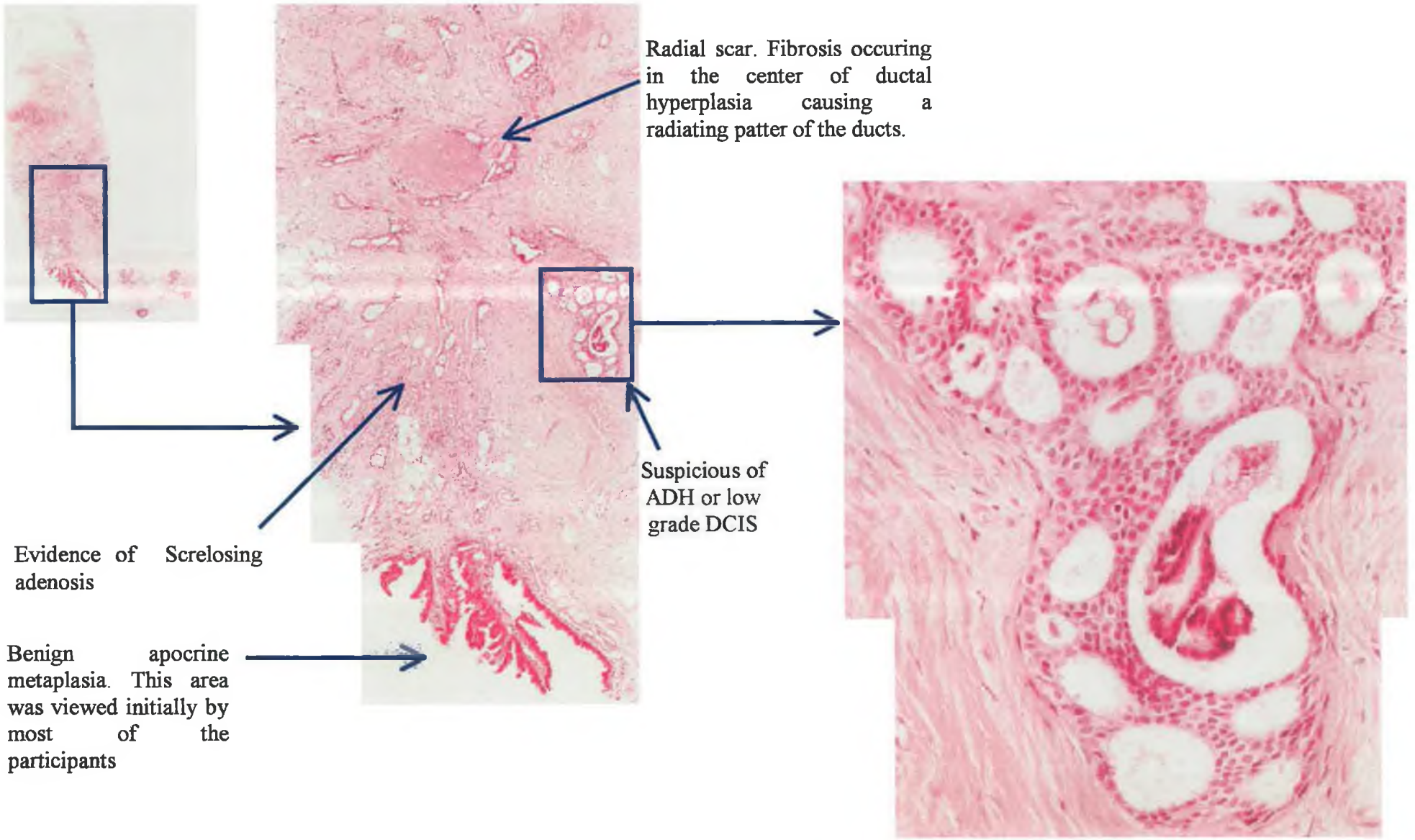


Figure 7.11 Fields of view selected from the hotspot region identified from the averaged bitmapper trace.

7.2.6 Examination Profiles of Slide 6

Slide 6 provides a unique case in that 100% diagnostic consensus amongst participants was achieved. A central region of interest is clearly identifiable from the bitmap diagnostic traces. This hotspot clearly corresponds to a darkened region of the core suggestive of carcinoma. All Participants navigated to this region. Apart from Participants 55 and 1 who examined the slide at a highest magnification of 125x, all other participants examined this hotspot at 500x and 15 out of a total of 20 examinations viewed this area at 2000x.

Slide 6 was clearly an 'easy' slide to diagnosis with an obvious hotspot discernible from the mid region of the Slide.

According to Professor Dervan, high magnification was not necessary to render a diagnosis with this slide. When presented with a 125x image depicting the hotspot region examined by all participants, Professor Dervan indicated he could render a diagnosis of B5 infiltrating lobular carcinoma.

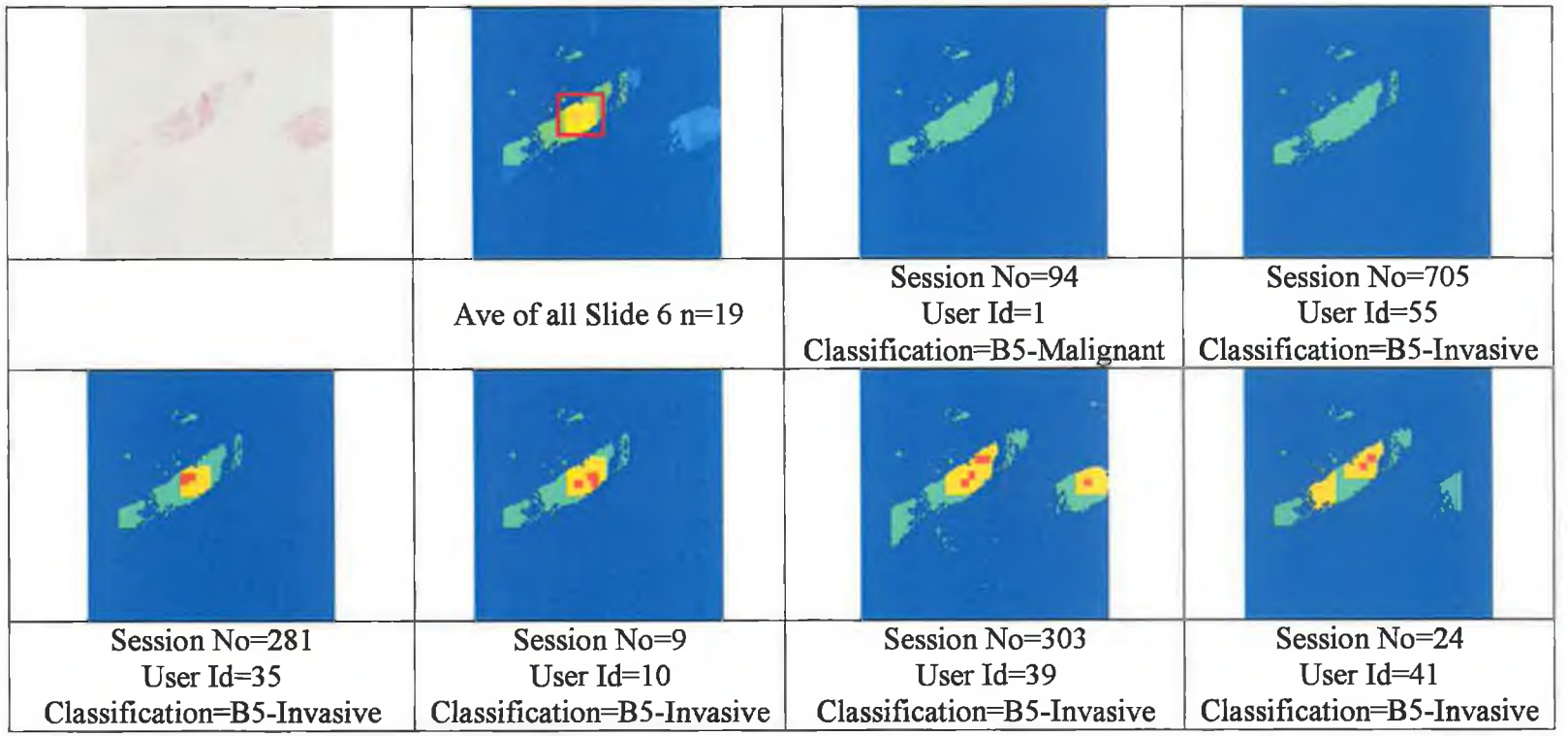
Participant 1 and 55 submitted a correct diagnostic classification without using a magnification higher than 125x when examining Slide 7.

Infiltrating lobular carcinoma accounts for up to 10% of invasive breast cancers. It is the second most frequent type of invasive breast cancer. The most common invasive breast cancer is infiltrating ductal carcinoma. Infiltrating lobular carcinoma originates in the lobules of the breast and progressively infiltrates the surrounding tissue. On mammography lobular carcinoma gives rise to spiculation, on physical examination it normally presents as a thickening of the breast tissue. All participants who submitted a diagnostic comment indicated lobular carcinoma. Images from Slide 6 illustrating infiltrating lobular carcinoma are shown in Figure 7.13

Professor Dervan indicated that when viewing 500x images from the 125x field of view he was not as sure of his diagnosis, 'it felt confusing', he was more capable of rendering a diagnosis on this specific slide at 125x.

Images used to compile Figure 7.12 and individual bitmaps for Slide 6 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=6.



Percentage Consensus for Slide 6: 100%.
Consensus Diagnosis: B5.

Figure 7.12 Bitmap depicting the average diagnostic trace for all examinations of Slide 6 in addition to the diagnostic traces of several other participants demonstrating participants interest in one particular region of the Slide.

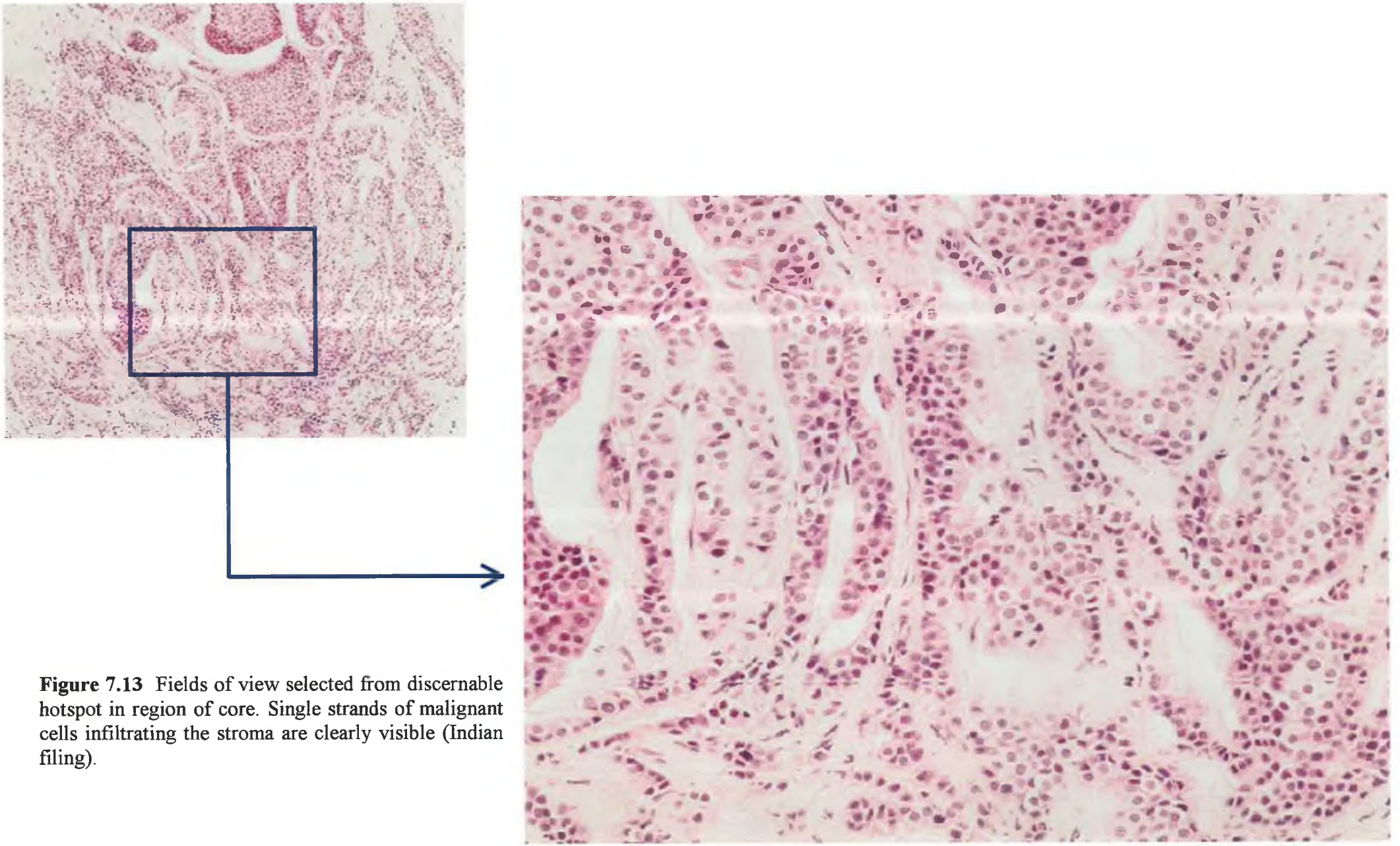


Figure 7.13 Fields of view selected from discernable hotspot in region of core. Single strands of malignant cells infiltrating the stroma are clearly visible (Indian filing).

7.2.7 Examination Profiles of Slide 7

The diagnostic consensus for Slide 7 was B2-Benign. The slide consensus for this diagnosis was 64.7%. A diagnostic hotspot is clearly identifiable in the upper region of the core. All participants examined this area at 500x and 2000x magnification except Participant 7 who examined the hotspot at 500x and submitted a consensus diagnostic decision of B2.

The most thorough examination was carried out by Participants 22 and 65 who submitted a consensus diagnosis of B2 and Participants 39 who submitted a discordant diagnosis of B3. It is reasonable to infer that participants who did not submit a consensus diagnosis of B2 due to misinterpretational error may have been over cautious in their interpretation of this case.

A cause for concern is the performance of Participant 1 in their examination of this Slide. Participant 1 performed two examinations, the first one was thorough and a B4 grade was submitted, The second examination was performed by Participant 1 in order to revise their diagnosis to a B5. Such an error could have serious consequences for a patient possibly resulting in unnecessary therapy and surgical intervention.

There was a clearly discernable hotspot region in Slide 7 that every participant examined. Professor Dervan reviewed static images of the region at low and high magnification and commented on its diagnostic value. When reviewing the 125x image Professor Dervan was of the opinion that the slide was benign but remained uncertain.

At 500x, Professor Dervan found evidence of sclerosing adenosis. By categorising this slide as B2 this patient will not undergo surgery. However, a categorisation of B3 or higher usually results in surgical excision. Participants 36, 39, 10 and 1 had submitted B3, B3, B3 and B4 classification respectively. Professor Dervan felt the categorisation by some participants of B3 was over cautious. This decision was most likely taken to exclude the possibility of a carcinoma.

Comments submitted by some of the above participants are as follows.

> *"Possibly sclerosing adenosis" (User 36- B3)*

"*DCIS*" (User 1- B4)

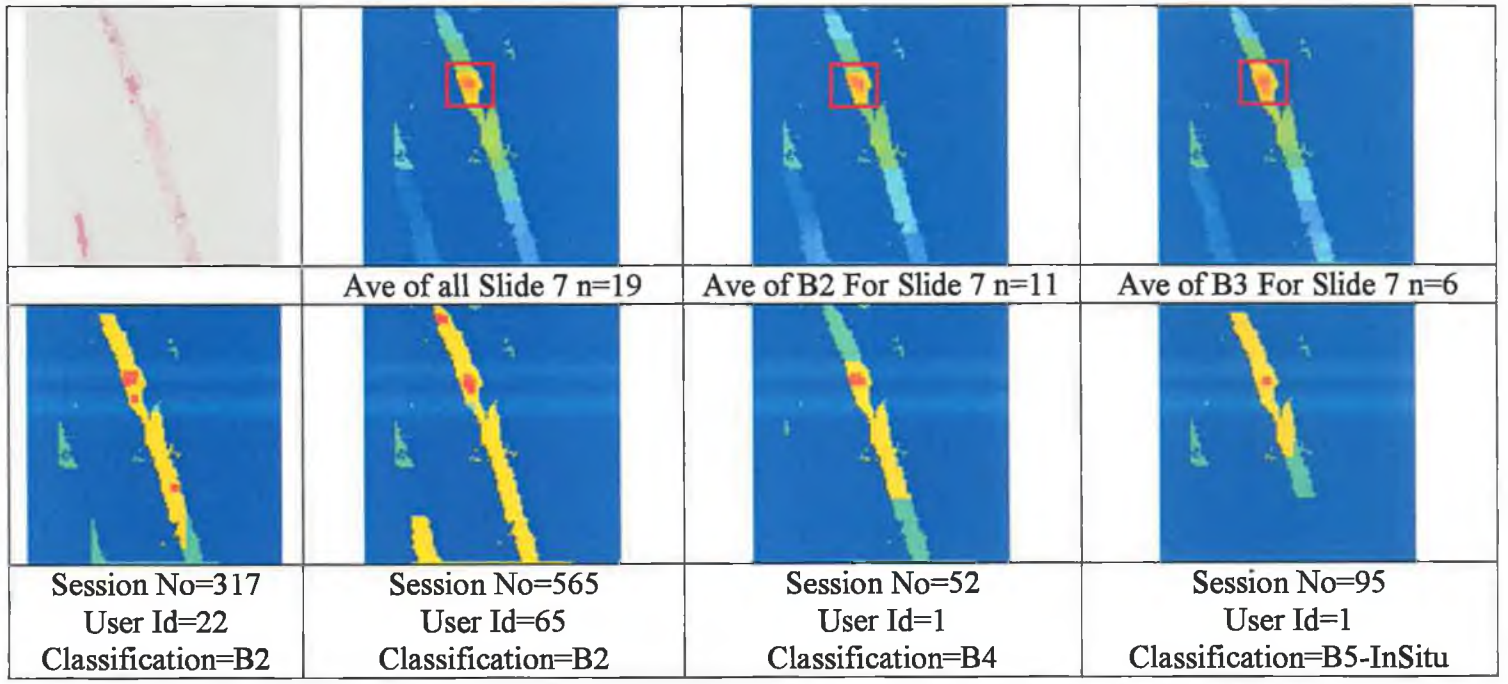
"*Atypical ductal hyperplasia/low grade DCIS*" (User 39- B3)

"*Best regarded as ADH*" (User 10- B3)

Professor Dervan indicated the tissue on the slide was to some degree architecturally distorted. The region surrounding the hotspot, which every participant examined, contained tissue with a lobular growth pattern consisting of a dual cell population with elongated cells comprised of indistinct borders giving rise to the misconception by some participants that they were viewing ADH or low grade DCIS. Professor Dervan was of the opinion that the participants who submitted a B4 and a B5 diagnosis may also have misinterpreted the presence of necrosis for carcinoma, and that inexperience was the principle reason for misinterpretation. Professor Dervan also indicated that if Participant 36 believed Slide 7 to be sclerosing adenosis, they should have submitted a B2 category.

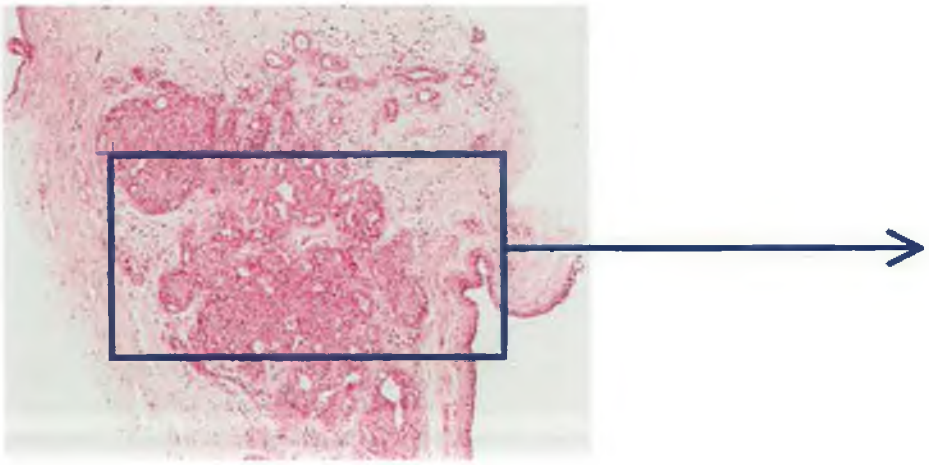
Images used to compile Figure 7.14 and individual bitmaps for Slide 7 are available at the following URL:

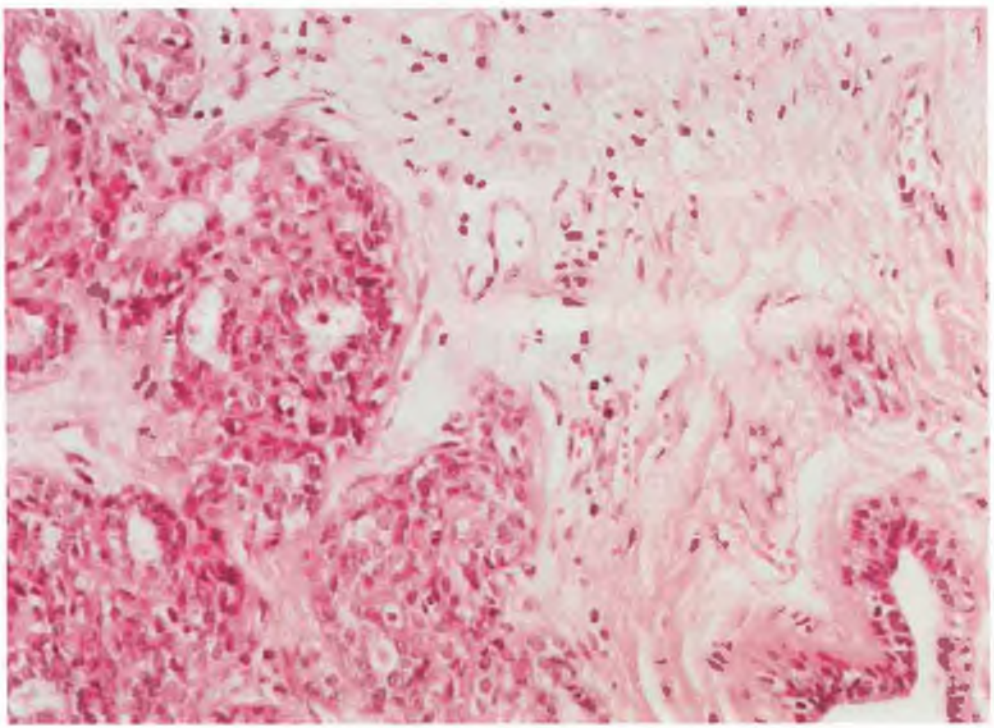
http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=7.



Percentage Consensus for Slide 7: 64.7%.
Consensus Diagnosis: B2.

Figure 7.14 Bitmaps depicting the average diagnostic trace for all examinations of Slide 7 and the averaged diagnostic trace for B2 and B3. The diagnostic trace of the most thorough examinations by Participants 22 and 65 are also illustrated in conjunction with the diagnostic traces of Participant 1 who submitted an incorrect diagnosis.





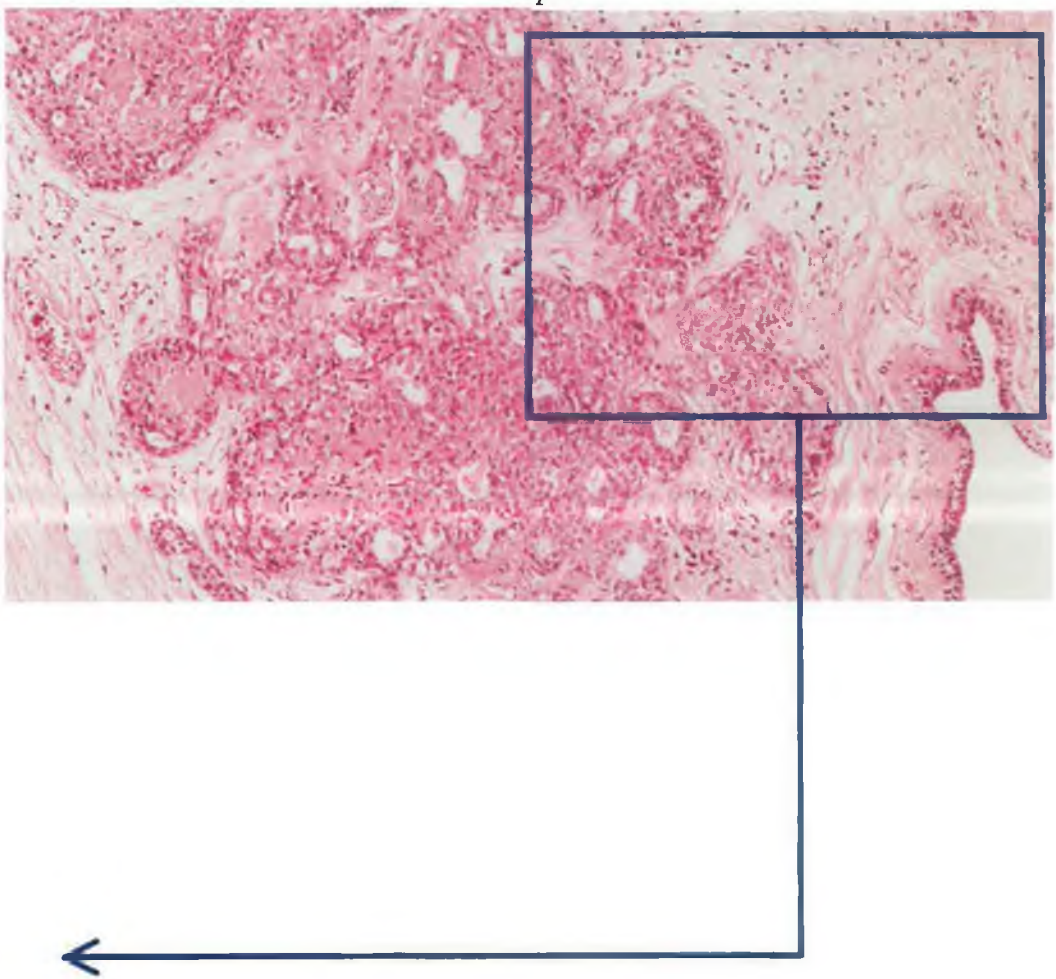


Figure 7.15 Fields of view selected from discernable hotspot in upper core. Dual cell population with elongated cells comprised of indistinct borders are clearly visible. This architecture was mistaken by some participants as ADH or DCIS.

7.2.8 Examination Profiles of Slide 8

Slide 8 achieved the lowest diagnostic agreement of 35.3%. It is also the only slide where initial VPS consensus slide diagnosis (B4) differed to that of the glass slide diagnosis (B3).

All participants examined regions of the slide using high power 2000x magnification except participant 36 and 75 who used a maximum of 500x magnification and submitted a discordant diagnostic grade of B2. However, at 125x Professor Dervan indicated he could observe lobular infolding indicative of an intraductal papilioma lesion. While he could make the correct diagnosis at the magnification, the difficulty was in determining its stage of progression, i.e. whether it was B3 or higher.

Intraductal papillomas are non-cancerous wart-like growths with a branching or stalk that has grown inside the breast. Occasionally, multiple papillomas may be found further from the nipple. Intraductal papilloma may be associated with a serous or bloody nipple discharge, or it may cause some nipple retraction. Of the benign conditions that cause nipple discharge, approximately half are due to papillomas. However, despite being a benign lesion, statistically they are known to be atypical or pre-cancerous and are therefore classified as B3 or higher.

Participant 1 initially graded the slide B1 stating they could not view the slide correctly. They subsequently re-examined the Slide and submitted a B4 grade.

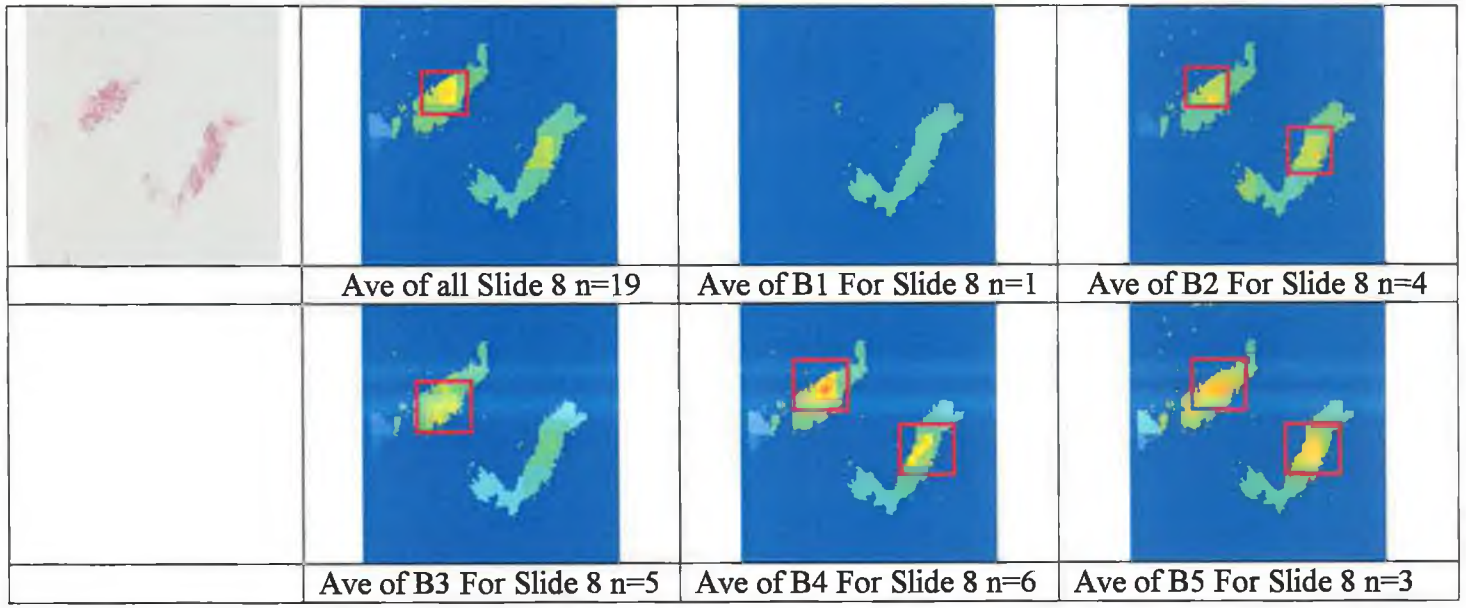
Two cores are presented in Slide 8. From the averaged diagnostic traces an observable 'hotspot' is evident in both cores. In particular, the 'hotspot' in the upper core is more readily discernible.

The most thorough examination was performed by Participant 62, who scanned much of the lower core at 2000x magnification and submitted a VPS consensus diagnostic grade of B4, (Figure 7.17). However, it is evident from individual bitmaps that despite poor consensus agreement in terms of the classification submitted, diagnostic traces for Slide 8 are very similar in terms of the volumes of tissue viewed, the regions of tissues viewed and the magnification that they were viewed at.

Professor Dervan indicated that the poor level of observational agreement was not as contentious as it appears. The therapeutic consequence for the patient whether categorised as B3, B4 or B5 would have been similar. The high level of discrepancy between the categories submitted by different participants was symptomatic of the difficulty in accurately categorising this slide. Professor Dervan indicated he would expect to see a high level of intra-observer variability amongst participants should they re-examine the Slide. The comments submitted by participants in examining slide 8 are discussed in greater detail in Chapter 4.

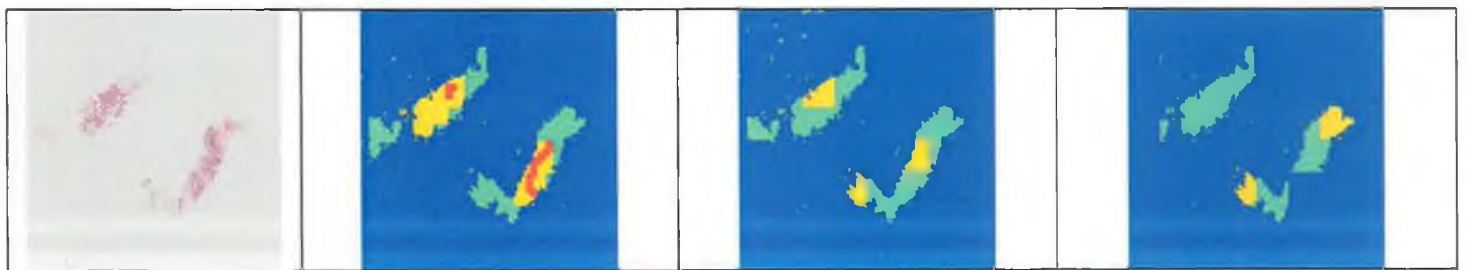
Images used to compile Figure 7.16-7.17 and individual bitmaps for Slide 8 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=8.



Percentage Consensus for Slide 8: 35.3%.
Consensus Diagnosis: B4.

Figure 7.16 Bitmaps depicting the average diagnostic trace for all examinations of Slide 8 and the averaged diagnostic trace for each diagnostic classification. Where possible observable hotspots are identified.



	Session No=496 User Id=62 Classification=B4	Session No=509 User Id=75 Classification=B2	Session No=121 User Id=36 Classification=B2

Percentage Consensus for Slide 8: 35.3%.
Consensus Diagnosis: B4.

Figure 7.17 Bitmaps of Slide 8 depicting the diagnostic trace of the most thorough examination in terms of magnification used and area examined by Participant 62 and the least thorough examinations by Participants 75 and 37.

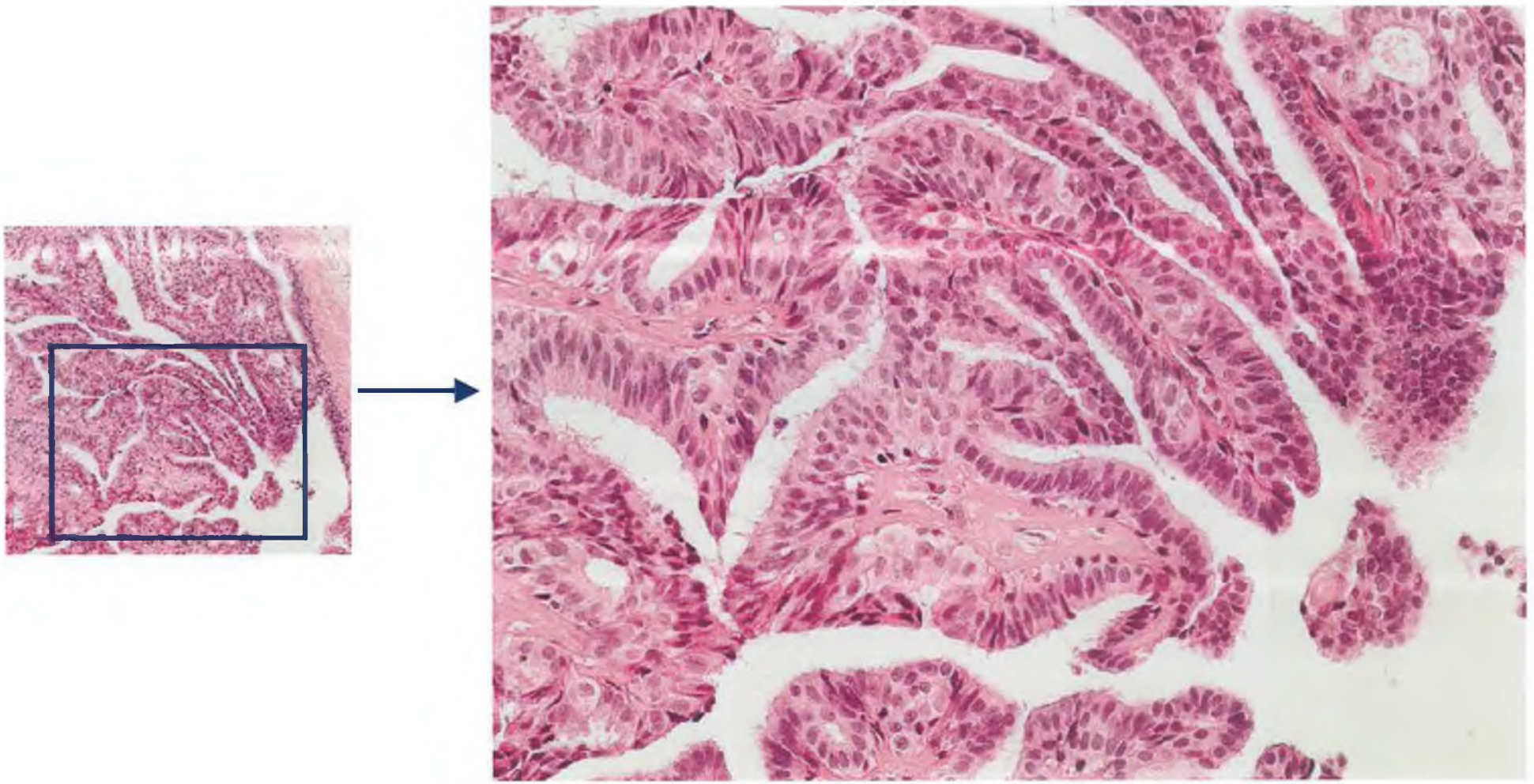


Figure 7.18 Fields of view selected from discernable hotspot in the upper core of Slide 8. Image shows characteristic infolding of tissue associated with Intraductal Pappilloma.

7.2.9 Examination Profiles of Slide 10

A number of participants who viewed Slide 10 reported the presence of calcification, All participants used a 2000x magnification to view some regions of the Slides, as this is critical in confirming the presence of micro-calcifications.

In is evident from averaged diagnostic traces of examinations where B1, B2 and B5 grades were submitted that 'hotspots' were identified, in particular in the upper region of the slide.

Participant 39 initially performed a thorough examination and submitted a B2 diagnosis, they upgraded this diagnosis to B3 in a subsequent examination. Participant 36 also performed a through examination scanning the upper regional 'hotspot' at 500x magnification and a lower regional 'hotspot' at 2000x. However they submitted a B4 classification.

There are three definable regions of tissue on Slide 10 in the upper, right and lower part of the slide. Professor Dervan reviewed each region individually and commented on their diagnostic value.

Upper Region: From the 125x field of view, Professor Dervan found the structure of the tissue ambiguous and impossible to determine the tissue type.

Right Region: When examining images from the hotspot on the right, Professor Dervan observed normal lobules with some big nuclei but did not see that as sufficient proof of carcinoma.

Lower Region: From the 125x image, Professor Dervan identified atypical hyperplasia. This is an abnormal growth of tissue. It is a benign abnormaility, although there is an increased risk of a patient identified with a form of atypical hyperplasia subsequently developing a carcinoma. Atypical hyperplasia is associated with fibrocystic change and microcalcification. Although microcalcification can be identified from the mammogram, Professor Dervan failed to find evidence of such within the specimen. While this can be due to biopsy sampling error more frequently the hard crystals simply fall out of the specimen when the slide is being prepared. On occasion, the gross biopsy

itself may be x-rayed to confirm that crystals are present and that correct sampling took place. Professor Dervan was satisfied he could submit a B2 categorisation for this slide.

Participants 6, 22, 75 and 36 had submitted B5, B5 B5 and B4 classifications respectively. This would have resulted in the patient undergoing unnecessary surgery and treatment. Comments submitted by some of the above Participants are as follows.

> *"difficult case. i think there is in situ and infiltrating lobular carcinoma here .however there is a problem withh the nuclear detail which appears washed out and poorly fixed making interpetation difficult!"* (Participant 6- Screen Resolution 1024x768 *Colour Depth 24- bit)*

Participant 6 was the most thorough in scanning a large proportion of the slide at 500x and 2000x magnification and still submitted a discordant grade of B5.

> *"Invasive" (*Participant 75 - Screen Resolution 800x600: *Colour Depth 24- bit*)

User 75 scanned the least area of the slide compared to other participants. However, they did zoom in to the hotspot of interest in the upper region of the Slide.

> *"At this power there is a suggestion of single file infiltration. Unfortunately the 2000x field will not load for me therefore I cannot be certain that these cells are epithelial - ie invasive lobular carcinoma (classical subtype). Nevertheless, I will report it as such. (I think a new B category should be introduced ie suspicious for malignancy but due to electronic failure, cannot be confirmed!!!!) Invasive lobular carcinoma (classical subtype)". (Participant 22 – Screen Resolution 1280x1024 Colour Depth 32- bit).*

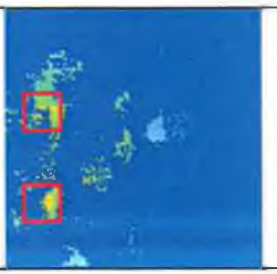
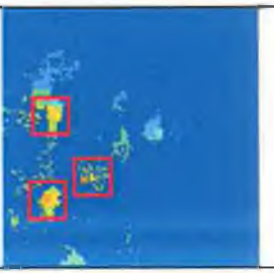
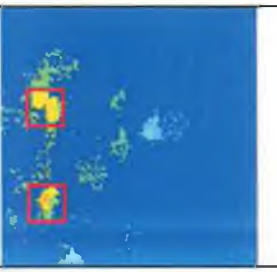
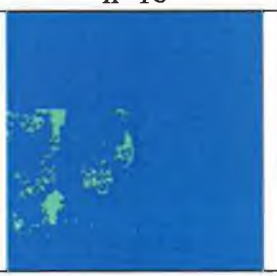
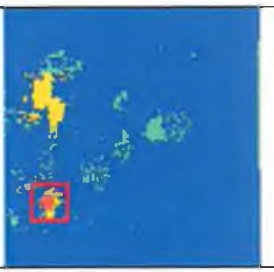
It is evident from the diagnostic trace of Participant 22 that they did not view regions of tissue similar to that of other participants and that their diagnostic grade relied on observations using low magnification.

It is a cause of concern that three participants classified this Slide B5 malignant. As stated previously such an error could have serious consequences for a patient resulting in unnecessary therapy and surgical intervention.

Professor Dervan reviewed the fields of view examined by Participants 6, 36, 75 and 22 and observed a prevalence of red blood cells due to extravasation. Professor Dervan believes the red blood cells were mistaken for cancer. Fields of view examined at 500x by participants 22 and 75 show in particular a line of red cells arranged in a manner commonly referred to as an 'Indian File' and usually suggestive of infiltrating lobular carcinoma of the breast. Professor Dervan was of the opinion that despite the Indian file arrangement of the red blood cells their distinctive red colour should not have been mistaken for carcinoma. He was also of the opinion that Participant 22 use of the term '*classical subtype*' was improper given that none of the subtypes lobular carcinomas are defined as 'classical'. Professor Dervan expressed an interest as to whether the examination time or screen resolution of the participants could have contributed to their mistake. All participants used a high resolution 32-bit monitor to render a diagnosis. It is possible that brightness and contrast settings may have rendered a darker image of the red blood cells making it easier to misinterpret the cells as cancerous.

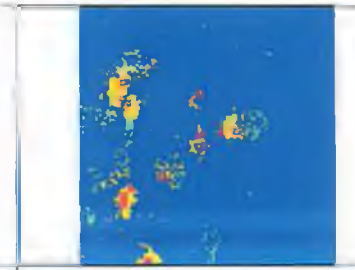
Images used to compile Figure 7.19-7.20 and individual bitmaps for Slide 10 are available at the following URL:

http://www.telepathology.dcu.ie/administration/study1/bitmapselect.php?slideno=10.

			
Slide 10	Ave of all Slide 10 n=18	Ave of B1 For Slide 10 n=4	Ave of B2 For Slide 10 n=9
			
Ave of B5 For Slide 10 n=3	Session No=692 User Id=39 Classification=B3	Session No=123 User Id=36 Classification=B4	

Percentage Consensus for Slide 10: 52.9%.
Consensus Diagnosis: B2.

Figure 7.19 Bitmaps depicting the average diagnostic trace for all examinations of Slide 10 and the averaged diagnostic trace for each diagnostic classification. Where possible observable hotspots are identified.

			
Slide 10	Session No=511 User Id=75 Classification=B5-Invasive	Session No=376 User Id=22 Classification=B5-Invasive	Session No=42 User Id=6 Classification=B5-Invasive

Percentage Consensus for Slide 10: 52.9%.
Consensus Diagnosis: B2.

Figure 7.20 Bitmaps depicting the average diagnostic trace for all examinations of Slide 10 and the averaged diagnostic trace for each diagnostic classification. Where possible observable hotspots are identified.

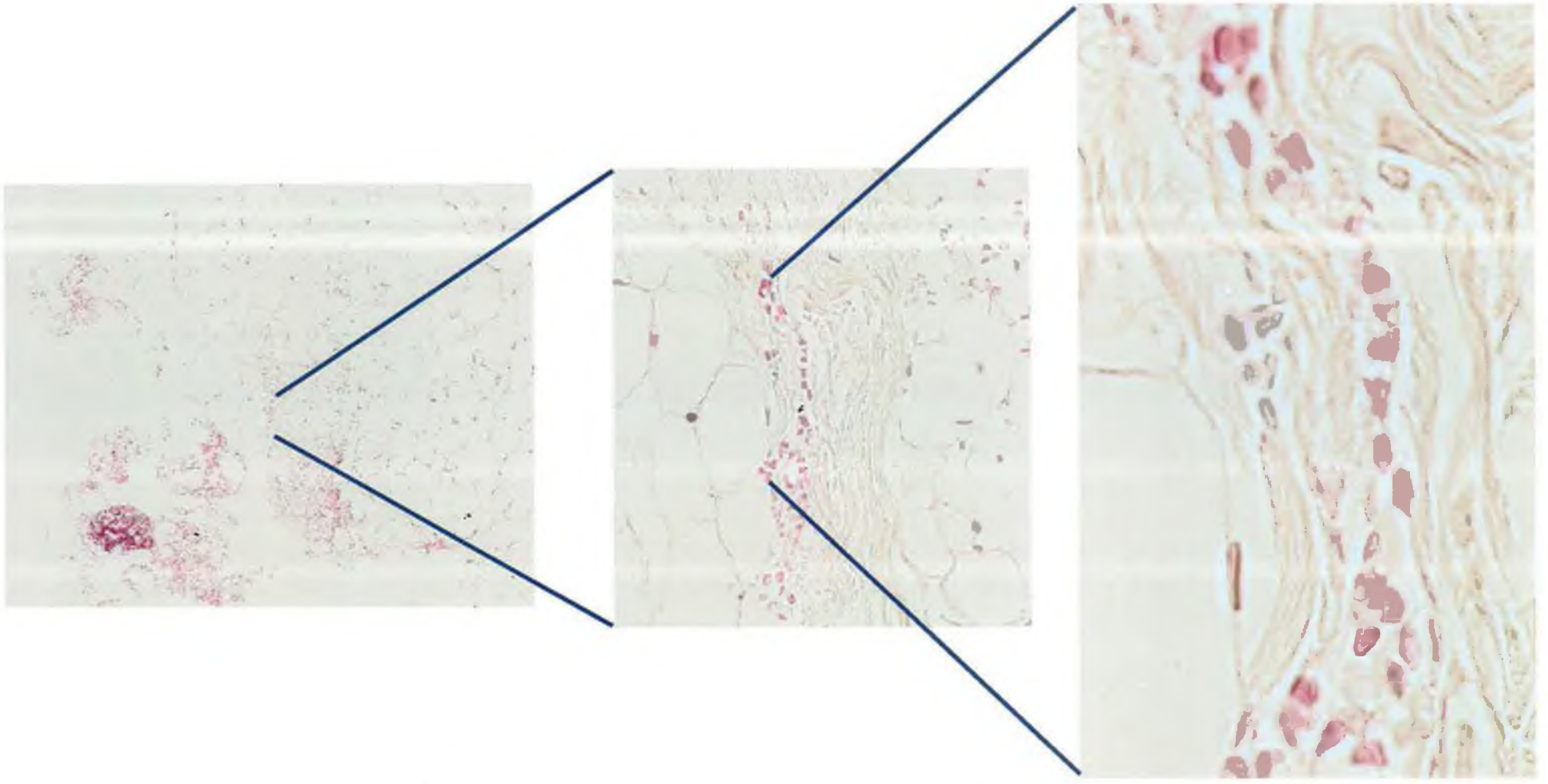


Figure 7.21 Fields of view selected from the diagnostic traces of Participant 6 and 22. Both participants viewed the 'Indian Trail' of red cells at 2000x It is probable that they misinterpreted this as invasive lobular carcinoma.

7.3 Conclusion

For each diagnostic grade submitted for Slide 1, distinctive hotspots could be identified from Bitmapper generated examination traces. However, presentation of images from these regions to Professor Dervan demonstrated that correct diagnosis of Slide 1 was not region specific. It depended on correctly identifying apocrine change and that the degree and extent of atypia indicated carcinoma.

Due to the thoroughness of slide examinations by participants in Slide 2, it was not possible to identify potential diagnostic hotspots. However, using Bitmapper, it was possible to identify the region of tissue examined by participant 6 who submitted a discordant classification and note that other participants who had examined identical fields of view had made a correct diagnosis. This indicated that Participant 6s diagnostic inconsistency was due to misinterpretation. Analysis of fields of view identified from a Bitmapper generated trace of Participant 6's slide examination by Professor Dervan in conjunction with there diagnostic comment confirmed this.

In Slide 3, potential diagnostic hotspots were not readily discernable. Again this was due to the thoroughness of slide examinations by participants who examined every region of the slide. According to Professor Dervan, Slide 3 was extremely difficult to diagnose correctly. This explains the extensive examination of the slide by participants. Regions of tissue examined by participants who submitted a discordant diagnosis were identified from Bitmapper examination traces and presented to Professor Dervan in conjunction with diagnostic comments. Based on this evidence, it was possible for Professor Dervan to attribute discordance to misinterpretation.

Slide 4 achieved a consensus grade of B2-benign. A review of individual diagnostic traces generated by Bitmapper shows readily discernable hotspots that correlate directly with regions in the tissue that contain breast ducts. It is obvious from the Bitmapper diagnostic traces that participants examined and based their diagnosis on these artefacts.

Examination traces generated by Bitmapper for Slide 5 show a clearly identifiable hotspot region examined by all participants. Fields of view from this region were

presented to Professor Dervan who identified a number of histological features (Figure 7.11), which, taken in conjunction with diagnostic comments submitted by discordant participants, could be used to explain reasons for diagnostic inconsistency.

A readily identifiable hotspot was visible from Bitmapper examination traces of Slide 6. This Slide achieved 100% diagnostic agreement amongst participants. This hotspot region was not critical for correct diagnosis. Professor Dervan examined the slide and indicated that there was extensive evidence of invasive carcinoma throughout the slide.

A readily identifiable hotspot was visible from Bitmapper examination traces of Slide 7. This region was examined by every participant. Professor Dervan was shown fields of view from this area and could identify the presence of sclerosing adenosis which gave rise to the consensus diagnosis of B2. Professor Dervan reviewed diagnostic comments submitted by participants who submitted a discordant diagnosis and felt those who submitted a B3 classification most likely took the decision to exclude the possibility of carcinoma. Professor Dervan was able to determine from images of the hotspot that there were some features that could be misinterpreted as ADH/Low grade DCIS and that this explained why some participants submitted a B4 or B5 diagnosis.

Slide 8 was the most controversial slide in the study and is discussed at length in Chapters 3,4 and 5. Based on diagnostic classification submitted it achieved the poorest consensus (35.3%). However, the consensus for Slide 8 based on diagnostic comments submitted was 64.5%. Readily identifiable hotspots were visible from Bitmapper examination traces of Slide 8. Professor Dervan examined fields of view from this area and determined that they unmistakably contained evidence of intraductal papilloma. (Figure7.17). Ambiguous instructions in the reporting guidelines coupled with the subjective difficulty in quantifying the degree and extent of atypia associated with a pappilloma lesion, was identified as the reason for diagnostic inconsistency in this slide.

The consensus diagnosis for Slide 10 was B2-benign. However, four Participants diagnosed it as B5. This would have resulted in the patient concerned receiving unnecessary surgery. Professor Dervan examined fields of view identified from

Bitmapper examination traces of the four participants who submitted a B5 classification. It was evident to Professor Dervan that the use of 2000x was required to examine nuclear detail and exclude the possibility of carcinoma. Professor Dervan was of the opinion that participants misdiagnosed the presence of red blood cells as carcinoma. This may have been caused by participants brightness and contrast settings being set dark.

In a number of cases such as Slides 4, 5 ,6, 7 and 8 it was possible to identify hotspots that proved to be diagnostically significant upon examination by Professor Dervan. However, where a hotspot is readily discernable on a slide, the overall histology and quality of the slide should first be determined before deciding on whether that hotspot is diagnostically significant. For example, hotspots were discernable in Bitmapper diagnostic traces for Slide 1, for each diagnostic classifications submitted. Professor Dervan's examination of these hotspot regions did not show them to be diagnostically significant. Bitmapper diagnostic traces can therefore identify potentially diagnostic hotspots, these should not be considered diagnostically important until the histology of the slide has been considered.

For some slides such as Slide 3 or Slide 10, it was not possible to readily identify a hotspot. This was indicative of the difficulty of the case where participants had to extensively examine the slide in order to render a diagnosis. Slides such as paraffin sections that contain more tissue would have been more beneficial in evaluating the potential of Bitmapper as a tool for identifying diagnostic hotspots.

The minimal tissue available in needlecores makes it difficult to attribute diagnostic inconsistency to observational error or insufficient field selection. The greater the number of slide examinations the greater ability of Bitmapper to identify diagnostic hotspots.

Magnification level used in diagnosis is important. However, it is specific to the histology of each slide. For example, it was possible to classify Slide 6 from 125x. However, 2000x was required in order to discriminate red cells from invasive epithelial cells in Slide 10. Bitmapper would have been more effective in evaluating the

importance of magnification used in the diagnosis process if the VPS used a greater number of intermediary magnification powers between 125x and 2000x.

Based on fields of view identified from Bitmapper diagnostic traces, Professor Dervan was able to provide a reason for diagnostic inconsistencies for each slide and each participant.

Despite the use of needlecore biopsies in the study, the limited number of participants and lack of additional intermediary magnification powers, Bitmapper proved to be extremely useful in understanding why discordant diagnosis were submitted for each slide.

Chapter 8: Conclusion

8.1 Conclusion

The process of microscopic diagnosis is a subjective skill. It is dependent on a pathologist's experience and their individual approach to slide examination. The multivariate nature of this skill gives rise to observer variation. While the degree of observer variation can be measured using conventional means, the exact cause of individual pathologists submitting a discordant diagnosis cannot.

Until now, pathologists have attempted to reduce diagnostic inconsistencies in a number of ways. This has included the formation of professional and specialist groups such as the European Working Group In Breast Screening Pathology to foster increased professionalism and expertise and improve training.

Reporting guidelines are reviewed on a regular basis to reduce ambiguities in the diagnostic process for various lesions and account for increased understanding of disease processes.

There are a number of organisations such as the "California Tumor Tissue Registry" that distribute interesting and rare cases to subscribed members for diagnosis. The names and diagnosis of subscribers who submitted a discordant diagnosis are subsequently published in a member's journal. This is not so much a "*name and shame policy*" as an acknowledgement that in the ambiguous field of histopathology, sometimes there is more than one right answer.

External quality assurance studies and training seminars are conducted on a regional, national and EU wide basis for different specialities. However, the inability to identify and define specific reasons for submitting an inconsistent diagnosis has implications for pathologist training in that it limits the benefits achieved through their continuous education and participation in EQA studies.

Telepathology has been used to support the education and training of pathologists. There are a number of telepathology learning environments such as '*www.raretumours.org*' that allow pathologists to examine and discuss difficult cases. The benefits of using telepathology in this role are numerous. It provide pathologists with the benefit of discussing and reviewing a greater number of cases with colleagues, while causing limited disruption to their normal work pattern.

In the US, the advent of virtual slides has brought about a migration in recent years towards the use of virtual slides for the teaching of histopathology. The virtual slide is replacing the microscope in a number of Universities such as the Medical University of South Carolina, University of Iowa and University of Pittsburgh.

There are an increasing number of studies reporting on the use of virtual slides for quality assurance and proficiency testing. Such studies are lending increased confidence towards the use of virtual slides in this role by demonstrating that the diagnostic accuracy of using virtual slides is comparable to that of using conventional glass slides.

However, if pathologists are to realise the true benefit of using this technology then virtual slides must do more than simply replicate the basic functionality of a conventional microscope. In order to '*learn from their mistakes*' pathologists require a tool that can measure their performance and provide data that is critical to identifying the reasons discordant diagnoses are made.

To provide such added value the Virtual Pathology Slide was developed. The Virtual Pathology Slide tracks pathologist's behaviour while examining a slide. The development of such a system entailed conceptualising and designing a telepathology system capable of emulating the functionality of a conventional microscope while incorporating the ability to record the spatial data of a user when examining a slide.

In order to elucidate reasons why a user submitted a particular disease classification, the data recorded for a slide examination had to be sufficiently detailed to allow a examination to be spatially mapped.

Analysis of slide examination data had to be readily accessible and easy to interpret in order to be able to determine reasons why a particular disease classification was submitted. To achieve this, a software tool was developed called 'Bittmapper' that visualises a pathologist's diagnostic trace. Bitmapper uses spatial descriptors of a slide examination to generate a graphical representation of regions of a slide that were examined by a pathologist and the magnifications used. This has proved useful for rapidly identifying regions on a slide frequented by an examining pathologist.

The VPS system is a web application delivered through a customised Internet Explorer browser that uses a number of client and server side internet technologies such as PHP, JavaScript, DHTML and Oracle 8*i* database management system. The technologies selected for use in the development of the VPS are widely available and platform independent. No proprietary development environment was required for programming in PHP, JavaScript or DHTML. The majority of the functionality of the VPS is powered through PHP. PHP is a high level interpreted programming language and although an open source language. It is very popular, is well supported and is becoming increasingly more sophisticated in its capabilities and applications. Oracle 8*i* was selected as the database management system because it was part of the existing IT infrastructure within DCU and could easily be integrated into the VPS application. Oracle has a worldwide reputation as a leading provider of enterprise level database solutions. The use of Oracle 8*i* provided a level of confidence in the security and integrity of VPS tracking data. The browser used in the VPS could only be used on a computer that had Internet Explorer 5.0 or higher installed. Although the vast majority of Internet users browse using Internet Explorer, this compatibility issue did prevent Apple Mac users and those with Netscape or Mozilla from using the VPS.

Bitmapper was developed in C++ and proved extremely useful for post study analysis. It is, however, currently limited in its use as a desktop application. The redevelopment of Bitmapper as a Java servlet represents a "best of breed" strategy in terms of technology choice and will lend itself to extending the functionality and capability of Bitmapper for automated real-time generation of diagnostic traces from a VPS webpage.

Data transmitted during a slide examination does not contain information that could identify a patient or participant and was therefore not encrypted or secured in any way. However there is a trend towards the use of HTTPS or SSL in telepathology. This is now standard in iPath, UICC TCC and AIFP. Future development of the VPS will involve a migration towards the use of HTTPS standards.

Crucial to the success of any telepathology system is the determination of whether a correct diagnostic decision can be made using the system. An evaluation study was performed in order to determine whether a correct diagnostic decision could be made

using the VPS by measuring the diagnostic performance of participants examining 10 needle core biopsies. The impact of known barriers to the acceptance and use of a telepathology system was also determined during the course of the study by measuring the attitudes and perception of participants towards the image quality and download speed of the system.

The evaluation of the VPS required participants to individually examine 10 slides and submit an electronic report for each one. The architecture of the examination process is illustrated in Chapter 3, Figure 3.1. Participants were required to select a slide for examination from a slide gallery, examine the slide and submit an electronic report. All participants who attempted to use VPS were subsequently invited to complete a VPS questionnaire.

The needlecore biopsies used in the study were randomly selected. They were not specifically prepared for use as a telepathology slide and are therefore be representative of slides that a pathologist would review on a daily basis both in terms of quality of preparation and histological content. In retrospect, the quality of some of the slides was poor, especially slide 10, 9 and 2.

The Guidelines For Non-Operative Diagnostic Procedures and Reporting in Breast Cancer Screening (NHS 2001) was selected as the classification protocol. This was considered appropriate as it is widely used in the UK, Ireland and commonwealth countries and has been adopted for the reporting of breast lesions by the EWGBSP.

The use of needle core biopsies for evaluation was suggested by Professor Dervan on the basis that they represented a diagnostic challenge in terms of being difficult to diagnose due to limited tissue and an intrinsic concern as to whether sufficient sampling has taken place.

It was convenient to use needlecore biopsies as the limited amount of tissue used in needlecores reduced the scanning time and memory requirements for storing a VPS. Up to five VPS needlecores could be stored on a 700MB CD.

All Participants who evaluated the VPS did so on a voluntary basis. Their contact details were supplied by Professor Dervan and were personally unknown to the author.

Eight of the 17 participants were members of the EWGBSP. They were therefore biased both in terms of experience and speciality in breast pathology.

One of the most challenging aspects of evaluating a telepathology system is separating the performance of a pathologist examining a slide using the VPS from their performance when examining a glass slide. In that context, the VPS evaluation study demonstrated that based on the diagnostic classifications submitted, consensus glass diagnosis agreed with consensus VPS diagnosis in 9 out of 10 cases. One of the cases (Slide 8) was out by one grade. Participants using the VPS achieved very strong individual performance with 10 of the 17 participants displaying excellent agreement with VPS consensus based on Kappa rating. Based on this performance, it is evident that a user can make a correct diagnostic decision using the VPS.

It was however evident from subsequent analysis of diagnostic comments submitted by participants, that Slide 8 was predominantly diagnosed correctly as intraductal papplioma but substantially categorised incorrectly. It is believed this is partly due to lack of familiarity with the study reporting guidelines and also an ambiguous description as to how intraductal papplioma should be classified. Modification of classifications submitted for Slide 8 resulted in its consensus increasing from 35.3% to 64.5%. Modification of classifications submitted for Slide 8 changed the VPS consensus diagnosis for Slide 8 from B4 to B3 increasing the agreement between glass slide and VPS diagnosis from 90% to 100%.

A number of other diagnostic categorisations were modified for Slide 1, 5 and 7 based on the diagnostic comment submitted. This resulted in the average percentage concordance for the ten slides increasing from 66.5% to 69.4%. It is of interest that diagnostic modifications occurred for slides with the three lowest consensuses. (Slides 1, 5 and 8 respectively). A recalculation of individual Kappa values based on the modified slide consensus results in a decrease from 0.76 to 0.72. The most notable change is for Participants 36. The diagnostic classifications of Participants 36 were changed in three out of the four slides where modifications took place. This resulted in individual percentage concordance increasing for Participant 36 from 40% to 70% while their Kappa increased from 0.26 to 0.46.

In conclusion, performance in terms of individual Kappa value decreased (0.76 to 0.72) when pathologist's text diagnosis was evaluated and classifications adjusted accordingly. However, the average overall slide consensus increased (66.5% to 69.4%).

Table 3.7 in Chapter 3 illustrates that participant's diagnostic performance is not related to their confidence in making a diagnostic decision using the VPS. For example Participant 18 indicated that they were '*Not-Confident*'. However, they achieved a Kappa score of 0.86. Conversely, Participants 36 and 6 indicated they were '*Reasonably-Confident*' and '*Confident*' respectively, despite achieving the lowest individual Kappa of 0.49 and 0.29 respectively .

Despite obtaining the fifth highest level of agreement (64.7%), the significance of discordance in agreement for slide 7 is greater than any other slide, given that the discrepancy in agreement lies predominantly between a B3 and a B2 category. This is because patients who receive a B3 categorisation will usually have further surgery and treatment while those who receive a B2 will not. The consensus agreement for Slide 7 was B2. It is believed that participants who submitted a B3 categorisation were being over cautious in their assessment.

In Chapter 5 perception of image quality and download speed were analysed. Perception of image quality was clearly dependent on a participant's screen resolution and colour depth. Almost all participants who indicated the image quality of a slide as "*Poor*" had a screen resolution of 800 x 600 and a colour depth of 16 bit. However, perception of image quality did not appear to substantially affect individual diagnostic performance. For example, Participant 55 rated image quality as "*Poor*" in 6 out of 10 slides. However, they achieved a Kappa of 0.9. Perception of image quality was better for some slides than for others. Slide 9 and 10 achieved the greatest number of "*Poor*" ratings, while Slides 1 and 2 achieved the greatest number of '*Excellent*' ratings.

Perception of download speed did not appear to be related to individual diagnostic performance. However, Table 5.3 in Chapter 5 does suggest that there is an increase in the number of fields of view examined by participants as their perception of download speed increases. Their was no noticeable difference in perception of download speed by participants who used a CD. However, it was felt that their expectation may have increased in comparison to participants who downloaded images over the Internet. It is

of interest that Participant 87 who examined five of the slides using a CD and the remainder by downloading images from the Internet did not appear to perceive a difference, having rated their experience of using both as 'Adequate'.

The number of fields of view examined for a slide appears to be representative of the histological difficulty in interpreting a case. Analyse performed in Chapter 5 on the number of fields of view examined for each slide demonstrated that, in general, as slide consensus decreases, the number of fields view examined for that slide increases. This relationship is illustrated in Figure 5.4.

The number of fields of view examined at a particular magnification is unique for each slide and dependent of the histological properties of each slide. For example, Slides 3, 7, 10, 1 and 5 had the highest number of fields of view examined. Analysis performed in Chapter 7 confirmed that these slides contained multiple histological features that were ambiguous or difficult to interpret, resulting in slide 10, for example, requiring examination at 2000x to submit a correct diagnosis while examination at 125x and 500x was sufficient to appreciate the complexity of the histology for Slide 3.

In order to exploit the data recorded by the VPS to its fullest potential and determine reasons for diagnostic inconsistency, a software tool called 'Bitmapper' was developed. This utilises VPS tracking data to create a graphic visualisation of an individual slide examination. This diagnostic trace was used to locate hotspot regions of potential diagnostic importance within a slide. A pathologist who was a specialist in breast disease, examined images from these hotspots, in order to determine their histological significance.

Using Bitmapper, it was possible to identify hotspots that proved to be diagnostically significant upon examination for Slides 4, 5, 6, 7 and 8. However, for some slides such as slide 3 or Slide 10, it was not possible to readily identify a hotspot. This was indicative of the difficulty of the slide where participants had to extensively examine the slide in order to render a correct diagnosis. Slide 3 achieved the third highest consensus agreement (82.4%), illustrating that sometimes slide consensus can conceal the difficulty in interpreting the histology of a case.

Hotspots were discernable in Bitmapper diagnostic traces for Slide 1, However evidence of carcinoma (masked by the presence of apocrine change) was found extensively throughout the slide. It is important therefore that hotspots identified using Bitmapper should only be considered as having potential diagnostic significance when the histology of the Slide has been appreciated.

Based on fields of view identified from Bitmapper diagnostic traces, a reason for diagnostic inconsistencies for each slide could be determined, demonstrating that Bitmapper is an extremely useful tool for determining reasons for observer variation.

This project has demonstrated that the use of the VPS for conducting EQA studies in pathology has several benefits such as:

- Complete elimination of study paperwork – all study data can be transmitted and received electronically.
- No data entry requirements - The system allows electronic collation and storage of report forms.
- Each pathologist reviews the same section from the same biopsy potentially at the same time - the benefit of virtual slides.
- Complete logging of each field of view examined by each pathologist for each slide.
- The capability to rapidly customise and design study templates.
- Ability to collate data for statistical analysis such as slide consensus, individual consensus or Kappa.
- The ability, using Bitmapper to review a graphical representation of diagnostic traces permitting identification of reasons for observer variability.
- No hardware requirements. The Medical Informatics Group at DCU could scan the slides and host the images and data on their servers, if required.

The development of the VPS and ancillary software tools was successful in that pathologists were willing to use the system. Pathologists could make a correct diagnostic decision using the system. The degree of observer variation could be quantified ands using Bitmapper, reasons for observer variation could be determined.

Appendix 1

Diagnostic Guidelines.

This document is an adaption of the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening, as used by the British National Co-ordinating Committee for Breast Screening Pathology (2002). This document was used by pathologists participating in the VPS Validation Study.

1. Background To the Virtual Pathology Slide

The medical Informatics group in DCU, telepathology@dcu.ie in conjunction with the Department of Pathology, Mater Hospital, has pioneered the development of the Virtual Pathology Slide (VPS). VPS is an image of a tissue section, which can be scaled within the normal working magnification limits of a pathologist, without loss of resolution at any magnification, within a conventional browser on the World Wide Web (WWW). With the VPS, there are no geographic or numerical barriers to case distribution.

We have currently generated 10 new VPS slides from breast needle core biopsies and are using these slides to validate the diagnostic accuracy and acceptability of the system with pathologists. The slides presented via the VPS were randomly selected. To learn more about the VPS access http://www.telepathology.dcu.ie.

Before participating in the study users are encouraged to become familiar with the VPS by visiting a demonstration page accessed by clicking the "Guest Access" hyperlink online at http://www.telepathology.dcu.ie.

2. Parameters To Be Measured

We have incorporated several novel features within the web site, which will allow us to assess and compare, in real time, user satisfaction with system performance.

Data is recorded for each examination of a VPS slide and stored on a server side database.

The Data recorded consists of the following three types:

- System analysis data defining the type of interface and connection used to view the VPS
- User-inputted data describing user experience with the VPS.
- User-inputted data in relation to diagnostic classification of the slide.

Upon examination of a VPS slide, this data may be viewed in the Summary Report Form.

3. Core Biopsy Reporting Guidelines Using the VPS.

3.1 Diagnostic Classification of Slide

The classification system used in the VPS summary report form is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer Screening, available online at: - http://www.cancerscreening.nhs.uk/breastscreen/publications/nhsbsp50.pdf-(pages 32-45). This is the recommended reporting guidelines for breast cancer screening in the United Kingdom, Republic of Ireland and most European Countries.

N.B. Participants who are unfamiliar with the classification system are advised to access this document on the web. A summary of the scheme is described below however, where lesions are encountered that presents diagnostic difficulties the above document should be referenced as the definitive reporting guideline.

3.2 B1-Normal Tissue

Indicates normal tissue whether breast parenchymal structures are present or not. Used to describe a core including normal breast ducts and lobules or mature adipose tissue or stroma only. B1 characterisation may include microcalcification. If calcification is present, the nature, size and site should be indicated.

The report should include a description of components present and a description of breast epithelial structures.

This classification may be used to describe un-interpretable cores, for example, due to excessive crush artefact or composition of blood clot only.

A B1 classification should be indicated where only very minor changes are present or if there is insufficient histopathological features to determine if a specific lesion is present, for example evidence of minor fibrocystic changes.

3.3 B2-Benign Lesion

Indicates a benign abnormality. Appropriate for a range of benign lesions such as

- Fibroadenomas.
- Fibrocystic changes.
- Sclerosing adenosis.
- Duct ectasia.
- Other non-parenchymal lesions such as abscesses and fat necrosis.

A B1 classification should be indicated where only very minor changes are present or if there is insufficient histopathological features to determine if a specific lesion is present, for example evidence of minor fibrocystic changes.

3.4 B3-Lesion of Uncertain Malignant Potential

Cases classified as B3 or B4 will result in either diagnostic excision of the area or repeat of core biopsy sampling to obtain a definitive diagnosis. Cases should be characterised using the following guidelines.

Atypical epithelial hyperplastic lesions where a uniform population of cells arranged in an appropriate manner involves one duct space or partially involves two or more duct spaces. These structures should be sufficiently structured to raise the possibility of Ductal Carcinoma In Situ (DCIS) but be of insufficient extent to fulfil the diagnostic criteria (Royal College of Pathologists Working Group for Breast Screening Pathology. *Pathology Reporting in Breast Cancer Screening* (2nd edition). Shef. eld, NHS Breast Screening Programme, 1997 (NHSBSP Publications No 3). The case should be classified as either B3 or B4 depending on severity and extent of lesion.

- Atypical Ductal Hyperplasia ADH. Accurate diagnosis of ADH by means of core biopsy alone is not possible. However cores which include atypical intraductal

epithelial proliferative foci showing features of low grade DCIS but in less than two duct spaces or less than 2mm in diameter should be classified as either B3 or B4 depending on severity and extent of lesion. However, if the cytology is high grade the above quantitative criteria is not necessary.

- Lobular neoplasia (LCIS and ALH) is included in the B3 category as it does not have the same management implications as DCIS or invasive malignancy.

- Low grade phyllodes tumour, fibroadenomatoid lesions with cellular stroma, stromal overgrowth and possibly mitotic activity indicating phyllodes tumour should all be catergoised as B3.

- Papillary lesions may show significant intralesional hetrogeneity. The area sampled with core biopsy may miss areas of *in situ* cancer. The majority of papillary lesions should therefor be classified as B3.

- Radial scar/complex sclerosing lesion such as areas of hyalinisation, elastosis, tubular entrapment with epithelial proliferation should be categorised as B3. This is because unless the lesion has been very widely sampled, the presence of DCIS or invasive carcinoma cannot be excluded. A decision on the presence of radial scar/complex sclerosing lesion should only be made after radiological correlation.

3.5 B4-Suspicious

Cases should be characterised as B4 using the following guidelines.

- Technical difficulties where the core is crushed or poorly fixed and contains probable carcinoma.

- Neoplastic cells contained within a blood clot or adherent to the outer aspect of the specimen.

- Very small foci of invasive carcinoma.

- A single or part of a duct space containing high-grade atypical epithelial process particularly if necrosis is present. Should be regarded as suspicious rather than malignant. Care should be taken to verify interpretation of cells of apocrine morphology, which may represent an atypical apocrine proliferation.

- Evidence of high grade intraductal proliferation with a significant degree of atypia possibly representing intermediate or low grade DCIS where relatively few duct spaces are represented in the biopsy. Report should indicate the presence of atypical intraductal proliferation and the degree of suspicion. Characterisation of the case as a B3 or a B4 should be based on the extent or severity of atypia.

3.6 B5-Malignant

Indicates unequivocal malignancy on core biopsy. Further classification into in situ or invasive should be made.

The report should include w here present, co-existing in situ and invasive carcinoma or other forms of malignancy such as lymphoma.

Where possible the nuclear grade, architecture and presence of necrosis should indicated. LCIS is included in the B3 category as it does not have the same management implications as DCIS or invasive malignancy. In circumstances where LCIS may be impossible to distinguish from small cell solid DCIS, the case may be classified as B5.

4. Registering For the VPS Study

Participants are required to register online for the VPS study. The registration form may be found by clicking on the "Registration" hyperlink located at http://www.telepathology.dcu.ie

Successful registration requires the following fields to be completed by the participant.

- Years of experience in pathology:

- Username: This is case sensitive.
- Password: This is case sensitive
- E-mail address: This is required to confirm registration details and notify the registrant of commencement of the study and how to access it online.

NB Participants are advised to note the username and password they use as this will be required every time they wish to access the needlecore slide library. Participants should choose a username and password that does not identify them personally.

4.1 Accessing The VPS Needlecore Slide Library.

Upon commencement of the study participants will be notified as how to access the needlecore slide library webpage on the world wide web.

4.2 Selecting a VPS Slide

Upon login access is permitted to the needlecore slide library. This is similar to the Guest demonstration slide library (available by clicking the "guest" hyperlink on www.telepathology.dcu.ie). A slide viewer presents an overview image of a slide. By clicking the relevant hyperlinks the user may navigate from one slide overview to the next. The user may select the slide they wish to view by clicking on the slide overview image presented. For each of the slides relevant case information is included.

4.3 Examining A VPS Slide

The VPS interface is designed as a user friendly, intuitive, microscope emulator.

The interface is comprised of the following three components:

- A field of view, this is an area of the screen presenting images of a tissue specimen to the user. A user may zoom to a higher level of magnification by clicking on a region of interest within the image.
- A control panel, this contains a number of simple utilities that allow a user to zoom to a higher or lower degree of magnification and directional buttons that allow the

user to navigate laterally within a given magnification. The control box also contains a button that permits the user to progress to a summary report form.

- A Comment box, this is an area where text may be submitted to the summary report form describing the field of view.

NB. When examining a VPS slide participants are expected to navigate to areas they consider pertinent to accurate classification and reporting of the case.

NB. Participants should comment on fields of view they feel are important to correct classification of a case. Participants may review such fields of view in the summary report page.

When participants are satisfied they have examined a VPS slide sufficiently to render a classification they should click on the "Finish" hyperlink located in the control panel on the left – hand side of the VPS interface.

4.4 Summary Report Form

The summary report form displays the following data.

Data submitted to the server in conjunction with the contents of the summary report form is as follows:

System Analysis Data

IP Number	Internet Protocol number describing the users network type and network address on the world wide web. This will be used to examine system performance variability between users.

Session Number:	Number assigned to each individual examination of a slide for database archiving purposes.
Screen Resolution:	Expressed as width and height of participants screen in pixels.
Time Stamp:	Record of date and time (GMT) when a slide examination took place.
No of Moves:	Record of the number of fields of view selected during the examination of a slide before submitting to the summary report form.
Bandwidth Speed test:	Measures rate of data transfer between server and client. Expressed as kilobytes per second. Kbs

System Performance Data	
Image Quality:	User Indicates their perception of image quality of slide. Op include: Poor, Average, Good, Excellent
User Satisfaction:	User Indicates their experience of using the VPS. Options inc Poor, Satisfactory, Good, Excellent.
Comment Field:	User contributes additional comments about their experien using the VPS
Slide Classification Data	User classifies the VPS they have just examined by adhering t core biopsy reporting guidelines for using the VPS.

Diagnostic Opinion:	User classifies the VPS they have just examined by adhering t core biopsy reporting guidelines for using the VPS.
Diagnostic Notes:	User contributes extra diagnostic information about the Slide

NB. The Summary report form should be fully completed before submission. Failure to do so will results in session data being discarded.

Upon submission the user is returned to the slide viewer page.

References.

1. www.telepathology.dcu.ie

2. http://www.cancerscreening.nhs.uk/breastscreen/publications/nhsbsp50.pdf

3. Royal College of Pathologists Working Group for Breast Screening Pathology.

Pathology Reporting in Breast Cancer Screening (2nd edition). Shef. eld, NHS

Breast Screening Programme, 1997 (NHSBSP Publications No 3).

Appendix 2

Diagnostic comment and classification submitted by participants for Slides 1 to 10. Where appropriate, a corrected classification based on a participant's diagnostic comment is also included.

Slide 1- Glass slide diagnosis B5 Invasive Ductal Carcinoma With Apocrine Change			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
41	B2		microglandular adenosis
6	B3		adenomyepethioloma apocrine adenosis
87	B3		Atypical apocrine hyperplasia ? in papilloma
5	B4		probably invasive carcinoma with apocrine change
7	B4		Based on the cellularity I am suspicious of malignancy but I was not able to see adequate cellular detail to make a confident diagnosis (see comment on VPS system)
35	B4	B3	Atypical ductal proliferation with apocrine change. Complete excision required
10	B5		In situ carcinoma on a background of a sclerosing lesion
62	B5		invasive lobular carcinoma
68	B5		Invasive ductal carcinoma
1	B5	B4	Atypical ductal proliferation, favor adenocarcinoma.
22	B5		Invasive carcinoma : possibly apocrine carcinoma

Diagnostic comment and classification submitted by participants for Slide 1. Where appropriate, a corrected classification based on participant's diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 2- Glass slide diagnosis B5 Invasive Ductal Carcinoma			
User ID	**SESSNO**	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
6	34	B2	multiple giant cells ? reaction to foreign material ?amyloid would like to see congo red stain would lijke to polarise as well
7	69	B5	In spite of poor focus and image degradation am confident of diagnosis as tissue architeture can be seen.
10	5	B5	Infiltrating ductal carcinoma
35	277	B5	Invasive ductal carcinoma (with features of invasive micropapillary carcinoma)
39	269	B5	Ductal carcinoma, low grade
39	684	B5	micropapillary carcinoma
41	20	B5	invasive mucinous carcinoma
62	490	B5	Invasive micropapillary carcinoma
68	542	B5	Invasive ductal carcinoma
87	649	B5	Invasive carcinoma ? apocrine ? micropapillary
1	90	B5	Invasive CA
22	311	B5	Invasive ductal carcinoma NOS

Diagnostic comment and classification submitted by participants for Slide 2. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 3 - Glass Slide Diagnosis B5-Invasive Ductal Carcinoma		
User ID	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
36	B2	Sclerosing adenosis
5	B5	mixed tubular ca and invasive ca NOS (NSP)
7	B5	Tubular carcinoma
10	B5	Not confident as unable to visualise cells adequately. My initial impression was that this was sclerosing adenosis.
35	B5	Tubular carcinoma
39	B5	tubular carcinoma
41	B5	invasive ductal carcinoma
62	B5	Invasive ductal carcinoma
68	B5	Invasive ductal carcinoma
1	B5	Infiltrating ductal CA
6	B5	infiltrating adenocarcinoma duct orgin grade 2 intermediate grade cannot see dcis on this slide
22	B5	Invasive ductal carcinoma NOS

Diagnostic comment and classification submitted by participants for Slide 3. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 4-Glass Slide Diagnosis B5 fibrocystic change		
User ID	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
39	B1	no calcification
41	B1	normal breast with slight cystic changes
1	B2	B9.
5	B2	fcd
6	B2	fibrous mastopathy only
7	B2	Fibrocystic change
75	B2	hamarthoma

Diagnostic comment and classification submitted by participants for Slide 4. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 5-Glass Slide Diagnosis B5 Invasive Ductal Carcinoma			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
7	B2		Area of sclerosis suggestive of radial scar.
36	B2	B3	Ductal papiloma in addition there is fibrocystic changes
1	B5		Favor DCIS with fibrocystic changes and sclerosis.
41	B4		suspicious of radial scar, rule out tubular carcinoma
68	B4		Radial scar and ADH.
5	B5		mixed tubular ca and nos
35	B5		Invasive ductal carcinoma
39	B5		?recurrence
6	B5		infiltrating adenocarcimoma low grade grade 1 with foci of dcis low grade
22	B5		Invasive ductal carcinoma NOS with associated low grade DCIS

Diagnostic comment and classification submitted by participants for Slide 5. Where appropriate, a corrected classification based on participant's diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 6 –Inv- Ductal Carcinoma		
User ID	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
1	B5	Ductal CA with lobular features.
5	B5	inv. lobular ca
6	B5	infiltrating adenocarcimoma Lobular type grade grade 2 intermediate grade with solid or alveolar fetures no definite in situ component identified orlthough one area suggestive
7	B5	Infilatrating ductal carcinoma
35	B5	Invasive carcinoma, ? lobular
39	B5	Lobular carcinoma
39	B5	lobular carcinoma
41	B5	in situ and invasive lobular carcinoma
62	B5	Combined ductal and lobular carcinoma
68	B5	Invasive ductal carcinoma
1	B5	Mammary CA with lobular features.
22	B5	Invasive ductal carcinoma NOS (also insitu component)

Diagnostic comment and classification submitted by participants for Slide 6. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 7 Glass slide diagnosis B2- Benign Usual Epitheial Hyperplasia			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
5	B2		sclerosing adenosis
6	B2		benign ductal epithelial proliferation i think it looks like blunt duct adenosis. foci of hemorrhage present consistent with previous fna
7	B2		fibrocystic disease with sclerosing adenosis
22	B2		Florid epithelial hyperplasia, of usual type.
41	B2		fibrocystic changes with epithelial hyperplasia of the usual type
55	B2		Hiperplasia usual type
10	B3		Best regarded as ADH
36	B3	B2	Possibly sclerosing adenosis
39	B3	B5	Atypical ductal hyperplasia/low grade DCIS
1	B5		DCIS

Diagnostic comment and classification submitted by participants for Slide 7. Where appropriate, a corrected classification based on participant's diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 8 B3 Glass slide diagnosis intraductal papilloma.			
User ID	**Classification Submitted by User**	**Classification Corrected by PA Dervan**	**Diagnostic Comment Submitted By User**
6	B2	B3	this looks like subareolar duct papillomatosis. what part of the breast is the biopsy from?
36	B2	B3	Ductal papillomatosis
68	B2	B3	Intraductal papilloma
7	B3		Papillary lesion - looks like a papilloma but needs biopsy
55	B3		papillary lesion
87	B3		Papilloma
1	B4	B3	Intraductal papilloma
5	B4	B3	intraduct papilloma with atypia. at least b3 probably b4. needs excision
10	B4		suspicious for intracystic papillary carcinoma
35	B4	B3	Intraductal papillary with atypia
41	B4	B3	papillary lesion, resection needed
62	B4	B5	Papillary carcinoma
22	B5		Intracystic papillary carcinoma. Advise excision.
39	B5		Intracystic papillary carcinoma
39	B5		Papillary carcinoma

Diagnostic comment and classification submitted by participants for Slide 8. Where appropriate, a corrected classification based on participant's diagnostic comment is also included. The correct classification is based on the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 9 B2 Benign		
User ID	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
22	B2	Apocrine papillary hyperplasia. No malignancy.
1	B2	Ductal hyperplasia.
41	B2	extensive benign apocrine change
55	B2	fibrocystic change with apocrine metaplasia
36	B3	Apocrine hyperplasia with cribiform glandular structures.
39	B3	Papillary lesion
6	B4	looks like it might be an encysted adenocarcinoma with apocrine features would recommend lumpectomy to see what it is high power images very blurred looks like oil imersion on slide

Diagnostic comment and classification submitted by participants for Slide 9. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

Slide 10 - Glass Slide Diagnosis B2-fibrocystic change		
User ID	**Classification Submitted by User**	**Diagnostic Comment Submitted By User**
7	B1	appears to be benign breast tissue but could not see any microcalcification (see comments about system) so this is then an inadequate biopsy.
35	B1	No microcalcifications identified
65	B1	No microcalcification
1	B2	Fibrocystic changes -adenosis, sclerosis, apocrine met.
1	B2	Fibrocystic changes.
1	B2	Fibrocystic changes.
5	B2	sclerosing adenosis
39	B2	calcification present
41	B2	focal benign apocrine change
55	B2	fibrocystic change
68	B2	Sclerosing adenosis
6	B5	difficult case. i think there is in situ and infiltrating lobular carcinoma here .however there is a problem withh the nuclear detail which appears washed out and poorly fixed making interpetation difficult!
22	B5	Invasive lobular carcinoma (classical subtype)

Diagnostic comment and classification submitted by participants for Slide 10. Based on participant's diagnostic comments, PA Dervan did not feel the classification submitted by users needed to be amended to comply with the Core Biopsy Reporting Guidelines for Non-operative Diagnostic Procedures and Reporting in Breast Cancer screening as used by the British National Co-ordinating Committee for Breast Screening Pathology.

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