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TuBaFrost 6: Virtual microscopy in virtual tumour banking ☆

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ABSTRACT

Many systems have already been designed and successfully used for sharing histology images over large distances, without transfer of the original glass slides. Rapid evolution was seen when digital images could be transferred over the Internet. Nowadays, sophisticated Virtual Microscope systems can be acquired, with the capability to quickly scan large batches of glass slides at high magnification and compress and store the large images on disc, which subsequently can be consulted through the Internet. The images are stored on an image server, which can give simple, easy to transfer pictures to the user specifying a certain magnification on any position in the scan. This offers new opportunities in histology review, overcoming the necessity of the dynamic telepathology systems to have compatible software systems and microscopes and in addition, an adequate connection of sufficient bandwidth. Consulting the images now only requires an Internet connection and a computer with a high quality monitor. A system of complete pathology review sup-

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porting bio-repositories is described, based on the implementation of this technique in the European Human Frozen Tumor Tissue Bank (TuBaFrost).

1. Introduction

Telepathology is the practice of 'pathology' at a distance, using telecommunications technology as a medium to facilitate transfer of images concerning pathology data between remote locations for the purposes of diagnosis, education and research.[1,2] Traditionally, telepathology is the practice of diagnostic pathology by a remote pathologist utilising images of tissue specimens transmitted over a telecommunications network. However, uses of telepathology also include remote discussion from pathologist to pathologist and from clinicians to pathologist, remote quality assurance involving pathologists and referral labs and research collaboration between research teams.[1,2]

Telepathology systems have been traditionally defined as either dynamic or static. 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.[3,4] Static (or store- and -forward) telepathology involves the capture and storage of images followed by transmission over the Internet via e-mail attachment, file transfer protocol, or a Web page, or distribution via CD-ROM. Dynamic hybrids also exist, which incorporate aspects of both technologies.[4]

Today, telepathology systems are divided into three major types: static image-based systems, real-time systems and virtual slide systems. To overcome problems attributable to sampling bias and interpretation resulting from limited field selection, telepathologists must be able to navigate to any field of view, at magnifications comparable to that of a conventional microscope, using images of sufficient resolution to render a correct diagnosis.[5–7] Real-time systems and virtual slide systems (the Virtual Pathology Slide)[8,9] meet these criteria. The Virtual Pathology Slide mimics the use of a microscope in both the stepwise increase in magnification and in lateral motion in the X and Y Cartesian directions. This permits a pathologist to navigate to any area on a slide, at any magnification, similar to a conventional microscope. This is discussed further in the next section.

Static image systems have the significant drawback in only being able to provide selected microscopic fields. Conversely, both real-time and virtual slide systems provide a consultant pathologist the opportunity to evaluate the entire microscopic slide. With real-time systems, the consultant actively operates a microscope located at a distant site, whereas virtual slide systems utilise an automated scanner that takes a virtual image of the entire slide, which can then be forwarded to another location.

While real-time and virtual slide systems appear ideal for telepathology, there are certain drawbacks to each. Real-time systems perform best on local area networks, but performance may suffer if employed during periods of high network traffic. The scanning of virtual slides can be a time-intensive operation requiring anywhere from minutes to hours to accurately scan a single slide. Also the large data size of the virtual slide means that a large data storage space is required as well as adequate archival and backup systems.

However, recent developments in virtual slide systems have resulted in a dramatic reduction of the time required for scanning a single slide, and also auto slide feeding technology has been developed in conjunction with these systems to allow batch slide scanning of a number of slides without the user having to manually feed a single slide one at a time onto the stage. Developments in software applications and compression methods have also allowed for a smaller data size compared to what was possible in the past.

Telepathology can also be classified according to the technologies employed. Each has its own characteristics: video conferencing (high cost, satellite or large bandwidth line, expertise); telepathology with motorised microscopy (high tech, costly, large bandwidth, internet speed limiting factor); full digitised image as movie (high tech, costly, large bandwidth, efficient connection); selected static images by e-mail/web (low cost, e-mail capability, speed is not critical, less accurate, repeat sending may be necessary).

The diversity in telepathology systems reflects growing technological advances in this area and the increasing importance of telepathology in education, training, quality assurance and teleconsultation.[10–15] 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.[13–15]

2. Virtual microscopy

Traditional histopathology diagnosis uses the standard microscope to observe prepared tissue sections on glass slides. If second opinion is required for the original diagnosis, it would be necessary to send the glass slides by post to another pathologist. This method takes time, risks permanent damage to the glass slides and incurs transport costs.

Classroom viewing of microscope slides in an educational environment would require an optical microscope with a projector operated by an instructor and would require the students to be physically present in the same classroom at the same time.

However, because of Virtual Microscopy technology and development of the broadband internet connection it is now possible for these microscope slide images to be digitised, placed on an image server and made available online via a (secure) website, overcoming time and physical constraints.

Virtual Microscopy (virtual slide system or Virtual Pathology Slide) is the technique of digitising an entire glass microscope slide at the highest resolution to produce a 'digital virtual microscope slide' with diagnostic image quality. This 'digital virtual slide' can be used in conjunction with image processing software tools to view, manipulate, position and specify the magnification of the image on screen as if using a regular microscope to view the original glass slide (see Fig. 1). As the glass slide is now in a digital or virtual format, it is possible to use the image for archival, replication, transferring over networks, remote consultation, integration with other media types for educational use on the web or DVD, integration into laboratory information systems and image analysis (reference article 'Nanozoomer/Medical Solutions user manual and website').

The whole virtual slide image is not sent to the user over the Internet. When a user selects a point and magnification on the virtual slide map, the image server will select that relevant field of view section (1–3 MB) from the full image stored on disk (300–500 MB) and will send it to the user over the Internet. This method is performed every time the user requests another location or magnification. This means that all the pathologists involved in reviewing a case would be able to log into this website, access these images and be able to decide on their histopathological diagnosis of the case due to the diagnostic image quality of the virtual slide image (see Fig. 2).

Students would also be able to access these images online and by using their computer can independently look at any image from a database containing thousands of slides. The viewing technology that is available with virtual microscopy technology allows the user to select magnifications and use UP/DOWN/LEFT/RIGHT arrow buttons to move the centre of field of view. The viewing software can also be navigated by clicking on the map or field of view image.

3. Application of telepathology and virtual microscopy in routine pathology

Current telepathology applications include intra-operative frozen sections services, routine surgical pathology services, second opinions, and subspeciality consultations. In this context, diagnostic accuracy of telepathology is comparable with that of conventional light microscopy for most diagnoses. Rapid and ultrarapid virtual slide processors may further expand the range of telepathology applications. Next-generation digital imaging light microscopes may make virtual slide processing a routine laboratory tool.[16]

Two kinds of virtual pathology laboratories are emerging: (a) those with distributed pathologists working in distributed (one or more) laboratories and (b) distributed pathologists working in a centralised laboratory. Both are under technical development. A virtual pathology institution (mode a) accepts a complete case with the patient's history, clinical findings, and virtual images for second opinion. The Internet serves as a platform for information transfer, and a central virtual slide server for coordination and performance of the diagnostic procedure. A group of pathologists is 'on duty', or selects one member for a predefined duty period. The diagnostic statement of the pathologist(s) on duty is retransmitted to the virtual slide sender who maintains full diagnostic responsibility. A centralised virtual pathology institution (mode b) depends upon the digitalisation of a complete slide, and the transfer of the virtual slide to different pathologists working in one institution.[17] In order to acquire uniform high quality in pathologic diagnostics and to have fast spread of cancer research knowledge, a Virtual Pathology Institution group is under development in Italy. It is composed of distributed pathologists and distributed laboratories working in the seven Italian Cancer Institutes within the Alliance Against Cancer (ACC) national project. Program implications of the ACC virtual pathology institution have included consensus sessions; quality control; education activities; second opinion and research. Virtual microscopy technology and Internet connection are used. Microscope slides images are digitised; virtual slides are then placed on a 'central' image server and made available on-line via a website. Since its implementation in 2003, the ACC virtual pathology institution has processed over 200 teleconsults, providing the communicating pathologists with diagnostic assistance on their most difficult cases. Today, the open access of the virtual microscopic images enables the user to send links of virtual microscopic images over e-mail. In addition, these links can be preset to a certain area and magnification of the image so allowing each pathologist to highlight interesting histology, whilst retaining the ability to view the complete histology of the scanned glass slide. Combining the use of other computer-aided communication

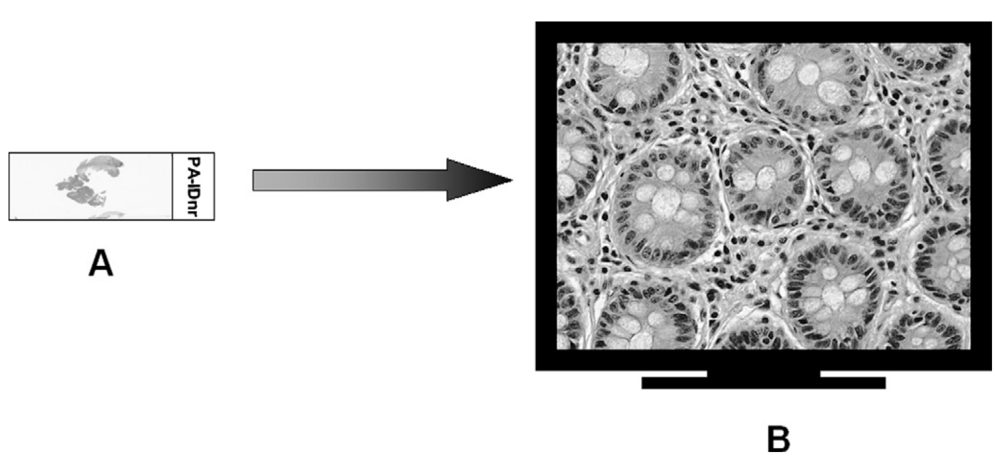

Fig. 1 – Virtual microscopy involves the digitisation of an entire glass microscope slide (A) to produce a digital virtual microscope slide with diagnostic image quality (B).

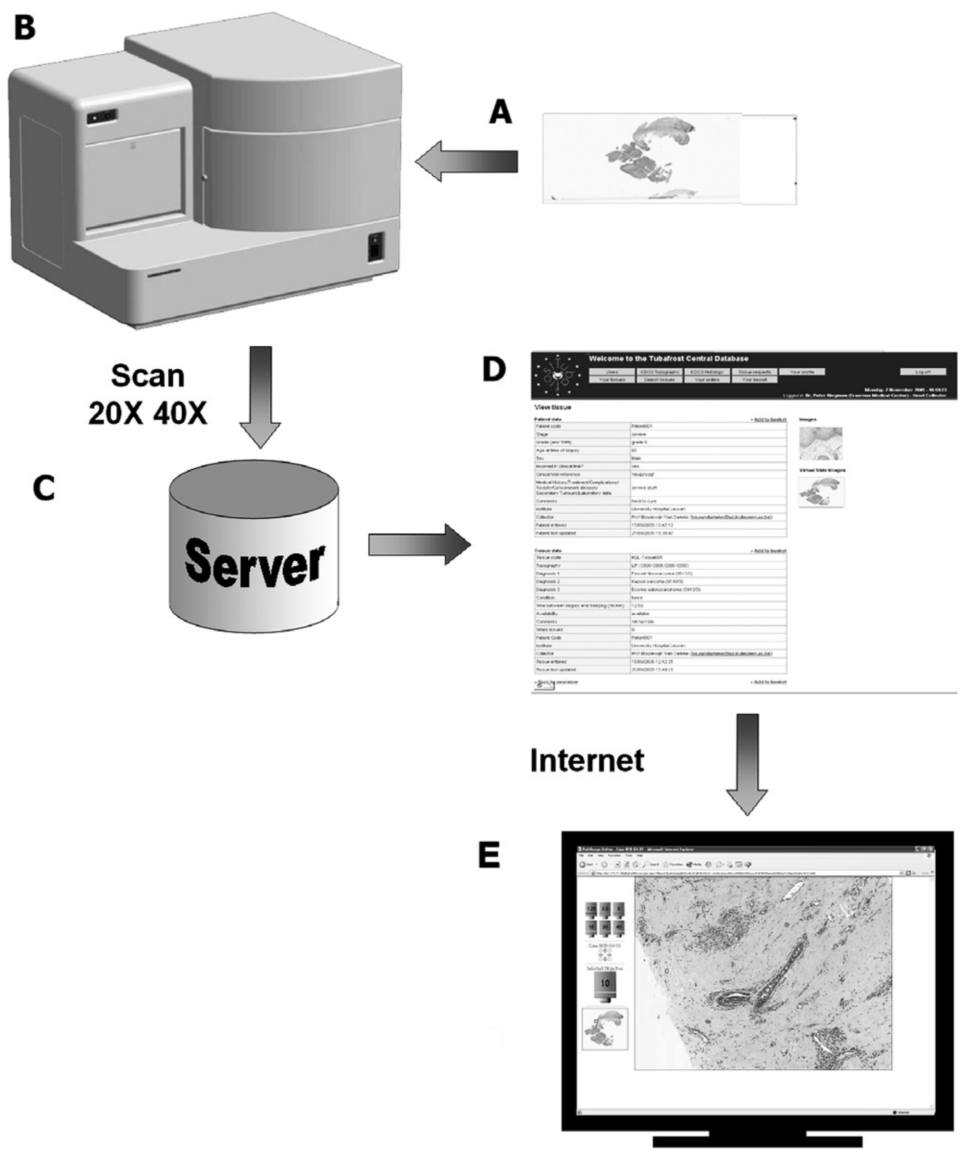

Fig. 2 – Once the tissue slide (A) is scanned by the virtual microscope (B), stored on the image server (C) and catalogued in the tissue sample database, users can access and view these images from the online tissue record (D) on their computer monitor using a website interface (E) and discuss the slide image with colleagues.

allows for a large variety of new forms of applications in consulting.

4. Virtual microscope system components

Fig. 3 portrays a general setup for a virtual microscope and telepathology system. The glass slide is placed on the microscope stage and the digital camera (3-chip 24 bit) takes high quality 'field of view' single images (usually at 20× objective but 40× is possible) as the motorised stage moves the glass slide left-to-right and up and down. Eventually, a collection of 'field of view images' are taken representing the entire glass slide. These images are saved to the PC that is connected to the digital camera and the image acquisition software installed on the PC will 'stitch' together these fields of view images to create an entire virtual slide image (between 300 and 600 MB in file size using jpeg image compression).

This virtual slide image can then be placed on the image file server and the server file address can be stored in a database under the tissue record (see article 'Central Database for an International Tumor Bank'). Client viewer software is installed on the web server so that when a user logs into the website and selects a tissue record, he/she can click on the low-objective slide image (taken, for instance, at 1/2× objective) which will load the client viewer software so that the user can view and navigate the matching virtual slide image that is stored on the image server (Fig. 2). The request is made to the image server, where the requested image is calculated and sent to the user as described in the section 'What is a Virtual Microscope'.

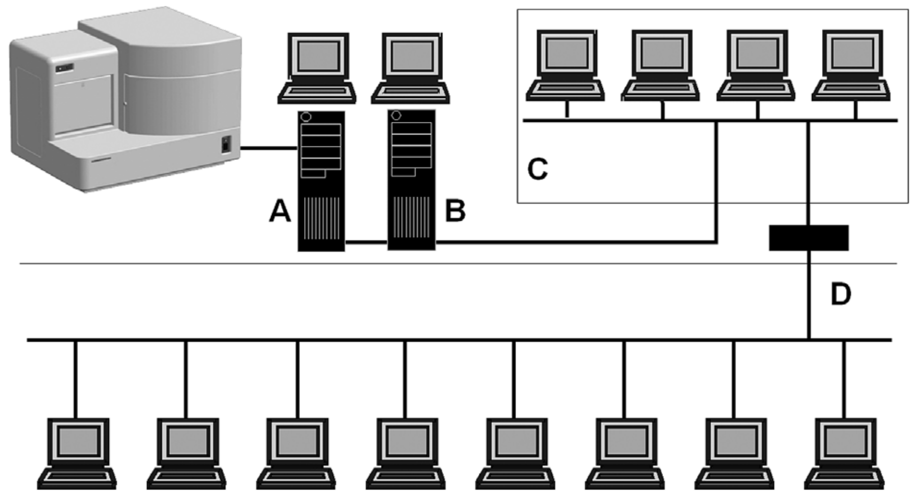

Fig. 3 – System setup for a virtual microscope and telepathology system. The virtual microscope network consists of a workstation (A) to operate the Virtual Microscope and an Image server (B) of large storage capacity and connected to the network. The network connection can involve a connection to the Intranet (C) enabling all institute computers with access to the image server to view the Virtual Microscopic images. It can also be connected to the Internet (D), enabling external users access to the images.

5. Investigation of commercial virtual microscope systems

During 2003–2004, as part of the TuBaFrost Project, 20 companies involved in virtual microscope (VM) systems around Europe were consulted and their systems evaluated.

From this evaluation the criteria for a good virtual microscope system were determined:

1. Good quality images (good resolution, focus and sharpness).
2. Adequate range of objectives/magnification.
3. Accurate focusing.
4. Fast scan speeds/low scan times.
5. Best compression rates and low image sizes (but maintaining good quality images – e.g. JPEG image compression).
6. Easy integration into existing software, databases and computer systems.
7. Affordable system.
8. Reliable.

The components of a typical Virtual Microscope system are

1. Microscope (standard or research) with XYZ motorised stage and controller (attached to PC). Includes focusing system.
2. Three-chip digital camera attached to a high performance PC with high resolution monitor.
3. High-resolution flat-screen monitor attached directly to the digital camera (for displaying current field of view).
4. Image acquisition software installed on PC that, through the motorised stage controller, allows the microscope to move across slide and collects frames of 'field of views' and then stitches these field of view images together to make a complete virtual slide image of the complete glass slide.
5. Image file server to hold these compressed virtual slide images.
6. Web server to hold the website files to provide an interface to the images and tissue information.
7. Pathology-tissue database system that holds the image server-file location for each virtual image within the tissue record.
8. Remote client viewing software installed on web server that works together with the tissue record database to allow pathologists and scientists to remotely access and navigate these virtual images.

Based on the criteria determined from the investigations and testing of some of the systems at that time, the Hamamatsu Nanozoomer Digital Pathology (NDP) system was selected for the TuBaFrost Project. The hardware for this system is developed by Hamamatsu Photonics.

6. Application of virtual microscopy in the TuBaFrost network

The addition of the virtual microscope tool to the tissue bank model was primarily to assist when tissues with a difficult diagnosis are selected for experimental purposes. In this way, the user is aware of the exact constitution of the sample so samples are not transported unnecessarily at cost to the requestor and samples are not unjustifiably unavailable. In addition, for very problematic cases a diagnosis review system can be easily established.

As detailed in the associated article 'Central database for an International Tumor Bank', a central database was developed for the TuBaFrost network into which locally collected sample information is uploaded and is searchable by the scientific community in order to locate tissue material for their research projects. Associated with each tissue record in the central database is the diagnosis. In order to back up this diagnosis as well as provide images of the tumour sample for training, education and reference, a virtual microscope system was employed within the network so that virtual slide images could be made from the tissue material and linked to the tissue samples entered into the central database.

Therefore, during the development of the TuBaFrost central database system, web software (provided by Medical Solution Plc) was integrated into the system to allow users to view these virtual slides online along with the tissue record and patient case information.

A large image server (1 TB [= 1,000,000 MB]) was integral to the NDP system; however, since each image will be between 300 and 600 MB in file size the limiting factor is the storage capacity. The TuBaFrost consortium decided that it was not essential for all tumour samples to be supported with virtual slide images, support is only required for difficult to diagnose samples. In these cases, a local pathologist can determine the diagnosis by judging the virtual images. Less difficult cases can be supported by a normal representative image (0.2–1.0 MB), which can be uploaded into the central database (see article 'Central Database in International Tumor Banking'), whereas easily diagnosed cases need no image at all. In the future as storage media reduce in price, data capacity increases, and compression methods develop further, it will be possible to store more virtual slide images.

Pathologists associated with collecting institutes participating in the TuBaFrost network will select the slides required for virtual slide scanning (difficult cases) and send them to the Tumor Bank office at the EORTC in Brussels for digitisation. The virtual slide images will be stored on an image server linked to the tissue records and patient case within the central database. After digitisation is completed, the glass slides will be returned to the collector institute.

7. Conclusion and future prospects

A relevant application for virtual slide technology is the documentation of tissue samples in tissue banks. This technology in the tissue bank can enhance traditional sample annotation, assist in ensuring that remote tissue bank clients receive appropriate tissue for their research, and form the basis of quality assurance systems.[18] In this paper, the TuBaFrost methods for integration of whole slide imaging into the tissue bank workflow and information systems have been reported.

An additional application for this technology is the development of a virtual resource of histopathology images for educational purposes. Users of this resource can view any part of an entire specimen at any magnification within a standard web browser. The virtual slides can be supplemented with textual descriptions, but can also be viewed without diagnostic information for self-assessment of histopathology skills. Web based virtual microscopy will probably become widely used at all levels in pathology teaching.[19] Virtual microscopy has significant advantages over real microscopy in education, it enables learning and has been favourably received by students and teachers alike.[20]

In conclusion, as the acquisition of high-quality virtual slides is still a time-consuming task and the cost of storing the image electronically is still significant larger than storing the information on a glass slide, the application of virtual microscopy in routine diagnostics cannot be recommended at this time. With the arrival of fast and easy to use virtual slide systems, we expect a significant change in telepathology application. Whether this will be in the near or more distant future will be determined by the costs of digital storage and the scanning time. As with images from biomedical technologies (MRT, PET, sonography, CT, termography), histology images may be integrated into the digital information systems for medical diagnostics, making these data available as part of the patient's electronic file.

In the future the glass slide will be archived immediately following digitisation. The work, diagnosis, teaching and research will be done with the virtual slide.

Conflict of interest statement

None declared.

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