

Ultrastructural Telepathology: Remote EM Diagnostic via Internet

JOSEF A. SCHROEDER

14.1 Introduction

Pathology, a diagnostic medical discipline, is associated in the mind of most people with performing autopsies on human bodies to find out the reason for death. This traditional understanding of anatomical pathology is conveyed in numerous works of narrative and fine art, for example, the famous canvas “The Anatomy Lesson of Dr. Tulp” painted in 1632 by Rembrandt van Rijn [77] or the impressive contemporary “Body Worlds” exhibitions of Gunther von Hagens [39], which is attracting considerable interest and controversy.

However, in fact, modern pathologists are mainly occupied with the light microscopy examination of cells (e.g., smears of exfoliated cells and fine-needle aspirations) and tissue or organ excisions (surgical pathology) from living patients with a disease. The patient’s clinician needs a cytology or tissue-based diagnosis describing the nature of the lesion and an interpretation of the data providing advice for an individual therapeutic strategy. The precedent for the shift in the pathological focus to the “living patient” was Virchow’s cellular theory, dating from 1858: this inaugurated the change from the ancient Hippocratic body fluid “humoral pathology” to contemporary “cellular pathology” and provided explanations of several observations – then considered strange – such as inflammation, infection, or cancer at the light microscopic level (“think microscopic”) [98]. It has taken more than a century to develop the microscopy and laboratory technology (tissue fixation, cutting, and staining) and the interpretation of tissue and cellular structure in terms of diagnostic features.

Today, tissue-based diagnosis is the “gold standard” for all subsequent medical procedures, especially surgery or drug treatment, and has a pronounced specificity and sensitivity in terms of clinical significance. Recent achievements in molecular biology and genetics using the nucleic acid amplification technique (polymerase chain reaction, PCR), combined with microscopic investigation such as in situ hybridization (FISH) and tissue microarray (TMA) processing

[22, 27, 51, 78, 101], provide a more detailed and “time-related” insight into the pathways of disease: we can now discern classic and prognosis-, risk-, and treatment-associated diagnoses. The complexities of these new techniques in concert with modern information technology (IT) have had a great impact on current pathology practice and may well lead to a paradigm shift for the discipline, partly but not only due to this new methodology [1, 12, 65, 76, 79].

In contrast to the extended responsibilities of pathologists as a result of the new genomics, proteomics, and metabolomics, the histopathologic diagnosis is still primarily based on the light microscopic examination of a classical hematoxylin and eosin (H&E)-stained, 4–7 μm thin tissue section mounted on a glass slide. This tissue visualization technique, perfected over the years, “fixes” the spatial arrangement of a living biological system as tissue or organ morphology (architecture), which is the phenotypic expression of the genetic information coded in the DNA in the cell nucleus (= genotype). Using the H&E and special staining procedures, one can analyze the important biological structures in normal healthy tissues and their disturbance under conditions of abnormality (disease). Until now, particularly abnormal tissue growth could not be treated without knowledge of the tumor morphology [101]. If the analyzed features are smaller than the resolution limit of the light microscope (200 nm), the 1,000 times higher resolving power of electron microscope (EM) technology (0.2 nm) can provide ultrastructural data at the sub-cellular level to facilitate a diagnosis that might be uncertain by light microscopy [20, 28]. To assess the functional status of a lesion or abnormal growth, other techniques, for example, immunohistochemistry [101], can visualize the expression of specific “marker” macromolecules, providing a simple “yes–no” presence or absence of a characteristic molecule, for example, breast cancer receptor protein or antigen.

In both light and electron microscopic images, the information content is separated intuitively by the observer into two diagnostically relevant domains: an object-associated information and a nonobject-related part (=background). The objects usually display “abnormal” features (cancer or inflammatory cells, inclusions, and infectious agents), which are not present in the analyzed tissue under normal (healthy) conditions. Once detected, the specific object features allow, for example, a biological classification (cell type, bacterium, or virus) or identification of structural and functional units (glands, vessels, mitochondria, or filaments). In many samples, also the background and its context or texture can contain valuable information (grey values, basic pattern, and relations to the object) to help reach the right diagnosis. Because each pathologist has his/her own algorithm for the image analysis and interpretation, in difficult cases it can happen that different pathologists can give different diagnostic statements (interpretations) on the same specimen [49, 101].

This problem of interobserver variability can be solved by a group discussion of the case using a multiheaded microscope, as shown in Fig. 14.1, or a consensus



Fig. 14.1. Traditional local and simultaneous image sharing at a multiheaded light microscope during the intradepartmental daily case discussion. Every observer can see exactly the same tissue image area at the magnification chosen by the case-referring pathologist and make comments on the rendered diagnosis. (Courtesy of Pathology Department University Hospital/Regensburg)

report of a panel board discussion (mandatory in several countries, e.g., hematological tumors), or by consultation of a recognized expert or reference center for this special case/disease. The simple and traditional way of requesting a “second opinion” was to send the glass slides with the tissue sections (optionally also the paraffin blocks for preparing additional sections if necessary) by “snail” mail and wait for the expert’s statement. The answer was usually available after some days or weeks.

Another time delay aspect is related to “frozen-section diagnosis service,” which is usually requested to guide the surgeon intraoperatively [75]. Particularly in smaller hospitals, which cannot afford to employ a pathologist and which may be located in remote or impassable country areas, the excised tissue or tumor samples are transported by courier to an urban “hub” pathology center for cryostat sectioning and “crude” diagnosis (time can be crucial, because the patient is still under anesthesia). The instantaneous advice of the pathologist (e.g., in assessing excision margins for a tumor) is essential for the course and extension of the surgical treatment. Clearly, therefore, some diagnoses are very urgent or of great impact for the patient’s treatment, and a great deal of effort has gone into improving these procedures, which include digital technology.

14.2 The Digital Era

The real breakthrough in rapid sharing of medical information independent of place, distance, and time has been made possible by fast-paced computer, Internet, and telecommunication technologies. The digital revolution has given rise to new tools and techniques in different disciplines of science; mobile messaging and video-streaming has become a daily experience for ordinary people, but more exciting – during the last decade the Internet has enabled people to have firsthand worldwide access to information and, for example, appreciate remote operation of complex space mission devices such as the “Sojourner” (1996, Pathfinder Mars Mission) or the “Spirit” rover roboter (2004) performing microscopic and spectroscopic analyses of rocks on Mars [67]. Much of this new knowledge has been assimilated into different telemedicine applications (tele-diagnosis, teletherapy, telecare, tele-education, teleresearch, telemeasurement, and administration) [10,11,13,16,33,38,44,56,73,80,88,100] and has been implemented by morphologists to study microscopic samples. “Telepresence microscopy” [113] is a term coined in 1998 and includes the remote operation of microscopes using telecommunication links or the Internet, applied to pathology in the fields of light and electron microscopy (EM).

14.3 From Remote to Virtual Light Microscopy

Interestingly, the first concept of performing a diagnosis at a distance was born in the very early days of radio broadcast development and is illustrated in the “Radio News” of 1924 [107]. However, for regular distant expert consultations, live black-and-white images of skin and blood diseases were first transmitted from Logan Airport to the Massachusetts General Hospital in Boston using a broadband telecommunication system in 1968. In 1985, Dr. R.S. Weinstein demonstrated the high diagnostic accuracy of video microscopy, while in 1986 he installed the first “robotic” light microscope for transmission of real-time images from El Paso/Texas to Washington DC via an SBS-3Comsat satellite [102,103]. In 1989 in Europe, in the rural areas of Norway, two provincial hospitals, without their own pathologist, installed a robotic pathology diagnostic system (a telephone/video combination) and were linked to the University of Tromsø Pathology Department, providing them with intraoperative frozen-section support. In February 1990, the first remote expert consultation was performed in Germany, between Darmstadt, Hannover, and Mainz [46,102]. The rapid progress in digital sensor technique and digital image processing and increasing bandwidth of telecommunication links attracted increasing

numbers of researchers and institutions to use this new technology for collaboration in science and health; the collected experience was shared at the first European Telepathology Conference in Heidelberg in 1992.

Today, one can speak of fourth-generation telepathology systems (=diagnostic pathology service at a distance, SNOMED definition), the technology having matured to the point where light microscopy telepathology has become a standard tool in a number of clinics and hospitals worldwide, and its applicability to and suitability for diagnostic routine use are well documented [46,104,105]. It includes a wide range of possible telepathology applications from remote gross (macroscopic) specimen description [55], intraoperative frozen section service [6,14,15,17,43,68,106,109], request for a second opinion [21,72,91,92,103,115], and quality assurance in screening pathology [40,47] to distance learning [2,7,46,53,73], using commercial systems. They are working in a passive (store and forward) or active (real-time) telepathology mode. The passive systems acquire and digitize images from a microscope and transmit them to a remote site for a second opinion; the recipient has no control over the image sampling, magnification, and quality and must trust the transmitting participant to have selected an adequate area of specimen. The dynamic or real-time telepathology systems give the remote expert as much control over the image selection as the sender. By remote control of the microscope, the expert can get an overview of the specimen at low magnification, select the relevant areas of interest, and choose the appropriate magnification to render a diagnosis. In this new millennium, an automated procedure has been described [19], capable of managing a robotic microscope and rapid image acquisition for the construction of a “virtual case”: this consists of a “stitched-up” collection of digital images (“tiles”) from a histological/cytological slide at all magnification levels together with all relevant clinical data. This “digital slide” can be viewed on a computer using a user-friendly interface (“digital microscope”) and used for multiple purposes independent of time and space with respect to the observer. To reduce the time necessary for scanning the original glass slide, this trend culminated in the development of a miniaturized light microscope array (MMA), allowing ultrarapid virtual slide processing [37,49,105].

14.4 Implications for Diagnostic Electron Microscopy

These developments were not applied to the use of EM for remote diagnostic applications, and there are several reasons for this. On the one hand, this may be due to the general decline of the use of EM in recent decades due to major advances in immunohistochemistry and molecular techniques (in situ hybridization, PCR). On the other hand, in addition to applications in research, the diagnostic value of EM in surgical pathology [29,30,95] in such

areas as renal, muscle, nervous system, skin, ciliary defects, storage diseases, opportunistic infections, and rapid viral diagnosis is good [20,28,31,35,70]. Diagnostic EM possesses an approximately 100 times higher resolution as light microscopy, and so it visualizes structures not recognizable by light microscopy examination. In combination with rapid microwave-assisted tissue preparation procedures [25,50,84], it can deliver specific and exact findings on the same day as sampling and make therapeutic decisions that are much easier. It is important to realize that the ultrastructural examination of neoplasms, especially addressing diagnostic difficulties by light microscopy, is a complementary approach with other techniques such as immunohistochemistry [32,42,69] and can be a very valuable adjunct to diagnostic assessment (including samples retrieved from tissues previously embedded in paraffin).

Another example is rapid viral diagnosis: in the negative-staining method, viruses are applied directly to a small grid coated with a support film and can be visualized in approximately half an hour after sample arrival (Fig. 14.2). No agent-specific

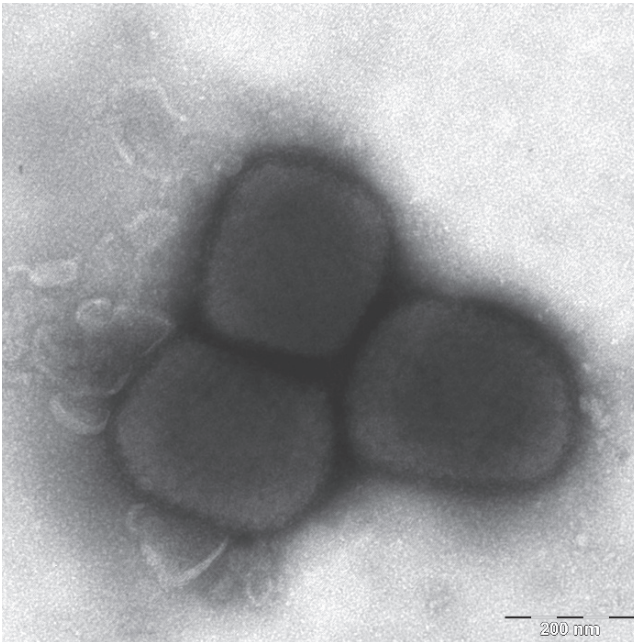


Fig. 14.2. Digital image of poxviruses, prepared with the rapid negative-staining method (2% PTA) and visualized half an hour later in the EM. This mouse ectromelia virus belongs to the poxvirus family, and its morphology and size are identical to the small-pox particles, which have a bioterrorism potential. Original magnification $\times 40,000$. (Sample courtesy of N. Bannert, Robert-Koch-Institute/Berlin)

solutions or antigens are necessary, and, owing to the “open view” of the experienced observer, multiple or totally unexpected infectious agents can be detected [58,70].

Second, owing to a reduction in the demand for diagnostic EM examinations in the last two decades and the need to limit laboratory running costs, the microscopy equipment is often technically not up to date in many laboratories. An analog-operated EM with a traditional photographic plate camera is not suitable for remote control and effective networking. This situation is slowly changing, because now (often for economic reasons) centralized EM units, equipped with modern digitally controlled EMs and digital image acquisition, are being established in life-science centers and clinics. Another point is the lack of automation and rationalization of the sample preparation and embedding methods. The implementation of micro-processor-controlled tissue processors and use of microwave-assisted procedures are considerably reducing sample turnaround times (from 3–4 days to a matter of hours); in short, “same day” diagnoses are becoming a clinical reality [84].

Third, the virtual slide concept [37,49] – so promising for light microscopy – can probably not be applied for virtual EM. The intrinsic problem is the grid (equivalent to the glass slide) carrying the tissue section, with its bars partitioning the section into translucent and obscured parts, and this creates currently a number of problems in correct image acquisition with a digital camera: alignment of multiple image “tiles” into one large picture (“virtual slide”) is very problematic. Another challenge is the different modes in which samples are examined in light microscopy and EM, the latter having an inherent and considerable magnification range. This means that, compared with virtual light microscopy, different technical solutions may be needed for EM.

14.5 The Need for Ultrastructural Telepathology

With the realization among many surgical pathologists that immunohistochemistry and the current rapidly evolving molecular procedures are not capable of resolving all diagnostic problems, there has been a resurgence of interest in transmission EM as an ancillary diagnostic tool [29,32,61]. In our opinion, the value and usefulness of ultrastructural examinations could be increased greatly by establishing a worldwide consultation network of experts or “national centers of excellence” to assist in obtaining and interpreting ultrastructural data of complex cases in real time. What is almost a consultation routine for light microscopy histopathologists (in specialized telediagnosis centers such as AFIP [108]; UICC, Berlin [21]; iPATH, Basel [8]; and WWM, Japan [66]) would be equally useful for ultrastructural pathology. The two main benefits would be time savings in “live” examination of the original specimen by remote experts and avoiding difficulties inherent in

the interpretation of either photographic prints (“snail posting”) or a still image collection (e-mail attachments), possibly captured from inadequate areas of the tissue in question. A significant advantage specifically of worldwide consultation networks for ultrastructural telepathology would be that expensive instrumentation would become available for remote experts or users [93]. This may be extremely useful in case of an unknown or epidemic viral outbreak (such as SARS, avian flu, and norovirus) as well as in potential bioterror scenarios [36,41,57,62,63].

14.6 Remote Electron Microscopy

The ability to examine original samples live can be realized by modern automated and digitally controlled EMs using telepresence microscopy and collaborative techniques. In materials science, several research groups around the world are already demonstrating the benefits of remote collaboration and consultation [99,111,114]. In 2000, the remote observation of thick biological samples across the Pacific was reported using the world’s unique 3MV EM (Hitachi H-3000) at Osaka University [93]. The first remote examination of pathological tissue and virus samples was demonstrated live at the G7SP4 conference (Global Health Care Applications Project, Sub-Project 4) in Regensburg, in November 1998, in collaboration with the Oak Ridge National Laboratory (ORNL) TE, USA, and Edgar Voelkl and Larry Allard [81,82]. For the transatlantic sessions, the material science-oriented Hitachi HF-2000 TEM in Oak Ridge was operated remotely via the Internet using the commercial collaborative software TimbuktuPro® (<http://www.Netopia.com>). This software effectively mirrors the remote computer, in this case a Macintosh running the DigitalMicrograph program at ORNL, to a Windows computer at the University of Regensburg, operated by the author, J. Schroeder. A technically updated and completely remote-operable system was recently reported by the L. Allard group at the MSA 2007 conference for functional remote microscopy via the Atlantic (“Global Lab,” Imperial College London, UK) [71].

14.7 The Ultrastructural Telepathology Setup

A different setup, designed in a server–client architecture and based on the LEO912AB transmission EM located at our Central EM-Lab at the University Hospital Regensburg, was introduced on 4–6 May 2000, at the Society for Cutaneous Ultrastructure Research (SCUR) Meeting in Bochum, Germany [97]. This digitally controlled EFTEM (LEO/Zeiss, Oberkochen, Germany) provides an in-column integrated energy filter, operates at 80–120 kV and is an

optimal instrument for biological and diagnostic sample examination. At this time, it was equipped with a bottom-mounted $1,024 \times 1,024$ pixel CCD slow-scan camera (TRS/Moorenweis, Germany) for image acquisition. The system was controlled by a Windows NT server and acquired the images from the camera by a frame grabber. The commercial software “analySIS” (EsiVision® Ver. 3.1, SIS/Muenster) was expanded with a dedicated TelePresence server module that controlled the microscope and handled the communication with the remote computer. At the “client site” in Bochum, a laptop equipped with an ISDN-PCMCIA card provided direct access to the Internet (a client “analySIS” software was installed on the laptop computer). A video beamer with a resolution of $1,024 \times 768$ pixels displayed the remote examination of a (routinely prepared) skin biopsy section from a CADASIL patient to the audience (CADASIL is a central nervous system disease with ischemic stroke; the definitive diagnosis is rendered by EM evidence of specific, very small deposits in the wall of skin vessels) (Fig. 14.3). The meeting participants could not only

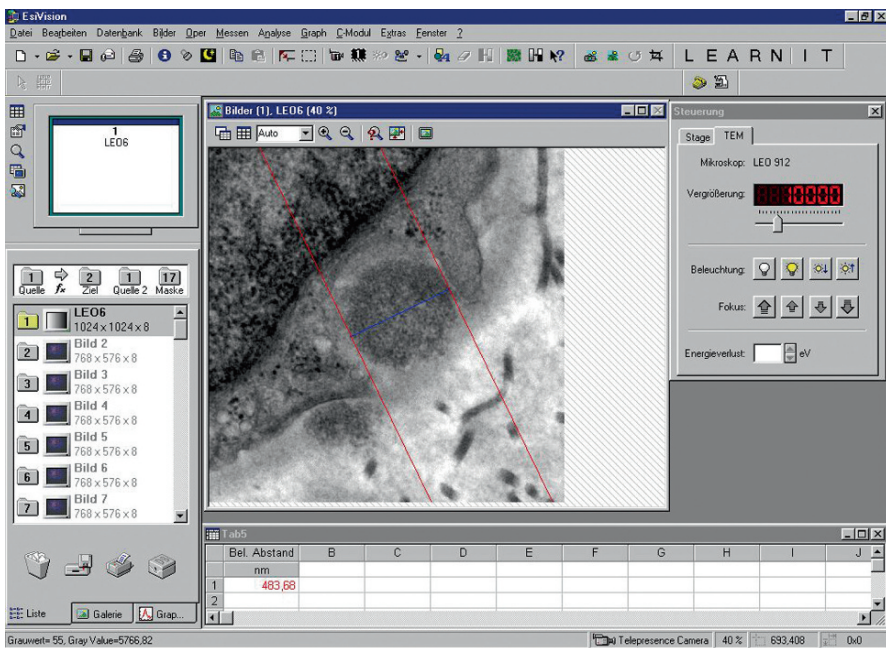


Fig. 14.3. Historical screenshot of the “client site” laptop monitor saved during the inaugural first “German” ultrastructural telepathology connection via the Internet between Regensburg and Bochum (May 6, 2000, SCUR-Meeting; approximately 500 km distance). Note the high-resolution “snapshot” image (original magnification $\times 10,000$) displaying a GOM deposit in a dermal blood vessel wall (diagnostic for CADASIL) and the immediately performed size measurement (red parallel lines and the result in the table below). Simple mouse clicks on the buttons visible in the EM-control panel allowed the magnification, beam blaster (on/off), illumination adjustment, control of the stage navigation, and coarse/fine focus adjustment

experience the live remote operation of the EM in Regensburg but also learn interactively how to make the diagnosis of this peculiar disease.

Since 2004, the microscope was retrofitted with a motorized and automatic adjustable special (seven-hole) objective aperture, which was necessary for remote low-magnification range observations (9–2,000 \times) (to avoid image area clipping by the aperture). In 2006, an additional 1 k \times 1 k fast frame-transfer CCD camera (5 MHz clock) with a fiber-optic-coupled YAG scintillator (TRS[®]/Moorenweis, Germany) was side-entry mounted to the EM column and USB connected to the server computer (Dell Precision 380, 3.2 GHz Pentium processor, 2 GB internal memory) (Fig. 14.4). This side-entry camera is placed above the fluorescent

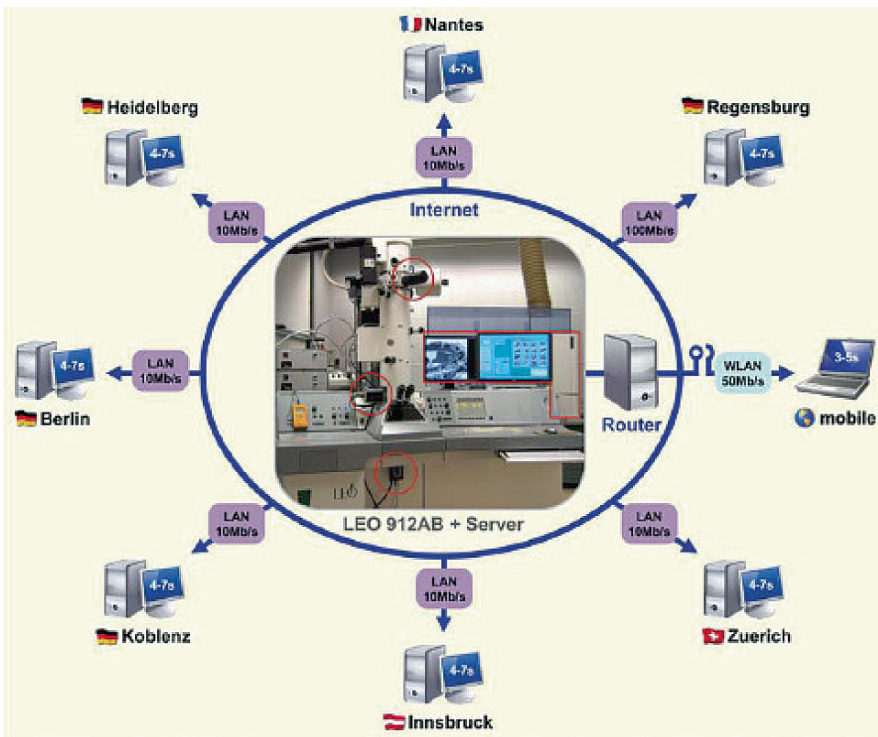


Fig. 14.4. Scheme of the server–client architecture of the electron microscopy telepathology consultation system at the University Regensburg. The LEO912 EFTEM is retrofitted with a motorized drift-minimized objective aperture, and a 1 k \times 1 k pixel CCD camera is bottom- and side-entry-mounted on the EM column (*red encircled*). The system is controlled by a Windows-XP server running the “iTEM” software and handling the communication via standard LAN or WLAN links to the Internet. The image transmission performance of the system in the “live mode” is 1–3 frames per second; in the “frozen snapshot mode” (uncompressed high-resolution 16-bit images for storage) the transfer needs 4–7 s (dependent on daytime bandwidth)



Fig. 14.5. The “server site” operator can flexibly control the EM alternatively by the software button controls and/or conventionally use the microscope panel knobset. The software supports a two-monitor image display; note the left screen showing the live image (abnormal cilia), the large right-hand screen displays the control panels (EM parameters, CCD camera, FFT-Image-Aid, and histogram), the images in the live computer memory, and the currently used database. The telepresence “feeling” in a telepathological consultation can be markedly increased by hooking it up to a parallel standard phone connection (a headset is a helpful tool enabling – here the author – freehand EM operation)

observation screen and is superior to the bottom-mounted camera for tissue-section examinations (comparable to a “wide-angle” lens effect in ordinary photography). The monitors were replaced by large, high-resolution, calibrated, flat-screen TFT monitors to improve the image display and databank handling, while, for the parallel verbal communication with the remote expert during the consultation, a headset for standard phone connection was used (Fig. 14.5).

14.8

Extension of the Server–Client Architecture

In the meantime, the client “analySIS” software (now upgraded by the “iTEM” software package, OSIS/Muenster, Germany; www.olympus-sis.com) was installed on Windows-XP running desktops in different locations (Fig. 14.4): Pathology Innsbruck/Austria, Thomas Mairinger; Pathology Zurich/



Fig. 14.6. The remote “client site” monitor displaying a transmitted high-resolution 16-bit image from the server and deposited on the local memory computer of the expert (abnormal cilium). Note the overlay discussion tools that can be synchronized with the image from both sites (server = *blue arrow*; remote client/expert = *red arrow*) (Image courtesy of B. Hauroeder, Bundeswehr/Koblenz)

Switzerland, Stefan Kolb; Bundeswehr (Central Institute of the Federal Armed Forces Medical Service) Koblenz/Germany, Baerbel Hauroeder (Fig. 14.6); Laboratoire de Therapie Genetique INCERM U649 Nantes/France, Fabienne Rolling (Fig. 14.7); Dermatology Reference EM-Lab Heidelberg/Germany, Ingrid Hausser (Fig. 14.8); and Robert-Koch-Institute (National Reference Lab Rapid Virus Diagnosis) Berlin/Germany, Norbert Bannert and Hans Gelderblom for feasibility testing and improvement in the remote EM operation under different Internet bandwidth settings and limitations (ISDN, LAN, ADSL, and WLAN). Since 2004, the server is connected by a 100 Mbps LAN to the Internet, and, for IT security reasons and Firewall restrictions, the clients get access to our server through a VPN channel.

Using the present system configuration, JPEG2000 data compression, and standard client LAN Internet access (10 Mbps), we obtained in the preview “live-image” mode (used for screening the sample; image size $1,024 \times 1,024$ pixel, 2 MHz pixel-clock at 16 bits, and 100 ms exposure time) at a frame rate of 1–3/s;



Fig. 14.7. Example of remote basic research consultation of a visual disorder project in collaboration with a French group in Nantes. The quality of the negatively stained recombinant virus particle preparation used in the experimental blindness therapy can be directly assessed on the “client” monitor and commented on through phone by the group leader. (Image courtesy of F. Rolling, Laboratoire de Thérapie Génétique, INSERM/Nantes, France)

for the transfer of the uncompressed high-resolution (16 bits, $1,024 \times 1,024$ pixel) “snapshot” image to the local hard disk, we experienced a transfer time of 4–7 s (depending on date and time of day). During the server–client connection, the remote control capabilities include stage navigation and search for area of interest at low or high resolution in the “live mode,” selection of adequate magnification (9–500,000 \times) in 38 steps, focus adjustment, beam blaster on/off, illumination intensity adjustment, exposure time setting, and image storage at full resolution (Figs. 14.3 and 14.8). On the “frozen snapshot” high-resolution image overlay, functions like discussion pointer (arrows) and annotation settings or distance measurements are synchronized between the two sites. To increase the telepresence feeling, the live telepathology consultation is usually backed up by a standard phone connection for verbal communication with the remote client/expert.

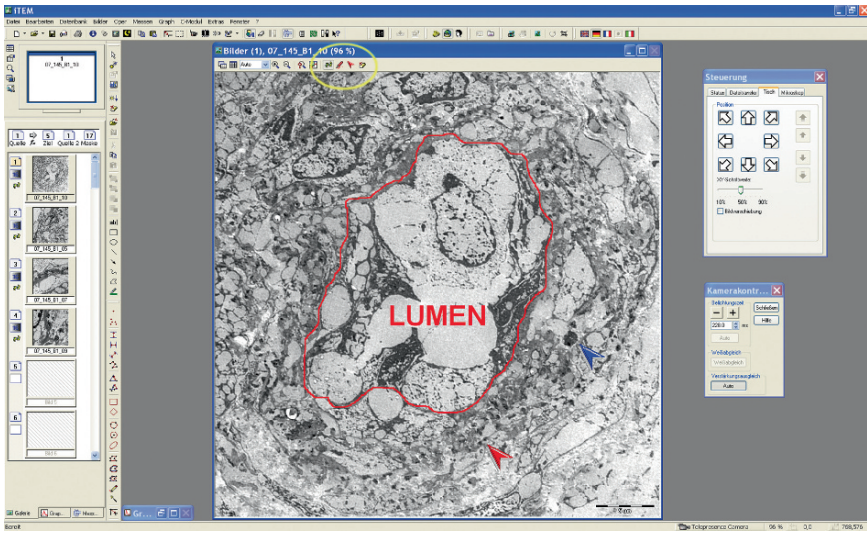


Fig. 14.8. Screenshot of the remote skin ultrastructure expert monitor in Heidelberg consulting a difficult fibrosing lesion in deparaffinized skin tissue of a patient with kidney insufficiency. Teleconference overlay discussion tools (*yellow encircled*: drawing pen, pointing arrows, and annotations) allow the expert to graphically delineate a blood vessel and direct the attention of the observer to important inclusions (*red arrow*); the expert also discusses immediately and by phone the structures pointed out on the server side in Regensburg (*blue arrow*). Note the microscope stage control panel enabling the remote expert to navigate the sample and to decide which section area needs to be examined at higher magnification. (Image courtesy of I. Hausser, Dermatology Department University Hospital/Heidelberg)

14.9

Performance of the Ultrastructural Telepathology

Ultrathin sections of selected diseases (skeletal muscle [minicore disease], skin granuloma [Histiocytosis-X], head tumor [oncocytoma], and skin lesion [Molluscum contagiosum]) as well as negatively stained adeno virus, birna virus, herpes virus, and rotavirus particles were examined remotely and consultation made with the aforementioned experts. The results were presented at the “Ultrapath-XI” Conference of the Society for Ultrastructural Pathology (August 2002, Aspen, CO) and the 15th International Congress on Electron Microscopy in Durban, SA (September 2002). A live ultrastructural telepathology demonstration of the EM examination of poxvirus (Fig. 14.2) and Anthrax spores using wireless Internet access was performed in a special ses-

sion during the 7th International Conference on the Medical Aspects of Telemedicine in Regensburg (September 2002) [83]. Mobile expert remote EM operation was also successfully carried out by wireless hotspot Internet access from the airports of Munich/Germany and Denver/USA using, on the client site, a notebook equipped with the Orinoco/Lucent Technology Wireless-PCMCIA-card (maximum 11 Mbps), revealing sufficient performance for the microscope control and only slightly prolonged image transfer in comparison to wired Internet connection. In summer 2006, we performed an outdoor test of the wireless new UMTS communication option (measured mean bandwidth 0.320 Mbps) offered by national telecommunication companies (UMTS PCMCIA-card, T-Mobile, and Vodafone) (Fig. 14.9). We successfully completed a remote microscope operation and experienced a stable but markedly slower image transfer rate (mean 0.2 fps for “live images” and 130–280 s for high-resolution “snapshots”).



Fig. 14.9. Demonstration of an outdoor wireless remote access on the telepathology system in Regensburg. The “client” expert notebook was equipped with a dedicated UMTS PCMCIA-card (*pink colored*), which uses the new telecommunication channel of T-Mobile and Vodafone company. We experienced stable connections, but the available bandwidth (0.320 Mbps) caused a significant image transfer delay. (UMTS-cards, courtesy of T-Mobile and Vodafone Regensburg)

14.10 Illustrative Telepathology Diagnostic and Basic Research Examples

Recently, a paraffin block containing a skin biopsy from an elderly patient with a suspicion of nephrogenic fibrosing dermopathy (NFD) was referred for EM examination, with special attention on the presence of gadolinium deposits. NFD (also termed NSF, nephrogenic systemic fibrosis) is a painful and disabling recently documented disease observed mainly in patients with renal insufficiency (dialysis or kidney transplant patients) with a previous gadolinium exposure, the etiology of the disease being so far elusive [90]. Gadolinium is a rare-earth element and, owing to its paramagnetic properties, it is widely used as a relatively safe contrast agent in clinical magnetic resonance imaging. The patient concerned had been dialysis-dependent and also had a history of gadolinium exposure; the light microscopy skin histopathology was suggestive for NFD, but other differential diagnoses like autoimmune disorders (such as scleroderma) had to be considered. In the deparaffinized skin sample, we found some tiny perivascular aggregates of undefined material and performed a telepathology “second-opinion” consultation with a skin pathology expert in Heidelberg to exclude possible artifactual findings. The direct telepathologic examination of the sections (choosing different section areas and magnifications) by the expert resulted in an instant confirmation of possible real deposits of inorganic material (Fig. 14.8). This immediately prompted us to perform an in situ elemental analysis using the ESI (electron spectroscopic imaging) and EELS (electron energy loss spectroscopy) methodology with our EFTEM to clear the nature of the deposits in question. This had a considerable impact for the patient’s further treatment and is probably also important for the etiologic understanding of the disease [85].

We are cooperating with a French INSERM molecular genetics research group concerning a study of visual disorder in Briard dogs, which are an important animal model for developing a therapy for human blindness caused by hereditary retinal degeneration. After the discovery that a microdeletion mutation in a defined gene (RPE65) is responsible for the disease in dogs and primates, a recombinant vector construct AAV-RPE65 (adeno-associated virus – missing cDNA of the RPE65 gene) was injected subretinally into the affected eyes, and restoration of vision was reported [4,89]. The quality of the AAV-vector particle preparations can be examined by the rapid negative staining procedure and remotely and directly assessed and discussed by all the cooperating participants (Fig. 14.7), saving time and traveling costs.

In rapid viral diagnosis by EM, which is based on the characteristic virus family particle morphology and size, some clinical samples can pose

diagnostic difficulties even for experienced investigators. This is a typical situation for a live “second-opinion” request during the daily routine case diagnosis with experts of the Robert-Koch-Institute Reference Laboratory in Berlin and/or the Bundeswehr Medical Centre in Koblenz. The telepresence sample examination and case discussion at the monitor with an expert mostly allow a clear-cut diagnosis and provide significant learning opportunities (for both sides). It is worth noting that the Bundeswehr EM laboratory has similar server telepathology equipment (sibling Zeiss-TEM and TRS-camera, iTEM software) and can provide remote telepathology support (Fig. 14.10).

The concept of remote diagnostic EM was presented to the public in the traditional so-called “Long Night of Science” event (Berlin, 15 June 2002). Numerous visitors of the Robert-Koch-Institute had the opportunity firsthand to remotely operate our TEM in Regensburg and observe different infectious agents (Fig. 14.11).

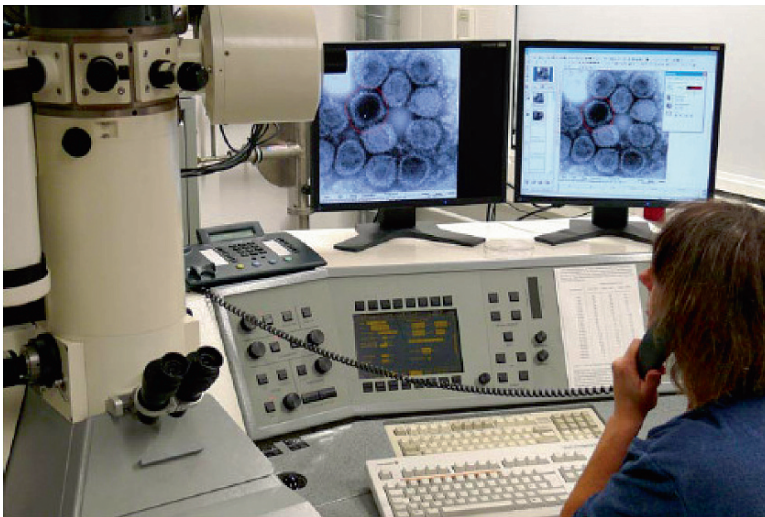


Fig. 14.10. Illustration of the “server site” of the similarly designed and equipped ultrastructural telepathology system of the Bundeswehr’s EM laboratory. This is an example of distant teaching: Regensburg was connected as a remote “client” to this system, learning about diagnostic peculiarities in herpes diagnostics. Note the use of discussion tools and the parallel voice support enhancing the telepresence effect for the session participants. (Image courtesy of B. Hauroeder, Bundeswehr/Koblenz)



Fig. 14.11. Documentation from the “Long Night of Science” in Berlin, 15 June 2002. Visitors interested in science of the Robert-Koch-Institute got an introduction to telepresence microscopy by H. Gelderblom (second from left) and operated firsthand our EM in Regensburg looking remotely at different infectious agents. (Image courtesy of H. Gelderblom, RKI/Berlin)

14.11 Discussion and Conclusions

The digital revolution and rapidly expanding telecommunication and computing ability have forced new developments like telepathology and “virtual microscopy” in the light microscopy pathologic practice. Competing immunohistochemical and molecular methods and other factors have tended to supplant EM in many diagnostic fields, but now a resurgence of this proven ancillary diagnostic tool is predicted [29,96]. This provides an opportunity to learn from the experiences collected during the evolution of light telemicroscopy technique – to adapt them or discover other solutions more suitable for the specificity of EM. Also, solutions established in material science and more advanced teleradiologic disciplines like teleradiology (similar black and white image features) [24,86,87] might lead to applications in EM.

Significant savings in time in managing difficult ultrastructural diagnoses are already achieved by sharing and discussing images via e-mail (still image, static telepathology), but reliability is limited. However, for effec-

tive EM diagnosis, only an interactive dynamic telepathology system can provide the external expert with the opportunity to select the correct details for an accurate diagnosis and avoid the pitfalls of preselected images. The recently established light microscopic “virtual slide” can indeed be extended to selected sample areas at the ultrastructural level [37,49]; this would probably be applied for electronic teaching using the “virtual microscope” rather than for diagnostic purposes. The “iTEM” software used includes an automated image “stitching” module (MIA, multiple image alignment), but the usability for ultrastructural diagnostic purposes is very limited.

Our national and international ultrastructural telepathology sessions demonstrate that available microscopes can be run remotely by desktop computers or workstations using standard Internet links and can provide a reasonably “smooth” operation of the instruments by “real-time” transmission of images via the Internet. To save bandwidth, the EM needs more automation (speed autofocus, constant illumination brightness at magnification changes, and robotic sample loader [54]). Now almost all EM providers offer an integrated remote operation option for their microscopes; unfortunately, interchangeability is still missing and is a heavy obstacle for collaboration between users of different systems. Depending on the individual need of the user, a range of high-resolution and fast, bottom- or side-entry CCD cameras can be chosen, but, currently for telemicroscopy settings, it is advisable to keep the pixel number of the transferred images as small as possible (camera pixel binning is a helpful adjunct). This limitation will probably be compensated by the rapidly growing Internet speed and bandwidth, as well as improved image compression algorithms in the near future.

The telepresence of an expert is a very effective way of obtaining a second opinion in solving diagnostic or research problems and has significant value for teaching and learning, on both server and client side. The influence of human factors is a very important aspect in establishing such new diagnostic techniques [23,52,59]. Psychological resistance or uncertainty in handling the new tools as well as rendering a diagnosis from a monitor can be a challenge, but this could be compensated by parallel voice assistance and graphic overlay discussion tools. A helpful measure for less experienced users is to hide all but present the only immediately necessary buttons on the graphic user interface. Stepwise and regular training increases familiarity with the system and markedly reduces remote examination time.

Telemicroscopy and the digital image have opened new doors for scientific cooperation, image sharing, consulting, and distant learning, with other microscopy experts. For light microscopy, specialized telediagnosis systems such as those of the AFIP/USA [64,108], UICC-TPCC/Berlin [21,45], and iPATH/Basel (Internet based on an open telemedicine platform) [8,49] with specific

performance have been developed. Now mainly for research purposes, a new kind of cooperation using worldwide-distributed hardware and software resources (nodes) have been linked together, and the so-called Grid technology will be established [5,26,34,60] (the name comes from the electrical power grid that works in a similar manner). This “distributed or parallel computing” uses the Internet as a communication layer and allows access to geographically widespread and sometimes unique resources – for example, dedicated light and electron microscopes, computing performance, information in databases, and services, respecting the rules of the economy of scale (which, e.g., determinate the relation between the size of a plant and the production costs). Different Grids are already established, for example, the Telescience Project at the NCMIR, San Diego, CA, for life science and microscopy [94] and the PRAGMA (Pacific Rim and Grid Middleware Assembly) [74]; the trend may culminate in the development of virtual institutions [48].

No doubt, in the globalized and digitized world of tomorrow and the stepwise population conversion into the “information era,” these new cyberspace structures and tools will change the face of future medical care, including pathology [3,65,110]. Old and new diseases and emerging infectious or adverse environmental agents need to be managed by raising costs for resources and energy worldwide. Telemedicine in concert with eScience and eHealth solutions has the potential to offer an efficient health service to everybody independently of geographical location and social status [112]. In trust of this knowledge and vision, we have to work in our subdiscipline of ultrastructural telepathology to establish practical solutions to manage the requirements of modern health-care systems for time and cost reduction [9,18,49] while maintaining the highest diagnostic standards. The telepathology motto, “move the image, not the patient,” will help to direct us to achieve these objectives.

14.12 Summary

- Electron microscopy – owing to its high-resolution power – can provide significant data at the ultrastructural level in tissue-based pathological diagnosis. In some subspecialties (kidney, skin, and ciliary disorders) EM is indispensable; virus diagnosis can be rendered with unprecedented speed.
- In 1999, we established an Internet-based interactive dynamic telepathology system combining remote operation of an EM, digital image acquisition, and software specifically tailored for collaborative needs.
- Remote experts can examine samples directly with adequate EM functionality, and, in case of diagnostic dilemmas, the consulting “second-opinion” expert is no longer constrained by problems inherent in preselected images.

- We demonstrated that the ultrastructural telepathology system can be remotely operated using standard workstations and Internet connections with experts in different localities; together with teleconferencing tools it provides increased effectiveness and support for diagnosis, research, and distant teaching.
- Further progress in EM automation and growing Internet bandwidth will foster such interactive telemicroscopy solutions and, through implementation into the developing Grid technology, enable new collaborative opportunities, saving time and resources.

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References

1. Aas IH (2002) Changes in the job situation due to telemedicine. *J Telemed Telecare* 8(1):41–47
2. Aas IH (2002) Learning in organizations working with telemedicine. *J Telemed Telecare* 8(2):107–111
3. Aas IH (2002) Telemedicine and changes in the distribution of tasks between levels of care. *J Telemed Telecare* 8(Suppl 2):1–2
4. Acland GM, Aguirre GD, Ray J, et al. (2001) Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* 28(1):92–95
5. Akiyama T, Teranishi Y, Nozaki K, et al. (2005) Scientific grid activities and PKI deployment in the Cybermedia Center, Osaka University. *J Clin Monit Comput* 19(4–5):279–294
6. Baak JP, van Diest PJ, Meijer GA (2000) Experience with a dynamic inexpensive video-conferencing system for frozen section telepathology. *Anal Cell Pathol* 21(3–4):169–175
7. Boutonnat J, Paulin C, Faure C, Colle PE, Ronot X, Seigneurin D (2006) A pilot study in two French medical schools for teaching histology using virtual microscopy. *Morphologie* 90(288):21–25
8. Brauchli K, Oberholzer M (2005) The iPath telemedicine platform. *J Telemed Telecare* 11(Suppl 2):S3–S7
9. Bryant J (2000) Cost minimization analysis of telepathology: a critical review. *Am J Clin Pathol* 113(6):902–905
10. Buzug TM, Handels H, Holz D (2001) *Telemedicine: medicine and communication*. Kluwer, New York
11. Coiera E (1997) *Guide to medical informatics, the Internet, and telemedicine*, 1st edn. Chapman & Hall Medical, London
12. Coma de Corral MJ, Pena HJ (1999) Quo vadis telemedicine? *Rev Neurol* 29(5):478–483
13. Dalla Palma P, Morelli L, Forti S, et al. (2001) Telemetric intraoperative diagnosis among hospitals in Trentino: first evaluations and optimization of the procedure. *Pathologica* 93(1):34–38

14. Dawson PJ, Johnson JG, Edgemon LJ, Brand CR, Hall E, Van Buskirk GF (2000) Outpatient frozen sections by telepathology in a veterans administration medical center. *Hum Pathol* 31(7):786–788
15. Della Mea V, Cataldi P, Pertoldi B, Beltrami CA (1999) Dynamic robotic telepathology: a preliminary evaluation on frozen sections, histology and cytology. *J Telemed Telecare* 5(Suppl 1):S55–S56
16. Della Mea V, Roberto V, Conti A, di Gaspero L, Beltrami CA (1999) Internet agents for telemedicine services. *Med Inform Internet Med* 24(3):181–188
17. Della Mea V, Cataldi P, Pertoldi B, Beltrami CA (2000) Combining dynamic and static robotic telepathology: a report on 184 consecutive cases of frozen sections, histology and cytology. *Anal Cell Pathol* 20(1):33–39
18. Della Mea V, Cortolezzis D, Beltrami CA (2000) The economics of telepathology – a case study. *J Telemed Telecare* 6(Suppl 1):S168–S169
19. Demichelis F, Barbareschi M, Dalla Palma P, Forti S (2002) The virtual case: a new method to completely digitize cytological and histological slides. *Virchows Arch* 441(2):159–164
20. Dickersin GR (2000) *Diagnostic electron microscopy: a text/atlas*, 2nd edn. Springer, New York
21. Dietel M, Nguyen-Dobinsky TN, Hufnagl P (2000) The UICC Telepathology Consultation Center. International Union against Cancer. A global approach to improving consultation for pathologists in cancer diagnosis. *Cancer* 89(1):187–191
22. Dolled-Filhart M, Ryden L, Cregger M, et al. (2006) Classification of breast cancer using genetic algorithms and tissue microarrays. *Clin Cancer Res* 12(21):6459–6468
23. Draper JV, Kaber DB, Usher JM (1998) Telepresence. *Hum Factors* 40(3):354–375
24. Dreyer KJ (2006) *PACS: a guide to the digital revolution*, 2nd edn. Springer, New York
25. Dykstra MJ, Reuss LE (2003) *Biological electron microscopy. Theory, techniques, and troubleshooting*, 2nd edn. Kluwer, New York
26. EGEE (2008) Enabling grids for E-Science. <http://www.eu-egee.org/>
27. Elles R, Mountford R (2004) *Molecular diagnosis of genetic diseases*, 2nd edn. Humana Press, Totowa, NJ
28. Erlandson RA (1994) *Diagnostic transmission electron microscopy of tumors: with clinicopathological, immunohistochemical, and cytogenetic correlations*. Raven Press, New York
29. Erlandson RA (2003) Role of electron microscopy in modern diagnostic surgical pathology. In: Cote W, Weiss S (eds) *Modern surgical pathology*. Saunders, Philadelphia, PA
30. Erlandson RA, Rosai J (1995) A realistic approach to the use of electron microscopy and other ancillary diagnostic techniques in surgical pathology. *Am J Surg Pathol* 19(3):247–250
31. Eyden B (1996) *Organelles in tumor diagnosis: an ultrastructural atlas*. Igaku-Shoin, New York
32. Eyden B (1999) Electron microscopy in tumour diagnosis: continuing to complement other diagnostic techniques. *Histopathology* 35(2):102–108
33. Ferrer-Roca O, Sosa-Judicissa MC (1998) *Handbook of telemedicine*. IOS Press, Amsterdam, Washington DC
34. Foster I, Kesselman C (2004) *The grid: blueprint for a new computing infrastructure*, 2nd edn. Morgan Kaufmann, Amsterdam, Boston
35. Ghadially FN (1997) *Ultrastructural pathology of the cell and matrix*, 4th edn. Butterworth-Heinemann, Boston
36. Goldsmith CS, Tatti KM, Ksiazek TG, et al. (2004) Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis* 10(2):320–326

37. Gu J, Ogilvie RW (2005) Virtual microscopy and virtual slides in teaching, diagnosis, and research. Taylor & Francis, Boca Raton
38. Hadida-Hassan M, Young SJ, Peltier ST, Wong M, Lamont S, Ellisman MH (1999) Web-based telemicroscopy. *J Struct Biol* 125(2–3):235–245
39. Hagens v.G (2007) Body Worlds. <http://www.bodyworlds.com/en.html>
40. Haroske G, Giroud F, Kunze KD, Meyer W (2000) A telepathology based virtual reference and certification centre for DNA image cytometry. *Anal Cell Pathol* 21(3–4):149–159
41. Hazelton PR, Gelderblom HR (2003) Electron microscopy for rapid diagnosis of infectious agents in emergent situations. *Emerg Infect Dis* 9(3):294–303
42. Herrera GA, Lowery MC, Turbat-Herrera EA (2000) Immunoelectron microscopy in the age of molecular pathology. *Appl Immunohistochem Mol Morphol* 8(2):87–97
43. Hufnagl P, Bayer G, Oberbamscheidt P, et al. (2000) Comparison of different telepathology solutions for primary frozen section diagnostic. *Anal Cell Pathol* 21(3–4):161–167
44. Hutarew G, Schlicker HU, Idriceanu C, Strasser F, Dietze O (2006) Four years experience with tele neuropathology. *J Telemed Telecare* 12(8):387–391
45. Kayser K (2002) Interdisciplinary telecommunication and expert teleconsultation in diagnostic pathology: present status and future prospects. *J Telemed Telecare* 8(6):325–330
46. Kayser K, Szymas J, Weinstein RS (1999) Telepathology: telecommunication, electronic education, and publication in pathology. Springer, Berlin, New York
47. Kayser K, Beyer M, Blum S, Kayser G (2000) Telecommunication – a new tool for quality assurance and control in diagnostic pathology. *Folia Neuropathol* 38(2):79–83
48. Kayser K, Kayser G, Radziszowski D, Oehmann A (2004) New developments in digital pathology: from telepathology to virtual pathology laboratory. *Stud Health Technol Inform* 105:61–69
49. Kayser K, Molnar B, Weinstein RS (2006) Virtual microscopy. VSV Interdisciplinary Medical Publishing, Berlin
50. Kok LP, Boon ME (2003) Microwaves for the art of microscopy. Coulomb Press Leyden, Leiden
51. Kricka LJ, Fortina P (2001) Microarray technology and applications: an all-language literature survey including books and patents. *Clin Chem* 47(8):1479–1482
52. Krupinski EA, Tillack AA, Richter L, et al. (2006) Eye-movement study and human performance using telepathology virtual slides: implications for medical education and differences with experience. *Hum Pathol* 37(12):1543–1556
53. Kumar RK, Freeman B, Velan GM, De Permentier PJ (2006) Integrating histology and histopathology teaching in practical classes using virtual slides. *Anat Rec B New Anat* 289(4):128–133
54. Lefman J, Morrison R, Subramaniam S (2007) Automated 100-position specimen loader and image acquisition system for transmission electron microscopy. *J Struct Biol* 158(3):318–326
55. Leong AS, Visinoni F, Visinoni C, Milios J (2000) An advanced digital image-capture computer system for gross specimens: a substitute for gross description. *Pathology* 32(2):131–135
56. Linderth HC (2002) Managing telemedicine: from noble ideas to action. *J Telemed Telecare* 8(3):143–150
57. Madeley CR (2003) Diagnosing smallpox in possible bioterrorist attack. *Lancet* 361(9352):97–98
58. Madeley CR, Field AM (1998) Virus morphology, 2nd edn. Churchill Livingstone, Edinburgh, New York

59. Mairinger T (2000) Acceptance of telepathology in daily practice. *Anal Cell Pathol* 21 (3–4):135–140
60. Martone ME, Gupta A, Ellisman MH (2004) E-neuroscience: challenges and triumphs in integrating distributed data from molecules to brains. *Nat Neurosci* 7(5):467–472
61. Mierau GW (1999) Electron microscopy for tumour diagnosis: is it redundant? *Histopathology* 35(2):99–101
62. Miller SE (2003) Bioterrorism and electron microscopic differentiation of poxviruses from herpesviruses: dos and don'ts. *Ultrastruct Pathol* 27(3):133–140
63. Morens DM, Folkers GK, Fauci AS (2004) The challenge of emerging and re-emerging infectious diseases. *Nature* 430(6996):242–249
64. Mullick FG, Fontelo P, Pemble C (1996) Telemedicine and telepathology at the Armed Forces Institute of Pathology: history and current mission. *Telemed J* 2(3):187–193
65. Murphy WM (2007) Anatomical pathology in the 21st century: the great paradigm shift. *Hum Pathol* 38(7):957–962
66. Nagata H, Mizushima H (1998) World wide microscope: new concept of internet telepathology microscope and implementation of the prototype. *Medinfo* 9(Pt 1):286–289
67. NASA (2004) Mars exploration rover mission. <http://marsrovers.jpl.nasa.gov/newsroom/pressreleases/20040305a.html>
68. Onguru O, Celasun B (2000) Intra-hospital use of a telepathology system. *Pathol Oncol Res* 6(3):197–201
69. Ordonez NG, Mackay B (1998) Electron microscopy in tumor diagnosis: indications for its use in the immunohistochemical era. *Hum Pathol* 29(12):1403–1411
70. Papadimitriou JM, Henderson DW, Spagnolo DV (1992) Diagnostic ultrastructure of non-neoplastic diseases. Churchill Livingstone, Edinburgh, New York
71. Perkins JM, Blom DA, McComb DW, Allard LF (2007) Functional remote microscopy via the AtlanTICC Alliance. *Microsc Microanal* 13(Suppl 2):1702–1703
72. Petersen I, Wolf G, Roth K, Schluns K (2000) Telepathology by the Internet. *J Pathol* 191(1):8–14
73. Picot J (2000) Meeting the need for educational standards in the practice of telemedicine and telehealth. *J Telemed Telecare* 6(Suppl 2):S59–S62
74. PRAGMA (2006) Pacific rim applications and grid middleware assembly. <http://www.pragma-grid.net/>
75. Ranchod M (2003) Intraoperative consultations in surgical pathology. In: Cote W, Weiss S (eds) *Modern surgical pathology*. Saunders, Philadelphia, PA
76. Rashbass J (2000) The impact of information technology on histopathology. *Histopathology* 36(1):1–7
77. Rembrandt vR (1632) *The Anatomy Lesson of Dr. Tulp*. <http://www.sgipt.org/kunst/medizin/sektion>
78. Sambrook J, Russell DW (2001) *Molecular cloning: a laboratory manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
79. Sawai T, Uzuki M, Watanabe M (2000) Telepathology at presence and in the future. *Rinsho Byori* 48(5):458–462
80. Schlag PM (1997) On the way to new horizons: telemedicine in oncology. *Oncologist* 2(2):III–IV
81. Schroeder JA, Voelkl E (1998) Electron microscopy examination of pathological samples in Oak Ridge/USA by remote control via Internet from Regensburg/Germany: a live ultrastructure-telepathology presentation. Abstracts G7SP4 Conference: the impact of telemedicine on health care management, Regensburg, Germany 1998:84

82. Schroeder JA, Voelkl E, Hofstaedter F (2001) Ultrastructural telepathology – remote EM-diagnostic via Internet. *Ultrastruct Pathol* 25(4):301–307
83. Schroeder JA, Voelkl E, Hofstaedter F (2002) Ultrastructural telepathology – an application of remote electron microscopy via Internet. *Eur J Med Res* 7(Suppl 1):74–75
84. Schroeder JA, Gelderblom HR, Hauroeder B, Schmetz C, Milios J, Hofstaedter F (2006) Microwave-assisted tissue processing for same-day EM-diagnosis of potential bioterrorism and clinical samples. *Micron* 37(6):577–590
85. Schroeder JA, Weingart C, Coras B, et al. (2008) Ultrastructural evidence of dermal gadolinium deposits in a patient with nephrogenic systemic fibrosis and end-stage renal disease. *Clin J Am Soc Nephrol* 3(4):968–975
86. Seiwerth S, Danilovic Z (2000) The telepathology and teleradiology network in Croatia. *Anal Cell Pathol* 21(3–4):223–228
87. Siegel EL, Kolodner RM (1999) *Filmless radiology*. Springer, New York
88. Stanberry B (2000) Telemedicine: barriers and opportunities in the 21st century. *J Intern Med* 247(6):615–628
89. Stieger K, Mendes-Madeira A, Meur GL, et al. (2007) Oral administration of doxycycline allows tight control of transgene expression: a key step towards gene therapy of retinal diseases. *Gene Ther* 14(23):1668–1673
90. Swaminathan S, Shah SV (2007) New insights into nephrogenic systemic fibrosis. *J Am Soc Nephrol* 18(10):2636–2643
91. Szymas J, Wolf G (1999) Remote microscopy through the internet. *Pol J Pathol* 50(1):37–42
92. Szymas J, Papierz W, Danilewicz M (2000) Real-time teleneuropathology for a second opinion of neurooncological cases. *Folia Neuropathol* 38(1):43–46
93. Takaoka A, Yoshida K, Mori H, Hayashi S, Young SJ, Ellisman MH (2000) International telemicroscopy with a 3 MV ultrahigh voltage electron microscope. *Ultramicroscopy* 83(1–2):93–101
94. Telescience Project (2006) <http://ncmir.ucsd.edu/>
95. Tucker JA (2000) The continuing value of electron microscopy in surgical pathology. *Ultrastruct Pathol* 24(6):383–389
96. Turbat-Herrera EA, D’Agostino H, Herrera GA (2004) The use of electron microscopy to refine diagnoses in the daily practice of cytopathology. *Ultrastruct Pathol* 28(2):55–66
97. Ultrastructural Telepathology - EM Diagnostic via Internet (2000) SCUR 2000 Meeting: histo-morphology, today and tomorrow. Bochum, Germany
98. Virchow RLK (1858) *Die cellularpathologie in ihrer begründung auf physiologische und pathologische gewebelehre*. A. Hirschwald, Berlin
99. Voelkl E, Allard LF, Nolan TA, Hill D, Lehman M (1997) Remote operation of electron microscopes. *Scanning* 19:286–291
100. Walter GF, Matthies HK, Brandis A, von Jan U (2000) Telemedicine of the future: teleneuropathology. *Technol Health Care* 8(1):25–34
101. Weidner N (2003) *Modern surgical pathology*. 1st ed. Saunders, Philadelphia
102. Weinstein RS, Bloom KJ, Rozek LS (1987) Telepathology and the networking of pathology diagnostic services. *Arch Pathol Lab Med* 111(7):646–652
103. Weinstein RS, Bhattacharyya A, Yu YP, et al. (1995) Pathology consultation services via the Arizona-International Telemedicine Network. *Arch Anat Cytol Pathol* 43(4):219–226
104. Weinstein RS, Bhattacharyya AK, Graham AR, Davis JR (1997) Telepathology: a ten-year progress report. *Hum Pathol* 28(1):1–7

105. Weinstein RS, Descour MR, Liang C, et al. (2001) Telepathology overview: from concept to implementation. *Hum Pathol* 32(12):1283–1299
106. Wellnitz U, Binder B, Fritz P, Friedel G, Schwarzmann P (2000) Reliability of telepathology for frozen section service. *Anal Cell Pathol* 21(3–4):213–222
107. Wells CA, Sowter C (2000) Telepathology: a diagnostic tool for the millennium? *J Pathol* 191(1):1–7
108. Williams BH, Mullick FG, Butler DR, Herring RE, O’Leary TJ (2001) Clinical evaluation of an international static image-based telepathology service. *Hum Pathol* 32(12):1309–1317
109. Winokur TS, McClellan S, Siegal GP, et al. (2000) A prospective trial of telepathology for intraoperative consultation (frozen sections). *Hum Pathol* 31(7):781–785
110. Wootton R (2006) Realtime telemedicine. *J Telemed Telecare* 12(7):328–336
111. Wright M (1999) Publication of the Material Microcharacterisation Collaboratory ORNL. <http://www.ornl.gov/sci/doi2k/MICSReview/99/publications.html>
112. Yogesan K (2006) *Teleophthalmology*. Springer, Berlin, New York
113. Zaluzec NJ (1998) Tele-presence microscopy: a progress Report. *Microsc Microanal* 4(Suppl 2):18–19
114. Zaluzec NJ (2007) TelePresence Microscopy Collaboratory. <http://tpm.amc.anl.gov/>
115. Zhou J, Hogarth MA, Walters RF, Green R, Nesbitt TS (2000) Hybrid system for telepathology. *Hum Pathol* 31(7):829–833