Ultrastructural Telepathology—Remote EM-Diagnostic via Internet

Joseph A. Schroeder, PhD  
Department of Pathology, University Clinics, Regensburg, Germany

Edgar Voelkl, PhD  
Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

Ferdinand Hofstaedter, Prof. MD  
Department of Pathology, University Clinics, Regensburg, Germany

New collaborative opportunities in ultrastructural research and diagnostics are now available on the Internet through the combination of digital image acquisition, remote operation of modern digitally controlled and automated electron microscopes, and the development of software specifically tailored for collaborative needs. Remote experts can examine samples directly, and unique instruments can be utilized from anywhere. In the case of diagnostic dilemmas, the second-opinion expert is no longer constrained by problems inherent in the interpretation of preselected images. The remote examiner can independently choose the area of interest on the sample as well as select the appropriate magnification for an accurate diagnosis. With these capabilities together with teleconferencing tools and securely accessible databases on-line, telepathology can provide increased effectiveness and support for diagnostics, research, and teaching in many areas. The authors report their experience with remote electron microscope diagnoses of pathological samples using two different dynamic imaging systems and discuss the main technical issues encountered. It appears that only minor technical issues need to be resolved before ultrastructural telepathology can be promoted for routine use in areas with high-speed Internet access.

Keywords diagnostic, dynamic imaging, electron microscopy, Internet, remote, ultrastructural telepathology

Telepresence microscopy includes the remote operation of microscopes by using available telecommunication links or the Internet, and when applied to pathology it can be related to light and electron microscopy. Today, the term “telepathology” (the performance of pathology at distance) is well established, although the first concept was already illustrated in 1924 in the magazine Radio News [1]. Over the years, as technology improved, telepathology evolved. In 1968, live black-and-white images were transmitted from the Logan Airport to the Massachusetts General Hospital in Boston. In 1985, R. S. Weinstein demonstrated high diagnostic accuracy of video microscopy, and in 1986, he installed the first “robotic” microscope for transmission of real-time images from El Paso, Texas to Washington, DC. In February 1990, the first remote expert consulting was performed in Europe between Darmstadt, Hannover, and Mainz by K. Kayser [2–4], and in 1993, the Arizona International Telemedicine Network established telepathology services between the United States, Mexico, and China [5].

Today, the technology has matured to the point where telepathology could become a standard tool. At the Fifth European Congress on Telepathology held in Aurich, Germany, July 2000, a forum demonstrated the use of light microscopy in different settings and social contexts around the globe [6]. Optical telepathology is well described and documented in literature [2, 7–10]. It discloses a wide range of possible telepathology applications from remote gross (macroscopic) specimen description [11], intraoperative frozen section service [12–14], second-opinion retrieval [15–18], quality assurance in screening pathology [19], to remote teaching [2, 20] by using commercial systems.

ULTRASTRUCTURAL TELEPATHOLOGY

Not considered to date was the use of electron microscopy (EM) in remote diagnostic application. This may be due to the general decline of the utilization of this technique over the last decade [21, 22]. However, the diagnostic value of EM in surgical path-
ology [23] as well as in other strong indications, like renal, muscle, nervous system, skin, ciliar defects, storage diseases, opportunistic infections, and rapid viral diagnosis (negative staining) is documented in detail [24–30]. It is important to realize that the ultrastructural examination, especially addressing diagnostic difficulties by light microscopy in neoplasms, is a complementary approach to immunohistochemistry [23, 25, 31] and mostly placed at the very end of the microscopic examinations (including sample retrieval from paraffin block).

We suggest that 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 for complex cases in real time. What is almost a routine for a light histopathologist (AFIP [32], UICC/Berlin [16, 33], WWM/Japan [34]) would be equally useful for ultrastructural pathology. The two main benefits would be the time savings in “live” examination of the original specimen by remote experts and the avoidance of the difficulties inherent in the interpretation of either photographic prints (which must travel by “snail mail”) or of a still image collection (sent by 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. This may be extremely useful in case of an unknown or epidemic viral outbreak.

REMOTE ELECTRON MICROSCOPY

The capability to examine original samples live can now 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 providing the benefits of remote collaborative and consulting [35–39]. Remote observation of thick biological samples across the Pacific was recently demonstrated using the world’s unique 3MV electron microscope (Hitachi H-3000) in Osaka University (40). To our knowledge, the first remote examination of pathological samples was presented live to a large audience at the G7SP4 (Global Health Care Applications Project, Sub-Project 4) Conference held in Regensburg in November 1998 [41].

ACTUAL REMOTE-EM EXAMINATIONS

Transatlantic Connection

For all of our transatlantic sessions, we used the computer controlled Hitachi HF-2000 TEM at the Oak Ridge National Laboratory (ORNL) in the United States. This microscope was operated via the Internet from Regensburg, Germany. It is a materials science oriented TEM (smallest magnification available X2000), is operated at 200 kV, and the images are recorded through a Gatan 794 multiscan camera with 1024 by 1024 pixels. The microscope is controlled through an RS232 interface from the same computer and program (DigitalMicrograph) that acquires the digital images. To run the microscope remotely, significant efforts for automating the instrument had to be made. For example, the illumination is adjusted automatically and all apertures of the microscope were retrofitted for computer control. Since images of the size of 1024 by 1024 pixels are rather large data sets (1 MB) they presently can not be used for “live” image transfer through the Internet. Therefore, for live imaging, a binning factor of 4 was used to reduce the amount of data to 64 kB. In this way, the field of view remains constant, but the image size is reduced to 256 by 256 pixels. From our experience, this image size is just sufficient to search the sample. Once an area of interest is found, the extended trans-mission time for large (1 MB) images (presently several seconds) is quite acceptable. As can be seen from Figure 1, the graphical user interface (GUI) allows one to collect the full-size image at request while the smaller “preview” image is transmitted either at selected time intervals or after each request to the microscope (e.g., after changing magnification). ORNL has a high-speed Internet connection to the “ESnet” and can provide large data streams in excess of several 100 kbps.

At the “client site” (University of Regensburg), access to the Internet was provided through the German Internet 2 (GWIN). This wide-area network (WAN) connects through Frankfurt directly via cable to Washington and into the ESnet, and thus provides a fast connection to ORNL. We experienced a total data throughput in the range of 100–500 kbps, depending on date and time. At both locations, the local area network (LAN) was carefully designed to keep the local “traffic” below 10%. Since the transfer rates on the LAN are usually specified at 10 Mbps or higher, this criterion assures that bandwidth limitations for remote collaboration are imposed by the Internet connection only.

As collaborative software, the commercial, platform independent software package TimbuktuPro (http://www.Netopia.com) was used. This software effectively mirrors the remote computer, in this case a Macintosh computer at ORNL, to a Windows computer at the University of Regensburg. The transmitted data are compressed during transmission using a “lossless” compression (“run-length encoding”) algorithm [42]. This has the advantage that the data remain fully intact, but the required bandwidth can be significantly higher than if a “lossy” compression algorithm would be selected (this is true in general because a “lossy” algorithm provides a much higher compression than a “lossless” algorithm).

With this transatlantic setup, we examined conventionally prepared ultrathin Epon-embedded sections of a kidney with an immunotactoid glomerulopathy, a sural nerve biopsy specimen with
primary demyelinating polyneuropathy, and a herpes-virus grid prepared from routine negative-staining diagnostic (Figures 1–3). The remote control capabilities included stage navigation and search of area of interest at low magnification in “preview” mode; selection of adequate magnification (2,000–200,000), astigmatism correction, focus adjustment, and image documentation at full resolution (1024×1024 pixel). This ultrastructural telepathology session was presented via a high-resolution video-projector to the conference audience [41]. The live images from the EM at ORNL were bright and crisp. Selected images were saved onto the local computer in Regensburg for print on demand. In addition to the live connection, a videoconference link (Intel Team Station) was provided and backed by standard phone connection for verbal communication. This arrangement provided a convincing telepresence demonstration to the participants.

Connections Within Europe
At the Society for Cutaneous Ultrastructure Research (SCUR) Meeting in Bochum, Germany, 4–6 May 2000, we were able to test a different setup. From one of the meeting rooms in Bochum, we controlled our LEO912AB electron microscope located at our lab at the University of Regensburg, Germany [43]. This new generation, digitally controlled TEM (Zeiss/Oberkochen) provides an in-column integrated energy filter, operates at 120 KV and thus provides an ideal instrument for biological samples. It is equipped with a bottom-mounted 1024 by 1024-pixel CCD slow-scan camera (Proscan/Scheurich, Germany) for image acquisition. The system is controlled by a Windows NT server and requires a high-speed processor, i.e., a dual Pentium-III Processor (2×500 MHz, 128 MB RAM) and a frame grabber. The commercial software “analySIS” (EsiVision Ver. 3.1, SIS/Muenster) was expanded with a dedicated Telepathology-Server-Module that handles the communication between the microscope and the remote computer. At the “client site” in Bochum a 160-MHz Pentium II laptop offered sufficient speed for collaboration. An ISDN-PCMCIA card provided direct access to the Internet at 64 kbps and the same “analySIS” software was installed on the laptop computer. A video projector with a resolution of 1024 by 768 pixels displayed the remote operation to the audience.

Once the Internet connection to the EM server in Regensburg was established, the remote examination of a (routinely prepared) skin biopsy section from a CADASIL-patient was started. A live search at an overview magnification (×500) soon exposed a small arterial vessel. The basal membrane of the pericytes of the vessel wall was inspected, and some BM deposits were located. At ×4,000 and ×10,000 magnification, the pathognomonic GM deposits (=granular material) could be recognized clearly and were documented (saved on hard drive). With an image size of 512 by 512 pixels and an exposure time of 0.2 s, we obtained a frame rate of 0.2 fps, or about one image every 5 s. This transfer rate is clearly due to the slow uplink from the notebook. At 64 kbps, it is expected that image data of the size of 512 by 512 pixels per image (=256 kB) will be slow. Uncompressed, the transfer of these data through a 64-kbps bottleneck would require 32 s per image. Our measured data throughput suggests that the compression factor used by “analySIS” was about 16.

It should be mentioned that the telepathology system as demonstrated at Bochum was a preliminary test version and significant modifications (Figures 4 and 5 display the release of November 2000) to the graphical user interface are under way.

DISCUSSION
The advancement to documentation and archiving of EM images in digital form was a first important step for remote EM. The increasing number of digitally controlled EMs available, the growing telecommunication capabilities, and the availability of collaborative software have opened new ways for scientific cooperation, image sharing, consulting, and learning with other EM experts. Significant time savings are already achieved by sharing and discussing images via e-mail, but reliability is limited. However, only a dynamic telepathology system can provide the expert the opportunity to select the correct details for an accurate diagnosis and avoid the pitfalls of preselected images. Comparable to light microscopy examinations, it is preferable to get an idea of the whole specimen...
examined, also to assess for a possible tissue sampling error (tumor heterogeneity, artifacts).

Our national and international ultrastructural telepathology sessions demonstrate that available microscopes can be run remotely by using desktop computers and standard Internet links, and can provide a reasonable "smooth" operation of the instruments by real-time transmission of images via Internet. The small lower-resolution image (256\times 256 pixels) seems more suitable than higher resolution pictures for live navigation over the specimen and is sufficient for the orientation purposes. The large high-resolution (1024\times 1024 pixel) image meets the diagnostic requirements well, but at the expense of transmission time.

In our trials four main issues were encountered:

FIG. 2 BM with tubular protein deposits in an immunotactoid glomerulopathy of a 45-year-old patient. Examined and acquired remotely at an original magnification of \( \times 12,000 \), HF-2000 in Oak Ridge/ORNL, hard-drive print in Regensburg.
1. The specimen overview mode with a tracking and “position recall” operation is a central point in the remote EM examination for diagnostic purposes.

2. To save bandwidth, the EM needs to be automated (e.g., constant illumination conditions and autofocus capabilities when magnification is changed).

3. For widespread and ease of use, the expert or “client site” interface needs to be downloadable (for free) and should be platform independent (e.g., through Java applets). Additional teleconferencing tools such as a multi-user arrow pointer and a parallel running web-camera would significantly improve collaboration.

FIG. 3 Sural nerve biopsy of a 61-year-old patient, myelin sheath lesion in a demyelinating polyneuropathy. Captured remotely at an original magnification of $\times 20,000$ on HF-2000 in Oak Ridge/ORNL, hard-drive print in Regensburg.
4. An overall improvement in bandwidth and better implementation of data compression algorithms would be essential for routine collaboration.

As pointed out, the available bandwidth (transmission rate for images) on the Internet is crucial. We are presently in the process of introducing a wireless LAN network in Regensburg rated at 11 Mbps (Orinoco/Lucent Technologies), to provide better access to our instruments. The immediate access to structural and ultrastructural data is a very important health telematic tool, both in industrial countries as well as in developing countries [44–50]. Here, remote EM offers new collaborative ways in medical science and especially in ultrastructural pathology. All manufacturers of electron microscopes are presently implementing remote operation capabilities by fully digitizing their latest instruments. However, collaborative software, i.e., the GUI for remote instrument operation, and other collaborative tools like video conferencing tools or data compression algorithms need to be developed or improved. Also, problems concerning data security remain wide open [51]. On the positive side, ultrastructural telepathology already has become a reality and been proven to increase the effectiveness of the electron microscopic diagnostic work. Depending on the demands of given clinical settings, telepathology can offer remote diagnostic at the grossly, histologic, and ultrastructural level. The requirements of modern health care systems for time and cost reduction [52–54] while maintaining the highest diagnostic standards is the challenge of our time. The telepathology motto “move the image, not the patient” can help to manage the challenge.

REFERENCES

1. Wells CA, Sowter C. Telepathology: a diagnostic tool for the millennium? J Pathol. 2000;191:1–7.
2. Kayser K, Szymas J, Weinstein R. Telepathology: Telecommunication, Electronic Education and Publication in Pathology. Berlin: Springer; 1999.
3. Weinstein RS, Bloom KJ, Rozek LS. Telepathology and the networking of pathology diagnostic services. Arch Pathol Lab Med. 1987;111:646–652.
4. Weinstein RS, Bhattacharyya AK, Graham AR, Davis JR. Telepathology, a ten year progress report. Hum Pathol. 1997;28:1–7.
5. Weinstein RS, Bhattacharyya AK, Yu YP, et al. Pathology consultation services via the Arizona International Telemedicine Network. Arch Anat Cytol Pathol. 1995;43:219–226.
6. Kayser K. Abstracts of Vth European Congress on Telepathology, July 20th–23rd 2000; Aurich, Germany. Electr J Pathol. 2000;6:2.
7. Coiera E. Guide to Medical Informatics, the Internet and Telemedicine. London: Chapman & Hall Medical; 1997.
8. Dervan PA, Wootton R. Diagnostic telepathology. Histopathology. 1998;32:195–198.
9. Bidgood WD, Horii SC, Prior FW, Van Syckle DE. Understanding and using DICOM, the data interchange standard for biomedical imaging. J Am Med Inform Assoc. 1997;4:199–212.
10. Saltz JH. Digital pathology: the big picture. Hum Pathol. 2000;31:779–780.
11. Leong AS, Visioni F, Visioni C, Milios J. An advanced digital image-capture computer system for gross specimens: a substitute for gross description. Pathology. 2000;32:131–135.
12. Onguru O, Celasun B. Intra-hospital use of a telepathology system. Pathol Oncol Res. 2000;6:197–201.
13. Della Mea V, Cataldi P, Pertoldi B, Beltrami CA. Dynamic robotic telepathology: a preliminary evaluation on frozen sections, histology and cytology. J Telemed Telecare. 1999;5 (Suppl. 1):55–56.

14. Dawson PJ, Johnson JG, Edgemont LJ, Brand CR, Hall E, Van Buskirk DF. Outpatient frozen sections by telepathology in a Veterans Administration medical center. Hum Pathol. 2000;31:786–788.

15. Szymas J, Wolf G. Remote microscopy through the internet. Pol J Pathol. 1999;50:37–42.

16. Petersen I, Wolf G, Roth K, Schlüns K. Telepathology by the Internet. J Pathol. 2000;191:8–14.

17. Zhou J, Hogarth MA, Walters RF, Green R, Nesbit TS. Hybrid system for telepathology. Hum Pathol. 2000;31:829–833.

18. Winokur TS, McClellan S, Siegal GP, Redden D, Gore P, Lazenby A, et al. A prospective trial of telepathology for intraoperative consultation (frozen sections). Hum Pathol. 2000;31:781–785.

19. Kayser K, Beyer M, Blum S, Kayser G. Telecommunication—a new tool for quality assurance and control in diagnostic pathology. Folia Neuropathol. 2000;38:79–83.

20. Picot J. Meeting the need for educational standards in the practise of telemedicine telehealth. J Telemed Telecare. 2000;6 (Suppl. 2):59–62.

21. Hammar SP. Is electron microscopy dying? Ultrastruct Pathol. 1986;10:3–5.

22. Dardick I, Herrera GA. Diagnostic electron microscopy of neoplasms. Hum Pathol. 1998;29:1335–1338.

23. Tucker JA. The continuing value of electron microscopy in surgical pathology. Ultrastruct Pathol. 2000;24:383–389.

24. Erlandson RA, Rosai J. A realistic approach to the use of electron microscopy and other ancillary techniques in surgical pathology. Am J Surg Pathol. 1996;19:247–250.

25. Ordóñez NG, Mackay B. Electron microscopy in tumor diagnosis: indications for use in the immunohistochemical era. Hum Pathol. 1998;29:1403–1411.

26. Mierau GW. Electron microscopy of tumor diagnosis: not redundant—resurgent! Histopathology. 1999;35:99–101.

27. Eyden B. Electron microscopy in tumor diagnosis: continuing to complement other diagnostic techniques. Histopathology. 1999;35:102–108.

28. Dickersin GR. Diagnostic Electron Microscopy: A Text/Atlas, ed 2. New York: Springer; 2000.

29. Papadimitriou JM, Henderson DW, Spagnolo DV, eds. Diagnostic Ultrastructure of Non-neoplastic Diseases. New York: Churchill Livingstone; 1992.

30. Erlandson RA. Diagnostic Transmission Electron Microscopy of Tumors with Clinicopathological, Immunohistochemical, and Cytogenic Correlations. New York: Raven; 1994.

31. Herrera GA, Lowery MC, Turbat-Herrera EA. Immunoelectron microscopy in the age of molecular pathology. Appl Immunohistochem Mol Morphol. 2000;8:87–97.

32. Mullick FG, Fontelo P, Pembble C. Telemedicine and telepathology at the Armed Forces Institute of Pathology: history and current mission. Telemed J. 1996;2:187–193.

33. Dietel M, Nguyen-Dobinsky TN, Hufnagl P, The UICC Telepathology Consultation Center: a global approach to improving consultation for pathologists in cancer diagnosis. Cancer. 2000;89:187–191.

34. Nagata H, Mizushima H. World wide microscope: new concept of internet telepathology microscope and implementation of the prototype. Medinfo. 1998;9:286–289.

35. Voelki E, Allard LF. Das Materials Microcharacterisation Collaboratory: mehr als nur die Fernbedienung von Instrumenten. Optik. 1997;106 (Suppl. 7):133.

36. Voelki E, Allard LF, Nolan TA, Hill D, Lehmann M. Remote operation of electron microscopes. Scanning. 1997;19:286–291.

37. Ellisman MH, Hadida MH, Greer D, et al. Telemicroscopy: development of a collaboratory for microscope digital anatomy. Proceedings of the 1998 MSA Meeting. Atlanta, Georgia. Microsc Microanal. 1998;4 (Suppl 2):12–13.

38. Hadida MH, Young SJ, Pel'tier ST, Wong M, Lamont S, Ellisman MH. Web-based telemicroscopy. J Struct Biol. 1999;125:235–245.

39. Zaluzec NJ. Tele-presence microscopy: a progress report. Proceedings of the 1998 MSA Meeting. Atlanta, Georgia. Microsc Microanal. 1998;4 (Suppl 2):18–19.

40. Takaoka A, Yoshida K, Mori H, Hayashi S, Young SJ, Ellisman MH. International telemicroscopy with a 3 MV ultrahigh voltage electron microscope. Ultramicroscopy. 2000;83:93–101.

41. Schroeder J, Voelki E. Electron microscopic examination of pathological samples in Oak Ridge/USA by remote control via Internet from Regensburg/Germany: a live ultrastructural-telepathology presentation. Abstracts of the G7SP4 Conference: The Impact of Telemedicine on Health Care Management. Regensburg; 1998:84.

42. Nelson M. Data Compression Book. New York: Springer; 1998.

43. Schroeder J, Voelki E, Hofstaedter F. Ultrastructural Telepathology—EM-Diagnostic via Internet. Abstracts of the SCUR 2000 Meeting: Histo-Morphology, Today, and Tomorrow. Bochum; 2000:58.

44. Draper JV, Kaber DB, Usher JM. Telepresence. Hum Factors. 1998;40:354–375.

45. Della Mea V, Roberto V, Conti A, diGiseppe L, Beltrami CA. Internet agents for telemedicine services. Med Inform Internet Med. 1998;4(Suppl.2):12–18.

46. Sawai T, Uzuki M, Watanabe M. Telepathology at presence and in the future. Oncologist. 2000;6(Suppl.1):59–62.

47. Rashbass J. The impact of information technology on histopathology. Histopathology. 2000;36:1–7.

48. Schlag PM. On the way to new horizons: telemedicine in oncology. Oncologist. 1997;2:3–4.

49. Walter GF, Matthies HK, Bradis A, van Jan U. Telemedicine of the future: teleneuropathology. Technol Health Care. 2000;8:25–34.

50. Savai T, Uzuki M, Watanabe M. Telepathology at presence and in the future. Rinsho Byori. 2000;48:458–462.

51. Stanberry B. Telemedicine barriers and opportunities in the 21st century. J Intern Med. 2000;247:615–628.

52. Agha Z, Weinstein RS, Dunn BE. Cost minimization analysis of telepathology. Am J Clin Pathol. 1999;112:470–478.

53. Bryant J. Cost minimization analysis of telepathology: a critical review. Am J Clin Pathol. 2000;113:902–905.

54. Della Mea V, Corteijez D, Beltrami CA. The economics of telepathology: a case study. J Telemed Telecare. 2000;6 (Suppl 1):168–169.