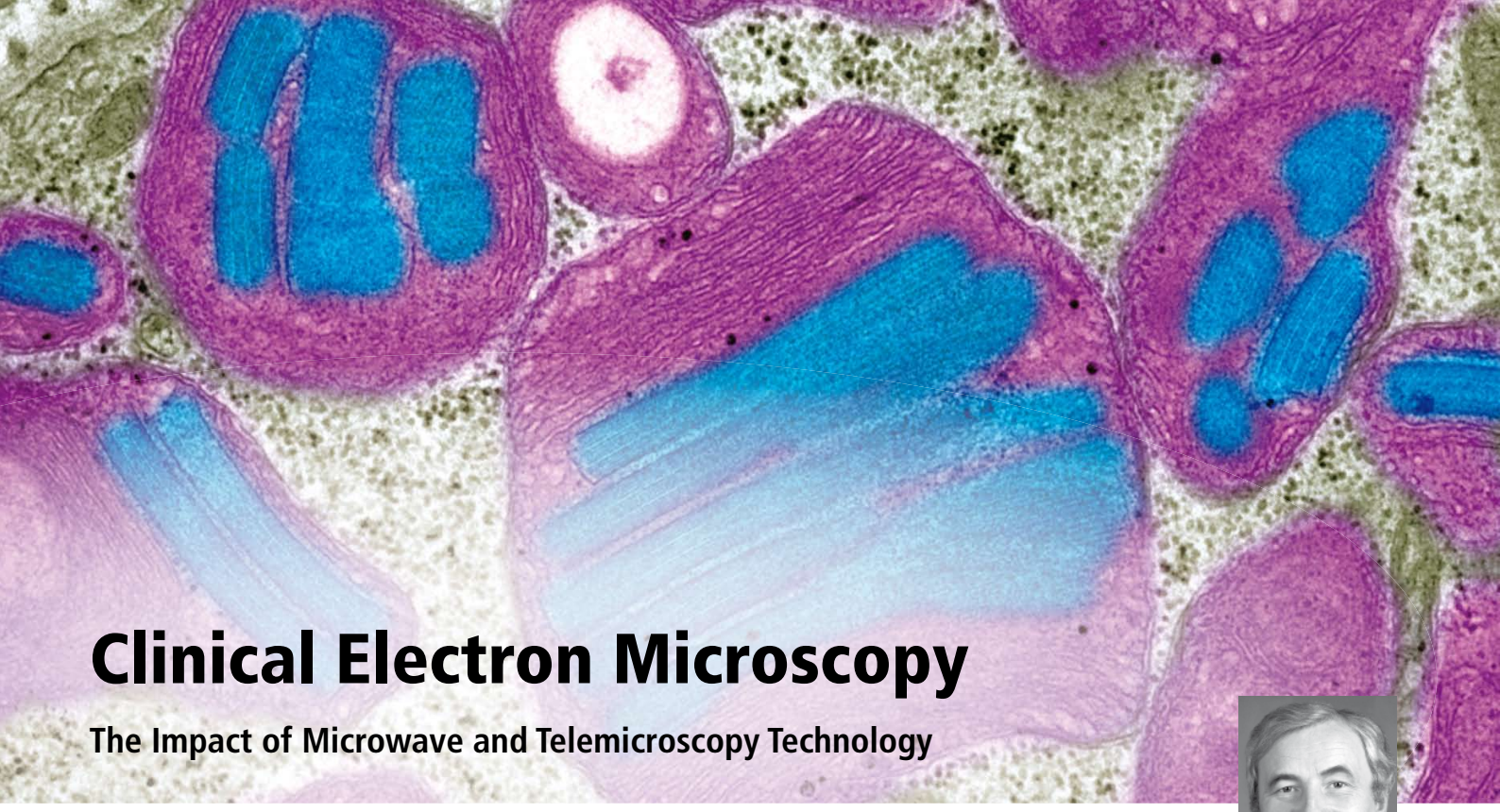

Clinical Electron Microscopy

The Impact of Microwave and Telemicroscopy Technology

Microchondriopathy of eyelid muscle in a ptosis patient

Dr. Josef A. Schröder

The high resolution and sensitivity of electron microscopy is a valuable ancillary tool or gold standard in pathological diagnosis. The conventional sample turnaround time for processing in the lab can be significantly reduced from days to hours by the microwave technology.

Microwave-assisted tissue processing, in combination with digital image acquisition, enables a "same-day" diagnosis in urgent clinical cases. Ultrastructural telepathology allows instant and live second opinion retrieval from a remote expert worldwide.

Introduction

The primary basis for pathologic tissue diagnosis is the morphological analysis: a pathologist assesses by light microscopy cell and matrix appearances including their spatial architecture in an H&E-stained section of tissue embedded in paraffin wax, in the context of gross findings and clinical data, to render a diagnosis. Additional stains and techniques, like immunohistochemistry (IHC), flow cytometry, cytogenetics and "molecular" techniques (gene rearrangement analysis, fluorescence in situ-hybridisation and polymerase chain reaction (PCR) analysis) provide additional information to refine the understanding of disease and diagnosis.

Electron microscopic (EM) examination of pathological samples is a method of extending morphologic analysis to the ultrastructural level providing information not discernible by the other methods, e. g. on the basis of the antigens expressed by a neoplasm [1].The advent and continual development and automation of the ancillary techniques, especially in IHC and now in molecular biology, resulted in reduced importance of EM in pathology since the early 1980s. Also a number of limitations of EM (e. g. poor sampling, need for adequate tissue fixation, expensive and sophisticated instrumentation, long turnaround time, high level of staff skills and interpretation expertise) need to be negotiated. Furthermore, the last EM technology milestones (cryotechniques, tomography/3D-visualisation, aberration correctors) which had a great impact on the basic research, show low if any benefit for diagnostic EM, with one exception – the digitalisation of the EM control and image acquisition. Today EM-diagnostic expertise is generally available only in larger laboratories or centres with specific interest in EM [2, 3].

Spectrum of EM-diagnosed Clinical Samples

It is interesting to recall, that one of the first samples visualized by Borries and Ruska in the very early days of EM ("Übermikroskopie") were poxvirus samples [4]. Today the negative-staining method is still a very efficient and rapid EM diagnostic (approx. 30 minutes) procedure when applied to potential infectious suspensions: it allows a rapid morphological identification and classification of different agents contained in the specimen, this can be crucial in emerging situations (e.g. SARS, bird flue, anthrax-attack) [3]. Based on the example of our centralised EM unit in a clinical context, we confirm the continuing value of EM diagnosis in surgical pathology of tumours [5] and numerous non-neoplastic indications like renal, muscle, nervous system, skin, ciliar defects, storage diseases, toxic lesions, male infertility (centriolopathy), and opportunistic infections, as already documented by others in detail [6,7].

In the clinical setting of the above mentioned diseases EM can be utilised as an ancillary tool, quality control method, or gold standard, to complement, support, or confirm the results of light histopathological diagnoses. This potential is often insufficiently used, either for budgetary constraints, strategic reasons, or too long turnaround sample prepara-

tion and examination time. To overcome the impact of the main EM-technology limitations we introduced the use of microwave technology to reduce the sample preparation time and improve the ultrastructure preservation quality; for fast examination result delivery and second opinion retrieval - aside the digital image acquisition – we developed a remote microscopy operation system for ultrastructural telepathology via the Internet.

Microwave–assisted Sample Preparation

The microwave technology is very successfully applied in organic chemistry, and the use of technical low microwaves "ovens" in the histopathology laboratory had started already in the early 1970s to speed up a range of processes: tissue fixation, decalcification, antigen retrieval, section staining, immunolabeling, and *in situ*-hybridisation [8]. The effect of microwave irradiation on polar substances is mainly well understood. It is attributed to dielectric heating – also called "thermal effect" – causing a temperature rise in the whole sample ("internal heating"; in contrast to conventional heating which starts at the specimen surface). The existence of an additional "non-thermal" direct microwave energy effect, which may be particularly effective in biological hydrated material (and hence relevant for tissue fixation), is still a matter of controversy.

We reported the results of microwave-assisted rapid tissue sample processing collected with a semi-automatic (Milestone/Sorisole, Italy, fig. 1) and the latest, fully-automatic (Leica/Vienna, Austria, fig. 2) microwave tissue processor for routine use. This technology cuts the usual three to five days turnaround time down to approx. three to six hours, enabling a "same-day" EM-diagnosis in urgent clinical settings or potential bioterrorism and/or emerging infectious agents (e.g. anthrax, SARS) scenarios [9]. Examples of microwave-assisted processed tissues showing excellent preservation of ultrastructure are comparatively presented in figure 3.

In the Milestone Rapid Electron Microscope microwave device (REM) the vial, containing baskets with the samples immersed in the process solution, is placed in a specially designed carrier which locates the vial in a defined position in the microwave cavity. A non-contact infrared temperature sensor measures the current solutions temperature in the vial, which is the critical parameter to monitor the magnetron wattage power output (max. 700W). This is controlled via a feedback loop during the continuous microwave irradiation of the sample. The slope of the temperature rise/stabilisation and the time for each processing step can be defined on a dedicated touch screen monitor. Each solution change to the next process step must be done manually by the user.

Fig. 1: Rapid Electron Microscope microwave device (Milestone REM)

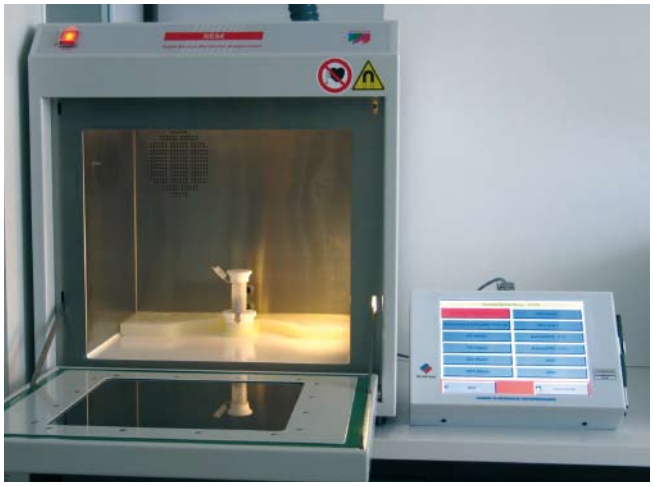

Fig. 2: Automatic microwave tissue processor (Leica EM AMW).

This change is carried out automatically by a robotic reagent system in the Leica automatic microwave tissue processor (AMW) which is a great benefit for saving laboratory time. The mono-mode microwave chamber provides homogeneous microwave distribution at the sample location without hot and cold spots. Thus water loads are not required and virtually 100% of the MW-radiation energy (restricted to 30W) is absorbed by the processing fluids and the specimens. Additionally, a dedicated pulse mode is available to maximise the benefits of the microwave-assisted processing.

In both devices, the complete microwave-assisted process is controlled by a microprocessor and dedicated software, both operating with the same sample baskets as used in standard EM tissue processors (e.g. LYNX, Leica/Vienna) for routine conventional tissue embedding. We observed that this shared equipment is a benefit for the EM-lab workflow because one can combine microwave-assisted steps (fixation 20 minutes, resin block polymerisation 80 minutes!) with conventional overnight processing generating more flexibility in handling urgent clinical samples.

Ultrastructural Telepathology via Internet

As in light-microscopy, the consultation of experts is essential for complex EM cases or controversial findings and original specimen sections need to be examined directly instead of interpreting pre-selected images. We established a dynamic remote EM-diagnostic system for the "second opinion" consultation based on the LEO912AB transmission electron microscope (Zeiss/Oberkochen) equipped with a side- and bottom-mounted 1k x 1k-pixel CCD cameras (TRS/Moorenweis), controlled by the "iTEM" software (OSIS/Muenster) from a server and linked via Internet to locations throughout Europe (fig. 4). This server-client architecture EM-telepathology system enables the remote expert to: perform stage navigation and live searching for the area of interest at low magnification, selection of adequate magnification (18–400,000x), focus adjustment, beam brightness and exposure time control, and image storage at full resolution on the local and remote computer hard-drive. Overlay features like direct structure size measurement utility, and the implemented discussion tools (arrows, drawing marks, annotations; fig. 5) used

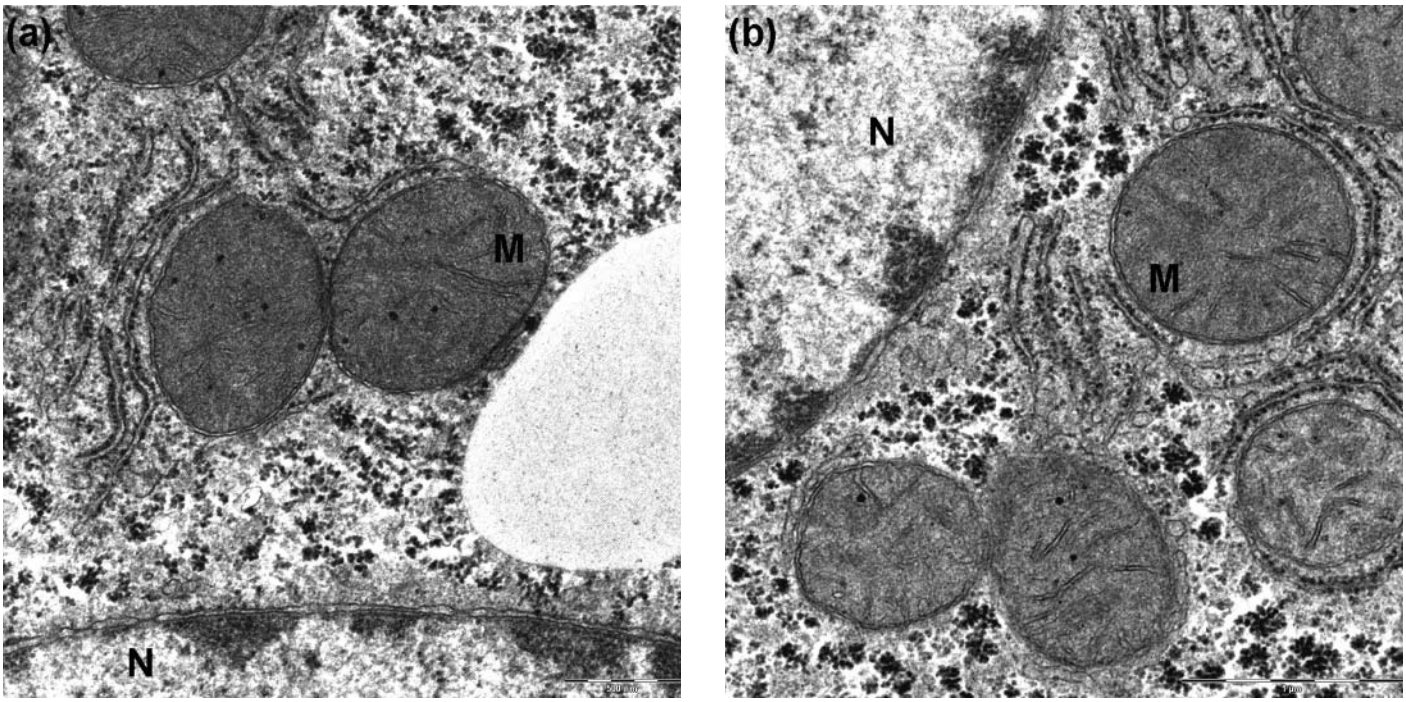

Fig. 3: Liver cell detail. (a) Microwave embedding. (b) Conventional embedding. Note the excellently displayed membranes of the mitochondria (M) and nucleus (N) in both images. Original magnification: 5,000x.

in a remote session hooked up by a parallel phone connection, ensure a real telepresence feeling at both collaborative locations [10].

Conclusions

Electron microscopy, with the potential of its 1000x higher resolving power compared with light microscopy, is still used as an ancillary tool, quality control method or gold standard to complement, support, or confirm the result of histopathological diagnoses. The microwave technology can significantly reduce the sample turnaround time from days to hours providing excellent ultrastructure preservation. Rapid microwave-assisted tissue processing combined with digital image acquisition make the "same-day" EM-diagnosis a reality, which can be crucial in urgent clinical cases. Ultrastructural telepathology bridges space and time, and is a novel tool for instant live second opinion retrieval and to share interesting findings worldwide. The rapid advances in Internet technology and speed enable a high level of telepresence collaboration in EM-diagnostics, research, and teaching.

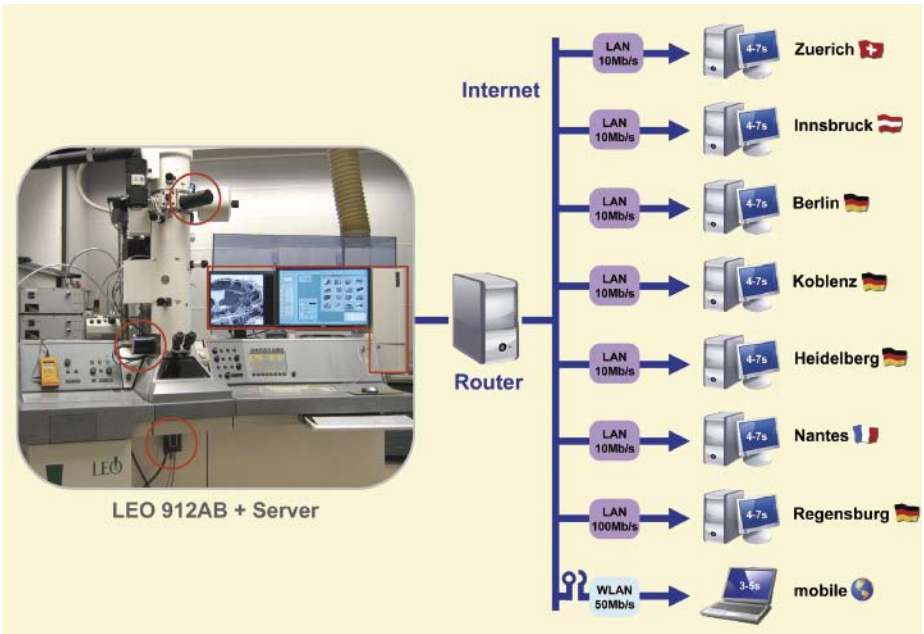

Fig. 4: Scheme of the server-client architecture of the electron microscopy telepathology consultation system at the Central EM-Lab/University Regensburg. The LEO912 EFTEM is retrofitted with a motorised drift-minimised objective aperture, and a 1kx1k pixel CCD-camera is bottom- and side-entry-mounted 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 "snapshot mode" (uncompressed high-resolution 16-bit images for storage) the transfer needs 4-7 seconds (dependent on Internet daytime bandwidth).

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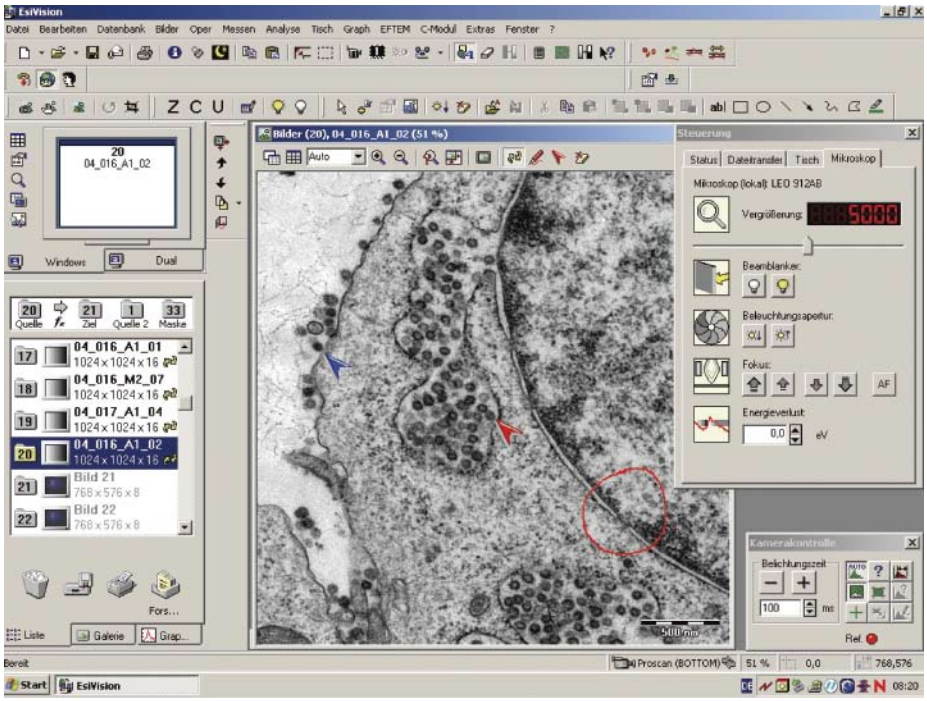

Fig. 5: Screenshot of the "client site" monitor visible to the remote located expert. Note the live transmitted image (SARS-virus propagation in cell culture, "Same-Day diagnosis", mag. 5,000x, arrows=discussion tools) and two panels for remote EM control.

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