

## Digital Slide and Virtual Microscopy-Based Routine and Telepathology Evaluation of Gastrointestinal Biopsy Specimen

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### 2.1 Introduction

Medical digital image analysis, following the rapid developments in the information technology industry, is getting more and more an accepted method [21]. Digital techniques and laboratories [7] already exist for the radiology applications. New standalone automated microscope systems and recently fully automated digital slide scanners were developed for the histo/cytology applications in the last decade and years as well.

The automated rescreening of cervical smears is now available for routine [14, 17]. Several new semiautomatic microscopes were developed for the quality control of the cytotechnologists' work [1, 2, 12, 13]. These systems notify the scanned area and images, and additional electronic recording of selected images is also available. The automated histological analysis is important research project for several years, but the results were unsatisfactory for routine applications [3, 19]. However, the histological diagnosis can be supported already today by new electronic techniques like the TV image cytometry and teleconsultation based on histological images rather than entire samples [6].

Telepathology services were built in the last decade around two technology platforms. The dynamic telepathology includes remote-controlled microscope systems with high-throughput online image transport channels [8, 27]. This method has the advantage of entire slide access and lacks the error source of preselected microscopic frames; however, the costs of the system and working are relatively high. The application of static preselected images for teleconsultation needs much less hardware investment; however, a sampling error can occur [25, 26]. Using Internet as a telecommunication pathway for static images is a low-cost widely available alternative [9, 11].

The application of a digital slide should be considerable as a source and target of the telepathology and automated histological analysis. In the last years, attempts to use an electronic or digital slide for education, teleconsultation, and immunohistochemical analysis are growing [4, 5, 15, 23]. The storage capacity and speed of personal computers became applicable only recently for handling the huge amount of image information stored on a slide.

Low-cost, commercial, motorized microscopes were marketed by several manufacturers in late 1990s. Recently, several automated digital slide scanning systems became widely available and used for transmitted light and multichannel fluorescence. They are using either optical elements of the microscopy with a CCD camera or a line sensor. A special alternative could be the multilens slide scanning systems.

Working on digital slides should require virtual or digital microscopy. Preliminary positive results on a limited number of mosaicked microscopic images have been reported recently [22].

The aim of the study was the evaluation of a digital slide scanning system and a virtual microscope (VM) on gastrointestinal biopsy specimen in a routine environment. A comparison was performed between the optical microscope (OM) and digital microscope evaluation in local and remote mode.

## 2.2

### Materials and Methods

#### 2.2.1

##### Gastric Routine Biopsy Specimen Analysis

Biopsy specimens were placed in buffered formalin, routinely processed and stained with hematoxylin and eosin. Altogether 103 specimens were selected from the files of cases seen at the Ist Department of Pathology. Single representative slide was evaluated from each case. The distribution of the cases is listed in Table 2.1.

The histological sections were evaluated in separate setting on the VM and OM without the knowledge of the previous analysis by two independent, board-certified histologists. First, the evaluation of the glass slides on optical microscopy was done. The paper copies of the clinical histories and the evaluation report were collected. In several weeks, the slides were digitized by our system described in later sections by an assistant.

The slide digitization was done in the Digital Microscopy Laboratory. The scanning computer was used as a digital slide server too. The virtual microscopy evaluation was done on a separate local area network (LAN) workstation in the

**Table 2.1.** Diagnoses

Organ	Diagnoses	No. of cases
Gastric	Healthy	5
	Chronic gastritis without intestinal metaplasia	16
	Chronic gastritis with intestinal metaplasia	12
	Chronic gastritis with atrophy	3
	Adenocarcinoma well differentiated, intestinal type	9
	Adenocarcinoma, nondifferentiated	6
	Sigillocellular carcinoma	4
	Anaplastic carcinoma	2
	Adenopapillar carcinoma	1
	Hyperplastic polyp	1
	MALT lymphoma	2
Colon	Healthy	5
	Colitis ulcerosa	14
	Crohn's disease	7
	Chr. Asp. colitis	4
	Hyperplastic polyp	2
	Adenoma tubulare with severe dysplasia	2
	Adenoma tubulovillosum with severe dysplasia	1
	Adenoma papillare	1
	Adenoma tubulopapillare	1
	Adenoma villosus with dysplasia	1
	Adenocarcinoma	4

Pathology Department with access to the slide server computer using the Internet (Fig. 2.1).

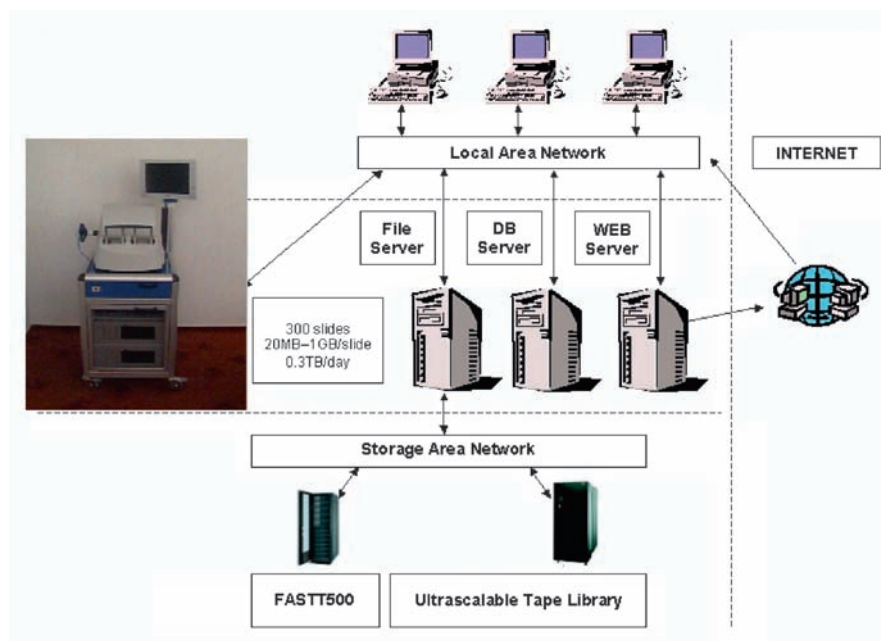
### 2.2.2

#### Data Analysis

At the conclusion of the study, having all the optical and virtual microscopy results and the consensus data, concordance and source of diagnostic discordance were determined.

Concordance was designated level “a.” Levels “b” and “c” were either clinically unimportant or clinically important discordance, as suggested by Weinberg et al. [25].

The cases where discordant results were observed were collected together with the optical and electronic data. The final diagnosis and definition of the source of the discordant data were brought by consensus diagnosis (CON) on a common session in the consultation room. Here, the access to the digital slides was also available through a computer workstation and Internet.



**Fig. 2.1.** Databank behind the Mirax digital slides. Slides can be stored on the scanning PC, on a dedicated slide server, dispatched through the Web directly. Slide images and patient information can be stored separately. Long-term storage application is included on tape libraries

The reasons for discordance were classified by the consensus meeting as to inadequate image quality (class I), interpretation (class II), and insufficient clinical information (class III). The significance of the concordance was determined using Kendall's concordance coefficient determined by the Statistica program package (V.4.3, Statsoft, USA).

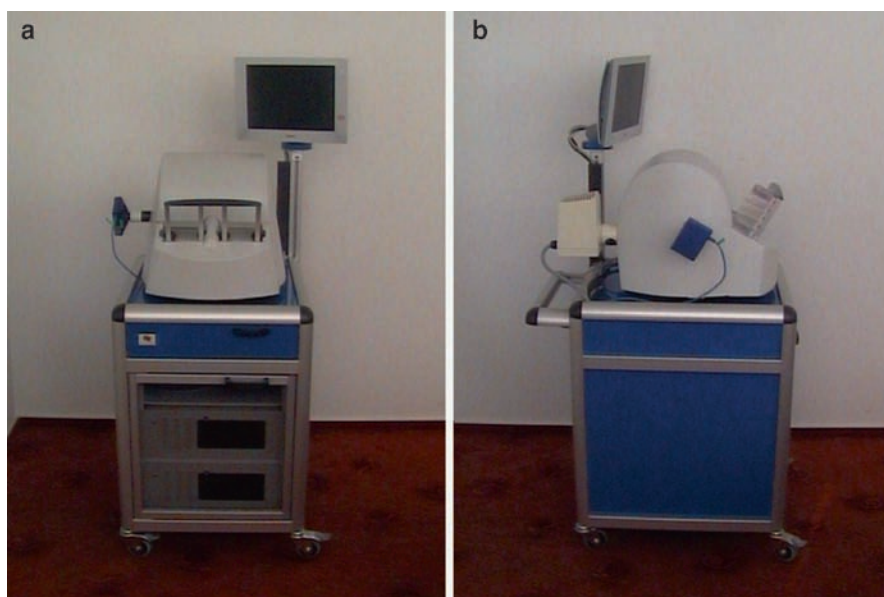
### 2.2.3

#### Slide Digitization and the VM System

##### 2.2.3.1

##### *Used Hardware Tools*

We used a Mirax Scan digital slide scanner (developed and produced by 3DHISTECH Ltd, Budapest; distributed worldwide by Carl Zeiss, Jena, Germany) (Fig. 2.2). This scanner allows the fully automated scanning of up to 300 slides in a batch, using slide magazines each for 50 slides. The automated identification of the slides is done through the barcode label. Automated identification of area of interest is done through a preview camera. The microscope functions (objectives, stage, focus, illumination, and filters) can be controlled and changed



**Fig. 2.2.** The Mirax scanner developed for high-throughput automated scanning of large-volume slides. (a) Frontal view with the stage and control computers. (b) Side view: view of the camera, fluorescent illumination. Note the slide magazines on the loading rack

through the RS232 interface from an application program. The mechanic accuracy of the motorized scanning stage for  $X/Y$  and  $Z$  directions was  $0.3\text{ }\mu\text{m}$ .

In our work, we used the Allied Vision Technologies Marlin 1,4 MP one-chip CCD camera with  $1,380 \times 1,030$  pixels. The integration time of the camera can be controlled through the computer interface RS485. The programs were running on computers with a double-core Intel processor, 128 MB RAM, and 2 GB hard disk. The scanning time for a biopsy, including loading, barcode identification, scanning, and slide repositioning into the slide holding magazine, is between 2 and 4 min.

### **2.2.3.2**

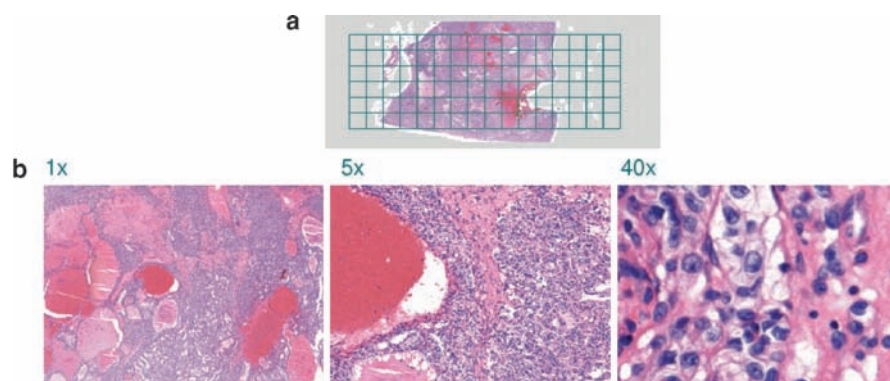
#### ***Features of the Digital Slide Scanning Program***

1. Calibration of the microscope, scanning stage, camera resolution.
2. Slide loading.
3. Barcode reading and identification.
4. Scanning area determination after preview scanning.
5. Autoscanning was started after setting the stage at zero position. In the scanning process, autofocusing was done only at each 3–5 field of view. Each of the images was compressed in JPEG and stored in the slide databank in the corresponding position. Autofocusing was done using Brenner's algorithm [10]. At  $40\times$  objective, 125,856 frames would have to be stored for the entire slide. However, a threshold filter was used to store only frames with image content. This way only the area of a biopsy was stored, which means 160–700 frames per slide.
6. First, the mosaicking of the field of views was done using mathematical algorithms [20]; however, the error of the stage between the required and real position was proved to be less than  $+0.5\text{ }\mu\text{m}$ . A high-precision software mosaicking alignment was used for stitching of the single field of views (Fig. 2.3).

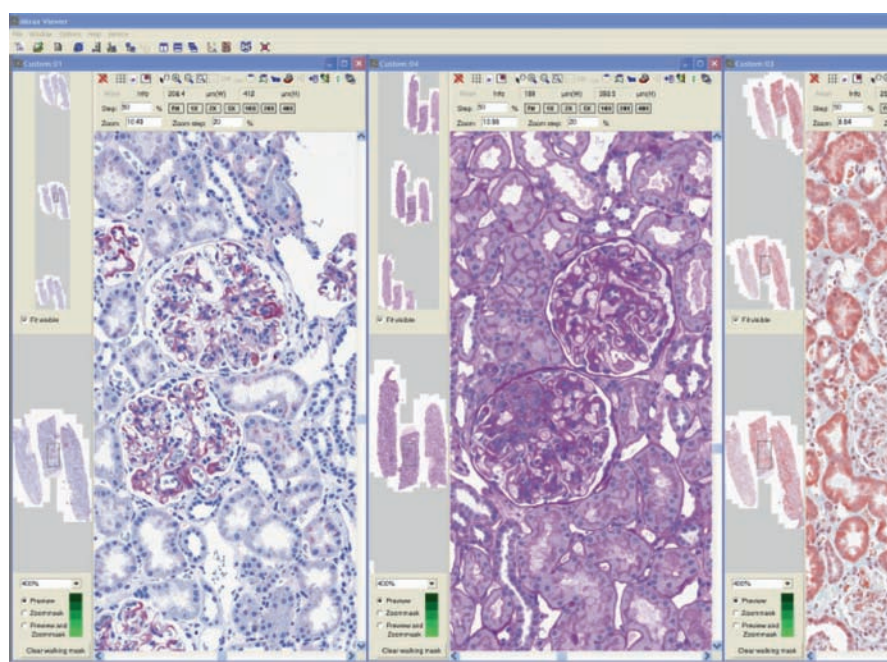
### **2.2.3.3**

#### ***Features of the VM Program***

1. *Slide selection.* After scanning, the slides are stored in subdirectories called *projects* for a higher ordering. After selection, an electronically minimized slide image is shown on the screen (Fig. 2.4).
2. *Slide orientation maps.* This map represents the whole slide, where one pixel on the screen corresponds to one field of view. The recorded segments are labeled with white pixels on a gray background.



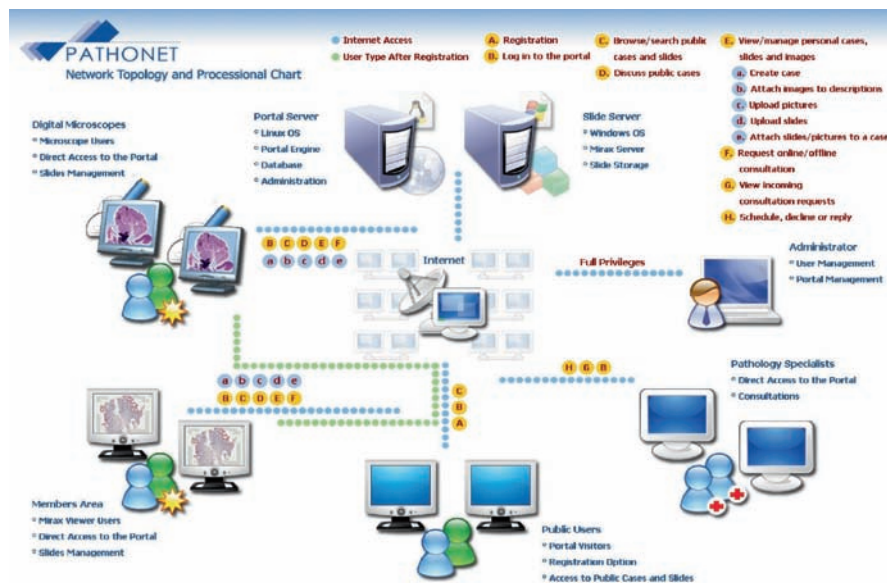
**Fig. 2.3.** (a) Scanning principle of the Mirax system: the sample on the slide is recorded in stitched mosaic of single field of views. (b) Digital magnification of a digital slide



**Fig. 2.4.** User interface of the MIRAX viewer software. Image of the slide, intermedier magnification of the slide, and virtual microscopy are available for perfect orientation. Parallel viewing of the same specimen is available with different sections and staining in oriented and multiple windows



3. *Applicable electronic magnifications.* After finding the interested area, the user has several options for magnification of the selected segments. One can use the prepared magnification steps of 100× and 200× or special “+” and “−” mouse arrows to change the magnification.
4. *Moving and scrolling the slide.* If the interested area is not on the screen in the required magnification on the slide, then the moving arrows (up, down, left, and right) can be used to move the slide into any directions.
5. *Labelling of interested frames for re-evaluation, consultation, and reporting.* Up to ten colored labels can be placed on the digital slide in the software. This option can also be used for reconsultation by experts via LAN or in specific cases via Internet.
6. *Internet access.* The scanning computer can be used as a slide server too. However, uploading to a central digital slide repository with safety options can be performed automatically. We used the PATHONET portal for digital slide storing and dispatch through the Internet (<http://www.pathonet.com>) (Fig. 2.5). This way homework and consultation became easily available.



**Fig. 2.5.** Concept and functions of the PATHONET portal (<http://www.pathonet.com>) for users with scanner or viewer. Active (up- and download of slides) and passive (viewing, library access) usages are available. Parallel viewing for closed participant network is available with options for publication-related digital slide handling, quality-control networks, and education applications



In the case of direct access to the slide server, the slide server's IP address has to be defined on the remote workstation. After connecting to the server computer, the list of the available slides and their information is transported to the client workstation. After selecting a slide, its electronically compressed low-resolution image is transferred to the workstation. Every image that is transported during the evaluation will be stored on the local machine for safety reasons.

## 2.3 Results

### 2.3.1 Concordance

In altogether seven cases (6.7%), discordance was found between VM or OM and the CON. In four cases the OM and in three cases the VM yielded the correct diagnosis.

Out of the 103 cases, in 96 (93.2%) concordance and in 98 (95.1%) clinically important concordance were found. OM yielded similar (100/103, 97%) concordance with the consensus results than the VM (99/103, 96.1%). For the clinically important diagnosis, a similar concordance was also observed (Table 2.2).

### 2.3.2 Reasons for Diagnostic Discordance

In our study due to the fact that the entire specimen was digitized, we could not have sampling error. However, the other common sources of errors in telepathology, interpretation (4/7), or insufficient clinical information (3/7)

**Table 2.2.** Concordance between optical microscopy, virtual microscopy, and the consensus diagnosis

	Concordance all types (%)	Concordance clinically important (%)
OM vs. VM	93.2 (96/103)	95.1 (98/103)
OM vs. Con	97.0 (100/103)	98.0 (101/103)
VM vs. Con	96.1 (98/103)	97.1 (100/103)

OM optical microscope, VM virtual microscope, Con consensus

**Table 2.3.** Reasons of diagnostic discordance

Level discordance	Reason of discordance	VM diagnoses	Optical microscopy diagnosis	Review consensus diagnosis
B	II	Chronic gastritis	Acute and chronic gastritis	VM
C	III	Gastric adenoma	Gastric hyperplastic polyp	VM
C	II	Chronic atrophic gastritis	Chronic gastritis with low-grade dysplasia	OM
B	II	Hyperplastic polyp	Hyperplastic mucosa	OM
C	II	Mild colitis	Normal colon	OM
C	III	Colitis ulcerosa with dysplasia	Colitis ulcerosa	VM
C	III	Adenoma papillare	Hyperplastic polyp	OM

Discordance: B clinically not important, C clinically important

Reason of discordance: I image quality, II interpretation, III insufficient clinical information

could be observed (Table 2.3). With the advances in optical imaging (higher-resolution, multiple focus levels), no image quality errors were found.

### 2.3.3

#### Technical and Practical Data at the Application of VM

The hard-disk volume of a microscopic field of view is between 60 and 100 KB after JPEG compression. The overall hard-disk place for a gastric biopsy is between 30 and 50 MB, and the scanning time is between 20 and 40 min, depending on the number and area of sections on a slide. The evaluation of the specimen on the monitor is more comfortable and more reproducible and documentable, as compared to the optical way (Table 2.4).

In the Internet remote access, the speed of the transfer was relatively fast. The first minimized image was uploaded in the real time. As the user switched over

**Table 2.4.** Comparison between optical and virtual microscope evaluation methods

Microscopy feature	Optical evaluation	Electronic evaluation
Speed	4–5 min	2–3 min
Costs	Every user, high-quality microscope	One high-quality scanning microscope Image server Evaluation terminal
Working conditions	Only limited number of slides can be evaluated because of fatigue	Normal monitor work
Consultation	With slide transportation or with image transfer through ISDN, telephone line, and Internet	Remote access to the image server through LAN or Internet
Quality control	With special additional hardware	By recording of evaluated image segments

to high magnification, the single field of views was demonstrated on the screen in seconds. The application of portal technology allowed the parallel viewing of the slides in different geographical locations, yielding CON opportunities through the Web.

## 2.4 Discussion

In this work, the technical feasibility and user acceptance of a digital histological section evaluation system were shown. Using high-end automated digital slide scanners, advanced PC technology, now a real-life application of digital histology, became available.

These developments and results prove that in the near future, we can have microscope-free virtual microscopy workstations with the function of the OM. On one side, it would mean more working comfort for the histologists. On the other side, it would also support quality-control techniques and consultation

with remote experts as well. The primary application of digital slides and virtual microscopy should be now the routine sign-out and not only the consultation.

Remote evaluation of the slides through Internet includes the advantages of the previously used static or dynamic telepathology methods (entire slide is available in high magnification, microscope, and remote assistance-free evaluation) [24].

The observed concordance with optical microscopy was higher than in previous static image-based telepathology studies [25–27]. This can be explained by the fact that this technology eliminates the sampling error. Evaluation of certainty, time constraints, and fatigue was not done. It is considered to be done in a larger interlaboratory study.

The digitization of the routine specimen for Intranet or telediagnosics could be performed directly in the H/E sample preparation laboratory. The Mirax scanner has slide magazines with 50 slides each. In the routine workflow in the biopsy laboratory, the coverslipping is done also in batches of 50–60 slides. In our laboratory, the first set of H/E-stained, coverslipped slides are ready at 11:00 a.m. Using the high-speed scanning, the first set of slides are on the workstations monitor at between 11:20 and 11:30. The routine sign-out can happen directly, and an electronic report could be prepared on the monitor and sent out even in an email in seconds.

O'Brien and Sotnikov [18] defined in their 1996 review article about the computerization of the histology laboratory as a desirable but far future. Leong and McGee [16] published recently that automated complete slide digitization has influence on all levels of the clinical practice and education. They emphasized the importance of a dedicated software technology for the practical use.

The results of our study showed that the digital slide and virtual microscopy technology can be used in selected cases for telepathology consultation, but not for everyday routine use.

## 2.5 Summary

- Automated digital slide scanning became available in the last years through digital slide scanners.
- Their pilot application on gastrointestinal specimen is rational due to the volume of the biopsy sections.
- Teleconsultation through Intra- and Internet are both applicable alternatives.
- The concordance between optical and virtual microscopy results to concordance diagnosis is similarly high due to image quality developments in the digital slide scanning technology.

- The application of virtual microscopy with digital slide scanners for gastrointestinal biopsy specimen telepathology is now supported by the results of our study.

## References

1. Anderson TL, Nelson AC (1995) Quality control and proficiency testing of cytological smear screening: an integrated approach using automation. In: Wied GL, Keebler CM, Rosenthal DL, Schenck U, Somrak TM, Vooijs GP (eds) Compendium on quality assurance, proficiency testing and workload limitations on clinical cytology. Tutorials of Cytology, Chicago, IL, pp. 83–287
2. Baker RW, Wadsworth J, Brugal G, Coleman DV (1998) An evaluation of 'rapid review' as a method of quality control of cervical smears using the AxioHOME microscope. *Cytopathology* 8:85–95
3. Belhomme P, Elmoataz A, Herlin P, Bloyet D (1996) Generalised region growing operator with optimal scanning: application to segmentation of breast cancer images. *J Microsc* 886:41–50
4. Burns BF (1997) Creating low power photomicrographs using a 35 mm digital slide scanner. *Am J Surg Pathol* 21:865–866
5. Dee FR (2006) Virtual microscopy for comparative pathology. *Toxicol Pathol* 34:966–973
6. Dictor M (1997) The surgical pathologist in a client/server computer network: work support, quality assurance, and the graphical user interface. *Mod Pathol* 10:259–266
7. Dooley RL, Engel C, Muller ME (1997) Automated scanning and digitizing of roentgenographs for documentation and research. *Clin Orthop* 274:113–119
8. Dunn BE, Almagro UA, Choi H, et al (1997) Dynamic-robotic telepathology: department of Veteran Affairs feasibility. *Hum Pathol* 28:8–12
9. Eusebi V, Foschini L, Erde S, et al (1997) Transcontinental consults in surgical pathology via the Internet. *Hum Pathol* 28:13–16
10. Firestone L, Cook K, Culp K (1991) Comparison of autofocus methods for automated microscopy. *Cytometry* 12:195–206
11. Gombas P, Szende B, Stotz G (1996) Support by telecommunications in diagnostic pathology. Experience with the first telepathology system in Hungary. *Orv Hetilap* 137:2299–2303
12. Grohs DH, Gombrich PP, Domanik RA (1996) AccuMed International, Inc. Meeting the challenges in cervical cancer screening: the AcCell Series 2000 automated slide handling and data management system. *Acta Cytol* 40:26–30
13. Grohs DH, Dadeshidze VV, Domanik RA, Gombrich PP, Olsson LJ, Pressman NJ (1997) Utility of the TracCell system in mapping *Papanicolaou*-stained cytologic material. *Acta Cytol* 41:144–152
14. Hailey DM, Lea R (1995) Prospects for newer technologies in cervical cancer screening programmes. *J Qual Clin Pract* 15:139–145
15. Helin HO, Lundin ME, Laakso M, Lundin J, Helin HJ, Isola J (2006) Virtual microscopy in prostate histopathology: simultaneous viewing of biopsies stained sequentially with hematoxylin and eosin, and alpha-methylacyl-coenzyme A racemase/p63 immunohistochemistry. *J Urol* 175:459–504
16. Leong FJWM, McGee O'D (2001) Automated complete slide digitization: a medium for simultaneous viewing by multiple pathologists. *J Pathol* 195:508–514

17. Mango LJ, Ivasauskas EZ (1995) Computer assisted cervical cytology using the PAPNET testing. In: Wied GL, Keebler CM, Rosenthal DL, Schenck U, Somrak TM, Vooijs GP (eds) Compendium on quality assurance, proficiency testing and workload limitations on clinical cytology. *Tutorials of Cytology*, Chicago, IL, pp. 155–167
18. O'Brien MJ, Sotnikov AV (1996) Digital imaging in anatomic pathology. *Am J Clin Pathol* 106:25–32
19. Ong SH, Jin XC, Sinniah R (1996) Image analysis of tissue sections. *Comput Biol Med* 26:269–279
20. Ott SR (1997) Acquisition of high-resolution digital images in video microscopy: automated image mosaicking on a desktop microcomputer. *Microsc Res Tech* 38:335–343
21. Shotton DM (1995) Robert Feulgen Prize Lecture 1995. Electronic light microscopy: present capabilities and future prospects. *Histochem Cell Biol* 104:97–137
22. Singson RPC, Natarajan S, Greenson JK, Marchevsky AM (1999) Virtual microscopy and the Internet as telepathology consultation tools. A study of gastrointestinal biopsy specimens. *Am J Pathol* 111:792–795
23. Stewart J III, Myazaki K, Bevans-Wilkins K (2007) Virtual microscopy for cytology proficiency testing: are we there yet? *Cancer* 111:203–212
24. Weinberg DS (1996) How is telepathology being used to improve patient care. *Clin Chem* 42:831–835
25. Weinberg DS, Allaert FA, Dusserre P, et al (1996) Telepathology diagnosis by means of digital still images: an international validation study. *Hum Pathol* 27:111–118
26. Weinstein RS (1996) Prospects for telepathology. *Hum Pathol* 17:433–434
27. Weinstein RS, Battacharayya AK, Graham AR, et al (1997) Telepathology a ten-year progress report. *Hum Pathol* 28:1–7



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