medicinska revija
medical review

UDK : 616-07

Kayser K. et al. MD-Medical Data 2011;3(2): 151-157 *MEDICAL DATA / Vol. 3. No 2 / Juni2011.*

Opšti pregledi/
General reviews

VIRTUAL SLIDES IN TISSUE – BASED DIAGNOSIS – A REVIEW*

DIJAGNOSTIČKA TELEPATOLOGIJA – REVIJSKI PRIKAZ*

Klaus Kayser[1], Stephan Borkenfeld[2], Gian Kayser[3]

[1] UICC-TPCC, Institute of Pathology, Charite, Berlin Germany
[2] IAT, Heidelberg, Germany
[3] Institute of Pathology, University Freiburg, Freiburg, Germany

Correspondence to:

Prof. **Klaus Kayser**, MD, PhD
UICC-PTCC, Institute of Pathology, Charite
Charite Platz 1
D-10118 Berlin.

Email: klaus.kayser@charite.de

*Invited paper/Rad po pozivu

Abstract

Virtual slides (VS) or digitized whole histological images (WHI) are digital images that are completely acquired from histological glass slides. They can be considered as basic elements of a new performance in tissue – based diagnosis or diagnostic surgical pathology which is called digital pathology. From the mathematical point of view the world of digital pathology includes several matrices with different meanings, such as VS itself, virtual immunohistochemistry, syntactic structure analysis, digital data obtained from molecular pathology investigations (virtual molecular biology), virtual clinical data stored in a hospital information system (HIS)), and data for quality evaluation (statistical information). These different tools interact by appropriate surfaces, which are usually regulated and controlled by internet standards. Historically, digital pathology has its roots in visual electronic communication of diagnostic pathology (telepathology). At present, telepathology has been embedded in specific forums if it is used for expert consultation, or is bound to virtual microscopy (specific VS viewers) if applied for frozen section services. A virtual microscope has to be equipped with different features if it will be used interactively, i.e., by human control, or in an automated manner. The viewers and their included tools play a significant role for diagnosis purposes. They are still in an experimental phase because VS have only rarely been used for routine diagnostic purposes until today. It can be expected that additional tools (diagnosis assistants) such as automated selection of regions of interest (ROI), automated artificial coloring of VS, and automated selection of diagnosis – dependent additional tissue examinations (essential immunostains to confirm/redefine a proposed diagnosis) will be commercially available in the near future. The significance of content based image analysis to automatically derive a diagnosis from VS is discussed as well as the implementation of content - based image information algorithms to be applied for predictive tissue – based diagnoses and image/case retrieval.

Key words
Virtual slide, telepathology, predictive diagnosis, tissue – based diagnosis.

Ključne reči
Virtualni slajdovi, telepatologija, osnovna dijagnoza, hirurška patologija

INTRODUCTION

It is still thought in the public and by numerous colleagues that surgical pathology is only an "appendix" in medical diagnosis and treatment. This opinion reflects to the low reputation of pathology in developing countries and to the steep decrease of autopsies in all pathology institutions, especially after live imaging procedures such as high resolution computed tomography (HRCT) or magnetic resonance imaging (MR) have been developed and implemented.

The analysis of biological structures at the cellular level and the consecutive construction of a "diagnostic building" which has been founded by Virchow's cellular pathology (Zellularpathologie) have certainly reached their limitations as well as the clinical (not public health related) significance of post mortem investigations [1-4]. On the other hand, diagnostic pathology is now-a-days involved in modern technologies such as electronic communication, molecular genetics, and molecular biology to a great extent [5, 6] [7]. Its clinical significance is now-a-days steeply increasing because it offers a reliable platform to incorporate molecular biological pathways as well as digital examination and communication procedures at the most applicable level, the cellular functions [5, 8]. The latest outcome is the so-called predictive diagnosis, which is an analysis of intra-cellular pathways in combination with expression of cellular membrane receptors and classical diagnosis [9]. Examples are the analysis of k-ras mutations in human colon carcinomas together with expression of the epithelial growth receptor (EGFR)[8], or the immune histochemically evaluation of the her2-new receptors in combina-

tion with in situ hybridization in advanced breast carcinoma stages [10]. Predictive diagnosis is more than the conventional one of colon carcinoma or invasive ductal breast carcinoma. It describes and accurately predefines the routes how to treat the individual patient.

In order to come up with these changes, we prefer the term tissue – based diagnosis which is more complete and includes all the different examinations that contribute to a clinically and therapeutic trendsetting diagnosis, instead of using the term surgical pathological diagnosis.

What are the reasons of, and why are digital technologies involved in these challenging diagnostic and therapeutic issues? What are the perspectives? To which extent can the "virtual world" explain or even foresee the development in the real world, and, for example, name the most promising successful treatment of a malignant tumor?

We will start to answer these and similar questions from the beginning which is the development and implementation of telepathology, work out the specific conditions of digital microscopy, describe its present stage, and analyze the perspectives of tissue – based diagnosis.

History, features, and present stage of telepathology

It was about in the 1950s when technology was able to transfer visual information with cameras and display systems (TV screens) of sufficient resolution and velocity [11, 12]. This was the beginning of the so-called communication era with worldwide electronic connection, which is most frequently performed with passive acoustic – visual communication systems (public TV) now-a-days. In addition, interactive systems such as fixed and mobile social networks, forums, etc. are available and partly already replace the "common TV".

In addition to NASA (National Air and Space Agency, USA) trials, the first approach in using electronic visual communication technology for medical diagnostic purposes was probably performed by Weinstein et al. in the late 1960s using an interactive multispecialty telemedicine system to connect the Logan International Airport in Boston with the Massachusetts General Hospital, Boston, USA [13-16]. It took additional 20 years until a more comprehensive service was started by Eide and Nordrum for routine frozen section services in Tromsö, Norway [11, 16]. At the same time extended expert consultation trials using histological images were performed by Kayser in Heidelberg, Germany [17]. Numerous investigations followed until to the end of the last century, which all used closed systems with specific end-to-end nodules (for details see: [11]).

The world wide web (or internet standard) was introduced at the beginning of this century, which was immediately used for the development and implementation of so-called open platforms. These systems can be considered as an open communication network with multifunctional end users such as clients, experts, analysis systems, teachers, controllers, etc. [18, 19]. An extension is called Grid technology. It is widely used in high energy experiments, molecular design, and astrophysics [20]. The most frequently used communication system (iPATH) has been developed by Brauchli and Oberholzer in 2000 [21, 22]. Others are the Campus Medicus which is now-a-days replacing the iPATH to some degree, or the MECES (www.diagnomx.eu) systems which is still under development.

All these systems are designed for expert consultation and related aims such as education, and, to a lesser degree, for quality evaluation, whereas frozen section services still use either fixed still image systems or VS in combination a virtual microscope (VM) [23-26].

Virtual microscopes, features and application

A virtual slide (VS) is primarily a matrix which corresponds to a microscopic image. Its visualization is performed by an associated program (viewer) that, in addition, includes interactive (or automated) functions to manipulate the visualization. These functions are derived from a conventional microscope, and the system is consecutively called virtual microscope (VM) [26, 27]. Two different types of functions can be distinguished in a virtual microscope, namely a) those that are present in a conventional microscope, and b) those that are specific for a VM and cannot be applied to a conventional microscope. The details are depicted in (Figure 1). Navigation, image magnification, focussing, correction of brightness and color balance are functions of a conventional microscope. They are also included in all commercially available VMs. Other functions such as contemporary display of several images, labelling, loupe magnification within a VS, artificial coloring, or quantitative analysis of certain image compartments (for example nuclei) are specific features of a VM. At present, only a few companies have included such features in their viewer.

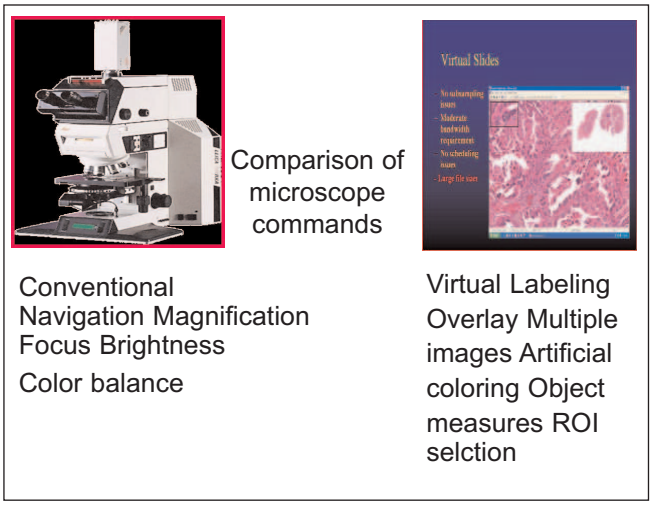

Figure 1: Comparison of conventional microscope and virtual microscope commands: Commands to be applicable include the conventional microscope ones and can be extended by additional features such as overlay, labels, multiple images and artificial colors.

The future development of VM focuses on digital archives, the integration of VM into hospital information systems (HIS), which requires additional standards such as DICOM 3 (Digital Imaging and Communication in Medicine) and PACS (Picture Archiving and Communication System) [28, 29]. It still remains an open issue whether the diagnostic performance with a "one screen" or "two screens" system is preferable in routine work. The tuning of DICOM and PACS for application in VM is still on its way. A final and definite obligatory standard has not been developed to our knowledge [30].

The integration of diagnostic assistants in VMs has also not been realized to our knowledge until today. However, several companies offer quantitative analysis of VS on specific commands, especially measurements of VS acquired from

immunohistochemically stained slides (3DHistech, Aperio, Bioimaging, Olympus) see: Appendix, selected scanner companies). The measurements include, for example, nuclear measurements (Ki-67, hormones), and membranes (Her2_neu). A FDA (Food and Drug Administration, USA) certificate has been given to some systems.

Development of virtual microscope assistants

A VM is a diagnostic tool that can replace a conventional microscope in daily diagnostic routine of tissue – based diagnosis [26, 27, 29]. Its main compartment is the VS viewer. The viewer can be constructed to view and judge

1. Either VS of individual cases; or
2. Routine diagnostic cases in a continuous workflow.

Both applications can, in addition, be performed interactively, i.e., by human commands, or embedded in an automated environment. A VM with automated assistants is commercially not available to our knowledge. All commercially available VMs address to a specific viewer. Such a viewer is solely considered to be a mandatory component of the corresponding virtual slide scanner [31, 32]. However, it seems to be of advantage if VS viewers and VMs are developed and implemented independently from each other. Potential customers usually work under specific conditions which a VM should address to [27]. Such a VM has to fulfil several mandatory conditions:

1. It has to automatically recognize the different VS formats;
2. It has to provide a set of default values (focus, brightness, color balance, navigation velocity, speed and range of magnification levels, still image capture);
3. The gateways to automated and non-automated assistant programs must be defined;
4. Internal quality control and potential warnings have to be embedded.
5. Acoustic information acquisition and transfer is probably a "must" for routine diagnostic application.
6. It must be open for future developments and should be assembled by different compartments.

VM assistants are programs or program tools that support and realize certain functions of the VM either dependent upon or independent from the wanted usage [14, 19, 24, 27, 33]. They can be compared with "assistants" in a computerized typing program (for example tools to write serial letters in Windows word). Such assistants will include

1. Assistants for diagnostic consultation, which require embedded tools for image quality control and still image export, dial tools, notification tools, and reference tools (for example automated access to the library of the National Institute of Health (pubmed).
2. Assistants for diagnostic procedures, which will include instructions for the laboratory to perform additional laboratory investigations (specific additional stains, immunohistochemical procedures, in situ hybridisation, additional tissue blocks, etc.);
3. Assistants for either content – based, or "labelled" image information (search for the best fitting image (or images with the proposed and/or closest differential diagnosis) in an image library),
4. Assistants to find the region(s) of interest (ROI). These assistants require for themselves segmentation assistants, assistants for syntactic structure analysis, texture analysis or other image transformation algorithms [31, 34-36]. Modules that assure a constant image quality are prerequisite too.
5. Assistants that support and automatically create primary diagnosis. These assistants require measurement and classification modules in addition to those that calculate the ROIs.

The inner hierarchy and communication pathways of the mentioned assistants are depicted in (Figure 2). The basic tools include modules that ascertain a constant image quality in terms of homogenous and constant brightness, and gray value range [27, 37-39]. These modules have to equalize the different staining intensities of the glass slides as well as potential shading or incorrect color acquisition. The underlying mathematical algorithms measure the "distances" of pixel gray values between the original VS and the transformed (corrected) one [27, 37].

Color correction is a tool of the next higher level in order to assure the optimum segmentation of nuclei (in HE stains) and detection of immunohistochemical reaction (DAB, AP, etc.). These assistants are of less importance if the image information content is displayed in the HSI (hue, saturation, intensity) color space because the intensity is projected to a singular intensity coordinate. The intensity coordinate can serve for segmentation "in the first line", which is not possible when using the commonly rbg (red, green, blue) space.

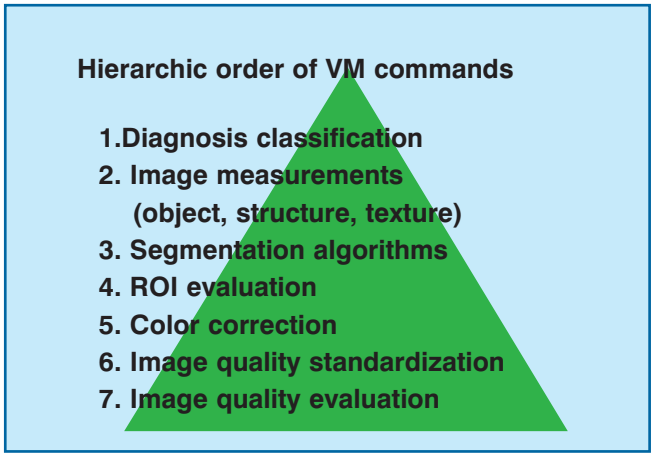

Figure 2: Hierarchic order of virtual microscope commands. The basic modules include programs of image quality assessment and quality standardization. The next higher levels color correction and definition of ROI. The highest level is the automated statement of a diagnosis.

The measurement assistants form the next level, and include algorithms that require a segmentation (measurements of objects and structures, i.e., measurements that prerequisite external information such as nuclei, chromosomes, membranes, etc.), and algorithms that are solely based upon pixel gray values and their spatial distribution (so called texture analysis) [5, 40]. Both methods can be combined to a reliable and automated ROI assistant [36, 41].

The next higher level includes algorithms that investigate in the association of image measurement data with the diagnosis. These assistants are composed of several information classification components applied to quantified VS data, additional external information (patient's presentation, radiological images, serum findings, etc.), and the "projection set" of potential diagnoses [41]. Numerous investigations have been published to derive certain diagnoses from still image (and VS) measurements, which could confirm a strong association of image parameters to a certain diagnosis [42-45]. The

application in diagnostic practice, however, failed due to the following reasons:

1. The images have not been standardized prior to the measurements;

2. Only a fixed set of parameters (for example nuclear size, moment of gray value distribution within a nucleus) have been used for classification (and not a dynamically selected, variable set of parameters).

3. The performance of these supportive measurements was not adequate for routine diagnostics, i.e., too time consuming [40, 46].

Now-a-days, these constraints seem to be solved by new strategies as described by [40].

The assistants which support fast and easy expert consultation, communication with the laboratory information system (LIS), and search for references can be implemented at different levels, and are not bound to a VM. In addition, they have not necessarily to be embedded in the VM or in a pathology information system; they can be a part of the HIS too.

Retrieval and content based image information

Obviously, the pathologist's duty is primarily the assessment of a tissue – based diagnosis that serves for communication within the clinical world, and gives directives for specific treatments. All textbooks, electronic education system in pathology, atlases etc. allow a retrieval or search by diagnosis classification only, and not by included labels or by direct comparison of images [27, 35]. The diagnostic pathologist wants to compare the images of his problematic case with the most similar (or even identical) images in order to ensure his diagnosis and discuss potential differential diagnoses. Certainly, this objective is a feature of digital pathology or digital atlases, and cannot be provided by paper prints at an appropriate level. The search for content based image information is a difficult approach, and has been in the field of scientific interest since a few years only [34, 39].

The problem starts with the definition of information, which is dependent upon the search items [47]. The simplest example is the identification of VS areas that contain tissue, and to separate these areas from those "that are empty". This aim is implemented in all commercially available scanner software in order to save image acquisition time. It is usually based upon gray value thresholds. From the theoretical point of view, it requires a definition of "tissue" (all pixels above a predefined gray value threshold) which cannot be derived from the image itself [47]. The derivatives from these considerations are:

1. Any VS can be divided into two non-overlapping areas, namely the ROI (or object space) and the background;

2. ROI depends upon a set of VS features that are related to pixel coordinates and gray values.

3. The limitations (selection limits) of the features have to be defined by external observers at the beginning.

4. In extension of point No 3, they can be calculated for all possible assignments because their number is limited.

Indeed, first experiments indicate that a flexible association of texture, object and structure features is sufficient to reproducible calculated ROIs of different diagnoses and organs [35, 40, 47]. In contrast to the ROI algorithms that are in use of digital consumer cameras (face recognition) ROIs of VS are characterized by a broad variance in biological meaningful objects and their structures. An additional problem is the (nearly) randomly distributed search starting point.

Examples of successfully detected ROIs are given in (Figure 3, Figure 4). The algorithms are either applied with a constant ROI frame that shifts along the VS (Figure 3), or with flexible ROI frames obtained from graph theory based entropy calculations (MST) (Figure 4).

Automated derivation of morphological diagnosis

The replacement of the pathologist's work (i.e., to judge VSs and to derive a diagnosis) is a complicated task that has to take into account the following:

1. There are different levels of diagnoses. Some diagnoses are crude and just distinguish between different classes of diseases such as normal, cancer, inflammation, infection, inborn errors, or infarction. The next level would be diagnoses which include more morphological details, for example adenocarcinoma, tuberculosis, etc. The introduction of different diagnosis levels permits quantitative evaluation of diagnostic errors, for example the comparison of experts' diagnosis with the preliminary diagnosis, or a "conventional glass slide diagnosis with the VS diagnosis. An appropriate definition of diagnosis levels has been described by Mireskandary et al., who

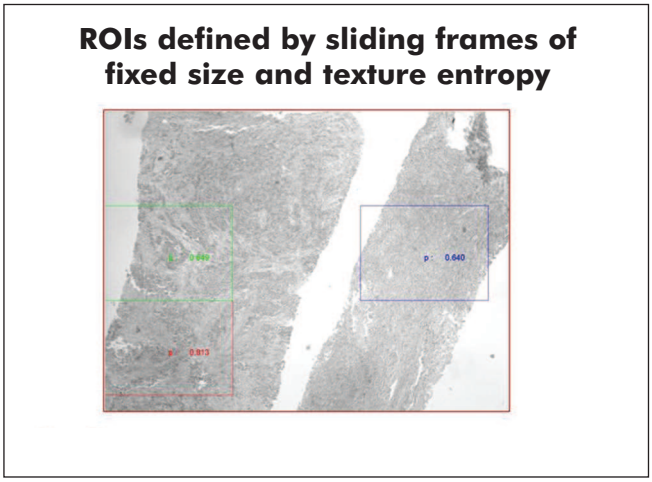

Figure 3: Example of a ROI defined by graph theory approach in a pleural biopsy showing tumor infiltrates of a metastatic adenocarcinoma. Note the correct area of metastatic tumor cells.

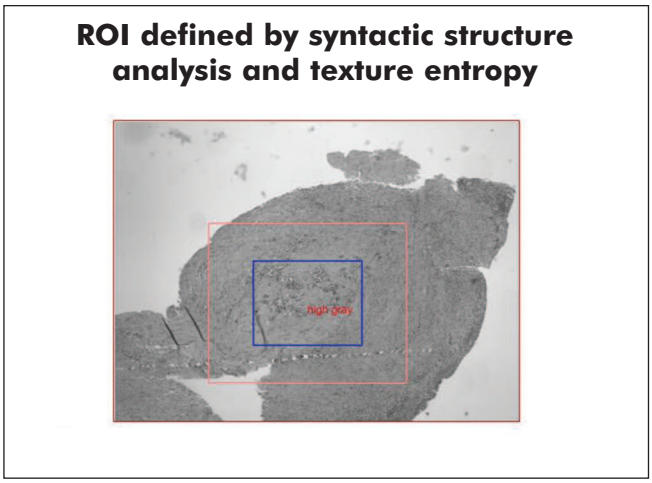

Figure 4: Example of a ROI defined by an approach of sliding squares with fixed area across the whole biopsy. The labelled percentages correspond to the probability to find diagnostic significant features in the marked area.

defined 10 levels of diagnosis related to the clinical significance of diagnostic error [48].

2. From HE stained VS only diagnoses belonging to a certain level can be derived. For example, the classification of malignant tumors usually requires additional stains such as PAS, or immunohistochemistry in order to state a more detailed diagnosis, or to estimate the patient's prognosis [7].

3. Most laboratories provide the pathologist with a HE stain, and only perform additional stains on specific request of the pathologist, because a HE stain is cheap and sufficient to state a crude diagnosis. Such a crude morphological diagnosis is usually the prerequisite to work out more diagnostic details.

At present, successful trials to automatically state a diagnosis are limited to the HE stained derivatives. One of the most advanced experiments that took into account the staining variation between different laboratories, different diseases and organs as well as learning and test sets has been recently reported by [40]. The results are listed in (Figure 5). The obtained discrimination power and diagnostic accuracy of the reported approach are convincing, sufficient for screening applications in HE stained tissues, and in a range to those that have been applied in cytology [49].

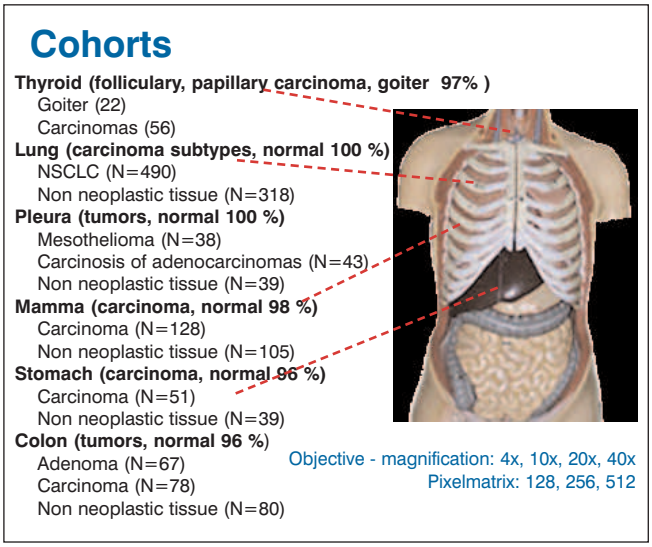

Figure 5: Result of automated diagnostics described by Š35Ć. The different cohorts and images are all classified by the same "open algorithm" that permits flexible classification features with statistically optimized classifiers.

Perspectives

Communication in diagnostic pathology with open access and diagnostic relevance has already left its childhood, if applied for expert consultation, teaching or multidisciplinary case discussions [11]. The prerequisites are digitized images and electronic communication standards as well as open access forums (iPATH, Campus Medicus, MECES) [15, 22, 41]. VS are in use for intra-institutional discussions and diagnostic assistances as well as for frozen section services [11, 12, 39, 50, 51]. The improvement of VM in terms of performance and implemented diagnosis assistants has not reached a level of appropriate user-friendly use despite several companies offer telecommunication and archiving services as well as quite sophisticated measurements tools (for example, see Aperio, Leica, 3DHistech).

At present, the situation is similar to that of the application of stereology into routine diagnosis in the 1980s [47]. To our opinion the relatively poor application in routine tissue-based diagnosis is not that much a question of difficulties in buying quite expensive equipment as a question of performance and embedding the new technology in a continuous workflow. Further more, advanced user-friendly assistants as described above are still missing. They are considered to be separate tools that require a specific handling. They should be taken as integrative compartment of a VM, which is on the way to induce remarkable changes in diagnostic pathology, and to open new pathways for integrative tissue – based diagnosis.

According to our observations and experience the perspectives of this route are promising and irreversible. There is only one question left: How fast the final aim can be reached, which is, at present, a clinically integrated and extensively automated tissue – based diagnosis.

Home pages of selected VS scanner companies:

3D-HISTECH (www.3dhistech.com)

Aperio (www.aperio.com)

Bioimagene (www.bioimagene.com)

Claro (www.claro-inc.jp)

Hamamatsu (www.hamamatsu.com)

Leica (www.leica-microsystems.com

Olympus (www.olympus.de

Philips (www.research.philips.com)

Apstrakt

Virtualni slajdovi (VS) digitalizovanih celih snimaka (WHI = whole slide imaging) su digitalne slike koje su kompletno preuzete sa klasičnih histoloških staklenih pločica. Oni mogu biti posmatrani kao osnovni elementi novog opisa tkiva – osnovne dijagnoze ili dijagnostičke hirurške patologije koja je poznata i kao dijagnostička patologija. Sa matematičkog stanovišta, svet digitalne patologije uključuje nekoliko matrica različitog značenja, kao što je sam virtuelni slajd (VS), virtuelna imunohistohemija, analiza sintetičke structure, digitalni podaci dobijeni od istraživanja molekularne patologije (virtual molecular biology), istraživanja virtuelnih kliničkih podataka sadržanih u bolničkom informacionom sistemu (HIS), i podacima za analizu kvaliteta (statističke informacije). Ovi različiti alati se stiču u raznim ravnima, koje su obično regulisane i kontrolisane međunaraodnim standardima. Istorijski, digitalna patologija ima korene u vizuelnoj elektronskoj komunikaciji sa dijagnostičkom patologijom (telepatologijom). Danas, je telepatologija ugrađena u specijalizovane forume ako se koristi za ekspertske konsultacije, ili je ugrađena u virtuelnu mikroskopiju (za posebne korisnike) ako se primeni za servise sa fiksnim snimcima. Virtuelna mikroskopija đe biti opremljena različitim opcijama ukoliko se koristi interaktivno, tj. kontrolom od strane čoveka ili na automatski način. Korisnici i njihove potrebe imaju značajnu ulogu u procesu dijagnoze. To je još uvek u eksperimentalnoj fazi, jer se VS još uvek nedovoljno koristi u rutinskoj dijagnozi. Očekuje se da đe tradicionalni alati (dijagnostički asistenti) kao što je automatska selekcija regije od interesa (ROI), automatsko veštačko bojenje VS, i automatska selekcija dijagnoza – zavisno od dodatnog ispitivanja tkiva (dodatno imunohistohemijsko) ispitivanje radi potvrde/redefinisanja predložene dijagnoze), biti komercijalno dostupni u bliskoj budućnosti. Razmatra se značaj analize slike na bazi sadržaja, radi automatskog određivanja dijagnoze iz VS, kao i ugradnja algoritama o sadržaju slike radi predviđanja dijagnoze tkiva na osnovu snimaka.

REFERENCES

1. Adappa, R., et al., Perinatal and infant autopsy. Arch Dis Child Fetal Neonatal Ed, 2007. 92(1): p. F49-50.

2. Dietel, M., ŠPost-mortems with organ and tissue retentionĆ. Z Evid Fortbild Qual Gesundhwes, 2008. 102(3): p. 189-93; discussion 199.

3. Knoke, M., H. Bernhardt, and G. Schwesinger, Is there a need for autopsies in the management of fungal disease? Mycoses, 2008. 51(4): p. 291-300.

4. Virchow, R., Die Cellularpathologie in ihrer Begründung auf die physiologische und pathologische Gewebelehre. 1859, Berlin: Verlag von August Hirschwald.

5. Kayser, K. and H.J. Gabius, Graph theory and the entropy concept in histochemistry. Theoretical considerations, application in histopathology and the combination with receptor-specific approaches. Prog Histochem Cytochem, 1997. 32(2): p. 1-106.

6. Kayser, K., et al., Association of prognosis in surgically treated lung cancer patients with cytometric, histometric and ligand histochemical properties: with an emphasis on structural entropy. Anal Quant Cytol Histol, 1998. 20(4): p. 313-20.

7. Kayser, G., et al., Numerical and structural centrosome aberrations are an early and stable event in the adenoma-carcinoma sequence of colorectal carcinomas. Virchows Arch, 2005. 447(1): p. 61-5.

8. Mandrekar, S.J. and D.J. Sargent, Clinical trial designs for predictive biomarker validation: one size does not fit all. J Biopharm Stat, 2009. 19(3): p. 530-42.

9. Mao, Z.G., et al., Differential expression of microRNAs in GH-secreting pituitary adenomas. Diagn Pathol. 5(1): p. 79.

10. Nitta, H., et al., Development of automated brightfield double in situ hybridization (BDISH) application for HER2 gene and chromosome 17 centromere (CEN 17) for breast carcinomas and an assay performance comparison to manual dual color HER2 fluorescence in situ hybridization (FISH). Diagn Pathol, 2008. 3: p. 41.

11. Kayser, K., J. Szymas, and R.S. Weinstein, Telepathology and Telemedicine - Communication, Electronic Education and Publication in e-Health. 2005, Berlin: Veterinärspiegel Verlag.

12. Weinstein, R.S., et al., Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. Hum Pathol, 2009. 40(8): p. 1057-69.

13. Elford, D.R., Telemedicine in northern Norway. J Telemed Telecare, 1997.3(1): p. 1-22.

14. Kayser, K., et al., From telepathology to virtual pathology institution: the new world of digital pathology. Rom J Morphol Embryol, 1999. 45: p. 3-9.

15. Williams, B.H., et al., A national treasure goes online: the Armed Forces Institute of Pathology. MD Comput, 1998. 15(4): p. 260-5.

16. Weinstein, R.S., et al., Telepathology overview: from concept to implementation. Hum Pathol, 2001. 32(12): p. 1283-99.

17. Kayser, K., Progress in telepathology. In Vivo, 1993. 7(4): p. 331-3.

18. Kayser, K., Interdisciplinary telecommunication and expert teleconsultation in diagnostic pathology: present status and future prospects. J Telemed Telecare, 2002. 8(6): p. 325-30.

19. Kayser, K., et al., New developments in digital pathology: from telepathology to virtual pathology laboratory. Stud Health Technol Inform, 2004. 105: p. 61-9.

20. Gortler, J., et al., Grid technology in tissue-based diagnosis: fundamentals and potential developments. Diagn Pathol, 2006. 1: p. 23.

21. Brauchli, K., et al., Diagnostic telepathology: long-term experience of a single institution. Virchows Arch, 2004. 444(5): p. 403-9.

22. Brauchli, K., et al., iPath - a Telemedicine Platform to Support Health Providers in Low Resource Settings. Stud Health Technol Inform, 2005. 114: p. 11-7.

23. Helin, H.O., et al., 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, 2006. 175(2): p. 495-9.

24. Kayser, G., et al., Standards in virtual microscopy: from tissue processing to image acquisition and visualization. Diagnostic Pathology, 2010. 5(Suppl 1):: p. S10.

25. Leong, F.J. and J.O. McGee, Automated complete slide digitization: a medium for simultaneous viewing by multiple pathologists. J Pathol, 2001. 195(4): p. 508-14.

26. Wienert, S., et al., Integration and acceleration of virtual microscopy as the key to successful implementation into the routine diagnostic process. Diagn Pathol, 2009. 4: p. 3.

27. Kayser, K., B. Molnar, and R.S. Weinstein, Virtual Microscopy – Fundamentals – Applications – Perspectives of Electronic Tissue - based Diagnosis. 2006, Berlin: VSV Interdisciplinary Medical Publishing.

28. Rocha, R., et al., Digital slides: present status of a tool for consultation, teaching, and quality control in pathology. Pathol Res Pract, 2009. 205(11): p. 735-41.

29. Rojo, M.G., G. Bueno, and J. Slodkowska, Review of imaging solutions for

integrated quantitative immunohistochemistry in the Pathology daily practice. Folia Histochem Cytobiol, 2009. 47(3): p. 349-54.

30. Garcia Rojo, M., et al., Digital pathology in Europe: coordinating patient care and research efforts. Stud Health Technol Inform, 2009. 150: p. 997-1001.

31. Roa-Pena, L., F. Gomez, and E. Romero, An experimental study of pathologist's navigation patterns in virtual microscopy. Diagn Pathol. 5: p. 71.

32. Evans, A.J., et al., Primary frozen section diagnosis by robotic microscopy and virtual slide telepathology: the University Health Network experience. Semin Diagn Pathol, 2009. 26(4): p. 165-76.

33. Roux, L., et al., A cognitive virtual microscopic framework for knowledge-based exploration of large microscopic images in breast cancer histopathology. Conf Proc IEEE Eng Med Biol Soc, 2009. 2009: p. 3697-702.

34. Oger, M., et al., Automated region of interest retrieval and classification using spectral analysis. Diagn Pathol, 2008. 3 Suppl 1: p. S17.

35. Kayser, K., et al., AI (artificial intelligence) in histopathology--from image analysis to automated diagnosis. Folia Histochem Cytobiol, 2009. 47(3): p. 355-61.

36. Kayser, K., et al., Theory of sampling and its application in tissue based diagnosis. Diagnostic Pathology 2009. 4:: p. 6.

37. Kayser, K., et al., Image standardization in tissue - based diagnosis. Diagnostic Pathology, 2010. 5(Suppl 1):: p. S13

38. Conway, C.M., et al., The development and validation of the Virtual Tissue Matrix, a software application that facilitates the review of tissue microarrays on line. BMC Bioinformatics, 2006. 7: p. 256.

39. Ortiz, J.P., V. Ruiz, and I. Garcia, Virtual slide telepathology systems with JPEG2000. Conf Proc IEEE Eng Med Biol Soc, 2007. 2007: p. 880-3.

40.Kayser, K., et al., Texture- and object-related automated information analysis in histological still images of various organs. Anal Quant Cytol Histol, 2008. 30(6): p. 323-35.

41. Kayser, K., et al., Towards an automated virtual slide screening: theoretical considerations and practical experiences of automated tissue-based virtual diagnosis to be implemented in the Internet. Diagn Pathol, 2006. 1(1): p. 10.

42. Garcia, I., et al., ŠUsefulness of nuclear morphometry as predictive factor of progression in bladder papillary carcinomaĆ. Actas Urol Esp, 1994. 18(10): p. 937-41.

43. Kavantzas, N., et al., Nuclear/Nucleolar morphometry and DNA image cytometry as a combined diagnostic tool in pathology of prostatic carcinoma. J Exp Clin Cancer Res, 2001. 20(4): p. 537-42.

44. Kayser, K., B. Kiefer, and H.U. Burkhardt, Syntactic structure analysis of bronchus carcinomas - first results. Acta Stereol, 1985. 4/2: p. 249-253.

45. Kayser, K. and W. Schlegel, Pattern recognition in histo-pathology: basic considerations. Methods Inf Med, 1982. 21(1): p. 15-22.

46. Collan, Y., Stereology and morphometry in histopathology. Principles of application. Anal Quant Cytol Histol, 1985. 7(4): p. 237-41.

47. Kayser, K., Quantification of virtual slides: Approaches to analysis of content-based image information J Pathol Inform, 2011. 2011(2:2).

48. Mireskandari, M., et al., Teleconsultation in diagnostic pathology: experience from Iran and Germany with the use of two European telepathology servers. J Telemed Telecare, 2004. 10(2): p. 99-103.

49. Nemec, E., et al., Ploidy and chromatin pattern analysis as an aid for cervical smear diagnosis. Histol Histopathol, 2002. 17(2): p. 403-9.

50. Molnar, B., et al., Digital slide and virtual microscopy based routine and telepathology evaluation of routine gastrointestinal biopsy specimens. J Clin Pathol, 2003. 56(6): p. 433-8.

51. Mori, I., et al., Issues for application of virtual microscopy to cytoscreening, perspectives based on questionnaire to Japanese cytotechnologists. Diagn Pathol, 2008. 3 Suppl 1: p. S15.

The paper was received and accepted on 28.02.2011.