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Methodology

Guidelines Digital Pathology for Diagnosis on (and Reports of) Digital Images Version 1.0 Bundesverband deutscher Pathologen e.V. (Federal Association of German Pathologist)

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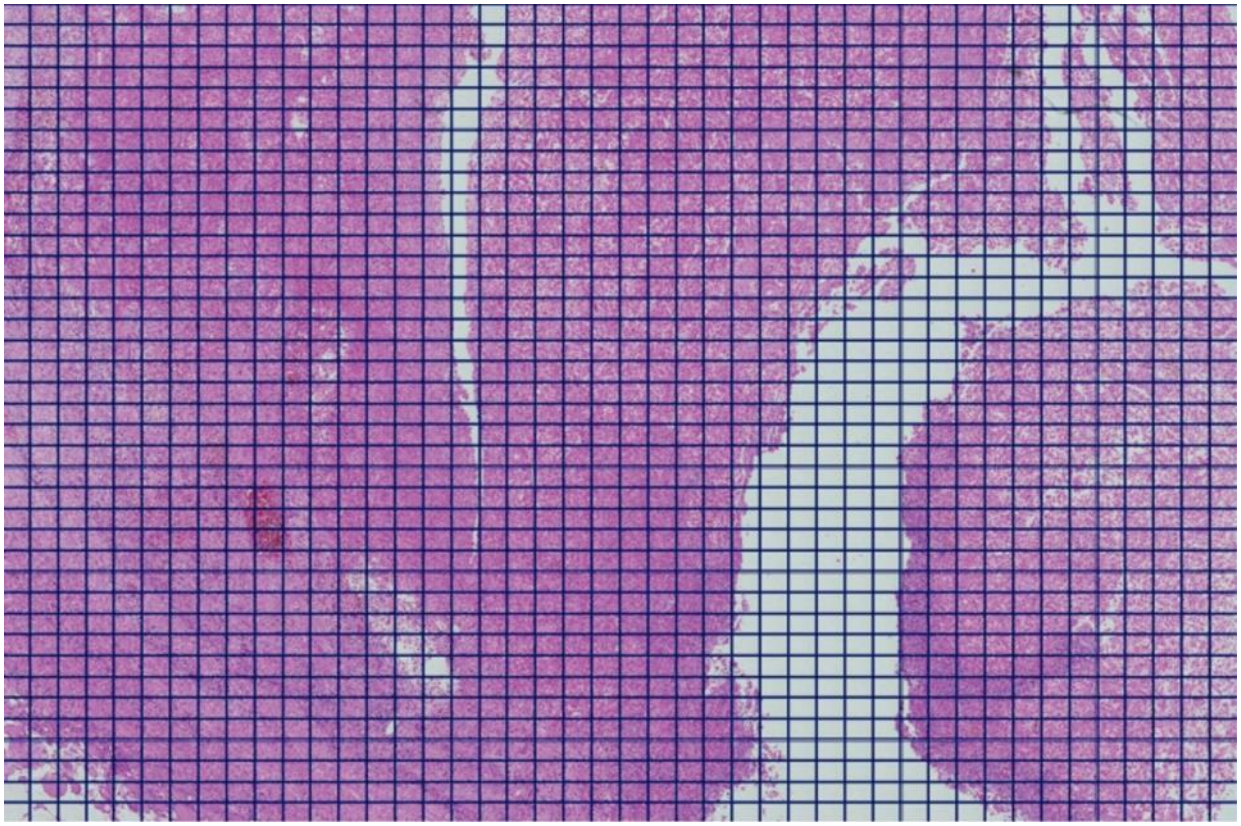

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Abstract

Digitalization is entering the medical fields with increasing velocity and impact on diagnostic and therapeutic actions. In addition, it matures to a mandatory tool of quality assurance, reliable inter-disciplinary communication, and promotion of research.

The Professional Association of German Pathologists wants to support their members in their thoughts and potential implementation of virtual microscopy and related issues. It founded a committee of digital pathology. Colleagues experienced in routine surgical pathology, information technology and practice have been asked to investigate prerequisites, actual technology stages and financial considerations, and to formulate their recommendations and guidelines.

Herein, the official guidelines of the Professional Association of German Pathologists are presented. The guidelines focus on practical issues, Pathologists as well as IT experts or interested researchers are invited to make use of these guidelines. Our readers are also invited to inquire specific tasks or discuss their ideas and experiences. They might either contact the committee directly, or discuss specific points of view by writing a letter to the editor, or by submission of, and to formulate a corresponding interactive publication.

Keywords: Computer-assisted image interpretation, Microscopy, Pathology, Reproducibility of results, Workflow.

Content

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Preface

These guidelines address to pathologists who intend to completely or partly diagnose their routine cases by use of virtual slides, and, in addition, to manufacturers of components or of complete diagnostic digital pathology systems.

Routine diagnosis of digitized microscopic images is still in its childhood in Germany. It raises several technical and legal questions which these guidelines response to. In Germany, medical practitioners are free of applying their own diagnostic and therapeutic methods. This principal statement is also valid for diagnostic procedures which are based upon digital images of real histological and cytological preparations. These statements still remain the gold standard of surgical pathology diagnoses and material archives.

Virtual microscope and virtual microscopy can be considered a diagnostic tool that is equal to the conventional light microscope. Any pathologist might use virtual microscopy as long as he/she can proof that his/her knowledge and abilities are at least equal to the performance in conventional microscopy.

Therefore, the validation technique has been developed. The application safety of digital methods increases, if additional minimum technical requirements are fulfilled. These address to the producers of devices and systems, and are described in the guidelines too.

Finally, the guidelines recommend the implementation of international standards in generation, distribution, and archiving of digital images. The performance assures the most available safety of investment, selection of the most appropriate system, of its components, and provider in relation to local clinical and laboratory conditions. The Recommendations are numbered, included in the text, ordered and summarized in a specific chapter.

Kommission Digitale Pathologie

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1. General remarks

1.1 Digital Pathology

Digital pathology is the integrated use of information technology (IT) which supports the complex laboratory workflow with idea, share and exchange of information including data and images.

The workflow starts with the submission of diagnostic material and ends with the delivery of the final report. Digital pathology is more than a simple connection of a slide scanner to the laboratory (pathology) management system. Digital pathology requires the development of an infrastructure, which manages the cooperation of different pathology institutions or health care systems, which should benefit from the access to their multimodal and multistage data.

The components of digital pathology include:

Digital process management

Digital pathology / diagnostics on digital images

Digital reporting

Gross and Microscopy imaging (Ocular image acquisition)

Whole slide images (WSI)

Integration of image measurements (Morphometry such as Ki-67, hormone receptors, Her2neu)

Image amendment

Molecular analysis data

Correspondence and consultation

Digital communication

Digital continuing education and training

Data security

The actual version of the guidelines focuses on the part of 'Digital pathology / diagnostics on digital images', specifically on 'Virtual slides /whole slide images (WSI). The section 'digital process management' is included if appropriate.

Virtual microscopy is the method which enables the digital images to be created, and to be seen by humans. It postulates the existence of digitized histological slides, the so-called virtual

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slides or Whole Slide Images (WSI). A slide scanner acquires and digitizes the microscopic images. The viewer (monitor and necessary programs) which is used to evaluate virtual slides is called virtual microscope.

1.2 Intention of the guidelines

The implementation of virtual microscopy in routine primary diagnostics is still limited in Europe as well as worldwide, despite of several advantages which include expert consultation, quality assurance, measurements and image analysis. The proposed limiting factors include the influence of different legal and professional determining issues.

In general, virtual microscopy can be successfully implemented in the clinical routine workflow which has already been confirmed by numerous studies [Campbell et al., 2014].

The primary diagnosis is evaluated from digital slides and no longer by use of a conventional microscope. The presented guidelines are aimed on directing the framework how to implement virtual microscopy in routine diagnosis in the Federal Republic of Germany. The legal denotation of the glass slide in terms of the diagnosed objects remained disregarded until today. Explicitly, the present guidelines do not regulate the practice of virtual microscopy to replacing the original glass slide.

Far more aspects are of influence on the virtual archive as an appropriate use of virtual microscopy in routine diagnosis. The glass slide will still remain the mandatory archive material. Its digitalization is a contemporary converting only, although herein some specific aspects of digital archives have to be discussed, too.

Digital pathology is in the childhood of its implementation, its clarification of legal aspects and quality assurance considerations when compared to the other digital medical systems. The development of new image processing techniques is a mandatory prerequisite. Therefore the already developed legal aspects of digital medicine, for example digital radiology, cannot be applied without adequate modifications. Therefore, the present guidelines start with the medical self care and freedom of choice of the applied techniques. The prerequisites of an accurate diagnosis, however, differ from one individual pathologist to the other because of different visual abilities. Several studies indicate that the same statement holds true for image magnification, color, focus, and image quality, and will probably also be valid in virtual microscopy.

Therefore, the present guidelines focus on

- Commitment of minimum requirements of acquisition and management of virtual slides (WSI)

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- Support of the pathologist in function and quality assurance of the established virtual microscopy system
- Support of the pathologist in performance of validation studies within his/her institution.

This means in practice to define:

- the minimum technical requirements of virtual microscopy (slide scanner, visualization pipeline, digital archive)
- the minimum requirements for the implementation in the pathology information system (LIS)
- useful standards, which regulate the access to certification and accreditation organizations
- a system of basic and easy to implement tests, which assure the adequate adjustment of digitalization and visualization pipeline as well as the correct adjustment of the monitor,

and to provide the pathologist with his own validation study that should acquaint him with the difference between conventional (light microscopic) and the implemented virtual microscopy system.

1.3. Outlines

The present guidelines do not present with instruction commands how to implement virtual microscopy in an institution, because the implementation strongly depends upon the local conditions and the aimed target. They do not give any instructions, how to technically realize the digital system demanded, and do not mention deviant specific requirements for telepathology, if telepathology is included in the diagnostic procedure.

1.4. Design of the Guidelines

The guidelines are grouped in seven main chapters. The first chapter contains general preliminary notes. The second chapter discusses the specificities of digital diagnosis in comparison to the conventional light microscopy and draws the related conclusions. The third chapter describes the demands on the slide scanner which have to be fulfilled in clinical practice. The fourth chapter lists the requirements of the visualization chain and the digital archive, which are mandatory to display the virtual slides by a virtual microscope. The fifth chapter focuses on the implementation of the slide scanner in the workflow and the laboratory information system. The sixth chapter mentions the supportive efforts of the Professional

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Association of German Pathologists. The seventh chapter gives a survey of the Recommendations of these guidelines, and focuses on the Recommendations which appeal to the pathologists.

1.5. Life cycle of the guidelines

The guidelines should be regularly revised to allow for the rapid technological development, and additional areas of digital pathology should be included or cited step by step.

2. Digital diagnostics in comparison of conventional primary light microscopy diagnosis

2.1 Background

Digital pathology comprises the evaluation of pathology-anatomical findings and derived diagnoses based upon digital images at different levels of complexity. Digital pathology excludes the investigation in the original glass slides using a conventional microscope.

Image facilities such as macro- and micro-cameras, slide scanners, are mandatory. They should deliver images of at least the same quality as a conventional light microscope.

Still images are not outstanding in routine microscopic diagnosis. Virtual slides (whole slide images) are essential for virtual microscopy and can only be applied in primary microscopic diagnosis if certain prerequisites are taken into account because of their complex image acquisition and visualization technique.

Information losses of conventional light microscopy images are technologically unavoidable and appear in the scanning, image preparation and storage procedures. However, the human eye should not recognize them.

The pathologist who wants to use virtual microscopy and abstain from conventional microscopy has to ensure that no quality losses occur in comparison with the conventional performance.

The medical product law (Medizinproduktgesetz, MPG) regulates the clinical application of slide scanners and virtual microscopy for in Vitro Diagnostics (MPG, §3, as at July 2017).

Devices which are certified for digital diagnosis can be used within the permitted framework for clinical diagnostics without limitations. This statement holds, for example, true for an

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approval of the American Food and Drug Administration (FDA), which still remains an exception until today.

The equivalence of diagnostic certainty between virtual and conventional microscopy has to be verified for all other system components and devices. The validation process follows the regulations of in vitro diagnostics. The following chapters 3 – 5 of the guidelines describe in detail the framework and minimum requirements of the systems and their components which should be equivalent in diagnostic certainty. Thereby, the pathologist receives a catalogue of criteria which are useful to negotiating with system providers.

Recommendation 2_1: All pathology institutions which want to establish virtual microscopy for primary diagnosis MUST conduct their own validation studies, if they want to use systems which are not explicitly certified for digital primary diagnostics.

2.2 Validation of digital diagnostics

2.2.1. Preliminary remarks – Guideline of the College of American Pathologists (CAP)

The American College of Pathologist has appointed an expert panel to working out the virtual microscopy guidelines in routine surgical pathology [Pantanowitz, et al., 2013]. The experts conducted a thorough literature search and put the result in the internet up for discussion. The commission digital pathology considers the Cap procedure and the derived ‘Guideline Statements’ trustworthy and a solid basis.

2.2.2. Premise

The diagnostic evaluation of WSI and of conventional glass slides is similar; however both methods differ in techniques of visualization and methods of performance. Therefore, it has to be confirmed that the diagnostic abilities of the pathologist do not significantly differ between both techniques for a specific diagnostic application. An appropriate tool are validation studies.

2.2.3. Study design

The actual diagnostic problem defines size and design of the validation study.

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Recommendation 2_2: The validation SHOULD be suitable for the clinical aim (kind of diagnosis) which is addressed by the WSI implementation. The preparation of the material (formalin fixation, paraffin embedding, frozen sections, immunohistochemical stains, cytology smears, etc.) should be included in the study. A renewed validation study is necessary if a new preparation technique is inaugurated which differs from the already proven method.

Recommendation 2_3: The validation study SHOULD reproduce the reality of the clinical – pathological environment which will be used for the virtual technology.

Recommendation 2_4: The validation study SHOULD include the complete WSI-System and its archive.

It is not necessary to validate individual components of the system.

Recommendation 2_5: The adequately trained pathologist, who will diagnose the WSI diagnostically in the routine, SHOULD be involved in the validation process.

Recommendation 2_6: The validation process SHOULD include a sample of at least 60 cases per application (Routine stains of fixed tissue), which are representative samples of the spectrum and complexity of routine diagnostics.

The validation process SHOULD each have 20 cases for each additional Application (e.g. immunohistochemistry, special stains). The validation is done by the diagnosis comparison of the associated glass slide and of archived WSI, or of the actual WSI, if the manufacturer provides this procedure as fallback option.

Recommendation 2_7: WSI and glass slides MAY be evaluated at random or systematic order.

Recommendation 2_8: A waiting period of at least two weeks between the assessment of the WSI and glass slides SHOULD be arranged.

2.2.4. Times of necessary revalidation

Recommendation 2_9: Revalidation MUST be performed as soon as a significant change to one components of the WSI system has occurred.

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2.2.5. Criteria for successful validation

Minimum values for the (intra - observer) reproducibility are currently not provided by the guidelines, because such values are not resilient available for conventional microscopy. Mismatch rates have been reported in the literature in a wide range (between 1.4% to 30% [Jukic, et al., 2011]). The digital diagnostics applying pathologist sets this acceptance threshold on its own responsibility.

Recommendation 2_10: The validation MUST confirm the diagnostic concordance between WSI and glass slides, and document the accepted intra - observer reproducibility.

Recommendation 2_11: The validation SHOULD confirm that all material, which is present on the glass slide, will be also present in the WSI. The original of these statements is described in Appendix A3.

3. Requirements for slide scanners

3.1. Completeness of the scan

3.1.1. Preliminary note

It is of vital importance for virtual diagnostics that all relevant tissue particles that are on microscopic glass slides can also be examined. There are two main reasons that particles relevant for diagnosis on glass slides are not or only insufficiently acquired on a virtual slide: an incorrect setting of the scan area and an erroneous scanning the glass slide.

There are different sizes of glass slides and of cover slips. In addition, different laboratory practices generate a wide range of tissue localizations on the glass slide (an example is depicted in Figure 1).

It is therefore necessary to correctly adjust the scan area. The settings of the scan area may well differ between the laboratories of the same institution, which then has to be regulated organizationally or technically. The validation study will handle and review the appropriate adjustment.

It is common practice for slide scanners, to first detect with an overview image (preview scan at low magnification) at which points of the slide, or, if at all, tissue is present. Only these areas are scanned with a significantly higher resolution for the definite image. The pathologist cannot examine these particles with his virtual microscope if they are not previously detected by the prescan. This case must be excluded with high probability because the missed tissue particles might be important for diagnosis,

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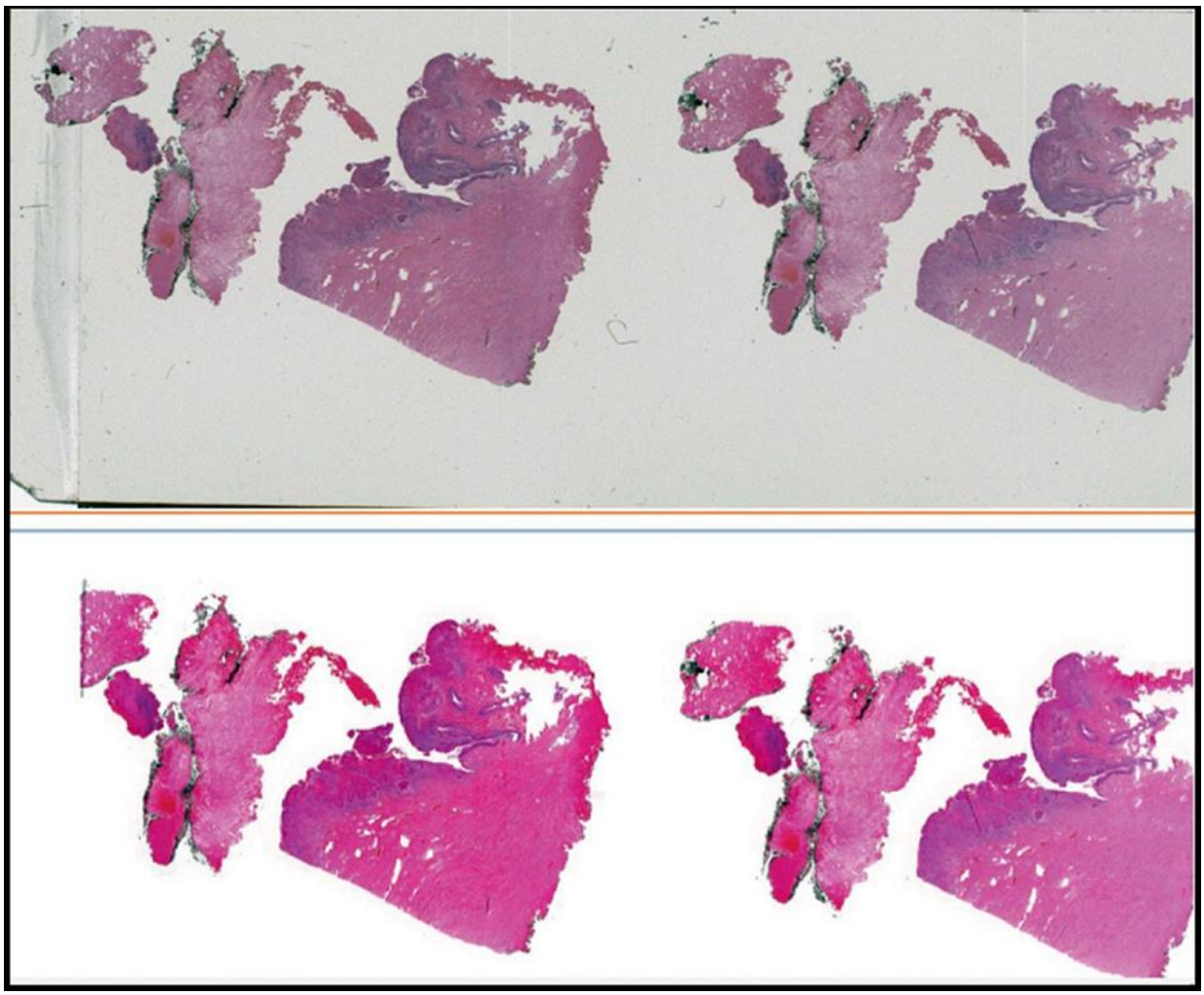

Figure 1: The compartments of the tissue on the glass slide which are not covered by the coverslip have not been scanned and are, therefore, not present in the virtual slide.

3.1.2. Size of relevant particles

A histological investigation is based on cellular agglomerations in contrast to cytological preparations. Herein we define the number of 3 x 3 = 9 cells as the smallest detectable cellular agglomeration. Based on different kinds of tissues, we alternatively define a minimum width of 30 µm for the area of detecting cellular agglomerations (structures) (see figure 2).

Proposing a circular tissue acquisition the minimum scanned area has to measure about 700 mm2, in order to detect the tissue. Relevant tissue particles have to be mapped in the virtual slide (i.e. scanned) as defined in section 3.1.1.

Recommendation 3_1: A slide scanner MUST scan all relevant particles.

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A comparative display of the preview image and the overview of the scanned slide are provided for each virtual slide. (see an example in Figure 3). The overview image is calculated by scanning the glass slide and generating the virtual slide. The comparative display allows the pathologist on duty to identify the non scanned areas.

Recommendation 3_2: Virtual microscopes SHOULD provide a control view of the photographed and scanned overview image. This requires appropriate Interfaces between the slide scanner, the virtual microscope and the pathology Information system. An adequate image analysis system might support the comparison of the preview and overview image.

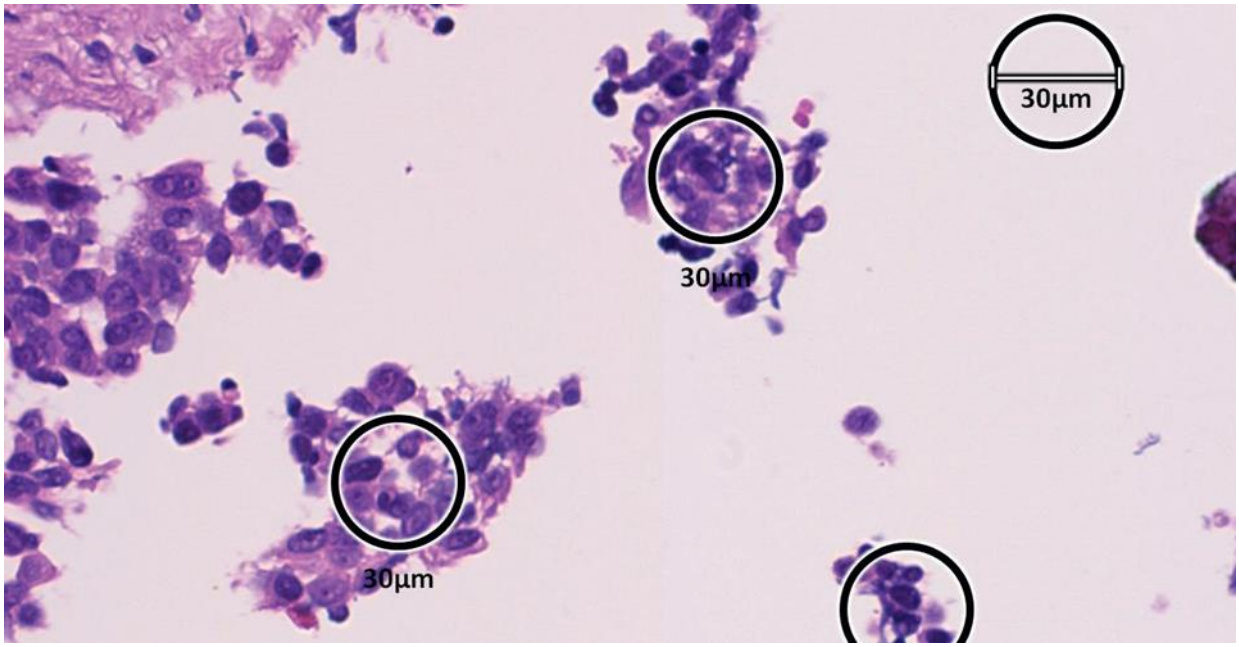

Figure 2: Visualization of the minimum tissue particle size.

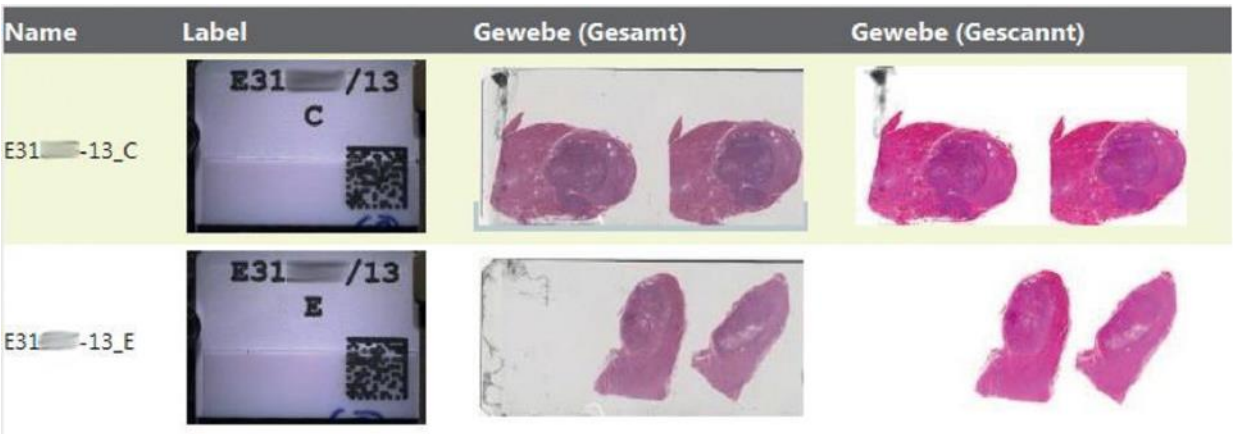

Figure 3 Comparative visualization of Preview (total) and Overview (scanned).

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3.2. Color fidelity, density resolution and area resolution

3.2.1. Preliminary remarks

Color fidelity is the ability of the scanner to correctly transfer color differences of the original microscopic image to a virtual microscope for display. This is independent of the monitor's display ability. These differences should also be adhered to in the display. A check of the color fidelity is only possible with a calibrated reference slide and with an appropriate program for quantitative evaluation of the image content. A lossy compression of the image format must not be switched off or reduced, and match to the values of routine operation.

Something similar is valid for the dissolution of density. It determines which differences in the brightness of the pixels a scanner can take up. It is different for different colors and must meet the requirements to the colored variability of the respective application.

An examination of the density dissolution is also possible with a calibrated reference slide as well as with a suitable program for the visualization of the image content. The minimum differences of the color densities which are just still distinguishable by the observer can be qualitatively evaluated. The lossy compression of the image format may not be switched off and/or reduced hereby.

The spatial resolution is the number of different pixels per unit of length which can be handled likewise differently by the scanner. The term of „still recognizable pairs of lines" with sufficiently small distance and small dimension has been established in image processing.

An evaluation of the spatial resolution is only possible with a suitable reference slide. It contains respective pairs of reference lines which can be evaluated by the observer. The observer might switch off and/or reduce a lossy compression of the image format, because the assigned compression algorithms cannot handle the common strong contrast at the edges well. The arising edge artifacts would negatively affect the contrast. The compression of the image format does not considerably influence the evaluation of the spatial resolution because spatial resolution is only of importance for technical assessment and not in clinical practice.

Color distortions of the scan process can affect the interpretation of biomarkers which has been demonstrated in the Scanner Contests 2010 and 2012. Therefore, such color distortions should be minimized by color calibration of the scanner and of the visualization chain, independently of the fact whether standardized or non standardized staining procedures have been applied out of the following reasons:

- The virtual sections of the same glass slide should be comparable if multiple scanners are used in an institution.
- Automated stainers are increasingly in use for HE stains. Standardized stains should also be scanned in a standardized procedure.

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- Quantifications and image registrations will only result in reproducible data if the evaluated original images have been scanned and compared with calibrated colors.
- Visualizations of different stains in an overlay mode will be easier comparable if the used individual dyes have been standardized and scanned. It is therefore important to implement a routine autocalibration of the applied systems as well as regular manual controls. The latter might include special test preparations and a subsequent software-based on the analysis of the corresponding virtual sections.

The described procedures will provide both a relative and an absolute impression of any divergence.

Recommendation 3_3: Virtual microscopes SHOULD support control views of density, location and color resolution test preparations and log the results. The corresponding interfaces between the slide canner and the virtual microscope must be included.

3.2.2. Test procedure

Test execution see appendix A1

Recommendation 3_4: A technical validation of all devices SHOULD be carried out annually. The manufacturer's intended tests SHOULD be carried out according to the manufacturer's prescriptions. The test procedure SHOULD in particular become dependent on the scan throughput. The new automatic or manual color calibration must be used by the above-mentioned methodology before the date of validation, if it is already supported by the manufacturer of the devices.

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3.2.3. Visualization of parallel staining

The contemporary visualization of different stains (e.g. markers) is a major advantage of virtual microscopy. As a rule, the visualization of the parallel staining is based on the HE-section Small particles may not be present in parallel stainings. One can recognize this fact by contemporary visualization of the HE section and may react according to the diagnosis relevance of the area, for example, by re-digitization of the parallel stain, if small particles are missing in the virtual section. See also section 2.2.

3.2.4. Image compression and data format

In virtual microscopy, image compression is not only an optional way to increase the capacity of the memory in terms of the number of images, but rather an essential part of the display chain. This is due to the fact that the large data volumes of the WSI require a lossy image compression in order to be able to distribute the data efficiently.

Only a reduced amount of image information can be transferred in sufficiently fast velocity. The transfer starts with the image generation, divided into individual camera images (tiles), which are then each compressed transferred from the scanner into the computers of the image processing, afterwards transferred to the archive, and finally to the representation in the virtual microscope.

A lossless reduction of the information can only be done to a certain extent. In addition, insignificant information must be removed for display. The original image can no longer be exactly reconstructed afterwards. The first component of the processing chain (i.e., which compresses) defines the loss of image information. It reduces the information that is no longer needed in the final use of the image, such as dense resolutions in color areas for which the human eye is less sensitive.

The degree of compression is approximated by the ratio of the sizes before and after the compression, (e.g. a ratio 20:1). Although this ratio describes the saving of storage space and the bandwidth required for transmission, it is only a limited indication of the actual loss of meaningful image information and, furthermore, its diagnostic influence. The quantitative loss of information is only partly related to the qualitative loss of information.

The quantitative loss of information can be measured by ratios, the qualitative loss only by comparative studies. Different compression methods differ in their efficiency, and, in addition, also in their application and results. The procedures vary considerably, especially at large compression rates, due to the appearance of "image faults", so-called artifacts in the form of block formation or lubrication effects. However, they all have in common the fact that the

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indicating component at the back end of the display chain must know the compression method in order to decode and display the image data.

In the expected amount of data, the demand to include as much as possible error redundancy and associated robustness against data loss or change has been completely unnoticed in the medical field. However, it represents an essential quality characteristic for the storage format. The very large images of the WSI increase significantly the likelihood of errors. Neither partial data loss nor accidental alteration within an image file should make the image unreadable. A partial loss of information should not result in a total loss of the WSI.

Closely related to this is an aspect of digital data organization within an image archive (PACS). WSI are internally organized as resolution pyramids. Significant advantages in terms of the computational overhead and access speed required for data organization can be obtained, if a compression method organizes the compressed data streams and automatically creates the progressive arrangement.

The separate management of both (e.g. organizing individually lossy compressed tiles in a TIFF resolution pyramid) is not only of disadvantage in data organization, it can even lead to edge artifacts at the interfaces between the tiles, because the information reduction does not take into account the relationship of the individual images. Finally, it has to be taken into account that a considerable number of software patents has been extended to storage formats of the WSI. As a result, the use of the data stored in the archive would then only be permitted under the license of a patent owner. Even though these permits are currently given generously, they may be withdrawn at any time. Of course, such a foreign determination possesses a threat to an archive of patient-related data. Such a situation should be checked before choosing a particular image format or manufacturer.

Thus, the decision for a storage format is fundamental. It influences the selected archive for its entire period and is the key feature for its future durability and security. Compression has to be considered as investment in which the future use of the database as a whole and the described general conditions determine the requirements.

Recommendation 3_5: The storage concept SHOULD take into account the compression methods until to the end of the visualization chain, an extensive error redundancy in storage, an automated progressive arrangement of the compressed data streams and the patentability of the storage format.

3.2.5. Archiving

The original glass slide of the diagnosis report must be archived. The stained glass slide remains the original data source in virtual microscopy. Images acquired from the same glass slide (WSI) are only to be treated as original if specific manipulations have led to diagnosis relevant findings, e. g. by detecting so-called hotspots in immunohistochemically stained

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preparations. However, long-term archiving of all or selected digital images is recommended for practical reasons.

The operator's duty on the care of a digital archive requires doing everything possible to ensure the changelessness, the guaranteed future and future-proofing of the data. It includes the unity of patient or case data and the actual image content, in addition to the statements of 3.2.4. Such a unit is guaranteed by the use of a DICOM-capable archive. On the other hand, file storage without redundant assignment options, such as those that are organized with the file or folder name of a file system only, is not recommended.

Although the type of storage media used is not the subject of this guide, it should be understood that the use of simple external or internal hard drive solutions can be considered negligent. Organized and redundant storage solutions should be used (NAS or similar with RAID-X) instead.

Lossy compression means a change in the image content. Therefore, the original compression method of the scanner manufacturer would have to be documented in the archive to keep an unchanged archiving. However, this is impossible if the diagnosis took place in an image distribution system of the manufacturer and the image distribution is based on a proprietary image format.

Once this format is converted to a common format (e.g. DICOM), lossy recompression occurs. Such recompression should be allowed if the image quality control (see 3.2.6.) and the archived image data are continuously checked and the images cannot qualitatively and quantitatively be distinguished. The qualitative test might only be done once during the validation.

The storage period of digital pathological data is currently not specifically regulated. This means that the general rules governing the filing of glass slides apply. Therefore, the storage of the data is mandatory for at least ten years. The years until to the legal age should be added to the retention period, if the patient was under the age of majority at the time of the data collection. It should be noted that the data have to be deleted at the expiration of the retention period, since the data storage period is not a minimum provision.

Selective archiving concepts may be used to reducing the amount of data to be stored. In this case the selection concerns content-related aspects.

Recommendation 3_6: All diagnosed images that are relevant for the diagnosis SHOULD be kept unchangeable for a complete ten-years period. Selection criteria and mechanisms for diagnostic relevance MUST be permanently documented and SHOULD work on the basis of algorithms.

Finally, it should be noted that these Recommendations do not allow the forensic archiving of the WSI in replacement of the original glass. The glass slide has still to be archived, because on the one hand, future conditions have to be met which may change the archiving of a WSI, and

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on the other hand, the glass slide might serve for additional (biological) investigations than a microscopic examination only.

3.2.6 Image Quality Control

Continuous review of the image quality is necessary to maintain its suitability, once the basic adequacy of the scanner / archive / viewer combination for the virtual microscopy has been tested in the test series. Hereby unnoticed changes should be excluded, for whatever reason.

Recommendation 3_7: The image quality check SHOULD take place during the test procedure (3.2.2.).

3.2.6.1. Content of the review

A compressed image must be compared to its uncompressed source data in order to eliminate the influence of negative image compression factors. The quadratic error is calculated logarithmically in the form of a PSNR and documented. The purpose of the scanners is to provide a feature that allows the output of these two virtual slide versions.

A lossless or nearly lossless (compressed) version must be delivered, if a scanner cannot create an uncompressed version of an image.

Images of the same glass slide can be acquired twice in fast succession, if a scanner cannot create the two wanted variants. Both image variants should be stored both in the image format of the scanner and in a free accessible trivial image format (e.g. PPM).

3.2.6.2. Performance of the review

The image contents are compared on ten randomly selected image sections. Each of them should cover an area of 1/100 of the whole image size (WSI). All ten PSNR values must be documented and prepared for analysis of potential changes which may appear in a longer period of time.

3.2.6.3. Achievement of the review

The findings must be documented and presented in an appropriate form over the entire period of use, so that a sudden deviation from the normal values can be detected. In case of a deviation, the cause of the fault must be immediately remedied before a continued use of the device’s image data occurs.

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3.2.6.4. Subject of the review

The original glass slides should be used for the test, and might be the same which have been recommended for the test series described in Chapter 2.2.

4. The visualization chain

4.1. Systematics of the influences on the display (presentation on the monitor)

The magnification of a conventional light microscope is calculated by multiplying the objective magnification with the projection magnification. For example, a 60x objective and a 10x ocular magnification results in a 600x overall magnification.

The magnification of virtual microscopy is calculated by the aperture of the lens, the resolution of the camera sensor, the resolution of the monitor and the distance of the eye from the monitor. These sizes must be coordinated for optimal presentation,

Recommendation 4_1: The spatial resolution of the image MUST exceed at least twice the value of the individual resolution which is according to the sampling theorem mandatory for the respective diagnostic approach.

4.2. Monitors and their attitude

There are no legal regulations for the display of WSIs on monitors, unlike in radiology. Based on past experience, however, Recommendations for the quality of the implemented display can be given.

4.2.1. Monitor quality

The monitor quality can be defined by the size of the image matrix, the color resolution, the spatial resolution, the pixel size (number of pixels and their respective distance) and by the absolute and relative color fidelity.

The size of the monitor is measured by the physical diagonal dimension of the screen. The color depth, the maximum brightness and the maximum contrast are additional quality features. Pixel errors and homogeneity of the image geometry are not considered herein, because their quality has been consistently sufficient since the introduction of flat screen technologies. The same statement holds true for the viewing angles and display times

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Recommendation 4_2: The use of flat screens (TFT) is recommended, CRTs are not sufficient.

4.2.1.1. Image size and resolution

The matrix size indicates how many pixels can be displayed simultaneously in horizontal and vertical directions of the display area. The spatial resolution indicates the number of pixels per track which is comparable to the pixels size in relation to the size of the display area. Unlike matrix and screen size, the spatial resolution is rarely specified because these values depend on each other. The ergonomic and needs-based ratio of matrix size and screen size is more important than the highest possible spatial resolution.

Flat panel displays are actually only suitable for their physical resolution unlike CRT monitors. A selected deviating row or column resolution has to be interpolated. The obtained intermediate values might induce a fuzzy display. This should be avoided. In addition, the best spatial resolution is directly dependent on the eyesight (sharpness) and the habits of the viewer. The chosen distance of the viewer to the front of the screen has also to be taken into account.

As a rule of thumb, the screen should be set up at a distance to displaying a complete and sharp vertical copy of an A4 document of a normal letter or of text and tables of laboratory report without additional magnification. Therefore, several factors influence this arrangement and generally accepted Recommendation is hard to be set up. The distance of the display differs from viewer to viewer, as well as its pleasant and sufficiently sharp perception. This also applies to the screen size, although a trend to larger monitors is noticeable.

Recommendation 4_3: The following default values MIGHT be recommended for the combination of screen size and resolution:

WQXGA 16: 10 27 "diagonal matrix 2560 x 1600 (4 MP) UHD 16: 9 32" diagonal matrix 3840 x 2160 (4 K or 8 MP) A projective magnification which is comparable to the conventional microscope cannot be realized downstream of the monitor, because the digitization interrupts the optical beam path. In other words, the image itself must already contain the resolution which is mandatory for diagnostics. The spatial resolution of the monitor cannot create additional levels of image details.

Recommendation 4_4: The spatial resolution of the monitor SHOULD be sufficiently high and adapted to the distance of the observer in front of the monitor or its visual capabilities only.

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4.2.1.2. Brightness and contrast

The pixel spacing (the grid) indicates the size of the (unlit) columns between the pixels. The raster is indirectly a measure of the pixel size and thus of luminosity and contrast, since the maximum size of a pixel depends directly on the spatial resolution. This value has lost its importance due to the technology of flat screens compared to the CRT screens, since modern screens present with a value which is sufficient in almost all applications.

The same statement applies to the possible contrasts and brightness settings. No absolute requirements can be defined for these three values because surgical pathology diagnostics normally take place in an ill defined "office environment" (and not under dimmed conditions). As stated in the chapter of spatial resolution, the eyesight and the habits of the viewer are also decisive here.

In general, the setup of a monitor with the default values for brightness and contrast should allow a sufficient representation of all image content which are necessary for comfortable working under normal external illumination (no direct sunlight).

Recommendation 4_5: The screen SHOULD have a minimum contrast ratio of 1000: 1 and a maximum brightness of 300 cd / m². The minimum brightness SHOULD be displayed at 0.5 cd / m² or more intensively.

4.2.1.3. Color depth

The color depth is the number of color values, which can be represented simultaneously and be differentiated. The color depth is defined for each color channel of the display (e. g red, green, blue) and for the simultaneous display of all channels (gray). The minimum requirement of 256 density values per each color and gray channel is currently being realized by every available device.

Recommendation 4_6: A 24 bit color (true color) and 8 bit gray scale SHOULD be implemented as displayable color space. However, these values are not sufficient, if the display is also used for radiographic grayscale image viewings.

4.2.1.4. Color fidelity

Color fidelity is the ability of the monitor to maintain the luminosity levels of the different primary colors for a defined time interval or to exactly reproduce standardized colors under defined lighting conditions.

Absolute color fidelity refers to the exact reproduction of defined color values. It is important for the printing industry. This requirement would be equivalent with the demand that each

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microscope should display a glass slide exactly in the same colors. At present, this is not the case. Self-calibrating monitors should be considered an investment in the future, since most medical reporting monitors are already equipped with an automated calibration,

Relative color fidelity denotes the same representation of identical color values at different times. Herein, consistent but no exactly identical color values are expected. This capability is also achieved by automated calibration of the monitors. Digitized images do not lose color information over time, in reverse to glass slides. This statement should also apply to the screens which are used for display.

Recommendation 4_7: The SOLAR screen should have a color calibration option. An automated self-calibration is recommended. Manual calibration SHOULD be performed at time intervals according to the manufacturer's Recommendations.

4.2.1.5. Color profiles

Color profiles are gradients of correction values which are stored in the monitor. They can be used to achieve a better image adaptation to its content, ambient lighting and personal vision, as well as to calibrate its relative density profiles. They allow an optional adjustment of contrast, brightness and color fidelity; however, they are not of mandatory demand at the current state of the art.

Recommendation 4_8: If color profiles can be assessed to the screen, the selected color depth REQUIRES to meet the profiles after application in accordance with the postulate 4.2.1.3.

For this purpose, most of these devices are internally equipped with a 10-bit color depth before the profile is applied. (A corresponding example is the DICOM Grayscale Standard Display Function in radiology.) A comparable color variant is currently in its phase of definition).

5. Integration of the slide scanner into the pathology information system (Patho-LIS), see also the guideline "Pathology: Workflow in Digital Medicine"

5.1. Introduction and standards

Analogous to the development in digital radiology, manufacturers of slide scanners have developed their own image formats for the virtual tissue sections and use them in their proprietary viewers (virtual microscopes).

The same holds true for the interfaces to pathology information systems. This behavior prevents a flexible distribution of image data between software products from different

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manufacturers. It lacks of independent color calibration and reduces the diagnostics to a fixed scanner-viewer combination, instead of an optional n:m distribution.

The determination of a proprietary storage format or system for image distribution impedes the integration of scanners from different manufacturers or even prevents it.

Thus, a central archive (PACS) is of central importance for an independent, hospital-wide image distribution. Standards simplify the introduction and operation of integrated IT solutions by minimizing the number of interface solutions required. They secure the investment and contribute significantly to quality assurance. Most standardization are implemented by joint projects of industrial companies, potential user groups and scientific participation. Examples include the common in medicine standards DICOM and HL7.

HL7 standardizes the hospital-wide information exchange, as far as it does not directly refer to image or video data. In addition to patient management, these are messages and documents for order, management, billing and examination findings.

The DICOM standard is the accepted worldwide standard for medical image processing and the associated workflow organization. In particular, it is expandable and, within certain limits, changeable, which explains its permanent functionality.

An imaging or processing device has to support DICOM in order to integrate DICOM into the clinical environment. An integration of proprietary (manufacturer-specific) image formats or communication protocols is now rejected by most operators and sometimes even manufacturers. Additional discussions are presented in Annex A2.

Recommendation 5_1: A scan workflow SHOULD be included in a Patho-LIS and an image archive, which should meet both the HL7 and the DICOM standard. Conformity to the IHE profile APW or its current successors SHOULD be achieved.

Recommendation 5_2: All image data in free formats (e.g. JPEG 2000) SHOULD be sent to the PACS and retrieved from there by use of a corresponding streaming mechanism (e.g. JPIP).

All these standards are publicly available and the least legal issues are to be utilized.

Recommendation 5_3: Macroscopic images (macro photos) SHOULD be stored in the DICOM class Visible Light Photographic Image Storage. The storage of eyepieces (microscope photos) SHOULD be performed in the DICOM class Visible Light Microscopic Image Storage. The overview picture of a WSI scan (label picture) MAY also be stored in this picture class.

Recommendation 5_4: The storage of findings or other text-based documents SHOULD occur in the DICOM class Encapsulated PDF Storage.

Recommendation 5_5: The storage of findings or other structured documents SHOULD be performed in the DICOM class Encapsulated CDA Storage.

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Recommendation 5_6: The storage of other image data (letter of submission or similar) SHOULD occur in the DICOM class Secondary Capture Image Storage or Multi-frame True Color Secondary Capture Image Storage.

The integration of slide scanners and virtual microscopes into a Patho-LIS must be done via interfaces that should be designed according to existing standards.

For these purposes, IHE, the international organization „Integrating Health Care Enterprises“ defines characteristic profiles for so-called use cases, which define specific limitations of the standards and which are thus clearly applicable, testable and certifiable.

The desired interoperability between the systems under discussion achieves high investment security in terms of system portability. The IHE profile for this purpose is the IHE-PaLM APW (Anatomic Pathology Workflow). However, there exist currently only a few interfaces from the manufacturers which are based on IHE profiles or use the standards in a genuine way.

Recommendation 5_7: The purchase of systems which use the standards DICOM, HL7, CDA for communication with third-party systems (e.g. the hospital information system) or whose systems meet the IHE's conformity criteria is particularly important and sustainable in the environment of larger facilities (e.g. Universities).

This applies both to the adaptability to software developments in the environment and to the certifiability of facilities. Image archives are preferable to organize their data with free data formats and to allow free, streaming capable and sufficiently efficient access to their image data.

5.2. Basic requirements

The following basic requirements for the integration of a slide scanner must be implemented by standard-based software interfaces, because an implementation with organizational "manual" tools is only feasible for a low number of cases per day. At the time of diagnostics on a virtual slide, the following data have to be immediately visualized or visualized at any time:

1. Accession number (ID) of the case

2. Related sections (additional tissue blocks and stains, biomarkers)

3. ID of the associated tissue block

4. Stain or marker of the associated glass slide

5. Pictogram of the slide label

6. Resolution or amplification of the WSI

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7. Degree of lossy compression

8. In progressive presentation: Display of present stage of progression, or at least, whether the image section with all progression levels has already been displayed or not.

Recommendation 5_8: Interfaces between the slide scanner and Patho-LIS according to requirements 1 to 8 SHOULD be implemented and tested by the responsible pathologist within the scope of the required validation study (see section 2.2.). The identification of the glass slide with a barcode (see section 5.3) usually forms the basis for an automated, correct linking of the glass slide and its virtual equivalent.

Recommendation 5_9: All imaging software systems SHOULD be able to obtain the DICOM header data as SCU for the creation of correct DICOM objects via the DICOM service Modality Worklist. The relevant image object has to be identified b use of a query parameter in the tag Specimen Identifier (0040,0551) in these cases,

Recommendation 5_10: An information system which manages the patient and case data SHOULD support the DICOM-Service Modality Worklist as SCP and also support its object identifications as single value matching in the tag (0040,0551) Specimen Identifier.

5.3. Correct assignment of glass slides and virtual slides

The correct, automatic assignment of glass slides and virtual slides is essential for histological diagnosis. The interaction of several manufacturers (e.g.: manufacturer A: barcode printer, manufacturer B: stainer, manufacturer C: slide scanner, manufacturer D: pathology information system) can lead to problems of the correct assignment. This may, for example, be due to limited readability of the barcode as a result of the laboratory process. The following alternatives can be chosen:

5.3.1. Assurance of correct recognition by the manufacturer

If all the systems involved in a digital pathology solution are matched, tested, and delivered by the same manufacturer, the manufacturer may guarantee that the barcodes are correctly recognized and assigned. (Of course, human or technical failure cannot be completely ruled out.) ´

Remark: For example, several technical solutions already use a barcode which is clearly assigned by the manufacturer and laser-engraved into the slide.

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5.3.2. Comparison of barcode and alphanumeric code

Recommendation 5_11: In addition to the barcode on the slides, the alphanumeric code of the glass slide (eg E-20564/2017-BL2-Giemsa) SHOULD be printed. Thus a read error can be identified by comparing the recognized barcode with the recognized alphanumeric label, and, for example, corrected manually by comparison with the original glass slide.

5.3.3. Manual assignment of glass slides and associated virtual slides

The technical staff might also manually assign the mandatory identification codes, if the virtual microscopy solution is only used to a limited extent in clinical routine. An example would be the use of virtual microscopy to quantify Ki67 in selected tumors.

5.4. Visualization of serial sections

The quality of the parallel visualization of stains also depends on the distance between the corresponding serial sections in the block. The quality of image registration can be considerably increased if the position of the serial cut in the block is known and can be retrieved for every virtual slide.

A systematic approach to block processing and cross-institution definition of preparation and documentation are prerequisite. A practical solution would be to define a constant order for standard staining procedures in the laboratory and to label the sections correspondently.

Recommendation 5_12: The different stains SHOULD be registered on the HE-section, so that one can compare any position in the HE section to the analogue position in a different stained series section at any magnification.

6. Supporting services of the Federal Association of German Pathologists e. V.

6.1. Planning and evaluation of a validation study

The Federal Association assists pathologists in the configuration, the selection of cases as well as diagnostic assessment schemes and in the statistical evaluation of the outcome of the validation study.

For the planning and evaluation of the study, the Federal Association provides a set of documents that supports the planning and evaluation of the validation study. It is planned to provide this functionality in electronic form via an interactive website.

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6.2. Provision of IT8 target and color calibration evaluator

The Federal Association provides all the information needed to obtain an IT8 target for analyzing the color calibration of a scanner. At the same time, the website of the Federal Association links to a website that allows the upload of a scanned IT8 target as WSI and its analysis. The result of the analysis can be printed as a document or downloaded as a PDF file.

7. Survey of Recommendations

7.1. Recommendations predominantly for pathologists

Recommendation 2_1: All pathologists who wish to introduce virtual microscopy for primary diagnostics MUST conduct their own validation studies, unless they explicitly use digitally certified systems.

Recommendation 2_2: The validation SHOULD be appropriate for the clinical purpose (diagnostic problem) which the WSI deployment addresses.

Recommendation 2_3: The validation study SHOULD replicate the reality of the clinic-pathological environment in which the technology will be implemented.

Recommendation 2_4: The validation study MUST include the entire WSI system, including archiving.

Recommendation 2_5: The adequately trained pathologist who will evaluate the WSI in routine diagnostics SHOULD be involved in the validation process.

Recommendation 2_6: The Validation Process SHOULD include a sample of at least 60 cases per application (routine glass slides of fixed tissue) which are representative of the range and complexity of sample types and routine diagnoses.

Recommendation 2_7: WSI and Glass Slides MAY be assessed in random or in a systematic order.

Recommendation 2_8: A waiting period of at least two weeks SHOULD be arranged between the assessment of WSI and glass slides.

Recommendation 2_9: Revalidation MUST be performed once any of the components of the WSI system changed significantly.

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Recommendation 2_10: The validation MUST confirm the diagnostic concordance between WSI and glass slides in the form of accepted intra-observer reproducibility, which should be documented.

Recommendation 2_11: The validation SHOULD confirm that all material on the glass slide is also present in the WSI.

Recommendation 3_1: The slide scanner MUST scan all relevant particles.

Recommendation 3_6: All diagnosed images that are diagnosis relevant SHOULD kept unchangeable for the full ten-years period. Selection criteria and mechanisms for diagnostic relevance MUST be permanently documented and SHOULD be based on algorithms.

Recommendation 5_1: A scan workflow SHOULD be included in the Patho-LIS and in the image archive. It should meet both the HL7 and the DICOM standards. A conformity to the IHE profile APW or its current successors SHOULD be achieved.

Recommendation 5_7: Sustainable and particularly important in the environment of larger facilities (e.g. Universities) is the acquisition of systems which use the standards DICOM, HL7, CDA for communication with third-party systems (e.g. the hospital information system) or whose systems meet the IHE's conformity criteria.

7.2. Recommendations for the Components of the Digital Pathology Solution

Recommendation 3_1: The slide scanner MUST scan all relevant particles.

Recommendation 3_2: Virtual microscopes SHOULD support a control view between the photographed and scanned overview image. For this purpose, appropriate interfaces between the slide scanner, the virtual microscope and the pathology information system MUST be implemented. Useful is the assistance of an image-analysis system which compares the preview image and the overview image.

Recommendation 3_3: Virtual Microscopes SHOULD support the control views of density, location and color resolution of test specimens and log the results. Appropriate interfaces between the slide scanner and the virtual microscope MUST be implemented.

Recommendation 3_4: The technical validation of all devices SHOULD be performed annually. The tests provided by the manufacturer SHOULD be carried out according to the instructions of the manufacturer.

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Recommendation 3_5: The memory concept SHOULD consider the compression methods until to the end of the visualization chain, and include an extensive error redundancy in the archive, an automatic progressive arrangement of the compressed data streams and the patentability of the storage format.

Recommendation 3_7: The image quality SHOULD be checked during the test procedure (3.2.2.).

Recommendation 4_1: The spatial resolution of the image MUST exceed at least twice the value of the individual resolution which is according to the sampling theorem mandatory for the respective diagnostic approach.

Recommendation 4_2: It is recommended the use of flat screens (TFT), CRTs are not sufficient.

Recommendation 4_3: The following default values MAY be recommended for the combination of screen size and resolution:

WQXGA 16: 10 27 "diagonal matrix 2560 x 1600 (4 MP) UHD 16: 9 32" diagonal matrix 3840 x 2160 (4 K or 8 MP)

Recommendation 4_4: The spatial resolution of the monitor SHOULD be sufficiently high and adapted to the distance of the observer in front of the monitor or its visual capabilities only.

Recommendation 4_5: The screen SHOULD have a minimum contrast ratio of 1000: 1 and a maximum brightness of 300 cd / m². The minimum brightness SHOULD be displayable at 0.5 cd / m² or higher.

Recommendation 4_6: The displayable color space SHOULD include 24 bit color (true color) and 8 bit gray scale. However, these values are not sufficient to display and view radiographic grayscale images.

Recommendation 4_7: The SOLAR screen should have a color calibration option. An automated self-calibration is recommended. Manual calibration SHOULD be performed at time intervals according to the manufacturer's recommendations.

Recommendation 4_8: If color profiles can be assessed to the screen, the selected color depth MUST meet the profiles in accordance with the postulate 4.2.1.3 after application. For this purpose, most of these devices are internally equipped with a 10-bit color depth before the profile is applied.

Recommendation 5_7: The acquisition of systems which use the standards DICOM, HL7, CDA for communication with third-party systems (e.g. the hospital information system) or whose systems meet the IHE's conformity criteria is sustainable and particularly important in the environment of larger facilities (e.g. Universities).

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Recommendation 5_9: All imaging software systems SHOULD be able to purchase the DICOM header data as SCU for the creation of correct DICOM objects via the DICOM service Modality Worklist. The identification of the relevant image object serves as query parameter in the tag 'Specimen Identifier' (0040.0551) in this case.

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7.3. Recommendations for IT interfaces, standards and workflow

Recommendation 3_2: Virtual microscopes SHOULD support a control view between photographed and scanned overview image. For this purpose, appropriate interfaces between the preparation scanner, the virtual microscope and the pathology information system MUST be created. Useful is an image-analysis system which compares the preview image with the overview image.

Recommendation 3_3: Virtual microscopes SHOULD support inspection views of density, location and color resolution test specimens and log the results. Appropriate interfaces between the slide scanner and the virtual microscope MUST be included.

Recommendation 3_4: The technical validation of all devices SHOULD be repeated annually. The tests provided by the manufacturer SHOULD be performed according to the manufacturer's instructions.

Recommendation 3_5: The memory concept SHOULD investigate the compression methods until to the end of the visualization chain, and take into account an extensive error redundancy in its storage, an automated progressive arrangement of the compressed data streams and the patentability of the storage format.

Recommendation 5_1: A scan workflow SHOULD be integrated into a Patho-LIS and an image archive. The work flow should meet both the HL7 and the DICOM standard. The conformity to the IHE profile APW or its current successors SHOULD be achieved.

Recommendation 5_2: All image data in free formats (e.g. JPEG 2000) SHOULD be sent to the PACS and retrieved from there using a corresponding streaming mechanism (e.g. JPIP). As these standards are open and publicly available, the least legal issues are to be expected.

Recommendation 5_7: The acquisition of systems which use the standards DICOM, HL7, CDA for communication with third-party systems (e.g. the hospital information system) or whose systems meet the IHE's conformity criteria is sustainable and particularly important in the environment of larger facilities (e.g. Universities).

Recommendation 5_8: Interfaces between the slide scanner and Patho-LIS according to requirements 1 to 8 SHOULD be established and tested by the responsible pathologist within the scope of the required validation study (see section 2.2.).

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Recommendation 5_11: The alphanumeric code of the tissue cut (e.g. E-20564/2017-BL2-Giemsa) SHOULD be printed in addition to the barcode on the slides. Thus a read error can be identified by comparing the recognized barcode with the recognized alphanumeric label, and, for example, corrected manually by comparison with the original glass slide.

Recommendation 5_12: The different stains SHOULD be registered on the HE-section, so that one can compare any position in the HE section with the analogue position in a different stained series section at any magnification.

7.4. Recommendations for archiving

Recommendation 3_5: The memory concept SHOULD investigate the compression methods until to the end of the visualization chain, and take into account an extensive error redundancy in its storage, an automated progressive arrangement of the compressed data streams and the patentability of the storage format.

Recommendation 3_6: All diagnosed images that are relevant for the diagnosis SHOULD kept unchangeable for a complete ten-year period. Selection criteria and mechanisms for diagnostic relevance MUST be continuously documented and SHOULD be based on algorithms.

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Recommendation 5_3: Macroscopic images (macro photos) SHOULD be stored in the DICOM class Visible Light Photographic Image Storage. The storage of eyepieces (microscope photos) SHOULD be performed in the DICOM class Visible Light Microscopic Image Storage. The overview picture of a WSI scan (label picture) MAY also be stored in this picture class.

Recommendation 5_4: The storage of findings or other text-based documents SHOULD occur in the DICOM class Encapsulated PDF Storage.

Recommendation 5_5: The storage of findings or other structured documents SHOULD be performed in the DICOM class Encapsulated CDA Storage.

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Recommendation 5_6: The storage of other image data (letter of submission or similar) SHOULD occur in the DICOM class Secondary Capture Image Storage or Multi-frame True Color Secondary Capture Image Storage.

Recommendation 5_7: The acquisition of systems which use the standards DICOM, HL7, CDA for communication with third-party systems (e.g. the hospital information system) or whose systems meet the IHE's conformity criteria is sustainable and particularly important in the environment of larger facilities (e.g. Universities).

Recommendation 5_9: All imaging software systems SHOULD be able to purchase the DICOM header data as SCU for the creation of correct DICOM objects via the DICOM service Modality Worklist. The identification of the relevant image object serves as query parameter in the tag ‘Specimen Identifier’ (0040.0551) in this case.

Recommendation 5_10: An information system that manages the patient and case data SHOULD support the DICOM-Service Modality Worklist as SCP and support its object identifications as single value matching in the Tag (0040,0551) Specimen Identifier.

Recommendation 5_11: The alphanumeric code of the tissue cut (e.g. E-20564/2017-BL2-Giemsa) SHOULD be printed in addition to the barcode on the slides. Thus a read error can be identified by comparing the recognized barcode with the recognized alphanumeric label, and, for example, corrected manually by comparison with the original glass slide.

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Attachments

A1 Test procedure for technical validation of the scanner and visualization chain

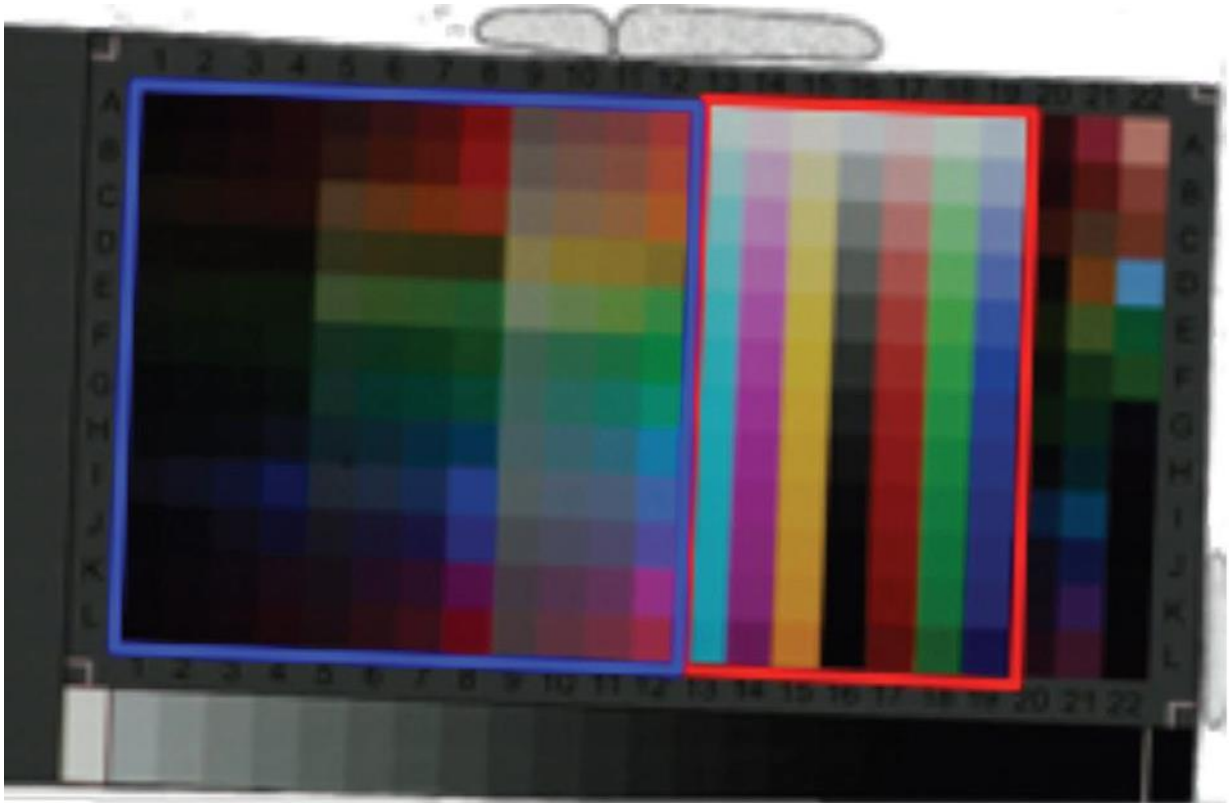

Figure 4 IT8.7 / 1 target; outlined in blue is the color matrix for assessing color fidelity.

The test preparation consists of a standard slide which presents with an IT8.7 / 1 Color Target. The specific absorption spectra colorimetric coordinates of the tiles are known and can be compared after the digitization. The target itself consists of 264 color and 24 gray fields.

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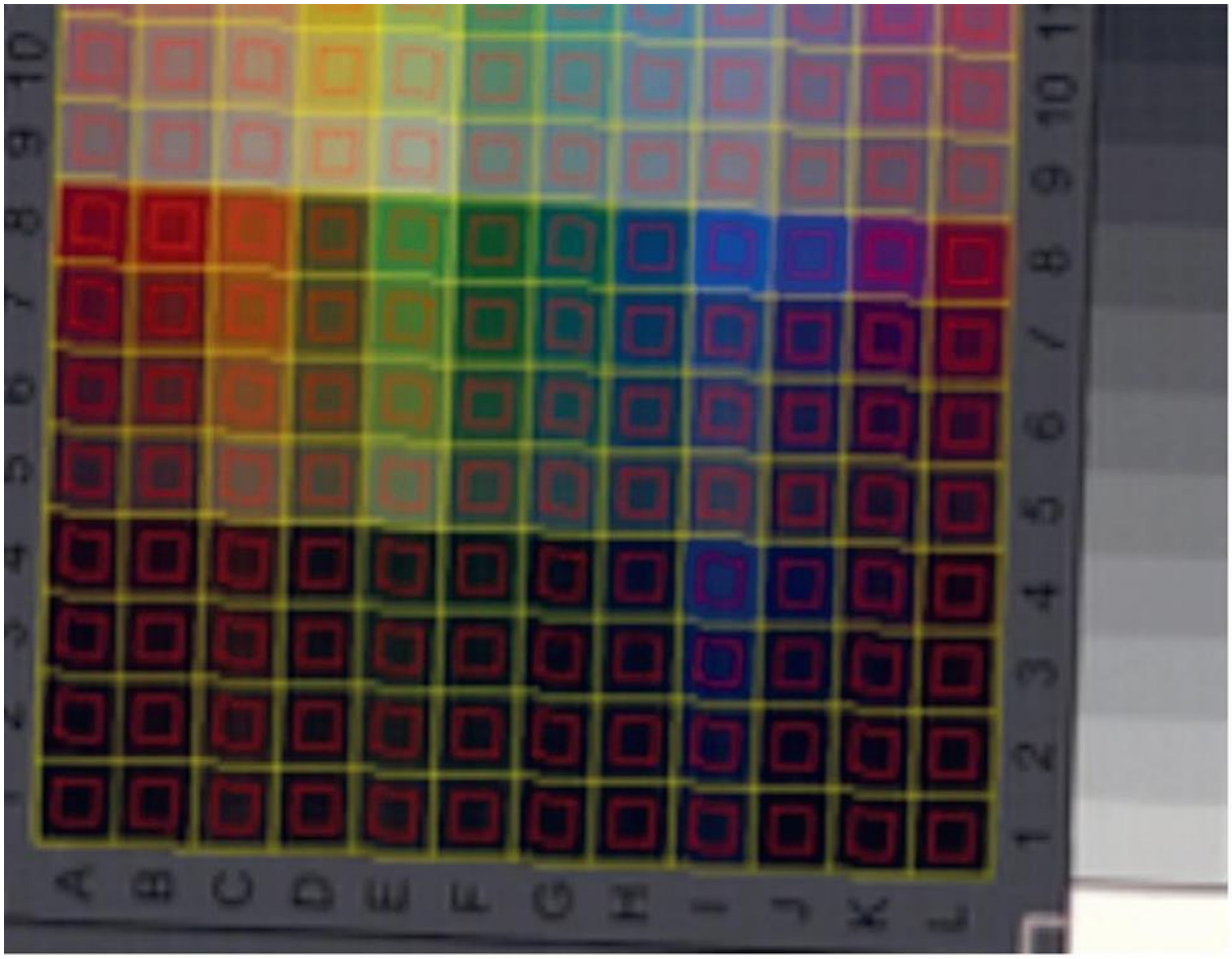

Figure 5 Red squares mark the ranges for CIEDE2000. (see Figure 4).

For judgment, the color differences are calculated according to CIEDE2000 (see equations 1 and 2). This is done after previous registration of the target, based on the mean value of all pixels of the interior 50% of the respective color tile (see Figure 5).

A2 Explanations to the DICOM standard

The DICOM standard includes not only the image formats, i.e. how an image is displayed in its digital form; in addition, it includes the message formats which are mandatory for its creation, archiving, query and distribution.

DICOM starts with the client's work list (usually the pathological information system) which is addressed to the image modality (e.g. a slide scanner), and which ensures the identity of image and patient.

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It continues with potential searches (queries) of image display systems (Viewer, herein the Virtual Microscope), which retrieve the necessary information from the archive (e.g. PACS). In addition to these basic functions (services),

DICOM also offers a wide range of optional additional definitions such as additional image content (annotations, overlays, etc.), structured reports (SR) or the management of requirements (MPPS).

The DICOM standard has been from the beginning developed for practical use. Therefore, it is possible to integrate other standards, rather than reinvent them. These are predominantly compressive image formats and protocols for request-related image distribution (streaming). However, this is only possible if these formats and protocols are open to be used by everybody, e.g., "royalty-free".

Nobody can claim a right in any form for the any use of DICOM, i.e., its use is of limited costs and claim-free. The open or completely free implementation of DICOM is a recipe for the success and stabilization of the standard in the past 20 years. Access rights and access to the stored data are included in the medicolegal aspects, which is important in particular for large archives.

The operator fulfils his duty of care to the patient by maintaining his own independence from established systems only if a patent-free, vendor-independent and sufficiently fast access to the entire dataset is guaranteed.

However, this aspect has been disregarded so far by the Supplement 145 in the integration of WSIs (Whole Slide Images) in the DICOM standard.

The storage of digital slides is integrated into the DICOM standard in a way that the application of free formats becomes unattractive. In addition, it later turned out that one of the involved companies possesses a patent on the defined mechanisms. This fact violates clearly the rules of the Standardization Committee; however, a widespread rejection of the DICOM extension by the other manufacturers did not occur.

Although the group in question has now granted a far-reaching assignment of all rights to all users of the DICOM standard, it did so only after a few years of waiting and under the influence of the other committee members. This behavior induced a loss of trust on the part of users and manufacturers, because the legal situation, especially in the US, is now difficult to assess.

This statement does explicitly not apply to the remainder and predominant part of the DICOM standard. Both the workflow services (Modality Worklist, MPPS) and the storage, search and retrieval of other pathological image information (macroscopy, microscope images, etc.) are sufficiently integrated into the standard (DICOM Supplement 122). Only the application of supplement 145 for the integration of WSIs into a DICOM archive represents a technological

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dead-end and a legal risk. It can therefore only be recommended after a substantial modification, being under way.

A3 Statements for the Validation of Diagnostic Digital Pathology

(Guideline Statements of the College of American Pathologists)

1. (R) All pathology laboratories implementing WSI technology for clinical diagnostic purposes should carry out their own validation studies.

2. (R) Validation should be appropriate for and applicable to the intended clinical use and clinical setting of the application in which WSI will be employed. Validation of WSI systems should involve specimen preparation types relevant to intended use (e.g. formalin-fixed paraffin embedded tissue, frozen tissue, immunohistochemical stains, cytology slides, haematology blood smears). Note: If a new intended use for WSI is contemplated, and this new use differs materially from the previously validated use, a separate validation for the new use should be performed.

3. (R) The validation study should closely emulate the real-world clinical environment in which the technology will be used.

4. (R) The validation study should encompass the entire WSI system. Note: It is not necessary to validate separately each individual component (e.g. computer hardware, monitor, network, scanner) of the system nor the individual steps of the digital imaging process.

5. (E) Revalidation is required whenever a significant change is made to any component of the WSI system.

6. (R) Pathologist(s) adequately trained to use the WSI system must be involved in the validation process.

7. (R) The validation process should include a sample set of at least 60 cases for one application (e.g., H & E stained sections of fixed tissue, frozen sections, cytology, haematology) that reflects the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine practice. Note: The validation process should include another 20 cases for each additional application (e.g., immunohistochemistry, special stains).

8. (S) The validation study should establish diagnostic concordance between digital and glass slides for the same observer (i.e. intra-observer variability).

9. (R) Digital and glass slides can be evaluated in random or non random order (as to which is examined first and second) during the validation process.

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10. (R) A washout period of at least two weeks should occur between viewing digital and glass slides.

11. (E) The validation process should confirm that all of the material present on a glass slide to be scanned is included in the digital image.

12. (E) Documentation should be maintained recording the method, measurements, and final approval of validation for the WSI system to be used in the clinical laboratory.

Guideline Statements of the College of American Pathologists; E – Expert Consensus Opinion, R – Recommendation, S – Suggestion

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Glossary

Image registration: In the context of histology, image registration refers to the determination of a transformation between two or more serial sections of one or more stains, with the aim of parallel visualization at arbitrary locations at any desired magnification.

CDA: Clinical Document Architecture. Standard for the structure of any clinical documents based on HL7 Version 3.

DICOM: Digital Imaging and Communication in Medicine. Standard for the management of digital images and related meta-information, including structured findings.

IHE: Integrating Health Care Enterprises. Most prominent international profile-developing organization in the medical field.

IT8: Summary of ANSI Color Control Standards. According to these standards, scanners, cameras, monitors etc. are calibrated to ensure color accuracy.

IT8.7 / 1 target: Specific target for color calibration. It is provided as glass slide to check the color calibration of slide scanners.

PACS: Picture Archiving and Communication System. Term derived from digital radiology to describe an archive and communication system for digital image objects.

Patho-LIS: Derived from LIS - Laboratory Information System for Pathology Information System.

Label image: When scanning a glass slide, usually two images are created, the label image containing the label of the slide and the actual virtual slide. The label image may also exist as part of an overview image of the entire slide.

Slide scanner: Device for scanning stained histological or cytological glass slides at conventional resolution comparable to conventional transmission of fluorescent microscopy.

Virtual Microscope: Software used to visualize WSI on a computer monitor.

Virtual Microscopy: Visualization of microscopic images which are digitally available.

Virtual Section: Two- or three-dimensional pixel object of a microscopic image of a stained histological or cytological specimen.

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WSI: Whole Slide Image. Corresponds to a completely digitized glass slide (virtual cut).