Review Article

Quantification of virtual slides: Approaches to analysis of content-based image information

Klaus Kayser

UICC-TPCC, Institute of Pathology, Charite, Charite Platz, D-10118 Berlin, Germany

E-mail: *Klaus Kayser - klaus.kayser@charite.de
*Corresponding author

Abstract

Virtual microscopy, which is the diagnostic work on completely digitized histological and cytological slides as well as blood smears, is at the stage to be implemented in routine diagnostic surgical pathology (tissue-based diagnosis) in the near future, once it has been accepted by the US Food and Drug Administration. The principle of content-based image information, its mandatory prerequisites to obtain reproducible and stable image information as well as the different compartments that contribute to image information are described in detail. Automated extraction of content-based image information requires shading correction, constant maximum of grey values, and standardized grey value histograms. The different compartments to evaluate image information include objects, structure, and texture. Identification of objects and derived structure depend on segmentation accuracy and applied procedures; textures contain pixel-based image information only. All together, these image compartments posses the discrimination power to distinguish between object space and background, and, in addition, to reproducibly define regions of interest (ROIs). ROIs are image areas which display the information that is of preferable interest to the viewing pathologist. They contribute to the derived diagnosis to a higher level when compared with other image areas. The implementation of content-based image information algorithms to be applied for predictive tissue-based diagnoses is described in detail.

Key words: Content-based image information, image standardization, object, predictive diagnosis, structure, texture

INTRODUCTION

Virtual slide technology is already leaving its childhood. The velocity of scanning a whole glass slide reaches now in less than one minute with a sufficient number of focus points, and will probably further decrease with the new scanner generation.[1,2] In addition to its main applications which are research (such as tissue micro arrays (TMA), interdisciplinary case discussion as well as education in anatomy and pathology, the aims listed below are already under consideration: digital storage of routine cases, replacement of the conventional microscope by virtual microscopy, adjuvant automated measurements of stains and *in situ* hybridization (immunohistochemistry; IHC, fluorescent *in situ* hybridization (FISH), necessary for predictive pathological diagnosis), as well as the development of so-called diagnosis assistants to further support the pathologist in his/her daily work.[3−6]

In addition to precise, robust, and standardized hardware

(scanners and their components such as optics, inbuilt cameras, image standards, and display stations), the software must communicate with databank systems and fulfill image-specific properties.[7–10] Unfortunately, generally accepted standards do not exist neither for the hardware, nor for display of colored images or monitors. Databank handling mainly addresses problems and standardization of storage, velocity, and retrieval of patients' data, whereas, image-specific properties address features such as brightness, illumination, focus, and image size.[11–13] The personal adjustment or general standardization of these parameters is mandatory for human–machine interaction, that is, a pathologist who wants to derive a diagnosis from an electronically presented histological image. This standardization is, however, not sufficient to investigate in analysis and potential-automated quantification and extraction of any image information.[14,15]

What is the information of a histological image? How can it be described and measured? Finally, what are the basic algorithms to proceed to an automated information extraction? Or does this approach belong to the set of questions that cannot be answered in principle?

Basic Considerations

Image information in surgical pathology (or tissue-based diagnosis) is the morphology of a disease that can be "read by a trained person (pathologist)". According to this definition, it can be defined as "the contribution of a microscopic image to the pathologist's diagnosis" (or to limit the pathologist's "freedom" to state any diagnosis). Obviously, not every image possesses information according to this definition. In addition, there are images which display "a strong association with a certain disease", or allow an "easy" detection of information, others "might be related with a broad variety of diseases", and it is difficult to derive a diagnosis. Commonly, an image is called to be of "good quality", if its presented information can be "easily" derived.[14–16] On the other hand, those images are of "poor quality" if it is difficult (or even impossible) to detect a correlation with a diagnosis.[17] Thus, image quality is a main feature that influences the detection of image information. It is usually evaluated by comparing a derived image with the original one [Figure 1].

The standardization of image quality is necessary for any reproducible measurement or interpretation, and the first step if we want to develop a quantitative method on image information. Having images of good quality, we could then try to look for image properties that are related to information. In any image these properties are color features in relation to their image position, and include objects, structures, and texture.[15,18,19] The principle is exemplarily explained in Figure 2. Objects are items that can be identified (interpreted) and "directly
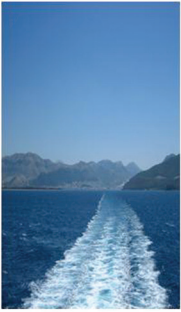

Figure 1: Survey of basic image quality measurements - Machine-oriented methods commonly deal with signal/noise ratio and compare image copies with the original one, whereas, subjective methods try to evaluate the influence of certain image parameters (brightness, contrast, etc.) on the interpretation of the viewer. In interactive virtual microscopy, image parameters can be modified according to the individual "taste" of the viewing pathologist. In automated application, an original image that serves for virtual slides derived from glass slides of different laboratories does not exist and has to be computed by normalization of grey value range and distribution
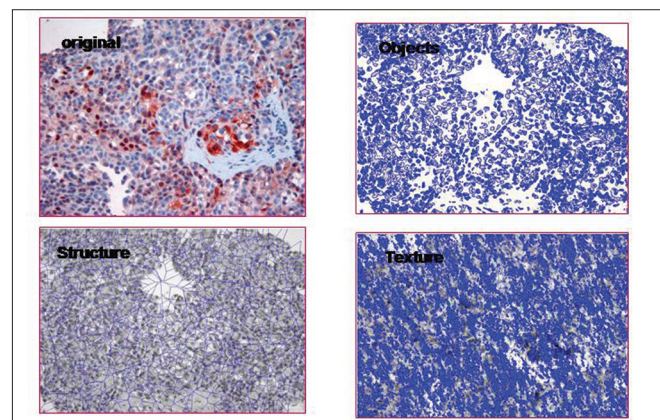

Figure 2: Illustration of an original image (epithelioid mesothelioma, immunohistochemically stained with Calretinin – a positive marker for mesothelioma), segmented objects, derived structure, and image texture (computed with an autoregressive algorithm)

associated" with a meaning, for example, trees, animals, buildings, or biological meaningful units such as vessels, cells, nuclei, chromosomes, genes, membranes, etc. If they are not known to the pathologist (i.e. cannot be associated with a diagnosis), they can only be described (or measured). Often these objects are multiple in an image, and their spatial relationship can be analyzed which are called structures.[15,20] Naturally, structures can be "condensed" to a new object, if they aggregate and display in translational symmetries such as rings, tubes, lines, etc.[21–24] The described classic (commonly applied by pathologists) "information" approach correlates image features that are mainly "detected" objects and structures, with external information. The "association function" is the knowledge of the viewing pathologist. In other words, an object has to be known by the viewer in order to be

identified.[25] It is the implementation of the well-known sentence: "*You only do see what you see, i.e., what you have seen and identified previously, and, therefore, you do already know*" (<Man erblickt nur, was man schon weiß und versteht>, in a letter of J.W. Von Goethe to F. V. Müller, dated 24.4.1819).

A different approach is the analysis of the basic image elements which are the pixels (or voxels) and their individual grey values.[14,15,19] The result of pixel-based image analysis is called image texture.[15] It is crudely defined, and includes terms like *Coarseness, Contrast, Directionality, Line-likeness, Regularity, and Roughness.*[26] Voss[27] calculated image texture by an autoregressive algorithm which results in reproducible and stable extraction of image information.[28,29] The prerequisite for a reproducible and stable automated application of any of the mentioned algorithms is an image that has been normalized in order to minimize the influence of different laboratories (fixation, tissue processing, staining intensity, etc.).

Image Quality and Standardization

It is not a simple task to describe image quality, although every pathologist intuitively can judge the quality of a histological image. In the literature, two different terms of image quality are distinguished: a) that of individual (emotional) judgment and b) that of parameter-based measurements.[3,13,16,25,30,31] In addition, the influence of the image display (monitor) plays an important role and should be discussed separately. Individual judgment of histological virtual slides has been performed in relation to image compression, to brightness, focus, and field of view.[25,32] An image compression of 1:20 (in jpg compression technique) has been considered not to affect the pathologist's ability to state a diagnosis.[13,14,30,33]

Image presentation on a monitor should take into account Nyquist's theorem (relationship between color wavelength and the resolution of the human eye) as well as the natural field of view which is the angle of overlapping fields of view of the two human eyes).[13,31,34] The computation yields a maximum display of about 25 cm × 35 cm (height × length) on a monitor at a distance of 1 m to the observer.[25,34] To evaluate a diagnosis from larger fields of view takes more time and is more tiring, that is, one has to move the eyes or head, smaller ones are felt to have lost information.[25,34]

The color display of virtual slides is of additional significance if a diagnosis should be evaluated, although images derived from histological glass slides do not correspond to an original, that is, natural image.[25,35] Therefore, a standardized glass slide with several main colors has been developed.[36,37] Investigations of color measurements between the standard glass slide and the acquired virtual slides revealed striking differences in color presentation between scanners of different

companies and even between different scanners of the same company. An analysis of a "standard image" derived from a color standardized hematoxylin/eosin (H&E) stained glass slide could allow an adjustment of display in order to correct electronically the variation in color, etc., of the glass slides. Such a still missing standardized virtual H and E slide could allow corrections in virtual microscopy prior to being viewed by a pathologist.

For other than human–machine interaction purposes such as automated feature extraction, etc., virtual slides have to be spatially standardized for background grey values (vignetting), the range of grey values in each color space, and for the normalization of the grey value histogram (having approximately the same grey values in the same number of pixels).[15,25,35] These image corrections should be done for the original virtual slide. It is also useful to perform the corrections after a gradient transformation on the original image (differentiated image). The work on a differentiated image is an appropriate technique to search for membranes or other objects automatically.[25,38] An example on how image standardization affects segmentation and grey value distribution is shown in [Figure 3].

The average grey value distance between the standardized and the original image can serve as a measure of quality of the original image.[14] Kayser *et al*, have shown that the measured corrections are smaller in fluorescent images compared to H&E stained or IHC (diaminobenzidine; DAB, alkaline phosphatase; AP) stained images.[15] The documentation of the measured aberrations allows the construction of so-called running curves.[35] These can be used to evaluate or control the constancy or potential dynamics of image quality [Figure 4]. An internet assessable automated IHC, FISH, nuclear organizer regions (AGNoR), and Comet FISH measurement system (EAMUS™, www.Diagnomx.eu) has already implemented the described quality evaluation algorithms.[35]

Having assessed the basic image quality, the next steps of image quality control depend upon the aims of the virtual microscopy system. Most of the systems are constructed to segment and identify objects such as nuclei or membranes. The segmentation of objects only makes sense if object features can be derived from the detected objects. These include at the basic level area, mean grey value content, grey value distribution within the object, and the form factor.[25] Additional features such as moments, entropy, etc., are derivatives from the basic features.[25] Thus, the amount of pixels that present an object is closely related to the statistical accuracy to measure the basic and derived features. The magnification of a virtual slide should be relative to the size of the objects to be measured. An object should cover about 1000 pixels in order to allow a feature extraction with an accuracy of 3%.[17]

All object segmentation algorithms are based upon grey value thresholds. The detection of maxima and minima in the grey value histogram is, therefore, an indicator of the accuracy to potentially segment objects.[17] In addition to the normalization of the grey value histogram, the number and significance of grey value maxima and minima are characteristics that describe suitable potential thresholds to separate an object area from the background.[2,14,17,18,38–40] The grey value difference between a maximum and the associated minimum should amount to at least 5% of the histogram peak, if reproducible object segmentation is aimed.[20,25,40,41] An example of evaluated potential thresholds in a standardized histological image (epithelioid mesothelioma) together with the summary of object segmentation prerequisites is depicted in [Figure 5].

An additional important, however not frequently implemented, image information parameter is the spatial arrangement of objects, which is called image structure.[23,25,29,42] The majority of automated (or semi-automated) image analysis systems do not analyze structures, although the spatial arrangement of objects such as cancer cells is of great biological significance. It reflects, for example, to the approved grading of cancer cell types.[43–45] A reproducible measurement of an image structure requires a) reproducible identification of objects, b) a sufficient number of objects, and c) a reproducible (and spatial independent) definition of the applied neighborhood condition.[25]

Independent from the applied neighborhood condition, at least 100 objects should serve to construct an image structure.[15] If substructures (clusters) should be detected, the minimum number of image objects should amount to 1000 or even greater. Virtual slides can meet these conditions as they allow investigations of different magnifications of the same selected tissue area. Voronoi's neighborhood condition (and the derived Dirichlet's tessellation) is the most frequently used one.[25,27,34,46] The application of graph theory and the construction of the minimum spanning tree (with or without distance limitations), or O'Callaghan's approach are also useful, especially in detection of clusters within the obtained structure.[28,43,47]

Extraction of Image Information

Potential histological image features that "contain" all image information are the described texture, objects, and structure. The viewing pathologist associates a certain "meaning" with the image information, which can be called the "reaction" of the pathologist. In mathematical terms, the pathologist's meaning is a set of discrete items, such as diagnosis and differential diagnosis. The items are overlapping, if the pathologist is "only" able to derive a differential diagnosis. The maximum of the mapping procedure is directly related to the final or definite
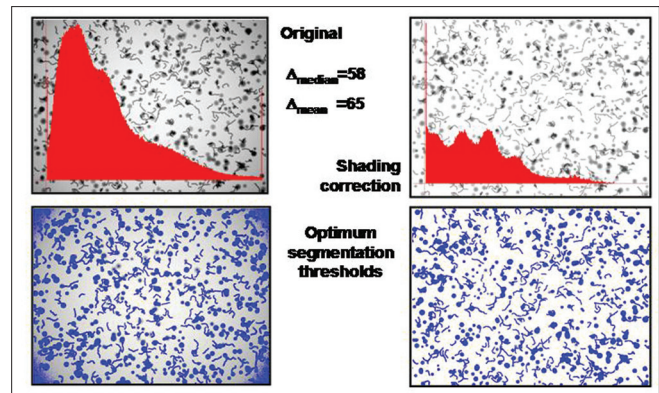

Figure 3: Example of influence of image standardization (shading correction) on the derived grey value histogram and accuracy of object segmentation (artificial image consisting of randomly distributed balls and fibers)
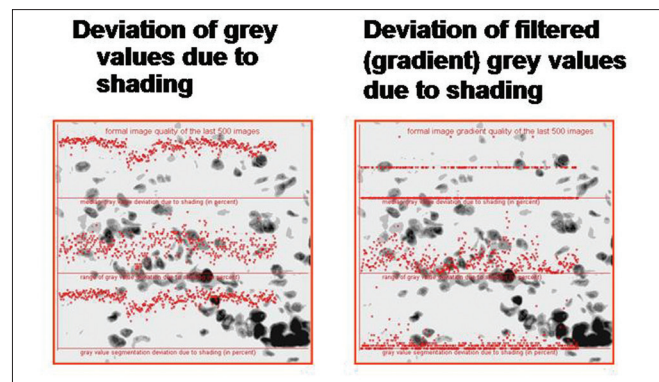

Figure 4: Example of monitoring formal image quality (mean grey value distance of the original image from the "corrected" one)
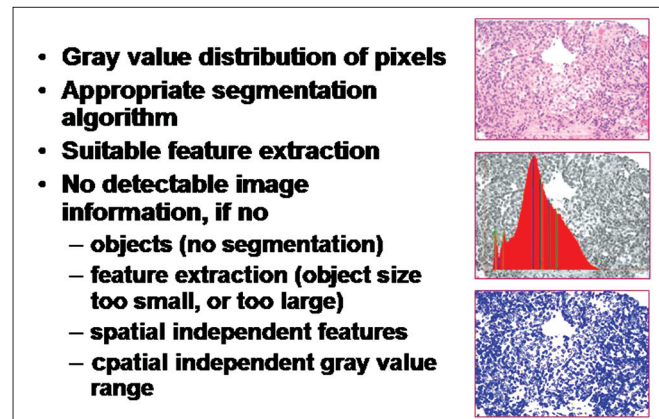
Figure 5: Illustration of grey value histogram, derived segmentation thresholds, and segmented objects (epithelioid mesothelioma, H and E, same case as displayed in Figure 2)

diagnosis.[19,20,25,38] In a next step, the different contributors to the set of diagnosis (texture, object, and structure) can be separately analyzed. A consideration of the functions' extremes reveals: a) a detectable texture is a prerequisite to segment objects, and b) segmented objects are a prerequisite to measure the built structure.[19,20,25,38]

To furthermore tune the algorithm, a virtual slide can

be spatially analyzed for regions which display with no or only a few textures, and consequently do not contain objects and structures. These areas are called background. Obviously, they do not contribute to image information. Thus, the image can be divided into two non-overlapping areas, namely, the object space and the background. The same procedure can then be applied for the object space only, and sub-areas displaying with the "maximum of textures, objects, or structures" can be separated.[15,18,19,25] These areas are called "busy" and correspond to ROI. They can be reproducible identified by measures of the texture entropy and structural entropy based upon segmented objects [Figure 6],[38] as well as by application of spectral analysis,[48] or by mimicking the pathologist's pathway through a histological image.[49,50]

Applications

The traditional histological diagnosis which comprises a reproducible and detailed morphological description of the underlying disease has been extended by the terms "predictive diagnosis" and "risk-associated diagnosis".[51,52] Risk-associated diagnosis is related to the individual risk of developing a certain disease (for example, breast cancer associated with the BRCA1/2 gene expression), whereas, predictive diagnosis guides the individual patient's therapy by analyzing the expression of certain genes in association with that of associated cellular or nuclear receptors.[53] Both diagnoses require precise and reproducible measurements or morphology-associated object features,[54,55] either in the complete virtual slide or in carefully selected ROIs.

Algorithms to reproducibly measure the ROIs and to verify the correct selection have been successfully tested.[48,49] Experienced pathologists have been asked to state a diagnosis viewing the ROIs only, and, in addition, independently state the diagnosis by viewing the whole image. Concordant diagnoses have been obtained in 97−100% of the cases. ROIs are, in addition, useful to select the biological important areas for TMA, which is another technique to invest in predictive diagnosis or so-called individual cancer therapy.[56]

The described issues are the main promoters of virtual microscopy. Its present stage can be described as follows:

Commercially available scanners to acquire virtual slides and to perform virtual microscopy are in use for image storage and for educational purposes for several years.[25] Although they have not been approved by the FDA to our knowledge, they have been remarkably matured, and are currently already equipped with software that addresses to reproducible measurements of image objects, especially for application in predictive diagnosis.[19,53]

The implementation of structure analysis is still in its childhood as well as the analysis of textures.[18] Image standardization focuses on the implementation of medical
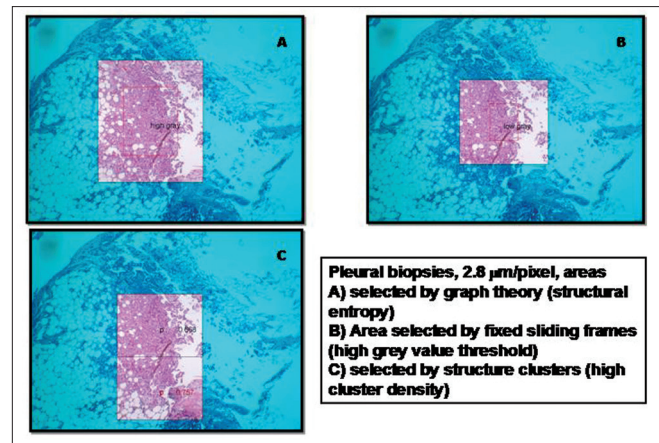

Figure 6: Demonstration of automated selection of ROI in a pleural biopsy (epitheloid mesothelioma, H & E). The accuracy of the tested algorithm (2227 slides, biopsies obtained from colon, lung, pleura, and stomach) revealed >97%)

standards such as (digital imaging and communications in medicine, DICOM3), and pathology-related changes of picture archiving and communication systems (PACS). The necessary working groups have announced a release of a pathology addressed PACS in the near future.[18]

Investigations on additional standardization of virtual slides to be applied for automated object segmentation and derived image information are in the stage of individual tests at present.[50]

CONCLUSION

Diagnostic surgical pathology or tissue-based diagnosis is changing its face as it combines the latest progress and knowledge of molecular biology and genetics with innovative development of computerized information technology.[18,19] Images of cellular morphology are still its basis. They serve, however, only as starting point to invest in biological properties and gene expressions of individual patient's disease, especially cancer.[15,25] Tissue-based diagnosis is now-a-days the trusted guide in the treatment of a broad variety of cancer such as breast, colon, or lung cancer.[25] Quantification of content-based image information is the contribution of information technology to furthermore develop and expand the combination of alteration, expression, and disease-associated impact of genes and their interaction.[19]

The analysis of this information is based upon three main components only, namely image texture, objects, and structure.[15,38] The most interesting component is the structure, which probably frequently precedes gene expressions. It could, therefore, serve for reliable forecasting of disease development.[38]

A precise definition of a suitable neighborhood condition that includes functional properties and the evaluation of "rare events", which act only as catalytic converters are

still missing to our knowledge. Therefore, the described, already implemented tools of tissue-based diagnosis and content-based image information analysis can be considered as now-a-days detected entrance into a new world of disease understanding and treatment.

As a result, diagnostic surgical pathology is getting more responsibility and closer to the treatment and fate of patients, because it is incorporating recently developed biology and IT technology.

REFERENCES

1. Grabe N, Lahrmann B, Pommerencke T, von Knebel Doeberitz M, Reuschenbach M, Wentzensen N. A virtual microscopy system to scan, evaluate and archive biomarker enhanced cervical cytology slides. Cell Oncol 2010;32:109-19.

2. Molnar B, Berczi L, Diczhazy C, Tagscherer A, Varga SV, Szende B, et al. Digital slide and virtual microscopy based routine and telepathology evaluation of routine gastrointestinal biopsy specimens. J Clin Pathol 2003;56:433-8.

3. Mori I, Nunobiki O, Ozaki T, Taniguchi E, Kakudo K. Issues for application of virtual microscopy to cytoscreening, perspectives based on questionnaire to Japanese cytotechnologists. Diagn Pathol 2008;3:S15.

4. Rocha R, Vassallo J, Soares F, Miller K, Gobbi H. Digital slides: Present status of a tool for consultation, teaching, and quality control in pathology. Pathol Res Pract 2009;205:735-41.

5. Słodkowska J, Pankowski J, Siemiatkowska K, Chyczewski L. Use of the virtual slide and the dynamic real-time telepathology systems for a consultation and the frozen section intra-operative diagnosis in thoracic/pulmonary pathology. Folia Histochem Cytobiol 2009;47:679-84.

6. Gongora Jara H, Barcelo HA. Telepathology and continuous education: Important tools for pathologists of developing countries. Diagn Pathol 2008;3:S24.

7. Chen YK, Hsue SS, Lin DC, Wang WC, Chen JY, Lin CC, Lin LM. An application of virtual microscopy in the teaching of an oral and maxillofacial pathology laboratory course. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:342-7.

8. Helin HO, Lundin ME, Laakso M, Lundin J, Helin HJ, Isola J. 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;175:495-9.

9. Sims MH, Mendis-Handagama C, Moore RN. Virtual microscopy in a veterinary curriculum. J Vet Med Educ 2007;34:416-22.

10. Słodkowska J, Chyczewski L, Wojciechowski M. Virtual slides: Application in pulmonary pathology consultations. Folia Histochem Cytobiol 2008;46:121-4.

11. Albin S, Rougeron G, Péroche B, Trémeau A. Quality image metrics for synthetic images based on perceptual color differences. IEEE Trans Image Process 2002;11:961-71.

12. Doolittle MH, Doolittle KW, Winkelman Z, Weinberg DS. Color images in telepathology: How many colors do we need? Hum Pathol 1997;28:36-41.

13. Evered A, Dudding N. Accuracy and perceptions of virtual microscopy compared with glass slide microscopy in cervical cytology. Cytopathology 2010.[In Press].

14. Kayser K, Görtler J, Metze K, Goldmann T, Vollmer E, Mireskandari M, et al. How to measure image quality in tissue-based diagnosis (diagnostic surgical pathology). Diagn Pathol 2008;3:S11.

15. Kayser K, Hoshang SA, Metze K, Goldmann T, Vollmer E, Radziszowski D, et al. Texture- and object-related automated information analysis in histological still images of various organs. Anal Quant Cytol Histol 2008;30:323-35.

16. Nascimento SM, Foster DH, Amano K. Psychophysical estimates of the number of spectral-reflectance basis functions needed to reproduce natural scenes. J Opt Soc Am A Opt Image Sci Vis 2005 ;22:1017-22.

17. Kayser G, Görtler J, Kluge N, Wiech T, Werner M, Kayser K. Standards in virtual microscopy: From tissue processing to image acquisition and visualization. Diagn Pathol 2010;5:S10.

18. Kayser K, Borkenfeld S, Görtler J, Kayser G. Image standardization in tissue - based diagnosis. Diagn Pathol 2010;5:S13.

19. Kayser K, Görtler J, Bogovac M, Bogovac A, Goldmann T, Vollmer E, et al. AI (artificial intelligence) in histopathology--from image analysis to automated diagnosis. Folia Histochem Cytobiol 2009;47:355-61.

20. Kayser K, Schultz H, Goldmann T, Görtler J, Kayser G, Vollmer E, et al. Theory of sampling and its application in tissue based diagnosis. Diagn Pathol 2009;4:6.

21. Kayser K, Schlegel W. Pattern recognition in histo-pathology: Basic considerations. Methods Inf Med 1982;21:15-22.

22. Kayser K, Shaver M, Modlinger F, Postl K, Moyers JJ. Neighborhood analysis of low magnification structures (glands) in healthy, adenomatous, and carcinomatous colon mucosa. Pathol Res Pract 1986;181:153-8.

23. Kayser K, Stute H. Minimum spanning tree, Voronoi's tesselation and Johnson-Mehl diagrams in human lung carcinoma. Pathol Res Pract 1989;185:729-34.

24. Kayser KK, Modlinger F, Postl K. Quantitative low-resolution analysis of colon mucosa. Anal Quant Cytol Histol 1985;7:205-12.

25. Kayser K, Molnar B, Weinstein RS. Virtual Microscopy – Fundamentals – Applications – Perspectives of Electronic Tissue - based Diagnosis. Berlin: VSV Interdisciplinary Medical Publishing; 2006.

26. Tamura H, More S, Yamawaki T. Texture features corresponding to visual perception. IEEE Transactions on Systems, Man, and Cybernetics (SMC) 1978;8:460-73.

27. Voss K, aüße H. Praktische Bildverarbeitung. München, Wien: Carl Hanser Verlag; 1991.

28. Kayser K, Gabius HJ. Graph theory and the entropy concept in histochemistry. Theoretical considerations, application in histopathology and the combination with receptor-specific approaches. Prog Histochem Cytochem 1997;32:1-106.

29. Kayser K, Kiefer B, Burkhardt HU. Syntactic structure analysis of bronchus carcinomas - first results. Acta Stereol 1985;482:249-53.

30. Bertulis A, Glezer V. Colour-spatial vision. Int J Psychophysiol 1984;2:147-65.

31. Bubka A, Bonato F. Natural visual-field features enhance vection. Perception 2010;39:627-35.

32. Kayser K, Szymas J, Weinstein RS. Telepathology and Telemedicine - Communication, Electronic Education and Publication in e-Health. Berlin: Veterinärspiegel Verlag; 2005.

33. Ionita MC, Corcoran P, Buzuloiu V. On color texture normalization for active appearance models. IEEE Trans Image Process 2009;18:1372-8.

34. Leong FJ, McGee JO. Automated complete slide digitization: A medium for simultaneous viewing by multiple pathologists. J Pathol 2001;195:508-14.

35. Kayser K, Radziszowski D, Bzdyl P, Sommer R, Kayser G. 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;1:10.

36. Weinstein RS, Graham AR, Richter LC, Barker GP, Krupinski EA, Lopez AM, et al. Overview of telepathology, virtual microscopy, and whole slide imaging: Prospects for the future. Hum Pathol 2009; 40:1057-69.

37. Yagi Y, Gilbertson J. The importance of optical optimization in whole slide imaging (WSI) and digital pathology imaging. Diagn Pathol 2008;3:S1.

38. Kayser K, Kayser G. Virtual microscopy and automated diagnosis, in virtual microscopy and virtual slides in teaching. In: Gu J, Ogilvie R, editors. Diagnosis and research. Boca Raton: Taylor and Francis; 2005.

39. Lian NX, Zagorodnov V, Tan YP. Edge-preserving image denoising via optimal color space projection. IEEE Trans Image Process 2006;15:2575-87.

40. Nedzved A, Ablameyko S, Pitas I. Morphological Segmentation of Histology Cell Images. 15th International Conference on Pattern Recognition (ICPR'00). 2000;1:1500.

41. Nock R, Nielsen F. Statistical region merging. IEEE Trans Pattern Anal Mach Intell 2004;26:1452-8.

42. Kayser K, Stute H, Tacke M. Minimum spanning tree, integrated optical density and lymph node metastasis in bronchial carcinoma. Anal Cell Pathol 1993;5:225-34.

43. Kayser K, Hoeft D, Hufnagl P, Caselitz J, Zick Y, André S, et al. Combined analysis of tumor growth pattern and expression of endogenous lectins as a prognostic tool in primary testicular cancer and its lung metastases. Histol Histopathol 2003;18:771-9.

44. Kayser K, Kayser G, Eichhorn S, Biechele U, Altiner M, Kaltner H, 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;20:313-20.

45. Zink S, Kayser G, Gabius HJ, Kayser K. Survival, disease-free interval, and

associated tumor features in patients with colon/rectal carcinomas and their resected intra-pulmonary metastases. Eur J Cardiothorac Surg 2001;19:908-13.

46. Voronoi G. Nouvelles applications des paramêtres continus à la théorie des formes quadratiques, dexièmes memoire: Recherches sur les parallèloedres primitifs. J Reine Angew Math 1902;134:188-287.

47. Kayser K. Application of structural pattern recognition in histopathology, in Syntactic and structural pattern recognition. In: Ferraté TP, Sanfeliu A, Bunke H, editors. Ney York: Springer: Berlin Heidelberg New York; 1988. p. 115-35.

48. Oger M, Belhomme P, Klossa J, Michels JJ, Elmoataz A. Automated region of interest retrieval and classification using spectral analysis. Diagn Pathol 2008;3:S17.

49. Schrader T, Niepage S, Leuthold T, Saeger K, Schluns K, Hufnagl P, et al. The diagnostic path, a useful visualisation tool in virtual microscopy. Diagn Pathol 2006;1:40.

50. Wienert S, Beil M, Saeger K, Hufnagl P, Schrader T. Integration and acceleration of virtual microscopy as the key to successful implementation into the routine diagnostic process. Diagn Pathol 2009;4:3.

51. van der Groep P, van Diest PJ, Menko FH, Bart J, de Vries EG, van der Wall E. Molecular profile of ductal carcinoma in situ of the breast in BRCA1 and BRCA2 germline mutation carriers. J Clin Pathol 2009;62:926-30.

52. Mandrekar SJ, Sargent DJ. Clinical trial designs for predictive biomarker validation: One size does not fit all. J Biopharm Stat 2009;19:530-42.

53. Stevens E, Kimler BF, Davis MK, Fan F, Thomas P, Wang XY, et al. A newly proposed semi-automated method of grading invasive lobular carcinoma: A unifying concept and correlation with prognostic markers and patient survival. Ann Clin Lab Sci 2009;39:25-31.

54. Tamimi RM, Baer HJ, Marotti J, Galan M, Galaburda L, Fu Y, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. Breast Cancer Res 2008;10:R67.

55. Kayser G, Gerlach U, Walch A, Nitschke R, Haxelmans S, Kayser K, et al. Numerical and structural centrosome aberrations are an early and stable event in the adenoma-carcinoma sequence of colorectal carcinomas. Virchows Arch 2005;447:61-5.

56. Marchiò C, Iravani M, Natrajan R, Lambros MB, Savage K, Tamber N, et al. Genomic and immunophenotypical characterization of pure micropapillary carcinomas of the breast. J Pathol 2008;215:398-410.