International Journal of Emerging Trends in Health Sciences

Volume 01, Issue 2, (2017) 49-55

www.ijeths.eu

Advances in digital pathology

Evgin Goceri*, Engineering Faculty, Akdeniz University, Antalya, 07058, Turkey.

Suggested Citation:
Goceri, E. (2017). Advances in digital pathology. *International Journal of Emerging Trends in Health Sciences*. *1*(2),49-55

Received August 15, 2017; revised October 19, 2017; accepted November 5, 2017.
Selection and peer review under responsibility of Prof. Dr. Nilgün Sarp, Uskudar University, Istanbul, Turkey.
©2017 Academic World Education & Research Center. All rights reserved.

Abstract

Characterization of cancer diseases and preparation of diagnostic reports after analyzing tissue specimens and several cell samples are provided by pathologists. One of the most successful strategies in pathology is to divide tumors into different subtypes and to adapt the treatment for each tumor. However, this approach has put a big burden on pathologists, who are reviewing tissue samples under the light of the microscope. Because, tumors have about 200 subtypes and pathologies are facing a growing demand for accurate and fast diagnosis and also patient safety. Therefore, digital pathology has been important and growing rapidly. Advances in computer technology such as computing power, faster networks and cheaper storage have enabled pathologists to manage images more easily than in the last decade. Novel pathology tools have a potential for automated and faster diagnosis and also better management of data. Moreover, it enables re-reducibility, validation of results, quality assurance and sharing of new ideas at anywhere and anytime. Advances in digital pathology have been reviewed in this paper. It seems that innovations in technologies will not only provide important improvements in pathology service, but also they will change healthcare and research fundamentally despite some challenges.

Keywords: Cell detection, computer assisted diagnosis, digital pathology, image analysis, nuclei segmentation, tissue classification.

* ADDRESS FOR CORRESPONDENCE: **Evgin Goceri,** Engineering Faculty, Akdeniz University, Antalya, 07058, Turkey
E-mail address: evgin@akdeniz.edu.tr / Tel.: +0-90-242-310-4393

1. Introduction

Pathology laboratories and pathologists play a vital role in cancer care. Characterization of cancer diseases and preparation of diagnostic reports after analysing tissue specimens and several cell samples are provided by them. Oncologists make their decision on treatment alternatives for patients (e.g., chemo-therapy, radiation therapy or immune targeted therapy) according to the information in these reports.

In pathology, one of the most successful strategies is to divide tumors into different subtypes and to adapt the treatment for each tumor. Currently, tumors are categorized into about 200 subtypes. However, this approach has put a big burden on pathologists, who are reviewing tissue samples under the light of the microscope. Patients have to wait for diagnosis, which delays their treatment and leads to unnecessary worries. However, this is changing since digital pathology has been growing due to the advances in software and computer technologies and also usage of whole slide scanners. Nowadays, digital scanners are able to create gigapixel-sized digital images of the tissue in a few minutes. Images can be shown in real time. Cells can be detected and counted automatically. Also, the importance of tissue-based studies to discover a biomarker has increased.

Although there are many challenges in digital pathology, it has important advantages. For instance; 1) It avoids problems caused by glass slide storage, such as loss, breakage, staining attenuation. 2) It reduces time to recover slides from archives and helps research and education. 3) It provides retrieval and reproducibility of images and helps quality control and research. 4) Reduces intra-/inter-observer variability.

The editors in Scientific American review of digital pathology pointed that; 1) there are promising methods, which allow images to be manipulated, 2) integrating of digital images and pathology is overdue, 3) digital pathology will allow more accurate diagnoses [May, 2010].

Advances in digital pathology have been reviewed in this paper and we organized this as follows. An introduction to the diagnostic work of pathologists and description of digital pathology is presented in Section 2. Also, image analysis algorithms and applications implemented to solve problems related with digital pathology are given in this section. The importance of software for pathological applications is explained in Section 3. Challenges and opportunities are explained in Section 4. Finally, conclusions are presented in Section 5.

2. Digital pathology

2.1. Background and visual analysis

In pathology, tissue samples are collected and sent to laboratories for analysis. Glass slides with pieces of the specimens are created and the content of the glass slide is reviewed by a pathologist in the pathology laboratories to write a report.

Tissue specimens arrive at the laboratory in a plastic box filled with formalin. A pathologist or technician performs a grossing examination on specimens. This examination is performed by sketching, measuring or taking photos of specimens. Small pieces of tissues can be cut to use in further analysis. These pieces are further processed by an automatic processing machine and embedded into paraffin blocks. Small sections of tissues are sliced from these paraffin blocks by a microtome, which is able to create micrometer thin sections. After this step, the resulting sections seems as almost transparent. To make clear these tissue structures, the glass slides are stained with different colors. In this stage, many glass slides can be obtained for each specimen. After this process, the pathologist writes a report according to the content of the glass slide, which is reviewed under a microscope (Figure 1).

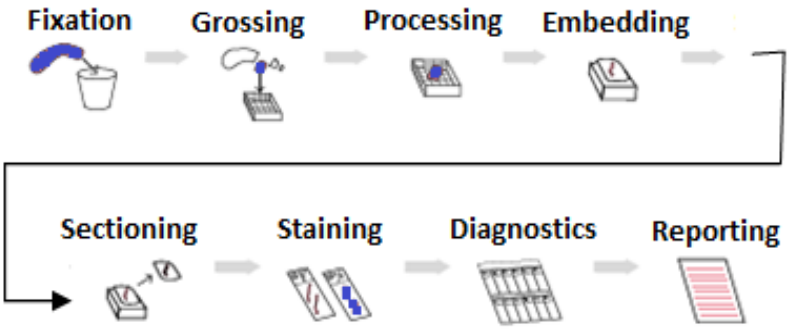

Figure 1. Work-flow in pathology

The image analysis is based on visual features that are associated with different conditions. The pathology's report includes a description of both microscopic, macroscopic findings and a diagnosis. Histological grades (according to texture of the cancer cells and their growth), surgical margins, predictive values, estimates on overall prognosis for a patient's survival can also be written in these reports. Therefore, in pathology, which is a visual science, the quality and interpretation of digital images has vital importance.

In a work (Randell,Randell, Ruddle & Quirke, 2012; Randell, Ruddle & Thomas, 2011), pathologists' review process was analyzed with recorded videos. The authors highlighted the requirements of pathologist' in terms of collection and organization of information generated from different sources in order to collaborate with colleagues by double checking the findings and results on final reports.

The glass slides and the embedded blocks can be stored for future works and follow-up purposes, if patients have given consent. Currently, although the initial processing and staining are performed automatically by machines, the workflow is still performed by a laboratory technician. However, in a digital workflow, the glass slides are scanned in a digital scanner to create digital slides for review by digital workstations.

In pathology, reviewing glass slides is still performed with microscope mainly due to limited storage capacity and the technology used for scanning. Because, the size of an image generated by scanning of a slide is considered as very large. The images are sampled at a pixel density of 0.25 microns per pixel in the maximum scanning magnification. As a result of this, a glass slide (75x25mm) requires an image of 30 gigapixels. This size is 3 orders of magnitude that is bigger than a high-end camera. However, it is possible to obtain an image with a lower size (almost 1 gigabyte per scanned slide) since mostly glass slides include regions without pieces of tissue, and lossy image compression algorithms can be applied. Even though this size is still big, management is possible since storage prices are decreasing.

Whole Slide Imaging (WSI), which is a wide-field microscopy, produces digital slides. Before the usage of WSI, only taking snapshots of small regions had been possible. WSI can create digital images of glass slides with high resolution. WSI files contain thousands of small image parts, in multiple magnifications. Therefore, it provides fast retrieval and enable to show images in any field of view. Multiple magnification levels allow quick zooming and panning similar to the navigation experience of a microscope. Because of this, the software of these scanners has been referred to as virtual microscopes.

WSI is useful in education since 1) it provides sharing images at anywhere and anytime, 2) it provides accessing to a slide to help giving response to "on-the-spot" questions, 3) it involves less preparation time, 4) it helps to prepare teaching materials (i.e., virtual slides) using different cases, which do not disappear or break. Therefore, WSI is being preferred and used increasingly in several

examinations, such as American Board of Pathology. Also, adoptability of WSI in many medical schools has allowed to abandon to use microscopes (Weaker et al., 2009).

2.2. Image analysis in digital pathology

Image processing and analysis has been applied for many years for better interpretation and to increase accuracy. The key advantages come from image analysis in digital pathology are (i) reproducibility, (ii) standardization of measurement, (iii) reduced cost, (iv) improved productivity, (v) automation, and (vi) efficiency in terms of processing time and accuracy.

In the literature, works on pathological image analysis have been reviewed in different aspects. For instance, in (Cataldo, Ficarra & Macii, 2012), the authors have reviewed common stages involved in scoring of IHC slides as well as methods used to score slides. The use of image analysis in a clinical setting has been reviewed in [Hansen et al., 2012]. In another work [Hamilton et al. 2014], usage for biomarkers in image analysis was reviewed. The authors, in (Veta et al. 2014), presented a review on image analysis for breast cancer pathology. Nudei detection and classification methods have also been reviewed in (Irshad, HVeillard & Roux, 2014). Studies on tissue classification can be divided into two broad groups; 1) un-supervised and 2) hand-crafted feature based algorithms. Un-supervised feature based methods (e.g., deep learning) are rely on filter responses obtained from training data and also less intuitive. Hand-crafted feature based methods use attributes in images and have a level on image interpretability. We can group works on image analysis in digital pathology as follows:

1) Nuclei Detection Methods: Immunohistochemistry (IHC) can stain the cytoplasm, nucleus and cell membrane. Nuclei detection and scoring of cell membrane algorithms are mostly applied according to the staining. Nuclei detection from IHC stained images results in accuracy about 80-90%. For instance, deep learning based methods have been applied in (Sirinukunwattana et al., 2016). According to their results, an F1-score of 0.88 has been obtained for epithelial nuclei. In (Qi et al., 2012), different segmentation scores have been compared and F1-score of 0.83 has been found. In the literature, there are also several approaches proposed to estimate the accuracy rate without applying any nuclei detection process as an intermediate stage. For instance, in (Gudlaugsson et al., 2012), the authors proposed a system, which uses well calibrated nudei area ratio. In (Vilppu et al., 2010) pixels belong to epithelial nudei have been detected without segmenting them, and then used the ratio of negative and positive pixels to find the Ki-67 positivity. Although this approach produces an abstract result since it is not directly connected to manual cell counting, it decreases the sensitivity of the algorithm.

2) Mitosis Detection Methods: Mitosis detection are applied for diagnosis of different cancer types. Algorithms for detection and quantification of mitoses from histology images have been evaluated in a recent work (Veta, Diest, Willems, Wang, Madabhushi, Cruz-Roa, Gonzalez & Larsen, 2015). In the MITOS-challenge (Roux et al. 2012), the method that gives the best performance has reached an F1-score with the detection rate of 0.78. In another work represented by the AMIDA13 challenge (Veta et al., 2015), 14 different research groups have been used to evaluate with a common dataset. A convergent approach by combining deep learning features and domain inspired features has been proposed to detect mitoses (Wang et al. 2017). The authors showed that their technique results in superior detection than other hand-crafted or deep learning feature based methods alone.

3) Tumor Heterogeneity and Hotspot Selection Methods: Tumor heterogeneity provides an important contribution to interpretations of pathological images. For example, in breast cancer tissue samples, Her2 heterogeneity may account for the 30% of patients that may not give response or have strength for herceptin therapy. However, visual evaluation of the degree of tumor heterogeneity is a challenging task. Image analysis enables useful tools for quantitative evaluation of spatial heterogeneity in tissue samples for biomarker expressions. The number of nuclei calculated from a small region may change according to the location and size of the selected region (Besusparis et al., 2016). Heterogeneity can be modelled and hotspot selection can be performed once the nuclei have

been detected by a software, e.g. in (Stålhammar et al., 2016). Also, hotspot selection can be made using the staining component, which is much faster than detecting every nucleus individually. The authors in (Laurinavicius, Plancoulaine & Rasmusson, 2016); proposed to use calibrated nucleus detection to investigate other heterogeneity measurements and to model the tumor. According to their results, bimodal positivity is an independent predictor of overall patient survival.

4) Tissue Characterization Methods: Methods mentioned in the previous paragraphs present accuracy as a number of detection. However, these results are not encouraging enough since they present the accuracy in case of pixels are turned into cells or objects. Therefore, more advanced methods based on improvements in image analysis have been proposed. For instance, the authors in (Stålhammar et al. 2016) proposed to create a proliferation score for nuclei detection. A prognostic model (without the steps of cell morphology and glandular structures) using image features has been proposed in (Beck, Sangoi & Leung, 2011). In another work (Laurinavicius et al., 2016), bimodality features have been built from non-perfect nuclei detectors and prognostic information has been added. However, these approaches are semi-automated. Therefore, user interaction is required and it is still not clear whether usability of these methods is sufficient or not.

Also, it should be noted here that the accuracy of all semi- or fully-automated methods mainly depends on the evaluation techniques and the datasets that were used for evaluation.

3. The importance of software

Software is crucial in digital pathology area because digital slides are large (there might be hundreds of digital slides) and mostly an image cannot be loaded into a memory in a computer. Therefore, a software is required to manage (to search, archive, analysis and report) digital resources and to view digital slides without any loading/transferring process for an image. Metadata, which are associated with digital slides, should also be recoded when digital slides are used since it gives important information such as unique identifier, bar code, tissue type, date of scan, biomarker name, date of scan, clinical stage, responses to therapy, scan operator, histological score, treatment, survival, mutation analysis, etc. Therefore, a useful user-interface is required. In (Goode et al. 2013), OpenSlide, which is a widely used software to read and manipulate slides in industry and academy, has been presented. Its user interface allows to handle multiple vendor formats transparently. The authors in (Rojo et al., 2016), evaluated 31 different digital microscopies. Properties of the scanning devices (such as imaging or camera qualities), compression methods, file formats and visualization of digital slides are described. It has been identified that if digital microscopies are used efficiently then they can provide important benefits for works in pathology departments.

4. Challenges and opportunities

Although there are many advances in digital pathology and WSI, their implementation in clinic is still slow. The largest pathology laboratory, which is the first laboratory in pathology, the Lab. for Pathology East Netherlands Foundation, began to 100% digital diagnosis in 2015 (Cheng et al., 2016). Feedbacks of this kind of laboratories will be important and contribute to reduce fears in next years.

Although there have been many improvements in digital pathology and WSI, a guideline for better quality control (from preparation of sample to WSI) are still required. Also, independent principles to evaluate qualities of different softwares are required to make quantification automatically and also to make a uniform evaluation for image analysis (Cheng et al., 2016; Higgins et al., 2015). Other important challenges are universally accepted file formats, data storage capacities and the cost for implementation of a digital pathology workflow.

The digitization of tissue glass slides brings challenges in image computation as well as exciting opportunities. In the near future, computerized analysis of images will be very important for clinical decision making in digital pathology. Also, the tools used for image computation can help pathologists for risk characterization of diseases (Veta et al., 2016; Lewis et al., 2014).

5. Conclusion

Visual assessments are subjective and changes from person to person and time to time. Therefore, the traditional approach in pathology leads to intra-/inter-observer variability. Although, traditional diagnosis in pathology has been regarded as ground truth for many years, mostly accepted that it is no longer sufficient in tissue-based biomarker research works and clinical usage. Thanks to computer assisted methods, which can provide quantitative, fast, accurate, reproducible and objective results.

An important change in pathology is implementation of virtual microscopy, which enables digitization of microscopy slides and presents many opportunities for analysis of digital images. Digitization of images in pathology enables automated diagnosis, improvement of the quality obtained from tissue samples and image analysis.

Different imaging systems and image analysis approaches have been proposed to solve problems in pathology since histological images present a wide variety of visual information. Also, these images are complex due to different stains, type of tissues and magnifications.

In digital pathology, robust and automatic combination of information from multiple slides will be important. With advanced imaging tools and computer assisted diagnosis, digital pathology will provide more accurate, fast, objective and consistent assessments in the coming years. Digital pathology is becoming common since technology is becoming more cost effective. However, more integration with computer systems (i.e. PACS), images and standards (i.e., DICOM) for imaging analysis are still required.

References

Beck, A. H., Sangoi, A. R. & Leung, S. (2011). Systematic analysis of breast cancer morphology uncovers stromal features associated with survival. *Science Translational Medicine, 3,* 108-113.

Besusparis, J., Plancoulaine, B. & Rasmusson, A. (2016). Impact of tissue sampling on accuracy of Ki67 immunohistochemistry evaluation in breast cancer, *Diagnostic Pathology, 11*(1), 82-92.

Cataldo, S. D., Ficarra, E. & Macii, E. (2012). Computer-aided techniques for chromogenic immunohistochemistry: Status and directions. *Computers in Biology and Medicine, 42,* 1012–1025.

Cheng, C. L. & Tan, P. H. (2016). Digital pathology in the diagnostic setting: Beyond technology into best practice and service management. *Journal of Clinical Pathology, 70,* 1-13.

Goode, A., Gilbert, B., Harkes, J., Jukic, D. & Satyanarayanan, M. (2013). OpenSlide: A vendor-neutral software foundation for digital pathology, *J Pathol Inform, 4,* 27-36.

Gudlaugsson, E., Skaland, I. & Janssen, E. M. (2012). Comparison of the effect of different techniques for measurement of Ki67 proliferation on reproducibility and prognosis prediction accuracy in breast cancer, *Histopathology, 61,* 1134–44.

Hamilton, P. W., Bankhead, P. & Wang, Y. (2014). Digital pathology and image analysis in tissue biomarker research. *Methods, 70,* 59–73.

Hansen, R. R., Vainer, B. & Steiniche, T. (2012). Digital image analysis: A review of reproducibility, stability and basic requirements for optimal results. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica, 120,* 276–289.

Higgins, C. (2015). Applications and challenges of digital pathology and whole slide imaging, *Biotechnic & Histochemistry, 90,* 5-11.

Irshad, H., Veillard, A. & Roux, L. (2014). Methods for nuclei detection, segmentation, and classification in digital histopathology: A review current status and future potential. *IEEE Reviews in Biomedical Engineering, 7,* 97–114.

Laurinavicius, A., Plancoulaine, B. & Rasmusson, A. (2016). Bimodality of intratumor Ki67 expression is an independent prognostic factor of overall survival in patients with invasive breast carcinoma, *Virchows Archiv, 468,* 493–502.

Lewis, J. S., Ali, S., Luo, J., Thorstad, W. L. & Madabhushi, A. (2014). A quantitative histomorphometric classifier (QuHbIC) identifies aggressive versus indolent p16- positive oropharyngeal squamous cell carcinoma. *Am. J. Surg. Pathol., 38,* 128–137.

May, M. (2010). A better lens on disease. *Sci. Am., 10,* 74-77.

Qi, X., Xing, F. & Foran,D. J. (2012). Robust segmentation of overlapping cells in histopathology specimens using parallel seed detection and repulsive level set, *IEEE Transactions on Biomedical Engineering, 59,* 754–765.

Randell, R., Ruddle, R. A. & Quirke, P. (2012). Working at the microscope: Analysis of the activities involved in diagnostic pathology. *Histopathology, 60*(3), 504–510.

Randell, R., Ruddle, R. A. & Thomas, R. (2011). Diagnosis at the microscope: A workplace study of histopathology. *Cognition, Technology & Work, 14,* 319–335.

Rojo, M. G., García, G. B., Mateos, C. P., García, J. G. & Vicente, M. C. (2016). Critical comparison of 31 commercially available digital slide systems in pathology, *International Journal of Surgical Pathology, 14*(4), 285-305.

Roux, L., Racoceanu, D. & Lomenie, N. (2013). Mitosis detection in breast cancer histological images an ICPR 2012 contest, *Journal of Pathology Informatics, 4,* 8-17.

Sirinukunwattana, K., Raza, S. & Tsang, Y. (2016). Locality sensitive deep learning for detection and classification of nuclei in routine colon cancer histology images. *IEEE Transactions on Medical Imaging, 35,* 1196–1206.

Stalhammar, G., Fuentes, Martinez, N. & Lippert, M. (2016). Digital image analysis outperforms manual biomarker assessment in breast cancer. *Modern Pathology, 29,* 318–329.

Veta, M., Pluim, J. P. W. & Van Diest, P. J. (2014). Breast cancer histopathology image analysis: A review. *IEEE Transactions on Biomedical Engineering, 61,* 1400–1411.

Veta, M., Diest, P. J., Willems, S. M., Wang, H., Madabhushi, A., Cruz-Roa, A., Gonzalez, F. & Larsen, A. B. L (2015). Assessment of algorithms for mitosis detection in breast cancer histopathology images, *Med. Image Anal., 20,* 237–248.

Veta, M., Van Diest, P. J. & Willems, S. M. (2015). Assessment of algorithms for mitosis detection in breast cancer histopathology images. *Medical Image Analysis, 20,* 237–248.

Veta, M., Kornegoor, R., Huisman, A., Verschuur-Maes, A. H. J., Viergever, M. A., Pluim, J. P. W. & Can Diest, P.J. (2012). Prognostic value of automatically extracted nuclear morphometric features in whole slide images of male breast cancer. *J. U. S. Can. Acad. Pathol. Inc., 25,* 1559–1565.

Vilppu, J., RuotTuominenoistenmaki, S. & Viitanen, A. (2010). ImmunoRatio: A publicly available web application for quantitative image analysis of estrogen receptor (ER), progesterone receptor (PR), and Ki-67. *Breast Cancer Research, 12,* 22-31.

Wang, H., Cruz-Roa, A., Basavanhally, A., Gilmore, H., Shih, N., Feldman, M., Tomaszewski, J., Gonzalez, F. & Madabhushi, A. (2014). Mitosis detection in breast cancer pathology images by combining handcrafted and convolutional neural network features. *J. Med. Imaging Bellingham Wash, 1,* 34-43.

Weaker, F. J. & Herbert, D. C. (2009). Transition of a dental histology course from light to virtual microscopy. *J. Dent. Educ., 73,* 1213-1221.