REVIEW

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Pathology on the edge of interdisciplinarity. A historical epitome

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Abstract

Pathology is a bridging discipline that involves both basic and clinical biomedical sciences. In this context, it includes both descriptive and mechanistic approaches, with the final goals of further understanding the anatomic and functional changes and underlying molecular events involved in disease-related processes. Pathology studies mainly comprise macroscopic and microscopic examinations, and involve the visual recognition of different patterns in cells and tissues. In time elapsed to this end, it has adopted a dazzling array of methods and techniques from the most varied fields of natural and engineering sciences in order to optimize tissue analysis, which will be summarized in this article.

Keywords: pathology, techniques, methods, biomedicine, interdisciplinarity.

Introduction

Herophilos of Alexandria (ca. 335–280 BC), together with his younger contemporary, Erasistratos (ca. 304–245 BC), did the first ever scientific human cadaveric dissections for a short period of no more than around 40 years. The latter is credited for his description of the valves of the heart, and he also concluded that the heart was not the center of sensations, but instead it functioned as a pump. Erasistratos was among the first to distinguish between veins and arteries. It seems that only these two physicians ever performed human dissections until the renaissance. The former was a famous teacher in Alexandria who wrote a treatise on human anatomy, describing among other things the structure which still carries his name, the torcular Herophili (*confluens sinuum*) The anatomic and physiologic discoveries of Herophilos were phenomenal. As Hippocrates is called the Father of Medicine, Herophilos is called the Father of Anatomy. At that time, in Alexandria, it is generally accepted that human dissections were performed with official approval, both to determine the normal structure and the changes made by disease. The only person who might challenge him in this assessment is Vesalius, who worked during the 16th century. However, in regard to pathology Herophilos accepted the dominant humoral theories and did not place much emphasis on morphologic disturbances. But, some years later, Erasistratos carried out dissections and made observations about the effects of disease [1]. He noted, for example, that the liver of a man who died from abdominal dropsy was hard as stone but in a man who died of snake bite, the liver was soft. He largely abandoned the prevalent humoral theory in favor of a solidist theory and realized that diseases were associated with changes in the solid organs. The study of these was therefore worthwhile, a point of view that eventually gave significance to the autopsy. Nevertheless this knowledge that changes in the shape of organs are related to functional disturbances dwindled away in subsequent times. This lasted mainly until the end of the 15th century when Pope Sixtus 4th (1471–1484) allowed anatomical studies of corpses; this permission was confirmed by Pope Clement 7th (1523–1534) and ultimately sanctified by the Roman Catholic Church in 1556 [2]. It was Giovanni Battista Morgagni (1682–1771), often named the Father of Pathologic Anatomy, who introduced the mechanistic concept in human physiology and pathology and thus paved the way to modern scientific pathology. Morgagni's practice based on a rigorous observational approach through macroscopic anatomy, and by his successful attempt to relate these organic findings to the clinical presentation of diseases, rightly his work is paid an extraordinary attention in the origin of modern scientific pathology. Moving away from the macroscopical dimension, like in other scientific disciplines, novel techniques and instrumentation was essential for further advancement of pathologic anatomy. Far away from the claim for completeness, in the following a very truncated and subjective bias through the history of some methodological, and/or technical highlights for improving tissue analysis is presented.

Impact of the microscope for tissue analysis

Implementation of microscopic tissue analysis for scrutinizing diseases, correlations of disturbed forms with function, and formal pathogenesis lent the fundament to medical science. Although the microscope was already known and used since the middle of the 17th century, there was a gap concerning its practical application in pathology. Despite the microscopic investigation of cells, discovery of capillaries, and e.g. the inkling of main features in the process of fertilization, for almost 250 years, the microscope was more or less a kind of toy rather than a serious auxiliary

scientific device. So, it is justified to feature the prominence of the microscope in the history of medicine, especially in the history of pathology which developed under the influence of varied geopolitical factors diverse in different European countries and the USA. In Germany it was Rudolph L. K. Virchow (1821–1902) studying the work of Morgagni and being convinced of the advantages of the microscope founded the medical fields of cellular pathology [3]. Even nowadays, it is a corner stone on which the entire building of pathology, including molecular pathology, is based on.

As indicated it was the use of the microscope that totally changed concepts of disease from whole organs, to focus upon cells; it enabled the practice of histopathology and spawned numerous attendant advances in technique necessary for modern practice [4]. Thus in the beginning slices of fresh tissue were cut by hand and examined unstained. By contrast, in the last decades of the 19th century this crude approach had given way to fixed tissues, embedding techniques, microtomes, a plethora of biological stains, and greatly improved microscopes.

Fixation is the first step in tissue preservation for pathological diagnosis. Use of formaldehyde in histopathology has a long history; 1894 it was introduced by Ferdinand Blum (1865–1959) [5]. The man who introduced paraffin embedding in 1869 was Edwin Klebs (1834–1913) [6]. To improve the embedding process, hardening and dehydration were necessary. Among others chromic acid, chrome–osmium–acetic acid and Zenker's fluid entered routine use for this purpose. Fixation arrests autolysis and putrefaction, coagulates soluble and structural proteins, fortifies tissues against the deleterious effects of subsequent processing, and facilitates staining. Fixation of tissues can be accomplished by formalin to form cross-links in tissue [7]. Conventional tissue fixation method involves placing tissue samples in 10% neutral-buffered formalin, followed by overnight processing. This standard formalin fixation and paraffin embedding practice has not changed over the past decades and is used in over 90% of cases in hospitals and clinical settings because it provides superior morphological details and high consistency under various conditions, and offers simple and economical processing and handling.

In Heidelberg, a precision engineer, Rudolf Jung (1845–1900), and a pathologist, Richard A. Thoma (1847–1923), developed the first microtome that could be mass produced in series 1923. This marked the beginning of a new era in histology. It became possible to cut tissue samples in reproducible thin sections for microscopic studies [8, 9].

Ernst Abbe (1840–1905) a brilliant mathematician and physicist made several of the most important contributions to the design of lenses for optical microscopy. He taught physics in Jena where he met Carl Zeiss (1816–1888) in 1866 and became very interested in the optical problems surrounding mid 19th century microscopy. They formed a partnership and he was made the research director of Zeiss Optical Werke. Zeiss and Abbe worked intensively to lay the scientific foundations for the design and fabrication of advanced optical systems. In 1869, they introduced a new "illumination apparatus" that was designed to improve the performance of microscope illumination. Three years later, in 1872, Abbe formulated his wave theory of microscopic imaging and defined what would become known as the "Abbe Sine Condition". In 1881, Ernst Abbe met Otto Schott (1851–1935), who was a glass chemist with a doctorate in Physics from the University of Jena. Over the next years, Abbe and Schott developed several new glass formulas and made adjustments to the mixing and annealing process to eliminate internal defects and produce optical-grade glass having a uniform refractive index. In 1884, Schott, Abbe, and Zeiss formed a new company known as Schott and Sons in Jena, Germany. Continued experimentation with glass recipes and preparation techniques yielded good results and in 1886, they introduced a new type of microscope objective, the apochromat. These were the most precise and highest performance microscope lenses ever built. They eliminated chromatic aberration and brought the resolving power of the microscope to the limit that it enjoys today [10]. Besides that, appropriate illumination of the specimen is the most important variable in achieving high-quality images in microscopy and critical photomicrography. It was August Köhler (1866–1948) who introduced in 1893 a method of providing the optimum specimen illumination by aligning the light path of a microscope to ensure the highest quality images [11].

New dimensions by electron microscopy

By the middle of the 19th century, microscopists had accepted that it was simply not possible to resolve structures of less than half a micrometer with a light microscope because of the Abbe's formula, but the development of the cathode ray tube was literally about to change the way one looked at things, by using electrons instead of light. The possibility for high magnifications has made the transmission electron microscope (TEM) a valuable tool in both medical, biological and materials research. TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Electrons as "light source" and their much lower wavelength make it possible to get a resolution a thousand times better than with a light microscope. In 1931, Ernst Ruska (1906–1988) and Maximilian Knoll (1897–1969) [12] succeeded in magnifying and electron image. This was, in retrospect, the moment of the invention of the electron microscope (EM), but the first prototype was actually built by Ruska in 1933 and was capable of resolving to 50 nm. Although it was primitive and not really fit for practical use, Ruska was recognized some 50 years later by the award of a Nobel Prize. In 1937, the Siemens Company financed the development work of Ernst Ruska and Bodo von Borries (1905–1956), and employed Helmut Ruska (1908–1973), a physician and Ernst's brother, to develop applications for the microscope, especially with biologic respectively medical specimens. Also in 1937, Manfred von Ardenne (1907–1997) pioneered the scanning electron microscope (SEM) [13]. Although

modern EM can magnify objects up to about two million times, they are still based upon Ruska's prototype and the correlation between wavelength and resolution. It must be emphasized from the outset that every electron micrograph is, in a sense, an artifact. Changes in the ultra-structure are inevitable during all the steps of processing that samples must undergo: material is extracted, dimensions are changed, and molecular rearrangement occurs. The best thing we can do is to keep these changes to a minimum by understanding the processes involved so that we make informed choices of the best preparative procedures to use for each sample. As a chemical fixative for EM, glutaraldehyde is often used to crosslink protein molecules and osmium tetroxide to preserve lipids. Instead of paraffin, in EM epoxy resins are used. Staining uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens can be stained "en bloc" before embedding and also later after sectioning. Typically, thin sections are stained for several minutes with an aqueous or alcoholic solution of uranyl acetate followed by aqueous lead citrate. Nowadays EM is an integral part of many scientific laboratories yet. In life sciences, it is still mainly the specimen preparation, which limits the resolution of what we can see in the EM, rather than the microscope itself. Materials to be viewed on the ultra structural level generally require a special processing to produce a suitable sample. This is mainly because the whole of the inside of an EM is under high vacuum in order to enable the electron beam to travel in straight lines. SEM usually image conductive or semi-conductive materials best. Non-conductive materials can be imaged, either by an environmental SEM or more usually by coating the sample with a conductive layer of metal. A common preparation technique is to coat the sample with a layer of conductive material, a few nanometers thick, such as 10nm of gold, from a sputtering machine. This process does, however, have the potential to disturb delicate samples and cover some detail. Because the SEM image relies on surface processes rather than transmission, it is able to image bulk samples up to many centimeters in size and (depending on instrument design and settings) has a great depth of field, and thus it can produce images that are good representations of the three-dimensional shape of the sample. In addition to the above mentioned chemical fixatives there is a diversity of other suitable sample preparations like: cryofixation, freeze-fracture and freeze-etch, freeze drying etc. Nonetheless, there are some limitations in everyday and general use of EM in pathology, since EM are comparably expensive to buy and maintain. They are dynamic rather than static in their operation: requiring extremely stable high voltage supplies, extremely stable currents to each electromagnetic coil/lens, continuously-pumped high/ultra-high vacuum systems and a cooling water supply circulation through the lenses and pumps. As they are very sensitive to vibration and external magnetic fields, microscopes aimed at achieving high resolutions must be housed in buildings with special services. In addition, a significant amount of training is required in order to operate an EM successfully and electron microscopy is considered a specialized skill.

Despite of these disadvantages, EM can reach a far greater magnification than conventional microscopes that are limited up to max. 2000 times and EM also show a much higher resolution thus enabling the pathologist to investigate tissue in expanded dimensions. TEM helps to study the interior structure of cells whereas in SEM the specimens' surface can be examined with minimal preparation activity. Here the thickness is not a consideration. Therefore, bulk samples can be examined in a SEM with a size only limited by the dimensions of the test specimen compared to the dimensions of the SEM specimen stage within the vacuum enclosure. Thus, SEM is becoming one of the most unique and also versatile instruments available for the non destructive inspection, evaluation, examination or analysis of the microstructural surface condition, configurational, and point-to-point characteristics of solid objects. SEM's great advantage is the ultra high resolution providing down to 10 nm.

In 2000, Lloreta-Trull J *et al.* [14] scrutinized the percentage of EM-containing articles in three subject-specific journals with respect to the total number of published articles that ranged from 9.6 to 21%, and the percentage of articles not including potentially useful EM information ranged from 2 to 8.7%. Out of 2531 articles, a total of 448 (17.6%) were the subject of this study. From these, 345 (77%) contained relevant ultra-structural information and the remaining 103 (23%) contained none, in spite of being potentially indicated. The authors listed in their retrospective appraisal the general settings and indications using EM in pathological tissue analysis: (1) renal biopsy (glomerular and transplant pathology); (2) muscle biopsy for evaluation of myopathy; (3) ciliary dyskinesia (nasal or bronchial mucosa, spermatozoa); (4) metabolic storage diseases (skin, conjunctiva, liver, etc.); (5) identification of microorganisms, particularly in AIDS virus, protozoa, and some bacteria (e.g., bacillary angiomatosis, intestinal spirochaetosis, Whipple disease); (6) skin diseases (Bullous epidermolysis, Ehlers–Danlos, cutis laxa, etc.); (7) differential diagnosis of carcinoma/melanoma/ sarcoma; (8) differential diagnosis of adenocarcinoma/ mesothelioma; (9) differential diagnosis of tumors in anterior mediastinum; (10) differential diagnosis of blue round small cell tumors (Ewing sarcoma, PNET, lymphoma, etc.); (11) differential diagnosis of spindle cell tumors; (12) differential diagnosis of endocrine vs. non endocrine tumors; (13) differential diagnosis of meningeal tumors; (14) metastatic tumors with unknown primary origin; (15) hematologic disorders and neoplasms (histiocytic proliferations, leukemias, granulocytic sarcoma; megakaryocytic, platelet, and red blood cell abnormalities). There is a wide consensus on the value of EM for teaching residents and students in pathology, and the use of EM as a tool in basic research has been lowly affected by the development of other modern techniques and methods. However, practical or

economic reasons are often adduced to explain the decreasing demand for EM in diagnostic pathology. Many situations in which EM was formerly the only available aid could be successfully managed nowadays using other new tools, but pathologists should be aware that there are still many situations in which EM can provide crucial or contributive complementary information especially when combined with them.

Cyto-/histochemistry, immunology and other novel techniques

The impressive possibilities of tissue analysis by histochemistry have led to a broad application especially in clinical pathology. This technique is used for studying chemistry of tissues and cells and it comprises enzyme histochemistry, immunocytochemistry and *in situ* hybridization (ISH) as a bridge to molecular pathology. The former serves as a link between biochemistry and morphology. It is based on metabolization of a substrate provided to a tissue enzyme in its orthotopic localization. Visualization is accomplished with an insoluble dye product. It is a sensitive dynamic technique that mirrors even early metabolic imbalance of a pathological tissue lesion, combined with the advantage of histotopographic enzyme localization. Enzyme histochemical insight into development of a pathologic tissue lesion and evaluation of function and vitality of tissue enhance our understanding of the pathophysiology of diseases. In this context it must suffice to mention as one innovative example the introduction of horseradish peroxidase as a tracer for light microscopy studies by William Strauss in 1957 [15] and in 1966 by Graham RC and Karnovsky MJ for electron microscopy [16]. In surgical pathology there is on the light microscopic level a variety of mucin stains used in demonstrating the different types of mucopolysaccharides in tissue, e.g. Alcian Blue, PAS and Mucicarmine, which has a firm practical impact on everyday pathological tumor diagnostics. Other examples are stains for biogenic amines, iron (hemosiderin), calcium, urates, etc. In this process, histochemistry constitutes a valuable complement to conventional histology [17–19].

The objective of immunohistochemistry is using antibodies in order to identify antigens, imparting a much greater specificity of the stain to the tissue with which it reacts. In doing so, immunohistology has transformed surgical pathology from a highly subjective discipline into a much more objective science.

In 1941, Albert H. Coons (1912–1978) and his colleagues [20] were the first to use fluorescent dye labeled antibodies to identify antigens in tissue sections. Subsequently, enzyme labels such as peroxidase by Paul K. Nakane and Barry G. Pierce Jr. in 1966 [21] and application for the localization of alkaline phosphatase by David J. Mason and Randolph E. Sammons in 1978 [22] were introduced. The original immunoenzyme bridge method using enzyme-specific antibody has been superseded by an improved and more sensitive technique of Ludwig A. Sternberger's team in 1970, the so-called PAP technique [23]. Even as early as 1959 Jonathan S. Singer published in *Nature* a short paper which he heralded the birth of immunohistochemistry for electron microscopical immunocytochemistry [24]. In 1971, colloidal gold was utilized by Page W. Faulk and Malcolm G. Taylor on the ultrastructural level [25]. A multitude of different signal amplification techniques and antigen retrieval methods had been adapted for immunohistochemistry thus improving tissue analysis tremendously in both diagnostics and research [26].

It was in the late 1970s that the discovery of monoclonal antibodies by Georges Köhler (1946–1995) and César Milstein (1927–2002) [27] gave the much needed impetus for ushering objectivity in surgical pathology diagnosis. Initially begun as a diagnostic tool, immunohistochemistry today has far surpassed its initial expectations. Newer bio-molecules which have a role in prognostication or which form the basis of justification of expensive targeted therapy have increased the demands from surgical pathology services [28, 29]. Today we are indeed in an era of "translational crossroads for biomarkers" and immunohistochemistry will remain center stage in the demonstration of newer monoclonal antibodies [30].

Molecular biomedicine

The use of the EM has revolutionized histology and anatomic pathology because of its ability to see beyond the resolution of the light microscope. Beyond the resolution of the EM is the molecular level. Virtually like microscopy on the light and ultrastructural level, as well as immuno-/histo-chemistry, molecular pathology in the context of biomedicine cannot exist in an isolated manner. It roots in the tradition of the subject pathology and gains its particular significance in the clinical context. It is an integral part of pathology and accounts for further progress in tissue analysis. The development of ISH in the late 1960's by Mary L. Pardue and Joe G. Gall [31], the introduction of a common laboratory procedure for detection of a determined gene sequence in a complex DNA mixture by Edwin M. Southern 1975 [32] and the revolutionary discovery of the polymerase chain reaction (PCR) by Kary B. Mullis and colleagues in 1985 [33], had an enormous impact on tissue analysis and have shaped today's pathology practice. DNA and RNA analysis (genomics) and total protein analysis (proteomics) are now at the forefront of molecular pathology as new tools for diagnosis. State-of-the-art technology allows the pathologist a view of the suborganelle and molecular levels.

PCR has revolutionized molecular biology by allowing the amplification and characterization of minute amounts of nucleic acids. The widespread success of PCR comes from the speed, efficiency, and reproducibility of the reaction, making it suitable for many procedures in the basic science or pathology laboratory. ISH, as the name suggests, is a method of localizing and detecting specific nucleic acid sequences in morphologically preserved tissues sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest in contrast to normal hybridization that requires the

isolation of DNA or RNA, separating it on a gel, blotting it onto e.g. nitrocellulose and probing it with a complementary sequence. Therefore, besides immunohistochemistry, ISH is playing an increasingly important role in diagnostic pathology, as well as in translational research. Combined methods that allow researchers to localize sites of gene expression at both the mRNA and protein level, within histological sections enable the identification of the cells e.g. within a tumor specimen that expresses a specific mRNA (assessed by hybridization *in situ*) or protein (assessed by immunohistochemistry) [34]. This is a significant step toward understanding tumor behavior, which is not possible using conventional methods when tissues are homogenized before analysis. Thus, combined detection of mRNA and protein may permit effects of subtle regulatory processes such as translational repression to be observed and allow co-expression of genes to be detected. Application of such combined techniques to tissue microarrays of tumor tissues from cohorts of patients with known clinical outcome would allow the predictive value of specific patterns of expression to be tested retrospectively. With the advent of tissue microarrays the way for a wide range of molecular techniques has been paved [35–37]. They are usually produced by re-locating tissues from conventional histological blocks. Typically, 40 to 1000 cylindrical formalin-fixed and paraffin-embedded tissue cores can be densely and precisely arrayed into a single paraffin block. From the block, a vast number of serial sections can be produced and placed on individual slides. Tissue microarrays enable parallel in situ detection of DNA, RNA or protein targets in each specimen on an array at cellular and tissue levels in a uniform way. The large number of available consecutive arrays allows rapid analysis of multiple molecular markers at the same time and in the same set of specimens. Essentially the same tests are performed on conventional histological samples, including conventional staining, immunohistochemistry and ISH; this all can be done separately, or in parallel, using tissue microarrays. Tissue microarray is a high-throughput technique that allows rapid gene expression and copy-number surveys of large numbers of different tissue specimens. In addition, it represents a technical revolution for the effective use and analysis of human tissue specimens and helps to increase our understanding of basic mechanisms in diseases like cancer and eventually, it may be able to lead to better treatment and increase the survival rate of patients. It provides a more effective way to determine the diagnosis and prognosis in patients. So, it is not amazing that microarray analysis is now at the forefront of clinical molecular pathology.

The microarray technique is employed to compare normal and cancerous genes, which can be tested on a vast number or "array" of test materials quickly and efficiently. Microarray technology is also being used for translational medicine like screening for newborn genetic diseases and the study of infectious diseases. It also has its applications in diagnostic and forensic pathology. In summary, tissue microarrays are a cost effective technique when e.g. applied for a typical cohort analysis; while enabling more assays less reagents are needed. ISH has emerged over the past decade as an extraordinarily sensitive technique for the detection of gene expression at the cellular level. Advances in probe preparation and labeling methods have facilitated the transfer of this technology from the research laboratory to the clinical arena. In contrast to immunohistochemistry, which is dependent on the protein content of cells, ISH analyses permit the identification of cells on the basis of their contents of specific mRNAs encoding the products of interest. A well known example for the application of the ISH is the determination of the Her2 status in breast cancer patients either by fluorescence (FISH-Test) or by a chromogenic (CISH-Test) assay. These methods provide a critical approach for the analysis of heterogeneity in tumors that typically contain cells at different phases of neoplastic progression and at multiple levels of differentiation and functional activity. ISH methods have been of particular value for studies of mRNAs encoding oncogenes, hormones, secretory proteins, cytokines, and a wide variety of other cellular products. Advances in ISH technology, including PCR based methods, offer particular promise for examining genes with low levels of expression at the cellular level. In addition, the advent of Laser capture microdissection technique has brought about this reliable procurement of pure population of cells from tissue sections under direct microscopic visualization and bridged a very significant technical gap between the histopathologist's microscope and the molecular biologist's workbench [38]. This has now opened the doors to enhancing our understanding of molecular mechanisms regulating cellular developments and its functioning both in normal and diseased states.

Several different new technologies are summarized under the name "Omics", which stands for the parallel analysis of many, ideally all, events taking place on a certain biologic level. Firstly, transcriptomics, which analyze the transcriptomes of certain materials, are capable to deliver the complete information on the mRNA level by using high-density arrays [39]. Novel approaches even go further by applying parallel sequencing of transcriptomes; these carry the "deep sequencing" designation. Proteomics are applied to gain information about the proteomes of certain samples. The classical way to achieve this is the two-dimensional gel-electrophoresis, which increasingly is applied even to archived specimens [40], and is complemented by new, gel-free techniques. All these "Omics" offer powerful screening capabilities, which increase the broadness of understanding the complex events taking place e.g. in the sites of a disease in a tissue. One can speculate that such techniques will also get a part of diagnostics in the near future.

In order to successfully implement the advances in biotechnology, as illustrated above, such as DNA microarrays and proteomics to clinical and molecular medicines the parameters of procurement and fixation that affect the quality of the tissues at the molecular level have to be kept in mind. The Achilles' heel is the

preservation of the tissue's proteins as well as of nucleic acids under investigation. In this context, we refer to a recognized review article from 2002 [41]. In most pathology laboratories worldwide formalin fixed paraffin embedded samples are used for diagnostic tissue analyses, which allow the accomplishment of many of these modern molecular techniques only in parts and in a restricted manner. In addition, there is a need for standardization in collecting and processing procedures of pathologic anatomic tissue specimens [42]. The HOPE-fixation (Hepes-glutamic acid buffer-mediated organic solvent protection effect) avoids all these obstacles and presents an outstanding formalin-like morphology, excellent preservation of protein antigens for immunohistochemistry and enzyme histochemistry, distinguished RNA and DNA yields and absence of cross-linking proteins [43–45]. Summarized, all common molecular techniques can be applied to HOPE-fixed materials, the HOPE-technique is the most intensively studied alternative fixation procedure, allows long storability of paraffin blocks and can be applied to tissue and cells (e.g. BAL, sputum, etc.). It is ideal for biobanking and suits methodologically as a crucial base of ex vivo systems for functional studies [46], thus allowing the entire application of the so-called "Omics" for screening.

Telemedicine and automation

Telemedicine is the use of telecommunications to provide medical information and services at a distance. Pathologists, similar to radiologists, visually examine images to provide other clinicians with diagnoses. In today's health care system, one use of telepathology is to provide urgent services at sites either without a pathologist (e.g. intra-operative rapid frozen section diagnoses) or with a pathologist requiring back-up. Secondly, telepathology can provide immediate access to subspecialty pathology consultants. For example, if a primary care pathologist in a rural area needs a specialist to diagnose a (rare/uncommon) disease, and the nearest pathologist is far away, telemedicine can be an excellent alternative. Thirdly, and probably the one most often used, is to generate a second opinion. If physicians are not sure if their diagnosis is correct and in order to confirm their decision, they can contact another telepathologist *via* telemedicine. Additionally, it can assist pathologists in completing or refining a differential diagnosis. Finally, telepathology can be used to continue medical education, proficiency, testing, and recertification of pathologists as well as other laboratory personnel. This can be beneficial in rural areas thus avoiding long distance traveling. Telepathology has become an increasingly important aspect of telemedicine in the past few years and it will even more in the years to come [47].

There are different types of telepathology. The first one is called dynamic imaging telepathology (also known as real time telepathology). Here a microscope is used along with a personal computer to send images. With real time systems, the consultant actively operates a robotically controlled motorized microscope located at a distant site – changing focus, illumination, magnification, and field of view – at will. Either an analog video camera or a digital video camera can be used for robotic microscopy. This method is very beneficial because it is almost just the same as the usual technique of pathological tissue examinations. The second type of telepathology is the static imaging telepathology. In this form, pathologists select images, store them on a computer, and upload the image to other pathologists. Although this may seem practical, there are two main downsides on it. For one, only a selected number of images can be transmitted, and two, the consulting telepathologist is prompted to rely exclusively on these. Nevertheless, static image systems have benefits of being the most reasonably priced and usable systems. But, they have the significant drawback in only being able to capture a selected subset of microscopic fields for off-site evaluation. In contrast to this, real time robotic microscopy systems and virtual slides allow a consultant pathologist the opportunity to evaluate histopathology slides in their entirety from distance. The latter utilize automated digital slide scanners that create a digital image file of an entire glass slide (whole slide image). This file is stored on a computer server and can be navigated at a distance via the internet, using a browser. Digital imaging is required for virtual microscopy. Real time and virtual slide systems offer higher diagnostic accuracy compared with static image telepathology. Expense is an issue with real-time systems and virtual slide systems as they can be costly. Virtual slide telepathology is emerging as the technology of choice for telepathology services. However, high throughput virtual slide scanners (those producing one virtual slide or more per minute) are currently expensive. Also, virtual slide digital files are relatively large, often exceeding one gigabyte in size. Storing and simultaneously retrieving large numbers of telepathology whole slide image files can be cumbersome, introducing their own workflow challenges in the clinical laboratory.

An eminent point in telepathology is the indemnification of providing a good image quality in order to make a reliable diagnosis. Whole slide imaging is an emerging telepathology technology, which can produce accurate and detailed diagnostic reports. So, telepathology could improve efficiency and quality of care in manners similar to teleradiology.

Historically, histopathologic evaluation is performed by a pathologist generating a qualitative assessment on thin tissue sections on glass slides. In the past decade, there has been a growing interest for tools able to reduce human subjectivity and improve workload. So far, no computer-driven system can match the human eye in its ability to recognize complex patterns. However, computers can surpass the human eye in their ability to detect rare events and recognize subtle variations in color and intensity. Computer scientists first approached the challenge of automating the reading of samples on glass slides from a pattern-recognition angle. Digital pathology is an emerging clinical practice in which a pathologist makes diagnoses using digital images of tissues acquired with an automated high-throughput microscope and stored and viewed using a medical image database [48, 49].

Current whole slide scanners are 2D bright field scanners, envisioned follow-up scanners would broaden to 3D bright field imaging and 3D fluorescence imaging for FISH-analyses related to e.g. Her2-diagnosis for breast cancer treatment. Whole slide scanning technology combined with object orientated image analysis can offer the capacity of generating fast and reliable results. For evaluation of signals from immunohistochemistry or ISH, automated stand-alone tissue microarray detection systems (e.g. "Spot Browser") can be used to analyze stained tissue microarrays [50]. The combination of whole slide digital scanning and image analysis can be fully automated and in the end might deliver even more descriptive and biologically relevant data over traditional methods in evaluating sole histopathological tissue analysis [51].

There is no doubt that microscopy on the light and ultrastructural level represents a first major breakthrough in tissue analysis. From early days of the 20th century to the present, the pace of discovery and change has accelerated still more. Ongoing advances in the fields of fixation, embedding, cutting, immunohistochemical staining, molecular methods, confocal microscopy, and image processing have continued to yield better diagnostic tools, and new, better, more precise diagnoses. Thus, the pathologist's armamentarium has been greatly enriched and the addition of more promising new instruments including computational procedures will provide the pathologist a glimpse of disease at the molecular level with important clues in understanding the mechanisms of diseases (e.g. the origin of malignant cell transformation). Recent advances in various fields of science and technology and the incorporation of these to pathology back up the impact of the scientific breakthroughs in tissue analysis. This can be said confidently when we talk about the contribution of the gene array technology, which allows gene expression measurements of thousands of genes in parallel, providing a powerful tool for pathologists seeking new markers for diagnosis (gene expression profiling) [52]. This holds major promise for optimizing the management of patients with cancer. There is evidence that microarray based gene expression profiling enables and supports tumor classification and can be a very helpful diagnostic tool. Molecular profiling has also given way to histoclinical classifications since such a classification would be expected to corroborate more accurately with the functional behavior of a tumor. Molecular classification systems have been attempted extensively in different organ systems. Whereas genomic studies are establishing new molecular classifications, genetic alterations are also being identified and characterized, generating new targets for therapy and new tools to predict disease recurrence and response to therapy. This combined molecular approach is expected to have an impaction on individual so called tailored therapy for cancer patients [53]. Overall, molecular profiling is a fascinating and promising technology, but its incorporation into clinical decision making nevertheless requires careful planning and robust evidence.

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Received: November 5th, 2010

Accepted: December 10th, 2010