Current and emerging imaging applications in pathology: From microscopy to digital pathology, clinical perspectives

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ABSTRACT
Pathology is the study of the causes and consequences of disease or injury and those morphological, functional, and molecular changes. This paper reviews current and emerging imaging applications, including optical/light microscopy and molecular testing, used for routine pathological diagnosis in clinical settings, focusing on cancer diagnosis.

1 Introduction
In clinical practice, pathologists diagnose macroscopic, microscopic, and molecular changes in diseases or injuries of patients, contributing to treatment decision-making and assessments of the efficacy or effectiveness of therapies, including surgery and drugs. In the field of cancer, pathological diagnosis is performed based on the World Health Organization (WHO) Classification of Tumours, which provides an international consensus on the classification and diagnostic criteria of tumors for each anatomical site (organ). The classification comprises a unique synthesis of scientific evidences (both written and illustrated) that underpins the diagnosis of individual tumor types. It is published as a series of books, known to pathologists as the WHO Blue Books, and is now also available as a website (see https://tumourclassification.iarc.who.int). The evidence base for each tumor type comprises peer-reviewed publications in multiple fields. In this paper, I review the current and emerging imaging applications, including light microscopy and molecular testing, used for routine pathological diagnosis based on the WHO classification in clinical settings and their prospects in the near future.

2 WHO classification of Tumours
The international standards for cancer diagnosis are contained within the World Health Organization (WHO) Classification of Tumours, published by the International Agency for Research on Cancer (IARC), and known worldwide as the WHO Blue Books. In addition to their relevance to individual patients in clinical practice, these volumes provide a valuable contribution to cancer research and surveillance, fulfilling an important role in scientific evidence synthesis and international standard setting [1].

In recent years, precision medicine and targeted therapies, and their increased adoption in clinical practice, have had a major impact in the field by complementing the role of histopathology in the prognostication and prediction of cancer. The application of molecular profiling has also made a substantial impact on tumor taxonomy and the classification of human malignancies. Thus, in the current fifth edition series of WHO classification, while morphology with H&E stain observation under bright-field microscopy remains the foundation for the taxonomy, there is in addition a group of molecularly defined tumor entities [2-6].

The increasing emphasis on molecular classification underscores the importance of having appropriate ancillary technology in our pathology laboratories. Immunohistochemistry and molecular testing have become crucial for accurate cancer diagnosis, prognosis, and prediction. This has important implications for low- and middle-income countries, where it may be difficult to get high-quality routine histology, let alone contemporary ancillary testing. Even in high-income countries, the availability of immunohistochemistry and molecular testing may be limited because of geographical, fiscal, and human resource issues. These facts have implications for the WHO Blue Books because they are written for worldwide use. Therefore, major emphasis is placed on histopathological criteria, and essential and desirable diagnostic criteria are listed within each entity section [2-6]. These include clinical, radiologic, and histologic criteria with optical microscopic observation for hematoxylin & eosin (H&E) stained tissues (Fig. 1).
immunohistochemistry, and electron microscopy findings as well as and molecular biomarkers [2,3], such as fluorescence in situ hybridization (FISH) with fluorescence microscopy (Fig. 2) or DNA sequencing with next-generation sequencing (NGS) to improve diagnostic accuracy. For molecularly defined tumors that were newly introduced entities in the current edition series of the WHO classification, molecular findings are essential for the diagnosis [3-6]. Each independent histological tumor type classified according to the WHO classification diagnostic criteria exhibits distinctive clinicopathological features, including common age of onset, prognosis, risk factors, underlying diseases, and familial predisposition. Furthermore, the optimal treatment may also differ. Therefore, it is important to determine the histologic type by accurate pathological diagnosis [1-6].

3 Next-generation sequencing (NGS)

In the standard Sequencing by Synthesis methods for NGS DNA sequencing of Illumina platform, a powerful camera captures 4-color, 2-color, or 1-color fluorescence high-magnification images to identify fluorescent dye-labeled nucleotides - adenine, cytosine, guanine, thymine, and the image processing provides the sequence data [7,8]. In a sense, the basic process of analysis in the NGS system platform is the acquisition of digital images of optical fluorescence microscopy. With the emergence of NGS-based comprehensive genome profiling (CGP) testing in routine clinical practice, NGS technologies will become more accessible and widespread, promising to unlock new frontiers of advancements in cancer medicine.

4 Digital pathology for remote pathology diagnosis in current pathology laboratories

‘Telepathology’ and Digital pathology is a practical alternative to on-site intraoperative frozen section diagnosis and utilizes digitized image transfer to allow remote/off-site reporting of histopathology slides. This digital service is being used to cover a large, sparsely populated rural area, and this technology can facilitate the centralization of pathology services, improving their quality, cost-effectiveness, and time efficiency. For example, from tissue samples taken during surgery, frozen sections and H&E stains are prepared by clinical laboratory technologists, mounted on a motorized stage at a remote hospital, Sado General Hospital on Sado Island, and digital images on the monitor are viewed and controlled via the Internet by pathologists at Niigata University Hospital 70 km away (3 hours by ship) to provide rapid intraoperative microscopic analysis that helps surgeons determine the surgical procedure (Fig. 3).

5 Prospects: Digital pathology and artificial intelligence (AI) in translational medicine

The growth of digital pathology and whole-slide imaging have created the opportunity to extract more information from histological samples through image analysis. Digital pathology analysis can be broadly categorized into two main approaches: quantitative analysis and AI-driven assessment [9]. The use of digital image analysis in pathology can identify and quantify specific cell types quickly and accurately and can

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**Fig. 2** The representative fluorescence microscopy image of fluorescent in situ hybridization with red and green fluorescence labeling of specific genetic loci. Multiple signals are observed, suggesting gene amplification.

**Fig. 3** ‘Telepathology’ in Niigata area in Japan, using whole slide image of H&E-stained frozen section.
quantitatively evaluate histological features, morphological patterns, and biologically relevant regions of interest (e.g., tumoral or peritumoral areas, relationships between different immune cell populations, areas of expression, presence of metastasis). AI approaches are built to initially extract appropriate image representations and then to train a machine classifier for a particular segmentation, diagnostic, or prognostic task using a supervised or unsupervised approach [10]. Thus, AI applications in pathology improve quantitative accuracy and enable the geographical contextualization of data using spatial algorithms. Despite the advances in the application of AI in digital pathology and medical workflow, there remain several challenges. Many of the AI approaches, particularly deep-learning based systems, are criticized for not being able to explain how they arrive at their decisions, hence called “black boxes.” [10,11]; however, there’s active research to make the algorithms easier to interpret by humans and provide insight on how they work, e.g. by providing some of the features that the algorithm is focusing on or by dividing the AI algorithm execution into steps, each of which make logical sense to a human expert. These may provide some transparency to AI algorithms. Although this research field still stand at the dawn of the innovating era, the power of AI to analyze large amounts of bioinformatic data with the integration of AI with digital pathology, molecular, and other clinical data quickly would significantly speed up the discovery of novel histopathology features that may aid our understanding of or ability to predict how a patient’s disease will progress and how the patient will likely respond to a specific treatment.

6 Conclusions

In modern clinical practice, various microscopic imaging techniques and applications are used for pathological diagnosis and therapeutic decision-making, including conventional manual brightfield microscopy, fluorescence microscopy, electron microscopy, and even molecular testing NGS methodology. A combination of pathologists and current and future advances in digital pathology and AI/machine learning/bioinformatics approaches can yield more accurate, consistent, and useful results beyond a human’s ability and may offer practical advantages in precision cancer medicine.

References


