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BRIEF COMMUNICATION

The Potential of Digital Microscopy in Breast Pathology

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Abstract The rapidly evolving field of digital microscopy supports the efficient exploitation of inherent information from stained glass slides to offer widespread utilization in breast histopathology. Digital image signals can be accurately measured, integrated into databases and shared through computer networks. Therefore, digital microscopy can boost telepathology-consultation, gradual- and postgradual teaching, proficiency testing, intra- and interlaboratory validation of biomarker screening interpretation, and automated image analysis of biomarker expression for both diagnostics and research applications. This is a brief highlight of the potential of digital microscopy in breast pathology applications.

Keywords Digital microscopy · Breast pathology · Teleconsultation · Proficiency testing · Biomarker quantitation

Digital Microscopy

Digital microscopy, allowing prompt and dynamic access to any part of stained slides at any microscopic magnification as driven with a mouse through the computer monitor, can be perfectly exploited in breast histopathology for diagnostics, research and quality assurance. Digital slides can be shared through computer networks by many pathologists worldwide either online with others or by navigating independently, offering an efficient tool for telepathology yielding primary diagnosis, teleconsultation for second opinion, teaching, proficiency testing, external quality assurance and interlaboratory process validation [6].

Digital slides are built up as pyramids of microscopic image series allowing in focus navigation even at continuously changing magnifications. Upgraded features of digital slides to traditional microscopy include easy annotations, measurements and image archiving at perfect color fidelity and illumination [7]. Simultaneous running of low power previews and navigation of linked serial slides stained for different markers for comparative analysis e.g. for viewing ER and HER-2 side-by-side are also specific for digital microscopy. Z-stacking of optical layers ensures high resolution and accurate tracing of small signals such as those of HER-2 FISH or cytoplasmic details in aspiration cytology samples. Serial digital slides can also be assembled into a three-dimensional structure for reconstructing tissue architecture, e.g. for studying tumor invasion. In addition, digital signals permit image segmentation along color, intensity and size for automated object quantification, where digital slides facilitate batch processing of many slides [7].

System integration of histopathology workflow is a recent trend serving improvement of standards, traceability and reproducibility i.e. quality assurance of diagnostic procedures. System integration is based on network linking of procedures and databases of all units within histopathology laboratories for tracing of diagnostic samples/slides from entry to reporting and monitoring indicators of quality. With proper informatics support digital slides perfectly fit into diagnostic and research databases for system integration.

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Digital Slides in Breast Pathology

The potential of digital microscopy at several fields of breast pathology has gained attention recently and been substantiated by published data, some of which are briefly referred to below along with some local experience of the authors.

Education, Teleconsultation and Quality Assurance

Digital slides have been used for pathology teaching and assessment including breast pathology for several years, e.g. at University of South Wells, Australia [8]. 1st Department of Pathology at Semmelweis University, Budapest, Hungary introduced fully digital teaching of graduate pathology in September 2007 [4]. The digital slide repository used can also be accessed by students from home through http://www.pathonet.com portal, which also offers teleconsultation service. Furthermore, digital slide series used for national slide seminars for monitoring diagnostic skills including breast pathology and for sharing knowledge in external quality assurance (samples from QualiCont and NEQAS ICC EQA schemes) of diagnostic techniques are also available at the same web-site supervised by the Hungarian Society of Pathologists.

The European Working Group for Breast Screening Pathology (EWGBSP) have run pilot quality assurance circulation in diagnostic breast pathology using virtual slides accessed through a server at Queen Mary Hospital, University of London (UK; http://www.telepathology.qmul.ac.uk). The College of American Pathologists (http://www.cap.org) also offer "Online Digital Slide Program in Surgical Pathology" including breast pathology for proficiency testing of diagnostic interpretation skills. A telepathology consultation service was developed at the Institute of Pathology, Charite, Berlin and tested with success by the German Association of Pathologists for supporting second opinion in breast cancer screening [10].

Digital microscopy has also been involved in generating a web-based digital atlas of breast histopathology

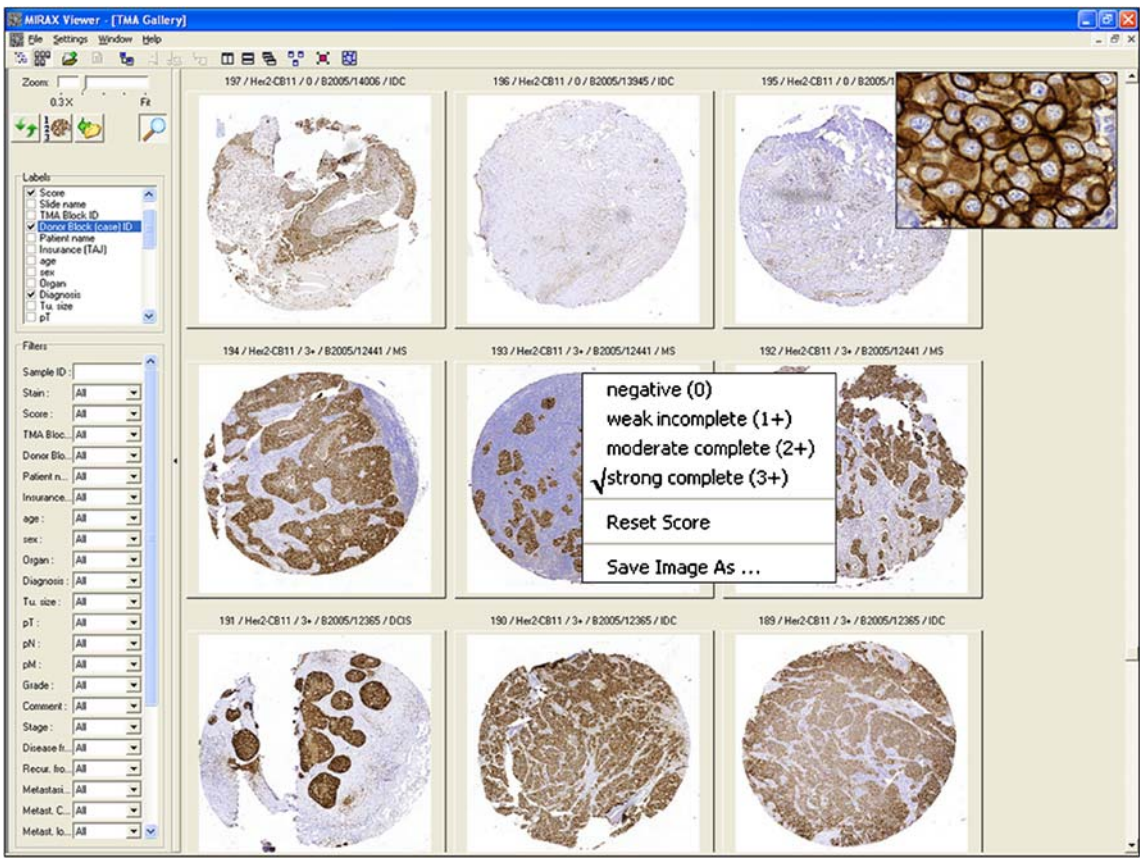

Fig. 1 The on-screen scoring of HER-2 immunostaining of digital tissue microarray (TMA) slides of breast cancer is highly facilitated by gallery filtering of immunostained spots, which allow free sample sorting and validation of scores. A typical view of gallery scoring with label and filter options (on the *left*) which are identical with the imported Excel columns, the drop-down scoring scheme (*center*) and the immunostained spots specified with labels above them. Mirax TMA Module software

(http://www.webmicroscope.net/breastatlas), an excellent tool for both graduate and postgraduate teaching [9]. Its advantages can also be exploited for virtual tumor banking offering a digital slide catalogue and database of large repositories of tumor samples stored at distantly situated laboratories of consortium members [12].

Diagnostics and Research

Standardized image acquisition (exposure time, color fidelity etc…) is a key requirement for consistency in analyzing comparative biomarker expression with quantitative image analysis, which is readily met in digital microscopy.

Automated image analysis using digital slides can substantially improve consistency in predictive marker scoring and interpretation, such as that of HER-2 [11]. Systems for reproducible digital image analysis have been recently available, e.g. the ACIS II, which is approved by the FDA for diagnostic testing of HER-2, ER and PR immunostaining results [2, 11]. Digital microscopy can also improve accuracy and reliability of HER-2 immunohistochemical scoring/analysis resulting in significant improvement of interobserver agreement and better correlation with FISH amplification results than subjective manual scoring [2, 11].

Digital microscopy may significantly increase the chance for detecting rare metastatic breast cancer cells in the bone marrow [3], and in the blood of metastatic breast cancer patients [13].

Tissue microarray (TMA) studies, widely used for high throughput testing of breast cancer biomarkers, can be significantly catalyzed and eased with digital microscopy, particularly when supported with dedicated software tools for intelligent data management [1, 7, 11]. TMAs can be used for saving diagnostic budget of routine HER-2 assessment by concentrating IHC and FISH in once a week runs. TMAs are also well-suited for intra- and inter-laboratory validation of prognostic and predictive markers of target therapy in breast cancer [5].

We have initiated interlaboratory validation for HER-2 scoring by monitoring interobserver agreement between seven experts from five major breast cancer centers in Hungary using digital microscopy on 210 samples from 150 consecutive cases of breast cancer in 2 mm cores included in TMAs (Fig. 1). Since interpretation of HER-2 immunohistochemistry can be highly subjective, quantitative image analysis with the Mirax HistoQuant software (3DHistech, Budapest) is used (Fig. 2.) in difficult cases besides FISH for gold standard of HER-2 status.

In conclusion, digital slides can boost gradual-, post-gradual teaching, life-long professional education, tele-

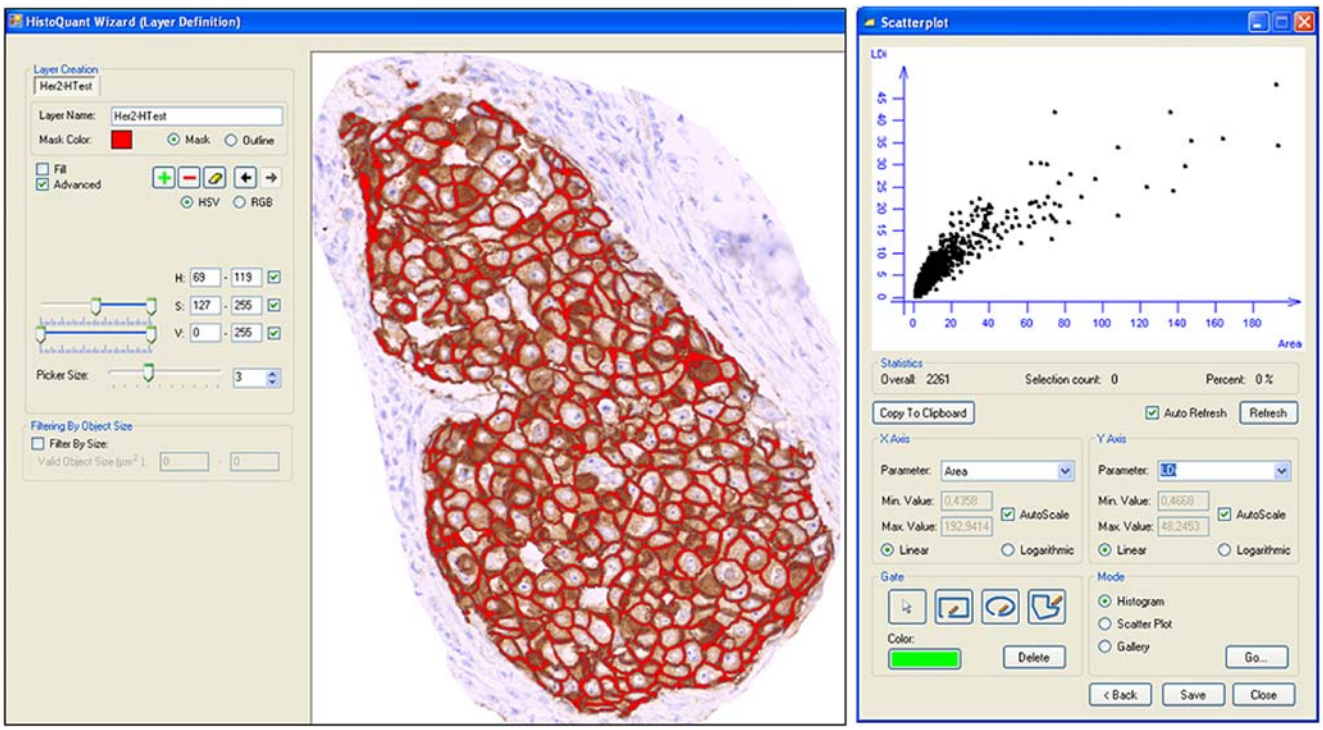

Fig. 2 Selected area from a digital slide of breast cancer is analyzed with the HistoQuant software using image color segmentation for HER-2 cell membrane staining (highlighted in *red*, *middle panel*) for automated quantification and further analysis using a scatter plot (*right panel*)

pathology and -consultation, external quality assurance, process validation and research in breast pathology. In the forthcoming era of digital pathology traditional slides do not loose importance rather their inherent information can fully be exploited and made more easily accessible to pathologists in the form of digital slides. Thus, the most critical factors in digital microscopy are the same as for physical/optical slides, i.e. tissue quality, section thickness and staining quality, which again underline the importance of preanalytics and histotechnology as key preconditions for gaining high quality slides properly supporting all aspects of breast histopathology.

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