

**A web-based diagnostic reference center
for the European Reference Network “EpiCare”:
Recommendations of the eNeuropathology working group**

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Summary

Epilepsy surgery is a valuable treatment strategy for a selected group of patients with drug-resistant focal epilepsy. While reliable disease classification is essential for the optimal management of patients in general and crucial for the development of more personalized therapies in the future, arriving at a precise diagnosis often poses considerable difficulties due to the broad and variant-rich spectrum of epilepsy-associated brain lesions. Given the scarcity of European institutions diagnostically focusing on the histopathology of epilepsy surgicals the readily available subspecialty expertise as well as training opportunities remain logistically and financially challenging. To improve this situation, the European Reference Network’s (ERN) epilepsy care program (EpiCare) has set out to develop a web-based microscopy referral and teaching framework. This paper reviews the aspects of digital microscopy, data storage and image analysis technology relevant to the practice of neuropathology. Cognizant of the European data security requirements and regulations we propose a diagnostic eNeuropathology reference center initiative and delineate a roadmap for its implementation favouring open-source, vendor-independent browser platforms.

Introduction

Global digitization continues to change medical research and practice across all specialties. Currently pathologists in general face substantial challenges to adopt novel microscopic methods, to securitize large image repositories and to standardize analytical pipelines. Neuropathologists in particular are required to integrate various pre- and postoperative imaging modalities into their macroscopic, histological and molecular reports to provide optimal guidance for anti-epileptic therapies. Ideally all these tasks, like most critical digitization efforts should be tackled in an internationally concerted action. Reviewing the current state of digital technology with respect to neuropathology is a necessary step towards developing guidelines within the emerging European Reference Network's epilepsy care program (ERN EpiCare) and beyond. Digital technology is well established across all medical disciplines and has become essential to daily routines and specialist activities alike. Whole slide imaging (WSI), affordable storage solutions and burgeoning computation power are driving image analysis routines into the methodologic core of pathology. Undoubtedly progress in automation and algorithmization will bring about considerable benefits as well as novel threats to the welfare of patients and practitioners. The torrents of data created by high-throughput processes can easily overwhelm even the most conscientious diagnosticians, if sufficiently detailed methodological knowledge is not provided in the framework of postgraduate training and continuous medical education. This calls for updated curricula, detailed guidelines and accreditation procedures encompassing the relevant aspects of digital technology to avoid jeopardizing pathology's essential function as the foundation of medical practice. Neuropathologists who by default are constantly interact with neurophysiologists, neuroradiologists and neurosurgeons have developed some familiarity with the data formats current in their neighboring disciplines. The ability to integrate findings, modalities and procedures across different fields of expertise is essential to building a truly networked version of medicine. Neuropathology is therefore ideally positioned to play a major role in shaping the multimodal pathology of the fully digitized future.

Virtual microscopy: Tiled high-resolution pictures

The currently available and increasingly affordable combinations of large sensor multimegapixel sCMOS cameras, ultra-high resolution (up to 8K) desktop monitors and

graphics processing units supporting frame rates above 60Hz are providing detailed, jitter-free and wide-screen visual experiences now surpassing those delivered through the eyepieces of traditional microscopes. Only long entrained habits, not picture quality may slow the adoption of “screen scoping” in pathology diagnostics. Mere generational shift and a growing familiarity with augmented reality (AR) technology will bring about this change of pathologists’ view. Already commercially available “smart glasses” (<https://tech.moverio.epson.com/en/bt-300/>) can deliver live pictures of a quality sufficient for morphologic diagnosis. Just as ocular lenses glass slides are on the retreat as the medium of long term storage, a trend first gaining momentum in morphology-based pathology research as a growing number of automated microphotography devices sporting multiple dry and immersion objectives, motorized stages, monochrome and color cameras and slide loaders. Virtual slides are now permeating not only pathologic diagnosis, consultation, review, publication and training but also all morphology-based branches of the life sciences.

WSI-scanners have considerably evolved from those envisaged in the first “virtual microscopy” patents (Bacus & Bacus, 2001) and now able to digitize standard and fluorescence microscopic sections in various formats (Table 1). It currently takes under 2 minutes to scan conventional and special stains of a 15x15 mm² section area under bright field illumination with a 20x objective; recording z-stacks and several fluorescence channels will multiply the scan times required. Individual sections are identified by their 1D- or 2D-bar coded tabs, while each partially overlapping „slide shot“ is exposure corrected and tiled by instrument-specific proprietary or open-source software in realtime or post-acquisition. Depending on tissue area, magnification, number of scanning levels and compression formats (non-lossy or high efficiency lossy, such as JPEG XR) used the resulting image files can vary in size, reaching hundreds of GB in multi-channel fluorescence slides. Consequently the rapid and general introduction of glass-less pathology archives even at academic centers is faced with considerable costs and the complex infrastructural demands necessary to maintain sufficiently performant retrieval and back-up logistics (Stathonikos et al. 2013).

Diverse proprietary formats vs the DICOM standard

WSI files are generally formatted in a pyramidal fashion encompassing several levels of resolution to allow continuous and rapid zooming through fields of view/regions of interests

at minimized data transfer rates and RAM requirements. Unfortunately many microscope manufacturers and biomedical software providers have developed proprietary versions of the generic pyramidal format in an attempt to entrap the user in their ecosystems („walled gardens“) of locally installed or server-based applications. These irritant business policies in blatant disregard of the true scientific spirit have spawned a number of initiatives which develop platform-spanning alternative applications for different operating systems (Windows, Mac OS, Linux) under the GNU General Public License (GPL) model. Exemplarily OpenSlide developed by Carnegie Mellon University School of Computer Science (<http://openslide.org/>) offers C libraries with Java and Python bindings to convert the WSI formats of most scanner manufacturers for viewing in standard browsers supporting HTML5. Ideally, however, WSI files should comply with the „Digital Imaging and Communications in Medicine“ standard, synonymous with ISO (International Organization for Standardization) standard 12052 whose supplement 145 defines objects containing very large image data sets common to pathology. The creation of interoperability with the „Picture Archiving and Communications System (PACS)“ long established in most hospitals for radiology images represents a major integrative effort which notwithstanding the abortive attempts of a few manufacturers has not been addressed in a coordinated fashion. Currently most WSI are generated by brightfield instruments capable of scanning standard, special and immunohistochemical stains as well as chromogenic in-situ hybridisations (CISH), all modalities which can pose considerable challenges for downstream image analysis. More recently the more expensive fluorescence-enabled scanners have become more popular which can provide image data for more discriminatory and reliable analyses of multi-channel immunofluorescence and fluorescence in-situ hybridisations (FISH). Multispectral scanners additionally allow the unmixing of spectrally overlapping chromophores and thereby analyses of an even greater number of fluorescent markers or probes in the same section <http://www.perkinelmer.com/product/vectra-3-0-200-slide-cls142338>).

Slide archiving beyond Petabytes

As X-ray archiving has moved from film to PACS pathology institutes are now en route to divest their slide cabinets for data troves of truly gigantic capacities. The resultant demands on storage media, networking infrastructure and data security are immense. In view of the growing importance of global access to medical image for reproducible

subtyping of large collectives, reference pathology of rare entities, training medical professionals and international research communication in general investing in adequate hardware and resilient infrastructure for medical purposes should have considerable priority in global policy decisions. If medical data can be entrusted in the long term to commercial cloud services or only to institutional agencies remains to be seen given the ever increasing exfiltration risks and criminal threats to large data storage installations of all kind. Fortunately browser-based “open source” solutions like OMERO (<http://www.openmicroscopy.org/site/products/omero>) have sufficiently matured now offering all pathology-relevant functions such as granular access rights management, platform-independent WSI-rendering, slide mark-up and annotation on a par with their commercial alternatives (Allan et al. 2012).

Image analytical pipelines

Quite early on WSI has been recognized as the critical enabling technology for the effective application of image analytical routines in diagnostic pathology . Digitized high resolution images of large tissue areas can effect significantly increased statistical power by analyzing pathologic changes in their entirety or on the basis of randomly selected regions of interest (subsampling). Several commercial (www.definiens.com/solutions/diagnostics, www.visiopharm.com/solutions) and open-source (<http://cellprofiler.org/>) programs for the automatic quantification of absolute or relative cell numbers, cell densities, tissue areas and staining intensities based on conventional stains, immunologic labelling or hybridizations are available . They provide modules for a wide range of image analytical routines, such as noise reduction and object identification which must be combined, adapted and iteratively optimized for each specific task. The latter procedure regularly proving the most demanding and time consuming part of the whole process. Just as current neuropharmacological research into the efficacy of targeted immunotherapy routinely employs image analysis for quantitative histology , eventually neuropathological diagnostic practice will profitably adopt substantial parts of the toolkit already established in computational neuroscience . Naturally high-throughput metricization of even easily recognizable and well defined morphologic features such as nuclear diameter, circumference, roundness or excentricity used for neuroglial differentiation will exceed the patience and focus of even the most experienced pathologist. When even more complex algorithms are used to derive prognostic

information from conventionally or immunohistologically stained sections the cognitive and integrative capacity of human diagnosticians is no match for image analytical pipelines. Similarly the current predominance of brightfield microscopy will be lost to multichannel fluorescence which allows for more exact segmentation of cell populations and more reliable quantification of staining intensities in digitized formats.

A roadmap for a diagnostic reference center for ERN “EpiCare”

Future navigation of the digital route towards web-based slide review and pathology teaching platforms will face a number of technological challenges for which technical and financial support will have to be obtained from the EU. The roadmap towards an internationally integrated European e-Neuropathology reference center will encompass

Freedom of data and software:

Without doubt every procedure in medicine should allow for its stringent and effective control. In the context of computerized applications and algorithmic diagnostics this requires complete transparency regarding source codes, data formats, processes and results. Existing initiatives advocating the global availability of the results of scientific research and the preferential use of open-source over proprietary software

(<https://stallman.org>) will eventually be difficult to oppose at least in the medical field.

Of foremost importance is the effective implementation of the DICOM Standard which has been developed with an emphasis on diagnostic medical imaging including pathology. It is already actively endorsed by the EU (https://ec.europa.eu/eip/ageing/standards/ict-and-communication/other-ict/dicom_en), however, the urgent need for its adoption needs to be impressed more forcefully on manufacturer of WSI equipment worldwide.

Integration of multiple imaging modalities:

The portability and convertibility of digital data formats will enable pathologists to fuse 2D- and 3D-renditions of diagnostic samples visualized by different imaging technologies (e.g. fluorescence, CT, MRI) into multimodal composites.

Rapid 3D-scanning technology can provide volumetric data sets of diagnostic specimens at various stages of processing which are prerequisite to fusion images combining macro- and microscopic details. Collectively multimodal imaging will improve morphological

correlations and the interdisciplinary communication of radiological, electrophysiological and pathological findings.

Securitization of data and networks:

Local and global exchange of medical data is critically dependent on a performant network and storage infrastructure, which must be provided by the ERN hosting organization of the EU. The European Parliament has adopted the *General Data Protection Regulation (GDPR EU 2016/679)*, that will be directly applicable in all Member States of the EU and effective from May 25th, 2018 replacing *Directive 95/46/EC*. Equally relevant in this context is the EU's *Medical Device Regulation (EU) 2017/745 (MDR)* which will be in effect on 26 May 2020. To meet the current and future e-Health requirements of European cybersecurity frameworks (<http://www.consilium.europa.eu/media/31666/st14435en17.pdf>) all pathology-related metadata and image files will have to be end-to-end encrypted using current state-of-the-art cryptography.

Postgraduate medical education and specialist training require only network infrastructure of sufficient bandwidth whereas internet-based image data exchange for diagnostic or review purposes needs sufficiently securitized end-to-end encryption protocols. Currently protection from unauthorized interception can only be assured by the use of auditing the communication protocols' source code and full disclosure of the type of cryptography used. A heightened awareness for the importance of strong encryption and its reliable implementation in all medical communications will be essential for the productive use of all types of data formats in digital pathology.

Increased performance analytics:

Developments in the microscopic visualization of molecular details will reveal additional layers of complexity directly impacting on the practice of personalized medicine and theranostic capabilities of every pathologist. The recognition of morphologic and mutational diversity will require a dramatic increase in diagnostic power which will only be attained in a concerted European effort to fully incorporate computerized image analysis, machine learning and diagnostic algorithms into practice. The need to derive prognostic information from conventionally or immunohistologically stained sections will necessitate the use artificial intelligence (AI) techniques, such as as deep neural networks (DNNs) generated complex multi-parametric decision algorithms which can match and even outperform expert-level human diagnosticians (Djuric et al. 2017).

Establishing a European e-Neuropathology reference center will undoubtedly help in the promotion and validation of these emerging technologies across ERN neuropathology groups and accelerate their adoption in the pathology community at large (Fig. 1).

References

1. Allan C, Burel J-M, Moore J et al. (2012) OMERO: flexible, model-driven data management for experimental biology. *Nat Meth* 9:245-253
2. Bacus J, Bacus J (2001) Method and apparatus for creating a virtual microscope slide. U.S. Patent 6,272,235
3. Bhargava R, Madabhushi A (2016) Emerging Themes in Image Informatics and Molecular Analysis for Digital Pathology. *Annual Review of Biomedical Engineering* 18:387-412
4. Djuric U, Zadeh G, Aldape K, Diamandis P (2017) Precision histology: how deep learning is poised to revitalize histomorphology for personalized cancer care. *npj Precision Oncology* 1:22
5. Fine JL, Grzybicki DM, Silowash R et al. (2008) Evaluation of whole slide image immunohistochemistry interpretation in challenging prostate needle biopsies. *Human Pathology* 39:564-572
6. Goode A, Gilbert B, Harkes J et al. (2013) OpenSlide: A vendor-neutral software foundation for digital pathology. *Journal of Pathology Informatics* 4:27-27
7. Heindl A, Lan C, Rodrigues DN et al. (2016) Similarity and diversity of the tumor microenvironment in multiple metastases: critical implications for overall and progression-free survival of high-grade serous ovarian cancer. *Oncotarget* 7:71123-71135
8. Kamentsky L, Jones TR, Fraser A et al. (2011) Improved structure, function and compatibility for CellProfiler: modular high-throughput image analysis software. *Bioinformatics* 27:1179-1180
9. Kong J, Cooper LaD, Wang F et al. (2013) Machine-based morphologic analysis of glioblastoma using whole-slide pathology images uncovers clinically relevant molecular correlates. *PLOS ONE* 8:e81049
10. Meyer J, Paré G (2015) Telepathology Impacts and Implementation Challenges: A Scoping Review. *Archives of Pathology & Laboratory Medicine* 139:1550-1557
11. Nawaz S, Heindl A, Koelble K et al. (2015) Beyond immune density: critical role of spatial heterogeneity in estrogen receptor-negative breast cancer. *Mod Pathol* 28:1621-1621
12. Pantanowitz L, Sinard JH, Henricks WH et al. (2013) Validating Whole Slide Imaging for Diagnostic Purposes in Pathology: Guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Archives of Pathology & Laboratory Medicine* 137:1710-1722
13. Sevigny J, Chiao P, Bussi re T et al. (2016) The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature* 537:50-56
14. Singh R, Chubb L, Pantanowitz L et al. (2011) Standardization in digital pathology: Supplement 145 of the DICOM standards. *Journal of Pathology Informatics* 2:23-23
15. Stathonikos N, Veta M, Huisman A et al. (2013) Going fully digital: Perspective of a Dutch academic pathology lab. *Journal of Pathology Informatics* 4:15-15
16. Valous NA, Lahrmann B, Zhou W et al. (2013) Multistage histopathological image segmentation of Iba1-stained murine microglia in a focal ischemia model: Methodological workflow and expert validation. *Journal of Neuroscience Methods* 213:250-262

Table

Tab. 1: Technical features of commercial WSI-scanners

| Company | Model | Mode | Capacity | SS | File formats |
|-----------|--------------------------------|--------|-----------|-----------|--------------|
| 3DHistech | Pannoramic 250 Flash III, 1000 | BF, FL | 250, 1000 | st | mrxs |
| Hamamatsu | S60 | BF | 60 | St, J | ndpi |
| Leica | Versa | BF, FL | 200 | st | scn |
| Aperio | ScanScope AT2 | BF | 400 | st | svs |
| Olympus | VS120-L100-W | BF, FL | 100 | st, J | vsi |
| Zeiss | Axio Scan.Z1 | BF, FL | 100 | st, J | czi |
| Huron | TissueScope LE120 | BF | 120 | st, J, wm | hdf |

Abbreviations: BF – bright field, FL – fluorescence, SS – slide size, st – standard (25mm x 75mm), J – jumbo (50mm x 75mm), wm - whole mount (150mm x 200mm)

Figure

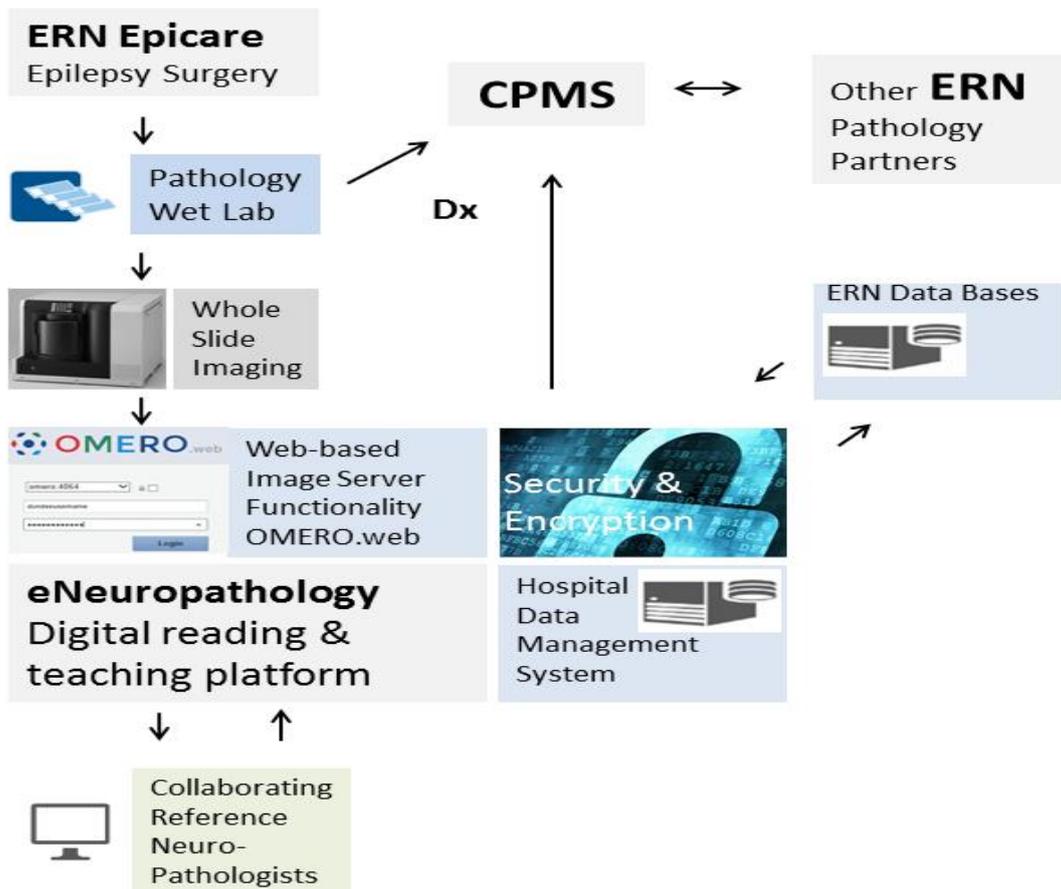


Fig. 1: Flowchart of the referral procedures to be implemented in the proposed European e-Neuropathology reference center. Postsurgically clinical centers collaborating within the European Reference Network's (ERN) Epicare program will have their surgical specimens processed for routine diagnostic work-up by their local neuropathology services; initial diagnoses (Dx) will be entered into the clinical patient management systems (CPMS). After whole slide imaging of the relevant conventionally stained or immunohistological slides the digitized images are uploaded via https to the open-source OMERO platform providing encrypted long-term storage and secure browser-based access to all ERN participants. The panel of ERN neuropathologists will provide consensus diagnoses of necessary involving additional collaborating neuropathologists. All diagnostic and morphological annotations will be synchronized with the ERN data bases, CPMS and participating hospital data management systems.