

# A web-based diagnostic reference centre for the European Reference Network “EpiCare”: recommendations of the eNeuropathology working group

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**ABSTRACT** – 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 surgery cases, the provision of subspecialty expertise as well as training opportunities remains logistically and financially challenging. To improve this situation, the European Reference Network’s (ERN) epilepsy care program (EpiCare, <http://epi-care.eu>) 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 collaborative, diagnostic network initiative (the eNeuropathology reference centre) and delineate a roadmap for its implementation favouring open-source, vendor-independent browser platforms.

**Key words:** epilepsy surgery, diagnosis, European Reference Network, EpiCare, teaching, web-based, neuropathology

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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 antiepileptic therapies (Blümcke *et al.*, 2017). 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, <http://epi-care.eu>) 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 (Pantanowitz *et al.*, 2013). Neuropathologists who, by default, are constantly interacting with neurophysiologists, neuroradiologists, and neurosurgeons have developed some familiarity with the data formats current in their neighbouring 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 60 Hz 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 colour 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 and Bacus, 2001) and are now able to digitize standard and fluorescence microscopic sections in various formats (*table 1*). It currently takes under two minutes to scan conventional and special stains of a 15 × 15 mm<sup>2</sup> 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 real-time or post-acquisition. Depending on tissue area, magnification, number of scanning levels, and compression formats used (non-lossy or high efficiency lossy, such as JPEG XR), 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 centres 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

**Table 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, FL	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

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

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 (Goode *et al.*, 2013). Ideally, however, WSI files should comply with the “Digital Imaging and Communications in Medicine” standard, synonymous with the ISO (International Organization for Standardization) standard 12052 whose supplement 145 defines objects containing very large image data sets common to pathology (Singh *et al.*, 2011). 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. These issues have impact also for eNeuropathology with regard to training and agreement studies for rare epilepsy-associated brain tumours or cortical dysplasias. 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 (Fine *et al.*, 2008). Digitized high-resolution images of large tissue areas can effect significantly increased statistical power by analysing pathological changes in their entirety or on the basis of randomly selected regions of interest (subsampling). Several commercial ([www.definiens.com/solutions/diagnostics](http://www.definiens.com/solutions/diagnostics), [www.visiopharm.com/solutions](http://www.visiopharm.com/solutions)) and open-source (<http://cellprofiler.org/>; Kamensky *et al.*, 2011) programs for the automatic quantification of absolute or relative cell numbers, cell densities, tissue areas, and staining intensities, based on conventional stains, immunological 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 (Valous *et al.*, 2013); 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 (Sevigny *et al.*, 2016), 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 morphological 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 (Kong *et al.*, 2013). 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 (Nawaz *et al.*, 2015; Heindl *et al.*, 2016). Similarly, the current predominance of brightfield microscopy in routine diagnostics will have to cede ground 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 centre network for ERN “EpiCare”

Future navigation of the digital route towards web-based slide review, clinical audit, international agreement studies, 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 centre will encompass these features.

### 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](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.

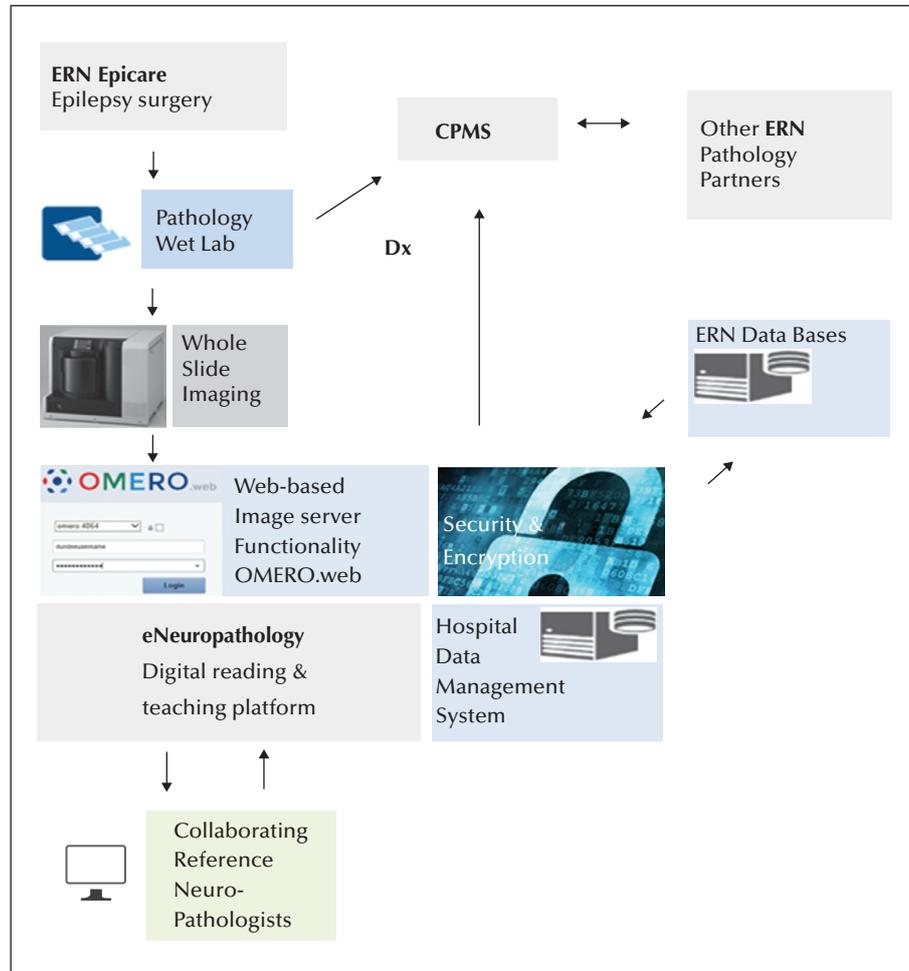
### Integration of multiple imaging modalities

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 (Magee *et al.*, 2015).

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

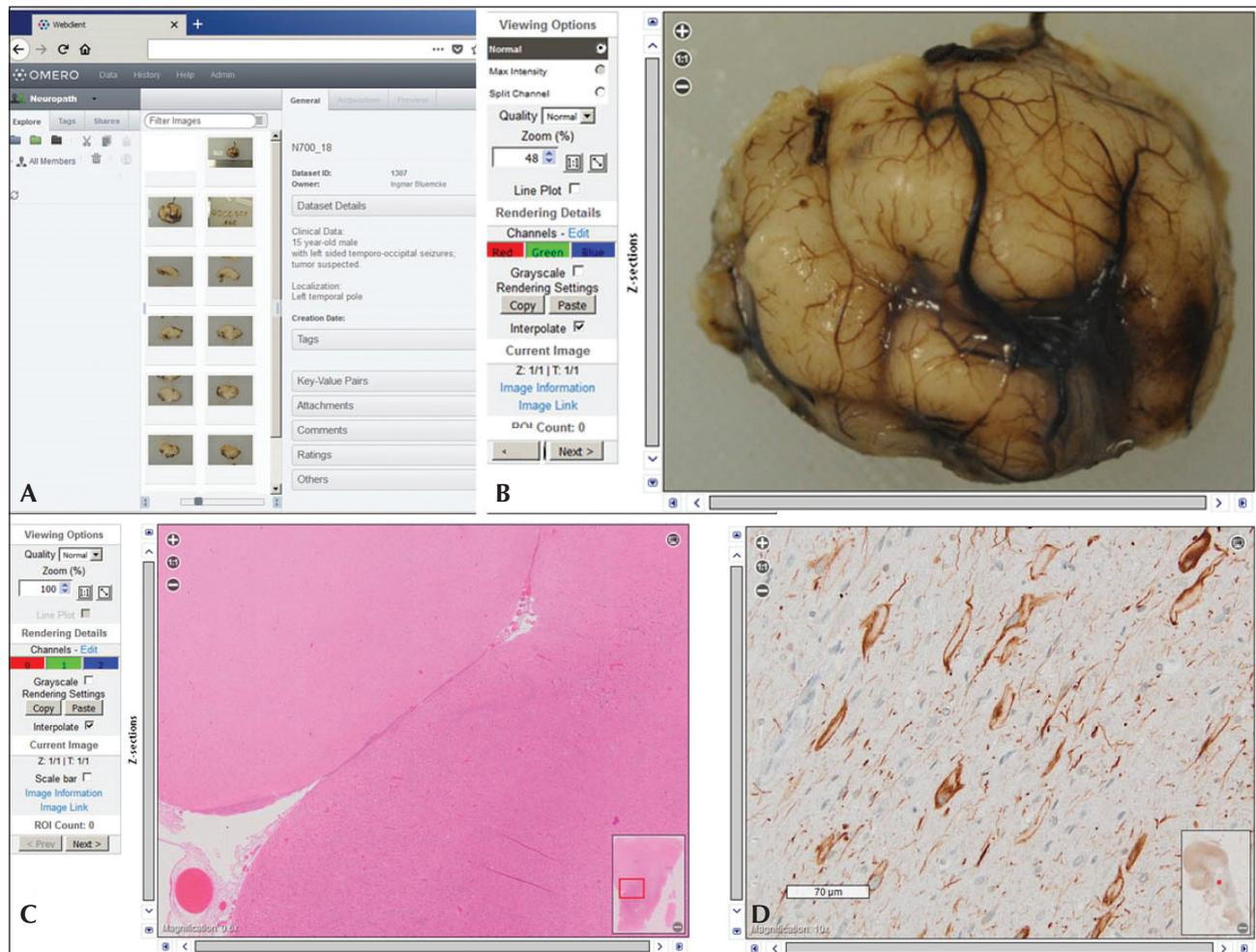
Local and global exchange of medical data is critically dependent on a performant network and storage



**Figure 1.** Flowchart of the referral procedures to be implemented in the proposed European eNeuropathology reference centre. Postsurgically, clinical centres 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 immunohistochemical 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, if necessary, involving additional collaborating neuropathologists. All diagnostic and morphological annotations will be synchronized with the ERN databases, CPMS, and participating hospital data management systems.

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/st14435/en17.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.



**Figure 2.** Case report for reference eNeuropathology using the open microscopy environment platform, OMERO.

### 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 morphological 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 of artificial intelligence (AI) techniques, such as deep neural networks (DNNs) generating complex multi-parametric decision algorithms which can match and even outperform expert-level human diagnosticians (Bhargava and Madabhushi, 2016; Djuric *et al.*, 2017). AI-assisted pathology will only be successful, however, if accompanied by

intensified efforts to standardize immunohistological staining protocols between laboratories (Lin and Chen, 2014). Several quality assurance programs in eyepiece-based diagnostic immunohistochemistry, such as NEQAS (<https://ukneqas.org.uk/programmes/result/?programme=neuropathology>), NordiQC (<http://www.nordiqc.org/about.php>) and the Canadian Immunohistochemistry Quality Control (ciQC; <http://cpqa.ca/main/>) are already well established.

Only immunofluorescence provides superior segmentability, signal quantitation, spatial resolution, and colocalization information to ensure the image quality mandatory for meaningful automated analyses. Therefore, future standardization efforts will have to incorporate multi-channel immunofluorescence imaging. Input into DNN will also require rich metadata encompassing methodological details on tissue fixation, processing, and staining conditions embedded in each digitized image format.

Establishing a European diagnostic reference network, i.e. eNeuropathology, 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 (figure 1).

## Case report (figure 2)

The open-microscopy-environment software OMERO as a platform for secure web-based neuropathological review and consultation: (A) Authenticated user's view of the OMERO landing page which lists the relevant clinical information as well as the macro- and microscopic images available for a 15-year-old male patient with left-sided focal seizures originating from the temporo-occipital lobe. (B) Macroscopic image of the surgical specimen submitted after formalin fixation showing the resected left temporal pole. The OMERO viewer allows zoomified magnification via key stroke or mouse wheel. (C) Magnification of an H&E stain demonstrating an anatomically and surgically well preserved human neocortex with inset on lower right providing whole-slide overview for orientation. (D) Immunohistochemistry using antibodies (clone SMI32) specific to the non-phosphorylated neurofilament protein, confirming the presence of enlarged dysmorphic neurons; vimentin-positive balloon cells were also identified (not shown here). The histopathological diagnosis transmitted was focal cortical dysplasia (FCD) IIb (ILAE classification 2011). □

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