Spotlight on
Digital Pathology

I saw the future of pathology and it's digital, says **Peter Hufnagl** ≫ Mobile technology could enhance digital pathology, **Johan Lundin** is convinced ≫ **Marcial Garcia Rojo** proposes options for storing digital images which reduce costs and improve efficiency ≫ Goodbye to the microscope says **Paul van Diest** as the digital age is in diagnostics dawns ≫ **and many more**

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Johan Lundin, M.D., Ph.D., Karolinska Institute Stockholm, Sweden
and Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland

I saw the future
of pathology – and it's digital

Healthcare is going digital. No doubt about it, Prof. Hufnagl predicts. Information and communication technologies have gone beyond moving data from one place to the other; they are triggering stellar improvements in healthcare: diagnoses are becoming ever more precise, therapies ever more personalised. The extent to which the individual clinical disciplines have progressed in their technological development varies greatly. In pathology, for example, change has only just set in. However, it is already obvious, that digitisation will change the discipline forever.

Scepticism towards digital systems is still widespread and fundamental questions regarding the sense and purpose of the new technology in the pathology department continue to be raised. These concerns are primarily caused by the significant necessary financial investments in hardware and software.

Basic equipment encompasses slide scanners, data storage devices and an image management system. There are many opinions as to whether and when the purchase of these digital pathology devices makes economic sense. Clinicians, normally not interested in viewing histological slides, are sometimes enthusiastic if they get brilliant overview images via DICOM access. However, there are – throughout Europe – very encouraging examples that demonstrate the successful step-by-step transformation from microscope-based histopathological diagnostics to digital diagnostics.

'Unfortunately, these positive examples are not sufficiently published in the pathology community', says Professor Peter Hufnagl, Head of Digital Pathology at the Institute of Pathology at Charité, Berlin, and President of the 13th European Congress on Digital Pathology in Berlin in 2016. 'The two types of workflow, digital and analogue, can co-exist very well and, for some institutions, such a two-pronged approach might be the best solution in the long run. That means you don't have to wait until you can afford fully-fledged digitisation. Start small!'

Workflows in the pathology lab will benefit with only one third of the diagnostic task being digitised. Communication with clinicians will become faster, simpler and more effective. For large institutes involved in research and teaching complete digitisation makes sense, Professor Hufnagl underlines: 'The biobanks being established in the academic environment particularly profit from a digital slide archive, since it allows larger sample groups to be selected and to check whether certain slides are suitable for a research project.'

Peter Hufnagl, Head of Digital Pathology, Charité Berlin

Many pathology labs are still hesitant to jump on the digital bandwagon although the industry has recognised the market potential long ago and is actively driving its rapid growth through technological innovation. The fact that the use of a virtual microscope saves a lot of money on expensive samples is only one of the arguments manufacturers use to lure new customers. More importantly, however, digital pathology optimises quality assurance and thus contributes to a lab's competitiveness. Digitisation provides a better overview of comparable cases; it allows the application of quantitative methods to check diagnoses and accelerates obtaining second opinions.

Hufnagl is convinced that it is only a matter of time before digital pathology will prevail. In radiology, he points out, there were very similar concerns regarding on-screen readings – today this is standard operating procedure. The fact that, due to image volumes in pathology – 150.000 x 300.000 = 45000Mpixels – digitisation is taking root 20 years later than in radiology, is an advantage. We can learn from radiologists' experiences and from many of their solutions, because they can be translated into the questions that need answering in pathology.' Having said that, there are indeed also fundamental differences between the

two image-based disciplines. The different CT slices, for example, are automatically adjusted – a step that is not so easy with histopathological samples that consist of three-dimensional tissue, such as a tumour. 'The registration of slides with different stains from the identical block is sometimes difficult. Differences between slides, such as pressure points, tiny fissures etcetera are originated by mechanical alterations in the lab or switching the lab during processing. Software programmers have not yet found a solution to deal with all different artefacts in a sufficient manner.'

In addition to diagnostics with the virtual microscope, digital pathology is being pushed ahead by other technological innovations, such as molecular pathology, particularly Next Generation Sequencing (NGS). This procedure can detect gene mutations much faster and simpler than before. This allows inter alia precise characterisation of tumours, which in turn translates into customised cancer therapies. Today NGS is considered the path-breaking technology towards personalised healthcare, because it is expected to have a crucial impact on diagnosis and prognoses of cancer and other diseases.

'Thus digitisation makes pathology much more visible in the landscape of clinical disciplines', Peter Hufnagl confirms, adding: 'This development is being supported on the organisational level, for example by the establishment of tumour boards that include pathologists and it will be accelerated by the new technologies.'

Consequently not only the work environment and the 'job description' of a pathologist are undergoing radical transformation; the image of the pathologist is also changing. For years, pathology has been struggling with recruiting problems. Digitisation is giving the field a fresh, modern look and more junior physicians are becoming interested in this field.

KAROLINE LAARMANN

>> **Wednesday, May 24th ● 5.30 PM–7 PM ● Room: Hörsaal**
The 13th ECDP – a scientific overview
P. Hufnagl, Berlin, Germany

Morphological medicine and pathology will boom

Professor Klaus Kayser, former Head of the Institute of Pathology at Heidelberg University Hospital's Thorax Clinic, may be retired but he continues to be a leading figure in his discipline, a visionary, famous for this critical and 'out of the box' thinking. During the run-up to the European Congress on Digital Pathology (ECDP), Ralf Mateblowski asked the expert about telemedicine and standards and, even more importantly, a discipline in transition.

As a mere communication tool, telepathology crosses time and space barriers by enabling data analysis from anywhere and at any time, no matter when and where the data were generated. The crucial advantages of digital pathology (DP) lie in its time independence and the ability to 'turn back the hands of time': the evaluation of histological specimens involves much more than looking at the slide and wobbling it back and forth; rather, it requires returning to the original, switching between the original specimen and its stained or marked version.

A modern routine lab that processes 50,000 to 60,000 cases per year covers two major areas: lab and pathology. At first, digitisation creates more work in the lab, since the scanner has to be loaded with slides, a cumbersome procedure that requires some getting used to. The process is speeded up when the pathologist receives his specimens pre-sorted, e.g. the liver and lung specimens. If – and only if – DP is optimally organised, the lab technician no longer needs to move around, but can perform all necessary steps at the workstation, such as transferring the digitised slides, including suggestions for analysis, to the pathologist. Ideally, results are made available to the departments via the HIS.

Efficiency, however, is not only a matter of the degree of digitisation and organisation; to a large extent it is a matter of having access to pathologists who are specialised in certain organs. They can be found in large institutions such as Charité in Berlin, or the University Hospital in Heidelberg. Currently, 'human' routine pathology, unlike experimental pathology in the pharmaceuticals industry, it is struggling with standardisation even though defined strategies are available for all parameters in order to reduce imprecise measurements, such as thickness, intensity of the dye, or correct lighting in order to be able to assess suspect areas in a tumour specimen.

In Heidelberg, algorithms were developed that provide high sensitivity and specificity (95%) for difficult-to-diagnose tumours such as mesothelioma, or metastatic adenocarcinoma, and can be applied for breast and lung carcinoma. Even if the software programme itself is not yet perfect, the algorithms work and routine usage is around the corner with confirming parallel studies the only component missing.

Nevertheless, morphological medicine and pathology will experience an enormous boom as scanners with a €100,000 price tag are becoming obsolete since US-American drones feature entirely new optics. Cheap iPhone lenses can be combined with scanners – such projects are underway in China, Finland and the US. If these ideas really pan out, scanner prices will drop by factor 10 and high investment
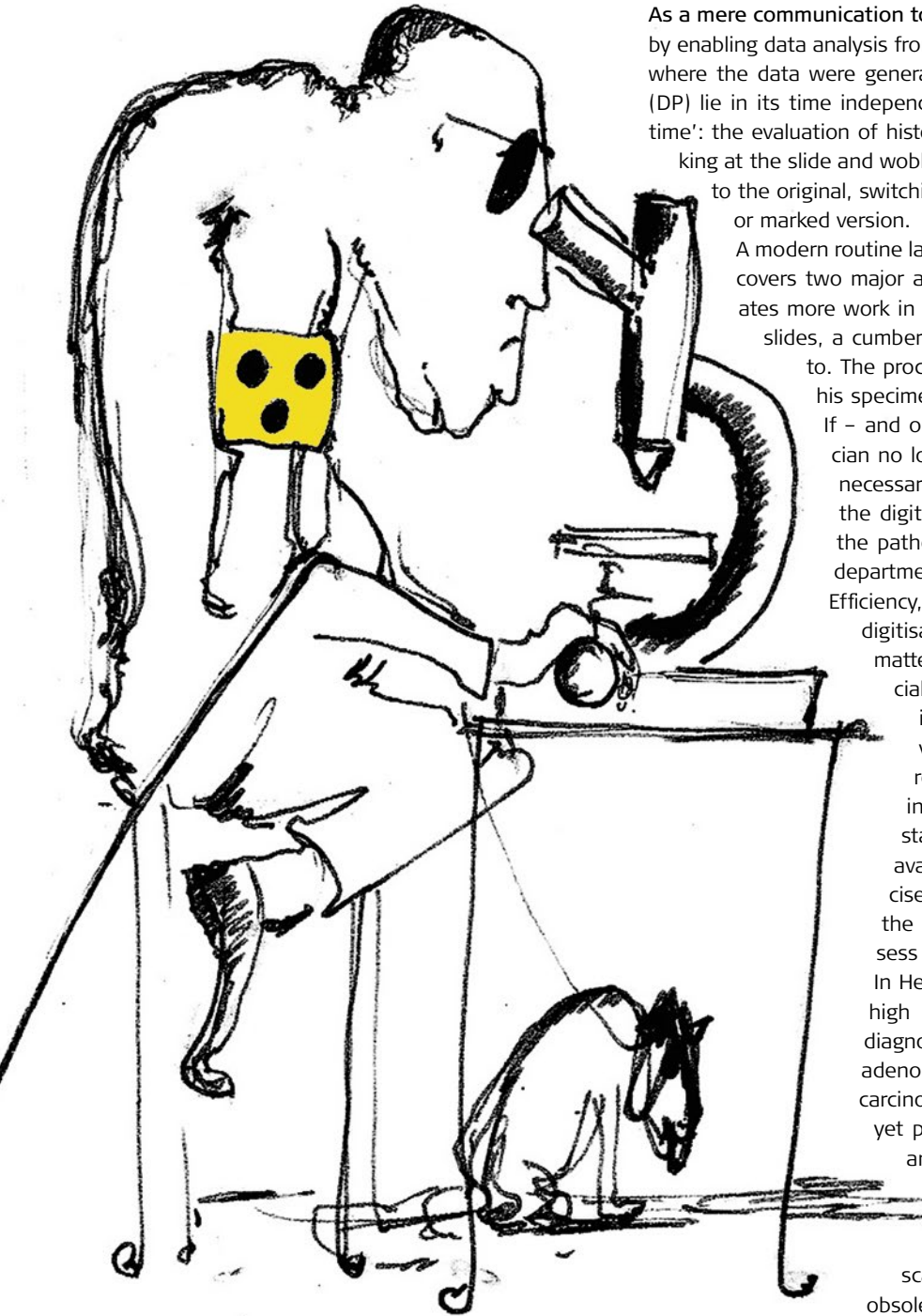
costs will be a thing of the past. While developments in terms of data transmission are stagnating – whether the hoped-for revamping of the internet with fibre optics, or the use of satellite telephones. for example in Africa, will spell progress remains to be seen – the imaging market is immense with hardware and software solutions becoming more and more affordable.What's really missing is an umbrella organisation bringing together pathologists and lab and IT experts. The wait-and-see attitude of the industry is a definite obstacle: many companies have excellent IT staff who potter about without understanding the work of the pathologist.

On the other hand there are many specialist physicians who are highly interested in IT but lack the necessary knowledge. Communication does not really happen!

In the now defunct GDR mathematicians and physicists frequently worked in institutes of pathology alongside their colleagues in medicine and to a large extent it was this direct access that enabled them to develop innovations. This very effective cooperation was abolished and today in Germany there is a slew of insti-

tutes – Fraunhofer, Max-Planck and the German Cancer Research Centre – all of which work in different and distinct areas. However, when it comes to application-specific issues, close spatial proximity is the non plus ultra. The almost philosophical contemplation of the relationship between structure and function is a topic only very few pathologists are interested in. In biology it is a matter of the inside and the outside. When observed long enough, a structure will turn into a function. This approach opens a different view on pathology: Today, no theory, be it energy balance, metabolism, or any one of the common physical-chemical concepts, can explain why a cancer lesion of 2 cm diameter can destroy the entire system and kill the patient. 'Cardiovascular failure' is nothing but a catch-all phrase because, in the end, structure-associated functions defined on the gene level determine what does not function and why. Which parameter is it that triggers a domino effect that causes the human system to collapse? Digital pathology may well help to understand the construction of 'life as such'.

RALF MATEBLOWSKI

Klaus Kayser MD PhD, Professor of Pathology and Epidemiology, Dr. rer.nat. (Physics), Dr. med, Dr. honoris causae mult. headed the Institute of Pathology, Thoraxklinik, University of Heidelberg until 2005. The former faculty member of Heidelberg and Berlin University (now Charite Berlin) is a pioneer in electronic medical communication, research on image analysis, lectins, structural entropy, and lung cancer.

» **Wednesday, May 24th ● 5.30 PM–7 PM ● Room: Hörsaal**
History of the European conference series on digital pathology: memories and perspectives
K. Kayser, Heidelberg, Germany

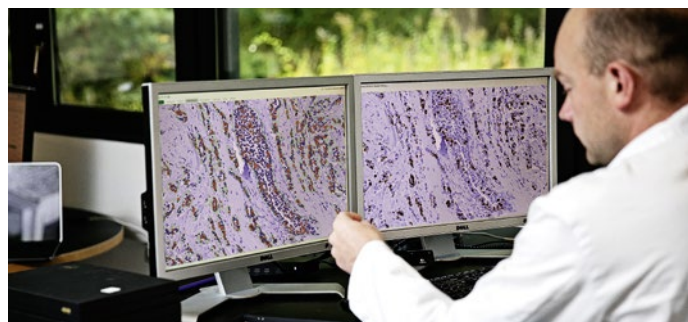# New study strongly encourages the use of digital image analysis in Breast Cancer
– the most common cause of cancer death among women worldwide

In a study of 436 breast cancer cases with 28 years of survival data histo-pathologists from Karolinska Institute, Stockholm, Sweden shows that protein tissue-based biomarker data are at least as good as gene expression assays.

When summarizing the study from Karolinska Institute, Stockholm, Sweden, manual assessment of the biomarkers ER, PR, HER2, and Ki67, with an emphasis on the latter, is in most aspects an inferior alternative to Digital Image Analysis (DIA). When comparing sensitivity and specificity to PAM50 subtype classification and prognostic power DIA outperforms manual scoring. Being able to reduce time to diagnosis as well as the overall cost for breast cancer testing are good news. What makes it even more convincing is that all subtypes including the Luminal B, which is widely considered being the most challenging distinction in surrogate sub-classification, produced a slightly better concordance and Cohen's agreement.


Digital Image Analysis (DIA) a viable and competitive, if not superior, alternative for gene expression testing in breast cancer

The cost of combining automated DIA with biomarker analysis is a fraction of the cost of a gene expression test, which is time consuming to conduct and often has to be done outside the histopathology lab. Combining biomarker analysis with DIA creates value for all parties involved: patients, pathologists and payers. For patients it is to receive a faster treatment, for pathologists an opportunity to reduce time consumption and allocate precious resources to more qualified tasks and for payers a much more cost efficient diagnostic method. An operator of the Visiopharm DIA system has the option to manually evaluate the digitized slide image and perform the diagnosis based on this, but DIA also allows the computer to conduct an automated analysis avoiding subjective assessments of biomarker positivity. This implicates that an approach like DIA to count full tumor cross-sections or to make automated 'hot spot' analysis could free up time for histopathologists, biomedical scientists or other laboratory personnel. "The desire for faster and more cost-effective solutions for the histopathology laboratories need no longer be a dream. In complex heterogeneous tumors DIA in combination with immunohistochemistry has shown to be a viable competitive option for laboratories lacking in-house gene expression testing," says Helle Fisker, CMO at Visiopharm.

The DIA system used in the study was the Visiopharm DIA software VIS 4.6.3
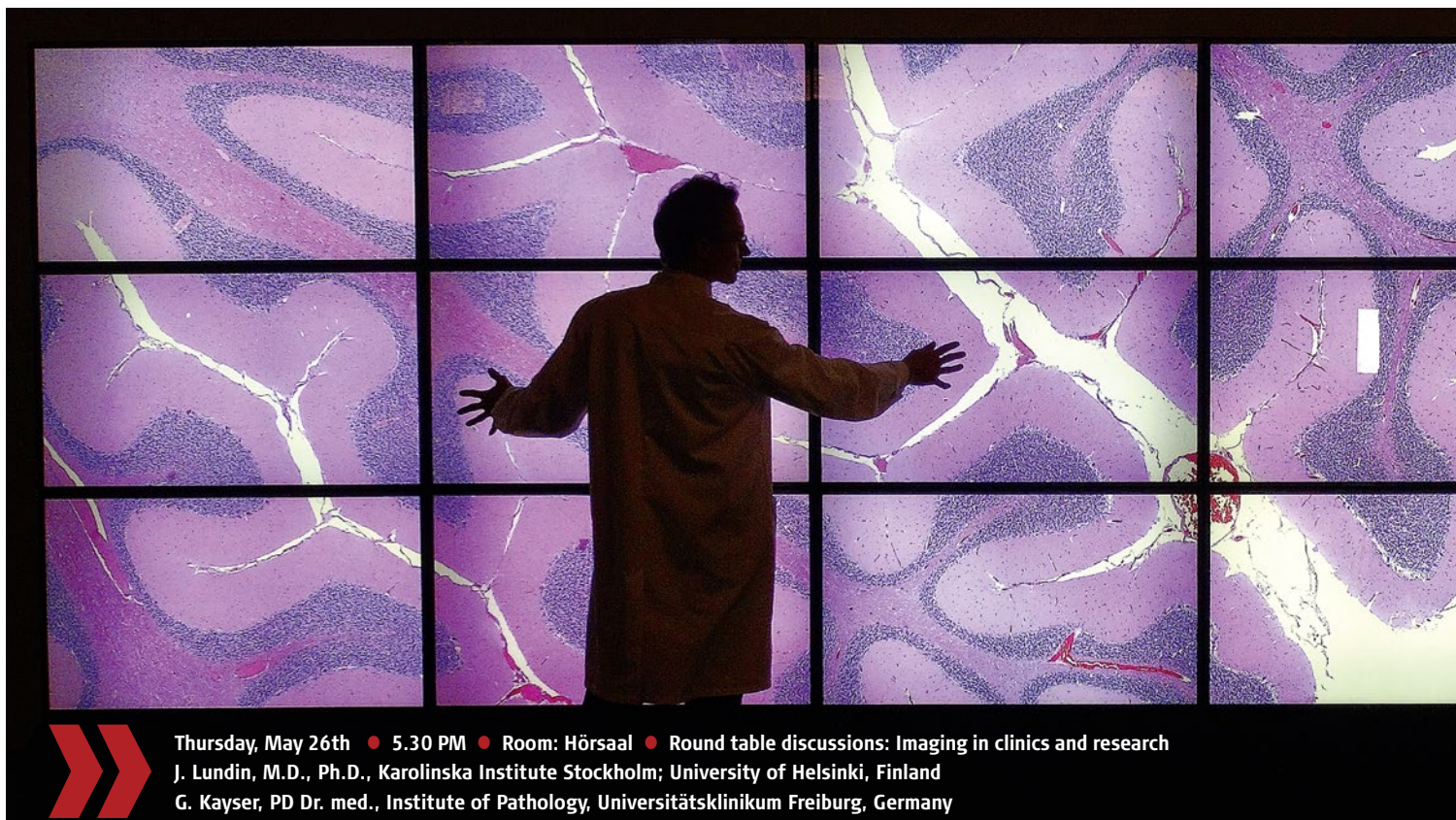
Mobile technology could enhance digital pathology

Mobile technology could prove an important catalyst in helping take digital pathology onto a new level in delivering clinical diagnostics.

For a number of years, pathology has moved more slowly than other fields that have embraced digitisation, primarily because of the costs and scale of the required equipment, according to Professor Johan Lundin, who is Research Director at the Institute for Molecular Medicine Finland (FIMM), hosted by the University of Helsinki.

An inability to take digital microscopy out of the laboratory as a portable tool has also been a consideration, however, that is about to change with a number of mobile digital microscopes in development.

Among them is Professor Lundin's research group at FIMM and Karolinska Institutet in Stockholm, which has been working to pioneer the MoMic portable scanner, currently undergoing tests in Finland and Tanzania.

He explained: "Having the microscope closer to patient, or where the samples are obtained, would improve the way you can perform diagnostics, making it more rapid and more accessible. It would improve the workflow of diagnostics, not just in pathology but also microbiology, cytology and forensic medicine."

The low-cost, easily transported mobile microscope and scanner developed by the Finnish researchers enabling the use of rapid, computer-assisted remote diagnostics, uses cheap components originally intended for mobile phones and connects wirelessly to a cloud server to which it transmits images for machine vision analysis.

"Digital pathology, and digital microbiology, can make much use of technology from the mobile phone industry if combined with the optical components you need for microscopy," added Professor Lundin, who is chairing sessions at the 13th European Congress on Digital Pathology in Berlin from May 25-28.

MoMic is still an academic study, but what differentiates it from similar mobile microscopy projects is the scanner facility it incorporates, which is vital as it enables the digitisation of the whole sample.

"If you cannot digitise the whole sample it is very difficult to use it for medical diagnostics because it is extremely difficult to control where the important part of the sample is on the slide," he pointed out.

The advantage of MoMic is that it becomes a point-of-care device and can attain laboratory-levels of microscopic resolution and scan a sufficient area of the sample to enable diagnosis. Once a sample is obtained it can facilitate faster digital diagnosis or the ability to consult remotely.

Professor Lundin said the MoMic project started with the goal of creating an instrument for low resource settings and currently five prototypes are being tested.

Field studies are under way in a small hospital in a provincial area of Tanzania, a country where there are less than 20 pathologists among a population of 45 million people.
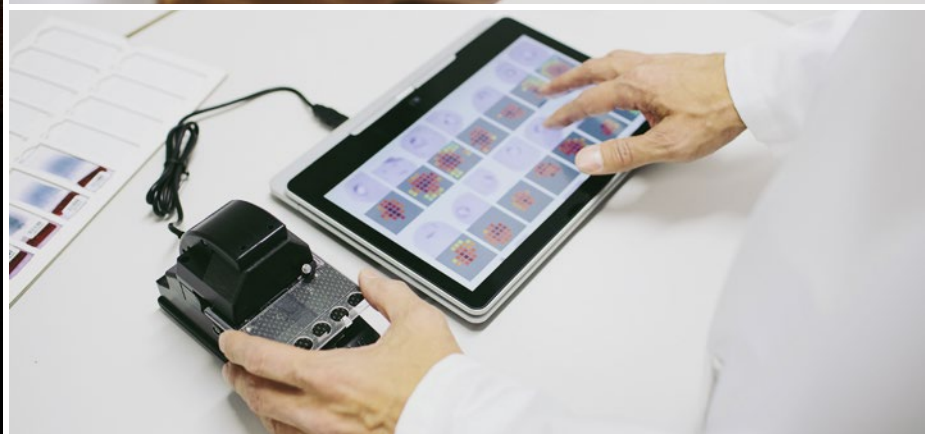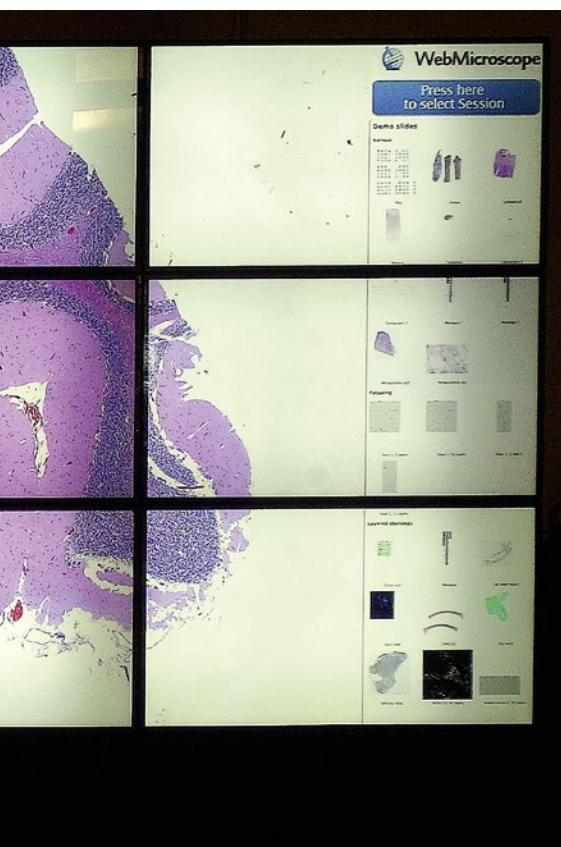
In the three-year pilot programme, patient samples will be diagnosed both on location and remotely based on the digitised sample. In such countries, he believes MoMic will make an impact in the diagnosis of cancer as well as infectious diseases including malaria, tuberculosis or stool parasites.

Other tests are under way in Finland for cancer diagnostics during surgery, using the device away from the lab for pathologists to view samples to check tumour margins and ensure the lymph nodes are clear from cancer

The next steps, supported by a government innovation grant, are to establish routes to commercialisation with partners having been identified to transform it into a product from the prototypes, with large-scale validation studies planned for next year.

Professor Lundin also sees portable digital pathology microscopy being used in parallel with larger high throughput scanners, supporting single pathologists in smaller units, a move he believes would open the world of digital pathology to more practitioners.

The mobile microscope would also be well suited for research work, including the study of tissue and cell samples to identify the expression of biomarkers in cancer research, believes Professor Lundin.

MARK NICHOLLS

Professor Johan Lundin is Research Director at the Institute for Molecular Medicine Finland (FIMM) hosted by the University of Helsinki and Guest Professor in Medical Technology at Karolinska Institutet, Stockholm, and also Associate Professor in Biomedical Informatics at the University of Helsinki. His key research aims are to fully utilize development within information and communication technologies for improvement of diagnostics and care of the individual patient.

We expect to
see some changes

Strictly speaking, digital pathology has not yet resulted in any groundbreaking changes for clinical diagnostics. The conventional light microscope introduced to pathology around 100 years ago continues to be the most important tool for pathologists. Nevertheless, in the future, according to private lecturer Dr Frederick Klauschen, Head of the Molecular and Systems Pathology Group and Consultant at the Institute for Pathology at the Charité Clinic Berlin, we can expect to see some changes from the introduction of digital technology.

'Digital pathology is supporting us already in making information from tissue sections more easily quantifiable with the help of computer assisted systems,' says pathologist Dr Frederick Klauschen. 'However, when it comes to pattern recognition, and therefore tumour typing and classification into malignant or non-malignant tumours, the pathologist will remain superior to the computer for the foreseeable future.'

Let the computer do the counting
To date, the biggest innovation is a more objective and standardised view and quantification of certain tissue characteristics facilitated by image analysis procedures. 'One example of this is the measurement of the proliferation index,' Klauschen says. This can be determined via the immunohistochemical detection of the protein Ki67, which is found in the cell nucleus of proliferating cells. The result normally shows some individual cell nuclei in the normal histological colour (blue) and other cell nuclei where Ki67 is detected in brown or red shades.
Counting the frequency of the brownish

colours was once something the pathologist had to do 'manually'. Now, however, the counting can be done with the help of a computer, using representative image regions. 'We have developed a specific programme for this purpose, the so-called Ki67 Quantifier. This software supports us with the counting and determination of the proliferation index. It facilitates standardised, automated and precise quantification,' Klauschen explains.
'We work very closely with the German Breast Group with regards to breast cancer, for the validation of such procedures.' Tumour samples that arrive, from all over Germany and other countries, are examined in the Institute for Pathology at the Charité Clinic, which acts as a pathology reference centre. The above-mentioned software is then utilised in the context of these studies and validated based on clinical data.
Digital procedures are currently being developed and tested to examine different types of tissue characteristics and markers. 'However, many of these procedures are not yet ready for use,' the pathologist points

out, adding: 'although some Institutes are already using these software solutions it will take some time before all areas of diagnostics will benefit from them.'

Common problem – lack of standards
One big problem with the practical application of image analysis procedures is the lack of standards. Although there is a dialogue and exchange between the various areas of pathology, the analysis of tissue slides from different labs is difficult, the specialist regrets. There are no unified standards regarding histological colourings. 'Each laboratory produces slightly different depths of colours, so the sections differ from one another. Pathologists are not normally worried by a stronger pink or blue – a computer, however, then may have problems with the classification. The objective is the development of procedures which are independent of colour,' he emphasises. Although previously not an important topic in pathology, these types of standardisation are works in progress, having gained importance only with the introduction of digitisation.
Klauschen sees a further difficulty in the practical implementation of digitisation in day-to-day pathology. 'Although we have the technology available, scans of sections are only compiled for special studies, conferences, or for research projects. The actual pathology workflow is not digitised.' One reason for this is the vast number of tissue sections produced at the Institute every day and the related expenditure of time for the digitisation of each section.
The data volume of the images still represents a big problem as pathological images have much larger file sizes than those in radiology, for instance. 'We have amounts of data which simply represent big challenges for the IT infrastructure. There is room for development here, particularly regarding the archiving of all this data.' Klauschen is aware of only two institutes where the workflow
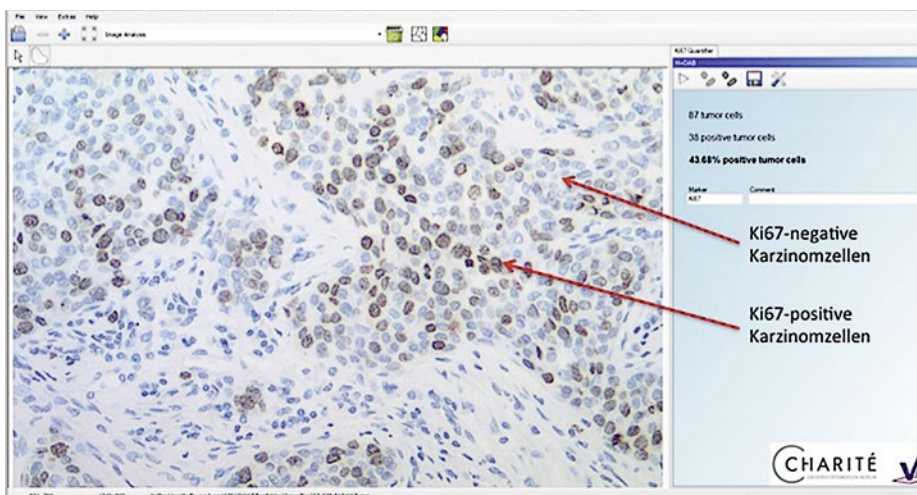
1. Ki57 Quantifier: Determination of the Ki67 proliferation index in breast cancer. The image shows the original immunohistological image (brown: Ki67-positive (proliferating) cells, blue: Ki67-negative (non-proliferating cells)
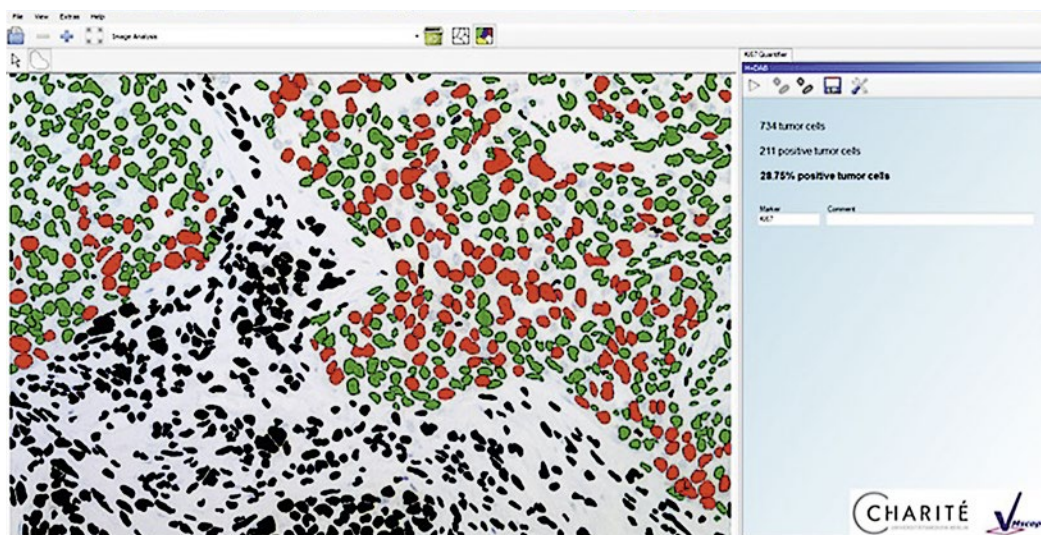
2. Ki67 Quantifier: Computer-assisted diagnosis for the determination of the Ki67-proliferation index in breast cancer. This shows the analysis result of the original image 1. Black: Stroma, red: Ki67-positive tumour cells, green: Ki67-negative tumour cells

Frederick Klauschen MD is a pathologist, physicist, lecturer and consultant as well as Head of the Molecular and Systems Pathology Group in the Institute for Pathology at the Charité University Clinic in Berlin. Up to 2009 he worked at the National Institutes of Health in Bethesda, USA and he is currently a Junior Fellow at the Einstein Foundation in Berlin.

is completely digitised – one in the Netherlands, the other in Sweden. Despite the numerous challenges the pathologist believes that digital pathology means progress, which, in future, will also play an important part in the integration of conventional-morphological and molecular pathology. 'Fluorescence-in-situ hybridisation visualises genetic changes in the tissue. Digitisation and computer-assisted evaluation can be of enormous benefit here.

'I also believe that digital pathology will become important for the interpretation of molecular profiles in the tissue context.'

MARCEL RASCH

Digital pathology: a new diagnostic technology

Histopathologists play key roles in diagnosing disease entities and determining biomarkers related to the prognosis and response to specific therapy of malignant tumours.

Histopathology is still firmly based on cell and tissue morphology supplemented with in situ molecular information and these together can be studied using an optical microscope.

Digital microscopy creates a digital representation of the whole microscopic slides at decent quality, which can be dynamically viewed, navigated and magnified via a mouse and computer monitor, and shared though computer networks without spatial and temporal limitations.

Digital slides can be integrated into the hospital information system (HIS) and accessed through intra- or internet for teaching, primary diagnosis, teleconsultation and quality assurance.

Discrete pixels of calibrated qualities allow automated image analysis and signal quantification for drawing unbiased conclusions in diagnostic and research applications. Therefore, utilisation of the full power of computer technology to access multiple functions and the internet grants digital microscopy great potential to upgrade the efficiency of pathology workflow and pathologists. By resolving critical issues, including
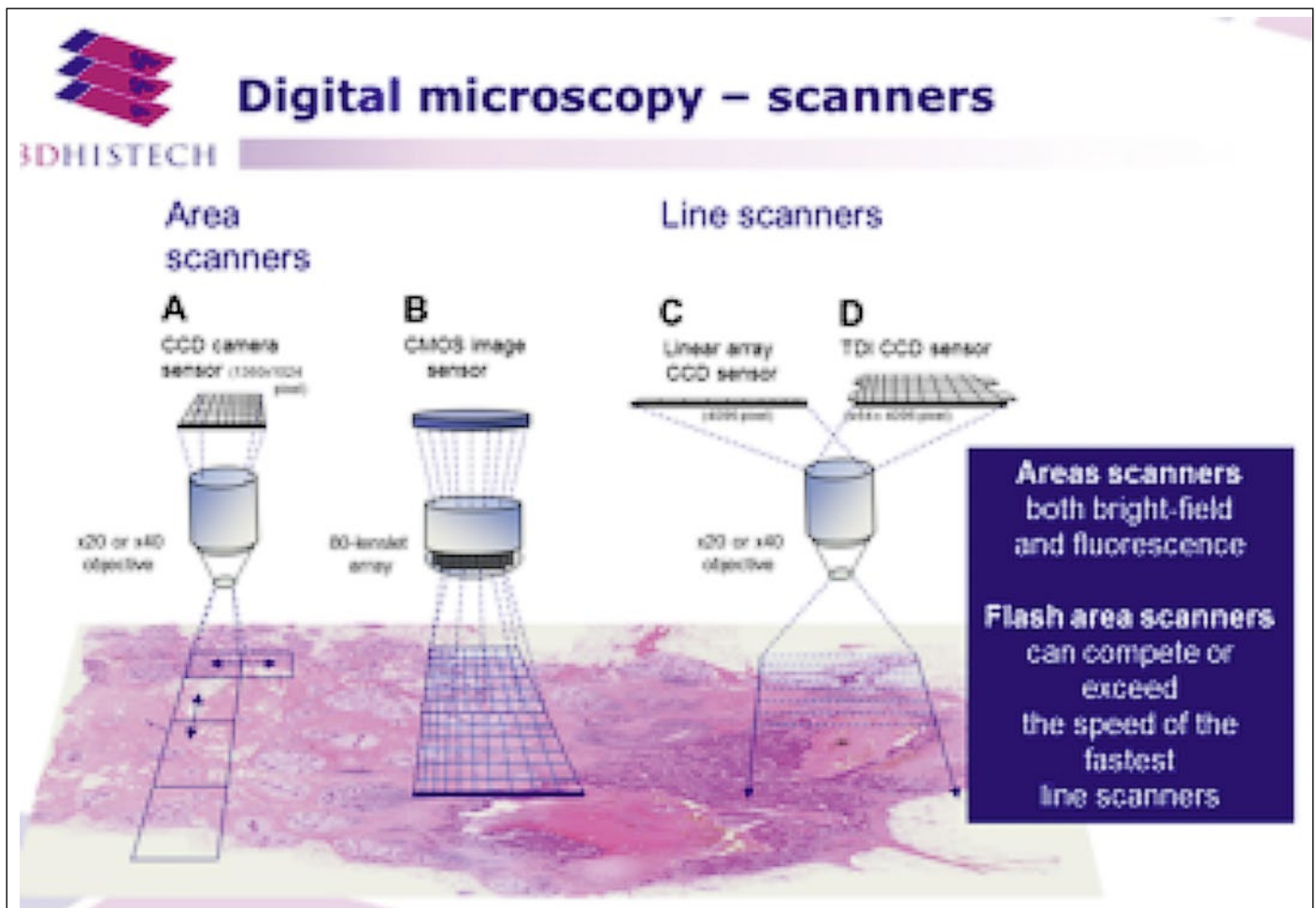

Fig. 2 Schematic representation of area scanning (A, B) and line scanning (C, D) techniques used by available slide scanners. A) Classical area scanners collect large series of images at x-y dimensions through a microscope objective with a CCD camera, either in bright-field or fluorescence mode. B) The area scanner combining an 80-element lenslet array with complementary metal oxide semiconductor (CMOS) sensor can cover large section areas at once. C) Typical line scanners can collect image strips from the continuously moving slides through an objective using a linear array light sensor, which is, however, not sensitive enough for fluorescence signals. D) Combination of 64 or more of linear array sensors permits TDI (time delay and integration) scanning, where consecutive sensors cumulate the signals making TDI appropriate also for fluorescence scanning
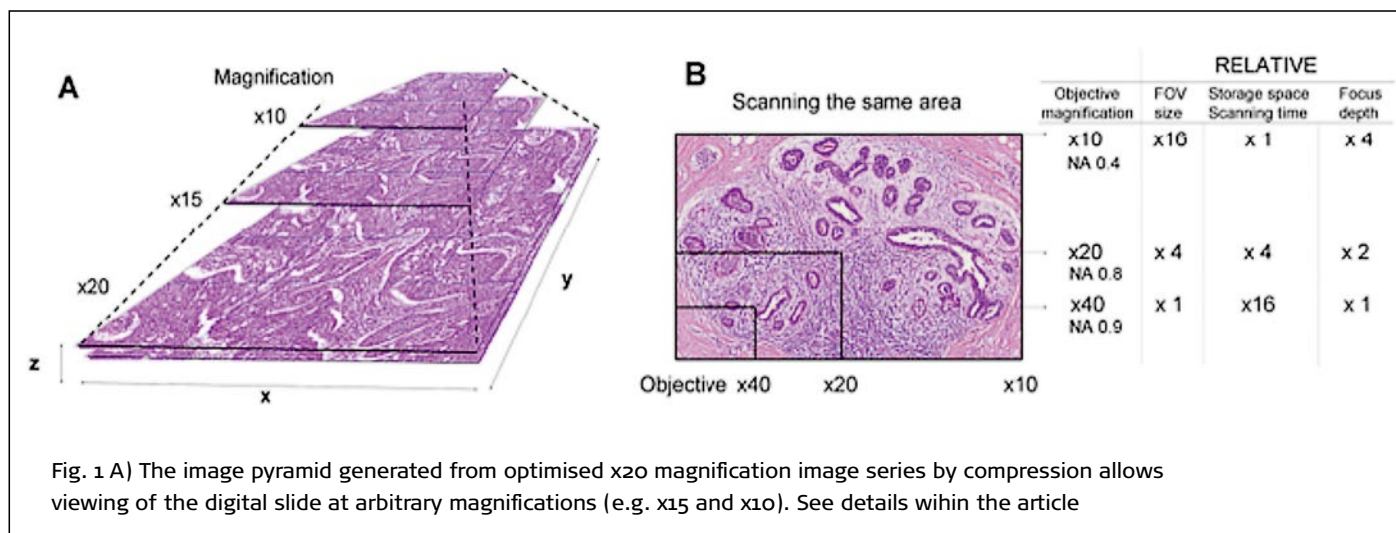
Fig. 1 A) The image pyramid generated from optimised x20 magnification image series by compression allows viewing of the digital slide at arbitrary magnifications (e.g. x15 and x10). See details wihin the article

	RELATIVE		
Objective magnification	FOV size	Storage space Scanning time	Focus depth
x10 NA 0.4	x16	x 1	x 4
x20 NA 0.8	x 4	x 4	x 2
x40 NA 0.9	x 1	x16	x 1

standardisation of data formats, secure and fast internet communication and medico-legal aspects, digital microscopy is expected to play a revolutionary role in future histopathology.

Digital slides created by slide scanners

Digital microscopy creates large digital files representing all crucial details of stained tissue sections with decent resolution and high colour fidelity achieved using automated focusing and white balance. Digital slides are made up of giant arrays of rectangular pixels organised along x-y coordinates, each of which is characterised by size, colour and intensity values.

Produced either by area or line scanning, digital slides are built up as pyramids of microscopic image series where low power views are generated by compressing the original sharp and optimally lit images (Fig. 1A). Scanning through several focal levels within the usual 3-8µm sample thickness offers access to the z dimension used for emulating fine focusing of the optical microscope. Fig. 1 A) The image series by compression allows viewing of the digital slide at arbitrary magnifications (e.g. x15 and x10). Scanning at different focal levels within the sample thickness (~3-8µm) offers access to fine details in the z-dimension.

Using x10 lens offers high field of view (FOV) size and focal depth, while requiring small storage space. A x20 objective allows double the optical resolution than that of x10, but at the expense of revealing smaller FOV and focal depth, while increasing the storage need. x40 objective does not offer significant improvement in optical resolution compared to x20 (NA=0.9 vs.0.8) despite needing large storage space and long scanning time.

Bela Molnar MD DSc is CEO of 3DHISTECH Ltd, which has developed high-performance hardware and software products for digital pathology since 1996. A medical graduate from Semmeweis University, Budapest, and the Hungarian Academy of Sciences, he gained qualifications as an internist and gastroenterologist, followed by a post doctoral 2-year fellowship with Boehringer Ingelheim GmbH. Scientific/industrial cooperation arose with Roche Diagnostics, Epigenomics Inc (Seattle/Berlin), and Carl Zeiss MicroImaging. Today, Molnar's main research areas include biomarkers of colorectal cancer development, molecular biology applications, virtual microscopy, and quantitative image analysis, and his publications, memberships of scientific bodies and professional awards are numerous.
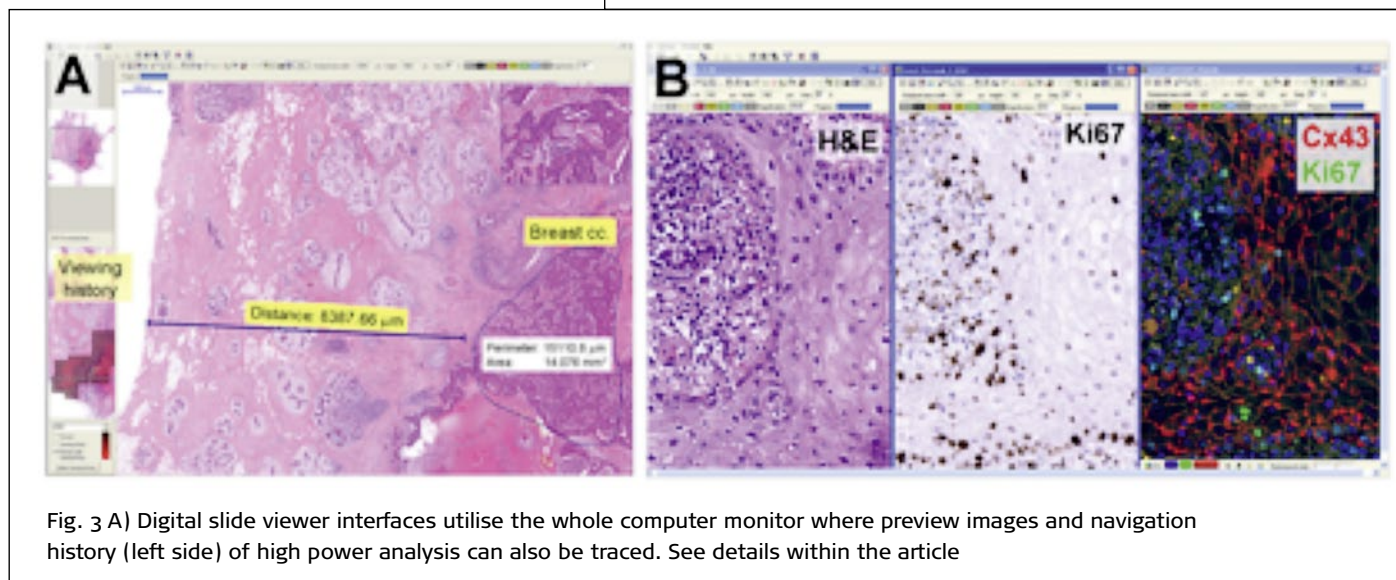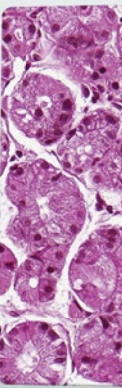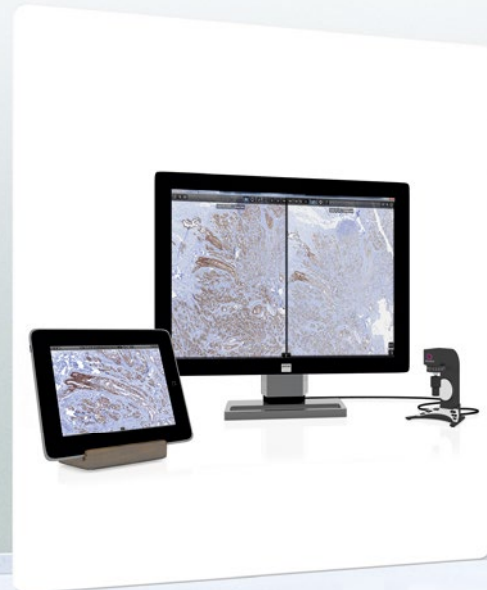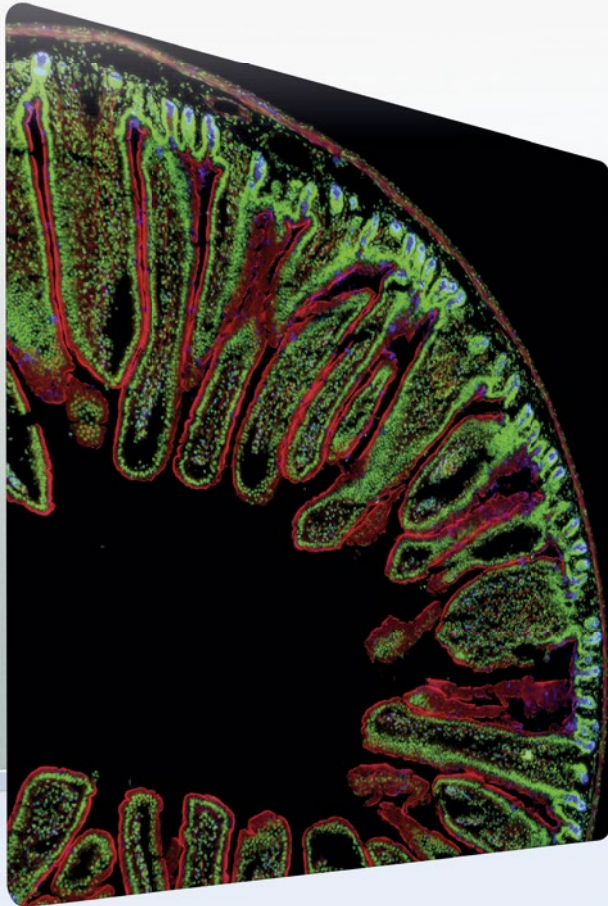
Fig. 3 A) Digital slide viewer interfaces utilise the whole computer monitor where preview images and navigation history (left side) of high power analysis can also be traced. See details within the article
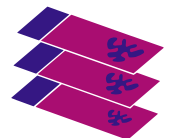
Unique features of digital slides

Seeing slides on a monitor, with easy access to a computer's multi-functionality, is far more ergonomic than peering though an ocular lens of an optical microscope. Even pathologists with high affection for conventional microscopy respect digital microscopy benefits if they practise enough. The computer-generated image pyramid format of digital slides allows in-focus navigation through continuously changing magnifications, without changing objectives, or re-aligning the focus or lighting conditions. Digital magnifications beyond that used for scanning still reveal fine microscopic details hidden in the original magnification.

Slides can be tilted arbitrary for proper orientation and preview images of the whole slide are available simultaneously on the monitor where navigation history of high power analysis can also be traced (Fig. 3 A). Digital slide viewer interfaces utilise the whole computer monitor where preview images and navigation history (left side) of high power analysis can also be traced. Calibrated pixels allow straight measurements of object distance, perimeter or area highlighted by permanent annotations. The monitor can be shared for several digital slides for comparative studies, as shown by the same area of serial slides stained for H&E, the proliferation marker Ki67 (brown; middle) and the gap junction connexin-43 (red immunofluorescence) combined with the Ki67 protein (green; right), respectively in oral epithelial hyperplasia. (Fig. 3B)

Permanent annotations and text put on digital slides, straight measurements of object distance, perimeter or area and prompt still-image archiving at publication quality all support the pathology workflow. Several digital slides can be opened side-by-side on the monitor for comparative analysis of serial slides of a sample stained for different biomarkers. Even samples of immunohistochemistry and FISH (fluorescence in situ hybridisation) can be opened, linked and navigated alongside, which normally need consecutive steps or even separate microscopes.

Pixels making up the images have calibrated dimension, colour and intensity, features that can be used for colour separation-based automated quantification and measurements of image-objects.

Furthermore, pattern recognition of morphological and functional units within the tissue, such as glands, or hyperplastic or abnormally arranged epithelial nests, can be automatically made based on shape, size and texture identification.

Serial digital slides can also be assembled into a 3-D structure for reconstructing tissue architecture, e.g. to study tumour invasion or re-orient colorectal biopsies. Digital data, including whole digital slides with annotations and measurements, can be integrated into digital databases and shared though intra- or internet with unlimited partners, even simultaneously. The freedom to access digital slide archives for re-review, and the logistics of slide storage and sorting are
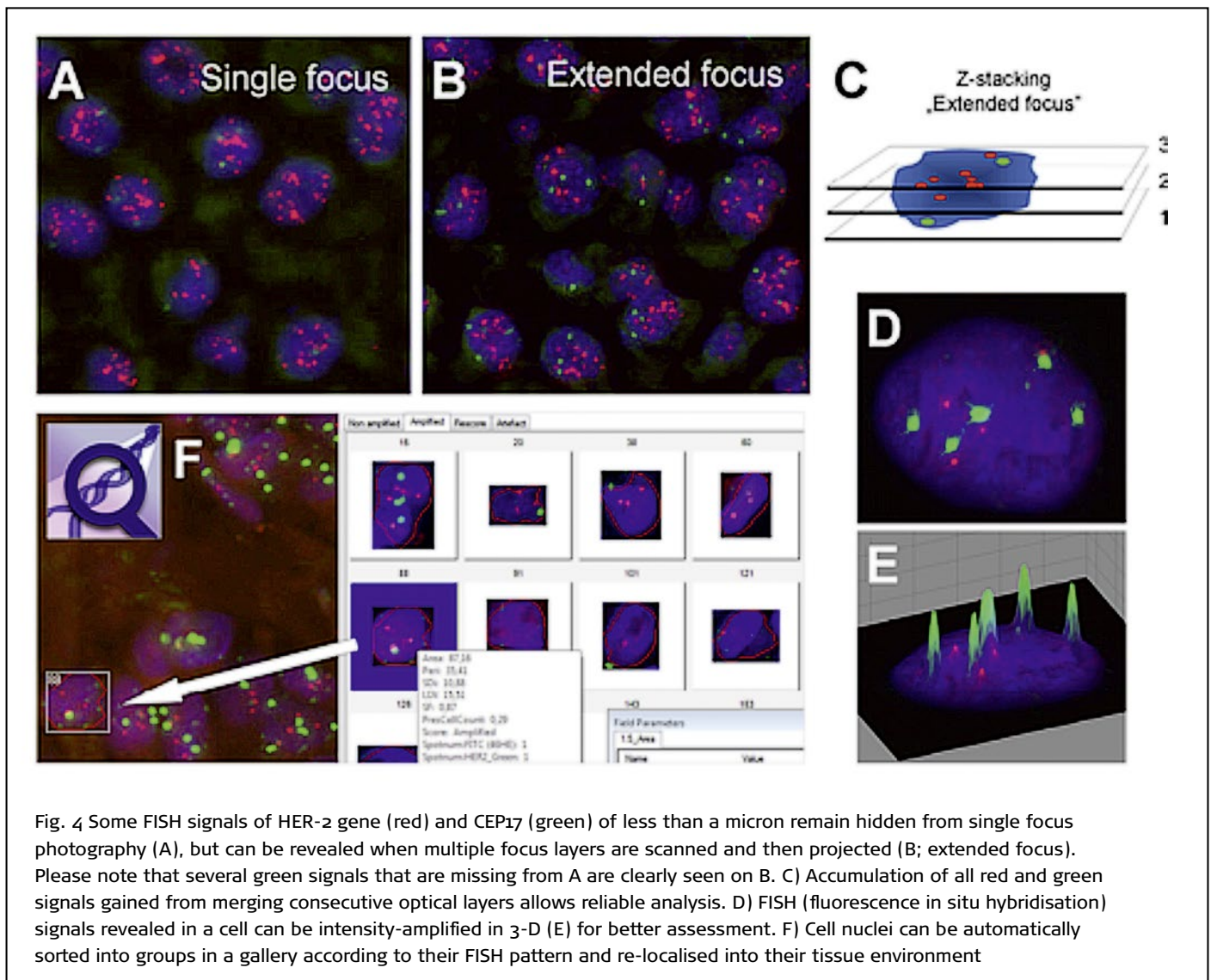
Fig. 4 Some FISH signals of HER-2 gene (red) and CEP17 (green) of less than a micron remain hidden from single focus photography (A), but can be revealed when multiple focus layers are scanned and then projected (B; extended focus). Please note that several green signals that are missing from A are clearly seen on B. C) Accumulation of all red and green signals gained from merging consecutive optical layers allows reliable analysis. D) FISH (fluorescence in situ hybridisation) signals revealed in a cell can be intensity-amplified in 3-D (E) for better assessment. F) Cell nuclei can be automatically sorted into groups in a gallery according to their FISH pattern and re-localised into their tissue environment

Thursday, May 26th ● **12:15 PM–12:35 PM** ● **Room: Robert Koch** ● **Digital pathology for and in the routine:new generation systems**
B. Molnár, Budapest, Hungary

15

simple tasks managed through a computer. All these, of course, need advanced IT, including high speed computers, massive storage capacity, safe data handling using backup storage, and wide-range internet access and dedicated software tools with a user-friendly graphic interface.

Molecular pathology visualisation

Fluorescence microscopy detects fluorophores used to label molecules in cells and tissues with the techniques of molecular morphology. Fluorophores are activated at UV or visible wavelength to emit light of lower frequency, usually in blue, green or red, which can be collected through emission filters in a dark background.

Samples targeted with fluorescing labels, particularly genomic FISH, must be studied within a short time-frame to avoid false ne-gative results due to rapid signal fading (fading artefacts).

In addition, small signals of a few hundred nm size, such as those of gene and chromosome probes in FISH, or those of its chromogenic version (CISH), are randomly placed throughout the whole 3-8 μm thickness of tissue sections, or cells, and thus some remain hidden from conventional single focus photography. These signals can only be revealed with confidence by scanning several focal planes through the sample (z-stacking, or extended focus) for proper quantification of gene/chromosome gain, such as that of HER2 on chromosome17 (CEP17) in breast cancer (Fig. 4A-F); or for the fine spatial localisation of signals proving gene-translocations, such as that of t (9;22) resulting in the BCR-ABL gene fusion in a case of chronic myeloid leukaemia. Gene abnormalities may determine a specific diagnosis and the concomitant treatment options in an increasing number of malignant tumours.

Digital pathology within routine sign out processes

Once the digital slide scanners digitise the hematoxilin eosin, surgical pathology specimen, and then immunohistochemical second round and FISH third round slides are also available in the slide holder/server solution, a pathologist can evaluate them from the laboratory's digital workplace. (Fig. 5)

In addition to slide viewing, the software supports quantification of identified alterations. The digital report can be signed out with detailed image and quantitative data.

BELA MOLNAR

Fig.5 Digital pathology integrated in the routine workflow processes. Imaging of the grossing, different slide stainings are combined into one digital case, which is then later evaluated on a digital pathology workplace. The quantitative analysis is an integrated part of the digital pathology report

The digital age in diagnostics dawns

Pathologists in Utrecht step away from the microscope as the first fully digital workflow goes live for primary diagnostics.

Paul J van Diest MD, took charge of the Department of Pathology at the Utrecht University Medical Centre in 2003. He is a full professor at the university's medical school where, to date, he has personally supervised 57 PhD theses. He is also an Adjunct Professor of Oncology at the Sidney Kimmel Oncology Centre at Johns Hopkins in Baltimore, Maryland, USA, and serves on the editorial board of 23 international journals. Professor van Diest has also served as president of several international societies and published more than 600 papers in peer-reviewed journals.

'**The whole world wants** to stop by and see the show,' said Paul van Diest MD, who leads the Department of Pathology at the Utrecht University Medical Centre. Why? In March 2016 the centre began to run a fully digital workflow for primary diagnostics. The centre is now overwhelmed with requests for visits.

It was a bold move into the digital age where van Diest convinced the University Medical Center to roll forward the next five years of capital spending for the pathology department to pay for the new system in the first year. 'We replaced the old scanners, we set up a completely new server architecture and a workflow system, so that we can now do the diagnostics in a different way,' he said. 'For primary diagnosis we no longer use the microscope, we are looking at a digital image on a screen.'

'I can not give you a precise percentage because, during this transition period, we haven't logged any numbers, but my gut sense is that at least 90%, possibly as much as 95% of the diagnostic work, is now done in a completely digital way, which I think is pretty good for the first month.'

In May 2016, a highlight of the European Congress of Digital Pathology in Berlin will be a Roundtable Session on 'Digital Pathology Workflow Integration,' where van Diest will share with fellow pathologists his pioneering experience and some lessons learned.

'Change management will be my first bullet point,' he said during our interview. 'Stepping away from the microscope is a revolution for the average pathologist, so you have to make sure you have everyone on board to do something this radical. This means influence and involvement, the key terms.'

'My second point will be to make sure you get the right stuff, because it's difficult to make an optimal system with the wrong stuff,' he said with a laugh, adding that 'It's not just about the hardware, it's also about the people from the company, who you will need to have on-board to help with project management, because it is very complicated to bang this down.'

'Then it becomes important to design upfront exactly what you want,' van Diest advises. 'This sounds obvious, but you need to get the design really right – the architecture for the system. To know what you want to have in the end becomes critical at the beginning.'

Following a pan-European tender, the Utrecht pathologists selected a line of scanners from Hamamatsu Photonics of Japan and a workflow informatics system from Sweden's Sectra.

The conversion, over five months, to digital for diagnostics was less a revolution than an evolution for the pathology group in Utrecht, which has been regularly handling digital images with an archiving system set up in 2007 for the retrieval of old cases, whether for comparative study, for research or education.

Thanks to this early experience with archiving, the Utrecht group was also prepared for the massive storage challenge the new digital diagnostics system presents. Reaching 800 terabytes the archival system was pushing the internal capacity. 'We had a chance to be part of a bulk central storage initiative at this big academic we are part of, so we joined as a customer,' the professor explained. 'The archives were transferred to the new system, which he said is safer, more secure and more affordable than maintaining a dedicated storage facility. Also, it is faster: flashing requested images on the screen in seconds, rather than in minutes.

Challenges and compromises

If the transition to working digitally has been fairly smooth, there remain both challenges and compromises. 'There are things that are simply not possible for digital processes yet,' van Diest pointed out. 'Here we simply go back to the slides. It is always possible to return to the slides. This does not mean the images are not good. It may be a difficult case. We certainly don't force someone to do a diagnosis in a digital way only to make a wrong diagnosis.'

There also remains the controversy of depth-of-view on digital slides versus the microscope view, which he said is 'a compromise we have to live with today'.

'We'd prefer to have three to five focus layers within every image. Yet that increases scan time by a factor of three to five, and increases storage requirements by a factor of three to five. This is something we simply cannot afford, both in terms of time and financially in terms of storage.'

Now that the diagnostic system is operational, the next stage is optimisation, working through what he called a long wish list that will keep the vendor companies busy for the next three to five years. The group, he said, is also keen to begin implementation of image analysis algorithms.

'The one we will start with, one we have developed ourselves, are algorithms for the recognition of mitosis, something very important in cancer diagnostics that is now done in a subjective way. By using algorithms it can be done more quickly and more reliably,' van Diest pointed out.

Pleased by the high level of interest in visits, van Diest added: 'We are pioneers here. We had to invent the wheel, which means that other people who plan to do this will probably be able to do it slightly faster, perhaps better, and likely in a cheaper way than us.'

JOHN BROSKY

Friday, May 27th ● 5.30 PM ● Room: Hörsaal ● Round table discussions: Digital pathology workflow integration
P. van Diest, Prof. Dr., Dept. of Pathology, University Medical Centre Utrecht, Netherlands
L. Pantanowitz, M.D., Medical Center, University of Pittsburgh

Spanish pathologist to highlight image storage issues at ECDP

Compressing and storing digital images in pathology remains technically challenging but there are many options that can both help reduce costs and improve efficiency, a Spanish expert will explain in a dedicated talk during ECDP in Berlin.

High-resolution digital pathology images can weigh up to 1GB. Loaded with information on the patient's clinical situation, these pictures are a goldmine for investigation and can help tailor treatment to each patient. Digital pathology image analysis already helps quantify biomarkers for cancer treatment and could soon enable to detect tumor areas just like mammography, according to Marcial García Rojo, director of the pathology department at General Hospital (GH) Jerez de la Frontera in Cadiz, Spain. "Microscope images already enable to identify suspicious areas. The big plus is efficiency; image analysis saves us a lot of time," he said.

These possibilities trigger a growing need for images. But as more and more pictures are generated, they occupy more and more space and current storage systems offered by the industry are unsustainable, Rojo believes. "For example our team generates about 150 digital slides a day. 150GB of images multiplied by 200 working days a year, that's about 20TB of information volume per year. It's impossible to sustain this rhythm much longer if we can't find cheaper storing solutions. Besides manufacturers only reduce the size of images from 1GB to 800MB without losing important information; that's good but insufficient," he said.

Rojo, a pathologist who trained in medical IT extensively, has been working on digital pathology scanners since 2005. He now focuses on transforming pathology images to DICOM to move them onto standardized DICOM servers and repositories, one of the best options to reduce space and costs. "Images generated with DICOM can be even smaller than those generated in owner format," he explained ahead of his talk about standardization in pathology with DICOM and IHE.

Following the radiology example, pathologists can store images in a dedicated picture archiving computer system (PACS). They can also save images on discs and films, a slower but significantly cheaper alternative. "The point is to use something that is economically relevant to store the huge amount of information and images we need today," he said.

Pathologists can also use low cost vendor-neutral archive (VNA) systems to store images in a repository cheaper than the one used for radiology images. Several companies, including Agfa HealthCare, Carestream Health, GE Healthcare and Merge Healthcare, now offer VNA products either in-house or through acquisitions.

Dr. Marcial García Rojo is director of the pathology department at Hospital de Jerez de la Frontera in Cádiz, Spain. From 2009 to 2013 he was head of the pathology department at Ciudad Real University Hospital and associate professor of pathology at the Medical School of Castilla-La Mancha University.
Dr Garcia Rojo is president of the Ibero-American Association for Telemedicine and Telehealth (IATT) for the term 2015 -2017. He is also vice-president of the Spanish Society of Health Informatics (SEIS), Spanish representative on the International Medical Informatics Association (IMIA) board and past-president of the Internet Association for Biomedical Sciences (INABIS).
He is collaborating as a researcher in European projects on digital pathology such as AIDPATH (Academia and Industry Collaboration for Digital Pathology).
His main research areas are medical informatics standards in digital pathology and molecular pathology.

Another possibility is to erase the images after a given period of time. Pictures do not need to be saved forever; the paraffin block, from which images and slices can be generated anytime, always remains as a back up for future investigation in molecular pathology or genomics.
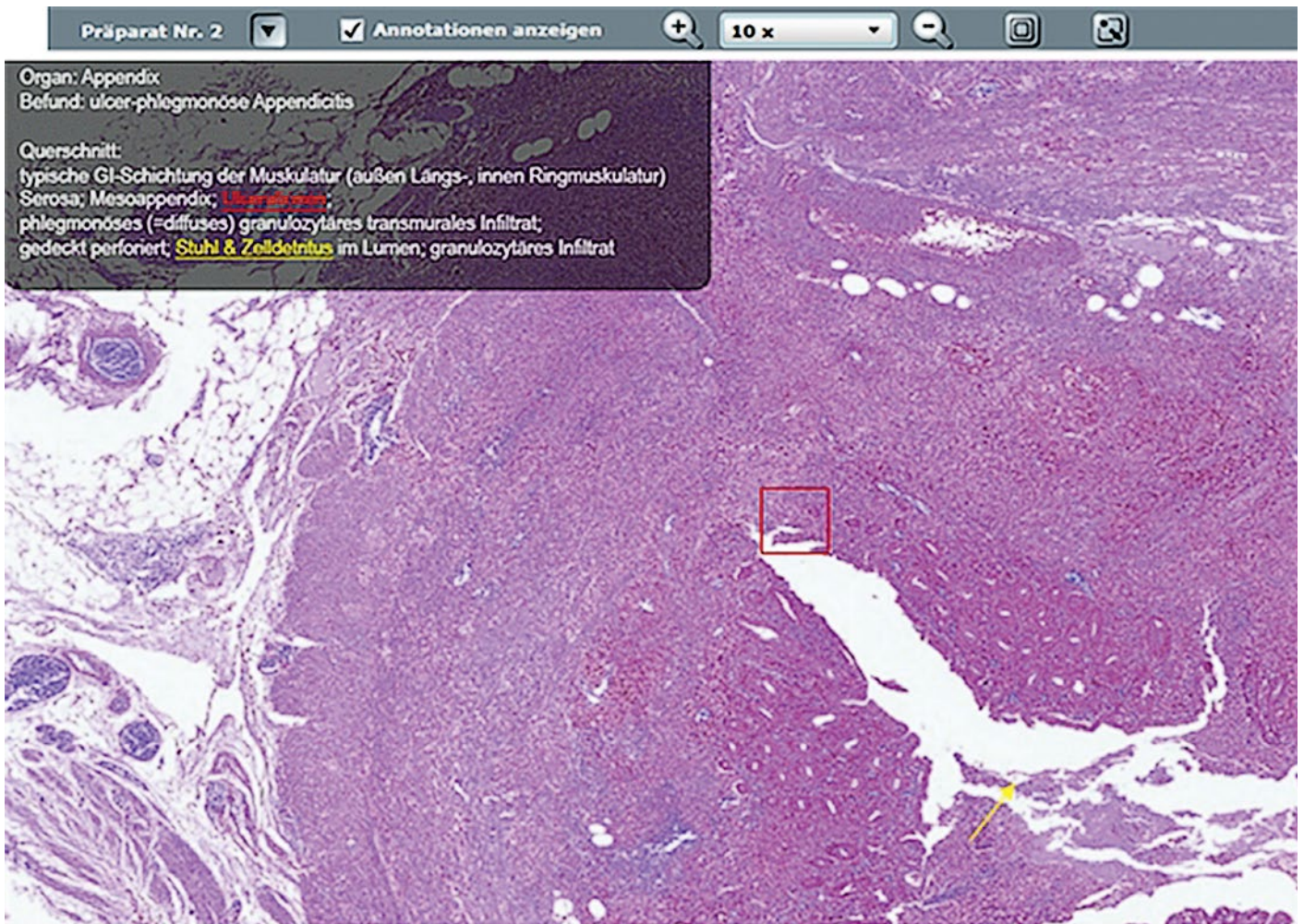
25 public hospitals are equipped with digital pathology scanners in Spain, but only five use them in clinical practice: GH Jerez de la Frontera, University Hospital Arnau de Vilanova in Lerida, University Hospital in Ciudad Real, and Barcelona's Del Mar Hospital and University Hospital Clínic. The latter leads the way with up to 300 preparations a day. Several digitalization projects for private clinical practice are underway across Spain. Santa Lucia University Hospital in Murcia offers telepathology services to the rest of the country.

"Spanish digital pathology is doing well. Almost every pathology department now digitalizes preparations. Sweden, Norway and the Netherlands are still leaders in clinical practice, but the situation across Europe is very irregular. We're doing what we can to stay ahead of the curve," Rojo said.

MÉLISANDE ROUGER

» **Friday, May 27th** ● **1:30 PM–1:45 PM** ● **Room: Hörsaal** ● **A review on international guidelines for digital pathology**
M. García-Rojo, Hospital de Jerez de la Frontera, Pathology Department, Jerez de la Frontera, Spain

Präparat Nr. 2 ▼   ☑ Annotationen anzeigen   ⊕ 10 x ▼ ⊖   ⊡ ⊡

Organ: Appendix
Befund: ulcer-phlegmonöse Appendicitis

Querschnitt:
typische GI-Schichtung der Muskulatur (außen Längs-, innen Ringmuskulatur)
Serosa; Mesoappendix; Ulcerationen
phlegmonöses (=diffuses) granulozytäres transmurales Infiltrat;
gedeckt perforiert; Stuhl & Zelldetritus im Lumen; granulozytäres Infiltrat

Goodbye to the microscope? Not yet!

Carol-Immanuel Geppert MD, from the Institute for Pathology at Erlangen
University Hospital, Friedrich Alexander University Erlangen-Nuremberg,
debates the impact of digitisation on pathology.

Digital pathology is already an important part of teaching at the Institute for Pathology, Erlangen University Hospital. Via the internet, students can access all course contents, browser-based with annotations and texts, as well as for monitoring achievements (online microscope based on development in cooperation with Fraunhofer IIS Erlangen, www.pathoskopieren.de)

Digital Pathology (DP) is the fastest growing area of pathology, along with molecular pathology. However, the use of digital tools such as scanners and analysis software, as mentioned in a previous article, is mainly limited to academia (volume 24. issue 6/15). Academic medicine benefits most in teaching and research where larger investments can be applied for without the pressure of daily clinical routine, or the economic pressures of the health system.

In Erlangen, DP has been an established part of teaching in human, molecular, and dental medicine. Using an online-microscope, students from Würzburg, Regensburg and Erlangen (Cooperation partners: the universities and university hospitals as well as the Fraunhofer Institute Erlangen) can access digital slides from their respective courses browser-based via the internet, from anywhere and can study with superimposed texts and annotations (image 2). In addition, in recent research some projects have also been driven with the help of DP within the Comprehensive Cancer Centre Erlangen–Nuremberg, addressing problems from different clinical fields.

DP already plays an important part in national and international cooperation projects. Naturally, new and fast-paced technology also has its limitations, which need to be clearly stated. The error rate of the scanners with slides or low contrast, or errors in digital image analysis (DIA) caused by artefacts can negatively impact on trust in this new technology. Therefore, there is also some criticism and scepticism amongst pathologists.

Furthermore, next to the many advantages of DP there are also clear disadvantages, such as high initial investment costs for scanners and data storage, as well as the on-going costs for maintenance and support. Pathology will be confronted with si-

milar problems to those seen in radiology years ago, during the initial conversion to digital image processing. Although terabyte (TB) mass storage is now comparatively affordable, the amount of data involved in DP is extremely high. One case with five sections can take up between 2.5 to 20 gigabytes (GB), i.e. the equivalent of a vast storage requirement of several 1,000 TB, which converts to several PB per year, assuming all cases from one large centre were to be digitally stored every year. This new dimension of data exceeds the requirements in radiology by tenfold and would therefore be unprecedented in clinical medicine.

By contrast, there is the new dimension of the interpretability of an immeasurable number of cells with their colourings for immunohistochemistry, CISH or FISH. New analysis software – digital image analysis (DIA) – from companies such as Definiens or Indicalab, can quantitatively evaluate all cells from one section. This can be a routine section for one patient or samples from many patients via tissue micro arrays (TMAs), where more than 400 samples can be put on one slide (such as GrandMaster, 3-D-histech, Hungary). This will deliver completely new and detailed views of tumours and their specific biomarkers. Although previously we could gain good and realistic estimates with the bare eye, we can now generate far more exact evaluations of hundreds of thousands of tumour cells in one section, for instance for each individual tumour cell. Until recently this was completely impossible.

The integration of individual parts of DP into clinical routine is already happening in interdisciplinary tumour boards and conferences, in telepathology and through the transmission of digital slides in the context of studies or consultations. It has facilitated a much faster, worldwide exchange between experts and colleagues without the need to physically send sections. As there has been an abundance of new technologies and innovations over the last few years and a continuously expanding market for imaging equipment (scanners and the camera technology used in them) for image processing (reconstruction and visualisation software) as well as for digital image analysis (DIA) the need for standards for diagnosis and research has become equally imperative.

If, one day, DP was to become established in daily routine it will have to withstand comparison with the microscope in daily practice, in terms of practicability, sturdiness and, most importantly, speed. The microscope is unlikely to disappear. Some areas, such as quick section diagnosis where a diagnosis has to be achieved within

15 minutes during on-going surgery, there can be no digitisation. However, we (including Professor Peter Hufnagel at the Charité Berlin and Professor Gian Kayser at Freiburg University Medical Centre) believe that, more and more pathologists will mainly work digitally, and also beyond university settings, in the distant future, because the requirements from this field will continue to grow and the investment costs for scanners and data storage are likely to fall due to increased competition. In our view the full potential of DP, which is as versatile as it is promising, has not yet been exhausted, by far. However, high quality standards in image generation, processing and analysis must be established independent of manufacturers. They should be the basis for the continuously growing and ambitious community of pathologists in diagnostics, research and teaching. DP can help achieve a new measure of quality, particularly in the growing field of cancer diagnostics (companion diagnostics) with immunohistochemical biomarkers such as Her2 or PD1/PD-L1, which are decisive for treatment.

The tool will become a robust, reproducible, secure, comprehensively quantitative and observer-independent aid for diagnosis. With the help of DIA, important biomarkers, such as prognosis parameters, can be completely, quantitatively, digitally evaluated (e.g. Her2-FISH in Z-stack) even three-dimensionally. DIA is therefore superior to the previous methods, as it makes millions of tumour cells analysable, if necessary even in several layers (for FISH signals). Furthermore, observer-independent, digital evaluations will lead to a location-independent, comprehensive increase in diagnostic quality for certain problems, be it in a large centre or a peripheral practice.

Along with many well-known pathologists we believe that DP will have become an established part of a hybrid workflow consisting of DP and conventional microscopy in clinical routine within the next 10 years. The advantages speak for themselves and there is no end in sight for the rapid developments and resulting opportunities for application.

I believe many pathologists are following the rapid developments in digital pathology with excitement and interest, as well as with scepticism. Most of them are not yet happy to swap their microscopes for computers. However, within the next ten years I believe that a hybrid diagnostics workflow, consisting of conventional microscopy and digital image analysis, will be established. A comprehensive change to purely digital diagnostics is still a long time coming.

WALTER DEPNER

Carol-Immanuel Geppert MD from the Institute for Pathology at Erlangen University Hospital

The Digital Path (ology) to Personalized Medicine

Though it is the underlying science that drives diagnosis and treatment decisions, pathology is an often overlooked field. As part of the health continuum, as the turning point for treatment, as a new source for research and discovery – in all these ways, the power of pathology has gone unnoticed. However, the industry is slowly coming to realize its potential in transforming care.

Personalized medicine offers the promise of individualizing care – a particularly strong motivator in the field of oncology. By harnessing the data that pathology already has, we can be the ones to put the industry on the path to making personalized medicine a reality.

Size Does Matter...

In research, size matters. The more information you have, the better; it gives you raw material to work with and it validates your finding. When it comes to pathology, size matters both in terms of how many samples a pathology repository may contain and the size and quality of each piece of data related to those samples.

The Mount Sinai Health System in New York, USA is comprised of seven hospital campuses serving approximately 170,000 inpatients and 2.6 million outpatients annually. Over the years, these facilities have collectively accumulated hundreds of thousands of tissue samples – all existing in the form of glass tissue slides.

Though they have historically been used to diagnose and then archived away, these slides hold clues to diseases, treatments and potential breakthroughs. This sheer quantity of data that has been hiding away is the epitome of untapped data. Last year Philips and Mount Sinai announced to create a comprehensive digital image repository containing the digital scans of all these glass tissue slides.

"Big data" may be taking center stage today, but pathology is filled with something that may be even more critical in healthcare – "fat data." Digitized slides are huge files with extremely high-quality, detailed images that capture secrets of the human body from blood and tissue across a range of diseases. The insights they hold don't offer the big picture worldview that big data promises, but they are invaluable about a very specific item that could transform care for an individual – or a population.

...But Context Matters More

However, sheer numbers are not enough. How can this data be translated into knowledge? By creating a comprehensive digital image repository of all these slides, researchers and clinicians suddenly have access to a huge library of source material. While having millions of digital pieces of tissue has its own value, that value is multiplied when that data is correlated to other clinical data for a given patient, with that patient's history in mind. That leads to a much more comprehensive view and analysis of results, treatment protocols and behavior at a personalized level. By taking this information and integrating it; analyzing it; looking at whole slide pathology images from clinical laboratory services, genetic analysis, radiology, and surgical and molecular pathology, the deep and complex puzzle pieces about individualized patient care start to come together.

Unlocking its Secrets

Now take pathology data, in context with healthcare data, and multiply it by
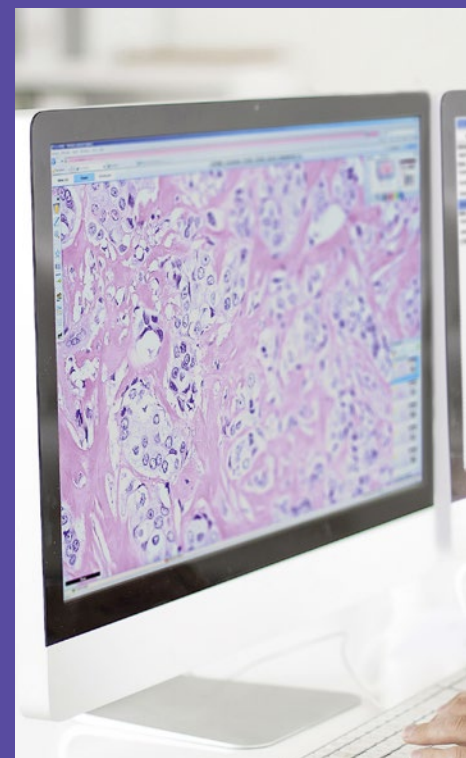
hundreds of thousands of cases. The combination of quantity and quality will undoubtedly unlock unique insights that will enable the development of predictive analytics to help personalize patient care. By understanding how diseases and treatments work and being able to look for distinguishing clues, researchers will be able to pursue the discovery of new tissue-based tests and uncover specialized treatments. At the same time, having ready access to high quality information on diseases will allow pathologists to make more confident diagnoses and enable faster intervention. Together, this offers the potential for pathology to drive a new paradigm in healthcare that includes the optimization of treatment efficacy and superior clinical outcomes to maximize personalized patient management.

Additionally, we can start looking at data outside the traditional health continuum. When pharmaceutical companies conduct drug trials, they not only have to track, data to get a drug approved and released but then they track it over time to measure its effectiveness. We have all this data now.   Forward-thinking institutions are realizing the promise of digital pathology, and will put the industry on a path to endless opportunities in genomics and personalized medicine. Though pathology has often worked in the background of healthcare, new technologies are enabling a new and exciting opportunity for us to show our value and transform care.

BY HANS DRIESSEN, PHILIPS DIGITAL PATHOLOGY SOLUTIONS

Three-step process for digital pathology

As laboratories in Europe shift to systems for digital pathology, they must ensure the technology not only works, but works for them, says Dr Liron Pantanowitz, director of pathology informatics at University of Pittsburgh Medical Center (UPMC).

On 27 May, at the 13th European Congress on Digital Pathology in Berlin, pathologist Dr Liron Pantanowitz, from the Pittsburgh Medical Center will give the keynote address 'Strategies and demands for digital pathology workflow integration', discussing how to bring digital technologies into a laboratory without disrupting the processes already set up. 'Just because you're bringing in new technology doesn't mean you're going to do a better job,' Pantanowitz confirms. 'The people have to be willing to work with the technology and be efficient.'

Later in that 3rd day of the congress, Pantanowitz will also participate in a roundtable discussion on Digital Pathology Workflow Integration.

In his keynote address, the pathologist will go over the three-step process for digital pathology: pre-imaging, imaging and post imaging, providing strategies for incorporating scanning of slides into a lab's workflow, and discussing the importance of training people to do high-quality imaging to create the best slides and how laboratories can make decisions about saving immense amounts of data.

Going digital could have a negative affect on a laboratory's workflow if, for example, employees batch all the work of scanning slides, or if there's downtime, Pantanowitz points out.

If laboratories follow the right integration strategies, he adds, they can take advantage of the many benefits of a digital system -- using computer-aided diagnostic tools and conducting image-based searches,

Pantanowitz will also touch on his work with Onyx, a company that provides

Dr Liron Pantanowitz is Director of Cytopathology at UPMC Shadyside. He is also Director of the Pathology Informatics Fellowship Programme and Associate Director of the Pathology Informatics Division.

digital pathology technology and is a joint venture between GE Healthcare and UPMC

Digital pathology is gaining ground in Europe faster than in the USA, Pantanowitz points out, with less stringent regulations governing its use.

The country's Food and Drug Administration (FDA), which regulates medical devices, states that digital pathology cannot be used for primary diagnosis. While the USA's laboratories can use digital pathology technology 'off label', they risk being held liable if there should be a malpractice case. As a result, three laboratories in Europe have gone fully digital, according to Pantanowitz, while he is not aware of any fully digital laboratories in the USA.

UPMC runs a digital pathology consultation service, providing second opinions to pathologists, clinicians and patients around the world, which provides the facility with an additional revenue stream.

The Pittsburg Medical centre is in the process of collecting data regarding the use of digital methods for primary diagnosis to help make the case for using digital technologies for primary diagnosis, Pantanowitz adds.

LISA CHAMOFF

>> Friday, May 27th ● 9:00 AM–10:00 AM ● Room: Hörsaal
Strategies and demands for digital pathology workflow integration
L. Pantanowitz, University of Pittsburgh, Medical Center, Pittsburgh, United States

Taking biomarker research to a new level

Harnessing the potential of digital pathology is taking research into new and more efficient biomarkers to a new level.

By combining strategic planning with the latest digital pathology technology, high quality tissue microarrays for biomarker research are being produced. This December, at the Digital Pathology Congress in London, the application of digital pathology in this area was highlighted by Professor Inti Zlobec from the Translational Research Unit at the Institute of Pathology, University of Bern, in Switzerland,

Her presentation on the 'Application of digital pathology to the construction of high-quality tissue microarrays for biomarker research: The next-generation tissue microarray (ngTMA) approach' demonstrate how digital pathology can be applied to construction of tissue microarrays. 'The inclusion of digital pathology into our tissue microarray workflow means we can have very high quality tissue microarrays that also allow us to address research questions we could have never answered before,' she told European Hospital, ahead of the international meeting.

Zlobec explained that by using digital pathology constructing tissue microarray becomes more efficient and precise. In an example where a biomarker needs to be examined on 500 tissue blocks, small cores can be punched out from each tissue and repeatedly transferred into an empty paraffin block to produce a tissue microarray. 'Tissue microarrays are a way of putting together an archive of all of these different patients material into one single block,' she added.

The process effectively creates a tissue microarray that contains dozens, or even hundreds, of different tissue cores, ranging in size from 0.6 to 2.0mm in diameter that contains selected regions for transfer into the TMA. 'This would have been impossible without digital pathology,' she confirmed. 'Before, we had to approximate from where we would be taking out those tissue cores, but now, because we have digital pathology, we know exactly which region we want transferred out from that block.'

This is a 'more specific, targeted and efficient' approach. However, Professor Zlobec believes at Bern they take the approach one step further by defining their ngTMA approach by three pillars: planning and design; application to digital pathology; and automated tissue micro-arraying.

'The planning phase makes or breaks the TMA quality, so regardless of how big the project size is – 10 patients or 1,000 – the planning is a critical point,' she explained.

Examples of key questions to keep in mind are: does the research question require a targeted ngTMA approach or can it be achieved without digital pathology; what histological regions need to be captured to answer the research question; how many patients does the project need to achieve the required statistical power; and how many cores should you use to account for the possible heterogeneity in the tissue?

'These are questions we never really asked before, when we did not have this new ngTMA approach,' Zlobec added. 'Once we have the plan and a corresponding slide we scan it, view it, annotate it, and finally align it with the actual tissue block from which it came. The annotated regions are cored out and transferred so, in the end, we have this amazing and precise ngTMA block, which can be cut and prepared for biomarker analysis.'

The Bern team also creates ngTMAs of animal models of human disease for comparison. There are significant benefits for clinicians, researchers and patients.

'On the one hand, we have an ngTMA approach that allows for high throughput biomarker screening and, on the other, allows us to go in-depth to answer targeted research questions that can only be addressed because we have this digital pathology aspect.

'We can apply our approach to a large number of patients in a way that is very cost-effective and resource sparing in terms of tissues, consumables and time in the lab. 'Because we can make these digital annotations on scanned slides we can construct ngTMA in a very precise manner and for the first time we can study important interfaces inside the tissue.'

Additionally, in cancer biomarker research, for example, intra-tumoural heterogeneity can be explored with ngTMA by annotating different regions within the tumour.

Also, because the slides are digital, the pathologists can go back to the annotation and see what exactly was cored out from that block, ensuring high levels of quality control.

Such use of ngTMA helps to create standards for biomarker research and, because tissues that are included in a block undergo the same experimental conditions, this means that variability from batch processing can be considerably reduced.

'All of these aspects combined mean we achieve high quality biomarker research and validation that can hopefully be more effectively translated back into patient management,' she said.
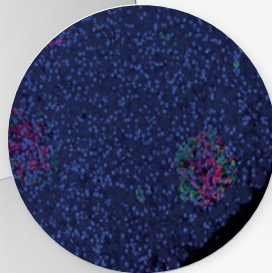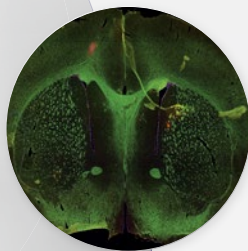
The next step – as well as challenge – is the quantification of the biomarkers using image analysis software to objectively measure the biomarkers, which in effect 'opens a whole field of digital pathology research'.
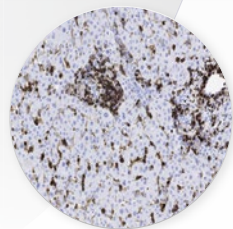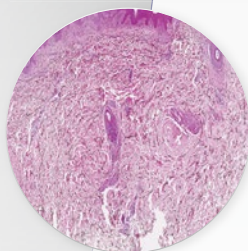
MARK NICHOLLS

Inti Zlobec PhD is professor and head of the Translational Research Unit at the Institute of Pathology, University of Bern, where she is involved in inter-disciplinary translational research that aims to improve the clinical management of patients with colorectal cancer. Her research also focuses on histomorphological biomarkers as prognostic and predictive features of tumour response to therapies.

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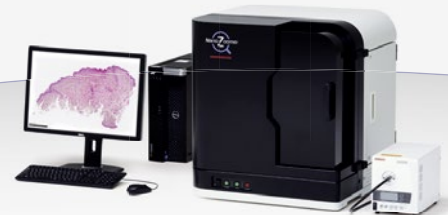
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